

# MMPC Laser capture

Version: 1

Modified from: Laser Capture Microscopy Protocol- Van Winkle Lab

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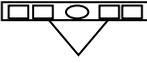
**Summary:** *To quantify regions or cell type specific alterations in RNA expression. Laser capture microscopy is utilized to isolate region or cell-specific regions in the brain and qualitative real time-PCR (qRT-PCR) is used to assess relative or semi-quantitative RNA expression of genes of interest.*

## Reagents and Materials:

Reagent/Material	Vendor	Stock Number
PEN Membrane Glass Slides	Life Technologies	LCM0522
RNAlater®-ICE	Life Technologies	AM7030
RNase Away spray	Molecular BioProducts	
Nuclease-free water		
Kimwipes		
nuclease-free pipette tips		
Dry ice		
Gloves		
Lidded Glass slide box w/desiccant		
Coplin jar		
Desiccant		
Forceps		
0.5 mL microfuge tubes (nuclease-free)		
PicoPure RNA Isolation Kit	Applied Biosystems	12317-03

## Protocol:

1. Perform an RNA necropsy (under RNase-free conditions) on tissue (e.g. mouse brain) of interest; mold tissue immediately and place cryomold on dry ice; OR flash freeze tissue and put in -80C for cryomolding later. NOTE: Tissue should not be fixed for sectioning.
2. Before cryosectioning, make sure you have prepared RNase-free slide boxes and membrane slides. For slide boxes, spray with RNase Away and rinse with nuclease-free water. For membrane slides, place in the oven at 175C for about an hour. Do not keep membrane slides in the oven for an extended period of time.
3. Cryosection tissue (8 µm.) Make sure the cryostat is as RNase-free as possible. Wipe blade with a little RNase Away solution. Keep slides in RNase-free box until ready to use. Keep an extra slide box in the cryostat in which to place the sectioned tissue so the RNA in the sectioned tissue will not degrade.

4. Return to lab or go directly to LCM. If you return to the lab, store slides at -80C until ready to use. Cover the sections with RNAlater®-ICE (and then remove excess) to prevent RNA degradation before storage at -80°C. If going directly to LCM, keep slides on dry ice until ready to use.
2. Turn on components in this order: computer, microscope, and laser (turn the key clockwise or to the “|” position.) Open the computer program “Leica Laser Microdissection V 6.5 software.”
3. Prepare sample slides: pour nuclease-free water into the Coplin Jar and put 3-4 slides from the sample slide box into water. Set timer for 5 minutes. In the computer program, open “Microscope Control” (☐menu ☐options ☐microscope control) and “Laser Control” (☐menu ☐laser ☐control). Adjust laser settings as needed (usually defaults are okay, but play with laser amplitude and focus area to fine-tune as needed.)
4. Dehydrating tissue: after 5 minutes, remove slides individually from nuclease-free water and gently tap each slide on a Kimwipe to remove excess water. Put slides into the glass slide box and put the lid on (the glass box should have a small amount of desiccant at the bottom for moisture absorption.) Leave slides in glass box for 5-10 minutes or until residual water is COMPLETELY evaporated and slides and totally dry. Check slides periodically to see if water has pooled at the bottom edge of the slide. If so, blot with a Kimwipe. Do not touch the membrane area.
5. Once slides are dry, in the LCM computer program, click  to load slides; position tissue/membrane side facing down. Click  and set-up the collection tube(s) by inserting the nuclease-free 0.5 mL tube in the “holder” and pipetting 50 µL of RNA extraction reagent into the cap of the tube.
6. Click on “Collector Device: Tube Caps” (bottom left corner) and select position where cap is located. Give the sample a name.
7. Click on slide holder (bottom middle) and select slide to view. Click on “Create Fast Overview.” This will generate a quick scan of the entire slide at 1.25X.
8. Once the fast overview is complete, scan around each tissue slice at 6.3X; if you see a feature that may be a vessel increase magnification to identify (20X or 40X.)
9. When you are ready to clip away a vessel, you must first calibrate the laser. To calibrate the laser, select the “Draw and Cut” option (upper right) and then ☐menu ☐laser ☐calibrate. MOVE AWAY from your area of interest to another, unimportant area of tissue. The laser will make a cross (+) cut in four different places on the tissue. Your mouse cursor will also turn into a cross shape. After each cross cut is made by the laser, match the mouse cross shape up with the laser cross cut and click.
10. To cut an area (vessel) of interest, select “Move and Cut” (upper right) and trace around the vessel. The magnification must be at least 20X cutting objective. The laser will cut through the section and the tissue will fall into the designated sample cap.
  - a. To avoid future RNA amplification for the synthesis of cDNA to be used in qRT-PCR, a minimum of approximately 25-30 vessels must be harvested to achieve an adequate yield.
11. Put labeled sample tubes on dry ice until you are back at the lab.
12. When finished with LCM, shut down the components in this order: laser, microscope, and computer.
13. Proceed with RNA extraction and cDNA synthesis protocol or store at -80C until ready to process.