

Microdissection Manual Microdissection

This method was successful in our lab using prostate tissue and for our specific objectives. Investigators must be aware that they will need to tailor the following protocol for their own research objectives and tissue under study.

About Manual Dissection

There are several manual methods that can be used to perform tissue microdissection. Techniques using hand-held tools as well as mechanical micromanipulator-based approaches have been described. However, speed and precision are the most important parameters and any method that achieves these is adequate. Investigators should also expect to invest time initially by practicing on 10 to 20 cases to begin to feel comfortable with the technique.

Method

The approach of the NCI Prostate Group is to dissect on a standard inverted microscope using a 30 gauge needle on a syringe as the microdissecting tool.

TIP: To stabilize the dissecting hand, the dissector should prop their elbow on a solid surface adjacent to and at the same height as the stage of the microscope. It is helpful to rest the ulnar aspect of the dissecting hand on the stage of the microscope and move the needle into the microscopic field, a few millimeters above the tissue. In this way, the dissecting arm and hand can be rested on solid support surfaces.

1. While viewing the tissue through the microscope, the cell population of interest should be gently scraped with the needle. The dissected cells will become detached from the slide and form small dark clumps of tissue that can be collected on the needle by electrostatic attraction. Several small tissue fragments can be procured simultaneously. Collection of an initial fragment on the tip of the needle will assist in procuring subsequent tissue. The tip of the needle with the procured tissue fragments should be carefully placed into a small PCR tube containing the appropriate buffer. Gentle shaking of the tube will ensure the tissue detaches from the tip of the needle.

TIP: Pressing down on the shaft of the syringe to inject an air bubble into the extraction solution helps to detach the tissue from the needle and prevents any fragments from remaining lodged in the barrel of the needle.

2. Placement of frozen tissue sections directly on agarose coated slides can be helpful in maintaining enzyme stability. Additionally, the agarose gels can be prepared or soaked in custom buffers that will bathe the frozen section prior to and during the microdissection, e.g., pH, salt concentration, proteinase inhibitors, etc., can be varied specifically for a given enzyme. Some members of the NCI Group also prefer to use the agarose coated slide microdissection approach for recovery of mRNA. Slides for microdissection are prepared by placing 200 μ l of warm agarose on standard uncoated glass slides, covering with a glass slip, and allowing the gel to polymerize. The glass slip is removed from the slide and the frozen tissue section is immediately placed onto the agarose gel. The freshly cut section should be transferred directly from the cryostat to the agarose coated slide.

Microdissection can be performed similar to the method described above. However, the dissector may find it easier to "tease" the tissue apart since the tissue remains bathed in the fluid from the gel and can be gently pulled apart. The tissue will also separate along tissue planes, e.g., stroma and epithelium will easily separate from each other. The dissected tissue can be gently picked up from the slide, or alternatively, the dissector can use the needle to physically cut the agarose and procure both the agarose and the tissue fragment together.

References:

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