DNA Analysis by Flow Cytometry

1. Begin with 5 x 10^5 to 1 x 10^6 cells per ml to be fixed and examined. Place the cells in a 6-ml polystyrene Falcon tube (#2058).

2. Pellet the cells and pour off the supernatant leaving a small amount of liquid in the bottom of the tube.

3. Gently vortex the cells and slowly (drop-wise) add 70% cold EtOH to approximately 2 ml (approximately 1 pasteur pipet worth of EtOH). This is to avoid clumping of the cells during fixation.

4. Add cold 70% EtOH to 5 ml and store the cells at 4^{o}C overnight. The cells can be stored at this point for up to 2 weeks at 4^{o}C or 6 months at -20^{o}C, however it is best to atleast let the cells fix overnight in EtOH.

5. To prepare the cells for flow cytometry, pellet the EtOH fixed cells and wash once in approximately 3-ml of cold 1X PBS.

6. Add 1-ml of PI solution (20 ug/ml PI/ 10 Units/ml Rnase One -Promega in 1X PBS) to each sample. Incubate in the dark for 20 minutes. Additional PI solution may be needed depending in order to control the flow rate of the cells.

7. Examine by flow cytometry by initially gating on an area versus width PI dot plot, used as doublet discrimination. Proceed to examine the cells in this gate on a forward-scatter versus side-scatter dot plot, a forward-scatter histogram, and a PI histogram to analyze the cell cycle. Collect at least 7,500 cells in a LO flow rate (the rate of cells should be less than 400 cells/second).