

## Scat Collection for Host/Prey DNA Protocol

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### Sample Tube Preparation:

- Scat can be collected and stored in the following options:
  - 15mL plastic tube with 7mL of color indicating drierite/silica desiccant (standard species, individual identification, and diet projects or studies where space is limited).
  - 50mL plastic tube or specimen collection cup (or larger) half filled with drierite/silica (23mL or equivalent) (standard species, individual identification, and diet analysis projects where additional extractions needed).
    - Notes: Various preservatives can be used to store scat samples -
      - While effective, ethanol is discouraged as it can leak out of the tubes and wash off identifying markings on the tube, and creates difficulties when shipping samples.
      - DET buffer must be prepared freshly and accurately, which may not be logistically feasible in remote field conditions.
    - A Kimwipe/tissue is recommended to separate desiccant from the scat sample in smaller sized tubes to avoid the silica/drierite from getting on the scat and/or contaminating it.

### Scat Collection:

#### Sample Labeling –

- Label tube or cups with permanent marker using the following nomenclature [YEAR-MONTH-DAY-COLLECTOR'S INITIALS-SCAT NUMBER].
- Example:
  - First scat collected on June 1, 2020 by Charlotte Hacker: 2020-06-01-CH-01
  - Second scat collected on June 1, 2020 by Charlotte Hacker: 2020-06-01-CH-02
    - The numeric order of scats starts over each new day of collection.
    - Each collector uses their own numeric scale starting from 01.
- Use the unique sample label on the cup to label a waypoint for the scat location in the GPS.
- This system ensures that each scat has its own unique field name and provides basic information for reference later on if needed, such as date and collector.



*Labeling for sampling collection.*

*Filling Out Sample Sheet –*

- Fill out the corresponding sample sheet with the information pertinent to the sample being currently collected.
- Set an empty labeled tube near scat, but not touching it, with the label facing upwards, and take a picture prior to collection.



*Recording information prior to sample collection.*









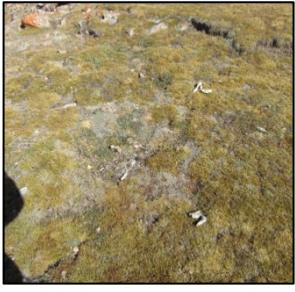
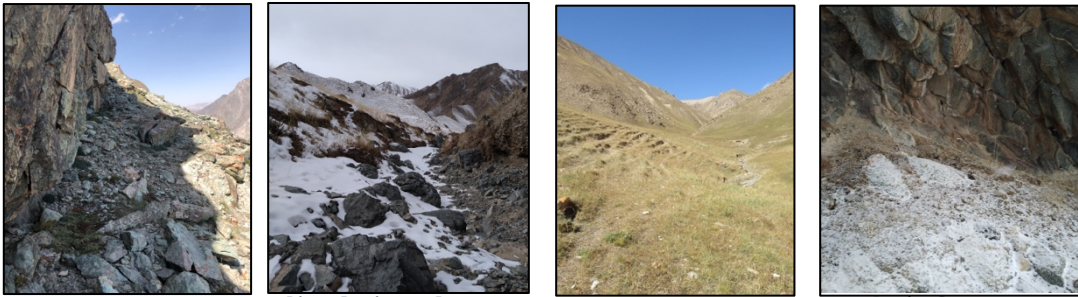
*Picture of scat sample with tube and name adjacent.*

**Filling out sampling sheet example:**

Date	Sample ID	Transect	Species	Diameter	Age	Sign, Age, Proximity	Feature	Nearest Landmark	GPS/ Elevation	Notes
2020/06/01	2020-06-01-CH-01	01	Snow leopard	~3cm	old	Fresh scrap 1m away	Ridge-line	Boulder ~3m high	35.513 98.347 4442m	By yak skull
2020/06/01	2020-06-01-CH-02	01	Snow leopard	~5cm	fresh	Old pugmark 2m away	Rocky Outcrop	Set of prayer flags	35.512 98.252 4472m	Grass in scat

Definitions of each sampling sheet heading:

Heading	Definition
<i>Date</i>	Date the sample was collected.
<i>Sample ID</i>	The field name of the sample as written on the tube.
<i>Transect</i>	The transect number of which samples are being collected on.
<i>Species</i>	The species the collector believes the scat to originate from.
<i>Diameter</i>	The size in cm of the scat across its width as judged by eyesight.
<i>Scat Age</i>	<p>The relative age of the scat.</p> <p><u>Fresh</u> – high or some moisture content, dark in color, may or may not have odor.</p> <div style="display: flex; justify-content: space-around; align-items: center;">   </div> <p><u>Old</u> – no moisture, discolored (faded or bright white), outside casing may be missing.</p> <div style="display: flex; justify-content: space-around; align-items: center;">   </div>

<p><i>Sign, Age, Proximity</i></p>	<p>Signs of carnivore presence near the sample, how old the signs appear, and their distance to the sample. Examples include scrape, urine, latrines, claw rake, pugmark, and scent spray.</p> <p><u>Scrape</u> – Results from snow leopards digging their hind legs down and back into the ground, displacing soil and loose earth.</p>  <p><u>Pugmark</u> – A distinct footprint in the surface of the ground. Can be identified down to species if clear enough.</p>  <p><u>Latrine</u> – A site used by multiple snow leopards or other carnivores. Beneficial for detecting multiple individuals living in an area at one site.</p> 
<p><i>Feature</i></p>	<p>A geographic feature for the landscape where the scat was found. Examples include ridgelines, saddles, river drainage bottoms, flat grasslands, and rocky outcrops.</p>  <p style="text-align: center;"> <span><i>Ridgeline</i></span>      <span><i>River Drainage Bottom</i></span>      <span><i>Flat Grassland</i></span>      <span><i>Rocky Outcrop</i></span> </p>
<p><i>GPS and Elevation</i></p>	<p>The GPS coordinates of where the scat was collected in decimal degrees and its elevation in meters.</p>
<p><i>Notes</i></p>	<p>Any additional information that may be useful.</p>

### Collection –

- It is imperative that bare hands do not touch the scat. This could lead to contamination of the next scat sample with the previous one. A new tool for collection should be used for every scat sample. Latex gloves are ideal, but rocks and sticks can also be used if gloves are unavailable.
- Using sterile tweezers, fresh gloves, or rocks and sticks, break off/cut a full-cross-section of the scat. Chunks of scat (full circumference and diameter) that appear freshest/less dry are best as the DNA is less likely to be degraded and are more useful for diet analysis. Place the scat into the tube.



*Scat being broken apart and collected with gloves.*



*Scat being broken apart and collected with a rock.*



*Scat being broken apart and collected with tweezers.*

- Do not stuff the scat tightly into the tube. Make sure there remains airspace between the scat and the lid of the tube. This is so the desiccant can properly dry out the scat to preserve the DNA inside the scat.
- Remove gloves or set down tools used to handle the scat.
  - Dispose of gloves properly by putting them in a receptacle bag and then putting that bag in the appropriate bin after the sampling session (label and store separately from clean gloves).
- Place the cap back on the tube securely.
- If using tweezers, sterilize between samples with fire by using a lighter to burn off any scat or hair on the tweezer for at least 5 seconds (let cool before packing up tools). Do not touch the end of the tweezers (the part used to collect the scat) at any time with your bare hands.
- Place the tube in the collection bag.
- Avoid collecting the entire scat if not necessary in order to maintain the chemical and visual cues scat provides within a habitat.
- If sampling the same transect repeatedly, mark the remainder of the scat with nail polish so that it is not collected during the next sampling session.



*Sample put into tube with airspace to ensure it dries properly.*



*Sample marked with nail polish to avoid recollection in future sampling efforts.*

### Data Entry –

- Entering data from field sheets into an electronic format relatively soon after sampling sessions is helpful for organizing samples and preparing them for transport.
- At the end of every field day, take a photo of the datasheet for temporary backup in case the datasheet is lost or destroyed. This covers the lag time between field collection and entering the data electronically.
- Open up an excel spreadsheet and enter in the information on your sampling sheets as written. Save file.
  - Note: the names labeled on the collection cups in the field are helpful for discerning date and collector, but are not practical for lab work later on when sample names must be written repeatedly on the small surfaces of microcentrifuge tubes. It is helpful to add an associated lab name to the collection tubes with the samples.

- Line up your cups on a flat surface in order of date, collector, and then collection number.
- Relabel each tube in order with a short prefix related to the sample site followed by a number. Write this name on both the side of the tube and its cap. Continue onward for the entire sampling session, picking up each numeric value from where you left off prior.
- Add a “Lab Name” column into your Excel spreadsheet and enter in the name of each sample corresponding with its field name.
- Example:

Field Name	Lab Name
2020-06-01-CH-01	DUL01
2020-06-01-CH-02	DUL02
2020-06-01-IC-01	DUL03
2020-06-01-IC-02	DUL04
2020-06-01-IC-03	DUL05
2020-06-02-CH-01	DUL06
2020-06-02-CH-02	DUL07
2020-06-02-CH-03	DUL08

### Sample Transport:

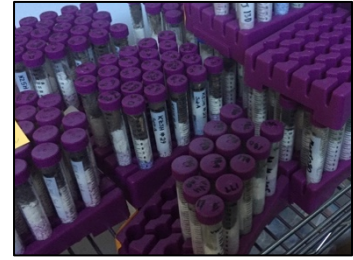
- Collected samples should be kept in a cool, dry place after collection until they can be transported.
- Transport of samples should adhere to local government and country regulations.
- Place sample tubes in a sealed bag. Label the outside of the bag with name, material, and number of samples in bag.
- If multiple bags accumulate, place them in a larger box or sturdy bag.
- All boxes should be reinforced with adequate tape to ensure samples are secured and arrive safely.



*Samples organized, labeled, and packed for transport.*

### Sample Storage:

- Upon return to the laboratory space, proper sample storage ensures DNA degradation will not occur.
- Room temperature storage is possible so long as the ambient temperature is stable.
  - Labs that do not have reliable temperature control, or those which are kept above room temperature ( $\sim 20^{\circ}\text{C}$ ), are not adequate for room temperature storage
  - Samples as old as 10 years stored at stable room temperatures have resulted in successful DNA extraction when stored in this manner
- Refrigerator or freezer storage should be considered if the laboratory does not have consistent temperature control.
  - Freezing samples halts microbial activity that could further degrade DNA.



*Samples stored in organized rows at stable room temperature.*



*Samples stored in laboratory freezer.*



*Samples stored in urine specimen cups with lab and field ID.*