

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

**Cartilage sampling protocol**

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This protocol describes the anatomical procedure to isolate cartilage.

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## Fr-AgEncode - Cartilage sampling protocol

This protocol applies to cartilage which is considered to be homogenous enough so that any piece of cartilage is representative of the whole cartilage.

### Required reagents and instruments

Liquid nitrogen in a storage tank (usually 25 L)

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

A pair of cryogloves

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

Sterile disposable Petri dishes

Disposable scalpels

A rack for 15 mL tubes

Surgeon gloves

Pre-labelled 15 mL and 2 ml cold-resistant 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.

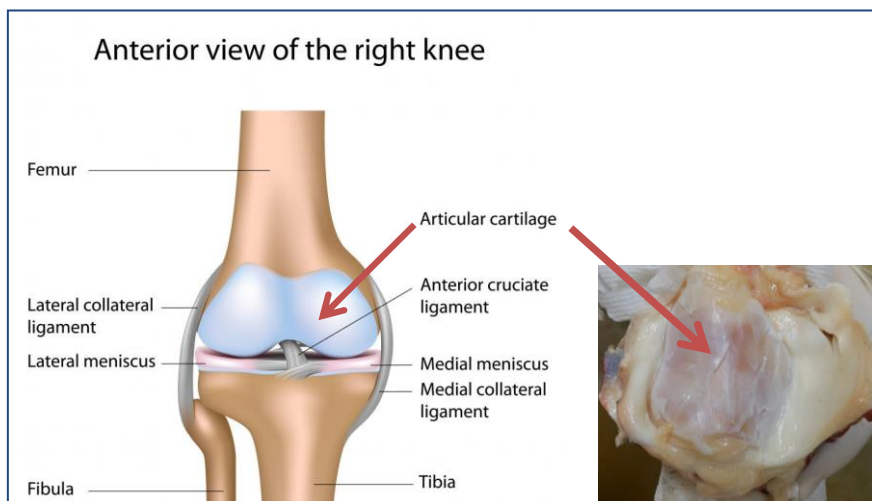
Paper towels

Waste bucket

### 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.

For cartilage sampling we used the knee and dissect the articular cartilage from femur.



### Cartilage processing

Once the femur is in the tray, the cartilage harvest should be performed with an instrument able to obtain full-thickness cartilage pieces.

We used disposable scalpels and we harvested small piece of 0.5 cm long.

The pieces of cartilage were either transferred into cryotubes or 15 ml tube and snap frozen in liquid nitrogen before final storage in a -80° freezer.

The detailed Snap-freezing procedures are described in dedicated FAANG protocols (Fr-AgEncode\_sampling\_protocol\_1 and Fr-AgEncode\_sampling\_protocol\_2a).