GENE-SWitCH

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

Protocol WP1 T1.1
Pooling of tissues from D30 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.

To increase the quantity of available material for ChIP-Assay, we decided to pool D30 pig foetuses.

We describe here the procedures used to sample tissues from D30 pig foetuses.

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

We used 6 unrelated sows inseminated with 6 unrelated boars.

We sampled 6 fetuses per sow and each fetus has been sampled individually and sexed by PCR according to Kim et al (2016)[1].

A small piece of tissue has been lysed in 60µl of 50mM NaOH during 1hour at 95°C with agitation. DNA is quantified by spectrophotometry (Nanodrop) and 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).

For each sow (6 sows in total), we sampled 6 fetuses to get at least one female and one male. Pools have been performed in a second time, after sexing.

Sow 1	Fetus1	Fetus2	Fetus3	fetus4	Fetus5	Fetus6
Sow 2	Fetus7	Fetus8	Fetus9	Fetus10	Fetus11	Fetus12
Sow 3	Fetus13	Fetus14	Fetus15	Fetus16	Fetus17	Fetus18
Sow 4	Fetus19	Fetus20	Fetus21	Fetus22	Fetus23	Fetus24
Sow 5	Fetus25	Fetus26	Fetus27	Fetus28	Fetus29	Fetus30
Sow 6	Fetus31	Fetus32	Fetus33	Fetus34	Fetus35	Fetus36

[1] Kim, Yoo-Kyung & Kang, Yong-Jun & Kang, Geun-Ho & Seong, Pil-Nam & Kim, Jin-Hyoung & Park, Beom-Young & Cho, Sang-Rae & Jeong, Dong & Oh, Hong-Shik & Cho, In-Cheol & Han, Sang-Hyun. (2016). Molecular Sexing and Species Identification of the Processed Meat and Sausages of Horse, Cattle and Pig. Journal of Embryo Transfer. 31. 61-64. 10.12750/JET.2016.31.1.61.

2.3 Pooling strategy

After sexing, four pools of D30 Large White fetuses (2 pools of females and 2 pools of males) have been produced.

We determine the pools according to the following table, in order to balance the genetic diversity between pools. Pooling was done as follow, to get 2 pools for male and 2 pools for females.

	Female 1	Female 2	Female 3	Female 4	Female 5	Female 6
Pool1	Fetus1 Fetus 6	Fetus8 Fetus 10	Fetus18	Fetus19 Fetus 22	Fetus25 Fetus 27	Fetus31 Fetus 33
Pool2	Fetus2 Fetus5	Fetus9 Fetus12		Fetus21 Fetus23 Fetus24	Fetus26	Fetus 32 Fetus34 Fetus35
Pool3	Fetus4	Fetus11	Fetus17 Fetus13	Fetus20	Fetus30	Fetus36
Pool6	Fetus3	Fetus7	Fetus14 Fetus15 Fetus16		Fetus28 Fetus29	

2.4 Pooling procedure

Working on the cryotable (at -25°C), 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.

For example, liver from Fetus 4, 11, 13, 17, 20, 30 and 36 (corresponding to Pool3) were placed on a Petri Dish, cutted in small pieces using a scalpel blade, mixed and distributed randomly into 4 different tubes, constituting the 4 aliquots of the Liver_Pool3 sample.

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