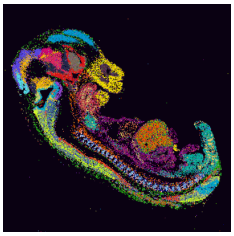

Trekker™ Library Preparation with BD Rhapsody scTCR/BCR Next and mRNA Whole Transcriptome Analysis User Guide

I. Introduction

This protocol provides instructions for performing single-nucleus TCR/BCR-Seq and mRNA-seq on the BD Rhapsody platform after performing the workflow and nuclei isolation in the Trekker U Single-Cell Spatial Mapping Kit User Manual. The final library products include the WTA library, the TCR and/or BCR library, and the Trekker library, which contains the spatial location of each nucleus.

NOTE: This protocol is provided for general information only and has not been validated by Takara Bio.

II. Additional Materials Required (Not Provided)

Trekker PCR Primer and Index Primer Sequences

Name	Sequence	Concentration
Trekker PCR Primer	GTGACTGGAGTTCAGACGT	100 µM
Trekker i7 Index Primer	CAAGCAGAAGACGGCATACGAGAT **XXXXXXXX**GTGACTGGAGTTCAGACGTGT	10 µM

Refer to the Illumina® Index (i7) sequences table in Appendix C of the BD Rhapsody Single-Cell Analysis System User Guide for recommended index sequences for Trekker i7 Index Primers.

Reagents

- BD Rhapsody WTA Reagent Kit - 8 Pack (BD Biosciences, P/N 666620)
- Choose one of the following:
  - Human: BD Rhapsody TCR/BCR Next Amplification Kit (BD Biosciences, P/N 667058)
  - Mouse: BD Rhapsody Mouse TCR/BCR Next Amplification Kit (BD Biosciences, P/N 667059)

- Other kits, reagents and equipment listed in the BD Rhapsody User Guides: Human, Mouse

III. Protocol

A. Single Nuclei Capture

Perform single nuclei capture as described in the Rhapsody HT Single-Cell Analysis System - Single-Cell Capture and cDNA Synthesis Protocol (23-24252(01)), with the following modifications based on the BD technical note 'Processing nuclei samples on the BD Rhapsody Single-Cell Analysis System':

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

Sample buffer-RI:	
10 µl	RNase inhibitor
2,000 µl	Sample buffer
2,010 µl	**Total per sample**

2. Use the Sample buffer-RI throughout the BD Rhapsody Cartridge workflow before lysis, including for 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.

3. In the protocol section, "Lysing cells":

   a. After adding DTT to the lysis buffer (Lysis Buffer + DTT) in Step 1, add an aliquot of the mixture to a new Eppendorf tube and add Proteinase K, following the table below:

400 µl	Lysis Buffer + DTT
20 µl	Proteinase K
420 µl	**Total per sample**

   - In Step 5, load the cartridge with Lysis Buffer + DTT + Proteinase K.

NOTE: The addition of Proteinase K is not needed for the Lysis Buffer used in Step 6 of "Retrieving BD Rhapsody Enhanced Cell Capture Beads".

   - In Step 6, incubate the cartridge at room temperature for 5 min.

B. Perform Reverse Transcription, Template Switching, and Exonuclease I Treatment and Library Preparation

Perform reverse transcription, template switching and exonuclease I treatment and library preparation as described in the appropriate protocol:

- Human: BD Rhapsody TCR/BCR Next, mRNA Whole Transcriptome Analysis (WTA), and BD AbSeq Library Preparation Protocol (23-24510(01))

- Mouse: BD Rhapsody Mouse TCR/BCR Next, mRNA Whole Transcriptome Analysis (WTA), and BD AbSeq Library Preparation Protocol (23-24518(01))

(OPTIONAL) To enhance TCR/BCR detection, you may modify Step 8 in Section 1.1, (cDNA synthesis and template switching) of the protocol by extending the reverse transcription incubation time from 30 min to 90 min.

C. Generate the Trekker Library

Follow the instructions for AbSeq library amplification to generate the Trekker library, with the following modifications:

1. In Step 1 of the 'AbSeq PCR1' section, make the AbSeq PCR1 Mix according to the table below:

AbSeq PCR1 Mix:

100 µl	PCR MasterMix (BD)
10 µl	Universal Oligo (BD)
4 µl	Trekker PCR Primer (100 µM)
18 µl	Nuclease-Free Water
132 µl	**Total per reaction**

2. In the ' AbSeq index PCR cleanup and quality check' section, the expected Trekker product size is ~174 bp. See Figure 1 for an example library trace on the Tapestation (D5000):

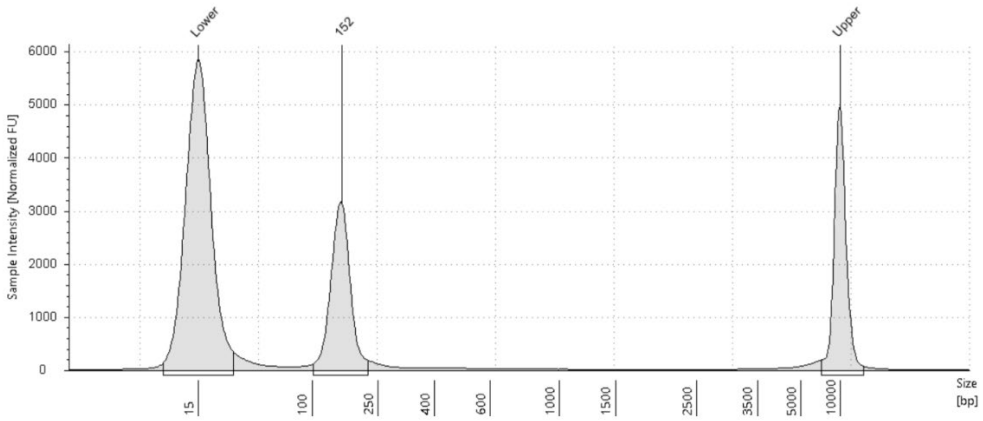

Figure 1. Example Tapestation D5000 library trace for quantifying Trekker PCR1 products.

3. In Step 1 of the '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
47 µl	**Total per reaction**

4. In Step 16 of the 'Purifying AbSeq index PCR products' section, the expected Trekker product size is ~256 bp. See Figure 2 for an example Trekker library trace on the Tapestation (D5000):

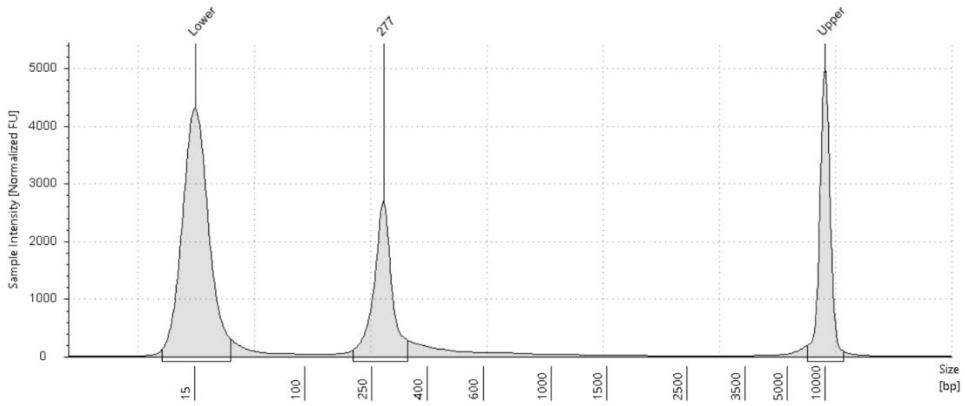

Figure 2. Example Tapestation D5000 library trace after purifying Trekker index PCR products.

D. Sequencing

- Sequence the WTA and TCR/BCR libraries according to the BD Rhapsody user guide.
- Sequence the Trekker library at ~1,000 read pairs per nucleus captured. For example, if 10,000 nuclei were captured, allocate 1 x 10^7 reads for the Trekker library.
  - Read 1: 70 bp
  - i7 index: 8 bp
  - i5 index: 8 bp
  - Read 2: 50 bp

IV. Bioinformatics

For details on data processing and output interpretation, refer to the Trekker Primary Analysis Pipeline for local analysis, and the Takara Bio Spatial Bioinformatics Portal for cloud analysis, which can both be found at takarabio.com.

Products

Cat. #	Product	Size
SK017	Trekker U 10x10 Bundle	4 Rxns

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
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