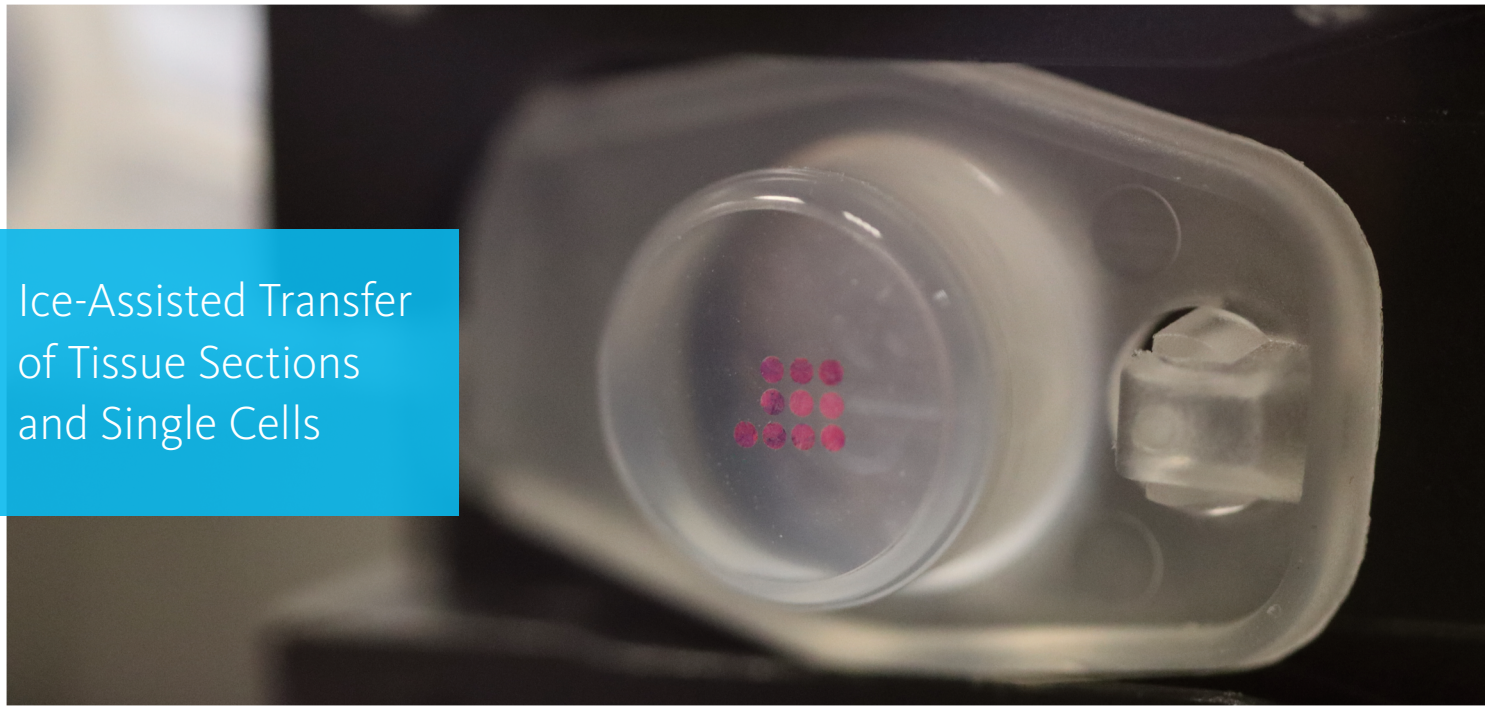
MMI Protocol

Sample Lysis Using Isolation Caps and CapStrips



Ice-Assisted Transfer of Tissue Sections and Single Cells

Introduction

The following protocol describes the lysis of tissue sections or single cells isolated using the CellCut laser microdissection system.

Principle of the ice-assisted transfer

For the ice-assisted transfer, tissue sections or single cells are frozen together with either water or lysis buffer. Upon freezing, ice formation leads to the establishment of strong hydrogen bonds between the ice and the water-containing tissue. As a result, the tissue adheres much more strongly to the ice than to the silicone membrane, allowing it to be completely detached.

Note: The ice-assisted transfer works reliably with bidistilled water. The water can subsequently be removed by evaporation, allowing the desired volume of lysis buffer to be added. The direct use of a specific lysis buffer for the ice-assisted transfer should be tested beforehand.

Materials

- Isolation Caps (0.2, 0.5 or 1.5 ml)
- 8-CapStrips
- 96-well plate (e.g. Eppendorf PCR Plate 96 LoBind, skirted, 150 µl)
- Bidistilled water or lysis buffer (composition depending on your downstream application)
- Laboratory freezer (-20 °C)

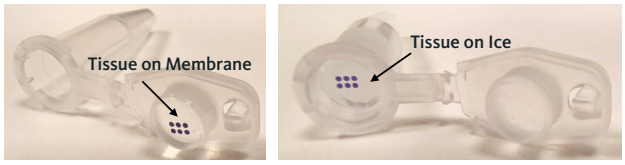


Fig. 1: Transfer of FFPE tissue using the ice-assisted transfer. **Left:** Tissue on a 0.5 ml Isolation Cap after laser microdissection. **Right:** Transferred tissue on ice.

Method

ROI Transfer using MMI Isolation Caps

1. Collect tissue or cells into MMI Isolation Caps using the MMI CellCut.
2. Add the following volumes of bidistilled water or lysis buffer to the bottom of the Isolation Cap:
    - 0.2 ml Isolation Cap: 30 µl
    - 0.5 ml Isolation Cap: 40 µl
    - 1.5 ml Isolation Cap: 100 µl
3. Gently close the Isolation Cap, invert the tube, and ensure that the water or lysis buffer is positioned directly on top of the silicone membrane.
4. Place the tube upside down at –20 °C for at least 1.5 hours.
5. Open the tube lid; the dissected tissue has now been transferred into the frozen water/lysis buffer (Fig. 1)

ROI Transfer using MMI CapStrips

1. Collect tissue or cells into MMI Isolation CapsStrips using the MMI CellCut.
2. Add 30 µl of bidistilled water or lysis buffer to the bottom of a compatible 96-well plate.
3. Gently attach the CapStrips to the 96-well plate ensuring proper sealing.
4. Turn the plate upside down.
5. Ensure that the water or lysis buffer is positioned directly on top of the silicone membrane.
6. Place the plate upside down at –20 °C for at least 1.5 hours.
7. Remove the CapStrips from the 96-well plate; the tissue is now transferred into the wells of the plate (Fig. 2).

Recommendations

- Keep the time between removal from the freezer and tube/plate separation as short as possible to prevent thawing.
- After successful transfer, the water can be evaporated and replaced with the appropriate lysis buffer for downstream sample preparation.
- Ensure that the freezer temperature is at least as low as –20 °C. If the temperature is higher, the storage time must be extended.

Verification of Transfer Efficiency

- Transfer efficiency can be verified by examining the MMI Isolation Caps or CapStrips under a microscope after separation
- Successful transfer is indicated by the presence of the laser ablation mark while no tissue or cell material remains at the collection site.
- If residual tissue is still visible at the collection site, the transfer can be repeated before proceeding with downstream sample preparation.



Fig. 2: After freezing, the CapStrip is removed, resulting in complete transfer of the tissue onto the ice.

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