STOmics

Stereo-seq OMNI FFPE Library Preparation

USER MANUAL

with Stereo-seq 16 Barcode Library Preparation Kit V1.0

Cat. No.: 111KL160 (16 RXNs)
Kit Version: V1.0
Manual Version: B

STUM-LP001

REVISION HISTORY

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

Manual Version:	B
Kit Version:	V1.1
Date:	Mar. 2025
Description:	• Updated the libraries input parameters for SAW v8.1.3 and above. • Extended the transferring and storage temperature. • Modified the library structure in chapter 3.

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

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NOTE: Additional operation tips and guidance.

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

CHAPTER 1
INTRODUCTION

1.1. Intended Use

Stereo-seq OMNI FFPE Library Preparation requires the usage of Stereo-seq 16 Barcode Library Preparation Kit, which is designed for samples utilizing Stereo-seq technology, including fresh frozen transcriptome cDNA samples, multi-protein antibody-derived tags (ADT) samples, and FFPE transcriptome cDNA samples. It enables the addition of sample barcodes and library construction, suitable for multi-sample mixed sequencing, supporting up to 16 samples for mixed sequencing.

All reagents provided within this kit have passed stringent quality control and functional verification, ensuring performance stability and reproducibility of library construction, as well as the uniformity and accuracy of sequencing data splitting.

1.2. Sequencing Guideline

Sequencing libraries produced via the Stereo-seq Transcriptomics Set require the DNBSEQ sequencing platform For details, please refer to Library Construction for Stereo-seq FFPE Transcriptome Library.

Necessary input parameters for Stereo-seq FFPE transcriptome libraries for the Stereo-seq Analysis Workflow (SAW) bioinformatics pipelines:

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

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

or `--sequencing-type` = 'PE75_25+62' (applicable for SAW v8.1.3 and above)

1.3. List of Kit Components

Each Stereo-seq 16 Barcode Library Preparation Kit consists of:

Table 1-1

Stereo-seq 16 Barcode Library Preparation Kit V1.0		Cat. No.: 111KL160	
Component	**Reagent Cat. No.**	**Cap Color**	**Quantity (tube)**
KMB	1000047709	○	160 μL × 1
KME	1000047770	○	80 mg × 1
PCR Barcode Primer Mix1	1000043201	●	25 μL × 1
PCR Barcode Primer Mix2	1000043202	●	25 μL × 1
PCR Barcode Primer Mix3	1000043203	●	25 μL × 1
PCR Barcode Primer Mix4	1000043204	●	25 μL × 1
PCR Barcode Primer Mix5	1000043205	●	25 μL × 1
PCR Barcode Primer Mix6	1000043206	●	25 μL × 1
PCR Barcode Primer Mix7	1000043207	●	25 μL × 1

Stereo-seq 16 Barcode Library Preparation Kit V1.0		Cat. No.: 111KL160	
Component	**Reagent Cat. No.**	**Cap Color**	**Quantity (tube)**
PCR Barcode Primer Mix8	1000043208		25 μL × 1
PCR Barcode Primer Mix9	1000043209		25 μL × 1
PCR Barcode Primer Mix10	1000043210		25 μL × 1
PCR Barcode Primer Mix11	1000043211		25 μL × 1
PCR Barcode Primer Mix12	1000043212		25 μL × 1
PCR Barcode Primer Mix13	1000043213		25 μL × 1
PCR Barcode Primer Mix14	1000043214		25 μL × 1
PCR Barcode Primer Mix15	1000043215		25 μL × 1
PCR Barcode Primer Mix16	1000043216		25 μL × 1
PCR Amplification Mix	1000043217		800 μL × 1
Storage Temperature: -25°C~-15°C	Transported by cold chain	Expiration Date: refer to label	

Please ensure that a substantial amount of dry ice remains with the kits upon arrival.

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.

1.4. Additional Equipment and Materials

The table below lists equipment and materials needed for this protocol. Some common laboratory equipment not named in Table 1-2 are expected to be accessible by the user, for instance, an ice maker, biological safety cabinet, freezers, etc.

Table 1-2

Equipment		
Brand	**Description**	**Cat. No.**
-	PCR thermocycler	-
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 (or similar)
-	Vortex mixer	-
Agilent Technologies™	Agilent 2100 bioanalyzer	G2939AA (or similar)

Reagents		
Brand	**Description**	**Cat. No.**
Ambion	Nuclease-free water	AM9937
	1X TE buffer, pH 8.0	AM9858
-	100% Ethanol (Analytical grade)	-
*Beckman Coulter	AMPure® XP	A63882
	SPRIselect	B23317/B23318/ B23319
*VAZYME	VAHTS™ DNA Clean Beads	N411-02
Invitrogen	Qubit dsDNA HS Assay Kit	Q32854
	Qubit ssDNA Assay Kit	Q10212
Agilent Technologies™	High sensitivity DNA kit	5067-4626

Consumables		
Brand	**Description**	**Cat. No.**
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
	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

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

CHAPTER 2

LIBRARY PREPARATION FOR FFPE SAMPLES

For guidelines on sample embedding, sectioning and mounting, please refer to Sample Preparation, Sectioning, and Mounting Guide for Fresh Frozen Samples on Stereo-seq Chip Slides (Document No.: STUM-SP001).

2.1. Experiment 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

2.2 cDNA Amplification and Purification cDNA

a. Use 20 ng cDNA sample for the amplification reaction according to Table 3-1.

Table 2-1 Amplification Reaction Mix

Components	1X (μL)
PCR Amplification Mix	50
PCR Barcode Primer Mix	25
cDNA Product	X
Nuclease-free water	25-X
Total	100

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

b. Program a thermocycler according to Table 3-2. When the module starts to heat up, put the reaction tube into the thermocycler.

Table 2-2 Reaction program (100μL)

Temperature	Time	Cycle
(Heated lid) 105 °C	on	-
95°C	5 min	1
98°C	20 sec	8
58°C	20 sec	
72°C	3 min	
72°C	5 min	1
12°C	Hold	-

c. Take 1 μL of the PCR product and use the Qubit dsDNA HS Kit to measure the concentration. The concentration is usually more than 5 ng/μL.

d. Purify the above reaction products with **0.8x** magnetic beads.

1) Mix the cDNA PCR product (100 μL) with beads in a ratio of 1 : 0.8. Vortex the mixture then incubate it at room temperature for 10 min.

2) Spin down and place the sample tube onto a magnetic separation rack for **3-5 min** until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

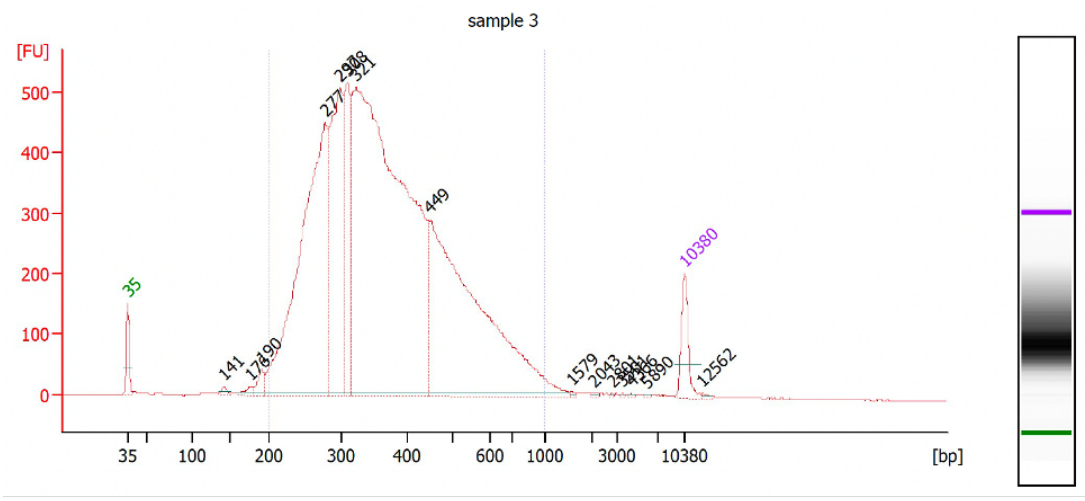
3) Add **200 μL** of 80% ethanol for washing, using freshly prepared and equilibrated 80% ethanol at room temperature. During the washing process, the tube should always be placed on the magnetic rack, and the pipette tip should operate on the wall of the tube away from the magnetic rack. Do not aspirate or disturb the magnetic beads. Keep the tube on the magnetic rack.

4) Repeat step 3) one more time.

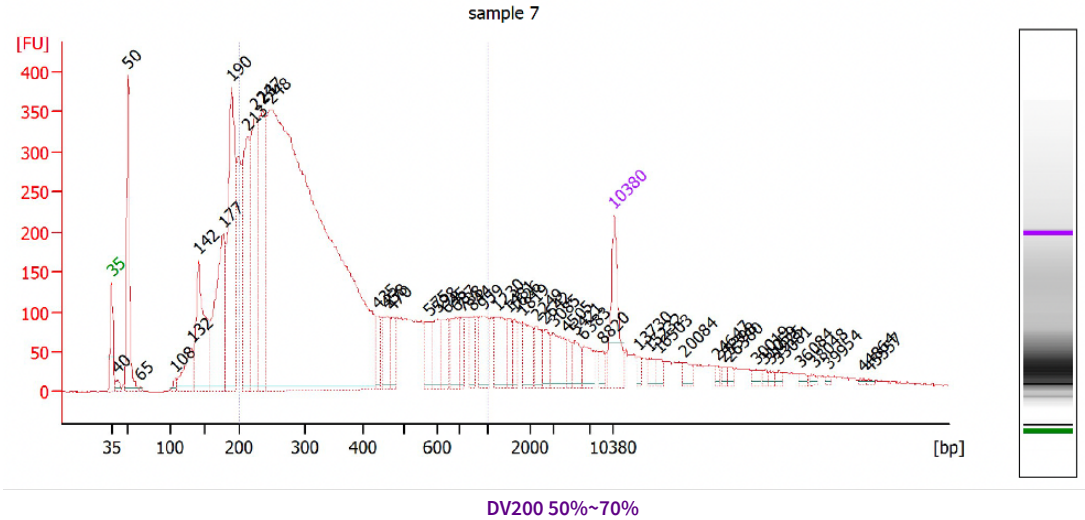
5) Keep the tube on the magnetic rack, and open the lid to air-dry the beads at room temperature until no wetness (reflectiveness) is observed. Drying times will vary but will be approximately **5-8 min**.

6) Add **42 μL** of TE buffer 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 to a new 1.5 mL tube.

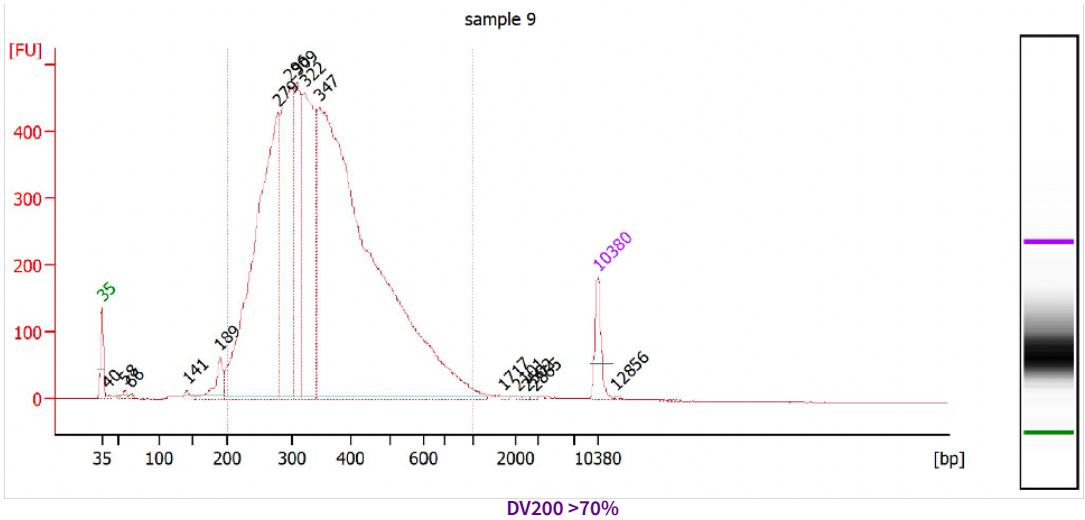
e. Take 1 μL of purified PCR product and measure the concentration with Qubit dsDNA HS Kit. Use Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer), Fragment Analyzer™ Advanced Analytical) or other equipment based on the principle of electrophoretic separation to detect the fragment distribution of the purified PCR products. The main peak of fragment distribution is around **100-1000 bp** (Figure1). Normally, the PCR yield is higher than **100 ng**.



DV200 30%~50%



DV200 50%~70%



DV200 >70%

Figure 1. Agilent 2100 Bioanalyzer fragment size distribution of the FFPE Libraries from samples with different DV200 score

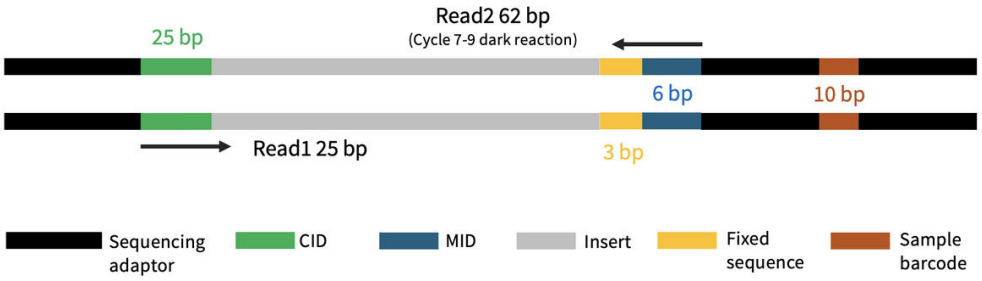
CHAPTER 3

LIBRARY CONSTRUCTION FOR Stereo-seq FFPE TRANSCRIPTOME LIBRARY

The structure of the Stereo-seq OMNI FFPE library structure is shown in the figure below.

Refer to the user manual of **DNBSEQ-G400RS Stereo-seq Visualization Reagent Set, 940-001886-00 (MGI)/940-001885-00 (CG)** or **DNBSEQ-T7RS Stereo-seq Visualization Reagent Set, 940-001895-00 (MGI)/940-001889-00 (CG)** for DNB preparation. Recommended library input is **60ng**.

Please read the corresponding user manual carefully before performing sequencing and strictly follow the instructions. If you have any questions about sequencing, please contact your local technical support for DNBSEQ platforms.



Use the following parameters to perform the sequencing run:

• Choose paired-ended mode with 25 cycles of Read 1 and 62 cycles of Read 2 with dark reaction of 7-9 bp, and then an additional 10 cycles of sample barcode.

Appendix A: PCR Barcode Primer Mix Use Rules

The Stereo-seq 16 Barcode Library Preparation Kit V1.0 provides 16 types of PCR Barcode Primer Mix, designed to meet the needs of high-throughput library construction for a large number of samples and multiplex sequencing of multiple samples. This kit is based on the design principle of base balance and has been tested by repeated experiments. To ensure the best results, please read the following usage rules carefully.

Any sample with the same barcode cannot be sequenced in the same lane.

Based on the base balancing principle, the PCR Barcode Primer Mix should be used in groups with the following grouping rules:

4 PCR Barcode Primer Mixes in groups: 1~4, 5~8, 9~12, 13~16, a total of 4 sets.

Make sure to centrifuge the PCR Barcode Primer Mix thoroughly before use. Gently open the lid during use, to prevent the liquid from splashing and avoid cross-contamination. Pipetting to mix well and shortly centrifuge while using. Close the lid in time after use.

N types of PCR Barcode Primer Mix Method: take the same volume of each; mix them and add the mixture to the sample.

When the data amount requirements for each sample are the same:

For different sample sizes, please refer to the recommended Barcode combinations shown below:

Sample/lane	Method 1	Method 2	Method 3	Method 4
1	1 to 4	5 to 8	9 to 12	13 to 16
2	Sample 1: 1 and 2 Sample 2: 3 and 4	Sample 1: 5 and 6 Sample 2: 7 and 8	Sample 1: 9 and 10 Sample 2: 11 and 12	Sample 1: 13 and 14 Sample 2: 15 and 16
3	Sample 1: 1 Sample 2: 2 Sample 3: 3 and 4	Sample 1: 5 Sample 2: 6 Sample 3: 7 and 8	Sample 1: 9 Sample 2: 10 Sample 3: 11 and 12	Sample 1: 13 Sample 2: 14 Sample 3: 15 and 16
4	Sample 1: 1 Sample 2: 2 Sample 3: 3 Sample 4: 4	Sample 1: 5 Sample 2: 6 Sample 3: 7 Sample 4: 8	Sample 1: 9 Sample 2: 10 Sample 3: 11 Sample 4: 12	Sample 1: 13 Sample 2: 14 Sample 3: 15 Sample 4: 16
5	Sample 1: 1 Sample 2: 2 Sample 3: 3 Sample 4: 4 Sample 5: select any group from the remaining three groups	Sample 1: 5 Sample 2: 6 Sample 3: 7 Sample 4: 8 Sample 5: select any group from the remaining three groups	Sample 1: 9 Sample 2: 10 Sample 3: 11 Sample 4: 12 Sample 5: select any group from the remaining three groups	Sample 1: 13 Sample 2: 14 Sample 3: 15 Sample 4: 16 Sample 5: select any group from the remaining three groups

Sample/lane	Method 1	Method 2	Method 3	Method 4
6	Sample 1: 1 Sample 2: 2 Sample 3: 3 Sample 4: 4 Sample 5 and 6: select any two groups from the remaining three groups	Sample 1: 5 Sample 2: 6 Sample 3: 7 Sample 4: 8 Sample 5 and 6: select any two groups from the remaining three groups	Sample 1: 9 Sample 2: 10 Sample 3: 11 Sample 4: 12 Sample 5 and 6: select any two groups from the remaining three groups	Sample 1: 13 Sample 2: 14 Sample 3: 15 Sample 4: 16 Sample 5 and 6: select any two groups from the remaining three groups
7	Sample 1: 1 Sample 2: 2 Sample 3: 3 Sample 4: 4 Sample 5 and 7: select groups by referencing the methods used for 3 samples/lane	Sample 1: 5 Sample 2: 6 Sample 3: 7 Sample 4: 8 Sample 5 and 7: select groups by referencing the methods used for 3 samples/lane	Sample 1: 9 Sample 2: 10 Sample 3: 11 Sample 4: 12 Sample 5 and 7: select groups by referencing the methods used for 3 samples/lane	Sample 1: 13 Sample 2: 14 Sample 3: 15 Sample 4: 16 Sample 5 and 7: select groups by referencing the methods used for 3 samples/lane
8	Select any two groups from the four groups.			
N= 9~16 **(N, number of mixed samples)**	Perform the following steps: 1. Classify samples 1 to 8 as a group, and add the PCR Barcode Primer Mix by referencing the methods used for 8 samples/lane. 2. Classify the remaining samples as a group, and correspondingly add different groups of PCR Barcode Primer Mix based on a value of (N-8) by referencing the methods used for 1 to 8 samples/lane.			

Examples of the mixture with different PCR Barcode Primer Mix:

Example one, 2 samples /lane (refer to Method 1):

1. Take 12.5 μL of PCR Barcode Primer Mix 1 and 2, mix them in equal volumes, and add them to Sample 1.
2. Add 12.5 μL of PCR Barcode Primer Mix 3 and 4 to Sample 2.

Example two, 13 samples/lane:

1. Add 25 μL of PCR Barcode Primer Mix 1 to Sample 1, 25 μL of PCR Barcode Primer Mix 2 to Sample 2, ..., and 25 μL of PCR Barcode Primer Mix 12 to Sample 12.
2. Take 6.25 μL of PCR Barcode Primer Mix 13, 14, 15, and 16, respectively, and mix them in equal volumes and add them to Sample 13.

When the library data amount requirements are different:

Libraries that require more than 20% data in a lane are required to use grouped PCR Barcode Primer Mix

Example:

If there are 9 samples pooled in a lane, and 1 of them requires 30% data, the following scheme is required: if the other 8 samples each use PCR Barcode Primer Mix 1~8, this sample should not use a single PCR Barcode Primer Mix, but use a non-duplicative and grouped PCR Barcode Primer Mix 9~12 or 13~16.