STOmics

Stereo-seq Sample Preparation, Sectioning and Mounting Guide for Formalin-fixed and Paraffin-embedded (FFPE) Samples on Stereo-seq Chip Slides

USER MANUAL

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REVISION HISTORY

Manual Version:	A
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Description:	Added US use only catalog numbers.

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

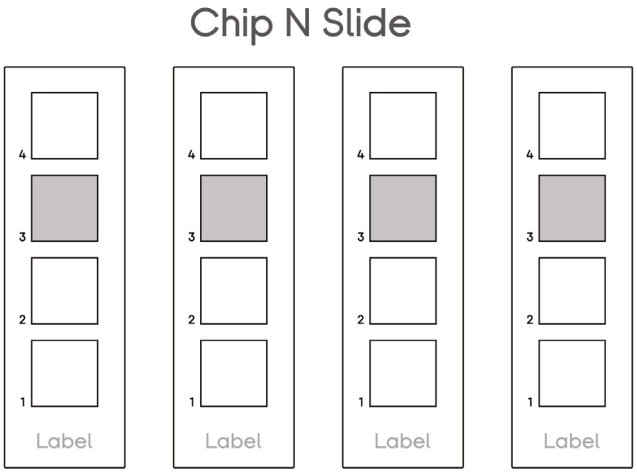
The STOmics Stereo-seq Transcriptomics Set for FFPE is intended for generating a spatially-resolved total RNA library from formalin-fixed and paraffin-embedded (FFPE) biological tissue sections that requires a Stereo-seq Chip Slide with intact tissue sections as input. This guide provides general guidelines 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 Transcriptomics Set for FFPE (211SN114 / 211SN114-CG)

Stereo-seq Chip N Slide

- Includes 4 Stereo-seq Chip N Slides containing one Chip N (1cm*1cm) on each slide.
- Stereo-seq Chip P Slides, Stereo-seq Chip T Slides, and Stereo-seq Chip N Slides are differentiated by a laser-engraved label at the end of the slide.



CHAPTER 2
SAMPLE PREPARATION

2.1. Reagent Preparation

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

Reagent Preparation Master Planning Table

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

Prep Day	Reagent	Workflow Section	Preparation Steps	Maintenance
Day 1	400 mL 30% ethanol	Tissue sectioning and mounting	Dilute anhydrous ethanol to 30% using ddH_2O.	Room Temperature up to 1 day
Day 2	100 mL 96% ethanol	Deparaff-inization	Dilute anhydrous ethanol to 96% using ddH_2O.	Room Temperature up to 1 day
	50 mL 90% ethanol	Deparaff-inization	Dilute anhydrous ethanol to 90% using ddH_2O.	Room Temperature up to 1 day
	50 mL 80% ethanol	Deparaff-inization	Dilute anhydrous ethanol to 80% using ddH_2O.	Room Temperature up to 1 day
	50 mL 70% ethanol	Deparaff-inization	Dilute anhydrous ethanol to 70% using ddH_2O.	Room Temperature up to 1 day
	50 mL 50% ethanol	Deparaff-inization	Dilute anhydrous ethanol to 50% using ddH_2O.	Room Temperature up to 1 day
	50 mL 30% ethanol	Deparaff-inization	Dilute anhydrous ethanol to 30% using ddH_2O.	Room Temperature up to 1 day
	Hematoxylin (Solarbio, G4470)	H&E staining	Take it out of 4°C in advance and equilibrate to room temperature at least 5 min prior to use.	Room Temperature up to 2 hr
	Eosin Solution	H&E staining	Eosin was diluted with 70% ethanol at a 2:1 volume ratio.	Room Temperature
	50 mL 5X SSC	H&E staining	Add 12.5 mL 20X SSC into 37.5 mL ddH_2O and mix well.	Room Temperature up to 1 week
	200 mL 0.1X SSC	H&E staining	Add 1 mL 20X SSC into 199 mL ddH_2O and mix well.	Room Temperature up to 1 week

Day 2	200 µL 5X SSC	ssDNA staining	Add 50 µL 20X SSC into 150 µL ddH_2O and mix well.	Room Temperature up to 1 week
	50 mL 0.1X SSC	ssDNA staining	Add 250 µL 20X SSC into 49.75 mL ddH_2O and mix well.	Room Temperature up to 1 week
	FFPE Mounting Medium	H&e staining and ssDNA staining	Take it out of -20°C in advance and equilibrate to room temperature until it thaws.	Room Temperature
	Fluorescent Staining Solution	ssDNA staining	Add 1 µL Qubit ssDNA Reagent and 10 µL RI into 189 µL 5X SSC and mix well.	Room Temperature in the dark up to 1 hr
	TE buffer (pH=9.0)	H&E decolorization	Take it out of 4°C in advance and equilibrate to room temperature at least 5 min prior to use.	Room Temperature up to 2 hr
	FFPE Decross-linking Reagent	Decrosslinking	Take it out of -20°C in advance, equilibrate to room temperature until it thaws, then mix well to ensure that no white precipitates are visible.	Room Temperature
	If white precipitates are visible in the reagent, dissolve them by heating the buffer at > 50 °C and equilibrate to room temperature before mixing.			
	0.01N HCl	Permeabi-lization	Prepare at least 2 mL of 0.01N HCl per sample. Configure HCl to 0.01N. Measure and make sure 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.			
	10X Permeabi-lization Reagent Stock Solution	Permeabi-lization	Add 1 mL of freshly prepared 0.01N HCl to dissolve PR Enzyme (red cap, in powder form), and thoroughly mix the reagent through pipetting.	-20°C up to 1 month
	DO NOT vortex the permeabilization enzyme. Mix by pipette before using. Aliquot this 10X stock solution to avoid freeze-thaw cycles.			

Day 2	1X Permeabilization Reagent Solution	Permeabilization	Make 1X PR solution (200 µL / chip) by diluting 10X PR stock solution with 0.01N HCl.	On ice until use, up to 6 hr
	0.1X SSC	Permeabilization	Add 10 µL 20X SSC into 1990 µL nuclease-free water and mix well.	Room Temperature up to 1 week
	0.1X SSC (with 5% RI)	Permeabilization	Add 10 µL RI into 190 µL 0.1X SSC and mix well	On ice until use
	FFPE RT Buffer Mix	RT and ligation	Take it out of -20°C in advance, equilibrate to room temperature until it thaws, then mix well to ensure that no white precipitates are visible.	On ice until use
	FFPE Dimer	RT and ligation	Take it out of -20°C in advance, and thaw on ice.	On ice until use
	FFPE RT Oligo	RT and ligation	Take it out of -20°C in advance, and thaw on ice.	On ice until use
Day 3	cDNA Release 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
	If white precipitates are visible in the buffer, dissolve them by heating the buffer at 55 °C again and equilibrate to room temperature before mixing.			
	Magnetic beads	cDNA Purification	Take it out in advance and equilibrate to room temperature at least 30 min prior to use.	4°C
	10 mL 80% Ethanol	cDNA Purification	Dilute 100% ethanol to 80%.	Room temperature up to 1 day
	cDNA Amplification Mix	cDNA Amplification	Take it out of -20°C in advance, and thaw on ice.	On ice until use
	FFPE cDNA Primer Mix	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

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 preferable to use 10% formalin for fixation.
- To prevent drying, corrosion, and mildew, seal 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 slices without cracks or gaps (apart from the tissues themselves), ensuring that the specimen remains embedded in the wax during sectioning.
- Paraffin block samples can be sent directly. For additional details, please refer to Appendix I: Shipping Preservation and Transportation.

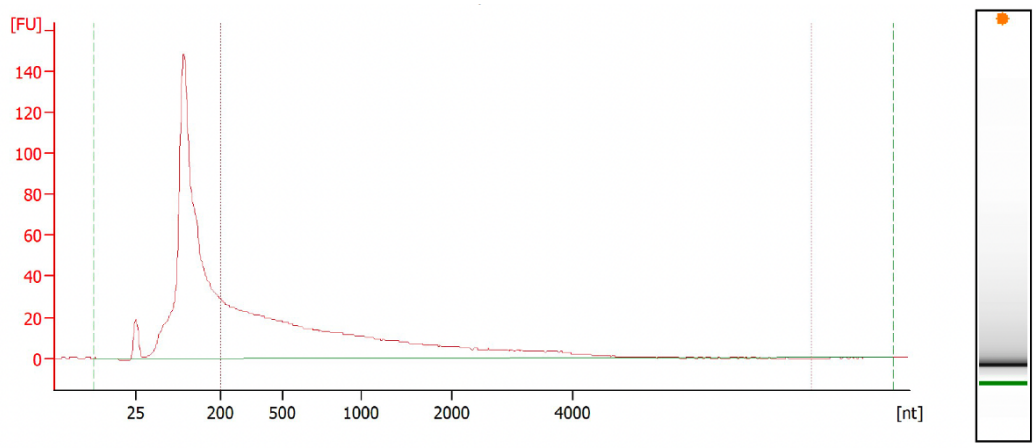
FFPE Sample RNA Quality Control (QC) by DV200 Value

Prepare the following:

Reagent	Preparation Steps	Purpose
RPE buffer	Strictly follow the instructions from Qiagen, and add ethanol in advance	RNA extraction
DNAse I	Strictly follow the instructions from Qiagen. Dissolve the powder, aliquot the solution, then store it at -20°C . Take it out in advance and keep it on ice until use.	RNA extraction

It is recommended that you check the RNA quality (DV200 value) of a 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 1.5mL EP tube for up to 1 month . Refer to Figure 1 for examples of DV200 distribution ranges after quality control of different RNA samples, using Agilent High-Sensitivity RNA Analysis Kit (Agilent 5067-1513).

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



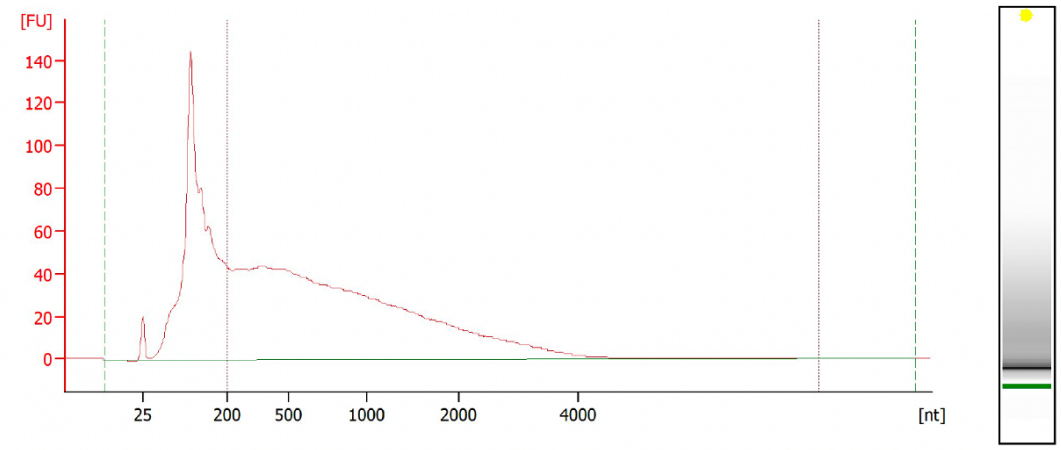
Overall Results for sample 8 : **Sample 8**

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%



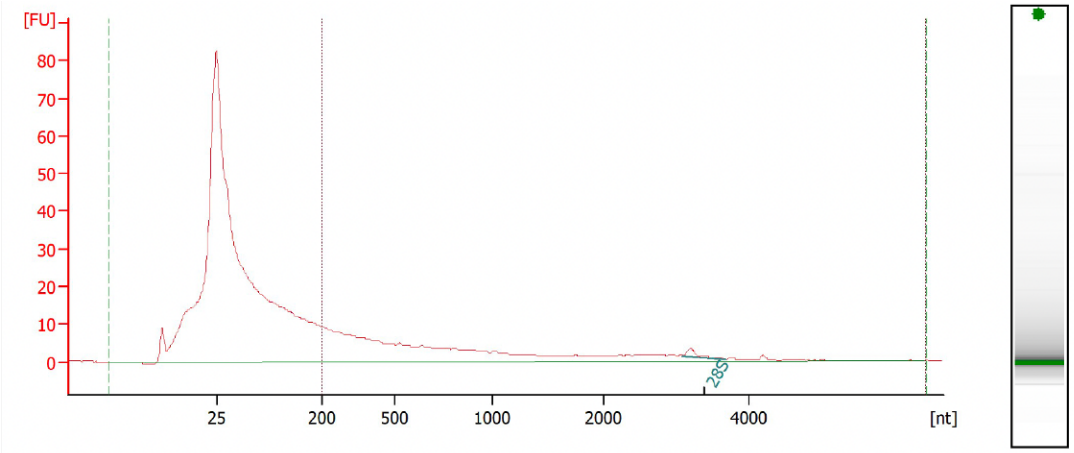
Overall Results for sample 10 : **Sample 10**

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 5 : **Sample 5**

RNA Area:	128.8	Result Flagging Color:	
RNA Concentration:	1,000 pg/µl	Result Flagging Label:	RIN: 1.20 ; DV200 > 70%
rRNA Ratio [28s / 18s]:	0.0		
RNA Integrity Number (RIN):	1.2 (B.02.10)	Corr. Area 1:	116.2

Fragment table for sample 5 : **Sample 5**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
28S	3,098	3,691	2.5	1.9

Region table for sample 5 : **Sample 5**

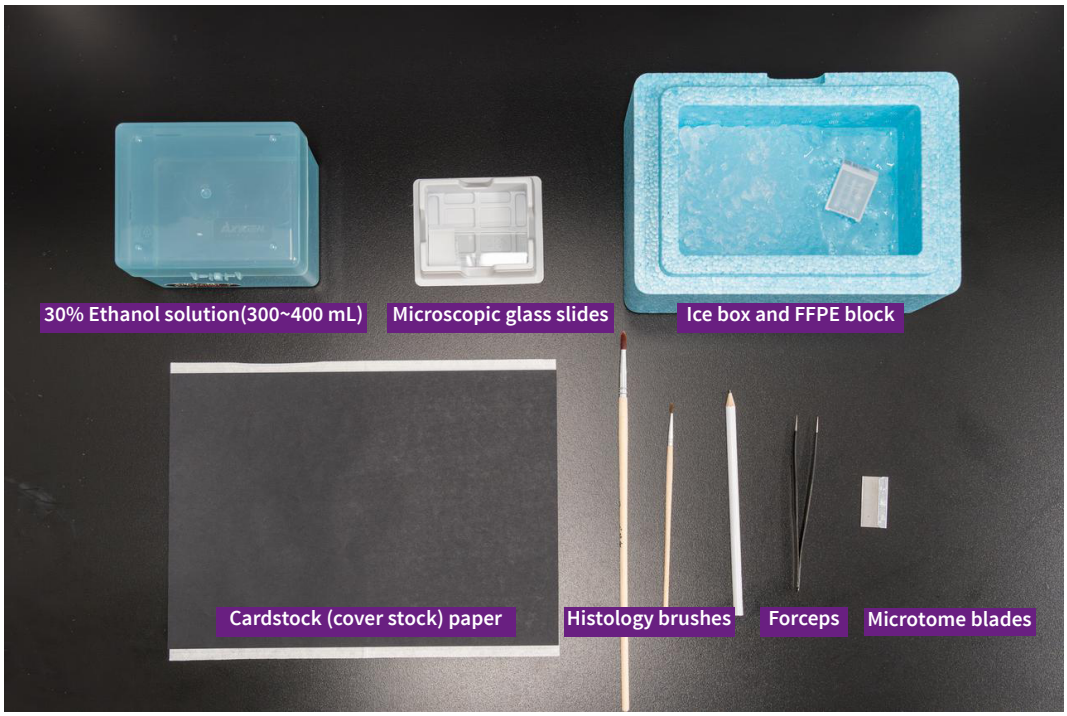
Name	From [nt]	To [nt]	Corr. Area	% of Total	Color
DV200	200	6,452	116.2	90	

DV200: >70%

Figure 1. Examples of DV200 distribution intervals after different RNA quality control

2.3. Tissue Sectioning and Mounting

For a demonstration video of the 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 ddH_2O. 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 your 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 baking machine in advance, and set the PCR thermal cycler according to the following incubation protocol.

Program selection: Incubate

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 paraffin 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**.

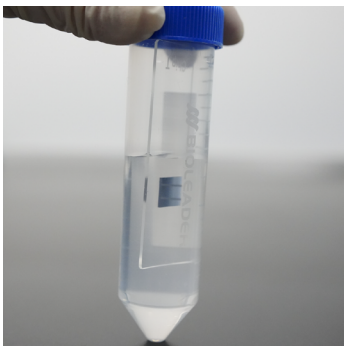
For tissues with high-fat content, such as breast tissue samples, freeze the FFPE block at -20°C for 10 min before sectioning.

d. Take the Chip Slide out of the vacuum-sealed aluminum bag and record the Chip ID (SN) number located on the back side of the slide. Do not touch the front side of the chip.

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

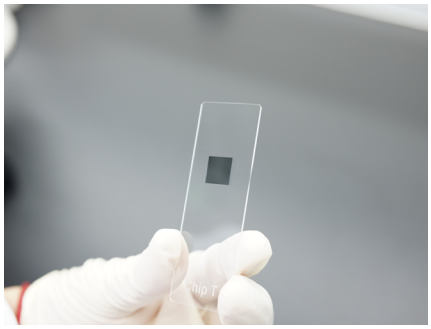
e. Make sure the PCR thermal cycler has been turned on and set to 42°C.

f. Equilibrate the Stereo-seq Chip Slide to room temperature for **1 min** on the benchtop, then rinse with 100 µL nuclease-free water **twice** with a pipette or rinse the slide up and down **twice** in a 50 mL centrifuge tube with sufficient nuclease-free water.



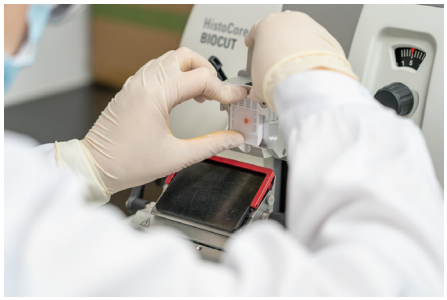
Store unused slides in the original packaging (first in the slide container and then in the sealable aluminum bag) and sealed at -25°C ~ 8°C. KEEP the desiccant in the aluminum bag.

g. Remove excess water from the chip by blowing gently with a power dust remover (MATIN, M-6318). Wipe off excess water from around the chip and on the slide with dust-free paper.

h. Only when the chip is completely dry and without wavy white stains is it ready for tissue mounting.

i. 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, approach the block with an old trimming blade and cut a few thin sections to ensure that the positioning is correct. Adjust if necessary.



j. 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**.

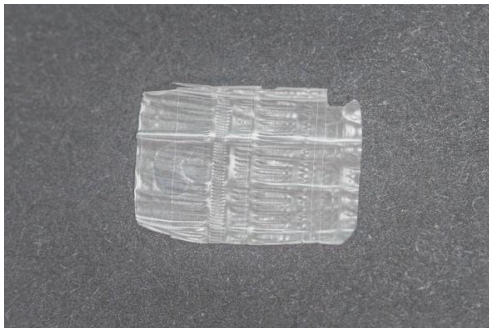
k. Adjust the section thickness to **5 μm** for regular tissue and **4 μm** for high-fat-content tissue to reduce the possibility of section detachment in subsequent operations.

l. Discard the first section if the thickness does not meet the requirements. Select the desired section during subsequent sectioning and use it for subsequent trimming and mounting on the Stereo-seq Chip Slide. Using clean forceps, carefully transfer the selected section onto cardstock paper for trimming.

To preserve adjacent sections, make a ribbon of three continuous sections. Use the first and third sections for subsequent H&E staining on microscopic glass slides. Carefully separate the middle section from the other two sections and use it for Stereo-seq Chip Slide mounting later.

Refer to Appendix I: Shipping Preservation and Transportation.

A paraffin section is divided into two sides, side A and side B. The side facing the microtome 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 mounted onto the Stereo-seq Chip Slide and microscopic glass slide to prevent the section detachment.

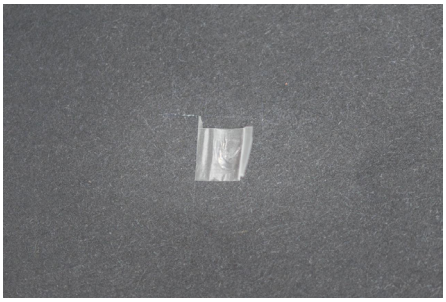


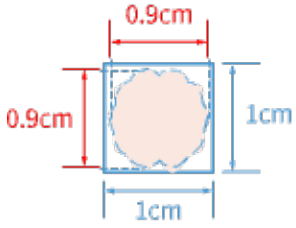
Side A

Side B

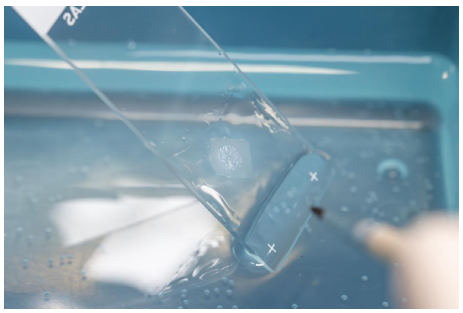
m. Trim the edges of the targeting section to fit the standard Stereo-seq Chip size (1 cm × 1 cm), and carefully transfer the section with side B facing down to the container filled with 30% ethanol using a histology brush or clean forceps.

After trimming, the section should not exceed 0.9 cm x 0.9 cm because the tissue section should not exceed 80% area coverage of the chip. An area exceeding 80% area coverage of the chip will interfere with image and gene expression matrix registration, resulting in misaligned results during bioinformatics analysis.

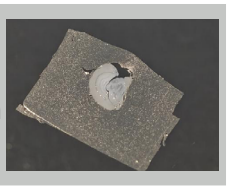




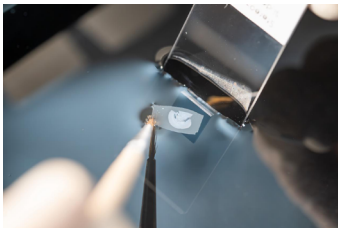
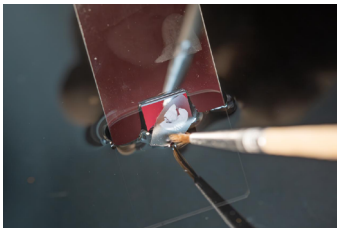
n. Use a clean microscopic glass slide to pick up the section and float it on the surface of the water in the preheated water bath. Make sure side B is facing down.



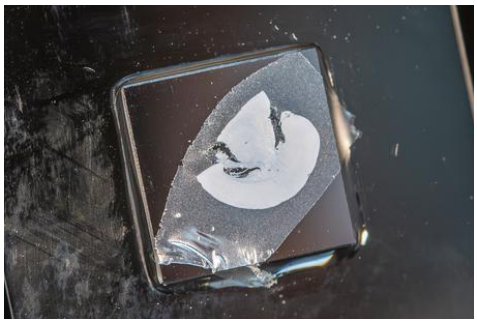
To prevent mix-ups when transferring adjacent sections, it is recommended that you transfer the adjacent sections to 30% ethanol and water bath separately and mount them onto pre-labeled microscopic glass slides in subsequent operations.

Tissue Section State	Possible Cause	Operation	
The section is completely flattened without folds.	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 then scoop out the section immediately.	

o. 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 with the assistance of a histology brush by gently touching the corner of the flattened section, ensuring that side B is in contact with the Stereo-seq Chip.

p. Wipe off excess liquid from around 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 on a PCR thermal cycler, the slides can be stored in a slide container placed in an oven at 25°C or 37°C for 72 hr or at 4°C with desiccant for up to 1 week.**

For detailed shipping guidance of 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.
- Please 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 at a constant rate.

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

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

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

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

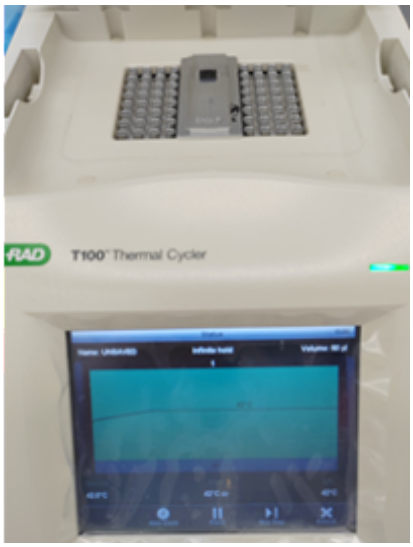
c. Use no fewer than 6 ice packs (13 cm × 23 cm) for a 24-hour shipment.

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

e. In summer, 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 for up to 1 week.

f. 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.



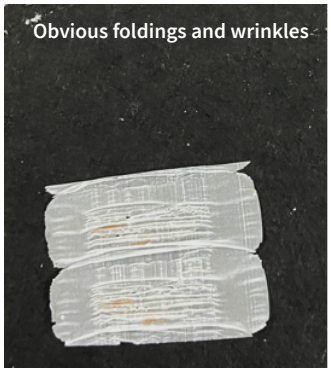
The temperature difference in the room during ice pack transportation may cause water vapor to form on the chip's surface; therefore, it is important to dry the chips as soon as possible.

g. After drying the chips at 42°C, 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** before proceeding to the subsequent Transcriptomics experiments.

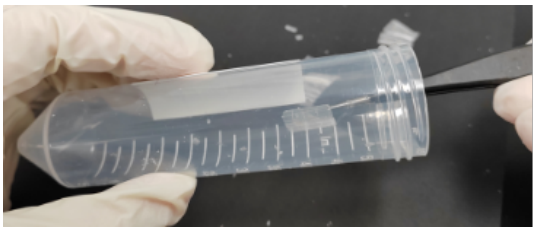
Preservation and Transportation of Paraffin Sections

a. Perform sectioning according to the instructions provided in Section 2.3. Choose the paraffin section that has the entire flat tissue surface revealed so that the section is complete and representative.

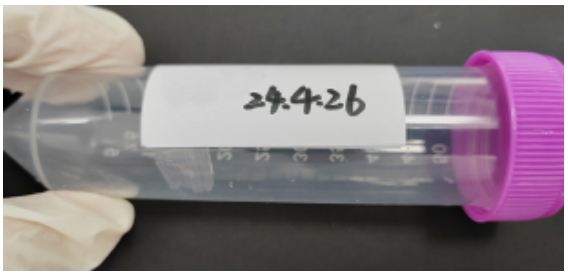
Sections that are incomplete, falling apart, or have obvious folds and wrinkles are not acceptable for shipping and subsequent Transcriptomics experiments.



b. Pick up the section with clean forceps or a histology brush and place it in a properly labeled 50 mL centrifuge tube. Continue until all sections are collected. To prevent sections from colliding during shipment and causing tears, no more than 5 sections should be placed in one tube.



c. Close the lid and place the pre-labeled centrifuge tube in a sealable bag with a sufficient number of desiccants.



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

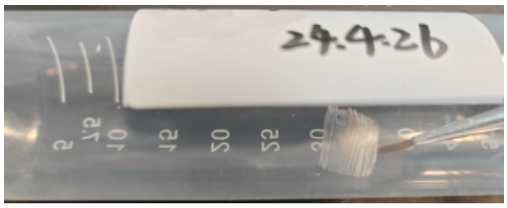
e. e. Use no fewer than 6 ice packs (13 cm × 23 cm) for a 24-hour shipment.

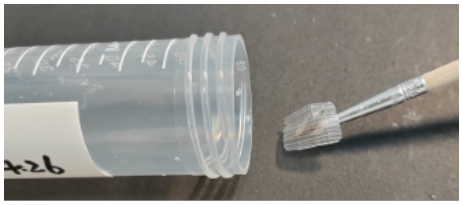
f. f. Use foam, foam paper, and multiple layers of packaging to prevent sample damage during transportation.

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

When received, the paraffin sections can be stored in the original packaging at 4°C in dry conditions for up to 1 week.

h. 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.





i. Flatten out the section in the water bath and then pick up the section with a microscopic glass slide. Before proceeding to the subsequent experiments, perform a routine H&E staining to ensure that the tissue has not been deformed.

j. After the morphology of the tissue has been confirmed, follow the instructions starting from **Step h.** in **Section 2.3, Tissue Sectioning and Mounting**, to float the paraffin section in 30% ethanol and then in a water bath.

k. When the section is picked up and mounted on the Stereo-seq Chip Slide, proceed with section drying and baking in **Section 2.3, Tissue Sectioning and Mounting**.