TECHNICAL NOTE

Sequencing Metrics & Base Composition of Single Cell Multiome ATAC Libraries

Introduction

The Chromium Next GEM Single Cell Multiome ATAC + Gene Expression workflow produces sequencing-ready Multiome ATAC and Gene Expression libraries from the same single nuclei, enabling simultaneous epigenomic and gene expression profiling. This Technical Note presents a comparison of sequencing metrics for a Single Cell Multiome ATAC library across multiple Illumina® platforms. Individual results may vary depending on the specific sequencing instrument and/or particular sample and loading characteristics.

As the Multiome Gene Expression library is identical to the Chromium Single Cell 3' Gene Expression (dual index) library, see the Gene Expression Dual Index Library data (Table 1) in the Technical Note– Sequencing Metrics & Base Composition of Single Cell 3' Gene Expression and Feature Barcode Dual Index Libraries (CG000374).

Single Cell Multiome Libraries

Chromium Next GEM Single Cell Multiome ATAC + Gene Expression assay produces two library types from the same single nuclei:

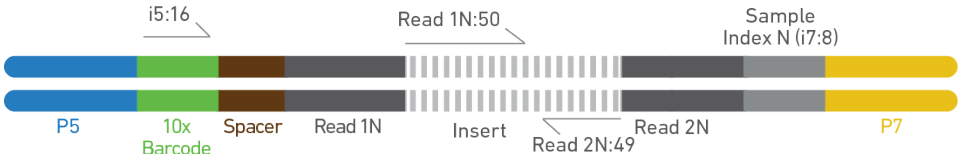
1. Chromium Single Cell Multiome ATAC Library

The library includes transposed DNA flanked by P5 and P7 sequences, necessary for binding to the Illumina flow cell. Read 1N and Read 2N sequences are used for priming and sequencing the DNA insert. The 8 bp sample index is sequenced in the i7 read and the 16 bp 10x Barcode is sequenced in the i5 read.

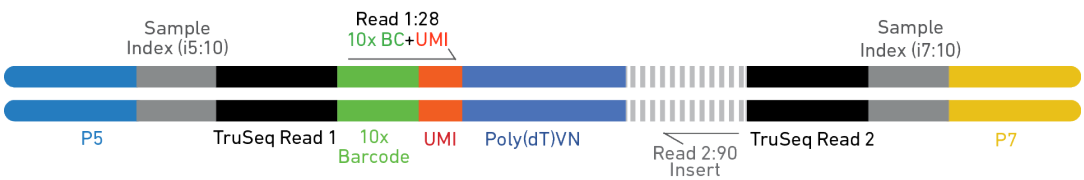
2. Chromium Single Cell Multiome Gene Expression Library

The library includes cDNA insert flanked by P5 and P7 sequences, necessary for binding to the Illumina flow cell. TruSeq Read 1 is used to sequence the 16 bp 10x Barcodes and 12 bp UMI, and TruSeq Read 2 is used for priming and sequencing the cDNA insert. The two 10 bp sample indexes are sequenced in the i5 and i7 read respectively.

Chromium Single Cell Multiome ATAC Library



Chromium Single Cell Multiome Gene Expression Library



Methods Overview

Multiome Single Cell ATAC and Gene Expression libraries were generated from one sample (PBMCs) as described in the Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338). Approximately 500 nuclei were targeted using the Chromium Next GEM Chip J to generate the libraries used for this Technical Note.

As the Multiome Gene Expression library is identical to the Chromium Single Cell 3' Gene Expression (dual index) library, see the Gene Expression Dual Index Library data (Table 1) in the Technical Note–Sequencing Metrics & Base Composition of Single Cell 3' Gene Expression and Feature Barcode Dual Index Libraries (CG000374).

The Multiome ATAC library was quantified and sequenced with 1% PhiX on various Illumina® sequencers and their respective dual index workflows as listed below.

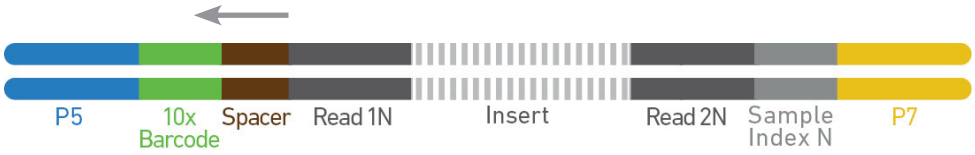
1. **Forward Strand:**
   The i5 index Read occurs before Read 2 resynthesis, so the i5 index is sequenced on the forward strand. The sequencer will read the i5 index and capture the 10x Barcode in the Multiome ATAC library.
2. **Reverse Complement:**
   The i5 index Read occurs after Read 2 resynthesis, which creates the reverse complement of the i5 index adapter sequence. Sequencers that run this workflow will read through the Spacer before reading through 10x Barcode in the Multiome ATAC library. This workflow requires a longer i5 index read length (24nt).
3. **Custom Reverse Complement:**
   For sequencers that cannot support a longer i5 read of 24 cycles, a custom recipe is necessary. In this custom workflow, dark cycles skip sequencing the Spacer before reading the 10x Barcode in the Multiome ATAC library.

Results Overview

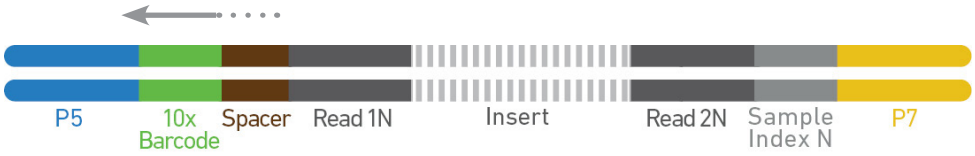
Tables 1-3 show representative sequencing metrics and base composition data derived from Multiome ATAC library sequenced using the indicated sequencers/ workflows. The Q30 quality scores, representative Data by Cycle plots, and other metrics for each sequencer/ workflow is shown. Individual results may vary depending on the specific sequencing instrument and/or particular sample and loading characteristics.

1. Forward Strand Workflow Index primer binds P5 and reads towards Read 1N

2. Reverse Complement Workflow Index primer binds Read 1N and reads towards P5



3. Custom Reverse Complement Workflow Index primer binds Read 1N, performs 8 dark cycles (not reads), & sequences 10x BC



For more information about the workflows, visit the Illumina website and refer to the Indexed Sequencing Overview Guide (15057455 v07).

Forward Strand Illumina® Workflow

Chromium Single Cell Multiome ATAC libraries were sequenced on Illumina sequencers with Forward Strand Workflow. 'Data by Cycle' plot from the Illumina software displaying the percentage of base calls and Q30 quality scores along with additional metrics are shown in Table 1.

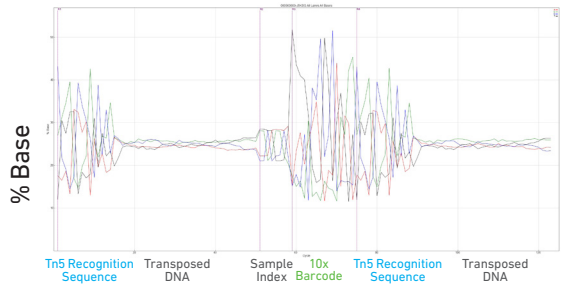
Sequencing configuration & run parameters:

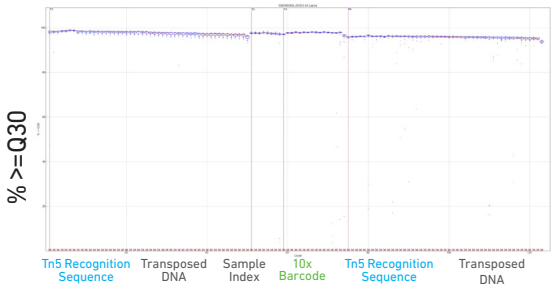
Paired-end, dual indexing

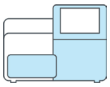
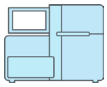
- Read 1: 50 cycles
- i7 Index: 8 cycles
- i5 Index: 16 cycles
- Read 2: 49 cycles

Table 1: Representative Plots and Sequencing Data

Representative plots show the base distribution that reflects the Tn5 transposase recognition sequence, transposed DNA, sample index, and 10x Barcode.





		% ≥Q30				Yield per Lane (Gb)		% ≥ mapQ30
		R1N	i7	i5	R 2N	R 1N	R 2N	
MiSeq™								
	Loading Conc. (pM): 10 Cluster Density: 900 K/mm^2 Phix (%): 1	97.72	97.61	97.00	95.39	0.99	0.97	86.69
HiSeq™ 2500								
	Loading Conc. (pM): 10 Cluster Density: 1,0416 K/mm^2 Phix (%): 1	97.35	96.28	95.84	94.30	8.67	8.49	85.70
NovaSeq™ (S2 flow cell; v1 kits; v1.6 software)								
	Loading Conc. (pM): 300 % PF: 79.70 Phix (%):1	94.29	89.88	87.97	92.85	112.51	110.21	87.11

Reverse Complement Illumina® Workflow

Chromium Single Cell Multiome ATAC libraries were sequenced on indicated Illumina® sequencer with Reverse Complement workflow. 'Data by Cycle' plot from the Illumina® software displaying the percentage of base calls and Q30 quality scores along with additional metrics are shown below.

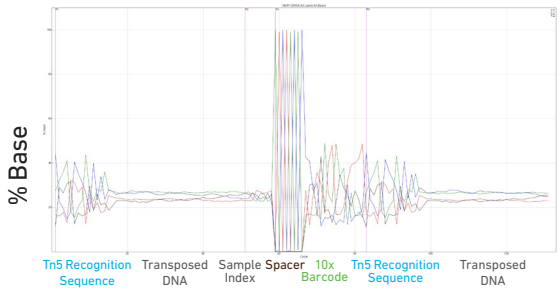
Sequencing configuration & run parameters:

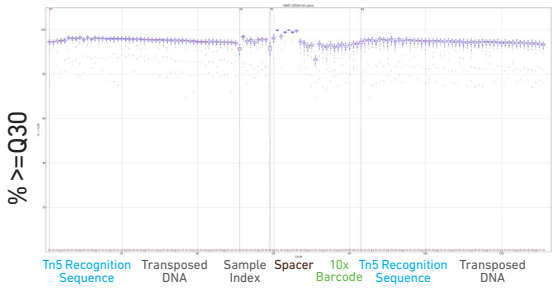
Paired-end, dual indexing

- Read 1: 50 cycles
- i7 Index: 8 cycles
- i5 Index: 24 cycles
- Read 2: 49 cycles

Table 2: Representative Plots and Sequencing Data

Representative plots show the base distribution that reflects the Tn5 transposase recognition sequence, transposed DNA, sample index, and 10x Barcode. A distinct 8 bp Spacer sequence is observed before reading the 10x Barcode.





		% ≥Q30				Yield per Lane (Gb)		% ≥ mapQ30
		R 1N	i7	i5	R 2N	R 1N	R 2N	
NovaSeq™ (SP flow cell; v1.5 kits; v1.7 software *released August 2020*)								
	Loading Conc. (pM): 300 % PF: 72.27 Phix (%):1	95.42	94.84	94.43	94.68	22.63	22.16	85.37

Custom Reverse Complement Illumina® Workflow

Chromium Single Cell Multiome ATAC libraries were sequenced on the indicated Illumina® sequencer with Custom Reverse Complement workflow. 'Data by Cycle' plot from the Illumina software displaying the percentage of base calls and Q30 quality scores along with additional metrics are shown below.

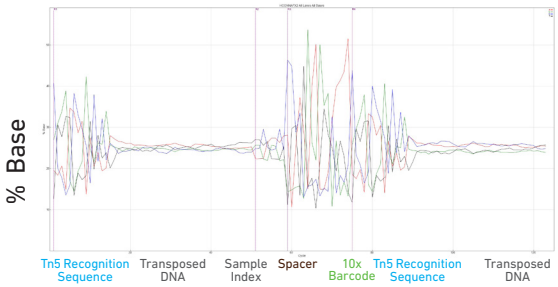
Sequencing configuration & run parameters:

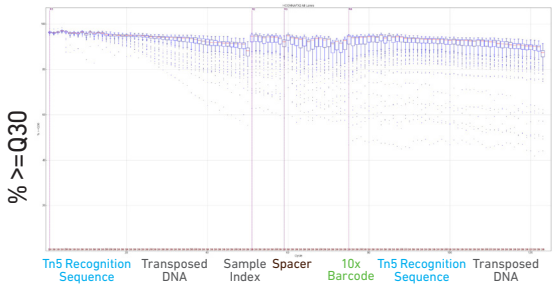
Paired-end, dual indexing

- Read 1: 50 cycles
- i7 Index: 8 cycles
- i5 Index: 8 **dark** cycles and 16 cycles
- Read 2: 49 cycles

Table 3: Representative Plots and Sequencing Data

Representative plots show the base distribution that reflects the Tn5 recognition sequence, transposed DNA, sample index and 10x barcode. A distinct 8 Spacer sequence is not observed prior to the 10x Barcode as dark cycles are not read by the sequencing instrument.





		% ≥Q30				Yield per Lane (Gb)		% ≥ map**Q30**
		R 1N	**i7**	**i5**	**R 2N**	**R 1N**	**R 2N**	
NextSeq™ 500/550								
	Loading Conc. (pM): 1.5 Cluster Density: 205 K/mm^2 Phix (%):1	93.87	92.60	90.81	91.01	1.85	1.81	85.30

Conclusions

In summary, 'Data by Cycle' plots displaying the percentage of base calls and Q30 quality scores% show highly consistent profiles for all sequencing platforms and workflows tested. The data serve as guidelines for assessing the quality of Single Cell Multiome ATAC 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 nuclei suspension.
- Final libraries with fragment length of 200-2,000 bp and a significant number of inserts between 200-600 bp 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.

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