Collection and storage of non-invasive DNA samples from wild primates

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Genetic analyses have become an extremely valuable tool to study multiple aspects of primate evolution. Sources of DNA usually vary between organisms, but usually researchers prefer to obtain DNA samples through non-invasive means, such as fecal material or shed hair. Several studies have shown that storage methods could potentially have a great effect on DNA quality and yield. Here, we describe four storage methods for fecal DNA that have been used by various research groups. We do not intend this list to be all inclusive, but rather focus on methods that allow storage of DNA material in difficult field conditions.

Important aspects for consideration are

a) Availability of reagents and storage vials in the field
b) Storage conditions in the field
c) Transport of samples from field to laboratory
d) Cost of reagents

We have identified five different protocols. The first three are suited for fecal material, while the forth one works well with small pieces of tissue, if for instance a dead animal is encountered. The last protocol describes the storage and preservation of shed hair.

Some general comments:

Fecal material should be collected as soon as possible after defecation. As a rule of thumb, all samples should be stored as cool as possible (preferably frozen, but < 4°C works well, too). Unfortunately, in many field situations this is not possible. A possible solution is a so-called “dry-shipper” (sometimes called a “vapor-shipper”). These are fabricated from durable, lightweight aluminium, containing a hydrophobic absorbent that traps the liquid nitrogen. If properly filled, a dry shipper can cool specimens to -150°C for three weeks, even at high ambient temperatures. Dry-shippers are also approved my most airlines. If refrigerated storage is not possible in the field, the very least that should be done is storing the specimens at a cool and dry place. In areas of high humidity, this could be achieved by placing the samples in a sealable plastic container that contains silica gel to absorb the moisture. Generally, samples should be transferred to a lab as soon as possible.

Collection of fecal material:

IMPORTANT: Most of the shed cells of the target organism are found at the 'front end' and the outside of the fecal material. Hence, it is important to scrape as much as possible from the outside of the material in order to maximize DNA yield.
To avoid contamination with human DNA, gloves should be worn at all times when handling samples (although we know that this is sometimes difficult to do in tropical environment). For each sample, a new pair of gloves should be used to minimize the risk of cross-contamination.

1) Storage in 95 % Ethanol

Materials required:

- 8 ml polypropylene tube, lid with o-ring (for instance Sarstedt 60.542.007)
- 95% Ethanol or Methanol
- Parafilm (www.parafilm.com)

Procedure:

- Fill half of polypropylene tube with ethanol or methanol
- Place a grape-sized sample in the tube, make sure that it is fully submerged
- Seal tube with Parafilm
- Label the tube with relevant information
- Record relevant information (date, GPS coordinates, sample ID, sex, age, name of location, collector's name, etc) in a datasheet

Advantages:

- Cheap
- Quick

Disadvantages:

- DNA appears to degrade more quickly compared to other methods
- Flammable, might lead to transport problems

2) Alcohol, followed by silica gel desiccation

Materials required:

- 15 ml polypropylene tube with spoon in lid (eg. Sarstedt
- 8 ml polypropylene tube, lid with o-ring (eg. Sarstedt 60.542.007)
- 95% Ethanol or Methanol
- Parafilm (http://www.parafilm.com)
- Silica Gel
- KimWipes (http://www.kimberly-clark.com/)

Procedure:

- Collect fecal sample directly in approximately 15 ml tubes with built-in spoon filled with 5-7 ml alcohol (95%)
- Label the tube with relevant information
- Record relevant information (date, GPS coordinates, sample ID, sex, age, name of location, collector's name, etc) in a datasheet
- Let sample soak in alcohol for 24-36hrs
- After 24-36hrs, transfer 2g fecal sample to two 8 ml tubes
- Place small square of KimWipe on sample to separate silica from sample
- Add 4g of silica, so silica ends up between sample and lid
- Cover silica tubes with Parafilm
- Leave remainder of fecal sample in alcohol, add a little extra alcohol to tube for preservation if necessary
- Check after 2-3 days whether sample is dry.
  If yes: Monitor silica gel every two-three weeks for change in color, replace silica gel ONLY if necessary
  If not dry: replace silica gel, check again after two to three days.

Advantages:

- DNA yield and quality is higher compared to method 1
- Samples are dry and non-flammable

Disadvantages:

- Repeated handling increases contamination risk
- More expensive than method 1
- Several different tubes and containers are required

3) RNA later

RNA later (manufactured by several companies, eg. Ambion, Sigma-Aldrich, Qiagen and others!) is a specialized storage solution that was originally designed to preserve RNA in samples collected in the field. It appears to preserve DNA very well, and seems to have a superior performance compared to methods 1 and 2. Unfortunately, it is not cheap.

Materials required:

- 8 ml polypropylene tube, lid with o-ring (eg. Sarstedt 60.542.007)
- RNA later solution (Ambion # 7020, Sigma-Aldrich #R0901)

Procedure:

- Add 3ml of RNA later to the tube
- Place a grape-sized sample in the tube, make sure that it is fully submerged
- Seal tube with Parafilm
- Label the tube with relevant information
- Record relevant information (date, GPS coordinates, sample ID, sex, age, name of location, collector's name, etc) in a datasheet

Advantages:

- DNA yield and quality higher than for methods 1 and 2
- Simple
- Small contamination risk
- Non flammable

Disadvantages:

- Expensive (~US$ 2.50 / sample)

4) 20% DMSO / saturated $NaCl_2$

This method works very well to preserve small quantities of tissue at room temperature. It is basically like pickling meat. The DMSO helps to rupture cell structures, so the salt can penetrate the cells much quicker to denature proteins.

Materials required:

- 8 ml polypropylene tube, lid with o-ring (eg. Sarstedt 60.542.007)
- 20% (v/v) di-methyl sulphoxide (DMSO) / saturated (5 M) $NaCl_2$ solution (needs to made before sampling)
- Parafilm

Procedure:

- Add 4ml of DMSO/sat. $NaCl_2$ to tube
- Place a grape-sized sample in the tube, make sure that it is fully submerged
- Seal tube with Parafilm
- Can be stored at room temperature for several months
- Label the tube with relevant information
- Record relevant information (date, GPS coordinates, sample ID, sex, age, name of location, collector's name, etc) in a datasheet

Advantages:

- Cheap
- Non-flammable

Disadvantages:

- Needs to be prepared ahead of field work
- Not suitable for fecal material

5) Storage techniques for shed hair:

Materials required:

- Gloves (one pair per sample)
- Tweezers
- Lighter
- Paper envelope
- Silicagel

Procedure:

- Wear gloves
- Collect the hairs with tweezers that are sterilized with a flame between each use.
- Place hairs into a clean paper envelope
- Place the envelopes in a plastic bag (Ziploc) together with silicagel (if possible)
- Record the following information: date, GPS coordinates, sample ID, nest age, height of nest from ground, collector's initials, and reference to any faecal samples collected nearby.

We thank Todd Dissotell for providing information regarding the RNA later storage method.

Suggested reading:

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