

## FACS cell prep for lungs (AT2 and BASCs)

BEFORE BEGINNING, you will need:

- Avertin (1X stock is 20 mg/mL)
- Forceps, surgical scissors, pins, dissecting platform (Styrofoam lid wrapped in foil, covered with a paper towel)
- PBS on ice
- 30, 3, and 1 mL syringes
- 25G and 18G needles
- Dispase (BD 354235, 50 U/mL)
- Collagenase/dispase (Roche 10 269 638, dilute to 100mg/mL in cold PBS)
- DNase (SIGMA D4527, made to 1% solution in sterile water)
- PF10 (10% FBS in PBS)
- 100 and 40  $\mu$ m filters
- Bucket with ice
- 50 mL conical tubes
- 0.25% Trypsin-EDTA
- Eppendorfs and flow cytometry tubes
- Antibodies

### Dissection

1. Anesthetize mouse with 1 mL avertin per average adult mouse (25G needle on 1mL syringe, IP injection). Once mouse is unconscious, pinch foot firmly with forceps to ensure lack of sensation/reflex.
2. Affix mouse to dissecting platform with tape or pins and spray down mouse fur with 70% ethanol.
3. Open the skin on the stomach to expose the base of the sternum. Hold the base of the sternum with forceps and carefully cut up the ribcage to the neck. Cut skin from center line right and left along diaphragm edge. Fold the two sides of the rib cage open and secure with pins.
4. Using a butterfly needle on a 30 mL syringe, perfuse 10-15 mL of PBS (ice cold) through right ventricle (on your left) until lungs cleared of blood.
5. Remove heart to euthanize mouse, then cut up the neck (through collar bone) to the chin of the mouse.
6. Expose trachea, place forceps under trachea to keep exposed.
7. Using an 18G needle on a 3 mL syringe, inject 2mL dispase into trachea (go between the cartilage rings) just until the lungs inflate.
8. Dissect out lungs by gently tugging on the trachea while snipping away the connective tissue; leave lungs intact and place in small Petri dish on ice with PBS.
  - You may leave lung tissue on ice while dissecting other mice and before proceeding to digestion.

### Lung Tissue dissociation

1. Trim other tissues off lung, dissect off each lobe, and mince lung tissue into small pieces inside of a 50 mL conical tube. **For each lung sample, add 3 mL PBS.**
2. Add **60uL collagenase/dispase for every 3mL of PBS.** Tightly cap the tube and place in rotisserie to rotate 45 min @ 37°C.
3. After 45 minutes, place the tubes on ice. Add **7.5 uL DNase per 3 mL** of digest solution and swirl to mix.
4. Into a 50 mL tube, filter digested tissue through a 100 um filter, then a 40 um filter using excess cold PBS to wash cell through filter and the plunger end of a 1mL syringe to mechanically push the cells over the filter.
5. Spin 6 min @ 800 rpm in Beckman centrifuge.
6. Aspirate supernatant being careful not to aspirate cell pellet, which may not be tightly adhered to the bottom of the tube. Resuspend the cell pellet in 1 mL of lysing solution (0.15 M NH<sub>4</sub>Cl, 10mM KHCO<sub>3</sub>, 0.1 mM EDTA, in 1L distilled H<sub>2</sub>O; filtered with 0.45 um filter and stored at RT).
7. Lyse 90 sec at RT.
8. Add 6 mL of PF10 and mix to end lysis, then centrifuge for 4 min @ 800 rpm.
9. Aspirate the supernatant; the pellet should now be white in color and well packed and is now ready to be stained.

#### Surface staining

1. Resuspend the cell pellet in 150uL PF10 and move to a clean eppendorf tube. Combine 20uL of solution from each mouse in a separate tube, then aliquot the mix evenly to tubes for single stains and unstained controls.
2. For the BD antibodies, add 3uL of each antibody per 100uL of PF10 (or 2 uL for each single stain) and let sit at room temperature for 10 minutes.
3. Fill tube to brim with fresh PF10 and invert to wash the cells, then pulse spin to pellet the cells and aspirate the media.
4. Resuspend each sample in 200 uL PF10+DAPI and move to a flow cytometry tube.

Antibodies we use currently:

- 1° CD31-APC BD #551262
- 1° CD45-APC BD #559864
- 1° EpCAM-PE-Cy7 BioLegend #118216
- 1° SCA-1-APC-Cy7 BD #560654

#### Sorting cells

Sort into a tube and then plate by hand

For sorting into tubes, prepare collection tubes ahead of time:

3% BSA in PBS, made fresh or filtered, fill up the collection tube and let stand at least one hour (or overnight in 4°C). Aspirate this solution, add about 200 uL of media into which to sort the cells.

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*Technique was based on:*

Kim, C.F., Jackson, E.L., Woolfenden, A.E., Lawrence, S., Babar, I., Vogel, S., Crowley, D., Bronson, R.T., and Jacks, T. (2005). Identification of Bronchioalveolar Stem Cells in Normal Lung and Lung Cancer. *Cell* 121, 823-835.

Rock, J.R., M.W. Onaitis, E.L. Rawlins, Y. Lu, C.P. Clark, Y. Xue, S.H. Randell, B.L.M. Hogan. 2009. Basal cells as stem cells of the mouse trachea and human airway epithelium. *PNAS*. 106(31):12771-12775.