Harvest and culture of Mouse Embryonic Fibroblasts (MEFs)

Preparation

- Prepare PBS by adding 5 mL Pen-Strep (PS) per 500 mL
- Make 20% serum MEF media
- Prepare or thaw enzyme mix (50% ES trypsin, 50% EB dissociation mix)
- Absolute ethanol for utensil sterilization

Mouse House

- Bring falcon tubes on ice with PBS+PS
- Start with pregnant (E13.5) mice
- Euthanize by CO^2 followed by cervical dislocation
- Lay mice on their backs and soak with ethanol
- Remove embryos
    - Use scissors to open the skin across the abdomen
    - Rip open the skin across the front of the mouse
    - Use scissors to cut carefully into the abdominal cavity, not nicking any organs
    - Use forceps to grasp one of the uterine horns between embryos and remove it gently, snipping away all tissue connecting it to the mouse
    - Place the uterine horns in PBS+PS in a petri dish on ice

Sterile Tissue Culture Hood

- Pour PBS into a petri dish
- Briefly soak intact uterine horns in 100% ethanol, then wash in PBS
- Separate embryos from uterus and placenta
    - Embryos will be inside multiple membranes. They will be generally bean shaped with a single large red dot (liver/guts). The placenta is semi-triangular and nestles against the embryo.
    - Place embryos into the fresh dish of PBS
    - If it is hard to determine which part is which, wash it off in the clean PBS to get a clearer look

- Prepare PBS for next step
    - If genotyping, number multiple 6 cm dishes and corresponding eppendorf tubes for head collection
    - If not genotyping, just use another 10 cm dish
- Remove the heads
    - Use forceps to separate the heads of the embryos from the bodies
    - The bodies are the half of the embryo that contain the dark red spot
    - If using head for genotyping, place in corresponding eppendorf
    - Wash the bodies 3x in PBS (to minimize contamination, refresh this after every 3-4 embryos)
    - Place body in PBS (either 10 cm or 6 cm)
- Disembowel the bodies
    - Use forceps to remove the red spot from the bodies of the mice
    - Wash 3x in fresh PBS (to minimize contamination, refresh this after every 3-4 embryos)
- Mince bodies
    - Soak new razorblade(s) in 70% ethanol, allow to dry
    - Use blade to fragment embryos to a slurry
    - To speed things along, can add a few drops of enzyme mix after initial fragmentation
- Get to single(ish) cell
    - Add 1 mL enzyme mix to each embryo and transfer embryo(s) to conical tubes
    - Incubate in 37 degree waterbath for 15 minutes, vortexing halfway through
    - Pipette to mechanically dissociate
    - Inactivate enzymes by adding 1 mL filtered serum

- Plate
  - Spin at 1200rpm for 5 minutes to pellet your cells
  - Remove media and resuspend in 1 mL MEF media
  - Filter the cells through a 70μM filter into one 10 cm dish per embryo
- Split
  - Split cells the next day if confluent
  - Two days later, cells can be frozen

EB dissociation mix

250 mg collagenase IV (Gibco #17104-019)
100 mg Hyaluronidase V (Sigma H-6254)
6.8 mg DNAse I (Sigma D-4527)
50 mL DMEM

For MEFs:

Add 1:1 0.25% trypsin

Filter and aliquot.