

SCE protocol

This is the protocol I use for wildtype MEFs. It may require some adjustments for other genotypes or cell types.

1. Plate cells and grow to about 50-60% confluence.
2. Remove medium and add fresh medium containing 10 μ M BrdU.
3. Grow 18 hours. Its probably best to choose a few timepoints that bracket 18 hours if possible.
4. Add colcemid (1:1000 KaryoMAX) directly to culture dish and swirl. Incubate 30 min to 2 hours.
 - Metaphases can be prepared without colcemid. Colcemid should increase the number of metaphase chomosomes but longer incubation times will result in shorter, more compact chromosomes.
5. Trypsinize cells as normal and wash 1X 10ml PBS. At this point it is no longer necessary to be sterile.
6. Remove as much PBS as possible and gently resuspend the cells in the residual.
7. Slowly add 0.075M KCl **dropwise** to 10ml. I add 1-2 drops then invert the tube. As soon as there are about 3 ml of KCl in the tube addition can become faster.
8. Incubate at 37°C (in a water bath) for EXACTLY 6 minutes.
9. Centrifuge at 900rpm for 5 minutes.
10. Remove as much KCl as possible and gently resuspend the cells in the residual.
11. SLOWLY add 5 ml of fixative (3:1 Methanol/Acetic acid; prepared fresh) **dropwise** and carefully mix the whole time. Adding fixative too quickly will result in clumping.
12. Centrifuge at 900rpm for 5 minutes and remove fixative
13. Slowly add 2 ml fixative **dropwise**.
14. Centrifuge 900rpm 5 minutes and remove all but 200-500 μ l of the fixative. Cells are stable for extended times in fixative. If desired, store at 4°C.
15. Drop a few drops from about 18 inches high onto angled, humidified microscope slide.
16. IMMEDIATELY blow on the slide very gently.
17. Air dry at least 10 minutes. Slides are now stable for a very long time.

18. Place slides into a staining jar containing about 70 ml 1XPBS+250 μ l Acridine Orange (20mg/ml in H₂O). Incubate 5 minutes in the dark.
19. Wash with several changes of 1XPBS, incubating about 5 minutes per wash.
20. Allow excess liquid to run off and tap slide a few times on a paper towel to remove residual liquid.
21. Place a few drops of Vectashield I (without DAPI) onto slide. Place coverslip on slide and press gently.
22. Seal coverslip at short edges with nail polish and remove excess Vectashield by aspirating.

Store slides at 4°C. They should be stable for a couple of weeks if stored in the dark.

Solutions

- 0.075M KCl. Not necessary to filter or autoclave.
- Freshly prepared Methanol/Acetic acid fixative. Add 1 part glacial Acetic acid to 3 parts Methanol and chill at -20°C.
- 10mg/ml BrdU in PBS. Dissolve BrdU in PBS then filter using a 0.2 μ m filter. Store in dark at -20°C
- 20mg/ml Acridine Orange in H₂O. Not necessary to filter. Store in dark at -20°C