

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

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Informed Consent Form for Participation in Research

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

IRB Protocol: 27363

Principal Investigator(s): Nathan Crook, nccrook@ncsu.edu, 919-513-2429

Funding Source: National Institutes of Health

Collaborating Researchers: Abdulkерim Eroglu, North Carolina State University, aeroglu@ncsu.edu, 704-250-5455

You are invited to take part in a research study. Here are some important things to know:

- Your participation in this study is voluntary. You can choose not to participate without penalty. If you decide to participate and change your mind, you can stop participating at any time without penalty.
- The purpose of this research study is to gain a better understanding of how microbes interact with the food we eat.
- You will be asked to donate a stool sample. The estimated time to perform the study is 50 minutes.
- You are not guaranteed any personal benefits from being in this study. Research studies may pose risks to those who participate.
- You may want to participate in this research because you wish to advance our understanding of gut microbes. You may not want to participate in this research because you do not want others to study your stool, or you are uncomfortable with the risk that future technologies could identify you based on the microbes present in your stool sample.
- If you have questions about your participation in this research at any time, do not hesitate to contact the researcher(s) named above.
- If you have questions about your rights as a research participant, do not hesitate to contact the NC State IRB office via email at IRB-Director@ncsu.edu or via phone at 1-919-515-8754.

Please read the rest of this consent form for more specific details of this research. If you do not understand something, please ask the researcher for clarification or more information.

What do some of these terms below mean?

Throughout this consent form there are some repeated words or phrases being used that you might not be familiar with and you may ask the researcher for any additional clarification.

- **Bio-specimen:** This is any feces that has been collected from you for this research study.
- **Microbe:** A microscopic organism, including members of the fungi, bacteria, archaea, or virus groups.
- **DNA:** The molecules that contain your unique genetic code, as well as the genetic code of the microbes living in your body.
- **RNA:** The molecules which contain the instructions that a cell follows to make proteins (see below).
- **Proteins:** The molecules which are produced by the cell using the instructions in RNA. These molecules can perform different tasks in the cell, including the production of metabolites (see below), DNA, RNA, or other proteins.

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- **Metabolites:** These are the molecules (excluding DNA, RNA, and Proteins) that cells make.
- **De-identified.** De-identified data is information or bio-specimen(s) that at one time could directly identify you, but that we have recorded so that your identity is separated from the data. We will not collect or record your real name; only your signature will be retained as evidence of your consent. As a result, there is no master list linking your identity to the research data. The data made available to the public or other researchers will remain fully de-identified. However, please be aware that future technological advancements may potentially increase the risk of re-identification, even when names are not collected.

What is the purpose of this study?

The purpose of the study is to recover microbial DNA from human fecal material and study it to determine if it contains genes that allow microbes to modify carotenoids, which are antioxidant molecules contained in many red and orange foods.

How many people will be in the study?

There will be approximately 39-60 participants in this study.

Am I eligible to be a participant in this study?

In order to be a participant in this study, you must agree to be in the study and be between 18 and 65 years of age.

You cannot participate in this study if you do not want to be in the study or if you have taken antibiotics, antifungals, or probiotic supplements in the past three months. Probiotics consumed as a part of food material is OK. You cannot participate in this study if you have had a diarrheal episode in the previous month. You also cannot participate in this study if your body mass index (BMI) is over 25, or if you know you are pregnant.

What will happen if you take part in the study?

If you agree to participate in this study, you 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 this consent form to you. If you agree to the terms in this form and wish to participate in the study, you will sign the consent form. Next, a member of the research team of your gender will obtain your height and weight fully-clothed. The conference room is a private space and two members of the research team will be present with you at all times during this meeting. Next, we will ask you about your age, any dietary restrictions you follow, and whether you have eaten certain foods or taken certain medications recently. Next, you will receive sample collection materials. These materials will consist of a stool collection device, secondary containment, cold packs 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.
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.
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.
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.
8. Notify us via email (zli49@ncsu.edu) when you are ready to transport the samples back to NCSU.
9. Bring the sample 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.

The total amount of time that you will be participating in this study is 50 minutes.

Benefits to participating in this research

There are no direct benefits to your participation in the research. The indirect benefits are an increased understanding of how microbes live in the gut by the scientific community, which could be used to design better probiotic therapies.

Risks to participating in this research

The risks to you, as a result of participating in this research, include:

1. Contact with your own stool sample.
2. Re-identification of you via the microbes present in your stool sample.

These risks are mitigated through:

1. Washing the contacted area with warm soapy water for 2 minutes.
2. The inability of current technologies to link the microbes present in your samples to you as an individual. However, future technological advances may allow this to happen. If you are linked to the data present in these samples, the risks to you as a result of this research include:
 - Employment: 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 the microbes present in 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 the microbes found in 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.

3. If you provide broad consent, we will store information linking your samples to your name and email address so that, if you choose to withdraw your consent in the future, we can destroy your samples. This information will be stored in an encrypted file to prevent unauthorized access.

Emergency medical treatment

If you are hurt or injured during the study session(s), the researcher will call 911 for necessary care. There is no provision for compensation or free medical care for you if you are injured as a result of this study.

What data will be collected about me and are there risks associated with that?

The data that are collected about you include the DNA, RNA, microbes, and metabolites present in your fecal material. These data are not connected to your name or other information that could easily identify you. The risks to you as a result of collecting this information include risks to employment, insurability, legal, and private behavior, as described above. These risks will be mitigated through implementing data protections in accordance with NC State data protection standards. Additionally, we note that in the process of collecting fecal DNA, RNA, and proteins, it is possible to also recover DNA, RNA, and proteins from your own body. We will use automated computer programs to remove any DNA or RNA data that is human-derived, thus removing your unique genome sequence from the data we collect and only keeping the microbial data.

Note: if you provide broad consent then your name will be directly linked to the samples you provide. There is a small risk that the file containing the linking information could be breached.

How will my identity and the data about me be stored and protected?

If you do not provide broad consent: After all data is collected, the researchers will go through the data and remove all direct and indirect identifiers so that the dataset can no longer be connected to your identity. Your samples will be recorded as coming from Subject 1, Subject 2, etc.

If you provide broad consent: we will still assign your samples a number, but we will also have a file linking that ID to your name in case you decide to withdraw your consent at a later date.

Who can access my data and how will my data be shared and used in the future?

Because this study is funded by the NIH, data about you including the DNA and RNA present in your stool samples will be shared with the larger research community. Your data will not be directly identifiable, but we cannot guarantee anonymity now or in the future.

Additional analysis: To help maximize the benefits of your participation in this project, by further contributing to science and our community, the microbes present in your stool will be saved for future research. These analyses could include:

- Sequencing the genomes of these microbes
- Measuring the behavior of these microbes in the lab and in the gut of research animals

General storage: Your biospecimens and the microbes we extract from them will be stored in freezers located at 840 Main Campus Dr, Room 2109, Raleigh NC 27606, which is locked. If Dr. Crook moves to a different laboratory at NCSU, or a different academic institution, he will follow NC State's regulations following data sharing and transfer. For current information as to the location of the Crook Lab, you can email crook.nathanc@gmail.com. Stool samples will be destroyed within one year of collection.

Microbe sharing: Microbes from your stool may be shared with other people without additional consent from you, and may be placed in a public microbial repository.

Microbe storage: Microbes from your stool, as well as DNA extracted from these isolated microbes, will be stored in our laboratory freezers and refrigerators indefinitely.

Data sharing and storage: Your de-identified data, including metadata and DNA/RNA/protein sequences from the microbes we recover, may be included in scientific publications and/or presentations. This data will be stored indefinitely on University-owned computer clusters, which are password-protected and encrypted. This data may also be stored in public databases, such as the National Center for Biotechnology Information, Data Dryad, ProteomeXchange, and/or Github, which are public databases.

Re-identifiability: The data that will be shared about you is de-identified, but in the near future it may be possible to re-identify you from the DNA contained in your fecal sample. However, we aren't seeking to re-identify you and we will not keep your stool sample for more than one year.

How will the data about me be reported to the public and are there risks associated with that?

We may share specific genes contained in your stool microbes in our publications and presentations but we will not include your name or any other information that could easily identify you. As a result, there are minimal risks to you as a result of how we report the data.

Right to withdraw your participation

Your participation is voluntary. Even if you agree initially, consent is an ongoing process. You can stop participating at any time for any reason. To do so, tell the researcher you are interacting with. You can contact the faculty advisor for this research, Nathan Crook, at nccrook@ncsu.edu and 919-513-2429.

If you withdraw, we will stop any procedures or data collection that may be happening. We will also delete any data that's already been collected from you whenever possible. We will not be able to delete your data if we cannot identify which responses are yours (e.g. if you do not provide broad consent) or if the data has already been published. Data will be de-identified after collection. After this point, we may not be able to identify which samples are yours.

Compensation

For your participation in this study, you will receive a \$60 Amazon gift card.

If you withdraw from the study prior to its completion, you will be allowed to keep any materials you have been given.

What if you are a student?

Your participation in this study is not a course requirement. Your participation or lack thereof will not affect your class standing, grades, or relationship with your instructors or advisors.

What if you are an employee?

Your participation in this study is not a requirement of your employment. Your participation or lack thereof will not affect your job.

Sponsorship and funding

This research is funded by the National Institutes of Health. This means that the sponsor is paying the research team for completing the research. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study. If you would like more information, please ask the researcher(s) listed at the top of this form about the funding and sponsorship.

What if you have questions about this study?

If you have questions at any time about the study itself or the procedures implemented in this study, you may contact the faculty advisor for this research, Nathan Crook, at nccrook@ncsu.edu and 919-513-2429.

What if you have questions about your rights as a research participant?

If you feel you have not been treated according to the descriptions in this form, or your rights as a participant in research have been violated during the course of this project, you may contact the NC State IRB (Institutional Review Board) office. An IRB office helps participants if they have any issues regarding research activities. You can contact the NC State University IRB office at IRB-Director@ncsu.edu, 919-515-8754, or [fill out a confidential form online](https://research.ncsu.edu/administration/participant-concern-and-complaint-form/) at <https://research.ncsu.edu/administration/participant-concern-and-complaint-form/>

Consent to participate

By signing this consent form, I am affirming that I have read the above information. All of the questions that I had about this research have been answered. If I consent to participate, I understand that I can stop participating at any time without penalty or loss of benefits to which I am otherwise entitled. I am aware that I may revoke my consent at any time.

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Yes, I want to be in this research study.

Name _____ Today's Date _____

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No, I do not want to be in this research study.

Thank you for your consideration.

Participant Broad Consent Form

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

IRB Protocol: 27363

Principal Investigator(s): Nathan Crook, nccrook@ncsu.edu, 919-513-2429

Funding Source: National Institutes of Health

Collaborating Researchers: Abdulkemir Eroglu, North Carolina State University, aeroglu@ncsu.edu, 704-250-5455

In this study, we are collecting data, including your fecal biospecimen samples, that are considered identifiable and private. Your data and samples are identifiable because they contain your genomic DNA. They are considered identifiable even though they do not include your name and other direct identifiers. We are unable to remove every piece of information that would allow your identity to be figured out.

We would like to keep, use, and share these identifiable, private data and samples for future unspecified research. The specific research is currently unknown and could be unrelated to the purposes of the current study. This is why we need to ask you for additional consent, called “broad consent.”

Please know that you do not have to provide broad consent in order to be in the original study. Your decision will not affect your participation or compensation in the current study. It is not a condition of your enrollment or employment at any institution. Providing broad consent is completely optional and there is no personal benefit for you. You may indirectly benefit as your data will be used for research that can support or improve many areas of life.

If you provide broad consent:

We will do the following with your identifiable, private data:

- Store a link between your data, samples, and your name and email address.
- Store and maintain your data and samples (for an indefinite period of time)
- Use your data and samples for future research purposes (for an indefinite period of time)
- Share your data and samples with other researchers or entities for their research use. The other entities may include research, academic, and medical institutions, Drug and device companies, Biotechnology companies, and others

Your data and samples will be used for medical, scientific, and other research. However, you will not be told the purposes, results, or any other details of the specific research studies that will use your data and samples. You will also not be asked for consent each time your data and samples are used or shared. This means your data and samples could be used for studies that you may not have consented to if you were asked.

Your data and samples may also be used to create products or to deliver services. These products or services may be sold and/or make money for others. If this happens, there are no plans to tell you, pay you, or give any compensation to you or your family.

The main risks in providing broad consent are that in the future, you may not agree with how your data and samples will be used, your identity could be figured out, and your data and samples could

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be accessed by unintended parties. We note that in the process of collecting fecal DNA, RNA, and proteins, it is possible to also recover DNA, RNA, and proteins from your own body. We will use automated computer programs to remove any DNA or RNA data that is human-derived, thus removing your unique genome sequence from the data we collect and only keeping the microbial data. We will manage who can access your data and samples, and store it using regularly updated security plans. However, we will be unable to control what happens to the data and samples after we share it with others.

If you don't provide broad consent or complete this form:

We will not store, use, or share the identifiable, private data and samples collected from you beyond the purposes stated in the previous consent form that you agreed to and signed for the "Phage-Enabled Mining of Gut Metagenomes for Terpenoid and Carotenoid Metabolism" study. If you do not complete this form, we will assume you are not providing broad consent.

If you want to withdraw your broad consent

Please contact the researcher, Nathan Crook at nccrook@ncsu.edu and 919-513-2429. You can also contact the faculty advisor for this research, Nathan Crook at nccrook@ncsu.edu and 919-513-2429.

If you want to withdraw your broad consent and have your permission revoked for the use of your data and samples for unspecified future research, we will delete your data from our records and destroy the samples so that we can no longer use them for future research. If your data and samples have been shared with others for other research studies, we will ask them to destroy your data and samples as well.

If you have questions:

Please ask the research team to explain anything in this form that you do not clearly understand. Please think about this broad consent, ask questions, and/or discuss it with family or friends before making the decision to say "Yes" or "No" to broad consent.

If you want to discuss your rights as a person who has agreed to, refused, or declined to respond to an offer of broad consent or believe that your rights were violated as a result of your agreeing to this broad consent, please contact the NC State IRB Director at IRB-Director@ncsu.edu, 919-515-8754, or [fill out a confidential form online](https://research.ncsu.edu/administration/participant-concern-and-complaint-form/) at <https://research.ncsu.edu/administration/participant-concern-and-complaint-form/>.

Please select one option and provide your name and today's date

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Yes, I would like to provide broad consent. My identifiable, private information and biospecimen samples can be kept and used for future research studies. I acknowledge that I have read this form and any questions have been answered. I have been informed that I can withdraw my consent at any time without any penalty or loss of benefits to which I am otherwise entitled.

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No, I do *not* want to provide broad consent. I do not give permission for my identifiable, private data or biospecimen samples to be kept or used for other research studies. I acknowledge that I have read this form and any questions have been answered. **I know I can still participate in this research if I say No.**

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Name

Today's Date