

## Macrodissection of FFPE Tissue for Nucleic Acid Purification

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### OVERVIEW

Macrodissection of FFPE tissue prior to nucleic acid purification allows the operator to select tissue of interest (typically tumor tissue) and minimize background tissue. This in turn can enrich the extracted nucleic acid for variants of interest.

The Ionic Purification System from Purigen Biosystems is an excellent choice for macrodissected tissue. It offers a simple, automated work-flow and typically generates yields that are 2-4x higher than other methods, maximizing the amount of nucleic acids extracted from macrodissected tissue.

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## Macrodissection Technique

Macrodissection consists of 3 primary steps: marking, scraping, and transferring for downstream analysis. Care must be taken at each step to ensure the desired tissue is safely captured and unwanted tissue is appropriately disposed.

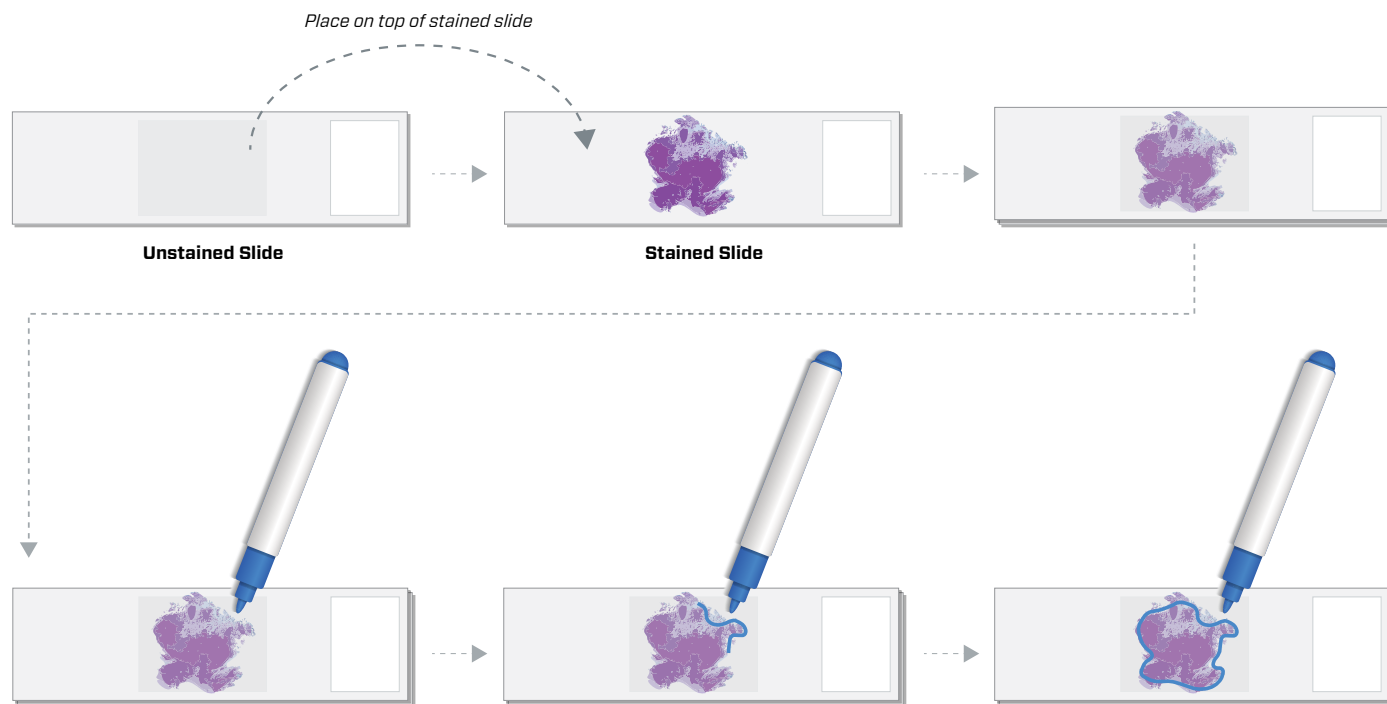
### Marking

Macrodissection starts by defining an area of interest. Typically, a pathologist will mark one stained slide (e.g. with H&E). The markings can then be transferred to the unstained slides to be used for nucleic acid collection.

The precision of the marking process can impact the degree of enrichment in the nucleic acid preparation. To get the most precise area selection:

- **Mark with a thin-tip sharpie.**
- **Consider the width of the line while marking. In our lab, we assume that material covered by the line will be scraped away and only the material inside the line will be collected.**
- **Be precise in transferring the line from the stained section to the unstained sections. Align the slides carefully, and be sure to trace while looking from above the stack, not at an angle.**

In some cases, it is desirable to simply remove paraffin from the sample and collect only the tissue. In these cases, it may be possible to mark directly on the unstained slide, without using a stained section.



**FIGURE 1:** Place a slide with unstained tissue on top of a slide with stained tissue and mark the desired area on the unstained slide. Both slides should be oriented with the FFPE slice on the bottom and the area of FFPE between the two slides should be aligned.

## Scraping

Once the desired tissue section is marked, the next steps are to trim the waste material, then collect the desired material. Both steps are typically performed by hand with a sharp, disposable blade.

Safety is of critical importance at this stage because there are two sharps hazards in play during the operation – the blade, and the potential for a broken glass slide.

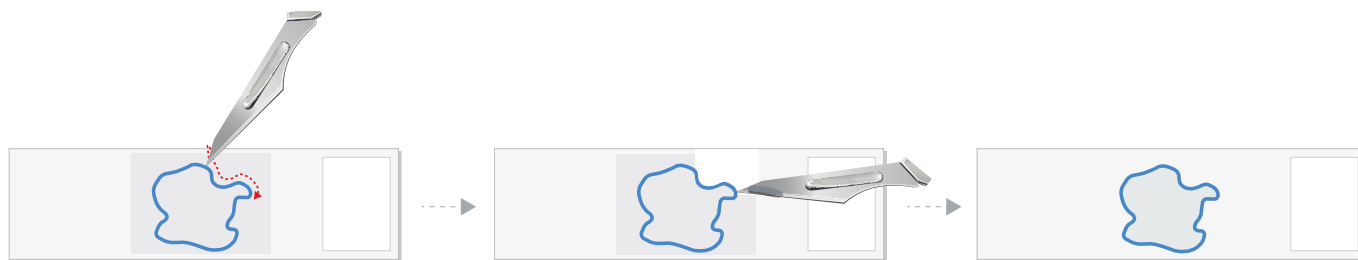
Start by using appropriate PPE: safety glasses, disposable gloves, and a lab coat. Make sure that each element fits properly and does not obstruct movement.

Use the appropriate technique during scraping:

- **Select a comfortable sitting or standing position that minimizes body movement. It can help to rest your elbows on the bench to stabilize yourself.**
- **Create a clean space to work – this will aid cleanup of any excess tissue.**
- **Support the glass in the area where you scrape. This minimizes the risk of slide breakage. The best way to do this is to use a soft cutting mat under the slide.**
- **Never direct the blade toward your fingers or body. Always scrape so that if the blade slips, it does not cut you.**

We recommend practicing on non-critical FFPE sections before performing critical work.

Once the technique is clear, proceed to the macrodissection. First, scrape away the tissue not desired for the purification, leaving only the desired tissue. Then, come back and collect the desired region.



**FIGURE 2:** Carefully scrape away undesired material and discard in an appropriate disposal receptacle

## Transfer for Downstream Analysis

This scraping of the desired tissue is the most important step. It is ideal to scrape it in a single pass if possible, because this results in the most compact piece of tissue to transfer into the tube. However, this is not always possible, and will depend strongly on the tissue in the block and thickness of the section.

Transferring the collected tissue to a tube can be a challenge for several reasons.

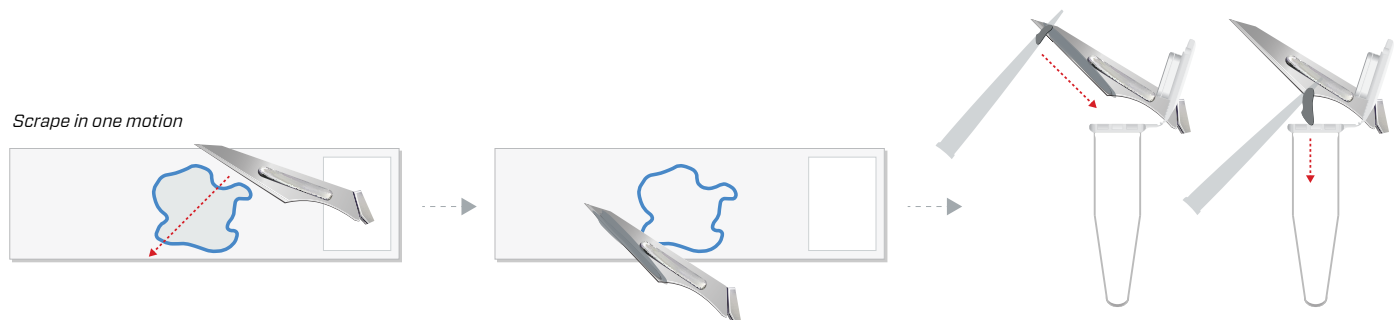
- **Their mass is very small, so they don't fall with gravity.**
- **FFPE is typically stored dry, so it can easily become statically charged.**
- **Paraffin tends to stick to surfaces.**

A couple of techniques help mitigate the challenge.

- **Always use a tube rack to hold your collection tube. This is one fewer object for you to hold.**
- **Use a pipette tip to push the tissue off the blade and into the interior of the tube.**

These operations usually still result in tissue stuck to the side of the tube. One way to encourage them into the bottom of the tube is to centrifuge the tube. Centrifugation at maximum speed for 2 minutes is usually sufficient to move most of the material to the bottom.

It is critically important that the tube you transfer to at this stage is the tube intended for lysis. Any additional transfer after this stage is likely to result in lost tissue.



**FIGURE 3:** Create a “scroll” with the desired tissue by scraping in a smooth motion that curls the tissue section around itself. Ideally this is done in a single scrape. Use a pipette tip to transfer the scroll to an Eppendorf tube.

## Extract Nucleic Acid

Select the appropriate Ionic Purification Kit depending on your needs and follow the protocol provided with the kit:

- **Ionic FFPE to Pure DNA Kit to maximize DNA yields**
- **Ionic FFPE to Pure RNA Kit to maximize RNA (including miRNA) yields**
- **Ionic FFPE Complete Kit to extract RNA and DNA with a single workflow**

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