

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

USER MANUAL



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NOTE: Additional operation tips and guidance.



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



QUALITY CHECK POINT



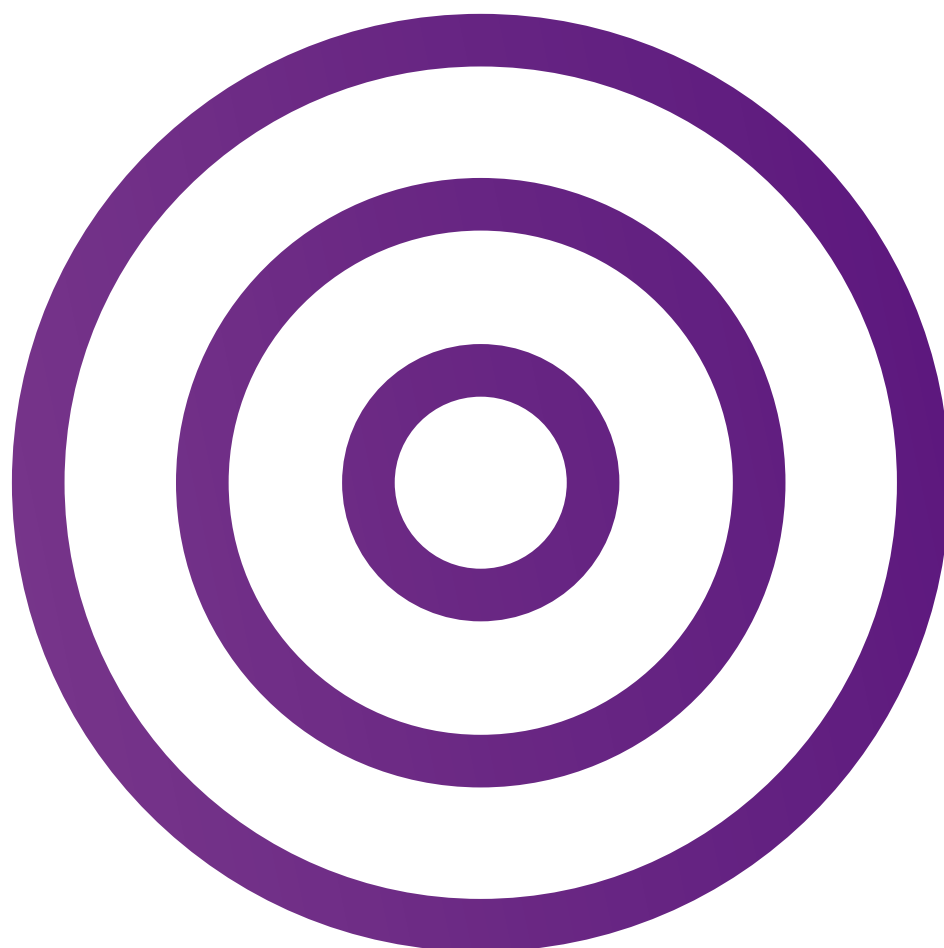
CAUTION: Proceed with extra care; improper handling or carelessness may cause experimental failure or accidents.



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

CHAPTER 1

INTRODUCTION



1.1. Introduction

The STOmics Stereo-seq Transcriptomics Set is intended for generating a spatially-resolved 3' mRNA library from biological tissue sections and a Stereo-seq Chip Slide with intact tissue sections as input. Prior to the Stereo-seq Transcriptomics workflow, optimal permeabilization time needs to be determined using the Stereo-seq Permeabilization Set for fresh frozen tissues. This guide provides general guidelines on how to properly perform tissue embedding, sectioning, and mounting to better preserve the morphological quality of the tissue sections and the integrity of mRNA transcripts.

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

Stereo-seq Permeabilization Set for Chip-on-a-slide (211SP118)

Stereo-seq Transcriptomics Set for Chip-on-a-slide (211ST114)

CHAPTER 2

SAMPLE

PREPARATION

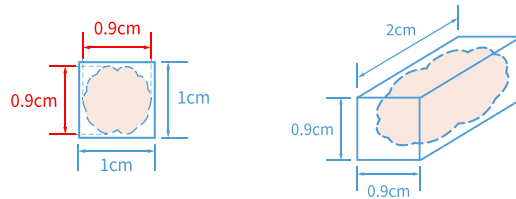


2.1. Sample Requirements for Fresh Frozen Tissue



To prevent RNA degradation, we recommend performing tissue embedding **within 30 min** of harvesting.

The tissue size should not exceed **0.9 cm x 0.9 cm x 2 cm**. The tissue section should not exceed 80% area coverage of the chip.



Sample Types

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

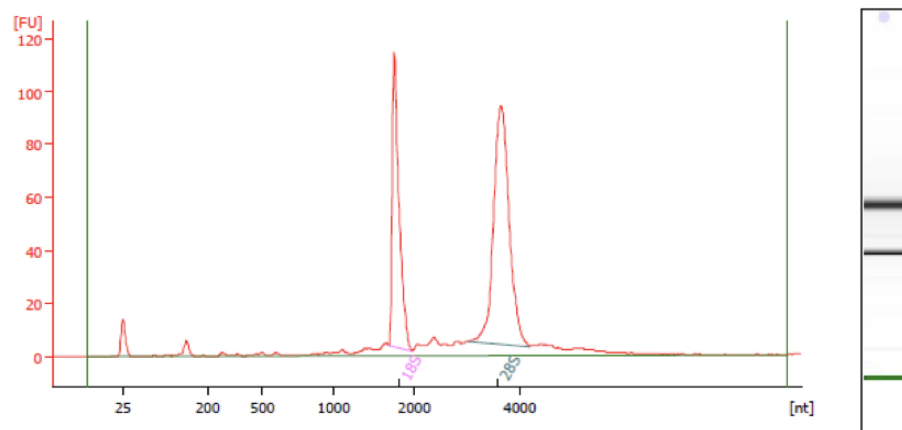
For details, refer to the list: <https://en.stomics.tech/resource/stomics-validated-tissue-list?lang=en#>

Fresh Frozen Sample RNA Integrity Number (RIN) Value

It is recommended that you check the RNA quality (RIN value) of a tissue sample before proceeding to the Stereo-seq experiment. Total RNA can be extracted from 10-20 slices of 10 µm-thick tissue sections and stored at -20°C in a pre-cooled 1.5mL EP tube. Refer to Figure 1 for the peak of RNA RIN value in mouse brain tissue sections.



It is strongly recommended that you proceed only with tissue samples that have a RIN value ≥ 7 .



Overall Results for sample 6 : 8522203007050

RNA Area:	568.4	RNA Integrity Number (RIN):	9.8 (B.02.11, Anomaly Threshold(s) manually adapted)
RNA Concentration:	281 ng/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.8	Result Flagging Label:	RIN: 9.80

Fragment table for sample 6 : 8522203007050

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,675	1,964	151.3	26.6
28S	3,006	4,187	238.4	41.9

Figure 1. Example of RNA size distribution and RIN value measurement of mouse brain tissue sections

2.2. Sample Embedding for FF Samples



For a demonstration video of tissue embedding, refer to the following link or scan the QR code: <https://en.stomics.tech/resources/videos>

Preparation list:



Materials

Brand	Description	Quantity
-	Crushed ice in a box	1
-	Dry ice in a box	1
-	Aluminum foil	1
-	Sealable plastic bag	1
BIOSHARP/Metal Coolbox/BC032	Metal Block	1
-	Sterile gauze	2
Corning	Corning® 35 mm TC-treated Culture Dish (353001)	1
Sakura/Base Molds/4583	OCT	1
Sakura/Base Molds/4162	Stainless-steel base mold A	1
Sakura/Base Molds/7055	Stainless-steel base mold B (slightly larger than mold A)	1
-	Blunt end forceps	1
-	Syringe	1
-	Spatula	1
-	Scissors	1

a. Prepare these materials in advance:

a1. A box of crushed ice and pre-cool OCT on ice for **10 min** in advance.

a2. **2** pieces of stainless-steel base molds slightly larger than the tissue of interest - **mold A** and **mold B** (slightly larger than **mold A**).

a3. Add a few drops of pre-cooled OCT in **mold A** until it reaches approximately 2/3 of the mold and pre-cool on ice for **> 10 min** (remove introduced air bubbles using a syringe).

a4. A petri dish filled with OCT and pre-cool the OCT on ice for **> 10 min** (remove introduced air bubbles using a syringe).

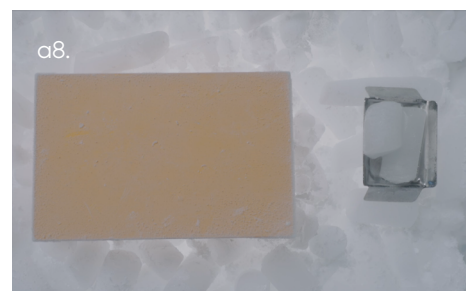
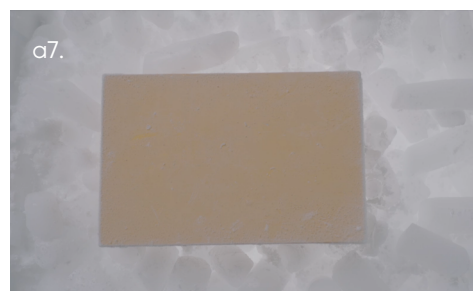


a5. A box of dry ice.

a6. A metal block that has a flat surface to support the stainless-steel base mold when placed on dry ice. The size of the metal block should be larger than the stainless-steel base mold.

a7. Place the metal block on dry ice and pre-cool for **> 5 min** with the flat surface facing up.

a8. Place **mold B** on dry ice and pre-cool for **> 5 min**.



b. Upon harvesting within **30 min**, use sterile gauze or dust-free paper to absorb excess liquid on the tissue surface to prevent ice formation in later steps.



- c. Place the tissue in pre-cooled OCT and wrap the tissue evenly with OCT using a spatula without introducing air bubbles.



- d. Remove any air bubbles using a syringe.



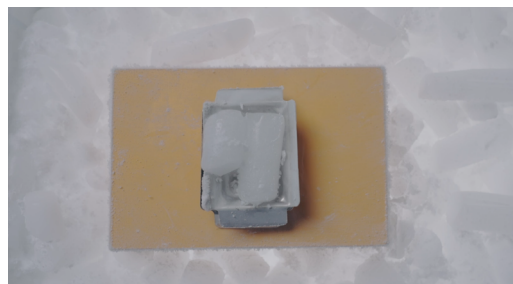
- e. Orient the tissue with the side intended to be sectioned facing down and then place it into **mold A** . Ensure that the tissue is at the bottom of **mold A** and fill the mold with chilled OCT, without introducing bubbles, until the tissue is fully covered.



- f. Place the tissue containing **mold A** onto the metal block that was placed on dry ice.



g. Use **mold B** as a lid with the opening facing up, place it on top of **mold A** gently, and then place a few dry ice cubes on top of **mold B** . Ensure that the two stainless-steel base molds can be covered with enough dry ice cubes.



h. After **5 min** , remove **mold B** and determine if the OCT is completely frozen and has turned opaque. If it is not completely frozen and opaque, repeat step f.



i. If the tissue block has solidified and turned opaque, grip the two edges of **mold A** and press down the edges to detach the tissue block from the mold.



j. Determine if the sectioning side of the tissue has been completely covered by OCT. If not, place the tissue block on the metal block, sectioning side facing up, add a few drops of the OCT, and then wait until it solidifies and turns opaque.



k. Label the tissue block to mark the orientation of the tissue.

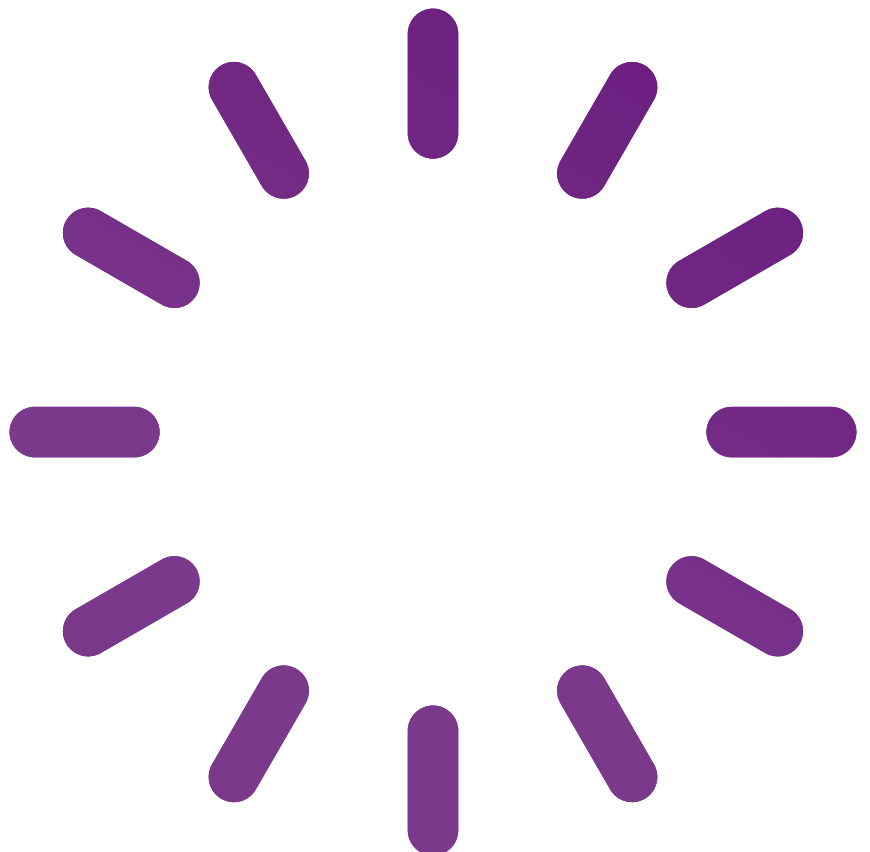


2.3. Sample Storage and Transportation

To store samples, wrap the tissue block with aluminum foil and keep it in a properly-labeled sealable plastic bag to prevent dehydration and damage, and then store it at -80°C . For transportation, ship samples on dry ice according to local policy.

CHAPTER 3

CRYOSECTION PREPARATION



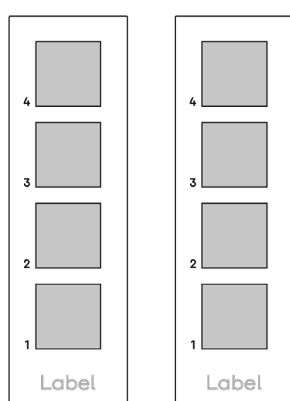
3.1. Stereo-seq Chip Slides

Stereo-seq Chip P Slide

Includes 2 Stereo-seq Chip P Slides containing **four** Chip P (1cm*1cm) on each slide.

Stereo-seq Chip P Slides and Stereo-seq Chip T Slides are differentiated by a laser-engraved label at the end of the slide.

Chip P Slide

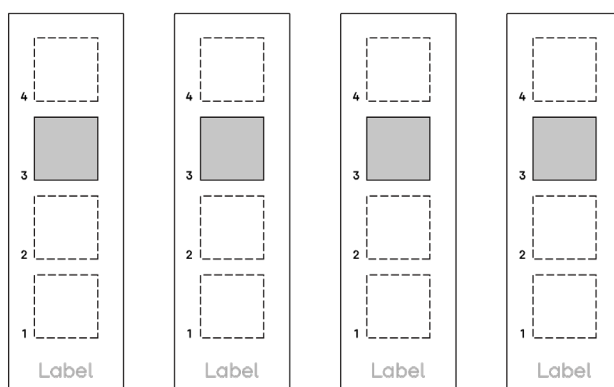


Stereo-seq Chip T Slide

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

Stereo-seq Chip P Slides and Stereo-seq Chip T Slides are differentiated by a laser-engraved label at the end of the slide.

Chip T Slide



Stereo-seq Chip P Slide Storage

Always store unused slides in their original slide container and then the aluminum bag at $-25^{\circ}\text{C} \sim 8^{\circ}\text{C}$. Keep sealed with tape or another re-sealable bag.

Always KEEP the desiccant in the bag.

3.2. Cryosection Preparation

Equipment	Preparation	Notes
Cryostat	Set the cryostat chamber temperature to -20°C and specimen disc temperature (object temperature) to -10°C~-15°C.	The specimen disc temperature depends on the tissue type.

a. Set a PCR Thermal Cycler to 37°C with the heated lid set to 42°C in advance with a PCR adaptor.

b. Set the cryostat chamber temperature to -20°C and the specimen disc temperature (object temperature) to -10°C~-15°C.



If the specimen disc is over-cooled, it could result in tissue section cracking during sectioning, and sections will wrinkle when the disc temperature is too high. Optimal specimen disc temperature depends on the tissue type.

c. Place the forceps, brushes, and razor blades inside the chamber for pre-cooling.

d. Take the OCT-embedded tissue sample out of the -80°C freezer and place it in the cryostat chamber for **30 min** to allow it to equilibrate to cryostat chamber temperature.

e. Remove the sample outer covers (aluminum foil) and trim the embedded tissue block to the appropriate size (sectioning area smaller than 0.9 cm x 0.9 cm).

f. By using OCT, mount the embedded tissue block onto the specimen disc/holder of the cryostat chamber.

g. If necessary, trim the tissue block again to ensure a good fit between the tissue section and the Stereo-seq Chip. The specimen is ready for cryosection.

3.3. Tissue Mounting



For a demonstration video of tissue mounting onto the Stereo-seq Chip Slide, refer to the following link or scan the QR code:

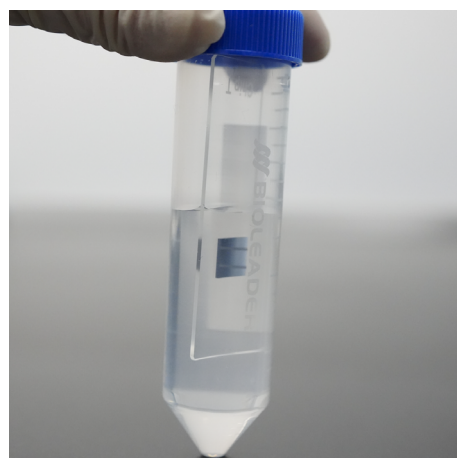
<https://en.stomics.tech/resources/videos/list.html>



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

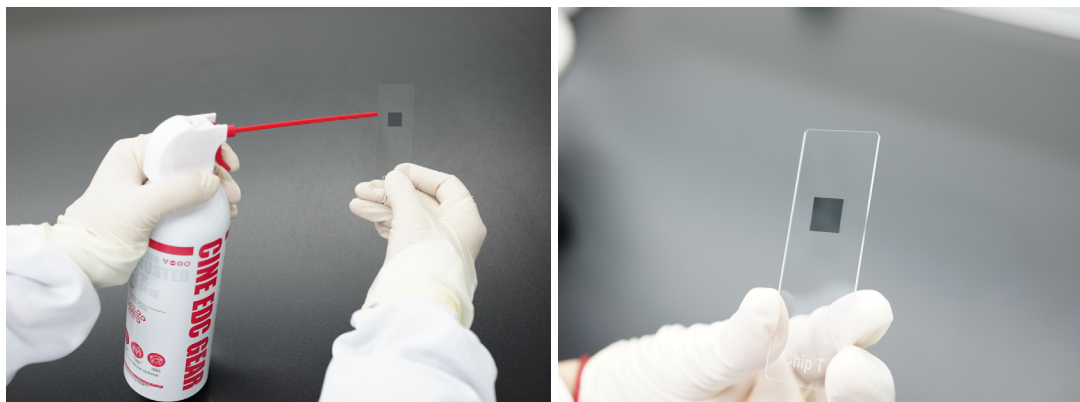
After opening the bag, confirm that all Stereo-seq Chip Slides in the slide container are oriented with the front facing up. The front of a chip has a shiny surface which contains DNB-probes for mRNA capture. DO NOT scratch the surface.

- b. Ensure that the PCR Thermal Cycler has been turned on and set to 37°C.
- c. Equilibrate 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 Corning tube with nuclease-free water.



Store unused slides in original packaging (first in the slide container and then in the sealable aluminum bag) and keep sealed at -25°C ~ 8°C. KEEP THE DESICCANT IN THE ALUMINUM BAG.

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



- e. The chip is ready for tissue mounting only when the chip is completely dry and there are no wavy white stains.
- f. Prepare enough methanol in a 50 mL Corning tube or an empty slide container at a volume that could submerge all of the chips on the slide. Immerse a regular glass slide in the methanol containing tube to confirm that the volume is enough. Close the lid and pre-cool the methanol for **5-30 min** at -20°C .
- g. Place the tissue-mounted specimen disc/holder onto the cryostat head and adjust the angle accordingly.
- h. Tissue mounting can be achieved using either the cold method (option A) or the warm method (option B). We recommend practicing tissue mounting and section placement on plain glass slides first.

A. Cold Method

- 1) Place Stereo-seq Chip Slide inside the chamber with the front facing up and pre-cool inside the cryostat chamber for **1-6 min**.



Prolonged cooling for longer than 6 min may cause mist to form on the chip surface.

- 2) Perform cryosection, then carefully flatten the tissue section by gently touching the surrounding OCT with cryostat brushes. Place the tissue section onto the chip center carefully with forceps and brushes. Ensure that the tissue section is complete and without wrinkles.
- 3) Immediately pick up the Stereo-seq Chip Slide and place a finger on the back of the Stereo-seq Chip Slide directly under the chip for a few seconds to allow the section to adhere to the chip.
- 4) Place the tissue-mounted Stereo-seq Chip Slide back inside the chamber and move on to the second tissue slicing and mounting. Continue transferring sections on the remaining chips.
- 5) When all tissue mounting is completed, immediately dry the Stereo-seq Chip Slide at 37°C in the PCR Thermal Cycler with PCR Adaptor for **5 min** (without heated-lid).



When performing cold mounting, mind the time interval between each tissue section placement. Longer time intervals (>5 min) could result in tissue wrinkle formation.

B. Warm Method

- 1) Perform cryosection and obtain two or four consecutive tissue sections (depending on the number of chips on the Stereo-seq Chip Slide), carefully flatten the tissue sections by gently touching the surrounding OCT with cryostat brushes.
- 2) Move the tissue sections to the edge and place each tissue section at a distance greater than the chip spacing on the Stereo-seq Chip Slide.
- 3) Flip the Stereo-seq Chip Slide and aim the tissue section within a chip area on the Stereo-seq Chip Slide by gently touching the section with the front-side of the chip.
- 4) Repeat step 3) until all of the tissue sections have been mounted onto the chips of the Stereo-seq Chip Slide.
- 5) Turn the Stereo-seq Chip Slide over, and immediately dry it the PCR Thermal Cycler at 37°C with PCR Adaptor front up for **5 min** (without heated-lid).

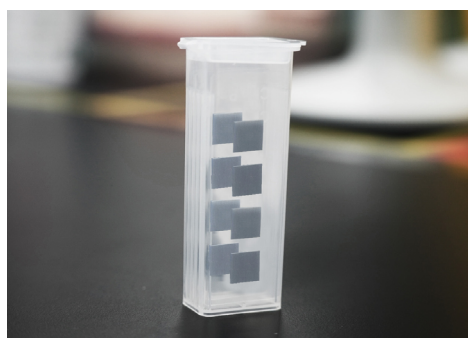


If two different tissue blocks need to be cryosectioned and mounted onto the same Stereo-seq Chip Slide, it is recommended that you first trim both tissue blocks. Perform tissue sectioning and mounting for one tissue block first with the warm method, and then place the tissue-mounted Stereo-seq Chip Slide in the PCR Thermal Cycler for no longer than 5 min while preparing the second tissue block. Perform tissue sectioning and mount the second tissue block using the warm method, then place the tissue-mounted Stereo-seq Chip Slide in the PCR Thermal Cycler to dry for 5 min.



Stop Point:

- After drying the tissue containing Stereo-seq Chip Slides in a PCR Thermal Cycler, they can be stored in a slide container then transferred to a -80°C freezer on dry ice.
- Store the sealed slide container containing Stereo-seq Chip Slides with tissue at -80°C for up to **four weeks**.
- When retrieving Stereo-seq Chip Slides with tissue from the freezer, transfer out the slide container on dry ice, and take out the tissue containing Stereo-seq Chip Slides then immediately incubate at 37°C with PCR Adaptor for **5 min**.





Shipping Guidance for the Tissue Mounted Stereo-seq Chip Slide:

- After drying the tissue containing Stereo-seq Chip Slides on a PCR Thermal Cycler, transfer the Stereo-seq Chip Slide into a slide container 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.
- Prepare a styrofoam shipping container filled with dry ice. Allow 0.5 kg of dry ice for every 2 hours in transport. For example, for a 3-day shipping duration, $0.5\text{kg} * 12\text{hr} * 3 = 18\text{kg}$ of dry ice can be prepared.
- Place the ziplock bag at the bottom layer of the shipping box. If necessary, use sheets of bubble wrap to ensure the slide container remain in a vertical position.
- Fill the empty space in the box with bubble wrap or paper. This will help prevent shifting of the slide container when the ice dissipates.
- Close the styrofoam lid. DO NOT tape the styrofoam lid to the box.
- Secure the outer lid of the shipping box with tape. When using dry ice, it is recommended that you leave an air gap when taping to ensure that carbon dioxide can be released. This can prevent a buildup of pressure that could rupture the package.
- Label the shipping box with a dry ice sticker and then adhere the pre-printed label showing both the recipient's address and the return address. Contact the recipient lab before shipping to ensure that the staff is prepared to receive the shipment.
- When retrieving the Stereo-seq Chip Slides with tissue from the carrier, store the Stereo-seq Chip Slides at -80°C (less than 4 weeks from mounting) until you are ready to process the slide. Before processing, take out the tissue containing Stereo-seq Chip Slides, immediately incubate at 37°C on the PCR Adaptor for 5 min, and then proceed to tissue fixation.



Shipping guidance is based on STOmics in-house validation and general shipping guidance provided by CDC www.cdc.gov.