1. Section Tissue and Transfer ROI

This section describes the process of sectioning FFPE tissue, mounting the sections onto gel pads, punching out the Region-of-Interest (ROI), and transferring the ROI to the sample slide. For required reagents and consumables, see *Supplies–Section Tissue and Transfer ROI* on page 9.

Before transferring tissue to the sample slide, note the following:

Sample slide

- Obtain one sample slide for each flow cell you intend to prepare.
- Record the slide ID (flow cell ID) for tracking and documentation purposes. The slide ID should follow the format of HRXXXXY, where X represents a digit (0-9) and Y is a letter (A-Z).

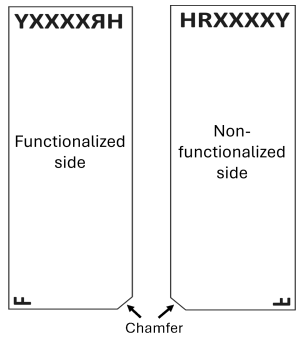

Figure 14 Location of slide ID

Sample slide sides

The sample slide has a propietary functionalized surface coating on one side, and a non-functionalized surface on the other side. Here is how you can identify the slide surfaces:

Side	Slide ID	Chamfer location (see Figure 14)
Non-functionalized surface	Etched, readable	Bottom left
Functionalized surface	Mirrored	Bottom right

Storage and handling of new slides

- The product label will indicate the expiry date of the slide.
- Slides are functionalized with a propietary surface coating and should be stored at 4°C in original sealed packaging. **Do not open packaging until you are ready to do tissue transfer to prevent contamination.**
- Allow slides to equilibrate to room temperature for 15 minutes before opening packaging to prevent condensation.

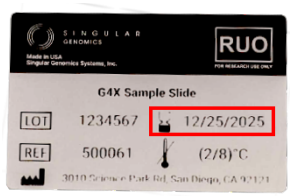

Figure 15 Sample slide production label

Handling and storage of slides with mounted tissue

Sample slides that are mounted with tissue may be stored in a slide box with a desiccant packet at room temperature. Ensure desiccant packet is not saturated with moisture. Use within 1 month of tissue transfer.

Do not touch, wipe, or otherwise disturb the tissue or the functionalized surface of the slide to avoid contamination or damage. Always handle the Sample Slide by the sides or coners to avoid touching the surface.

1.1 Set Up Equipment

This topic describes how to set up equipment for slicing sections from the FFPE tissue block.

1. Place a HistoCool block filled with **RNase-free MilliQ water** in the -20°C freezer overnight or until water is completely frozen.

   NOTE
   If you don't own a HistoCool, you can prepare a frozen ice bath by using **RNase-free MilliQ ice chips**.

2. Remove gel pad from 4°C fridge and allow to equilibrate to room temperature for at least 30 minutes.
   - Do not remove sticker from the bottom of the gel pad tray.
   - Gel pad can be kept at room temperature for up to 4 hours, provided the lid and sticker are securely in place. Do not remove the lid until ready to mount tissue.
3. Clean equipment with 70% ethanol before tissue sectioning, including water bath, microtome, and forceps.
   - Use gauze instead of a Kimwipe to avoid leaving fibers. Ensure no paraffin or other debris is visible on the stage, specimen holder clamp, flywheel handle, forceps, and other high-touch areas to minimize contamination.
   - Set micron thickness to 3 µm for lymphoid tissues (tonsil, lymph node, spleen, and thymus). Set micron thickness to 5 µm for other tissue types. Refer to *FAQ: Why do we recommend cutting non-lymphoid tissues at 5µm and lymphoid tissues at 3µm?* for more information about the recommended thickness for different tissue types.
4. Fill water bath with **RNase-free MilliQ water** and turn the water bath on.
5. Ensure water bath is set to the optimal temperature. Standard temperature range is 37 - 42°C. **Set the water bath slightly cooler (37 - 40°C)** to accommodate extra floating time while mounting on gel. Temperature should be optimized based on histotechnologist's ability and tissue specifications.
6. Remove prepared HistoCool block or ice bath from the freezer and place on benchtop.
7. Proceed with the *Section Tissue* procedure.

1.2 Section Tissue

This topic describes how to section Formalin-Fixed, Paraffin-Embedded (FFPE) tissue.

1. Place FFPE blocks on ice bath for 10-20 minutes prior to sectioning.

   NOTE
   Do not leave blocks unattended while tissue is in contact with water. Tissue overhydration and swelling may occur which will result in wrinkled sections and tissue loss. If leaving blocks unattended for more than 20 minutes, place blocks cassette side down to prevent tissue overhydration.

2. Carefully place a low-profile blade into the stage clamp and secure with the tightening lever.

   NOTE
   For sections that are bony, brittle, or otherwise difficult to cut, high-profile blades may be used instead.

3. Remove desired block from the ice bath and place in the specimen holder clamp on the miroctome.
4. Use coarse driving wheel to carefully move the block closer to the blade edge. Do not allow the blade to touch the block face.

5. Rotate the flywheel at a slow and steady pace to advance the block in 5 micron increments until the blade begins to lightly trim the block.
6. To achieve a full-face section faster and minimize tissue loss, use the adjustment levers to align the angle of the block face to the blade. Make small adjustments and increase as needed.
7. After making angle adjustments, always use the coarse driving wheel to retreat the block before resuming sectioning. This will prevent chunking the block, which results in tissue loss and damage.
8. Proceed with the *Mount Sections on Gel Pad* procedure.

1.3 Mount Sections on Gel Pad

This topic describes how to mount tissue sections on the gel pad. Also watch Training Video: Tissue Sectioning and Mounting on Gel Pads.

When working with the gel pads, be aware of the following:

- Always use tray handle to move gel pads.
- When re-applying lid or sticker, apply pressure along the seam. Do not press down on the center to avoid applying pressure to tissue sections.
- Do not stack gel pads directly on top of each other. Use the gel pad storage box to store gel pads.
- Fresh gel pads should be stored at 4°C, and can be used until the expiration date printed on the label.

Figure 16 Label on the underside of the gel pad tray handle

1. Briefly place tips of forceps (or other desired transfer tool) in the ice bath prior to floating paraffin sections to prevent paraffin sticking to the forceps.
2. Once a ribbon of sections has been cut, float sections onto the nuclease-free hot water bath using forceps.
3. Allow sections to float until small wrinkles in tissue or paraffin have relaxed and sections are visibly smooth.

   NOTE
   Avoid floating sections for more than 2 minutes to prevent overhydration, which can lead to tissue swelling and separation, and risk delamination once mounted onto the glass slide.
4. Remove the lid from the gel pad tray by holding the handle down with one hand and carefully lifting the lid starting from a corner with the other hand. Do not remove the sticker on the bottom of the gel pad tray.
5. Gently lower the gel pad into the water bath. Align sections to gel pad using forceps or other desired tool.

   NOTE
   Touch only the paraffin to avoid damaging tissue. If excess paraffin is present, trim the section to fit the gel pad using a reverse pinching motion with the curved edge of the forceps.

   Each gel pad can fit 2-3 sections depending on the size of the section.
6. Slowly lift the gel pad out of the water. Use a shallow angle so that sections do not slide off the pad.

   If sections float away easily, use forceps or other tool to guide sections during this step.

7. Carefully tilt the tray vertically to drain excess water from the gel surface, keeping the tissue in place. If needed, you may pin sections to the pad by pressing onto the paraffin with forceps or another tool while simultaneously lifting out of the water bath. Do not touch the tissue during this step.

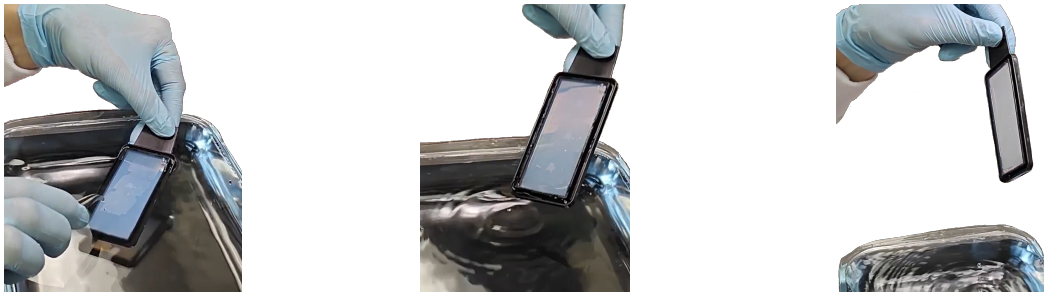

Figure 17 Float tissue sections to the gel pad, lift the pad out of the water bath, and remove excess water adhering to the gel pad.

CRITICAL STEP
Tissue sections should be free of wrinkles, cracks, and bubbles. If tissue sections are not smooth or needs realignment, re-submerge gel pad in the water bath and adjust the section as needed.

8. Label the handles of gel pad trays with sample ID and section number.

If immediately transferring tissue to slides, skip to step 3 in *Select ROI and Transfer Tissue* on page 26.

If you plan to store tissue sections on gel pads prior to proceeding with tissue transfer, proceed with step 9 below.

9. Drain water from underneath the tissue sections by placing the gel pad on the drying rack. Insert gel pad to a slot on the drying rack with sticker tab at the top, facing the user. Do not remove the sticker. Allow to drain for 15 minutes at room temperature.

Figure 18 Place gel pad tray in gel pad drying rack.

Figure 19 Example of a poorly drained and well drained tissue section

After 15 minutes of draining, confirm that there is no water underneath the tissue sections. Refer to Figure 19 for examples of drained tissue sections. If there is water, return to the drying rack until all water is drained from the tissue, checking every 5 minutes. Water on the pad surface or trough is acceptable.

CRITICAL STEP
Do not leave gel pads unsupervised in the drying rack for extended periods of time, as they may begin to shrink once dry.

10. Place gel pad tray on a flat surface and align lid with the tray edges. Press firmly along one short edge, then along both long edges, and finally on the last short edge to ensure a complete seal. Do not press down on the center of the lid.
11. Confirm that the lid sits flush with the tray edges and it is not loose.

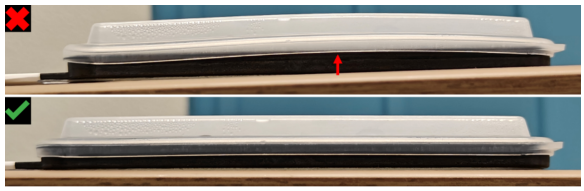

Figure 20 Lid should be flat and sits flush with the tray edges.

SAFE STOPPING POINT
The gel pad can be stored at 2°C to 8°C. Use the mounted tissue sections within 1 month from date of mounting or the expiration date of the gel pad, whichever is sooner.

12. Proceed with the *Select ROI and Transfer Tissue* procedure.

1.4 Select ROI and Transfer Tissue

This topic describes how to punch a Region-of-Interest (ROI) out of the tissue on the gel pad and transfer it to a sample slide. Also watch Training Video: Tissue Transfer.

1. Make sure you have enough tissue punch blades to match the amount of tissue sections you want to punch. Visually inspect blades to make sure there is no dust inside and outside the blades.

   NOTE
   You can mount up to 32 small sections on a 4-lane flow cell or 10 large sections on a 2-lane flow cell.

2. If previously stored, obtain gel pads from storage. Remove the lid from each Gel Pad Tray by holding the handle down and gently lifting the lid from one corner.
3. Remove the sticker by pulling downward from the tab.

4. Place gel pads into the slots of the Gel Pad Drying Rack. If drying multiple gel pads at once, space them evenly by every other slot as shown in Figure 21. This allows for adequate air flow between the pads to ensure even drying.

NOTE
Do not place more than 3 gel pads per drying rack.

Figure 21 Evenly spaced out Gel Pads in the drying rack

5. Allow gel pad to dry for 60 minutes at room temperature. Once the gel pad and tissue sections are dry, remove gel pad from the rack and place it onto a cutting mat.

NOTE
If mounting and transferring tissue on the same day following guidelines outlined in step 8 of *Mount Sections on Gel Pad*, dry gel pad for a total of 75 minutes to account for draining and drying time.

The optimal drying time may vary depending on several factors, including the duration of pad storage after tissue mounting and environmental conditions. Before proceeding with tissue punching, ensure that there is no condensation or water droplets present on either the tissue sections or the gel pad surface.

Example of an under-dried gel:
- Condensation on tissue sections is appreciable
- Water droplets are visible on the gel surface

Example of a well-dried gel:
- No moisture is visible on the pad or tissue sections
- The gel mostly extends to the edges of the tray

Example of an over-dried gel:
- The gel has significantly shrunk around the edges
- The gel pad falls out of the tray encasing

Figure 22 Examples of under-dried, well-dried, and over-dried gel