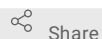


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# Dietary Sample for Phytochemical Assays

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In Development



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## ABSTRACT

All of the methods and protocols for this project have been reviewed and approved by the Otterbein University Institutional Animal Care and Use Committee, Otterbein University's Environmental Health and Safety Officer, the AZA Prosimian Taxonomic Advisory Group, and the individual collaborating AZA Institutions.

Many plants produce chemicals known as phytoestrogens that activate estrogen receptors. The phytoestrogen-fertility relationship is well-documented in livestock, rhinoceroses, cheetahs, and pandas, and this study will characterize the pattern in primates, especially pygmy slow lorises, gorillas, and pottos. We will adapt existing biomedical methods to investigate dietary estrogens and reproductive outcomes in captive primates. Gene expression profiles will serve as proxies for in vivo estrogen receptor activity, and our heterologous cell-based receptor reporter assay will quantify in vitro estrogen receptor activation. Generalized linear mixed-effects models will integrate these parameters with reproductive outcomes to test the effect of gene expression profiles and diet estrogenicity on fertility. This long-term project will ultimately provide one of the first deep physiological examinations of phytoestrogen's proximate and ultimate influences on primate reproduction.

## PROTOCOL CITATION

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## CREATED

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## LAST MODIFIED

May 24, 2021

## PROTOCOL INTEGER ID

50188

## GUIDELINES

Thank you in advance for agreeing to participate in this project on Primates, Dietary Estrogens, and Health. This study aims to identify endocrine-active compounds in primate foods, which may be contributing to reproductive complications or other chronic health outcomes in captivity. We know that everyone helping us collect these samples is already incredibly busy, and truly appreciate your contribution to what we hope will benefit zoos and the primates.

If you have any questions about this protocol or the long-term study, or you would like to inquire about more formal collaboration on the project, please contact the principal investigator, Dr. Alicia Rich, an Assistant Professor at Otterbein University ([rich2@otterbein.edu](mailto:rich2@otterbein.edu)).

This protocol does not involve any direct animal handling, and we will only need one of these samples for each participating zoo in our large-scale study. It should only take you a few minutes to set aside these samples.

#### MATERIALS TEXT

- Plastic bag
- Styrofoam cooler
- Gel ice packs
- Documents for shipment
- Labels for shipment
- Box for shipment

#### BEFORE STARTING

Make arrangements with Dr. Rich to plan the day that you will gather this sample so that she can make sure you are able to hand it off to her or a Fedex carrier at the end of that day or on the following morning.

Dr. Rich will send you all of the materials you need, so make sure you have those available to begin setting aside food first thing in the morning.

#### Set aside food

- 1 For one full day, each time you prepare the diet for our target animal, set aside one extra portion of food in a plastic bag or styrofoam crate. Keep this food in the same location or under the same conditions as you would just before you feed it to the primate.

Our goal will be to extract chemicals from the food items when they are in the same state they would be right when the animal eats them. So if they are typically frozen and thawed, that is what we want to happen. If they sit at room temperature, then that is what we will replicate.

For some large herbivores (e.g., gorillas) the total volume of food consumed in one day may be unreasonably large for shipment. In those cases, try to take a representative portion of each food item (e.g. 1/2 or 1/4 of the usual portion for every food, making note of this). Our goal is to have all of the food items in representative portions.

If you have detailed diet documentation of some kind that your zoo uses already and are willing to share it, we would find it very helpful. This is not required though.

#### Package diet sample for transport

- 2 At the end of the day, prepare all of the food for immediate pickup and transport to our lab or for shipment, depending on your institution's location.

- 2.1 Combine all items into the plastic bag provided. Close the bag as shown below.

#### Bag Closure Instructions



Twist end of bag tightly.

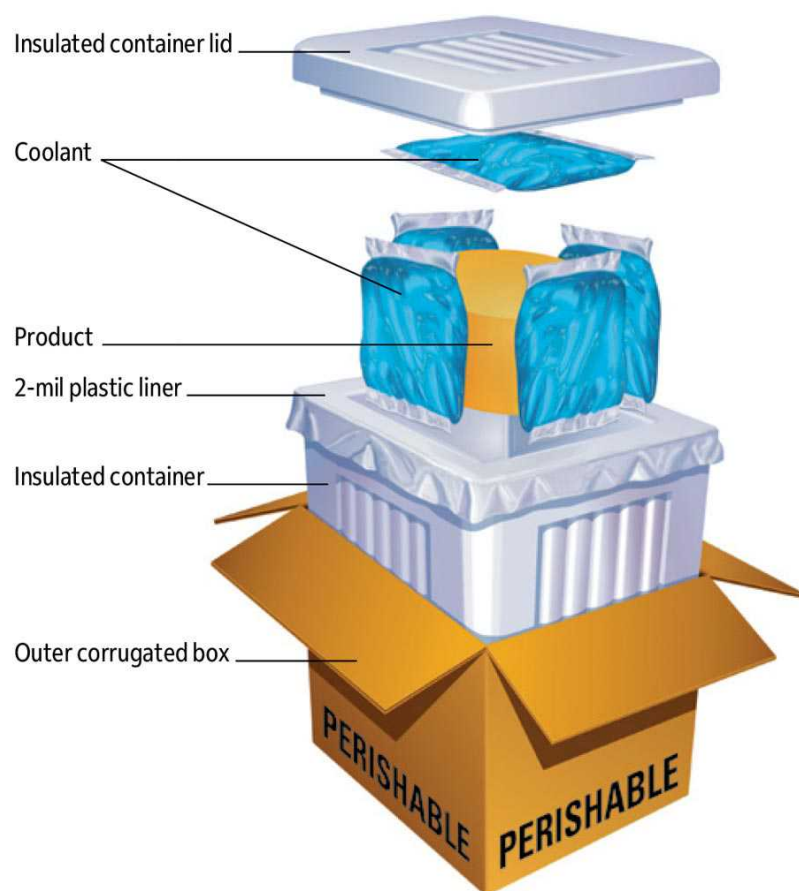


Fold over.



Wrap rubber band securely around fold-over to ensure closure.

- 2.2 Put the plastic bag and the ice packs (provided by us) into the styrofoam cooler that we sent. Try to position it similar to the image below. Make sure the outside of the styrofoam cooler is labeled with your information (we should have sent it to you this way).



Note that if you are preparing this for pickup, you do not need to position things as carefully as this. In fact, you may only need to use the plastic bag with a clear label of some kind and hand that off to Dr. Rich. She will take care of long term storage and come prepared with her own cooler for transport.

- 3 Place the styrofoam cooler inside the cardboard box we sent. Either prepare this box for shipment or pickup, depending on your location.

Step 3 includes a Step case.

**Box for Pickup**

**Box for Shipment**

Sample Pickup

step case

**Box for Pickup**

Follow these directions if you are at one of the zoos where we will be picking up the box directly (Cleveland Metroparks, Cincinnati, Columbus, Akron, Pittsburgh, Buffalo)

- 4 Send a text message or email to Dr. Rich or the other contact you were given to let us know the box is ready. We will arrive shortly to receive the box at the most convenient time/location for you.
- 5 We will follow up with you as soon as we have results! Thank you!

