

Visium Spatial Gene Expression for FFPE – Deparaffinization, H&E Staining, Imaging & Decrosslinking

Introduction

The Visium Spatial Gene Expression for FFPE is designed to measure mRNA in tissue sections derived from formalin fixed & paraffin embedded (FFPE) tissue samples and requires a Visium Spatial slide with intact tissue sections as input. This protocol outlines deparaffinization, Hematoxylin & Eosin (H&E) staining, imaging, and decrosslinking of tissue for use with 10x Genomics Visium Spatial Gene Expression for FFPE assay. Deparaffinized, stained, and decrosslinked tissue sections are inputs for the downstream Visium Spatial Gene Expression for FFPE workflow.

Additional Guidance

Consult the Visium Spatial Gene Expression for FFPE - Tissue Preparation Guide (Document CG000408) for complete information on sectioning FFPE tissue blocks and placing sections on Visium Spatial slides. Ensure that tissue sections have been placed onto the appropriate slide prior to starting this Demonstrated Protocol. Consult the Visium Spatial Gene Expression for FFPE Imaging Guidelines (Document CG000436) to verify imaging settings prior to starting this Demonstrated Protocol. After completing this Demonstrated Protocol (CG000409), proceed with the Visium Spatial Gene Expression for FFPE - User Guide (CG000407).

Contents

| | | | |
|-----------|--|-----------|---|
| 2 | Reagent Kits | 21 | 2. Tissue Imaging |
| 3 | Recommended Thermal Cyclers | 21 | 2.1 Imaging System Recommendations |
| 3 | Workflow Overview | 22 | 2.2 Imaging |
| 4 | Specific Reagents & Consumables | 22 | 2.3 Coverslip Removal |
| 5 | Tips & Best Practices | 23 | 3. Decrosslinking for H&E Stained Sections |
| 14 | 1. Deparaffinization & H&E Staining | 23 | 3.1 Preparation - Buffers |
| 14 | 1.1 Preparation - Buffers | 24 | Protocol Overview |
| 15 | Protocol Overview | 25 | 3.2 Decrosslinking for H&E Stained Sections |
| 17 | 1.2 Deparaffinization | 26 | Troubleshooting |
| 19 | 1.3 H&E Staining | | |
| 20 | 1.4 Coverslipping | | |

Reagent Kits

Visium Spatial Gene Expression for FFPE Reagent Kits

Refer to SDS for handling and disposal information

Visium Spatial Gene Expression Slide Kit, 16 rxns PN-1000185

| Visium Spatial Gene Expression Slide Kit | | |
|---|---|---------|
| 16 rxns, PN-1000185 | | |
| <i>store at ambient temperature</i> | | |
| | # | PN |
| Visium Spatial Gene Expression Slide | 4 | 2000233 |
| *Visium Slide Seals, 40-pack | 1 | 2000284 |
| Visium Cassette & Gasket Assembly, 4-pack | 1 | 2000282 |

10x
GENOMICS

Visium Spatial Gene Expression Slide Kit, 4 rxns PN-1000188

| Visium Spatial Gene Expression Slide Kit | | |
|---|---|---------|
| 4 rxns, PN-1000188 | | |
| <i>store at ambient temperature</i> | | |
| | # | PN |
| Visium Spatial Gene Expression Slide | 1 | 2000233 |
| *Visium Slide Seals, 12-pack | 1 | 2000283 |
| Visium Cassette & Gasket Assembly, 1-pack | 1 | 2000281 |

10x
GENOMICS

*Visium Slide Seals may come in varying dimensions and quantities in different lots. Check the number of slide seals in the kit. Additional seals may be required. Refer to page 13 ([Visium Slide Seal Application & Removal](#)) of this Demonstrated Protocol for instructions on how to resize seals or cut additional seals..

Visium Tissue Section Test Slides, 4 Pack PN-1000347

| Visium Tissue Section Test Slides | | |
|--|---|---------|
| 4 Pack, PN-1000347 | | |
| <i>store at ambient temperature</i> | | |
| | # | PN |
| Visium Tissue Section Test Slide | 4 | 2000460 |

10x
GENOMICS

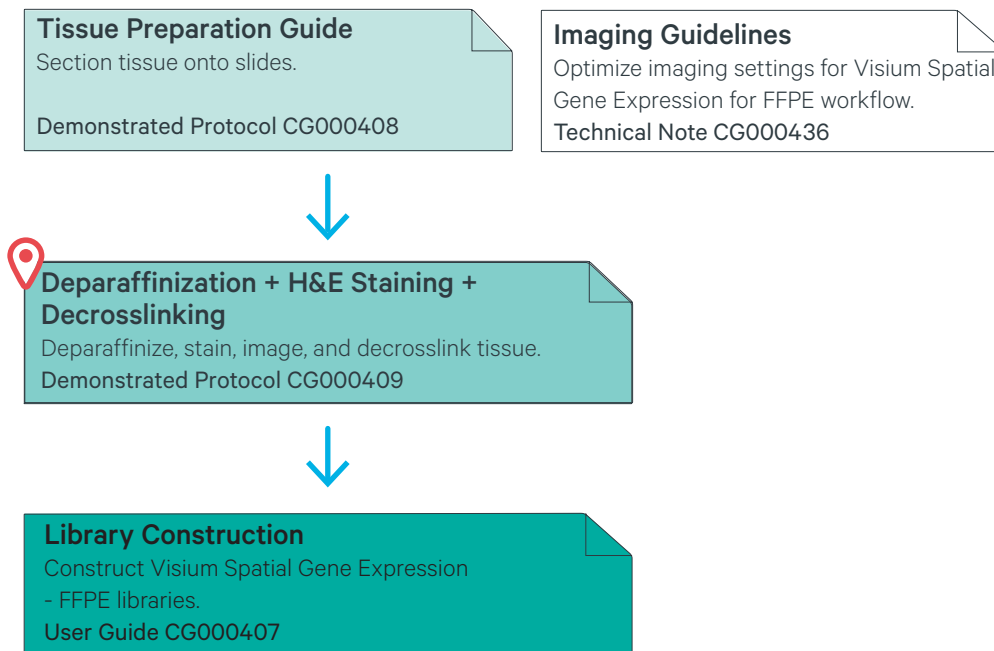
10x Genomics Accessories

| Product | Part Number (Kit) | Part Number (Item) |
|-----------------------------------|-------------------|--------------------|
| Thermocycler Adaptor | 1000194 | 3000380 |
| Visium Spatial Imaging Test Slide | | 2000235 |
| 10x Magnetic Separator | | 230003 |
| Slide Alignment Tool | | 3000433 |

Recommended Thermal Cyclers

| Supplier | Description | Part Number |
|---------------------------------|--|---|
| Bio-Rad | C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module | 1851197 |
| Eppendorf | MasterCycler Pro (discontinued) | North America 950030010 International 6321 000.019 |
| Thermo Fisher Scientific | Veriti 96-Well Thermal Cycler | 4375786 |

Workflow Overview



Visit the 10x Genomics Support website for the most current documentation.

Specific Reagents & Consumables

For each item, a number of vendor options are listed. Choose item based on availability and preference.

| Item | Alternatives/Options | Vendor | Part Number |
|---|--|--------------------------|-------------|
| Xylene | Xylene, Reagent Grade | Millipore Sigma | 214736 |
| | Xylene, Histological Grade | Millipore Sigma | 534056 |
| Ethanol | Ethyl Alcohol, 200 Proof | Millipore Sigma | E7023 |
| | Ethanol absolute ≥99.5%, TechniSolv, pure (Europe Only) | VWR | 83813.360DP |
| Eosin | Eosin Y-solution, Alcoholic | Millipore Sigma | HT110116 |
| | Eosin Y Solution (Modified Alcoholic) | Abcam | ab246824 |
| | Eosin Y with Phloxine 1% alcoholic solution | VWR | 10143 |
| Hematoxylin | Hematoxylin Solution, Mayer's | Millipore Sigma | MHS16 |
| | Hematoxylin Solution According to Mayer | Millipore Sigma | 51275 |
| | Hematoxylin, Mayer's | Agilent | S330930-2 |
| Bluing reagent | Bluing Reagent, Dako | Agilent | CS70230-2 |
| | Thermo Scientific Shandon Bluing Reagent | Fisher Scientific | 6769001 |
| | Scott's Bluing Agent | Ricca Chemical Company | 6697 |
| Glycerol | Glycerol Solution | Millipore Sigma | 49781 |
| | Glycerol | Acros Organics | 327255000 |
| 0.1 N HCl | Hydrochloric Acid Solution, 0.1 N <i>Or any equivalent HCl</i> | Fisher Chemical | SA54-1 |
| TE Buffer (pH 9.0) | TE Buffer (pH 9.0) <i>Alternatively, prepare TE buffer using Tris and EDTA and adjust the pH to 9.0</i> | GeneMed | 10-0046 |
| Or Prepare using Tris and 0.5 M EDTA | Tris Base <i>For preparing TE buffer (pH 9.0), alternative to Genemed product</i> | Fisher Scientific | BP152-500 |
| | UltraPure 0.5 M EDTA, pH 8.0 <i>For preparing TE buffer (pH 9.0), alternative to Genemed product</i> | Thermo Fisher Scientific | 15575020 |
| | 1.0 M HCl <i>For preparing TE Buffer (pH 9.0), alternative to Genemed product</i> | Millipore Sigma | 258148 |
| Staining jar/ dishes | Coplin Jar | VWR | 100500-232 |
| | Staining Dishes | VWR | 25608-906 |
| Section dryer oven | Epredia High Capacity Section Dryer <i>Or any equivalent product</i> | Fisher Scientific | A84600051 |
| Slide holders | Slide Holders, 24-place | VWR | 25608-868 |
| Coverslips | Fisherbrand Cover Glasses: Rectangles | Fisher Scientific | 12-544-EP |
| | Cover Glasses, Rectangular | VWR | 16004-322 |
| Sealing film | Microseal 'B' PCR Plate Sealing Film, adhesive | Bio-Rad | MSB1001 |

Additional Materials

| | | |
|---|---|---|
| Beakers | - | - |
| Ultrapure/Milli-Q Water, <i>from Milli-Q Integral Ultrapure Water System or equivalent</i> | - | - |

Tips & Best Practices



Tips & Best Practices section includes additional guidance



Signifies critical step requiring accurate execution



Troubleshooting section includes additional guidance

General Reagent Handling

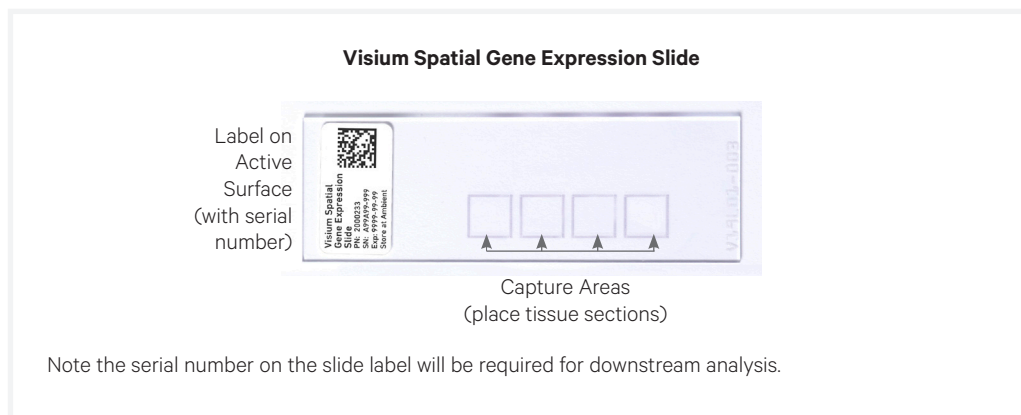
- Thoroughly mix reagents before use.
- Use a pH meter to adjust pH as necessary during buffer preparation.

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.

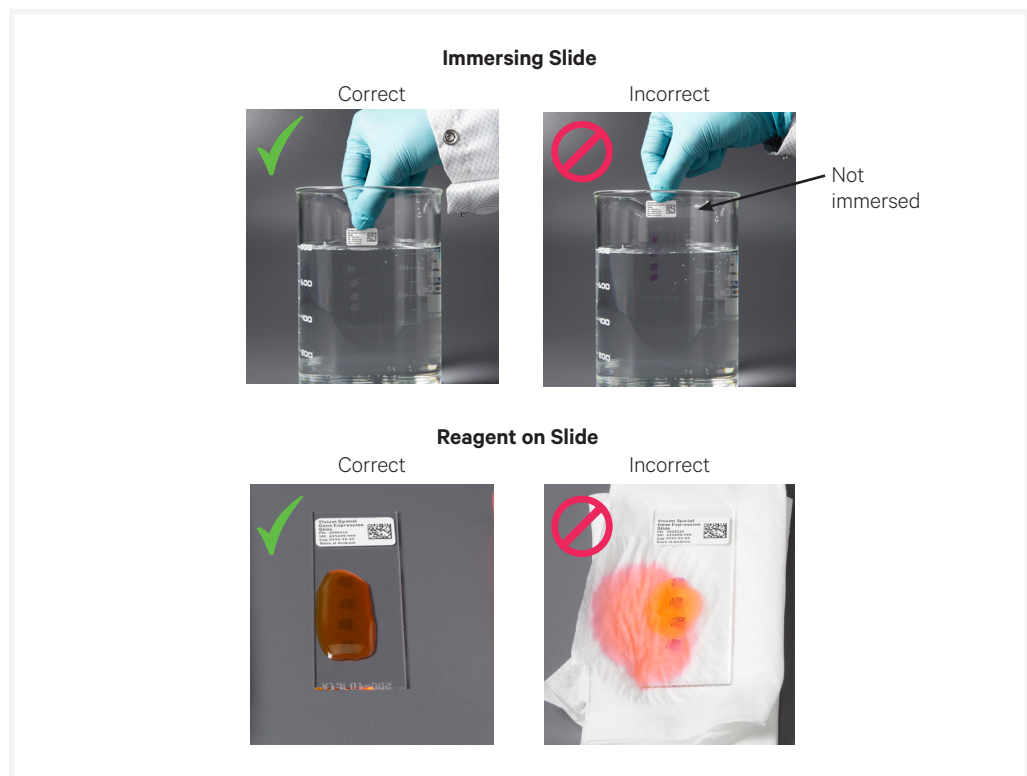
Visium Spatial Gene Expression Slide

- Visium slides include 4 Capture Areas (6.5 x 6.5 mm), each with ~5,000 unique gene expression spots.
- Each gene expression spot includes primers with a unique Spatial Barcode.
- The active surface of the slide is defined by a readable label that includes the serial number.
- The tissue sections are always placed on the active surface of the Capture Areas. For more information, consult the Visium Spatial Gene Expression for FFPE – Tissue Preparation Guide (Demonstrated Protocol CG000408).
- Always store slides in a cool, dry environment. After tissue placement, store the slides at room temperature in a low moisture environment such as a desiccator.



Slide Handling

- Always wear gloves when handling slides.
- Ensure that the active surface of a slide faces up and is never touched. The orientation of the label on the slide defines the active surface.
- The tissue sections should always be on the active surface of the slide. DO NOT touch the tissue sections on the slide.
- Minimize exposure of the slides to sources of particles and fibers.
- When immersing slides in deparaffinization solutions and water, ensure that the tissue sections are completely submerged.
- Xylene and ethanol may cause the slide label to come off. Keep the label above the surface of the liquid when immersing in xylene and ethanol.
- Keep the slide flat on the bench when adding reagents to the active surface.
- Ensure that no absorbent surface is in contact with the reagents on the slide during incubation.



Slide Incubation Guidance

Incubation at a specified temperature

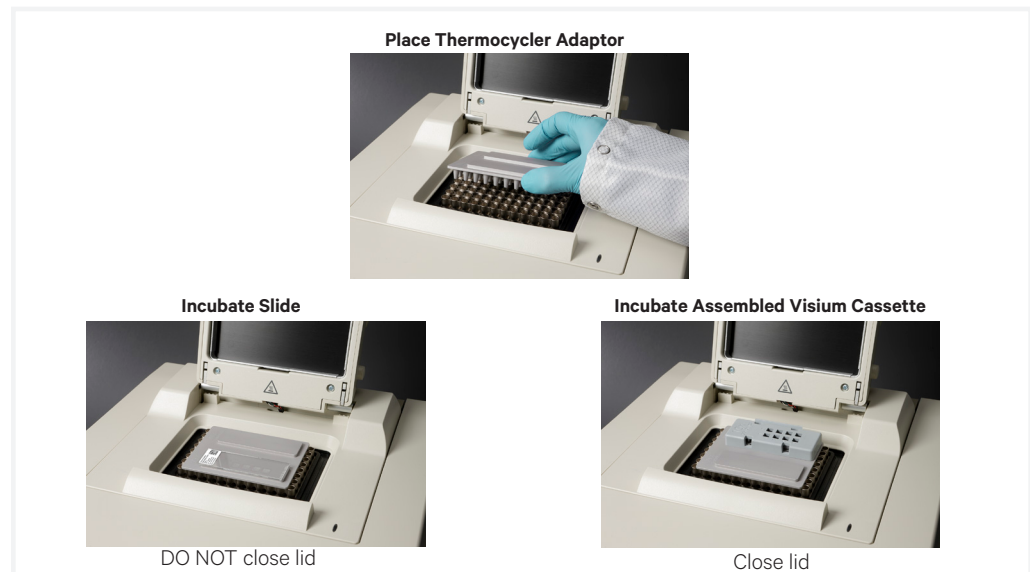
Incubation using a Section Dryer Oven:

- Place the slides in a slide drying rack.
- Close the lid when incubating the slide in the oven.



Incubation using a Thermal Cycler:

- Position a Thermocycler Adaptor on a thermal cycler that is set at the incubation temperature.
- Ensure that the Thermocycler Adaptor is in contact with the thermal cycler surface uniformly.
- When incubating a slide, position the slide on the Thermocycler Adaptor with the active surface facing up.
- Ensure that the entire bottom surface of the slide is in contact with Thermocycler Adaptor.
- When incubating a slide encased in a Visium Cassette, place the assembled unit on the Thermocycler Adaptor with the wells facing up. The Visium Cassette should always be sealed when in the Thermocycler Adaptor.

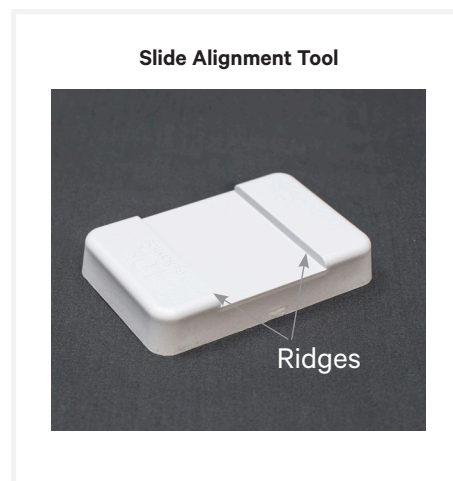
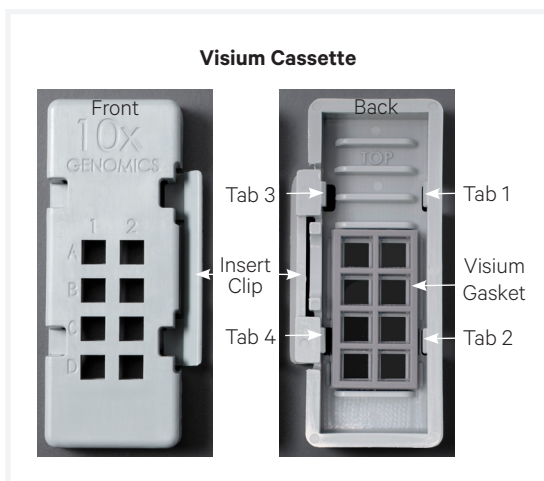


Incubation at room temperature

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

Visium Cassette

- The Visium Cassette encases the slide and creates leakproof wells for adding reagents.
- Place the slides in the Visium Cassette only when specified.
- The Visium Cassette includes a removable Visium Gasket.
- An Insert Clip and four tabs at the back of the Visium Cassette are used for holding the slide in the cassette, as shown.
- The removable Visium Gasket corresponds to the Capture Areas on the slides.
- The Visium Cassette may be assembled using the Slide Alignment Tool or manually. Instructions for both are provided in the following section.
- See [Visium Cassette Assembly & Removal](#) instructions for details.
- Ensure that the back of the Visium Cassette is facing the user prior to assembly. The active surface of the slide with tissue sections will face down such that the slide label is no longer readable.
- Practice assembly with a plain glass slide (75 x 25 x 1 mm).
- Applying excessive force to the slide may cause the slide to break.

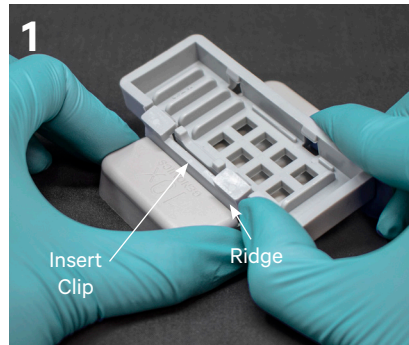


Visium Cassette Assembly



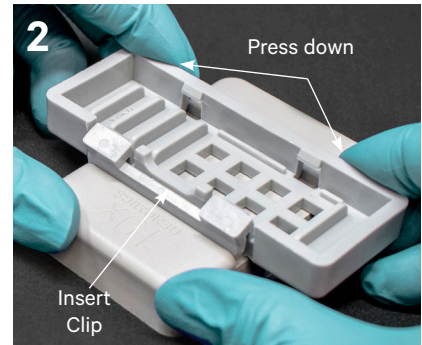
Exercise caution when handling slide edges to prevent injury.

Position Visium Cassette along alignment tool ridges



Visium Cassette secured on alignment tool

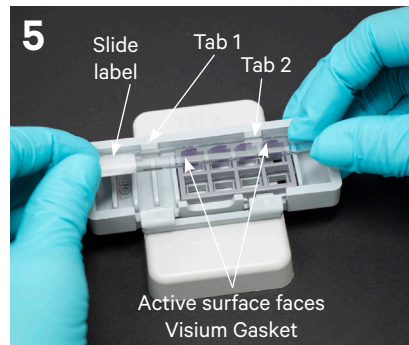
Push Insert Clip along the ridge & press Visium Cassette down



Position Visium Gasket to align with Visium Cassette cutouts



Insert long edge of slide under tabs 1 & 2; ensure slide is flush



Press slide down until it is flush with the Visium Gasket and under tabs 3 & 4



Slide insertion may push Visium Gasket out of alignment with slide cutouts. Adjust if necessary.

Remove Visium Cassette while pressing slide against the Visium Gasket



Slides in images are representative.

Visium Cassette Removal

Position Visium Cassette along alignment tool ridges



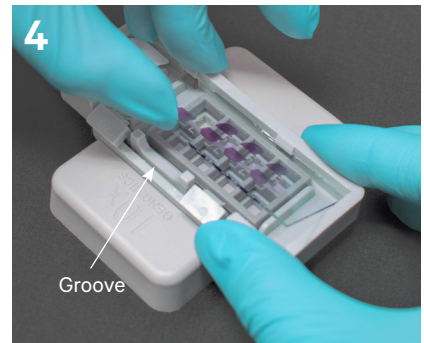
Push Insert Clip along the ridge & press down



Visium Cassette sits securely on alignment tool



Lift slide at Visium Cassette groove



Slides in images are representative.

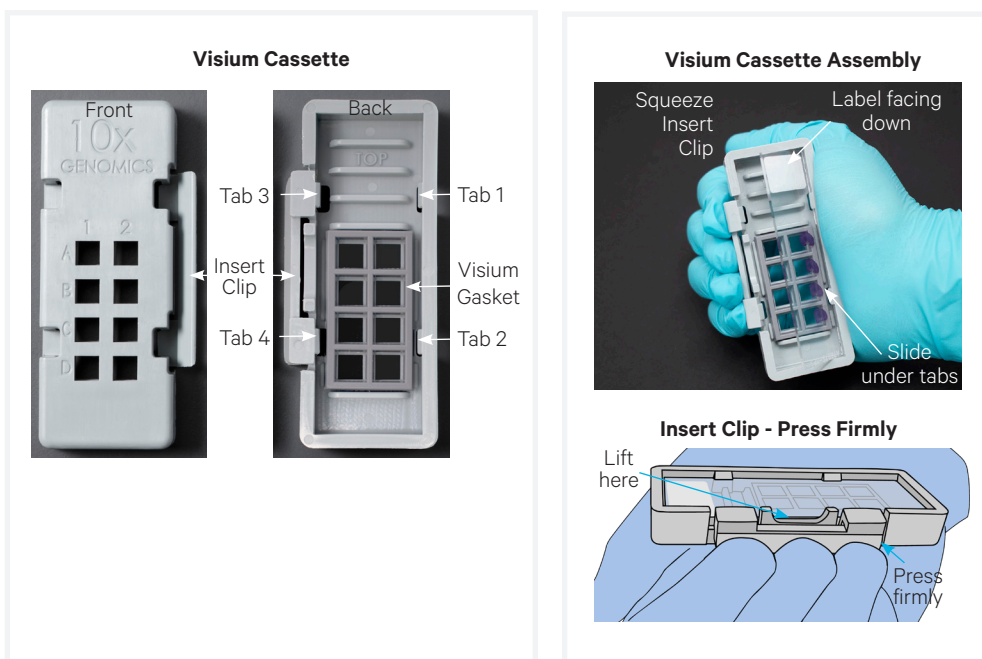
Manual Visium Cassette Assembly & Removal

Assembly

- a. Remove the Visium Gasket from the Visium Cassette and re-insert the Visium Gasket, ensuring that the Visium Gasket and Visium Cassette cutouts are aligned.
- b. Align the label on top of the slide to the top of the Visium Cassette, as shown.
- c. Insert the slide under tabs 1 and 2. Ensure that the long edge of the slide is flush with the side of the Visium Cassette.
- d. Press the insert clip very firmly by applying even force on the lower part of the insert clip.
- e. Place a finger in between tab 3 and the top of the cassette, and one finger between tab 4 and the bottom of the cassette. Press down on the slide evenly until the slide is under each tab and release the insert clip.

Removal

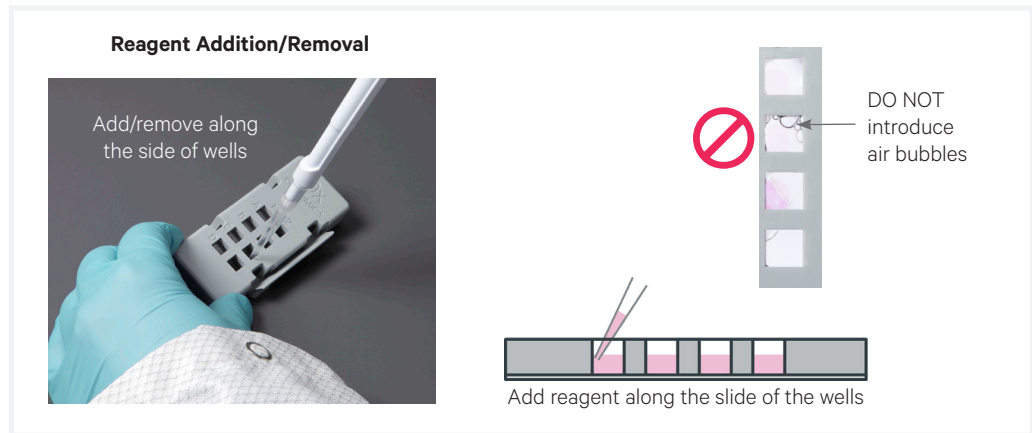
- a. Press the insert clip very firmly to release the slide from the cassette.
- b. Lift slide at Visium Cassette groove between tabs 3 and 4 until the slide can be removed.



Reagent Addition to Wells

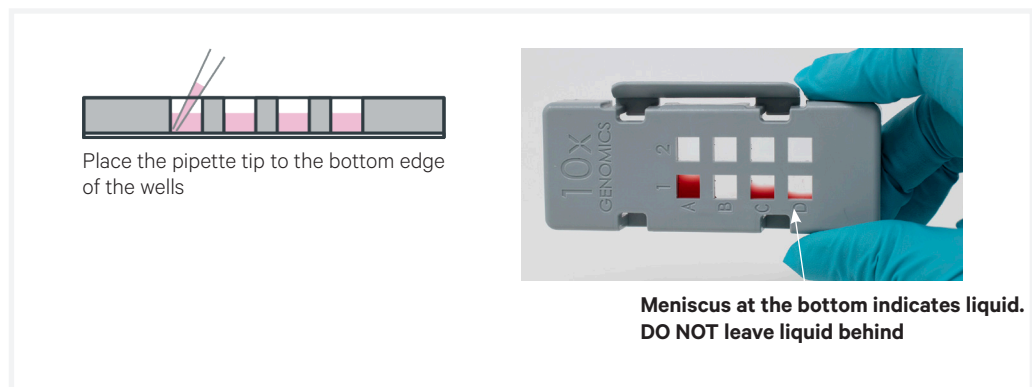


- Place the assembled slide in the Visium Cassette flat on a clean work surface.
- Dispense reagents along the side of the wells without touching the tissue sections and without introducing bubbles.
- Always cover the tissue section completely when adding reagents to the well. A gentle tap may help spread the reagent more evenly.
- Ensure that no bubbles are introduced in the process.



Reagent Removal from Wells

- Place the assembled slide in the Visium Cassette flat on a clean work surface.
- Slightly tilt the Visium Cassette while removing the reagent.
- Place the pipette tip to the bottom edge of the wells.
- Remove reagents along the side of the wells without touching the tissue sections and without introducing bubbles.
- Ensure that no bubbles are introduced in the process.
- Remove all liquid from the wells in each step. To ensure complete removal, check the bottom of the well by tilting the cassette slightly. A meniscus at the bottom of the well will indicate the presence of liquid in the well.



Visium Slide Seal Application & Removal

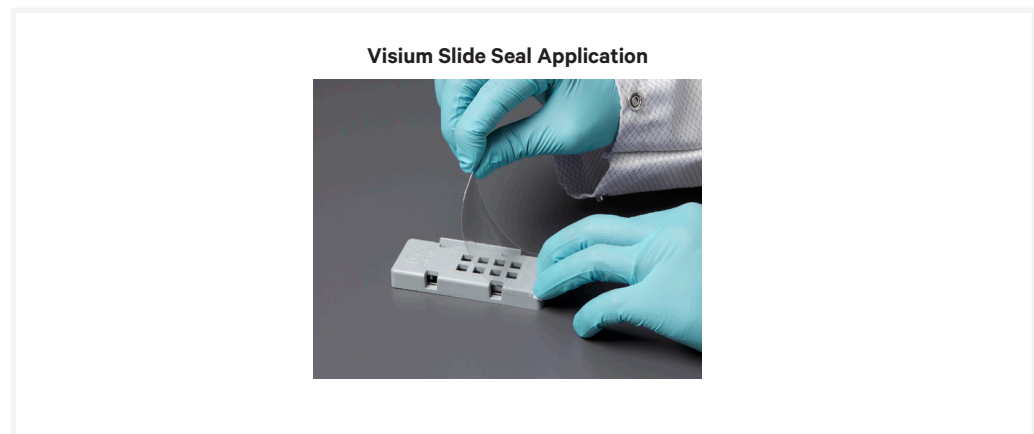
To generate new or resize Visium Slide Seals, use one of the provided seals (PN-2000283/2000284) as a template to cut additional seals from MicroSeal 'B' PCR Plate Sealing Film (PN-MSB1001; listed in Specific Reagents & Consumables). Contact support@10xgenomics.com if assistance is required.

Application

- Place the Visium Cassette flat on a clean work surface.
- Remove the back of the adhesive Visium Slide Seal.
- Align the Visium Slide Seal with the surface of the Visium Cassette and apply while firmly holding the Visium Cassette with one hand.
- Press on the Visium Slide Seal to ensure uniform adhesion.

Removal

- Place the Visium Cassette flat on a clean work surface.
- Pull on the Visium Slide Seal from the edge while firmly holding the Visium Cassette.
- Ensure that no liquid splashes out of the wells.



Tissue Detachment on Visium Slides

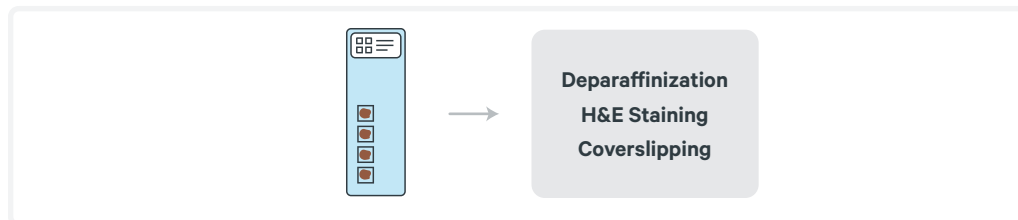


- Monitor section adhesion on the Visium slides throughout the workflow. For more information, consult the Visium Spatial Gene Expression for FFPE – Tissue Paraffin Guide (Demonstrated Protocol CG000408).
- Tissue detachment during the workflow can impact performance.

1. Deparaffinization & H&E Staining

1.0 Overview

This chapter provides guidance on deparaffinization and H&E staining of Visium slides containing FFPE tissue sections that are dried overnight in a desiccator. Ensure that microscope settings have been verified and imaging programs have been created prior to starting this program. Consult the Visium Spatial Gene Expression for FFPE Imaging Guidelines Technical Note (CG000436) for more information.



1.1 Preparation - Buffers

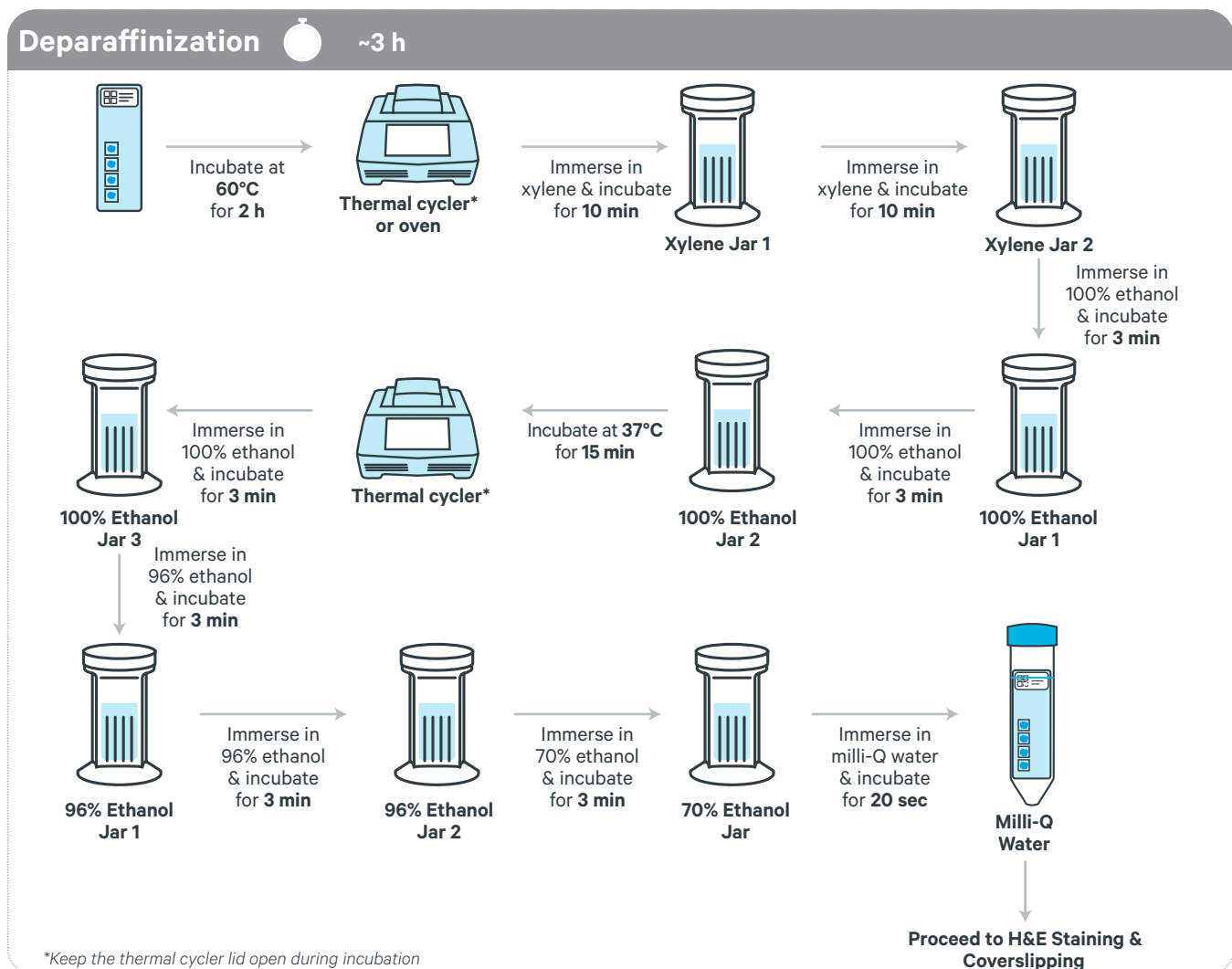
| For Deparaffinization | |
|--|--|
| Items | Preparation & Handling |
| <input type="checkbox"/> Xylene | Label two coplin jars as Xylene Jar 1 and 2. Dispense 30 ml xylene in each. |
| <input type="checkbox"/> 100% Ethanol | Label three coplin jars as 100% Ethanol Jar 1, 2, and 3. Dispense 30 ml 100% ethanol in each. |
| <input type="checkbox"/> 96% Ethanol | Label two coplin jars as 96% Ethanol Jar 1 and 2. Dispense 30 ml 96% ethanol in each. |
| <input type="checkbox"/> 70% Ethanol | Label one coplin jar as 70% Ethanol Jar. Dispense 30 ml 70% ethanol. |
| <input type="checkbox"/> Milli-Q Water | Label one coplin jar as Milli-Q Water Jar. Dispense 30 ml milli-Q water. Alternatively, use a 50-ml centrifuge tube or a beaker. |



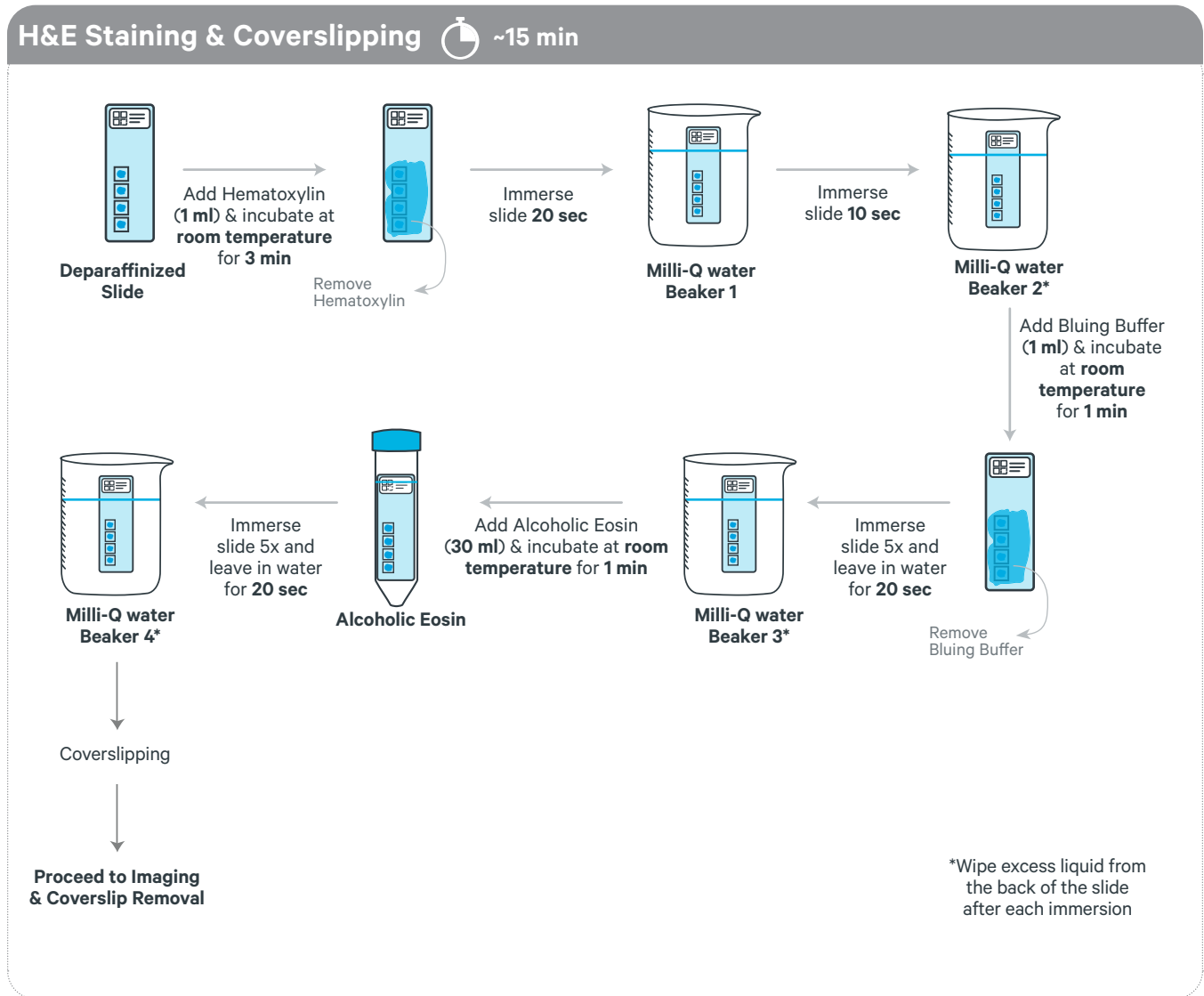
Alternatively, a slide staining dish can also be used in place of a coplin jar. Adjust the volumes of deparaffinization solutions and water, accordingly. Use xylene-resistant dishes for immersion in xylene. Use xylene-resistant gloves or forceps for deparaffinization. Prepare fresh reagents every week.

| For H&E Staining | |
|--|---|
| Items | Preparation & Handling |
| <input type="checkbox"/> Milli-Q Water | Label four 1000-ml beakers as Water Beakers 1, 2, 3, and 4. Dispense the following volumes of Milli-Q water. 800 ml in Beaker 1 800 ml in Beaker 2 800 ml in Beaker 3 800 ml in Beaker 4 Dispensed volumes in each beaker can be used for two slides. Alternatively, use four 50-ml centrifuge tubes. |
| <input type="checkbox"/> Alcoholic Eosin | 30 ml in a 50-ml centrifuge tube |
| For Coverslipping | |
| Items | Preparation & Handling |
| <input type="checkbox"/> 85% Glycerol | Prepare 85% Glycerol using Nuclease-free Water. Invert mix. Prepare 150-200 µl per slide. Briefly centrifuge before using to remove bubbles. |

Protocol Overview



Protocol Overview



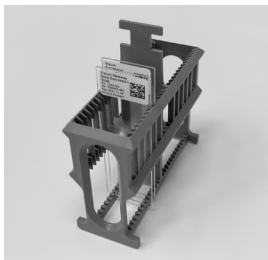
1.2 Deparaffinization

Deparaffinization steps should be performed in a fume hood due to the hazardous nature of xylene.

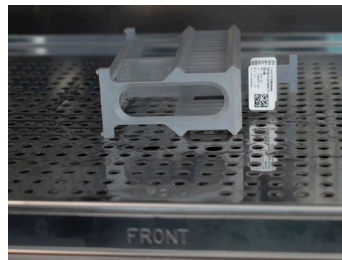
Visium Spatial slide contains a readable label with a serial number on the active surface of the slide. The label may come off during deparaffinization steps. In such cases, the serial number etched on the slide can be used.

- a. Retrieve the slide with tissue sections from the desiccator after overnight drying.
- b. Place slides in a Section Dryer Oven and incubate uncovered at **60°C** for **2 h**. Keep the oven lid closed during incubation.

Incubation in a Section Dryer Oven



Place slide in a rack



Place slide sideways and keep the oven door closed during incubation

Alternatively, place a Thermocycler Adaptor on a thermal cycler set at **60°C**. Place slide on the Thermocycler Adaptor with the active surface facing up and incubate **2 h** at **60°C**. **DO NOT** close the thermal cycler lid.



Incubation in a Thermal Cycler



Place Thermocycler Adaptor



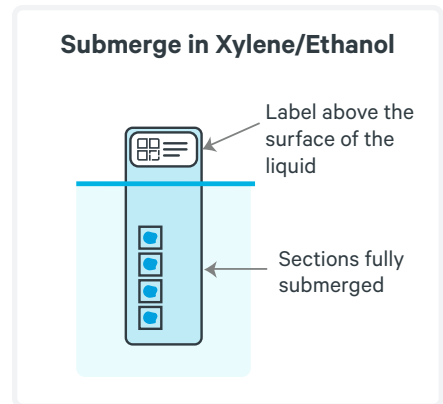
Incubate Slide for 2 h at 60°C

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

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

When immersing slides in xylene, ensure that the tissue sections are completely submerged and the xylene doesn't reach the label.

- e. Incubate for **10 min**.
- f. During incubation, set up a thermal cycler to **37°C**. Place a Thermocycler Adaptor on the thermal cycler.



- g. Gently immerse slide in the xylene in Xylene Jar 2 and incubate for **10 min**.

- h. Gently immerse slide in the 100% Ethanol Jar 1 for **3 min**.

When immersing slides in ethanol, ensure that the tissue sections are completely submerged and the ethanol doesn't reach the label.

- i. Gently immerse slide in the 100% Ethanol Jar 2 for **3 min**.

- j. Discard ethanol by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe.

- k. Wipe excess liquid from the back of the slide without touching the tissue section.

- l. Place slide on the Thermocycler Adaptor with the active surface facing up and incubate for **15 min** at **37°C**.



DO NOT close the thermal cycler lid.

- m. Gently immerse slide in the 100% Ethanol Jar 3 for **3 min**.

- n. Gently immerse slide in the 96% Ethanol Jar 1 for **3 min**.

- o. Gently immerse slide in the 96% Ethanol Jar 2 for **3 min**.

- p. Gently immerse slide the 70% Ethanol Jar for **3 min**.


- q. Gently immerse slide in the water in the tube/beaker and incubate for **20 sec**.

- r. Proceed **immediately** to H&E Staining & Coverslipping. *DO NOT let the slides dry.*

1.3 H&E Staining

- a. Place on a flat, clean, non-absorbent work surface. Some residual droplets may remain.
- b. Add **1 ml** Hematoxylin to uniformly cover all tissue sections on the slide.
- c. Incubate **3 min** at **room temperature**.
- d. Discard reagent by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe.



- e. Gently immerse the slide for **20 sec** in the water in Beaker 1.
- f. Gently immerse the slide for **10 sec** in the water in Beaker 2.
- g. Wipe excess liquid from the back of the slide without touching the tissue section.
- h. Place on a flat, clean, non-absorbent work surface. Some droplets may remain
- i. Add **1 ml** Bluing Buffer to uniformly cover all tissue sections. Incubate **1 min** at **room temperature**.
- j. Discard reagent by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe.
- k. Gently immerse the slide 5x in the water in Beaker 3 and leave the slide in the water for **20 sec**.
- l. Wipe excess liquid from the back of the slide without touching the tissue section. Place on a flat, clean, non-absorbent work surface. Some droplets may remain.
- m. Gently immerse the slide in alcoholic Eosin solution in a 50-ml centrifuge tube. Incubate **1 min** at **room temperature**. **DO NOT** use diluted Eosin. Discard reagent by draining and/or holding the slide at an angle with the bottom edge in contact with a laboratory wipe.
- n. Gently immerse the slide 5x in the water in Beaker 4 and leave the slide in the water for **20 sec**.
-  o. Wipe excess liquid from the back of the slide without touching the tissue section. **DO NOT air dry the slide.**

1.4 Coverslipping

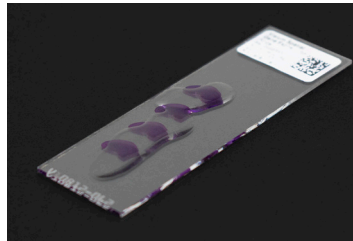
- a. Place slide on a flat, clean, non-absorbent work surface. Some residual droplets may remain.
- b. Using a **wide-bore** pipette tip, add **150-200 μ l** 85% Glycerol to uniformly cover all tissue sections on the slide.
- c. Apply the coverslip at an angle on one end of the slide. Slowly lower the coverslip, without introducing bubbles. Allow glycerol to spread and settle.
- d. If needed, remove any large excess of glycerol by carefully wicking away from the edge of the coverslip with a laboratory wipe. Be careful not to move coverslip and disturb the tissue.
- e. Once coverslipping is complete, **immediately** proceed with imaging.



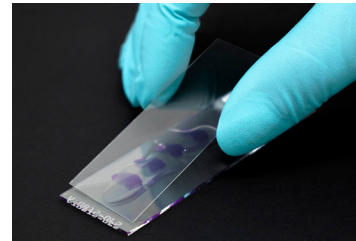
DO NOT let the attached coverslip dry.

DO NOT use Cytoseal or nail polish for securing the coverslip.

**Cover uniformly with 85%
Glycerol**



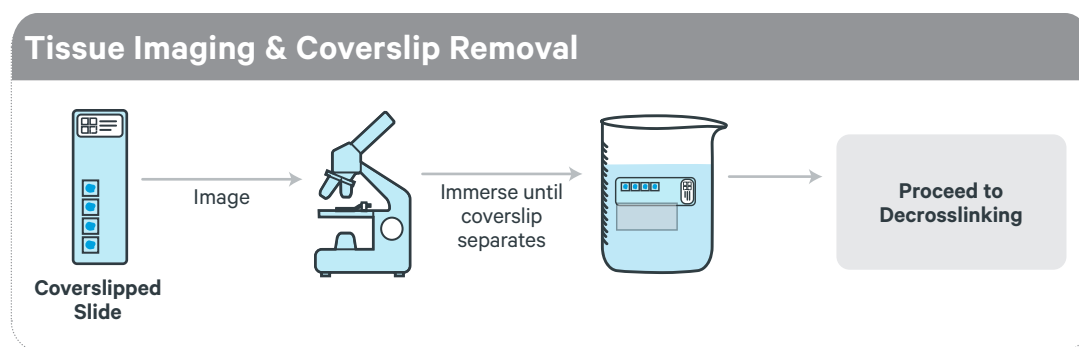
Apply coverslip



2. Tissue Imaging

2.0 Overview

This chapter provides guidance on imaging Visium slides containing H&E stained FFPE sections and coverslip removal.



2.1 Imaging System Recommendations

The following table shows imaging systems used by 10x Genomics in the development of this protocol. Any equivalent imaging system can be used as an alternative.

| Supplier | Model | Configuration |
|--------------------------|-------------------|---------------------|
| Thermo Fisher Scientific | EVOS M7000 | Inverted |
| Leica | Aperio Versa 8 | Upright |
| | Leica DMI8 | Inverted |
| MetaSystems | Metafer | Upright |
| Nikon | Nikon Eclipse Ti2 | Inverted |
| BioTek | Cytation 7 | Inverted or Upright |
| Keyence | Keyence BZX800 | Inverted |

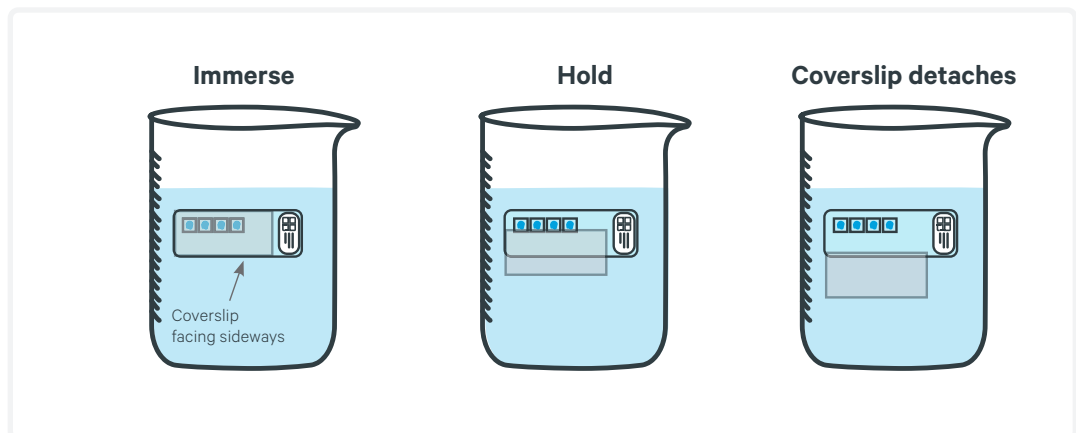
| Brightfield Recommended Configuration |
|--|
| Color camera (3 x 8 bit, 2,424 x 2,424 pixel resolution) |
| White balancing functionality |
| 2.18 µm/pixel minimum capture resolution |
| Exposure times 2-10 milli sec |

2.2 Imaging

- a. Image all Capture Areas individually at the desired magnification using brightfield imaging settings. Ensure that fiducial frames are captured.
- b. Consult the Visium Spatial Gene Expression for FFPE Imaging Guidelines Technical Note (CG000436) for additional information.
- c. After imaging, proceed **immediately** to the Coverslip Removal.

2.3 Coverslip Removal

- a. Dispense **800 ml** Milli-Q water in a beaker.
- b. Remove the coverslip immediately after imaging is complete.
- c. Immerse the slide sideways/horizontal in the beaker containing **800 ml** water with the coverslipped surface fully sideways.
- d. Hold the slide in water until the coverslip slowly separates away from the slide.
To avoid damaging the tissue sections and Capture Areas or causing tissue detachment, DO NOT move the slide up and down, shake forcibly or manually move the coverslip.



- e. Gently immerse 15x in the water to ensure all glycerol is removed.
- f. Wipe the back of the slide with a laboratory wipe. Place on a flat, clean, non-absorbent work surface and air dry.
- g. Incubate slide on the Thermocycler Adaptor with the thermal cycler lid open for **3 min** at **37°C**.
- h. Proceed **immediately** to Decrosslinking or store in a slide mailer or 50-ml tube at **4°C** for up to **2 weeks**.



3. Decrosslinking for H&E Stained Sections

3.0 Overview

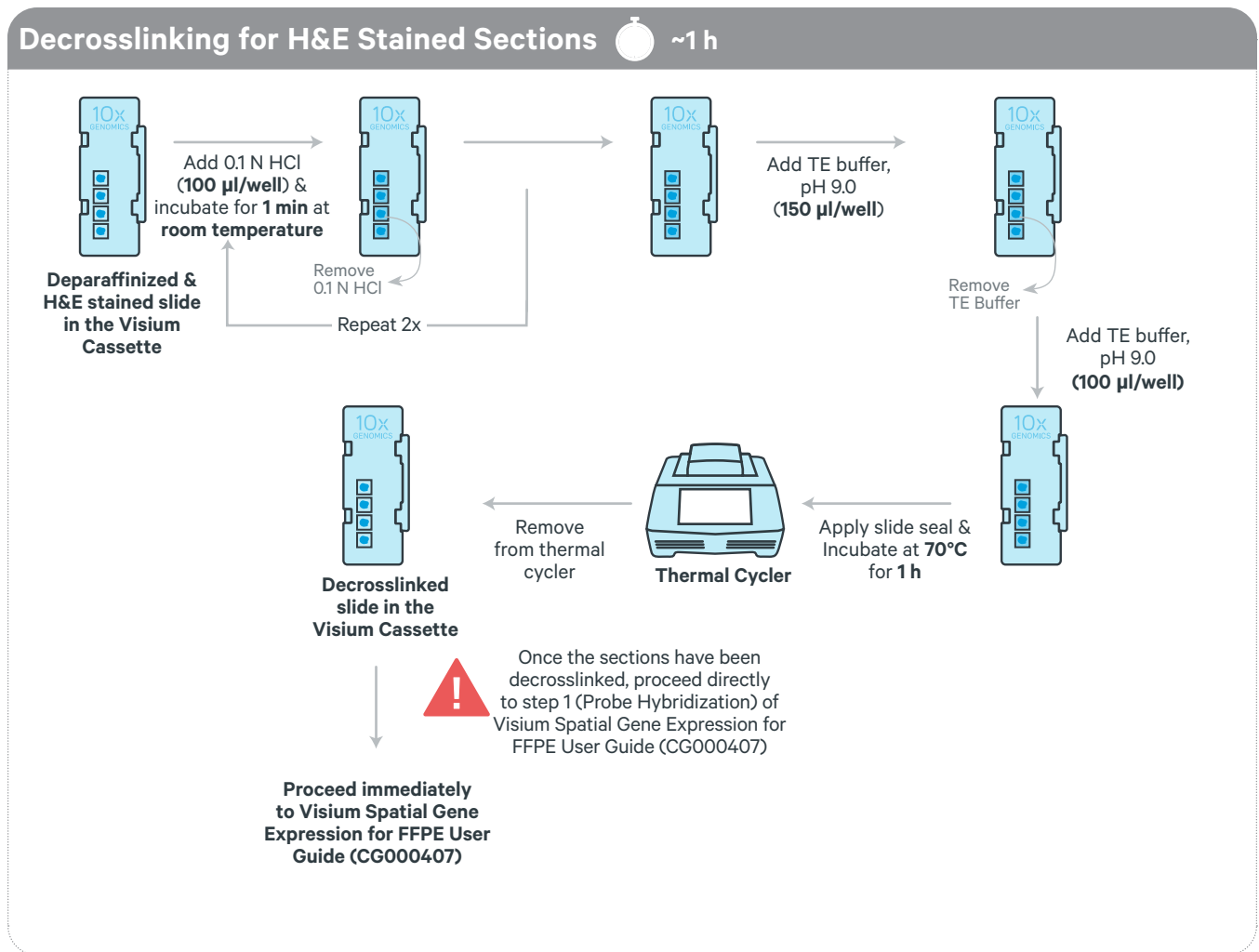
This chapter provides guidance on performing decrosslinking to release RNA that was sequestered by the formalin fixation. Ensure that the coverslip is removed before starting decrosslinking. Once the sections have been decrosslinked, step 1 (Probe Hybridization) of Visium Spatial Gene Expression for FFPE User Guide (CG000407) should be immediately performed.



3.1 Preparation - Buffers

| For Decrosslinking | |
|--|--|
| Items | Preparation & Handling |
| <input type="checkbox"/> TE Buffer, pH 9.0 | |
| Or | |
| <input type="checkbox"/> Prepare TE Buffer, pH 9.0 | TE Buffer Preparation (Prepare fresh and maintain at room temperature) <ul style="list-style-type: none"> • Dissolve 1.21 g Tris base in 950 ml nuclease-free water. • Adjust the pH to 9.0 with 1.0 M HCl. • Add 2 ml of 0.5 M EDTA and adjust the pH to 9.0 using 1.0 M HCl. • Bring the volume to 1000 ml using nuclease-free water. |
| <input type="checkbox"/> 0.1 N HCl | |

Protocol Overview




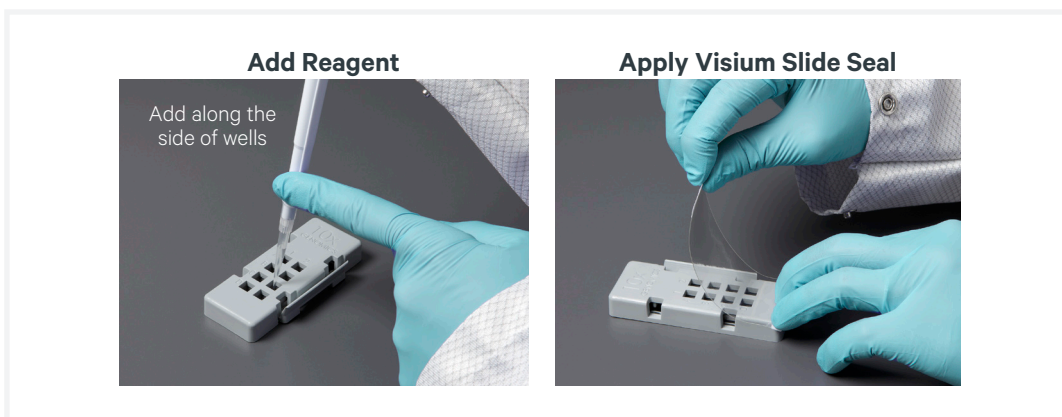
3.2 Decrosslinking for H&E Stained sections

- a. Place a Thermocycler Adaptor in the thermal cycler. Prepare the thermal cycler with the following incubation protocol and start the program.
- b. Prepare the thermal cycler with the following incubation protocol and start the program.

| Lid Temperature | Reaction Volume | Run Time |
|-----------------|-----------------|----------|
| 70°C | - | 60 min |
| Step | Temperature | Time |
| Pre-equilibrate | 70°C | Hold |
| Decrosslinking | 70°C | 00:60:00 |
| Hold | 22°C | Hold |

TIPS

- c. Place the slide in the Visium Cassette.
See Tips & Best Practices for assembly instructions. Practice assembly with a blank slide.
- d. Add **100 µl** 0.1 N HCl along the side of the wells to uniformly cover the tissue sections, without introducing bubbles. Tap Visium Cassette gently to ensure uniform coverage.
- e. Incubate for **1 min** at **room temperature**.
- f. Using a pipette, remove all the HCl from the well corners.
- g. Repeat d-f 2x for a total of 3 washes.
- h. Add **150 µl** TE Buffer (pH 9.0) along the side of the wells.
- i. Remove all TE Buffer from the wells.
- j. Add **100 µl** TE Buffer (pH 9.0) along the side of the wells.
- k. Apply Visium Slide Seal on the Visium Cassette and place the cassette on the Thermocycler Adaptor at **70°C**.
- l. Close the thermal cycler lid. Skip Pre-equilibrate step and initiate Decrosslinking.
- m. Once the Decrosslinking is complete, remove the cassette from the Thermocycler Adaptor and place on a flat, clean work surface.
-  n. Proceed **immediately** to Visium Spatial Gene Expression for FFPE User Guide (CG000407).



Troubleshooting

| STEP | Notes |
|--------------------------|---|
| Tissue Detachment | <ul style="list-style-type: none">If tissue detachment is observed during the workflow, contact support@10xgenomics.com. |

Document Revision Summary

| | |
|-------------------------|---|
| Document Number | CG000409 |
| Title | Visium Spatial Gene Expression for FFPE – Deparaffinization, H&E Staining, Imaging & Decrosslinking |
| Revision | Rev A to Rev B |
| Revision Date | October 2021 |
| General Changes | Updated for general minor consistency of language and terms throughout |
| Specific Changes | Added guidance on using xylene-resistant gloves and forceps for deparaffinization. Updated alcoholic Eosin staining process in H&E staining |

LEGAL NOTICE © 2021 10x Genomics, Inc. (10x Genomics). All rights reserved. Duplication and/or reproduction of all or any portion of this document without the express written consent of 10x Genomics, is strictly forbidden. Nothing contained herein shall constitute any warranty, express or implied, as to the performance of any products described herein. Any and all warranties applicable to any products are set forth in the applicable terms and conditions of sale accompanying the purchase of such product. 10x Genomics provides no warranty and hereby disclaims any and all warranties as to the use of any third-party products or protocols described herein. The use of products described herein is subject to certain restrictions as set forth in the applicable terms and conditions of sale accompanying the purchase of such product. A non-exhaustive list of 10x Genomics' marks, many of which are registered in the United States and other countries can be viewed at: www.10xgenomics.com/trademarks. 10x Genomics may refer to the products or services offered by other companies by their brand name or company name solely for clarity, and does not claim any rights in those third-party marks or names. 10x Genomics products may be covered by one or more of the patents as indicated at: www.10xgenomics.com/patents. The use of products described herein is subject to 10x Genomics Terms and Conditions of Sale, available at www.10xgenomics.com/legal-notice, or such other terms that have been agreed to in writing between 10x Genomics and user. All products and services described herein are intended FOR RESEARCH USE ONLY and NOT FOR USE IN DIAGNOSTIC PROCEDURES.

The use of 10x Genomics products in practicing the methods set forth herein has not been validated by 10x Genomics, and such non-validated use is NOT COVERED BY 10X GENOMICS STANDARD WARRANTY, AND 10X GENOMICS HEREBY DISCLAIMS ANY AND ALL WARRANTIES FOR SUCH USE. Nothing in this document should be construed as altering, waiving or amending in any manner 10x Genomics terms and conditions of sale for the Chromium Controller or the Chromium Single Cell Controller, consumables or software, including without limitation such terms and conditions relating to certain use restrictions, limited license, warranty and limitation of liability, and nothing in this document shall be deemed to be Documentation, as that term is set forth in such terms and conditions of sale. Nothing in this document shall be construed as any representation by 10x Genomics that it currently or will at any time in the future offer or in any way support any application set forth herein.

Contact:support@10xgenomics.com

10x Genomics

6230 Stoneridge Mall Road

Pleasanton, CA 94588 USA

