The GOODELL laboratory

Author	Grant Challen	1/30/09
Title	**BrdU Labeling of Hematopoietic Stem Cells**	
Introduction	This protocol describes the use of BrdU incorporation to determine the rate of turnover of hematopoietic stem cells (HSCs). BrdU is incorporated into the DNA of replicating cells so the percentage of BrdU+ cells over a time period can be used to determine the relative rate of turnover. For this assay, mouse HSCs are labelled with BrdU *in vivo*, the HSCs are purified (along with a carrier cell population to minimize cells loss when dealing with limiting numbers of HSCs), and then fixed and reanalyzed for BrdU incorporation.	
Materials	1. BrdU (Sigma; B5002-500MG) 2. PBS 3. HANKS+ buffer = Hanks Balanced Salt Solution (Gibco #14170) + 2% FBS + 10 mM HEPES (Gibco #15630) 4. DMEM+ buffer for Hoechst staining = DMEM (Gibco #11965) + 2% FBS + 10 mM HEPES (Gibco #15630) 5. Hoechst 33342 (Sigma; B2261-500MG) 6. Sca-1 or c-Kit microbeads (Miltenyi Biotec) 7. Fluroescent-conjugated antibodies for Sca-1, c-Kit, and Lineage markers 8. Propidium iodide (PI) stock = 200 µl / mL. Dissolve 10 mg PI (Sigma; P1470-25mg) in 50 mL dI H_2O. 9. PI solution = 1:100 dilution of PI stock in HANKS+ buffer. 10. BD Biosciences BrdU-FITC analysis kit (cat. # 559619).	
Protocol		*Notes*
1.	Make up BrdU solution for injection. Need 3.33mg BrdU dissolved in 500µL PBS per mouse (e.g. for 5 mice dissolve 0.0167g BrdU in 2500µL PBS, inject 500µL). This is for an average 8-week old C57Bl/6 mouse. This amount works well for most applications but for large variations in weight the amount of BrdU injected must be adjusted accordingly.	*BrdU takes up to a few hours to dissolve at 37^oC depending on amount.*
2.	Prepare BrdU for supplementation in drinking water (if necessary). Make up solution of 0.8mg/mL BrdU in water (e.g. 0.240g in 300mL).	

3.	Inject mice intraperitoneally with 500 µL of BrdU / PBS solution and substitute BrdU-drinking water.	*Supplementation of drinking water not necessary for timepoints less than 12 hours.*
4.	Sac mice and prepare bone marrow at desired timepoints.	*Typical timepoints are 12 hours, 3 days and 6 days.*
5.	Prepare spleenocytes from unlabelled mouse for carrier cells.	*Prepared by manual dissociation with scissors and filtration.*
6.	Prepare bone marrow for HSC isolation using the Hoechst staining protocol. Perform positive-selection magnetic enrichment if desired using Sca-1+ or c-Kit+ microbeads and AUTOMACS magnetic separation. Following enrichment, stain the cells with the following antibodies - Sca-1 / c-Kit-PE, Sca-1 / c-Kit-APC, Lineage-PeCy5. Resuspend cells in PI for FACS sorting.	*For Sca-1 enrichment, use c-Kit-PE and Sca-1-APC;*

14.	Determine rate of turnover of HSCs by determining percentage of BrdU-FITC+ HSCs.	*Approximate normal HSC rate of turnover 4*