Hair follicle bugle stem cell FACS analysis

(prepared by B. Lee, amended by P.Sun)

Materials:
Isoflurane (Fisher scientific, 100 ml, NC9732036)
2% FBS(HyClone, Ca# SH30071.03) in PBS
6cm TC dishes
forceps (sterilize in 70% EtOH before using)
razor blade (sterilize in 70% EtOH before using)
0.25% trypsin (Invitrogen Corp., Cat# 15050-065)
70um filter (BD falcon, Cat# 352350)
FACS tube (5 ml Polystyrene Round-bottom tube, BD Falcon, Cat# REF352052)
biotin-anti-mouse CD34 (eBioscience Inc., Cat# 13-034-81)
biotin-rat IgG2a k Isotype, (BDPharmingen Cat# 553928)
PE-alpha6 integrin (CD49) (BD bioscience, Cat# 555736)
PE-Isotype Control: Rat IgG2b, Kappa, (BD Pharmingen, Cat# 553989)
Streptavidin-APC (BD Pharmingen, Cat# 554067)
Mouse: in telogen (typically p49), anagen works too but needs to be incubated (0.25% trypsin treatment—see step 7) for an extra hour or so.

1. Pipet 0.5 ml of isoflurane into a 1 L beaker and cover the liquid with several paper towels. Place the mouse inside the beaker and cover the beaker with foil. The mouse should lose consciousness within a minute or so. (This procedure should be performed in hood.)

2. Shave the whole back really well and carefully.

3. Kill mouse and cut off all of the skin from the back of the mouse.

4. Pin skin with dermis facing up on a styrofoam plate and scrape off fat, muscle, etc using a razor blade (you may need to be rough with the scraping).

5. Place skin piece in 6cm plate with epidermis facing up.

6. Add 5mL 0.25% trypsin. Lift up skin so trypsin goes underneath it.

7. Incubate for 2 hours in 37C incubator without CO_2 in the lab (increase in CO_2 will result in pH change of trypsin). This incubator is OK for FACS, but not for primary culture (which will need to be done in a TC incubator with proper CO2 concentration – needs to be worked out).

8. Place skin in lid of plate with epidermis facing up and scrape off epidermis with razor blade while holding down one edge with forceps. (Don't blot dry epidermis, this way you will have a bit of trypsin left upon transfer to keep the tissue moist)

9. Transfer scraped epidermis to a new dish and mince with scissors into pieces smaller than 0.5 mm.

10. Transfer to 15mL tube and add 5mL 2% FBS in PBS.

11. Add another 5mL 2%FBS PBS to wash dish and pour into tube.

12. Pipette up and down 20 times with moderate force and avoid bubbling.

13. Add contents to a 50mL tube with 70um filter on top.

14. Add 5mL of 2%FBS PBS to wash the rest of the cells out of the 15mL tube and add again to the 50mL tube with filter.

15. Transfer filtrated from above to 15mL tube and spin 1000g for 5 min.

16. Remove supernatant and add 2-5mL new 2% FBS PBS. (The amount added depends on the size of the pellet.)

17. Count cells using 10uL cell suspension + 10uL trypan blue. Count the four corners and average. Total cell number = # cells total for all corners / 4 (to average the four corners) X 2 (for trypan blue dilution). You may also need to dilute cells in order to obtain an accurate count.

18. Add about 1 million of those cells to a FACS tube and bring volume up to 2mL with 2% FBS PBS.

19. Spin 1000g for 5 min.

20. Remove supernatant and add 50uL 2%FBS PBS (This will make roughly 100uL total. Not necessary to resuspend here).

21. Add 1uL biotin-IgG or biotin-CD34 and 0.5uL PE-IgG or PE-alpha6 integrin. Resuspend pellet and mix well, and incubate for 30 min in the dark at RT. (1 tube for IgG and 1 for experimental)

22. Add 2mL 2%FBS PBS directly into tube with antibodies and cells.

23. Spin down 1000g for 5 min and remove supernatant.

24. Add 50uL 2% FBS PBS again (Not necessary to resuspend here).

25. Add secondary antibodies - 0.5uL strep-APC into the tube containing ~100uL total, resuspend pellet and mix well. Incubate for 30 min in the dark at RT.

26. Add 2mL 2% FBS PBS directly into tube again and spin down 1000g for 5 min.

27. Remove supernatant and add 300uL PBS without the FBS to pellet, resuspend.

28. Cover tubes with foil and proceed to FACS analysis.

Alternative:
29. If you need to fix the cells, add 4% PFA to make 1% (final concentration) with a total of 200uL solution in tube and incubate in the dark at RT for 10 minutes. (fixed cells can be stored at 4 C for up to 1 day before FACS analysis. Note that fixation seems to reduce signal/noise ratio so try to avoid it if at all possible)

30. Add 2mL fresh PBS and spin down 1000g for 5 minutes.

31. Add 300uL fresh PBS to pellet, resuspend, and proceed to analysis.

FACS
Instrument name: Becton-Dickinson FACSCalibur cell analyzer (FL2: PE, FL4: APC)
Location: Institute for Immunology (Hewitt Hall, Room 3101).

Instrument name: BD LSR II
Location: Stem Cell Core Facility (Gross Hall, First Floor, Room 1302)

Analysis software: Flowjo 7.5