The normal mouse cell contains 40 acrocentric chromosomes. The procedure described below is suitable for chromosome counting as an indication of the potential of mouse ES cell clones for generating germ line chimeras.

**Materials**

- Microscopic slides (e.g. Fisherbrand Superfrost catalog #12-550-12 or #12-549)
- Microscopic cover glass, 22 x 50mm (e.g. ESBE Scientific catalog #ESO225OB)
- Coplin staining jars
- Microscope with 100X oil immersion and 40X phase contrast objectives
- Hypotonic solution (0.075M potassium chloride – 0.559g KCl in 100ml water) prewarmed to 37°C before use
- Colcemid, 10ug/ml (Gibco catalog #1512-012)
- Gurr’s buffer tablets, pH 6.8 (Gibco catalog #10582-013 or BDH catalog #33-193)
- Giemsa stain (Gibco catalog #10092-013)
- Fixative (3 parts methanol: 1 part glacial acetic acid). Freshly prepared for each use and stored at 40C before use

**Method**

1. To an actively growing ES cell culture (i.e. 24 or 48 hours after usual 1:3 – 1:5 passage on gelatinized 60mm plate), add fresh media and incubate at 370C for 1 hour. Aspirate the media, add fresh media containing colcemid (25ul of 10ug/ml stock colcemid solution per 5ml of cell media to final concentration of 0.05ug/ml) and incubate at 370C for 1-1.5 hours. To accumulate very large numbers of mitotic figures, it is possible to incubate the culture in colcemid for 3-4 hours but chromosome will be short and suitable only for counting and not for identification.
2. Remove the media, wash with PBS, trypsinize for 3-5 minutes in the usual way to obtain a single cell suspension and then stop the reaction with 3-5ml cell media.
3. Harvest cells in a 15ml centrifuge tube, centrifuge for 5 minutes, aspirate the supernatant and flick the pellet to resuspend the cells in 1.5ml of fresh media.
4. Add 10ml of warmed KCl solution slowly, drop by drop, along the wall of the tube, while flicking the tube gently at the same time to resuspend the pellet. Do not pipette up and down or all the cells will stick to the inside of the pipette. Invert the tube several times and incubate at 370C for 15-20 minutes (the length of time in KCl is crucial).
5. Add 2-3 drops of cold fixative, invert the tube and then centrifuge at 1000rpm for 5 minutes.
6. Aspirate the supernatant but leave 1ml behind in which to resuspend the pellet by flicking the tube.
7. Work in the fume hood from this step. Place the tube in a vortex and add up to 10ml of cold fixative drop by drop while vortexing the tube to disperse the cells thoroughly in the fixative. Centrifuge at 1000rpm for 5 minutes, aspirate the supernatant but leave 1ml behind in which to resuspend the pellet by flicking the tube.
8. Repeat step 7 at least 2 or 3 times for a total of 3-4 fixations.
9. Slides are best made within 3 hours of the final fixation, but can be made later if the cells are kept in fixative at 40C and resuspended in cold fresh fixative before making spreads.
10. Resuspend the cells in a small volume of cold fixative (~0.5ml) that can be diluted later if the suspension is too dense (4-5 slides should be made from each sample).
11. Prepare slides for the spreads by washing them in cold fixative and then soaking in ice cold water in Coplin jars. It is important for the slides to be both cold and wet just when ready for use.

12. Remove the slide from the Coplin jar. Hold the wet slide at an angle of about 150 from the horizontal and make spreads by using a pipette with a 200ul tip to slowly disperse 80ul of cell suspension drop by drop from at least 6 inches above the surface of the slide (2-3 drops per slide). If the cells are dropped from too close a distance they will not burst. Start at one end of the slide and slowly make one’s way to the opposite end of the slide.

13. End by putting a few drops of fixative on top of the slide. Air-dry the slides in a moist environment. A home-made water-bath (container with hot water and empty pipette tip racks placed inside) kept in the fume hood would help to maintain the humidity during slide preparation. To facilitate drying of the slides, a desk lamp bulb may be used to heat them briefly while air is blown onto them at the same time.

14. Scan the first slide with a 40X objective. Dilute the cell suspension if necessary.

15. Stain slides for 10 minutes in Coplin jar with freshly made Giemsa stain (2.5ul of Giemsa stain stock in 47.5ml of Gurr’s pH 6.8 buffer). Rinse the slide with several changes of water in a Coplin jar until the water remains clear. Air dry the slide.

16. Apply a cover slip using mounting media (e.g. Entellan BDH catalog #65037-71).

17. Count chromosomes with 100X oil immersion objective. Look for spreads in widely separated fields. Ignore spreads with fewer than 39 chromosomes. 20+ good spreads is an adequate sample size. ES cell lines in which a minimum of 70% of the spreads contain 40 chromosomes are acceptable.