

Supplementary Material

Capturing environmental DNA in snow tracks of polar bear, Eurasian lynx and snow leopard towards individual identification

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1. Supplementary Data

S1: Protocol for collection and post-collection processing of snow tracks

1 Sampling instructions snow tracks

1.1 Preparation for field collection

- 1.1.1 Clean large metal ladle (for smaller animals - tablespoons) by immersing them in a household bleach solution (1 bleach:4 water). Use gloves and mouth protection – clean the rubber boots you are using with the bleach solution and rinse.
- 1.1.2 Let soak for an hour.
- 1.1.3 Pick up the spoons and transfer to a container with distilled water.
- 1.1.4 Let dry and wipe with 70% EtOH with a cloth, let dry.
- 1.1.5 Transfer the spoons to a clean container, alternatively one spoon per kit.
- 1.1.6 Recommended 5 - 10 L sterile sample bags (Whirl-Pak®) for polar bears and 3 L for smaller animals. If plastic containers with lids are used instead of sampling bags - clean the containers by wiping them inside out with the bleach solution using a cloth. Rinse with distilled water, wipe off with 70% EtOH. Wipe dry with household paper or let air dry.

1.2 Assembly of field kits:

- 1.2.1 Choose unique sample names for each sample. For example, PB_21_01 (Polar Bear 2021, sample 01).
- 1.2.2 Mark two adhesive labels with the sample name.

Supplementary Material

1.2.3 Put one label on a Whirl-Pak® bag and 1 label on a resealable bag, alternatively use precleaned plastic containers with lids and mark the container with a label.

1.2.4 If the weather is warmer and the collections are done when mouth not covered, use surgical face masks to avoid breathing on the tracks.

Each kit contains:

- 1 x Whirl-Pak® bag with label inside
- 1 x sterile spoon or ladle
- 1 x surgical mask
- 2 x sterile gloves

1.3 Field Sampling:

1.3.1 Set the camera on GPS before heading out, if too cold, use a GPS.

1.3.2 Identify polar bear tracks, preferably tracks used by a single individual.

1.3.3 Place the marked sample bag next to the track, take a picture in order to receive coordinates and sample name. Take a picture of the track.

1.3.4 Put on the surgical mask provided in the kit.

1.3.5 Put on gloves (if needed, otherwise be careful not to touch the samples with hands)

1.3.6 Take out the metal spoon/ladle from the kit and open the labelled Whirl-Pak® bag OR the labelled plastic container.

1.3.7 Scrape the polar bear track from the snow and place in the provided Whirl-Pak® bag OR plastic container.

1.3.8 Collect as many tracks as possible. If more than one container is needed from one individual, make a note.

1.3.9 Record number of tracks per sample, and if possible, male/female or cub (M, F, C)

1.3.10 Place the Whirl-Pak® bag inside the resealable bag OR close the lid of the container. Pack the bags for transport to the laboratory.

1.3.11 Record temperature, wind and type of snow, hard, soft, ice etc.

1.3.12 Make an estimate of the freshness of the tracks.

1.3.13 Put the used spoons in a separate bag for cleaning and reuse after the field trip.

1.3.14 Take a negative field sample, i.e., collect snow without tracks to control for field contamination.

1.3.15 Fill in the field protocol after arrival to the lab.

1.3.16 If samples will be filtered later, pack the snow, seal the bags and place in -20C freezer. If samples need to be transported to other laboratories, please Otherwise go to step 17.

1.3.17 Place the snow containers at a distance from each other indoors and allow the snow to melt in the bags OR plastic containers, it can take several hours.

Note.

- It is very important that the samples do not get contaminated by biological materials from other individuals. Be vigilant in snow collections and during processing after collection.
- Make sure not to use the same spoon for more than one set of tracks, change spoons for each sample.
- Do not collect tracks from many individuals in the same bag.
- Hair samples can be collected, keep them dry and in an envelope in room temperature after collection (no freezing).
- Try to keep samples and containers away from other animal materials in the field.

2 Post-collection processing of footprints in snow: filtration and fixation

2.1 Materials:

- Dishwashing cloths x 2
- Chlorine (household bleach)
- 70% EtOH
- Distilled water
- 50 ml luer-lock syringe or peristaltic pump with hose
- 2 mL syringe
- Filter (disc filter or Sterivex filter 0,22 or 0,45 (Merck-Millipore, Germany)
- Ethanol 99% 200 proof Molecular Biology Grade (Thermo Fisher Scientific Inc., Waltham, MA, USA).
- Small resealable plastic bags
- Luer lock inlet and outlet caps
- Surgical mask
- 1 pair of nitrile gloves for each sample order sizes M and XL
- Tissue paper
- Parafilm
- 1 x larger Ziplock bag or plastic container with lid for sample storage
- Pre-filled labels (with sample name) and marker pens
- Measuring cup

2.2 Preparation of workspace:

2.2.1 Change to clean clothes

2.2.2 Tie long hair

2.2.3 Wash hands with soap and dry

2.2.4 Put on surgical mask

2.2.5 Put on clean lab gloves

2.2.6 Make a chlorine solution 1 chlorine:3 water in a container

2.2.7 Use a garbage bag put on the floor/table and wipe with chlorine solution with moist cloth

2.2.8 Wipe with 70% EtOH using moist cloth

Supplementary Material

2.3 Preparation of filtration kits:

Prepare all the kits before starting the filtering procedure.

Each kit contains:

- 1 x pair of gloves
- 1 x filter
- 1x 60 mL sterile luer-lock syringe (you can keep them in the main box and take them out for each sample) OR clean tubing for peristaltic pump
- 50 mL sterile tube with lid
- 1 x 2 mL syringe
- Female cap
- Male cap
- Molecular grade 95% (or higher) ethanol 200 proof (- call it DNA preservative.
- 1 x 2 cm parafilm
- Surgical mask (same can be used throughout the procedure)

2.4 Filtration and fixation instructions

2.4.1 Put on a surgical mask.

2.4.2 Put on clean gloves.

2.4.3 Pour the EtOH from the main bottle into a 50 mL tube with lid, count on 2 mL per sample, include the negative filter control and add 2 mL extra.

2.4.4 Fill the 2 mL syringes with 1.5 mL ethanol and close the tip of the syringe with a female cap. Prepare syringes for all the samples you will process for the day. Make an extra one for negative filter control.

2.4.5 Place one male cap into one small pre labelled plastic zip lock bag for each sample

2.4.6 Add a parafilm strip to each kit.

2.4.7 Take out the filters and labels.

2.4.8 Put the prefilled 2 mL syringe, the labelled filter and the small, labelled bag with caps into a larger Ziplock bag.

2.4.9 Bring out melted snow sample

2.4.10 Filter the sample using a syringe (step 2.4.11 and 2.4.12) or a peristaltic pump with sterile tubing (step 2.4.13)

2.4.11 Fill the syringe with the melted snow and push through the filter into the measuring cup.

2.4.12 Filter until all the snow has gone through the filter.

2.4.13 Alternatively use a peristaltic pump

2.4.14 Record the volume of melted snow filtered in mL

- 2.4.15 Flush the filter with air by filling the syringe with air, until filter is dried (3 x air flush)
- 2.4.16 Take out the 2 mL EtOH filled syringe, attach to the inlet side of the filter. Inject the liquid from below. When liquid comes through the filter, use the cap on the syringe and close the inlet end.
- 2.4.17 Turn the filter and inject the rest of the preservative.
- 2.4.18 Cap the other end of the filter.
- 2.4.19 Wipe dry with tissue paper.
- 2.4.20 Seal the closed caps with parafilm.
- 2.4.21 Place the labelled filter into the pre-marked small bag.
- 2.4.22 Place in a container.
- 2.4.23 Put the used syringes etc., in a container for disposables.
- 2.4.24 Clean the workspace following steps 7 and 8 under section 4.2.
- 2.4.25 Prepare a negative filter control where you filter 500 mL distilled water through a filter, fix as the real samples.
- 2.4.26 Store the filtered and fixated samples in their individual sealed small Ziplock bags in an airtight container in +4°C or in -20°C until downstream applications are applied.

3 Supplementary Figures and Tables

Supplementary tables:

Table S1: Collection and filtration information and microsatellite genotyping success for snow tracks and positive controls from wild and captive polar bears, *U. maritimus*, Eurasian lynx, *L. lynx* and captive snow leopards, *P. uncia* (F footprints in snow, N/A not applicable).

| ID | Species | Source | Type | Location | Air temperature (°C) | Collection date | No. footprints | Volume snow filtered (ml) | No. loci genotyped/ Total no. tested |
|--------|------------|--------|------|----------|----------------------|-----------------|----------------|---------------------------|---|
| PBA_01 | Polar bear | Wild | F | Alaska | -33.0 | 4/01/2019 | 20 | 580 | 3/5 |
| PBA_02 | Polar bear | Wild | F | Alaska | -33.0 | 4/01/2019 | 25 | 690 | 5/5 |
| PBA_03 | Polar bear | Wild | F | Alaska | -32.0 | 5/01/2019 | 14 | 780 | 5/5 |
| PBA_04 | Polar bear | Wild | F | Alaska | -32.0 | 5/01/2019 | 40 | 2160 | 5/5 |
| PBA_05 | Polar bear | Wild | F | Alaska | -32.0 | 5/01/2019 | 16 | 940 | 0/5 |
| PBA_06 | Polar bear | Wild | F | Alaska | -32.0 | 5/01/2019 | 40 | 1890 | 5/5 |
| PBA_12 | Polar bear | Wild | F | Alaska | -27.0 | 5/01/2019 | 30 | 880 | 5/5 |
| PBA_07 | Polar bear | Wild | F | Alaska | -32.0 | 5/01/2019 | 25 | 590 | 4/5 |
| PBA_08 | Polar bear | Wild | F | Alaska | -32.0 | 5/01/2019 | 20 | 810 | 0/5 |
| PBA_09 | Polar bear | Wild | F | Alaska | -32.0 | 5/01/2019 | 30 | 795 | 5/5 |
| PBA_10 | Polar bear | Wild | F | Alaska | -32.0 | 5/01/2019 | 30 | 840 | 4/5 |
| PBA_14 | Polar bear | Wild | F | Alaska | -27.0 | 5/01/2019 | 30 | 685 | 5/5 |
| PBA_15 | Polar bear | Wild | F | Alaska | -33.0 | 6/01/2019 | 20 | 630 | 4/5 |
| PBA_11 | Polar bear | Wild | F | Alaska | -33.0 | 6/01/2019 | 20 | 540 | 3/5 |
| PBA_18 | Polar bear | Wild | F | Alaska | -33.0 | 6/01/2019 | 20 | 420 | 5/5 |
| PBA_16 | Polar bear | Wild | F | Alaska | -33.0 | 6/01/2019 | 30 | 390 | 2/5 |
| PBA_22 | Polar bear | Wild | F | Alaska | -32.0 | 8/01/2019 | 20 | 695 | 5/5 |
| PBA_23 | Polar bear | Wild | F | Alaska | -32.0 | 8/01/2019 | 15 | 630 | 3/5 |
| PBA_24 | Polar bear | Wild | F | Alaska | -32.0 | 8/01/2019 | 13 | 470 | 5/5 |

| | | | | | | | | | |
|---------|---------------|-----------|------|-----------|-------|------------|-----|-----|-----|
| PBA_25 | Polar bear | Wild | F | Alaska | -32.0 | 8/01/2019 | 22 | 515 | 5/5 |
| PBA_28 | Polar bear | Wild | F | Alaska | -32.0 | 8/01/2019 | 12 | 300 | 0/5 |
| PB22_1 | Polar bear | Wild | F | Alaska | -2.0 | 9/05/2022 | - | - | 5/5 |
| PB22_2 | Polar bear | Wild | F | Alaska | -2.0 | 9/05/2022 | - | - | 5/5 |
| PB22_3 | Polar bear | Wild | F | Alaska | -2.0 | 9/05/2022 | - | - | 4/5 |
| PBO_04 | Polar bear | Captivity | Hair | Sweden | - | 3/18/2018 | NA | NA | 5/5 |
| PBO_05 | Polar bear | Captivity | F | Orsa Zoo | - | 3/18/2018 | - | - | 5/5 |
| PBO_7_8 | Polar bear | Captivity | F | Orsa Zoo | - | 3/18/2018 | - | - | 5/5 |
| PBR_12 | Polar bear | Captivity | F | Rauna Zoo | - | 4/18/2018 | - | - | 3/5 |
| PBR_13A | Polar bear | Captivity | Hair | Rauna Zoo | - | 4/18/2018 | NA | NA | 5/5 |
| PBR_13B | Polar bear | Captivity | F | Rauna Zoo | - | 4/18/2018 | - | - | 5/5 |
| LO_01 | Eurasian lynx | Wild | F | Sweden | 0 | 19/12/2018 | 9 | 150 | 2/3 |
| LO_02 | Eurasian lynx | Wild | F | Sweden | 0 | 19/12/2018 | 10 | 255 | 0/3 |
| LO_03 | Eurasian lynx | Wild | F | Sweden | 0 | 19/12/2018 | 10 | 432 | 3/3 |
| LO_04 | Eurasian lynx | Wild | F | Sweden | 0 | 19/12/2018 | 10 | 585 | 1/3 |
| LO_05 | Eurasian lynx | Wild | F | Sweden | 2 | 19/12/2018 | 15 | 158 | 1/3 |
| LO_6 | Eurasian lynx | Wild | F | Sweden | 2 | 19/12/2018 | 12 | 225 | 2/3 |
| LO_08 | Eurasian lynx | Wild | F | Sweden | 2 | 19/12/2018 | 12 | 135 | 0/3 |
| LO_09 | Eurasian lynx | Wild | F | Sweden | 2 | 19/12/2018 | 7 | 125 | 3/3 |
| LL015 | Eurasian lynx | Wild | F | Sweden | -6 | 24/01/2019 | 17 | 130 | 3/3 |
| LL016 | Eurasian lynx | Wild | F | Sweden | -6 | 24/01/2019 | 22 | 120 | 2/3 |
| LL017 | Eurasian lynx | Wild | F | Sweden | -4 | 24/01/2019 | 20 | 155 | 1/3 |
| LL018 | Eurasian lynx | Wild | F | Sweden | -4 | 24/01/2019 | 20 | 160 | 1/3 |
| LL019 | Eurasian lynx | Wild | F | Sweden | -4 | 24/01/2019 | 15 | 120 | 2/3 |
| LL020 | Eurasian lynx | Wild | F | Sweden | -4 | 24/01/2019 | 18 | 140 | 3/3 |
| LL023 | Eurasian lynx | Wild | F | Sweden | N/A | 27/01/2019 | N/A | 140 | 3/3 |
| LL025 | Eurasian lynx | Wild | F | Sweden | -4 | 31/01/2019 | 12 | 210 | 2/3 |
| LL026 | Eurasian lynx | Wild | F | Sweden | -2 | 31/01/2019 | 12 | 220 | 2/3 |
| LL033 | Eurasian lynx | Wild | F | Sweden | -4 | 31/01/2019 | 16 | 150 | 2/3 |

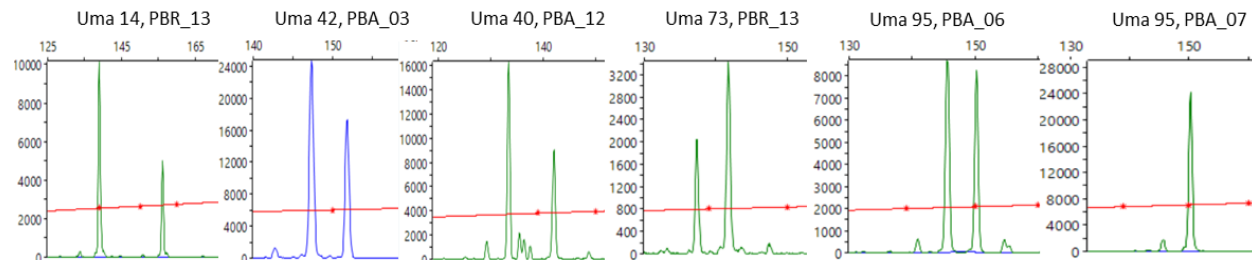
Supplementary Material

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|--------|---------------|-----------|--------|-------------|-----|------------|-----|-----|-----|
| LL035 | Eurasian lynx | Wild | F | Sweden | -4 | 31/01/2019 | 16 | 150 | 2/3 |
| LL039 | Eurasian lynx | Wild | F | Sweden | -4 | 31/01/2019 | 12 | 180 | 3/3 |
| LL046 | Eurasian lynx | Wild | F | Sweden | -3 | 31/01/2019 | 12 | 280 | 2/3 |
| LL048 | Eurasian lynx | Wild | F | Sweden | -1 | 31/01/2019 | N/A | 180 | 0/3 |
| LL049 | Eurasian lynx | Wild | F | Sweden | -1 | 31/01/2019 | N/A | 95 | 0/3 |
| LL050 | Eurasian lynx | Wild | F | Sweden | N/A | 31/01/2019 | N/A | 170 | 0/3 |
| LL054 | Eurasian lynx | Wild | F | Sweden | -3 | 31/01/2019 | 12 | 280 | 0/3 |
| LL_27 | Eurasian lynx | Wild | F | Sweden | - | 16/01/2021 | N/A | 170 | 0/3 |
| LL_28 | Eurasian lynx | Wild | F | Sweden | - | 16/01/2021 | N/A | 200 | 0/3 |
| Lx_01 | Eurasian lynx | Wild | F | Sweden | - | 05/02/2021 | 11 | 200 | 0/3 |
| Lx_03 | Eurasian lynx | Wild | F | Sweden | - | 05/02/2021 | 20 | 300 | 0/3 |
| Lx_04 | Eurasian lynx | Wild | F | Sweden | - | 05/02/2021 | - | 200 | 0/3 |
| Lx_06 | Eurasian lynx | Wild | F | Sweden | - | 05/02/2021 | 21 | 480 | 3/3 |
| Lx_07 | Eurasian lynx | Wild | F | Sweden | - | 05/02/2021 | - | 400 | 3/3 |
| Lx_08 | Eurasian lynx | Wild | F | Sweden | - | 05/02/2021 | 18 | 180 | 0/3 |
| Lx_09 | Eurasian lynx | Wild | F | Sweden | - | 05/02/2021 | 11 | 240 | 0/3 |
| Lx_11 | Eurasian lynx | Wild | F | Sweden | - | 05/02/2021 | - | - | 0/3 |
| Lx_15 | Eurasian lynx | Wild | F | Sweden | - | 09/02/2022 | - | - | 0/3 |
| Lx_27 | Eurasian lynx | Wild | F | Sweden | - | 04/02/2021 | 7 | 320 | 3/3 |
| Lx_28 | Eurasian lynx | Wild | F | Sweden | - | 04/02/2021 | 5 | 185 | 0/3 |
| Lx_31 | Eurasian lynx | Wild | F | Sweden | - | - | 8 | 350 | 1/3 |
| LyU_10 | Eurasian lynx | Wild | F | Sweden | -4 | 17/01/2021 | 32 | 200 | 0/3 |
| LyU_12 | Eurasian lynx | Wild | F | Sweden | -4 | 17/01/2021 | 11 | 95 | 1/3 |
| LyU_01 | Eurasian lynx | Wild | F | Sweden | -4 | 17/01/2021 | 14 | 300 | 2/3 |
| LyU_02 | Eurasian lynx | Wild | F | Sweden | -4 | 17/01/2021 | 14 | 300 | 2/3 |
| LyU_08 | Eurasian lynx | Wild | F | Sweden | -4 | 17/01/2021 | 14 | 600 | 0/3 |
| 4452 | Eurasian lynx | Captivity | Saliva | Nordens Ark | - | 18/03/2018 | N/A | N/A | 3/3 |
| 4454 | Eurasian lynx | Captivity | F | Nordens Ark | - | 18/03/2018 | 20 | 280 | 3/3 |

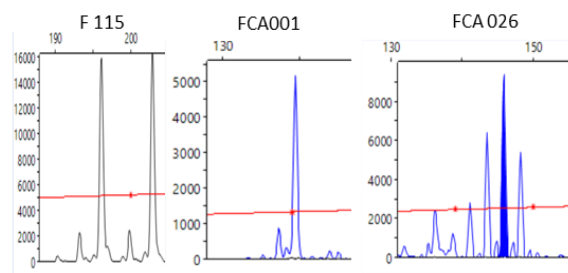
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|----------|---------------|-----------|----------------|-------------|---|------------|-----|-----|-----|
| 4456 | Eurasian lynx | Captivity | Hair | Rauna Zoo | - | 19/04/2018 | N/A | N/A | 3/3 |
| 4457 | Eurasian lynx | Captivity | F | Rauna Zoo | - | 19/04/2018 | 20 | 280 | 3/3 |
| SL-T_09 | Snow leopard | Captivity | Nasal mucus | Nordens Ark | - | 25/1/2021 | N/A | N/A | 3/3 |
| SL_Et_10 | Snow leopard | Captivity | F | Nordens Ark | - | 25/1/2021 | 13 | 190 | 3/3 |

3.1 Supplementary Figures

A. Polar bear



B. Snow leopard



C. Lynx

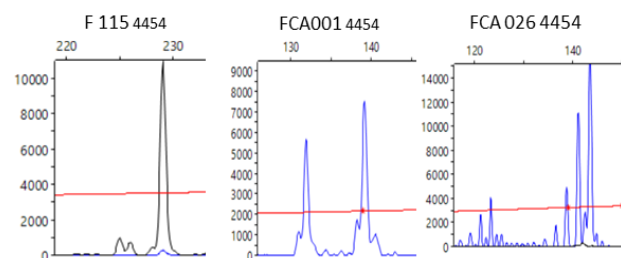


Figure S1: Example DNA electropherogram traces originating from snow tracks from a) wild polar bears (*U. maritimus*) at microsatellite loci Uma 14, Uma 40, Uma 42, Uma 73, Uma 95, b) captive snow leopard, (*P. uncia*) at F115, FCA001, FCA026, and c) wild Eurasian lynx (*L. lynx*) at F115, FCA001, FCA026.