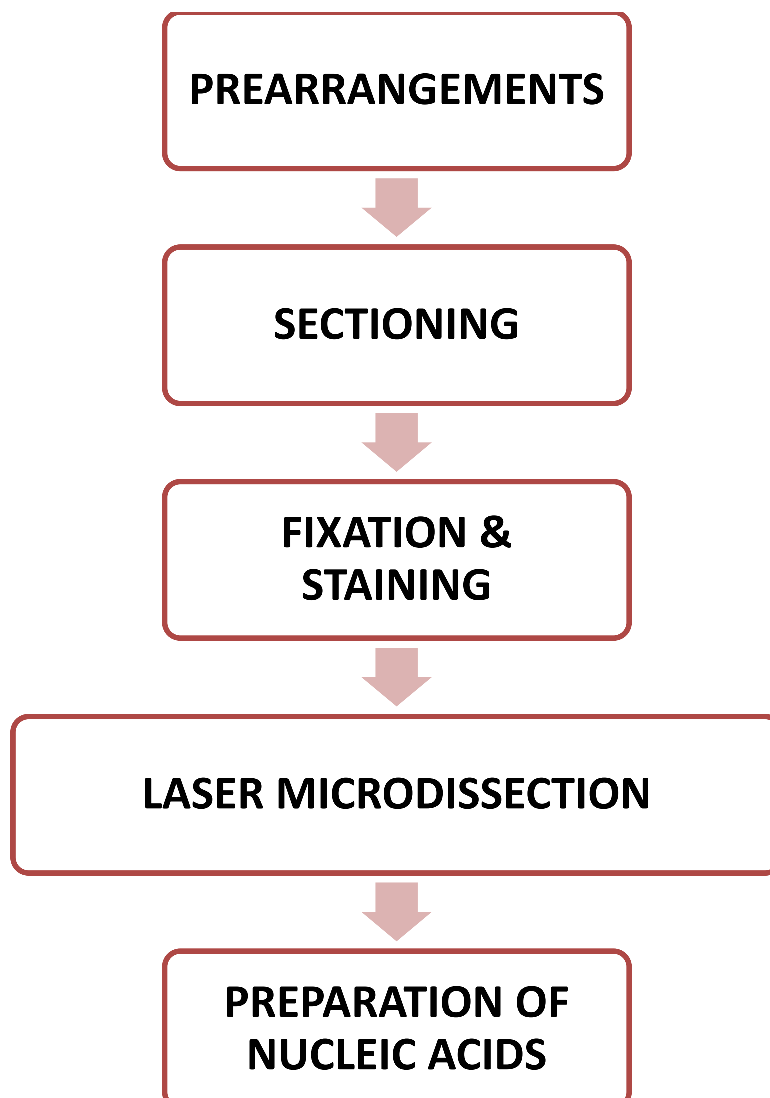
From Eye to Insight

Leica MICROSYSTEMS

Protocol: Laser Microdissection of Single Cells for RNA Purification



PREARRANGEMENTS

- Generally, fresh, frozen tissues are preferred for isolating RNA.
- Frozen sections can be obtained quickly on a cryostat.
- High quality RNA is achieved from tissues frozen immediately after surgery, which minimizes RNA degradation over time by ubiquitous RNases or heat.

Choice of consumable

Membrane based slides are the best choice for reliable Laser Microdissection. For RNA downstream analysis standard PEN glass slides (#11505189, #11505158 or #11600288) are recommended as these have the best price performance.

Sterilizing Membrane Slides

Several methods to sterilize LMD membrane slides are available: 1. autoclaving, 2. chemical treatment or 3. UV-C (254 nm) irradiation. Please note that sterilizing slides by autoclaving or UV-C-treatment does not guarantee complete destruction of RNases. For RNA preparation, especially from a single cell or from a small number of cells, RNase-free certified PEN membrane slides (#11505189) are recommended.

Method 1: Autoclaving

Place slides into a steel basket such as a slide holder for paraffinization and place the basket with the slides into a beaker or jar. Autoclave at 121 °C for 20 minutes.

Method 2: Chemical Treatment

Treat membrane Slide with RNase decontamination solution according manufacturer's instructions. Always rinse membranes and slides in RNase-free (molecular biology grade) water after chemical treatment to remove remaining chemicals.

Method 3: UV-Treatment

Incubate slides in a UV-C (254 nm) crosslink chamber and deliver at least 1 joule of energy (maximum power at 30 – 45 minutes, for #11505158 or #11600288 or max. 15 minutes for #11505189). Please refer to the manufacturer's information when using other UV-light sources.

UV-light sources from sterile flow hoods can also be used for sterilization instead of a cross linker.

Collection caps can be sterilized using the same UV-C light source: place the tubes with open cap facing the light source into the crosslink chamber/flow hood and at least 1 joule of energy (maximum power) for at least 45 minutes.

Note: Sterilizing methods can be combined and should be done shortly before membrane Slides are used.

The cap may be used dry, but can also be pre-filled with buffer to protect nucleic acids from degradation.

SECTIONING

Freezing the Samples

Cryo tissue or organs should be flash-frozen (snap-frozen) after surgery followed by cryo-sectioning. Sections should be stained within a very short time, best practice: not exceeding in total 30 minutes.

Method 1: Flash-freezing using 2-methyl-butane (synonym: isopentane)

1. Precool 2-methyl-butane in a beaker surrounded by dry ice. This prevents the 2-methyl-butane from bubbling over when the dry ice is added.
2. In a beaker or specimen container, add crushed dry ice to the 2-methyl-butane to make a slurry mixture (work in a hood).
3. When bubbling stops, the 2-methyl-butane is at the correct freezing temperature of approximately -90°C.
4. Immerse the embedded tissue slowly; eventually it will sink to the bottom of the 2-methyl-butane.

Safety Note: Be sure to fully evaporate the 2-methyl-butane after freezing to prevent the possibility of explosion in the freezer.

Method 2: Flash-freezing in liquid nitrogen

1. Place liquid nitrogen in a styrofoam container.
2. Place the styrofoam container inside a Petri dish lid; a support rack may be needed to hold the Petri dish lid.
3. Place the tissue into a disposable mold and embed it in the tissue freezing medium; or alternatively, place the tissue (embedded in the tissue freezing medium) on a coverslip and place into the liquid nitrogen.

Method 3: Flash-freezing using 2-methyl-butane and liquid nitrogen

1. Place a beaker with 2-methyl-butane into liquid nitrogen and wait until the 2-methyl-butane cools down to –80°C. This is the point when the wall of the beaker turns white, the 2-methyl-butane is now solid.
2. Insert the tissue into 2-methyl-butane and let it freeze. Alternatively, the tissue can be put onto a floating platform made of cork.

Method 4: Direct freezing at -80°C or using dry ice

1. Place fresh tissue in baked and pre-cooled aluminum foil or place the tissue onto a platform made of cork with some fresh OCT (cork allows easy re-store and re-use for cryostats later on).
2. Directly transfer tissue into -80°C (e.g. using a box with dry ice).

Note: Dry ice might increase the overall CO_2 concentration which can alter the pH, tissue and RNA quality.

Cryo-Sectioning

Cryo blocks can be directly sectioned (recommended for RNA preparation) or stored at –80°C. The temperature of the cryo blocks should be adjusted to –20°C in the cryostat before sectioning.

1. Clean the cryostat before sectioning to avoid contamination
2. Mount the cryo block onto the specimen clamp
3. Trim the sample to get a plane surface and an approach to the desired tissue
4. Cut the block into 5–25 µm sections (according desired single cell diameter) and immediately place them on the slides for LMD and let the sections briefly thaw to the membrane

Important: If you are using FrameSlides, the FrameSupport is strongly recommended. Sections can be fixed with ice-cold acetone for 2–3 minutes, 70% or 100% ethanol for 20 seconds or mixture of ethanol : acetic acid (19:1) to increase the adhesion of the tissue to the PPS-, PEN-, PET-, POL- or FLUO-membranes.

Sample slides should be fixed and stained right after cryo-sectioning.

FIXATION & STAINING

Quick Cresyl Violet Staining

Cresyl violet stain is commonly used for neuronal tissue. It is a basic stain that binds to acidic molecules of neuronal cytoplasm, such as RNA-rich ribosomes. Cresyl violet permanently stains a section and is suitable for both paraffin and frozen sections and subsequent RNA preparation.

Recommended Modified Cresyl Violet Staining for RNA-Research

For RNA protection Cresyl violet can be prepared and applied without PBS or water steps, preventing degradation of RNA by humidity activated RNases.

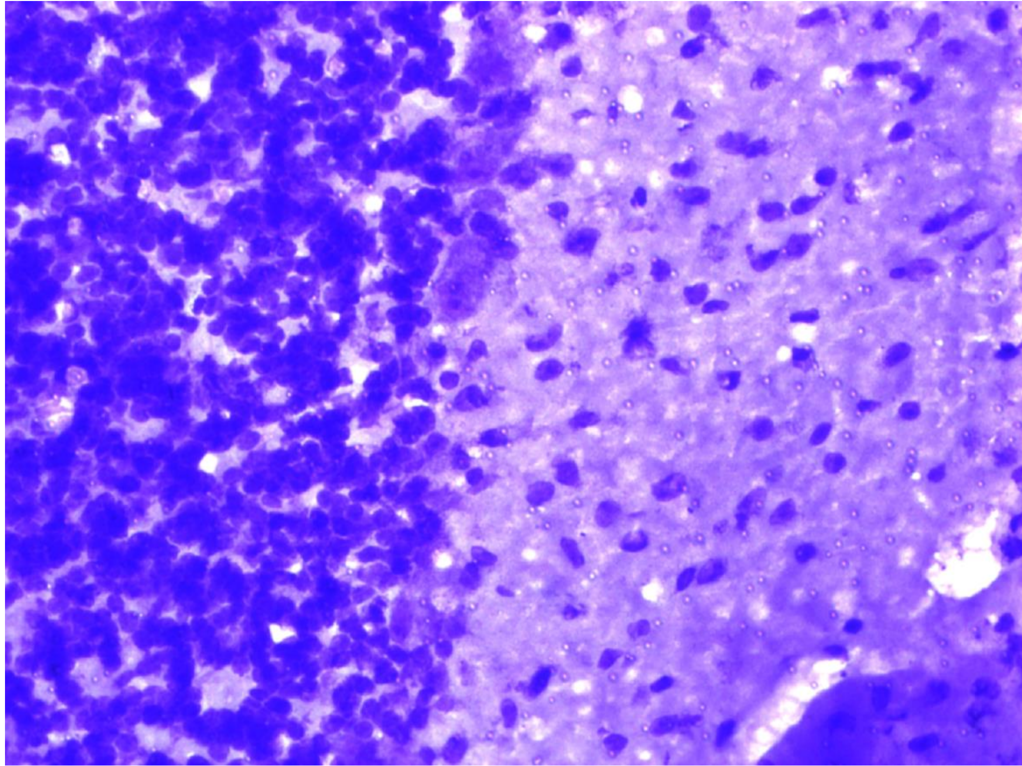
Modified Cresyl violet staining solution

1. Prepare Cresyl violet staining solution at least one week prior usage
2. Add 0.5 g Cresyl violet into 50 ml 100% ethanol
3. Mix solution and store at 4°C sealed air-tight and dark

<u>Procedure</u>

1. Mount section and fix in cold (-20°C) 75% ethanol for 2 minutes
2. Dip 2 x 1min in DEPC water in order to remove OCT
3. Apply Cresyl violet staining solution directly with syringe and sterile filter to the section and incubate for 1minute, swivel gently
4. Dip for 5 seconds in 75%, 90% and 100% ethanol
5. Final fixation is done in fresh 100% ethanol for 1 minute
6. Dry sample in drying chamber with desiccant

Adapted from Gründemann et al., NAR, 2008; Methods Mol Biol, 2011



LASER MICRODISSECTION

Recommended number of dissected cells for RNA research:

In general gene expression profiling of single cells or as few as 10-30 cells has been reported. If quality tests of each microdissected sample is desired (e.g. using a RIN value) many more (hundreds-thousands of cells) are required to meet the minimum amount of RNA (5 ng or 50 pg) of the Bioanalyzer system.

PREPARATION OF NUCLEIC ACIDS

Leica Microsystems recommends the high-quality QIAGEN kits (RNeasy® Micro Kit) for preparation of nucleic acids. They can be immediately used in downstream applications such as PCR, sequencing, quantitative, real-time PCR, or can be stored at –20°C until needed. Please refer to www.qiagen.com for details.

GENE EXPRESSION ANALYSIS

Gene expression analysis is classically done via qPCR. Technologies such as Microarrays or next generation sequencing (NGS) are applicable as well

REFERENCES

Bandyopadhyay et al., JoVE, 2014
Schlaudraff et al., Neurbiol of Aging, 2014
Gründemann et al., Methods Mol Biol, 2011
Gründemann et al., NAR, 2008

FEEDBACK

Do you miss anything in the protocol or do you have questions or suggestions?
Please contact us.

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