Visium Spatial Gene Expression for FFPE – Tissue Preparation Guide

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. Proper tissue handling and preparation techniques preserve the morphological quality of the tissue sections and the integrity of mRNA transcripts. Maintaining tissue adhesion and high-quality RNA is critical to assay performance.

The Tissue Preparation Guide provides guidance on:

- Best practices for handling tissue samples and Visium Spatial slides before and after sectioning.
- Sectioning of tissue samples and placement of sections on Visium Spatial slides.
- Performing RNA quality assessment of FFPE tissue blocks.

Contents

Additional Guidance

This protocol is compatible with most tissue types. Modifications to this protocol (e.g. section flotation time and water bath temperature) may be required for the preparation of certain tissue blocks and difficult tissue types, such as breast, colon, skin, and lungs. Refer to the 10x Genomics Support website for additional resources, including How-to Videos.

The slides prepared using this Tissue Preparation Guide can be used with:

- Visium Spatial Gene Expression for FFPE – Deparaffinization, H&E Staining, Imaging & Decrosslinking (CG000409)
- Visium Spatial Gene Expression for FFPE – Deparaffinization, Decrosslinking, IF Staining & Imaging (CG000410)

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
store at ambient temperature

	#	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
store at ambient temperature

	#	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 Tissue Section Test Slides, 4 Pack PN-1000347

Visium
Tissue Section Test Slides
4 Pack, PN-1000347
store at ambient temperature

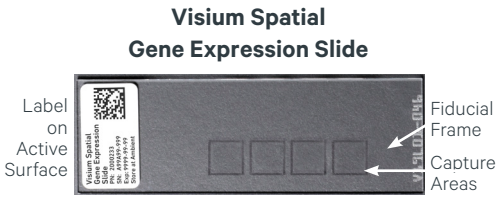
	#	PN
Visium Tissue Section Test Slide	4	2000460

10x GENOMICS

Visium Spatial Slides

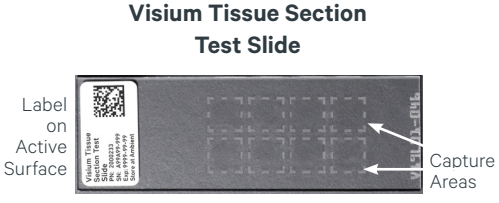
Visium Spatial Gene Expression Slide (PN-2000233)

- Used with Visium Spatial Gene Expression for FFPE User Guide (CG000407) to generate Visium Spatial Gene Expression – FFPE libraries.
- Includes 4 Capture Areas, each with ~5,000 unique gene expression spots.
- Each Capture Area is 6.5 x 6.5 mm and is surrounded by a fiducial frame for a total area of 8 x 8 mm.
- A readable label with a serial number defines the active surface of the slide. Tissue sections are always placed on the Capture Areas on the active surface.



Visium Tissue Section Test Slide (PN-2000460)

- Used to perform Tissue Adhesion Test.
- Includes 8 Capture Areas etched on the surface.
- Each Capture Area is 8 x 8 mm.
- The Capture Areas do not contain any oligonucleotides.
- A readable label with a serial number defines the active surface of the slide. Tissue sections are always placed on the Capture Areas on the active surface.



Tips & Best Practices

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Sample Preparation

- It is recommended to store FFPE tissue blocks at 4°C and to avoid exposure to direct light to ensure even chilling and to preserve RNA integrity.

RNA Quality Assessment

- Assess RNA quality of the tissue block before proceeding with sectioning by calculating the percentage of total RNA fragments >200 nucleotides (DV200) of RNA extracted from tissue sections. See RNA Quality Assessment for details on RNA extraction and DV200 calculation.
- Various factors could lead to variations in DV200 scores, such as specific tissue types, diseased or necrotic tissues, sample preparation, and handling.

Tissue Trimming & Scoring

A tissue section of ≤6.5 x 6.5 mm is compatible with Visium Spatial slides.

- **FFPE Tissue Blocks ≤6.5 x 6.5 mm:** Paraffin around the embedded tissue in the FFPE tissue block should not be trimmed, and the section will fit the Capture Areas.
- **FFPE Tissue Blocks >6.5 x 6.5 mm & Tissue ≤6.5 x 6.5 mm:** Paraffin around the embedded tissue in the FFPE tissue block can be trimmed for the section to fit the Capture Areas.
- **FFPE Tissue Blocks & Tissues >6.5 x 6.5 mm:** 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 Capture Areas.
- Use a razor blade for trimming and scoring the tissue Block. See Trimming/Scoring the Block for details.
- Once the tissue has been trimmed and scored, use extra care during sectioning and section handling.

Section Thickness

- Recommended section thickness is 5 µm.

Water Bath Temperature & Section Floating Time

- Optimal water bath temperature and section floating time are critical for tissue section expansion. 42ºC 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 before tissue placement on the Visium slides by practicing section placement on a plain glass slide.
- 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.

Practice Section Placement

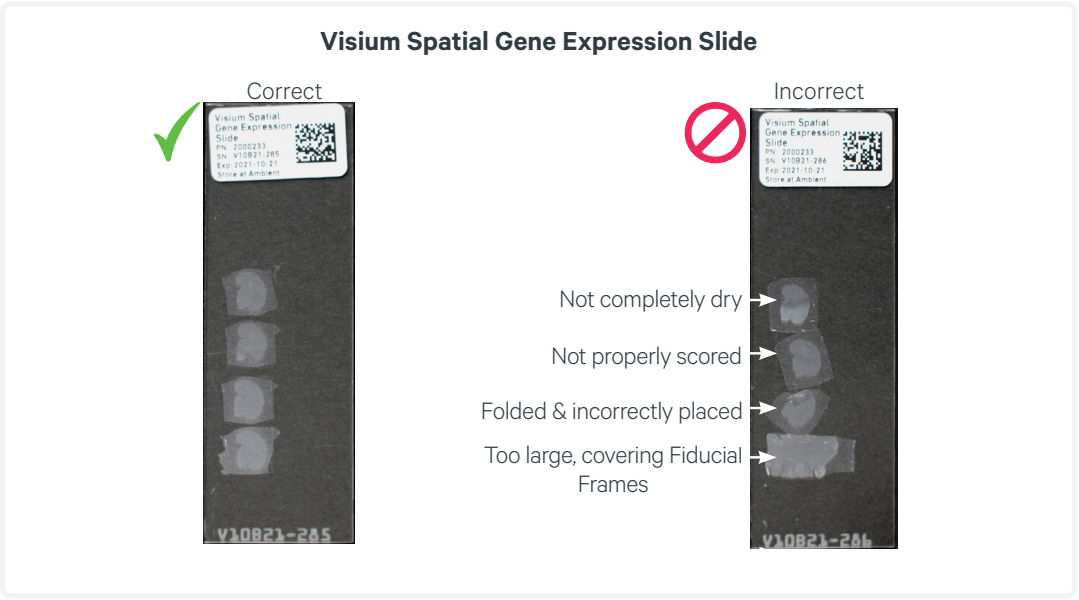
- Create representative frames on a 75 x 25 x 1 mm plain glass slide using the Visium Spatial Slide Layout (see Appendix).
- Frames should be drawn on the back of the slide.
- Practice correct section placement within the representative frames using non-experimental blocks.

Section Attachment on Visium Slides

- Section quality can affect the section attachment on the Visium slides.
- Carefully inspect the tissue block to gauge the extent of dehydration. Allow sufficient time in the ice bath to ensure proper hydration.
- Use a new, clean blade for sectioning. Inspect the blade after every 20-25 sections and adjust to the blade areas that are not nicked or rough. Replace the blade after ~50 sections.
- Perform sectioning in a continuous motion to get a ribbon of sections. The sections should be separated during floating in the water bath.
- Sections should have the same thickness.
- Allow the section to float in the water bath until it is free of folds and wrinkles.
- Tissue detachment may occur due to factors such as the quality of paraffin used during the tissue embedding process, the age of the tissue block, tissue section thickness, and the length of time used to infiltrate a tissue in paraffin.
- To easily visualize the Fiducial Frames on the Visium Slide during tissue section placement, trace the fiducial frame(s) on the back of the Visium slide with a permanent marker. After tissue drying, carefully remove trace with a laboratory wipe soaked in 100% ethanol. Avoid touching active surface of the slide.

Sections Placement on Visium Slides

- Place the tissue section within the fiducial frame or the etched frames of the Capture Area on the Visium Spatial slides. Avoid covering the frames of the Capture Areas with the tissue.
- The section on the slides should be uniform without any cracks, tears, or folds.
- Only one section should be placed within each Capture Area.
- Always start the section placement with the topmost Capture Area.



Handling Visium Slides

Handling Visium Spatial Slides Containing Tissue Sections

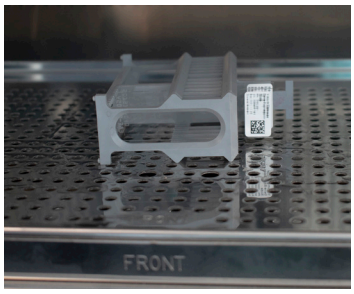
- Maintain slides containing tissue sections in a low moisture environment such as a desiccator and avoid exposure to direct light.
- Keep slides at room temperature.
- Slides containing the tissue sections that have been incubated at 42°C for 3 h and dried overnight at room temperature in a desiccator can be stored for up to 2 weeks at room temperature in a desiccator.

Visium Slides Incubation

Incubation using a Section Dryer Oven:

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

Place in a Rack

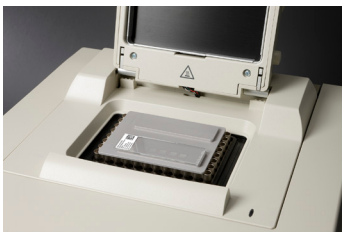


Incubation using a Thermocycler:

- 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.
- DO NOT close the thermal cycler lid when incubating the slide.

Place Thermocycler Adaptor

Incubate Slide



Specific Reagents & Consumables

For FFPE Tissue Sectioning & Section Placement			
Item	**Alternatives/Options**	**Vendor**	**Part Number**
Microtome	Epredia HM 355S Automatic Microtome *Or any standard histology grade microtome*	Fisher Scientific	23-900-672
Microtome blade	Epredia MX35 Premier Disposable Microtome Blades, Low Profile	Fisher Scientific	3052835
Cool-Cut, *Optional*	Thermo Scientific Cool-Cut, *Optional*	Fisher Scientific	77-112-0
Section transfer system (STS)	Thermo Scientific Section Transfer System (STS), *Optional - If using Section Transfer System*	Fisher Scientific	771200
Probes	Fisherbrand Fine Precision Probe	Fisher Scientific	12-000-153
Forceps	Fisherbrand Curved Medium Point General Purpose Forceps	Fisher Scientific	16-100-110
Microscope slides	Fisherbrand Premium Plain Glass Microscope Slides	Geyer	194242
Water bath	Premiere Tissue Floating Bath, Lighted *Or any equivalent water bath*	Fisher Scientific	A84600061
Section dryer oven	Epredia High Capacity Section Dryer	Fisher Scientific	A84600051
Brushes	Camel Hair Brushes, *Or any equivalent paintbrush*	Ted Pella	11859
Additional Materials			
Razor blades		-	-
Ice bucket		-	-
Ultrapure/Milli-Q Water for Water Bath, *from Milli-Q Integral Ultrapure Water System or equivalent*		-	-
For RNA Quality Assessment			
Ethanol	Ethyl Alcohol, Pure (200 Proof, anhydrous)	Millipore Sigma	E7023-500ML
RNA extraction kit	RNeasy FFPE Kit (50)	Qiagen	73504
Deparaffinization solution	Deparaffinization Solution	Qiagen	19093
RNase decontamination solution	RNaseZap RNase Decontamination Solution	Thermo Fisher Scientific	AM9780
Nuclease-free water	Nuclease-free Water (not DEPC-Treated)	Thermo Fisher Scientific	AM9937
1.5-ml centrifuge tubes	DNA LoBind Tubes, 1.5 ml	Eppendorf	022431021
Nanodrop/Qubit fluorometer	Nanodrop 2000c Spectrophotometers *Or any equivalent Nanodrop* *Alternative to Qubit Fluorometer*	Thermo Fisher Scientific	ND-2000C
	Qubit RNA BR Assay Kit	Thermo Fisher Scientific	Q10210
	Qubit Assay Tubes	Thermo Fisher Scientific	Q32856
	Qubit 4 Fluorometer	Thermo Fisher Scientific	Q33238
Bioanalyzer/Tapestation Choose Bioanalyzer or TapeStation based on availability & preference.	RNA 6000 Pico Kit	Agilent	5067-1513
	2100 Bioanalyzer Laptop Bundle	Agilent	G2953CA
	4200 TapeStation	Agilent	G2991AA
	High Sensitivity RNA ScreenTape	Agilent	5067-5579
	High Sensitivity RNA ScreenTape Ladder	Agilent	5067-5581
	High Sensitivity RNA ScreenTape Sample Buffer	Agilent	5067-5580

1. FFPE Tissue Sectioning & Section Placement

Overview

This chapter provides guidance on sectioning FFPE tissue blocks using a microtome and section placement on the Visium Spatial Slides using a water bath. A Section Transfer System (STS) can also be used for section placement.

Exposing the Tissue

FFPE tissue block is placed in a microtome and cut to expose the tissue or face the block.

RNA Quality Assessment

Once the tissue is exposed, RNA quality of the tissue is assessed by calculating DV200 of RNA extracted from freshly collected tissue sections.

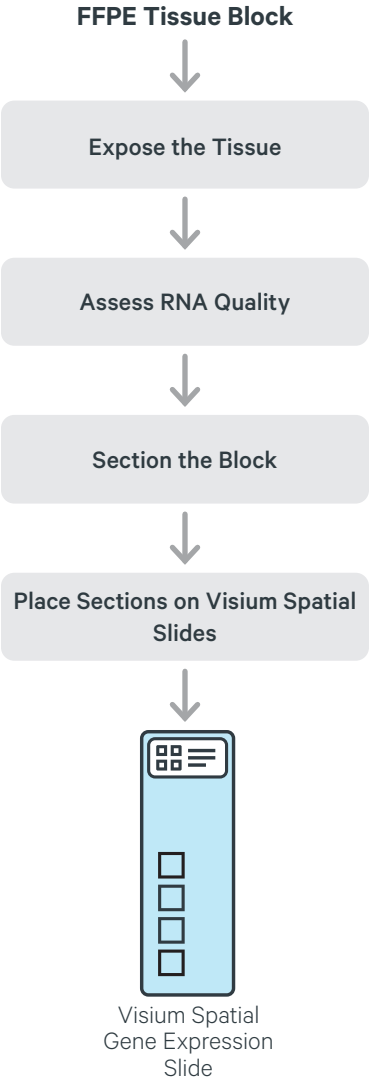
Sectioning

The tissue block with exposed tissue is trimmed or scored to make it compatible in size to the Capture Areas on Visium slides. The tissue block is then sectioned by a microtome to generate appropriately sized sections for Visium slides.

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 sections are then placed within the frames of Capture Areas on Visium Spatial slides.

Practice section placement on a plain glass slide before proceeding with Visium slides. Only one section should be placed within each Capture Area.



1.1 Exposing the Tissue or Facing the Block

Before starting, wipe down all the surfaces and work areas with RNase Zap RNase decontaminating solution.

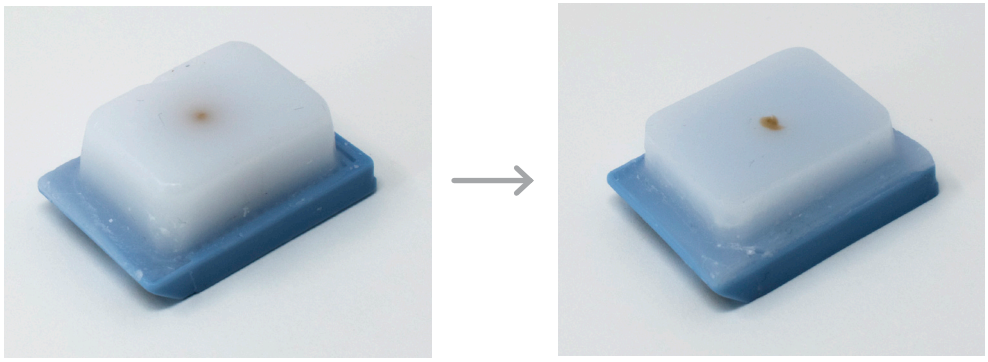
a. Remove tissue blocks from storage. For a tissue block with already exposed tissue, proceed directly to RNA Quality Assessment.

b. Set the microtome to the 15 µm setting.

c. Place tissue block on the specimen clamp.

d. Cut the tissue block at 15 µm until all of the edges of the tissue are exposed or until the region of interest is exposed. The block should be at **room temperature** during cutting.

Exposing the Tissue Block



1.2 RNA Quality Assessment of FFPE Tissue Block

This section provides guidance on determining the RNA quality of the tissue by calculating DV200 of RNA extracted from freshly collected tissue sections.

Mean RNA fragment size is a reliable determinant of RNA quality. This requires a measurement of the percentage of total RNA fragments >200 nucleotides (DV200) for RNA quality assessment upstream of library preparation. See the Appendix for details on the impact of DV200 on library sensitivity.

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. Set the microtome to the 10 µm setting and collect tissue sections of 10 um thickness for RNA extraction. Discard the first few sections if the block was already exposed. The number of sections needed depends upon the tissue size. Refer to the Qiagen RNeasy FFPE Kit protocol to determine the appropriate number of sections. See below for guidance:

- Collect ~4 sections for smaller tissues (≤6.5 x 6.5 mm)
- Collect 1-2 sections for larger tissues (≥6.5 x 6.5 mm)

b. Place the sections inside a **pre-cooled** microcentrifuge tube.

c. Proceed to RNA extraction using Qiagen RNeasy FFPE Kit. Follow the manufacturer's recommendation for RNA extraction.

d. Using a Nanodrop or Qubit Fluorometer, measure RNA concentration to determine the appropriate dilution for DV200 evaluation using the RNA 6000 Pico Kit.

e. Store purified RNA at **–80°C** for **long-term** storage or **immediately** proceed to DV200 evaluation using either Agilent RNA 6000 Pico Kit or TapeStation. Follow manufacturer's instructions (Agilent) for DV200 evaluation.

1.3 Sectioning

Trimming/Scoring the Block

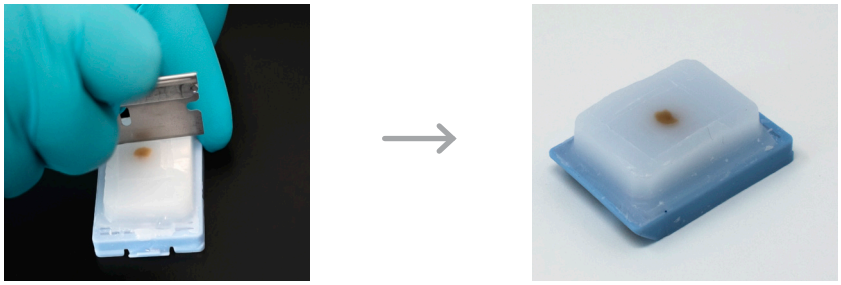
Before starting, wipe down all the surfaces and work areas with RNaseZap RNase decontaminating solution. A tissue section of ≤6.5 x 6.5 mm is compatible with Visium Spatial slides.

FFPE Tissue Block >6.5 x 6.5 mm & Tissue ≤6.5 x 6.5 mm

For tissue samples that fit the Capture 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 Capture Areas.

- After facing, remove the tissue block from the microtome and start trimming.
- Using a razor blade, remove excess paraffin from around the tissue, so it is not larger than the Fiducial Frame.
- Retain paraffin edges around the tissue samples to enhance tissue attachment on the slide.

Trimming large tissue block containing tissues that fit the Capture Areas



FFPE Tissue Block & Tissue >6.5 x 6.5 mm

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

- Remove the tissue block from the microtome and start scoring.
- To score, glide a razor blade over the surface of the tissue to introduce a shallow cut. A deep incision may lead to tissue damage and disintegration.

Scoring tissues larger than the Capture Areas

Section Collection:

a. Place blocks in the ice bath, ensuring that the tissue part is fully submerged.

b. Incubate on the ice bath for **10-30 min**. The incubation time depends upon the tissue type and the extent of dehydration.

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.

FFPE Tissue Block in Ice Bath

c. Carefully wipe off the excess oils from a 35X Ultra disposable blade using a laboratory wipe with 100% ethanol. Let the ethanol evaporate before proceeding. Always use new blades for sectioning.

d. Secure the blade in the disposable blade holder of the microtome and place the knife guard over the blade to minimize injury. Ensure that the clearance angle is set to 10°.

e. After hydration is complete, place the tissue block in the specimen clamp and align it with the blade.

f. Fill up a water bath with Milli-Q water and ensure that the temperature is set at **42°C** and free from particulates by gliding a laboratory wipe over the water surface. Repeat this step between sectioning as and when necessary.

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 before tissue placement on the Visium slides by practicing section placement on a plain glass 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 Particulates

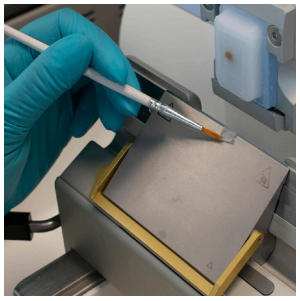
g. Set the microtome to 5 µm for tissue sectioning and begin sectioning. For tissue blocks with exposed tissue, discard the first few sections and start collection on the subsequent sections.

h. To collect sections, place the paintbrush tip slightly above and parallel to the blade. Lift the section by lightly touching the edge with the paintbrush while rotating the wheel handle.

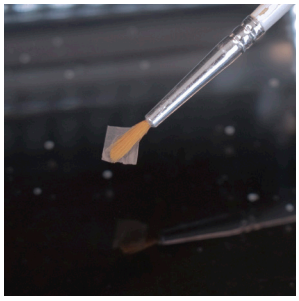
i. With the help of the brush, pick the section up. Immediately place it on the water surface of the water bath, making sure that the brush tip goes underneath and away from the section.

j. Let the section float on the water bath surface until most of it is flat and without wrinkles. The floating time depends upon the sample type. If the section is too creased or puckered, let it float longer.

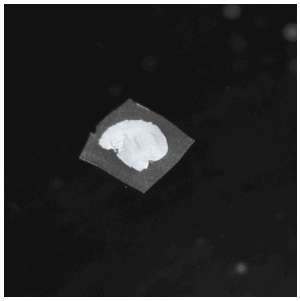
Lift the section with the paint brush



Place section in the water bath



Let the section float in the water bath



k. Float the section on a plain glass slide and perform a quality check under a microscope. Use the following table for guidance:

Section Appearance	Floating Time
Section is flat with no wrinkles	Sufficient floating
Section has wrinkles	Insufficient floating; increase the floating time
Section is torn	Section left too long; decrease the floating time

See Troubleshooting section for additional phenotypes.

l. After determining the appropriate floating time, proceed to Section Placement on Visium slides.

Some tissue sections may take more or less time to smooth out, and some may never be 100% wrinkle-free. The section can disintegrate if left for an extended period in the water bath.

1.4 Section Placement on Visium Spatial Slides

a. Before proceeding with Visium Spatial slides, practice section placement on plain glass slides (see Appendix for Visium Spatial slide layout).
To enhance the Capture Areas Visibility, trace fiducial frames on the back of the slide with a permanent marker to mimic the Visium Spatial Gene Expression slides. Carefully remove trace with a laboratory wipe soaked in 100% ethanol prior to Deparaffinization. Avoid touching active surface of the slide.

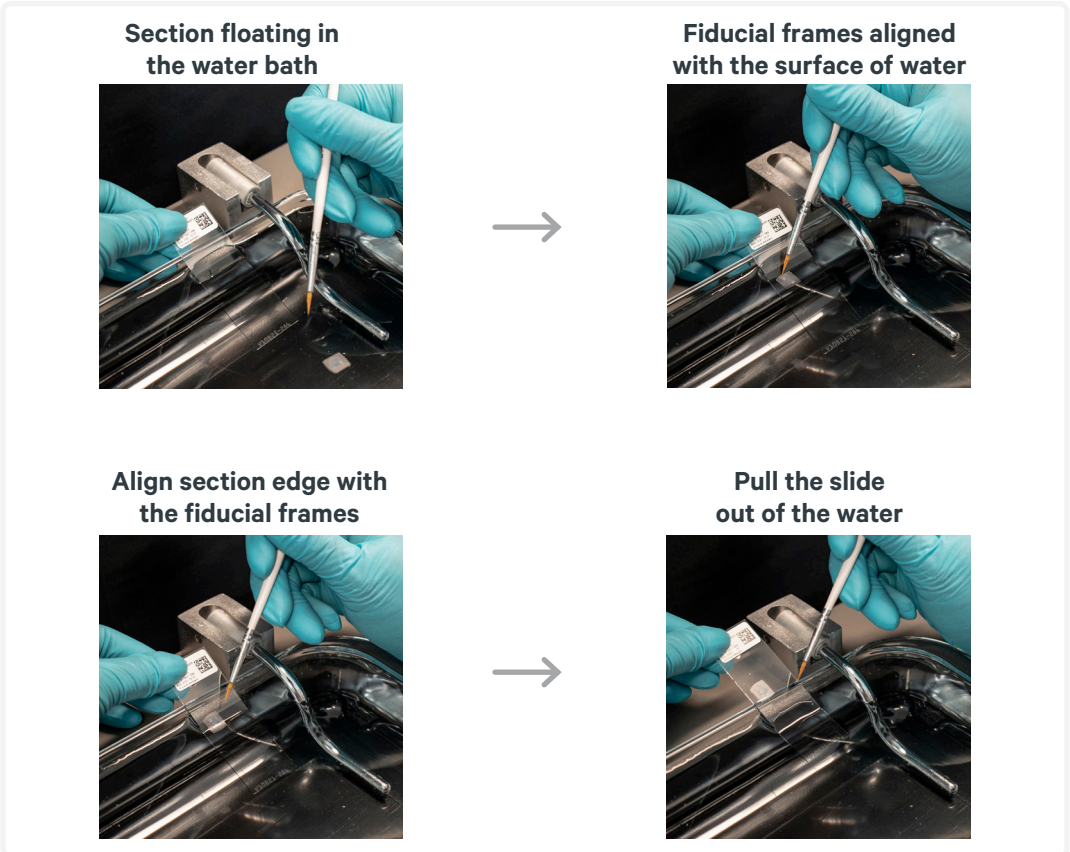
b. Transfer sections to the water bath and let them float for the time determined previously.

c. Insert the slide into the water and align the fiducial frames with the surface of the water while keeping the slide straight.

d. Using the paintbrush or the probe, maneuver the section to the top of the Capture Area on the slide. The edge of the section must align with the fiducial frame, which is aligned with the water surface.
If sections float away from the slide, Visium slide can also be dipped into the water bath before section placement.

e. Pull the slide up and out of the water, ensuring there are no air bubbles trapped underneath and set aside. Place sections on the topmost Capture Area first.

f. Repeat for the rest of the Capture Areas, ensuring that the previous sections do not get re-submerged in the water.



g. Place the slides in a slide drying rack and incubate for **3 h** in an oven at **42°C.** Alternatively, a slide warmer or a thermo cycler set at **42°C** can be used for drying.

See Tips & Best Practices for guidance on slide incubation.

h. Place in a desiccator and keep overnight at **room temperature** to ensure proper drying.

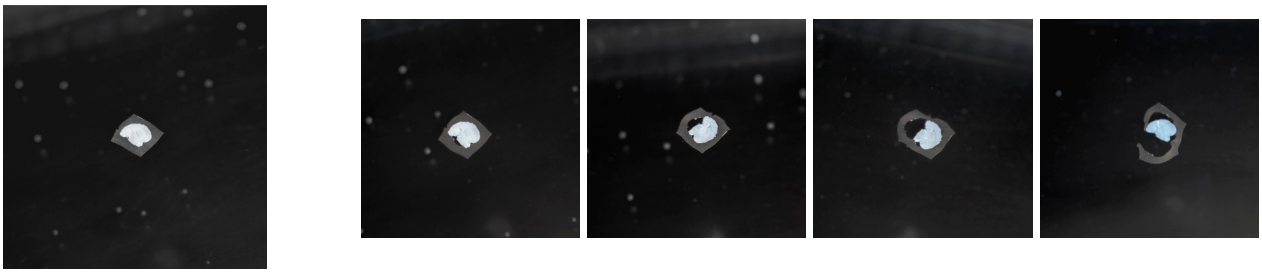
i. After overnight drying, proceed to deparaffinization and staining protocols (see References) or store the slide containing dry tissue sections at **room temperature** in a desiccator for up to **2 weeks**.

Troubleshooting

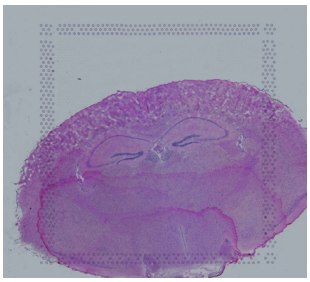
Ideal Floating Time Determination

Ideal Floating Time

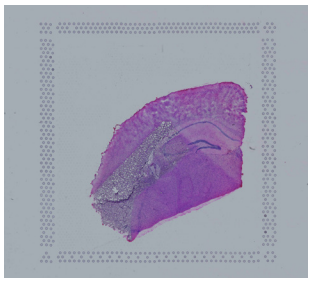
Section disintegration due to increased floating time



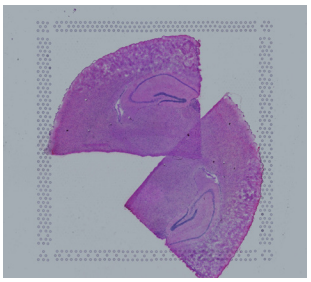
Incorrect Placement of Tissue Sections



Fiducial frames covered



Folded tissue section

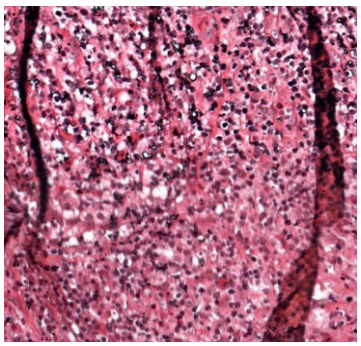


Overlapping sections

Practice correct section placement on blank glass slides before proceeding with Visium Spatial slides.
See Appendix for Visium Spatial slide layout.

Common Artifacts that cause Detachment or Misleading Data

Wrinkles



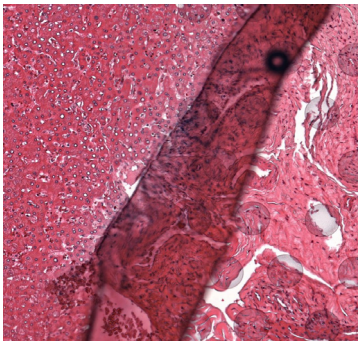
Causes

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

Troubleshooting

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

Folds



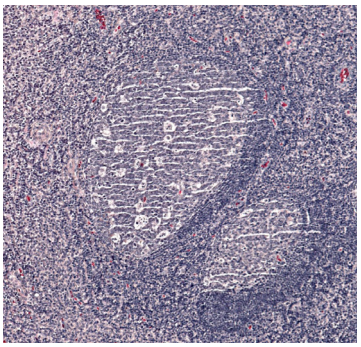
Causes

- Mostly happens when a wavy section is placed on the water bath.
- Folds at the edge mostly happen during sectioning or section placement on the slide.

Troubleshooting

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

Venetian Blinds or Shatter



Causes

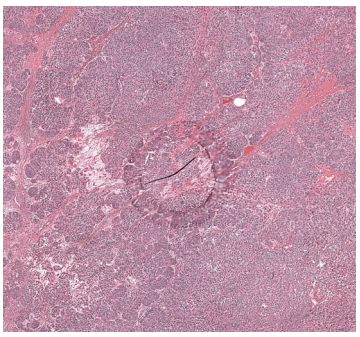
- Parallel lines in the section mostly appear due to dry tissue because of under-hydration of the block in the ice bath.
- Less likely due to dull blade or loose parts of the microtome.

Troubleshooting

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

Common Artifacts that cause Detachment or Misleading 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.

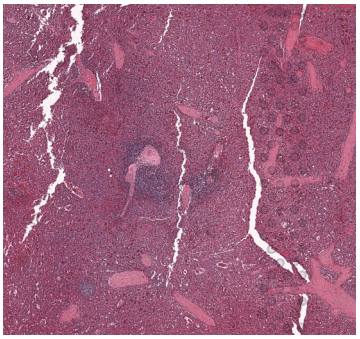
Causes

- 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

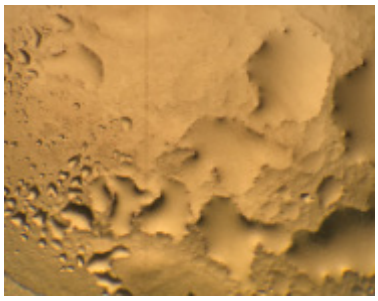
- Dry and over-processed tissue can crack during sectioning.
- The cracks that are created before tissue embedding will be filled with wax when section is observed under the microscope after sectioning and wax will be washed away after deparaffinization or H&E staining.

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 Data

Sweating



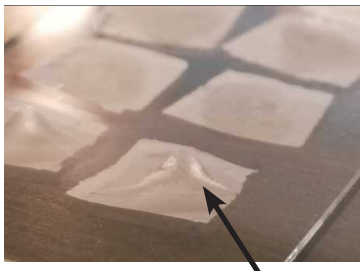
Causes

- 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



Water retention under the section

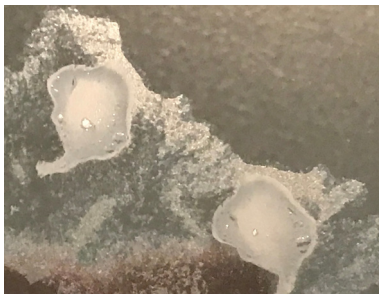
Causes

- Sections from tissues that are under-processed or excessively hydrated before sectioning may retain water under the section.
- Water will accumulate under the 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 cause 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).
- Water bath temperature should be lowered to 38-40°C.

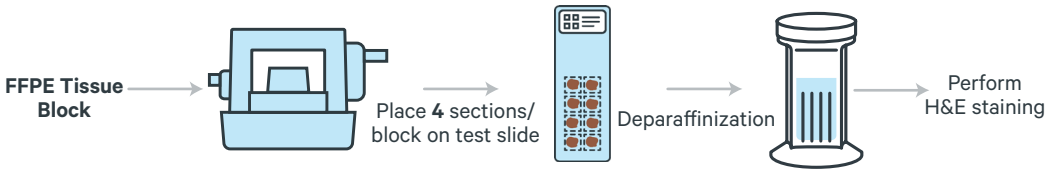
Tissue Adhesion Test

Tissue adhesion to the surface of the Visium Spatial slides may be different than is observed with other tissue-based assays. Fixation and embedding, tissue type, sectioning, and section placement can affect the adhesion of tissue during the Visium Spatial Gene Expression for FFPE workflow. If needed, the Tissue Adhesion Test can be used to troubleshoot detachment of FFPE sections on Visium slides. The Tissue Adhesion Test is not an optimization experiment where protocol steps can be modified to improve attachment, as the protocol is already optimized for tissue adhesion.

Tissue Adhesion Test:

The Tissue Adhesion Test involves placing four sections per tissue block onto the Visium Tissue Section Test Slide and performing deparaffinization and H&E staining. Stained slides are then visually inspected to assess tissue section adherence on the test slide. This test is only compatible with H&E staining.

Tissue Adhesion Test Workflow



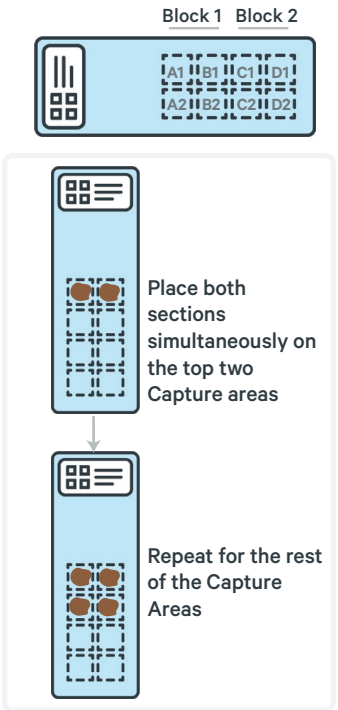
a. Set the microtome to the 5 µm setting.

b. Collect tissue sections and transfer two sections to a water bath set to **42°C.** Let the sections float for a predetermined time.
See Section Collection for guidance on collecting and transferring sections and on determining appropriate floating time.

c. Place both sections nearly simultaneously on the top two Capture Areas (A1 and A2) on a Visium Tissue Section Test Slide.
See Section Placement for details on how to place sections on the slide. Pull the slide up and out of the water, ensuring there are no air bubbles trapped underneath and set aside.

d. **Repeat** b-d for the rest of the Capture Areas, ensuring that the previous sections do not get re-submerged in water. Place the next two sections on the Capture Areas B1 and B2.

e. Repeat for the next block. Place sections on C1 and C2, followed by D1 and D2 Capture Areas.



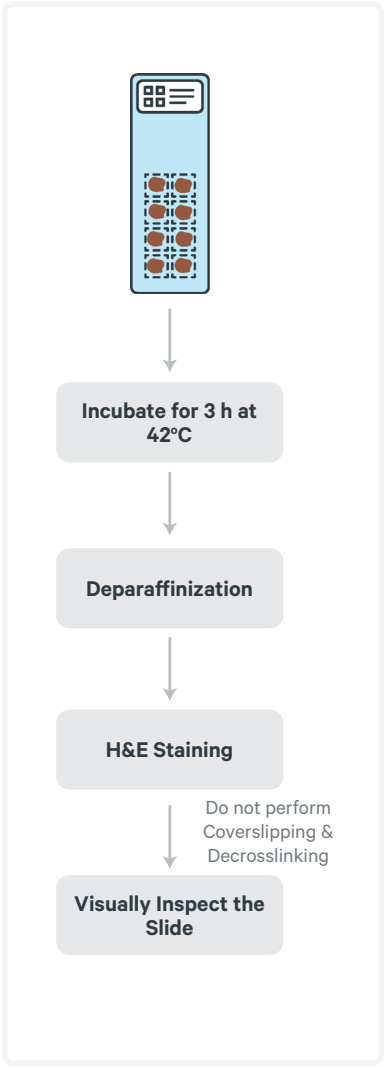
f. Place the slides containing sections in a slide drying rack and incubate for **3 h** in an oven at **42°C**.

See Tips & Best Practices for guidance on slide incubation.

g. Place in a desiccator and keep overnight at **room temperature** to ensure proper drying.

h. After overnight drying, perform deparaffinization and H&E staining. Refer to Demonstrated Protocol Deparaffinization, H&E Staining, Imaging & Decrosslinking for Visium Spatial for FFPE (CG000409) for guidance on deparaffinization and H&E staining.
After H&E staining, do not proceed to Coverslipping and Decrosslinking.

i. Perform qualitative assessment of tissue adhesion by visually inspecting the slide for tissue detachment.
See Appendix for details on qualitative assessment of tissue adhesion. Tissue Adhesion Test results should be interpreted by the naked eye. A picture can be taken to record the results.



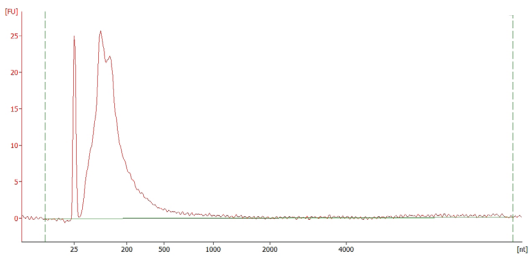
Appendix

Impact of DV200 on Library Sensitivity

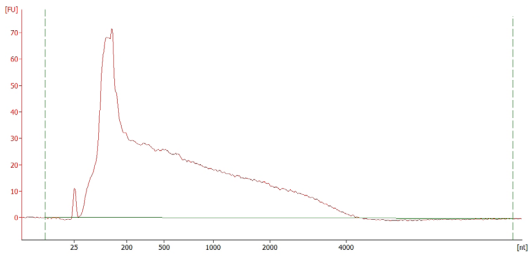
- Tissue with DV200 >50% are more likely to generate successful Visium Spatial Gene Expression – FFPE libraries.
- Shown below are bioanalyzer traces of RNA extracted from different tissue types for which DV200 was calculated.

Bioanalyzer Traces for Samples with Different DV200 Counts

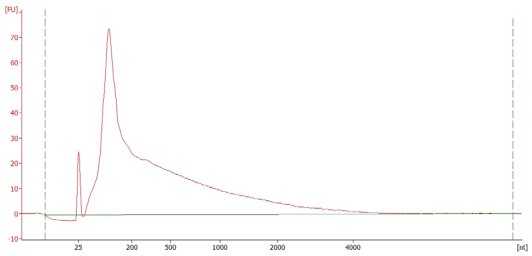
A. DV200 = 20%



B. DV200 = 61%



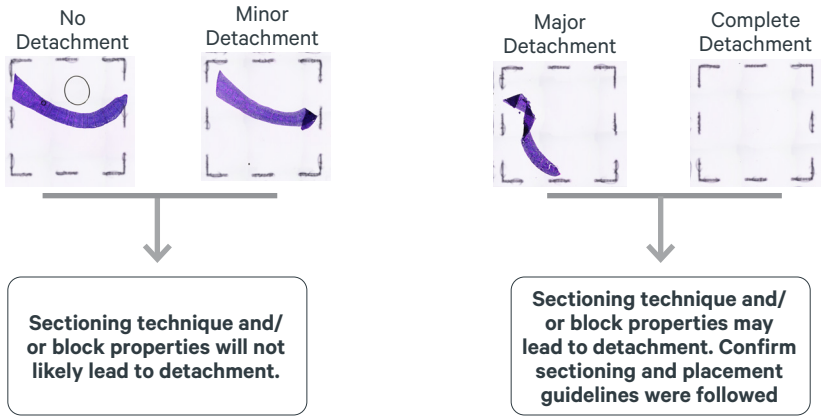
C. DV200 = 51%



Samples displayed are from DV200 <50% (A) and DV200 >50% (B and C). The Agilent RNA 6000 Pico kit was used for preparing the samples.

Qualitative Assessment of Tissue Adhesion

Qualitative Assessment of Tissue Adhesion



Interpretation of Results

Sections can experience no detachment or detachment (minor, major, and complete).

- If all sections from a block experience no or minor detachment, sample handling may have contributed to detachment on the Gene Expression Slide.
- If any number of sections from a block experience major or complete detachment, and best practices were followed, the block may be more prone to detachment.
- Contact 10x Genomics Support at support@10xgenomics.com if there is detachment on the Gene Expression Slide.

Impact of Tissue Detachment on Sequencing Data

The tissue detachment can result in:

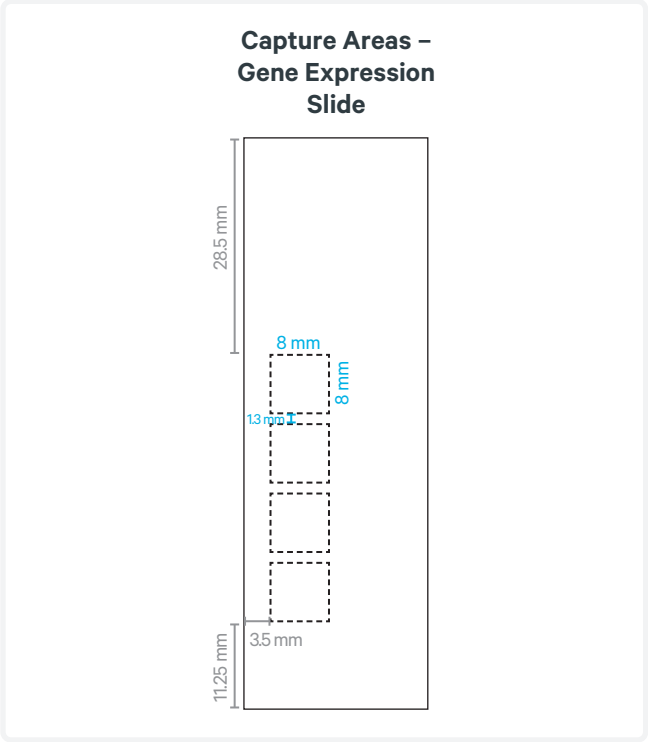
- A loss of gene expression complexity
- A loss of spatiality in Visium Spatial Gene Expression – FFPE libraries

Visium Spatial Slide Layout

A layout of Capture Areas of Visium Spatial slides is shown below and can be used to create representative frames on plain glass slides with dimensions (75 x 25 x 1 mm) similar to Visium slides to practice tissue section placement. The frames should be drawn on the back of the slide.

The slide dimensions represent a 75 x 25 x 1 mm laboratory glass slide; printer settings may impact the image scaling.

For Visium Spatial Gene Expression Slide, each Capture Area is 6.5 x 6.5 mm and is surrounded by a fiducial frame for a total area of 8 x 8 mm.



References

- Visium Spatial Gene Expression for FFPE – Deparaffinization, H&E Staining, Imaging & Decrosslinking (CG000409).
- Visium Spatial Gene Expression for FFPE – Deparaffinization, Decrosslinking, IF Staining & Imaging (CG000410).

Document Revision Summary

Document Number	CG000408
Title	Visium Spatial Gene Expression for FFPE – Tissue Preparation Guide
Revision	Rev C
Revision Date	February 2022
General Changes	Updated for general minor consistency of language and terms throughout
Specific Changes	Provided additional troubleshooting guidance

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