Metaphase spread of mouse oocytes

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Method Article

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Abstract

this protocol briefly described the procedure of performing metaphase spreads on single mouse MII (metaphase-II) oocytes.

This will allow the user to count the chromosomes as well as perform additional immunofluorescence based stains for protein localization on fixed samples. the assay is however prone to false positive whole chromosome aneuploidies due to the nature of dropping the oocytes onto the etched glass slides. Hence, some skill development by repetition is required to ensure consistent outcomes. The whole procedure from obtaining oocytes to imaging happens over two days.

Introduction

This protocol will allow the user to perform a metaphase chromosome spread of oocytes and fix them on a glass slide, making it amenable to immunostaining protocols downstream

Reagents

- Paraformaldehyde (Sigma P6148- 500g)
- 1 M NaOH (stock is fine)
- Boric acid (50 mM; stock is fine, but check pH. From Sigma).
- Triton-X100 (100%; Sigma)
- 500 mM DTT (Sigma D9779- 10g). Keep in -20 °C freezer. Made in up water.
- Tyrode Solution (Sigma)
- PBS
- Na_2citrate (Sigma T8787)
- Photoflo (Kodak 146 4510)
- Ethanol or IMS to clean slides

Equipment

- Slides (Superfrost Plus, ThermoFisher)
- Coplin Jar

· Micropipettor (100 µm diameter).

· Culture dish

· Gilson (P10; P20; P200; P1000).

· Pipette tips

· Timer

· Humidifier

· Diamond pen for etching slides

e.g. http://www.thermoscientific.com/content/tfs/en/product/diamond-point-marker.html

· Tissue paper for slides – ordinary tissue paper is fine

Procedure

<u>On the day preparations: (allow 2-3 hours)</u>

<u>Solutions & preparation of materials:</u>

1. 1 % formaldehyde solution– made freshly from paraformaldehyde (PFA). Note that PFA is a polymer of formaldehyde that must be hydrolyzed to be functional. This is done by heating the aqueous solution (60 °C) and increasing the pH (NaOH).

a. Add 0.25 g of PFA *(Sigma P6148 – 500g)* to 22.5 ml of double-distilled water in a 50 ml conic tube. Add one drop of 1M NaOH. Incubate in 60 °C water bath for at least 20 minutes. Invert every 5 min. or so to ensure all PFA is dissolved.

b. Allow solution to cool to room temperature before adjusting the pH to 9.2 using 50mM boric acid (usually takes about 0.5 ml).

c. Add 35 µl of Triton X-100 (0.15% final v/v). Make sure is completely dissolved before use. Triton X-100 is a nonionic detergent that bursts the nuclear envelope. This is done concomitantly with the fixation step (formaldehyde in this case).

d. Add 150 µl of a 500 mM solution of DTT *(Sigma D9779 – 10g)* to the fix (final concentration: 3mM DTT)

Note: good for 2 weeks in the refrigerator at 4 °C.

2. Thaw out Tyrode's solution.

3. Prewarm sodium citrate solution to 37 °C.

4. Pre-warm a staining humid chamber for 10 minutes before fixing eggs.

5. Etch and clean microscope slides. Use ethanol or IMS to wipe slides to remove grease using standard laboratory tissue wipes. Use a diamond pen to i) draw a circle where you will place the oocyte; ii) label slide with patient ID and oocyte number.

Procedure:

The following steps are carried out under the stereomicroscope on a heated stage – We use a minitub heated stage for the stereo miscoscope with an HT50 control unit.

http://www.minitube.de/Products-Services/Canine/Heated-Stages-and-Warm-Plates/Control-Unit-HT50-for-heating-systems

1. Place the slides in 1% formaldehyde solution in a coplin jar to coat. **Low humidity conditions are bad; if you work in a dry environment use a humidifier in the room.**

2. Using an 100µm micropipette, transfer the MI or MII oocytes to a 50µl drop of Tyrode's solution on the lid of a culture dish. Allow the eggs to sit in the drop for approximately 1 minute. Watch for loosening of the zona under a stereomicroscope.

3. When the zona begins to slough off, carefully transfer the eggs though 2-3 drops of 0.9% sodium citrate to rinse off the Tyrode solution. The zona should be completely removed by the time the egg is in the last drop (pipetting the egg up and down to help zona removal). At this point the eggs will become quite sticky - use care while handling them to prevent bursting.

4. Remove slide from PFA, dab the end on a paper towel to remove excess solution, and place on stereomicroscope.

5. Take the oocyte from the Na_2citrate solution using the micropipette and pipette onto the PFA-soaked slide within the etched circle (do this under a stereomicroscope). **It is important to limit the amount of media that is transferred, as it will dilute the formaldehyde solution solution.** The eggs should be seen bursting in the formaldehyde solution. If the eggs don't start lysing you can agitate the surrounding liquid with the pipette until you see the egg start to lyse. Do not agitate too much as it will cause the chromosomes to spread excessively on the slide and cause unintended chromosomal loss.

6. Taking care to keep the slide absolutely flat, transfer slides to a humidity chamber (prewarmed) for gradual drying for several hours to overnight (we've tested 2 hour to overnight and there's no differences).

7. After slides have dried, add photoflo *(Sigma 146 4510)* (500 µL in 50 mL of water) to a Coplin jar. Then add your slides for 1 minute.

8. Take slides out and air dry at room temperature on the bench for 10-20 min.

9. The slides can be scanned under phase or you can put DAPI on them to see the chromosomes. The slides must be stored at -20°C until staining.

Troubleshooting

Torubleshooting during dropping oocytes has been described in the procedure as a comment

Time Taken

2 working days

Anticipated Results

References

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