Study Title: Phage-Enabled Mining of Gut Metagenomes for Terpenoid and Carotenoid Metabolism.

NCT Number: Not yet assigned

Document Date: 7/22/2025

NORTH CAROLINA STATE UNIVERSITY

INSTITUTIONAL REVIEW BOARD FOR THE USE OF HUMAN SUBJECTS IN RESEARCH

SUBMISSION FOR NEW STUDIES

Protocol Number 27363

Project Title

PHAGE-ENABLED MINING OF GUT METAGENOMES FOR TERPENOID AND CAROTENOID METABOLISM

IRB File Number:

Original Approval Date:

08/23/2024

Approval Period

07/22/2025 - 07/21/2026

Source of funding (provide name of funder not account number):

National Institutes of Health

NCSU Faculty point of contact for this protocol:NB: only this person has authority to submit the protocol

Nathan Crook: Chemical and Biomolecular Engineering

Does any investigator associated with this project have a significant financial interest in, or other conflict of interest involving, the sponsor of this project? (Answer No if this project is not sponsored)

No

Is this conflict managed with a written management plan, and is the management plan being properly followed?

No

Preliminary Review Determination

Category:

In lay language, briefly describe the purpose of the proposed research and why it is important. Provide a brief synopsis of the study including who is targeted to participate and the data collection methods employed (limit text to 1500 characters)

The purpose of the study is to recover microbes from human fecal material and study them to determine how they interact with carotenoids present in the diet. This is important because while it is known that many microbes live in the human gut, it is not known at a genetic level how or if these microbes can metabolize carotenoids. No study has developed a repository of fungal carotenoid-active enzymes, which is what we intend to do here. To do this, we will collect fecal samples from >30 adult (age 18-65), healthy (BMI < 25, no antibiotics or probiotics in the past 3 months), non-pregnant volunteers. We will determine whether the BMI of our participants is less than 25 through weight and height measurements during enrollment after consent has been obtained. We will administer a food frequency questionnaire to the participants. Then, participants will bring a stool collection device to their home and deposit a sample in the device at their convenience. Subjects will be asked to place the collection device in secondary containment, and put this in a styrofoam box containing single-use cold packs and transport the box to us. With these, our lab will be able to study many important facets of carotenoid biology, including what carotenoids are being produced by gut microbes and their potential impacts on human health.

We received the NIH award and are submitting this amendment to finalize study procedures for IRB review.

Does any member of the project team who is responsible for the design, recruitment, consent, implementation of intervention, interaction with participants, or those handling identifiable private information under this IRB protocol - or any members of their immediate family (defined as spouse, dependent children - have any Significant Financial Interest or other types of conflict of interest (as described in SOP 14.3.a) related to the protocol?

If the answer is "yes," please provide the name of the investigator(s) with the potential or actual conflict and confirm that the relationship has been fully disclosed in the investigators most recent COI disclosure filed with NC State or disclosed through the collaborative research process. If there is a COI management plan in place with NC State University, please upload it with this application to ensure the IRB protocol meets the expectations of the COI plan and the COI is properly considered in the IRB review process. If you are uncertain how to respond or have questions, please contact COI-NOI-Compliance@ncsu.edu.

This research qualifies for Exemption. Review NC State's Exemption Research SOP for studies that may qualify. If you want to apply for an Exemption, download the Exemption Request Form and complete it. To the eIRB, upload the completed Exemption Request Form, all instruments, and if applicable a Data Access and Security Plan and the edited Consent/Opt-Out forms modified to fit the study design. Only complete the "Title" and "Description" tabs in the eIRB, upload the aforementioned documentation, and submit the eIRB application. Do not complete any other tabs within the eIRB system.

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Is this research being conducted by a student?

No

Is this research for a thesis/disseration/capstone?

Yes

Is this research for a dissertatiion?

No

Is this independent research?

No

Is this research for a course?

No

Do you currently intend to use the data for any purpose beyond the fulfillment of the class assignment?

No

Please explain

If so, please explain

If you anticipate additional NCSU-affiliated investigators (other than those listed on the Title tab) may be involved in this research, list them here indicating their name and department.

Abdulkerim Eroglu (Molecular and Structural Biochemistry Department)
Zidan Li (Chemical and Biomolecular Engineering)

Will the investigators be collaborating with researchers at any institutions or organizations outside of NC State?

No

List collaborating institutions and describe the nature of the collaboration. If researchers from both institutions are doing any of the following activities: recruitment, consent process, data collection or handling of identifiable information/specimens a reliance agreement may be appropriate. For more information, please contact irb-coordinator-admin@ncsu.edu

What is NCSU's role in this research?

Describe funding flow, if any (e.g. subcontractors)

Is this international research?

No

Identify the countries involved in this research

An IRB equivalent review for local and cultural context may be necessary for this study. Can you recommend consultants with cultural expertise who may be willing to provide this review? Consultants may not be a part of the research team or have a stake in the research project. Provide email contact information for consultant(s). A local context review may lengthen the time it takes for your approval.

Adults 18 - 64 in the general population?

Yes

NCSU students, faculty or staff?

Yes

Adults age 65 and older?

No

Minors (under age 18--be sure to include provision for parental consent and/or child assent). If minors are included in your research, please read through the NC State University Regulation for your additional responsibilities. Following this regulation is a requirement of your affiliation with NC

State.?

No

List ages or age range:

Could any of the children be "Wards of the State" (a child whose welfare is the responsibility of the state or other agency, institution, or entity)?

No

Please explain:

Does this study involve people who are also incarcerated, involuntarily detained or committed, or are in a program or hospital as an alternative form of sentencing?

No

Pregnant women?

No

Are pregnant women the primary population or focus for this research?

No

Provide rationale for why they are the focus population and describe the risks associated with their involvment as participants

Fetuses?

No

Students?

Yes

Does the research involve normal educational practices?

No

Is the research being conducted in an accepted educational setting?

No

Are participants in a class taught by the principal investigator?

No

Are the research activities part of the required course requirements?

No

Will course credit be offered to participants?

No

Amount of credit?

No

If class credit will be given, list the amount and alternative ways to earn the same amount of credit.Note: the time it takes to gain the same amount of credit by the alternate means should be commensurate with the study task(s)

How will permission to conduct research be obtained from the school or district? IRB approval is not permission to conduct the research. You need to access a gatekeeper. If you are implementing a survey with NC State populations, please make sure you follow the NC State survey regulation.

After NC State IRB approval, we will place advertisements on the NC State campus. Due to this and our inclusion/exclusion criteria, we will likely recruit mostly students. We will not be seeking approval from a gatekeeper beyond the IRB because this research is not part of a class, we are not accessing any student records, and the students themselves will be opting into the research on their own time and of their own free will.

Will you utilize private academic records?

No

Explain the procedures and document permission for accessing these records.

Employees?

Yes

Describe where (in the workplace, out of the workplace) activities will be conducted.

Consent will be obtained and information/study materials will be provided in the 3rd floor conference room of Partners II, which may be in the workplace for some NCSU employees. Participants will collect their stool sample at home. The stool sample will be returned by the participants to 3rd floor conference room for immediate placement in a lab freezer.

From whom and how will permission to conduct research on the employees be obtained?

Once we have IRB approval, employees can choose to self-select into the research. We will not be researching employees while they are at work.

How will potential participants be approached and informed about the research so as to reduce any perceived coercion to participate?

The attached flyer will be posted around campus, and those who email us will be informed further about the study. The flyer is designed to reduce any perceived coercion to participate. The script which will be read to the participants will state that participants are responsible for securing the time needed to participate in the study (if necessary), thereby separating this study from any employment-related activities.

Is the employer involved in the research activities in any way?

No

Please explain:

Will the employer receive any results from the research activities (i.e. reports, recommendations, etc.)?

No

Please explain. How will employee identities be protected in reports provided to employers?

Impaired decision making capacity/Legally incompetent?

No

How will competency be assessed and from whom will you obtain consent?

Mental/emotional/developmental/psychiatric challenges?

No

Identify the challenge and explain the unique risks for this population.

Describe any special provisions necessary for consent and other study activities (e.g., legal guardian for those unable to consent).

People with physical challenges?

No

Identify the challenge and explain the unique risks for this population.

Describe any special provisions necessary for working with this population (e.g., witnesses for the visually impaired).

Economically or educationally disadvantaged?

No

Racial, ethnic, religious and/or other minorities?

No

Non-English speakers?

No

Describe the procedures used to overcome any language barrier.

Will a translator be used?

No

Provide information about the translator (who they are, relation to the community, why you have selected them for use, confidentiality measures being utilized).

Explain the necessity for the use of the vulnerable populations listed.

We are targeting NCSU students and employees for convenience. In order to determine if the BMI requirement is met, we will be measuring the subject's height and weight (fully clothed) during the initial meeting and after consent has been obtained. Further information on this is available in the "procedures" tab.

State how, where, when, and by whom consent will be obtained from each participant group. Identify the type of consent (e.g., written, verbal, electronic, etc.). Label and submit all consent forms. Adult Non-Exempt Consent Form Template Exemption Consent Form Templates

Consent will be obtained via hard-copy forms which have been uploaded. This study includes two levels of consent. The first level allows the Crook Lab to isolate microbial DNA from stool samples, study it, and share the genes encoding carotenoid-active enzymes to other researchers. The second level allows the Crook Lab to store the fecal samples

indefinitely for use in other experiments, including the isolation of microbes, DNA/RNA/protein sequencing and metabolomics. The second level also allows the Crook Lab to share the fecal samples, any extracts derived from the fecal samples (including DNA, RNA, protein, and metabolites), microbes isolated from the fecal samples, and measurement data pertaining to the above material with other researchers. In order to access the fecal samples or their extracts, researchers must obtain a separate IRB approval.

Participants will be asked to meet with a member of the lab in the 3rd floor conference room of Partners II. At this time, participants will be provided with the consent form and the form will be explained to them. If the participant agrees to participate, their signature will be collected, subject metadata will be collected (height, weight, dietary information), a food frequency questionnaire will be administered, and stool sample collection devices will be provided to the participant. If they provide broad consent, their email address and name will also be collected.

If any participants are minors, describe the process for obtaining parental consent and minor's assent (minor's agreement to participate).Parent/Guardian Permission FormMinor Assent Forms

N/A

Are you applying for a waiver of the requirement for consent (no consent information of any kind provided to participants) for any participant group(s) in your study?

No

For each participant group that you are requesting a waiver of consent for, please state what method this waiver is needed for, why it is needed and address each of the above 5 criteria to justify why your study qualifies for a waiver of consent.

Are you applying for an alteration (exclusion of one or more of the specific required elements) of consent for any participant group(s) in your study?

No

Identify which required elements of consent you are altering, describe the participant group(s) for which this waiver will apply, and justify why this waiver is needed.

Are you applying for a waiver of signed consent (consent information is provided, but participant signatures are not collected)? A waiver of signed consent may be granted only if: The research involves no more than minimal riskThe research involves no procedures for which consent is normally required outside of the research context.

No

Would a signed consent document be the only document or record linking the participant to the research?

No

Is there any deception of the human subjects involved in this study?

No

Describe why deception is necessary and describe the debriefing procedures.Does the deception require a waiver or alteration of informed consent information?Describe debriefing and/or disclosure procedures and submit materials for review.Are participants given the option to destroy their data if they do not want to be a part the study after disclosure?

For each participant group please indicate how many individuals from that group will be involved in the research. Estimates or ranges of the numbers of participants are acceptable. Please be aware that participant numbers may affect study risk. If your participation totals differ by 10% from what was originally approved, notify the IRB.

We will involve 60-39 total subjects in this study.

How will potential participants be be found and selected for inclusion in the study?

We will post flyers around centennial and main campus. See attached recruitment material. After consent has been obtained, we will obtain height and weight measurements to compute a BMI. We will also ask the participants to disclose whether their age is between 18 and 65, whether they have taken antibiotics, antifungals, or probiotics in the past three months, and whether are pregnant. If the subjects are between 18 and 65 years of age, the BMI of the subjects is less than 25, and if the subjects have not taken antibiotics, antifungals, or probiotics in the past three months, have not had a diarrheal episode in the prior month, and are not pregnant, then they will be selected for participation in this study.

For each participant group, how will potential participants be approached about the research and invited to participate? Please upload necessary scripts, templates, talking points, flyers, blurbs, and announcements.

We will post flyers around centennial and main campus. See attached recruitment material.

Describe any inclusion and exclusion criteria for your participants and describe why those criteria are necessary (If your study concentrates on a

particular population, you do not need to repeat your description of that population here.) Inclusion and exclusion criteria should be reflected in all of your recruitment materials and consent forms.

We wish to obtain fecal samples from healthy adults (18-65). A BMI of less than 25 broadly indicates a non-overweight individual, and so is consistent with our interest in healthy individuals. Furthermore, probiotics, antibiotics, and antifungals can alter the composition of the microbiota, and so we do not wish to study subjects which have taken these within the past 3 months. Diarrhea is also indicative of an abnormal microbiota state, and so we do not wish to study individuals which have recently had a diarrheal episode. Finally, we do not wish to study pregnant individuals.

Is there any relationship between researcher and participants - such as teacher/student; employer/employee?

No

What is the justification for using this participant group instead of an unrelated participant group? Please outline the steps taken to migitate risks to participants from the pre-existing relationship, including power dynamics of this relationship and/or perceived coercion.

Describe any risks associated with conducting your research with a related participant group.

Describe how this relationship will be managed to reduce risk during the research.

How will risks to confidentiality be managed?

Address any concerns regarding data quality (e.g. non-candid responses) that could result from this relationship.

In the following questions describe in lay terms all study procedures that will be experienced by each group of participants in this study.For each group of participants in your study, provide a step-by-step description of what they will experience from beginning to end of the study activities. Should you prefer, you can upload a detailed study procedure packet and refer us to that document in this text box. If you choose to upload a procedures packet, do not discuss procedures in the below text box.

Subjects will be asked to do all of the following:

1.Come to the 3rd floor conference room of Partners II at NCSU.

2.A member of the research team of your gender will read and explain the consent form and broad consent addendum.

We will note that in addition to isolating individual microbes from these samples, a portion of the fecal sample will be archived for follow-on analysis at a future date. These analyses could include recovery of additional bacteria, archaea, fungi, and/or viruses which infect these microbes. Analyses could also include analysis of DNA sequences, RNA sequences, proteins, or metabolites present in these fecal samples. We will note, however, that while measurement techniques for DNA, RNA, and proteins can also recover DNA, RNA, and proteins derived from the subject, it is possible to delete subject-derived data in an automated fashion, which we will do. Under no circumstances will we attempt to re-identify subjects from these samples, nor will we release data that we believe enables subject re-identification, nor will we release fecal samples to researchers who do not agree to the same.

If the participants agree to the terms in this form and wish to participate in the study, they will sign the consent form (and maybe the broad consent addendum). Next, a member of the research team of your gender will obtain the subject's height and weight fully-clothed. The conference room is a private space and two members of the research team will be present at all times during this meeting. Next, we will ask you about the subject's age, any dietary restrictions you follow, and whether you have eaten certain foods or medications recently. The subject will be administered a food frequency questionnaire. Next, the subject will receive sample collection materials. These materials will consist of a stool collection device, secondary containment, cold packs, a Styrofoam box and a pair of gloves. We expect this meeting to last less than 30 minutes.

3.At home, place cold packs in freezer at least 8 hours before sample collection (less than 1 minute).

4.When ready to provide a stool sample, remove the specimen collection container from its packaging. Lift the toilet seat and place the collection container on the rim of the toilet bowl (less than 1 minute).

5.After the stool has been provided, put on the provided gloves and place the included cap on the collection container. Avoid direct contact with stool (2 minutes).

6.If you do touch your own stool with your skin, wash the area for at least 2 minutes with warm, soapy water.

7.Place the stool-containing collection container in the secondary container. Remove your gloves and thoroughly wash your hands, then place frozen cold packs and the secondary container into the provided styrofoam box (2 minutes).

8.Notify us via email (zli49@ncsu.edu) when you are ready to transport the samples back to NCSU (2 minutes).

9.Bring the box back to the 3rd floor conference room of Partners II during normal business hours (8 am to 5 pm Monday-Friday).
10.A member of the research team will be present to receive the stool samples, ask follow-up dietary questions, and provide compensation. We expect that this process will take less than 10 minutes.

Are you requesting the use of secondary information to be used as data for this research project? The secondary information can either currently exist or be generated in the future.

Discuss the following: permission to access the information (direct permission from the participant or records release), how researchers will access, transfer, store, and destroy the data.
Discuss the identifiable/re-identifiable nature of the data through either direct IDs, indirect IDs, or triangulation of datasets, data points, researcher access/expertise, or analysis. .
List all data categories to be requested (ex: age, race, student ID, GPA, ACT, Medical ID, diagnosis).
Discuss if the data requires a Data Use Agreement.
Discuss if the data are subject to FERPA, HIPAA, or the GDPR.

No existing information or secondary data will be used for this research project. All data necessary for this project will be collected as part of this research project.

Social/Reputational?
No

Psychological/Emotional?
No

Financial/Employability?
No

Legal?
No

Physical?
No

Academic (affect grades, graduation)?
No

Employment (affect job)?
Yes

Financial (affect financial welfare)?
No

Medical (harm to treatment)?
No

Insurability (harm to eligibility)?
Yes

Legal (reveals unlawful behavior)?
Yes

Private behavior (harm to relationships/reputation)?
Yes

Religious Issues/Beliefs?
No

Describe the nature and degree of risk that this study poses. Describe the steps taken to minimize these risks. You CANNOT leave this blank, say 'N/A', none' or 'no risks'. You can say "There is minimal risk associated with this research." For each 'Yes' selected above, describe the probability of the risk occuring and the magnitude of harm should the risk occur. Discuss how you are mitigating those risks through participant selection, study design, and data security.
There is minimal risk to this research currently.
For all subjects, there is a risk that their skin can contact their stool sample during collection. If this occurs, participants are instructed to wash the affected area with warm soapy water for 2 minutes.

While not possible currently, if it becomes possible in the future to identify the subjects to which these samples are

derived, the following risks are possible with all participants and particularly with participants who agree to broad consent. Participants who agree to broad consent could also be re-identified if the excel sheet linking participant IDs to their name/email is breached.

Employment: Human and microbial genetic information has been linked to the human health status, including predisposition to certain diseases. If prospective employers know the health status of a job applicant, they could use this information to discriminate between potential hires.

Insurability: For the reasons described above in Employment, prospective insurance providers may deny coverage, or charge higher rates, for individuals with certain health conditions.

Legal: The abundance and types of microbes present in the gut have been linked to the activities that their human host participates in. While not possible currently, in the future it might be possible to predict whether an individual has engaged in illegal activity (drug use, for example) based on DNA data from stool samples.

Private behavior: Gut microbes have been shown to be more similar between individuals in close proximity, such as family members. While not possible currently, in the future it might be possible to predict whether two individuals have cohabitated based on DNA data from stool samples. Furthermore, the health and illegal activity data mentioned above, if technology progresses to the extent that this data becomes available, has the potential to harm an individual's relationships or reputation.

These risks have been put forth in the informed consent document prior to participants signing the broad consent.

If you are accessing private records, describe how you are gaining access to these records, what information you need from the records, and how you will receive/record data. Private records may include: educational, medical, financial, employment. Some of these private records may be subject to laws such as FERPA and HIPAA. Your content here should match what you've discussed on the procedures tab.

We will not be accessing private records.

Are you asking participants to disclose information about other individuals (e.g., friends, family, co-workers, etc.)?

No

You have indicated that you will ask participants to disclose information about other individuals (see Populations tab). Describe the data you will collect and discuss how you will protect confidentiality and the privacy of these third-party individuals.

If you are collecting information that participants might consider personal or sensitive or that if revealed might cause embarrassment, harm to reputation or could reasonably place the subjects at risk of criminal or civil liability, what measures will you take to protect participants from those risks?

For participants that do not give broad consent, unique random numbers will be assigned to each subject (Subject 1, Subject 2, etc) and no links between a subject's identity and either their biospecimens, data, or metadata will be saved. Stool samples contain human DNA, which while not sufficient to identify an individual currently, may be sufficient in the future.

For participants that do give broad consent, the above applies, and in addition they could be re-identified if the excel sheet linking their name to their sample is breached. We will safeguard against this possibility by encrypting the excel file and keeping it on a university owned computer to which only Dr. Crook has access.

If any of the study procedures could be considered risky in and of themselves (e.g. study procedures involving upsetting questions, stressful situations, physical risks, etc.) what measures will you take to protect participants from those risks?

There is a risk that participants skin may contact their own stool sample. If this occurs, participants will be instructed to wash the affected area with warm soapy water for 2 minutes.

Describe the anticipated direct benefits to be gained by each group of participants in this study (compensation is not a direct benefit).

None

If no direct benefit is expected for participants describe any indirect benefits that may be expected, such as to the scientific community or to society.

The scientific community will gain an increased understanding of how gut microbes interact with dietary carotenoids, which could be used to design health supplements in the future.

Will you be receiving already existing data without identifiers for this study?

No

Will you be receiving already existing data which includes identifiers for this study?

No

Describe how the benefits balance out the risks of this study.

Will data be collected in a way that would not allow you to link any identifying information to a participant?

No

Will any identifying information be recorded with the data (ex: name, phone number, IDs, e-mails, etc.)?

Yes

Will you use a master list, crosswalk, or other means of linking a participant's identity to the data?

Yes

Will it be possible to identify a participant indirectly from the data collected (i.e. indirect identification from demographic information)?

No

Audio recordings?

No

Video recordings?

No

Images?

No

Digital/electronic files?

Yes

Paper documents (including notes and journals)?

Yes

Physiological Responses?

No

Online survey?

No

Restricted Access (who, what, when, where)?

Yes

Password Protection (files, folders, drives, workstations)?

Yes

Suggestion of anonymous browsing?

No

Locks (office, desks, cabinets, briefcases)?

Yes

VPN (transfer, upload, download, access)?

No

Encryption (files, folders, drives)?

Yes

Describe all participant identifiers that will be collected from each data collection method (surveys, interviews, focus groups, existing data, background data collected via host site or software). Discuss why it is necessary to record identifiers at all and describe the deidentifying process

We will collect the signature of the participants for the consent process, and we will record that the participants meet the study criteria. We will also correspond with the subjects via email to coordinate obtaining consent and delivery of the samples to the lab.

For participants that do not give broad consent:
When we record the subject's metadata (age, gender, height, weight, dietary info, and what foods they have recently eaten), we will assign this data to a random number, and we will not record any links between the signed consent forms and this metadata. Recording of this metadata is necessary because these variables have been shown in prior work to impact microbiota composition.

For participants that do give broad consent:
We will do the above, but we will keep a list that maps participant IDs to their name and email address. This allows the participant to withdraw their consent at a later date.

If recording identifiable information about participants, discuss any links between the data and the participants and why you need to retain them. Discuss destruction of links or removal of identifers.

Signed consent forms will be stored in a locked drawer of the the PI's (Nathan Crook's) office. Email correspondence with subjects will be performed only using ncsu gmail systems, and upon sample deposition all emails with that subject will be permanently deleted within 24 hours. Upon obtaining consent, a random subject identifier will be assigned to that subject. The subject's metadata and subject number will be input into an encrypted excel sheet on a university-owned computer. This excel sheet will also include who we have shared the sample with, and their IRB approval information (if the subject has given broad consent). The subject will be provided a cup which contains the subject's identifier written on it. After sample return, these labeled cups will be placed in our freezer.

For those that have given broad consent, we will additionally keep an encrypted excel sheet on a university owned computer that links their participant ID to their name and email address. This is necessary to destroy a participant's samples if they withdraw their broad consent at a later date.

Discuss if you'll be working with your departmental IT to create a data management plan and if you're using NC State managed devices, NC State Google Drive or other NC State non-networked device. If using a personal device, discuss data protection.

We will be using NC State-managed devices to store all data, including desktop or laptop computers and computing clusters (BRC Cluster). We have confirmed with our departmental IT that encryption options are available for these computers. All data relating to these samples will be stored in encrypted folders on the desktops/laptops, or on encrypted drives on the BRC cluster. We have worked with our departmental IT to create a data management plan.

If Dr. Crook moves to a different academic institution, then he will follow NC State's regulations following data sharing and transfer.

All biospecimens will be handled and stored in accordance with IBC.

Describe any ways that participants themselves or third parties discussed by participants could be identified indirectly from the data collected, and describe measures taken to protect identities. (Data can be reidentified by researcher access, technology employed, researcher expertise, and triangulation of data or other information. Discuss the probability of reidentification and the magnitude of harm to participants should the data be reidentified. Discuss the probability of reidentification occuring and the magnitude of harm should it occur).

Human DNA, RNA, and protein is present in fecal samples. During whole-metagenome sequencing, whole-metatranscriptome sequencing, or whole metaproteome sequencing of feces, some data will be derived from the subject and could potentially reveal the subject's ethnicity and gender. However, while it is not currently possible to identify a subject using this data alone, future technological developments could potentially enable subject re-identification from human or microbial genomic data. To safeguard against subject re-identification, all human reads will be identified computationally and deleted after DNA sequencing.

Subject age, gender, height, weight, and dietary info alone are not sufficient to identify a subject without additional information. If, however, other databases containing subject identifiers and this information is breached, then the metadata associated with these samples might be sufficient to link these samples to individuals.

If the excel sheet linking names/emails to IDs is breached, then those that have given broad consent may be linked to their samples.

If subjects are linked to the data present in these samples, the risks to the subjects as a result of this research include:

â€¢Employment: Human and microbial genetic information has been linked to the human health status, including predisposition to certain diseases. If prospective employers know the health status of a job applicant, they could use this information to discriminate between potential hires.

â€¢Insurability: For the reasons described above in Employment, prospective insurance providers may deny coverage, or charge higher rates, for individuals with certain health conditions.

â€¢Legal: The abundance and types of microbes present in the gut have been linked to the activities that their human host participates in. While not possible currently, in the future it might be possible to predict whether an individual has

engaged in illegal activity (drug use, for example) based on DNA data from stool samples.

â€¢Private behavior: Gut microbes have been shown to be more similar between individuals in close proximity, such as family members. While not possible currently, in the future it might be possible to predict whether two individuals have cohabitated based on DNA data from stool samples. Furthermore, the health and illegal activity data mentioned above, if technology progresses to the extent that this data becomes available, has the potential to harm an individual's relationships or reputation.

Subjects who have provided either general or broad consent have been informed of the risks associated with re-identifiability of stool samples from the human DNA present within, and have agreed to them.

For all recordings of any type:Describe the type of recording(s) to be made Describe the safe storage of recordings Who will have access to the recordings? Will recordings be used in publications or data reporting? Will images be altered to de-identify?Will recordings be transcribed and by whom?

No recordings will be made.

Describe how data will be reported (aggregate, individual responses, use of direct quotes) and describe how identities will be protected in study reports. Reporting data may sometimes reidentify your participants. If needed, you can adjust how you report your data to protect the identities of your participants. Discuss.

Upon consent, each subject will be assigned a random number identifier. All data about that subject will be recorded and reported in terms of this number.

Data will consist of microbial isolates or their genomes from the fecal samples, as well as raw metagenome, metatranscriptome, or metaproteome sequences from fecal samples. No human identifiable material is expected to be present in microbial isolates or their genomes, and all metagenome reads will have human reads removed as described above. Data on these DNA/RNA/protein sequences or the properties of the microbial isolates will be published in de-identified format. For example, subject 1, subject 2, etc.

Data also consists of subject metadata, which includes whether they met the inclusion criteria at the time of the study, age, weight, height, gender, date of sample deposition, dietary information, and what foods the subject has recently consumed. The subject's age, weight, height, and gender will be reported upon publishing the data in a scientific journal and deposition of the sequence data into public repositories. Other metadata may be reported to other researchers, upon request. A subject's identity will not be able to be deduced from this metadata.

Will anyone besides the PI or the research team have access to the data (including completed surveys) from the moment they are collected until they are destroyed? This includes sharing data with sponsors, journals, or using the data for future research endeavors. If you are sharing the data, this should be in your consent form.

Yes.

Microbial isolates and the genetic information from microbial isolates will be deposited in public repositories or shared with other researchers or commercial entities upon request, provided a material transfer agreement is in place.

Sequencing data (including DNA, RNA, protein, and metabolite, with human data removed) will be placed in a public repository, or shared with other researchers upon request, provided a material transfer agreement is in place.

Any human fecal samples, or physical samples derived from human feces, including DNA, RNA, protein, and metabolites, will be shared with other researchers upon request provided that the researcher has obtained IRB approval to use the samples. If a subject who has provided broad consent decides to withdraw this consent at a later date, we will instruct other investigators to destroy these samples.

IRB approval will be tracked by Dr. Crook, who will verify that IRB approval has been obtained for each requesting researcher, and copies of the approved IRB protocol will be stored by Dr. Crook.

We will keep the fecal samples and any physical samples derived from them (e.g. DNA, RNA, proteins, and metabolites) in refrigerators and freezers in our lab for one year (if limited consent is given) or until they have been completely used/indefinitely (if broad consent has been given). We will keep microbial isolates from these fecal samples in our

laboratory refrigerators and freezers indefinitely. We have noted in our consent form that researchers with access to stool or extracted DNA will also have access to the subject's DNA. While this information is currently insufficient to identify the subject, subject identification from microbial or human DNA might be possible in the future. We will have informed participants who provide us with broad consent of that reality.

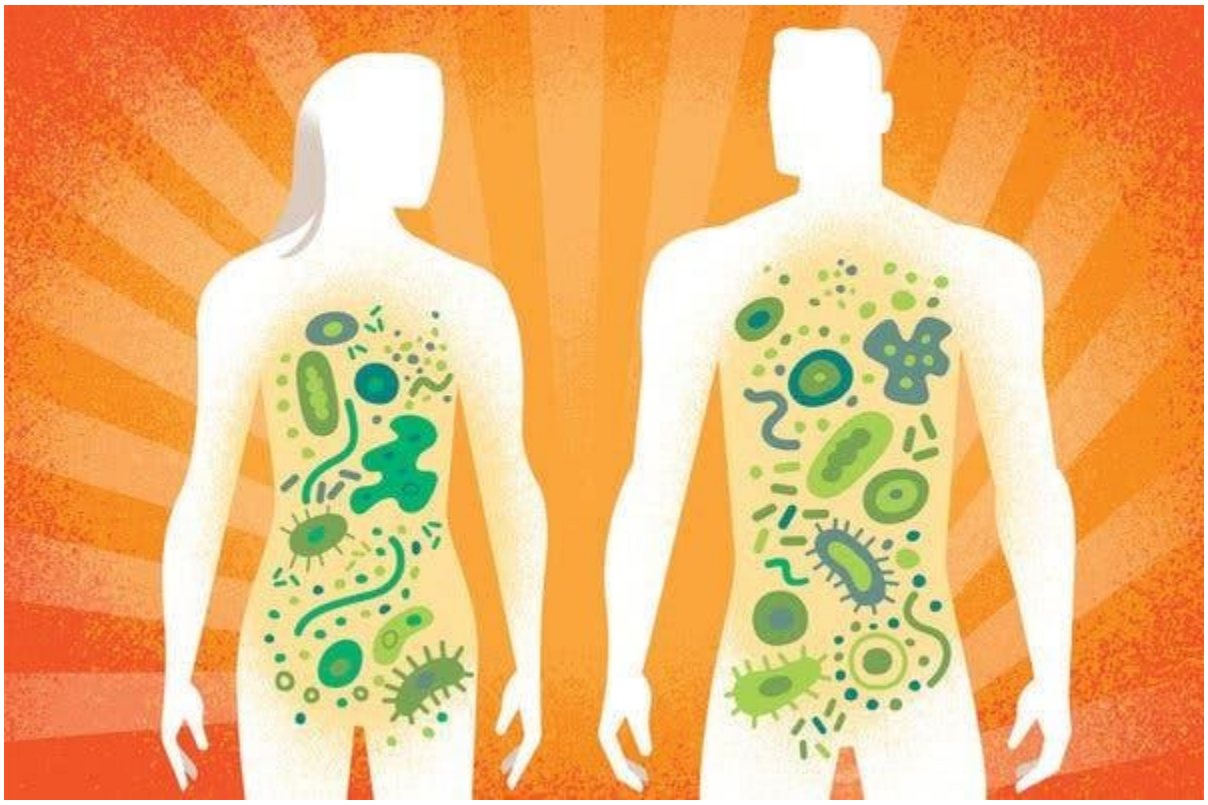
Describe any compensation that participants will be eligible to receive, including what the compensation is, any eligibility requirements for that compensation, and how that compensation will be delivered. Examples of compensation include: monetary compensation, research credits, raffle/drawing, novel items. Make sure to check with your department regarding issues of tracking payments as your department accounting office may have requirements that affect your human subjects privacy (such as the mandatory tracking of anyone who receives compensation). This tracking may influence the confidentiality/anonymity of your research and must be addressed in this application.

Participants which provide a fecal sample will be provided a physical $20 amazon gift card at the same time as the sample is delivered.

Explain compensation provisions if the participant withdraws prior to completion of the study.

Participants may keep the study materials provided to them at the start of the study, if they choose to withdraw before providing a sample. No gift card will be provided in this case.

Subjects Needed for Human Microbiome Research!



We are looking for individuals who:

- Are between the ages of 18 and 65,
- Have a body mass index of less than 25 (see https://bit.ly/2NxNKH6 for how to compute your BMI),
- Have not taken probiotic supplements, antibiotics, or antifungals in the past 3 months,
- Have not had a diarrheal episode in the past month, and
- Are not currently pregnant.

If you meet these criteria, we need your stool! Please email zli49@ncsu.edu for more information.

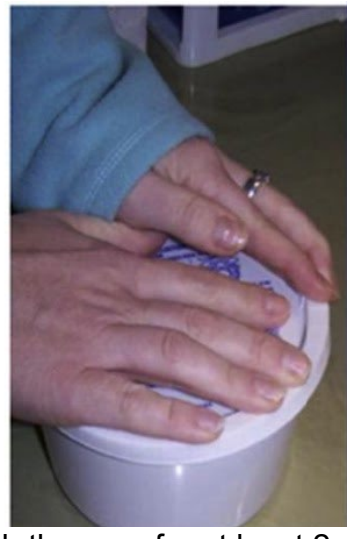
Study participants will receive a $60 Amazon gift card for donating a stool sample.

Participant Instructions (Last updated 5/9/2025)

1. At home, place cold packs in freezer at least 8 hours before sample collection
2. Since cold packs only last 24 hours, please do not collect your sample on a Friday or Saturday because you won't be able to reach us on the weekend.
3. When ready to provide a stool sample, remove the specimen collection container from its packaging. Lift the toilet seat and place the collection container on the rim of the toilet bowl.

4. After the stool has been provided, put on the provided gloves (note the individual in the picture is not wearing gloves) and place the included cap on the collection container. Avoid direct contact with stool.





5. If you do touch your own stool with your skin, wash the area for at least 2 minutes with warm, soapy water.
6. Place the stool-containing collection container in the secondary container. Remove your gloves and thoroughly wash your hands, then place frozen cold packs and the secondary container into the provided styrofoam box.

7. Notify us via email (zli49@ncsu.edu) when you are ready to transport the samples back to NCSU.
8. Bring the box back to the 3rd floor conference room of Partners II during normal business hours (8 am to 5 pm Monday-Friday).
9. A member of the research team will be present to receive the stool samples, ask follow-up dietary questions, and provide compensation. We expect that this process will take less than 10 minutes.