Peripheral blood-derived erythroblasts from fetal liver (Fetal PBDE)

A. Collection of cells (16-19 weeks human fetal liver was obtained from the Einstein fetal tissue core facility and processed by the laboratory of Eric Bouhassira)

1. Using sterile forceps, take the fetal liver and place in a Petri dish. Rinse a few times w/ PBS containing penicillin and streptomycin (PBS/PS).
2. Cut the fetal liver into tiny pieces with sterile scissors or scalpels.
3. Dilute the pieces w/ PBS/PS
4. Collect the cells in a 50 ml tube and filter them with a 70um filter to remove the larger pieces.
5. Centrifuge the cells at 1200 rpm for 4 min. Discard the supernatant and dilute the pellet in PBS/ 2% FBS.
6. Dilute the cell suspension with 2 volumes of PBS/ 2% FBS. Slowly overlay 30 ml of cell dilution over 15 ml of Histopaque (Sigma, cat # 10771, density=1.077 g/ml)
7. Centrifuge w/o brake at 400 g for 35 min at RT. Aspirate the upper level leaving the mononuclear layer undisturbed at the interface.
8. Carefully transfer the mononuclear cells to a 50 ml tube and add PBS to wash cells with the final volume of 50 ml.
9. Centrifuge at 300 g for 15 min at RT.
10. Discard the supernatant and resuspend the cell pellet in 20 ml of PBS.
11. Centrifuge at 300 g for 15 min at RT.
12. Discard the supernatant and resuspend the cells. Count the cells and proceed to isolate the CD34+ cells or culture the mononuclear fraction.

B. Purification of CD34+ cells from fetal liver mononuclear cells using EasyStep® Human CD34 Positive Selection Kit

1) Incubate 8 x 10^6 fetal liver mononuclear cells in 100 ul of PBS/2%FBS with 20 ul CD34+cocktail in a polysteyrene tube for 15 min at RT.
2) Add nanoparticles:cocktail mix in 1:2 ratio (add 10 ul nanoparticles); incubate for 10 min at RT
3) Bring cell suspension to a total volume of 2.5 ml by adding 2% FBS/PBS and mix well
4) Place tube in magnet holder for 5 minutes (w/ cap loosened)
5) Pick magnet up and invert in one continuous action (invert for 2-3 sec)
6) Return the magnet in the upright position
7) Remove tube from magnet and resuspend cells in 2.5 ml of 2% FBS/PBS, mix well
8) Repeat 5x from step 6.
9) Count CD34+ cells
10) Verify purity by FACS (should be at 90-95% CD34+)

C. Culturing fetal liver cells (StemSpan can be purchased at http://www.stemcell.com)
Note: Cell culture can be initiated either with the mononuclear cells or with the CD34+ cells. Erythroblasts obtained from cultures seeded with mononuclear cells or with CD34+ cells are indistinguishable by FACS or after Giemsa Staining.

Week 1. Culture 10^4 $CD34^+$ cells/ml or $2X10^5$ mononuclear cells/ml in Stemspan supplemented with:

10^{-6} M Hydrocortisone
300ng/3mL SCF
120ng/3mL Flt-3L
40ng/3mL IL-3
40ng/3mL BMP-4
8U/3mL EPO

Replace the medium on day 4 with fresh Stemspan; keep cell density at ~2-3x10^5 /mL.

Week 2. Culture the cells in Stemspan supplemented with:

10^{-6} M Hydrocortisone
120ng/3ml SCF
120ng/3ml IGF1
40ng/3ml BMP4
40ng/3ml IL-3
40ng/3ml IL-11
10U/3ml EPO

Change the medium every 2-3 days keeping the cell density under 10^6 cells/ml.

D. Crosslinking protocol

At the end of week 2, the cells are collected by centrifugation at 2000-2500 x g for 10 min and crosslinked in PBS containing 1% formaldehyde for 10 min at room temperature on oscillating platform shaker. The reaction is stopped by adding glycine to a final concentration of 0.125 M. After 5 min, cells are washed twice with ice-cold PBS (Ca+2/Mg+2 free) and centrifuged at 1,500-2,000 x g for 5 min at 4°C. Supernatant is discarded, and cell pellets are flash frozen in liquid nitrogen and stored at -80°C.