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Evercode Single Index PCR

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We use this protocol and it's working

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Abstract

This protocol describes the **single-index** PCR procedure for Parse Biosciences Evercode WT and WT Mega v2 kits. Each subpool is barcoded with a single Illumina index on the 3' end of the cDNA library. This acts as the fourth "round" of cell barcoding and **must be included in the final cell ID/barcode in order to ensure unique barcodes across subpools within an experiment.** The numerical ID and sequence of the Illumina barcode used for each subpool are recorded in experiment metadata and used downstream for subpool demultiplexing after the sequencing run.

Appendix D2: Sublibrary Single Index PCR

1 If using unique dual indexes (UDIs) instead of sublibrary single index primers for indexing, see Section 3.5. Otherwise, replace the entirety of Section 3.5 with the following steps.

Multiple thermocyclers may be needed for this section depending on the amount of cDNA added to each sublibrary during the fragmentation reaction. Refer to step next page to determine how many thermocyclers are needed.

2 Using a new 1.5 mL tube, combine the **Universal Index Primer** and **Index Primer Mi**x to make the **Sublibrary Amplification Mi**x. Mix well by pipetting and store on ice.

	A	B	C	D	E	F	G	H	I	J
	# Sublibraries	1	2	3	4	5	6	7	8	16
	Index PCR Mix	27.5	55	82.5	110	137.5	165	192.5	220	440
	Universal Index Primer	2.2	4.4	6.6	8.8	11	13.2	15.4	17.6	35.2
	Total	29.7	59.4	89.1	118.8	148.5	178.2	207.9	237.6	465.2

3 Add **2 µL of different index primers to each sublibrary** ensuring that no two sublibraries contain the same sublibrary index primer. Make sure to record which sublibrary contains which index primer.

4 Add **27 µL Sublibrary Amplification Mix** to the 23 µL sublibrary from the previous step. Pipette up and down 10x with the pipette set to 27 µL to ensure proper mixing, followed by brief centrifugation (~2 sec).

5 Place the samples(s) into a thermocycler and run the program below. The number of cycles (X) should be adjusted based on the amount of cDNA added to the fragmentation reaction.

	Run Time	Lid Temperature	Sublibrary Volume
	~30 min	105C	50 uL

Sublibrary Index Amplification Overview

	Step	Time	Temperature
	1	3 min	95C
	2	20 sec	98C
	3	20 sec	67C
	4	1 min, then go to step 2, repe at X-1 times (X cycles tota l)	72C
	5	5 min	72C
	6	Hold	4C

Sublibrary Index Amplification

	A	B	C	D	E	F	G
	cDNA in Fragmentation (ng)	10-24	25-49	50-99	100-299	300-999	1,000+
	Total PCR cycles required (X)	13	12	11	10	9	7

PCR Cycles based on cDNA in Fragmentation

Note: cDNA concentration was recorded in step 2.5.18, and 10 µL from each sublibrary should have been added into the fragmentation reaction (step 3.1.2).

6 Sublibraries can be stored at this point at 4°C overnight. If you wish to continue, proceed directly to Section 3.6: Post-Amplification Double-Sided Size Selection.
[STOPPING POINT]