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Golden monkey fecal sampling protocol

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Protocol status: Working
We use this protocol and it's working, but improvements are still being made

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Abstract

This project aims to use data collected from fecal samples to asses the effects of habitat loss on two populations of endangered monkey. The protocol outlines the steps for collecting and processing fecal samples from groups of habituated and un-habituated monkey groups suitable for multiple habitat types. The sub-samples processed from the feces will be used for host (monkey) DNA extraction, helminth DNA extraction, and morphological parasite ID using mini-FLOTAC and sedimentation.

Guidelines

Overview:
This protocol describes procedures for collecting and processing 30-50 (target: **48**) fecal samples from golden monkeys in each of two populations, VNP and GMNP. Samples will be collected opportunistically during golden monkey group follows when team members observe defecation by a monkey from whom no samples have yet been collected.

In **VNP**, only one sample will be taken per identifiable individual (8-15 per group).

In **GMNP** we also aim to collect one sample per unique individual (4-12 per group) but because these monkeys are not individually recognized we will use a combination of age-sex class and any uniquely identifying characteristics such as scars or bent digits to distinguish individuals.

Materials

Materials for field collection:

- gloves
- masks
- 8ml tubes filled with 4 ml RNAlater
- wooden tongue dispenser
- labeling tape
- sharpie marker
- ballpoint pen
- data sheets

Materials for lab preparation (per field site):

- digital scale
- weigh paper
- labeling tape
- 750 ml 96% ethanol
- 1500 ml 10% formalin
- 8 ml sample tubes
- wooden tongue dispenser
- spatula
- alcohol (for sterilization)
- lighter
- tissues
- sharpie marker
- ballpoint pen
- notebook
- data sheets
- parafilm

Safety warnings

Safety information

When handling fecal samples, always wear a mask and gloves. Discard gloves immediately after they come in contact with fecal matter or anything carrying human DNA such as skin, saliva, or hair. Discard wooden sticks after collecting each sample. Similarly, flame sterilize any equipment (spatula) that comes in contact with fecal matter between uses by wiping off any fecal matter with a tissue, dousing in alcohol, and then lighting using a cigarette lighter, being careful not to burn yourself.

Before start

Before going into the Field:
Check the field materials list. Make sure to have at least 15 pairs of gloves and 15 tubes filled with 4 ml RNAlater, enough to be able to collect samples from an entire group. Before going into the field, use labeling tape to label the caps of the tubes filled with RNAlater with 1 through 15. Then, weigh the mass of each each tube, including the RNAlater and the cap. Record the mass for each vial in a notebook, using a new page each day. Each page should contain only the date, the list of numbers 1-15, and the corresponding mass for each numbered vial. It is ok to re-use vials between days, just be sure that no numbers are repeated within a day.

Boots and hands need to be sanitized with alcohol before entering the forest and a mask must be worn at all times in the forest except when you are eating or drinking.

Each sample you collect will be assigned a unique ID code which you will record on the data sheet and on each collection container before filling them with fecal matter. These instructions therefore begin with how to fill out the data sheet and assign the ID code, and then explain how to collect the samples.

Data recording

1 You should use one set of data sheets (field, lab, and note) per group. If you fill up a data sheet set, start a new set for that group if necessary and mark 1/2 on the first set and 2/2 on the new datasheets. Make all notations in pen. If you make a mistake, cross it out it and then begin again on the next line. Do not write over a previous incorrect entry to try and fix it. Relevant abbreviations are defined in later steps. When preparing samples, double check that the sample's ID code on your containers matches the ID code across all data sheets.

2 Below is the heading of the field data sheet. Site refers to the specific park, VNP or GMNP, or pine forest outside GMNP. Group ID refers to the identity of the group if known and should include all groups observed that day, separated by a comma. If the group does not have a name (GMNP) note the approximate location of the group's home range (ex. in GMNP: core forest south-eastern edge)

Make note of any abnormalities in collected feces such as presence of blood, mucus, or worms. Unique marks used to identify individuals (ex. bent digits or tail, bald spots) should also be recorded. If you need more space, you can record additional notes on the notes sheet. Be sure that you match the sample ID across sheets.

	A	B	C	D	E	F	G	H
	Observer name(s): Alex							
	Site: VNP							
	Group ID: Musango							
	Sample ID	Date:	Time	Latitude	Longitude	age/sex class	Tube #	Notes:
	V-04APR-1	04-04-22	15:30	1.24 S	25.39 E	AF	1	stool in 2 pieces

3 The unique ID code consists of three parts with a hyphen separating each:

1. The first letter of the site
2. The date (month and day) written as 2 digits and a 3 letter month abbreviation (ex. 8 May would be 08-MAY)
3. The sample number where 1 is the first sample collected that day and each following sample is assigned the next consecutive number (2,3,4, and so on)

Site and team codes:
V = VNP
G = GMNP
P = Pine Forest

Note

Example: If Team A is sampling in VNP on June 3, the 4th fecal sample they collect will have an ID of: V-03JUN-4

For each team, sample numbers increase consecutively on each **day**, no matter how many groups you follow. They reset to 1 only on a new day.

Field sample collection and sub-sample preparation

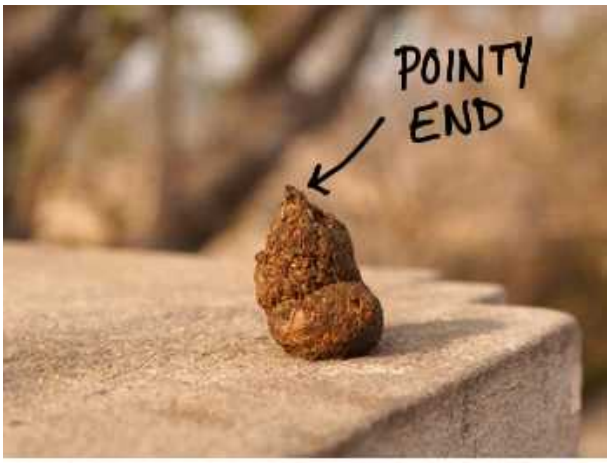
4 After locating the fecal bolus, use a wooden spatula to remove leaves, sticks, vegetative debris, and soil as much as possible from the outside of the bolus.

Note

Some fecal matter is not suitable for collection:

- If you cannot distinguish the interior of the fecal bolus from the exterior, or it cannot be separated from leaves, soil, or other organic matter without loosing large amounts of sample (ex. if stepped on or splattered on impact with ground, branch, etc.), do not collect it.
- Additionally, if the fecal bolus (or cumulative fecal bolus if it is in 2-3 large pieces) is shorter than the length between your middle knuckle and the tip of your pointer finger, do not collect it (it will be too small to provide the material needed).

5 Look for the pointy end of the fecal bolus (this is the last part of the feces to leave the monkey). From the outside layer of the pointy end, use a wooden spatula (tongue dispenser) to scrape off 2 mL of feces (this is half the volume of the liquid in the tube). Place this fecal matter in 4 mL of **RNAlater** in an 8 ml tube.



Note

If feces are minimally misshapen or broken (i.e., you can't distinguish one end from the other but fecal bolus is still in 1-2 large pieces) during or after defecation, prioritize taking a sub-sample from the outer-most layer of the fecal bolus.

6 Use labeling tape to label the sub-sample tube with the ID code followed by a D for DNA. Also record the tube number (from when you measured the mass of the sample tubes with RNAlater) on the **field** data sheet.

Note

Example: the DNA sub-sample of the fecal bolus collected in the example above would be: VA-03JUN-4D

7 Fill in the **field** data sheet, recording the time, GPS location, and age/sex class of the individual, and in VNP the identity of the individual if possible. Also note any identifying characteristics of the monkey (ex. bent digits or tail, bald spots) and irregularities of the fecal sample such as presence of blood, mucus, or worms. Any information that does not fit on the field data sheet can be added to the **note** data sheet.

Age/sex class abbreviations:
AM = adult male
AF = adult female
SM = subadult male
J = juvenile

8 Seal the remaining feces in the glove for transport to the field station and use a sharpie marker to label the glove with the ID code.

Note

Some deformation of the sample during transport is acceptable, but try to avoid squashing the sample as much as possible as this will make it easier to take sub-samples.

Station lab sub-sample preparation

9 Prepare the **lab** data sheet (example below) by filling in the ID codes for all samples collected on that day, the dates, and the tube # and the tube + RNAlater mass that corresponds with each sample.

	A	B	C	D	E	F	G	H	I	J	K	L
	Observer name(s): Alex											
	Site: VNP											
	Group ID: Musango											
	Sample ID	Date:	Tube #	Tube + RNAlater mass (g)	DNA - tube w/ sample mass (g)	DNA sample mass (g)	Fecal bolus mass (g)	Total mass (g)	Mass OH (g)	Mass F1 (g)	Mass F2 (g)	Notes:
	V-04A PR-1	04-04-22	1	12	13		7		0.95	3.05	2.96	

Note

The DNA sample mass and the total mass will be left empty.

10 Weigh the tube containing the DNA fecal sample, and record the DNA-tube w/ sample mass (tube, cap, RNAlater and feces) on the lab data sheet. Then subtract the tube + RNAlater mass from the DNA-tube w/ sample mass.

11 Place a sheet of weigh paper on the scale and tare it. Then carefully invert the fecal bolus in the glove onto the weigh paper to measure the total mass of the fecal bolus. Record that number on the lab data sheet under bolus mass.

12 Use labeling tape to label 3 collection tubes for parasite sub-samples with the ID code followed by the reagent code. For samples in formalin, add a 1 or 2 after the reagent code (F) for the first and second 3 g sub-samples.

Reagent codes:

- OH = 96% ethanol
- F = 10% formalin

Note

Example: Our fecal sample from the example above (VA-06034) would have 3 different labels for the parasite sub-samples:
1 g in 96% ethanol: VA-03JUN-4OH
3 g in 10% formalin #1: VA-03JUN-4F1
3 g in 10% formalin #2: VA-03JUN-4F2

Note

When using ethanol, try to avoid getting it on the outside of the collection tube and smearing the label. If some of the ink comes off, dry the collection tube and then re-label with the correct ID.

13 Tare the 8 ml collection tube labeled with OH on the scale and use a flame sterilized spatula to measure 1 g of feces into it, removing or adding fecal matter as necessary to reach 1 g within ± 0.1 g. Record the final mass of the sub-sample on the **lab** data sheet under Mass OH. Then, fill the collection tube with **96% ethanol** so that the tube is filled. Note any major sample loss during measurement or sample abnormalities in the notes section of the **lab** data sheet.

Note

All measurements of mass may have an error of up to ± 0.1 g, so a 1g sample may weigh within 0.9 - 1.1 g.

14 In a similar way, measure two 3 g sub-samples into the appropriately labeled collection tubes (F1 and F2) and record each sample mass on the **lab** data sheet under Mass F1 and Mass F2 respectively. Fill each tube with **10% formalin** so that there is no air.

Note

With error a 3 g sample may weigh within 2.9 - 3.1 g.

14.1 If you cannot measure out 2 full 3 g samples, measure one full 3 g sample into the tube labeled F1. Then, measure the remaining feces into the tube labeled F2 and record the mass (which will be less than 3 g) under Mass F2 on the **lab** data sheet.

Sample/data storage

15 Seal sample tube caps with a strip of parafilm (as shown below) before storing. Store samples in sample boxes labeled with the project name (Johnston GM fecal samples), location, and reagent code (D, OH, F1, F2). Boxes should be kept at Room temperature and in a dark location. Make sure that all samples are stored upright so that any air pockets are at the top of the tube, minimizing the likelihood fecal matter will be exposed to air.

Parafilm strips should be approximately 1.5 in long and 1 in wide, enough to cover the side of the cap and approximately 0.5 inches of the sample tube. To get a proper seal, stretch the parafilm slightly as you rotate it around the cap.

16 After a set of data sheets are complete (all information about all samples listed is filled out), take a picture or scan them. Once a week, email all new digital copies to project

leads. Physical data sheets should be stored together in chronological order in a dry location and duplicated by photocopying when complete.