STOmics

Stereo-seq Sample Preparation, Sectioning and Mounting Guide for FFPE Samples on Stereo-seq Chip Slides

USER MANUAL

Cat. No.:
211SN11114 (4 RXN) / 211SN11114-CG (4 RXN)/
211SN11004 (4 RXN) / 211SN11004-CG (4 RXN)
Kit Version: V1.1
Manual Version: B

STUM_SP003

REVISION HISTORY

Manual Version:	A
Kit Version:	V1.0
Date:	Jul. 2024
Description:	Initial release

Manual Version:	A_1
Kit Version:	V1.0
Date:	Jul. 2025
Description:	Added US use only catalog numbers.

Manual Version:	B
Kit Version:	V1.1
Date:	Nov. 2025
Description:	Compatible with Stereo-seq OMNI Transcriptomics Set V1.1.

TABLE OF CONTENTS

NOTE: Additional operation tips and guidance.

CRITICAL STEPS: Pay extra attention for these steps to avoid experimental setbacks or problematic results.

QUALITY CHECK POINT

CAUTION: Proceed with extra care; improper handling or carelessness may cause experimental failure or accidents.

STOP POINT: Here you may pause your experiment and store your sample.

CHAPTER 1
INTRODUCTION

1.1. Introduction

The STOmics Stereo-seq Transcriptomics Set for FFPE is intended for generating a spatially-resolved total RNA library from formalin-fixed and paraffin-embedded (FFPE) biological tissue sections that requires a Stereo-seq Chip Slide with intact tissue sections as input. This guide provides general guidelines for properly performing tissue sectioning and mounting to better preserve the morphological quality of the FFPE tissue sections.

The Stereo-seq Chip Slides prepared in this guide are part of these products:

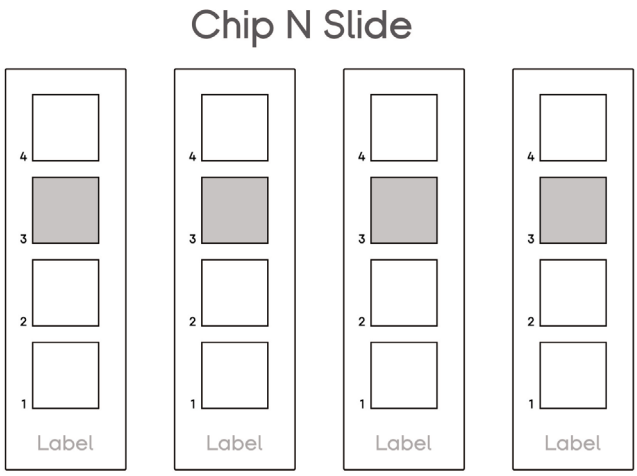
Stereo-seq OMNI Transcriptomics FFPE Set for Chip-on-a-slide (1cm*1cm) V1.1 (211SN11114 / 211SN11114-CG)

Stereo-seq OMNI Transcriptomics FFPE Set for Chip-on-a-slide (0.5cm*0.5cm) V1.1 (211SN11004 / 211SN11004-CG)

1.2. Stereo-seq Chip N Slide Information

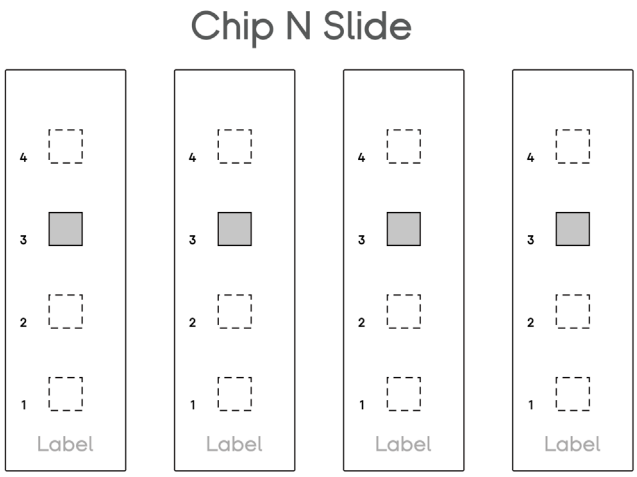
Stereo-seq Chip N Slide (1cm * 1cm)

- Includes 4 Stereo-seq Chip N Slides containing **one** Chip N (1cm * 1cm) on each slide.



Stereo-seq Chip N Slide (0.5cm * 0.5cm)

- Includes 4 Stereo-seq Chip N Slides containing **one** Chip N (0.5cm * 0.5cm) on each slide.



Stereo-seq Chip N Slide Storage

Upon receiving the Stereo-seq Chip N Slide (0.5cm * 0.5cm) or (1cm * 1cm), follow the instructions in Stereo-seq Chip Slide Operation Guide For Receiving, Handling And Storing to properly store unused Stereo-seq Chip N Slides.

Always store unused slides in their original slide container and keep them sealed in a sealable aluminum bag at -25°C ~ -15°C. Keep sealed with tape or another re-sealable bag. **Always KEEP the desiccant in the bag.** Resealed Stereo-seq Chip N Slides should be used within 2 weeks.

1.3. Stereo-seq Transcriptomics N Kit

Table 1-1 Stereo-seq Transcriptomics N Kit Components

Stereo-seq Transciptomics N Kit	Cat. No.:211KN11114 / 211KN11114-CG		
Component	**Reagent Cat. No.**	**Cap Color**	**Quantity (tube)**
Coating Buffer I	1000064221	●	220 μL × 1
Coating Buffer II	1000064222	●	10 μL × 1
Mounting Medium	1000064223	●	100 μL × 1
Decrosslinking Reagent	1000064224	●	1700 μL × 1
PR Enzyme	1000028500	●	10 mg × 1
RI	1000028499	●	300 μL × 1
RT Buffer I	1000064225	●	800 μL × 1
RT Buffer Mix	1000047911	○ (transparent)	731 μL × 1
RT Oligo	1000047913	○ (transparent)	44 μL × 1
RT Enzyme Mix	1000047914	○ (transparent)	88 μL × 1
RT Plus	1000047912	●	18 μL × 1
Elute Buffer	1000064226	●	1725 μL × 2
Elute Additive	1000064227	●	88 μL × 1
4X cDNA PCR Mix	1000047916	●	220 μL × 1
cDNA Primer	1000047917	●	36 μL × 1
Storage Temperature: -25°C ~ -15°C		Transportation Temperature: -25°C ~ -15°C	Expiration Date: refer to label

CHAPTER 2
SAMPLE PREPARATION

2.1. Reagent Preparation

Unless otherwise specified, use nuclease-free water for all reagents prepared prior to this experiment.

*Only the row bolded in red will be used in this guide. Please use the following master planning table as your reference when planning for subsequent experiments.

Table 2-1 Reagent Preparation Master Planning Table

Prep Day	Reagent	Workflow Section	Preparation Steps	Maintenance
Day 1	**400 mL 30% Ethanol**	Tissue sectioning and mounting	Dilute anhydrous ethanol to 30% with ddH_2O.	Room temperature up to 1 day
	Coating Buffer I	Chip pre-treatment	Take it out of -20°C in advance, thaw on ice and store at 4°C. If precipitates are visible, vortex at maximum speed until they disappear.	4°C up to 1 day
	Coating Buffer II	Chip pre-treatment	Take it out of -20°C in advance, and store on ice.	On ice until use, up to 2 hr
Day 2	100 mL 96% Ethanol	Deparaff-inization	Dilute anhydrous ethanol to 96% with ddH_2O.	Room temperature up to 1 day
	50 mL 90% Ethanol	Deparaff-inization	Dilute anhydrous ethanol to 90% with ddH_2O.	Room temperature up to 1 day
	50 mL 80% Ethanol	Deparaff-inization	Dilute anhydrous ethanol to 80% with ddH_2O.	Room temperature up to 1 day
	50 mL 70% Ethanol	Deparaff-inization	Dilute anhydrous ethanol to 70% with ddH_2O.	Room temperature up to 1 day
	50 mL 50% Ethanol	Deparaff-inization	Dilute anhydrous ethanol to 50% with ddH_2O.	Room temperature up to 1 day
	50 mL 30% Ethanol	Deparaff-inization	Dilute anhydrous ethanol to 30% with ddH_2O.	Room temperature up to 1 day
	400 μL 5X SSC	ssDNA Staining	Dilute 100 μL of 20X SSC to 400 μL.	Room temperature up to 1 week
	Mounting Medium	ssDNA Staining	Take it out of -20°C in advance and equilibrate to room temperature until it is thawed.	Room Temperature
	Fluorescent Staining Solution	ssDNA Staining	Dilute 1 μL of Qubit ssDNA Reagent to 200 μL with 5X SSC.	Room temperature in the dark up to 1 hr

Day 2	0.1X SSC	ssDNA Staining, Decrosslinking, cDNA Release	Dilute 250 µL of 20X SSC to 50 mL.	Room temperature up to 1 week
	0.01N HCl	Permeabi-lization	Prepare at least 2 mL of 0.01N HCl per sample. Measure and ensure that the pH = 2.	Room temperature for 48 hr **(Storing longer than 48 hr will affect the desired pH. Use within 48 hr of preparation)**
	Always use freshly prepared 0.01N HCl (pH = 2.0 ± 0.1). For pre-made 0.1N HCl and newly purchased HCl, check the pH prior to the experiments.			
	5X SSC (with 5% RI)	Permeabi-lization	Add 20 µL RI and 100 µL 20X SSC into 280 µL nuclease-free water and mix well, maintain on ice.	On ice until use, up to 2 hr
	Decrosslink-ing Reagent	Decrosslinking	Take it out of -20°C in advance, and equilibrate to room temperature until no white precipitates are visible.	Room temperature
	Methanol	Fixation	Prepare 30 mL of methanol in a staining jar and pre-cool it for 5-30 min at -20°C.	-20°C
	10X Permeabi-lization Reagent Stock Solution	Permeabi-lization	Briefly centrifuge the PR Enzyme (red cap, in powder form) for 2-3 sec. Add 1 mL of freshly prepared 0.01N HCl and thoroughly mix the reagent by pipetting. Store at -20°C for up to 1 month.	On ice until use, up to 2 hr
	Do not vortex the permeabilization enzyme. Mix by pipette before using. **Aliquot the 10X PR Stock Solution to avoid freeze-thaw cycles.**			
	1X Permeabi-lization Reagent Solution	Permeabi-lization	Dilute 20 µL of 10X PR stock solution to 200 µL with 0.01N HCl.	On ice until use, up to 6 hr
	RT Buffer I	RT	Take it out of -20°C in advance, and thaw on ice.	On ice until use
	RI	RT	/	-20°C
	RT Enzyme Mix	RT	/	-20°C
	RT Buffer Mix	RT	Take it out of -20°C in advance, and thaw on ice. If precipitates are visible, equilibrate at room temperature for 5 min, mix well, and vortex for use.	On ice until use

Day 2	RT plus	RT	Take it out of -20°C in advance, and thaw on ice.	On ice until use
	RT Oligo	RT	Take it out of -20°C in advance, and thaw on ice.	On ice until use
	Elute Buffer	cDNA Release	Take it out in advance and heat the buffer for 5 min at 55°C to dissolve the precipitate. Equilibrate it to room temperature prior to use.	Room temperature
	Elute Additive	cDNA Release	/	-20°C
Day 3	Magnetic beads	cDNA Purification	Take it out in advance and pre-heat at 42°C at least 30 min prior to use.	4°C
	30 mL 80% Ethanol	cDNA Purification	Dilute anhydrous ethanol to 80%.	Room temperature up to 1 day
	4X cDNA PCR Mix	cDNA Amplification	Take it out of -20°C in advance, and thaw on ice.	On ice until use
	cDNA Primer	cDNA Amplification	Take it out of -20°C in advance, and thaw on ice.	On ice until use
	TE Buffer, pH 8.0	cDNA Purification	Set it aside at room temperature until use.	Room temperature

Other Preparation

Equipment	Setup	Notes
PCR Thermal Cycler	Set the temperature in the following order: 1. 95°C for decrosslinking (heated lid at 85°C). 2. 37°C for slide baking and permeabilization (heated lid at 42°C). 3. 42°C for slide baking and FFPE Mix reactions (heated lid at 45°C). 4. 55°C for cDNA release (heated lid at 60°C). 5. 95°C, 98°C, 58°C, 72°C for PCR amplification (heated lid at 105°C).	Check the PCR thermal cycler for any abnormalities, and replace it if necessary.
Fluorescence Microscope	For fluorescent imaging: Set the epi-fluorescence channel to FITC mode	Check the microscope for any abnormalities, and replace it if necessary.
Slide Dryer	Set the baking temperature to 60°C	Check the slide dryer for any abnormalities, and replace it if necessary.

2.2. Sample Requirements for Formalin-fixed and Paraffin-embedded (FFPE) Tissue

Sample Types

This set of kits can be used for samples from all common animals, including but not limited to human, monkey, and mouse.

Sample Preparation

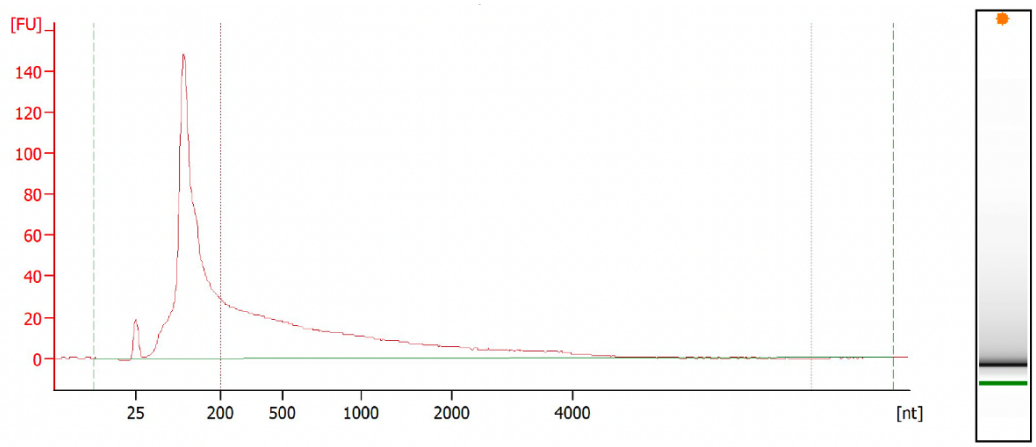
- Ensure that tissue samples are promptly fixed with 10% formalin or 4% paraformaldehyde (PFA) for 12 to 48 hr after sampling. It is recommended that use 10% formalin for fixation.
- To prevent drying, corrosion, and fungal contamination, seal the wax blocks properly.
- When processing samples, it is important to maintain the original tissue structure.
- Refer to your institution's pathology/histology labs for guidance on making paraffin blocks. This process requires adequate dehydration and wax immersion to produce continuous sections without cracks or gaps (apart from inherent tissue structures), ensuring that the specimen remains embedded in the wax during sectioning.

Paraffin block samples can be shipped directly. For details, refer to Appendix I: Shipping Preservation and Transportation.

FFPE Sample RNA Quality Control (QC) by DV200 Value

It is recommended that you assess the RNA quality (DV200 value) of a paraffin tissue sample before proceeding to the Stereo-seq experiment. Total RNA can be extracted from 3-5 slices of 5 μm-thick FFPE sections (Deparaffinization Solution, Qiagen 19093, and RNeasy FFPE Kit, Qiagen 73504), and the RNA can be stored at -80°C in a 1.5 mL EP tube for up to 1 month. Refer to **Figure 1** for examples of DV200 distribution ranges of different RNA samples, analyzed through Agilent High-Sensitivity RNA Analysis Kit (Agilent 5067-1513).

It is strongly recommended that you proceed only with tissue samples with a DV200 ≥ 30%.



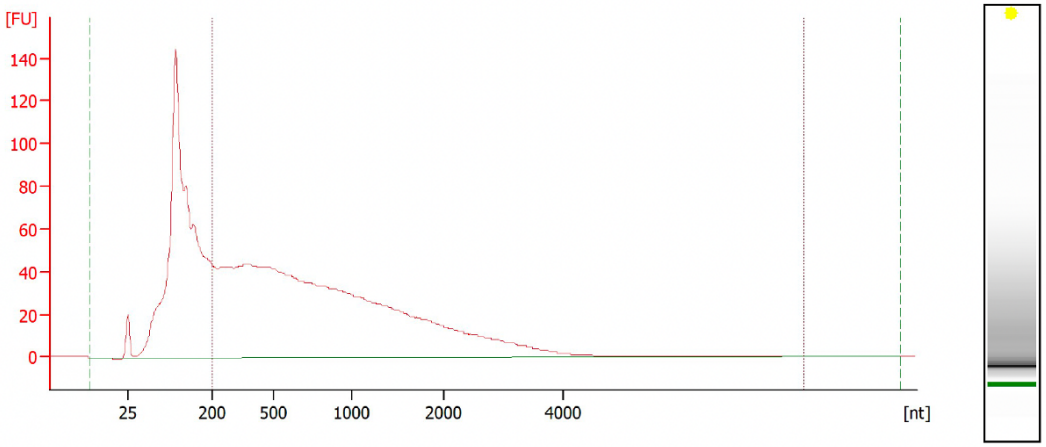
Overall Results for sample 8 : **Sample 8**

RNA Area:	1,490.5	Result Flagging Color:	
RNA Concentration:	5,968 pg/µl	Result Flagging Label:	RIN: 2.10 ; DV200 30-50%
rRNA Ratio [28s / 18s]:	0.0		
RNA Integrity Number (RIN):	2.1 (B.02.10)	Corr. Area 1:	682.5

Region table for sample 8 : **Sample 8**

Name	From [nt]	To [nt]	Corr. Area	% of Total	Color
DV200	200	8,000	682.5	46	

DV200: 30%~50%



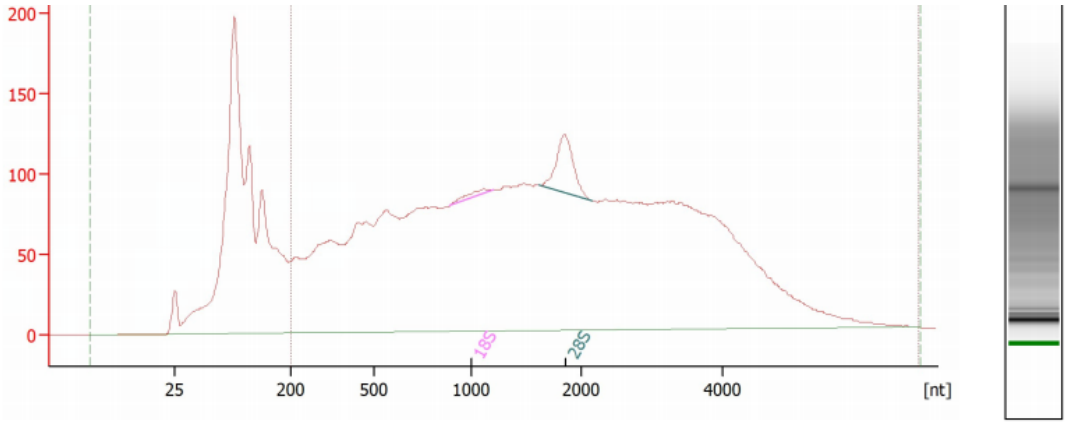
Overall Results for sample 10 : **Sample 10**

RNA Area:	2,395.0	Result Flagging Color:	
RNA Concentration:	9,590 pg/µl	Result Flagging Label:	RIN: 1.90 ; DV200 50-70%
rRNA Ratio [28s / 18s]:	0.0		
RNA Integrity Number (RIN):	1.9 (B.02.10)	Corr. Area 1:	1,507.8

Region table for sample 10 : **Sample 10**

Name	From [nt]	To [nt]	Corr. Area	% of Total	Color
DV200	200	8,000	1,507.8	63	

DV200: 50%~70%



Overall Results for sample 2 : **Sample 2**

RNA Area:	6,296.1	Result Flagging Color:	
RNA Concentration:	40,314 pg/µl	Result Flagging Label:	RIN: 2.40 ; DV200 > 70%
rRNA Ratio [28s / 18s]:	6.8		
RNA Integrity Number (RIN):	2.4 (B.02.11)	Corr. Area 1:	4,902.0

Fragment table for sample 2 : **Sample 2**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	890	1,195	13.3	0.2
28S	1,613	2,143	90.9	1.4

Region table for sample 2 : **Sample 2**

Name	From [nt]	To [nt]	Corr. Area	% of Total	Color
DV200	200	6,797	4,902.0	78	

DV200: >70%

Figure 1. Examples of DV200 Distribution Intervals of Different RNA Samples for Quality Control

2.3. Chip Pre-treatment

Chip pre-treatment is necessary for Stereo-seq Chip N Slide. After treating the chip with the coating solution, use it immediately. If you do not perform section mounting on the Stereo-seq Chip N Slide, do not perform pre-treatment for the Stereo-seq chip.

For FFPE sections that have been transported (or stored after transportation), if you are unsure if they can be successfully expanded, you can test it by placing one section in 30% ethanol solution to evaluate the sample condition before chip pre-treatment.

Reagent	Preparation Steps	Maintenance
400 mL 30% Ethanol	Dilute anhydrous ethanol to 30% using ddH_2O.	Room temperature up to 1 day
Coating Buffer I (1000064221)	Take it out of -20°C in advance, thaw on ice, and store at 4 °C. If precipitates are visible, vortex at maximum speed until they disappear.	4°C up to 1 day
Coating Buffer II (1000064222)	Take it out of -20°C in advance, and store on ice.	On ice up to 2 hr

a. Take out Coating Buffer I and Coating Buffer II from the Stereo-seq Transcriptome N Kit (Cat. No.: 211KN11114 / 211KN11114-CG).

If you will not be performing a transcriptome experiment immediately after tissue mounting, promptly store remaining reagents at -20°C.

b. Prepare the chip coating solution according to Table 2-2 and mix (for Stereo-seq chip of 1cm * 1cm or 0.5cm * 0.5cm) :

Table 2-2 Chip coating solution

Components	1X + 10% (μL)	2X + 10% (μL)	3X + 10% (μL)	4X + 10% (μL)
Nuclease-free Water	162.8	325.6	488.4	651.2
Coating Buffer I (1000064221)	55	110	165	220
Coating Buffer II (1000064222)	2.2	4.4	6.6	8.8
Total	220	440	660	880

c. Take the stere-seq Chip N slide out of the vacuum-sealed aluminum bag and record the Chip ID (serial number) from the back of the chip. Be careful not to touch the chip surface.

After opening the bag, check all Stereo-seq Chip Slides in the slide container and place the slide with the front of the chip facing up. The front of a chip has a shiny surface that contains DNB-probes for RNA capture. DO NOT scratch the surface.

Store unused slides in the original packaging (first in the slide container and then in the sealable aluminum bag) and seal at -25°C ~ -15°C. KEEP the desiccant in the aluminum bag.

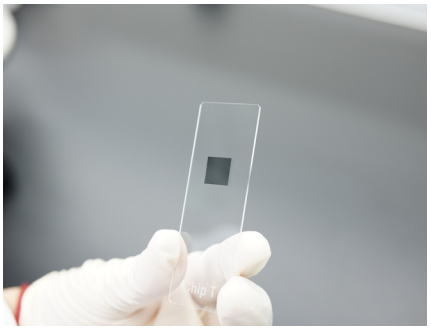
d. Place the Stere-seq chip N Slide flat on the bench for **1 min** to equilibrate to room temperature.

e. Assemble the cassette, add **200 μL** of chip coating solution, add it into the cassette from the four corners of the chip, and incubate at room temperature for **10 min**.

f. Discard the liquid and add **200 μL** nuclease-free water from the four corners of the chip into the Cassette to clean the chip.

g. Repeat **step f**.

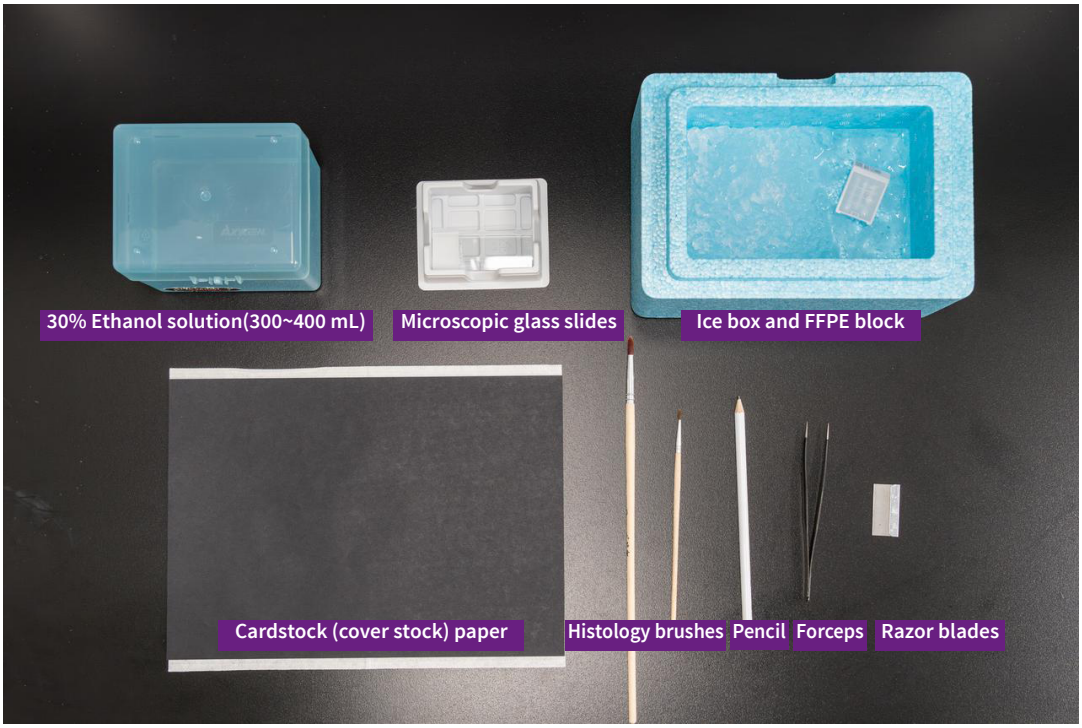
h. Remove the nuclease-free water, discard the cassette, and use a power dust remover to dry the chip surface for later use;



After chip pre-treatment, if the tissue mounting can be completed within 1 hr, it can be stored at room temperature.

If the waiting time is greater than 1 hr, put the chip into a slide container, and store at 4°C with desiccants for up to 1 day.

2.4. Tissue Sectioning and Mounting



For a demonstration video of paraffin section mounting on the Stereo-seq Chip Slide, refer to the link below or scan the QR code:
https://en.stomics.tech/resources/videos/list.html

Prepare the following:

Reagent	Preparation Steps	Purpose
400 mL 30% Ethanol	Dilute 120 mL of anhydrous ethanol to 30% using 280mL ddH2O. Always use freshly prepared 30% ethanol.	Paraffin block sectioning and mounting

This procedure should be carried out by technicians who are experienced in performing paraffin sectioning.

a. Make sure the water bath and baking machine have been turned on and set to 40°C ~ 48°C and 42°C, respectively.

If a different water bath temperature has been employed at your institution's pathology/histology laboratories, always adhere to the established protocols.

If a PCR thermal cycler is used in place of a baking machine, place the PCR Adaptor on the PCR thermal cycler in advance, and set the PCR thermal cycler according to the following incubation protocol.

Table 2-3 Baking Program

Temperature	Time
(Heated lid) 45°C	on
42°C	∞
42°C	3 hr
37°C	∞

b. Prepare the microtome, histology brushes, forceps, new microtome blades, and a container filled with **300 ~ 400 mL** of 30% ethanol.

c. Place the FFPE block face down in an ice water mixture for **10 ~ 30 min**, or cool the tissue surface on a cooling platform for **5 ~ 10 min**.

For tissues with high-fat content, such as breast tissue samples, freeze the FFPE block at -20°C for 10 min before sectioning.

d. Make sure the baking machine (or PCR thermal cycler) has been turned on and set to 42°C.

e. Before sectioning, wipe away any liquid from the FFPE block surface and its surroundings.

f. Insert the paraffin block on the microtome and orient it such that the blade will cut straight across the block. If the tissue is deeply embedded, process the block with an old trimming blade and cut a few thin sections to ensure that the positioning is correct. Adjust if necessary.

g. Trim the block to expose enough tissue surface from which a representative section can be cut. Trimming is normally done at a thickness of **10~30 μm**. Once the tissue is exposed, switch to a new blade for sectioning.

h. Adjust the section thickness to **5 μm** for regular tissue and **4 μm** for high-fat-content tissue to reduce the possibility of section detachment in subsequent operations.

i. Discard the first section as its thickness generally does not meet the requirements due to sectioning artifacts. Select the desired section during subsequent sectioning and trim it to a suitable size for mounting on the Stereo-seq Chip Slide. Using clean forceps, carefully transfer the selected section onto cardstock paper for trimming.

If adjacent sections need to be saved for H&E staining, prepare three serial sections Use the first and third sections for H&E staining on microscope glass slides. Carefully separate the middle section from the other two and prepare it for Stereo-seq Chip Slide.

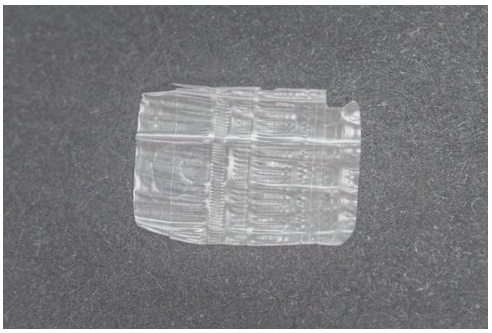
For details on shipping paraffin sections, refer to Appendix I: Shipping Preservation and Transportation.

For a demonstration video, refer to the links below:

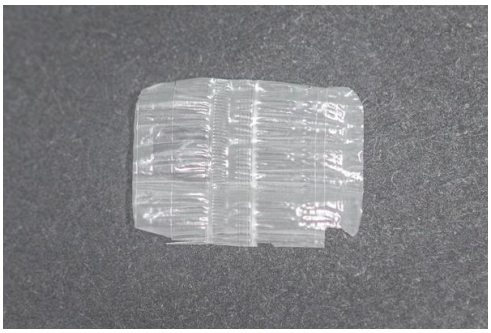
FFPE Section (Sample Selection) Preparation and Shipping Guidelines (For Section Preparation)

FFPE Section H&E Staining and Selection Guidelines (For the Recipient)

A paraffin section has two sides, side A and side B. The side facing the operator is side A (matte side), and the side touching the blade is side B (smooth side). Always keep side B facing down when it is floating in the water bath, or keep it contacting the Stereo-seq Chip surface and microscope glass slide for mounting to prevent the section detachment.



Side A

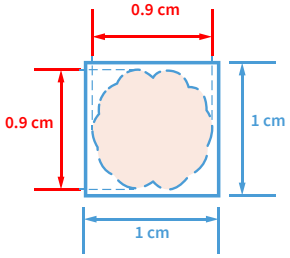


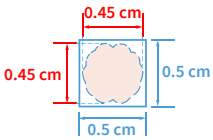
Side B

j. Trim the edges of the targeting section to fit the Stereo-seq Chip, and carefully transfer the section. Make sure the side B is in contact with the liquid surface of 30% ethanol using a histology brush or clean forceps.

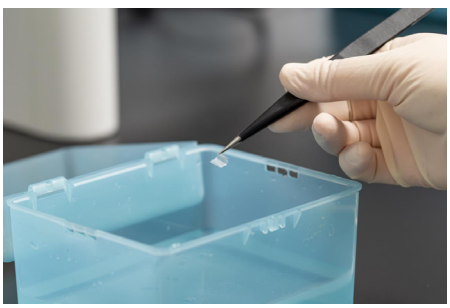
Chip Size	Tissue Section Size
1cm * 1cm	≤0.9 cm * 0.9 cm
0.5cm * 0.5cm	≤0.45 cm * 0.45 cm

After trimming, the tissue section should not exceed the recommended size, as a coverage beyond 80% of the chip area will interfere with image registration. This can cause misalignment between the image and gene expression matrix, leading to errors in subsequent bioinformatics analysis.

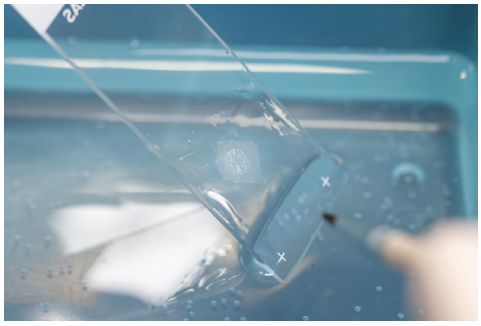


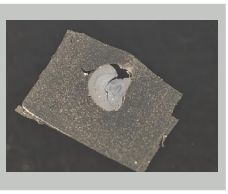


To prevent mix-ups when transferring adjacent sections, it is recommended that you transfer the adjacent sections to 30% ethanol and water bath separately and mount them onto pre-labeled microscope glass slides in subsequent operations.

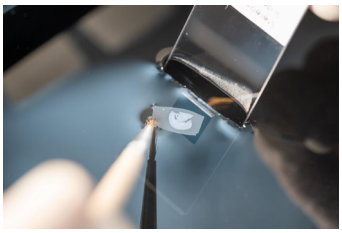
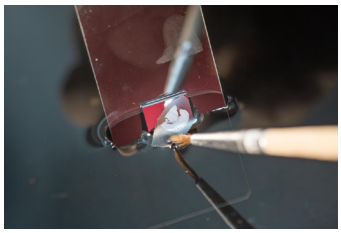


k. Using a clean microscope slide, pick up the section and float it on the pre-heated water bath with Side B facing down.

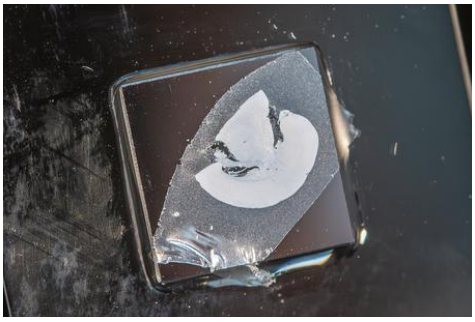


Tissue Section State	Possible Cause	Operation	
The section is completely flattened without folds.	The temperature is normal/ideal.	Proceed with the experiment by following the instructions.	
The section appears to be wrinkled.	The temperature is too low.	Increase the water bath temperature by 0.5°C ~ 1°C, and observe continuously until the tissue is fully flattened.	
The paraffin around the section shows signs of melting.	The temperature is too high.	Stop heating, add a small amount of cold water, and mount it immediately.	

l. Wait until the tissue section is completely flattened in the water bath. Place the Stereo-seq Chip Slide into the water bath and place the section near the chip. Using a histology brush, pick up the section by gently touching the corner of the flattened section, ensuring that side B is in contact with the Stereo-seq Chip.

m. Wipe off excess liquid from around the edges and the back of the slide using dust-free paper without touching the tissue, and then place it on the Stereo-seq PCR Adaptor in the PCR thermal cycler. Dry the slide at **42°C** for **3 hr** and then bake it **overnight** at **37°C** according to the following incubation protocol.



Temperature	Time
(Heated lid) 45°C	on
42°C	∞
42°C	3 hr
37°C	∞

Stop Point:

After drying the tissue containing Stereo-seq Chip Slides at 42°C for 3 hr, the slides can be stored in a slide container stored at 4°C with desiccant for up to 1 month.

After drying the tissue-containing Stereo-seq Chip Slides at 42°C for 3 hr, the slides can also be transported. For details on shipping paraffin-section-mounted Stereo-seq Chip Slides, refer to Appendix I: Shipping Preservation and Transportation.

- **A technician with extensive experience in paraffin block sectioning can perform section trimming and mounting according to their personal preferences.**
- **Pay attention to prevent air bubbles from forming when mounting the tissue section onto the chip surface.**
- **The heating and baking process can also be substituted with a metal bath or a heating module that can be heated accurately and maintained at a constant rate.**

For information on the Stereo-seq OMNI for FFPE V1.1 Transcriptome workflow, refer to STUM-TT007: Stereo-seq Transcriptomics Set for FFPE V1.1 (0.5cm * 0.5cm) User Manual or STUM-TT004: Stereo-seq Transcriptomics Set for FFPE V1.1 User Manual.

Appendix I: Shipping Preservation and Transportation

Preservation and Transportation of FFPE Blocks

Store FFPE blocks at room temperature or at 4°C ~ 8 °C.

a. Place the paraffin block in a sealed bag, then put the sealed bag into a foam box with a wall thickness of at least 3 cm, ensuring a tight seal.

b. Use no fewer than 6 ice packs (13 cm × 23 cm) for a 24-hour shipment.

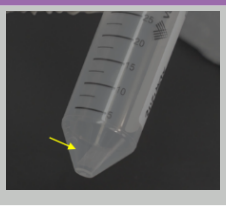
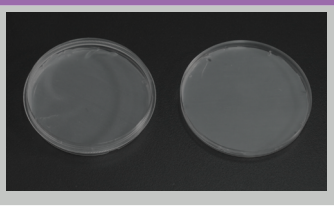
c. Use foam, foam paper, and multiple layers of packaging to prevent sample damage during transportation.

d. In summer or in unusually warm or hot weather conditions, increase the number of ice packs to maintain sample temperatures between 2°C ~ 8°C.

Preservation and Transportation of Paraffin Sections

a. Prepare the container for the sections according to the tissue size and requirements of the experiment. You can use 50 mL centrifuge tubes or appropriately-sized Petri dishes.

If a Petri dish is used to transport sections, line the Petri dish base and lid with appropriately sized Parafilm tape circles to avoid tissue adhesion during transport.

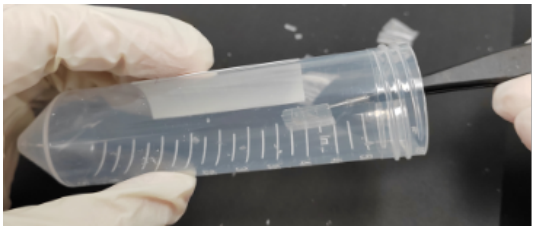
Container	50 mL centrifuge tube	Petri dish with parafilms
Example		
Pros	• No pre-treatment for containers • Suitable for FFPE sections of regular size and small size	• Open container with a wide opening and shallow depth, for easy access • The FFPE section can be kept flat during transportation • Available in different sizes • 1 section/container, several consecutive sections
Risk	Only 1 section/container	Need to prepare and place the parafilm

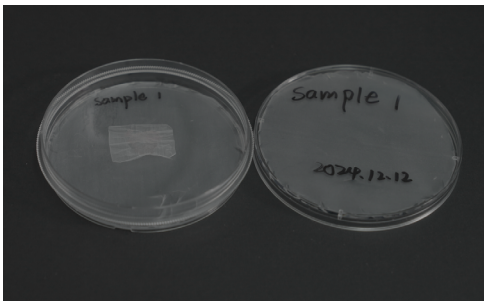
b. Perform sectioning according to the instructions in Section 2.4,**Tissue Sectioning and Mounting**. Choose the paraffin section with the entire flat tissue surface revealed so that the section is complete and representative.

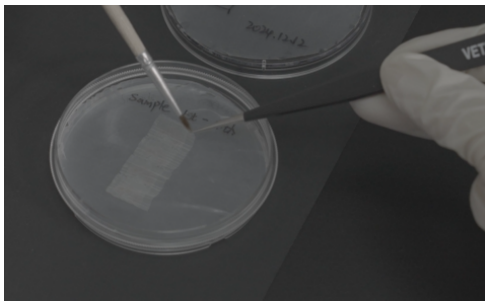
Sections that are incomplete, fragmented, or have obvious folds and wrinkles are not acceptable for shipping and subsequent transcriptomics experiments.

c. Pick up the section with clean forceps or a histology brush and place it in a properly labeled 50 mL centrifuge tube or a well-prepared Petri dish.

To prevent sections from colliding during shipment and causing tears, no more than one section should be placed in one tube. If a well-prepared Petri dish is chosen, place one section per dish or several consecutive sections per dish as illustrated below.



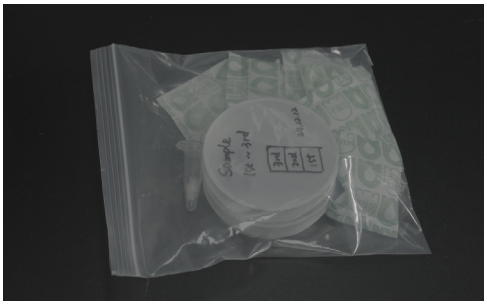




To minimize tissue loss, prepare 3 to 5 sections from each QC-passed sample at a time for transportation.

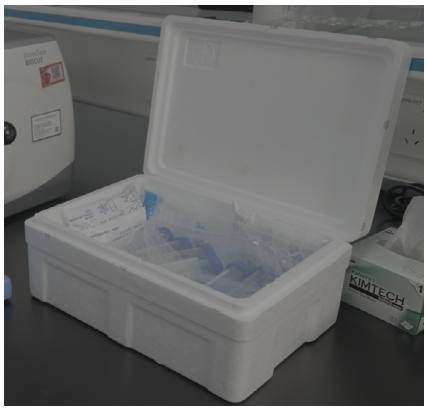
d. Seal the container with parafilm and record the sample information and date. If using a Petri dish, mark both the culture dish and the lid to prevent confusion. If you need to send serial sections, be sure to distinguish the order of sections and mark the containers to prevent confusion.

e. After all sections are collected, place the pre-labeled centrifuge tube or Petri dish in a sealable bag with a sufficient number of desiccants.



f. Place the bag into a foam box with a wall thickness of at least 3 cm, ensuring a tight seal. If using petri dishes with parafilm for transportation, be sure to place them flat in a foam box.

g. Use no fewer than 6 ice packs (13 cm × 23 cm) for a 24-hour shipment.



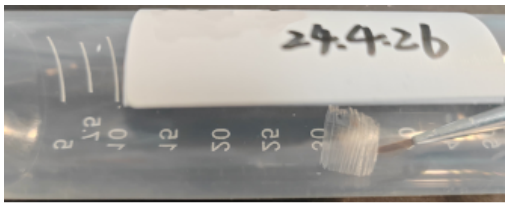
h. Use foam, foam paper, and multiple layers of packaging to prevent sample damage during transportation.

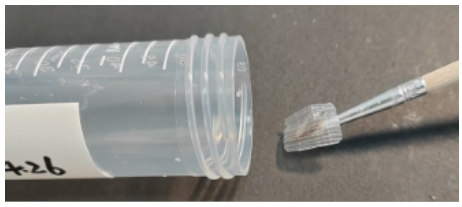
i. In summer, increase the number of ice packs to maintain sample temperatures between 2°C ~ 8°C.

When received, the FFPE sections can be stored in the original packaging at 4°C in dry conditions; transportation time plus storage time is up to 4 weeks.

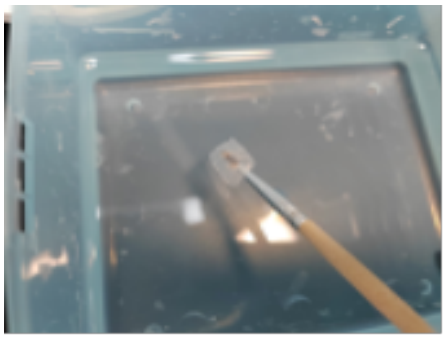
j. After shipment and storage, and before proceeding to the subsequent experiments, if you are concerned about the physical state of the sections, perform a routine H&E staining to ensure that the tissue has not been deformed (not mandatory).

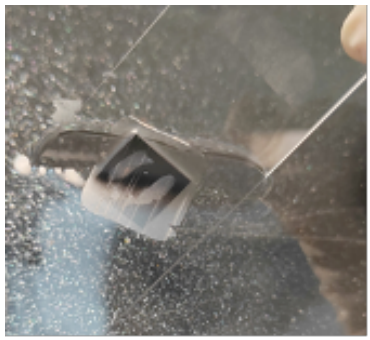
Static electricity may be generated during transportation, causing the sections to adhere to the tube wall. Therefore, it is important to conduct a histology examination before proceeding. When retrieving the sections, place the 50 mL centrifuge tube at -20°C for 5 min, then pick up the section with clean forceps or a histology brush.





k. After the morphology of the tissue has been confirmed, follow the instructions starting from **Step i**. in Section 2.4, Tissue Sectioning and Mounting, to float the paraffin section in 30% ethanol and then in a water bath.





For a demonstration video, refer to the links below:

- FFPE Section (Sample Selection) Preparation and Shipping Guidelines (For Section Preparation)
- FFPE Section H&E Staining and Selection Guidelines (For the Recipient)

Preservation and Transportation of Paraffin-section-mounted Stereo-seq Chip Slides

a. After baking the tissue-containing Stereo-seq Chip Slide at 42°C for 3 hr, transfer the Stereo-seq Chip Slide into a slide container.

b. Place the slide container in a sealable plastic bag. Place one desiccant pack per Stereo-seq Chip Slide into a sealable plastic bag, push out as much air as possible, and seal the bag tightly.

c. Place the bag into a foam box with a wall thickness of at least 3 cm, ensuring a tight seal.

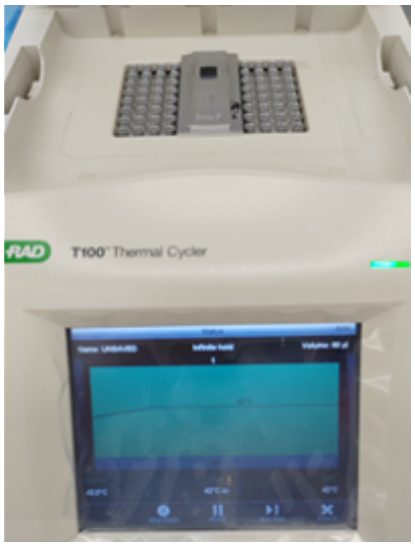
d. Use no fewer than 6 ice packs (13 cm × 23 cm) for a 24-hour shipment.

e. Use foam, foam paper, and multiple layers of packaging to prevent sample damage during transportation.

f. In summer or in unusually warm or hot weather conditions, increase the number of ice packs to maintain sample temperatures between 2°C ~ 8°C.

When received, the paraffin-section-mounted Stereo-seq Chip Slides can be stored in the original packaging at 4°C in dry conditions, transportation time plus storage time is up to 1 month.

g. When retrieving the paraffin-section-mounted Stereo-seq Chip Slide from the shipping container or storage, take it out of the slide container and immediately incubate it at 42°C with PCR Adaptor for 3 hr, and then at 37°C overnight.



When transitioning from cold storage or transport to room temperature, vapor may form on the chip surface due to temperature differences; therefore, it is important to dry the chips as soon as possible.

h. After drying the chips, transfer the paraffin-section-mounted Stereo-seq Chip Slide to a baking machine or a PCR thermal cycler and bake it at 60°C for **1 hr** and proceed to the transcriptomic experiment according to STUM-TT007: Stereo-seq Transcriptomics Set for FFPE V1.1 (0.5cm * 0.5cm) User Manual or STUM-TT004: Stereo-seq Transcriptomics Set for FFPE V1.1 User Manual.