



StrataMap Spatial Transcriptome

User Guide

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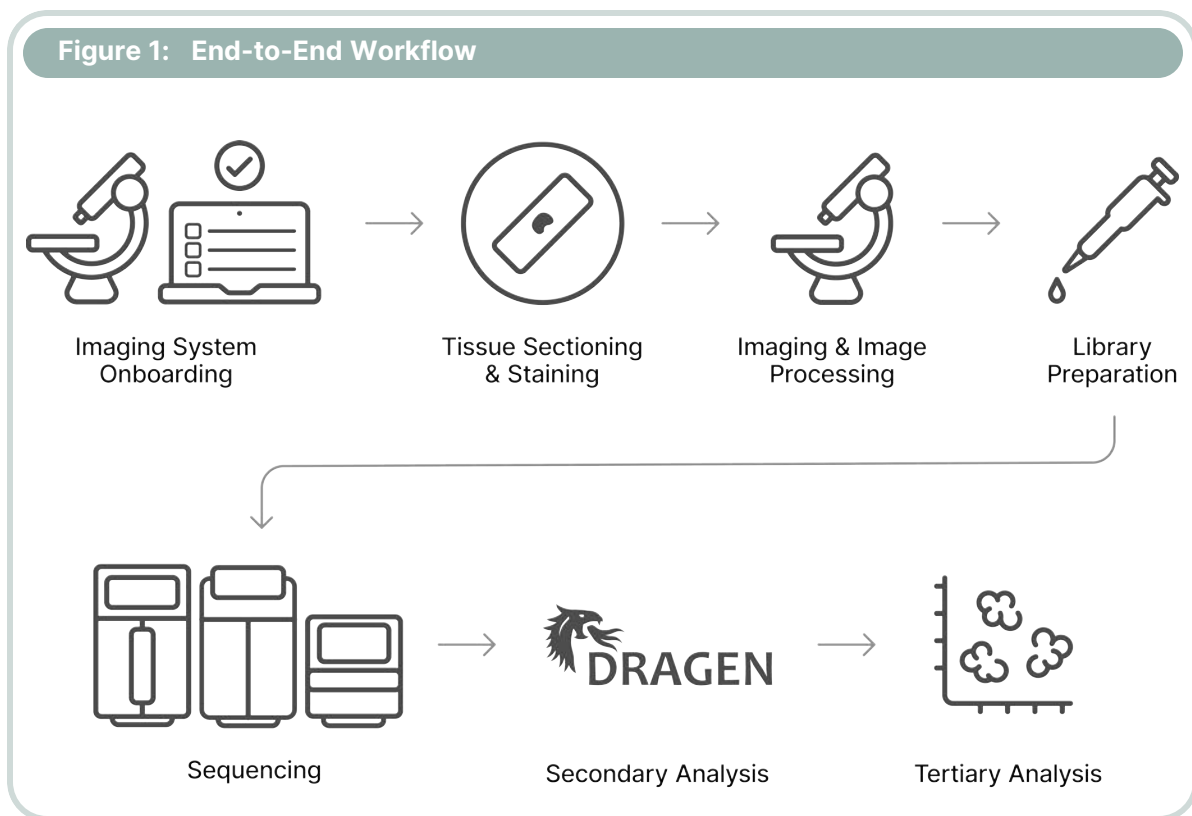
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1. Overview

This resource explains how to prepare libraries using the StrataMap Spatial Transcriptome assay. The entire assay workflow consists of the following actions:

- Assess your imaging system to confirm that it can work with the assay protocol
- Prepare and image tissue sections
- Create spatial libraries for sequencing
- Sequence using a NovaSeq X Series, NovaSeq 6000, or NextSeq 2000 system
- Secondary analysis using the DRAGEN StrataMap analysis software, BaseSpace Sequence Hub, and Illumina BioInsight Platform Core (Platform Core)
- Tertiary analysis using Illumina Connected Multiomics (ICM)

Figure 1 shows the end-to-end workflow for the StrataMap Spatial Transcriptome assay.



Imaging Assessment

- Before you begin the protocol, evaluate your imaging system by performing *Imaging Assessment* in the *StrataMap Spatial Transcriptome Imaging Guide* (document # 200081305) on the [Illumina support site](#).
 - This test confirms that fiducial markers are accurately imaged and compatible with analysis.
 - For more information on configuring Illumina StrataMap Image Tool (ISIT) for this test, refer to *Imaging Assessment* in the [Illumina StrataMap Spatial Software documentation](#).

Protocol

- The protocol uses the StrataMap Spatial Transcriptome kit to prepare tissue sections for imaging and create spatial libraries for sequencing.
 - For more information on the StrataMap Spatial Transcriptome kit, refer to [2.1 Product Contents on page 7](#).
 - For additional consumables and equipment needed for the protocol, refer to [2.2 User-Supplied Consumables and Equipment on page 10](#) and [2.3 Illumina Consumables and Equipment on page 14](#).
- The protocol is performed over the course of three days:
 - **Day 1**—The first day involves preparing the tissue and taking images of the tissue sections for analysis using your qualified imaging system. After imaging, you permeabilize the tissue, which releases the RNA from the cells, and then you can create cDNA that complements the RNA transcripts.
 - **Day 2**—The second day involves refining and isolating the cDNA to create the libraries needed for sequencing, which includes adding a second strand of cDNA. Then, you can elute and purify the cDNA before determining the optimal amplification cycle number.
 - **Day 3**—The third day is used to complete the sequencing preparation. Use the optimal amplification cycle number to amplify your library, which creates copies of each unique template for sequencing. After purifying the amplified library, check the quantity and quality of the library so that you can dilute it to the starting concentration needed for sequencing.

Sequencing

- The StrataMap Spatial Transcriptome assay is compatible with the following flow cells and workflows:
 - NovaSeq X Series 5B, 10B, and 25B flow cells using the NovaSeq X Series protocol
 - NovaSeq 6000 S2 and S4 flow cells using the Standard workflow
 - NovaSeq 6000 S4 flow cell using the Xp workflow
 - NextSeq 2000 P4 flow cell using the XLEAP-SBS reagents

- The sequencing system that should be used is dependent on the tissue amount and sequencing depth. To estimate the sequencing data output needed for your experiment, refer to [5.22 Sequencing and Analysis on page 103](#).
- If you are using the NovaSeq X Series system, you can also do run planning per lane.
- StrataMap Spatial Transcriptome libraries can be sequenced across all lanes of a flow cell or in individually addressable lanes.
 - If the library originates from one slide, sequencing data from multiple flow cells can be combined into one analysis.
 - If combining with other library types on the same flow cell, refer to [Illumina StrataMap Spatial Software documentation](#) for specific instructions.
 - For sequencing depth recommendations, refer to [3.2 Sequencing Recommendations on page 20](#).

Analysis

Secondary and tertiary analysis is used to process spatial sequencing data and images, perform additional analyses, and view the data.

Secondary Analysis

- Secondary analysis is done with the DRAGEN StrataMap analysis software, along with BaseSpace Sequence Hub and Platform Core.
- Secondary analysis consists of the following steps in the [Illumina StrataMap Spatial Software documentation](#):
 - **[Optional]** Cell segmentation can be initiated independently using the Illumina StrataMap Cell Segmentation Tool. This tool can be used after image acquisition and before proceeding with the assay protocol. You can confirm if the cell segmentation meets your experimental needs or if the images should be retaken.
 - Run setup and planning using auto or manual launch with Platform Core.

Tertiary Analysis

- Tertiary analysis is done using Illumina Connected Multiomics (ICM) and consists of the following steps in the [Illumina StrataMap Spatial Software documentation](#):
 - Data input into ICM.
 - Creating the Spatial Report in the Spatial Data Viewer.
 - Spatial analysis and performing additional analyses.
 - Exporting plots and images, such as PNG, SVG, or PDF files.

1.1 Warnings, Cautions, and Notes

Icons are used for warnings, cautions, and notes across this documentation and are defined in [Table 1](#).

Table 1: Icons

Icon	Definition
	Warnings are used for actions that can result in harm to person or damage to the instrument.
	Cautions are used for actions that can lead to unexpected or detrimental results, or loss of data.
	Notes are used for supplemental information that is required for a step, including Illumina recommendations and references to related documentation.

1.2 Safety Considerations

- Before performing the protocol, review the following applicable safety documentation:
 - Safety Data Sheets (SDSs)
 - Laboratory safety procedures
 - Equipment manufacturer instructions
- Personnel performing the protocol must be trained on the applicable laboratory safety practices and the operation of the equipment used in the steps.
- If compressed gas is used to dry slides, ensure that the gas source is properly regulated and used in accordance with local compressed gas safety requirements.
- Cryostat operation involves hazardous sharp edges and cold surfaces. Follow all safety precautions provided by the cryostat manufacturer and your facility safety procedures.
- Adhere to the following warnings and cautions when using samples and the reagents with this protocol.



This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Ventilation should be appropriate for handling of hazardous materials in reagents. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.



Handle all samples as if they are potentially infectious agents.



Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wash hands thoroughly after handling specimens and assay reagents.



Exercise caution when handling sharps, including cryostat blades, razor blades, and scalpels. Follow institutional sharps safety procedures and dispose of used blades in approved sharps containers.



Cryostat operation involves hazardous sharp edges and cold surfaces. Follow all safety precautions provided by the cryostat manufacturer and your facility safety procedures.



Do not use any assay components beyond their stated expiration date on the assay box label. Do not interchange assay components from different assay lots. Assay lots are identified on the assay box label. Store the assay components at the specified temperature.

Failure to follow the procedures as outlined can result in erroneous results or significant reduction in library quality.

2. Consumables and Equipment

This section lists all components included in the StrataMap Spatial Transcriptome assay reagent kit, with storage conditions. This section also details the ancillary consumables, equipment, and other prerequisites needed to complete the protocol.

The protocol has been validated using the listed items. Before starting the protocol, make sure that you have the required items. Using alternate consumables and equipment can impact performance.

2.1 Product Contents

- The StrataMap Spatial Transcriptome assay protocol does not require index adapters.
- You can order the StrataMap Spatial Transcriptome (L) kit (catalog # 20146113) and the StrataMap Spatial Transcriptome (S) kit (catalog # 20146112) based on your experiment needs.



Both kits come with the same reagent boxes (StrataMap Spatial Transcriptome Box 1 and 2) and cassette kit, but the contents of StrataMap Spatial Transcriptome Box 3 differ based on if you are ordering the (L) or (S) kit.

The (S) kit also comes with the StrataMap Spatial Stencil Kit. For instructions on how to use the stencil, refer to [3.1 Tissue Placement Guidelines on page 15](#).

- The StrataMap Spatial Transcriptome (L) kit consists of the following contents:
 - StrataMap Spatial Transcriptome Box 1 ([Table 2](#))
 - StrataMap Spatial Transcriptome Box 2 ([Table 3](#))
 - StrataMap Spatial Transcriptome Box 3 ([Table 4](#))
 - StrataMap Spatial Single-Well Cassette Kit ([Table 5](#))
- The StrataMap Spatial Transcriptome (S) kit consists of the following contents:
 - StrataMap Spatial Transcriptome Box 1 ([Table 2](#))
 - StrataMap Spatial Transcriptome Box 2 ([Table 3](#))
 - StrataMap Spatial Transcriptome Box 3 ([Table 4](#))
 - StrataMap Spatial Single-Well Cassette Kit ([Table 5](#))
 - StrataMap Spatial Stencil Kit ([Table 6](#))

Table 2: StrataMap Spatial Transcriptome Box 1

Quantity	Reagent
1	Enhanced PCR Mix (EPM)
1	First Strand Enzyme (FSE)
1	First Strand Reagent 1 (FSR1)
1	First Strand Reagent 2 (FSR2)
1	First Strand Reagent 3 (FSR3)
1	First Strand Supplement (FSS)
3	Spatial Amplification Mix (SAM)
1	Spatial Particle Mix (SPM)
1	Spatial Primer Cocktail (SPC)
1	Spatial PCR Reagent (SPR)
1	Spatial Surface Reagent (SSR)
1	Tissue Digestion Enzyme (TDE)
1	Tissue Permeabilization Enzyme (TPE)
1	Universal Cleave Mix (UCM)

Table 3: StrataMap Spatial Transcriptome Box 2

Quantity	Item
1	Illumina Purification Beads (IPB)
1	Resuspension Buffer (RSB)
1	Tissue Digestion Buffer (TDB)
20	Sealing tape

Table 4: StrataMap Spatial Transcriptome Box 3

Quantity	Item
1	StrataMap Spatial Slide (L) ¹
1	StrataMap Spatial Slide (S) ²

¹ The L slide is specific to the StrataMap Spatial Transcriptome (L) kit (catalog # 20146113).

² The S slide is specific to the StrataMap Spatial Transcriptome (S) kit (catalog # 20146112).

Table 5: StrataMap Spatial Single-Well Cassette Kit

Quantity	Item
1	Spatial cassette base
1	Spatial cassette lid

Table 6: StrataMap Spatial Stencil Kit³

Quantity	Item
1	Stencil

³ The stencil kit is specific to the StrataMap Spatial Transcriptome (S) kit (catalog # 20146112).

Kit Storage

Kit contents must be stored at the following temperatures ([Table 7](#)):

Table 7: Storage Temperatures

Temperature	Kit Contents
-25°C to -15°C	StrataMap Spatial Transcriptome Box 1 (Table 2)
15°C to 30°C	StrataMap Spatial Transcriptome Box 2 (Table 3)
	StrataMap Single-Well Cassette Kit (Table 5)
	StrataMap Spatial Stencil Kit (Table 6)
2°C to 8°C	StrataMap Spatial Transcriptome Box 3 (Table 4)

Store all reagent kit boxes in an upright position.



Make sure that the kit boxes are stored at the correct temperature.

The StrataMap Spatial slides (L and S) ship at room temperature. After receiving the slides, they must be stored upright at 2°C to 8°C.

2.2 User-Supplied Consumables and Equipment

Table 8 and Table 9 provide information on the required user-supplied consumables and equipment for the StrataMap Spatial Transcriptome assay.



Unless otherwise specified, using substitute consumables and equipment can impact performance or cause failures.

Table 8: Consumables

Consumable	Supplier
0.1 N HCl	Fisher Scientific, catalog # SA541 (or equivalent)
10 N sodium hydroxide (NaOH), molecular biology grade	General lab supplier
Absolute ethanol (EtOH), molecular biology grade	- Decon, catalog # 2716 - Sigma, catalog # E7023 (or equivalent)
Absolute methanol (MeOH), molecular biology grade	Sigma, catalog # 34860-4L-R (or equivalent)
Alcoholic eosin Y ¹	- Sigma, catalog # HT110132 - Abcam, catalog # AB246824 - Leica, catalog # 3801615 or 3801616 - Epredia, catalog # 71204
Bluing reagent ¹	- Epredia, catalog # 6769001 - Sigma, catalog # S5134-6X100ML - Agilent, catalog # CS70230-2 - Leica, catalog # 3802901
Conical tubes, 50 ml (qty 20–25)	General lab supplier
Coverslip, no 1.5 (22 or 24 x 60 mm is recommended)	- Mercedes Scientific, catalog # MER R24601.5 - Fisher Scientific, catalog # 12541037 (or equivalent)
Dry ice	General lab supplier
Glycerol	General lab supplier

Consumable	Supplier
H&E Staining Kit with the following consumables ¹ :	Abcam, catalog # ab245880
- Eosin Y solution, 30 ml - Bluing reagent, 30 ml - Hematoxylin, 30 ml	
Hematoxylin ¹	- Electron Microscopy Sciences, catalog # 26043-05 - Sigma, catalog # MHS1, MHS16, MHS32, MHS80, MHS128, or 51275 - Agilent, catalog # S330930-2 - Leica, catalog # 3801520 - Abcam, catalog # ab220365
High sensitivity D5000 ladder	Agilent, catalog # 5067-5594
High sensitivity D5000 reagents	Agilent, catalog # 5067-5593
High sensitivity D5000 screentape	Agilent, catalog # 5067-5592
High sensitivity RNA ladder	Agilent, catalog # 5067-5581
High sensitivity RNA sample buffer	Agilent, catalog # 5067-5580
High sensitivity RNA screentape	Agilent, catalog # 5067-5579
Isopropanol	Sigma, catalog # PX1835-6 (or equivalent)
Lint-free wipes	General lab supplier
KAPA Library Quantification Kit	Roche, catalog # 07960140001 or # 07960166001
KAPA SYBR FAST qPCR Master Mix (2X) Kit	Roche, catalog # 07959362001
Microcentrifuge tubes, 1.5 ml	General lab supplier
Microcentrifuge tubes, DNA LoBind, 2 ml	Eppendorf, catalog # 022431048 (or equivalent)
Nuclease-free water, molecular grade (700 ml)	General lab supplier
For OCT compound embedding, the following consumables:	General lab supplier
- Liquid nitrogen (with dewar) - Dry ice (with foam container) - Isopentane - Disposable cryomold	
PCR plate	General lab supplier

Consumable	Supplier
PCR plate seal	General lab supplier
Pipette tips, aerosol-resistant	General lab supplier
[Optional] QIAshredder	QIAGEN, catalog # 79656
qPCR plate	General lab supplier
Razor blade (or scalpel)	General lab supplier
RNase decontamination solution	General lab supplier
RNeasy Plus Mini Kit	QIAGEN, catalog # 74134
Saline-sodium citrate (SSC) buffer, RNase-free, 20X	Sigma, catalog # S6639 (or equivalent)
Slide mailers, LockMailer	Simport, catalog # M9504MA
Tissue-Tek OCT compound	VWR, catalog # 25608-930
Trough, disposable plastic	General lab supplier

¹ Use either the H&E Staining Kit or the individual catalog numbers for alcoholic eosin Y, bluing reagent, and hematoxylin.

Table 9: Equipment

Equipment	Supplier
Benchtop mini-centrifuge	General lab supplier
Benchtop vortexer	General lab supplier
Cryostat, one of the following (or equivalent): • Epredia Cryostar NX70 • Epredia Cryostar NX50	Epredia, one of the following: • catalog # 957000K • catalog # 957260
Forceps	General lab supplier
Heat block (for microcentrifuge tubes)	General lab supplier
Magnetic stand (for microcentrifuge tubes)	Invitrogen, catalog # 12321D (or equivalent)
Imaging system that meets minimum specifications in <i>StrataMap Spatial Transcriptome Imaging Guide</i> (document # 200081305)	Varies
Pipettor (10–1000 µl)	General lab supplier
[Optional] Plate shaker (BioShake iQ) with 96-well PCR plate adapter	Q Instruments, catalog # 1808-0506 and 1808-1041
Plate spinner (or swinging bucket centrifuge), compatible with PCR plates	General lab supplier

Equipment	Supplier
[Optional] Pure compressed inert gas (for example, nitrogen gas)	General lab supplier
Real-time qPCR system, one of the following systems (or equivalent): - Bio-Rad CFX384 Touch Real-Time PCR System - Bio-Rad CFX Opus 96 Real-Time PCR System - Bio-Rad CFX Opus 384 Real-Time PCR System	Bio-Rad, one of the following: - catalog # 1855484 - catalog # 12011319 - catalog # 12011452
TapeStation, one of the following systems: 4150 TapeStation 4200 TapeStation	Agilent, one of the following: - catalog # G2992AA - catalog # G2991BA
Thermal cycler with 96-well block, any of the following (or equivalent) that can accommodate skirted PCR plates, has a minimum inside height of 14 mm, and a maximum lid pressure of 80 pounds of force: - Bio-Rad C1000 - Bio-Rad PTC Tempo - Analytik Jena Biometra TAdvanced 96 SG - Eppendorf Mastercycler x50	- Bio-Rad, catalog # 12015382 - Bio-Rad, catalog # 12015392 - Analytik Jena, catalog # 846-4-070-241 - Eppendorf, catalog # 6313000018
Timer	General lab supplier

2.3 Illumina Consumables and Equipment

[Table 10](#) identifies the consumables that can be ordered from Illumina. The kit that should be used is dependent on the sequencing system ordered in [Table 11](#).

Table 10: Sequencing Consumables

Consumable	Catalog Number
NovaSeq X Series 5B Reagent Kit (100 cycles)	20145965
NovaSeq X Series 10B Reagent Kit (100 cycles)	20085596
NovaSeq X Series 25B Reagent Kit (100 cycles)	20125967
NovaSeq 6000 S2 Reagent Kit v1.5 (100 cycles)	20028316
NovaSeq 6000 S4 Reagent Kit v1.5 (200 cycles)	20040719
NovaSeq 6000 Xp 4-lane Kit v1.5	20043131
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (100 cycles)	20100994

[Table 11](#) identifies the sequencing systems that can be ordered from Illumina. The sequencing system that should be used is dependent on the tissue amount and sequencing depth.



To estimate the sequencing data output needed for your experiment, refer to [5.22 Sequencing and Analysis on page 103](#).

Table 11: Equipment

Equipment	Catalog Number
NovaSeq X Sequencing System	20084803
NovaSeq X Plus Sequencing System	20084804
NovaSeq 6000 Sequencing System	20023471
NextSeq 2000 Sequencing System	20038897

3. Before You Begin

Before starting the protocol, review the following information:

- Tissue section requirements
- Sequencing recommendations
- Instructions for embedding tissue in Optimal Cutting Temperature (OCT) compound
- RNA quality assessment requirements
- Instructions for extracting RNA from tissue sections
- Information on RIN analysis and how to get the RIN value
- Specimen transportation and storage requirements



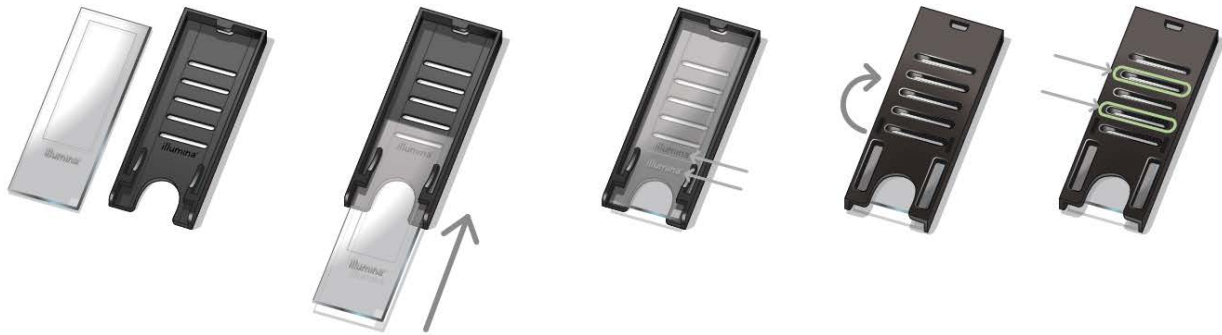
The StrataMap Spatial Transcriptome assay is compatible with high-quality fresh frozen tissue. The tissue must contain polyadenylated RNAs so that these transcripts can be captured during library preparation.

3.1 Tissue Placement Guidelines

- Illumina has currently demonstrated protocol performance with human, mouse, pig, and rat tissue.
 - Broader compatibility with many animal tissues is expected.
 - Untested species and tissue with rigid extracellular structures, such as those with cell walls (for example, plant tissue) or calcified matrices (for example, bone), have not been tested and may require customization and further optimization.
- Illumina offers a large active area (L) and small active area (S) version of the spatial transcriptome slide. Both versions of the slide have the same slide dimensions, but have different active area dimensions.
 - The L slide active area size is 50 mm × 15 mm.
 - The S slide active area size is 17 mm × 15 mm.
- When performing the assay with the S slide, additional setup is required so that tissue sections are placed within the usable region of the slide.
 - The S slide includes a stencil that defines the boundaries of the active area.

- Insert the slide into the stencil so that the Illumina logos align vertically. Use the stencil to identify the region of the slide where tissue sections can be placed to generate a spatial library ([Figure 2](#)).

Figure 2: Stencil and Slide Alignment



- Make sure that the region is properly aligned and fill in the second and fourth stencil lines completely (Figure 3).

Figure 3: Stencil Marking



- Make sure that the slide is properly marked before mounting tissue ([Figure 4](#)).

Figure 4: Marked Slide



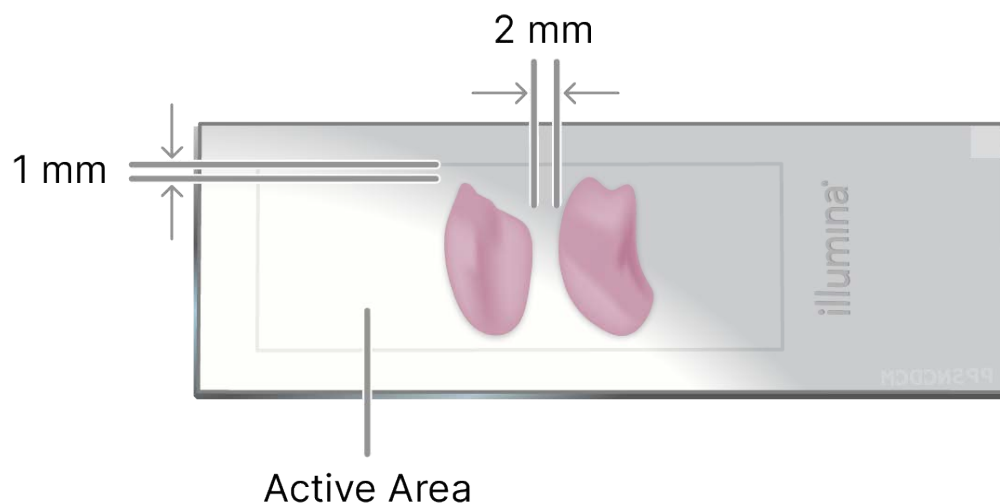
- Tissue sections should be placed at least 2 mm apart (Figure 5).



Illumina recommends having a margin of 1 mm between the tissue edge and active area in order to capture an adequate number of fiducials (Figure 5).

For more information on handling and tissue placement, refer to [4.2 Handle Tissue and Slide](#) on page 27.

Figure 5: Tissue Section Spacing



- Illumina has successfully processed slides containing up to 12 tissue sections. For slides containing more than 12 tissue sections or tissue sections larger than 13 mm × 33 mm (or an equivalent area, such as 429 mm²), image processing in ISIT and downstream secondary analysis can take longer to complete.



For guidance in applications that exceed these recommendations, contact Illumina Support.

- Illumina recommends that all tissue sections on the slide are of a similar type and approximately the same size if equal sequencing representation across samples is required.
 - Large tissue naturally generates more sequencing output when placed with smaller tissue.
 - Similar type refers to tissues that originate from the same species and the same organ or anatomical region.

- Individual images can be taken for each tissue section or images can contain multiple sections and samples separated during image processing. Memory requirements scale with image size and resolution. For example, a 10 mm x 10 mm image with 0.4 pixels/ μm and three color channels occupies ~1.7 GB of memory. Recommended memory for image processing is 32 GB or higher with a minimum of 16 GB.

3.2 Sequencing Recommendations

- Illumina recommends sequencing to a depth of 5000 raw reads per 10 x 10 μm tissue area.
 - This sequencing depth recommendation is a starting point and is provided as general guidance. It can be further adjusted based on your needs.
 - Actual reads per cell reported in the analysis output can vary based on tissue type and cell size.



For more information, refer to [5.22 Sequencing and Analysis on page 103](#).

3.3 Embed Tissue in OCT Compound

Tissue samples must be frozen and embedded in Optimal Cutting Temperature (OCT) compound for optimal section quality. The following instructions describe a recommended method for freezing and embedding tissue samples in OCT compound and are intended as general guidance.

1. Add liquid nitrogen to a dewar.
2. Fill a container (for example, a stainless steel beaker) with isopentane and place it in the liquid nitrogen.
3. Place the fresh tissue sample into a cryomold.
4. Fill the cryomold with OCT compound until the tissue sample is covered.
5. Using forceps, lower the cryomold into the isopentane. Hold until the OCT compound is opaque and solidified.



Do not let the isopentane enter the cryomold.

6. Remove the OCT compound-embedded tissue sample from the cryomold.
7. Store the tissue sample at -80°C until you are ready to start the protocol.

3.4 Extract RNA for Quality Assessment

This section describes the process for collecting tissue sections in preparation for RNA extraction and RIN analysis. Before placing tissue sections on a slide, assess RNA quality by extracting RNA from fresh tissue and calculating the RNA integrity number (RIN).

- RIN scores are indicative of RNA quality. Low scores indicate that RNA is degraded, which results in fewer RNA molecules available for capture.
- The RNA quality within a tissue block impacts the sensitivity of the StrataMap Spatial Transcriptome assay. For best results, use tissue blocks with RNA that has a RIN greater than or equal to 7.



For tissue-specific recommendations, refer to the RNA extraction kit protocol.

Consumables

- QIAGEN RNeasy Plus Mini Kit
- [Optional] QIAGEN QIAshredder
- Nuclease-free water
- 2 ml DNA LoBind microcentrifuge tube
- Dry ice
- Forceps
- Razor blade or scalpel
- RNase decontamination solution

Preparation

1. Turn on the cryostat to cool the chamber.



Make sure that the cryostat reaches operating temperature before sectioning.

2. Remove the tissue block from -80°C storage.
3. Place the tissue block into the cryostat for 30 minutes to equilibrate to the cryostat chamber temperature.

4. Clean forceps with RNase decontamination solution, and then rinse with nuclease-free water. Let the forceps air dry.
5. Place the 2 ml microcentrifuge tube and forceps on dry ice for at least 5 minutes.



The tube and forceps must be chilled to prevent thawing and degradation of tissue sections.

Procedure

1. Adjust the temperature of the cryostat blade and specimen holder based on the tissue type.



Illumina recommends -20°C for the blade holder and -10°C for the specimen head as starting points.

For more information on adjusting temperature, refer to the cryostat manufacturer manual.

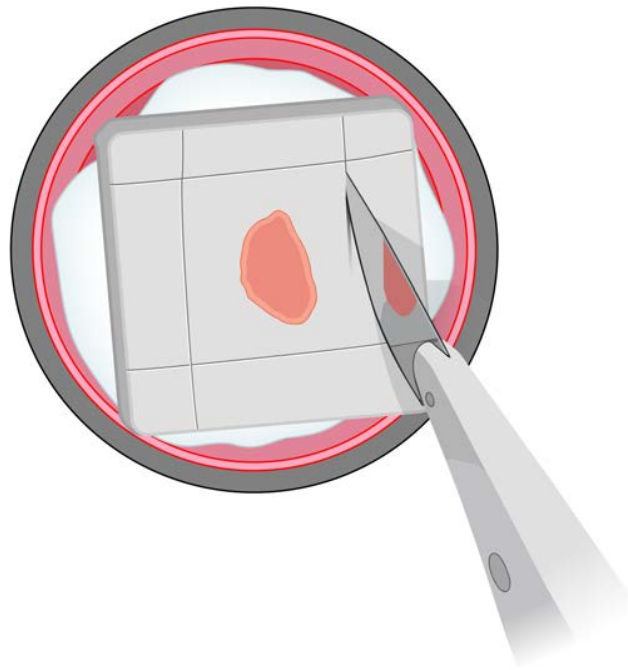
2. Place the specimen chuck with your tissue block into the specimen head of the cryostat.

3. If the OCT compound extends more than ~1 mm beyond the tissue edge, use a razor blade or scalpel to trim around the tissue carefully to remove excess OCT ([Figure 6](#)).



Excess mounting media can negatively affect the RIN scores.

Figure 6: OCT Trimming



4. Install the cryostat blade. Use a new blade for each tissue type.



To avoid injury when using the cryostat blade, make sure to follow all safety precautions and instructions in the cryostat manufacturer manual.

5. Slice tissue into 10 μ m sections.



For instructions, refer to the cryostat manufacturer manual.

6. Remove the forceps and microcentrifuge tube from the dry ice.
7. Using the chilled forceps, collect two 10 µm tissue sections.



Illumina recommends discarding the first three sections cut from the tissue block so that highest quality tissue is available for mounting.

For instructions, refer to the cryostat manufacturer manual.

8. Place the tissue sections in the chilled microcentrifuge tube.



Make sure that the tissue sections do not thaw or melt.

9. Place the microcentrifuge tube on dry ice.



If you are stopping here, refer to the [SAFE STOPPING POINT on page 24](#) at the end of this procedure. Otherwise, proceed to step 10.

10. Perform the Purification of Total RNA from Animal Tissues protocol from the QIAGEN RNeasy Plus Mini Kit.



If you are stopping here, refer to the [SAFE STOPPING POINT on page 24](#) at the end of this procedure. Otherwise, proceed to [3.5 RIN Analysis on page 25](#).

SAFE STOPPING POINT

If you are stopping after placing the tissue sections in the microcentrifuge tube in step 9, then store the tube at -80°C until you are ready to proceed with step 10.

If you are stopping after extracting RNA in step 10, then you can store the extracted RNA at -80°C until you are ready to proceed to [3.5 RIN Analysis on page 25](#).

3.5 RIN Analysis

This step quantifies the amount of RNA from two tissue sections and determines the RIN for the tissue block.

Consumables

- High sensitivity RNA screentape
- High sensitivity RNA screentape ladder
- High sensitivity RNA screentape sample buffer

Procedure

1. Quantify the extracted RNA and determine the RIN value for the tissue block.



For instructions, refer to the RNA screentape assay documentation on the Agilent website.

3.6 Specimen Transport and Storage

- Transportation of samples must comply with all applicable governing regulations for the transport of etiologic agents.



Handle all samples as if they are potentially infectious agents.

- Expedited shipping and transportation methods are recommended.



Exposure to elevated temperatures during transportation can negatively impact individual sample failure rates and sample performance.

- Use a conical tube or slide mailer to transport the spatial slide.



If tissue is mounted, do not use a slide mailer to transport the spatial slide. Minimize movement during transport. Otherwise, slide damage can occur.



If you are transporting the spatial slide to another lab space after mounting tissue slices, but before tissue fixation, Illumina recommends transporting the slide in a pre-chilled, empty conical tube on dry ice.

If you are transporting the spatial slide to another lab space after tissue fixation, Illumina recommends placing the slide in a clean, empty conical tube at room temperature during transport.

4. Tips and Techniques

This section identifies tips and techniques that can be used to perform actions in the protocol.

General Tips and Techniques

- Follow the protocol steps precisely.



Changes in volumes, timing, or steps can cause compromised results or assay failure.

- When sectioning tissue, embedding tissues in OCT compound, or sequencing, make sure that you are following the guidance outlined in [3. Before You Begin on page 15](#).
- Unless a safe stopping point is specified in the protocol, proceed immediately to the next step.

4.1 Avoid Contamination

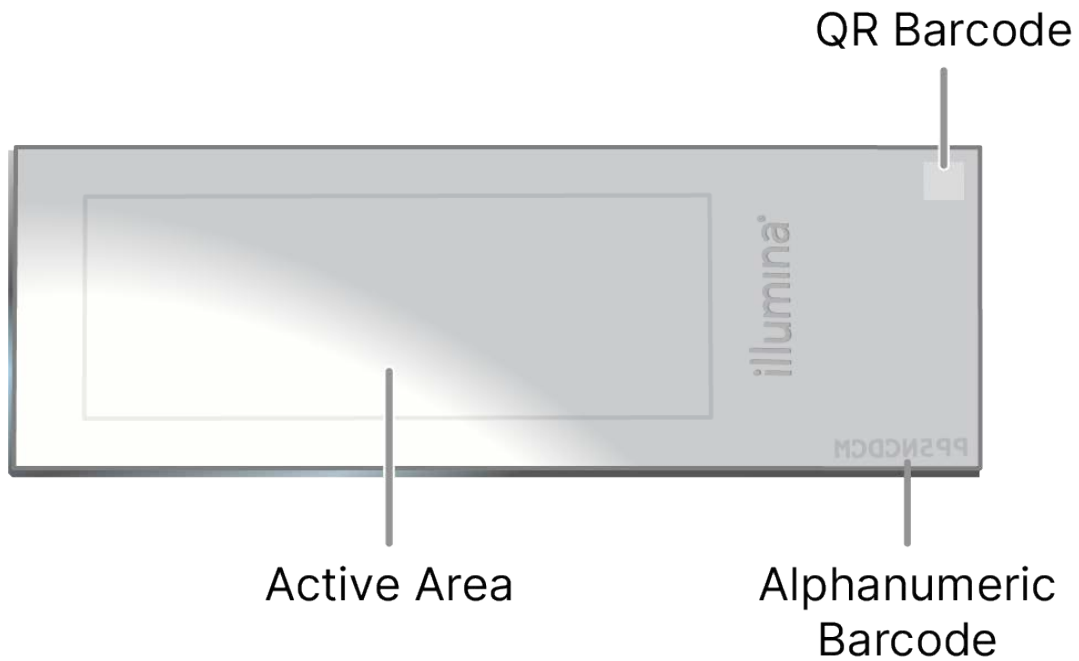
- Wear properly fitted gloves and change them frequently.
- Use filter tips only.
- If you are adding or transferring samples or reagent master mixes, change the tips.
- When you are between experiments, sterilize the bench and equipment to reduce the risk of carryover contamination as follows.
 - Wipe surfaces with 70% EtOH and let dry.
 - Wipe surfaces with 10% sodium hypochlorite and let stand for at least 1 minute.
 - Wipe surfaces with sterilized, laboratory-grade water to remove any residual sodium hypochlorite, and then let dry.

4.2 Handle Tissue and Slide

- Identify the active side of the slide ([Figure 7](#)).
 - When the active side is facing up, the Illumina logo is legible.
 - The alphanumeric barcode is etched in the bottom-right corner of the slide. The barcode is not legible on the active side. Tissue sections are placed on this side of the slide.

- If the barcode is legible, turn the slide over so that the active area is facing up.
- If you are placing the slide on a surface, make sure that the active side is facing up.

Figure 7: Active Side of Slide



- When handling the slide, avoid touching the active area. Touch the edges of the slide only ([Figure 8](#)).

Figure 8: Slide Handling Example



- The slide is fragile. When handling the slide, do not apply excessive pressure.
- To avoid cross-sample contamination, make sure that tissue samples are placed at least 2 mm away from each other.
- If possible, place the tissue in the middle of the capture area and away from the edges.

4.3 Section Tissue

- Adjust the temperature of the cryostat blade and specimen holder based on the tissue type.



Illumina recommends -20°C for the blade holder and -10°C for the specimen head as starting points.

For more information on adjusting temperature, refer to the cryostat manufacturer manual.

- Avoid touching the active area of the slide to the surface of the cryostat. Sensitivity can be reduced.
- To avoid decreased performance, make sure that the shortest amount of time possible (less than one hour) is used between mounting the first tissue section and fixing tissue.
- If you are stopping at the **SAFE STOPPING POINT** after [5.2 Section and Place Tissue on page 43](#), store the mounted slide at -80°C within one hour of mounting the first tissue section.
- If needed, excess OCT compound can be scored to prevent excessive OCT compound overlap with adjacent tissue sections placed on the slide.



Illumina recommends that you do not score within the tissue before placing it on the slide.

4.4 Apply and Remove Coverslip

- When placing a coverslip:
 - Use 75 µl 85% glycerol.
 - Pipette slowly to avoid bubbles.
 - If there are any bubbles on top of the tissue sections, gently press the coverslip onto the slide to remove them.



Do not shift the coverslip after mounting. Damage to the underlying tissue can occur.

- Remove a coverslip as follows.
 1. Place the slide in a prepared 50 ml conical tube with 45 ml nuclease-free water.
 2. Incubate at room temperature for 5 minutes.
 3. Using forceps, slowly remove the slide from the conical tube. As you lift the slide, make sure that the coverslip slides off.

4. If the coverslip does not slide off, submerge the slide in the nuclease-free water as much as possible and gently pull the coverslip parallel to the slide surface to separate it from the slide (Figure 9).



Do not pry or lift the coverslip at an angle or scrape the tissue sample.

Figure 9: Coverslip Removal



4.5 Wash Slide

- The slide must be washed in nuclease-free water at specific points in the protocol (for example, during steps in [5.3 Stain Tissue on page 49](#)).
- Wash the slide with nuclease-free water as follows.
 1. Carefully place the slide into one of the prepared conical tubes with 45 ml nuclease-free water.



When handling the slide, use gloves and touch the edges only. Do not touch the active area.



For more information on how to handle the slide, refer to [4.2 Handle Tissue and Slide on page 27](#).

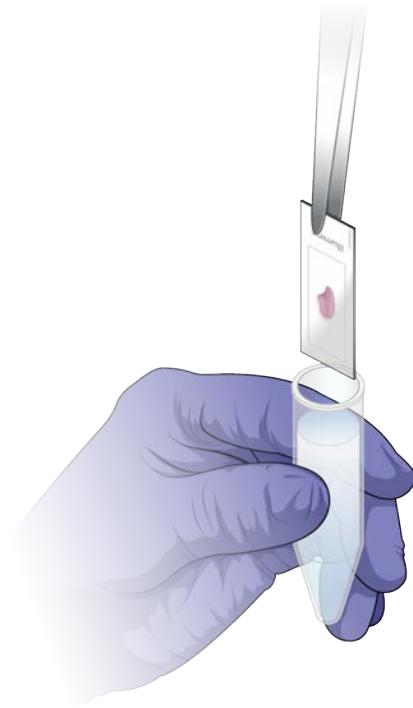
2. Invert conical tube 15 times to wash.
 3. Using forceps, remove the slide from the conical tube.
- If you are having issues removing the slide, the following methods can help with removal:
 - Use the forceps to move the slide gently from side to side to loosen it from the tube wall.
 - Recap and invert the conical tube, and then gently tap the lid on the benchtop to release the slide from the bottom of the tube.

- Gently squeeze the sides of the conical tube to widen the area inside where the slide is stuck, and then use the forceps to remove the slide (Figure 10).



Make sure that the slide is not lodged in the cap before twisting to open the conical tube. Damage to the slide can occur.

Figure 10: Slide Removal



4.6 Handle Beads

Store and Prepare Beads

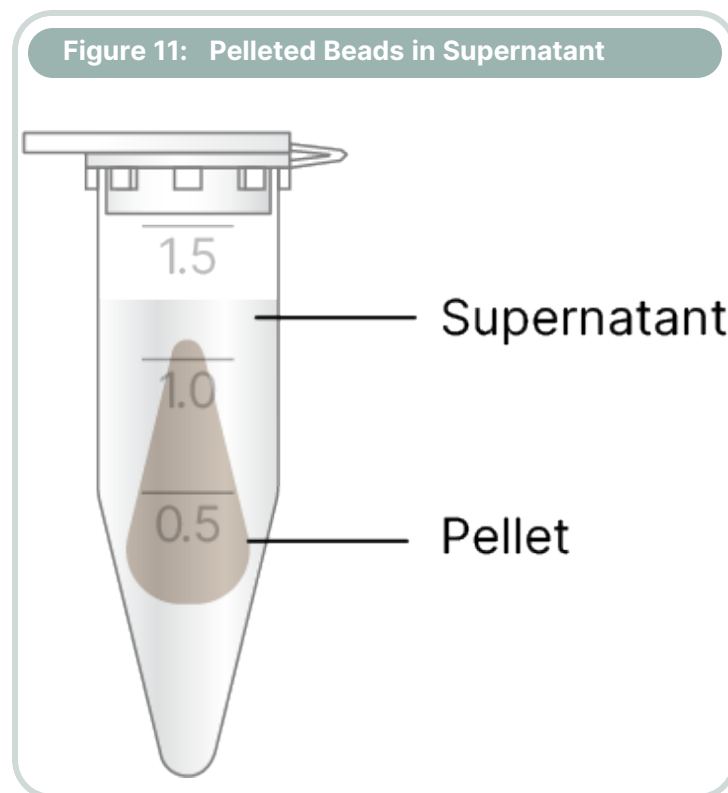
- Store purification beads upright so that beads are always submerged in the buffer.
- Vortex stock bead tubes for 1 minute until the beads are resuspended. To avoid resettling the beads, vortex before pipetting.

Use Beads During Cleanup

- Use the magnetic stand to pull bead pellets to the bottom of the tubes so that they are fully pelleted (Figure 11).



Do not agitate the tubes while they are on the stand. Remove them only when instructed.



- Pipette slowly to minimize bubbles.
- Do not disturb the bead pellets.
- If beads are aspirated into the pipette tips, dispense them back into the tube or onto the plate that is on the magnetic stand.
- Wait until the liquid is clear before proceeding.
- If the liquid adheres to the side or top of the tube, centrifuge at $280 \times g$ for 3 seconds.

5. Protocol

This section describes the protocol for the StrataMap Spatial Transcriptome assay.

- Review the complete assay workflow.
- Make sure that products and experiment parameters are compatible with the assay.
- Before proceeding, confirm the kit contents and make sure that you have the required components, equipment, and consumables.
- Make sure that the consumables are fresh, sealed, and stored properly and that containers are clean and RNase-free.
- Follow the protocol steps in the order shown, using the specified volumes and incubation parameters. Changes in volumes, timing, or steps can cause compromised results or assay failure.



For a comprehensive overview of the protocol, refer to [5.1 Workflow on page 38](#).

For support videos, refer to the [Spatial Transcriptomics playlist](#).

- The StrataMap Spatial Transcriptome assay requires a third-party imaging system. The imaging system should be qualified before beginning the StrataMap Spatial Transcriptome assay workflow.



For more information, refer to the [StrataMap Spatial Transcriptome Imaging Guide](#) (document # 200081305) on the [Illumina support site](#).

- Permeabilization times from third-party spatial transcriptomics kits cannot be used with the StrataMap Spatial Transcriptome assay.

5.1 Workflow

The following diagrams illustrate the assay workflow.

- Protocol steps are organized by day.
- Each step has a section number identified for navigation.
- The total and hands-on time estimates have been included.
- Applicable reagents for each protocol step are listed.
- Safe stopping points are included along with general instructions.

Day 1

Protocol steps 5.2 - 5.7

 15°C to 30°C -25°C to -15°C**5.2 Section and Place Tissue** Total time: 60 min / Hands-on: Varies

MeOH

 **Safe Stopping Point** Up to 7 days**5.3 Stain Tissue** Total time: 45 min / Hands-on: 30 min

EtOH Alcoholic Eosin Y Bluing reagent Glycerol Isopropanol Mayer's hematoxylin

5.4 Imaging Total time: 60 min / Hands-on: 15 min **Safe Stopping Point** Overnight, or up to 48 hours**5.5 Prepare for Steps:**

Permeabilize Tissue, Synthesize First-Strand cDNA



FSE FSR1 FSR2 FSR3 FSR3 FSS SPM TPE

5.6 Permeabilize Tissue Total time: 40 min / Hands-on: 30 min

TPE



HCl SSC

5.7 Synthesize First-Strand cDNA Total time: 5 min / Hands-on: 3 min (overnight incubation)

First Strand Master Mix

Day 2

Protocol steps 5.8 - 5.13

 15°C to 30°C -25°C to -15°C**5.8 Prepare for Steps:**

Digest Tissue, Remove RNA

 TDE RSB TDB**5.9 Digest Tissue** Total time: 60 min / Hands-on: 5 min TDE TDB**5.10 Remove RNA** Total time: 12 min / Hands-on: 7 min RSB NaOH**5.11 Prepare for Steps:**

Synthesize Second-Strand cDNA, Elute cDNA

 SAM SSR UCM RSB**5.12 Synthesize Second-Strand cDNA** Total time: 75 min / Hands-on: 25 min SAM SSR RSB NaOH**5.13 Elute cDNA** Total time: 7 min / Hands-on: 3 min SAM SSR RSB

Day 2

Protocol steps 5.14 - 5.16



15°C to 30°C



-25°C to -15°C

5.14 Prepare for Steps:

Clean Up Elution, Identify Amplification Cycle Number



SPC SPR



IPB RSB

5.15 Clean Up Elution

⌚ Total time: 60 min / Hands-on: 12 min



IPB RSB EtOH

|| Safe Stopping Point Up to 3 days

5.16 Identify Amplification Cycle Number

⌚ Total time: 90 min / Hands-on: 30 min



SPC SPR

|| Safe Stopping Point Up to 3 days

Day 3

Protocol steps 5.17 - 5.22



15°C to 30°C



-25°C to -15°C

5.17 Prepare for Steps:

Amplify Library, Clean Up Library



EPM SPC SPR



IPB RSB

5.18 Amplify Library

⌚ Total time: 60 min / Hands-on: 20 min



EPM SPC SPR

5.19 Clean Up Library

⌚ Total time: 40min / Hands-on: 20 min



IPB RSB EtOH

|| Safe Stopping Point Up to three weeks

5.20 Library Quantification

⌚ Total time: 115 min / Hands-on: 40 min

5.21 Dilute Libraries to the Starting Concentration

⌚ Total time: 30 min / Hands-on: 20 min

5.22 Sequencing and Analysis

5.2 Section and Place Tissue

This step sections and transfers fresh frozen tissue onto the slide.

Consumables

- Absolute MeOH
- Nuclease-free water
- Slide mailer
- 50 ml conical tube (qty 3–4)
- Disposable trough
- **[Optional]** Pure compressed inert gas (for example, nitrogen gas)
- Spatial transcriptome slide
- Razor blade (or scalpel)

Preparation

1. Turn on the cryostat to cool the chamber.



For instructions, refer to the cryostat manufacturer manual.

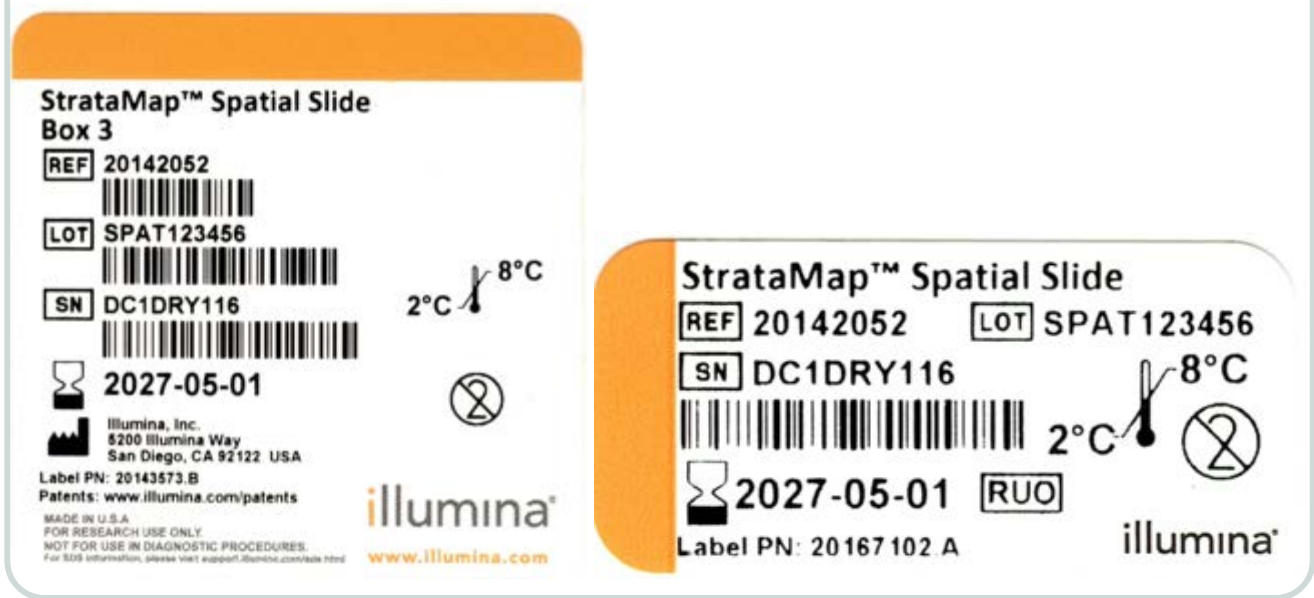
2. Remove the tissue block from -80°C storage.
3. Place the tissue block into the cryostat for 30 minutes to equilibrate to the cryostat chamber temperature.
4. Prepare the MeOH as follows.
 - a. Add 14 ml of absolute MeOH to a slide mailer.
 - b. Place the slide mailer in the -20°C freezer for at least 30 minutes.

- Record the serial number of the slide packaging (Figure 12).



This serial number is used for image processing with ISIT and is identified as SN on the label. If you do not record the SN, issues with cloud upload and run planning can occur.

Figure 12: Example Packing Labels with SN



- Remove the slide from the storage buffer.



When handling the slide, make sure that you are using a sterile RNase-free technique to avoid contamination. Make sure that you are wearing gloves and only touch the edges of the slide to avoid the active area.

- Gently wash the slide three times in nuclease-free water. Use a new conical tube for each wash.



For instructions, refer to [4.5 Wash Slide on page 33](#).

- If you have pure compressed inert gas, use it to dry the slide.

9. If you do not have pure compressed inert gas, use a swinging bucket centrifuge to spin the slide in the conical tube at 250 x g for 30 seconds to remove excess water.



When using the swinging bucket centrifuge, do not exceed 250 x g. Otherwise, the slide can break or become damaged.

10. Remove the slide from the conical tube and identify the active area.



For more information, refer to [4.2 Handle Tissue and Slide on page 27](#).

11. Place the slide active side up and let it air dry until no water droplets remain.

12. If you are using the S slide, make sure that the active area is properly marked using the stencil.



For more information, refer to [3.1 Tissue Placement Guidelines on page 15](#).

13. Place the slide into the cryostat on top of a disposable trough with the active area facing up for a minimum of 5 minutes before starting tissue sectioning.

Procedure

1. Set the temperature of the cryostat blade and specimen holder.



Make sure that the cryostat reaches operating temperature before sectioning.



Illumina recommends -20°C for the blade holder and -10°C for the specimen head as starting points.

Adjust the temperatures as needed based on the tissue type or previous experience with using a cryostat for sectioning. For more information on adjusting temperature, refer to the cryostat manufacturer manual.

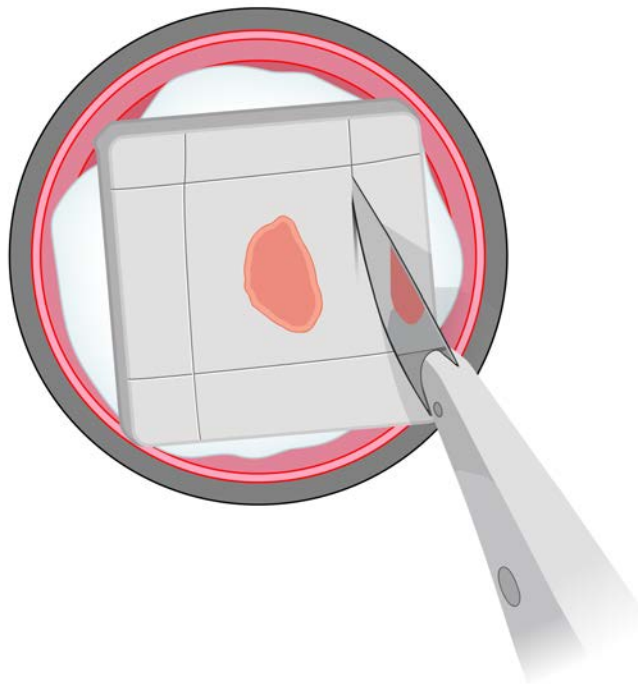
2. Place the specimen chuck with your tissue block into the specimen head of the cryostat.

3. Make sure that the OCT compound does not extend more than ~1 mm beyond the tissue edge. If it does, then use a razor blade or scalpel to carefully score so that excess OCT compound around the tissue can be removed ([Figure 13](#)).



Trimming excess OCT compound allows tissue to be positioned together closely, which maximizes the capture area.

Figure 13: OCT Trimming



4. Install the cryostat blade.



To avoid injury when using the cryostat blade, make sure to follow all safety precautions and instructions in the cryostat manufacturer manual.



Use a new blade for each tissue type.

5. Slice tissue into 10 µm sections.

Make sure that the slide remains in the cryostat while working to avoid tissue thawing and degradation.



To avoid decreased performance, make sure that the shortest amount of time possible (less than one hour) is used between mounting the first tissue section and fixing tissue.

If you stop at the [SAFE STOPPING POINT on page 48](#), store the mounted slide at -80°C within one hour of mounting the first tissue section.



Illumina recommends discarding the first three sections cut from the tissue block so that highest quality tissue is available for mounting. For instructions, refer to the cryostat manufacturer manual.

6. Place the tissue section on the slide surface as follows.



Make sure that you are placing the tissue section on the active side of the slide and avoid overlapping OCT with any neighboring tissue sections.

Tissue sections cannot be adjusted or removed from the slide after placement. All tissue slices placed on the slide are part of the single final library.

- a. Pick up the slide and place a gloved finger on the back of the slide (the non-active side) where you are planning on placing the tissue section.
- b. Hold your finger in place for at least 5 seconds to warm the slide before transferring the tissue section.

- c. Lower the warmed active area of the slide onto the tissue section to start adhesion.



Do not touch the slide to the cryostat surface.



The warmed area attracts the tissue section to the slide.

- d. Place a gloved finger on the back of the slide until the tissue section and OCT melts fully onto the slide.
- e. Repeat steps [a–d](#) for the remaining tissue sections that must be added to the slide. Make sure that tissue sections are at least 2 mm apart.



If you are stopping here, refer to the [SAFE STOPPING POINT on page 48](#). Otherwise, proceed to step [7](#).

7. Immediately place the slide into the slide mailer with pre-chilled MeOH. Make sure that the tissue sections are fully submerged.
8. Place the slide mailer upright in the -20°C freezer for 30 minutes for tissue fixation.

SAFE STOPPING POINT

If you are stopping before step [7](#), store the slide as follows.

- Pre-chill a 50 ml conical tube on dry ice or at -80°C.
- Place the slide in the pre-chilled, empty conical tube and seal tightly.
- Store the slide at -80°C for up to 7 days.

5.3 Stain Tissue

This step stains tissue with hematoxylin and eosin before imaging.

Consumables

- Absolute EtOH
- Alcoholic eosin Y
- Bluing reagent
- Glycerol
- Isopropanol
- Hematoxylin
- Nuclease-free water
- 1.5 ml microcentrifuge tubes
- Slide mailer (qty 4)
- 50 ml conical tube (qty 10)
- Lint-free wipes
- No 1.5 coverslip
- Disposable trough
- Cassette base

Preparation

1. If you stored the slide after [5.2 Section and Place Tissue on page 43](#), prepare it as follows.
 - a. Place the slide into the slide mailer with pre-chilled MeOH. Make sure that the tissue sections are fully submerged.
 - b. Place the slide mailer upright in the -20°C freezer for 30 minutes for tissue fixation.
2. Prepare four slide mailers with reagents as follows.
 - a. Add 14 ml isopropanol to a slide mailer.
 - b. Add 14 ml hematoxylin to a slide mailer.
 - c. Add 14 ml bluing reagent to a slide mailer.
 - d. Add 14 ml alcoholic eosin Y to a slide mailer.
3. Add 45 ml nuclease-free water to six 50 ml conical tubes.

4. Combine the following volumes to create 100 ml fresh 70% EtOH, including overage. Vortex to mix.
 - Absolute EtOH (70 ml)
 - Nuclease-free water (30 ml)
5. Add 45 ml 70% EtOH to two conical tubes.
6. Combine the following volumes to create 100 ml fresh 50% EtOH, including overage. Vortex to mix.
 - Absolute EtOH (50 ml)
 - Nuclease-free water (50 ml)
7. Add 45 ml 50% EtOH to two conical tubes.
8. Combine the following volumes to create 300 μ l 85% glycerol. Pipette slowly to avoid bubbles.
 - Absolute glycerol (255 μ l)
 - Nuclease-free water (45 μ l)
9. Preheat the thermal cycler to 37°C. Set the lid temperature to 47°C.
10. When prompted to select a reaction volume, set it to the maximum volume.



The volume is not applicable when using the cassette adapter.

11. Remove the cassette from the packaging.



The cassette packaging includes the cassette base and lid. The cassette does not come pre-assembled.

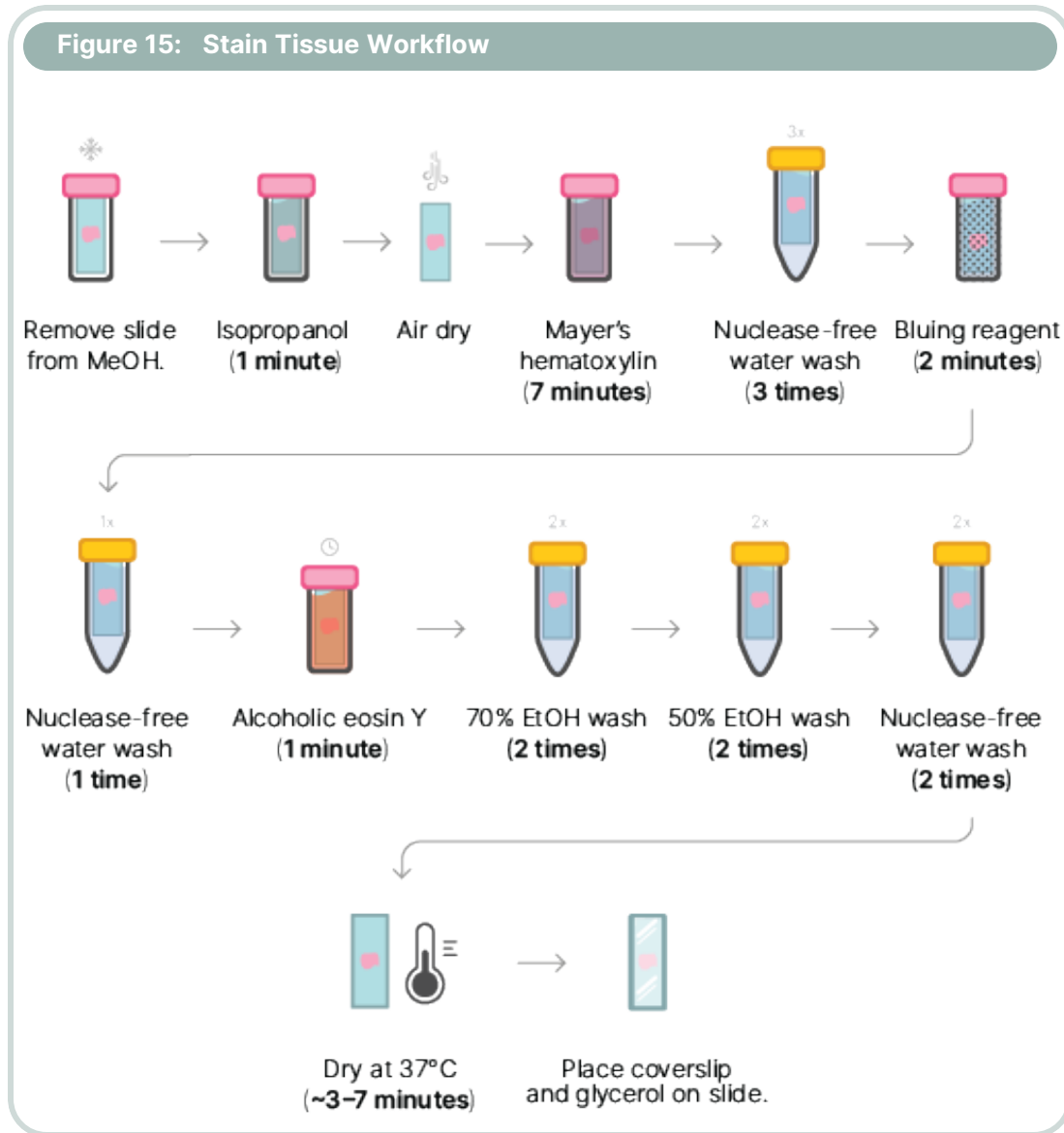
- 12.** Place the cassette base onto the thermal cycler. Do not close the thermal cycler lid.
- The cassette base is used to dry the slide when it is placed in the thermal cycler.
 - Make sure that the cassette is flat and that the cassette legs are within the wells of the thermal cycler ([Figure 14](#)).

Figure 14: Cassette Placement onto Thermal Cycler



Procedure

This procedure involves incubating the slide in multiple reagents, as shown in [Figure 15](#).



1. Remove the slide from the MeOH.



Do not touch the active surface. Damage to the sample or image quality issues can occur.



If the slide has excess MeOH on the surface after removal, it is safe to proceed with staining.

2. Incubate the slide in isopropanol as follows.
 - a. Carefully place the slide into the prepared slide mailer with 14 ml isopropanol.
 - b. Incubate at room temperature for 1 minute.
 - c. Using forceps, remove the slide from the slide mailer.
3. Place the slide active-side up on top of a trough and let air dry for 5–10 minutes. Inspect after 5 minutes.



Do not let the slide air dry for longer than 10 minutes. Damage to the sample or imaging quality issues can occur.



The tissue appears white when it is dried.

4. Incubate the slide in hematoxylin as follows.
 - a. Carefully place the slide into the prepared slide mailer with 14 ml hematoxylin.
 - b. Incubate at room temperature for 7 minutes.
 - c. Using forceps, remove the slide from the slide mailer.



When using hematoxylin, avoid inhaling vapor or skin contact.

For more information, refer to the SDS at support.illumina.com/sds.html.

5. Wash the slide in nuclease-free water three times. Use a new conical tube for each wash.



For instructions, refer to [4.5 Wash Slide on page 33](#).

If there is excess water on the surface after washing, you can proceed.

6. Incubate the slide in bluing reagent as follows.
 - a. Carefully place the slide into the prepared slide mailer with 14 ml bluing reagent.
 - b. Incubate at room temperature for 2 minutes.
 - c. Using forceps, remove the slide from the slide mailer.

7. Wash the slide in nuclease-free water one time.



For instructions, refer to [4.5 Wash Slide on page 33](#).

8. Using forceps, remove the slide from the slide mailer.
9. Incubate the slide in alcoholic eosin Y as follows.
- Carefully place the slide into the prepared slide mailer with 14 ml alcoholic eosin Y.
 - Incubate at room temperature for 1 minute.
 - Using forceps, remove the slide from the slide mailer.



When using alcoholic eosin Y, avoid inhaling vapor or skin contact.

For more information, refer to the SDS at support.illumina.com/sds.html.

10. Wash the slide in 70% EtOH as follows.
- Carefully place the slide into the prepared conical tube with 45 ml 70% EtOH.
 - Invert 15 times to wash.
 - Using forceps, remove the slide from the conical tube.
 - Repeat steps a–c one time for a total of two washes. Use a new conical tube for each wash.
11. Wash the slide in 50% EtOH as follows.
- Carefully place the slide into the prepared conical tube with 45 ml 50% EtOH.
 - Invert 15 times to wash.
 - Using forceps, remove the slide from the conical tube.
 - Repeat steps a–c one time for a total of two washes. Use a new conical tube for each wash.
12. Wash the slide in nuclease-free water two times.



For instructions, refer to [4.5 Wash Slide on page 33](#).

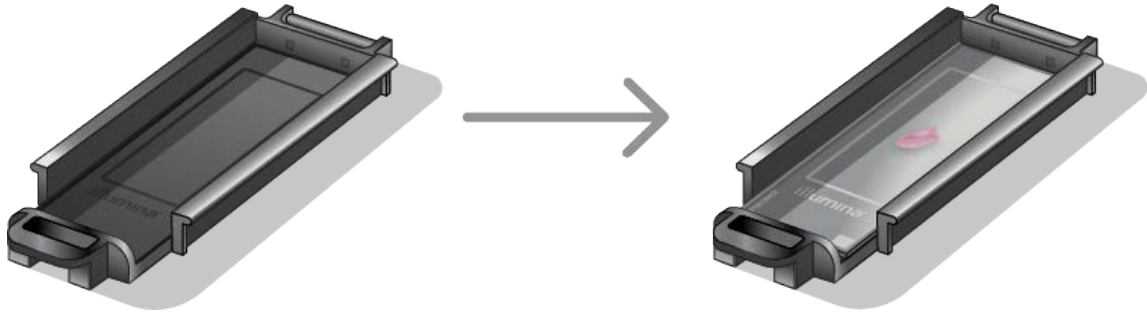
13. Tilt the edge of the slide onto a lint-free wipe to remove excess water. Wipe the back of the slide until it is dry.



Make sure that the lint-free wipe does not touch the active area of the slide.

14. Place the slide active-side up into the base of the cassette so that the Illumina logos on the slide and cassette base align. Make sure that the slide is flat and level in the base of the cassette. (Figure 16).
 - Make sure that you are wearing gloves and only touch the edges of the slide to avoid the active area.
 - Make sure that the slide is flat and level in the base of the cassette.

Figure 16: Slide Placement in Cassette Base



15. If the cassette base was removed from the thermal cycler, place the cassette base with the slide onto the thermal cycler.



Do not close the thermal cycler lid.

16. Dry the slide at 37°C for 3–7 minutes or until there is no visible moisture.



Monitor the slide closely during the drying process. Insufficient drying can lead to bubble formation when applying the coverslip. Excessive drying can damage the tissue and affect image quality.

17. Remove the cassette base with the slide from the thermal cycler.
18. Remove the slide from the cassette base and place it on a lint-free wipe. Set the cassette base aside for later use.
19. Using glycerol, place the coverslip on the slide.



For instructions, refer to [4.4 Apply and Remove Coverslip on page 31](#).

- 20. [Optional]** If a significant amount of bubbles form on the area covered by tissue, remove the coverslip, wash the slide two times in nuclease-free water, and apply a new coverslip.



For instructions, refer to [4.4 Apply and Remove Coverslip on page 31](#) and [4.5 Wash Slide on page 33](#).

- 21.** Incubate the slide at room temperature for at least 10 minutes to allow the glycerol to settle evenly.
- 22.** Pick up the slide and lightly press it at an angle against a lint-free wipe to remove any excess glycerol that is seeping out from under the coverslip.
- 23.** Using a lint-free wipe moistened with absolute EtOH, clean the back of the slide.



Excess glycerol must be removed before imaging. If glycerol is not removed, damage to the imaging system or image quality issues can occur.



After staining, proceed directly to [5.4 Imaging on page 57](#).

For imaging requirements and recommendations, refer to the StrataMap Spatial Transcriptome Imaging Guide (document # 200081305) on the [Illumina support site](#).

5.4 Imaging

Imaging is done with a third-party imaging system that is used to take images of the stained tissue on the slide. Before imaging, make sure that your system has been assessed and that the same setup (including the objective lens) during the assessment is used for this step. After imaging, the Illumina StrataMap Image Tool (ISIT) is used to assess images.

For more information, including example imaging systems, assessment instructions, and guidance on good image quality, refer to the StrataMap Spatial Transcriptome Imaging Guide (document # 200081305) on the [Illumina support site](#).



For setup instructions and imaging guidance, refer to the manufacturer instructions for your system.

For more information on ISIT, refer to the Illumina StrataMap Image Tool section of the [Illumina StrataMap Spatial Software documentation](#).

Preparation

1. Make sure that the imaging system is set to 20X magnification.
2. Position the slide in the slide holder so that it is properly oriented for the imaging system.
 - For more information, refer to the imaging system manufacturer instructions.
 - If the objective is in the top of the imaging system, place the slide active side up.
 - If the objective is in the bottom of the imaging system, place the slide active side down.



If you are using a spring-loaded slide holder, release the spring slowly so that it does not crack the slide.

3. Make sure that the imaging system is stabilized.



Do not move or shake the table or touch the imaging system during imaging. Imaging errors can occur.



For more information, refer to the manufacturer instructions.

Procedure

1. Image the slide using the imaging procedure from your imaging system manufacturer.
 - If the image contains more than one tissue section, crop the image so that there are individual images for each tissue section.
 - Include one Field of View (FOV) border around the tissue edge.
2. Using the software provided with your imaging system, stitch the FOVs together.



You can also use a third-party application for FOV stitching.

3. Save the brightfield colored image as a single-page, uncompressed TIFF file with at least 8 bit depth.
4. Assign a unique name for the image (for example, slide ID_RepX).



This name is used across all workflow steps. After the name is assigned, it cannot be changed.

5. Record the image pixel size in microns for use when processing the image with ISIT.
6. After imaging, assess the image visually and make sure that they have the following attributes:
 - In focus with no excessive blur
 - Clearly defined nuclear borders and cellular/tissue structures
 - Balanced exposure



Good image quality is necessary for accurate image alignment and downstream analysis.



For more information and examples of good image quality, refer to Image Quality Standards in StrataMap Spatial Transcriptome Imaging Guide (document # 200081305) on the [Illumina support site](#).

7. After visually inspecting the image, process the image using ISIT.



Individual tissue sections must be registered as separate samples in ISIT. For more information, refer to Image Processing in the [Illumina StrataMap Spatial Software documentation](#).

8. Make sure that the image passes the Image QC step in ISIT, and then proceed to [5.6 Permeabilize Tissue on page 62](#).



Always visually inspect the image. An image passing the QC step in ISIT does not guarantee high-quality downstream analysis.

9. [Optional] Use the Illumina StrataMap Cell Segmentation Tool to initiate cell segmentation before proceeding with the remainder of the assay protocol.



The Illumina StrataMap Cell Segmentation Tool can confirm if the cell segmentation meets your experimental needs or if the images should be retaken.

For more information, refer to the [Illumina StrataMap Spatial Software documentation](#).

SAFE STOPPING POINT

- Slides can be stored in the following conditions:
 - Room temperature (~20–22°C) overnight (up to 16 hours)
 - 4°C for up to 48 hours
- Slides must be kept flat.
- Keep the coverslip and glycerol on the slide.

5.5 Prepare for Steps

Reagents from the StrataMap Spatial Transcriptome kit must be prepared for the following protocol steps:

- [5.6 Permeabilize Tissue on page 62](#)
- [5.7 Synthesize First Strand cDNA on page 72](#)



During these steps, make sure that reagents are pipetted onto and off of the slide promptly to minimize drying on the slide surface.



For support videos, refer to the [Spatial Transcriptomics playlist](#).

1. Prepare the applicable reagents for each step ([Table 12](#)).

Table 12: Reagent Preparation Instructions

Step	Reagent	Box Number	Instructions
5.6 Permeabilize Tissue on page 62	Tissue Permeabilization Enzyme (TPE)	1	Thaw at room temperature.
5.7 Synthesize First Strand cDNA on page 72	First Strand Enzyme (FSE)	1	Do not thaw. Store on ice.
	First Strand Reagent 1 (FSR1)	1	Thaw at room temperature.
	First Strand Reagent 2 (FSR2)	1	Do not thaw. Store on ice.
	First Strand Reagent 3 (FSR3)	1	Thaw at room temperature.
	First Strand Supplement (FSS)	1	Thaw at room temperature.
	Spatial Particle Mix (SPM)	1	Thaw at room temperature.

2. Prepare the First Strand Master Mix for [5.7 Synthesize First Strand cDNA on page 72](#) as follows.
 - a. Prepare the following consumables:
 - FSE—Centrifuge briefly, pipette to mix, and centrifuge briefly again. Store on ice.
 - FSR1—Vortex for 3 seconds to mix, and then centrifuge briefly.

- FSR2—Centrifuge briefly, pipette to mix, and centrifuge briefly again. Pipette slowly to avoid bubbles. Store on ice.
- FSS—Vortex for 3 seconds to mix, and then centrifuge briefly.
- FSR3—Centrifuge briefly, pipette to mix, and centrifuge briefly again.
- SPM—Vortex for up to 30 seconds. Make sure that particles are homogenous immediately before preparing the First Strand Master Mix.



Make sure that FSR1, FSS, and the 1.5 ml microcentrifuge tube are at room temperature before proceeding.

Make sure that the SPM particles are homogenous immediately before preparing the First Strand Master Mix.

- b. Combine volumes in the following order into a 1.5 ml microcentrifuge tube to prepare 935 µl First Strand Master Mix (Table 13). Pipette 10 times to mix.

Table 13: First Strand Master Mix Preparation

Reagent	Volume (µl)
FSR1	520
FSS	25
FSR2	18
FSR3	44
FSE	108
SPM	220



The First Strand Master Mix can appear light brown after mixing. This coloration does not affect assay performance.

FSR2 can contain minor particulates that do not affect assay performance.

An excess of FSS is expected after the required volume is dispensed.

- c. Store the First Strand Master Mix at room temperature and set aside for [5.7 Synthesize First Strand cDNA on page 72](#).

5.6 Permeabilize Tissue

This step permeabilizes tissue so that the RNA is released from cells.

Illumina recommends a permeabilization time of 10 minutes for optimal performance in the spatial transcriptome assay across a wide range of tissue types.



Previously determined permeabilization times from third-party transcriptomics kits are not applicable to the StrataMap Spatial Transcriptome assay and can result in suboptimal performance.

Consumables

- Tissue Permeabilization Enzyme (TPE)
- 0.1 N HCl
- 20x SSC buffer
- Nuclease-free water
- 50 ml conical tube (qty 3)
- 1.5 ml microcentrifuge tube
- Lint-free wipes
- Cassette base
- Cassette lid
- Sealing tape

Preparation

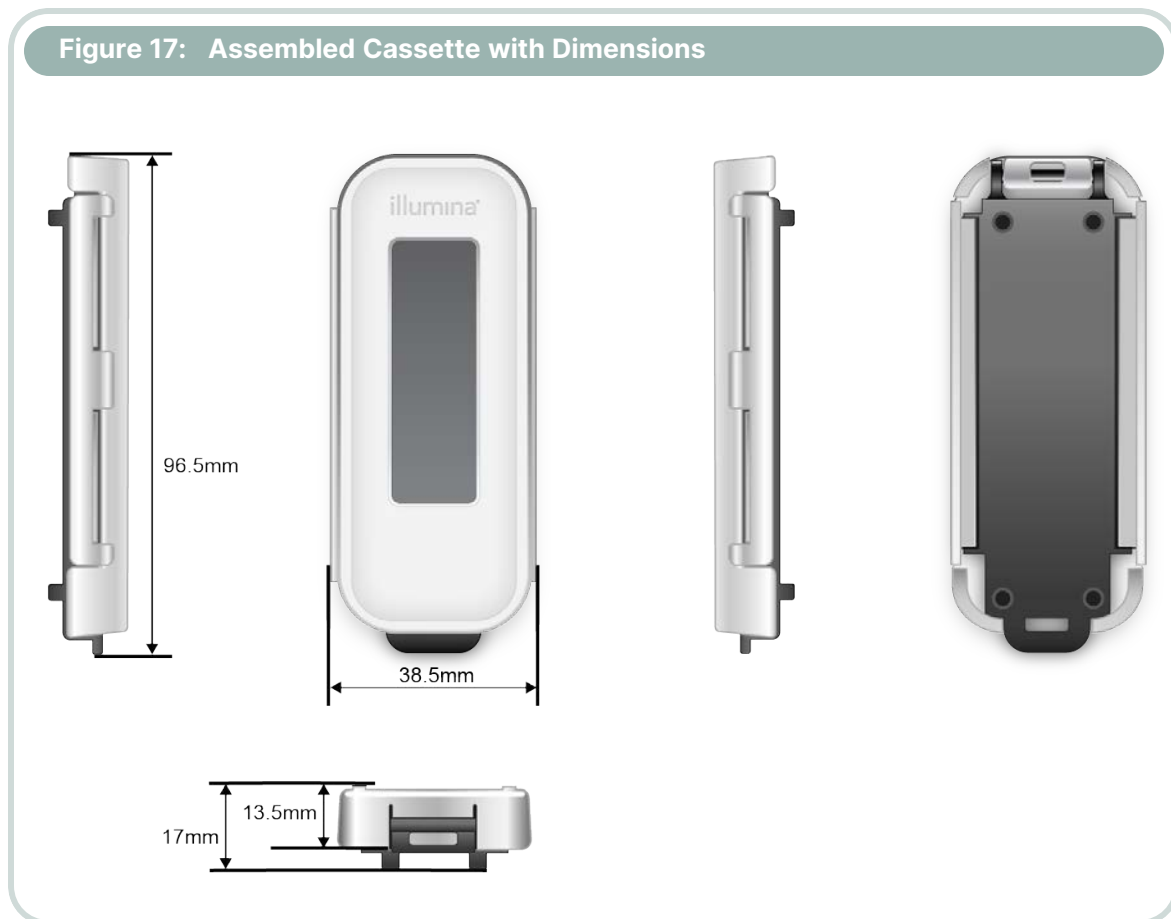
1. If the slide was stored at 4°C, let the slide equilibrate to room temperature for a minimum of 5 minutes.
2. Preheat the thermal cycler to 37°C. Set the lid temperature to 47°C.
3. Preheat the heat block for the microcentrifuge tubes to 37°C.
4. Add 45 ml nuclease-free water to three 50 ml conical tubes.
5. Combine the following volumes to prepare 7 ml 0.1x SSC wash buffer. Vortex to mix.
 - 20x SSC buffer (35 µl)
 - Nuclease-free water (6.97 ml)

6. Place the following consumables and equipment near the thermal cycler:

- Sealing tape
- 1000 µl pipettor with tips
- Timer (set to 10 minutes)
- Tip waste container (non-biohazardous)

Procedure**Prepare Slide and Assemble Cassette**

Use the following instructions to wash the slide and assemble the cassette for permeabilization. [Figure 17](#) shows the assembled cassette and its dimensions.



1. Remove the coverslip from the slide.



For instructions, refer to [4.4 Apply and Remove Coverslip on page 31](#).

2. Wash the slide two times in nuclease-free water. Use a new conical tube for each wash.



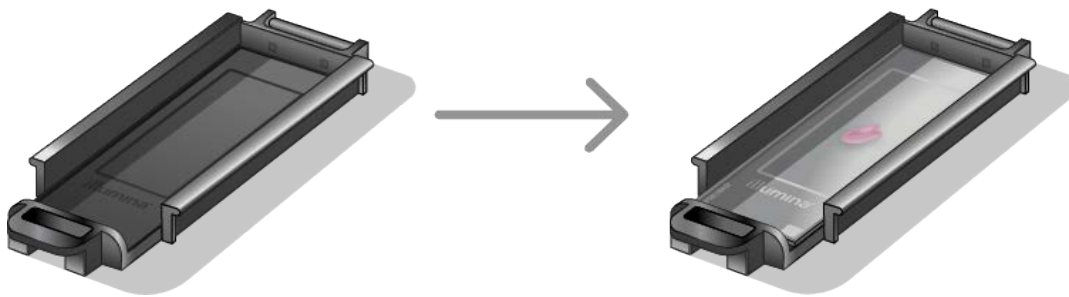
For instructions, refer to [4.5 Wash Slide on page 33](#).

3. Using a lint-free wipe, remove any excess liquid from the back of the slide.
4. Place the slide into the base of the cassette so that the active area is facing up and the Illumina logo on the cassette base and slide are aligned ([Figure 18](#)).



Make sure that you are wearing gloves and only touch the edges of the slide to avoid the active area. Make sure that the slide is flat and level in the base of the cassette.

Figure 18: Slide Placement in Cassette Base



5. Place the cassette base with the slide into the thermal cycler. Do not close the thermal cycler lid.
6. Dry the slide at 37°C for 5–10 minutes or until there is no visible moisture.



Do not turn off the thermal cycler. The thermal cycler temperature must be 37°C for [step 3](#).

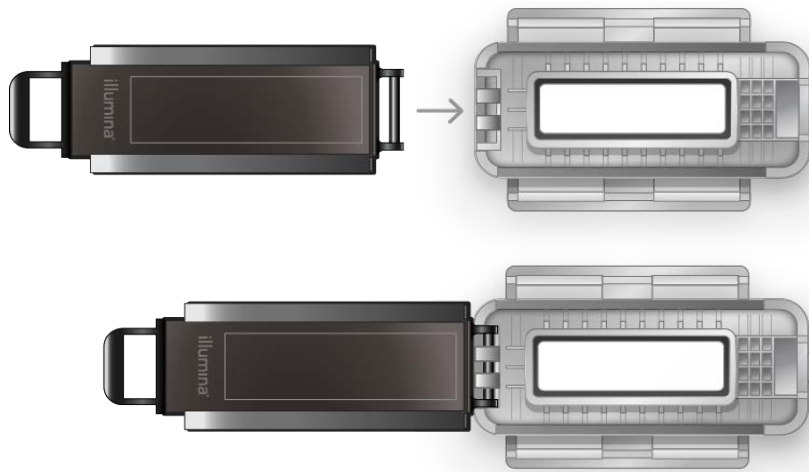
7. Remove the cassette base with the slide from the thermal cycler.
8. Remove the slide from the cassette base and place it on top of a clean lint-free wipe or a trough.
9. Place the cassette base and white cassette lid flat on the benchtop.



For optimal performance, use the cassette as intended. To prevent damage, avoid excessive handling when assembling and using the cassette.

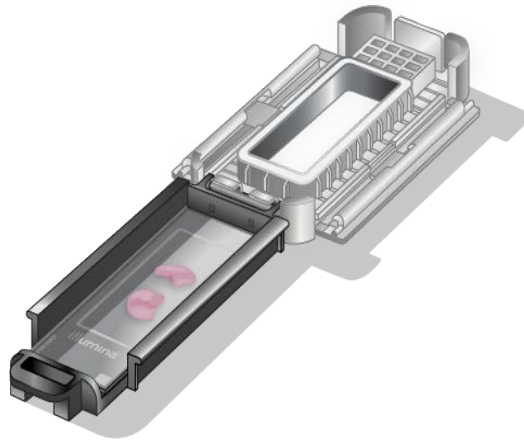
10. Attach the cassette lid to the cassette base at the hinge (Figure 19).

Figure 19: Cassette Hinge Attachment



11. Place the slide into the cassette so that the active area is facing up and the barcode aligns with the Illumina logo on the cassette base (Figure 20).

Figure 20: Slide Placement in Cassette



12. Close the cassette lid as follows.
 - a. Place the cassette flat on the workbench.
 - b. Close the cassette lid. Press down on top of the closed lid so that the lid is properly seated.

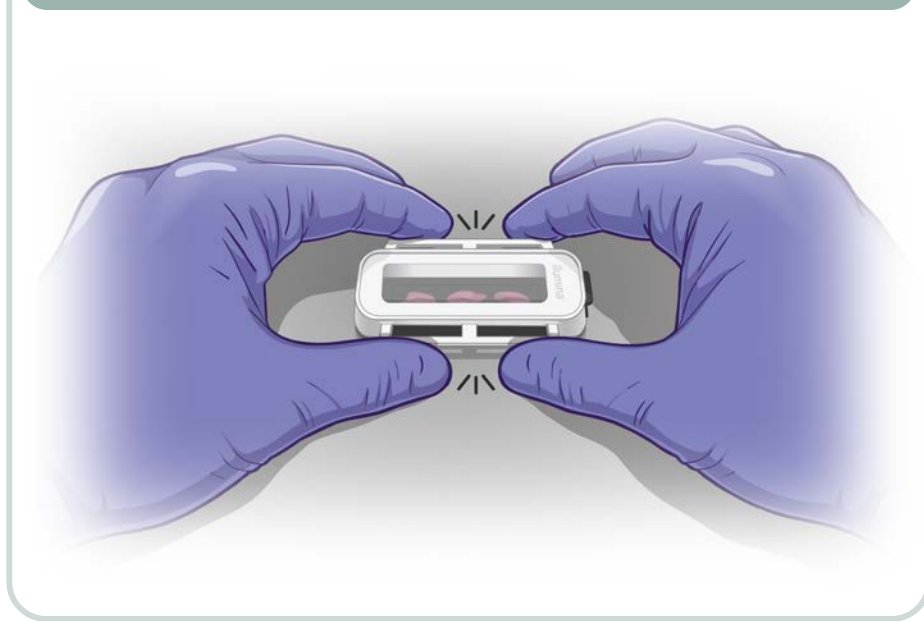
- c. While pressing down on the lid, close the two clips on each side of the cassette lid so that the lid is secured to the base and the cassette is sealed ([Figure 21](#)).



When closing the clips, apply even pressure with all fingers simultaneously. Otherwise, twisting at the hinge and damage to the cassette and slide can occur.

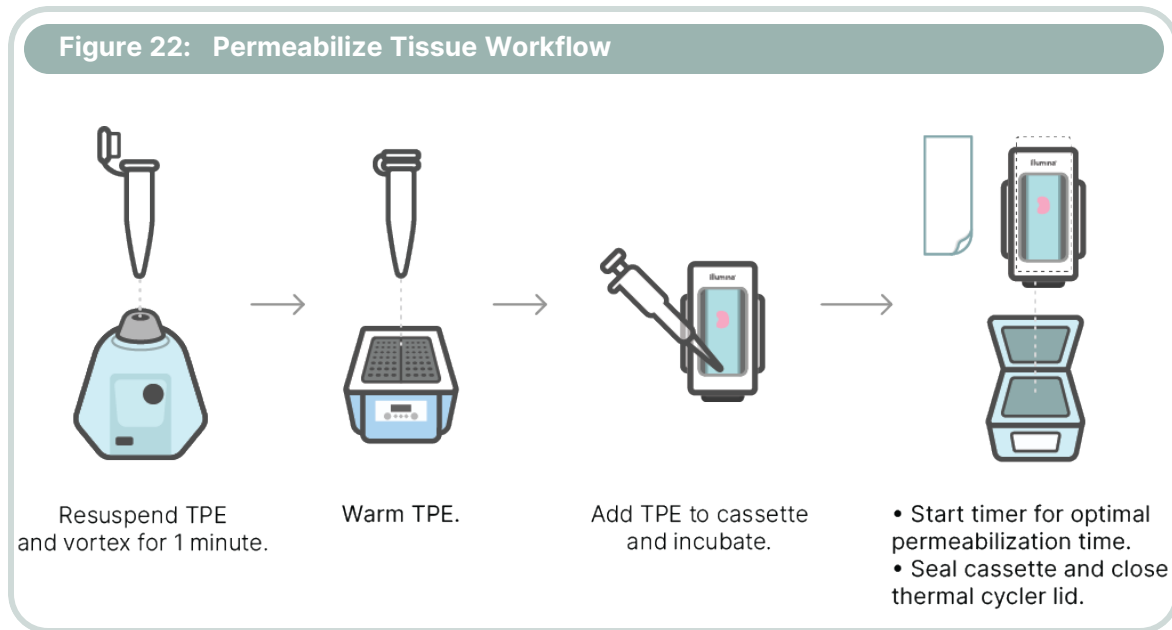
Make sure that you close the side clips to seal the cassette. Otherwise, leaks can occur.

Figure 21: Cassette Side Clips



Permeabilize Tissue

Use the following instructions to permeabilize the tissue. For an overview of these steps, refer to [Figure 22](#).



1. Resuspend the TPE as follows.
 - a. Centrifuge TPE briefly.
 - b. Add 1.67 ml 0.1 N HCl to the TPE tube.
 - c. Vortex for 1 minute and centrifuge briefly.
 - d. Store at room temperature until you are ready to preheat.
2. Using a heat block, warm the resuspended TPE at 37°C for 15 minutes. While the TPE is warming, you can proceed with step 3.



Each resuspended TPE tube must be preheated for at least 15 minutes before use. Do not warm the TPE for more than 20 minutes.

3. Place the cassette with the slide onto the thermal cycler that is preheated to 37°C.
4. Pipette 1.2 ml nuclease-free water into the cassette well to wet the slide surface. Make sure that the tissue is covered.
5. After resuspended TPE is warmed for 15 minutes, use a pipettor to remove and discard the water wash from the cassette well.

! *Do not touch the tissue with the pipette tip.*

6. Immediately pipette 1.6 ml TPE into the cassette well. Pipette from the corner of the cassette well.

! *Avoid pipetting bubbles from the TPE tube.*

7. Start the timer to begin the incubation time needed for permeabilization.
8. Seal the cassette with sealing tape. Make sure that you are handling the cassette properly when applying the sealing tape ([Figure 23](#)).

Figure 23: Cassette Handling



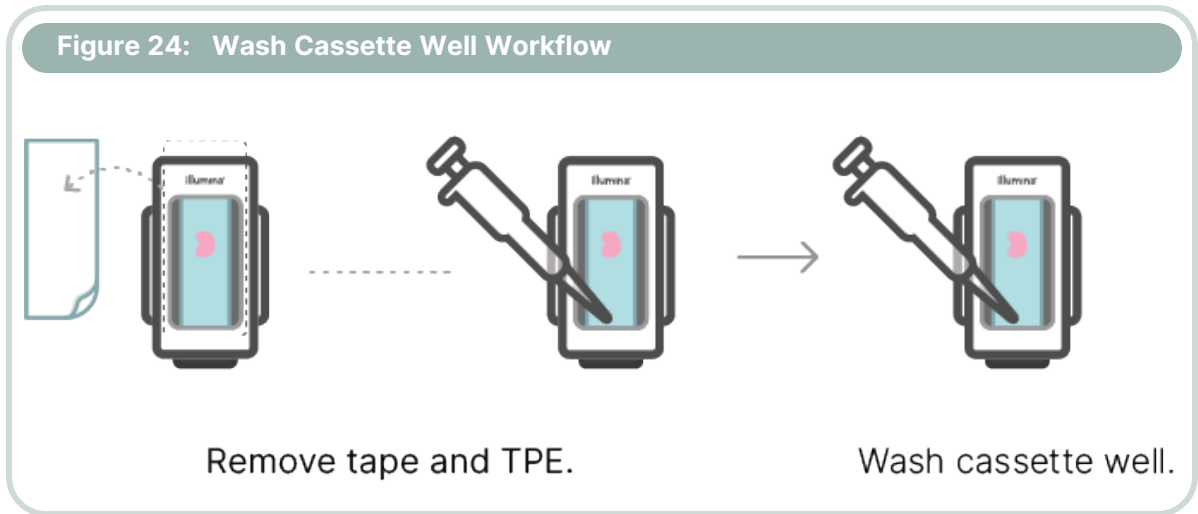
9. Close the thermal cycler lid. If the thermal cycler has an adjustable lid, gently tighten it.
10. After permeabilization, remove the cassette from the thermal cycler. Keep the cassette near the thermal cycler and proceed to [Wash Cassette Well on page 70](#).



Do not transport the cassette and minimize cassette movement while TPE is in the cassette well.

Wash Cassette Well

Use the following instructions to wash the cassette well after permeabilization. For an overview of these steps, refer to [Figure 24](#).



1. Remove the sealing tape from the cassette.
2. Using a pipettor, remove the TPE from the cassette well. Discard the TPE and pipette tip.
3. Immediately wash the cassette well with 0.1x SSC wash buffer as follows.
 - a. Pipette 1.8 ml 0.1x SSC wash buffer into the cassette well. Pipette from the corner of the cassette well.

- b. Using a pipettor, remove and discard the 0.1x SSC wash buffer. Tilt the cassette so that the liquid pools in the corner of the cassette well ([Figure 25](#)).

Figure 25: Pipetting Location



- c. Repeat steps [a](#) and [b](#) two times for a total of three washes.
4. Leave the last 0.1x SSC wash in the cassette well until you are ready to add the First Strand Master Mix in [5.7 Synthesize First Strand cDNA on page 72](#).

5.7 Synthesize First Strand cDNA

This step uses reverse transcriptase to synthesize DNA that complements the RNA transcripts on the surface of the slide.

Consumables

- First Strand Master Mix
- Sealing tape

Procedure

1. Preheat the thermal cycler to 20°C. Set the lid temperature to 20°C.



If your thermal cycler lid cannot be set to 20°C, then set it to the lowest temperature setting available.

2. Place the cassette with the 0.1x SSC wash onto the thermal cycler.
3. Using a pipettor, remove the 0.1x SSC wash buffer from the cassette well. Discard the buffer and pipette tip.
4. Pipette First Strand Master Mix prepared in [5.5 Prepare for Steps on page 60](#) 10 times to mix and homogenize.
5. Pipette 850 µl First Strand Master Mix into the cassette well.



After adding First Strand Master Mix, do not tilt or move the cassette.

6. Seal the cassette thoroughly with sealing tape to prevent evaporation.
7. Close the thermal cycler lid.
8. Incubate the cassette at 20°C for 5 minutes.
9. While the cassette is in the thermal cycler, change the temperature to 42°C. Set the lid temperature to 52°C.

10. Incubate the cassette at 42°C overnight (approximately 12–18 hours).



Do not exceed 18 hours.

5.8 Prepare for Steps

Reagents from the StrataMap Spatial Transcriptome kit must be prepared for the following protocol steps:

- [5.9 Digest Tissue on page 75](#)
- [5.10 Remove RNA on page 77](#)



For support videos, refer to the [Spatial Transcriptomics playlist](#).

1. Prepare the applicable reagents for each step ([Table 14](#)).

Table 14: Reagent Preparation Instructions

Reagent	Box Number	Instructions
Tissue Digestion Enzyme (TDE)	1	Do not thaw. Store on ice.
Resuspension Buffer (RSB)	2	Store at room temperature.
Tissue Digestion Buffer (TDB)	2	Store at room temperature.

5.9 Digest Tissue

This step digests and removes tissue and cell debris from the surface of the slide.

Consumables

- Resuspension Buffer (RSB)
- Tissue Digestion Buffer (TDB)
- Tissue Digestion Enzyme (TDE)
- 1.5 ml microcentrifuge tube
- Sealing tape

Preparation

1. Prepare the following consumables:
 - TDB—Vortex for 3 seconds to mix, and then centrifuge briefly.
 - TDE—Centrifuge briefly, pipette to mix, and centrifuge briefly again. Store on ice.
2. Combine the following volumes into a 1.5 ml microcentrifuge tube to prepare 720 µl Tissue Digestion Master Mix, including overage ([Table 15](#)). Pipette mix 10 times.

Table 15: Tissue Digestion Master Mix Preparation

Reagent	Volume (µl)
TDB	705
TDE	15

3. Store Tissue Digestion Master Mix at room temperature.

Procedure

1. After overnight incubation, remove the sealing tape from the cassette.
2. With the cassette still on the thermal cycler, use a pipettor to remove the First Strand Master Mix. Discard the pipette tip and First Strand Master Mix.



Even though the tissue slices can appear less visible, avoid touching the pipette tip to the area where they were placed. The tip can damage the library surface.

3. Wash the cassette well with RSB as follows.

- a. Pipette 1 ml RSB into the cassette well.
- b. Using a pipettor, remove the RSB from the cassette well. Discard the RSB and pipette tip.
- c. Repeat steps **a** and **b** two times for a total of three RSB washes.



Particles can adhere to the cassette surface during the second day of the protocol. These particles do not affect assay performance.

- 4.** Pipette 650 μ l Tissue Digestion Master Mix into the cassette well.
- 5.** Seal the cassette with sealing tape to prevent evaporation and close the thermal cycler lid.
- 6.** Change the thermal cycler temperature to 56°C and the lid temperature to 66°C.
- 7.** Incubate at 56°C for 1 hour.

5.10 Remove RNA

This step removes RNA from the surface of the slide.

Consumables

- Resuspension Buffer (RSB)
- 10 N NaOH
- Nuclease-free water

Preparation

1. Combine the following volumes to prepare 5 ml 0.1 N NaOH:
 - Nuclease-free water (4.95 ml)
 - Stock 10 N NaOH (50 µl)

Procedure

1. Remove the cassette from the thermal cycler.
2. Remove sealing tape from the cassette.
3. Using a pipettor, remove and discard Tissue Digestion Master Mix from the cassette well.
4. Wash the cassette well with RSB as follows.
 - a. Pipette 1 ml RSB into the cassette well.
 - b. Using a pipettor, remove the RSB from the cassette well. Discard the RSB and pipette tip.
 - c. Repeat steps [a](#) and [b](#) two times for a total of three RSB washes.
5. Wash the slide in 0.1 N NaOH as follows.
 - a. Pipette 650 µl 0.1 N NaOH into the cassette well.
 - b. Incubate at room temperature for 5 minutes.



While you are incubating, you can start preparing the reagents for [5.12 Synthesize Second Strand cDNA on page 80](#). For more information, refer to [5.11 Prepare for Steps on page 79](#).

- c. Using a pipettor, remove the 0.1 N NaOH from the cassette well. Discard the pipette tip and 0.1 N NaOH.
6. Pipette 1 ml RSB into the cassette well to wash away residual NaOH. Leave the RSB on the slide until you are ready to begin .
7. Store the remaining 0.1 N NaOH at room temperature.



This NaOH is used during [5.12 Synthesize Second Strand cDNA on page 80](#).

5.11 Prepare for Steps

Reagents from the StrataMap Spatial Transcriptome kit must be prepared for the following protocol steps:

- [5.12 Synthesize Second Strand cDNA on page 80](#)
- [5.13 Elute cDNA on page 84](#)



For support videos, refer to the [Spatial Transcriptomics playlist](#).

1. Prepare the applicable reagents for each step ([Table 16](#)).

Table 16: Reagent Preparation Instructions

Reagent	Box	Instructions
Spatial Amplification Mix (SAM)	1	Thaw three tubes at room temperature.
Spatial Surface Reagent (SSR)	1	Thaw at room temperature.
Universal Cleave Mix (UCM)	1	Thaw at room temperature.
Resuspension Buffer (RSB)	2	Store at room temperature.

5.12 Synthesize Second Strand cDNA

This step adds a second strand of cDNA.



For workflow videos, refer to the [Spatial Transcriptomics support playlist](#).

Consumables

- Spatial Amplification Mix (SAM)
- Spatial Surface Reagent (SSR)
- Resuspension Buffer (RSB)
- Universal Cleave Mix (UCM)
- 0.1 N NaOH
- Sealing tape

Preparation

1. Prepare the following consumables:
 - SSR—Pulse vortex three times to mix, and then centrifuge briefly. Store on ice.
 - SAM—Centrifuge briefly, pipette to mix, and centrifuge briefly again. Store on ice.
2. Program the thermal cycler to run the SAM program as follows.
 - a. Set the temperature and time for each cycle ([Table 17](#)).

Table 17: SAM Program Cycles

Number of Cycles	Temperature	Time
1	65°C	10 minutes
1	22°C	1 minute

- b. Set the lid temperature to 75°C.
 - c. When prompted to select a reaction volume, set it to the maximum volume.
 - d. Save the SAM program.
3. Preheat the thermal cycler to 40°C. Set the lid temperature to 50°C.

4. Preheat the heat block for microcentrifuge tubes to 37°C.



The heat block is used to preheat the UCM tube used in [5.13 Elute cDNA on page 84](#).

Procedure

Surface Conditioning

1. Using a pipettor, remove the RSB from the cassette well. Discard the RSB and pipette tip.
2. Pipette 650 µl SSR into the cassette well.
3. Seal the cassette with sealing tape.
4. Place the cassette onto the thermal cycler. Close the thermal cycler lid.
5. Incubate at 40°C for 10 minutes.
6. Remove the cassette from the thermal cycler.
7. Remove the sealing tape from the cassette.
8. Using a pipettor, remove the SSR from the cassette well. Discard the SSR and pipette tip.
9. Pipette 1 ml RSB into the cassette well.

First SAM Incubation

1. Using a pipettor, remove the RSB from the cassette well. Discard the RSB and pipette tip.
2. Pipette 650 µl SAM into the cassette well.
3. Seal the cassette with sealing tape.
4. Place the cassette onto the thermal cycler and close the lid.
5. Start the SAM program that was saved on the thermal cycler in [Preparation on page 80](#).
6. After the SAM program ends, remove the cassette from the thermal cycler.
7. Remove the sealing tape from the cassette.
8. Using a pipettor, remove SAM from the cassette well. Discard SAM and the pipette tip.

First NaOH Incubation

1. Add 650 µl 0.1 N NaOH into the cassette well.
2. Incubate at room temperature for 5 minutes.
3. Using a pipettor, remove 0.1 N NaOH from the cassette well. Discard NaOH and the pipette tip.
4. Pipette 1 ml RSB into the cassette well to wash.

Second SAM Incubation

1. Using a pipettor, remove the RSB from the cassette well. Discard the RSB and pipette tip.
2. Pipette 650 µl SAM into the cassette well.
3. Seal the cassette with sealing tape.
4. Place the cassette onto the thermal cycler and close the lid.
5. Start the SAM program that was saved on the thermal cycler in [Preparation on page 80](#).
6. After the SAM program ends, remove the cassette from the thermal cycler.
7. Remove the sealing tape from the cassette.
8. Using a pipettor, remove SAM from the cassette well. Discard SAM and pipette tip.

Second NaOH Incubation

1. Add 650 µl 0.1 N NaOH into the cassette well.
2. Incubate at room temperature for 5 minutes.
3. Using a pipettor, remove 0.1 N NaOH from the cassette well. Discard NaOH and the pipette tip.
4. Pipette 1 ml RSB into the cassette well to wash.

Third SAM Incubation

1. Using a pipettor, remove the RSB from the cassette well. Discard the RSB and pipette tip.
2. Pipette 650 µl SAM into the cassette well.
3. Seal the cassette with sealing tape.
4. Place the cassette onto the thermal cycler and close the lid.
5. Start the SAM program that was saved on the thermal cycler in [Preparation on page 80](#).
6. While the SAM program is running, use the heat block to warm the UCM tube at 37°C for 15 minutes.



The UCM tube is used in [5.13 Elute cDNA on page 84](#).

7. After the SAM program ends, remove the cassette from the thermal cycler.
8. Remove the sealing tape from the cassette.
9. Using a pipettor, remove SAM from the cassette well. Discard SAM and pipette tip.
10. Pipette 1 ml RSB into the cassette well. Leave the RSB on until you are ready to start [5.13 Elute cDNA on page 84](#).

5.13 Elute cDNA

This step elutes the cDNA from the slide surface.

Consumables

- Universal Cleave Mix (UCM)
- 2 ml DNA LoBind microcentrifuge tube
- Sealing tape

Preparation

1. Label one 2 ml microcentrifuge tube as UCM Elution.
2. Prepare UCM by centrifuging briefly, pipetting to mix, and then centrifuging briefly again. Make sure that UCM is at 37°C before use.

Procedure

1. Using a pipettor, remove RSB from the cassette well. Discard RSB and pipette tip.
2. Pipette 650 µl warmed UCM into the cassette well.



An excess of UCM is expected after the required volume is dispensed.

3. Incubate at room temperature for 5 minutes.
4. Gently move the cassette from side to side to mix the liquid across the surface for 5 seconds.
5. Pick up and carefully tilt the cassette so that the elution moves to one corner.
6. Using a pipettor, remove 640 µl elution and transfer it to the UCM Elution tube. Place the tube on ice until you are ready to start [5.15 Clean Up Elution on page 86](#).



Do not discard the elution in the UCM Elution tube. This tube contains the assay library.

7. Discard the assembled slide and cassette.

5.14 Prepare for Steps

Reagents from the StrataMap Spatial Transcriptome kit must be prepared for the following protocol steps:

- [5.15 Clean Up Elution on page 86](#)
- [5.16 Identify Amplification Cycle Number on page 89](#)



For support videos, refer to the [Spatial Transcriptomics playlist](#).

Prepare the applicable reagents for each step ([Table 18](#)).

Table 18: Reagent Preparation Instructions

Reagent	Box	Instructions
Spatial Primer Cocktail (SPC)	1	Thaw at room temperature.
Spatial PCR Reagent (SPR)	1	Thaw at room temperature.
Illumina Purification Beads (IPB)	2	Store at room temperature.
Resuspension Buffer (RSB)	2	Store at room temperature.

5.15 Clean Up Elution

This step purifies the eluted DNA with a 1.5x bead cleanup using Illumina Purification Beads (IPB).



For more information on using IPB, refer to [4.6 Handle Beads on page 35](#).

Consumables

- Illumina Purification Beads (IPB)
- Resuspension Buffer (RSB)
- Absolute EtOH
- Nuclease-free water
- 1.5 ml microcentrifuge tube
- 50 ml conical tube
- PCR plate

Preparation

1. Combine the following volumes into a conical tube to prepare 18 ml 80% EtOH wash solution, including overage. Vortex to mix.
 - Absolute EtOH (14.4 ml)
 - Nuclease-free water (3.6 ml)



This solution can be used now and in later parts of the protocol. If you are continuing past the [SAFE STOPPING POINT on page 88](#) and proceeding immediately to [5.16 Identify Amplification Cycle Number on page 89](#), you can retain the solution for use in that protocol step.

2. Vortex IPB for one minute to disperse the beads. Make sure that the IPB is mixed thoroughly for bead binding efficiency.

Procedure

Bind

1. Add 960 μ l IPB to the UCM Elution tube containing elution.
2. Using the same pipette tip, pipette at least 10 times to mix.
3. Incubate at room temperature for 8 minutes.

Wash

1. Place the tube on a magnetic stand for 12 minutes or until the beads are fully pelleted.
2. Remove and discard the supernatant.
3. While the tube is on the magnetic stand, add 1.6 ml 80% EtOH to the tube.



Do not disturb the bead pellet.

4. After 30 seconds, remove and discard 80% EtOH.



Make sure that the beads are not disrupted during this process.

5. Repeat steps 3 and 4 one time for a total of two 80% EtOH washes.
6. Centrifuge at $280 \times g$ for 10 seconds to collect the remaining EtOH.
7. Place the tube back on the magnetic stand for 1 minute or until the beads are fully pelleted.
8. Using a P20 pipettor with fine tips, remove the residual supernatant.
9. While the tube is on the magnetic stand, let the beads dry at room temperature for up to 10 minutes until they are no longer glossy.



Do not let the beads dry to the point where they crack or become lighter brown.

First Elution

1. Remove the tube from the magnetic stand.
2. Add 154 μ l RSB to the tube.
3. Pipette at least 10 times to mix. Make sure that the beads are completely resuspended.

4. If any liquid remains on the walls, centrifuge at $280 \times g$ for 5 seconds.
5. Incubate at room temperature for 5 minutes.
6. Place the tube on the magnetic stand for 2 minutes or until the beads are fully pelleted.
7. Transfer 153 μl elution from the tube into a new microcentrifuge tube. Place the tube on ice and proceed to [Second Elution on page 88](#).

Second Elution

1. Remove the tube from the magnetic stand.
2. Add 154 μl RSB to the tube.
3. Pipette at least 10 times to mix. Make sure that the beads are completely resuspended.
4. If any liquid remains on the walls, centrifuge at $280 \times g$ for 5 seconds.
5. Incubate at room temperature for 5 minutes.
6. Place the tube on the magnetic stand for 2 minutes or until the beads are fully pelleted.
7. Transfer 153 μl elution from the tube into the same elution tube so that the final elution volume is ~306 μl . Place the tube on ice.
8. Store the remaining 80% EtOH at room temperature.



This EtOH can be used in [5.19 Clean Up Library on page 95](#).

SAFE STOPPING POINT

If you are stopping, store the eluted DNA in -80°C freezer for up to 3 days.

5.16 Identify Amplification Cycle Number

This step identifies the optimal amplification cycle number for library amplification.

Consumables

- Spatial Primer Cocktail (SPC)
- Spatial PCR Reagent (SPR)
- KAPA SYBR FAST qPCR Master Mix (2X) kit
- Nuclease-free water
- 1.5 ml microcentrifuge tube
- PCR plate seal
- qPCR plate

Preparation

1. Prepare the following consumables:
 - SPC—Pulse vortex three times to mix, and then centrifuge briefly. Store at room temperature.
 - SPR—Pulse vortex three times to mix, and then centrifuge briefly. Store at room temperature.
2. Combine the following volumes to prepare 40 µl Spatial qPCR Master Mix ([Table 19](#)). Pipette to mix thoroughly.

Table 19: Spatial qPCR Master Mix Preparation

Reagent	Volume (µl)
KAPA SYBR FAST qPCR Master Mix (2X) kit	22
Nuclease-free water	12
SPR	4.5
SPC	1.5

3. Place master mix on ice until it is ready to use.
4. Store SPR and SPC until the amplification cycle number is identified.



These reagents are used again in [5.18 Amplify Library on page 93](#).

For storage information, refer to [SAFE STOPPING POINT on page 91](#).

Procedure

1. Set up the No Template Control (NTC) as follows.
 - a. Aliquot 9 μ l Spatial qPCR Master Mix into one well of the qPCR plate.
 - b. Add 1 μ l nuclease-free water to the well with the Spatial qPCR Master Mix.
2. Combine the following volumes in a microcentrifuge tube to prepare the 25 μ l sample reaction mix. Pipette to mix.
 - Spatial qPCR Master Mix (22.5 μ l)
 - Eluted DNA (2.5 μ l)
3. Pipette 10 μ l sample reaction mix into two wells of the qPCR plate to create technical duplicates.
4. Store the remaining eluted DNA until the amplification number is identified.



For storage information, refer to [SAFE STOPPING POINT on page 91](#).

5. Seal the qPCR plate and shake at 2000 rpm for 1 minute.
6. Using a plate spinner, briefly centrifuge the plate.
7. Place the qPCR plate in the thermal cycler.
8. Run the following program on the thermal cycler:
 - a. Choose the preheat lid option and set to 105°C.
 - b. Set the reaction volume to 10 μ l.
 - c. Set the temperature and time for each set of cycles ([Table 20](#)).

Table 20: Thermal Cycler Program Cycles

Number of Cycles	Temperature	Time
1	95°C	3 minutes
25	95°C	10 seconds
	60°C	1 minute

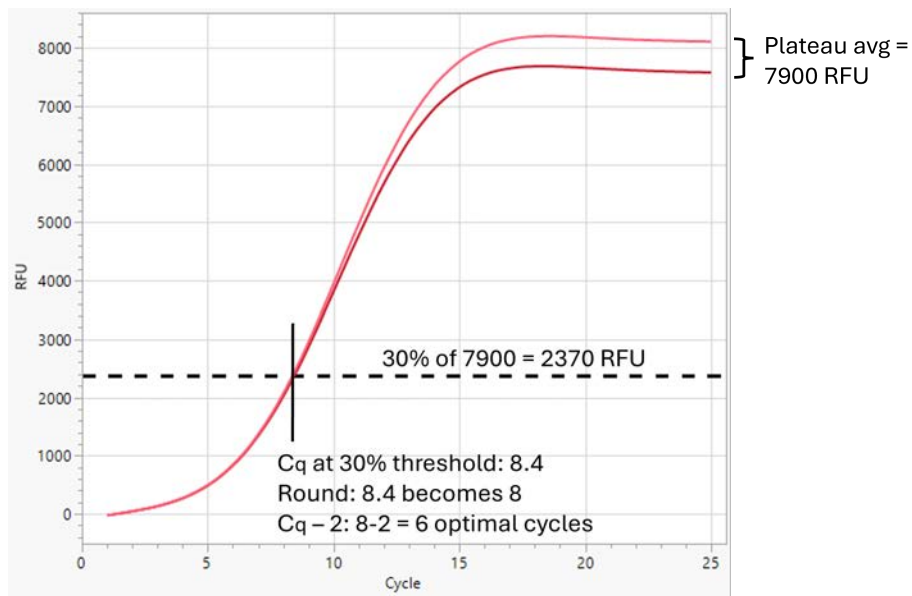
- d. Use the SYBR channel to read the signal.
9. Identify the optimal amplification cycle number as follows.
 - a. Calculate the average RFUs at the plateau phase.
 - b. Calculate where 30% of the plateau average is.
 - c. Identify the cycle number that corresponds with the 30% plateau average. Find the average C_q of the two technical qPCR replicates and round the C_q value to the nearest whole number.

- d. Subtract 2 from the identified C_q . Refer to the example in [Figure 26](#).



If the calculated optimal value is less than 1, use 1 so that there is sufficient amplification of the final library.

Figure 26: Cycle Number Identification Example



SAFE STOPPING POINT

Store the eluted DNA at -80°C for up to three days.

If you are stopping here, store SPR and SPC at -20°C .

If you are amplifying the library immediately after identifying the amplification number, then store the eluted DNA, SPR, and SPC at 4°C for up to 2 hours during qPCR.

5.17 Prepare for Steps

Reagents from the StrataMap Spatial Transcriptome kit must be prepared for the following protocol steps:

- [5.18 Amplify Library on page 93](#)
- [5.19 Clean Up Library on page 95](#)



For support videos, refer to the [Spatial Transcriptomics playlist](#).

Prepare the applicable reagents for each step ([Table 21](#)).

Table 21: Reagent Preparation Instructions

Reagent	Box	Instructions
Enhanced PCR Mix (EPM)	1	Thaw at room temperature.
Spatial Primer Cocktail (SPC)	1	Thaw at room temperature.
Spatial PCR Reagent (SPR)	1	Thaw at room temperature.
Illumina Purification Beads (IPB)	2	Store at room temperature.
Resuspension Buffer (RSB)	2	Store at room temperature.

5.18 Amplify Library

This step amplifies libraries to create copies of each unique template.

Consumables

- Enhanced PCR Mix (EPM)
- Spatial Primer Cocktail (SPC)
- Spatial Primer Reagent (SPR)
- Resuspension Buffer (RSB)
- 1.5 ml microcentrifuge tube
- PCR plate seal
- PCR plate

Preparation

1. Prepare the following consumables:
 - EPM—Pulse vortex three times to mix, and then centrifuge briefly. Store on ice.
 - SPC—Pulse vortex three times to mix, and then centrifuge briefly.
 - SPR—Pulse vortex three times to mix, and then centrifuge briefly.
2. Combine volumes in the following order in a 1.5 ml microcentrifuge tube to prepare the PCR Master Mix on ice ([Table 22](#)).
 - These volumes produce 825 μ l PCR Master Mix for 16 PCR reactions, including overage.
 - Pipette mix thoroughly.

Table 22: PCR Master Mix Preparation

Reagent	Volume (μ l)
EPM	333
SPC	25
SPR	83
RSB	82
Eluted DNA	302

Procedure

1. Pipette 50 µl PCR Master Mix into 16 wells of a PCR plate to create 16 PCR reactions.
2. Seal the plate with the PCR plate seal.
3. Centrifuge at 280 × g for 10 seconds.
4. Run the following PCR program:
 - a. Choose the preheat lid option and set to 105°C.
 - b. Set reaction volume to 50 µl.
 - c. Set the temperature and time for each set of cycles ([Table 23](#)).

Table 23: PCR Program Cycles

Number of Cycles	Temperature	Time
1	98°C	30 seconds
4	98°C	30 seconds
	58°C	1 minute
	72°C	1 minute
X ¹	98°C	30 seconds
	70°C	1 minute
	72°C	1 minute
1	72°C	2 minutes
1	10°C	Infinite hold ²

¹ The number of cycles (X) is the optimal cycle number identified in [5.16 Identify Amplification Cycle Number on page 89](#). If the calculated optimal amplification cycle number is less than 1, then use 1 for the number of cycles so that there is sufficient amplification of the final library.

² Press the ∞ key to set infinite hold.

5. After running the PCR program, centrifuge the plate at 280 x g for 10 seconds.

5.19 Clean Up Library

This step purifies the amplified library with a 0.6x bead cleanup using Illumina Purification Beads (IPB).

Consumables

- Illumina Purification Beads (IPB)
- Resuspension Buffer (RSB)
- Absolute EtOH
- Nuclease-free water
- 50 ml conical tube
- 1.5 ml microcentrifuge tubes

Preparation

1. **[Optional]** Combine the following volumes in a 50 ml conical tube to prepare 6 ml 80% EtOH, including overage. Vortex to mix.
 - Absolute EtOH (4.8 ml)
 - Nuclease-free water (1.2 ml)



If you retained 80% EtOH during [5.15 Clean Up Elution on page 86](#) and did not stop at the SAFE STOPPING POINT, you can use that for this step and do not have to prepare additional 80% EtOH.

2. Vortex IPB for 1 minute to disperse the beads fully. Make sure that IPB is mixed thoroughly for bead-binding efficiency.
3. Combine the 16 PCR reactions from [5.18 Amplify Library on page 93](#) by pipetting them into a 1.5 ml microcentrifuge tube. Pipette to mix thoroughly.

Procedure

Bind

1. Using a pipettor, measure the total volume of the combined PCR product.

2. Calculate the required volume of IPB for a 0.6x bead cleanup by multiplying the PCR product volume by 0.6.



For example, if there is 720 μ l PCR product, you need to add 432 μ l IPB.

3. Add the calculated amount of IPB to the combined PCR product in the 1.5 ml microcentrifuge tube. Pipette at least 10 times to mix.
4. Incubate at room temperature for 8 minutes.

Wash

1. Place the microcentrifuge tube on the magnetic stand for 5 minutes or until the beads are fully pelleted.
2. Remove and discard the supernatant.
3. Wash the beads as follows.
 - a. While the microcentrifuge tube is on the magnetic stand, add 1 ml 80% EtOH.
 - b. After 30 seconds, remove and discard the supernatant.



Make sure that the beads are not disrupted during this process.

- c. Repeat steps [a](#) and [b](#) one time for a total of two 80% EtOH washes.
4. Centrifuge at $280 \times g$ for 10 seconds to collect the remaining EtOH.
 5. Return the microcentrifuge tube to the magnetic stand for one minute or until the beads are fully pelleted.
 6. Using a P20 pipettor with fine tips, remove the residual supernatant.
 7. While the microcentrifuge tube is on the magnetic stand, let the beads dry at room temperature for up to 5 minutes until they are no longer glossy.



Do not let beads dry to the point where they crack or become lighter brown.

Elute

1. Remove the microcentrifuge tube from the magnetic stand.

2. Add 432 μ l RSB to each sample. Pipette mix at least 10 times and make sure that the beads are completely resuspended before proceeding.
3. If any liquid remains on the walls, centrifuge at $280 \times g$ for 5 seconds.
4. Incubate at room temperature for 5 minutes.
5. Place the microcentrifuge tube on the magnetic stand for 2 minutes or until the beads are fully pelleted.
6. Transfer 425 μ l elution into a new 1.5 ml microcentrifuge tube to create the final purified library.

SAFE STOPPING POINT

If you are stopping, store at -20°C for up to three weeks.

5.20 Library Quantification

This step checks the quantity and quality of the purified library in preparation for library dilution and sequencing.

When performing library quantification, expect a wide distribution of fragment sizes and library concentrations that are greater than or equal to 2 nM. The average fragment size and concentration varies based on various factors, such as tissue type and the initial RNA quality in the tissue block.

Consumables

- KAPA Library Quantification Kit
- High sensitivity D5000 screentape
- High sensitivity D5000 reagents
- High sensitivity D5000 ladder

Procedure

qPCR Library Quantification

1. Use the KAPA Library Quantification Kit to quantify your library.



For instructions, refer to the KAPA Library Quantification Kit documentation.

Library quantity cannot be determined using the TapeStation quantification values.

TapeStation Library Analysis

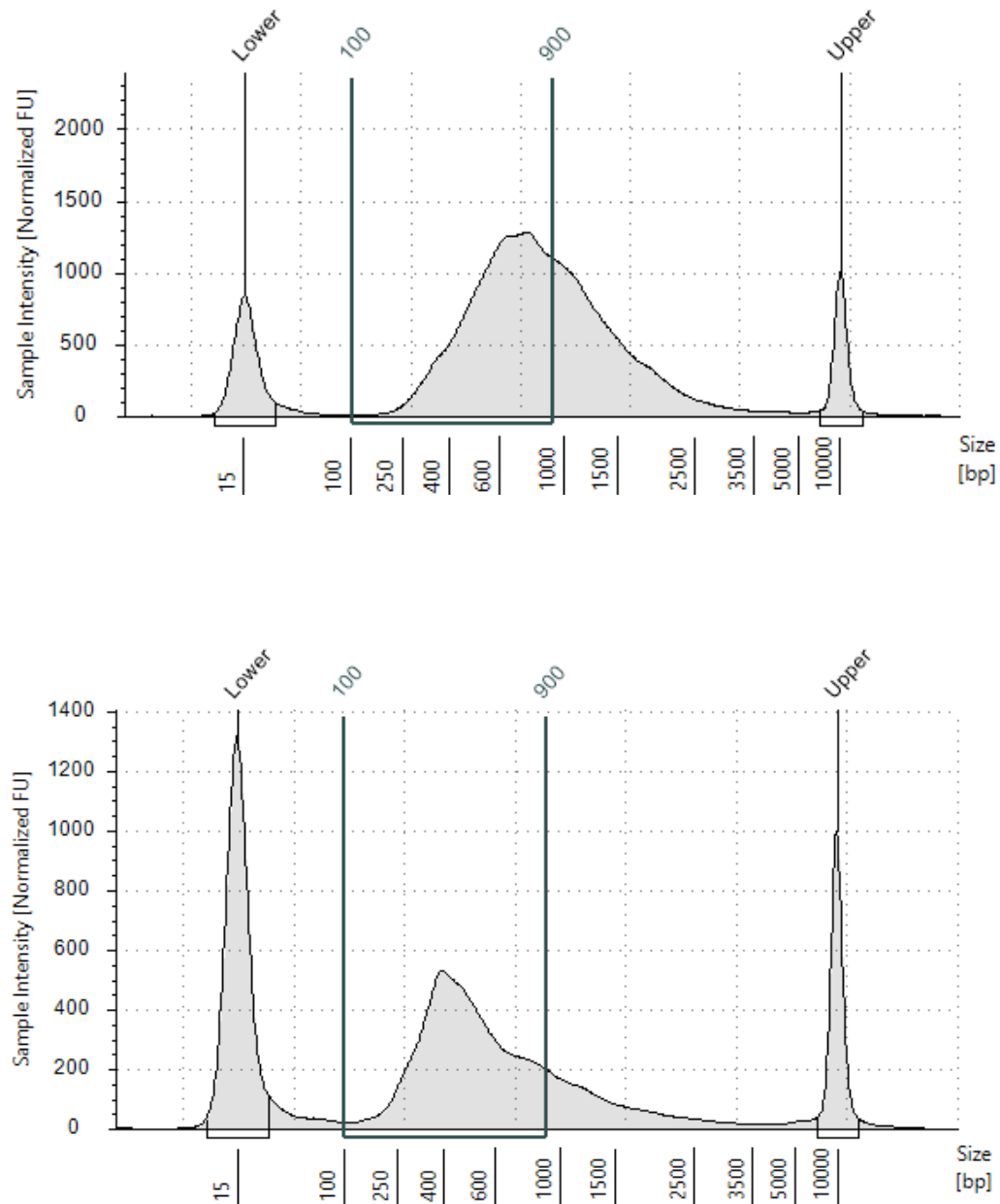
1. Prepare the high sensitivity D5000 reagents according to the manufacturer recommendations.
2. Using the high sensitivity D5000 screentape, analyze 2 µl library.



If the library is not within the quantification range of the D5000 high sensitivity kit, then you can dilute the library until it is in the range.

- Using the TapeStation analysis software, edit the region boundaries to define an analysis window of 100–900 base pairs (bp). Refer to the examples in [Figure 27](#).

Figure 27: TapeStation Trace Examples



4. Within the 100–900 bp analysis region, calculate the average fragment size.



If you do not use the 100–900 bp region for size normalization, inaccurate library quantifications and reduced sequencing performance can occur.

A long tail of fragments with less than 300 bp can indicate an error in size selection during cleanup, which can result in reduced performance.



Spatial libraries have fragments that extend beyond 900 bp that should not be included when determining the average fragment size for loading.

5.21 Dilute Libraries to Starting Concentration

This step dilutes the libraries to the starting concentration for the sequencing system and is the first step in a serial dilution. After diluting to the starting concentration, the libraries are ready to be denatured and diluted to the final loading concentration.

The final loading concentration is a starting point and is provided as general guidance. Optimize concentrations for your workflow and quantification method over subsequent sequencing runs or by flow cell titration.

Consumables

- RSB
- 1 N NaOH
- PhiX Control v3

Procedure

1. Calculate the molarity value of the library using the following formula:

$$qPCR\ concentration \times \frac{452\ bp}{average\ fragment\ length\ in\ 100-900\ bp\ region} \times dilution\ factor = Adjusted\ library\ molarity$$



Use the qPCR KAPA library quantification kit and TapeStation to analyze each library. For instructions, refer to [5.20 Library Quantification on page 98](#).

- Using the molarity value, calculate the volumes of RSB, PhiX Control v3, and library needed to dilute the library to the starting concentration for your system ([Table 24](#)).



The following final loading concentrations are used as a general guideline. Determine the optimal loading concentrations for your workflow using the preferred quantification method with sequencing runs or through flow cell titration.

Table 24: Final Loading Concentrations

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)	PhiX (%)
NovaSeq X Series 5B flow cell	2	250	1
NovaSeq X Series 10B flow cell	2	250	1
NovaSeq X Series 25B flow cell	2	250	1
NovaSeq 6000 S2 and S4 flow cells	0.5	100	1
NextSeq 2000 P4 flow cell	2	800	1

- Follow the denature and dilute instructions from the [Denature and Dilute Protocol Generator](#) to dilute each library to the starting concentration.
- Set up a single-end run for your system.

Illumina recommends setting up a single-end run with 130 cycles for Read 1 and 8 cycles for Index Read 1.



For NovaSeq X Series, NovaSeq 6000, and NextSeq 2000, Illumina recommends a single library (single slide) with the library loaded across one flow cell.

For NovaSeq 6000 and NovaSeq X Series, Illumina recommends multiple spatial transcriptome libraries (multiple slides) with individual libraries loaded by lane across one flow cell. The libraries are separated by individual lanes.

5.22 Sequencing and Analysis

After diluting your libraries to the starting concentration, you can start sequencing using a NovaSeq 6000, NovaSeq X Series, or NextSeq 2000 system.

For more information on the sequencing systems, refer to the following documentation:



- [NovaSeq X Series Product Documentation](#)
- [NovaSeq 6000 Sequencing System Guide](#)
- [NextSeq 1000/2000 Product Documentation](#)

StrataMap Spatial Transcriptome assay libraries can be sequenced across all lanes of a flow cell or in individually addressable lanes.

- If the library originates from one slide, sequencing data from multiple flow cells can be combined into one analysis.
- If combining with other library types on the same flow cell, refer to the [Illumina StrataMap Spatial Software documentation](#) for specific instructions.

Sequencing Planning

The spatial transcriptome assay is compatible with the following flow cells and workflows:

- NovaSeq X Series 10B and 25B flow cells using the NovaSeq X Series protocol
- NovaSeq 6000 S2 and S4 flow cells using the Standard workflow
- NovaSeq 6000 S4 flow cell using the Xp workflow
- NextSeq 2000 P4 flow cell using the XLEAP-SBS reagents

If you are using the NovaSeq X Series instrument, you can also do per-lane loading run planning.

Recommended Sequencing Depth

- Illumina recommends sequencing to a depth of 5000 raw reads per 10 x 10 μm tissue area.
 - This sequencing depth recommendation is a starting point and is provided as general guidance. It can be further optimized based on your needs.
 - Actual reads per cell reported in the analysis output can vary based on tissue type and cell size.

Calculate Sequencing Amount Using Tissue Area

The area of each tissue section (in mm²) can be found in the local ISIT export file (for example, `tissue_area_for_substrate_[Slide ID].csv`). The area for each tissue section is also shown on the Refine tissue masks page. Use the following formula to calculate the recommended sequencing depth in number of raw sequencing reads using the total area:

$$\text{sum of sample area (mm}^2\text{)} \times 10,000 \times 5000 \text{ raw reads} \times 1.10$$

This equation assumes that there is a 10% overage for conservative sequencing estimation. The unit conversion is 10,000 for converting mm² to a 10 x 10 µm tissue area.

For example, if there are three samples with tissue areas of 41.8, 38.8, and 38.7 mm², then ~6.56 billion reads are needed to achieve ~5,000 raw reads per 10 x 10 µm tissue area in each sample:

$$119.3 \times 10,000 \times 5,000 \times 1.10 = 6,561,500,000$$

Primary and Secondary Analysis

The DRAGEN StrataMap analysis software is used to process sequencing data along with the images taken during the protocol. Secondary analysis with this software and the DRAGEN StrataMap pipeline consists of the following steps:

- **[Optional]** Cell segmentation can be initiated independently using the Illumina StrataMap Cell Segmentation Tool. This tool can be used after image acquisition and before proceeding with the assay protocol. You can confirm if the cell segmentation meets your experimental needs or if the images should be retaken.
- Run setup, including run planning, and manual launch with Platform Core.
- If you are using the NovaSeq X Series system, you can also do run planning per lane.
- Uploading the following analysis inputs:
 - BCL folders
 - Sample sheets
 - Reference genomes
 - OME-TIFF files from ISIT
 - **[Optional]** Analysis configuration file
 - **[Optional]** Cell segmentation file from the Illumina StrataMap Cell Segmentation Tool

- Generating the outputs from the Intermediates folder.



For a list of the files with descriptions, refer to the [Illumina StrataMap Spatial Software documentation](#).

- Generating the Run Summary report and information (for example, contour CSV files and binned data) that is used by ICM for downstream analysis.



For more information, refer to the DRAGEN StrataMap in the [Illumina StrataMap Spatial Software documentation](#).

Tertiary Analysis

ICM is used to create and update studies that can be used for spatial analysis. ICM also generates the Spatial Report, which contains graphs and plots of the bin and cluster data. In this report, you can do the following actions:

- Navigate and adjust the spatial image
- Configure the cluster and bin plots
- Highlight clusters across plots



For more information, refer to the Illumina Connected Multiomics in the [Illumina StrataMap Spatial Software documentation](#).

6. Resources and References

This section contains reference documentation and the revision history.

6.1 Reference Documentation

Table 25 identifies documentation that is used with the StrataMap Spatial Transcriptome assay or can be referred to for additional information on Illumina software.

Table 25: Reference Documentation

QR Code	Title
	StrataMap Spatial Transcriptome Imaging Guide
	StrataMap Spatial Transcriptome Permeabilization Optimization Kit User Guide
	Illumina StrataMap Spatial Software documentation

6.2 Revision History

Document	Date	Description of Change
Document # 200073552 v00	August 2026	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com

Email: techsupport@illumina.com

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download from support.illumina.com.



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