GENE-SWitCH

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

Protocol WP5 T5.1
Sampling of tissues from piglets and foetuses

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1 Protocol description

1.1 Overview of tissue samples

For both piglets and foetuses: heart, duodenum, jejunum, ileum, colon, distal lung, kidney (cortex), liver, skeletal muscle = 9 tissues.

Piglets: faeces at dissection

For the target tissues (liver and skeletal muscle) we collect 6 aliquots: (2) for DNA and RNA extractions (same protocol as WP4); (2) for ATAC-seq libraries (to be delivered to DIAGEN); (2) for safety storage.

For the other 7 tissues, we should store 3 aliquots per tissue.

At Sampling site															At lab (-80C):
sows	diet	**piglets**	Target tissues	other tissues	aliquots/ target tissues	aliquots/ other tissues	**# Petri dishes**	**Liquid N2 (L)**	**Dry ice (Kg)**	**Water (# L)**	**# PBS bottles (500 ML)**	**# cryovial tubes**	**Ziplock bags target tissues**	**Ziplock bags other tissues**	**# boxes** (96 tubes)
4	3	4	2	7	6	3	672	25	20	12	12	1584	96	336	16.5
3	3	4	2	7	6	3	504	25	20	9	9	1188	72	252	12.375
						Totals:	1176								
sows	diet	**fetuses**	Target tissues	other tissues	aliquots/ target tissues	aliquots/ other tissues	**# 6-wells plates**								**# boxes** (96 tubes)
4	3	4	2	7	6	3	112	25	20	12	12	1584	96	336	16.5
3	3	4	2	7	6	3	84	25	20	9	9	1188	72	252	12.375
						Totals:	196	100	80	42	42	5544	336	1176	57.75

Table: overview of number of samples

Note

- Extra samples from gut (4) and liver (3) were cryopreserved for one male and one female foetus per sow.

1.2 Required reagents and instruments

- Liquid nitrogen in a storage tank (usually 25 L per day of sampling)
- Dry ice (20 kg in a large box per day)
- 1 small styrofoam box (30 x 20 x 15) for temporary storage of liquid nitrogen
- A pair of cryogloves
- Sterile disposable Petri dishes (100 mm): at minimum 1/piglet tissue (see Table)
- 6-well plates for fetuses (see Table)
- Zip lock bags: 1 for each Piglet/target tissues and 1 for each piglet/other tissues (see Table)
- Pre-labelled 2 mL cryotubes with animal number, tissue code, aliquot number; use cold-resistant labels label! (see Table)
- Water to wash tissues from faeces (for gut) and blood (see Table)
- Dulbecco's Phosphate-Buffered Saline (DPBS): at minimum 1 bottle (500 ML) for each 4 piglet (see Table) + PBS in squeeze bottles
- 50 falcon tubes (50 mL) to use for washing tissues
- Latex gloves
- Paper coats
- 3 permanent markers to label the zip lock bag.
- Paper towels

- Waste bag
- Ethanol spray bottle
- A cleaning spray against RNAse
- Digital Camera
- Weighting scales

Per each tissue-dissecting person:

- A wooden clip
- 1 cold plate (per each user doing dissections) (Leica), approximate size 20 x 30 cm
- Petri dishes and/or 6-wells plates
- 1 Racks for pre-labeled 2 mL tubes
- 1 Racks for 50 mL Falcon tubes
- 4 falcon tubes (50 ml) filled with water, ethanol (100%), PBS, solution against RNAse.
- Beakers of water or PBS
- 2 Disposable scalpels
- 2 Sterile clamps with smooth ends, 10cm long and 15cm long
- 1-2 Scissors
- 2 forceps (10 and 15 cm long)
- Water and PBS bottles at hand

1.3 Preparatory step

Prepare the workplace by putting aluminum foil and paper towel on the working bench. Place on each workplace a cold plate (kept cold on ice), 2 scalpels, 2 forceps (10 and 15 cm long), 2 scissors, 2 racks (for 2 ml tubes and 50 ml falcon) and Petri dishes (100 mm); for fetuses (smaller organs) use 6-wells plates. Furthermore, to better clean the organs from remaining blood and wastes, we prepare beaker and falcon tubes filled with PBS.

1.4 Animal dissection

Piglets:

- They are sedated with Zoletil followed by a T61® injection directly into the heart.
- Each dissected organ is put in 100 mm Petri Dishes filled with PBS – pre-labelled with tissue name and animal number.

Foetuses:

- They are extracted directly from the reproductive tract, which is first dissected. At this stage the foetuses are approximately 15-20 cm long, and the uterus is very large. 2 **males and 2 females (the closest to the uterus bifurcation!) per sow** are taken out, weighed and dissected. Target samples are snap-frozen asap.
- Each dissected organ is put in 6 multi-well plates filled with PBS (for large organs petri dishes could be used) - pre-labelled with tissue name and animal number.

Tissue processing:

Each organ is rapidly extracted from the carcass, in a pre-determined order. The following order allows proceeding rapidly for fetuses: heart, distal lungs, **liver**, digestive tract (duodenum, jejunum, ileum and proximal colon), kidney (cortex), and **skeletal muscle**. The whole digestive tract can be extracted as a whole from the carcass and laid down into large petri dishes to separate gut regions. From the piglets luminal content/faeces from the colon is collected.

Once the organ, or piece of organ, is on the cold plate, little cubes of 0.5 cm long edges are cut, washed in PBS and individually stored in one empty 2 mL cryotube (6 or 3 aliquots per tissues). The cap is securely tightened and the whole tube is snapfrozen into the liquid nitrogen. The tubes are then put in a zip lock bag, which is 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 PBS.

2 Metadata

The day of sampling it is important to take a picture of each animal and to fill the Correlation Table (below). This table is used for tracking the animals and to start filling the FAANG Metadata file.

2.1 Correlation Table

Heart	Distal Lung	Liver	Duodenum	Jejunum
Ileum	Proximal Colon	Skeletal Muscle	Kidney (cortex)	

Order of slaughter	Gene Switch Name	ID	ID Mother	ID Father	Weight (g)	slaughter time	Sex
1	WP5_FT70_1				250	13 h 00	Male
2	WP5_FT70_2				275	13 h 10	Female
3	WP5_FT70...				….	….	

2.2 Metadata file

Sample Name		**Sex**	
ID (if he has one)		**ID mother**	
Project		**ID Father**	
Health status		**Mother Parity**	

Birth date **Sampling date** **Birth location** **Birth weight**		**Mother Weight** **Insemination Date** **Animal Age At Collection** **Gestational Age At Sample Collection**	
Weight **Diet mother**		**Pregnancy length** **Location in uterus (foetus only)**	