Trekker Q Library Preparation with Parse Evercode WT v3 Kits Protocol-At-A-Glance

I. Introduction

This protocol provides instructions for performing single-nucleus RNA-seq on the Parse Evercode platform after performing the Trekker™ workflow and nuclei isolation using the Trekker Q Single-Cell Spatial Mapping Kit User Manual. The final library products include the single-nucleus whole-transcriptome library, which contains 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 Q Single-Cell Spatial Mapping Kit (Cat. No. SK027)

Additional Materials Required (Not Provided):

- Evercode WT Mini v3 (Parse Biosciences, Part Number ECWT3100) or
  - Evercode WT v3 (Parse Biosciences, Part Number ECWT3300) or
  - Evercode WT Mega v3 (Parse Biosciences, Part Number ECWT3500 or ECWT3530)
- Evercode Low Input Cell or Nuclei Fixation v3 (Parse Biosciences, Part Number ECLC3301 or ECLC3501) or
  - Evercode Cell or Nuclei Fixation v3 (Parse Biosciences, Part Number ECFC3300 or ECFC3501)
- CloneAmp™ HiFi PCR Premix (Cat. No. 639298) or
  - KAPA HiFi HotStart ReadyMix (Roche, Material Number 07958927001)
- Other kits, reagents, and equipment listed by the vendor (Parse 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 Q PCR Primer sequence.

Name	Sequence	Concentration
Trekker Q PCR Primer	CTACACGACGCTCTTCCGATCT	100 µM

III. Protocol

A. Single-nucleus RNA-seq with Parse Evercode

1. Perform nuclei fixation following instructions in the Evercode Low Input Cell or Nuclei Fixation v3 User Guide (UMLCN3301).

   NOTE: The low-input fixation kit is recommended for better nuclei recovery, but you may use the non-low-input kit if your nuclei yield is higher than 100,000 post isolation.

2. Perform single nuclei barcoding following the instructions described in the appropriate Evercode WT v3 User Guide (Table 2, next page), with modifications described in Step A.3.

Table 2. Parse Biosciences User Guides.

Kit	Parse Bioscience User Guide
Evercode WT Mini	Evercode WT Mini v3 User Manual v1.5
Evercode WT	Evercode WT v3 User Manual v1.5
Evercode WT Mega	Evercode WT Mega v3 User Manual v1.5

3. Modifications for the appropriate Evercode WT v3 User Guide:
   a. In Step 2.3.2, spike-in the Trekker Q PCR Primer. Add:
      i. 0.6 µl for WT Mini v3
      ii. 2.4 µl for WT v3
      iii. 4.8 µl for WT Mega v3
   b. In Steps 2.4.4 and 2.4.5, follow these instructions:
      i. Transfer 50 µl of cDNA to a new tube.
      ii. Combine the cDNA with 40 µl of SPRI beads (0.8X) for the WT cDNA library.
      iii. Proceed to the rest of the appropriate Evercode WT v3 User Guide to generate the gene expression library.
4. Transfer 40 µl of cDNA to a new tube and perform dual-sided bead cleanup of the Trekker cDNA products following the steps below.
   a. Vortex to resuspend the SPRIselect reagent.
   b. Add 24 µl SPRIselect reagent (0.6X) to each sample and mix by vortexing.
   c. Incubate 5 min at room temperature.
   d. Place on the magnet until the solution clears.
   e. Transfer and save 60 µl supernatant in a new tube strip and discard the pellet.
   f. Add 22 µl SPRIselect reagent (~1.2X) to 60 µl of the transferred supernatant and vortex briefly.
   g. Incubate for 5 min at room temperature.
   h. Briefly centrifuge the tube and place on the magnet until the solution clears.
   i. Remove supernatant.
   j. Follow Steps 2.4.10–2.4.18 of the Parse protocol to complete purification and quantification. Elute the Trekker cDNA library in 20 µl of nuclease-free water in 2.4.18.

SAFE STOPPING POINT: Store at 4°C overnight.

5. Perform Trekker Index PCR following Section 3.5 of the appropriate Evercode WT v3 User Guide with the following modifications:
   a. Prepare 50 ng of Trekker cDNA library in 21 µl of nuclease-free water in a new 0.2 ml PCR tube.
   b. Transfer 4 µl from the chosen UDI plate to the PCR tube in Step 3.5.5.
   c. Add 25 µl of CloneAmp HiFi PCR Premix (Takara Bio) or KAPA HiFi HotStart ReadyMix (Roche) to the PCR tube in Step 3.5.7.
   d. Perform 8 cycles of PCR in Step 3.5.9.
6. Perform Trekker library bead purification following Section 3.6 of the appropriate Evercode WT v3 User Guide with the modifications below:
   a. Add 20 µl of SPRI beads to 75 µl of supernatant in Step 3.6.8 (1X-0.6X SPRI cleanup).
7. Perform Trekker library quantification following Section 3.7 of the appropriate Evercode WT v3 User Guide. See below for an example Trekker library trace on the TapeStation (D1000, Figure 1, next page).

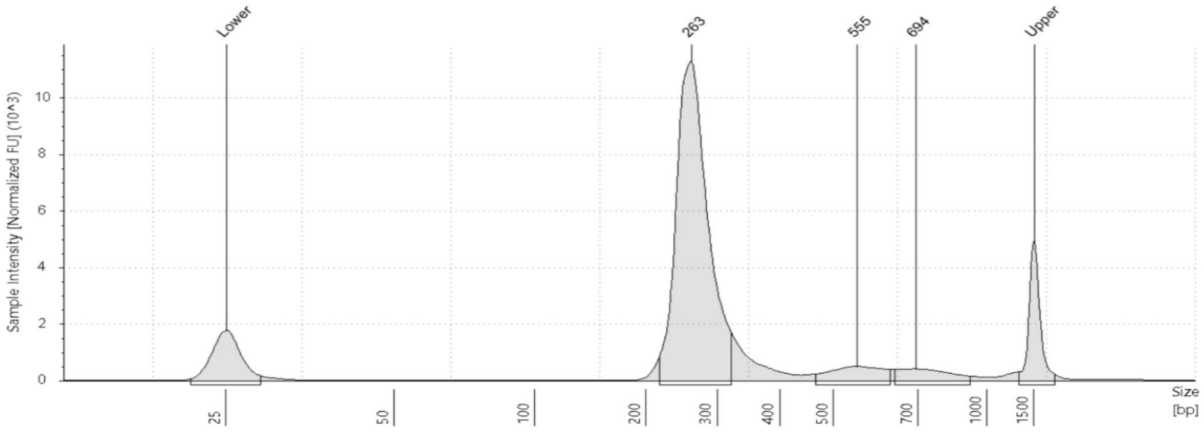

Figure 1. Example Trekker library trace on the TapeStation (D1000).

B. Sequencing

1. Sequence the gene expression library according to the appropriate Parse Evercode WT v3 User Guide.
2. Sequence the Trekker library at ~5,000 read pairs per nucleus captured. For example, if 10,000 nuclei were captured, aim for 5 x 10^7 reads for the Trekker library.

Table 3. Recommended read lengths for Trekker library sequencing reads.

Sequencing read	Recommended read length (bp)
Read 1	min 32
i7 index	8
i5 index	8
Read 2	58

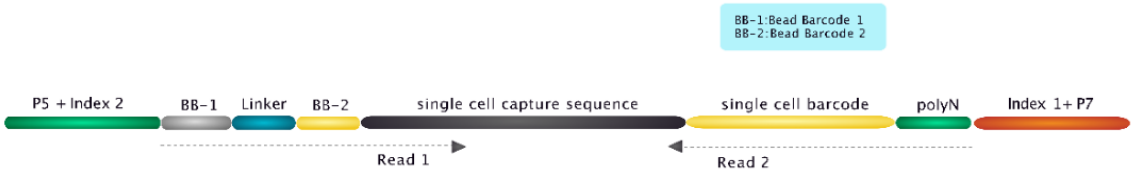

Figure 2. Trekker library structure.

IV. 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.

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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