Fecal sampling protocol for population genetics

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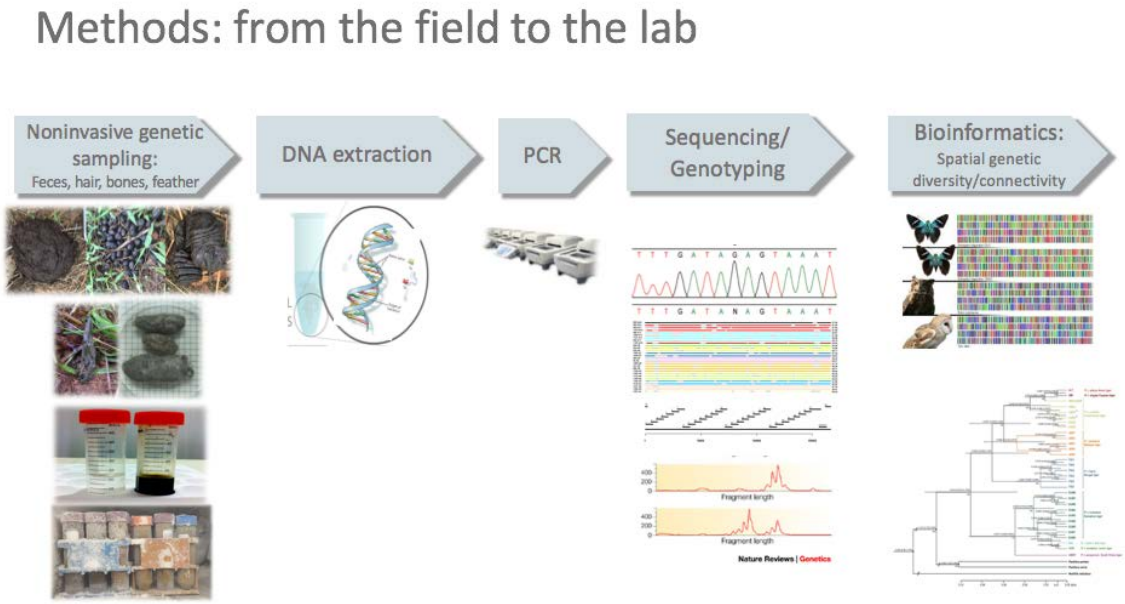
Fecal sampling protocol for population genetics

This preservative buffer and sampling method are suitable for addressing phylogeographic and population genetics questions including 1) species identification, 2) sex ratio estimation, 3) impacts of human disturbance on population demography (effective population size, *Ne*), population genetic diversity (observed & expected heterozygosity, H_O & H_E, number of allele, *A*, and allelic richness, A_r) and connectivity (gene flow, recent migration rate, the number of migrant, N_m). Schematic diagram from field sampling to the lab is illustrated below. Feel free to leave questions or comments by emailing Pai (worata@gmail.com), the PI of CEG's Conservation Genetic team.



Equipment

1) 50 mL sterile polypropylene centrifuge tube containing 25mL NaCl-saturated DMSO buffer

2) Examination Gloves (Latex Powder free)

3) Plastic fork or spoon

4) Record sheet:

- Sample ID: Initial of genus and species followed by locality and number XXX, i.e. LPTR001 for *Lutrogale perspicillata* (Smooth-coated Otter), Trung Province, Thailand. In case of ambiguous scat morphology among Mustelidae, please label MT001 or among *Prionailurus viverrinus* (Fishing Cat) vs. *P. bengalensis* (Leopard Cat), please label PN001.
- UTM coordinates: WGS84 datum
- Date & time of collection
- Fresh samples (<3d, shinny surface, pleasant smelling, near fresh track/scrape)
- Environmental condition: rained on vs. exposed to strong sunlight vs. under the shade (which may affect DNA quality/quantity)
- Name of collector

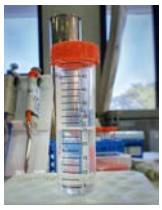
Fecal sampling protocol

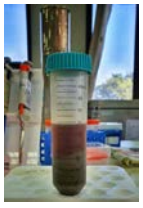
**Jelly samples from anal gland secretion are of higher DNA quality compared to fecal DNA. For otter sampling, please look for these jelly-like substance along the latrines. Please store anal jelly in a separate tube from fecal sample. Sampling protocol for fecal and jelly samples are the same.

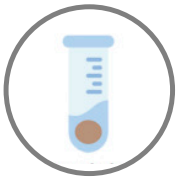
1) Wear gloves to prevent cross contamination between samples and achieve reliable sex identification

2) Use plastic fork/cotton bud to scrape the surface of fecal sample (gut epithelial cells are more abundance on the outside and serve as the source of fecal DNA)

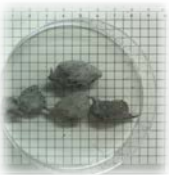
- ~5g or the level of preservative buffer rises from **25 mL** → **30 mL**







- For dry samples, cut the tip, transfer it to the dry tube/dry envelope, then keep dry at room temperature or freezer (-20C) is fine.

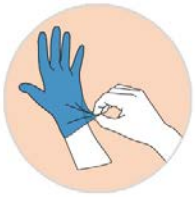


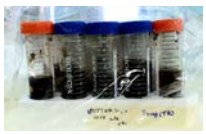
- Preservative buffer acts as DNA stabilizer and prevent microbial growth, therefore slowing down DNA degradation. Appropriate ratio between buffer and sample is needed, so please do not exceed >35mL level

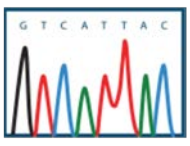
Fecal sampling protocol (continued)

3) Mix well by shaking the tube a~ 4 times to make sure buffer comes into contact with the sample

4) Record : Sample ID, UTM coordinates, Date & time of collection, fresh vs. old sample (see definition in the equipment page), environmental condition, collector and other remarks (i.e. diet composition based on bone fragment/hair morphology.







5) Discard gloves and fork. Do not use the same sampling equipment twice. **Storage**: Avoid direct sunlight and moist condition by covering the tubes with black plastic bags or boxes. Samples in the preservative buffer can be kept at an ambient temperature for 2-3 weeks. However, if you plan to work on metabarcoding diet identification & population genomics (hybridization, gene flow and fine-scale detection of population substructure using genome-wide data), the preferred condition is to transfer samples back to the lab (4°C or -20°C) once a week.