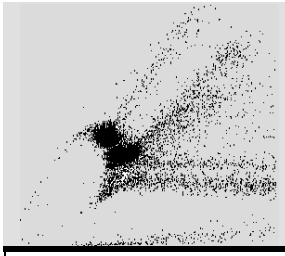

THE GOODELL LABORATORY

Author	Nathan Boles	Feb.5, 2009
Title	**Peripheral Blood Analysis**	
Introduction	This protocol describes the analysis of mouse peripheral blood	
Materials	HBSS+ : Hanks Balanced Salt Solution (from Gibco) with 2% Fetal Calf Serum and 10 mM 2% dextran in PBS Heparin Solution Antibody Cocktail (In HBBS+) Antibody — Concentration B220 - Pac Blue — 1:100 B220 - Pe-Cy7 — 1:100 CD4 - Pac Blue — 1:100 CD8 - Pac Blue — 1:100 Gr-1 - Pe-Cy7 — 1:200 Mac-1 – Pe-Cy7 — 1:200 CD45.1 - Fitc — 1:100 CD45.2 – APC — 1:100	
Protocol		*Notes*
1.	Prepare 1.5 mL tubes to receive blood by pipeting 150µL of 2% dextran in PBS and 150µL of Heparin Solution.	
2.	Retro-orbitally bleed mice using heparin coated capillary tubes and place blood into prepared 1.5mL tubes. Also obtain spleenocytes to use as a control at this time.	
3.	Allow blood to settle for at least 20 minutes (until supernatant appears almost white) then pipet off supernatant into an appropriate FACS tube.	
4	Add 1 mL of RBC lysis buffer to each tube.	…

5	Wait at least ten minutes.	*Best to wait around 20 minutes*
6	While you are waiting, prepare the antibody cocktail shown in the Materials section of this protocol(enough to make a final solution of 100µL per sample).	*CD45.1 or CD45.2 can be substituted for an antibody of your choice. Also PE is still open for the use of an antibody of your choice.*
7	Place antibodies into an appropriate amount of Hank's+ (enough to make 100µL per tube) to finish your antibody cocktail.	
8	Stain spleenocytes with B220+ in each color to act as controls	
9	Wash tubes with 2 mL of Hank's+, then spin down for 8 min. at 2000 rpm.	
10	Discard supernatant (I personally use the 'net method' in combo with the centrifuge holders)	*In order to discard supernantant of all the tubes together, you can use a plastic web to cover the tubes and turn it down together with the centrifuge holders.*
11	Resuspend cells with 100µL of the antibody cocktail	
12	Wait 10 minutes, then wash with 2 mL of Hank's+ and spin down for 8 min. at 2000 rpm.	
13	While your samples are spinning prepare a PI solution by using the lab stock of PI (1:100concentration) and Hank's+.	
14	Discard supernatant.	
15	Resuspend cells with 300 µL of PI solution.	
16	Go do the analysis on the LSRII.	

References.

1. Challen G, Boles NC, Lin KY, Goodell MA. Mouse Hematopoietic Stem Cell Identification And Analysis. Cytometry A. 2009 Jan;75(1):14-24.

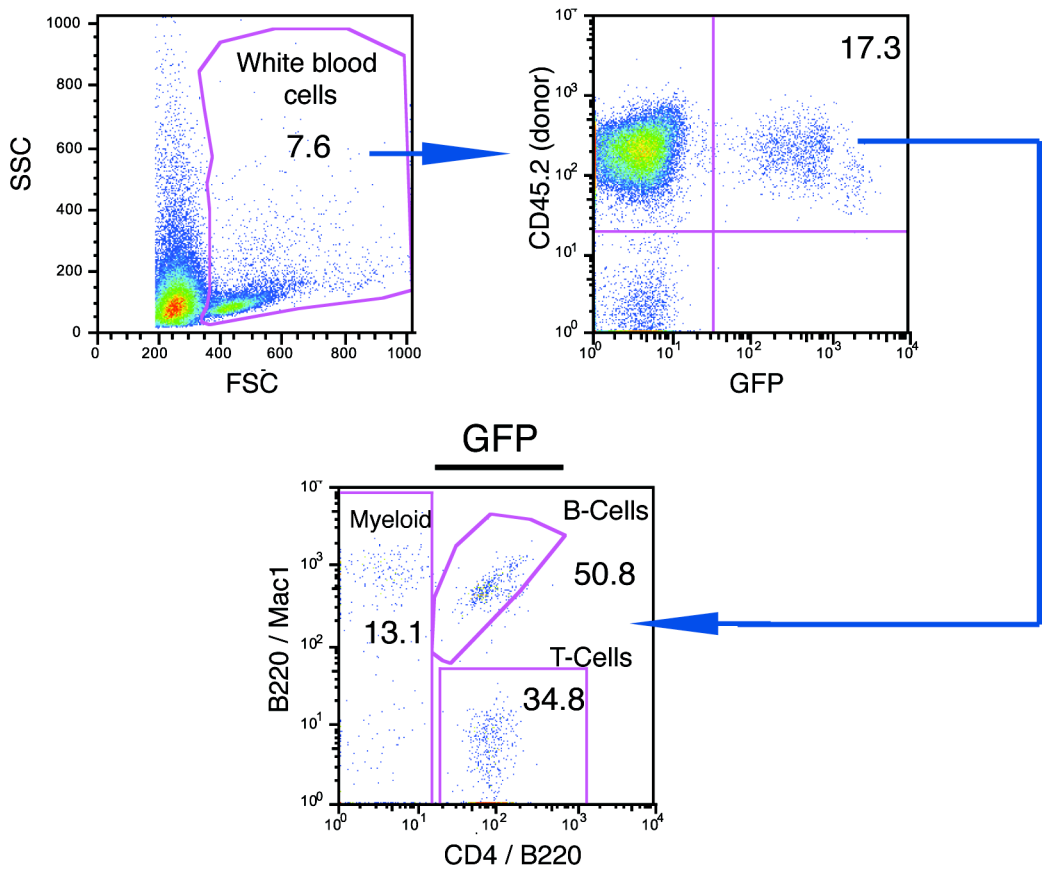

Figure 1. Example of Retroviral transduction and lineage analysis. At twelve weeks after transplant/transduction mice were bled and engrafted/transduced cells were identified using CD45.2-APC (donor background) and eGFP expression. B-cells were dual stained with B220-Pacific Blue and B220-PE-Cy7. T-cells were single labelled with CD4- and CD8-Pacific Blue. Myeloid cells were Pacific Blue negative. Transduced and non-transduced cells were simultaneously examined using a FacsAria (BD).