Isolation of HSC, MEP, CMP, & GMP from Mouse Bone Marrow

1. Harvest bone marrow cells from 20-25 C57BL6J mouse femurs and tibias (via standard flush protocol) into 11 mL PBS+5%FCS
2. Cell Count:

3. Dilute cells to 50 mL with ACK Lysing Buffer
4. Incubate 20 min on ice with occasional mixing
5. Spin 2100 rpm (850 x g), 5 min @ 4°C
6. Resuspend in 8 mL PBS+5%FCS
7. Add 200** μL of each purified antibody: anti-

CD4	IL-7Ra		Ter119	Mac-1
CD8	B220	Gr-1		

8. Incubate 30 min on ice with occasional mixing

---While cells are incubating---

9. Place 7 mL magnetic beads into 8 conical tubes, mix, and apply to magnet, 5 min @ 4°C
10. Wash magnetic beads x2 with 8 mL PBS+5%FCS (add wash, vortex, apply to magnet for 5 min, and remove supernatant); leave final wash on until ready to apply cells

---Following 30 min incubation---

11. Divide cells into 2x 50 mL conicals
12. Add 45 mL PBS+5%FCS
13. Spin 2100 rpm, 5 min @ 4°C
14. Resuspend cells in each tube in 32 mL PBS+5%FCS
15. Add 8 mL cells+Ab suspension to tubes with magnetic beads and mix
16. Incubate 30 min on ice with occasional mixing
17. Mix, apply to magnet for 5 min @ 4°C
18. Remove sup to fresh 15 mL culture tube
19. Mix, apply to magnet for 5 min @ 4°C
20. Collect supernatant into fresh 50 mL conical (Lin-depleted cells)

21. Spin, 2100 rpm, 5 min @ 4°C
22. Resuspend in 8 mL PBS+5%FCS in 15 mL Falcon tube
23. Apply to slightly stronger magnet while counting
24. Cell Count:

25. Transfer cells to a 15 mL conical containing:

    150 μL IgG2ak

    150 μL IgG2bk

    150 μL IgG1k

26. Incubate for 20 minutes on ice, mixing occasionally
27. Pellet sample by spinning at 2100 rpm, 5 min @ 4°C
28. Set aside 7x 5 mL FACS tubes, each with 125 μL PBS+5%FCS, labeled and containing 3 μL of each of the following:

    No stain (cells alone)

    APC anti-cKit

    PE anti-Sca-1

    V450 anti CD16/32

    FITC anti-CD34

    cKit+Sca-1+CD16/32

    cKit+Sca-1+CD34

29. Add $2x10^5$ cells into each of the 7 control-stain tubes and incubate for 30 minutes on ice

    _____ μL cells (usually just add 30 μL of cells to each control stain)

30. Resuspend in 8 mL PBS+5% FBS containing 150 μL of each of the following:

    APC anti-cKit

    PE anti-Sca-1

    V450 anti CD16/32

    FITC anti-CD34

31. Incubate on ice, 30 minutes, mixing occasionally
32. Add 3 mL PBS+5% FBS to each of the control tubes. Pellet by centrifuging at 2100 rpm, 5 min @ 4°C
33. Pour off supernatant and resuspend in 300 μL PBS + 5% FBS for analysis

34. Pellet sample by centrifuging at 2100 rpm, 5 min @ 4°C

35. Resuspend in 4 mL PBS+5% FBS for analysis

Cell Sorting:

Megakaryocyte/Erythroid Progenitors (MEP):

Lin^{-} $cKit^{+}$ $CD34^{Lo}$ $CD16/32^{-}$

Hematopoietic Stem Cells (HSC):

Lin^{-} $cKit^{+}$ $Sca\text{-}1^{+}$

Granulocyte Macrophage Progenitor (GMP):

Lin^{-} $cKit^{+}$ $CD34^{+}$ $CD16/32^{+}$

Common Myeloid Progenitor (CMP):

Lin^{-} $cKit^{+}$ $CD16/32^{+}$ $Sca\text{-}1^{-}$ $CD34^{-}$