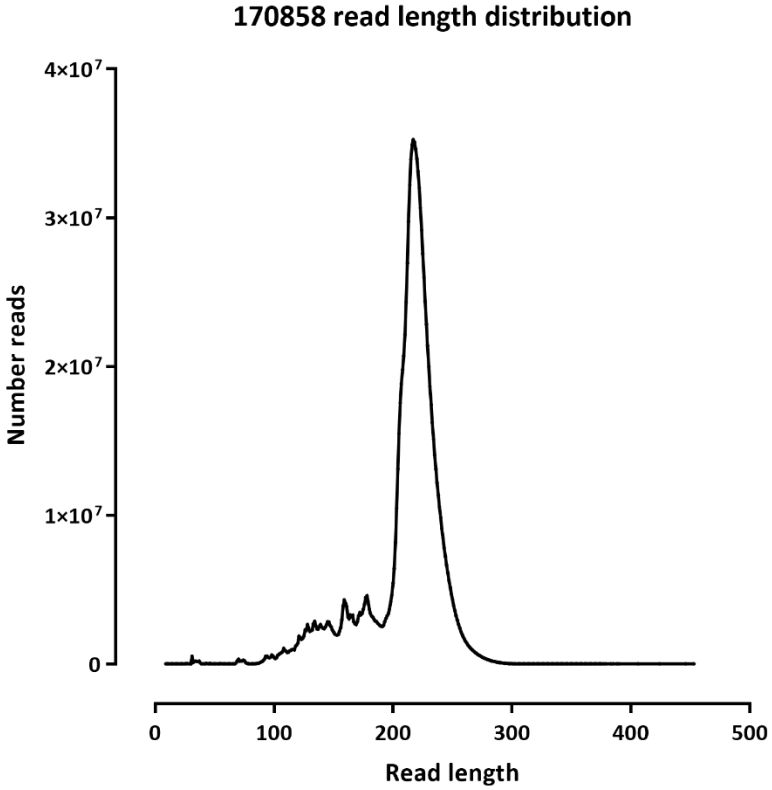
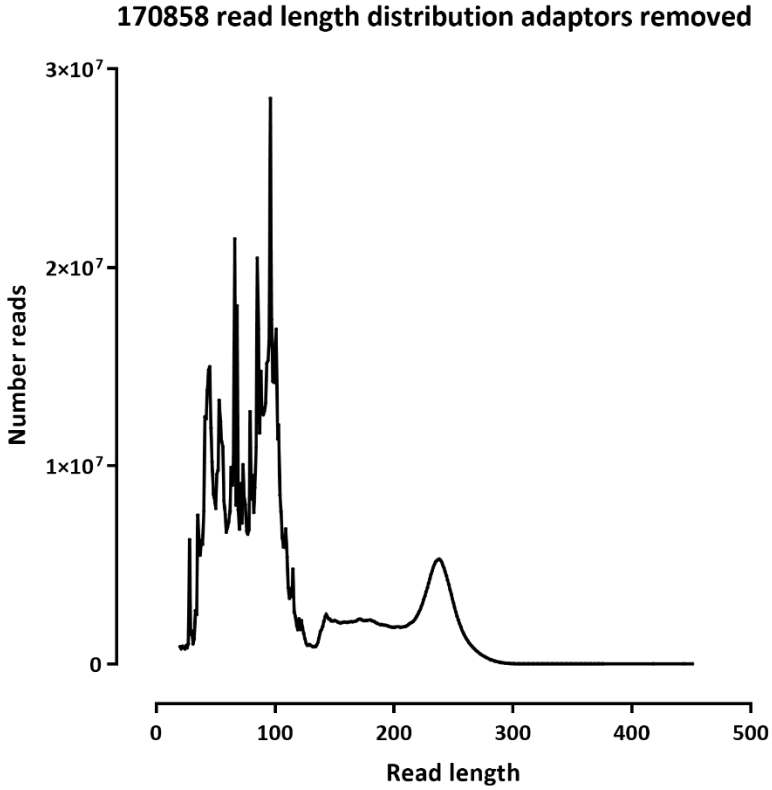

mRNA:
5'- AATGATACGGCGACCACCGAGATCTACACNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAGXXX...XXXBAAAAAAAAAAAAAAAANNNNNNNNNCCGAGCCCACGAGACTCGGACGATCATGGGNNNNNNNNCAAGTATGCAGCGCGCTCAAGCACGTGGATNNNNNNNNAGTCGTACGCCGATGCGAAACATCGGCCACTAGNNNNNNNCACGTCAGCCTCTGTGCGTCCCTACTCTA -3'
3'- TTACTATGCCGCTGGTGGCTCTAGATGTGNNNNNNNNAGCAGCCGTCGCAGTCTACACATATTCTCTGTCXXX...XXXVTTTTTTTTTTTTTTNNNNNNNNNGGCTCGGGTGCTCTGAGCCTGCTAGTACCCNNNNNNNNGTTCATACGTCGCGCGAGTTCGTGCACCTANNNNNNNNTCAGCATGCGGCTACGCTTTGTAGCCGGGTGTAGNNNNNNNCACGTCAGCCTCTGTGCGTCCCTACTCTA -5'
Illumina P5
8-bp i5
s5
ME
cDNA
10-bp UMI
linker1
Barcode1
linker2
Barcode2
linker3
Barcode3
Illumina P7
30
8
30
8
30
ATAC:
5'- AATGATACGGCGACCACCGAGATCTACACNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAGXXX...XXXCTGTCTCTTATACACATCTCCGAGCCCACGAGACTCGGACGATCATGGGNNNNNNNNCAAGTATGCAGCGCGCTCAAGCACGTGGATNNNNNNNNAGTCGTACGCCGATGCGAAACATCGGCCACTAGNNNNNNNCACGTCAGCCTCTGTGCGTCCCTACTCTA -3'
3'- TTACTATGCCGCTGGTGGCTCTAGATGTGNNNNNNNNAGCAGCCGTCGCAGTCTACACATATTCTCTGTCXXX...XXXGACAGAGAATATGTGTAGAGGGCTCGGGTGCTCTGAGCCTGCTAGTACCCNNNNNNNNGTTCATACGTCGCGCGAGTTCGTGCACCTANNNNNNNNTCAGCATGCGGCTACGCTTTGTAGCCGGGTGTAGNNNNNNNCACGTCAGCCTCTGTGCGTCCCTACTCTA -5'
P5
ME
gDNA
ME
linker1
Barcode1
linker2
Barcode2
linker3
Barcode3
UGv3 PA
19
30
8
30
8
30

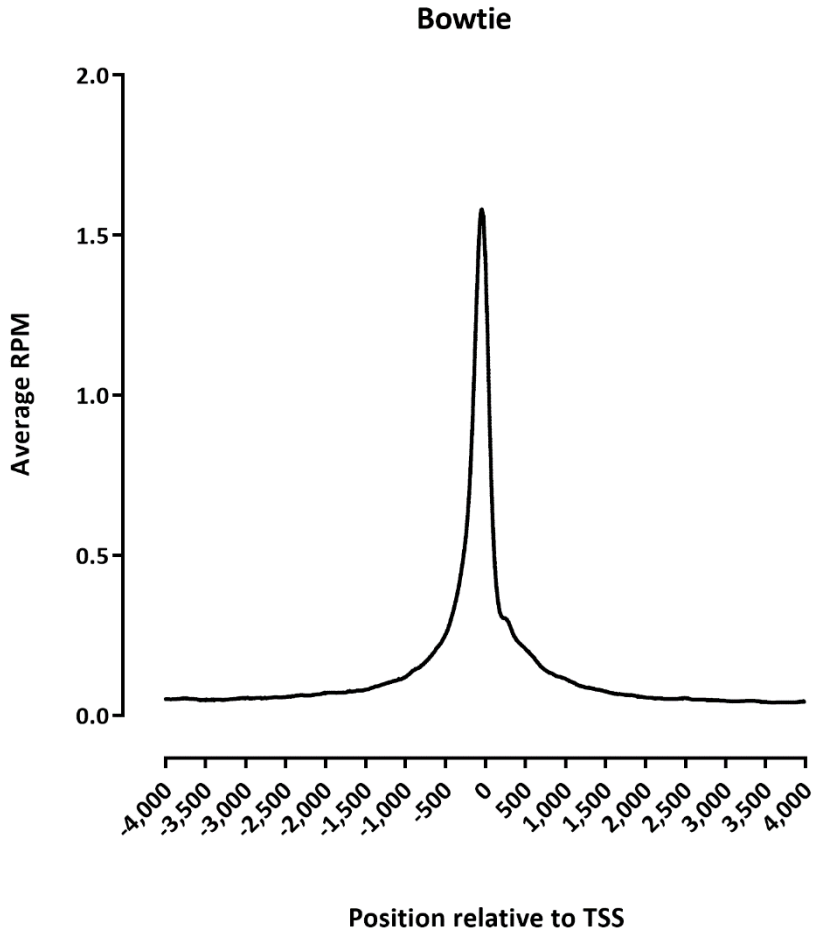
1

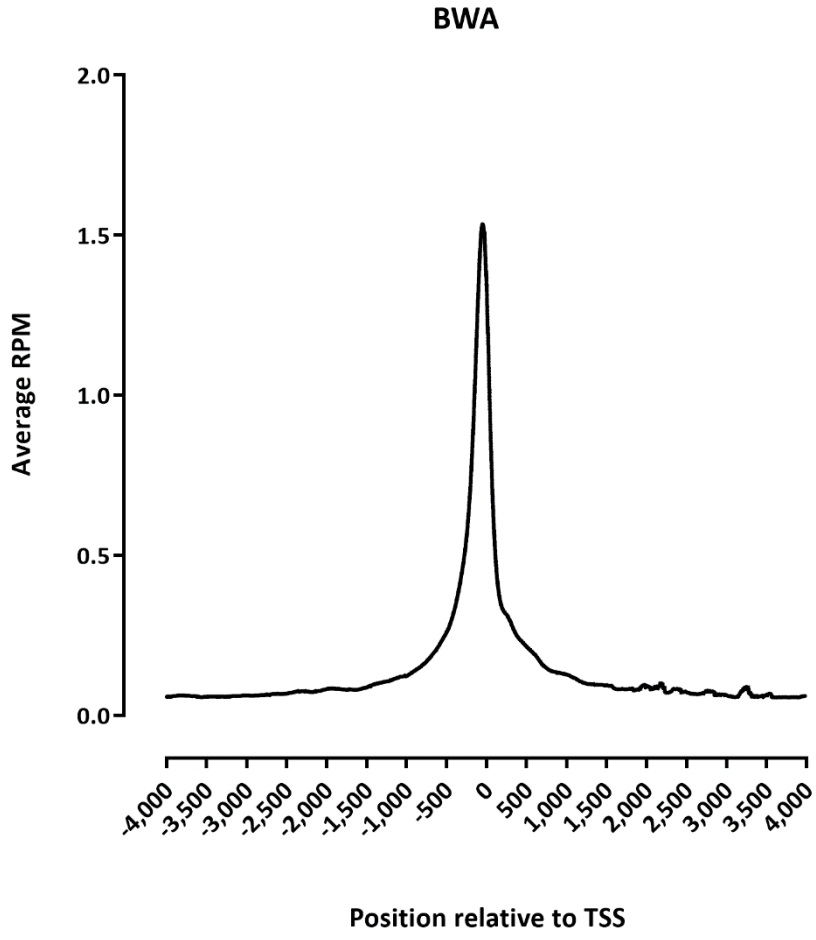

170858 read length distribution
4×10^7
3×10^7
2×10^7
1×10^7
0
Number reads
0
100
200
300
400
500
Read length


170858 read length distribution adaptors removed
3×10^7
2×10^7
1×10^7
0
Number reads
0
100
200
300
400
500
Read length

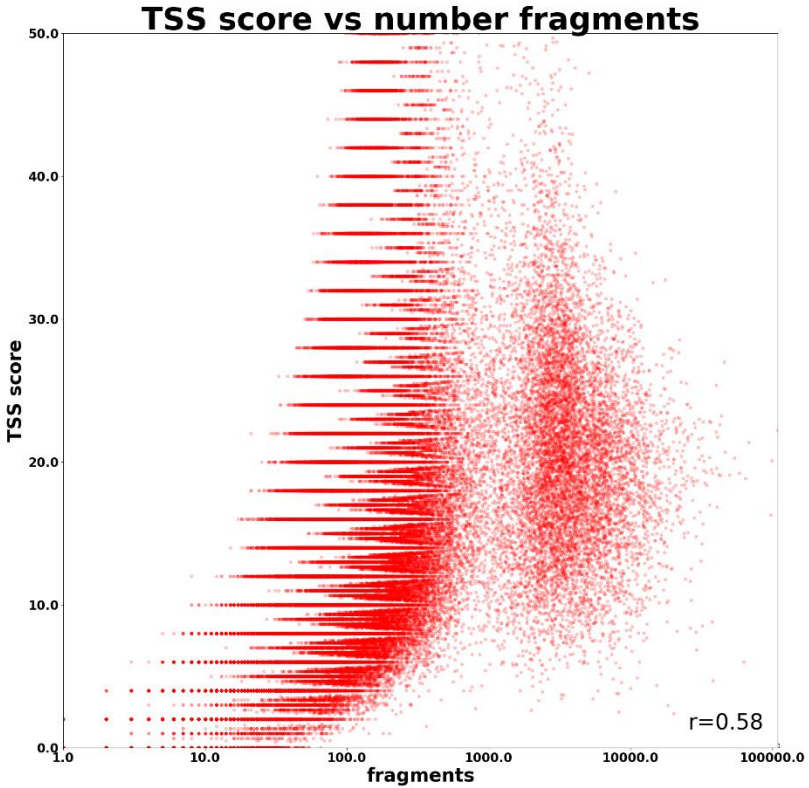
Aligned all reads as ATAC:

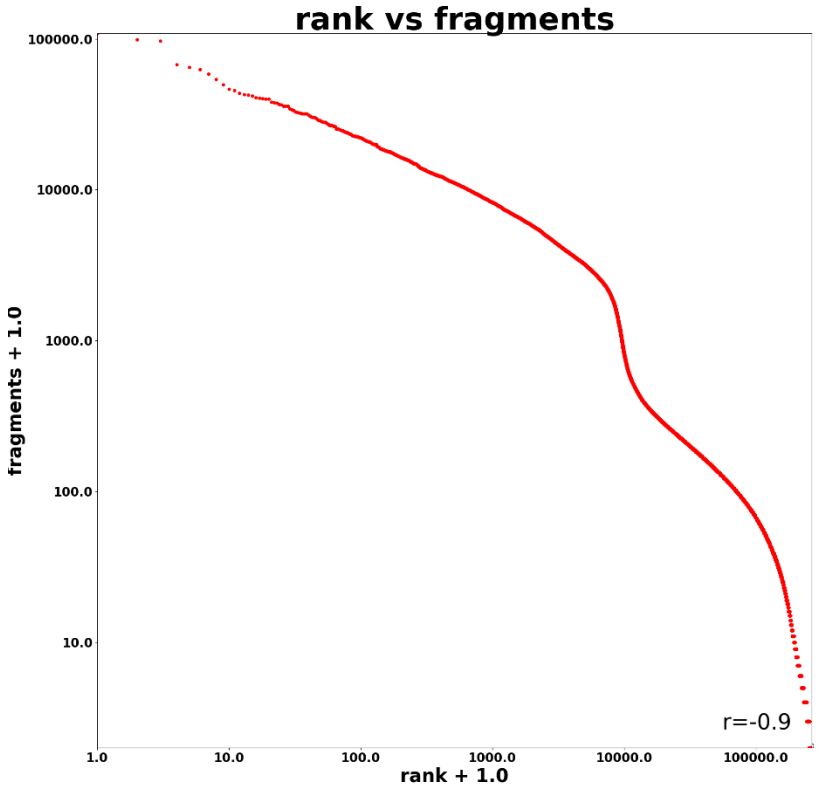
Species	Read Length	Library	Raw fragments	Unique non-chrM reads	Alignment fraction	Complexity	chrM reads	chrM fraction	scATAC dedup reads	scATAC dedup Complexity	TSS ratio	Cells >500 fragments
hg38	**UG, BT**	170858	1,490,206,546	271,069,843	0.10	0.31	16,348,826	0.06	123,596,172	0.43	19.54	11,901
hg38	**UG, BWA**	170858	1,490,206,546	680,093,055	0.23	0.68			383,527,036	0.47	14.02	32,530


Bowtie
Average RPM
2.0
1.5
1.0
0.5
0.0
-4,000
-3,500
-3,000
-2,500
-2,000
-1,500
-1,000
-500
0
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
Position relative to TSS

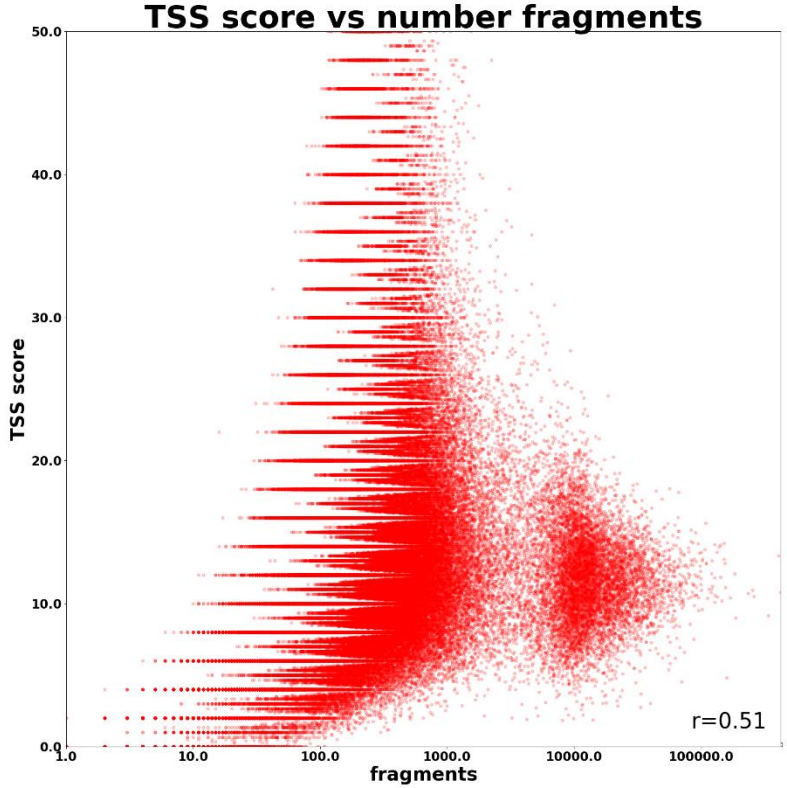

BWA
Average RPM
2.0
1.5
1.0
0.5
0.0
-4,000
-3,500
-3,000
-2,500
-2,000
-1,500
-1,000
-500
0
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
Position relative to TSS

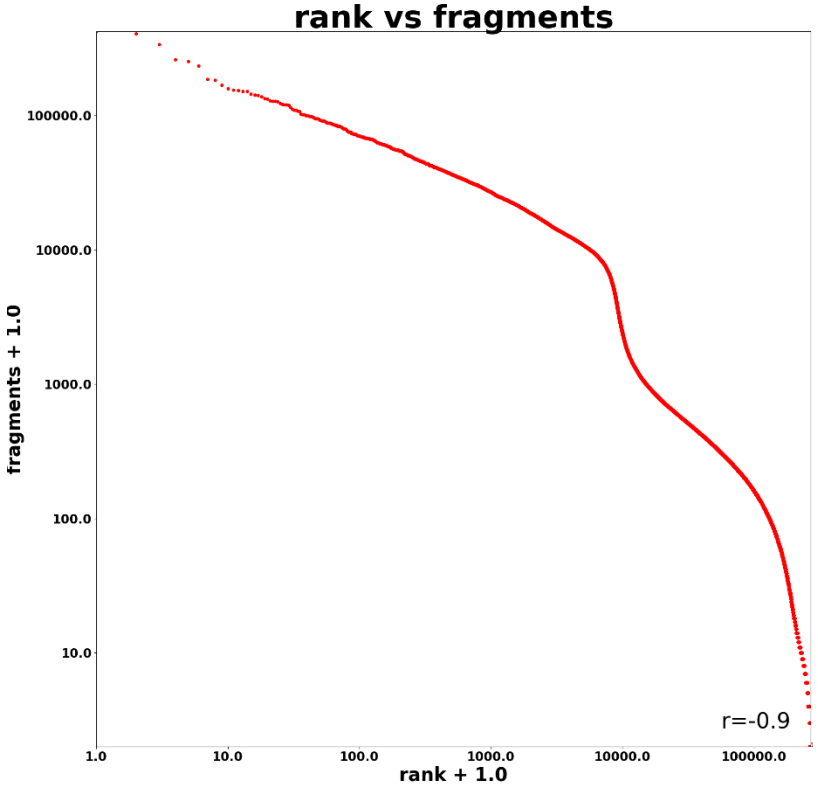
Bowtie


TSS score vs number fragments
TSS score
fragments
r=0.58
50.0
40.0
30.0
20.0
10.0
0.0
1.0
10.0
100.0
1000.0
10000.0
100000.0


rank vs fragments
fragments + 1.0
rank + 1.0
r=-0.9
100000.0
10000.0
1000.0
100.0
10.0
1.0
10.0
100.0
1000.0
10000.0
100000.0

BWA


TSS score vs number fragments
TSS score
fragments
r=0.51
0.0
10.0
20.0
30.0
40.0
50.0
1.0
10.0
100.0
1000.0
10000.0
100000.0


rank vs fragments
fragments + 1.0
rank + 1.0
r=-0.9
10.0
100.0
1000.0
10000.0
100000.0
1.0
10.0
100.0
1000.0
10000.0
100000.0

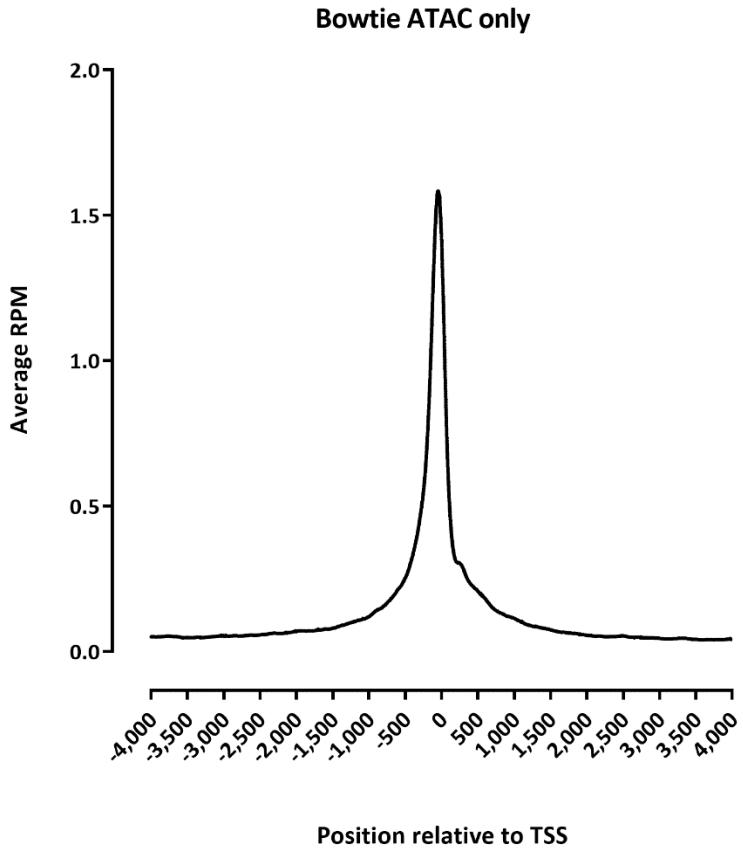
Split into RNA/ATAC

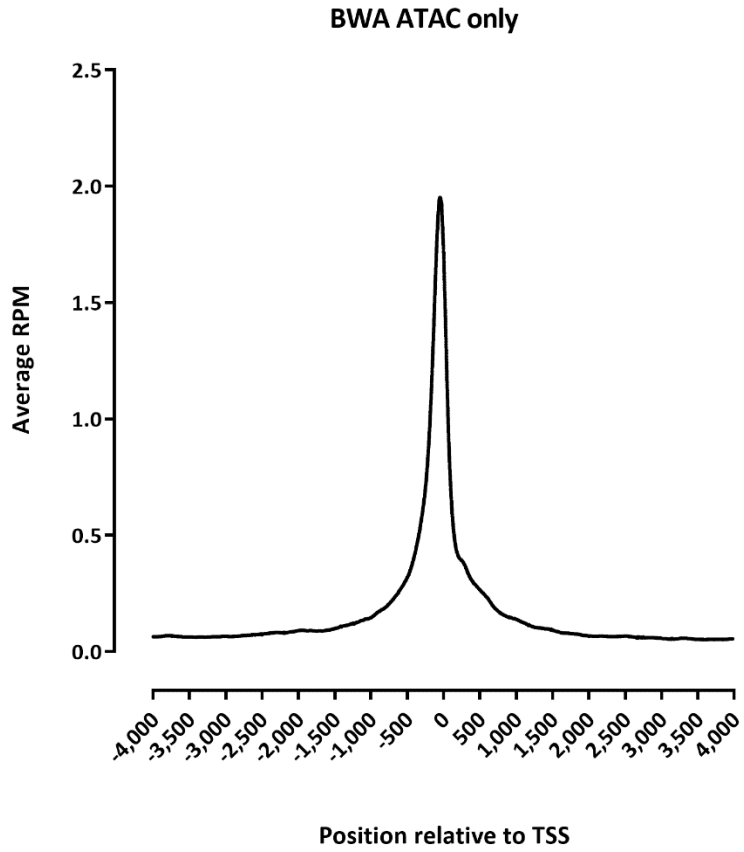
- Looked for polyT(10) – if present -> RNA, if absent -> ATAC

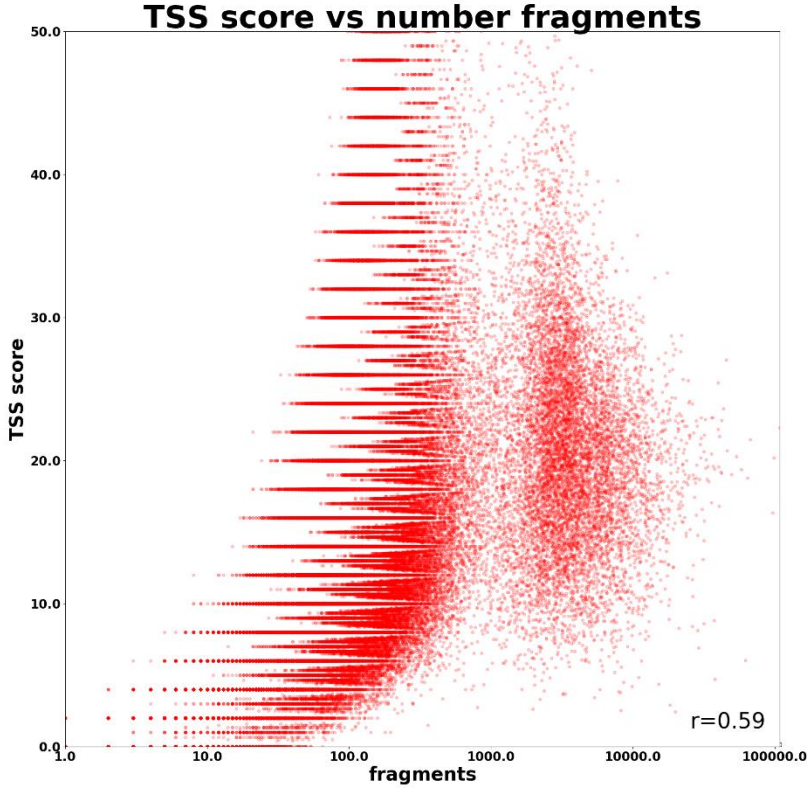
ATAC stats:

Species	Read Length	Library	Raw fragments	Unique non-chrM reads	Alignment fraction	Complexity	chrM reads	chrM fraction	scATAC dedup reads	scATAC dedup Complexity	TSS ratio	Cells >500 fragments
hg38	**UG, BT**	170858	1,490,206,546	271,069,843	0.10	0.31	16,348,826	0.06	123,596,172	0.43	19.54	11,901
hg38	**UG, BWA**	170858	1,490,206,546	680,093,055	0.23	0.68			383,527,036	0.47	14.02	32,530
hg38	**UG, BT**	170858, no polyT(10)	970,819,214	270,386,524	0.15	0.31	16,348,691	0.06	123,123,294	0.43	19.61	11,890
hg38	**UG, BWA**	170858, no polyT(10)	970,819,214	522,470,475	0.27	0.61			212,717,376	0.47	19.25	19,872

*RNA is to be processed at the single-cell level once the UMI assignment is fully worked out, but we have plenty of sequence after the polyT in this run, so there should be no issues


Bowtie ATAC only
Average RPM
2.0
1.5
1.0
0.5
0.0
-4,000
-3,500
-3,000
-2,500
-2,000
-1,500
-1,000
-500
0
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
Position relative to TSS


BWA ATAC only
Average RPM
2.5
2.0
1.5
1.0
0.5
0.0
-4,000
-3,500
-3,000
-2,500
-2,000
-1,500
-1,000
-500
0
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
Position relative to TSS


TSS score vs number fragments
TSS score
fragments
r=0.59
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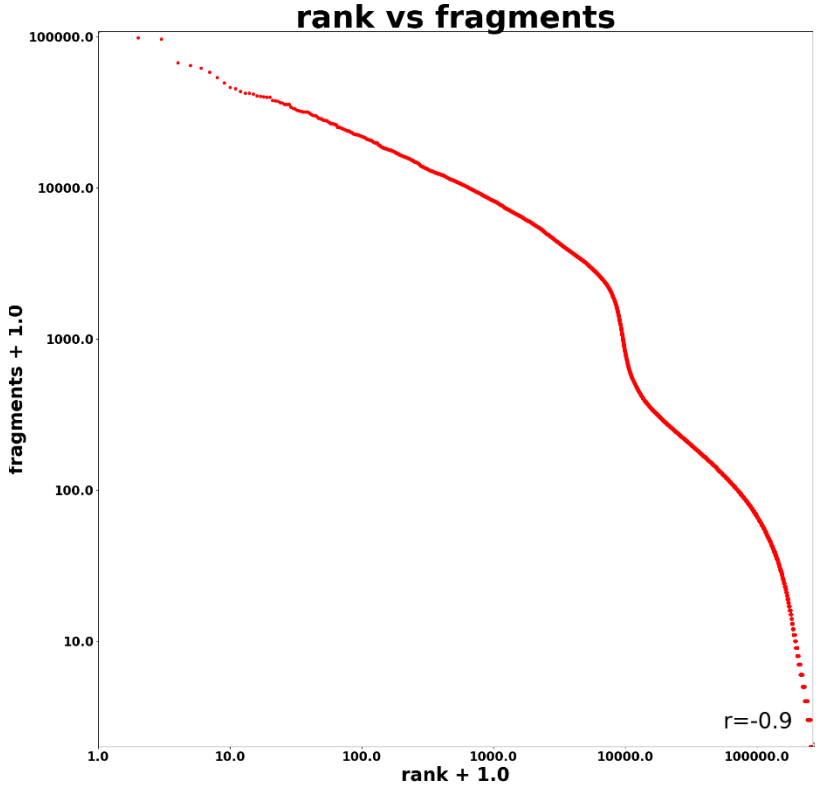
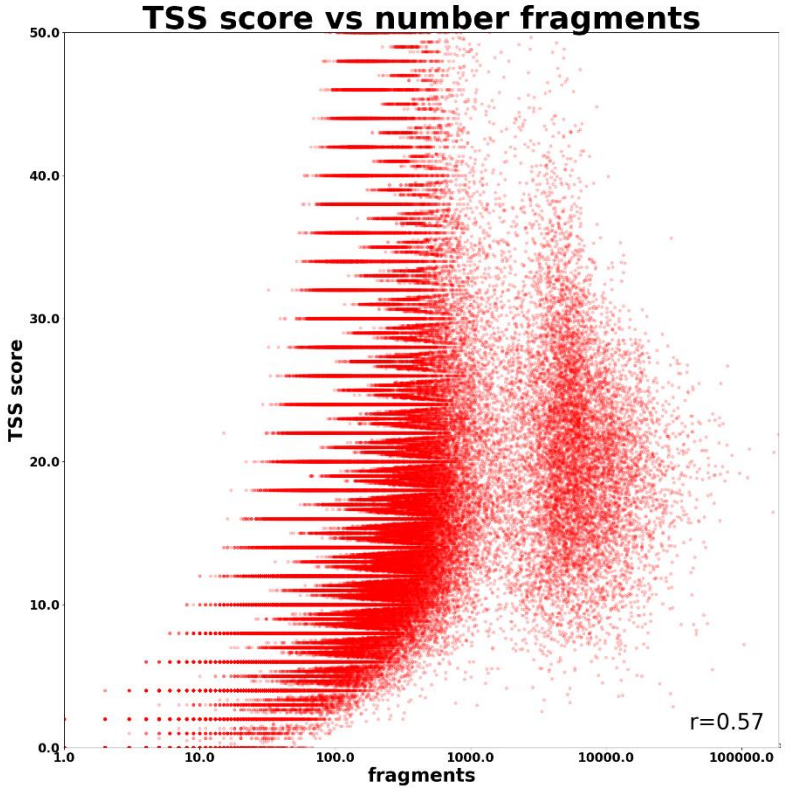
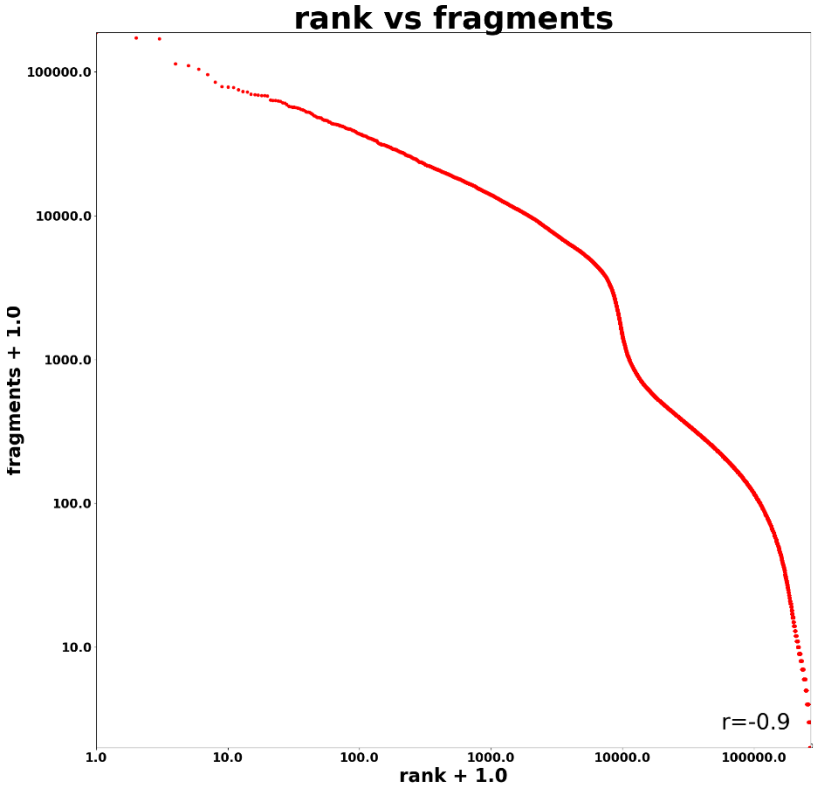
10.0
20.0
30.0
40.0
50.0
1.0
10.0
100.0
1000.0
10000.0
100000.0


rank vs fragments
fragments + 1.0
rank + 1.0
r=-0.9
10.0
100.0
1000.0
10000.0
100000.0
1.0
10.0
100.0
1000.0
10000.0
100000.0


TSS score vs number fragments
TSS score
fragments
r=0.57
0.0
10.0
20.0
30.0
40.0
50.0
1.0
10.0
100.0
1000.0
10000.0
100000.0


rank vs fragments
fragments + 1.0
rank + 1.0
r=-0.9
10.0
100.0
1000.0
10000.0
100000.0
1.0
10.0
100.0
1000.0
10000.0
100000.0