



## **standard operating procedure sampling animal material**

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## Sampling animal tissue and storage

Tissues must be cryo-frozen and stored in RNA-later as soon as possible after an animal 's death to preserve RNA. Therefore large numbers of tissues are to be sampled in a short time with a large group of people. They should be assembled in the following teams /roles

- Pre-sampling team. Makes all preparation at the location of slaughtering. Prepares the list of samples to be collected, all tubes, and equipment needed at location.
- Dissection team. Consisting at least of one veterinarian, persons who are responsible for the euthanasia of the animals, persons responsible for the identification of the tissues and dissection.
- Tissue tracker. Person who checks if all the tissues are sampled and stored in the wright way.
- Tissue handling team. Persons who will receive the tissues and will perform downstream processing for further storage.

One animal can be euthanized and dissected at a time.

### *Warning:*

1. This procedure uses the following hazardous chemicals:

- Liquid nitrogen
- Dry ice

Both liquid nitrogen and dry ice displace oxygen and are therefore asphyxiants. Both can cause cold burns. All staff using these chemicals are required to undergo cryogenics training. Both should be used in a well ventilated area and stored in such a way to prevent accidents. The Pre-sampling team is responsible for sampling location set-up.

1. This procedure has the following manual handling risks:

- Cutting from sharp knives
- Injury from large animals

Cut resistant gloves must be worn on the non-cutting hand. Only farm staff trained or slaughterhouse trained personal in animal handling will handle the animals.

#### Requirements:

- Pre-labelled aluminium cryogenic tubes
- Pre-labelled RNA-later 1.5 ml tubes filled with 1ml RNA-later
- Printed labels for bags
- Zip lock bags (20 x20 cm)
- Plastic petri-dishes (9cm and 15cm) (x per animal/tissue)
- Ethanol spray bottles
- Aluminium foil
- Gloves
- Markers
- Liquid nitrogen canister
- Cryo gloves, face shield, cryo tongs
- Waste bags
- Kim wipes/paper towels
- Surgical blades, tweezers, surgical scissors
- Replacement blades
- Operation clothes
- Shoe-protection
- Hair-net

#### Protocol:

#### Dissection team:

1. Euthanasia of animal according to slaughterhouse protocol.
2. Animal will be dissected , organs will be removed and transported to the tissue handling team.
3. Specific carcass samples will be collected in petri-dishes/plastic bags and handed over to the tissue handling team.
4. Animal head will be opened according to plan and handed over to the tissue handling team.

#### Tissue tracker:

5. checks the tissue sample list per animal.

5. Record tissue collection and place the samples in Liquid nitrogen and for the RNA later samples in specific animal box.
6. Record missing samples.
7. Make sure that all samples will be stored after sampling in the right environment.

Tissue dissection team:

1. Put new household foil on the working bench
2. Record of tissue
3. Makes a picture of tissue
4. Select the pre-defined sampling location of the tissue
5. Places the parts in plastic bags/or 15cm petri dishes
6. Cut smaller pieces in 9cm petri dish for further dissection
7. Prepare sampling for cryo conservation and for RNA-later conservation.
8. Checks every tube for correct animal id, and tissue id.
9. Freeze cryo samples according to protocol
10. Fill up tubes and hand tubes over to the tissue tracker
11. Record progress to tissue tracker
12. Clean down bench
13. Clean knives, tweezers and scissors with ethanol before re(use)
14. Repeat for next tissue





