Automating single cell transcriptomics with the Parse Biosciences Evercode™ WT v3 kit on the Opentrons Flex™

Written by
Vasudha Nair, PhD,[1] Hoai Nguyen,[2] Devon Davenport,[2] Frances Slater, PhD,[1] Kinnari Watson, PhD[1]
[1]Opentrons Labworks, Inc., [2]Parse Biosciences

ABSTRACT

Single-cell RNA-sequencing (scRNA-seq) techniques have been useful for teasing apart the heterogeneity between cells in a population and identifying subtypes within a sample. The Parse Biosciences, Evercode WT v3 kit for single cell transcriptomics, uses four rounds of barcoding to uniquely label up to 100,000 individual cells. We demonstrate how automation on the Opentrons Flex can save time and generate high quality libraries using the Parse Biosciences Evercode scRNA-seq kit in peripheral blood mononuclear cells (PBMCs).

Key Features

- This study demonstrates the efficacy of automating Evercode WT v3 kit using PBMC samples in conjunction with the Opentrons Flex Platform. Automation minimizes operator variability, promoting consistency across the entire protocol.
- A maximum of 48 samples can be processed using this kit and is particularly advantageous for medium- to large-scale projects.
- Generated sublibraries exhibit quality comparable to those prepared manually, and are compatible with downstream sequencing platforms.
- Automating the entire Evercode WT v3 workflow from fixed cells to prepared libraries reduces the needed hands-on time from approximately 12 hours to approximately 2 hours.

INTRODUCTION

Single cell RNA sequencing (scRNA-seq) methods that involve combinatorial barcoding use multiple rounds of barcoding and pooling to create cells that are uniquely and individually labeled. These methods help scientists understand patterns of gene expression at the individual cell level compared to bulk RNA sequencing which gives the average gene expression across all cells in the population.[1]

While scRNA-seq experiments of the past required microfluidics equipment to compartmentalize individual cells, combinatorial barcoding methods eliminate the need for specialized instrumentation. However, scRNA-seq methods can still be lengthy involving multiple rounds of barcoding before amplification and library preparation. These protocols can involve many hours of hands-on time and generating libraries for scRNA-seq can take days. Automating these processes save time and provide more reproducibility and reduced variability between samples. With the Parse Biosciences Evercode WT v3 kit, preparing samples for scRNA-seq becomes even more user friendly and can be done with fixed samples so that cells across time-points can be stored and then processed all at once. Here, we describe how scRNAseq samples can be prepared from PBMCs using the Opentrons Flex and the Evercode WT v3 kit from Parse Biosciences.

METHODS

Samples

PBMCs were fixed and permeabilized with the Evercode Cell Fixation v3 kit using the standard manual method from Parse Biosciences (Cat. No. ECFC3300).

In Situ Cell Barcoding of PBMCs:

RNA from PBMCs was reverse transcribed and barcoded using the Evercode WT v3 kit (Cat. No. ECWT3300). Cells were split into 8 sublibraries and lysed to release barcoded cDNA. The process was automated using the Evercode Whole Transcriptome (WT) kit Parse Biosciences Protocol 1 which can be found on the Opentrons Protocol Library. https://library.opentrons.com/.

cDNA Capture and Amplification:

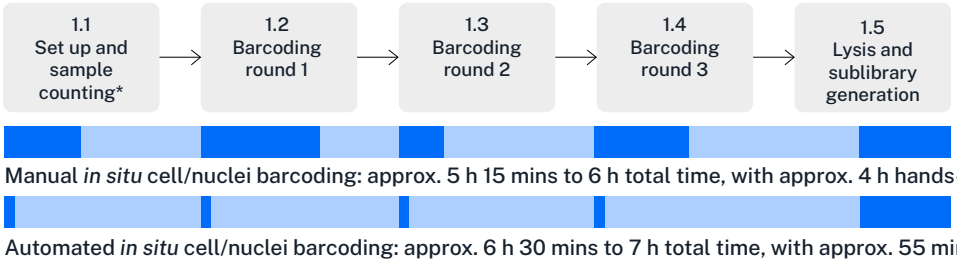
The sublibrary lysates are processed with Streptavidin beads to capture barcoded cDNA. An adapter is added to the 3' end of the cDNA by a template switch reaction. The cDNA is amplified using primers specific to template switch adapter and Illumina Truseq Read 2. This workflow was automated using the Evercode Whole Transcriptome (WT) kit Parse Biosciences Protocol 2 which can be found on Opentrons Protocol Library.

Sequencing Library Preparation:

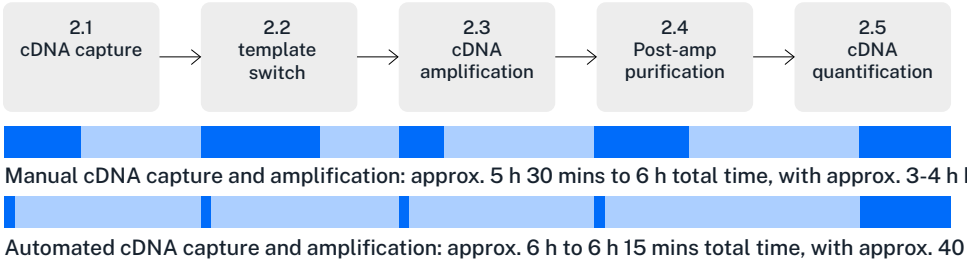
The cDNA is fragmented, ends are repaired and A-tailed. Trueseq Read 1 adapter from Illumina is ligated to the 5' end of the fragmented DNA. This followed by Round 4 of barcoding via the UDI-WT plate. This workflow was automated using the Evercode Whole Transcriptome (WT) kit Parse Biosciences Protocol 3 published on Opentrons Protocol Library. The libraries obtained from this workflow were sequenced on NextSeq® 550 instrument (2 X 75 bp).

The workflow for all three sections are shown in Figure 1.

1. *In Situ* Cell Barcoding of PBMCs



2. cDNA Capture and Amplification:



3. Sequencing Library Preparation:

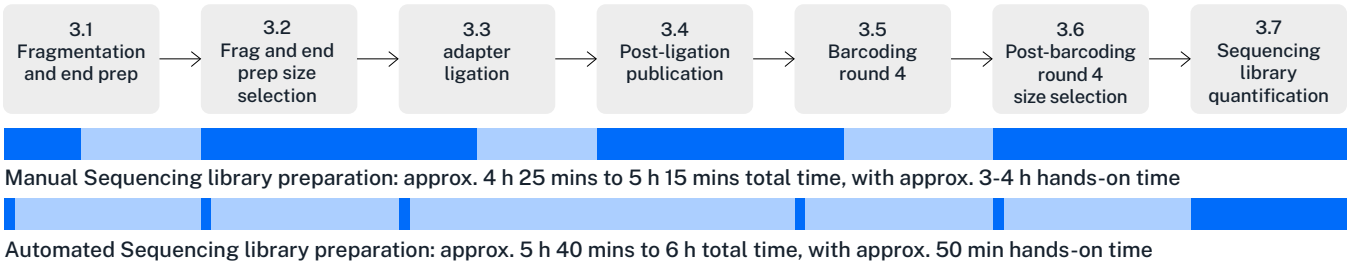


Figure 1. Fully Automating the Parse Evercode WT v3 workflow from fixed cells to prepared libraries on the Opentrons Flex reduces hands-on time by 10 hours. Timings are approximate for 48-sample throughput. Hands-on time is indicated by the dark blue bars and light blue bars indicate time on instrument.

Data Analysis:
Raw sequencing data (FASTQ files) were processed by the pipeline module of Trailmaker™ (cloud-based software from Parse Biosciences) analysis platform to generate quality control reports which include sequence saturation plots, cluster visualization of genes, and the Insights module allows for further processing of the analyzed data to generate figures such as the violin plot (Figure 4).

RESULTS

Single cell sequencing reads generated using our automated method achieved expected sequencing saturation for both transcripts and genes (Figure 2) and show similar sequencing saturation as compared to performing the protocol manually.[2] The metrics comparable are median transcripts/cell, median genes/cell, mean reads/cell and transcriptome map fraction.

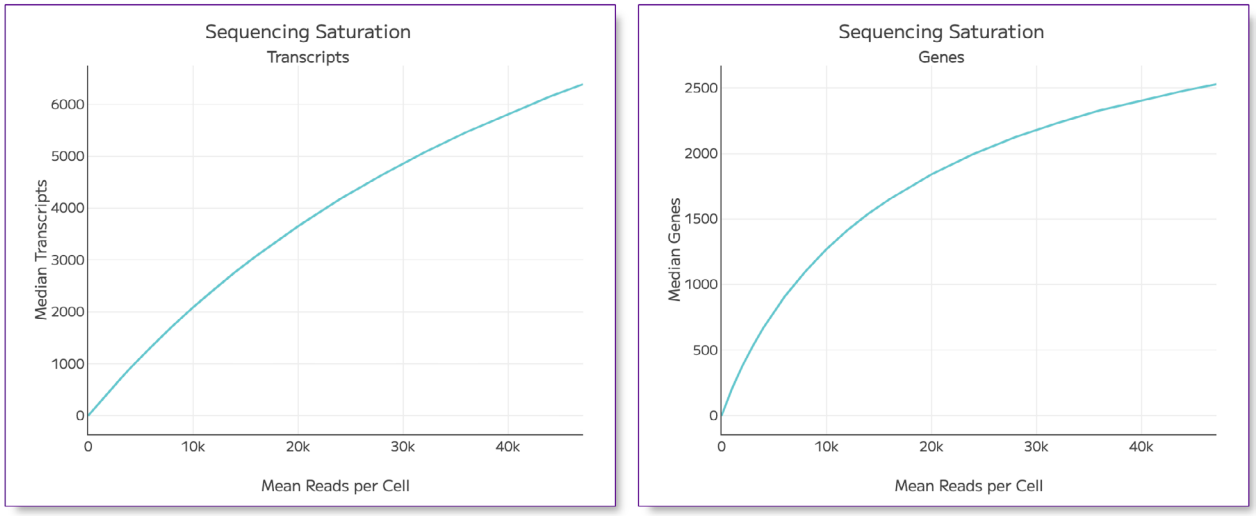


Figure 2. Transcript and gene detection sensitivity. Median A) transcripts and B) genes detected per cell across multiple sequencing depths in PBMCs.

To identify the different cell types in the PBMC sample, we used the Trailmaker software to cluster cell types based on marker expression (Figure 3). The clusters generated are as expected for PBMCs.

Cluster	Gene name	Score	Log2 FC	Pval adj
1	ENSG00000290577	41.5	4.2	4.6E-14
1	EDAR	33.6	3.9	3.4E-123
1	SORCS3	31.9	3.9	3.9E-26
1	RNF175	31.2	3.8	1.6E-38
1	LRP6	27.7	3.6	7.3E-22
1	ANKRD36BP2	24.8	3.5	1.1E-02
1	ANKRD55	24.1	3.5	1.2E-28
1	CACHD1	23.8	3.4	1.6E-20
1	LEF1-AS1	23.0	3.4	3.5E-136
1	TMEM272	22.0	3.3	1.7E-08
1	DACT1	21.0	3.7	1.8E-01
1	CA6	19.6	3.2	5.3E-05
1	NPAS2	18.8	3.1	5.3E-04
1	ENSG00000290109	18.0	3.5	6.1E-01
1	TMEM45B	17.5	3.2	2.3E-04
1	ADTRP	17.5	3.2	3.7E-08

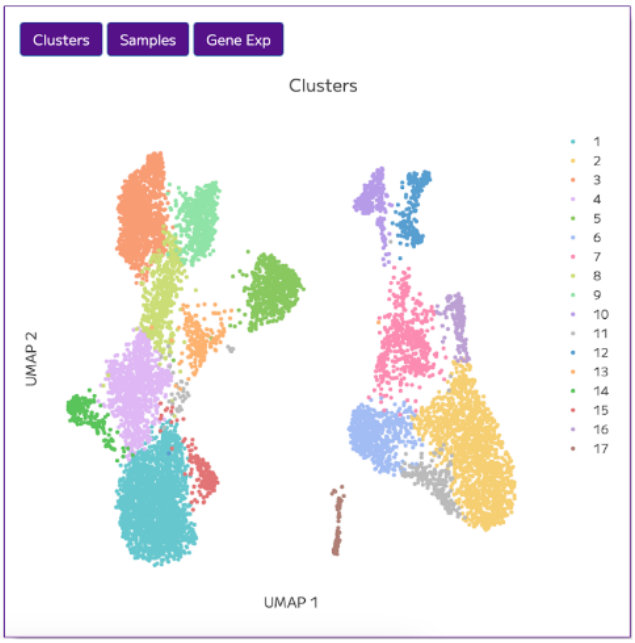


Figure 3. Differentially expressed genes and clusters visualized as UMAP. scRNA-seq data from PBMCs were analyzed using the Trailmaker software. (Left) Gene expression in Cluster 1. (Right) Cluster visualization.

We then looked at the expression of individual markers. Below, CD86 expression occurred as expected within each cell type (Figure 4).

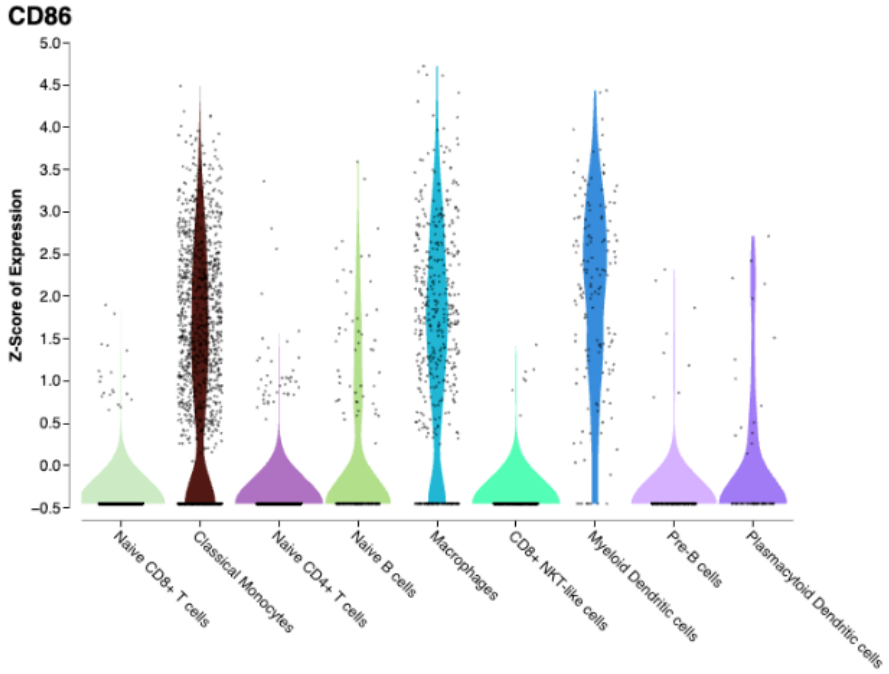


Figure 4. Expression profile of CD86 marker in different immune cell types. The violin plot shown here was generated by the Trailmaker software.

DISCUSSION

Combinatorial barcoding scRNA-seq methods are cost-effective and require no specialized instrumentation, allowing researchers to scale their transcriptomic studies. However sample prep is still a bottleneck, requiring multiple rounds of barcoding followed by amplification and library preparation, that together require many hours of hands-on time. To address this, we automated the Parse Biosciences Evercode WT V3 workflow on the Opentrons Flex.

Here we demonstrate that the automated workflow takes what would have been a protocol requiring 12 hours of hands-on time and shortens that to approximately 2 hours (Figure 1). Libraries produced with automated methods result in high quality data similar to the data resulting from manual methods. The automated protocols developed to support this workflow are freely available on the Opentrons Protocol Library and are easy to implement by lab scientists looking to streamline their single-cell workflows.

REFERENCES

1. Rosenberg AB , Roco CM , Muscat RA, Kuchina A, Seelig G. SPLiT-seq reveals cell types and lineages in the developing brain and spinal cord. Science 2018, 360(6385): 176-182

2. Comparison of Evercode WT V3 and Evercode WT V2 in Human Immune Cells. Dataset from Parse Biosciences.