B Sullivan July 2003

Preparation of Unfixed Metaphase Chromosomes for Immunofluorescence

1. Wash cells once with 1X PBS, Hanks or other buffered solution. Cells that grow attached to flasks should be trypsinized and collected into a 50 ml tube and then washed once with PBS.

2. Count cells using hemocytometer.

3. Spin at 1200 rpm x 5 minutes at RT to pellet cells.

4. Resuspend pellet in hypotonic (75mM KCl, 0.8% Na Citrate, or 1:1:1 75mM KCl: 0.8% Na Citrate: dH20) to the following concentrations:

   $2x10^5$ cells/ml for drosophila S2 or Kc cells - single chamber

   $3.5X\ 10^5$ cells/ml for drosophila S2 or Kc cells - double chamber

   1.8×10^5 cells/ml for human lymphoblasts - single chambers

   2.2×10^5 cells/ ml for human lymphoblasts - double chambers

   4×10^4 cells/ml for mammalian primary fibroblasts - single chambers

   $6\text{-}7 \times 10^4$ cells/ml for mammalian primary fibroblasts - double chambers

   6.1×10^4 cells/ml for mammalian immortalized fibroblasts - single chambers

   $8.0\text{-}8.2 \times 10^4$ cells/ml for mammalian immortalized fibroblasts - double chambers

5. Add 500 ul per cytofunnel (single) or 250 ul (per side of double funnels).

6. Spin at 1900-2000 rpm x 10 minutes with high acceleration.

7. After cytospinning, incubate slides in PBST (PBS + 0.1% Triton X-100 **OR** 0.1% Tween-20) or KCM (120mM KCl, 20mM NaCl, 10mM Tris-HCl, pH 7.7, 0.1% Triton X-100) for 5 minutes.

8. Block for 10-30 minutes in blocking solution (PBS + 0.5% Triton X-100 + 1% BSA + 0.02% Na Azide). Cover slides with a parafilm coverslip during incubation.

9. Add 5-15 ul of antibody in blocking solution per area of cells, cover with parafilm coverslip cut to fit just larger than cell area. Incubate 30-60 minutes at 37 degrees C, 1-2 hours at RT, or O/N at 4 degrees C.

10. Carefully remove parafilm coverslip and immerse slides in PBST. Remember that cells are unfixed so parafilm must be removed carefully to prevent removing or scraping cells.

11. Wash 3 x 5 minutes in PBST.

12. Add 15-20 ul of secondary antibody. Incubate 30 minutes at 37 degrees C or 1 hour at RT.

13. Wash as in 10.

14. OPTIONAL Fix in 6-10% formalin in PBST. This step must be done if FISH is to be subsequently performed.

15. Counter-stain with the appropriate dye (DAPI in antifadent/PI in antifadent etc.)