The
GOODELL laboratory

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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 100 L 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 100 L 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*

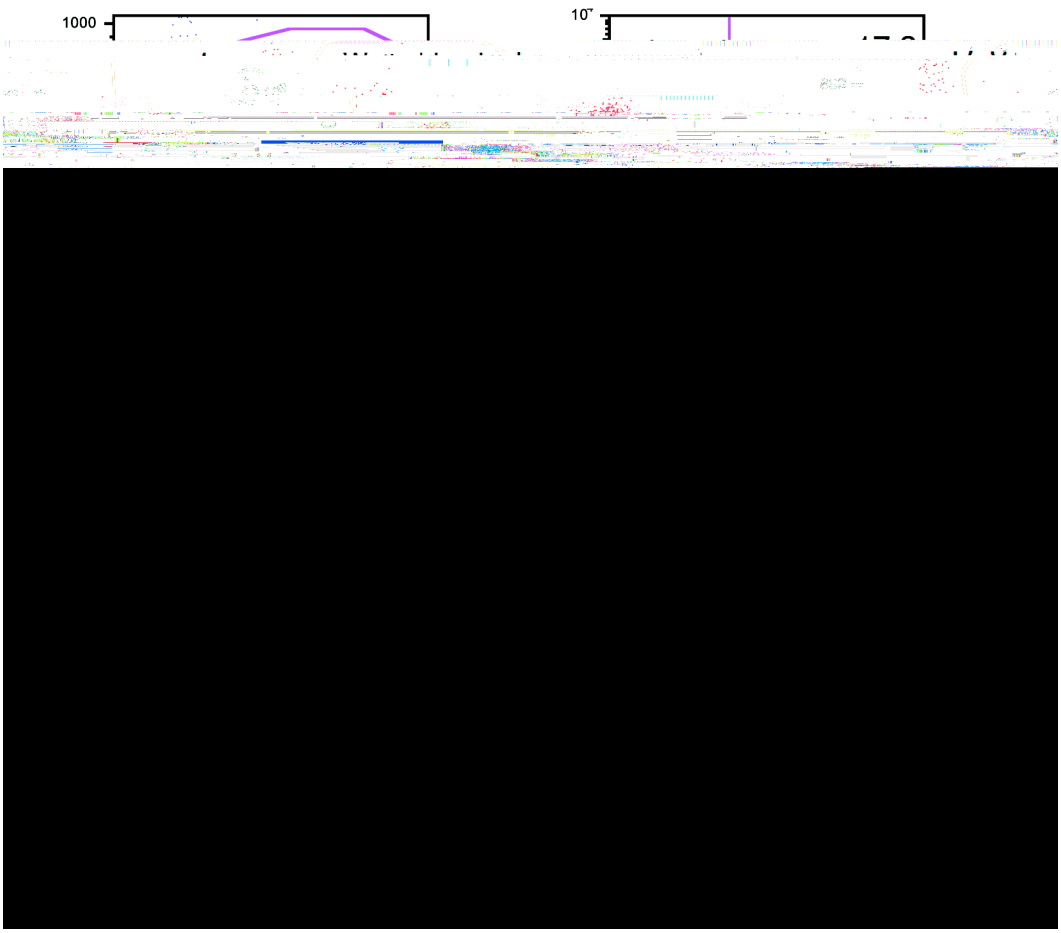

Figure 1. Example of Retroviral transduction and lineage analysis. At twelve weeks after transplant/transduction mice were bled and engrafted/transduced cells were identified using CD45.2-APC (donor background) and eGFP expression. B-cells were dual stained with B220-Pacific Blue and B220-PE-Cy7. T-cells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