TECHNICAL NOTE

Sequencing Metrics & Base Composition of Single Cell 3' v3.1 Dual Index Libraries

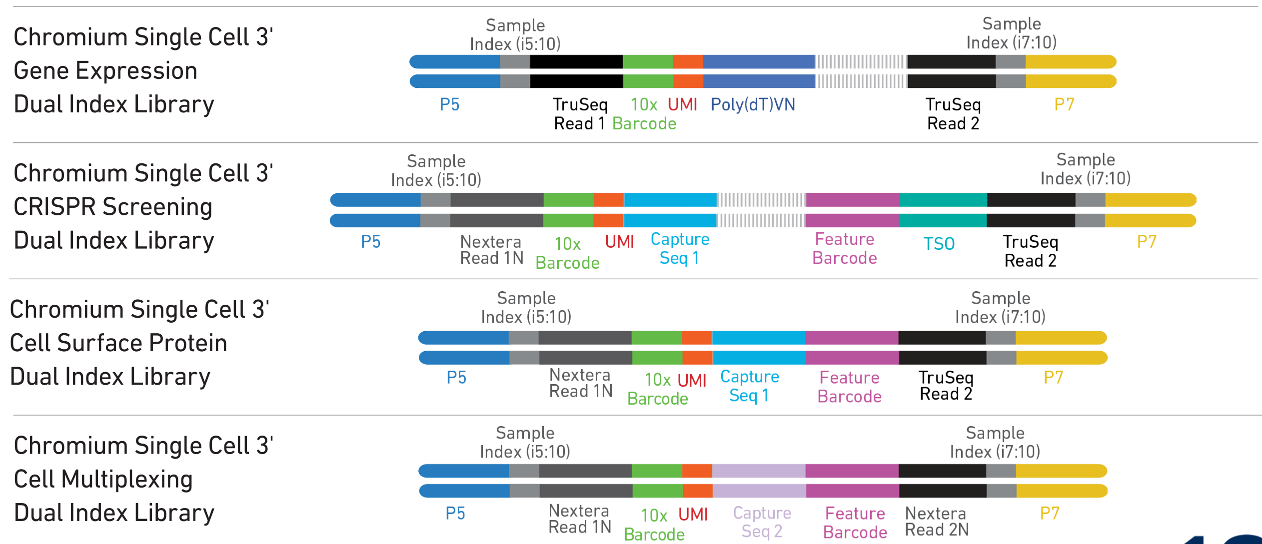
Introduction

The Chromium Next GEM Single Cell 3' v3.1 (dual index) standard and high throughput (HT) workflows produce sequencing-ready Gene Expression libraries alone or in combination with Cell Surface Protein, CRISPR Screening, or Cell Multiplexing libraries from the same single cells. This enables simultaneous profiling of cellular features in combination with gene expression profiling. This Technical Note presents a comparison of sequencing metrics for various Single Cell 3' Dual Index library types across Illumina platforms. Individual results may vary depending on the specific sequencing instrument and/or particular sample and loading characteristics.

Single Cell 3' Dual Index Libraries

Four dual index library types can be generated using either the Chromium Next GEM Single Cell 3' HT v3.1 or the standard Single Cell 3' v3.1 (dual index) reagents and protocols. The four library schematics are shown below.

The libraries include cDNA insert or Feature Barcode constructs which begin with P5 and end with P7, sequences necessary for binding to the Illumina flow cell. Read 1 is used to sequence 16 bp 10x Barcodes and 12 bp UMI and Read 2 is used for priming and sequencing the cDNA insert or the Feature Barcode as illustrated above. The two 10 bp sample indexes are sequenced in the i5 and i7 reads.



10x GENOMICS

Methods Overview

Single Cell 3' Gene Expression libraries alone or in combination with Cell Surface Protein, CRISPR, or Cell Multiplexing libraries were generated from a variety of sample types (Peripheral Blood Mononuclear Cells, dissociated mouse embryonic brain tissue, dissociated human lung tumors, and A375 and A549 cells transduced with CRISPR machinery) as described in the respective user guides (see References).

For the standard Single Cell 3' v3.1 assay, 1,000-5,000 cells were targeted using the Chromium Next GEM Chip G to generate the libraries. For Cell Multiplexing experiments, 1,000 to 30,000 cells were targeted. The libraries were quantified and sequenced as indicated in the results (Tables 1-7; Figure 1).

For the Single Cell 3' HT v3.1 assay, 20,000 cells were targeted using the Chromium Next GEM Chip M to generate the libraries. For HT Cell Multiplexing experiments, 60,000 cells were targeted. The libraries were quantified and sequenced as indicated in Figure 1.

Results Overview

Tables 1-7 show representative sequencing metrics and base composition data derived from the indicated libraries. The Q30 quality scores, representative Data by Cycle plots, and other metrics for each sequencer/workflow is shown for the standard Single Cell 3' v3.1 assay. Figure 1 shows representative 'Data by Cycle' plots from the Illumina SAV software displaying the percentage of base calls and Q30 quality scores of Single Cell 3' v3.1 standard and the HT assay derived libraries run on the NovaSeq.

Individual results may vary depending on the specific sequencing instrument and/or particular sample and loading characteristics.

Conclusions

In summary, % Bases by cycle and % ≥Q30 Quality Score distribution showed highly consistent profiles for all sequencing platforms and workflows tested. Furthermore, sequencing performance between the library types generated using the Single Cell 3' v3.1 standard and the HT assay are comparable. These data serve as guidelines for assessing the quality of Single Cell 3' Dual Index library sequencing. Additional factors that may contribute to overall success of a sequencing run and impact downstream application performance metrics include:

- Sample preparation to obtain a high quality single cell/nuclei suspension.
- Final libraries with fragment lengths within the expected size range for each library type, for optimal cluster formation on Illumina flow cells.
- Reliable and accurate library quantification using the KAPA DNA Quantification Kit using the average insert size determined by Agilent Bioanalyzer QC.
- Sequencing platform loading concentration.

Gene Expression Dual Index Library

Three Chromium Single Cell 3' Gene Expression (dual index) libraries were pooled and sequenced on indicated Illumina sequencers. 'Data by Cycle' plots from the Illumina SAV software displaying the percentage of base calls and Q30 quality scores along with additional metrics are shown in Table 1.

Sequencing configuration & run parameters:

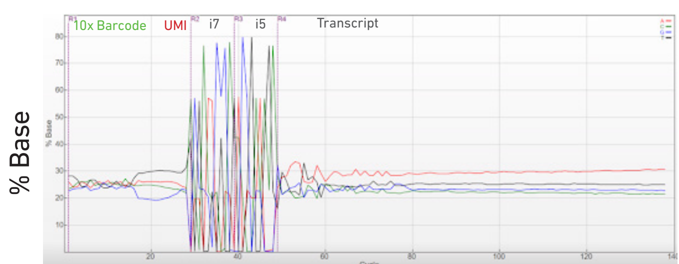
Minimum sequencing depth 20,000 read pairs per cell

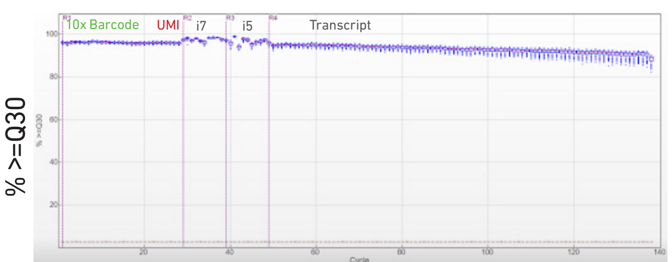
Paired-end, dual indexing

- Read 1: 28 cycles
- i7 Index: 10 cycles
- i5 Index: 10 cycles
- Read 2: 90 cycles

Table 1: Representative Plots and Sequencing Data

Plots shown are from a pool of three Gene Expression libraries sequenced on a NovaSeq SP flowcell.





		% ≥Q30				Yield per Lane (Gb)		Reads Mapped to Genome (%)
		R1	i7	i5	R2	R 1	R 2	
NextSeq 550								
	Loading Conc. (pM): 1.8 Cluster Density: 235 K/mm^2 PhiX (%): 1	96.0	91.6	96.2	89.7	3.7	12.1	95.7
HiSeq 2500 RR								
	Loading Conc. (pM): 10 Cluster Density: 1047 K/mm^2 PhiX (%): 1	97.6	96.4	97.7	89.0	4.9	16.2	95.4
NovaSeq (SP flow cell)								
	Loading Conc. (pM): 300 % PF*: 80.9 PhiX (%) 1	96.2	96.7	97.7	93.1	14.0	46.1	96.5
NextSeq 2000								
	Loading Conc. (pM): 650 % PF*: 81.7 PhiX (%) 1	95.4	96.6	97.3	94.5	14.7	48.4	95.9

*Percent Pass Filter (% PF) is reported for NovaSeq and NextSeq 2000 instead of cluster density due to the patterned flow cell

Cell Surface Protein Dual Index Library

Three Chromium Single Cell 3' Cell Surface Protein (dual index) libraries were pooled and sequenced on indicated Illumina sequencers. 'Data by Cycle' plots from the Illumina SAV software displaying the percentage of base calls and Q30 quality scores along with additional metrics are shown below.

Sequencing configuration & run parameters:

Minimum sequencing depth 5,000 read pairs per cell

Paired-end, dual indexing

- Read 1: 28 cycles
- i7 Index: 10 cycles
- i5 Index: 10 cycles
- Read 2: 25 or 90 cycles

Read 2 can be sequenced at either 90 or 25 cycles.
Sequencing to 90 bp leads to a drop in quality scores after the first 25 Read 2 cycles due to sequencing through the Capture Sequence. However, this does not impact assay performance. This skewed base distribution can be normalized by pooling the Cell Surface Protein library with Gene Expression libraries (see Table 3).

Table 2: Representative Plots and Sequencing Data

Read 2: 25 cycles (HiSeq 2500)

Read 2: 90 cycles (NovaSeq)

		% ≥Q30				Yield per Lane (Gb)		CSP Fraction Antibody Reads (%)
		R1	i7	i5	R2	R1	R2	
NextSeq 550								
	Loading Conc. (pM): 1.8 Cluster Density: 172 K/mm^2 PhiX (%): 1	97.4	97.4	96.8	96.1**	2.8	2.5	93.6
HiSeq 2500 RR								
	Loading Conc. (pM): 10 Cluster Density: 1,094 K/mm^2 PhiX (%): 1	97.7	95.7	98.1	96.5**	5.2	4.6	94.0
NovaSeq (SP flow cell)								
	Loading Conc. (pM): 300 % PF*: 81.3 PhiX (%): 1	97.1	96.9	97.1	75.6***	14.0	46.3	93.8

*Percent Pass Filter (% PF) is reported for NovaSeq instead of cluster density due to the patterned flow cell
**Read 2: 25 cycles; ***Read2: 90 cycles

Gene Expression + Cell Surface Protein Dual Index Libraries

Three Chromium Single Cell 3' Gene Expression and three Cell Surface Protein (dual index) libraries were pooled (3:1 ratio) and sequenced on indicated Illumina sequencers. 'Data by Cycle' plot from the Illumina SAV software displaying the percentage of base calls and Q30 quality scores along with additional metrics are shown below.

Sequencing configuration & run parameters:

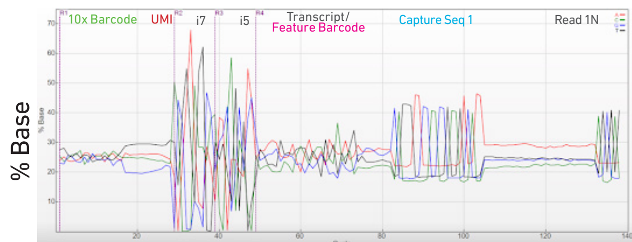
Minimum sequencing depth 20,000 read pairs per cell for Gene Expression and 5,000 read pairs per cell for Cell Surface Protein library

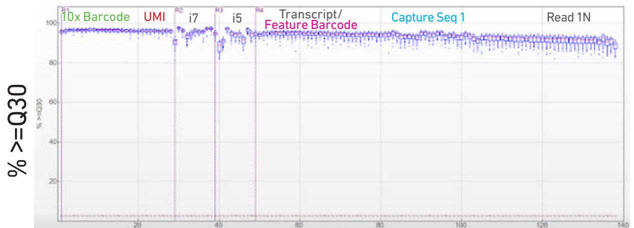
Paired-end, dual indexing

- Read 1: 28 cycles
- i7 Index: 10 cycles
- i5 Index: 10 cycles
- Read 2: 90 cycles

Table 3: Representative Plots and Sequencing Data

Plots shown are from a pool of three Gene Expression libraries and three Cell Surface Protein libraries sequenced together on a NovaSeq SP flowcell.





		% ≥Q30				Yield per Lane (Gb)		Reads Mapped to Genome (%)	CSP Fraction Antibody Reads (%)
		R1	i7	i5	R2	R1	R2		
NextSeq 550									
	Loading Conc. (pM): 1.8 Cluster Density: 222 K/mm^2 PhiX (%): 1	96.3	93.6	94.4	91.1	3.5	11.4	95.6	93.4
HiSeq 2500 RR									
	Loading Conc. (pM): 10 Cluster Density: 1,030 K/mm^2 PhiX (%): 1	97.9	96.2	97.2	90.1	4.9	16.0	95.2	94.1
NovaSeq (SP flow cell)									
	Loading Conc. (pM): 300 % PF*: 81.8 PhiX (%): 1	96.4	93.4	95.3	93.3	14.1	46.5	96.2	93.9

*Percent Pass Filter (% PF) is reported for NovaSeq instead of cluster density due to the patterned flow cell

Gene Expression + CRISPR Screening Dual Index Libraries

Two Chromium Single Cell 3' Gene Expression and two CRISPR Screening (dual index)libraries were pooled (3:1 ratio) and sequenced on indicated Illumina sequencers. 'Data by Cycle' plots from the Illumina SAV software displaying the percentage of base calls and Q30 quality scores along with additional metrics are shown below.

Sequencing configuration & run parameters:

Minimum sequencing depth 20,000 read pairs per cell for Gene Expression and 5,000 read pairs per cell for CRISPR Screening library

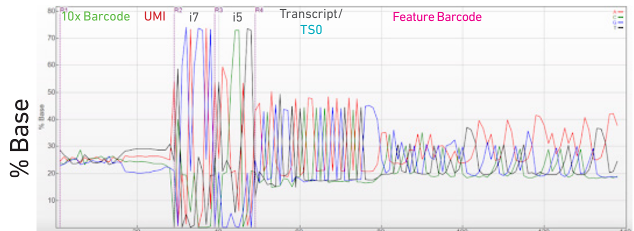
Paired-end, dual indexing

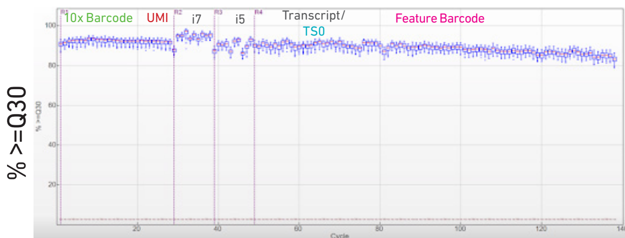
- Read 1: 28 cycles
- i7 Index: 10 cycles
- i5 Index: 10 cycles
- Read 2: 90 cycles

Pooling Single Cell 3' Gene Expression & CRISPR Screening dual index libraries is recommended for sequencing to maintain nucleotide diversity.

Table 4: Representative Plots and Sequencing Data

Plots shown are from a pool of two Gene Expression libraries and two CRISPR Screening libraries sequenced together on a NovaSeq SP flow cell.





		% ≥Q30				Yield per Lane (Gb)		GEX Reads Mapped to Genome (%)	CRISPR Fraction Guide Reads (%)
		R1	i7	i5	R2	R1	R2		
NextSeq 550									
	Loading Conc. (pM): 1.8 Cluster Density: 223 K/mm^2 PhiX (%): 1	96.3	94.0	95.9	91.2	3.5	11.6	96.0	67.0
HiSeq 2500 RR									
	Loading Conc. (pM): 10 Cluster Density: 951 K/mm^2 PhiX (%): 1	98.0	96.3	97.6	88.1	4.5	14.9	95.0	65.3
NovaSeq (SP flow cell)									
	Loading Conc. (pM): 300 % PF*: 76.9 PhiX (%): 1	92.3	89.7	94.2	88.6	13.3	43.7	96.3	66.1

*Percent Pass Filter (% PF) is reported for NovaSeq instead of cluster density due to the patterned flow cell

Gene Expression + Cell Multiplexing Dual Index Libraries

Three Chromium Single Cell 3' Gene Expression and three Cell Multiplexing (dual index) libraries were pooled (4:1 ratio) and sequenced on indicated Illumina sequencers. 'Data by Cycle' plot from the Illumina SAV software displaying the percentage of base calls and Q30 quality scores along with additional metrics are shown below.

Sequencing configuration & run parameters:

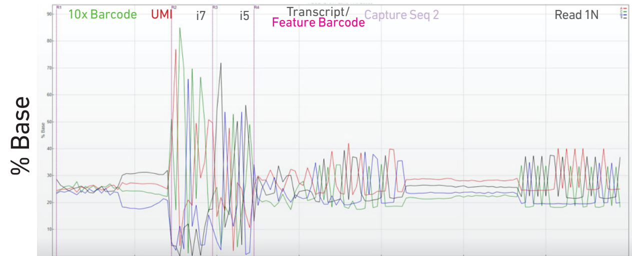
Minimum sequencing depth 20,000 read pairs per cell for Gene Expression and 5,000 read pairs per cell for Cell Multiplexing library.

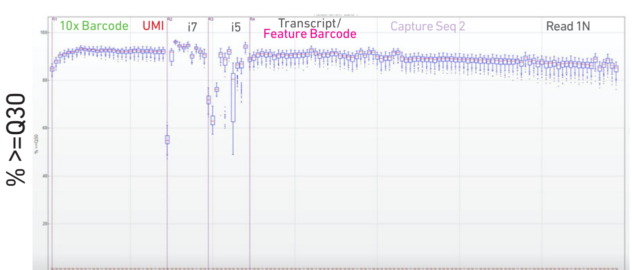
Paired-end, dual indexing

- Read 1: 28 cycles
- i7 Index: 10 cycles
- i5 Index: 10 cycles
- Read 2: 90 cycles

Table 5: Representative Plots and Sequencing Data

Plots shown are from a pool of three Gene Expression libraries and three Cell Multiplexing libraries sequenced together on a NovaSeq SP flowcell.





		% ≥Q30				Yield per Lane (Gb)		Reads Mapped to Genome (%)	Fraction Cell Multiplex Reads (%)
		R1	i7	i5	R2	R1	R2		
NextSeq 550									
	Loading Conc. (pM): 1.8 Cluster Density: 290 K/mm^2 PhiX (%): 1	93.6	89.7	90.7	86.8	16.0	52.8	94.5	96.8
NovaSeq (SP flow cell)									
	Loading Conc. (pM): 300 % PF*: 69.4 PhiX (%): 1	91.7	80.5	88.9	88.9	12.0	39.5	95.0	98.1
NextSeq 2000									
	Loading Conc. (pM): 650 % PF*: 79.4 PhiX (%): 1	94.3	94.7	94.8	94.2	14.3	47.1	95.6	98.0

*Percent Pass Filter (% PF) is reported for NovaSeq and NextSeq 2000 instead of cluster density due to the patterned flow cell

Gene Expression + Cell Multiplexing + Cell Surface Protein Dual Index Libraries

Two Chromium Single Cell 3' Gene Expression, two Cell Multiplexing, and two Cell Surface Protein (dual index) libraries were pooled (4:1:1 ratio) and sequenced on indicated Illumina sequencers. 'Data by Cycle' plot from the Illumina SAV software displaying the percentage of base calls and Q30 quality scores along with additional metrics are shown below.

Sequencing configuration & run parameters:

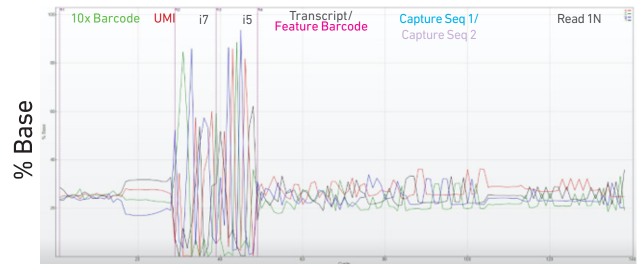
Minimum sequencing depth 20,000 read pairs per cell for Gene Expression and 5,000 read pairs per cell for Cell Multiplexing and Cell Surface Protein library.

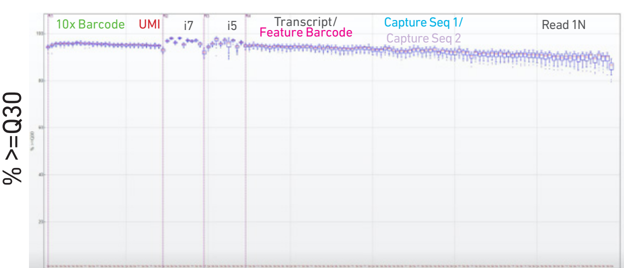
Paired-end, dual indexing

- Read 1: 28 cycles
- i7 Index: 10 cycles
- i5 Index: 10 cycles
- Read 2: 90 cycles

Table 6: Representative Plots and Sequencing Data

Plots shown are from a pool of two Gene Expression libraries, two Cell Multiplexing, and two Cell Surface Protein libraries sequenced together on a NovaSeq SP flowcell.





		% ≥Q30				Yield per Lane (Gb)		Reads Mapped to Genome (%)	Fraction Cell Multiplex Reads (%)	CSP Fraction Antibody Reads (%)
		R1	i7	i5	R2	R1	R2			
NextSeq 550										
	Loading Conc. (pM): 1.8 Cluster Density: 208 K/mm^2 PhiX (%): 1	96.4	94.4	96.4	91.2	13.1	43.2	95.6	97.4	90.2
NovaSeq (SP flow cell)										
	Loading Conc. (pM): 300 % PF*: 78 PhiX (%): 1	95.4	95.5	96.5	92.3	13.4	44.3	96.4	98.2	89.8
NextSeq 2000										
	Loading Conc. (pM): 650 % PF*: 80.9 PhiX (%): 1	94.8	94.2	94.7	94.1	14.6	48.0	97.1	98.0	88.9

*Percent Pass Filter (% PF) is reported for NovaSeq and NextSeq 2000 instead of cluster density due to the patterned flow cell

Gene Expression + Cell Multiplexing + CRISPR Screening Dual Index Libraries

Two Chromium Single Cell 3' Gene Expression, two Cell Multiplexing, and two CRISPR screening (dual index) libraries were pooled (4:1:1 ratio) and sequenced on indicated Illumina sequencers. 'Data by Cycle' plot from the Illumina SAV software displaying the percentage of base calls and Q30 quality scores along with additional metrics are shown below.

Sequencing configuration & run parameters:

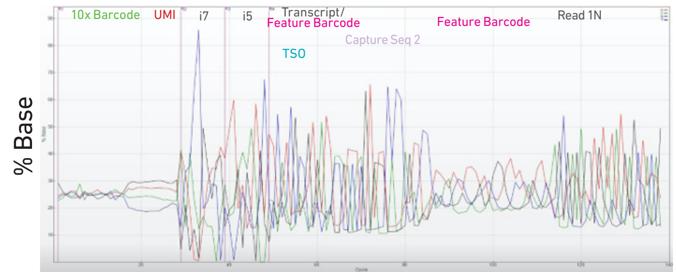
Minimum sequencing depth 20,000 read pairs per cell for Gene Expression and 5,000 read pairs per cell for Cell Multiplexing and CRISPR Screening library.

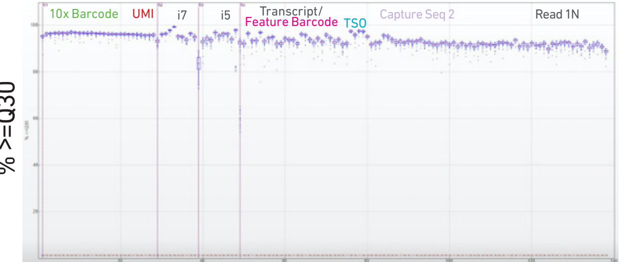
Paired-end, dual indexing

- Read 1: 28 cycles
- i7 Index: 10 cycles
- i5 Index: 10 cycles
- Read 2: 90 cycles

Table 7: Representative Plots and Sequencing Data

Plots shown are from a pool of two Gene Expression libraries, two Cell Multiplexing, and two CRISPR Screening libraries sequenced together on a NovaSeq SP flowcell.





	% ≥Q30				Yield per Lane (Gb)		Reads Mapped to Genome (%)	Fraction Cell Multiplex Reads (%)	CRISPR Fraction Guide Reads (%)
	R1	i7	i5	R2	R1	R2			
NextSeq 550									
Loading Conc. (pM): 1.8 Cluster Density: 225 K/mm² PhiX (%): 1	96.0	94.5	95.8	90.2	13.9	45.8	96.6	97.5	64.4
NovaSeq (SP flow cell)									
Loading Conc. (pM): 300 % PF*: 81.9 PhiX (%): 1	96.3	93.6	95.9	92.8	14.1	46.5	97.3	98.2	64.8
NextSeq 2000									
Loading Conc. (pM): 650 % PF*: 84.9 PhiX (%): 1	95.5	91.3	94.4	93.6	15.3	50.3	97.6	98.1	64.0

*Percent Pass Filter (% PF) is reported for NovaSeq and NextSeq 2000 instead of cluster density due to the patterned flow cell

Gene Expression Dual Index Libraries from Single Cell 3' v3.1 standard & HT assays

A pool (1:1) of two Chromium Single Cell 3' Gene Expression (standard assay) libraries were compared to a pool (1:1) of two Chromium Single Cell 3' Gene Expression (HT assay) libraries. 'Data by Cycle' plot from the Illumina SAV software displaying the percentage of base calls and Q30 quality scores are shown in Figure 1.

Sequencing configuration & run parameters:

Minimum sequencing depth 20,000 read pairs per cell
Paired-end, dual indexing

- Read 1: 28 cycles
- i7 Index: 10 cycles
- i5 Index: 10 cycles
- Read 2: 90 cycles

Figure 1: Representative Plots

Plots shown are from a pool (1:1) of two Gene Expression libraries, sequenced together on a NovaSeq S1 flowcell.

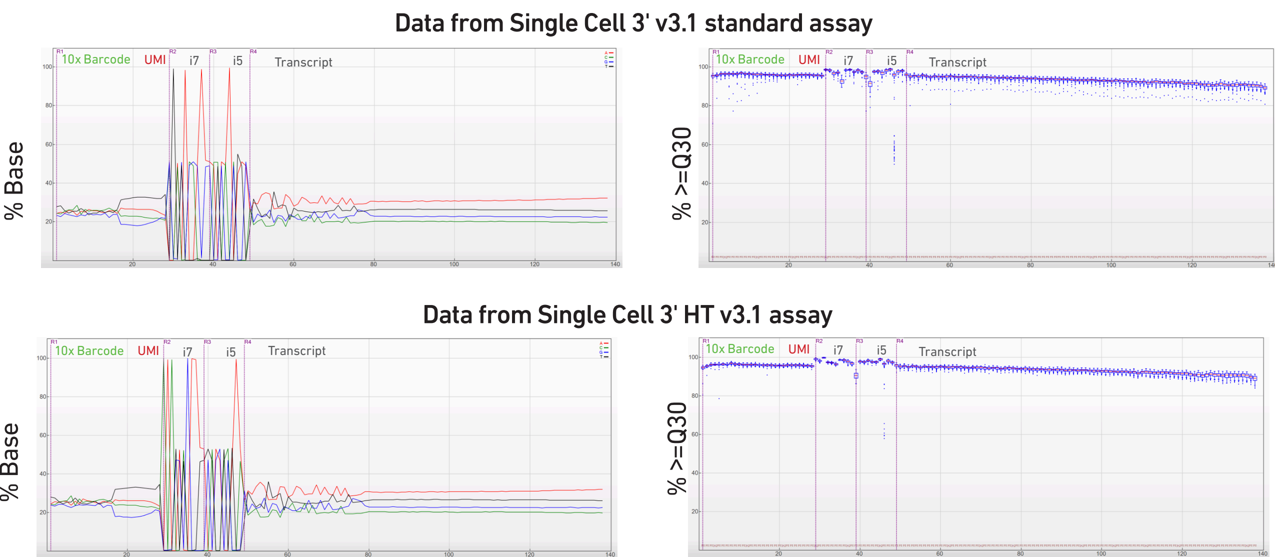


Figure 1. Representative plots derived from Single Cell 3' v3.1 standard assay (top panel) and HT assay (bottom panel) libraries sequenced on a NovaSeq S1 flowcell. Metrics were comparable across all other sequencers tested (data not shown).

References

- Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) User Guide (CG000315)
- Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for CRISPR Screening User Guide (CG000316)
- Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Surface Protein User Guide (CG000317)
- Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Multiplexing (CG000388)
- Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for CRISPR Screening and Cell Multiplexing (CG000389)
- Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Surface Protein and Cell Multiplexing (CG000390)
- Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 (Dual Index) (CG000416)
- Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Surface Protein (CG000417)
- Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for CRISPR Screening (CG000418)
- Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Multiplexing (CG000419)
- Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Cell Multiplexing (CG000420)
- Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for CRISPR Screening & Cell Multiplexing (CG000421)

© 2022 10x Genomics, Inc. (10x Genomics). All rights reserved. Duplication and/or reproduction of all or any portion of this document without the express written consent of 10x Genomics, is strictly forbidden. Nothing contained herein shall constitute any warranty, express or implied, as to the performance of any products described herein. Any and all warranties applicable to any products are set forth in the applicable terms and conditions of sale accompanying the purchase of such product. 10x Genomics provides no warranty and hereby disclaims any and all warranties as to the use of any third-party products or protocols described herein. The use of products described herein is subject to certain restrictions as set forth in the applicable terms and conditions of sale accompanying the purchase of such product. A non-exhaustive list of 10x Genomics' marks, many of which are registered in the United States and other countries can be viewed at: www.10xgenomics.com/trademarks. 10x Genomics may refer to the products or services offered by other companies by their brand name or company name solely for clarity, and does not claim any rights in those third-party marks or names. 10x Genomics products may be covered by one or more of the patents as indicated at:www.10xgenomics.com/patents. The use of products described herein is subject to 10x Genomics Terms and Conditions of Sale, available at www.10xgenomics.com/legal-notices, or such other terms that have been agreed to in writing between 10x Genomics and user. All products and services described herein are intended FOR RESEARCH USE ONLY and NOT FOR USE IN DIAGNOSTIC PROCEDURES.

The use of 10x Genomics products in practicing the methods set forth herein has not been validated by 10x Genomics, and such non-validated use is NOT COVERED BY 10X GENOMICS STANDARD WARRANTY, AND 10X GENOMICS HEREBY DISCLAIMS ANY AND ALL WARRANTIES FOR SUCH USE. Nothing in this document should be construed as altering, waiving or amending in any manner 10x Genomics terms and conditions of sale for the Chromium Controller or the Chromium Single Cell Controller, consumables or software, including without limitation such terms and conditions relating to certain use restrictions, limited license, warranty and limitation of liability, and nothing in this document shall be deemed to be Documentation, as that term is set forth in such terms and conditions of sale. Nothing in this document shall be construed as any representation by 10x Genomics that it currently or will at any time in the future offer or in any way support any application set forth herein.

Contact:
support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA