mirxes

TO KNOW. TO ACT.

FFPE Stereo-seq

Sample Preparation Guidelines

Dec 2025

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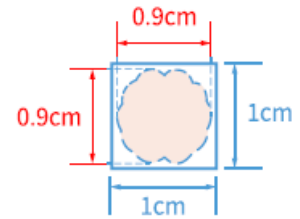
Precautions

- 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 Stereo-seq chip for FFPE sample (referred as “Stereo-seq N-chip” in this document).

Section 1. Sample requirements

- The sectioning plane should not exceed **0.9 cm x 0.9 cm** (refer to the figures on the right).
- It is highly recommended to choose tissues that can cover **at least 50%** of the chip area.
- To achieve optimum tissue condition, please ensure the tissue is promptly fixed with 10% formalin (preferable) or 4% paraformaldehyde for 12-48 hours after sample collection.
- It is important that the original tissue structure is well preserved during the embedding process.
- Please ensure the tissue blocks are stored in properly sealed plastic bags to avoid drying, corrosion, or mildew.
- Refer to your institution's pathology/histology labs for guidance on making paraffin blocks. Adequate dehydration and wax immersion are required to produce FFPE sections without cracks or gaps, ensuring that the specimen remains embedded in the wax during sectioning.
- It is required to submit the DV200 value and H&E staining image of the sample (please refer to section 2 for details).



Section 2. DV200 determination, H&E staining and FFPE block image

- It is recommended to measure DV200 **BEFORE** sample submission/shipment.
- Total RNA can be extracted from 3-5 slices of 5 µm-thick tissue sections to determine the DV200 value (DV200≥30% is required). Please refer to the manufacture's protocol for the detailed process (Deparaffinization Solution – QIAGEN 19093; RNeasy FFPE Kit – QIAGEN 3504).
- Agilent High-Sensitivity RNA Analysis Kit (Agilent 5067-1513) can be used for DV200 measurement.
- H&E staining of a tissue section **of the same tissue block** is required to check sample condition.
- Please send us the SOF - Service Order Form (with DV200 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 submission/shipment.
- Please include images of the FFPE block in SOF and clearly indicate the sectioning side on the image. Cutting would start from the indicated side.

Section 3A. Sample shipment for FFPE block submission

- Store the FFPE blocks preferably at 4°C ~ 8 °C.
- 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.
- Maintain sample temperature **between 4°C ~ 8 °C** during shipment: use no fewer than 6 ice packs (13 cm × 23 cm) for a 24-hour shipment. Increase the number of ice packs accordingly if the shipment is taking longer than 24 hours.
- Use foam, foam paper, and multiple layers of packaging to prevent sample damage during transportation.

Section 4. Stereo-seq FFPE tissue QC and tissue-mounting workflow overview

Tissue QC: DV200 and H&E image

- DV 200: total RNA will be extracted from 5 slices of 5 µm FFPE sections for DV200 QC check
(provided by customer in 50 mL centrifuge tube if the FFPE block can not be processed at Mirxes lab)

- QC criteria: DV200 ≥ 30%

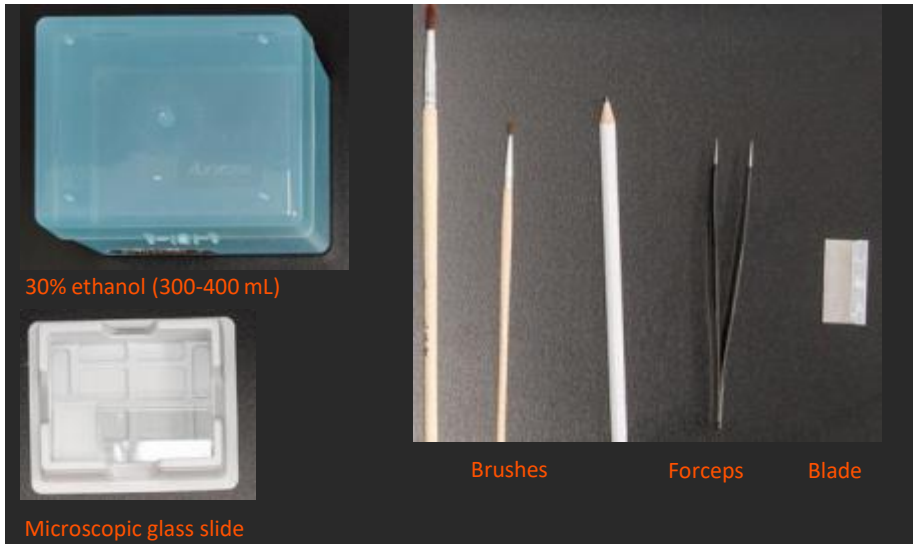
- FFPE image to be provided by customer. Otherwise, one FFPE section on microscopic glass slide is required for H&E staining.
(provided by customer on microscopic glass slide if the FFPE block can not be processed at Mirxes lab)

Tissue-mounting at Mirxes lab or at customer designated sectioning site*

- A few FFPE sections will be used to optimize the tissue flattening and mounting conditions on glass slide
- One FFPE section will be used to test tissue mounting condition on Stereo-seq Test-chip
- One FFPE section will be mounted on the actual Stereo-seq N-chip for FFPE workflow
- At least one adjacent section mounted on microscopic glass slide will be preserved for H&E staining

* If the FFPE block can not be processed at Mirxes lab, Mirxes scientist will be on-site at customer designated sectioning site (for SG Only) to support the sectioning and mounting workflow. Please refer to Section 5 in the next few slides for detailed sectioning procedures.

Equipment/Reagent/Consumable	Remarks
Microtome	Other accessories: forceps, brushes, dust-free paper (Kimwipes), cardstock paper
Microtome blade	To separate FFPE sections
Water bath	Make sure the water bath is clean and is filled with fresh clean ddH2O; If multiple samples are processed, please clean the water bath and refill it with fresh ddH2O after completing each sample; Set at 40°C ~ 48°C (adhere to the established protocol of the sample)
Slide dryer	Set at 42°C to dry the slide
Anhydrous ethanol	Freshly prepared 30% ethanol in ddH2O is required
Nuclease-free water	To rinse the Stereo-seq chip
Microscopic glass slide	To scoop out FFPE sections; or for tissue mounting
Stereo-seq N-chip and Test-chip	Prepared by Mirxes lab



Section 5. FFPE sectioning and mounting workflow (Caution: Do not touch the front side of the chip!)

Step 1: Prepare the microtome, histology brushes, forceps, new microtome blades, and a container filled with 300~400 mL of 30% ethanol.

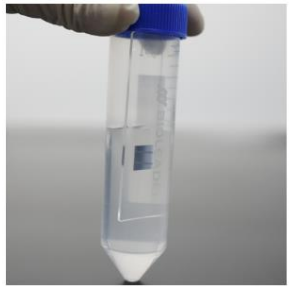
Step 2: Place the paraffin block face down in an ice water mixture for 10 to 30 min, or cool the tissue surface on a cooling platform at ~4°C for 5 to 10 min. For tissues with high-fat content, such as breast tissue samples, freeze the FFPE block at -20°C for 10 min before sectioning.

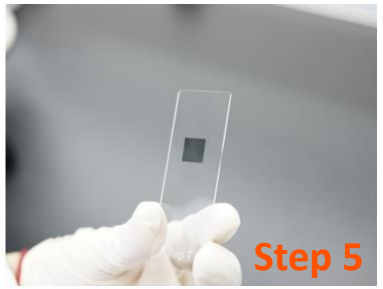
Step 3 – Step 5: Stereo-seq Chip preparation

Step 3: Take out the Stereo-seq Chip Slide and record the Chip ID (SN) number located on the back side of the slide (**by Mirxes Scientist**). Set the slide dryer at 42°C.

Step 4: 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. Make sure the chip is fully covered with nuclease-free water during washing step.

Step 5: 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. Only when the chip is completely dry and without wavy white stains is it ready for tissue mounting.





Section 5. FFPE sectioning and mounting workflow (Caution: Do not touch the front side of the chip!)

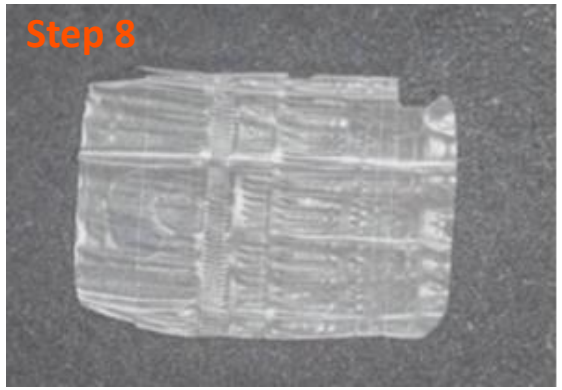
Step 6: Insert the paraffin block on the microtome and orient it such that the blade will cut straight across the block. If the tissue is deeply embedded, approach the block with an old trimming blade and cut a few thin sections to ensure that the positioning is correct. Adjust if necessary. Trim the 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.

Step 7: Adjust the section thickness to 5 µm for regular tissue and 4 µm for high-fat-content tissue.

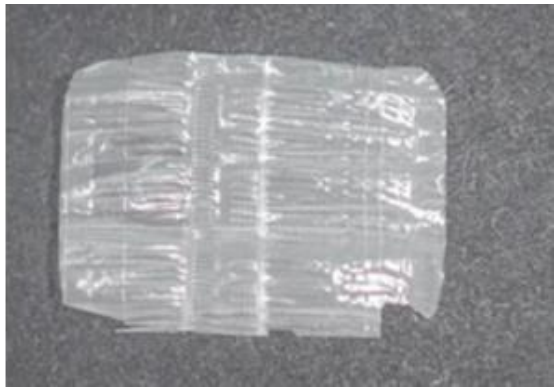
Step 8: Identify Side A and Side B of the selected paraffin section. The side facing the microtome is side A (matte side), and the side touching the blade is side B (smooth side). Always keep side B facing down when it is floating in the water bath, or keep it mounted onto the Stereo-seq chip and microscopic glass slide to prevent section detachment.

Step 9: Cut and select the desired section. If adjacent sections are required, make a ribbon of continuous sections. Using clean forceps, carefully transfer the selected section onto cardstock paper for trimming and separation. To preserve adjacent sections, make a ribbon of three continuous sections. Using clean forceps, carefully transfer the sections onto cardstock paper for trimming.

Step 10: Carefully separate the middle section from the other two sections and use it for Stereo-seq Chip Slide mounting. Use the first and third sections for subsequent H&E staining on microscopic glass slides.



Side A



Side B

Section 5. FFPE sectioning and mounting workflow (Caution: Do not touch the front side of the chip!)

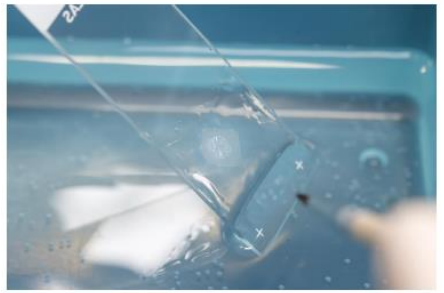
Step 11: Trim the edges of the targeting section by removing excess paraffin in order to fit the standard Stereo-seq Chip size (1 cm × 1 cm). It is important to still have sufficient paraffin surrounding the tissue area to improve attachment to the Stereo-seq Chip. After trimming, the section should not exceed 0.9 cm x 0.9 cm to ensure that the section does 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.

Step 12: After trimming, carefully transfer the section with side B facing down to the container filled with 30% ethanol using a histology brush or clean forceps.

Step 13: Use a clean microscopic glass slide to scoop out the section and float it on the surface of the water in the preheated water bath. Make sure side B is facing down.

Putting into 30% ethanol

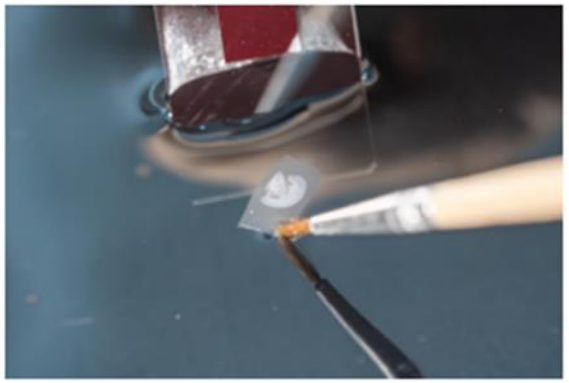
Pre-flattening in 30% ethanol

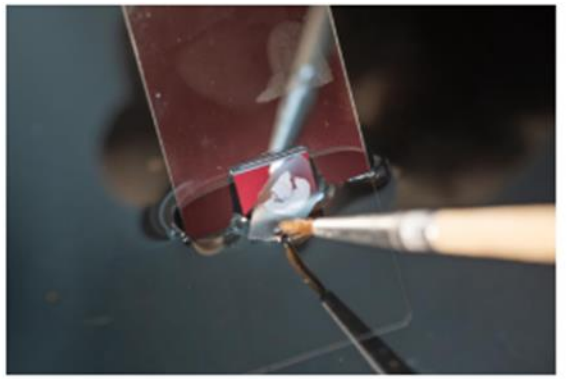


Scoop out and transfer to water bath

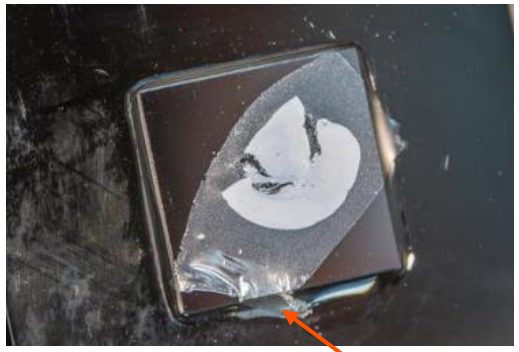
Section 5. FFPE sectioning and mounting workflow (Caution: Do not touch the front side of the chip!)

Step 14: Wait until the tissue section is completely flattened in the water bath. Place the Stereo-seq Chip or glass 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 or glass slide.





Step 15: Wipe off excess liquid from around and the back of the slide using dust-free paper without touching the tissue or the front of the chip. Poke a small hole in the paraffin area outside the edge of the chip (using forceps) and use dust-free paper to drain off the excess liquid trapped between the section and the chip. Then dry the Stereo-seq Chip at 42°C for 3 hr.



Note:

1. Follow step 3 to step 15 for both Test-chip and Stereo-seq N-chip.
2. For a demonstration video of the paraffin section mounting on the Stereo-seq Chip Slide, please refer to the link: Demonstration video on FFPE Sample Sectioning and Mounting

Section 5 Appendix:

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.

Please use one of the following PCR thermal cyclers and PCR adaptor:

PROFLEXTM PCR 3X 32-well PCR system (Applied biosystem)

T100TM PCR instrument (Bio-rad)

Stereo-seq PCR adaptor (BGI 301AUX001)

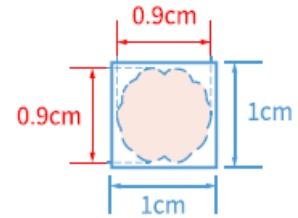
Temperature	Time
(Heated lid) 45°C	On
42°C	∞
42°C	3 hr
37°C	∞

Section 6. 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 one FFPE block 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 four tissue samples on one block 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.

***Note**: if more than four tissues are to be embedded on one block, the spacing between every two tissues must be greater than the size of each individual tissue.



Please note that by embedding multiple tissues on one FFPE block, 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
- There is a risk of diffusion in downstream experiment and may affect the spatial transcriptomics results