10x GENOMICS®

TECHNICAL NOTE

Chromium™ Single Cell V(D)J Libraries – Sequencing Metrics for Illumina® Sequencers

INTRODUCTION

The Chromium™ Single Cell V(D)J Protocol (CG000086) produces Chromium™ Single Cell V(D)J libraries ready for Illumina® sequencing. The libraries have been validated on the following Illumina sequencing instruments: MiSeq®, NextSeq® 500/550, HiSeq® 2500 Rapid Run (RR) and High Output (HO), HiSeq® 3000/4000, and NovaSeq®. While Chromium Single Cell V(D)J libraries are run using paired-end sequencing with a single index read 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 V(D)J libraries across different Illumina sequencers and describes the differences seen for several key sequencing metrics. This document is intended to give general guidance of the expected range of sequencing metrics on multiple platforms, based on a set of controlled libraries. Individual results may still vary, depending on the specific sequencing instrument and/or particular sample and loading characteristics.

METHOD

We prepared Chromium™ Single Cell V(D)J libraries with cells listed in Table 1 following the *Chromium™ Single Cell V(D)J Reagent Kits User Guide* – CG000086. The pool of 14 libraries was originally sequenced on Illumina HiSeq 4000 with approximately 5,000 read pairs per cell (one lane). Libraries were run using paired-end sequencing (150 bp Read 1 and 150 bp Read 2) with a single index (8 bp). Raw and processed data from a subset of replicate libraries are freely available from: https://support.10xgenomics.com/single-cell-vdj/datasets.

Cell Type	# Libraries
Pan T cells	4
Peripheral blood mononuclear cells (PBMCs)*	2
Jurkat (lymphoblast cell line)*	4
Anti EBV specific T cells*	4

Table 1. Cells used to generate 14 Single Cell V(D)J libraries. *Data from replicate libraries are available on the 10x Genomics® Support website.

We assessed sequencing metrics as follows:

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

Table 2 provides the comparison of a pool of 14 libraries (Library ID 1) that was sequenced on NextSeq® 500, HiSeq® 2500 RR, HiSeq 2500 HO, and HiSeq 4000. A subset of libraries was pooled (Library ID 2) and sequenced on MiSeq® to achieve similar numbers of read pairs per cell. Finally, a pool of 72 libraries (Library ID 3, see *Technical Note*

Chromium™ Single Cell V(D)J Libraries – Sequencing Metrics for Illumina® NovaSeq® - CG000121 for more details) was sequenced on the NovaSeq to assess differences in sequencing performance across all different Illumina sequencing platforms.

Table 3 outlines variability in sequencing performance based on different loading concentrations (library and PhiX) for the same library pool that was sequenced on the same Illumina sequencer. Specifically, we loaded

- Two different library concentrations with two different % PhiX spike-in on the NextSeq 500
- Four different library concentrations with the same % PhiX spike-in on the HiSeq 2500 RR
- One library concentration with different % PhiX spike-in on the HiSeq 2500 HO
- Three different library concentrations with two different % PhiX spike-in on the HiSeq 4000

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 and NovaSeq, respectively. Note that HiSeq 4000/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 and Read 2 (R2)
- Productive V-J Spanning (TRA, TRB) Pair

Library ID	Loading Conc. (pM)	PhiX Spike-in (%)	Instrument	Cluster Density (%PF if HiSeq 4000)	Yield per Lane (Gb)		%>=Q30			Productive V-J Spanning (TRA, TRB) Pair (%)*
					R1	R2	R1	i7	R2	
2	10	1	MiSeq	1186 K/mm^2	3.0	3.0	96.2	95.2	87.4	61.7
1	1	10	NextSeq 500	170 K/mm^2	15.3	15.3	74.1	96.1	75.9	58.7
1	8	1	HiSeq 2500 RR	1101 K/mm^2	28.8	28.8	96.9	95.9	93.6	62.7
1	14	1	HiSeq 2500 HO	1102 K/mm^2	28.7	28.7	93.0	90.8	89.3	63.7
1	200	10	HiSeq 4000	81.00	58.4	58.5	92.8	92.2	89.1	63.7
3	300	10	NovaSeq	81.00	349	349	92.1	96.7	94.5	62.6

Table 2. Reported sequencing metrics for Chromium™ Single Cell V(D)J libraries across different sequencing instruments with recommended loading concentrations and PhiX spike-in concentrations. PhiX spike-in is recommended to increase sequence diversity of Read 1. * Only shown for Pan T cells and based on ~5,000 mean read pairs per cell. Metrics are sample dependent.

Library ID	Loading Conc. (pM)	PhiX Spike-In (%)	Cluster Density (%PF if HiSeq® 4000)	Yield per Lane (Gb)		%>=Q30			Productive V-J Spanning (TRA, TRB) Pair (%)*
				R1	R2	R1	i7	R2	
NextSeq® 500									
1	1.0	10	170 K/mm^2	15.3	15.3	74.1	96.1	75.9	58.7
1	1.3	1	213 K/mm^2	18.3	18.3	66.0	94.2	71.4	54.0
1	1.3	10	214 K/ mm^2	18.3	18.3	67.8	94.3	70.1	53.6
HiSeq 2500 RR									
1	6	1	781 K/mm^2	21.0	21.0	97.8	96.5	95.4	64.1
1	8	1	1101 K/mm^2	28.8	28.8	96.9	95.9	93.6	62.7
1	10	1	1269 K/mm^2	32.4	32.4	95.8	94.7	91.4	63.0
1	11	1	1332 K/mm^2	33.8	33.8	95.7	94.5	91.3	61.2
HiSeq 2500 HO									
1	14	1	1102 K/mm^2	28.7	28.7	93.0	90.9	89.4	63.7
1	14	5	1132 K/mm^2	29.4	29.4	92.5	87.1	89.0	62.6
1	14	10	1158 K/mm^2	29.9	29.9	92.2	83.4	88.5	62.6
1	14	20	1220 K/mm^2	31.0	31.0	91.3	74.7	86.7	62.9
HiSeq 4000									
1	100	1	75	54.4	54.4	92.7	95.8	89.7	63.0
1	200	1	80	58.0	58.0	93.1	96.0	89.6	63.1
1	300	1	80	57.9	57.9	93.1	96.0	89.4	62.9
1	100	10	79	57.3	57.3	93.2	86.0	91.0	64.0
1	200	10	81	58.4	58.5	92.8	92.2	89.1	63.7
1	300	10	80	57.6	57.6	92.3	93.5	88.5	62.7

Table 3. Reported sequencing metrics for Chromium™ Single Cell V(D)J libraries at different loading concentrations and different % PhiX spike-ins across different sequencing instruments. *Only shown for Pan T cells and based on ~5,000 mean read pairs per cell. Metrics are sample dependent.

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 300 pM on the HiSeq 4000 did reveal only minor variability in Q30 quality scores (R1: Δ0.9%, i7: Δ10.0%, R2: Δ2.5%) and productive V-J spanning pairs (Δ1.3%). Libraries sequenced on the HiSeq 2500 RR showed similar consistencies (R1: Δ2.1%, i7: Δ2.0%, R2: Δ4.1%).

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 R1 and R2 were lowest on NextSeq® 500 (~ 76%). In contrast, Q30 quality scores on all other sequencing platforms were generally very stable across both reads (> 86%). The percentage of cells detected with productive V-J spanning pairs remained relatively consistent across all sequencing platforms (~62%) with the exception of those libraries that were sequenced on NextSeq 500 (~58%).

CONCLUSION

We have discussed sequencing parameters for Chromium™ Single Cell V(D)J 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 V(D)J 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 (see Document CG00053)
- Final libraries with fragment length of 200 bp – 3000 bp and significant number of inserts between 400 bp – 1000 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

- *Single Cell Protocols – Cell Preparation Guide* (CG00053)
- *Chromium™ Single Cell V(D)J Reagent Kits User Guide* (CG000086)
- *Chromium™ Single Cell V(D)J Libraries – Sequencing Metrics for Illumina® NovaSeq®* (CG000121)

Notices

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