

Sampling protocol

1. Reminder of criteria to be used sample collection

- 33 unrelated animals per breed.
- 10-15 males.
- 3 unrelated animals (known different parents) per flock from 11 flocks covering the whole area of origin. Adults if possible.
- If sensible sample sheep and goats from the same area

Biological material:

- Target 20 micrograms of DNA (sample enough material!)
- Source: BLOOD, TISSUE and HAIR (the last for quality check). A detailed downloadable protocol for sampling and sample storage will be available on the WEB.

Additional data

- Filling of questionnaire regarding sampling addressed to sampler
- Filling of the socio-economic questionnaire addressed to farmers
- Filling of the questionnaire on breeds and on region addressed to Partners and Subcontractors

2. Materials needed

- Questionnaire regarding sampling
- Questionnaire regarding socio-economics addressed to farmer
- Vacuum vials containing 3.2% sodium citrate or EDTA as anticoagulant for blood samples
- Vials for tissue sample
- Plastic bag or falcon tube for hair sample
- Ethyl alcohol 96%
- Needle (e.g. vacutainer) for blood sample
- Needle for biopsy
- Gloves
- Marker pens
- 1.5 m Stick (10 cm graduation)
- Portable Freezer or thermos-bag
- Camera for photo-documentation.
- White paper sheets
- Note book
- Plastic folders
- GPS recorder

- Sampling protocol
 - Photodocumentation protocol
 - GPS position protocol and questionnaire
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3. Protocol

- Select and capture the animal (33 animal per breed, 10-15 males, no more than 3 unrelated animal per flock).
- Assign to the animal the ID code according to ECONOGENE rules (two digit for species, two digit for country according to EU nomenclature, three digit for the name of the breed, two digit for sample serial number (e.g. third animals sampled from an Italian goats breed named Bionda: CHI-BIO03; fifth animal sampled from a Greek sheep breed named Sfakia: OaELSFK05).
- Start filling the questionnaire and the parts related to the animal (section 1 to 7).
- Write the ID code in capital letters on a white sheet of paper for the photo documentation.
- Take a picture of the animal following the rules of the photo-documentation protocol (insert stick and ID code into the image).
- Start collecting biological materials wearing gloves and labeling the vials for blood, tissue and hair samples in capital letters with the ID of the animals.
- Collect at least 20 ml of blood divided into two or more vials containing Sodium citrate or EDTA (use a new needle for each animal).
- Mix the vials gently by using inversion group vials belonging to the same animal. Place masking tape or rubber and store them into the freezer or thermo-bag.
- Collect 100-200 mg of ear tissue and store it into a vial containing 95% of ethyl alcohol (tissue collection is mandatory if blood cannot be collected and highly desirable if blood is collected).
- Collect hair sample from a clean area in which contamination from other animals hair is unlikely (be sure to pick the bulbs) and store it into a falcon tube.
- Record GPS position according to the GPS user protocol.
- Complete the sampling questionnaire (section 8 to12).
- Prepare a folder containing the sampling questionnaire, photo-documentation, vials of tissue samples and vials for hair samples.
- Select a new animal and repeat the procedure again.
- Fill the socio-economic questionnaire together with the farmer.

3.1 In the laboratory:

- Store immediately blood and tissue samples at $-20\text{ }^{\circ}\text{C}$ (store tissue in >6 volumes of 95% ETOH at room temp or at $+4\text{ }^{\circ}\text{C}$ —for easier storage and shipping).
- Store the hair samples at room temperature (very dry, with silica drying beads).
- Complete the questionnaire filling the part related to morphological characteristics according to the instruction supplied.
- Insert all the information on the web-site.
- Fill the questionnaire on breeds and on region addressed to Partners and Subcontractors