Appendix III

SENCKENBERG
world of biodiversity

Centre for Wildlife Genetics

Information on sampling and preservation of sample material for genetic analyses of captive lynx

1. General information before sampling

Enclosed in this package you will find:

- ✓ Sealed and labelled plastic cups with 33 ml undenatured ethanol (96 %)
- ✓ Labelled ziplock bags with filter paper and desiccant
- ✓ Swabs (separat packaged)
- ✓ One-way tweezers (separately packaged)
- ✓ Nitrile gloves
- ✓ Scalpels
- ✓ Pencil (for labelling the samples)

The collection of samples from captive lynx and the professional and timely preservation of the collected material is of decisive importance for a successful genetic analysis. To avoid contamination with alien DNA, the sample collection should always be carried out with the highest care possible. Therefore, the following basic rules should be followed:

- Disposable gloves should be worn during all steps of sample collection to avoid contamination. Gloves should also be changed during sampling if potentially contaminated packaging, pens or other objects have been touched. Changing gloves between each sampling is strongly advised.
- Every sample container (cups/bags) and every sampling utensils (tweezers/swabs/scalpels) should only be used for ONE sample from A SINGLE individual.
- None of the substances in the sampling kit are hazardous to health.

2. Sampling

The following applies to all samples: Please completely fill out the labels attached to the sample containers (plastic cups/ziplock bags)! If possible, always give the **name of the animal** AND the **studbook number (ESB)** AND the **local ID**. If the husbandry conditions make it impossible to reliably assign a sample to an individual, please state all possible individuals from whom the sample could have originated (or, if possible, exclude individuals). This information is of great importance for genetic analyses.

In our many years of experience, conscientious observance of the instructions for handling samples leads to an increase in the success rates of the subsequent genetic analysis. After consultation, we will be happy to put together and send you further suitable sampling material. Please do not hesitate to contact us (even spontaneously by telephone) if you have any questions or are unclear about the sampling and treatment of sample material. You can find the contact on page 2.

Plastic cups with 33 ml undenatured ethanol (96 %)

Faeces is picked up with a glove or one-way tweezers and placed in the plastic cup filled with ethanol (see picture). The ethanol should completely cover the faecal sample. For larger amounts of faeces, a piece of 3-5 cm should be cut from one of the ends and placed in the cup. Often there is a shiny layer on the surface of the faeces shed by the intestinal mucous membrane, which is particularly suitable for DNA analyses. When separating large faecal pieces, those with a mucus layer, should preferably be sampled, if available. The plastic cubs should be labelled with a pencil. The yellow lid should be closed tightly.

If it is the case that **tissue samples** are available, these should also be placed in one of the plastic cups, also well covered by ethanol. To cut, please use one of the sterile scalpels provided.

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Ziplock bag with filter paper and desiccant

The ziplock bags are primarily intended for **hair** and **saliva samples**. The sampling of which is particularly useful for veterinary examinations or translocations of the animals. The samples are placed in the folded filter paper. The desiccant should be left in the ziplock bag.

To take a **hair sample**, you should ideally pull out around 30 hairs with your fingers (please wear gloves!). So that the hair follicles are preserved. Then place the hair in the folded filter paper within the bag. Please do not fix any hair on the adhesive surface, which will hinder the later analyses. Under certain circumstances, lynx can leave hair behind at their berths. Whenever possible, berths should be checked for hair left behind. Hair on rubbing areas or on walls in stables (if there are any) is also of interest.

Saliva (as an invasive oral swab sample) is preferably collected using the cotton swabs. To do this, please rub at least one swab vigorously back and forth on the oral mucosa and turn it around its own axis in order to absorb as much DNA-containing material as possible. After sampling, the swabs are placed in folded filter paper and stored in a ziplock bag with a desiccant.

Alternatives

If **blood samples** (e.g. EDTA blood samples in stock) are already available from previous or current treatments, these are also of interest for the analyses. We usually not advise collecting and sending blood samples - also in commercially available EDTA tubes - as it is difficult to send them in a cooled state and the DNA in the EDTA solution degrades very quickly. Otherwise, such samples should be sent in a well-cooled condition. Alternatively, cotton swab samples can be taken from such blood samples and preserved as described in the saliva sampling protocol above. Let the cotton swab fully soak up blood beforehand. If sedimented or clumped blood samples are available, please take up the cellular component (haematocrit).

3. After the sampling

After sampling, the samples should be preserved as quickly as possible, depending on the type of sample, by drying them or soaking them in 96% ethanol. Store all samples in or below room temperature and out of direct sunlight. Sample material should never be sent in an untreated condition. Alternative preservation methods should be discussed with us before use. All the waste generated can be disposed of as residual waste.

The samples including completely filled out labels will be sent to the following address:

Senckenberg Gesellschaft
Zentrum für Wildtiergenetik
Probenannahme
Clamecystraße 12
63571 Gelnhausen
GERMANY

Contact: Michelle Mueller
Phone: +49(0)6051-61954-3138
E-mail: forschung.wildtiergenetik@senckenberg.de