Experiment design:

- K562 H3K4me1 and H3K27ac ChIP using NspI for digestion instead of sonication
  - NspI is a 5-cutter leaving a 3-bp overhang
  - the overhangs are asymmetric, i.e. self-ligation should not be happening

- recovered ~500ng per reaction

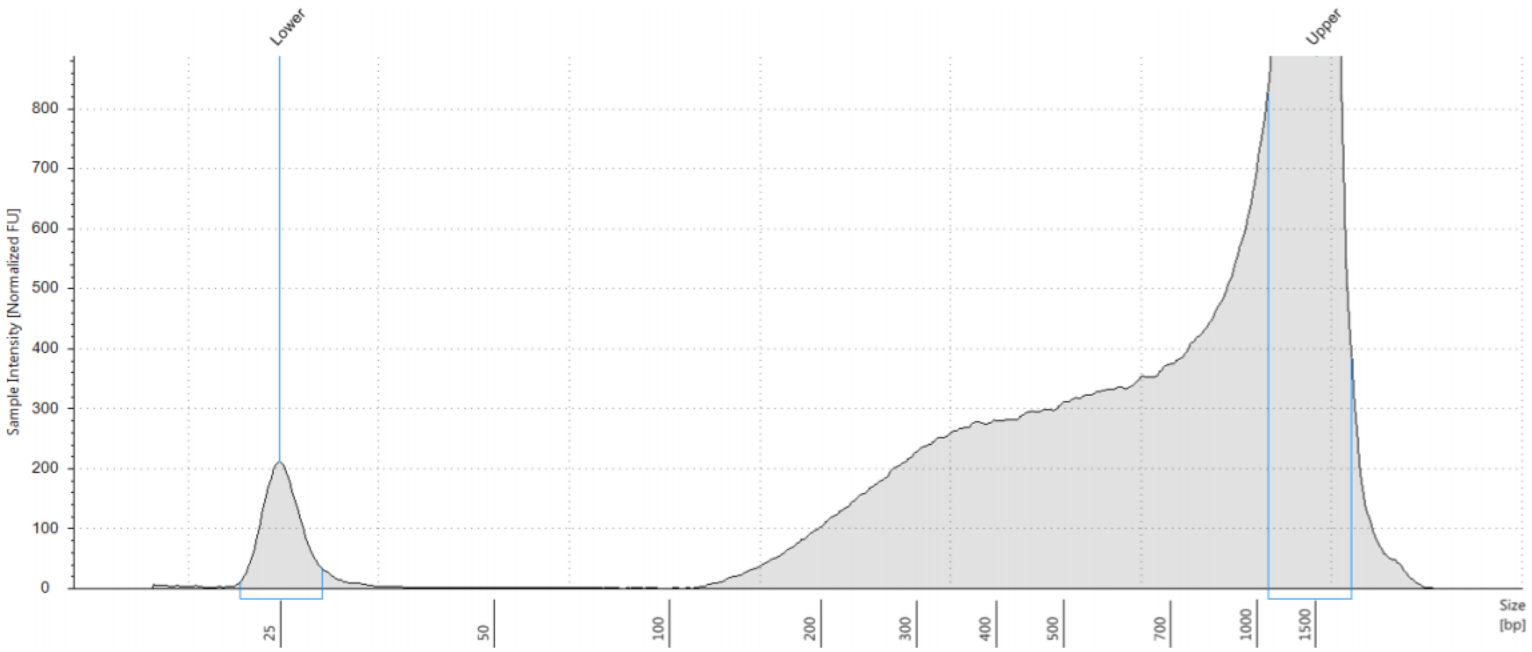
- pooled 2 reactions for each mark

- Ligated using T7 DNA Ligase (T7 requires overhangs) in order to increase the length of input molecules

- Nanopore sequencing using the transposition-based barcoding kit

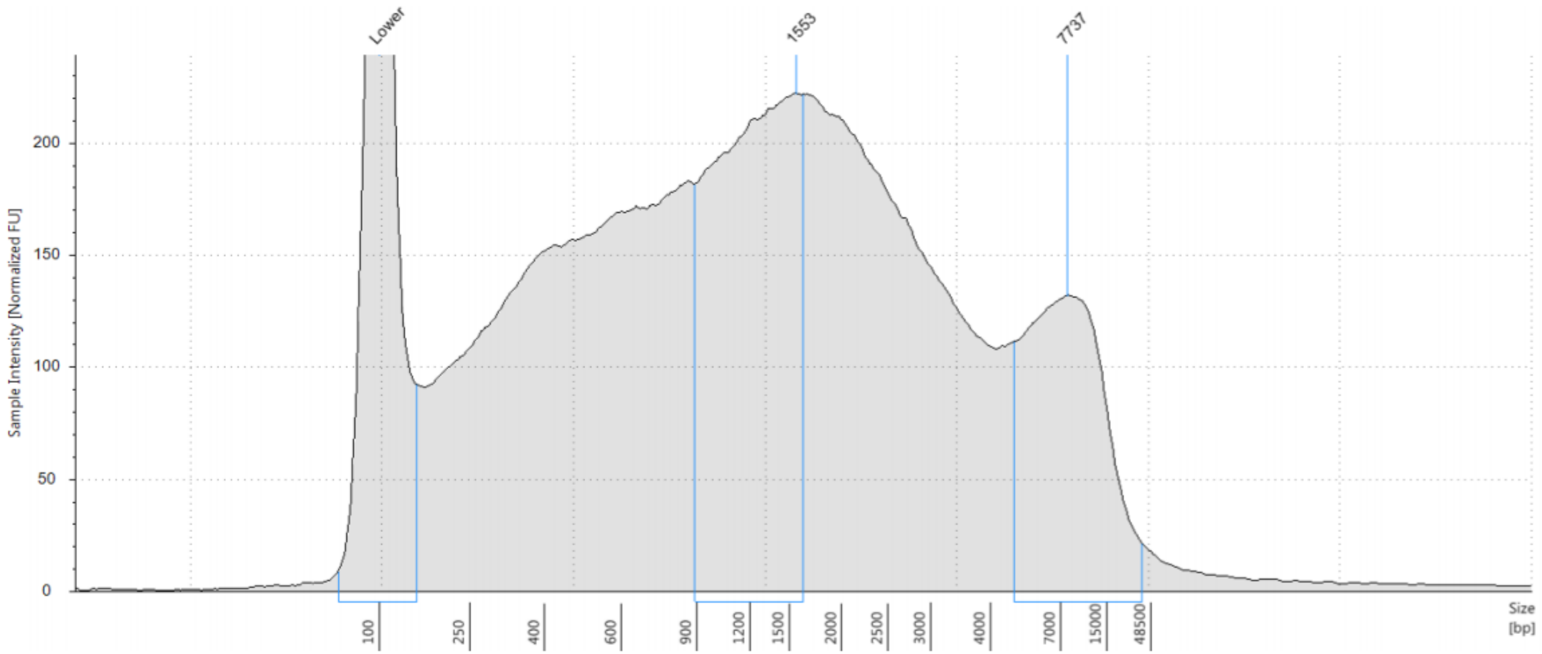
Before ligation:

B1: C190



After ligation:

C1: C189 (pre-clean-up)



Note: it is not clear whether the ligation actually ligated much, but we went ahead with the sequencing

Barcoding results:

```
Adapters detected in 776361 of 1857654 reads
       RBK001 776361: |          ######### |  41.79 %
         none 1004799: |        ########### |  54.09 %
Barcodes detected in 776361 of 1857654 adapters
    barcode01 293393: |              ### |  15.79 %
    barcode02  55906: |                  |   3.01 %
    barcode03  85476: |                  |   4.60 %
    barcode04 186713: |               ## |  10.05 %
    barcode05 154685: |                # |   8.33 %
    barcode06    102: |                  |   0.01 %
    barcode07     32: |                  |   0.00 %
    barcode08      3: |                  |   0.00 %
    barcode09     32: |                  |   0.00 %
    barcode10      1: |                  |   0.00 %
    barcode11      4: |                  |   0.00 %
    barcode12     14: |                  |   0.00 %
         none 1004799: |        ########### |  54.09 %
76494 reads were skipped due to the min. length filter.
```

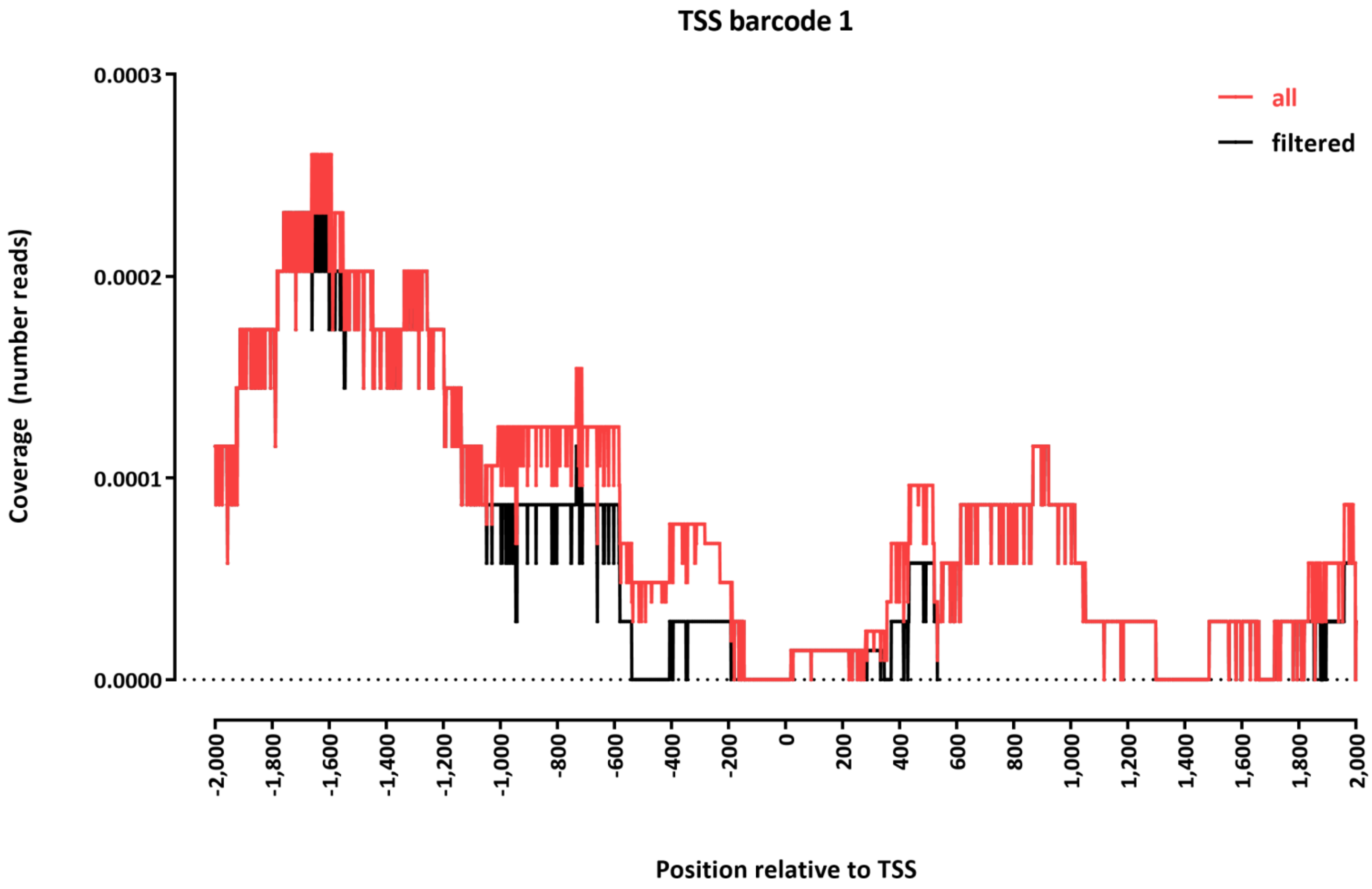
Barcodes 1-3 -- Lambda DNA

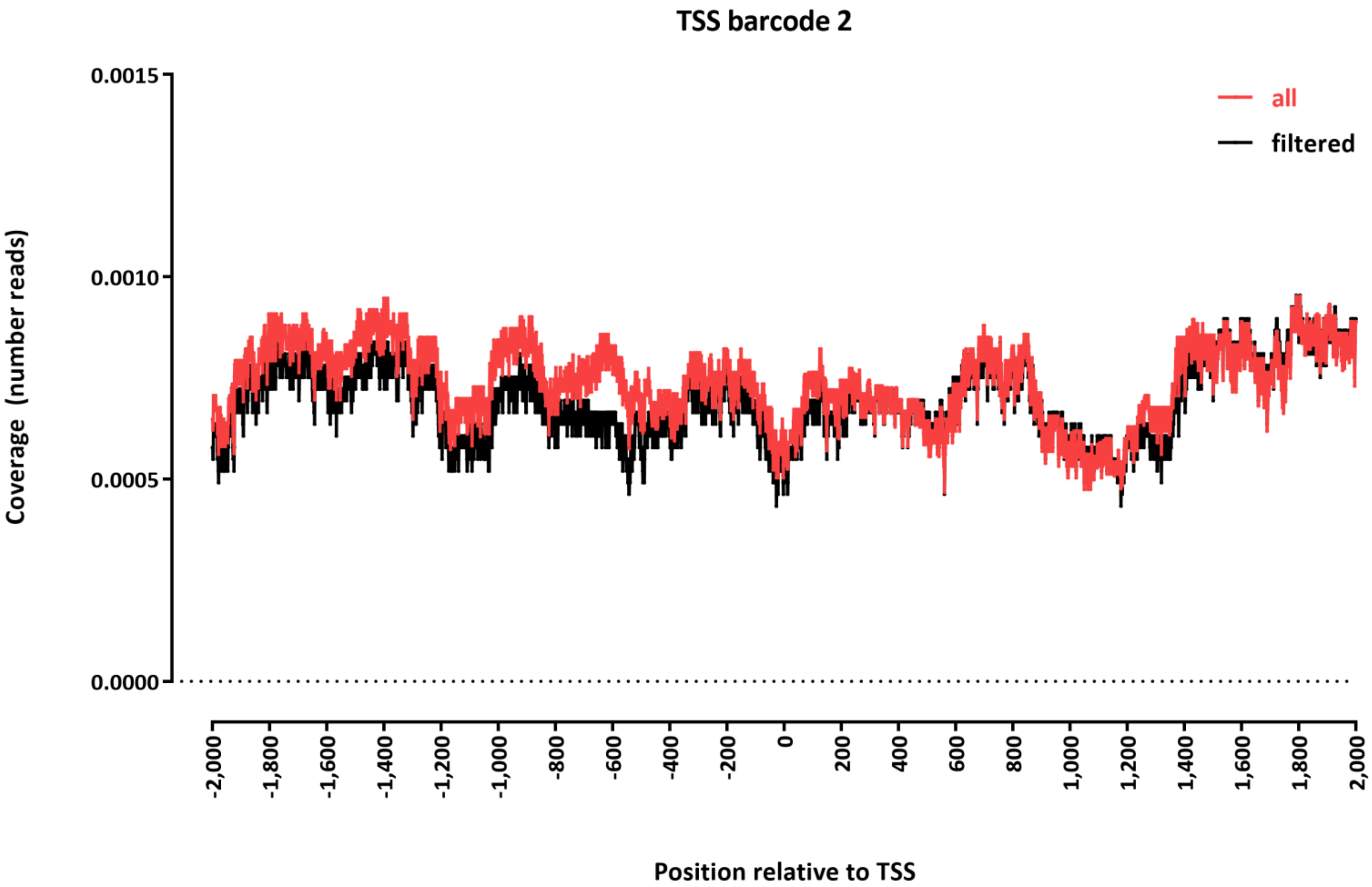
Barcodes 4-5 -- K562 H3K27ac and H3K4me1 ChIP

Mapping stats:

- Mapped with MiniMap 2.17 against hg38-female
- Note that because of the ligation, we expect to see different parts of reads mapping to different locations in the genome, including different chromosomes
- Minimap handles such cases well in terms of mapping, but the mapping stats code as currently written considers those as multimappers because they have the same read ID
- “F1804-q30” – alignments filtered with “samtools view -F 1804 -q 30”

Species	**alignment**	**#**	**Complexity**	**Average Read Length**	**Unique**	**Multi**	**Raw fragments**	**Alignment fraction**
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-barcode01	1.00	692	179	30	293,393	0.00
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-barcode01.F1804-q30	1.00	862	165	2	293,393	0.00
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-barcode02	1.00	826	1,757	200	55,906	0.04
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-barcode02.F1804-q30	1.00	901	1,657	9	55,906	0.03
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-barcode03	1.00	662	99	11	85,476	0.00
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-barcode03.F1804-q30	1.00	831	93	0	85,476	0.00
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-barcode04	1.00	733	151,954	14,180	186,713	0.89
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-barcode04.F1804-q30	1.00	767	142,497	1,476	186,713	0.77
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-barcode05	1.00	915	130,441	10,336	154,685	0.91
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-barcode05.F1804-q30	1.00	926	124,657	948	154,685	0.81
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-none	1.00	845	358,959	31,201	1,004,799	0.39
Homo sapiens hg38-female	MiniMap 2.17	2019_05_23_Barcoded_0_1_SDS-none.F1804-q30	1.00	874	332,422	2,723	1,004,799	0.33


TSS barcode 1
Coverage (number reads)
0.0003
0.0002
0.0001
0.0000
all
filtered
-2,000
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to TSS


TSS barcode 2
all
filtered
Coverage (number reads)
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0.0010
0.0005
0.0000
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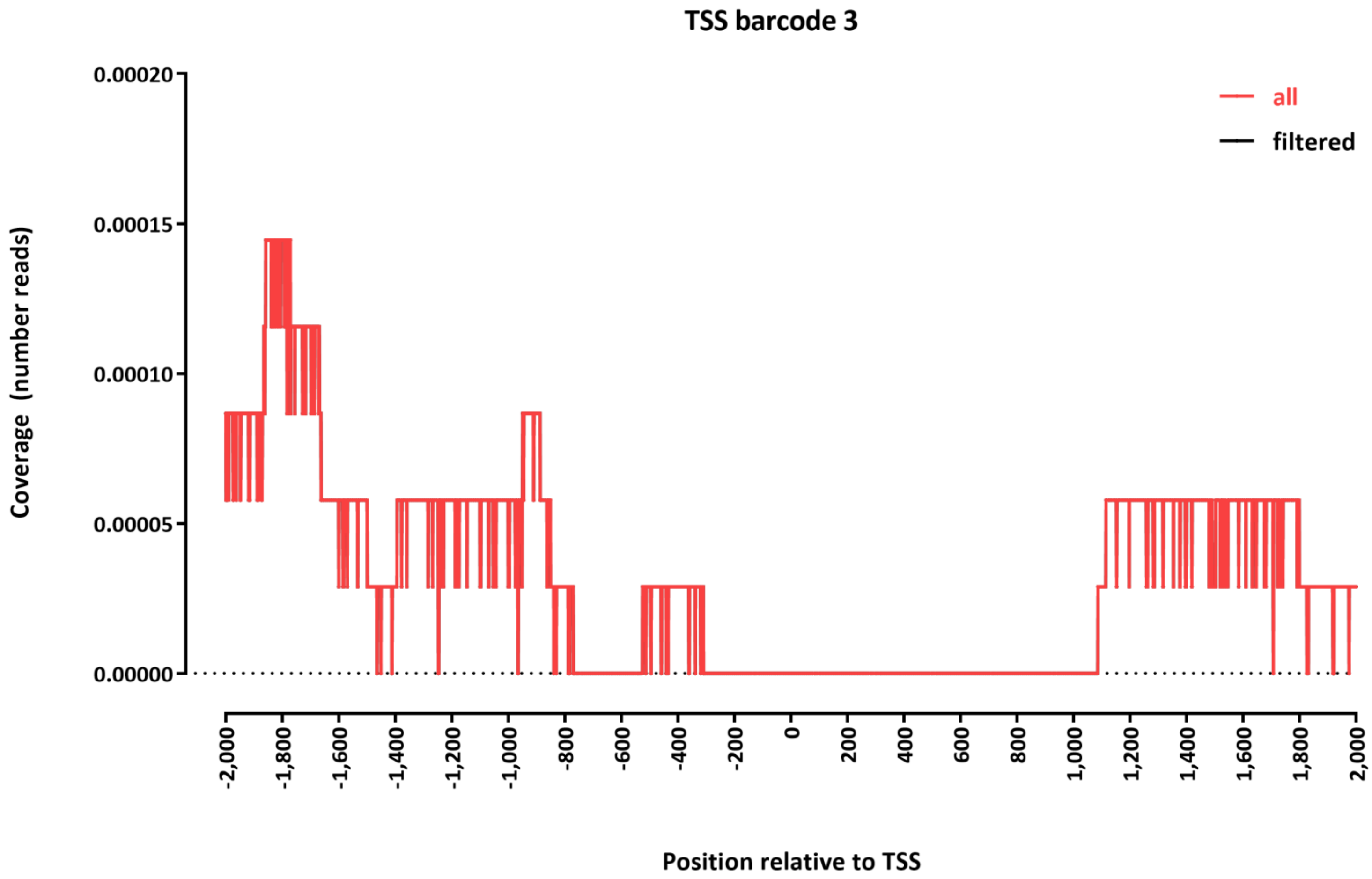
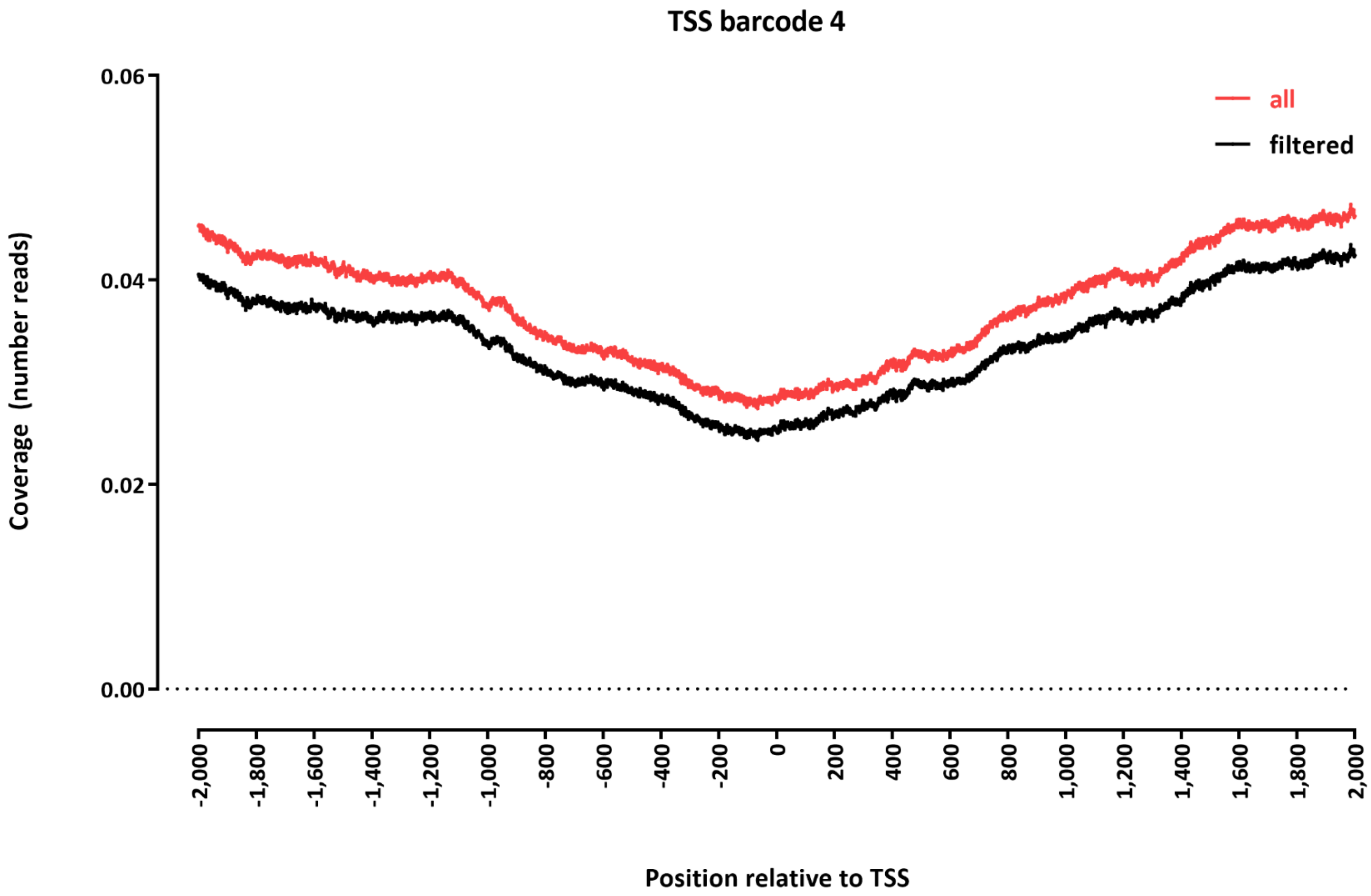
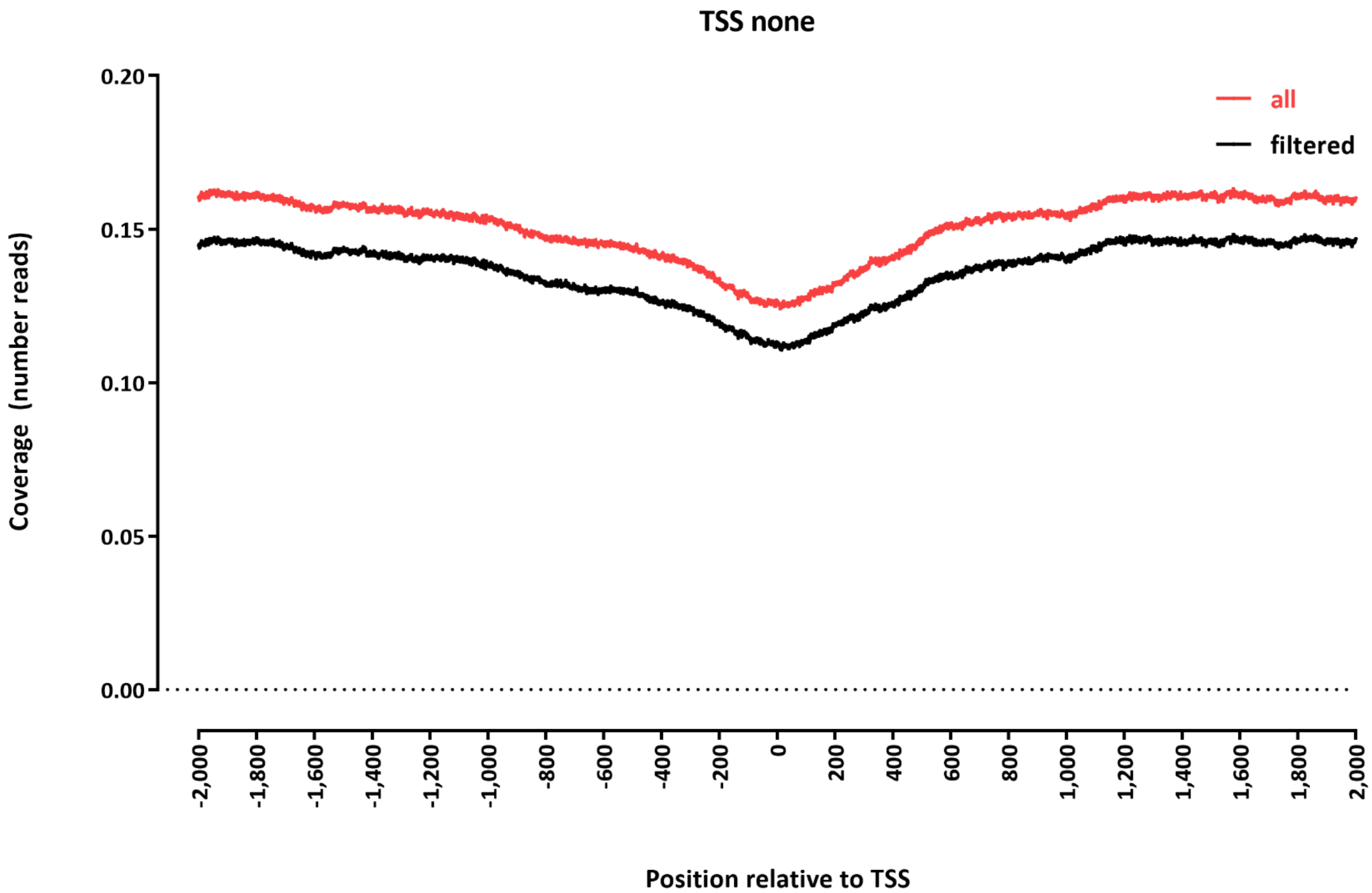
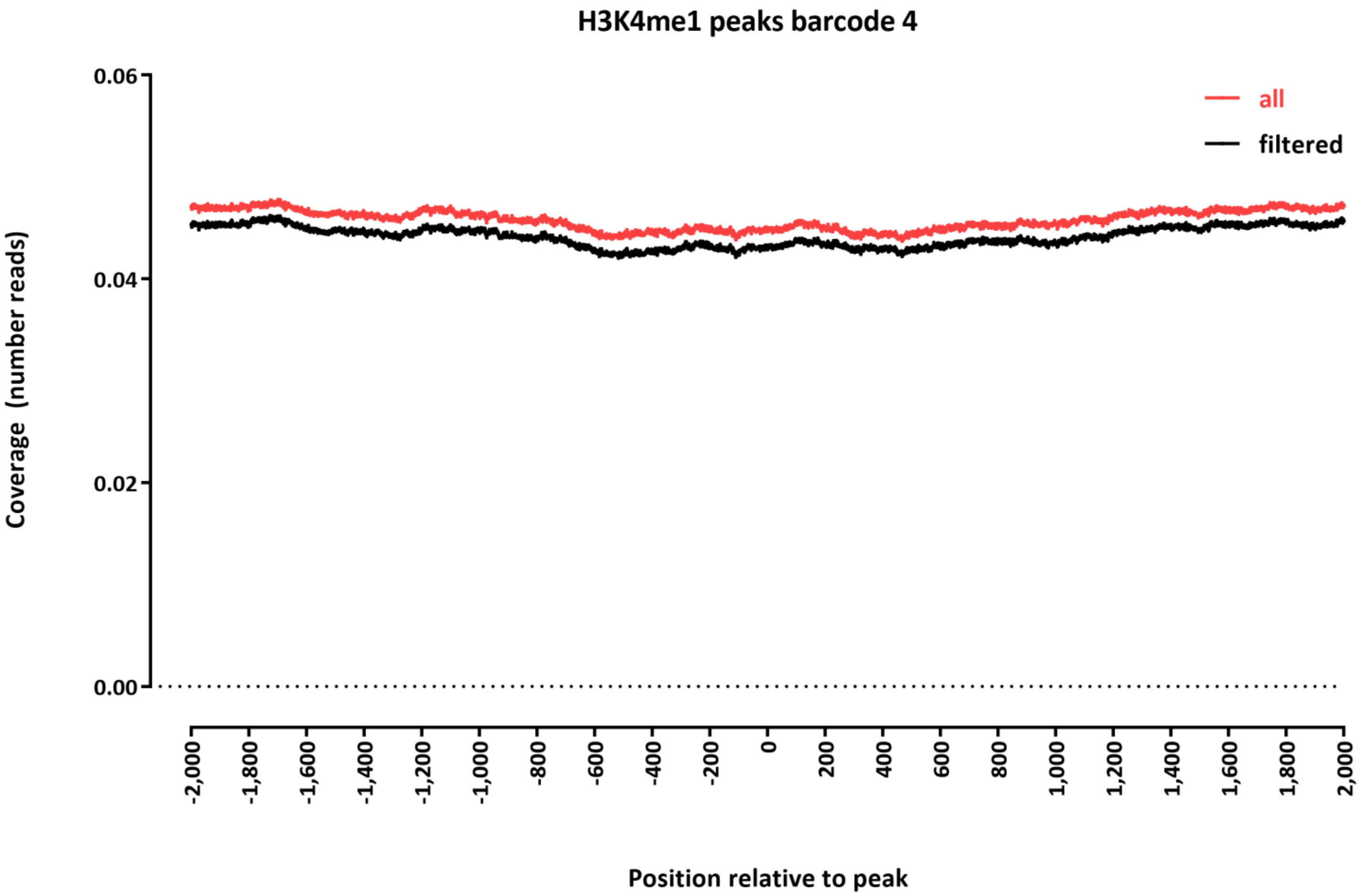
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to TSS

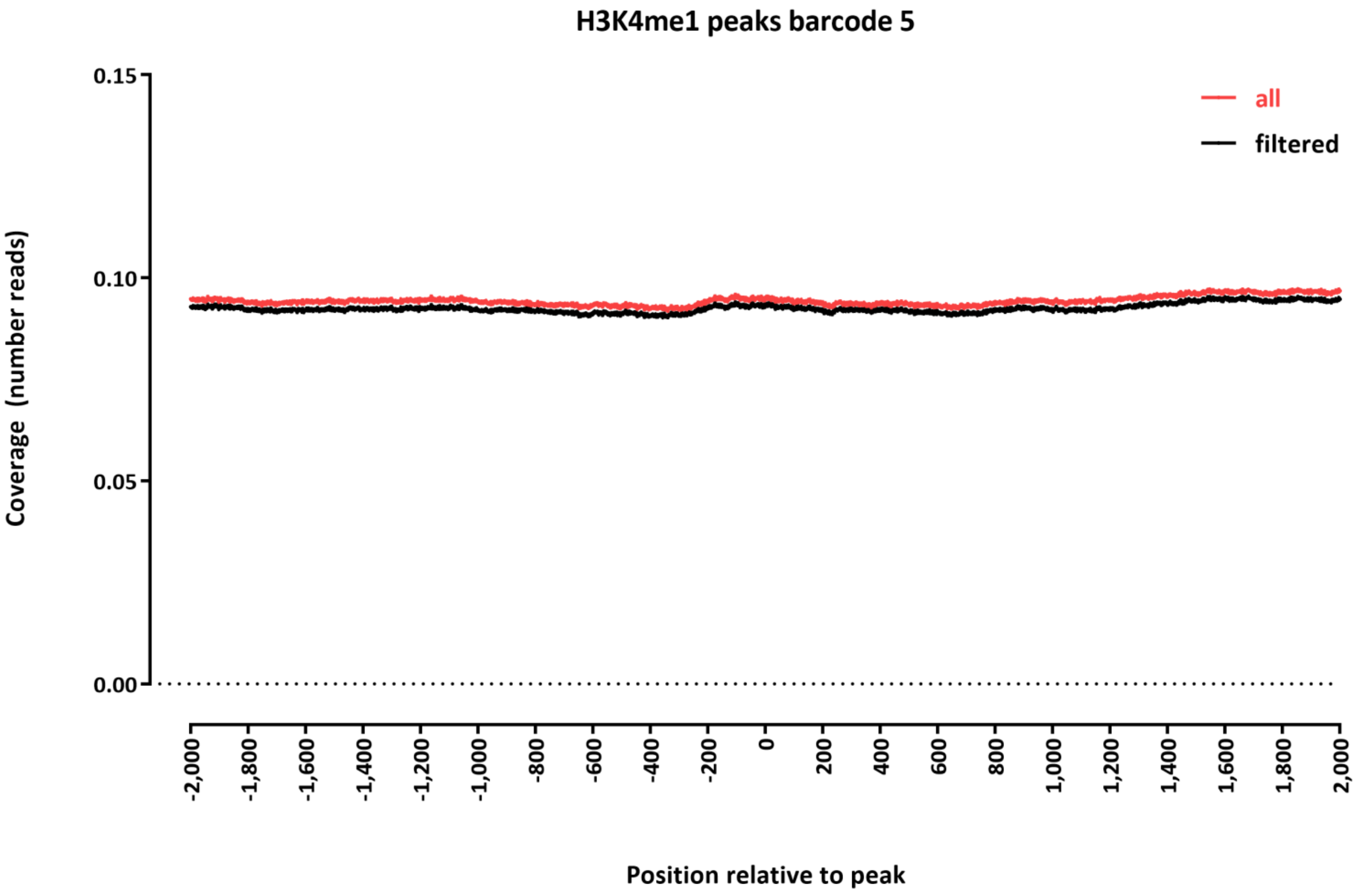

TSS barcode 3
Coverage (number reads)
0.00020
0.00015
0.00010
0.00005
0.00000
all
filtered
-2,000
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to TSS


TSS barcode 4
Coverage (number reads)
0.06
0.04
0.02
0.00
all
filtered
-2,000
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to TSS

TSS barcode 5


TSS none
all
filtered
Coverage (number reads)
0.20
0.15
0.10
0.05
0.00
-2,000
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to TSS


H3K4me1 peaks barcode 4
Coverage (number reads)
0.06
0.04
0.02
0.00
all
filtered
-2,000
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to peak


H3K4me1 peaks barcode 5
Coverage (number reads)
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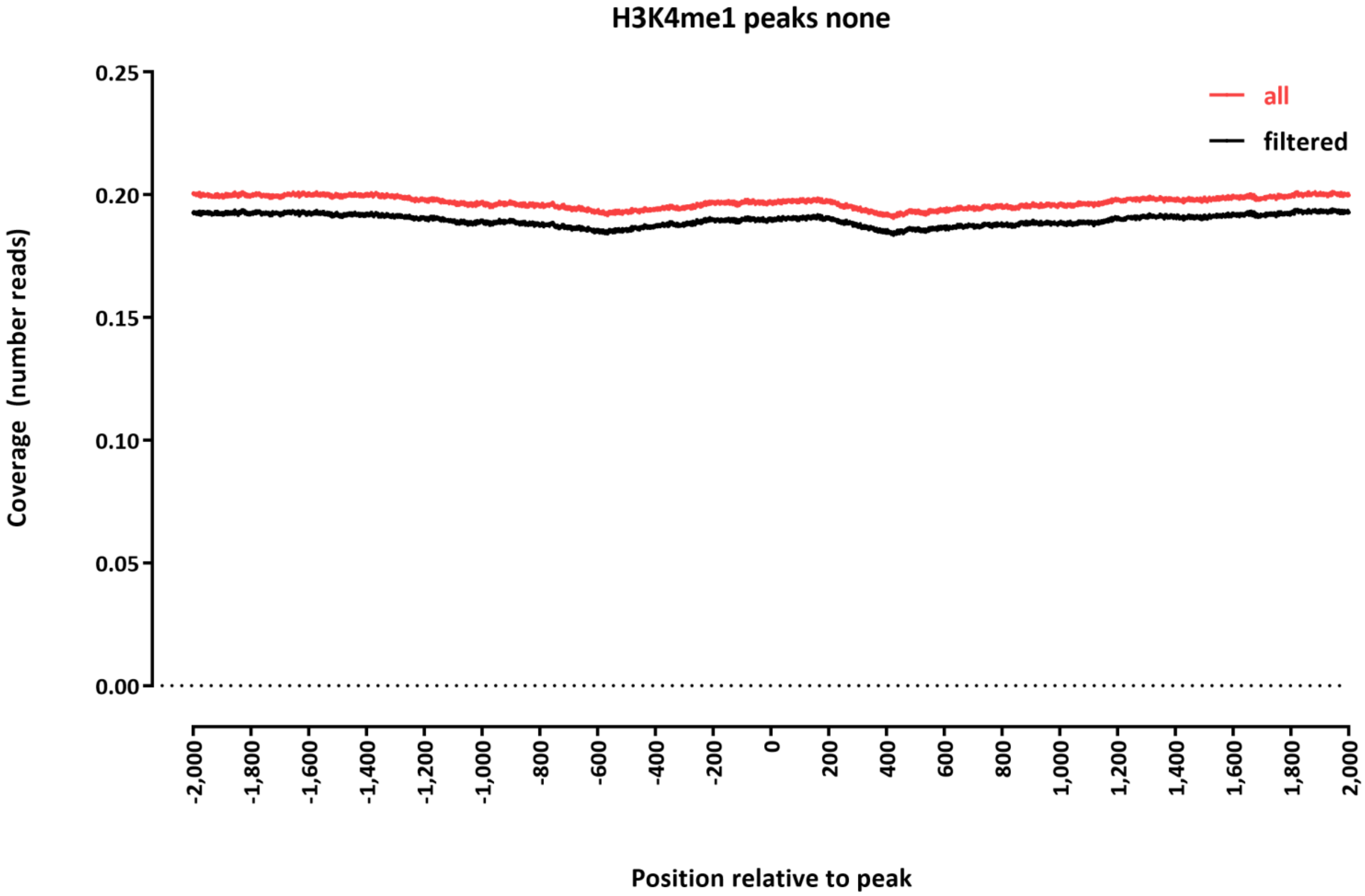
0.10
0.05
0.00
all
filtered
-2,000
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to peak


H3K4me1 peaks none
all
filtered
Coverage (number reads)
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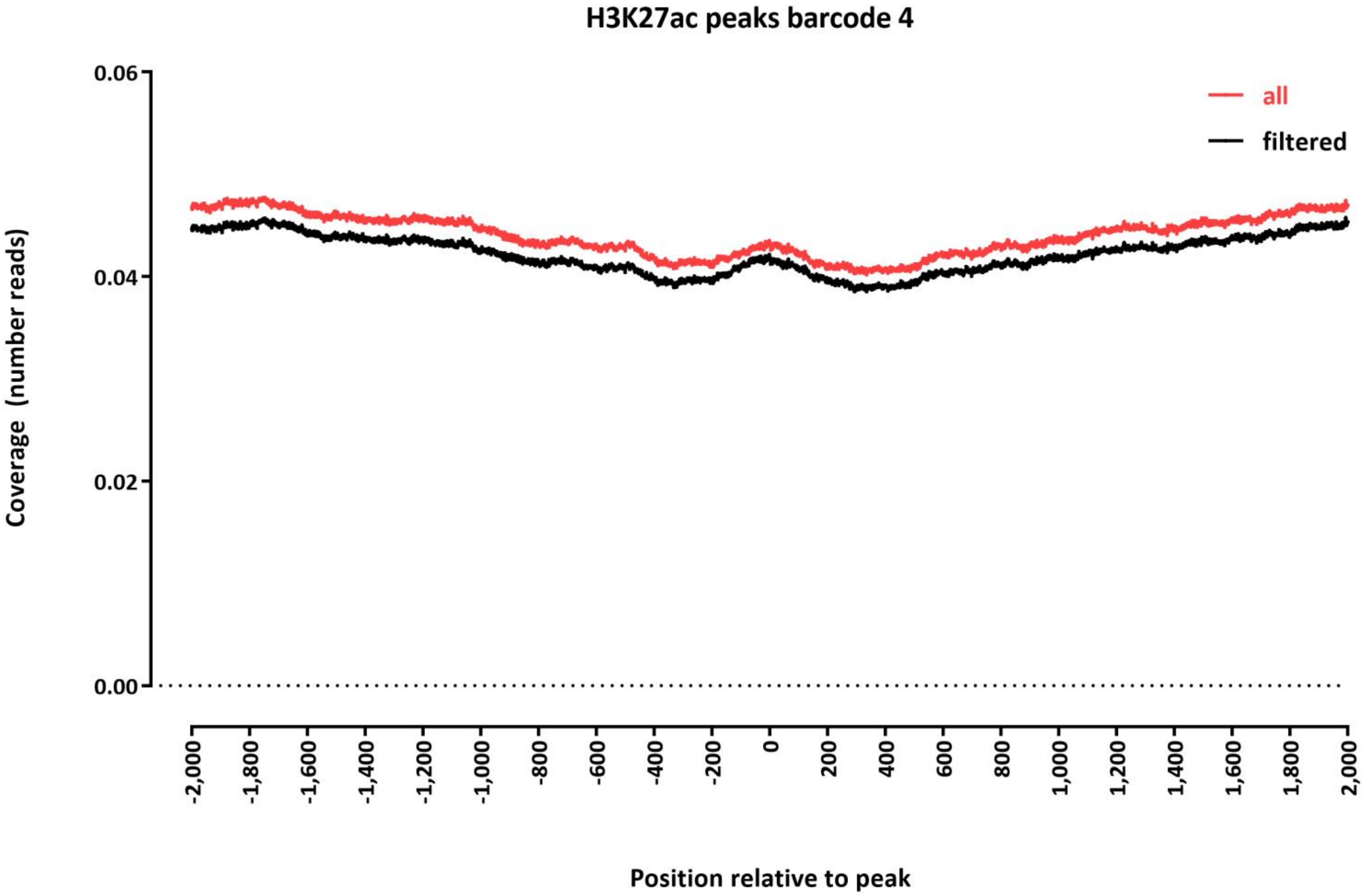
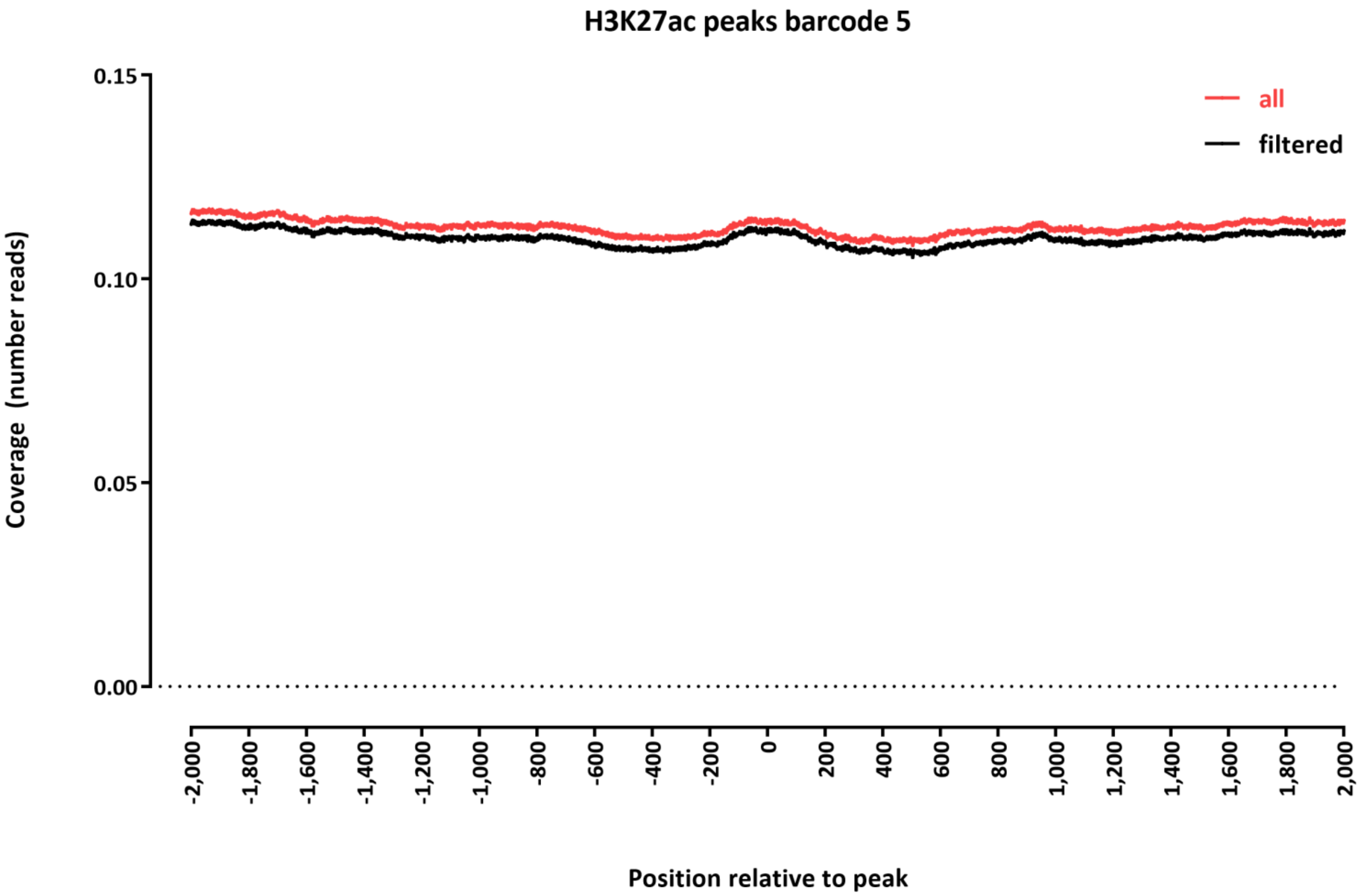
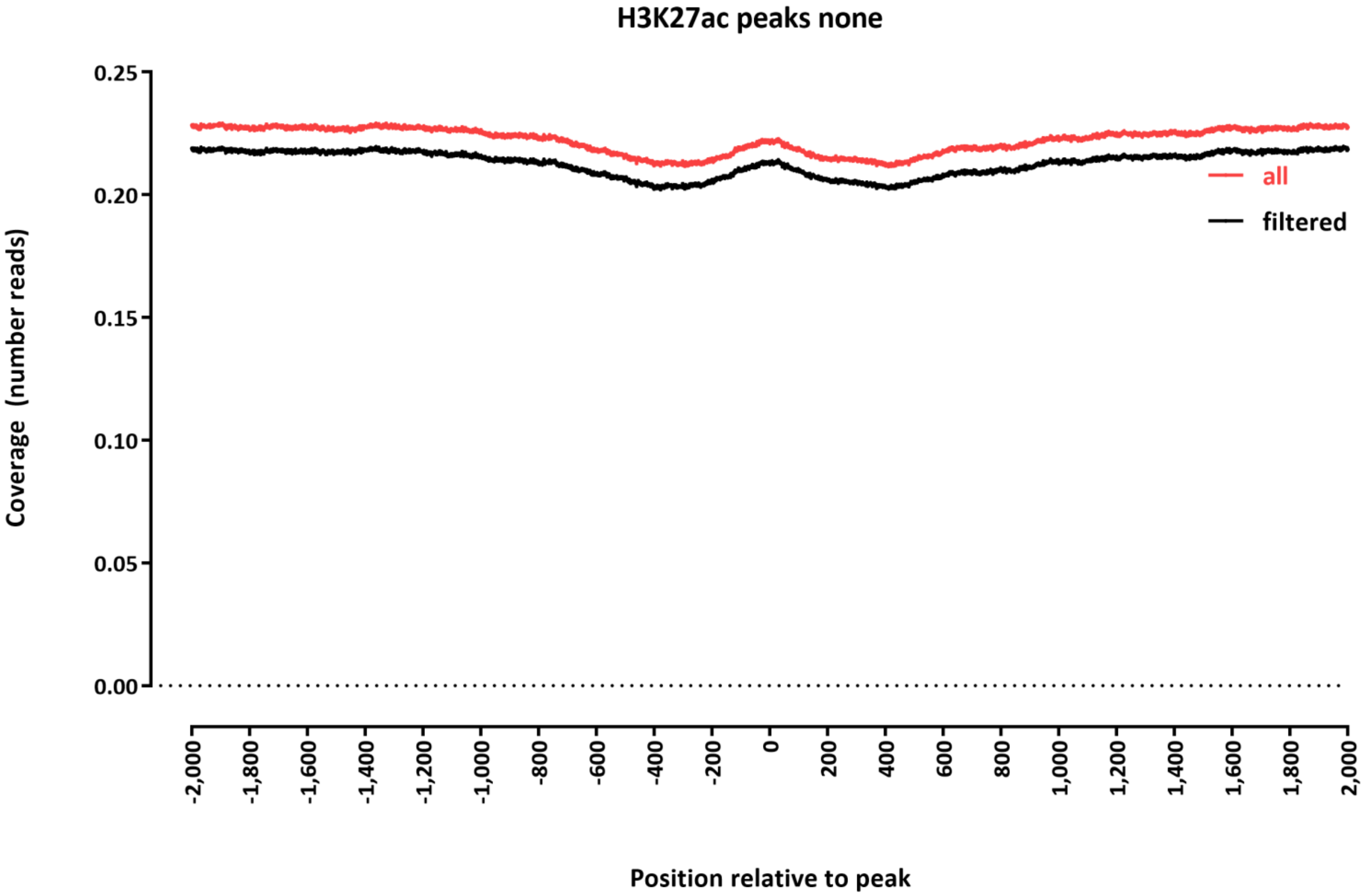
0.20
0.15
0.10
0.05
0.00
-2,000
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to peak


H3K27ac peaks barcode 4
all
filtered
Coverage (number reads)
0.06
0.04
0.02
0.00
-2,000
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to peak


H3K27ac peaks barcode 5
Coverage (number reads)
0.15
0.10
0.05
0.00
all
filtered
-2,000
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to peak


H3K27ac peaks none
Coverage (number reads)
0.25
0.20
0.15
0.10
0.05
0.00
all
filtered
-2,000
-1,800
-1,600
-1,400
-1,200
-1,000
-800
-600
-400
-200
0
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
Position relative to peak