North Carolina Genomic Evaluation by Next-generation Exome Sequencing, 2 (NCGENES 2)

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(NCGENES 2)

March 12, 2020

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ABOUT THE PROTOCOL

Protocol Formatting

Headings

Main: **CENTERED, BOLD, & UPPERCASE (SIZE 14 FT.)**

Sub-heading I: **Flushed left, bold, blue (Size 14 ft)**

Sub-heading II: **Flushed left, bold (Size 13 ft)**

Sub-heading III: ***Indented left, bold-italics, blue (Size 12 ft)***

Sub-heading IV: ***Indented left, bold-italics (Size 12 ft)***

Sub-heading V: Indented left, underlined (Size 12 ft)

Note: Boxed (Size 11 ft)

Emphatic Note: Boxed, blue-background (Size 11 ft)

Font

Type: Avenir Next Cyr (Body)
Size: 11 ft
Body: Flushed Left

Statuses should be referenced in upper case
References to pages in tracking should be italicized.

Study documents, especially mail merged documents, should be downloaded directly from **IRBIS** folder to ensure that the most recent edited and authorized document versions are being used. Other documents can be downloaded from IRBIS and copied on appropriately colored paper (see below), HOWEVER careful attention should be paid to use the current version of the document. IF ANY change to a copied form is made and subsequently approved by IRB, all old copies of the form must be discarded, and a new original obtained. Careful attention should be paid that current versions of all documents are being used.

The UNC Study Coordinator is responsible for ensuring that all other sites (ECU/Mission) have access to IRBIS.

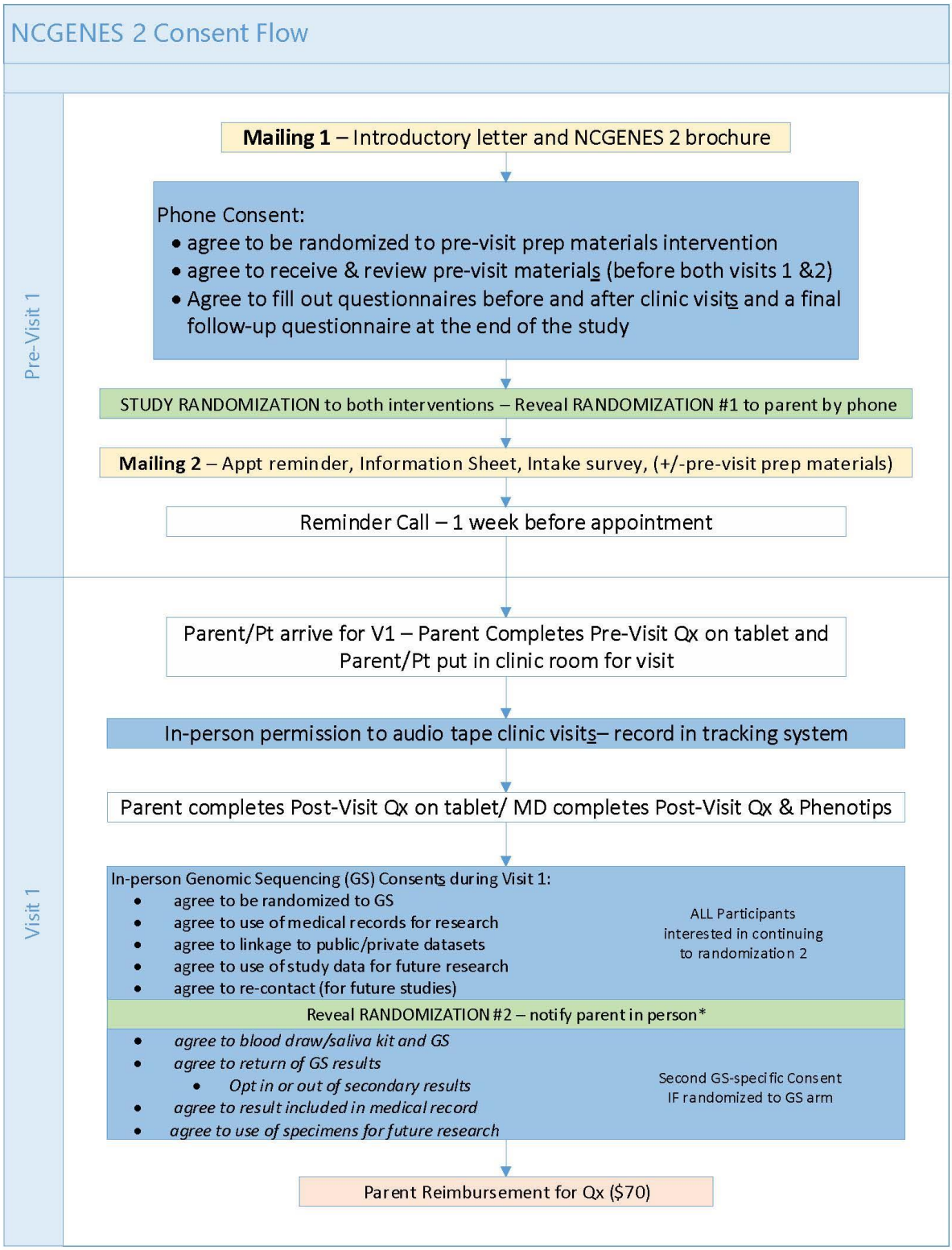
Abbreviations

BSP	Bio-Specimen Processing Facility
CDW	Carolina Data Warehouse for Health (sometimes referenced as CDWH)
CLIA	Clinical Laboratory Improvement Amendments Laboratory
DOB	Date of Birth
EMR	Electronic medical record
GC	Genetic Counselor
MRN	Medical Record Number
NC TraCS	North Carolina Translational & Clinical Sciences Institute
NCGENES 2	North Carolina Genomic Evaluation by Next-gen Exome Sequencing, phase 2
PI	Principle Investigator
QPL	Question prompt list
RA	Research Assistant
SC	Study Coordinator

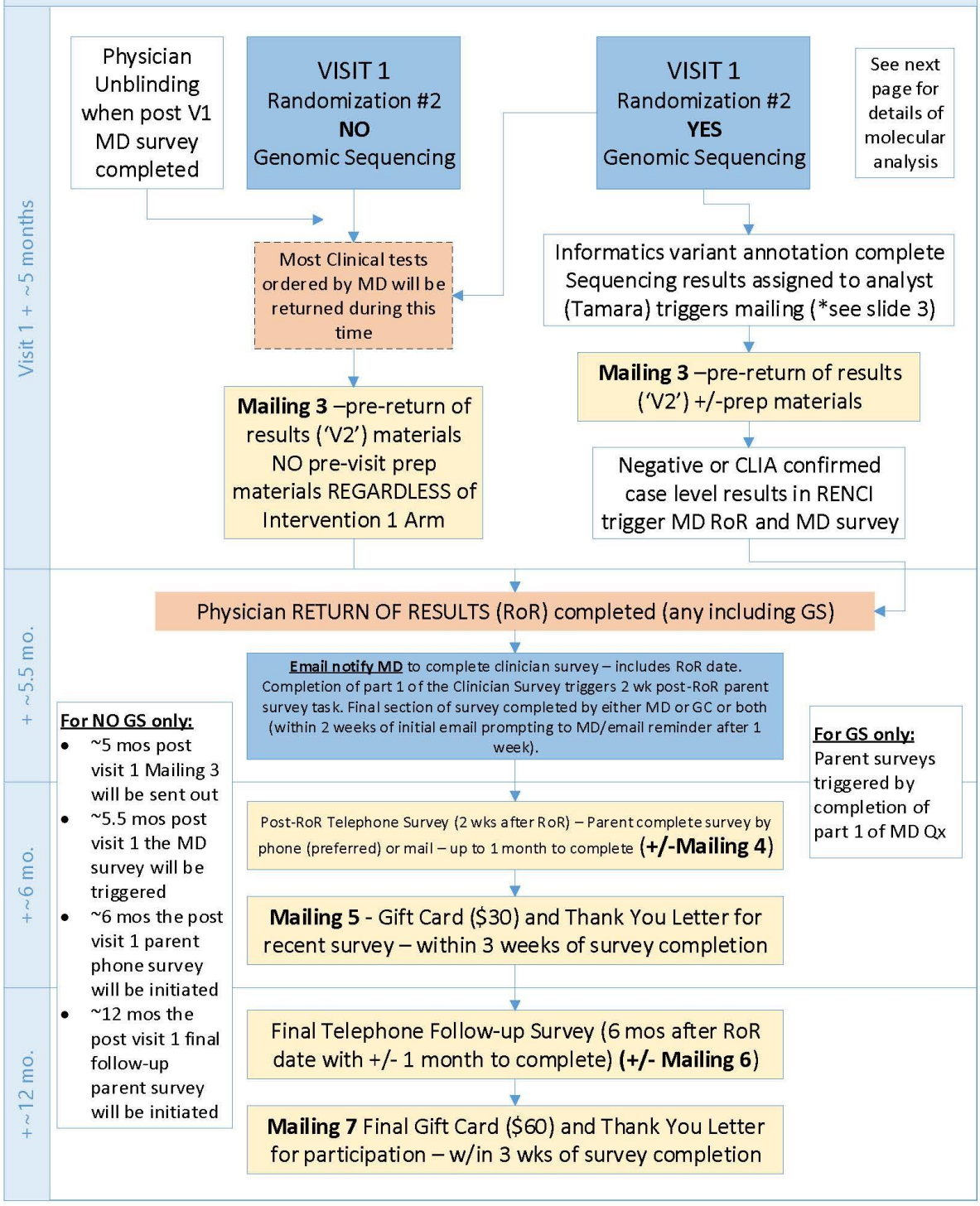
NCGENES 2 OVERVIEW

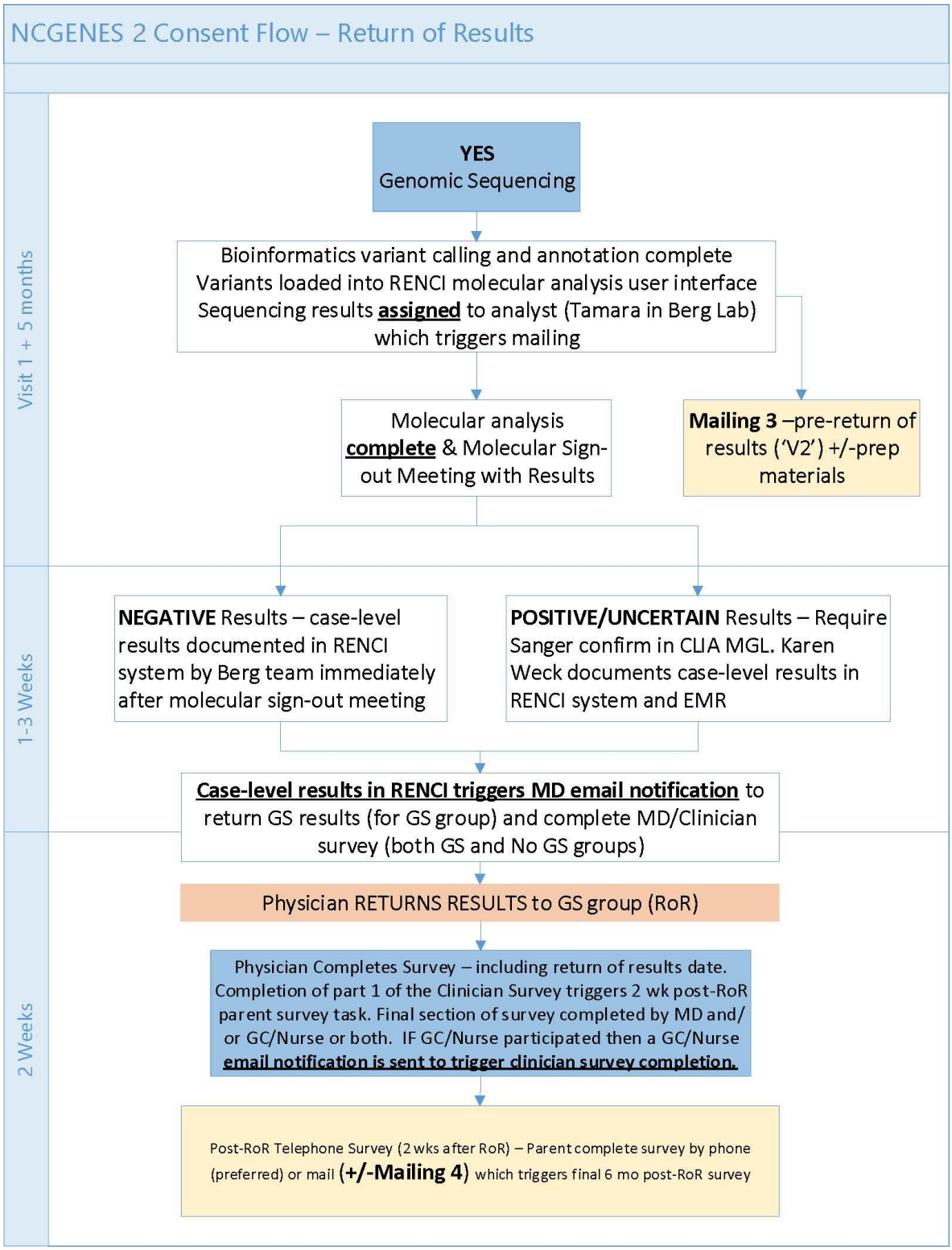
NCGENES 2 stands for the North Carolina Genomic Evaluation by Next-gen Exome Sequencing, phase 2. The research study investigates ways parents understand what to expect at their child's doctor visit and resources that might allow parents to speak more easily with their child's doctor. Parents who decide to take part in this research will be paid up to $170 for their time and their child may be offered a special test called exome sequencing, free of charge. This special test may help clinicians identify a reason for their child's condition. Understanding a child's condition may, in turn, help providers and parents/legal guardians plan for the child's care. Importantly, this study also attempts to understand differences between people who have an easy time getting health care and people who do not. The diagrams on the next few pages illustrate the overall flow of the NCGENES 2 study, from participant referral to follow-up surveys. New users should study these pages in detail to understand the structure of the study.

Study Flow - Enrollment, Consent, Visit 1, Return of Results and Follow-Up

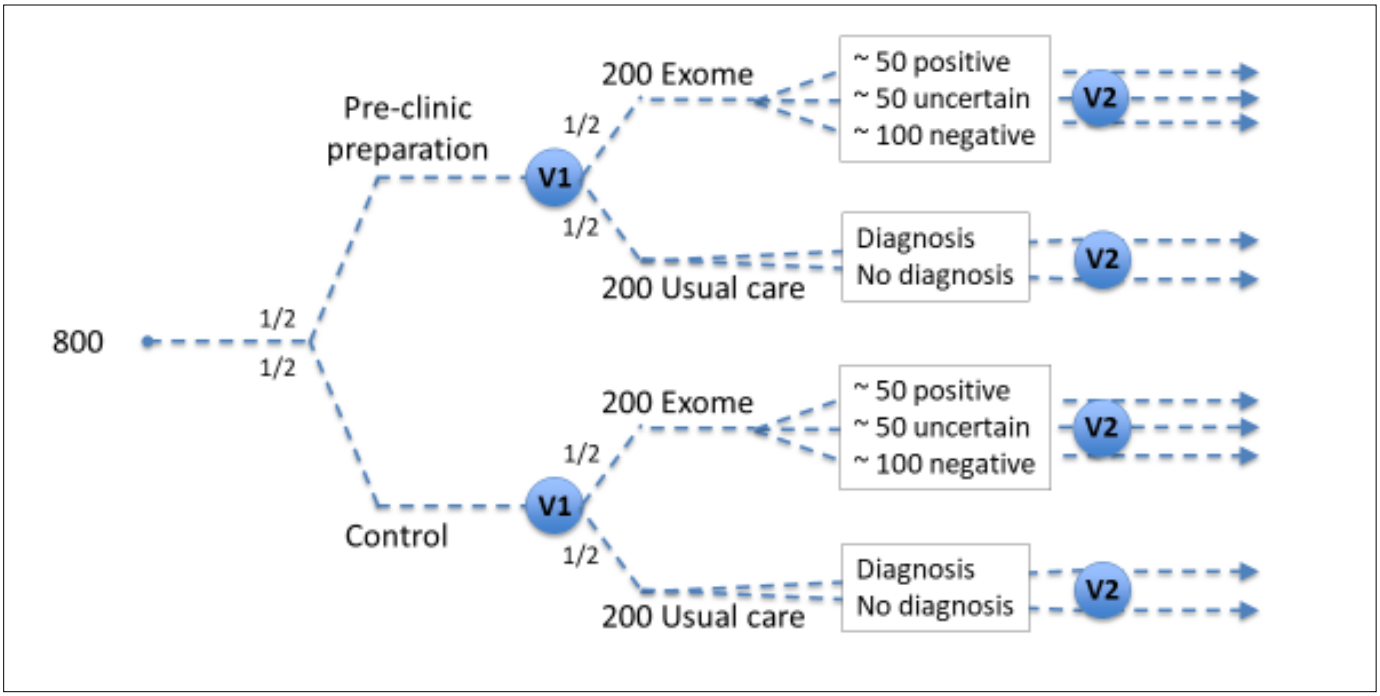


NCGENES 2 Consent Flow – Return of Results




NCGENES 2 Consent Flow – Return of Results
Visit 1 + 5 months
1-3 Weeks
2 Weeks
YES
Genomic Sequencing
Bioinformatics variant calling and annotation complete
Variants loaded into RENCI molecular analysis user interface
Sequencing results assigned to analyst (Tamara in Berg Lab) which triggers mailing
Molecular analysis complete & Molecular Sign-out Meeting with Results
Mailing 3 –pre-return of results ('V2') +/-prep materials
NEGATIVE Results – case-level results documented in RENCI system by Berg team immediately after molecular sign-out meeting
POSITIVE/UNCERTAIN Results – Require Sanger confirm in CLIA MGL. Karen Weck documents case-level results in RENCI system and EMR
Case-level results in RENCI triggers MD email notification to return GS results (for GS group) and complete MD/Clinician survey (both GS and No GS groups)
Physician RETURNS RESULTS to GS group (RoR)
Physician Completes Survey – including return of results date. Completion of part 1 of the Clinician Survey triggers 2 wk post-RoR parent survey task. Final section of survey completed by MD and/or GC/Nurse or both. IF GC/Nurse participated then a GC/Nurse email notification is sent to trigger clinician survey completion.
Post-RoR Telephone Survey (2 wks after RoR) – Parent complete survey by phone (preferred) or mail (+/-Mailing 4) which triggers final 6 mo post-RoR survey

Overview of NCGENES 2 Clinical Protocol Interventions



*NOTE: Planned enrollment is 850, for ease of distribution across groups 800 was used here.

**NOTE: Clinic Visit 2 (V2) is now called Return of Results (RoR) because the vast majority of participants do not return to clinic for a second visit, instead results are provided by phone.

NCGENES 2 Clinical Study Roles

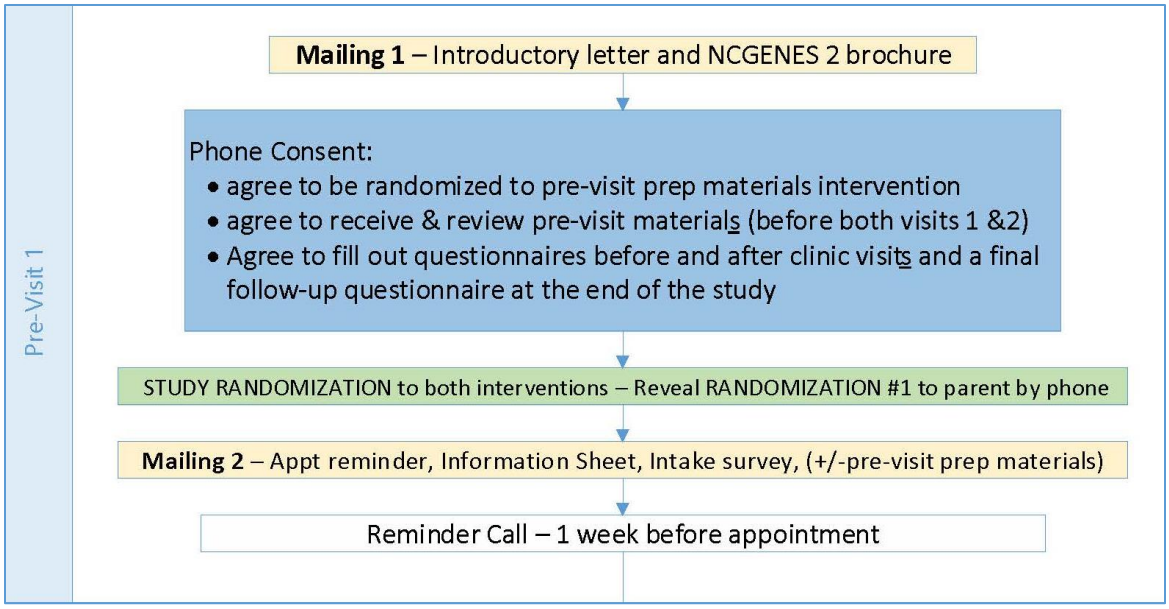
Study task in NCGENES are distributed between team members with distinct roles. These roles are based in part on the various blinding statuses necessary to perform each task. Different roles also have access to different data within the study tracking system. The principal distinction in roles exists between the Study Coordinator (SC) and Research Assistant (RA). The table below lists all the clinical roles, their level of blinding, and the tasks they perform.

Role[1]	**Blinding Status**	**Tasks (Not exhaustive, may vary by site)**
Study Coordinator	Unblinded to participant randomizations as the occur	Eligibility and Selection, Enrollment, Mailing 2 & 3, Consents/Assents, Biospecimen Collection
PI/Clinical Project Director	Unblinded throughout	Oversee study design, resolve implementation issues
Research Assistant	Blinded to Intervention 1 randomization through clinic visit Unblinded to intervention 2	Mailing 1, Pre- and Post-Visit Surveys, Audio Recordings, RoR Parent Mailings, Biospecimen Collection
Data Analyst/Programmer	Unblinded throughout	Generates enrollment reports, ensures clean data
Lab Staff	Blinded to identifying information on participants	Analyzes biospecimens and records results
Genetic Counselor	Blinded to Intervention 1 randomization through clinic visit Unblinded to intervention 2	Post-RoR Provider surveys, Consents/Assents (if necessary)
Clinicians	Blinded to Intervention 1 randomization through clinic visit Unblinded to intervention 2	Post-Visit and Post-RoR Provider Surveys, PhenoTips, Return results

[1] The same person can hold multiple different roles across sites and therefore have different blinding statuses at each.

ENROLLMENT OVERVIEW

This section of the protocol describes the participant enrollment process for NCGENES 2 up to the 1st clinic visit.



Participant Referral

The set of all potential participants in the NCGENES 2 study is comprised of children (less than 16 years of age) referred for a disorder with unknown but suspected genetic etiology, who are scheduled in the Pediatric Neurology or Pediatric Genetics and Metabolism clinics at the University of North Carolina at Chapel Hill, the Pediatric Genetics clinic at East Carolina University in Greenville, NC, or the Pediatric Genetics and Metabolism clinic at Mission Health in Asheville, NC. Each participant will have an initial status of "Referred" in the NCGENES 2 Tracking System. Referred participants' scheduling and demographic information will either be populated nightly from the UNC Carolina Data Warehouse for Health (CDW)[1] or will be entered manually into the study's tracking system following review of scheduling information in the Electronic Medical Record (EMR). Study staff will use this tracking system throughout the enrollment and data collection process, starting with manual entry or auto-population of patient scheduling data and the identification of eligible participants.[2]

Participant Eligibility

Eligible participants must:

- Be less than 16 years of age at the time eligibility is determined
- Be referred to a participating clinic with an appointment at least 3 weeks away with one of the study physicians
- Have an unknown but likely genetic etiology for their disorder (See **Appendix I** on page 185)

If the study coordinator cannot determine the eligibility of a child due to inconclusive data on their condition, they should consult directly with the primary study physician at their respective site. If eligibility is still in question, staff should contact Dr. Jonathan Berg (**jonathan_berg@med.unc.edu**) for a final determination of eligibility. Once a determination

[1] If the participant data is uploaded to the tracking system automatically from the EMR, the primary guardian is set as the primary guarantor (i.e. the insurance policy holder). For this study, the primary guardian must be the consenting parent/caregiver. If the consenting parent is not the primary guarantor, this information should be corrected by study staff during the introduction to the Enrollment Call (see page 67) task by entering the correct parent's name in the field following "May I Speak with."

[2] The process for entering participants manually into the tracking system is explained in detail in the "Using the Tracking System" section of the protocol on page 54.

is made, participant eligibility information should be entered in the tracking system by completing the "Patient Eligibility and Selection" task for that participant. Detailed instructions on completing this ask can be found in the "**Tracking System Tasks**" section on page 80.

Status Update: Participants who meet the eligibility criteria will be given the status of "Participant eligible." Participants who do not meet the criteria will be given the status of "Participant ineligible." **Appendix II** on page 188 provides a flowchart of study statuses.

Mark Participant as Ineligible Manually[1]

In some cases, a participant status may need to be changed to "Ineligible" manually. Detailed instructions for changing a participant's status manually can be found in the "**Using the Tracking System**" section of the protocol on page 70.

The following situations require a participant be marked ineligible manually:

1. If prior to completion of the "Patient eligibility and selection" task,[2] EMR review reveals the patient/family
   a. permanently canceled their visit to UNC
   b. has been seen previously at any genetic clinic (regardless of how long ago that appointment occurred)
2. If after completion of the "Patient eligibility and selection" task, but prior to enrollment,[3] it is discovered the patient/family
   a. permanently canceled their visit to UNC
   b. needs an interpreter
   c. other
      i. has been seen at any genetic clinic
      ii. has received a diagnosis

If the participant is marked "ineligible – permanently cancelled visit 1" due to impacts of the COVID-19 pandemic and suspension of research, the corresponding note should begin with the following: *"During the time period of Covid-19 the participant's clinic appointment was cancelled. Per EMR review it was determined the participant is no longer coming to the study clinic."* Additional explanations of the specific situation should also be noted (e.g. parent/guardian declined rescheduling participant's {INSERT DATE} clinic appointment)

Participant Selection

Eligible participants are 'selected' in or out of the study using a method called randomized recruitment (**See Appendix III** on page 189). Selection into the study will be based on an algorithm designed to create a cohort with 60% from under-served or under-represented populations and 40% from served populations at enrollment. In NCGENES 2, under-served and under-represented populations are defined by race, ethnicity and insurance status. Specifically, under-served and under-represented are defined as either Non-White OR Hispanic OR having Medicaid, charity care, or no insurance. Participants with one or more of the defining characteristics will automatically be 'selected' to the study. Randomized recruitment is used to designate all other eligible participants as "SELECTED" or "NOT SELECTED" and is done in the background of the tracking system. This process is prompted by completion of the "Patient eligibility and selection" task. Detailed instructions for completing this task can be found in the "**Tracking System Tasks**" section of this protocol on page 80.

For Participant's with a status of 'Not Selected' or 'Participant Ineligible'

This is a final study status for this participant and no further action is required.

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[1] All statuses entered manually are ***final statuses***. Only tracking system administrators can change these statuses once they are set.
[2] This set of circumstances applies only at sites where patients are automatically referred into the tracking system. At sites where patient data is entered into the tracking system manually, such situations should not arise, as these cases will not be entered into tracking in the first place. See Eligibility Criteria for more detail.
[3] Such situations usually occur during the enr

For Participant's with a status of 'Selected'

Mail the Introductory Letter (See **Mailing 1** in the participant mailings section on page 51). Participants that have been selected into the study will have a status of "pending" in the tracking system for their enrollment call (or for their enrollment call with selection) until they are approached for enrollment into the study. Enrollment calls with and without selection should then be placed about 7 days after Mailing 1 to obtain initial consent to randomization 1. In some cases, enrollment calls may be placed closer to Mailing 1 (i.e. less than 7 days after) in order to allow sufficient time to for recruitment.

For Participant's with missing selection criteria that must be obtained on the enrollment call

When participant study selection criteria are completely unknown or the known information is insufficient to determine whether the participant is under-served/under-represented, participants will still have the status of "Participant Eligible". In this case, the Introductory Letter and Study Brochure should be mailed (See **Mailing 1** in the participant mailings section on page 51). An "**Enrollment Call with selection**" should then be placed 7 days after Mailing 1 has been sent. During this call, the parents of these participants will be asked to provide the missing selection criteria and selection will be completed during the call. The participant's status will change when selection is completed. If the participant is selected, the tracking system will prompt the staff member to proceed with enrollment. If the participant is not selected, the tracking system will prompt the staff member to conclude the call.

Enrollment Call

The Study Coordinator will use the parent's contact information from the tracking system to facilitate the enrollment call to determine parent eligibility and invite the parent and child into the study. In NCGENES 2, both the parent and child are participants.

The primary components of the enrollment call are to:

1. Determine the parent's eligibility for the study
2. Verbally consent the parent into the study – focused on intervention 1
3. Randomize the parent to intervention 1 (pre-visit education or no pre-visit education)
4. Confirm the parent's mailing address
5. Inform the parent of parking/travel assistance to the study-related clinic visit, and of compensation for completion of study surveys.

This section details practical instructions for completing the enrollment call. The enrollment call should be completed in concert with the "Enrollment call (with selection)" task in the tracking system. A step-by-step guide to completing that task can be found in the "**Tracking System Tasks**" section of this protocol on page 82.

(See **IRBIS** for the Enrollment Call Scripts)

Enrollment Call Strategy

Attempts to reach the parent or legal guardian should be spread throughout the day with calls during the morning (9 AM to Noon, afternoon (Noon to 5PM), and evening (7 to 8 PM) – in some cases weekend calls may be needed and should focus on Saturdays, 9 AM to 4 PM. Over a period of approximately 5 - 14 days, 1-5 attempts should be made to reach the parent on file. Once an enrollment call is made, the participant's status becomes "APPROACHED". This status will serve as the overall denominator for much of the reporting.

If the study staff member does not feel comfortable contacting a person because of personal conflict, it is their responsibility to find another study staff member who is willing to approach the participant and follow the enrollment protocol.

Early Phase Enrollment

During roll-out at each site, enrollment may need to be restricted based on the capacity of the clinic to handle multiple participants in a short time period. In these cases, all eligible participants should be screened as such. From the list of eligible participants, the study coordinator should begin contacting parents gradually. If the study coordinator reaches their enrollment capacity, they should immediately stop contacting eligible participants. If a previously approached parent calls the study coordinator back expressing interest after this point, the study coordinator should say the following:

> "Thank you very much for returning our call. Unfortunately, at this time, we are only able enroll one patient each day in this research study and someone else has been scheduled to participate on the same day as your appointment. Again, we really appreciate your call back. Please remember that your child is still scheduled to see Dr. {NAME} on {VISIT DATE/TIME} in the Genetics Clinic. Dr. {NAME} and the clinic staff are looking forward to seeing you and your child on that date.
>
> Goodbye."

Mark Participant as Failed Approach Manually[1]

Participants will have a status of "FAILED APPROACH" if the participant's parents are:

- not able to be reached (e.g. bad contact information)
- not reached within the number of attempts per protocol (max=5 calls)
- not reached by phone 3 or more weeks prior to the participant's outpatient clinic visit.

Mark Participant as No Approach Manually

Participants will have a status of "NO APPROACH" if study staff does not attempt to enroll an *eligible* participant. This can happen if

- insufficient staffing prevents enrollment from being conducted 3 or more weeks before the visit
- the patient completes their visit before being enrolled due to
    - the visit being rescheduled unbeknownst to staff
    - the visit being scheduled as telemedicine or while staff cannot perform in-clinic research activities, e.g. during the COVID-19 pandemic suspension of research activities.

If a participant is marked "No Approach," a note should be created in the tracking system explaining the reason for this status change.

If the participant is marked "No Approach" due to impacts of the COVID-19 pandemic and suspension of research, the corresponding note should begin with the following: *"No Approach due to COVID-19: Patient completed visit during suspension of in-person clinic research activities."* Additional explanations of the specific situation (e.g. telemedicine visit) should also be noted. **IF PARENT WAS APPROACHED, BUT ENROLLMENT WAS INTERRUPTED:** the note should begin with the following: "*Interrupted enrollment due to Covid-19. See communication log/notes section in tracking for details."*

The "FAILED APPROACH" status should be manually applied in the tracking system by the study coordinator. Detailed instructions for changing a participant's status manually can be found in the "**Using the Tracking System**" section of the protocol on page 70.

Parent Eligibility

The study coordinator will use the participant tracking system and enrollment script to determine eligible parents and enroll parents into the study. Parents/legal guardians eligible for invitation to the NCGENES 2 study will be:

1. 18 years of age or more
2. able to sign legal documents for your child (excludes foster parents)
3. willing and able to attend the study-related clinic visit

[1] All statuses entered manually are ***final statuses***. Only tracking system administrators can change these statuses once they are set.

4. able to complete surveys in English (NOTE: Spanish enrollment is planned for the future)

If the study coordinator reaches the parent and performs the parent eligibility screening, the enrollment status will automatically update to 'PARENT ELIGIBLE' or 'PARENT INELIGIBLE'. 'PARENT INELIGIBLE' is a final study status and no further action is required. Those parents determined to be eligible will continue to the next step: parent consent to intervention/randomization 1.[1]

Mark Participant as Refused Manually[2]

A participant's status should be manually marked refused when a participant refuses to complete a study activity. This is initiated by the participant and occurs during a data collection activity (e.g., enrollment call, pre-visit survey, post-visit survey). Detailed instructions for changing a participant's status manually can be found in the "**Using the Tracking System**" section of the protocol on page 70.

Parent Consent

Eligible parents will be invited to participate in the study and consented. If the study coordinator consents the parent, the enrollment status will be updated to 'PARENT CONSENTED'. Parents who refuse or decline to consent will have an updated status of 'REFUSED-AT ENROLLMENT (INTERVENTION I)'. The status change is triggered when a parent answers "no" to the question: Do you want to join the first part of the NCGENES research study? All parents who decline/refuse participation will be asked if they would like to indicate a reason for their decision. If a reason is provided, it should be recorded in the tracking system when the participant's status is changed.

Randomization 1

Parents who consent to be in the first part of the study will be randomized into either the pre-visit education or no pre-visit education groups (i.e. intervention 1). The group assignment will be based on a block randomization scheme. A participant is randomized into a group when the study coordinator clicks "Yes" and "Next" in the consent part of the enrollment call that is captured in the tracking system (See on page 90). The status determination will automatically appears on the next screen in the tracking system.[3] Based on this status (and after conclusion of the call), the study coordinator or another unblinded study staff person should mail a Pre-Visit Packet to the participant with or without Prep materials (See **Mailing 2** in the participant mailings section on page 51).

Protocol if unable to complete enrollment call

For any attempts to contact the parent for the enrollment call, the following statuses should be recorded in the communication log[4] as appropriate, along with any additional relevant information:

- Parent not available
- No one answers the telephone
- Call disconnected
- Wrong phone number
- Phone disconnected

Provider Notification of NCGENES 2 Participant

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[1]If the parent is determined to be eligible **but** the enrollment call is not completed, the participant's status will be "PARENT ELIGIBLE" until the call is completed. This situation can occur, when the parent does not have time to complete the enrollment call, so the call is scheduled for a future date/time.

[2] All statuses entered manually are ***final statuses***. Only tracking system administrators can change these statuses once they are set

[3]If the tracking system fails at this point in the call, the study coordinator should complete the call and inform the parent they will learn their group assignment when they receive the study materials. Then the coordinator should inform the Sheps team of the tracking system failure and ask that they provide the intervention 1 assignment. Once this information is obtained study Mailing 2 should occur and the tracking system updated once the failure has been resolved.

[4]For detailed instructions on using the communication log, see the "Using the Tracking System" section of this protocol on page 60.

After a participant has been enrolled, study staff must notify the clinician of their patient's status as an NCGENES 2 participant. The protocol for this notification varies by clinic.

UNC Genetics and Metabolism Clinic

For patients seen in the UNC Genetics and Metabolism Clinic, the Study Coordinator will place the phrase "NCGENES Participant" in the clinic calendar in MEDGIS (the Genetics and Metabolism EMR system).

UNC Pediatric Neurology Clinic

For patients seen in the UNC Pediatric Neurology clinic, the study coordinator emails the neurology nurse at least two times a month, informing them of the patients whose parents have agreed to participate in NCGENES 2. These actions allow clinicians and clinical teams to know that the patient-parent pair are NCGENES 2 participants.

Mission Health Fullerton Genetics Clinic

For patients seen in the Mission Health Fullerton Genetics Clinic, the Study Coordinator will enter the phrase "NCGENES2 Participant" in the clinic calendar in Cerner (the Mission EMR system).

Reminder Call – 1 week prior to visit

Study staff should call to remind a participant about their study research visit approximately 1 week prior to the visit. A version of this script can be found in **IRBIS**. This event will be captured in the participant's **Communication Log** (See page 68).[1]

The purpose of this call is to:

1. Remind the parent about their research visit
2. Remind the parent to complete the documents mailed to them and bring them to the clinic
3. Discuss travel to the clinic
4. Answer any questions the parent may have about their pending study visit

Call Strategy

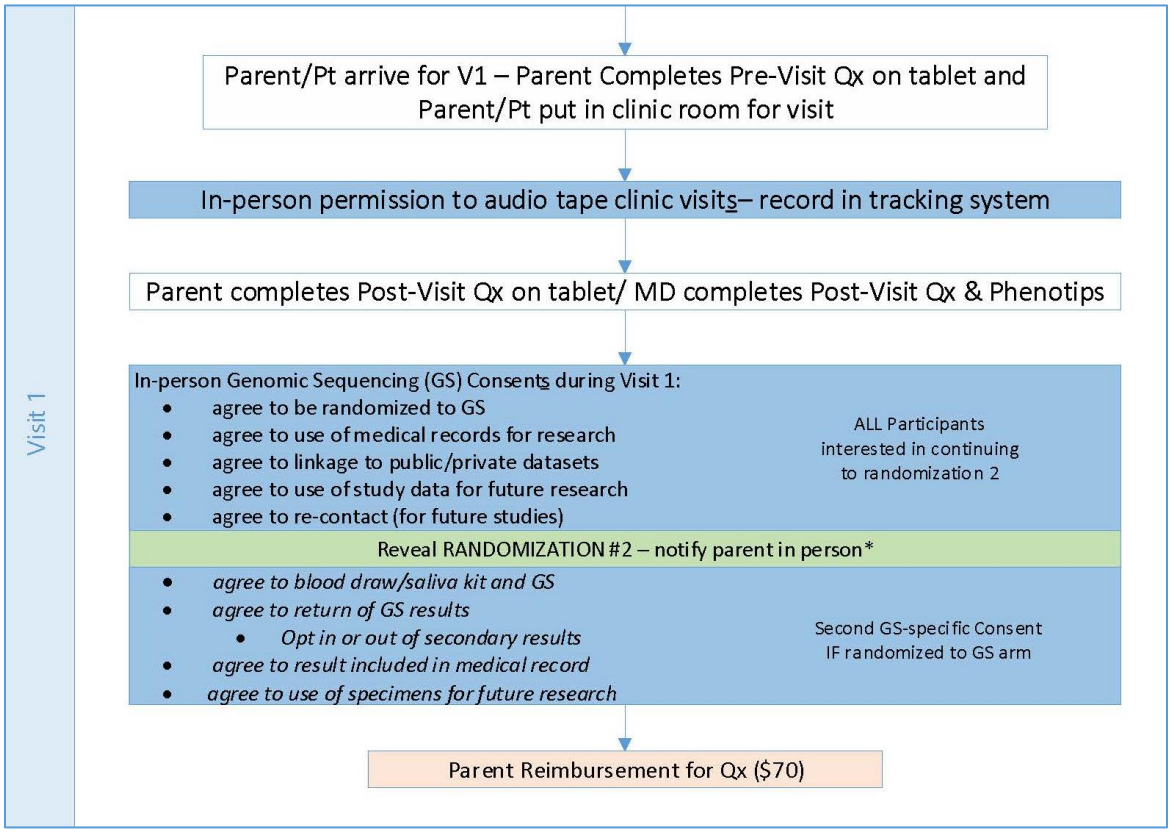
Calls to the parent should be spread through the day with calls during the morning (9 AM to Noon), afternoon (Noon to 5PM), and evening (7 PM to 8 PM) – in some cases weekend calls may be needed and should focus on Saturdays, 9 AM to 4 PM. Staff should make 1-2 calls in a 5 day period with the latest one happening 1 or 2 business days before the visit.

[1] In the tracking system, the reminder call task is displayed as "Completed" if the study staff member reached the parent **or** left a message for the parent. The reminder call can also have the statuses of "Canceled" or "Not Done". The "Not Done" status could result if a reminder call is not completed at all.

VISIT 1

Overview

This section of the protocol outlines the procedures that should be followed before, during and after the clinic visit. The Visit 1 protocol includes information for preparing for a clinical visit, confirming participant clinic date, documentation collection, conducting pre-visit surveys, facilitating audio recording of clinic visit, conducting post-visit surveys, invitation to and consenting for intervention/randomization #2 (genomic sequencing), and distribution of incentives. This section also covers special topics for clinic visit 1 which include participant distress and no-show protocols and information for physician surveys and entry of PhenoTips information.



Most of the tasks in visit 1 are conducted by the study RA and Study Coordinator. The RA greets the families and handles all tasks up to the post-visit survey after which the RA transitions the participants to the SC.

Preparation for Visit 1

Detailed task lists for the study coordinator and research assistant can be found in **Appendix IV** (see page 191. To prepare for a clinic visit, both the Study Coordinator and RA should ensure that all electronic devices needed for clinic are fully charged, including audio recorders, tablets and iPads.

Additionally, study staff should download movies/cartoon content on the iPad/tablet for child participants weekly. Prior to the clinic visit, the Study Coordinator compiles a hard copy folder for each participant and, if necessary, books a consultation room in the clinic where randomization, payment, child gift/snack distribution and genomic sequencing-related consenting will take place.

Compiling the Participant Folder

The Participant Folder principally contains a complete set of Visit 1 documents (used if electronic system fails). ***No indication of study randomization status should be included in or on the participant's folder.*** This folder is then given to the RA so that it can be brought to the clinic for use. The participant folder should be compiled as follows:

1. Label a Manila Folder with the participant's NCG ID.
2. With the folder open flat and label positioned in the top right corner, place a sticky note on the left side of the folder with the parent and child's name, child's age, and provider's name.
3. On the right side of the folder, clip the following items:
    a. Envelope with parent participant cash compensation ($70)
    b. Blood draw bag with the following items:
        i. Study ID Labels (5)
        ii. 2 Lab requisition form (BSP should be printed on Blue Paper and CLIA will be printed on white paper)
        iii. Blood draw tubes (2)
        iv. Additional blood draw bag (1)
4. Gather the following study documents and place them in the order indicated:
    a. Intake Questionnaire[1] with stamped return envelope and ID label
    b. Parent Pre-visit survey
    c. Permission to Audio Tape form
    d. Parent Post-visit survey
    e. Clinician Post-Visit Survey
    f. Blank Blue Sheet to Separate RA and SC forms
    g. Consent to Randomization 2 form (3)
    h. If necessary, Assent to Randomization 2 (3)
    i. HIPAA Authorization (3)
    j. Yellow Paper with reminder to get signature for compensation[2]
    k. Consent to Genomic Sequencing (3)
    l. If necessary, Assent to Genome Sequencing (3)
    m. Post-genomic sequencing consent brochure
    n. Blank CLIA Order Form
5. Once compiled, these documents should be placed inside the Manila folder, and the folder should be closed and secured with a binder clip.

What to Bring to Clinic

In addition to the participant folder, there are several items that the RA and the Study Coordinator should bring with them to the clinic. These items are placed in rolling bags for secure transport to clinic.

RA Clinic Bag Contents

- iPad tablet for child participants with updated videos/cartoons
- Study Tablet, Power cord and Mouse (ensure stylus is in tablet – bring an extra stylus)
- Copy of Intake Survey and a postage paid envelope with address for mail return, if necessary
- Charged, numbered audio recording device & charger
- Participant research folder secured with binder clips (**See above**)
- Laminated card of instructions for Clinician survey and PhenoTips
- Laminated sheet with MDs and genetic counselor names (Optional)
- Hard copy RA checklists (Optional)
- Post-it notes

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[1] **Note:** Intake form must be for the age that the child was at the time of eligibility. Children 24 months old should get the toddler 2-3 year old survey and NOT the 12-24 months form

[2] The envelope with parent compensation and assent should be pulled out immediately after flipping the yellow sheet over. These documents should be put out in the open where they can be seen and not missed when the visit ends.

- Pens

Study Coordinator Clinic Bag Contents

- Study tablet with stylus (more than one if needed)
- iPad with updated videos/cartoons (more than one if needed)
- numbered audio recorder with case (more than one if needed)
- Chargers for all 3 electronic devices listed above
- Extra stylus
- Snacks
- Hard copy receipt book for parent compensation in the event electronic tablet version fails
- Pens (for completion of forms if tracking fails)
- Post-it notes
- Children's gifts – age appropriate

To make sure the audio recorder is charged:
1. Turn on the recorder by sliding the POWER/HOLD switch toward "POWER."
2. Check that the battery has three bars.
3. If the battery doesn't have three bars, charge the battery.

To charge the battery:
1. Connect the USB cable to the USB port of a computer.
2. While the recorder is turned off, connect the USB cable to the bottom of the recorder.
3. Press the PLAY/OK button to start charging, which takes about three hours.
4. Charging Is complete when the battery says "F".

Visit 1 Procedures

Arrival to Visit 1

The RA, blinded to the participant's randomization statuses (pre-visit preparation and genomic sequencing), will coordinate with anyone who will be assisting with the clinic/research visit (e.g., study coordinator, student volunteer, and/or other research and clinical staff). Prior to leaving for the research appointment, if the RA does not see the SC in the NCGENES office, they should inform the SC by text or phone that they are on their way to clinic or have arrived at clinic. The RA should arrive at the clinic at least 15 - 20 minutes prior to the research visit. Upon clinic arrival, the RA should begin looking for the patient and their parent. About 20 - 30 minutes before the research appointment, the SC will check EPIC or Cerner to learn if the patient has arrived and will continue to monitor EPIC/Cerner until the patient arrives. By text, the SC will inform the RA of the patient's status per EPIC at the time of the initial EPIC review and periodically until the patient's arrival.

The first portion of the research visit is typically conducted in the waiting room. Upon participant arrival and after the parent checks the child in at the clinic reception desk, the RA should greet the participants (child and parent) in the clinic waiting room. The RA should introduce themselves and confirm the parent's and child's names. When the RA is with the family in the waiting area or has escorted the family to a consultation room (depending on clinic), they should ask the parent if they may offer pre-recorded videos to the child – prior to the child seeing the iPad. The RA may have a student volunteer who accompanies them to the clinic to entertain the child and any sibling(s) with the video/waiting room toys.

Wrong parent? If the parent who brings the child to the clinical encounter is NOT the parent who consented by phone, the parent who is with the child MUST be consented to the study to complete the pre- and post-visit surveys. A note must be placed in the tracking system on participant's individual page to indicate that this has happened (See "**Notes**" section on page 72), and an email sent to the Study Biostatistician (Laura Farnan at UNC: **laura_farnan@med.unc.edu**) to explain this.

Participant Late? If a participant shows up 20 minutes late for a clinic appointment, the clinic check-in staff must obtain provider approval for the participant to have the clinic appointment. Therefore, if a participant is 15-20 minutes late for their clinic appointment, the RA should ask the provider/genetic counselor if the participant will be seen upon arrival. If the answer is "Yes", the RA should ask if they can also complete the study pre-visit survey. If the answer is "Yes", the RA should continue to wait for the participant. And upon arrival, complete their usual study actions as quickly as possible. The RA should inform the SC of the provider/genetic counselor's response to these questions, either by text or in-person. If neither of these options are possible the RA should send the SC an email.
If the provider/genetic counselor informs the RA that a participant will not be seen or that the study pre-visit survey cannot be completed, the RA should obtain the reason for this event and inform the SC by either text or in-person of this event and the related reason. If neither of these options are possible the RA should send the SC an email.[1]

Participant Missed Clinical Appointment? See **No Show Protocol.**

Confirm Visit 1 appointment date and provider

When parent has checked into the clinic and the child has been offered the iPad (if parents allow), the RA should confirm the Visit 1 details. The purpose of this task is to confirm the participant's appointment date and provider. This is particularly important if a participant ends up being seen by a different physician in a particular clinic. Confirmation of this sets the NCGENES visit 1 date for the study. Detailed instructions on completing this task can be found in the "**Tracking System Tasks**" section on page 95. Following the appointment date and provider confirmation, the RA should turn their attention to the parent and remind the parent about what will happen during the visit and should ask the parent if they have brought their completed intake survey.

Collecting the Intake Survey

Participants should have completed the Intake Survey prior to their research visit and should have brought the completed document with them to the clinic. If the participant has their completed form, collect it at the beginning of the research appointment. When you collect the document briefly check for its completion (especially for the child's partial social security number)-this step can also be done while the parent is completing the pre-visit survey. Record the collection of the completed Intake Survey in the participant tracking system by completing the "**Visit 1-Collect Info about Intake Completion**" task.

Parent forgot their completed intake survey? If the participant completed the survey at home but failed to bring it with them to the study visit, provide the parent with a labeled envelope with prepaid postage and a blank copy of the intake form (in case the parent cannot locate the completed form). Inform the parent that they should use the provided envelope to return the survey by mail.

Parent didn't complete intake survey? If the participant did not complete the Intake Survey, two things can be done:
1. If the participants arrive with substantial time before their doctor visit, parent can complete intake form in the clinic after completing the pre-visit survey.
2. If the participant cannot complete the survey in clinic, parent should be provided with another Intake Survey with a labeled envelope with prepaid postage. Inform the parent that they should use the provided envelope to return the Intake Survey by mail.

The collection status of the intake survey should be recorded in the tracking system. This is done by completing the "Visit 1-Collect Info about Intake Completion" task in the tracking system. Detailed information on completing this task can be found in the "**Tracking System Tasks**" section on page 104. Additionally, if the intake survey is *not* collected during the clinic visit, a note should be made (using the **Notes** tab in the participant's tracking system page (see page 72) that includes information about whether the intake survey was completed or not prior to the visit.

[1] When possible the SC will contact the RA via text or phone, to remind them to check with the provider/genetic counselor to learn if the participant will be seen and if so, can the pre-visit survey occur.

Additional Notes on Document Collection

The task "Questionnaires-Collection Method" allows for tracking the type of questionnaire collection for the Baseline/Intake, Pre-visit Parent, Post-visit Parent, and Post-Visit Clinician surveys. This task should be completed by the RA but may be completed by other study staff members. The RA or staff member will note if the questionnaire was completed by phone, on the tablet, or by paper. For details on completing this task, see the "**Tracking System Tasks**" section on page 145.

Parent attempts to give RA QPL? The parent has been provided with instructions to give their question prompt list (QPL) ("Visiting a pediatric specialist: It's OK to ask questions.") to their child's physician. If the parent tries to hand the QPL to the RA, the RA should inform them to give the QPL to their child's physician. If this happens, the RA may say the following: "You may ask anyone on your child's medical team these questions, but *please make sure to give this list to the doctor*."

Pre-visit Survey

After addressing the Intake Survey, the RA will administer the Pre-Visit Questionnaire using the tablet. Instructions on accessing the Pre-Visit survey can be found in the "**Tracking System Tasks**" section on page 96. Before handing the tablet to the parent, read the instructions on the initial page of the survey and let them know they can consult you if they have any questions. The pre-visit survey should take most participants about 15-20 minutes. When the parent has finished the survey, the system should automatically log off the user so that the parent cannot get to other software to on the tablet. The RA should ensure that the parent has clicked on finish and finalize and close the survey window. The RA should then access the tracking system and go to the participant's individual page to ensure that the Pre-visit survey status is completed (if you fail to close the initial participant task list menu you will need to refresh the screen to see the completed status for that task).

If tracking system is not available, the parent should complete a paper copy of the survey that has a participant study ID label placed in the upper right-hand corner. Read the instructions on the initial page of the survey and let them know they can consult you if they have any questions. Hard copies of completed surveys should be manually entered within 24 hours of completion. However, if a hardcopy is received on a Friday, it must be manually entered by 5:00 pm the following Monday. When the parent has finished the survey the RA should check to see that the survey has been finished and the tablet is back on the appropriate home screen. For instructions on uploading documents to the tracking system, see the "**Using the Tracking System**" section on page 74.

Audio Recording and the Clinic Visit

Prior to the visit, the RA should ensure the audio recorder is properly charged and in the clinic bag (See page 22).

Consenting the Parent to Audio Recording

The RA should consent the parent to audio recording by completing the "Parent Permission to Audio Tape Clinic Visit" task. This consenting should occur in the clinic waiting room or after you enter the clinic room with the participants for the appointment. For detailed information on completing this task, see the "**Tracking System Tasks**" section 121.

Tracking system down? If the tracking system is down, consent the parent to audio recording using the paper version of the consent in the participant's folder. After the visit, when the tracking system is working again, upload this paper version to the **participant documents tab** (see page 74). Then, complete the "Parent Permission to Audio Tape Clinic Visit" task and, if the parent consented, write "Completed Document on Paper" in the signature field.

Once the consent process is complete, the RA should remind the parent of what to expect after their clinic visit before setting up the audio recording. To do so, the RA should tell the parent the following:

> "*When you are done, I'll bring you to meet with Tracey (or whoever is helping that day), the study coordinator/genetic counselor, where you will receive your reimbursement, some snacks and a gift for [child's name]. This is also where you will get a chance to hear more about the special research test that your child may have a chance to get. You might remember that we mentioned this test to you when we spoke to you by phone a few weeks ago*."

Setting-up in the Clinic Room to Collect Audio

If the parent consents to audio recording, the RA will be responsible for initiating audio recording in the clinic room ideally immediately before the genetic counselor OR the physician if no counselor will be seeing the patient. If, however, this is not possible, audio recording can be initiated after the patient has been placed in a clinic room.

To begin recording with an Olympus DS-2500 recorder:
1. Turn on the recorder by sliding the POWER/HOLD switch toward "POWER."
2. Press the NEW button on the side of the recorder to create a new file.
3. Press the REC button to start recording.
4. The indicator light will glow orange and the record symbol will appear on the display.
5. Place the recorder near the sound source.

The RA should state the audio recorder number immediately after beginning recording. The RA should exit the room once the audio recording has been set up and should wait outside the room until the end of the visit.

During the visit, regardless of whether the parent has consent to audio recording, the RA should text or call the Study Coordinator to let them know the parent has completed the pre-visit activities.

At the Conclusion of the Clinic Visit

Once the visit is over and all medical staff has left the room, the recorder should be turned off and stored in the RA rolling bag. At the earliest, this can occur after the physician leaves the room but before they return with the hard copy version of the patient's clinic summary.

To stop recording with an Olympus DS-2500 recorder:
1. Press the STOP button to stop recording.
2. If you want to append additional recordings to the same file, press the REC button again.
3. Turn off the recorder by sliding the POWER/HOLD switch toward "POWER" and holding it for 0.5 seconds or longer.

If you have any additional questions, please consult the instruction manual for the recorder:
http://www.olympusamerica.com/cpg_section/cpg_support_product.asp?id=1620

After returning to the office, the audio recording content should be uploaded to the secure folder accessible via the participant tracking system. Detailed instructions on uploading these files can be found in the "**Using the Tracking System**" section on page 66. If the battery power is low (<2 bars), upload file after recording device has been charged.

Post-visit survey

Once the audio recording has been completed, the RA will administer the post-visit survey. Based upon available space, the post-visit survey will be completed in the exam room, clinic waiting room or the assigned NCGENES consultation room. By working with the clinic nurse/genetic counselor/observing overall clinic flow, the RA will establish the location for the post-visit survey. The RA will escort the family to this location and then provide a tablet with pre-recorded videos to the child. Next, the RA will log in to tablet and provide the survey to the parent. Instructions on accessing the Pre-Visit survey can be found in the "**Tracking System Tasks**" section on page 123. Before handing the tablet to the parent, read the instructions on the initial page of the survey and let them know they can consult you if they have any questions.

Before the RA leaves the parent to complete their survey, they should remind them of the study visit that will follow its completion by saying the following:

> "When you are done, I'll bring you to meet with Tracey (or whoever is helping that day), the study coordinator/genetic counselor, where you will receive your reimbursement, some snacks and a gift for patient's name. This is also where you will get a chance to hear more about the special research test that your child may have a chance to get. You might remember that we mentioned this test to you when we spoke to you by phone a few weeks ago."

The RA should stay nearby as the parent completes the survey should they have questions or difficulties. While the parent is completing the survey, the RA should notify the study coordinator that the participants will soon be ready (10 – 20 mins) to complete the second part of the research visit (i.e. meet with the SC). When the parent has finished the survey, the system should automatically log off the user so that the parent cannot get to other software to on the tablet. The RA should ensure that the parent has clicked on finish and finalize and close the survey window. The RA should then access the tracking system and go to the participant's individual page to ensure that the Post-visit survey status is completed (if you fail to close the initial participant task list menu you will need to refresh the screen to see the completed status for that task).

When the post-visit survey is completed, the RA will escort the family to the assigned NCGENES consultation room if necessary. While walking to this room, the RA can orient the family to the next steps of the research visit (e.g., introduce opportunity to be in the second part of the study, compensation, etc.).

Once the SC, RA, and family are all in the consultation room, the RA will introduce the study coordinator (or unblinded study staff member) and give them the participant's research folder. If necessary (and there are no scheduling conflicts) the student volunteer or RA will stay to help entertain the child/sibling while the SC talks with the family and/or help escort the family to phlebotomy.

RA Notifications

Before the SC begins their portion of the research visit, the RA must notify the SC either via text or in-person of the following:

1) If the physician has ordered bloodwork for the patient
2) Of any non-standard clinic operations, such as time sensitive blood work or another clinic appointment that is scheduled for the same day as the research visit
3) The physician's determination of the participant's developmental age (for participants 7 years of age or older)
4) Feedback provided by any of the providers regarding the project

Determining Developmental Age

Since participants who are developmentally age 7 years or older[1] are asked by the SC to provide assent to participate in intervention 2 of the NCGENES study, the RA and SC must collect the developmental age of any child participant chronologically age 7 or older at the time of their first visit. Developmental age *must be collected* before the family is consented to study intervention 2 (i.e. genomic sequencing).

It is up to the RA to collect the developmental age of relevant participants. When the RA confirms the Visit 1 Appointment and Provider in the tracking system, a "Collect Developmental Age" task will appear for any participant 7 years or older chronologically. This indicates that a developmental age should be collected for this participant. The RA will need to consult with the provider after the visit to collect the developmental age of the child from them. The RA should record this developmental age by completing the "**Collect Developmental Age**" task in the tracking system (see page 146) and by making a note of the participant's developmental age in the participant's folder. If the participant is over 7 developmentally, the tracking system will automatically generate the required assent forms for the SC/GC to review with the participant. The SC/GC should also verbally confirm the developmental age with the RA.

[1] Pediatric participants in NCGENES 2 must be <16 years at eligibility determination. The age-specific study documents sent to parent participants are based on the age determined at eligibility. Pediatric participants may age-up while actively in the study, but age is treated as static in the study. Assent is based on the child's *developmental* age at Visit 1.

Developmental age not available? If the provider does not inform the RA of a child's developmental age, the RA should ask the counselor (for genetics patents) or Betty (for neurology patients) if the provider has given them this information. If not, the RA should ask the counselor/Betty if the doctor can be reached so that the developmental age can be obtained. The RA should inform the person obtaining consent for study intervention 2 of the above ASAP.

The person obtaining consent for study intervention 2 will inform the parent of the following:

- a developmental age from the provider is required for continued participation
- unfortunately, this information is currently unavailable, so the study team is working to contact the provider
- once this information is obtained, they (i.e. the person who is obtaining consent) will contact the parent

After informing the parent of the situation, provide the family with snacks as appropriate and walk them to the phlebotomy area or exit as necessary.

If the developmental age is determined after the parent and child have concluded their visit and left the clinic, next steps will depend on the developmental age of the child. If the provider says the participant is developmentally age 7 or older, the SC should inform the family by phone that they are unable to participate in the second part of the study as both parental consent and their child assent are required for participation and the time period for obtaining this permission has passed. If the provider says the child is developmentally less than age 7, the SC will inform the family they will able to participate in the second part of the study, and that the necessary forms for this participation will be sent to them. Then follow the protocol for **Phone Consent to Intervention 2** on page 36.

Study Intervention 2

When the parent and child arrive in the consultation room, the study coordinator will thank the parent for participating in the study and remind them that they have spoken previously on the phone during the enrollment call (or state that they are the person mentioned during this call that would speak with them about the second part of the NCGENES study). Then the SC will ask that the family make themselves comfortable in the room and inform the parent of the next steps (i.e. discuss the second part of the study, escort them to check-out/phlebotomy). The SC will ask the parent for permission to offer the child an iPad with pre-recorded videos and a snack. The SC should inform the parent of the types of snacks (in order to avoid providing something that the child is allergic to or the parent would not like for them to have). This will be done before the child sees the iPad and snacks. If the parent gives permission for the iPad/snacks, the SC should provide the child with these items. The coordinator will then begin reviewing consent form 1 with the parent (i.e. Consent to Randomization).

Parent cannot extend visit to provide consent? In some cases, a parent and child may not have the time and/or ability to extend their visit and complete the consenting process in person. While it is strongly preferred for consents to occur in person, such cases may necessitate a phone consent to intervention 2. If the parent cannot consent in person immediately following their clinic visit, but would still like to remain in the study, the SC/GC can offer them the option of a phone consent. The SC/GC should still attempt to complete as much of the study visit as possible, namely distribution of reimbursement.[1] If the option of a phone consent is requested by a parent, the SC/GC should note this in the tracking system and follow the **Phone Consent to Intervention 2** protocol on page 36 to complete the consent.[2]

The SC will discuss consent form 1 (i.e. Consent to Randomization to Genome Sequencing) with the parent by reviewing this form screen-by-screen on the SC's laptop and in general conversation. For detailed instructions on this task in the tracking system, see the "**Tracking System Tasks**" section on page 146. The parent should be offered several opportunities to ask any questions and receive an answer from the SC. The SC should remind the parent of their ability to ask questions periodically during their discussion of the consent. The SC also informs the parent that (1) they will receive

[1] Reimbursement may also be completed by mail if absolutely necessary and should not, in any circumstances, be withheld should a parent decline the offer of consent. Reimbursement is for study activities already completed (i.e. surveys, Visit 1) and is not contingent upon a parent hearing an explanation of consent.

[2] Only child participant's with a developmental age less than 7 are eligible for phone consent. Any participant requiring assent cannot give that assent by phone and must do so in person to continue in the study.

a paper copy of any consent form discussed with them (and their child) and (2) they may contact the SC via the study number and email address that is on these forms if they have any questions following the study visit.

Both parents present? If both parents are present, the SC will inform the parents that for consistency the parent who provided consent via telephone must also sign the consent forms for the second part of the study should the family decides to continue with study participation. The non-consenting parent will be informed they can also ask any questions they have during this time and can look at the laptop or follow along via a paper version of the consent.

If the Parent Consents

If consent form 1 is signed, the SC will review the HIPAA consent following the same procedure as consent form 1 (see above). For detailed instructions on this task, see the "**Tracking System Tasks**" section on page 148.

All participants who consent to the genomic sequencing randomization MUST also have a signed HIPAA document to continue participation in the study. If a participant refused to sign the HIPAA document, they must be manually given the final status of REFUSED and a note detailing the reason for this change should be added to the participant's log.

After the HIPAA consent is signed, the parent/family will be informed of the intervention 2 assignment (i.e. genomic sequencing or non-genomic sequencing). This status will appear on the **participant's individual page** (see page 67).

If the participant is assigned to non-genomic sequencing, the parent/family will be informed that have not been assigned to be offered genomic sequencing. The SC should inform the parent/family that their remaining study participation will consist of completing follow-up surveys and having their child's information obtained until they are 18 years of age as discussed during the consent process. The SC should then thank the family for their time, provide parent and child compensation as described **below**, and escort them to either phlebotomy (for provider bloodwork), check-out, or towards the appropriate hospital exit.

If the participant is assigned to genomic sequencing, the SC will inform the parent/family that they have been assigned to be offered genomic sequencing. Then the SC or Genetic Counselor will review consent form 2 (i.e. Consent to Genomic Sequencing) following the same procedure as consent form 1 (see above). For detailed instructions on this task in the tracking system, see the "**Tracking System Tasks**" section on page 149. If the parent signs consent form 2, The SC/GC will proceed with biospecimen collection and parent compensation. See below sections entitled "**Biospecimen Collection**" on page 32 and "**Distribution of Parent and Child Compensation**" on page 32 for further detail.

If the Parent Declines

If the parent declines to sign either consent form 1 <u>or</u> the HIPAA consent, the parent/family will be informed their study participation ends at the conclusion of the study visit. In the tracking system, the SC should exit whichever consent form they are viewing with the parent and mark the participant "Refused" manually with the reason "Declined at Visit 1 – Intervention 2" and a short explanation.[1] The SC should thank the family for their time, provide parent and child compensation as described **below**, and escort them to either phlebotomy (for provider bloodwork), check-out, or towards the appropriate hospital exit.

If the parent declines to sign consent form 2, the parent/family will be informed that their remaining study participation will consist of completing follow-up surveys and having their child's information obtained until they are 18 years of age as discussed during the consent process. The SC should then thank the family for their time, provide parent and child compensation as described **below**, and escort them to either phlebotomy (for provider bloodwork), check-out, or towards the appropriate hospital exit. These participants will have a status of "Declined Consent/Assent to GS."

[1] See page 18 for more information on marking participants refused.

Tracking system down? If the tracking system is down, all consents/assents should be administered using the paper versions in the participant's folder. After the visit, when the tracking system is working again, upload these paper versions to the **participant documents tab** (see page 74). Then, complete the corresponding tracking system tasks and, if the parent consented, write "Completed Document on Paper" in the signature fields.

Obtaining assent

If the child's developmental age is 7 or greater (**see above**), the SC/GC must collect assent from that child for the parent and child to continue in the study. Assent for randomization (i.e. Assent form 1) and assent for genomic sequencing (i.e. Assent form 2) are obtained by the SC/GC in the same manner as outlined above for obtaining parental consent for intervention 2. Further details on these tasks can be found in the "**Tracking System Tasks**" section on page 150. Assent forms are typically discussed once the parent consent process is complete. In rare occasions, assent form 1 is discussed after the parent signs the HIPAA consent form, and then assent form 2 is discussed after the parent signs the parent consent form 2.

These alternative time periods for obtaining assent are necessary because parents may be more interested in hearing about the second part of the study, while participants are tired and/or need time to become engaged in the process or are focused on the iPad/snacks. The SC should use their judgement as to when to begin the assent process. The SC should inform the parent of the timing of the assent process and check their agreement. If they agree, the SC can proceed with that timing. If there is disagreement, the SC should defer to the parents preferences.

Regardless of the timing of the assent process, the SC starts the process by re-introducing themselves to the participant. Then, the SC asks the participant if they have any questions about why they are seeing the SC or what they heard the SC discuss with their parent(s). After questions are answered, the SC tells the participant what will happen next (i.e. they will talk about the study by looking at the laptop and talking) and that they can ask the SC questions, their parent(s) questions about their talk with the SC or ask their parent(s) to call the SC if they have questions later. Then, the SC begins the assent process.

Note: The assent process should involve more discussion than a screen by screen review on the SC's laptop, especially with younger participants.

If the participant signs assent form 1, they are told if they are in the group of kids that are asked to give a little bit of their blood (about 1-2 teaspoons) or if they are in the group of kids that do not have to do anything extra then what the doctor has already talked to their parent(s) about during their doctor's visit. The participant is asked if they have any questions, and if so, the questions are answered. Then depending upon the intervention 2 assignment the SC proceeds with assent 2 or with providing child and parent compensation as described **below**.

Note: parent(s) are given a copy of the assent forms that are discussed with their child.

If the participant declines signing assent form 1, the SC informs the parent(s) and participant that study participation for intervention 2 cannot proceed. In the tracking system, the SC should exit (without saving) the current task and mark the participant "Refused" manually with the reason "Declined at Visit 1 – Intervention 2" and a short explanation.[1] Then the SC thanks the family for their time, provides parent and child compensation as described below, and escorts them to either phlebotomy (for provider bloodwork), check-out, or towards the appropriate hospital exit.

If the participant declines assent form 2, the SC informs the participant that no genetic sequencing will be done but the participant would continue to be in the NCGENES 2 study and the parent would be provided with follow-up surveys. Then the SC thanks the family for their time, provides parent and child compensation as described below, and escorts them to either phlebotomy (for provider bloodwork) or check-out, or towards the appropriate hospital exit. These participants will have a status of "Declined Consent/Assent to GS"

[1] See page 18 for more information on marking participants refused.

Cases of discordant consent/assent. If a child can assent to participate in Intervention 2, both the parent and child must agree to continue participation. Further, both parent and child must have concordant consent/assent for the check boxes related to the study's continued access to the child's medical records and for the disclosure of medically actionable secondary findings. If the child is 7 years or older chronologically and developmentally, the parent must consent AND the Child must assent for the participants (parent-child pair) to remain in NCGENES.

If the consent and assent are discordant, a participant should be manually given the final status of REFUSED and a note detailing the reason for this change should be added to the participant's log in the study's tracking system.

Distribution of Parent and Child Compensation

At the completion of the consent/assent process parents will sign for compensation through the "Visit 1-Parent Reimbursement" task. For detailed instructions on this task see the "**Tracking System Tasks**" section on page 152. If the tracking system does not work, the SC will use a receipt book to complete this task. The study coordinator fills in the amount provided to the parent according to what was completed before and during visit 1. Child participants in the study will be offered a nonmonetary gift at the end of visit 1. For more information on participant compensation, see the "**Tracking Participant Compensation**" section on page 35.

Biospecimen collection

After the appropriate forms are signed (i.e. parental consent form 2 and if necessary, assent form 2) and the parent and child compensation have been distributed, the SC escorts the family to the phlebotomy area appropriate to that clinic. At UNC, the SC will direct the participants to UNC's Women's Hospital for phlebotomy check-in. If the patient is having provider bloodwork, the SC escorts the parent to the individual check-in area. If the patient is not having provider bloodwork, the SC escorts the family to the area where they can pull a number for general check-in. At Mission, the SC will direct the family to the phlebotomy area of the Mission Children's Hospital.

When the participant is called for blood draw, the SC should escort the family to the blood draw area and inform the phlebotomist of the need to use the research blood tubes immediately after the provider's blood order is filled. The SC will pass the phlebotomist the labeled research blood tube either right before they are to be filled or places them next to the tubes for the provider's bloodwork, depending upon the level of SC assistance needed.

SC assistance

When necessary, the SC assists the phlebotomist/parent in holding the child for blood draw, as well as trying to keep them calm for this procedure. Assistance is typically needed, however the SC should take care not to obstruct the phlebotomist.

See biospecimen protocol regarding sample labeling, collection, lab distribution, processing, and reporting.

Study Withdrawal & No Show Protocol

The protocols in this section involve changing a participant's status using the "Change Status" button on the individual participant's page in the tracking system. Further instructions on this process, can be found in the "**Using the Tracking System**" section on page 70.

Withdrawal

A participant could be considered withdrawn under two circumstances:

1. When a participant asks to be withdrawn from the study. When this request occurs, a withdrawal form is completed by the study coordinator or RA. This request is initiated by the participant and occurs at any time.
2. Or, during study contact for non-data collection activities, such as making the reminder call. This action is initiated by a member of the study team.

In either case, the participant should be marked "Withdrawn."

Investigator Withdrawal

Participants are subject to investigator withdrawal when:

1. A pediatric patient is found to have a confirmed genetic related diagnosis during Visit 1. This would be considered "Investigator Withdrawal-At Clinic Visit 1 (Intervention 1)". These participants will/can complete the Intake Survey, Pre- and Post-Visit Surveys but are not considered for Genetic Sequencing part of the study (i.e. intervention 2). Parents can receive up to $70 in compensation, and the child will receive a nonmonetary gift.
2. NCGENES study staff unable to attend the research/clinic appointment (e.g. due to illness or other conflict). Participants that attend their clinical appointments, can receive up to $40 ($10 for parking and $30 if they submit a completed Intake Survey).
3. A participant (child or adult) was ineligible at the time of enrollment BUT this was only discovered after participant was already enrolled (e.g at Visit 1). For example, a participant has a confirmed genetic diagnosis prior to the enrollment (i.e. this diagnosis does not occur at Visit 1), but this diagnosis was not disclosed during the enrollment call and was not found in the patient's medical record. In these cases, all the data associated with this participant will be removed for the analytic database.
    - If the participant is found to be ineligible for reasons which preceded their enrollment, the following phrase should be copied into the reason text box when changing the participant's status: "Participant was ineligible at the time of enrollment BUT this was only discovered after participant was already enrolled, thus participant coded as 'Investigator Withdrawal' and data removed from analytic database."

No Shows

Participants are considered a *No Show* if the study staff are unable to complete the research visit with a consented participant.

No Show – Research Visit ONLY

A participant should be considered a *"No Show – Research Visit ONLY"* if there is not enough time for the study staff to complete the research visit tasks (e.g. pre- and post- survey or consenting process) at the time of the clinic visit AND the participant must be assented and is thus ineligible for phone consent. This can happen if a participant arrives to the clinic at or after their scheduled clinic appointment time or if the participant has multiple clinical visits that conflict with the research visit protocol. In this case, participants are censored from the study.

No Show – Research and Clinic Visit

A participant should be considered a *"No Show – Research and Clinic Visit"* if they miss their clinical appointment completely. In this case, the participant is given 30 days to reschedule their appointment.[1] If they do reschedule their appointment in 30 days to some date in the future, they can still participate in the study. Participants may be rescheduled up to 3 times.

If the participant is not rescheduled for an appointment within 30 days after their original appointment date, the participant is coded with a final status of *"No Show"*. The SC will periodically monitor EPIC to determine if the participant has been rescheduled and work with the tracking system team and other study team members to complete necessary steps based upon the participant's reschedule status. The study biostatistician will generate regular reports to indicate participants whose statuses should be changed to a *"No Show."*

Alert Protocol for NCGENES 2 Distressed Adult Participants

Parent/Caregiver participant distress is evaluated through the parent's or caregiver's completion of study surveys. When responses are entered into the tracking system, the system will score the GAD7 (anxiety measure) and the PHQ8 (depression measure). The measures appear on the questionnaires listed below:

- Pre-clinic visit 1 parent survey:

[1] The appointment does not need to occur within 30 days; it must be rescheduled within 30 days.

    - **Question 6, A-G** (GAD7, anxiety, 7 items)
    - **Question 6, H-O** (PHQ8, depression, 8 items)
- Post-Return of results (2 weeks after RoR) parent survey:
    - **Question 21***, **A-G** (GAD7, anxiety, 7 items)
    - **Question 21***, **H-O** (PHQ8, depression, 8 items)
- Final Follow up (6 months after RoR) parent survey:
    - **Question 14***, **A-G** (GAD7, anxiety, 7 items)
    - **Question 14***, **H-O** (PHQ8, depression, 8 items)

*Question numbers subject to change

Both the GAD7 and the PHQ8 should be scored (separately) as follows:

- Not at all=0, Several days=1, More than half the days=2, Nearly every day=3
- Sum the items to create measure score
- If a participant scores 15 to 21 on the GAD7 (indicating severe symptoms) AND/OR scores 20 to 24 on the PHQ8 (indicating severe symptoms), the system will create an alert.

IMPORTANT NOTE: For any surveys administered by paper or over the phone, the data should be entered directly into the electronic data collection system within 24 hours of collection or first thing Monday morning if the data is collected late on Friday afternoon. Expedient data entry will allow for rapid distress score calculation, alert reporting, and appropriate follow-up action according to the protocol described above.

Immediately upon data entry, the tracking system will score each measure. If scores meet the thresholds specified above, the system will automatically send an email alert to the study coordinator and study clinical project director, notifying them of the participant's alert status and distress score(s). The system will also generate the "Parental Distress Follow-up" task to appear in the participant's individual page in the tracking system.

The clinical project director will:

1. Share this information with the study coordinator within 24 hours of email receipt.

The study coordinator will:

1. Complete the "Parental Distress Follow-up" task by selecting the "Get Started" button.
2. Complete the first section of the Distress Call Form (**See Appendix VII**) and email it to the study clinical psychologist within 24 hours of receiving the alert notification by the Director and via the tracking system. The email should be sent with delivered and read request. If the read request is not returned within 24 hours, the study coordinator will also call the study psychologist to confirm that they have received the emailed form. If the alert is received on a Friday afternoon, the study coordinator will contact the study psychologist by end of day on the following Monday.
3. Provide resources to distressed participant via email or regular US mail, using the appropriate distress documents (see **IRBIS**) as requested by the study psychologist.
4. Email the study PIs to inform them that the psychologist has been notified about a severely distressed participant.
5. Be responsible for recording all alert protocol actions taken in the tracking system under the participant's alert task.
6. Report on the status of action taken for severely distressed NCGENES 2 caregivers/adult participants at Steering Committee meetings (when relevant).

The study psychologist will:

1. Follow up with the participant (e.g., by phone, or in-person) within two weeks, making a minimum of three call attempts, to assess the report of distress and determine if any relevant support/resources need to be provided. If these resources are needed, the study psychologist will inform the study coordinator.
2. The study psychologist will follow the Distress Screening Script (see **IRBIS**) developed for this study to evaluate the participant.

NOTE: The NCGENES 2 study does not include items about suicidality, and thus individuals flagged for high distress may or may not be experiencing suicidal ideation. One goal of screening high distress individuals is to determine if people are experiencing suicidal ideation or other symptoms that may require referral to specific resources for suicide prevention or therapy. A suicide screening script is provided for use in cases that a participant signals possible suicidal ideation during study interactions or in this screening call.

Complete the bottom section of the Distress Call Form (regardless of whether they were able to reach the participants of not) and return it via email to the study clinical Project Director (Jeannette Bensen, **jeannette_bensen@med.unc.edu**) and the Study Coordinator (Tracey Grant, **traceyg@unc.edu**).

Visit 1 Post-visit Physician Survey and PhenoTips

The physician will complete the MD survey on their own using their login in for the tracking system. They will receive an email notification the patient's physician survey is ready for completion with an email reminder every 24 hours after the clinic visit (due date).

NCGENES 2 Patient Tracking Instructions for Physicians/Providers

1. Login to **https://ncgenes2.sirs.unc.edu** by entering your email and password
2. You will land on a page automatically that is the MD Survey tab
    a. Click on the 'GO TO SURVEY' for the relevant patient
    b. Complete the Survey
        i. Click Save – this will return you to the MD Survey landing page. You will note that the patient whose survey you completed no longer appears on this screen
3. Click on the PhenoTips tab
    a. Click on the PhenoTips button for the relevant patient
    b. PhenoTips will open in a new window
    c. Login with your PhenoTips username and password
        i. NOTE: this is not necessarily the same as your login for the Patient Tracking System
    d. Complete PhenoTips entry of patients – save and logout
    e. You must also logout of the patient tracking system which is still open in another window
4. If you cannot access PhenoTips from the tab in the patient tracking system, try copying and pasting this link into a web browser:
    https://phenotips.med.unc.edu/

Phone Consent to Intervention 2

In some cases, a parent and child pair may not have the time and/or ability to extend their visit and complete the consenting process in person. While it is strongly preferred for consents to occur in person, such cases may necessitate a phone consent to intervention 2. If the parent cannot consent in person immediately following their clinic visit, but would still like to remain in the study, the SC/GC can offer them the option of a phone consent. The SC/GC should still attempt to complete as much of the study visit as possible, namely distribution of reimbursement.[1] If the option of a phone consent is requested by a parent, the SC/GC should do as follows.[2]

Scheduling Call

Within a week from the completion of Visit 1, the SC will call the parent participant to explain the process of phone consent. The SC will explain that certain forms will need to be reviewed, signed by the parent, and returned before the child and parent can continue with the study, and that reviewing these forms will require an additional phone call. The SC will ask whether that call can be scheduled and, if the participant agrees, attempt to schedule a follow-up call 1 to 2 weeks from the current date. This call should be recorded in the **Communication Log** and the follow-up call entered into the NCGENES Outlook Calendar. The SC should also complete **Mailing 4** at the conclusion of the call and record this in the communication log.

Call for Consent(s) to Intervention 2

In many ways, the flow of the phone consent process mimics that of the in-person consent. When the SC reaches the parent participant, they will first confirm that they have received hard copies of the consent and HIPAA forms.

Mailing not received? Phone consent may not proceed without the parent possessing physical copies of the consent/HIPAA forms. If they have yet to receive the mailing, schedule a future call with the parent.

The SC will ask them to collect their forms so that they can review them and answer any questions they might have. The SC will discuss consent form 1 with the parent by reviewing the form and by general conversation. The parent will be offered several opportunities to ask and have answered any questions and during the discussion by the SC. The SC also informs the parent that 1) they should keep one copy of the consent form and 2) they can contact the SC via the study number and email address that is on these forms if there are any questions following the study visit. For detailed instructions on completing this task in the tracking system, see the "**Tracking System Tasks**" section on page 146.[3]

Both parents present? If both parents are present, the SC will inform the parents that for consistency the parent who provided consent via telephone must also sign the consent forms for the second part of the study should the family decides to continue with study participation. The non-consenting parent will be informed they can also ask any questions they have during this time and can look at the laptop or follow along via a paper version of the consent.

If the Parent Consents

[1] Reimbursement may also be completed by mail if absolutely necessary and should not, in any circumstances, be withheld should a parent decline the offer of consent. Reimbursement is for study activities already completed (i.e. surveys, Visit 1) and is not contingent upon a parent hearing an explanation of consent.

[2] Only child participant's with a developmental age less than 7 are eligible for phone consent. Any participant requiring assent cannot give that assent by phone and must do so in person to continue in the study.

[3] The tracking system should be used to record the parents stated decision. If the parent consents, the SC should record "Consent by Phone" in the parent's signature box and place their own signature in the box for witness. When the hard copy of the consent is received from the parent by mail, it should be scanned and uploaded to the participant's document page. If there are any discrepancies between the hard copy consent and the tracking system, the hard copy takes precedence. Should significant differences exist, the SC should consult the study director, Jeannette Bensen.

If consent form 1 is signed, the SC will review the HIPAA consent by following the same procedure outlined above. For detailed instructions on this task in the tracking system, see the "**Tracking System Tasks**" section on page 148.[1] After the HIPAA consent is signed, the parent/family will be informed of the intervention 2 assignment (i.e. genomic sequencing or non-genomic sequencing). This status will appear on the **participant's individual page** (see page 67).

All participants who consent to the genomic sequencing randomization MUST also have a signed HIPAA document to continue participation in the study. If a participant refused to sign the HIPAA document, they must be manually given the final status of REFUSED and a note detailing the reason for this change should be added to the participant's log.

If the participant is assigned to non-genomic sequencing, the parent/family are informed that have not been assigned to be offered genomic sequencing. Then, the SC informs the parent/family, their remaining study participation will consist of completing follow-up phone interviews and having their child's information obtained until they are 18 years of age as discussed during the consent process. Next, the SC should thank the family for their time and ask that they place any signed forms in the prepaid envelope they received and mail them as soon as possible.

If the participant is assigned to genomic sequencing, the SC will inform the parent/family that they have been assigned to be offered genomic sequencing. Then the SC or Genetic Counselor, depending on clinic, will review consent form 2 (i.e. Consent to Genomic Sequencing) by following the same procedure outlined for consent form 1. For detailed instructions on completing this task in the tracking system, see the "**Tracking System Tasks**" section on page 149.[2] If the parent signs consent form 2, the SC/GC will explain that the next step involves collecting a sample of the child's saliva for analysis. In order to do this, the SC will be mailing a saliva kit to the parent as soon as they (the SC) receive the signed consent forms from the parent. The SC should ask the parent to place all signed forms in the prepaid envelope they received and mail them as soon as possible. When the forms are received,

If the Parent Declines

If the parent declines signing either consent form 1 <u>or</u> the HIPAA consent, the parent/family should be informed that they will no longer be participating in the study. In the tracking system, the SC should exit (without saving) the current task and mark the participant "Refused" manually with the reason "Declined at Visit 1 – Intervention 2" and a short explanation.[3] The SC should then thank the family for their time and ensure they have received proper reimbursement for their visit, either in person or by mail.

If the parent declines signing consent form 2, the parent/family should be informed that their remaining study participation will consist of completing follow-up phone interviews and having their child's information obtained until they are 18 years of age as discussed during the consent process. The SC should then thank the family for their time and ensure they have received proper reimbursement for their visit, either in person or by mail. These participants will have a status of "Declined Consent/Assent to GS."

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[1] The tracking system should be used to record the parents stated decision. If the parent consents, the SC should record "Consent by Phone" in the parent's signature box and place their own signature in the box for witness. When the hard copy of the consent is received from the parent by mail, it should be scanned and uploaded to the participant's document page. If there are any discrepancies between the hard copy consent and the tracking system, the hard copy takes precedence. Should significant differences exist, the SC should consult the study director, Jeannette Bensen.

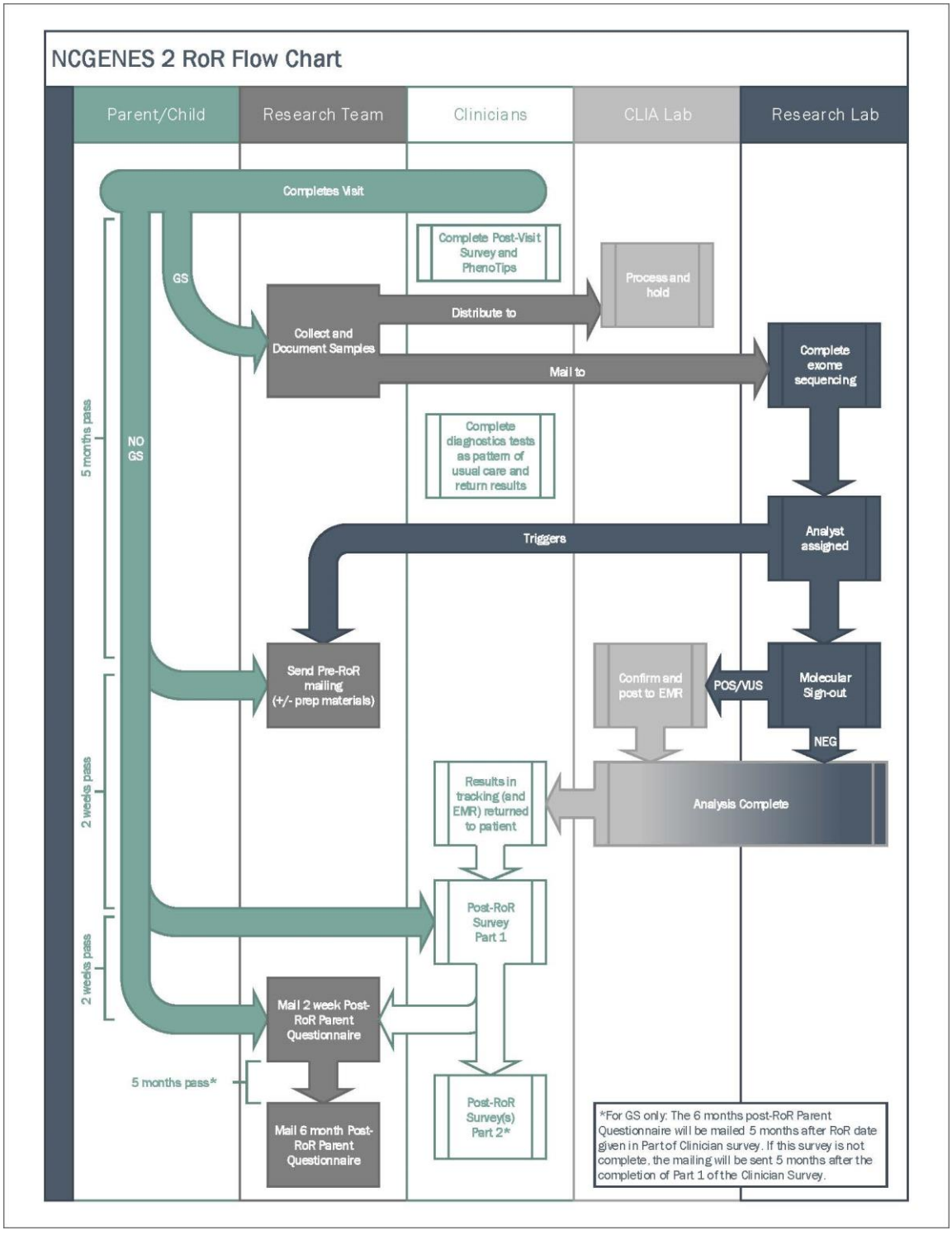
[2] See above note.

[3] See page 18 for more information on marking participants refused.

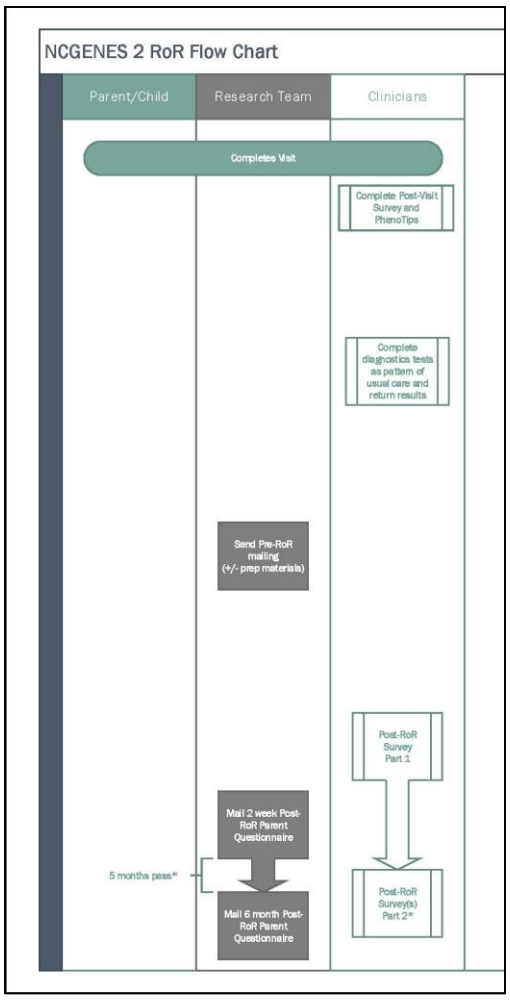
RETURN OF RESULTS

Overview

Currently in NCGENES 2, the term “Return of Results” (RoR) is used in two senses: (1) to refer to the general time period following Visit 1/Biospecimen Collection and (2) to refer to the process/task of returning results to participants who were randomized and consented to the genomic sequencing group. The RoR process flow can be seen in the diagram below. This diagram is broken apart and explored in detail in the sections that follow.



After Visit 1: All Participant Groups



At Visit 1, NCGENES participants are randomized into two groups, Genome Sequencing (GS) and No Genome Sequencing[1] (No GS). Much to the RoR process differs depending on the randomization arm of the participant. However, a few things can be generally stated about the process:

1. **Post-Visit Provider Activities** – Providers will be asked to completed a Post-Visit 1 Clinician Questionnaire and enter patient clinical data in the NCGENES2 PhenoTips Web Software regardless of the participant's randomization.

2. **Usual care** – Providers should *not* adjust their plan of care for a participant based on their participation in NCGENES 2 or their randomization. Providers should complete and return the results of any non-NCGENES 2 diagnostic tests according to their usual practices and timelines.

3. **Pre-RoR Mailing** – All participants will receive a "Pre-RoR" mailing of some type. The contents and timing of that mailing depend and the participant's randomization. See below for more detail.

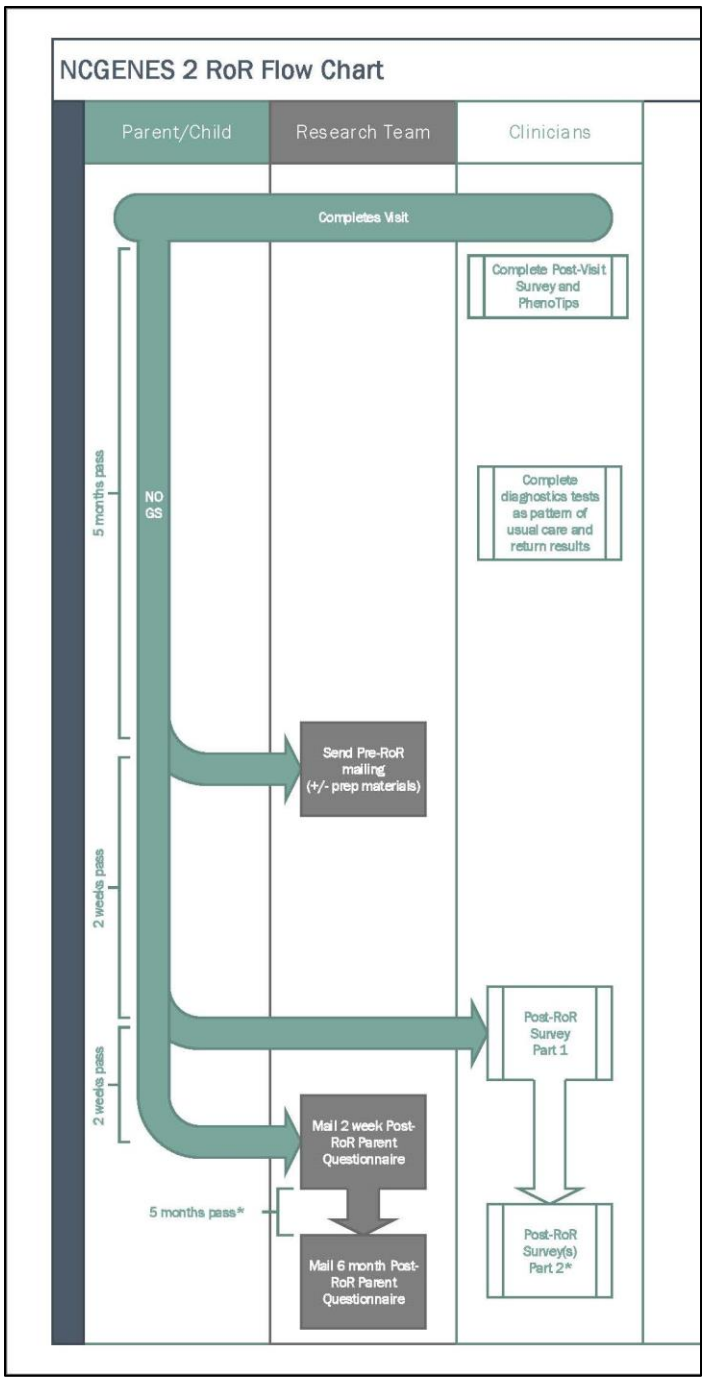
4. **Post-RoR Provider Survey(s) –** Providers will be asked to complete a 2-part post-RoR questionnaire for each NCGENES 2 participant. The content and timing of these questionnaires depends upon the randomization and documented treatment of the participant.
    a. *Part 1* addresses the utility of diagnostic tests (including genome sequencing, if applicable) and plan for continued care of the patient. The participant's primary genetic/neurology provider, usually a physician, will always complete this part of the survey.
    b. *Part 2* addresses the actual process of returning results, i.e. the interaction with the participant's caregiver(s). Whoever completes part 1 of the survey for a participant will indicate all parties that took part in returning results, e.g. genetic counselors or nurses. Each of those participating providers will complete a copy of part 2 of the survey for that participant.

5. **Post-RoR Parent/Caregiver Survey(s)** – Parent participants will be asked to complete a post-RoR questionnaire 2 weeks and 6 months after results have been returned. The content and exact timing of these questionnaires depends upon the randomization, documented treatment, and age of the participant. Questionnaires will be mailed to participants, but may also be completed by phone on request.

[1] This group also includes participants who are randomized to genome sequencing, but decline to receive it.

RoR for No GS Participants

The Return of Results process for No GS participants is generally simpler than that for GS participants. That being said, certain linitation of this clincial trial do create quriks that may be confusing to staff, providers, and participants.



Defining Results for No GS

Unlike their GS counterparts, GS participants have no standardized diagnostic test (i.e. exome sequencing performed by NCGENES 2) that might reliably produce results returned to the patient's caregivers. Instead, "results" for No GS participants should be understood as the results of all diagnostic tests ordered (if any[1]) by the participant's provider *during their first visit*.

Timing of RoR Tasks for No GS

Because the actual timelines for return of results for No GS participants will vary widely, the timelines surrounding RoR research tasks for these participants have been set chronologically to mimic, as best as possible, the timelines expected for participants in the other arm of the study. This means that the timing will rarely correspond to usual clinical practice in terms of returning results of diagnostic tests ordered at Visit 1. This is a necessary limitation of the study that should be understood by study staff should other stakeholders express confusion at the process.

Pre-RoR Mailings – No GS

Pre-RoR Mailings for No GS participants will always be sent exactly 5 months after the participant's Visit 1. For more information on completing this mailing, see Mailing 3 in the Mailings section on page 53. Staff should complete the "Pre-RoR Packet - No GS without Pre-Visit Prep" task in the tracking system upon completion of this mailing.

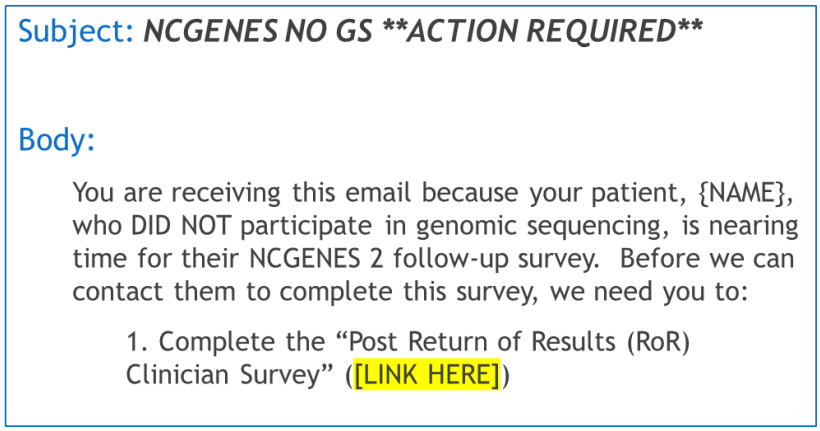
RoR Date – No GS

Functionally, the RoR Date for No GS participants is always set at 6 months after Visit 1. This date determines the windows in which post-RoR Parent Surveys are sent and received. Providers may also indicate in their Post-RoR surveys a date when results from ***all*** diagnostic tests ordered at Visit 1 where returned to the participants (i.e. the date the final result from these tests was returned). While this information is important to the study, it does not determine timelines for No GS participants.

[1] In some cases, the provider may not have ordered any diagnostic tests during the first visit. Even in these situations, the participant (and their provider) will receive post-RoR questionnaires tailored to that specific case.

Provider Post-RoR Surveys – No GS

Providers of No GS participants will receive an email notification (Fig. 1) exactly 5.5 months after visit one that informs them a Post-RoR survey is due. Providers can access this survey using the link in the email or from the MD surveys tab in their tracking system account. Once Part 1 of the provider survey is complete, Part 2 will be triggered automatically. If the initial provider is completing Part 2 of the survey, they will be automatically directed to it upon completion of Part 1. Any other provider designated to complete Part 2 will receive an email notification. Surveys should be completed as quickly as possible to minimize recall bias.


Subject: *NCGENES NO GS **ACTION REQUIRED***

Body:

You are receiving this email because your patient, {NAME}, who DID NOT participate in genomic sequencing, is nearing time for their NCGENES 2 follow-up survey. Before we can contact them to complete this survey, we need you to:

1. Complete the "Post Return of Results (RoR) Clinician Survey" ([LINK HERE])

FIGURE 1

Parent Post-RoR Surveys – No GS

Each parent will be sent two surveys after the RoR Date. These surveys will be mailed to the parents, filled out, and returned. Parent's also have the option of completing surveys by phone.

Post-RoR 2 Week Parent Survey

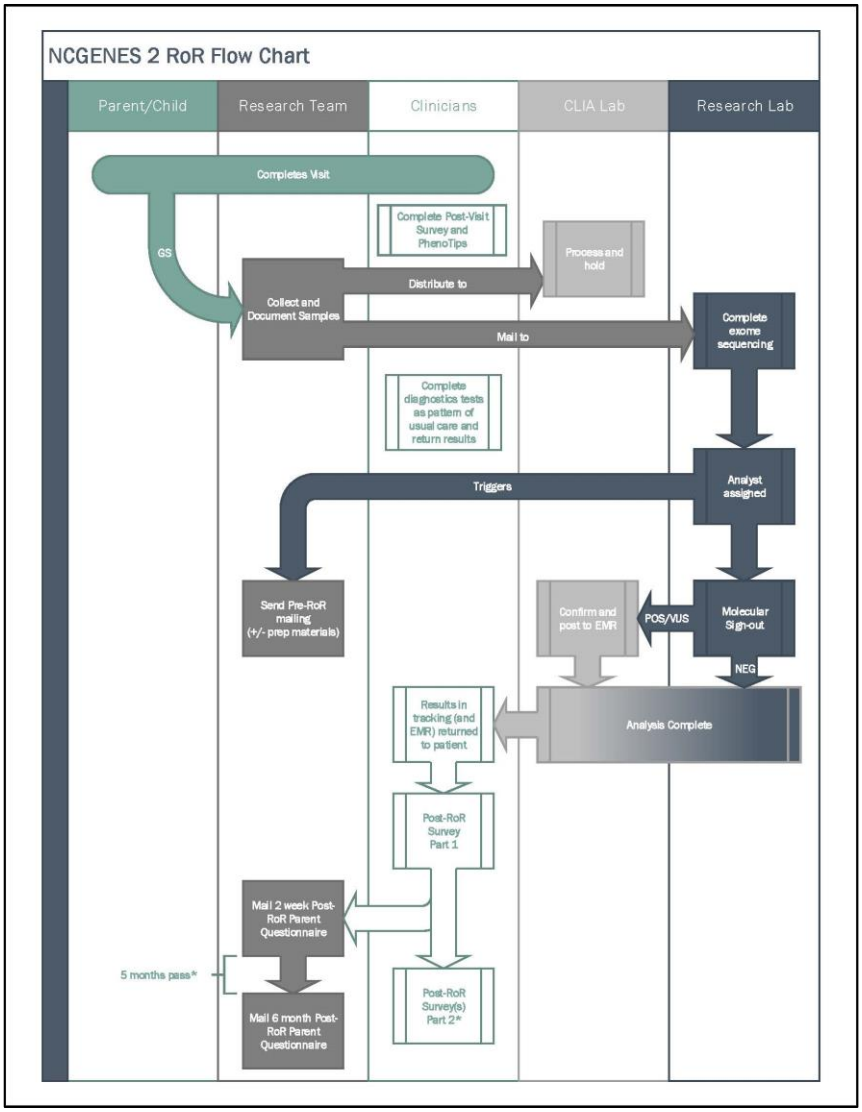
The first parent survey should be completed roughly two weeks after the RoR date. The window for completing the Post-RoR 2 week Parent survey is 0-6 weeks after return of results. For No GS participants, this means the 2 week survey should be completed between 6-7.5 months after Visit 1. Exactly six months after Visit 1, study staff will mail a survey to parents of participants not randomized to GS. For more details on this mailing, see Mailing 4 in the mailings section. Staff should complete the "Post-RoR - Parent 2 Week Questionnaire Mailing" task in the tracking system upon completion of this mailing. When the parent returns a completed Post-RoR 2 week survey by mail, staff should complete the "Post-RoR - Parent 2 Week Questionnaire Mailing - Received" task in the tracking system, the paper copy of the survey should be scanned and uploaded in the participants documents, and the data in the survey should be entered in the "Post-RoR – Parent 2 Week Questionnaire" task in the tracking system. This data entry should be done regardless of whether the survey was completed within the given window. Finally, staff should send a thank you note with gift card to the parent (See Mailing 5).

Post-RoR 6 Month Parent Survey

The first parent survey should be completed roughly six months after the RoR date. The window for completing the Post-RoR 6 month Parent survey is 5-7 months after return of results. For No GS participants, this means the 6 month survey should be completed between 11-13 months after Visit 1. Exactly eleven months after Visit 1, study staff will mail a survey to parents of participants not randomized to GS. For more details on this mailing, see Mailing 6 in the mailings section. Staff should complete the "Post-RoR - Parent 6 month Questionnaire Mailing" task in the tracking system upon completion of this mailing. When the parent returns a completed Post-RoR 6 month survey by mail, staff should complete the "Post-RoR - Parent 6 month Questionnaire Mailing - Received" task in the tracking system, the paper copy of the survey should be scanned and uploaded in the participants documents, and the data in the survey should be entered in the "Post-RoR – Parent 6 month Questionnaire" task in the tracking system. This data entry should be done regardless of whether the survey was completed within the given window. Finally, staff should send a thank you note with gift card to the parent (See Mailing 7).

RoR for GS Participants

The Return of Results process for GS participants is complex and depends upon multiple teams contributing to perform tests, return results, and document the process.



Results for GS

"Results" for GS participants refers=to the results of NCGENES 2 exome sequencing. NCGENES 2 staff will collect two biospecimens from each participant randomized to GS. One specimen will be sent to a CLIA lab to process and hold for potential confirmation. The other sample will be sent to UNC's Biospecimen Processing Facility. From there, DNA will be extracted and sent for sequencing. When data from sequencing returns, an analyst will be assigned to review any reported variants and perform a preliminary assessment of clinical significance. This report will be presented at regular Molecular Sign-Out committee meetings. If no variants are deemed clinically significant (i.e. results are negative), the results will be reported directly to the child's provider. If results are positive or uncertain, variants will be sent to the CLIA lab for confirmation. Confirmed positive/uncertain results will be posted in the patient's EMR.

Timing of RoR Tasks for GS

Unlike no GS participants, the timelines for RoR tasks for GS participants are variable and depend on upon the completion of tasks by a variety of NCGENES 2 teams. Because of this, timely completion of tasks is critical, particularly to ensure Post-RoR parent surveys can be collected within an acceptable timeframe.

Pre-RoR Mailings – GS

Pre-RoR Mailings for GS participants will always be sent as soon as an analyst is assigned to interpret that participant's sequencing data. For more information on completing this mailing, see Mailing 3 in the Mailings section on page 53. Staff should complete the "Pre-RoR Packet - GS without Pre-Visit Prep" or "Pre-RoR Packet - GS with Pre-Visit Prep" task in the tracking system upon completion of this mailing.

RoR Date – GS

The RoR Date for GS participants is set in one of two ways.

Prior to August 2020, the RoR Date was only set by the provider(s) during Part 2 of the Post-RoR Clinician Questionnaire. In that portion of the survey, the provider(s) will be asked to indicate when all planned communications associated with returning the exome sequencing results for the participant were complete. This date determines the windows in which post-RoR Parent Surveys are sent and received. However, because there may be a delay between the provider completing their survey(s) and the actual RoR date, NCGENES also uses the date of completion of Part 1 of the Post-RoR survey as a temporary RoR date until Part 2 of the survey is complete. Since Part 1 of the survey can only be

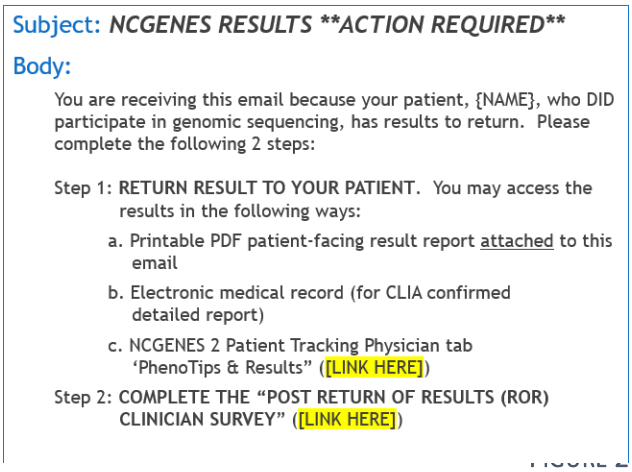
completed after results are returned, this allows study staff to quickly move forward on administering parent surveys without interfering with standard clinical practice.

As of August 2020, NCGENES staff will perform weekly checks of the EMR for any participant for whom results have been posted to the tracking system. If these checks reveal that NCGENES 2 results have been returned, the staff member will enter that RoR Date into the "Edit Info" page on the participant's page in tracking. This will trigger the post-RoR Parent survey tasks.

The Post-RoR Provider Part 1 trigger will remain in place. However, if any Parent RoR tasks trigger on that basis, UNC staff will immediately review the patient's EMR, determine the RoR Date, and enter it into tracking to trigger the remaining Post-RoR parent tasks.

Provider Post-RoR Surveys – GS

Providers of No GS participants will receive an email notification (Fig. 2) as soon as the molecular analysis for that participant is complete that informs them they have results and that a Post-RoR survey is due. Providers can access this survey using the link in the email or from the MD surveys tab in their tracking system account. Once Part 1 of the provider survey is complete, Part 2 will be triggered automatically. If the initial provider is completing Part 2 of the survey, they will be automatically directed to it upon completion of Part 1. Any other provider designated to complete Part 2 will receive an email notification. Surveys should be completed as quickly as possible to minimize recall bias.



Subject: ***NCGENES RESULTS **ACTION REQUIRED*****

Body:

You are receiving this email because your patient, {NAME}, who DID participate in genomic sequencing, has results to return. Please complete the following 2 steps:

Step 1: **RETURN RESULT TO YOUR PATIENT.** You may access the results in the following ways:

a. Printable PDF patient-facing result report attached to this email

b. Electronic medical record (for CLIA confirmed detailed report)

c. NCGENES 2 Patient Tracking Physician tab 'PhenoTips & Results" ([LINK HERE])

Step 2: **COMPLETE THE "POST RETURN OF RESULTS (ROR) CLINICIAN SURVEY"** ([LINK HERE])

Parent Post-RoR Surveys – GS

Each parent will be sent two surveys after the RoR Date. These surveys will be mailed to the parents, filled out, and returned. Parent's also have the option of completing surveys by phone.

Post-RoR 2 Week Parent Survey

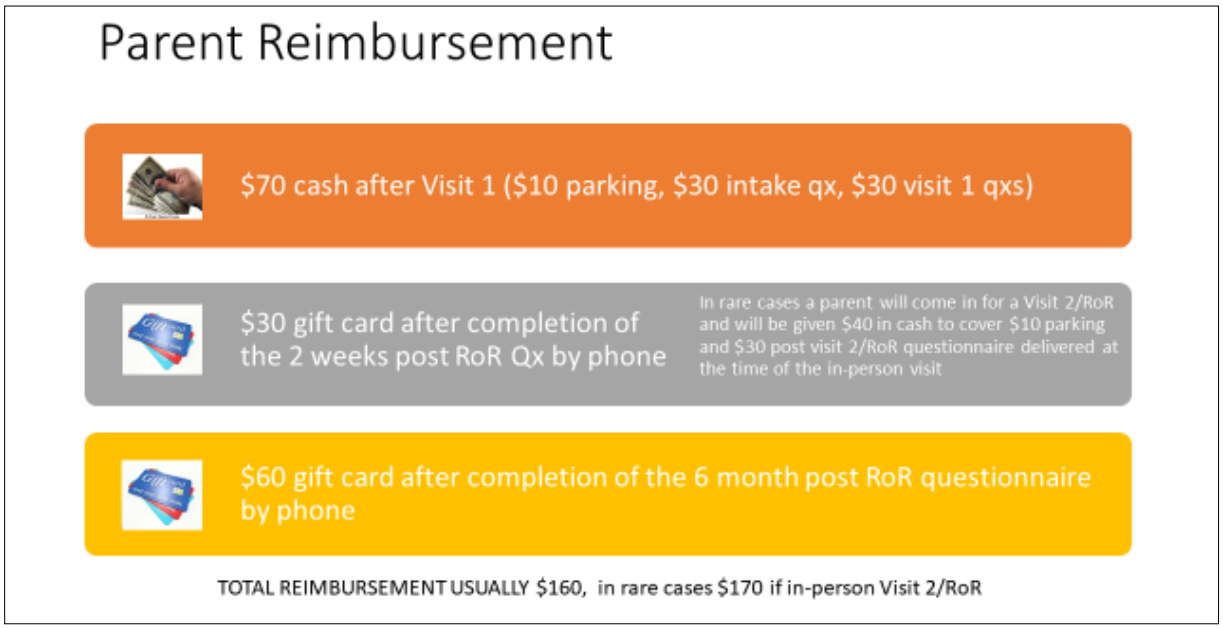
The first parent survey should be completed roughly two weeks after the RoR date. The window for completing the Post-RoR 2 week Parent survey is 0-6 weeks after return of results. As soon as an RoR Date is confirmed, study staff will mail a survey to participants randomized to the GS Group. For more details on this mailing, see Mailing 4 in the mailings section. Staff should complete the "Post-RoR - Parent 2 Week Questionnaire Mailing" task in the tracking system upon completion of this mailing. When the parent returns a completed Post-RoR 2 week survey by mail, staff should complete the "Post-RoR - Parent 2 Week Questionnaire Mailing - Received" task in the tracking system, the paper copy of the survey should be scanned and uploaded in the participants documents, and the data in the survey should be entered in the "Post-RoR – Parent 2 Week Questionnaire" task in the tracking system. This data entry should be done regardless of whether the survey was completed within the given window. Finally, staff should send a thank you note with gift card to the parent (See Mailing 5).

Post-RoR 6 Month Parent Survey

The first parent survey should be completed roughly six months after the RoR date. The window for completing the Post-RoR 6 month Parent survey is 5-7 months after return of results. Exactly 5 months after the RoR Date, study staff will mail a survey to parents of participants not randomized to GS. For more details on this mailing, see Mailing 6 in the mailings section. Staff should complete the "Post-RoR - Parent 6 month Questionnaire Mailing" task in the tracking system upon completion of this mailing. When the parent returns a completed Post-RoR 6 month survey by mail, staff should complete the "Post-RoR - Parent 6 month Questionnaire Mailing - Received" task in the tracking system, the paper copy of the survey should be scanned and uploaded in the participants documents, and the data in the survey should be entered in the "Post-RoR – Parent 6 month Questionnaire" task in the tracking system. This data entry should be done regardless of whether the survey was completed within the given window. Finally, staff should send a thank you note with gift card to the parent (See Mailing 7).

TRACKING PARTICIPANT COMPENSATION

The study coordinator will distribute compensation at the appropriate study time points. The breakdown for cash compensation to the parent can be seen below.



This section explains how compensation is acquired and tracked by NCGENES study staff.

Requesting Cash and Gift Cards at UNC

1. Staff should request 2 weeks before a need cash advance.
2. Once a cash advance is deposited to the clinical director's personal checking account (auto-deposit), another cash advance should be immediately initiated. Up to 2 cash advances may be outstanding when submitting new cash advance requests.
3. To complete a cash advance request, ask for the cash advance request form from Minh Quach, tell him the amount needed, receive via email, print, sign and return the originally signed doc to Minh (no scanned docs). Minh will get Jonathan Berg's co-signature.
4. Receipt the distribution of money by ID#, date, amount with participants' signature. This is done in the tracking system (receipt book used if tracking system does not work). The tracking system will create a printable spreadsheet of current charges (date to date) that I can be signed and given to Minh to justify each cash advance.
5. Study cash must be spent within 60 days, otherwise staff will need to provide justification of unspent money (e.g., participant no shows etc.)

Gift cards will be purchased with cash requested following the protocol above.

Purchasing Gift Cards for Mission Participants

Gift cards will be purchased by a Mission study team member and maintained in a locked box until needed.

Requesting Child Nonmonetary Gifts at UNC

The study coordinator will:

1. Request the P-Card from Laura Milko, NCGENES 2 Project Manager to make gift purchases
2. Purchase gifts and make two copies of the receipt
3. Submit the original receipt to Minh
4. Keep an Excel spreadsheet that tracks the distribution of each purchased item

5. Submit the tracking spreadsheet to Minh Quach along with one copy of the corresponding receipt, when all of the items on a receipt have been distributed
6. Keep one copy of the receipt for the NCGENES team records

Requesting Child Nonmonetary Gifts at Mission

The study coordinator will be responsible for purchasing gifts, storing in secure location, and having appropriate gift available for each participant (by gender and age). Purchase/reimbursement processes have been determined and agreed upon with the Principal Investigator and the Mission Research Institute.

Tracking of Parent and Child Compensation

Following the completion of visit 1, the SC will complete the Excel tracking spreadsheets to indicate distribution of parent compensation and child gift selection (i.e. child compensation), and periodically update the clinical project director on the status of compensation distribution. The Excel document tracks the Participant ID#, date of compensation distribution, amount distributed, and whether the parent's signature was obtained (Y/N). After all the funds from each cash/gift card request have been distributed (typically to the amount of $700 or compensation for 10 participants), the SC will inform the clinical project director of completion of fund distribution. Additionally, the SC will inform the clinical project director when a purchase is needed for nonmonetary gifts and when related submissions to Minh Quach occur.

Compensation for Follow-up tasks

After completion of the Return of Results and Final Follow-up surveys, the parents will be mailed gift cards. The gift cards will be tracked by recording the card amount, type and number of the gift card.

INTERNAL REPORTING/TRACKING

To assist with providing study updates to senior study staff/study sponsor, the below spreadsheets/reports are completed.

Enrollment Report

The NCGENES Steering Committee meets twice a month to discuss overall study activities. Prior to each meeting, the biostatistician, Laura Farnan, creates a pre-visit 1 enrollment, visit 1 participation, and participation report. Each of these reports are stratified by served and underserved. The biostatistician works with the clinical project director and the study coordinator to identify and resolve any concerns regarding these reports. Then the clinical project director presents these reports during the Steering Committee meeting. If the director is unable to attend the meeting, the reports are presented by the study coordinator.

Provider Report

Beginning in February 2019, the biostatistician creates a report demonstrating the completion rate of provider surveys and PhenoTip data entry. Data in this report is displayed per provider in each UNC clinic. The SC works with the biostatistician to resolve any questions regarding this report. Then the SC submits this report on a weekly basis to the project PI and clinical project director.

Participant Eligibility Tracking

During the participant eligibility screening process, if the SC is unable to determine eligibility, they discuss the situation with the clinical project director/project PI. Based upon these discussions, eligibility is determined, and the decision is reflected in the tracking system/eligibility report.

Participants that are discussed with the PI, the biostatistician includes in the eligibility report. For this report the SC provides the biostatistician the following information to be included in the report: the participant's name, medical record number, a summary of the situation, and the eligibility decision. For eligible participants, the SC also provides the biostatistician the NCGENES study ID number.

This report is used by the SC and clinical project director as a resource when discussing future participants with unclear eligibility and to review the completeness of the study inclusion criteria.

PARTICIPANT MAILINGS

This section contains step-by-step guides for completing all study mailings. First, this section addresses general mailing procedures (e.g. mail-merge, postage). Then, it details the steps for each specific mailing, starting with normal study mailings and moving to biospecimen collection mailings

Study documents, especially mail merged documents, should be downloaded directly from **IRBIS** to ensure that the most recent edited and authorized document versions are being used. Other documents can be downloaded from IRBIS and copied on appropriately colored paper (see below), HOWEVER careful attention should be paid to use the current version of the document. IF ANY change to a copied form is made and subsequently approved by IRB, all old copies of the form must be discarded and a new original obtained. Careful attention should be paid that current versions of all documents are being used.

The UNC Study Coordinator is responsible for ensuring that all other sites (ECU/Mission) have access to IRBIS.

General Mailing Procedures

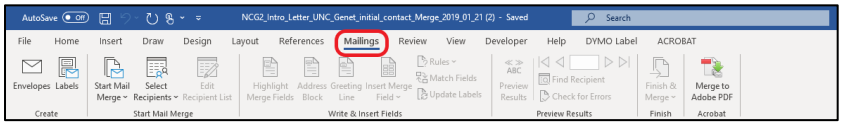
Tracking System Mailings Page

All study mailings begin from the Mailings Page in the tracking system. This page generates mail merge lists (excel files) with participant contact information and tracks when mailings get sent. A detailed description of the Mailings Page can be found in the "**Using the Tracking System**" section on page 65.

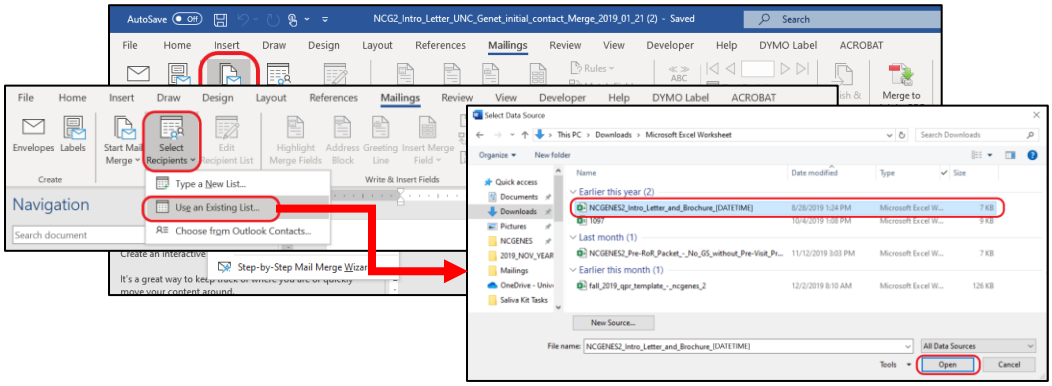
Completing the Mail Merge

Once a mail merge excel document was been generated by the tracking system and downloaded to a user's computer, staff must use the mail merge function in Microsoft Word to print the letter(s) specific to each mailing. The process to complete these mail merges is largely the same for each mailing

1. Download the appropriate letter from **IRBIS** and open it in Word.[1]
2. In Word, select the "Mailings" tab from the ribbon.[2]



3. Select "Start Mail Merge" and then "Letters."

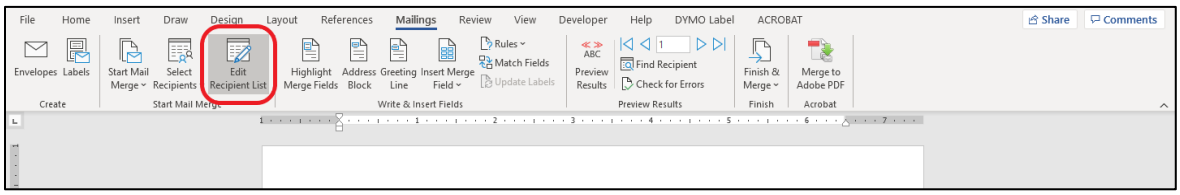


4. Click "Select Recipients," "Select an existing list," and then find and select the appropriate excel sheet downloaded from the tracking system.

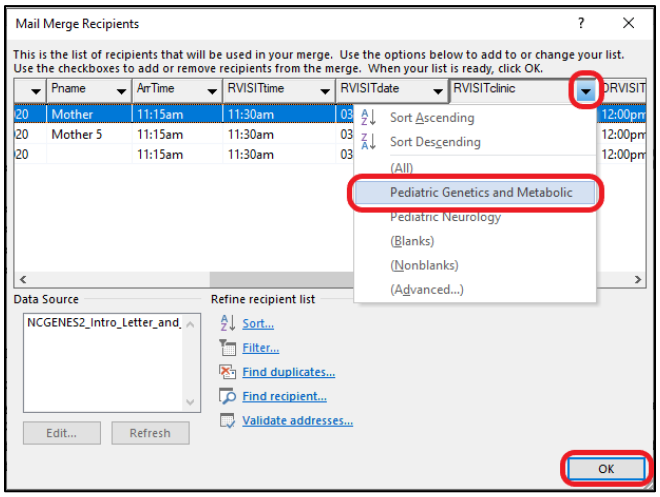
[1] The letters necessary for each mailing are listed in that mailing's section below. You may also consult the "IRBIS – Cheat Sheet" for clarification on the appropriate file to download. This is a separate file; contact Peter Newman-Matthews if you do not have a copy.
[2] Screenshots from Microsoft Office display Windows version of these programs. Other versions may appear differently.

(Optional: Some letters differ depending on clinic. For these letters, only relevant participants should be included in the merge. Steps 5-6 demonstrate the process of filtering by clinic)

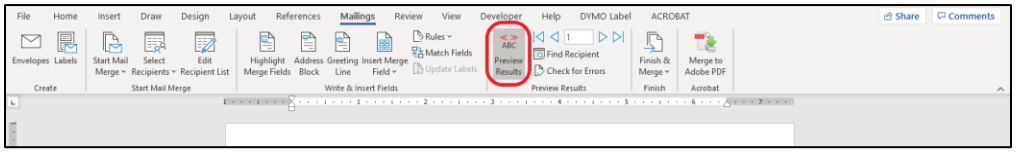
5. Click “Edit Recipient List”



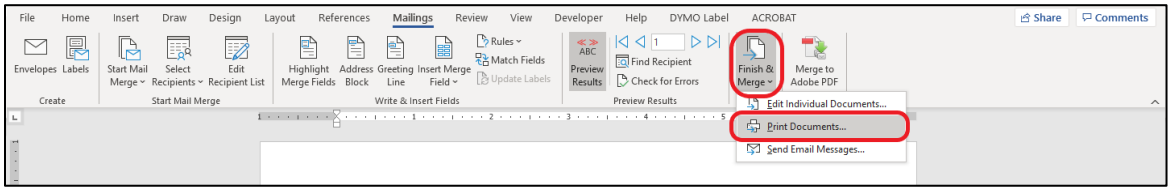
6. Sort by the field RVISITclinic and select only the appropriate clinic for the specific letter.



7. Select “Preview Results.”



8. Click “Finish & Merge” and then “Print documents.”



In most cases, a mailing will contain at least one mail merge document. If a staff member encounters any issues with the mail merge function or cannot locate the required documents, please contact Peter Newman-Matthews (**peter@med.unc.edu**) for assistance.

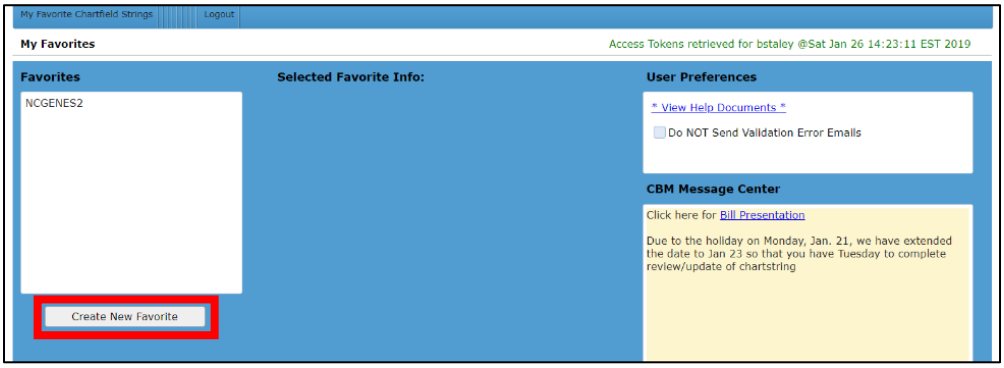
Mailing-Pre-postage protocol

Each study site follows a different process in terms of ordering/printing postage. This section outlines these processes.

UNC

1. Go to site: **https://portal.facilities.unc.edu/masterpg/slipMain.aspx**

2. **If you are visiting this site for the first time, you will need to associate the project account with your onyen. <u>If you have already done this, skip to step 3</u>:**
   a. Click the green "+" sign to add the NCGENES 2 account to your onyen
   b. Click "Create New Favorite"

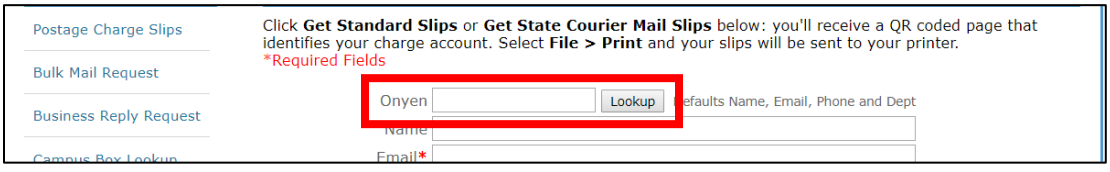


   c. Enter content listed below in the appropriate boxes

Favorite Name:	NCGENES 2
Business Unit:	UNCCH
Fund:	25210
Department:	423501 *(updated 3/31/15)*
Source:	49000
PC Bus Unit:	CHOSR
Project:	5113853 *(updated 6/12/2019)*
Activity:	1

   d. Slick the "Save New Favorite" button.
   e. Close the window.

3. Type your onyen and click "lookup" and the account information will automatically populate.



4. For the chartfield string click the drop down arrow and select the account.

5. For the campus box, you will initially need to manually enter: “7240” then going forward this number will be available via the dropdown arrow.
6. Click “get standard slips”
7. On the next page, enter today’s date and click 1st Class full Charge and then enter the # of items you are mailing.
8. Use snipping tool to get “snip” of postage authorization slip. Save it and email it to Minh Quach at the end of each the month **(mquach@email.unc.edu)**
9. Print the postage slip and cut along the dotted lines Paper clip it to the top of the mail and take it to the Bioinformatics Building mailroom on the Basement floor. The mailroom closes at 4:00pm; therefore, ideally all mail should be taken to the mailroom by 3:30pm to increase the likelihood of same day processing.

Note:
University Mailing Service Contact
Nicole Ferrell
Quality Assurance Specialist
Located in the Bioinformatics Building
Email: colee84@email.unc.edu
Office #: 919.962.5075

ECU (TBD)

Mission

Once the Mailing 2 packet is assembled and placed in the envelope, stamps (purchased and held by the Study Coordinator) are placed on the envelope according to weight; calculator can be accessed on USPS site if needed. For example:

- A Pre-Visit Prep envelope weighing 3.8 oz would require $1.45 in postage.
- An envelope without the Pre-Visit Prep weighing 2.8 oz would require $1.30 in postage.

Study mailings are then placed with other outgoing mail in the Laboratory reception area.

Mailing 1 – Intro Letter and Brochure

All participants who are selected or pending selection into the study will have a clinic-specific introductory letter and study brochure mailed to the parent's address recorded in the tracking system. This mailing should be processed daily.

To complete this mailing:

1. Download the clinic-specific Intro letter from **IRBIS**, complete the **mail merge**, and print.
2. Print 1 Introductory Brochure for each mailing.
3. Affix **postage** and **address labels**.
4. Deliver to appropriate mailing deposit/box.

Once the letters are printed, they should be placed in standard envelopes with appropriate labels. There are more steps here.

Relevant Documents

Document	**File Name (*Names in italics vary by site/clinic*)**
Introductory Brochure	NCGENES_2_Study_Brochure_Recruitment_[DATE]
Intro Letter	*NCG2_Intro_Letter_UNC_Genet_initial_contact_Merge_[DATE]*
Mail merge excel sheet	NCGENES2_Intro_Letter_and_Brochure_[DATESTAMP].xlsx

Mailing 2 – Pre-Visit Packet

All parents who consent to participate in the study will be sent a study mailing that includes:

- Reminder letter for research and doctor appointment with a map of the UNC Health Care Main Medical Campus
- Instruction sheet for that describes the order in which forms in the study mailing packet should be completed
- Information sheet (essentially a summary of the phone consent)
- Intake Survey (Versions of survey vary by age[1])
- Pre-Visit Materials (Dependent on randomization 1 assignment - These additional documents are for parents randomized to receive pre-visit educational materials.)
  - Pre-Visit Guide
  - Question Prompt List

This mailing should be packaged after completing an enrollment call and sent the day it is put together.

Note: Except for the reminder letter, all of the other previously listed items should be pre-printed in bulk on the appropriately colored paper.

[1] Pediatric participants in NCGENES 2 must be <16 years at eligibility determination. The age-specific study documents sent to parent participants are based on the age determined at eligibility. Pediatric participants may age-up while actively understudy, but age is treated as static in the study. However, for assent, age must be based on the child's age while at visit 1.

To Complete this Mailing:

1. Download the Visit 1 Appointment Reminder letter from **IRBIS**, complete the **mail merge**, and print.
2. Print/collect and package the following attachments:
   a. **For participants randomized *into* the pre-visit prep arm**
      - Instruction Sheet A (on white paper)
      - Information Sheet A (on blue paper)
      - *Age-appropriate* Intake Survey (on yellow paper)
      - Pre-Visit Prep Envelope with
        - Pre-visit Guide
        - Question Prompt List

   b. **For participants randomized *out of* the pre-visit prep arm**
      - Instruction Sheet B (on white paper)
      - Information Sheet B (on blue paper)
      - *Age-appropriate* Intake Survey (on yellow paper)
3. Affix **postage** and **address labels**.
4. Deliver to appropriate mailing deposit/box.

To simplify Mailing 2, study coordinators are encouraged to create separate files/sectionals for the two versions of the Visit 1 appointment packets (i.e. pre-visit prep and no pre-visit prep). The Pre-Visit Guide and Question Prompt List can be pre-printed together and enclosed in an individual envelope with a pre-visit envelope label. Those envelopes can be stored alongside the Instruction sheet A and Information sheet A. A separate file/sectional can house the contents of the Version B packet for the packets without Pre-Visit Preparation: Instruction Sheet-B and Information Sheet-B.

The Intake Survey is **child-age specific** and does not differ across pre-visit prep assignments. Be sure to include the correct version of the survey for the participant. Include the survey version for the age of the participant at the time that their eligibility was determined.

Important notes about age in NCGENES 2: Pediatric participants in NCGENES 2 must be <16 years at eligibility determination. Intake surveys sent to parent participants are based on the child's age at their determination of eligibility. The following table clarifies the age ranges found on intake surveys:

Survey Age Bracket	Clarified Age Range
1-12 mos	From birth until 1 day prior to 1st birthday (includes infants <1 month old)
13-24 mos	From 1st birthday until 1 day prior to 2nd birthday
2-4 yrs	From 2nd birthday until 1 day prior to 5th birthday
5-7 yrs	From 5th birthday until 1 day prior to 8th birthday
8-12 yrs	From 8th birthday until 1 day prior to 13th birthday
13-18 yrs	From 13th birthday until 1 day prior to 16th birthday (excludes patients >16 years old)

Relevant Documents

Document	File Name (*Names in italics vary by site/clinic*)
Visit 1 Appointment Reminder Letter	*NCGENES_2_V1_Appt_Reminder_Letter_Mail_Merge_[DATE]*
Intake Survey	Intake Survey [DATE] for [Age Range] clean
Information Sheet	*NCGENES_2_Information_sheet_Version_A_clean_[DATE]* *NCGENES_2_Information_sheet_Version_B_clean_[DATE]*
Instructions Sheet	NCGENES 2 Instructions Sheet with Previsit_[DATE] NCGENES 2 Instructions Sheet without Previsit_[DATE]
Pre-Visit Booklet	Pre-Visit Booklet_post CCT input -- FINAL [DATE]
Question Prompt List	NCGENES QPL_1 page flyer [DATE]-jsb cr.pptx
Mail Merge Excel Document	NCGENES2_Visit_1_Appointment_Packet_-_without_Pre_Visit_Prep_[DATESTAMP].xlsx NCGENES2_Visit_1_Appointment_Packet_-_with_Pre_Visit_Prep_[DATESTAMP].xlsx

Mailing 3 – Pre-RoR Packet

Adult participants who consent (with assent as necessary) to the Randomization to Genome Sequencing will receive some version of a Pre-Return of Results Packet regardless of their randomization to intervention 1 or 2. This mailing contains a letter reminding parents that they will receive a survey after they've received results. Participants randomized to exome sequencing and pre-visit prep will also receive some pre-return of results educational materials.

To Complete this Mailing:

1. **For participants randomized to receive Exome Sequencing and Pre-Visit Prep:**
   a. Download the "pre-ROR Packet Letter – GS and Prep" letter from **IRBIS**, complete the **mail merge**, and print.
   b. Print and attach the Pre RoR prep brochure with Question Prompt List
2. **For all other participants,** download the "pre-ROR Packet Letter – NO RoR" letter from **IRBIS**, complete the **mail merge**, and print.
3. Affix **postage** and **address labels**.
4. Deliver to appropriate mailing deposit/box.

Relevant Documents:

Document	File Name (*Names in italics vary by site/clinic*)
Pre-RoR Packet Letter	NCGENES_2_pre-RoR_PACKET Letter_GS_and_Prep_Arm [DATE] NCGENES_2_pre-RoR PACKET_Letter_NO_RoR_Prep [DATE]
Pre RoR Prep brochure	NCGENES 2 pre ROR prep_FINAL
Mail Merge Excel Document	NCGENES2_Pre-RoR_Packet_-_No_GS_without_Pre-Visit_Prep_[DATESTAMP].xlsx NCGENES2_Pre-RoR_Packet_-_ GS_without_Pre-Visit_Prep_[DATESTAMP].xlsx NCGENES2_Pre-RoR_Packet_-_ GS_with_Pre-Visit_Prep_[DATESTAMP].xlsx

Mailing 4 – 2 Week Post-RoR Parent Survey

Adult participants who consent their children (with assent as necessary) to the Randomization to Genome Sequencing will receive some version of a Post-RoR 2 week survey regardless of their randomization to intervention 1 or 2. This mailing contains a cover letter and one of four surveys depending upon the participant.

To Complete this Mailing:

1. Download the RoR 2 Week Survey Letter from **IRBIS**, complete the **mail merge**, and print.
   a. **For participants consented to genome sequencing:**
      i. *If the participant was randomized to Pre-Visit Prep*, attach the "RoR 2 Week Parent Survey [DATE] Paper ES and Previsit Prep" Survey
      ii. *If the participant was randomized out of Pre-Visit Prep*, attach the "RoR 2 Week Parent Survey [DATE] Paper ES and NO Previsit Prep" Survey
   b. **For participants who did not receive genome sequencing:**
      i. *If the participant received some diagnostic testing*, attach the "RoR 2 Week Parent Survey [DATE] Paper No ES and Diagnostic Testing" Survey
      ii. *If the participant did not receive any diagnostic testing*, attach the "RoR 2 Week Parent Survey [DATE] Paper No ES and No Diagnostic Testing" Survey
2. Create a return envelope and include in the mailing
3. Affix **postage** and **address labels**.
4. Deliver to appropriate mailing deposit/box.

Relevant Documents:

Document	**File Name (*Names in italics vary by site/clinic*)**
RoR 2 Week Survey Letter	RoR 2 week survey cover letter_[DATE]
RoR 2 Week Surveys	RoR 2 Week Parent Survey [DATE] Paper ES and Previsit Prep RoR 2 Week Parent Survey [DATE] Paper ES and NO Previsit Prep RoR 2 Week Parent Survey [DATE] Paper No ES and Diagnostic Testing RoR 2 Week Parent Survey [DATE] Paper No ES and No Diagnostic Testing
Mail Merge Excel Document	NCGENES2_Post-RoR_- _Parent_2_Week_Questionnaire_Mailing_[DATESTAMP].xlsx

Mailing 5 – Gift Card and Thank You for 2 Week Survey

Whenever a completed 2 week Post-RoR Parent Questionnaire is returned by the parent, a thank you card should be sent to the parent along with a gift card for $30 (See **Tracking Participant Compensation**). This mailing should be sent even if the survey was completed outside the technical window for completion.

To Complete this Mailing:

1. In a thank you card, write the following message:
   "Thank you for completing and returning your survey! It will be a few months before the next study step which is a survey. Please let us know if you have any questions or changes to your contact information. Thank you for your continued participation in NCGENES!
   [include a name and best contact method #]"
2. Place a gift card in the thank you card and place both in an envelope.
3. Affix **postage** and **address labels** (using **mail merge** from tracking system).
4. Deliver to appropriate mailing deposit/box.

Relevant Documents:

Document	**File Name (*Names in italics vary by site/clinic*)**
Mail Merge Excel Document	NCGENES2_Post-RoR_- _Parent_2_Week_Questionnaire_Gift_Card_Mailing_[DATESTAMP].xlsx
Thank You Text	Text_inside_Thank_You_Notes_NCGENES_[DATE]

Mailing 6 – 6 Month Post-RoR Parent Survey

Adult participants who consent their children (with assent as necessary) to the Randomization to Genome Sequencing will receive some version of a Post-RoR 6 month survey regardless of their randomization to intervention 1 or 2. This mailing contains a cover letter and one of several surveys depending upon the participant's age and clinical history.

To Complete this Mailing:

1. Download the RoR 6 month Survey Letter from **IRBIS**, complete the **mail merge**, and print.
   a. **For participants consented to genome sequencing,** attach the "RoR 6 Month Parent Survey [DATE] [AGE RANGE] Paper Diagnostic Testing" Survey for the age of the participant.
   b. **For participants who did not receive genome sequencing:**
      i. *If the participant received some diagnostic testing*, attach the "RoR 6 Month Parent Survey [DATE] [AGE RANGE] Paper Diagnostic Testing" Survey for the age of the participant.
      ii. *If the participant did not receive any diagnostic testing*, the "RoR 6 Month Parent Survey [DATE] [AGE RANGE] Paper **NO** Diagnostic Testing" Survey for the age of the participant.
2. Create a return envelope and include in the mailing
3. Affix **postage** and **address labels**.
4. Deliver to appropriate mailing deposit/box.

The 6 month Post-RoR Survey is **child-age specific**. Be sure to include the correct version of the survey for the participant. Include the survey version for the age of the participant *at the time of the survey's mailing*.

Important notes about age in NCGENES 2: Pediatric participants in NCGENES 2 must be <16 years at eligibility determination. Intake surveys sent to parent participants are based on the child's age at their determination of eligibility. The following table clarifies the age ranges found on intake surveys:

Survey Age Bracket	Clarified Age Range
1-12 mos	From birth until 1 day prior to 1st birthday (includes infants <1 month old)
13-24 mos	From 1st birthday until 1 day prior to 2nd birthday
2-4 yrs	From 2nd birthday until 1 day prior to 5th birthday
5-7 yrs	From 5th birthday until 1 day prior to 8th birthday
8-12 yrs	From 8th birthday until 1 day prior to 13th birthday
13-18 yrs	From 13th birthday until 1 day prior to 16th birthday (excludes patients >16 years old)

Relevant Documents:

Document	File Name (*Names in italics vary by site/clinic*)
RoR 6 Month Survey Letter	RoR 6 month survey cover letter_[DATE]
RoR 6 Month Surveys	RoR 6 Month Parent Survey [DATE] [AGE RANGE] Paper NO Diagnostic Testing RoR 6 Month Parent Survey [DATE] [AGE RANGE] Paper Diagnostic Testing
Mail Merge Excel Document	NCGENES2_Post-RoR_- _Parent_6_Month_Questionnaire_Mailing_[DATESTAMP].xlsx

Mailing 7 – Final Gift Card and Thank You for 6 Month Survey

Whenever a completed 6 month Post-RoR Parent Questionnaire is returned by the parent, a thank you card should be sent to the parent along with a gift card for $60 (See **Tracking Participant Compensation**). This mailing should be sent even if the survey was completed outside the technical window for completion.

To Complete this Mailing:

1. In a thank you card, write the following message:
   "Thank you for completing your final survey with NCGENES 2!
   [include a name and best contact method #]"
2. Place a gift card in the thank you card and place both in an envelope.
3. Affix **postage** and **address labels** (using **mail merge** from tracking system).
4. Deliver to appropriate mailing deposit/box.

Relevant Documents:

Document	**File Name (*Names in italics vary by site/clinic*)**
Mail Merge Excel Document	NCGENES2_Post-RoR_-_Parent_6_Month_Questionnaire_Gift_Card_Mailing_[DATESTAMP].xlsx
Thank You Text	Text_inside_Thank_You_Notes_NCGENES_[DATE]

USING THE TRACKING SYSTEM

A web-based tracking system has been developed for the management of the NCGENES 2 project. The tracking system originates, facilitates and/or records every study activity. In other words, it is the central tool of study staff. The tracking system can be accessed in several different ways. Users should be cautious of which site they are using, as any data entered on the live site will be part of the study record.

Each study location (UNC, Mission, ECU) has a corresponding tracking site independent from the others. While the URLs for each are identical, a user's log-in information will dictate which tracking site they view. The study location of a tracking site will be indicated in its header (See **Homepage** on page 61). If a study staff member has different roles at multiple sites, they will need different log-in credentials in order to access each site separately.

Live Site: ***https://ncgenes2.sirs.unc.edu/***
The live site is the main hub for study operation.

Demo Site I: ***https://ncgenes2.sirsdemo.unc.edu/***
This demo version of the site was used for development of pre-visit and visit 1 tasks and can be used for training purposes. Data input here does not enter the study record. Additionally, alert protocols created by the Demo Site will not trigger emails to the clinical project director.

Demo Site II: ***https://ncgenes2-ror.sirsdemo.unc.edu/***
This demo version of the site is used for development of return of results tasks and can also be used for training purposes. Data input here does not enter the study record. Additionally, alert protocols created by the Demo Site will not trigger emails to the clinical project director.

Survey Site: ***https://ncgenes2.sirs.unc.edu/surveys***
The survey site is used to complete parent surveys. Each survey requires a unique code and parent's completing surveys using this site cannot access other data in the tracking system.

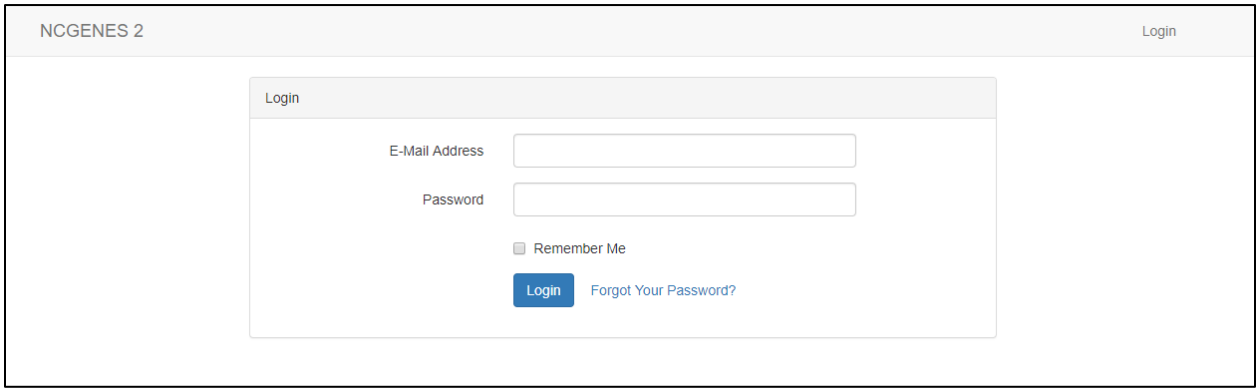
Provider Site: ***https://ncgenes2.sirs.unc.edu/***
Provider's share a log-in page with other study staff but see a unique version of the site. Provider's only access surveys and result information for their patients.

This section covers the basic functions of the tracking system, key terminology, important tools within the tracking system and tracking system roles. Later on, the protocol provides more detailed instructions for completing specific tasks within the tracking system.

Logging In

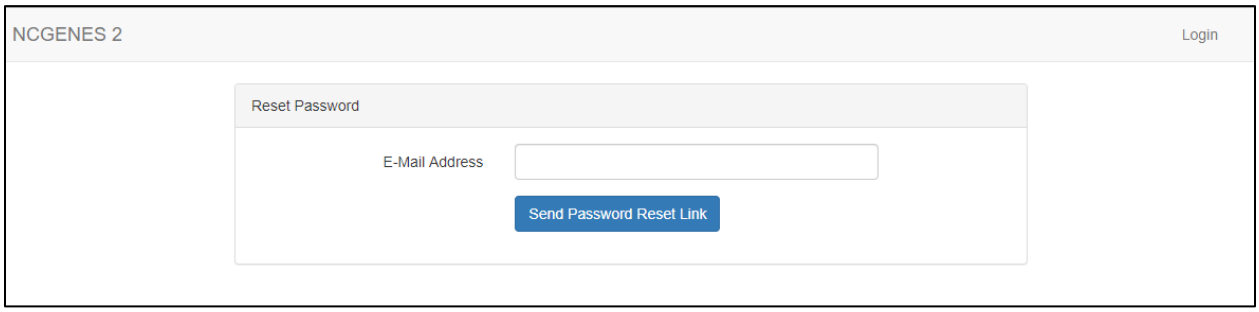
The following covers logging in to the live tracking site with appropriate screenshots. The same procedures can be used to access either demo version of the site.

1. In a web browser, navigate to ***https://ncgenes2.sirs.unc.edu/***. The following page will appear:



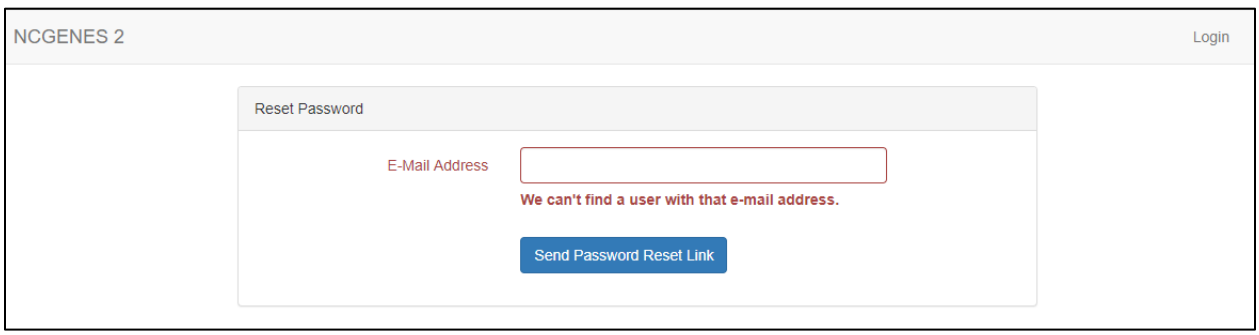
2. If this is your first time accessing the system, you'll need to create a password. Steps 3 through 7 will cover this process. If you already have a password, simply enter your email and password in the appropriate boxes and click the blue "Login" button to log in.

3. If you need to create a password, or if you cannot remember your password, click the "Forgot Your Password?" link just to the right of the "Login" button. The following page will appear:

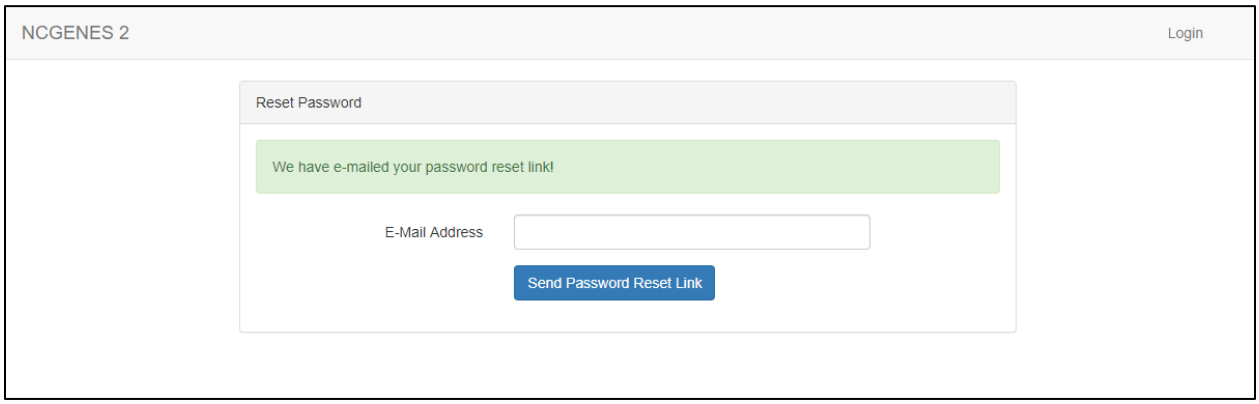


4. Enter your email address into the appropriate box, and click the "Send the Password Reset Link." One of two things will happen at this point.

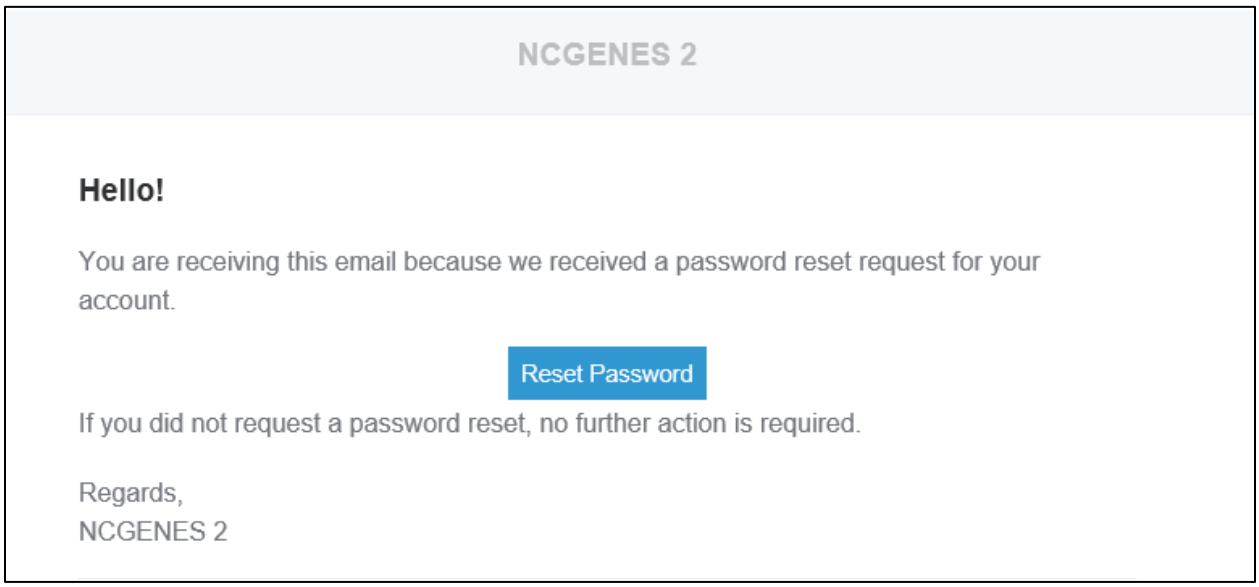
    a. If the following screen appears (next page), you may not have been added as a user to the tracking system. If you receive this message, contact the project coordinator (**pnewmatt@med.unc.edu**) or study director (**jeannette_bensen@med.unc.edu**) to resolve the issue.



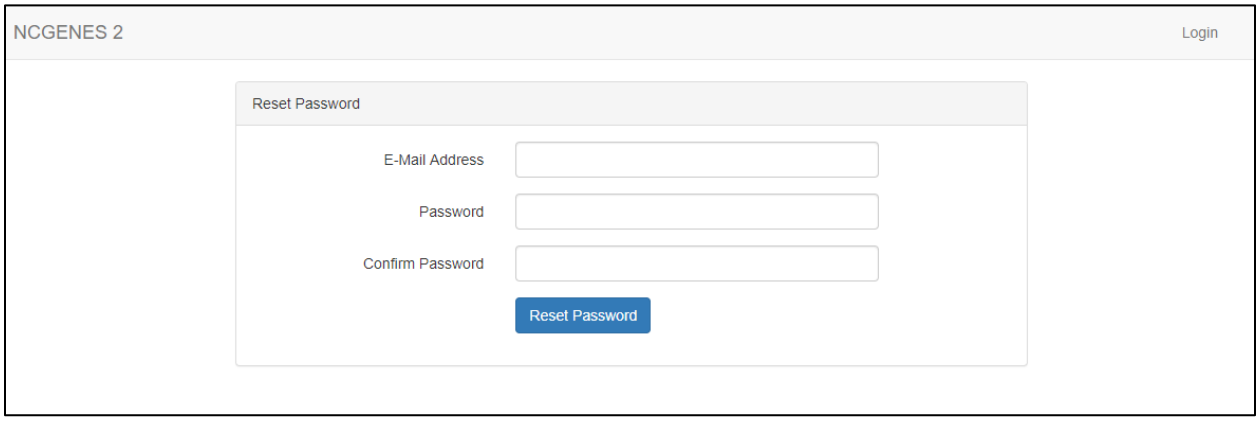
b. In most cases, the following screen will appear:



5. If you receive this message, proceed to your email inbox and look for an email from **jknop@unc.edu**. The email should look like this:



6. Click the blue "Rest Password" button, which will open a new browser window (or tab) displaying the following page:



7. Enter your email, new password twice in the appropriate boxes. Then press the blue "Reset Password" button. You will be automatically logged in at that time. Remember to keep track of your password for future logins.

Key Terminology: Task v. Status

Two terms, task and status, are central to the functioning of the tracking system. Unfortunately, these terms are also easily conflated.

Tasks

Study activities are captured in the tracking system as tasks. A task is completed by study staff in accordance with wider protocol. For example, the study coordinator will complete the "Enrollment Call" task while they call a parent and attempt to enroll them. As a participant moves through the study, the completion of tasks will trigger subsequent tasks. At a given time, a participant may have several tasks pending. A participant's tasks are listed on their **individual page** (see page 67).

Statuses

Unlike tasks, a participant can only have one status at a time. Statuses capture a participant's progress through the study. For instance, a participant whose parent has completed the enrollment call and consented will have the status of "Parent Consented." Participants with similar statuses are in the same phase of the study. Several statuses in the study are final statuses. As the name indicates, final statuses do not change and indicate a participant's final state in relationship to NCGENES. **Appendix II** contains a full diagram of study statuses. A participant's current status is listed on the **Participants List** (see page 62) or on the participant's **individual page** (see page 67).

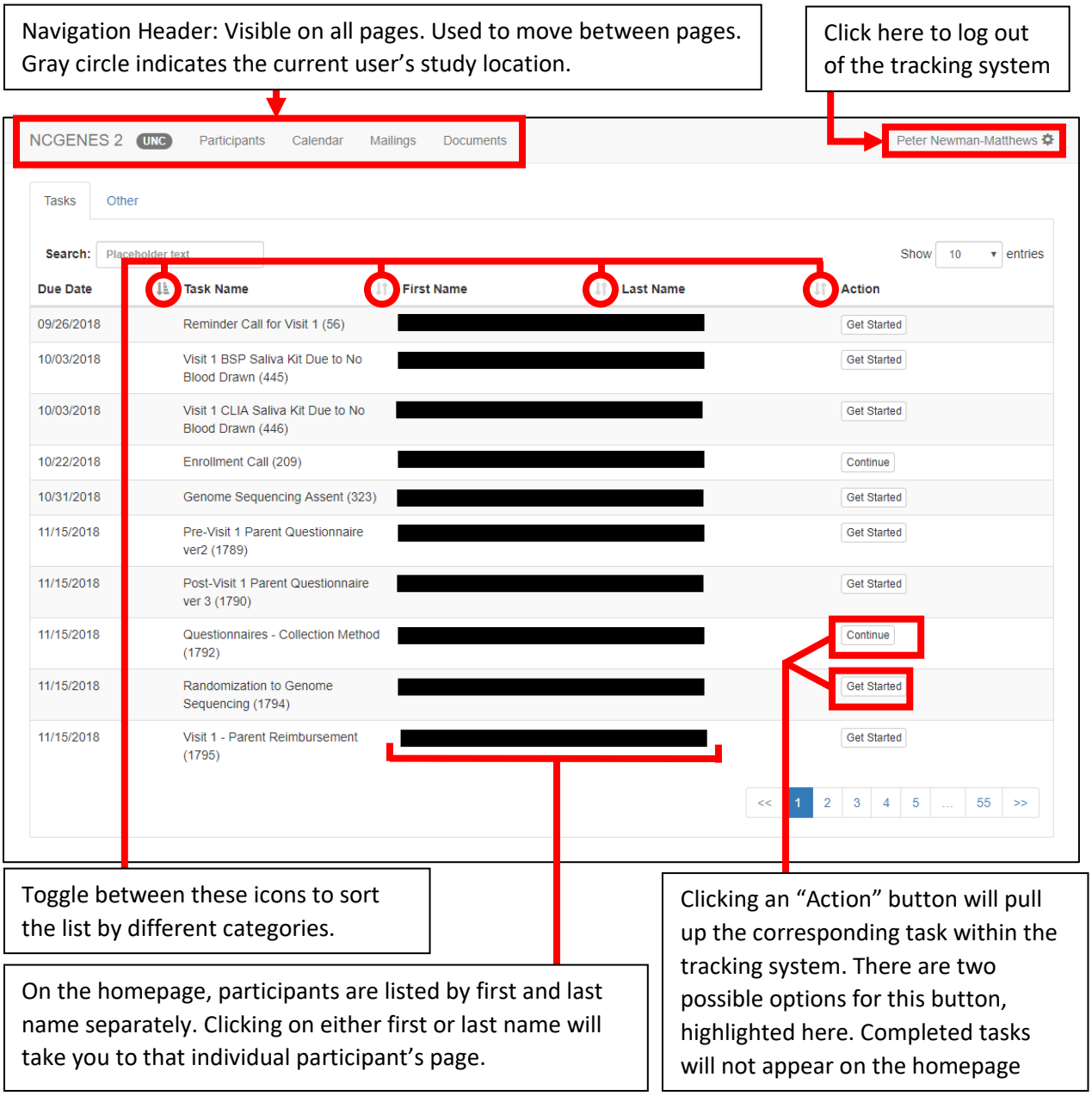
Status v. Assignment Statuses are distinct from selection and randomization assignments. NCGENES 2 has 1 selection and 2 randomizations. For each participant that reaches those points of the study, their selection or randomization assignment will appear to the right of their name on their **individual page** (see page 67). A participant can have more than one assignment at one time (in addition to their status) and keeps any assignment throughout the study.

Main Tracking System Pages

Every tracking system user will frequently encounter a few main pages. In order to orient a potential user, this section contains a screenshot of each page with detailed breakdown of their components. The pages covered are the homepage, participant list, mailings page, documents page, and individual participant interface.

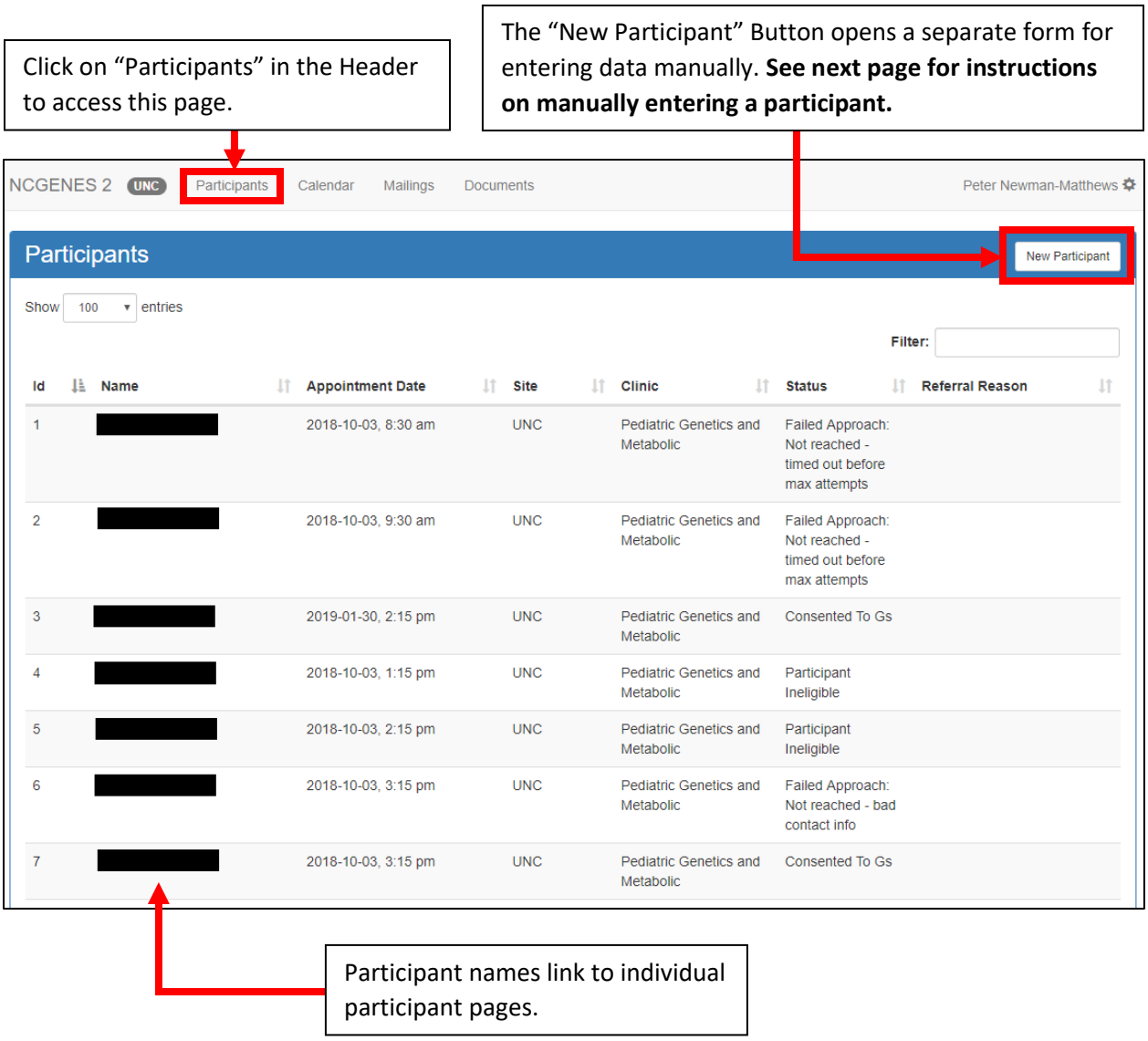
Homepage

Upon logging in to the tracking system, users are directed to the homepage. The homepage lists incomplete study tasks by order of their due date. All study activities logged in the tracking system are captured as **tasks**. As tasks are completed, new tasks are assigned and given due dates by the system based on study protocol.



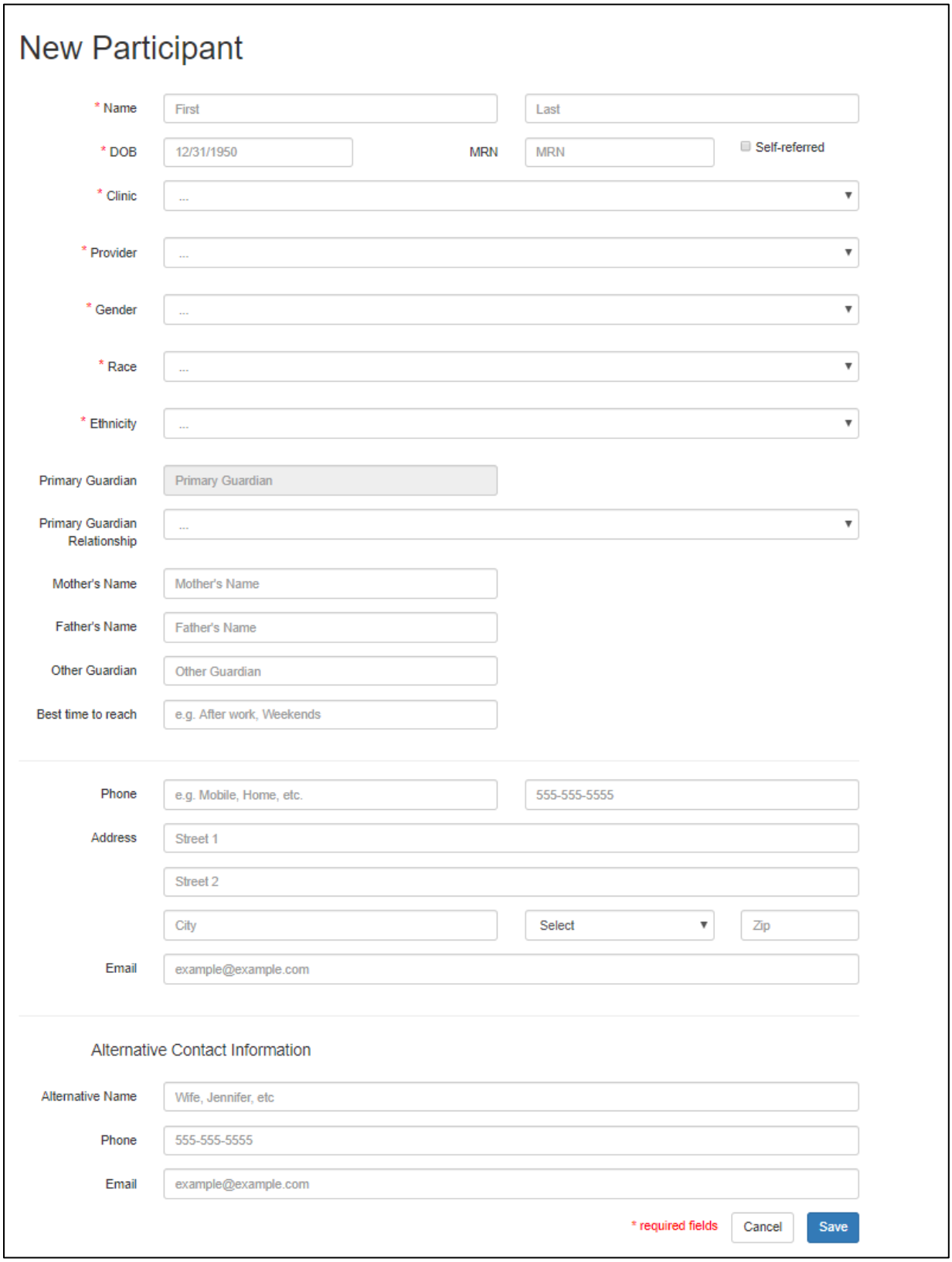
Participants List

The Participants List is accessible from the Navigation Header. As opposed to the homepage, the Participants List displays each participant by order of their ID number. In addition, the Participants List shows each participant's most recent status. Importantly, the "New Participant" button is accessed from the Participant List, which allows for the manual entry of new participants.



Manually Adding a Participant

1. After clicking the "New Participant" button (see above), the following dialog box will appear.



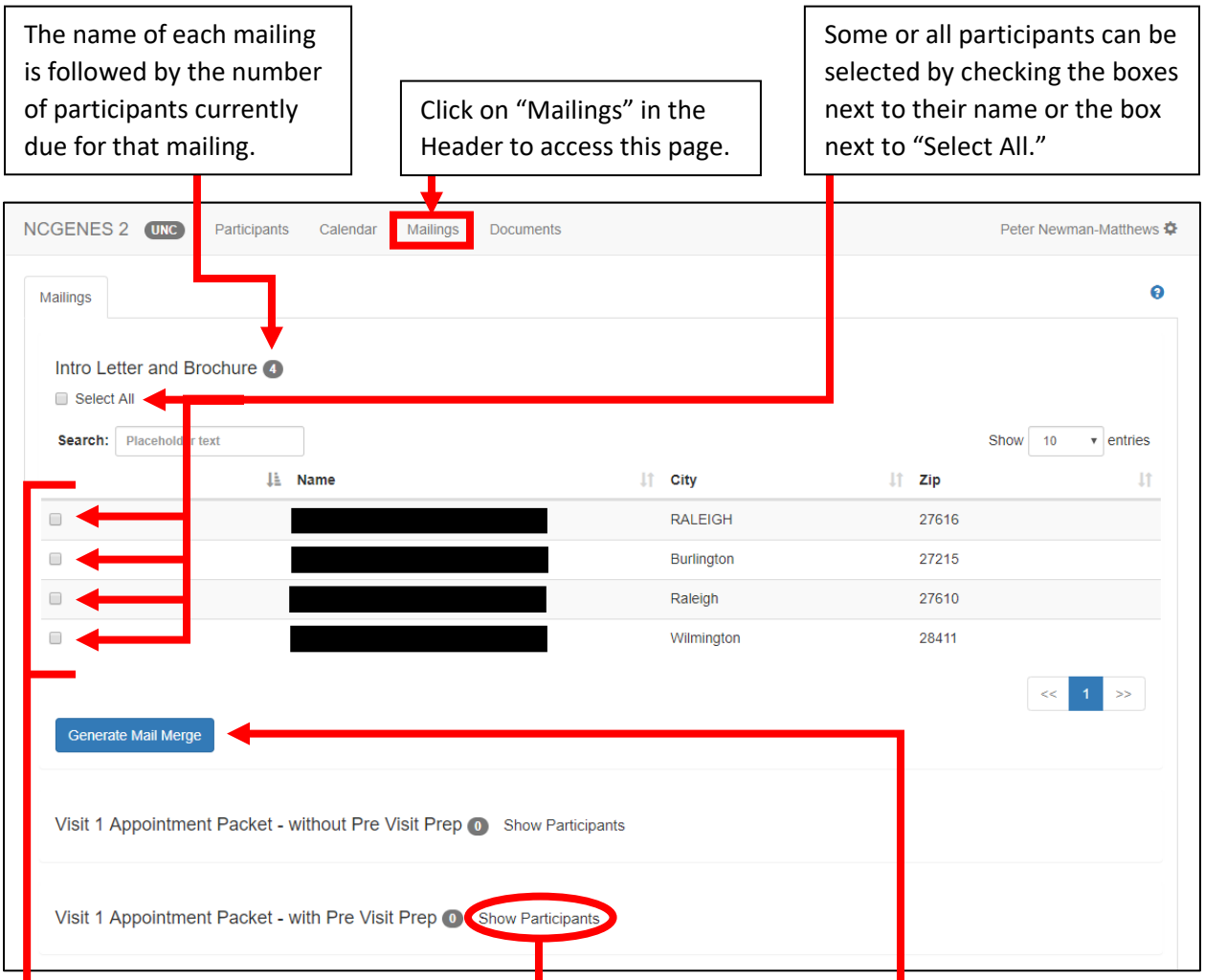
2. On this form, enter in as much information as possible about the participant. At minimum, the following fields are required:
    a. Name
    b. DOB
    c. Clinic
    d. Provider
    e. Gender
    f. Race
    g. Ethnicity

3. Once all available information has been input, click the blue “Save” button in the bottom corner of the form to enter the participant.

Need to edit information on an automatically referred participant? Once a participant has been referred, demographic and contact information can be edited from that participant’s individual page. For more see the **Changing Participant Info** section.

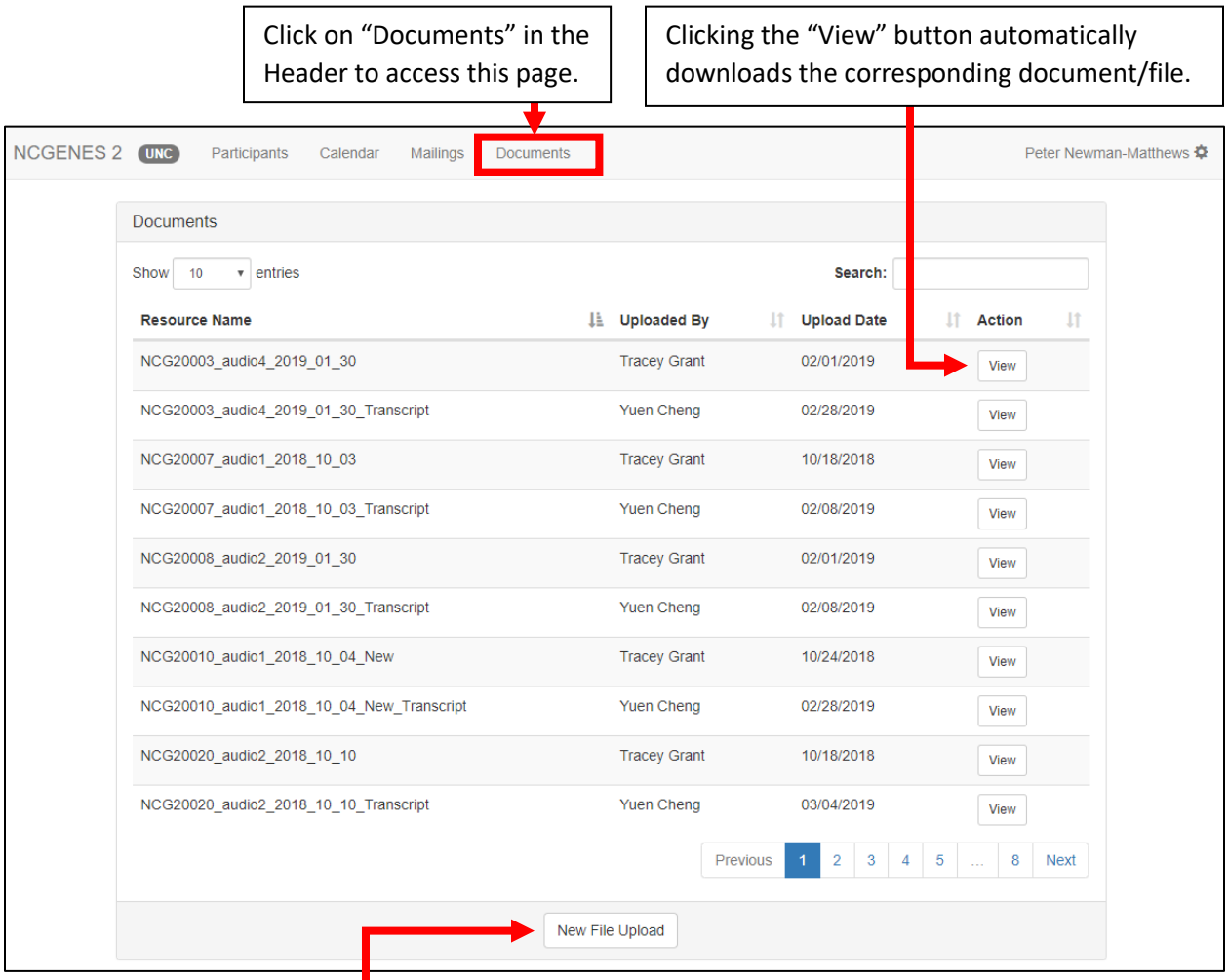
Mailings Page

The Mailings Page provides lists, organized by mailing, of which participants are due to receive each mailing. This page can be accessed from the Navigation Header and by starting any mailing task.



General Documents Page (Not Participant Specific)

The Documents Page houses all the audio files and transcripts generated by audio-recorded clinic visits. Files can be uploaded and downloaded from this page, which is accessed using the Navigation Header. **Apart from audio files, all participant files should be uploaded on the individual participant's page at the Documents tab (See page 74).**

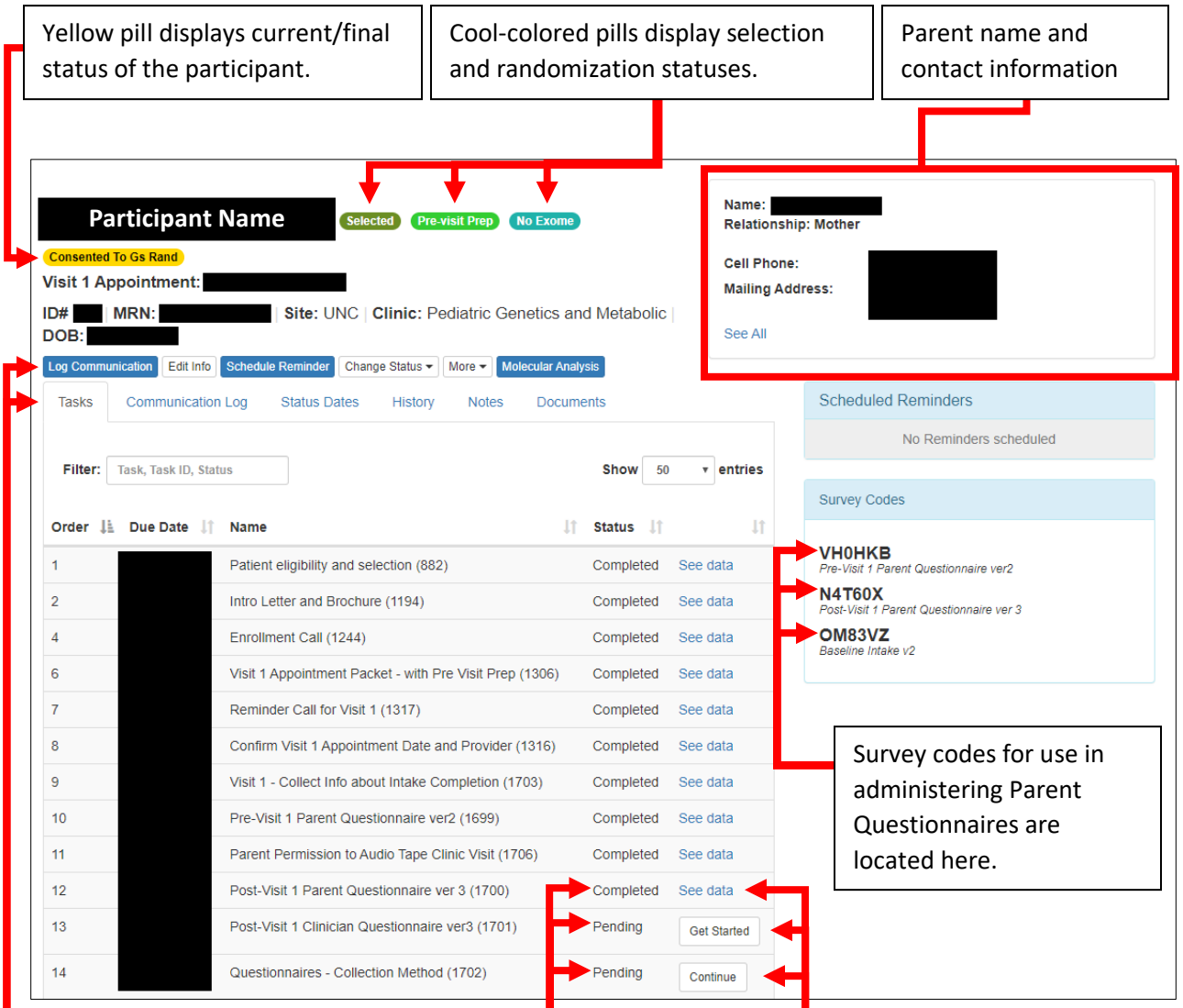


Individual Participant Page

Each participant that is referred as potentially eligible or manually entered into the tracking system has an individual page which tracks all the data regarding that participant. This page also contains several tools for completing study tasks or logging contact with the participant. It can be accessed from the **Homepage** or **Participants List**.

Participant Tasks

The standard view of the participant page displays all pending and completed tasks associated with that participant listed by their order in study protocol.

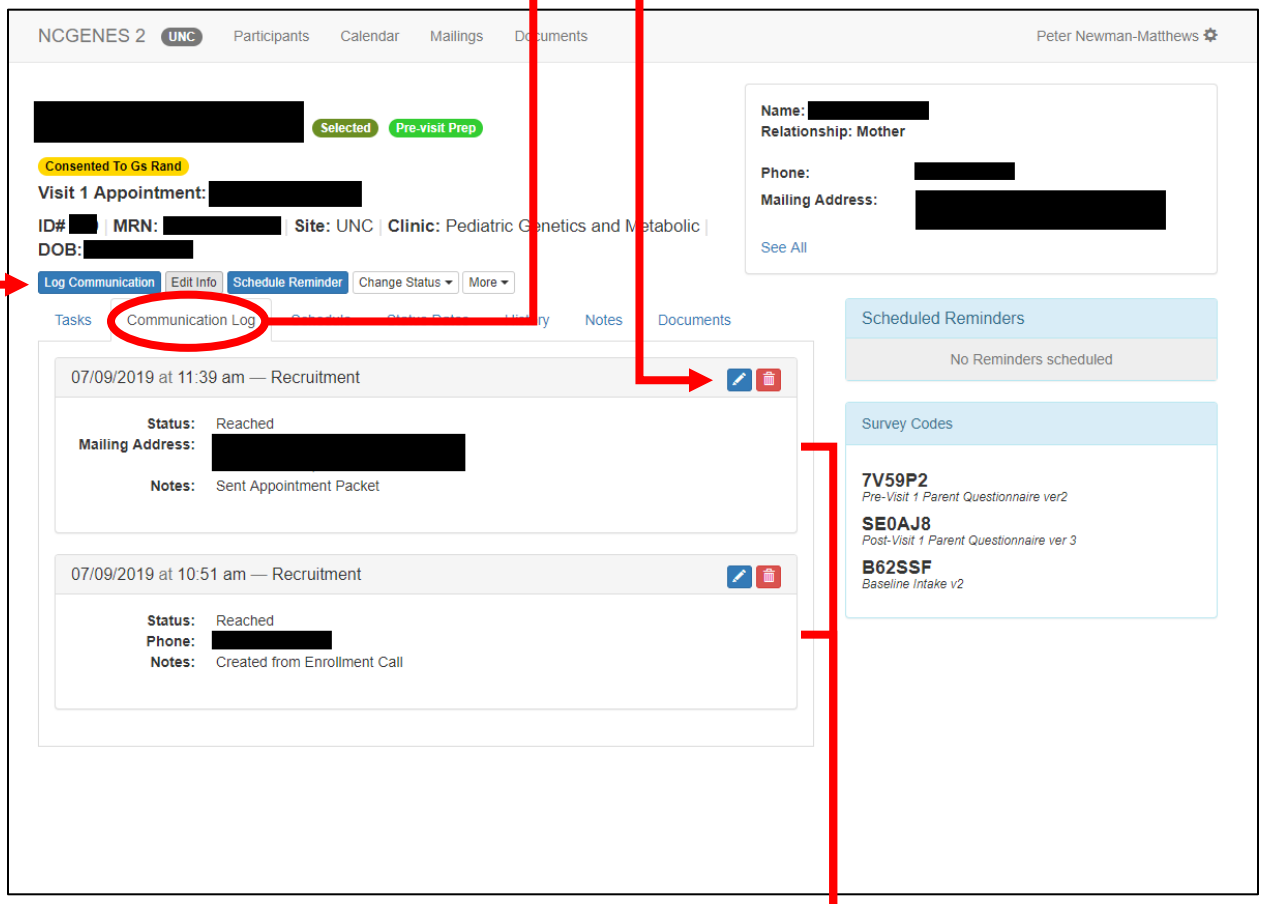


Communication Log

The study tracking system has a built-in communication log. Communications with participants (calls, mailings, emails, etc.) should be tracked in the communication log.

The Communication Log is accessed by selecting its tab on the Sub-menu

Existing logs will be displayed by date and can be edited or deleted using the pen or trash buttons respectively

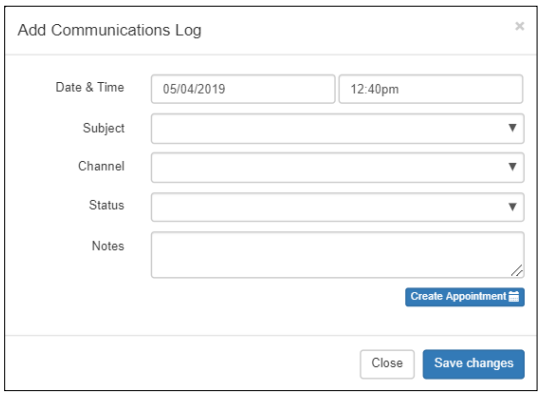


New Communication Logs can be created by clicking the "Log Communication" button. **See next page for step-by-step instructions for logging communication.**

The Communication Log is ordered in reverse-chronological order with the most recent log appearing at the top of the list.

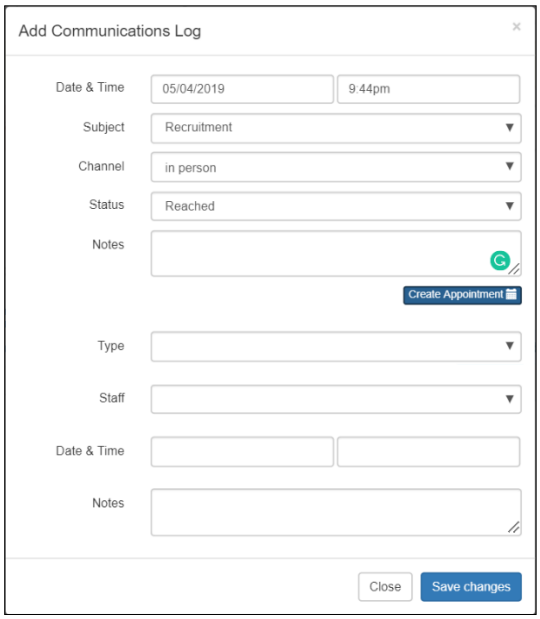
To log a communication:

1. After clicking the "Log Communication" button on the appropriate participant's page, the following dialog box will appear:



2. Enter the following data in the communication:
   - Date & Time: These fields will auto-populate to the time you log the communication
   - Subject: Recruitment, Scheduling, Compensation, Contact Check, Birthday
   - Channel: In-Person, Phone, E-mail, Mailing Address
   - Status: Reached, Left Message, Bad Contact Information, No English, No Answer
   - Notes

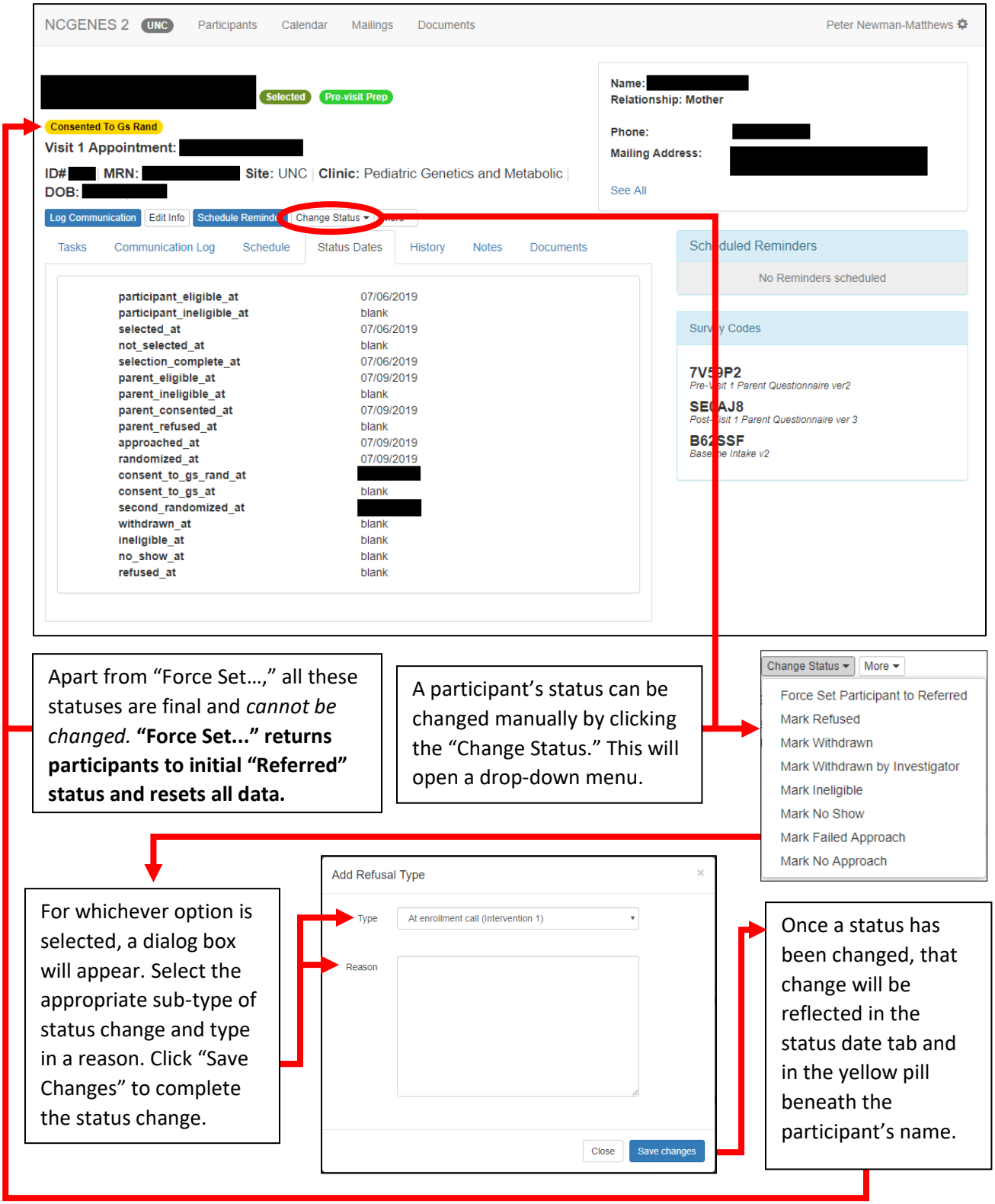
3. If necessary, select the "Create Appointment" button to set an appointment in conjunction with the communication log.



4. In either case, click the "Save changes" button at the bottom of the box. The communication will then be saved and visible from the Communication Log tab on the participant's page.

Status Dates

The Status Dates tab displays all the statuses associated with the study. If a participant currently has or has previously had that status, the date of that status will be displayed. Otherwise, the status will display as "blank."



Apart from "Force Set…," all these statuses are final and *cannot be changed.* **"Force Set…" returns participants to initial "Referred" status and resets all data.**

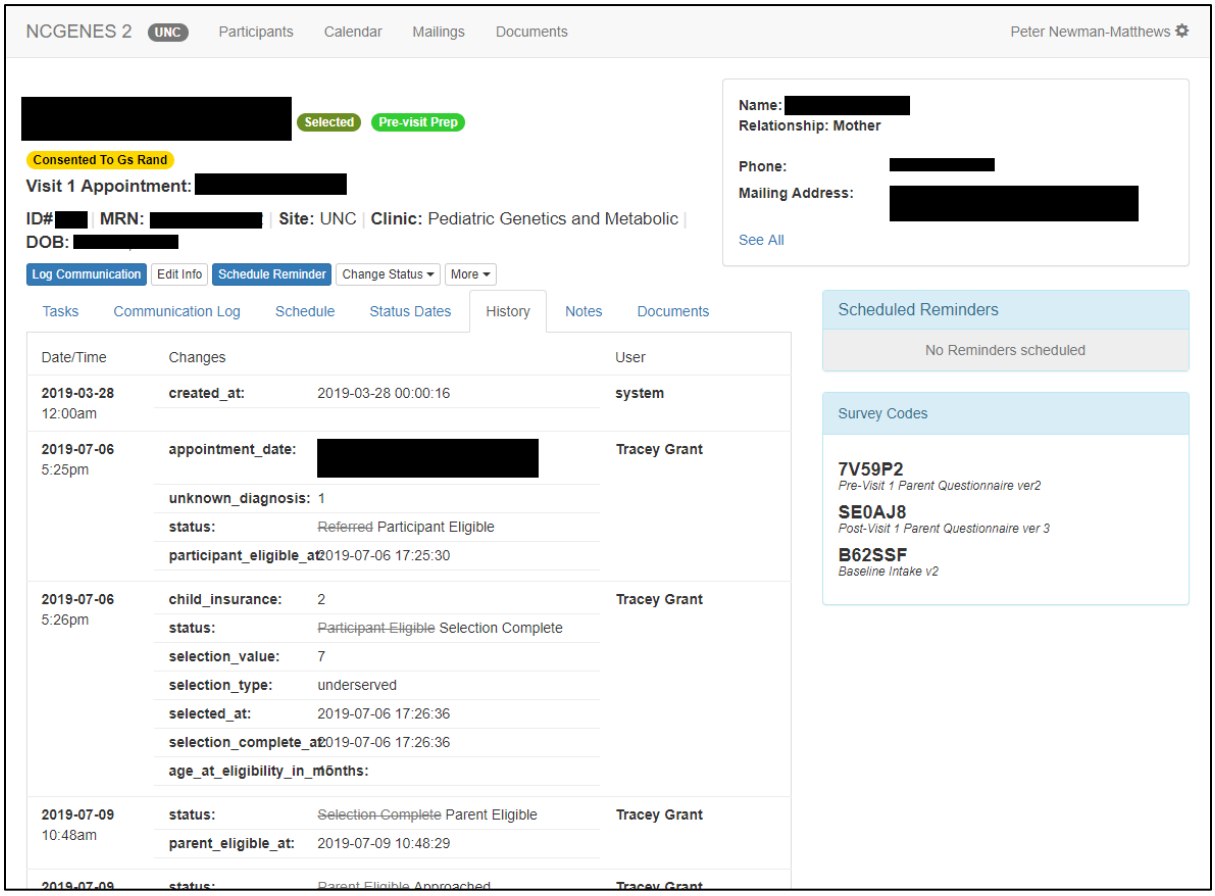
A participant's status can be changed manually by clicking the "Change Status." This will open a drop-down menu.

For whichever option is selected, a dialog box will appear. Select the appropriate sub-type of status change and type in a reason. Click "Save Changes" to complete the status change.

Once a status has been changed, that change will be reflected in the status date tab and in the yellow pill beneath the participant's name.

History

The History tab displays all the tracking system activity for a given participant. Most changes to participant data, including participant status, are captured here. This tab is useful for reviewing a participant's case in more depth than might be afforded by the task page.

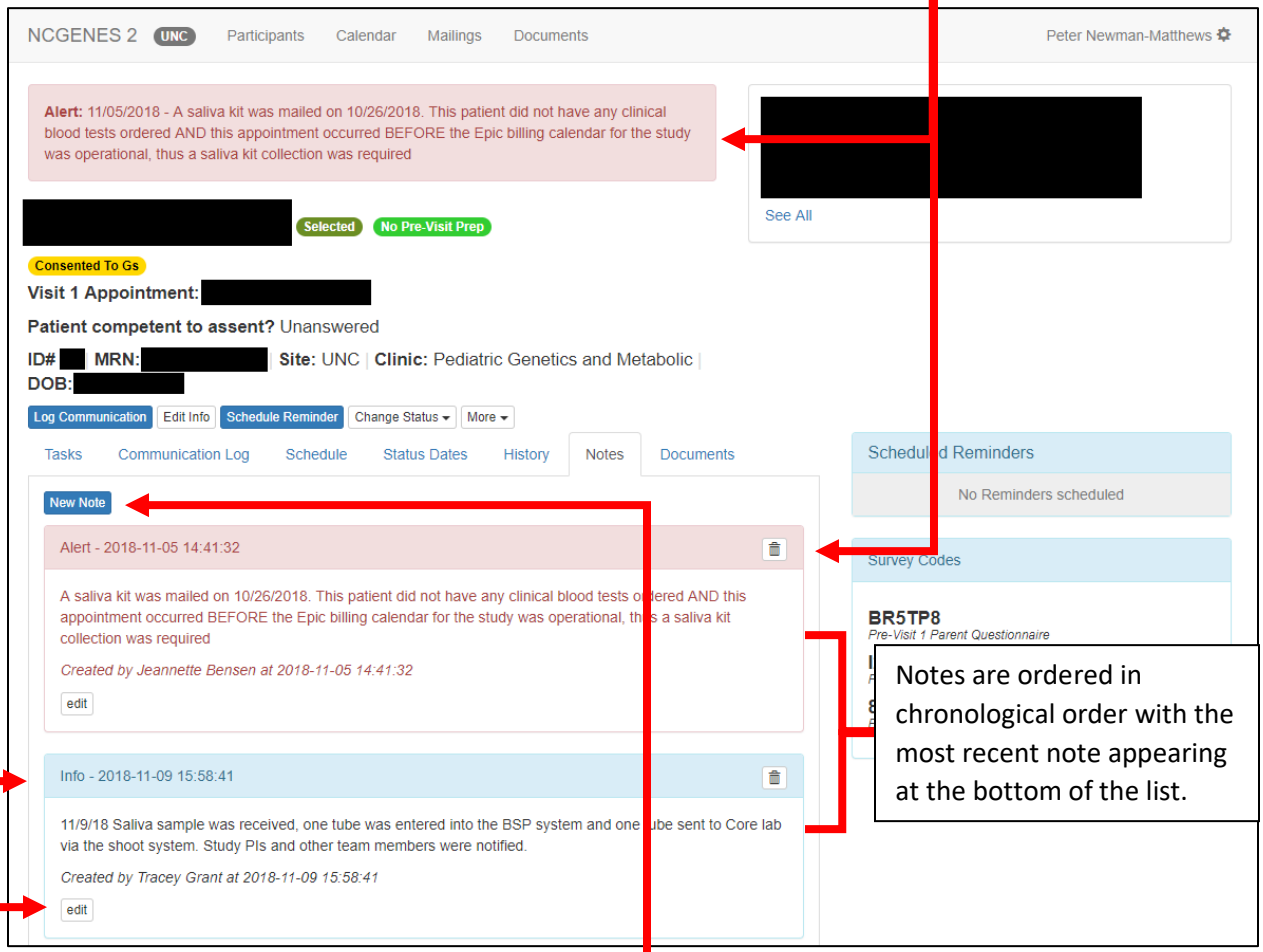


Notes

Notes are used for participant-specific internal communication between NCGENES study staff. There are two types of notes in the system: 1) Information notes and 2) Alerts.

Information notes remain in the notes tab and should be used for general and internal communication/reminders between study staff.

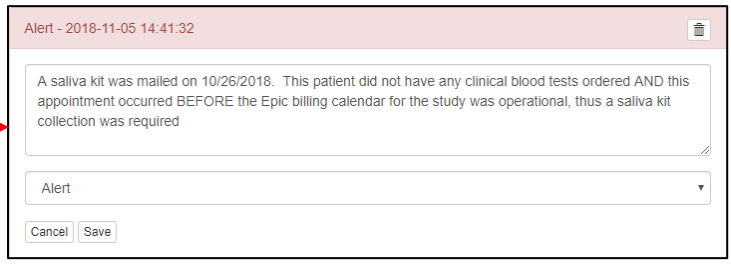
Alerts are used for time-sensitive/important information that study staff should know about the participants. Different from an information note, alert notes appear on the *Participant's Individual Page* above the participant's name.



Notes are ordered in chronological order with the most recent note appearing at the bottom of the list.

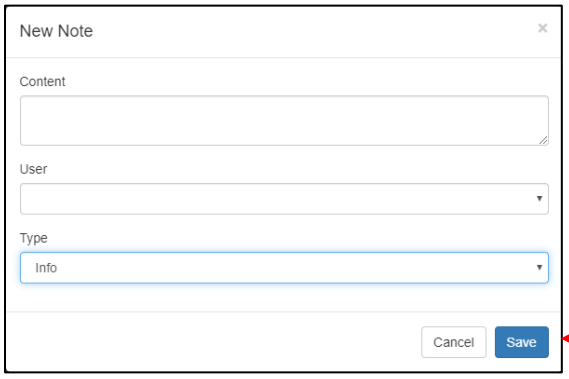
New Notes are created by click the "New Note" button on the Notes tab. This opens a dialog box shown on the next page.

When an alert is no longer necessary or a note needs to be changed, a note can be edited by clicking the "edit" button on that note and updating its text and/or changing its type using the dropdown menu and clicking "Save."

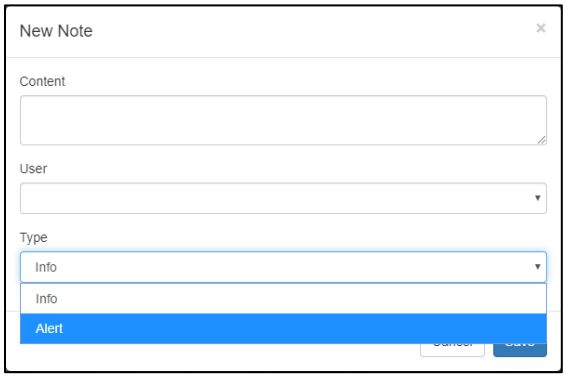


To create a note:

1. After clicking the "New Note" button on the Notes tab of a participant's page (see previous page), the following dialog box will appear:



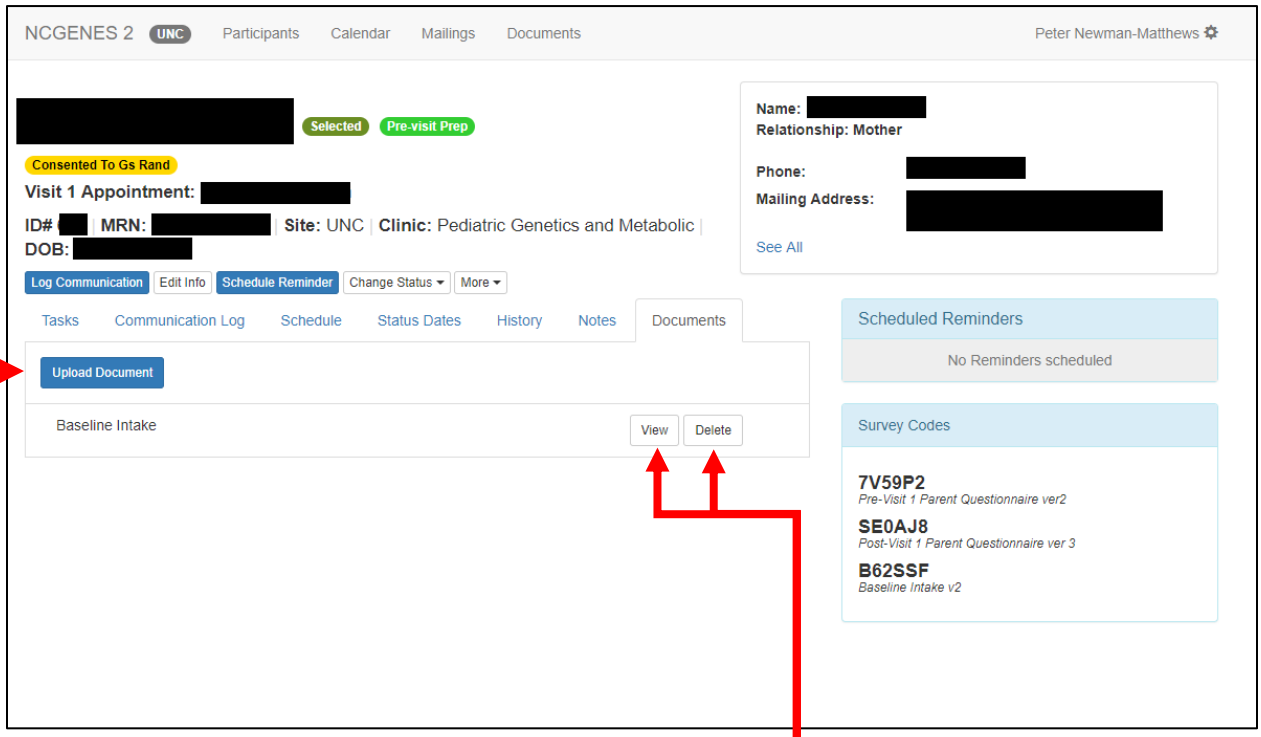
2. Enter the Content, and select your Username and desired note type from the corresponding dropdown menus (Second dropdown shown here).



3. Click the "Save" button
4. Check that the note has saved in the Notes tab

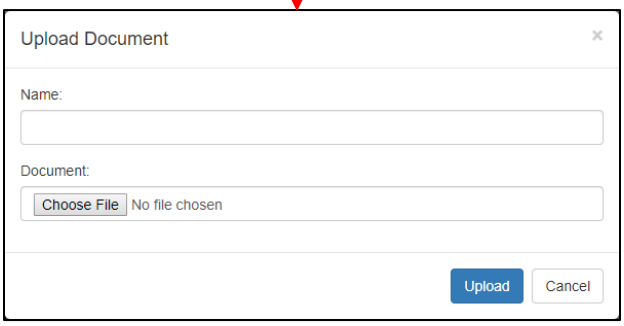
Participant Documents

The Participant Documents tab houses electronic copies of any forms or surveys completed by the participant on paper.[1] While many forms and surveys can be completed within the tracking system, technical disruptions or other extenuating circumstances may require the participant or parent complete forms on paper. Such forms should be uploaded into the system at the participant's Documents tab.



Files can be added to a participant's Documents tab by clicking the "Upload Document" tab, which opens the following dialog box. After naming and selecting the file to upload, click the "Upload" document to upload the file.

Clicking the "View" button will automatically download a copy of the corresponding document. Documents can also be deleted by clicking the "delete" button.



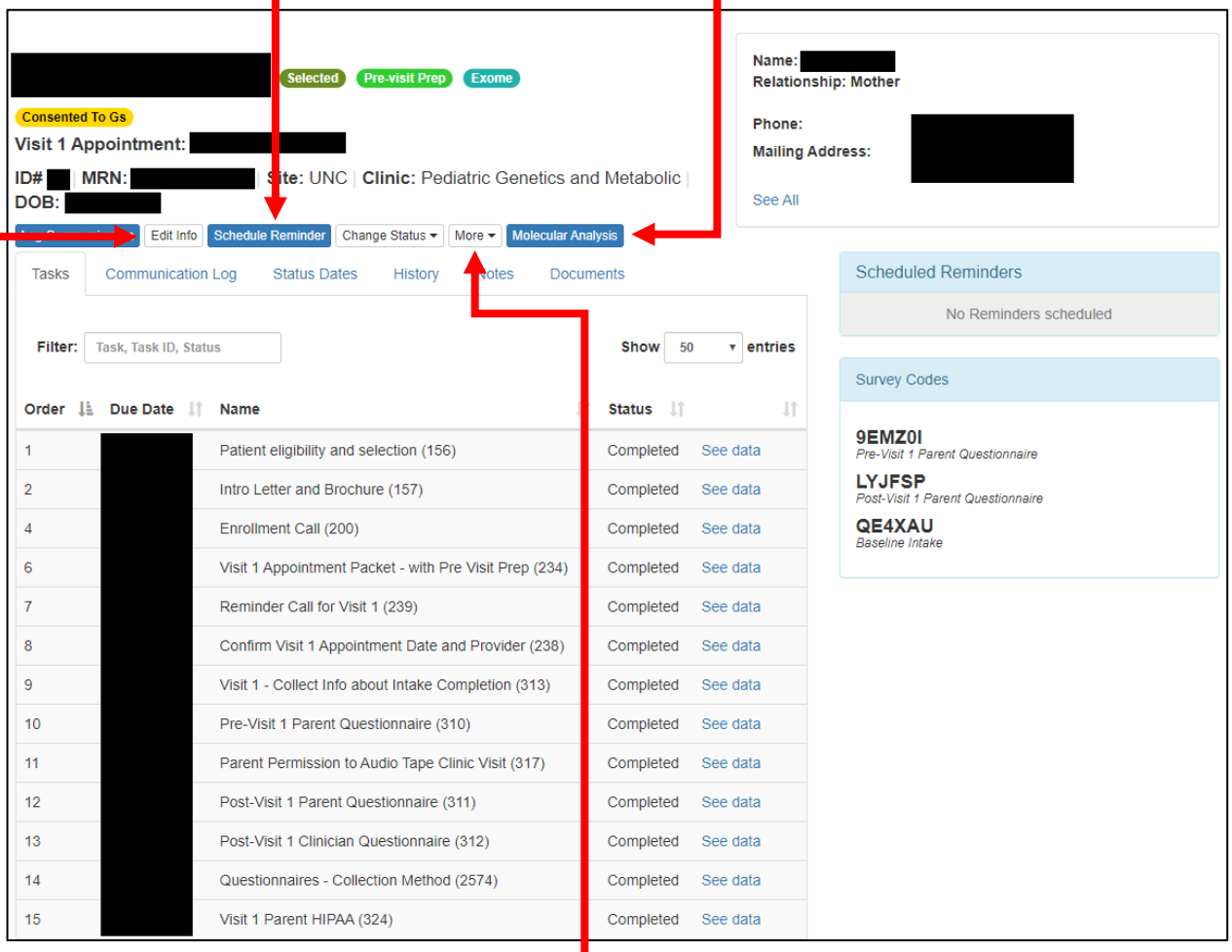
[1] Audio recordings and transcripts will be found on the main documents page accessible from the Navigation Header. See Documents Page

Other Items on the Individual Participant Page

The Individual Participant Page contains some features in addition to the tabs and functions already mentioned. These are addressed here.

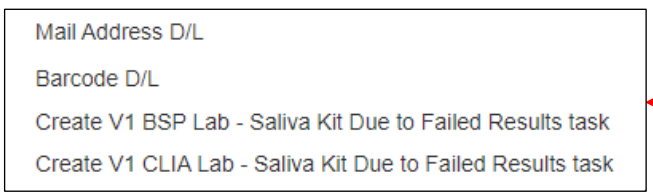
The "Schedule Reminder" button is not currently part of study protocol. See Calendaring

The "Molecular Analysis" button opens a separate page which displays results of a participant's exome sequencing.



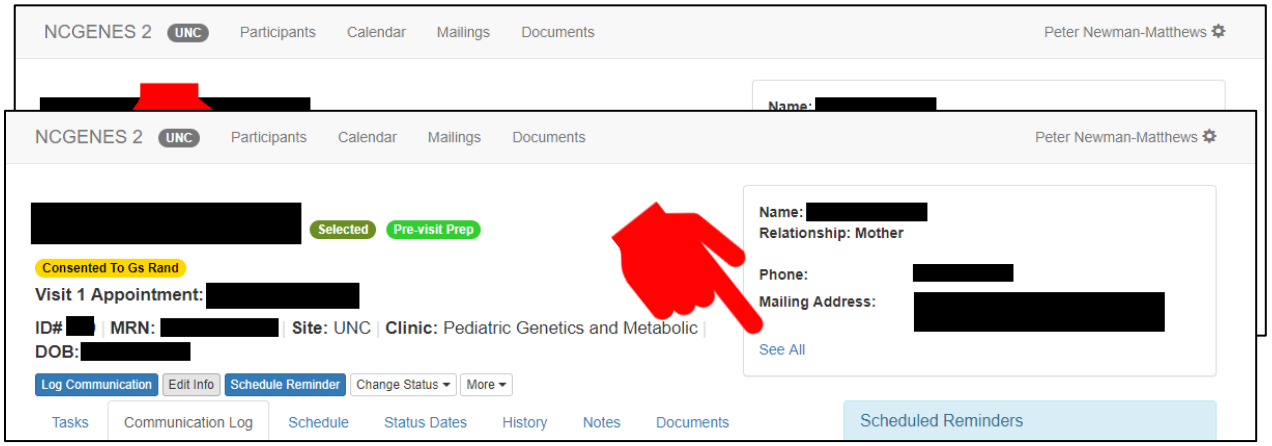
The "Edit Info" button allows the user to edit any demographic information about the participant in the dialog box it opens. See below.

The "More" button opens a dropdown menu. The options of this menu will change depending on various participant data. All possible options are depicted here. The first two options download a excel document with the participant's address and NCGENES2 barcode, respectively. The last two create the listed task in the tracking system.



Changing Participant Info and Contact

As mentioned above, participant info can be changed by clicking the "Edit Info" button on an individual participant's page. This opens a new page where the participant information can be edited/updated.

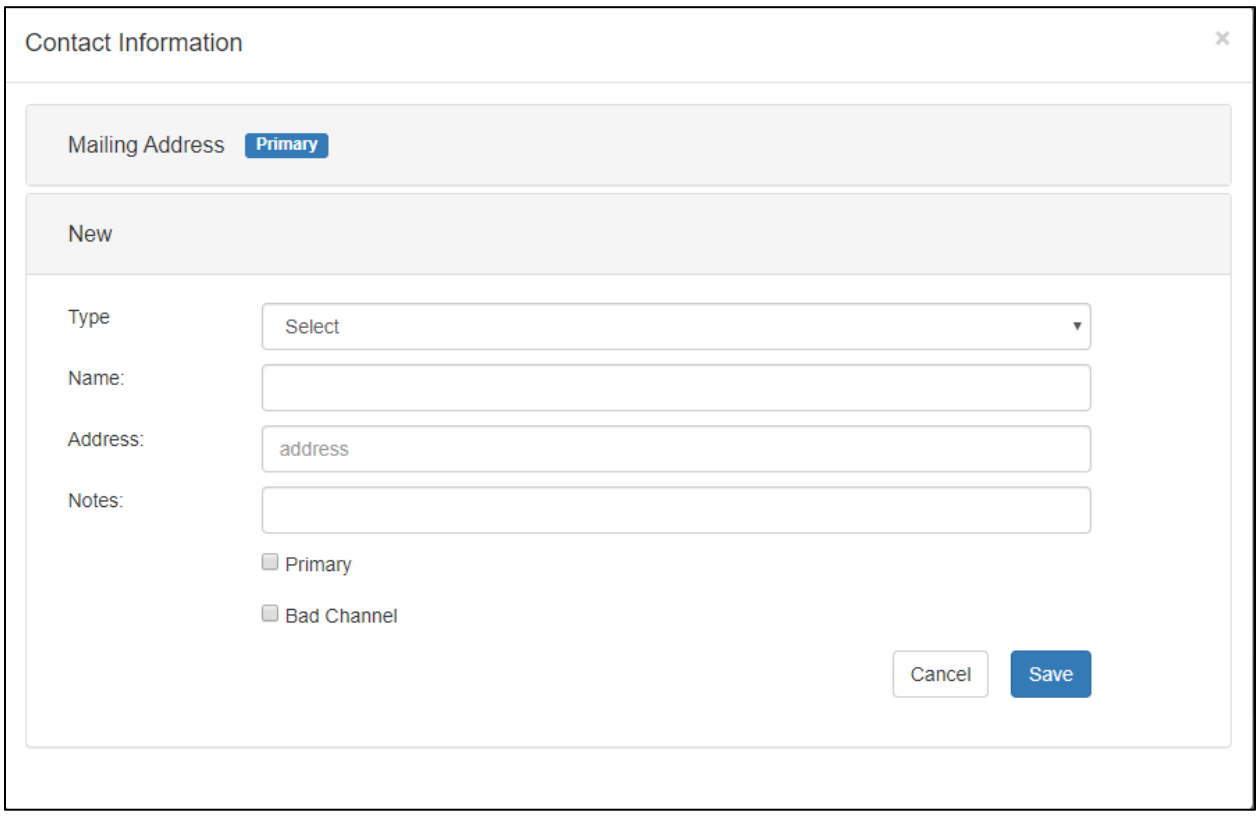


If a participant's contact information needs to be edited/added, the user should instead click on the "See all" located within the parent contact box in the upper-right corner of the individual participant's page.

This will open the dialog box shown below. All contact channels currently listed in tracking will appear in this box.

Primary channels will be tagged as shown.

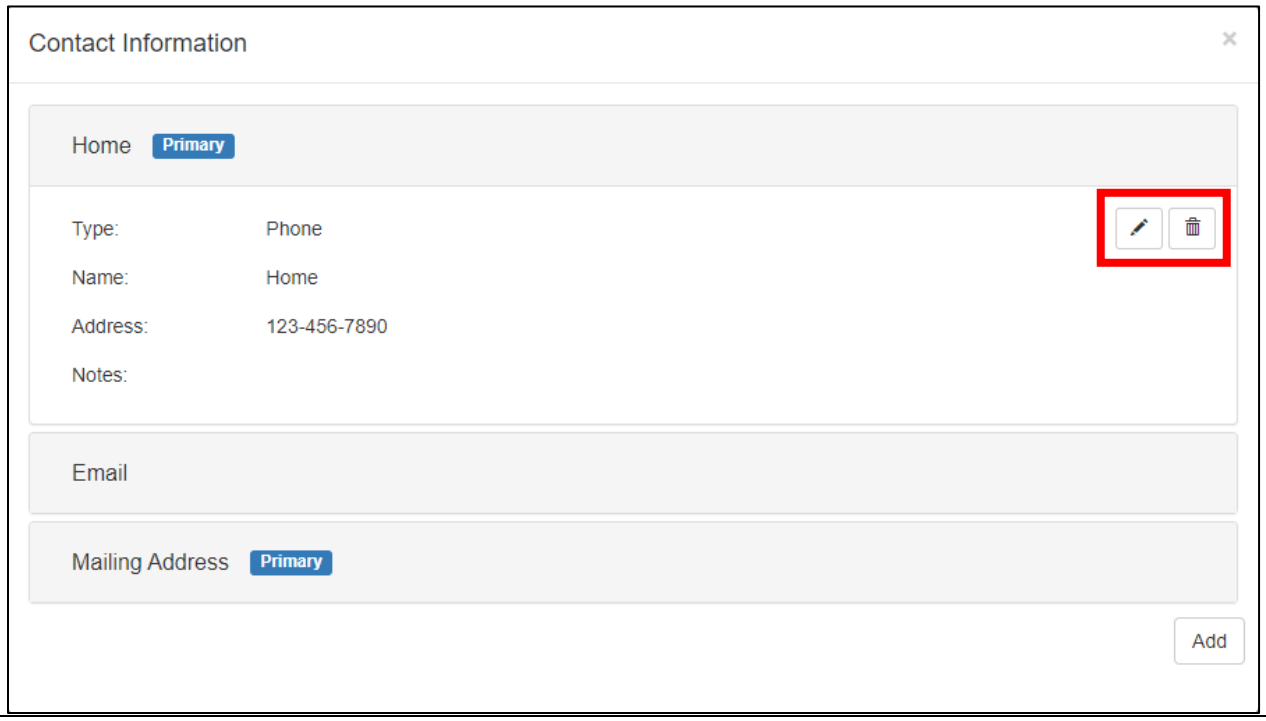
To add a new contact channel, click the “Add” button in the bottom right of the box shown above. This will open the following dialog.



Select the appropriate channel type from the dropdown list shown below.

Then, enter in the name of the channel (e.g. Home Phone), “Address” (e.g 123-456-7890), and any notes as appropriate. If the channel is the primary contact of that type or if the channel is defunct, indicate this by checking the appropriate boxes.

Any existing contact information can be edited by selecting it in the dialog box and clicking the pencil icon as show below. A contact channel can also be deleted by clicking on the trash icon, if necessary.

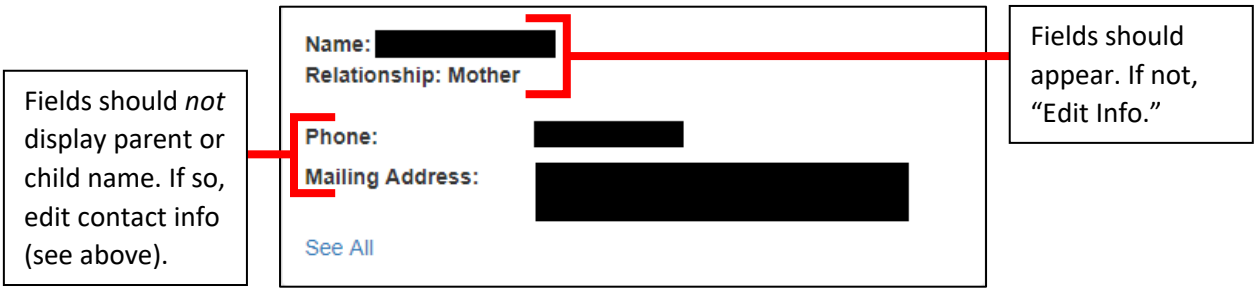


Conventions for Contact Information

If the contact information for the parent is entered correctly, the parent info box in the top right corner to the participant's individual page should appear as shown below. Two common errors may skew the appearance of this box.

Parent Info Missing: If the "Name:" and "Relationship:" fields do not appear AND the primary guardian is known, ensure the "Primary Guardian Relationship" dropdown has a value selected using the "Edit Info" button.

Name listed before Contact Info: If one of the contact fields (e.g. Phone, Mailing Address) is instead listing the legal guardian or participant name, this is a misnomer. In order to resolve this issue, that contact channel's info should be edited so that the "Name" field lists the name of the contact channel, rather than the name of the legal guardian or participant.



Tracking System Roles

Each use in the tracking system is assigned a role that determines what pages and data that user can access. These roles are related to **clinical study roles,** but are not identical.

id	name	Description
1	Programmer	Programmer working on the system. Has all privileges.
2	Study Manager	Study Manager has administrative permissions, but should not be able to make configuration level changes
3	Primary Investigator	Primary investigator has full access to the system.
4	Interviewer	This role is blinded for randomization groups across the whole system.
5	Provider	Provider doing select surveys.
6	Interviewer Transcriber	This role is blinded to randomization groups for participants until Visit 1 is completed. This role is unblinded to randomization groups for participants after post visit 1 is completed.
7	Transcriber	This role only has access to documents screen.
8	CLIA	CLIA labs

TRACKING SYSTEM TASKS: STEP-BY-STEP GUIDES

For every study activity, there is a corresponding task in the tracking system. Some tasks, such as mailings, originate in the tracking system and are completed in paper form. Others, such as enrollment calls, are conducted in concert with the tracking system. In this section of the protocol, we provide step-by-step instructions for completing every task within the tracking system. These guides will omit explanations of exceptional situations which might require deviation from standard procedures. Such explanations are addressed in the narrative section of the protocol.

Patient eligibility and selection

1. From the Homepage (Fig. 1) or an Individual Participant's Page (Fig. 2), find the "Patient eligibility and selection" task and select the "Get Started" or "Continue" button.[1]

Fig. 1

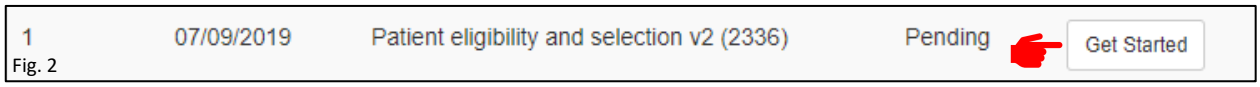
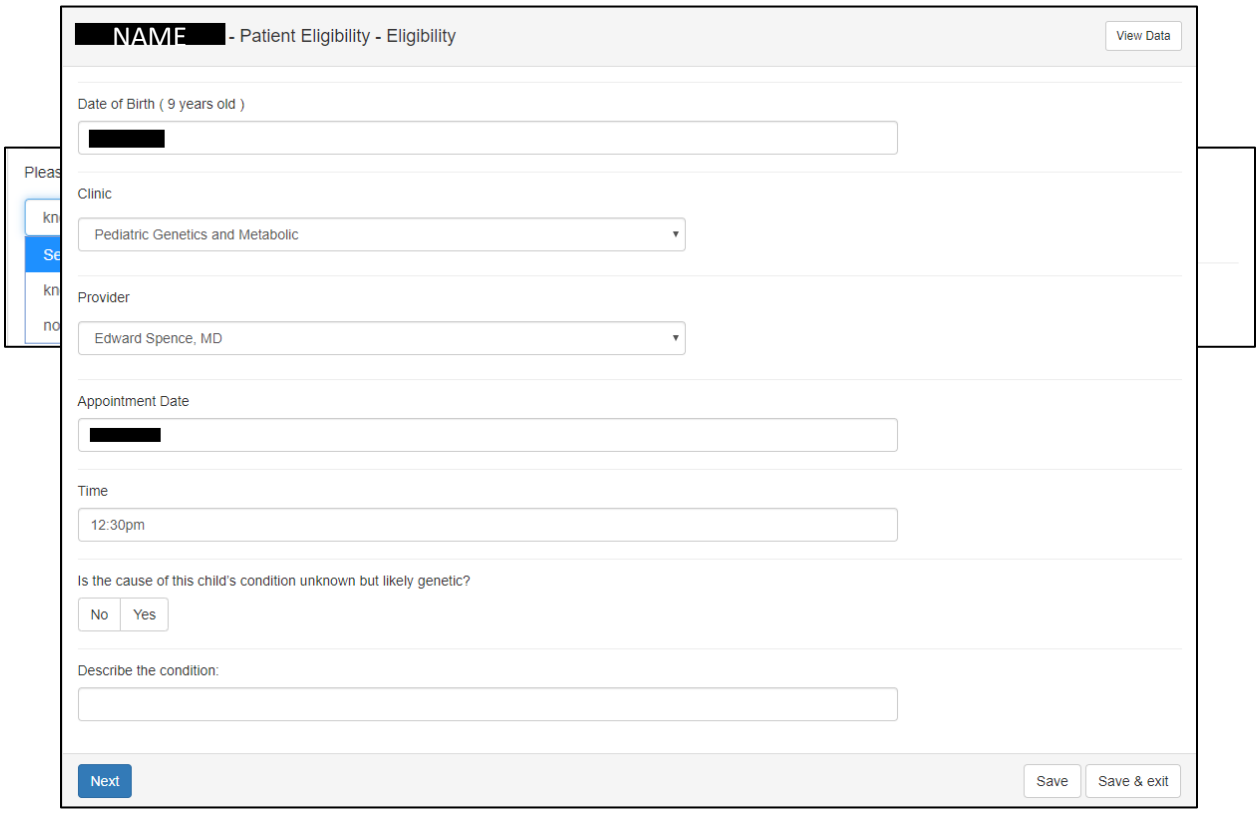


Fig. 2

2. The dialog box pictured below will appear. DOB, Clinic, Provider, Appointment Date and Time, should be pre-populated on the E*ligibility Page.* Any missing information should be entered.

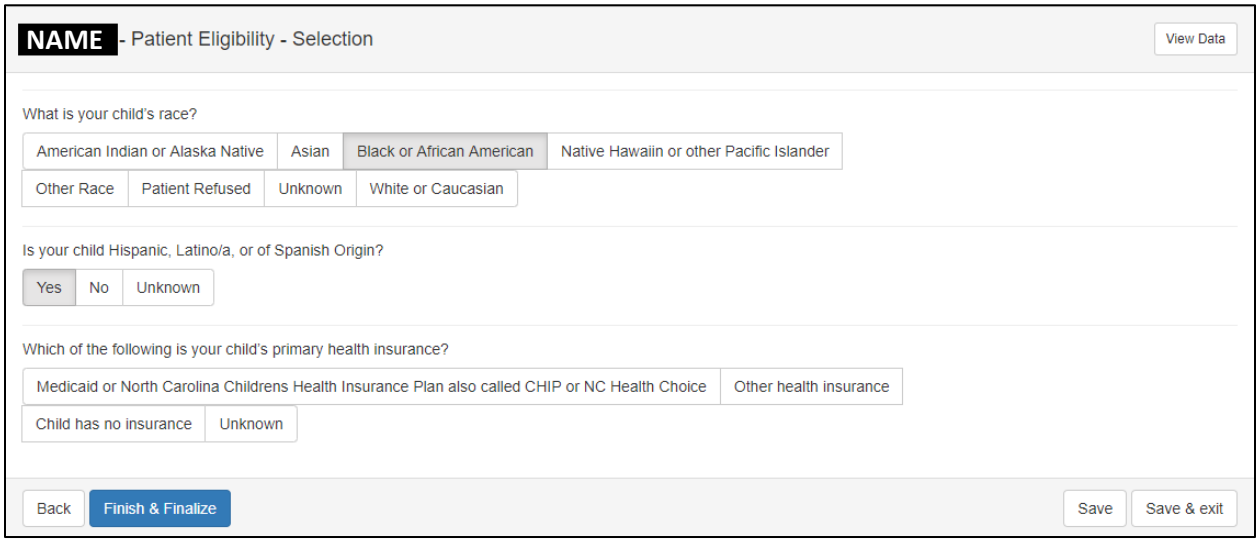


3. Based on **etiology criteria**,[2] click "No" or "Yes" for the question: "Is the cause of this child's condition unknown but likely genetic?" If "No" the following question will appear. Select the appropriate reason for ineligibility from the dropdown.

[1] At any point, clicking "Save" or "Save & Exit" allows the user to leave the task and return later to continue.
[2] A list of inclusion and exclusion clinical criteria has been developed by the NCGENES 2 physician team and should be referred to when evaluating a participant's eligibility Appendix I. Etiology Criteria.

4. In either case, input the referral reason/clinical information/diagnosis under “Describe the Condition.”

5. Once all data has been entered, click the “Next” button to proceed. If the participant is ineligible, this action will take the user to the participant’s individual page and the task will be complete. Otherwise, the following page will appear for participant selection.



6. Choose the pediatric participant’s status for each of the following: race, ethnicity, and type of insurance. This information, if available, should be prepopulated from the auto-feed or manually entered patient data.

7. Even if all fields cannot be completed, click the blue “Finish & Finalize” button. This will complete the task and redirect the user to the participant’s individual page. **This step is critical because it triggers the task to mail the Introductory Letter and Brochure.**

Intro Letter and Brochure

The “Intro Letter and Brochure” task is a mailing task. This (and all) mailing task(s) can be started from the homepage (Fig. 1) or individual participant page (Fig. 2) by clicking the “Get Started” button for the appropriate participant. Either action will redirect the user to the “Mailings” page, where they can generate a mail merge document for a particular

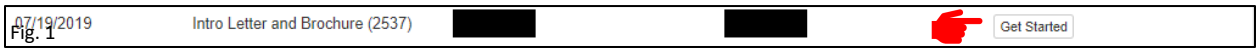


Fig. 1

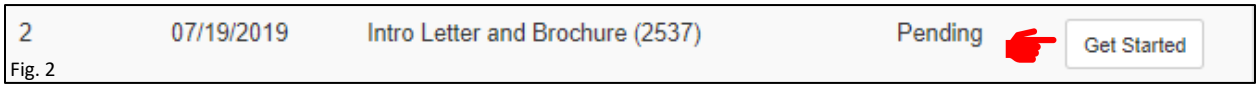


Fig. 2

participant. The “Intro Letter and Brochure” task will be marked complete in the tracking system when this mail merge is generated.

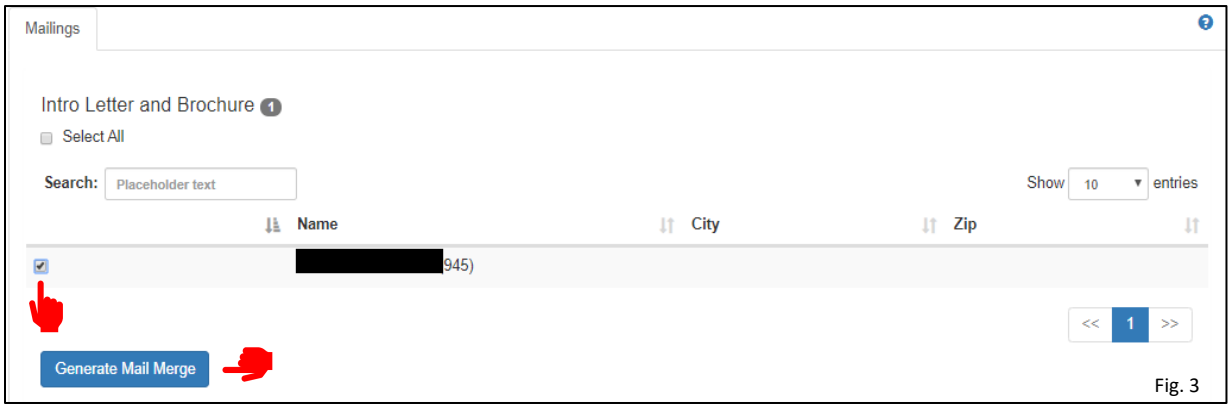


Fig. 3

Mailings should be completed on the same day they are originated to ensure accurate tracking of this task. For detailed instructions on completing this mailing, go to the **Mailing 1** section under **Mailings**.

Enrollment Call (with Selection)

Introduction

1. To begin the enrollment call, select the “Get Started” or “Continue” button next to the “Enrollment Call” or “Enrollment Call with Selection” task for the appropriate participant. This can be done from either the Home Page (Fig. 3) or the Individual Participant Page (Fig. 4).

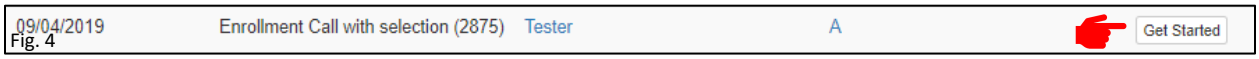


Fig. 4

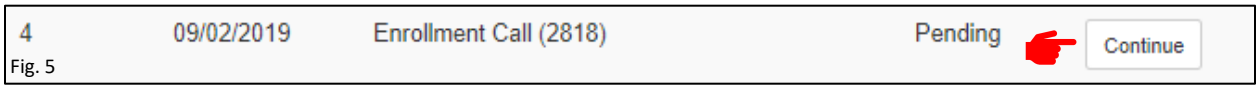
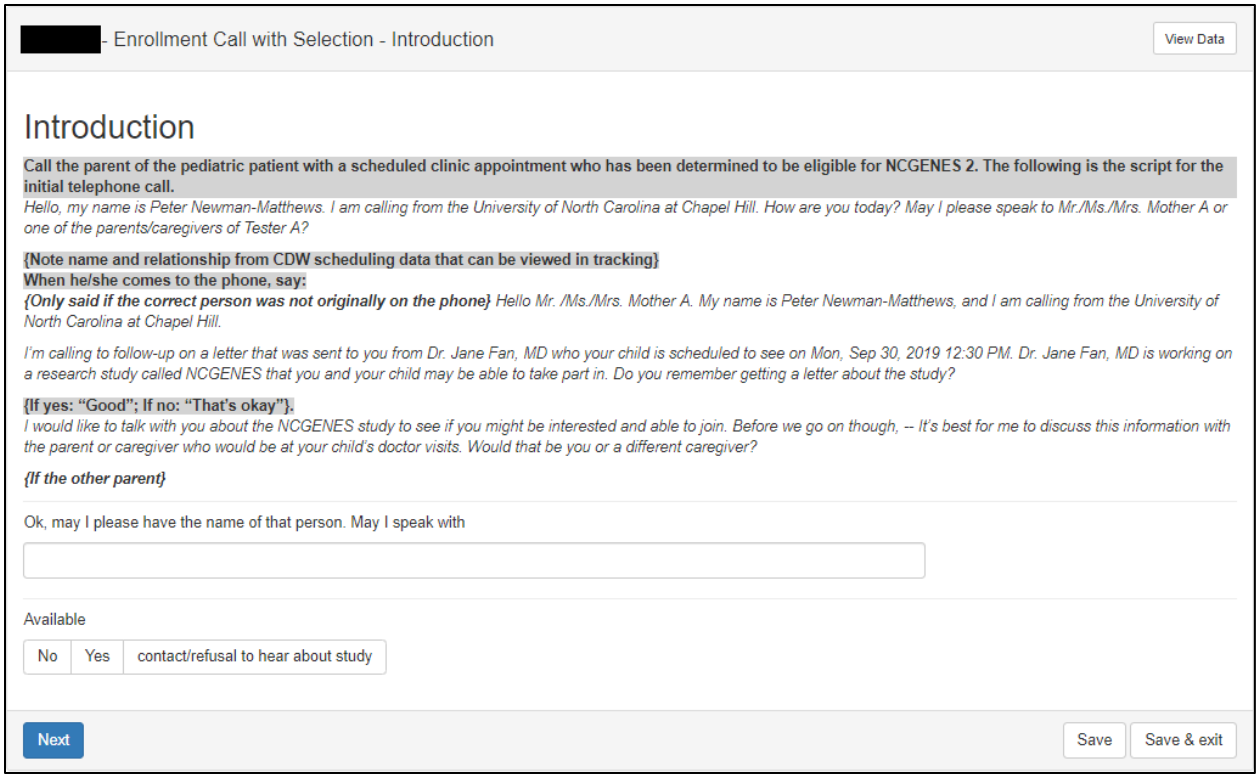


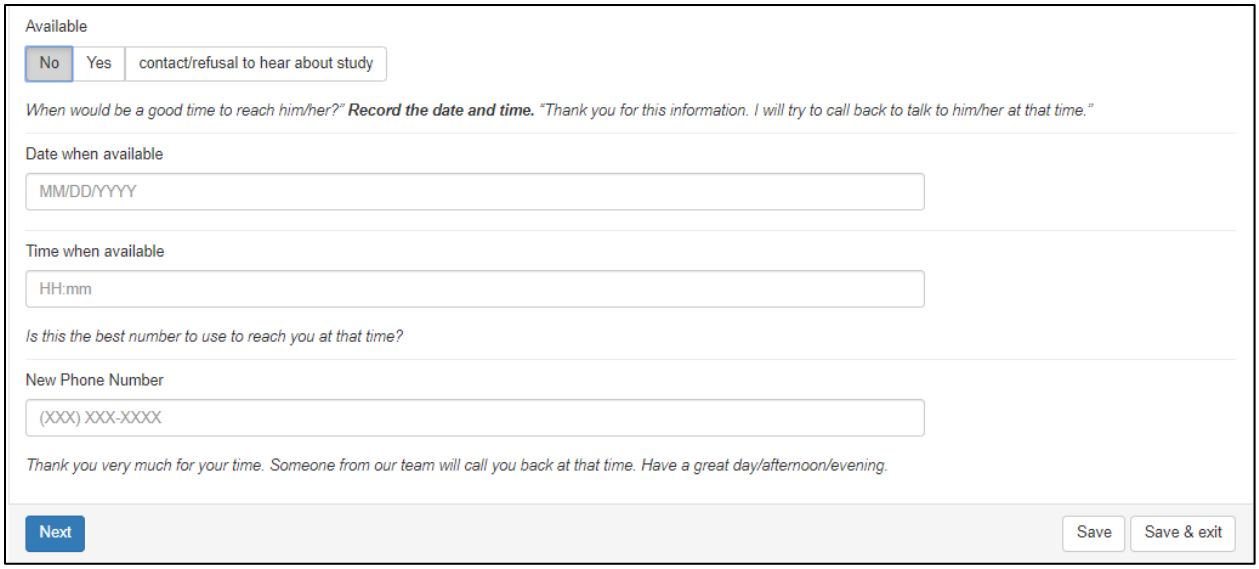
Fig. 5

2. The dialog box shown below will appear. Throughout the call the script will displayed in a series of dialog boxes. The user should follow along with the script in each dialog box. Actions and reminders will be bolded and bracketed and should not be spoken aloud. Call the parent and walk through the scripts, following prompts appropriately. Under "Available," select whether the parent is available and willing to speak over the phone at that time.[1]

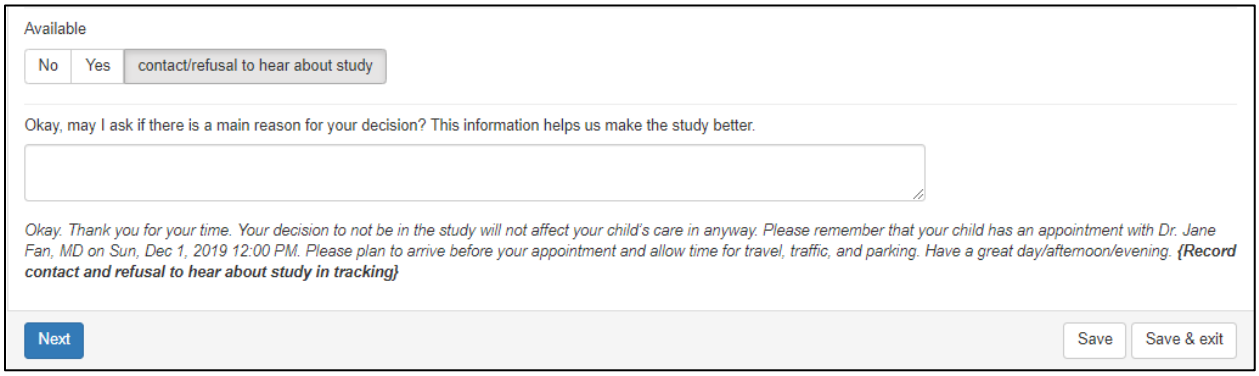


[1] At any point, clicking "Save" or "Save & Exit" allows the user to leave the task and return later to continue.

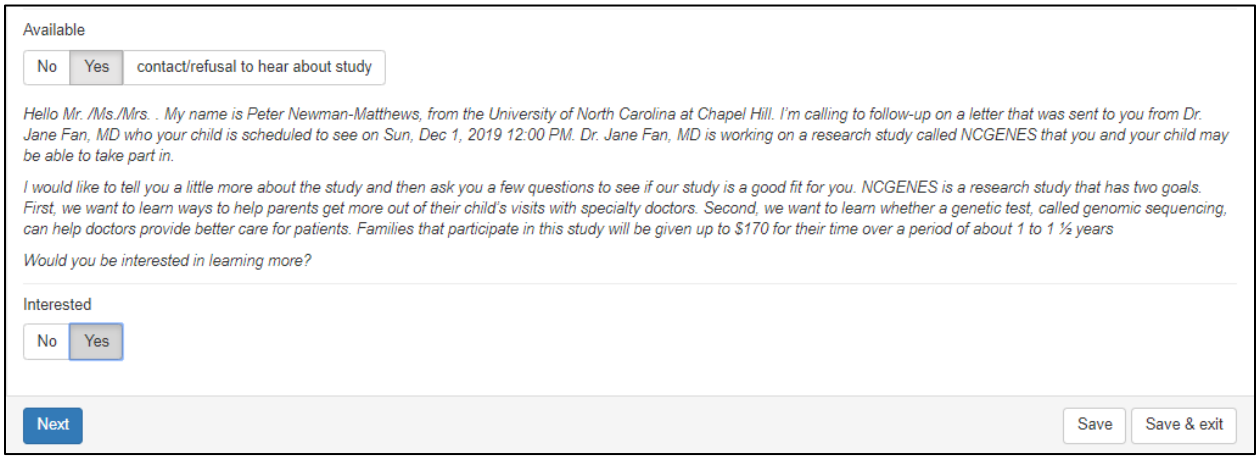
a. If the parent is not available, the following section will expand within the dialog box. Enter a date, time, and phone when the parent will be available. After concluding the call, click the blue “Next” button. This will end the task for the time being, which can be restarted when the parent is called back. (See step 1)



b. If the parent declines, the following section will expand within the dialog box. If the parent gives a reason, enter it here. After concluding the call, click the “Next” button. This will complete the task and the participant’s status will be changed to “Refused.”

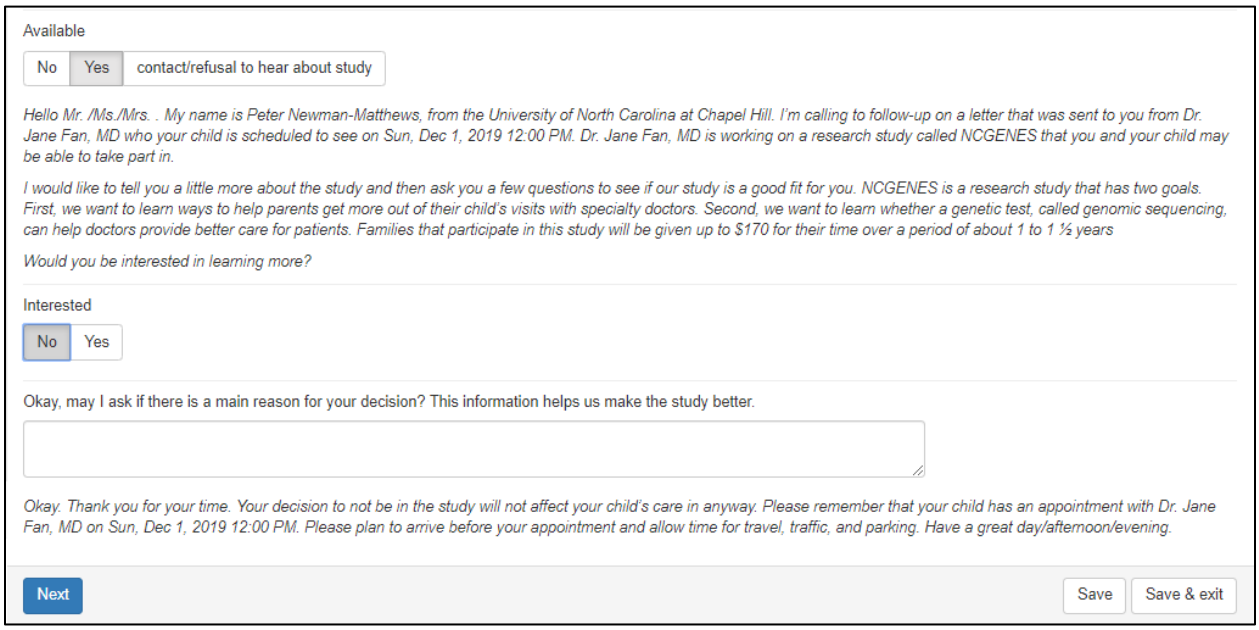


c. If the parent is available, the following section will expand within the dialog box. Continue with the call.



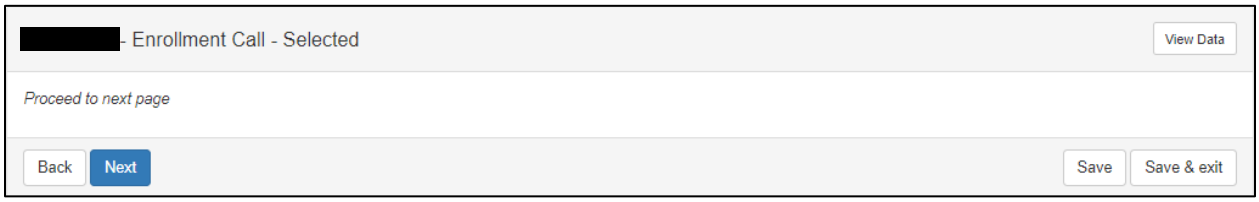
i. If the parent is interested in the study, select "Yes" under "Interested." Then, click the blue "Next" button. The user will be directed to the Selection page.

ii. If the parent is not interested in the study, select "No" under "Interested." The dialog box will expand as shown below. If the parent provides a reason for their lack of interest, enter it here. Then, after concluding the call, click the blue "Next" button. This will close the dialog box, complete the task, and change the participant's status to "Refused."

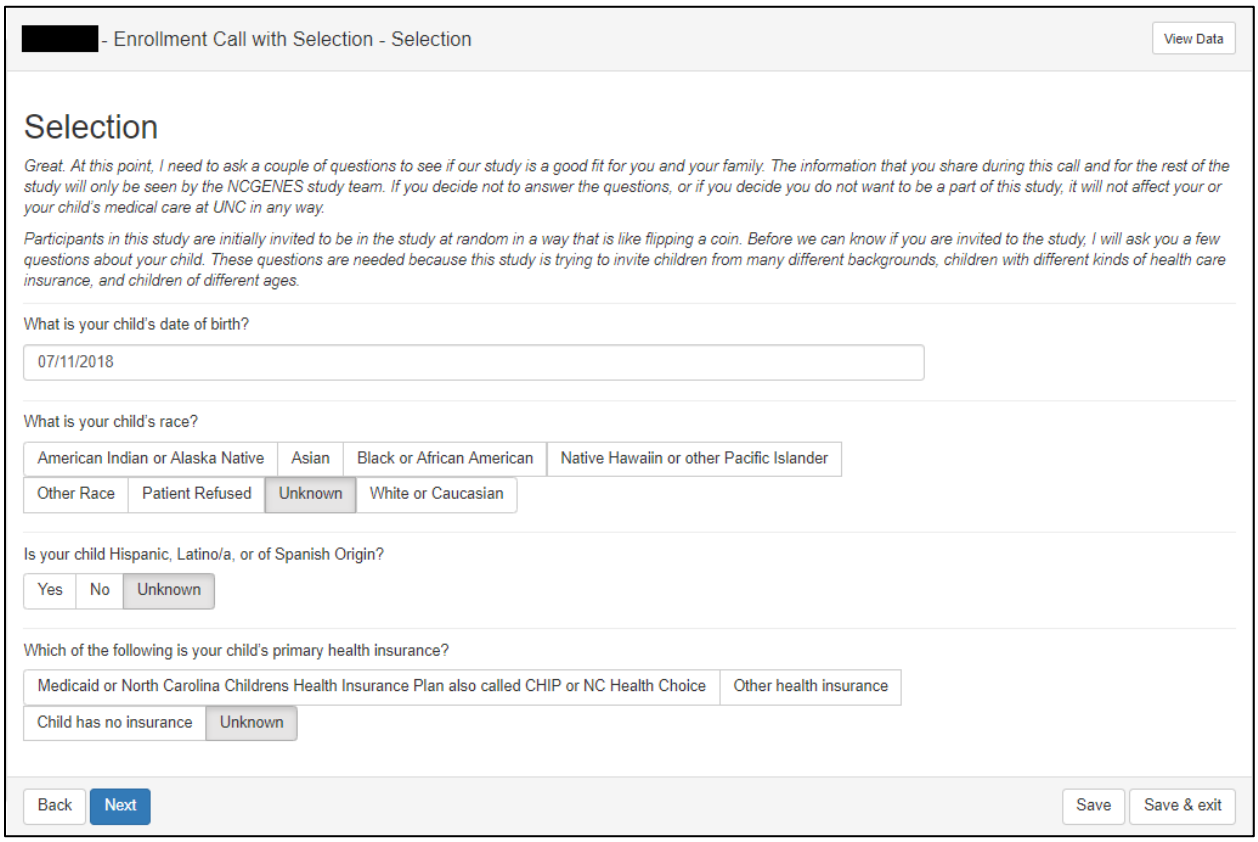


Selection

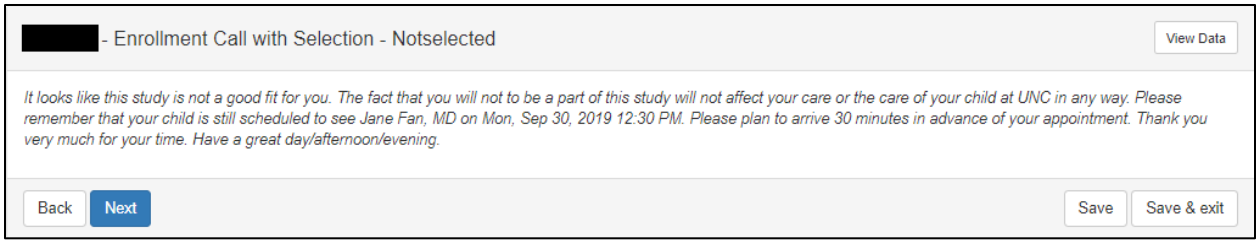
1. If a participant's selection was completed at the time eligibility was determined, the following dialog box will appear. Click the blue "Next" button and proceed to **Parent Eligibility**.



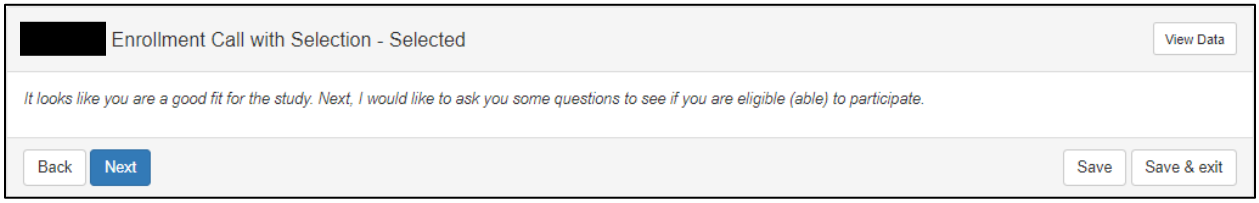
2. If, however, the participant's selection has not occurred, the following dialog box will appear. After reading the script, enter the parent's answers to the child's DOB, race, ethnicity, and health insurance. Once all fields are entered, click the blue "Next" button.



a. If the participant is <u>not</u> selected, the following dialog box will appear. Conclude the call with the parent and click the blue "Next" button. This will complete the task and change the participant's final status to "Participant Not Selected."

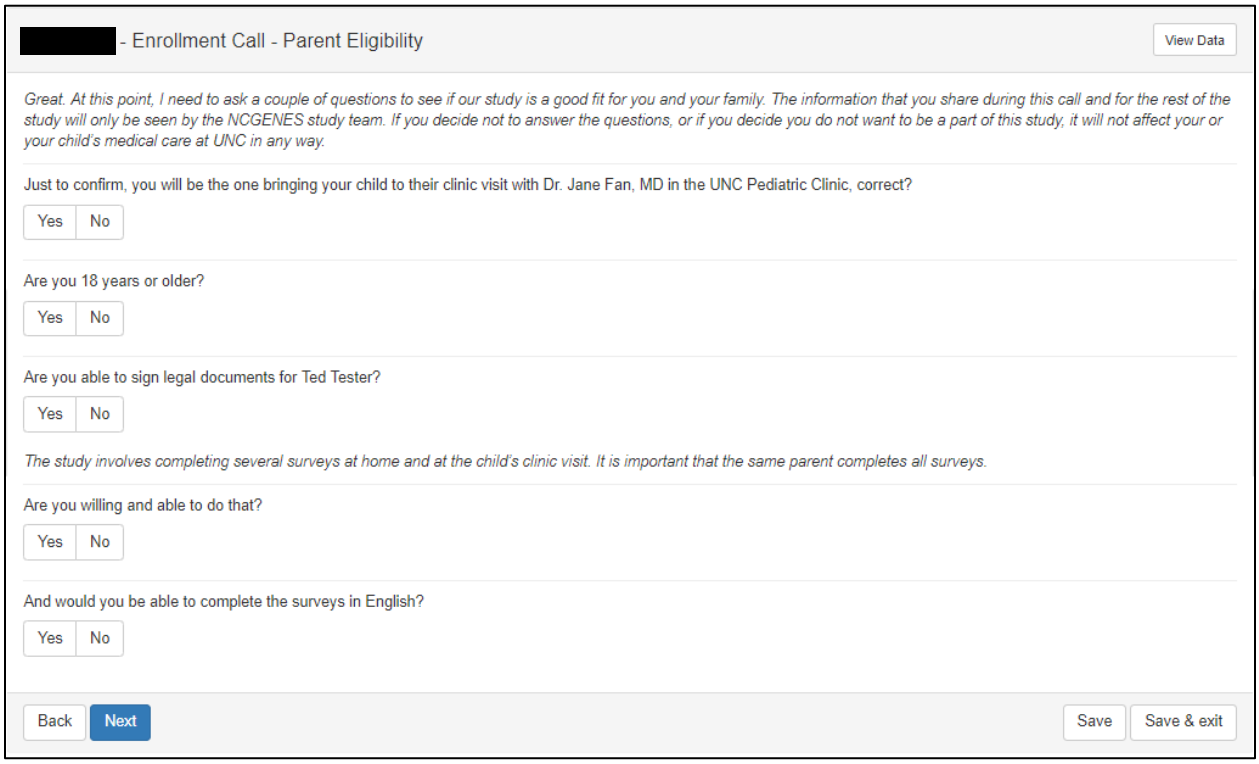


b. If the participant is selected, the following dialog box will appear. After reading the script, click the blue "Next" button. The system will automatically proceed to Parent Eligibility.

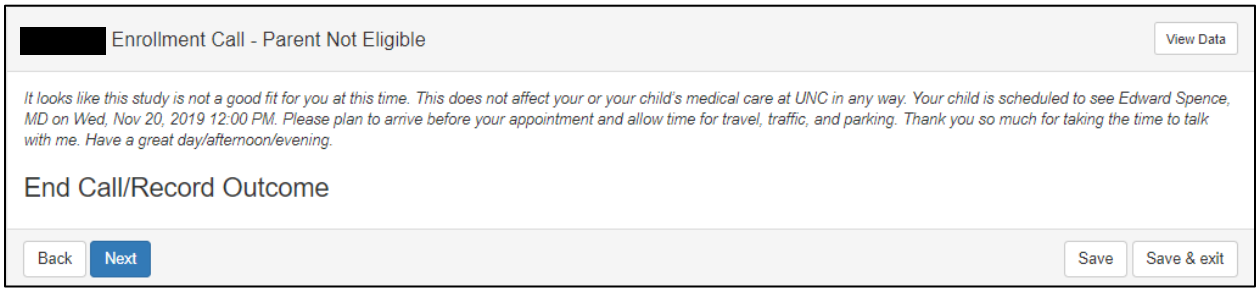


Parent Eligibility

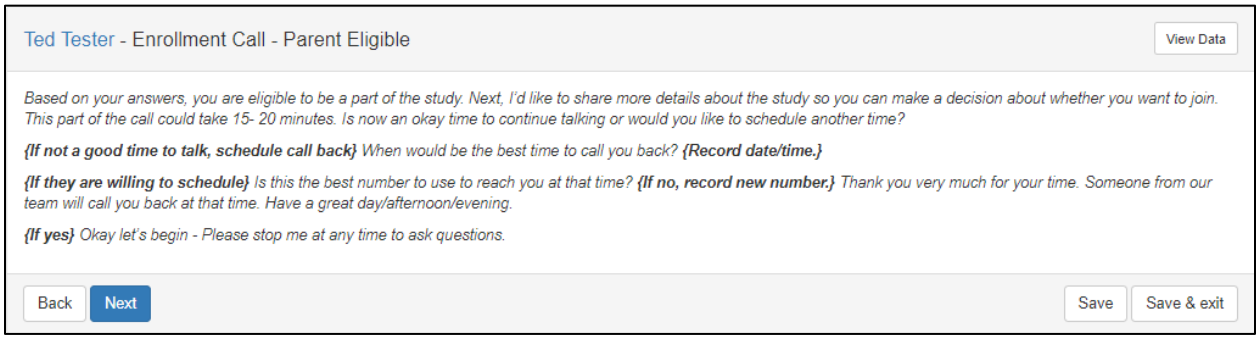
1. When the Parent Eligibility page is reached, read through the script and record the parent's answers to each question where appropriate. Once every question has been answered, click the blue "Next" button.



   a. If the parent answers "No" to any of the questions, they will be ineligible. The following dialog box will appear. Read the script, conclude the call, and click the blue "Next" button. This will complete the task and change the participant's status to "Parent Ineligible – [Reason for Ineligibility]."

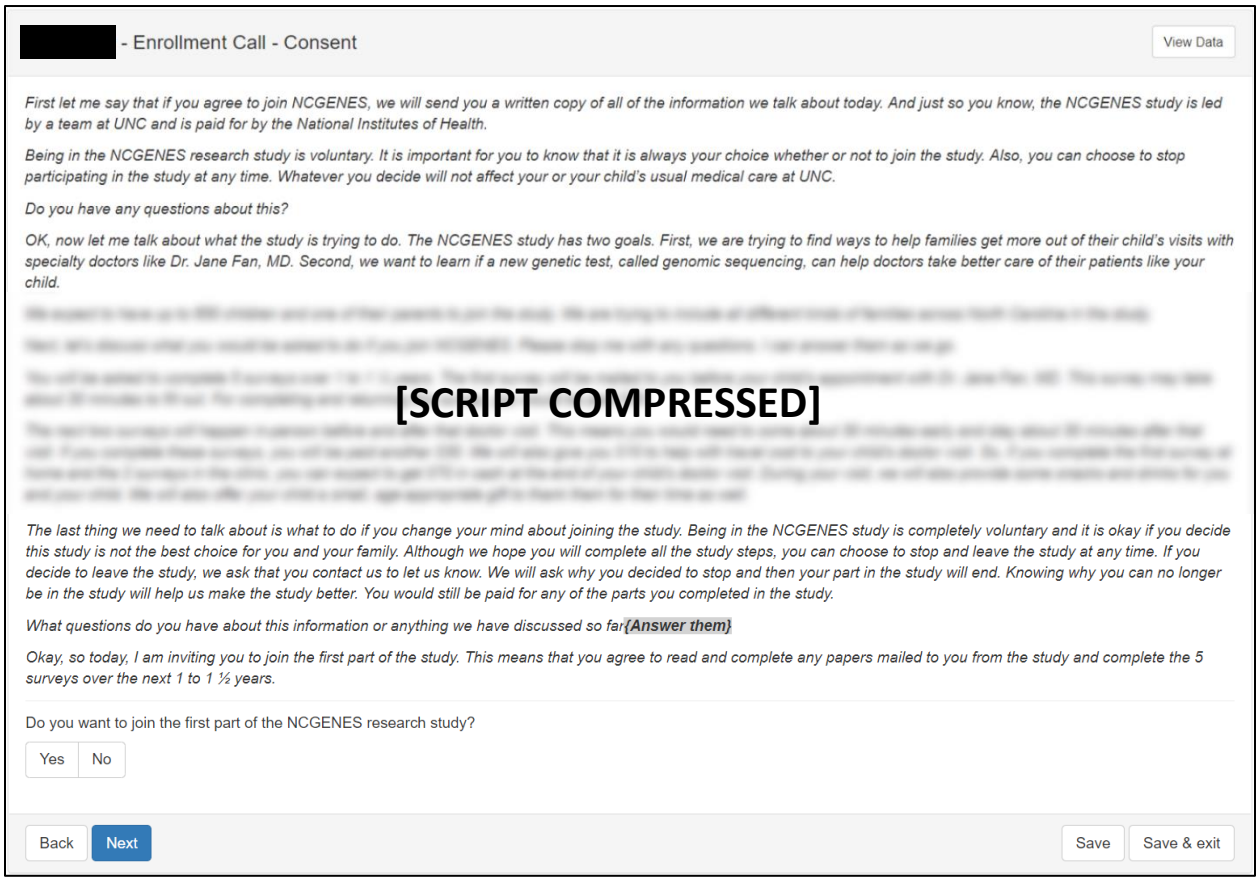


b. If the parent answers “Yes” to all questions, they will be eligible. The following dialog box will appear. If the parent cannot continue the call, record a call-back time, conclude the call, and click “Save & Exit” in the bottom right corner of the box. If the parent can continue the call, click the blue “Next” button to proceed to Consent.

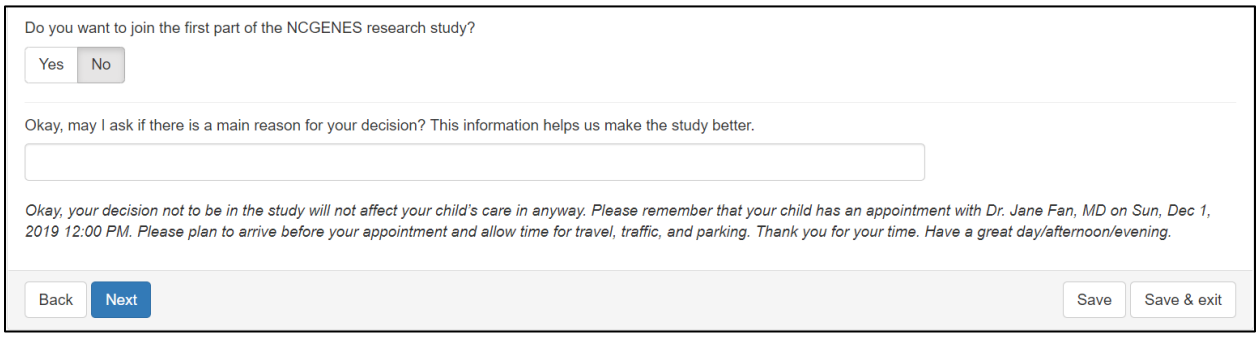


Consent

1. The consent page (shown below) is the most extensive of any page during the enrollment call. After carefully explaining the study to the participant following the script, ask the parent if they agree to the first part of the study.



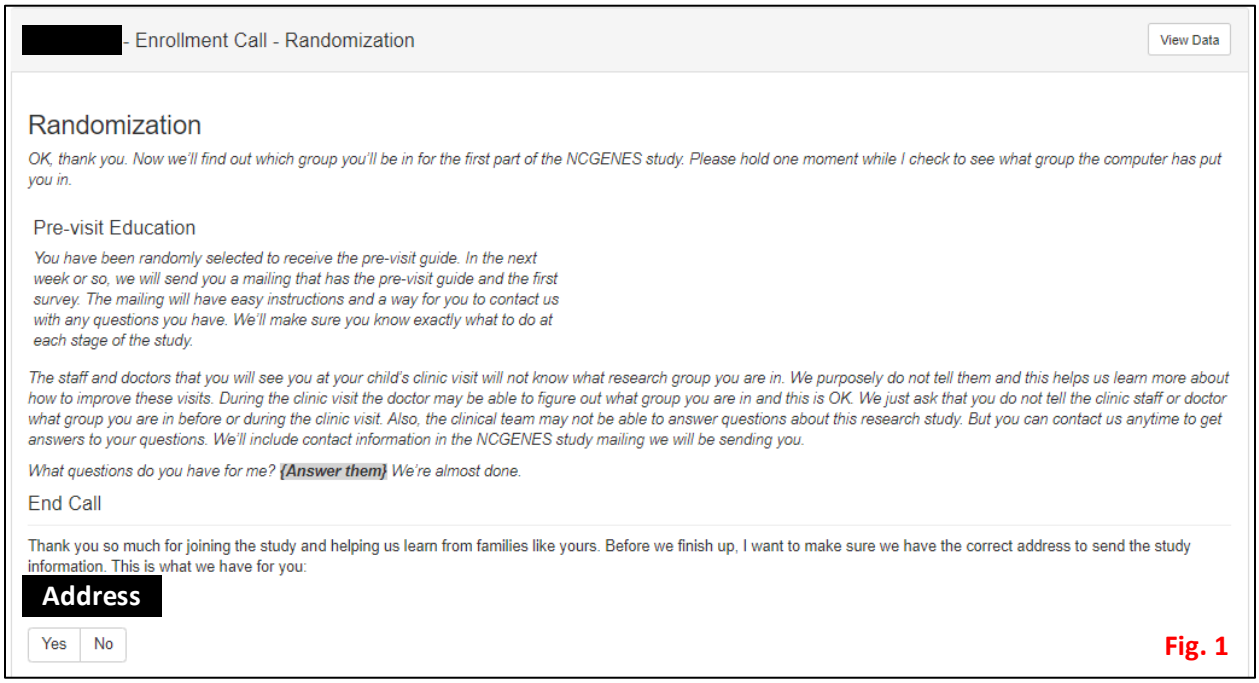
   a. If the parent declines, select "No." The following section will expand. If the parent offers a reason for declining, enter it here. Conclude the call and press the blue "Next" button. This will complete the task and give the participant a status of "Refused."

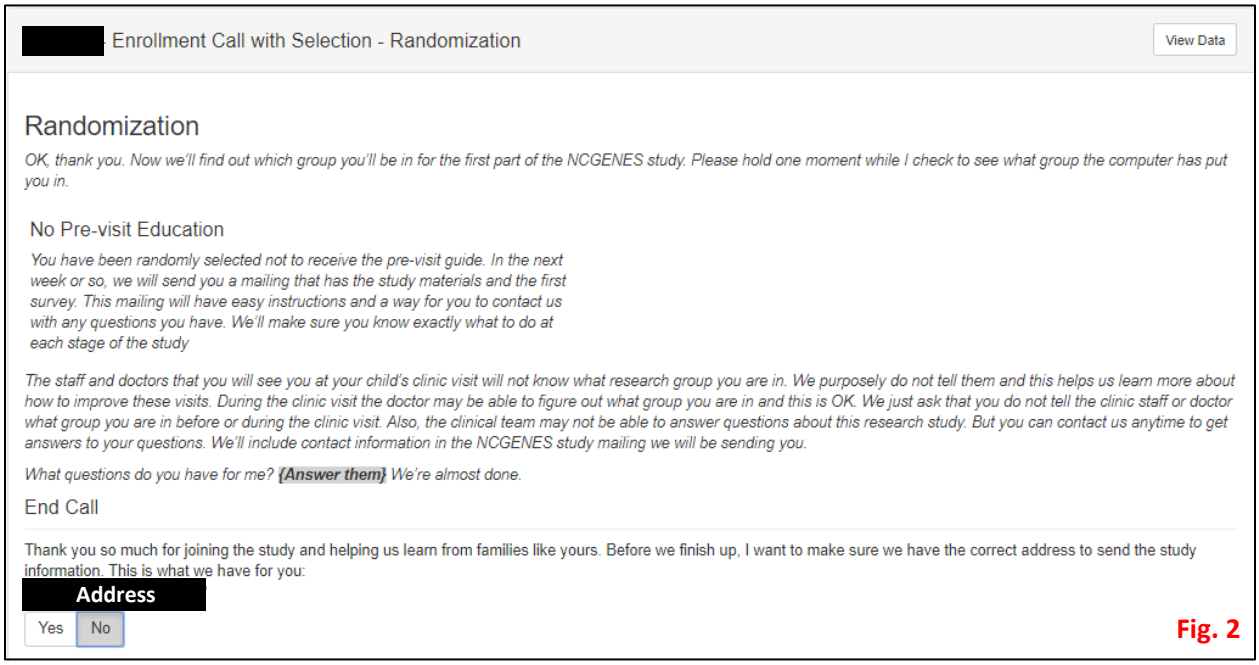


   b. If the parent agrees to join the study, select "Yes" and click the blue "Next" button. The system will automatically continue to Randomization.

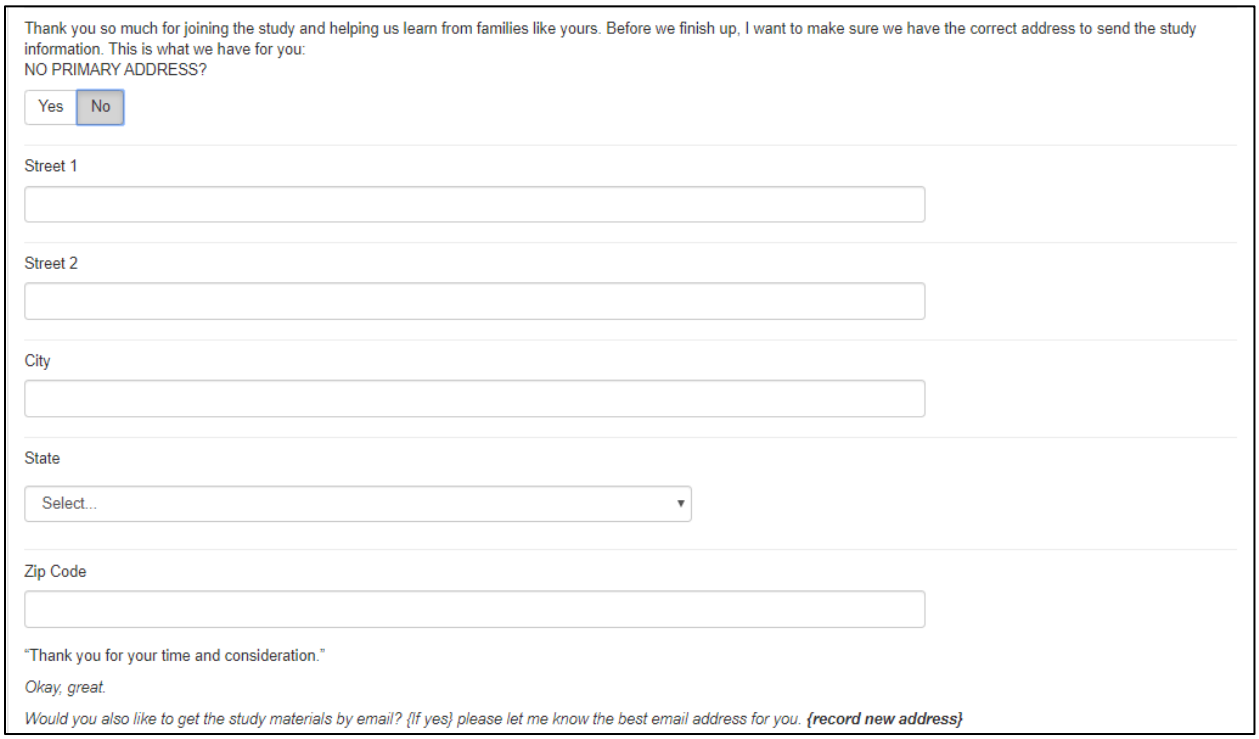
Randomization

1. The Randomization page has two versions, with (Fig. 1) and without (Fig. 2) pre-visit education. Read through the script and verify the mailing address for the parent.

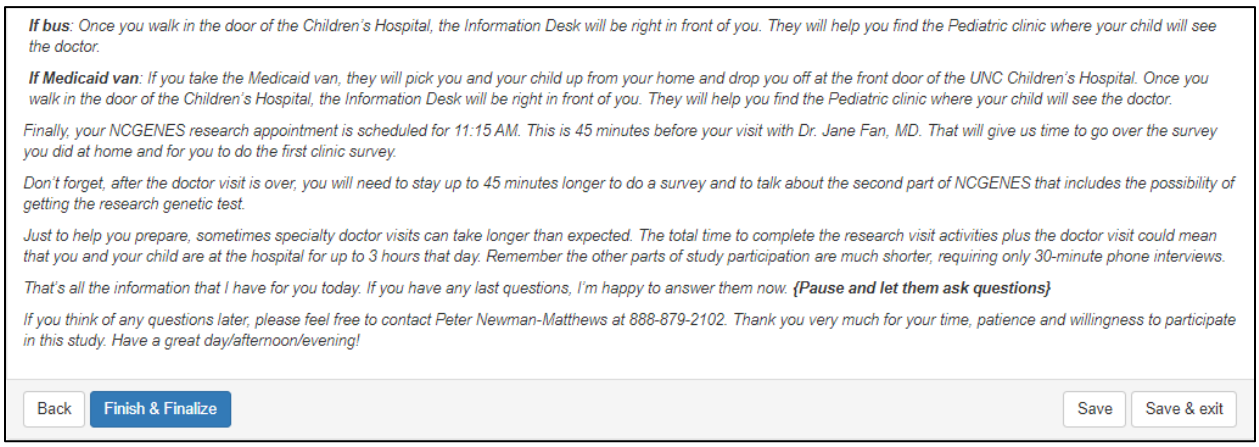




2. If the parent indicates the address is incorrect, click "No." The box will expand as shown below. Enter the parent's corrected address and continue with the call.



3. Once the script is concluded, end the call with the parent and click the blue "Finish & Finalize" button. This will complete the task and automatically redirect the user to the individual participant's page.



Visit 1 Appointment Packet – with(out) Pre-Visit Prep

The "Visit 1 Appointment Packet – with(out) Pre-Visit Prep" task is a mailing task. This (and all) mailing task(s) can be started from the homepage (Fig. 1) or individual participant page (Fig. 2) by clicking the "Get Started" button for the appropriate participant.

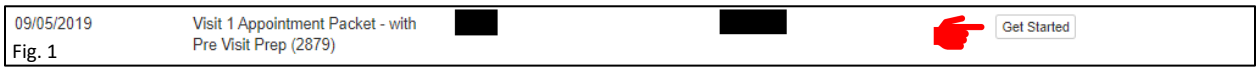


Fig. 1

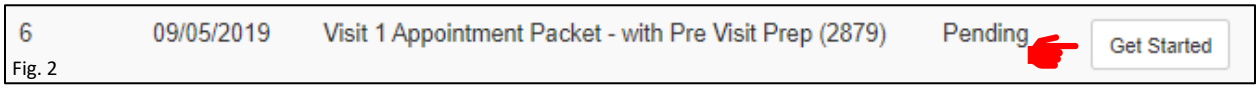


Fig. 2

Either action will redirect the user to the "Mailings" page, where they can generate a mail merge document for a particular participant. The "Visit 1 Appointment Packet – with(out) Pre-Visit Prep" task will be marked complete in the tracking system when this mail merge is generated (Fig. 3).

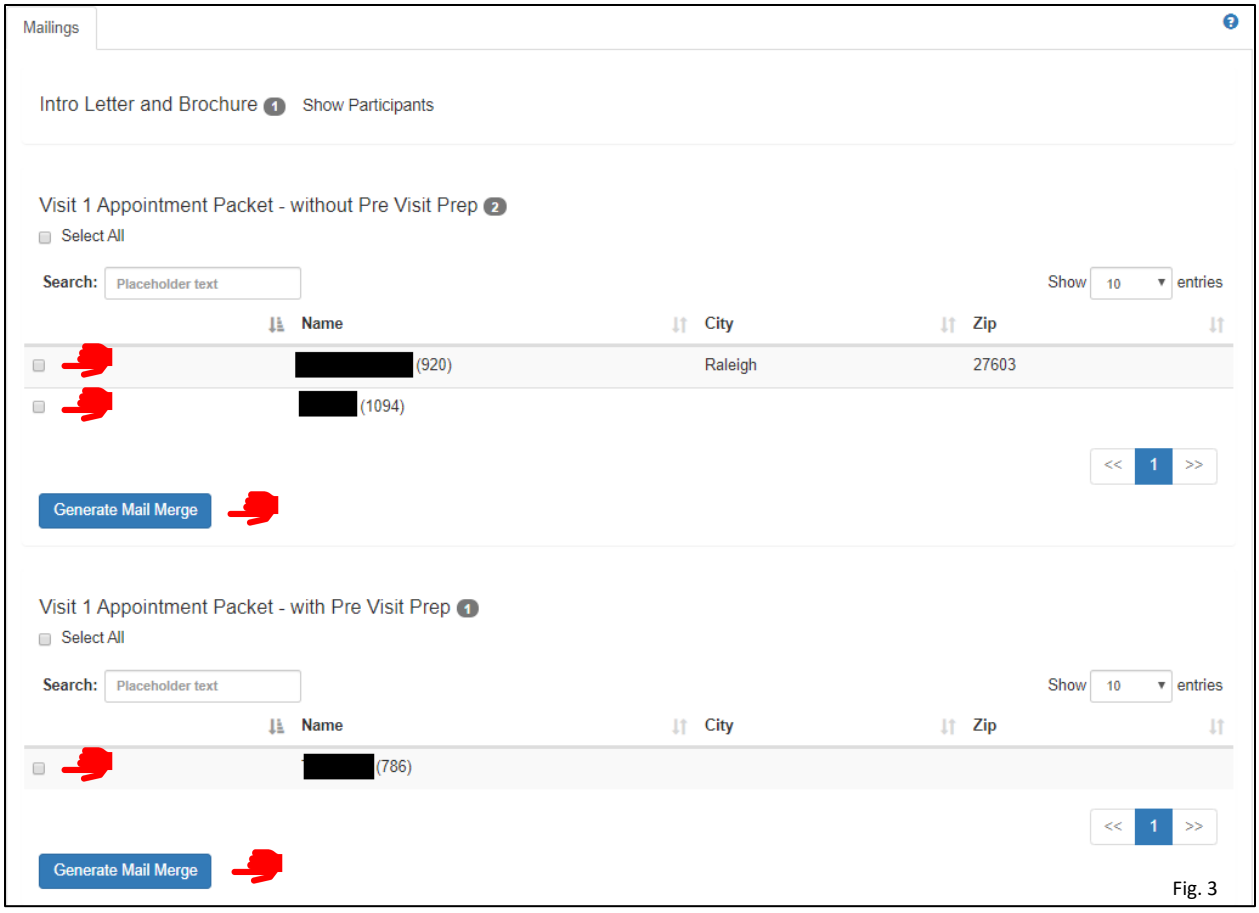


Fig. 3

Mailings should be completed on the same day they are originated to ensure accurate tracking of this task. For detailed instructions on completing this mailing, go to the **Mailing 2** section under **Mailings**.

Reminder Call for Visit 1

1. To begin the Reminder call, select the "Get Started" button next to the "Reminder Call for Visit 1" task for the appropriate participant. This can be done from either the Home Page (Fig. 1) or the Individual Participant Page (Fig. 2).

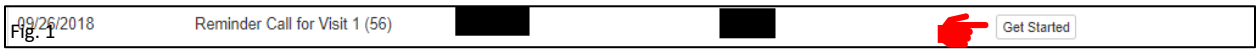
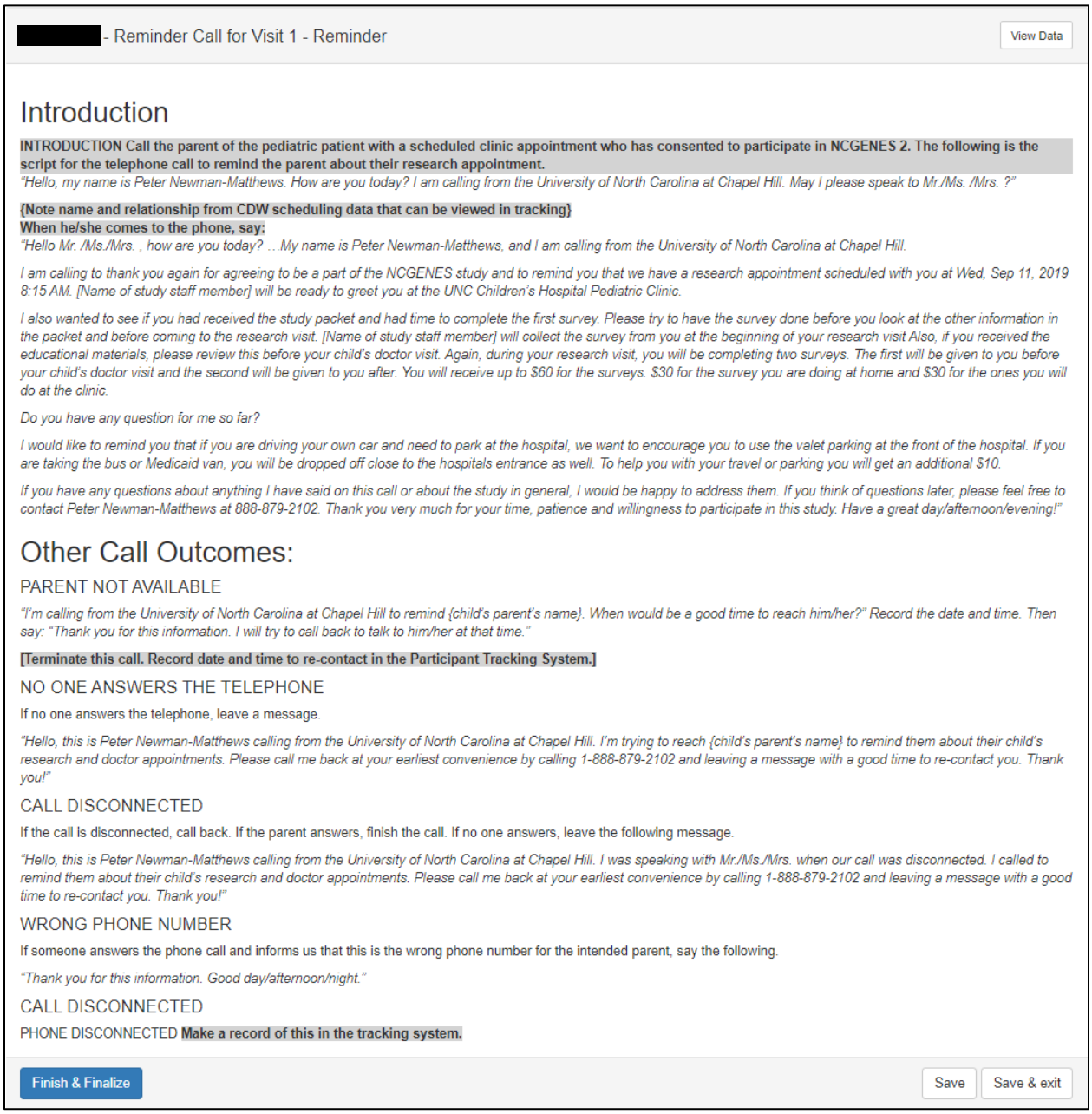


Fig. 1

Fig. 2

2. Walk through the script as prompted. If the call cannot be completed, click the "Save & Exit" button and **log the communication** as directed. If the call is completed, click the blue "Finish & Finalize" button to complete the task.



Confirm Visit 1 Appointment Date and Provider

1. To confirm the visit, select the "Get Started" button next to the "Confirm Visit 1 Appointment Date and Provider" task for the appropriate participant. This can be done from either the Home Page (Fig. 1) or the Individual Participant Page (Fig. 2).
2. Confer with the participant to confirm all the appointment details auto-populated by the tracking system are

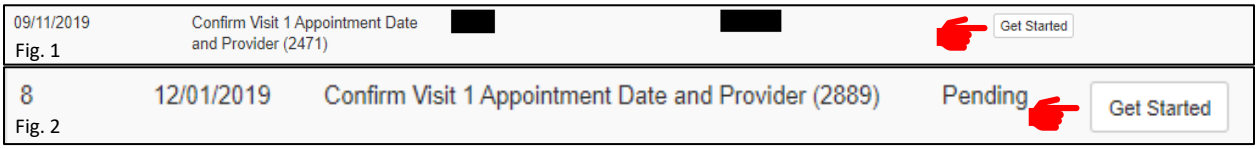
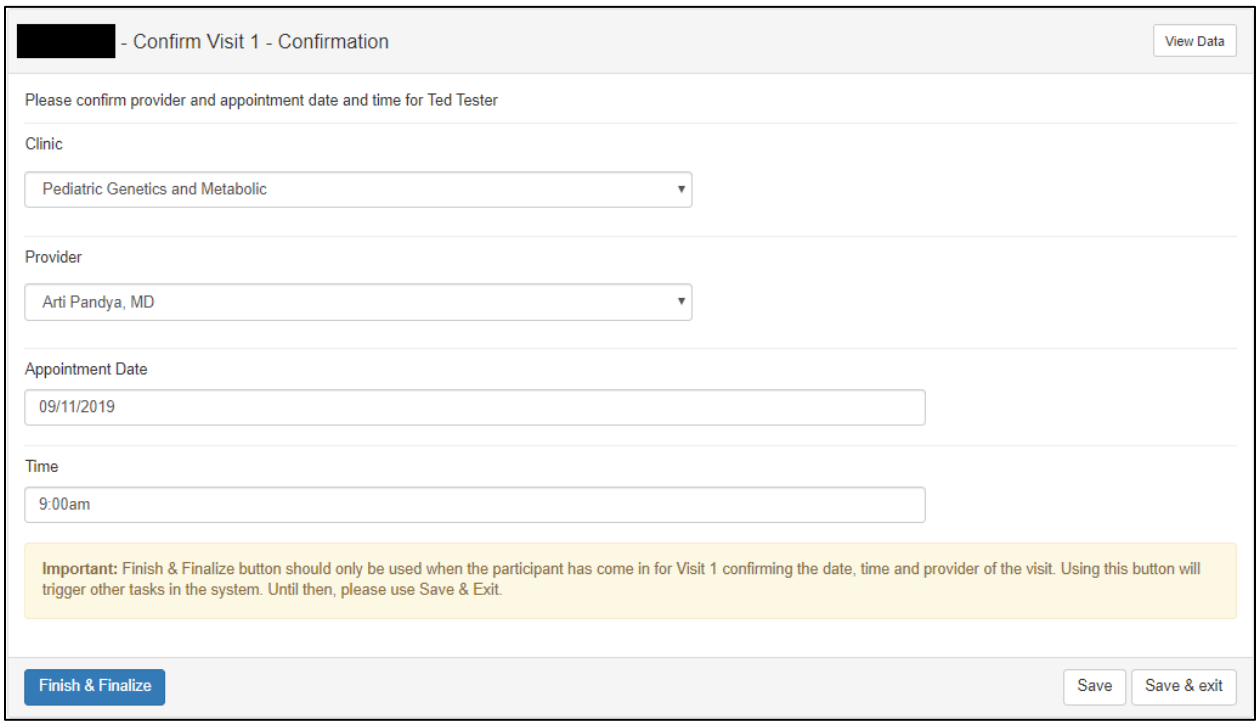


Fig. 1
Fig. 2

correct. If necessary, change the clinic, provider, appointment date, and time. Once this has been confirmed with the participant in the clinic, click the blue "Finish & Finalize" button. This will complete the task.

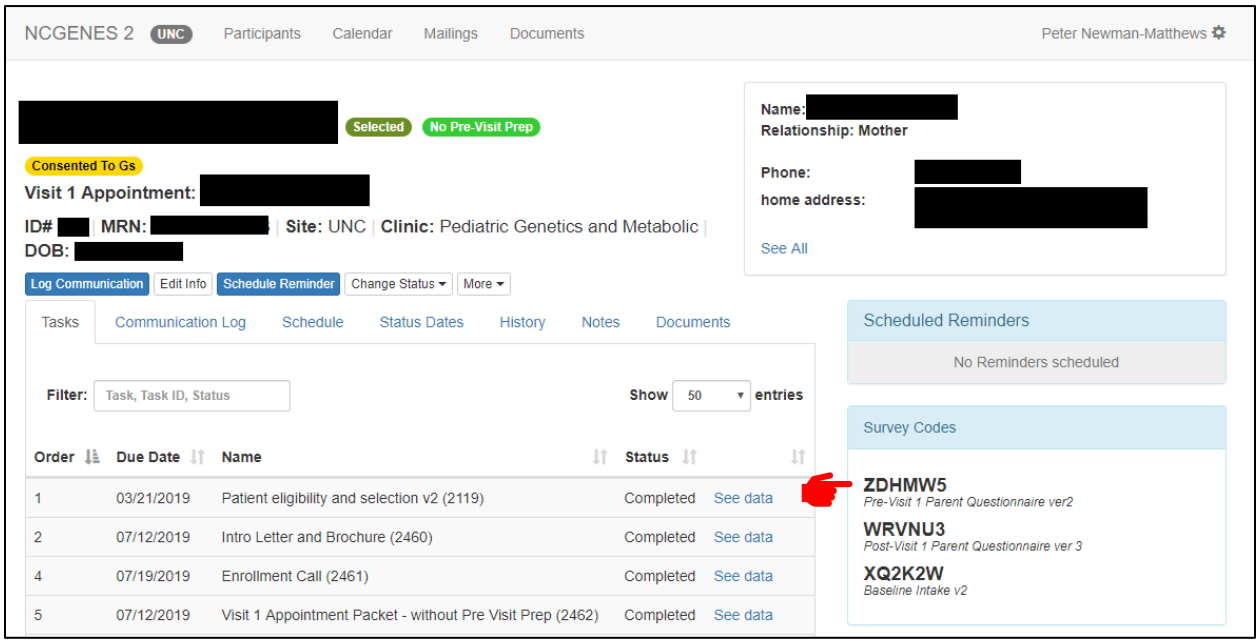


Pre-Visit 1 Parent Questionnaire

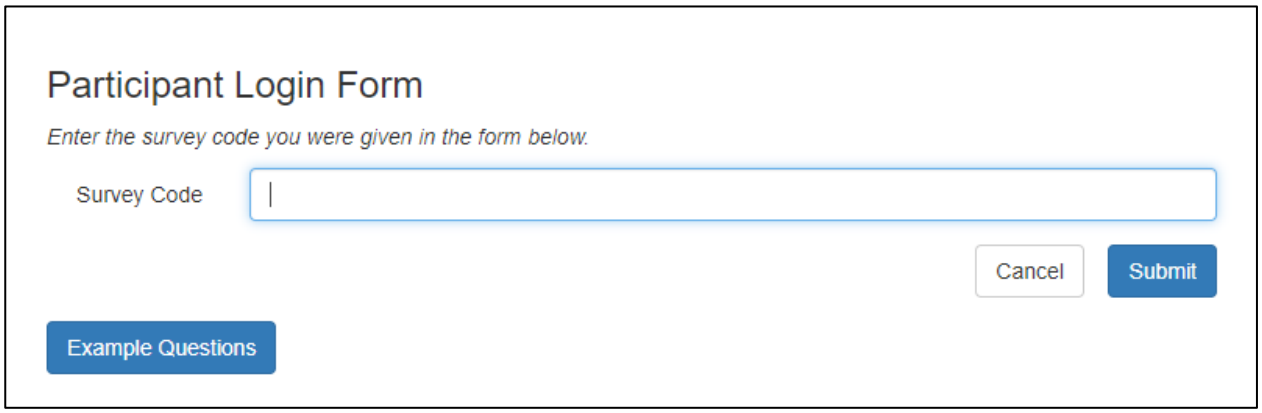
The Pre-Visit 1 Parent Questionnaire will in most cases be administered in the clinic and completed by the parent themselves. In cases where the tracking system is not accessible in clinic, the parent may fill out the survey on paper and a study staff member, usually the RA, will enter the survey information into the tracking system after the fact. This step-by-step guide covers both scenarios.

Completion in the Tracking System

1. First copy the "Pre-Visit 1 Parent Questionnaire" code from the individual participant's page (Shown Below).
2. In a new window, navigate to the surveys version of the tracking system: ***https://ncgenes2.sirs.unc.edu/surveys***.



The following screen will appear.



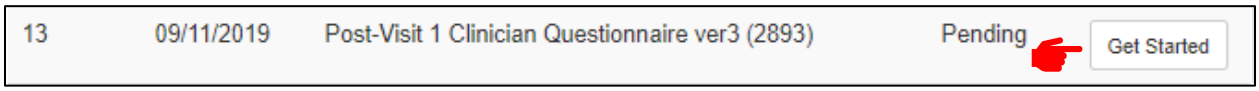
3. Paste the code into the text box, click the blue "Submit" button. Ensure any other tracking system windows in the browser are closed and hand the tablet over to the parent. When the parent finishes the survey, ensure that the blue "Finish & Finalize" button has been clicked. This will complete the task and redirect to the survey login page.

Entry of Paper Form in the Tracking System

If the parent completes the Pre-Visit 1 Questionnaire on paper, it should be entered into the tracking system within 24 hours. The survey must be scanned and uploaded as well as entered via the task.[1]

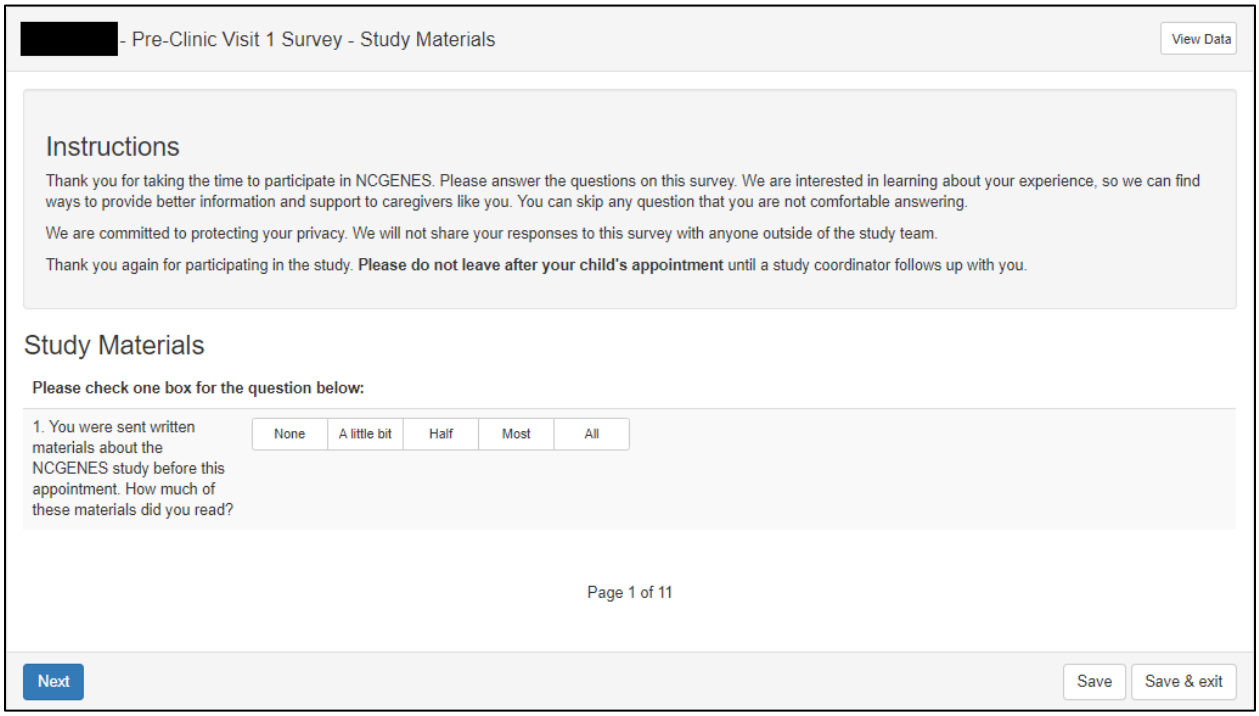
1. First, upload a scan of the paper questionnaire to the **participant documents** page and label it "Pre-Visit 1 Parent Questionnaire_ID#"

2. From the individual participant's page, click "Get Started" next to the Pre-Visit 1 Parent Questionnaire task, as shown below.



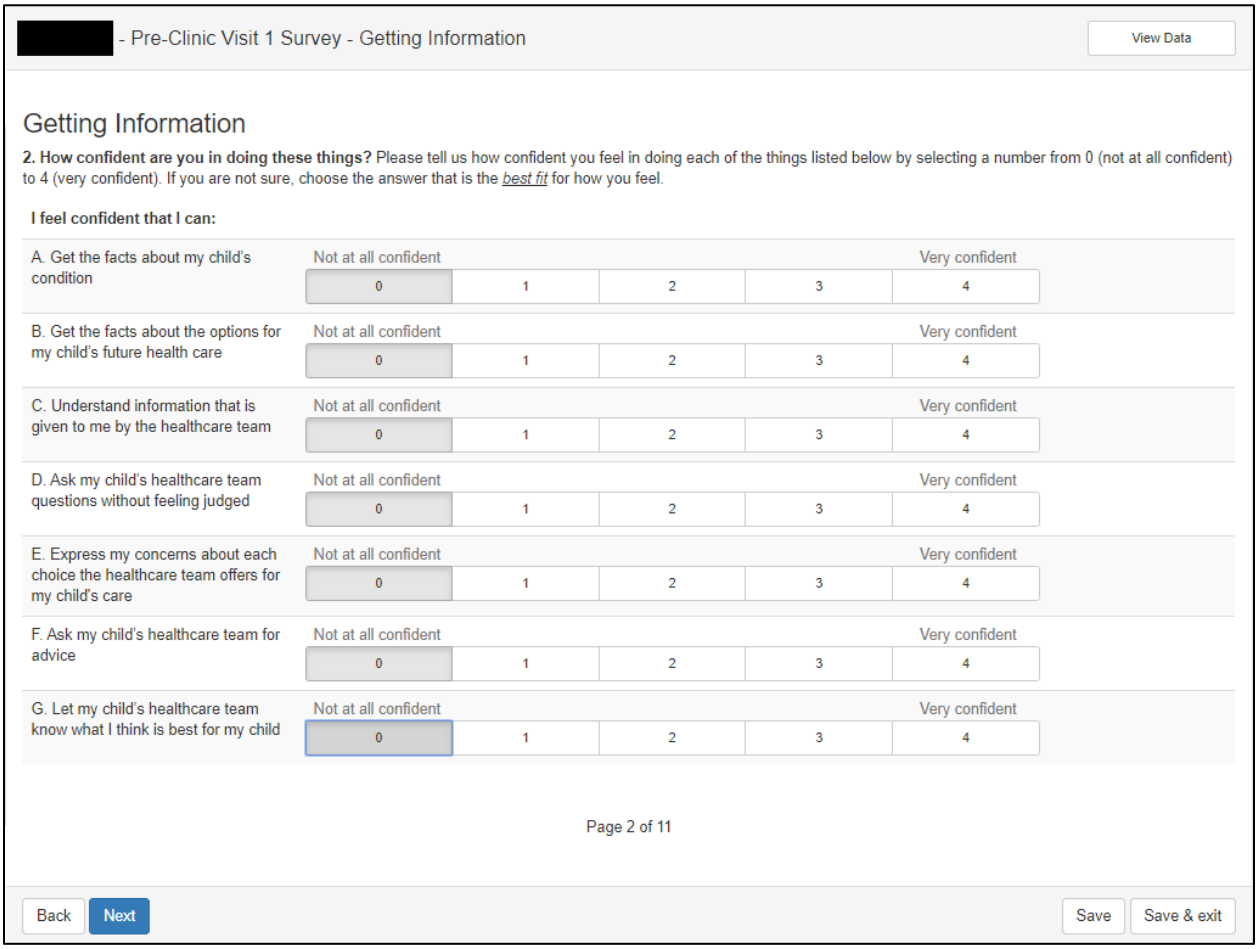
3. The following pages will appear in succession. After entering the parent's answers at each page, click the blue "Next" and repeat for subsequent pages. When all data is entered, click the blue "Finish & Finalize" button to complete the task (See **Page 11**).

Page 1



[1] If you encounter any ambiguity in an answer to one of the survey questions that requires interpretation, please consult with the Clinical Study Director, Jeannette Bensen, by emailing her at Jeannette_bensen@med.unc.edu.

Page 2


[redacted] - Pre-Clinic Visit 1 Survey - Getting Information
View Data
Getting Information
2. How confident are you in doing these things? Please tell us how confident you feel in doing each of the things listed below by selecting a number from 0 (not at all confident) to 4 (very confident). If you are not sure, choose the answer that is the best fit for how you feel.
I feel confident that I can:
A. Get the facts about my child's condition
B. Get the facts about the options for my child's future health care
C. Understand information that is given to me by the healthcare team
D. Ask my child's healthcare team questions without feeling judged
E. Express my concerns about each choice the healthcare team offers for my child's care
F. Ask my child's healthcare team for advice
G. Let my child's healthcare team know what I think is best for my child
Not at all confident
Very confident
0
1
2
3
4
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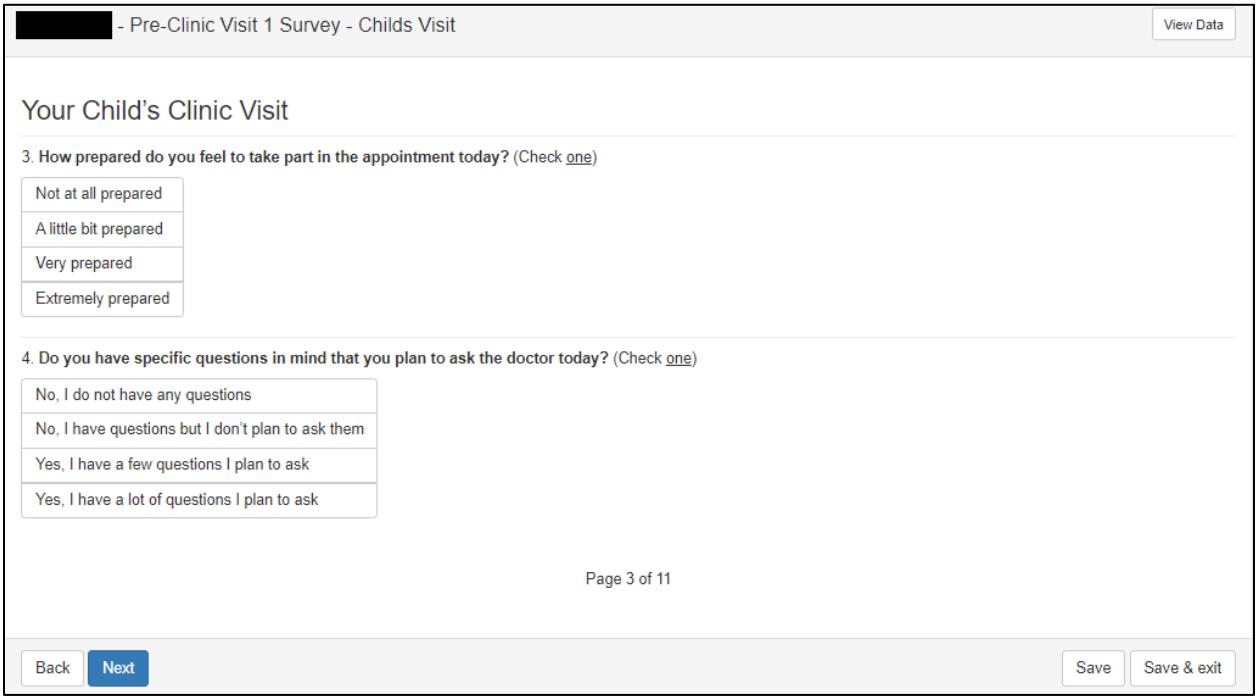
Save
Save & exit

Page 3


[redacted] - Pre-Clinic Visit 1 Survey - Childs Visit
View Data
Your Child's Clinic Visit
3. How prepared do you feel to take part in the appointment today? (Check one)
Not at all prepared
A little bit prepared
Very prepared
Extremely prepared
4. Do you have specific questions in mind that you plan to ask the doctor today? (Check one)
No, I do not have any questions
No, I have questions but I don't plan to ask them
Yes, I have a few questions I plan to ask
Yes, I have a lot of questions I plan to ask
Page 3 of 11
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Save
Save & exit

[redacted]- Pre-Clinic Visit 1 Survey - How You Feel

View Data

How You Feel

5. Over the last 2 weeks, how often have you been bothered by the following problems? Please check one box to tell us how often you have experienced each thing listed below.

	Not at all	Several days	More than half the days	Nearly every day
A. Feeling nervous, anxious or on edge				
B. Not being able to stop or control worrying				
C. Worrying too much about different things				
D. Trouble relaxing				
E. Being so restless that it is hard to sit still				
F. Becoming easily annoyed or irritable				
G. Feeling afraid as if something awful might happen				
H. Little interest or pleasure in doing things				
I. Feeling down, depressed, or hopeless				
J. Trouble falling or staying asleep, or sleeping too much				
K. Feeling tired or having little energy				
L. Poor appetite or overeating				
M. Feeling bad about yourself — or that you are a failure or have let yourself or your family down				
N. Trouble concentrating on things, such as reading the newspaper or watching television				
O. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual				

Back Next Save Save & exit

[redacted] - Pre-Clinic Visit 1 Survey - How You Feel Continued

View Data

6. **How do you feel right now?** These emotions can be about anything going on in your life right now. Please check one box after each of the words listed below.

	Not at all	A little	Moderately	Quite a bit	Extremely
A. Hopeful			■		
B. Unhappy	■				
C. Determined			■		
D. Anxious	■				
E. Curious			■		
F. Angry	■				
G. Panicky	■				
H. Relieved					■
I. Confused	■				
J. Helpless	■				
K. Excited			■		
L. Guilty	■				
M. Happy			■		
N. Tense	■				
O. Uncertain	■				
P. Joyful			■		
Q. Sad	■				
R. Carefree			■		
S. Hopeless	■				
T. Relaxed			■		
U. Nervous	■				
V. Interested			■		

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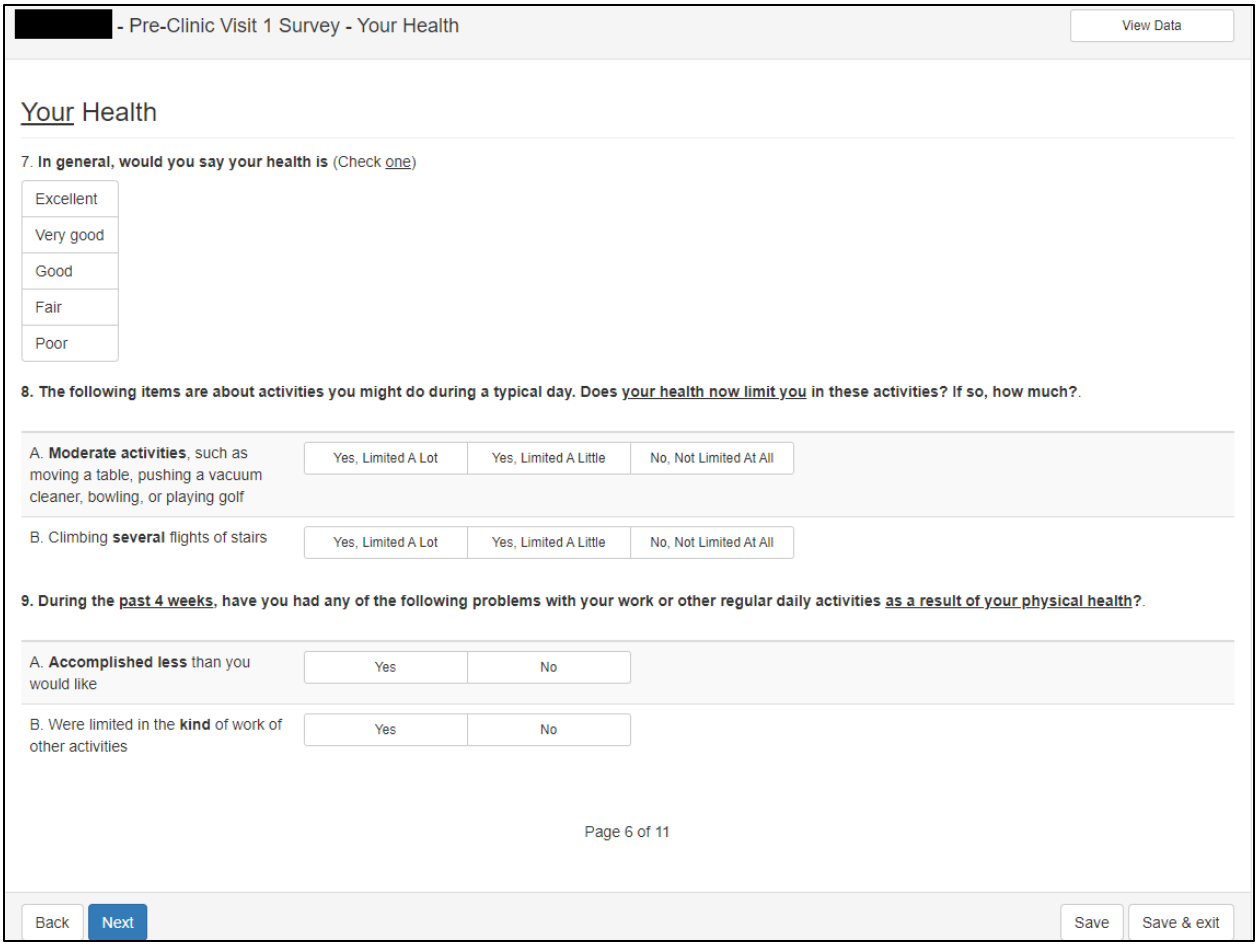
Page 6


[redacted] - Pre-Clinic Visit 1 Survey - Your Health
View Data
Your Health
7. In general, would you say your health is (Check one)
Excellent
Very good
Good
Fair
Poor
8. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?.
A. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf
Yes, Limited A Lot
Yes, Limited A Little
No, Not Limited At All
B. Climbing several flights of stairs
Yes, Limited A Lot
Yes, Limited A Little
No, Not Limited At All
9. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?.
A. Accomplished less than you would like
Yes
No
B. Were limited in the kind of work of other activities
Yes
No
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Save & exit

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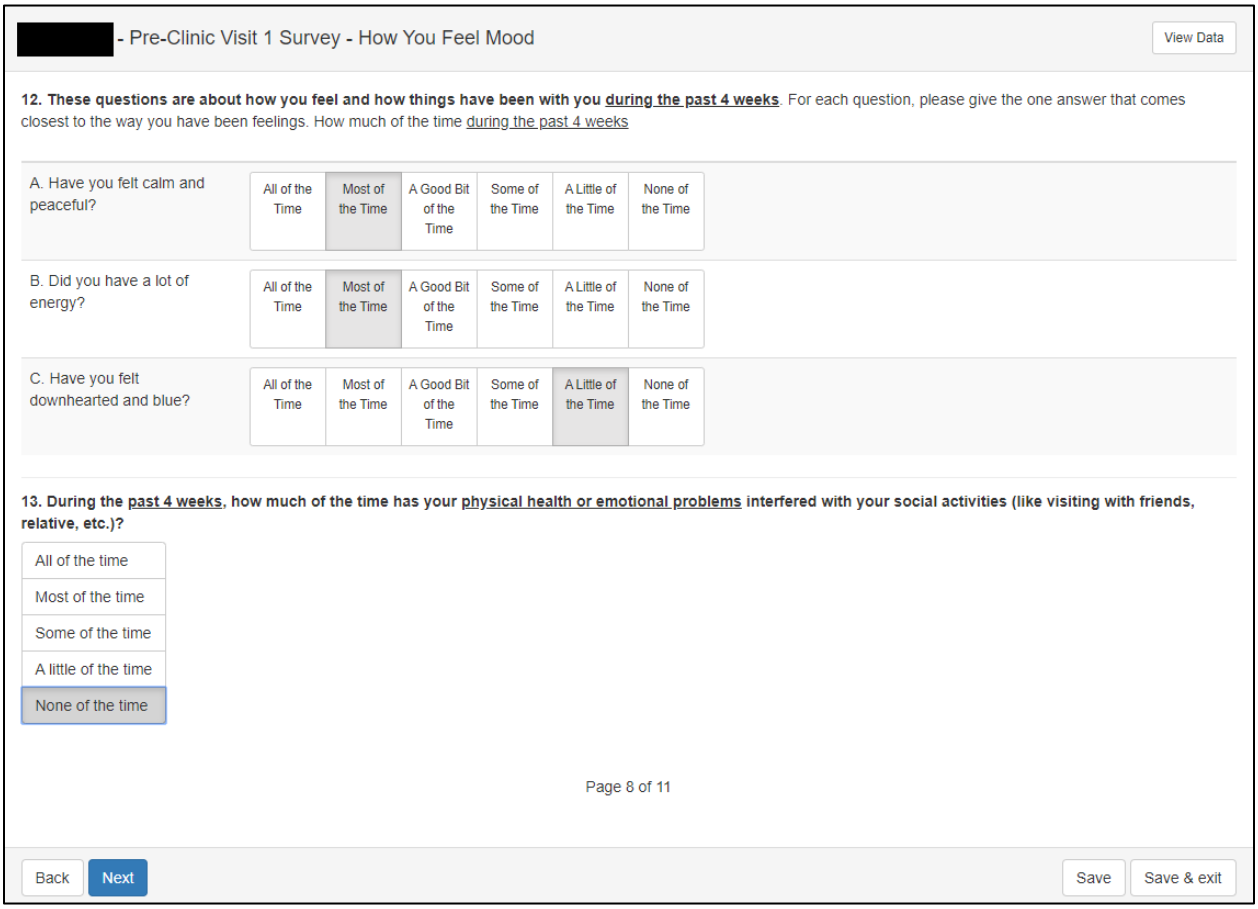
Page 8


[redacted] - Pre-Clinic Visit 1 Survey - How You Feel Mood
View Data
12. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feelings. How much of the time during the past 4 weeks
A. Have you felt calm and peaceful?
All of the Time
Most of the Time
A Good Bit of the Time
Some of the Time
A Little of the Time
None of the Time
B. Did you have a lot of energy?
All of the Time
Most of the Time
A Good Bit of the Time
Some of the Time
A Little of the Time
None of the Time
C. Have you felt downhearted and blue?
All of the Time
Most of the Time
A Good Bit of the Time
Some of the Time
A Little of the Time
None of the Time
13. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relative, etc.)?
All of the time
Most of the time
Some of the time
A little of the time
None of the time
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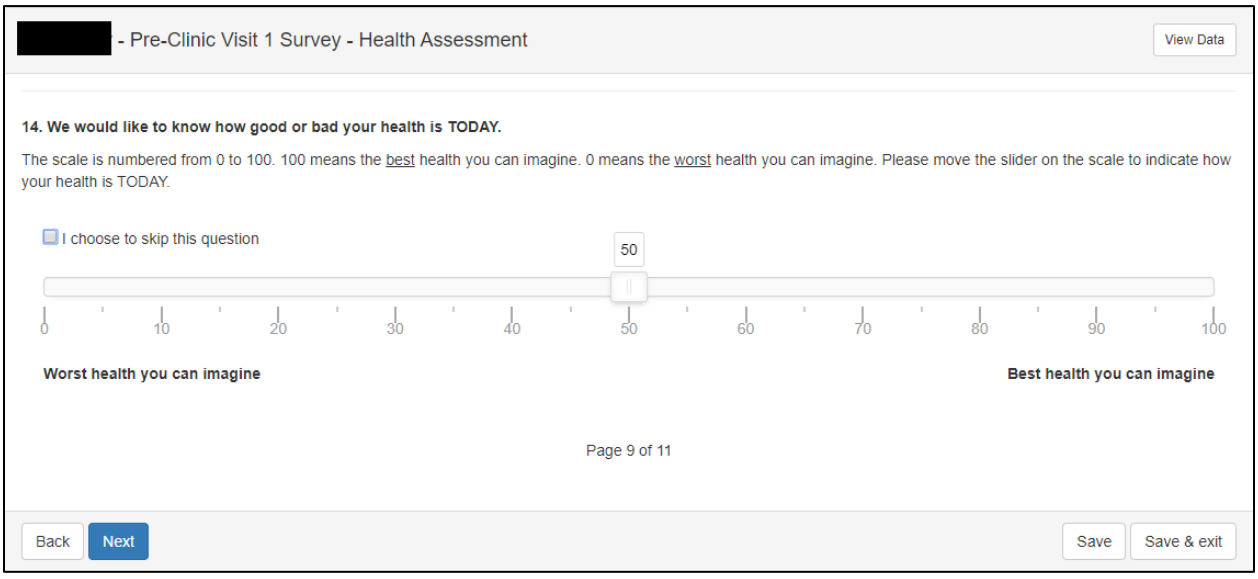
Save & exit

Page 9


[redacted] - Pre-Clinic Visit 1 Survey - Health Assessment
View Data
14. We would like to know how good or bad your health is TODAY.
The scale is numbered from 0 to 100. 100 means the best health you can imagine. 0 means the worst health you can imagine. Please move the slider on the scale to indicate how your health is TODAY.
I choose to skip this question
50
0
10
20
30
40
50
60
70
80
90
100
Worst health you can imagine
Best health you can imagine
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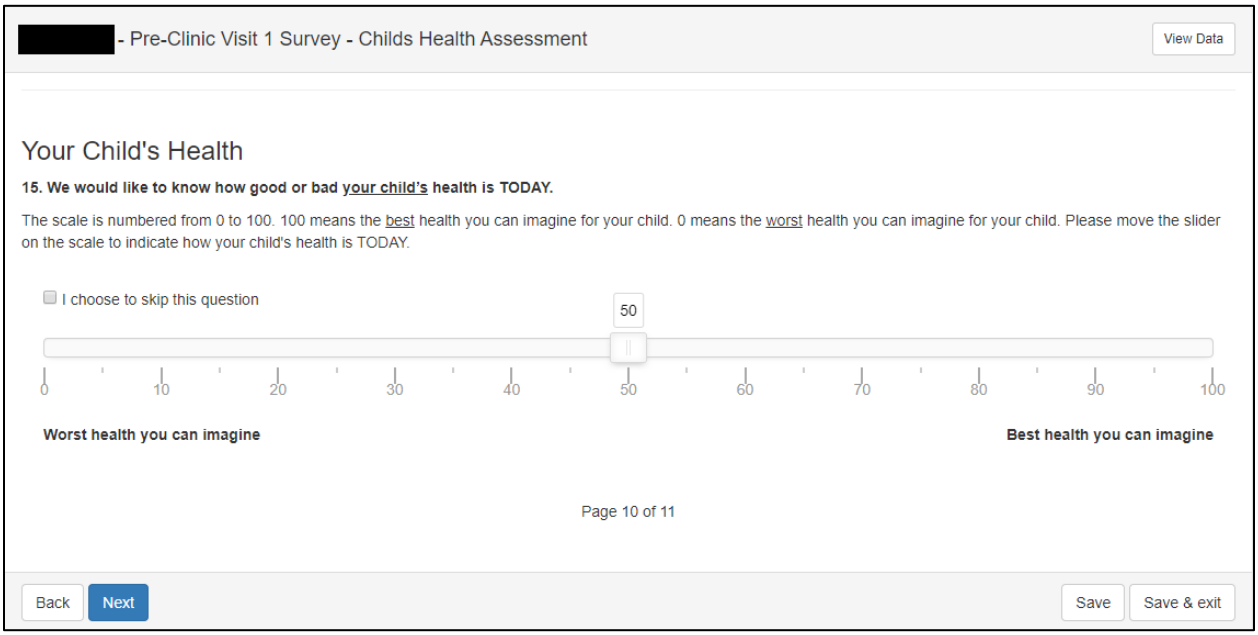
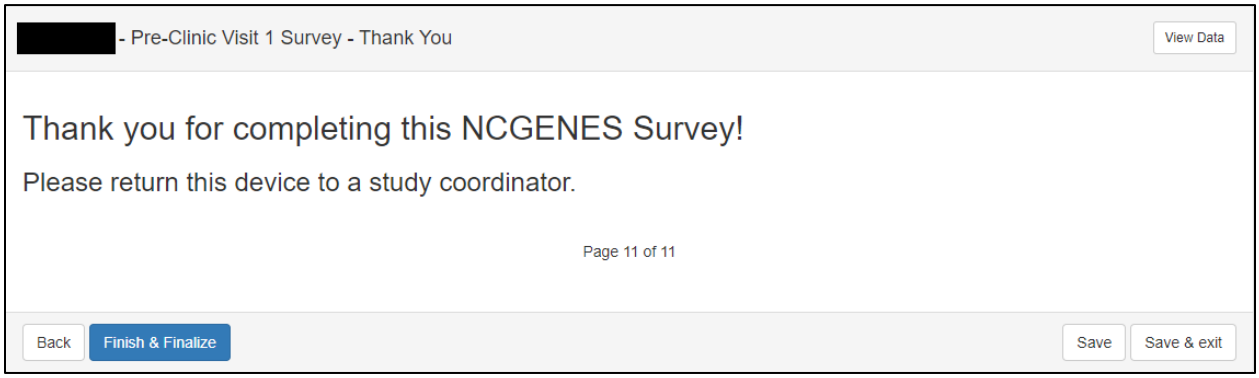
Save & exit

Page 10



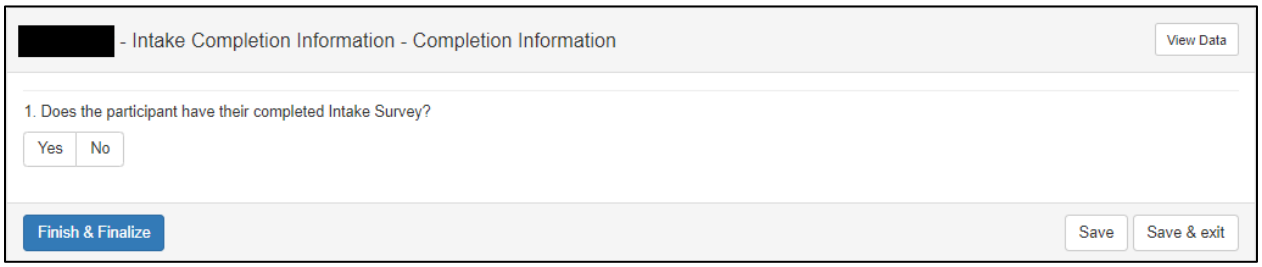
Page 11



Visit 1 – Collect Info about Intake Completion

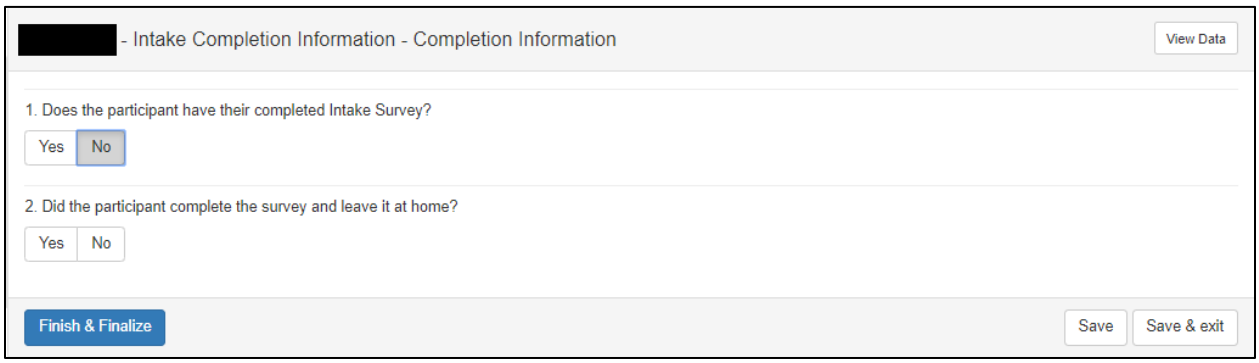
1. To begin the "Visit 1 – Collect Info about Intake Completion" task, click the "Get Started" button next to the task on individual participant's page. (Shown below)

2. The following page will appear.



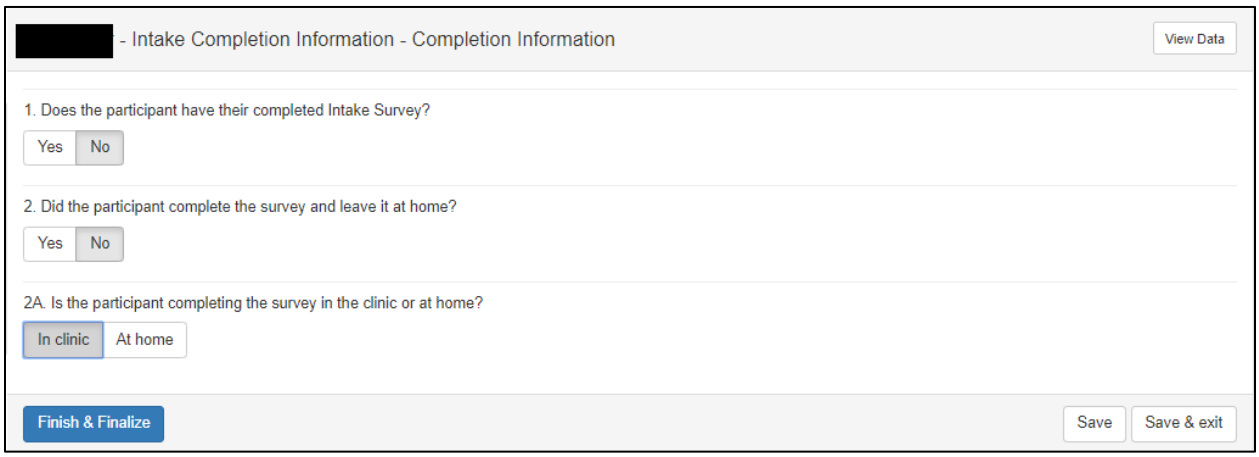
   a. If the parent has their completed Intake Survey, select "Yes" for Question 1 and then complete the task by clicking the blue "Finish & Finalize" button.

   b. If the parent does not have the intake survey, select "No" for Question 1. The dialog box will expand as shown below.



      i. If the parent completed their survey, but left it at home, select "Yes" for Question 2. A reminder to provide the parent with a prepaid envelope will appear. Click the blue "Finish & Finalize" button to complete the task.

ii. If the parent has not completed the survey, select “No” for Question 2. The dialog box will expand as shown below.



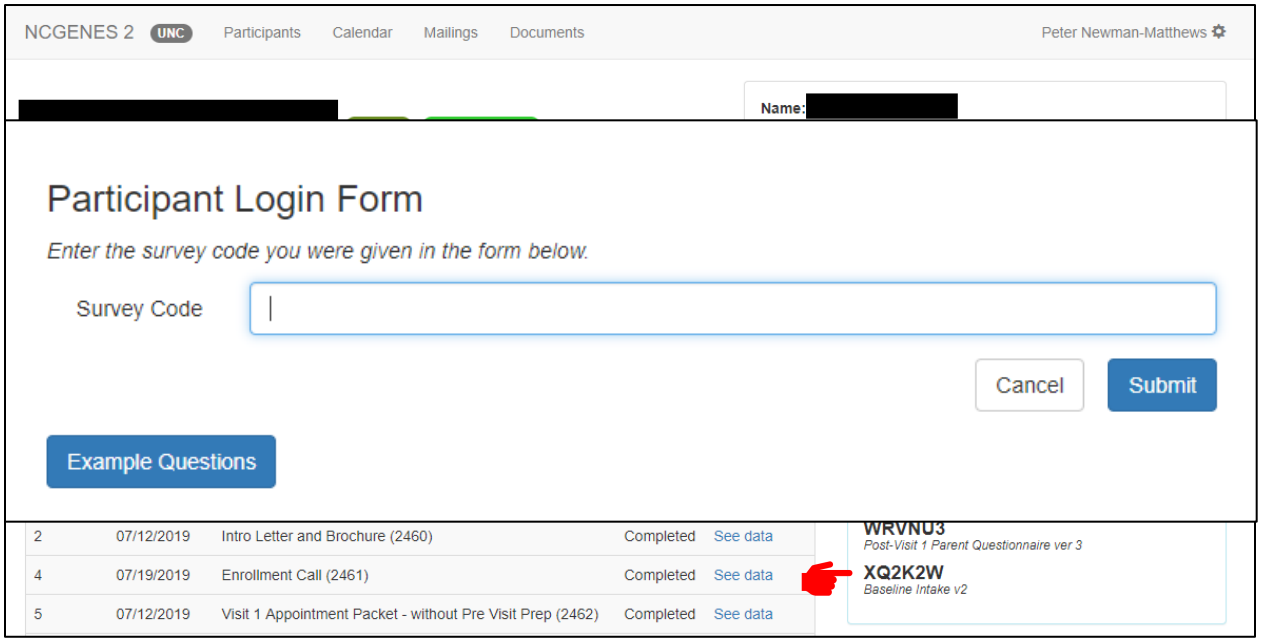
iii. Indicate whether the participant will complete the survey at the clinic or at home. If at home, provide the parent with a prepaid envelope as prompted. In any case, click the blue “Finish & Finalize” button to complete the task.

Baseline Intake

The Baseline Intake will in most cases be completed by the parent on paper before they arrive to Visit 1. After collecting the paper copy, a study staff member, usually the RA, will enter the survey information into the tracking system after the fact. In cases where the parent has not yet completed the Intake and time allows, the parent may fill out the survey on the tablet in the clinic before their visit. This step-by-step guide covers both scenarios.

Completion in the Tracking System

1. First copy the "Baseline Intake" code from the individual participant's page (shown below).



2. In a new window, navigate to the surveys version of the tracking system: ***https://ncgenes2.sirs.unc.edu/surveys***. The following screen will appear.

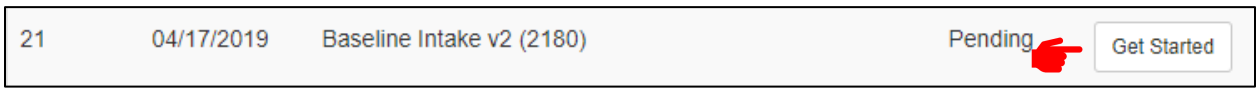
3. Paste the code into the text box, click the blue "Submit" button. Ensure any other tracking system windows in the browser are closed and hand the tablet over to the parent. When the parent finishes the survey, ensure that the blue "Finish & Finalize" button has been clicked. This will complete the task and redirect to the survey login page.

Entry of Paper Form in the Tracking System

If the parent completes the Baseline Intake on paper, it should be entered into the tracking system within 24 hours of receipt. The survey must be scanned and uploaded as well as entered via the task.[1]

1. First, upload a scan of the paper questionnaire to the **participant documents** page and label it "Baseline Intake."

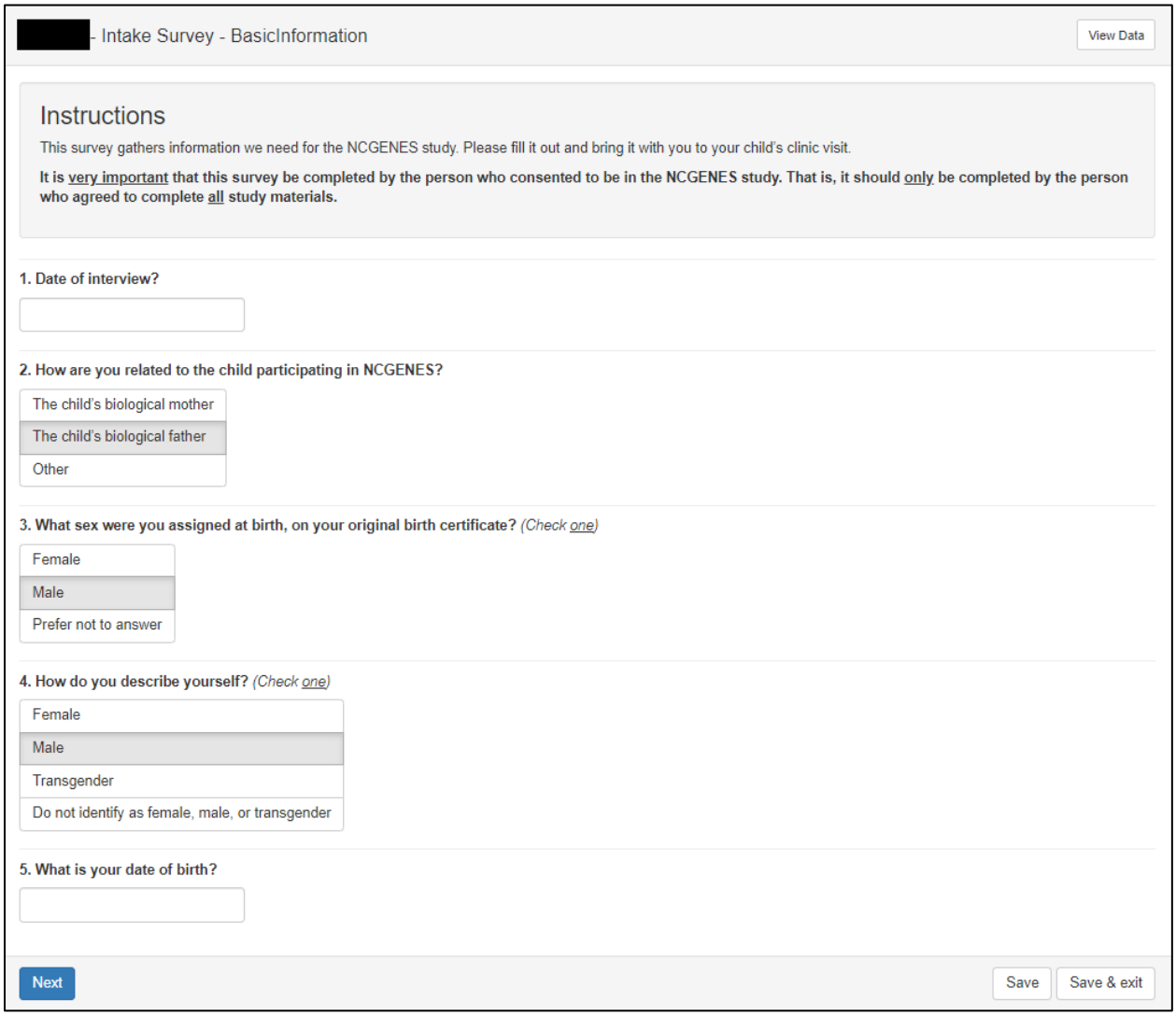
2. From the individual participant's page, click "Get Started" next to the "Baseline Intake" task, as shown below.



3. The following pages will appear in succession. After entering the parent's answers at each page, click the blue "Next" and repeat for subsequent pages. When all data is entered, click the blue "Finish & Finalize" button to complete the task (See **Page 15**).

[1] If you encounter any ambiguity in an answer to one of the survey questions that requires interpretation, please consult with the Clinical Study Director, Jeannette Bensen, by emailing her at Jeannette_bensen@med.unc.edu.

Page 1



[redacted] - Intake Survey - BasicInformation

View Data

Instructions

This survey gathers information we need for the NCGENES study. Please fill it out and bring it with you to your child's clinic visit.

It is very important that this survey be completed by the person who consented to be in the NCGENES study. That is, it should only be completed by the person who agreed to complete all study materials.

1. Date of interview?

2. How are you related to the child participating in NCGENES?

- The child's biological mother
- The child's biological father
- Other

3. What sex were you assigned at birth, on your original birth certificate? *(Check one)*

- Female
- Male
- Prefer not to answer

4. How do you describe yourself? *(Check one)*

- Female
- Male
- Transgender
- Do not identify as female, male, or transgender

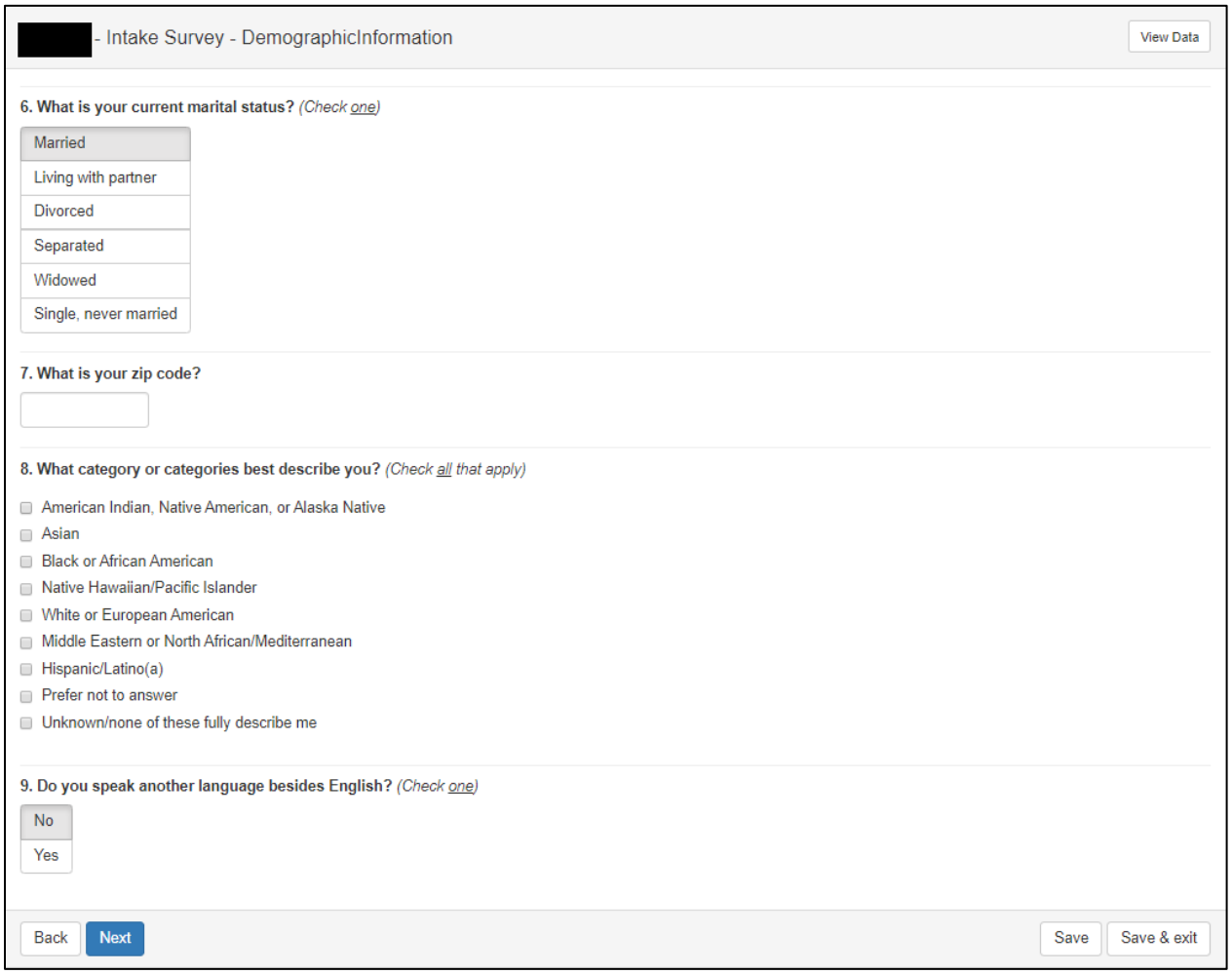
5. What is your date of birth?

Next

Save

Save & exit

Page 2



[redacted] - Intake Survey - DemographicInformation

View Data

6. What is your current marital status? *(Check one)*

- Married
- Living with partner
- Divorced
- Separated
- Widowed
- Single, never married

7. What is your zip code?

8. What category or categories best describe you? *(Check all that apply)*

- ☐ American Indian, Native American, or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Native Hawaiian/Pacific Islander
- ☐ White or European American
- ☐ Middle Eastern or North African/Mediterranean
- ☐ Hispanic/Latino(a)
- ☐ Prefer not to answer
- ☐ Unknown/none of these fully describe me

9. Do you speak another language besides English? *(Check one)*

- No
- Yes

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Page 3

[redacted] - Intake Survey - EmploymentInformation

View Data

10. Do you currently work for pay? *(Check one)*

- No
- Yes

11. What was your household's total family income (before taxes) from all sources in the last year? *(Check one)*

- $5,000 to $9,999
- $10,000 to $14,999
- $15,000 to $19,999
- $20,000 to $24,999
- $25,000 to $29,999
- $30,000 to $39,999
- $40,000 to $49,999
- $50,000 to $59,999
- $60,000 to $69,999
- $70,000 to $79,999
- $80,000 to $99,999
- $100,000 to $119,999
- $120,000 to $139,999
- $140,000 or more

12. How many people (children and adults) were supported by this income in the last year?

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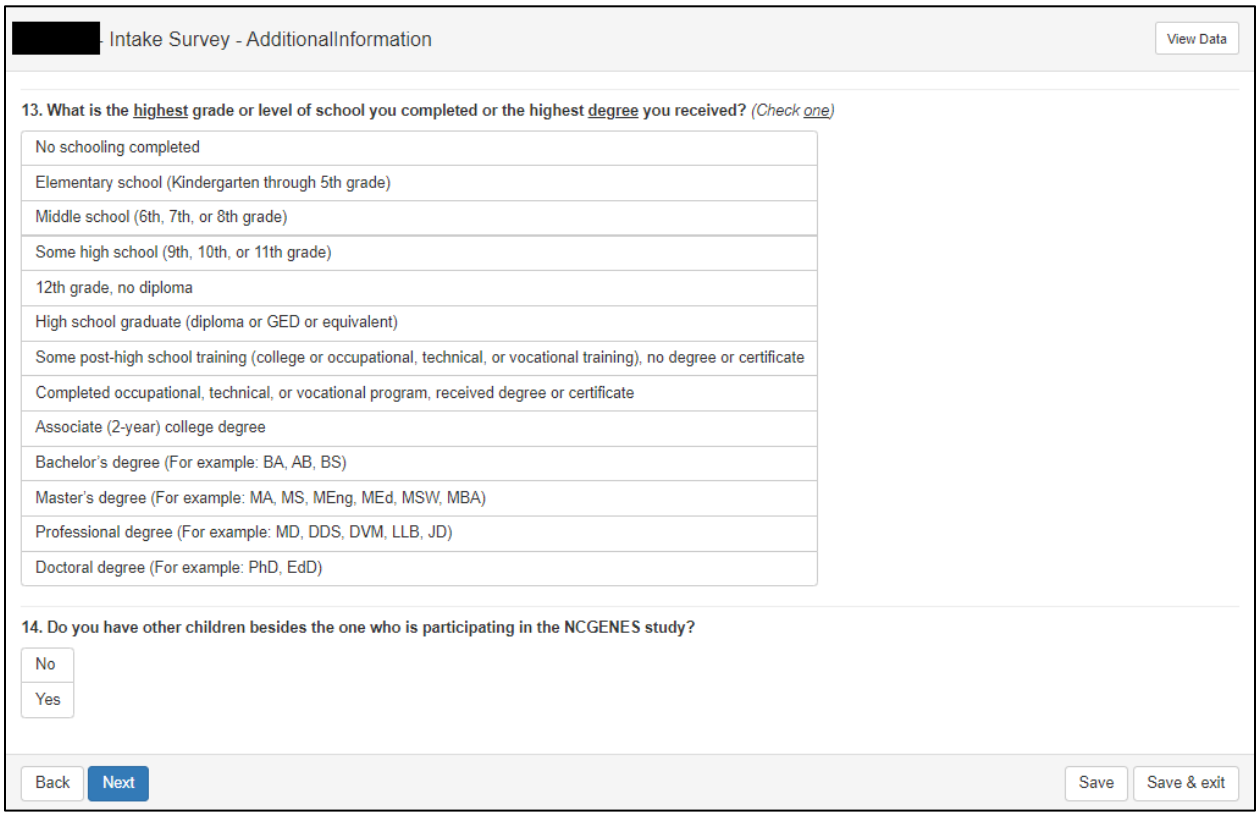

Question 14 **refers to all persons the respondent considers to be their child, biological or otherwise.

Page 5

[redacted] - Intake Survey - ChildBasicInformation

View Data

15. What is your child's gender? *(Check one)*

- Female
- Male
- Transgender
- Something else
- Prefer not to answer

16. What is your child's date of birth?

07/11/2018

17. What category or categories best describe your child? *(Check all that apply)*

- [ ] American Indian, Native American, or Alaska Native
- [ ] Asian
- [ ] Black or African American
- [ ] Native Hawaiian/Pacific Islander
- [ ] White or European American
- [ ] Middle Eastern or North African/Mediterranean
- [ ] Hispanic/Latino(a)
- [ ] Prefer not to answer
- [ ] Unknown/none of these fully describe my child

18. What is your child's primary spoken language? *(Check one)*

- English
- Spanish
- Other
- Not applicable

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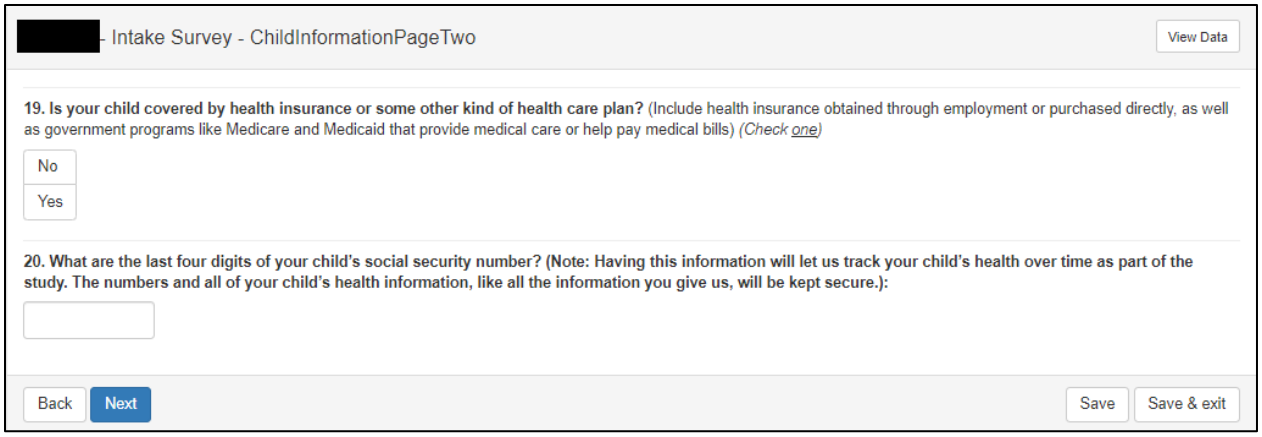
Page 6



[redacted] - Intake Survey - ChildInformationPageTwo

View Data

19. Is your child covered by health insurance or some other kind of health care plan? (Include health insurance obtained through employment or purchased directly, as well as government programs like Medicare and Medicaid that provide medical care or help pay medical bills) *(Check one)*

- No
- Yes

20. What are the last four digits of your child's social security number? (Note: Having this information will let us track your child's health over time as part of the study. The numbers and all of your child's health information, like all the information you give us, will be kept secure.):

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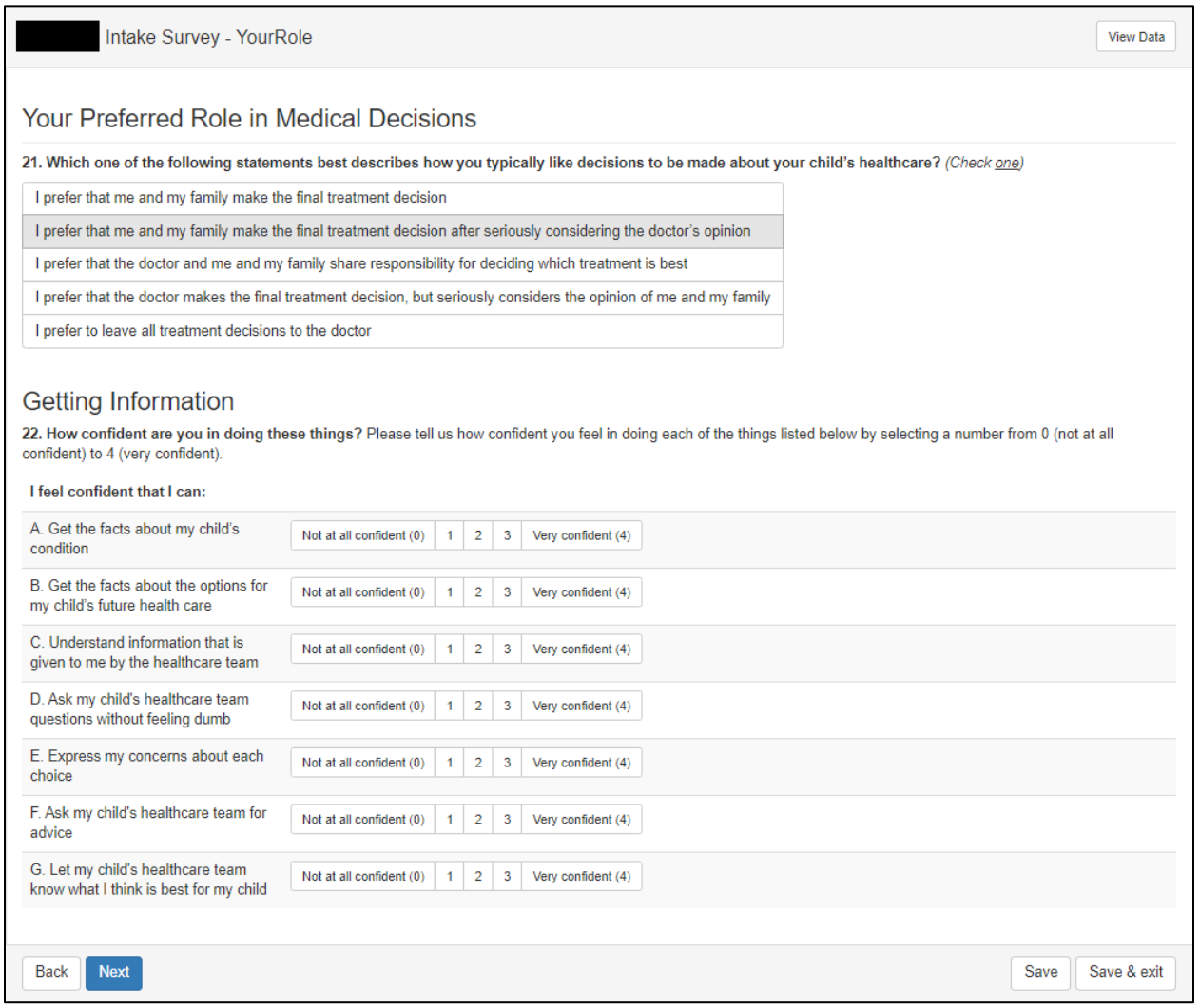
Page 7



[redacted] Intake Survey - YourRole

View Data

Your Preferred Role in Medical Decisions

21. Which one of the following statements best describes how you typically like decisions to be made about your child's healthcare? *(Check one)*

- I prefer that me and my family make the final treatment decision
- I prefer that me and my family make the final treatment decision after seriously considering the doctor's opinion
- I prefer that the doctor and me and my family share responsibility for deciding which treatment is best
- I prefer that the doctor makes the final treatment decision, but seriously considers the opinion of me and my family
- I prefer to leave all treatment decisions to the doctor

Getting Information

22. How confident are you in doing these things? Please tell us how confident you feel in doing each of the things listed below by selecting a number from 0 (not at all confident) to 4 (very confident).

I feel confident that I can:

	Not at all confident (0)	1	2	3	Very confident (4)
A. Get the facts about my child's condition					
B. Get the facts about the options for my child's future health care					
C. Understand information that is given to me by the healthcare team					
D. Ask my child's healthcare team questions without feeling dumb					
E. Express my concerns about each choice					
F. Ask my child's healthcare team for advice					
G. Let my child's healthcare team know what I think is best for my child					

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[redacted] - Intake Survey - HealthcareandYourFamily

View Data

Healthcare and Your Family

23. How long have you been searching for an answer to your child's symptoms?

24. Has there been any time in the last 12 months when you wanted or needed your child to see a doctor or health care professional and did not?

- No
- Yes

25. In the past 3 months, how much work have you missed because of your child's condition or treatments? *(Check one)*

- None
- Less than a week
- Between 1 and 4 weeks
- Between 4 and 8 weeks
- Between 8 and 12 weeks
- I stopped working altogether

26. In the past 3 months, how much did your child's condition or treatments make it hard to finish your normal work (including both work outside of the home and housework)? *(Check one)*

- Not at all
- A little bit
- Somewhat
- Quite a bit
- Very much

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[redacted] - Intake Survey - HealthcareandYourFamilyPageTwo

View Data

27. Please read each statement below and check the box that indicates your response as it applies to the past 7 days.

A. I know that I have enough money in savings, retirement, or assets to cover the cost of my child's treatment.	Not at all	A little bit	Somewhat	Quite a bit	Very much
B. My out-of-pocket medical expenses are more than I thought they would be.	Not at all	A little bit	Somewhat	Quite a bit	Very much
C. I worry about the financial problems I will have in the future as a result of my child's condition or treatment.	Not at all	A little bit	Somewhat	Quite a bit	Very much
D. I feel I have no choice about the amount of money I spend on care.	Not at all	A little bit	Somewhat	Quite a bit	Very much
E. I am frustrated that I cannot work or contribute as much as I usually do.	Not at all	A little bit	Somewhat	Quite a bit	Very much
F. I am satisfied with my current financial situation.	Not at all	A little bit	Somewhat	Quite a bit	Very much
G. I am able to meet my monthly expenses.	Not at all	A little bit	Somewhat	Quite a bit	Very much
H. I feel financially stressed.	Not at all	A little bit	Somewhat	Quite a bit	Very much
I. I am concerned about keeping my job and income, including work at home.	Not at all	A little bit	Somewhat	Quite a bit	Very much
J. My child's condition or treatment have reduced my satisfaction with my present financial situation.	Not at all	A little bit	Somewhat	Quite a bit	Very much
K. I feel in control of my financial situation.	Not at all	A little bit	Somewhat	Quite a bit	Very much

Back | Next | Save | Save & exit

[redacted] - Intake Survey - MedicalCare

View Data

Medical Care

28. These questions are about how you feel. There is no right or wrong answer, and your responses are confidential. Please read each statement below and check the box that indicates how much you agree or disagree with it.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
A. Doctors and health care workers sometimes hide information from patients who belong to my ethnic group.					
B. Doctors have the best interests of people of my ethnic group in mind.					
C. People of my ethnic group should not confide in doctors and health care workers because it will be used against them.					
D. People of my ethnic group should be suspicious of information from doctors and health care workers.					
E. People of my ethnic group cannot trust doctors and health care workers.					
F. People of my ethnic group should be suspicious of modern medicine.					
G. Doctors and health care workers treat people of my ethnic group like "guinea pigs."					
H. People of my ethnic group receive the same medical care from doctors and health care workers as people from other groups.					
I. Doctors and health care workers do not take the medical complaints of people of my ethnic group seriously."					
J. People of my ethnic group are treated the same as people of other groups by doctors and health care workers.					
K. In most hospitals, people of different ethnic groups receive the same kind of care.					
L. I have personally been treated poorly or unfairly by doctors or health care workers because of my ethnicity.					

Medical Care

29. Thinking about how much you TRUST your child's doctors, how strongly do you agree or disagree with each of the following statements. Remember that your answers are confidential.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
A. I can tell my child's doctors anything.					
B. My child's doctors sometimes pretend to know things when they are really not sure.					
C. I completely trust my child's doctors' judgement about my child's medical care.					
D. My child's doctors care more about holding costs down than about doing what is needed for my child's health.					
E. My child's doctors would always tell me the truth about my child's health, even if there was bad news.					
F. My child's doctors care as much as I do about my child's health.					
G. If a mistake was made in my child's treatment, my child's doctors would try to hide it from me.					

H. All things considered, how much do you trust your child's doctors? *(Check one number from 0 (Not at all) to 10 (Completely)*

0 (Not at all)	1	2	3	4	5	6	7	8	9	10

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[redacted] - Intake Survey - Genes

View Data

Instructions

These questions will help us learn what information NCGENES participants need to understand their test results.

Before you begin, you should know that we are using the term "***gene variant***" to mean a version of a gene. Sometimes two people have the same version of the gene (that is, they have the same gene variant) and other times two people have different versions of a gene (that is, they have different gene variants).

The following statements are either true or false. For each statement, circle the response ("True" or "False") that you think is correct, or circle "Not Sure/Don't Know."

Please answer all of these questions. ***Don't worry if you don't know the right answers!*** We do not expect you to get them all right. Your answers will help us develop the right educational materials for families like yours.

Information About Genes

1. Genes are made of DNA.	True	False	Not sure/ don't know
2. Genes affect health by influencing the proteins our bodies make.	True	False	Not sure/ don't know
3. All of a person's genetic information is called his or her "genome."	True	False	Not sure/ don't know
4. A person's genes change completely every 7 years.	True	False	Not sure/ don't know
5. The DNA in a gene is made of four building blocks (A, C, T, and G).	True	False	Not sure/ don't know
6. Everyone has about 20,000 to 25,000 genes.	True	False	Not sure/ don't know

Genes and Health

7. Gene variants can have positive effects, harmful effects, or no effects on health.	True	False	Not sure/ don't know
8. Most gene variants will affect a person's health.	True	False	Not sure/ don't know
9. Everyone who has a harmful gene variant will eventually have symptoms.	True	False	Not sure/ don't know
10. Some gene variants have a large effect on health while others have a small effect.	True	False	Not sure/ don't know
11. Some gene variants decrease the chance of developing a disorder.	True	False	Not sure/ don't know
12. Two unrelated people with the same genetic variant will always have the same symptoms.	True	False	Not sure/ don't know

How Genes Are Inherited in Families

13. Genetic disorders are always inherited from a parent.	True	False	Not sure/ don't know
14. If only one person in the family has a disorder it can't be genetic.	True	False	Not sure/ don't know
15. Everyone has a chance for having a child with a genetic disorder.	True	False	Not sure/ don't know
16. A girl inherits most of her genes from her mother while a boy inherits most of his genes from his father.	True	False	Not sure/ don't know
17. A mother and daughter who look alike are more genetically similar than a mother and daughter who do not look alike.	True	False	Not sure/ don't know
18. If a parent has a harmful gene variant, all of his or her children will inherit it.	True	False	Not sure/ don't know
19. If one of your parents has a gene variant, your brother or sister may also have it.	True	False	Not sure/ don't know

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[redacted] - Intake Survey - WorkingWithHealthInformation

View Data

30. How often do you have someone (like a family member, friend, hospital/clinic worker, or caregiver) help you read medical materials? *(Check one)*

- Always
- Often
- Sometimes
- Occasionally
- Never

31. How often do you have problems learning about your medical conditions because of difficulty understanding written information? *(Check one)*

- Always
- Often
- Sometimes
- Occasionally
- Never

32. How often do you have a problem understanding what is told to you about your medical condition? *(Check one)*

- Always
- Often
- Sometimes
- Occasionally
- Never

33. How confident are you filling out medical forms by yourself? *(Check one)*

- Not at all
- A little bit
- Somewhat
- Quite a bit
- Extremely

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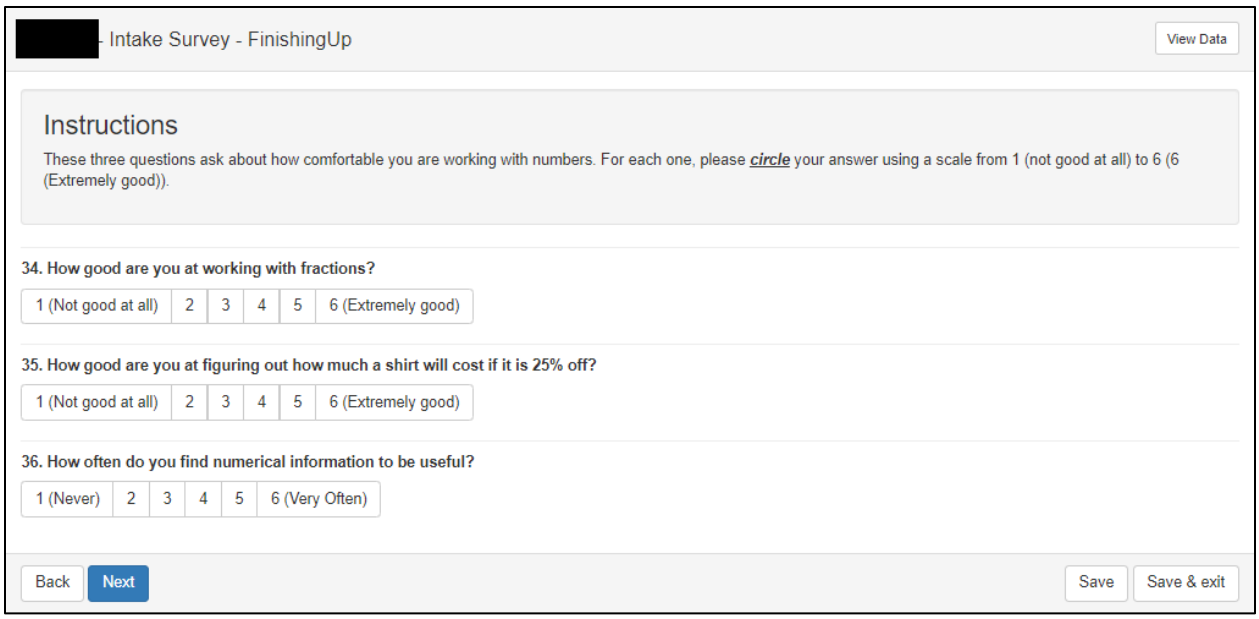
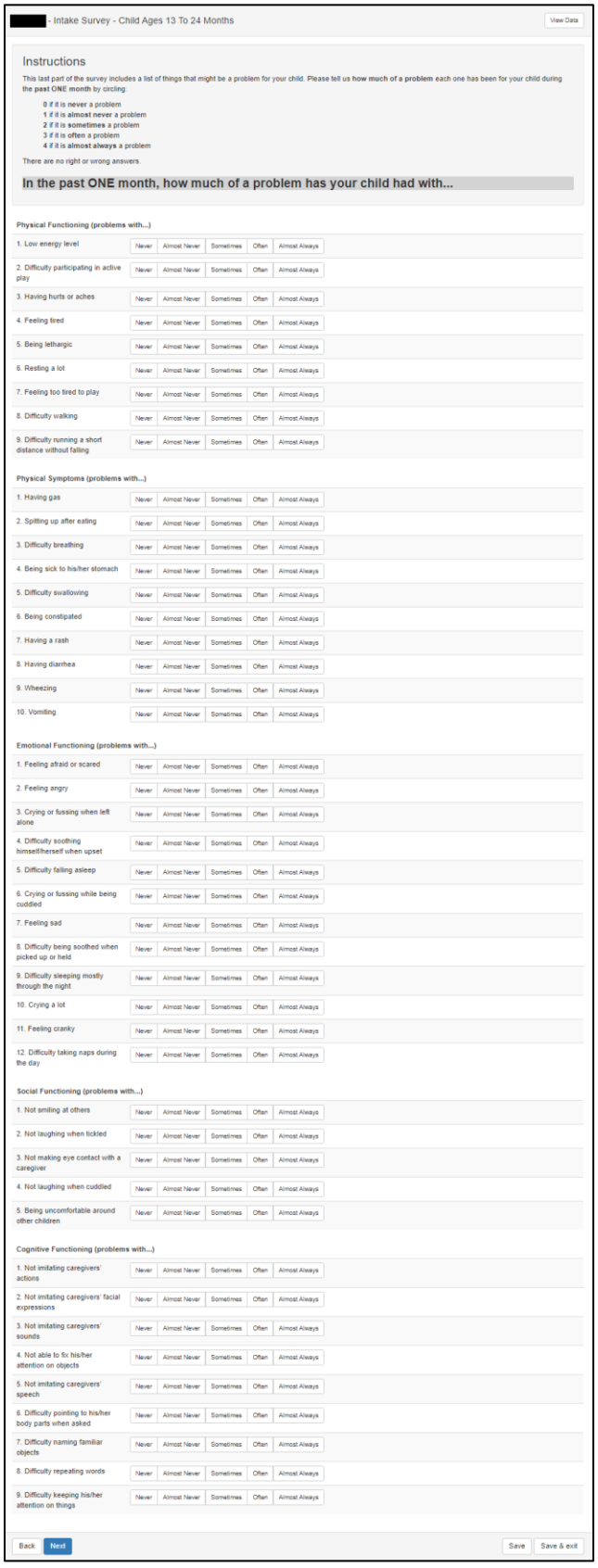

[redacted] - Intake Survey - FinishingUp
View Data
Instructions
These three questions ask about how comfortable you are working with numbers. For each one, please circle your answer using a scale from 1 (not good at all) to 6 (6 (Extremely good)).
34. How good are you at working with fractions?
1 (Not good at all) 2 3 4 5 6 (Extremely good)
35. How good are you at figuring out how much a shirt will cost if it is 25% off?
1 (Not good at all) 2 3 4 5 6 (Extremely good)
36. How often do you find numerical information to be useful?
1 (Never) 2 3 4 5 6 (Very Often)
Back
Next
Save
Save & exit



[redacted] - Intake Survey - Child Ages 13 To 24 Months

View Data

Instructions

This last part of the survey includes a list of things that might be a problem for your child. Please tell us **how much of a problem** each one has been for your child during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

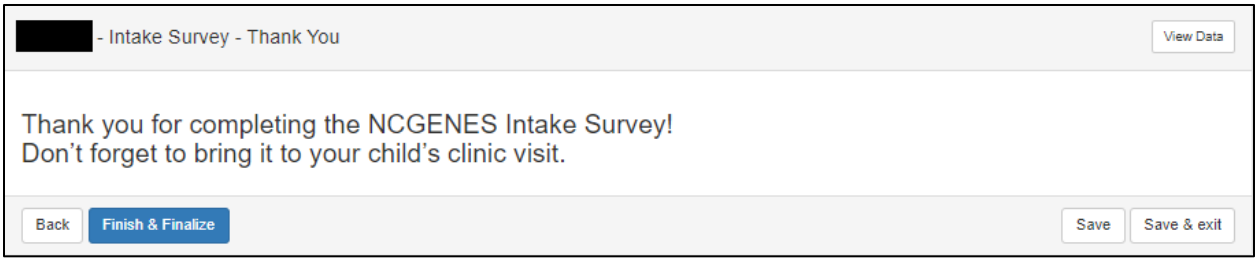
There are no right or wrong answers.

In the past ONE month, how much of a problem has your child had with...

	Never	Almost Never	Sometimes	Often	Almost Always
Physical Functioning (problems with...)					
1. Low energy level					
2. Difficulty participating in active play					
3. Having hurts or aches					
4. Feeling tired					
5. Being lethargic					
6. Resting a lot					
7. Feeling too tired to play					
8. Difficulty walking					
9. Difficulty running a short distance without falling					
Physical Symptoms (problems with...)					
1. Having gas					
2. Spitting up after eating					
3. Difficulty breathing					
4. Being sick to his/her stomach					
5. Difficulty swallowing					
6. Being constipated					
7. Having a rash					
8. Having diarrhea					
9. Wheezing					
10. Vomiting					
Emotional Functioning (problems with...)					
1. Feeling afraid or scared					
2. Feeling angry					
3. Crying or fussing when left alone					
4. Difficulty soothing himself/herself when upset					
5. Difficulty falling asleep					
6. Crying or fussing while being cuddled					
7. Feeling sad					
8. Difficulty being soothed when picked up or held					
9. Difficulty sleeping mostly through the night					
10. Crying a lot					
11. Feeling cranky					
12. Difficulty taking naps during the day					
Social Functioning (problems with...)					
1. Not smiling at others					
2. Not laughing when tickled					
3. Not making eye contact with a caregiver					
4. Not laughing when cuddled					
5. Being uncomfortable around other children					
Cognitive Functioning (problems with...)					
1. Not imitating caregivers' actions					
2. Not imitating caregivers' facial expressions					
3. Not imitating caregivers' sounds					
4. Not able to fix his/her attention on objects					
5. Not imitating caregivers' speech					
6. Difficulty pointing to his/her body parts when asked					
7. Difficulty naming familiar objects					
8. Difficulty repeating words					
9. Difficulty keeping his/her attention on things					

Back Next

Save Save & exit


[redacted] - Intake Survey - Thank You
View Data
Thank you for completing the NCGENES Intake Survey!
Don't forget to bring it to your child's clinic visit.
Back
Finish & Finalize
Save
Save & exit

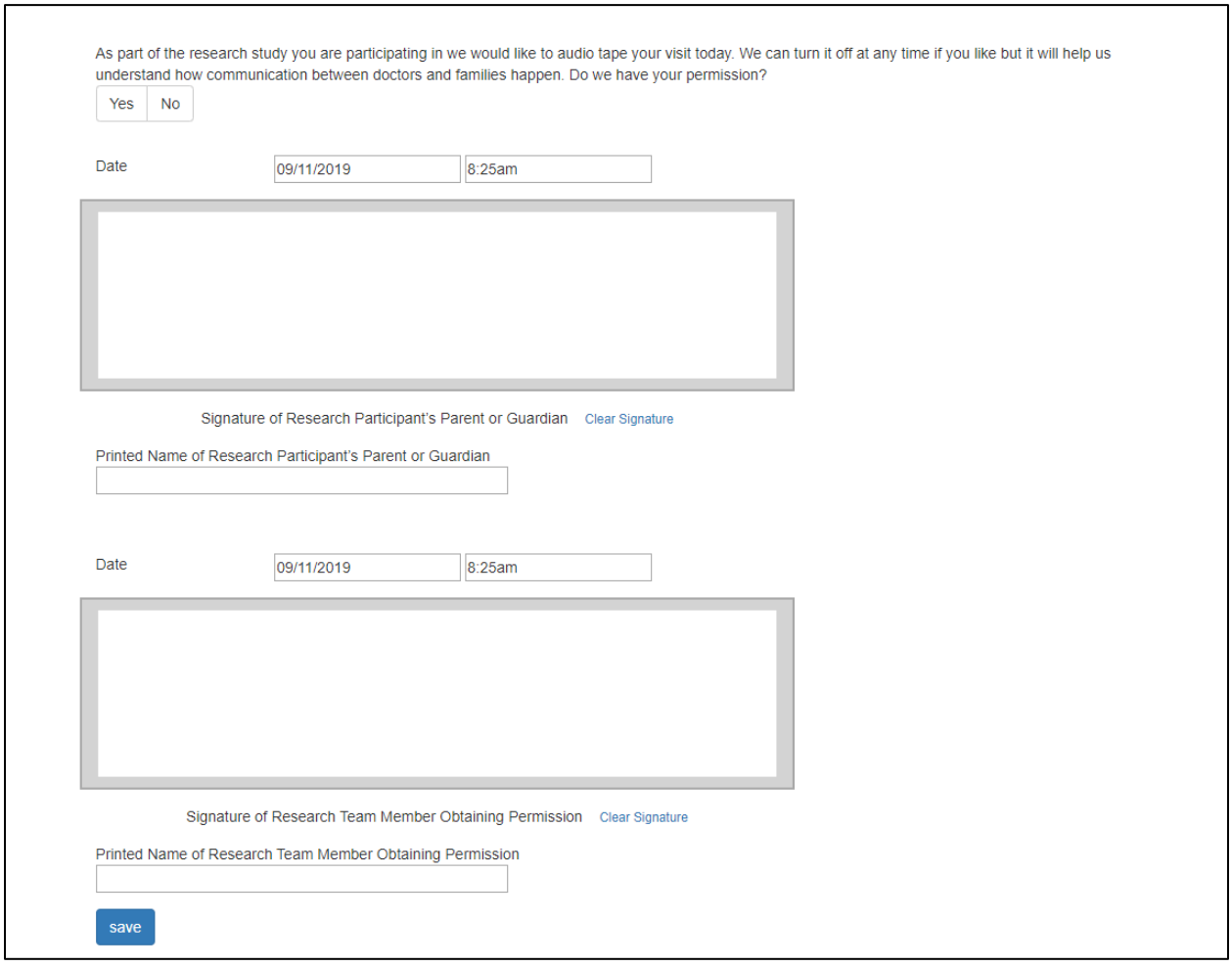
Parent Permission to Audio Tape Clinic Visit

1. To begin the "Parent Permission to Audio Tape Clinic Visit" task from the participant page, click the "Get Started" button next to the task (shown below).

2. After reading the initial prompt on the page shown below, record the parent's response ("Yes" or "No") to the request to audiotape.

   a. If the parent declines, skip the signatures and click the blue "save" button to complete the task.

   b. If the parent consents, the parent and study staff should sign in the appropriate boxes, and the names of each should be printed below each signature. Once this is done, click the blue "save" button to complete the task.

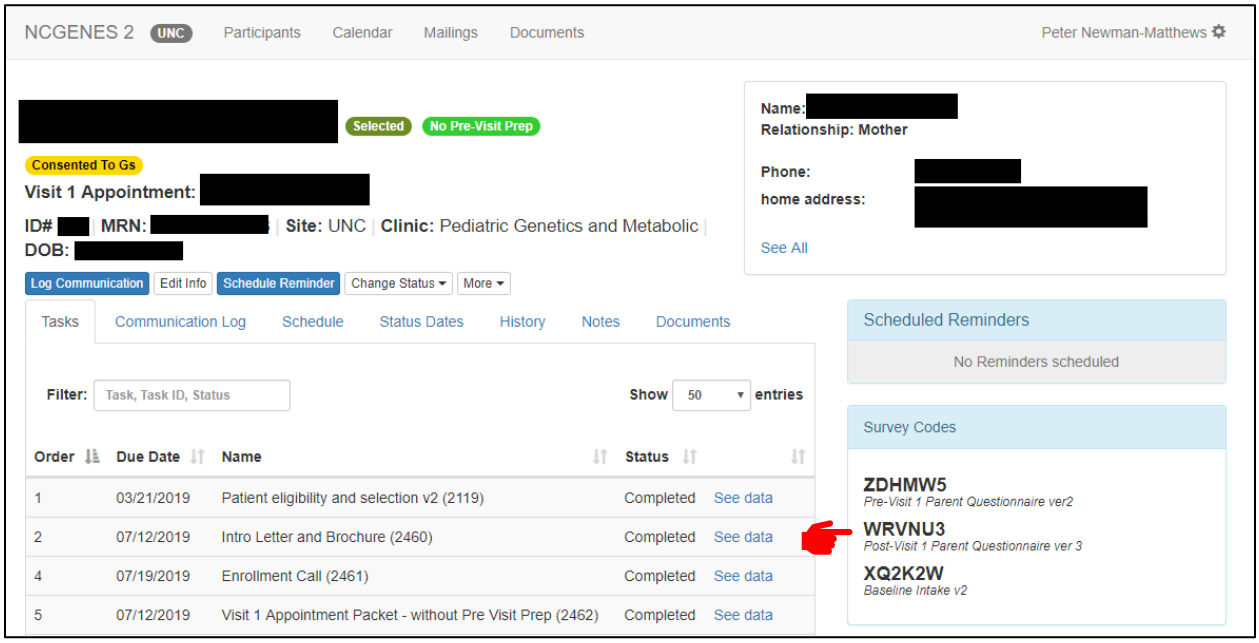


Post-Visit 1 Parent Questionnaire

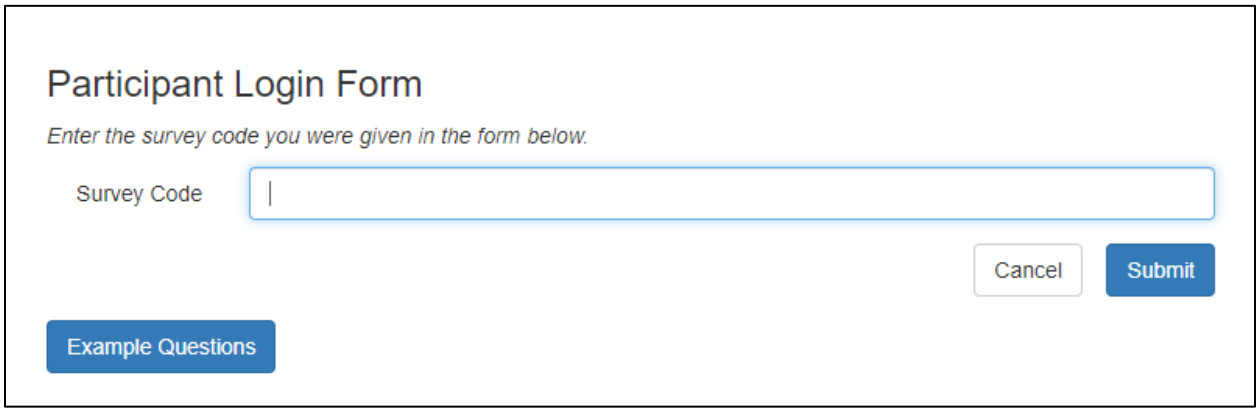
The Post-Visit 1 Parent Questionnaire will in most cases be administered in the clinic and completed by the parent themselves. In cases where the tracking system is not accessible in clinic, the parent may fill out the survey on paper and a study staff member, usually the RA, will enter the survey information into the tracking system after the fact. This step-by-step guide covers both scenarios.

Completion in the Tracking System

1. First copy the "Post-Visit 1 Parent Questionnaire" code from the individual participant's page (Shown Below).
2. In a new window, navigate to the surveys version of the tracking system: ***https://ncgenes2.sirs.unc.edu/surveys***.



The following screen will appear.



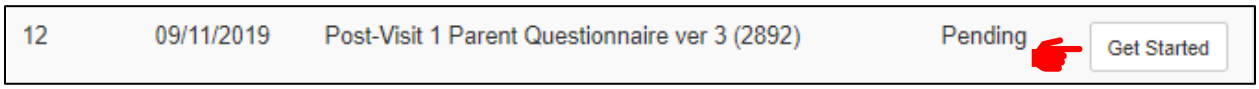
3. Paste the code into the text box, click the blue "Submit" button. Ensure any other tracking system windows in the browser are closed and hand the tablet over to the parent. When the parent finishes the survey, ensure that the blue "Finish & Finalize" button has been clicked. This will complete the task and redirect to the survey login page.

Entry of Paper Form in the Tracking System

If the parent completes the Post-Visit 1 Questionnaire on paper, it should be entered into the tracking system within 24 hours. The survey must be scanned and uploaded as well as entered via the task.[1]

1. First, upload a scan of the paper questionnaire to the **participant documents** page and label it "Post-Visit 1 Parent Questionnaire."

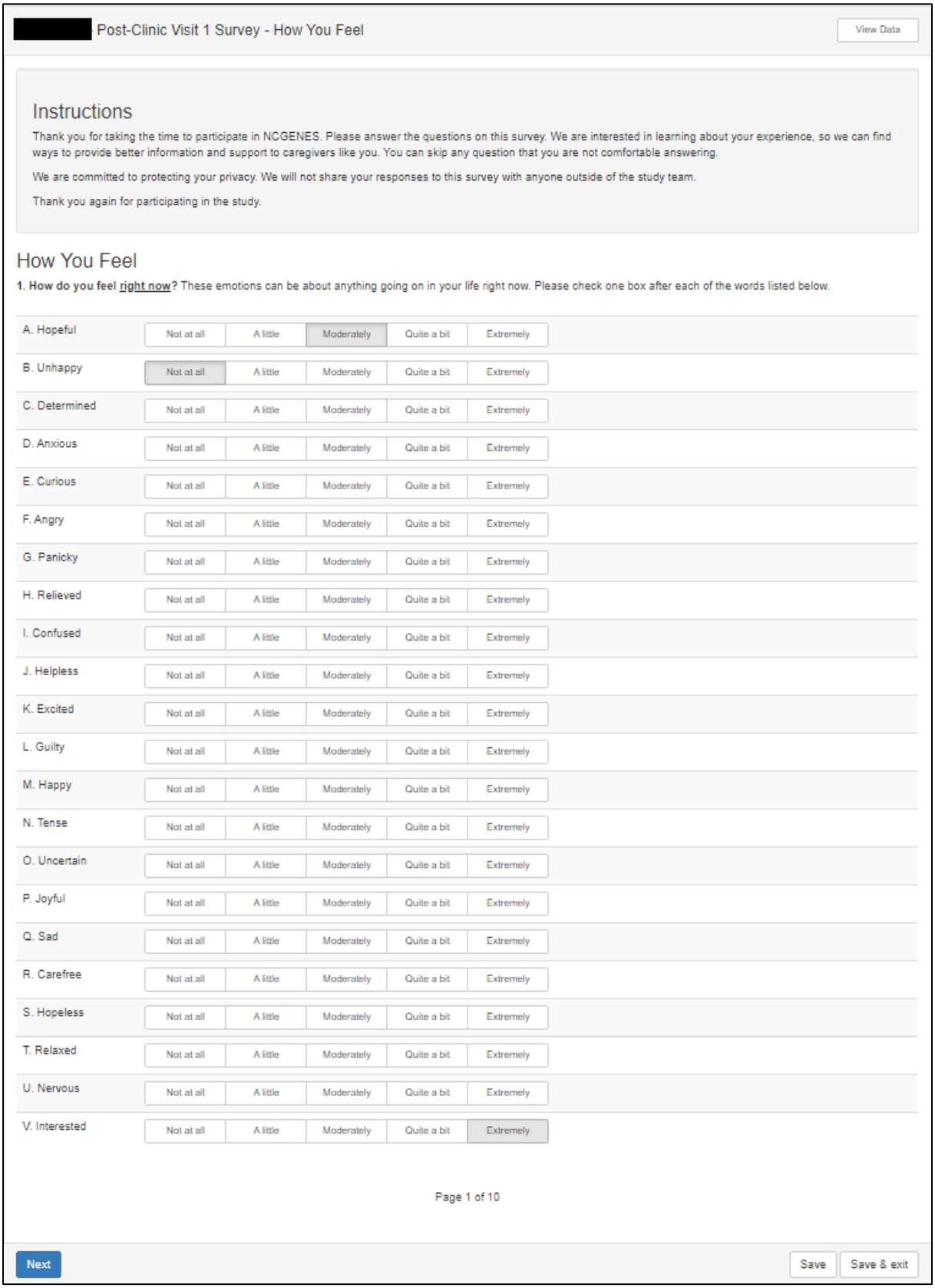
2. From the individual participant's page, click "Get Started" next to the Post-Visit 1 Parent Questionnaire task, as shown below.



3. The following screens will appear in succession. After entering the parent's answers at each page, click the blue "Next" and repeat for subsequent screens. When all data is entered, click the blue "Finish & Finalize" button to complete the task (See **Page 10**).

[1] If you encounter any ambiguity in an answer to one of the survey questions that requires interpretation, please consult with the Clinical Study Director, Jeannette Bensen, by emailing her at Jeannette_bensen@med.unc.edu.

Page 1



[redacted] Post-Clinic Visit 1 Survey - How You Feel

View Data

Instructions

Thank you for taking the time to participate in NCGENES. Please answer the questions on this survey. We are interested in learning about your experience, so we can find ways to provide better information and support to caregivers like you. You can skip any question that you are not comfortable answering.

We are committed to protecting your privacy. We will not share your responses to this survey with anyone outside of the study team.

Thank you again for participating in the study.

How You Feel

1. How do you feel right now? These emotions can be about anything going on in your life right now. Please check one box after each of the words listed below.

	Not at all	A little	Moderately	Quite a bit	Extremely
A. Hopeful			■		
B. Unhappy	■				
C. Determined					
D. Anxious					
E. Curious					
F. Angry					
G. Panicky					
H. Relieved					
I. Confused					
J. Helpless					
K. Excited					
L. Guilty					
M. Happy					
N. Tense					
O. Uncertain					
P. Joyful					
Q. Sad					
R. Carefree					
S. Hopeless					
T. Relaxed					
U. Nervous					
V. Interested					■

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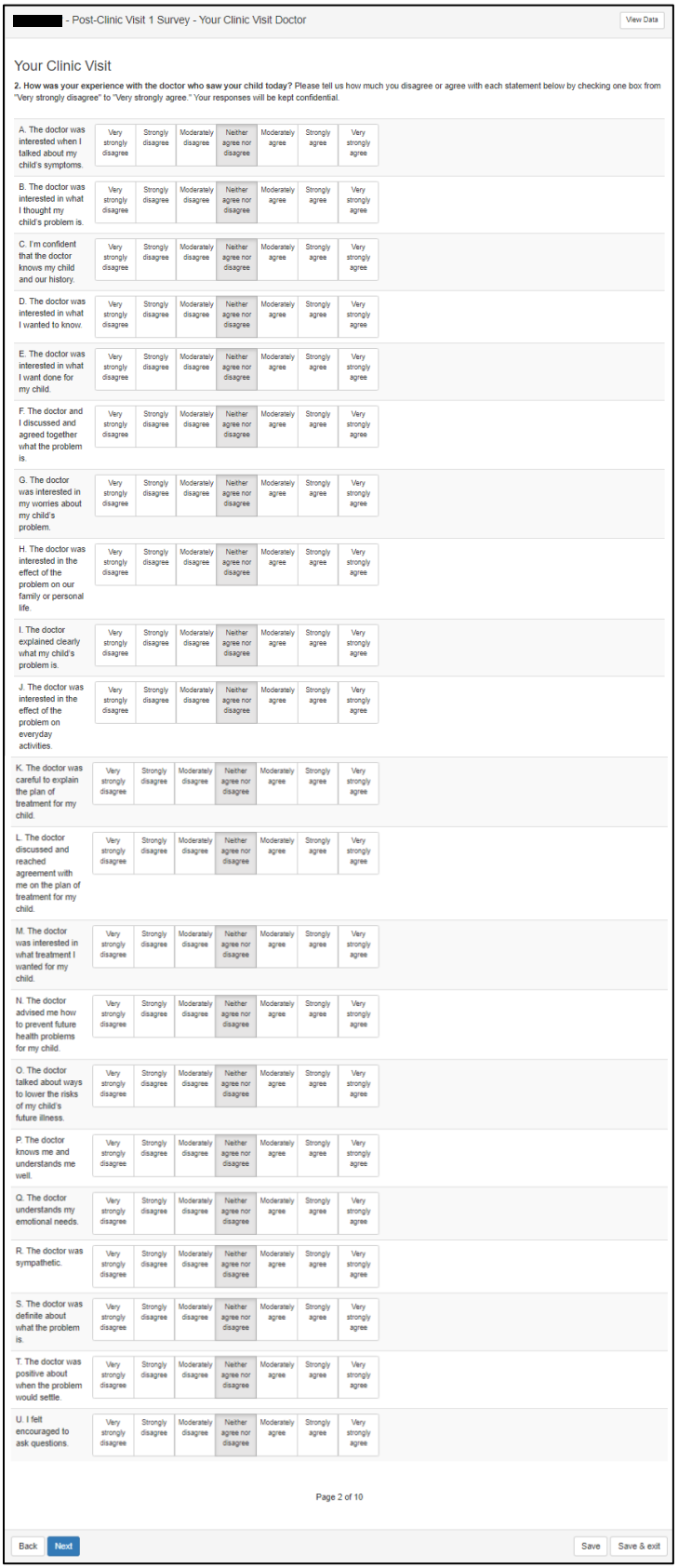
Next

Save

Save & exit

Page 2



[redacted] - Post-Clinic Visit 1 Survey - Your Clinic Visit Doctor

View Data

Your Clinic Visit

2. How was your experience with the doctor who saw your child today? Please tell us how much you disagree or agree with each statement below by checking one box from "Very strongly disagree" to "Very strongly agree." Your responses will be kept confidential.

	Very strongly disagree	Strongly disagree	Moderately disagree	Neither agree nor disagree	Moderately agree	Strongly agree	Very strongly agree
A. The doctor was interested when I talked about my child's symptoms.							
B. The doctor was interested in what I thought my child's problem is.							
C. I'm confident that the doctor knows my child and our history.							
D. The doctor was interested in what I wanted to know.							
E. The doctor was interested in what I want done for my child.							
F. The doctor and I discussed and agreed together what the problem is.							
G. The doctor was interested in my worries about my child's problem.							
H. The doctor was interested in the effect of the problem on our family or personal life.							
I. The doctor explained clearly what my child's problem is.							
J. The doctor was interested in the effect of the problem on everyday activities.							
K. The doctor was careful to explain the plan of treatment for my child.							
L. The doctor discussed and reached agreement with me on the plan of treatment for my child.							
M. The doctor was interested in what treatment I wanted for my child.							
N. The doctor advised me how to prevent future health problems for my child.							
O. The doctor talked about ways to lower the risks of my child's future illness.							
P. The doctor knows me and understands me well.							
Q. The doctor understands my emotional needs.							
R. The doctor was sympathetic.							
S. The doctor was definite about what the problem is.							
T. The doctor was positive about when the problem would settle.							
U. I felt encouraged to ask questions.							

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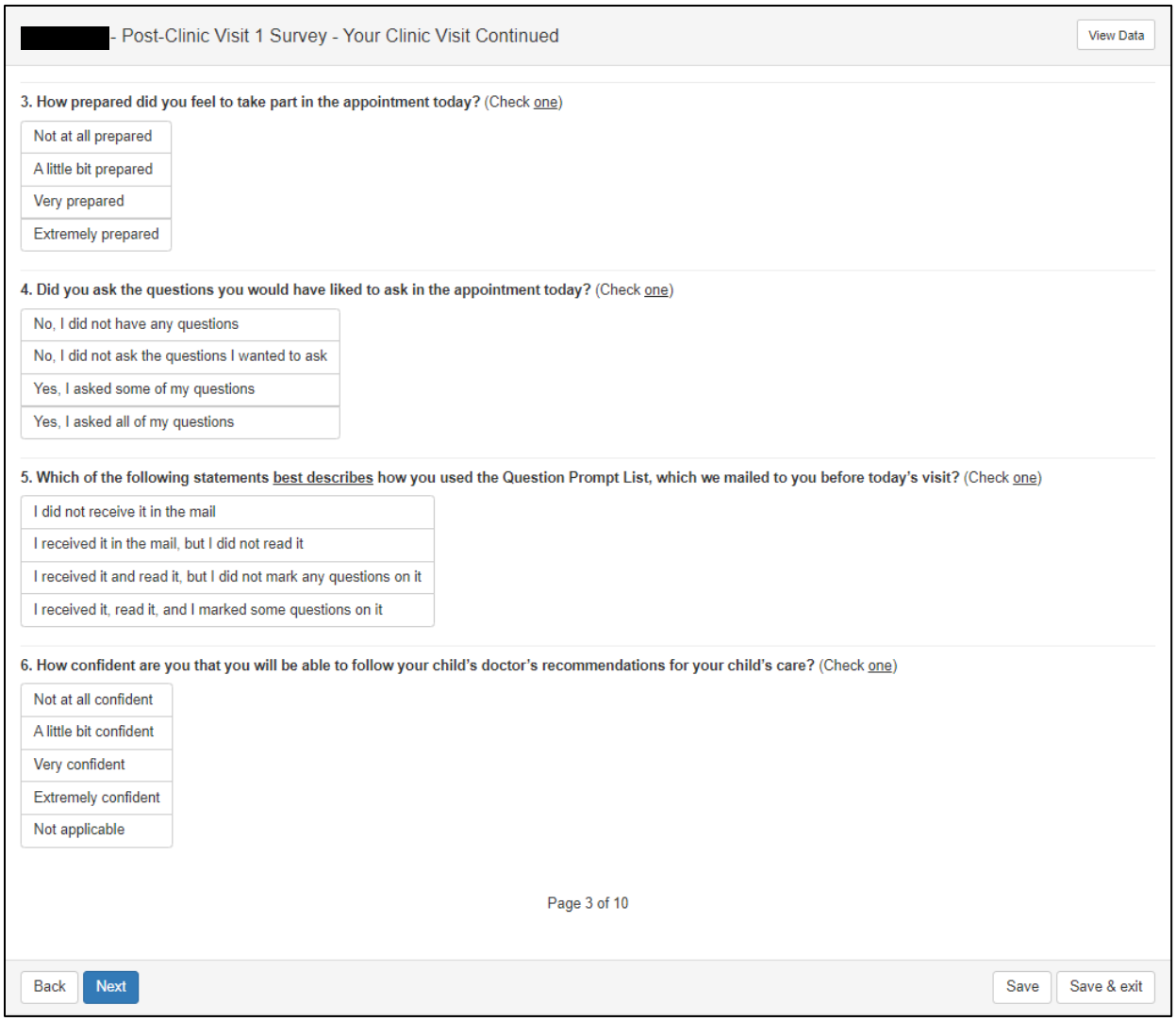
Page 3



[redacted] - Post-Clinic Visit 1 Survey - Your Clinic Visit Continued

View Data

3. How prepared did you feel to take part in the appointment today? (Check one)

- Not at all prepared
- A little bit prepared
- Very prepared
- Extremely prepared

4. Did you ask the questions you would have liked to ask in the appointment today? (Check one)

- No, I did not have any questions
- No, I did not ask the questions I wanted to ask
- Yes, I asked some of my questions
- Yes, I asked all of my questions

5. Which of the following statements best describes how you used the Question Prompt List, which we mailed to you before today's visit? (Check one)

- I did not receive it in the mail
- I received it in the mail, but I did not read it
- I received it and read it, but I did not mark any questions on it
- I received it, read it, and I marked some questions on it

6. How confident are you that you will be able to follow your child's doctor's recommendations for your child's care? (Check one)

- Not at all confident
- A little bit confident
- Very confident
- Extremely confident
- Not applicable

Page 3 of 10

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[redacted]- Post-Clinic Visit 1 Survey - Your Clinic Visit Recommended

View Data

7. Did your child's doctor that you saw today recommend or order any of the following for your child?

A start, stop, or change in medication (not including vitamins)?

- Start
- Stop
- Change (for example, stop taking one medication and start another one; or increase or decrease the dose or frequency)
- Not applicable

A start, stop or change to any non-genetic tests for screening, monitoring, or diagnosis (for example, blood test, imaging such as e-ray, MRI, etc.)

- Start
- Stop
- Change (for example, increase or decrease the frequency)
- Not applicable

Consult with other doctors or specialists

- No
- Yes
- Stop seeing other doctors or specialists
- Not applicable

Consult with one or more of the following non-MD health professionals (Check all that apply)

- [ ] Audiology
- [ ] Dental
- [ ] Genetic counselor
- [ ] Psychologist
- [ ] Other
- [ ] Stop seeing other non-MD health professional
- [ ] Not applicable

See someone for mental health support for your child (Check all that apply)

- [ ] Mental health
- [ ] Social support
- [ ] Palliative care
- [ ] Not applicable

See someone for therapeutic services for your child (Check all that apply)

- [ ] Speech therapy
- [ ] Occupational therapy
- [ ] Physical therapy
- [ ] Not applicable

Make lifestyle changes for your child (Check all that apply)

- [ ] Change diet
- [ ] Change exercise
- [ ] Start taking vitamins and supplements
- [ ] Change alcohol consumption, if your child uses alcohol
- [ ] Stop smoking, if your child smokes
- [ ] Other
- [ ] Not applicable

Other?

No | Yes

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[redacted] - Post-Clinic Visit 1 Survey - What Is Diagnostic Test

View Data

What is a "diagnostic test?"

A "diagnostic test" is any test or evaluation the doctor uses to help find the cause of your child's health condition. The medical word for finding this cause is "diagnosis." There are many kinds of "diagnostic tests." For example, some tests use blood, some use saliva (spit), and others use urine (pee). Other tests take pictures of your child's bones or organs, like an X-ray or MRI.

8. According to the doctor, what is the chance that your child's health concern is caused by their genes? (You can check any box along the scale)

Definitely not caused by their genes						Definitely caused by their genes
0	1	2	3	4	5	6

9. According to the doctor, how likely is it that the diagnostic testing the doctor is ordering will reveal the cause of your child's condition? (You can check any box along the scale)

Not at all						Definitely	Not applicable
0	1	2	3	4	5	6	7

10. According to the doctor, how likely is it that the diagnostic testing you discussed with the doctor will change how your child is being medically treated? (You can check any box along the scale)

Not at all						Definitely	Not applicable
0	1	2	3	4	5	6	7

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Page 6

[redacted] - Post-Clinic Visit 1 Survey - Beliefs

View Data

Sometimes we have different beliefs than our doctors based on things we know about our children and our families. Considering your beliefs, please answer the following questions.

11. Considering your beliefs now, not those of the doctor, what is the chance that your child's health concern is caused by their genes? (You can check any box along the scale)

Definitely not caused by their genes						Definitely caused by their genes
0	1	2	3	4	5	6

12. Considering your beliefs now, not those of the doctor, how likely is it that the diagnostic testing the doctor is ordering will reveal the cause of your child's condition? (You can check any box along the scale)

Not at all						Definitely	Not applicable
0	1	2	3	4	5	6	7

13. Considering your beliefs now, not those of the doctor, how likely is it that the diagnostic testing you discussed with the doctor will change how your child is being medically treated? (You can check any box along the scale)

Not at all						Definitely	Not applicable
0	1	2	3	4	5	6	7

14. Please read each statement below and check one box to tell us if you think the statement is true or false.

A. Diagnostic tests will always find the cause of a condition.	True	False
B. Diagnostic tests could have results that cannot be interpreted at the present time.	True	False
C. Diagnostic tests don't always have results that could improve one's health.	True	False

Back Next Save Save & exit

[redacted] - Post-Clinic Visit 1 Survey - Clinic Visit Discussions

View Data

Your Clinic Visit

15. Please read each statement below and check one box to tell us whether or not you discussed it with the doctor today:

In the appointment, the doctor and I discussed:

A. How to get the medical services my child needs	Yes	No
B. How to get the non-medical help my child needs (e.g., educational, financial, or social support)	Yes	No
C. How to get the support my child's caregiver(s), siblings, and family need (e.g., support group or counseling)	Yes	No
D. How to explain what the condition means to people outside my family who may need to know (e.g., teachers, social workers)	Yes	No
E. How to talk to my child about their condition	Yes	No
F. How to talk about my child's condition to other people	Yes	No
G. The drawbacks of each option for diagnosing my child's condition	Yes	No
H. The drawbacks of each option for treating and managing my child's condition	Yes	No
I. The benefits of each option for diagnosing my child's condition	Yes	No
J. The benefits of each option for treating and managing my child's condition	Yes	No
K. Whether my relatives may be at risk for this condition	Yes	No
L. The impact of the condition on my other child(ren) or any other child I may have	Yes	No
M. What to expect for my child in the future	Yes	No
N. How new medical knowledge or treatments that become available may change how this condition affects my child	Yes	No

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[redacted] - Post-Clinic Visit 1 Survey - Talking To Others

View Data

Talking to Others

Sometimes parents want or need to talk to other people about their child's diagnostic tests. These questions will help us understand the ways talking to others might be hard for parents.

16. How confident are you in explaining diagnostic testing to others? Please check one box for each person listed below.

I feel confident that I can explain diagnostic testing to:

	Not at all confident	A little confident	Somewhat confident	Very confident	Not applicable
A. My child					
B. My other children					
C. My family members					
D. My child's teachers or therapists					
E. Other doctors or specialists that treat my child					

17. How confident are you in explaining the kinds of diagnostic tests your child is having to others? Please check one box for each person listed below.

I feel confident that I can explain diagnostic testing to:

	Not at all confident	A little confident	Somewhat confident	Very confident	Not applicable
A. My child					☑
B. My other children					
C. My family members					
D. My child's teachers or therapists					
E. Other doctors or specialists that treat my child					

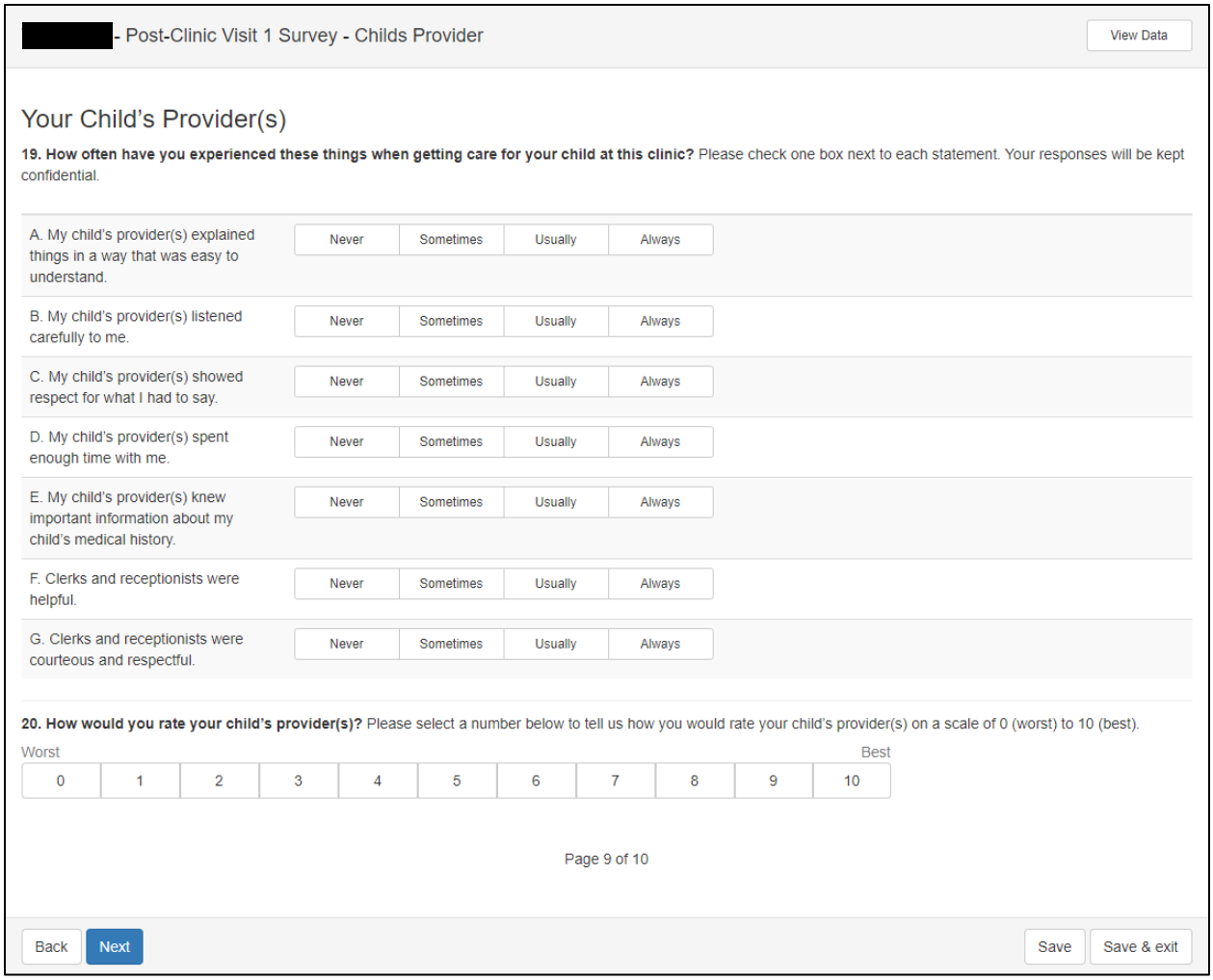
18. How stressful would it be to do following things? Please check one box for each person listed below.

How stressful would it be to:

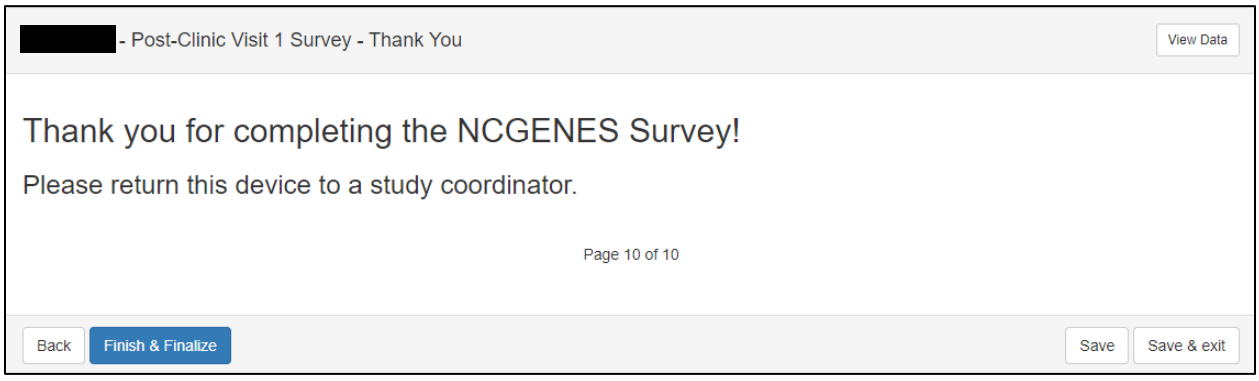
	Not at all stressful	A little stressful	Somewhat stressful	Very stressful	Not applicable
A. Explain what diagnostic testing is to other people?					
B. Explain the kinds of diagnostic tests your child is having to other people?					
C. Explain what steps other family members should take to learn whether they are at risk for a health problem like your child's?					

Back | Next | Save | Save & exit

Page 9



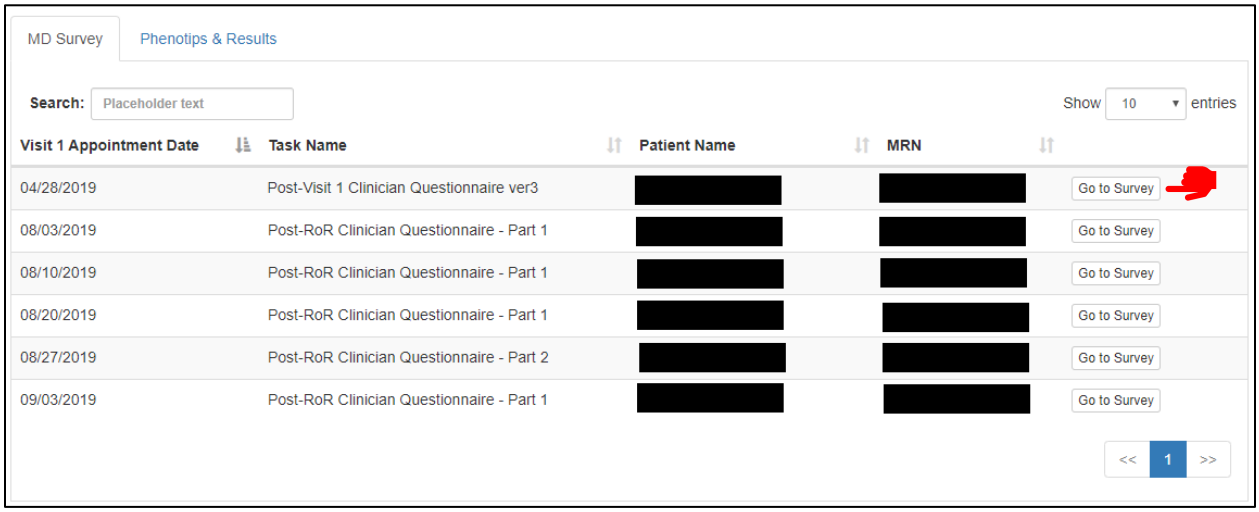
Page 10



Post-Visit 1 Clinician Questionnaire

The Post-Visit 1 Clinician Questionnaire should be completed by the relevant clinician after Visit 1. Study staff will, in most cases,[1] not be involved in the completion of this task. The following guide will serve as a reference for clinicians completing the Post-Visit 1 survey.

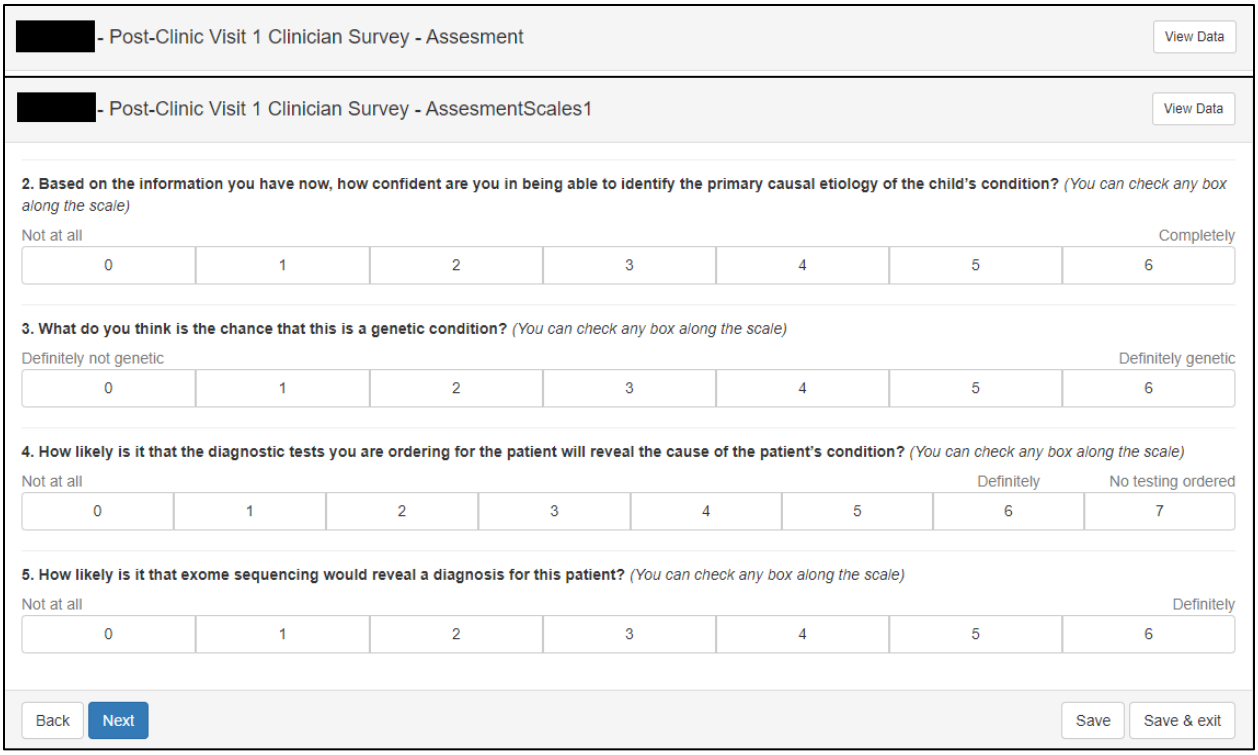
1. To begin the "Post-Visit 1 Clinician Questionnaire," click the appropriate "Go to Survey" button for the participant as it appears on the MD Surveys tab (shown below).



2. The survey consists of a series of pages (shown below). After completing the questions on each page, click the blue "Next" button in the bottom left-hand corner of the page. When you reach **the final page**, click the blue "Finish & Finalize" button to complete the survey.

[1] In extremely rare situations, the clinician may complete the Post-Visit 1 Questionnaire on paper. In such scenarios, the paper form should be scanned and uploaded at the participant documents page, and the answers should be copied into the tracking system by completing the task from the individual participants page. See instructions for the Parent Post-Visit 1 Questionnaire for comparison.

Page 1


[redacted] - Post-Clinic Visit 1 Clinician Survey - Assesment
View Data
[redacted] - Post-Clinic Visit 1 Clinician Survey - AssesmentScales1
View Data
2. Based on the information you have now, how confident are you in being able to identify the primary causal etiology of the child's condition? (You can check any box along the scale)
Not at all
Completely
0 1 2 3 4 5 6
3. What do you think is the chance that this is a genetic condition? (You can check any box along the scale)
Definitely not genetic
Definitely genetic
0 1 2 3 4 5 6
4. How likely is it that the diagnostic tests you are ordering for the patient will reveal the cause of the patient's condition? (You can check any box along the scale)
Not at all
Definitely
No testing ordered
0 1 2 3 4 5 6 7
5. How likely is it that exome sequencing would reveal a diagnosis for this patient? (You can check any box along the scale)
Not at all
Definitely
0 1 2 3 4 5 6
Back
Next
Save
Save & exit

Page 2

Page 3

[redacted] - Post-Clinic Visit 1 Clinician Survey - AssesmentScales2 View Data

6. In an ideal scenario, what do you think would be the most appropriate initial genetic test for this patient? *(Check one)*

- Single gene test
- Multigene panel test
- Exome/genome
- No genetic test

7. To what extent can this patient's symptoms be improved with available treatments? *(You can check any box along the scale)*

Not at all						Completely	Not applicable
0	1	2	3	4	5	6	7

8. How likely is it that learning a diagnosis for this patient will change your clinical management? *(You can check any box along the scale)*

Not at all						Definitely	Not applicable
0	1	2	3	4	5	6	7

9. How likely is it that learning a specific diagnosis for this patient would improve this patient's health outcomes? *(You can check any box along the scale)*

Not at all						Definitely	Not applicable
0	1	2	3	4	5	6	7

Back Next Save Save & exit

[redacted] - Post-Clinic Visit 1 Clinician Survey - Caregiver

View Data

10. In your opinion, how prepared was the patient's caregiver(s) for today's clinical visit? *(Check one)*

- Not at all prepared
- A little bit prepared
- Very prepared
- Extremely prepared

11. In your opinion, how well did the patient's caregiver(s) understand the information you provided? *(Check one)*

- Not at all
- A little bit
- Somewhat
- Very much
- Completely

12. To what extent was the patient's caregiver(s) prepared to be a partner with the child's care team? *(Check one)*

- Not at all prepared
- A little bit prepared
- Very prepared
- Extremely prepared

13. In your opinion, how likely is it that this patient/caregiver will be fully compliant with medical recommendations? *(Check one)*

- Extremely unlikely
- Very unlikely
- A little bit unlikely
- Equally likely and unlikely
- A little bit likely
- Very likely
- Extremely likely

Back | Next | Save | Save & exit

[redacted] - Post-Clinic Visit 1 Clinician Survey - Caregiver2 View Data

14. How would you rate the number of questions asked by the caregiver(s)? *(Check one)*

- Far too many
- A little too many
- Just right
- A little too few
- Far too few

15. To what extent did the caregiver's questions influence the appointment? *(Check one)*

- Very disruptive
- A little disruptive
- Neither disruptive nor conducive
- A little conducive
- Very conducive

16. How would you rate the overall relevance of the caregiver's questions? *(Check one)*

- All or nearly all questions were relevant
- Most questions were relevant
- Some questions were relevant
- Few questions were relevant
- Not applicable

17. Did the caregiver hand you a list of pre-prepared questions during the visit?

- No
- Yes

19. Today, were you able to articulate a clear next step to establish a diagnosis? *(Check one)*

- No
- Yes

20. Today, were you able to give the patient a clear recommendation for management of symptoms? *(Check one)*

- No
- Yes
- Not Applicable

Back Next Save Save & exit

[redacted] - Post-Clinic Visit 1 Clinician Survey - Appointment Recommendations 1 View Data

21. Did you order or recommend any of the following for the patient related to today's appointment?

A cytogenetic test (*Check one*)

- Karyotype only
- Karyotype and microarray
- Microarray only
- Other
- I did not order/recommend

A molecular genetic test (*Check all that apply*)

- ☐ Single gene test or small panel test (<10 genes)
- ☐ Large panel test (>10 genes)
- ☐ Exome sequencing
- ☐ Mitochondrial DNA testing
- ☐ Other
- ☐ I did not order/recommend

A metabolic lab test (*Check all that apply*)

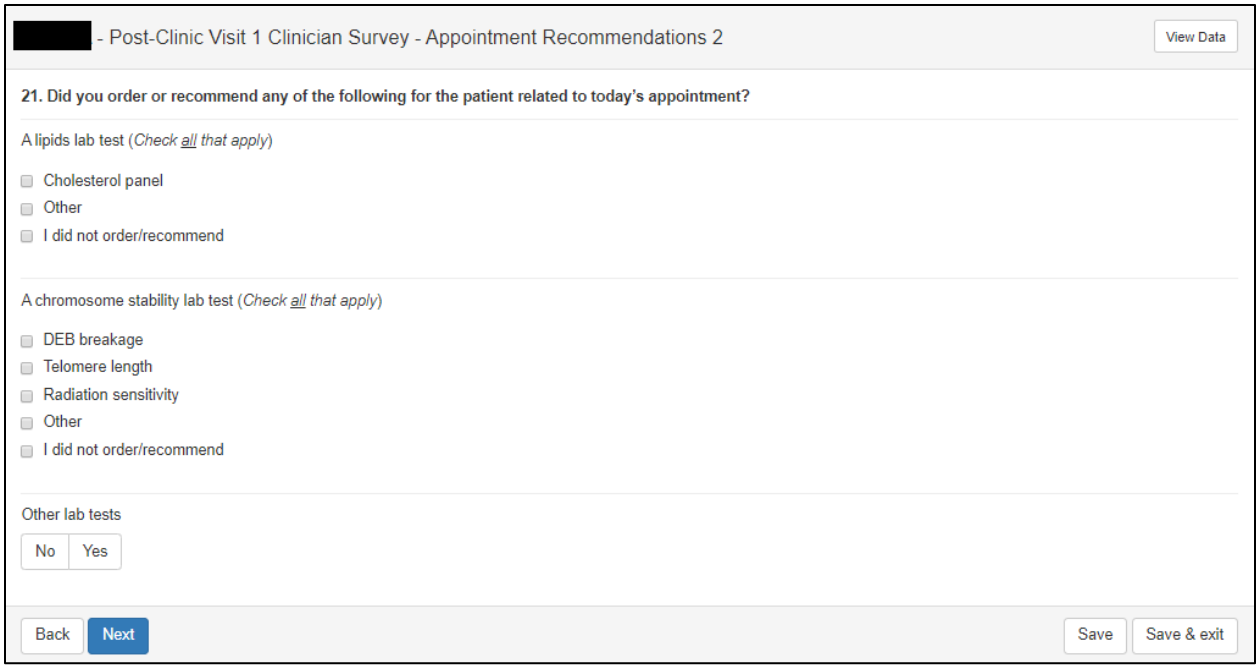
- ☐ Plasma amino acids
- ☐ Urine organic acids
- ☐ Acylcarnitine panel
- ☐ Lactate
- ☐ Ammonia
- ☐ Metabolomic panel
- ☐ Transferrin isoelectric focusing
- ☐ Guanidinoacetate
- ☐ I did not order/recommend

An endocrine lab test (*Check all that apply*)

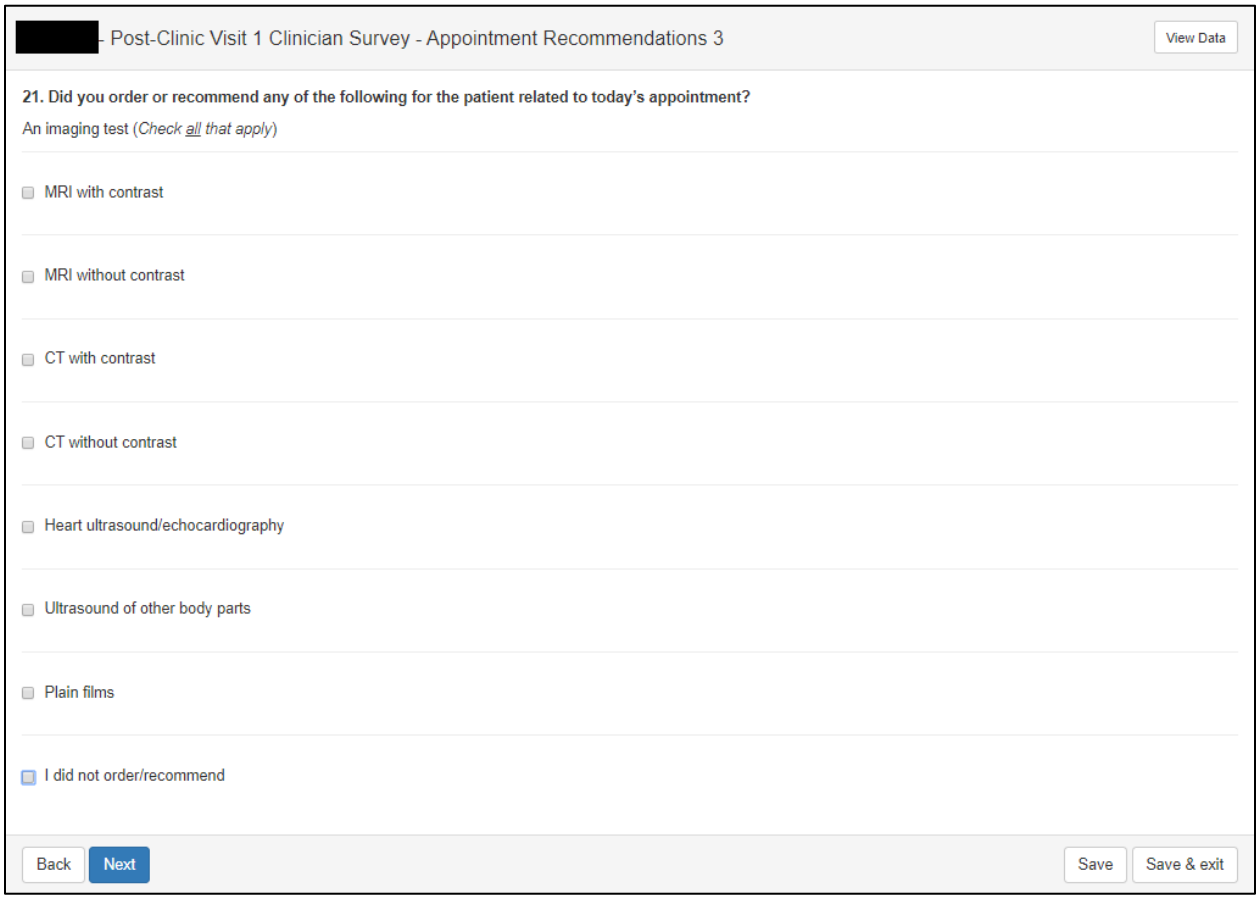
- ☐ Thyroid
- ☐ Diabetes related
- ☐ Adrenal axis
- ☐ Other
- ☐ I did not order/recommend

Back Next Save Save & exit

Page 7


[redacted] - Post-Clinic Visit 1 Clinician Survey - Appointment Recommendations 2
View Data
21. Did you order or recommend any of the following for the patient related to today's appointment?
A lipids lab test (Check all that apply)
Cholesterol panel
Other
I did not order/recommend
A chromosome stability lab test (Check all that apply)
DEB breakage
Telomere length
Radiation sensitivity
Other
I did not order/recommend
Other lab tests
No
Yes
Back
Next
Save
Save & exit

Page 8


[redacted] - Post-Clinic Visit 1 Clinician Survey - Appointment Recommendations 3
View Data
21. Did you order or recommend any of the following for the patient related to today's appointment?
An imaging test (Check all that apply)
MRI with contrast
MRI without contrast
CT with contrast
CT without contrast
Heart ultrasound/echocardiography
Ultrasound of other body parts
Plain films
I did not order/recommend
Back
Next
Save
Save & exit

[redacted] Post-Clinic Visit 1 Clinician Survey - Appointment Recommendations 4 View Data

21. Did you order or recommend any of the following for the patient related to today's appointment?

A procedure to obtain a tissue sample for testing (*Check all that apply*)

- ☐ Muscle biopsy
- ☐ Lumbar puncture
- ☐ Skin biopsy
- ☐ Other
- ☐ I did not order/recommend

Prophylactic surgery to reduce disease risk

No | Yes

Non-invasive electrophysiology (*Check all that apply*)

- ☐ EKG
- ☐ EEG
- ☐ Other
- ☐ I did not order/recommend

Invasive electrophysiology (*Check all that apply*)

- ☐ EMG
- ☐ Nerve conduction
- ☐ Other
- ☐ I did not order/recommend

Referral for therapeutic services (*Check all that apply*)

- ☐ Speech therapy
- ☐ Occupational therapy
- ☐ Physical therapy
- ☐ Other
- ☐ I did not order/recommend

Referral to a non-MD health professional (*Check all that apply*)

- ☐ Audiology
- ☐ Dental
- ☐ Other
- ☐ I did not order/recommend

Back Next Save Save & exit

[redacted] - Post-Clinic Visit 1 Clinician Survey - Appointment Recommendations 5 View Data

21. Did you order or recommend any of the following for the patient related to today's appointment?

Referral to another medical specialty for evaluation or management (*Check all that apply*)

- ☐ Cardiology
- ☐ Neurology
- ☐ Genetics and Metabolism
- ☐ Ophthalmology
- ☐ Nephrology
- ☐ Dermatology
- ☐ Pulmonology
- ☐ Immunology/Allergy
- ☐ Rheumatology
- ☐ Hematology
- ☐ Oncology
- ☐ Other
- ☐ I did not order/recommend

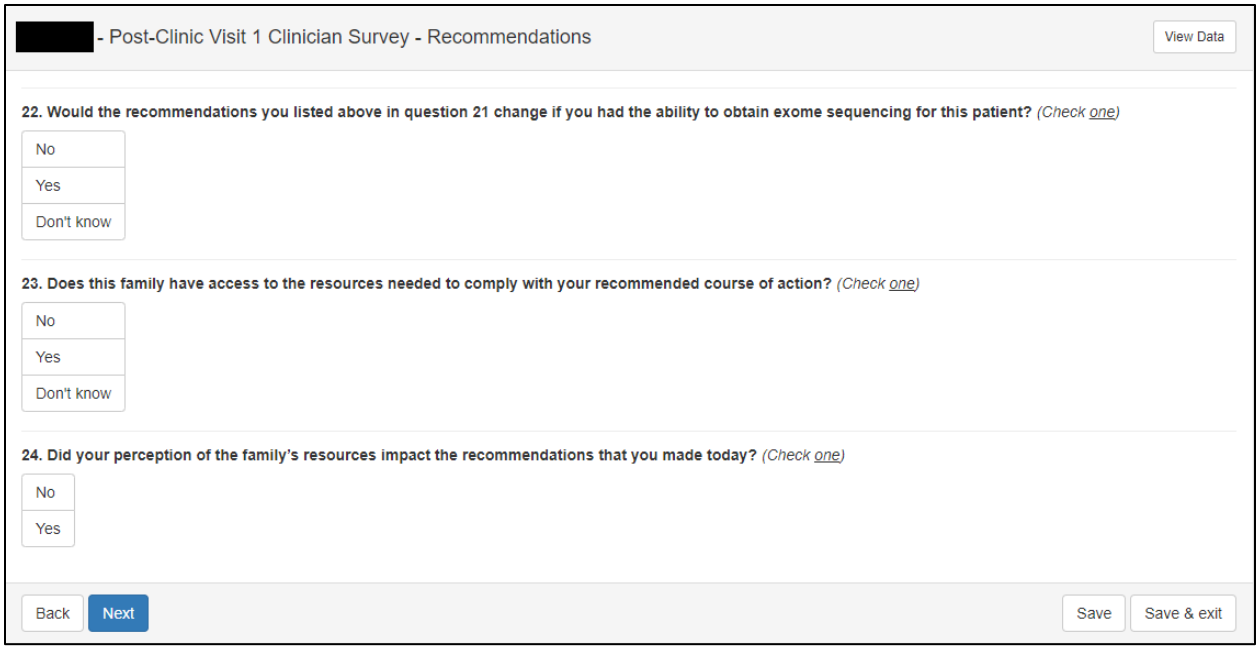
Referral for mental health support (*Check all that apply*)

- ☐ Mental health
- ☐ Social support
- ☐ Palliative care
- ☐ Other
- ☐ I did not order/recommend

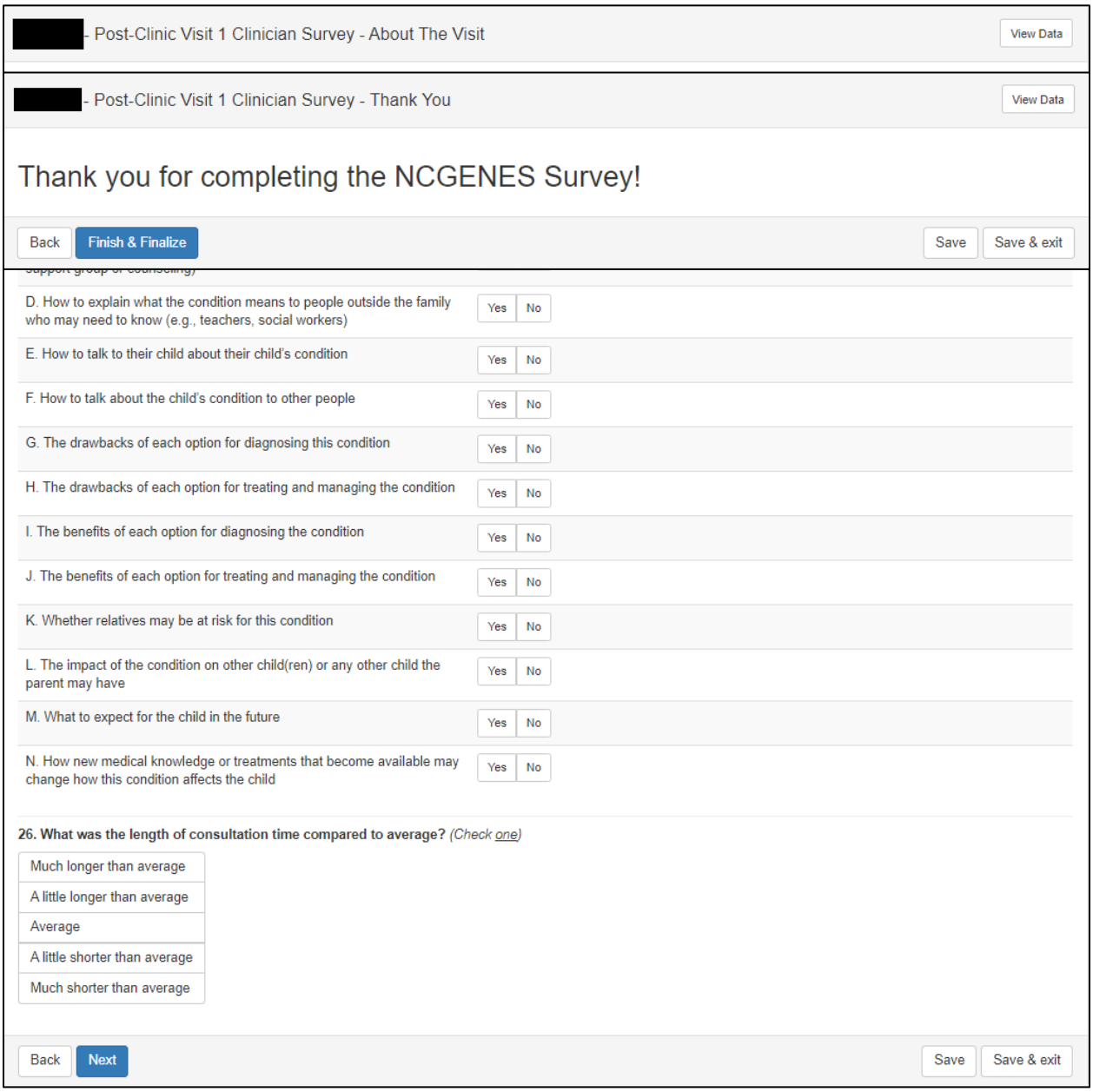
Other changes to management (*Check all that apply*)

- ☐ Recommended a new medication
- ☐ Recommended a change of dose of an existing medication
- ☐ Recommended discontinuation of an existing medication
- ☐ Changes to over the counter (OTC) medicines or supplements
- ☐ Medical/metabolic diet
- ☐ General dietary recommendations
- ☐ Recommended change in exercise or level of activity
- ☐ Other types of lifestyle changes
- ☐ Any other recommendations not covered above
- ☐ I did not order/recommend

Back Next Save Save & exit


[redacted] - Post-Clinic Visit 1 Clinician Survey - Recommendations
View Data
22. Would the recommendations you listed above in question 21 change if you had the ability to obtain exome sequencing for this patient? (Check one)
No
Yes
Don't know
23. Does this family have access to the resources needed to comply with your recommended course of action? (Check one)
No
Yes
Don't know
24. Did your perception of the family's resources impact the recommendations that you made today? (Check one)
No
Yes
Back
Next
Save
Save & exit

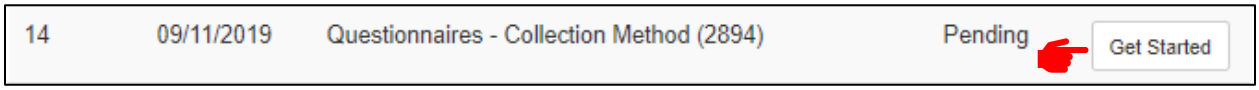
Page 12



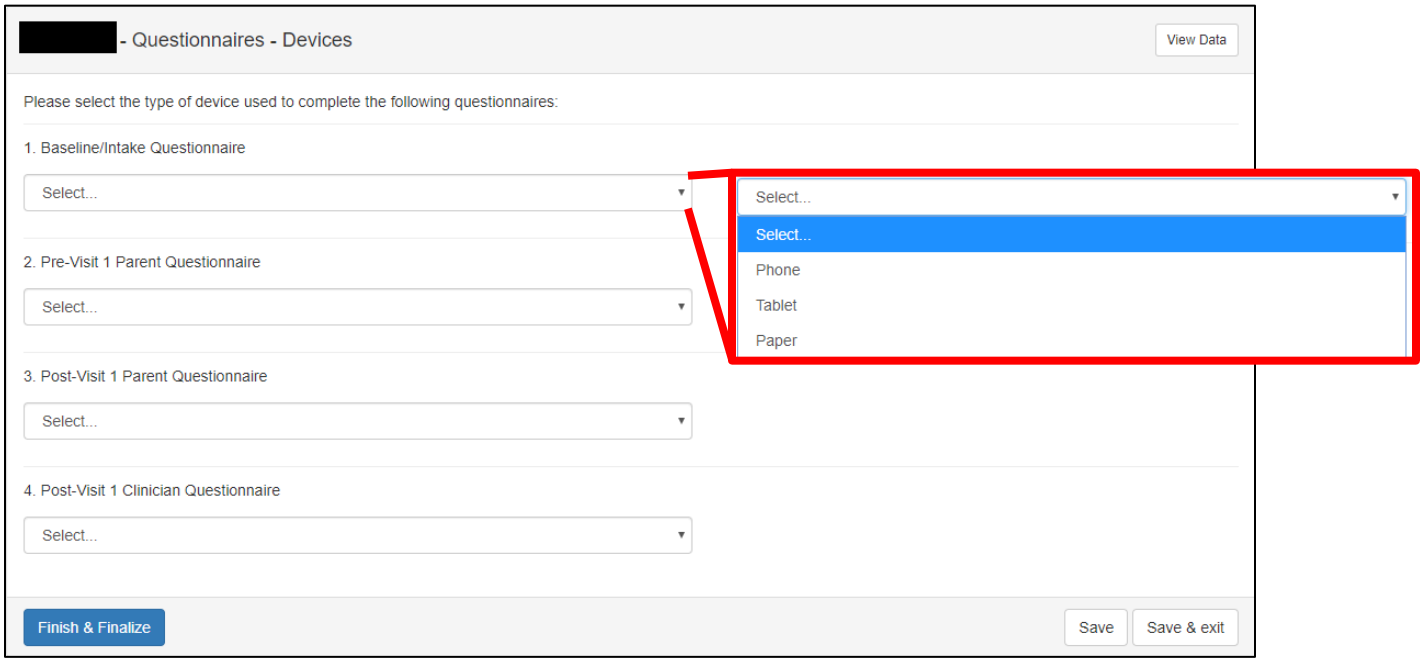
Page 13

Questionnaires – Collection Method

1. Begin the "Questionnaires – Collection Method" task by clicking the "Get Started" button next to the task on the individual participant's page.



2. The following dialog box will appear. For each questionnaire, select from the dropdown whether that questionnaire was completed by phone, tablet (or computer), or paper. Once all four questions are answered, click the blue "Finish & Finalize" button to complete the task.



Collect Developmental Age

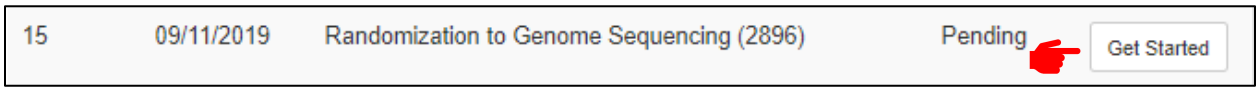
1. To complete the “Collect Developmental Age” task, click the “Get Started” button next to the task on the individual participant page.

2. The following screen will appear. From the dropdown menu, select the developmental age of the participant as provided by the child’s provider.

3. Once the age has been selected, click the blue “Finish & Finalize” button to complete the task.

Randomization to Genome Sequencing

1. To complete the "Randomization to Genome Sequencing" task, click the "Get Started" button next to the task on the individual participant page.



2. The screen to the right will appear. **This screen is the consent form for this randomization. The user should read through and discuss the form with the parent in detail.**

3. Indicate whether the parent consents for their child's data to be used in future studies.

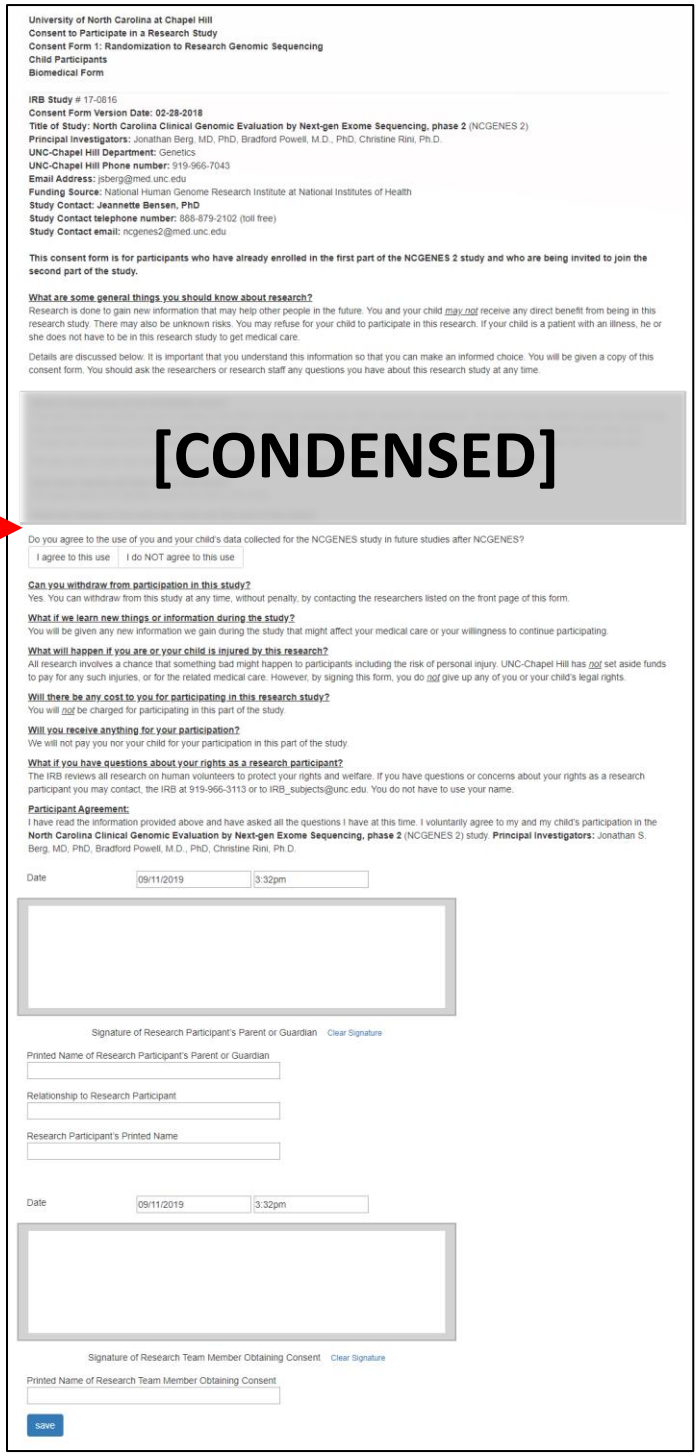
4. If the parent *does not* consent to randomization, click the blue "save" button to complete the task.[1]

5. The date and time will auto-populate above both signature fields. If the parent consents, direct them to sign in the appropriate gray box.

6. Record the parent's name, their relationship to the participant, and the participant's name in the appropriate boxes below the signature.

7. Sign and print your name in the second set of boxes.

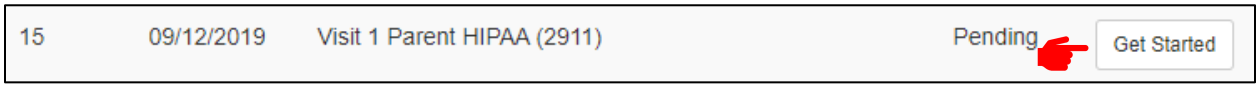
8. Click the blue "save" button to complete the task.



[1] Making ANY mark in EITHER signature field will cause the system to register the parent as consented. If the parent does not consent, be particularly careful to not make any mark in these fields.

Visit 1 Parent HIPAA

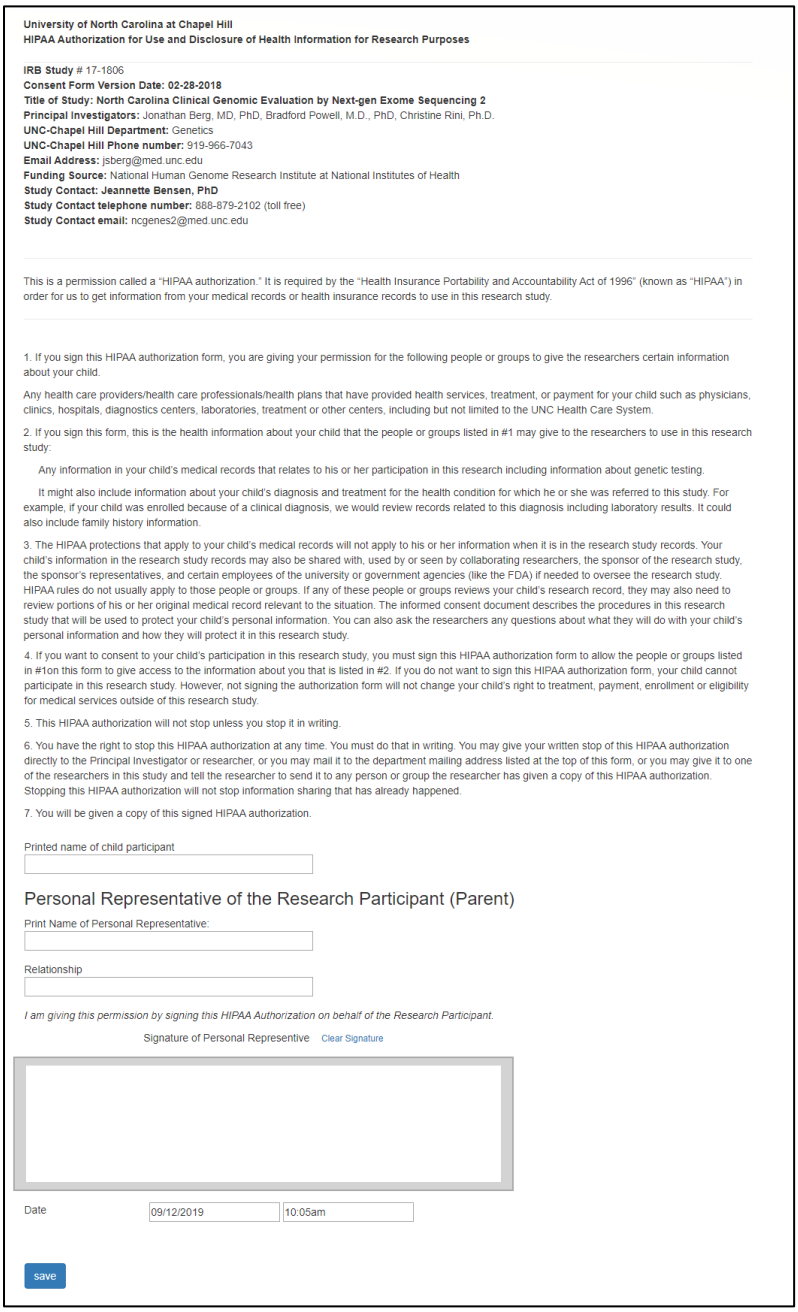
1. Begin the "Visit 1 Parent HIPAA" task by clicking the "Get Started" button next to the task on the individual participant's page.



2. The screen on the right will appear. **This screen is the HIPAA form for this study. The user should read through and discuss the form with the parent in detail.**

3. If the parent agrees to sign the HIPAA consent form, record their child's preferred name, their name, and their relationship to the child in the appropriate boxes. Then, have the parent sign within the gray box. To complete the task, click the blue "save" button.

4. If the parent **does not** consent to HIPAA, skip all data entry (including signature[1]) and click the blue "save" button to complete the task.



University of North Carolina at Chapel Hill
HIPAA Authorization for Use and Disclosure of Health Information for Research Purposes

IRB Study # 17-1806
Consent Form Version Date: 02-28-2018
Title of Study: North Carolina Clinical Genomic Evaluation by Next-gen Exome Sequencing 2
Principal Investigators: Jonathan Berg, MD, PhD, Bradford Powell, M.D., PhD, Christine Rini, Ph.D.
UNC-Chapel Hill Department: Genetics
UNC-Chapel Hill Phone number: 919-966-7043
Email Address: jsberg@med.unc.edu
Funding Source: National Human Genome Research Institute at National Institutes of Health
Study Contact: Jeannette Bensen, PhD
Study Contact telephone number: 888-879-2102 (toll free)
Study Contact email: ncgenes2@med.unc.edu

This is a permission called a "HIPAA authorization." It is required by the "Health Insurance Portability and Accountability Act of 1996" (known as "HIPAA") in order for us to get information from your medical records or health insurance records to use in this research study.

1. If you sign this HIPAA authorization form, you are giving your permission for the following people or groups to give the researchers certain information about your child.

Any health care providers/health care professionals/health plans that have provided health services, treatment, or payment for your child such as physicians, clinics, hospitals, diagnostics centers, laboratories, treatment or other centers, including but not limited to the UNC Health Care System.

2. If you sign this form, this is the health information about your child that the people or groups listed in #1 may give to the researchers to use in this research study:

Any information in your child's medical records that relates to his or her participation in this research including information about genetic testing.

It might also include information about your child's diagnosis and treatment for the health condition for which he or she was referred to this study. For example, if your child was enrolled because of a clinical diagnosis, we would review records related to this diagnosis including laboratory results. It could also include family history information.

3. The HIPAA protections that apply to your child's medical records will not apply to his or her information when it is in the research study records. Your child's information in the research study records may also be shared with, used by or seen by collaborating researchers, the sponsor of the research study, the sponsor's representatives, and certain employees of the university or government agencies (like the FDA) if needed to oversee the research study. HIPAA rules do not usually apply to those people or groups. If any of these people or groups reviews your child's research record, they may also need to review portions of his or her original medical record relevant to the situation. The informed consent document describes the procedures in this research study that will be used to protect your child's personal information. You can also ask the researchers any questions about what they will do with your child's personal information and how they will protect it in this research study.

4. If you want to consent to your child's participation in this research study, you must sign this HIPAA authorization form to allow the people or groups listed in #1on this form to give access to the information about you that is listed in #2. If you do not want to sign this HIPAA authorization form, your child cannot participate in this research study. However, not signing the authorization form will not change your child's right to treatment, payment, enrollment or eligibility for medical services outside of this research study.

5. This HIPAA authorization will not stop unless you stop it in writing.

6. You have the right to stop this HIPAA authorization at any time. You must do that in writing. You may give your written stop of this HIPAA authorization directly to the Principal Investigator or researcher, or you may mail it to the department mailing address listed at the top of this form, or you may give it to one of the researchers in this study and tell the researcher to send it to any person or group the researcher has given a copy of this HIPAA authorization. Stopping this HIPAA authorization will not stop information sharing that has already happened.

7. You will be given a copy of this signed HIPAA authorization.

Printed name of child participant

Personal Representative of the Research Participant (Parent)

Print Name of Personal Representative:

Relationship

I am giving this permission by signing this HIPAA Authorization on behalf of the Research Participant.

Signature of Personal Representive Clear Signature

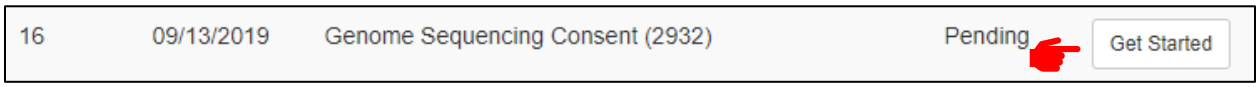
Date 09/12/2019 10:05am

save

[1] Making ANY mark in EITHER signature field will cause the system to register the parent as consented. If the parent does not consent, be particularly careful to not make any mark in these fields.

Genome Sequencing Consent

1. To complete the "Genome Sequencing Consent" task, click the "Get Started" button next to the task on the individual participant page.



2. The screen to the right will appear. **This screen is the consent form for undergoing exome sequencing. The user should read through and discuss the form with the parent in detail.**

3. Indicate whether the parent consents to secondary analysis of their child's genes.

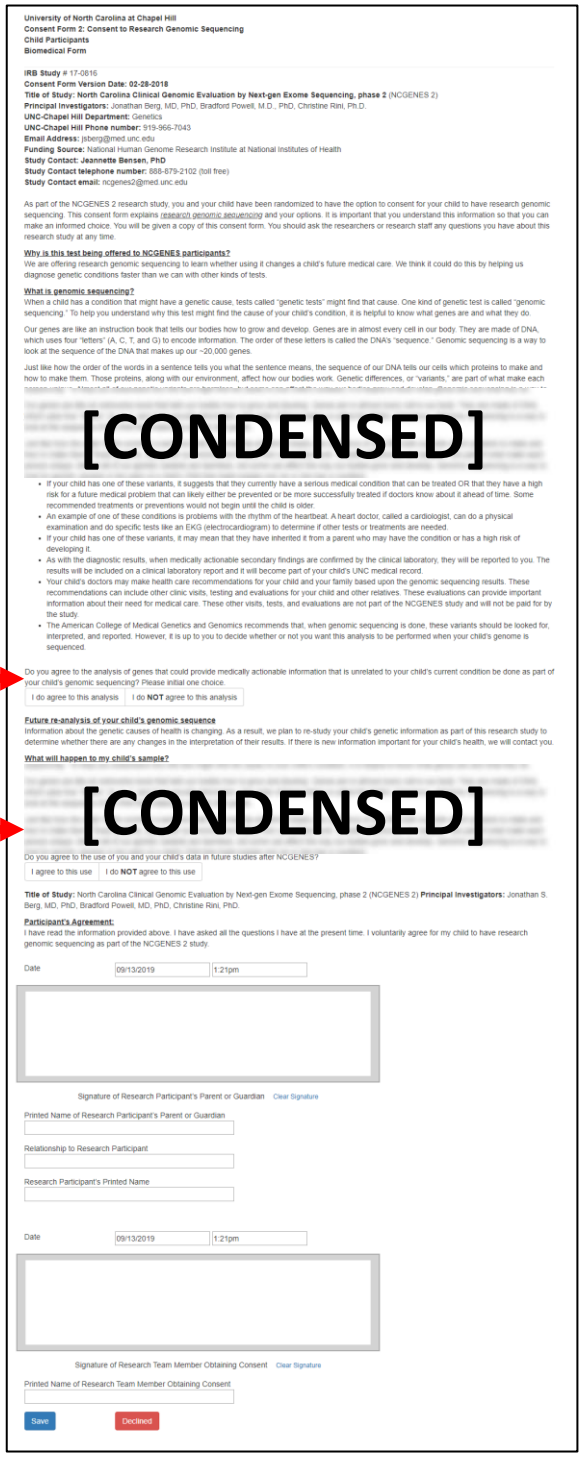
4. Indicate whether the parent consents for their child's data to be used in future studies.

5. If the parent *does not* consent, click the red "Declined" button to complete the task.

6. If the parent consents, direct them to sign in the appropriate gray box. The date and time will auto-populate above both signature fields. Record the parent's name, their relationship to the participant, and participant's name in the appropriate boxes below the signature.

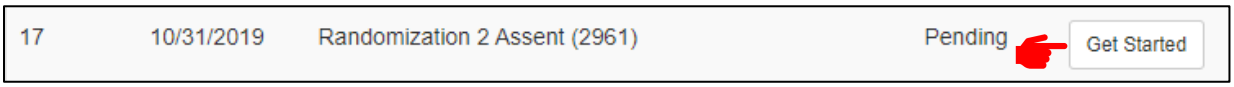
7. Sign and print your name in the second set of boxes.

8. Click the blue "Save" button to complete the task.



Randomization 2 Assent

1. To complete the "Randomization 2 Assent" task, click the "Get Started" button next to the task on the individual participant page.



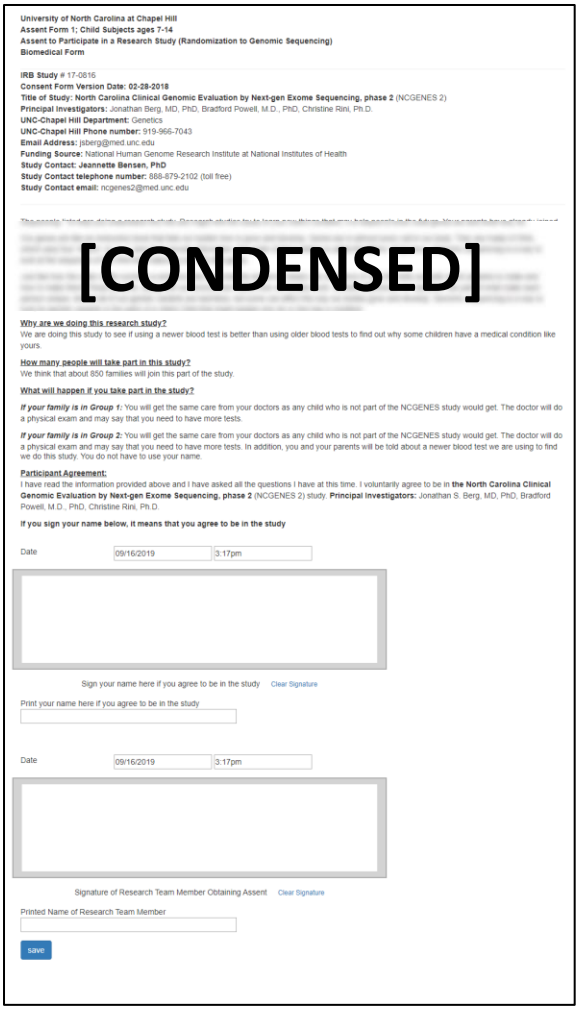
2. The screen to the right will appear. **This screen is the assent form for Randomization 2. The user should read through and discuss the form with the participant in detail.**

3. If the participant *does not* assent, click the blue "save" button to complete the task.[1]

4. If the participant assents, direct them to sign in the appropriate gray box. The date and time will auto-populate above both signature fields. Record the participant's name in the appropriate box below the signature.

5. Sign and print your name in the second set of boxes.

6. Click the blue "save" button to complete the task.



[1] Making ANY mark in EITHER signature field will cause the system to register the parent as consented. If the parent does not consent, be particularly careful to not make any mark in these fields.

Genome Sequencing Assent

1. To complete the “Genome Sequencing Assent” task, click the “Get Started” button next to the task on the individual participant page.

2. The screen to the right will appear. **This screen is the assent form for undergoing exome sequencing. The user should read through and discuss the form with the parent in detail.**

3. If the participant *does not* assent, click the red “Declined” button to complete the task.

4. If the participant assents, direct them to sign in the appropriate gray box. The date and time will auto-populate above both signature fields. Record the participant’s name in the appropriate box below the signature.

5. Sign and print your name in the second set of boxes.

6. Click the blue “Save” button to complete the task.

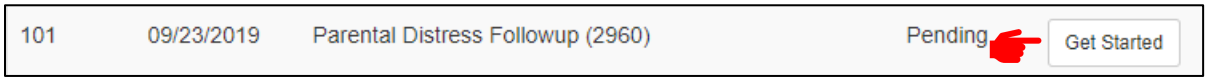
Visit 1 – Parent Reimbursement

1. From the individual participant page, click “Get Started” next to the “Visit 1 – Parent Reimbursement” task.

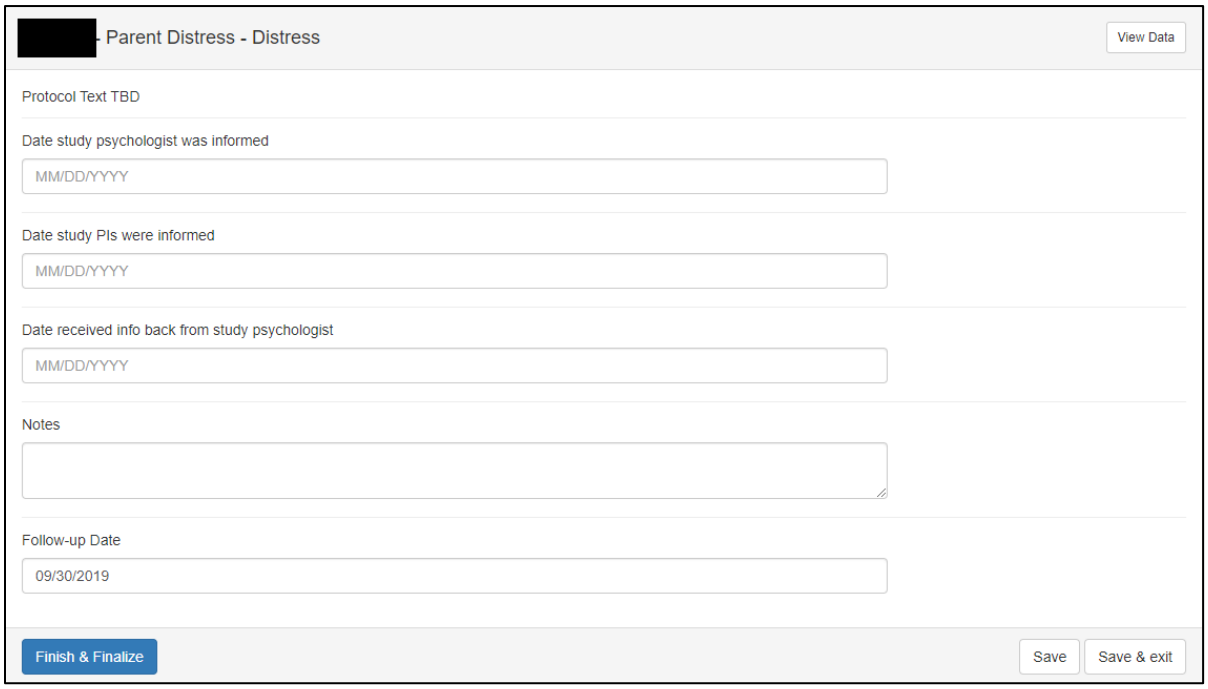
2. The page shown below will appear. Enter the amount of compensation and allow the parent to sign within the gray box for their receipt. (Date and time will auto-populate). When this is complete, click the blue “save” button to complete the task.

Parental Distress Followup

1. Begin the “Parental Distress Followup” task by clicking the “Get Started” button next to the task on the individual participant’s page.
2. The following screen will appear. Record the date that the psychologist and PIs were informed, as well as the date

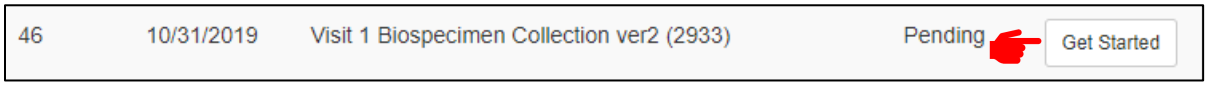


the psychologist responded and any notes in the appropriate spots. Then, click the blue “Finish & Finalize” button to complete the task.

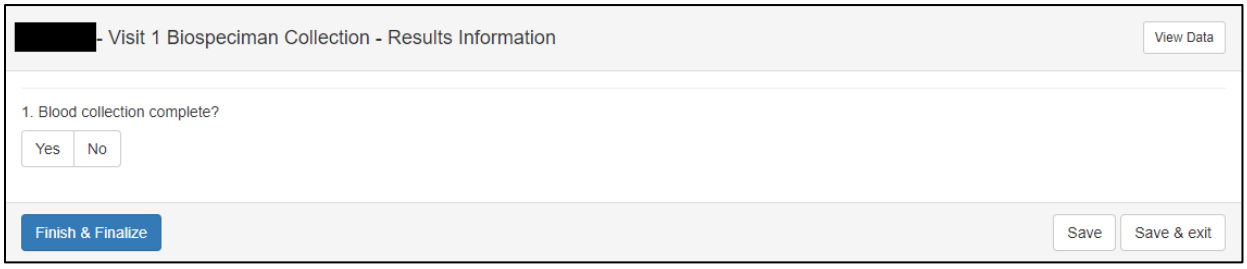


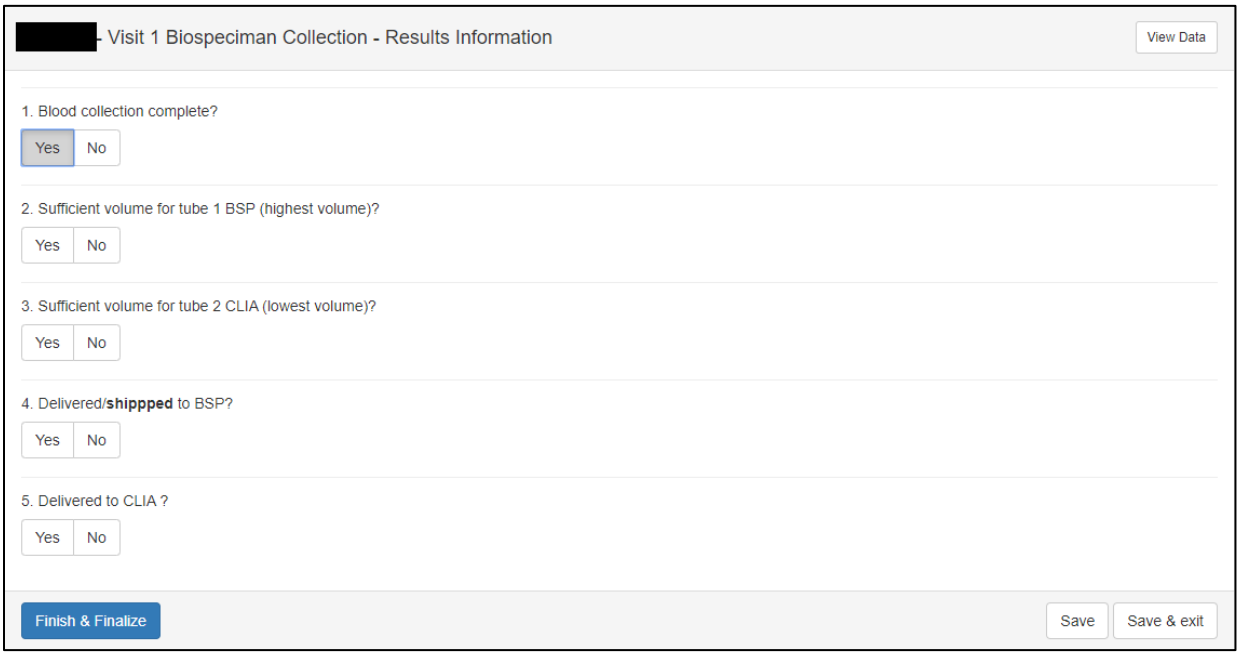
Visit 1 Biospeciman Collection

1. Begin the “Visit 1 Biospeciman Collection” task by clicking the “Get Started” button next to the task on the individual participant’s page.
2. The following screen will appear. If the blood collection is *not* complete, click “No” and then the blue “Finish & Finalize” button to complete the task.



3. If the specimen collection is complete, click “Yes.” The screen will expand as shown below. Answer the subsequent questions “Yes” or “No” as applicable and then click the blue “Finish & Finalize” button to complete the task.

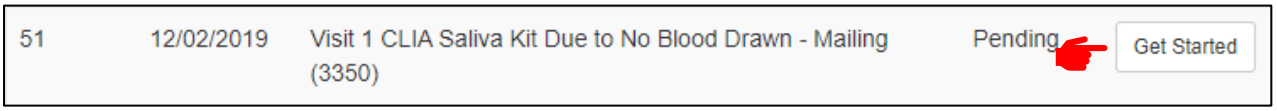




Saliva Kit Tasks

Visit 1 CLIA Saliva Kit Due to No Blood Drawn - Mailing

The “Visit 1 CLIA Saliva Kit Due to No Blood Drawn - Mailing” task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the “Get Started” button for the appropriate participant.

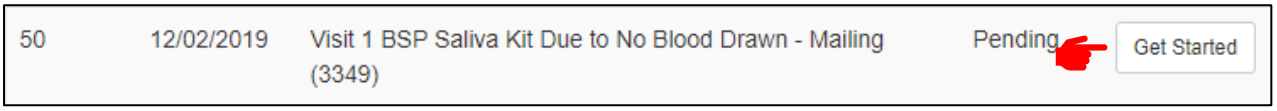


This will redirect the user to the “Mailings” page, where they can generate a mail merge document for a participant. The “Visit 1 CLIA Saliva Kit Due to No Blood Drawn - Mailing” task will be marked complete in the tracking system when this mail merge is generated.

Mailings should be completed on the same day they are originated to ensure accurate tracking of this task. For detailed instructions on completing this mailing, go to the **Mailing 5** section under **Mailings**.

Visit 1 BSP Saliva Kit Due to No Blood Drawn - Mailing

The “Visit 1 BSP Saliva Kit Due to No Blood Drawn - Mailing” task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the “Get Started” button for the appropriate participant.

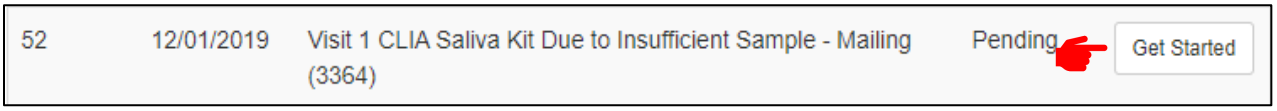


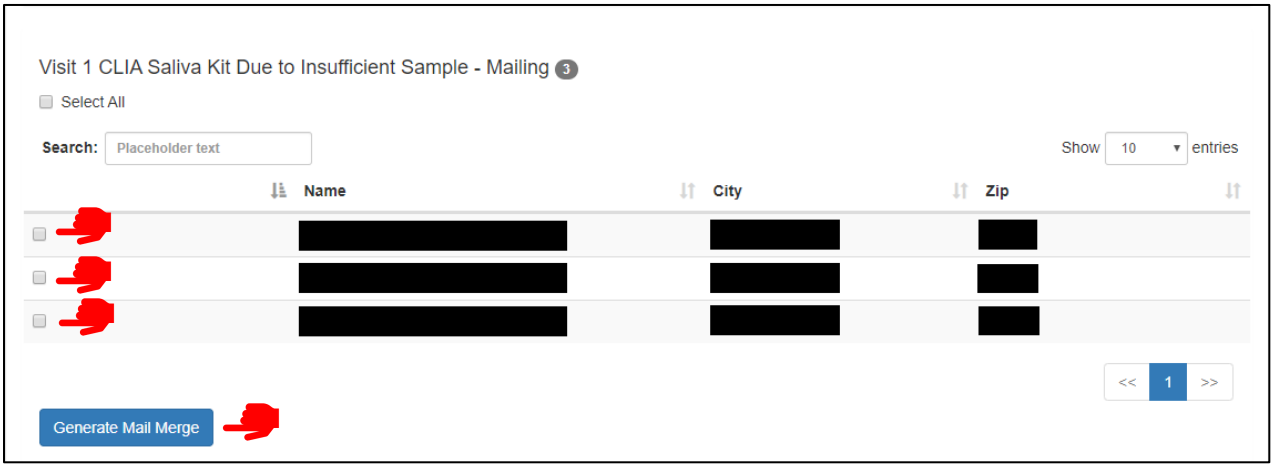
This will redirect the user to the “Mailings” page, where they can generate a mail merge document for a participant. The “Visit 1 BSP Saliva Kit Due to No Blood Drawn - Mailing” task will be marked complete in the tracking system when this mail merge is generated.

Mailings should be completed on the same day they are originated to ensure accurate tracking of this task. For detailed instructions on completing this mailing, go to the **Mailing 5** section under **Mailings**.

Visit 1 CLIA Saliva Kit Due to Insufficient Sample - Mailing

The “Visit 1 CLIA Saliva Kit Due to Insufficient Sample - Mailing” task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the “Get Started” button for the appropriate participant.



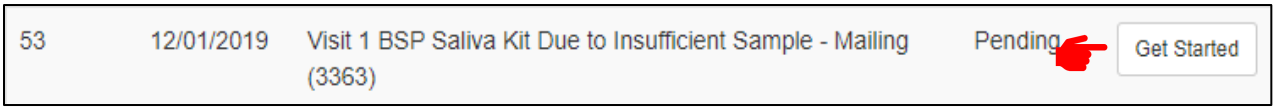


This will redirect the user to the “Mailings” page, where they can generate a mail merge document for a participant. The “Visit 1 CLIA Saliva Kit Due to Insufficient Sample - Mailing” task will be marked complete in the tracking system when this mail merge is generated.

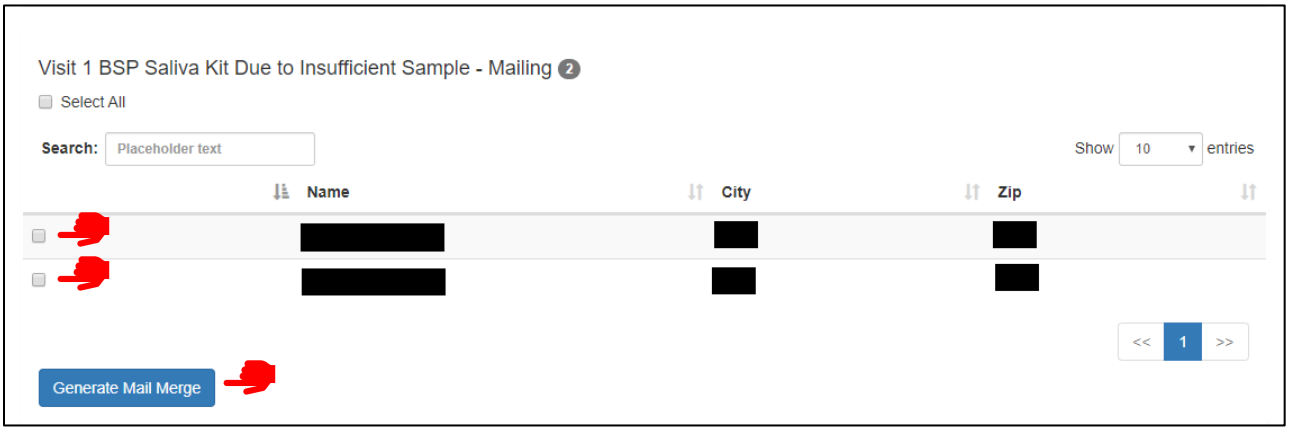
Mailings should be completed on the same day they are originated to ensure accurate tracking of this task. For detailed instructions on completing this mailing, go to the **Mailing 5** section under **Mailings**.

Visit 1 BSP Saliva Kit Due to Insufficient Sample - Mailing

The "Visit 1 BSP Saliva Kit Due to Insufficient Sample - Mailing" task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the "Get Started" button for the appropriate participant.



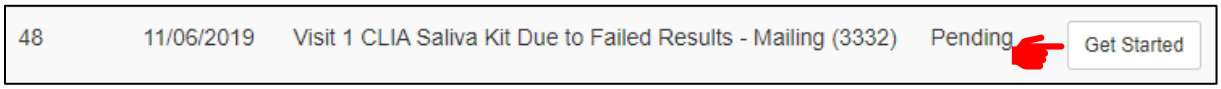
This will redirect the user to the "Mailings" page, where they can generate a mail merge document for a participant. The "Visit 1 BSP Saliva Kit Due to Insufficient Sample - Mailing" task will be marked complete in the tracking system when this mail merge is generated.



Mailings should be completed on the same day they are originated to ensure accurate tracking of this task. For detailed instructions on completing this mailing, go to the **Mailing 5** section under **Mailings**.

Visit 1 CLIA Saliva Kit Due to Failed Results - Mailing

The “Visit 1 CLIA Saliva Kit Due to Failed Results - Mailing” task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the “Get Started” button for the appropriate participant.



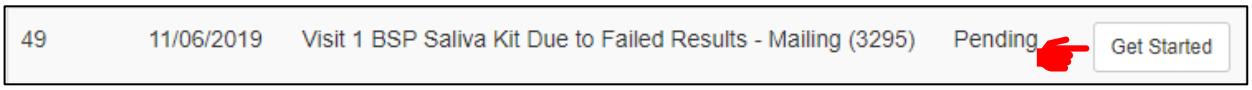
This will redirect the user to the “Mailings” page, where they can generate a mail merge document for a participant. The “Visit 1 CLIA Saliva Kit Due to Failed Results - Mailing” task will be marked complete in the tracking system when this mail merge is generated (see below).

Mailings should be completed on the same day they are originated to ensure accurate tracking of this task. For

detailed instructions on completing this mailing, go to the **Mailing 5** section under **Mailings**.

Visit 1 BSP Saliva Kit Due to Failed Results - Mailing

The “Visit 1 BSP Saliva Kit Due to Failed Results - Mailing” task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the “Get Started” button for the appropriate participant.



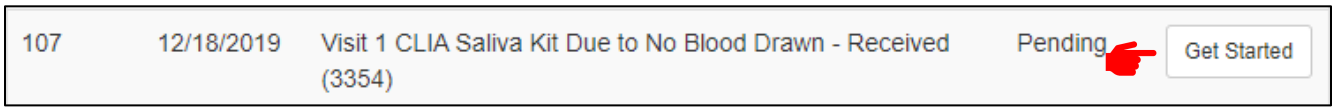
This will redirect the user to the “Mailings” page, where they can generate a mail merge document for a participant. The “Visit 1 BSP Saliva Kit Due to Failed Results - Mailing” task will be marked complete in the tracking system when this mail merge is generated (see below).

Mailings should be completed on the same day they are originated to ensure accurate tracking of this task. For

detailed instructions on completing this mailing, go to the **Mailing 5** section under **Mailings**.

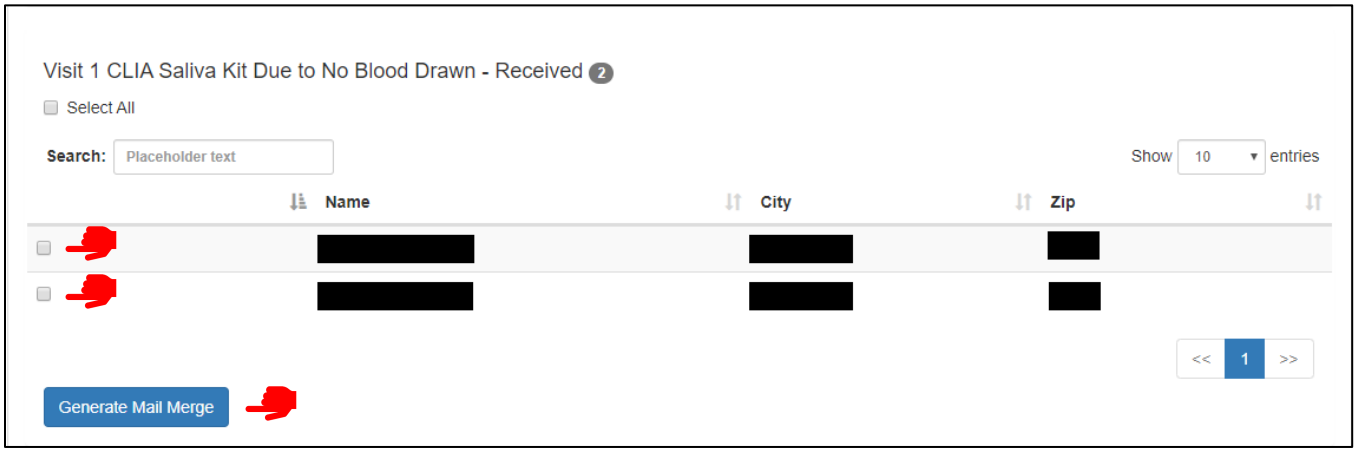
Visit 1 CLIA Saliva Kit Due to No Blood Drawn - Received

The "Visit 1 CLIA Saliva Kit Due to No Blood Drawn - Received" task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the "Get Started" button for the appropriate participant.



This will redirect the user to the "Mailings" page, where they can generate a mail merge document for a participant. The "Visit 1 CLIA Saliva Kit Due to No Blood Drawn - Received" task will be marked complete in the tracking system when this mail merge is generated (see below).

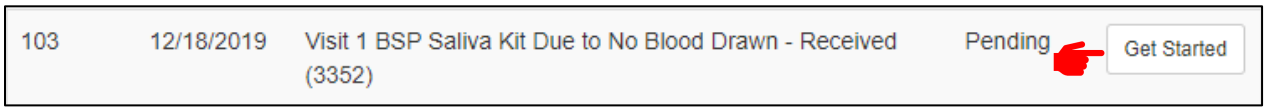
While the Saliva Kit receipt tasks are technically mailing tasks, they are not completed in conjunction with outgoing mail. Because of this, the generation of a mail merge document is only for the purpose of completing this task. The mail merge document itself will not be used.



This task should be completed on the same day that a kit is reported received by the BSP to ensure accurate tracking of this task. For detailed instructions on completing receipt of this mailing, go to the **Mailing 5** section under **Mailings**.

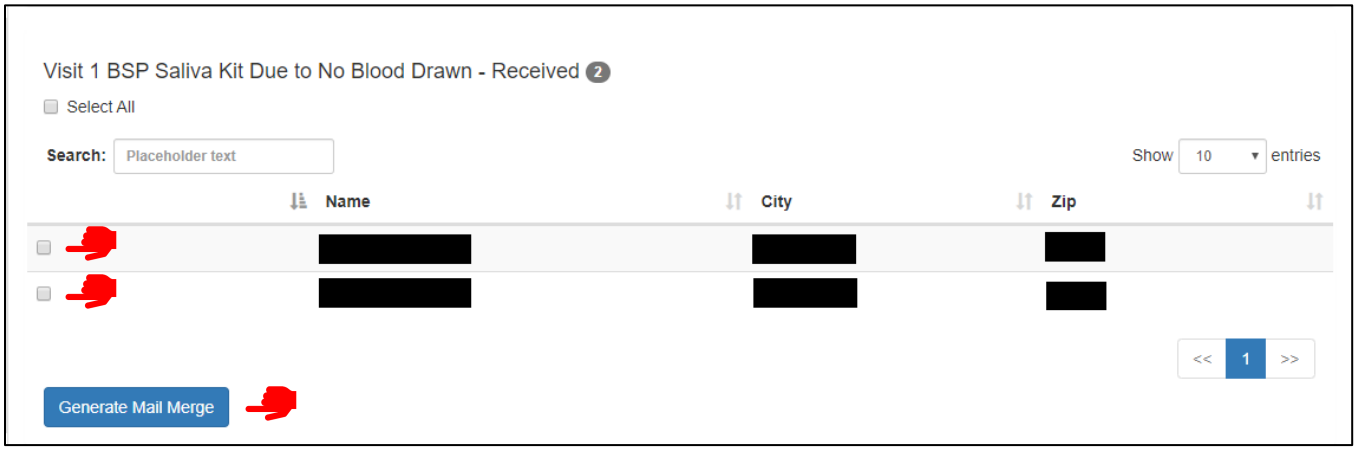
Visit 1 BSP Saliva Kit Due to No Blood Drawn - Received

The "Visit 1 BSP Saliva Kit Due to No Blood Drawn - Received" task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the "Get Started" button for the appropriate participant.



This will redirect the user to the "Mailings" page, where they can generate a mail merge document for a participant. The "Visit 1 BSP Saliva Kit Due to No Blood Drawn - Received" task will be marked complete in the tracking system when this mail merge is generated (see below).

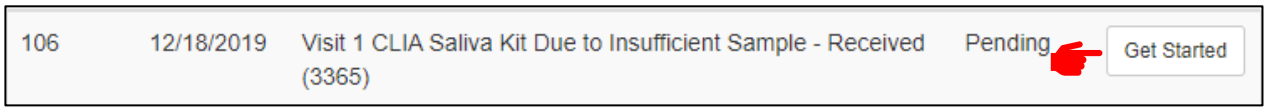
While the Saliva Kit receipt tasks are technically mailing tasks, they are not completed in conjunction with outgoing mail. Because of this, the generation of a mail merge document is only for the purpose of completing this task. The mail merge document itself will not be used.



This task should be completed on the same day that a kit is reported received by the BSP to ensure accurate tracking of this task. For detailed instructions on completing receipt of this mailing, go to the **Mailing 5** section under **Mailings**.

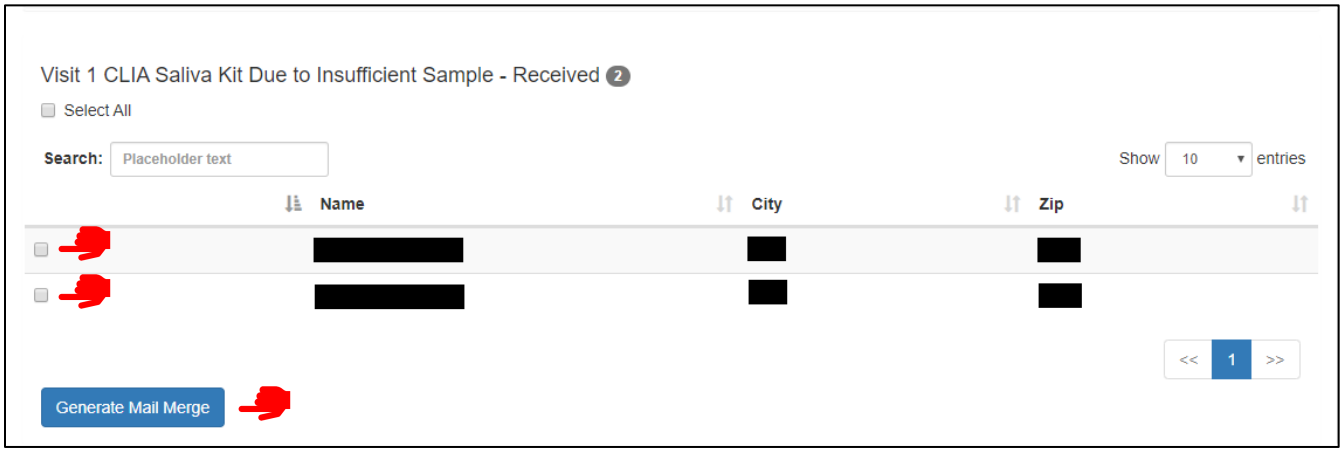
Visit 1 CLIA Saliva Kit Due to Insufficient Sample - Received

The "Visit 1 CLIA Saliva Kit Due to Insufficient Sample - Received" task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the "Get Started" button for the appropriate participant.



This will redirect the user to the "Mailings" page, where they can generate a mail merge document for a participant. The "Visit 1 CLIA Saliva Kit Due to Insufficient Sample - Received" task will be marked complete in the tracking system when this mail merge is generated (see below).

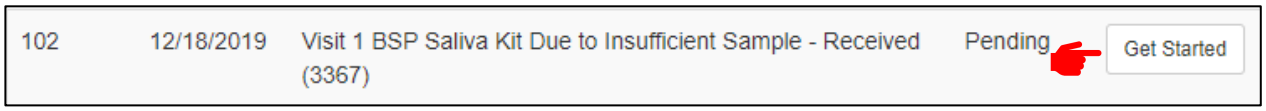
While the Saliva Kit receipt tasks are technically mailing tasks, they are not completed in conjunction with outgoing mail. Because of this, the generation of a mail merge document is only for the purpose of completing this task. The mail merge document itself will not be used.



This task should be completed on the same day that a kit is reported received by the BSP to ensure accurate tracking of this task. For detailed instructions on completing receipt of this mailing, go to the **Mailing 5** section under **Mailings**.

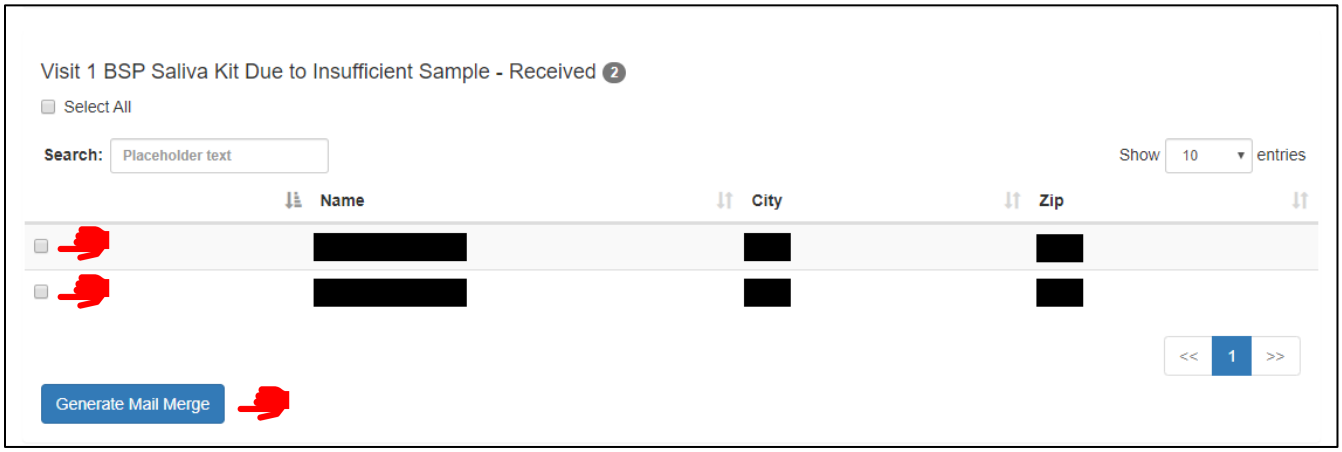
Visit 1 BSP Saliva Kit Due to Insufficient Sample - Received

The "Visit 1 BSP Saliva Kit Due to Insufficient Sample - Received" task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the "Get Started" button for the appropriate participant.



This will redirect the user to the "Mailings" page, where they can generate a mail merge document for a participant. The "Visit 1 BSP Saliva Kit Due to Insufficient Sample - Received" task will be marked complete in the tracking system when this mail merge is generated (see below).

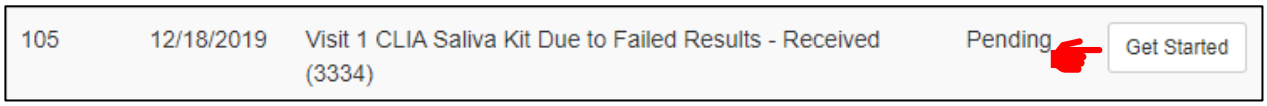
While the Saliva Kit receipt tasks are technically mailing tasks, they are not completed in conjunction with outgoing mail. Because of this, the generation of a mail merge document is only for the purpose of completing this task. The mail merge document itself will not be used.



This task should be completed on the same day that a kit is reported received by the BSP to ensure accurate tracking of this task. For detailed instructions on completing receipt of this mailing, go to the **Mailing 5** section under **Mailings**.

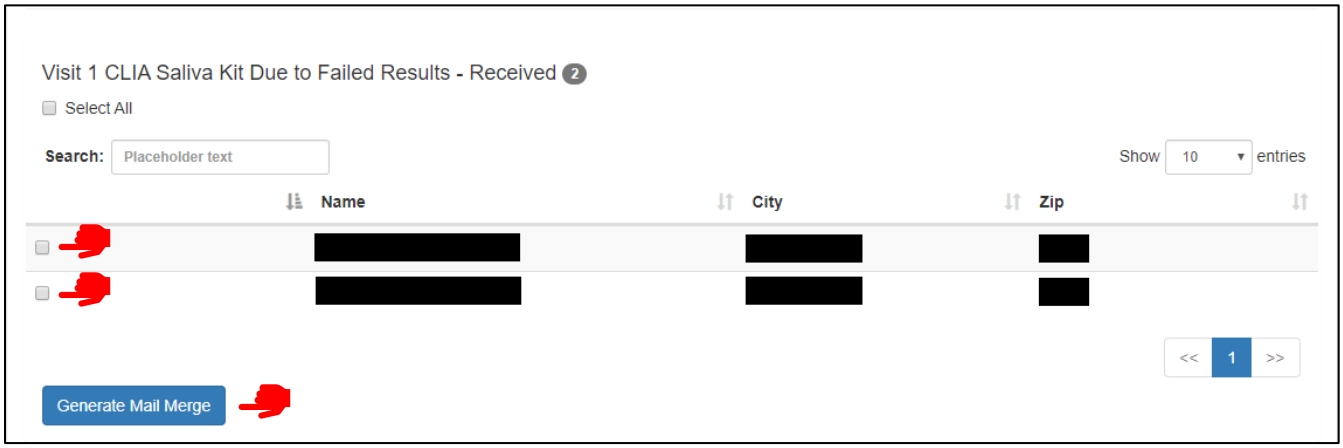
Visit 1 CLIA Saliva Kit Due to Failed Results - Received

The "Visit 1 CLIA Saliva Kit Due to Failed Results - Received" task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the "Get Started" button for the appropriate participant.



This will redirect the user to the "Mailings" page, where they can generate a mail merge document for a participant. The "Visit 1 CLIA Saliva Kit Due to Failed Results - Received" task will be marked complete in the tracking system when this mail merge is generated (see below).

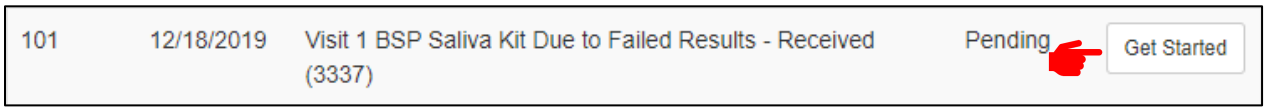
While the Saliva Kit receipt tasks are technically mailing tasks, they are not completed in conjunction with outgoing mail. Because of this, the generation of a mail merge document is only for the purpose of completing this task. The mail merge document itself will not be used.



This task should be completed on the same day that a kit is reported received by the BSP to ensure accurate tracking of this task. For detailed instructions on completing receipt of this mailing, go to the **Mailing 5** section under **Mailings**.

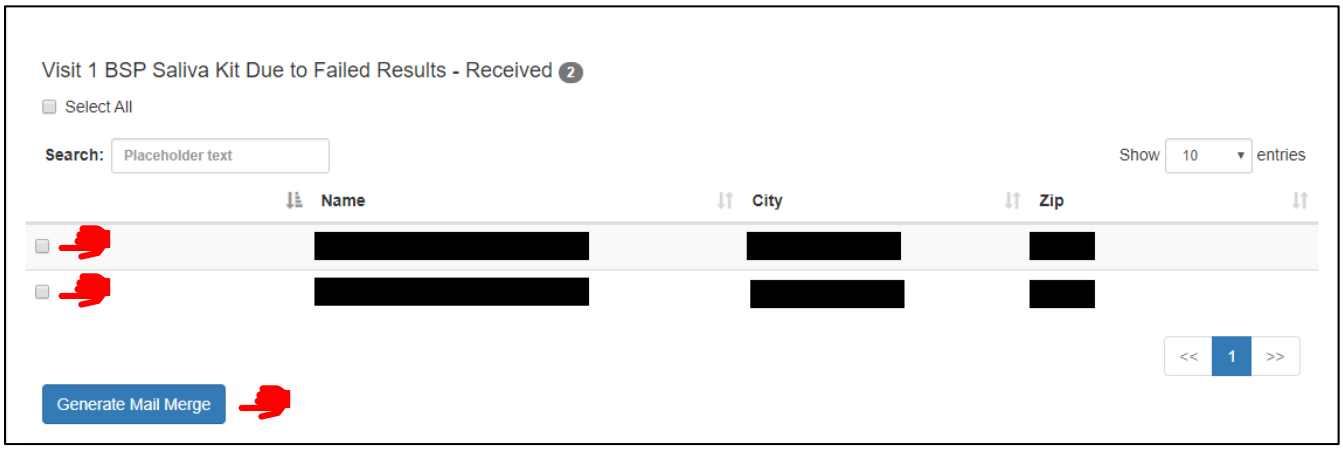
Visit 1 BSP Saliva Kit Due to Failed Results – Received

The "Visit 1 BSP Saliva Kit Due to Failed Results - Received" task is a mailing task. This (and all) mailing task(s) can be started from the individual participant page by clicking the "Get Started" button for the appropriate participant.



This will redirect the user to the "Mailings" page, where they can generate a mail merge document for a participant. The "Visit 1 BSP Saliva Kit Due to Failed Results - Received" task will be marked complete in the tracking system when this mail merge is generated (see below).

While the Saliva Kit receipt tasks are technically mailing tasks, they are not completed in conjunction with outgoing mail. Because of this, the generation of a mail merge document is only for the purpose of completing this task. The mail merge document itself will not be used.



This task should be completed on the same day that a kit is reported received by the BSP to ensure accurate tracking of this task. For detailed instructions on completing receipt of this mailing, go to the **Mailing 5** section under **Mailings**

Pre-RoR Packet – No GS without Pre-Visit Prep

Pre-RoR Packet – GS with Pre-Visit Prep

Pre-RoR Packet – GS without Pre-Visit Prep

CALENDARING

Each NCGENES 2 site will maintain its own system for keeping track of tasks not captured by the tracking system. These systems (will be) explained in this section of the protocol.

NCGENES 2 BIOSPECIMEN PROTOCOL

Overview

For participants randomized to genome sequencing as part of the NCGENES 2 study, blood is drawn at the first clinical visit (evaluation) for DNA extraction, library preparation, sequencing, identity check genotyping and clinically significant genetic variant confirmation for positive or uncertain results (with respect to their association with the patient's clinical phenotype). Specifically, one blood tube is sent to the Biospecimen Processing Facility (BSP) and one is sent to UNC Clinical Molecular Genetics Laboratory (CLIA labs) for processing. All laboratories initially extract the DNA.

The CLIA lab will also genotype 8 Single Nucleotide Polymorphisms (SNPs) via Sanger sequencing in all participants, which will later be compared to genotypes obtained via research GS. This comparison is used as an identity check to identify any potential sample mix-ups. The CLIA lab additionally stores the remaining DNA for later confirmation of clinically significant research results that may arise during the course of the study.

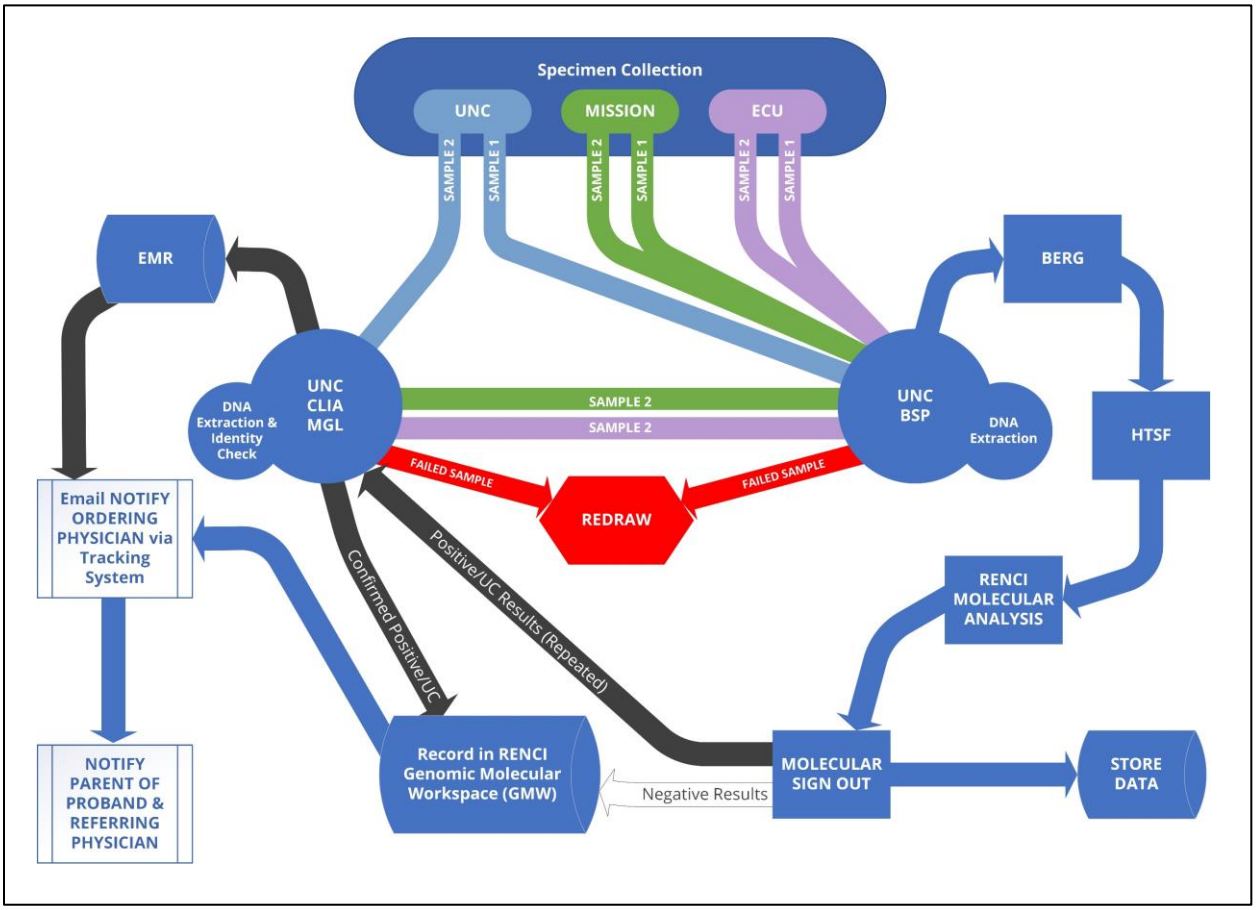
The BSP sends a portion of the extracted DNA to the Berg Laboratory for library preparation. The sequencing libraries are then sent to the High Throughput Sequencing Facility (HTSF) for research GS. The genetic data is annotated, undergoes molecular analysis and is reviewed in conjunction with clinical/phenotypic data at a molecular sign-out meeting.

If any clinically significant primary or secondary findings are identified, the CLIA Lab is notified to request confirmation and reporting. Confirmation is conducted via Sanger sequencing. CLIA confirmed results are placed in the patient's medical record which automatically notifies the onsite physician. If the CLIA confirmation is being conducted for an offsite institution, then the CLIA lab results will be sent via fax to the ordering physician. Negative research GS results cannot be confirmed in the clinical lab. All results (CLIA confirmed and negative) are reported by the research team to the ordering physician via email notification. Additionally, physicians are notified by CLIA confirmed results via the electronic medical records (EMR) system. The research email notification asks the physician to return results of testing and complete the NCGENES 2 post return of results (RoR) provider survey (the email subject line indicates "ACTION REQUIRED"). A patient friendly research report regarding the results of genomic sequencing is attached to the email notification to the physician. Additionally, the physician email notification includes a link to the patient tracking system (for participants in the GS arm). Once at the physician landing page in tracking, the MD clicks on the '*PhenoTips & Results'* tab, where there is a link to the '*Molecular Analysis*' tab for each participant who is in the GS arm. Results on the molecular analysis tab may indicate that the test results are pending, negative or positive/uncertain (CLIA confirmed). There is also a link on this page to a patient's research result report (Word document that can be printed).

Remaining DNA can be used for future research with parental consent and in rare cases with required concordant child assent (which is obtained at the time of GS randomization consent/assent). DNA will be stored indefinitely, but linked data will be destroyed on the patient's 18th birthday rendering all remaining samples anonymized.

In some cases, the physician may determine that parental DNA confirmatory testing is needed to interpret the genomic sequencing results of the child. In this case, the genetic counselor or nurse notifies the family and the research team of the need to collect parental DNA. The research team contacts the patient's parent to let them know that parental/relative genetic testing consents and saliva kits are being mailed (one for each parent). The research study coordinator will wait approximately one week and phone the parents to consent them by phone, have them sign the consents and return them along with their saliva samples to the BSP lab in an addressed/postage paid envelope. Extra consents will be provided so that the parents may keep copy for their records. In rare cases other adult relatives may need to be tested. This will be determined by the ordering physician. NCGENES 2 will pay for additional confirmatory testing in the CLIA of the physician determines that it is necessary for the interpretation of the results for the child.

FIGURE 1. Overview of sample flow through study laboratories, molecular sign-out, CLIA confirmation, and reporting



Collecting Blood Samples at Visit 1

NCGENES 2 prioritizes collection of blood for research biospecimens. As often as possible, NCGENES 2 blood specimens will be collected immediately following Visit 1 from participants consented to exome sequencing. This section outlines the protocol for collecting blood samples at each NCGENES 2 site.

Pre-Visit 1: Preparation of the Blood Collection Kit

All sites will use a generally uniform procedure for preparing blood collection kits. A blood kit is created for each participant and clipped to that participant's folder for use in the clinic (see "**Preparation for Visit 1**" on page 22). In addition, a second blood kit (without forms) should always be brought to the clinic should a redraw become immediately necessary due to failed collection.

Kit contents

Each blood collection kit will include:

- 2 biohazard bags
- 2 purple top 3ml EDTA vacutainers **(ALWAYS CHECK EXPIRATION DATE)**
- 5 ID barcode labels (includes 1-2 extra)
- [1]1 CLIA Molecular Genetics Test Request Form signed/provider-specific[2] – printed on white paper (See **Appendix XXXIV** on page 281)
- 1 BSP Requisition Form printed on blue paper[3] (see **Appendix XXXIII** on page 279)

Kit assembly before the visit

Place print patient-specific barcode ID labels, both blood tubes, one folded biohazard bag and a folded Molecular Genetics Test Request Form and/or BSP Requisition Form into the second biohazard bag. ALWAYS bring a pair of gloves should you need to handle the blood tubes. A biohazard-labeled cooler bag should also be brought to the clinic appointment for transporting/mailing blood tubes to the BSP post-Visit 1.

After the Visit: Collection and Distribution

Each site will follow a slightly different protocol for collecting and distributing blood samples. This section describes the protocol for creating a phlebotomy order, assisting with blood collection, assembling the blood collection kits, and distributing blood samples at each site.

University of North Carolina at Chapel Hill – UNC

Creating EMR Research Phlebotomy Order

Following reveal of randomization #2 to receipt of genomic sequencing and completion of GS consent, but prior to walking the patient to phlebotomy area, the study coordinator will complete an Epic EMR Research Phlebotomy blood draw order ONLY IF a Clinical Phlebotomy order has NOT been initiated by the physician. If clinical draw HAS BEEN ordered, then the research blood collection is piggybacked on the clinical draw. Detailed instructions for creating a blood draw order can be found in **Appendix XXVII** on page 263.[4] EPIC orders require a 3-step process: 1) creation of the order, 2) approval by the ordering physician, and 3) approval by phlebotomist before the phlebotomist draws the blood.

Blood Collection

Once an order has been created, the SC should assist the participant and parent with navigating the process of the blood draw.

---

[1] **ONLY** for ECU and UNC. Mission creates Molecular Genetic Test Request electronically

[2] A UNC MGL form will need to be signed by each physician on the study team one time – then these can be photocopied for use throughout the study. The study coordinator will select the appropriate MGL/CLIA lab physician's form to include with the blood kit.

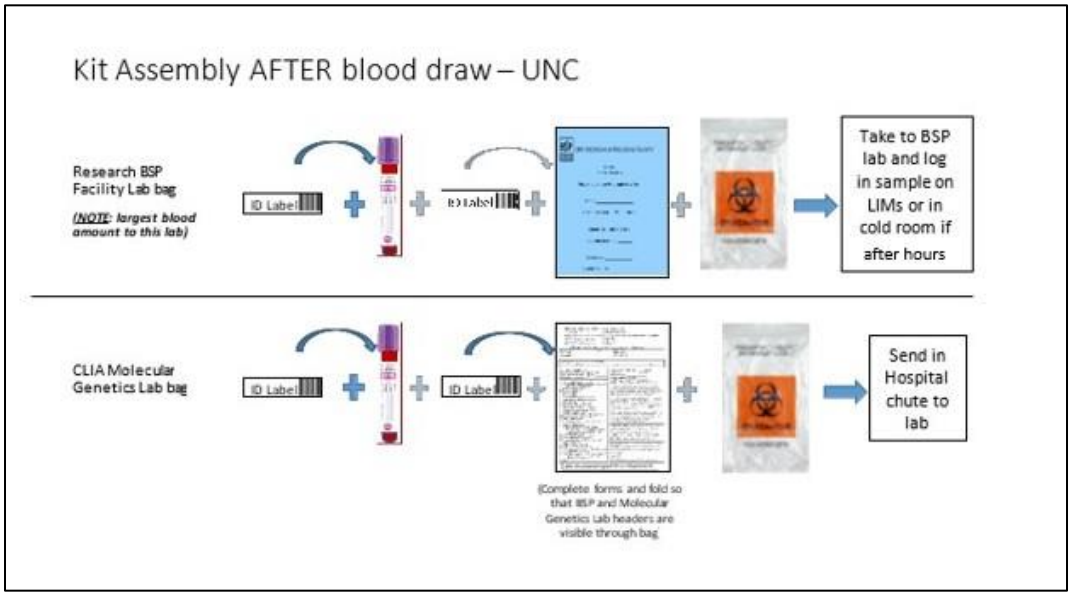
[3] The BSP Requisition Form is printed on blue paper to easily distinguish which form is which when both are folded and placed in separate blood bags

[4] All staff placing orders must comply with Epic training and receive authorization for placing research phlebotomy orders (See Appendix XXVI on page 252).

1. Walk patient and parent/relative to main lab in the UNC Women's Hospital lobby. Get a number from the clinic dispenser.
2. The parent will inform the phlebotomy worker at the check-in desk that their child needs labs. The SC or the parent should notify the worker that one of the labs is for research.
3. Accompany the family to the blood drawing area/station when their number is called.
4. Provide the phlebotomist with the tubes from the phlebotomy kit.
5. Put on gloves
6. Record the time and date blood is drawn on the relevant lab forms.
7. Receive the blood tubes from the phlebotomist and assemble the blood collection kits (see **below**)
8. Thank the family and guide them to the hospital exit.
9. Immediately, complete the "Visit 1 Biospecimen Collection" task (see page 154 **for more info**) using the tablet computer (e.g. indicating successful collection of both tubes and mailing to the labs).

Assembling the Blood Collection Kit for Distribution

Following the Epic EMR research phlebotomy order placement and blood draw,[1] label each blood tube with a barcode label. Label and complete the UNC BSP Requisition Form. Fold and insert it into one of the biohazard bags along with the 3ml EDTA vacutainer tube **with greatest blood volume**, ensuring that the header of the form is visible through the bag.



Label and complete the UNC Molecular Genetic Lab (MGL) Test Request Form by placing a participant-specific barcode label in the upper right-hand corner of the form and recording the date. Fold and insert the form into the other biohazard bag with the other 3ml EDTA vacutainer tube, ensuring that the header of the form is visible through the bag. The different colored forms (blue BSP and white CLIA) will facilitate distribution to the correct laboratory.

[1] Labeling of tubes and forms follows blood collection to avoid wasting pre-labeled tubes and forms if blood collection is declined or unsuccessful.

Blood Sample Distribution

The blood tubes for the MGL may be tubed/chuted (using the tubing station in the Phlebotomy area or behind the Pediatric Check-out desk (near the pediatric consult room). One specimen within the biohazard bag that includes the correct MGL request form should be placed in the tube and sent to the Core lab (tube station #30) or delivered to Core Lab by hand (they will deliver it to the MGL).[1]

Tubes for research exome sequencing should be delivered to the BSP, located on 3rd floor of Michael J. Hooker Building/Gilling School of Public Health. Study staff delivering specimens to the BSP will need access to this building on their UNC one card. At the BSP, blood samples should be logged in using the LIMS system (for detailed instructions on entering blood samples into the LIMS, see **Appendix XL** on page 286)

How is delivery tracked? The NCGENES 2 study coordinator logs that the samples have been delivered to each lab in the Tracking System by completing the "Visit 1 Biospecimen Collection" task (See page 154 **for more info**). The entry of the specimens into the BSP LIMs will be the indication that the BSP lab has received the sample. The entry of the specimen into RENCI Genomic Molecular Workbench by the MGL/CLIA lab will indicate that the CLIA lab has received the sample.

East Carolina University – ECU

Creating EMR Research Phlebotomy Order

Following reveal of randomization #2 to receipt of genomic sequencing and completion of GS consent, but prior to walking the patient to phlebotomy area, complete an Epic EMR Research Phlebotomy blood draw order ONLY IF a Clinical Phlebotomy order has NOT been initiated by the physician. If clinical draw HAS BEEN ordered, then the research blood collection is piggy backed onto the clinical draw. Detailed instructions for creating a blood draw order can be found in **Appendix XXVII** on page 263.[2] ECU EPIC orders require a 3-step process: 1) creation of the order, 2) approval by the ordering physician, and 3) approval by phlebotomist before the phlebotomist draws the blood.

Blood Collection

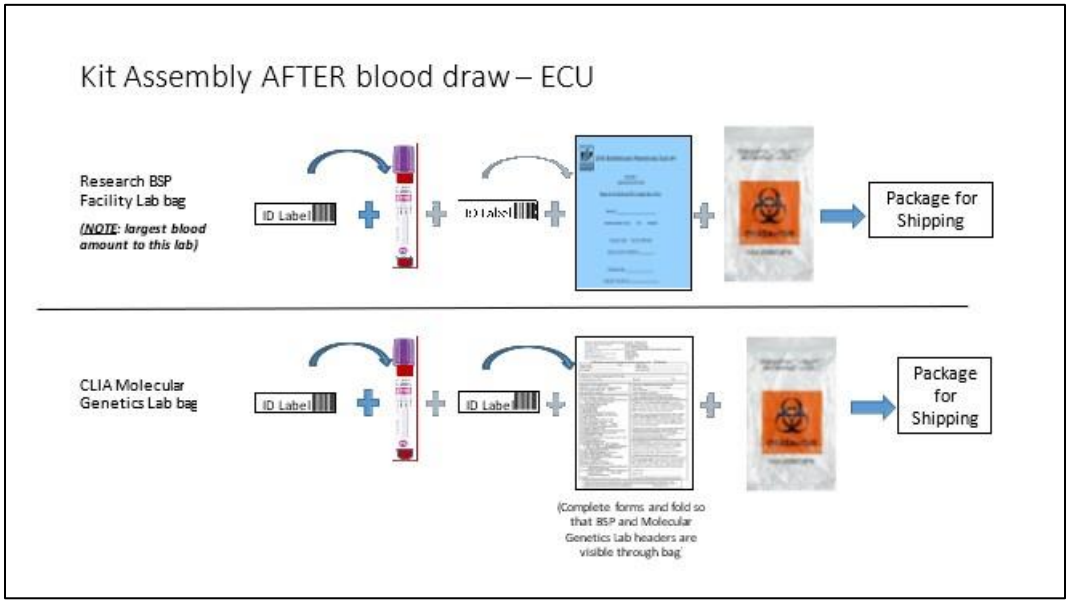
1. Walk patient and parent to main lab in the UNC Women's Hospital lobby. Get a number from the clinic dispenser.
2. The parent will inform the phlebotomy worker at the check-in desk that their child needs labs. The SC or the parent should notify the worker that one of the labs is for research.
3. Accompany the family to the blood drawing area/station when their number is called.
4. Provide the phlebotomist with the tubes from the phlebotomy kit.
5. Put on gloves
6. Record the time and date blood is drawn on the relevant lab forms.
7. Receive the blood tubes from the phlebotomist and assemble the blood collection kits (see **below**)
8. Thank the family and guide them to the hospital exit.
9. Immediately, complete the "Visit 1 Biospecimen Collection" task (see page 154 **for more info**) using the tablet computer (e.g. indicating successful collection of both tubes and mailing to the labs).

[1] The MGL staff enters sample receipt once/week (and other data as sample is processed) into the Genomic Molecular Workbench hosted by RENCI (see Appendix XXV on page 248) for details of data entry process and content)

[2] All staff placing orders must comply with Epic training and receive authorization for placing research phlebotomy orders (See Appendix XXVI on page 252).

Assembling the Blood Collection Kit for Distribution

Following the Epic EMR research phlebotomy order placement and blood draw,[1] label each blood tube with a barcode label. Label and complete the UNC BSP Requisition Form. Fold and insert it into one of the biohazard bags along with the 3ml EDTA vacutainer tube **with greatest blood volume**, ensuring that the header of the form is visible through the bag.



Label and complete the UNC Molecular Genetic Lab (MGL) Test Request Form by placing a participant-specific barcode label in the upper right-hand corner of the form and recording the date.

Note: Per CLIA standards, ECU must include ordering physician name and fax# on the Molecular Genetics Lab Test Request Form for results reporting.

Fold and insert the form into the other biohazard bag with the other 3ml EDTA vacutainer tube, ensuring that the header of the form is visible through the bag. The different colored forms (blue BSP and white CLIA) will facilitate distribution to the correct laboratory.

Blood Sample Distribution

After collection, both blood tubes should be sent in a mailer to the UNC BSP Laboratory. The mailer consists of an outer box and an inner biohazard bag and a cotton 4-tube, tube holder. The tubes will be placed in the tube holder and then this holder placed in the biohazard bag. The biohazard bag containing the blood tubes and the two labeled requisition forms (one for BSP and one for CLIA MGL) are placed in the box. The PRE-CHILLED gel-pack will be placed in the foil bubble pouch and placed on top of the tube containing bag in the box. This cold gel pack keeps the samples at approximately 2-10 degrees C during shipment. The tubes/forms will be separated and grouped appropriately upon arrival in the UNC BSP lab. A member of the BSP staff will walk the CLIA laboratory sample to the Molecular Genetics Laboratory (CLIA lab) drop-off window, indicating to the clerk that 'the sample is for the DNA Lab'. No prior notification is necessary. The BSP lab will record receipt of the BSP samples in the BSP LIMS and this status can be reported by the study analyst on a daily basis. In this way centers will know when mailed samples have arrived. Immediate notification to center-specific study coordinator by the BSP lab is necessary if samples arrive and tubes are broken or otherwise produce unsatisfactory DNA yield. This will initiate the **saliva collection** protocol at the participant's site. The BSP address for shipment is located in **Appendix XXX** on page 274.

Upon mail receipt the BSP will EMAIL notify the UNC Study Coordinator (Tracey Grant) and Co-Investigator (Jeannette Bensen) of the receipt, ID# and study site (ECU/Mission) for all NCGENES 2 samples. The entry of the

[1] Labeling of tubes and forms follows blood collection to avoid wasting pre-labeled tubes and forms if blood collection is declined or unsuccessful.

specimens into the BSP LIMS upon arrival will be the indication that the BSP lab has received the sample. The entry of the specimen into RENCI by the MGL/CLIA lab will indicate that the CLIA lab has received the sample.

NOTE: UNC staff may assist ECU in the clinic and thus transport all samples back to the respective BSP and CLIA labs (therefore mailing of specimens by ECU to UNC BSP may not be required).

Mission Health - Asheville

Creating EMR Research Phlebotomy Order – Mission Health

Following reveal of randomization #2 to receipt of genomic sequencing and completion of GS consent, but prior to walking the patient to phlebotomy area, complete a Cerner EMR GENE Order with comments of "NCGENES 2 Research Phlebotomy blood draw - see kit for instructions and tubes- genetics to pick up". Detailed instructions for creating a blood draw order can be found in **Appendix XXVIII** on page 272.[1]

Note: this may not be necessary if a clinical phlebotomy blood draw has been ordered and the research team is allowed to piggyback on to the clinical draw to collect the 2 research blood tubes.

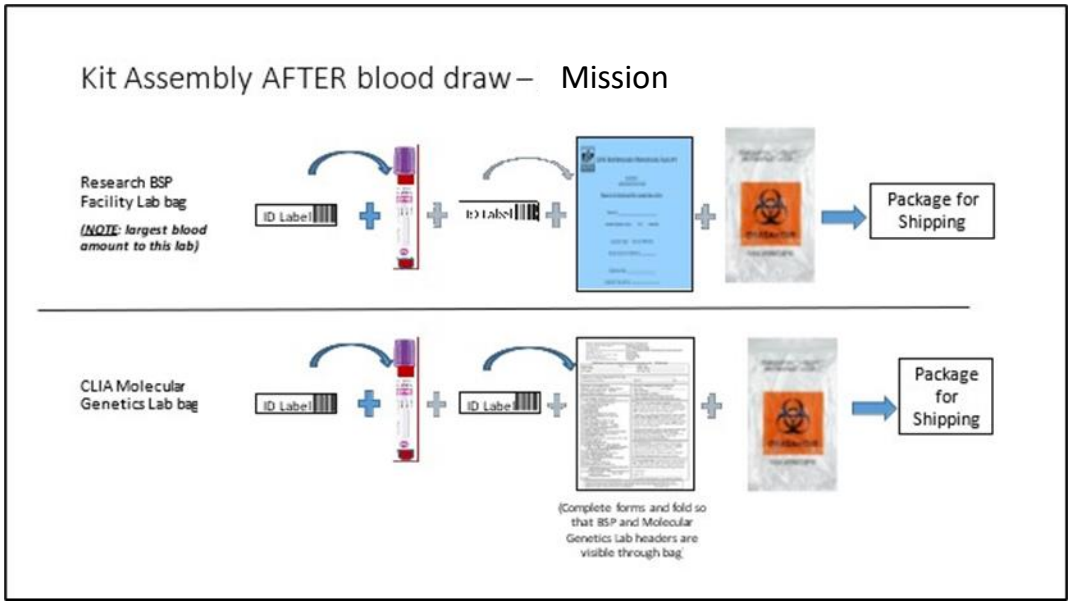
Blood Collection

1. Walk patient and parent to Mission Children's Phlebotomy Laboratory.
2. The parent will inform the phlebotomy worker at the check-in desk that their child needs labs. You or the parent should notify the worker that one of the labs is for research.
3. Accompany the family to the blood drawing room when they are called.
4. Provide the phlebotomist with the tubes from the phlebotomy kit.
5. Put on gloves
6. Record the time and date blood is drawn on the relevant lab forms.
7. Receive the blood tubes from the phlebotomist and assemble the blood collection kits (see **below**)
8. Thank the family and guide them to the hospital exit.
9. Immediately, complete the "Visit 1 Biospecimen Collection" task (see page 154 **for more info**) using the tablet computer (e.g. indicating successful collection of both tubes and mailing to the labs).

[1] All staff placing orders must comply with Cerner training and receive authorization for placing research phlebotomy orders (See Appendix).

Assembling the Blood Collection Kit for Distribution

Following the Cerner EMR research phlebotomy order placement and blood draw, label each blood tube with a barcode label. Label and complete the UNC BSP Requisition Form. Fold and insert it into one of the biohazard bags along with the 3ml EDTA vacutainer tube **with greatest blood volume**, ensuring that the header of the form is visible through the bag.



Label and complete the UNC Molecular Genetic Lab (MGL) Test Request Form by placing a participant-specific barcode label in the upper right-hand corner of the form and recording the date.

Blood Sample Distribution

After collection, both blood tubes should be sent in a mailer to the UNC BSP Laboratory. The mailer consists of an outer box and an inner biohazard bag and a cotton 4-tube, tube holder. The tubes will be placed in the tube holder and then this holder placed in the biohazard bag. The biohazard bag containing the blood tube and the BSP lab requisition form is placed in the box. The PRE-CHILLED gel-pack will be placed in the foil bubble pouch and placed on top of the tube containing bag in the box. This cold gel pack keeps the samples at approximately 2-10 degrees C during shipment. The BSP lab will record receipt in the BSP LIMs system and this status can be reported by the study analyst on a daily basis. In this way centers will know when mailed samples have arrived. Immediate notification to center-specific study coordinator by the BSP lab is necessary if samples arrive and tubes are broken or otherwise produce unsatisfactory DNA yield. This will initiate the **saliva collection** protocol at the participant's site. The BSP address for shipment is located in **Appendix XXX** on page 274.

Upon mail receipt the BSP will EMAIL notify the UNC Study Coordinator (Tracey Grant) and Co Investigator (Jeannette Bensen) of the receipt, ID# and study site (ECU/Mission) for all NCGENES 2 samples. The entry of the specimens into the BSP LIMs upon arrival will be the indication that the BSP lab has received the sample.

Saliva Collection for Child Participants

Overview

NCGENES 2 prioritizes collection of blood samples from child participants consented to exome sequencing. A family should be offered saliva collection only if one of the following scenarios occurs:

1. *No Blood* was collected at visit 1: Neither tube of blood was able to be collected
    a. Attempted blood collection was not successful[1]
    b. Parent refused blood collection for child
2. *Insufficient blood* was collected at visit 1: One or both tubes have < 1ml of blood (1 ml is minimum volume for each lab) at collection
3. *Samples failed processing (FAILED RESULTS)*: One or both labs (BSP & CLIA/MGL) notify NCGENES 2 Study Coordinator/Co-Investigator that completely or partially and saliva kit(s) are needed to obtain DNA for testing
    a. Sample tube(s) arrive broken
    b. A sample is dropped in the processing lab
    c. The volume is 1ml or more at collection but yields an insufficient amount of DNA

Scenarios 1 and 2 should be identified by the SC at the time of the blood draw. Scenario 3 will occur after the conclusion of Visit 1. The protocol in each scenario is described below.

Saliva Collection Initiated at Visit 1 Phlebotomy

Scenario 1: No Blood Drawn

The Study Coordinator may encounter a scenario in which blood collection fails entirely at phlebotomy or the parent declines blood draw for the child at Visit 1. In these cases, the parent is offered the option of saliva collection by mail. In the tracking system, this will be recorded in the "**Visit 1 Biospecimen Collection**" task (see page 154) with "Question 1. Blood collection Complete?" Answering this question "No" will *autogenerate* two tasks: "**V1 BSP Saliva Kit Due to No Blood Drawn**" (see page 156) and "**V1 CLIA Saliva Kit Due to No Blood Drawn**" (see page 155). Because DNA will be needed for both labs, two saliva kits are mailed to the parent.

Scenario 2: Insufficient Blood Collection at Visit 1

The Study Coordinator may encounter a second scenario of insufficient sample collection (<1ml in either tube). If this occurs the Study Coordinator will ask the phlebotomist to attempt to draw an additional tube (or attempt a second stick if the parent agrees) to see if enough blood may be collected to satisfy each lab's requirements (at least 1 ml for each lab). If the phlebotomist is unable to obtain two 3ml tubes (or a set of 3 tubes that provides at least 1 ml for each lab), the Study Coordinator will inform the family that they will receive 1-2 saliva kits by mail depending on the need, e.g. if enough blood was collected (1 ml) to satisfy the needs of one of the labs, then only 1 kit would be needed (see **Saliva Collection section** on page 178). In the tracking system, this will be recorded in the "**Visit 1 Biospecimen Collection**" task (see page 154) with Questions 2-3. Answering Question 2 "No" will *autogenerate* the "**V1 BSP Saliva Kit Due to No Insufficient Sample**" task (see page 158) and answering Question 3 "No" will *autogenerate* the "**V1 CLIA Saliva Kit Due to Insufficient Sample**" task (see page 157).

Scenario 3: Saliva Collection Initiated by Lab (BSP/CLIA/Mission CLIA)

Failed Results

If insufficient specimen is received by mail (either blood (<1ml) or saliva (<0.5ml)) in the lab(s) OR if DNA extraction fails to produce sufficient quantities of DNA for study needs at either lab, then the lab staff must notify Jeannette Bensen, **jbensen@med.unc.edu** AND 919-801-9267) and the Study Coordinator by **email and phone** (Tracey Grant (**traceyg@unc.edu** AND 919-306-1410) within 24 business hours of sample receipt/sample extraction failure to allow for recollection by saliva kit mailing. The UNC research team will notify the partner site if relevant and work together to ensure that the tracking system is updated and the saliva kit(s) are properly mailed (in most cases the UNC staff will be

[1] In some cases an extra tube or second stick (after the clinical draw) may be necessary to acquire a sufficient volume of blood, so the SC should always have an extra set of tubes/labels should this be necessary at blood draw

responsible for mailing saliva kit(s) to collect a pediatric patient's (Child's) specimen).[1] If the need for saliva collection arises after the conclusion of Visit 1, the study coordinator or other study staff under the direction of the study coordinator will notify the parent by phone of the need for a saliva kit collection. This contact is recorded in **communication log** in the tracking system. Additionally, the local Study Coordinator must record the failed results from blood collection (failed extraction/insufficient blood) reported by the lab(s) by creating "V1 BSP/CLIA Saliva Kit Due to Failed Results" task for one or both labs. For detailed instructions on how to create these tasks, see the "**Using the Tracking System**" section on page 75.

Note: ALL POST-VISIT 1 Phlebotomy sample scenarios such as insufficient samples received by mail and failed extractions are designated in the patient tracking system as FAILED RESULTS by Creating the V1 lab – saliva kit due to failed results task. Note to complete that task go to the task list for the patient and click 'pending', this will take you to a mail merge to create the label for the kit. Once the mail merge is completed the date associated with this task will be the date the kit was mailed.

Sample Fails Identity Check

If the identity check genotypes assayed in the CLIA Lab do not match the identity genotypes assayed by the High Throughput Sequencing Facility, a recollection of DNA via mailed saliva kit will be required. The Molecular Analysis group (Bradford Powell, **bradford.powell@unc.edu**) will notify the study coordinator if discordance in identity genotyping occurs. In the event of failed identity check, one or two DNA saliva kits will be mailed, one for DNA extraction in the UNC CLIA laboratory and possibly one for DNA extracted in the BSP (the need for one vs. two saliva kits will be determined by the molecular analysis team at the time of identify check discordance). Upon receipt, the CLIA laboratory will perform genotyping of the identity-check SNPs on their received sample, and the decision for additional processing of the BSP sample will depend on whether the new CLIA genotyping establishes sample identity.

Saliva Kit Mailing Preparation

A patient saliva kit[2] includes: one large bubble mailer with one or two saliva kit(s) (swab and case), one or two Ziploc bag(s), and one folded, pre-posted and addressed bubble mailer (this bubble mailer will be smaller than the larger one that the rest of the supplies are mailed in). One saliva kit is mailed if only one lab needs a sample, but 2 kits are mailed if both BSP and CLIA labs need samples. ***Sample collection swabs/swab tubes are removed from the plastic kit box(es) and are labeled with patient-specific barcode labels (generated from tracking system) and then replaced back into the plastic kit box(es) prior to mailing.*** An example of a pediatric participant (child) ID barcode label is shown below:
Patient barcodes will always end with -00.

[1] If insufficient sample is identified by one lab, the UNC Study Coordinator should email the other study lab to confirm that the other sample is sufficient prior to mailing the saliva collection kit in case 2 saliva kits are needed.
[2] See Appendix XXXII on page 268 for information on ordering supplies

One plastic box/kit will go inside each provided Ziploc bag to prevent leakage during transport. The large bubble mailer will also include three sheets of paper:

1. an NCGENES 2 participant saliva letter (see **Appendix VIII** on page 203)
2. an NCGENES 2 saliva kit mailing instructions sheet that outlines how to return the saliva back to the BSP (see **Appendix IX** on page 204) and
3. enlarged Oragene OG 575 saliva kit instructions printed on a colored sheet of paper (yellow; these instructions are the same as the ones inside the saliva kit, however this form additionally collects the data and time of saliva collection and is returned to UNC along with the saliva samples. (see **Appendix X** on page 205)

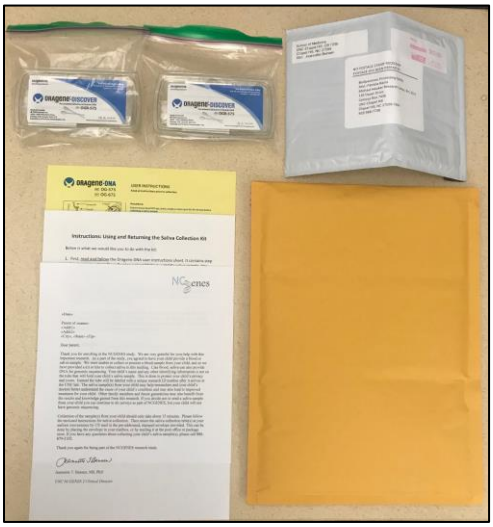
**All relevant paperwork can be found on IRBIS.*

Example return labels[1] and saliva mailing contents/package for 2 kits shown here.

School of Medicine
UNC-Chapel Hill, CB 7295
Chapel Hill, NC 27599
Attn: Jeannette Bensen

NO POSTAGE STAMP NECESSARY
POSTAGE HAS BEEN PREPAID BY

BioSpecimen Processing Facility
Attn: Patricia Basta
Michael Hooker Research Center, Rm. 3213
135 Dauer Drive
Campus Box 7406
UNC-Chapel Hill
Chapel Hill, NC 27599-7406
919-966-7738



For Mission saliva samples, the Mission SC will address the return envelope to the UNC BSP.

Mailing and receipt of saliva kits as well as distribution to relevant lab(s) is recorded in the patient tracking system by the Study Coordinator. Routine reports are run by the study analyst to ensure that the loop is closed from sample collection to receipt and processing.

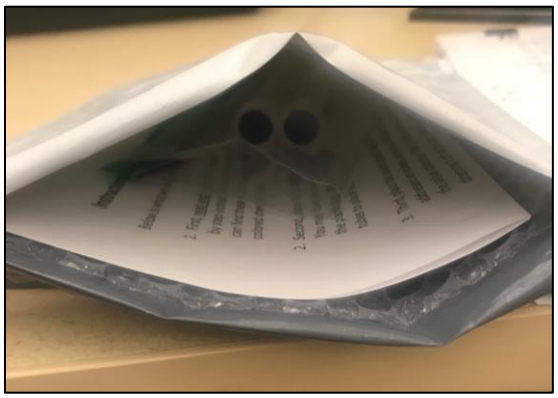
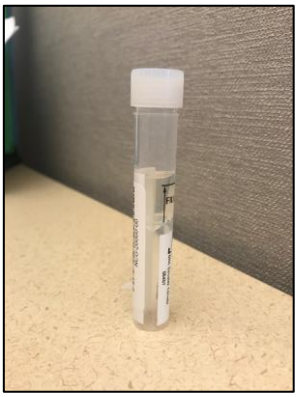
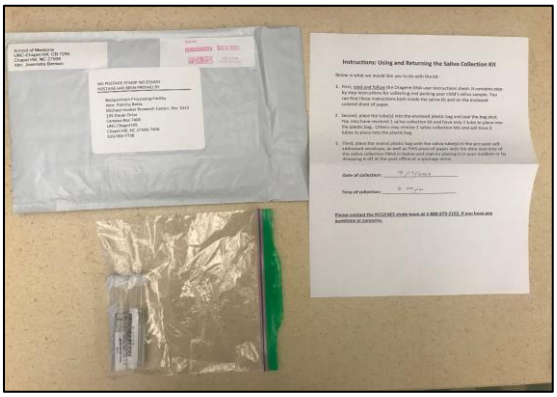
The participants will be instructed on the NCGENES 2 saliva kit collection process and how to return the completed saliva kit(s) by mail to the UNC BSP. The parent will include the following in the returned pre-posted and addressed bubble mailer:

1. NCGENES 2 saliva kit mailing instructions with the date and time of the saliva collection completed by the parent,

[1] Use the Dymo label maker to print the return address and the 5264 shipping labels to print the BSP address

2. the Ziploc bag with one or two saliva tube(s).

Example return mailings shown here.

Upon receipt of returned saliva kits to the lab, the BSP staff will notify Jeannette Bensen (jbensen@med.unc.edu/919-801-9267) and Tracey Grant (traceyg@unc.edu). Returned kits will follow the same sample distribution as outlined for blood specimens above and will depend on site (UNC, ECU, Mission Health) and the lab reporting the sample failure (UNC BSP lab, UNC CLIA/MGL lab, Mission Health CLIA lab). The UNC Study Coordinator/staff will go to the BSP when notified of saliva sample receipt and will take the appropriate request forms and ensure distribution to the correct labs:

1. Blue BSP request form – be sure to indicate that saliva is the "TYPE" of sample on the form and place an appropriate label on the form
2. MGL/CLIA request form – be sure to complete the signed version (correct ordering physician) of the appropriate child/pediatric patient saliva request for exome sequencing form.

Saliva Collection For Parents/Relatives

Overview – Parent/Relative

To initiate the parent/relative saliva kit collection the clinical team (MD/GC) must contact the local SC, who must in turn notify the UNC SC (Tracey Grant) and Jeannette Bensen of the need to collect a DNA sample from parents/relatives to resolve interpretation of the child's exome sequencing results. Confirmatory DNA tests will be paid for by NCGENES 2 research funds, performed in the MGL/CLIA lab (UNC), and reported to the child's medical record. Any adult relatives (e.g. parents) must be consented in advance of saliva collection. The general procedure for this will be to mail the consents (2 copies of each for each parent so that one can be signed and returned to UNC/Mission and one kept by the parent).[1] This can be done in conjunction with the saliva kit mailing, i.e. each parent is mailed 1 saliva kit with an accompanying consent and other standard documents that are contained in the saliva kit (described below). The Study Coordinator/Genetic Counselor who typically consents the participants to genomic sequencing should call the parent to consent them to the parent/adult relative DNA testing. Following this phone consent, the parent signs and dates the form and places it in the addressed/postage paid envelope.

DNA Sample needed from child relative? Relatives under the age of 18 must provide assent and have a parent/legal guardian consent in advance of saliva collection. ***Assenting by phone is not approved.*** All assents to relative testing must be made in person for NCGENES 2 to cover family testing.

Saliva Kit Mailing Preparation

A parent/relative saliva kit[2] includes: one large bubble mailer with one saliva kit (swab and case), one Ziploc bag, and one folded, pre-posted and addressed bubble mailer (this bubble mailer will be smaller than the larger one that the rest of the supplies are mailed in). ***The saliva tube is removed from the kit and the appropriate participant label is placed directly on the tube. Labels are generated from the tracking system. The labeled tube is then replaced back inside its plastic kit box.*** Examples of participant ID barcode labels for mom and dad are shown below:

Mom – 01 Dad – 02

Parent barcodes will always end with -01 for mom/parent 2 and -02 for dad/parent 2. The indication that the saliva kit is for -01 mom/parent 1 and -02 dad/parent 2 should be explicit, with a barcode on the saliva tube, a label that says "mom - 01" or "dad - 02" on the outside of the plastic casing holding the saliva kit, and a label that says "mom - 01" or "dad - 02" on the outside of the plastic Ziploc bag.[3]

The plastic box/kit will go inside the Ziploc bag to prevent leakage during transport. The saliva tube will be pre-labeled with a parent appropriate barcode with the correct participant ID ending in "-01" for mom/parent 1 and "-02" for dad/parent 2. Inside the large bubble mailer will also include four sheets of paper:

1. an NCGENES 2 parent saliva letter (see **Appendix XI** on page 206)
2. Parent/Relative DNA testing consent (two copies per parent, one to return and one to keep)
3. an NCGENES 2 saliva kit mailing instructions sheet that outlines how to return the saliva back to the BSP[4] (see **Appendix XII** on page 207), and

[1] In some cases, consent and/or saliva collection can occur in the clinic should the family be returning for another reason. A clinic visit should not be scheduled solely for the purpose of parent or relative consent/collection.
[2] See Appendix XXXII on page 268 for information on ordering supplies
[3] Note: -03 will designate other relatives as needed – the relative type (mom, dad, maternal aunt, etc should be written on the MGL forms that go to the CLIA lab)
[4] These instructions are the same as the ones inside the participant saliva kit outside of additionally recording the data and time of saliva collection. These should be returned to UNC along with the saliva samples.

4. enlarged Oragene OG 575 saliva kit instructions printed on a yellow sheet of paper (see **Appendix X** on page 205)

**All relevant paperwork can be found on IRBIS.*

Parents/relatives will be instructed on the NCGENES 2 saliva kit collection process and how to return the completed saliva kit by mail to the UNC BSP. The parent will include the following in the returned pre-posted and addressed bubble mailer:

1. NCGENES 2 saliva kit mailing instructions with the date and time of the saliva collection completed by the parent,
2. the Ziploc bag with one or two saliva tube(s).

Upon receipt of returned saliva kits to the UNC lab, the BSP staff will notify Jeannette Bensen (**jbensen@med.unc.edu**; 919-801-9267) and Tracey Grant (traceyg@unc.edu). Samples collected from Mission participants may be mailed to the UNC BSP and then brought to the UNC CLIA lab if necessary. The UNC RA will go to the BSP lab when notified of saliva sample receipt and will take the appropriate request forms OR actions (enter into an electronic system) and ensure logging of sample receipt and distribution (UNC MGL CLIA lab). Two forms for parental/relative collection are needed by the UNC MGL CLIA lab (2 below):

1. MGL/CLIA request forms:
    a. Be sure to complete (label, etc.) the signed version (with correct ordering physician) of the appropriate child/pediatric patient saliva request for exome sequencing form.
    b. Label the Phenotypic information MGL request form with a parent-specific bar code (generated from the tracking system). Ask the genetic counselor to complete the MGL request form with phenotypic information on each parent when the initial request for parental saliva kits is made. Hold the form until sample arrival and then combine it with the first form/MGL confirmatory testing request form (custom seq family variant) and send it with the sample to the MGL/CLIA lab.

Mailing and receipt of the saliva kit is tracked in the communication log of the tracking system by the SC or designee. Any consents should be uploaded to the appropriate participant's document tab. Receipt of the sample is also tracked in the RENCI molecular workspace and in the MGL/CLIA laboratory LIMs. Routine reports are run by the study analyst to ensure that the loop is closed from sample collection to receipt and processing.

Mailed Saliva Kit Tracking– for samples processed and logged into the BSP LIMs

The UNC BSP will notify a member of the NCGENES 2 team when saliva samples have arrived to the BSP. The NCGENES SC/RA will transport the saliva kit to the CLIA lab along with the appropriately completed MGL forms. For returned parental saliva kits, an additional MGL form titled, "NCGENES Request for Family Studies: Molecular Genetics Laboratory" will be completed by the participant's physician or more likely genetic counselor. The study team will request that this form be completed when the request is made for parental testing. The genetic counselor will return the completed form to the study team who will hold it until sample arrival in BSP. At that time the study team will complete the MGL request form (pre-signed by the relevant ordering physician) for parental saliva confirmatory testing and will combine that form with the Family Studies form completed by the counselor. Both forms will be sent with the saliva sample to the MGL/ CLIA lab by the hospital tube system. Each parent's saliva sample will have their own set of 2 MGL forms. The MGL will log receipt of saliva kit in the RENCI workflow system, extract DNA from the saliva kit (See **Appendix XXII** on page 238), and perform the requested confirmatory testing.

ECU – Saliva Sample Request/Need

The local Study Coordinator is responsible for coordinating saliva kit mailing for the UNC MGL Lab. The saliva kit is mailed from the UNC Study Team (including appropriate lab request forms) for ECU to the family and is/are returned to the UNC BSP. If two kits are mailed both are returned to the UNC BSP lab return address. The BSP will notify the study team who will be responsible for completing appropriate forms and distributing the saliva samples to the appropriate UNC lab for processing. The UNC Study Coordinator records and logs the contact with the parent, as well as the kit mailing/blood recollection and receipt and distribution of the sample in the patient tracking system.

Mission Health – Saliva Sample Request/Need

The Mission Study Coordinator is responsible for coordinating saliva kit mailing. The saliva kit is mailed from the Mission Health team to the family and is returned to the Mission Health Study Coordinator. The kit will have the Mission health CLIA Lab return address. The Mission Study Coordinator records and logs the contact with the parent, as well as the kit mailing and receipt and distribution of the sample in the patient tracking system. The Mission team will also perform the phone consent and upload signed consent forms to the tracking system. The MGL will log receipt of saliva kit in the RENCI workflow system and extract DNA from the saliva kit (See **Appendix XXII** on page 238). As with blood DNA, the CLIA MGL will perform identify check genotyping and store/hold remaining DNA for confirmation of positive findings if needed.

Note: Saliva collection kits will be ordered centrally by the Biospecimen Processing Facility. Saliva kits will be supplied to Mission Health. [1]

Biospecimen Processing, Distribution and Reporting

All specimen processing protocols are available in the Appendix. This section provides an overview of the general process:

Samples processed and distributed by the UNC Biospecimen Processing Facility (BSP)

Blood or Saliva Specimen Processing

For pediatric participants enrolled at either UNC, ECU or Mission Health in the genomic sequencing arm of the clinical trial, either a 3ml blood specimen or saliva swab kit is sent to the UNC BSP laboratory where DNA is extracted and quantitated. The UNC BSP Lab sample flow overview and blood and saliva DNA extraction and quantitation protocols are available in the appendix. Following DNA extraction and quantitation, the sample is sent to the Berg Lab for library preparation prior to high-throughput sequencing.

Distribution of blood or saliva DNA to Berg Lab

After BSP processing (DNA extraction), quantitation and aliquoting the samples are placed into the protocol "BSP_TO_BERG". The BSP lab staff will send the Berg lab (specifically Alicia Brandt) an email indicating that a set of samples is ready and provide the list of sample IDs in the batch. Batches are approximately 10 or more samples per transfer. Berg lab staff (Alicia Brandt or technician TBD) pick up the samples in the BSP and sign a copy of the sample manifest (hard copy of the BSP_TO_BERG worksheet). This signed copy goes into the BSP transfer log and the Berg lab takes a second copy. The BSP staff input the pickup information (courier, date, time) into the BSP LIMS and then close out the worksheet. This then allows the Berg lab to perform a core transfer of the samples into their (Berg) LIMS.

Samples processed and POSITIVE OR UNCERTAIN RESULTS reported by the UNC Molecular Genetics CLIA Lab (UNC MGL)

Blood or Saliva Specimen Processing

For pediatric participants in the genomic sequencing arm of the clinical trial enrolled at UNC, Mission, or ECU either a 3ml blood specimen or saliva swab kit is sent to the UNC Molecular Genetics Lab (UNC MGL) where DNA is extracted, quantitated and identity check genotype completed (used to resolve potential sample mix-ups). The UNC MGL blood and saliva DNA extraction, quantitation and identity check protocols are available in the appendix. Following DNA extraction and quantitation, the DNA sample undergoes genotyping for 8 single nucleotide polymorphisms to be used for comparison to data obtained through GS. The remaining DNA sample is held until notification at the molecular sign-out meeting that positive or uncertain research findings must be confirmed.

Confirmation of Positive or Uncertain Research Genomic Sequencing Findings

[1] See the Supplies list in Appendix XXXII on page 268.

Research sequencing confirmation is conducted via Sanger sequencing in the UNC MGL CLIA lab (protocol available in the Appendix). CLIA confirmed results are placed in the patient's medical record which automatically notifies the UNC ordering physician. If the CLIA confirmation is being conducted for an ECU or Mission study participant, then the CLIA lab results will be sent via fax to the ordering physician. Negative research GS results are NOT confirmed in the CLIA clinical lab. Negative results notification is done by the NCGENES 2 Patient Tracking System that generates an email notification to the ordering physician indicating results are available. This notification includes a patient friendly research result report and points the MD to the molecular analysis tab to see the negative result report and requests the physician to complete the physician post-RoR survey. All results (positive, negative or uncertain) are reported to the family by the clinical genetic counselor or ordering physician by phone or in-person.

Sample reporting of NEGATIVE RESULTS by UNC NON-CLIA RENCI System - All Sites

Return of NEGATIVE RESEARCH results to NCGENES 2 patients (All Sites)

Negative research results cannot be confirmed, thus negative results will not be confirmed by either UNC CLIA Molecular Genetics Labs.

Instead negative results notification is done by the NCGENES 2 Patient Tracking System that generates an email notification to the ordering physician indicating results are available. This notification includes a patient friendly research result report and points the MD to the molecular analysis tab to see the negative result report and requests the physician to complete the physician post-RoR survey. All results (positive, negative or uncertain) are reported to the family by the clinical genetic counselor or ordering physician by phone or in-person.

Final Genomic Sequencing Report to Parent/Caregiver

At the end of the study, after the final 12-month telephone survey, parents will be mailed a final thank you letter for their participation (a copy of the final genomic sequencing results for their records MAY accompany their thank you note).

APPENDICES

Appendix I. Etiology Criteria

CLINICAL CRITERIA
Research Project: NCGENES 2
Updated 2/21/2020

Clinical screening criteria to determine pediatric patient NCGENES 2 eligibility

Instructions: Eligibility screening has two phases. First, use the "Initial (Non-Phenotypic) Eligibility Criteria" (Page 1) to determine if the patient should be screened by phenotype. Then, if the participant meets the first set of criteria, use the "Phenotypic Eligibility Criteria" (Page 2-3) to determine whether the patient qualifies by phenotype. ***Both sets of criteria include separate inclusion and exclusion criteria.***

It is anticipated that this document will evolve as the study unfolds. Likely new criteria both inclusion and exclusion will emerge as we begin to screen potential patient records at all sites.

Contact person for questions: Jeannette Bensen, PhD (**jbensen@med.unc.edu**, 919-801-9267 (cell))

Initial (Non-Phenotypic) Eligibility Criteria

1. **Inclusion criteria:**
   a. Age at the initial evaluation: less than 16 years old at NCGENES 2 study eligibility determination.
   b. The following types of pediatric patients with *first visit* to either outpatient Pediatric Neurology or Pediatric Genetics and Metabolism clinic will be are considered eligible for phenotypic screening and if phenotypically eligible are invited to enroll in NCGENES 2:
      i. Patients who have never been worked up and who do not currently have a known etiology for their condition but it is suspected to be genetic
      ii. Patients who are referred from an outside UNC/NCGENES 2 Study Site neurologist - 'second opinion'/patient has had some work up
      iii. Patients initially seen as an inpatient at UNC/NCGENES 2 Study Site, with subsequent first visit to Pediatric Neurology or Pediatric Genetics and Metabolism clinic
      iv. Patients who have previously been seen in Pediatric Neurology (1 or more times) and are referred as a New patient to Pediatric Genetics are considered eligible for phenotypic screening and if phenotypically eligible are invited to enroll in NCGENES 2

2. **Exclusion criteria:**
   a. Patients who are 16 years or older at NCGENES 2 study eligibility determination
   b. The following types of pediatric patients with a 'New' visit to either outpatient Pediatric Neurology or Pediatric Genetics and Metabolism clinic are considered NOT eligible for phenotypic screening:
      i. Patients designated as 'New visit' to either (Peds Genetics or Peds Neuro) clinic because it has been > 3 years since their last visit to the clinic (essentially, they are a return patient to the same clinic)
      ii. Patients designated as 'New visit' to either (Peds Genetics or Peds Neuro) clinic but have been seen in <=3 years ago in that same clinic. This can be determined by a review of the medical record where it can be clearly seen that this patient has been seen by the same or a different doctor in that same clinic in the past. For example, a patient has been seen 2 times in a clinic and then calls needing an immediate appointment and the MD tells the scheduler to put them in ANY type of clinic appointment available and the scheduler places the return patient in a "New visit' slot.

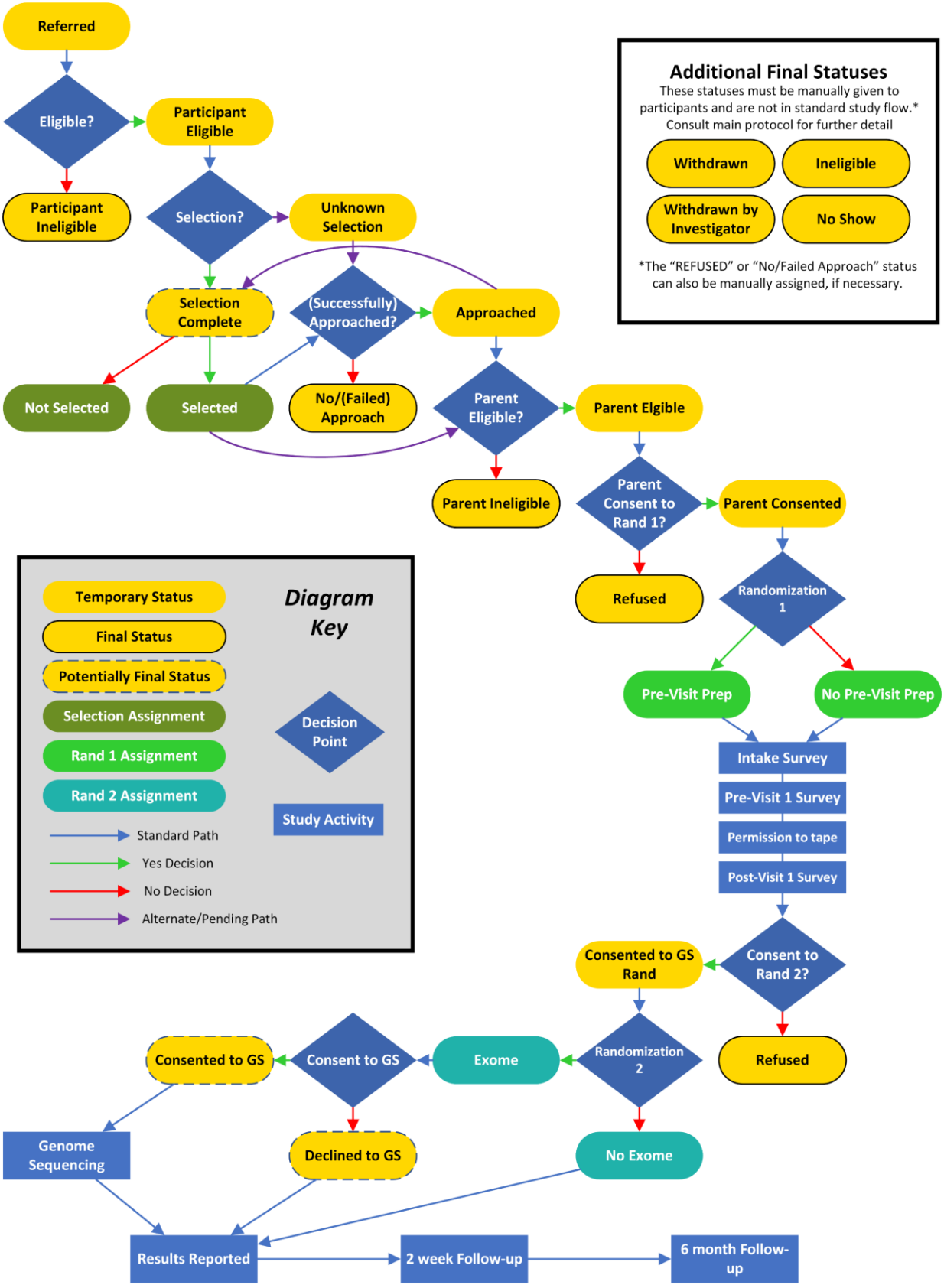
Phenotypic Eligibility Criteria

1. **Inclusion Criteria**
   a. One of the following conditions/initial referral reasons:

i. Epilepsy and seizures (Any seizures if with developmental delays. Any seizures onset prior to 6 months old. But not for example febrile seizures or a single afebrile seizure in a child with typical development).
ii. Neuromuscular disorder (this may include muscle weakness, elevated CKs, neuropathies, arthrogryposis, etc)
iii. Brain malformation (this includes lissencephaly, schizecephaply, megaencephaly, polymicrogyria, pachygyria, etc)
iv. Intellectual disability/autism spectrum disorder with intellectual disability/developmental delay
v. Hypotonia (unexplained)
vi. Inborn errors of metabolism suspected
vii. Movement disorder (dystonia, ataxia, etc)
viii. Other conditions that are considered have a genetic etiology (such as severe congenital central hypoventilation syndrome).
ix. Microcephaly/Macrocephaly with developmental delays or any that are >2.5 SD from normal.
x. Metabolic indications that WILL be included [represents 30% of metabolic patients] are:
    1. R/O mitochondrial disorder
    2. R/O metabolic with neurologic component like;
        a. Hypotonia
        b. Ketosis
        c. Hypoglycemia
        d. Hepatomegaly
        e. Cyclic vomiting
        f. Splenomegaly
xi. Chromosome Abnormality (some select cases see below)
    1. Note: A microarray VUS would still effectively be in a "diagnostic odyssey" and be eligible, however it could also be a real "positive" in which case the referral would be for post-test counseling and the case would be ineligible.
xii. Hearing loss
xiii. Dysmorphic features
xiv. Skeletal Dysplasia (e.g., achondroplasia, OI, Unknown)
xv. Multiple congenital anomalies
    1. Includes multiple congenital anomaly syndromes with no etiology (e.g., VATER, CHARGE (make sure not related to CHD 7 gene mutation), Pierre Robin
xvi. Rhabdomyolysis
xvii. Hereditary/Childhood Cancer while typically out of scope for NCGENES 2, should not be an absolute exclusion criterion. There are some multisystemic genetic syndromes where cancer can be seen (e.g., Wilms tumor in the setting of overgrowth, neonatal hypoglycemia, and exomphalos would suggest Beckwith-Wiedemann syndrome). When such a condition is described provide as much detail as possible related to any other clinical findings, path report and send to Jonathan for determination of eligibility.
xviii. Fetal Alcohol Syndrome – while an entirely non-genetic disorder BUT may be confused with other genetic syndromes. Many cases of FAS are challenging for referring pediatricians to diagnose and thus in some cases these referrals will be considered eligible. Specifically, if *developmental delay* is included as a finding in the child with FAS referral we will consider them ineligible. We will let the geneticist rule the child in or out during the clinic visit. If the clinical geneticist diagnosis FAS then this will be a 'diagnosis at visit 1' and thus these patients will be investigator withdrawals with the typical ineligibility status sentence.

2. **Exclusion criteria**
    a. Any known history of the following acquired conditions such as ischemic encephalopathy (including perinatal stroke), CNS infection (such as meningitis or encephalitis), brain trauma, autoimmune/inflammatory disorder or other acquired injury to brain/nerve/muscle. Macrocephaly in a patient with known hydrocephalus/shunt.
    b. Patients who have a known genetic diagnosis that explains the clinical presentation.
    c. Most metabolic will NOT be included (e.g., abnormal newborn screen) [represents 70% of metabolic patients]
    d. Café au lait (isolated)
    e. Trisomy 21, 18, 17, 16p
    f. Microdeletion 22q
    g. Family history of a known genetic condition (e.g., CF, being referred for genetic counseling)
    h. Short stature (isolated)
    i. Obesity (isolated)
    j. Failure to thrive (isolated)
    k. Cleft lip and/or cleft palate (isolated - not part of a syndrome)
    l. R/O connective tissue disorder/hypermobility
        i. R/O Ehlers-Danlos Syndrome
        ii. R/O Marfan Syndrome
    m. Congenital nevus (isolated)
    n. Congenital scoliosis (isolated)
    o. Isolated Attention Deficit/Hyperactive Disorder (ADHD)

Appendix II. Study Statuses



Appendix III. Randomized Recruitment

Recruitment sampling and randomization in NCGENES
2/14/2018

1. **Recruitment Sampling** (randomization recruitment) – Using the last full year (2016) of patients coming to relevant UNC clinics where NCGENES2 patients are seen identify the frequency of the following groups (% of total patients). Operationally recruit all African Americans/Hispanic/Medicaid (under-served/under-represented) patients and calculate sampling proportions for the remaining group (served). Randomly assign a sample weight to patients in this group and recruit only if the randomly assigned proportion is at or below the sampling weight.

Randomized Recruitment Charts for UNC NCGENES 2 Clinics

Pediatric Genetics and Metabolism Clinic

Per yr*	Project number in UNC clinics	% selected randomly for study	Number selected	Minus 10% ineligible	Eligible	Minus 30% non-responders or refusals	Projected cases enrolled	Proportion
AA -Hispanic-Medicaid	1438	100%	1438	144	1294	388	906	60%
Non-AA - Non-Hispanic - Non-Medicaid	1500	64%	960	96	864	259	605	40%
ALL							1511	100%
Pediatric Neurology Clinic								
AA -Hispanic-Medicaid	3035	100%	3035	304	2731	819	1912	60%
Non-AA - Non-Hispanic - Non-Medicaid	2640	77%	2033	203	1830	549	1281	40%
ALL							3193	100%
Both Clinics Combined								
AA -Hispanic-Medicaid	4473	100%	4473	447	4025	1208	2818	60%
Non-AA - Non-Hispanic - Non-Medicaid	4140	71%	2993	299	2694	808	1885	40%
ALL							4703	100%

**Assumes proportions (served/under-served) of ALL clinic cases (shown here) is equal to the proportion of cases who fit clinical criteria

*** Likely non-response rates will differ by group and will need to be adjusted over time to allow better % selected estimates

2. Study intervention randomization #1 (Pre vs. No Pre-visit prep) – Create randomly permuted blocks of size 2-8 for each of the 4 sample groups above until the targeted number of enrolled is achieved.
3. Study intervention randomization #2 (Genomic Sequencing (GS) vs. No GS) – Should make sure that those in pre vs No pre-visit prep are equally distributed into +/- GS groups
    a. I agree – per Fang-Chang Lin
4. Both interventions are randomized 1:1
5. Concern and Question:
    a. While we will apply randomized recruitment (sampling/selection) to balance proportion of underserved to served at enrollment this will not ensure balance of served and underserved within intervention arms, true? Is there any way to ensure balance of served underserved within intervention arms?
        i. I thought it would be balanced. If I understand it correctly, we are going to randomize intervention/control assignments 1:1 ratio in each of the 4 groups above. Therefore, the underserved and served ratio is still 3:2 in the intervention group, as well as in the control group. Per Feng-Chang Lin
    b. Should subjects be randomized to both arms at the same time. Concern that some participants in arm 1 with pre-visit prep of certain demographic group will be burdened and drop out before GS randomization.

i. I feel drop-out is probably the main reason for unbalance. If we do have the same drop-out rate between underserved and served, then 3:2 ratio remains, as shown in your table. If we have the same drop-out rates between intervention and control, then 1:1 ratio between intervention and control remains, as well as the 3:2 ratio between underserved and served.- per Dr. Lin

c. Should we do something at block randomization to account for these concerns. Dr. Lin would not recommend any other adjustments at randomization to address this Indeed. The block randomization can guarantee the balance between intervention and control, but without balanced drop-out, the final sample cannot reach the ideal ratio. However, we will do intention-to-treat analysis, so the intervention and control ratio should still be balanced.-per Dr. Lin

d. Per Dr. Lin, he will generate trial randomization list but wants to be sure that the numbers on the list are assigned sequentially and matched to a specific ID#. The patient tracking system will handle this. He will send randomization list when we let him know that we are ready to go. The randomization scheme will be applied to individuals in the study as they enrolled. There will be a different randomization list for each site.

REFERENCES: **Weinberg CR**[1], **Sandler DP**. Randomized recruitment in case-control studies. **Am J Epidemiol.** 1991 Aug 15;134(4):421-32.

Appendix IV. Pre-Clinic and In-Clinic Activities list for Research Assistant and Study Coordinator

RA Pre-Clinic and In-Clinic Activities
(updated 10/7/2019)

Before clinic visit

1. Check NCGENES calendar for clinic location and study ID of scheduled participants
2. Obtain clinic consult room
3. Charge all electronic devices (audio recorder, iPad, tablet)
4. Be sure that all movies and cartoons on Netflix have been refreshed each week
5. Pack the following:
   - All chargers for electronic devices
   - All electronic devices (tablet should have stylus)
   - Create participant study folders (see chart order document)[1]
   - Pen
   - Laminated instructions for physicians
   - Laminated sheet with MDs and genetic counselor names

To do in clinic

1. Sit outside Pediatrics clinic to look for relevant patient check in[2]
2. Correspond with the study coordinator by text regarding arrival of patient noted in the EMR
3. Login to UNC-guest/relevant hospital internet access (set up so automatic)
4. Open VPN and connect, which ensures secure access
5. Once connected, open Chrome and click on NCGENES tab
6. Click on 'participants' and in the URL line enter participant ID number or order participant list by ID and click on participant's name to open task list
7. Approach patient in clinic waiting area, confirm parent's/patient's name and introduce yourself and your relationship to the NCGENES
8. Confirm appointment in tracking system
9. Collect intake from parent, place in participant's folder and indicate receipt of form in tracking.
   a. If patient doesn't have form, provide paper intake with postage paid return envelope and complete necessary items in the tracking system for that scenario
   b. If there is 45 minutes prior to the clinic appointment AFTER completion of all pre-clinic visit research and clinical activities (e.g. height and weight), the parent can complete the intake form on paper in the clinic room
   c. A note must be made in the participant's chart in the tracking system if any part of the Intake form is completed AFTER the clinic visit
10. Open a second web page and navigate to ***https://ncgenes2.sirs.unc.edu/surveys***[3]
11. Go back to specific patient's 'task' page and copy the survey code for pre-visit survey and go to second page and paste in survey code
12. Go back to patient task page and close it - leaving only the survey page open. This ensures that the parent cannot see other study participant's details/files
13. Open survey on tablet and hand to parent for completion (with or without stylus or touch). Let parent know to hand you the tablet when done. Stay nearby for questions/tablet challenges.
14. Obtain tablet from parent (click finish, if needed)
15. Re-open a patient specific 'task' page and confirm pre-visit 1 parent survey now has a 'completed' status
16. Click on parent permission to audiotape task and read/state that study is trying to learn about parent and physician communication and would like to audiotape the clinic visit and that if the parent consents they will check "yes" and sign
17. Hand the tablet to the parent to indicate "yes" or "no" and sign

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[1] SC or unblinded member of the study team will provide RA with the appropriate post-visit survey for each participant.
[2] If the patient is approached prior to check in, just do a brief introduction and inform them you will speak with them after check-in - do not want to delay check-in process)
[3] Tasks 10-18 may be completed after patient has been weighed, etc. by nurse and placed in exam room.

18. When they return tablet to you, type in the parent's name, then sign and date and type your name as witness before clicking "Finish and finalize"
19. Prior to genetic counselor (or physician) entering the room, turn on audio recorder (side bar), then press record and say the recorder number and participant's study ID number[1]
20. Set recorder and case on shelf in clinic room out of reach of patient
21. When done, let the genetic counselor or physician (they sit in work room) know that you are finished, and the patient is ready for clinical visit to begin[2]

(Both the counselor and physician will need to see patient next)

22. Go to waiting area/consultation room and, if possible, enter the paper intake form into the tracking system,[3] while monitoring whether the clinician has concluded their visit
23. At the end of the visit, turn off and collect tape recorder and provide post-visit survey (ideally in the exam room, though the post-visit survey can be administered in the waiting/consultation room if needed)
24. Provide the post-visit parent survey as you did pre-visit survey via a new surveys tab, BUT use the appropriate survey code for the post-visit survey on the participant page
25. While parent is doing post-visit survey, consult with the physician regarding developmental age of child (only if 7 years of age or older chronologically) and any planned blood orders. Also identify if any time sensitive blood collections are needed (e.g., those needed by 3pm, etc.). Relay this information to the Study Coordinator in-person or by text.
26. When parent is done with the survey let them know that you will now take them to the Study Coordinator (who they may have spoken with by phone before today's visit) who will talk to them about a potential next test and provide snacks/reimbursement. When the SC is finished seeing the parent they will direct them to the check-out area and then take them to the phlebotomy area if their child needs to have any blood drawn.
27. Collect the iPad and walk patient and parent to consultation room and hand off folder and patient to Study Coordinator[4]
28. Double check to be sure that you have all tablets, iPads and audio recorders that were brought to the clinic
29. Return to the office and place all electronic devices on chargers in preparation for the next clinic

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[1] If unable to record the study ID number, then on the intake form, you received from the parent, write the number of the recorder that was placed in the room.

[2] NEVER unblind the physician or genetic counselor to the randomization status of the patient (either pre-visit prep or genomic sequencing). If this happens a NOTE should be placed in the participant's tracking system record. The physician and genetic counselor can ONLY be unblinded to patient's randomization status AFTER the physician has completed the post-visit 1 physician survey

[3] If the Intake form was not entered by the RA during the clinic visit, it should be entered after the Study Coordinator returns with the folder from the clinic or immediately the next morning. Intake forms should be entered within 24 hours of collection.

[4] To date we have used the UNC consultation room in Pediatrics clinic (behind check out) primarily, but have also used the cardiology conference room

Study Coordinator Pre-Clinic and In-Clinic Activities
(10/7/2019)

Before clinic visit:

- Log into the NCGENES tracking system[1] to review potential study participants
- Once in tracking, select a referred patient scheduled to visit the clinic three or more weeks from the date of review, starting with the closest appointment date
- Log into EPIC and navigate to the selected patient's medical record information.
- Review this information to confirm the patient's DOB, MRN#, appointment information, and language spoken is the same as that listed in the tracking system.
- If any of the data is different, revise the tracking system to contain the same information that is listed in EPIC and as necessary add a note in tracking to document any changes.
- Review the patient's medical record information in EPIC to determine if the patient meets study's clinical inclusion criteria.
- Make appropriate selections in the tracking system to indicate the patient's study eligibility, per the above EPIC review.
- For ineligible patients, no further action is needed
- For eligible patients:
    - Ask an RA to send the study introduction letter (SC will serve as their back-up)
    - Make enrollment calls
    - Update tracking system to reflect the outcome of enrollment calls
- For participants that agree to be in the study:
    - Based upon randomization for intervention 1, have an unblinded RA create and mail enrollment packet (SC will serve as their back-up)
    - Add participant to NCGENES calendar
    - For participants attending the genetics & metabolism clinic, make a note in MEDGIS that patient is an NCGENES participant
    - For patients attending the neurology clinic, email Betty to inform her of their participation[2]
    - Inform RA, student volunteer, and Jeannette of upcoming clinic dates and times
    - Make reminder calls and update tracking system to reflect call outcomes
- 1 to 2 days before clinic:
    - Provide RA attending clinic with the appropriate post-clinic visit survey for each participant
    - Check SC clinic bag to ensure it contains all needed supplies and age appropriate participant gifts (generally there are 2 gift choices per participant)

[1] All actions before and during clinic are in the production version of the NCGENES tracking system.
[2] The email should contain the patient name, MRN#, appointment date & time, and provider name (generally, SC sends Betty an email twice a month regarding study participants)

To do in clinic:

During initial meeting:

- Introduce yourself
    - Typically, parents are told on the phone that they will meet with Tracey or another member of the team in clinic. If you spoke with the family on the phone, introduce yourself accordingly
    - If not, tell the parent you work with Tracey
- Tell them what will happen during this time (i.e. discuss the second part of the study, take them to check-out /blood draw). Also tell them at check-out they can get a note for school or work if needed. While the parent is at check-out the SC should stand either outside the consult room door or by the tiles with handmade pictures, etc., to meet the parent and take them to phlebotomy/direct them how to exit the hospital.
- Remind they will receive a copy of the forms discussed (also remind them of this point during/at the end of each consent discussion, depending upon how overwhelmed they seem)
- If assent is needed:
    - Tell parent you will need to talk to their child about the study, since they are at least 7 years old. Also, because of this, both parent and child must agree to participate. Generally, try to say this without the child hearing as they may get excited/make a comment that they don't want to participate (and at this point they have not heard anything about the study). Also, this facet of the study tends to make parents tense up. If so, explain to the parent that this is part of standard practice, is required, and is a way to ensure that kids understand what is taking place.
    - Explain to the parent that you'll discuss the study with them first and then their child, and that they will get a copy of everything that is discussed with them and their child.

Consent form 1:

1. At the top of the form and on all the forms we discuss today is the name of the study investigators and contact information. Pointing out that the phone number is the same one that they have received before in the packet. And that this number and email is what they can use to reach members of the study team.
2. They can decline at any time and that it will not impact their care. Whatever was decided/is done during the study will not change what the Dr. (say provider name) wants to have done.
3. If they decide to continue in the study, regardless of if they are assigned to GS or no GS that we will study their child until they are 18 by looking at their medical records and insurance information. Once they turn 18 the link between their name and study ID number will be removed.
4. We are looking at medical records and insurance information for items around why they came to clinic today. That when we use this information to report on study findings, we report in generalities, like for example we saw 850 families and from 50 we learned X and from 150 we learned Y. Also mention that we place information in public databases, which is something that researchers do to learn from each other, but this information is included without any personal information on study participants.
5. Participation will not involve an additional trip to UNC, we understand that no one lives close, so the study was designed in such away as to not have them make a trip to UNC only for the study. Which is why we try to have study activities done when they are already coming.
6. What will happen today is, if they are assigned to have testing and they decide to do so, that for their child participation means giving a little extra blood today. For them it means doing a phone interview about 6-8 months from today and then another phone interview about 6-months after that. If they are assigned to no GS or decide not to have testing, then there are no activities for their child, but that they will still have the phone interviews.
7. The study's funder is part of the NIH (the part of government that funds most research).
8. The IRB is the Institutional Review Board, which is the organization that approved us to do this study and that they make sure researchers do what they say they will do. If they do not think this has occurred, they can contact the IRB with or without giving their name to discuss the issue.
9. The IRB's number is included in the consent, and that they will get a paper copy of the consent after signing.
10. The IRB requires that we have information in the consent about future use of the data, so this section discusses this use and that they can still be in the study regardless of if they decide to have or not have their information stored in the separate database.
11. Discuss the future use section of the consent and offer to let the parent read it on the screen.

12. At the end of reviewing consent form, inform the parent that in by signing this form they are agreeing to allow us to
    - 1) flip the coin to see if they are or are not offered GS testing
    - 2) to look at their child's medical and insurance information regarding information around why they came to clinic today, until their child is 18
    - 3) that we will have to obtain HIPPA consent, since this consent is what allows us to look at their child's medical and insurance records.
13. Ask if they will sign consent form 1.

HIPPA Consent:

1. Acknowledge they are likely familiar with HIPPA due to previous trips to the doctor/hospital.
2. Their signature is needed because it allows us to receive and share information with other HIPPA covered entities.
3. Point out below differences with giving us HIPPA consent versus other times they typically encounter HIPPA:
    - Although they can withdraw from the study at any time by calling, that to withdraw HIPPA authorization requires notification in writing by email or letter, because it involves medical records, etc. The phone number and email address are at the top of the consent forms.
    - Because of the role of NIH and the IRB they can audit the project and that during this audit they may look at their child's research records. This look may reveal some of their child's medical/insurance information or they may ask to see this information to clarify a question. They are not HIPPA covered entities, but we expect them to treat the information in the same manner as HIPPA entities. Records chosen for an audit are random, and that their child's record may or may not be selected.
    - If they give HIPPA authorization to others, after this project, and there is project information in their child's medical record, that these others will have the project information (unless they restrict access)
4. Offer to let parent read HIPPA consent on the screen

Informed consent 2:

1. GS testing is not a new test, but we are trying to determine if having the test sooner rather than later in the process of learning a reason for why their child came to clinic today, impacts care they receive down the road. For example, if they have more or less additional test then children who do not have GS testing.
2. Test results typically come back into one of three categories:
    - Positive, meaning the change we see is the cause for why their child came to clinic today.
    - Uncertain, means that there is not enough data yet to know if the change we see causes health issues or is part of the many changes that people just have.
    - Negative, means at this time we did not find a change that is related to why their child came to clinic today.
    - As more research is done, an uncertain or negative result could change to something else. And if we learn of a change, or of a reason that may impact their wanting to continue study participation that we will contact them.
3. If the results are positive or uncertain, the test is confirmed in the hospital lab, and this confirmation allows the results to become part of their child's medical record.
4. Once information is placed in the medical record it cannot be removed. So, if they call after this has occurred, we can stop research but cannot remove the information from their child's medical record. Usually give an example of what happens if they withdraw before and after the 6-month time point of receiving results.
5. When discussing medically actionable items:
    - Remind them that since this GS test is not new, there are some changes that we know cause health issues and that these issues are typically something that happens as an adult. Therefore, the professional genetics organization, has made it optional for parents to learn this information. Regardless of what they decide, it will not change the care they receive or what happens to study participation (i.e. We still follow until their child is 18, phone interviews still occur.
    - Because these conditions usually happen as an adult, finding them may mean that they, the child's other parent or other family members may need to be tested. The study does not cover these tests; the cost is billed to them/their insurance.
    - Offer to let parent read this section of the consent on the screen

6. Ask if they will sign consent form 2.

Assent form 1 and 2:

1. Intro yourself again to the child
2. Tell them you're going to talk to them about the study, but first you would like to know if they have any questions about what they overheard you telling their parent
3. Tell them their parent will get a copy of what we talk about and that a phone number is on it. So, if they have a question later, they can ask their parent to call and their question(s) will be answered.
4. That although you may like too, you can't change anything that the Dr. (state their name) wants to happen.
5. Tell them that like in school they have to study to learn things, that we are doing this study to learn if a test, will help doctors learn why they and other kids have to come see them.
6. That we are seeing 850 kids and that we ask half the kids if we can look at their medical records until they are 18, and the other half of the kids we ask if we can look at their medical records and that they give a little extra blood (like 1-2 teaspoons). Giving this extra blood just means adding small tubes to the ones the doctor wants. They will not get stuck another time.
7. Us looking at their records does not mean they have to come back to see us, it just means work for us to do.
8. Ask if it's okay for you to flip the coin to see which group they are in
9. Pause and tell them which group they are in
10. Tell them the test is looking to see if there is a change in their genes that is the reason for why they came to clinic today.
11. The test will either say "yes" we see a change that is the reason, "no" we don't see a change that is the reason, or "we see a change" but don't know what it means. That we all have changes, and some cause people to come to the doctor and others don't.

Blood Draw:

- On side pocket of red bag are gloves and extra blood supplies.
- While family is waiting for blood draw to occur (or sooner if possible), do the following:
    - Label the tubes
    - In upper right- hand corner of the white provider signed MGL form, place study label and hand write date and time of blood draw
    - After tubes are obtained, complete the BSP form
    - After blood draw is done, walk them back to the area outside of phlebotomy and give them directions on how to exit the hospital. Then prepare the biohazard bags as below. If they valet parked, they go to either the hospital staff member at the front of the phlebotomy section or the information desk and they contact the valet service.
    - In one biohazard bag place the MGL form and the blood tube with the smaller amount of blood and send this bag to the CORE lab via the shoot system. Number for the core lab is 30. There is long delay between when select enter, for the tube to be sent and it actually goes. Stay there until the tube is gone and the screen is as it was before you sent the tube.
    - In the other biohazard bag, place the tube with the higher amount of blood and the completed BSP form into the red biohazard bag and take it to the BSP.
    - If needed help the parent hold/calm the child during blood draw.

In the phlebotomy area, take the family to the desk area closest to where you enter the phlebotomy section. The parent gives the child's name and DOB and the name of the doctor who saw them. They do not need to take a number or see someone in the glass office area, because they have already checked into clinic.

Appendix V. Address, Barcode, and Shipping Label Information and Instructions

Required label printers and labels[1]

Each product name links to the manufacture for specifications. Choice of vendor depends upon the preference of the buyer and available prices. Suitable alternatives may be substituted at buyer's discretion.

Printer Hardware:

For Barcodes *and* Address Labels:	**LabelWriter® 450 Twin Turbo**	SKU: 1752266
For Barcodes *or* Address Labels:	**LabelWriter® 450 Turbo**	SKU: 1752265
For Large Shipping Labels:	**Any Standard Printer**	

Labels:

For Address Labels:	**LW Address Labels 1 1/8" x 3 1/2"**	SKU: 30252
For Barcodes:	**LW Multi-Purpose Labels, Small 1" x 2 1/8"**	SKU: 30336
For Large Shipping Labels:	**Avery® Shipping Labels, 3-1/3" x 4"**	Prod #5264

Printer Software:

For Dymo Printers: *Mac:* DYMO Label v8.7.3 Download *Windows:* DYMO Connect for Desktop v1.2 Download
All other printers: Contact your institution's IT/Helpdesk

DYMO LabelWriter Setup Instructions

Follow these steps to configure a DYMO LabelWriter for printing.

1. Download the software for your computer by clicking the appropriate link above and obtaining IT approval as necessary.
2. Download the following documents and store them somewhere on your PC. These files are available in Teams or Sharepoint. You may also contact Peter Newman-Matthews if you have trouble locating these files.
    a. NCGENES 2 Barcode.label
    b. NCGENES 2 Address.label
    c. Barcode Data Template.xlsx
    d. Address Data Template.xlsx
3. Load your Dymo printer with the appropriate size labels per the above specifications. If using a twin turbo, load one type of label in each spool.

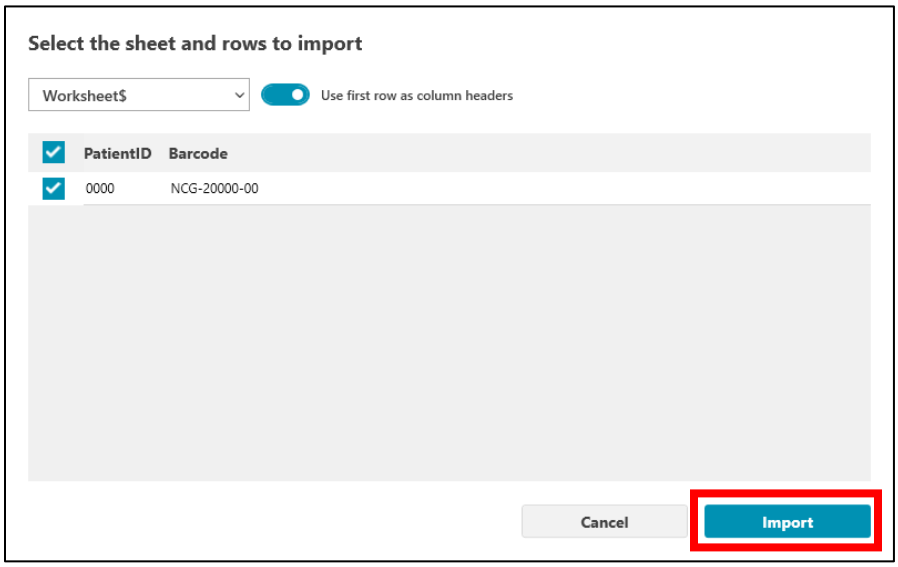
[1] Ordered by clinical team, not lab

Printing Barcode Labels

(If using a single DYMO, make sure to spool LW Multi-Purpose Labels, Small 1” x 2 1/8”)

1. Open “NCGENES 2 Barcode.label” using the DYMO Connect application.
2. Click the purple “Import Data” button on new the top of the window.

3. Find the barcode excel merge document (use “Browse…”) and then import the desired records. Settings should be preset. If not, mirror the image below.



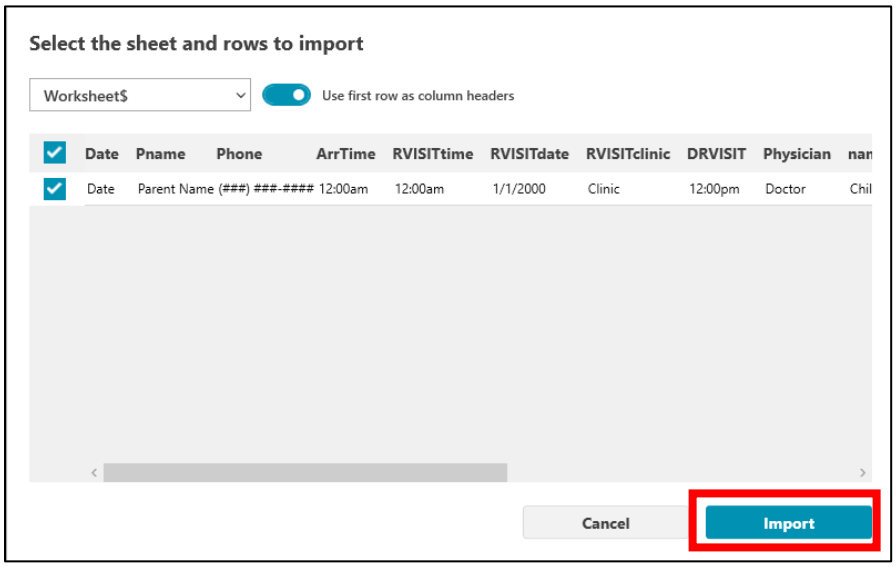
4. If necessary, adjust the fields on the barcode (this should not be necessary). If you need to print multiple copies of a barcode, first select the settings icon next to the print button and indicate the number of copies you wish to print. Then, click the blue “Print” button in the bottom right-hand corner.

Printing Address Labels

(If using a single DYMO, make sure to spool LW Address Labels 1 1/8" x 3 1/8")

1. Open "NCGENES 2 Address.label" using the DYMO Connect application.
2. Click the purple "Import Data" button on new the top of the window.

3. Find the address excel merge document (use "Browse…") and then import the desired records. Settings should be preset. If not, mirror the image below.



4. If necessary, adjust the fields on the address label (this should not be necessary). If you need to print multiple copies of a barcode, first select the settings icon next to the print button and indicate the number of copies you wish to print. Then, click the blue "Print" button in the bottom right-hand corner.

Appendix VI. Shopping List for ECU and Mission

Shopping Lists for ECU and Mission
(May 24, 2019)

1. Lenovo study tablet computers…THEY ARE HAVING A SALE IN MARCH SO ORDER SOON:
    a. See specification on next sheet/back sheet. You will need to purchase 2 one will be used for recruitment and one for clinic activities. You will not be able to see 2 participants at the same time unless the Study Coordinator who is recruiting is willing to give up the tablet to the clinic staff (RA and Genetic Counselor).
2. Additional Tablet ThinkPad Pro pens (in case they get lost) – ONLY if using a tablet-otherwise use mouse
3. Two audio recorders and cases (For UNC four Olympus DS-2500 recorders were purchased.: (get box Link to recorder instruction manual: **http://www.olympusamerica.com/cpg_section/cpg_support_product.asp?id=1620**
4. Two double port USB wall charger (for UNC Staples Dual USB wall charger were purchased at a cost of $13.19 each)
5. Need one iPad AND cases – via Netflix download free movies and cartoons onto the iPads for kids to view during the visit (optional – only if institution allows purchase)Two
6. Two rolling carts (the carts for UNC were purchased from Staples, the specific item information is: GP 80783 Embassy Plus Rolling Briefcase for 16" Laptops, Black. Cost $57.96.)
7. Printer with the ability to print in black and white and in color, and on dual sides
8. See Supplies and Ordering Information section of the Biospecimen Protocol for descriptions/specifications of items that need to be purchased for both blood and saliva collection, as well as, materials needed to mail samples to UNC. **Note:** Due to expiration dates ordering blood tubes and saliva collection kits should occur a month before the first participant is seen in clinic. This will allow for maximum self-life. Also, once materials are received confirm expiration dates are at least 12-months from the date of receipt.
9. One biohazard transport bags (For UNC these were purchased from Amazon. Product: Biohazardous Transport Insulated bag. Brand name: Hopkins Medical Products Part #: Ho539656. Cost: $7.95)
10. Gloves for handling blood and possibly saliva samples (if your hospital allows you to collect them onsite)
11. Snacks for participants and other children with the family (fruit gummies and animal crackers go over big with families)
12. File folders and related labels (Avery labels were purchased for UNC with the following dimensions:
    a. Mailing introduction letter: Avery #5160 1" x 2 5/8"
    b. Mailing enrollment packets: Avery #5264 3 1/3" x 4"
    c. For file folders: Avery #8366 1/3 Cut 2/3" x 3 7/16"
13. Thank you and birthday cards
14. Non-monetary child compensation gifts (and storage container if they do not have a locked storage area). (For UNC all of these gifts contained the school logo, footballs and water bottles were the family preferred items however, it was difficult to include a football in the rolling cart.)
15. Laptop protector for each laptop (for UNC Belkin Notebook Sleeve for 14" Laptop/Chromebook, black were purchased at a cost of $16.49 each)
16. Lock box to store cash/gift cards for study visits and phone interviews (For UNC a Barska extra small cash box with combination lock was purchased at a cost of ($26.61)
17. Barcode label printer and labels (For UNC these items were purchased at Staples and the specifications are: printer: DYMO Label Writer 450, labels: DYMO 45010 ½-inch high-performance permanent self-adhesive polyester label tape for label makers, black on clear (sold by the role).

Item:	Ships In:	Qty:	Price:
ThinkPad X1 Yoga 3G Part No: 20LDCTO1WW	Ships in 5-7 business days	1	$1,701.00

- 8th Generation Intel® Core™ i5-8250U Processor (1.60GHz, up to 3.40GHz with Turbo Boost, 6MB Cache)
- Windows 10 Pro 64
- Windows 10 Pro 64 English
- 14.0" FHD (1920 x 1080) IPS anti-reflective, anti-smudge, multi-touch, 270 nits
- 8 GB LPDDR3 2133MHz (Onboard)
- Integrated Intel® UHD Graphics 620
- 256 GB Solid State Drive, PCIe-NVMe OPAL2.0 M.2
- Black
- 720p HD Camera with ThinkShutter and microphone
- ThinkPad Pen Pro (Garaged)
- Backlit Keyboard - US English
- Fingerprint Reader
- Hardware dTPM
- Hardware dTPM2.0 Enabled
- 4 cell Li-Ion 54Wh
- 65W AC Adapter (2pin) - USB Type C
- Intel Dual Band 8265 Wireless AC (2 x 2) & Bluetooth 4.1 with vPro
- 14.0" FHD (1920x1080), IPS, 300nits, Touch, WLAN, No WWAN, 720p HD Camera with shutter, Mic, Black
- Intel UHD Graphics 620
- Publication-English
- Retail Packaging
- 1 Year Depot or Carry-in

Sub total:	$1,701.00
Estimated total:	$1,275.75
You Saved:	-$425.25

Appendix VII. Participant Distress Call Form

This is a study document and should be downloaded directly from **IRBIS** to ensure that the most recent edited and authorized document versions is being used. This form is reproduced here as a reference only and should not be copied for use in the study.

The UNC Study Coordinator is responsible for ensuring that all other sites (ECU/Mission) have access to IRBIS.

NCGENES PARTICIPANT DISTRESS CALL FORM
CONFIDENTIAL

These forms include protected health information (PHI) as defined by the Privacy Rule of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). The forms and the information on them should be stored and transmitted in accordance with HIPAA rules regarding *Confidentiality* (PHI should not be available or disclosed to unauthorized people) and *Integrity* (PHI should not be altered or destroyed in an unauthorized manner).

********** **To be completed by study team** **********

Date of referral: _____

Participant name: _____

Participant phone numbers:

Cell: _____

Home: _____

Participant address: _____

_____

Reason for referral (check one):

☐ Anxiety/GAD7 score above 15

☐ Depressive symptoms/PHQ8 score above 20

********** **To be completed by study psychologist** **********

INSTRUCTIONS: Please contact participant within 2 weeks, making a minimum of 3 call attempts. If possible, try both weekdays and weekends at different times of day. Regardless of whether you were able to contact the participant after 2 weeks, complete this form and return it to the study team.

Contacted participant? (check one): ☐ Yes ☐ No

Date contact made: _____

Wanted referral? ☐ Yes ☐ No

Brief description of problem and action taken during contact: _____

_____

_____

_____

_____

_____

Appendix VIII. NCGENES 2 Participant Saliva Letter

This is a study document and should be downloaded directly from **IRBIS** to ensure that the most recent edited and authorized document versions is being used. This form is reproduced here as a reference only and should not be copied for use in the study.

The UNC Study Coordinator is responsible for ensuring that all other sites (ECU/Mission) have access to IRBIS.

«Date»

Parent of «name»
«Addr1»
«Addr2»
«City», «State» «Zip»

Dear parent,

Thank you for enrolling in the NCGENES study. We are very grateful for your help with this important research. As a part of the study, you agreed to have your child provide a blood or saliva sample. We were unable to collect or process a blood sample from your child, and so we have provided a kit or kits to collect saliva in this mailing. Like blood, saliva can also provide DNA for genomic sequencing. Your child's name and any other identifying information is not on the tube that will hold your child's saliva sample. This is done to protect your child's privacy and yours. Instead the tube will be labeled with a unique research ID number. The saliva sample(s) from your child may help researchers and your child's doctors better understand the cause of your child's condition and may also lead to improved treatment for your child. Other family members and future generations may also benefit from the results and knowledge gained from this research. If you decide not to send a saliva sample from your child you can continue to do surveys as part of NCGENES, but your child will not have genomic sequencing.

Collection of the sample(s) from your child should only take about 15 minutes. Please follow the enclosed instructions for saliva collection. Then return the saliva collection tube(s) at your earliest convenience by US mail in the pre-addressed, stamped envelope provided. This can be done by placing the envelope in your mailbox, or by mailing it at the post office or package store. If you have any questions about collecting your child's saliva sample(s), please call 888-879-2102.

Thank you again for being part of the NCGENES research study.

Jeannette T. Bensen, MS, PhD

UNC NCGENES 2 Clinical Director

Appendix IX. NCGENES 2 Saliva Kit Mailing Instructions (Participant)

This is a study document and should be downloaded directly from **IRBIS** to ensure that the most recent edited and authorized document versions is being used. This form is reproduced here as a reference only and should not be copied for use in the study.

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Saliva Collection for Pediatric Participant (Child)

Instructions: Using and Returning the Saliva Collection Kit

Below is what we would like you to do with the kit:

1. First, read and follow the Oragene DNA user instructions sheet. It contains step by step instructions for collecting and packing your child's saliva sample. You can find these instructions both inside the saliva kit and on the enclosed colored sheet of paper.

2. Second, place the tube(s) into the enclosed plastic bag and seal the bag shut. You may have received 1 saliva collection kit and have only 1 tube to place into the plastic bag. Others may receive 2 saliva collection kits and will have 2 tubes to place into the plastic bag.

3. Third, place the sealed plastic bag with the saliva tube(s) in the pre-paid self-addressed envelope, as well as THIS piece of paper with the *date* and *time* of the saliva collection filled in below and mail by placing it in your mailbox or by dropping it off at the post office or a package store.

Date of collection: _____

Time of collection: _____

Please contact the NCGENES study team at 1-888-879-2102, if you have any questions or concerns.

Appendix X. Enlarged Oragene OG 575 Saliva Kit Instructions

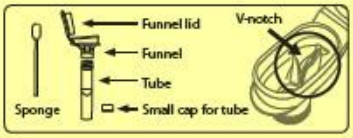
This is a study document and should be downloaded directly from **IRBIS** to ensure that the most recent edited and authorized document versions is being used. This form is reproduced here as a reference only and should not be copied for use in the study.

The UNC Study Coordinator is responsible for ensuring that all other sites (ECU/Mission) have access to IRBIS

ORAgene·DNA

REF OG-575

REF OG-675



Collection precautions:

Do NOT remove the plastic film from the funnel lid.

Check sponge for damage each time before inserting into donor's mouth. Use second sponge if first sponge shows any signs of wear or tear.

Do NOT substitute with other sponges or swabs.

Intended use: For the assisted collection of human DNA from saliva samples.

Contents: Kit contains stabilizing liquid.

Warnings and precautions:

Choking hazards:

- Small cap in collection kit.
- Plastic bag containing sponges.
- Caution should be used when inserting sponge into donor's mouth.

For supervised collections:

- Do NOT leave donor unattended.
- Do NOT allow donor to handle the sponge, small cap or packaging.
- Wash with water if stabilizing liquid comes in contact with eyes or skin. Do not ingest. See MSDS at www.dnagenotek.com.

Storage: 15°C – 30°C

Summary and explanation of the kit:

Oragene·DNA is an assisted collection kit that provides the materials and instructions for collecting and stabilizing saliva specimens.

Label legend:

- Consult package insert
- Collect saliva by (Use by)
- In vitro diagnostic medical device
- Catalog number
- CE Marking
- Caution, consult instructions for use
- Storage instructions
- Authorized Representative
- Manufacturer
- Lot number

USER INSTRUCTIONS

Read all instructions prior to collection

Procedure:

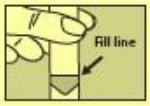
Ensure donor does NOT eat, drink, smoke or chew gum for 30 minutes before collecting a saliva sample.

Ensure donor is in an upright position during sample collection.

It may take up to 15 minutes to collect a saliva sample following steps 1 to 7.

1 Place one sponge in cheek pouch. Gently move the sponge along the gums and inner cheeks for 30 seconds to soak up as much saliva as possible.

2 Once sponge is saturated with saliva, insert sponge in V-notch of funnel. Wring saliva out of sponge using a twisting and pushing motion against the inner wall of the V-notch. Saliva will flow into tube.



3 Repeat these steps (1 to 2) USING THE SAME SPONGE until the liquid saliva (not bubbles) reaches the fill line. Check sponge for damage each time before inserting into donor's mouth. Use second sponge if first sponge shows any signs of wear or tear.

Tap tube bottom against hard surface to reduce number of bubbles.

4 Hold the tube upright with one hand. Close the lid with the other hand (as shown) by firmly pushing the lid until you hear a loud click. The liquid in the lid will be released into the tube to mix with the saliva. Make sure the lid is closed tightly.

5 Hold the tube upright. Unscrew the funnel from the tube.

6 Use the small cap to close the tube tightly.

7 Shake the capped tube for 5 seconds. Discard the funnel and sponges.

CE IVD

For In Vitro Diagnostic Use

DNAgenotek

Superior samples
Proven performance

Made in Canada
DNA Genotek Inc.
3000 - 500 Palladium Drive
Ottawa, ON, Canada K2V 1C2

Toll-free (North America): 1.866.813.6554
Tel: +1.613.725.5757 · Fax: +1.613.725.5057
info@dnagenotek.com
www.dnagenotek.com

EC REP Emergo Europe, Prinsessegracht 20, 2514 AP The Hague, The Netherlands
Australian Sponsor: Emergo Australia, Level 20, Tower II, Darling Park, 201 Sussex Street, Sydney, NSW 2000 Australia

Oragene®DNA is not available for sale in the United States.
®Oragene is a registered trademark of DNA Genotek Inc.
Some DNA Genotek products may not be available in all geographic regions, contact your sales representative for details.
All DNA Genotek protocols, white papers and application notes, are available in the support section of our website at www.dnagenotek.com.

PD-PR-154 Issue 5/2017-05

Appendix XI. NCGENES 2 Parent Saliva Letter

This is a study document and should be downloaded directly from **IRBIS** to ensure that the most recent edited and authorized document versions is being used. This form is reproduced here as a reference only and should not be copied for use in the study.

The UNC Study Coordinator is responsible for ensuring that all other sites (ECU/Mission) have access to IRBIS.

NCGenes

«Date»

Parent of «name»
«Addr1»
«Addr2»
«City», «State» «Zip»

Dear parents of «name»,

Thank you for enrolling in the NCGENES study. We are very grateful for your help with this important research. As we discussed, you agreed to provide a saliva sample to help your child's doctors better understand your child's genetic results. Like blood, saliva can also provide DNA for genetic sequencing. To protect your privacy, your name is not on the saliva tube. The tube is labeled with a research ID number instead. The results from the testing done on your saliva sample will be added to your child's test report and shared with your child's doctor. The doctor or genetic counselor you saw in clinic will review the results with you.

Collection of the sample(s) from you should only take about 15 minutes. Please follow the enclosed instructions for saliva collection, then return the saliva collection tube(s) at your earliest convenience by US mail in the pre-addressed, stamped envelope provided. This can be done by placing the envelope in your mailbox, or by mailing it at the post office or package store. If you have any questions about collecting your child's saliva sample(s), please call 888-879-2102.

Thank you again for being part of the NCGENES research study.

Jeannette T. Bensen

Jeannette T. Bensen, MS, PhD

UNC NCGENES 2 Clinical Director

Appendix XII. NCGENES 2 Saliva Kit Mailing Instructions (Parent)

This is a study document and should be downloaded directly from **IRBIS** to ensure that the most recent edited and authorized document versions is being used. This form is reproduced here as a reference only and should not be copied for use in the study.

The UNC Study Coordinator is responsible for ensuring that all other sites (ECU/Mission) have access to IRBIS

Saliva Collection for Parent(s)/Relatives

Instructions: Using and Returning the Saliva Collection Kit

Below is what we would like you to do with the kit:

1. First, place the signed and dated "Consent to Participate in a Research Study Adult Subjects – Relatives of Study Subject Biomedical Form" into the pre-paid self-addressed envelope enclosed inside the yellow folder.

2. Second, read and follow the Oragene DNA user instructions sheet. It contains step by step instructions for collecting and packing your saliva sample. You can find these instructions both inside the saliva kit and on the enclosed colored sheet of paper.

3. Third, place the tube into the enclosed plastic bag and seal the bag shut.

4. Fourth, place the sealed plastic bag with the saliva tube in the pre-paid self-addressed envelope, as well as THIS piece of paper with the *date* and *time* of the saliva collection filled in below and mail by placing it in your mailbox or by dropping it off at the post office or a package store.

Date of collection: _____

Time of collection: _____

Please contact the NCGENES study team at 1-888-879-2102, if you have any questions or concerns.

Administrative Note: NCGENES 2 staff member must sign and date consent upon receipt.

Appendix XII. Parent Consent to DNA Collection

This is a study document and should be downloaded directly from **IRBIS** to ensure that the most recent edited and authorized document versions is being used. This form is reproduced here as a reference only and should not be copied for use in the study.

The UNC Study Coordinator is responsible for ensuring that all other sites (ECU/Mission) have access to IRBIS.

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Adult Subjects - Relatives of Study Subject
Biomedical Form

IRB Study # 17-0816
Consent Form Version Date: 12/7/2018
Title of Study: North Carolina Clinical Genomic Evaluation by Next-gen Exome Sequencing, phase 2 (NCGENES 2)
Principal Investigators: Jonathan Berg, MD, PhD, Bradford Powell, M.D., PhD, Christine Rini, Ph.D.
UNC-Chapel Hill Department: Genetics
UNC-Chapel Hill Phone number: 919-966-7043
Email Address: jsberg@med.unc.edu
Funding Source: National Human Genome Research Institute at National Institutes of Health
Study Contact: Jeannette Bensen, PhD
Study Contact telephone number: 888-879-2102 (toll free)
Study Contact email: ncgenes2@med.unc.edu

It is important that you understand the information in this consent form so that you can make an informed choice about joining this research study.

What are some general things you should know about research studies?
You are being asked to take part in a research study. Joining the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason.

Research studies are designed to learn new knowledge that may help other people in the future. You may not receive any direct benefits and there may be risks from being in the research study.

Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill.

You will be given a copy of this consent form. You should ask the researchers or staff members any questions you have about this study at any time.

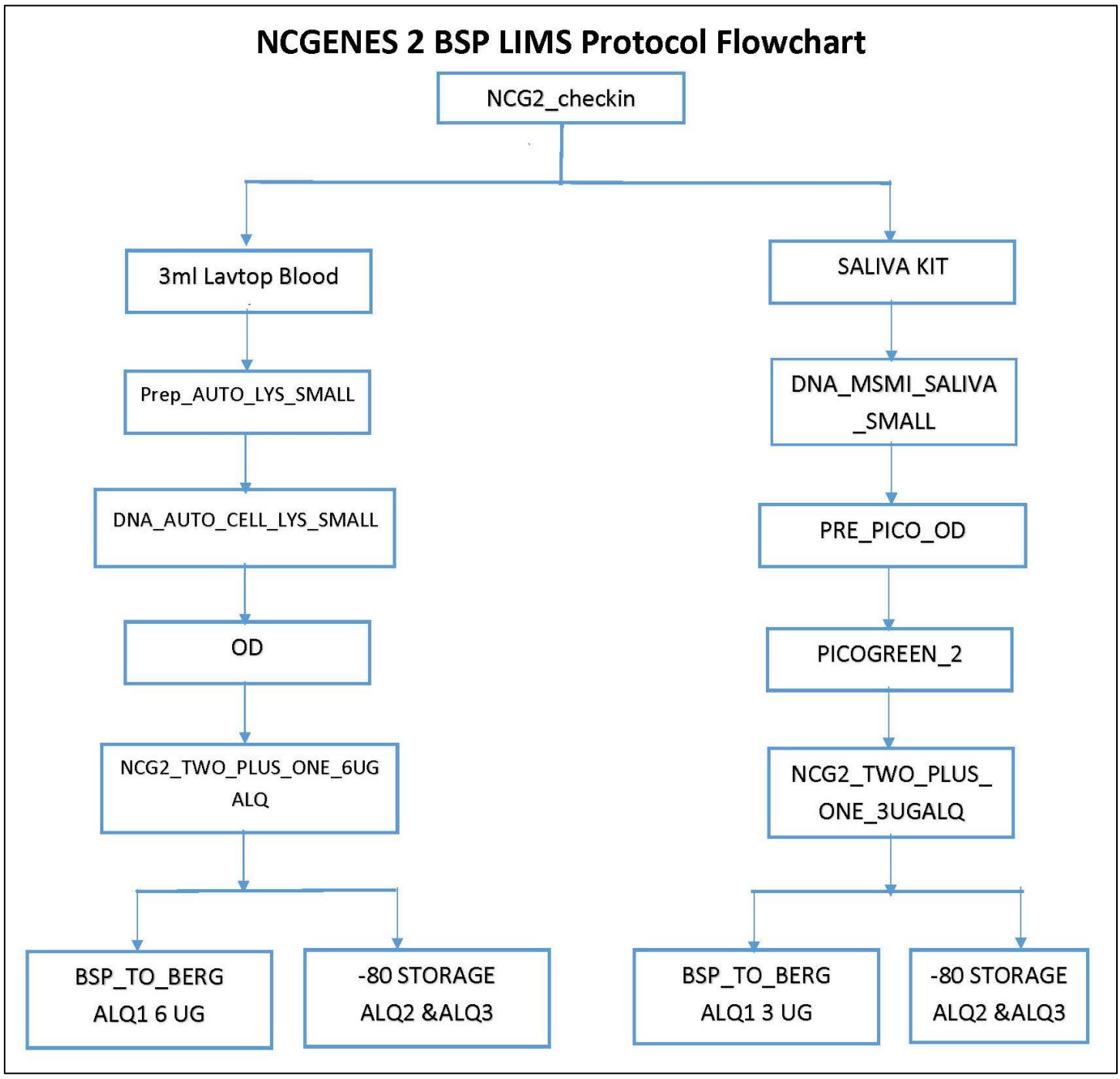
Why are you being invited to participate in this study?

One of your relatives has had genetic testing done as part of the research study called NCGENES. This testing looks for differences in genes called "genetic variants." Most genetic variants are harmless, but some are harmful because they can cause specific diseases.

Sometimes, we cannot say for sure whether a genetic variant or combination of variants explains a person's symptoms. In some cases, testing for the presence of genetic variant(s) in one or more family members can improve our understanding of the patient's results.

You are being invited to join this study because your relative's genetic testing was inconclusive for one of these reasons. If we test you to see whether you have the same genetic variant or not, we may be able to learn information that could improve our understanding of your relative's result.

Appendix XIII. Biospecimen Processing Facility (BSP) – NCGENES 2 Overview



Appendix XIV. BSP Blood Processing for NCGENES 2 – includes DNA Extraction

UNC BIOSPECIMEN PROCESSING FACILITY

BSP Facility Autopure Automated Whole Blood Plasma and DNA two step extraction.
protocol ver. 04_25_18

I Purpose: To insure high molecular weight DNA that is free from contaminants and suitable for sequencing and genotyping.

II Materials:

- Field sample delivered either by study staff or overnight courier
- Gloves
- 50 ml Autopure input qubes (pink caps)
- 50 ml Autopure output qubes (blue caps)
- Auotpure and all Puregene reagents
- 5 ml serologic pipettes
- Screw top 1.5-2.0ml microcentrifuge tube
- -80º C freezer

III Procedure:

Collection

- Universal precautions for working with human specimens should be followed during all stages of specimen handling. Universal (or Standard) precautions such as hand washing, contaminated needle and sharps disposal, use of personal protective equipment, decontamination of equipment and work surfaces, and labeling of specimen containers are described in the UNC Laboratory Safety/Exposure Control Plan (located on lab bookshelf).
- Receive whole blood into the BSP facility by courier or overnight carrier and log into BSP facility's Laboratory Information Management System (LIMS).
- Blood tubes will automatically be planned into the LYS_PL_EXT general or equivalent study-specific protocol. If plasma is not required for the project blood tubes will be routed to a Cell lysate preparation in the LIMS. The specific protocol will depend on the blood volume and whether manual or an automated protocol will be used.

Plasma Extraction

- Within 0.5 hours, begin processing sample.
- Process each blood tube per lab/specimen ID separately.
- Open LYS_PL_EXT protocol in the LIMS and create a workset.
- Follow LIMS directed protocol which is summarized below
- Spin blood tube using Program1 (3250 x g for 10 minutes at 15°C) in the Sorvall centrifuge in the tissue culture area using bucket covers.
- In a biological safety cabinet remove the plasma without disturbing the buffy coat layer with a 5ml serological pipette, with the pipette aid set on low.
- Aliquot plasma equally into four pre-labeled Screw top 1.5-2.0ml microcentrifuge tubes. Note some study-specific protocols may require more than four aliquots, which will be noted in the LIMS protocol.
- Place aliquots into their LIMS directed redundant storage locations.

Cell Lysate Preparation

- The remainder of the whole blood after removal (or the entire blood tube if plasma was not collected) is then processed to cell lysate either in an automated fashion on the Autopure LS, (if either 8 or 16 samples are ready for processing) or manually performed using the same procedures that the Autopure uses. These procedures are briefly described below.

Manual Lysate Preparation

- Note manual lysate preparation was performed in the LIMS
- Pour remainder of the whole blood after plasma removal (or the entire blood tube if plasma was not collected) into a pre-labeled Pink qube.
- Add 30mls (for 5-10 ml of whole blood) or 15mls (for 1-5mls of whole blood) of Puregene RBC lysis, close tube and invert slowly 50 times.
- Incubate at room temperature (RT) for 6.5 minutes
- Spin on protocol 2 (3,000 x g for 5 minutes at 15°C) in Sorvall centrifuge in the tissue culture area using bucket covers.
- Remove PinkQube(s) from centrifuge; note the size of the WBC pellet. If there is no pellet or the pellet is extremely small, make a comment in the open protocol in the LIMS.
- Pour supernatant into biohazardous waste. Place cap back on tube and briefly vortex pellet to loosen.
- Add 10 ml of Puregene cell lysis solution to loosened pellet. Replace top, vortex, and place into LIMS directed RT lysate storage. Store lysate for at least 1 day and up until 1 month before proceeding to the DNA lysate preparation.

Automated Lysate Preparation

- Enter rack set-up menu.
- Choose the "lysate prep" protocol.
- Choose number of tubes (8 or16).
- Enter run name date.run#of the day
- Enter rack number
- Enter Qube lot #
- Scan PinkQubes
- Place rack on machine
- Press start run
- At end of run, vortex PinkQubes and place into LIMS directed room temperature lysate storage. These container IDs will be automatically placed in the next appropriate protocol in the LIMS. Store lysate for at least 1 day and up until 1 month before proceeding to the DNA lysate preparation.

DNA Extraction Autopure Run

- Add glycogen to BlueQubes if samples arrived >4 days after collection or if original sample was frozen. Note some studies may not request this step because of cost and will accept a lower DNA yield.
- If multiple tubes arrive for one lab/specimen ID, process these lysate tubes on separate DNA extraction Autopure runs
- Enter rack set-up menu.
- Choose the "Cell lysate 3X spin EtOH stop" protocol.
- Choose number of tubes (8 or16).
- Choose re-hydration volume (500ul for a 1-5ml blood prep or 1000ul for a 5-10ml blood prep). Choose 0 for hydration volume and manually add specific volumes when the run is finished.
- Enter run name
- Enter rack number
- Enter Qube lot #
- Scan input and output Qubes
- Place rack on machine
- Press start run

Post Autopure Run

- Run ends with DNA spun in 70% ethanol, this needs manual finishing. Carefully pour off ethanol and leave tube to air dry upside down for 10 minutes. Then add hydration solution. Unless otherwise specified by the project use 500ul of hydration buffer for 1-5ml starting blood tube or 1000ul for 5-10ml starting blood tube.
- After manual resuspension of hydration buffer incubate Pink qubes at 65°C for 1 hour (30min for a 500ul prep). Samples must be vortexed thoroughly (at least 1 minute), prior to being placed in the water bath and at least 1 time during the hour incubation, and at the end of the incubation.

- After incubation in the water bath samples are placed on the Fisher Scientific multi-tube shaker, set at 2500 RPM for 1 hour.
- After shaking leave DNA on bench at RT overnight.
- Evaluate DNA solution after overnight incubation, if DNA is not in solution more rehydration buffer may be added followed by additional incubations at 65ºC and/or RT.
- After hydration combine any samples that had been placed into multiple Qubes prior to Autopure run.
- Place DNA at 4°C prior to quantitation, gel analysis (if requested by project), and aliquoting.

DNA Quantitation

- DNA extracted from either blood is quantitated using UV absorbance on either the Denovix instrument or the Dropsense. Project specific needs may require Picogreen quantitation also. See specific SOPs for the use of these pieces of equipment.
- Prior to quantitation and/or aliquoting, mix samples on the multi-tube shaker set at 2500 RPM for 5 minutes, briefly spin DNA and proceed to next LIMS-directed procedure.

Appendix XV. BSP Saliva Processing – DNA extraction

UNC BIOSPECIMEN PROCESSING FACILITY

Procedure: MSMI 2ml Saliva Protocol Version: 04.27.18

Prepared By: Hendrick DeJong (Author's name) _____ (Signature) 6/12/2014 (Date)

Preparer's Title: Lab Manager

Adopted: _____ (Date)

Updated: 4/27/2018 (Date)

BSP Sign Off

Reviewed:

_____ Signature	_____ Date
_____ Signature	_____ Date
_____ Signature	_____ Date
_____ Signature	_____ Date
_____ Signature	_____ Date

UNC BIOSPECIMEN PROCESSING FACILITY

PROCEDURE CHANGE LOG

Procedure: MSMI 2ml Saliva Protocol

Date	Version	Changes Made	Edited By (initials)	Reviewed By (initials)	Date Reviewed
1/12/14	1.12.14	Changed elution volume to 300 µl (from 500 µl) to increase concentration. Incorporated spin step immediately after MSM1 elution. Added 20 µl elution buffer to final elution volume to account for transfer loss.	HD		
3/3/16	3.3.16	Added cover sheet, procedure change log, reference, safety, and change log sections. Added footer (File Name)	AR		
6/14/17	06.14.17	Minor procedural additions and clarifications	PS		
4/27/2018	04.27.18	Expanded on Supplies, Procedure, MSM1 Run (pre-run checks), and Post Run (waste disposal).	PVB		

UNC BIOSPECIMEN PROCESSING FACILITY

MSMI 2ml Saliva Protocol - Ver. 03.03.16

Purpose High throughput isolation of nucleic acid from 2ml Oragene saliva samples or buccal swabs using the Magnetic Separation Module I

Equipment and Supplies

- Magnetic Separation Module I (MSMI)
- Dedicated PC with Chemagic software with Code II scanner
- Positioning frame for Block 8
- Gloves
- Tissue Lysis Buffer – Perkin Elmer Art No. 805
- Chemagic Saliva Kit Number CMG-1081
- 4 ml MSMI elution tubes
- 24 well deep-well blocks
- 24 rod sheaths
- Snapcap 1.5 ml microcentrifuge tubes
- Screwtop 1.5 microcentrifuge tubes
- Microcentrifuge
- Pipettors:20ul, 200ul, 1000ul pipette and tips
- -80° Freezer

Reference

- Purification Protocol for 2ml of Oragene Saliva Using the Chemagic Magnetic Separation Module I
- M:\epid_bsp\BSP Administration\Maintenance\MSMI\Chemagic Protocols\ Microsoft Word - US - chemagic DNA Saliva Kit special 2 ml H24 VD120214.doc
  www.chemagen.com

- Oragene • Discover DNA Collection Kits
  OGR-675 for assisted collection
  OGR-575 for assisted collection
  OGR-250 2ml sample volume
  OC-175 for assisted collection
  www.dnagenotek.com

Safety Precautions

- Wear appropriate Personal Protective Equipment whenever handling chemicals or human body fluid
- CDC Universal precautions should be used when working with any human specimen. Precautions such as hand washing, contaminated needle and sharps disposal, decontamination of equipment and work surfaces, and labeling of specimen containers are described in the UNC Laboratory Safety/Exposure Control Plan (located on bookshelf in MHRC Room 3213)

- Read and understand the MSDS sheets for all of the reagents

Procedure

Collection

- Receive Sample into the BSP facility by courier or overnight carrier and log into BSP facility's Laboratory Information Management System (LIMS)
- Prior to extraction on the MSMI, saliva samples need to be incubated at 55º C for at least 1 hour (Following instructions from DNAgenotek)
- **If the volume is *less than* 1mL, bring total volume up to 1.5mL by adding tissue lysis buffer (Perkin Elmer Art. No. 805)**
- Samples with volumes up to 2.5mL can be processed with this protocol.
- Place frozen (-80º C) samples on bench for 5 minutes, and then thaw immediately in a 37º C water bath for 3 minutes
- Duplicate samples should be processed in separate runs to avoid loss through machine failure

Pre MSMI Run

LIMS Protocol: DNA_MSM1_Saliva_Small

- Turn on MSMI and Computer
- Open Chemagic software
- Connect lines for reagents 2-6 and verify sufficient volume for run (~250ml)
- Select protocol "Prime Lines 1-6" and verify that liquid is being dispensed correctly.
    - If nozzles are clogged, use Kimwipe or remove nozzle and clean with warm water.
- Select protocol "Check Manifolds 1-6" and verify that liquid is being dispensed correctly.
- Check waste container and empty if needed

MSMI Run

- Select protocol "8 saliva 2ml autofill h24 4ml.che"
- Place 24 disposable rod sheaths in the 24 Tip Rack and place in Position 1
- First pipette up and down to homogenize samples, then transfer saliva samples into deep well block in Position 2
- Mix magnetic beads by shaking bottle or gently vortex for 1 minute
- Pipet 240 µl magnetic beads in the deep well block in Position 3
- Place empty deep well racks in Position 4, 5 And 6
- Place elution tubes in tube holder in Position 7
- Fill elution tubes with 170 µl elution buffer (assuming 20 µl loss)
- Place empty deep well plate **in positioning frame** in Position 8 for waste collection
- Scan input and output tubes
- Start run (run time approximately 1hr. 15mins.)
- Disconnect lines from reagents 2-6 after dispensing in completed

Post MSMI Run

- After run has finished, remove elution tubes
- Transfer samples to 1.5ml snapcap tubes

- Spin the tubes for 5 minutes at 14000xg
- Transfer supernatant to a sterile 1.5ml screwcap tube (Do not transfer pellet)
- For short term storage: Place DNA at 4°C in coldbox until OD readings are taken in the next protocol
- For long term storage: Store sample in LIMS assigned -80°C storage
- WASTE DISPOSAL:
    - P2 (binding buffer) and P6 (mag beads) - MSM1 waste container
    - P3, P4, P5 - down sink with lots of water
    - Empty P8 (waste tips) and place used deep well blocks from P2-P6 into biohazard waste container.

DNA Quantitation

- Extracted DNA from Oragene saliva or buccal swabs is quantitated using UV absorbance on either the DeNovix instrument or the Dropsense. See specific SOPs for the use of these instruments
- After ODs are uploaded into LIMS, follow study-specific protocols for either aliquoting, additional quantification, or storage.

Change Log

- Ver. 01.12.14
- Ver. 06.12.14
- Ver. 03.02.15
- Ver. 03.03.16
- Ver. 06.14.17
- Ver. 04.27.18

Appendix XVI. CLIA Clinical Lab – Processing, Storage, Genetic Analysis and Reporting

UNC Molecular Genetics Laboratory

I. Procedure for Receipt of Samples for NCGENES2

1. NCGENES2 is a research study on the implementation of genomic sequencing. Samples are sent to the clinical laboratory for Research DNA Extraction and will be used for CLIA confirmation of mutations identified on a research basis. Samples (primarily blood, although saliva is a secondary option if blood collection or DNA extraction fail – see Insufficient Blood Sample Protocol) are collected from patients enrolled at three sites (UNC, ECU and Mission Health). The UNC MGL acts as the CLIA confirmatory lab for any positive findings (per research protocol, positive or uncertain findings potentially contributing to the child's disorder are confirmed as are any clinically actionable secondary sequencing findings) for both UNC and ECU. Mission Health will perform its own confirmatory testing in its own CLIA-certified lab.
2. One 3mL tube of blood will be sent with a Molecular Genetics Test Request Form. Under Patient Information there should be a unique NCGENES number (NCG_2XXXX), and a test request for "other" with Whole Exome Sequencing written in.
3. Confirm the number on the requisition is the same as the number on both tubes. Write the date received on the requisition.
4. Accession in EPIC for tests: Research Extraction (LAB13783) and Research Identity Genotyping (LAB13803) using the "Requisition Entry" function.
    a. Use "Genetics department-participating" as the submitter
    b. Click on the "Patient" Box. Close the patient demographic window that opens up. Then click on "Non - Human" button on the tool bar.
    c. Go to the "Patient" box again and Enter the name as the described format**: "NCG, 20046-00"**
    d. Use Z13.79 as the Diagnosis code
    e. Order both tests on the same Case#
5. Perform two DNA extractions of 100 uL each on the QiaSymphony. Label the two tubes of extracted DNA with the NCG number plus A or B (example: NCG_00001A and NCG_00001B). Nanodrop both DNA extractions and staple the results to the requisition. Freeze both DNA tubes at -70^{o}C.
6. Add the sample information to the spreadsheet found on the L drive at Molecular>NCGENES>NCGENES sample log.xls.
7. Make a new folder for the requisition/paperwork and place it in the NCGenes file cabinet.
8. The research specialist (Sayanty Roy) will Login to the RENCI system and record sample receipt, date and time.
9. When a batch of 12 samples is available, the research specialist (Sayanty Roy) will perform identity genotyping and enter the results in the MGL NCGENES2 database and Login to the RENCI system to enter the results of the identify check.

10. **Insufficient Blood Sample Protocol** If the UNC MGL determines that insufficient sample has been provided they are to notify the UNC study coordinator at 919-801-9267. The study coordinator will contact the family and mail a saliva collection kit (one per lab that reports insufficient sample). The saliva kit will be returned to the UNC BSP research lab where it will be couriered to the UNC MGL, along with a MGL test request form, for DNA extraction, identity genotyping and potential mutation confirmation. The research specialist (Sayanty Roy) will complete DNA extraction and items 6-9 above.

II. Receipt of Samples for NCGENES2 Family members (NCG_2XXXX-1,-2, etc.)

When a sample arrives from a family member of an NCGENES participant (labeled NCG_2XXXX-1,-2, etc.):

1. Follow steps 1-6 above.

2. Give the paperwork to Dr. Weck so that she can assign variant confirmatory testing immediately.

III. Accessioning NCGENES2 samples with positive results for UNC Patients into Medical Records (EPIC)

1. For all patients with positive or uncertain research results, confirmatory sequence testing will be performed by the research specialist (Sayanty Roy) in the CLIA-certified MGL. After confirmatory testing is complete, these samples will be re-accessioned into EPIC/ beaker using the patient's MR# so that the results can be transmitted to EPIC and become part of the patient's medical record.
2. Dr. Weck will provide the necessary paperwork with all of the patient information needed for accessioning (this information will be retrieved from the RENCI system and will include the patient's name, MRN and DOB). A medical record number is required. If one cannot be found within this paperwork, notify Dr. Weck so she can ask the study coordinator to provide one.
3. If a medical record number is provided but the patient is not found in the EPIC/Beaker system, notify Valerie in the LIS department to update this patient into the system.
4. Accession the specimen using the Requisition Entry function, with "Genetics department-participating" as the submitter.
5. Select blood as the specimen type. Select NCGENES research sequence confirmation as the procedure (LAB13793). Use the ordering physician listed on the MGL Test Request Form as the Ordering provider and Dr. Jonathan Berg as the Authorizing provider. Use Dr. Weck as the case pathologist.
6. Ensure that the Bill to box is selected Client Bill and **not** Patient Bill. This is important so that the NCGENES2 study will be billed for the testing and not the patient (this will be the case for all tests both at UNC and ECU – they will be paid centrally at UNC by the study).
7. Save the changes and be sure to indicate the Case# (i.e. MLM #) -number on the paperwork. Return all paperwork to Dr. Weck and let her know that the accessioning is complete.
8. Sayanty will perform Sanger research confirmation and Dr. Weck will sign out the case in EPIC
9. Dr. Weck will log in to RENCI System and enter the research results category.

IV. Reporting positive clinical results for NCGENES2 ECU Patients and Faxing to the Ordering Physician

1. For all ECU patients with positive or uncertain research results confirmed in the UNC MGL, it will be necessary to fax (using the fax number on the request form) the generated report to the ordering physician listed on the MGL Test Request Form received with the sample.

Appendix XVII. UNC MGL – Blood DNA Extraction for NCGENES 2

Molecular Genetics Laboratory Department Manual			
UNC HEALTH CARE	Policy Name/Procedure Name	**DNA Extraction using QIASYMPHONY DNA Mini Kits**	
	Author/ Revision Date	**Sherry Nelson, Revision date 07/01/17**	
	Date this Version Effective	**July 2017**	

SPECIMENS:

DNA can be extracted from whole blood, stem cells, cultured cells, and CD3 fractionated cells. Whole blood is collected in pale yellow-top ACD or purple-top EDTA tubes. Other anticoagulants such as heparin should be used only with the knowledge and consent of the directors, and documented on worksheets. Cultured cells such as amniocytes are processed by centrifugation at 3000rpm for 5 minutes. RPMI buffer is then removed by pipette. The cell pellet is re-suspended with 800-1000 uL PBS buffer. Excess sample not used for extraction is stored at –20°C until final reports are generated and then it is discarded. Extracted DNA not used for requested testing is stored at 4°C short term and then at –20°C for a minimum of two years.

APPARATUS/REAGENTS:

1. QIAsymphony DSP DNA mini kit – Qiagen (937236)
2. Sample Prep Cartridges, 8 well – Qiagen (997002)
3. 8 Rod Covers – Qiagen (997004)
4. Filter Tips, 1500 uL – Qiagen (997024)
5. Filter tips, 200uL – Qiagen (990332)
6. 1.5mL and 2.0mL Sarstedt tubes
7. 01, 02 and 3B tube adaptors

PROCEDURE:

System Set-Up

1. Turn on the instrument - the power button is located on the lower left side of the machine, in the front.

2. Log in on the main screen.

Preparation for Operation

1. Prepare the reagent pack:
   a. When opening a fresh extraction kit:

Remove the reagent pack from the pre-packaged white base and insert the reagent pack into the grey support base. Attach the Proteinase K rack to the side of the reagent pack. Place the piercing lid on top of the reagent pack and push down lightly until both ends make a snapping sound. Remove and vortex the magnetic bead cartridge for approximately 1 minute. Place the magnetic beads cartridge back onto the reagent pack and peel back the foil cover. Uncap both Proteinase K vials.

   b. When using a pre-opened extraction kit:

Remove and vortex the magnetic bead cartridge for approximately 1 minute. Remove the seal lids from each position and place on a clean absorbent pad. Uncap both Proteinase K vials. Place the caps on a clean absorbent pad.

2. Open the reagents and consumables drawer and slide the reagent pack into either the front or back slot with the magnetic beads facing outward.

3. Fill Sample Prep Cartridges and 8 Rod Covers as necessary.

4. Close the reagents and consumables drawer and, when prompted, select "Yes" for all components of the drawer and then select "Scan" at the bottom of the window to start a full inventory scan.

5. Extractions are maximized by processing samples in groups of 8. Gather all samples to be extracted and print labels using the Labjet printer.

6. Print out a Qiasymphony set-up worksheet from the L drive and place the labels in the appropriate spots on the worksheet template. Fill in the technologist's initials, extraction date and DNA Mini Kit lot number on the worksheet.

7. While referencing the Qiasymphony set-up worksheet, uncap each blood tube and place it in the corresponding slot on the sample rack. For collection tubes that are not 16 X 100 in size, place 3B adapters containing 2mL sarstedt tubes into the appropriate position in the sample rack. Pipet 500uL of blood (a minimum sample volume of 400uL is required) into each 2mL tube. **NOTE:** If for any reason a space in the sample rack is skipped, a tube must be placed in that position to prevent throwing off all the following samples.

8. For the pediatric ACD tube (3mL yellow top), place a 3B adaptor into the appropriate position on the sample rack. For the 3mL EDTA tube, place a 01 adaptor; and if there are any 6mL ACD or EDTA tube (s), place a02 adaptor into the appropriate position(s) on the sample rack.

9. Pull down to open the sample drawer, and slide the sample rack up to the black line of any slot labeled 1-4.

10. Wait for the barcode reader to move out into position near the sample rack, and then in one continuous motion, slide the sample rack completely into the slot.

11. If the rack was successfully inserted, the green indicator light of that slot will turn an amber color. A light blue "Batch" button will also light up on the main screen in the corresponding slot. The status line, to the right of the batch button, should update to "Loaded". If the slot turns a red color, an error message will pop up on the main screen. If this happens, pull the sample rack completely out of the slot, touch "OK" on the main screen, and repeat steps 8-9.

12. Touch the light blue "Batch" button on the main screen.

13. The sample tube selection screen will appear, and a dark blue "Sample Tubes" button should be lit on the right side of the screen. All positions occupied by blood tubes should also be lit. If a barcode was scanned, the position will be light blue, and if no barcode was scanned, it will be orange.

14. Samples requiring a 3B adapter were scanned as the sample rack was inserted into the instrument and should have the appropriate tube size listed on the sample selection screen. All 16X100 collection tubes should also have the appropriate tube size listed on the sample selection screen.
15. Touch the space for slots where a 3B adaptor was placed for the pediatric ACD tubes.

16. Touch the “ID/Type” button on the right hand side of the sample tube selection screen. Choose Tube 3B option. From the drop down menu, select “BD#364012 T2.6 glass 10.25x64”.
17. Touch “Next” when finished.

18. On the next screen, highlight all samples. On the right side of the screen, touch the “DNA Blood” protocol button. From the drop down menu, select “Blood_200_V7_DSP_100 Elution”, then touch “Next”.

19. Label one 1.5mL Sarstedt tube for each sample. Remove tube caps and using the set-up worksheet as a reference, place each tube in the correct position in the cooled elution rack.

20. Open the Qiasymphony elution drawer and place the elution tube rack in position 1.

21. Close the elution drawer, and touch slot 1 on the elution screen. A □ icon will appear to the left of slot 1 indicating the elution rack is cooled.

22. On the right side of the elution screen, touch “Tube_1.5mL AdapterV1 (no BC)”. On the drop down menu, select “SAR#72.607 **T1.5 Screw”. Touch “OK” and an inventory scan of the elution drawer will begin.

23. Touch the “Queue” button on the right side of the screen.

24. Open and close the waste drawer, and when prompted, touch “Scan” to start an inventory scan of the waste drawer.

25. When the inventory scan is complete, touch “Run” on the main screen.

26. When the run is complete, a blue status bar will flash on the front of the instrument. Open the sample drawer and slide the sample rack out to remove it from the slot. The “Batch” button will disappear from the main screen.

27. Cap each blood tube and store appropriately.

28. Open the elution drawer, and remove the elution rack from slot 1. Touch slot 1 on the elution screen, and select “Remove” and then “OK”. Close the elution drawer and wait while the instrument performs an inventory scan.

29. Open the reagents and consumables drawer and slide out the reagent pack. Cap each reagent using the seal lids. Cap the Proteinase K vials as well. The reagent pack can be stored at room temperature until next use.

Procedure Revisions:

January 2015: added that a sample must not be skipped in the rack.

July 2016: No changes made

July 2017: No changes

Appendix XVIII. UNC MGL – Saliva DNA Extraction for NCGENES 2

Molecular Genetics Laboratory Department Manual		
UNC HEALTH CARE	Policy Name/Procedure Name	**Newborn Saliva DNA Extraction using the Qiagen BioRobot® EZ1**
	Author/ Revision Date	**Kay Chao, 08/20/15**
	Date this Version Effective	**September 2015**

REFERENCES:

1. BioRobot EZ1 User Manual, Version 1.1, July 2005

2. EZ1 DNA Handbook, 3rd Edition, February 2008
   (EN-EZ1-DNA-Blood-Handbook, April 2010)

PRINCIPLE:

Magnetic particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. DNA is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotrophic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted in elution buffer.

SPECIMENS:

DNA is extracted from newborn saliva sample collected from prototype P-118 (DNA Genotek, Inc. Ottawa, ON, Canada) with swabs and preservation buffer in collection tube. After collection, ensure the collected sample is in an upright position to keep swab in the preservation solution and store at 15°C to 25°C.
Extracted DNA not used for requested testing is stored at 4°C short term and then at –20°C for a minimum of two years.

APPARATUS/REAGENTS:

1. BIOROBOT® EZ1 – Qiagen
2. EZ1 DNA Blood 350 µl Kits – Qiagen (cat# 951054)

PROCEDURE:

Proteinase K pretreatment:
Add 10 µl of 20 mg/mL Proteinase K solution to 350 µl of saliva sample. Mix the sample by inverting the sample tube 5 times and incubate at 56°C hybridization oven for overnight. Proteinase K-treated samples may be stored at room temperature until ready for purification.

EZ1 purification:

1. Insert the appropriate EZ1 Card (EZ1 DNA blood card) completely into the EZ1 Card slot.
2. Switch on the BioRobot EZ1.
3. Press “Start” to display the “Protocols” menu.

4. For worktable setup, press "**2**" to start for the **350** µl **sample protocol**. Select"**2**" to **elute in 100 µl** elution volume. **Pure ethanol wash, select** "**2**" **Yes**.
5. Press any key to proceed through the text displayed in the LCD.
6. Open the workstation door.
7. Invert 1-6 reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.
8. Load the reagent cartridges into the cartridge rack.

   Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

9. Load 1-6 opened elution tubes in to the first row.
10. Load 1-6 tip holders containing filter-tips into the second row.
11. Load 1-6 each of 2 mL tube with 1800 µl of **80% Ethanol** (1440 µl of 100% Ethanol and 360 µl of molecular grade water) into the 3rd row.
12. Load 1-6 opened sample tubes containing 200 µl l saliva samples into the fourth row.
13. Close the workstation door.
14. Press "Start" to start the protocol.
15. When the protocol ends (~20 min), the LCD displays "Protocol Finished". Open the workstation door.
16. Remove the elution tubes containing the purified DNA. Discard the sample preparation waste.
17. To run another protocol, press "ESC", prepare samples as described in step 1, and follow the procedure from step 5 onward. Otherwise, press "Stop" twice to return to the first screen of the LCD, close the workstation door, and switch off the BioRobot EZ1.
18. Clean the BioRobot EZ1with Sani wipes.

Appendix XIX. UNC MGL - Identity Testing Protocol for NCGENES 2

STEP 1: PCR

REAGENTS:

Platinum Blue PCR SuperMix (Invitrogen)
PCR primers (10µM working stocks):

SNP	Oligo	Sequence 5'-3'
rs600859	rs600859_for	GGGATTCATCTCCCACAAAA
	rs600859_rev	CCCAGCAGATGACTTCTTCC
rs1800351	rs1800351_for	TTCCAGGATCTGTAACAATGGA
	rs1800351_rev	ATGCAAAGCACCATGATGAA
rs3746438	rs3746438_for	GACTCACGTCCTCCACCTGT
	rs3746438_rev	GGTGAAGAACCTGACGGAAG
rs2236181	rs2236181_for	TTGAGGGATTCGGGACAATA
	rs2236181_rev	ACAGGCCATCCTCACTGTTC
rs11734372	rs11734372_for	CACAAAATTGGTTTAAAGCTGATG
	rs11734372_rev	ACCCGGCCTGAATACTCTTT
rs2229992	rs2229992_for	TTGGTACCAGTTTGTTTTATTTTAGA
	rs2229992_rev	CCTTGTTGGCTACATCTCCA
rs10835051	rs10835051_for	GGGCCTCCACTCTTTGATCT
	rs10835051_rev	CTGATTTCAAAGAGGCAACTGA
rs12129650	rs12129650_for	GCATCATAGGAAACCACAGG
	rs12129650_rev	GAGAAATTGTAGCCGTATGAAGC

PCR AMPLIFICATION

1. For high-throughput workflow (i.e., runs of multiples of 12 samples), for each SNP, combine the For & Rev oligos to a final concentration of 10uM each. Plate 1uL of mixed primers in a 96-well plate, so that each column of 8 wells contains each primer pair, cover with a kimwipe and dry overnight. Dried primer plates can be stacked and stored in plastic bags at 4C for at least 6 months.

2. For each sample, create the following PCR mix:

Platinum Blue SuperMix	118µL
DNA (10-50ng/uL)	9uL

3. Add 15uL of PCR mix to each well in a column on the PCR plate.

4. Vortex briefly, buzz spin to ~1200rpm, and incubate the samples in the thermocycler using the following conditions:

   94°C, 2:00

   94°C, 0:30 (
   55°C, 0:30 (35 cycles of these 3 steps)
   72°C, 1:00 (

   72°C, 3:00

4°C, ∞

When finished, perform check gel with 5uL product if desired.

PCR CLEANUP

Use AMPure PCR cleanup according to manufacturer's instructions

STEP 2: CYCLE SEQUENCING

REAGENTS:

Lab-grade water
Big-Dye Terminator

SEQUENCING AMPLIFICATION

1. For high-throughput workflow, create sequencing plates by plating 1uL of FOR primer (10uM working stock) in a 96-well plate, so that every column contains 1 well for each of the 8 FOR primers, cover with a kimwipe and dry overnight. Dried primer plates can be stacked and stored in plastic bags at 4C for at least 6 months.

2. Create BigDye master mix, multiplying the values below by the number of samples:

Reagent:	Volume per sample (8 reactions):	For 12 samples:
BigDye	42µL	504µL
Water	52.8µL	634µL

3. Using an 8-channel pipet, add 10µL to each appropriate well of the sequencing plate.
4. Using an 8-channel pipet, transfer 1uL of the cleaned PCR amplicon to the sequencing plate. Apply adhesive plateseal.

5. Vortex briefly, buzz spin to ~1200rpm, and incubate the samples in the thermocycler using the following conditions:

96°C, 0:05
96°C, 0:10 (
50°C, 0:05 (25 cycles of these 3 steps)
60°C, 4:00 (

60°C, 10:00
4°C, ∞

SEQUENCING CLEANUP

Purify sequencing reactions with Centrisep columns or plates according to manufacturer's instructions.

PERFORM SANGER DIDEOXY SEQUENCING PER PROTOCOL

ENTER IDENTITY CHECK GENOTYPE DATA INTO GENOMIC MOLECULAR WORKBENCH

The resulting genotype data from the identity testing protocol described above is entered into the Genomic Molecular Workbench hosted by RENCI (see Appendix section: CLIA Lab Tracking in NCGENES 2 Genomic Molecular Workbench (GMW) for details of data entry process and content)

Appendix XX. UNC MGL - CLIA Confirmation of Genetic Variant for NCGENES 2

Molecular Genetics Laboratory Department Manual		
UNC HEALTH CARE	Policy Name/Procedure Name	**Custom DNA Sequencing**
	Author/ Revision Date	**Ferrin Wheeler, Review date 12/5/17**
	Date this Version Effective	**December 2017**

This is a general procedure for sequence-based testing of a known/familial mutation.

Assay Design

Identify the exon and flanking intronic sequence that harbors the known mutation using Ensembl, UCSC Genome Browser or NCBI. Typically, amplification of the entire exon will be the best assay design, with primers placed in the flanking introns. Choose primers by hand or use a primer design website such as Primer3 (http://bioinfo.ut.ee/primer3/). Ideal amplicon size is between 200 and 400 bp. In most cases, PCR primers will also be used as sequencing primers. Ideal primer Tm values are 55-60 ºC, with GC content of 50%.

PCR Amplification

For previously unanalyzed specimens: In addition to the patient sample, include a positive control (family member with mutation, when available), normal, and no template control in every run.

For specimens run to confirm the presence of a previously identified mutation (such as information from a research assay): In addition to the patient sample, include a no template control in every run.

PCR Master Mix	1 Reaction (in µl)
Platinum Blue PCR Master mix	22
Forward Primer (10 □□)	1
Reverse Primer (10 □□)	1
DNA or Water	1

94ºC for 2 min.

94ºC for 30 sec.
55ºC for 30 sec. } X 35
72ºC for 1 min.

4ºC hold

Check Gel

Run 5 µl of PCR product on an e-gel and proceed if DNA samples amplified and the water control is blank.

Purification of PCR Product

Add 2 µl l of ExoSAP-IT to 5 µl of PCR product.

Incubate in PE 9700 or MJ thermocycler using **EXOSAP-IT** program:
37 °C for 30 minutes, then 80 °C for 15 minutes. Product can be stored at –20°C if necessary.

Cycle Sequencing Reaction

1. The sequencing reaction is set up on ice as follows:
   * All volumes are in microliters.

Sequencing Reaction	12.5 uL reaction*
ExoSAP-IT treated PCR product	1.125
Primer (10 µM)	0.75
Autoclaved ddH_20	5.625
BigDye Terminator	5

2. Amplify in 9700 thermocycler using the following program:
   9700 user: cx; program: cx26-seq

   96°C for 5 sec.

   96°C for 10 sec.
   50°C for 5 sec. } X 25
   60°C for 4 min.

   60°C for 10 min.

   4°C hold

   Samples can be stored at –20°C if not used immediately

Purification of Cycle Sequencing Product

1. Separate the required number of 8-well strips from the package, cut the bottom plugs and remove the foil covers.

2. Place the strips in the wash trays, making sure to balance the trays, using old strips if necessary. Centrifuge at 750 g for 2 minutes.

3. Place the strips into the ABI 3130 Genetic Analyzer 96-well plate, in the wells to be used for the run. If the run is larger than 16 samples, use two plates for the run so the spins are balanced. If not, use old columns for the balance plate in the centrifuge.

4. Using a P20 pipette set at 15 µl, add the sequencing reaction to the center of the column. Be careful to keep track, taking care to add one sample to each tube, in the correct order. Keep the empty reaction tubes in the order used to set up the sample sheet.

5. Secure samples with parafilm and centrifuge at 750g for 2 minutes.

6. Runs are done in groups of 16 (two rows of 8 from the 96-well plate). If fewer than 16 samples are to be run, the remaining wells are filled with 20 µl of water. Add the water to the side of the well, to avoid having air trapped below.

7. Cover the plate(s) with the 96-well septa and place the sample tray(s) in a 9700 thermocycler at 95 °C for 3 minutes to denature the samples.

ABI 3130 Operation

1. Turn on the computer before turning on the instrument.

2. Open **3130 Data Collection** from the desktop, click the **Plate View** tab and click **New** or **Edit**. This process is analogous to creating a sample sheet and will identify which sample is in each well. Name the plate with the date followed, if necessary, by a letter suffix (a, b, c, etc.), and select **Sequencing**.

3. Use the **Plate Editor** as a template for the plate and enter sample information for the wells containing samples. If a full plate is not needed, mark on the plate which wells have been used so the remaining wells can be used for subsequent runs. Enter the following information using the pull-down window when present:

   Sample Name: Sample and primer (i.e., MLM#-exonF). Spaces are not allowed in this window.
   Comment: Gene name and exon number
   Results Group: Whatever folder you want the results to be saved to
   Instrument Protocol: SeqE
   Analysis Protocol: SeqE

   When the plate record is complete, click **OK**. The plate record will now appear in the **Plate View** tab as a Pending Plate Record.

4. Place the 96-well plate, with the 96-well septa in place, into the plate base and fit the plate retainer over the top. Make sure the plate retainer holes are aligned with the holes in the septa. Place the entire plate assembly onto the autosampler.

5. When the plate is in place, the plate position indicator in the **Plate** View will change from gray to yellow, indicating it is ready to be linked to a pending plate record.

6. Click on the appropriate plate record in **Pending Plate Records** and then click the plate position indicator that contains the plate to be linked.

7. Once the plate has been linked to a plate record, the green **Run Instrument** button on the toolbar is enabled. Click the **Run** button to start run. The run will be separated into groups of 16.

8. During a run **Status View** can be used to monitor the run. **Array View** and **Capillary View** can be viewed but these windows should not be left open as they can cause unrecoverable screen update problems.

Run Analysis

1. Open ABI Sequencing Analysis Software from the **Start** menu:
   Start
   Applied Biosystems
   Sequence Analysis 5.2
   Sequencing analysis

2. In the **Sample Manager** window, click **Add Files**. Under Desktop, select **Shortcut to Data Extractor**. Select the appropriate run and select **Add All**.

3. Individual files can be viewed and printed.

Procedure Revisions
November 2016: Replaced the MO# with MLM#
December 2017: Procedure reviewed, no change made

ENTER CLIA GENETIC VARIANT RESULTS INTO GENOMIC MOLECULAR WORKBENCH

The resulting genotype data from the identity testing protocol described above is entered into the Genomic Molecular Workbench hosted by RENCI (see Appendix section: CLIA Lab Tracking in NCGENES 2 Genomic Molecular Workbench (GMW) for details of data entry process and content)

Appendix XXI. Mission Health MGL – Blood DNA Extraction for NCGENES 2

POLICY: ML606 NUCLEIC ACID ISOLATION USING MAXWELL RSC
POLICY VERSION: D
POLICY EFFECTIVE DATE: 6/6/2018

SCOPE
For use in the Molecular section of the Fullerton Genetics Laboratory

PRINCIPLE
The Promega Maxwell® Rapid Sample Concentrator (RSC) Instrument provides automated nucleic acid purification for a range of sample types. The purification methods use sample lysis and paramagnetic particles that bind nucleic acids to a solid mobile plunger as the primary separation principle. Up to 16 samples can be prepared in a single run using preprogrammed kit specific run methods (approximately 40 minutes). This approach to magnetic capture avoids common problems such as clogged tips or partial reagent transfers, which result in suboptimal purification processing by other commonly used automated systems.
The automated purification performed by the Maxwell® RSC Instrument include:

1. Sample lysis in the presence of a specially formulated Lysis Buffer
2. Binding of nucleic acids to paramagnetic particles
3. Washing of the bound target molecules away from other cellular components
4. Elution of the product

The Promega Maxwell® RSC Instrument is supplied with preprogrammed purification procedures and is designed for use with predispensed reagent cartridges. Four kits are currently being used:

1. The Maxwell® RSC Whole Blood DNA Kit is used for purification of genomic DNA (gDNA) from whole blood samples. The Maxwell® RSC Whole Blood DNA Kit purifies samples using a silica-based paramagnetic particle, called the MagneSil® particle. The Maxwell® RSC Whole Blood DNA Kit is designed to purify genomic DNA from 50µl to 500µl of whole blood.
2. The Maxwell® RSC Blood DNA Kit is used for purification of genomic DNA (gDNA) from samples. The Maxwell® RSC Blood DNA Kit purifies samples using a novel paramagnetic particle, called the MagnaCel™ particle. This particle utilizes cellulose-based binding of nucleic acids and provides a higher bind capacity and cleaner eluate than traditional DNA purification. The Maxwell® RSC Blood DNA Kit is designed to purify genomic DNA from up to 300µl of whole blood. In addition to whole blood, FGL has validated this kit to isolate DNA from OraCollect Buccal Swabs, Tissues, Amniotic Fluid, and Cultured Cells.
3. The Maxwell RSC DNA FFPE Kit purifies nucleic acid form FFPE tissue using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of gDNA following a manual deparaffinization and tissue lysis procedure.
4. The Maxwell® RSC simplyRNA Blood kit is used for isolation and purification of total RNA from fresh whole blood collected in EDTA tubes. Prior to loading the samples onto the Maxwell® RSC, blood samples are preprocessed by selective red blood cell lysis, white blood cell isolation, followed by white blood cell lysis. The lysed samples are transfered into the provided cartridge and RNA is captured using paramagnetic particles that bind RNA and provide a solid mobile phase that carries the sample through a series of washes for sample purification.

POLICY
The Nucleic Acid Isolation and Quantitation process is documented in the Laboratory Information System.

SPECIMEN REQUIREMENTS

See appendices for sample specific requirements

EQUIPMENT, REAGENTS, AND SUPPLIES

Equipment and Instrumentation:

- Maxwell® RSC (Promega)

- Maxwell® RSC 16 sample deck tray
- Lab Armour Bead Bath set to 53°C
- Microcentrifuge
- Magnetic microcentrifuge tube rack
- Vortex
- NanoDrop® ND-1000 Spetrophotometer
- P1000, 200, and 20 Pipettes
- Heat Block
- Dead Air Box
- Auto Pipette

Reagents or Media:

- Maxwell® RSC Blood DNA Kit (Promega)
- Maxwell® RSC Whole Blood DNA Kit (Promega)
- Maxwell® RSC DNA FFPE kit (Promega)
- Maxwell® RSC simplyRNA Blood kit (Promega)

Reagent Preparation: Only the Maxwell® RSC simplyRNA Blood kit. See ML606 Appendix F for instructions.

Storage Requirements: Store the Maxwell® RSC kits at room temperature (15–30°C). See ML606 Appendix F for instructions and Maxwell® RSC simplyRNA Blood Reagent storage requirements.

Supplies:

1. 1.5 ml screw top microcentrifuge tubes
2. Filtered Pipette Tips: Long P1000, Standard P1000, P200, P20
3. 1.5 mL Flip Cap tubes
4. 10 mL Serological Pipettes
5. Disposable Transfer Pipettes

QUALITY CONTROL

QC Material: Nuclease Free Water, Elution Buffer from the Extraction Kit.
Preparation and Handling: QC material kept at room temperature.
Frequency of Performance: For every sample.
Acceptable Limits: $OD_{260/280}$ between 1.6 and 2.00 for DNA. $OD_{260/280} > 1.6$ and $OD_{260/230} > 1.2$ for RNA
Corrective Action (if fail): Re-extraction of patient sample if possible. DNA Clean-up protocol.
Recording QC Data: The following QC information is documented in the LIS:

6. Proteinase K added (check if added)
7. Lot number and Expiration dates of Maxwell kit and dilution solution used
8. Type of Maxwell kit used
9. Volume of Elution Buffer used
10. Initial and final concentration reading of Nucleic Acid
11. 260/280 Values. 260/230 Values for RNA Isolation
12. Total yield of Nucleic Acid
13. Initials of technologist isolating nucleic acid and date performed
14. Deparaffinization Date (for FFPE tissue)
15. Reagent Preparation Dates for simplyRNA Blood kit

PROCEDURE

Maxwell® RSC Instrument Operation
Refer to Appendices for sample specific procedures and preprocessing requirements.
Refer to the *Maxwell® RSC Instrument Operating Manual* for detailed information.

1. Turn on the Maxwell® RSC Instrument and Tablet PC. Log in to the Tablet PC, and start the RSC software on the Tablet PC. The instrument will power up, proceed through a self-check and home all moving parts.
2. Press **Start** to access the extraction method selection screen.
3. On the extraction method selection screen, select the method that matches the kit being used.
4. Verify the correct method is selected and press the **Proceed** button
5. Use the bar code reader to scan the 2D bar code on the kit box to enter the kit lot and expiration information.
6. On the cartridge setup screen, touch the cartridge positions to deselect any positions that will not be used. Selecting or deselecting any cartridge position is only used for reporting purposes and does not affect the way the instrument processes samples.
7. Enter the Elution tube identifier by scanning the barcode of the corresponding side label (optional).
8. Press **Proceed** button to continue
9. Confirm all checklist items
    a. cartridges are loaded on the instrument with the seals completely removed
    b. samples were added to well #1 of the cartridges
    c. uncapped elution tubes are present with Elution Buffer
    d. plungers are in well #8
10. Transfer the deck tray containing the prepared cartridges onto the Maxwell® RSC Instrument platform by angling the back of the tray into the platform and pressing down on the front until the tray snaps into place.
11. Ensure the deck tray is placed in the Maxwell® RSC Instrument with the elution tubes closest to the door. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation.
12. Ensure the deck tray is level on the instrument platform and fully seated.
13. Touch the **Start** button to begin the extraction run. The tray will retract, and the door will close.
14. The Maxwell® RSC Instrument will immediately begin the purification run. The screen will display information including the user who started the run, the current method step being performed, and the approximate time remaining in the run.
15. Pressing the **Abort** button will abandon the run. The samples will be lost for all aborted runs. If the run is abandoned before completion, you will be prompted to check whether plungers are still loaded on the plunger bar. If plungers are present on the plunger bar, you should perform Clean Up when requested. If plungers are not present on the plunger bar, you can choose to skip Clean Up when requested.
16. Follow on-screen instructions at the end of the method to open the door. Following the automated purification procedure, the deck tray will be warm. It will not be too hot to touch. To remove the deck tray from the instrument platform, hold onto the sides of the deck tray
17. Verify that plungers are located in well #8 of the cartridge at the end of the run. If plungers are not removed from the plunger bar, follow the instructions in the *Maxwell® RSC Instrument Operating Manual* #TM411 to perform a Clean Up process to attempt to unload the plungers.
18. Cap the elution tubes
19. Remove the deck tray from the instrument.
20. Remove the elution tubes from the tray.
21. The extraction run report will be displayed. From the report screen, you can print or export this report or both.
22. Remove the cartridges and plungers from the deck tray and discard as hazardous waste. Do not reuse reagent cartridges, plungers or elution tubes.

PROCEDURE NOTES

- If you are processing fewer than 16 samples, center the cartridges on the deck tray.
- Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bactericidal spray or wipe, then water. Do not use bleach on any instrument parts.
- Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® RSC Instrument.
- If a larger volume of eluate is desired, the chemistry can be eluted in up to 150µl of Elution Buffer. Elution with 60µl of Elution Buffer will result in the greatest final **concentration** of purified nucleic acid, while elution with a higher volume will result in higher **yield** of purified nucleic acid
- The starting volume of Elution Buffer will not result in the same elution volume after running the method. Typically, the resulting elution volume will be approximately 10–25µl less than the starting volume.
- Resin carryover into the Elution Tube is typical. Using a larger starting volume of Elution Buffer and/or a smaller volume of sample will reduce this carryover

- RNA isolation is carried out using equipment and consumables dedicated explicitly for RNA isolation under aseptic conditions.

Nucleic Acid Concentration and Working Solution Preparation

Reference Document ML607- Nucleic Acid Concentration and Working Solution Preparation.

REFERENCES

Promega/Madison, WI, “Operating Manual Maxwell® RSC Instrument Operating Manual”, TM411 Revised 7/16.
Promega/Madison, WI, “Technical Manual Promega Maxwell® RSC Whole Blood DNA Kit”, TM455 9/15.
Promega/Madison, WI, “Technical Manual Promega Maxwell® RSC Blood DNA Kit”, TM419 Revised 1/15.
Promega/Madison, WI, “Technical Manual Promega Maxwell® RSC DNA FFPE Kit”, TM437 Revised 11/17.
Promega/Madison, WI, “Technical Manual Promega Maxwell® RSC simplyRNA Blood Kit”, TM417 Revised 11/17.

POLICY: ML606 APPENDIX A BLOOD
POLICY VERSION: A
POLICY EFFECTIVE DATE: 12/15/2016

SPECIMEN REQUIREMENTS

Patient Preparation: No special patient preparation required.

Specimen Collection Method: Peripheral blood in lavender top EDTA vacuum tube obtained using standard venipuncture techniques. Other anticoagulants may be acceptable, including Sodium Heparin (green top), ACD Solution A (yellow top), and Sodium Citrate (light blue top). A minimum of 1 ml can be drawn, but the optimal amount is 3 ml or greater.

Special Handling or Storage Conditions: Upon receipt, store tube at 4°C until processed.

PROCEDURE

Using Whole Blood DNA Kit for Blood Specimens

1. Open the LIS and build an isolation batch.
2. Print two labels for the specimens in the batch.
3. Double check the LIS labels with the specimen container.
4. Change gloves before handling Cartridge, Plunger and Elution Tube.
5. Place the cartridge on the deck tray with the printed end opposite the numbers on the tray. Reference the Manufacturer's Quick Start Guide for a visual.
6. Press down on the cartridge to snap it into position.
7. Place an empty elution tube into the elution tube position for the cartridge on the deck tray.
8. Add 125µl (60 – 150µl) of Elution Buffer to the elution tube. Close the lid.
9. Place a label on the top of the elution tube and the reagent cartridge.
10. Verify the name on labeled cartridge corresponds to the name on the blood tube. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
11. Transfer 500µl (50–500µl) of mixed blood sample to well #1 of the corresponding cartridge.
12. Tip-mix the blood sample in Well #1 by pipetting up and down several times.
13. Verify the number on the specimen container matches the number on the label of the corresponding elution tube.
14. Refer to ML606 for the Maxwell Instrument Operations.
15. Label 1.5ml screw-top tube during the Maxwell run.
16. Upon completion of the run, open the Maxwell and remove the elution tube.
17. Vortex and centrifuge the elution tube in the microcentrifuge for 1 minute at full speed.
18. Place the elution tube on the magnetic rack to pull any resin that remains in the tube to one side.
19. Transfer the supernatant to the labeled screw-top tube that corresponds with the accession number on the elution tube. Make note of the elution volume that is recovered from each elution tube.
20. Enter the actual elution volume in the Elution Volume column on the LIS Isolation worksheet (ex. 125uL elution buffer typically gives 109ul after run is complete)
21. Enter/Verify kit lot number on the LIS Isolation worksheet

Using <u>Blood DNA Kit</u> for <u>Clotted</u> Blood Specimens

Pretreatment

- Mix blood sample for at least 5 minutes at room temperature.
- Prepare and label 1.5mL flip-top tube
- Add 300µl of Lysis Buffer to the flip-top tube.
- Add 30µl of Proteinase K (PK) Solution to flip-top tube.
- Add liquid blood 50-300µl to the flip-top tube.
- Vortex flip-top tube for 10 seconds.

- Incubate flip-top tube at 56°C for 20-30 minutes.

Processing

- Change gloves before handling Cartridge, Plunger and Elution Tube.
- Place the cartridge on the deck tray with the printed end opposite the numbers on the tray. Reference the Manufacturer's Quick Start Guide for a visual.
- Press down on the cartridge to snap it into position.
- Place an empty elution tube into the elution tube position for the cartridge on the deck tray.
- Add 100µl (range 40 – 100µl) of Elution Buffer to the elution tube. Close the lid.
- Place a label on the top of the elution tube and the reagent cartridge.
- Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
- Verify the number on the specimen tube matches the number on the label of the corresponding elution tube.
- Transfer the blood lysate sample from the flip-top tube to well #1 of the cartridge.
- Tip-mix the sample in Well #1
- Refer to ML606 for the Maxwell Instrument Operations.
- Label 1.5ml screw-top tube during the Maxwell run.
- Upon completion of the run, open the Maxwell and remove the elution tube.
- Vortex and centrifuge the elution tube in the microcentrifuge for 1 minute at full speed.
- Place the elution tube on the magnetic rack to pull any resin that remains in the tube to one side.
- Transfer the supernatant to the labeled screw-top tube that corresponds with the accession number on the elution tube. Make note of the elution volume that is recovered from each elution tube.
- Enter the actual elution volume in the Elution Volume column on the LIS Isolation worksheet (ex. 100uL elution buffer typically gives 90ul after run is complete)
- Enter/Verify kit lot number on the LIS Isolation worksheet

Appendix XXII. Mission Health MGL – Saliva DNA Extraction for NCGENES 2

POLICY: ML606 APPENDIX G SALIVA
POLICY VERSION: A
POLICY EFFECTIVE DATE: 9/18/2018

SPECIMEN REQUIREMENTS

Patient Preparation: It is preferable that the patient has no food or drink for 30 minutes prior to collection.
Specimen Collection Method:

Collection instructions given to physicians/practitioners:

Step 1: Gently move sponge along gums and inner cheek for 30 seconds to soak up saliva.
Step 2: Wring saliva out of sponge using the v-notch of the funnel. Repeat until liquid saliva reaches the fill line on the collection tube.
Step 3: Holding the tube upright, close the lid firmly until you hear a clicking sound. The liquid in the lid will then be dispensed into the tube.
Step 4: Holding the tube upright, unscrew the lid and replace with cap provided.
Step 5: Shake the capped tube for 5 seconds, discard the funnel.

Special Handling or Storage Conditions: Upon receipt, store tube containing saliva sample at room temperature until processed.

PROCEDURE

Using Blood DNA kit for ORAGENE Discover Saliva Specimens
Pretreatment

1. Vortex the ORAGENE Discover Saliva Specimens for 10 seconds
2. Incubate the ORAGENE Discover Saliva Specimens at 50-53°C in a bead bath for 1 hour.

Processing

3. Change gloves before handling Cartridge, Plunger and Elution Tube.
4. Center the cartridges on the deck tray. Press down on the cartridge to snap it into position.
5. Place an empty elution tube into the elution tube position for the cartridge on the deck tray. Label both cartridge and corresponding elution tube.
6. Add 68µl of Elution Buffer to the elution tube. Close the lid.
7. Verify the name and number on the specimen container matches the name and number on the label of the corresponding cartridge.
8. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
9. Transfer all of the sample to well #1 of the corresponding cartridge.
10. Mix sample in Well #1 by pipetting up and down several times.
11. Refer to ML606 for the Maxwell Instrument Operations
12. Label 1.5ml screw-top tube during the Maxwell run.
13. Upon completion of the run, open the Maxwell and remove the elution tube.
14. Vortex and centrifuge the elution tube in a microcentrifuge for 1 minute at full speed.
15. Place the elution tube on the magnetic rack to pull any resin that remains in the tube to one side.
16. Transfer the supernatant to the labeled screw-top tube that corresponds with the accession number on the elution tube.

REFERENCES

Promega/Madison, WI, “Operating Manual Maxwell® RSC Instrument Operating Manual”, TM411 Revised 7/16.

Promega/Madison, WI, “Technical Manual Promega Maxwell® RSC Blood DNA Kit”, TM419 Revised 1/15.

ORAGENE DISCOVER Product Insert. PD-PR-00619 Issue. DNA Genetec Inc. Ottawa, Canada. 1/2016-09.

Appendix XXIII. Mission Health MGL - Identity Testing Protocol for NCGENES 2

POLICY: ML809 MATERNAL CELL CONTAMINATION TESTING (PROMEGA POWERPLEX16 KIT)

POLICY VERSION: B

POLICY EFFECTIVE DATE: 6/5/2018

SCOPE

This procedure is intended for use by the Molecular section of the Fullerton Genetics Laboratory.

PRINCIPLE

STR (short tandem repeat) loci consist of short, repetitive sequence elements 3 to 7 base pairs in length. These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers. Alleles of STR loci are differentiated by the number of copies of the repeat sequence. Analysis of STR loci creates a unique genetic profile of an individual and can be used in many different human identification applications. Prenatal samples are assessed for maternal cell contamination (MCC) by comparing the STR profiles of DNA isolates from a fetal sample and a maternal blood sample. MCC occurs from poor collection technique of the fetal sample or, in the case of product of conception sample types, the inability to differentiate fetal tissue from maternal tissue during sample processing. MCC testing ensures that any results from downstream testing are a product of the fetal sample and not of contaminating maternal DNA.

The Promega PowerPlex® 16 HS System is a kit that is designed to amplify 16 STR loci in a multiplex PCR fashion using fluorescently labeled primers. The amplified products are separated by size using capillary electrophoresis and the raw data is analyzed on the GeneMapper Software.

Fluorescent Dye/Color of peaks	Loci
Fluorescein (FL)/ Blue	**Penta E**
	D18S51
	D21S11
	TH01
	D3S1358
Carboxy-tetramethylrhodamine (TMR)/ Black	**FGA**
	TPOX
	D8S1179
	vWA
	Amelogenin
6-carboxy-4′,5′-dichloro-2′,7′-dimethoxy-fluorescein (JOE)/ Green	**Penta D**
	CSF1PO
	D16S539
	D7S820
	D13S317
	D5S818

POLICY

This assay is not considered a diagnostic test. Rather, it is used as a prerequisite for downstream testing of prenatal sample types. All patient and assay information is documented and stored in the Laboratory Information System.

SPECIMEN REQUIREMENTS

Specimen:

- Prenatal Sample: DNA isolated from Amniotic Fluid, Product of Conception (POC), Tissue, Chorionic Villi, or cultured cells in a buffered solution.
- Maternal Sample: Whole Blood or Buccal Swab.

EQUIPMENT, REAGENTS, AND SUPPLIES

Equipment or Instrumentation:

- ABI 9700 thermal cycler
- ABI 3500 Genetic Analyzer with POP-7 Polymer (or equivalent)
- Centrifuge
- Minicentrifuge
- Vortex
- Pipettes (P-2, P-20, P-200, P-1000)

Reagents:

- Promega PowerPlex® 16 HS System

Contains: Water, Amplification Grade
PowerPlex® HS 5X Master Mix
PowerPlex® 16 HS 10X Primer Pair Mix
2800M DNA Control
ILS 600
Allelic ladder control

- HiDi Formamide

Storage Requirements:

All PowerPlex® 16 HS reagents are to be stored at -20□C (no defrost).

Consumables

- 0.2 ml thin-walled PCR tubes
- 1.5 mL tubes
- Half skirted 96-well reaction plate
- Clear adhesive film plate cover
- Aerosol resistant tips for pipettes
- Septa

Special Supplies:

GeneMapper Software

QUALITY CONTROL

QC Material:

- Allelic Ladder control (provided with kit)
- 2800M DNA control (provided with kit), diluted to 0.5 ng/µL in PCR grade water
- No DNA control run with each experiment.
- Internal Lane Standard (ILS) 600 (provided with kit)

Frequency of Performance:

Testing of controls is carried out each time an experiment is performed

Acceptable Limits:

- The PowerPlex® 16 HS Allelic Ladder mix contains all possible allele sizes for each marker.

- 2800M Control DNA allele sizes:

Marker	Expected Size(s)	Marker	Expected Size(s)
D3S1358	17, 18	D16S539	9, 13

THO1	6, 9.3	CSF1PO	12
D21S11	29, 31.2	Penta_D	12, 13
D18S51	16, 18	AMEL	X, Y
Penta_E	7, 14	vWA	16, 19
D5S818	12	D8S1179	14, 15
D13S317	9, 11	TPOX	11
D7S820	8, 11	FGA	20, 23

- The No DNA control should only have the ILS 600 visible, no blue, green or black peaks above a peak amplitude of 200.
- The Internal Lane Standard (ILS) 600 contains 22 DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases in length

Corrective Action (if fail):

Repeat assay, check DNA quality, if necessary.

PROCEDURE

Refer to ML809F1 Maternal Cell Contamination Worksheet.

Creating GeneMapper Project

1. Open the GeneMapper program. Under the File menu, choose New Project.

2. Choose the project type “Generic”. In the File menu, select “Add Samples to Project”. When the “Add Samples” window opens, choose the “Files” tab and navigate to the samples, typically found in: Computer/AB SW & Data (D:)/Applied Biosystems/3500/Data/(folder containing data).

3. Highlight the samples to be added to the project and click "Add To List" button. Once samples (or the folder they are in) appear in the right side of the window, click the "Add" button.

4. Once the samples open in the window, find the Allelic Ladder on the list. Ensure the sample type is set to "Allelic Ladder".

5. In the "Analysis Method" column, click on the box for the sample at the top of the list and choose "GM4.1 Analysis Method 16 HS" analysis method. Click on the heading to highlight the entire column, and press Ctrl D to fill down the column.

6. In the "Panel" column, click on the box for the top sample and open the folder "PowerPlex_16_Panels_v2.0". Choose "PowerPlex_16_v2.0" and double click to add it. Highlight the entire column and fill down.

7. In the "Size Standard" column, click the top sample box and choose "CXR_ILS_600" and fill down the entire column.

8. Locate the green arrow at the top of the GeneMapper window. Click on the arrow to analyze the samples. Save the project, using the experiment number (i.e. MCC-7) as part of the name.

Analysis

1. Highlight the 2800M control, the allelic ladder and the No DNA to check each control. Go to the "Analysis" menu at the top of the GeneMapper window and select "Display Plots" to view the electropherograms. Make sure the Plot Setting on the new window is set at "HID plot setting". Set the number of panes to view to the desired number. To change the Y-axis, click on it when the cursor is a magnifying glass and slide the line down to the desired height.

2. Confirm the run meets the QC requirements as listed in this protocol. Look to be sure each dye has clean peaks.

3. Save an image of the QC sample’s electropherograms. Click out of window.

4. Highlight the fetal and maternal samples and view the electropherograms. Deselect all but one dye color. Adjust image and save. In the "View" menu, choose “Zoom” and then “Zoom To Marker” to view each marker individually. View the peaks for each marker and document the alleles for the fetal and maternal samples. Repeat for all dyes.
    a. Off-ladder allele calls (labels OL) can be deleted and not analyzed further.
    b. All on-ladder allele calls should be scrutinized carefully. Peaks below 1000 may represent artifacts due to stutter PCR products, fluorescent background peaks or contaminating maternal alleles in the fetal sample. If these peaks align with either or both of the maternal alleles then they should be noted and flagged for further review.
    c. Document if the marker is informative. A marker set is informative if there is a unique maternal allele that is not in

the stutter position of the fetal or shared allele. A marker set is uninformative if 1) alleles are completely shared between maternal and fetal samples, 2) if the maternal sample is homozygous (only 1 peak), or 3.) if the maternal allele is in the stutter position of the fetal or shared allele.

d. Document if there is evidence of contamination. Evidence of contamination includes either the presence of 3 peaks in the fetal sample if the fetal sample marker is heterozygous, or if there is a large difference in fetal peak heights of a likely homozygous allele and a maternal allele in the fetal sample.
e. If an informative marker shows evidence of contamination, determine which of the peaks in the fetal sample fit the descriptions of Fetal, Maternal, and Shared allele. Document the marker name, allele identifications, allele sizes (# of repeats), and peak heights.
f. Calculate estimated % Maternal Cell Contamination for each contaminated marker as well as the Average % Maternal Cell Contamination for the fetal Sample (if applicable).

CALCULATIONS

1. The GeneMapper software performs the calculations necessary for viewing the data collected by the Genetic Analyzer. The allele sizes and peak height values are used to estimate the percentage of maternal contamination, if applicable.
2. All other calculations are performed the LIS.

% MCC for a Marker with a Heterozygous Fetal Sample
Maternal Allele Peak Height / (Maternal Allele Peak Height + Fetal Allele Peak Height)

% MCC for a Marker with a Homozygous Fetal Sample
2 x Maternal Allele Peak Height / (Maternal Allele Peak Height + Fetal Allele Peak Height)

Average % Maternal Cell Contamination of Fetal Sample
Sum of the % MCC for Contaminated Informative Markers / Total # of Informative Markers

REPORTING RESULTS

Reporting Format:

Results are reported as either "No Contamination" or "Contamination present" and the Average % MCC is given.

Reference Intervals:

- At least 2 informative alleles must be present for analysis.
- The lower limit of detection of maternal cell contamination (MCC) using the PowerPlex® 16 HS kit is ~5%.
- Normal (Uncontaminated) result: <5% maternal cell contamination detected.
- Contaminated result: ≥5% maternal cell contamination detected.
- FGL has shown that Chromosomal Microarray results for prenatal samples with up to 20% MCC can be distinguished from contaminating maternal DNA. Results for such samples may be reported at the Director's discretion.

Note: The sensitivity of different test methodologies to MCC varies, and the effects of MCC levels on result interpretation should be determined for each test procedure.

LIMITATIONS OF PROCEDURE

Interferences:

The STR loci and primers have been selected to avoid or minimize artifacts. The artifacts often associated with Taq DNA polymerase include repeat slippage and terminal nucleotide addition.

Repeat slippage (called stutter or shadow bands) is due to the loss of a repeat unit during DNA amplification, somatic variation within the DNA, or both. The amount of slippage depends on the locus and the DNA sequence.

Terminal nucleotide addition occurs when Taq DNA polymerase adds a nucleotide (usually adenine) to the 3' ends of amplified DNA fragments. This addition is not dependent on the template sequence. The efficiency with which this occurs varies with different primer sequences, thereby occasionally creating an artifact band that is one base shorter than expected. Primer sequences and a lengthy final extension in the amplification parameters provide conditions to complete the terminal nucleotide addition when the template DNA amounts are optimal.

The presence of microvariant alleles (alleles differing from one another by lengths other than the repeat length) complicates interpretation and assignment of alleles. There appears to be a correlation between a high degree of polymorphism, a tendency for microvariants and increased mutation rate. Thus, FGA and D21S11 display numerous, relatively common microvariants. For reasons yet unknown, the highly polymorphic Penta E locus does not display frequent microvariants.

See the PowerPlex® 16 HS System technical manual for full explanations.

REFERENCES

Promega Corporation/ Madison, WI, Technical Manual "PowerPlex® 16 HS System Instructions for use of products DC2100 AND DC2101", Part # TMD022, Revised 5/16.

www.promega.com

Applied Biosystems/Foster City, CA, "GeneMapper Software Version 4.1, Microsatellite Analysis Getting Started Guide", Part Number 4403672, Rev. A, 04/2009.

Nagan, N., *et al*., J Mol Diagn. 2011 Jan;13(1):7-11.

Lamb, A., *et al*., Genet Med. 2012 Nov;14(11):914-21.

Coleman, WB, Tsongalis, GJ. Molecular Diagnostics for the Clinical Laboratorian. 2nd Edition. Humana Press. New York, New York. 2006.

Appendix XXIV. Mission Health MGL - CLIA Confirmation of Genetic Variant for NCGENES 2

POLICY: ML901 APPENDIX O-WHOLE EXOME SANGER CONFIRMATION SEQUENCING
POLICY VERSION: A
POLICY EFFECTIVE DATE: 9/30/2017

SCOPE
This document is intended for use in the Molecular section of the Fullerton Genetics Laboratory

PRINCIPLE
Confirmation testing by Sanger sequencing will be performed after the whole exome sequencing (WES) data are analyzed. This testing is initiated by the director analyzing the WES data via exporting the variants of interest from the Cartagenia Bench Lab NGS software (see ML408-Appendix A) and the SeqMera application (see ML406). Primers are designed as needed after consulting the database of amplification primers already in-house. The primer sequences used for variant confirmation are stored in an Excel sheet on the Genetics Lab shared drive.

POLICY
- Confirmation testing is performed to confirm a potentially reportable sequence change that has been found using the WES workflow.
- Sanger confirmation of variants found using the NGS workflow are sequenced in the forward and reverse direction unless one of those reactions does not give clear results due to the nature of the sequence (i.e. homopolymer region).

REAGENTS
Amplification:
- Primers (containing M-13 sequence) for confirmation are designed as needed. The primer sequences are stored in an Excel file that is updated as more primers are ordered.

Diluted to 10 □M (Stock=100□M, dilute each primer 1:10)
Stored in -80□C freezer.

QUALITY CONTROL
Confirmation primers are considered to be validated if the NGS change is confirmed. This method ensures that subsequent testing using those primers (including family members of the patients with confirmed changes) are performed with validated primers. A record of the validated primers is located in the CRE Sanger Primers Record, located in the Genetics Lab share drive.

PROCEDURE
1. Check the NM number that was exported from Cartagenia with the NM number for the gene as listed in HGMD. Primers should be designed using the NM number listed in HGMD.
    a. If the NM number from Cartagenia does not match the one in HGMD, check to see if Cartagenia also lists the other available NM numbers by pulling up the variant of interest and expanding the NM number tract (click the arrow next to the top NM number; boxed in red in snapshot below). The export only pulls the top line from Cartagenia; therefore, the HGMD NM number may also be listed in Cartagenia but it may not be located on the top line.
    b. If the HGMD NM number is listed, check the exon number for which primers need to be designed, as it may not be the same as the exon listed on the top line (see below)
    c. If the HGMD NM number is not listed, check the exon sequence of the HGMD NM transcript number against the surrounding sequence provided in Cartagenia associated with the variant of interest to ensure that the exon listed on the export matches that exon sequence in HGMD. Otherwise, the primers for an incorrect exon might be designed. The HGMD NM number sequence and exon number can be accessed via UCSC (http://genome.ucsc.edu/cgi-bin/hgGateway).
        -In Cartagenia, click on the chromosome and genomic position link under the heading “position” (see snapshot below).
        -A window will pop up that indicates the variant with a red line and the surrounding sequence (see snapshot below).

2. Use the HGMD listing to create a cDNA file for the gene. Alternatively, follow the steps below to create a cDNA file that can be viewed in Sequencher.
    a. Copy the NM number determined above, open NCBI.

b. In the drop-down, select 'Nucleotide'.
c. Search using the NM number.
d. When the gene file opens, select 'Send to' in the drop-down menu on the top right (boxed in red in snapshot). Click the radio button beside 'File'. Then click "Create File."
e. When the file downloads, drag it into the NCBI file folder (\\Mh\ss\Genetics Office\genetics lab\Data_Architecture\Molecular\Sequencing\Sanger\CRE Sanger Confirmations\Sequencing Reads\cDNA files\NCBI files) and rename it with the gene name and NM number.
f. The file can now be dragged into or saved in the Sequencher project when needed for analysis of the patient sequence.
g. To use the cDNA file, drag the file into Sequencher and double click to open it.
h. Find the 1st base of the coding sequence. It will be the A of the ATG start codon and will have an M (indicating methionine) under it. Highlight the A.
i. Go to the 'Sequence' menu at the top of the page and hover over 'Set Base Number' then select 'As Base 1'. The highlighted base (A of the ATG) now becomes c.1 and is designated "residue 1" at the top of the cDNA window (see boxed items below). The c. number is on the left margin with the p. number below it in parentheses.
j. The sequence can be manipulated in this file to see how the change affects the cDNA. For example, if a C changes to a T in the patient, the user can change the base to a T in that position to see the amino acid change, or can be used to name frameshifts by counting the amino acid residues until the novel stop codon (designated as ".").
k. Exons can be visualized by going to the 'Sequence' menu and choosing 'Edit Features'.

Designing a Sequencher reference file for Sanger confirmation

1. Open Sequencher and select "New Project."
2. In the File menu, choose Import and then Sequencher Project from the 2nd menu that opens.
3. Find the project for the 1st gene exon that needs to be analyzed and import it. Files are stored in Genetics Lab share drive. If the exon(s) of interest is there, press the Ctrl button and click on it to remove the highlight. Then right click and select "Remove from project" to delete the unneeded sequences. Repeat as necessary for other gene exons.
4. If the exon of interest has not been added to the control project for the gene, follow the steps above to remove all the exons from the new project.
5. Save the new project using the patient accession number. The project should be saved Genetics Lab share drive.
6. Create a new sequence for the exon of interest.
    a. Open the control project for the genes beginning with that letter (i.e. A gene files, C gene files, etc.) found in the CRE Sanger folder.
    b. Under the Sequence menu select "New Sequence". Name the sequence as "GENE exon #".
    c. Obtain the NM # for the gene from the cDNA file, Sanger confirmation file or primer list.
    d. Navigate to UCSC Genome Browser. Search for the gene using the NM#.
    e. Select the highlighted gene on the browser.
    f. Click on the link below "mRNA/Genomic Alignments". This opens the gene sequence.
    g. On the left side of the screen, select the block number that corresponds with the exon of interest (i.e. if exon 25 is desired, select block 25).
    h. Copy the 10 intronic bases before the exon, all of the exon and 10 bases after the exon. Paste the sequence in the window that has opened in Sequencher.
    i. Highlight the exon by counting in 10 bases from the beginning and end of the newly pasted sequence. Confirm the exon sequence by comparing to the UCSC file.
    j. Right click on the highlighted exon sequence and select Edit Features.
    k. On the new window, click the Add button. Type the gene and exon# in the Feature Name box. Click Display Style to choose a color to display the exon. Blue is typically used for the exons, red for UTR sequence. Introns are left black.
    l. Click the Done button and close the sequence window. If the sequence is complete, click "Record as Experimental Data" in the window that opens.
    m. The exon is now added to the list of exons in the gene project. Save the project before closing the gene project.
7. Return to the patient project. Add the exon you just created to the patient project by following steps 2-4 above.
8. Import the sequencing raw data for the patient. Assemble the contigs. The contigs should be named by right clicking on each one and selecting 'Rename Contig'.
9. Analyze as usual and document results on the file created by the director containing the confirmation exons.

REFERENCES

Sequencher® version 5.4.6 DNA sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA
Cartagenia Bench lab NGS (Agilent Technologies, Santa Clara, CA)

POLICY: ML901 SANGER SEQUENCING
POLICY VERSION: R
POLICY EFFECTIVE DATE: 1/22/2018

SCOPE

This procedure is intended for use by the Molecular section of the Fullerton Genetics Laboratory

PRINCIPLE

See appendices for principle of each test

POLICY

For procedures utilizing Polymerase Chain Reaction (PCR), the PCR only pipettes and plugged tips are used. PCR products are not opened in the PCR set-up areas of the lab.

Specimen:

- Isolated DNA in buffered solution

Equipment:

- Thermal cycler (ABI 9700, ABI 2720 or other)
- Microcentrifuge (Biofuge 13 or other)
- Tabletop Centrifuge (Sorvall Legend T, Heraeus Megafuge 16 or other)
- SpeedVac (with rotors for microcentrifuge tubes and 96-well plates)

Reagents: *(See Appendices for Test Specific Reagents)*

- AmpliTaq Gold 360 Master Mix
  Stored in lab refrigerator (4□C)

- AmpliTaq Gold 360
  Stored in lab freezer (-20ºC)

  - GC Enhancer
    Stored in lab freezer (-20ºC)

    - 6M Betaine.
      Stored in lab freezer (-20□C)

  - Primers M-13-Forward and M-13-Reverse (if applicable)
    Diluted to 10 □M (Stock=100 □M, dilute 1:10 for sequencing).
    Stored in -80□C freezer.
    M13 For-short: GTAAAACGACGGCCAG
    M13 Rev-short: CAGGAAACAGCTATGAC

  - Exo-SAP IT
    Stored at –20□C

  - ABI prism BigDye Terminator v1.1 Cycle Sequencing Kit with AmpliTaq DNA Polymerase, FS (Ready Reaction Kit)
    Stored in lab freezer (-20□C)

  - BigDye Terminator 5X Sequencing Buffer
    Stored in lab refrigerator (4ºC)

  - DyeEx 2.0 Spin Kit
    Stored at room temperature on top shelf of molecular workbench.

- Performa DTR V3 96-Well Short Plates
  Stored in lab refrigerator (4ºC)

- Hi-Di Formamide
  Stored in lab freezer (-20□C). Aliquot into 1.5 ml tubes for long term storage.

Reagent Preparation:

- Mix the 6M Betaine. Weigh 0.7g of Betaine and mix with 1000µl of sterile deionized water. The Betaine solution can be aliquoted into 1.5 ml tubes and frozen at -80°C.

Note: Do not use Betaine with AmpliTaq Gold 360 Master Mix as it inhibits amplification.

Materials:

- 0.2 ml thin walled PCR tubes
  Stored in cabinet of molecular workbench

- 1-20 □l filter pipette tips
  Stored in the molecular lab

- 1-200 □l filter pipette tips
  Stored in the molecular lab

- 1.5 ml microcentrifuge tubes
  Stored in cabinet of molecular work bench

- Pipette tips (1-200 □l)
  Stored in the molecular lab

- Blue pipette tips (200-1000□l)
  Stored in the molecular lab

- Half-Skirted 96-well plates
  Stored in the molecular lab

Controls:

One tube containing no DNA

POLYMERASE CHAIN REACTION AMPLIFICATION

PROCEDURE

NOTE: See Appendices and Worksheets for Test Specific Details

1. See the attached amplification worksheets as listed under "Other Related Documents"

2. Mix the components as listed, multiplying by the number of samples to be tested plus at least 1.

3. Program a thermal cycler to the conditions as listed and place the samples in the sample block to run.

4. Remove the PCR products from the thermal cycler when cycling is complete. One or 2 □l of the PCR product can be run on a 1-3% gel to confirm amplification, if desired.

5. PCR products must be cleaned prior to sequencing using Exo-SAP It. For each amplification, label one 200 □l PCR tube or 96-well plate. The "No DNA" tube(s) does not need to be cleaned.

6. Aliquot 5 □l of the PCR products into the corresponding tube. Add 2 □l of Exo-SAP It and pipet up and down to mix. Volumes may be increased in this proportion if more volume is needed for sequencing reactions (i.e. 10 □l of PCR products and 4 □l of Exo-SAP It).

7. Place tubes in a thermal cycler programmed at 37□C for 15 minutes, 80□C for 15 minutes, hold at 4□C.

8. The purified DNA should be stored at 4□C until sequencing is performed. Run 2 □l of the purified products on a 1-3% gel to confirm that all primers have been removed, if necessary.

CYCLE SEQUENCING REACTION

NOTE: See Appendices and Worksheets for Test Specific Details

PROCEDURE

1. See the attached sequencing worksheets as listed under "Other Related Documents"

2. Mix the components as listed, multiplying by the number of samples to be tested plus at least 1.

3. Place into the thermal cycler programmed for the proper conditions and start.

4. When the thermal cycler is finished running the sequencing cycles, remove the sequenced products and proceed to Clean-up of Sequencing Reactions.

CLEAN-UP OF SEQUENCING REACTIONS

DyeEx Spin Kit (tubes)

PRODEDURE

1. Prepare one DyeEx spin column and label one 1.5 ml centrifuge tube for each sequenced patient and control.

2. Gently vortex the spin column to resuspend the resin. Loosen the cap of the column (to prevent a vacuum inside the column), and snap off the bottom tip of the column. Place the column in the 2ml collection tube provided with the kit.

3. Set the speed of the Biofuge 13 microcentrifuge to 3000 rpm for 3 minutes and spin the column inside the collection tube.

4. Transfer the spin column to the labeled 1.5 ml centrifuge tube. Slowly transfer the sequenced products to the gel bed. Pipet the products in the center of the slanted gel bed, and allow them to absorb into the gel bed. It is not necessary to replace the lid on the column.

5. Again, spin the column inside the centrifuge tube at 3000 rpm for 3 minutes.

6. Discard the column. The cleaned sequencing products are in the 1.5 ml centrifuge tube. Dry the products in the SpeedVac on medium for 20 minutes or until dry.

7. When the samples are completely dry, remove from SpeedVac and add 15 □l of Hi-Di Formamide. Set tubes aside while preparing the Genetic Analyzer. See procedure ML311--ABI Genetic Analyzer Use.

Performa DTR V3 96-Well Short Plates

(For use when cleaning ≥24 samples)

PROCEDURE

1. Remove the Performa plate from the refrigerator and unwrap the foil packaging.

2. Peel the bottom adhesive tape from the Performa plate and stack it on top of an empty half-skirted 96-well plate. This 96-well plate will receive the waste eluate from the Performa plate. Use small pieces of scotch tape to attach the 2 layers on the sides.

3. Peel the top adhesive tape from the Performa plate.

4. Balance the Performa plate assembly using an empty assembly. Add water to the wells of the empty assembly as necessary to balance the two.

5. Remove all the carriers from the tabletop centrifuge buckets. Place the cushion from the plate carrier directly into the bucket designed to spin plates. Place the assemblies directly into the bucket (not using the carrier).

Note: To prevent damage to the centrifuge carriers, it is very important to remove all carriers from the centrifuge before spinning at the speeds required for the Performa plates.

6. Spin at 850 x g for 3 minutes (program 2).

7. Remove the Performa plate assembly. Carefully cut or peel the scotch tape to remove the waste plate and discard the waste.

8. Place the Performa plate into a clean half-skirted 96-well plate. Attach the assembly using scotch tape.

9. Using a multichannel pipette, transfer the sequencing reaction products into the center of the wells of the Performa plate, taking care not to touch the sides of the wells.

10. Balance the assembly using another empty assembly, adding water as necessary. Place directly into the centrifuge buckets.

11. Spin at 850 x g for 5 minutes (program 3).

12. Remove the assembly and carefully cut or peel the scotch tape. Retain the 96-well plate containing the cleaned sequencing products. There will be some liquid in all of the plates, regardless of whether all the wells were loaded with sequencing products.

13. Change the SpeedVac rotor to the plate adaptor. Place the 96-well plate into the carrier and balance with an empty plate. Dry on high for 35 minutes or until all liquid has evaporated.

14. When the samples are completely dry, remove from SpeedVac and add 15 □l of Hi-Di Formamide. Set tubes aside while preparing the Genetic Analyzer. See procedure ML311--ABI Genetic Analyzer Use.

REFERENCES

Applied Biosystems/ Foster City, CA, "Automated DNA Sequencing Chemistry Guide", Part Number 4305080B, 2000.
Qiagen/ Valencia, CA, "DyeEx Handbook for DyeEx 2.0 Spin Kit, DyeEx 96 Kit", May 2002.
EdgeBio/ Gaithersburg, MD, "Product Summary-Performa DTR V3 96-Well Short Plates" v.3.

OTHER RELATED DOCUMENTS

Appendix O-Whole Exome Sanger Confirmation Sequencing
ML901F59 NGS Confirmation-Sanger Sequencing-AMPLIFICATION
ML901F56 NGS Sanger Sequencing-Recurrent Drop-outs and Confirmation
ML316 ABI Genetic Analyzer Use - 3500

POLICY: ML901F59 NGS Confirmations – Sanger Sequencing Amplification Worksheet
POLICY VERSION: B
POLICY EFFECTIVE DATE: 7/11/2016

Set-up Date: _____ Tech Initials: _____ Experiment #:_____

Used plugged pipette tips? _____ Used "PCR Only" Pipettes? _____

PROCEDURE

1. Paste the Sample #, Gene, Exon and Mutation to Confirm (HGVS) columns from the CT panel Sanger confirmation log into the chart on page 2 of this form.
2. Label PCR plate or tubes for each patient or control to be tested and each gene/exon to be tested. Circle the "No DNA" control well.
3. Add 1 □l of the appropriate primer set into each well using multichannel pipette. Add one primer set to the "No DNA" control for each exon.
4. Make up the appropriate reaction stock volume based on the components below without primers or DNA. Mix gently and spin down.
5. Aliquot 18 □l of reaction mix into the "No DNA" control well(s).
6. Add DNA to the remaining reaction stock, mix gently and spin down. Aliquot 19□l of reaction mix to each of the remaining wells.
7. Place in a thermal cycler and program for the conditions listed below.
8. After PCR, run 4 □l on a 3% NuSeive agarose gel and clean with Exo-SAP-It (2.5 □l of PCR product + 1 □l of Exo-SAP-It). Continue with sequencing reaction using cleaned PCR products.

Master Mix 1		
1X PCR Reaction Volumes (µl)	**Component**	**X Reaction Volumes (□l)**
10	AmpliTaq Gold 360 Master Mix **Lot #:**	
8.5	Sterile deionized H_2O	
0.5	Patient DNA @ working solution stock (115 □g/ml)	**(X-1)**
1	Forward and Reverse Primer Mix for each exon (stock=100□M) dil 1:10 each (10 □l of each primer stock + 80 □l diH_2O=100□l of primer mix @ 10□M)	In wells
20 □l	**Total Volume**	
Optional (for primer sets that are difficult to amplify initially)	Add 1 µl GC Enhancer (as needed) **Lot #:**	

Control & Patient Samples Tested:
See chart on page 2.

Temp. Profile A	
Temp x Time	**Cycles**
95°C x 10 min	1
95°C x 30 sec 60°C x 45 sec 72°C x 1 min	35
72°C x 7 min	1
4ºC x ∞	1

Thermal Cycler Model (circle one): 9700 or 2720 Thermal Cycler #: _____

Primers used--Copied from CT panel Sanger confirmation log				
Amp Plate Well #	**Sample #**	**Gene**	**Exon**	**Mutation to confirm (HGVS)**

POLICY: ML901F56 NGS Sanger Sequencing Worksheet
POLICY VERSION: A
POLICY EFFECTIVE DATE: 2/16/2015

Reference:

Set-up Date: _____ Tech Initials: _____ Experiment #:_____

Used plugged pipette tips? _____ Used "PCR Only" Pipettes? _____

PROCEDURE

1. Label half-skirted 96-well plate and aliquot 1□l of cleaned PCR product into each well of a 96-well plate. One set of PCR products will be sequenced in the forward direction, a second set will be sequenced in the reverse direction.
2. Label two 1.5 ml tubes: one for the forward primer, one for the reverse primer.
3. Mix the components (listed below) and add 9 □l to each well, ensuring the forward and reverse cocktails are added to the correct wells.
4. Seal the plate using film and spin down. Place in a thermal cycler and program for the conditions listed below. Place a compression pad on top of the plate to ensure proper sealing of plate.
5. Clean sequencing products using the Performa 96-well plates or Dye-Ex Spin kit. Run on one of the ABI capillary sequencers.

Lot #'s: ExoSap-it: _____

DyeEx kit or Performa plate: _____

HiDi formamide: _____

Sequencer Used:_____

Reaction Volumes (µl)	Component	X Reaction Volumes (□l)	
		Forward	Reverse
1	Cleaned PCR product (dil 1:3)	In wells	In wells
6	Sterile deionized water		
1	Big Dye Terminator 5X Sequencing Buffer **Lot #:**		
1	Big Dye Terminator Ready Reaction Mix **Lot # :**		
1	M13 Primer Forward and Reverse (stock= 100□M--dil 1:10 for 10□M working stock)		
10 □l	**Total Volume**		

Control & Patient Samples Tested:

Temp x Time	Cycles
94°C x 5 min	1
94°C x 10 sec	25
50°C x 5 sec	
60°C x 2 min	
4ºC x ∞	1

Thermal Cycler Model (circle one): 9700 or 2720 Thermal Cycler #: _____

POLICY: ML316 GENETIC ANALYZER USE (3500)
POLICY VERSION: C
POLICY EFFECTIVE DATE: 2/12/2018

SCOPE

This procedure is intended for use by the Molecular section of the Fullerton Genetics Laboratory

PRINCIPLE

The 3500 Genetic Analyzer automates fragment analysis by separating DNA fragments according to length using capillary electrophoresis. The fragments are fluorescently tagged to produce different wavelengths of light when excited by the laser. The CCD camera converts the light (fluorescence) into electronic data for the computer to analyze and provide a profile of the separation.

REAGENTS AND SUPPLIES

Reagents:

- Anode Buffer Container, 3500 Series (Applied Biosystems®)
- Cathode Buffer Container, 3500 Series (Applied Biosystems®)
- Conditioning Reagent, 3500 Series (Applied Biosystems®)
- POP-7 Polymer pouch (384 samples), 3500 Series (Applied Biosystems®)
- DS-30 Matrix Standard (Dye Set D) (Applied Biosystems®)
- BigDye® Terminator v1.1 Sequencing Standard Kit 3500 Series Genetic Analyzers (Dye Set E) (Applied Biosystems® P/N 4404314)
- PowerPlex® 4C Matrix Standard (Dye Set F) (Promega)
- Hi-Di™ formamide (Applied Biosystems®)

Storage Requirements*:* Store at 4°C with the exception of the Hi-Di™ formamide and BigDye® Terminator v1.1 Sequencing Standard which should be stored at -20°C. Store PowerPlex® Matrix Standard at -20°C upon arrival and then 4°C after first use. Expiration dates on containers.

Special Supplies:

- 8-capillary array, 50 cm (Applied Biosystems® P/N 4404685)
- Cathode Buffer Container Septa (3500)
- Half skirted 96-Well Reaction Plate
- Clear Adhesive Film for 96 well plate
- Genetic Analyzer Plate Septa, 96-Well
- 20 ml, all plastic Luer-lock syringe
- P20, P200, and P1000 pipettes and aerosol-resistant tips

PROCEDURE

1. Start-up the computer. Enter “Administrator” for both user and password. Wait for computer to start-up.
2. After entering the username immediately turn on the 3500 instrument. The Daemon will automatically start and the computer will connect to the instrument. This may take a few minutes.
3. Click (on the desktop) on the “3500” icon to start the software. Enter the password “Administrator1” for the Data Collection software.
4. The Dashboard will open. The gauges show the status of the polymer, buffers and capillary. From the Dashboard, the other functions of the instrument can be accessed by clicking on either the buttons across the top of the screen, or the menu headings in the top right of the screen.

Preparing a run

1. From the Dashboard, determine if any maintenance of the instrument needs to be done prior to the run. Click on the “Refresh” button to ensure the information is up to date.

2. Check the number of runs remaining for the polymer and capillary, as the instrument will not link a plate that exceeds those numbers of runs.
3. Click on the Create New Plate button at the top of the Dashboard screen.
4. In the next window, assign a name to the plate and choose the plate type (Sequencing, Fragment, or Mixed). You may also fill in the Owner Name and Description, but these are optional.
5. Click the Assign Plate Contents button to move to the next window.
6. You may use the Plate View or Table View to name the samples. List the sample names in the correct wells.
7. The lower window on the screen shows 3 columns: Assays, File Name Conventions and Results Group. Click on "Add From Library" for each of these to choose the file(s) that will be used for the plate.

Assays	File Name Conventions	Results Group
FastSeq50_E (Sequencing)	Sequencing	Sequencing
PowerPlex (MCC)	Fullerton Sample Naming	Fragment Analysis
MSI (MSI)		Fragment Analysis
Fragment_Analysis_Assay (FMR1)		Fragment Analysis
MLPA_POP7-50cm-FRAG-ANALYSIS (MLPA)		MLPA RESULTS

8. The corresponding columns on the Table View must be filled in. A drop-down menu will appear when clicking on the cells. Choose the Assay, File Name Conventions and Results Group for each sample. When the top box is filled in for all 3, highlight the boxes of interest underneath and hold Ctrl D to fill them all in simultaneously.
9. Press the Tray button on the front of the instrument. Place the plate in either position A or B (left or right). Wait for the green light before proceeding to the next step.
10. Once all columns are filled in, click on the "Link Plate for Run" button at the bottom of the screen. If a plate has been previously run but is still linked, a message window will appear asking if you want to link the plate in position A (the left side) or leave the previous plate in A and link the new plate to B. Make the appropriate choice, and click "Okay".
11. On the next screen, if all plates to be run have been linked, click the "Start Run" button at the bottom of the screen to begin the run. The RFID tags will be read for all reagents before the run actually begins. It is best to wait to insure that no message appears.

NOTE: If the polymer has been on the instrument more than 7 days, the instrument will prompt you to choose if you want to continue the run. The run will not begin until after the message window is addressed.

12. The run can be monitored on the next screen. The column of the plate that is currently being run is highlighted in green. The instrument light will flash green when running until complete. When finished the light will remain solid green.

Cleaning and Maintenance

Buffer/Flush Water Trap (approximately Biweekly)

1. The Anode Buffer Container and the Cathode Buffer Container must be changed after it has been installed on the instrument for 14 days (hard stop). The 3500 monitors the time the containers have been on the instrument using RFID tags on all reagents.
2. Remove a new set of buffer containers from the refrigerator and allow to warm to room temperature.
3. Press the "Tray" button on the front of the instrument. Wait for the autosampler to move to the front.
4. Open the door, the light will become orange and flash, remove any plate that may be on the autosampler. Remove the Cathode Buffer Container from the autosampler by pushing it slightly back until it unclips from the 2 notches on the front of the autosampler. Remove both septa and install on the new Cathode Buffer Container. Set the new Cathode Buffer Container in place on the autosampler until it clicks into position.
5. The Anode Buffer Container must be pulled slightly forward before it can be lowered away from the electrode block and removed. Before the seal is removed on the new Anode Buffer Container, tilt the container to drain the small reservoir and pour any buffer that has overflowed back into the main container. Peel off the seal and lower the Anode Buffer Container under the electrode block. Lift the container until it can be slid into place toward the back of the instrument and held by the frame above the electrode block.
6. After the buffers have been changed, the water trap should be flushed using the plastic syringe.
7. Close the door, allow the autosampler to move back into position, the instrument light will turn solid green.
8. Document maintenance on ML316F1-ABI Genetic Analyzer Use-3500 Cleaning Record.

Polymer/Wash pump and channels-As Needed (approximately monthly)

1. The polymer has a soft stop that will flag the user after 7 days on the instrument. However, our experience shows that the polymer is stable for up to/exceeding 1 month on the instrument. The hard stop for the polymer is **384 samples**, which equals **48 injections**. Once the hard stop is reached, the instrument will not run until the polymer is changed.
2. Remove the used polymer pouch by lowering the arm that is to the right of the pouch. The pouch will click as it is lowered or raised. Once the pouch lowers, it can be pulled forward to slide the pouch fitment out of the bracket. Discard a completely used pouch in the biohazard waste.
   Note: If replacing a Polymer Pouch of the same Lot as the used Pouch, Proceed directly to "Replenish Polymer" Wizard under the Maintenance tab and do not run Conditioning Reagent.
3. Install a new pouch of Conditioning Reagent on to the instrument by sliding the pouch fitment into the bracket and raising the arm to click the pouch into place. Click on the Maintenance heading at the top left of the screen.
4. The actions available under the Maintenance menu are listed on the left of the screen. Click on Maintenance Wizards, and choose the "Wash Pump and Channels" button. The wizard will list the steps to follow to perform the wash and install a new pouch of polymer. This wizard will only work if you are using the same type of polymer. There is a separate wizard for changing polymer type.
5. Document maintenance on ML316F1-ABI Genetic Analyzer Use-3500 Cleaning Record.

Capillary Array-As needed (160-200 injections)

1. The capillary array has a soft stop of 160 injections but may be successfully used for many injections after this point. If quality begins to diminish after 200 injections, the capillary array may need to be changed.
2. To change the capillary array, click on the Maintenance menu at the top right of the Dashboard.
3. The actions available under the Maintenance menu are listed on the left of the screen. Click on Maintenance Wizards, and choose the "Change Capillary Array" button.
4. This wizard will list the steps to follow to change the capillary array and fill it with polymer.
5. When a new capillary array has been installed, both the Spatial and Spectral calibrations must be performed.
6. First, perform the Spatial Calibration:
   a. In the Maintenance menu, under the Calibration section (on Left), choose Spatial.
   b. Choose either the Fill or No-Fill option to either fill the capillary array with polymer or not.
   c. Click the Start Calibration button.
   d. When the calibration is complete, look at the peaks to ensure that they are approximately the same height and the blue plus-mark is at the top, center of each peak. If not, click the Reject Results button and repeat the calibration.
   e. If the calibration is good, click the Accept Results button to complete the Spatial Calibration.
7. Second, perform the Spectral Calibration:
   a. In the Maintenance menu, under the Calibration section (on Left), choose Spectral.
   b. Click on the Calibration Run tab to set up a run. The Chemistry Standard (Sequencing Standard or Matrix Standard), the Dye Set, and starting position must be selected.
   c. The chemistries/dye sets that are used by FGL are:
      i. Matrix Standard DS30=dye set D (for MLPA)
      ii. BigDye® Terminator v1.1 Sequencing Standard Kit = dye set E (for sequencing)
      iii. Matrix Standard for PowerPlex® 4C=dye set F (for Maternal Cell Contamination, and MSI testing)
   d. Refer to ML316 Appendix A for dye specific Spectral Calibration protocols.
   e. Remove the sealing film and replace with a 96-well septa. Place the plate in the plate holder for the 3500.
   f. Press the "Tray" button; wait for the autosampler to move forward. Remove any plate that is installed, and place the plate containing the spectral calibration standard in position A. Close the doors and wait for the green light.
   g. Click the "Start Run" button and wait for the run to be completed.
   h. If the data passes, click the Accept button at the bottom of the screen.
   i. Repeat for each dye set that may be run on the instrument. Only 1 plate containing 1 spectral calibration standard may be run at a time.
8. Document maintenance on ML316F1-ABI Genetic Analyzer Use-3500 Cleaning Record.

- Note: Spectral Calibration should be run when a new Dye Set or Polymer type is used on the instrument, or if the CCD camera has been realigned/replaced.

REFERENCES

Life Technologies/ Foster City, CA, “Applied Biosystems 3500/3500xL Genetic Analyzer User Guide”, Part Number 44011661, Rev. B, 08/2009.

Technical Manual: PowePlex® 4C Matrix Standard TMD048. October 2015. Promega Corporation. Madison, WI.

Product Information Sheet: DS-30 Matrix Standard Set (Dye Set D). Rev B. April 2017. Life Technologies Corporation. Carlsbad, CA.

Product Insert: BigDye® Terminator v1.1 Sequencing Standard Kit 3500 Series Genetic Analyzers. Rev A 6 March 2009. Applied Biosystems. Foster City, CA.

Appendix XXV. CLIA Lab Tracking in NCGENES 2 Genomic Molecular Workbench (GMW)

NCGENES2 Protocol for Tracking of Clinical Laboratory Samples, Sequencing Confirmation, and Reports

Version 1.0

Overview

Participants who are randomized to and consent to having genomic sequencing performed as part of the NCGENES2 project will provide paired samples: one to undergo genomic sequencing through the research lab, and a second which will be received in a clinical laboratory (monitored under CLIA). The clinical laboratory will receive and extract DNA from the sample, perform genotyping of a set of polymorphic single-nucleotide variants (the "identity-check" SNVs) and hold extracted DNA until confirmation of research results (pathogenic, likely pathogenic, or reportable variant of uncertain significance) is requested by the molecular sign-out committee. After attempt at confirmation, the clinical laboratorian will indicate whether this confirmation was successful. If confirmation was successful, then the clinical lab director will prepare a clinical report to be included in the participant's medical record, and will also record the report text as part of the study data set.

System of record

The system of record for these activities within the NCGENES2 project is the **NCGENES2 Genomic Molecular Workbench (GMW),** located at **https://ncg2.unc.edu** with authentication using a UNC onyen (**https://onyen.unc.edu**). Access to this system is restricted to computers on the UNC School of Medicine network or through the UNC Campus VPN. Authorization to access this system is controlled by study coordinators.

Upon login to this system, the user is presented with a role-based list of pending tasks that can be performed (Figure 1)

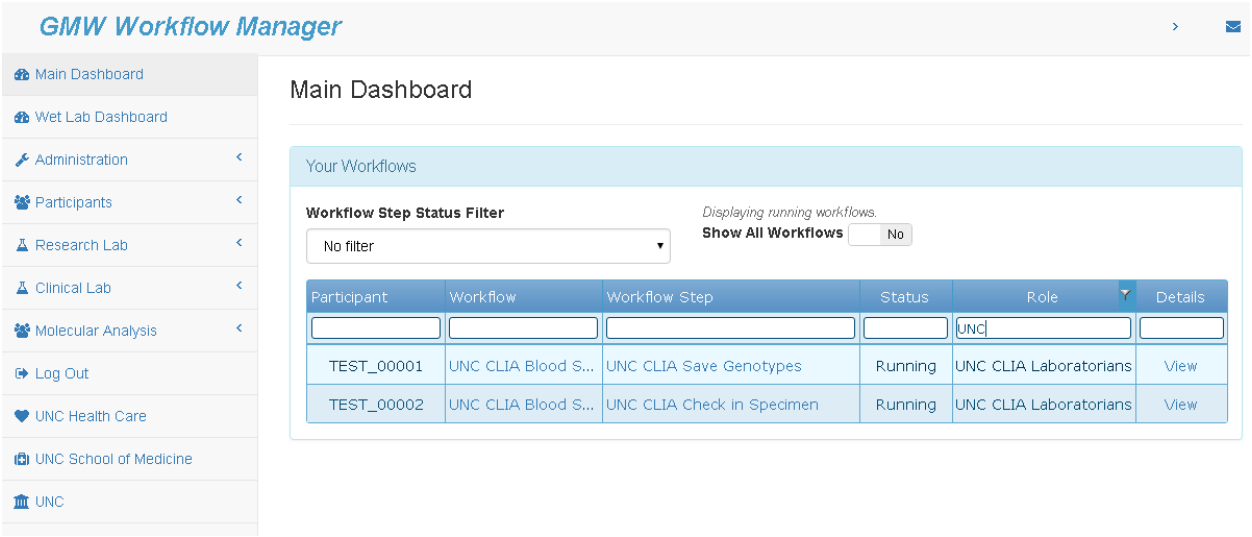


FIGURE 3: WHEN LOGGING INTO THE MOLECULAR WORKBENCH, THE USER IS PRESENTED WITH A ROLE-BASED LIST OF AVAILABLE TASKS. IN THIS EXAMPLE, THE SAMPLE FOR PARTICIPANT TEST_00002 IS AWAITING SPECIMEN CHECK-IN, AND THE SAMPLE FOR PARTICIPANT TEST_00001 HAS B

Recording receipt of sample

There are two options for indicating receipt of sample. Option 1 below is preferred since it reduces the possibility of improperly indicating sample receipt by allowing for entry of the sample identifier using a barcode scanned

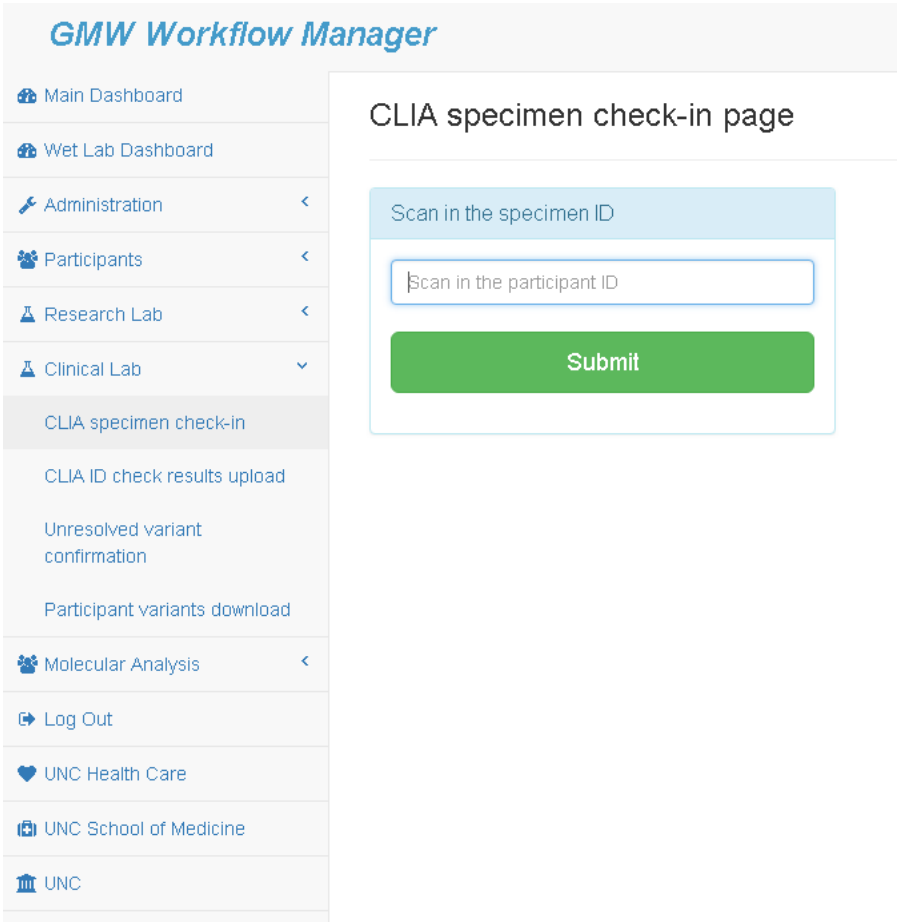


FIGURE 4: CLIA SPECIMEN CHECK-IN PAGE. NOTE THAT THE MENU OPTIONS ON THE LEFT WILL VARY BASED ON THE USER ROLE, BUT FOR CLIA LABORATORIANS THE "CLINICAL LAB" GROUP WILL ALWAYS BE AVAILABLE.

Option 1: Entry of sample ID using barcode reader

Selection of "Clinical Lab" and then "CLIA specimen check-in" from the menu on the left of the page displays a "CLIA specimen check-in" page (Figure 2). The technician will verify that the sample identifier on the tube and on the requisition form match, and use a barcode scanner to enter the specimen ID. This page then remains active and will allow for the entry of additional specimen IDs.

Option 2: Identification of the sample in the list of pending tasks.

The laboratory technician may identify samples awaiting check-in in this list as having the pending workflow step of "UNC CLIA Check in Specimen" or "Mission CLIA Check in Specimen" for the relevant lab where the sample is expected. The laboratory technician can indicate sample receipt by matching the participant number from the sample label and requisition form to a pending task in the GMW. Clicking on that task will present a form in which the technician provides a response to whether the sample received was adequate or not.

Recording genotypes of polymorphic single nucleotide variants

Following DNA extraction, Sanger sequencing is performed on a select set of polymorphic single nucleotide variants. These genotypes will be compared to genotypes of the same sites performed in the research labs by genomic sequencing as a control against sample swap.

After a specimen has been accessioned into the system, the next pending task related to this specimen for the CLIA Laboratorian is "UNC CLIA Save Genotypes" or "Mission CLIA Save Genotypes" for the respective laboratories (for example, as seen for participant TEST_00001 in Figure 1).. Clicking on the text of this pending workflow step will display the interface (Figure 3) for entry of genotypes of these "identity check" polymorphisms. Genotypes for each polymorphism (indicated by dbSNP RS identifier) are entered using a drop-down box of options.

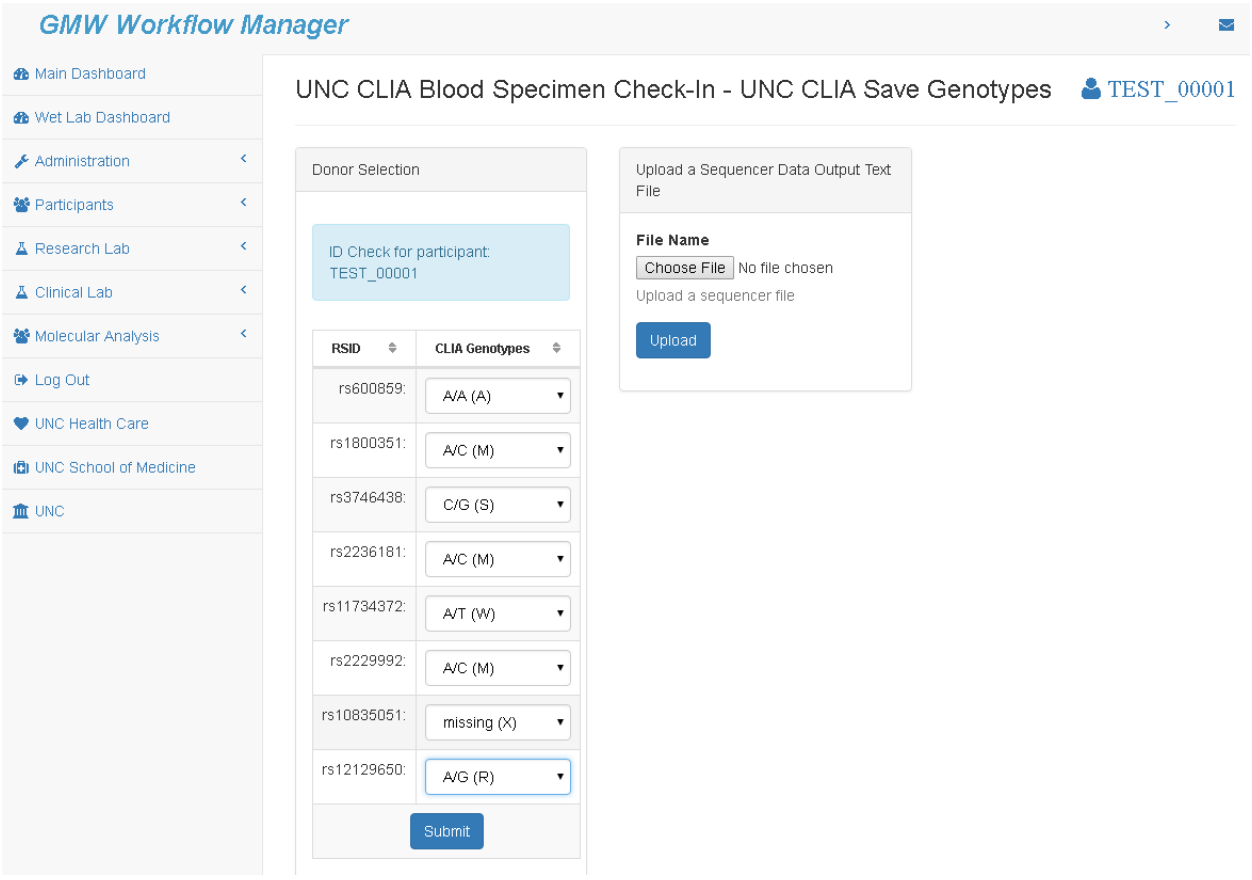


FIGURE 3: INTERFACE FOR RECORDING SANGER GENOTYPING OF "IDENTITY CHECK" GENOTYPES

Requests for confirmation of genomic sequencing results

Variants which are identified as reportable by the molecular sign-out committee (Pathogenic, Likely pathogenic, or reportable Variants of Uncertain Significance) will be marked by the committee within the GMW. This creates a notification to individuals with the "CLIA Laboratorian" role, and also adds the relevant task ("CLIA Variant Analysis Results") to their dashboard.

Primer design

Selecting "Clinical Lab" and then "Unresolved variant confirmation" from the menu to the left provides a list of all variants for which CLIA confirmation is pending. This list includes a column for amplicon design. The laboratorian will click in this column to obtain the sequence surrounding the variant to be confirmed as well as suggested PCR primers.

Recording of variant confirmation

The results of variant confirmation are recorded in the workflow step for "CLIA Variant Analysis Results". These are recorded in the column labeled "Confirmed" using one of the following codes:

- P: Pending (used for variants for which confirmation has not been attempted, so will not be a final code)
- U: Unknown- unable to demonstrate presence or absence of the variant (e.g., unable to design primers to amplify the relevant region, or unable to define primers that differentiate between this region and paralogous sequence)
- N: No, not confirmed- Sanger sequencing indicates that the variant is not present
- Y: Yes, confirmed- Sanger sequencing indicates that the variant is present.

When all of the variants have been assigned to one of the final categories (U, N or Y), the laboratorian presses the submission button to send the results on to the molecular pathologist for final interpretation.

Molecular pathologist determination of final result and reporting

After Sanger sequencing of the requested variants is performed, the molecular lab director determines the final case-level result. This includes marking whether the variants requested by the sign-out committee were confirmed, and if so, the generation of the text of the report. When no reportable findings are identified in the research lab (or none of the requested findings are confirmed in the clinical lab) then a negative report is generated directly by the GMW. When there are

findings to report that do confirm, then the laboratory director prepares a report according to their usual clinical practice. In addition, the laboratory director provides, within the GMW, the text of the clinical report (without any identifiable information) and the classification of the case-level result which can include one or more of the following categories:

- Definitive positive:
    - One or more Pathogenic variants in a gene, phenotype consistent with condition, with known phase or *de novo* inheritance consistent with expected inheritance pattern
- Probable positive:
    - One or more Likely Pathogenic variants in a gene, phenotype consistent with condition, with known phase or *de novo* inheritance consistent with expected inheritance pattern
- Inconclusive - due to unknown phase:
    - At least two Pathogenic/Likely Pathogenic variants in AR condition or X-linked recessive condition in female with phase not determined
- Inconclusive - due to variant uncertainty:
    - Case that would otherwise meet Definitive Positive or Probable Positive but at least one of the involved variants is a VUS
- Inconclusive - due to insufficient zygosity:
    - Single Pathogenic or Likely Pathogenic variant identified in AR condition or in female with X-linked condition
- Inconclusive - phenotype mismatch:
    - Case that would otherwise meet Definitive Positive or Probable Positive but where the condition would not explain all of the patient's phenotype, where the patient does not exhibit all characteristics expected of the condition, or where there is discordant co-segregation of phenotype with the variant within a pedigree (e.g., implicating incomplete penetrance or phenocopy)
- Inconclusive - novel gene:
    - Case where the association of the implicated gene with the patient's phenotype has not been definitively established
- Negative

Appendix XXVI. Registering for UNC LMS and UNC EPIC Training

Below is the process for getting write access in EPIC in order to pend research phlebotomy orders (for non-clinicians):

1. Must complete UNC HIPAA training. Need HIPAA certificate to send to Joe Baker
2. Must complete UNC Citi trainings (1) GCP for US FDA trials, and 2) Human Subjects Biomedical Group 1). Need both certificates to attach and send to Joe Baker
3. Must be on an approved IRB protocol. Need IRB approval letter to send to Joe Baker (You need HIPAA and both Citi trainings recorded in order to be approved on a UNC IRB protocol)
4. Must complete and sign a UNC Health Care Confidentiality Statement (ask Study Coordinator)
5. Must complete and have signed an University Employee EPIC Access Request Form (ask Study Coordinator)

All completed/signed copies of above documents should be sent to Joe Baker (Baker, Joseph J. **Joseph.Baker@unchealth.unc.edu**). There should be a total of 6 documents you send to Joe. You should request a UNC LMS account for EPIC training/access. Within 48 hours you will get an email from Joe with your "u" number and instructions on how to log in to LMS the first time. Once in LMS, you will need to take the following trainings:

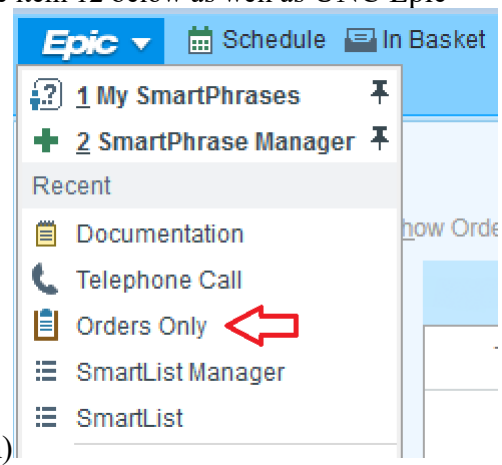
1. Go to Library (book icon)
2. Click on ISD Training-EPIC@UNC and get drop-down menu
3. Go to Research
4. Go to Research Assistant (Read-Only Access) and take this online course for read-only access to EPIC
5. Go to Research Coordinator Oncology (Non-CPO) and register for this curriculum.
    - There are 4-5 courses that need to be taken. Some are onsite in Morrisville and some are here at TraCs

6. Once you have completed the Research Coordinator (Non-CPO) curriculum you are technically able to pend orders, but you will need to contact EPIC admin first who has to set you up in EPIC. They should tell you who to contact at one of these courses.
7. Once you pend an order, you will need to show the provider how to find it in EPIC (see attached quick reference).

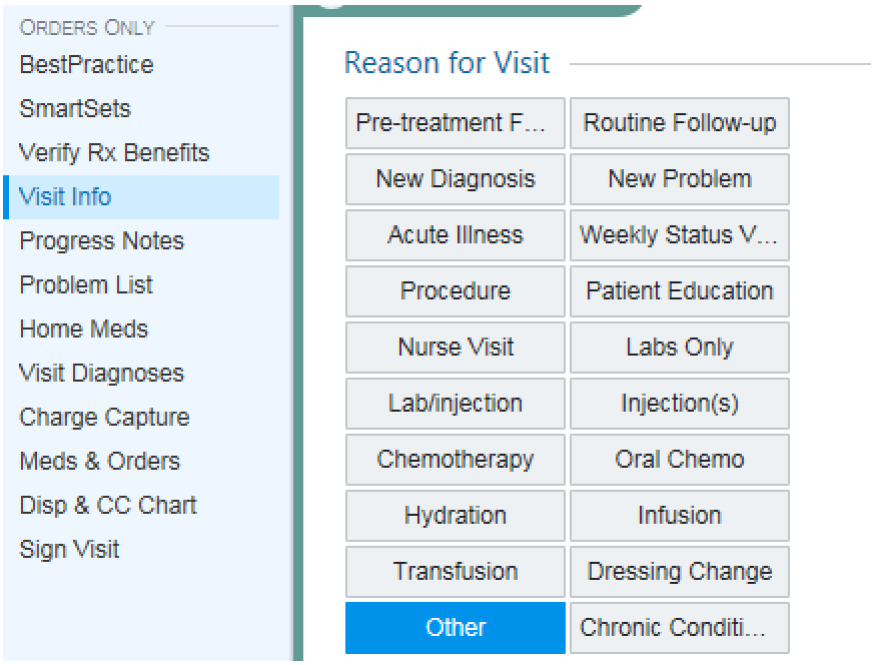
Stephanie Mascaro is a good contact if you happen to have any trouble once in EPIC.

Appendix XXVII. Creating a UNC EPIC Order

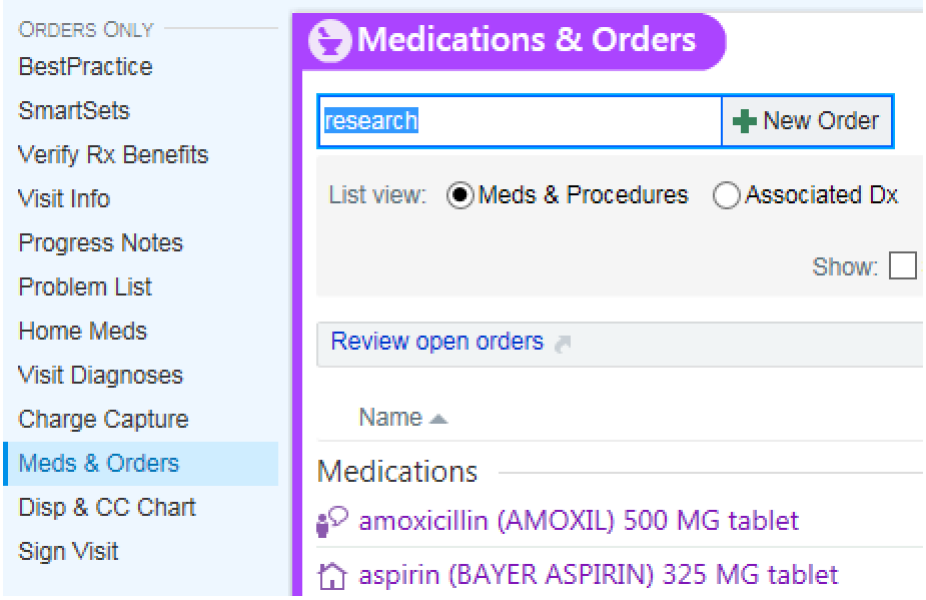
1. In Epic, create an orders only encounter for the participant. (See item 12 below as well as UNC Epic TRAINING REQUIREMENTS – in the Appendix section)



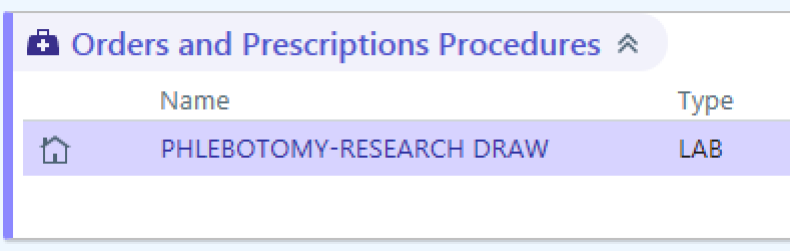
2. Choose "Other" as the reason for visit in the visit info section.

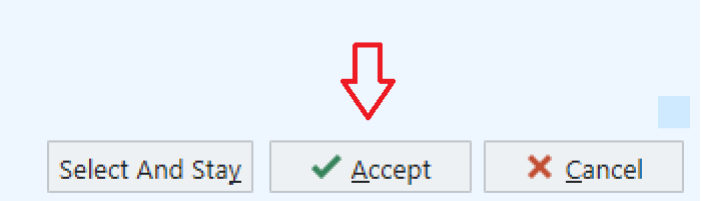


3. Go to the Meds & Orders section and begin searching for your lab by typing “research.” Hit enter to search.

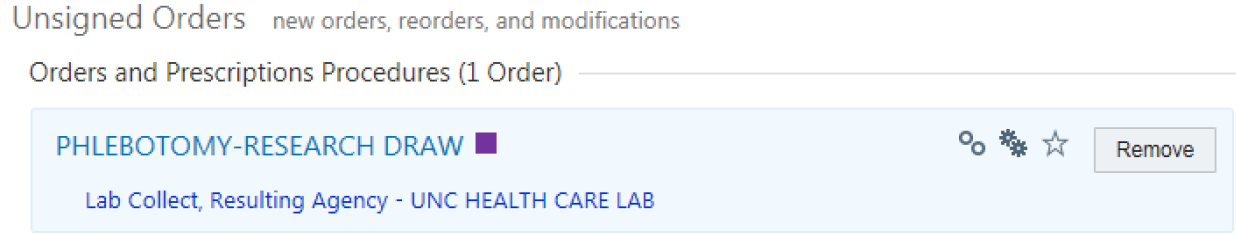


4. Select “Phlebotomy- Research Draw” and then click “Accept.”

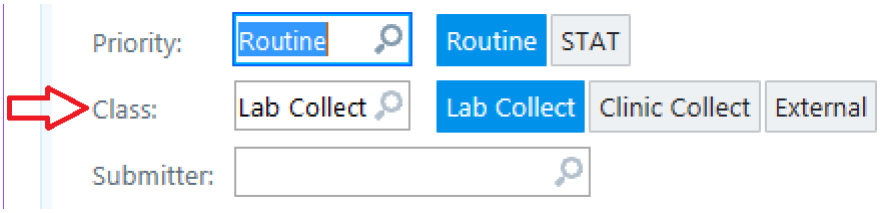




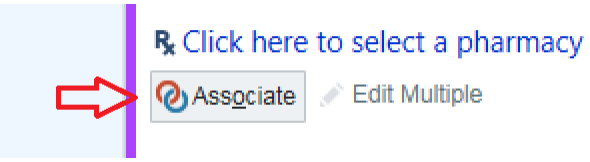
5. The order should now appear as an “unsigned order.” Click on the order name to open it. You need to open the order to check on the collection methodology and associate it with a diagnosis code.



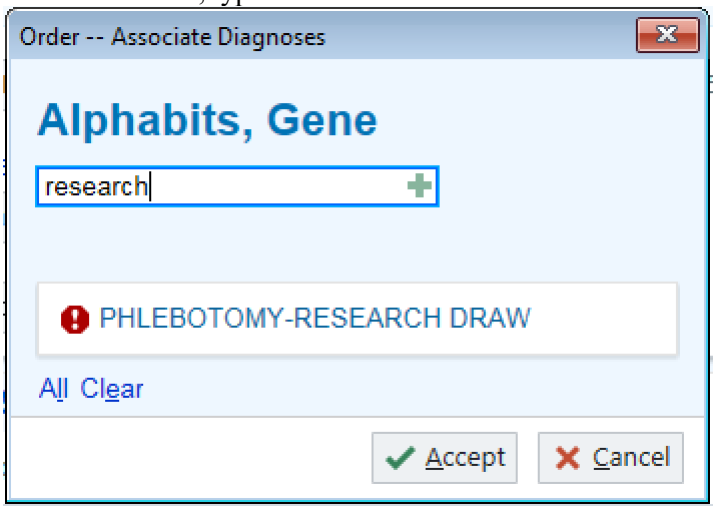
6. Make sure that the “Class” is Lab Collect.



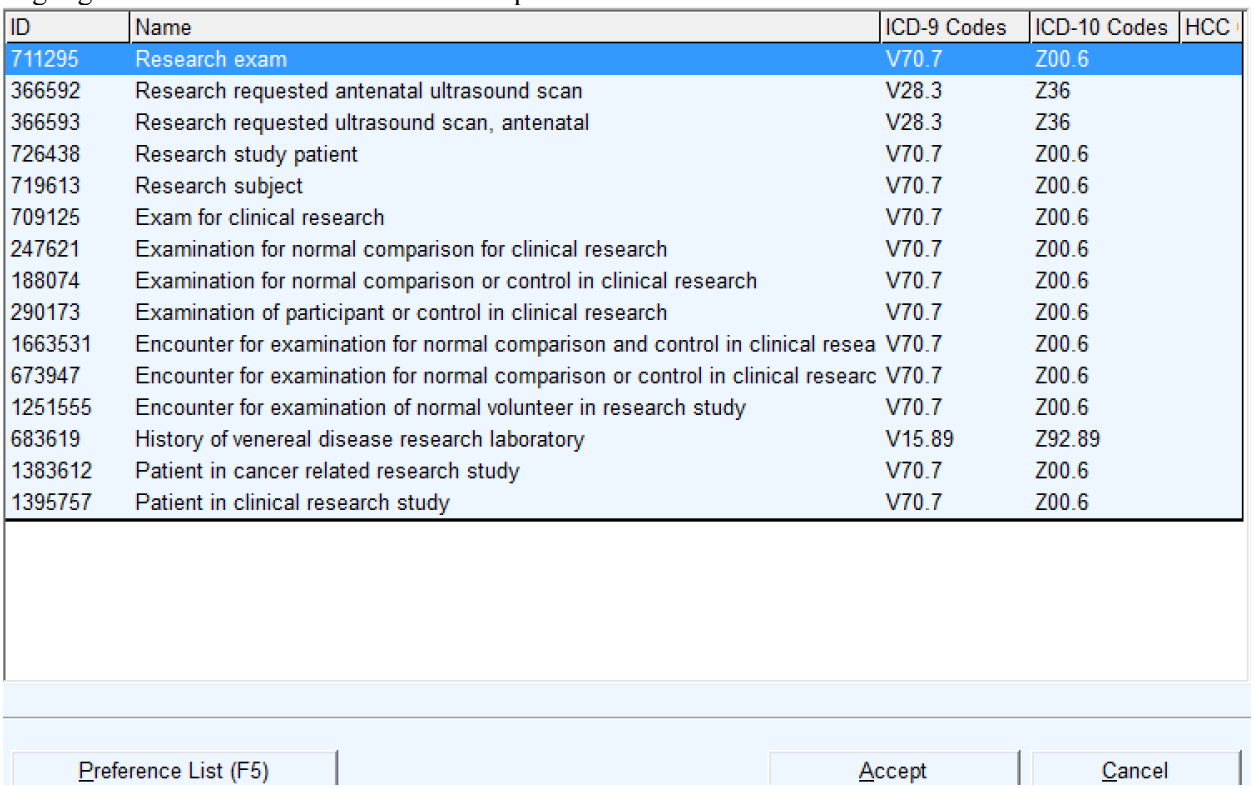
7. Click the “Associate” button.



8. In the search bar, type “research” and hit Enter.

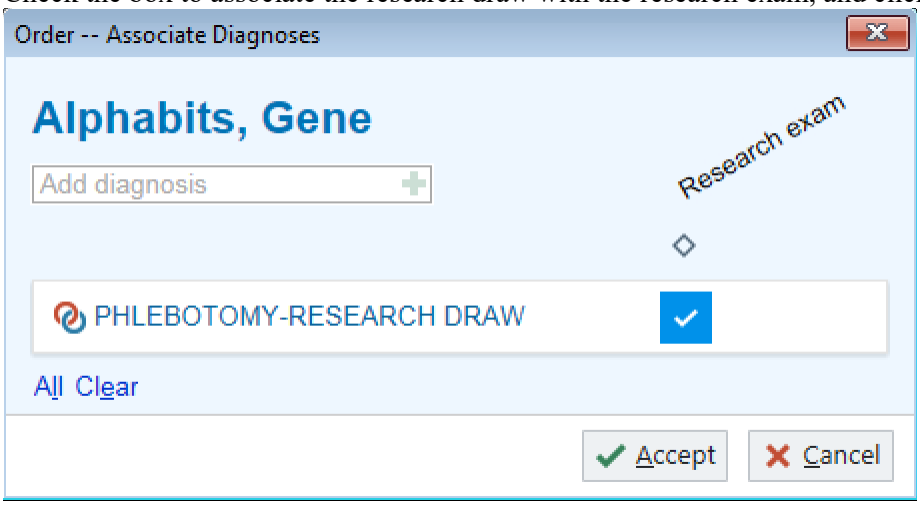


9. Highlight “Research Exam” and click Accept.

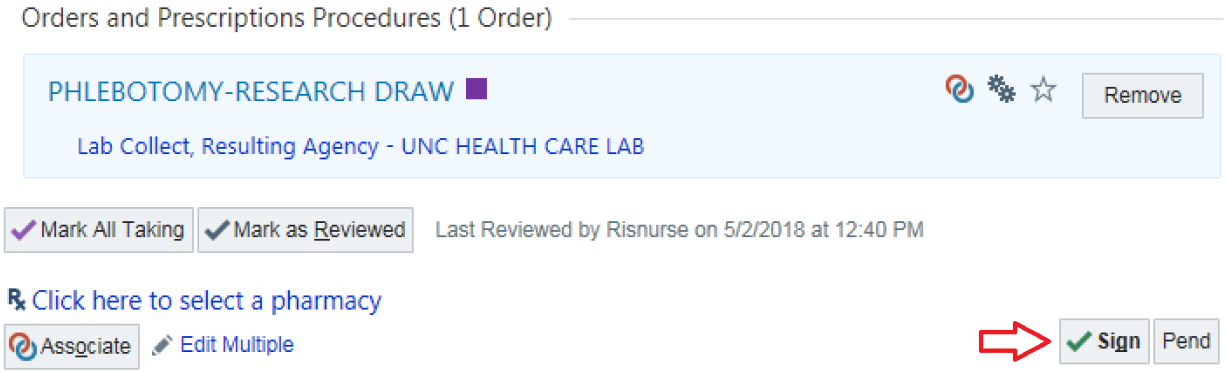


ID	Name	ICD-9 Codes	ICD-10 Codes	HCC
711295	Research exam	V70.7	Z00.6	
366592	Research requested antenatal ultrasound scan	V28.3	Z36	
366593	Research requested ultrasound scan, antenatal	V28.3	Z36	
726438	Research study patient	V70.7	Z00.6	
719613	Research subject	V70.7	Z00.6	
709125	Exam for clinical research	V70.7	Z00.6	
247621	Examination for normal comparison for clinical research	V70.7	Z00.6	
188074	Examination for normal comparison or control in clinical research	V70.7	Z00.6	
290173	Examination of participant or control in clinical research	V70.7	Z00.6	
1663531	Encounter for examination for normal comparison and control in clinical resea	V70.7	Z00.6	
673947	Encounter for examination for normal comparison or control in clinical researc	V70.7	Z00.6	
1251555	Encounter for examination of normal volunteer in research study	V70.7	Z00.6	
683619	History of venereal disease research laboratory	V15.89	Z92.89	
1383612	Patient in cancer related research study	V70.7	Z00.6	
1395757	Patient in clinical research study	V70.7	Z00.6	

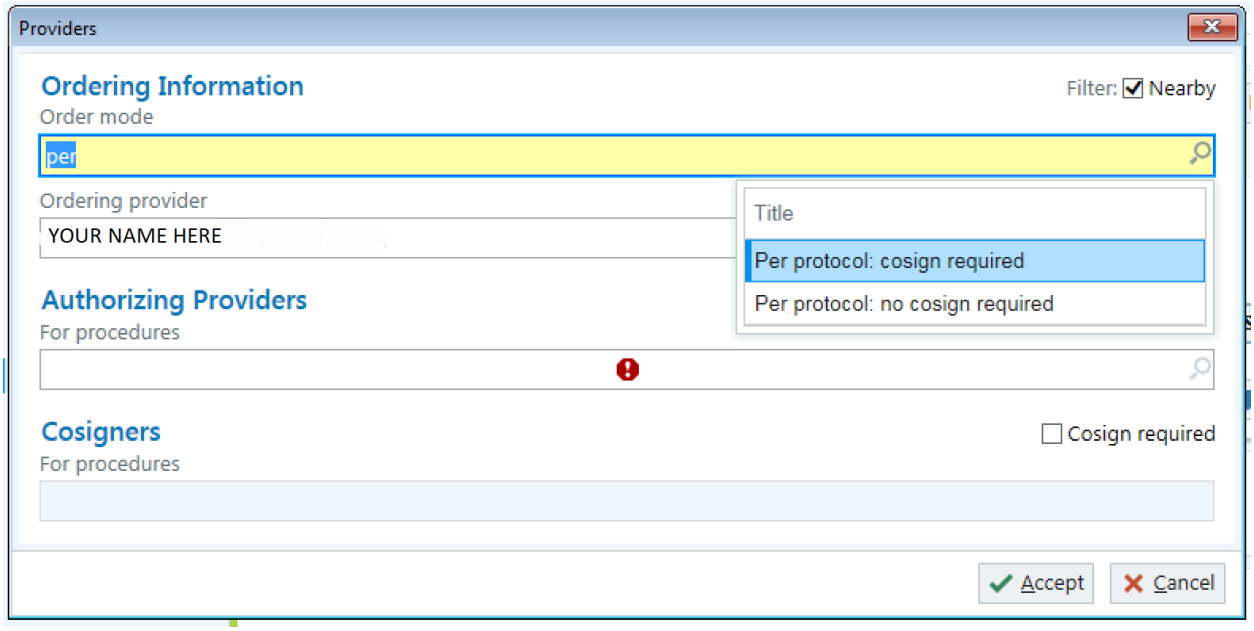
10. Check the box to associate the research draw with the research exam, and click Accept.



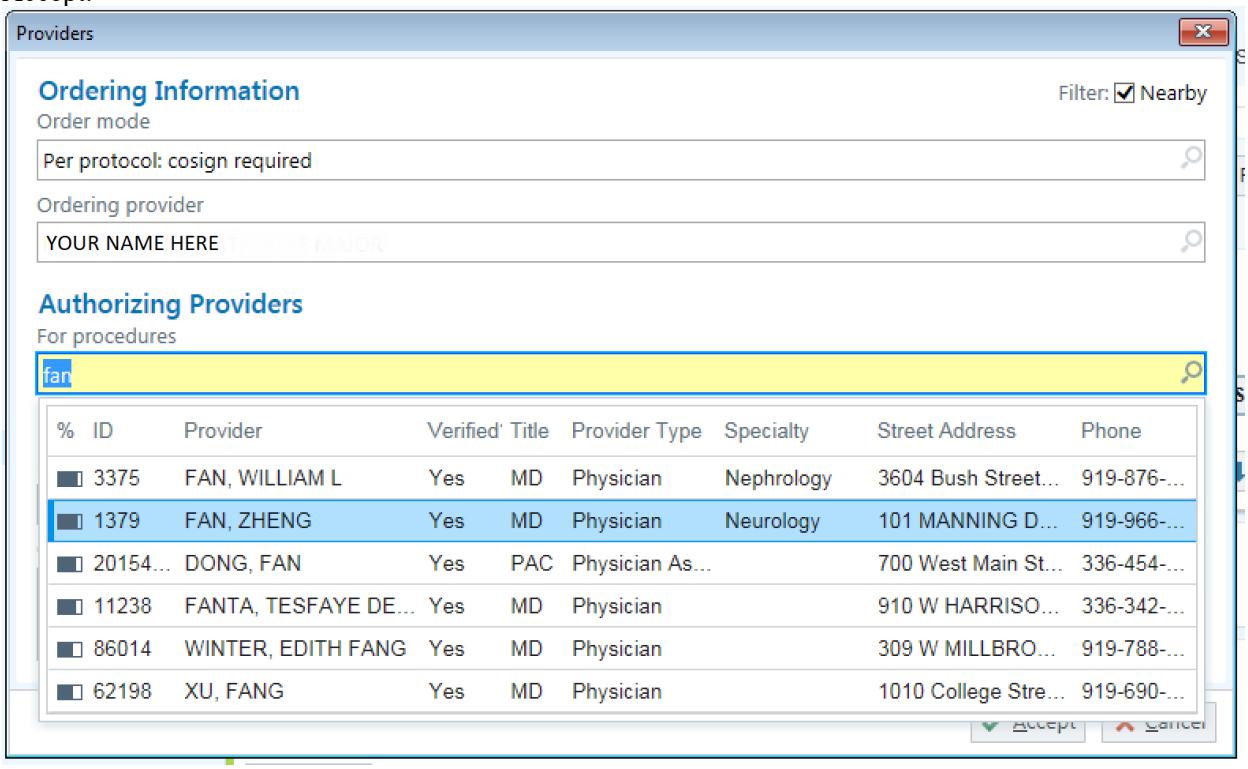
11. Click the Sign button in the Unsigned Orders section.



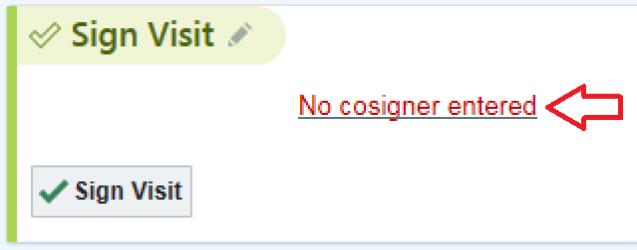
12. In the Order Mode box, type “per” and hit enter. Then choose “Per protocol: cosign required” by clicking on that option. NOTE: there are 3 courses (2 in person) for EPIC research training that will allow a staff member to pend research blood order. The research blood draw order (8243) must be set up in EPIC ahead of the appointment so that the physician can sign it. It has to be put in as a “LAB” not as a “CLINIC” order if phlebotomy is drawing the blood which will always be the case. If the patient is randomized to no sequencing or doesn’t show/cancels, the Epic blood order can be cancelled.)



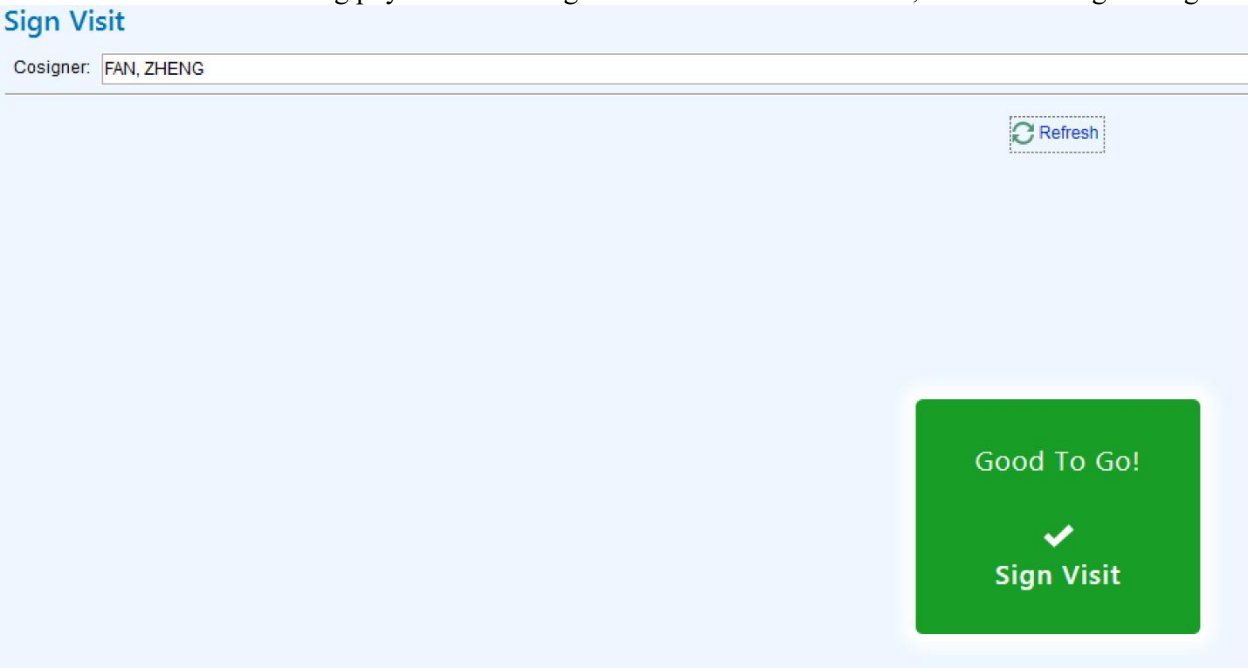
13. In the Authorizing provider box, begin typing the name of the attending physician that the participant is seeing in a UNC Pediatric Specialty Clinic, and hit Enter. Select the correct physician by clicking on their name. Then click Accept.



14. Click on the red text “No cosigner entered.”



15. Search for the same attending physician to co-sign the visit. Select their name, then click the green sign visit box.



QUICK REFERENCE:
Finding Pending Orders

Epic @ UNC
one patient ID, one problem list, one medication list, one bill

WHY DO I NEED TO KNOW THIS?

If you have Research Coordinators (RCs) on your staff who are not licensed providers, they have the ability to "pend" orders in Epic, but cannot sign them. Having RCs pend orders for research patients ahead of time can be a time saver for the provider, but RCs cannot carry out an order until you (the provider) sign off. So, once a RC pends an order, where can you find it so you can sign it?

THE INFORMATION YOU NEED

In order for you to locate a pended order in Epic, you need to know:

- The patient (MRN, name, etc.)
- The provider for the encounter and type of encounter, which could either be an Orders Only encounter or a regular face-to-face encounter (more on this in the next section)
- The date of the encounter

WHICH ENCOUNTER TYPE SHOULD STAFF BE USING?

A RCs decision to place an order in an Orders Only encounter versus a face-to-face encounter follows this logic:

RC should use a **Future or Standing** order in an **Orders Only encounter** when they:

- Know about the patient's future research encounter ahead of time, and already know what orders need to happen at that visit.
- Have the patient in front of them, but need to have a blood draw or lab test completed outside of the clinic where a new encounter will be created (i.e., somewhere where the patient will be scheduled as a walk-in, like a phlebotomy station), even if it's just five minutes from now.

RC should use a **Normal** order in **today's encounter** when they:

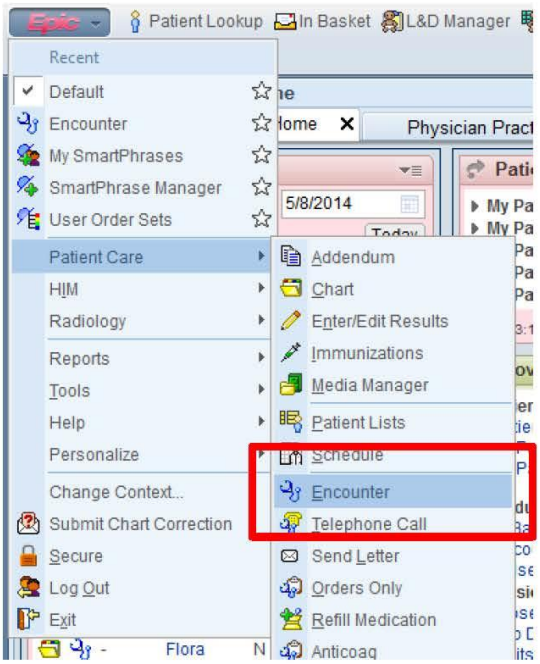
- Did not know about the patient's visit ahead of time (such as when they unexpectedly recruit, consent, and screen a patient during a regular clinic visit), and need to execute the order right then and there.
- Plan to carry out the order in the same encounter in which they're ordering it.

SO HOW DO I SIGN OFF?

RC Mary Smith knows that patient Brian Ray is coming in for a visit next week. Mary wants to be efficient, so she pends a Future order for Brian's CBC in an Orders Only encounter. The Orders Only encounter has today's date, because she's making it today.

Now it's time for you (the provider) to sign off. **Pended orders will not automatically route to your In Basket**; rather your study staff will need to communicate with you directly when orders need to be signed. So, Mary calls you and tells you to sign off on the CBC for patient Brian Ray in an Orders Only encounter on 5/8/2014, with Mary listed as the provider.

With this information, you log in to Epic and navigate to your **Epic Button > Patient Care > Encounter**.

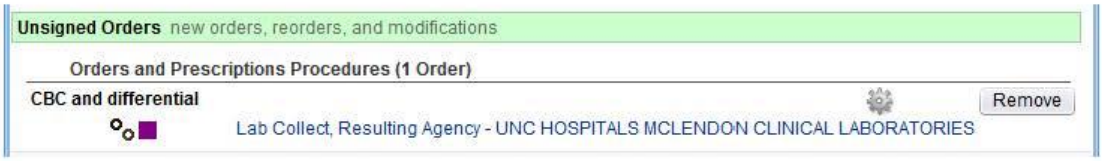


Type the patient's name or MRN in the dialog box, then select the appropriate encounter from the list (based on the encounter type, date, and provider the coordinator has given you).

Once you're in the navigator for the correct encounter, navigate to the **Meds & Orders** section.

Once you're in Meds & Orders, you'll see that you have an unsigned order waiting for you.

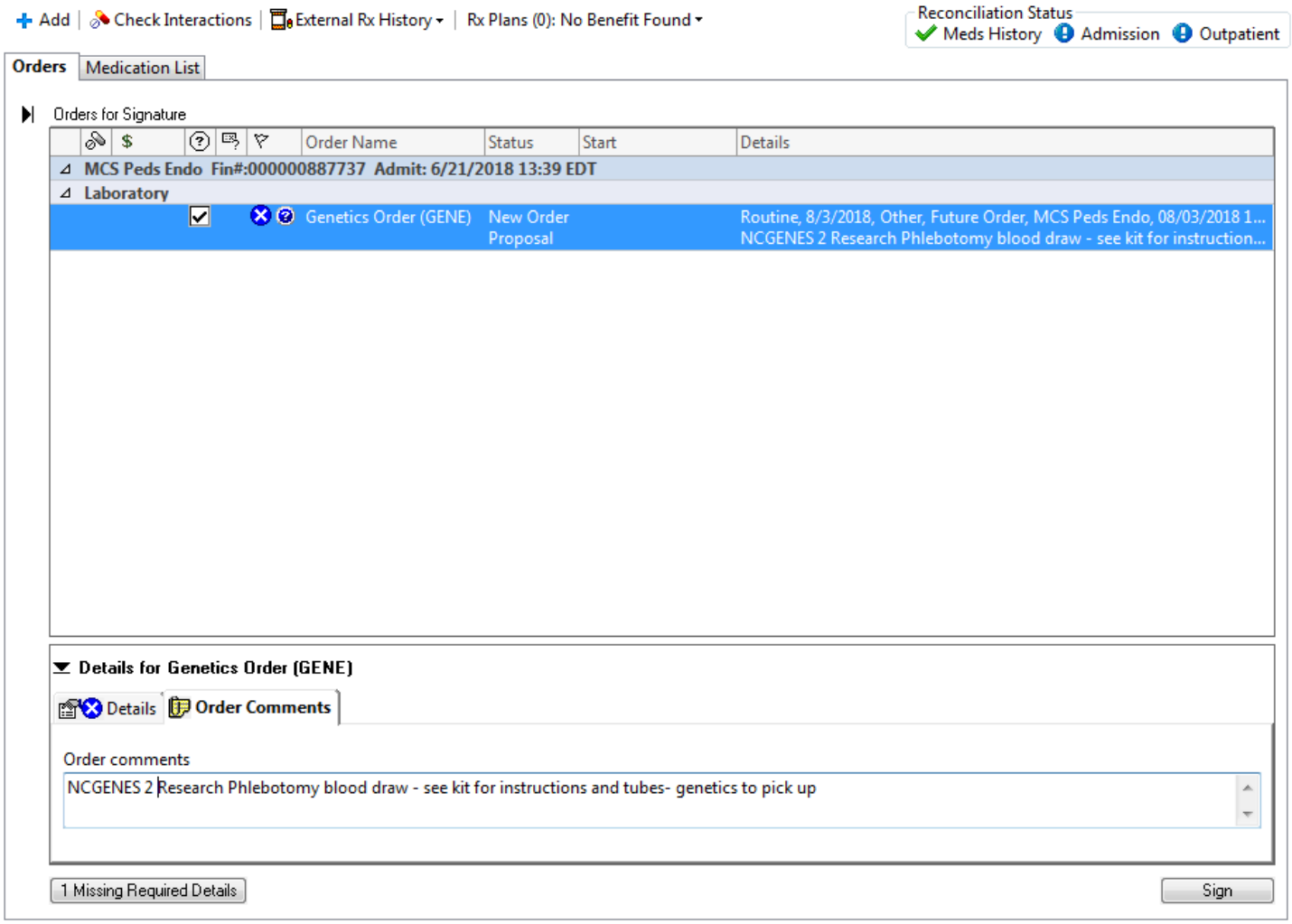
Click the **Unsigned Order** notification to be taken to the coordinator's pended order, which you can then sign as usual.



Here we've shown how it works when Mary uses an Orders Only encounter, but the process works the same way if she places a Normal order in today's encounter. The rule of thumb is that in order for you to see the order Mary pended, you need to actually jump into the encounter in which she placed the order (using the Encounter menu option), and navigate to your Meds and Orders section to sign off.

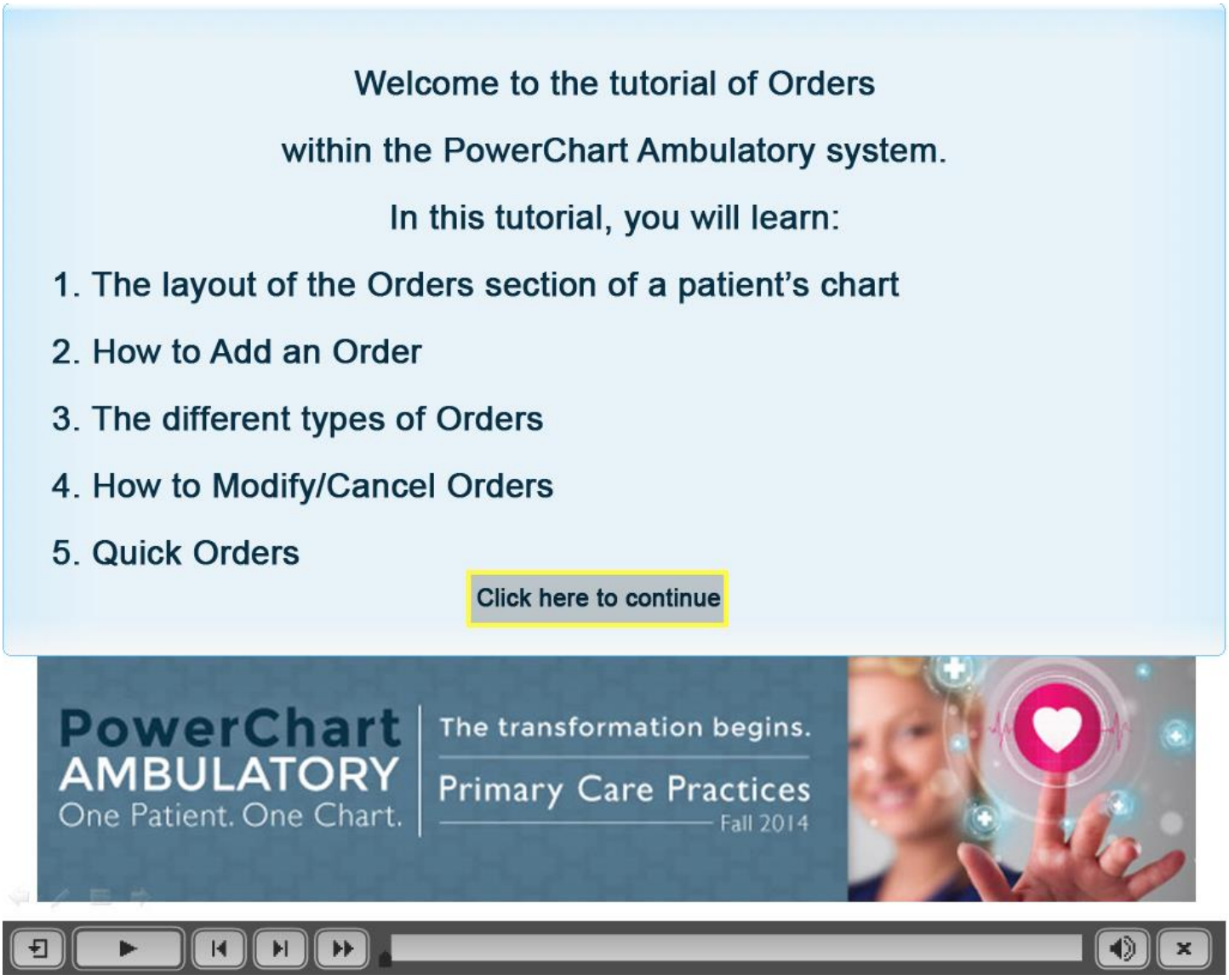
Appendix XXVIII. Creating a Mission Health Cerner Order

1. Enter GENE Order.
2. Under order comments section type “NCGENES 2 Research Phlebotomy blood draw- see kit for instructions and tubes”.
3. Sign order.



Appendix XXIX. Mission Health Cerner EHR Training Requirements

See training video on MissionOnDemand.
\\mh\ss\Learning and Development\Completed Captivates\PCA\PCA Orders\PCA Orders.htm



Appendix XXX. Laboratory Directory

UNC Biospecimen Processing Facility (BSP)

1. Shipping Address and Lab Directory

Shipping Address:
Attn: Dr. Patricia Basta, Lab Director
BioSpecimen Processing Facility
Michael Hooker Research Center, Rm 3213
135 Dauer, Drive
Campus Box 7406
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27599-7435
Phone: 919-966-7738
Website: http://bsp.web.unc.edu

Paige Schmadeke
Research Specialist
UNC Biospecimen Processing Facility
Chapel Hill, NC
Lab Phone: (919) 966-7738

UNC Hospital CLIA Molecular Genetics Lab

1. Lab UNC Hospital Location and Directory

Location:
McLendon Clinical Laboratories
Room 1046 Anderson Pavilion
CB 7600 (if you need this)
Fax: 984-974-2496

Karen Weck
Karen.Weck@unchealth.unc.edu
Professor and Director of Molecular Genetics
984-974-1825

Howard Parker
Howard.Parker@unchealth.unc.edu
Supervisor, UNC Molecular Genetics Laboratory
984-974-1825

Roy, Sayanty
sayanty@email.unc.edu
Research Specialist, Dept of Pathology
984-974-1825

Mai Xiong, PhD
Mai.Xiong@unchealth.unc.edu
Developmental Specialist

Appendix XXXI. Study Coordinator Contact Information

University of North Carolina at Chapel Hill (UNC)

Jeannette Bensen, MS, PhD, NCGENES 2 Clinical Implementation Director
Bioinformatics Building, Room 3130
Campus Box 7295
Chapel Hill, NC 27599-7295
Work Phone: 919-843-1017
Cell Phone: 919-801-9267
Email: jbensen@med.unc.edu

Tracey Grant, MS, NCGENES 2 Study Coordinator
Bioinformatics Building
Campus Box 7295
Chapel Hill, NC 27599-7295
Work Phone: 919-445-2841
Cell Phone: 919-306-1410
Email: traceyg@unc.edu

Mission Health – Asheville

Ellen Rowe, Study Coordinator
Fullerton Genetics Laboratory
Mission Health
9 Vanderbilt Park Drive
Asheville, NC 28803
Direct: 828-213-6817
Fax: 828-213-6987
Email: Ellen.Rowe@HCAHealthcare.com

East Carolina University (ECU)

Appendix XXXII. Supplies and Ordering Information

Blood Collection Kit Supply Ordering: (NEED ORDER INFO FROM PAIGE)

3ml LavTop Vacutainers
Fisher 23-021-016 (BD cat. no. 368054)
$30.53, pack of 100

Biohazard bags – 2 per patient blood
Fisher 01-800-05
9 x 6 in. w/ document compartment + absorbent pad
$52.87, pack of 500

Blood tube mailers: VWR 76203-882 at $123.16/case of 20

Cold packs – included in mailer above

Gloves – purple nitrile (to handle filled blood tubes)

DNA Genotek Saliva Kits

1) OGR-575 - same as above but the tube is round-bottom and there is no barcode. Price right now is the same as OGR-675.
2) OC-175 - contains I sponge for assisted collection of saliva for infants <6 months old. Company stated yield is 2ug.
3) The option to purchase FDA approved saliva kits is not necessary and not preferred for NCGENES 2. The saliva kits designed for research are preferred.

Barcode label printer and labels (ordered by clinical team not lab):

DYMO LabelWriter 450 – available at Staples
DYMO 45010 1/2-Inch High-Performance Permanent Self-Adhesive Polyester Label Tape for Label Makers, Black on Clear Sold at Staples by the roll

Avery 5364 Shipping Labels 3 1/3” x 4” (for the pre-addressed small bubble mailer for return of saliva to BSP).

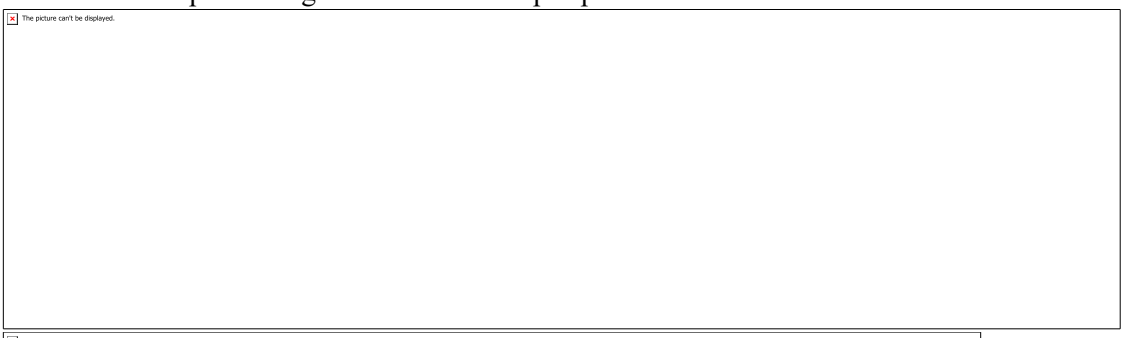
Mailers and cold packs for blood transport (each NCGENES site has funding to support postage so each site will link to their institution-specific billing account):
VWR 76203-882 – See picture below

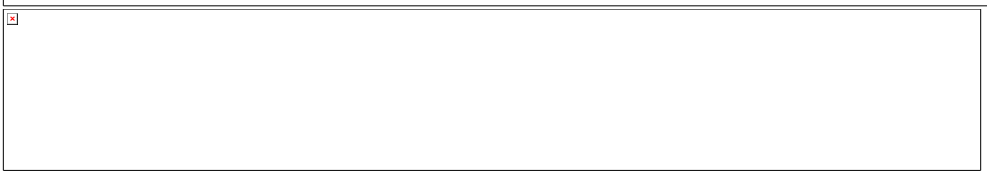
BLOOD MAILER KIT with Cold Packs (put in refrigerator)

Once blood is placed in the packaging above (sleeve, pouch and box), the box is placed in the FedEx Clinical Pak (described below) for ease of mailing

Mailers for saliva kits (mail out to patient and return to BSP lab)

Fold the pre-paid mailer – the smaller bubble mailer – and place it inside the larger bubble mailer. Instruct parent to return swabs to plastic bag and then insert in pre-paid mailer.





Appendix XXXIII. NCGENES 2 BSP Lab Requisition Form

UNC BIOSPECIMEN PROCESSING FACILITY

NCGENES II
LAB REQUISITION FORM

Please do not include any PHI on sample tube or form.

Donor ID _____(PLACE ID LABEL HERE)

Location (please circle one): UNC ECU Mission

Container Type: 3ml LAV TOP EDTA

Blood volume (in milliliters): _____

Collection Date: _____(day/month/year)

Collection Time: _____ am pm (circle one)

Collected By (NCGENES 2 staff): _____

Appendix XXXIV. NCGENES 2 UNC MGL Patient Blood CLIA lab form

MOLECULAR GENETICS TEST REQUEST FORM
University of North Carolina Hospitals
101 Manning Drive
Molecular Genetics Laboratory, Rm. 1046 Anderson Pav.
Chapel Hill, NC 27514
Phone: (984) 974-1825 Fax: (984) 974-2496
http://labs.unchealthcare.org/
MIM #963, Chart Location: Physician Orders

PATIENT INFORMATION
Full Name (Last, First, M.I.):
UNC Medical Record Number:
NOT a UNC Hospitals Patient? Add'l Information Needed for Registration
Date of Birth:
Patient Address:
City / State / Zip:
Telephone:

☐ **Bill Patient's Insurance (Attach copy of both sides of insurance card)** ☐ **Bill Facility**

Requested By: _____ **Date:** _____ **Facility Name:** _____
Phone Number: _____ **Facility Address:** _____
Fax Number: _____ **City / State / Zip:** _____

Indication for Testing and Supporting ICD-10 Code(s): _____
Ordering Physician (Print) _____ **Signature:** _____ **Date:** _____

SPECIMEN TYPE SUBMITTED:
☐ **Blood (ACD or EDTA tube)** ☐ **Bone Marrow**
☐ **Cerebrospinal Fluid (CSF)** ☐ **Other:** _____
Date and Time of Collection: _____

PARAFFIN EMBEDDED TISSUE SUBMITTED:
Tissue Type: _____ **Case Number:** _____
Date of Collection: _____
Archived Specimen Located at:
☐ **UNC Hospitals Surgical Pathology Department**
☐ **Other Institution (Provide Facility Information Above)**

☐ **A1AT deficiency (*SERPINA1 Z and S*)**
☐ ***BRCA1* & *BRCA2* Mutation Panel (Call laboratory before ordering. Additional Information needed)**
☐ **B-cell clonality (*IgH* and *IgK*)**
☐ **T-cell clonality (*TRG*)**
☐ **BCR/ABL1 p210**
☐ **BCR/ABL1 p190**
☐ **BCR/ABL1 mutations (*TKI resistance*)**
☐ **Connexin panel (includes *GJB2* and *GJB6*)**
☐ **BKV viral load**
☐ **CMV from Guthrie Card (*UNC Only*)**
☐ **EBV viral load (*Epstein-Barr Virus*)**
☐ **Extract and Hold** ☐ **DNA** ☐ **RNA**
☐ ***FLT3* Internal Tandem Duplication (ITD)**
☐ **Fragile X syndrome (*FMR1*)**
☐ **Nephrotic Syndrome Genetic Panel (17 gene panel)**
☐ **Hemochromatosis (*HFE C282Y and H63D*)**
☐ **JAK2 1849G>T [V617F]**
☐ **MCAD (Med.-Chain Acyl-CoA Dehydrogenase, *K329E / Y42H*)**
☐ ***MTRNR1* (1555A>G)**
☐ **Lymphoid Mutation Panel**
☐ **Myeloid Mutation Panel - Select Indication:**
☐ **AML** ☐ **MDS** ☐ **Myeloproliferative Neoplasm**
☐ **With *FLT3* internal tandem duplication (ITD)**
☐ ***NPM1* Quantitative RNA PCR**
☐ **Plavix response genotyping (*CYP2C19*)**
☐ **Prader Willi/Angelman syndromes**
☐ **Primary ciliary dyskinesia (PCD) (37 gene panel))**
☐ ***UGT1A1* genotyping**
☐ **Factor V Leiden (*FV1691G>A*)**
☐ **Prothrombin (Factor II, 20210G>A)**
☐ **DNA fingerprinting (marrow engraftment/chimerism)**
☐ **With CD3 Fractionation**
☐ **Cystic Fibrosis mutations. Ashkenazi Jewish Descent?**
☐ **Carrier Screen** ☐ **Yes** ☐ **No**
☐ **Diagnostic/Symptomatic**
☐ **Other:** _____

☐ **MSI DNA Assay (Microsatellite Instability):** 10 unstained sections of tumor tissue 5-10 micron thickness (preferably greater than 70% tumor on the slide) plus 1 "H&E recut" of the same section *AND* 10 unstained sections of any non-tumor tissue plus 1 "H&E recut" of the same non-tumor tissue (22 slides total)

☐ **MSI DNA Assay with Immunohistochemistry (IHC) staining* (MLH1, MSH2, MSH6, PMS2):** 15 unstained sections of tumor tissue 5-10 micron thickness (preferably greater than 70% tumor on the slide) plus 1 "H&E recut" of the same section *AND* 10 unstained sections of any non-tumor tissue plus 1 "H&E recut" of the same non-tumor tissue (27 slides total).

☐ **GastroGenus Panel for Gastric Cancer (Includes Solid Tumor Mutation Panel, MLH1 methylation, EBV Viral Load):** 15 unstained sections of tumor tissue 4 – 5 micron thickness with greater than 20% tumor on the slide **plus** 1 "H&E recut"
**IHC performed by UNCH Histology Laboratory.*

For the following tests: 10 unstained sections of tumor tissue 4 – 5 micron thickness with greater than 20% tumor on the slide **plus** 1 "H&E recut" of the same section for 11 total slides. Additional slides are not necessary for multiple tests in this section.

☐ *IDH1& IDH2* ☐ *TERT* ☐ *MLH1* **methylation** ☐ *MGMT*

☐ **Solid Tumor Mutation Panel (Excluding Glial Neoplasms)**

☐ **ALK and ROS1 FISH:** 4 unstained slides 4-5 micron thickness **plus** 1 "H&E recut" (5 total slides). These slides must be reviewed for suitability & marked by a pathologist. Signature required from pathologist who marked the relevant regions for testing. See below.

Percentage tumor: _____

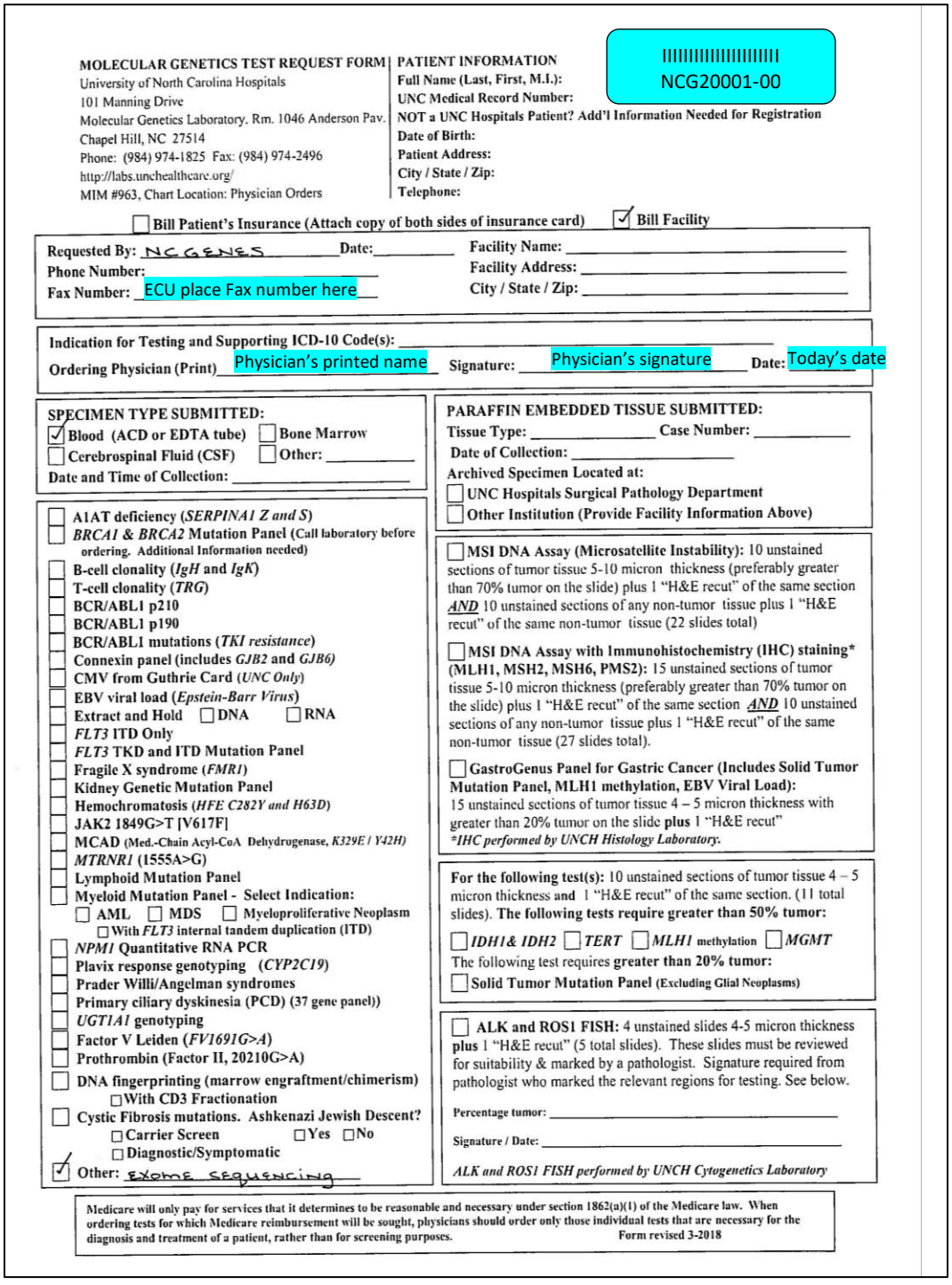
Signature / Date: _____

ALK and ROS1 FISH performed by UNCH Cytogenetics Laboratory

Medicare will only pay for services that it determines to be reasonable and necessary under section 1862(a)(1) of the Medicare law. When ordering tests for which Medicare reimbursement will be sought, physicians should order only those individual tests that are necessary for the diagnosis and treatment of a patient, rather than for screening purposes. **Form revised 6-7-2017**

Appendix XXXV. Example of Completed UNC MGL Patient Blood CLIA Lab Form

(Blue items are patient or physician specific. A set of MGL CLIA forms can be created for each study Physician and should include printed name, signature and fax if relevant (ECU), the date of the blood collection and the patient's label is placed on the form after randomization and consent to genomic sequencing.) *UNC MGL MD Order Forms for patient blood, patient saliva, and parent/relative saliva can all be found in Microsoft Teams.*



MOLECULAR GENETICS TEST REQUEST FORM
University of North Carolina Hospitals
101 Manning Drive
Molecular Genetics Laboratory, Rm. 1046 Anderson Pav.
Chapel Hill, NC 27514
Phone: (984) 974-1825 Fax: (984) 974-2496
http://labs.unchealthcare.org/
MIM #963, Chart Location: Physician Orders

PATIENT INFORMATION
IIIIIIIIIIIIIIIIIIII
NCG20001-00
Full Name (Last, First, M.I.):
UNC Medical Record Number:
NOT a UNC Hospitals Patient? Add'l Information Needed for Registration
Date of Birth:
Patient Address:
City / State / Zip:
Telephone:

☐ Bill Patient's Insurance (Attach copy of both sides of insurance card) ☑ Bill Facility

Requested By: NC GENES Date:
Phone Number:
Fax Number: ECU place Fax number here
Facility Name:
Facility Address:
City / State / Zip:

Indication for Testing and Supporting ICD-10 Code(s):
Ordering Physician (Print) Physician's printed name Signature: Physician's signature Date: Today's date

SPECIMEN TYPE SUBMITTED:
☑ Blood (ACD or EDTA tube) ☐ Bone Marrow
☐ Cerebrospinal Fluid (CSF) ☐ Other:
Date and Time of Collection:

PARAFFIN EMBEDDED TISSUE SUBMITTED:
Tissue Type: Case Number:
Date of Collection:
Archived Specimen Located at:
☐ UNC Hospitals Surgical Pathology Department
☐ Other Institution (Provide Facility Information Above)

☐ A1AT deficiency (*SERPINA1 Z and S*)
☐ *BRCA1* & *BRCA2* Mutation Panel (Call laboratory before ordering. Additional Information needed)
☐ B-cell clonality (*IgH* and *IgK*)
☐ T-cell clonality (*TRG*)
☐ BCR/ABL1 p210
☐ BCR/ABL1 p190
☐ BCR/ABL1 mutations (*TKI resistance*)
☐ Connexin panel (includes *GJB2* and *GJB6*)
☐ CMV from Guthrie Card (*UNC Only*)
☐ EBV viral load (*Epstein-Barr Virus*)
☐ Extract and Hold ☐ DNA ☐ RNA
☐ *FLT3* ITD Only
☐ *FLT3* TKD and ITD Mutation Panel
☐ Fragile X syndrome (*FMR1*)
☐ Kidney Genetic Mutation Panel
☐ Hemochromatosis (*HFE C282Y and H63D*)
☐ JAK2 1849G>T [V617F]
☐ MCAD (Med.-Chain Acyl-CoA Dehydrogenase, *K329E / Y42H*)
☐ *MTRNR1* (1555A>G)
☐ Lymphoid Mutation Panel
☐ Myeloid Mutation Panel - Select Indication:
☐ AML ☐ MDS ☐ Myeloproliferative Neoplasm
☐ With *FLT3* internal tandem duplication (ITD)
☐ *NPM1* Quantitative RNA PCR
☐ Plavix response genotyping (*CYP2C19*)
☐ Prader Willi/Angelman syndromes
☐ Primary ciliary dyskinesia (PCD) (37 gene panel))
☐ *UGT1A1* genotyping
☐ Factor V Leiden (*FV1691G>A*)
☐ Prothrombin (Factor II, 20210G>A)
☐ DNA fingerprinting (marrow engraftment/chimerism)
☐ With CD3 Fractionation
☐ Cystic Fibrosis mutations. Ashkenazi Jewish Descent?
☐ Carrier Screen ☐ Yes ☐ No
☐ Diagnostic/Symptomatic
☑ Other: EXOME SEQUENCING

☐ **MSI DNA Assay (Microsatellite Instability):** 10 unstained sections of tumor tissue 5-10 micron thickness (preferably greater than 70% tumor on the slide) plus 1 "H&E recut" of the same section ***AND*** 10 unstained sections of any non-tumor tissue plus 1 "H&E recut" of the same non-tumor tissue (22 slides total)

☐ **MSI DNA Assay with Immunohistochemistry (IHC) staining* (MLH1, MSH2, MSH6, PMS2):** 15 unstained sections of tumor tissue 5-10 micron thickness (preferably greater than 70% tumor on the slide) plus 1 "H&E recut" of the same section ***AND*** 10 unstained sections of any non-tumor tissue plus 1 "H&E recut" of the same non-tumor tissue (27 slides total).

☐ **GastroGenus Panel for Gastric Cancer (Includes Solid Tumor Mutation Panel, MLH1 methylation, EBV Viral Load):** 15 unstained sections of tumor tissue 4 – 5 micron thickness with greater than 20% tumor on the slide **plus** 1 "H&E recut"
**IHC performed by UNCH Histology Laboratory.*

For the following test(s): 10 unstained sections of tumor tissue 4 – 5 micron thickness **and** 1 "H&E recut" of the same section. (11 total slides). **The following tests require greater than 50% tumor:**
☐ *IDH1& IDH2* ☐ *TERT* ☐ *MLH1* methylation ☐ *MGMT*
The following test requires **greater than 20% tumor:**
☐ **Solid Tumor Mutation Panel** (Excluding Glial Neoplasms)

☐ **ALK and ROS1 FISH:** 4 unstained slides 4-5 micron thickness **plus** 1 "H&E recut" (5 total slides). These slides must be reviewed for suitability & marked by a pathologist. Signature required from pathologist who marked the relevant regions for testing. See below.
Percentage tumor:
Signature / Date:
ALK and ROS1 FISH performed by UNCH Cytogenetics Laboratory

Medicare will only pay for services that it determines to be reasonable and necessary under section 1862(a)(1) of the Medicare law. When ordering tests for which Medicare reimbursement will be sought, physicians should order only those individual tests that are necessary for the diagnosis and treatment of a patient, rather than for screening purposes. Form revised 3-2018

Appendix XXXVI. Example of Completed UNC MGL Patient Saliva CLIA Lab Form

MOLECULAR GENETICS TEST REQUEST FORM
University of North Carolina Hospitals
101 Manning Drive
Molecular Genetics Laboratory, Rm. 1046 Anderson Pav.
Chapel Hill, NC 27514
Phone: (984) 974-1825 Fax: (984) 974-2496
http://labs.unchealthcare.org/
MIM #963, Chart Location: Physician Orders

PATIENT INFORMATION
Full Name (Last, First, M.I.):
UNC Medical Record Number:
NOT a UNC Hospitals Patient? Add
Date of Birth:
Patient Address:
City / State / Zip:
Telephone:

IIIIIIIIIIIIIIIIIIIII
NCG20001-00

☐ Bill Patient's Insurance (Attach copy of both sides of insurance card) ☑ Bill Facility

Requested By: NC GENES Date:
Phone Number:
Fax Number: ECU place Fax number here
Facility Name:
Facility Address:
City / State / Zip:

Indication for Testing and Supporting ICD-10 Code(s):
Ordering Physician (Print) Physician's printed name Signature: Physician's signature Date: Today's date

SPECIMEN TYPE SUBMITTED:
☐ Blood (ACD or EDTA tube) ☐ Bone Marrow
☐ Cerebrospinal Fluid (CSF) ☒ Other: Saliva
Date and Time of Collection:

PARAFFIN EMBEDDED TISSUE SUBMITTED:
Tissue Type: Case Number:
Date of Collection:
Archived Specimen Located at:
☐ UNC Hospitals Surgical Pathology Department
☐ Other Institution (Provide Facility Information Above)

☐ A1AT deficiency (*SERPINA1 Z and S*)
☐ *BRCA1 & BRCA2* Mutation Panel (Call laboratory before ordering. Additional information needed)
☐ B-cell clonality (*IgH* and *IgK*)
☐ T-cell clonality (*TRG*)
☐ BCR/ABL1 p210
☐ BCR/ABL1 p190
☐ BCR/ABL1 mutations (*TKI resistance*)
☐ Connexin panel (includes *GJB2* and *GJB6*)
☐ CMV from Guthrie Card (*UNC Only*)
☐ EBV viral load (*Epstein-Barr Virus*)
☐ Extract and Hold ☐ DNA ☐ RNA
☐ *FLT3* ITD Only
☐ *FLT3* TKD and ITD Mutation Panel
☐ Fragile X syndrome (*FMR1*)
☐ Kidney Genetic Mutation Panel
☐ Hemochromatosis (*HFE C282Y and H63D*)
☐ JAK2 1849G>T [V617F]
☐ MCAD (Med.-Chain Acyl-CoA Dehydrogenase, *K329E / Y42H*)
☐ *MTRNR1* (1555A>G)
☐ Lymphoid Mutation Panel
☐ Myeloid Mutation Panel - Select Indication:
☐ AML ☐ MDS ☐ Myeloproliferative Neoplasm
☐ With *FLT3* internal tandem duplication (ITD)
☐ *NPM1* Quantitative RNA PCR
☐ Plavix response genotyping (*CYP2C19*)
☐ Prader Willi/Angelman syndromes
☐ Primary ciliary dyskinesia (PCD) (37 gene panel))
☐ *UGT1A1* genotyping
☐ Factor V Leiden (*FV1691G>A*)
☐ Prothrombin (Factor II, 20210G>A)
☐ DNA fingerprinting (marrow engraftment/chimerism)
☐ With CD3 Fractionation
☐ Cystic Fibrosis mutations. Ashkenazi Jewish Descent?
☐ Carrier Screen ☐ Yes ☐ No
☐ Diagnostic/Symptomatic
☑ Other: EXOME SEQUENCING

☐ **MSI DNA Assay (Microsatellite Instability):** 10 unstained sections of tumor tissue 5-10 micron thickness (preferably greater than 70% tumor on the slide) plus 1 "H&E recut" of the same section *AND* 10 unstained sections of any non-tumor tissue plus 1 "H&E recut" of the same non-tumor tissue (22 slides total)

☐ **MSI DNA Assay with Immunohistochemistry (IHC) staining* (MLH1, MSH2, MSH6, PMS2):** 15 unstained sections of tumor tissue 5-10 micron thickness (preferably greater than 70% tumor on the slide) plus 1 "H&E recut" of the same section *AND* 10 unstained sections of any non-tumor tissue plus 1 "H&E recut" of the same non-tumor tissue (27 slides total).

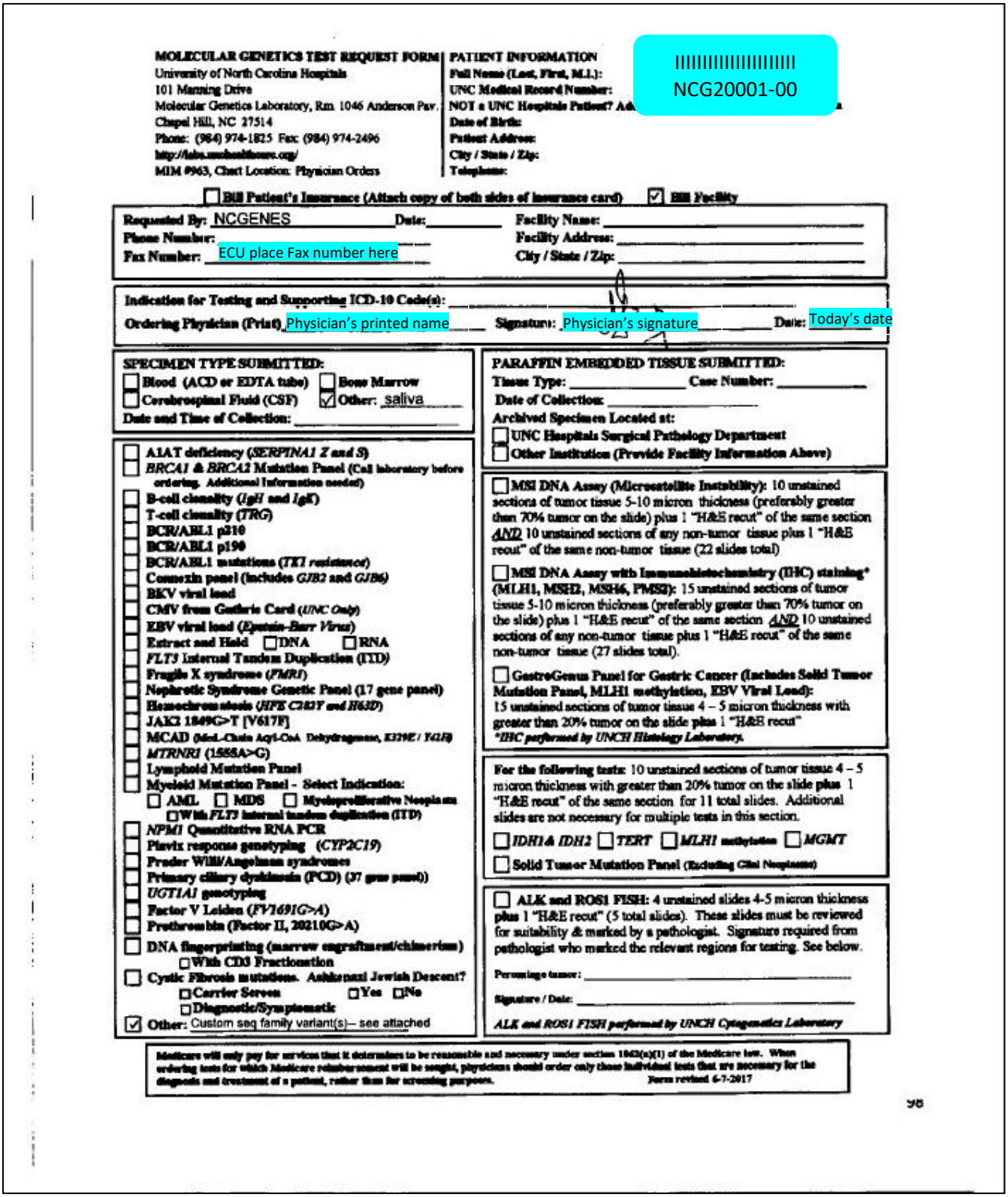
☐ **GastroGenus Panel for Gastric Cancer (Includes Solid Tumor Mutation Panel, MLH1 methylation, EBV Viral Load):** 15 unstained sections of tumor tissue 4 – 5 micron thickness with greater than 20% tumor on the slide plus 1 "H&E recut"
**IHC performed by UNCH Histology Laboratory.*

For the following test(s): 10 unstained sections of tumor tissue 4 – 5 micron thickness and 1 "H&E recut" of the same section. (11 total slides). **The following tests require greater than 50% tumor:**
☐ *IDH1& IDH2* ☐ *TERT* ☐ *MLH1* methylation ☐ *MGMT*
The following test requires greater than 20% tumor:
☐ Solid Tumor Mutation Panel (Excluding Glial Neoplasms)

☐ **ALK and ROS1 FISH:** 4 unstained slides 4-5 micron thickness plus 1 "H&E recut" (5 total slides). These slides must be reviewed for suitability & marked by a pathologist. Signature required from pathologist who marked the relevant regions for testing. See below.
Percentage tumor:
Signature / Date:
ALK and ROS1 FISH performed by UNCH Cytogenetics Laboratory

Medicare will only pay for services that it determines to be reasonable and necessary under section 1862(a)(1) of the Medicare law. When ordering tests for which Medicare reimbursement will be sought, physicians should order only those individual tests that are necessary for the diagnosis and treatment of a patient, rather than for screening purposes.
Form revised 3-2018

Appendix XXXVII. Example of Completed UNC MGL Parent/Relative Saliva CLIA Lab Form



MOLECULAR GENETICS TEST REQUEST FORM
University of North Carolina Hospitals
101 Manning Drive
Molecular Genetics Laboratory, Rm. 1046 Anderson Pav.
Chapel Hill, NC 27514
Phone: (984) 974-1825 Fax: (984) 974-2496
http://labs.unchealthcare.org/
MIM #963, Chart Location: Physician Orders

PATIENT INFORMATION
Full Name (Last, First, M.I.):
UNC Medical Record Number:
NOT a UNC Hospitals Patient? Ad
Date of Birth:
Patient Address:
City / State / Zip:
Telephone:

IIIIIIIIIIIIIIIIIIIII
NCG20001-00

☐ Bill Patient's Insurance (Attach copy of both sides of insurance card) ☑ Bill Facility

Requested By: NCGENES Date:
Phone Number:
Fax Number: ECU place Fax number here
Facility Name:
Facility Address:
City / State / Zip:

Indication for Testing and Supporting ICD-10 Code(s):
Ordering Physician (Print) Physician's printed name Signature: Physician's signature Date: Today's date

SPECIMEN TYPE SUBMITTED:
☐ Blood (ACD or EDTA tube) ☐ Bone Marrow
☐ Cerebrospinal Fluid (CSF) ☑ Other: saliva
Date and Time of Collection:

PARAFFIN EMBEDDED TISSUE SUBMITTED:
Tissue Type: Case Number:
Date of Collection:
Archived Specimen Located at:
☐ UNC Hospitals Surgical Pathology Department
☐ Other Institution (Provide Facility Information Above)

☐ A1AT deficiency (*SERPINA1 Z and S*)
☐ *BRCA1 & BRCA2* Mutation Panel (Call laboratory before ordering. Additional information needed)
☐ B-cell clonality (*IgH and IgK*)
☐ T-cell clonality (*TRG*)
☐ BCR/ABL1 p210
☐ BCR/ABL1 p190
☐ BCR/ABL1 mutations (*TKI resistance*)
☐ Connexin panel (includes *GJB2* and *GJB6*)
☐ BKV viral load
☐ CMV from Guthrie Card (*UNC Only*)
☐ EBV viral load (*Epstein-Barr Virus*)
☐ Extract and Hold ☐ DNA ☐ RNA
☐ *FLT3* Internal Tandem Duplication (ITD)
☐ Fragile X syndrome (*FMR1*)
☐ Nephrotic Syndrome Genetic Panel (17 gene panel)
☐ Hemochromatosis (*HFE C282Y and H63D*)
☐ JAK2 1849G>T [V617F]
☐ MCAD (Med.-Chain Acyl-CoA Dehydrogenase, *K329E / Y42H*)
☐ *MTRNR1* (1555A>G)
☐ Lymphoid Mutation Panel
☐ Myeloid Mutation Panel - Select Indication:
☐ AML ☐ MDS ☐ Myeloproliferative Neoplasm
☐ With *FLT3* internal tandem duplication (ITD)
☐ *NPM1* Quantitative RNA PCR
☐ Plavix response genotyping (*CYP2C19*)
☐ Prader Willi/Angelman syndromes
☐ Primary ciliary dyskinesia (PCD) (*37 gene panel*)
☐ *UGT1A1* genotyping
☐ Factor V Leiden (*FV1691G>A*)
☐ Prothrombin (Factor II, 20210G>A)
☐ DNA fingerprinting (marrow engraftment/chimerism)
☐ With CD3 Fractionation
☐ Cystic Fibrosis mutations. Ashkenazi Jewish Descent?
☐ Carrier Screen ☐ Yes ☐ No
☐ Diagnostic/Symptomatic
☑ Other: Custom seq family variant(s)– see attached

☐ **MSI DNA Assay (Microsatellite Instability):** 10 unstained sections of tumor tissue 5-10 micron thickness (preferably greater than 70% tumor on the slide) plus 1 "H&E recut" of the same section *AND* 10 unstained sections of any non-tumor tissue plus 1 "H&E recut" of the same non-tumor tissue (22 slides total)

☐ **MSI DNA Assay with Immunohistochemistry (IHC) staining* (MLH1, MSH2, MSH6, PMS2):** 15 unstained sections of tumor tissue 5-10 micron thickness (preferably greater than 70% tumor on the slide) plus 1 "H&E recut" of the same section *AND* 10 unstained sections of any non-tumor tissue plus 1 "H&E recut" of the same non-tumor tissue (27 slides total).

☐ **GastroGenus Panel for Gastric Cancer (Includes Solid Tumor Mutation Panel, MLH1 methylation, EBV Viral Load):** 15 unstained sections of tumor tissue 4 – 5 micron thickness with greater than 20% tumor on the slide **plus** 1 "H&E recut"
**IHC performed by UNCH Histology Laboratory.*

For the following tests: 10 unstained sections of tumor tissue 4 – 5 micron thickness with greater than 20% tumor on the slide **plus** 1 "H&E recut" of the same section for 11 total slides. Additional slides are not necessary for multiple tests in this section.

☐ *IDH1& IDH2* ☐ *TERT* ☐ *MLH1* methylation ☐ *MGMT*
☐ **Solid Tumor Mutation Panel** (Excluding CNS Neoplasms)

☐ **ALK and ROS1 FISH:** 4 unstained slides 4-5 micron thickness **plus** 1 "H&E recut" (5 total slides). These slides must be reviewed for suitability & marked by a pathologist. Signature required from pathologist who marked the relevant regions for testing. See below.

Percentage tumor:
Signature / Date:
ALK and ROS1 FISH performed by UNCH Cytogenetics Laboratory

Medicare will only pay for services that it determines to be reasonable and necessary under section 1862(a)(1) of the Medicare law. When ordering tests for which Medicare reimbursement will be sought, physicians should order only those individual tests that are necessary for the diagnosis and treatment of a patient, rather than for screening purposes.
Form revised 6-7-2017

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Appendix XXXVIII. NCGENES 2 Parent/Relative Saliva UNC MGL Additional Form

NCGENES Request for Family Studies
Molecular Genetics Laboratory

Family Member Study ID: NCG- 2 ___ ___ ___ ___ - ___ ___
*NOTE: By convention, a mother's ID should end in -01 and a father's ID should end in -02.

Date of Sample Collection: _____

Requesting Clinician: _____

Proband Study ID: NCG- 2 ___ ___ ___ ___ - 00

Relationship to Proband:
☐ Mother ☐Father ☐Other (specify): _____

Reason for Study:
☐ Determine phase ☐ Determine if variant is *de novo* or inherited
☐ Other (specify below)

_____

Request Testing of Variants in Which Gene(s):

_____

_____

*NOTE: Testing will be performed for all variants in the requested gene(s) that were previously reported in the proband. If more than one variant was reported in a given gene and you want testing for only one, please specify the gene and variant.

Phenotype of This Person:
☐ Unaffected ☐ Affected (provide details below)

_____

_____

Additional Family History: (please provide pedigree on back of form or attached as appropriate)

_____

_____

Appendix XXXIX. Mission FGC Laboratory Requisition Form (3 Pages)

Fullerton Genetics Center - Laboratory Requisition Form

MISSION HOSPITALS

9 Vanderbilt Park Drive ¤ Asheville, NC 28803
Phone (828) 213-1015 ¤ Fax (828) 213-6987
Website: http://www.mission-health.org/centers-and-services/programs-service/genetics/genetics-physicians

Highlighted fields are required

Patient Information (Please print):

Last name	First	MI	Address:
Medical Record#		Sex ❒M ❒F	
DOB	SS#		Phone#

Referring Physician:

Name		Address	
Institution		City, State, Zip	
NPI#		Phone#	Fax#
CC report copies to:	Name	Institution	Fax#

Genetic Counselor/Care Coordinator:

Name	Phone#	Fax#

Billing Information:

❒Bill patient (Please attach copy of insurance card)		❒Bill physician office or laboratory	
Name of insured		Institution/Organization	
DOB	SS#	Address	
Relationship to Patient		City, State, Zip	Telephone #
Phone#		Fax#	Auth/Precert#

Sample Information:

Specimen Type	Collection date
❒Blood ❒Buccal Swab ❒Amniotic Fluid ❒CVS ❒Tissue :_____ ❒Other:_____	

Clinical Indications for Study:

❒ Symptomatic_____

❒ Family History/Known Mutation(s)_____

❒ Carrier Screening _____

❒ Parental Study / Proband Name:_____

Proband MR#:_____CNV/Mutation:_____

Is the patient currently pregnant?

❒Yes ❒No If yes, provide gestational age:_____

If known, provide fetal sex: ❒M ❒F

Ultrasound Abnormalities_____

ICD10 codes	Definition

Informed consent: ❒Yes ❒No

For Chromosomal Microarray Testing, please list any suspected syndromes or genes:

Additional comments:

Please attach clinical information/family history. A pedigree can be drawn on p.2 or attached separately.

Fullerton Genetics Center - Laboratory Requisition Form

9 Vanderbilt Park Drive ▫ Asheville, NC 28803
Phone (828) 213-1015 ▫ Fax (828) 213-6987
Website: http://www.mission-health.org/centers-and-services/programs-service/genetics/genetics-physicians

Highlighted fields are required

Patient information:

Last name	First	MI	DOB	SS#

Cytogenetic Test Menu

Karyotype (check one)
- ❒ Standard study (20 cells)
- ❒ Mosaic study (50 cells)
- ❒ Abbreviated study (5 cells) (only with microarray or known abnormality)

***Chromosomal Microarray Analysis** (check one)
- ❒ Chromosomal Microarray only
- ❒ Perform with abbreviated karyotype
- ❒ Karyotype previously performed, Results:_____

***For Chromosomal Microarray studies, please select whether the patient had a previous karyotype or if an abbreviated karyotype is required.**

Fluorescence In Situ Hybridization (FISH) (check one)
- ❒ Prenatal aneuploidy screen (chromosomes X, Y, 13, 18, 21)
- ❒ X/Y chromosome identification/enumeration
- ❒ Parental FISH Analysis
- ❒ Other:_____

Cell culturing only
- ❒ Send out cells (provide referral lab information)
- ❒ Freeze cells
- ❒ DNA isolation and banking

Prenatal studies
- ❒ Amniotic Fluid AFP
- ❒ Amniotic Fluid Acetylcholinesterase, AChE
- ❒ Maternal Cell Contamination (MCC studies) – required with CMA order

Pedigree Information:

Molecular Test Menu

- ❒ **Angelman syndrome (methylation analysis)**
- ❒ **Connexin-26/30 related hearing loss (*GJB2* and *GJB6*)**
- ❒ **Cystic Fibrosis (*CFTR*), includes ACMG/ACOG panel**
- ❒ **DNA isolation**
- ❒ **Duchenne or Becker muscular dystrophy** (DMD del/dup analysis)

Dravet syndrome (check one)
- ❒ *SCN1A* gene sequencing and deletion/duplication analysis
- ❒ *SCN1A* gene sequencing only
- ❒ *SCN1A* deletion/duplication analysis only

- ❒ **Fragile X syndrome (*FMR1*)**
- ❒ **Hemochromatosis (*HFE*)**
- ❒ **Marfan syndrome (*FBN1*)** - FBN1 deletion/duplication
- ❒ **Maternal Cell Contamination (MCC studies)**
- ❒ **Microsatellite Instability (MSI)**

Myotonia Congenita (check one)
- ❒ Full Myotonia Congenita panel
  -includes CLCN1 seq, del/dup, and SCN4A seq
- ❒ *CLCN1* gene sequencing and deletion/duplication analysis
- ❒ *CLCN1* gene sequencing only
- ❒ *CLCN1* deletion/duplication analysis only
- ❒ *SCN4A* gene sequencing only (Paramyotonia Congenita)

- ❒ **Myotonic Dystrophy (DM1)**

Next Generation Sequencing
- ❒ Connective Tissue Panel
- ❒ Osteogenesis Imperfecta (*COL1A1, COL1A2, IFITM5*)
- ❒ Maran Syndrome (*FBN1* sequencing)
- ❒ Ehlers Danlos Type IV (*COL3A1*)
- ❒ Stickler Syndrome (*COL2A1*)
- ❒ Loeys Dietz Syndrome (*TGFBR1, TGFBR2*)
- ❒ Moya Moya (*ACTA2*)

- ❒ **Prader-Willi syndrome (methylation analysis)**
- ❒ **Periodic Paralysis Screen (CACNA1S,SCN4A, KCNJ2, KCNJ18)**

***PTEN*-related disorders** (check one)
- ❒ *PTEN* gene sequencing and deletion/duplication analysis
- ❒ *PTEN* gene sequencing
- ❒ *PTEN* deletion/duplication analysis

Rett syndrome (check one)
- ❒ *MECP2* gene sequencing and deletion/duplication analysis
- ❒ *MECP2* gene sequencing only
- ❒ *MECP2* deletion/duplication analysis only

- ❒ **Spinal Muscular Atrophy (*SMN1* and *SMN2*)**
- ❒ **Targeted Sequencing Analysis (Indicate gene and mutation on page 1)**
  - ❒ Previously reported pathogenic alteration
  - ❒ Previously reported likely pathogenic alteration &/or VUS
- ❒ **Thrombosis Multiplex PCR (FactorV-Leiden/Prothrombin)**
- ❒ **X-inactivation analysis**

For Lab Use only	Medical Record #	Account#

MISSION HOSPITALS

Fullerton Genetics Center - Laboratory Requisition Form

9 Vanderbilt Park Drive ▫ Asheville, NC 28803
Phone (828) 213-1015 ▫ Fax (828) 213-6987
Website: http://www.mission-health.org/centers-and-services/programs-service/genetics/genetics-physicians

Highlighted fields are required

Specimen Collection Guidelines

All specimen tubes should be labeled with patient full name, medical record# or DOB, date and time drawn and the initials of the person collecting sample. Preferably, all samples should be delivered to the laboratory within 24hrs.

Chromosome (Karyotype) Studies

Peripheral Blood:
- Sodium Heparin (green top) tube.
- 3-6 ml for adults and children, 1 to 2 ml for newborn infants.
- Maintain at room temperature.

Amniotic Fluid:
- Collect 20-30 ml of sterile amniotic fluid in 3-4 sterile tubes.
- Label tubes to indicate 1st through 3rd/4th tube drawn to assist lab in reducing the risk of maternal cell contamination.
- Maintain at room temperature and protect from light.

Products of Conception/ Abortus Tissue:
- Use sterile technique to obtain a minimum 5mm biopsy of unmacerated fetal tissue. Fetal membranes or chorionic villi are preferred placental tissues. Deep tissues (organ/tendon) are preferred if autopsy is performed.
- Place POC or abortus tissue in sterile container with POC transport media or sterile saline. Tubes containing transport media available upon request.
- Individually identified tissues should be placed in separate containers.
- If specimen is to be delivered immediately, maintain at room temperature. Specimen may be refrigerated and sent overnight to arrive at the laboratory within 24 hours.
- DO NOT PLACE SPECIMENS IN FORMALIN OR OTHER FIXATIVES.

Skin Biopsy:
- Use sterile technique to obtain a 5mm biopsy (note: use ONLY alcohol to prep skin surface before biopsy).
- Place in sterile container containing skin biopsy transport media or sterile saline.
- If specimen is to be delivered immediately, maintain at room temperature. Specimen may be refrigerated and sent overnight to arrive at the laboratory within 24 hours.
- DO NOT PLACE SPECIMENS IN FORMALIN OR OTHER FIXATIVES.

Chorionic Villi (CVS):
- Collect a minimum of 30mg of chorionic villi and place in sterile tube containing CVS transport media
- Maintain at room temperature.

Molecular (DNA) Studies

Peripheral Blood:
- 3 to 6 ml in EDTA (lavender top) tube - Other anti-coagulants may be acceptable.
- Maintain at room temperature.

Buccal Swab:
- Collect with ORACollect® swab provided by Fullerton Genetics.
- Maintain at room temperature.

Cultured cells:
- 2 confluent T-25 flasks (Also maintain 1 flask at requesting laboratory as back up.)
- Ship at ambient temperature.

Chromosomal Microarray Analysis (CMA)

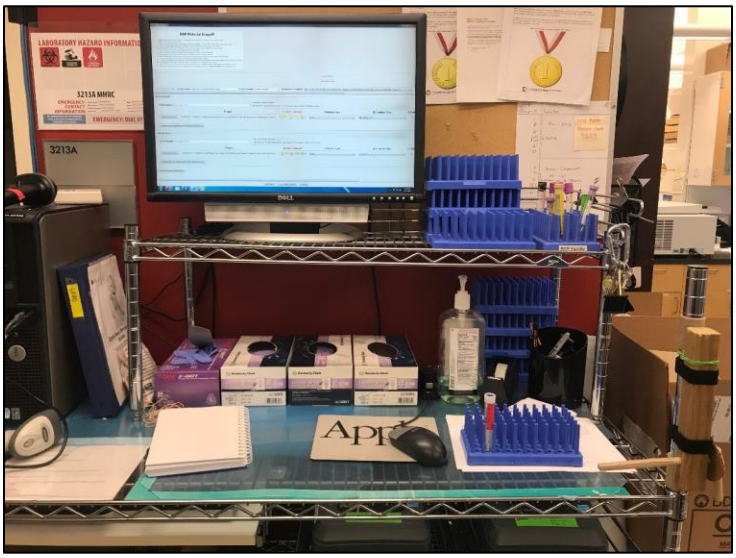
Peripheral Blood:
- 3 to 6 ml in EDTA (lavender top) tube AND if abbreviated karyotype is requested, a 3 to 6 ml in Sodium Heparin (green top) tube
- Maintain at room temperature.

Buccal Swab:
- Collect with ORACollect® swab provided by Fullerton Genetics.
- Maintain at room temperature.

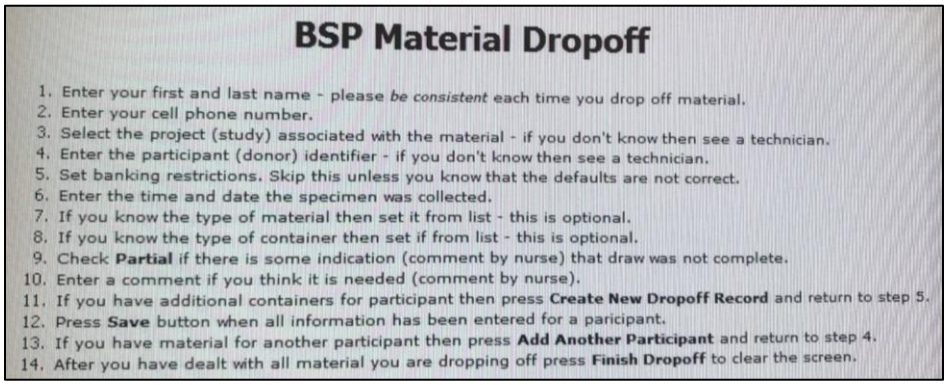
Amniotic Fluid, CVS or Products of Conception:
- Follow specimen collection guidelines above for Chromosome Studies.
- Requires a 3 to 6 ml in Sodium Heparin (green top) tube for Maternal Cell Contamination (MCC) studies – maintain at room temperature.

Appendix XL. Using the LIMS system at the BSP

1. At the BSP, use the LIMS system (pictured below) to log the samples.



a. The LIMS system provides instructions for specimen delivery shown here.

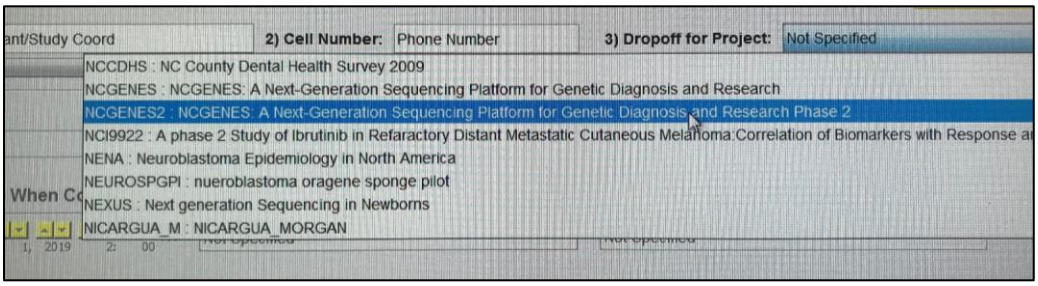


BSP Material Dropoff

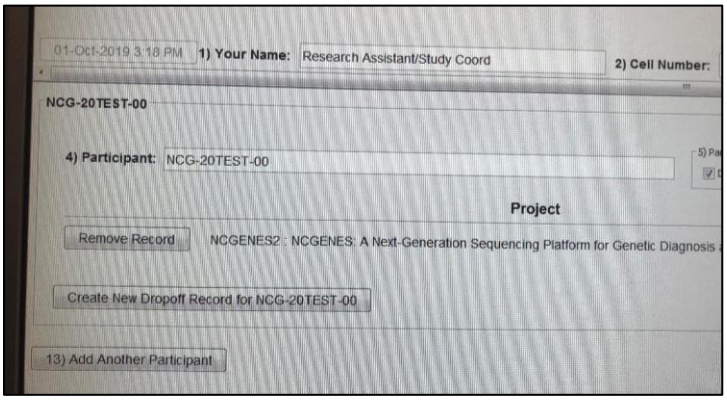
1. Enter your first and last name - please *be consistent* each time you drop off material.
2. Enter your cell phone number.
3. Select the project (study) associated with the material - if you don't know then see a technician.
4. Enter the participant (donor) identifier - if you don't know then see a technician.
5. Set banking restrictions. Skip this unless you know that the defaults are not correct.
6. Enter the time and date the specimen was collected.
7. If you know the type of material then set it from list - this is optional.
8. If you know the type of container then set if from list - this is optional.
9. Check **Partial** if there is some indication (comment by nurse) that draw was not complete.
10. Enter a comment if you think it is needed (comment by nurse).
11. If you have additional containers for participant then press **Create New Dropoff Record** and return to step 5.
12. Press **Save** button when all information has been entered for a paricipant.
13. If you have material for another participant then press **Add Another Participant** and return to step 4.
14. After you have dealt with all material you are dropping off press **Finish Dropoff** to clear the screen.

b. First, the study person delivering the sample should enter their name and cell phone number.

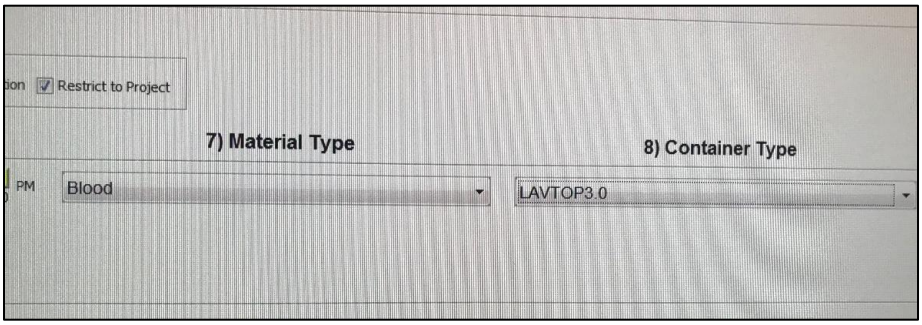
c. Next, select NCGENES 2 from the project name dropdown shown below.



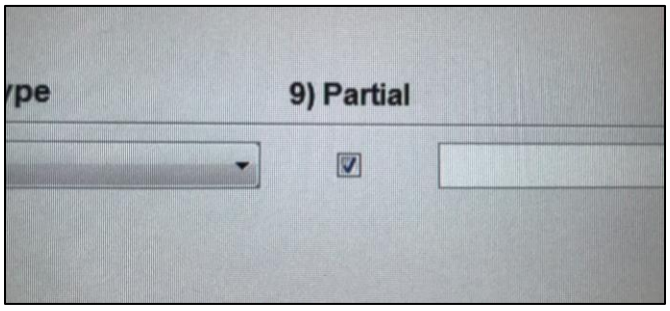
d. Whenever possible, sample barcodes should be scanned to auto-populate the "Participant" field in the LIMS system. If manual entry the participants barcode ID is required, the research assistant should type that ID into the LIMS system.[1]



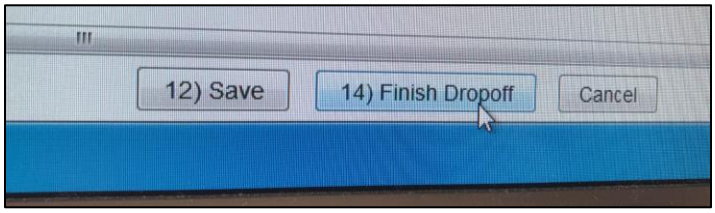
e. Manually enter the time of collection, and approximate volume (by comparing to pre-measured vacutainer available in BSP), as well as tube type (LAVTOP3.0), and sample type (e.g., blood).



f. If the tube is not completely filled, mark the "Partial" box. (shown below).



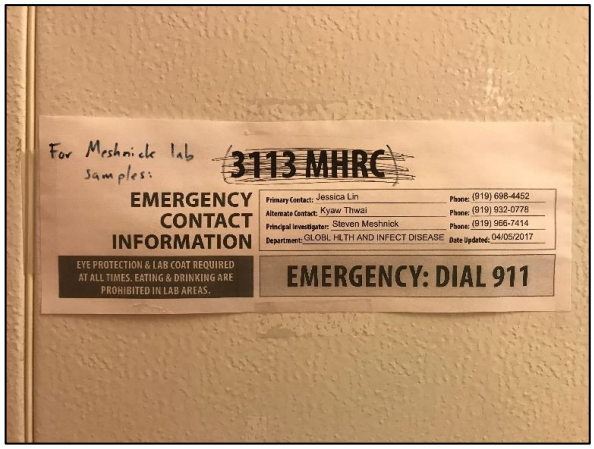
g. When the form is complete, click the "Finish Dropoff" button to finalize the LIMS entry.



[1] The participant ID for the BSP is the longer "NCG-2####-00" and not shorter number used in the Tracking System.

2. Once entered into LIMS, place the blood tubes in the refrigerator (tube rack in the refrigerator directly across from the LIMs computer) designated for sample deliveries.

3. ***If it is after 4:30 pm***, **FOR UNC BSP** - place the NCGENES 2 sample in the cold room (rm 3209) in the hallway (outside of the BSP and about half way down the hallway – metal door) and log the sample in on the form on the clipboard in the cold room. The samples will be processed the next day.



Appendix XLI. Tips for Interviewing

Being an interviewer

(Adapted from https://www.institute.org/wp-content/uploads/2011/06/Interviewer_Training_Manual.pdf.pdf)

You are the key to the success of this study, including

1) Getting people to participate
2) Helping people do a good job answering the interview questions
3) Recording their responses clearly and accurately

Getting people to participate:

Some people will agree to participate and be interviewed right away. But there will also be people who are suspicious or who, at first, do not want to participate. Getting them to participate can be difficult, but is one of the most important jobs of the interviewer. Here are some techniques that have worked with other surveys:

- Be positive and upbeat, and act confident. Act like you are certain that they will want to participate. Be friendly.
- Know why this project is important.
- The more you know, the more convincing you can be.

Getting people to do a good job answering the interview questions:

- You set the tone of the interview, and you can affect the quality of the answers you receive.
- For example, if you rush through the questions, the people you are interviewing may rush through their answers.
- If you seem bored, the person answering the questions may not want to spend the time thinking through how they respond.
- If you are confused, the person answering will be confused.
- Make sure you understand the study and the questions, and always feel free to ask the team questions.

Recording the answers clearly and accurately:

- You may do a great job interviewing, but if you don't record the answers accurately, we will never know that. For us to use the interviews, you need to keep track of everything clearly and accurately.
- Be sure that you mark down an answer for every question, unless it is missed through a skip pattern. Some questions have a list of possible answers that you are supposed to read to the person you are interviewing. Follow the directions for each of these questions.

How to Interview

You will be asking people questions that have a lot of detail and are somewhat personal. Here is some advice about how you can keep control of the interview and collect good information.

Practice:

- Be very familiar with the questionnaire, skip patterns, and the entire interview process before doing the first interview.
- If you don't understand a question, the person you are interviewing will also be confused. Practice asking the difficult questions until you can ask them in a simple, matter-of-fact way.

Asking the Questions:

- Don't Show Your Own Feelings or Give Away the "Correct" Answer
    - It is important not to influence how people reply to the questions. Be careful not to show any personal feelings and judgments.
    - Do not show surprise, approval, or disapproval.

- Ask the Questions in the Exact Order that they appear
    - In writing the questionnaire, we purposely put the questions in a certain order. Some questions are placed after others on purpose. So please don't change the order of the questions when you are interviewing people.

- Ask All of the Questions (Unless You Are Told Not To)

- Sometimes in answering one question, the person you are interviewing may also give an answer to a later question that you haven't asked yet.
- Do not fill in that answer. Wait until you get to that question and then ask it exactly as it is written on the form.
- If the person becomes annoyed at being asked a question again (and may say something like "I just told you that"), respond pleasantly by saying: "I need to ask all of these questions in the exact order to make sure we have complete information."
- Always feel free to blame the researchers for requiring you to ask repetitive questions or to be very clear about the answers. You can say: "I know it may sound like I'm being repetitive, but I have to follow the instructions I have from the researchers."

- Ask Each Question Exactly as it is Worded
  - Each interviewer needs to ask the questions in exactly the same way.
  - Any change, even of one or two words, can change the meaning of the question.
  - After all of the interviews are complete, all of the answers will be combined and studied as a group. Therefore, all of the questions need to be asked in exactly the same way by all of the interviewers.

- Keep the Focus on the Interview Questions
  - The person you are interviewing may describe things from the past or tell a long story. If they ramble or discuss irrelevant topics, you should politely interrupt and get them back on track. This is a very important part of your job. It can be hard to keep the person focused on the questions without being rude.
  - Here are some suggestions. Try saying:
    - "That sounds very interesting. So [repeat the question…"]
    - "I see what you mean. Let me repeat the last question…"

- What if the Person Says Things That Don't Makes Sense or Are Inconsistent?
  - People often have opinions that may not be accurate or may not make sense. We are interested in learning about those views – even if they are incorrect. So do not try to correct people or point out their mistakes.
  - If you know something about the person you are interviewing and you think they have answered a question incorrectly, do not correct them or change the answer

- Make the Person You are Interviewing Feel Comfortable
  - Your goal should be to make the person you are interviewing feel as comfortable as possible. Be friendly and calm.
  - If the person seems nervous or says that they feel uncomfortable answering a question, remind them that all of their answers will be kept confidential.
  - Also remind them that there are no right or wrong answers to these questions. We are interested in their opinions.
  - But remember that the person you are interviewing does have the right to refuse to answer a question.

- Getting a Clear Answer ("Probing")
  - One of the hardest and most important parts of your job will be getting the person you are interviewing to answer the question the way it was asked. People may give only part of an answer or they may misunderstand the question or go off on another topic. Sometimes they will be difficult to understand. It is your job to get them back on track and to "probe" for a clear answer to the question.
  - When should you probe?
  - Probe when the person misunderstands the question:
    - This usually happens when the person you are interviewing didn't hear or missed a key word or phrase in a question.
    - Here is an example:
      - Question: Do you smoke cigarettes on a daily basis?
      - Response: "I usually smoke about a pack a week."

- In this example, the person did not hear that the interviewer asked him about his daily smoking habits. The interviewer should probe by saying something like, "I see. Well, do you smoke cigarettes on a daily basis?"
- If you stress the key words the second time, the person will understand and give you the answer you need.

- Probe when the person finds it hard to remember something:
  - Some questions require the person you are interviewing to remember events that took place some time ago. If they have a hard time remembering, ask them for their best guess.
  - Sometimes it helps to ask if it occurred before or after a holiday or other date.

- Probe when the person says they don't know:
  - Sometimes when a person says that they don't know, they may really mean that they don't understand the question or that they need some time to think about it.
  - Some people may say that they don't know because they are afraid of giving the wrong answer or because the question seems too personal.
  - So if the person answers "I don't know," do not be in too big of a rush to settle for the "Don't Know" response. If you just wait quietly as if you are expecting an answer, they will usually think of something to say. This is usually the best approach for a "Don't Know" response.

- How do you probe?
  - Here is what not to do:
    - Don't change anything about the question. If you think that the question is confusing, make a note in the margin and go to the next question. Discuss this with the study team, if possible, before conducting the next interview.
    - Don't show that you expect a certain answer or that one answer is better than another.
    - Don't assume anything
      - Here is an example of what can go wrong if you assume something:
        - Question: "In general, would you say that your health is excellent, very good, good, fair, or poor?"
        - Response: "I already told you I have diabetes."
        - Improper Probe: "Then would you say your health is fair or poor?"
    - That probe forces the person who is being interviewed to decide between two choices (fair or poor). But she may feel that despite her diabetes, her health is excellent.
    - The interviewer should have just said, "Yes, I remember that," and then simply repeated the question.
  - Here is what you should do:
    - Wait as if you are expecting an answer and want the person you are interviewing to continue.
    - Show your interest and understanding by saying thing like "Uh-huh" or "I see" or "Yes."
    - Repeat the question or the possible answers. Many people hearing the question for a second time realize what kind of answer is needed. They may not have heard the question fully the first time or they might have missed the point of the question.
    - Some questions are open-ended. This means that there is no set of answers to choose from or to circle. Instead, you are asked to write down the person's words exactly. For these kinds of questions, you can probe for more information simply by repeating what the person has already said. You can also say, "Is there anything else you would like to add?"
    - If the person's answer to an open-ended question is confusing you can use questions like these:
      - "What do you mean exactly?"
      - "What do you mean by…?"

    - "Could you please explain that a little? I don't think I quite understand."
- For open ended questions, sometimes you will need to ask them to be more specific. You can use questions like these: "Could you be more specific about that?" "Tell me more about that: What/who/how/why…?"
- Sometimes people will be vague about numbers. If they give a range of numbers (for example, five to ten), you can probe by saying "I can only put down one number. Can you tell me what the best answer is?" If they refuse to choose a number, you should select the number in the middle.
- Sometimes you will need to need to pull them back on track. You can use phrases like these:
    - "Generally… (and then repeat the exact question)"
    - "Usually…(and then repeat the exact question)"
    - "What is your best guess?"
    - "Which choice comes closest to how you feel?"
    - "I see. Well, let me ask you again…(and then repeat the exact question)"

Interviewer training

(Adapted from: **http://www.hcsrn.org/en/Tools%20&%20Materials/Plan_Field/HCSRN_InterviewerTraining.pdf**)

Individual Responsibility

Interviewers must make every effort to protect the anonymity and confidentiality of respondents. Interviewers perform a professional function when they obtain information from individuals in personal interviews, and they are expected to maintain professional ethical standards of confidentiality regarding what they hear about a respondent.

1. All information about respondents obtained during the course of the research is privileged information whether it relates to the interview itself or includes extraneous observations concerning the respondent's home, family, and activities.
2. Such information is not to be discussed with anyone but project staff.
3. A breach of confidentiality is a serious violation.
4. It is very important that you never conduct an interview with someone you know as a: relative, friend, neighbor, or someone you remotely know. The respondent may feel you are judging him and therefore not respond honestly to the questions. Everyone is entitled to a confidential interview. Knowing the respondent could compromise confidentiality and the integrity of the interview.

Basically, if you know the respondent either personally or through a friend or relative, you can not interview that person.

For your own safety:

- NEVER give out personal information about yourself.
- NEVER agree to meet a respondent in person.
- NEVER reveal the actual location from which you are interviewing

Inadvertent disclosure

Often you talk to interesting people on the phone. Sometimes you have a conversation that upsets or disturbs you. You may be tempted to talk to someone about it – a close friend or partner, or another interviewer. It is important that you do not do this. You may think that you can describe the conversation without revealing someone's identity, but consider how very little you must say before someone can be identified. For example, if you were interviewing parents of kids with asthma, and you were amazed by one parent's account of raising 7 children, 2 of whom have asthma. The parent is doing this alone, because the other parent was killed tragically in an auto accident. Suddenly, your friend says, "I heard about a tragedy like that on the news about 6 months ago…" Suddenly, there is enough information revealed to do very little research to find this person.

Confirmation that the Respondent Understands Informed Consent

What if you are not convinced that the adult or other respondent understands their rights and responsibilities? We need to be aware of factors that may contribute to miscommunication and misunderstanding; the respondent may be agreeing to participate without fully understanding the risks and benefits.

Suggested Language

INTERVIEWER: "Before we go on, I need to be sure I've told you everything about the study. Would you mind telling me in your own words what I've told you?" (BE SURE TO THANK THE RESPONDENT.)

If the respondent is able to summarize in a meaningful way, you may proceed. If you get into the interview, though, and find that the questions are not understood, use the language below to terminate the contact.

Also, if it is clear that the respondent does not understand, say:

INTERVIEWER: "Thank you so much for your time. Those are all of the questions I have for you."

Reaffirming informed consent when calling back

Sometimes the respondent will need to be called back after they've heard the Risks and Benefits Section of the interview. The next time the respondent is contacted before beginning or resuming an interview, you must remind the respondent that:

1. All information is confidential, and will not be identified with the respondent.
2. Her/his relationship with her health plan will not be affected, and
3. S/he may skip any question.

Introduction to interviewing

Interviewing is a conversation with a purpose. As a professional interviewer, your goal is to collect accurate information in accordance with sound interviewing practices. Interviews are designed to obtain accurate and complete information, but to be effective the information must be collected in a uniform manner from all respondents. The people interviewed must be asked the same questions in the same way. It is only when each interviewer conducts the interview in the same fashion as all other interviewers that the information will be uniformly accurate and ensure that the study produces meaningful and useful results.

It is important for you to develop your own style of interviewing. Confidence and sincerity can be heard. All respondents should be treated with respect and appreciation for their time. Respondents will be more likely to participate if they perceive you as knowledgeable, respectful, and sincerely interested in what s/he has to say. You will find that the majority of participants are willing to be interviewed. A confident, enthusiastic approach which assumes people are willing to be interviewed is a most effective technique.

In general, consult your supervisor if you have any questions about the interview or about how a situation you're unfamiliar with should be handled.

Active Listening

Good interviewers are skilled professionals and their skills enable participants to give frank, complete, relevant answers to questions. The interviewer must convey that s/he is an understanding person capable of accepting information in a nonjudgmental manner and that she has an interest in what the participant is saying. A good interviewer is an active listener, one who always listens very carefully, respectfully and politely. The participant should enjoy the interview.

Studies have shown that a participant will often remember more about the interviewer and how the interview was conducted than about the topics covered during the interview. The interviewer is a representative of the study team, and the impression the interviewer leaves on the participant may be the impression the participant will then have of the study in general. .

For telephone interviewing you do not have the advantage of talking to the person face-to-face. Your ears will have to be your eyes. Listen very carefully when the respondent speaks. Listen for distractions such as running water, a baby crying, a child asking questions, or another conversation. Before the interview can begin you must first determine if you have the respondent's attention. If you have a respondent on the phone, it is often best to try to complete the interview, because you may not reach him again. On the other hand you do not want to compromise the quality of the data because the respondent is not paying attention. Listen very carefully and always offer to either hold (or call back).

Although it is important that all interviewers be trained to administer the questions of a survey over the telephone in the same way to every respondent, it is also important that we recognize that by interviewing over the telephone, we have the benefit of interacting with a respondent. You are not merely a talking survey. Sometimes what a respondent says is not what we expected to hear, or else the answers they give do not fit exactly into our pre-coded response categories. It is at these times that it is important for the interviewer to listen carefully to what the respondent is saying and to respond in a way that reflects that you HEARD what the respondent said. For example, you may be asking a series of questions with the response categories of "Strongly agree, agree, disagree, or strongly disagree." During the course of the interview, perhaps the respondent says, "Sure," instead of giving one of the pre-coded responses. Instead of reading the entire list again, you might just ask, "Would that be strongly agree or agree?" There is no need to say, "Disagree or strongly disagree," because the respondent has already indicated she is in agreement with the synonym, "Sure." But it is still important that you do not interpret "Sure" to be "Strongly agree" or "Agree," but rather give the respondent the choice of the most reasonable response categories closest to the answer she gave in the first place. Even if the respondent has already talked about a situation, when it comes time to ask the question, which addresses the same issue, ask the question and take their response.

Suggestions for Active Listening

• Listen attentively without interrupting.

• Exude warmth, curiosity, interest, and caring - qualities that will make respondents want to talk to you, to share their thoughts and feelings.

• Be present – avoid daydreaming; focus on the respondent and what s/he is saying. Control distractions.

• Become a "whole body" listener – sit with good posture, use verbals – "uh-huh, hmmm" – to let the speaker know you are hearing them.

• Maintain Neutrality" – it is your job to make your own experiences and opinions of no interest to the respondent, so it is important that your reactions to the answers given remain neutral but show that you are listening and paying attention. Avoid words that imply value or reaction, like "Good; Oh really?; No!; You're kidding!; etc.

• Be aware of your emotions – if someone is saying something that triggers your emotions, it is important that your interactions with her remain neutral and do not reflect those emotions.

• For open-ended questions: Summarizing, paraphrasing and asking for clarification – if the respondent has shared a great deal of information with you, you might pull it all together and summarize it or paraphrase it by restating the message in a summary form. This is an effective way to demonstrate that you have been listening actively to the respondent and you are now asking for affirmation that you heard what you think you heard or that you need clarification of the respondent's intended message.

Always keep in the back of your mind: Has this question been answered adequately, do I understand what the respondent has said, does the answer make sense, is it an answer to this question, and did I get all the information necessary to be able to code this question for data entry?

General Interviewing Practice

The First 10 Seconds

The first ten seconds after someone answers the phone is the precious time when people decide what to do with your call. Each time you reach a household, you must be in a "ready" position – speak your name confidently, ask for the designated respondent by name, and be prepared to answer any questions that may arise.

When we are nervous, our voices tend to end every sentence in a higher pitch – like a question. Avoid this – it conveys that you are reluctant or uncertain about what you are telling the respondent. Actively listen for distractions in the background, reluctance in the voice of the person you are talking to, and let the person on the phone dictate the volume and pace of your speech.

Asking the Questions

Read Exactly as Written

- Before fielding a survey, time should be allowed to test the questions for flow, verbal presentation, and understanding. Because this time and energy is spent pre-testing the questionnaire, it is mandatory that each interviewer read each question EXACTLY as it is written. The slightest change in wording can bias, or change the meaning of the question for different respondents. Each respondent must hear the entire question read exactly as it is written.

Read in the Order Indicated

- Questions are ordered in a certain way to prevent some answers from influencing other answers. Information is asked in logical progression. Any alteration of the order of questions could bias a respondent's answers. All questions must be read in the order they appear on the questionnaire.

Use a Conversational Tone

- Interviewers must be careful to avoid behavior, conscious or unconscious, spoken or unspoken, which could affect the way a respondent answers a question. It is important that the facts and opinions a respondent gives are his/her own. Questions should be read in a conversational tone without intonation that may change the meaning or bias the response. Only those words underlined in the questionnaire should be emphasized.

Read the Entire Question

- Each respondent must hear the entire question before they answer. If a respondent answers before the entire question has been read, there is a chance his/her response would be different if he/she heard the entire question. If the respondent interrupts, you can say, "I want to make sure you hear the entire question before you answer".

Don't Skip Questions

- Sometimes respondents mention information that answers a question that you will ask later in the survey. Don't skip a question because the answer was given earlier or because you "know" the response. <u>Although it is tempting to skip a question because you feel the respondent has already answered it, always ask each question it its entirety.</u> The respondent must hear each question. However, in those situations in which the respondent has already provided information that probably answers the next question, you may preface the question with some combination of the following phrases:
    - "I know we've talked about this" or "I know you just mentioned this, but I need to ask each question as it appears in the questionnaire".
    - "You have already mentioned this, but let me ask you..."

Do Not Assume You Know the Answer

- Even if the respondent has already talked about a situation, when it comes time to ask the question, which addresses the same issue, ask the question anyway and take their response.

<u>Probing</u>

When a respondent answers the question in a way that does not fit one of the categories, you need to probe for a correct answer. Probing is a prompt which encourages further conversation without biasing the response. There are three types of probing:

1. Probing for correctness
2. Probing for clarity
3. Probing for completeness

<u>Probing for Correctness</u>

Probing for correctness is used on pre-coded questions where you have categories that the respondent is to choose from. Sometimes reading the entire question again is an excellent probe to let the respondent know that the answer they gave was incorrect. Pausing and asking, "Your answer is," is a very good probe. If the respondent's answer does not fit into the categories you have, the best probe is to repeat the categories. When you repeat the categories always read ALL of the categories. Tell the respondent, "I only have these categories" and read them all again.

- Question 4a
- Respondent: Oh it is a problem.
- Interviewer: Should I mark "A big problem, a small problem, or not a problem at all"?

Always read the entire question and all of the categories when repeating. Never help the respondent by telling them which category is closest to their response. Be careful never to criticize the survey or express negativity to the respondent. Always let the respondent choose even if they are asking for your help.

Examples of Probes for Correctness:

- Which one response is closest to your answer?
- I don't have a category in between. I only have "yes" or "no".
- What would your answer be?
- If you had to choose, which one would you choose?

- Why don't I read the question and possible answers again and you can tell me what is closest to your situation or opinion.
- I only have these categories. Let me read them again and tell me which one is closest to your answer.
- There is no right or wrong answers only your opinions.
- The questionnaire does not offer an explanation or definition, so whatever "[term]" would mean to you. Let me read the categories again.
- I really can't choose for you, this is your opportunity to be heard and tell us what is important to you or how satisfied you are.
- Sometimes fitting into a category is difficult. Let me read them again
- These categories are a range of opinions, which make it easier for analysis. I realize we speak to many respondents in different situations.
- Let me read the categories again and if you would like I would be happy to record a comment with your response.

When probing with a scale the same standards would apply. Think of each number as a category. Here is an example.

- Question Please rate your current health plan. On a scale of 0 to 10 where 0 means the worst health plan possible and 10 means the best health plan possible, how would you rate your current health plan? You can use any number: 0,1,2,3,4,5,6,7,8,9 or 10.
- Respondent: Pretty good…probably one of the best.
- Interviewer: What number would that be? 0 means the worst health plan possible and 10 means the best health plan possible. You can select any number in between.
- Respondent: What would a five mean?
- Interviewer: I only have labels for 0 and 10 - 0 means the worst health plan and 10 means the best health plan; you can select any number zero through ten.
- Respondent: I would like to give them an above average rating but I am not sure which number.
- Interviewer: Scales can be difficult. I'm going to read you the scale again while you think about it. The lowest point of the scale is 0 meaning the worst, we move up the scale to 1,2,3,4,5,6,7,8,9,and 10 which means the best.
- Respondent: Let's go with an 8.

<u>Probing for Clarity</u>

A probe for clarity is often asking the respondent for a more detail about their response, or an explanation of their answer. Open-ended questions tend to be very general (what do you think, why do you feel that way, etc.). Respondents tend to answer these questions in a general way and use general adjectives to describe situations and options. Thus, probing for clarity is often a matter of asking for a more specific response, or an explanation of an answer.

Examples of Probes for Clarity:

- What do you mean?
- Could you be more specific?
- Could you say more?
- Could you explain?
- I don't understand.
- What about that?

The best probes for clarity are ones which tell the respondent exactly what you want to know. When probing for clarity, always help the respondent know what you don't understand and what you want clarified. Do not assume you understand - ask for an example or an explanation.

- Question: Is there anything in particular that you didn't like about the experience?
- Respondent: The wait is awful.
- Interviewer: Please tell me what you mean by the wait?
- Respondent: When I call for an appointment I usually have to wait 90 days before I am seen in the clinic.
- Interviewer: Is that for any particular type of an appointment or for all appointments?
- Respondent: No, they are good when I need to get in for my allergies, but for a physical I have to wait 90 days.
- Interviewer: Is there anything else you would like to comment on?
- Respondent; No, everything else is fine.

Here is another example with the same question.

- Question: Is there anything in particular that you didn't like about the experience?
- Respondent: Cost is very good.
- Interviewer: Could you give me an example of what you mean by costs?
- Respondent: The co-pays are much lower with this plan than the plan I had last year. I am very pleased with that.
- Interviewer: Anything else?

- Respondent: Yes, the costs for prescriptions are wonderful. I take a lot of allergy medication and I am very happy with the low co-pay for these medications.
- Interviewer: Anything else?
- Respondent: The people are great!
- Interviewer: Anyone in particular?
- Respondent: I'd have to say the office staff that check you in and the nurses. They are always so kind and thoughtful they go out of their way to make you feel welcome.

Probing for Completeness

Some questionnaires will have questions where the interviewer is instructed to "check all that are mentioned," or probe for "others." This is where you would use your probes for completeness.

Once a clear answer has been obtained, the interviewer should probe for additional responses to the question. The best way to do this is to repeat the substance of the question as part of a request for further information.

Examples of Probes for Completeness:

- What else do you like?
- What other reason did you have?
- Anything else?
- Any other comments?

Each additional response should be probed for clarity as necessary. The interviewer should continue probing for additional responses until the respondent indicates that he/she has nothing else to say on the subject.

Recording Data

Types of Questions

- Close-ended questions with pre-coded responses
- Open-ended questions – record verbatim responses
- Open-ended questions with pre-coded responses that are not read to the respondent

Recording Pre-coded Responses to Close-ended Questions

Pre-coded questions have response categories that are part of the question; there is a set of pre-planned response options and the respondent's answer must be coded into one of them:

Example:

Q. A9. In general, would you say your health is excellent, good, fair or poor?

- <1> Excellent
- <2> Good
- <3> Fair
- <4> Poor
- <8> DK (Don't know)
- <9> REF (Refused)

If the respondent answers "excellent," record 1 as the answer.

If the respondent answers "good," record 2, etc.

Be sure to enter the correct number for the respondent's response! If you need to look down at the keyboard while typing it in, go ahead. You can fill any brief "dead air" by simply repeating their answer, which is a good thing to do anyway. It helps the respondent know you're paying attention.

Below are examples of different pre coded questions.

Q. B10. In general, how would you say you are these days? Would you say you are very happy, pretty happy, or not too happy?

- <1> Very happy
- <2> Pretty happy
- <3> Not too happy
- <8> DK (Don't know)
- <9> REF (Refused)

If respondent says "Pretty happy," enter 2.

If respondent says, "Yes, I am happy" you must try to get respondent to fit the answer into one of the listed categories by repeating the responses closest to what the respondent said (probing).

You should probe by saying: "Would that be closer to very happy or pretty happy?"

Show your probe in your F-1 note pad as x and record what the respondent says in the box provided using the pre code.

Recording Verbatim Responses to Open-ended Questions

Sometimes researchers want to know exactly what respondents say when asked a question. When this occurs, the interviewer records WORD FOR WORD, or verbatim, what the respondent says. The interviewer should not paraphrase or summarize the respondent's answers unless instructed specifically to do so. You will record the respondent's verbatim comments, and your probes should be recorded in parentheses.
Most respondents will talk faster than you can record. It will be up to you to slow them down or ask them to wait while you catch up. Techniques for recording quickly and for slowing the respondent without inhibiting him/her will be different for each interviewer. Some examples of the techniques are:

- Begin writing or typing as soon as the respondent starts answering.
- Use standard abbreviations.
- Repeat what you are writing/typing while you are writing/typing.
- Slow the respondent down as soon as you get lost. Go back to the beginning and let him/her take it from there.

When you are recording the respondent's comments, be careful not to have too much dead time in the conversation. If the respondent is just sitting there while you write, they will be bored.
Use small talk to maintain their attention. Such as:

- O.K., I need to record all of your responses.
- This is helpful.
- I'm sorry I'm typing so slowly, but I want to make sure I write down your comments correctly.

Other examples of open-ended questions:
Q.B11. How many years have you been married?

- 98 DK
- 99 REF

___ ___Enter number of years <1 - 80>
This question shows you a range of appropriate answers and the pre-codes to be used for DK (don't know) and REF (refused) options. Acceptable ranges should be large enough to accommodate any reasonable, realistic response. In the example above an acceptable range on ages is 1 to 80. If a legitimate response falls outside the acceptable range, probe to make sure the respondent understand the question. If the response is still outside the acceptable range then record it in a note with the verbatim response and proceed to the next question.
Recording Verbatim Responses to Open-ended Questions
Sometimes researchers want to ask a question in an open-ended fashion, but they have a pretty good idea what the top 5 or 6 responses are likely to be. In this case, the interviewer listens to the answer, and if appropriate, records the number corresponding to the pre-coded response (usually presented in CAPITAL LETTERS, indicating they are NOT to be read to the respondent). If the answer does not fit into one of the pre-coded responses, the interviewer should record the number corresponding with "other," then record verbatim the respondent's answer.
Example:
What is the main reason why you do not eat as many fresh vegetables as you would like or think you should?

- <1> COST TOO MUCH
- <2> NOT AVAILABLE WHERE I SHOP
- <3> TOO DIFFICULT TO PREPARE
- <4> DON'T KNOW HOW TO PREPARE
- <5> SPOIL QUICKLY
- <7> OTHER - SPECIFY
- <88> DON'T KNOW
- <99> REFUSED

Handling Difficult Situations

People Who Talk Too Much
There are some respondents who may want to give more information than the interviewer needs or asks for, or who are not able or willing to stick to the point of the question. Some may be so concerned with being thorough that they provide answers to questions not yet asked. Others may be lonely and view the interview as an opportunity for companionship and want to engage the interviewer in social conversation. Still, other respondents may be naturally chatty and want to talk on and on about themselves. Although much of the information these respondents give may be interesting, an interview that normally takes five minutes should not be allowed to go on for fifteen. It is the interviewer's responsibility to rein the respondent back in by refocusing his attention while remaining respectful of his needs. If the respondent tends to give long explanations for every answer, politely cut him off, and direct his attention to the next question after you've gotten an acceptable response.
Examples of Ways to Redirect

- Say, "oh, I see, OK. . ." then immediately proceed to the next question.

- Say, "isn't that interesting -- now let me ask you . . ." and repeat the question.
- If the respondent is very talkative and doesn't answer the question, interrupt at an appropriate point and say "given what you've just told me, would you say . . ." or "given all that, if you had to choose, would you say . . ." and repeat the choices.
- Offer, “So that I don’t take any more of your time than is needed, let’s go to the next question…”
- Say "let me stop you so I can write this all down . . . now, given what you've said . . ." and repeat the choices or the question.
- If the respondent jumps ahead of the question, tell him you will be "asking about this later" and suggest he wait until you get to that part of the interview so you can record the information accurately at that time. Say "we're going to cover that a little later" or "we'll get back to that in a bit," then read the next question, or repeat the question you already asked, or repeat the choices you already gave.

People in a Hurry to Get Off the Phone

Sometimes the respondent is in a hurry to get off the phone or wants to cut the interview short. This can be a problem with long interviews. If you are almost done with the interview, let the respondent know, and usually you'll be allowed to finish. It helps to say "just ____ more questions" or "we'll be through in about ____ minutes." If you are not almost done, or if the respondent becomes truly weary of the interview, indicate how much more time you think you'll need to finish and let the respondent decide whether to continue or to reschedule another time to complete the interview. Many research centers allow interviews to be completed in multiple sittings. It is preferable to complete an interview in one sitting, because you never know if you will actually be able to contact the respondent again. But situations arise – someone comes to the door, something burns on the stove, a child needs attention, or the respondent simply has another appointment to get to, and you should have instructions on how to deal with the interview should such a situation arise. If you can resume the interview, try to schedule a specific day and time to call back; if the respondent cannot commit to an appointment readily, find out the best days and times to call back and call the respondent until you reach him/her and are able to complete the interview.

People with Difficulty Hearing or Understanding a Question

If the respondent has difficulty hearing or understanding a question, repeat the question slowly and clearly without raising your voice. For some people the louder the voice, the more distorted it sounds and the harder it is to hear, especially over the phone. A louder voice may convey the impression that the interviewer is getting impatient. Do not reword, explain or interpret the question; simply repeat it and encourage the respondent to do the best she can. If she cannot come to an understanding of the question, move on to the next and note in the margin or comments section of the CATI program what happened.

People Who Are Defensive

Health research telephone interviews often involve personal or sensitive questions. Sometimes they are conducted with patients who participated in a behavior change program in the past to follow up and see how they are doing now. People who try to quit smoking, lower cholesterol, or lose weight but do not succeed may feel that they have failed. They may be unwilling to participate in an interview, or they may answer questions defensively. It is imperative that the interviewer provide a safe, nonjudgmental environment where the respondent feels safe sharing the results of his/her experience, even if it didn’t result in the desired outcome. To minimize the respondent’s defensiveness, remain neutral; ask all questions in a direct, matter-of-fact tone of voice, especially when asking questions about income, education, weight, etc. Be careful to avoid criticism or even praise.

Suggestions for Reducing Defensiveness

- "There are no right or wrong answers"
- "Your medical care will not be affected if you participate.”
- Explain, "it is important to talk to you and get information from you because your experience may differ from the experience of others we talk to.”
- “I understand that you may not want to participate, but we need the information only you can give us" and that "others may benefit from your experience."

People Who Are Upset with the Health Plan or Angry in General

If the respondent is mad at the study or angry about something else, listen to what s/he is saying and acknowledge -- rather than deny or minimize -- the anger. A statement like "I can understand why you're upset" will validate the respondent’s feelings and help establish a trust between interviewer and respondent. Accept all opinions without showing surprise, approval or disapproval, agreement or disagreement. Telling the respondent "your opinions are very important to us -- we want your feedback - we can benefit from your experience" may diffuse the respondent’s anger and enlist his/her participation. Often the respondent just needs to vent frustration with the health plan in general and nothing more, but if you feel the respondent would benefit by discussing the complaint with someone else, consider referring the respondent to the health plan’s customer service/consumer helpline. If the respondent has a complaint about the research process – the advance letter or the phone call – note the details and inform your supervisor immediately. The research center’s supervisory staff and/or the project staff may decide to follow up with the respondent.

Suggestions for Interviewing Angry Respondents

- Say "I can understand why you're upset"
- Explain, “your opinions are very important to us -- we want your feedback - we can benefit from your experience."
- “I can pass on your comments about your experience to the study leaders or I can provide you with the number if you wish to talk with them.”

People who are Difficult to Reach

A household member may try to keep the interviewer from connecting with the respondent.
Some examples include:

- The household member wants more information about the purpose of your call than you can comfortably give.
- The household member is reluctant to put you through to the respondent.
- The household member tells you the respondent is not interested in participating.

In these situations, you need to be extremely diplomatic to avoid losing the respondent completely.
If the respondent is not home, ask when a good time to call back would be, thank the household member, and hang up. If asked, say you will call back, that there is no need for your call to be returned, and there's no message. If pressed, it's OK to explain that you're calling from [name your institution] and that you'll call back, and that it's not urgent and there is no problem (a call from health care institution makes many people wonder what's wrong). Due to HIPAA privacy issues, we must reveal minimal information to anyone other than the respondent, but we also do not wish to alarm the household.
If the household member says he can answer the questions for you --"I'm her husband" -- say you appreciate his interest and offer, but explain that you really need to speak to the respondent directly. If you sense after repeated attempts to reach the respondent that a household member is intentionally blocking your access to the respondent, say that you need to speak to the respondent "for just a minute, for one quick question" that it's "very important;" or try calling at different times when you suspect the household member might not be there.
If you're told by a household member that the respondent is not interested in participating, thank him for the information and let him know that participation is voluntary so it's OK if the respondent is not interested, but that you would like to speak with the respondent briefly anyway. The interviewer's best strategy for reaching the respondent is to remain polite but persistent and try to gain the trust of the resistant household member.

Voice mail Protocols -- Example

Most people have voice mail and some people use it to screen calls. Here is a sample protocol written for one study that provides guidelines for working with voice mail and leaving messages.
Sample Protocol for Leaving Messages:
We use messages to let respondents know we are trying to reach them.
For instance, a study could decide to leave up to 3 messages. The case is eligible to be re-called on the next "shift."
You can count the shifts as:

- Day, 9am-5pm = 1 shift.
- Evenings = 1 shift,
- Saturday = 1 shift,
- Sunday = 1 shift.

The first message is left on the first call attempt after all numbers are dialed and voice mail or no contact is encountered. This is so the respondent connects the advance materials to the call. Try to leave the first message at the home number. If you don't know which phone number is the home number, leave the message on the voice mail where the respondent's name is listed if it does not appear to be a work number.
If no name is listed on any of the messages, you choose one of the numbers to leave the message. This is <INTV NAME>from "The Health Institution"< MR/S FNAME LNAME > we sent you a letter about a study and will call you back soon. Our voice mail number is ……..
The 2nd message should be left in 5 days or the next call after 5 days, or on the 4th day if, in the interviewer's judgment, the situation calls for it. This message gives more information along with the phone number asking the respondent to return the call. It is your choice to include or not include day, date & time.
Leave this message as the 2nd message: This is <INTV NAME>from <SITE/HEALTH PLAN> Mr/s. FName LName we sent you a letter about a study. (Today is DAY/DATE/TIME) We return calls days, evenings and week-ends Please call our voicemail at …….leave your first & last name and several good times to reach you.
(If the situation calls for it go ahead and add thanks for returning our last call, we are sorry we missed you or Sorry we are playing phone tag, we will keep trying Again our Voicemail number is …….. .
3rd Message:
The third message is left after the supervisor/sample manager directs it or, if in the interviewer's judgment, the situation calls for it. The third message always occurs by the 10th call if there is constantly no-answer. If you are unsure of what to do, then wait for the supervisor to direct you when/if to leave the third and final message.
The parameters are to leave a message that encompasses the situation. There is no script.
Talking points for the final message are:

- Leave your name
- The study voice mail number
- Where you are calling from/or on behalf
- Address the person whom we are calling

- Tell them we have been trying to reach them about a study.
- Leave neutral information regarding 'study letter' and ask them to call back with times & numbers where they can be reached
- It is okay to say to a live contact I can call right back and leave this on the voice mail for the respondent if you'd like.
- It is okay to say that this is the only number we have and ask them to call us back with additional numbers and best times to be reached.

People with a Language Barrier

The respondent must understand both the consent and content of the survey. Where English is not the first language, children sometimes serve as translators for the adults in official interactions. We cannot accept a translated consent process. Some research organizations have large enough populations of non-English speaking respondents to go through a translation and re-translation of survey instruments; others choose to complete the interview or intervention with only English-speaking respondents. The study protocol will tell you more specifically how to address language barriers.

People Who are Deceased

Occasionally you may contact a household to find the person you are trying to reach has died. Sometimes the death is recent, but sometimes it occurred in the not-so-recent past. It is wise to express sympathy, indicate you will note this and we will not call again. If you have indicated that you are calling from the health plan, be prepared to answer questions about why you didn't KNOW the respondent was deceased, especially if s/he died at a health plan facility. You might offer, "I do not actually have access to the medical record. Sometimes it takes awhile for this to be recorded in the record. I will note this, and we will not contact you again."

If the respondent was already enrolled in a study, it may be important to the IRB to record when the death occurred so the researcher can take steps to explore if it was related to study participation. In this case, you might offer sympathy, indicate that you don't have access to the medical record, could the person you are talking with please tell you when this happened so you can note it in your records?

Preventing Refusals

A refusal is a person who states that they do not want to do the interview or do not want to participate further. Then, after you have taken the opportunity to explain the importance of the study, probed for concerns and responded to their individual situation, the person still states that they do not want to participate.

First, try to prevent a firm refusal. Figure out why the respondent does not want to do the interview, or why the respondent wants to leave the study, and be prepared with a response that addresses the issue. If the respondent says s/he does not have time, offer that you can call back any time that is convenient, and the interview can be completed in more than one sitting, if needed (and if possible at the respective site or within the study protocol). If the respondent says s/he is too old, offer that it is important that you speak with respondents of all ages.

Before you begin interviewing on any study, try to think of all of the reasons that a reluctant or refusing respondent might give you, and craft a response that is appropriate for each reason. These are often called "recommended responses," and study staff may provide them to you. However, it is important that your responses to the respondent concerns flow naturally from you, so practice them, and be prepared.

Preparation will allow you to speak with confidence about the study. Be yourself! Relax before you make the call, and then assert yourself confidently. Refusals seldom reflect the respondent's feelings about YOU in particular – they usually result because you have called at a bad time, the respondent does not have enough information to make an informed decision, or the respondent just does not participate in surveys. Do not take refusals personally.

If the respondent does not agree to participate immediately, try to end the conversation with, "I'm sorry I caught you at an inconvenient time. Take some time to think about the study."

Seldom must you force a reluctant respondent to make a decision immediately, but rather, allow some time to review the advance letter and think about your conversation, and perhaps a follow up phone call will be in order.

When a potential respondent says s/he is not interested, try to determine if s/he really has enough information about the study. It is legitimate for the respondent to be reluctant to share personal information by phone, so s/he may want to verify the study, verify your identity as an interviewer for [health plan], etc. Be polite, confident, and clear and answer all questions.

No Contacts

If someone says "Do not call back," "Please don't call this number again," or "Take me off your list," be sure to note the verbatim in the notes and inform the study team. If you contact a respondent who tells you:" I am not supposed to be called I was put on your list and they promised me I would not be bothered again," say "I am sorry to have bothered you. I will refer this to the supervisor and we will not call you again."

In-Person Interviewing

There are many similarities between in-person and telephone interviewing. Chiefly, data collection for the study is dependent on your interaction with the respondent. However, in-person interviewing differs from telephone interviewing a few key ways, which are described below.

Attend to the Respondent

Allow enough time to attend to the person in front of you, the interviewee. You should plan so you are not rushed either in reality or having the appearance of being rushed. Organize your study materials before the interviewee is in the room. Become familiar with

your surroundings, where the lights are, how to adjust the blinds, etc…. Know how to direct the respondent to restrooms or drinking fountains if necessary.

Introduce the Study

You can use the basic techniques for introducing the study discussed previously. In addition, you can show the respondent study materials to help convince her to participate. For example, the interviewer can show a copy of the advance letter or a description of the study.

Identification

As an interviewer and representative of the project, you should wear your ID badge with your name, picture, and the institution. The ID shows the respondent, or any other family member, that you are legitimate.

Be Aware of Non-Verbal Cues

Interaction goes beyond voice. Be aware of your own non-verbal communication such as smiling, raising your eyebrows, grimacing, yawning. You will also have the opportunity to monitor the respondent's non-verbal cues. Do they look tired? Are they distracted?

Setting

Once you have introduced yourself, you are ready to begin the interview by first arranging for a proper setting. The following two conditions are important:

1. Avoid an Audience: It may require ingenuity and tact to obtain privacy. The following are some suggested approaches you could use:

Example:

- "Could we go into another room so we can have privacy?"
- "If your husband will excuse us, maybe we could go into another room. The interview will go much faster."
- "It's best if you answer these questions privately. Could we go into another room?"

2. Arrange for Suitable Seating:

The interviewer should suggest sitting at a table, if one is available. Sit across from the respondent—not side-by-side. The respondent's attention will be too divided with trying to read ahead on the questionnaire if they can see it.

Appendix XLII. SC and RA Checklists

The following checklists were created by Ellen Roe at Mission health and may be useful to all NCGENES 2 sites.

Appt Date: _____
Patient Name_____

SC Tasks Prior to Visit 1

_____ Determine patient eligibility
_____ Enter in Tracking System
_____ Sent Introductory Letter
_____ Call parent ~ a week after mailing Intro Letter
_____ If agreeable, proceed with Randomization 1 for Pre-Visit Prep
_____ Add note on appointment in Cerner "NCGENES 2 patient"
_____ Email Paula to note appointment in Allscripts
_____ Send Outlook invite from NCG calendar to Steph and Ellen to block appt time
_____ Note phone call reminder on Outlook calendar 1 week prior to appt
_____ Mail Pre-Visit packet
_____ Phone call reminder ~ 1 week before appt (note: let parent know other reminders will be sent from Genetic Center; we need them there 30 min prior to appt time for study related tasks)
_____ Note NCG patient on 2 white boards in clinic (routinely done every Monday)

SC Task Checklist for Visit 1

_____ Prepare RA bag for visit

(RA will text SC while parent completing post-visit survey)

_____ Discuss Randomization 2 and consent process; mention gift card as a thank you at completion of this visit.
  _____ If not interested, thank them for participation in the pre-visit portion; present gift card and get signature of receipt; give child their gift.
  _____ If interested, proceed with Consent process.
_____ SC completes Consent to Randomization (assent also if applicable)
_____ Randomization completed in Tracking System; parent informed.
  _____ No GS – thank parent and give gift card; get receipt signature; give child gift.
  _____ GS – continue with process for next consent.
_____ SC notifies GC that parent is ready for Consent to GS.
_____ GC completes Consent to GS on laptop (and assent if applicable)
_____ Give parent gift card, get receipt signature, and give child their gift.
_____ Proceed to MCS Lab for blood draw with specimen collection baggie.
  _____ Let phlebotomist know: a) 2 add'l tubes needed for research study OR
    b) blood draw for research study only

_____ Remind MD to complete Post-Visit survey and PhenoTips!

_____ Prepare and mail one tube (with most volume) to UNC
_____ Enter order in Helix and give other tube to accessioning for processing.
_____ Upload audio recording per protocol (to GENERAL Documents/not PT SPEC Documents)
_____ Note upload on file from Maggie (Google doc).

Date: _____

Patient Initials/ID #:_____

NCGENES 2

RA Task Checklist

RA via laptop

Parent via laptop

_____ Set up in consult room; pull patient up in Tracking System.

_____ Greet parent/patient after they are registered and seated; escort to consult room.

_____ Explain what their visit will involve today (pre-visit survey….clinic visit….post-visit survey, information/randomization to next part of study if interested).

_____ Confirm appointment details in Tracking System.

_____ Retrieve Intake Survey (or give them envelope and/or survey to take home)

_____ Record receipt of survey in Tracking System (or whether it was sent home with them).

_____ Copy/paste Survey Code on right side of Tracking screen on the Survey tab screen.

_____ Have parent complete the Pre-Visit Survey on laptop.

_____ Offer iPad to child for entertainment if needed (passcode 121110).

_____ Ask permission for audiotaping; if agreed, get signature in Tracking System.

_____ Remove recorder from bag.

_____ Notify Med Asst that patient is ready; ask what room they will be in.

_____ Place audio recorder in room while Med Asst retrieves patient; turn on recorder; state recorder # and patient ID #; leave in room.

_____ Text/call SC to let her know pre-visit activities have been completed.

_____ When doc in with patient, place post-it note on his computer as a reminder to complete survey and PhenoTips

_____ When clinic visit complete, get info from doc:

_____ Blood draw ordered?

_____ Developmental age of child (only ask if patient is ≥ 7)

_____ Remove audio recorder from room (stop recording) and place back in bag.

_____ Escort parent/patient back to consultation room.

_____ Parent completes Post-Survey visit on laptop while child is occupied with iPad and offered a snack.

_____ **Notify SC when the parent is completing survey; should be ready in a few min.**

_____ **Give SC patient folder and iPad (child may be using iPad)**

_____ Share info with SC that doc provided – along with any feedback or clinic ops information.

_____ Enter Intake Survey answers in Tracking System (if parent brought survey in).

(*This can be done anytime during or after the visit when time allows.)*

Appendix XLIII. CLIA Template for Reporting Positive Results

Diagnostic Testing Report

Confirmatory Testing of Whole Exome Sequencing Analysis

INDICATION FOR TESTING:
The UNC Hospitals Clinical Molecular Genetics Laboratory performed Sanger DNA sequencing analysis to confirm the presence of genetic variants identified through the NCGENES2 research project at the University of North Carolina by massively parallel exome sequencing analysis.

RESULT:
Homozygous/Heterozygous/Hemizygous for a INSERT GENE VARIANT variant/variant of uncertain significance.

OR if KP:
Positive for a homozygous/heterozygous/hemizygous INSERT GENE VARIANT mutation.

INTERPRETATION:

REFERENCES:

COMMENTS:
The nucleotide and protein numbering for the human *XXXXX* gene are according to the current entries for this gene in the NCBI RefSeq database (NM_XXXXX and NP_XXXXXX). The genomic coordinates for the reported variants in the hg38 reference genome are NC_YYYYYYYY

METHOD:
Bi-directional Sanger sequencing of approximately 200 base pairs surrounding the relevant variants was performed using genomic DNA extracted from a peripheral blood or saliva sample.

This test was developed and its performance characteristics determined by the UNC Hospitals Molecular Genetics Laboratory. It has not been approved by the US Food and Drug Administration. However, such approval is not required for clinical implementation. This laboratory is CAP accredited and CLIA certified to perform high complexity testing.