PROTOCOL: FACS – PRIMARY MOUSE MAMMARY CELLS

Notes

- Thaw and/or prepare prior to start of experiment
  - Mouse media + supplements
    - DMEM/F12 (1:1)
    - 5% CS
    - 10 µg/mL insulin
    - 5 ng/mL mEGF
    - 0.5 µg/mL hydrocortisone
  - Digestion Mixture (10 mL/sample)
    - Mouse Media
    - 1.5 mg/mL collagenase (100 uL of 150 mg/mL stock)
    - 125 U/mL hyaluronidase (100 uL of 12500 U/mL stock)
  - Dispase Mixture (10 mL/sample)
    - Mouse Media
    - Dispase II (50 mg/sample)
    - Dnase I (200 uL of 5 mg/mL stock)
    - NOTES: Mix media & Dispase II, then filter sterilize (40 uM). Do not add Dnase I until just before use.
  - 0.25% trypsin-EDTA
  - PBS + 2% CS

Mammary Epithelial Cell Dissociation

- Gland dissection
  - For dissection prior to:
    - Thaw samples in water bath for 1-3 min
    - Add to 10 mL of digestion mixture
  - For dissection day of:
    - Sacrifice mice and fill out mouse sample info sheet
    - Dissect mammary glands (#3-5), removing lymph node on abdominal gland, and place in PBS on ice
    - Transfer all glands from one mouse to a 10-cm plate and chop briefly with scissors
    - With a cut p1000 tip on a pipette, resuspend glands with 1 mL of Digestion Mixture, transfer to flat-bottomed conical tubes, and add remaining 10 mL of Digestion Mixture
- Allow samples to digest for 30-90 min (usually 60 is sufficient) at 37°C on a rotator, checking periodically (every 30 min) to prevent over-digestion
- Pellet digested glands at 1200 rpm for 5 min
- Transfer fatty layer at top of supernatant to another tube with 10 mL of PBS and shake/invert to dislodge trapped organoids
- In originally pelleted tube, aspirate and discard supernatant and resuspend organoids in 10 mL PBS
- Spin original and fatty tubes for 1 min at high speed to settle organoids, then aspirate supernatant
- Pool organoids together, resuspend in 5 mL of red blood cell lysis buffer, and incubate for 2 min
- Add an additional 5 mL of PBS and pellet
- Wash with 10 mL PBS and pellet
- Resuspend in 1 mL 0.25% trypsin-EDTA and pipette up and down vigorously for ~1 min
- Incubate at 37°C water bath for 4 min, then repeat pipetting for ~1 min
- Add 10 mL of Dispase Mixture per sample and mix for ~1 min by pipetting
- Filter digested cells through a 40 uM mesh filter into 50-mL tube, transfer to 15-mL tube, & pellet at 1200 rpm for 5 min
- Resuspend cells in 1 mL of PBS + 2% CS and count cells

FACS Staining

- **If not sorting** Make aliquots of 400-500,000 cells in FACS tubes for unstained/control samples and 200,000 cells for stained samples, spin down, resuspend each in 200 uL of PBS + 2% CS.

- **If sorting:** From sample with largest cell #, make aliquotes of 200k cells for unstained/control samples, spin down, resuspend in 200 uL of PBS + 2% CS. Leave samples that are to be sorted in original volume in 15-mL tubes when applying stains.
- Add antibodies (turn off hood light) and let sit for 20 min at 4°C (or 10-15 min at RT) in the dark (wrap in aluminium foil)
    - We use EpCAM and CD49f to separate cells into luminal ($EpCAM^{hi}/CD49f^{lo}$) and basal ($EpCAM^{lo}/CD49f^{hi}$) populations – stromal cells will be negative for both
- Wash x1-2 with 3 mL PBS + 2% CS per tube
- Resuspend samples in 200 uL PBS + 2% CS
- Keep on ice and in dark until analysis