

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

Tissue sampling protocol 6

INRA
Division of Animal genetics

This protocol describes the embedding of heterogenous tissue samples in individual molds for further microdissection of specific cell types. It does not describe the anatomical procedure to isolate a specific organ or tissue.

Author: Michèle Tixier-Boichard, michele.boichard@jouy.inra.fr
Validation: Stéphane Fabre, stephane.fabre@toulouse.inra.fr

May 2016

Fr-AgEncode - Tissue sampling protocol 6

Required reagents and instruments

Dry ice in a large styroform box, from 3 to 10 kg according to number of samples to be collected
A large stainless steel tray or enamel tray, about 40 x 30 cm
A pair of cryogloves
20 cm x 20 cm zip lock bags
Sterile disposable Petri dishes
Disposable scalpels
2 sterile clamps with smooth ends, 10 cm long and 15 cm long
Surgeon gloves
A cold plate, stainless steel, 40 cm x 30 cm to be stored in the styroform box with dry ice
100 ml bottle of OCT embedding medium
Pre-labelled plastic molds of 3 cm x 2 cm and 0.7 cm depth,
Use cold-resistant labels, which will have been checked before, label shows animal number, tissue code, protocol number, aliquot number, stick the label on the bottom of the mold
Prepare 1 to 2 molds per tissue per animal,
A permanent marker to label the zip lock bag and the top of the mold
Paper towels
Waste bucket
Detergent
Ethanol spray bottles
A cleaning spray against RNase

Preparatory step

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 procedures for specific tissues (brain, digestive tract, reproductive tract, kidney, skin, cartilage...) are described in dedicated FAANG protocols.

Tissue processing

In case a cold plate would not be available, prepare a tray with a flat layer of dry ice.
Set a thin layer of OCT medium in the mold, avoiding bubbles, just before cutting the tissue.
Once the organ, or piece of organ, is collected, cut a slice of 1.5 cm long, 1 cm width and maximum 0.5 cm thickness. Put the slice into the mold on the layer of OCT, and completely cover the tissue slice with OCT, avoiding bubbles. The tissue slice should not touch the edge of the mold. If there is a specific orientation of the tissue slice regarding the organ anatomy, record it in the sample file. Generally, muscle samples will be laid with the fibers parallel to the bottom of the mold.
Put the mold with the tissue on the cold plate, or on the flat layer of dry ice.

Once the OCT has turned completely white, store it in the zip lock bag containing the other aliquots from the same animal and tissue and store the bag in dry ice. After transportation to the resource center, the molds are stored in appropriate boxes in a -80°C freezer.

Example of a mold filled of OCT with 2 pieces of black skin before freezing.

