Omnition Analysis Software: Understanding the ATAC Report

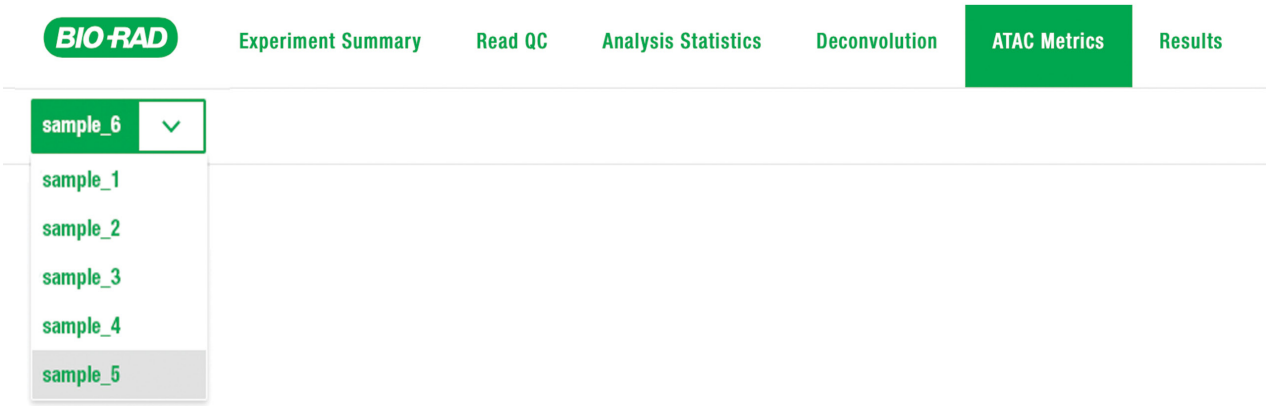
The Omnition Analysis Software outputs a single HTML report containing all samples that are processed in a run of the pipeline. This guide explains metrics for each plot and table reported by Omnition. Tooltips are also accessible by hovering over the ⓘ next to the headers of plots and tables.

Overview of Report Tabs

The report includes 6 sections:

- **Experiment Summary**
- **Read QC**
- **Analysis Statistics**
- **Deconvolution**
- **ATAC Metrics**
- **Results**

Follow the tabs on the top of the report page to view each section for a given sample. Switch between samples via the sample ID dropdown list found in the following sample-level sections: **Analysis Statistics**, **Deconvolution**, **ATAC Metrics**, and **Results**.



Experiment Summary

1 Summary Table

The Experiment Summary table provides an at-a-glance summary of key metrics and statistics for each sample analyzed in the same pipeline run.

Total Cells Recovered:
Calculated cell count after quality control and deconvolution.

Mean Valid Read Pairs per Cell:
Average number of read pairs containing a complete barcode sequence for each called cell.

Median Unique Nuclear Fragments per Cell:
Median number of unique fragments that aligned to nuclear DNA in a cell.

Median Estimated Library Size per Cell:
Median predicted number of unique nuclear fragments per cell in the sequencing library.

Aligned Read Pairs (%):
Percentage of read pairs that aligned to the genome and mitochondrial DNA.

Aligned Duplicate Read Pairs (%):
Percentage of proper read pairs with similar sequences and alignment positions.

Aligned Mitochondrial Read Pairs (%):
Percentage of proper read pairs that aligned to mitochondrial DNA.

Transcription Start Site (TSS) Enrichment Score:
The average ratio of coverage at the TSS normalized to coverage at a set distance from the TSS.

Peaks Called:
The number of unique accessible chromatin regions identified in the sample.

Fraction of Reads in Peaks (FRiP):
The fraction of unique reads that aligned to identified accessible chromatin regions (peaks).

2 Messages

The Messages section shows status messages output by Omnition.

1

Sample	Total Cells Recovered	Mean Valid Read Pairs per Cell	Median Unique Nuclear Fragments per Cell	Median Estimated Library Size per Cell	Aligned Read Pairs	Aligned Duplicate Read Pairs	A
BR_DMF_S6	1,967	19,325.697	9,737	18,576	94.4%	47.2%	
BR2_S5	2,333	31,552.041	15,555	25,176	97.8%	57.3%	
sw_15_55_S1	2,420	30,086.055	13,320.5	20,507	97.1%	55.0%	
sw_30_37_S3	2,865	22,176.284	7,739	10,106	94.2%	66.5%	
sw_30_55_S2	2,434	36,341.431	20,138	39,247.5	96.1%	46.8%	
sw_60_37_S4	2,879	20,737.293	10,359	19,475	94.6%	48.0%	

2

Messages

INFO: [ATAC] Executing mixed species workflow.
INFO: [ATAC] Executing analysis workflow.
WARN: [ATAC] Undetermined FASTQ read files will be ignored.

RETURN

Read QC

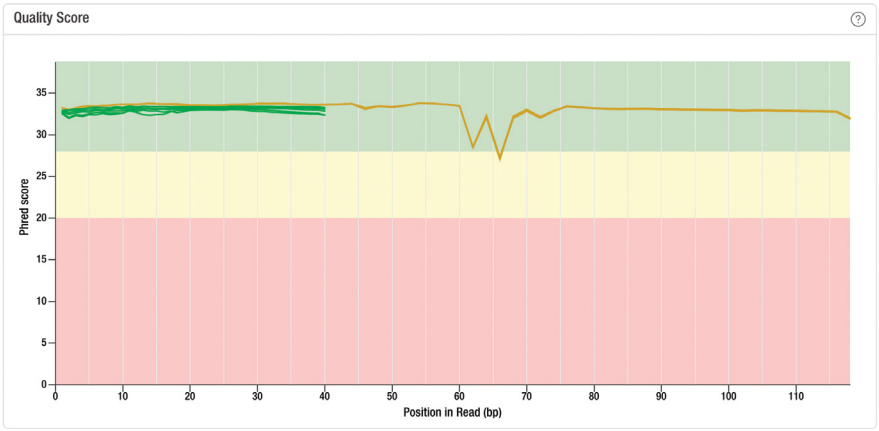
1 Quality Score Plot

The Quality Score plot shows the mean Phred quality score at each base position as an indication of the base call accuracy.

2 Sequence Trace Heat Map

The Sequence Trace heat map shows the proportion of each of the four DNA bases being called at each base position.

1



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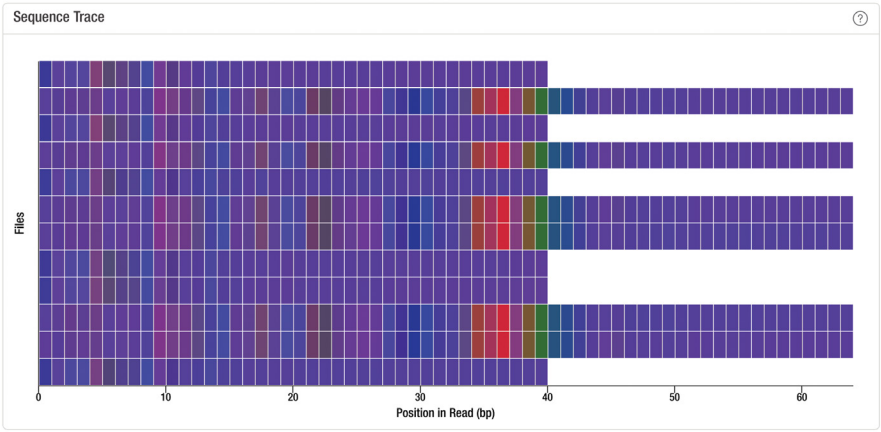
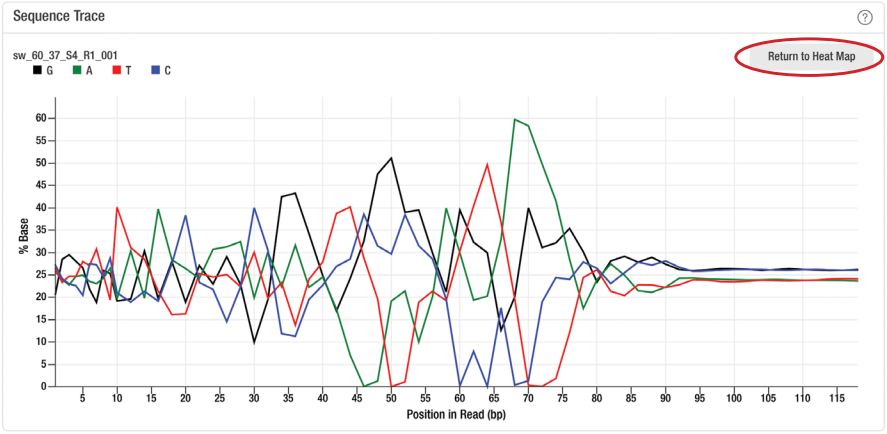


3 Sequence Trace Plot

The Sequence Trace plot shows the mean nucleotide composition at each base position.

Click each tile in the heat map to switch to the trace plot. Click “Return to Heat Map” (circled) to return.

3



RETURN

Analysis Statistics

1 Alignment Metrics Table

Input Read Pairs:
Number of read pairs input into alignment.

Aligned Read Pairs:
Percentage of read pairs that aligned to the genome and mitochondrial DNA.

Aligned Proper Read Pairs:
Percentage of read pairs that align to the genome and mitochondrial DNA in the correct orientation within a reasonable distance of each other.

Aligned Duplicate Read Pairs:
Percentage of proper read pairs with similar sequences and alignment positions.

Aligned Mitochondrial Read Pairs:
Percentage of proper read pairs that aligned to mitochondrial DNA.

2 Pipeline Summary Table

Pipeline Summary table shows the proportion of read pairs retained throughout the analysis.

Read Pairs with Valid Barcodes:
Read pairs containing complete barcode sequences.

High-Quality Read Pairs:
The percentage of deduplicated proper read pairs that met the quality threshold and insert size requirements.

High-Quality Pairs Remaining after Blocklist Removal:
The percentage of deduplicated proper read pairs aligned with high-quality in regions of interest.

Deduplicated Above-Knee Read Pairs:
The percentage of unique high-quality read pairs in regions of interest associated with called cells.

1

Alignment Metrics

Input Read Pairs	38,013,646
Aligned Read Pairs	95.4%
Aligned Proper Read Pairs	94.4%
Aligned Duplicate Read Pairs	23.5%
Aligned Mitochondrial Read Pairs	0.0%

2

Pipeline Summary

Read Pairs with Valid Barcodes	38,013,646
High-Quality Read Pairs	85.33%
High Quality Pairs Remaining after Blocklist Removal	85.23%
Deduplicated Above-Knee Read Pairs	53.90%

3 Read Pairs per Index Table

For single cell combinatorial indexing ATAC data with the Tagmentation Index (TI), the Read Pairs per Index table reports the proportion of read pairs with valid barcodes attributed to each unique combination of FASTQ and TI in a sample.

3

Read Pairs Identified per Index Metrics

FastQ-TI Pair	Count	% Read Pairs Identified
anchor_foot8A_3C6_S3-TI15	1,972,172	4.09%
anchor_foot8A_3C6_S3-TI21	5,032,253	10.43%
anchor_foot8A_3C6_S3-TI19	1,914,675	3.97%
anchor_foot8A_3C6_S3-TI24	4,461,358	9.25%
anchor_foot8A_3C6_S3-TI23	1,840,069	3.82%
anchor_foot8A_3C6_S3-TI16	6,341,968	13.15%
anchor_foot8A_3C6_S3-TI13	5,778,748	11.98%
anchor_foot8A_3C6_S3-TI20	4,935,085	10.23%
anchor_foot8A_3C6_S3-TI14	8,282,065	17.17%
anchor_foot8A_3C6_S3-TI18	2,013,479	4.17%
anchor_foot8A_3C6_S3-TI22	1,758,277	3.65%
anchor_foot8A_3C6_S3-TI17	3,901,497	8.09%

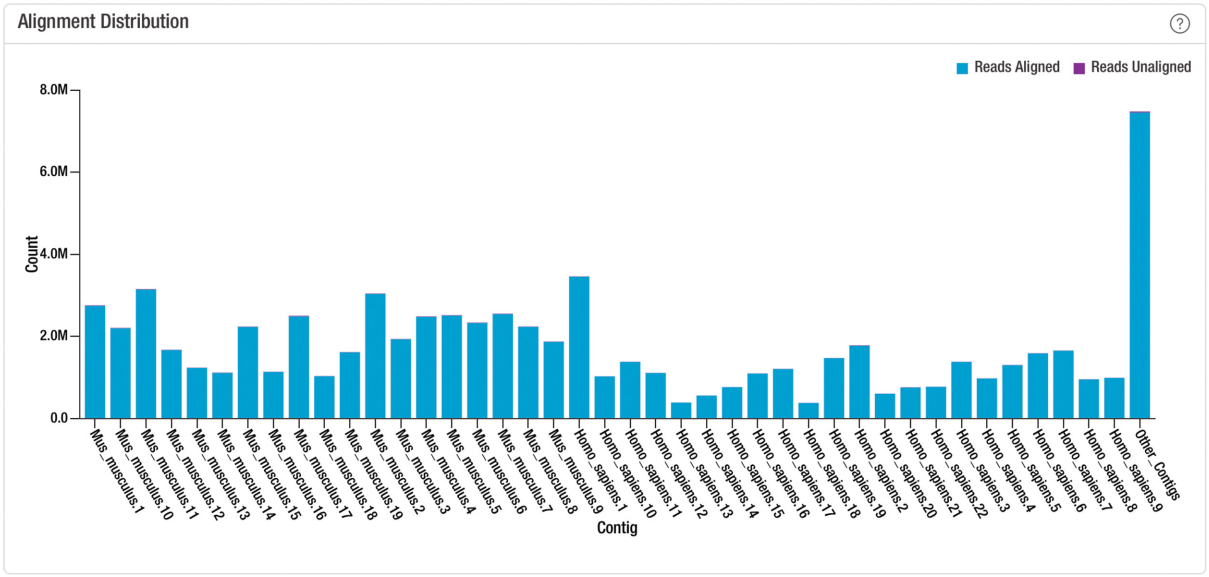
4 Alignment Distribution Plot

The Alignment Distribution plot shows chromosome locations of aligned and unaligned reads.

5 Sequence Saturation Plot

The Sequence Saturation plot shows the number of unique fragments identified per cell compared to the number of valid reads in a sample, as a function of sequencing saturation.

4



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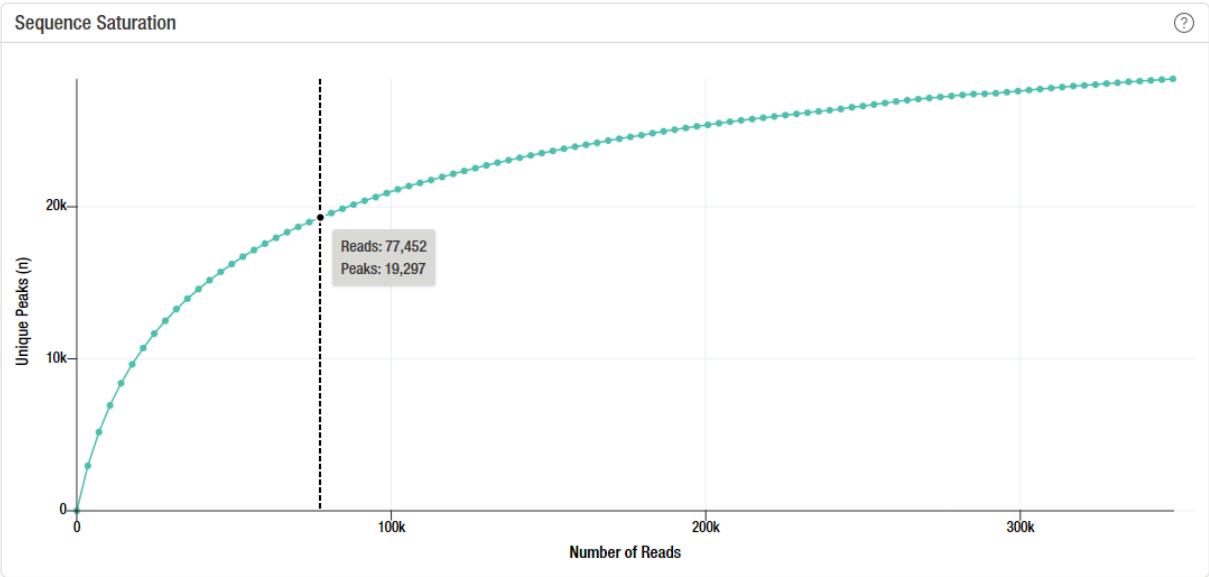


RETURN

Deconvolution

1 Bead Filtration Table

The Bead Filtration table displays a quantification of what happened in the bead filtration step.

Total Barcodes Observed:
The number of bead barcodes identified.

Barcodes Above Knee:
The number of bead barcodes associated with unique nuclear fragments that is greater than the unique nuclear fragment threshold at the knee.

Unique Nuclear Fragment Threshold at Knee:
The minimum number of unique nuclear fragments a bead barcode must be associated with to be considered true signal.

Median Unique Nuclear Fragments per Bead Above the Knee:
The median number of unique nuclear fragments per bead barcode that is greater than the threshold at the knee.

Total Cells Recovered:
Calculated cell count after quality control and deconvolution.

Mean Valid Read Pairs per Cell:
Average number of read pairs containing a complete barcode sequence for each called cell.

Median Unique Nuclear Fragments per Cell:
Median number of unique fragments that align to nuclear DNA in a cell.

Median Estimated Library Size per Cell:
Median predicted number of unique nuclear fragments per cell in the sequencing library, calculated using number of unique nuclear fragments and duplication rate.

2 Bead Filtration Plot

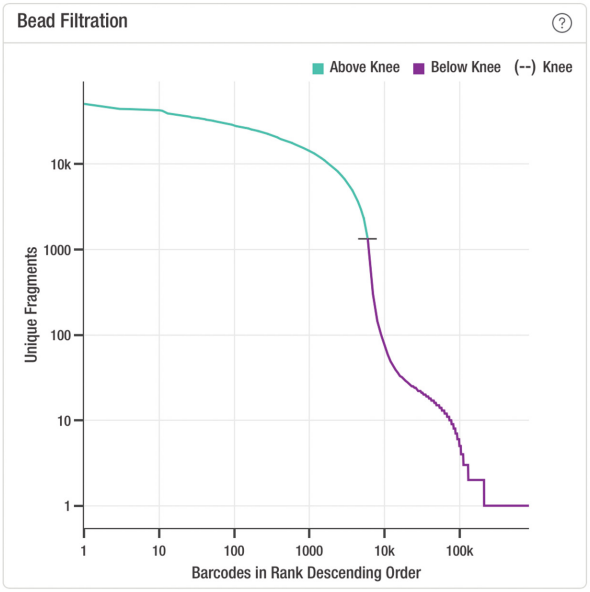
The Bead Filtration plot shows the distribution of unique nuclear fragments across individual bead barcodes. The signal below the inflection point is assumed to be noise.

1

Bead Filtration

Unique Nuclear Fragment Threshold at Knee	283
Total Barcodes Observed	1,210,624
Median Unique Fragments per Bead Above the Knee	4,289
Barcodes Above Knee	12,184
Total Cells Recovered	4,952
Mean Valid Read Pairs per Cell	23,942
Median Unique Nuclear Fragments per Cell	10,899
Median Estimated Library Size Per Cell	26,771.5

2



3 Jaccard Index Plot

The Jaccard Index plot shows the distribution of Jaccard similarity index scores across bead barcode pairs. Pairs with scores above the inflection point are assumed to be originating in the same partition.

4 Beads per Partition Plot

The Beads per Partition plot shows the frequency of different numbers of bead multiplets merged during deconvolution.

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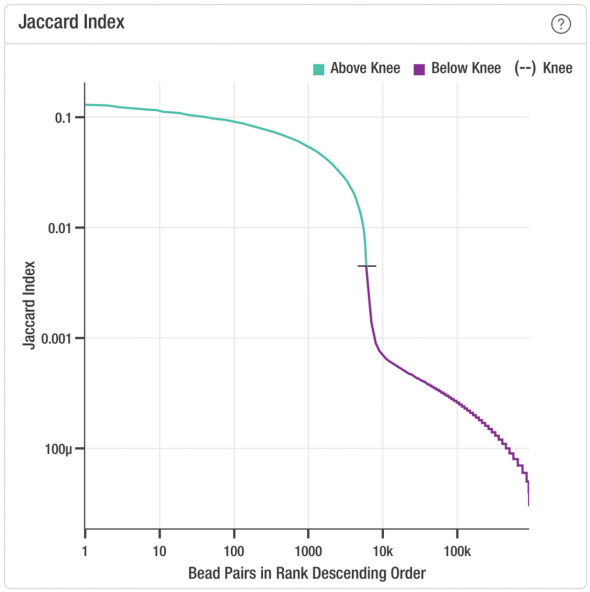

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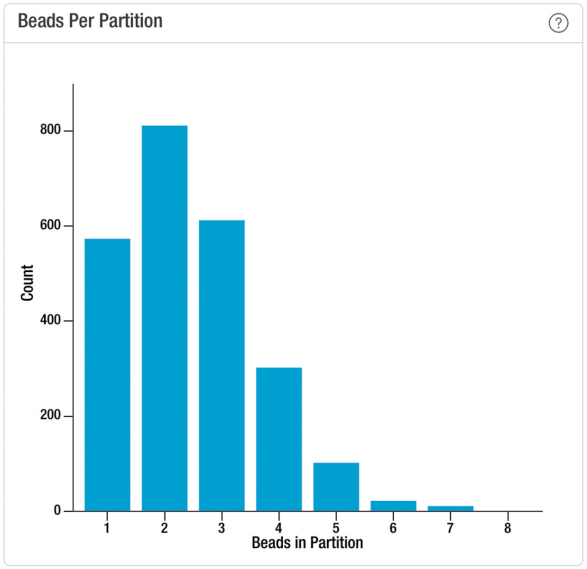

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ATAC Metrics

1 ATAC-Seq Metrics Table

Transcription Start Site (TSS) Enrichment Score:
The average ratio of coverage at the TSS normalized to coverage at a set distance from the TSS.

Total TSS Reads:
The number of proper read pairs that aligned to a TSS.

Peaks Called:
The number of unique accessible chromatin regions identified in the sample.

Fraction of reads in peaks (FRiP):
The fraction of unique reads that aligned to identified accessible chromatin regions (peaks).

TSS Read Percentage:
The percentage of proper read pairs that aligned to a TSS.

1

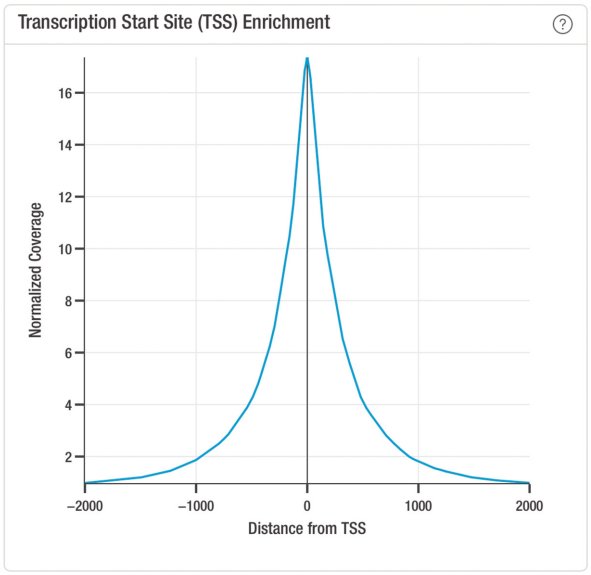
ATAC-Seq Metrics

Transcription Start Site (TSS) Enrichment Score	17.369
Total Transcription Start Site (TSS) Reads	20,149,131
Peaks Called	126,946
Fraction of reads in peaks (FRIP)	0.385
Transcription Start Site (TSS) Read	50.3%

2 TSS Enrichment Plot

The distribution of peaks relative to known transcription start sites (TSS).

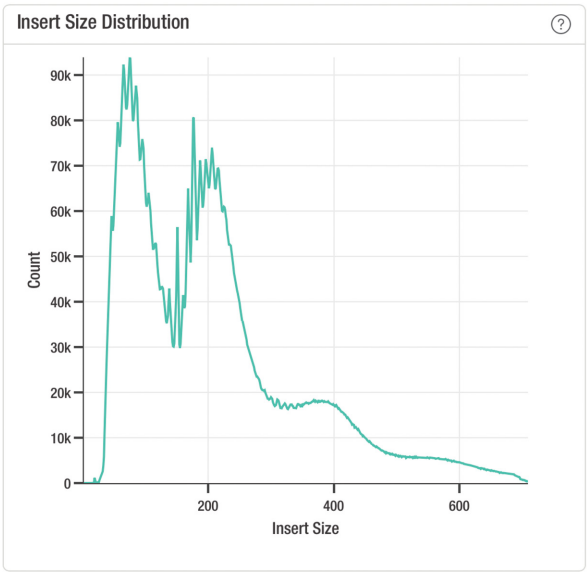
2



3 Insert Size Distribution Plot

The distribution of sequenced fragment sizes, indicative of nucleosome positioning periodicity.

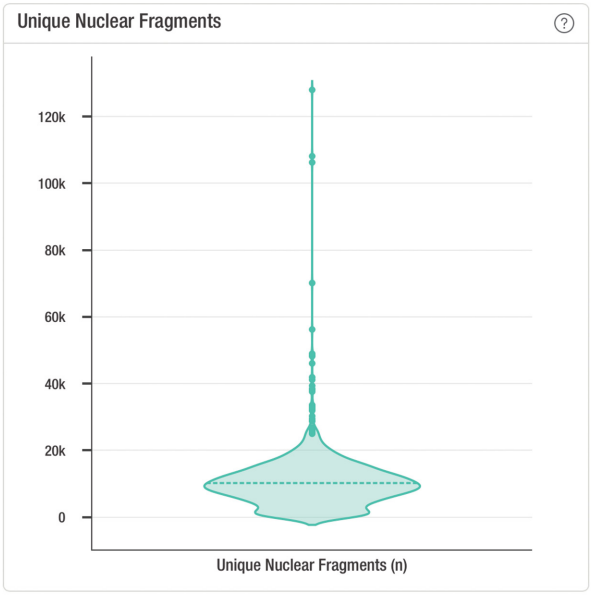
3



4 Unique Nuclear Fragments Plot

The distribution of unique nuclear fragments per cell.

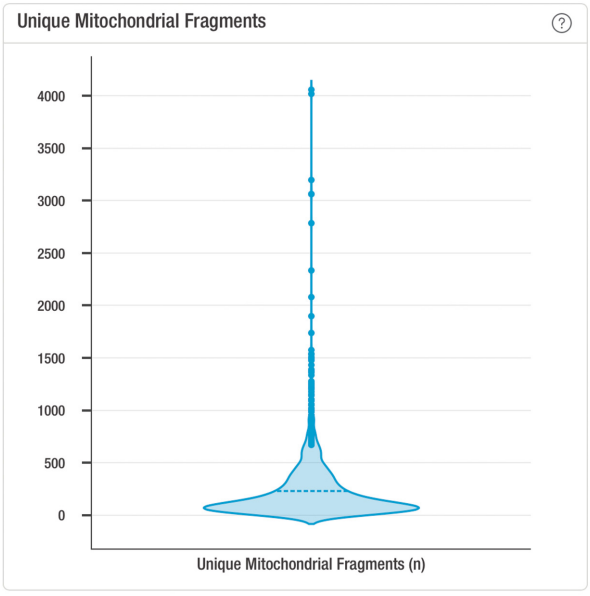
4



5 Unique Mitochondrial Fragments Plot

The distribution of unique mitochondrial fragments per cell.

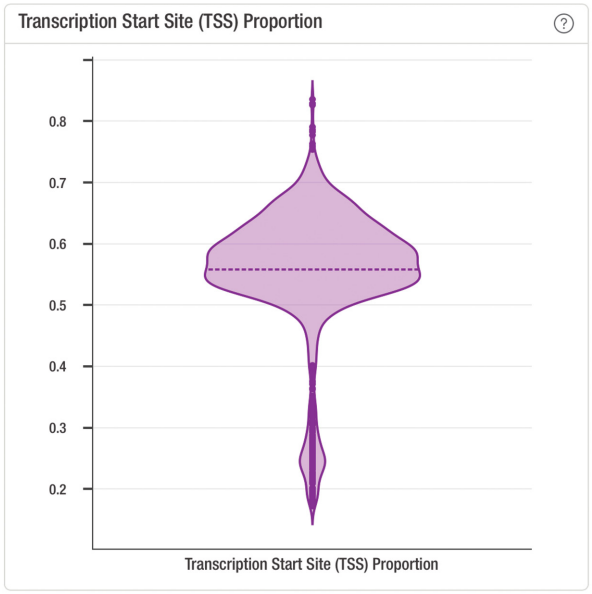
5



6 TSS Proportion Plot

The aggregate distribution of proper read pairs aligned to a TSS per cell.

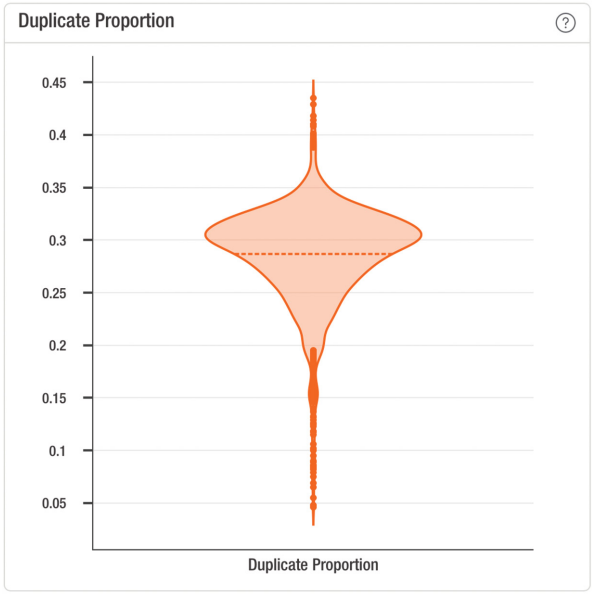
6



7 Duplicate Proportion Plot

The distribution of aligned duplicate read pairs per cell.

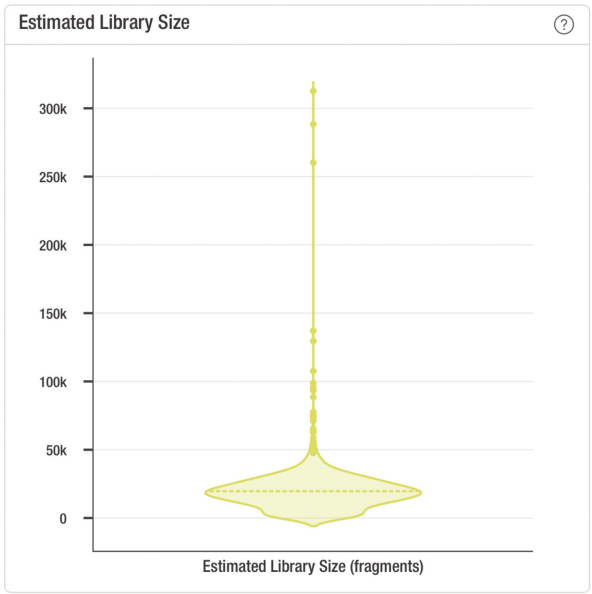
7



8 Estimated Library Size Plot

Predicted number of unique nuclear fragments per cell in the sequencing library, calculated using number of unique nuclear fragments and duplication rate.

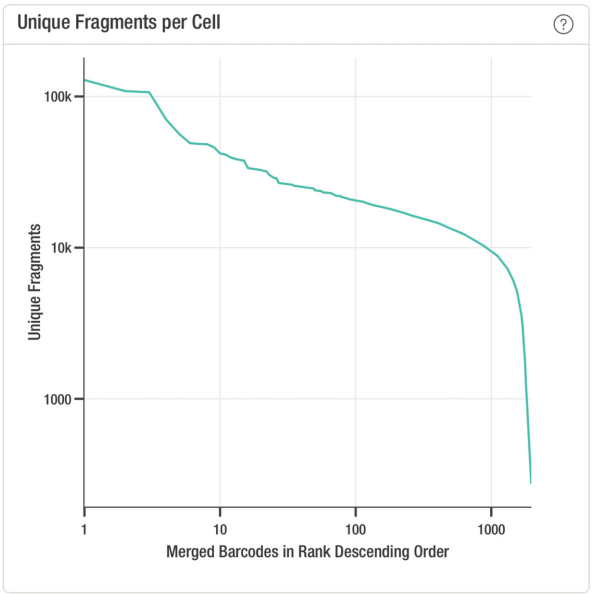
8



9 Unique Fragments per Cell Plot

The distribution of unique nuclear fragments across cells identified after merging of above-knee bead barcodes.

9



RETURN

Results

1 Highlights

Total Cells Recovered:
Calculated cell count after quality control and deconvolution.

Median Unique Nuclear Fragments per cell:
Median number of unique fragments that align to nuclear DNA in a cell.

Transcription Start Site (TSS) Enrichment Score:
The average ratio of coverage at the TSS normalized to coverage at a set distance from the TSS.

Fraction of Reads in Peaks (FRiP):
The fraction of unique reads that aligned to identified accessible chromatin regions (peaks).

2 Crosstalk Statistics

Total Cells Recovered:
Calculated cell count after quality control and deconvolution.

[Genome1] Cells:
The number of cell barcodes that have ≥90% (default) of proper read pairs from [genome1].

[Genome2] Cells:
The number of cell barcodes that have ≥90% (default) of proper read pairs from [genome2].

Mixed Cells:
The number of cell barcodes that have <90% (default) of proper read pairs from a single species.

Observed Mixed-Species Multiplets:
The percentage of cell barcodes that have <90% (default) of proper read pairs from a single species quantifying detectable multiplets.

Estimated Total Multiplets:
The percentage of the estimated total multiplets, including same-species multiplets, calculated by doubling the percentage of mixed-species multiplets.

Cell Purity:
The percentage of proper read pairs that are correctly assigned with the cell barcodes' species out of the total proper read pairs.

1

2,333	15,555	16.3	0.389
Total Cells Recovered	Median Unique Nuclear Fragments per Cell	Transcription Start Site (TSS) Enrichment Score	Fraction of Reads in Peaks (FRiP)

2

Crosstalk Statistics

Total Cells Recovered	2,290
homo_sapiens Cells	1,089
mus_musculus Cells	1,201
Mixed Cells	109
Observed Mixed-Species Multiplets	4.5%
Estimated Total Multiplets	9.0%
Cell Purity	99.6%

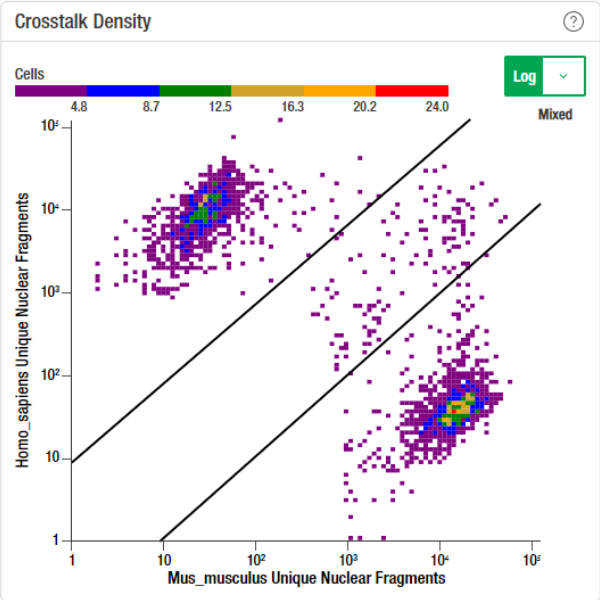
3 Crosstalk Density Plot

The Crosstalk Density plot shows the number of unique nuclear fragments from each cell barcode aligned to a particular species. Mixed cells are those with unique nuclear fragments aligned to both species.

4 UMAP Cluster Plot

The UMAP Cluster plot displays Identification of clusters based on differential chromatin accessibility across the genome.

3



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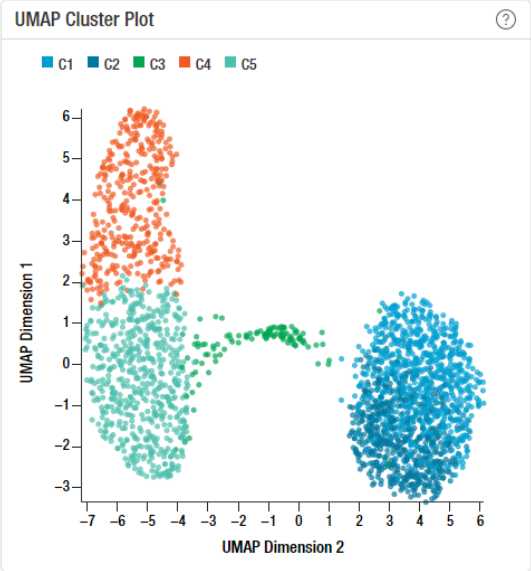

RETURN

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