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

Tissue sampling protocol 3c

INRA
Division of Animal genetics

This protocol describes the preparation and storage of isolated cells from tracheal epithelium. It has been applied to cattle, goat and pig.

Authors: Sophie Pollet (sophie.pollet@jouy.inra.fr)
Emmanuelle Rebours (emmanuelle.rebours@jouy.inra.fr)
Adeline Goubil (adeline.goubil@jouy.inra.fr)
Nicolas Bertho (Nicolas.bertho@jouy.inra.fr)

May 2015

Fr-Agencode – isolation of tracheal epithelial cells
Tissue sampling protocol 3c

Materials

- 4 large sterile beakers for trachea washing.
- scalpel blades,
- scissors
- pliers with smooth ends, one of it with curved end
- a cell counter
- 4 trays for dissection
- 4 cell strainer of 100 µm
- small Petri dishes, disposable
- 50ml Falcon tubes
- cryovials labeled with cold-resistant labels, showing animal number, tissue code, protocol number, aliquot number

Reagents

- 8 bottles of 500 ml PBS-EDTA 2 mM.
- PBS for rinsing instruments
- Ethanol for rinsing instruments
- RPMI/Glu/10%FCS/AB
- DMSO
- Enzyme mix already prepared and kept at 37°C for cell dissociation :

  10 mg Collagenase)
  5 mg Dispase) for each aliquot
  10 ml RPMI/Glu/10%FCS/AB)

Operating mode.

I- Sampling the epithelium

1- cut the trachea in 2 or 3 segments
2- open the tracheal tube with scissors, do not touch the epithelium inside
3- rinse the open segments with PBS
4- pull the epithelium with the curved pliers in order to get it in a single piece, proceed slowly from one end to the other end of the segment

II Dissociating the cells

1- mince the epithelium into a Petri dish containing the enzyme mix
2- collect the resulting mixture in a Falcon 50 ml
3- incubate at 37°C with regular and gentle stirring
4- Filter through a cell strainer of 100µm and rinse the strainer with 10 ml PBS/EDTA
5- Centrifuge 1800 rpm for 7 min at 4°C
6- Remove the supernatant and add 10 ml PBS/EDTA
7- Count cells
8- Centrifuge 1800 rpm for 7 min at 4°C
9- Remove the supernatant and collect the cells in 500 µl FCS medium, then progressively add, drop by drop, 500 µl of cold FCS with DMSO20%
10- Store about 15.10^6 cells per cryovial