

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

## Tissue sampling protocol 1a

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INRA  
Division of Animal genetics

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

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

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### 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 2 mL tubes  
Surgeon gloves  
Pre-labelled 2 mL cryotubes, use cold-resistant labels, which will have been checked before, the label shows animal number, tissue code, protocol number, aliquot number,  
Take 2 to 10 tubes per tissue per animal, according to the organ volume  
A permanent marker to label the zip lock bag.  
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 procedure for specific tissues (brain, digestive tract, reproductive tract, kidney, skin, cartilage...) is described in dedicated FAANG protocols.

### Tissue processing

Once the organ, or piece of organ, is in the tray, little cubes of 0.5 cm long edges are cut and individually stored in one empty 2 mL cryotube. The cap is securely tightened and the whole tube is immersed into liquid nitrogen stored in the styroform box. After a minimum of 15 minutes, the tubes are stored in a zip lock labeled with animal number and tissue code. The bag is stored and transported in dry ice to the resource center before final storage in a cryotube storage box in a -80° freezer.