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Mouse ES Cell Chromosome Spread Counting

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We use this protocol and it's working

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Abstract

The study of genetically engineered mouse models as surrogates for human genetic disease and to gain insights into pathophysiological mechanisms has a long and productive history for which Nobel prizes have been awarded. A key tool used to generate engineered mouse models is gene targeting in mouse embryonic stem (ES) cells. Mouse embryonic stem (ES) cell chromosome counting is a standard assay. Correctly used, it can reduce the numbers of mice used in research. ES cell clones that are shown to be aneuploid by chromosome counting are excluded from ES cell-chimera production workflows. After the desired mutation is introduced into an ES cell clone it is used to form mouse ES cell – mouse chimeras. In successful experiments, male chimeras are mated with wild type mice. Sperm derived from ES cell clones will transmit the engineered mutation to their mouse pups. The gene targeted mouse pups generate mouse for lines for study. Germline transmission of engineered genes requires euploid ES cell clones for the production of ES cell - mouse chimeras. ES cell clones with abnormal chromosomes can contribute to coat color and internal organs but will not form the male gametes (sperm) that are essential for these experiments to succeed.

Image Attribution

Example of mouse ES cell chromosome spread. This spread shows an aneuploid result due to the presence of an abnormal (circular) chromosome as indicated by the arrowhead.



Materials

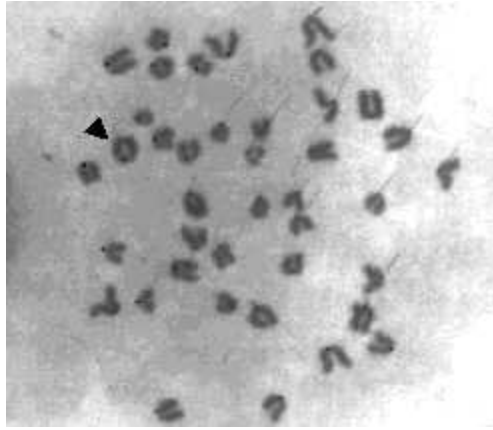
ES Cell Growth Media: Gibco™ DMEM, high glucose Cat. No. 11965092, 15% ES-qualified fetal bovine serum, 0.1 mM Beta-mercaptoethanol (Millipore Sigma Cat. No. #M-7522), 1000 units/ml ESGRO® Recombinant Mouse LIF Protein (Millipore-Sigma Cat. No. ESG1107), 4 mM glutamine (Gibco™ L-Glutamine Cat. No. 25030149). Gelatin Coated 10 cm culture dish. 10 ug/ml Gibco™ KaryoMAX™ Colcemid™ Solution in PBS Cat. No. 15212012. 0.075 M KCl Hypotonic Solution. Colony Counter Research Products International SKU no. 283133. 3:1 Methanol: Acetic Acid Fixative. Make fresh before use. Only use methanol stored in glass bottles. Glass Slides Pre-Cleaned by soaking overnight in 70% ethanol and wiped with a Kimwipe. Gibco™ KaryoMAX™ Giemsa Stain Solution Cat. no. 10092-013. Gibco™ Gurr Buffer Cat. no. 10582-013. Gibco™ DPBS, no calcium, no magnesium Cat. No. 14190144. Sterile 5 ml pipettes. Sterile Cotton Plugged Pasteur Pipettes. Wheaton Coplin staining jars Millipore-Sigma Cat. No. S5891. Immersion Oil Millipore-Sigma Cat. No. 56822. Research microscope with oil immersion lens reaching 1000X magnification, camera, and printer.

Procedure

- 1 Plate 5×10^6 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.
 - 2.1 Add 20 ul colcemid directly to the plate (final concentration 0.02 ug/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 for one to two hours.
 - 2.2 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.
 - 3.1 Pipet off the media and place into a 15 ml centrifuge tube.
 - 3.2 Rinse the plate with DPBS, place DPBS wash in the same tube as media, and centrifuge tube at 200 RCF for five minutes. Aspirate supernatant, leaving 4 ml in the tube.
 - 3.3 Add ~4 ml of trypsin to the plate and incubate 5 min at 37°C until the cells are dispersed.
 - 3.4 Pipet the cells up and down with a cotton plugged Pasteur pipet to make a single cell suspension. Transfer the cells into the centrifuge tube prepared with DBPS rinse.
 - 3.5 Centrifuge tube at 200 RCF for 5 min.
 - 3.6 Aspirate supernatant, leaving a small volume (0.1 ml). Resuspend pellet in this remaining fluid.
- 4 Add 10 ml of warm hypotonic solution (37°C). Gently pipette up and down one time and incubate 15 min at 37°C. The cells will swell in this hypotonic solution and become very fragile.

- 5 Centrifuge at 200 RCF for 5 minutes.
- 5.1 While the cells are in the centrifuge prepare the fixative solution by adding 30 ml of methanol to 10 ml of glacial acetic acid.
- 6 Aspirate the supernatant leaving a small volume (at least 2 pellet volumes, up to about 0.5 ml).
- 6.1 Gently resuspend the cells by flicking the tube. Make sure that the complete pellet is resuspended in order to prevent the formation of clumps of cells when the fixative is added.
- 6.2 Add 7 ml of fixative. Use a Pasteur pipet to add the first 1 ml drop by drop while agitating the tube. The remainder of the fixative can be added more quickly.
- 7 Incubate on ice for 30 - 60 minutes.
- 8 Change the fixative two more times by spinning the cells. There is no need to add the fixative drop by drop after the first fixation.
- 9 The final volume of the cell suspension should be approximately 1 ml. Excess cell solution can be stored at -20°C in ~10 ml of fixative. Stored preparations should be resuspended in fresh fixative before dropping new slides.
- 10 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.
- 11 Dried slides can be examined with the microscope using phase contrast to determine if the spreads are suitable for staining and counting.
- 12 Slides are stained by Giemsa stain in Coplin staining jars.
- 12.1 Incubate dried slides in 4% Gurr's Giemsa in Gurr's Buffer for 15 min.
- 12.2 Rinse slides in Gurr's Buffer for 10 min.

- 12.3 Rinse slides in distilled water for 5 minutes.
- 12.4 After air drying, chromosomes are counted using the 100X oil immersion objective of a research microscope. Final magnification 1,000X.
- 13 Use the camera connected to the microscope to photograph chromosome spreads.
- 13.1 Print the chromosome spread images.
- 13.2 Count the number of chromosomes in 20 to 40 spreads with the colony counting pen.
- 14 Identify the ES cell clones that contain 70% or more euploid chromosome counts. The euploid chromosome count of mouse cells is 40 chromosomes, including the sex chromosomes.
- 14.1 Mouse chromosomes are telocentric. Exclude ES cell lines with obvious chromosome aberrations (circular chromosomes, metacentric chromosomes, extra-long chromosomes, etc.).
- 14.2 ES cell clones that contain 70% euploid chromosome counts are suitable for microinjection into mouse blastocysts to produce ES cell-mouse chimeras for germline breeding.
- 14.3 ES cell clones with 50% euploid chromosome counts may form germline chimeras. Clones with less than 50% euploid chromosomes rarely form germline chimeras.
- 14.4 If necessary, ES cell lines can be subcloned to produce gene-targeted subclones with high euploid chromosome count numbers.
- 15 Results of chromosome counting.



Sample chromosome count. In this spread we see an abnormal (circular) chromosome as indicated by the arrowhead. This spread would be scored as aneuploid. 19 more spreads would be counted and then the percentage of euploid spreads with 40 normal chromosomes would be used to score the ES cell clone.