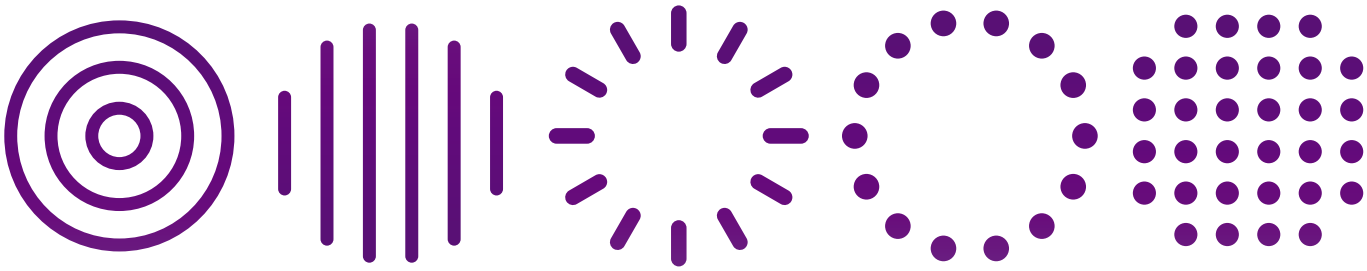
STOmics

# Stereo-seq TRANSCRIPTOMICS SET FOR CHIP-ON-A-SLIDE (0.5cm * 0.5cm)

USER MANUAL



Cat. No.: 211ST004 (4 RXNs)
Kit Version: V1.2.1
Manual Version: A

# REVISION HISTORY

**Manual Version:** A
**Kit Version:** V1.2.1
**Date:** Aug. 2023
**Description**:

- Initial release

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

# WORKFLOW


**TOTAL TIME:** ~1.5 ~2 DAYS

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**NOTE:** Additional operation tips and guidance.

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


**QUALITY CHECK POINT**

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

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

# CHAPTER 1
# INTRODUCTION

## 1.1. Intended Use

STOmics Stereo-seq Transcriptomics Set for Chip-on-a-slide is intended for generating a spatially-resolved 3' mRNA library from biological tissue sections. Built upon DNA Nanoball (DNB) technology, STOmics Stereo-seq Transcriptomics Set for Chip-on-a-slide enables a "tissue-to-data" solution through *in situ* capture of the whole transcriptome, at nanoscale resolution and centimeter-sized field of view. This kit utilizes DNB patterned array chips loaded with spatially-barcoded probes that capture and prime poly-adenylated mRNA from tissue sections *in situ*. Each cDNA synthesized from mRNA 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 within this kit have passed stringent quality control and functional verification, ensuring performance stability and reproducibility.

## 1.2. Sequencing Guideline

Sequencing libraries produced via the Stereo-seq Transcriptomics Set for Chip-on-a-slide requires the DNBSEQ sequencing platform. For details, please refer to **Chapter 5: Library Construct and Sequencing** of this manual.

## 1.3. List of Kit Components

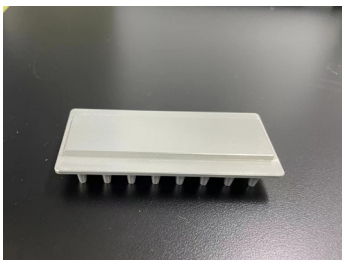
Each Stereo-seq Transcriptomics Set for Chip-on-a-slide (0.5cm * 0.5cm) consists of:

- Stereo-seq Transcriptomics Kit *1 (4 RXN)
- Stereo-seq Chip T Slide (0.5cm * 0.5cm) *1 (4 EA)
- STOmics Stereo-seq Accessory Kit *2 (5 PCs)

The Stereo-seq Library Preparation Kit is not included in Stereo-seq Transcriptomics Set for Chip-on-a-slide kit and needs to be purchased separately. If you wish to construct Stereo-seq Libraries in-house, please refer to **Chapter 4: Library Preparation** for more detail.


Compatible auxiliary but not included:

- (Optional) Stereo-seq PCR Adaptor *1 (2EA)

Further information on catalog numbers, kit components and specifications are listed below (next page).

**Upon receiving the Stereo-seq Chip T Slide (0.5cm * 0.5cm), please follow the instructions in Stereo-seq Chip P Slide  Stereo-seq Chip T Slide Operation Guide For Receiving, Handling And Storing to properly store unused Stereo-seq Chip T 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 Transciptomics T Kit Cat. No.:111KT114 | | | |
|---|---|---|---|
| **Component** | **Reagent Cat. No.** | **Cap Color** | **Quantity (tube)** |
| RI | 1000028499 | | 300 μL × 1 |
| PR Enzyme | 1000028500 | | 10 mg × 1 |
| PR Rinse Buffer | 1000042897 | | 880 μL × 1 |
| Glycerol | 1000031615 | | 50 μL × 1 |
| RT Reagent | 1000042898 | (transparent) | 720 μL × 1 |
| RT Oligo | 1000028508 | (transparent) | 1 OD × 1 |
| RT Additive | 1000028502 | (transparent) | 44 μL × 1 |
| ReverseT Enzyme | 1000042899 | (transparent) | 44 μL × 1 |
| TR Buffer | 1000028505 | | 1725 μL × 2 |
| cDNA Release Enzyme | 1000028511 | | 88 μL × 1 |
| cDNA Release Buffer | 1000028512 | | 1725 μL × 2 |
| cDNA Primer | 1000028513 | | 36 μL × 1 |
| cDNA Amplification Mix | 1000028514 | | 220 μL × 1 |
| Storage Temperature: -25°C~-18°C | Transported by cold chain | Expiration Date: refer to label | |

Table 1-2

| Stereo-seq Chip T Slide (0.5cm * 0.5cm) Cat. No.: 210CT004 | | | |
|---|---|---|---|
| **Component** | **Reagent Cat. No.** | **Cap Color** | **Quantity (per kit)** |
| Stereo-seq Chip T (0.5cm * 0.5cm) | - | - | 4 EA |
| Storage Temperature: -25°C~-18°C | Transported by cold chain | | Expiration Date: refer to label |

Table 1-3

| STOmics Accessory Kit Cat. No.: 1000033700 | | | |
|---|---|---|---|
| **Component** | **Reagent Cat. No.** | **Cap Color** | **Quantity (per kit)** |
| Cassette | 1000033699 | - | 1 EA |
| Gasket | 1000033698 | - | 4 EA |
| Sealing Tape | 1000042970 | - | 6 EA |
| Storage Temperature: Room Temperature | Transported at room temperature | | Expiration Date: refer to label |

Table 1-4

| Stereo-seq PCR Adaptor Cat. No.: 301AUX001 | | | |
|---|---|---|---|
| **Component** | **Reagent Cat. No.** | **Cap Color** | **Quantity (per kit)** |
| Stereo-seq PCR Adaptor | - | - | 2 EA |
| Storage Temperature: Room Temperature | Transported at room temperature | | Expiration Date: refer to label |

## 1.4. Additional Equipment and Materials

TheTable below lists equipment and materials needed for this protocol. Some common laboratory equipments not named in Table 1-5 are expected to be accessible by the user, for instance, an ice maker, biological safety cabinet, freezers, etc. For specific microscope requirements, please refer to **STOmics Microscope Assessment Guideline.**

Table 1-5

| Equipment | | |
|---|---|---|
| **Brand** | **Description** | **Catalog Number** |
| - | Cryostat | - |
| - | Benchtop centrifuge | - |
| - | Pipettes | - |
| - | pH meter | |
| - | Metal heating block dry bath (optional) | - |
| - | Vortex mixer | - |
| **Bio-Rad*** | T100 Thermocycler | 1861096 |
| **ABI*** | ProFlex 3 x 32-well PCR System | 4483636 |
| Labnet | Slide Spinner (optional) | C1303-T |
| NEBNext® | Magnetic Separation Rack for <200 µL tubes | S1515S |
| Thermo Fisher Scientific | Magnetic rack DynaMag™-2 for 1.5-2mL tubes | 12321D |
| | Qubit™3 fluorometer | Q33216 |
| Agilent Technologies™ | Agilent 2100 bioanalyzer | G2939AA (or similar) |

**Choose either one of the listed brands (with * mark). Suitable PCR adaptor will be needed.**

| Reagents | | |
|---|---|---|
| **Brand** | **Description** | **Catalog Number** |
| - | 100% Ethanol (Analytical grade) | - |
| Ambion | Nuclease-free water | AM9937 |
| | 20X SSC | AM9770 |
| | 1X TE buffer, pH 8.0 | AM9858 |
| *Agencourt | AMPure® XP | A63882 |
| *Beckman Coulter | SPRIselect | B23317/B23318/ B23319 |

| | | |
|---|---|---|
| *VAZYME | VAHTSTM DNA Clean Beads | N411-02 |
| Sigma Aldrich | Hydrochloric acid, HCl(0.1N) | 2104-50ML |
| | Methanol | 34860-1L-R |
| SAKURA | SAKURA Tissue-Tek® O.C.T. Compound | 4583 |
| Invitrogen | Qubit ssDNA Assay Kit | Q10212 |
| | Qubit dsDNA HS Assay Kit | Q32854 |
| Agilent Technologies™ | High sensitivity DNA kit | 5067-4626 |
| | High sensitivity RNA kit | 5067-1513 |

Choose either one of the listed brands (with * mark).

| Consumbales | | |
|---|---|---|
| **Brand** | **Description** | **Catalog Number** |
| - | Stainless-steel base mold | - |
| - | Aluminum foil | - |
| - | Forceps | - |
| - | Slide Staining Rack | |
| - | Microscope glass coverslip (area: 18 mm x 18 mm, thickness: 0.13 - 0.16 mm) | - |
| Corning | Corning® 100 mm TC-treated Culture Dish | 353003 |
| | 50 mL centrifuge tubes | 430829 |
| | 15 mL centrifuge tubes | 430791 |
| Kimtech | Kimwipes™ delicate task wipes | 34155 |
| MATIN | Power dust remover | M-6318 |
| Axygen | 1.5 mL centrifuge tubes | MCT-150-A |
| | 0.2 mL PCR tubes* | PCR-02-C |
| | 96-well PCR plate* | PCR-96M2-HS-C |
| | 1,000 µL filtered tips | TF-1000-L-R-S |

| | | |
|---|---|---|
| Axygen | 200 µL filtered tips | TF-200-L-R-S |
| | 100 µL filtered tips | TF-100-R-S |
| | 10 µL filtered tips | TXLF-10-L-R-S |
| | 0.5 mL thin wall PCR tubes | PCR-05-C |
| Invitrogen | Qubit Assay Tubes | Q32856 |
| BIOSHARP | Metal Block | - |

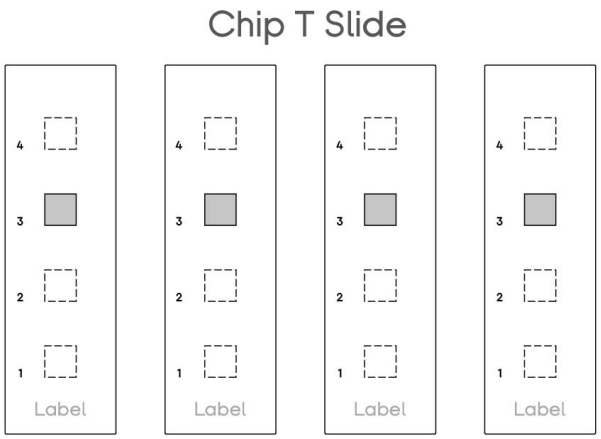
**Choose either one of the listed brands (with * mark).**

## 1.5. Practice Tips

### Stereo-seq Chip T Slide

Includes **4** Stereo-seq Chip T Slides, containing **one** Chip T (0.5cm * 0.5cm) on each slide.

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

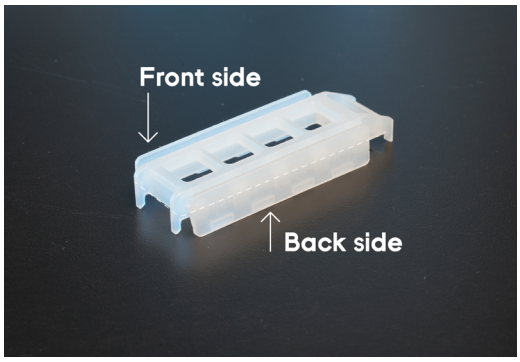


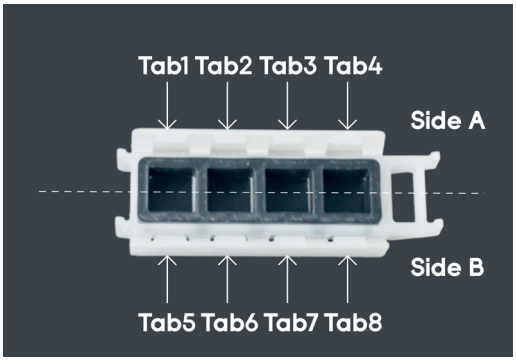

### Stereo-seq Chip T Slide Storage

- Always store unused slides in their original slide container and keep them sealed with a sealable aluminum bag at -25°C ~ 8°C. Keep sealed with tape or another re-sealable bag. **Always KEEP the desiccant within 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.

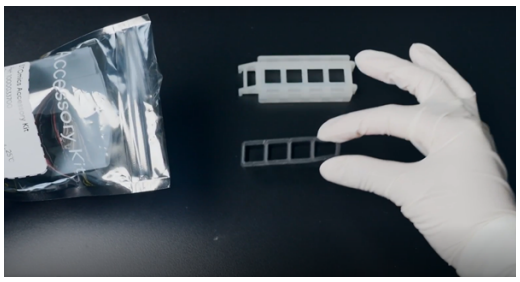


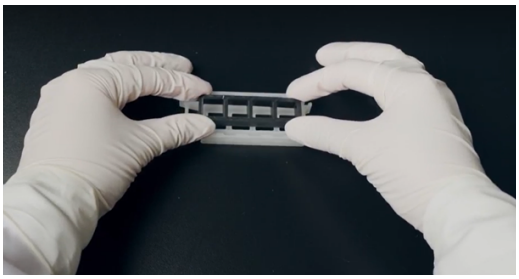

For a demonstration video of Stereo-seq Slide Cassette assembling and removal, please refer to the link or by scanning the QR code:
**https://drive.google.com/drive/folders/1_ty31lBo03rK9ux0xMY8xVQKIlOaulfm?usp=sharing**

## Stereo-seq Slide Cassette Assemble

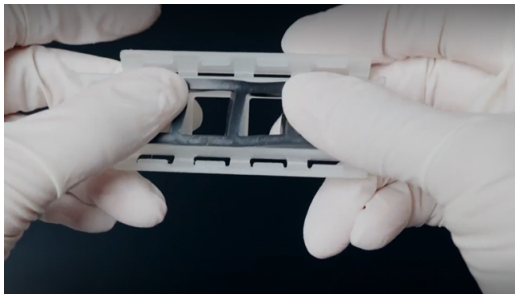
a. Take the Stereo-seq Slide Cassette and gasket out of the STOmics Stereo-seq Accessory Kit.



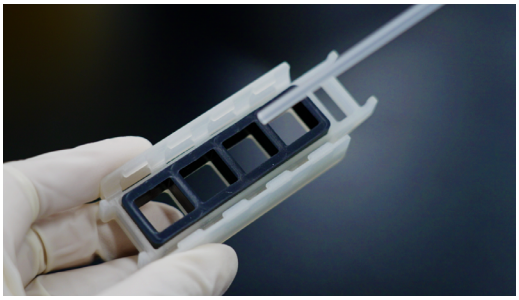
b. Pick up the Stereo-seq Slide Cassette and flip over. Insert the gasket into Stereo-seq Slide Cassette, ensuring the cutouts are aligned.



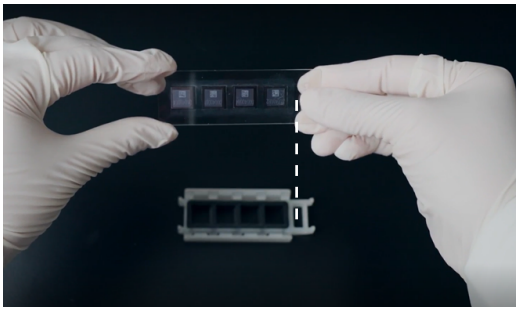
c. Press down the gasket to better fit the cassette.



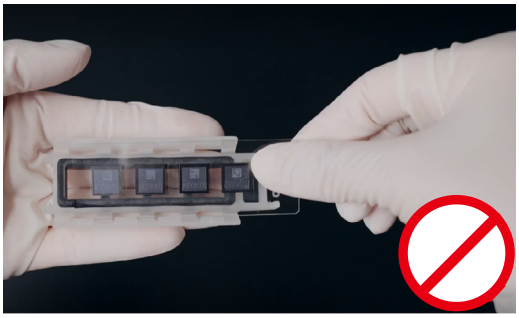
d. Use a power dust remover to blow off any debris on the gasket if necessary.



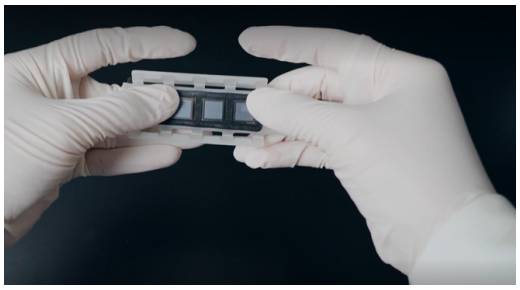
e. Pick up the Stereo-seq Chip Slide and flip over with the chip surface facing down. Align the engraved label with the long edge of the Stereo-seq Slide Cassette.



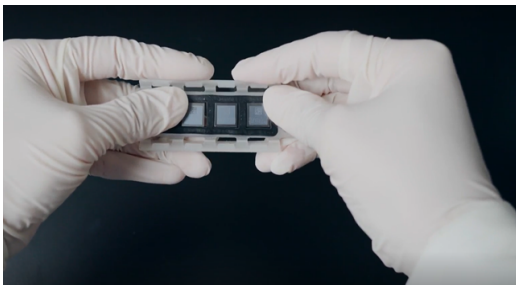
a. Make sure the chips are aligned within the empty space of the gasket and avoid touching the chip surface with the gasket or cassette during slide placement. Insert Stereo-seq Chip Slide under the bottom 4 tabs.



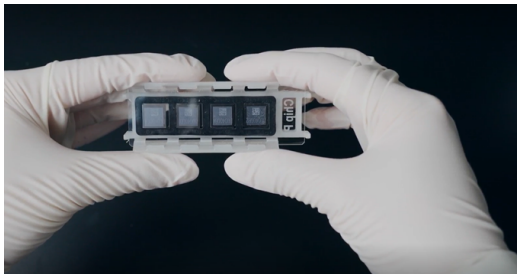
b. Support the back of the cassette with both middle fingers. Place left thumb between tab 1 and tab 2 while right thumb between tab 3 and tab 4.



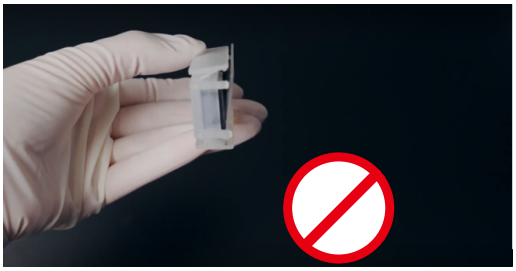
c. Press down evenly on the upper side (A side) of the slide (near the edge) and then simultaneously press down the top edge firmly with both index fingers to clip the slide in place until you hear a clicking sound.



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



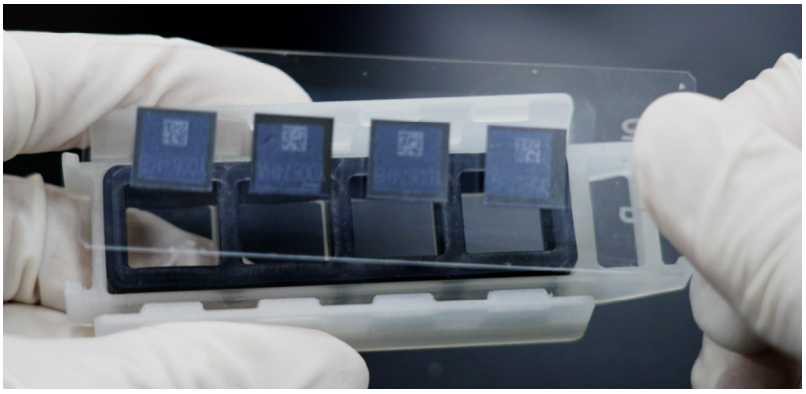
e. Take a final look at the Stereo-seq Slide Cassette to make sure the slide is clipped in place.



### Stereo-seq Slide Cassette Removal

a. Flip the cassette over and firmly press down the upper side to release the slide from the tabs, while gently supporting the back of the Stereo-seq Chip Slide with both thumbs to prevent the Stereo-seq Chip Slide from falling off.

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



**Stereo-seq Slide Cassette removal is not needed for the Stereo-seq Transcriptomics Set for Chip-on-a-slide.**

## 1.6. Precautions and Warnings

- This product is intended for research use only, not for use in diagnostic procedures. Please read all instructions in this manual carefully before using the product.
- Before performing experiments with the kits, users are recommended to ensure that they are familiar with related instruments, and operate them according to manufacturer's instructions.
- Instructions provided in this manual are intended for general use only and 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 further use. For other reagents, thaw them first at room temperature followed by inverting several times to mix them properly, and centrifuge them briefly before placing on ice for further use.
- mRNA capture will be compromised or absent for any scratched areas on the front-side surface of the chip.

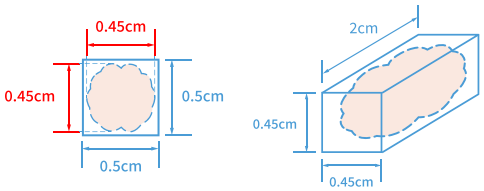
- To prevent cross-contamination, we recommend the use of filtered pipette tips. Use a new tip each time for pipetting different solutions.
- We recommend using thermal cycler with heated lids for PCR reactions. Pre-heat the thermal cycler to reaction temperature before use.
- Improper handling of samples and reagents may contribute to aerosol contamination of PCR products, resulting in data inaccuracy. Therefore, we recommend two distinctly separated working areas in the laboratory for PCR reaction preparation and PCR product cleanup tests. Use designated pipettes and equipments for each area and execute 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 PREPARATION

## 2.1. Sample Requirements for Fresh Frozen Tissue

To avoid RNA degradation, we recommend performing tissue embedding of fresh frozen tissues within 30 min upon harvesting.

The tissue size should not exceed **0.45 cm x 0.45 cm x 2 cm**, as the tissue section should not exceed 80% area coverage of the chip (0.5cm * 0.5cm).




### Sample Types

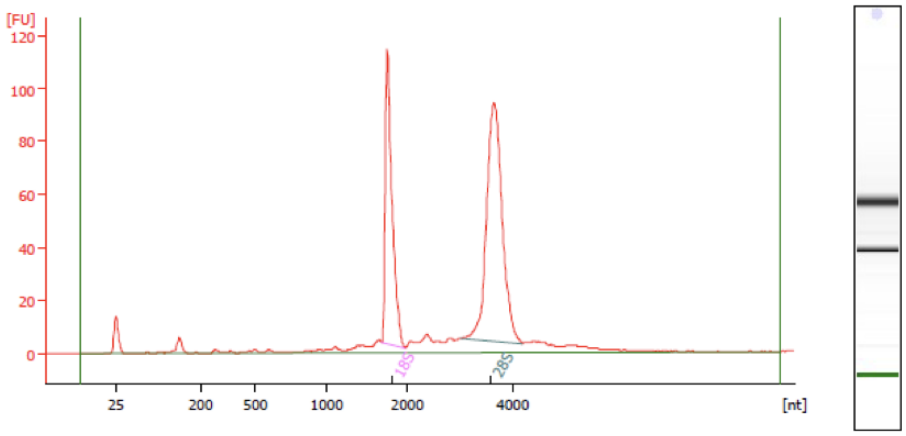
This set of kits can be used for samples from all common animals, including but not limited to human, monkey, and mouse.

For details, please refer to the list: **https://en.stomics.tech/resource/STOmicsTestedTissueList?lang=en#**

### Fresh Frozen Sample RNA Integrity Number (RIN) Value

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

**It is strongly recommended to proceed only with tissue samples with a RIN value ≥7.**




**Overall Results for sample 6 : 8522203007050**

| | | | |
|---|---|---|---|
| RNA Area: | 568.4 | RNA Integrity Number (RIN): | 9.8 (B.02.11, Anomaly Threshold(s) manually adapted) |
| RNA Concentration: | 281 ng/µl | | |
| rRNA Ratio [28s / 18s]: | 1.6 | Result Flagging Color: | |
| | | Result Flagging Label: | RIN: 9.80 |

**Fragment table for sample 6 : 8522203007050**

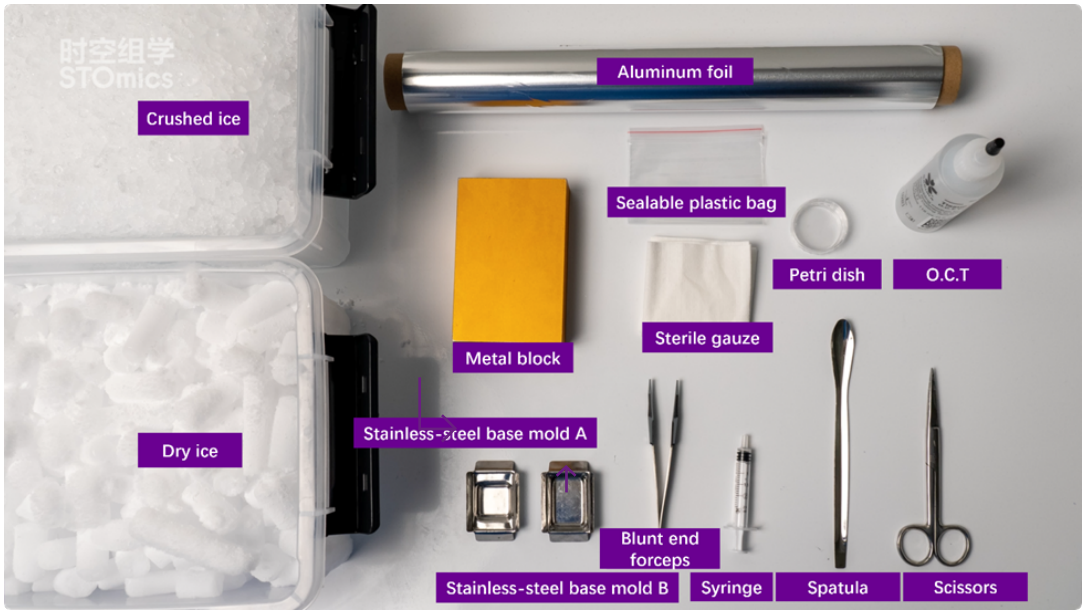
| Name | Start Size [nt] | End Size [nt] | Area | % of total Area |
|---|---|---|---|---|
| 18S | 1,675 | 1,964 | 151.3 | 26.6 |
| 28S | 3,006 | 4,187 | 238.4 | 41.9 |

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

## 2.2. Sample Embedding

For a demonstration video of tissue embedding, please refer to the link or by scanning the QR code: **https://drive.google.com/drive/folders/1O138SbfP8lKkYLaScnPkU3pOvwMf0NTW?usp=sharing**

a. Prepare these apparatuses/materials in advance:




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

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

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

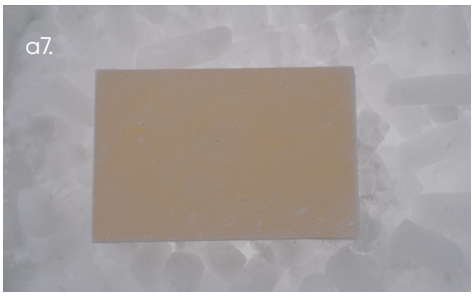
a3. Add a few drops of pre-cooled OCT in the mold A until it reaches approximately 2/3 of the mold and pre-cool on ice for **> 10 min** (remove introduced air bubble using a syringe).


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

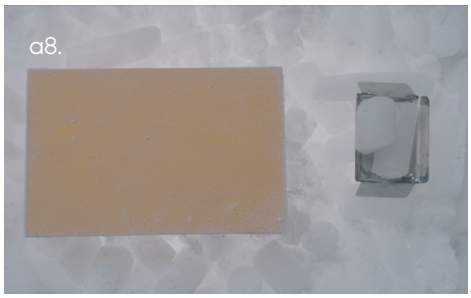

a5. A box of dry ice.

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

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

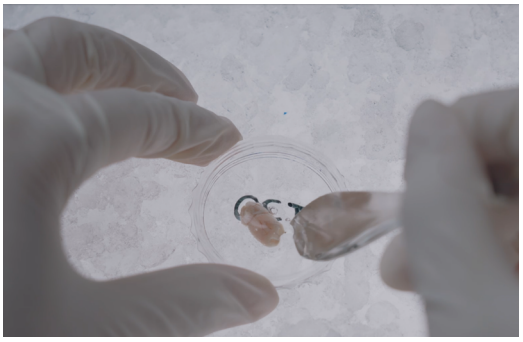



a8. Place mold B on dry ice and pre-cool for **> 5 min**.



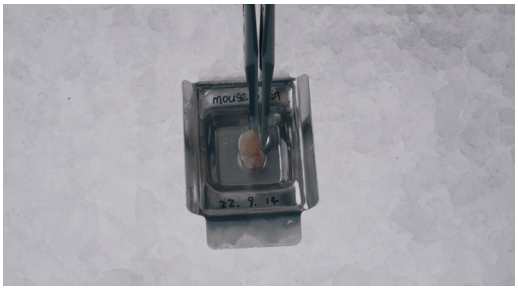

b. Upon harvesting within **30 min**, use sterile gauze or dust-free paper to absorb excess liquid on the tissue surface to avoid ice formation in later steps.

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

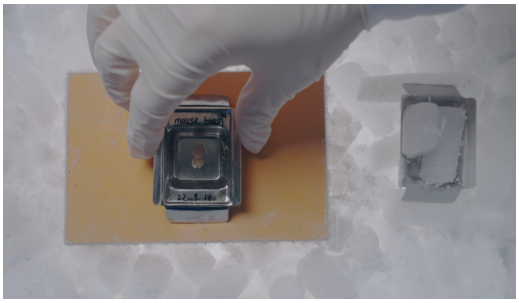


d. Remove any air bubbles using a syringe.

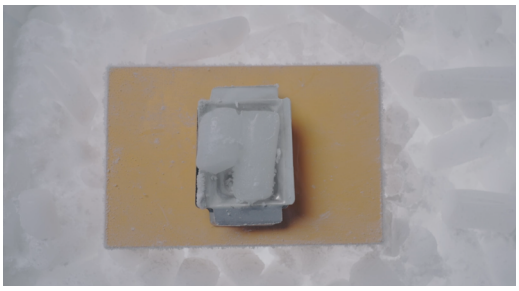
e. Orient the tissue to have the side intended to be sectioned facing downwards and then place into mold A. Make sure the tissue is at the bottom of mold A and fill the mold with chilled OCT without introducing bubbles until the tissue is fully covered.



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



g. Use mold B as a lid with 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 can be covered with enough dry ice cubes.



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

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

j. 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, add a few drops of the OCT and then wait till it solidifies and turns opaque.

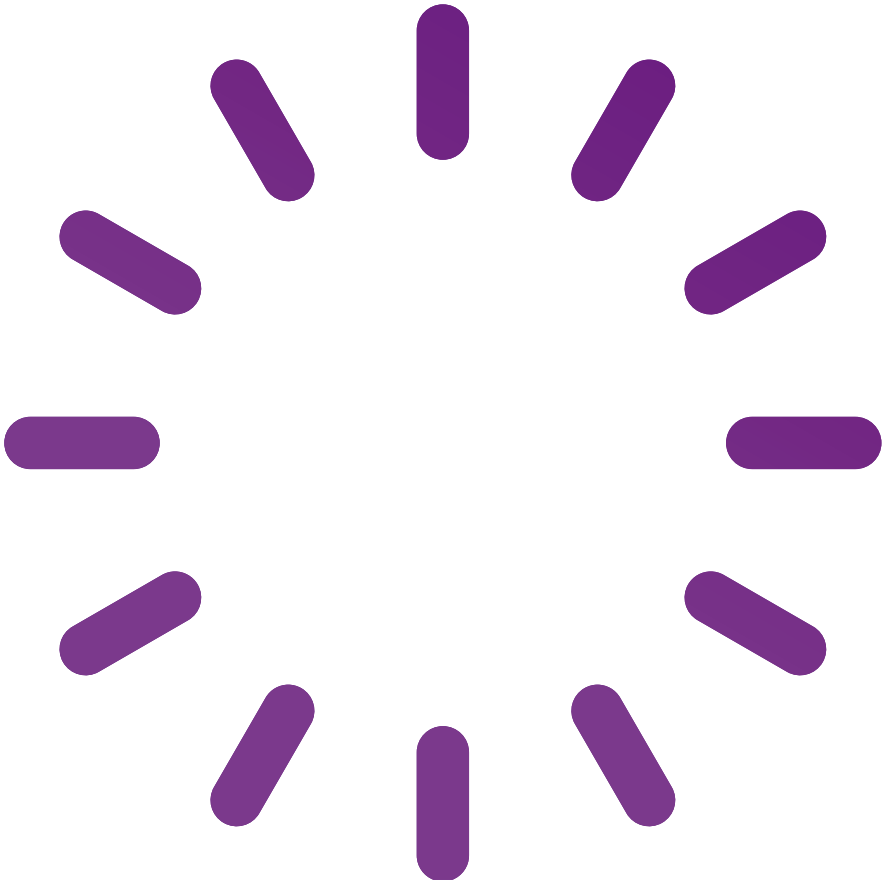
k. Label the tissue block to mark the orientation of the tissue.

## 2.3. Sample Storage and Transportation

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

# CHAPTER 3

# Stereo-seq TRANSCRIPTOMICS SET FOR CHIP-ON-A-SLIDE STANDARD OPERATING PROCEDURE



## 3.1. Experimental Preparation

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

| Prep Day | Reagent | Preparation Steps | Maintenance |
|---|---|---|---|
| Day 1 | 5X SSC | Dilute 5 mL of 20X SSC to 20 mL. | Room Temperature |
| | 0.1X SSC | Dilute 100 µL of 20X SSC to 20 mL; Dilute 250 µL of 20X SSC to 50 mL | Room Temperature |
| | Wash Buffer | Prepare at least 100 µL per chip (95 µL 0.1X SSC with 5 µL RI). | On ice until use |
| | 0.01N HCl | Prepare at least 2 mL of 0.01N HCl per sample. Configure HCl to 0.01N. Measure and make sure the pH = 2 (Prepare at least 2 mL / sample). | 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 Permeabilization Reagent Stock Solution | 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 |
| | **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 | 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 |
| | RT Oligo | Short spin the primer tube, dissolve RT Oligo in 79 µL TE buffer. Close the lid, vortex the tube for 15 sec at highest speed and short spin the tube. | -20°C |
| | **Aliquot the unused RT Oligo to avoid freeze-thaw cycles and store at -80°C.** | | |
| Day 1 | Glycerol | Take it out in advance and equilibrate to room temperature at least 5 min prior to use. | Room Temperature |
| | PR Rinse Buffer | Take it out in advance and equilibrate to room temperature at least 5 min prior to use. | Room Temperature |

| Prep Day | Reagent | Preparation Steps | Maintenance |
|---|---|---|---|
| Day 1 | PR Rinse Buffer (with 5% RI) | Prepare at least 100 μL per chip (95 μL PR Rinse Buffer with 5 μL RI) | On ice until use |
| Day 2 | 80% Ethanol | Dilute 100% ethanol to 80% | Room temperature up to 1 day |
| | Magnetic beads | Take it out in advance and equilibrate to room temperature at least 30 min prior to use. | 4°C |

| Other Preparation | | |
|---|---|---|
| **Equipments** | **Set up** | **Note** |
| Cryostat | Set the cryostat chamber temperature to -20°C and specimen disc temperature (object temperature) to -10°C ~ -15°C. | The specimen disc temperature depends on the tissue type. |
| PCR Thermal Cycler | Set the temperature in the following order:<br>37°C for slide drying and permeabilization (heating lid at 42°C);<br>42°C for reverse transcription (heating lid at 47°C);<br>55°C for tissue removal and cDNA release (heating lid at 60°C). | Check if there is any abnormality with the PCR thermal cycler and replace it if necessary. |
| Fluorescence Microscope | Set the epi-fluorescence channel to FITC mode. | Room Temperature |

## 3.2. Cryosection Preparation

a. Set PCR Thermal Cycler to 37°C in advance and place the PCR adaptor for pre-heating in advance.

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

**If the specimen disc is over-cooled, it could lead to tissue section cracking during sectioning, while sections would wrinkle when the disc temperature is too high. Optimal specimen disc temperature depends on the tissue type.**

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

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

e. Remove the sample outer covers (aluminum foil) and trim the embedded tissue block into appropriate size (sectioning area smaller than 0.45 cm x 0.45 cm).

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

g. Do a final trimming if necessary to ensure a good fit between the tissue section and Stereo-seq Chip T later. Now, the specimen is ready for cryosection.

## 3.3. Tissue Mounting

For a demonstration video of tissue mounting onto the Stereo-seq Chip Slide, please refer to the link or by scanning the QR code:
**https://drive.google.com/drive/folders/1mKOipoJdY_uDF-VA9I-githgVdrFeBcF**

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

**Once opened, please check if all the Stereo-seq Chip Slides in the slide container are well orientated with the front-side facing upward. The front-side of a chip has a shiny surface which contains DNB-probes for mRNA capture. DO NOT scratch the surface.**

b. Make sure the PCR thermal cycler has been turned on and set to 37°C.

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

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

d. Remove excess water on the chip by blowing gently with a power dust remover (MATIN, M-6318) from one side of the chip at a 30~45-degree angle horizontal to the plane of the chip. Wipe away excess water around the chip and on the slide with dust-free paper.

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

f. Prepare enough methanol in a 50 mL corning tube or an empty slide container at a volume that could submerge all the chips on the slide. Immerse a regular glass slide in the methanol containing tube to check if the volume is enough. Close the lid and pre-cool methanol for **5-30 min** at -20°C.

g. Place the tissue mounted specimen disc/holder onto the cryostat head and adjust the angle accordingly.

h. Tissue mounting can be achieved via either cold method (option A) or warm method (option B). We recommend practicing tissue mounting and section placement on plain glass slides first.

**A. Cold Method**

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

**Prolonged cooling for over 6 min may cause mist formation on the chip surface.**

2) Perform cryosection, then carefully flatten the tissue section out by gently touching the surrounding OCT with cryostat brushes. Place a tissue section onto the chip center carefully with forceps and brushes. Make sure the tissue section is complete and without wrinkles.

3) Immediately pick up the Stereo-seq Chip Slide and place a finger on the backside of the Stereo-seq Chip Slide directly under the chip for a few seconds to allow the section to adhere to the chip.

4) Place the tissue mounted Stereo-seq Chip Slide back inside the chamber and move on to the second tissue slicing and mounting. Continue transferring sections on remaining chips.

5) Once complete all tissue mounting, immediately dry the Stereo-seq Chip Slide on a PCR thermal cycler with PCR adaptor at 37°C for **5 min.**

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

**B. Warm Method**

1) Perform cryosection and obtain two or four consecutive tissue sections (depending on the number of chips on the Stereo-seq Chip Slide), carefully flatten the tissue sections out by gently touching the surrounding OCT with cryostat brushes.

2) Move the tissue sections to the edge and place each tissue section at a distance greater than the chip spacing on the Stereo-seq Chip Slide.

3) Flip the Stereo-seq Chip Slide and aim the tissue section within a chip area on the Stereo-seq Chip Slide by gently touching the section with the front-side of the chip.

4) Repeat step 3) until all the tissue sections have been mounted on to the chips of the Stereo-seq Chip Slide.

5) Turn the Stereo-seq Chip Slide over, and immediately dry it on a PCR thermal cycler with PCR adaptor front-side up for **5 min**.

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

**Stop Point:**

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

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

- After drying the tissue containing Stereo-seq Chip Slides on a PCR Thermal Cycler, transfer the Stereo-seq Chip Slide into a slide container then place it in a sealable plastic bag. Place one desiccant pack per Stereo-seq Chip Slide into a ziplock bag, push out as much air as possible and seal the bag tightly.
- Prepare a styrofoam shipping container filled with dry ice. Allow 0.5kg of dry ice for every 2 hours in transport. For example, for a 3-day shipping duration, 0.5kg * 12hr * 3 = 18kg of dry ice can be prepared.
- Place the ziplock bag at the bottom layer of the shipping box. If necessary, use sheets of bubble wrap to ensure the slide container remain in a vertical position.
- Fill empty space in the box with bubble wrap or paper. This will help prevent shifting of the slide container when the ice dissipates.
- Close the styrofoam lid. DO NOT tape the styrofoam lid to the box.
- Secure the outer lid of the shipping box with tape. When using dry ice, it is recommended to leave an air gap when taping to ensure that carbon dioxide can be released. This can prevent a buildup of pressure that could rupture the package.
- Label the shipping box with a dry ice sticker and then stick the pre-print label with both recipient address and return address. Contact the recipient lab before shipping to ensure that the staff is prepared to receive the shipment.
- When retrieving Stereo-seq Chip Slides with tissue from the shipping container, transfer out the slide container and take out the tissue containing Stereo-seq Chip Slides then immediately incubate at 37°C with PCR Adaptor for **5 min**.

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

## 3.4. Tissue Fixation

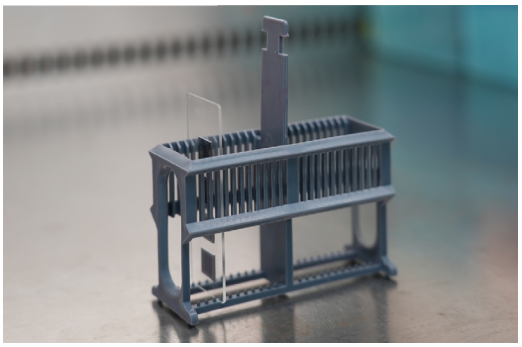
a. After drying, immediately immerse the tissue-mounted Stereo-seq Chip Slide in pre-cooled methanol prepared in section 3.3-f for a **30-min** fixation at -20°C. When immersing Stereo-seq Chip Slide in methanol, ensure that all the tissue sections are completely submerged.

**Meanwhile, make sure your microscope has been turned on, switched to FITC-mode and is ready for imaging.**

b. After fixation, move the **50 mL** corning tube or slide 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. While waiting, prepare fluorescent staining solution according to Table 3-1 and store it **in the dark**. **【PREPARE AHEAD】**

**Table 3-1 Tissue fluorescent staining solution**

| Components | 1X ( μL) | 2X + 10% ( μL) | 3X + 10% ( μL) | 4X + 10% ( μL) |
|---|---|---|---|---|
| 5X SSC | 28.34 | 62.37 | 94.5 | 124.74 |
| Qubit ssDNA Reagent | 0.15 | 0.33 | 0.5 | 0.66 |
| RI | 1.5 | 3.3 | 5 | 6.6 |
| **Total** | 30 | 66 | 100 | 132 |

f. Once methanol is fully evaporated, transfer the Stereo-seq Chip Slide on to a flat and clean bench top surface.

## 3.5. Fluorescent Staining

a. Place the Stereo-seq Chip Slide in a clean 10 cm petri dish, add **30 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 the droplets.

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

b. Cover the petri dish with aluminum foil and incubate it for **5 min** at room temperature **in the dark.**

c. Once 5 minute incubation is complete, slightly tilt the Stereo-seq Chip Slide while gently removing the staining solution from the corner of the chip using a pipette. Try to remove as much solution as possible.

d. Add **30 µL** of Wash Buffer per chip.

e. Slightly tilt the Stereo-seq Chip Slide while gently removing the Wash Buffer from the corner of the chip using a pipette. Try to remove as much solution as possible.

f. Transfer the Stereo-seq Chip Slide onto dust-free paper. Hold on to the slide with one hand and completely dry the chips further with a power dust remover in the other hand at a distance 2-3 cm away from the chip surface by blowing gently from one side of the chip at a 30-degree angle horizontal to the plane of the chip.

**Alternatively, centrifuge the Stereo-seq Chip Slide for 10 sec in a slide spinner to completely dry the chips (Labnet Slide Spinner, C1303-T ).**

**Ensure no residual staining solution is left on the chip.**

g. Pipette **1.2 µL** glycerol gently onto the center of the tissue on each chip without introducing air bubbles.

h. With a pair of forceps, place one end of the coverslip onto the chip while holding the other end and then gradually lower the coverslip onto the chips. Ensure that the chips are completely covered by glycerol and the coverslip. To avoid fluorescent bleaching, **IMMEDIATELY proceed to imaging**.

**Make sure the coverslip is clean without any dust or debris. Wiping with an alcohol swab or blowing with a power dust remover could be used for cleaning.**

## 3.6. Imaging

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

**Only use letters, numbers, and underscores in folder naming. Special characters and spacings are not allowed.**

**Example: B00249A1**

b. 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 onto the imaging platform.

c. Remove the light shield and select the chip area of interest.

d. Take fluorescence images from the chip with the following microscope setting: FITC channel, 10X objective lens, full scan on capture area.

**Take fluorescent images immediately to avoid prolonged fluorescent exposure which causes bleaching.**

e. Save original tile (FOV) images files and stitched images.

**Glycerol mounted chips could be stored for a maximum of 4 hr after imaging.**

f. Open the ImageStudio software and the Image Quality Control functional module within the software. Upload your nuclei-stained (ssDNA) image and run Image QC according to the ImageStudio User Manual within the software.

**The captured ssDNA-stained image needs to pass ImageQC in order to proceed to further image analysis (image "register") in Stereo-seq Analysis Workflow (SAW) pipelines.**

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

g. After imaging, gently push the coverslip with a pair of forceps until it is slightly beyond the edge of Stereo-seq Chip Slide.

h. Grip onto the coverslip with the pair of forceps and pull it to slide over the Stereo-seq Chip Slide slowly until the chips and the coverslip are fully separated.

i. Place the Stereo-seq Chip Slide in a corning tube filled with at least **30 mL** of 0.1X SSC and immerse it for **3-5 sec**.

**Ensure that all the chips on the Stereo-seq Chip Slide have been submerged in SSC solution.**

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

k. Assemble the Cassette and Gasket then place the Stereo-seq Chip Slide in the Cassette according to guidance written in 1.5 Practice Tips. It is recommended to practice with a regular blank glass slide.

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

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

## 3.7. Tissue Permeabilization

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

b. Make sure your PCR thermal cycler has been switched on and set to 37°C and the heating lid has been set to 42°C. Place the PCR adaptor in the PCR thermal cycler, then close the heated lid to pre-heat the adaptor for **3 min**.

| Temperature | Time | Cycle |
|---|---|---|
| (Heated lid) 47°C | on | - |
| 37°C | *Determined by Stereo-seq Permeabilization user manual | 1 |
| 37°C | Hold | - |

c. Warm up the 1X Permeabilization Reagent Solution inside the 37°C PCR thermal cycler or Metal Block for **>10 min**.

d. Thaw RT Reagent, RT Additive and RT Oligo on ice.

e. Warm up the assembled Stereo-seq Slide Cassette in the 37°C PCR Thermal Cycler for **3 min**, then 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.

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

f. Place unpeeled sealing tape on the Stereo-seq Slide Cassette and let the chip incubate inside the PCR thermal cycler at 37°C for the optimal permeabilization time.

**Optimal permeabilization time is pre-determined by Stereo-seq Permeabilization Kit (111KP118). For more information, please refer to the Stereo-seq Permeabilization Set for Chip-on-a-slide user manual.**

g. While waiting for permeabilization to be done, prepare RT mix according to Table 3-2 and leave it on ice until use. 【**PREPARE AHEAD**】

**Table 3-2 RT Mix**

| Components | 1X ( µL) | 2X + 10% ( µL) | 3X + 10% ( µL) | 4X + 10% ( µL) |
|---|---|---|---|---|
| RT Reagent | 160 | 352 | 528 | 704 |
| RT Additive | 10 | 22 | 33 | 44 |
| RI | 10 | 22 | 33 | 44 |
| RT Oligo | 10 | 22 | 33 | 44 |
| ReverseT Enzyme | 10 | 22 | 33 | 44 |
| **Total** | 200 | 440 | 660 | 880 |

h. Place the PCR Adaptor in another PCR Thermal Cycler in advance and set the temperature to 42°C with heated lid set to 47°C.

i. Once complete, remove the Stereo-seq Slide Cassette from the PCR Adaptor (37°C).

j. Slightly tilt the Stereo-seq Slide Cassette, remove 1X Permeabilization Reagent Solution with a pipette from the corner of each well without touching the chip surface.

k. Add **200 µL** of PR Rinse Buffer (with 5% RI) per chip from the corner of each well.

l. Slightly tilt the Stereo-seq Slide Cassette, remove PR Rinse Buffer with a pipette from the corner of each well without touching the chip surface. Keep the chip surface moisturized.

**Make sure to not dry the chip completely.**

m. Continue with reverse transcription immediately to avoid RNA degradation.

## 3.8. Reverse Transcription

a. Make sure the temperature of the PCR Thermal Cycler with PCR Adaptor has been set to 42°C in advance.

b. Pipette up and down, then short spin the prepared RT Mix. Gently add **200 µL** of RT Mix per chip along the side of each well, ensuring that the well surface is uniformly covered with RT Mix.

c. Apply sealing tape to Stereo-seq Slide Cassette and make sure it is sealed tightly. Incubate the Stereo-seq Slide Cassette at 42°C for **3 hr** or longer (no longer than 16 hr) with the following incubation protocol.

| Temperature | Time | Cycle |
|---|---|---|
| (Heated lid) 47°C | on | - |
| 42°C | 180 min | 1 |
| 42°C | Hold | - |

## 3.9. Tissue Removal

| Prepare | | |
|---|---|---|
| **Reagent** | **Preparation Steps** | **Maintenance** |
| TR buffer | Heat the buffer for **5 min** at 55 °C to dissolve the precipitate. Equilibrate it to room temperature prior to use. | Room Temperature |
| 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 precipitate is observed in the buffer, dissolve them by heating the buffer at 55 °C again and equilibrate to room temperature before mixing.**

a. Check and make sure the PCR Thermal Cycler with PCR Adaptor has been set to 55°C and the heated lid has been set to 60°C.

b. Remove the Stereo-seq Slide Cassette from the 42°C PCR Adaptor and then remove the sealing tape.

**When removing the sealing tape, hold on to the Stereo-seq Slide Cassette with one hand without applying forces to Side A and Side B of the cassette in order to prevent the Stereo-seq Chip Slide from falling off of the cassette.**

c. Slightly tilt the Stereo-seq Cassette, remove RT Mix with a pipette from the corner of each well without touching the chip surface.

d. Add 400 µL TR Buffer into each well and incubate the Stereo-seq Slide Cassette at 55°C on the PCR Adaptor for **10 min** with the following incubation protocol.

| Temperature | Time | Cycle |
|---|---|---|
| (Heated lid) 60°C | on | - |
| 55°C | 10 min | 1 |
| 55°C | Hold | - |

e. While waiting, prepare cDNA Release Mix according to Table 3-3. **【PREPARE AHEAD】**

**Table 3-3 cDNA Release Mix**

| Components | 1X ( µL) | 2X + 10% ( µL) | 3X + 10% ( µL) | 4X + 10% ( µL) |
|---|---|---|---|---|
| cDNA Release Buffer | 380 | 836 | 1254 | 1672 |
| cDNA Release Enzyme | 20 | 44 | 66 | 88 |
| **Total** | 400 | 880 | 1320 | 1760 |

f. Slightly tilt the Stereo-seq Slide Cassette, remove TR Buffer with a pipette from the corner of each well without touching the chip surface.

**If tissue removal is not complete, add 400 µL of 0.1X SSC and pipette up and down the well to remove the remaining tissue on the chip. Slightly tilt the Stereo-seq Slide Cassette, remove 0.1X SSC solution from the corner of each well without touching the chip surface.**

## 3.10. cDNA Release and Collection

a. Add 400 µL of cDNA Release Mix per chip prepared in 3.9-e into each well of the Stereo-seq Slide Cassette.

b. Apply sealing tape to Stereo-seq Slide Cassette and make sure it is sealed tightly. Incubate the Stereo-seq Slide Cassette at 55°C for **3 hr** or longer (no longer than 18 hr) with the following incubation protocol.

| Temperature | Time | Cycle |
|---|---|---|
| (Heated lid) 60°C | on | - |
| 55°C | 180 min | 1 |
| 55°C | Hold | - |

**Stop Point:**

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

c. After the reaction, completely collect the cDNA Release Mix from each well into a new 1.5 mL tube.

d. Add 100 µL of 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 1.5 mL tube with the cDNA Release Mix.

**Make sure to collect as much volume as possible to retrieve enough cDNA from Chip T. cDNA Release Mix should be about 400 µL after incubation (the volume might be less than 400 µL). It is required to combine the collected cDNA Release Mix with the 100 µL nuclease-free water before proceeding to the next step.**

**The Stereo-seq Chip Slide may be discarded. Ensure all the chip ID numbers on the Slide have been recorded, as it is required for downstream analysis.**

## 3.11. 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 VAHTSTM 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 the beads are thoroughly mixed every time before use.
- The volume of magnetic beads added directly affects the distribution of purified DNA fragments.

### Operation Notes

- In the magnetic separation step, please 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.
- 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 directly contacting the beads. If the beads are mistakenly aspirated, dispense supernantant back into tube and redo the magnetic separation.
- 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.
- After the 2nd wash of beads with ethanol, try to remove all the liquid within the tube. You may centrifuge briefly to accumulate any remaining liquid at the bottom of the tube, then separate beads magnetically, and remove the remaining liquid by using a small volume pipette.
- 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. Watch closely until the pellet appears sufficiently dry with a matte appearance, then continue to the elution step with TE Buffer.
- 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 avoid 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.
- **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. Equilibrate the magnetic beads to room temperature for at least **30 min**.

b. If white precipitate is observed in the collected cDNA, dissolve it by heating at 55°C and equilibrate to room temperature before the purification step.

c. cDNA Purification Procedures with 0.8X Magnetic Bead

1) Mix the collected cDNA (450-490 μL) with the beads in a ratio of 1 : 0.8. 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 seen on the cap, discard them with a pipette).

4) Keep the tube on the magnetic separation rack and add 1 mL of freshly prepared 80% ethanol to 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 seen on the cap, clean the cap with 80% ethanol).**

5) Repeat step 4) one more time.

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

7) Add 22 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 (~ **21 μL**) into a new 0.2 mL PCR tube.

9) Add another 22 μL of nuclease-free water to the dried beads in step 7) for a second elution. 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.

10) Transfer the supernatant (~ **21 μL**) into the 0.2 mL PCR tube in step 8) and obtain a combined eluted cDNA (~**42 μL**)

d. If collected eluted cDNA is less than 42 μL, simply top it up with nuclease-free water.

Resuspend the beads with 42 uL of nuclease-free after cDNA collection. Store resuspended beads at 4 C until the cDNA final product has passed QC.

e. Prepare PCR Mix by referring to Table 3-4. The total volume for the PCR reaction is 100 μL.

Table 3-4 PCR Mix

| Components | 1X ( μL) | 2X + 10% ( μL) | 3X + 10% ( μL) | 4X + 10% ( μL) |
|---|---|---|---|---|
| cDNA Amplification Mix | 50 | 110 | 165 | 220 |
| cDNA Primer | 8 | 17.6 | 26.4 | 35.2 |
| Eluted cDNA | 42 | 2 x 42 | 3 x 42 | 4 x 42 |
| **Total** | 100 | 2 x 100 | 3 x 100 | 4 x 100 |

f. Mix gently and short spin before placing the reaction tube in a thermal cycler. Amplify the eluted cDNA based on the PCR program stated in Table 3-5.

Table 3-5 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 | - |

g. Prepare Qubit dsDNA Mix and record the concentration of the PCR product according to Table 3-6.

Table 3-6 Qubit dsDNA Mix

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

h. Vortex the mix and then take 1 μL of the PCR product and measure its concentration using Qubit dsDNA HS Kit. The DNA concentration is usually more than 5 ng/μL.

QC

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

i. Use magnetic beads to purify the PCR product in a volume ratio of 1 : 0.6 (DNA : beads).

1) Mix the cDNA PCR product (100 µL) with beads in a ratio of 1 : 0.6. Vortex the mixture then incubates it at room temperature for **10 min**.

2) Spin down and place the sample tube onto a magnetic separation rack for **3 min** until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3) Keep the tube on the magnetic separation rack and add 200 µL of freshly prepared 80% ethanol to wash the beads by rotating the tube on the magnetic separation rack. Incubate for **30 sec** then 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.**

4) Repeat step 3) one more time.

5) Keep the tube on the magnetic rack, and open the lid to air-dry the beads at room temperature until no wetness (reflectiveness) is observed. Drying times will vary but will be approximately **5-8 min**.

6) Add 42 uL of TE buffer to tube and resuspend bead by vortexing. Incubate at room temperature for **5 min**. Spin down briefly and place the sample tube onto a magnetic rack for **3-5 min** until the liquid becomes clear. Transfer 40 µL supernatant to a new 1.5 mL centrifuge tube.

**Stop Point:**

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

**For troubleshooting purposes, after purification we recommend resuspending the beads with 42 uL nuclease-free water and store the mixture at 4 C until the cDNA final product has passed QC.**

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

k. 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 appearing at around 1,000- 1,500 bp (Figure 2), and a yield higher than 20 ng.**

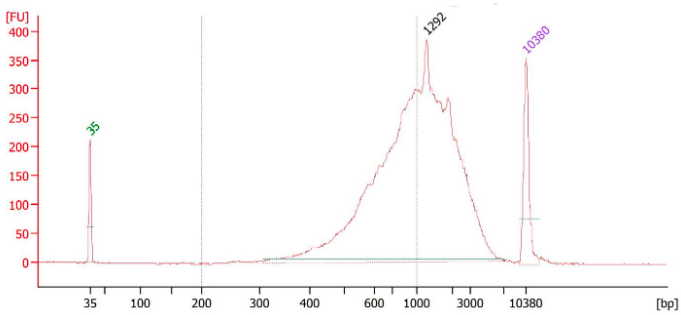
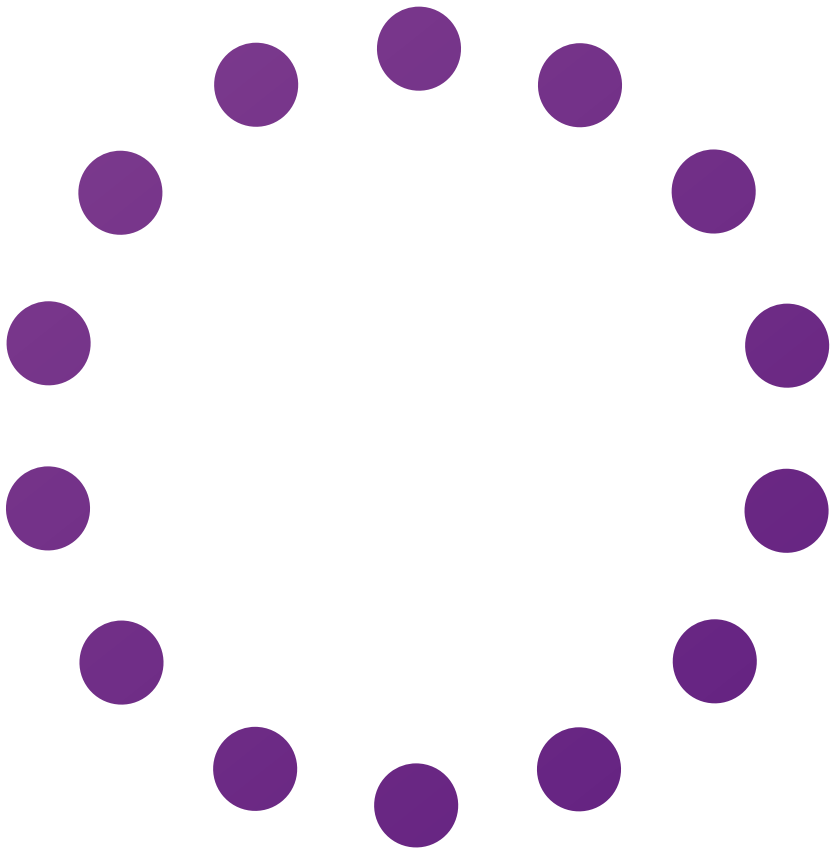



Figure 2. A representative Agilent Bioanalyzer 2100 analysis result of a PCR amplified cDNA sample

# CHAPTER 4
# LIBRARY PREPARATION



**The later steps will require Stereo-seq Library Preparation kit. Please make sure you have purchased the kit separately and prepare reagents in advance before constructing your library.**

| Stereo-seq Library Prep Kit Cat. No.: 111KL114 | | | |
|---|---|---|---|
| **Component** | **Reagent Cat. No.** | **Cap Color** | **Quantity (tube)** |
| TME | 1000028515 | ○ (white) | 4 μL × 1 |
| Stop Buffer | 1000028516 | ○ (white) | 40 μL × 1 |
| TMB | 1000028517 | ○ (white) | 40 μL × 1 |
| PCR Barcode Primer Mix (Barcode 57-64) | 1000028519 | ● | 50 μL × 1 |
| PCR Barcode Primer Mix (Barcode 81-88) | 1000029088 | ● | 50 μL × 1 |
| PCR Barcode Primer Mix (Barcode 89-96) | 1000029089 | ● | 50 μL × 1 |
| PCR Barcode Primer Mix (Barcode 97-104) | 1000029180 | ● | 50 μL × 1 |
| PCR Amplification Mix | 1000029181 | ● | 400 μL × 1 |

Storage Temperature: -25°C~-18°C | Transported by cold chain | Expiration Date: refer to label

**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.**

## 4.1. Experimental Preparation

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

| Reagent | Preparation Steps | Maintenance |
|---|---|---|
| 80% Ethanol | Dilute 100% ethanol to 80% | Room temperature up to 1 day |
| Magnetic beads | Take it out in advance and equilibrate to room temperature at least 30 min prior to use. | 4°C |
| 10-fold diluted TME | Dilute 1 μL of TME to 10 μL with TE buffer | On ice up to 1 hr |
| **DO NOT dilute all the TME at once. Volume provided should be enough for 4 dilutions.** | | |
| Stop Buffer | Take it out in advance and equilibrate to room temperature at least 30 min prior to use | Room temperature up to 1 day |

## 4.2. cDNA Fragmentation and Amplification

a. Use 20 ng cDNA sample prepared in section 3.11 for the following fragmentation reaction.

b. Prepare the Fragmentation Reaction Mix on ice through gentle pipetting according to Table 4-1. Pipette 10-fold diluted TME up and down before mixing with the rest. After a short spin, gently mix the solution through pipetting while keeping the tube on ice.

**Avoid vortexing TME.**

**Table 4-1 Fragmentation Reaction Mix**

| Components | 1X ( μL) |
|---|---|
| TMB | 4 |
| 10-fold diluted TME | 1 |
| cDNA Product | X |
| Nuclease-free water | 15-X |
| **Total** | 20 |

**cDNA Input: X (μL) = 20 ng/Concentration of cDNA (ng/μL)**

c. Program a thermocycler according to Table 4-2. When the module starts to heat up, put the reaction tube into the thermocycler.

Please DO NOT leave the reaction tube on ice after taking it out of the thermocycler.

Table 4-2 Fragmentation reaction program

| Temperature | Time |
|---|---|
| (Heated lid) 60°C | on |
| 55°C | 10 min |
| 4°C | Hold |

d. After the fragmentation reaction program is done, take out the reaction tube and leave it at room temperature. Add 5 μL of Stop Buffer to the fragmentation reaction mix to terminate the fragmentation process, and then pipette to mix thoroughly. Incubate the mix at room temperature for **5 min**.

e. Set up PCR Library Mix according to Table 4-3 to start the amplification process of fragmented cDNA.

Table 4-3 PCR Library Mix

| Components | 1X ( μL) |
|---|---|
| Fragmentation product | 25 |
| PCR Barcode Primer Mix | 25 |
| PCR Amplification Mix | 50 |
| **Total** | 100 |

Please refer to Appendix A for guidelines for using PCR Barcode Primer Mix.

f. Vortex and spin down briefly the reaction mix prepared above. Incubate it in a thermocycler with the following incubation protocol (Table 4-4) and start the program.

Table 4-4 PCR Amplification Program (for 100μL)

| Temperature | Time | Cycle |
|---|---|---|
| (Heated lid) 105 °C | on | - |
| 95°C | 5 min | 1 |
| 98°C | 20 sec | 13 |
| 58°C | 20 sec | |
| 72°C | 30 sec | |
| 72°C | 5 min | 1 |
| 12°C | Hold | - |

g. Take 1 μL of the PCR product and use the Qubit dsDNA HS Kit to measure the concentration. The concentration is usually around 10-100 ng/μL.

## 4.3. PCR Product Size Selection

a. Mix the PCR product obtained above with the magnetic beads in a volume ratio of 1 : 0.55 (PCR product : beads = 100 µL : 55 µL) in a PCR tube. Vortex the mixture then incubate it at room temperature for **5 min**.

b. Short spin the reaction mix and place the tube onto a magnetic separation rack for **3 min** until it becomes clear. Then, carefully transfer the supernatant to a new PCR tube.

**Keep the supernatant and discard the beads.**

c. Add **15 µL** of beads to the new PCR tube with the supernatant from step b. Vortex to mix thoroughly. Incubate at room temperature for **5 min**.

d. Spin down and place the tube onto a magnetic separation rack for **3-5 min** until it becomes clear. Carefully discard the supernatant with a pipette.

e. Keep the tube on the magnetic separation rack and add **200 µL** of freshly prepared 80% ethanol to wash the beads by rotating the tube on the magnetic rack. Incubate for **30 sec** and carefully remove the supernatant with a pipette.

f. Repeat step e. one more time.

g. Spin-down the tube and put it on the magnetic rack to extract the beads out of the liquid. Use a smaller pipette tip to remove the remaining liquid and discard it.

h. Air-dry the beads for **3-5 min** until the bead surface is not reflective or cracked.

i. Mix the dried beads with **20 µL** of TE buffer, vortex to mix and incubate at room temperature for **5 min**. Spin down briefly and place the centrifuge tube onto a magnetic separation rack for **3 min** until the liquid becomes clear. Transfer the supernatant to a new **1.5 mL** tube.

**Keep the supernatant.**

j. Take **1 µL** of purified PCR product and measure the concentration with Qubit dsDNA HS Kit. Use Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer), Fragment Analyzer(TM) (Advanced Analytical) or other equipment based on the principle of electrophoretic separation to detect the fragment distribution of the purified PCR products. The main peak of fragment distribution is required to be 200-600 bp (Figure 4). Normally the PCR yield is higher than 100 ng. QC

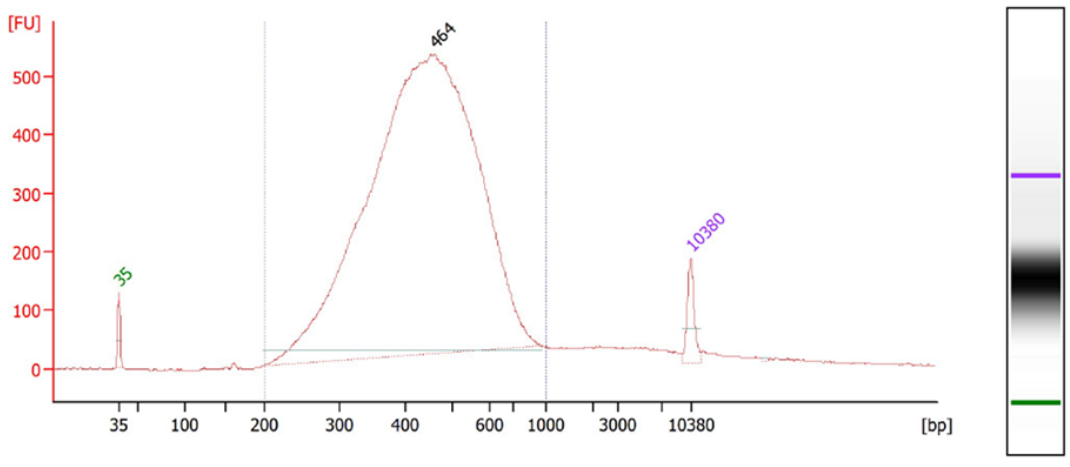
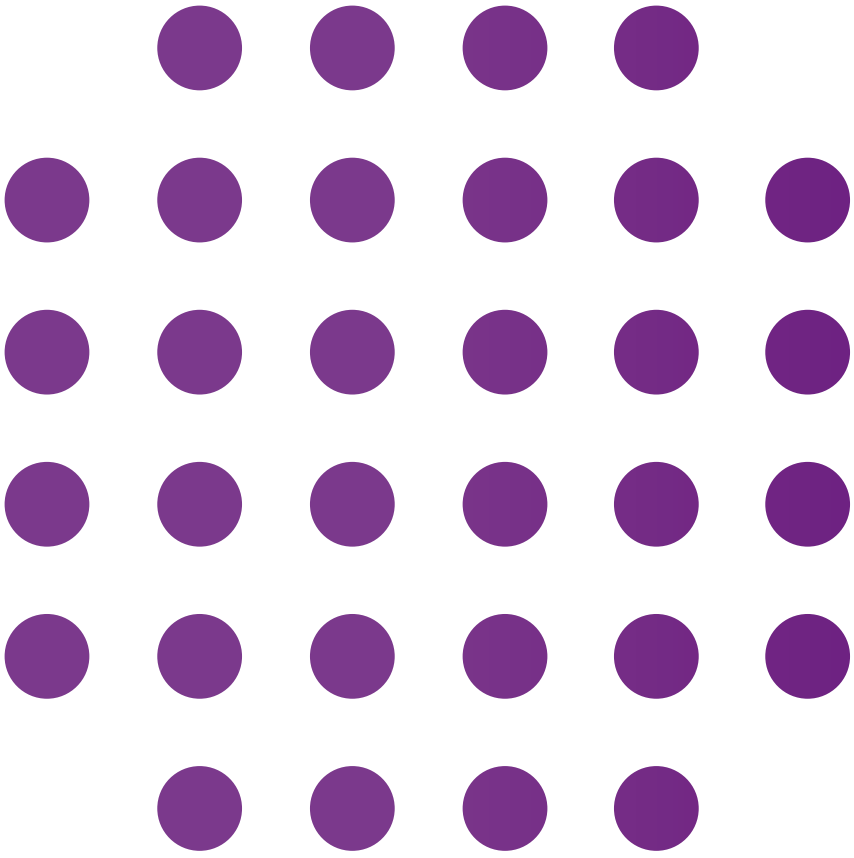



Figure 3. Agilent 2100 Bioanalyzer fragment size distribution of the purified PCR product

# CHAPTER 5
# LIBRARY CONSTRUCT & SEQUENCING



This chapter introduces the compatible sequencing instruments and sequencing reagents for the Stereo-seq system. The library construct is illustrated in Figure 4.

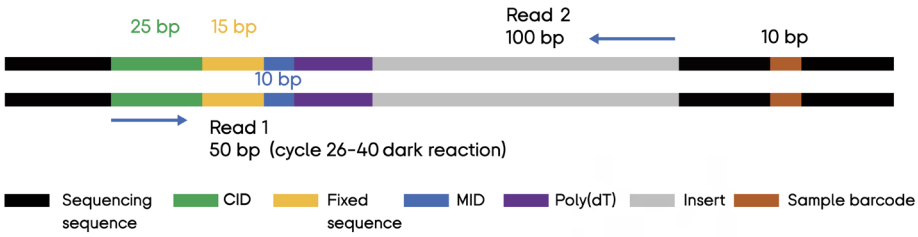



Figure 4. Stereo-seq Transcriptome Library Construct

**Please refer to the user manual of High-throughput Sequencing Primer Kit (STOmics), Cat. no. 940-000037-00 for DNB preparation.**

Use the following parameters to perform the sequencing run:

- Without sample barcode sequenced (for only one sample): choose paired-ended mode with 50 cycles of Read 1 and 100 cycles of Read 2. Use dark cycles on Read 1 from 26 to 40 cycles.
- With sample barcode sequenced (for two or more samples): choose paired-ended mode with 50 cycles of Read 1 and 100 cycles of Read 2 and an additional 10 cycles of sample barcode. Use dark cycles on Read 1 from 26 to 40 cycles.

**Please read the corresponding user manual High-throughput Sequencing Primer Kit (STOmics), Cat. no. 940-000037-00 carefully before performing sequencing and strictly follow the instructions. If you have any questions about sequencing, please contact your local MGI account manager or technical support.**

## Appendix A: PCR Barcode Primer Mix Use Rules

The PCR Barcode Primer Mix in this kit is a pre-mixed barcode combination with a balanced set of bases, which can be randomly selected for use by the customer. Splitting barcode is required in sequencing for two or more samples in the same lane (in order to distinguish your sequencing samples). If there is only one sample, do not split barcode for sequencing. The following table is the barcode sequence number in each pre-set PCR Barcode Primer Mix.

| PCR Barcode Primer Mix Name | Contains the Barcode Sequence Number | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PCR Barcode Primer Mix (Barcode 57~64) | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 |
| PCR Barcode Primer Mix (Barcode 81~88) | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 |
| PCR Barcode Primer Mix (Barcode 89~96) | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 |
| PCR Barcode Primer Mix (Barcode 97~104) | 97 | 98 | 99 | 100 | 101 | 102 | 103 | 104 |

# Appendix B: Stereo-seq Transcriptomics Set for Chip-on-a-slide Experimental Record

For recording and self-checking experimental procedures, users can access and download the Stereo-seq Transcriptomics Set Experimental Record that we provided here: **https://drive.google.com/drive/folders/18Od8IPO-H1805iycXlZtdTjTtGXIVGex?usp=sharing**