Protocol

Karyotyping Mouse Cells

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This protocol was adapted from “Detection and Analysis of Mouse Genome Alterations and Specific Sequences,” Chapter 12, in *Manipulating the Mouse Embryo*, 3rd edition, by Andras Nagy, Marina Gertsenstein, Kristina Vintersten, and Richard Behringer. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2003.

INTRODUCTION

The majority of mouse chromosome preparations for banding are now made by air-drying and, in essence, require the production of a cell suspension as a starting point. Some samples such as blood cultures, ascitic fluids, or cells growing in suspension will already be in suspension; others, such as bone marrow, solid tumors, or cells growing as attached layers in culture must be converted to suspensions. The basic steps in karyotyping and banding embryonal carcinoma cells are outlined below.

RELATED INFORMATION

Several methods for disaggregation of cells are described by Cronmiller and Mintz (1978).

Additional variations on the methods for karyotyping and banding embryonal carcinoma cells are found in McBurney and Rogers (1982) and Robertson et al. (1983). For guidance in trypsinization, see **Trypsinization of Cells Grown in Monolayer** (Darlington 2006).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <I>, and recipes for reagents marked with <R>.

Reagents

<R>Carnoy's fixative (freshly prepared and kept at room temperature)

<I>Ethanol, absolute:HCl, concentrated solution (1:1)

<I>Ethanol, absolute:diethyl ether solution (1:1)

Embryonic stem cells (in tissue culture dishes in growth medium)

Exponentially growing cultures will give best results.

<I>Giemsa stain (dilute 1:20 with H₂O) (Sigma)

Growth medium and serum

NaCl (0.85% [w/v])

Phosphate buffer (pH 6.8) (BDH)

Potassium chloride (KCl) (0.56% [w/v])

<R>SSC (20X)

Before use, dilute the SSC to a 2X solution.

<I>Trypsin (0.025% [w/v]) in 0.85% (w/v) NaCl

The age of the trypsin powder is not important, but the solution should be made ~30 min before use.

Equipment

Centrifuge
Coplin staining jars with lids (Lipshaw 107)
Desk lamp
Forceps
Glass slides (acid-washed, grease-free; for preparation, see Steps 5 and 6)
Glass tube (2 mL, conical)
Green filter for microscope
Kimwipes or any coarse, grease-free tissues
Microscope with 100X oil immersion objective and 40X phase-contrast objective
Pasteur pipettes (~0.2-mL capacity, drawn to deliver 10- μ L drops)
Water bath preset to 60°C-65°C
Whatman 3MM filter paper

METHOD

Preparation of Cells

1. Trypsinize cells that have been growing in tissue culture dishes with or without pretreatment with a mitotic arrestant. Pipette to give a single cell suspension. Add medium and serum and centrifuge at 1000 rpm in a 2-mL conical glass tube for 5 min.
*For guidance in trypsinization, see **Trypsinization of Cells Grown in Monolayer** (Darlington 2006).*
2. Resuspend the cell pellet gently in 1 mL of aqueous 0.56% (w/v) KCl by adding drops and flicking the tube. Do not pipette up and down. Add more KCl to a final volume of 4 mL. Leave at room temperature (21°C-23°C) for 6 min to swell the cells.
3. Centrifuge the cells again at 1000 rpm for 5 min. Carefully remove the supernatant, and fix the cell pellet in 1 mL of fresh Carnoy's fixative at room temperature. Resuspend by flicking the tube. Make up to 4 mL. Then centrifuge to pellet the cells. Repeat three times.
4. Centrifuge the cells at 1000 rpm for 5 min and then resuspend them in a small volume (0.5 mL) of fixative.

Slide preparations (see below) are best made within 0-3 h of the final fixation of the cells, but they can be made up to 7 d afterward if the material is kept at 4°C and the cell pellet is resuspended in fresh fixative before attempting to spread the cells. The longer the material is kept, the poorer the spreading quality of the mitotic cells. If possible, at least five slides should be made from each sample to guarantee success.

Slide Making

5. Clean commercially purchased, precleaned slides further by leaving them overnight in a mixture of absolute ethanol and concentrated HCl (1:1). The next day, wash the slides in running tap water, rinse in deionized H₂O, and store in absolute ethanol and diethyl ether (1:1).
6. Shortly before using the slides, use forceps to remove slides from the mixture of absolute alcohol and diethyl ether. Wipe the slides dry on Kimwipes or some other coarse, grease-free tissues, avoiding finger contact with the slide to prevent making fingerprints on the slide surfaces.
7. To make chromosome spreads on these slides, add a row of three drops of cell suspension from a prepared Pasteur pipette (~0.2-mL capacity, drawn to deliver 10- μ L drops), allowing them to spread to their maximum size. Let the drops dry until interference rings are visible at their periphery. Drying can be assisted by blowing on the slide while holding it up to the heat generated by a desk lamp bulb.
8. Once dry, inspect the slides under low-power phase-contrast microscopy (final magnification 160X). If the density of cells and chromosome spreads is insufficient, pellet the cells, resuspend in a smaller volume of fixative, and repeat with fresh drops.

9. For counting chromosomes without banding, stain in Giemsa for ~15 min and photograph with green filter.

G Banding

10. Place suitably aged slides (see note below) in 2X SSC in a Coplin staining jar with a lid in a water bath at 60°C–65°C for 1.5 h. Then cool the slides to room temperature by running tap water over the closed jar. Transfer the slides to 0.85% (w/v) NaCl at room temperature for 5 min.

Before G banding, slides should be “aged” for between 3 and 21 d by leaving them in a closed box at room temperature. Fresh slides give poor G-band resolution. Maximum G-band resolution is achieved at ~10 d after slide preparation. Beyond this time, resolution slowly decreases until, after several weeks in storage, the chromosomes either fail to band and stain uniformly or show “pseudobands” that are not significant to the standard idiogram.

11. Drain the slides by touching them onto filter paper. Place them on a flat surface and flood the chamber with 0.025% trypsin in 0.85% NaCl for 15–20 sec.

The trypsin exposure time is critical: Underexposure preserves chromosome morphology but gives poorly differentiated bands, and overexposure distorts morphology and eliminates most of the bands. Optimum trypsin times are known to vary among laboratories. A test slide should be treated for the minimal suggested time of 15 sec to establish the best treatment time for the rest of the slides.

In the laboratory of E.P. Evans, the optimal trypsin exposure time has been established as between 15 and 20 sec for mouse chromosomes, irrespective of the source of the mitotic cells.

12. Stop tryptic activity by placing the slides back into 0.85% NaCl. Then rinse slides in phosphate buffer (pH 6.8) and stain in fresh Giemsa stain in 5 mM phosphate buffer (pH 6.8). After 10 min in the stain, monitor wet slides under low-power, bright-field microscopy (160X) for staining intensity. Because, upon drying, wet slides gain contrast, care should be taken not to overstain the cells as this will reduce G-band differentiation. If necessary, repeat staining until adequate results are achieved and then quickly rinse slides in phosphate buffer (pH 6.8) and blow-dry with a current of cool air.

13. Examine unmounted slides with a 100X oil-immersion lens.

The majority of modern, readily available immersion oils that are declared “PCB-free” also have the unfortunate property of removing Giemsa stain after a few hours of exposure. Although direct viewing of slides under an oil immersion lens gives a higher optical resolution, it is wise to mount slides if they are to be kept.

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