Human peripheral blood-derived erythroblasts (PBDE)

1. Cell expansion and Differentiation

20mL blood was collected and the buffy coat isolated using ficoll-Pague.
Cells were expanded for 2 weeks as follows:

Week 1: Use StemSpan as basal medium and add:	10-6 M Hydrocortisone 300ng/3mL SCF 120ng/3mL Flt-3L 40ng/3mL IL-3 40ng/3mL BMP-4 8U/3mL EPO
Week 2: Use StemSpan as basal medium and add:	10-6 M Hydrocortisone 120ng/3mL SCF 120ng/3mL IGF1 40ng/3mL IL-3 40ng/3mL BMP-4 10U/3mL EPO

Feed the cells every other day. (Try to keep the density at ~2-3x10^5 /mL)

After 14 days, collect the cells, wash twice with PBS, and perform the crosslinking reaction.

2. Crosslinking reaction.

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.

Note: StemSpan can be purchased at http://www.stemcell.com.