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# Optimized Protocol for Mounting Tissue Sections onto Metal-Framed PEN Membrane Slides

PROTOCOL #9

## Introduction

Laser capture microdissection (LCM) technology allows the researcher to isolate pure cell populations from tissue sections for downstream molecular analysis. Traditional LCM is performed using an infrared (IR) laser which "captures" the cells of interest directly onto a specialized collection cap. Nucleic acids or proteins are then extracted and isolated from the collected cells for use in subsequent applications. The addition of a second, UV cutting laser, as in the Veritas™ Microdissection System, adds flexibility to the platform, increasing the speed and precision with which larger groups of cells can be microdissected.The system's utility is further enhanced as the dual laser system allows for the use of a wide variety of specimen types.

Metal-framed polyethylene naphthalate (PEN) membrane slides are ideal for use with the combined laser cutting and LCM platform. Properly prepared frozen or formalin-fixed, paraffin embedded tissues can be sectioned and mounted onto framed membrane slides. Flexibility in sample preparation is enabled as tissue sections can be dehydrated or non-dehydrated, thin (5µm) or thick (up to 100µm). The mounting technique used is specific to the sample type and the method used for preparation. This protocol specifically describes the mounting of tissue sections onto metal-framed PEN membrane slides for use in laser microdissection.

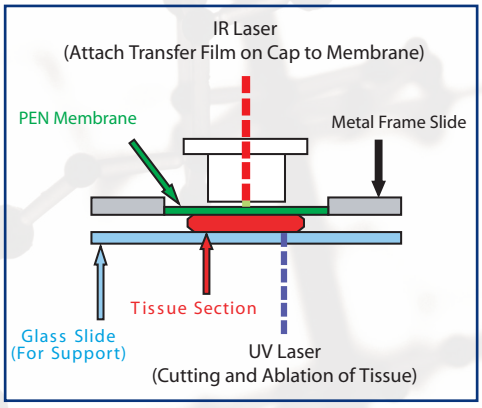


Figure 1. Laser Cutting Using Frame Membrane Slide

## Generally Required Materials and Equipment for Tissue Sectioning

The following laboratory materials are required to complete the protocol:

| Material |
|---|
| PEN Membrane Frame Slides (Arcturus Catalog # LCM0521) |
| RNase AWAY™ (Life Technologies, Cat. # 10328-011) |
| 100% Ethanol |
| Disposable gloves |
| Kimwipes or similar lint-free towels |
| Forceps and/or brushes |
| Microslide box, plastic (VWR Cat. # 48444-004) |
| Tissue Flotation Water Bath (FFPE sections) |
| RNase free water |
| Cryostat |
| OCT or similar compound (frozen sections) |
| Rotary Microtome |
| Disposable microtome blades |
| Dry ice |

## RNase-free Technique

RNase contamination of your samples and work area can cause experimental failure. Therefore, this protocol specifies the following technical guidelines for handling samples intended for RNA isolation:

- Wear disposable gloves and change them frequently.
- Use RNase-free solutions, glassware and plastic ware.
- Wash scalpels and forceps with detergent and bake at 210°C for four hours before use.
- Use RNase AWAY according to the manufacturer's instructions on the horizontal staining rack and any other surfaces that may come in contact with the sample.

## RNA Integrity Assessment

Arcturus recommends verifying the integrity of RNA in the tissue sample before proceeding with staining and laser microdissection procedures. This enables you to understand the quality of the RNA in the experimental sample before proceeding with further downstream processing. For frozen samples, refer to Arcturus Protocol #1 (Tissue Scrape Protocol for Verifying RNA Quality Using the PicoPure™ RNA Isolation Kit). If using paraffin tissue sections, refer to Appendix C of the Paradise Reagent System Whole Genome Expression User Guide (Sample Assessment Protocol).

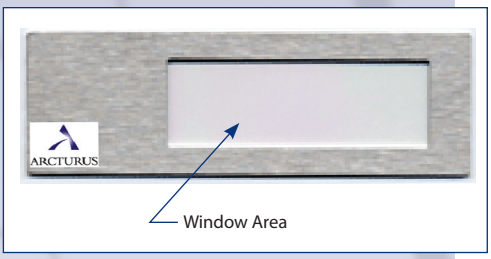


Figure 2. Metal-Framed PEN Membrane Slide

## Sectioning and Slide Preparation Procedure

Special Procedural Notes:

- Prior to use, frame membrane slides should be exposed to UV light (maximum=245nm) for 15-30 minutes. This process will increase the adherence characteristics of the membrane, especially for use with paraffin tissue sections. Irradiation with UV light also serves to sterilize the membrane slide by eliminating potential nuclease and nucleic acid contaminants.
- Tissue sections should be mounted only within the window area of the slide. Any tissue mounted outside of the window area will be obscured by the frame and will not be accessible for microdissection. Tissue sections should be oriented lengthwise within the window of the slide. This will maximize the area of the tissue accessible for microdissection.
- For best results, tissue sections should be mounted on the "flat" side (back) of the framed membrane slide. While the "well" side can be used, it is more difficult to mount sections on this side.
- Framed membrane slides should be kept at room temperature during the sectioning procedure.
- In order to maintain your frozen tissue sections at the coldest temperature possible during sectioning, place a microslide box in the cryostat or in a container filled with dry ice before beginning your sectioning. This box will be the repository for each slide that has tissue mounted on it while additional sections are being made.

## Frozen Tissue Sections

| Step | Procedure |
|---|---|
| 1. | Pre-cool the cryostat to the temperature recommended by the manufacturer for the specimen you are preparing. |
| 2. | Wipe down the knife holder and anti-roll plate of the cryostat with 100% ethanol to avoid sample cross-contamination. Allow the ethanol to completely evaporate, or dry surfaces with a clean kimpwipe, prior to initiating the sectioning process. |
| 3. | Install a new disposable microtome blade in the cryostat. |
| 4. | Set cutting thickness to 8µm. |
| 5. | Place a microslide box in the cryostat or on dry ice near the cryostat. |
| 6. | Transfer the cryomold containing the specimen from the –70°C freezer to the cryostat, transporting on dry ice if necessary. |
| 7. | Wait a minimum of 10 minutes for the specimen to equilibrate with the temperature of the cryostat. Using OCT, mount the specimen to the specimen holder. |
| 8. | Cut 8µm sections. As each section is cut from the block, lay the section flat on the metal plate just beneath the knife edge using the anti-roll plate or brushes. If you are cutting more than one specimen, move to a new, unused section of the blade, or use a new disposable blade for each one to avoid cross contamination. |
| 9. | To mount the section(s) onto the room temperature metal frame slide, slowly roll the flat side of the slide onto the sections and press gently down on the slide. Carefully lift the slide away from the knife holder. The sections should be loosely adhered to the membrane. Do not remove the slide from the interior of the cryostat during this process.<br>Note: Be sure that the tissue is mounted within the window area of the slide, and is not obscured by the metal frame. |
| 10. | To create a tight bond between the tissue sections and the membrane, gently place your index finger of your free hand on the "well" side of the slide for one to two seconds. This will cause the sections to "melt" and bind to the membrane. Do not remove the metal frame slide from the cryostat during this procedure. |
| 11. | After the frozen section has adhered to the membrane, immediately place the metal frame slide into the slide box in the cryostat or on dry ice. |

## Paraffin Tissue Sections

| Step | Procedure |
|---|---|
| 1. | Wipe all surfaces of the microtome that may come in contact with the tissue sections with RNase Away. |
| 2. | Set cutting thickness to 7 µm on the microtome. |
| 3. | Place paraffin block into specimen holder. Trim off any excess paraffin from the block face. Cut and discard the first five sections after trimming. |
| 4. | From the fresh surface, cut 7 µm sections from your specimen. If you are cutting more than one specimen, move to a new, unused section of the blade, or use a new disposable blade for each one to avoid cross contamination. |
| 5. | Remove section(s) from the microtome and float onto the heated water bath containing plain RNase free water (do not use adhesives such as gelatin in water bath). Allow section(s) to flatten. Minimize time in the water bath to no longer than one minute. |
| 6. | Mount each section on a room-temperature slide. When picking up section(s) from the water bath, collect the section(s) in the desired position within the usable area of the membrane. Either the "flat" or "well" side of the membrane may be used. However, the flat side is recommended for best performance during laser microdissection.<br>Note: If the "well" side of the membrane is used to collect paraffin sections, special care must be taken to ensure that all water is drained away from the area around the tissue. |
| 7. | Once the tissue section has been mounted, it cannot be repositioned to a different area on the membrane. |
| 8. | After the required number of paraffin sections has been collected onto the membrane, the frame slide should be placed in an upright position to facilitate draining away any water from the tissue area. |
| 9. | If water becomes trapped at the edge of the section, it should be wicked off with a clean kimwipe, taking care not to touch the tissue itself. |
| 10. | Air-dry the slides for a minimum of 30 minutes and a maximum of 3 hours at room temperature, then store in slide boxes until ready for use. Slides may be stored at room temperature in a dessicator for up to two weeks. For longer storage the slides should be placed at -70°C. |

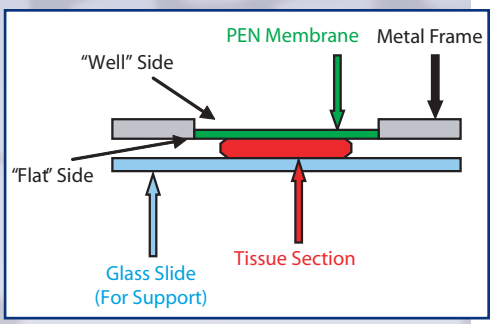


Figure 3. Frame Slide "Sandwich"

## Staining and Microdissection

| Step | Procedure |
|---|---|
| 1. | Once the tissue sections have been mounted onto the frame membrane slide, it is ready for the desired staining protocol, coupled with dehydration if necessary. See the HistoGene® LCM Frozen Section Staining Kit User Guide for use with frozen tissue sections. The Sample Preparation and Staining section of the Paradise™ Reagent System Whole Genome Expression User Guide should be referenced for use with paraffin tissue sections. |
| 2. | After staining and appropriate dehydration, the slide is ready for laser microdissection. It is important to note that the addition of a clean plain glass slide under the tissue is needed for the laser microdissection process. This glass slide serves as support for the tissue during this procedure. Place a clean glass slide on the "flat" side of the membrane slide to act as a solid support. The tissue section will be "sandwiched" between the membrane and the glass slide. |

## Troubleshooting:

If the section, or any portion of the section, detaches during or after the staining process, refer to the list below of troubleshooting items for possible resolution.

| Tip | |
|---|---|
| 1. | Coating of the slide with Poly-L-Lysine or silane may improve adherence of tissue to the membrane. |
| 2. | Surface properties of the membrane may have changed. Re-exposure of the slide to a UV light source (maximum=254nm) will correct the surface properties for proper adherence of section to the membrane. |
| | For Paraffin Sections only: |
| 3. | i. Water may have become trapped between the section and membrane. Remember to place the slide in an upright position after mounting sections. This allows the water to drain away from the sections.<br>ii. Drying time after mounting the sections may not have been adequate. The drying time may be increased from the initial time used.<br>Note: It is not recommended to dry slides in a heated oven.<br>iii. There may have been incomplete infiltration of paraffin into tissue specimen. Areas not embedded with paraffin will detach from the slide. When this occurs, the whole tissue sample will need to be reprocessed.<br>Note: Reprocessing of the sample may have a detrimental impact on RNA quality. It is highly recommend that a tissue scrape and an assessment of RNA integrity be completed on any tissue reprocessed prior to proceeding with any microdissection process. |

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