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Sample Collection: Primate Hair for RNA

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Works for me



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PAL Lab Otterbein

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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.

The pygmy slow loris (*Nycticebus pygmaeus*) captive population is declining at a rate that jeopardizes their future in the Species Survival Plan (SSP). Experts agree that diet is somehow compromising reproduction, and this study will investigate dietary estrogenicity. 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 slow lorises. We will adapt existing biomedical methods to investigate dietary estrogens and reproductive outcomes in captive pygmy slow lorises. 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.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

This protocol has been adapted from the following publication: Munds, Rachel A., Chelsea L. Titus, Lais A. A. Moreira, Lori S. Eggert, and Gregory E. Blomquist. 2021. "Examining the Molecular Basis of Coat Color in a Nocturnal Primate Family (Lorisidae)." *Ecology and Evolution*, no. ece3.7338 (March). <https://doi.org/10.1002/ece3.7338>.

PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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IMAGE ATTRIBUTION

<https://courses.lumenlearning.com/wm-biology2/chapter/hair/>

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GUIDELINES

Thank you in advance for agreeing to participate in this project on Pygmy Slow Lorises, Dietary Estrogens, and Reproductive Function. This study aims to identify endocrine-active compounds in pygmy slow loris foods, which may be contributing to reproductive complications 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 lorises.

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).

This protocol involves direct animal handling, so it should only be conducted by trained, experienced professionals and modified to fit their institution's guidelines for handling that animal. Collecting the hair should take <30 seconds, so you should not need to handle the animal for any more than 5 minutes.

MATERIALS TEXT

PPE:

- Thick, protective gloves
- Facial covering
- Nitrile examination gloves (included)
- Epipen (optional bite precaution)

Sample Collection:

- masking tape (included)

Sample Storage:

- Sterile tweezers (included)
- Sterile, RNase-free tubes (included)

 DNA/RNA Shield Zymo

- **Research Catalog #R1100-50** Step 2 (pre-filled in the tube)
- Sample bag (included)
- Envelope addressed to Rich Lab at Otterbein (included)

SAFETY WARNINGS

Follow all of your standard procedures at your institution for handling the animals. For slow lorises, this may include using thick gloves to protect you from venomous bites.

DISCLAIMER:

Monitor subjects during any and all sample collection procedures for signs of distress. For example, slow lorises emit a distinctive "chitter" vocalization when they are in distress. If collection causes an animal to repeatedly exhibit signs of distress, immediately suspend the protocol and reach out to the PI on this project to reassess the methods. Do not collect any samples from an animal if it will risk aggravating any existing wound, illness, or other condition. Let the PI know that you will need to exclude that animal from the study.

BEFORE STARTING

We will provide all of the necessary materials for sample collection. Make sure that nothing is missing from your kit and you understand these guidelines.

Animal Handling

5m

- 1 During routine handling procedures, fold one piece of masking tape around a tuft of ~20 hairs on the dorsal neck area^{5m} of the subject. As swiftly and firmly as possible, pluck the tuft of hairs in the masking tape. The most important part of the sample is the hair root at the end of the shaft, which is why it is important to pull rather than cut or shave the hair.

Safely release the animal as soon as you complete this step while taking care not to disturb the sample still attached to the tape. ⌚ **00:05:00 should take less than 5 minutes**

Sample Storage

5m

- 2 We have provided you with sterile, RNase-free tubes containing

 DNA/RNA Shield Zymo

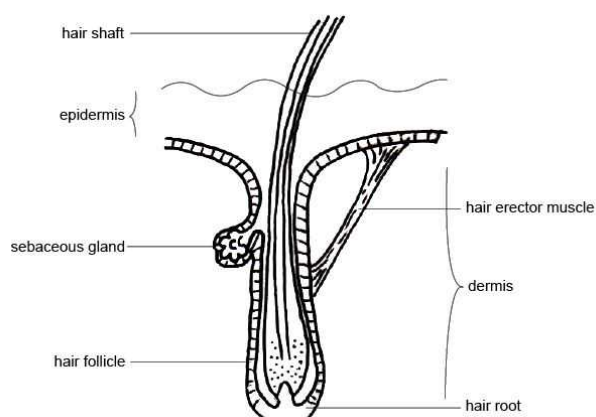
Research Catalog #R1100-50

and sterile tweezers.

Label the outside of the tube with today's date (YYYYMMDD), your initials, the time (HH:MM:SS), the sample type (hair or feces), the animal name or ID, and the species code (PSL for pygmy slow loris).

While still wearing sterile gloves (or after putting on a new pair), carefully open the tube, taking care not to touch the inside of the cap or the tube.

- 3 Using the sterile tweezers, gently remove each hair from the tape and put it into the tube. Be very careful not to disrupt the root portion of the hairs, so it is best to grip the tweezers as close to the tape as possible.



Basic hair anatomy. The living cells with plentiful mRNAs are at the root, not the shaft end.

- 4 Place this tube inside of a clearly labeled sample bag (also provided), and put that into the envelope with postage, already addressed to Alicia Rich at Otterbein University. Please drop this envelope in the mail as soon as possible (within 24-48 hours of sample collection).



Wash hands thoroughly after releasing the animal and monitor the subject for 20 minutes to ensure there are no negative welfare impacts from the procedure. If you observe any signs of distress then notify the PI immediately.