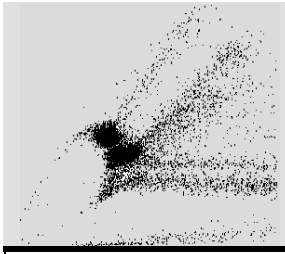

THE GOODELL LABORATORY

Author	Karen Lin	July 30, 2005
Title	**Transduction of Enriched Bone Marrow Cells with Retroviruses**	
Introduction	The objective of this procedure is to virally transduce freshly isolated and enriched bone marrow cells with least differentiation stress provided to the HSCs compartment during *in vitro* culture. All the viruses should contain a marker such as GFP in order to distinguish transduced cells from the non-transduced. After transduction, the infected bone marrow cell mixture or infected Sca-1+Lin- progenitor cells will be utilized as donor cells in subsequent transplantation experiments. Note that in our lab, this transduction protocol has accomplished ~60% transduction efficiency with MSCV-based retrovirus, and ~30% with pBabe-based retrovirus (measured in vivo).	
Materials	*Stempro34 transduction medium **Important: DO NOT filter nutrient supplement and cytokines** Stempro34 SFM (Gibco 10639-011) Nutrient supplement(40X) 1X Pen/strep (100X) (Gibco 10378-016) 1X L-Glutamine (Gibco 25030-081) 2mM SCF, mouse (R&Dsystems 455-MC-050/CF) 10ng/ml TPO, mouse (R&Dsystrems 488-TO-025/CF) 100ng/ml Polybrene (Sequa-brene, Sigma S2667) 4ug/ml **Stempro34 culture medium Nutrient supplement(40X) 1X Pen/strep (100X) 1X L-Glutamine 2mM SCF 10ng/ml TPO 100ng/ml	
Protocol		*Notes*
1.	**Day-6** Inject bone marrow donors with 150mg/kg 5-FU (Fluorouracil, American Pharmaceutical Partners Inc. NDC63323-117-10) to induce proliferation of hematopoietic stem cell. Dilute 5-FU into 10mg/ml, and intravenously inject mice: 300ul/20g.	*Weigh the mice before injection. For a 20g mouse, it requires 300ul of diluted 5FU (10mg/ml). See the appendix table for more detail.*
2.	**Day 0** Harvest Sca-1+ cells from whole bone marrow cells Harvest bone marrow cells from 5-FU treated mice (15-20 x10^6 cells/mouse on 5FU-Day6). Enrich Sca-	

	1+ cells as the protocol from lab archive described. Resuspend cells in the Stempro34 transduction media*.	
3.	Transduction-Spin infection Adjust cells to $5x10^5$ cells/ml, and plate 1ml/well into a 24 well plate. Apply freshly thawed virus supernatant into Sca-1+ cell suspension, and spin-infect at 1100rpm (230g) at room temperature for 2 hours. After spin-infection, incubate cells in 37^{o}C. Go to Step 4 if transplantations are planned. Otherwise, go to Step 5, in which cells are incubated overnight for further purification and experiments.	In our experience, progenitor cells lose their multipotency over the course of *in vitro* culture. To test the stem cell activity resulted from gene modification, it is highly recommended to transplant these cells at the same day of transduction.
4.	Bone marrow transplantation For bone marrow transplantation, further incubate cells for additional 3 hours after spin-infection. Briefly wash cells with cold HBSS and transplant mice with at least 2x10^4 cells per mouse.	
5.	**Day1** Replace medium with Stempro34 culture medium**.	Tilt the plate, take out ½ of the supernatant, and reaplce with warmed fresh Stempro34 culture medicum**.
6.	**Day2** Harvest infected progenitor cells 36 to 48 hours after transduction, harvest infected cells and stain cells with Sca-1-PE and Lin-cychrome cocktail antibodies (Lin cocktail is a combination of CD4, CD8, Ter119, Gr-1, and B220.) Infected progenitors will be sorted upon the expression of Sca-1+, Lin-, and GFP+ and transplanted into mice with competitor cells.	Cell death has been observed after in vitro cell culture. Propidium iodide (PI) exclusion is highly recommended when cultured cells are subjected for sorting or flow analysis.

Appendix – 5FU dose to Mouse Body Weight

body weight (gram)	5FU dose (g/mouse)	5FU injection (ul)
10	1.50	150
11	1.65	165
12	1.80	180
13	1.95	195
14	2.10	210
15	2.25	225
16	2.40	240
17	2.55	255
18	2.70	270
19	2.85	285
20	3.00	300
21	3.15	315
22	3.30	330
23	3.45	345
24	3.60	360
25	3.75	375
26	3.90	390
27	4.05	405
28	4.20	420
29	4.35	435
30	4.50	450