Synchronization of Cell Division

Lucho Fuentealba 2008

Note: This protocol is used to analyze daughter cell pairs

- Coat Lab-tek II 2-well chamber slides (Nalge Nunc international cat# 154461) with 0.05-0.1% Concanavalin-A (Sigma) in D-PBS (Dubelcco's-PBS Gibco #14190) for one hour

- Wash 1x with D-PBS, and allow chambers to dry for 10 min. Concanavalin-A improves the attachment of daughter cells and prevents them from migrating away from the site of division. The optimal concentration needs to be adjusted for each cell line. If Concavalin A is too high cells do not separate well, if too low they migrate away from each other. For example, to attach dissociated *Drosophila* blastoderm cells, 0.5% Concanavalin-A was required. During mitosis, cultured cells become rounded and attach more loosely to the culture dish plastic

- For mitotic shake-off cell cycle synchronization, Cos7 or L-cells Petri dishes are tapped gently ten times against a vertical surface (R. I. Freshney, *Culture of Animal Cells, a Manual of Basic Technique* (Wiley-Liss, New York, 1994, pp. 384-385) and the medium plated immediately into Concanavalin A-treated slide chambers. Shortly after plating most cells are single. Four hours after plating, 71% of cells (n=850) form pairs of daughter cells, which sometimes remain joined by mitotic microtubule midbodies. Two to four hours after synchronization, proteins targeted for degradation that have diffused from the pericentrosomal region during mitosis regain their pericentrosomal localization, providing the optimal time period for visualizing asymmetric mitotic distributions