PluS4PiGs: Cell tools for pig genetics

PluS4PiGs – Protocols

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Getting true Pluripotent Stem Cells in Pigs: a key step for large scale ex-vivo "Genotype to Phenotype" studies

Protocol 2
Sampling of embryos (without super ovulation and surgery)

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Version: 1.0

Submission date to FAANG:	**31/01/2023**

Project-ANR-19-CE20-0019
Duration of the project: 01 February 2020 – 31 January 2024, 48 months

This project has received funding from the French National Research Agency (ANR) under grant agreement No ANR-19-CE20-0019

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1 Summary

To address the current challenges of animal breeding, a better knowledge of the link between genotype and phenotype is necessary. The use of pluripotent stem cells is an interesting solution because these cells can be driven in vitro to differentiate towards all cell lineages and their genome can be easily manipulated. These characteristics make PSCs powerful tools to assess the causality of genetic variants associated with cellular intermediate phenotypes. We propose, within the framework of the PluS4PiGs project, to use the numerical dimension from multi-omics data at the single-cell and tissue scale to predict the molecules necessary and sufficient to maintain porcine pluripotency and transfer this knowledge for the production and use of porcine pluripotent stem cell lines (PSCs) for animal and human health applications.

We generated single-cell expression (scRNA-seq) and chromatin accessibility (scATAC-seq) data from four stages of embryonic development (early, expanded, spherical and ovoid blastocysts). All of these data will be used to identify and characterize the cell populations present in the embryo as well as the molecular interactions between cells of the same population or between cells of different populations.

To perform this project, we first sampled a collection of embryos corresponding to the selected developmental stages with associated metadata describing accurately the samples and the sampling process.

We describe here the procedures used to produce and sample embryos from pregnant sows after slaughtering.

2 Protocol description

2.1 Required reagents and instruments

- A slaughter house
- Sterile disposable Petri dishes (100 mm)
- 4-well culture plates (NUNC)
- Catheters
- Sterile clamps with smooth ends, 10 cm long and 15 cm long
- Pairs of fine dissecting forceps
- Scissors
- Pre-labelled 1.5ml and 2 mL tubes
- Racks for 50 mL Falcon tubes
- 50 Falcon tubes (50 mL)
- Latex gloves
- A dissection stereomicroscope with epi-illumination
- Paper towels
- Waste bag
- Sterile Saline solution for perfusion
- Dulbecco's Phosphate-Buffered Saline (DPBS)
- Embryo holding media IMV Technologies (Ref. 019449)
- Ethanol spray bottles
- Digital Camera
- P1000, P200 and P20 pipettes with tips
- Dry ice (when necessary)

2.2 Sow synchronisation and insemination

Estrous cycle of sows was synchronized using Altrenogest (Regumate), a synthetic progestin during 18 days. The fifth day after the end of the treatment with Regumate, the sows were artificially inseminated and the insemination was repeated the following day (see figure 1).

Figure 1: Timeline used for sow synchronization and insemination. D0 is the date that will be used to determine the sampling date of the embryos.

2.3 Blastocysts recovery

When the gestational time matches the embryonic stage to be sampled, the sows are transported to the slaughter house from INRAE (Nouzilly, France). They are stunned by electronarcosis and bled. The uterus is clamped and rapidly extracted from the abdominal cavity. Then, the embryos are collected into two tubes of 50 ml by retro-flushing of the uterine horns from the bottom of the horn (uterus) upwards (ovary) in 100 ml of physiological saline.

The saline solution is then transferred into petri dishes to be screened under the stereomicroscope. PBS can be used to dilute the saline solution if this latter is too cloudy.

The embryos are collected using a pipette and transferred into a 4-well plate. They are then counted, sorted regarding their morphology and pooled.

Embryos to be used for RNA extraction are immediately lysed in RLT plus buffer (Qiagen) and stored in dry ice.

Embryos to be used for single cell studies are kept alive and hold in Embryo holding media.