The University of Michigan
Transgenic Animal Model Core

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Chromosome Spread Preparation and Counting

Solutions and Materials:

ES Growth Media	DMEM+ 15%fcs +10^3 u/ml rLIF + 0.1mMβ-ME + 4mM Gln	
Gelatin Coated p100		
Colcemid Solution	10μg/ml	Invitrogen Cat. no. 15212-012
Hypotonic Solution	0.075 M KCl	
Colony Counting Pen		Bel-Art Products Cat. no. F378620000

Fixative	3:1 Methanol: Acetic Acid **Make fresh before use.** (Only use methanol stored in glass bottles.)
Glass Slides	Pre-Cleaned by soaking overnight in EtOH and wiping the surface with a Kimwipe.
Giemsa Stain	Invitrogen Cat. no. 10092-013
Gurr's Buffer	Invitrogen Cat. no. 10582-013, prepared according to product directions.

1. Plate 5 x 10^6 ES cells in 10 ml media onto a 100 mm gelatin coated tissue culture plate and culture overnight.

2. The next morning add colcemid to the plate

   Add 20μl colcemid directly to the plate (final concentration 0.02 μg/ml). Gently move the plate back and forth to mix the colcemid into the culture medium. Note the time on the plate and incubate at 37°C one to two hours.

Note: the longer the culture incubates in colcemid the more mitotic figures there will be. However, the chromosomes will get progressively more contracted with time and will be unsuitable for banding.

3. After 1 to 2 hours in colcemid, harvest the cells by trypsinization.

   Pipet off the media and place into a 15 ml centrifuge tube.
   Rinse the plate with PBS, place PBS wash in same tube as media, and spin at speed setting 3 for five minutes. Aspirate supernatant, leaving 4 ml in the tube. Add ~4 ml of trypsin to the plate and
   incubate 5 min at 37°C until the cells are well dispersed. Pipet the cells up and down with a plugged Pasteur pipet to make a single cell suspension and transfer the cells into the centrifuge tube you prepared with culture fluid.

4. Centrifuge on setting number 3 (RCF 200) for 5 min.

5. Aspirate supernatant, leaving a small volume (0.1 ml). Resuspend pellet in this remaining fluid.

6. Add 10 ml of pre-warmed hypotonic solution (37°) gently pipette up and down one time and incubate 15 min at 37°C. The cells swell in this hypotonic and become very fragile.

Protocol: **CHROMOSOME PREPARATION CONTINUED**

7. Centrifuge on setting number 3 for 5 minutes

   While the cells are centrifuging prepare the fixative solution.
   30 ml of methanol + 10 ml of glacial acetic acid

8. Aspirate the supernatant leaving a small volume (at least 2 pellet volumes, up to about 0.5ml). Gently resuspend the cells by flicking the tube. Make sure that the complete pellet is resuspended in order to prevent the formation of large clumps of cells when the fixative is added.

9. Add 7 ml of fixative. Use a Pasteur pipet to add the first 1ml drop by drop while agitating the tube. The remainder of the fixative can be added more quickly.

10. Incubate on ice for 30 - 60 minutes.

11. Change the fixative a further two times by spinning the cells. There is no need to add the fixative drop by drop after the first fixation.

    The final volume of the cell suspension should be approximately 1 ml. Excess cell solution can be stored at -20^{o}C in ~10 ml of fixative. Stored preparations should be resuspended in fresh fixative before dropping new slides.

12. Prepare mitotic spreads by dropping the fixed swollen cells onto glass microscope slides. The spreading of the fixative layer and subsequent evaporation causes the cell membranes to rupture and release the chromosomes.

13. Slides can be examined with the microscope using phase contrast to determine if the spreads are suitable for staining and counting.

14. Slides are stained by incubation in 4% Gurr's Giemsa in Gurr's Buffer for 15 min, followed by rinsing in Gurr's Buffer for 10 min and in distilled water for 5 minutes. After air drying, chromosomes are counted using the 100 X oil objective of a research microscope.

15. A video camera is connected to the microscope and spreads are photographed with a video graphic printer. Chromosomes on the prints are counted and marked off with a colony counting pen.