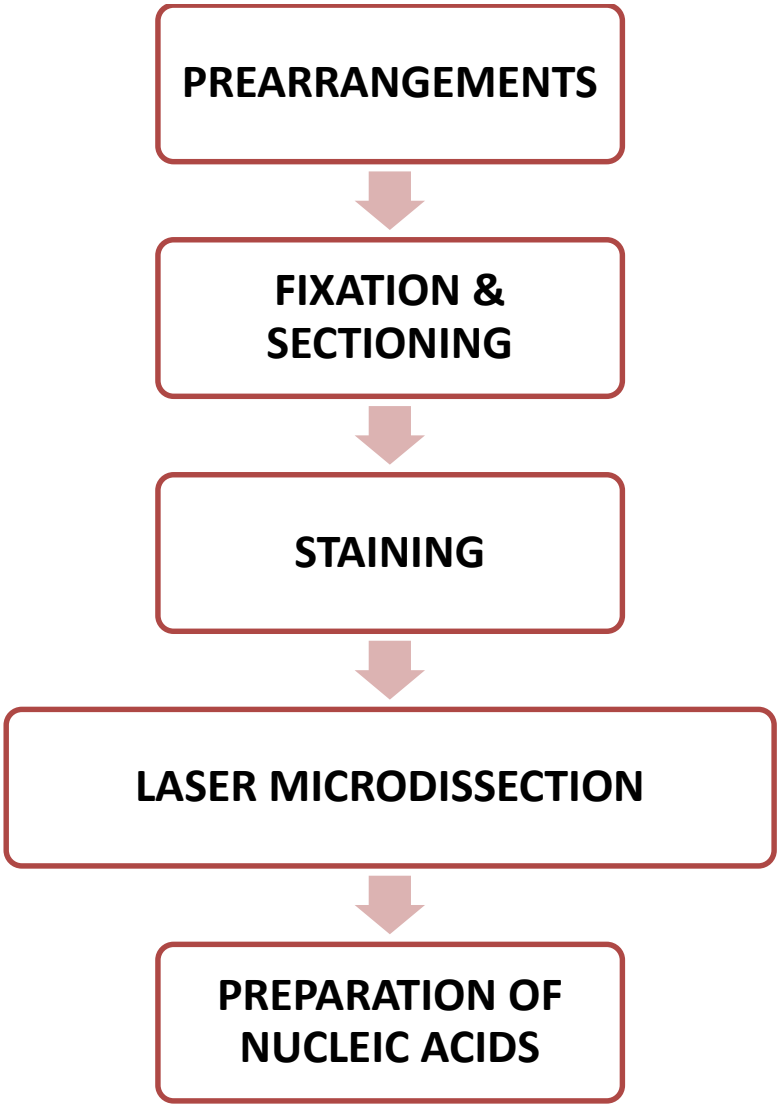
From Eye to Insight

Leica MICROSYSTEMS

Protocol: Laser Microdissection of Cancer Tissue for DNA Purification



PREARRANGEMENTS

- DNA can be prepared from both frozen (recommended) or formalin-fixed, paraffin-embedded (FFPE) material.
- The advantage of working with paraffin-embedded sections is superior morphology preservation as compared to frozen sections.
- The advantage of working with frozen sections is superior DNA preservation as compared to paraffin-embedded (FFPE) material.
- Preserving morphology can be critical for diagnostics and for final selection of specific cells for further analyses.

Choice of consumable

Membrane based slides are the best choice for reliable Laser Microdissection. For DNA 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 DNases. For DNA preparation, especially from a single cell or from a small number of cells, DNase-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: 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

Paraffin embedding is a standard process in pathology in which fixed tissue (utilizing neutral buffered formalin or other fixatives – see below) is infiltrated by paraffin to stabilize it for long term storage, easy sectioning and for histological examination. Most cancer tissue will be provided paraffin embedded. Paraffin may affect macromolecules such as RNA and DNA.

If fresh cancerous tissue is available, cryo-sectioning is recommended to preserve nucleic acids from degradation.

Preparation of Frozen Sections

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 and tissue 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.

FIXATION & STAINING

Fixation

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

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.

H&E Staining

Method 1: H&E (Hematoxylin and Eosin) Staining

H&E is a common histology stain. Hematoxylin stains the nuclei, and eosin stains the cytoplasm. H&E is suitable for both paraffin and frozen sections. Ready-to-use reagents are also commercially available.

Reagents

- 70% ethanol
- 95% ethanol
- 100% ethanol
- Hematoxylin (Hematoxylin Solution, Harris modified, Sigma Diagnostics, Order no. HHS-16)
- Eosin (Eosin Y solution, alcoholic with phloxine B, Sigma Diagnostics, Order no. HT110-3-32)
- 0,1 % NH4OH (blueing reagent): 200 µl NH4OH 100% + 200 ml H2O or Scot´s tape water substitute

Procedure

Place the slide in the following solutions for the designated time frames:

1.	Distilled H_2O	30 seconds
2.	Hematoxylin	1 minute
3.	H_2O	30 seconds
4.	Blueing reagent	30 seconds
5.	Eosin solution	10 seconds

6.	70% ethanol	30 seconds
7.	95% ethanol	30 seconds
8.	100% ethanol	30 seconds or air-dry at room temperature

Results

- Nuclei - blue-black
- Cytoplasm - varying shades of pink
- Muscle fibers - magenta
- Fibrin - deep pink
- Red blood cells - orange/red

Method 2: H&E Staining (additional version)

See the above application range and typical results of staining.

Reagents

- Gills Hematoxylin

Hematoxylin	6.0 g
Aluminium Sulphate	4.2 g
Citric Acid	1.4 g
Sodium Iodate	0.6 g
Ethylene Glycol	269 ml
Distilled Water	680 ml

- Eosin

Eosin Yellowish	1.0 g
Distilled Water	100 ml

- Lithium Carbonate 1 %

Lithium Carbonate	1 g
Distilled Water	100 g

- Acid Alcohol 1 %

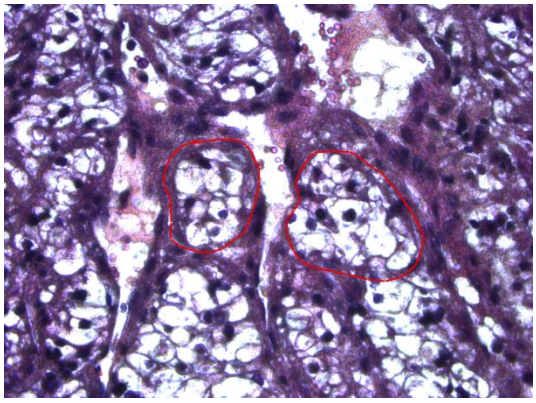
70% Alcohol	99 ml
conc. Hydrochloric Acid	1 ml

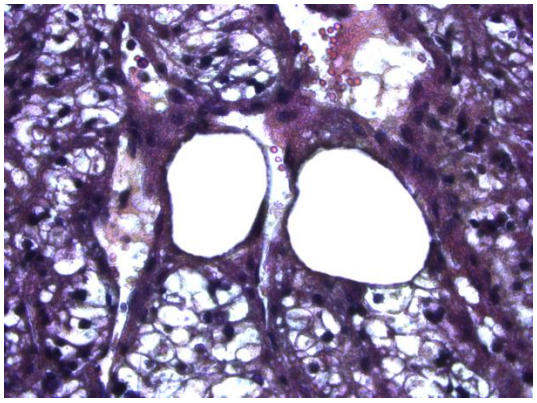
- Scott's tap water substitute

Before you begin, add 20 g sodium bicarbonate and 3.5 g magnesium sulphate to a beaker containing 1 L distilled water. Mix thoroughly with a magnetic stirrer to dissolve the salts, then pour into a storage container.

Procedure

1. Wash the slide with distilled water for 30 seconds
2. Place sections in the hematoxylin solution for 30 seconds
3. Wash in tap water
4. “Blue” sections in Scott's tap water
5. Wash in tap water
6. Place sections in 1% acid alcohol for a few seconds
7. Wash in tap water
8. Place sections in eosin for 15 ~ 30 seconds
9. Wash in tap water
10. Dehydrate with 95% ethanol for 30 seconds, 100% ethanol for 30 seconds, and air-dry 5 ~ 10 minutes





LASER MICRODISSECTION

Recommended number of dissected cells for DNA research:

Number of cells is not critical because DNA is easily amplified by PCR.
Usually up to 5000 cells can be dissected.

PREPARATION OF NUCLEIC ACIDS

Leica Microsystems recommends the high-quality QIAGEN kits (QIAamp® DNA 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.

REFERENCES

Ardighieri et al., Virchows Arch., 2016
Barrow et al., Int J Cancer, 2015
Begus-Nahrmann et al., Nat Genet, 2009

FEEDBACK

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

Leica Microsystems CMS GmbH | Ernst-Leitz-Strasse 17–37 | D-35578 Wetzlar (Germany)
Tel. +49 (0) 6441 29-0 | F +49 (0) 6441 29-2599

www.leica-microsystems.com/lmd-genotype