GENE-SWitCH

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

Protocol WP1 T1.2
Sampling of tissues from E15 chick embryos

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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 E15 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
- A dissection stereomicroscope with epi-illumination
- 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
- Weighting scales

2.2 Preparatory step

Fertilized eggs are incubated for 15 days at 37°C in a humidified incubator with automatic egg turning. The day of sampling, the workplace is prepared by putting aluminium foil and paper towel on the working bench. Place on each workplace a dissection stereomicroscope 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 12-well culture plates filled with cold PBS. Place the 2ml tubes on dry ice 30 minutes before starting.

2.3 Animal dissection

The incubated egg is opened by opening a small window in the shell at the level of the air chamber. The external membranes are removed and the embryo is recovered using a perforated spoon and placed into a 100mm Petri dish. Each embryo is immediately weighed, photographed and dissected under the stereomicroscope in a pre-determined order (heart, lungs, liver, spleen, gizzard, small intestine, large intestine, kidney, gonads, dorsal skin, hindlimb muscle, brain cortex and cerebellum).

2.4 Tissue processing

Once the organ is dissected, it is cleaved into small pieces (20-30mg), that are individually 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.

2.5 Sexing of embryo by PCR

We sampled up to 1-2 embryos per pen and each embryo has been sampled individually and sexed by PCR. Breast muscle tissue sample was taken from each day 15 embryo and used to determine sex after sampling. Tissue samples were lysed and gDNA was extracted using 'DNAEasy Blood and tissue kit' (Qiagen – 69504) following manufacturer's instructions.

Primers specific for GAPDH and W-chromosome were used in PCR experiments to determine sex of embryos. 'W-chromosome' is specific to short sequence on the W-chromosome that determines sex as female. Male chickens lack a W-chromosome, therefore negative (no band) and positive (band) results of PCR with W-chromosome primers indicate male and female DNA, respectively. GAPDH is expressed by male and female cells, therefore PCR for GAPDH controls for false negatives. PCR pre-mix (dNTP, Taq, water, buffer) was added tubes containing GAPDH and W-chromosome primer pairs (3' and 5', 0.4µl each per reaction) to give GAPDH and w-chromosome mastermixes. Both Mastermixes

were aliquoted into PCR tubes and extracted gDNA samples of embryos (1 µl) were added accordingly. As a control for background, a blank reaction was run where gDNA was replaced with water. Samples of confirmed male and female chickens were run for GAPDH and 'W' PCR for as controls for embryo test samples. All PCR's were run simultaneously on a thermocycler then reactions run on a 1% agarose gel and imaged on ultraviolet transilluminator.