TECHNICAL NOTE

Interpreting Cell Ranger ATAC Web Summary Files for Single Cell ATAC Assay

Introduction

The web summary file in the output folder of the Cell Ranger ATAC analysis software is the initial point of reference for determining sample performance in the Single Cell ATAC assay. This Technical Note presents an overview of web summary file interpretation, including the expected metrics and characteristic plots for libraries generated using the Single Cell ATAC workflow.

Interpreting Web Summary File Metrics

Representative summary files for Chromium Single Cell ATAC libraries and other Cell Ranger ATAC output files are available for download on the 10x Genomics Support website. The top of the web summary file displays key metrics (Figure 1). Green text indicates that the key metrics are in the expected range while red/yellow text indicates errors/warnings. Descriptions of the metrics can also be found by clicking the icon ⓘ next to the section header. The summary tab reports various metrics including sample, sequencing, cells, cell clustering, insert sizes, targeting, and library complexity.

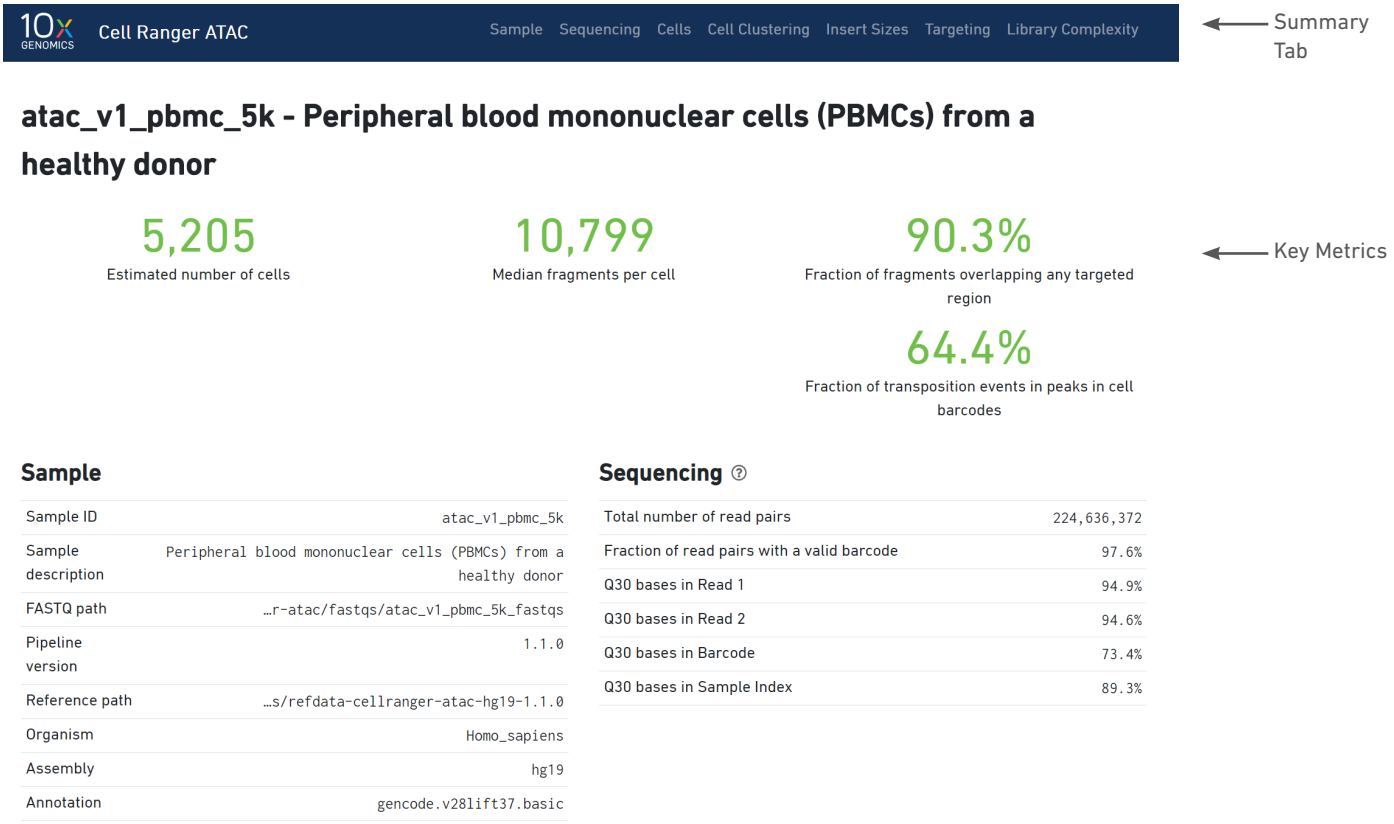


Figure 1. A representative web summary file (top section) for a PBMCs sample targeting 5,000 nuclei.

Table 1. Metrics in the ATAC web summary file.

Metrics	Definition	Expected Value	Notes
Sequencing Metrics			
Total number of read pairs	Total number of read pairs that were assigned to this library after demultiplexing	User defined	Suggested sequencing depth of 25,000 read pairs per cell.
Fraction of read pairs with a valid barcode	Fraction of reads with barcodes that match the whitelist after barcode correction	>75%	Low valid barcodes may indicate sequencing related problem or issues with library preparation.
Q30 bases in R1	Fraction of insert read bases (R1N) with Q-score ≥30	Sequencing platform dependent (ideally >65%)	Expected to be higher than Q30 Bases in barcode (i5 read) or Sample Index (i7 read) and is sequencing platform dependent. Low Q30 base percentages could indicate sequencing issue such as sub-optimal loading concentration of the library.
Q30 bases in R2	Fraction of insert read bases (R2N) with Q-score ≥30	Sequencing platform dependent (ideally >65%)	Expected to be higher than Q30 bases in barcode (i5 read) or Sample Index (i7 read) and is sequencing platform dependent. Low Q30 base percentages could indicate sequencing issue such as sub-optimal loading concentration of the library.
Q30 bases in barcode	Fraction of barcode read bases (i5) with Q-score ≥30	Sequencing platform dependent (ideally >65%)	Low Q30 base percentages could indicate sequencing issue such as sub-optimal loading concentration of the library.
Q30 bases in Sample Index	Fraction of sample index read bases (i7) with Q-score ≥30	Sequencing platform dependent (ideally >90%)	Low Q30 base percentages could indicate sequencing issue such as sub-optimal loading concentration of the library.
Cell Metrics			
Estimated Number of Cells	Total number of barcodes associated with cell-containing partitions estimated by ATAC Cell Ranger	500-10,000	± 20% expected value is acceptable. Higher or lower values outside of this range may indicate inaccurate nuclei count, nuclei lysis or failures during GEM generation.
Lower threshold on the number of fragments overlapping peaks per barcode to annotate barcode as cell	Minimum number of fragments overlapping peaks needed to be called as a cell	Dependent on cell type and sequencing depth	Number is determined through dynamic modeling for each sample (refer to "Cell Ranger ATAC Algorithms Overview").
Median fragments per cell barcode	Median number of transposase accessible fragments being assigned to cell barcodes	>500	Dependent on cell type and sequencing depth, will vary from sample to sample.
Median fragments per non-cell barcode	Median number of transposase accessible fragments being assigned to non-cell barcodes	Dependent on cell type and sequencing depth	Dependent on cell type and sequencing depth. A high number of fragments in non-cell barcodes may indicate high background and low sample quality.
Insert Size Metrics			
Fragments in nucleosome-free regions	Fraction of fragments passing all filters with a size smaller than 147 basepairs	>40%	Expected to be the highest proportion as compared to mononucleosome and dinucleosome fragment.
Fragments flanking a single nucleosome	Fraction of fragments passing all filters with a size between 147 and 294 basepair	Dependent on sample type	An increased proportion of mononucleosome fragments may indicate dead/dying cells or granulocyte contamination.

Table 1 contd. Metrics in the ATAC web summary file.

Metrics	Definition	Expected Value	Notes
Targeting Metrics			
Fraction of fragments overlapping any targeted region	Fragments overlapping any TSS, DNase HS, enhancer, or promoter regions	>55%	Dependent on quality of genome annotation.
Fraction of total read pairs mapped confidently to genome (>30 mapq)	Fraction of reads pairs with a 99.9% chance of mapping correctly	>80%	Reference quality and sequencing configuration (shorter than recommended cycles on Read 2) can impact mapping. May indicate the use of the wrong reference (wrong species, an improper build of custom reference, etc).
Fraction of total read pairs in mitochondria and in cell barcodes	Percent total read pairs that map to mitochondrial reads and are assigned as cell-associated barcodes	<40%	Using an alternative lysis buffer other than that indicated in the Demonstrated Protocols for isolating nuclei for ATAC Sequencing may increase this metric.
Fraction of transposition events in peaks in cell barcodes	Measures the abundance of transposition events in called peaks from cell-associated barcodes	>25%	Low percentage indicates that fragments are not coming from called peaks but rather from random regions of the genome. Causes include dead cells, or very low sequencing depth.
Enrichment score of transcription start sites	The summed number of cut sites per base in a window of 2,000 bases around all the annotated TSSs, normalized by the minimum signal in the window. This metric reports the maximum value in the profile	>5%	Low score may indicate sample quality issues such as loss of chromatin structure or improper cell lysis.

Interpreting the Web Summary File Plots

The summary file also contains multiple plots. Table 2 describes the six major plots that help in assessing assay performance.

Table 2. Plots in the ATAC web summary file.

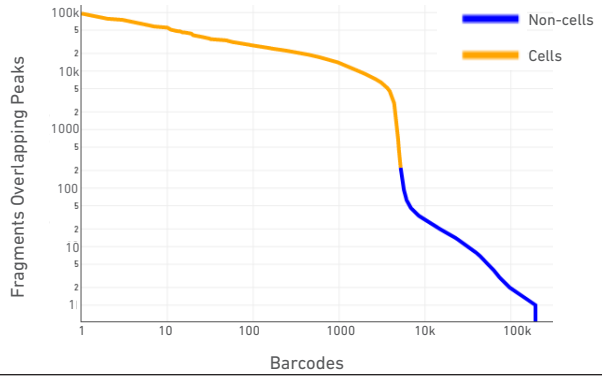
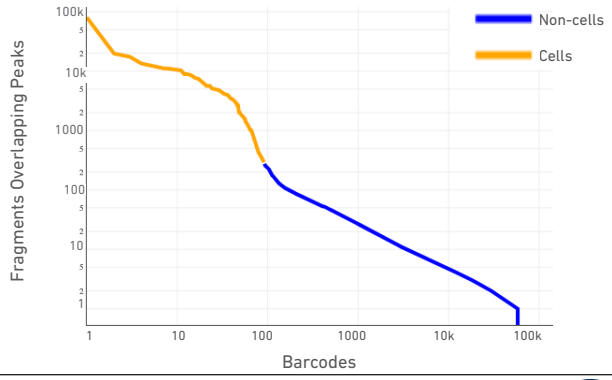
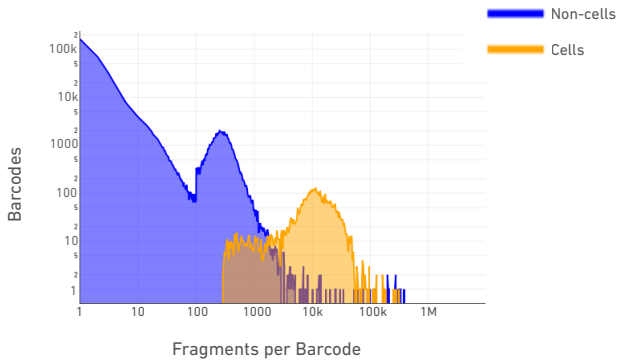
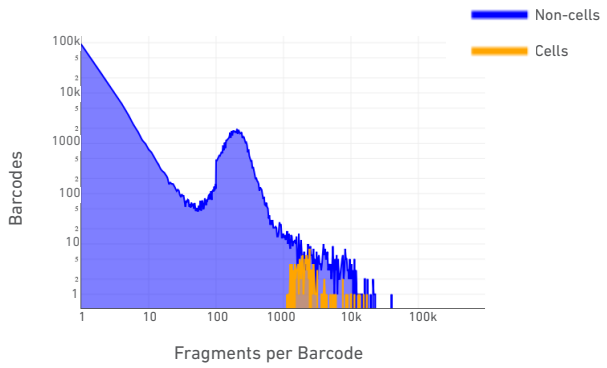
Plot & Interpretation	
Barcode Rank Plot: The Barcode Rank (or knee plot) for fragments overlapping peaks marks the barcodes that were inferred to be associated with cells.	
Example	
Ideal Sample: A steep drop-off is indicative of good separation between the cell-associated barcodes and the barcodes associated with empty GEMs.	**Compromised Sample**: Round curve and lack of steep drop-off may indicate low sample quality or loss of single-cell behavior.
	

Table 2 contd. Plots in the ATAC web summary file.

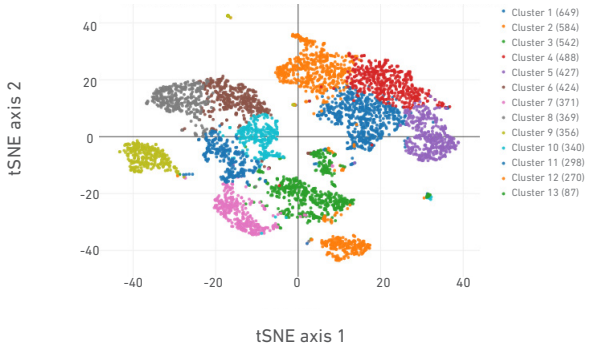
Plot & Interpretation
Fragment Distribution Plot: The distribution of the number of fragments per barcode for the non-cell and cell groups is displayed in the Fragment Distribution plot.
Example
Ideal Sample: A good separation between cell and non-cell groups indicate proper distinction between cells and non-cells.



Compromised Sample: Large overlap between cells and non-cells may indicate issues with sample quality.



Plot & Interpretation
Cell Clustering Scatter Plot: The Cell Clustering (colored by cluster) plot shows the cell-associated barcodes in a 2-D tSNE projection, with colors showing an automated graph clustering analysis which groups together cells with similar peak profiles.
Example
Ideal Sample: Structured clusters with good separation (for a sample with expected heterogeneous cell populations).



Compromised Sample: Lack of cluster structure, one large cluster or no separation (for a sample with expected heterogeneous cell populations) may indicate sample quality issue or loss of single cell behavior.

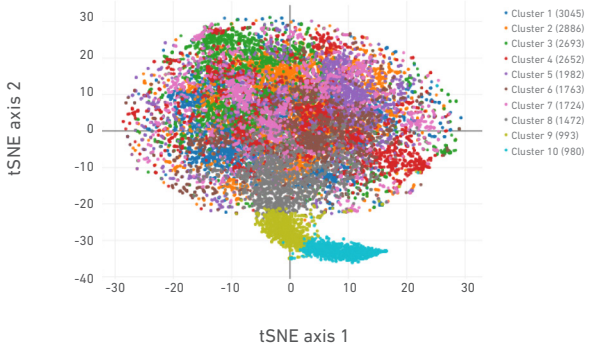
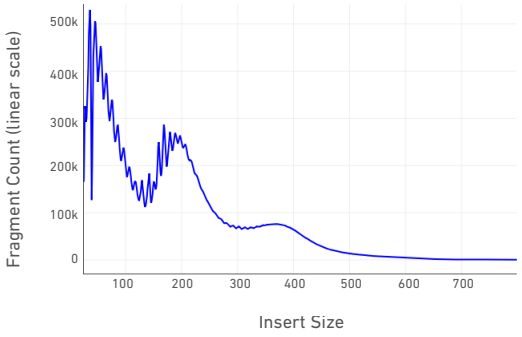
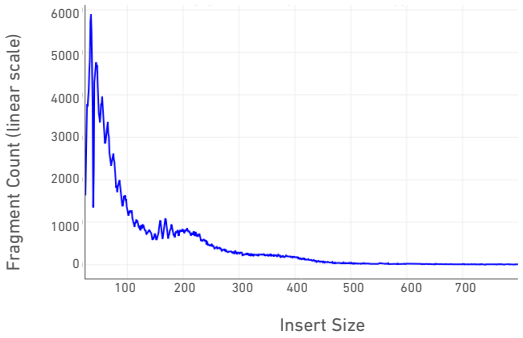


Table 2 contd. Plots in the ATAC web summary file.

Plot & Interpretation	
Insert Size Distribution Plot: Insert size distribution of transposase accessible fragments sequenced is displayed in the Insert Size Distribution plot.	
Example	
Ideal Sample: A periodicity of ~150 bp corresponds to the number of nucleosomes the transposase accessible fragments span (nucleosome free, mononucleosome, and dinucleosome fragments). Sawtooth pattern in fragments with insert size <200 bp corresponds to the helical pitch of DNA (~10.5 bp). 	**Compromised Sample:** Absence of periodicity may indicate loss of chromatin structure due to low sample quality. 

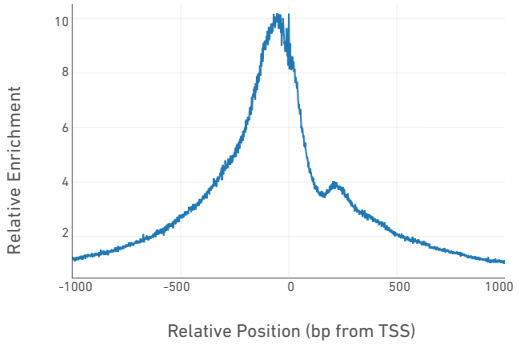
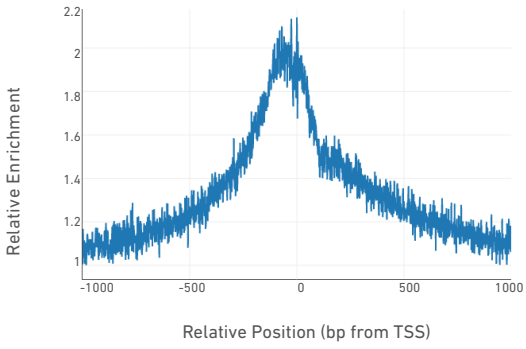
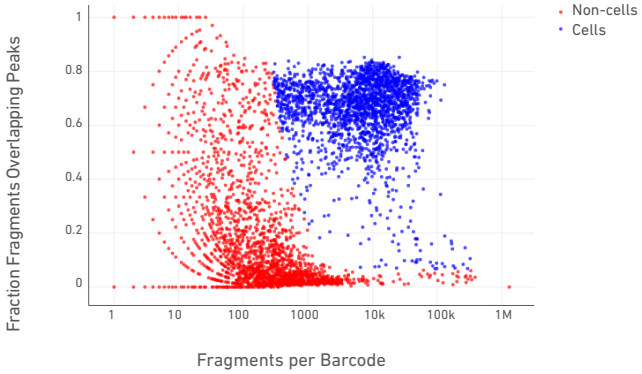
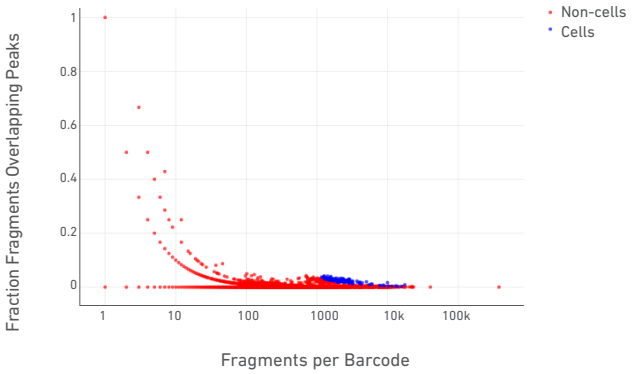
Plot & Interpretation	
Transition Start Site (TSS) Plot: The Transcription Start Site (TSS) profile, which is computed as the number of cut sites per base, of all the barcodes irrespective of cell versus non-cell assignment in a window of 2,000 bases around the full set of annotated TSSs is displayed in the Transcription Start Site plot. The y-axis scale is normalized by the minimum signal in the window.	
Example	
Ideal Sample: Large enrichment around TSS, as these regions are known to have a high degree of chromatin accessibility compared to the flanking regions. 	**Compromised Sample**: Low enrichment (<5%) around TSS sites may indicate improper lysis or loss of chromatin structure. 

Table 2 contd. Plots in the ATAC web summary file.

Plot & Distribution	
Single Cell Targeting Plot: A scatterplot displaying the number of fragments per barcode and the percent of fragments overlapping peaks.	
Example	
Ideal Sample: Cell-associated barcodes are expected to have a large number of fragments per barcode and a high percentage of fragments overlapping peaks (upper right corner). Non-cell associated barcodes are expected to have a small number of fragments per barcode and a low percentage of fragments overlapping peaks (lower left corner). An ideal sample should show good separation of cells and non-cells at the opposite ends.	**Compromised Sample:** Cell-associated barcodes have a low fraction of the barcode fragments overlapping peaks. Concentration of cell-associated and non-cell associated barcodes tightly concentrated in the same location may indicate issues with cell calling or sample preparation.
	

References

- Sequencing Metrics & Base Composition of Chromium Single Cell ATAC Libraries (Document CG000181)
- Chromium Single Cell ATAC Reagent Kits User Guide (Document CG000168)
- Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 User Guide (Document CG000209)

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