Preparing Fetal Liver Cells for ER-Hoxb8 GMP cell line production

Materials

- Pre-stimulation media
  - RPMI + 10% FBS + P/S + SCF (10 ng/ml) + IL-3 (5 ng/ml) + IL-6 (10 ng/ml)
- FACS buffer
  - PBS + 2% FBS + EDTA (1 mM)
- ACK red blood cell lysis buffer
  - E.g. Lonza (10-548E), though many manufacturers exist
- Acridine orange (10 ug/ml)
  - E.g. Invitrogen (A3568) sells a 1000x stock solution (10 mg/ml)
- 6 cm tissue culture dishes
- 6 well tissue culture plate
- 40-micron cell strainer (e.g. Fisher 22-363-547)
- 50 ml conical tubes
- 5-cc syringes

Protocol

1. Euthanize pregnant female and collect pups at day E15-16.5.
   a. The pups are larger and the fetal livers easier to dissect as they get closer to day E16.
2. Dissect the fetal liver and transfer to a 6 cm tissue culture dish with cold FACS buffer.
   a. Once all the fetal livers are collected, move to a tissue culture hood.
3. Aspirate the FACS buffer and transfer the fetal liver into a 40μ cell strainer with sterile tweezers.
4. Add 5 ml of ACK red blood cell lysis buffer to the dish.
5. Using the rubber plunger from a 5-cc syringe, macerate the fetal liver through the 40μ cell strainer into the ACK red blood cell lysis buffer.
   a. This will dissociate the cells into a single-cell suspension.
   b. The support cells (non-hematopoietic cells) will tend to lyse in the ACK lysis buffer.
6. Mix the cell suspension well and rinse through the cell strainer. Discard the cell strainer.
   a. Usually I will do this with a 5-ml pipet or a P1000.
7. Allow the RBC lysis to proceed for ~5 minutes time.
8. Transfer the cells through a 2nd 40μ cell strainer that you have placed on top of a 50 ml conical tube.
   a. This second filtration is important to get rid of most of the cell membranes that result from the red blood cell lysis.
9. Bring the cell suspension up to 50 ml with FACS buffer to neutralize the ACK lysis buffer.
10. Pellet the cells for 10' at 500g.
11. Using a P1000, resuspend the cells well in 1 ml of the pre-stimulation media.
12. Take 20 ul of the cell suspension and add to 20 ul of acridine orange to count the nucleated cells.
    a. The acridine orange helps avoid counting the remaining contaminating red blood cells.
13. Transfer the remaining cells to a 6-well plate with an additional 5 ml of pre-stimulation media.
14. Leave the cells in culture for 24-36 hours prior to retroviral transduction with the ER-Hoxb8 virus.
    a. The transduction can be carried out immediately but it seems more efficient once the cells are in culture overnight.