

FR-AgEncode: a French pilot project to enrich the annotation of livestock genomes

Tissue sampling protocol 1b

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This protocol describes the snap-freezing of pooled samples from homogenous tissues. It does not describe the anatomical procedure to isolate a specific organ or tissue.

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This protocol applies to tissues or organs and covers two main cases:

- i) homogenous tissues: as several aliquots can be obtained, multiple aliquots are stored as snap_frozen in a single large tube;
- ii) the organ/tissue is very heterogeneous: because a single aliquot cannot be considered representative of the tissue complexity, multiple aliquots are stored as snap_frozen in the same (large) tube.

Required reagents and instruments

Liquid nitrogen in a storage tank (usually 25 L)

Dry ice in a large styroform box, from 3 to 10 kg according to number of samples to be collected

1 small styroform box (30 x 20 x 15) for temporary storage of liquid nitrogen

A pair of cryogloves

20 cm x 20 cm zip lock bags

1 stainless steel tray or enamel tray, approximate size 20 x 30 cm

Sterile disposable Petri dishes

Disposable scalpels

2 sterile clamps with smooth ends, 10 cm long and 15 cm long

A rack for 15 mL tubes

A 500 ml glass beaker

Surgeon gloves

Pre-labelled 15 mL cold-resistant FALCON tubes, use cold-resistant labels, which will have been checked before, label shows animal number, tissue code, protocol number, aliquot number,

A permanent marker to label the zip lock bag.

Paper towels

Waste bucket

Detergent

Ethanol spray bottles

A cleaning spray against RNase

Preparatory steps

Animal is stunned before being slaughtered by bleeding. A professional butcher is in charge of the slaughtering and of extracting the organ from the carcass, in a pre-determined order.

The organ is laid down in the tray. For large organs, the butcher cuts a piece of tissue which is laid down in the tray. Whole tracts such as digestive tract or reproductive tract are extracted as a whole from the carcass by the butcher and laid down into large trays or dedicated table (cattle gut for instance) for experts to separate subsection. Dissection procedure for specific tissues (brain, digestive tract, reproductive tract, kidney, skin, cartilage...) is described in dedicated FAANG protocols.

Tissue processing

Place empty and open 15 mL storage tubes in the styrofoam box containing liquid nitrogen. Pour 200 mL liquid nitrogen in the beaker.

Once the organ, or piece of organ, is in the tray, little cubes of 0.5 cm long edges are cut and immersed in the liquid nitrogen temporarily stored in the beaker. Collect 10 aliquots per storage tube. After 10 minutes minimum, all tissue cubes are deep frozen and are then picked up with a clamp to be placed into the cold storage tube. The cap is securely tightened after checking that no liquid nitrogen remains in the tube. Then the tube is stored in the zip lock bag containing the other aliquots from the same animal and tissue, the bag is stored also in dry ice. After transportation to the resource center, the tubes are stored in appropriate storage boxes (for 15 mL tubes) in a -80° freezer.