GENE-SWitCH

The regulatory GENomE of SWine and CHicken: functional annotation during development

Protocol WP1 T1.1
Sampling of tissues from newborn piglets

Authors: Hervé Acloque and Mayrone Mongellaz (INRAE)

Workpackage: WP1

Version: 1.0

Protocol associated with Deliverable(s):	D1.1
Submission date to FAANG:	21/02/2020, month M8

Research and Innovation Action, SFS-30-2018-2019-2020 Agri-Aqua Labs
Duration of the project: 01 July 2019 – 30 June 2023, 48 months

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 817998

Table of contents

1 Summary

GENE-SWitCH aims at identifying functional elements located in the genomes of pig and chicken working on seven different tissues at three different developmental stages. It requires a collection of samples corresponding to the selected tissues and developmental stages with associated metadata describing accurately the samples and the sampling process.

The seven tissues analysed in GENE-SWitCH are:

- Cerebellum
- Lung
- Kidney
- Dorsal skin
- Small intestine
- Liver
- Skeletal muscle

Six additional tissues are also sampled for biobanking:

- Heart
- Gonads
- Cortex
- Spleen
- Colon
- Stomach/Gizzard

The three developmental stages are:

- Early organogenesis (E8 chick embryo and D30 pig foetuses)
- Late organogenesis (E15 chick embryo and D70 pig foetuses)
- Newborn piglets and hatched chicks

For each species and each developmental stage, 4 biological replicates (2 males and 2 females) are sampled.

We describe here the procedures used to sample tissues from newborn piglets.

2 Protocol description

2.1 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
- Zip lock bags
- 1 cold plate (Leica), approximate size 20 x 30 cm
- Sterile disposable Petri dishes (100 mm)
- Disposable scalpels
- Sterile clamps with smooth ends, 10cm long and 15cm long
- Scissors
- Racks for 2 mL tubes

- 100 pre-labelled 2 mL cryotubes showing animal number, tissue code, aliquot number; use cold-resistant labels label
- Racks for 50 mL Falcon tubes
- 50 falcon tubes (50 mL)
- Latex gloves
- A permanent marker to label the zip lock bag.
- Paper towels
- Waste bag
- Dulbecco's Phosphate-Buffered Saline (DPBS), 6 bottles for 4 animals.
- Water bottle (4 litres for 4 animals)
- Ethanol spray bottles
- A cleaning spray against RNAse
- Digital Camera
- Weighting scales

2.2 Preparatory step

Prepare the workplace by putting aluminum foil and paper towel on the working bench. Place on each workplace 2 scalpels, 2 forceps (10 and 15 cm long), 2 racks (2 ml tubes and 50 ml falcon) and Petri dishes (100 mm). Furthermore, to clean the organs from remaining blood and wastes, we prepare beaker and falcon tubes filled with PBS.

2.3 Animal dissection

Piglets are stunned by electronarcosis before being slaughtered by bleeding. Each organ is rapidly extracted from the carcass, in a pre-determined order (heart, lungs, liver, stomach, spleen, digestive tract, kidney, gonads, dorsal skin, skeletal muscle (gluteus medius), brain cortex and cerebellum). To extract the brain cortex and the cerebellum, we used a pair of scissors to open the skull. Whole tracts such as digestive tract are extracted as a whole from the carcass and laid down into large petri dishes to separate subsection (ileum and colon). Each dissected organ is put in Petri Dishes (100 mm), pre-labelled with the tissue name and animal number.

The Petri dish containing the organ is then laid down on the cold plate.

2.4 Tissue processing

Once the organ, or piece of organ, is on the cold plate, little cubes of 0.5 cm long edges are cut, washed in PBS and individually stored in one empty 2 mL cryotube (6 aliquots by tissues). The cap is securely tightened and the whole tube are stored into a zip lock bag labelled with animal number and tissue code. The zip lock bags are immediately snapfrozen by immersion into liquid nitrogen. The bag is then stored in dry ice and transported back to the laboratory. Samples are finally stored into a cryotube storage box at -80°C.

Between each tissues and between each animal, the forceps and the scalpel are washed in different falcons (50mL) which contained absolute ethanol, RNA away and water.