SAMPLE SUMMARY

Lambda DNA (https://www.thermofisher.com/order/catalog/product/SD0021?SID=srch-srp-SD0021) was incubated with methyltransferases:

M.TaqI (TCGA, N6-A)

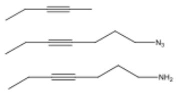
M2.BcnI (CCSGG, N4-C)

M.HhaI (GCGC, 5-C) (engineered variant)

and cofactors, each MTase with:

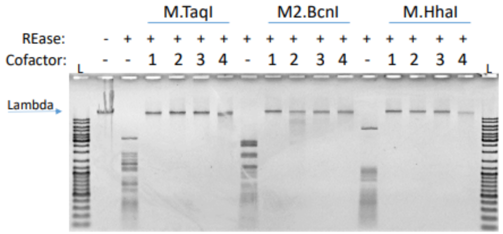
Transferable group

1. AdoMet -CH_3
2. AdoButyn
3. AdoHexynN_3
4. AdoHexynNH_2



The reaction mixtures were then treated with Proteinase K and column-purified (Zymo Research, genomic DCC), eluted with 100 µl Elution Buffer (10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA).

The extent of DNA modification was assayed by protection from the cognate restriction endonuclease (i.e. R.TaqI, R.BcnI and R.Hin6I, respectively)



Samples with complete protection (nearly all the target sites have an attached group): M.TaqI with cofactors 1, 2 and 3; M2.BcnI – 1, 3 and 4; M.HhaI 1, 2 and 3.

The rest (TaqI 4, Bcn 2 and HhaI 4) have incomplete protection, i.e. not all the recognition sites are modified.

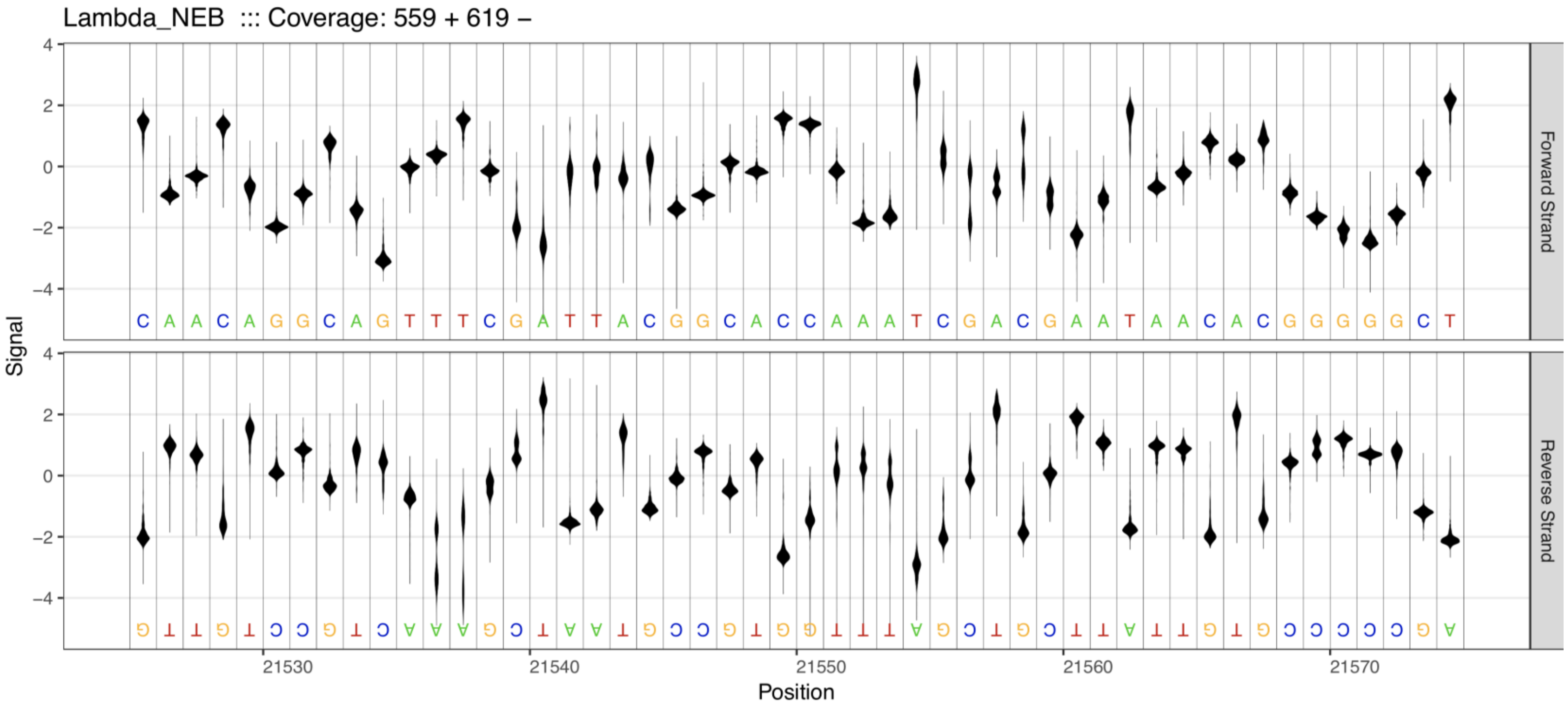
SEQUENCING SUMMARY

Note: we can only sequence one sample at a time at the moment, which we do as spike-ins into other runs; we have now obtained the ONT barcoding kit so we hope to be able to run all remaining samples soon in a single run

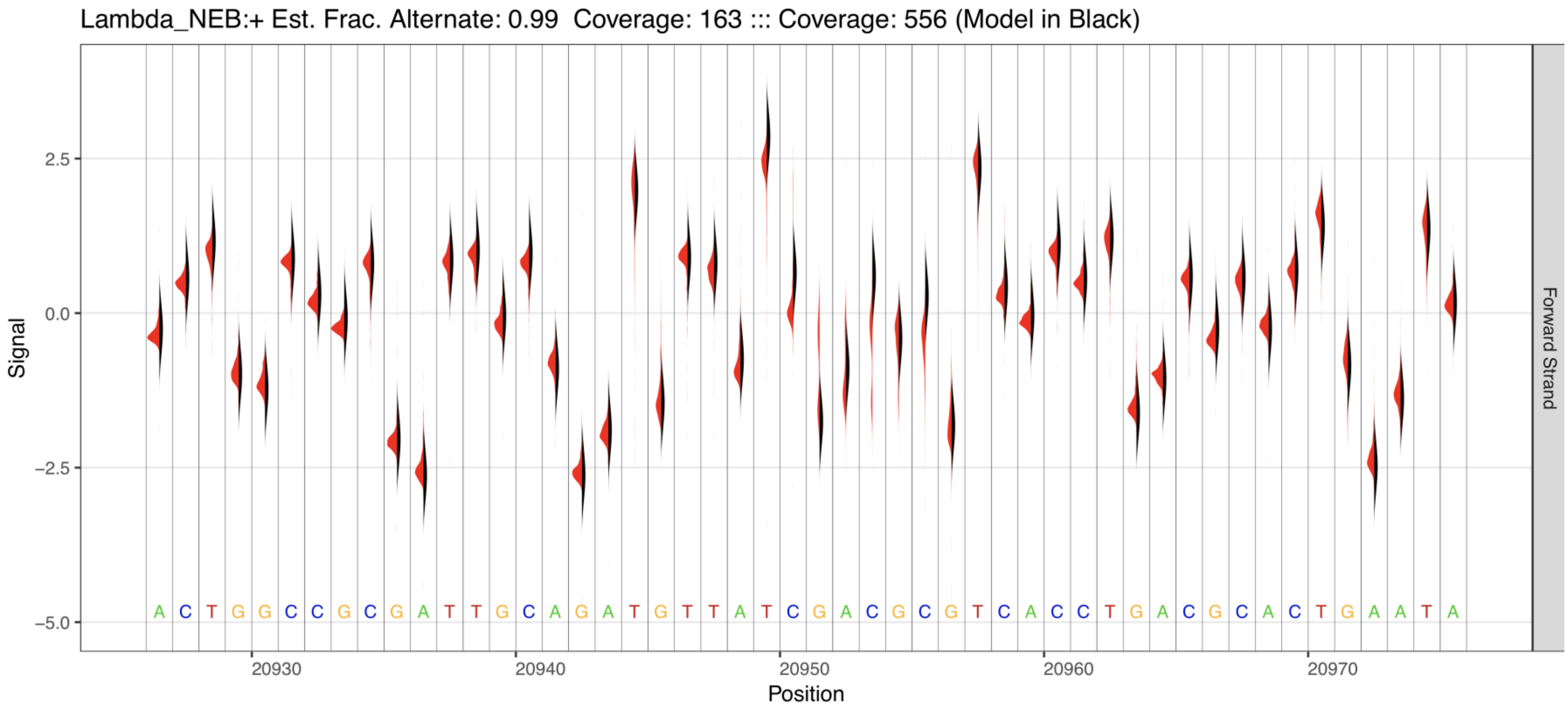
Species	Assay	#	Mapping	Base Contexts	Reads	Total Bases	Mean Read Length	Median Read Length
TaqI + HexynNH$_2$								
Lambda_NEB	**alkylated DNA**	2018_12_13_0h_Diamide_Lithu_TaqI_Hey-Lambda	**Tombo 1.3 denovo**	**CG-only**	31,857	139,682,998	4,385	2,998
Lambda_NEB	**alkylated DNA**	2018_12_13_0h_Diamide_Lithu_TaqI_Hey-Lambda	**Tombo 1.3 denovo**	**GC-only**	31,857	139,971,785	4,394	3,002
Lambda_NEB	**alkylated DNA**	2018_12_13_0h_Diamide_Lithu_TaqI_Hey-Lambda	**Tombo 1.3 denovo**	**T-only**	31,857	140,686,793	4,416	3,023
Lambda_NEB	**alkylated DNA**	2018_12_13_0h_Diamide_Lithu_TaqI_Hey-Lambda	**Tombo 1.3 denovo**	**all**	31,857	140,816,187	4,420	3,027
Lambda_NEB	**alkylated DNA**	2018_12_13_0h_Diamide_Lithu_TaqI_Hey-Lambda	**Tombo 1.3 denovo**	**m6A-GC-CG-only**	31,857	140,781,227	4,419	3,026
Lambda_NEB	**alkylated DNA**	2018_12_13_0h_Diamide_Lithu_TaqI_Hey-Lambda	**Tombo 1.3 denovo**	**m6A-only**	31,857	140,683,923	4,416	3,022
TaqI + CH$_3$								
Lambda_NEB	**alkylated DNA**	2019_01_16_60min_Diamide_Lithu_TaqI_Methyl	**Tombo 1.3 denovo**	**CG-only**	2,909	16,978,041	5,836	3,861
Lambda_NEB	**alkylated DNA**	2019_01_16_60min_Diamide_Lithu_TaqI_Methyl	**Tombo 1.3 denovo**	**GC-only**	2,909	17,000,315	5,844	3,865
Lambda_NEB	**alkylated DNA**	2019_01_16_60min_Diamide_Lithu_TaqI_Methyl	**Tombo 1.3 denovo**	**T-only**	2,909	17,064,980	5,866	3,897
Lambda_NEB	**alkylated DNA**	2019_01_16_60min_Diamide_Lithu_TaqI_Methyl	**Tombo 1.3 denovo**	**all**	2,909	17,076,734	5,870	3,901
Lambda_NEB	**alkylated DNA**	2019_01_16_60min_Diamide_Lithu_TaqI_Methyl	**Tombo 1.3 denovo**	**m6A-only**	2,909	17,064,555	5,866	3,894
TaqI + Butyn								
Lambda_NEB	**alkylated DNA**	2019_01_29_Lambda_Butyl	**Tombo 1.3 denovo**	**CG-only**	26,414	82,919,697	3,139	3,412
Lambda_NEB	**alkylated DNA**	2019_01_29_Lambda_Butyl	**Tombo 1.3 denovo**	**GC-only**	26,414	82,170,164	3,111	3,375
Lambda_NEB	**alkylated DNA**	2019_01_29_Lambda_Butyl	**Tombo 1.3 denovo**	**T-only**	26,414	83,287,319	3,153	3,421
Lambda_NEB	**alkylated DNA**	2019_01_29_Lambda_Butyl	**Tombo 1.3 denovo**	**all**	26,414	83,440,725	3,159	3,425
Lambda_NEB	**alkylated DNA**	2019_01_29_Lambda_Butyl	**Tombo 1.3 denovo**	**m6A-only**	26,414	83,336,353	3,155	3,423

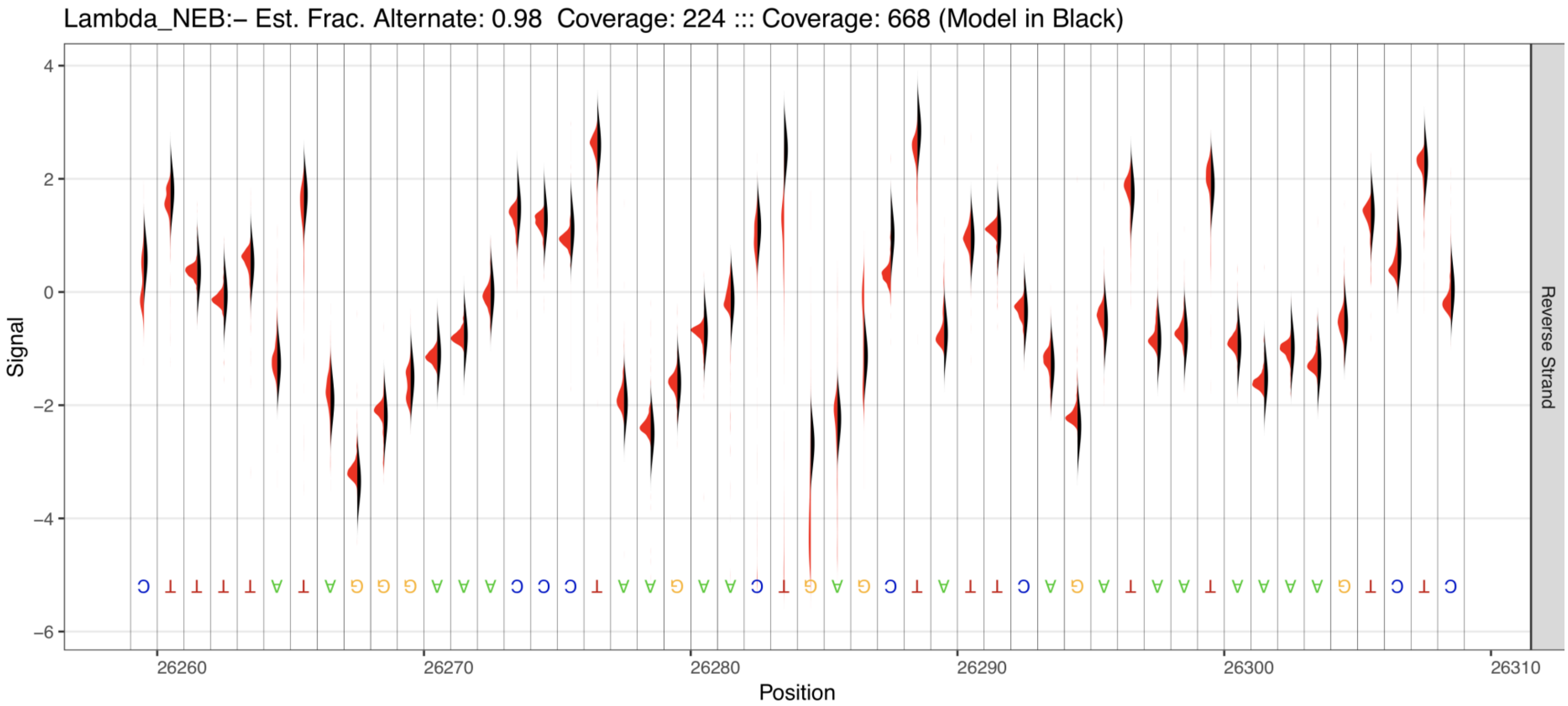
TAQI HexynNH$_2$ RESULTS

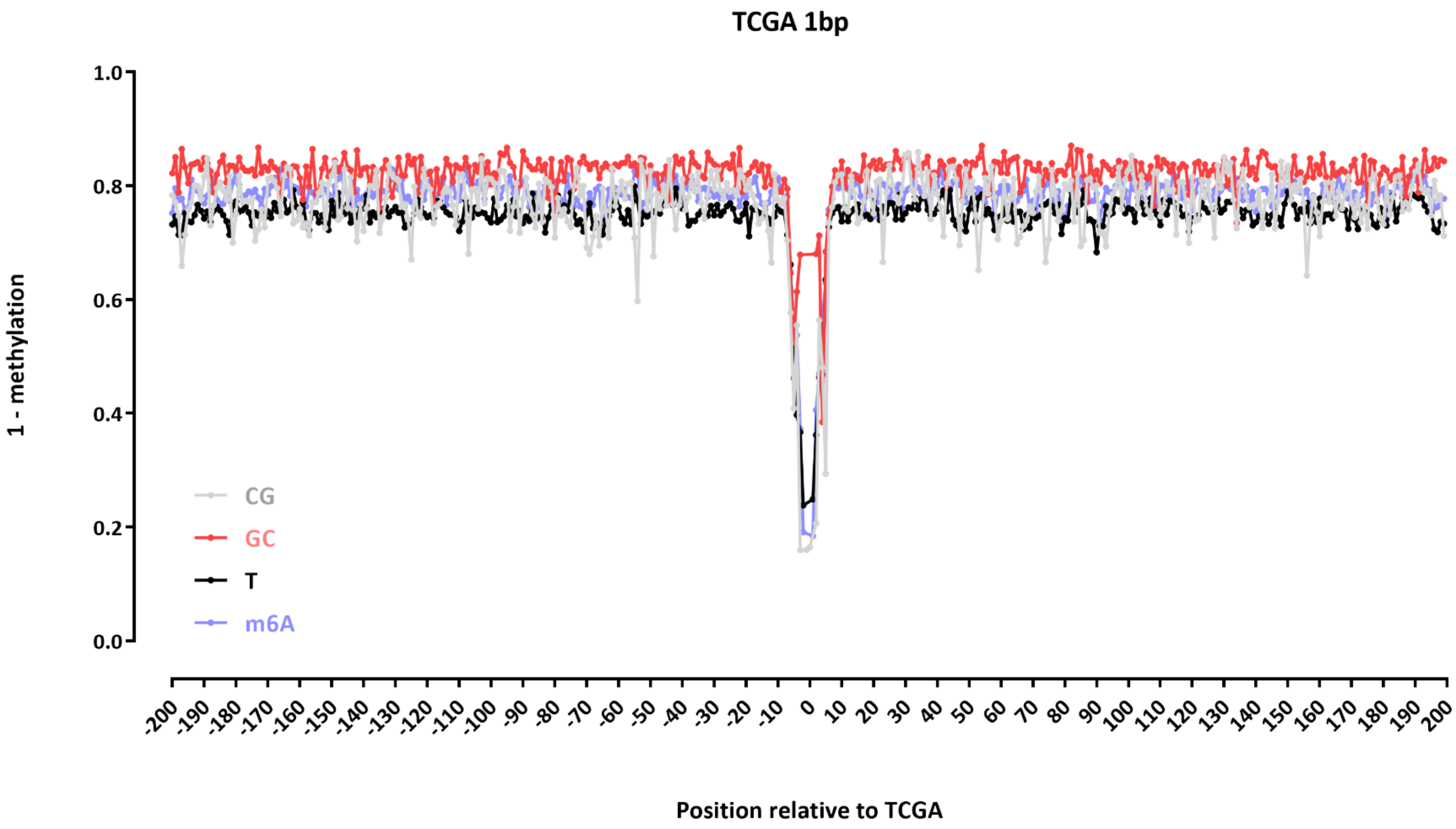
- High coverage (559X on the Plus strand and 619X on the Minus strand) with two TCGA sites
- Shown are the distributions of the Nanopore signal for each base. Most bases have a very tight distribution, the 2-3 bases around the A of TCGA have a bimodal distribution.
- We don't know if the bimodal distribution comes from less then 100% enzymatic efficiency of TaqI or bad base calling



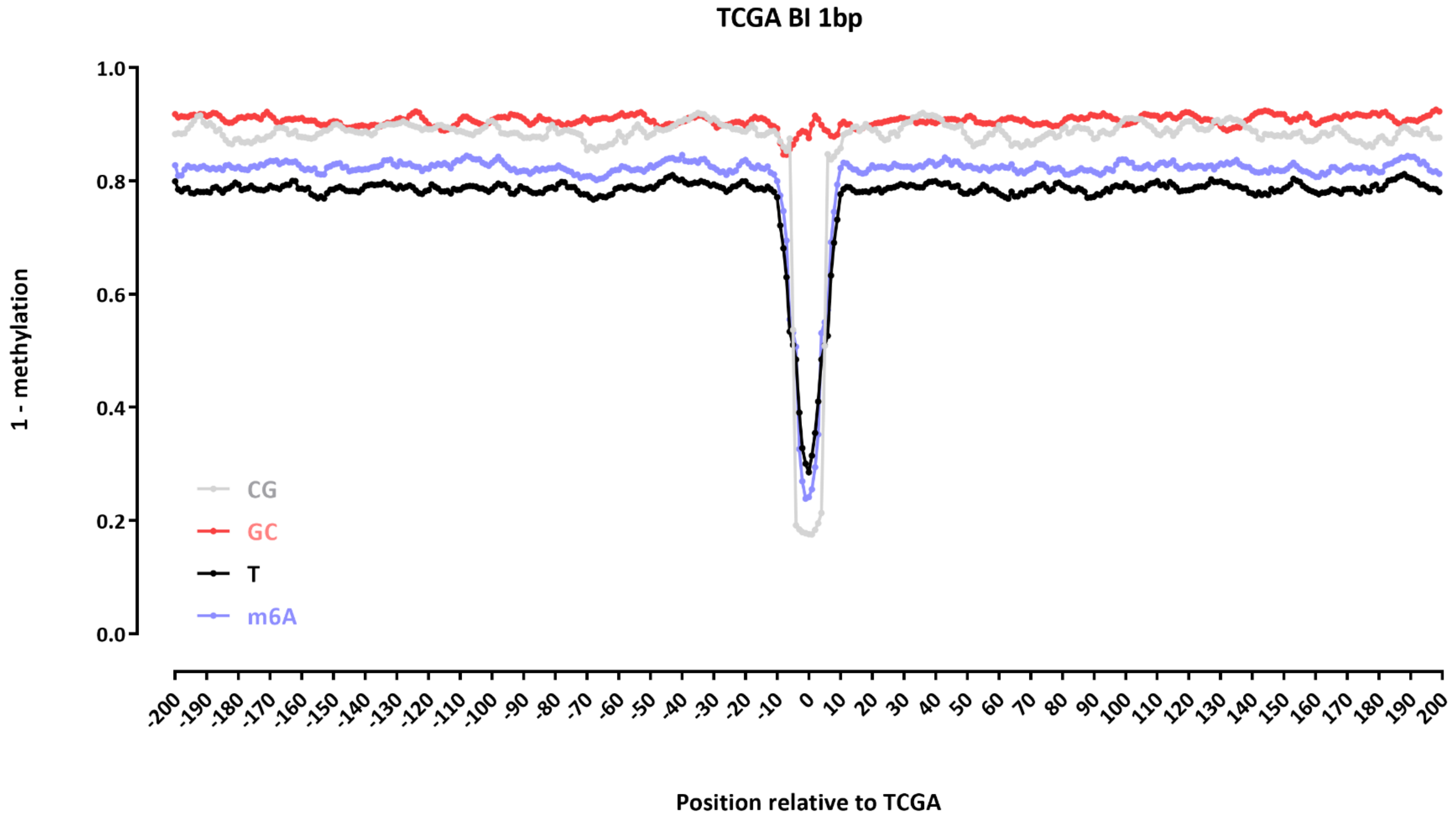
- Here we are looking at the distribution of signal for a high coverage region that has a TCGA motif in it. But now we compare the distribution to the model (the more off the model, the more modified the base is called)
- The modified A causes a big shift in the signal for the bases around it


Lambda_NEB:+ Est. Frac. Alternate: 0.99 Coverage: 163 ::: Coverage: 556 (Model in Black)

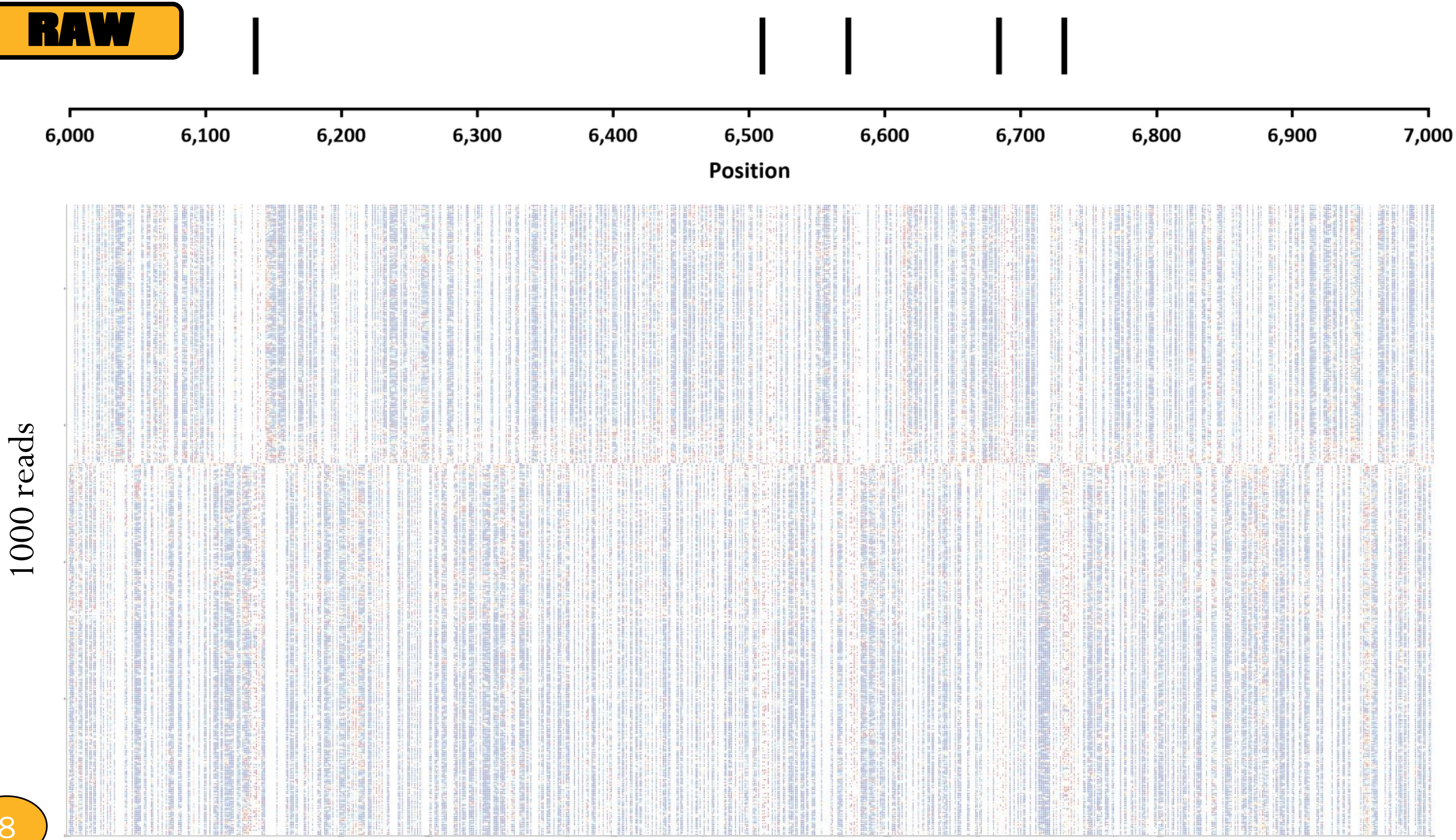

Lambda_NEB:– Est. Frac. Alternate: 0.98 Coverage: 224 ::: Coverage: 668 (Model in Black)
Signal
4
2
0
−2
−4
−6
Position
26260
26270
26280
26290
26300
26310
Reverse Strand
C T T T T A T A G G G A A A C C C T A A G A A C T G A G C T A T T C A G A T A A T A A A A G T C T C

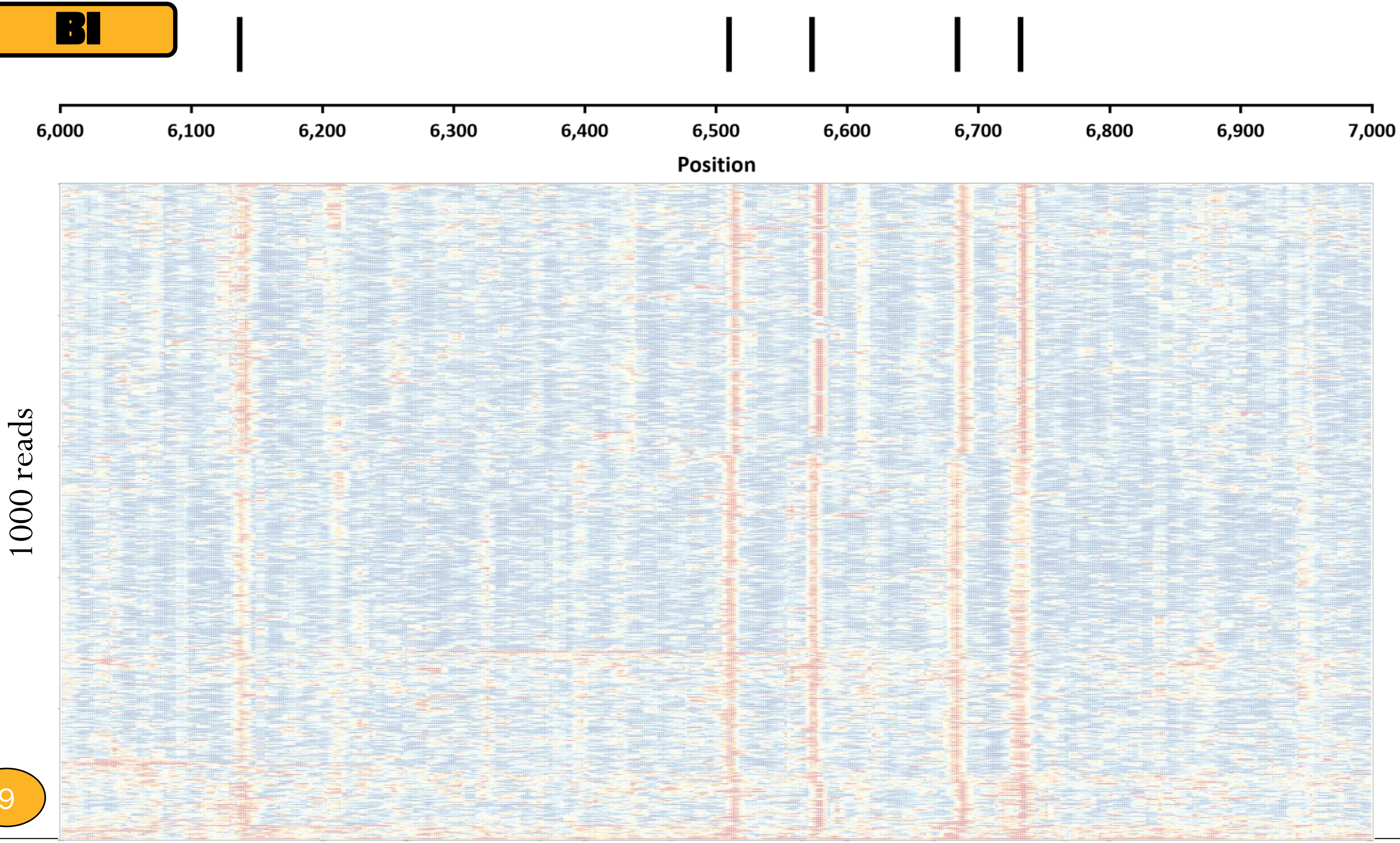

TCGA 1bp
1 - methylation
1.0
0.8
0.6
0.4
0.2
0.0
CG
GC
T
m6A
-200
-190
-180
-170
-160
-150
-140
-130
-120
-110
-100
-90
-80
-70
-60
-50
-40
-30
-20
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
Position relative to TCGA

- “BI” means “Bayesian integration”; we aggregate signal across neighboring bases using a Bayesian procedure to obtain more reliable calls of accessibility (in the context of chromatin). This is done over 10bp windows for every base in the sequence

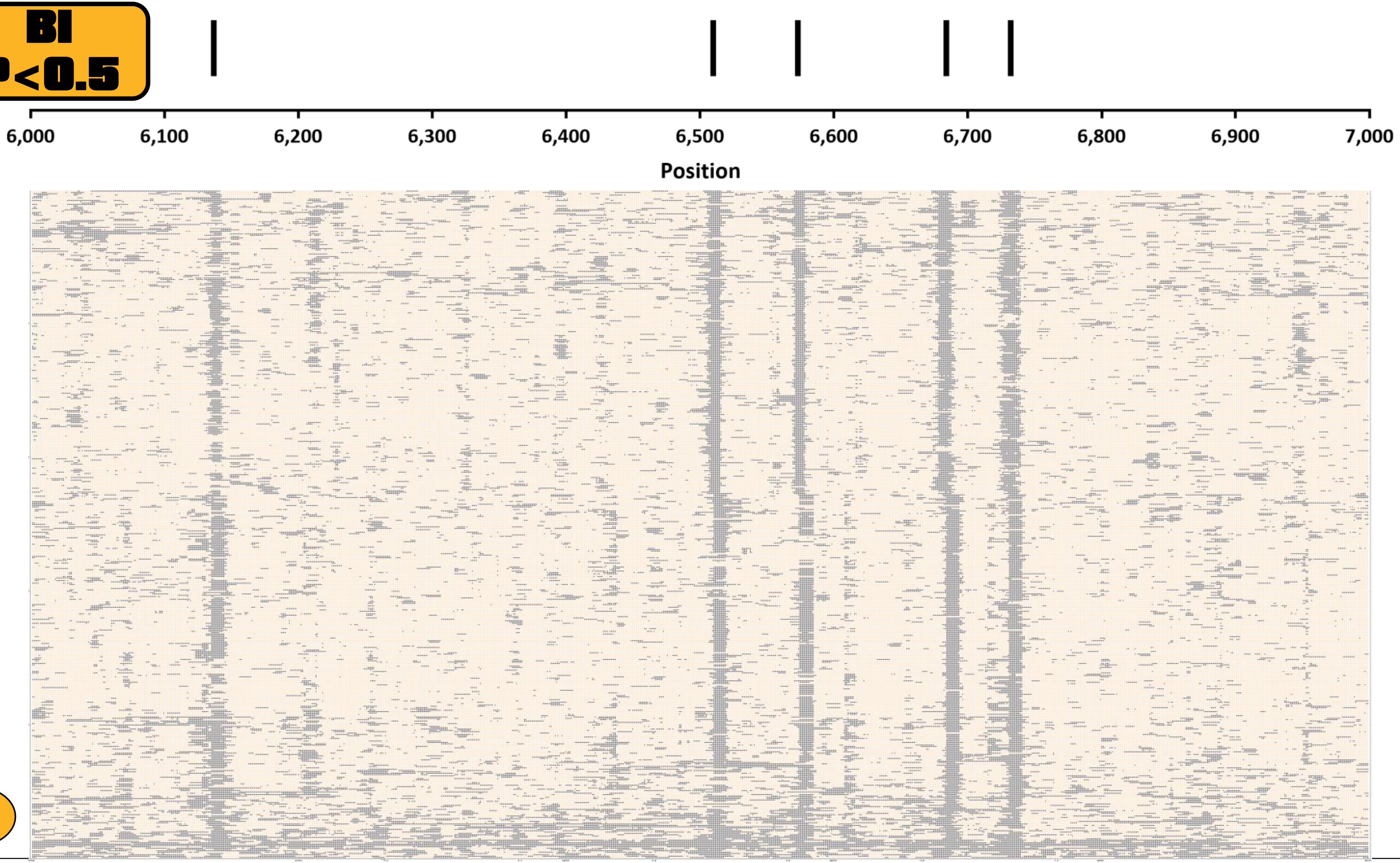


RAW

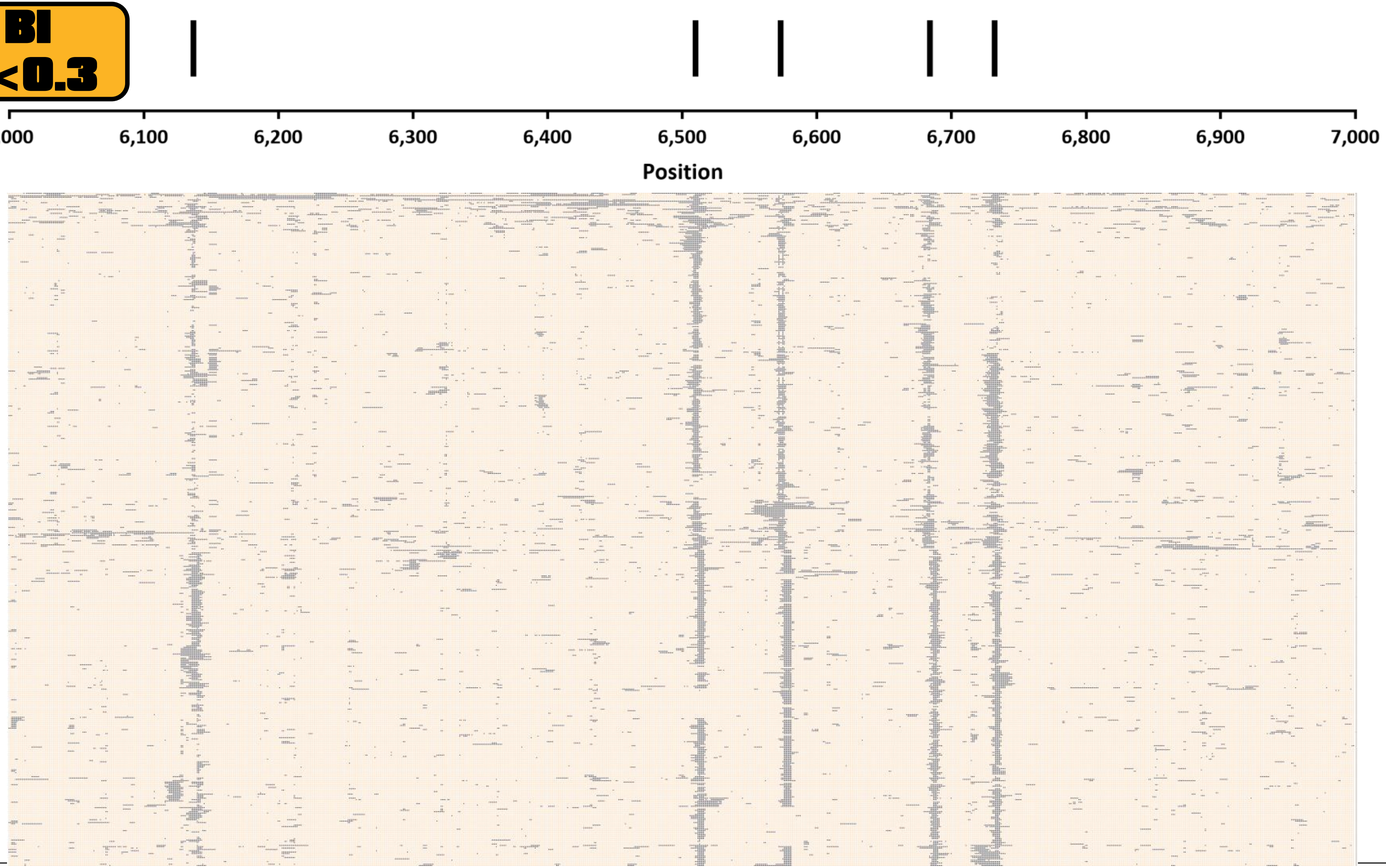

6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position
1000 reads


6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position
1000 reads

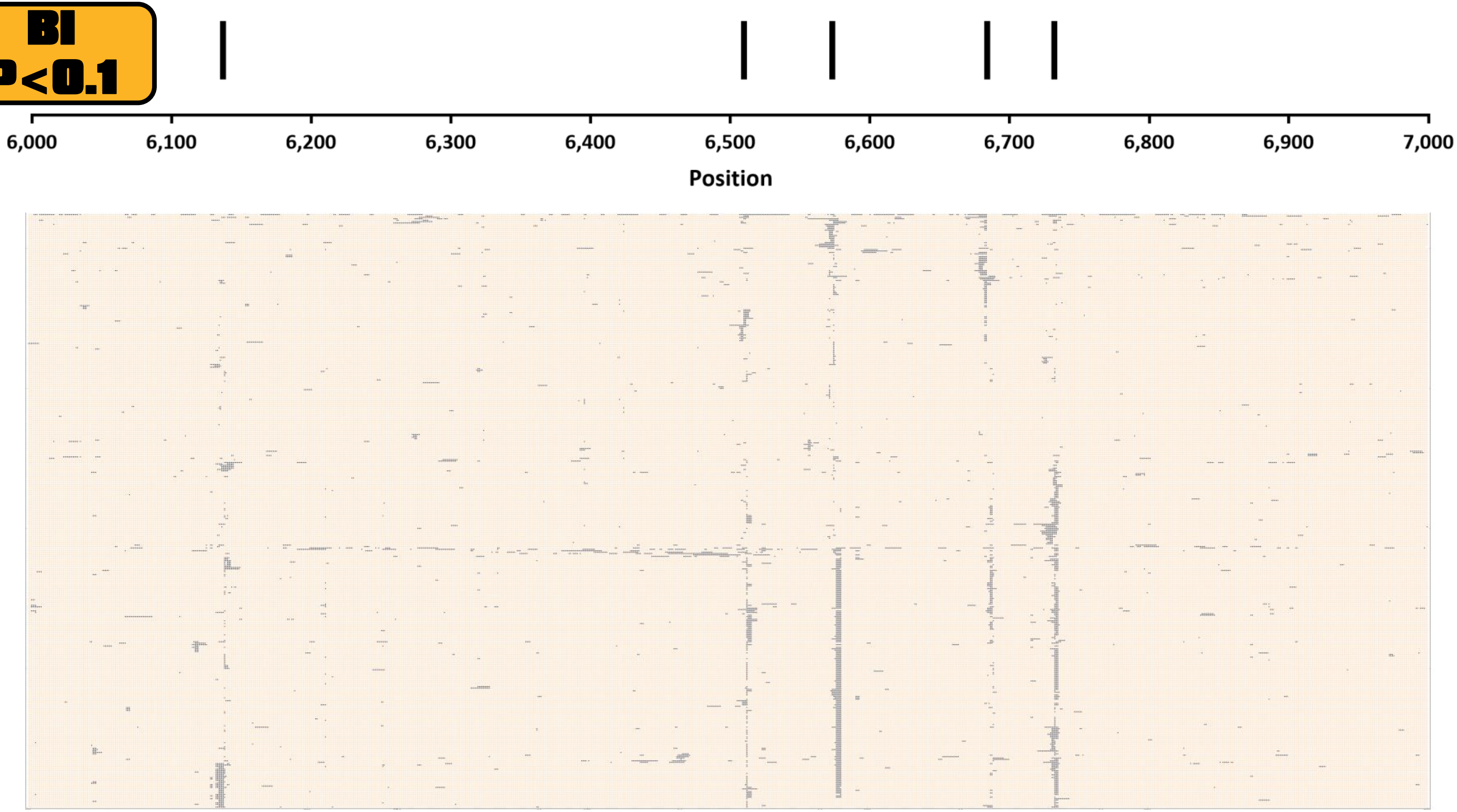
BI
P<0.5


6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position

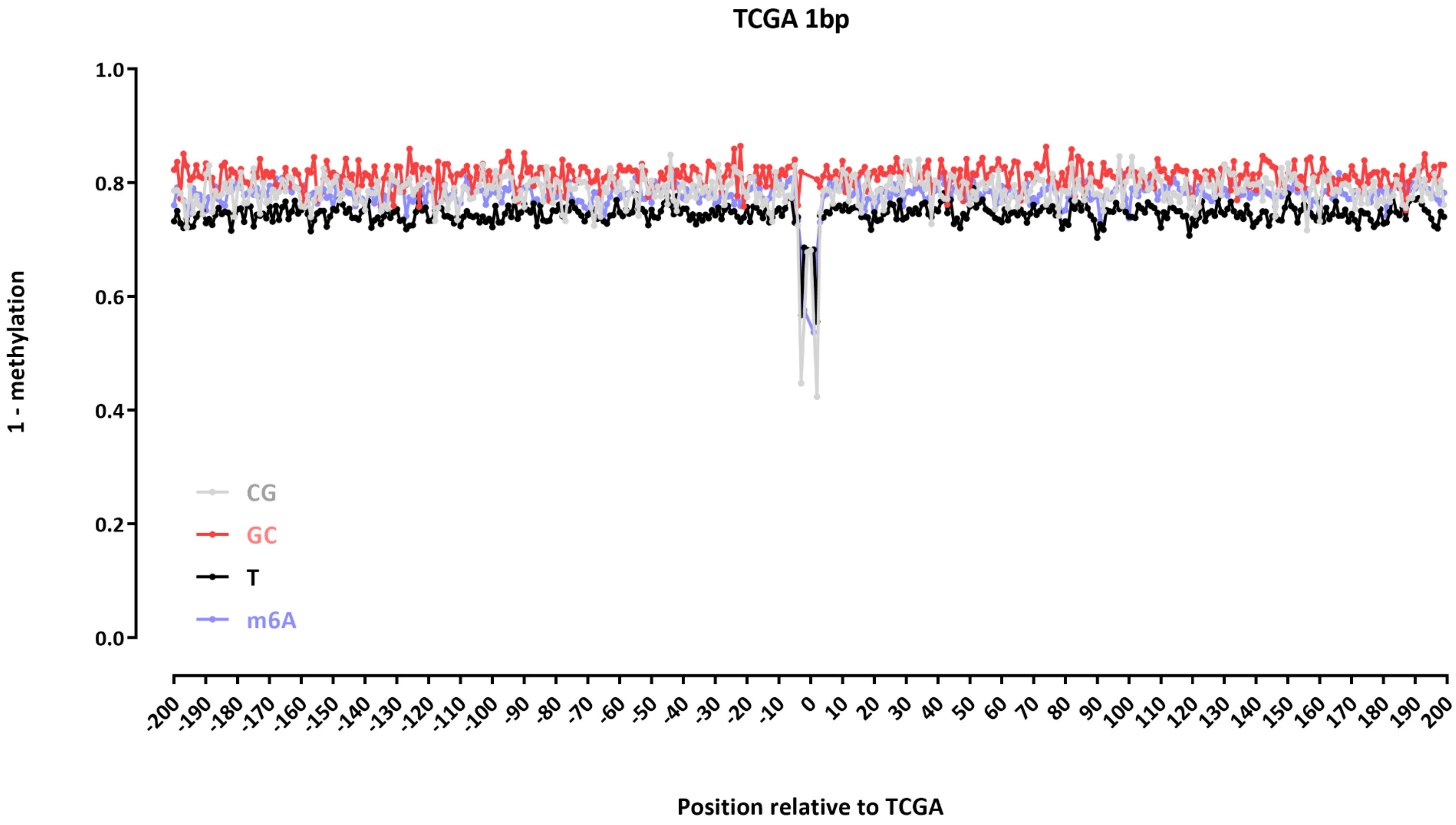
BI
P<0.3


6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position

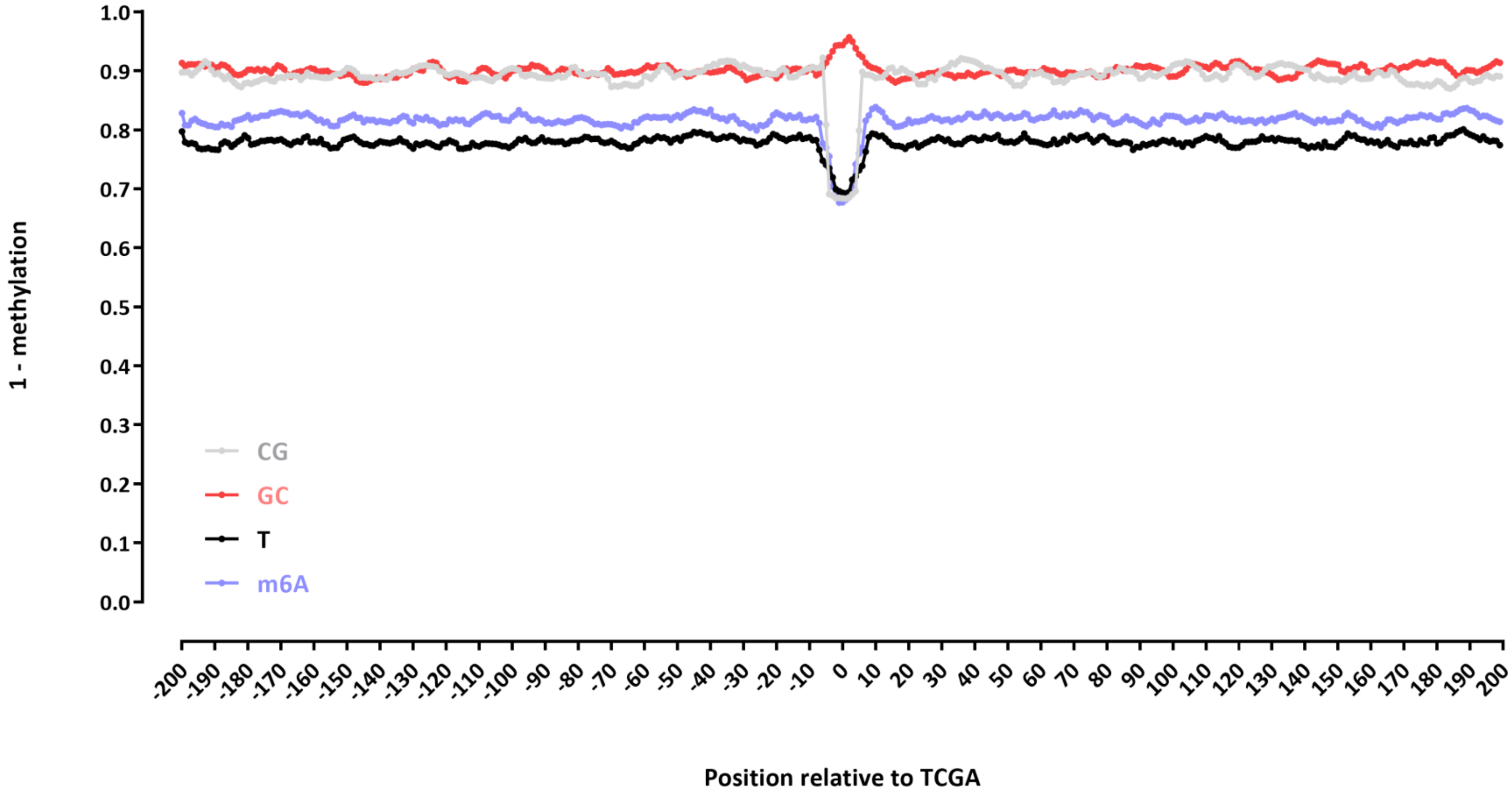
BI
P<0.1


6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position

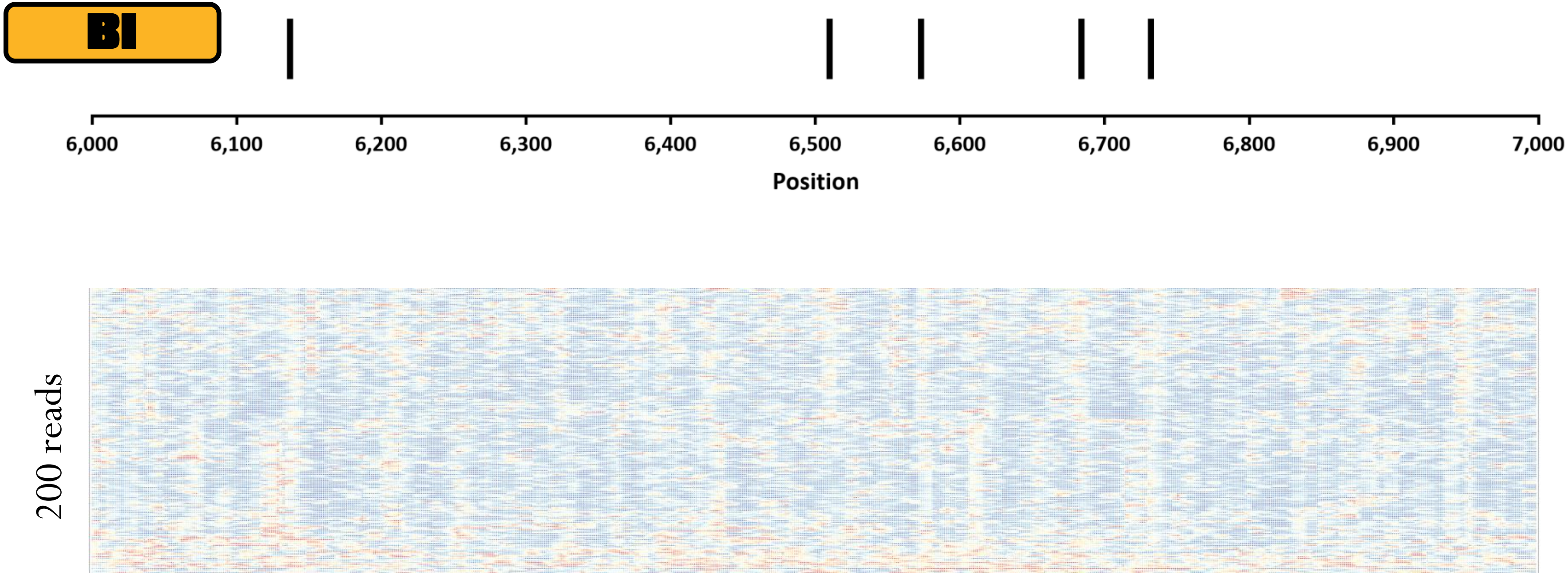
TAQI CH_3 RESULTS



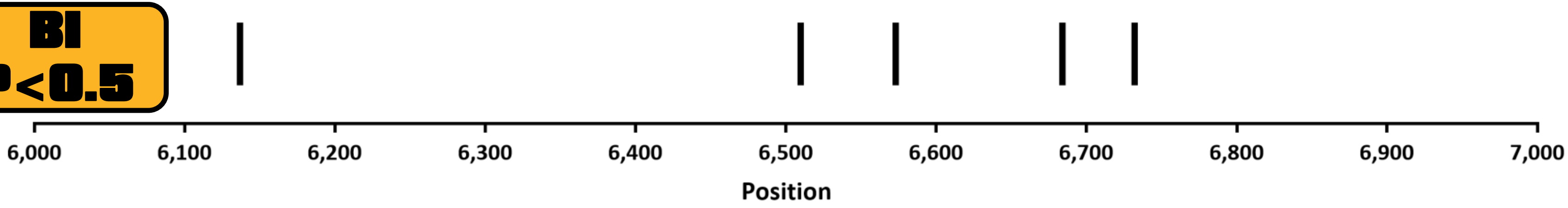
TCGA BI 1bp

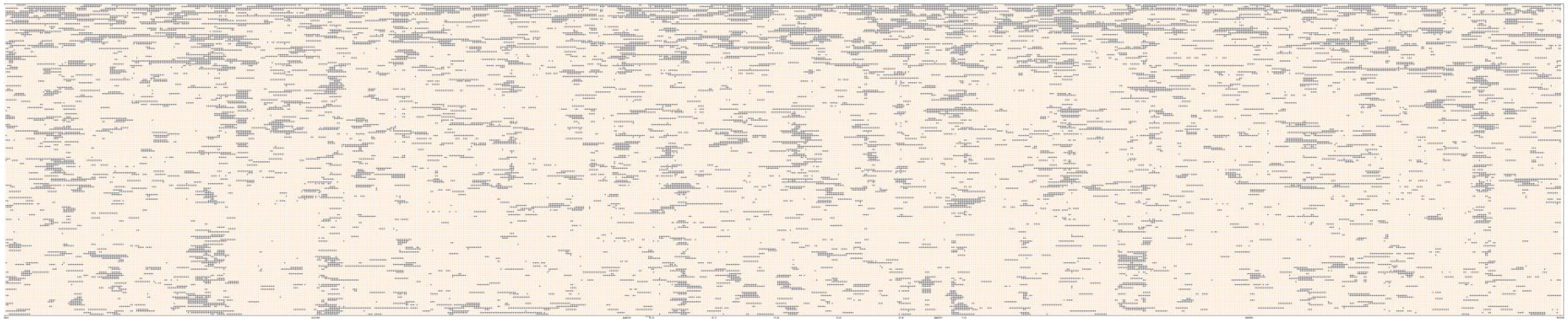

1 - methylation
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CG
GC
T
m6A
-200
-190
-180
-170
-160
-150
-140
-130
-120
-110
-100
-90
-80
-70
-60
-50
-40
-30
-20
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
Position relative to TCGA

BI

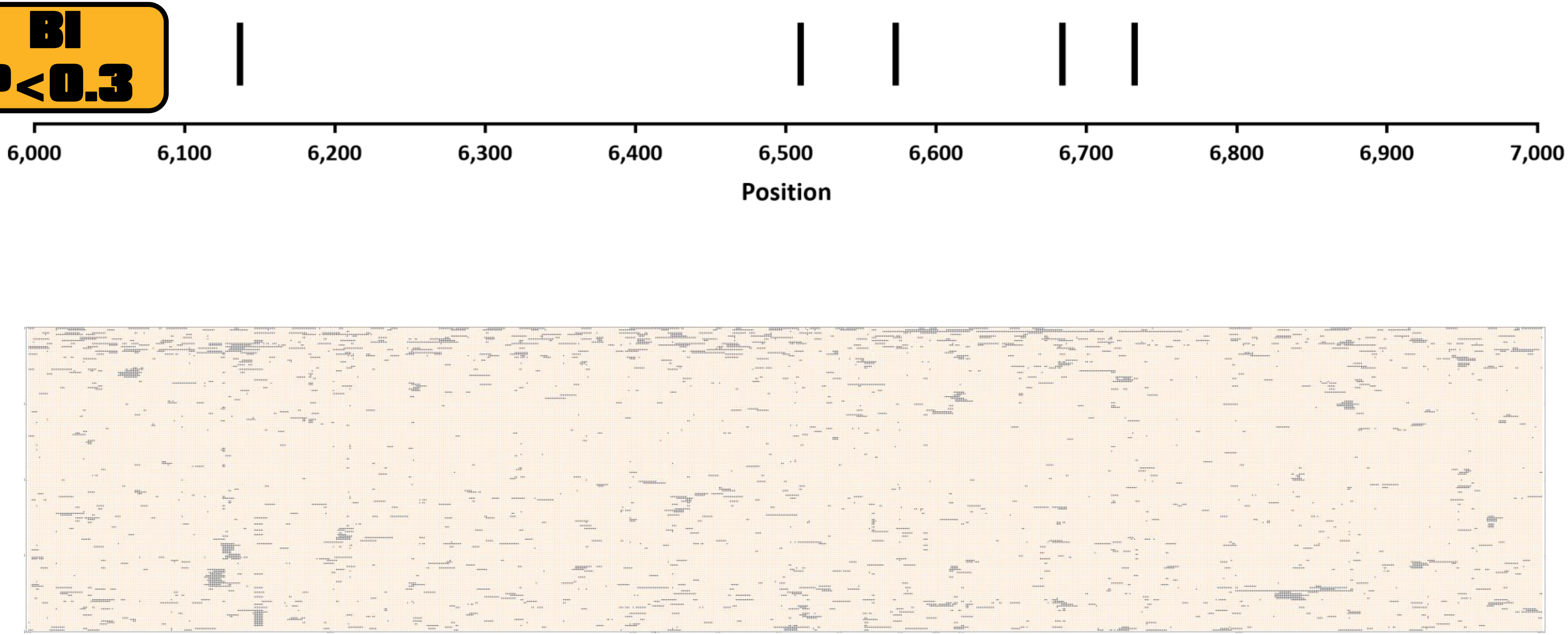

6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position
200 reads

BI
$P<0.5$

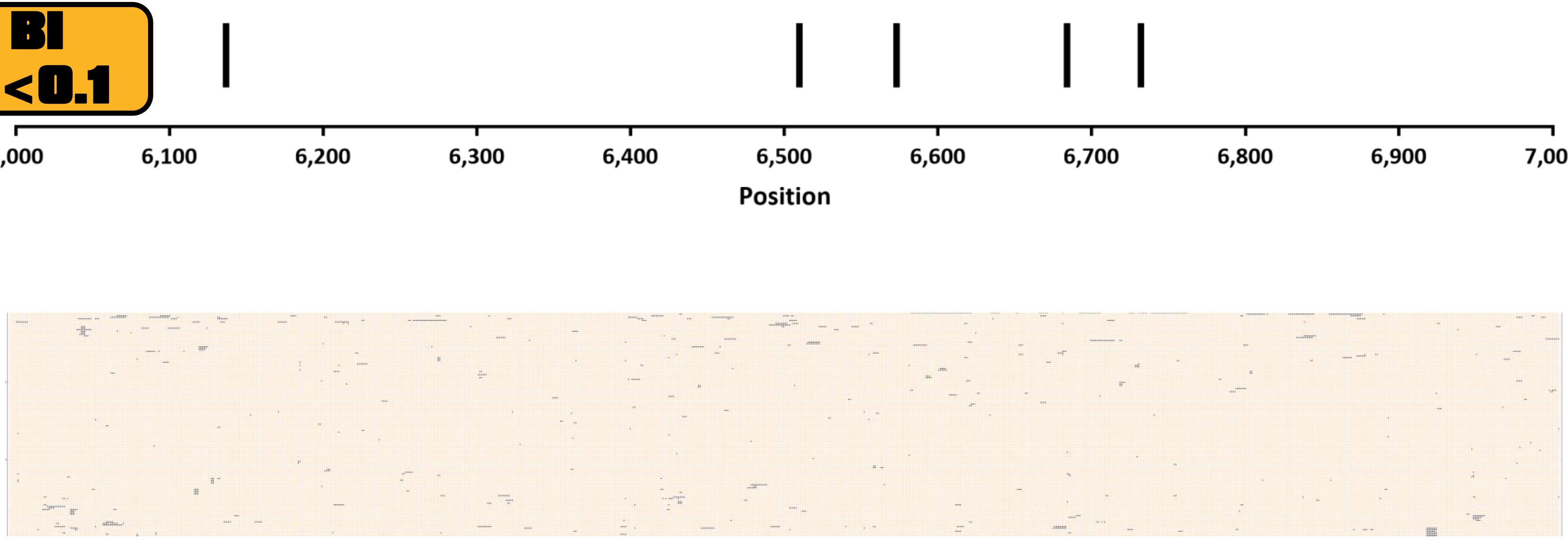

6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position



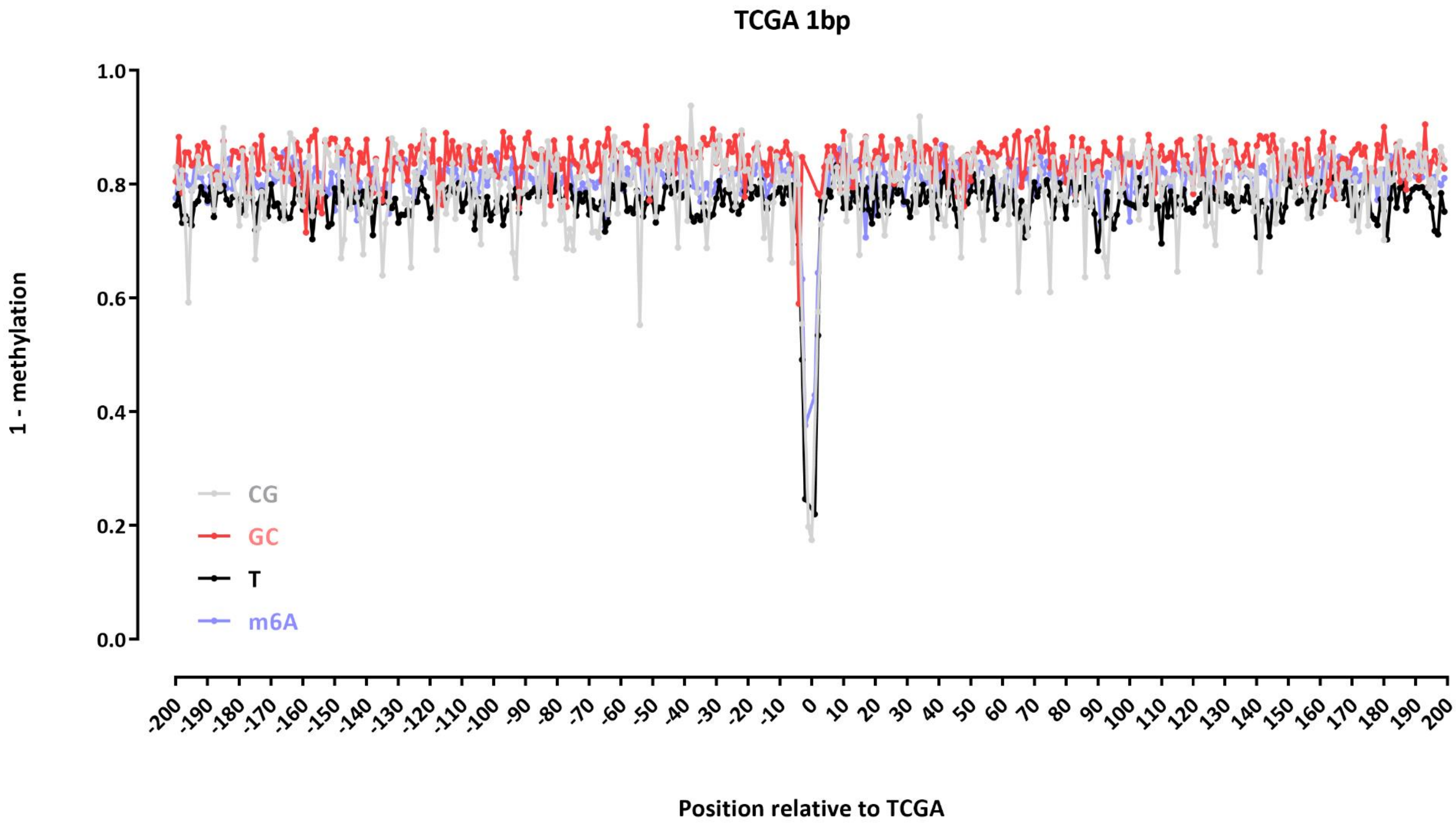
BI
P<0.3

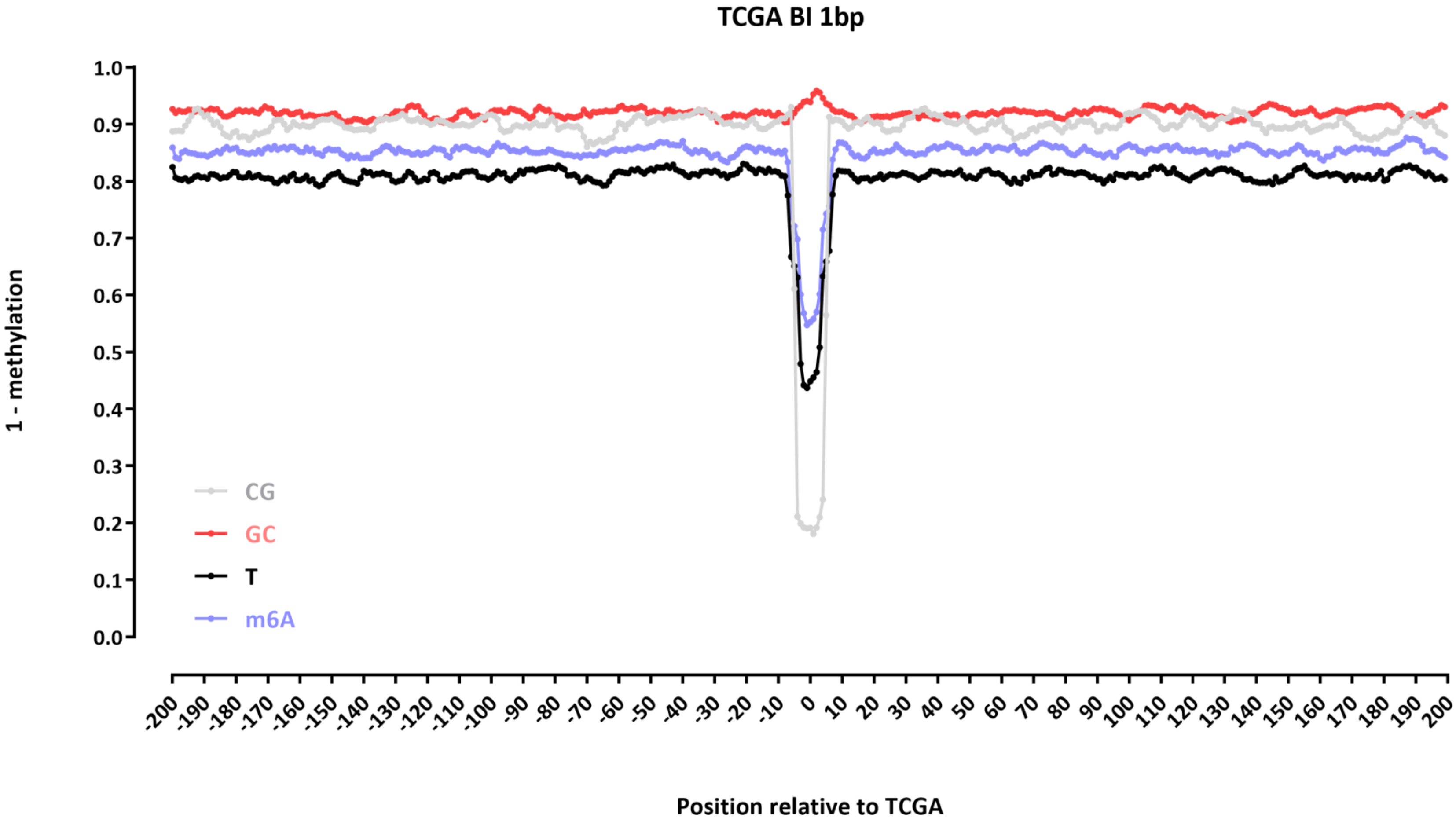

6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position

BI
P<0.1

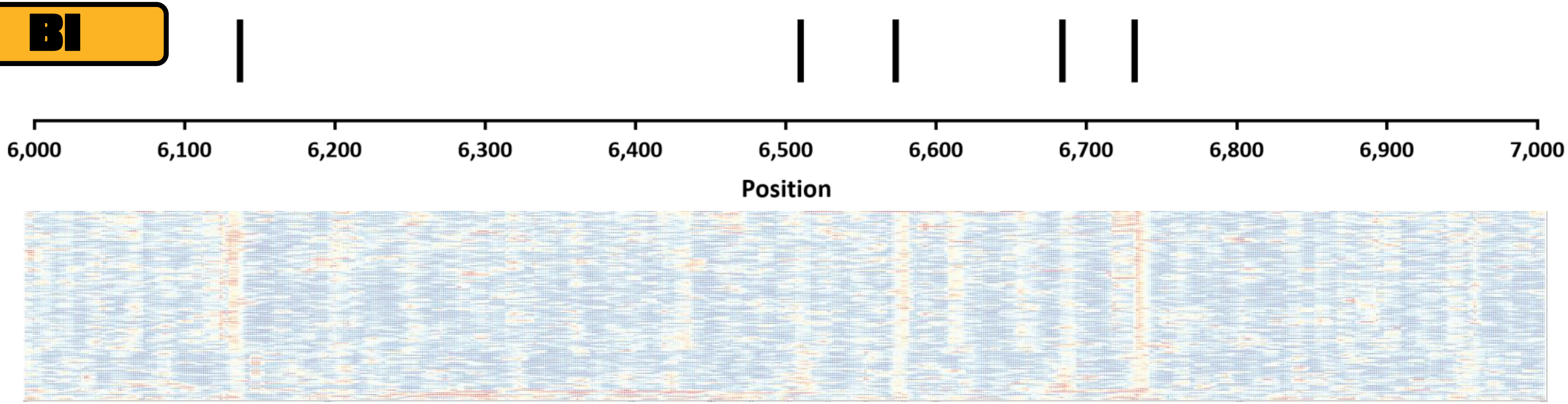

6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position

TAQI Butyn RESULTS

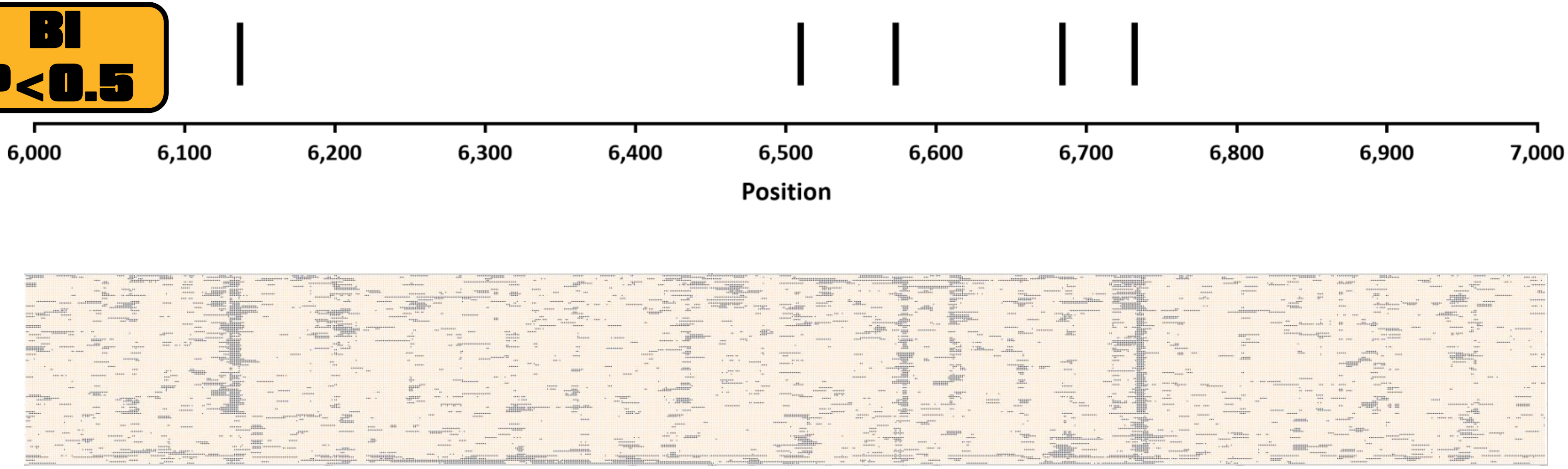



TCGA BI 1bp
1 - methylation
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CG
GC
T
m6A
-200
-190
-180
-170
-160
-150
-140
-130
-120
-110
-100
-90
-80
-70
-60
-50
-40
-30
-20
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
Position relative to TCGA

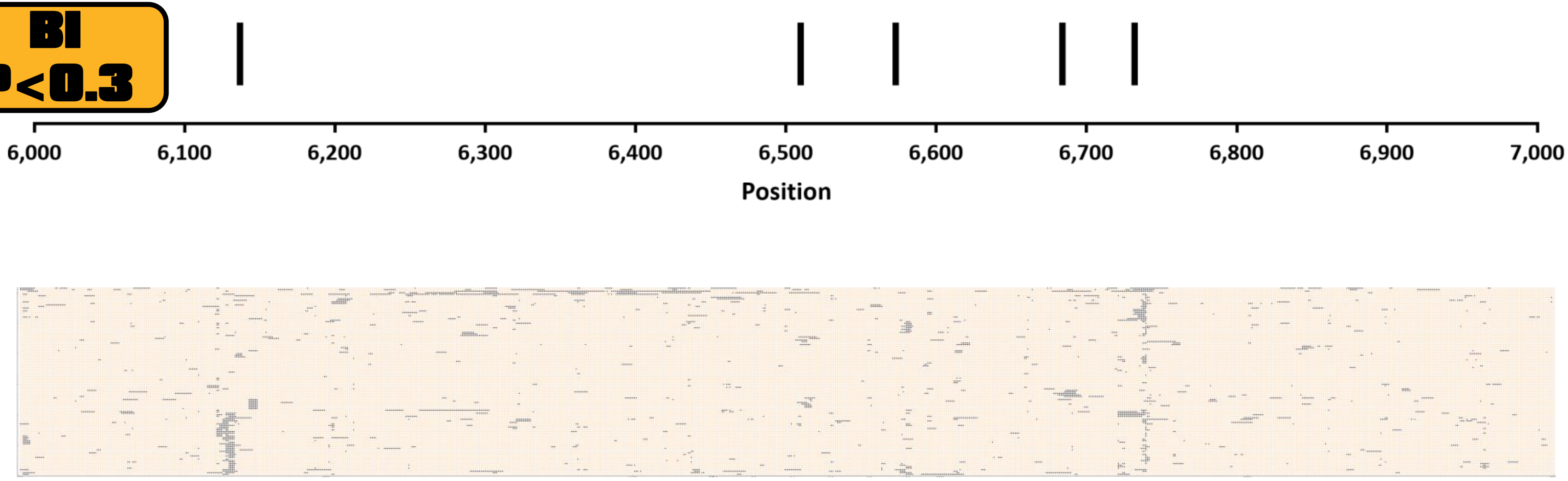
BI


6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position

BI
P<0.5


6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position

BI
$P<0.3$


6,000
6,100
6,200
6,300
6,400
6,500
6,600
6,700
6,800
6,900
7,000
Position