

## I. Introduction

This protocol provides instructions for performing single-nucleus RNA-seq on the 10x Chromium 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.

For use with 10x Chromium Next GEM Single Cell 3' Reagent Kits v3.1 and 10x Chromium GEM-X Single Cell 3' Reagent Kits v4.

## II. Required Materials

This protocol applies to the following Takara Bio products:

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

### Additional Materials Required (Not Provided):

- Dual Index Kit TT Set A (10x Genomics, Cat. No. PN-1000215)
- Other kits, reagents and equipment listed by the vendor

### For 10x Chromium v3.1

- Chromium Next GEM Single Cell 3' Kit v3.1 (10x Genomics, Cat. No. 4 rxns: PN-1000269, 16 rxns: PN-1000268)
- Chromium Next GEM Chip G Single Cell Kit (10x Genomics, Cat. No. 16 rxns: PN-1000127, 48 rxns: PN-1000120)
- 3' Feature Barcode Kit (10x Genomics, Cat. No. PN-1000262)

### For 10x Chromium v4

- Chromium GEM-X Single Cell 3' Kit v4 (10x Genomics, Cat. No. 4 rxns: PN-1000686, 16 rxns: PN-1000691)
- Chromium GEM-X Single Cell 3' Chip Kit v4 (10x Genomics, Cat. No. PN-1000690)
- Chromium GEM-X Single Cell 3' Feature Barcode Kit v4 (10x Genomics, Cat. No. PN1000702)

## III. Protocol

### A. Single-Nucleus RNA-seq on 10x Chromium

1. Perform single nuclei capture and library prep following instructions for
  - a. v3.1: [Chromium Single Cell 3' Reagent Kits User Guide \(v3.1- Dual Index\) with Feature Barcoding technology for Cell Surface Protein](#)
  - b. v4: [Chromium GEM-X Single Cell 3' v4 Gene Expression with Feature Barcoding technology for Cell Surface Protein User Guide](#)



**IMPORTANT:** Please ensure you are using the correct version of the 10x user guide. And confirm that the primer used for cDNA amplification in Section III.B, Step 2 (below) is Feature cDNA Primers 2 (10x Genomics, PN-2000097). **Using the incorrect protocol version or primer will result in failed Trekker library generation.**

## Trekker<sup>®</sup> Library Preparation with 10x Chromium 3' Reagent Kit Protocol-At-A-Glance

- Prepare the Trekker library following the instructions in the 10x Chromium User Guide for *Cell Surface Protein Library*. In the section titled "Step 4: Cell Surface Protein Library Construction", make the following modifications to the indicated steps below:
  - In Steps 4.0 and 4.1a in the user guide, use the Dual Index Plate TT Set A (10x Genomics, PN-3000431), which is the same index plate used for the gene expression library.  
**NOTE: DO NOT** use Dual Index Plate NT referenced in the user guide for this step.
  - In step 4.1c, combine 1  $\mu$ l of Trekker cDNA from step 2.3B ("Transferred Supernatant Cleanup for Cell Surface Protein library") with 4  $\mu$ l of Buffer EB for the 5  $\mu$ l input.
  - In step 4.1f, perform a total of 7 cycles (repeat six [6] times) of indexing PCR for the cell-surface protein library preparation (Step 4.1f). See Figure 1 for an example library trace on the Tapestation (D5000).

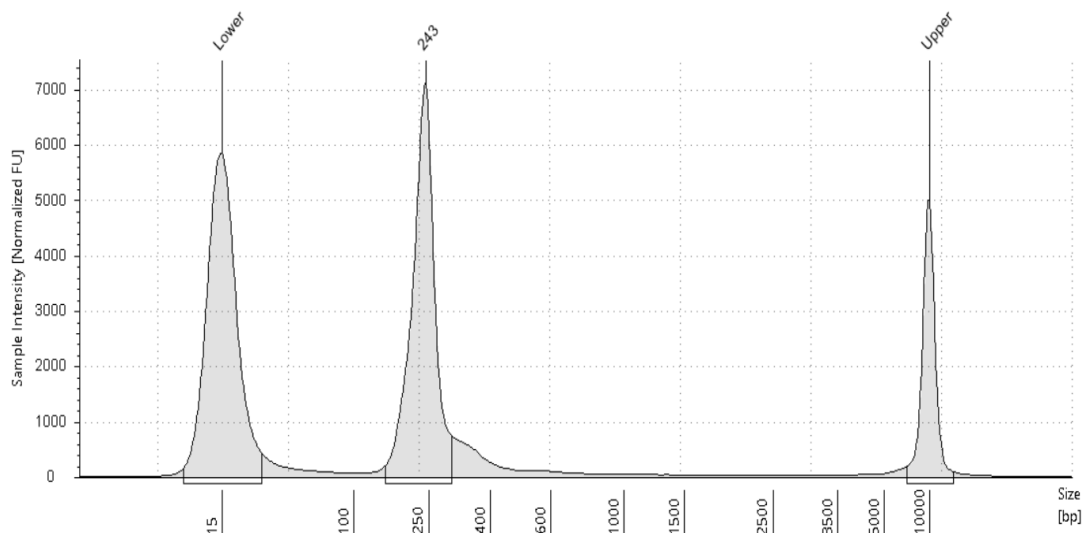


Figure 1. Example Tapestation library trace.

### B. Sequencing

- Sequence the gene expression library according to the 10x user guides listed in Section A (above).
- Sequence the Trekker library at  $\sim 5,000$  read pairs per nucleus captured. For example, if 10,000 nuclei were captured, allocate  $5.0 \times 10^7$  reads for the Trekker library.

Table 1. Read lengths for 10x Chromium.

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

- 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.

## Trekker® Library Preparation with 10x Chromium 3' Reagent Kit Protocol-At-A-Glance

- 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

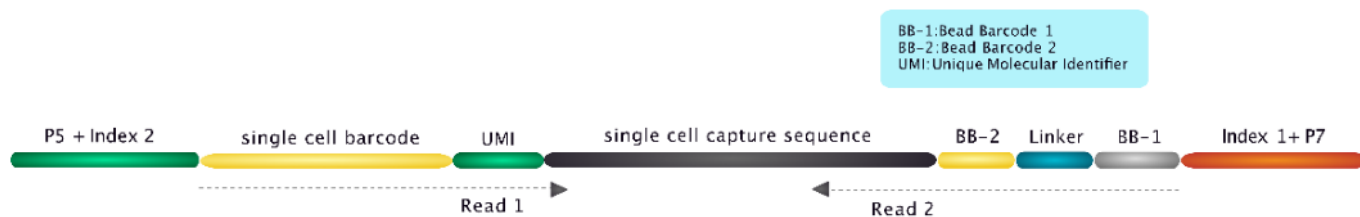


Figure 2. Trekker library structure.

## 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](http://takarabio.com).

## Appendix A. Troubleshooting Guide

Table 2. 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
Trekker final library contains significant off-target peaks	Over amplification	Ensure the correct input amount and cycle number (different from 10x user guide) was used for index PCR.

# Trekker® Library Preparation with 10x Chromium 3' Reagent Kit Protocol-At-A-Glance

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