Preparing Bacteria for Flow Cytometry. Protocol taken from Magdalena in Joe Kaguni's lab.

March 22, 2011

Reference: Steen, H.B., Skarstad K., Boye E. (1968) Flow Cytometry of bacteria: cell cycle kinetics and effects of antibiotics. Ann NY Acad Sci 468: 329-338.

- Pellet the cells by centrifugation, and discard supernatant.
- Resuspend the pellet in an equal volume of Steen Buffer (10mM Tris-HCl pH 7.8 **(Magdalena used pH 7.4)**, 10mM $MgSO_4$)
- Spin as above, discard supernatant.
- Resuspend the pellet in a small volume of Steen Buffer (concentrate bacteria about 10 times).
- Pass bacteria through a 22-gauge needle into 77% ice cold ethanol to dilute bacteria 10 times (e.g. 0.5ml to 4.5ml of ethanol). Final concentration of ethanol 70%, desired OD of bacteria 0.2 or more.
- Bacteria can be stored in ethanol for weeks.
- Prepare DAPI solution in Steen Buffer (1-2μg/ml).
- Remove 1.5ml bacteria from ethanol, pellet by centrifugation.
- Wash pellet in Steen Buffer, centrifuge.
- Resuspend pellet in 0.5ml of DAPI solution. Louis needs about $2*10^6$ cells in 0.5ml stain for at least 2 hours on ice, or overnight in a refrigerator.

Note: other protocols use 10mM Tris-HCl pH 7.5, 10mM $MgCl_2$ instead of Steen Buffer, and filter through 0.22μm filter.

Also see: Akerlund, T., Nordstrom, K., Bernander R. (1995) Analysis of Cell Size and DNA Content in Exponentially Growing and Stationary-Phase Batch Cultures of *Escherichia coli*. Journal of Bacteriology, Dec. 1995, Vol 177, No. 23, p.6791-6797.