Preparation of cells for FACS analysis: Farnham lab protocol; modified 02/26/08

Prepare cells (this can be done up to two weeks in advance)

1. Trypsinize adherent cells using standard protocol (non-adherent cells can be counted directly).
2. Determine cell number. Use 10^5-10^6 cells/sample.
3. Add appropriate volume of cells to a conical tube and spin them down at 1000 rpm for 3 min. Aspirate off the media.
4. Vortex pellet at low speed, add 0.5 ml of cold PBS, vortex again for 2-3 seconds. ***It is very important to achieve a single cell suspension.*** If cell clumping is a problem, you can use calcium- and magnesium-free PBS.
5. Resuspend the pellet in 5 ml of cold PBS. Centrifuge cells for 6 min at 1000 rpm.
6. Aspirate off the PBS.
7. Add 0.5 ml cold PBS and pipette up and down to achieve a single cell suspension.
8. Prepare a tube with 4.5 ml ice-cold 100% ethanol. Begin vortexing the tube with ethanol as you slowly add 0.5 ml of cells in PBS, dropwise.
9. Incubate on ice or at 4°C for at least 30 minutes. At this point, samples can be stored in the refrigerator for up to two weeks.
10. Make an appointment with the Flow Cytometry Facility!

Stain cells (this is done immediately before FACS analysis)

1. Prepare propidium iodide (PI)/Triton staining solution with RNAse A.
   Recipe: 10 ml of 0.1% (v/v) Triton X-100 (Sigma) of PBS (Triton is a viscous liquid), 2 mg DNAse-free RNAse A (Sigma), 200 microliters of 1 mg/ml PI (Molecular Probes). Prepare fresh!
2. Warm tubes to 37°C for 5-10 minutes (otherwise ice crystals can tear apart your cells).
3. Centrifuge the ethanol-suspended cell for 5 min at 200 g (=1000 rpm). Decant ethanol thoroughly.
4. Suspend cell pellet in 5 ml of PBS, wait about 1 min, centrifuge 5 min at 200 g (=1000 rpm) for 5 min. Discard the supernatant.
5. Suspend cell pellet in 1 ml PI/Triton X-100 staining solution with RNAse A. Keep in the dark at RT for 30 min or at 37°C for 15 min.
6. Immediately before analysis, vortex each sample and filter using a 5 ml polystyrene round-bottom tube with cell-strainer cap (Becton Dickinson, cat# 352235). Pour cell suspension onto the cap and centrifuge the tubes for a short period of time at 1000 rpm.
7. You are ready to do the FACS analysis.