Pullen Lab Protocol for FcεRI Probing of BMMC with a Countess FL II

This protocol is used to quickly phenotype bone marrow that has been skewed toward BMMC differentiation. For complete confirmation perform the same with the addition of a FITC anti-mouse CD117 (clone 2B8; BioLegend #105805), relevant isotype control (FITC Rat IgG2b, κ; BioLegend #400633), and a GFP light cube.

Materials:

- BMMC at least four weeks in culture
- Countess FLII with an RFP light cube
- Purified anti-mouse CD16/32 (clone 93; BioLegend #101301)
- PE anti-mouse FcεRIα (clone MAR-1; BioLegend #134307)
- PE Armenian hamster IgG isotype control (BioLegend #400907)
- Container with ice
- 2mL microcentrifuge tubes
- refrigerated microcentrifuge (set to 4°C)
- micropipettes with tips
- Incubation Buffer (0.5g BSA in 100mL 1X PBS)
- Blocking antibody solution (anti-CD16/32 dilutred 1:50 in Incubation Buffer)

Procedure:

1. Obtain enough cells for about $5x10^5$ cells/tube. You need at least two tubes for each cell lot to be tested: one that will receive the FcεRI antibody, and one that will receive the isotype control.
2. Centrifuge cells in tubes 500g for 5min.
3. Aspirate medium (carefully!).
4. Wash cells by resuspending pellet in 800μL of Incubation Buffer.
5. Centrifuge as in step 2.
6. Repeat steps 4 and 5.
7. Block FcγReceptors with anti-CD16/32 by adding 100μL of blocking antibody solution directly to pellet, mix, then incubate on ice for 5min.
8. Add 700μL of incubation buffer, then centrifuge 500g for 5min.
9. Aspirate supernatant.
10. Add PE-conjugated antibodies directly to their respective cell pellets (FcεRI or isotype control) and then bring to 100μL with Incubation Buffer.
    a. Refer to the data sheets for the amount to add, typically it will be 1.25μL.
11. Incubate on ice in the dark for 1hour.
12. Add 700μL of Incubation buffer, centrifuge then aspirate supernatant.
13. Wash again with incubation buffer and centrifuge.
14. Final resuspension should be 500μL of Incubation Buffer per pellet.
15. Inject 10μL of cell samples into a Countess slide, then place slide into the Countess FL II.
16. Turn on the RFP channel and adjust brightness to between 57 and 69.
17. BMMC with the FcεRI antibody will fluoresce, isotype control will not.*
18. Count to capture data. Export to USB drive if desired.

*another good negative control would be to use a cell line known to not express FcεRI, *e.g.* P815