mirxes

TO KNOW. TO ACT.

Stereo-seq

Sample Preparation Guidelines

Apr 2025

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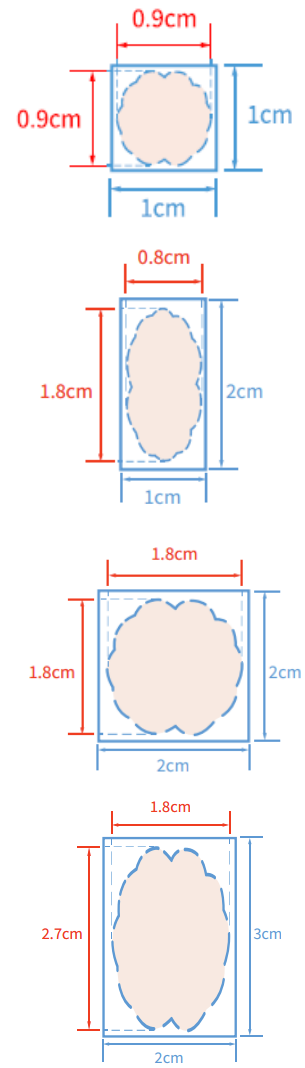
Precautions

- Instructions provided in this manual are intended for general use only and optimization may be required for specific applications.
- Instructions provided is applicable to 1cm*1cm, 1cm*2cm, 2cm*2cm and 2cm*3cm Stereo-seq chip sizes. Please refer to section 1 and section 2 for specific information on different chip sizes.

Section 1. Sample Requirements

- The sectioning plane should not exceed (refer to the figures on the right)
  - 0.9 cm x 0.9 cm x 1cm (for 1cm*1cm Stereo-seq Chip)
  - 0.8 cm x 1.8 cm x 1 cm (for 1cm*2cm Stereo-seq Chip)
  - 1.8 cm x 1.8 cm x 1 cm (for 2cm*2cm Stereo-seq Chip)
  - 1.8 cm x 2.7 cm x 1 cm (for 2cm*3cm Stereo-seq Chip)
- Appropriate cryomold size should be chosen based on the tissue size (please refer to section 2 for recommendations) .
- To avoid RNA degradation, we recommend performing tissue embedding within 30 min upon tissue harvesting. It is always recommended to embed **fresh tissues** directly with OCT for Stereo-seq workflow to achieve the best sample quality.
- Excess liquid on the tissue should be removed to avoid ice formation during embedding.
- Air bubbles should be avoided when filling the OCT in the cryomold.
- Please follow the steps in section 2 for sample embedding.
- It is recommended to submit the sample RNA Integrity Number (RIN) and a photo of H&E staining (please refer to section 3 for details).

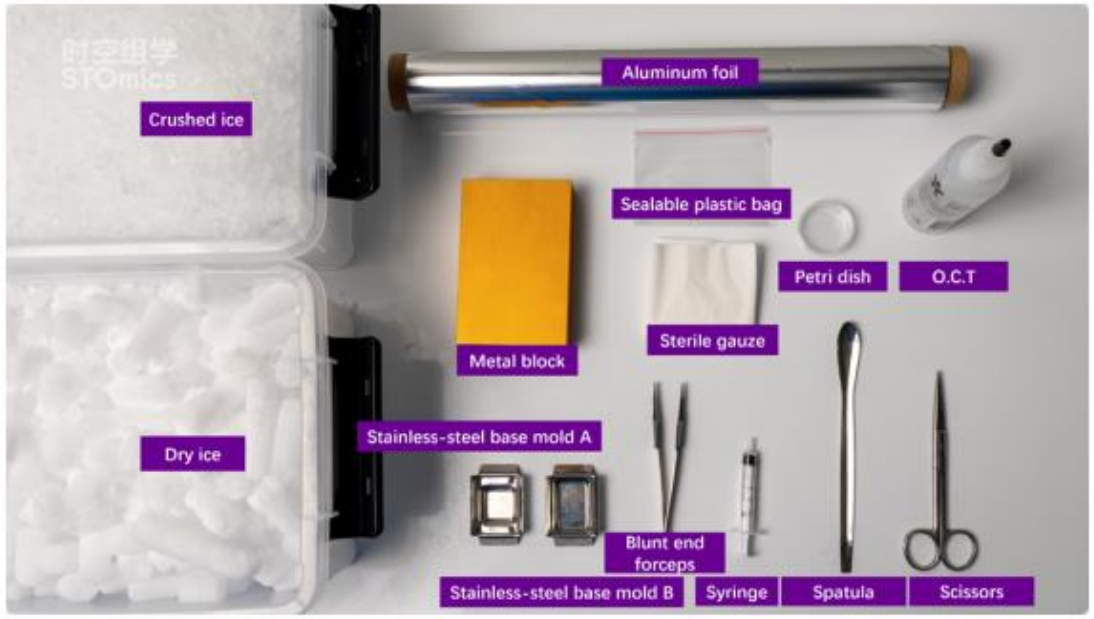


Section 2. Sample Embedding

2.1 Prepare these recommended apparatuses/materials in advance

Recommended Materials

Brand	Description	Quantity
-	Crushed ice in a box	1
-	Dry ice in a box	1
-	Aluminium foil	1
-	Sealable plastic bag	1
BIOSHARP BC032	Metal Coolbox	1
-	Sterile gauze	2
Corning 353001	Corning® 35 mm TC-treated Culture Dish	1
Sakura 4583	Tissue-Tek® O.C.T. Compound	1
Sakura Base Molds 4162/4131/4132/4133 or other brand that's suitable for the tissue size	Stainless-steel base mold A	1
Sakura Base Molds 7055/4133/4165/4124 or other brand that's suitable for the tissue size	Stainless-steel base mold B (slightly larger than mold A)	1
-	Blunt end forceps	1
-	Syringe	1
-	Spatula	1
-	Scissors	1
-	Stainless Steel Ruler	1



Section 2. Sample Embedding

2.1 Prepare these recommended apparatuses/materials in advance (continued)

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

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

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

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

5. A box of dry ice.

6. 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.

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

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

Section 2. Sample Embedding

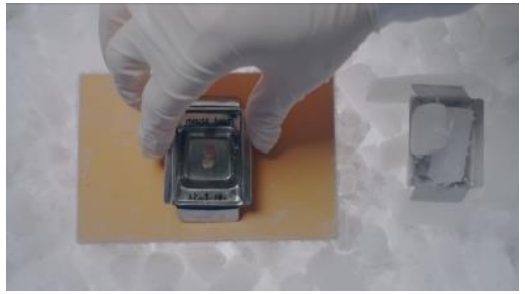
2.2 Sample embedding steps

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

2. Place the tissue in pre-cooled OCT and wrap the tissue evenly with OCT using a spatula without introducing air bubbles (remove any air bubble using a syringe).

3. Orient the tissue to have the side intended to be sectioned facing downwards and then place into mold A. Make sure 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.

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



Section 2. Sample Embedding

2.2 Sample embedding steps

5. Use mold B as a lid with opening facing up, place on top of mold A gently and then place a few dry ice cubes on top of mold B. Make sure the two stainless-steel base molds are nicely submerged in dry ice.

6. After **5 min**, remove mold B and check if the OCT is completely frozen and turns opaque, otherwise repeat step 5.

7. 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.

8. Check 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 and add a few drops of the OCT. Wait till it solidifies and turns opaque.

Section 2. Sample Embedding

2.2 Sample embedding steps

9. Label the tissue block to mark the orientation of the tissue: **ensure that the tissue is in the correct orientation after embedding in OCT. Label the sectioning side with a ★; cutting would be started from this side.**

10. Wrap the OCT-embedded tissue block with aluminum foil, properly label it and keep it in a sealable plastic bag. Store at -80 °C if cryosectioning is not performed immediately.

For a demonstration video of tissue embedding, please refer to the link: Guidance on Tissue embedding

Section 3. RIN determination and H&E staining

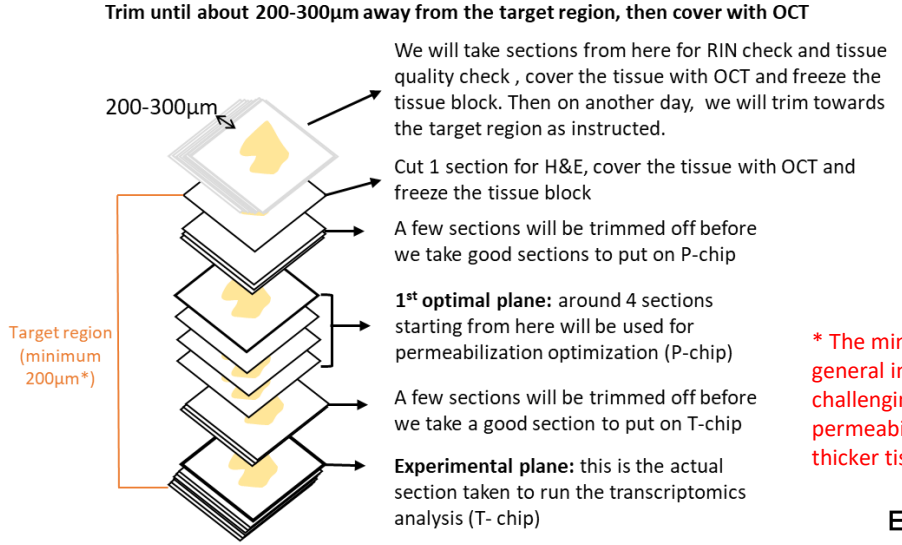
- It is recommended to measure RIN **after** OCT embedding***.**
- Total RNA can be extracted from 10-20 slices of 10 µm-thick tissue sections to determine the RIN value (RIN≥7 is required).
- H&E staining of a tissue section **of the same tissue block after** OCT embedding is required to check sample condition after embedding.
- The tissue must be coated with OCT immediately after trimming off sections for RIN or H&E staining. *Note: do not put excess amount of OCT.*
- **If you require a specific experimental region for analysis, please proceed to Section 4.**
- Please confirm that the OCT-embedded tissue block is wrapped with aluminum foil, properly labelled (**label the sectioning side with a ★ ; we will start to cut from the labelled side)**, kept in a sealable plastic bag and stored at -80 °C.
- Please send us the Service Order Form (with RIN value filled in), together with a photo of H&E staining, which can allow us to confirm the condition of the samples before arranging for sample shipment.

* If there are sample limitations, you may discuss with your Mirxes representative to skip this RIN determination step during pre-project planning phase.

Section 4. For customers who require a specific target region

Additional points to note:

- It is important to **ensure that the tissue is in the correct orientation after embedding in OCT.**
- After the RIN determination and H&E staining steps in section 3, the client should trim the OCT-embedded tissue block until it is close to the desired region for analysis. The client should leave about ~**200 to 300µm** of tissue above the desired experimental region.
- After this, coat the exposed tissue surface with OCT immediately. *Note: do not put excess amount of OCT*.
- Return to Section 3 for the labeling and packing instructions.
- Please provide images or guidelines for target region selection



* The minimum thickness mentioned is a general indication. For samples which are challenging in sectioning or requiring more permeabilization optimization timepoints, thicker tissues are required.

Section 5. Sample preparation for multiple tissues on one chip

Can multiple samples be mounted on one chip?

Yes, provided the multiple samples can be embedded on 1 cryomold and the samples are of the same tissue type, of similar size, and are embedded to the same plane. However, we recommend not to put more than 4 tissue samples on 1 cryomold to ensure adequate spacing (at least 0.1cm) between samples. It is equally important that the tissues are embedded in the center area of the cryomold to ensure a proper fit on the 1cm x 1cm chip.

Please note that by embedding multiple tissues on 1 cryomold, the customer should understand and bear the following risks:

- The tissue section used for the transcriptomics chip may not contain the desired plane of all tissue samples.
- The permeabilization time may not be optimal for all tissue samples, and therefore not all tissue samples will generate optimal gene counts. Requests for additional permeabilization time points are **chargeable**. We will work together with the customer to find out the most optimum permeabilization condition for multiple samples. This will be based on case-by-case discussions.
- There is a risk of diffusion in downstream experiment and may affect the spatial transcriptomics result

Section 6. Illustration of standard workflow

Tissue QC: sectioning for both RIN and H&E

Step 1: Trim off excess OCT and some tissue sections (min. 3-5 sections) to fully expose the tissue; cut 6x10µm sections for RIN check (customer will be notified on RIN value)
Step 2: Cut 10µm for H&E
- Tissue quality check by Mirxes; customer will be notified ONLY if the tissue does not pass quality check
- If no target region selection is required, this H&E image will be sent to customer for tissue morphology confirmation, and/or ROI selection (area/structure of interest within the tissue plane)

Step 3: Cover tissue with cold OCT and store in -80 freezer

Target region selection required

No target region selection

Target region selection and ROI selection (please refer to the next slide for details):

- Customer should provide images or guidelines for target region selection; SG customer can come to lab and do sectioning together (sectioning for P-chips can be completed on the same day in this case)
- Lab will follow the guidelines to trim off xxx µm to reach target region, and cut 1x10µm for H&E staining
- Lab will send H&E image* to customer for target region confirmation, and/or ROI selection (area/structure of interest within the tissue plane)

Note: * The H&E image is at least 100µm away from the T-chip section, thus the plane size and structure may not be 100% the same as the T-chip section

P-chip workflow:

Step 4: Trim off excess OCT and 3-5 sections; cut 4x10µm sections for P-chips (more trimming might be needed to get good quality sections)
Step 5: Cover tissue with cold OCT and store in -80 freezer
Step 6: Permeabilization time testing: 6min, 12min, 18min, 24min

Note:
1. Customer will be notified on the recommended permeabilization time and given 48 hours to raise concerns if any; we would proceed if no concerns received within 48 hours.
2. Due to the heterogeneity of cell type or tissue structure within the tissue plane, the permeabilization efficiency might not be uniform across the entire section. The recommended permeabilization time is intended to achieve the strongest signal with the lowest signal diffusion in the selected ROI.

T-chip section: actual transcriptomics chip

Step 7: Trim off excess OCT and 3-5 sections; cut 1x10µm section for T-chip (more trimming might be needed to get good quality sections)
Step 8: Cover tissue with cold OCT and store in -80 freezer
Step 9: Standard Stereo-seq workflow (ssDNA for nuclei staining)

cDNA and library:

Step 10: Permeabilization and Reverse Transcription
Step 11: cDNA* release and collection
Step 12: Library preparation
Step 13: Sequencing

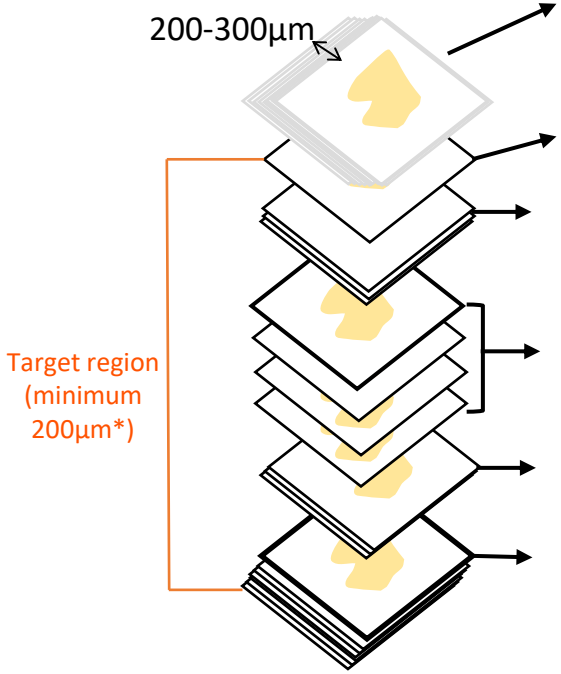
Note: * if cDNA does not pass QC criteria, customer will be notified, and consent is needed to proceed

Section 6. Illustration of standard workflow

If target region selection and/or ROI selection required

Trim towards the target region

Trim until about 200-300µm away from the target region, then cover with OCT



We will take sections from here for RIN check and tissue quality check , cover the tissue with OCT and freeze the tissue block. Then on another day, we will trim towards the target region as instructed.

Cut 1 section for H&E, cover the tissue with OCT and freeze the tissue block

A few sections will be trimmed off before we take good sections to put on P-chip

1st optimal plane: around 4 sections starting from here will be used for permeabilization optimization (P-chip)

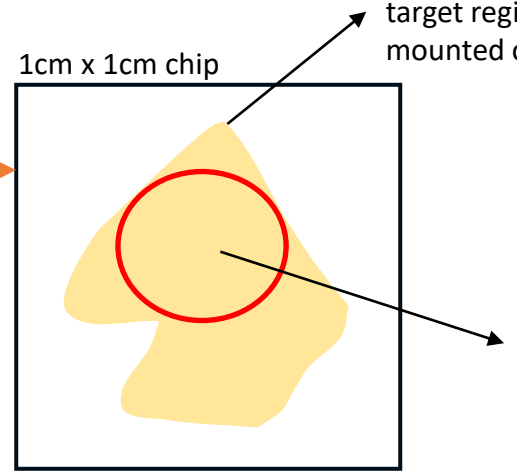
A few sections will be trimmed off, including one section for H&E staining, to get good section plane for T-chip.

Experimental plane: this is the actual section taken to run the transcriptomics analysis (T- chip)

* The minimum thickness mentioned is a general indication. For samples which are challenging in sectioning or requiring more permeabilization optimization timepoints, thicker tissues are required.

ROI selection if any (area/structure of interest within the tissue plane)

H&E image is sent tocustomer for target region confirmation, and/or ROI selection (area/structure of interest within the tissue plane)



If the customer has a specific area/structure of interest within the tissue plane, please mark it out on the H&E image and send back to us. We will try to place this region in the center of the P/T-chip, and also use this as reference during selection of permeabilization time

Section 7. Sample shipment

- If there is plastic mold available, you can place the OCT block inside the mold (of a suitable size) for shipment to provide extra protection to the tissue block. Otherwise, you can choose to place the OCT block inside pre-cooled, sealed small containers to minimize damage from collisions during shipment. Alternatively, wrap the OCT-embedded tissue block with aluminum foil.

- Put the sample in a sealable plastic bag. Place the sealed bag into a foam box with a wall thickness of at least 3 cm, ensuring a tight seal.

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

- Pack in sufficient dry ice (we recommend at least 4-5 days worth; the amount of dry ice depends on the expected duration of the shipment).

- Ensure package is tightly sealed prior to shipping

- Ensure you have confirmation to ship from your Mirxes representative