

Automation of Parse Biosciences Single Cell RNA Sequencing Evercode Workflow on the Opentrons Flex™



Written by

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ABSTRACT

The Parse Biosciences Evercode WT 3 Kit workflow for scRNA-seq incorporates efficient multiplexed split-pool combinatorial RNA barcoding, enabling hundreds of thousands of cells to be prepared and sequenced in a single experiment. All three stages of the Evercode sample preparation, including (1) in situ cell/nuclei barcoding, (2) cDNA capture and amplification and (3) sequencing library preparation, can be automated on the Opentrons Flex liquid handler. In terms of workflow time, over 85% of the workflow is automated, with less than 3 h of hands-on time required.

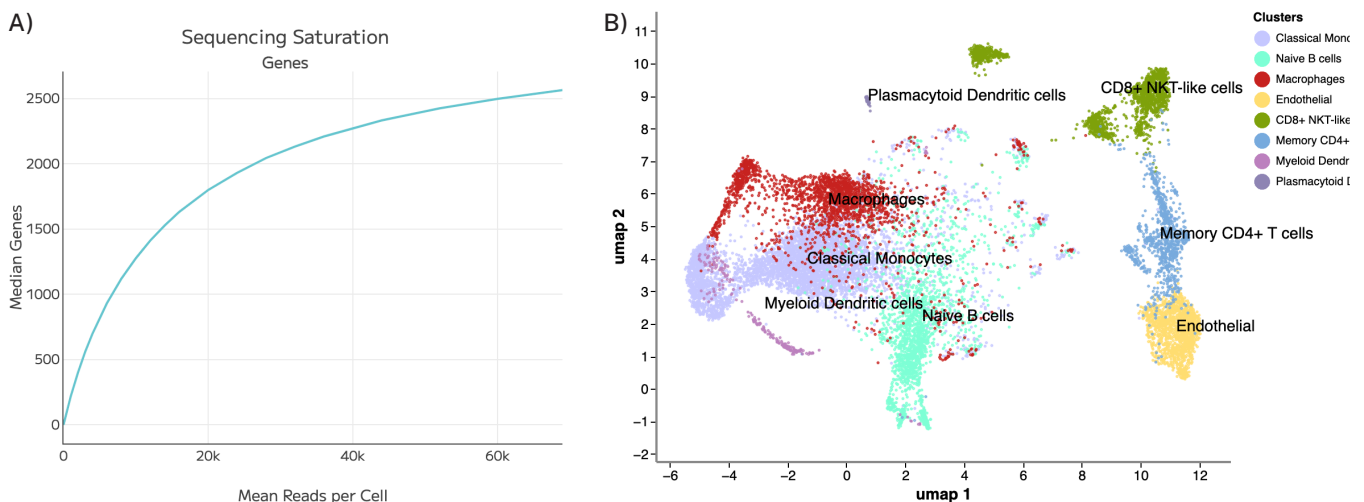
ROBOT SETUP

Evercode sample preparation can be automated on the Opentrons Flex robot, equipped with two pipettes (Flex 1-Channel 1000 μ L & Flex 8-Channel 50 μ L), a gripper, and the following on-deck modules: Thermocycler, two Temperature Modules, Heater-Shaker, and Magnetic Block.



RESULTS

Data generated from a sublibrary of fixed PBMC sample prepared using the automated workflow showed expected sequencing saturation across genes and transcripts (only genes shown below) (A) and population clustering of different immune cell types (B).



A) Gene detection. Median genes detected per cell across multiple sequencing depths in PBMCs.

B) 6679 PBMCs from Evercode WT v3 technology were clustered, annotated and visualized as UMAPs. Different immune cell types are shown here in PBMC sample.

EVERCODE WORKFLOW TIMING

Approximate timing for the three stages of the Evercode sample prep workflow automated on the Opentrons Flex. Of the 21 h 25 min workflow, less than 3 h is hands on, with the remainder carried out on the Opentrons Flex and centrifuge.

1. In situ cell/nuclei barcoding			2. cDNA capture and amplification		
	Time (mins)	Hands-on-time (mins)		Time (mins)	Hands-on-time (mins)
1.1	45	30	2.1	82	<5
1.2	150	<5	2.2	116	<5
1.3	152	<5	2.3	84	<5
1.4	160	<5	2.4	57	<5
1.5	122	35	2.5	30	30
Total	10 h 30 min	<1 h 20 mins	Total	6 h 15 min	<50 mins
3. Sequencing library preparation					
			3.1	64	<5
			3.2	34	<5
			3.3	30	<5
			3.4	35	
			3.5	46	<5
			3.6	41	<5
			3.7	30	30
			Total	4h 40 min	<50 mins