Trekker U Library Preparation with 10x Multiome Kit Protocol-At-A-Glance

I. Introduction

This protocol provides instructions for performing single-nucleus ATAC-seq (**A**ssay for **T**ransposase-**A**ccessible **C**hromatin) and RNA-seq on the 10x Chromium platform after performing the Trekker™ workflow and nuclei isolation using the Trekker U Single-Cell Spatial Mapping Kit User Manual. The final products include the ATAC library, single-nucleus gene expression library, and the Trekker library, which contains the spatial location of each nucleus.

Compatible with the 10x Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338 Rev G).

II. Required Materials

This protocol applies to the following Takara Bio products:

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

Additional Materials Required (Not Provided):

- Chromium Next GEM Single Cell Multiome ATAC Kit A (10x Genomics, Cat. No. 4 rxns: PN-1000281, 16 rxns: PN-1000280)
- Chromium Next GEM Single Cell Multiome Reagent Kit A (10x Genomics, Cat. No. 4 rxns: PN-1000284, 16 rxns: PN-1000282)
- Library Construction Kit (10x Genomics, Cat. No. 4 rxns: PN-1000279, 16 rxns: PN-1000190)
- Single Index Kit N Set A (10x Genomics, Cat. No. 96 rxns: PN-1000212)
- Dual Index Kit TT Set A (10x Genomics, Cat. No. 96 rxns: PN-1000215)
- Chromium Next GEM Chip J Single Cell Kit (10x Genomics, Cat. No. 16 rxns: PN-1000230, 48 rxns: PN-1000234)
- Other kits, reagents and equipment listed by the vendor (10x Genomics)
- NEBNext High-Fidelity 2X PCR Master Mix (NEB, Cat. No. M0541S)
- 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 U PCR Primer sequence.

Name	Sequence	Concentration
Trekker U PCR Primer	GTGACTGGAGTTCAGACGT	10 µM

III. Protocol

A. Single-nucleus ATAC-seq and RNA-seq on 10x Chromium

1. Perform transposition, GEM generation & barcoding, GEM incubation cleanup, pre-amplification, ATAC library and GEX library construction following instructions in Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338 Rev G) with the following modifications:

a. Make a dilution of the Trekker PCR Primer by combining 2 µl of 10 µM Trekker PCR Primer and 60.8 µl of nuclease-free water (0.329 µM final concentration).
b. In step 4.1, spike-in 1 µl of diluted (0.329 µM) Trekker PCR Primer.

2. Perform Trekker index PCR:
   a. Choose the appropriate sample index sets from Dual Index Plate TT (PN-3000431) to ensure no sample indices overlap in a multiplexed sequencing run.
   b. Prepare the Trekker index PCR reaction mix according to the table below:

Trekker index PCR reaction mix:

50 µl	NEBNext High-Fidelity 2X PCR Master Mix (NEB)
25 µl	Nuclease-free water
75 µl	**Total volume per reaction**

   c. Transfer 5 µl from the transferred supernatant cleanup (step 4.3 of Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338 Rev G)) to a new tube strip.
   d. Add 75 µl Sample Index PCR Mix to the 5 µl Transferred Supernatant Cleanup sample.
   e. Add 20 µl of an individual Dual Index TT Set A (10x Genomics PN-3000431) to each sample and record the well ID used. Pipette to mix and centrifuge briefly.
   f. Incubate in a thermal cycler with the following protocol:

Ramp rate: 3°C/sec ~35 min total run time

98°C		45 sec
15 cycles:		
	98°C	20 sec
	54°C	30 sec
	72°C	20 sec
72°C		10 min
4°C		Hold

3. Perform post sample index bead purification and quantification:
   a. Prepare fresh 80% ethyl alcohol.
   b. Vortex the SPRIselect reagent for 30 sec and add 120 µl to each sample (1.2X).
   c. Vortex to mix for 10-15 sec.
   d. Incubate at room temperature for 5 min.
   e. Briefly centrifuge the tubes and place the tubes on the magnetic rack. Once the solution is clear, carefully aspirate and discard the supernatant.
   f. Keeping the tube on the magnetic stand, add 300 µl of 80% ethyl alcohol.
   g. Wait 30 sec and remove the supernatant.
   h. Repeat steps f & g for a total of two (2) washes.
   i. Briefly spin the tube to collect the remaining ethyl alcohol at the bottom of the tube.
   j. Place the tube back on the magnetic rack and carefully remove the remaining ethyl alcohol.
   k. Remove the tube from the magnetic rack and add 40 µl of Buffer EB to the tube and pipette the beads to mix well.
   l. Incubate at room temperature for 2 min.
   m. Place the tube back on the magnetic rack.

n. Once the solution is clear, transfer the supernatant to a new 0.2 ml PCR tube. Discard the used beads.
o. Store at 4°C for up to 72 hr or at −20°C for long-term storage.
p. Quantify the Trekker library using Qubit (1X dsDNA HS assay kit) and a Bioanalyzer or TapeStation following the manufacturer's guidelines (Bioanalyzer High Sensitivity DNA assay or TapeStation High Sensitivity D5000 assay or TapeStation D5000 assay). See Figure 1 for an example library trace on the TapeStation system.

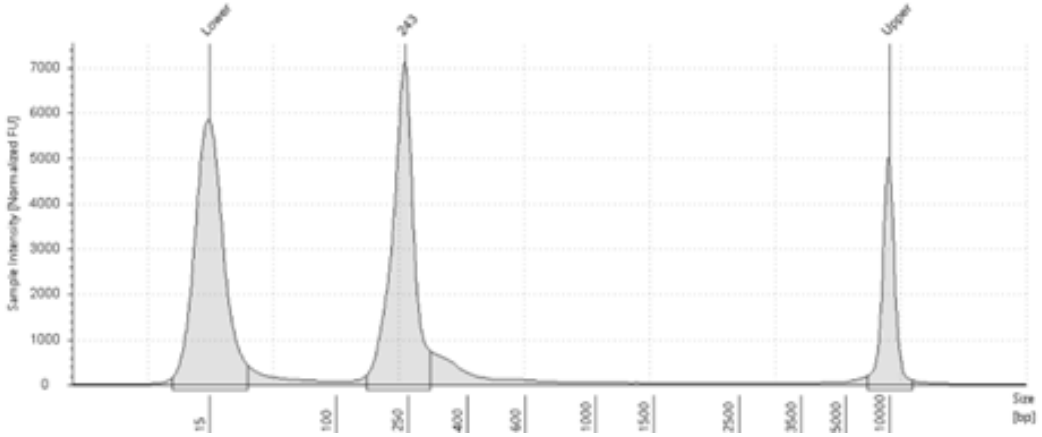

Figure 1. Example library trace on the TapeStation system (D5000).

B. Sequencing

1. Sequence the gene expression and ATAC libraries according to Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide
2. Sequence the Trekker library at 5,000 read pairs per nucleus captured. For example, if 10,000 nuclei were captured, allocate 5.0 x 10^7 reads for the Trekker library
3. Read lengths:

Table 2. Read lengths for 10x Multiome kit prep.

Sequencing read	Recommended read length (bp)
Read 1	28
i7 index	10
i5 index	10
Read 2	min 32

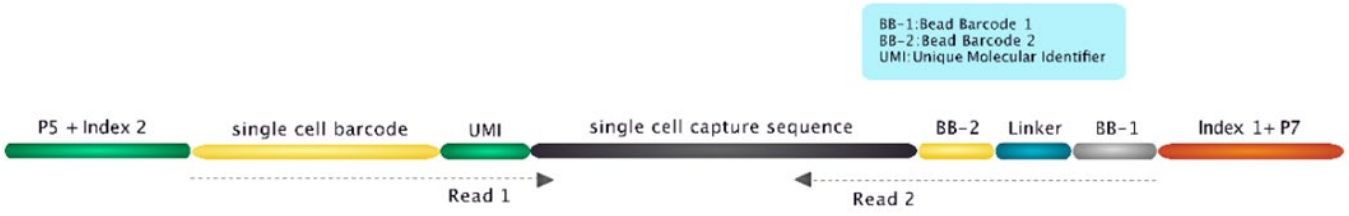

Figure 2. Trekker library structure.

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.

Appendix A. Troubleshooting Guide

Table 1. 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.

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