NORTHERN ARIZONA UNIVERSITY
College of Engineering, Forestry & Natural Sciences
School of Forestry
and Pathogen & Microbiome Institute

SPECIES FROM FECES
DNAwild.life

Standard Operating Procedure

Fecal Collection for Genetics

Note: This SOP is illustrated by bat guano collection;
Collection of feces from other taxa follows the same protocol

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Materials provided:

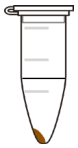
- 15mL conicals and/or 2mL tubes with RNAlater solution
- Parafilm
- Box to hold samples

Not provided:

- Powder-free nitrile gloves
- Permanent marker
- Ziploc bags (quart and gallon)
- Stainless steel tweezers (optional)

15mL conical for pooled guano

Multiple species



2mL tube for single pellet

Single species

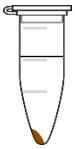
Great genetic testing begins with clean and careful sampling: Due to the sensitive nature of this genetic technique, avoid cross-contamination between guano samples or other mammalian DNA sources. This is your responsibility. Always use fresh gloves or tweezer/scoop between unique samples or sites, and do not set anything down before sample collection

unless it is still in its packaging. Always use different Ziploc bags to separate samples from different sites, and use parafilm to seal conicals. If there is reason to believe that cross-contamination has occurred, please note it and share with us.

Sampling protocols:

For pooling guano pellets across a roost into a 15mL conical: Ensure that you have a clean pair of gloves, tweezers (optional), scoop (optional) and the conical you are ready to use. The easiest collection method is to use a nitrile glove to pluck pellets and place them into the conical, and then swap gloves between conicals. If you use tweezers, these will need to be wiped off and flame-sterilized with a lighter between conicals. A scoop can be anything that has not been in contact with bat guano.

1. Try to target fresh-looking feces if you can. If you wish to sample broadly across a roost, make sure to collect pellets from all around the roost, either into a single conical, or multiple conicals. Depending on your study design, you may divide the roost into zones if collecting into multiple conicals.
2. Open the conical, making sure the cap does not touch anything. Either hold the cap between two unused fingers or have another gloved person hold it. Avoid setting it down if at all possible.
3. If you're interested in a particular guano pile, scoop or pluck horizontally across the top of the pile rather than digging vertically into it.
4. Fill the conical slightly less than half way (~1 gram), ensuring that all guano is submerged in the RNAlater solution. **Do not overfill conical.**
5. Place lid on conical and seal with parafilm. Stretch the parafilm around the lid area of the conical. Once parafilm is secured, invert the conical several times to submerge all contents with RNAlater solution and to test its seal for shipment.
6. Give it a clearly written and unique label with a permanent marker. If there are multiple replicates from the same guano pile, ensure that all samples are given a replicate ID (A, B, C, 1, 2, 3, etc.).



For collecting single fecal pellets, buccal swabs, or wing punches: Use clean gloves or rim of tube to transport a single sample into each tube, taking care not to spill the solution.

Other collection methods: While we recommend purchasing a sterile RNAlater sampling kit we provide in advance of a field outing, this may not be feasible in certain situations. We suggest purchasing 1.5 – 2 mL microcentrifuge tubes and having them on hand or available in your facility. You may collect dry but the samples must be frozen immediately (**dry ice only**) and delivered within the next two days to avoid DNA degradation. **We do not recommend collecting guano in a Ziploc bag** because this makes sub-sampling difficult and **may increase risk of cross-contamination**.

After collection:

Separate by site and by sample: Separate your samples or groups of samples by site or by what you define as site (roost, zone, grid cell, etc). **Each 15mL conical should be sealed in its own quart-sized Ziploc and conicals from each site should then be placed together in a gallon-sized Ziploc**. Do not use Ziplocs that have been used or opened previously in other sites. If you use the small 2mL tubes for single fecal pellets, they can be separated by spacing throughout the provided box. Ensure that these tubes are firmly sealed.

Storage until shipping: RNAlater is a salt-based DNA stabilizer that performs well under field conditions and is safe for air transport. **Do not consume or allow your pets to consume this solution**. After collection but while still in the field, place samples in a cooler or refrigerator if possible. If this is not possible, do not worry: just place in fridge after you return home. If you will be storing samples for weeks or months before shipping to us, place samples in a freezer.

Shipping: Please ship FEDEX to the address on Page 1. Dry ice and overnight or next day delivery is recommended for sensitive/rare samples; ice packs with next day or 2nd day shipment is fine for most samples. **Please also send a hard copy and a digital Excel spreadsheet (available from us)** of sample information. Sample information should include unique number or code for each conical and/or tube, county, state, GPS coordinate, sample

date, and any other relevant information. For single fecal pellets, buccal swabs, and wing punches, please also include species ID and sex if known.

We are happy to answer your questions.

Happy Collecting!