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 3
Pooling of embryos and dissociation to produce a single cell suspension

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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 dissociate pig embryos and to produce a single cell suspension ready to load for scRNA-seq and scATAC-seq assays.

2 Protocol description

2.1 Required reagents and instruments

- A fully equipped cell culture room
- Sterile disposable cell culture dishes
- 4-well culture plates (NUNC)
- 1.5 mL and 2 mL Eppendorf tubes
- 15 mL Falcon tubes
- Latex gloves
- A dissection stereomicroscope with epi-illumination
- An inverted cell culture microscope
- Dulbecco's Phosphate-Buffered Saline (DPBS)
- DMEM/F12
- Embryo holding media (IMV Technologies)
- Pronase (Merck P8811)
- Tyrode’s Solution, Acidic (Merck T1788)
- Accutase (Thermo)
- TrypLE (Thermo)
- P1000, P200 and P20 pipettes with tips
- Neubauer or Malassez cell counting chamber
- Cell strainer (70 µM)

2.2 Reception and pooling of embryos

Accutase, DPBS and DMEM/F12 are prewarmed at 37°C for 10 minutes.

At arrival, embryos are transferred to a 4-well dish and are checked under a microscope. When necessary, embryos of the same stage were then pooled together into a drop of DMEM/F12 or IMV Embryo holding media, washed in a drop of DMEM/F12 or IMV Embryo holding media and processed for cell dissociation.

2.3 Cell dissociation of D5 embryos

To remove the Zona Pellucida (ZP), embryos are transferred into drops of 0.5 % pronase for 5 minutes max. The ZP is removed by carefully aspirating/refulling the embryos using a pipette (P200). Embryos are washed into two drops of IMV Embryo holding media.

Then, embryos are transferred into drops of prewarmed Accutase for 5 minutes, followed by a transfer into drops of prewarmed TrypLE for 10 minutes. Mechanical dissociation is also performed in parallel to facilitate cell dissociation, by aspirating/refouling the embryos using a pipette (P200).

When the dissociation is complete, cells are washed in PBS; counted and resuspended in PBS containing 0.4% BSA according to 10X Genomics protocol: Chromium Next GEM Single Cell 3' Reagent Kits v3.1 CG000204 Rev D.

2.4 Cell dissociation of D7 and D9 embryos

Embryos are washed in DPBS and transferred into drops of prewarmed Accutase for 10 minutes, followed by a transfer into drops of prewarmed TrypLE for 10 minutes. Mechanical dissociation is also performed in parallel to facilitate cell dissociation, by aspirating/refulling the embryos using a pipette (P200).

When the dissociation is complete, cells are washed in DMEM/F12; counted and resuspended in PBS-0.4 % BSA according to 10X Genomics protocol: Chromium Next GEM Single Cell 3' Reagent Kits v3.1 CG000204 Rev D (for embryos sampled in 2021) or Chromium Single Cell 3' Reagent Kits v2 CG00052 Rev F (for embryos sampled in 2017).

2.5 Cell dissociation of D11 embryos

Embryos are washed in DPBS and transferred into drops of prewarmed Accutase for 10 minutes, followed by a transfer into drops of prewarmed TrypLE for 10 minutes. Mechanical dissociation is also performed in parallel to facilitate cell dissociation, by aspirating/refouling the embryos using a pipette (P200). The suspension is then filtered on a cell strainer (70 µm) to remove remaining aggregates.

When the dissociation is complete, cells are washed in DMEM/F12; counted and resuspended in PBS-0.4% BSA according to 10X Genomics protocol: Chromium Next GEM Single Cell 3' Reagent Kits v3.1 CG000204 Rev D.