STOmics

Stereo-seq TRANSCRIPTOMICS SET FOR FFPE

USER MANUAL

Cat. No.: 211SN114 (4 RXN) / 211SN114-CG (4 RXN)
Kit Version: V1.0
Manual Version: B_1

STUM-TT004

REVISION HISTORY

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

Manual Version: B
Kit Version: V1.0
Date: Oct. 2024
Description:

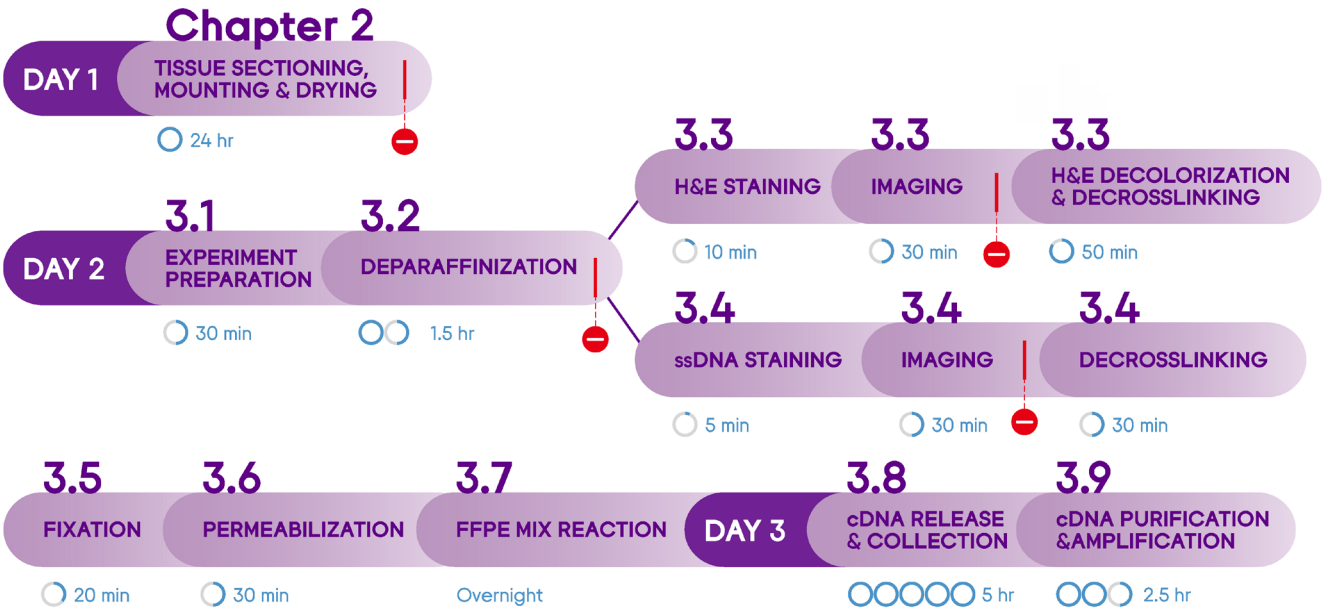
- Minor error fixation in reagent preparation tables.
- Updated the calculated total reagent tissue fluorescent staining solution.
- Revised for overall minor consistency in language.
- Updated the stop point descriptions.

Manual Version: B_1
Kit Version: V1.0
Date: Jul. 2025
Description:

- Added US use only catalog numbers.
- Adjusted the document hyperlinks to improve accessibility and user experience.
- Updated the storage and transportation temperature of STOmics reagent and chip.
- Updated reference to PE001 with note on upcoming replacement by PE002.

Note: Please download the latest version of the manual and use it with the corresponding Stereo-seq Transcriptomics N Kit.

WORKFLOW



TOTAL TIME: ~1.5 - 2 DAYS

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. Intended Use

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. Built upon DNA Nanoball (DNB) technology, STOmics Stereo-seq Transcriptomics Set for FFPE enables a “tissue-to-data” solution through *in situ* capture of the whole transcriptome, at nanoscale resolution and centimeter-sized field of view (FOV). This kit uses random primers to capture RNAs *in situ*. Each cDNA synthesized from RNA captured on a particular spot is linked to its spatially-barcoded probe, allowing subsequent gene expression mapping of a tissue section following sequencing and visualization analysis using the StereoMap visualization platform.

All reagents provided in this kit have passed stringent quality control and functional verification, ensuring performance stability and reproducibility.

1.2. Sequencing Guideline

Sequencing libraries produced using the Stereo-seq Transcriptomics Set require the DNBSEQ sequencing platform. For details, refer to **Stereo-seq OMNI FFPE Library Preparation User Manual (Document No.: STUM-LP001)**.

Required input parameters for Stereo-seq FFPE transcriptome libraries for the Stereo-seq Analysis Workflow (SAW) bioinformatics pipelines are as follows:

`--kit-version=` 'Stereo-seq N FFPE V1.0'

`--sequencing-type` = 'PE75_25+59'

1.3. List of Kit Components

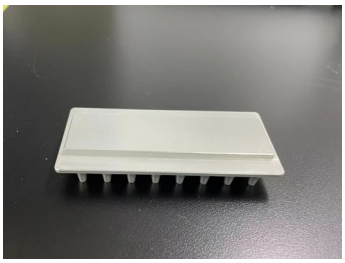
Each Stereo-seq Transcriptomics Set for FFPE consists of:

- Stereo-seq Transcriptomics N Kit V1.0 *1 (4 RXN)
- Stereo-seq Chip N Slide (1cm * 1cm) *1 (4 EA)
- STOmics FFPE Accessory Kit *3

Stereo-seq 16 RXN Library Preparation Kit is not included in the Stereo-seq Transcriptomics Set for FFPE and must be purchased separately. If you wish to construct Stereo-seq FFPE transcriptome libraries in-house, refer to **STUM-LP001 Stereo-seq OMNI FFPE Library Preparation User Manual** for details.

Compatible auxiliary but not included:

Stereo-seq PCR Adaptor *1 (2 EA)



Catalog numbers, kit components, and specifications are listed below (Table 1-1 to Table 1-4).

Upon receiving the Stereo-seq Chip N Slide (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.

Performance of products may only be guaranteed before their expiration date. Proper performance is also subject to the products being transported, stored, and used in appropriate conditions.

Table 1-1 Stereo-seq Transcriptomics N Kit

Stereo-seq Transciptomics N Kit	Cat. No.:211KN114 / 211KN114-CG		
Component	**Reagent Cat. No.**	**Cap Color**	**Quantity (tube)**
RI	1000028499	●	300 μL × 1
FFPE Mounting Medium	1000047466	●	100 μL × 1
FFPE Decrosslinking Reagent	1000047464	●	1725 μL × 2
PR Enzyme	1000028500	●	10 mg × 1
FFPE RT Buffer Mix	1000047460	○ (transparent)	700 μL × 1
FFPE RT Oligo	1000047461	○ (transparent)	44 μL × 1
FFPE RT Enzyme Mix	1000047462	○ (transparent)	132 μL × 1
FFPE Dimer	1000047463	●	10 μL × 1
cDNA Release Buffer	1000028512	●	1725 μL × 2
cDNA Release Enzyme	1000028511	●	88 μL × 1
cDNA Amplification Mix	1000028514	●	220 μL × 1
FFPE cDNA Primer Mix	1000047465	●	36 μL × 1
Storage Temperature: -25°C ~ -15°C	Transportation Temperature: -25°C ~ -15°C		Expiration Date: refer to label

Table 1-2 Stereo-seq Chip N Slide (1cm * 1cm) Components

Stereo-seq Chip N Slide (1cm * 1cm)	Cat. No.: 210CN114 / 210CN114-CG
Component	**Quantity (per kit)**
Stereo-seq Chip N (1cm * 1cm)	4 EA
Storage Temperature: 2°C ~ 8°C	Transportation Temperature: -25°C ~ -15°C	Expiration Date: refer to label

Table 1-3 STOmics FFPE Accessory Kit Components

STOmics FFPE Accessory Kit Cat. No.: 310AK002 / 310AK002-CG		
Component	**Reagent Cat. No.**	**Quantity (per kit)**
Cassette	1000033699	3 EA
Gasket	1000033698	3 EA
Sealing Tape	1000042970	/
Storage Temperature: 18°C ~ 25°C	Transportation Temperature: 0°C ~ 30°C	Expiration Date: refer to label

Table 1-4 Stereo-seq PCR Adaptor Components

Stereo-seq PCR Adaptor Cat. No.: 301AUX001 / 301AUX001-CG	
Component	**Quantity (per kit)**
Stereo-seq PCR Adaptor	2 EA
Storage Temperature: 18°C ~ 25°C	Transportation Temperature: 0°C ~ 30°C	Expiration Date: refer to label

1.4. Additional Equipment and Materials

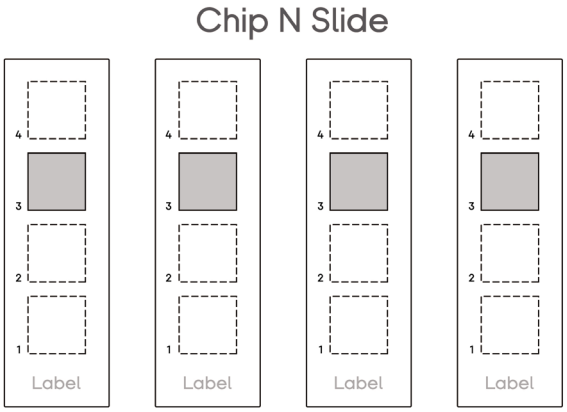
For equipment and materials needed for this protocol, refer to the **Experiment Preparation Checklist for FFPE**. The user is expected to have access to common laboratory equipment not named in the document (equipment such as an ice maker, biological safety cabinet, freezers, and so on). For specific microscope requirements, refer to **STOmics Microscope Assessment Guideline (Document No.: STUM-PE001)**, which will be superseded by **Stereo-seq Imaging Requirements and Guidelines (Document No.: STUM-PE002)** once available. Please refer to the same document link for the latest version.

1.5. Stereo-seq Chip Slide Information

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.

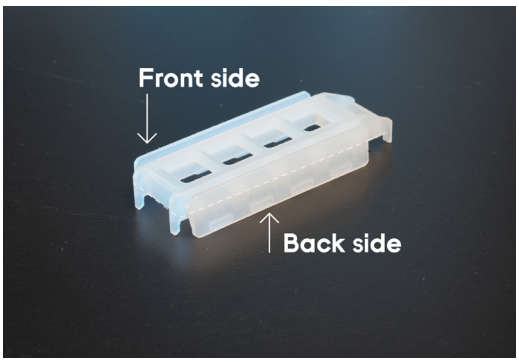


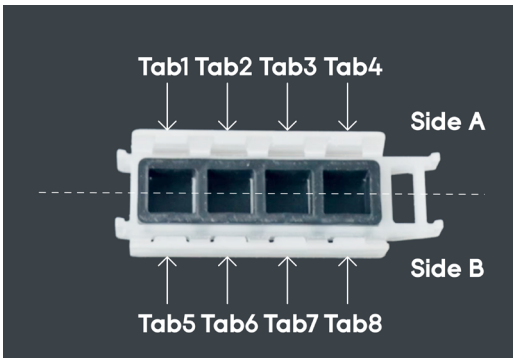
Stereo-seq Chip N Slide Storage

Always store unused slides in their original slide container and keep them sealed in a sealable aluminum bag at 2°C ~ 8°C. Keep sealed with tape or another re-sealable bag. **Always KEEP the desiccant in the bag.**

Stereo-seq Slide Cassette

STOmics Stereo-seq Accessory Kit contains a Stereo-seq Cassette and removable Gaskets which need to be assembled prior to use.





For a demonstration video of Stereo-seq Slide Cassette assembling and removal, please refer to the link or by scanning the QR code:
https://en.stomics.tech/resources/videos/list.html

Refer to Appendix I: Stereo-seq Slide Cassette Assembly

1.6. Precautions and Warning

- This product is intended for research use only, not for use in diagnostic procedures. Read all instructions in this manual carefully before using the product.
- Before performing experiments with the kits, ensure that you are familiar with all related instruments and operate them according to the manufacturers' instructions.
- Instructions provided in this manual are intended for general use only; optimization may be required for specific applications.
- Thaw reagents in the kits properly prior to use. For enzymes, centrifuge briefly and keep them on ice until use. For other reagents, thaw them first at room temperature, invert several times to mix them properly, and centrifuge them briefly. Place them on ice for future use.
- RNA capture will be compromised or absent for any scratched areas on the front-side surface of the chip.
- We recommend using filtered pipette tips to prevent cross-contamination. Use a new tip each time for pipetting different solutions.
- We recommend using a thermal cycler with heated lids for PCR reactions. Unless otherwise stated, pre-heat the thermal cycler to reaction temperature before use.
- Improper handling of samples and reagents may contribute to aerosol contamination of PCR products, resulting in data inaccuracy. Therefore, for PCR reaction preparation and PCR product cleanup tests, we recommend working in two distinctly separated working areas in the laboratory. Use designated pipettes and equipment for each area, and perform regular cleaning (with 0.5% sodium hydrochloride or 10% bleach) to ensure a clean and sterile working environment.
- Do not consume any sample or reagent, and avoid direct contact of reagents with skin and eyes. In case of an accident, immediately wash the affected area thoroughly with a large amount of water. Seek emergency medical assistance if needed.

CHAPTER 2
SAMPLE AND EXPERIMENT PREPARATION

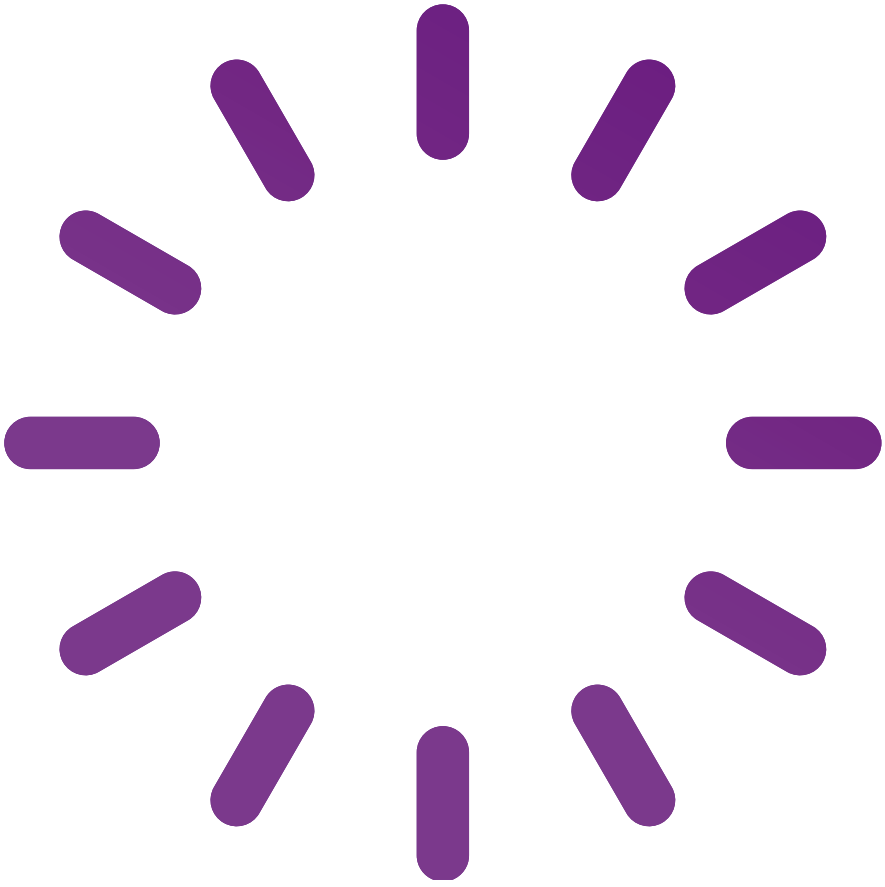
For paraffin sample sectioning and mounting guides, refer to **Sample Preparation, Sectioning, and Mounting Guide for Formalin-fixed and Paraffin-embedded (FFPE) Samples on Stereo-seq Chip Slides (Document No.: STUM-SP003)**.

This guide explained how to check the RNA quality (DV200 value) of a FFPE tissue sample before proceeding to the Stereo-seq experiment.

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

CHAPTER 3

Stereo-seq TRANSCRIPTOMICS SET FOR FFPE STANDARD OPERATING PROCEDURE



3.1. Experiment Preparation

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

Table 3-1 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% 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	Dilute 10 mL Eosin with 20mL 70% ethanol (Eosin solution can be re-used)	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. Please 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

Other Preparation		
Equipment	**Set up**	**Notes**
PCR Thermal Cycler	Set the temperature in the following order: 1. 85°C for H&E decolorization (heated lid at 85°C). 2. 95°C for decrosslinking (heated lid at 85°C). 3. 37°C for slide baking and permeabilization (heated lid at 42°C). 4. 42°C for slide baking and RT + ligation (heated lid at 45°C). 5. 55°C for cDNA release (heated lid at 60°C).	Check the PCR thermal cycler for any abnormalities, and replace it if necessary.
Fluorescence Microscope	For H&E imaging: Set to BF-Epi channel. For fluorescent imaging: Set the epi-fluorescence channel to FITC mode	Check the microscope for any abnormalities, and replace it if necessary.

3.2. Deparaffinization

a. Set aside the ethanol of different concentrations and ddH_2O you prepared in **3.1 Experiment Preparation**, turn on the Slide Dryer in advance (or Water bath-Slide Dryer, metal bath, or PCR thermal cycler with PCR Adaptor), and set the baking temperature to 60°C.

b. Set aside the hematoxylin solution from a 4°C refrigerator and equilibrate it to room temperature in advance.

c. Transfer the tissue-mounted Stereo-seq Chip Slide onto the Slide Dryer and bake at 60°C for **1 hr**.

d. Prepare the following reagents in staining jars, at least **30 mL** for each jar:

Description	Quantity
Histo-clear ①&②	2
100% ethanol ①&②	2
96% ethanol ①&②	2
90% ethanol	1
80% ethanol	1

Description	Quantity
70% ethanol	1
50% ethanol	1
30% ethanol	1
ddH_2O	1

Xylene can be used in place of Histo-clear

e. After baking, place the Stereo-seq Chip Slide into Histo-clear ① at room temperature for **20 min**, then transfer it to Histo-clear ② at room temperature for another **20 min**.

f. Take the Stereo-seq Chip Slide out from Histo-clear ② and remove the excess Histo-clear solution with dust-free paper.

g. Sequentially place the Stereo-seq Chip Slide into the following containers:

1. 100% ethanol ① for 5 min
2. 100% ethanol ② for 5 min
3. 96% ethanol ① for 5 min
4. 96% ethanol ② for 5 min
5. 90% ethanol for 2 min
6. 80% ethanol for 2 min
7. 70% ethanol for 2 min
8. 50% ethanol for 2 min
9. 30% ethanol for 2 min
10. ddH_2O for 1 min.

h. Take the Stereo-seq Chip Slide out and remove excess ddH_2O from around and the back of the slide with dust-free paper without touching the chip.

Stop Point:

After the depadraffinization step, let the Stereo-seq Chip Slides air-dry naturally, the slides can be stored in a slide container at 4°C with desiccant for up to 1 week.

3.3. H&E Staining, Imaging, Decolorization and Decrosslinking

Stereo-seq Transcriptomics for FFPE workflow offers two staining options: H&E staining or nuclei staining using ssDNA. Please choose one of the staining methods and proceed to section 3.3 for the H&E staining workflow or section 3.4 for the fluorescent staining workflow.

Reagent Required for Section 3.3	Purpose	Preparation
Hematoxylin (Solarbio, Cat. No.: G4470)	Staining	Prepare 1 mL per chip. Take it out of 4°C in advance and equilibrate to room temperature at least 5 min prior to use.
5X SSC	Washing	Dilute 12.5 mL 20X SSC with 37.5 mL ddH_2O in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube) and mix well.
Bluing reagent	Staining	Prepare 30~40 mL bluing reagent in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube).
0.1X SSC	Washing	Prepare 30~40 mL 0.1X SSC in two staining jars separately (alternatively, use a slide container or a 50 mL centrifuge tube).
70% ethanol	Eosin dilution	Dilute 14 mL 100% ethanol with 6 mL ddH_2O and mix well.
Eosin Solution	Staining	Dilute 10 mL Eosin with 20mL 70% ethanol (Eosin solution can be re-used) in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube) and mix well.
FFPE Mounting Medium	Chip Mounting	Take it out of -20°C in advance and equilibrate to room temperature until it thaws.
0.1X SSC	Washing and coverslip detachment	Prepare 30~40 mL 0.1X SSC in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube).
TE Buffer (pH9.0)	H&E decolorization	Take it out of 4°C in advance and equilibrate to room temperature at least 5 min prior to use.
FFPE Decrosslinking 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.

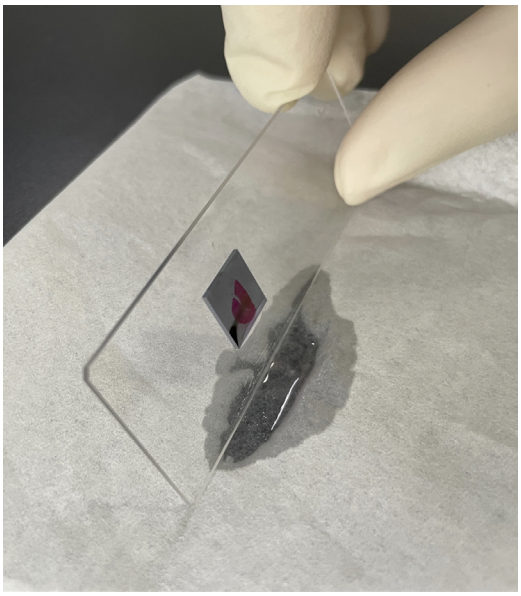
If white precipitates are visible in the reagent, dissolve them by heating the buffer at 55 °C and equilibrate to room temperature before mixing.

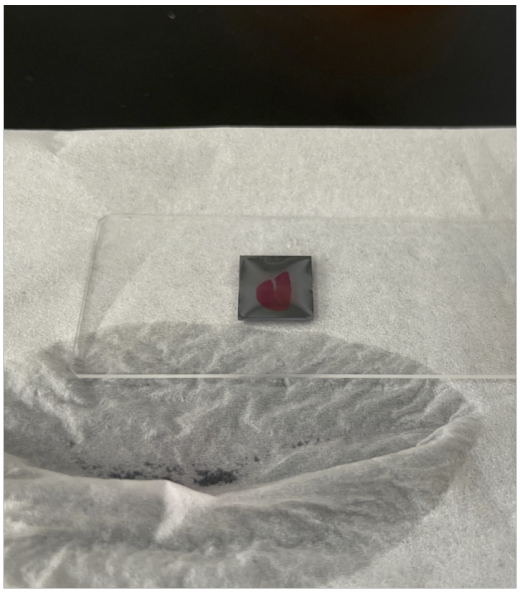
H&E Staining

a. To achieve optimal staining results, make sure the hematoxylin solution has been equilibrated to room temperature.

b. Pipette enough nuclease-free water on the Stereo-seq Chip then discard it to moisten the chip surface and facilitate H&E staining. Add **1 mL** of hematoxylin solution onto the Stereo-seq Chip, cover the chip surface completely, and stain for **2 min** at room temperature.

c. After the incubation, discard the hematoxylin solution by turning the Stereo-seq Chip Slide sideways while pouring out the solution onto dust-free paper.





d. Immerse the Stereo-seq Chip Slide in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube) with about 30 mL of 5X SSC, and then gently rinse the Stereo-seq Chip Slide up and down 5 times to wash away the hematoxylin solution.

e. Immerse the Stereo-seq Chip Slide in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube) with about 30 mL of the bluing reagent for **1 min** at room temperature.

f. Immerse the Stereo-seq Chip Slide in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube) with about 30 mL of 0.1X SSC, and then gently rinse the Stereo-seq Chip Slide up and down 5 times to wash away the bluing reagent.

g. Immerse the Stereo-seq Chip Slide in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube) with about 30 mL of Eosin solution for **1 min** at room temperature (the Eosin solution can be reused).

h. Immerse the Stereo-seq Chip Slide in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube) with about 30 mL of 0.1X SSC, and then gently rinse the Stereo-seq Chip Slide up and down 3 times to wash away the Eosin solution.

i. Securely hold the slide and completely dry the tissue using a power dust remover at a distance of 2-3 cm away from the tissue surface. Blow gently from one side at a 30-degree angle horizontal to the plane of the slide.

j. Pipette **5 µL** FFPE Mounting Medium onto the center of the tissue on the chip without introducing air bubbles.

Make sure the coverslip is clean and free of dust and debris. To clean the coverslip, wipe it with an alcohol swab or use a power dust remover.

k. Using clean forceps, place one end of the coverslip onto the tissue edge while holding the other end and then gradually lower the coverslip onto the tissue. Ensure that the tissue is completely covered by FFPE Mounting Medium and the coverslip. To ensure good image quality, **IMMEDIATELY proceed to H&E imaging.**

H&E Imaging

a. Create a new folder in a fluorescent microscope-connected PC, name the folder with the chip ID number and other essential information.

Use only letters, numbers, and underscores in the folder name. Special characters and spaces are not allowed.

Example chip ID number: B00249A1

b. Turn on the fluorescence microscope, select the transmitted fluorescence (color) mode and choose the BF-Epi channel. Push the color/black and white slider into the machine and insert the light source diffuser.

c. Place 1-2 µL of water on the imaging platform first, and then transfer and place the Stereo-seq Chip Slide onto the water drop. Water surface tension will grab onto the slide and adhere it onto the imaging platform.

d. Position the chip directly under the objective lens and adjust the appropriate Brightness, Gain, and Exposure parameters.

e. Adjust the coarse adjustment knob until the tissue appears within focus, and then use the fine adjustment knob to further fine-tune the focus until the tissue and cell contours can be clearly visualized.

f. Move to the tissue area first, focus on the tissue, determine whether the color of the tissue area is normal. If the color appears to be off, move the focus to a blank area on the chip to adjust the white balance first.

g. Box-select the chip area to fully enclose the chip boundaries (which should be slightly larger than the chip), and scan the map under the 4X objective lens (if the microscope does not have a map scanning function, skip to the next step).

h. After scanning is completed, adjust the objective lens to 10X, then adjust the parameters such as Brightness (usually around 30, [0, 225]), Gain (set to the minimum), and Exposure (be careful not to overexpose). The track lines and the tissue morphology should be clear.

i. Shift the focus to a blank area on the chip, and perform background balancing. Firstly adjust the focus to find a chip field of view without any tissue coverage or obvious impurities. Adjust the focusing knob to zoom out for defocusing. If there are small impurities in the previously selected area, they should be barely visible after defocusing. Finally, select "Background balancing".

j. Further adjust the box-selected area and the edge of the chip according to the map, move to the tissue area first, then fine-tune the focus until the tissue and cell contour can be clearly visualized.

k. Select the region of interest within the tissue area, then manually select and establish the model focal points. It is recommended that you first establish 3-5 model points in the blank areas of the chip (four corners of the chip).

l. After establishing the model focal points in blank areas, focus on the tissue area to establish the model focal point. It is suggested that you establish 4-6 model points in different tissue areas. When the model focal points have been established, perform a full scan using a 10X lens.

If multiple chips need to be imaged, image with the same background balancing parameters.

If the imaging is not satisfactory, retake the image.

m. Save the original tile (FOV) images, files, and stitched images.

n. Open the ImageMap software and the Image Quality Control functional module within the software. Upload your H&E-stained image and run Image QC according to the instructions in the **StereoMap User Manual** within the software.

If the image fails to pass Image Quality Control, continue with the experimental procedures and perform optimal image analysis later under the guidance of your local Field Application Scientist.

Stop Point:

After H&E imaging, the Stereo-seq Chip Slides can be kept at room temperature for 4 hr in a mounting state.

Decolorization and Decrosslinking for H&E-stained Tissue

a. After H&E imaging, immerse the coverslip-mounted Stereo-seq Chip Slide in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube) filled with at least 30mL of 0.1X SSC, allow the coverslip to naturally detach from the tissue-mounted chip, and gently rinse the Stereo-seq Chip Slide up and down to clean the chip surface.

b. Take out the Stereo-seq Chip Slide and wipe off the excess solution from around and the back of the slide with dust-free paper without touching the chip. Make sure there is no liquid residue around the chip. The chip does not need to be air-dried.

c. Assemble the Cassette and Gasket then place the Stereo-seq Chip Slide in the Cassette according to the guide written in **Appendix I: Stereo-seq Slide Cassette Assembly**. It is recommended that you practice with a regular blank glass slide.

d. Place the PCR Adaptor in the PCR thermal cycler and set the program as follows:

Table 3-2 Decolorization and decrosslinking incubation program for H&E-stained tissue

Program selection: Incubate

Temperature	Time	Steps
(Heated lid) 85°C	on	H&E Decolorization
85°C	∞	
85°C	20 min	
30°C	5 min	
30°C	∞	
95°C	30 min	H&E Decrosslinking
4°C	∞	

e. Grip along the Stereo-seq Cassette to make sure the Stereo-seq Chip Slide has been locked in place.

Do not touch the front of the chip while assembling the Stereo-seq Slide Cassette.

f. Add **400 µL** TE Buffer (pH 9.0) that has been equilibrated to room temperature into the well of the Stereo-seq Slide Cassette. Incubate at room temperature for **1 min**, then discard the TE Buffer using a pipette.

g. Add another **400 µL** TE Buffer (pH 9.0) into the well of the Stereo-seq Slide Cassette. Apply sealing tape to the Stereo-seq Slide Cassette and make sure it is sealed tightly.

h. When the PCR thermal cycler reaches 85°C (Time = ∞), place the Stereo-seq Chip Slide on the PCR Adaptor. Select **Edit**, and then select **Next Step** to skip the 85°C (Time = ∞) step.

Do not preheat the TE Buffer, which may cause the tissue section to detach from the chip.

i. Incubate the Stereo-seq Slide Cassette at 85°C for **20 min** followed by 30°C for **5 min**.

j. When the PCR thermal cycler reaches 30°C (Time = ∞), remove the Stereo-seq Slide Cassette from the PCR Adaptor, peel off the sealing tape, discard the TE Buffer (pH 9.0) using a pipette, and proceed to the decrosslinking steps.

k. Add **200 µL** FFPE Decrosslinking Reagent into the well of the Stereo-seq Slide Cassette. Incubate at room temperature for **30 sec ~ 1 min**, then discard the liquid using a pipette.

l. Add another **400 µL** FFPE Decrosslinking Reagent into the well of the Stereo-seq Slide Cassette. Apply sealing tape to the Stereo-seq Slide Cassette and make sure it is sealed tightly.

m. Place the Stereo-seq Slide Cassette on the PCR Adaptor in the PCR thermal cycler. Select **Edit**, and then select **Next Step** to skip the 30°C (Time = ∞) step.

n. Incubate at 95°C for **30min**. Meanwhile, prepare about 30 mL of methanol in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube), close the lid, and pre-cool it for **5-30 min** at -20°C.

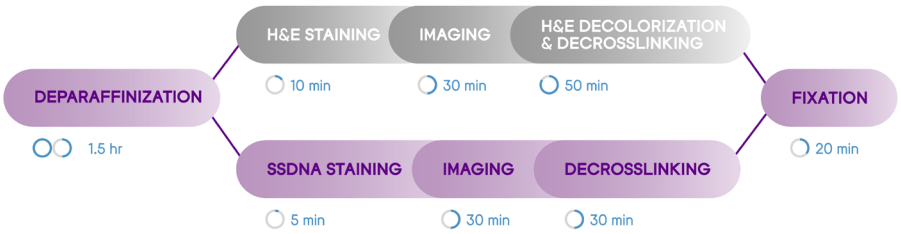
o. When incubation is completed, carefully transfer the Stereo-seq Slide Cassette to the nearest bench and peel off the sealing tape. Discard the FFPE Decrosslinking Reagent with a pipette.

p. Remove the slide from the Stereo-seq Slide Cassette according to the instructions in Appendix I: Stereo-seq Slide Cassette Assembly, discard the cassette and gasket, then immediately proceed to the fixation step (Section 3.5).

The cassette and gasket may deform after heating to 95°C. DO NOT reuse the cassette and gasket. Discard them after this step. Use a new cassette and a new gasket in the subsequent steps.

3.4. Fluorescent Staining, Imaging and Decrosslinking

Stereo-seq Transcriptomics for FFPE workflow offers two staining options: H&E staining or nuclei staining using ssDNA. Please choose one of the staining methods and proceed to section 3.3 for the H&E staining workflow or section 3.4 for the fluorescent staining workflow.



Reagent Required for Section 3.4	Purpose	Preparation
5X SSC	Dilution	Prepare 400 µL by diluting 100 µL 20X SSC with 300 µL ddH_2O and mix well.
FFPE Mounting Medium	Chip Mounting	Take it out of -20°C in advance and equilibrate to room temperature until it thaws.
0.1X SSC	Washing and coverslip detachment	Prepare 50 mL by diluting 250 µL 20X SSC with 49.75 mL ddH_2O and mix well.
FFPE Decrosslinking 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.

If white precipitates are visible in the reagent, dissolve them by heating the buffer at 55 °C and equilibrate to room temperature before mixing.

Fluorescent Staining

a. Prepare fluorescent staining solution according to Table 3-3 and store it **in the dark**.

Table 3-3 Tissue fluorescent staining solution

Components	1X (μL)	2X + 10% (μL)	3X + 10% (μL)	4X + 10% (μL)
5X SSC	189	396.9	585.9	774.9
Qubit ssDNA Reagent	1	2.1	3.1	4.1
RI	10	21	31	41
Total	200	420	620	820

b. Add **150 μL** of tissue fluorescent staining solution per chip by first pipetting one droplet at each corner of the chip and then adding the rest of the staining solution to the middle to merge all droplets.

Ensure that the chip is completely covered by tissue fluorescent staining solution.

c. Incubate the chip for **5 min** at room temperature in the dark.

d. Gently remove the staining solution from the corner of the chip using a pipette.

e. Add **150 μL** of 0.1X SSC per chip then discard using a pipette.

f. Repeat **step e**.

g. Gently dry the chip with a power dust remover.

h. Pipette **5 μL** FFPE Mounting Medium onto the center of the tissue on the chip without introducing air bubbles.

Make sure the coverslip is clean without any dust or debris. To clean the coverslip, wipe it with an alcohol swab or use a power dust remover.

i. Using clean forceps, place one end of the coverslip onto the tissue edge while holding the other end and then gradually lower the coverslip onto the tissue. Ensure that the tissue is completely covered by FFPE Mounting Medium and the coverslip. To avoid fluorescent bleaching, **IMMEDIATELY proceed to fluorescent imaging**.

Fluorescent Imaging

a. Create a new folder in a fluorescent microscope-connected PC, and name it with the chip ID number and other essential information.

Use only letters, numbers, and underscores in the folder name. Special characters and spaces are not allowed.

Example chip ID number: B00249A1

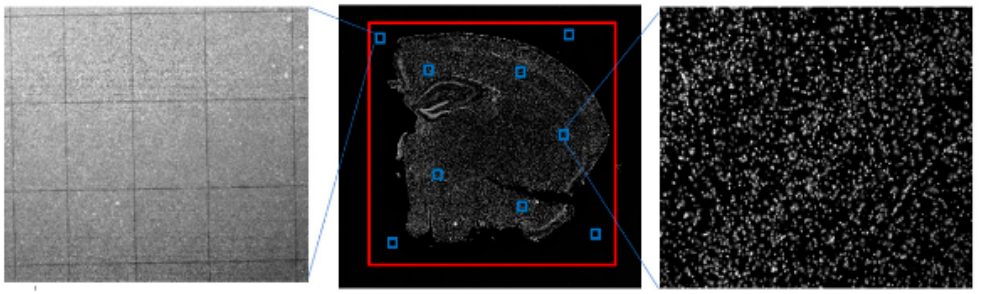
b. Turn on the fluorescence microscope and set the epi-fluorescence channel to FITC.

c. Place 1-2 μL of water on the imaging platform first, then transfer and place the Stereo-seq Chip Slide onto the water drop. Water surface tension will grab onto the Slide and adhere it to the imaging platform.

d. Remove the light shield, switch to 4X objective lens, select the FITC channel and select the chip area of interest. Adjust Brightness and Gain. The specific parameters will vary among different microscopes, as long as the tissue can be imaged clearly. However, the light intensity should be kept in the lower range to prevent fluorescence quenching.

e. Box-select the chip area to fully enclose the chip boundaries (should be slightly larger than the chip), and scan the map under the 4X objective lens (if the microscope does not have map scanning function, skip to the next step).

f. Shift the focus to a blank area on the chip, finely adjust the focus until the tracklines on the chip can be clearly visualized, and then manually select and establish the model focal points. It is recommended that you first establish 3-5 model points in the blank areas of the chip (four corners of the chip).



g. After establishing the model focal points in blank areas, focus on the tissue area to establish the model focal point. It is recommended that you establish 3-5 model points in different tissue areas. When the model focal points have been established, perform a full scan using a 10X lens.

h. Save the original tile (FOV) images, files, and stitched images.

i. Open the ImageMap software and the Image Quality Control functional module within the software. Upload your ssDNA-stained image and run Image QC according to the instructions in the StereoMap User Manual within the software.

The captured nuclei-stained image needs to pass Image QC before further image analysis (image "register") can be performed in Stereo-seq Analysis Workflow (SAW) pipelines.

If Image QC fails, continue with the experiment procedures and perform optimal image analysis later under the guidance of your local Field Application Scientist.

Stop Point:

After fluorescent imaging, the Stereo-seq Chip Slides can be kept at room temperature for 4 hr in a mounting state.

Decrosslinking for ssDNA-stained tissue

a. Equilibrate FFPE Decrosslinking Reagent to room temperature in advance.

b. After fluorescent imaging, immerse the coverslip-mounted Stereo-seq Chip Slide in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube) filled with at least 30mL of 0.1X SSC, allow the coverslip to naturally detach from the tissue-mounted chip, and gently rinse the Stereo-seq Chip Slide up and down to clean the chip surface.

c. Take out the Stereo-seq Chip Slide and wipe off the excess solution from around and the back of the slide with dust-free paper without touching the chip. Make sure there is no liquid residue around the chip. The chip does not need to be air-dried.

d. Assemble the Cassette and Gasket then place the Stereo-seq Chip Slide in the Cassette according to the guide written in **Appendix I: Stereo-seq Slide Cassette Assembly**. It is recommended that you practice with a regular blank glass slide.

e. Grip along the Stereo-seq Cassette to make sure the Stereo-seq Chip Slide has been locked in place.

Make sure not to touch the front-side of the chip while assembling the Stereo- seq Slide Cassette.

f. Place the PCR Adaptor in the PCR thermal cycler and set the program as follows:

Table 3-4 Decrossinglinking incubation program for ssDNA-stained tissue

Program selection: Incubate

Temperature	Time
(Heated lid) 85°C	on
30°C	∞
95°C	30 min
4°C	∞

g. Add **400 µL** FFPE Decrosslinking Reagent into the well of the Stereo-seq Slide Cassette. Apply sealing tape to the Stereo-seq Slide Cassette and make sure it is sealed tightly.

h. Place the Stereo-seq Slide Cassette on the PCR Adaptor in the PCR thermal cycler. Select **Edit**, and then select **Next Step** to skip the 30°C (Time = ∞) step.

i. Incubate at 95°C for **30min**. Meanwhile, prepare about 30 mL of methanol in a staining jar (alternatively, use a slide container or a 50 mL centrifuge tube), close the lid,and pre-cool it for **5-30 min** at -20°C.

j. When decrosslinking is completed, carefully transfer the Stereo-seq Slide Cassette to the nearest bench and peel off the sealing tape. Discard the FFPE Decrosslinking Reagent with a pipette.

k. Remove the slide from the Stereo-seq Slide Cassette according to instructions in **Appendix I: Stereo-seq Slide Cassette Assembly**, discard the cassette and gasket, then immediately proceed to the fixation step (**Section 3.5**).

The cassette and gasket may deform after heating to 95°C. DO NOT reuse the cassette and gasket. Discard them after this step. Use a new cassette and a new gasket in the subsequent steps.

3.5. Fixation

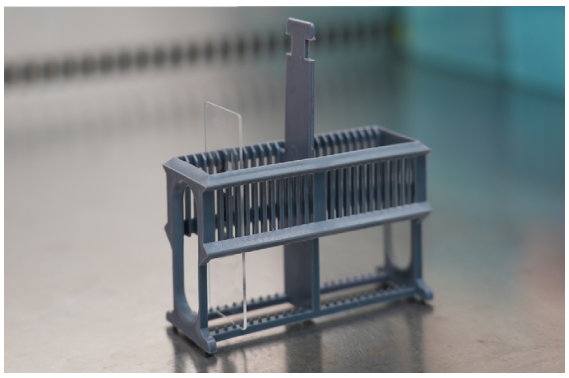
Reagent Required for Section 3.5	Purpose	Preparation
Methanol	Fixation	Pre-cooled for 5-30 min at -20°C.

a. Equilibrate the Stereo-seq Chip Slide to room temperature for **1 min**, then immerse the tissue-mounted Stereo-seq Chip Slide in the pre-cooled methanol for a **20 min** fixation at -20°C. Make sure that the entire tissue section is completely submerged.

b. After fixation is completed, move the container to a sterile fume hood.

c. Take out the Stereo-seq Chip Slide and wipe off excess methanol from around and the back of the slide with dust-free paper without touching the chips. Make sure there is no methanol residue between chips.

d. Place the Stereo-seq Chip Slide on a slide staining rack and leave it in the fume hood for **4-6 min** to let the methanol fully evaporate.



e. When the methanol is fully evaporated, transfer the Stereo-seq Chip Slide onto a flat and clean bench.

f. Assemble the Stereo-seq Slide Cassette with a new cassette and gasket.

g. Grip along the Stereo-seq Cassette to make sure the Stereo-seq Chip Slide has been locked in place.

Do not touch the front of the chip while assembling the Stereo-seq Slide Cassette.

3.6. Tissue Permeabilization

Reagent Required for Section 3.6	Purpose	Preparation
0.01N HCl	Permeabilization	Prepare at least 2 mL of 0.01N HCl per sample. Configure HCl to 0.01N. Measure and make sure the pH = 2.
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 Permeabilization Reagent Stock Solution	Permeabilization	Add 1 mL of freshly prepared 0.01N HCl to dissolve PR Enzyme (red cap, in powder form), and thoroughly mix the reagent by pipetting.
Do not vortex the permeabilization enzyme. Mix by pipette before using. **Aliquot this 10X stock solution to avoid freeze-thaw cycles.**		
1X Permeabilization Reagent Solution	Permeabilization	Make 1X PR solution (200 µL / chip) by diluting 10X PR stock solution with 0.01N HCl.
0.1X SSC	Dilution	Add 10 µL 20X SSC into 1990 µL nuclease-free water and mix well.
0.1X SSC (with 5% RI)	Washing	Add 10 µL RI into 190 µL 0.1X SSC and mix well.

a. Set aside the 2 mL of 0.01N HCl and 1X Permeabilization Reagent Solution you prepared in 3.1 Experiment Preparation.

b. Make sure your PCR thermal cycler has been switched on and set to 37°C with the heated lid set to 42°C. Place the PCR Adaptor in the PCR thermal cycler and set the program as follows:

Table 3-5 Tissue permeabilization incubation program

Temperature	Time
(Heated lid) 42°C	on
37°C	∞
37°C	30 min
4°C	∞

c. Add **200 µL** of 1X Permeabilization Reagent Solution onto the chip by first pipetting one droplet at each corner of the chip and then adding the rest of the solution to the middle to merge all the droplets. Apply sealing tape to the Stereo-seq Slide Cassette and make sure it is sealed tightly.

Make sure the chip is completely covered with 1X Permeabilization Reagent Solution.

d. Thaw FFPE RT Oligo and FFPE Dimer on ice.

e. Place the Stereo-seq Slide Cassette in the 37°C PCR thermal cycler and let the chip incubate inside the PCR thermal cycler at 37°C for **30 min**.

f. While waiting for permeabilization to be completed, prepare FFPE MIX Solution according to Table 3-6 and leave it on ice until use. **【PREPARE AHEAD】**

Table 3-6 FFPE MIX Solution

Components	1X (μL)	2X + 10% (μL)	3X + 10% (μL)	4X + 10% (μL)
FFPE RT Buffer Mix	158	331.8	489.8	647.8
FFPE RT Enzyme Mix	30	63	93	123
FFPE RT Oligo	10	21	31	41
FFPE Dimer	2	4.2	6.2	8.2
Total	200	440	620	820

g. When incubation is completed, remove the Stereo-seq Slide Cassette from the PCR Adaptor (37°C).

h. Remove 1X Permeabilization Reagent Solution with a pipette from the corner of each well without touching the chip surface.

i. Add **200 μL** of 0.1X SSC (with 5% RI) per chip from the corner of each well.

j. Remove 0.1X SSC (with 5% RI) with a pipette from the corner of each well without touching the chip surface.

3.7. FFPE MIX Reaction

a. Place the PCR Adaptor in the PCR thermal cycler in advance. Set the temperature to 42°C and the heated lid to 45°C according to the following program:

Program selection: Incubate

Table 3-7 FFPE MIX hybridization incubation program

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

b. Gently add **200 µL** of FFPE MIX Solution per chip along the side of each well, ensuring that the well surface is uniformly covered with FFPE MIX Solution. Apply sealing tape to the Stereo-seq Slide Cassette and make sure it is sealed tightly.

c. Place the Stereo-seq Slide Cassette in the 42°C PCR thermal cycler and let the chip incubate inside the PCR thermal cycler at 42°C for **5 hr** or longer (no longer than 24 hr).

3.8. cDNA Release and Collection

Prepare		
Reagent	**Preparation Steps**	**Maintenance**
cDNA Release Buffer	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.

a. Remove the Stereo-seq Slide Cassette from the PCR Adaptor, discard FFPE MIX Solution, then wash one time with **200 µL** 0.1X SSC per chip.

b. Prepare the cDNA Release Mix according to Table 3-8 and maintain the mix at room temperature.

Table 3-8 cDNA Release Mix

Components	1X (µL)	2X + 10% (µL)	3X + 10% (µL)	4X + 10% (µL)
cDNA Release Buffer	380	798	1178	1558
cDNA Release Enzyme	20	42	62	82
Total	400	840	1240	1640

c. Place the PCR Adaptor in the PCR thermal cycler in advance. Set the temperature to 55°C and the heated lid to 60°C according to the following program:

Table 3-9 cDNA Release Incubation Program

Temperature	Time
(Heated lid) 60°C	on
55°C	5 hr - 24 hr
55°C	∞

d. Add **400 µL** of cDNA Release Mix per chip into each well of the Stereo-seq Slide Cassette.

e. Apply sealing tape to the Stereo-seq Slide Cassette and make sure it is sealed tightly. Incubate the Stereo-seq Slide Cassette at 55°C for **5 hr** or longer (no longer than 24 hr).

Stop Point:

cDNA collection step may be left overnight. If it is left overnight, make sure the Stereo-seq Slide Cassette is sealed tightly with the sealing tape.

f. Before collecting the eluted cDNA, pre-heat 500 µL of nuclease-free water per chip at 55°C for **≥ 30 min**.

g. After the reaction, completely collect the cDNA Release Mix from each well into a new 2.0 mL centrifuge tube.

h. Add **350 µL** of pre-heated nuclease-free water per chip into each well. Pipette up and down to wash the chip surface thoroughly and then collect it into the same 2.0 mL centrifuge tube with the cDNA Release Mix.

Collect as much volume as possible to retrieve enough cDNA from the chip. cDNA Release Mix should be about 400 µL after incubation (the volume might be less than 400 µL). You must combine the collected cDNA Release Mix with the 350 µL nuclease-free water before proceeding to the next step.

The Stereo-seq Chip Slide may be discarded. Ensure that all of the chip ID numbers have been recorded as required for downstream analysis.

3.9. cDNA Purification and Amplification

Background Information

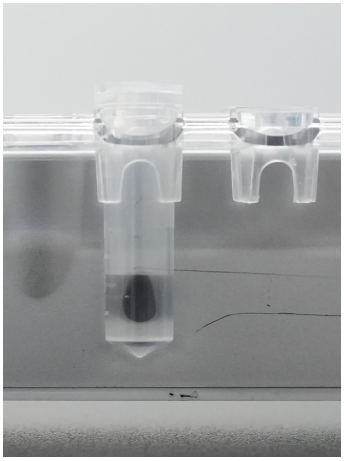
For bead-based purification, we recommend using DNA Cleanup Beads AMPure® XP(Agencourt, Cat. No. A63882), SPRIselect (Beckman Coulter, B23317/B23318/B23319) or VAHTS™ DNA Clean Beads (Vazyme, Cat. No. N411-02). *If magnetic beads from other sources are used, please optimize the cleanup conditions before getting started.*

Before Use

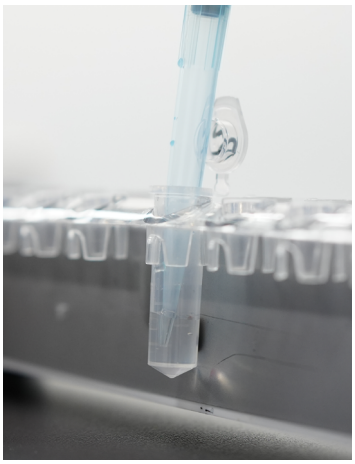
- To ensure the DNA capture efficiency of the magnetic beads, equilibrate the beads to room temperature **30 min before use**.
- Vortex or pipette up and down to ensure that the beads are thoroughly mixed every time before use.
- The number of magnetic beads directly affects the distribution of purified DNA fragments.

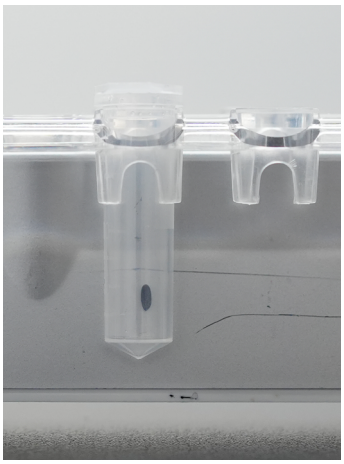
Operation Notes

1. In the magnetic separation step, allow the solution to become completely clear before removing the supernatant. This process usually takes approximately **2-3 min**, but can be longer or shorter depending on the type of magnetic separation rack being used.

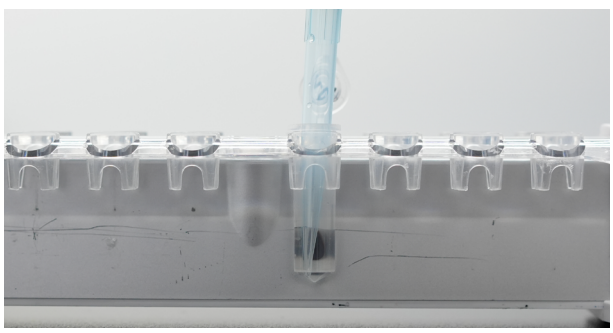


2. When collecting the supernatant with a pipette after magnetic separation, avoid taking up the beads. Instead of collecting the entire supernatant fraction, leave 2-3 μL in the tube to avoid the pipette from direct contacting the beads. If the beads are mistakenly taken up, dispense everything and redo the magnetic separation.

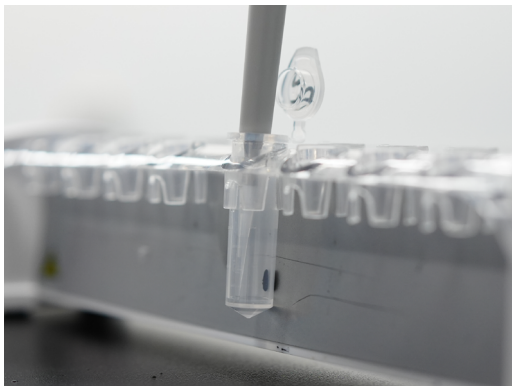




3. Use freshly prepared 80% ethanol (at room temperature) to wash the beads. Keep the sample tube on the magnetic separation rack during the washing step. Do not shake or disturb the beads.

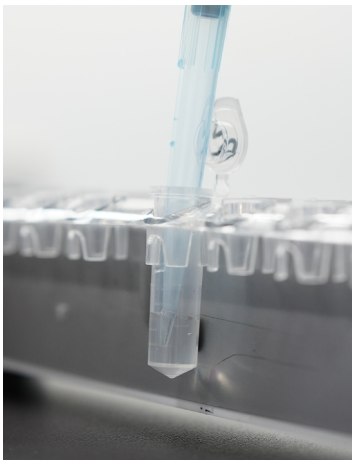


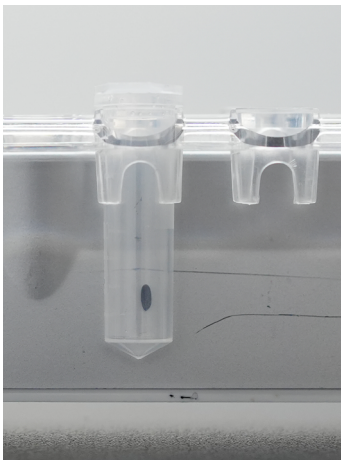
4. After the second washing of beads with ethanol, try to remove all of the liquid within the tube. You may centrifuge briefly to accumulate any remaining liquid at the bottom of the tube, then separate the beads magnetically, and remove the remaining liquid by using a small-volume pipette.



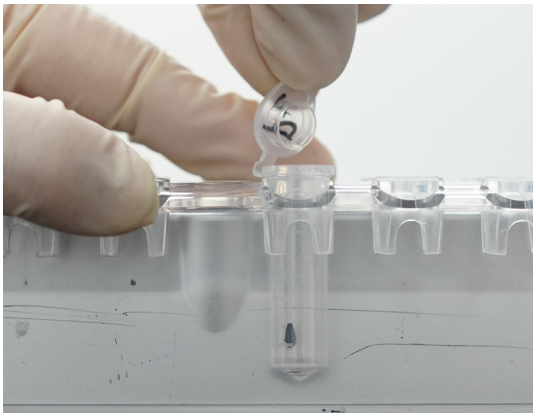
5. After washing twice with ethanol, air-dry the beads at room temperature. Drying usually takes approximately **5-10 min**, depending on the lab temperature and humidity level. Watch closely until the pellet appears sufficiently dry with a matte appearance, then continue to the elution step with TE Buffer.

6. During the elution step, do not touch the beads with the pipette tip when removing the supernatant. Contamination of a DNA sample with beads may affect subsequent purification steps. Therefore, to prevent the pipette tip from directly contacting the beads, always collect the eluate in 2 μL less than the initial volume of TE Buffer used for the elution.





7. **Pay attention when opening/closing the lid of a sample tube on a separation rack. Strong vibrations may cause samples or beads to spill from the tubes. Hold the body of the tube while opening the lid.**



a. If white precipitates are visible in the collected cDNA, dissolve them by heating at 55°C and equilibrate to room temperature before proceeding to the purification step. Equilibrate the magnetic beads to room temperature for at least **30 min**.

b. cDNA Purification Procedures with 1X Magnetic Bead

1) Mix the collected cDNA (about **750 µL**) with the beads in a ratio of 1 : 1. Vortex the mix then incubate it at room temperature for **10 min**.

2) Spin down and place the tube onto a magnetic separation rack for **3 min** until the liquid becomes clear.

3) Carefully remove and discard the supernatant with a pipette (if foam is visible on the cap, discard it with a pipette).

4) Keep the tube on the magnetic separation rack and add **1.5 mL** of freshly prepared 80% ethanol. Wash the beads by rotating the tube on the magnetic rack. Incubate for **30 sec** and carefully remove and discard the supernatant.

Always place the pipette tips on the tube wall and away from the magnetic beads. Do not disturb the beads while transferring the supernatant (if foam is visible on the cap, clean the cap with 80% ethanol).

5) Repeat **step 4**.

6) Keep the tube on the magnetic rack and open the lid to air-dry the beads at room temperature until no wetness (reflectiveness) or crack is visible. Drying times will vary but will take approximately **5-15 min**.

7) Add **44 µL** of nuclease-free water to the dried beads. Mix the beads and nuclease-free water by vortexing. Incubate at room temperature for **5 min**. Spin down briefly and place the sample tube onto a magnetic separation rack for **3-5 min** until the liquid becomes clear.

8) Transfer the supernatant (**~ 42 µL** cDNA) into a new 0.2 mL PCR tube.

c. If the volume of collected eluted cDNA is less than 42 μL, top it up with nuclease-free water.

Store the beads with 40 μL of nuclease-free water at 4°C after collecting the eluted cDNA untill your cDNA final product has passed QC.

d. Prepare PCR Mix according to Table 3-10. The total volume for the PCR reaction is 100 μL.

Table 3-10 PCR Mix

Components	1X (μL)	2X + 10% (μL)	3X + 10% (μL)	4X + 10% (μL)
cDNA Amplification Mix	50	105	155	205
FFPE cDNA Primers Mix	8	16.8	24.8	32.8
Eluted cDNA	42	2 x 42	3 x 42	4 x 42
Total (up to)	**100**	**2 x 100**	**3 x 100**	**4 x 100**

e. Mix gently and short spin before placing the reaction tube in a PCR thermal cycler. Amplify the eluted cDNA based on the PCR program shown in Table 3-11.

Table 3-11 PCR program for amplification (for 100 μL)

Temperature	Time	Cycle
(Heated lid) 105°C	on	-
95°C	5 min	1
98°C	20 sec	15
58°C	20 sec	
72°C	3 min	
72°C	5 min	1
12°C	Hold	-

f. Prepare Qubit dsDNA Mix and record the concentration of PCR product according to Table 3-12.

Table 3-12 Qubit dsDNA Mix

Components	1X (μL)
Invitrogen™ Qubit dsDNA HS Buffer	198
Qubit dsDNA HS Reagent 200X	1
PCR Product	1
Total	**200**

g. Vortex the mix, then take 1 μL of the PCR product and measure its concentration using Qubit dsDNA HS Kit. The DNA concentration is usually greater than 10 ng/μL.

For troubleshooting purposes, we recommend leaving about 2 μL of the PCR product in a PCR tube.

h. Use magnetic beads to purify the PCR product in a volume ratio of 1 : 1 (DNA : beads).

1) Mix the PCR product (100 μL) with the beads in a ratio of 1 : 1. Vortex the mix then incubate it at room temperature for **10 min**.

2) Spin down and place the tube onto a magnetic separation rack for **3 min** until the liquid becomes clear.

3) Carefully remove and discard the supernatant with a pipette (if foam is visible on the cap, discard it with a pipette).

4) Keep the tube on the magnetic separation rack and add **200 μL** of freshly prepared 80% ethanol. Wash the beads by rotating the tube on the magnetic rack. Incubate for **30 sec** and carefully remove and discard the supernatant.

Always place the pipette tips on the tube wall and away from the magnetic beads. Do not disturb the beads while transferring the supernatant (if foam is visible on the cap, clean the cap with 80% ethanol).

5) Repeat **step 4**.

6) Keep the tube on the magnetic rack, and open the lid to air-dry the beads at room temperature until no wetness (reflectiveness) or cracks are visible. Drying times will vary but will take approximately **5-8 min**.

6) Add **42 μL** of TE Buffer (pH 8.0) to the dried beads. Mix the beads and TE Buffer by vortexing. Incubate at room temperature for **5 min**. Spin down briefly and place the sample tube onto a magnetic separation rack for **3-5 min** until the liquid becomes clear. Transfer the supernatant (**~ 40 μL**) into a new 1.5mL PCR tube.

Stop Point:

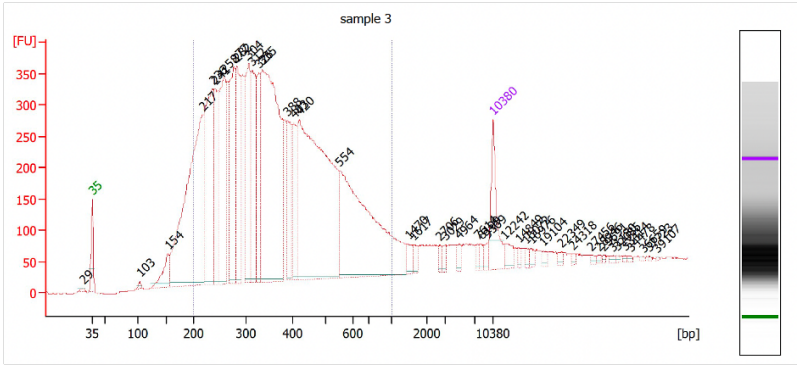
The purified cDNA sample can be stored at −20°C for up to 1 month.

For troubleshooting purposes, we recommend storing the beads with 40 μL of nuclease-free water at 4°C after purification until your cDNA final product has passed QC.

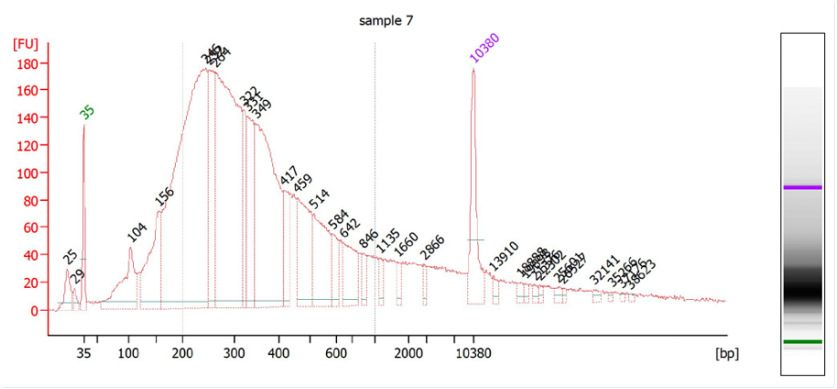
i. Take 1 μL of the cDNA sample and measure and record the concentration of the purified cDNA with Qubit dsDNA HS Kit.

j. Analyze the sample (dilution might be required) on an Agilent Bioanalyzer High Sensitivity chip or other library quality control platform such as TapeStation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer) and Fragment Analyzer™ (Advanced Analytical).

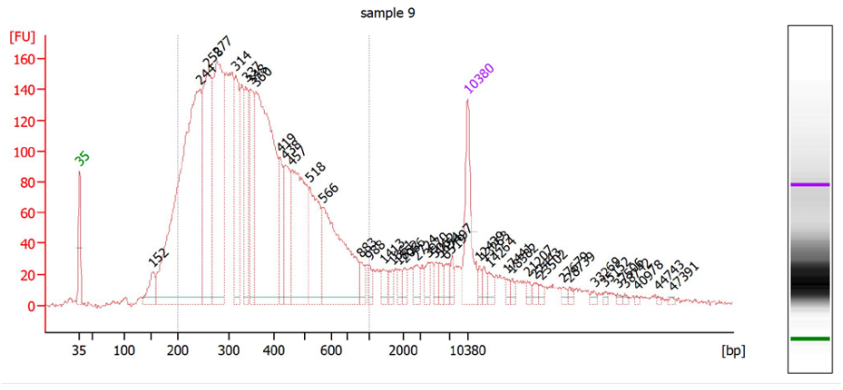
A qualified cDNA sample should have a main fragment distribution peak at about 150- 350 bp (Figure 1), and a yield that is higher than 300 ng.



DV200 30%~50%



DV200 50%~70%



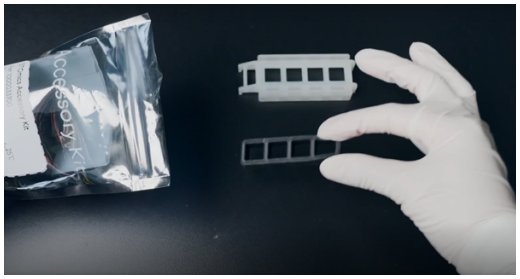
DV200 >70%

Figure 1. Representative Agilent Bioanalyzer 2100 analysis results of PCR amplified cDNA samples with different DV200 scores

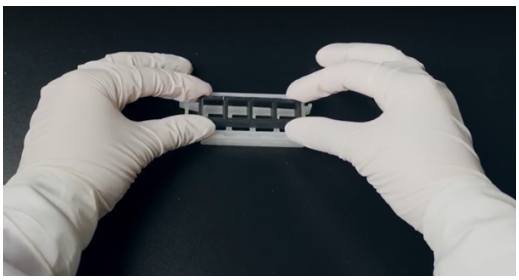
Appendix I: Stereo-seq Slide Cassette Assembly

For a demonstration video of Stereo-seq Slide Cassette assembling and removal, please refer to the link or by scanning the QR code:
https://en.stomics.tech/resources/videos/list.html

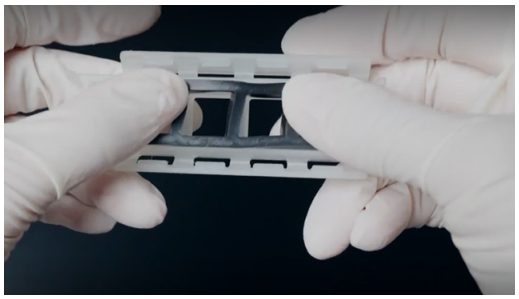
a. Take the Stereo-seq Slide Cassette and gasket out of the STOmics Stereo-seq Accessory Kit.



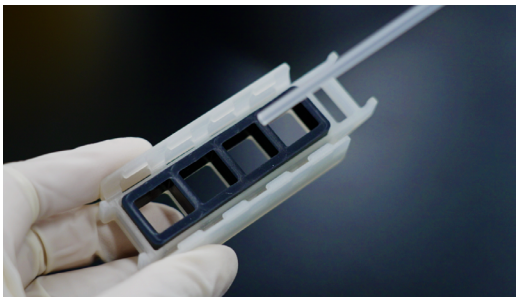
b. Pick up the Stereo-seq Slide Cassette and flip it over. Insert the gasket into the Stereo-seq Slide Cassette, ensuring that the cutouts are aligned.



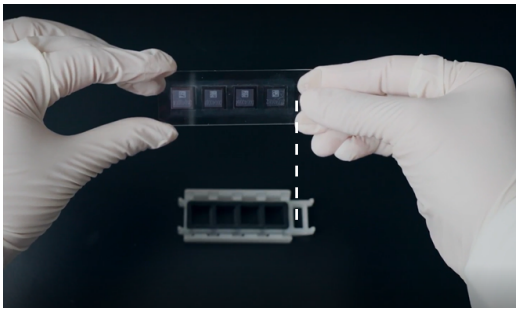
c. Press down on the gasket.



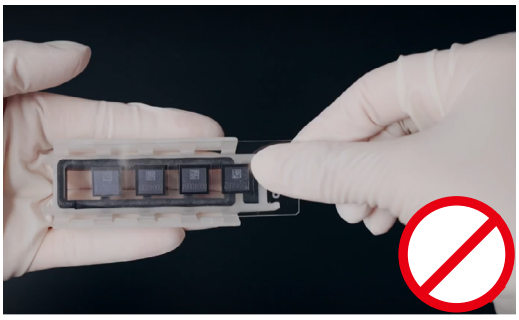
d. If necessary, use a power dust remover to blow any debris off the gasket.



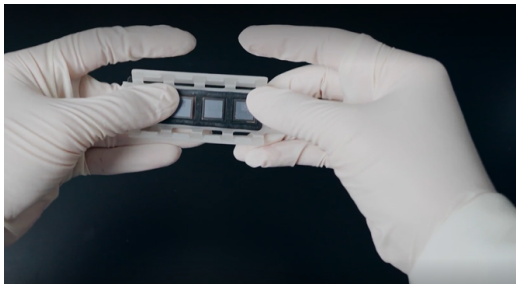
e. Pick up the Stereo-seq Chip Slide and flip it over with the chip surface facing down. Align the engraved label with the long edge of the Stereo-seq Slide Cassette.



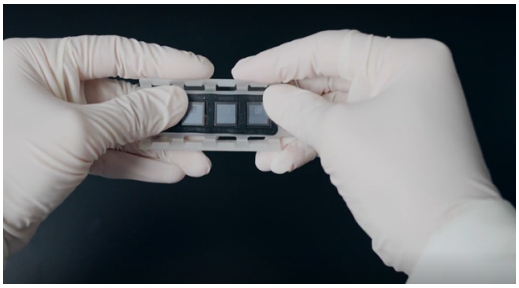
f. Make sure the chips are aligned within the empty space of the gasket and avoid touching the chip surface with the gasket or cassette during slide placement. Insert the Stereo-seq Chip Slide under the bottom 4 tabs.



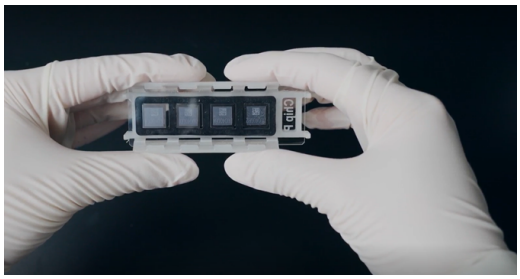
g. Support the back of the cassette with both middle fingers. Place your left thumb between tab 1 and tab 2 and your right thumb between tab 3 and tab 4.



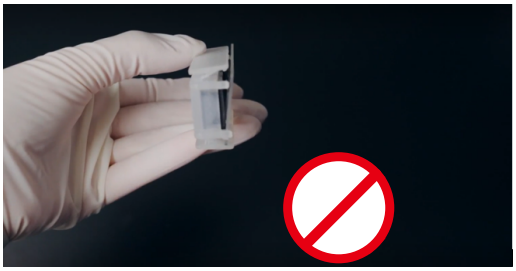
h. Press down evenly on the upper side (A side) of the slide (near the edge) and then simultaneously press down the top edge firmly with both index fingers to clip the slide in place until you hear a clicking sound.



i. Press along both edges of the Stereo-seq Slide Cassette to ensure that the Stereo-seq Chip Slide is locked in place.

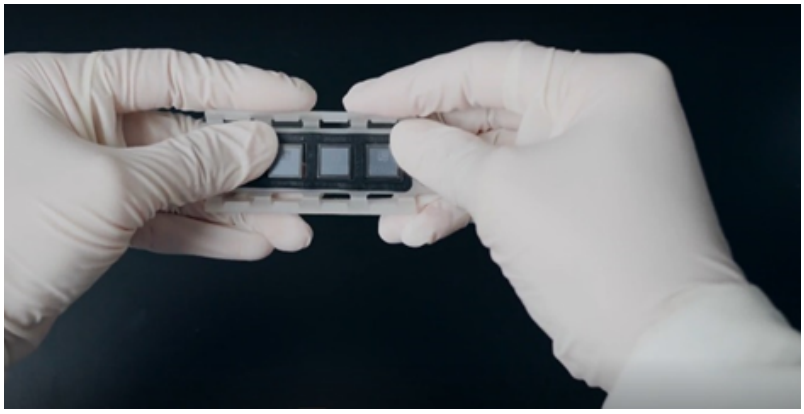


j. Recheck the Stereo-seq Slide Cassette and verify that the slide is clipped in place.



Stereo-seq Slide Cassette Removal

a. To release the slide from the tabs, first flip the cassette over, and then as you gently support the back of the Stereo-seq Chip Slide with both thumbs to prevent the Stereo-seq Chip Slide from falling, firmly press the upper side down.



b. Lift the Stereo-seq Chip Slide from the side showing the engraved label.