10x GENOMICS®

TECHNICAL NOTE

Chromium™ Genome v2 Libraries – Sequencing Metrics for Illumina® Sequencers

INTRODUCTION

The Chromium™ Genome v2 Protocol (CG00043) produces Genome v2 libraries ready for Illumina® sequencing. The libraries have been validated on the following Illumina sequencing instruments: HiSeq® 2500 Rapid Run, HiSeq® 3000/4000, HiSeq® X Ten/Five and NovaSeq®. While Genome v2 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 Genome v2 libraries across different Illumina sequencers 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 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 Genome v2 libraries with 1.25 ng of NA12878 DNA following the *Chromium™ Genome Reagent Kits v2 User Guide* - CG00043. The libraries were run using paired-end 2 x 150bp sequencing with a single sample index. Libraries were loaded according to Illumina's recommendations. We assessed sequencing metrics in two ways:

i. Sequence the same library on different Illumina sequencing instruments
ii. Sequence different libraries on the same Illumina sequencing instrument

Table 1 provides the comparison of four libraries (Library ID 1-4) that were sequenced on HiSeq 2500 RR, HiSeq 4000, HiSeq X Ten and NovaSeq to assess differences in sequencing performance across different Illumina sequencing platforms. Libraries ID 1 and ID 2 were sequenced on HiSeq 2500 RR in duplicate to illustrate technical reproducibility.

Table 2 outlines variability in sequencing performance for different libraries that were sequenced on the same Illumina sequencer. Specifically, nine Genome v2 libraries (Library ID 5 – 13) were sequenced on HiSeq 4000 and five Genome v2 libraries (Library ID 14 – 18) were sequenced on HiSeq X Ten.

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

- Cluster densities (K/mm^2) and "Percentage of Clusters Passing Filers (%PF)" for HiSeq 2500 RR and HiSeq 4000/ X Ten/ NovaSeq, respectively. Note that HiSeq 4000/ X Ten/ NovaSeq operate on patterned flow cells.
- Yield per Lane in Gb
- Phred quality scores (shown as %Q30) for Read 1 (R1) and Read 2 (R2)

Library ID	Loading Conc. (pM)	Instrument	Cluster Density (%PF if HiSeq® 4000, X Ten, NovaSeq®)	Yield per Lane (Gb)	R1 %>Q30	R2 %>Q30
Library ID 1						
1	11	HiSeq 2500 RR	1100 K/mm^2	56.9	94.4	88.1
1	11	HiSeq 2500 RR	1090 K/mm^2	56.4	94.3	88.5
1	180	HiSeq 4000	72.5	105	88.9	67.0
Library ID 2						
2	12	HiSeq 2500 RR	1328 K/mm^2	66	92.7	85.3
2	12	HiSeq 2500 RR	1332 K/mm^2	65	92.5	83.8
2	180	HiSeq 4000	73.9	107	87.9	70.7
2	130	HiSeq X Ten	66.5	125	90.3	79.0
Library ID 3						
3	180	HiSeq 4000	73.0	105	90.3	73.2
3	130	HiSeq X Ten	67.3	126	90.4	80.0
Library ID 4						
4	300	NovaSeq®	77.5	665	93.9	90.8

Table 1. Reported sequencing metrics for Chromium™ Genome v2 libraries across different sequencing instruments.

Library ID	Loading Conc. (pM)	Instrument	% of Clusters Passing Filters	Yield per Lane (Gb)	R1 %>Q30	R2 %>Q30
HiSeq 4000						
5	180	HiSeq 4000	73.5	106	88.9	71.6
6	180	HiSeq 4000	72.5	109	92.0	76.1
7	180	HiSeq 4000	69.1	100	89.8	74.4
8	180	HiSeq 4000	74.4	107	89.9	73.1
9	180	HiSeq 4000	73.3	106	91.8	76.3
10	180	HiSeq 4000	71.5	103	91.4	72.4
11	180	HiSeq 4000	73.4	106	91.1	72.8
12	180	HiSeq 4000	66.7	96	82.1	72.9
13	180	HiSeq 4000	71.3	103	86.6	76.1
HiSeq X Ten						
14	130	HiSeq X Ten	68.0	127	92.3	83.3
15	130	HiSeq X Ten	64.4	120	88.6	76.6
16	130	HiSeq X Ten	64.5	120	90.7	81.3
17	130	HiSeq X Ten	67.2	126	92.2	83.2
18	130	HiSeq X Ten	69.0	126	90.7	80.9

Table 2. Reported sequencing metrics for seven and five different Chromium Genome v2 libraries sequenced on HiSeq 4000 and HiSeq X Ten, respectively.

DISCUSSION

As expected, yield correlated with the amount of sequencing data that each sequencing platform can generate. Overall, libraries sequenced on the NovaSeq produced the highest Q30 quality scores, particularly for R2 (90.8%) compared to all other sequencing platforms. Q30 quality scores were higher for R1 compared to R2 on all sequencing platforms. Q30 quality scores for R2 were lowest on HiSeq 4000 (~ 70%) and were variable across different libraries (Δ6.2%). In contrast, Q30 quality scores on HiSeq X Ten were generally very stable across different libraries for both R1 and R2 (< Δ1%). We do recommend to spike-in PhiX to Chromium Genome v2 libraries at the following concentrations: 1% on the NovaSeq and HiSeq 2500 RR, 0.5% on the HiSeq 4000 and HiSeq X Ten. Sequencing metrics including %PF, Yield per Lane, and Q30 quality scores remained relatively consistent between different libraries sequenced on the same sequencing platform.

CONCLUSION

We have discussed sequencing parameters for Chromium™ Genome v2 libraries and different sequencing metrics that are typically obtained for Illumina® sequencing instruments. Libraries performed best on the NovaSeq and we encourage users to sequence their libraries on that platform, particularly if high sample sequencing throughput is required. Sequencing metrics reported in this Technical Note serve as guideline to assess sequencing run quality of Chromium Genome v2 libraries. Note that additional factors will contribute to overall success of a sequencing run and will have an impact on applications performance metrics such as haplotype phase block length and ability to call structural variants. These include:

- High quality of sample preparation to obtain high molecular weight (HMW) gDNA (Document CG00045)
- Final libraries with fragment length of 400 bp – 1000 bp and significant number of inserts between 400 bp – 600 bp in length for optimal cluster formation on Illumina flow cells (Document CG000066)
- Reliable and accurate library quantification using the KAPA DNA Quantification Kit using a fixed insert size of 550 bp (Document CG00048)
- Sequencing platform

REFERENCES

- *Chromium™ Genome Reagent Kits v2 User Guide* (CG00043)
- *Sample Preparation Recommendations for the Chromium™ Genome Kit* (CG00045)
- *QC of Chromium™ Genome v2 Libraries: Qualitative Evaluation Using Agilent Bioanalyzer* (CG000066)
- *QC of Chromium™ Genome Libraries: Quantitative Evaluation Using qPCR* (CG00048)

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