GENE-SWitCH

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

Protocol WP1 T1.1
Sampling of tissues from D70 pig fetuses

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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 (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 D70 foetuses.

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
- Cryoprotection gloves
- Zip lock bags
- 1 cold plate (Leica), approximate size 20 x 30 cm
- Sterile disposable Petri dishes (100 mm)
- 6-well culture plates
- Disposable scalpels
- Sterile clamps with smooth ends, 10cm long and 15cm long
- Pairs of fine dissecting forceps
- 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
- Cork plates (for dissection) and needles
- A permanent marker to label the zip lock bags
- 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 aluminium foil and paper towel on the working bench. Place on each workplace one cork plate together with 2 scalpels, 3 forceps (2 dissecting forceps and one of 10 cm long), 2 racks (2 ml tubes and 50 ml falcon) and 6-well culture plates filled with cold PBS.

2.3 Animal dissection

Pregnant sows are stunned by electronarcosis and bled. The uterus is rapidly extracted from the carcass and the fetuses are extracted from the amnios. Each fetus is immediately weighed, photographed, sexed by direct observation and placed on ice. The fetus is then fixed with needles on the cork plate and tissues are dissected in a pre-determined order (heart, lungs, liver, stomach, spleen, small intestine, colon, kidney, gonads, skin, hindlimb muscle, brain cortex and cerebellum). Each dissected organ is put in one well of the 6-well plates, pre-labelled with the tissue name and animal number.

The 6-well plate 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 0.3 cm long edges are cut and stored in empty 2 mL cryotubes (5 tubes per tissue). The cap is securely tightened and the whole tube is stored in a zip lock bag labelled with animal number and tissue code. The zip lock bag is 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.

2.5 Sexing of fetus by PCR

To confirm our sexing by direct observation, we also performed molecular sexing by PCR. For each fetus, a small piece of tissue is lysed in 60µl of 50mM NaOH during 1hour at 95°C with agitation. 10 µl of 1M Tris-HCl pH8.0 is then added and DNA is quantified by spectrophotometry (Nanodrop). 100ng of genomic DNA is then used to perform a PCR using the following primers (ZFX_ZFY_FW

ATCAAAACCTTCATGCCAATAGC and ZFX_ZFY_RV TCCGGTTTTCAATTCCATCAGAA) and the following amplification program (40 amplification cycles: 94°C 30s, 58°C 30s, 72°C 1min). PCR products are then separated by electrophoresis on 1.8% agarose gels. Resulting PCR products from male samples are represented by two bands (around 600bp and 500bp) while those from female samples are represented only by one (around 600bp).