# Trekker U Library Preparation with BD Rhapsody Single-Cell ATAC-Seq and mRNA WTA Kit Protocol-At-A-Glance

## I. Introduction

This demonstrated protocol provides instructions for performing single-nucleus ATAC-Seq (**A**ssay for **T**ransposase-**A**ccessible **C**hromatin using Sequencing) and mRNA-seq using the Trekker™ Single-Cell Spatial Mapping Kit (Cat. No. SK017) workflow described in the Trekker U Single-Cell Spatial Mapping Kit User Manual and the BD Rhapsody platform. The final library products include the whole transcriptome analysis (WTA) library, the ATAC library, 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):

- BD Rhapsody WTA Reagent Kit – 8 Pack (BD Biosciences, 666620)
- BD Rhapsody Tagmentation and Supplemental Reagents Kit (BD Biosciences, 41926)
- BD Rhapsody Multiomic ATAC-Seq Amplification Kit (BD Biosciences, 41928)
- Other kits, reagents and equipment listed by the vendor (BD Biosciences)
- Refer to the Illumina® Index (i7) sequences table in the 'Sequencing' section of the BD Rhapsody System Single-Cell ATAC-Seq and mRNA Whole Transcriptome Analysis Library Preparation Protocol (PDF, Rev. 23-24474(01)) for recommended index sequences for Trekker i7 Index Primers.

**Table 1. Trekker U PCR Primer and Index Primer sequences.**

| Name | Sequence | Concentration |
|---|---|---|
| Trekker U PCR Primer | GTGACTGGAGTTCAGACGT | 100 µM |
| Trekker i7 Index Primer | CAAGCAGAAGACGGCATACGAGAT**XXXXXXXX**<br>GTGACTGGAGTTCAGACGTGT | 10 µM |

## III. Protocol

### A. Single-Nuclei Capture and Library Prep

Perform single-nuclei capture and preparation of WTA and ATAC libraries following the instructions in BD Rhapsody System Single-Cell ATAC-Seq and mRNA Whole Transcriptome Analysis Library Preparation Protocol (PDF, Rev. 23-24118(02)) user guide.

### B. Trekker PCR1 on post-RPE Rhapsody beads

1. Make the Trekker PCR1 Reaction Mix according to the table below and keep on ice:

**Trekker index PCR reaction mix (per reaction):**

| | |
|---|---|
| 100 µl | PCR MasterMix (BD) |
| 10 µl | Universal Oligo (BD) |
| 4 µl | Trekker U PCR Primer (100 µM) |
| 12 µl | Bead RT/PCR Enhancer (BD) |
| 74 µl | Nuclease-free water |
| **200 µl** | **Total per reaction** |

2. Place the tube of beads saved from Step 20 of "Performing random priming and extension (RPE) on BD Rhapsody Enhanced Cell Capture Beads with cDNA" on a 1.5 ml magnet for <2 min.
3. Remove and discard the supernatant.
4. Remove the beads from the magnet and resuspend the beads in 200 µl of the Trekker PCR1 Reaction Mix.
5. Ensuring that the beads are fully resuspended, pipette 50 µl of the Trekker PCR1 Reaction Mix with beads into each of four 0.2 ml PCR tubes.
6. Run the Trekker PCR1 program in a preheated thermal cycler as follows:

| | | |
|---|---|---|
| 95°C | | 3 min |
| 10–14 cycles: | | |
| | 95°C | 30 sec |
| | 60°C | 30 sec |
| | 72°C | 1 min |
| 72°C | | 5 min |
| 4°C | | Hold |

**Table 1. Suggested number of PCR1 cycles.**

| Number of nuclei | PCR cycles |
|---|---|
| 1,000 | 14 |
| 2,500 | 13 |
| 5,000 | 12 |
| 10,000 | 11 |
| 20,000 | 10 |

**SAFE STOPPING POINT:** PCR can run overnight.

## C. Purify Trekker PCR1 Products

1. Prepare fresh 80% ethyl alcohol.
2. Combine the Trekker PCR1 products in a new 1.5 ml tube.
3. Place the tube on the magnet and transfer the supernatant to a new tube.
4. (Optional) Resuspend remaining BD Rhapsody beads in 200 µl cold Bead Resuspension Buffer and store at 4°C.
5. Vortex the AMPure XP magnetic beads for 30 sec and carefully add 280 µl (1.4X) to the tube containing Trekker PCR1 products.
6. Vortex to mix for 10–15 sec.
7. Incubate at room temperature for 5 min.
8. Briefly centrifuge the tubes and place the tubes on the magnetic rack. Once the solution is clear, carefully aspirate, and discard the supernatant.
9. Keeping the tube on the magnetic stand, add 500 µl of 80% ethyl alcohol.
10. Wait 30 sec and remove the supernatant.
11. Repeat Steps 9 and 10 for a total of two washes.
12. Briefly spin the tube to collect the remaining ethyl alcohol at the bottom of the tube.
13. Place the tube back on the magnetic rack and remove the remaining ethyl alcohol carefully.
14. Let the AMPure XP magnetic beads dry at room temperature until the beads appear matte (~5 min).

15. Remove the tube from the magnetic rack and add 30 µl of Elution Buffer to the tube and pipette the beads to mix.

**NOTE:** Beads may not fully resuspend.

16. Incubate at room temperature for 2 min.
17. Place the tube back on the magnetic rack.
18. Once the solution is clear, transfer the supernatant to a new 1.5 ml tube.

## D. Quantify Trekker PCR1 Products

1. Measure the yield of the Trekker PCR1 product using the Agilent Bioanalyzer with the High Sensitivity DNA Kit or the Agilent TapeStation system using the Agilent High Sensitivity (D1000 or D5000) ScreenTape assay. Follow the manufacturer's instructions.

**NOTE:** The expected size is ~174 bp. See Figure 1 (below) for an example library trace on the TapeStation (D5000).

2. After quantifying, dilute an aliquot of Trekker PCR1 products to 0.1–1.1 ng/µl with Elution Buffer for use in the next section.

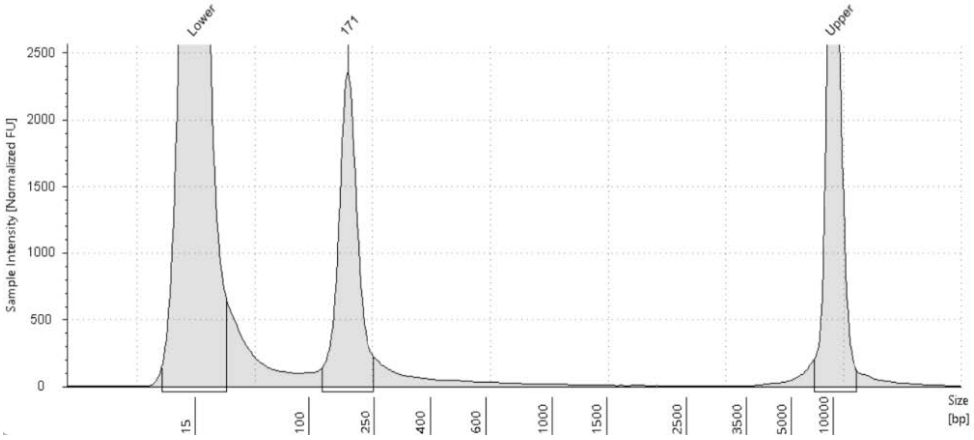


**Figure 1. Example TapeStation (D5000) trace of the Trekker PCR1 products.**

## E. Perform Trekker Index PCR

1. Make the Trekker Index PCR Mix according to the table below:

**Trekker 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** |

2. In a new 0.2 ml PCR tube, combine 47 µl of Trekker Index PCR Mix with 3 µl of diluted Trekker PCR1 products from Section III.D, Step 2.

3. Run the Trekker Index PCR program in a preheated thermal cycler as follows:

| | | |
|---|---|---|
| 95°C | | 3 min |
| 6–8 cycles: | | |
| | 95°C | 30 sec |
| | 60°C | 30 sec |
| | 72°C | 30 sec |
| 72°C | | 1 min |
| 4°C | | Hold |

**Table 2. Suggested number of Index PCR cycles.**

| Trekker library (ng/µl) | PCR cycles |
|---|---|
| 0.5–1.1 | 6 |
| 0.25–0.5 | 7 |
| 0.1–0.25 | 8 |

## F. Purify Trekker Index PCR Products

1. Prepare fresh 80% ethyl alcohol.
2. Vortex the AMPure XP magnetic beads for 30 sec and carefully add 40 µl (0.8X).
3. Vortex to mix for 10–15 sec.
4. Incubate at room temperature for 5 min.
5. Briefly centrifuge the tubes and place the tubes on the magnetic rack. Once the solution is clear, carefully aspirate, and discard the supernatant.
6. Keeping the tube on the magnetic stand, add 200 µl of 80% ethyl alcohol.
7. Wait 30 sec and remove the supernatant.
8. Repeat Steps 6 and 7 for a total of two washes.
9. Briefly spin the tube to collect the remaining ethyl alcohol at the bottom of the tube.
10. Place the tube back on the magnetic rack and remove the remaining ethyl alcohol carefully.
11. Let the AMPure XP magnetic beads dry at room temperature until the beads appear matte (~3 min).
12. Remove the tube from the magnetic rack and add 10 µl of Elution Buffer to the tube and pipette the beads to mix.
13. Incubate at room temperature for 2 min.
14. Place the tube back on the magnetic rack.
15. Once the solution is clear, transfer the supernatant to a new 1.5 ml tube.

## G. Quantify Trekker Index PCR Products

Measure the yield of the Trekker index PCR products using the Agilent Bioanalyzer with the High Sensitivity Kit or the Agilent TapeStation system using the Agilent High Sensitivity (D1000 or D5000) ScreenTape assay. Follow the manufacturer's instructions. The expected size is ~256 bp. See Figure 2 for an example library trace on the TapeStation system.

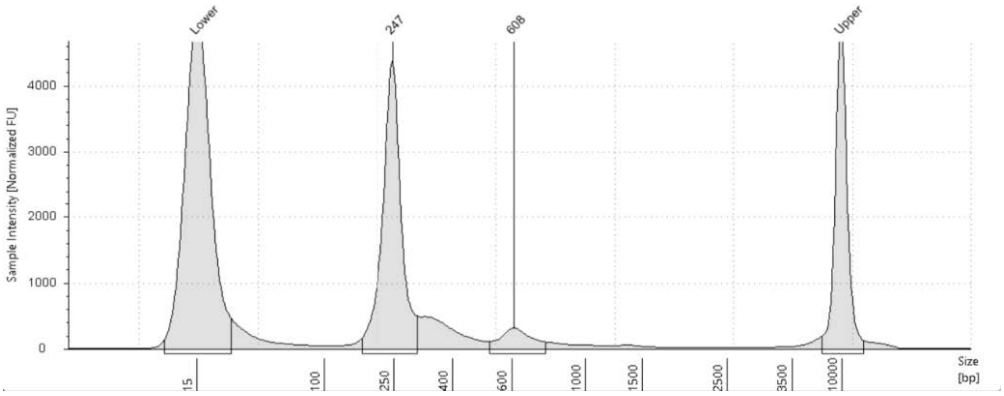


**Figure 2. Example library trace on the TapeStation system (D5000).**

## H. Sequencing

- Sequence the WTA and ATAC 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.

**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 |

# IV. Bioinformatics

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

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