Apr 18, 2024 Version 2

Parse Evercode WT v2 -- University of Minnesota TMCs V.2

DOI
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Protocol status: Working
We use this protocol and it's working

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Keywords: single nucleus rna, minnesota genomics center, sequencing library, parse evercode wt v2, barcoded cdna, 10x genomic, nucleus rna, rna, evercode wt system, well plates via the evercode wt system, sequencing, parse bioscience, nuclei from frozen tissue, single nuclei, university of minnesota tmcs combinatorial barcoding, minnesota tmcs combinatorial barcoding, pooling nuclei suspension, cdna, specific barcode, nuclei suspension, nuclei

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Abstract

Combinatorial barcoding is an instrument-free method used to generate single-cell and single-nucleus RNA-sequencing libraries. Outlined here are the methods used to isolate single-nuclei from frozen tissue; to fix nuclei following isolation; to ligate nuclei-specific barcodes to transcript cDNA by iteratively splitting and pooling nuclei suspensions between multiple 96-well plates via the Evercode WT system; and to prepare barcoded cDNA for sequencing by synthesis (SBS). The following protocol has been adapted from protocols developed by 10x Genomics, Parse Biosciences, and Illumina to be used at the University of Minnesota TMCs in collaboration with the University of Minnesota Genomics Center. These protocols are owned by their respective companies and are subject to periodic revision.

Single Nuclei Dissociation

1 CG000505_Rev-A.pdf 2.5MB

Nuclei Fixation Protocol

2 Evercode_Fixation_v2.1.1.pdf 3.1MB

Evercode WT Protocol

3 Evercode_WT_Mini_v2.2.1.pdf 5.2MB

Note

Sequence with the read format 66,8,8,86

Note

Sequencers used at UMN Genomics Center:
- Illumina NextSeq 2000
- Illumina NovaSeq 6000
- Illumina NovaSeq X Plus

FASTQ Generation

4 BCL data from Illumina sequencer is demultiplexed and converted into FASTQ format using bcl2fastq version 2.20.0.

Multiplexed FASTQ files were split by sample using a script provided by Parse Biosciences (parse_fastq_sep_groups.py, v0.4).