GENE-SWitCH

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

Protocol WP1 T1.6
Cell dissociation for Promoter Capture Hi-C

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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 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 promoter capture Hi-C, for each species and each developmental stage, only skeletal muscle and liver from 2 biological replicates (1 male and 1 female) were processed.

We describe here the procedure to prepare a cell suspension from skeletal muscle and liver to be used for the production of Capture Hi-C libraries. A snapfrozen pellet of around 1-2 million cells is required for each Hi-C experiment.

2 Protocol description

2.1 Required reagents and instruments

- PBS 1X for cell culture
- StemPro Accutase (Thermo Life Sciences, A1110501)
- Cryoprotection gloves
- Sterile disposable Petri dishes (100 mm and 60mm)
- Disposable scalpels
- Sterile clamps with smooth ends, 10cm long
- 50 mL Falcon tubes and racks for 50 mL Falcon tubes
- 15 mL Falcon tubes and racks for 15 mL Falcon tubes
- Racks for 2 mL tubes
- pre-labelled 2 mL cryotubes showing animal number, tissue code, aliquot number; use cold-resistant labels
- A permanent marker to label tubes
- Paper towels
- Waste bag
- Ethanol spray bottles

Prepare ice-cold PBS and prewarm Accutase at 37°C before processing.

2.2 Dissection of tissues

Skeletal muscle and liver from pig fetuses and newborns, chicken embryos and recently hatched chicks have been dissected following the protocoles publicly available on the FAANG Data Portal:

https://data.faang.org/api/fire_api/samples/INRA_SOP_GENESWITCH_D30_FETUS_SAMPLING_20200221.pdf
https://data.faang.org/api/fire_api/samples/INRA_SOP_GENESWITCH_D70_FETUS_SAMPLING_20200221.pdf
https://data.faang.org/api/fire_api/samples/INRA_SOP_GENESWITCH_PIGLET_SAMPLING_20200221.pdf
https://data.faang.org/api/fire_api/samples/ROSLIN_SOP_GENESWITCH_E8_EMBRYO_SAMPLING_20200915.pdf
https://data.faang.org/api/fire_api/samples/ROSLIN_SOP_GENESWITCH_E15_EMBRYO_SAMPLING_20200915.pdf

https://data.faang.org/api/fire_api/samples/ROSLIN_SOP_GENESWITCH_HATCHED_CHICK_SAMPLING_20200915.pdf

Tissues were kept on ice cold PBS during the journey from the slaughter house to the laboratory (~4 hours).

2.3 Single cell suspension (estimated time: 1 hour but may vary depending on the number of samples)

Once arrived in the laboratory, Accutase is prewarmed at 37°C.

We placed the tissue to be processed into a 100mm Petri dish. We dilacerated the tissue with scalpel blades as fine as you can (the finest the best to remove blood and optimize dissociation). The chopped liver should look like mashed tomatoes.

We washed the pieces of liver with PBS to remove blood as much as possible. For that, we add PBS to the pieces, agitate well, decant and remove the supernatant and we repeat until the supernatant is transparent. Remove the supernatant. *This step is not necessary for the muscle (except if it is contaminated with blood).*

We added 2ml of StemPro Accutase previously warmed at 37°C and incubated for 5 minutes at 37°C with moderate agitation.

After 5 minutes, using a 1ml pipet and a blue tip, we pipetted up and down as much as necessary (usually 10-20 times) times to homogenize the suspension. When necessary, incubate for 5 additional minutes and repeat the homogenization step. You need to get a cell suspension that easily flows in the blue tip.

We then filter the homogenate trough a cell strainer of 70µm into a 50ml tube and we added PBS (room temperature) up to 20ml.

We centrifugated the cell suspension at 300g (room temperature) for 5 minutes

We removed the supernatant (and remaining blood cells under or covering the pellet) and we added again 20 ml of PBS (at room temperature). At this step we counted the cells

We centrifugated the cell suspension at 300g (room temperature) for 5 minutes

We removed the supernatant (and remaining blood cells covering or under the pellet) and resuspended the cell suspension at a concentration of 2 million/cells per 1ml of PBS (1 million if less cells are recovered).

We dispatched 1ml of cell suspension to 2ml Eppendorf tube.

We centrifugated the tubes with cell suspension at 300g (room temperature) for 5 minutes.

We removed the supernatant and snapfroze the pellet in liquid nitrogen (pig cells) or dry ice (chicken cells)

Cell aliquots were stored at -80°C.

2.4 Sexing of individuals (estimated time: 3 hours)

Pig fetuses and chicken embryos have been sexed by PCR following the protocols publicly available on the FAANG Data Portal:

https://data.faang.org/api/fire_api/samples/INRA_SOP_GENESWITCH_D30_FETUS_SAMPLING_20200221.pdf
https://data.faang.org/api/fire_api/samples/ROSLIN_SOP_GENESWITCH_E8_EMBRYO_SAMPLING_20200915.pdf