PluS4PiGs: Cell tools for pig genetics

PluS4PiGs – Protocols

PluS4PiGs

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

Protocol 1
Sampling of pig blastocysts (with super ovulation and surgery)

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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 superovulation and surgery.

2 Protocol description

2.1 Required reagents and instruments

- A fully equipped surgery room
- 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 2 mL cryotubes
- 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)
- DMEM/F12
- Embryo holding media IMV Technologies (Ref. 019449)
- Ethanol spray bottles
- Digital Camera

- P1000, P200 and P20 pipettes with tips

2.2 Sow synchronisation and insemination

Estrous cycle of sows was synchronized using Altrenogest (Regumate), a synthetic progestin during 18 days. The day after the end of the Regumate treatment, sows were superovulated using a first injection of gonadotrophin (1,200 UI PMSG) followed 72h later by an injection of 500 UI hCG. The day after, sows were then artificially inseminated and the insemination was repeated the following day (see figure).

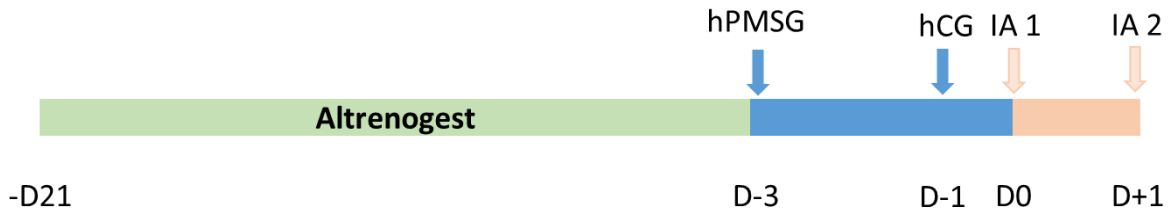

Figure 1: Timeline used for sow synchronization and insemination. D0 is the date that will be used to determine the sampling date of the embryos.

2.3 Blastocysts recovery

When the gestational time matches the embryonic stage to be sampled, surgery is performed by an authorized agent as follow. The morning of the surgery, the sow is showered, then taken to the pre-anesthesia room. There, she receives an intramuscular injection of anesthetic (Ketamine, 10 mg/kg) and analgesic (Xylazine, 2 mg/kg) to calm her down. When she stops moving, the anesthesia mask is placed on her snout, the evaporator is put into operation for diffusion of the volatile anesthetic (Isoflurane 2%). Once the effectiveness of the general anesthesia has been noted by the authorized agent for the surgery, the sow is hoisted onto the mobile operating table using an electric hoist, she is positioned in the supine position. The operating area is disinfected (vetedin, vetoquinol). A laparotomy is then performed, the uterus is extracted from the abdominal cavity, it is clamped and the embryos are collected by retro-flushing of the uterine horns from the bottom of the horn (uterus) upwards (ovary) in 100 mL of physiological saline. Once the sample has been taken, the clamps and catheters are removed and the uterus is replaced in the abdominal cavity. The wound is then sutured (aponeurotic closure with separate stitches and skin closure). The constants of the animal are checked continuously during the surgery (heart rate and breathing) until being placed back into the recovery room. This procedure was authorized by the French ministry of higher education, research and innovation under the authorization number: Apafis#10376-20170623130698.

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 to a 4-well multiplate. 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 DMEM/F12 medium.