

## I. Introduction

This protocol provides instructions for performing single-nucleus RNA-seq on the 10x Genomics platform with the 10x Flex assay after performing the Trekker® workflow and nuclei isolation using the [Trekker 5C Single-Cell Spatial Mapping Kit User Manual](#). The final products include the single-nucleus gene expression library, and the Trekker library containing the spatial location of each nucleus.

Compatible with the 10x Genomics [GEM-X Flex Gene Expression Reagent Kits for Multiplexed Samples with Feature Barcode technology for Protein using Barcode Oligo Capture User Guide \(CG000789\)](#).

**NOTE:** This User Guide is provided for general information only and has not been validated by Takara Bio USA, Inc.

## II. Required Materials

This protocol applies to the following Takara Bio products:

- Trekker 5C 10x10 Bundle (Cat. No. SK014)

### **Additional materials required (not provided):**

- GEM-X Flex Gene Expression Human 4-plex, 16 samples (10x Genomics, Cat. No. 16 rxns: PN-1000793) or
- GEM-X Flex Gene Expression Human 16-plex, 64 samples (10x Genomics, Cat. No. 64 rxns: PN-1000794) or
- GEM-X Flex Gene Expression Mouse 4-plex, 16 samples (10x Genomics, Cat. No. 16 rxns: PN-1000797) or
- GEM-X Flex Gene Expression Mouse 16-plex, 64 samples (10x Genomics, Cat. No. 64 rxns: PN-1000798)
- Flex Feature Barcode Kit (10x Genomics, Cat. No. 64 rxns: PN-1000628)
- GEM-X Flex Gene Expression Chip Kit (10x Genomics, Cat. No. 4 chips: PN-1000791)
- Dual Index Kit TS Set A (10x Genomics, Cat. No. 96 rxns: PN-1000251)
- Dual Index Kit TN Set A (10x Genomics, Cat. No. 96 rxns: PN-1000250)
- Other kits, reagents, and equipment listed by the vendor (10x Genomics)

## III. Protocol

### A. Single-nucleus RNA-seq with 10x Flex

1. Perform nuclei fixation following the Sample Fixation section in the 10x Genomics Demonstrated Protocol – [Fixation of Cells & Nuclei for GEM-X Flex Gene Expression \(CG000782\)](#).
2. Follow the GEM-X Flex Gene Expression Reagent Kits for Multiplexed Samples with Feature Barcode technology for Protein using Barcode Oligo Capture User Guide (CG000789) to perform probe hybridization, single-cell capture, and library prep with the following recommendation:
  - a. Perform Pooled Wash Workflow (Option 2.1 A) with  $\leq 50,000$  cells/hyb as outlined on page 56 to preserve the maximum number of nuclei.
3. Follow the steps for Protein Expression Library Construction to generate the Trekker library.
4. Quantify the WTA and Trekker libraries 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). For both libraries, select the region between 150–300 to determine the average size of the library. See below for an example Trekker library trace on the TapeStation (D5000):

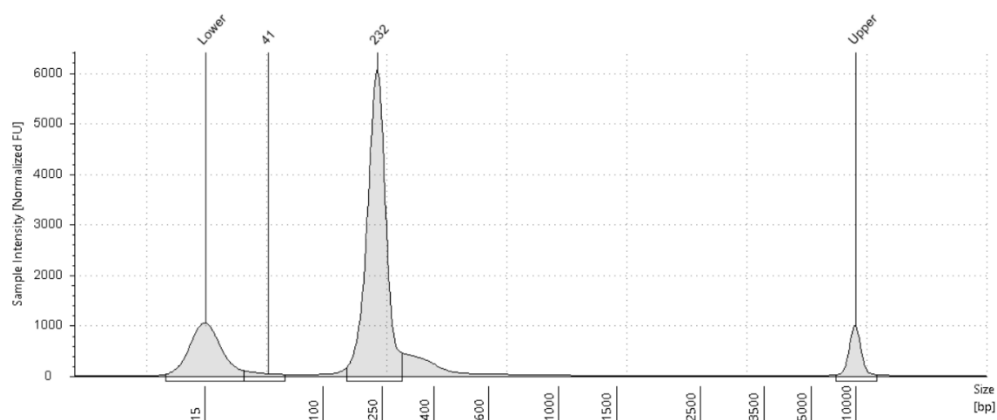


Figure 1. Example library trace on the TapeStation system (D5000).

## B. Sequencing

1. Sequence the gene expression library according to the GEM-X Flex Gene Expression Reagent Kits for Multiplexed Samples with Feature Barcode technology for Protein using Barcode Oligo Capture User Guide (CG000789).
2. Sequence the Trekker library at 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 Flex kit prep.

Sequencing read	Recommended read length (bp)
Read 1	28
i7 index	10
i5 index	10
Read 2	90

3. 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.
  - 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

## C. 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](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 are used

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