GENE-SWitCH

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

Protocol WP1 T1.2
Pooling of tissues from E8 chicken embryos

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Workpackage: WP1

Version: 1.0

Protocol associated with Deliverable(s):	D1.1
Submission date to FAANG:	2020/09/15, month M15

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

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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.

To increase the quantity of available material for ChIP-Assay, we decided to pool E8 chicken embryos.

We describe here the procedures used to pool tissues from E8 chicken embryos.

2 Protocol description

2.1 Required reagents and instruments

- PCR kit (dNTP, Taq, water, buffer)
- PCR tubes
- Pipets and tips
- 1.8% agarose gel
- Electrophoresis material
- Thermocycler
- Thermoblock
- Cryotable (or box of dry ice)
- Cryoprotection gloves
- Sterile disposable Petri dishes (100 mm and 60mm)
- Disposable scalpels
- Sterile clamps with smooth ends, 10cm long

- Racks for 2 mL tubes
- 100 pre-labelled 2 mL cryotubes showing animal number, tissue code, aliquot number; use cold-resistant labels label
- A permanent marker to label the zip lock bags
- Paper towels
- Waste bag
- Ethanol spray bottles
- A cleaning spray against RNAse
- Weighting scales

2.2 Sexing of fetus by PCR

Four pools of unrelated E8 chick embryos (2 pools of females and 2 pools of males) have been produced. Each embryo has been sampled individually and sexed by PCR (detailed procedures on the GENE-SWitCH sharepoint and the FAANG DCC). We used chick embryos from 4 pens composed of 4 hens and 1 cock. Eggs from each pen are traced but can not be associated to one specific hen from the pen.

We sampled up to 1-10 embryos per pen and each embryo has been sampled individually and sexed by PCR. Breast muscle tissue sample was taken from each day 8 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 adult 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.

First, for each pen, embryos were sexed by PCR (see Table, male in blue and female in pink)

Pen 1	Embryo 13				
Pen 2	Embryo 2	Embryo 6	Embryo 7	Embryo9	Embryo10
		Embryo 15	Embryo 16		
Pen 3	Embryo 3	Embryo 4	Embryo 5	Embryo 11	Embryo 12
			Embryo 14		
Pen 4	Embryo 1	Embryo 8			

2.3 Pooling strategy

After sexing, four pools of E8 chick embryos (2 pools of females and 2 pools of males) have been produced.

We determined the pools according to the following table, in order to balance the genetic diversity. Pooling was done as follows, to produce 2 pools of male and 2 pools of female samples for each tissue type.

	Pen 1	Pen 2	Pen 3	Pen 4
Pool1		Embryo 6 Embryo 16	Embryo 3 Embryo 4	
Pool2		Embryo 9 Embryo 10	Embryo 12	Embryo 1
Pool3	Embryo 13	Embryo 2	Embryo 5 Embryo 11	
Pool4		Embryo 7 Embryo 15	Embryo 14	Embryo 8

2.4 Pooling procedure

Working on dry ice, for each tissue of each pool, the different samples resulting from the pooling strategy were cutted into small pieces and distributed randomly into 4 different tubes.

Samples were finally stored into a cryotube storage box at -80°C.