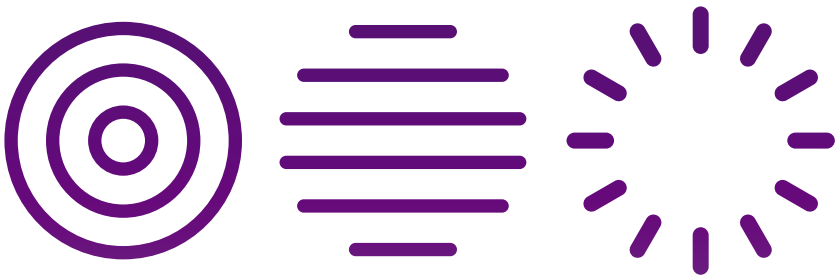
STOmics

GUIDE FOR rRNA REMOVAL IN Stereo-seq TRANSCRIPTOMICS APPLICATION OF SEQUOIA RIBODEPLETION KIT



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REVISION HISTORY

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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.

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

CHAPTER 1
INTRODUCTION

1.1. Background

In Stere-seq transcriptomics research, ribosomal RNA (rRNA) is the most abundant RNA type, typically accounting for 80-90% of total RNA in a sample. However, rRNA sequences do not provide valuable biological information for transcriptomic studies. Instead, they consume a significant portion of sequencing capacity, reducing the proportion of annotated reads and affecting the detection and quantification of other important transcripts. This issue is particularly pronounced in **Fresh Frozen (FF)** and **Formalin-Fixed Paraffin-Embedded (FFPE)** samples, where the high rRNA content poses a major challenge for transcriptomic analysis.

To improve the efficiency of **RNA sequencing (RNA-Seq)**, rRNA removal is a critical step that enables the enrichment of biologically relevant transcripts, enhances sequencing data quality, and reduces sequencing costs. By effectively depleting rRNA, the proportion of annotated reads is significantly increased, resulting in better transcript detection and more accurate biological insights.

1.2. Intended Use

To address the challenge of high rRNA content and to optimize sequencing performance for **FF and FFPE samples**, we use the **SEQuoia RiboDepletion Kit** from **Bio-Rad**. This kit employs specific probe hybridization and magnetic bead capture technology to efficiently remove abundant rRNA sequences from samples. By implementing this highly effective depletion strategy, the proportion of rRNA reads in sequencing data is significantly reduced, leading to a higher proportion of annotated reads, ultimately improving overall sequencing quality and data utility.

The SEQuoia RiboDepletion Kit is designed to target rRNA from **human**, **mouse** and **rat** samples, ensuring broad applicability across various **Stereo-seq transcriptomics library preparations**. The method is compatible with most library preparation workflows, making it a flexible and efficient solution for improving sequencing outcomes.

By integrating the **SEQuoia RiboDepletion Kit** into our workflow, we achieve cost-effective sequencing, improved data quality, and more accurate transcriptomic insights, enabling high-resolution spatial transcriptomic studies.

The operation steps in this guidance protocol are derived primarily from the instructions in this kit, but some steps have been adjusted according to the application requirements of Stereo-seq transcriptomics.

1.3. Applicable Species and Compatible Libraries

- **Applicable Species:** Designed for rRNA removal in **human**, **mouse** and **rat** samples.
- **Compatible Libraries:** Suitable for various Stereo-seq transcriptomic libraries, including **FF V1.3 library** and **FFPE library**.

1.4. Necessary Reagents from the Kits

Table 1-1 Kit Reagents

Reagent	Volume	Storage	Reagent Source
PCR Barcode Primer Mix	25 µL	-20°C	Stereo-seq 16 Barcode Library Preparation Kit (Cat.111KL160)
Amplification Mix	880 µL	-20°C	SEQuoia RiboDepletion Kit (Cat.17006487)
Probe Mix	110 µL	-20°C	SEQuoia RiboDepletion Kit (Cat.17006487)
Purification Beads	2.5 mL	4°C	SEQuoia RiboDepletion Kit (Cat.17006487)

This protocol uses only the reagents listed in the table within the SEQuoia RiboDepletion Kit and Stereo-seq 16 Barcode Library Preparation Kit.

1.5. Additional Equipment and Materials

Table 1-2 Recommended Equipment and Materials

Equipment		
Equipment and Consumables	**Recommended Brand**	**Cat. No.**
Thermal Cycler	Bio-Rad	/
Mini Centrifuge	Major Laboratory Supplier (MLS)	/
NEBNext® Magnetic Separation Rack	NEB	S1515S
Qubit® 3.0 Fluorometer	Thermo Fisher	Q33216
Vortex Mixer	MLS	-
2 °C to 8 °C Refrigerator	MLS	/
-25 °C to -15 °C Freezer	MLS	/

Table 1-3 Addition reagents required

Reagent		
Equipment and Consumables	**Recommended Brand**	**Cat. No.**
Nuclease-free Water	Ambion	AM9937
Anhydrous Ethanol (Analytical Grade)	MLS	/
SPRIselect Magnetic Beads	Beckman Coulter	B23317/B23318/ B23319
Qubit dsDNA HS Assay Kit	Invitrogen	Q32854
TE Buffer (pH 8.0)	Ambion	AM9858

Table 1-4 Additional consumables required

Consumables		
Equipment and Consumables	**Recommended Brand**	**Cat. No.**
0.2 mL PCR Tubes	Axygen	PCR-02-C
Filtered Pipette Tips (10 µL, 100 µL, 200 µL, 1000 µL)	Eppendorf	/
Qubit Assay Tubes	Invitrogen	Q32856

1.6. Precautions and Warnings

- This product is for **research use only** and **not intended for clinical diagnosis**. Please read this manual carefully before use.
- Before starting the experiment, familiarize yourself with the required instruments and ensure proper operation.
- The procedures described in this manual are **general guidelines**. Depending on specific experiment designs, sample characteristics, sequencing applications, and equipment, adjustments to experiment workflows and reaction parameters may be necessary to optimize performance and efficiency.
- Before use, remove all reagents from storage. **Centrifuge enzyme components briefly** and place them on ice, while allowing other components to thaw at room temperature. Gently invert several times to mix thoroughly, then briefly centrifuge and keep on ice until use.
- To prevent cross-contamination, use filter pipette tips and change tips between different samples.
- It is recommended that you **preheat the PCR thermal cycler** to the required reaction temperature before starting.
- Avoid direct contact of samples and reagents with skin and eyes. Do not ingest any samples or reagents. In case of accidental exposure, rinse immediately with plenty of water and seek medical attention if necessary.
- Dispose of all samples and laboratory waste in accordance with applicable regulations.

CHAPTER 2
RIBOSOMAL RNA (rRNA) REMOVAL PROCEDURE

2.1. Experiment Preparation

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

Reagent	Preparation Process	Storage
Library Products	Thaw on ice for 5 min	-20 °C
Amplification Mix	Thaw on ice for 5 min	-20 °C
Probe Mix	Thaw on ice for 5 min	-20 °C
PCR Barcode Primer Mix	Thaw on ice for 5 min	-20 °C
80% Ethanol	Prepare by diluting absolute ethanol to 80%	Store at room temperature for up to 1 day
Purification Beads	Remove from storage and equilibrate to room temperature	4 °C
SPRIselect beads	Remove from storage and equilibrate to room temperature	4 °C

Samples should use post-library construction products, refer to STUM-LP001 Stereo-seq OMNI FFPE Library Preparation User Manual, and STUM-LP002 Stereo-seq Transcriptomics FF Library Preparation User Manual.

2.2. First Hybridization and Capture

Reaction Mix Preparation

a. In a 0.2 mL PCR tube, prepare the reaction mix on ice according to Table 2-1.

Table 2-1 Hybridization Mix

Reagent	Volume (μL)
Amplification Mix	13.5
Probe Mix	2
Library Product (20 ng)	X
Nuclease-free Water	4.5-X
Total	20

The input library products volume X (μL) is calculated as:
X (μL) = Total library products (ng) / library products concentration (ng/μL)

b. Gently vortex the reaction mix for **5-10 sec**, then briefly centrifuge to collect the liquid at the bottom of the tube.

PCR Hybridization Reaction

a. Prepare the PCR thermal cycler and place the reaction tubes inside. Perform the reaction under the conditions listed in Table 2-2.

Table 2-2 PCR Program for Hybridization

Temperature	Time
(Heated lid) 105 °C	on
98°C	1 min
62°C	10 min
72°C	1 min
20°C	Hold

Magnetic Bead Capture of rRNA Fragments

a. Transfer **50 μL** of Purification Beads into a new 0.2 mL PCR tube, ensuring that the beads are evenly resuspended before use.

b. Place the PCR tube on a magnetic rack and let it stand for approximately **1 min,** until the beads have fully aggregated and the supernatant is clear.

c. Remove the supernatant, take the PCR tube off the magnetic rack, then add all PCR product to the bead-containing tube. Pipette up and down gently to ensure thorough mixing and binding.

d. Incubate the PCR tube at room temperature for **15 min**, flicking the tube every **5 min** to mix the beads and solution evenly.

e. Briefly centrifuge the tube to collect the beads at the bottom, then place the tube on the magnetic rack and let it stand for **1-2 min** until the solution is completely clear.

f. Gently remove **18 μL** of the clear supernatant without disturbing the beads, then transfer it to a fresh 0.2 mL PCR tube.

2.3. Second Hybridization and Capture

Second Hybridization Reaction Mix Preparation

a. In a 0.2 mL PCR tube, prepare the reaction mix on ice according to Table 2-3.

Table 2-3 Hybridization Mix

Reagent	Volume (μL)
Amplification Mix	8
Probe Mix	2
Library Product (from step 2.2)	18
Nuclease-free Water	2
Total	30

b. Gently vortex the reaction mix for **5-10 sec**, then briefly centrifuge to collect the liquid at the bottom of the tube.

PCR Hybridization Reaction

a. Prepare the PCR thermal cycler and place the reaction tubes inside. Perform the reaction under the conditions listed in Table 2-2.

Magnetic Bead Capture of rRNA Fragments

a. Transfer **50 μL** of Purification Beads into a new 0.2 mL PCR tube, pipetting gently to ensure that the beads are evenly resuspended.

b. Place the PCR tube on a magnetic rack and let it stand for **1 min**, until the beads have fully aggregated and the supernatant is clear.

c. Remove the supernatant, take the PCR tube off the magnetic rack, then add all PCR product to the bead-containing tube. Pipette up and down gently to ensure thorough mixing and binding.

d. Incubate the PCR tube at room temperature for **15 min**, flicking the tube every **5 min** to mix the beads and solution evenly.

e. Briefly centrifuge the tube to collect the beads at the bottom, then place it on the magnetic rack and let it stand for **1-2 min** until the solution is completely clear.

f. Carefully pipette out **27 μL** of the supernatant, avoiding any disturbance to the beads, and transfer it into a new 0.2 mL PCR tube.

2.4. PCR Amplification and Purification

PCR Amplification Mix Preparation

a. In a 0.2 mL PCR tube, prepare the reaction mix on ice according to Table 2-4.

Table 2-4 PCR Mix

Reagent	Volume (μL)
Amplification Mix	12
PCR Barcode Primers Mix	11
Library Product (from step 2.3)	27
Total	50

Refer to the STOmics Library Preparation User Manual for PCR barcode primer mix preparation.

b. Gently vortex the reaction mix for **5-10 sec,** then briefly centrifuge to collect the liquid at the bottom of the tube.

PCR Amplification Reaction

a. Prepare the PCR thermal cycler and place the reaction tubes inside. Run the amplification reaction according to the corresponding conditions based on the sample type:

For **FFPE samples**, refer to Table 2-5.

For **FF samples using the V1.3 protocol**, refer to Table 2-6.

Table 2-5 PCR Program for FFPE Samples

Temperature	Time	Cycles
(Heated lid) 105°C	on	-
98°C	1 min	1
98°C	10 sec	7
61°C	15 sec	
72°C	20 sec	
72°C	2 min	1
4°C	Hold	-

Table 2-6 PCR program for FF Samples (V1.3 Protocol)

Temperature	Time	Cycles
(Heated lid) 105°C	on	-
95°C	5 min	1
98°C	20 sec	7
58°C	20 sec	
72°C	3 min	
72°C	5 min	1
4°C	Hold	-

Magnetic Bead Purification (0.8x SPRI Cleanup)

Prepare **0.8x** magnetic bead purification on the PCR reaction product using the following steps:

a. Mix the PCR product (**50 μL**) with Purification Beads at a 1:0.8 ratio (**40 μL** beads) that have been equilibrated to room temperature. Gently vortex or pipette to mix thoroughly, then incubate at room temperature for 10 min.

b. Briefly centrifuge, then place the PCR tube on a magnetic rack and let it stand for **3-5 min**, until the liquid is clear. Carefully remove the supernatant.

c. Wash with **200 μL** of 80% ethanol. Use freshly prepared ethanol that has been equilibrated to room temperature. During washing, the tube should remain on the magnetic rack, and the pipette tip should be placed against the tube wall away from the magnetic beads. Do not pipette up and down or disturb the beads.

d. Remove the supernatant and **repeat step c.** for a second ethanol wash.

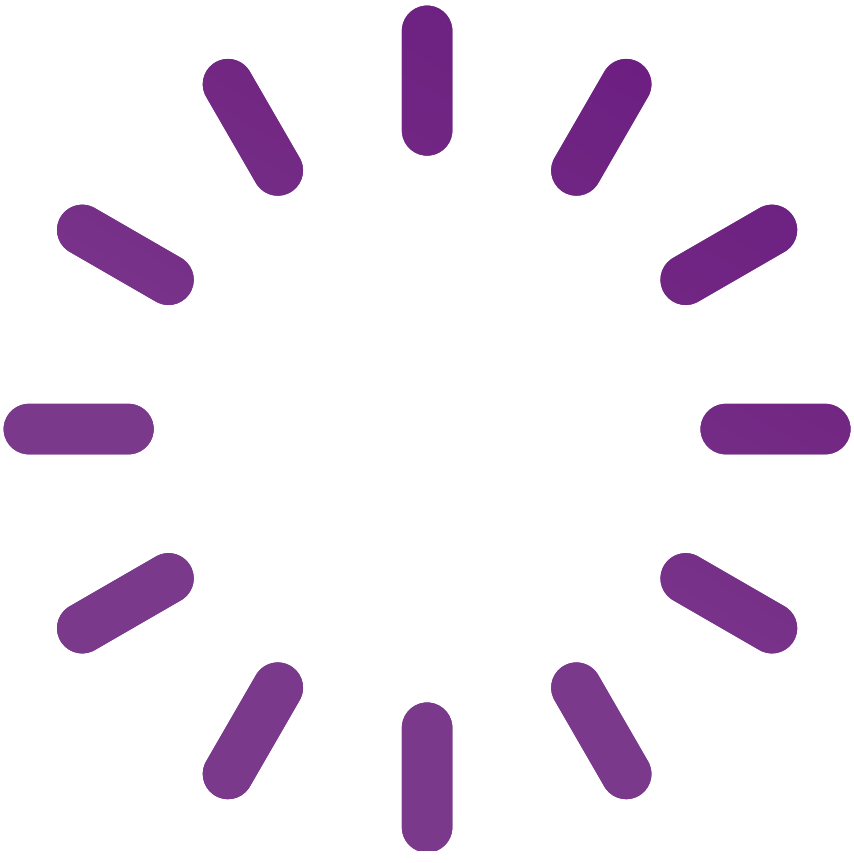
e. Remove the remaining ethanol and allow the beads to air-dry for **5-8 min** at room temperature, until the bead surface appears matte (non-reflective) and free of cracks.

f. Resuspend the purified DNA in **27 μL** of TE Buffer(pH 8.0). Gently vortex or pipette to mix, then incubate for **5 min** at room temperature. Briefly centrifuge, place on the magnetic rack for **3-5 min**, and recover the clear supernatant.

g. Take **1 μL** of the eluted product and measure the DNA concentration using the Qubit dsDNA Assay Kit. The expected concentration should typically be ≥5 ng/μL.

The purified PCR product can be stored long-term at -20°C.

CHAPTER 3
DNB Preparation



For DNB preparation, refer to the **DNBSEQ-G400RS Stereo-seq Visualization Reagent Set User Manual, 940-001886-00 (MGI)/940-001885-00 (CG)** or the **DNBSEQ-T7RS Stereo-seq Visualization Reagent Set User Manual, 940-001895-00 (MGI)/940-001889-00 (CG)**.

Follow the instructions in the respective manuals to prepare DNBs for sequencing.