Isolation of Ovarian Epithelial Cells

1. Remove ovaries from females
2. Label ___ eppendorfs
3. Add 300 µl DMEM (w/o serum) to each
4. Add 3 µl DiI-18* to all except the controls for each
5. Place an ovary in each tube
6. Short time point: Tubes _____

   --leave for ___ minutes in the 37°C incubator

   --fix the ovary from tube ___ and the control with 4%PFA for ___ hrs. (Eppendorf)

   --transfer ovary from tube ___ to 100 µl DMEM

   --trtiturate

   --fill the rest of the tube with 4%PFA

7. Long time point: Tubes _____

   --leave for ____ hours in the 37°C incubator

   --fix the ovary from tube ___ and the control with 4%PFA for ___ hrs. (Eppendorf)

   --transfer ovary from tube ___ to 100 µl DMEM

   --trtiturate

   --fill the rest of the tube with 4%PFA

8. After each time point:

   --Rinse triturated pieces and whole ovaries well with 1X PBS

   --Cut the whole ovaries in half on a flat surface

   --Return them to the PBS

   --Take one half to histology

   --Use the other for vibratomy

9. Use a transfer pipette to transfer the triturated pieces to a slide

   --Remove excess PBS

   --If pieces are too big, cut with a razor blade

   --Add a couple of drops of TOPO3: Vectashield mounting media (1:500)

   (Can also use 9 parts glycerol to 1 part PBS as mounting media)

   --View under confocal

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

Vibratome

1. 7% - 9% agarose (similar consistency to tissue)
   --Microwave – turn of 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
   --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 ~23-25°
    --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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