

I. Introduction

This protocol provides instructions for performing single-nucleus RNA-seq on the BD Rhapsody platform after performing the Trekker® workflow and nuclei isolation using the [Trekker Single-Cell Spatial Mapping Kit User Manual](#). The final library products include the single nucleus whole transcriptome library, which contains the gene expression information, and the Trekker library, which contains the spatial location of each nucleus.

II. Required Materials

This protocol applies to the following Takara Bio products:

- Trekker U 10x10 Bundle (Cat. No. SK017)

Additional Materials Required (Not Provided):

- RNase Inhibitor, Murine, 40,000 U/ml (NEB, M0314)
- Proteinase K, 800 U/ml (NEB, P8107S)
- BD Rhapsody WTA Reagent Kit - 8 Pack (BD Biosciences, 666620)
- Other kits, reagents and equipment listed by the vendor (BD Biosciences)
- DNA oligonucleotide primers should be ordered with desalted purification. If desired, primers can be ordered resuspended to a stock concentration of 100 µM in 1X TE Buffer, pH 8.0 or lyophilized. If primers are ordered lyophilized, resuspend primers to a stock concentration of 100 µM in 1X TE Buffer, pH 8.0 using best practices.

Table 1. Trekker PCR Primer and Index Primer sequences.

Name	Sequence	Concentration
Trekker PCR Primer	GTGACTGGAGTTCAGACGT	100 µM
Trekker i7 Index Primer	CAAGCAGAAGACGGCATAACGAGATXXXXXXXXXX GTGACTGGAGTTCAGACGTGT	10 µM

- Refer to the table below for recommended index sequences for Trekker i7 Index Primer.

Table 2. Recommended index sequences for Trekker i7 Index Primer.

Index name	Sequence in adapter
Nextera® N709	AGCGTAGC
Nextera N710	CAGCCTCG
Nextera N711	TGCCTCTT
Nextera N712	TCCTCTAC
TruSeq® D701	CGAGTAAT
TruSeq D702	TCTCCGGA
TruSeq D703	AATGAGCG
TruSeq D704	GGAATCTC

III. Protocol

A. Single nucleus RNA-seq on BD Rhapsody



IMPORTANT: The guidelines below are intended for use with the Rhapsody HT Single-Cell Analysis System. If you are using the non-HT version of the Rhapsody System, adjust volumes accordingly.

1. Perform single nuclei capture following instructions for Single-Cell Capture and cDNA Synthesis as described in the [Rhapsody HT Single-Cell Analysis System - Single-Cell Capture and cDNA Synthesis Protocol](#) (PDF, Rev. 23-24252(01)) with the following modifications based on the BD technical note [Processing nuclei samples on the BD Rhapsody Single-Cell Analysis System](#) (PDF, Rev. 23-23161-00):

- a. Before beginning, make Sample buffer-RI following the table below:

Sample buffer-RI (1 tile sample):

10 µl	RNase inhibitor
2,000 µl	Sample buffer
2,010 µl	Total volume

- b. Use Sample buffer-RI throughout the BD Rhapsody Cartridge workflow before lysis, including the sections:
 - "Counting and preparing single-cell suspension for cartridge loading"
 - "Preparing BD Rhapsody Enhanced cell capture beads"
 - "Loading and washing BD Rhapsody Enhanced cell capture beads"



IMPORTANT: Keep the isolated nuclei in Sample buffer-RI on ice or at 4 °C during the process. Use ice-cold buffer for all steps. Avoid unnecessary pipetting.

- c. In the protocol section "Lysing cells", follow the recommended modifications:
 - After adding DTT to the lysis buffer in step 1, set aside an aliquot in a new Eppendorf tube and add proteinase K following the table below.

400 µl	Lysis buffer with DTT
20 µl	Proteinase K
420 µl	Total volume
 - At step 5, load the cartridge with lysis buffer + DTT containing Proteinase K (The addition of Proteinase K is not needed for the lysis buffer used in step 6 of "Retrieving BD Rhapsody Enhanced Cell Capture Beads").
 - At step 6, incubate the cartridge at room temperature for 5 minutes
2. Perform library prep following instructions for [BD Rhapsody System mRNA Whole Transcriptome Analysis \(WTA\) and BD AbSeq Library Preparation Protocol](#) (PDF, Rev. 23-24118(02)). Follow the instructions for AbSeq library construction to generate the Trekker library, with the following modifications:

Trekker® Library Preparation with the BD Rhapsody WTA Kit Protocol-At-A-Glance

- a. In Step 1 of the "Performing AbSeq PCR1" section, make the PCR1 reaction mix according to the table below.

PCR1 reaction mix:

100 µl	PCR MasterMix (BD)
10 µl	Universal Oligo (BD)
4 µl	Trekker PCR Primer (100 µM)
18 µl	Nuclease-free water
<hr/>	
132 µl	Total volume per reaction

- b. In the "Quantifying BD AbSeq PCR1 products" section, the expected product size is ~174 bp. See Figure 1 for an example library trace on the Tapestation (D5000):

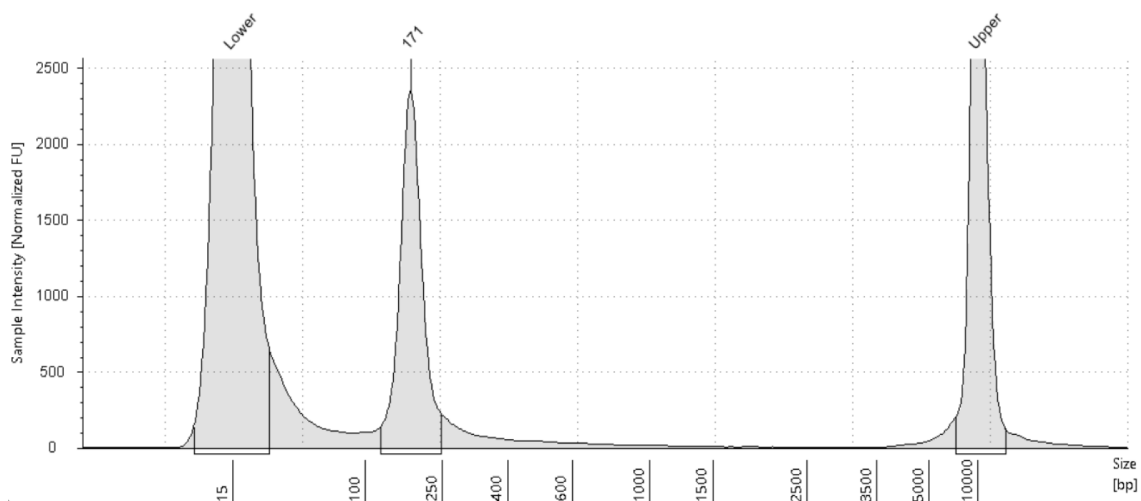


Figure 1. Example Tapestation library trace.

- c. In Step 1 of the "Performing AbSeq index PCR" section, make the index PCR mix according to the table below:

Index PCR mix:

25 µl	PCR MasterMix (BD)
2 µl	Library Forward Primer (BD)
1 µl	Trekker i7 Index Primer (10 µM)
19 µl	Nuclease-free water
<hr/>	
47 µl	Total volume per reaction

- d. In Step 14 of the "Purifying AbSeq index PCR products" section, the expected product size is ~256 bp. See Figure 2 for an example library trace on the Tapestation (D5000):

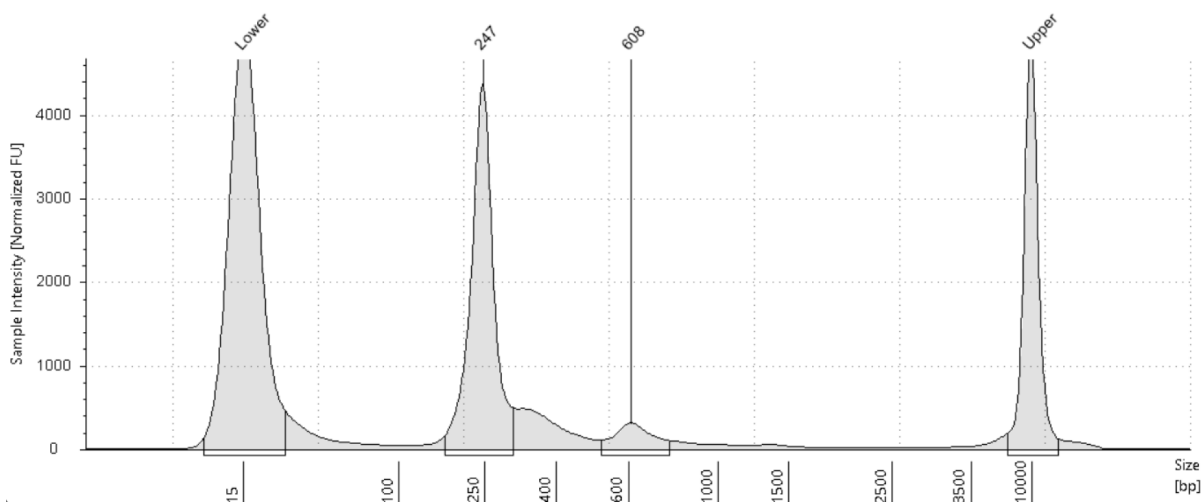


Figure 2. Example Tapestation library trace.

B. Sequencing

1. Sequence the gene expression library according to the BD Rhapsody user guide listed in Section III.A, Step 2 of this protocol.
2. Sequence the Trekker library at ~1,000 read pairs per nucleus captured. For example, if 10,000 nuclei were captured, allocate 1×10^7 reads for the Trekker library.

Table 3. Read lengths for BD Rhapsody WTA prep.

Sequencing read	Recommended read length (bp)
Read 1	70
i7 index	8
i5 index	8
Read 2	50

3. If the Trekker library is sequenced on its own, additional PhiX spike-in may be needed. If the Trekker library is sequenced with gene expression libraries, follow the sequencing recommendations in the Parse Evercode WT v3 User Guide for PhiX spike-in.
 - NextSeq 1000/2000: 10% PhiX spike-in when pooling with only Trekker libraries
 - NextSeq 500/550: 10% PhiX spike-in when pooling with only Trekker libraries
 - NovaSeq 6000 and Novaseq X:
 - 5% PhiX spike-in when pooling with non-Trekker libraries
 - 10% PhiX spike-in when pooling with only Trekker libraries

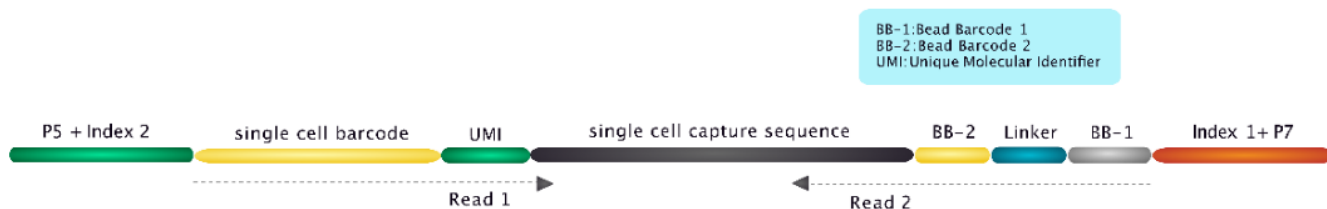


Figure 3. Trekker library structure.

IV. Bioinformatics

For details on data processing and output interpretation, refer to the [Trekker Bioinformatics Pipeline User Manual](#), which can be found at takarabio.com.

Appendix. Troubleshooting Guide

Table 4. Troubleshooting guide.

Problem	Possible Explanation	Solution
Trekker final library missing expected peak	UV cleavage unsuccessful	Ensure UV lamp settings are correct and the UV lamp is functioning
	Incorrect primers were used in library prep	Ensure correct primers and cycle numbers were used

Contact Us	
Customer Service/Ordering	Technical Support
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
web: takarabio.com/service	web: takarabio.com/support
e-mail: ordersUS@takarabio.com	e-mail: technical_support@takarabio.com

Notice to Purchaser

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

© 2026 Takara Bio Inc. All Rights Reserved.

All trademarks are the property of Takara Bio Inc. or its affiliate(s) in the U.S. and/or other countries or their respective owners. Certain trademarks may not be registered in all jurisdictions. Additional product, intellectual property, and restricted use information is available at takarabio.com.

This document has been reviewed and approved by the Quality Department.