TECHNICAL NOTE

Chromium™ Single Cell 3' v2 Libraries – Sequencing Metrics for Illumina® NovaSeq®

INTRODUCTION

The Chromium™ Single Cell 3' v2 Protocol (CG00052) produces Single Cell 3' libraries, ready for Illumina® sequencing. Single Cell 3' libraries incorporate standard Illumina paired-end constructs with P5 and P7 sequences at opposite ends. The 16bp 10x™ Barcode and the UMI is encoded at the start of Read 1, while sample index sequence information is incorporated into the i7 index read. Read 1 and Read 2 are standard Illumina sequencing primer sites used in paired-end sequencing (Figure 1). The libraries have been validated on the following sequencing instruments: MiSeq®, NextSeq® 500/550, HiSeq® 2500 (in Rapid Run (RR) and High Output (HO) mode), and HiSeq® 3000/4000. With the introduction of the Illumina NovaSeq® Series, we have validated the performance of Single Cell 3' libraries on the NovaSeq sequencing platform. This Technical Note describes key sequencing metrics of the Illumina NovaSeq sequencer and is intended to provide general guidance of the expected range of sequencing metrics. Individual results may still vary, depending on the particular sample and loading characteristics.

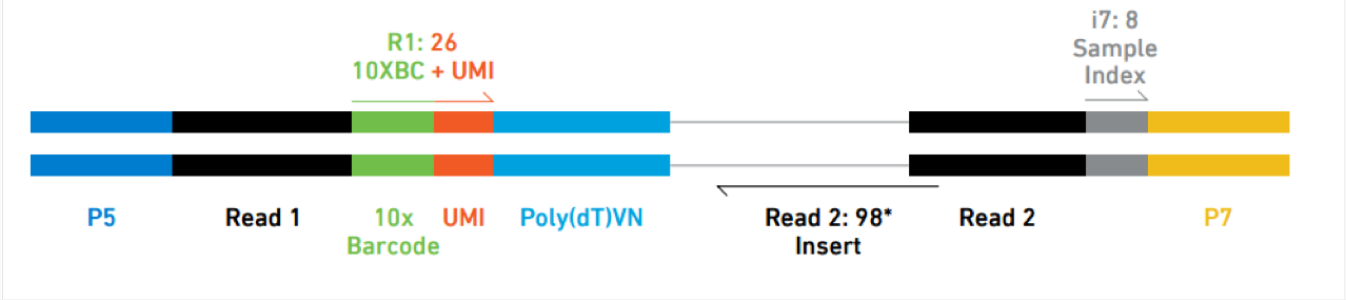

Fig. 1. Schematic of a fragment from a final Chromium™ Single Cell 3' v2 library. *Can be adjusted.

METHOD

Chromium Single Cell 3' v2 libraries were prepared for the following 8 samples (Table 1):

Library ID	Cell Type	# Detected Cells
1	1:1 Mixture of Fresh Frozen Human (HEK293T) and Mouse (NIH3T3) Cells	100
2		1,015
3		6,806
4		12,806
5	Peripheral blood mononuclear cells (PBMCs) from a healthy donor	4,342
6		8,403

Library ID	Cell Type	# Detected Cells
7	Pan T Cells from a healthy donor	3,549
8	Combined Cortex, Hippocampus and Subventricular zone of an E18 Mouse	9,099

Table 1. Chromium Single Cell 3' v2 libraries used in this study.

Libraries were prepared following the *Chromium™ Single Cell 3' Reagent Kits v2 User Guide* (CG00052). Note that libraries were initially sequenced on different Illumina® platforms (libraries 1-4 and 6-8: HiSeq® 4000, library 5: HiSeq 2500 RR). Results of the Cell Ranger™ analysis of these sequencing runs are freely available from: https://support.10xgenomics.com/single-cell-gene-expression/datasets. Libraries in this Technical Note were pooled at equimolar ratios and run on the Illumina NovaSeq® using paired-end sequencing with a single index read per sample.

RESULTS

Libraries were sequenced using a NovaSeq 5000/6000 S2 Reagent Kit (100 cycles) and the sequencing run parameters listed in Table 2. Note that the 100 cycle kit contains an additional 25 cycles of reagents allowing for a maximum of 125 total cycles. As a result, libraries were run using the following paired-end sequencing configuration, 26bp Read 1, 91bp Read 2 and a single sample index (8bp).

Sequencing Read	Recommended Number of Cycles
Read 1	26 cycles
i7 index	8 cycles
i5 index	0 cycles
Read 2	91 cycles

Table 2. Recommended NovaSeq® sequencing run parameter for Chromium Single Cell 3' v2 libraries.

We report the following sequencing metrics to assess sequencing run performance (Table 3):

- "Percentage of Clusters Passing Filers (%PF)"
- Yield per Lane for Read 1 and Read 2 in Gb
- Phred quality scores (shown as %Q30) for Read 1 (R1), i7 index and Read 2 (R2)
- Mapping rate of Read 2 transcript read to the appropriate reference in % (for each pooled sample)

Note that the pool of 8 libraries was sequenced on two different NovaSeq instruments (NovaSeq Instrument 1 and 2). Libraries were loaded at three different concentrations on NovaSeq Instrument 2.

Library ID	Loading Conc. (pM)	Instrument	%PF	Yield per Lane (Gb)		%>=Q30			Mapping Rate (%)
				R1	R2	R1	i7	R2	
NovaSeq Instrument 1									
1	300	NovaSeq	77.3	111.3	400.8	94.6	97.0	92.6	72.0
2									72.8
3									72.6
4									71.6
5									58.7
6									59.0
7									59.2
8									59.5

Library ID	Loading Conc. (pM)	Instrument	%PF	Yield per Lane (Gb)		%>=Q30			Mapping Rate (%)
				R1	R2	R1	i7	R2	
NovaSeq® Instrument 2									
1	200	NovaSeq	82.1	118.3	425.7	97.6	96.9	94.4	72.3
2									73.1
3									72.9
4									71.9
5									58.9
6									59.3
7									60.1
8									59.7
1	300	NovaSeq	80.0	115.2	414.8	96.9	96.7	93.9	72.0
2									72.9
3									72.8
4									71.6
5									58.6
6									59.0
7									59.8
8									59.6
1	400	NovaSeq	80.9	116.5	419.4	97.3	96.7	94.2	72.1
2									72.9
3									72.8
4									71.7
5									58.6
6									58.9
7									59.8
8									59.5

Table 3. Reported sequencing metrics for Chromium Single Cell 3' v2 libraries on two Illumina NovaSeq instruments with recommended loading concentration. Libraries were spiked with 1% PhiX.

Figure 2 illustrates the distribution of base composition along Read 1, the i7 index read, and Read 2 that we typically observe after a successful sequencing run of Chromium™ Single Cell 3' v2 libraries prepared according to the *Chromium™ Single Cell 3' Reagent Kits v2 User Guide* - CG00052. For additional details see also the Technical Note *Chromium™ Single Cell 3' v2 Libraries – Base Composition of Sequencing Reads of Chromium™ Single Cell 3' v2 Libraries* - CG000080.

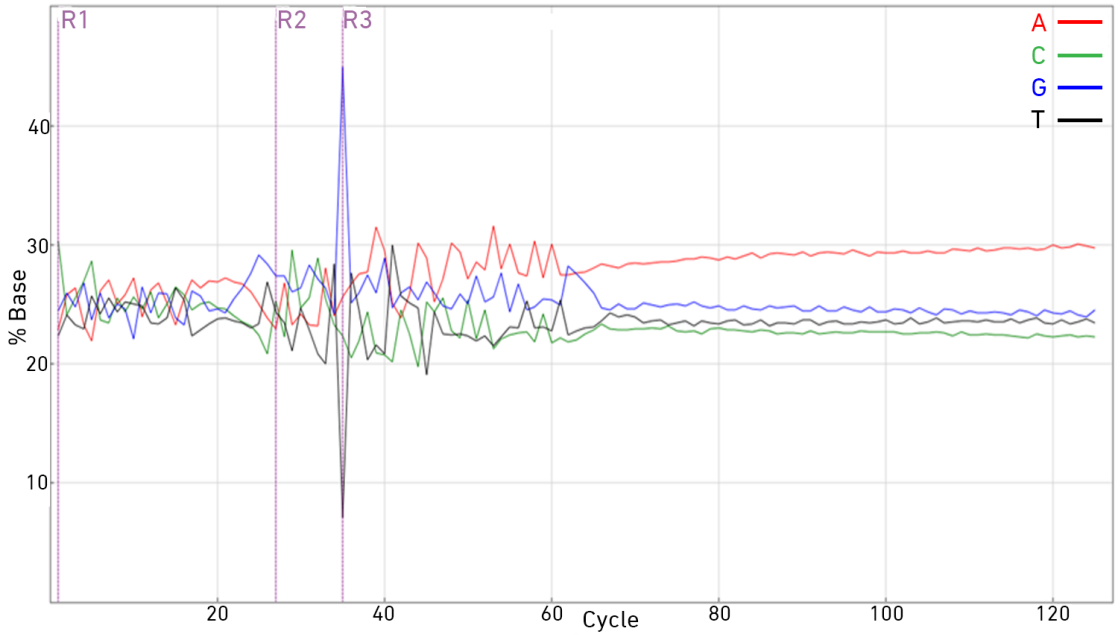

Fig. 2. Representative example of the 'Data by Cycle' plot in the Sequencing Analysis Viewer software (Illumina). Shown is the percentage of clusters for which the selected base has been called (% base: y axis) along the sequencing length (x axis). Profile is based on sequencing 10x library by itself with no other library type sequenced alongside.

The Phred quality score assesses base calling accuracy and is typically used to determine how much of the data from a given sequencing run can be used. Sequencing data with lower quality scores can result in a significant portion of reads being unusable. Figure 3 outlines the Q30 quality metrics that we typically achieve with Single Cell 3' v2 libraries run on the Illumina NovaSeq®. Percentages of Q30 are relatively stable across cycles for all three reads (Read 1, i7 and Read 2).

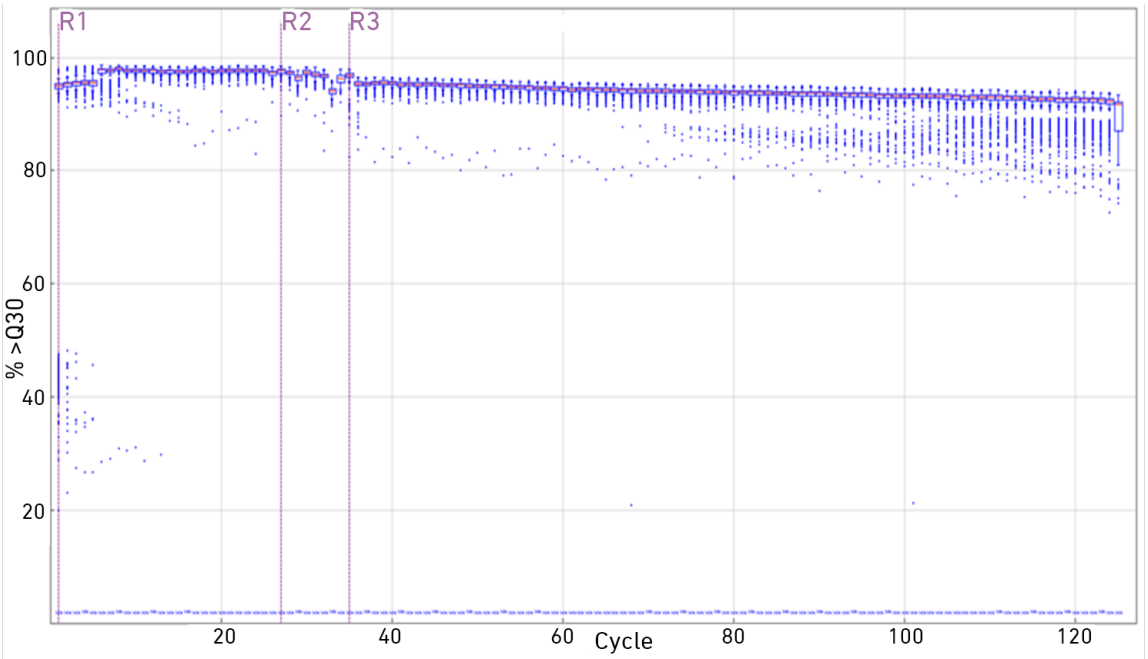

Fig. 3. Representative example of the 'Data by Cycle' plot in the Sequencing Analysis Viewer software (Illumina). Shown is the Q30 percentage along the sequencing length. Profile is based on sequencing 10x library by itself with no other library type sequenced alongside.

DISCUSSION

As expected, libraries sequenced on the NovaSeq® produced high quality data. Overall, sequencing quality is comparable to data obtained from the HiSeq® 2500 and HiSeq 4000 platforms. Q30 quality scores for R1 and the sample index read (i7) are stable at >90% and as expected decreased slightly towards the end of R2. We recommend loading Chromium™ Single Cell 3' v2 libraries at a concentration range of 200 – 400 pM. Sequencing metrics including %PF, Yield per Lane, and Q30 quality scores remained relatively consistent during the titration experiments. This is consistent with other sequencing platforms for our Chromium Single Cell 3' v2 libraries (see Technical Note *Chromium™ Single Cell 3' v2 Libraries – Sequencing Metrics for Illumina® Sequencers* – CG000089 for more details). As expected, mapping rates were comparable to the same libraries sequenced on the HiSeq 2500 or HiSeq 4000 (data not shown).

CONCLUSION

We have discussed sequencing parameters for Chromium Single Cell 3' v2 libraries sequenced on the Illumina NovaSeq. Illumina's NovaSeq sequencing platform is compatible with Chromium Single Cell 3' v2 libraries and may be used as an alternative for sequencing projects that require increased sample throughput. The representative example profiles and sequencing performance metrics of Chromium Single Cell 3' v2 libraries demonstrated here serve as a reference for what constitutes a successful sequencing run using this library type.

REFERENCES

- *Chromium™ Single Cell Reagent Kits v2 User Guide* (CG00052)
- *Chromium™ Single Cell 3' v2 Libraries – Base Composition of Sequencing Reads of Chromium™ Single Cell 3' v2 Libraries* (CG000080)
- *Chromium™ Single Cell 3' v2 Libraries – Sequencing Metrics for Illumina® Sequencers* (CG000089)

Notices

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