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Preparation of Chromatin Fibers from Tissue Culture Cells

This protocol requires a cytocentrifuge that dispenses unfixed cell or nuclei suspensions onto glass slides. (I use the Cytospin 4 from Shandon, Inc.)

1. Transfer cells from flask into a 50 ml tube and bring final vol to 40 mLs with 1X PBS.

2. Count cells using a hemacytometer.

3. Resuspend in hypotonic (75mM KCl or 0.8% Na Citrate) to 0.8-2 x10^5 cells/ml and centrifuge onto charged (Superfrost plus) slides. Use double funnels; 4 min at 800 rpm, high acceleration. For Drosophila cells, do not use superfrost plus (charged) slides; use regular glass slides.
4.
   2x10^5 cells/ml for drosophila S2 or Kc cells - single chamber

   3.5 x 10^5 cells/ml for drosophila S2 or Kc cells - double chamber

   0.5-1 x 10^5 cells/ml for human lymphoblasts - single chambers

   1-1.5 x 10^5 cells/ ml for human lymphoblasts - double chambers

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

   1 x 10^4 cells/ml for mammalian primary fibroblasts - double chambers

   1 x 10^4 cells/ml for mammalian immortalized fibroblasts - single chambers

   2 x 10^4 cells/ml for mammalian immortalized fibroblasts - double chambers

5. After cytospinning, immerse slides in a coplin jar of Lysis Buffer for 10-15 minutes at RT. You may have to vary the incubation time depending on the cell line (start with 8 minutes; if fibers are not stretched enough, increase time in Lysis Buffer. Alternatively, you can keep the shorter time (8m) but increase NaCl concentration to 625mM – be aware that you may lose proteins from the chromatin with increased salt.)

6. Slowly lift slide from lysis buffer; gravity helps buffer to pull chromatin down the slide. (Note: It usually takes me 15-20 seconds to pull a slide completely out of the coplin jar.)

7. Fix in 4% paraformaldehyde/formaldehyde diluted in PBS, PBS-Tween (0.05%) or PBST (0.1% Triton X-100) for 10 minutes at RT.

8. Rinse slides in 1X PBS+0.05% Tween-20 for 3 minutes at RT.

9. Block slides for 10-30 minutes at RT with PBS-Triton (1XPBS + 1%BSA + 0.5%Triton X-100 + 0.02% NaAzide) or your favorite blocking reagent.

10. Add primary antibody and incubate for 2-3 hours at RT or **overnight at 4°C.** *Overnight incubation at 4°C significantly decreases background; background needs to be minimized on the fiber preps since, ideally, the fibers should be very thin and antibody binding needs to be clearly distinguished from any background fluorescence.*

11. Wash slides in PBS-Tween (1X PBS + 0.05%Tween), 3 times for 5 minutes. Do not shake coplin jar as that will dislodge fibers from the slide.

12. Add secondary antibody and incubate for 1 hour at RT. I recommend using Alexa-Fluor conjugated secondaries – they give very strong fluorescence with little background. Ex. Alexa-488 (in place of FITC), Alexa-555, 568, 594 (in place of Rhod-amine or Texas Red). Cy3 is also a great conjugate for secondaries.

13. Wash as in step 7.

14. Counterstain with antifade (Vectashield) containing DAPI. The DAPI concentration should be 2-5ug/mL in antifade so that you can see the fibers.

15. If proceeding to do FISH or thymidine analog detection, crosslink antibodies and proteins in 4-10% formalin in KCM or PBS, 15 minutes at RT.

16. Rinse slides in PBS-T or 2XSSC (if necessary, slides can be stored for a few days at 4C in these buffers before doing FISH).

Salt-Detergent-Urea Lysis Buffer - make fresh each (important!)
25 mM Tris, pH7.5
0.5M NaCl
1% Triton X-100
0.5M Urea (Dilute Urea from 1M Urea that is made up fresh each time in dH_2O)