10x GENOMICS®

# TECHNICAL NOTE

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

## INTRODUCTION

The Chromium™ Single Cell V(D)J Protocol (CG000086) produces Single Cell V(D)J libraries, ready for Illumina® sequencing. Single Cell V(D)J 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 V(D)J libraries on the NovaSeq sequencing platform. This Technical Note describes key sequencing metrics on the Illumina NovaSeq platform and is intended to provide general guidance on the expected range of sequencing metrics. Individual results may still vary, depending on the particular sample and loading characteristics.

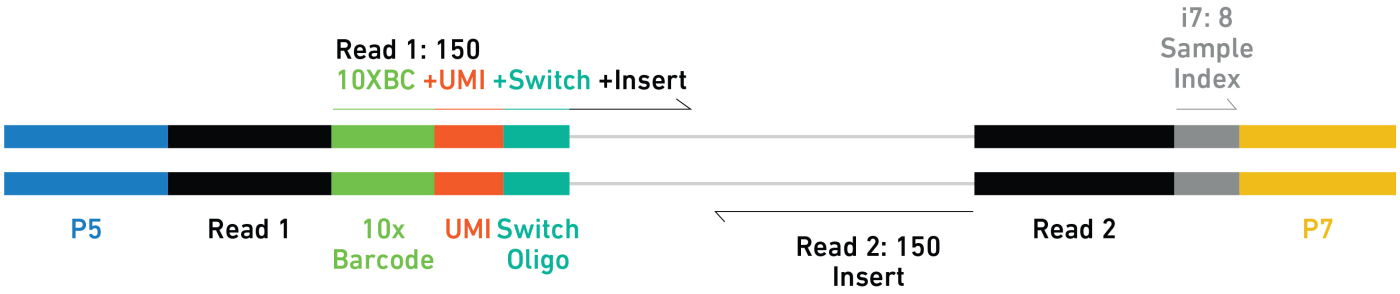


Fig. 1. Schematic of a fragment from a final Chromium Single Cell V(D)J library.

## METHOD

Chromium Single Cell V(D)J libraries were prepared for the following 72 samples (Table 1):

| Cell Type | # Libraries |
|---|---|
| Pan T cells | 8 |
| Peripheral blood mononuclear cells (PBMCs) | 2 |
| Jurkat (lymphoblast cell line) | 6 |
| Antigen specific T cells | 52 |
| CD4+ T cells | 2 |
| CD8+ T cells | 2 |

Table 1. Chromium Single Cell V(D)J libraries used in this study.

Libraries were prepared following the *Chromium™ Single Cell V(D)J Reagent Kits User Guide* - CG000068. Libraries were pooled at equimolar ratios and run on the Illumina® NovaSeq® using paired-end sequencing with a single index read per sample. We recommend the sequencing run parameters listed in Table 2.

## RESULTS

Libraries were sequenced using a NovaSeq 5000/6000 S2 Reagent Kit (300 cycles) and the sequencing run parameters listed in Table 2.

| Sequencing Read | Recommended Number of Cycles |
|---|---|
| Read 1 | 150 cycles |
| i7 index | 8 cycles |
| i5 index | 0 cycles |
| Read 2 | 150 cycles |

Table 2. Recommended sequencing run parameter for Chromium Single Cell V(D)J 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 (i7) and Read 2 (R2)
- Productive V-J Spanning (TRA, TRB) Pair

Note that the pool of 72 libraries was sequenced on two different NovaSeq instruments (NovaSeq Instrument 1 and 2).

| Library ID | Loading Conc. (pM) | Instrument | %PF | Yield per Lane (Gb) | | %>=Q30 | | | Productive V-J Spanning (TRA, TRB) Pair (%)* |
|---|---|---|---|---|---|---|---|---|---|
| | | | | R1 | R2 | R1 | i7 | R2 | |
| **NovaSeq Instrument 1** | | | | | | | | | |
| 1 | 300 | NovaSeq | 81 | 349 | 349 | 92.1 | 96.7 | 94.5 | 62.6 |
| **NovaSeq Instrument 2** | | | | | | | | | |
| 1 | 300 | NovaSeq | 79 | 338 | 338 | 92.7 | 96.5 | 92.5 | 62.6 |

Table 3. Reported sequencing metrics for Chromium Single Cell V(D)J libraries on two NovaSeq instruments with recommended loading concentration. Libraries were sequenced with 10% PhiX. *Only shown for Pan T cells and based on ≥5,000 mean read pairs per cell. Metrics are sample dependent.

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 V(D)J libraries that were prepared according to the *Chromium Single Cell V(D)J Reagent Kits User Guide* (CG000068). The profiles are characteristic for Chromium Single Cell V(D)J libraries that are sequenced with the recommended number of cycles (see Technical Note *Base Composition of Sequencing Reads of Chromium Single Cell V(D)J Libraries* – CG000103 for more details).

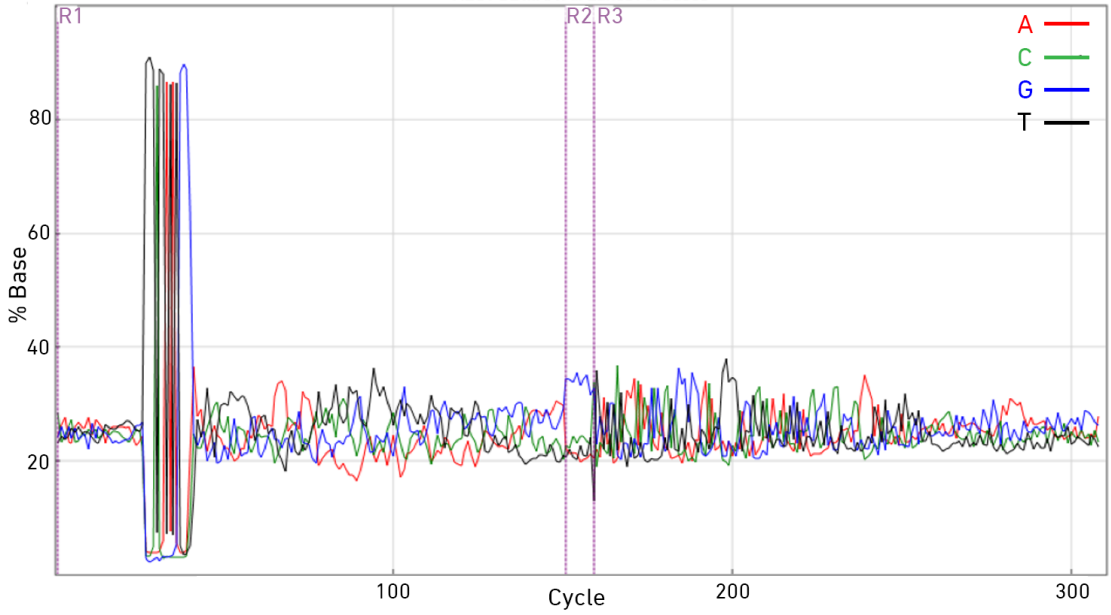


Fig. 2. Representative example of the 'Data by Cycle' plot in the Sequencing Analysis Viewer software (Illumina®). Shown is the percentage of clusters for which the selected base has been called (% base: y axis) along the sequencing length (x axis). Profile is based on sequencing 10x library by itself 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 V(D)J libraries run on the Illumina NovaSeq®. Shown are three plots that illustrate the Q30 percentage along the sequencing length for the top, the bottom and the combined flow cells. Occasionally, the Q30 profiles can differ between the top and the bottom flow cell surface which can be driven by Illumina consumables, fluidics and/ or optics. Percentages of Q30 are stable at the beginning of Read 1. The percentages drop at the beginning of the Switch Oligo sequence due to its low diversity but quickly recover after ~40 cycles. Percentages of Q30 remained stable for i7 and R2 with the bottom flow cell surface exhibiting overall lower quality scores.

## DISCUSSION

As expected, libraries sequenced on NovaSeq produced high quality data. Overall, sequencing data quality is comparable to data obtained from the HiSeq® 2500 and HiSeq 4000 instruments. Q30 quality scores for R1 and R2 are stable at >90% (with the exception of the Switch Oligo sequence) and as expected decreased only towards the end of each read. We do recommend to load Chromium™ Single Cell V(D)J libraries at a concentration of 300 pM with 10% PhiX spike-in. As expected, the percentage of productive V-J spanning (TRA, TRB) pairs was comparable to libraries that were sequenced on the HiSeq 2500 or HiSeq 4000 (see Technical Note *Chromium™ Single Cell V(D)J Libraries – Sequencing Metrics for Illumina® Sequencers* – CG000102 for more details). Note, that only metrics for Pan T cells are shown and will depend on the sample type.

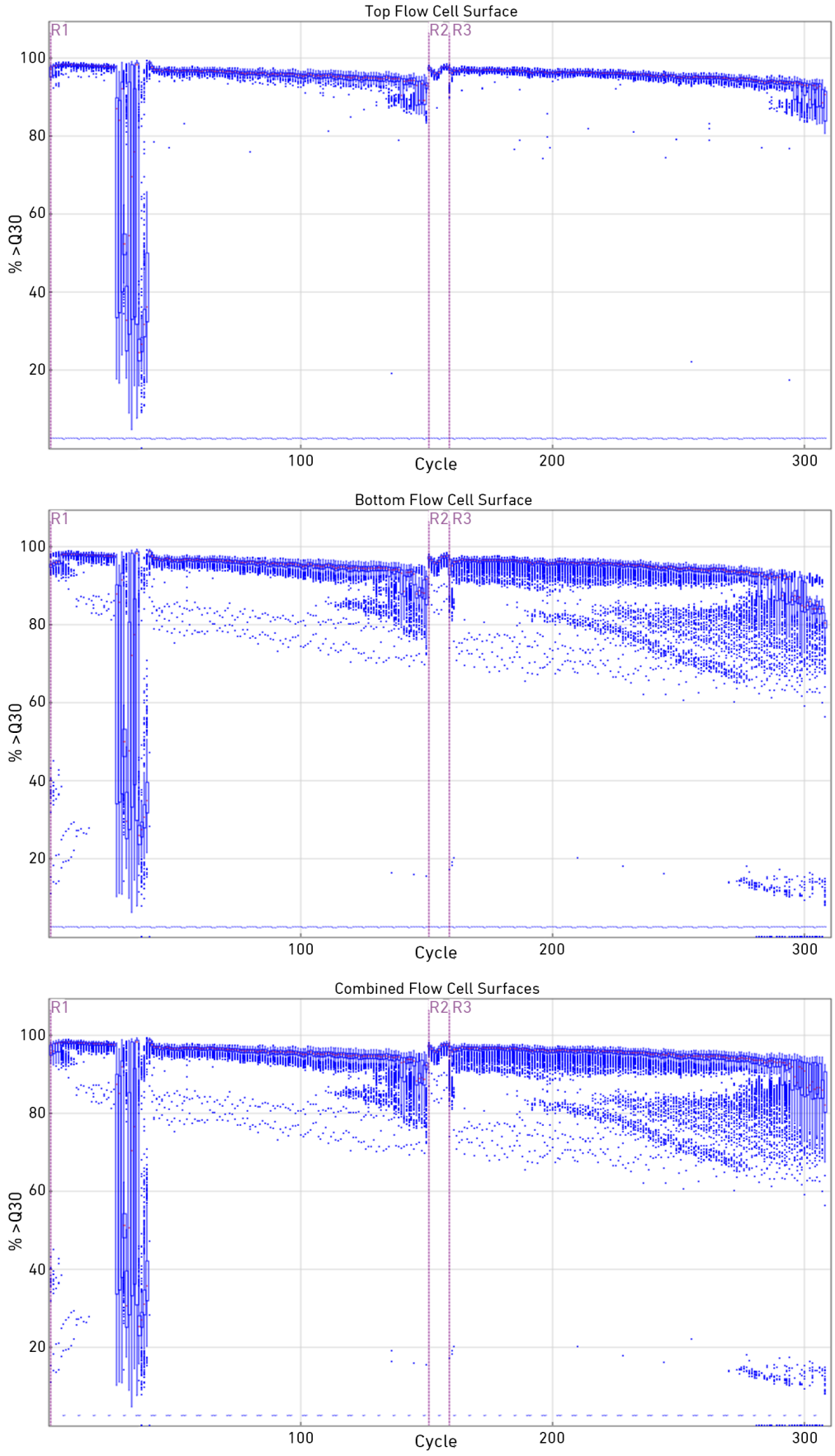


Fig. 3. Representative examples of the 'Data by Cycle' plot in the Sequencing Analysis Viewer software (Illumina®). Shown is the Q30 percentage along the sequencing length for the top, the bottom and the combined flow cells. Profiles are based on sequencing 10x library by itself with no other library type sequenced alongside.

## CONCLUSION

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

## REFERENCES


- *Chromium™ Single Cell V(D)J Reagent Kits User* Guide (CG000068)
- *Chromium™ Single Cell V(D)J Libraries – Sequencing Metrics for Illumina® Sequencers* (CG000102)
- *Base Composition of Sequencing Reads of Chromium™ Single Cell V(D)J Libraries* (CG000103)

# Notices

## Document Number

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## Legal Notices



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