

Atera Gene Expression – FFPE Tissue Preparation Handbook

Introduction

The Atera Gene Expression workflow is designed to measure mRNA in tissue sections derived from formalin fixed & paraffin embedded (FFPE) tissue samples and requires a slide with intact tissue sections as input. Proper tissue handling, storage, and preservation techniques preserve the morphological quality of tissue sections and integrity of mRNA transcripts.

This FFPE Tissue Preparation Handbook provides guidance on:

- Tissue fixation and embedding
- Best practices for handling tissue samples and slides before and after sectioning
- DV200, DAPI, and Hematoxylin and Eosin (H&E) staining to check tissue quality
- Tissue sectioning and placement
- Tissue deparaffinization and decrosslinking

Additional Guidance

Consult the 10x Genomics Support website for additional resources.

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2. FFPE Tissue Sectioning & Section Placement

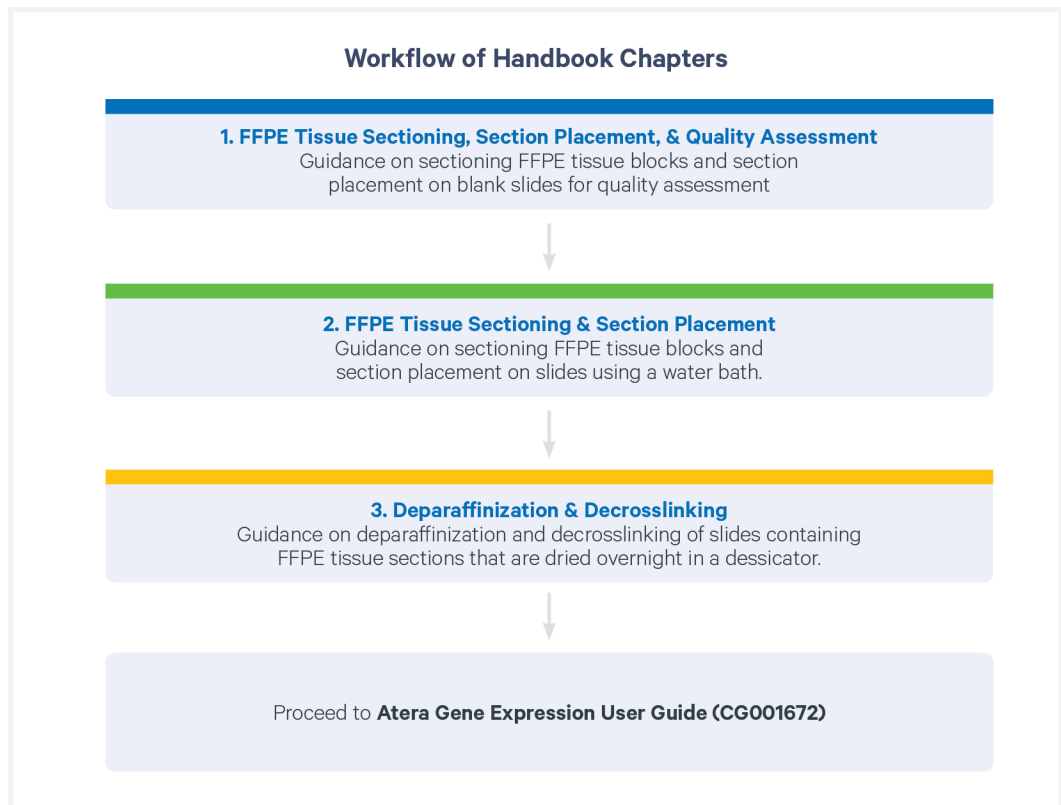
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Handbook Overview and Navigation

Overview

This Handbook describes FFPE tissue and sample preparation for the Atera Gene Expression workflow. Tabs on the right-hand side of the page denote different sections of this Handbook.



Atera Gene Expression Reagent Kits

Atera FFPE Sample Prep Reagents (2 reactions) PN-1001112

Consult SDS for handling and disposal information.

Atera FFPE Sample Prep Reagents (2 reactions*)					
PN-1001112					
Shipped on dry ice; Store at -20°C					
Cap Color	Reagent	PN	Cap Color	Reagent	PN
●	FFPE Tissue Enhancer	2000798	●	Perm Enzyme B	3000553
●	Atera Reagent S	2001913	●	Atera Wash Enhancer	2001914
○	Reducing Agent B**	2000087	○	Atera Hyb Buffer***	2001879
-	empty	-	-	empty	-
-	empty	-	-	empty	-

*If processing 4 slides, a second sample prep reagent kit is needed.



**Reducing Agent B is used in the Atera Gene Expression User Guide. Reagent should be thawed during this sample preparation workflow when indicated; DO NOT discard after thawing.

***Used in the Atera Gene Expression User Guide. Reagent should be thawed during this sample preparation workflow when indicated.

Atera Cassette Kit (2 cassettes) PN-1001153

Atera Cassette Kit	
PN-1001153	
Shipped at ambient temp	
Store at ambient temp	
	#
Atera Cassette Top	2
Atera Cassette Bottom	2
Atera Cassette Insert	4
Atera Cassette Lid	2

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Third-Party Items

Successful execution of this workflow requires third-party reagents, kits, and equipment in addition to those provided by 10x Genomics. All third-party reagents and consumables should be obtained before starting this workflow.

Consult the Atera Gene Expression Protocol Planner (CG001669) for a list of the following third-party items:

- Additional reagents, kits, and equipment
- Tested thermal cyclers
- Tested blank slides

10x Genomics has tested all items listed in the Protocol Planner. Unless otherwise noted, 10x Genomics recommends using these items to ensure optimal assay performance.



Substituting materials may adversely affect assay performance.

Tissue Handling, Fixation, and Embedding Guidance

Tissue Handling

Before fixation and embedding, tissues should be handled according to the following guidance to maximize RNA quality and prevent degradation.

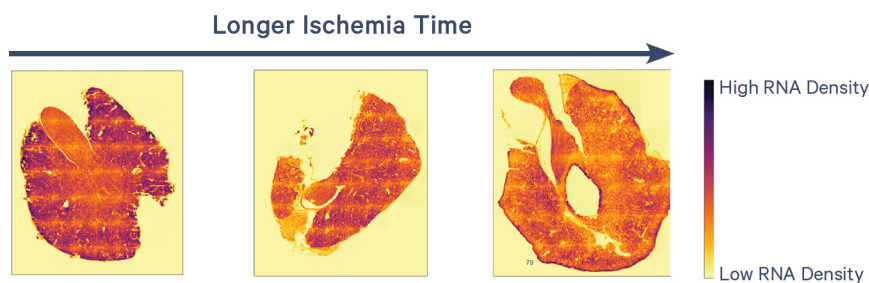
Gentle Handling

Tissues should be handled gently to avoid mechanical stress. Mechanical stress may damage tissue structure and prevent proper fixation. Examples of processes that introduce mechanical stress include ischemia, coagulative necrosis from electrocautery, and hemorrhages from surgical trauma.

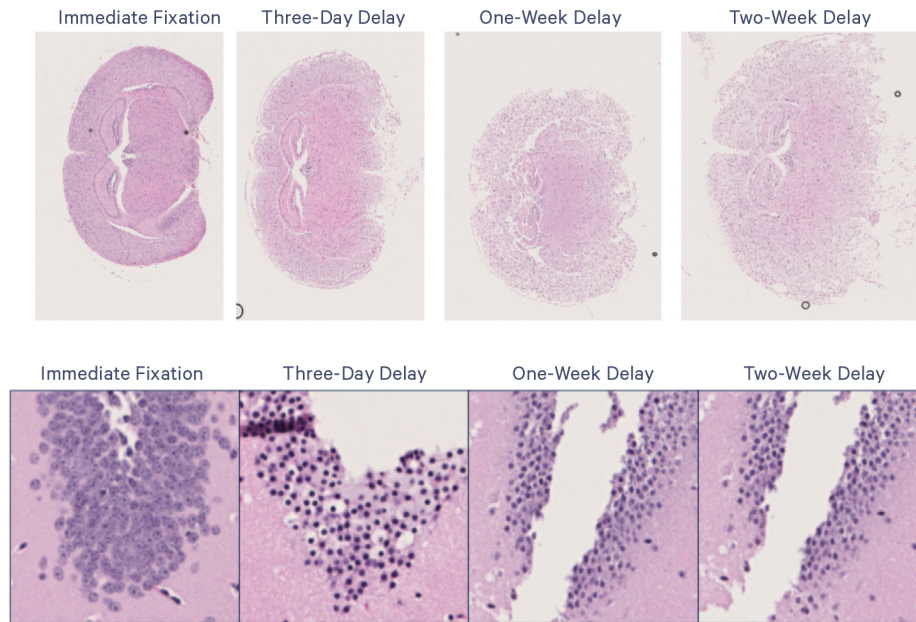
Minimizing Ischemia/Post Mortem Interval (PMI) and Fixation Timing

Prolonged ischemia and PMI can negatively affect tissue quality. If processing delays occur, store tissues in an isotonic solution and avoid exceeding four hours between tissue resection and fixation, though this time may be tissue-dependent. The images below show the negative effect of prolonged ischemia on RNA density.

Images below are for illustrative purposes.



Delayed tissue fixation may lead to autolysis, degrading tissue and negatively impacting results. Tissue samples should be fixed immediately after resection.



Tissue Storage

Store tissues in a cool and moist environment to prevent tissue drying and degradation. Tissues may be kept in an isotonic solution (e.g. RNase-free PBS) if tissue cannot be fixed immediately. Keeping tissues in isotonic solutions is not suitable for long-term storage. Avoid exceeding four hours. 10x Genomics cannot provide specific guidance on isotonic solutions or RNA preservation products.

Tissue Fixation and Embedding Guidance

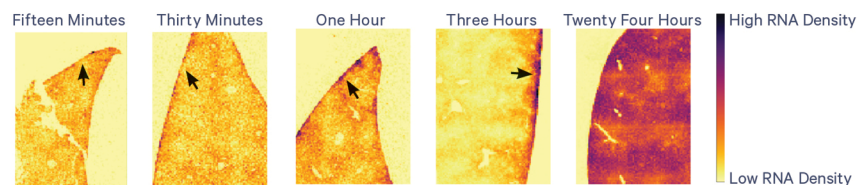
Before sectioning, tissues should be fixed and embedded according to the following guidance to maximize RNA quality and prevent degradation. These steps generate a formalin fixed & paraffin embedded block that is ready for sectioning.

Optimizing Fixation Time

Under or overfixation of tissues can negatively impact assay performance. Fixation time may need optimization for specific organisms, tissue types, and disease states.

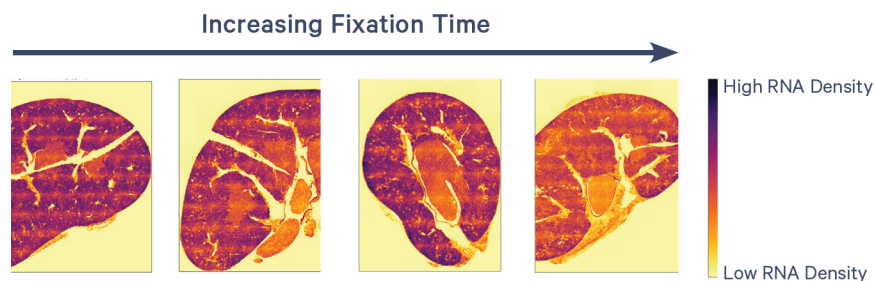
Underfixing tissues may result in continued enzyme activity after tissue resection, which may degrade proteins, nucleic acids, and lipids. RNA may also not be properly preserved in underfixed tissues. Lastly, underfixation can lead to tissue artifacts such as irregular chromatin patterns, overstained cytoplasm (eosin), and common autolysis artifacts such as separation of the epithelium from connective tissue. The images below show the negative impacts of underfixation in mouse liver on RNA density. Note the RNA density gradient that begins at the edge of the tissue.

Images below are for illustrative purposes.



Overfixation may lead to a loss of structural integrity of certain molecular features, lipid oxidation, excessive crosslinking, and decreased antigenicity. Additionally, overfixation may make sectioning more challenging due to tissue hardening. Overfixation can lead to tissue artifacts such as irregularly shaped/smaller cells or hyperchromatic nuclei staining. The images below show structural effects of overfixation in mouse kidney, as well as the negative impact of overfixation on RNA density.

Images below are for illustrative purposes.



To avoid under or overfixation, consider the following:

Tissue Size

The thickness of the tissue significantly affects fixative penetration. Tissue sections should be no larger than a standard tissue cassette and be between 5–10 mm.

Fixative and Fixative to Tissue Ratio

The use of 10% Neutral Buffered Formalin (NBF) at a fixative to tissue ratio of 20:1 is recommended for optimal performance.

Fixation Time and Temperature

Fixation should be carried out at 4°C for ~16–24 h to ensure proper fixative penetration. Fixation time may be tissue-dependent and require optimization. Ensure tissue is completely submerged and introduce light agitation.

Optimizing Embedding

Dehydration

After fixation, tissues are dehydrated in an ethanol series to displace any water remaining in the tissue. Inadequate dehydration may result in poor preservation of tissue structure and damage during sectioning.

Clearing

Tissues are "cleared" using a solvent to remove any remaining ethanol. Inadequate clearing may lead to poor wax infiltration, which can result in hard and brittle blocks that are difficult to section.

Infiltration

After clearing, wax is applied to the tissue and allowed to infiltrate. Inadequate wax infiltration may result in holes or gaps in the final block, which may lead to sectioning difficulties. Typically, wax is melted at 60–62°C before adding to the tissue. It is then allowed to cool to 20°C. Avoid excessive heat and check temperatures regularly during embedding. Use a high-quality wax with a low melting temperature.

Embedding

Finally, tissues are embedded in paraffin. Record orientation of the tissue during paraffin embedding to ensure proper spatiality for downstream region selection and analysis.

Additional Tips and Best Practices

- Regularly replace reagents used during the fixation and embedding process.
- DO NOT store or leave sample in fixative for an extended period of time, which will lead to overfixation.
- Proceed immediately from fixation to dehydration, clearing, and embedding. If proceeding to dehydration immediately is not possible, transfer tissue from fixative to 70% ethanol for short-term storage.
- Store FFPE blocks properly to maintain integrity. Avoid exposure to high temperatures or humidity. For optimal storage, store at 4°C.

Tips & Best Practices

Icons



Tips & Best Practices section includes additional guidance



Signifies critical step requiring accurate execution



Troubleshooting section includes additional guidance



Indicates a video is available for the specified technique

Videos

- Videos describing key steps in the protocol can be found [here](#).
- When a relevant video is available for a protocol step, it will be marked with a video icon.

General Reagent Handling

- Fully thaw reagents at indicated temperatures. Thoroughly mix reagents before use.
- When pipette mixing reagents, unless otherwise specified, set pipette to 75% of total volume.
- Promptly move reagents back to the recommended storage.
- In reagent preparation tables:
 - 1X, 2X, 4X refer to the number of slides/cassettes unless otherwise specified.
 - Volumes may not scale linearly as the number of reactions increases.

Pipette Calibration

Follow manufacturer's calibration and maintenance schedules.

Sample Preparation

Store FFPE tissue blocks at 4°C.

Sectioning Speed

- Sectioning speed is dependent on the desired thickness of the sections and the condition of the tissue. Harder and thicker sections require slow sectioning speed.
- Faster sectioning speed may lead to cracks or tears in the sections or damage to the tissue block.

Section Thickness

Recommended section thickness is 5 μm .

Optional Tissue Trimming & Scoring

FFPE tissue blocks can be trimmed or scored to fit multiple sections onto the Sample Area of the slide.

- **FFPE Tissue Block is smaller than Sample Area:** Paraffin around the embedded tissue in the FFPE tissue block should not be trimmed, and the section will fit the Sample Area.
- **FFPE Tissue Block is larger than Sample Area, but tissue is smaller than Sample Area:** Paraffin around the embedded tissue in the FFPE tissue block can be trimmed for the section to fit the Sample Area.
- **FFPE Tissue Block & Tissue are larger than Sample Area:** Paraffin around the embedded tissue in the FFPE tissue block can be trimmed, and the actual tissue can be scored to generate smaller sections to fit the Sample Area.
- Use a razor blade for trimming and scoring the tissue Block. See [Optional Trimming/Scoring the Block](#) for details. Alternatively, tissue sections may be sectioned and trimmed on a cutting mat.
- Once the tissue has been trimmed and scored, use extra care during sectioning and section handling.

Water Bath Temperature & Section Floating Time

- Optimal water bath temperature and section floating time are critical for tissue section expansion. 42°C is the recommended water bath temperature for most tissues. However, water bath temperature may need optimization based on the tissue type. Optimization should occur before section placement onto the slide that will be used for the assay.

- Determine optimal water bath conditions before tissue placement by practicing section placement on a slide that will not be used for the assay.
- If the tissue is taking too long to expand, turn the water bath temperature up by 1 or 2 degrees and let the section float for longer.
- If the tissue is expanding too quickly and dissociating, turn the water bath temperature down by 1 or 2 degrees and shorten the floating time.

Tissue Section Quality Assessment

Before beginning the assay, quality control assessments are recommended. Consult [FFPE Tissue Sectioning, Section Placement, & Quality Assessment on page 20](#) for more information.

Handling Slides Before and After Sectioning

Before Sectioning

- Always wear gloves when handling slides.
- Hold the slide on the label. DO NOT touch area where tissue sections will be placed on the slide.
- Minimize exposure of the slides to sources of particles and fibers.
- Applying excessive force to the slide may cause the slide to break.

After Sectioning

- Slides containing tissue sections that have been incubated at **42°C** for **3 h** and dried overnight at room temperature in a desiccator can be stored for up to **4 weeks** at room temperature in a desiccator.
- Keep the slide flat on the bench when adding reagents to the Sample Area.
- Ensure that no absorbent surface is in contact with the reagents on the slide during incubation.
- When pipetting reagent onto a slide, avoid generating bubbles.
- When labeling slides, use a xylene-proof marker to prevent the label from coming off during deparaffinization.

Section Placement

- Discard the first couple of sections after facing the block before section placement.
- Place the tissue section within the Sample Area on the slides.
 - For information on determining the Sample Area on a slide, consult the Atera Sample Placement Quick Reference Card (CG001675).

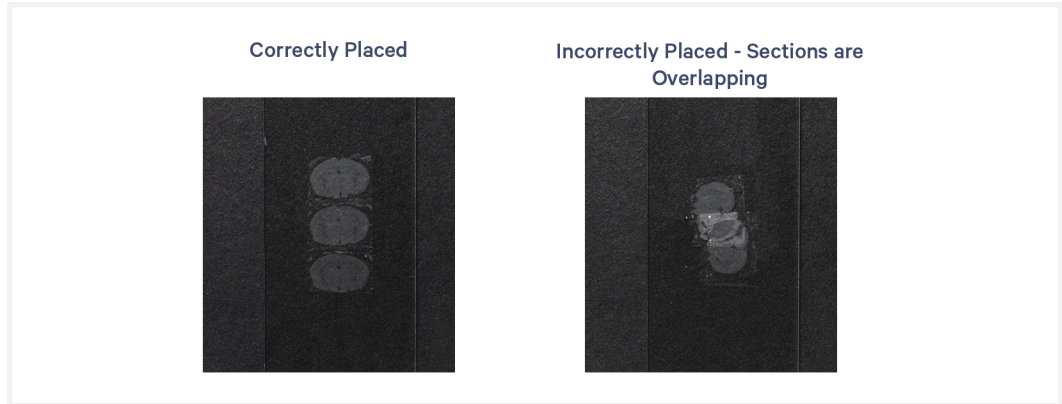


- The section on the slides should be uniform without any cracks, tears, or folds.
- After section placement, sections cannot be repositioned as this would compromise assay performance.
- 10x Genomics recommends using the tissue alignment tool to ensure tissue is placed within the Sample Area.
- Tissue outside the Sample Area will not be analyzed and may be damaged during the instrument run.
- Tissue underneath the gasket during cassette assembly does not require removal. DO NOT attempt to remove this tissue, which may cause tissue detachment.

Placing Multiple Sections

- Place sections at least 1 mm apart from one another to maximize the likelihood each can be selected as its own region during the instrument run. For more information on region selection, see the Atera Instrument User Guide (CG001673).

- The wax surrounding each tissue section should not overlap with the wax or tissue of an adjacent section.
- FFPE sections may be cut on a cutting mat prior to section placement to optimize section size.



Tissue Detachment

- Monitor section adhesion on slides throughout the workflow.
- Tissue detachment during the workflow can negatively impact performance.
- For more information, see [Troubleshooting](#).

Slide Storage

- After section placement, store slides for up to four weeks under the following conditions to maximize RNA integrity:
 - Below 30% relative humidity: use a desiccator to maintain 10–30% humidity. Very low humidity may damage sections.
 - A nonpowered desiccation system (e.g. desiccant in a centrifuge tube) may also be used. Ensure container is airtight and humidity can be monitored.
 - Ensure desiccant is fresh, is refreshed often, and monitored for saturation.
 - Avoid vacuum sealing slides with tissue sections, which can cause cracking.

- Room temperature: slides after section placement are stable at room temperature. Storing at 4°C is also possible and may benefit RNA integrity, but humidity may be more difficult to control.
- Avoid light: exposure to sunlight or indoor light can accelerate sample degradation.

Atera Cassette & Thermocycler Adaptor Handling

- Consult the Atera Cassette Assembly, Disassembly, and Use Quick Reference Card (CG001674) for information on:
 - Cassette assembly and disassembly
 - Atera Cassette Lid application and removal
 - Atera Cassette Insert application and removal
 - Thermocycler Adaptor handling

Slide Incubation

After sectioning, slides with tissue sections must be dried with either a section dryer oven (preferred) or a thermal cycler.

Incubation Using a Section Dryer Oven

- Before using a section dryer oven, ensure slides have been dried with a fan at room temperature to remove trapped water.
- Place slides in a slide drying rack sideways to minimize paraffin wax from entering neighboring tissue.
- Close the oven door when incubating the slide in the oven.

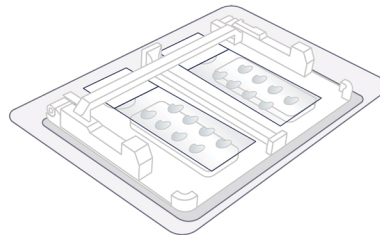
Incubation on a Thermal Cycler

- Position an Atera Thermocycler Adaptor on a thermal cycler that is set at the incubation temperature.
 - Ensure thermal cycler has reached appropriate temperature before starting incubation.
 - Ensure that the Atera Thermocycler Adaptor is in contact with the thermal cycler surface uniformly.
- Place the slide on the Atera Thermocycler Adaptor with the tissue facing up.

- Ensure that the entire bottom surface of the slide is in contact with Atera Thermocycler Adaptor.

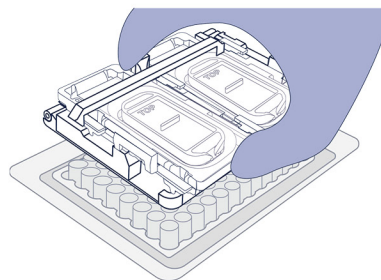
DO NOT close the thermal cycler lid when incubating the slide due to the risk of touching the tissue.

Slides on Thermocycler Adaptor



- When incubating a slide encased in an Atera Cassette, place the assembled unit on the Thermocycler Adaptor with the well facing up.
- The cassette should always be sealed with an Atera Cassette Lid when on the Thermocycler Adaptor unless indicated otherwise.

Atera Cassettes on Thermocycler Adaptor



Hold the adaptor at the center with the thumb and index finger, as illustrated

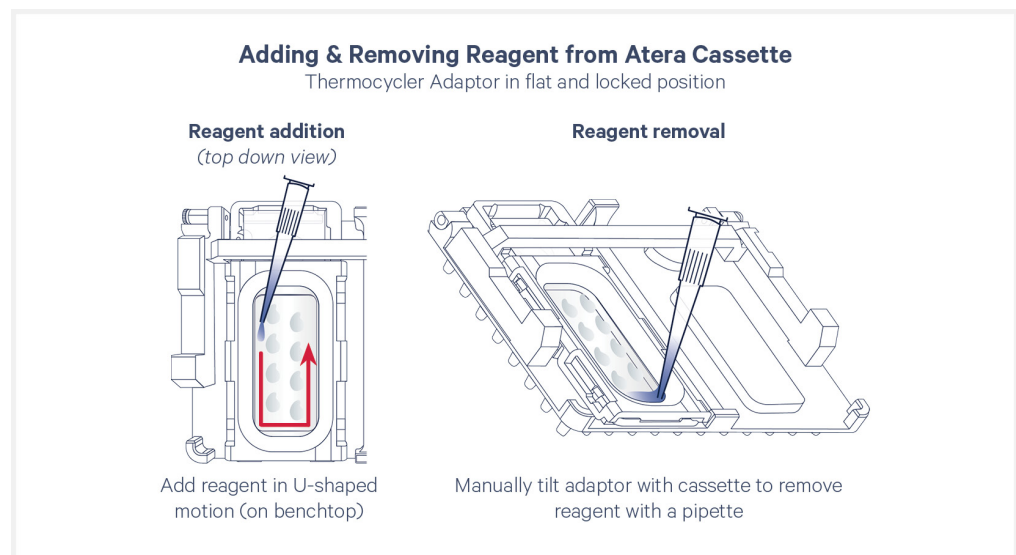
Incubation at Room Temperature

- Place the slide/Atera Cassette on a flat, clean, nonabsorbent work surface.
- Ensure that no absorbent surface is in contact with the reagents on the slide during incubation.

Adding & Removing Reagents

Adding Reagent to Well

- Ensure Atera Cassette is in Thermocycler Adaptor, the Thermocycler Adaptor is in the flat and locked position.
- Dispense reagents in a U-shaped motion along the side of the well unless otherwise specified.
- Gently tap cassette to disperse reagent evenly.
- Pipette slowly to avoid introducing bubbles.
- DO NOT let sample dry out during any wash steps. Add buffer back to the cassette as quickly as possible once buffer is removed.
- DO NOT touch tissue sections.



- If moving the adaptor with cassettes to the thermal cycler, ensure it stays level to avoid unnecessary reagent movement.

Removing Reagent from Well

- Slightly tilt the Thermocycler Adaptor with cassettes while removing reagent.
- Place pipette tip on bottom corner of the well while removing reagent.

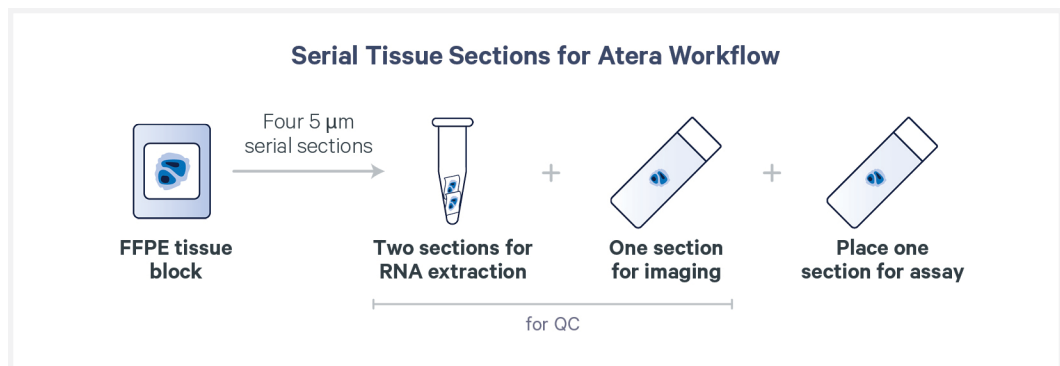
1. FFPE Tissue Sectioning, Section Placement, & Quality Assessment

Overview

This section provides guidance on choosing a quality assessment method, sectioning FFPE tissue blocks, section placement on blank slides using a water bath, and quality assessment.

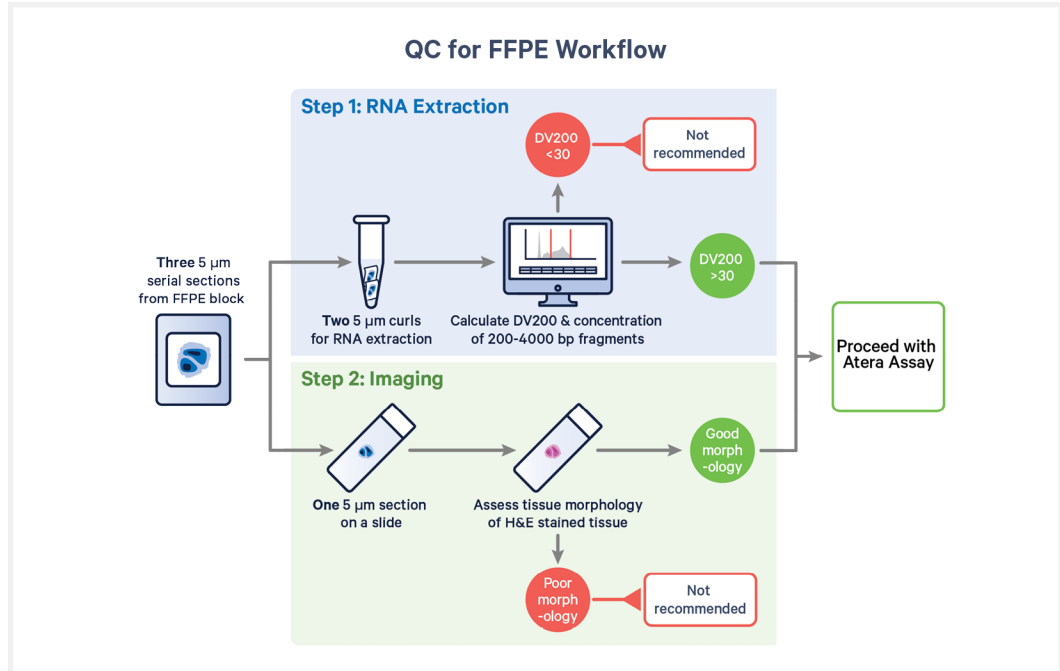


Quality assessment should be performed on a serial section that will NOT be used for the Atera assay.



Choose a Quality Assessment Method

Choose one or more quality assessment methods from the following options. Though quality assessment is optional, 10x Genomics strongly recommends performing DAPI and H&E quality assessment on one or more serial sections before starting the assay. Quality assessment is performed after sections are placed onto blank slides.



DAPI Staining

DAPI is a nuclear stain that enables assessment of nuclei quality, a predictor of assay success. However, good nuclei quality is not an absolute determinant of assay success.

H&E Staining

H&E staining allows for observation of tissue damage and artifacts that may result from poor tissue handling, sectioning, or fixation. These artifacts are correlated with poor assay outcomes. However, identification of artifacts may require histology expertise. Additionally, blood in the H&E image may result in high autofluorescence. High autofluorescence does not necessarily correlate with poor assay outcomes.

DV200

DV200 assesses RNA integrity, where the DV200 score indicates the percentage of RNA fragments over 200 nucleotides in length. A DV200 score of less than 30% is correlated with low transcript density.

Face the Block and Rehydrate

FFPE tissue block is placed in a microtome and cut to face the tissue. The block is rehydrated in an ice bath.

Sectioning

The tissue block is sectioned using a microtome. Sections may be saved at this step for optional DV200 assessment. See [DV200 Assessment on page 30](#) for more information.

Section Placement

Sections are collected in a water bath and are allowed to float on the water surface until they are flat and free from wrinkles and folds. The optimal floating time depends upon the specific tissue type and should be empirically determined when working with new tissue types. Expanded tissue sections are placed on a blank slide for DAPI and H&E staining.

Quality Assessment

Tissue sections on slides are deparaffinized before DAPI and H&E staining. A deparaffinization protocol is provided in the Appendix. Perform DAPI staining according to any preferred protocol. A DAPI protocol is provided in the Appendix. After DAPI staining and imaging, perform H&E staining according to any preferred protocol. An H&E protocol is provided in the Appendix. DAPI and H&E may be performed on the same tissue section or on serial sections.

DV200 analysis: RNA is extracted from tissue sections and analyzed as described in [DV200 Assessment on page 30](#).

1.0 Get Started

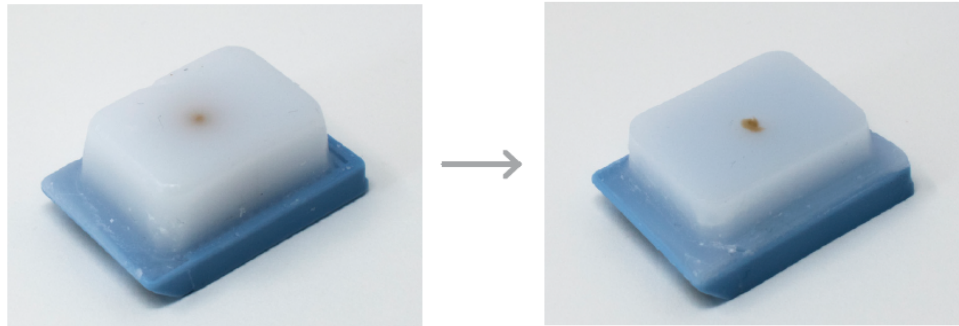
Items	10x PN	Preparation & Handling	Storage
Obtain			
☐ Optional - H&E Reagents	-	Consult Atera Gene Expression Protocol Planner (CG001669) for a list of recommended reagents for H&E staining and imaging	Ambient
☐ Optional - DV200 Reagents	-	Consult Atera Gene Expression Protocol Planner (CG001669) for a list of recommended reagents for DV200 analysis.	Ambient
☐ Optional - DAPI Reagents	-	Consult Atera Gene Expression Protocol Planner (CG001669) for a list of recommended reagents for DAPI staining and imaging	Ambient
☐ Milli-Q Water	-	-	Ambient
☐ Bucket with Ice	-	-	Ambient
☐ Water Bath	-	Equilibrate to optimal temperature.	Ambient
☐ Blank Slides	-		Ambient
☐ Brushes	-		Ambient
☐ Microtome	-		Ambient
☐ Microtome blade	-		Ambient
☐ 100% Ethanol	-		Ambient
☐ Fan (optional, but recommended)	-		Ambient
☐ Section Dryer Oven (optional, but recommended)	-	Equilibrate to desired temperature.	Ambient

1.1 Facing the Block

Before starting, wipe down all the surfaces and work areas with RNaseZap RNase decontaminating solution. If necessary, rewipe the area with 100% ethanol to quickly dry the surface.

- a. Remove tissue blocks from storage.
- b. Set microtome to 15 μm setting.
- c. Cut tissue block at 15 μm until all tissue edges are exposed or until the region of interest is exposed. Block should be at **room temperature** during cutting.

Exposing the Tissue Block

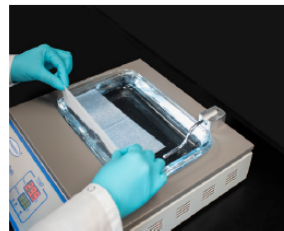


1.2 Sectioning

- a. Fill a water bath with Milli-Q water. Set temperature at **42°C** and ensure bath is free from bubbles & particulates by gliding a laboratory wipe over water surface. Repeat this step between sectioning as needed.

TIPS

42°C is the recommended water bath temperature for most tissues. However, water bath temperature may need optimization based on the tissue type. Determine optimal water bath conditions by practicing section placement on a blank slide. See Tips & Best Practices for guidance on optimizing water bath temperature. To better visualize the tissue sections, a Lighted Tissue Floating Bath with LED illumination can be used instead of standard water bath.

Remove Bubbles

- b. Place a clean pipette tip box on ice.

Ice should be from filtered water. If not available, freeze Milli-Q water ahead of tissue sectioning.

- c. Fill pipette tip box with 4°C Milli-Q or filtered water.
- d. Place blocks in the cold water, ensuring that the tissue/sectioning face is fully submerged.

For optimal hydration, using an ice bath is preferred to an ice block.

- e. Incubate on ice bath for **10–30 min**. Incubation time depends upon tissue type and extent of dehydration. Extent of dehydration depends on processing method and age of tissue block.



Monitor the exposed tissue every 5–10 min during the ice bath incubation. The tissue surface should be smooth and shiny and free of bumps at the end of the incubation. Sectioning compression and shattering are usually due to insufficient hydration. For more information on tissue hydration, see Troubleshooting section.

FFPE Tissue Block in Ice Bath



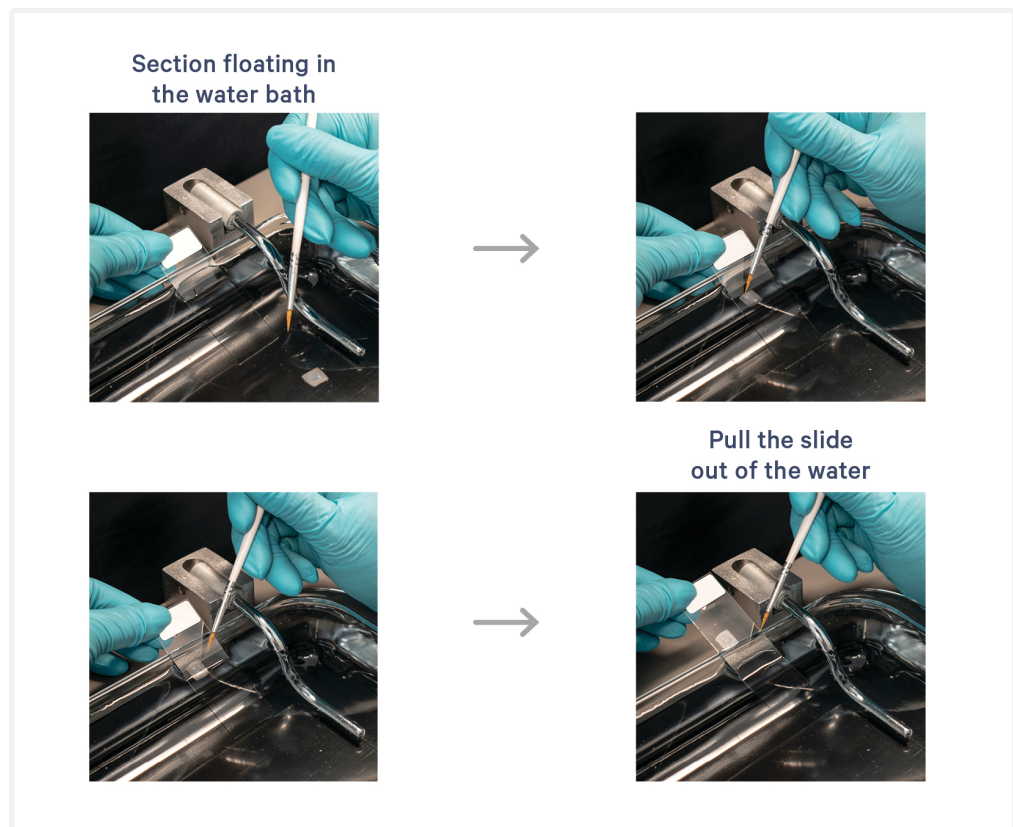
- f. Carefully wipe off the excess oils from a 35X Ultra disposable blade using a laboratory wipe with 100% ethanol. Let ethanol evaporate before proceeding. Always use new blades for sectioning.
- g. Secure blade in the disposable blade holder of the microtome and place knife guard over blade to minimize injury. Ensure that clearance angle is set to 10°. Set microtome to 5 μm (or desired cutting thickness) for sectioning.
- h. After hydration is complete, place tissue block in specimen clamp and align it with the blade. For tissue blocks with exposed tissue, discard the first few sections and start collection on subsequent sections.
- i. To collect sections, place paintbrush tip slightly above and parallel to blade. Lift section by lightly touching the edge with the paintbrush while rotating the wheel handle.
- j. With the help of the brush, pick section up. **Immediately** place it on the water surface of the water bath, making sure that the brush tip goes underneath and away from the section.
- k. If assessing RNA quality via DV200 is desired, collect two 5 μm tissue sections for RNA extraction during FFPE block sectioning.
 - Place the sections inside a **pre-cooled**, RNase-free microcentrifuge tube. Sections may be stored at **-80°C** for **long-term** storage. See [DV200 Assessment on page 30](#) for more information.
- l. Proceed to Section Placement.

1.3 Section Placement on Blank Slides

- a. Allow sections to float for previously determined optimal time.
- b. Insert slide into the water.
- c. Using a paintbrush or probe, maneuver section to slide.

If sections float away from the slide, dip the slide into water bath before section placement.

- d. Pull slide up and out of the water, ensuring there are no air bubbles trapped underneath and set aside.



- e. Repeat for remaining sections, ensuring that the previous sections do not get re-submerged in the water.
- f. Dry tissue sections upright in a drying rack at room temperature until tissue is opaque and no water remains on top of or under the section. A fan may be used to assist in drying.
- g. Place slides in a drying rack and incubate for **10 min** in an oven at **42°C**.

TIPS

See Tips & Best Practices for guidance on [slide incubation](#).

1.4 Deparaffinization & Quality Assessment

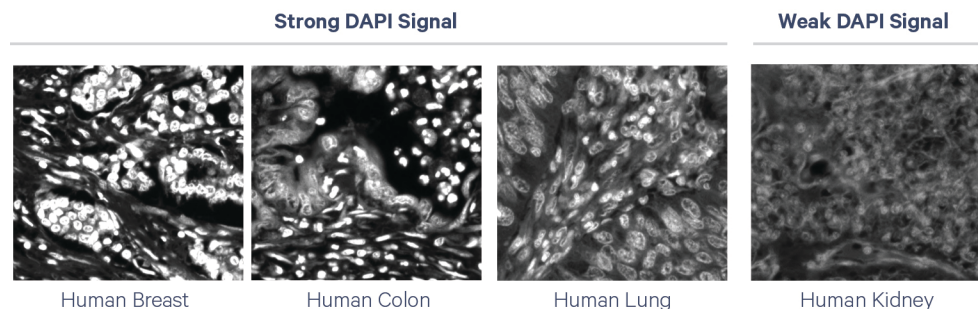
Imaging-based quality assessment is composed of two parts: DAPI staining and H&E staining. Perform DAPI staining according to any preferred protocol. A DAPI protocol is provided in the Appendix. After DAPI staining and imaging, perform H&E staining according to any preferred protocol. An H&E protocol is provided in the Appendix. DAPI and H&E may be performed on the same tissue section or on serial sections.

1.5 Optional DAPI Quality Assessment

Review the DAPI image under the microscope at 20x magnification. Examine several areas within a single DAPI-stained section to ensure assessment is comprehensive. Tissue sections suitable for the assay should have:

- Punctate nuclei that are clearly defined
- DAPI staining that does not appear washed out

Sections with poor DAPI staining are unlikely to generate good data. All samples in the images below were imaged under the same conditions (405 nm channel).

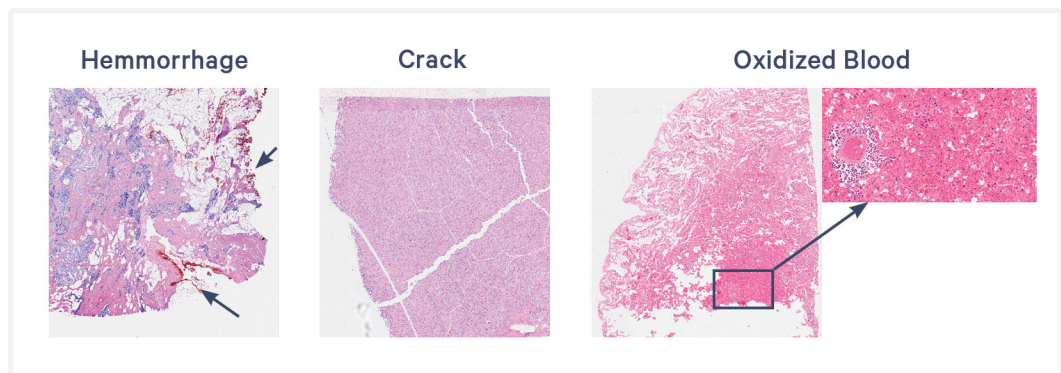


1.6 Optional H&E Tissue Quality Assessment

Review the H&E image thoroughly to assess tissue quality and select areas of interest. If scoring the block or section is necessary, see [Optional Trimming/Scoring the Block](#).

Ensure that the tissue section is free from tissue processing and sectioning artifacts. Tissue processing artifacts may include improper fixation, squeeze/crush artifacts, and hemorrhaging. Examples are shown in the images below. If H&E staining was performed on the same tissue section used for DAPI quality assessment (see previous section), ensure that DAPI staining overlaps well with nuclei seen during H&E imaging.

If imaging reveals satisfactory tissue morphology, proceed with placing sections on slides that will be used for the Atera Gene Expression assay. Otherwise, continue to [DV200 Assessment on the next page](#) to extract RNA for DV200.



1.7 DV200 Assessment

This section provides guidance on RNA extraction from freshly collected tissue sections and determining RNA quality of the tissue block by calculating DV200 of RNA. This requires a measurement of the percentage of total RNA fragments >200 nucleotides (DV200) for RNA quality assessment upstream of assay workflow. Tissues used in the Atera assay should have a DV200 of >30%.

DV200 is a broad measurement of RNA quality. Several factors influence DV200, including:

- Tissue block age, fixation method, and composition
- Region selected for RNA extraction
- Presence of diseased or necrotic regions
- Depth of section
- Miscellaneous upstream tissue handling and processing

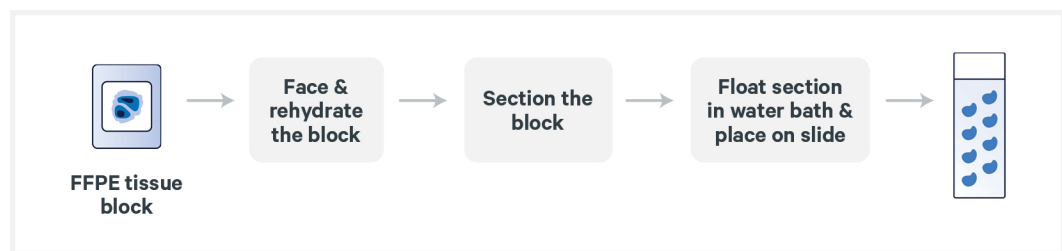
Before starting RNA extraction, apply RNaseZap to a paper towel and wipe work surface, centrifuge rotor, and shafts of the pipettes. Rinse with RNase-free water and then wipe dry. The electrodes of the Bioanalyzer can also be cleaned with RNaseZap by following Agilent decontamination procedure.

- a. Collect two 5 µm tissue sections for RNA extraction during FFPE block sectioning. Discard the first few sections if the block was already exposed.
- b. Place the sections inside a **pre-cooled**, RNase-free microcentrifuge tube. Sections may be stored at **-80°C** for **long-term** storage. For sections stored at **-80°C**, equilibrate to **room temperature** for **5 min** before adding the deparaffinization solution.
- c. Proceed to RNA extraction using RNeasy FFPE Kit and follow manufacturer's instructions.
- d. Using a Nanodrop or Qubit Fluorometer, measure RNA concentration to determine the appropriate dilution for DV200 evaluation using the RNA 6000 Pico Kit or TapeStation High Sensitivity Kit. RNA outside of the recommended concentration may lead to inaccurate DV200 evaluation.
- e. Store purified RNA at **-80°C** for **long-term** storage or immediately proceed to DV200 evaluation using either the Agilent RNA 6000 Pico Kit or TapeStation High Sensitivity Kit. Follow manufacturer's instructions (Agilent) for DV200 evaluation. Ensure gating region is between 200–4,000 bps.

2. FFPE Tissue Sectioning & Section Placement

Overview

This section provides guidance on sectioning FFPE tissue blocks and section placement on slides using a water bath. The resulting slide will be used for the Atera assay. If tissue sections are prone to detachment or contain high lipid content, consider sectioning slides onto a hydrogel slide such as Schott Nexterion Slide H - 3D Hydrogel Coated Slides (Schott North America, PN-1800434) or Tissue Adhesion Slides (10x Genomics, PN-1001228).



Face the Block and Rehydrate

FFPE tissue block is placed in a microtome and cut to face the tissue. Facing is only necessary for new, unused blocks. For tissue blocks with exposed tissue, discard the first few sections and start collection on subsequent sections. The block is rehydrated in an ice bath. Perform as described in step 1.1.

Sectioning

The tissue block with exposed tissue is optionally trimmed or scored to make it compatible in size to the Sample Area on the slides. The tissue block is then sectioned by a microtome to generate appropriately sized sections. Perform as described in step 1.2.

Section Placement

Sections are collected in a water bath and are allowed to float on the water surface until they are flat and free from wrinkles and folds. The optimal floating time depends upon the specific tissue type and should be empirically determined when working with new tissue types. Expanded tissue sections are placed on the slide of choice. Before section placement, practice section placement on a slide that will not be used for the assay.

A slide ID may be given to a slide for later input into the Atera Instrument to assist with sample tracking. IDs must not exceed 33 characters and cannot contain !@#\$%&*)+=.

2.0 Get Started

Items		10x PN	Preparation & Handling	Storage
Obtain				
☐	Tested Blank Slides	-	A list of tested glass slides can be found in the Atera Gene Expression Protocol Planner (CG001669).	Ambient
☐	Water Bath	-	Equilibrate to optimal temperature.	Ambient
☐	Brushes	-		Ambient
☐	Microtome	-		Ambient
☐	Microtome blade	-		Ambient
☐	Fan <i>(optional, but recommended)</i>	-		Ambient
☐	Section Dryer Oven <i>(optional, but recommended)</i>	-	Equilibrate to desired temperature.	Ambient
☐	Marker	-		Ambient

2.1 Section Placement

Optional: Before section placement, trace the Sample Area onto the back of the slide with a marker. Using an unsupported marker may compromise assay performance.

Before section placement, face, rehydrate, and section the block as described in step 1.

- a. Allow sections to float for previously determined optimal time.
- b. Insert slide into the water.
- c. Using the paintbrush or the probe, maneuver the section to the top of the Sample Area on the slide.

If sections float away from the slide, the slide can also be dipped into the water bath before section placement.

- d. Pull slide up and out of the water, ensuring there are no air bubbles trapped underneath and set aside.

If sections require repositioning and the sections have not dried onto the slide, it may be possible to repeat section placement by resubmerging the slide and refloating sections in the water bath. Ensure that minimal water is trapped beneath the section and that the section does not disintegrate from extended floating in the water bath.

- e. Repeat for the remaining sections, ensuring that the previous sections do not get re-submerged in the water.
- f. Dry tissue sections upright in a drying rack at room temperature until tissue is opaque and no water remains on top of or under the section. A fan may be used to assist in drying.

If no fan is used, slides may require extended drying time **overnight** at **room temperature** to ensure no water remains under the section or until dry (inspect visually, do not touch tissue). This method may be helpful for tissues prone to detachment. Ensure that after sectioning these sections, slides are flicked to remove excess water prior to drying.

- g. Place the slides in a slide drying rack and incubate for **3 h** at **42°C** in an oven or thermal cycler.



See Tips & Best Practices for guidance on [slide incubation](#).

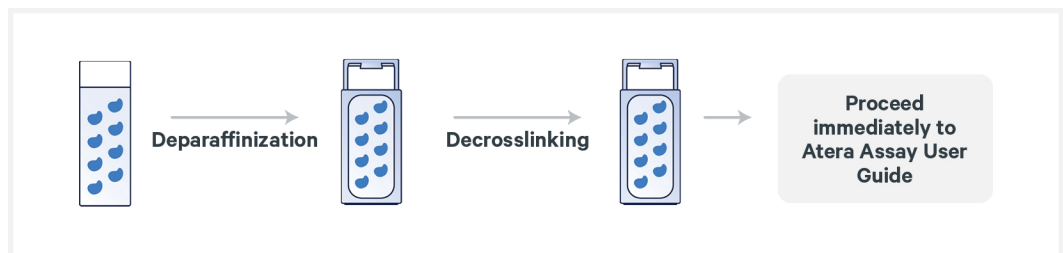


- h. After drying, proceed to [Deparaffinization & Decrosslinking](#) or store the slide containing dry tissue sections at room temperature in a desiccator for up to **4 weeks**.

3. Deparaffinization & Decrosslinking

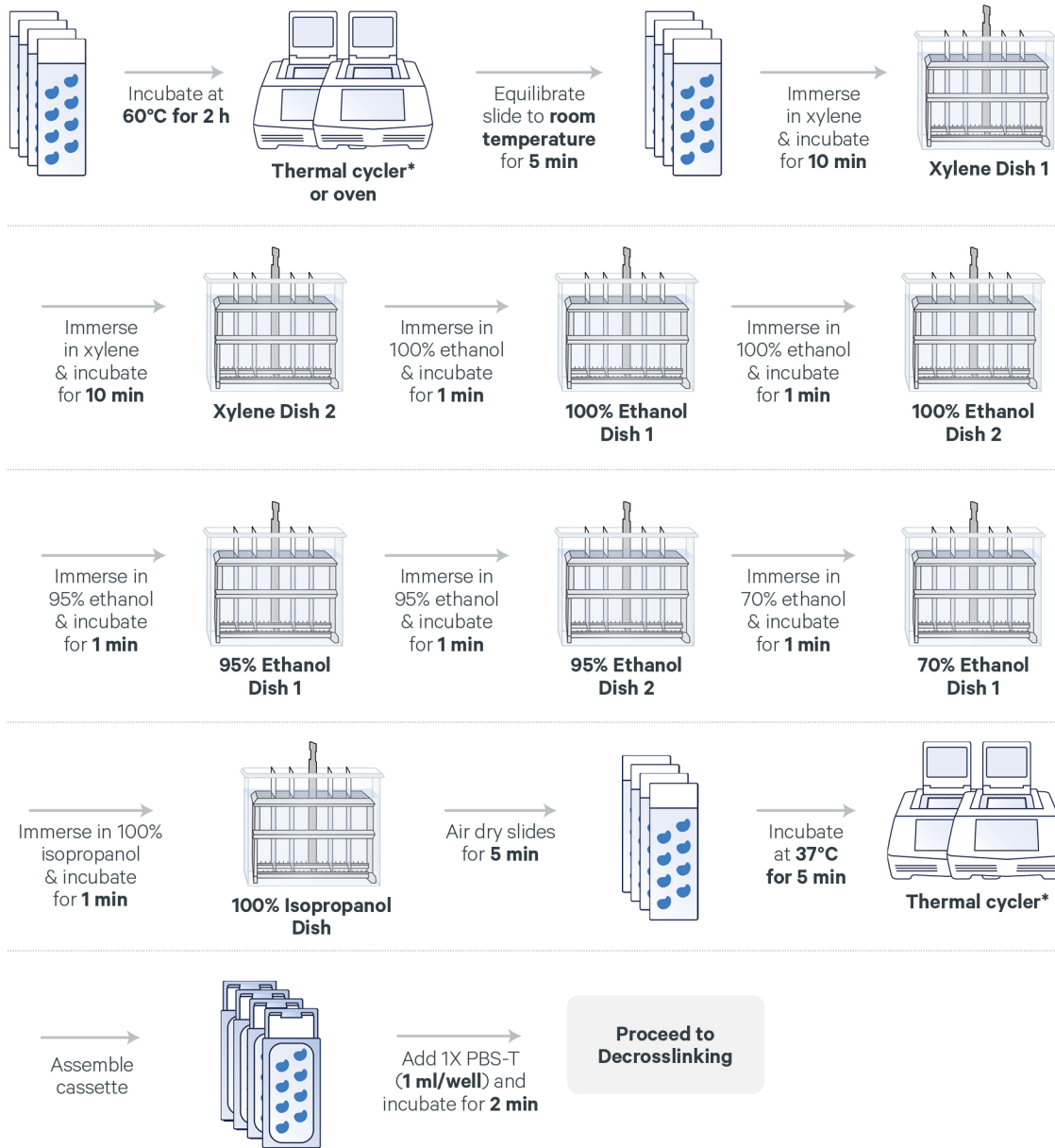
Overview

This chapter provides guidance on deparaffinization and decrosslinking of slides containing FFPE tissue sections that are dried overnight in a desiccator. After paraffin is removed from the tissue sections during the deparaffinization process, tissues are rehydrated and decrosslinked to release the sequestered RNA from the tissue. Following deparaffinization and decrosslinking, proceed immediately to the Atera Gene Expression User Guide (CG001672).

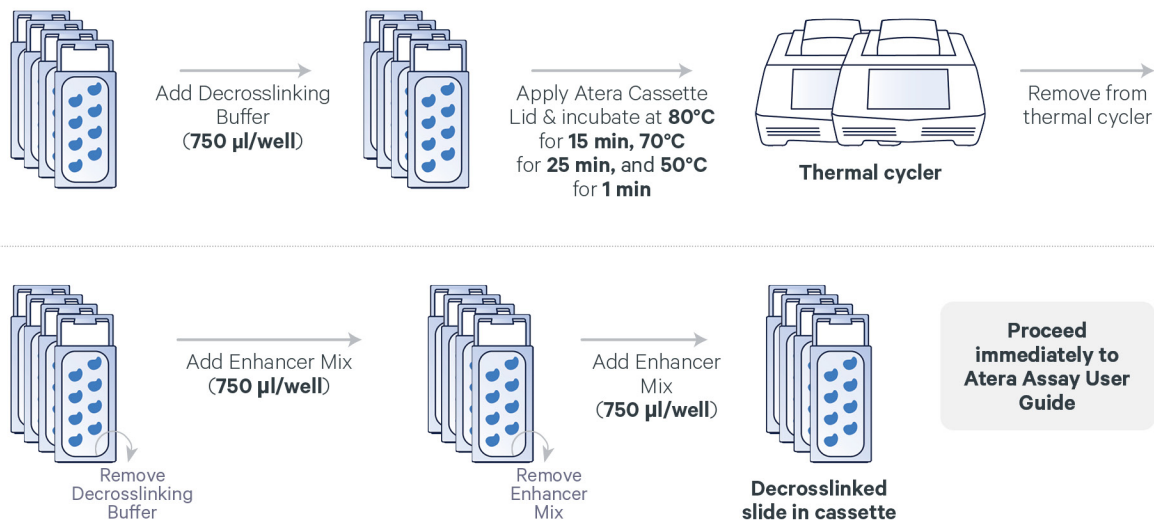


Protocol Overview

Deparaffinization (~3 h)



*Keep the thermal cycler lid open

Decrosslinking (~3 h)

3.0 Get Started – Deparaffinization & Decrosslinking

Each 10x Genomics reagent tube is good for 2 slides.

Deparaffinization Items		10x PN	Preparation & Handling	Storage
Obtain				
☐	Xylene	-	-	Ambient
☐	100% Ethanol	-	Prepare ethanol dilutions using Nuclease-free water.	Ambient
☐	100% Isopropanol	-	-	Ambient
☐	Nuclease-free Water	-	-	Ambient
☐	10X or 1X PBS	-	Preparing 1X PBS is only required if using 10X stock.	Ambient
☐	Forceps	-	Optional, if desired for slide handling	Ambient
☐	Slide Staining Rack	-	Only if using staining dishes. Slide rack should fit into chosen slide staining dish.	Ambient
☐	Coplin jars/Staining dishes	-	-	Ambient
☐	Slides with FFPE tissue sections	-	Prepared according to previous chapters in this Handbook.	Room temperature in a desiccator
☐	Atera Thermocycler Adaptor	-	See Tips & Best Practices.	Ambient
☐	Atera Cassette	-	See Tips & Best Practices. DO NOT open Atera Cassette Inserts. They are not used in this Handbook.	Ambient
☐	Atera Cassette Lids	-	See Tips & Best Practices. DO NOT open Atera Cassette Inserts. They are not used in this Handbook.	Ambient

			10x PN	Preparation & Handling	Storage
Equilibrate to room temperature					
☐	●	FFPE Tissue Enhancer	2000798	For optimal efficiency, thaw FFPE Tissue Enhancer one hour into Step 3.2b. Thaw in a thermomixer for 30 min at 37°C, 300 rpm with shaking. Cool to room temperature for 5 min. Vortex for 30 sec and centrifuge briefly. Alternatively, thaw in a water bath for 30 min at 37°C. Cool to room temperature for 5 min. Vortex for 30 sec and centrifuge briefly.	-20°C
☐	●	Perm Enzyme B	3000553	For optimal efficiency, thaw Perm Enzyme B one hour into Step 3.2b. Maintain at room temperature. Pipette mix, centrifuge briefly. DO NOT vortex.	-20°C
☐	●	Atera Reagent S	2001913	For optimal efficiency, thaw Atera Reagent S one hour into Step 3.2b. Maintain at room temperature. Vortex for 30 sec and centrifuge briefly.	-20°C
☐		Atera WTA, Select, and Custom Panel Probes	-	DO NOT thaw until called for during Decrosslinking. During Decrosslinking, thaw appropriate probe panels at room temperature based on experimental needs. Pipette mix 10x and centrifuge briefly. DO NOT vortex. Consult the 10x Genomics Support Website for the most updated list of available panels and part numbers, as well as information on panel compatibility.	-20°C
☐	○	Reducing Agent B	2000087	DO NOT thaw until called for during Decrosslinking. During Decrosslinking, thaw at room temperature. Vortex and centrifuge briefly.	-20°C
☐	○	Atera Hyb Buffer	2001879	DO NOT thaw until called for during Decrosslinking. During Decrosslinking, thaw at room temperature. Vortex and centrifuge briefly.	-20°C
Thaw at 65°C					
☐	●	Atera Wash Enhancer	2001914	Only thaw Atera Wash Enhancer when indicated in the protocol. If only processing one slide, aliquot half of the Atera Wash Enhancer and return the rest to storage. Use a tube that is just large enough to contain the leftover Atera Wash Enhancer to reduce the amount of air within the tube and prevent oxidation.	-20°C

		10x PN	Preparation & Handling	Storage
Obtain				
☐	Nuclease-free Water	-	-	Ambient
☐	10X or 1X PBS	-	Preparing 1X PBS is only required if using 10X stock.	Ambient
☐	10% Tween-20	-	-	Ambient
☐	Urea	-	-	Ambient
☐	Ice Bucket with Ice	-	-	Ambient
☐	Temperature-controlled Instrument		Appropriate instruments include bead baths, heat blocks, and thermomixers. Instrument should be able to be set to 37°C or 65°C. Instrument should be able to accommodate 1.5-ml or 2-ml centrifuge tubes.	

3.1 Preparation – Buffers

Prepare all buffers fresh according to the tables below. Some overage is included in all reagent preparations. The preparations below assume the user is processing four slides using a slide rack in a slide staining dish. If fewer slides are being processed, coplin jars or smaller staining dishes may be used. If using smaller containers, ensure reagents are scaled appropriately. Volumes should be sufficient to submerge the Sample Area on the slide, but not the slide label.

Ethanol preparations are only required if not using purchased ethanol that is already at the desired concentration.

Use xylene-resistant dishes and gloves during workflow. Prepare fresh reagents after every 20 slides or every week (whichever comes first).

- a. Label eight total staining dishes. Prepare ethanol dilutions using nuclease-free water. Preparation instructions are in subsequent steps.

For Deparaffinization	
Items	Preparation & Handling
Xylene	Label two staining dishes as Xylene Dish 1 and 2.
100% Ethanol	Label two staining dishes as 100% Ethanol Dish 1 and 2.
95% Ethanol	Label two staining dishes as 95% Ethanol Dish 1 and 2.
70% Ethanol	Label one staining dish as 70% Ethanol Dish.
100% Isopropanol	Label one staining dish as Isopropanol Dish.

- b. Prepare 1X PBS. Add reagents in the order listed. Invert gently to mix. Maintain at room temperature. This volume of 1X PBS is sufficient for washes in all subsequent steps of this Handbook, as well as the Atera Gene Expression User Guide (CG001672).

1X PBS					
Items	Stock	Final	1X (ml)	2X (ml)	4X (ml)
Nuclease-free water	-	-	24.6	46.8	92.7
RNase-free PBS, pH 7.4	10X	1X	2.7	5.2	10.3
Total	-	-	27.3	52.0	103.0

- c. Using 1X PBS from step 1.1a, prepare PBS-Tween (PBS-T). Add reagents in the order listed. Invert gently to mix. Maintain at room temperature. This volume of PBS-T is sufficient for washes in all subsequent steps of this Handbook, as well as the Atera Gene Expression User Guide (CG001672).

PBS-T					
Items	Stock	Final	1X (ml)	2X (ml)	4X (ml)
1X PBS	1X	~1X	24.9	49.7	99.5
Tween-20	10%	0.05%	0.1	0.3	0.5
Total	-	-	25.0	50.0	100.0

- d. Dispense **250 ml** Xylene into Xylene Dish 1 and 2.
- e. Dispense **250 ml** 100% ethanol into 100% Ethanol Dish 1 and 2.
- f. Dispense **250 ml** 100% isopropanol into Isopropanol Dish.
- g. Prepare 95% Ethanol. Add reagents in the order listed. Invert gently to mix. Fill each 95% ethanol staining dish with 250 ml 95% ethanol.

95% Ethanol			
Items	Stock	Final	2 Staining Dishes (ml)
Ethanol	100%	95%	475
Nuclease-free water	-	-	25
Total	-	-	500

- h. Prepare 70% Ethanol. Add reagents in the order listed. Invert gently to mix. Fill 70% ethanol staining dish with 250 ml 70% ethanol.

70% Ethanol			
Items	Stock	Final	1 Staining Dish (ml)
Ethanol	100%	70%	175
Nuclease-free water	-	-	75
Total	-	-	250

3.2 Deparaffinization

Deparaffinization steps should be performed in a fume hood due to the hazardous nature of xylene. Xylene jars should be covered at all times to prevent evaporation.

- a. Retrieve slides with tissue sections from desiccator after overnight drying.

Remove any marker annotations using a lint-free laboratory wipe and 70% ethanol or isopropanol.

- b. Place slides in a Section Dryer Oven and incubate uncovered at **60°C** for **2 h**.

Keep oven lid closed during incubation.

Alternatively, place a Thermocycler Adaptor on a thermal cycler set at **60°C**. Place slide on the Thermocycler Adaptor with tissue side facing up and incubate at **60°C** for **2 h**.



Keep the adaptor level to prevent slides from falling off.



DO NOT close thermal cycler lid. At one hour into incubation, thaw FFPE Tissue Enhancer as described in [Get Started – Deparaffinization & Decrosslinking](#) on page 38.

- c. Prepare Diluted Perm Enzyme B shortly before the next step. Add reagents in the order listed (some overage is included). Pipette mix thoroughly. Maintain at room temperature.

Diluted Perm Enzyme B					
Items	Stock	Final	1X (μl)	2X (μl)	4X (μl)
1X PBS	1X	-	21	42	84
Perm Enzyme B (Maintain at room temperature. Pipette mix, centrifuge briefly. DO NOT vortex).	-	-	4	8	16
Total	-	-	25	50	100

- d. Prepare Decrosslinking Buffer shortly before xylene immersions (step 3.2g). Add reagents in the order listed (some overage is included). Pipette mix thoroughly. Maintain at room temperature.

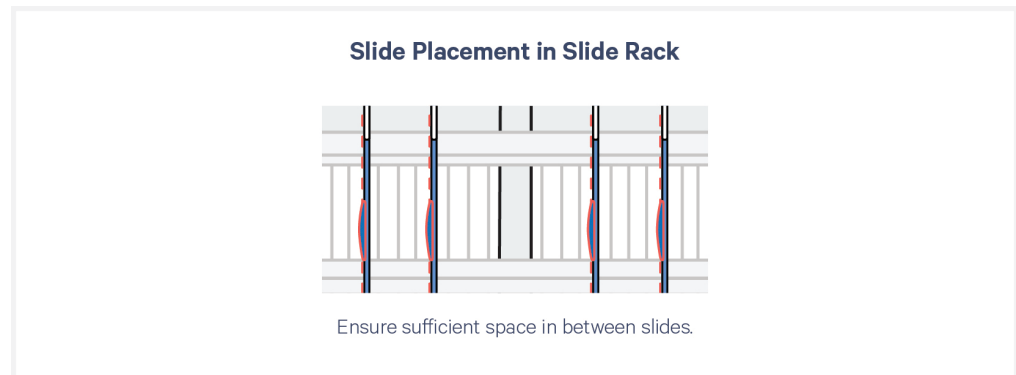
Decrosslinking Buffer					
Items	Stock	Final	1X (μl)	2X (μl)	4X (μl)
FFPE Tissue Enhancer	-	-	698.4	1,536.5	3,073.0
Urea	8 M	0.25 M	23.4	51.5	103.0
Atera Reagent S	-	-	15.0	33.0	66.0
10% Tween-20	10%	0.05%	3.8	8.4	16.7
Diluted Perm Enzyme B	-	-	9.4	20.7	41.4
Total	-	-	750.0	1,650.1	3,300.1

- e. Remove slides from oven or thermal cycler and allow to cool down to **room temperature** for **5 min**.



Optional: photograph the slide against a black background during 5 min cool down at room temperature. This image can be used for comparison purposes to identify tissue detachment downstream in the workflow. Work quickly as this is not a safe stopping point. See [Troubleshooting](#) for more details.

- f. Add slides to slide staining rack. Leave space between each slide.



- g. Gently immerse slides in Xylene Dish 1. Secure staining dish lid to prevent xylene loss.

When immersing slides in xylene, ensure that the tissue sections are completely submerged. DO NOT submerge any written labels.

- h. Incubate for **10 min.**

- i. Gently immerse slides in Xylene Dish 2 and incubate for **10 min.**

- j. Gently immerse slides in 100% Ethanol Dish 1 for **1 min.**

When immersing slides in ethanol, ensure that the tissue sections are completely submerged. DO NOT submerge any written labels.

- k. Gently immerse slides in 100% Ethanol Dish 2 for **1 min.**

- l. Gently immerse slides in 95% Ethanol Dish 1 for **1 min.**

- m. Gently immerse slides in 95% Ethanol Dish 2 for **1 min.**

- n. Gently immerse slides in 70% Ethanol Dish for **1 min.**

- o. Gently immerse slides in 100% Isopropanol Dish for **1 min.**

- p. Remove slides from 100% Isopropanol Dish and place in a slide drying rack.

- q. Air dry slides for **5 min.**

- r. While slides are drying, place Thermocycler Adaptors on thermal cyclers set to **37°C.**

- s. Place slides onto Thermocycler Adaptors and incubate at **37°C** for **5 min.** DO NOT close the lid.



Keep the adaptor level to prevent slides from falling off.

3.3 Cassette Assembly

Only one Atera Cassette per slide is provided for the workflow, from this step through instrument loading.

- a. Retrieve slides.
- b. Wipe back of slides with a lint-free laboratory wipe.
- c. Place slides into cassettes.



DO NOT open Atera Cassette Inserts.

Consult the Atera Cassette Assembly, Disassembly, and Use Quick Reference Card (CG001674) for guidance on assembly. Work quickly to avoid drying out of tissue sections.

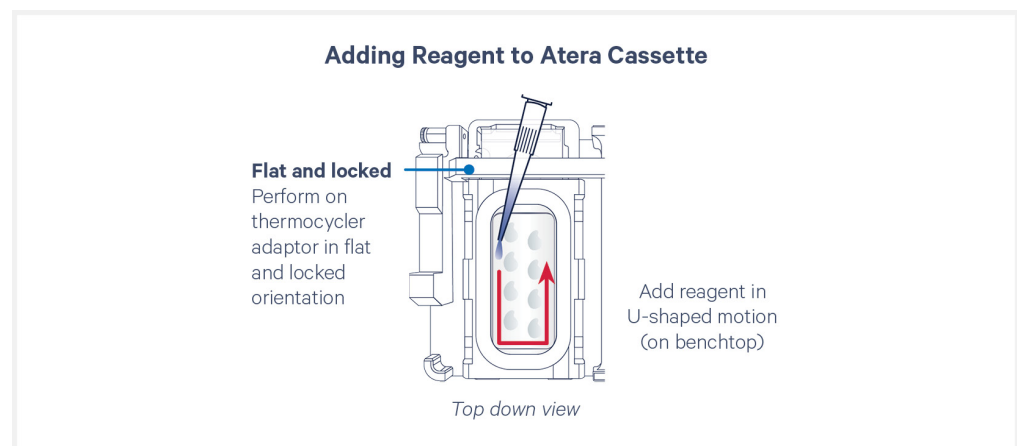


Photograph slides in the cassette without the gasket from above against a black background. This image can be used for comparison purposes to help identify potential issues encountered during the workflow. Work quickly as this is not a safe stopping point.

- d. Retrieve Thermocycler Adaptors for thermal cycler.
- e. Place cassettes on Thermocycler Adaptors and lock arms over cassettes.

***DO NOT** apply cassette lids.*

- f. Add **1 ml** 1X PBS-T to each well and incubate for **2 min.**



Observe the Atera Cassette for any leaks after PBS-T application. If leaks occur, slide may need to be reassembled into the cassette. Consult the Atera Cassette Assembly, Disassembly, and Use Quick Reference Card (CG001674) for cassette assembly and removal instructions.

- g. Proceed **immediately** to decrosslinking.

3.4 Decrosslinking

Reagent addition and removal should be done carefully. Do not touch tissue sections or introduce bubbles.

- a. Prepare two thermal cyclers with the following incubation protocol and start the program.

Only one thermal cycler is needed if running two samples or less.

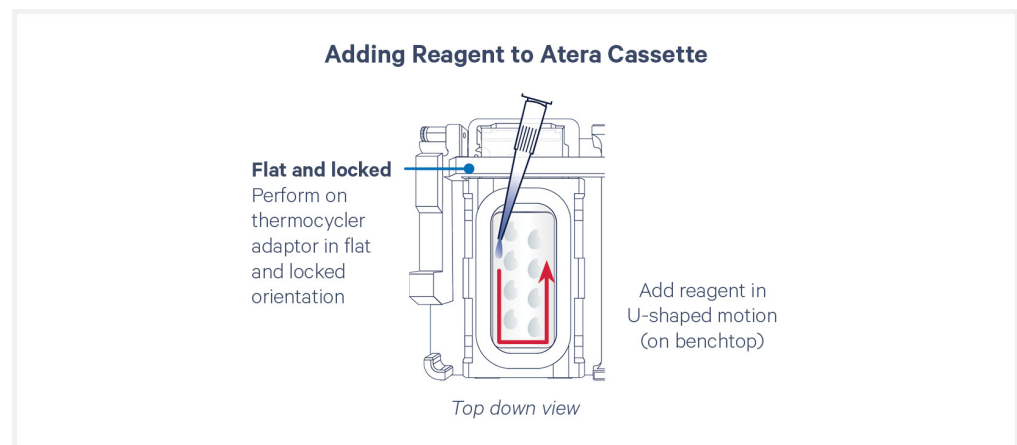


Ensure lid is at 70°C.

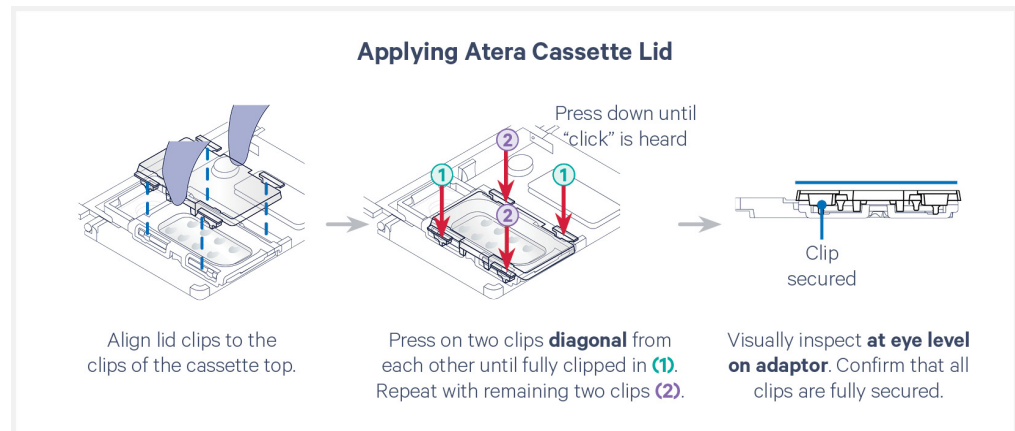
Lid Temperature	Reaction Volume	Run Time
70°C	100µL	-
Step	Temperature	Time
Hold	22°C	Hold
Decrosslinking Step 1	80°C	00:15:00
Decrosslinking Step 2	70°C	00:25:00
Decrosslinking Step 3	50°C	00:01:00
Hold	50°C	Hold*

DO NOT leave thermal cycler on 50°C Hold for more than **5 min.*

- b. Remove 1X PBS-T from cassettes.
- c. Add **750 µl** Decrosslinking Buffer to each well, without introducing bubbles. Tap cassette gently to ensure uniform coverage.



- d. Open adaptor arms and apply **new** Atera Cassette Lids on Atera Cassettes.



- e. Inspect cassettes at eye level and ensure all clips are secured. After inspection, lock adaptor arms over cassettes.



- f. Immediately place Thermocycler Adaptors with cassettes onto thermal cyclers and close lid. Tighten lid until resistance is felt.

- g. Skip Hold step and initiate Decrosslinking.



Begin thawing Atera Hyb Buffer, Reducing Agent B, and relevant probes during decrosslinking. These are needed for Probe Hybridization in Atera Gene Expression User Guide (CG001672). After the completion of Decrosslinking, the Probe Hybridization step in the User Guide must be started immediately.

- h. Incubate Atera Wash Enhancer at **65°C** for **10 min** or until solution is reconstituted to a dark green solution with no visible particulates. If using a temperature-controlled instrument with a 1.5 ml capacity, additional thawing time or mixing may be required.

DO NOT exceed 20 min at **65°C**. Store at room temperature until use. Gently invert before use.

Atera Wash Enhancer color change over time is normal.

Atera Wash Enhancer Before and After Incubation



- i. Prepare Enhancer Mix according to the table below, adding reagents in the order listed (some overage is included). Vortex. Maintain at room temperature.

Enhancer Mix	Stock	Final	1X (μl)	2X (μl)	4X (μl)
Atera Wash Enhancer	-	-	150	330	660
1X PBS-T	-	-	1,350	2,970	5,940
Total	-	-	1,500	3,300	6,600

- j. Remove Thermocycler Adaptors with cassettes from thermal cyclers and place on a flat, clean work surface.



Enhancer Mix washes should occur close to the thermal cycler to prevent the cassette from cooling down after decrosslinking.



DO NOT proceed with assay if slide is cracked or broken. Cracked or broken slides will result in assay failure.



DO NOT let slide dry out.

- k. Remove the Atera Cassette Lids and using a pipette, remove all Decrosslinking Buffer from each well. Save lids for later steps.



- l. Remove any condensation from the lids with a lint-free laboratory wipe.



See [Lid Condensation on page 57](#) for examples of acceptable and unacceptable condensation.

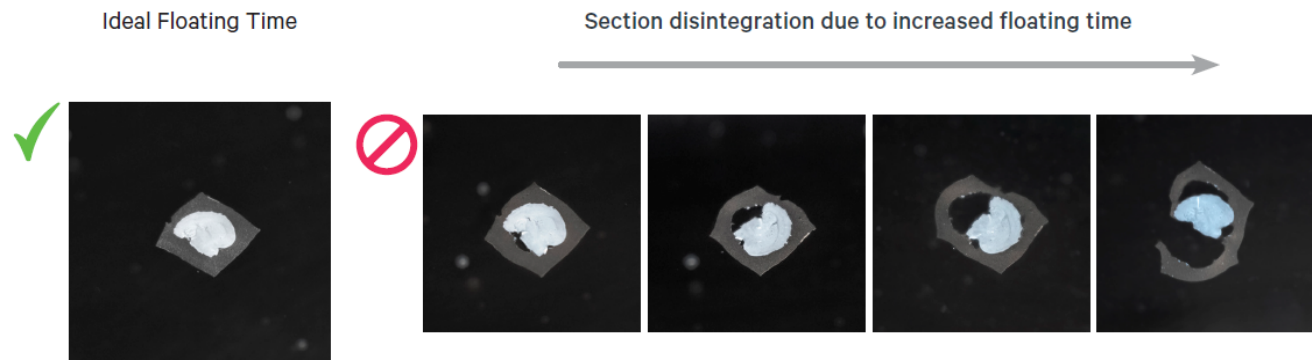
Two Enhancer Mix Washes:

Enhancer Mix washes DO NOT require sealing of the cassette with a lid.

- m. **Wash 1:** Add **750 µl** Enhancer Mix to each well. **Immediately** remove all Enhancer Mix.
- n. **Wash 2:** Add **750 µl** Enhancer Mix to each well.
- o. Proceed to the relevant Atera Gene Expression User Guide (CG001672). *This is not a safe stopping point.*

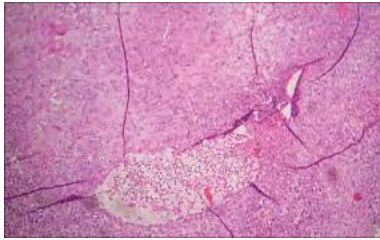
Troubleshooting

Ideal Floating Time Determination



Common Issues when Processing FFPE Tissue Blocks

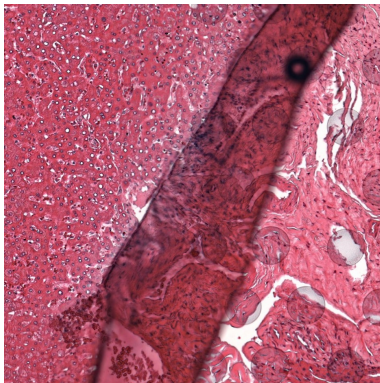
Section Phenotype	Tissue Characteristic	Guidance
Tissue is very dry	High Connective Tissue or High Cell Density Tissue	Soak block for an extended period of time. Re-soak tissue block every few sections.
Shattering, rapid expansion	High Adipose Tissue	If tissue is shattering, ensure tissue block is very cold by soaking the block in ice water for 10 min, re-soaking every few sections. If tissue expansion is occurring, lower water bath temperature to 37–39°C.
Rapid expansion	Low Cell Density Tissue	Lower water bath temperature to 37–39°C.

Common Artifacts that Cause Detachment or Misleading Spatial Data**Wrinkles****Causes**

- Section compression (due to warm block or dull blade) during sectioning leads to wrinkle formation. These wrinkles become permanent when placed in the water bath.
- Accumulated wax or static electricity on microtome parts also contribute to section compression.

Troubleshooting

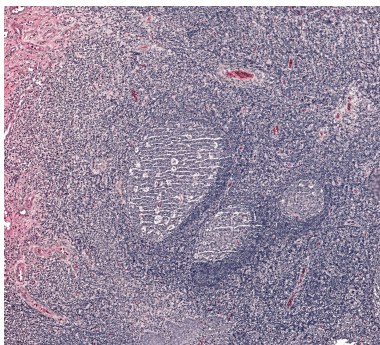
- Ensure that the block is well hydrated.
- Adjust temperature down and increase float time.
- Gently and gradually lay FFPE sections onto water bath surface, lengthwise.
- Utilize a new blade.
- Ensure microtome is cleaned with 100% ethanol to minimize static and section compression (bunching on blade).

Folds**Causes**

- Mostly happens when placing the section on the water bath especially when the section is uneven.
- If the fold is at the edge this most likely can happen during sectioning or mounting on the slide.

Troubleshooting

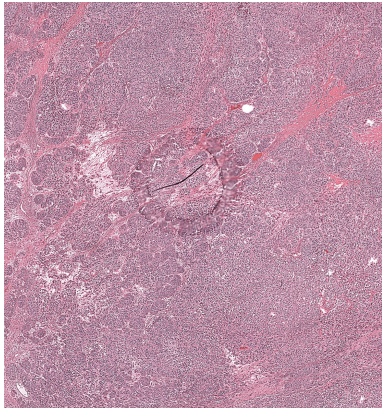
- Gently and gradually lay FFPE ribbons or sections onto water bath surface, lengthwise.
- If sections curl during sectioning, gently flatten them with a brush before floating.

Venetian Blinds or Shatter**Causes**

- Parallel lines in the section mostly appear due to dry tissue because of under-hydration of the block in the ice bath.
- Less likely due to dull blade or loose parts of the microtome.

Troubleshooting

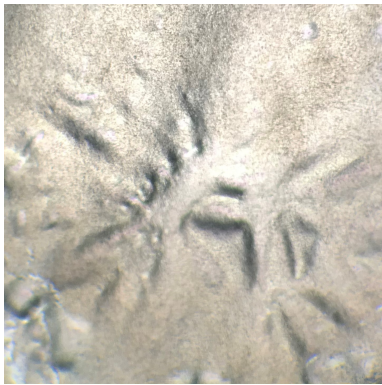
- Increase incubation time of the block in ice bath.
- Tighten down components of microtome and make sure the blade is at a correct angle.

Common Artifacts that Cause Detachment or Misleading Spatial Data**Air Bubbles****Causes**

- Air bubbles from the bottom of the water bath can rise and stick under the section.

Troubleshooting

- Using a brush, gently remove the bubbles from the bottom of the water bath before floating the sections.

Waves

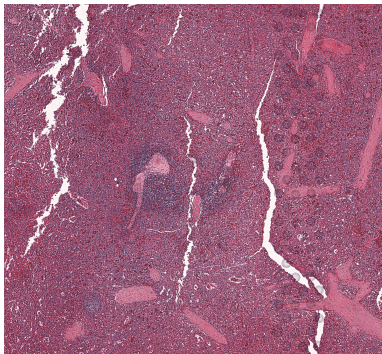
May be block-specific and easily observed under the microscope right after the section is picked up from the water bath. Minor waves can disappear after longer flotation in the water bath. Larger waves can create wrinkles or folds that are permanent after drying of the section.

Causes

- Tissue incompletely infiltrated with wax absorbs water faster during hydration step. When sections from such blocks are floated, the parts that absorbed enough water will have difficulty flattening and become wavy.

Troubleshooting

- Chill the block on a cold block or on ice avoiding contact with water.
- Submerge the block for 5–15 min in the ice bath for gentle hydration.
- Increase flotation times and/or temperature of the water bath.

Cracks**Causes**

- Dry and over-processed tissue can crack during sectioning.
- The cracks that are created before tissue embedding will be filled with wax when section is observed under the microscope after sectioning and wax will be washed away after deparaffinization or H&E staining.

Troubleshooting

- Prolonged hydration on the ice bath will most likely reduce the cracks.
- There is no solution for cracks created before tissue was embedded in wax.

Common Artifacts that Cause Detachment or Misleading Spatial Data**Sweating****Causes**

- Inadequate dehydration or under-processing of the tissue causes parts of the section to attract water and create this blistering effect. Such sections can disintegrate in the water bath.
- The blisters consist of:
 - Xylene or xylene substitutes if the cause is under-processing and insufficient removal of xylene or
 - Water droplets if the cause is inadequate dehydration.

Troubleshooting

- Be cautious about how long the block is kept in ice bath. Long incubation time in ice bath can impact section quality and thus should be avoided.
- Long flotation time in the water bath should be avoided.

Water Retention**Causes**

- Sections from tissues that are under-processed or excessively hydrated before sectioning may retain water under the section mounted on a slide.
- Water will accumulate under section and cause uneven drying ultimately leading to detachment.

Troubleshooting

- Gently flicking the slide will help to get rid of the water. This is one of the most overlooked causes of detachment.

Disintegrating/Exploding Section**Causes**

- Sections from tissues that are under-processed and not sufficiently infiltrated with wax can rapidly absorb water and explode or gradually disintegrate in the water bath.
- Tissues that are not sufficiently dehydrated can show similar phenotypes.
- Floating at high temperatures (42–50°C) can exacerbate the disintegration.

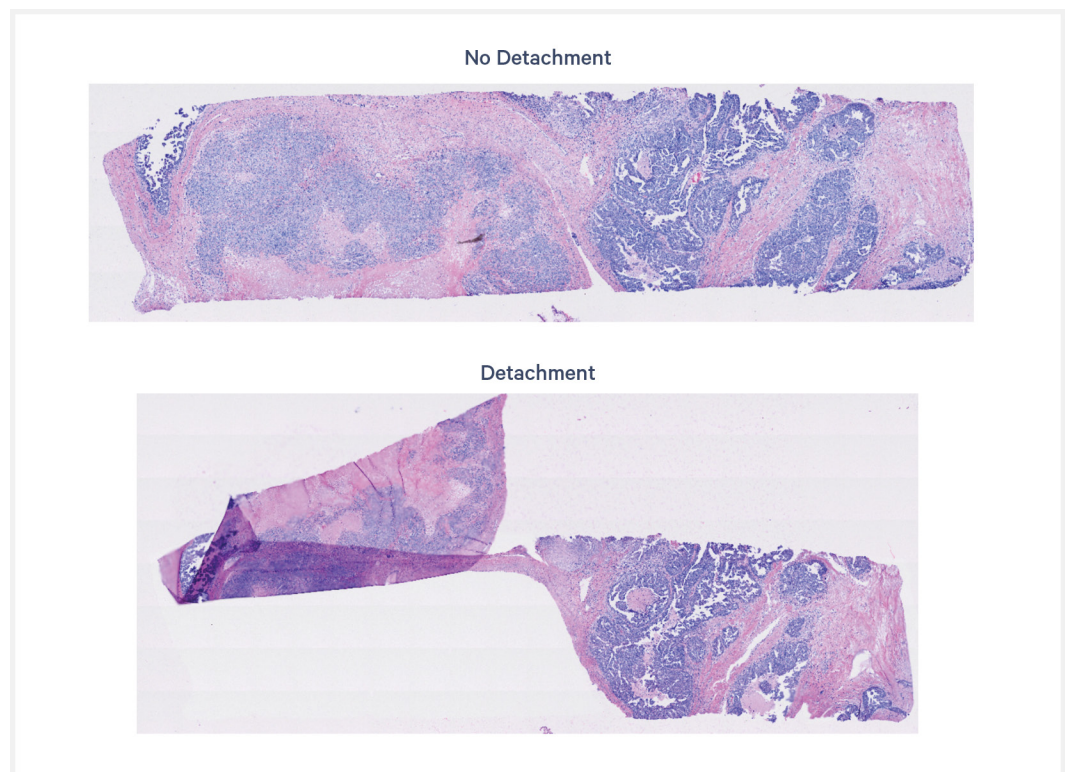
Troubleshooting

- Chilling of the block should be mostly performed on ice or cold block (30–60 min).
- Exposure to water in ice bath during chilling should be kept to a minimum (5–10 min).
- Flotation water bath temperature should be lowered to 38–40°C.

Tissue Detachment

Tissue detachment may result in a lack of decodable data in the region where detachment occurred. If the tissue has folded on itself, this may also cause elevated signal in the overlapping areas. To keep track of possible detachment, photograph the slides after decrosslinking.

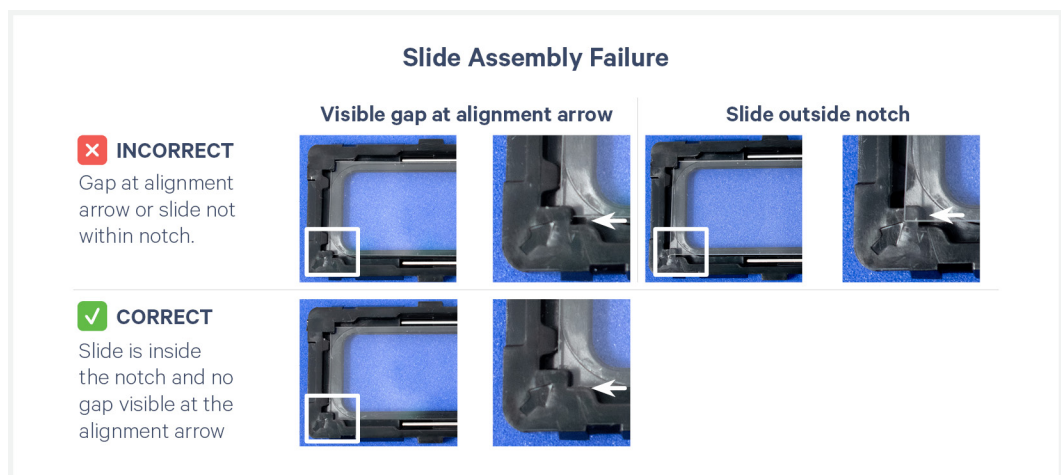
If detachment is observed during the workflow (>30% tissue detachment), 10x Genomics does not recommend proceeding with the workflow. To mitigate detachment, place difficult tissues (low quality samples, TMAs, etc.) on Schott Nexterion Slide H - 3D Hydrogel Coated Slides (Schott North America, PN-1800434) or Tissue Adhesion Slides (10x Genomics, PN-1001228).



Cassette Assembly Failure

Incorrect assembly of the Atera cassettes can negatively impact assay performance. Inspect the slide carefully to ensure it is seated fully within the cassette before assembly. Example scenarios that may indicate incorrect cassette assembly are described below:

- The cassette does not click shut or appears domed after assembly.
- Slide is not aligned to the long edge of the cassette.



Lid Condensation

Some condensation on the Atera Cassette Lid is normal. However, severe condensation may impact performance.

If severe condensation occurs:

- Do not proceed with the assay.
- Take photos of the cassette while on the thermal cycler showing condensation.
- Take a photo of the thermal cycler program.
- Remove the cassette from the Thermocycler Adaptors and take photos of the lid clips.
- Contact support@10xgenomics.com.



Appendix

Deparaffinization & Quality Assessment

Tissue slides should be deparaffinized before quality assessment (DAPI and/or H&E staining).

Deparaffinization Items		10x PN	Preparation & Handling	Storage
Obtain				
☐	Xylene	-	-	Ambient
☐	100% Ethanol	-	Prepare ethanol dilutions using Nuclease-free water.	Ambient
☐	100% Isopropanol	-	-	Ambient
☐	Nuclease-free Water	-	-	Ambient
☐	Forceps	-	-	Ambient
☐	Slide Rack	-	-	Ambient
☐	Coplin jars/Staining dishes	-	-	Ambient

Prepare eight total coplin jars for deparaffinization steps. Prepare ethanol dilutions using nuclease-free water.

For Deparaffinization				
Items		Preparation and Handling		
☐	Xylene	Label two coplin jars as Xylene Jar 1 and 2. Fill each with 50 ml xylene.		Ambient
☐	100% Ethanol	Label two coplin jars as 100% Ethanol Jar 1 and 2. Fill each with 50 ml 100% ethanol.		Ambient
☐	95% Ethanol	Label two coplin jars as 95% Ethanol Jar 1 and 2. Fill each with 50 ml 95% ethanol.		Ambient
☐	70% Ethanol	Label one coplin jar as 70% Ethanol Jar. Fill with 50 ml 70% ethanol.		Ambient
☐	100% Isopropanol	Label one coplin jar as 100% Isopropanol. Fill with 50 ml 100% isopropanol.		Ambient
☐	Coplin jars/Staining dishes	-		Ambient

DAPI Staining Items		10x PN	Preparation & Handling	Storage																									
Obtain																													
☐	DAPI Solution	-	Use any preferred DAPI stock solution. Dilute DAPI stock solution to 5 µg/ml in PBS-T to generate DAPI solution. 1.5 ml DAPI solution is enough for 2 slides.	Ambient																									
☐	10X PBS	-	Prepare 1X PBS using nuclease-free water.	Ambient																									
☐	Slide Mailer	-	-	Ambient																									
☐	Coverslips	-	-	Ambient																									
☐	Glycerol Mounting Medium	-	The dilution below is not necessary if stock glycerol is already at 85%. Invert to mix. Briefly centrifuge to remove bubbles.																										
			<table><tr><td>Glycerol Mounting Medium <i>1 slide = 1 Tissue Slide</i></td><td>Stock</td><td>Final</td><td>1 Slide (µl)</td><td>2 Slides +15% (µl)</td><td>4 Slides +15% (µl)</td></tr><tr><td>Glycerol</td><td>100%</td><td>85%</td><td>127.5</td><td>293.3</td><td>586.5</td></tr><tr><td>Nuclease-free Water</td><td>-</td><td>-</td><td>22.5</td><td>51.7</td><td>103.5</td></tr><tr><td>Total</td><td>-</td><td>-</td><td>150.0</td><td>345.0</td><td>690.0</td></tr></table>			Glycerol Mounting Medium <i>1 slide = 1 Tissue Slide</i>	Stock	Final	1 Slide (µl)	2 Slides +15% (µl)	4 Slides +15% (µl)	Glycerol	100%	85%	127.5	293.3	586.5	Nuclease-free Water	-	-	22.5	51.7	103.5	Total	-	-	150.0	345.0	690.0
Glycerol Mounting Medium <i>1 slide = 1 Tissue Slide</i>	Stock	Final	1 Slide (µl)	2 Slides +15% (µl)	4 Slides +15% (µl)																								
Glycerol	100%	85%	127.5	293.3	586.5																								
Nuclease-free Water	-	-	22.5	51.7	103.5																								
Total	-	-	150.0	345.0	690.0																								

Deparaffinization

Deparaffinization steps should be performed in a fume hood due to the hazardous nature of xylene. Xylene jars should be covered at all times to prevent evaporation.

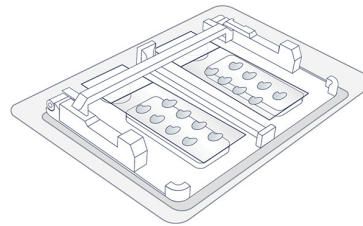
- Retrieve the slide with tissue sections from the desiccator after overnight drying.

Remove any marker annotations on slide using a lint-free laboratory wipe and 100% ethanol.

- Place slide in a Section Dryer Oven and incubate uncovered at **60°C** for **2 h**.
Keep the oven lid closed during incubation.

Alternatively, place a Thermocycler Adaptor on a thermal cycler set at **60°C**. Place slide on the Thermocycler Adaptor with the tissue side facing up and incubate at **60°C** for **2 h**.

Slides on Thermocycler Adaptor



DO NOT close the thermal cycler lid.

- c. Remove from the oven or thermal cycler and allow the slide to cool down to **room temperature** for **5 min.**

- d. Gently immerse slide in the Xylene Jar 1. Secure the jar cap to prevent xylene loss.

Hold slide at label with forceps for xylene immersion steps. When immersing slides in xylene, ensure that the tissue sections are completely submerged.

- e. Incubate for **10 min.**
- f. Gently immerse slide in the Xylene Jar 2 and incubate for **10 min.**
- g. Gently immerse slide in the 100% Ethanol Jar 1 for **1 min.**

Hold slide at label with forceps for ethanol immersion steps. When immersing slides in ethanol, ensure that the tissue sections are completely submerged.

- h. Gently immerse slide in the 100% Ethanol Jar 2 for **1 min.**
- i. Gently immerse slide in the 95% Ethanol Jar 1 for **1 min.**
- j. Gently immerse slide in the 95% Ethanol Jar 2 for **1 min.**
- k. Gently immerse slide in the 70% Ethanol Jar for **1 min.**
- l. Gently immerse slide in the 100% Isopropanol Jar for **1 min.**
- m. Remove slide from 100% Isopropanol Jar and place in a slide drying rack.
- n. Air dry slide for **5 min.**
- o. Proceed to DAPI staining.

DAPI Staining

DAPI staining is optional. If only performing H&E staining, skip to [H&E Preparation on page 63.](#)

- a. Place tissue slide on a flat, clean, nonabsorbent work surface.
- b. Add **750 µl** DAPI solution per slide to uniformly cover all tissue sections.
- c. Incubate **1 min** in the dark at **room temperature**.
- d. Discard reagent by holding slide at an angle with the bottom edge in contact with a laboratory wipe.
- e. Add **1 ml** 1X PBS to uniformly cover all tissue sections.
- f. Incubate **1 min** in the dark at **room temperature**.
- g. Discard reagent by holding slide at an angle with the bottom edge in contact with a laboratory wipe.
- h. Repeat steps e-g twice more for a total of three washes.
- i. Wipe excess liquid from the front and back of the slide without touching the tissue section.

Coverslipping

Before mounting the coverslip, ensure that the slide is dry. Moisture on the surface of the slide may result in faulty mounting. Wipe away any residual droplets with a lint-free laboratory wipe.

This coverslipping protocol requires Glycerol Mounting Medium to facilitate removal of the coverslip. A more permanent mounting media is used after H&E staining.

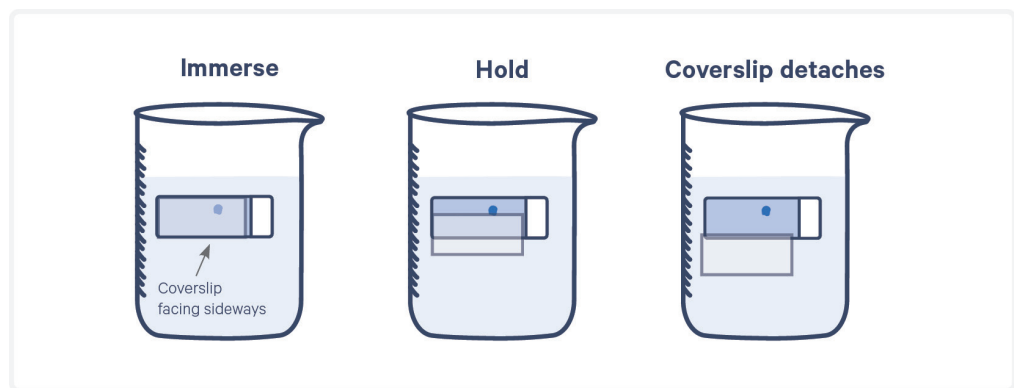
- a. Place slide on a flat, clean, nonabsorbent work surface.
- b. Using a **wide-bore** pipette tip, add **150–200 µl** Glycerol Mounting Medium to uniformly cover all tissue sections on the slide.
- c. Apply the coverslip at an angle on one end of the slide. Slowly lower the coverslip, without introducing bubbles. Allow mounting media to spread and settle.
- d. If needed, remove any large excess of mounting media by carefully wicking away from the edge of the coverslip with a lint-free laboratory wipe. Be careful not to move coverslip and disturb the tissue.
- e. Once coverslipping is complete, **immediately** proceed with imaging. See [DAPI Quality Assessment](#) for information on evaluating DAPI staining. Underexposure may result in inaccurate assessment of DAPI quality. Begin by overexposing samples, then reducing exposure until the resolution improves. If possible, process a control section in parallel from a block that has yielded good data.

Coverslip Removal

- a. Dispense **800 ml** Milli-Q water in a beaker. Up to 10 slides may be processed using this beaker.
- b. Immerse slides sideways in the beaker containing **800 ml** water with the coverslipped surface fully sideways to prevent the coverslip from dragging across the tissue.
- c. Hold slides in water until the coverslip slowly separates away from the slide.



To avoid tissue section damage or detachment, DO NOT move the slide up and down, shake forcibly, or manually move the coverslip.



- d. Gently immerse slides 30x in water to ensure all Mounting Medium is removed.
- e. Wipe the back of the slide with a laboratory wipe. Place on a flat, clean, nonabsorbent work surface and air dry for **5 min**.
- f. Proceed with H&E Staining.

H&E Preparation



Prepare buffers in appropriate sized conical tube or bottle and transfer carefully to corresponding coplin jar, if applicable. Filter Hematoxylin & Eosin solutions using filter paper before starting H&E Staining protocol.

Items	Preparation & Handling
☐ Milli-Q or UltraPure Water	Label seven 1000-ml beakers as Water Beakers 1–7. Dispense 800 ml of water into each beaker. Dispensed volumes in each beaker can be used for 2 slides. Alternatively, use 50-ml centrifuge tubes instead of beakers (1 tissue slide per 50-ml centrifuge tube).
☐ Alcoholic Eosin	Prepare 30 ml in a 50-ml centrifuge tube for each tissue slide.
☐ Gill II Hematoxylin	Prepare 30 ml in a 50-ml centrifuge tube for each tissue slide.
☐ Bluing Buffer	-
☐ 95% Ethanol	Label two coplin jars as 95% Ethanol Jar 1 and 2. Fill each with 50 ml 95% ethanol.
☐ 100% Ethanol	Label two coplin jars as 100% Ethanol Jar 1 and 2. Fill each with 50 ml 100% ethanol.
☐ Xylene	Label two coplin jars as Xylene Jar 1 and 2. Fill each with 50 ml xylene.
☐ Coverslips	-
☐ Mounting Medium	-

H&E Staining

H&E Staining steps should be performed in a fume hood due to the hazardous nature of xylene. Xylene jars should be covered at all times to prevent evaporation.

The protocol below is an example H&E protocol. Use any desired H&E protocol.

- a. Gently immerse slides in Hematoxylin in separate 50-ml centrifuge tubes.
- b. Incubate **1 min** at **room temperature**.
- c. Discard reagent by holding slides at an angle with the bottom edge in contact with a lint-free laboratory wipe.
- d. Immerse slides 10x in Water Beaker 1.
- e. Immerse slides 10x in Water Beaker 2.
- f. Immerse slides 10x in Water Beaker 3.
- g. Wipe excess liquid from back of slides with a lint-free laboratory wipe without touching the tissue section.

- h.** Place slide on a flat, clean, nonabsorbent work surface. Some droplets may remain.
- i.** Add **1 ml** Bluing Buffer per slide to uniformly cover all tissue sections.
- j.** Incubate **1 min** at **room temperature**.
- k.** Discard reagent by holding slides at an angle with the bottom edge in contact with a lint-free laboratory wipe.
- l.** Immerse slides 10x in Water Beaker 4.
- m.** Immerse slides 10x in Water Beaker 5.
- n.** Wipe excess liquid from back of slides with a lint-free laboratory wipe without touching the tissue section. Place on a flat, clean, nonabsorbent work surface. Some droplets may remain.
- o.** Gently immerse slides in alcoholic Eosin solution in separate 50-ml centrifuge tubes. DO NOT use diluted Eosin.
- p.** Incubate **2 min** at **room temperature**.
- q.** Discard reagent by holding slides at an angle with the bottom edge in contact with a lint-free laboratory wipe.
- r.** Immerse slides for **30 sec** in Water Beaker 6.
- s.** Immerse slides 10x in Water Beaker 7.
- t.** Gently immerse slide in the 95% Ethanol Jar 1 for **30 sec** at **room temperature**.
- u.** Gently immerse slide in the 95% Ethanol Jar 2 for **30 sec** at **room temperature**.
- v.** Gently immerse slide in the 100% Ethanol Jar 1 for **30 sec** at **room temperature**.
- w.** Gently immerse slide in the 100% Ethanol Jar 2 for **30 sec** at **room temperature**.
- x.** Gently immerse slide in the Xylene Jar 1 for **3 min** at **room temperature**.
- y.** Gently immerse slide in the Xylene Jar 2 for **3 min** at **room temperature**.
- z.** Wipe excess liquid from back of slides with a lint-free laboratory wipe without touching the tissue section.

Excess liquid may dilute the Mounting Medium, causing the coverslip to come loose.

DO NOT air dry the slides.

Coverslipping

Before mounting the coverslip, gently flick the slide to remove any droplets and ensure it is dry. Moisture on the surface of the slide may result in faulty mounting. Wipe away any residual droplets with a lint-free laboratory wipe.

Use permanent, organic solvent-based mounting media to apply the coverslip. Consult the Atera Gene Expression Protocol Planner (CG001669) for tested part numbers.



The mounting medium used for mounting H&E-stained sections is different than what was used to mount slides after DAPI staining. A more permanent mounting medium can be used in this step, as the coverslip does not need to be removed. Ensure that the appropriate mounting media is used.

- a. Place slides on a flat, clean, nonabsorbent work surface.
- b. Using a **wide-bore** pipette tip, add **150–200 µl** mounting media to uniformly cover all tissue sections on each slide.
- c. Apply the coverslip at an angle on one end of the slide. Slowly lower the coverslip, without introducing bubbles. Allow mounting media to spread and settle.
- d. If needed, remove any large excess of mounting media by carefully wicking away from the edge of the coverslip with a lint-free laboratory wipe. Be careful not to move coverslip and disturb the tissue.
- e. Dry the coverslipped slides for **30 min** at **room temperature**.
-  f. Once coverslipping is complete, proceed with imaging. Samples may be stored temporarily at **4°C** and imaged within 3 days after coverslipping. For long-term storage after imaging, maintain at **room temperature in the dark**.

Optional Trimming/Scoring the Block

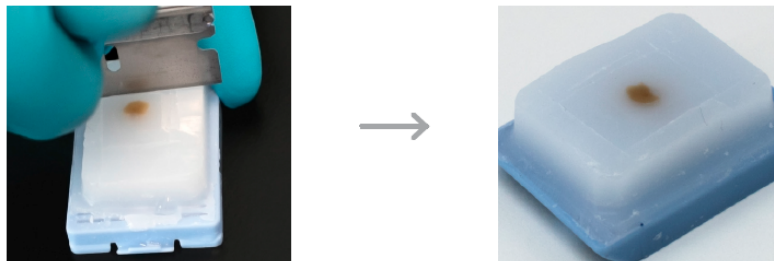
Before starting, wipe down all surfaces and work areas with RNaseZap RNase decontaminating solution. If necessary, rewipe the area with 100% ethanol to quickly dry the surface. To ensure proper fit for the slide Sample Area, the FFPE tissue block can be trimmed or scored as described below.

FFPE Tissue Block is larger than Sample Area, but tissue is smaller than Sample Area

For tissue samples that fit the Sample Area and are embedded in a large tissue block, the excess paraffin around the tissue can be trimmed to remove the excess and obtain smaller sections that fit the Sample Area.

- After facing, remove the tissue block from the microtome and start trimming.
- Using a razor blade, remove excess paraffin from around the tissue.
- Retain paraffin edges around the tissue samples to enhance tissue attachment on the slide.

Trimming large tissue block containing tissues that fit the Sample Area

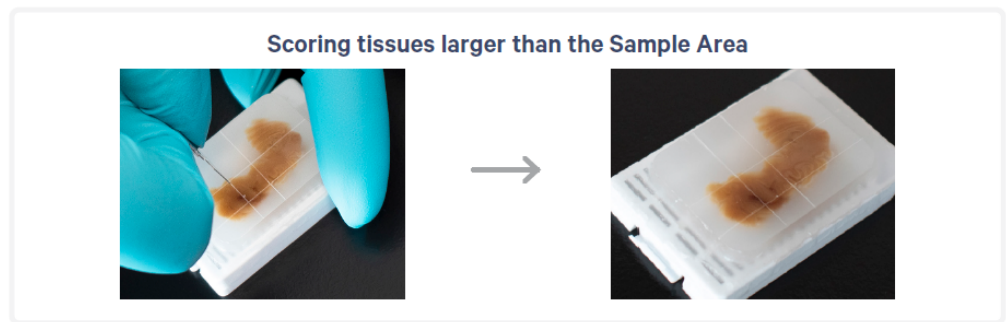


FFPE Tissue Block & Tissue are larger than Sample Area

Tissue samples larger than the Sample Area can be scored using a razor blade to generate smaller sections.

- Remove the tissue block from the microtome and start scoring.
- To score, lightly glide a razor blade over the surface of the tissue to introduce a shallow cut. This should yield approximately 10–15 trimmed

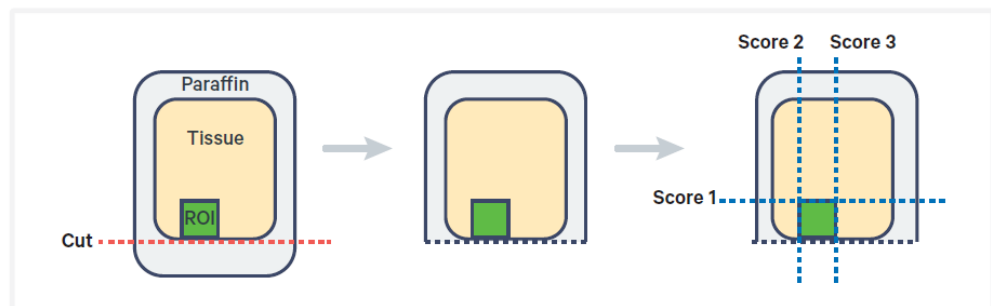
sections. A deep incision may lead to tissue damage and disintegration.



Scoring Configurations

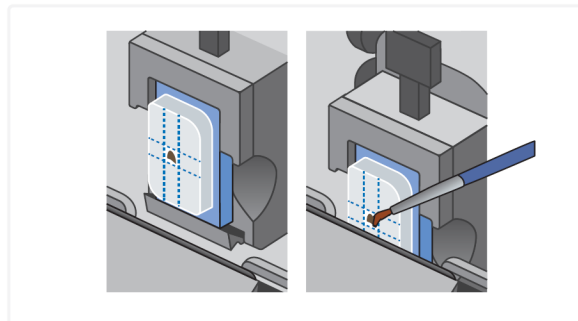
Region of Interest (ROI) is at the Top or Bottom of the Tissue

- If the ROI is at the bottom of the tissue, remove excess wax such that only three scoring cuts are necessary.
- This configuration may prevent curling of the ROI, as the excess wax at the top of the block may curl before affecting the ROI.
- Retain paraffin edges around the tissue samples to enhance tissue attachment on the slide.



ROI is in the Middle of the Tissue

- If the ROI is in the middle of the tissue, make four scoring cuts as shown below.
- To prevent curling, place the brush over the edge of the ROI as the blade crosses the section. Avoid cutting the brush bristles on the blade.



Shipping Guidance

- After sectioning, slides can be stored at **room temperature** in a desiccator.
- When ready for shipment, place slides in a slide mailer with desiccant in a sealed bag.
 - To avoid risk of scratching samples, a maximum of 2 slides per mailer is recommended. If adding more slides, ensure the mailer can accommodate extra space between the slides or exercise caution.
- Ship in a styrofoam or cooler box with cool packs.
- Upon arrival at destination, slides should be stored at **room temperature** in a desiccator.

Document Revision Summary

Document Number	CG001671
Title	Atera Gene Expression – FFPE Tissue Preparation Handbook
Revision	Rev A
Revision Date	August 2026
Specific Changes	Initial Release

Take 1 minute to evaluate this protocol. Scan this code or [click here](#).



Notices

Document Number

CG001671 | Rev A

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