Fr-AgENCODE : a French pilot project to enrich the annotation of livestock genomes

Brain tissues sampling protocol in cattle

Author Hugues Dardente, Laurence Dufourny, Martine Migaud, Catherine Taragnat

Preliminary comment

This protocol describes how to sample specific brain regions in cattle, it is also valid for pigs and goats.

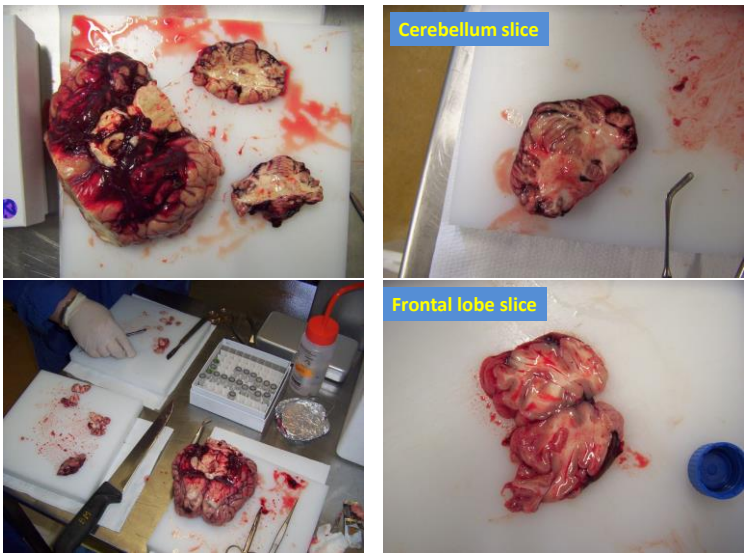
Required reagents and instruments

Liquid nitrogen; cold isopentan; styrofoam boxes
Pre-labeled cryotubes : 2ml, 15 ml, 50 ml.
Scalpels, forceps with smooth ends
Scissors

Procedure

Slaughtering : standard procedure for a slaughterhouse, except that the initial stunning is done so as to avoid damage to the brain as much as possible
The head is separated from the trunk as soon as possible after death
The butcher breaks the skull

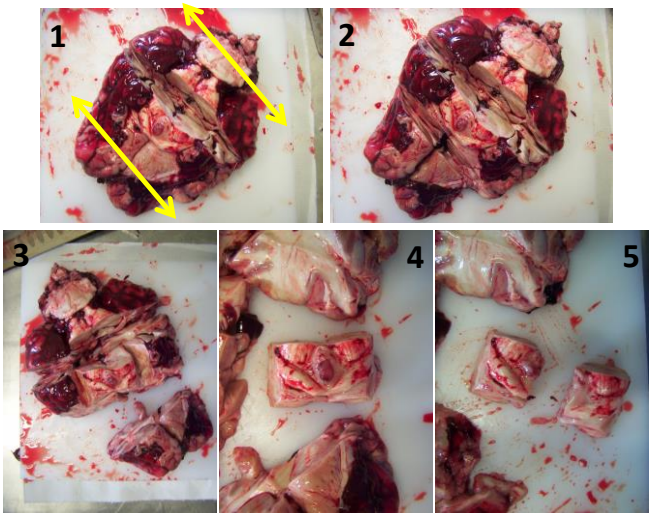
Tissue sampling
Removing the brain: from the anterior part, cut at the junction between olfactory bulbs and brain ; Olfactory bulbs are left *in situ* for sampling;
Brain is separated from the pituitary by cutting at the level of the pituitary stalk..
Pituitary is left *in situ* for sampling.
Frontal lobe (cortex): the most anterior part is removed (2-3 cms) and a 1-2 cms slice is taken and further cut in small cubes of 1 cm^3.
Cerebellum : cut from the rest of the brain; a slice is taken in its middle part and further cut in small cubes of 1cm^3 .
The approximate location of the slides for cortex and cerebellum is shown by the yellow arrows on slide 2.
NB: a lot of clotted blood must be removed before getting access to the brain *per se* ; on slide 1 the whole brain of animal 1 is not 'cleaned' and on slide 2, the whole brain of animal 2 has been cleaned;



Cerebellum and cortex (frontal lobe)

Hypothalamus : pos ior cutting 5mm-1cm ahead of the optic c they form the tractus optici (stripes) , see to 4).

This block is cut on its sagittal plan in 2 parts, the median eminence generally tears off, some may remain but its quantity can not be assessed. (photo 5). Then each half of the block is cut in small cubes of $1cm^3$



Hypothalamus

Freezing and preservation

For Frontal Lobe, Cerebellum:

- 10 individual cubes can be snapfrozen in liquid nitrogen and stored in individual cryotubes.
- Remaining cubes are frozen in cold isopentane on a bed of dry ice, to be stored altogether in a single 50ml tube as a 'stock';
- 2 cubes can be embedded in OCT on a bed of dry ice to make possible further morphological studies and/or microdissection of chosen cells
  (except for one male, no OCT bloc for frontal lobe)

For olfactory bulbs

- 5 individual cubes can be snapfrozen in liquid nitrogen and stored in individual cryotubes.
- Remaining cubes (4-6) are frozen in cold isopentane and stored altogether in a single 15ml tube as a 'stock';

For hypothalamus

All the cubes from a half hypothalamus are frozen in cold isopentane on a dry ice bed and stored in one 50 mL tube for further grinding, since it is hopeless to believe that specific subregions may be identified, so the recommendation is to grind all pieces in order to have an 'average' state of RNA expression. However, the grinding may be incompatible with some FAANG assays other than RNA-seq, in that case, samples may be used as such but users have to be aware that the sample representativeness within the hypothalamus will not be known.

Pituitary:

Cubes were snapfrozen in liquid nitrogen, same comment as for hypothalamus regarding representativeness of different cubes.