

Technical note

PREPARING KINNEX™ SINGLE-CELL LIBRARIES WITH PARSE BIOSCIENCES EVERCODE™ WT KITS

Overview

This technical note describes the experimental conditions of a modified workflow for preparing PacBio® Kinnex single-cell libraries with single-cell or single-nucleus cDNA generated using Parse Biosciences Evercode Whole Transcriptome products, the WT and WT Mini kits. This workflow has not been tested using the Evercode WT Mega kit.

Required materials and equipment

- Refer to all materials described in the Kinnex single-cell protocol.¹
- Single-cell or single-nucleus cDNA from the Parse Biosciences Evercode WT or Evercode WT Mini kits.
 - Note that the cDNA needs to be generated as described in the Parse protocols² for either the Evercode WT or Evercode WT Mini up through the end of those protocols' section 2. Then use either the 15 ng (from the WT protocol) or 60–75 ng (from the WT Mini protocol) of the generated cDNA as input into the workflow at right (see Figure 1).
- Parse Biosciences-specific amplification primers (Appendix 1).

Library preparation

Library preparation will follow the PacBio Kinnex single-cell protocol¹ with modifications outlined below. Workflow modifications begin with single-cell or single-nucleus cDNA produced by the Parse Biosciences Evercode WT and WT Mini kits.

1. Generate amplified cDNA per Parse Biosciences protocols. Complete the Evercode WT or Evercode WT Mini protocol up through section 2.
2. Carry out QC as described in the Parse Biosciences user manual (see the figure at the end of section 2, Expected post-amplification sublibrary cDNA size distribution). While cDNA size ranges can vary, there should not be an excess of shorter (<500 bp) material.

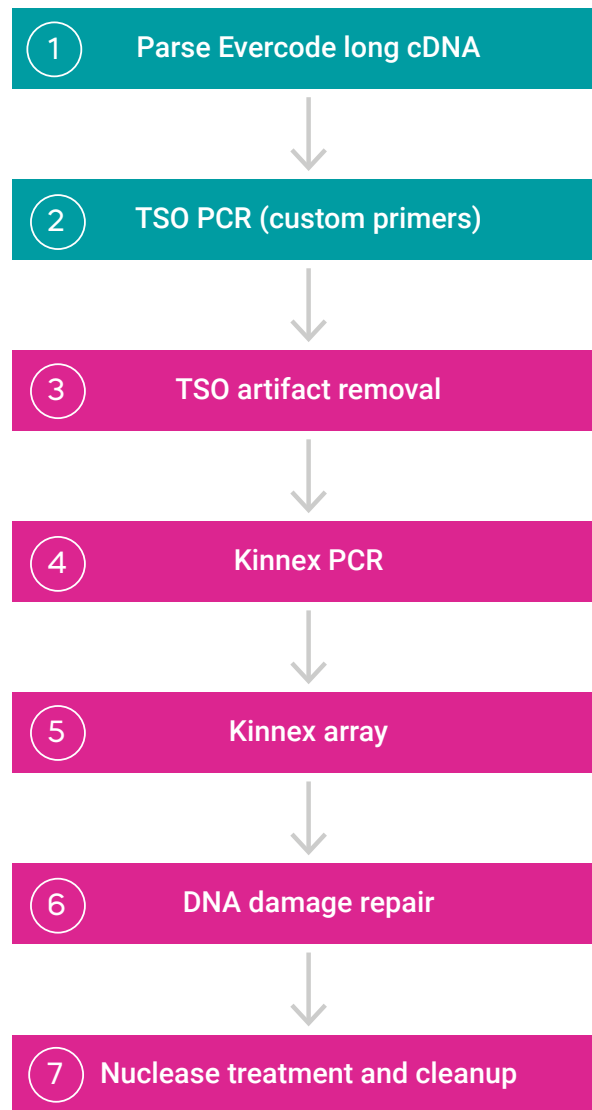


Figure 1. Library workflow for generating Kinnex single-cell libraries from single-cell or single-nucleus cDNA using Parse Biosciences Evercode WT kits.

3. Begin the Kinnex single-cell protocol¹ from step 2, TSO PCR with the following modification: follow step 2.1 and use 5 µL of the Parse Biosciences-specific amplification primers as described in Appendix 1 instead of Kinnex 5' or 3' capture primer mixes. This replacement is critical to adapt the Parse library termini to function with the Kinnex concatenation workflow.
4. After running the TSO PCR program, proceed with the subsequent Kinnex workflow from **cleanup with 1.5x SMRTbell® cleanup beads in the TSO PCR step through the rest of the protocol.**
5. Subsequent QC and library quantification can be carried out as described in the Kinnex single-cell protocol (step 7.21).¹ Proceed with the SMRT® Link Sample Setup for sequencing in the same manner as for standard Kinnex single-cell libraries

SMRT Link informatics and secondary analysis recommendations

Currently, the SMRT Link single-cell Iso-Seq® workflow does not support analysis of Parse Biosciences data. Instead, Read Segmentation can be run in SMRT Link (or skera in the command line) to generate segmented reads (S-reads). The S-reads will contain the original single-cell or single-nucleus cDNA-containing barcode information. Refer to Rebboah et al. (2021) (section Preprocessing of LR-Split-seq data)³ and Parse Biosciences long-read data processing guide⁴ (login required) for secondary analysis recommendations using command line and community tools.

Appendix 1

Order custom primers as described below from preferred oligo vendors. An example of IDT code is below. The primers should be HPLC purified.

Order these primers and reconstitute in TE buffer (10 mM Tris HCl pH 8, 0.1 mM EDTA). Use 10 µM solutions of each of these primers in place of the yellow-capped Kinnex 3' capture primer mix or the red-capped Kinnex 5' capture primer mix in the Kinnex single-cell RNA kit.

Parse Kinnex forward primer

/5Biosg/AA/ideoxyU/CTACACGACGCTCTTCCGATCTCAGACGTGTGCTCTTCCGATC

Parse Kinnex reverse primer

AAGCAGTGGTATCAACGCAGAG

REFERENCES

1. Procedure & checklist – Preparing Kinnex™ libraries using Kinnex single-cell RNA kit. PacBio documentation
2. Evercode-WT-v3-User-Guides. Parse Biosciences documentation.
3. Rebboah, E., et al. (2021). Mapping and modeling the genomic basis of differential RNA isoform expression at single-cell resolution with LR-Split-seq. *Genome biology*, 22, 1-28.
4. Workflow-for-Processing-Long-Read-Data-with-the-Parse-Biosciences-Pipeline. Parse Biosciences long-read data processing guide.



Questions? Visit pacb.com/contact or contact your local customer service representative

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