

August 12, 2002

Isolation of Ovarian Epithelial Cells

--using 5 μ M DiI-18 (green)

1. Remove ovaries from females
2. Add 300 μ l DMEM (w/o serum) to an Eppendorf
3. Add 6 μ l DiI-18* to one of the tubes (final DiI conc. is 0.1nM)
--The other tube will serve as a control (no DiI)
4. Place an ovary in each tube
5. Leave for 20 minutes in the 37°C incubator
6. Fix the ovaries with 4% PFA for 2 hours in a 14 ml Falcon tube
7. Rinse with 1X PBS (use DMEM when doing cell sorting)
8. Vibratome

Vibratome

1. 7% - 9% agarose (similar consistency to tissue)
--Microwave – turn off as soon as it starts to boil
--Set in ~38°C - 43°C water bath for 15 min.
2. Vibratome supplies:
--Ice bucket
--Chilled 1X PBS
--Blade (cut in half)
--Microslide (shelf above vibratome)
--Cover slip forceps (flat head)
--Vice adaptor
--Baster
--Kimwipes
--Superglue (Can get at storeroom – IRU 200)
3. Lower the stage and tighten using knob facing you
4. Clean a Microslide
5. Label the molding block w/ sharpie to keep track of your ovaries
--Keep the same orientation throughout
6. Transfer tissues from PBS to the microslide
7. Fill molding block w/ agarose ~3/4 full
8. Working quickly

*dioctadecyl-6,6'-di(4-sulfophenyl)-3,3,3',3'-tetramethylindocarbocyanine
(Molecular Probes)

- place tissue in agarose
- orient so the blade hits the narrower side first
- leave on ice for ~1 minute to solidify
- 9. Slide out the solidified agarose
- 10. Trim excess agarose w/ blade
- 11. Fill the black tray w/ ice
- 12. Make sure blade angle is $\sim 23-25^\circ$
 - avg. speed: 2-3
 - avg. amp.: 8-9

- 13. Superglue the small piece of agarose containing your tissue onto the metal tray
- 14. Place in front of fan to dry (only for ~ 5 min.)
- 15. Place tray in slot and tighten using knob on the right
- 16. Fill with chilled PBS
- 17. Advance (FFW) until you reach the tissue
 - slow down when you get close to the tissue
 - adjust speed/amp as needed

- 18. Keep the specimens separate in the tray as they're slivered off
- 19. Store in PBS or transfer directly to a microscope slide
 - use TOPO3:Mounting Media (1:500)

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