ADLER LAB

Laser Capture Micro-dissection

Cryo-embedding

The protocol below has guidelines for extracting RNA from cells collected by LCM. Unless otherwise stated, solutions should be made from DEPC treated solutions to minimize degradation of mRNA from contaminating RNAses.

1. Enucleate eye making sure to open the anterior chamber of the eye completely. I would recommend cutting away the anterior chamber with a pair of small scissors, then removing the lens and vitreous gently.
2. *Optional* - incubation in CMF (calcium/magnesium free HBSS) for 20 minutes at 37ºC for 20 minutes can be done to separate the RPE from the PhR's.
3. Cryoprotect tissue in the following gradient:
4. 6.75% sucrose/0.1M phosphate buffer for 10 minutes at 4ºC.
5. 12.5% sucrose/0.1M phosphate buffer for 20 minutes at 4ºC
6. 25.0% sucrose/0.1M phosphate buffer for 30 minutes at 4ºC
7. Equilibrate in a 2:1 ratio of 25%sucrose-0.1MPB/OCT (Tissue Tek),1 hour @ 4ºC
8. Snap-freeze tissue on dry ice with isopentane (methylbutane).
9. Store at -80 until needed.

Tissue handling

1. Cut 7uM sections at -30ºC and thaw mount on PEN foil slides.
2. Keep slides in cryostat until needed (use same day!).
3. Fix tissues in ice-cold, RNAse-free 70% ethanol for 30 seconds
4. Rinse in DEPC-H_2O for several dips.
5. ** If necessary stain for 10 seconds in Meyer's hematoxylin.
6. Dip in DEPC-H_2O several times (do only if doing H&E staining)
7. ** If necessary Stain in eosin-Y (in 95%EtOH) for 5 dips.
8. Dehydrate tissue in 95%EtOH for 30 seconds.
9. Immerse tissue in 100%EtOH for 1 minute.
10. Air dry for 2 minutes, then proceed directly to cutting the tissue with the LCM.

Using the LCM device (Leica LMD6000)

** Do not operate LCM without knowledge of the device. In addition- since this is an RNAse-free work area individuals should wear gloves when touching the LCM equipment including the touch-screen pen. The work area should be wiped down with RNaseZap before and after use..*

1. LMD 6000 laser startup procedure -
   - Turn laser power supply on with key switch. (LED on right will turn yellow)
   - Wait for both LED's to turn green (~5 min. which is enough to bring diode and crystal to operating temperature)
   - Once both LED's turn green press the red button to activate the laser.

     Note: Once the laser is active the laser is fully powered and time is being clocked on laser usage. When laser is not being used deactivate the laser by pressing the red button and turning both LED's green. This keeps the diode and crystal at temperature but saves laser time and usage. Turn off laser with keyswitch when not in use for extended periods and overnight. Turn on Laser (with key)
2. Turn on Microscope (bigger box on left)
3. Turn on Software

   ** from this step forward it is a good idea if you use automated features in the software and touch screens (ie- activate load/unload for both collector tubes and sample holder).
4. First load "collector" tubes onto tube holder then load tube caps with lysis soln. (65ul Qiagen "RLT" lysis soln. from RNeasy kit or 20ul global PCR lysis soln). Collect samples.

5. When turning off device turn off software first. Turn off laser with key and turn off microscope controller on left. Cover microscope when done.

Catalog #	Description	Size	Company	Contact
74004	RNeasy Microkit	50 pack	Qiagen	
710920	PCR tubes, 0.2ml, farbios		Leica Microsystems	
??	PCR tubes, 0.5ml, farbios		Leica Microsystems	
11505158	PEN-membrane slides 2,0 um	50 pack	Leica Microsystems	