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

## 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 | CAAGCAGAAGACGGCATACGAGAT**XXXXXXXX**<br>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 Analaysis 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:

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 |
| **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):

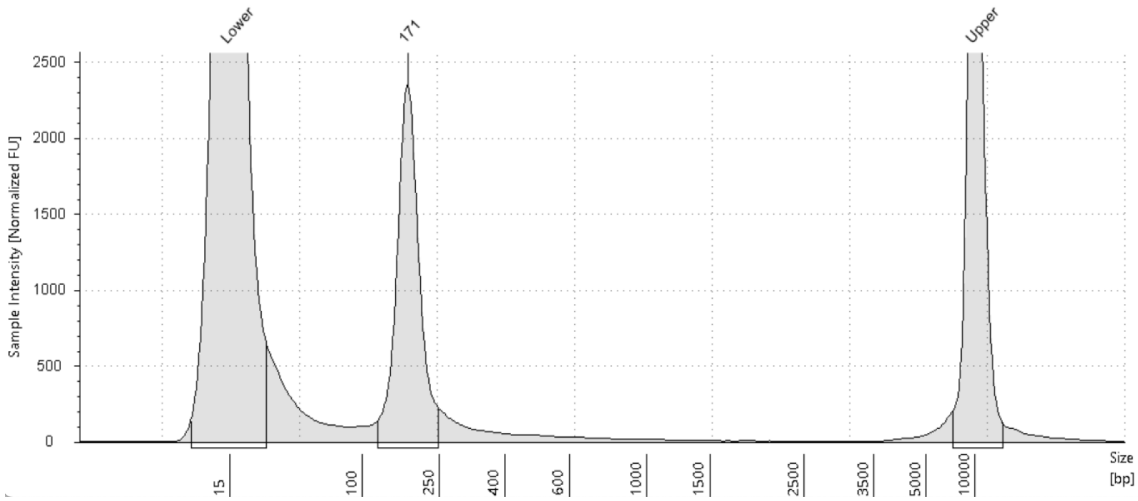


**Figure 1. Example Tapestation library trace.**

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 |
| **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):

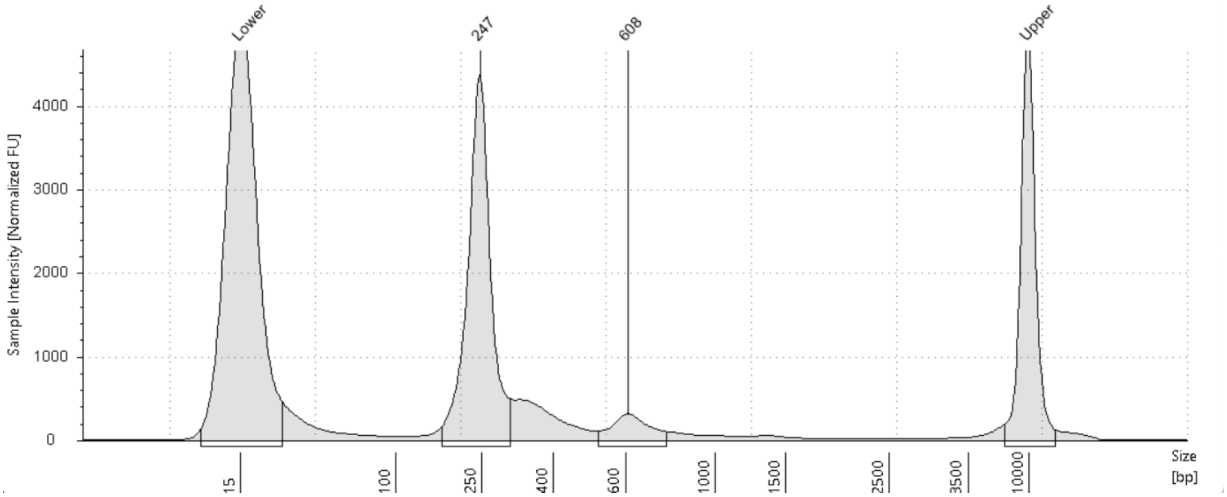


Figure 2. Example Tapestation library trace.

## 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 x $10^7$ 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 |

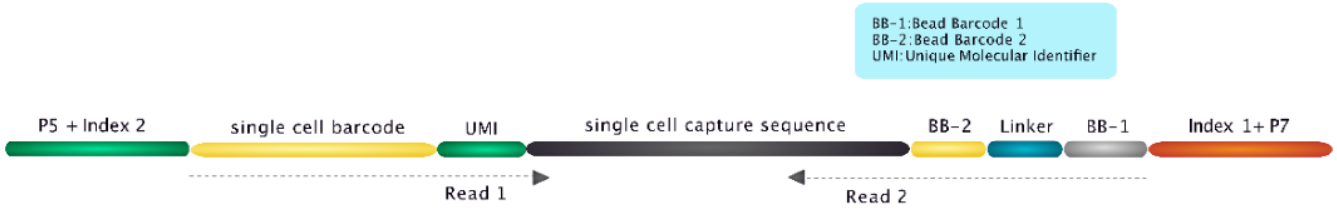


Figure 3. Trekker library structure.

# 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 |



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