Friend virus leukemia stem cell isolation

Paulson lab
http://vbs.psu.edu/research/labs/paulson

1. BALB/cJ mice 8-10 weeks old are infected with Friend virus polycythemia inducing strain (FVP)
2. 14 days after infection, mice are euthanized and spleen cells isolated.
3. Erythrocytes are lysed with Ammonium chloride solution and the cells are plated in IMDM +5% FCS+SCF (50 ng/ml) + BMP4 (15ng/ml) + Shh (200 ng/ml) + IL-3 (10 ng/ml) and grown for 7 days.
4. Cells were harvested and labeled with PKH26 (Sigma) according to manufacturers conditions. The cells were then cultured for 7 days in the same media.
5. After 7 days, the cells were harvested and labeled with anti-Kit, anti-Sca1, anti-m34 (Friend virus antibody) and anti-CD133 fluorescent antibodies.
6. The cells were gated on m34+Kit+Sca1+ cells and cells were sorted into $PKH26^{hi}CD133+$ and $PKH26^{lo}CD133-$ fractions.
7. M34+Kit+Sca1+ $PKH26^{hi}CD133+$ are Leukemia stem cells (Previous work showed that these cells are also CD34+) while M34+Kit+Sca1+ $PKH26^{lo}CD133-$ are progenitor cells. Functionally the difference is that progenitor cells form Epo-independent BFU-E when grown in methylcellulose containing IL-3 and SCF, while Leukemia stem cells do not form BFU-E. In addition, Leukemia stem cells will induce erythroleukemia when transplanted back into mice.