

Apr 18, 2024 Version 1

Parse Evercode WT -- University of Minnesota TMCs V.1

DOI

dx.doi.org/10.17504/protocols.io.dm6gpz8rplzp/v1

Laura J Niedernhofer¹, David A Bernlohr¹

¹University of Minnesota, Minneapolis, MN

Cellular Senescence Net...

UMN SenNet



Allie

Allie Pybas

UMN

OPEN  ACCESS



DOI: **dx.doi.org/10.17504/protocols.io.dm6gpz8rplzp/v1**

Protocol Citation: Laura J Niedernhofer, David A Bernlohr 2024. Parse Evercode WT -- University of Minnesota TMCs. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.dm6gpz8rplzp/v1>

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: March 26, 2024

Last Modified: April 18, 2024

Protocol Integer ID: 97413

Funders Acknowledgement:

NIH

Grant ID: 5U54AG076041-03

NIH

Grant ID: 5U54AG079754-02



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_v2.0.1.pdf 3.1MB

Evercode WT Protocol

3

 Evercode_WT_v2.0.1.pdf 5.5MB

Note

Sequence with the read format 29,10,10,89

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