TAPIR SPECIALIST GROUP

SAMPLING TECHNIQUES FOR GENETIC ANALYSIS

Introduction

The goal of the next few pages is to delineate sampling procedures to collect material that is suitable for use in genetic analysis. To a field biologist or veterinarian there are numerous possibilities of good samples for this type of analysis. Anything from fur to feces can yield good quality DNA, **if properly collected and preserved**.

General Sampling Considerations

The major difficulty in collecting biological material for genetic purposes is keeping the DNA from degrading. The main degrading agents of DNA in most samples are enzymes with DNAse activity. These can either be present in the sample, or be produced by growing microorganisms. Therefore, the chief goal is to inactivate or prevent these enzymes from acting.

There are several ways to inactivate enzymes. But, they are usually expensive, or require reagents that are not normally available to most field biologists and veterinarians. On the other hand, preventing its activity is much easier, and is generally carried out by dehydrating the sample. The most common, efficient and easy ways of doing this is by:

1. Dehydrating the sample using silica gel, or some other desiccant. These are usually readily available at supermarkets and crafts and flower stores (used to dry flowers). Drier samples are usually easy to transport, yet might face some restrictions from importing countries, for they might still contain viable spores, virus and bacteria that might infect people and livestock in those countries.

2. Dehydrating the sample using Ethanol (70-100%). The lower the concentration the easier to transport, for it becomes less flammable. Yet, the lower the concentration the more degraded the DNA gets. So, a concentration of less than 70% is not recommended. Additionally, anything other than Ethanol (such as Methanol) is not recommended either, for it might interfere with the DNA extraction, or amplification.

Therefore, most samples that you collect will either be preserved dry or in Ethanol. A part from this, you should also keep mind:

1. **Labeling:** Always label your samples, and keep an extra log. Sample labels should include a reference number, the date it was collected, the location, and species. The reference number should be used to reference the sample to a log with the same information on the label, plus the collector's name, the GPS measurement of the exact location of sampling, and any other observations deemed necessary. The GPS measurement is essential information for many genetic analyses, and should always be taken. Finally, when labeling use appropriate markers. For example, when labeling a tube with ethanol either use permanent ink pens, that don't come off with Ethanol, or use baking paper marked with a pencil, and put the paper inside the tube. Ideally, you should use both.

2. **Avoid contamination:** It is very easy to get human DNA on samples. For most analysis this is not an issue, because the analyses use methods that are specific to the species in consideration, or use procedures that don't pick-up human DNA from the sample. Nevertheless, for some applications this might be an issue, especially if we are trying to sex individuals. It is not necessary to wear gloves and a mask if you are out in the field, but you should be careful to avoid manipulating any sample with your hands. More importantly, we want to avoid cross-contamination among samples. Therefore, always sterilize your scalpel before taking a tissue sample, always use a new twig to manipulate a new dung sample, and always use a new syringe to collect a new blood sample.

3. **The more is often not the better**: It is sometimes tempting to collect as much material as possible. Yet, for example, filling up a sampling tube with tissue or feces is not the best way to preserve the genetic material. By filling up the sampling tube with biological material you leave very little space for the preserving agent. If you are preserving your feces sample in Ethanol, you want to make sure that there is enough Ethanol in the tube to cover the whole sample. That way you make sure that samples are preserved. Otherwise, we are left with a whole lot of useless samples! Now, if you do come across a nice pile of fresh dung, and you feel the need to collect as much as you can, you can always spread the sample into two or three different sampling tubes.

Types of Samples

Hair - One of the best samples

Hair is probably one of the best samples to collect. It is both easy to preserve and transport, and very easy to extract good quality DNA from. If you are trapping animals in the field, or can gain access to zoo animals from known origin, this is probably the best method. Also, if you are out in the field, and can easily find fur, say on scratching posts, this is probably the best sample, too.

If you are pulling hair samples out of an animal, look for coarse hairs that are clean and hard to remove. These hairs usually yield the hair follicles, or bulb, or root, which contains living tissue from which we can extract DNA. To obtain sufficient quantity of DNA a total of 5-10 hairs with bulbs from each individual are required. Always make sure the hairs contain the hair bulbs, otherwise they are of no use.

Hair samples are best preserved dry, in sealed unwaxed envelopes. You can label the sample by writing information on the envelopes. You can then keep several envelopes in a separated container or plastic bag with silica gel or some other desiccating agent to help keep the samples dry. Unwaxed envelopes are porous, so they allow the sample to breath.

Scats - Probably the next best thing

Scats, or feces are very easy to collect, and yield reasonably good DNA when collected fresh. The other great thing about feces is that we don't need CITES permits to transport them, and if they are preserved in Ethanol we don't need permission from most governments to import them. Although, we might still require export permits from some countries. Finally, they are great because it doesn't require any handling of the animals, so it avoids stressing them, and we usually can obtain more samples this way.

Two things to keep in mind when collecting feces is that we want it as fresh as possible, and we want only the outer layer. The outer layer was last in contact with the animals intestinal track, so this is the layer with the animal's cells. If the outer layer has any fungi, insects, or any other possible contamination DNA extraction probably won't work. On the other hand, if you are sampling in a really dry environment, and the outer layer is very dry, we should be able to get DNA from it, even it is a few weeks old. The other important thing to keep in mind is to avoid manipulating the feces with bare hands. It avoids contamination of the samples, and it preserves your own health. The best thing is to break-off a twig or branch from a nearby tree, and then you can break this twig in half, and use the newly broken portion to handle the dung.

To collect a sample you open the sample tube (ideally use 5ml tubes), and put the lid facing up. With the twig in your hand bring the mouth of the tube close to where you want to sample. You then use the twig to scrape off around 1cm^2 of the surface into the tube. You should then sample another 1cm^2 at some other portion of the dung. You should now have around 2ml of dung in the tubes. You should then fill the tube up with Ethanol (between 70-100%), this way you should have a ratio of 2:3 of dung to Ethanol. Close the tube, and wrapped the cap with parafilm to avoid leaks, throw away the twig and use a new one for each sampling tube – this is very important to avoid cross-contamination between tubes. Remember to label all your samples. Feces samples preserved in this manner can be stored at room temperature.

In the case that your animal defecates in water, there are a few studies that have successfully extracted DNA from feces samples of Dolphins and Dugongs. In the case of the dolphin, the researchers were with the animals when they defecated, and were able to sample immediately after the animal defecated. Therefore, the samples were very fresh. In the case of the Dugong, samples were found floating after an indeterminate amount of time, individually contained in plastic bags, and frozen until DNA extraction was possible. If you do find Tapir dung under, or floating in water, and it appears intact, we suggest removing a portion containing surface using a clean plastic bag (one per sample), and then use the procedure described above. Further testing is still required to evaluate if this protocol is adequate, but it should yield some DNA if the sample is relatively fresh.

As a final comment, the sampling should be done in the field. It is tempting to collect as much dung as you can in the field, and then sort it out in a lab. This should be avoided as much as possible, by undergoing the whole procedure in the field you avoid possible cross-contamination, and you it all gets done at once.

Tissue Samples - Good DNA, difficult to transport

Tissue samples can yield very good DNA material, yet they are hard to transport because of CITES and other regulations. Yet, if you are trapping animals, or work in an area that experiences road-kills or even hunting you might come across quite a few of these types of samples, and it would be a shame to let them go to waste. When collecting tissue samples always keep in mind that we need very little, and that you should always sterilize your scalpel before collecting a new sample.

If you come across a road-kill, or you are trapping animals the best part to sample is the ear. A small fragment of 1cm^2 or less provides excellent DNA in both quality and quantity. A small piece of the ear is much better than a portion of muscle, for example, because it doesn't rot as easily. Nevertheless, if you do come across fresh meat (say from some hunter), smell the meat, if smells bad then DNA extraction probably won't work. If it doesn't, you can cut out a few mm^2 of the tissue to be used.

The best way to preserve tissue samples is by immersing it in Ethanol. You have to be mindful that there is enough Ethanol in the sample tube to cover the whole sample. If you are only sampling a couple of mm^2 of tissue, you can use 2ml tubes, and fill them up with Ethanol. Again, you should wrap the cap with parafilm to avoid leakage, and label the tubes appropriately. Tissue samples preserved in this manner can be kept at room temperature.

Blood Samples - Excellent DNA, but costly

Blood produces probably the best quality DNA, yet is both expensive to obtain and to extract DNA from. Plus, it almost always involves knocking the animal down to be obtained, which is not ideal if you want large quantities of samples.

The best way to preserve blood is either using vacutainers (with EDTA - purple top) or Easy Blood Buffer (EBB). If using vacutainers, make sure to mix well so that the EDTA get in solution, and keep refrigerated. If using EBB you should mix one volume of blood with one volume of EEB, and also keep it refrigerated. We

usually only need very little blood to be able to extract DNA, therefore about 1ml of blood should be more than sufficient for genetic purposes.

Other Types of Tissue

Bones and teeth can yield some DNA, but it is difficult and an unreliable source. Additionally, bones can be hard to transport. Therefore, we don't recommend using this material for genetic analysis.

Shipping Considerations

When shipping your samples to another country we recommend that you use some sort of courier service, such as FedEx or UPS. This means that shipping costs are a little higher, but it does mean that samples are moved quickly. Also, declare a nominal value of $1 on customs forms, and state "Value for customs purposes only". Customs will interpret higher values to mean that we are buying your samples from you, and this will result in import duties and taxes being assigned. Remember also to include a copy of your export/import and CITES permits with the shipping invoice.

Further Reading

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Good Internet Resources

Field Vet Program (WCS) - www.fieldvet.org

Wildlife Genetics, Inc - www.wildlifegenetics.ca/pages/samples