GENE-SWitCH

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

Protocol WP1 T1.2
Sampling of tissues from hatched chicks

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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 (Embryonic day 8 (E8) chick embryo and 30 days old (D30) pig foetuses)
- Late organogenesis (Embryonic day 15 (E15) chick embryo and 70 days old (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 hatched chick embryos.

2 Protocol description

2.1 Required reagents and instruments

- 1 styroform box with lid filled with dry ice
- Sterile disposable Petri dishes (100 mm)
- 12-well culture plates
- Disposable scalpels
- Sterile clamps with smooth ends, 10cm long and 15cm long
- Pairs of fine dissecting forceps
- Scissors
- Perforated spoon
- 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
- Paper towels
- Waste bag
- Phosphate-Buffered Saline (DPBS), 2 bottles of 1liter
- Water bottle (4 litres for 4 animals)
- Ethanol spray bottles
- A cleaning spray against RNAse
- Digital Camera
- Weighting scales

2.2 Preparatory step

Fertilized eggs are incubated for 21 days at 37°C in a humidified incubator with automatic egg turning. The day of hatching, the workplace is prepared by putting aluminium foil and paper towel on the working bench. Place on each workplace 2 scalpels, 3 forceps (2 dissecting forceps and one of 10 cm long), 2 racks (2 ml tubes and 50 ml falcon) and 12-well culture plates filled with cold PBS. Place the 2ml tubes on dry ice 30 minutes before starting.

2.3 Animal dissection

The hatched chick is euthanized using cervical dislocation and placed into a 100mm Petri dish. Each chick is immediately weighed, photographed, sexed by direct observation and dissected in a pre-determined order (heart, lungs, liver, spleen, gizzard, ileum, colon, kidney, gonads, dorsal skin, thigh muscle from the chicken leg, brain cortex and cerebellum).

2.4 Tissue processing

Once the organ is dissected, it is cleaved into small pieces (20-30mg), that are stored into empty 2 mL cryotubes labelled with animal number and tissue code. The cap is securely tightened and the whole tube is immediately stored in dry ice. Samples are finally stored into a cryotube storage box at -80°C.

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