10x GENOMICS®

TECHNICAL NOTE

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

INTRODUCTION

The Chromium™ Single Cell 3' v2 Protocol (CG00052) produces Single Cell 3' v2 libraries ready for Illumina® sequencing. The libraries have been validated on the following Illumina sequencing instruments: MiSeq®, NextSeq® 500/550, HiSeq® 2500 (in both Rapid Run (RR) and High Output (HO) mode), HiSeq® 3000/4000, and NovaSeq®. While Chromium Single Cell 3' v2 libraries are run using paired-end sequencing with single indexing on these sequencers, they differ in loading concentration, resulting cluster densities, and sequencing quality metrics depending on which Illumina instrument was used. This Technical Note presents a comparison of Chromium Single Cell 3' v2 libraries across different Illumina sequencing platforms and describes the differences seen for several key sequencing metrics. This document is intended to provide general guidance on the expected range of sequencing metrics based on a controlled library. Individual results may still vary, depending on the specific sequencing platform and/or particular sample and loading characteristics.

METHOD

We prepared a Chromium Single Cell 3' v2 library with ~8,400 peripheral blood mononuclear cells (PBMCs) from a healthy donor by following the Chromium Single Cell 3' Reagent Kits v2 User Guide – CG00052. This library was initially sequenced using paired-end sequencing (26bp Read 1 and 98bp Read 2) with a single sample index (8bp) on an Illumina HiSeq 4000 with approximately 93,000 reads per cell (two lanes). Results of the Cell Ranger™ analysis of this sequencing run are available on our Support website as the 'pbmc8k' dataset (https://support.10xgenomics.com/single-cell-gene-expression/datasets/2.0.1/pbmc8k).

We next used the same library to evaluate sequence performance across Illumina sequencing platforms

We assessed sequencing metrics in multiple ways:

i. Sequence the same library on different Illumina sequencing instruments with our recommended loading concentrations.
ii. Sequence the same library on the same Illumina sequencing instrument at different loading concentrations.

Table 1 provides the comparison of one library (Library ID 1) that was sequenced on the MiSeq, NextSeq 500, HiSeq 2500 RR, HiSeq 2500 HO, HiSeq 4000 and NovaSeq to assess differences in sequencing performance across different Illumina sequencing platforms.

Table 2 outlines variability in sequencing performance based on different loading concentrations for the same library that was sequenced on the same Illumina sequencer. Specifically, we loaded

- Two different library concentrations on the HiSeq 2500 RR
- Eight different library concentrations on the HiSeq® 2500 HO

- Eight different library concentrations on the HiSeq 4000
- Three different library concentrations on the NovaSeq®

We report the following sequencing metrics to assess sequencing run performance:

- Cluster densities (K/mm^2) and "Percentage of Clusters Passing Filers (%PF)" for NextSeq® 500, HiSeq 2500 RR/HO and HiSeq 4000, respectively. Note that HiSeq 4000 and NovaSeq operate on patterned flow cells.
- Yield per Lane for Read 1 and Read 2 in Gb
- Phred quality scores (shown as %Q30) for Read 1 (R1), i7 index (i7) and Read 2 (R2)
- Mapping rate of Read 2 transcript read to reference (GRCh38) in %

Library ID	Loading Conc. (pM)	Instrument	Cluster Density (%PF if HiSeq 4000)	Yield per Lane (Gb)		%>=Q30			Mapping Rate (%)
				R1	R2	R1	i7	R2	
Library ID 1									
1	10	MiSeq	1188 K/mm^2	0.7	2.60	98.09	97.01	86.51	61.7
1	1.3	NextSeq 500	147 K/mm^2	2.2	8.4	95.96	87.62	55.48	52.3
1	8	HiSeq 2500 RR	877 K/mm^2	3.9	15.3	98.67	96.53	91.66	62.1
1	16	HiSeq 2500 HO	1049 K/mm^2	6.9	26.83	96.16	93.48	80.49	62.6
1	250	HiSeq 4000	81.45	9.8	38.21	97.82	93.41	80.43	62.2
1	300	NovaSeq	77.30	111.3	400.8	93.40	96.10	92.8	59.0

Table 1. Reported sequencing metrics for Chromium Single Cell 3' v2 libraries across different sequencing instruments with recommended loading concentrations.

Library ID	Loading Conc. (pM)	Cluster Density (%PF if HiSeq 4000)	Yield per Lane (Gb)		%>=Q30			Mapping Rate (%)
			R1	R2	R1	i7	R2	
HiSeq 2500 RR								
1	8	877 K/mm^2	3.9	15.3	98.67	96.53	91.66	62.1
1	12	1275 K/mm^2	5.4	21.1	96.16	95.13	86.28	62.1
HiSeq 2500 HO								
1	6	496 K/mm^2	3.4	13.14	98.83	94.70	83.97	63.1
1	8	634 K/mm^2	4.3	16.71	98.33	94.93	83.78	63.2
1	10	757 K/mm^2	5.1	19.86	97.91	94.92	83.74	63.1
1	12	877 K/mm^2	5.9	22.79	97.32	94.55	83.73	63.0
1	14	978 K/mm^2	6.5	25.22	96.67	94.03	81.81	62.3
1	16	1049 K/mm^2	6.9	26.83	96.16	93.48	80.49	62.6
1	18	1127 K/mm^2	7.3	28.46	95.34	92.57	81.22	62.9
1	20	1192 K/mm^2	7.7	29.70	94.49	91.71	80.21	62.8

Library ID	Loading Conc. (pM)	Cluster Density (%PF if HiSeq® 4000)	Yield per Lane (Gb)		%>=Q30			Mapping Rate (%)
			R1	R2	R1	i7	R2	
HiSeq 4000								
1	50	55.10	6.6	25.85	98.05	86.21	79.36	62.3
1	100	71.34	8.6	33.49	97.85	90.80	78.79	62.1
1	150	79.90	9.6	37.48	97.86	92.62	80.20	62.0
1	200	80.88	9.8	37.95	97.78	92.67	80.04	62.1
1	250	81.45	9.8	38.21	97.82	93.41	80.43	62.2
1	300	80.02	9.7	37.53	97.69	93.10	81.16	62.2
1	350	79.67	9.6	37.37	97.69	93.35	82.09	61.7
1	400	79.99	9.6	37.51	97.63	90.97	82.34	61.5
NovaSeq®								
1	200	82.1	118.3	425.7	97.60	96.80	94.30	59.3
1	300	80.0	115.2	414.8	96.70	96.70	93.90	59.0
1	400	80.9	116.5	419.4	97.20	96.50	94.10	58.9

Table 2. Reported sequencing metrics for Chromium Single Cell 3' v2 libraries at different loading concentrations across different sequencing instruments.

Sequencing metrics including %PF, Yield per Lane, and Q30 quality scores remained relatively consistent during the titration experiments. For instance, libraries loaded between 100 and 400 pM on the HiSeq 4000 did reveal only minor variability in Q30 quality scores (R1: Δ0.23%, i7: Δ2.61%, R2: Δ3.55%) and mapping rates (Δ0.5%). Libraries sequenced on the HiSeq 2500 HO and NovaSeq showed similar consistencies (R1: Δ3.34%, i7: Δ3.21%, R2: Δ3.76% and R1: Δ0.9%, i7: Δ0.3%, R2: Δ0.4%, respectively).

DISCUSSION

As expected, yield correlated with the amount of sequencing data that each sequencing platform can generate. Overall, Q30 quality scores were higher for R1 and the sample index read (i7) compared to R2 on all sequencing platforms. Q30 quality scores for R2 were lowest on NextSeq® 500 (~ 55%). In contrast, Q30 quality scores on all other sequencing platforms were generally very stable across R2 (> 80%). Libraries sequenced on the NovaSeq produced the highest sequencing quality, particularly for R2 (~94%). Mapping rates remained relatively consistent across all sequencing platforms (~62%) with the exception of those libraries that were sequenced on NextSeq 500 (~52%). Please refer to the Technical Note *Chromium™ Single Cell 3' v2 Libraries – Sequencing Performance on Illumina® NextSeq® 500 Flow Cells* - CG000085 for more details.

CONCLUSION

We have discussed sequencing parameters for Chromium Single Cell 3' v2 libraries and different sequencing metrics that are typically obtained for Illumina sequencing instruments. Sequencing metrics reported in this Technical Note serve as guideline to assess sequencing run quality of Chromium Single Cell 3' v2 libraries. Note that additional factors will contribute to overall success of a sequencing run and will have an impact on applications performance metrics. These include:

- High quality of sample preparation to obtain adequate single cell suspension (Document CG00053)

- Final libraries with fragment length of 300 bp – 1000 bp and significant number of inserts between 400 bp – 500 bp in length for optimal cluster formation on Illumina flow cells
- Reliable and accurate library quantification using the KAPA DNA Quantification Kit using an average library fragment size as derived from the Bioanalyzer or TapeStation
- Sequencer platform

REFERENCES

- *Chromium™ Single Cell 3' Reagent Kits v2 User Guide* (CG00052)
- *Demonstrated Protocol – Single Cell Protocols – Cell Preparation Guide* (CG00053)
- *Chromium™ Single Cell 3' v2 Libraries – Sequencing Metrics for Illumina® NovaSeq* (CG000120)
- *Chromium™ Single Cell 3' v2 Libraries – Sequencing Performance on Illumina® NextSeq® 500 Flow Cells* (CG000085)

Notices

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