Supplementary Material

Gyorgy B et al. Allele-specific gene editing prevents deafness in a model of dominant progressive hearing loss

Supplementary Notes

Supplementary Note 1. Correlating indel frequencies with AAV integration, we found the mean indel frequency of injected *Tmc1*$^{Bth/WT}$ animals (P42-P55) was 1.88% and this corresponded to a 0.4% AAV integration rate. In contrast, we estimated AAV integration in the WT allele was 0.0016%, corresponding to 0.0075% indel rate in the WT allele, which is much lower than the background indel frequency estimated from next-generation sequencing, which was found to be 0.05-0.06%. At 42-55 days after injection, the specificity of SaCas9-KKH was ~250-fold greater for the *Tmc1*^{Bth} allele than for the *Tmc1*^{WT} allele.

Supplementary Note 2. *In vivo*, we observed modest genomic editing (1-2%). For this assay we sequenced DNA harvested from whole cochleas, a small fraction of which includes sensory hair cells. Since the vector capsid we used preferentially targets cochlear hair cells[17], we suspect the apparent modest gene editing was due to sampling of DNA from non-transduced non-hair cells. When we sequenced *Tmc1* mRNA, which is preferentially expressed in sensory hair cells, we found a ~24% decrease in *Bth* mRNA in injected cochleas, confirming editing was concentrated in hair cells. Indel formation was detectable at the mRNA level; however, the decrease in read counts was more robust, suggesting nonsense mediated mRNA decay[30]. We did not find a complete loss of the *Bth* mRNA, perhaps due to the inability of the vector capsid, Anc80, to efficiently target hair cells at the basal, high frequency end of the cochlea. Silencing the *Bth* allele preserved hair cells and low frequency hearing up to 40 weeks, the latest time point tested. ABR thresholds were in the range of WT levels in injected heterozygous animals at lower frequencies, but elevated at higher frequencies. Interestingly, we note that the ~24% decrease in *Bth* mRNA may have originated from the apical quarter of the cochlea, which encodes frequencies in the 5-12 kHz range, consistent with the excellent ABR thresholds at these frequencies.

Supplementary Note 3. We observed that the in several cases, the ClinVar database is not correctly annotated when marking the dominant nature of the disease. Several diseases that are clearly dominant are not annotated, thus they were filtered out from our 'dominant' filtered table (Supplementary Table 3). Therefore we also performed the targetable space analysis on all ClinVar database entries regardless of whether it is dominant, recessive or not annotated. Readers should consult with this table as well in case a dominant disease is not listed in the table with only dominant entries. This dataset is presented as Supplementary Table 9 (variants targetable with SaCas9-WT) and Supplementary Table 10 (variants targetable with SaCas9-KKH).

Supplementary References

30. Popp, M. W. & Maquat, L. E. Leveraging rules of nonsense-mediated mRNA decay for genome engineering and personalized medicine. Cell 165, 1319–1332 (2016).

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Supplementary Dataset Files

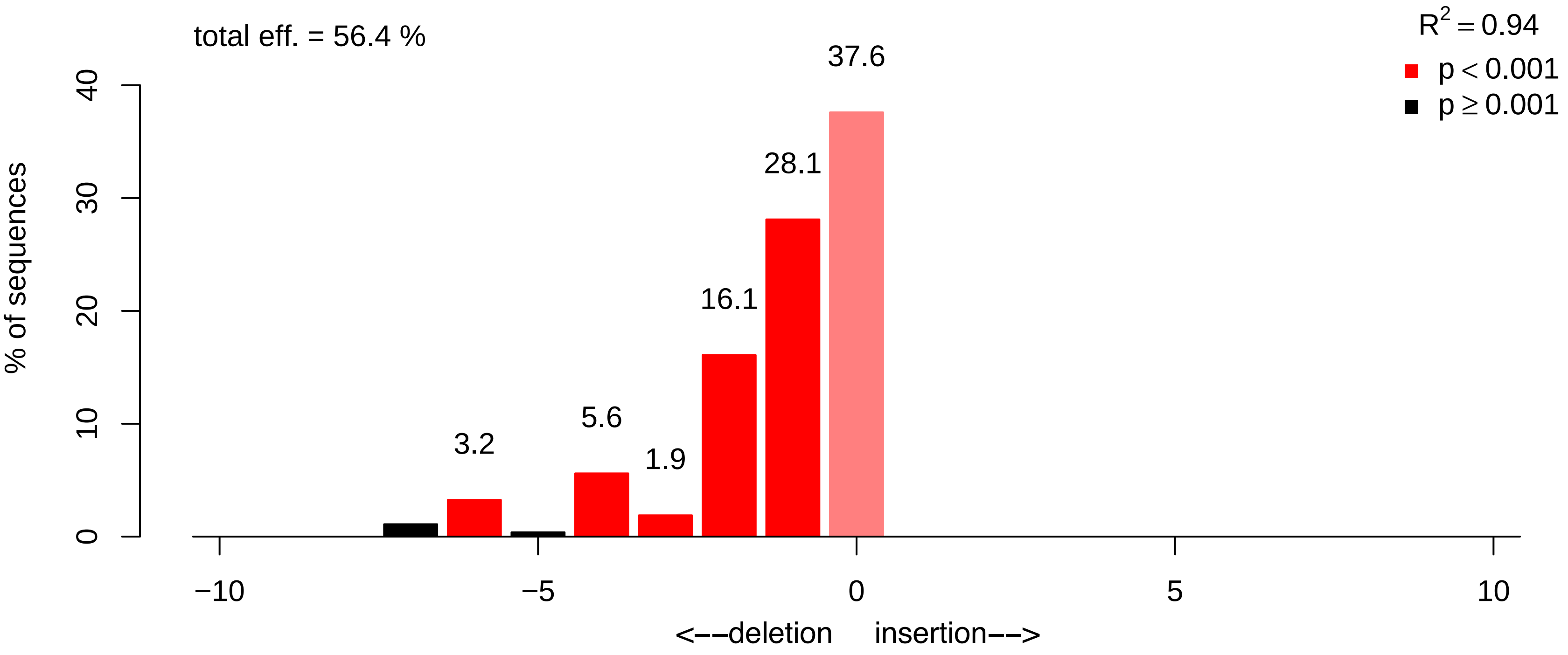
Supplementary Dataset 1. TIDE analysis of all Sanger sequencing samples from Extended Data Fig. 1. SpCas9 only (GFP) was used as a control for analysis (pages 1-96)

Supplementary Dataset 2. CRISPResso analysis on all samples from Extended Data Fig. 1e. In the case of *Tmc1*$^{Bth/WT}$ samples, reads were segregated to Bth and WT before CRISPResso analysis (pages 97-111)

Supplementary Dataset 3. CRISPResso analysis on all samples from Fig. 1b. In the case of *Tmc1*$^{Bth/WT}$ samples, reads were segregated to Bth and WT before CRISPResso analysis (pages 112-141)

Supplementary Dataset 4. Sequencing result from pBG201 empty vector (pAAV-CMV-NLS(SV40)-SaCas9(E782K/N968K/R1015H)-NLS(nucleoplasmin)-3xHA-bGHpA0-U6-BsaI-sgRNA). Plasmid DNA was sequences by next-generation sequencing using the Plasmid Sequencing Service of the MGH DNA Core (Cambridge, MA, USA) (pages 142-144).

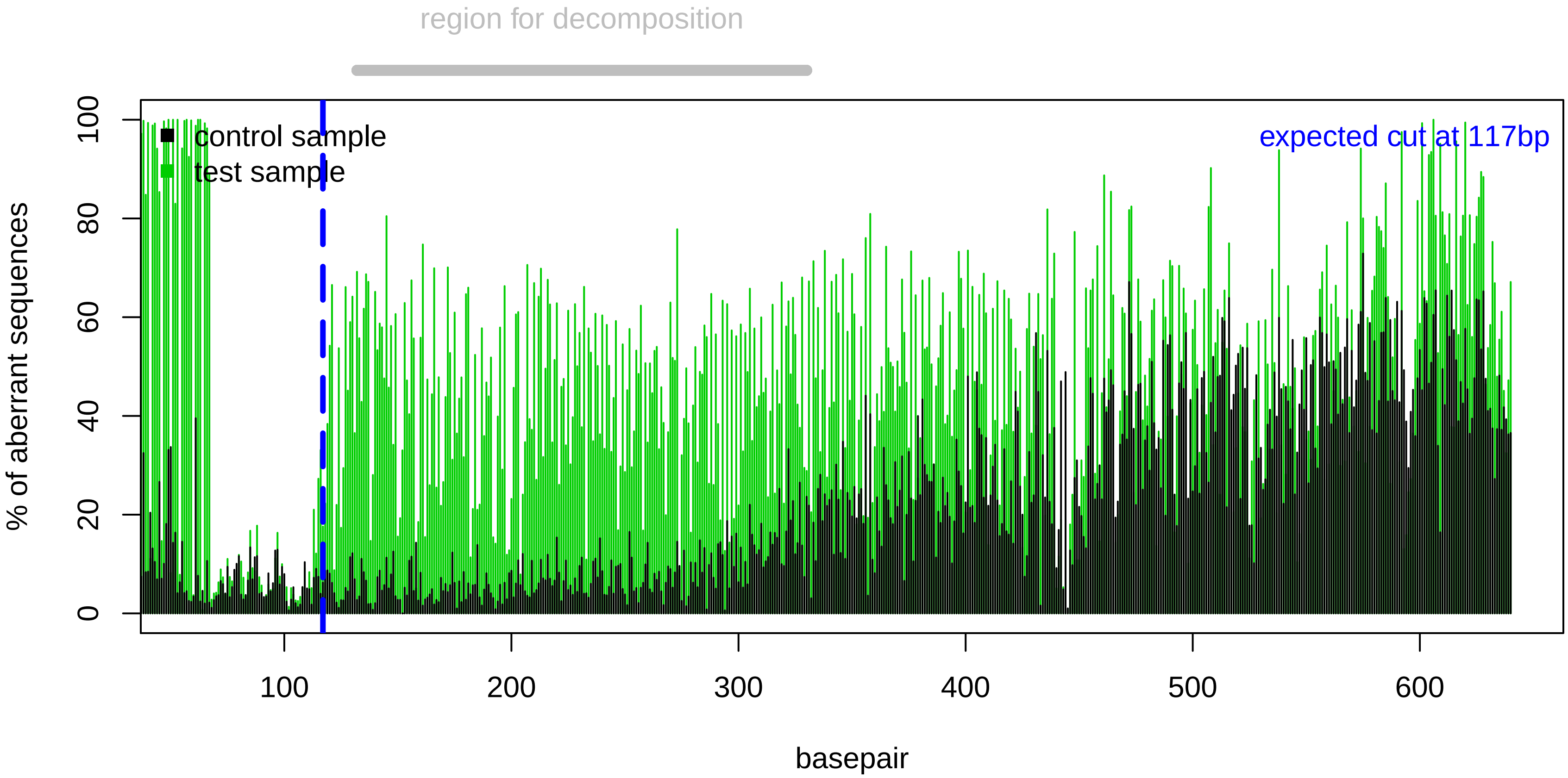
sample: WT/WT cell line, WT gRNA, reverse
guide: GGGTGGGACAGAACATCCCC



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

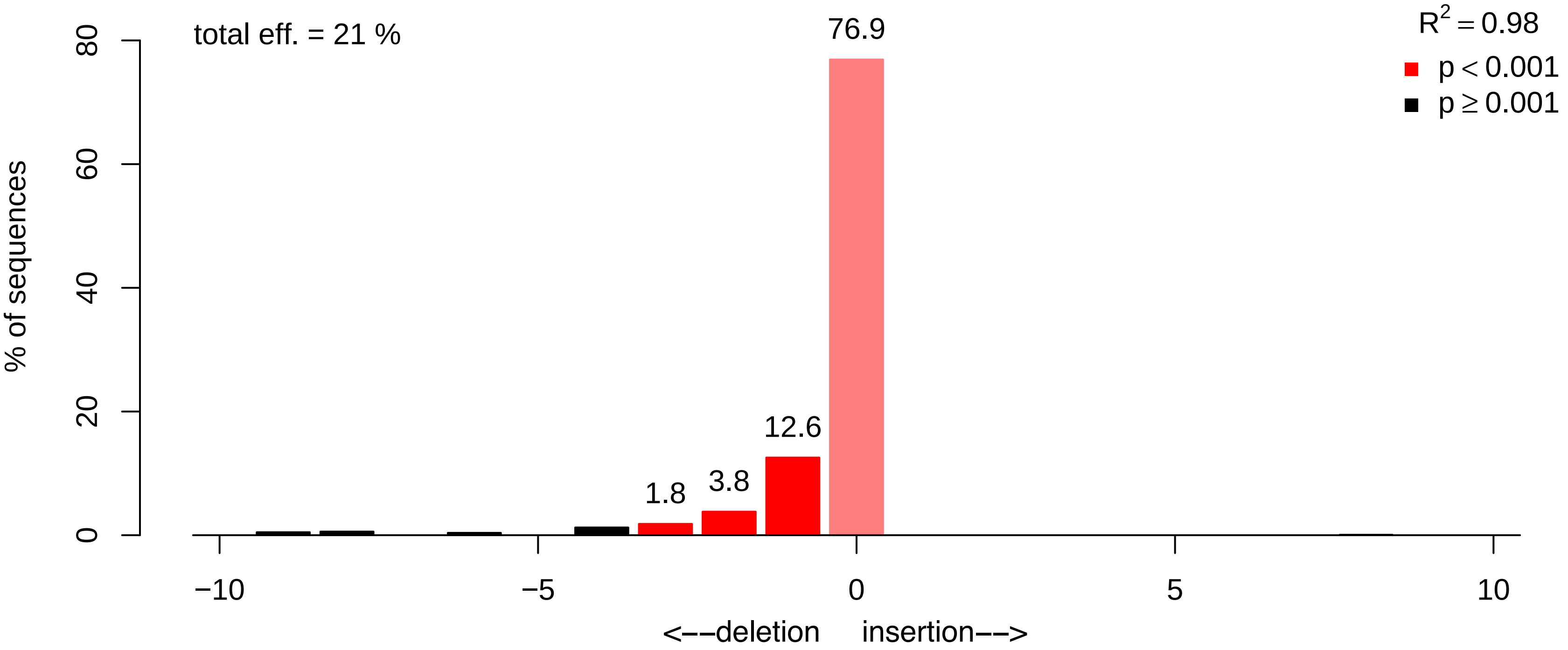
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: WT/WT cell line, WT gRNA, reverse
guide: GGGTGGGACAGAACATCCCC



Warning: left boundary of decomposition window was adjusted 132 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: WT/WT cell line, gRNA 1.1, reverse
guide: GGGTGGGACAGAACATCCCC



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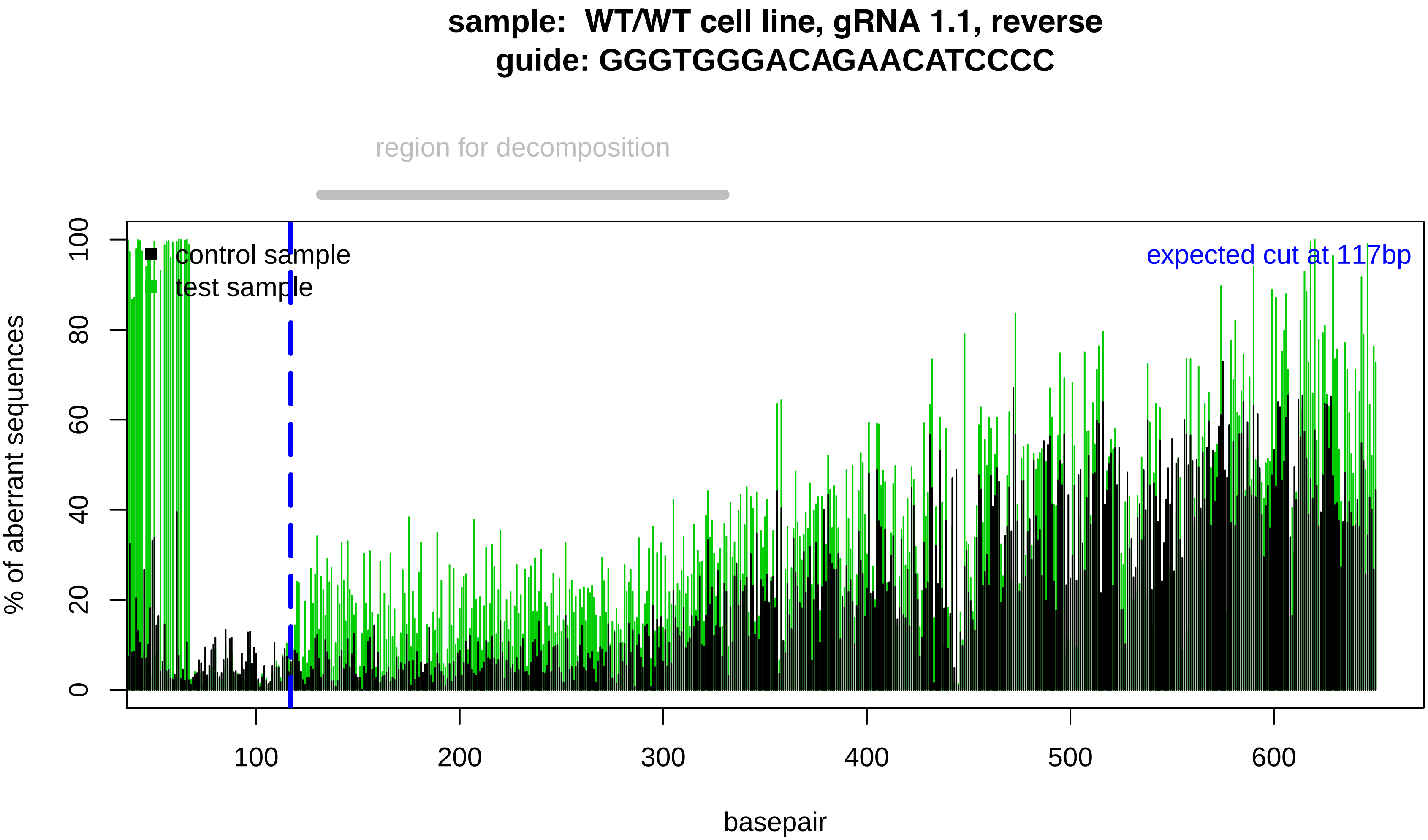

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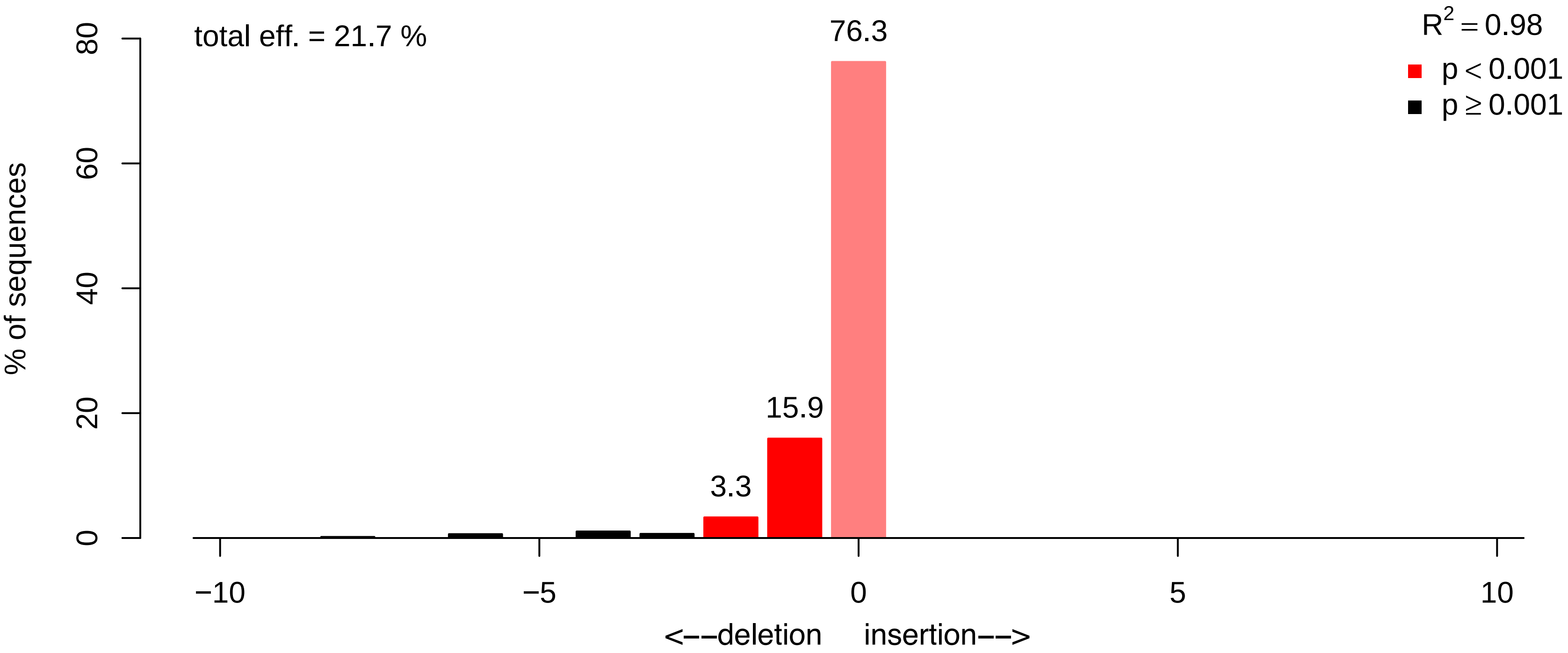

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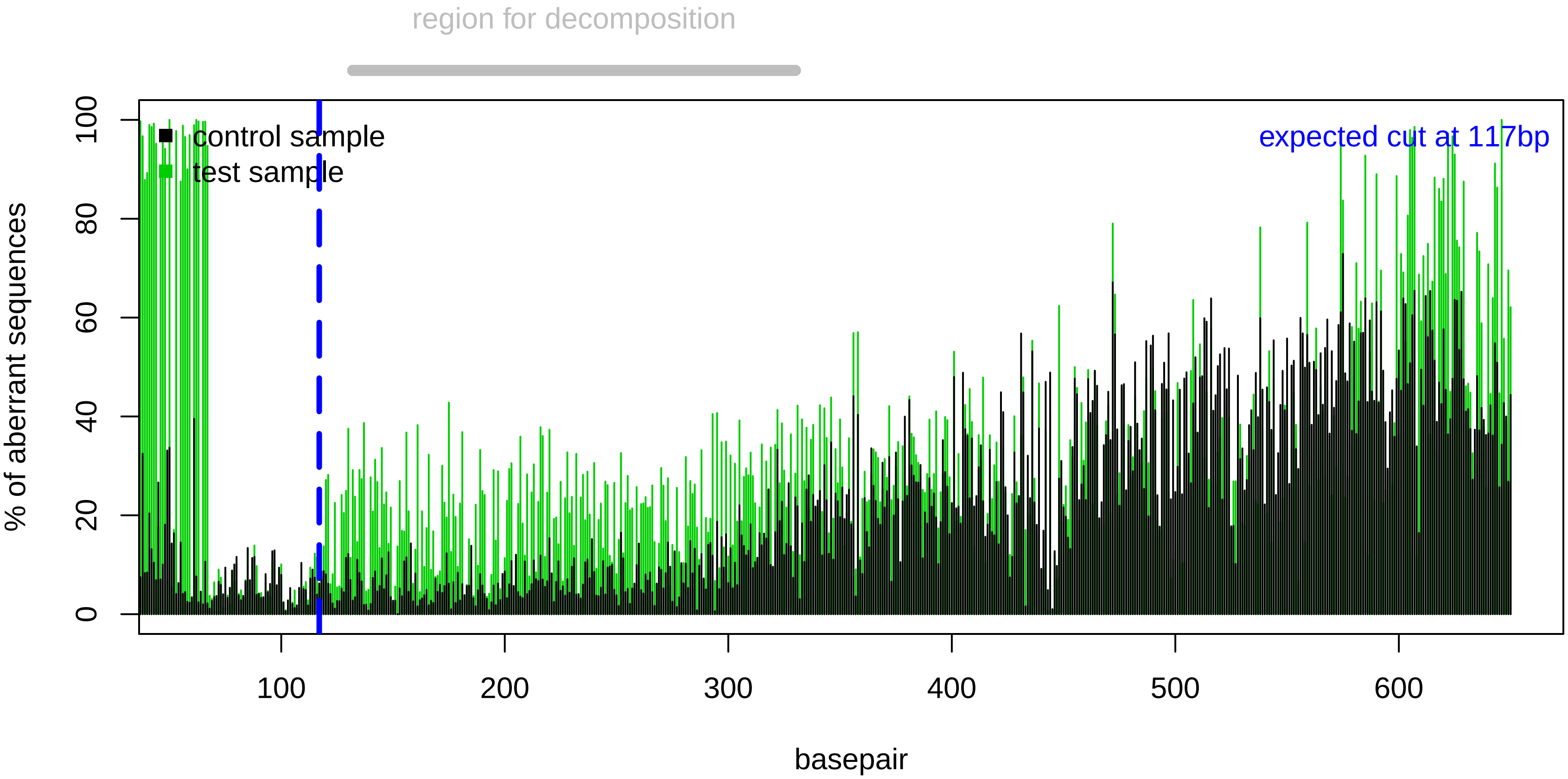
sample: WT/WT cell line, gRNA 1.2, reverse
guide: GGGTGGGACAGAACATCCCC



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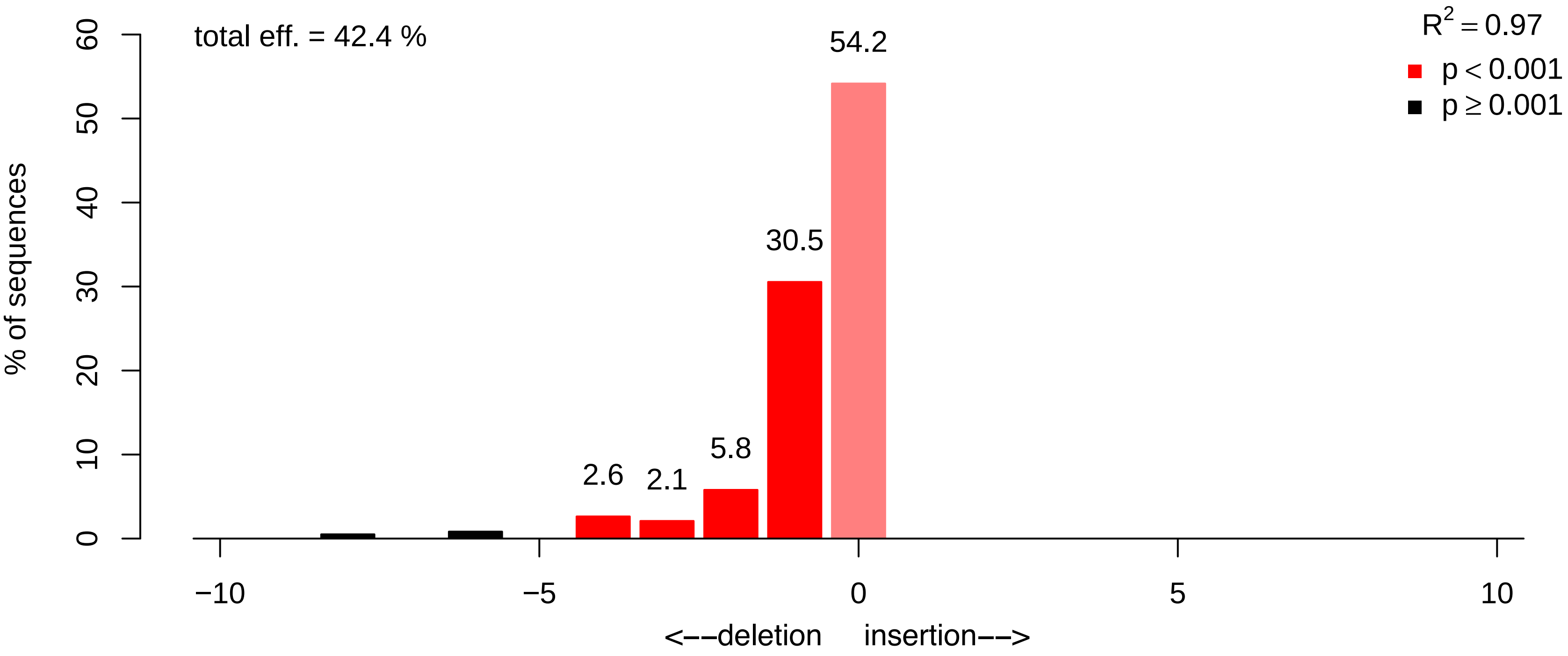

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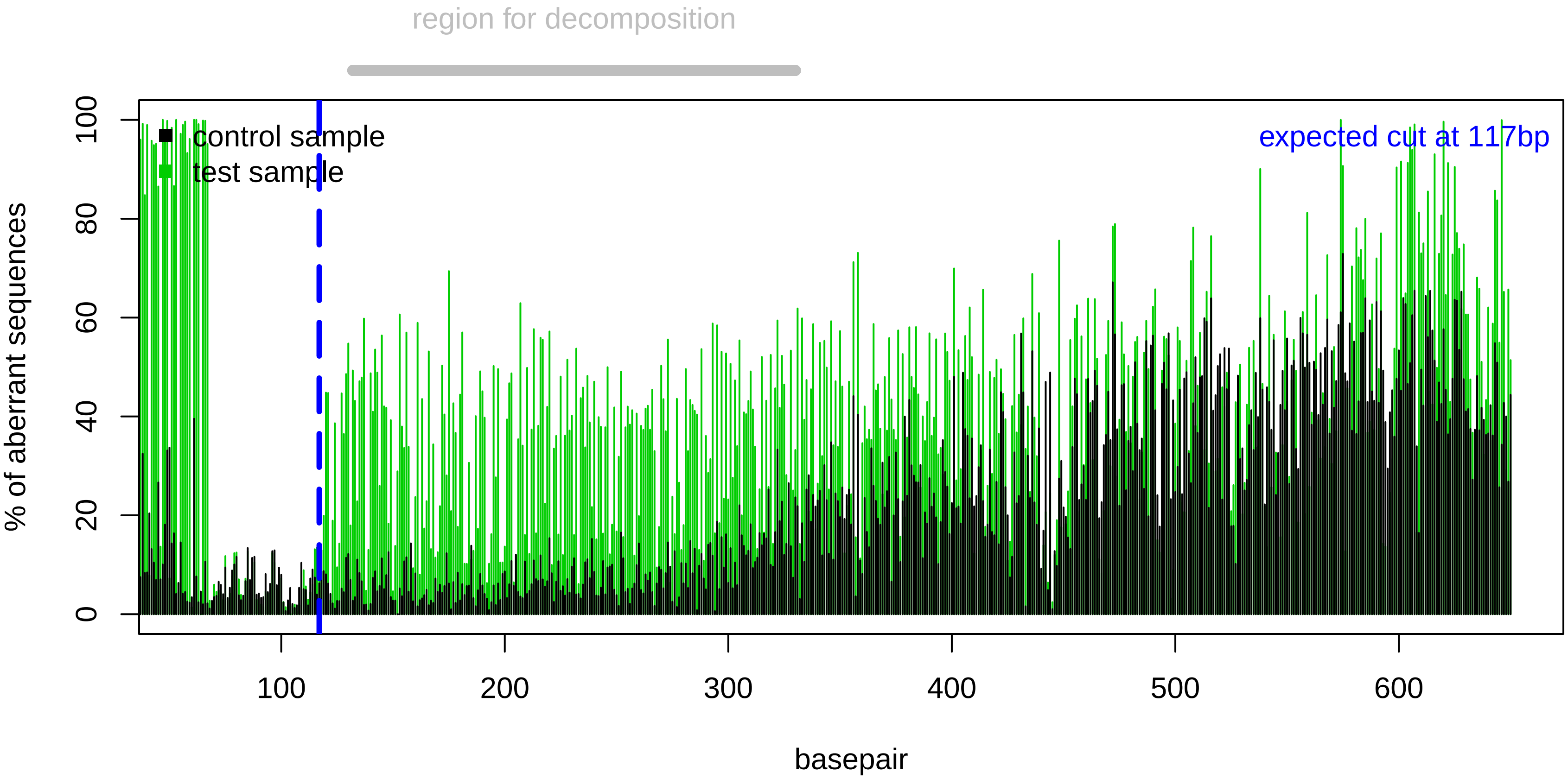

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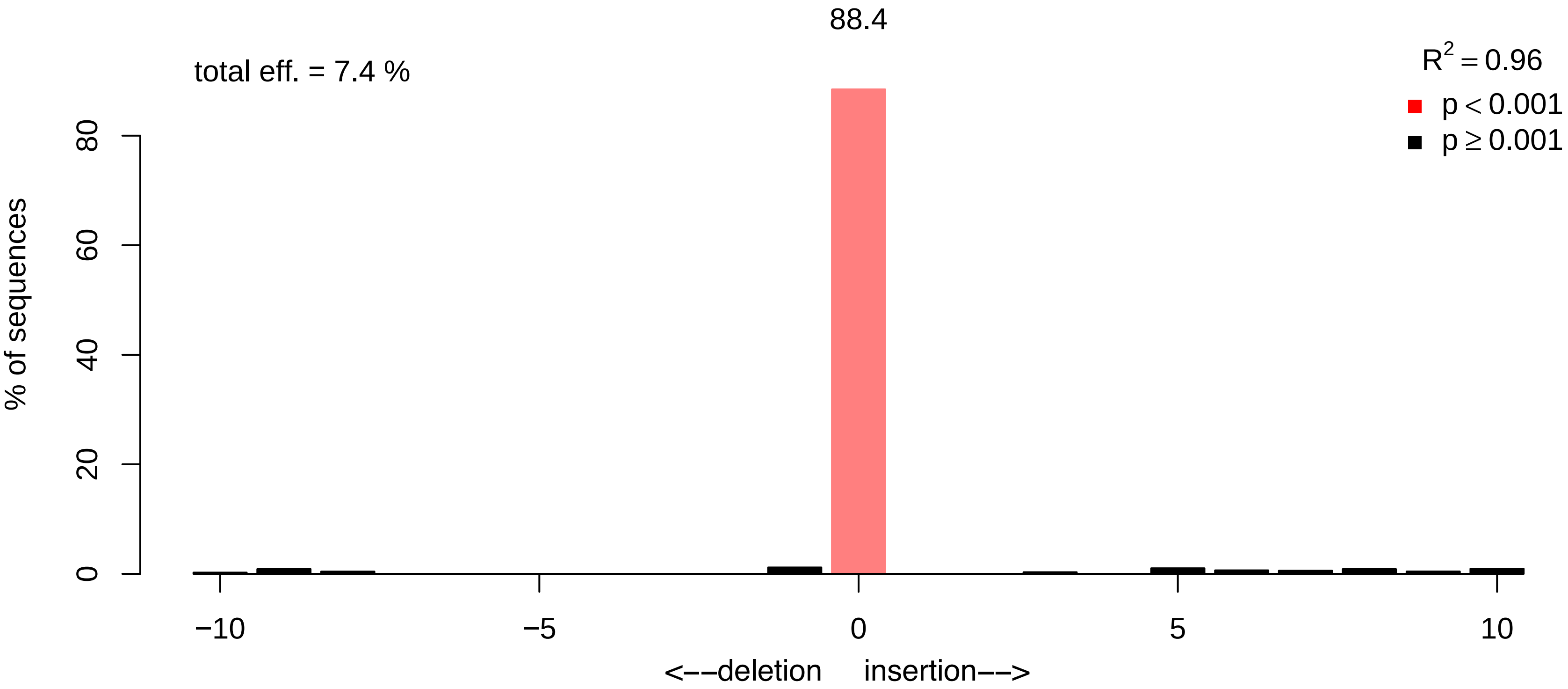
guide: TGGGACAGAACATCCCCAGG



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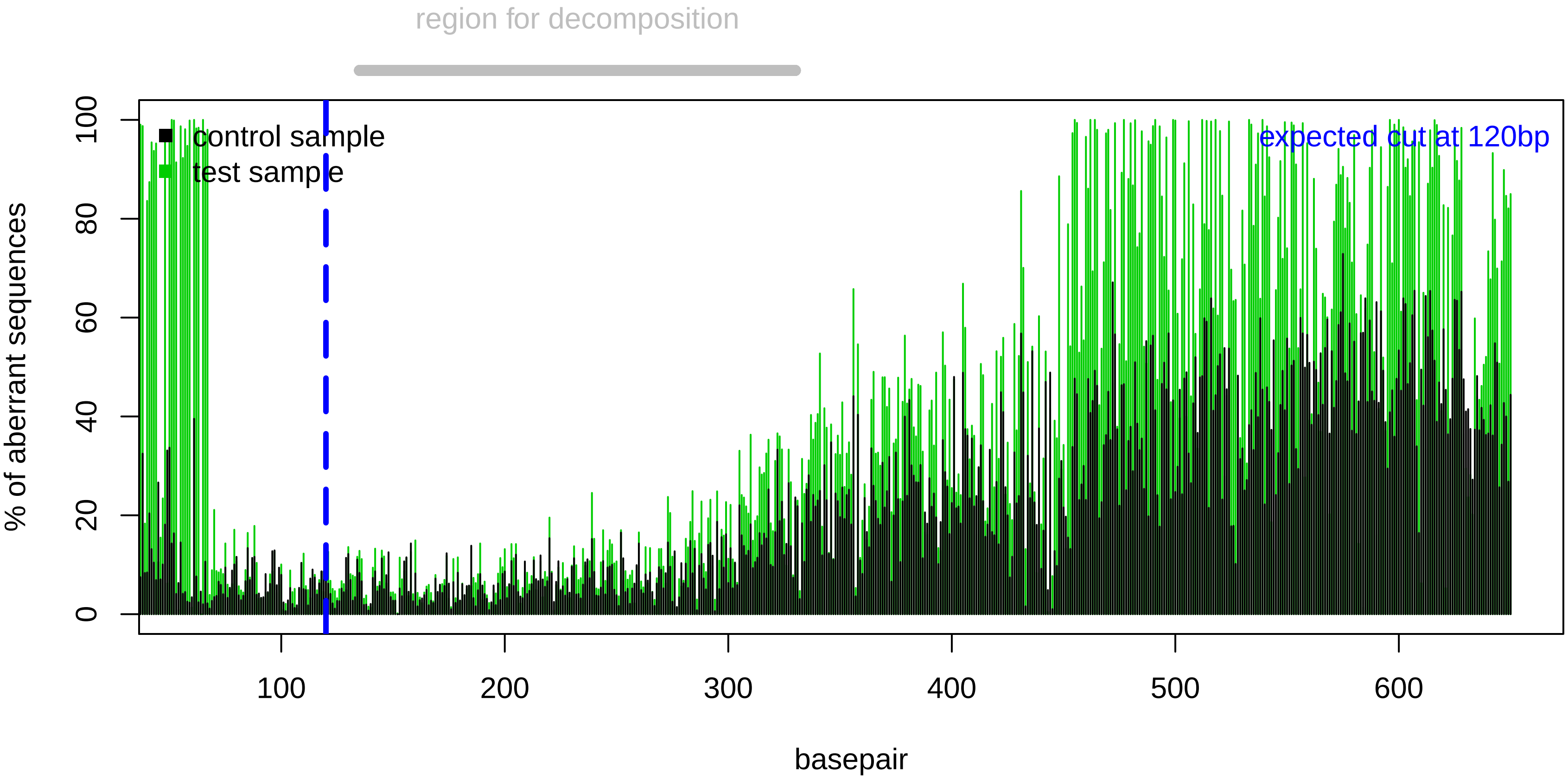
guide: TGGGACAGAACATCCCCAGG



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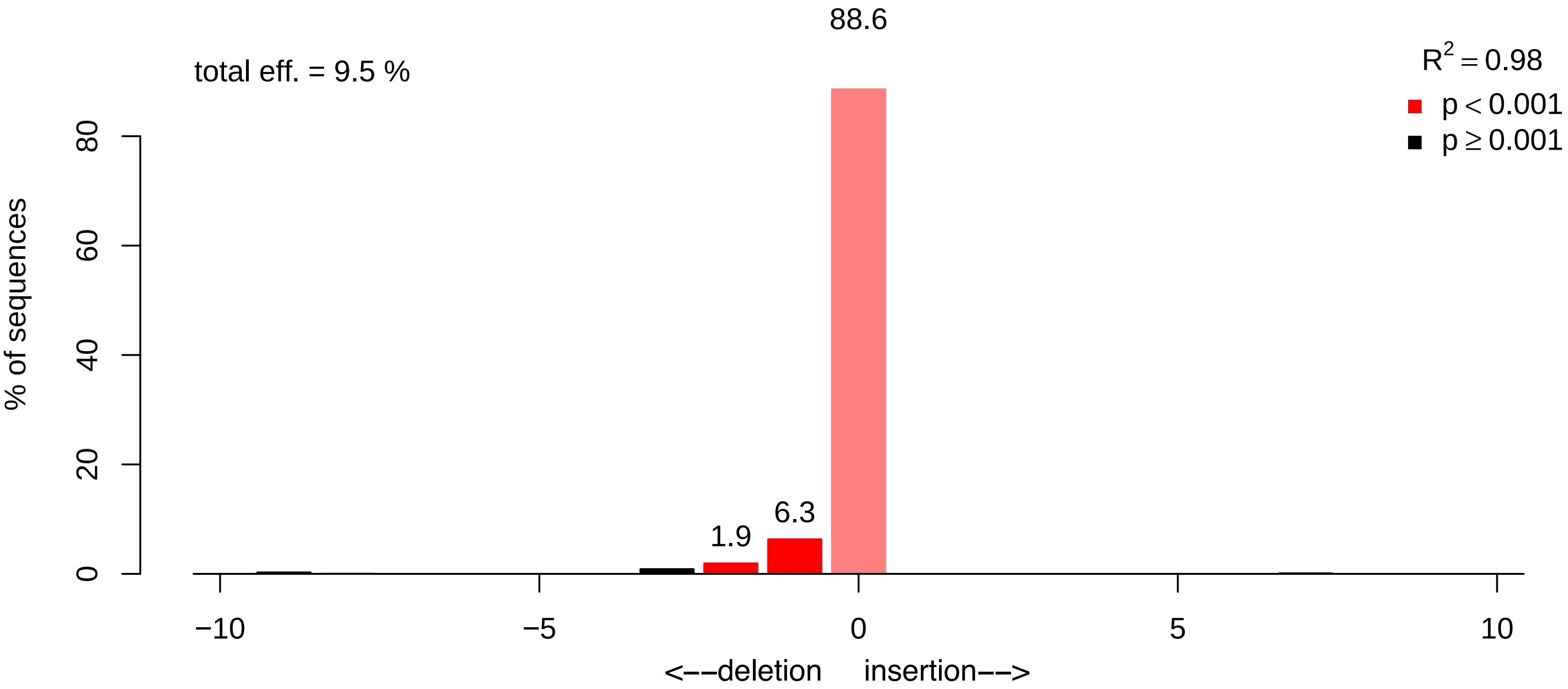
guide: TGGGACAGAACATCCCCAGG



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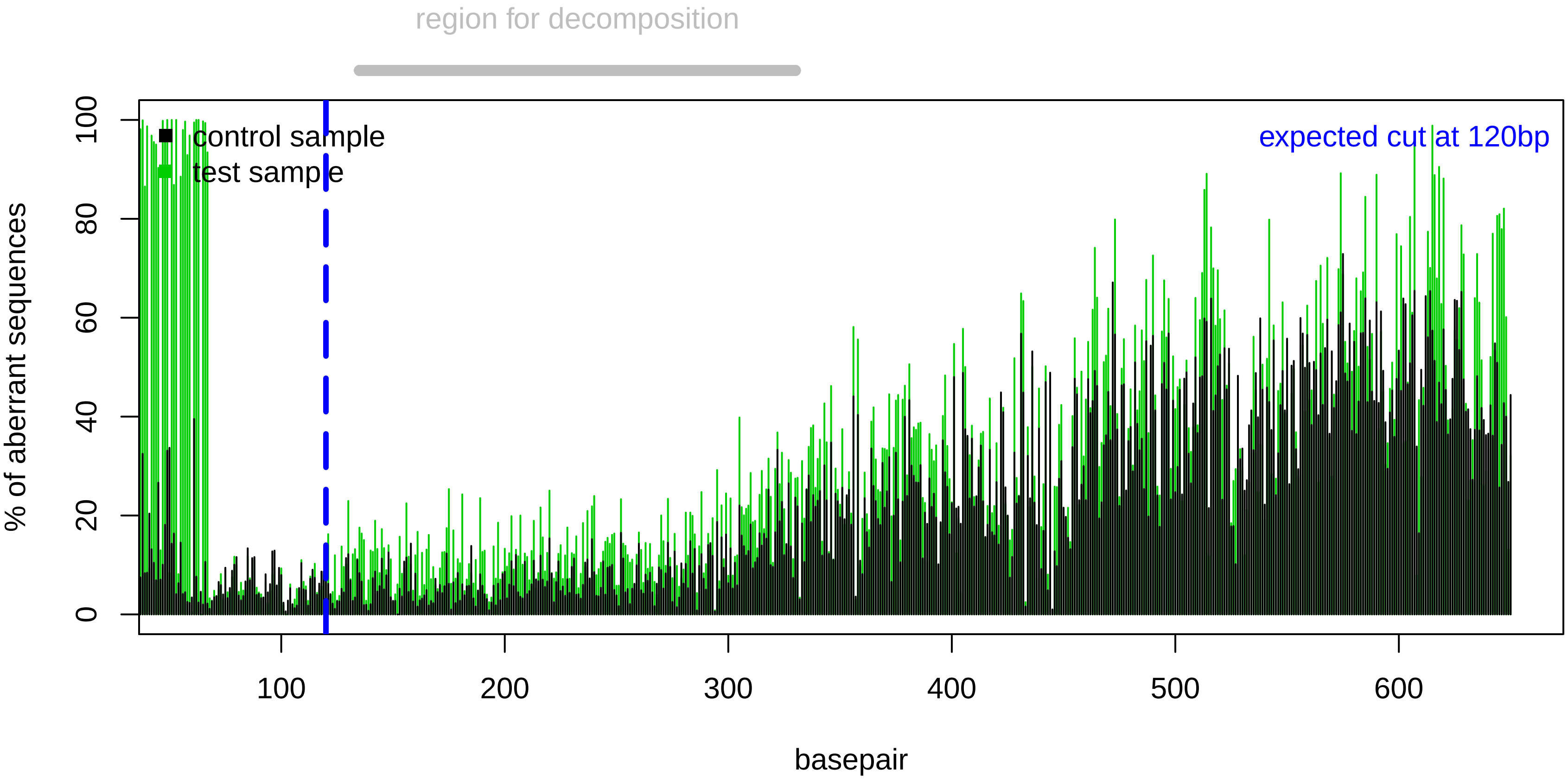
guide: TGGGACAGAACATCCCCAGG



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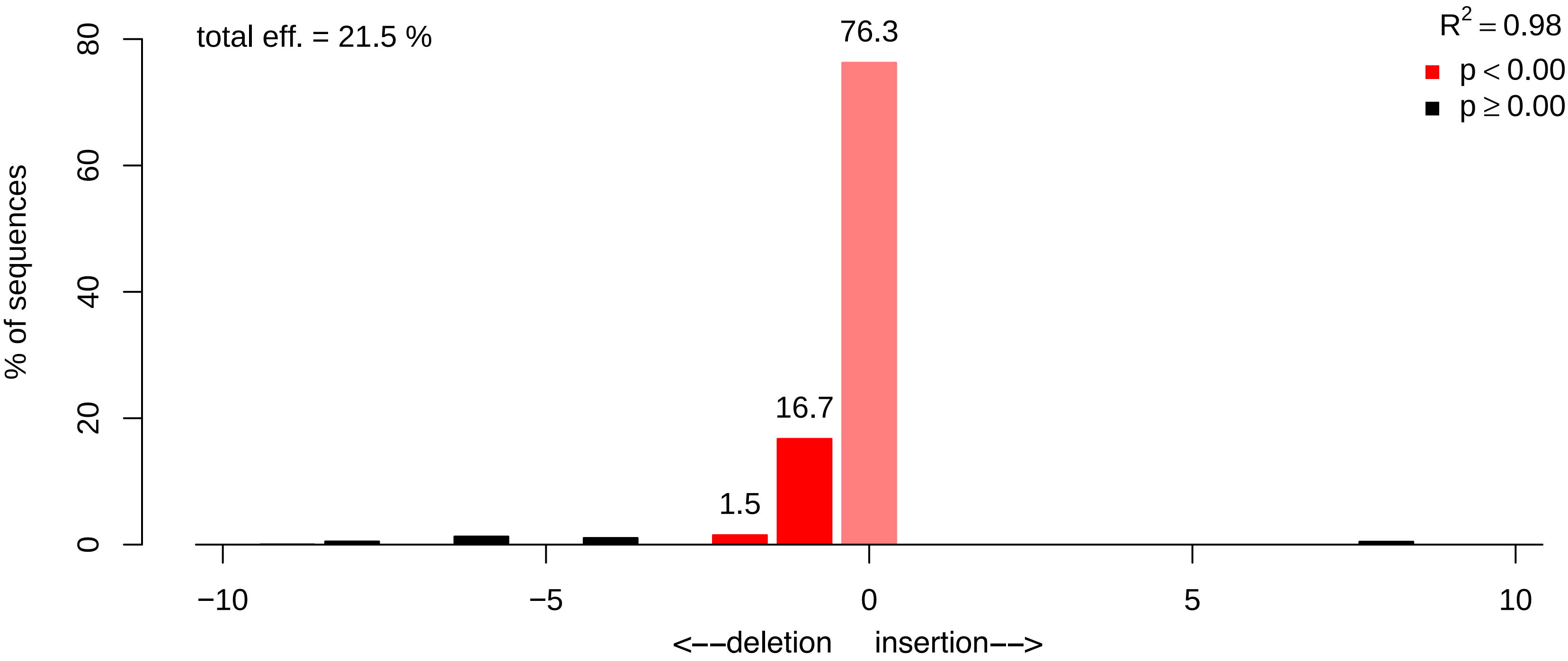

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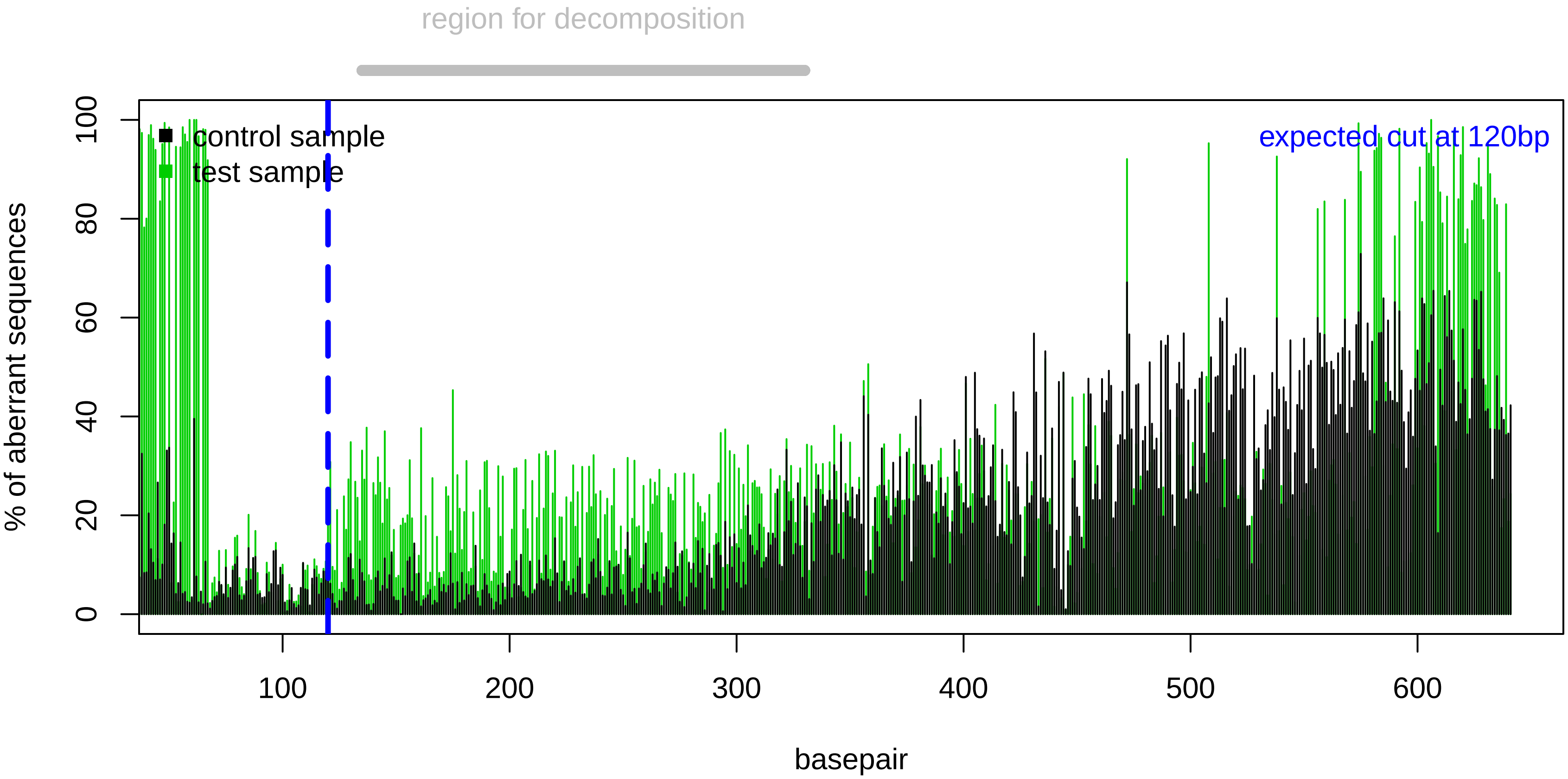

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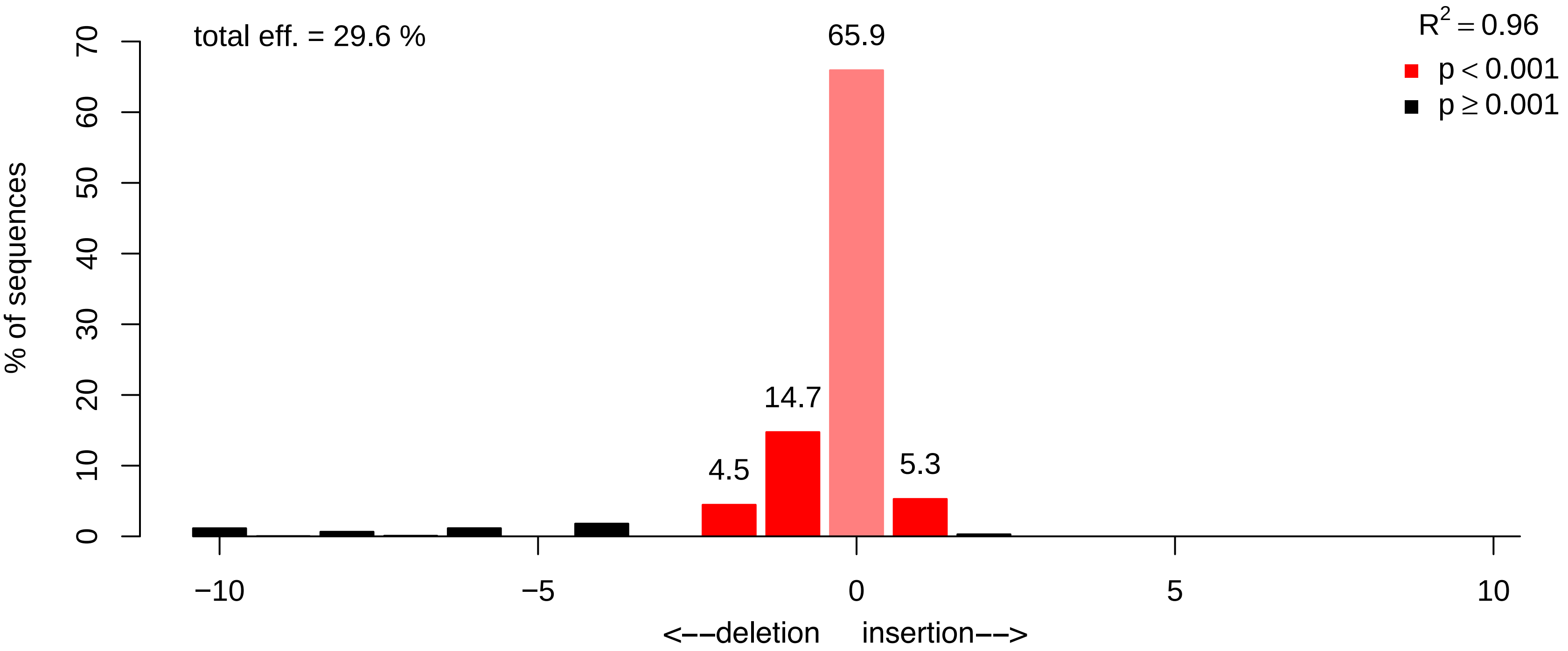

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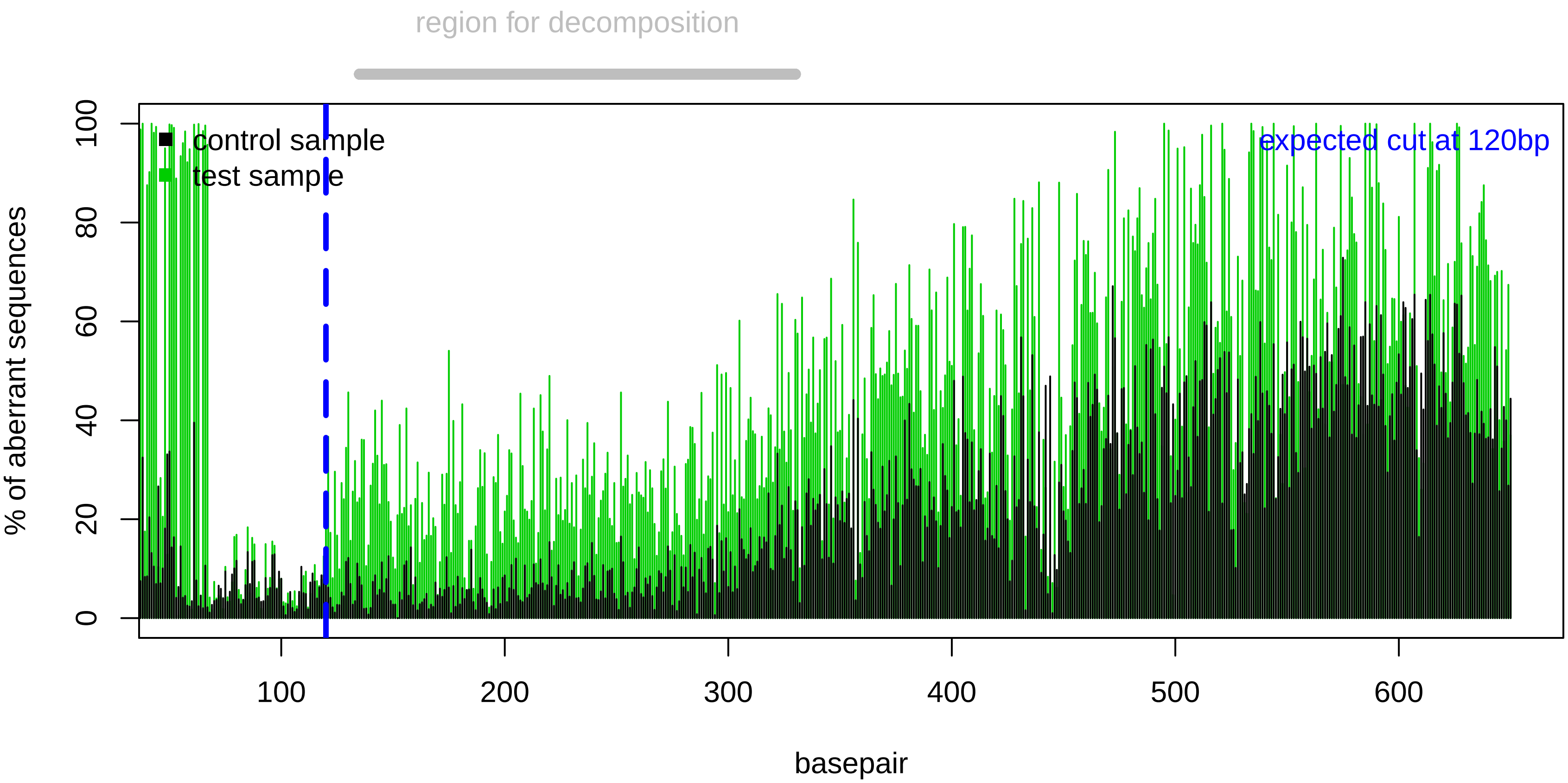

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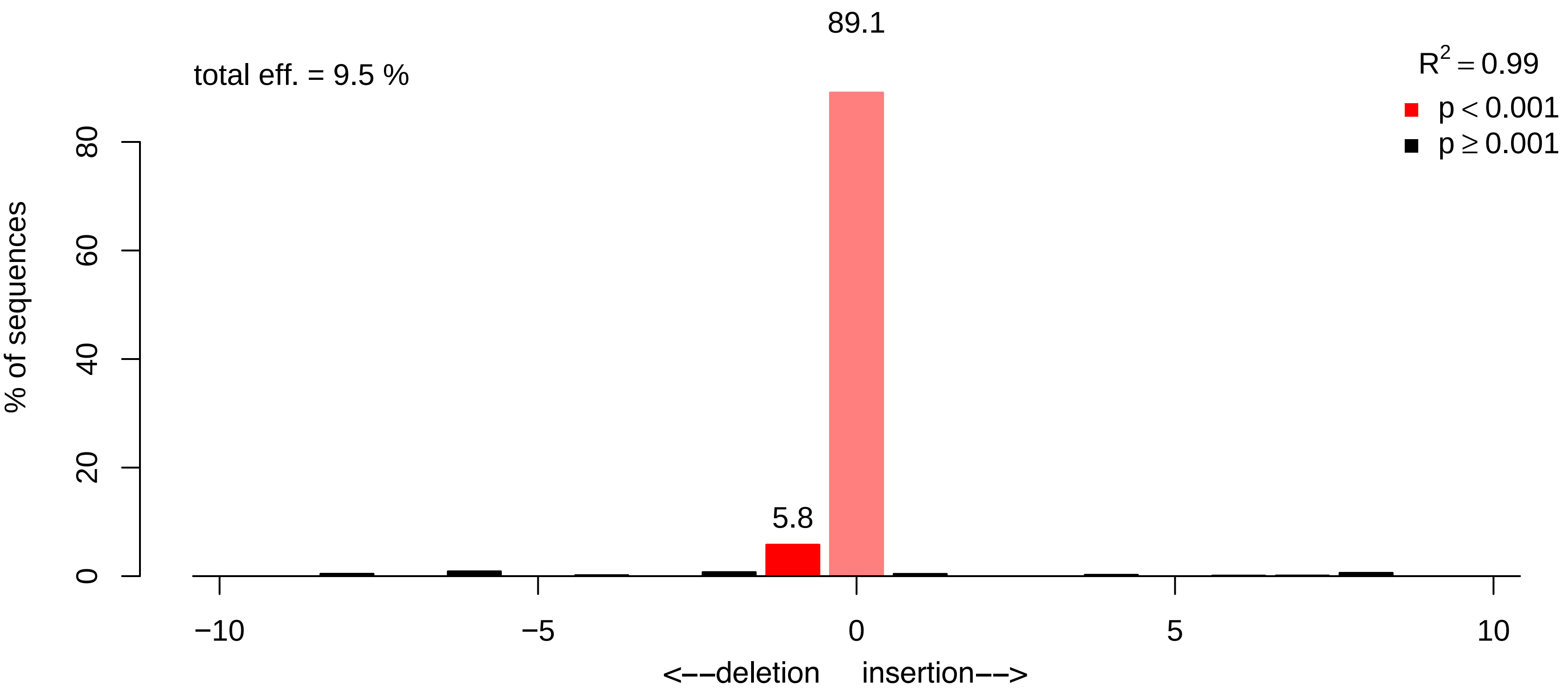

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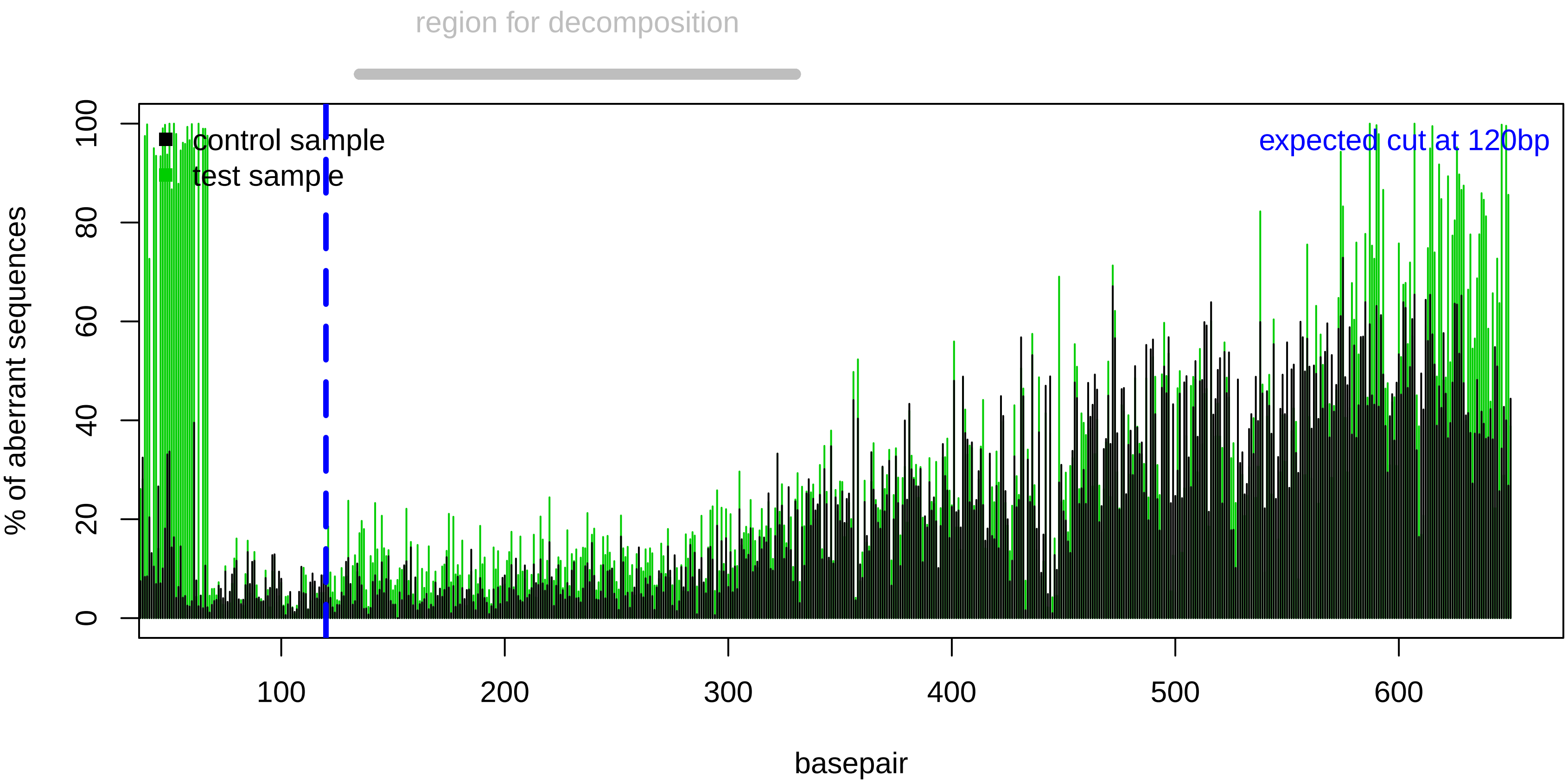

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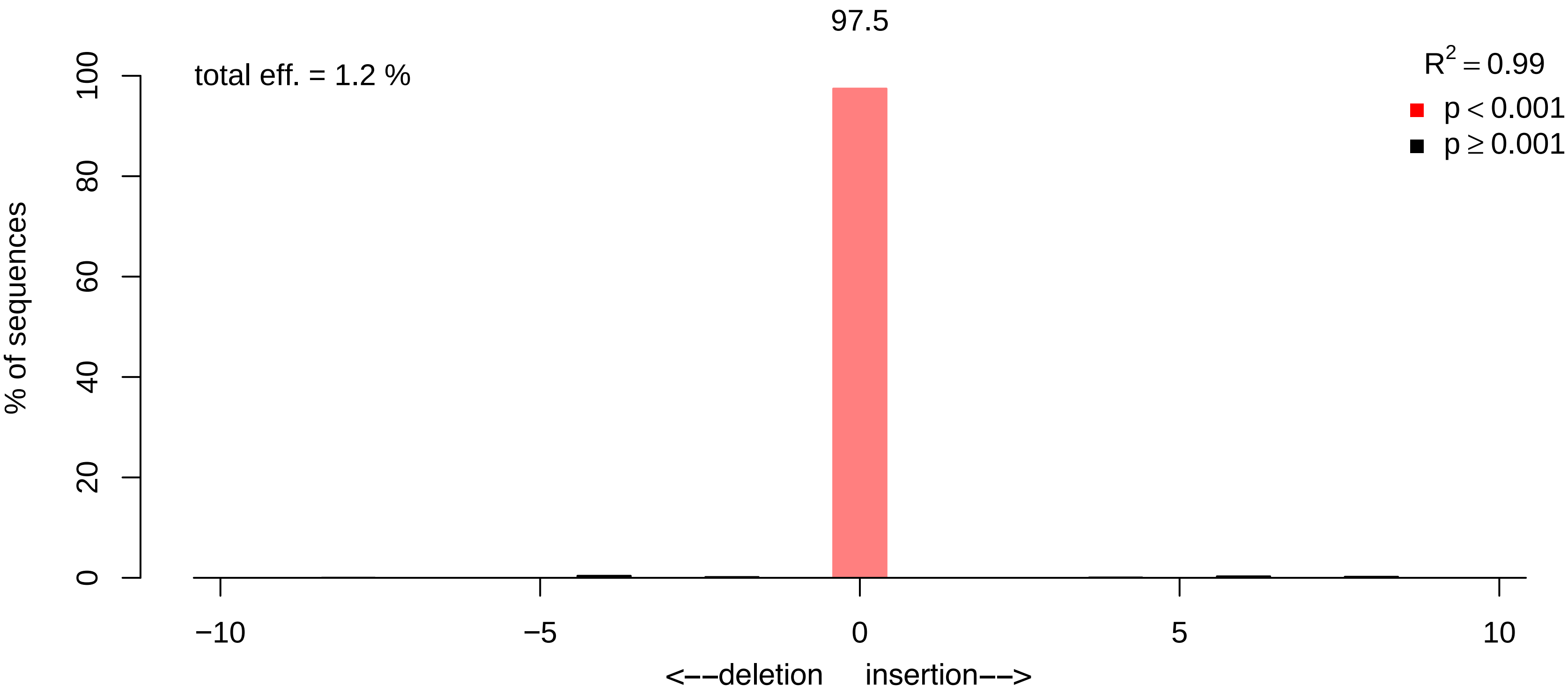

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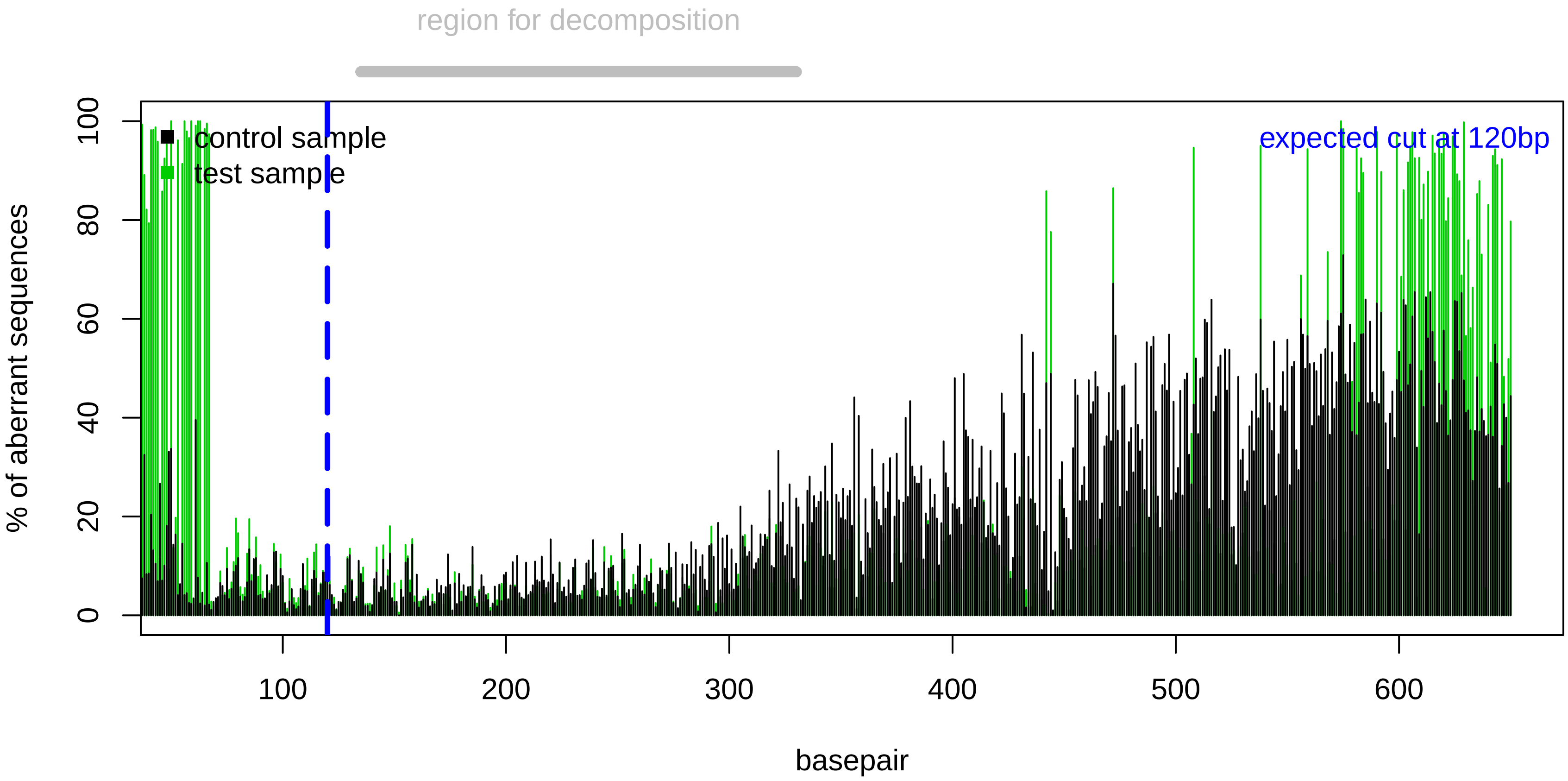

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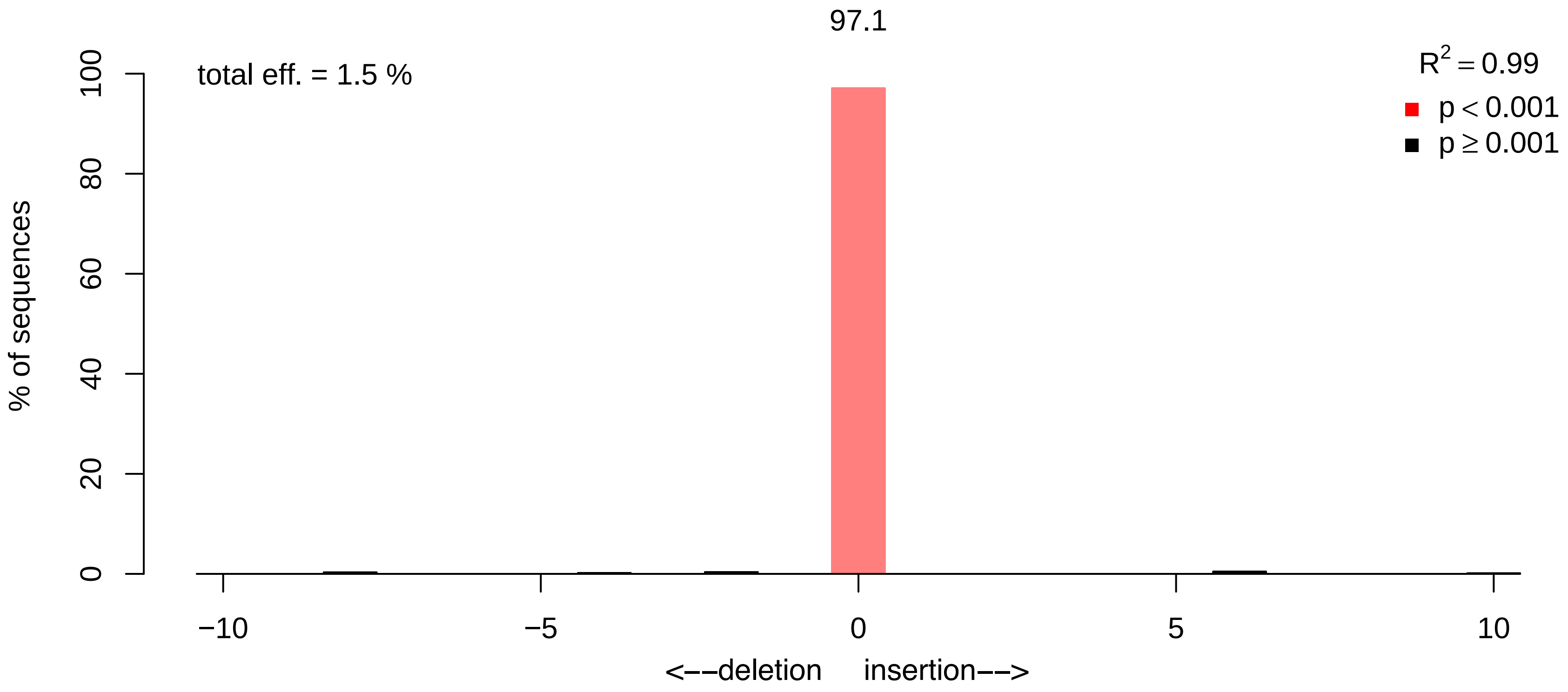

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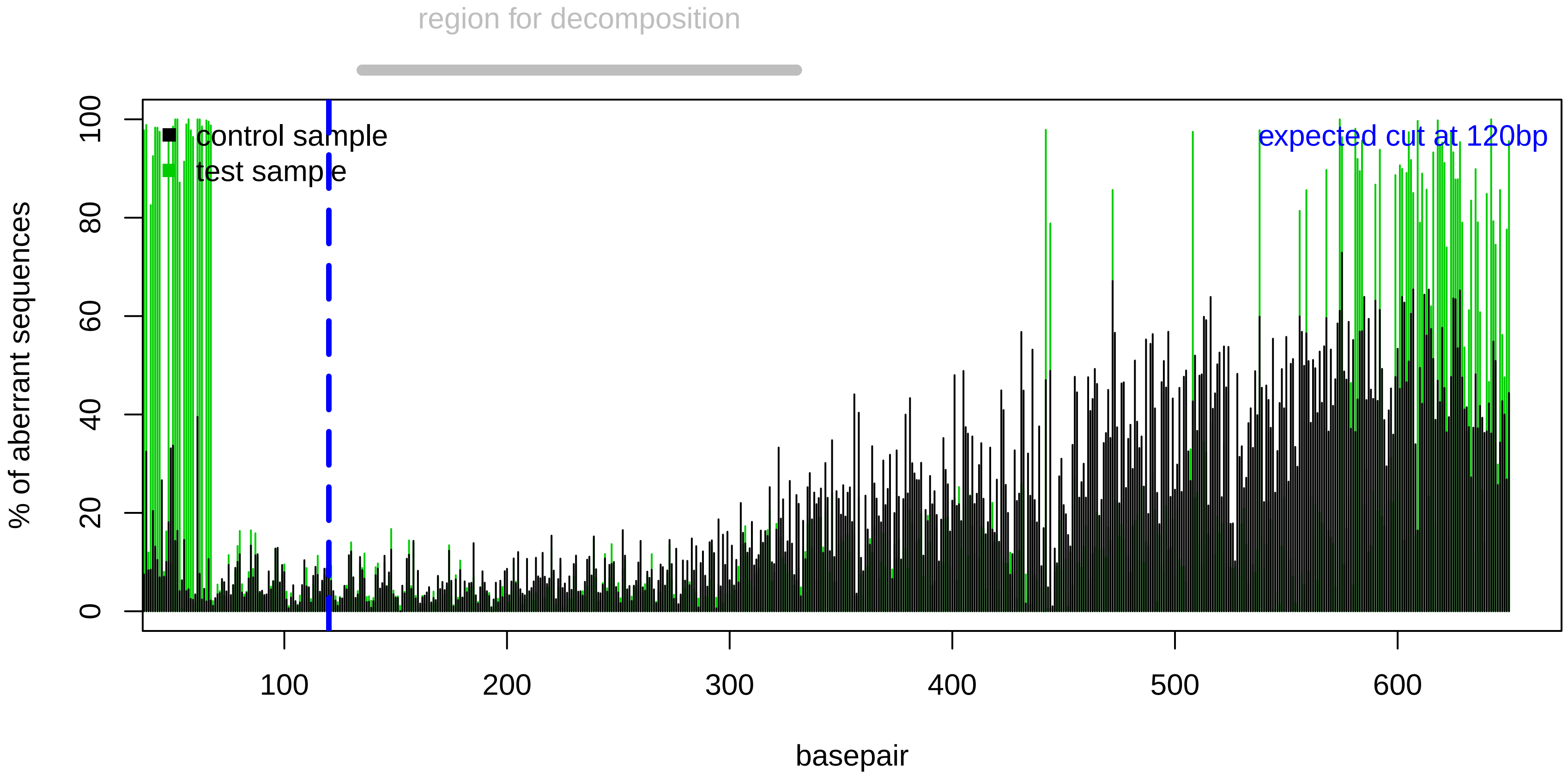

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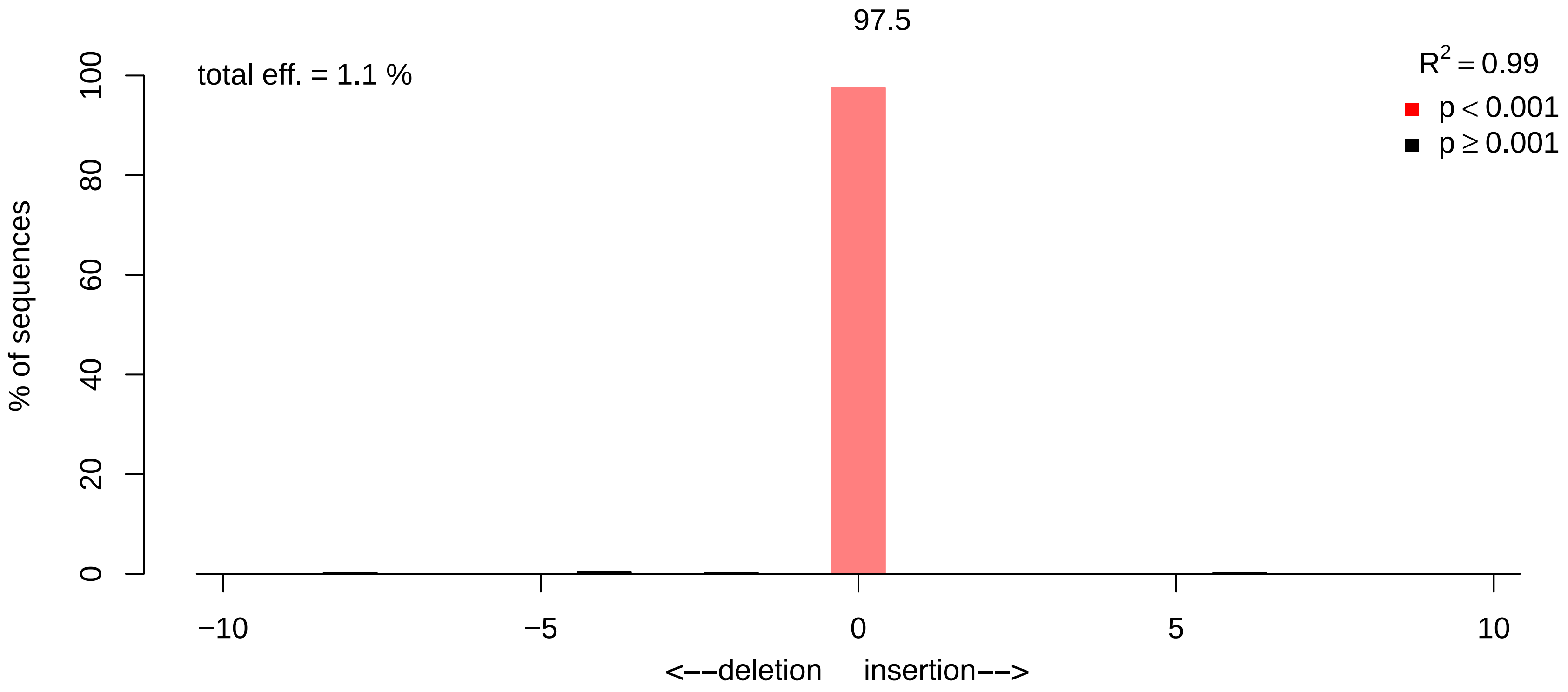

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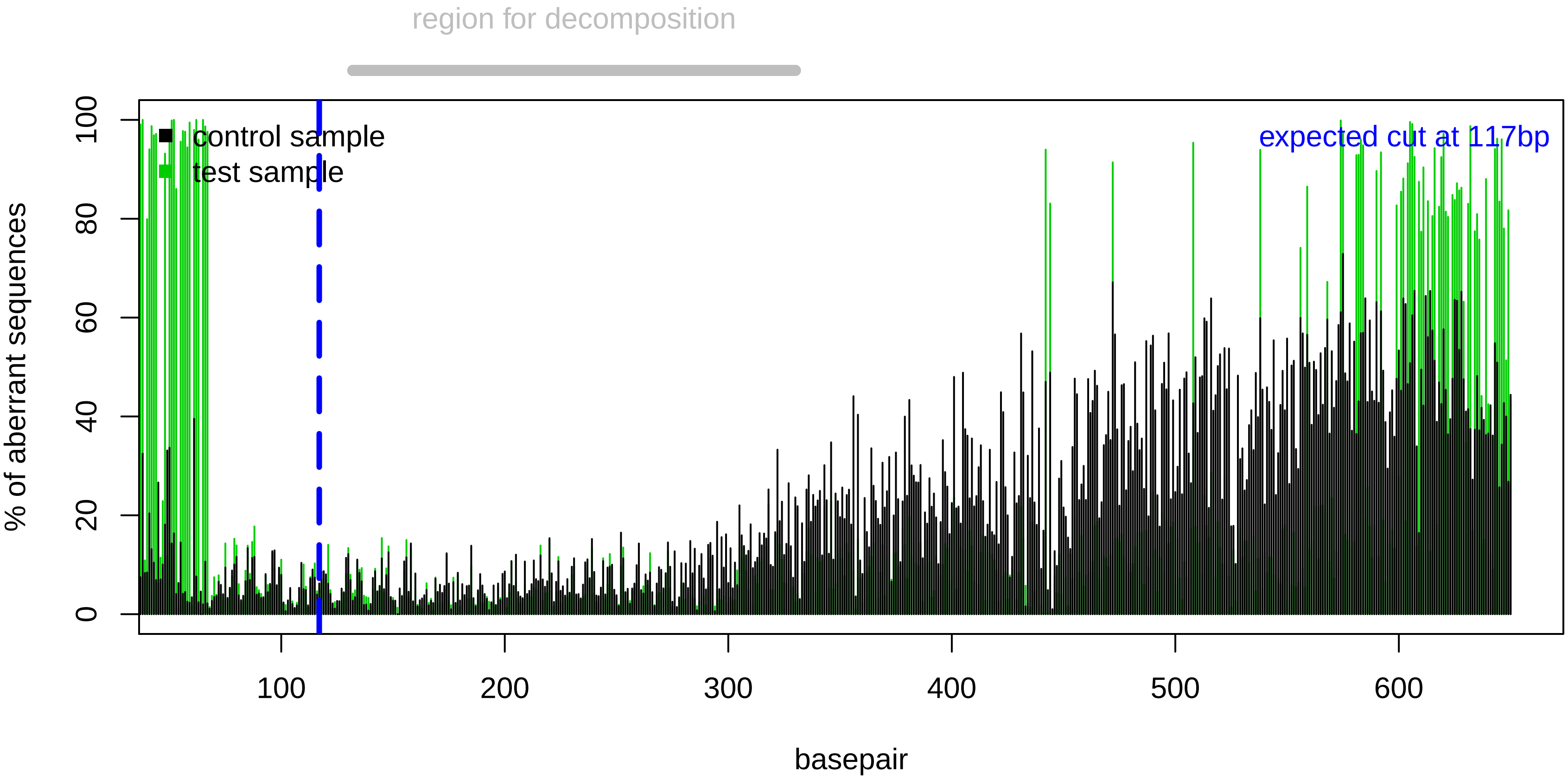

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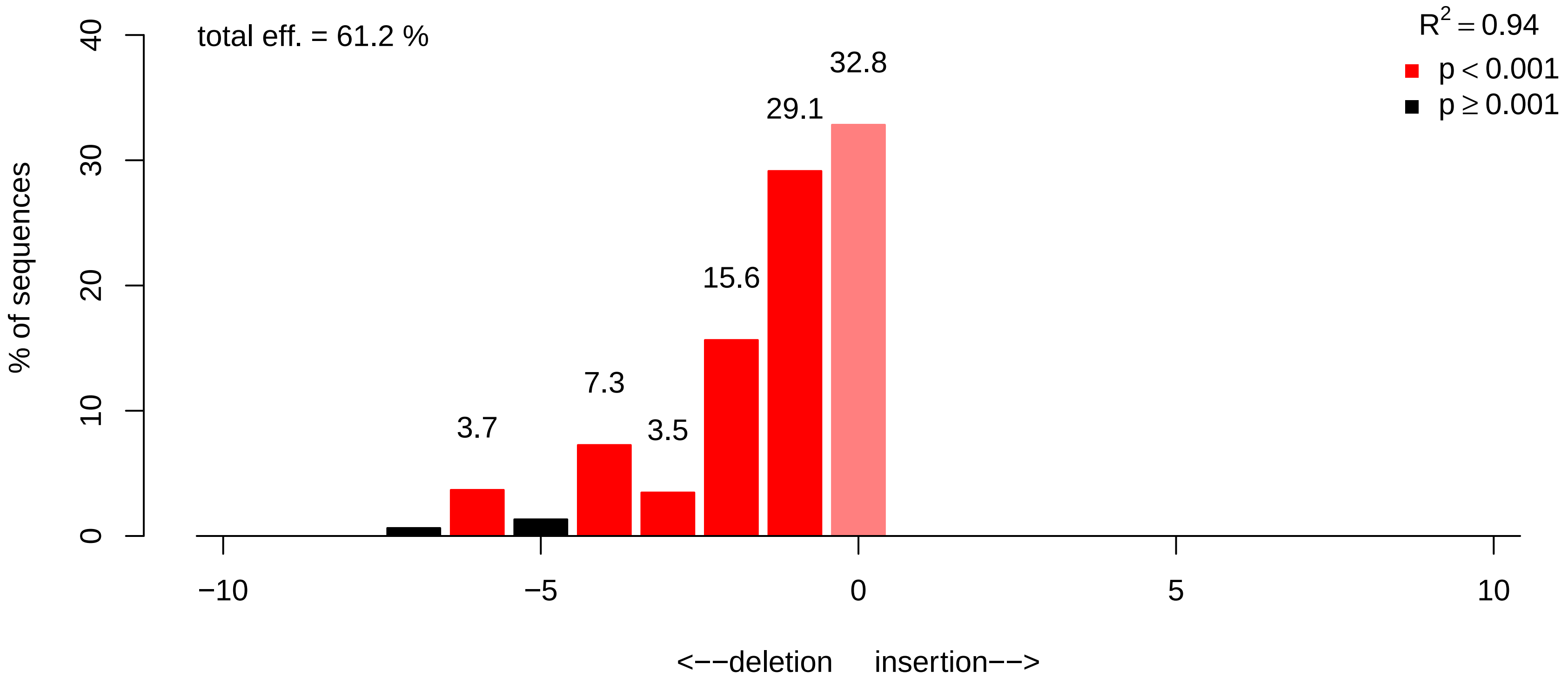

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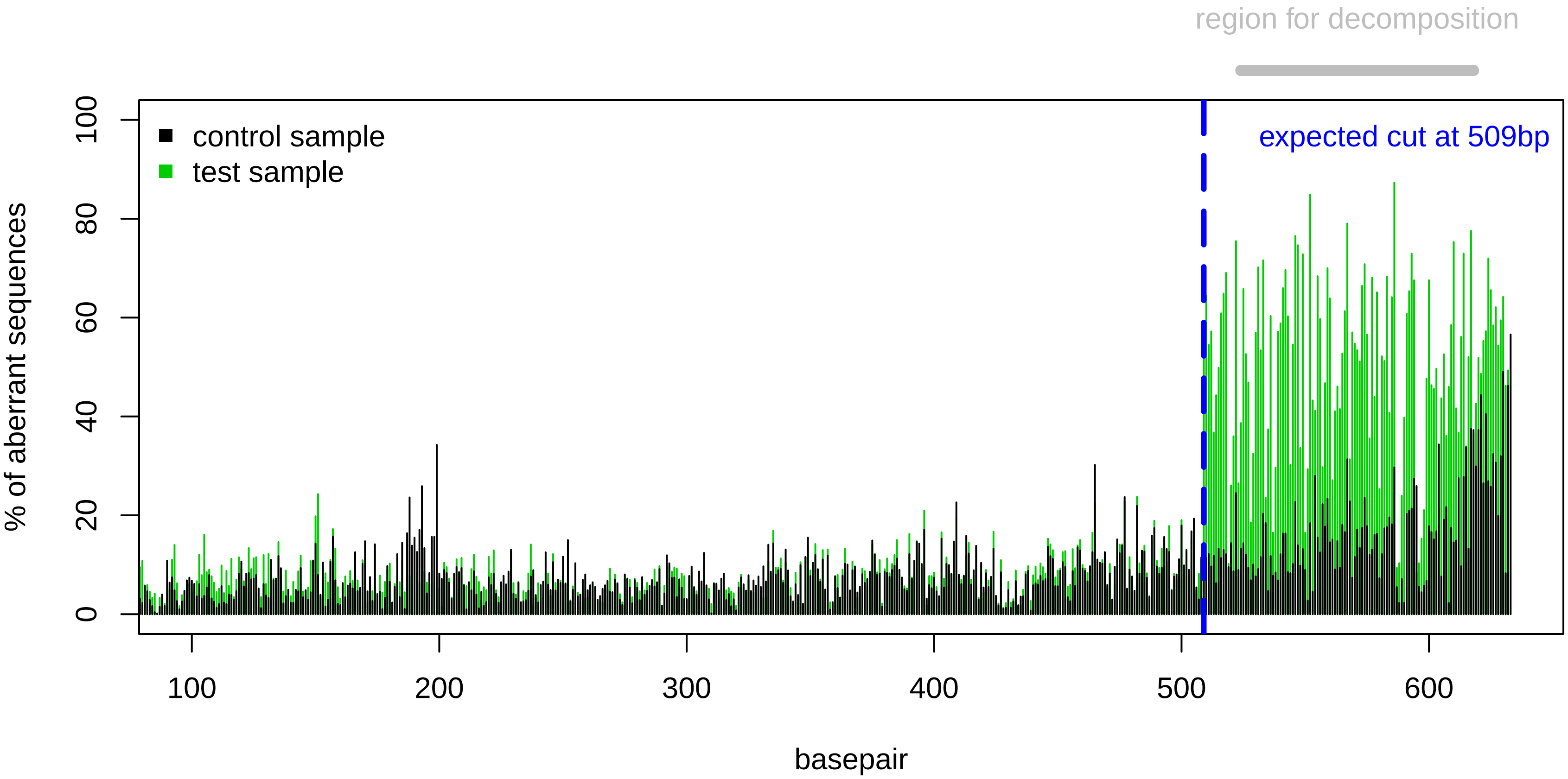
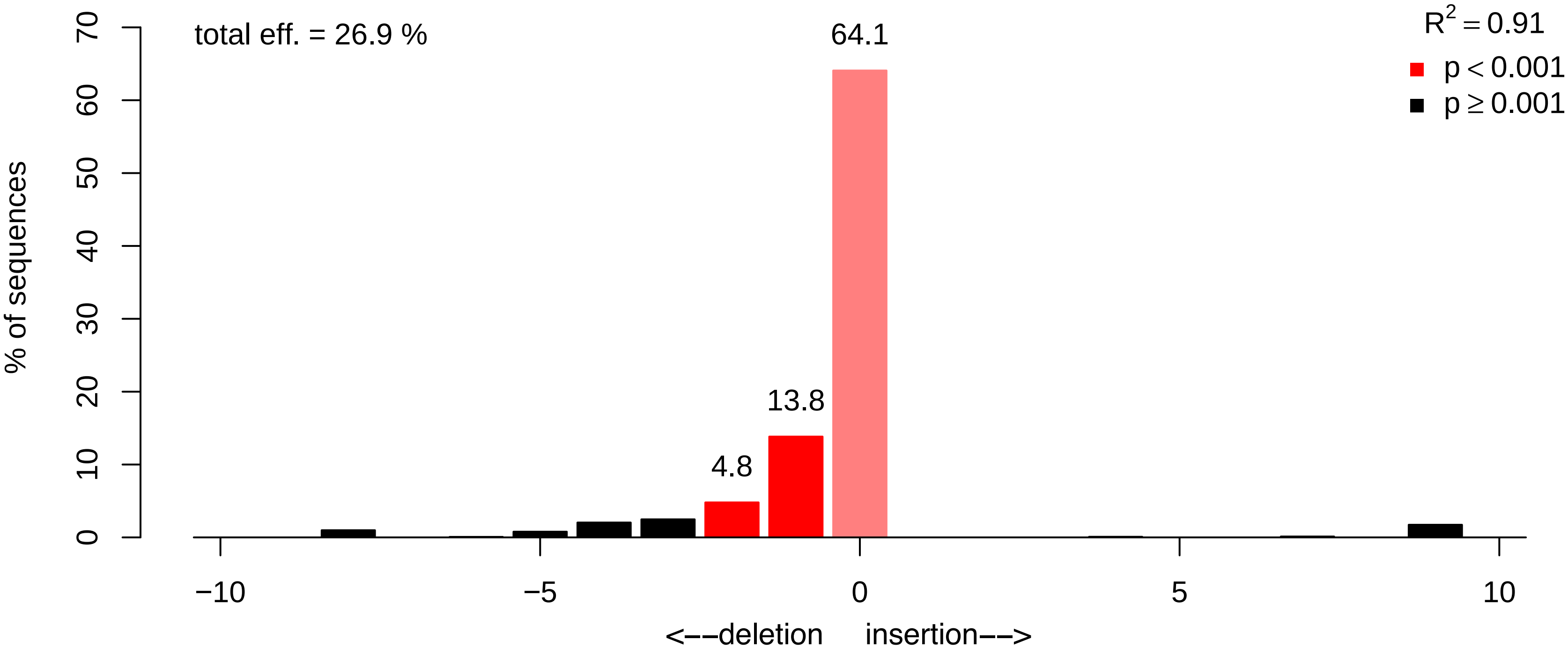
guide: GGGTGGGACAGAACATCCCC

sample: WT/WT cell line, gRNA WT, forward
guide: GGGTGGGACAGAACATCCCC



Warning: left boundary of decomposition window was adjusted 524 . It must be at least 5bp plus the maximum indel size downstream of the expected break site
Warning: right boundary of decomposition window was adjusted to 618 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: WT/WT cell line, gRNA 1.1, forward
guide: GGGTGGGACAGAACATCCCC



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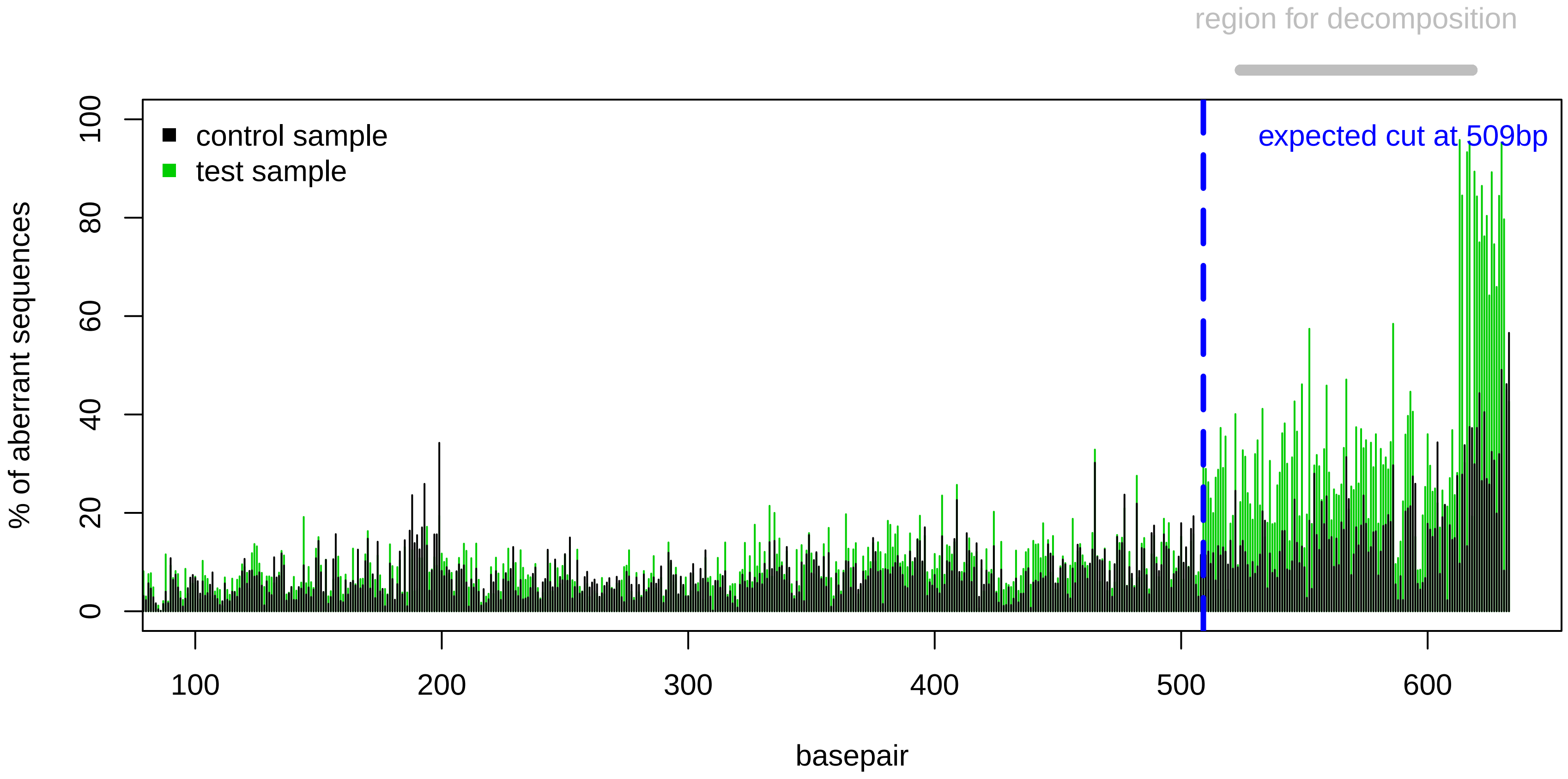
sample: WT/WT cell line, gRNA 1.1, forward
guide: GGGTGGGACAGAACATCCCC



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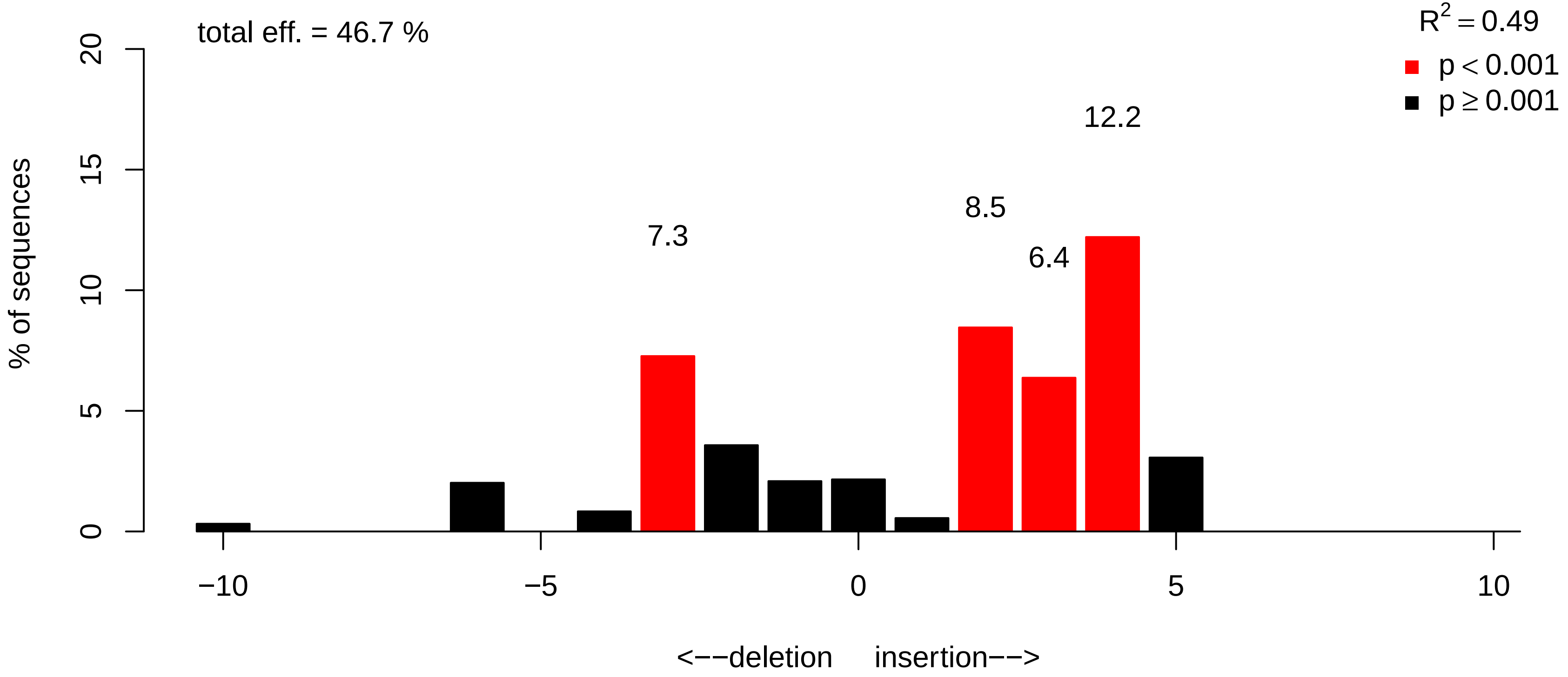

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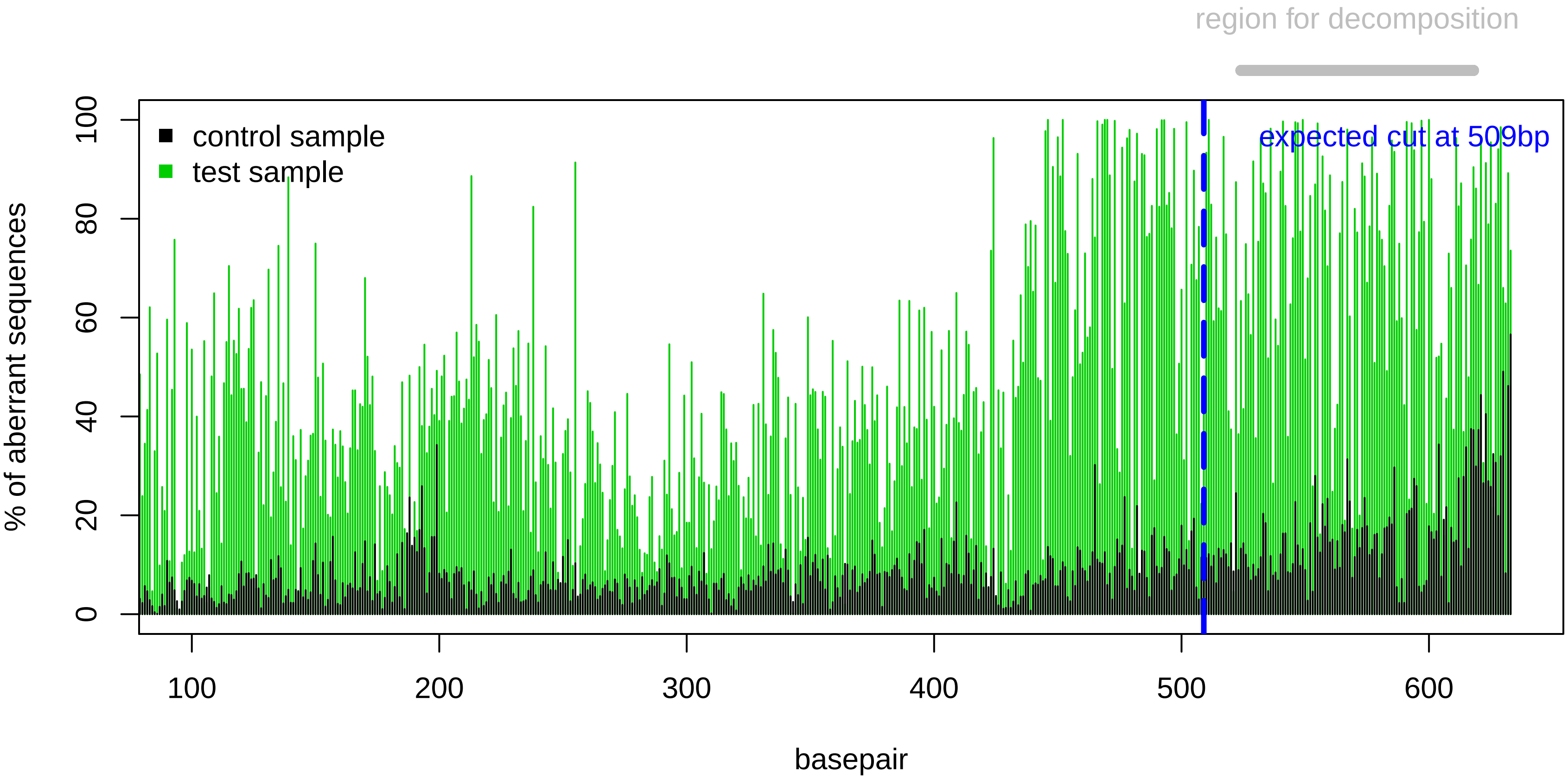

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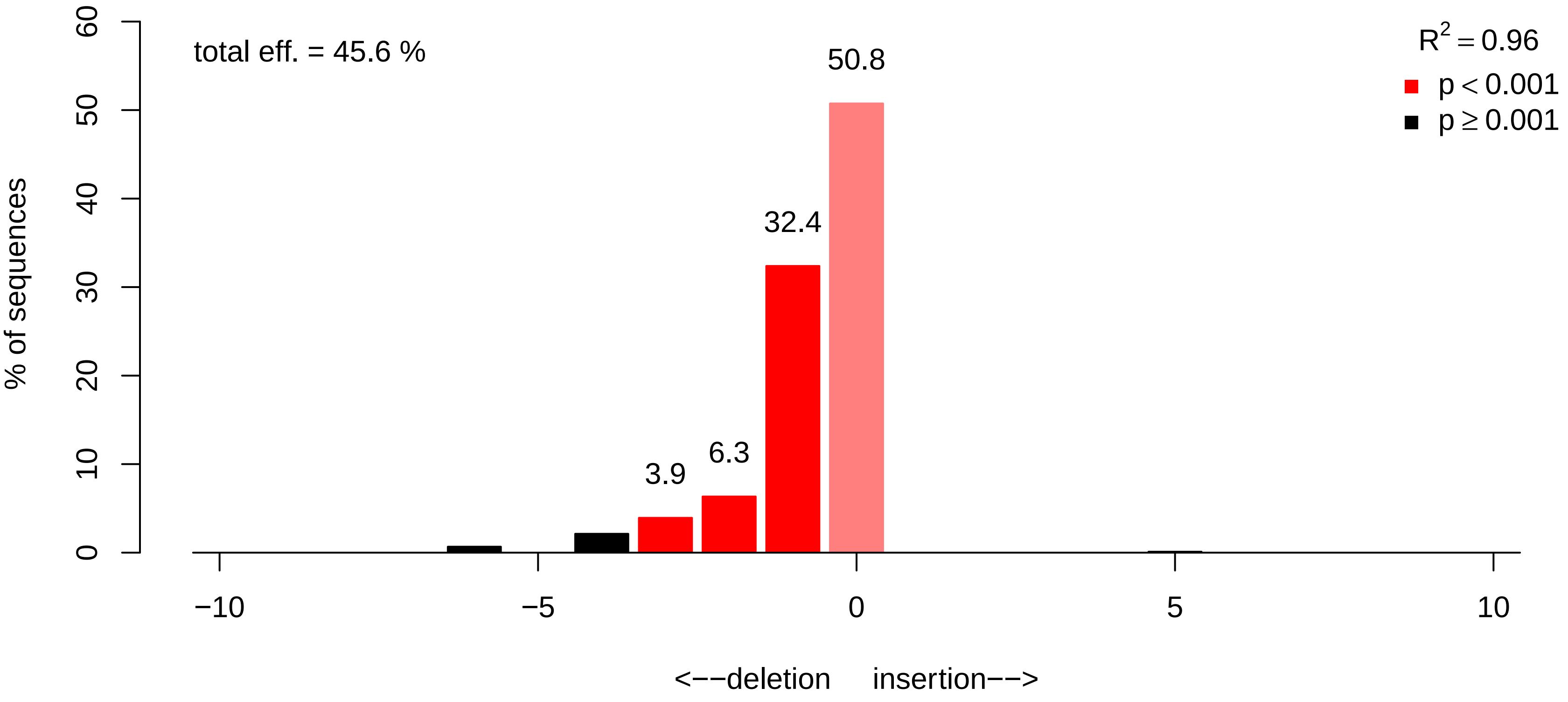

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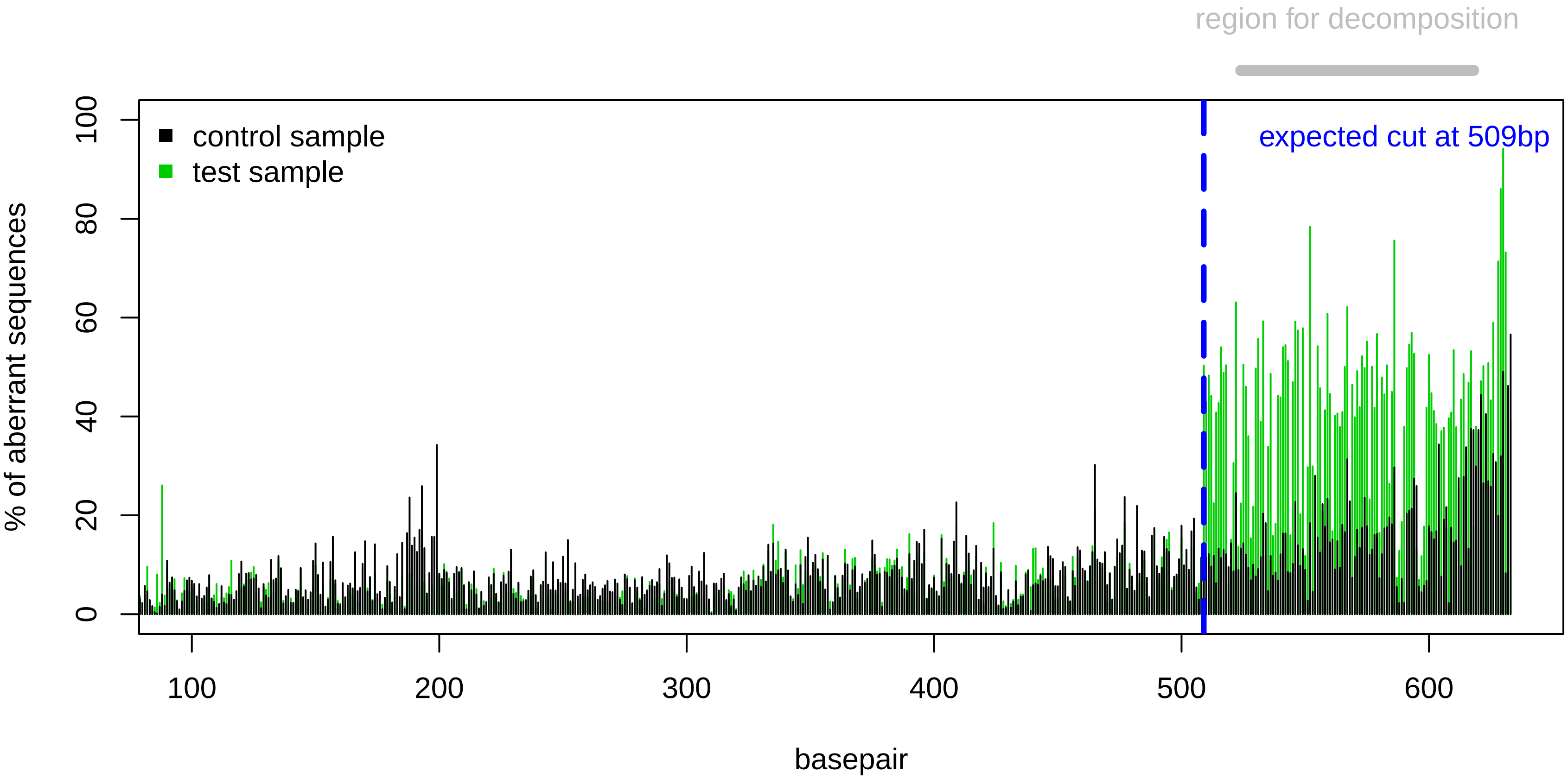

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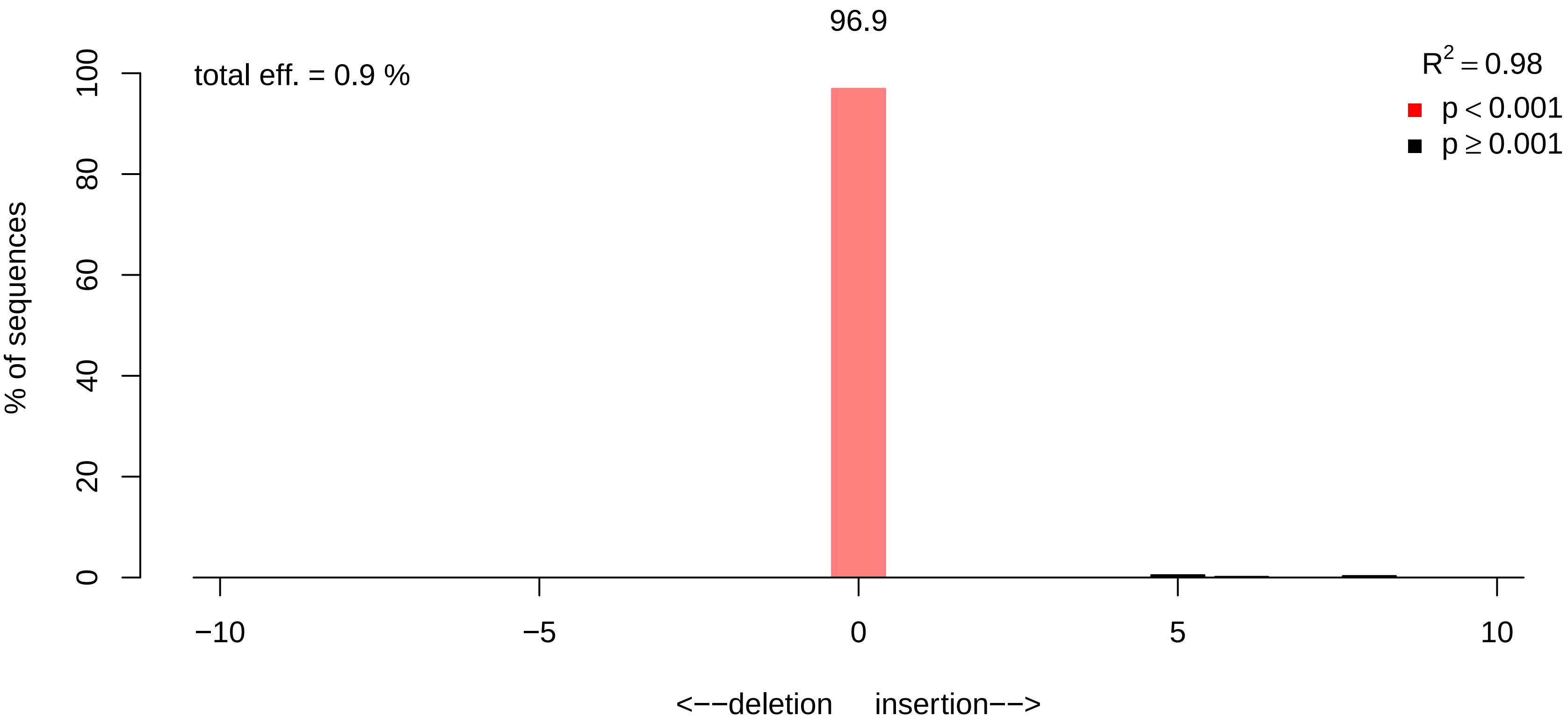

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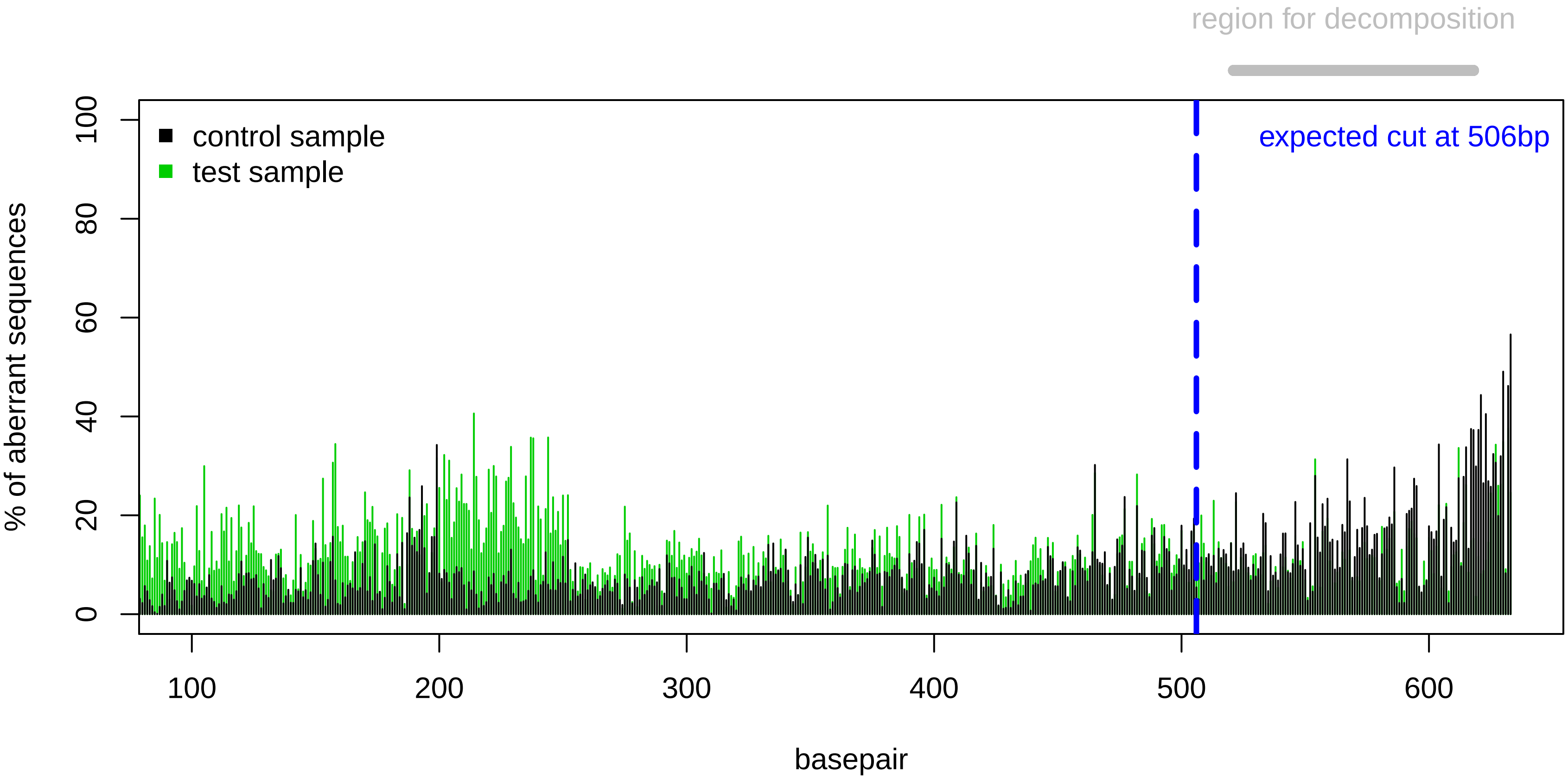

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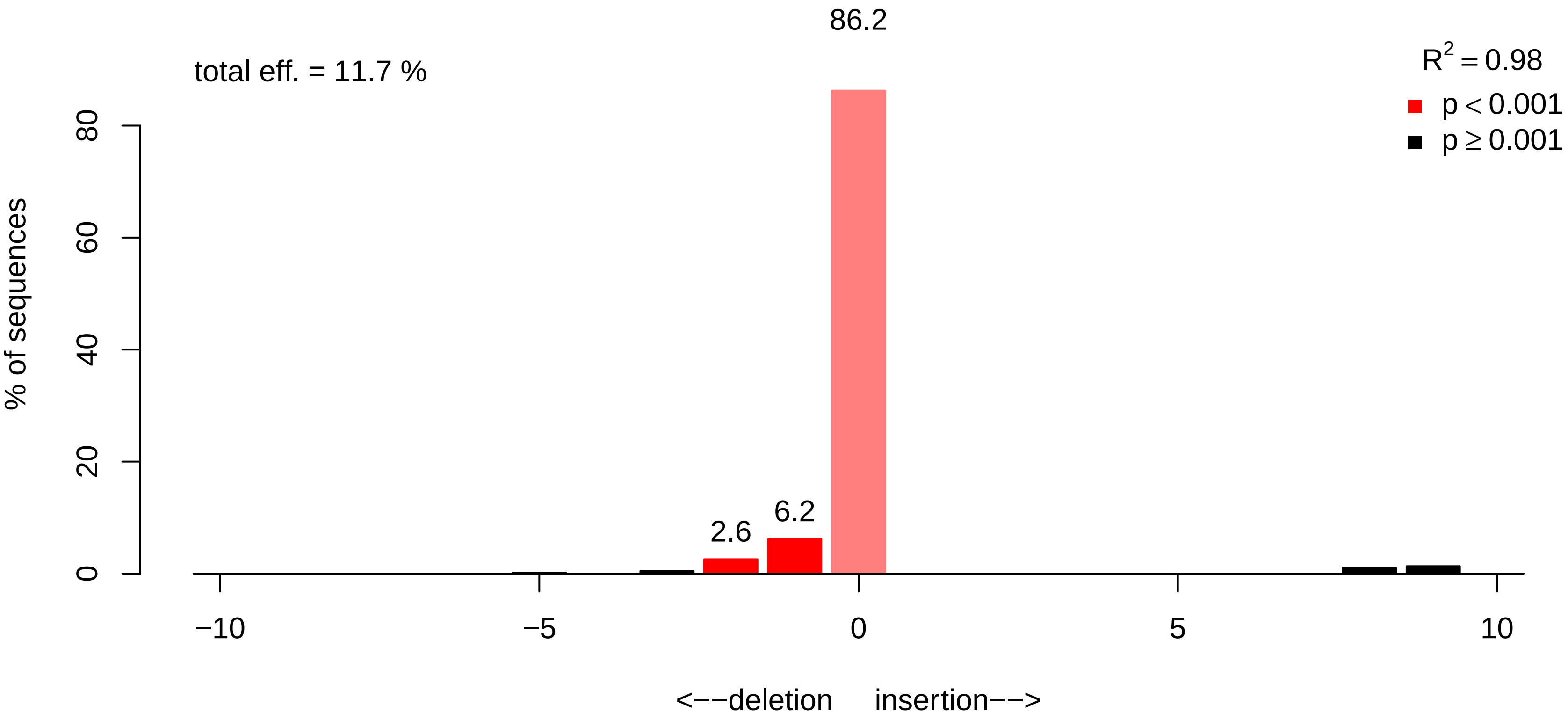

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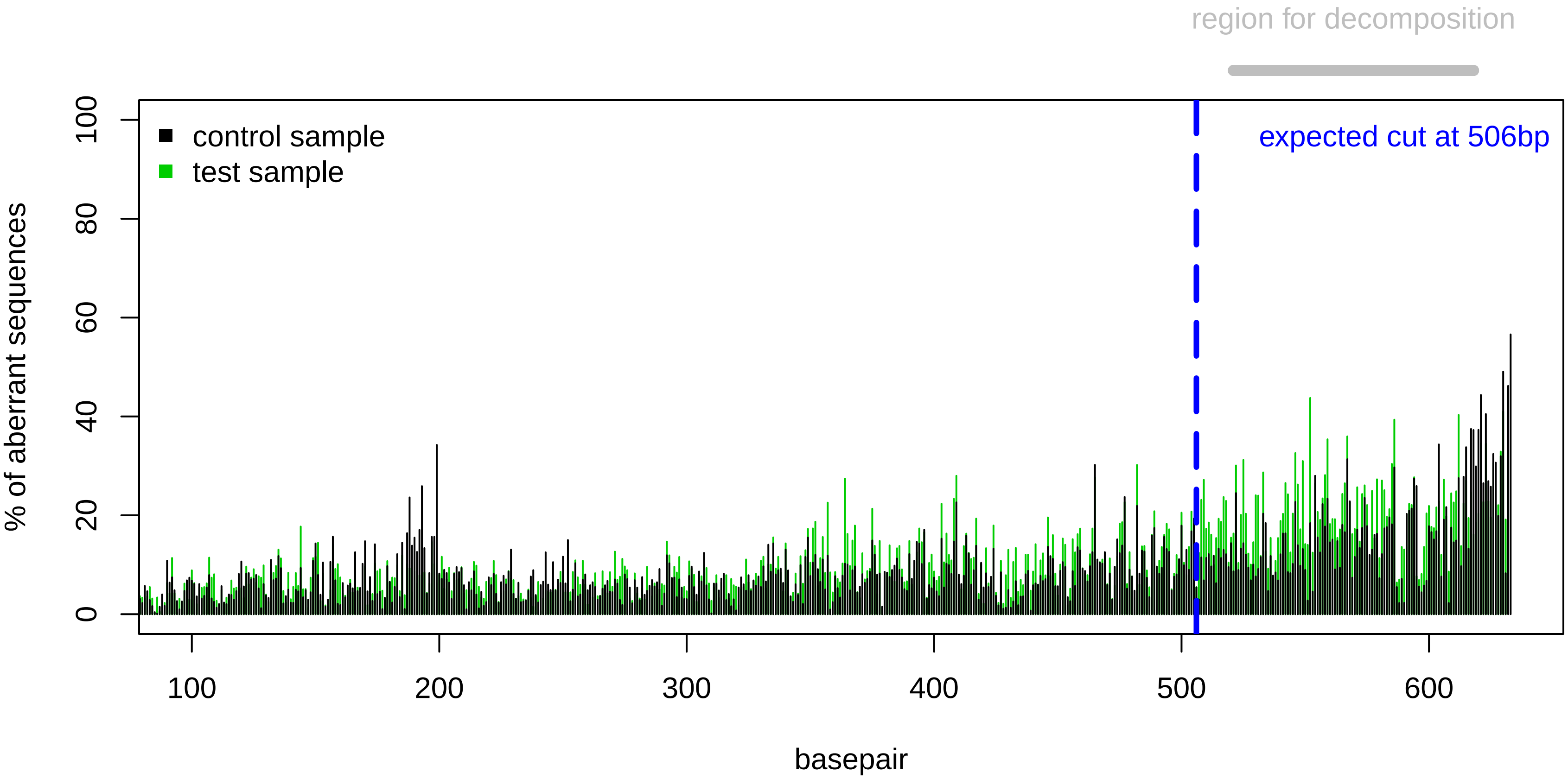

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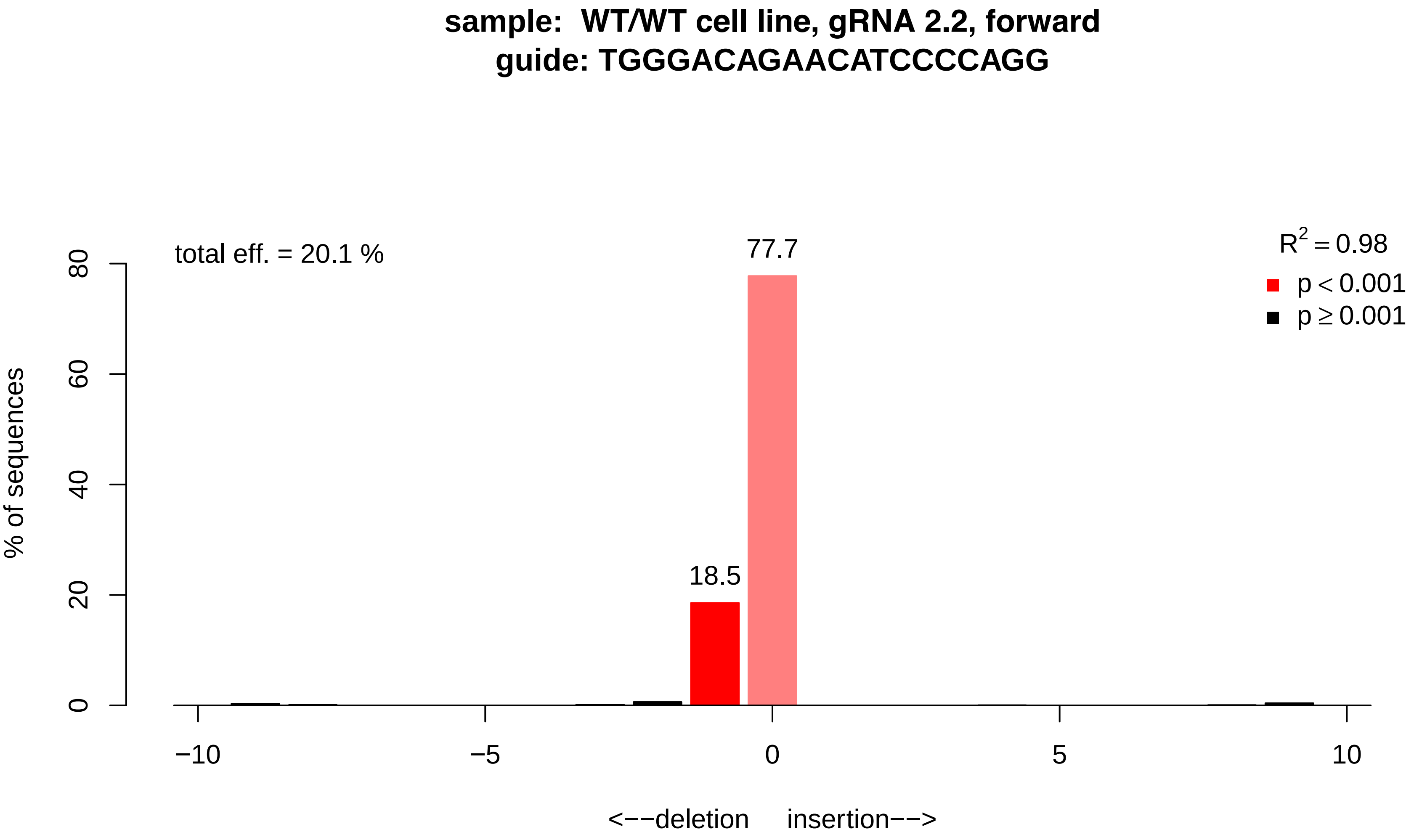
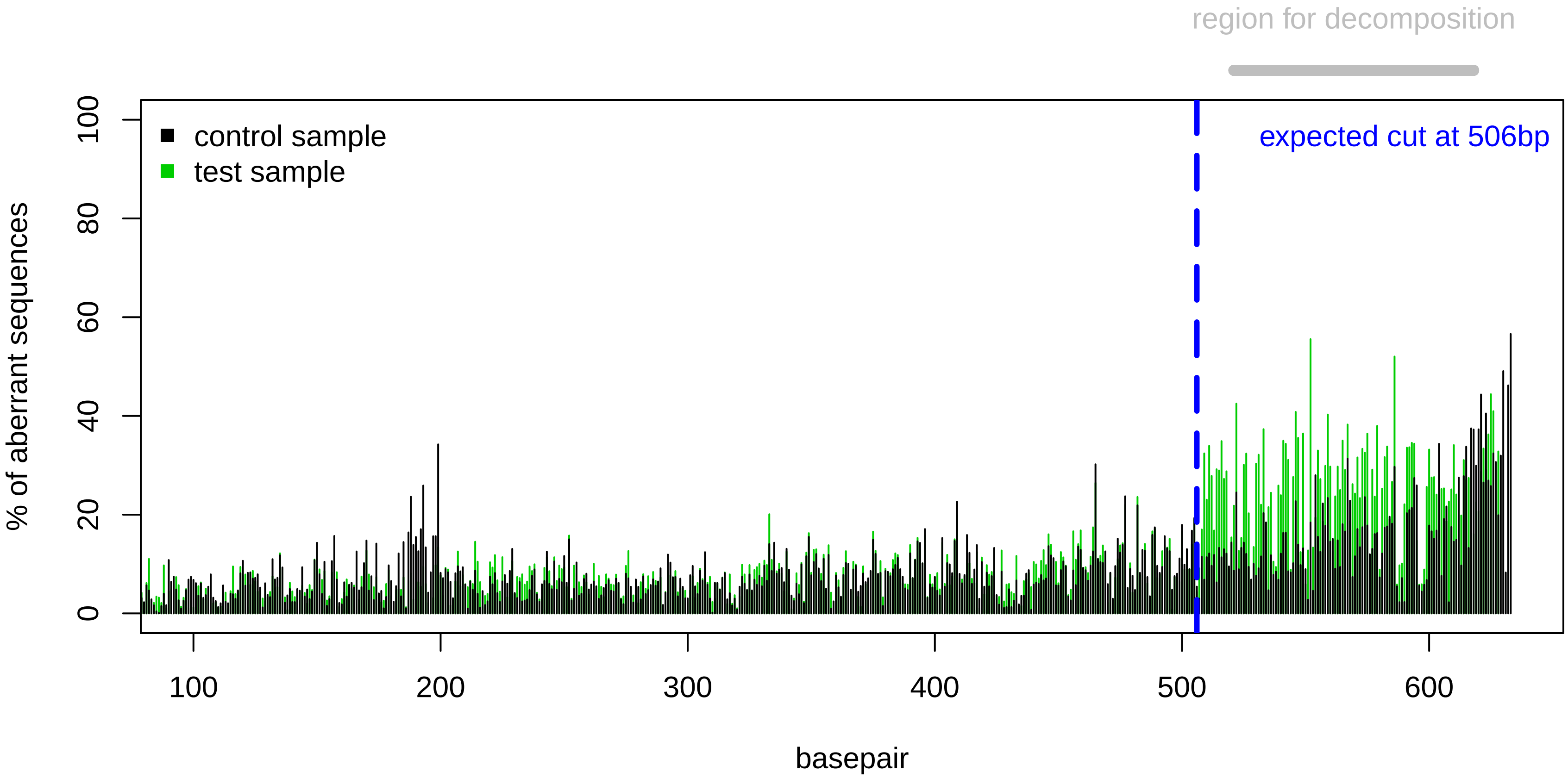

sample: WT/WT cell line, gRNA 2.2, forward
guide: TGGGACAGAACATCCCCAGG
total eff. = 20.1 %
$R^2 = 0.98$
🟥 $p < 0.001$
■ $p \geq 0.001$
77.7
18.5
% of sequences
0
20
40
60
80
−10
−5
0
5
10
<−−deletion insertion−−>

sample: WT/WT cell line, gRNA 2.2, forward
guide: TGGACAGAACATCCCCAGG



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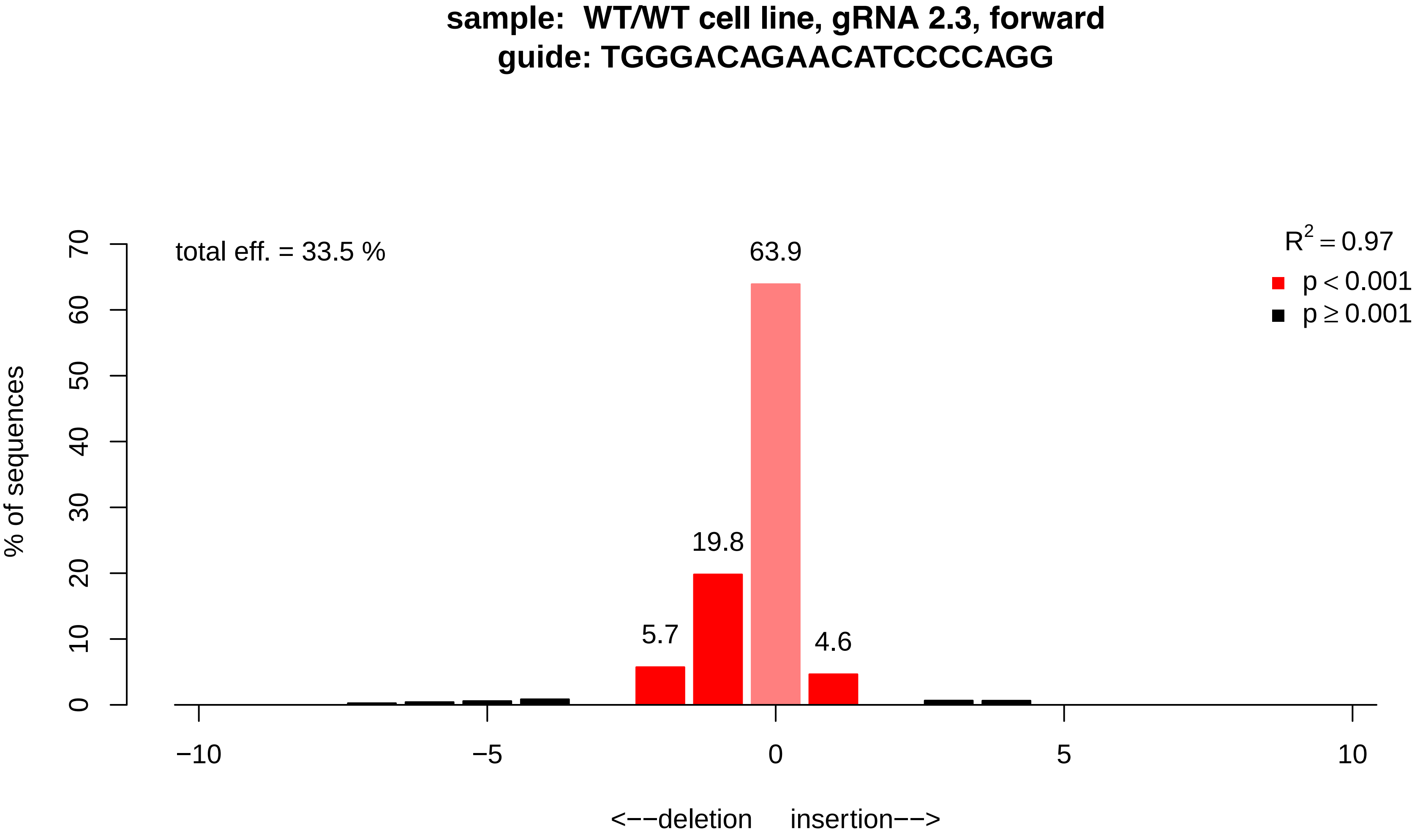
Warning: right boundary of decomposition window was adjusted to 618 . It must be at least 5bp plus the maximum indel size downstream of the expected break site



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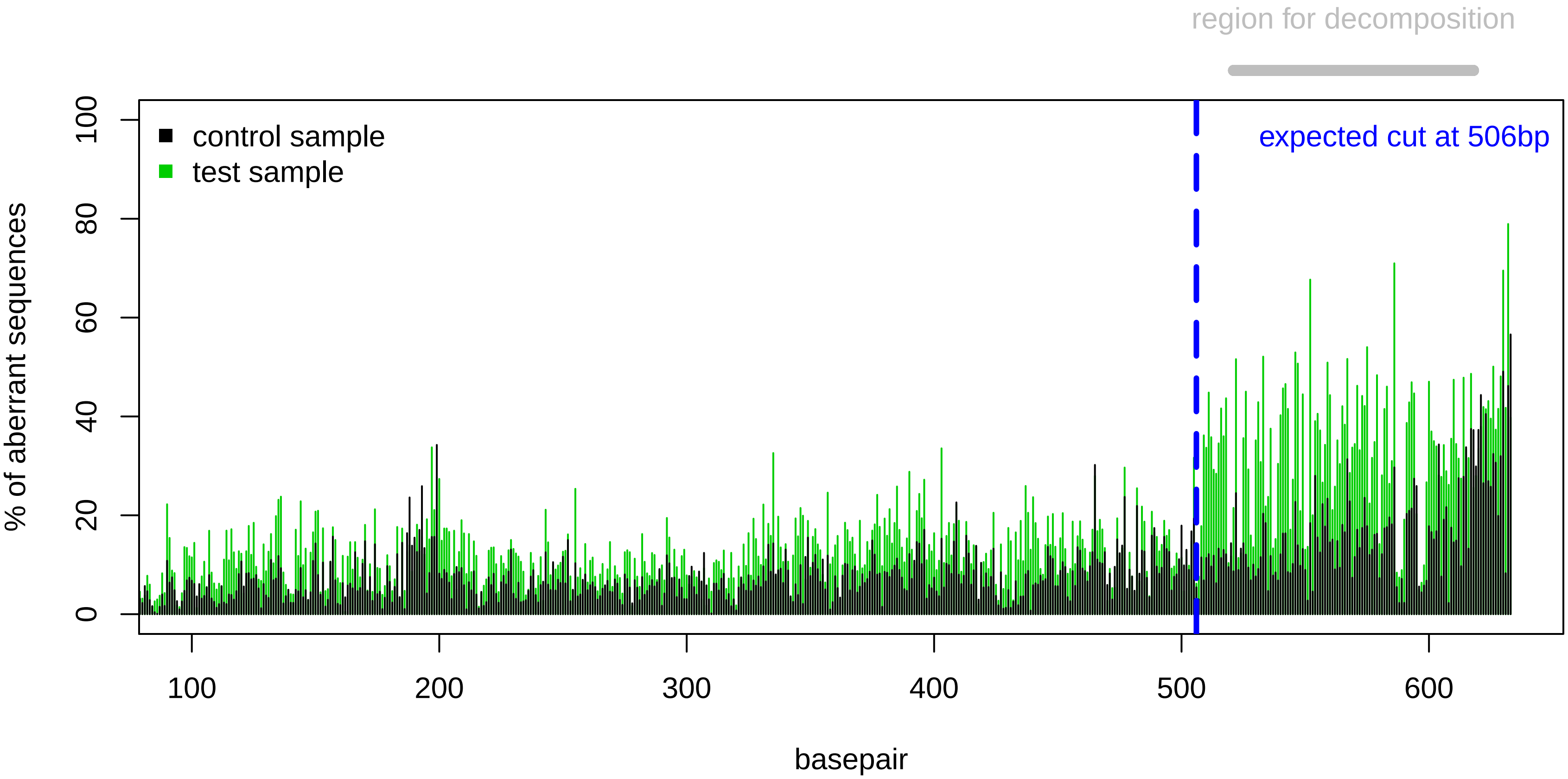
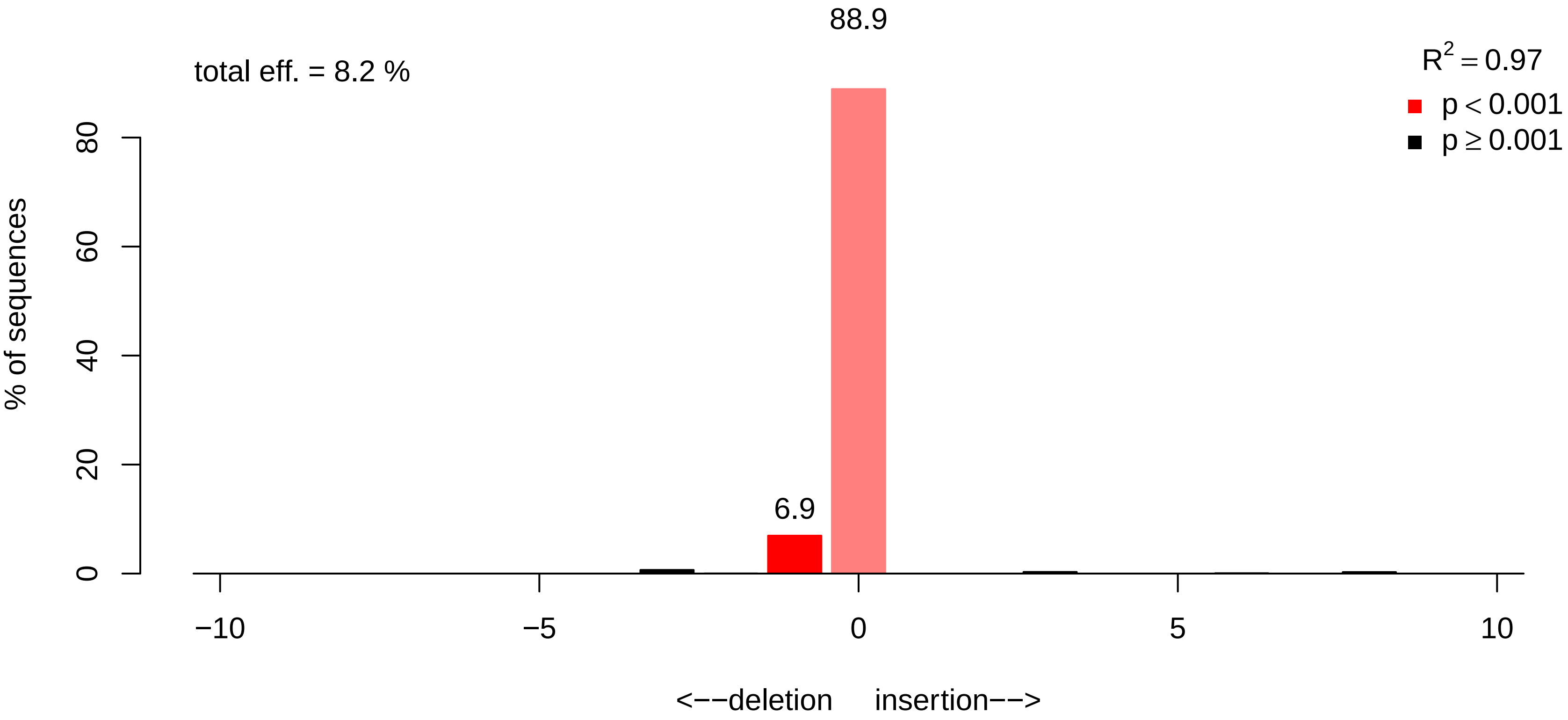
guide: TGGGACAGAACATCCCCAGG

sample: WT/WT cell line, gRNA 2.3, forward
guide: TGGGACAGAACATCCCCAGG



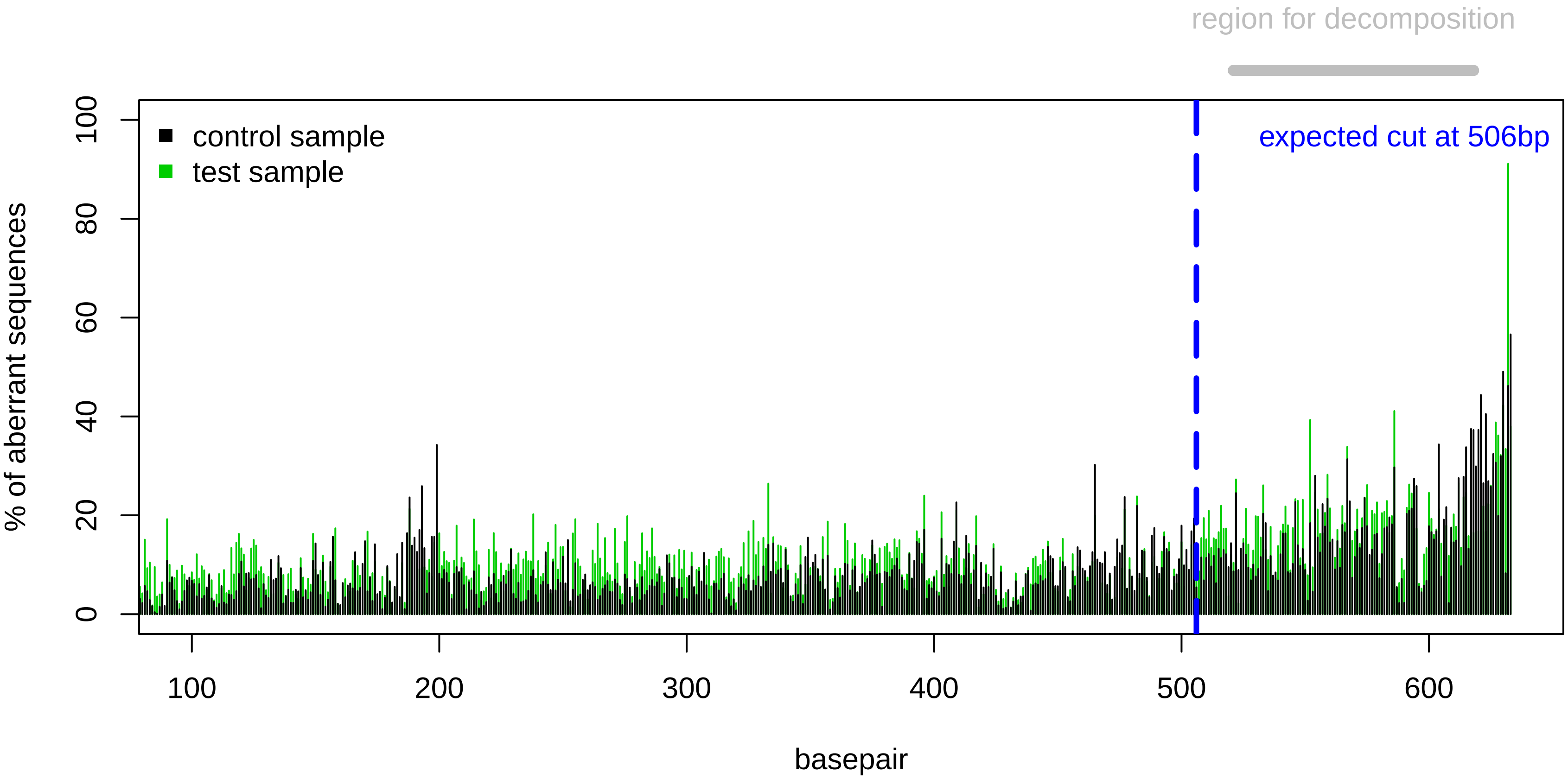
Warning: left boundary of decomposition window was adjusted 521 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 618 . It must be at least 5bp plus the maximum indel size downstream of the expected break site



sample: WT/WT cell line, gRNA 2.4, forward
guide: TGGTAATGTCCCTCCTGGGG

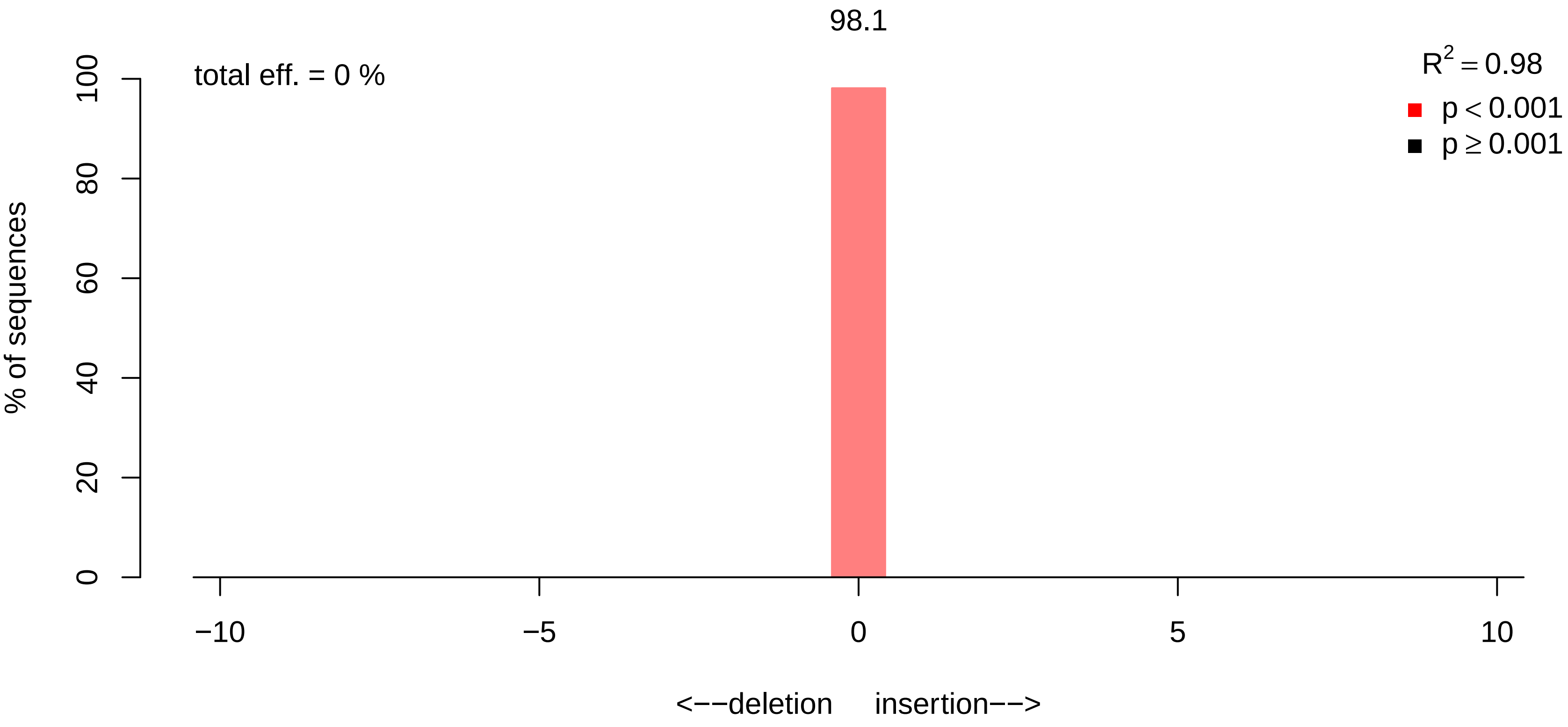
sample: WT/WT cell line, gRNA 2.4, forward
guide: TGGTAATGTCCCTCCTGGGG

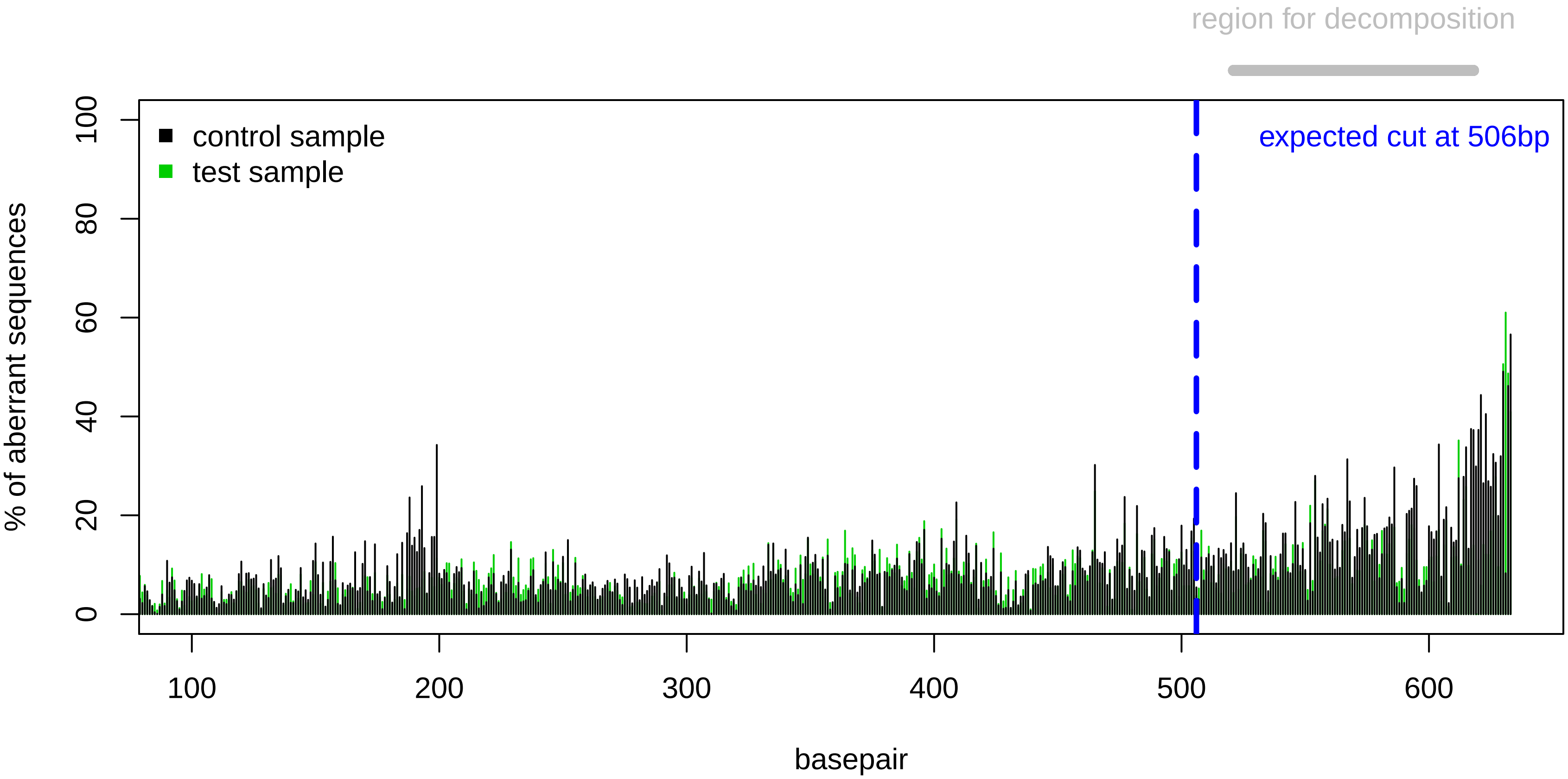


Warning: left boundary of decomposition window was adjusted 521 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 618 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: WT/WT cell line, gRNA 3.1, forward
guide: TGGTAATGTCCCTCCTGGGG



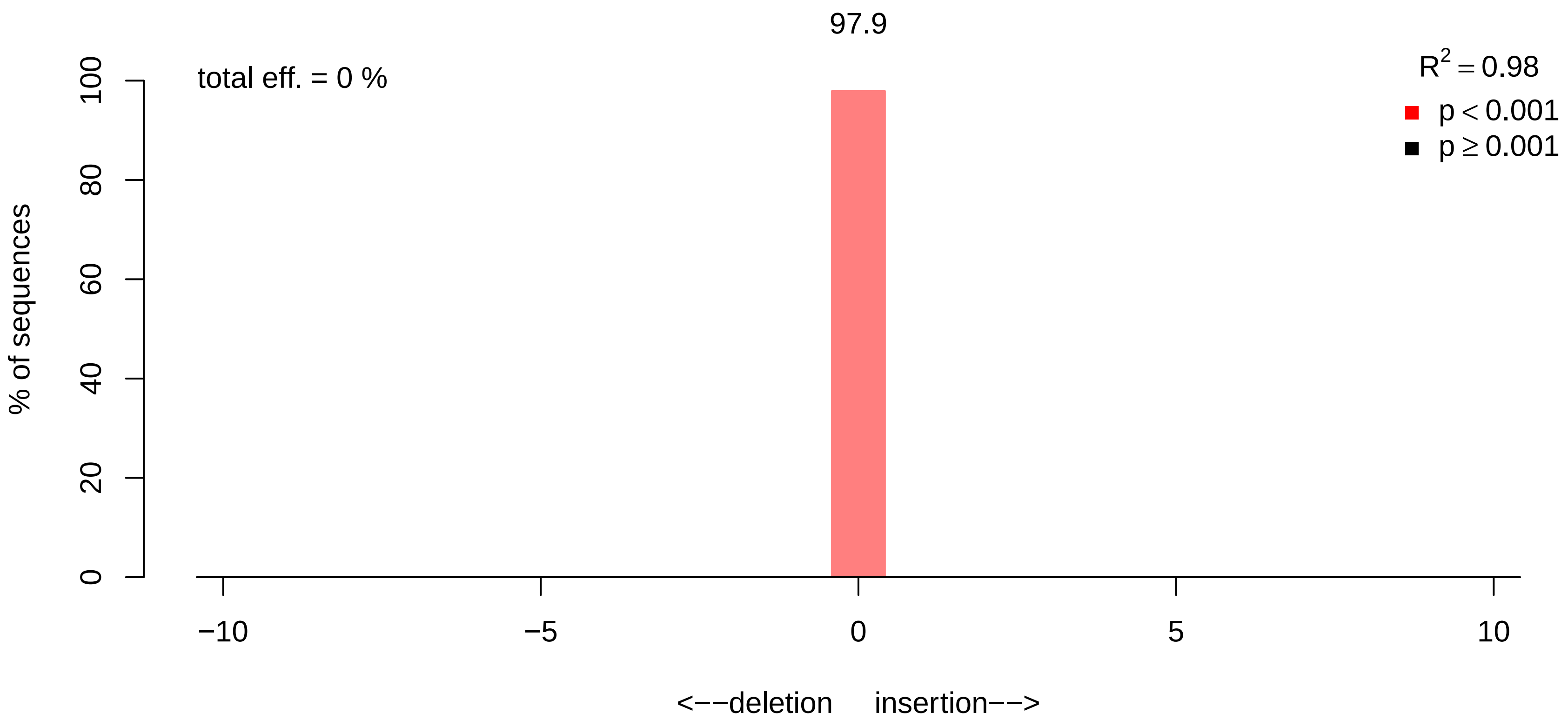


sample: WT/WT cell line, gRNA 3.1, forward
guide: TGGTAATGTCCCTCCTGGGG

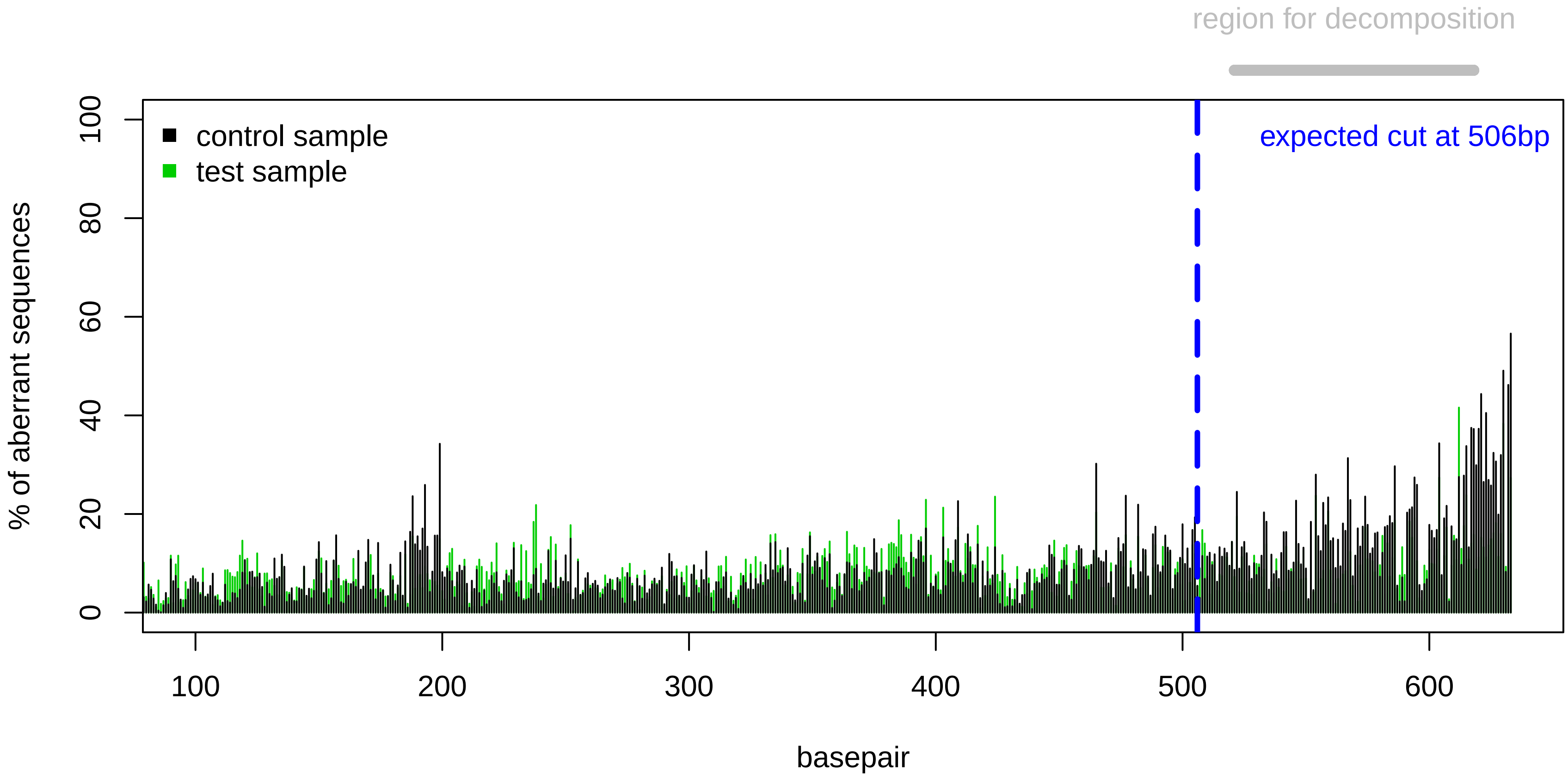
Warning: left boundary of decomposition window was adjusted 521 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 618 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: WT/WT cell line, gRNA 3.2, forward
guide: TGGTAATGTCCCTCCTGGGG



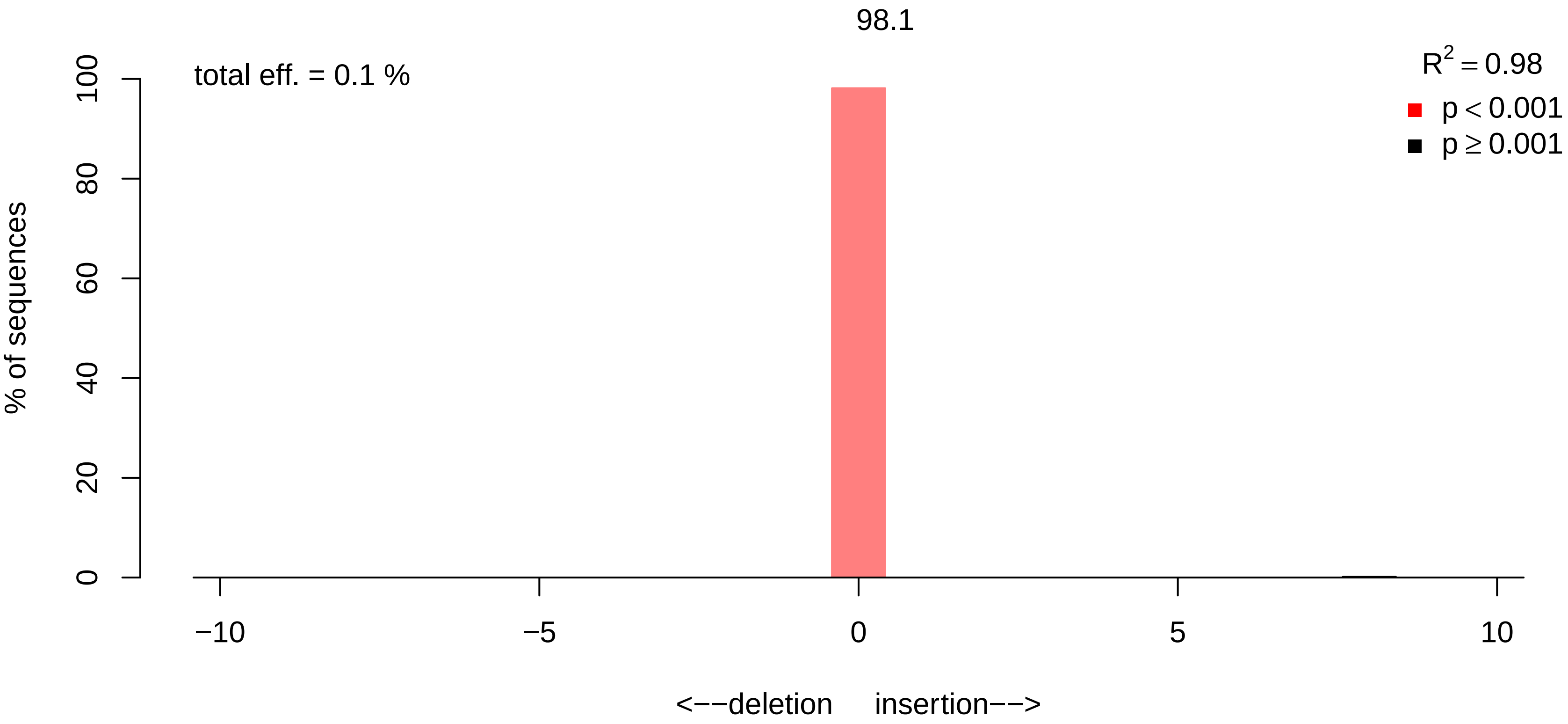
sample: WT/WT cell line, gRNA 3.2, forward
guide: TGGTAATGTCCCTCCTGGGG



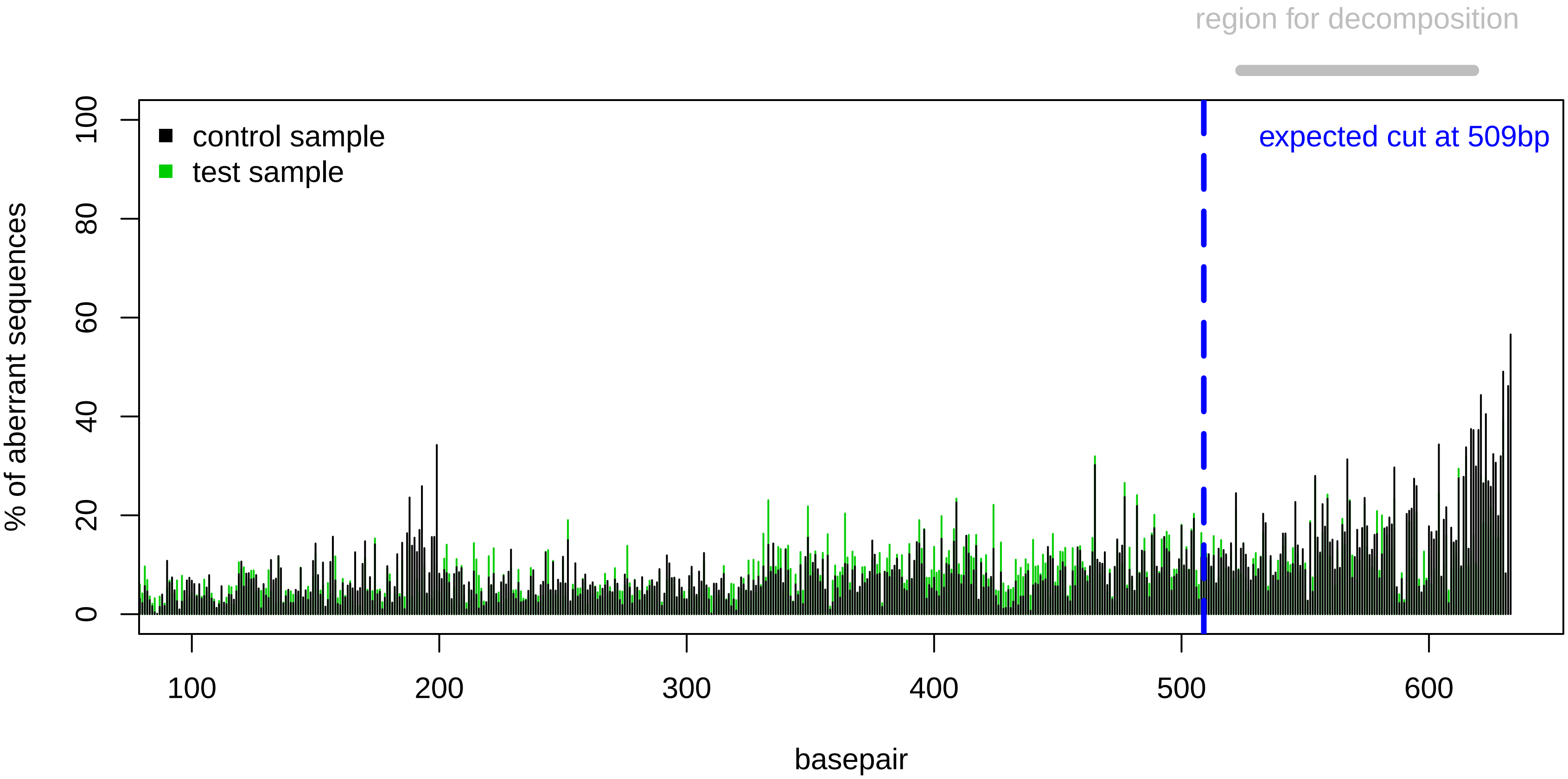
Warning: left boundary of decomposition window was adjusted 521 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 618 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: WT/WT cell line, gRNA 3,3, forward
guide: GGGTGGGACAGAACATCCCC



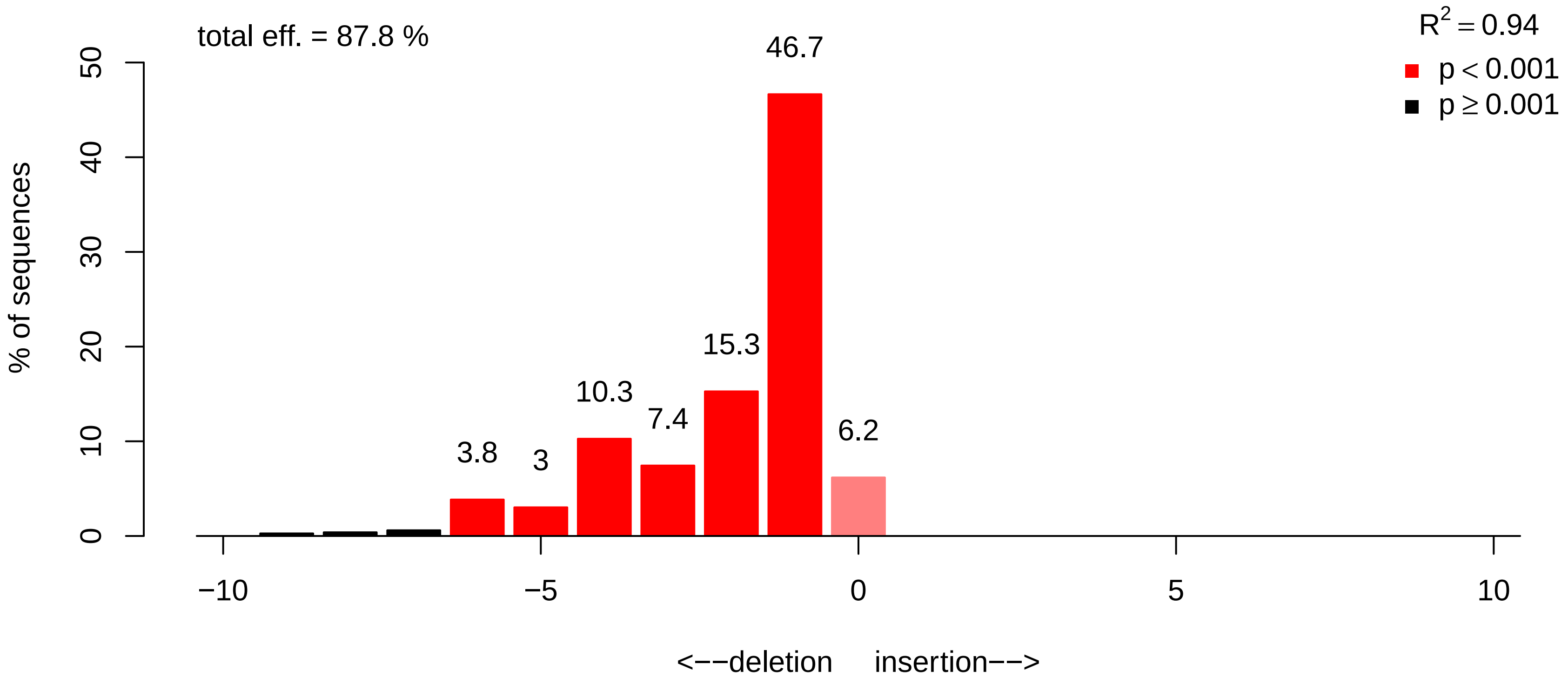
sample: WT/WT cell line, gRNA 3.3, forward
guide: GGGTGGGACAGAACATCCCC



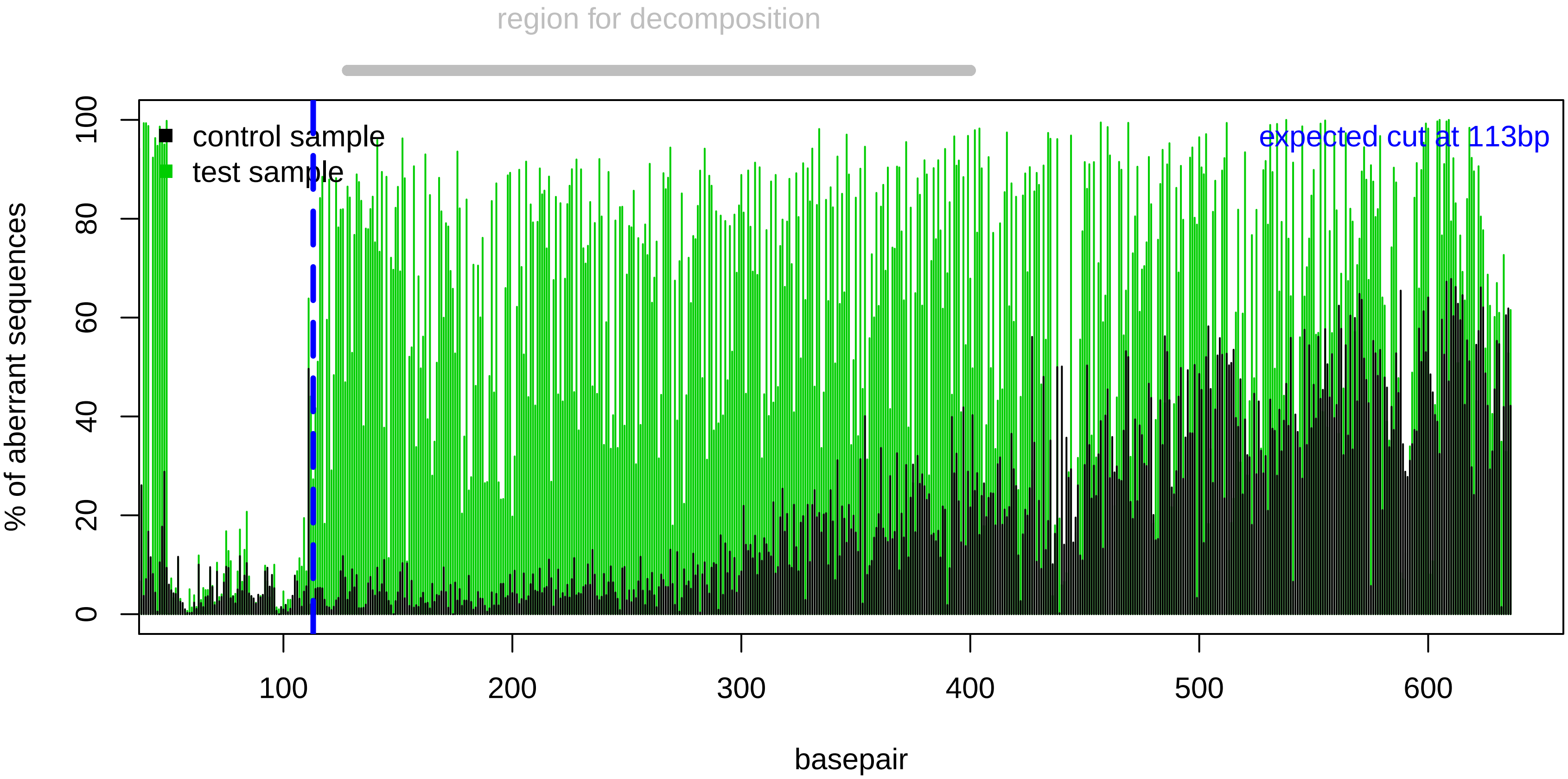
Warning: left boundary of decomposition window was adjusted 524 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 618 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: Bth/WT cell line, gRNA WT, reverse
guide: GGGTGGGACAGAACTTCCCC

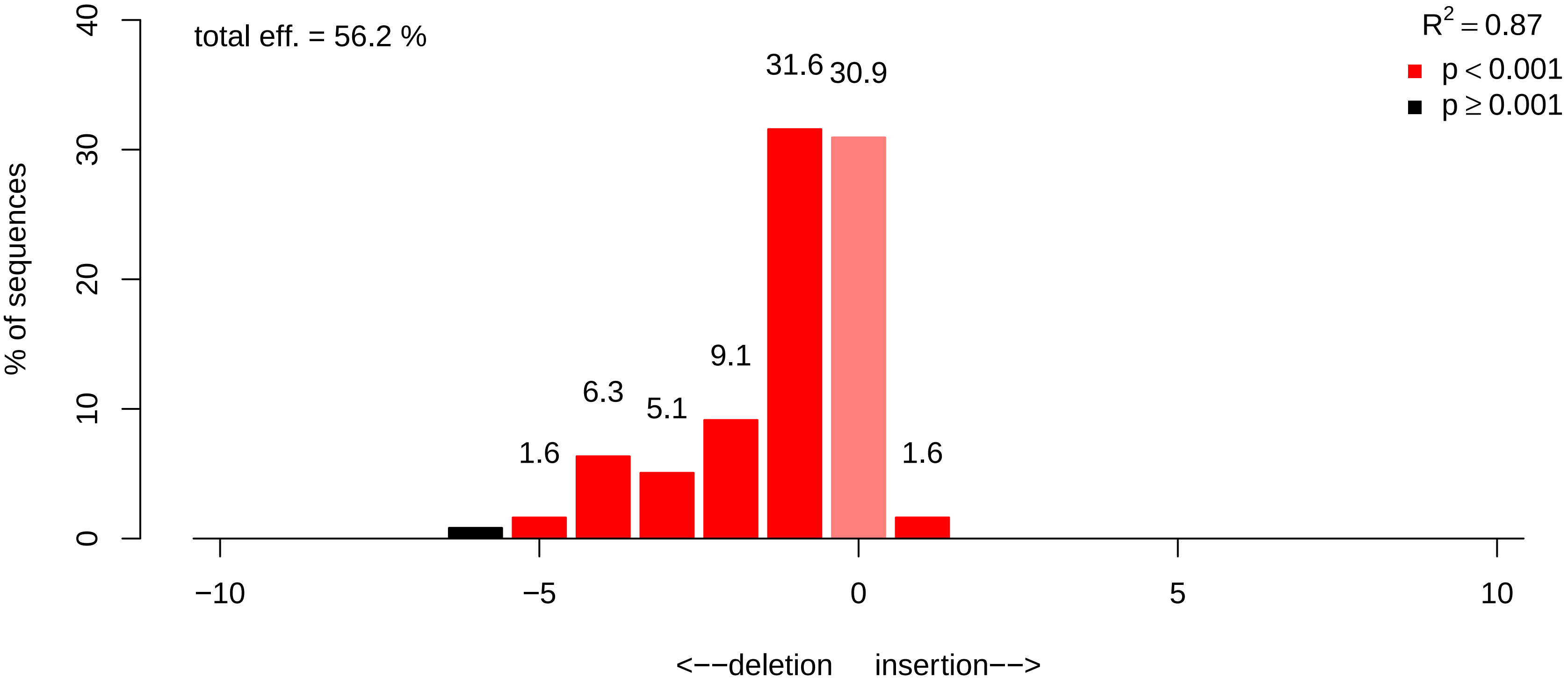


sample: Bth/WT cell line, gRNA WT, reverse
guide: GGGTGGGACAGAACTTCCCC

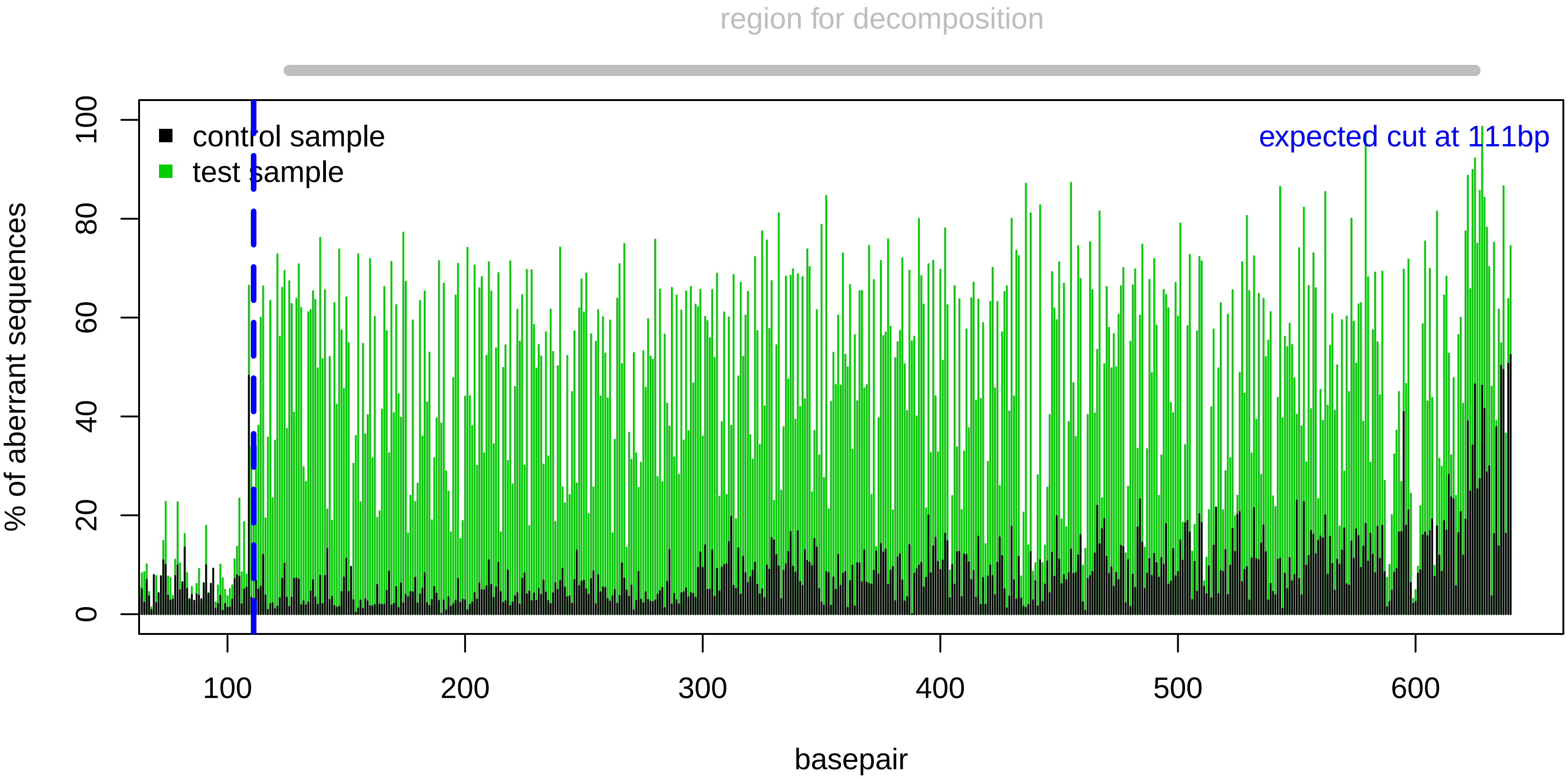


Warning: left boundary of decomposition window was adjusted 128 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: Bth/WT cell line, gRNA 1.1, reverse
guide: GGGTGGGACAGAACTTCCCC



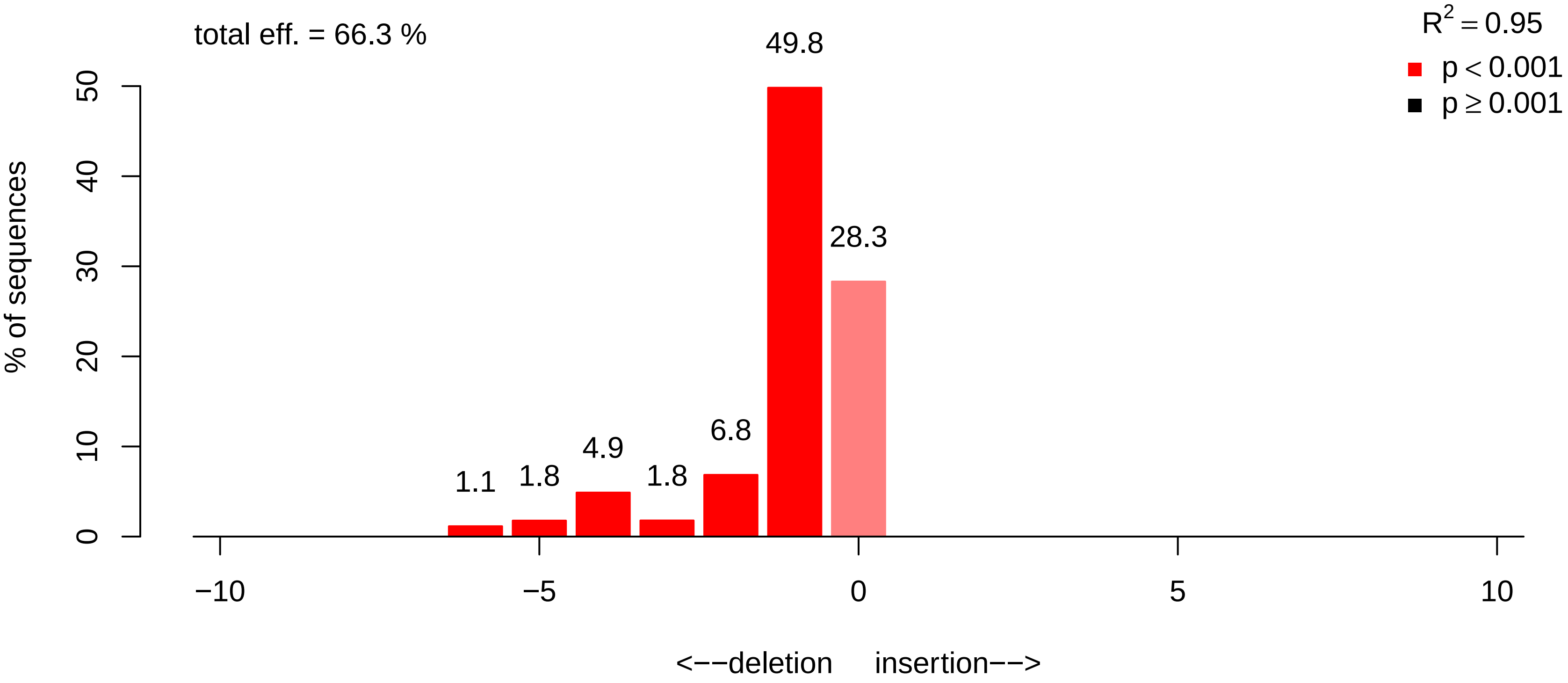
sample: Bth/WT cell line, gRNA 1.1, reverse
guide: GGGTGGGACAGAACTTCCCC



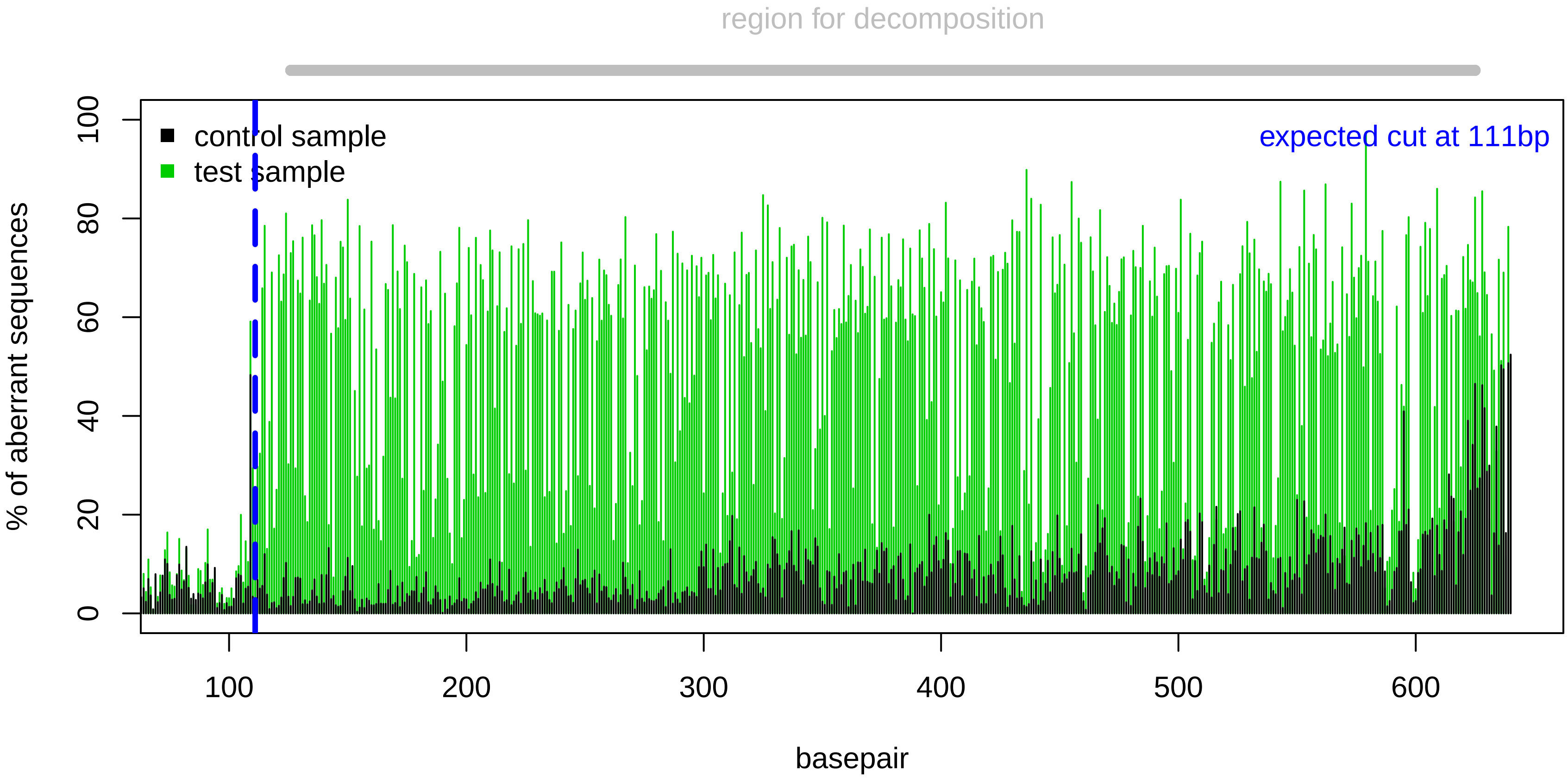
Warning: left boundary of decomposition window was adjusted 126 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 625 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: Bth/WT cell line, gRNA 1.2, reverse
guide: GGGTGGGACAGAACTTCCCC

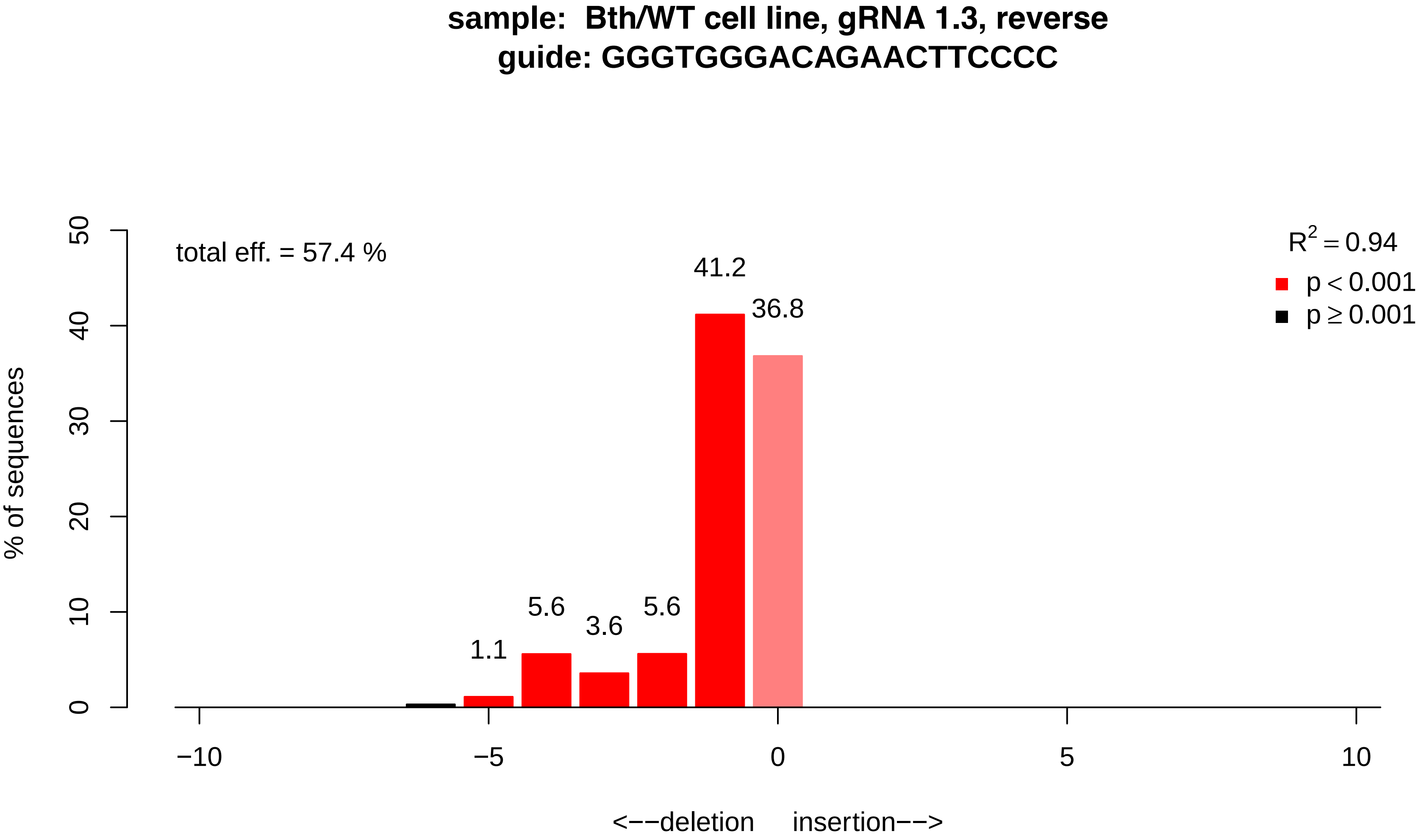


sample: Bth/WT cell line, gRNA 1.2, reverse
guide: GGGTGGGACAGAACTTCCCC

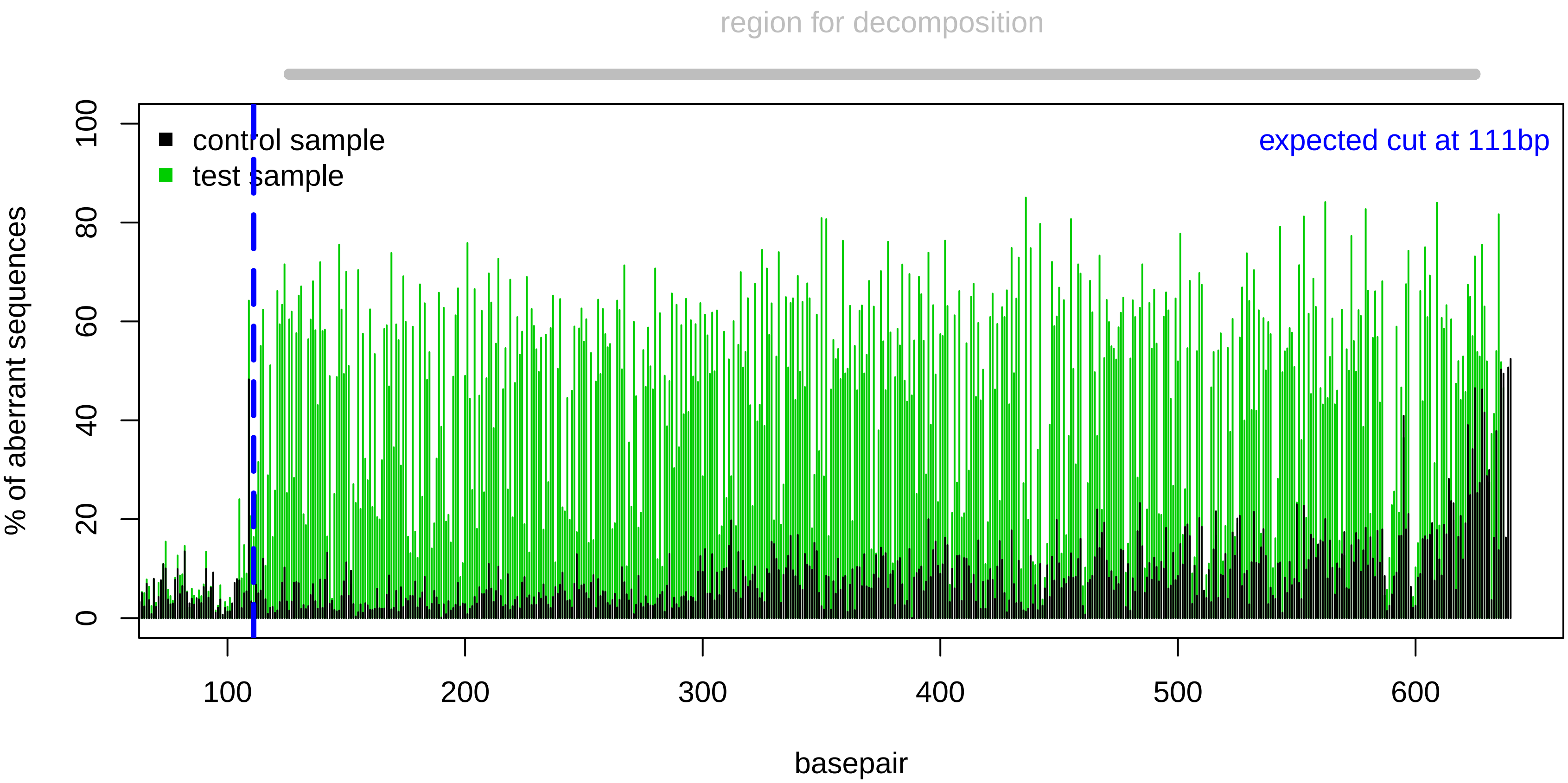


Warning: left boundary of decomposition window was adjusted 126 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 625 . It must be at least 5bp plus the maximum indel size downstream of the expected break site


sample: Bth/WT cell line, gRNA 1.3, reverse
guide: GGGTGGGACAGAACTTCCCC
total eff. = 57.4 %
$R^2 = 0.94$
$p < 0.001$
$p \geq 0.001$
41.2
36.8
5.6
3.6
5.6
1.1
% of sequences
0
10
20
30
40
50
−10
−5
0
5
10
<−−deletion insertion−−>

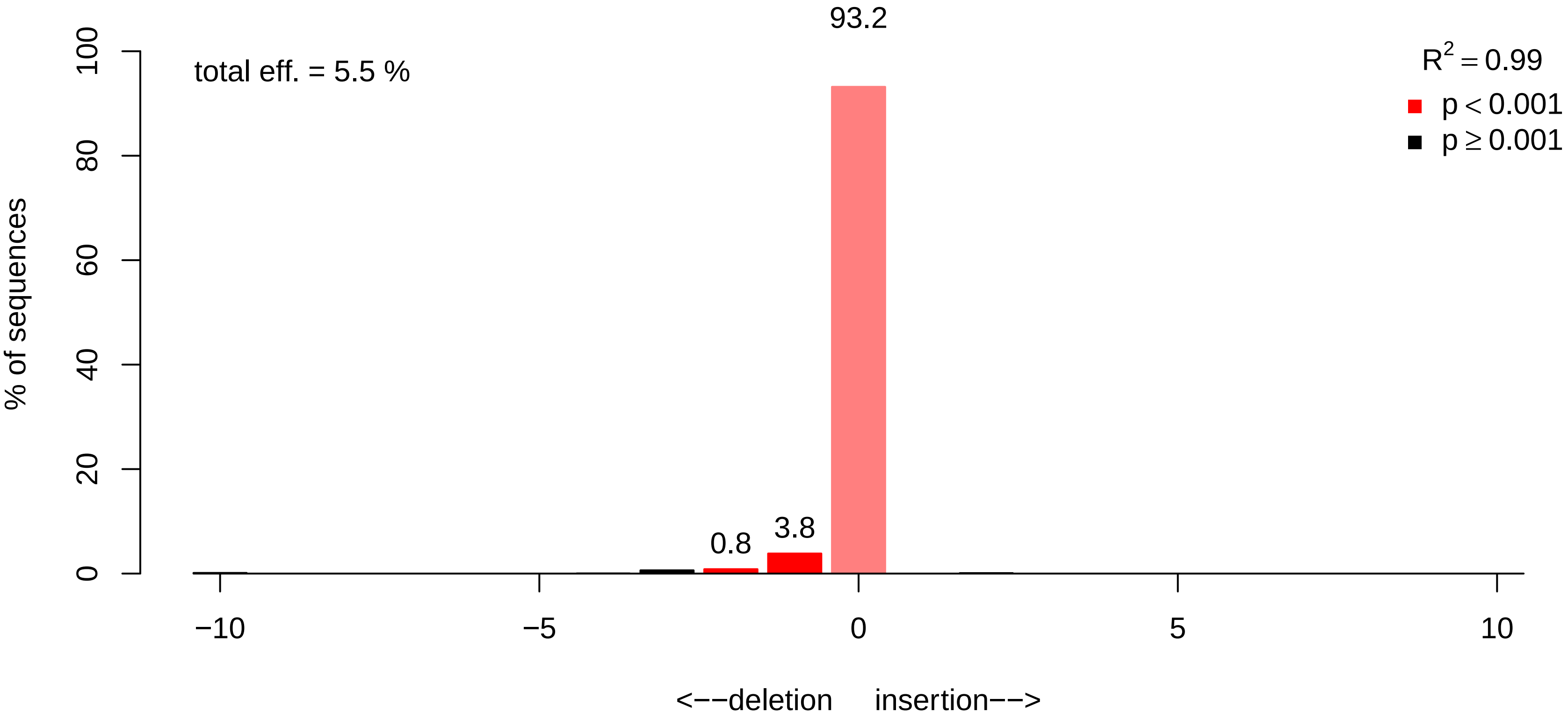
sample: Bth/WT cell line, gRNA 1.3, reverse
guide: GGGTGGGACAGAACTTCCCC



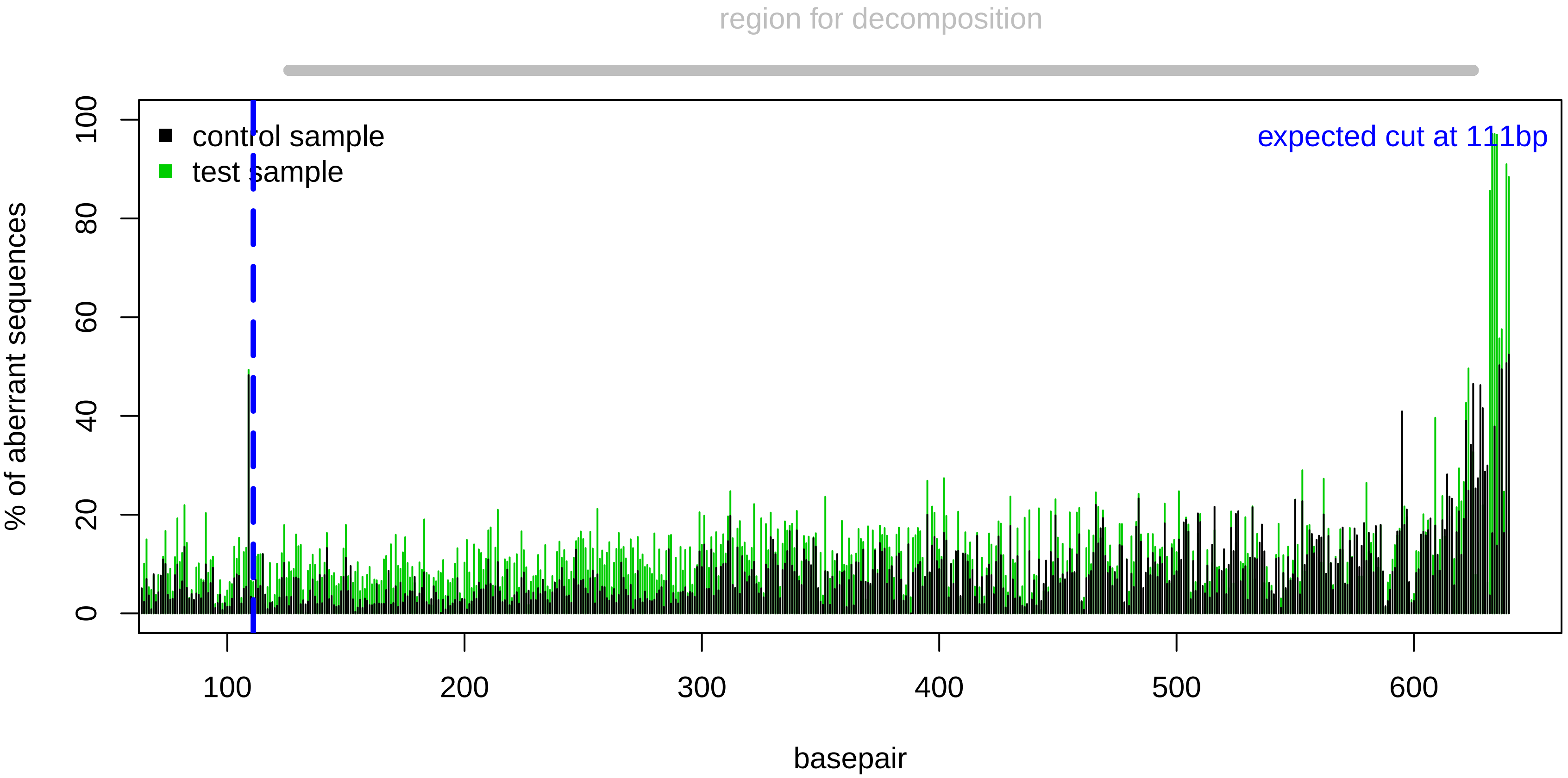
Warning: left boundary of decomposition window was adjusted 126 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 625 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: Bth/WT cell line, gRNA 1.4, reverse
guide: GGGTGGGACAGAACTTCCCC



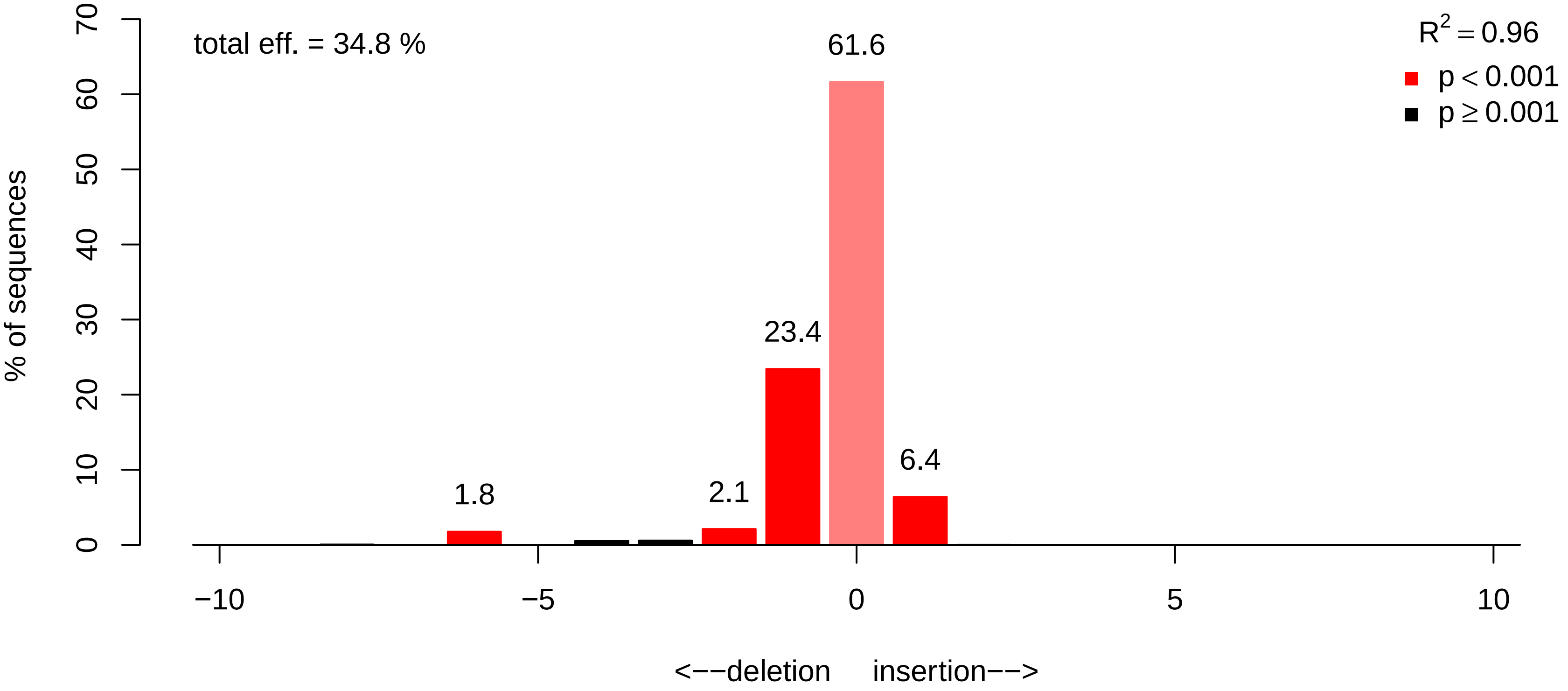
sample: Bth/WT cell line, gRNA 1.4, reverse
guide: GGGTGGGACAGAACTTCCCC



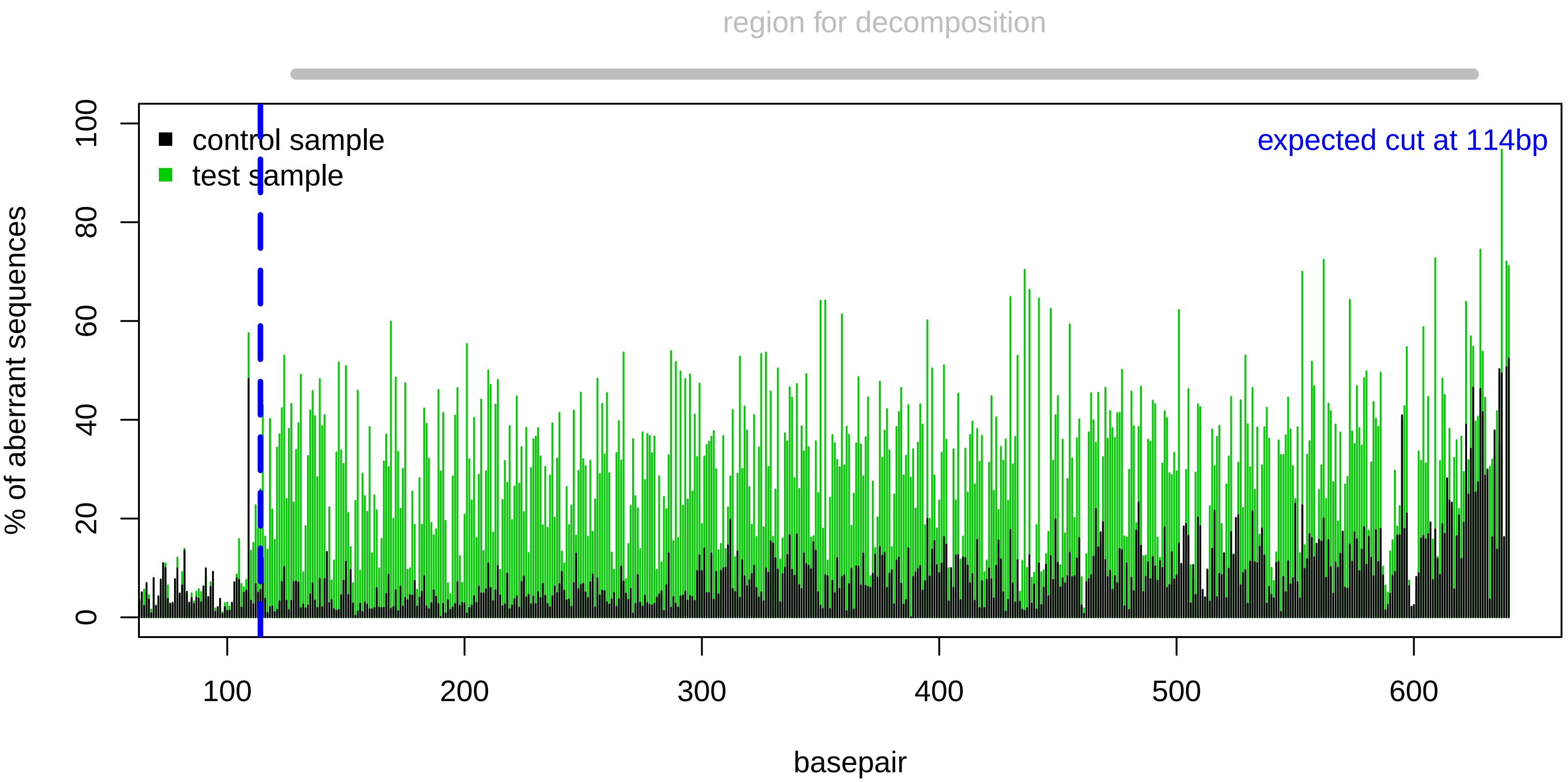
Warning: left boundary of decomposition window was adjusted 126 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 625 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: Bth/WT cell line, gRNA 2.1, reverse
guide: TGGGACAGAACTTCCCCAGG

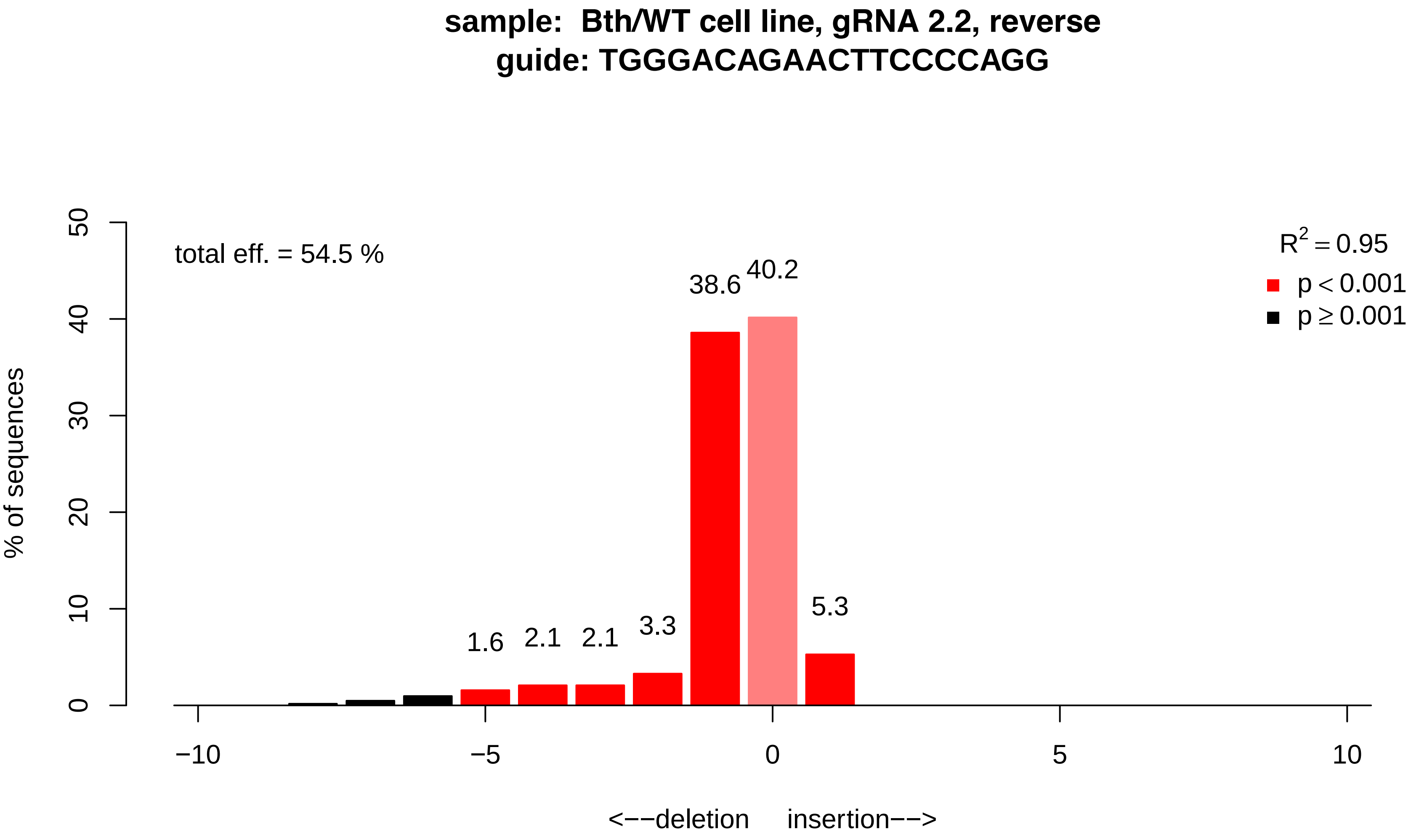


sample: Bth/WT cell line, gRNA 2.1, reverse
guide: TGGGACAGAACTTCCCCAGG

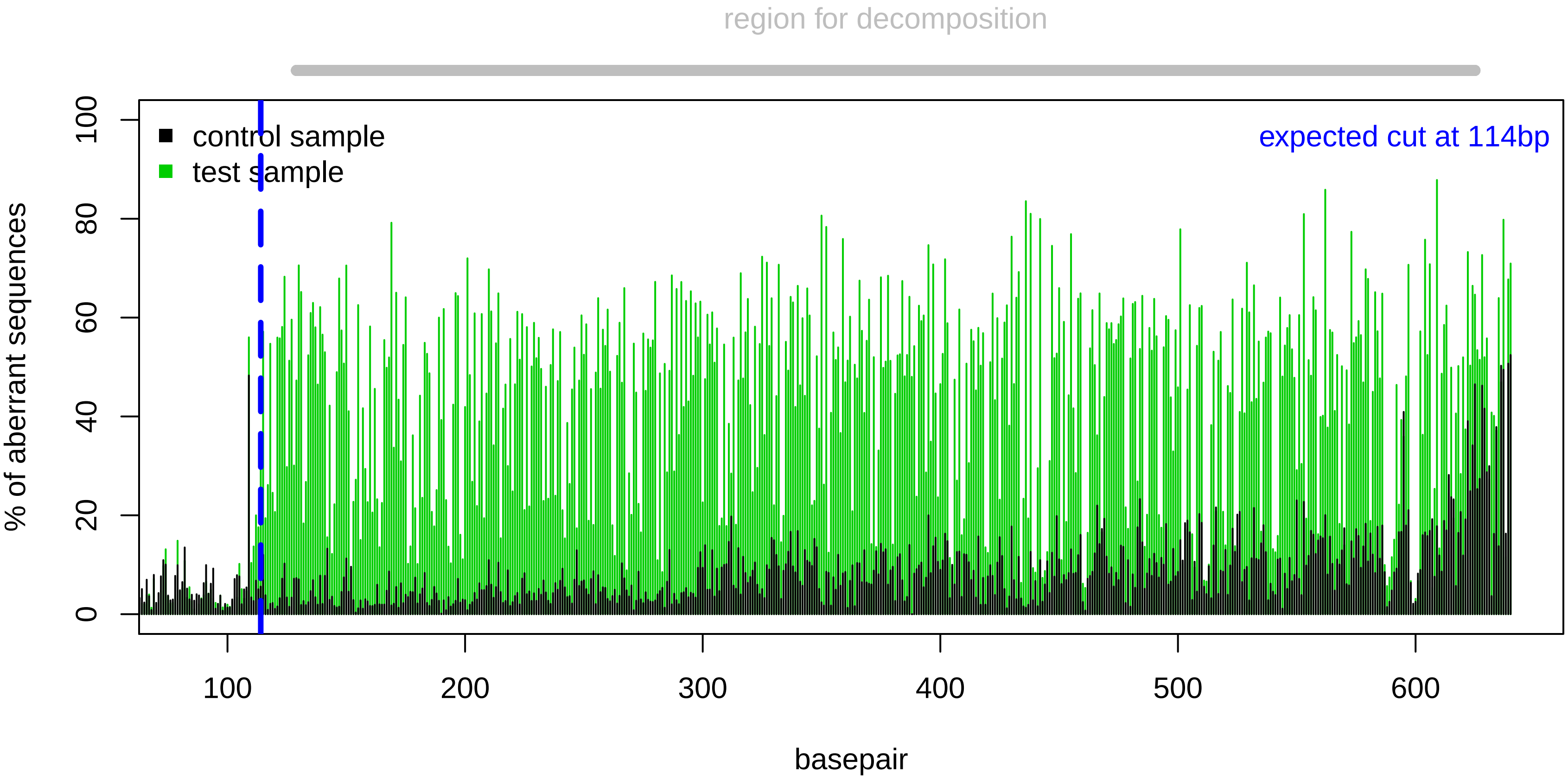


Warning: left boundary of decomposition window was adjusted 129 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 625 . It must be at least 5bp plus the maximum indel size downstream of the expected break site


sample: Bth/WT cell line, gRNA 2.2, reverse
guide: TGGGACAGAACTTCCCCAGG
total eff. = 54.5 %
$R^2 = 0.95$
🟥 $p < 0.001$
■ $p \geq 0.001$
% of sequences
0
10
20
30
40
50
1.6
2.1
2.1
3.3
38.6
40.2
5.3
−10
−5
0
5
10
<−−deletion insertion−−>

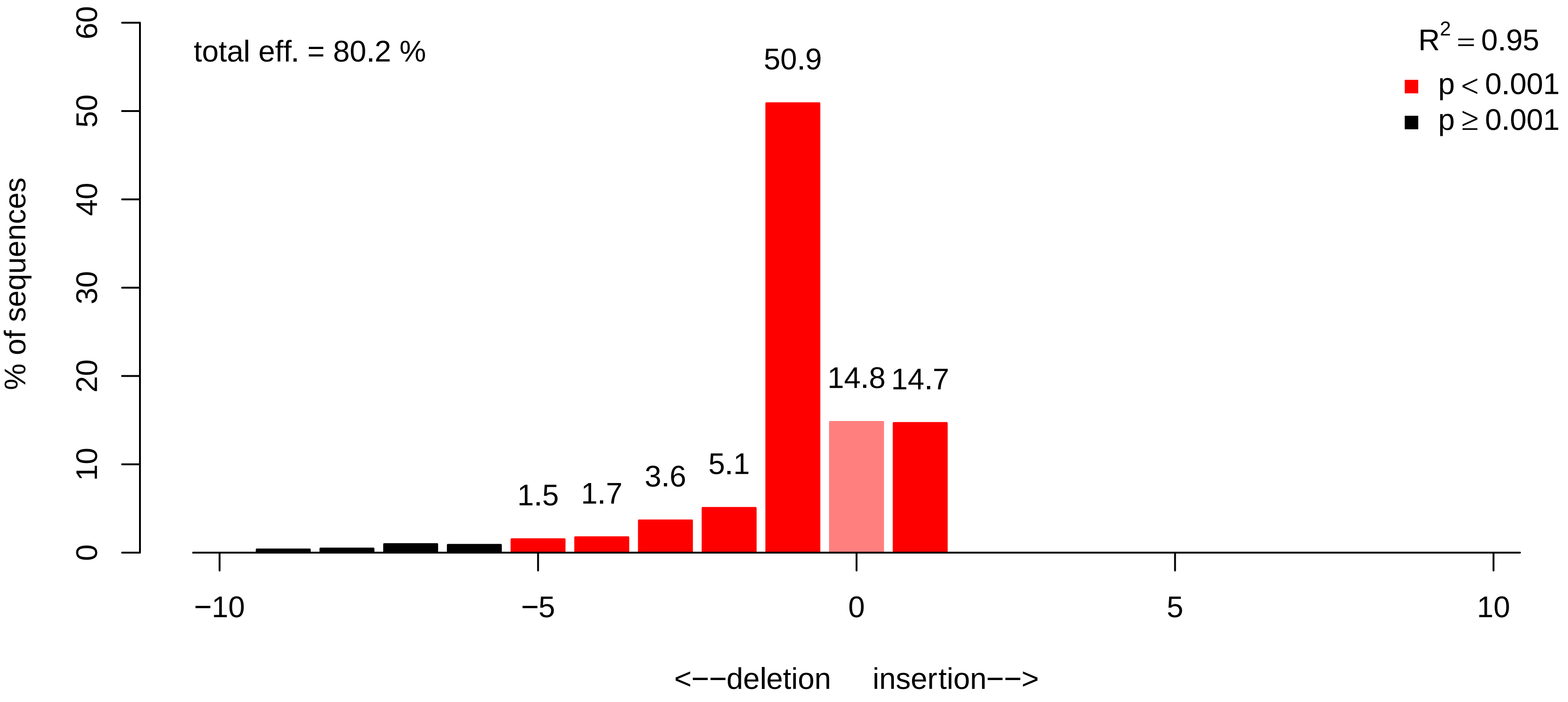
sample: Bth/WT cell line, gRNA 2.2, reverse
guide: TGGGACAGAACTTCCCCAGG



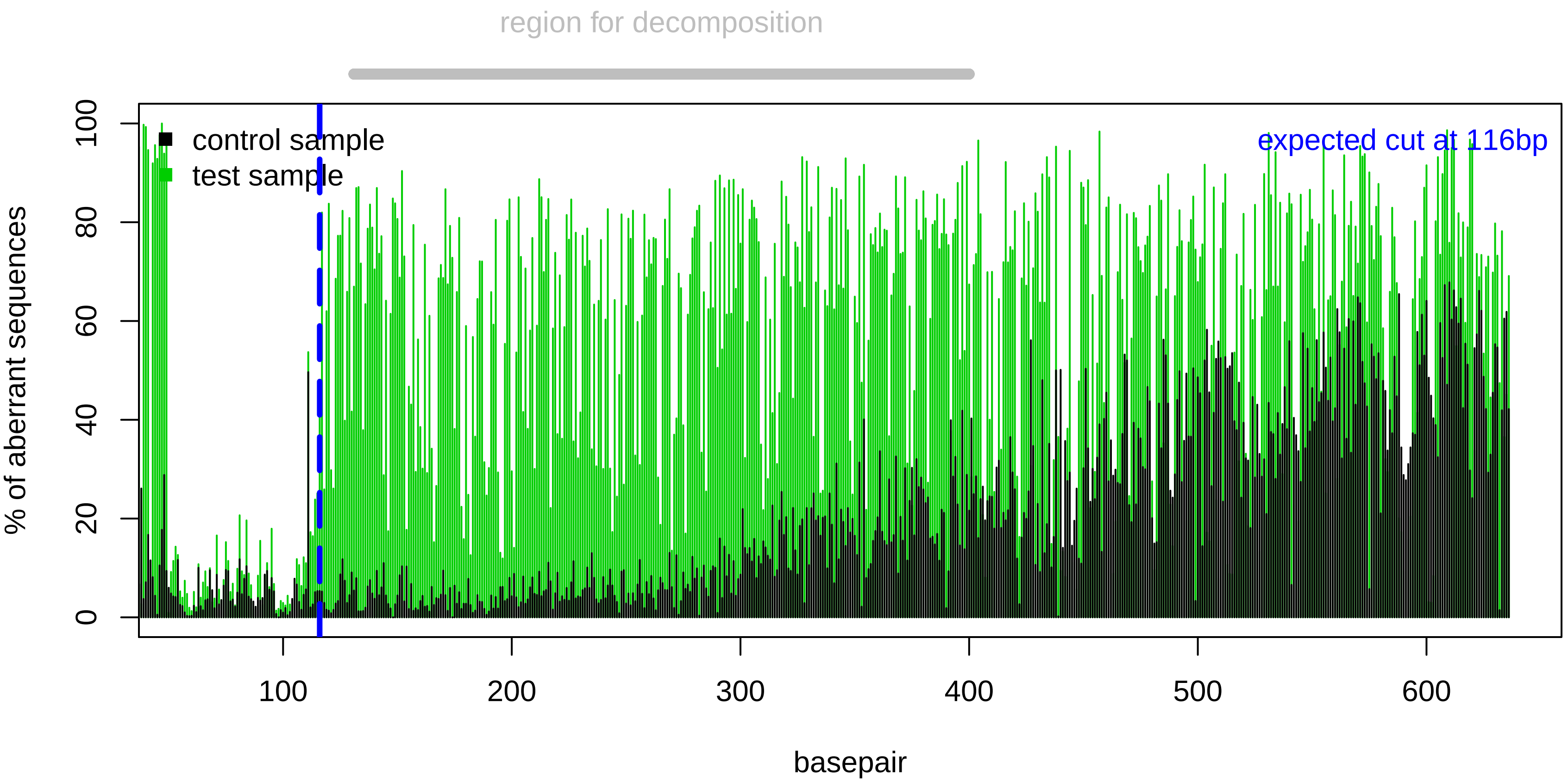
Warning: left boundary of decomposition window was adjusted 129 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 625 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

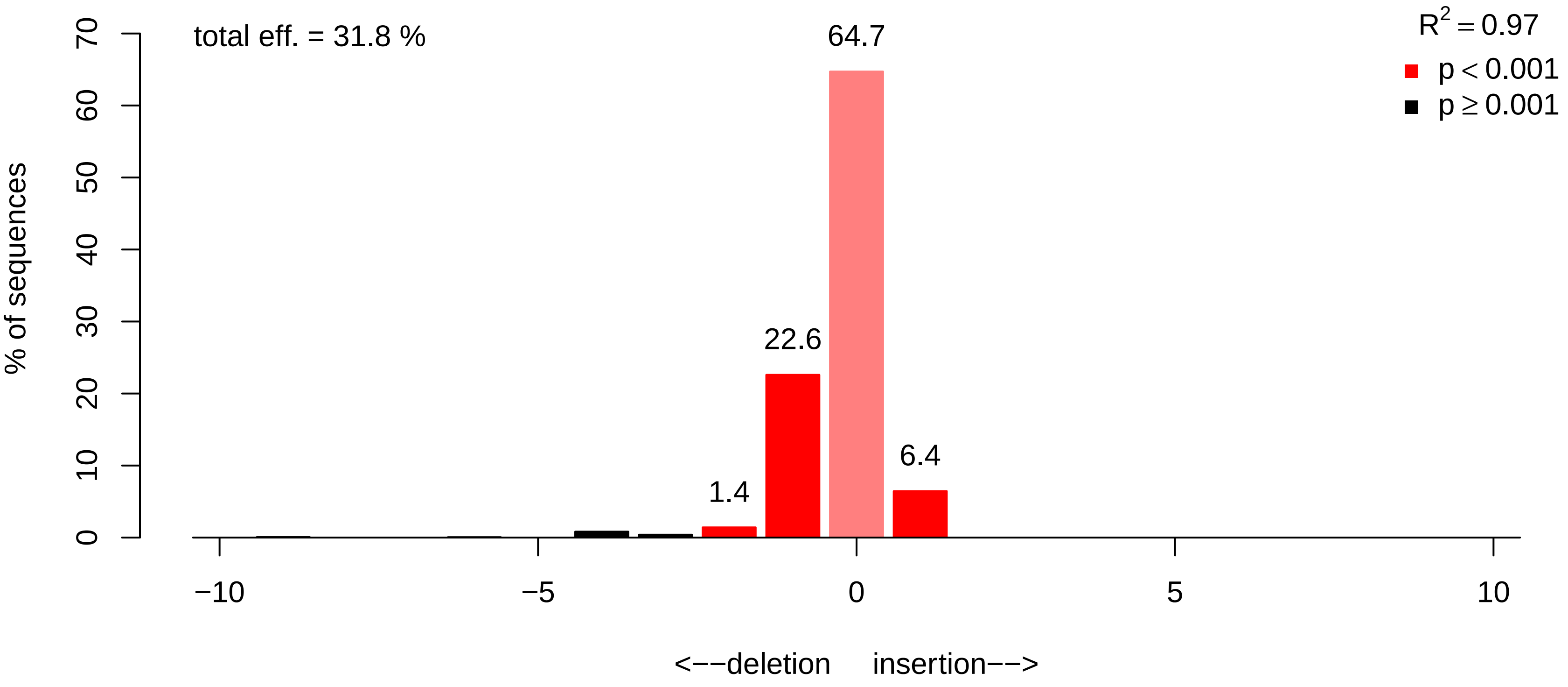
sample: Bth/WT cell line, gRNA 2.3, reverse
guide: TGGGACAGAACTTCCCCAGG



sample: Bth/WT cell line, gRNA 2.3, reverse
guide: TGGGACAGAACTTCCCCAGG

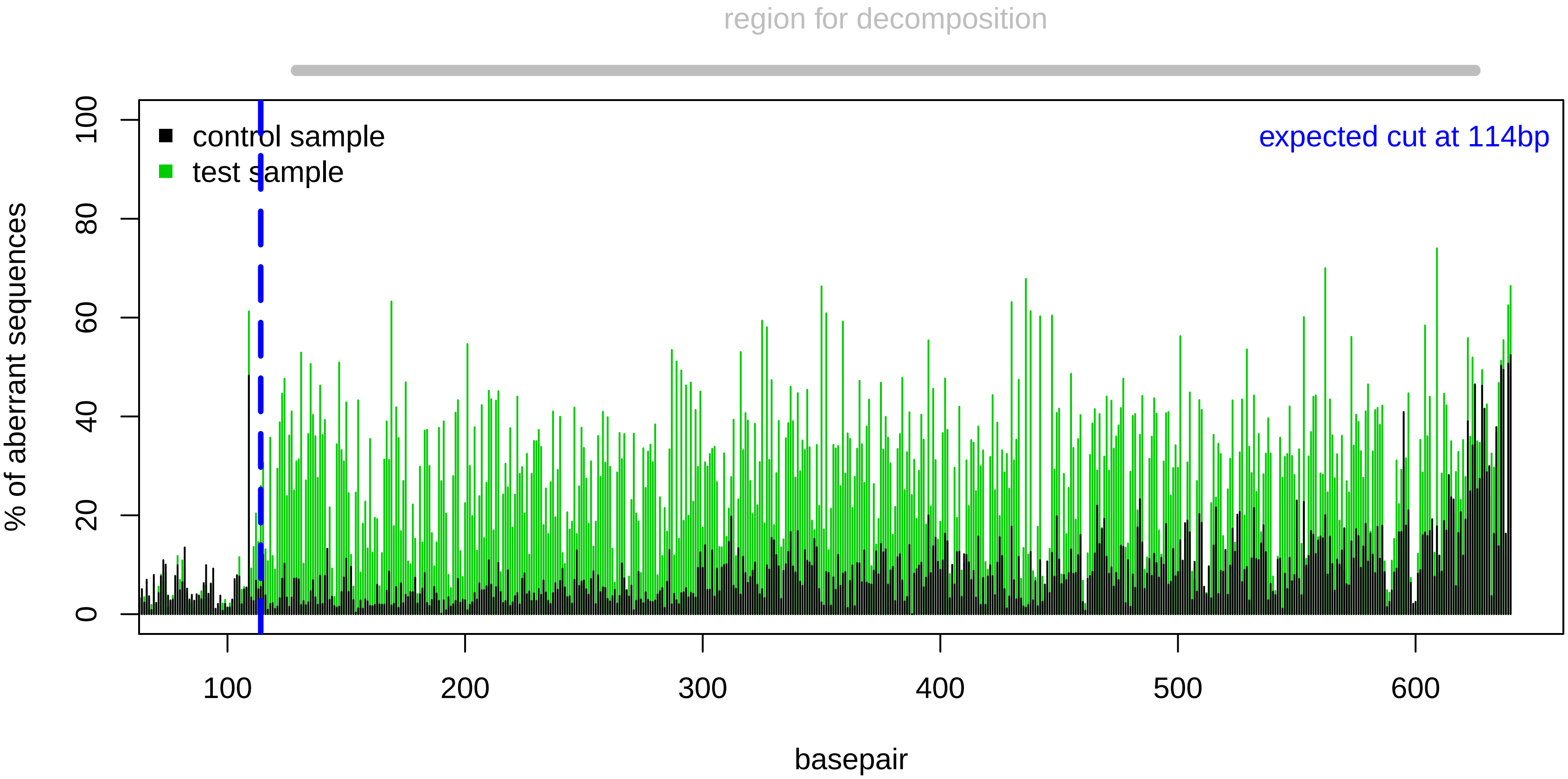


Warning: left boundary of decomposition window was adjusted 131 . It must be at least 5bp plus the maximum indel size downstream of the expected break site



sample: Bth/WT cell line, gRNA 2.4, reverse
guide: TGGGACAGAACTTCCCCAGG

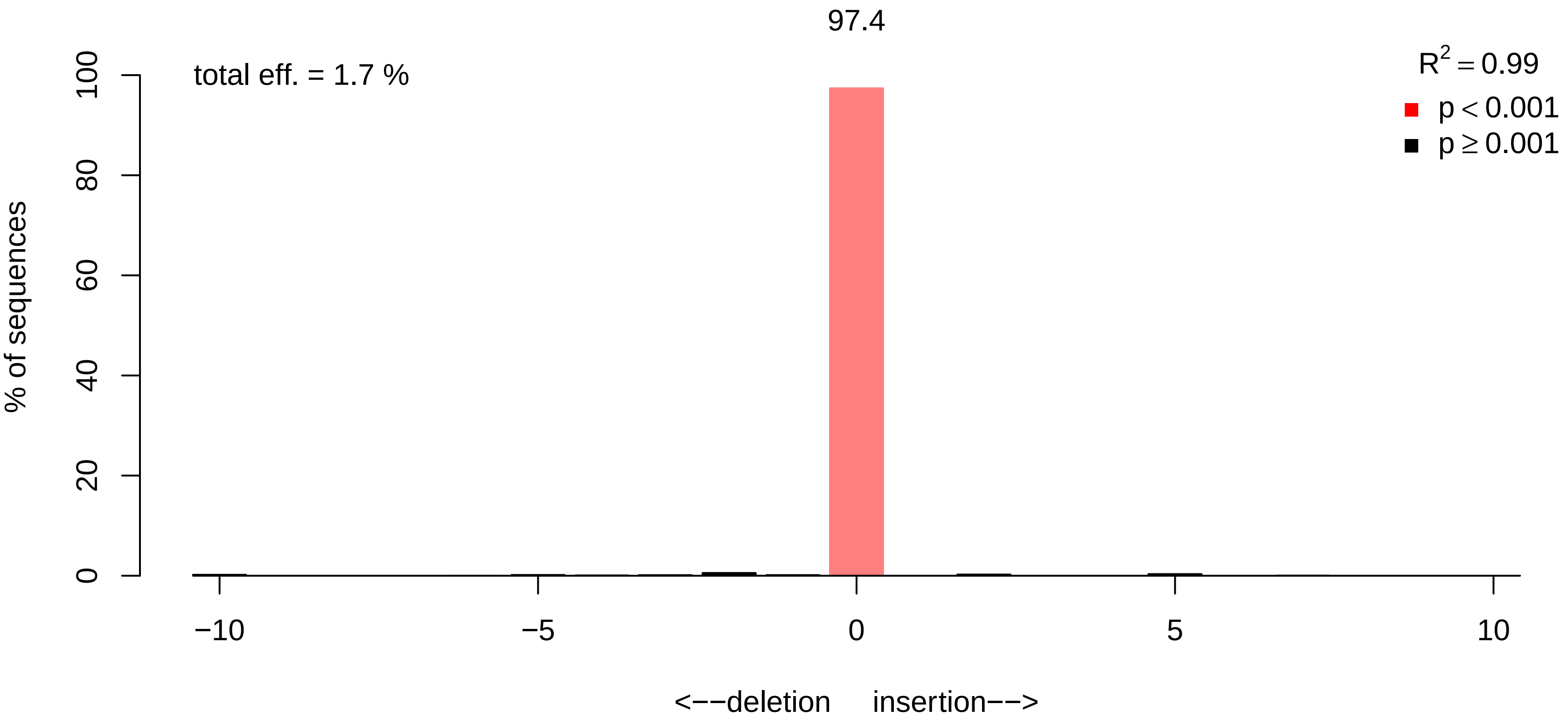
sample: Bth/WT cell line, gRNA 2.4, reverse
guide: TGGGACAGAACTTCCCCAGG



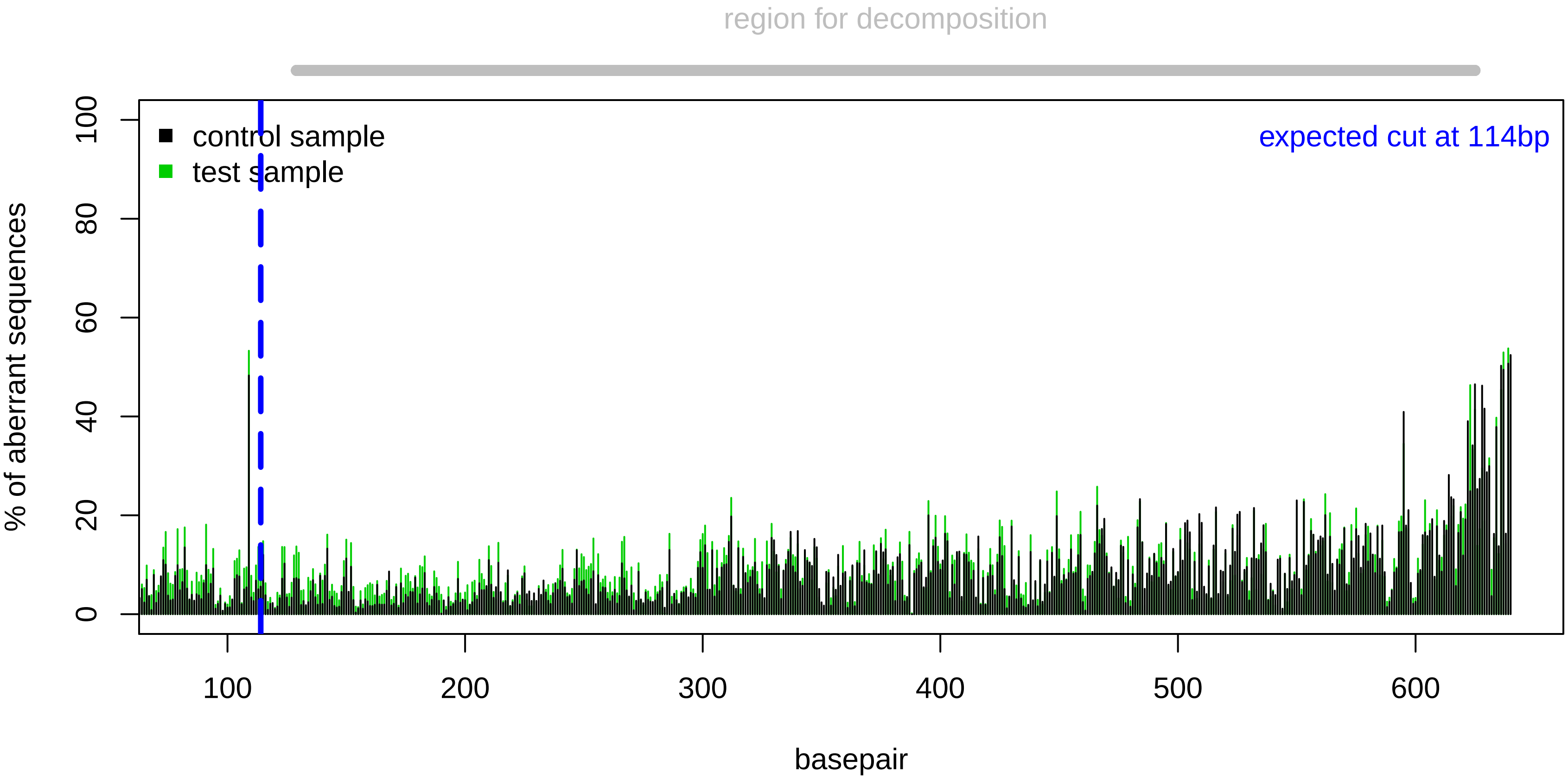
Warning: left boundary of decomposition window was adjusted 129 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 625 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: Bth/WT cell line, gRNA 3.1, reverse
guide: TGGTAATGTCCCTCCTGGGG

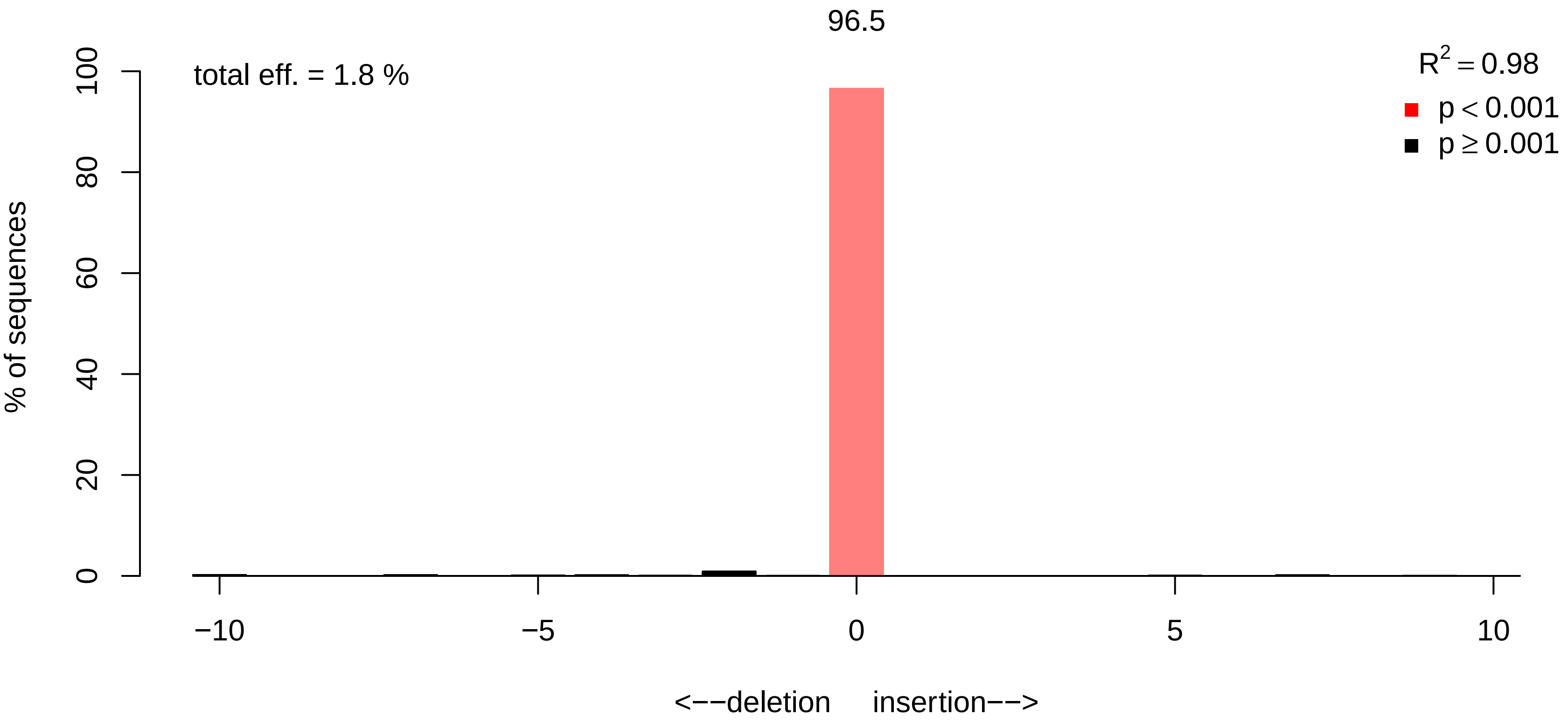


sample: Bth/WT cell line, gRNA 3.1, reverse
guide: TGGTAATGTCCCTCCTGGGG

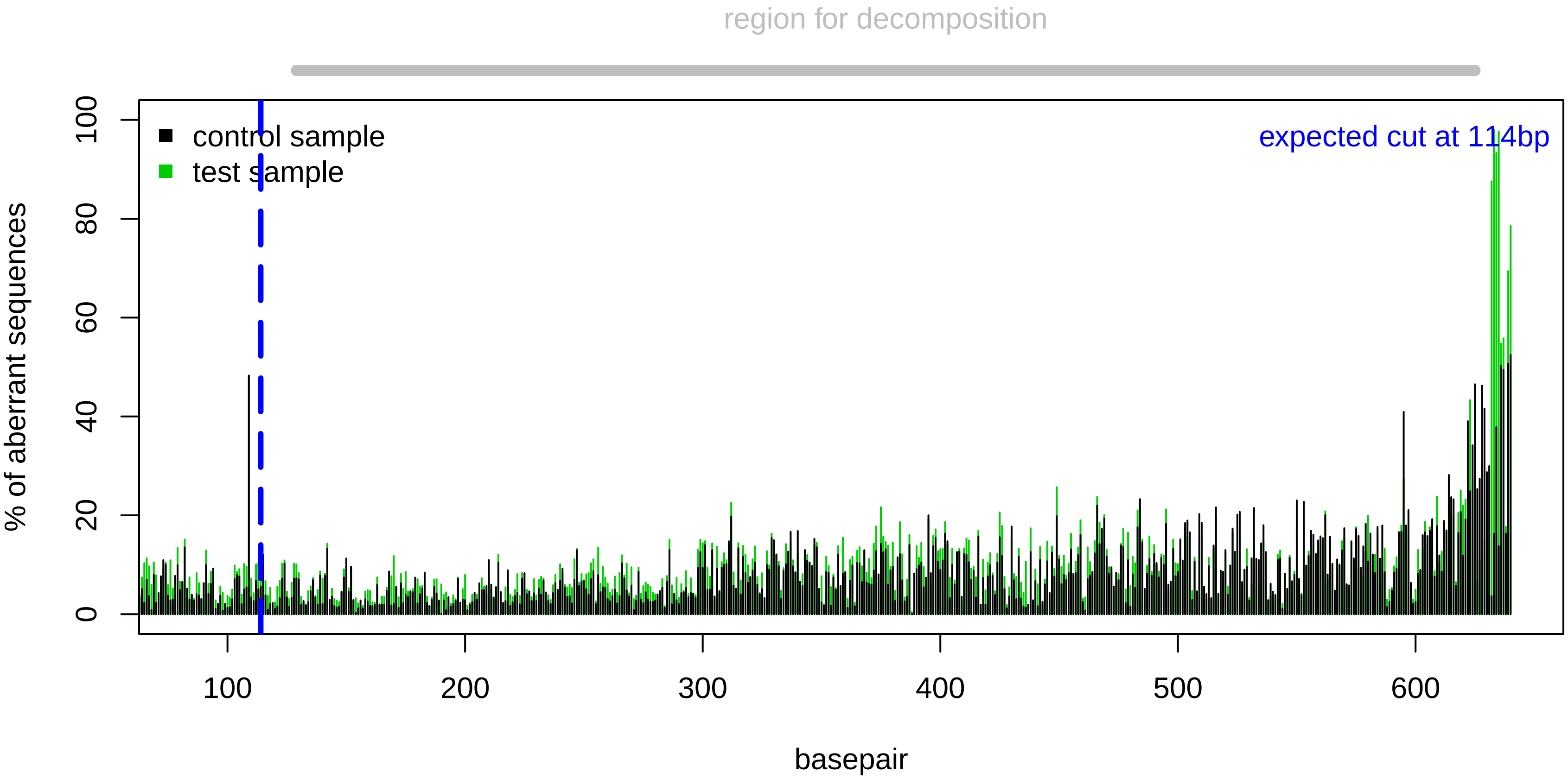


Warning: left boundary of decomposition window was adjusted 129 . It must be at least 5bp plus the maximum indel size downstream of the expected break site
Warning: right boundary of decomposition window was adjusted to 625 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: Bth/WT cell line, gRNA 3.2, reverse
guide: TGGTAATGTCCCTCCTGGGG



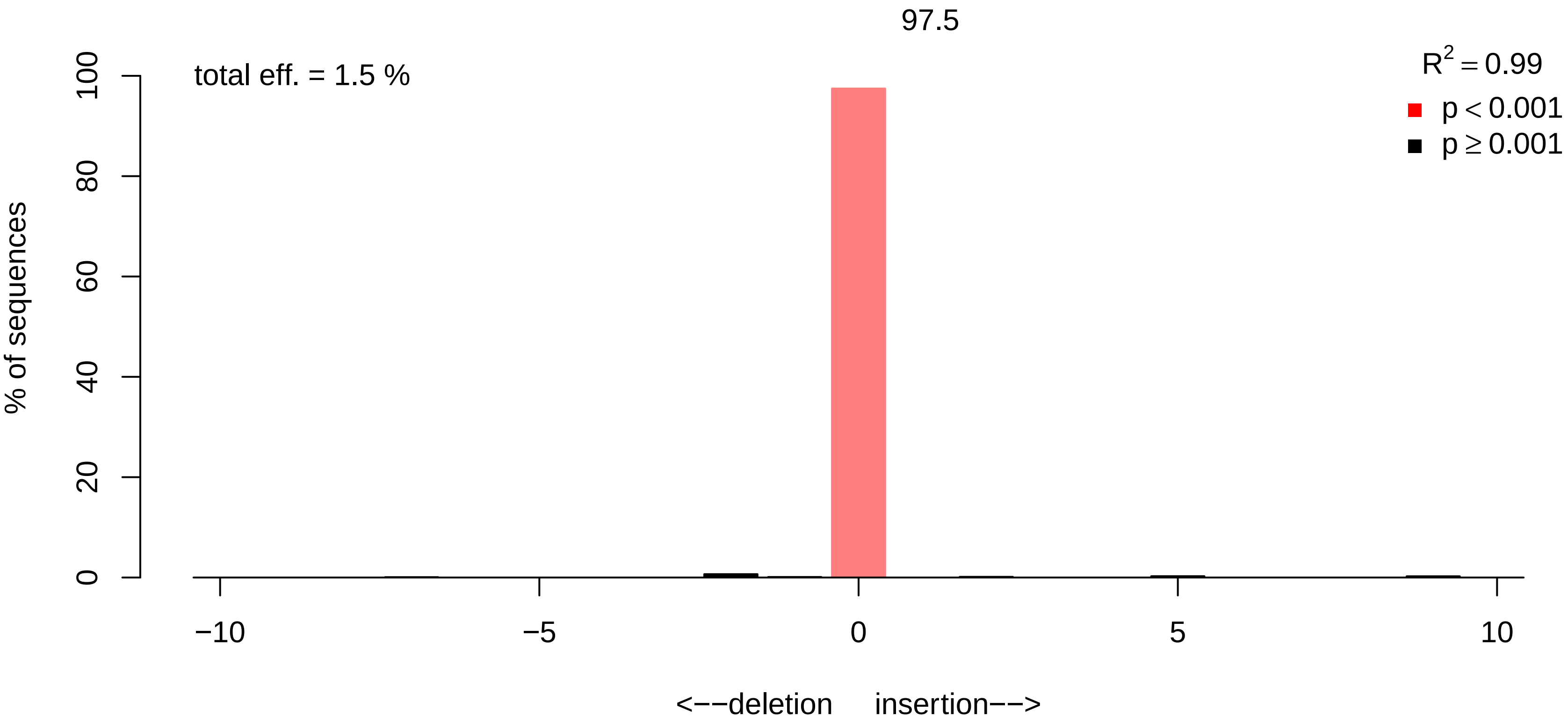
sample: Bth/WT cell line, gRNA 3.2, reverse
guide: TGGTAATGTCCCTCCTGGGG



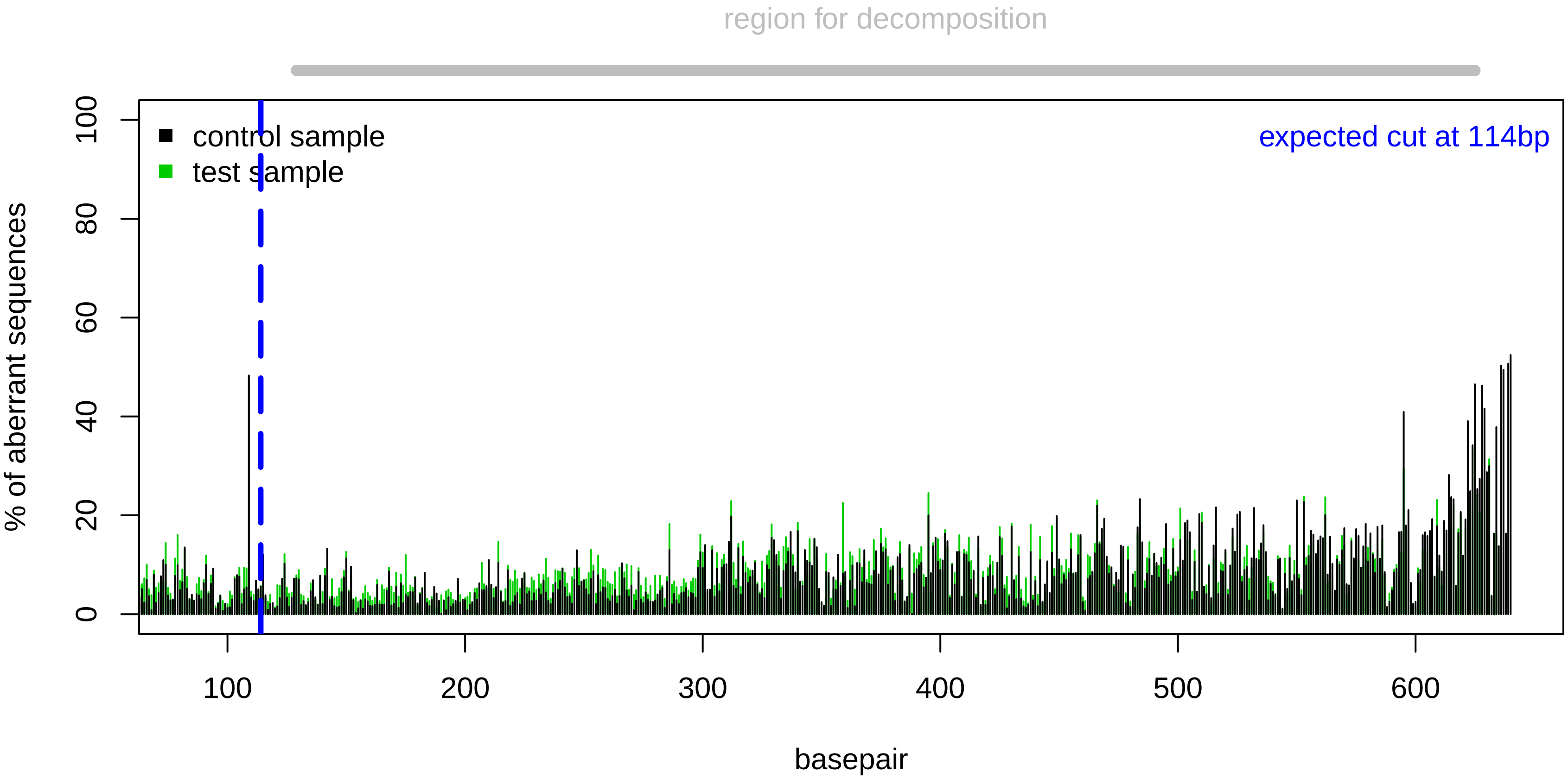
Warning: left boundary of decomposition window was adjusted 129 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 625 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: Bth/WT cell line, gRNA 3.3, reverse
guide: TGGTAATGTCCCTCCTGGGG



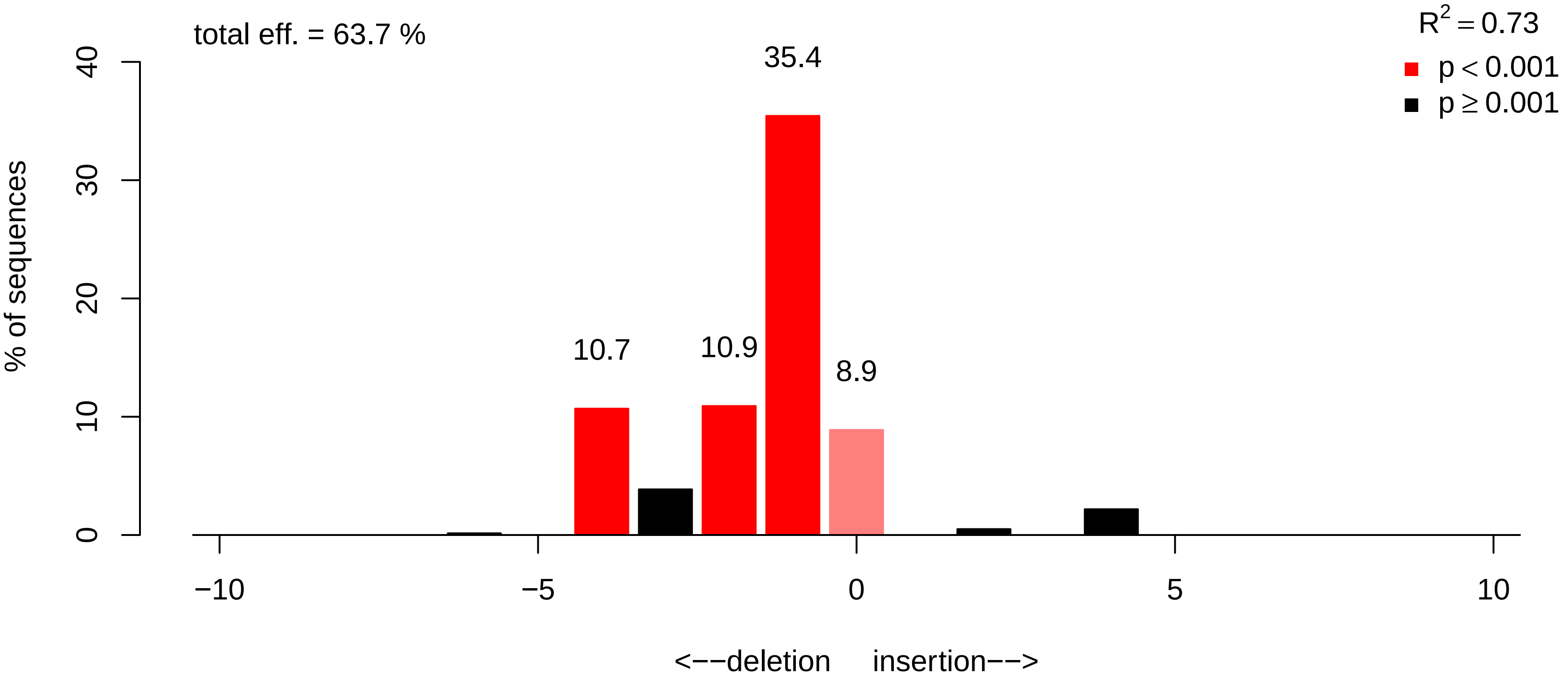
sample: Bth/WT cell line, gRNA 3.3, reverse
guide: TGGTAATGTCCCTCCTGGGG

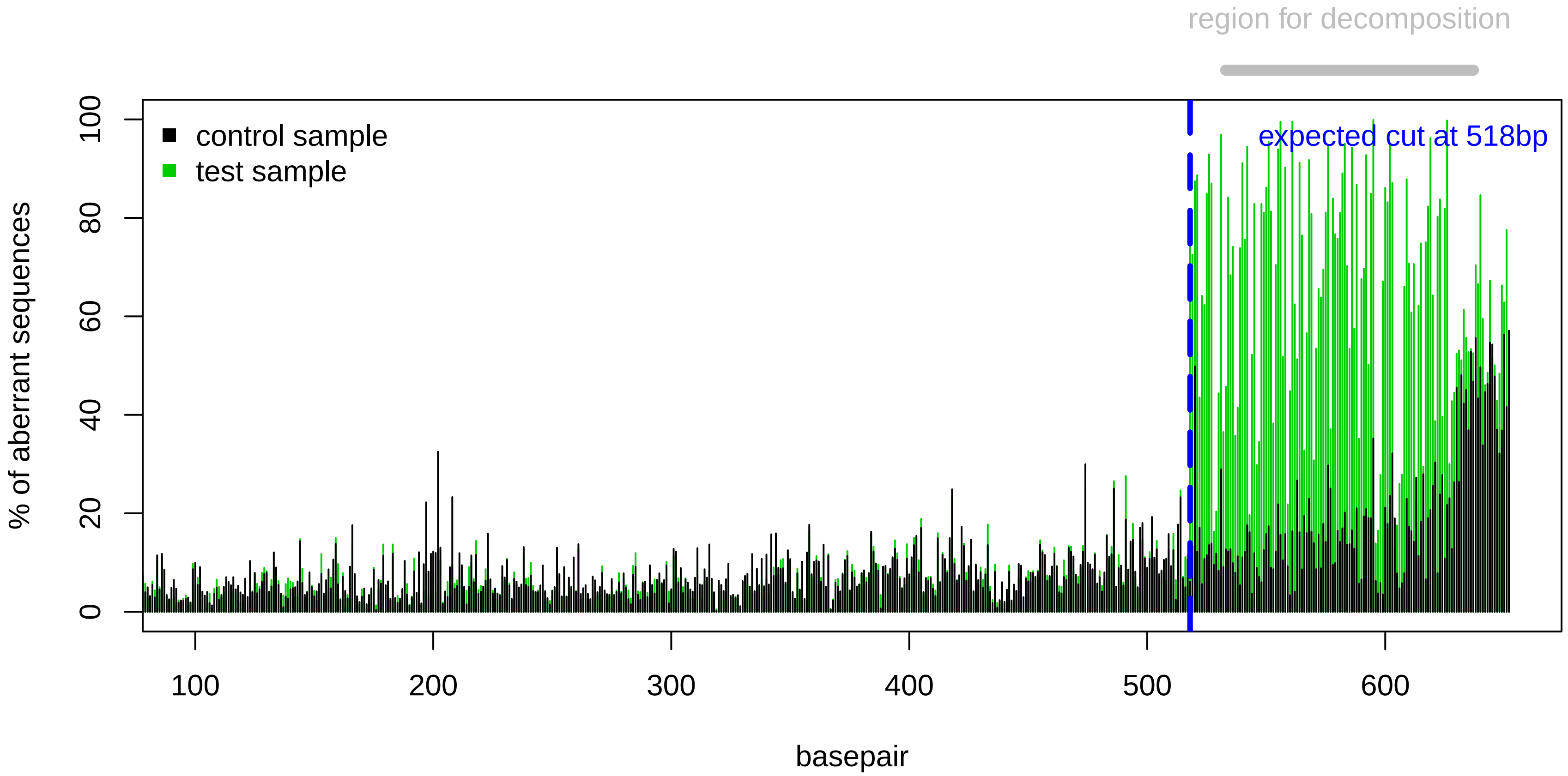


Warning: left boundary of decomposition window was adjusted 129 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

Warning: right boundary of decomposition window was adjusted to 625 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

sample: Bth/WT cell line, gRNA WT, forward
guide: GGGTGGGACAGAACATCCCC

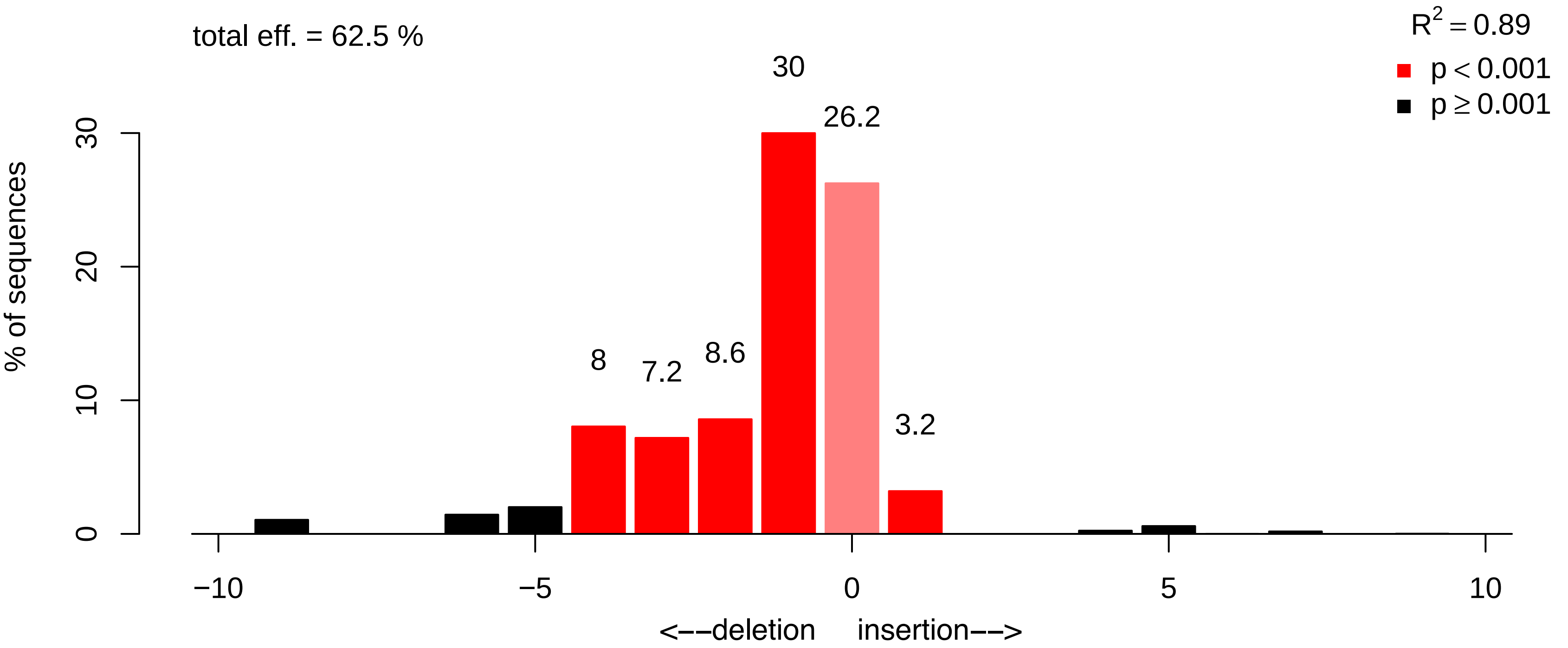




sample: Bth/WT cell line, gRNA WT, forward
guide: GGGTGGGACAGAACATCCCC

Warning: left boundary of decomposition window was adjusted 533 . It must be at least 5bp plus the maximum indel size downstream of the expected break site
Warning: right boundary of decomposition window was adjusted to 637 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

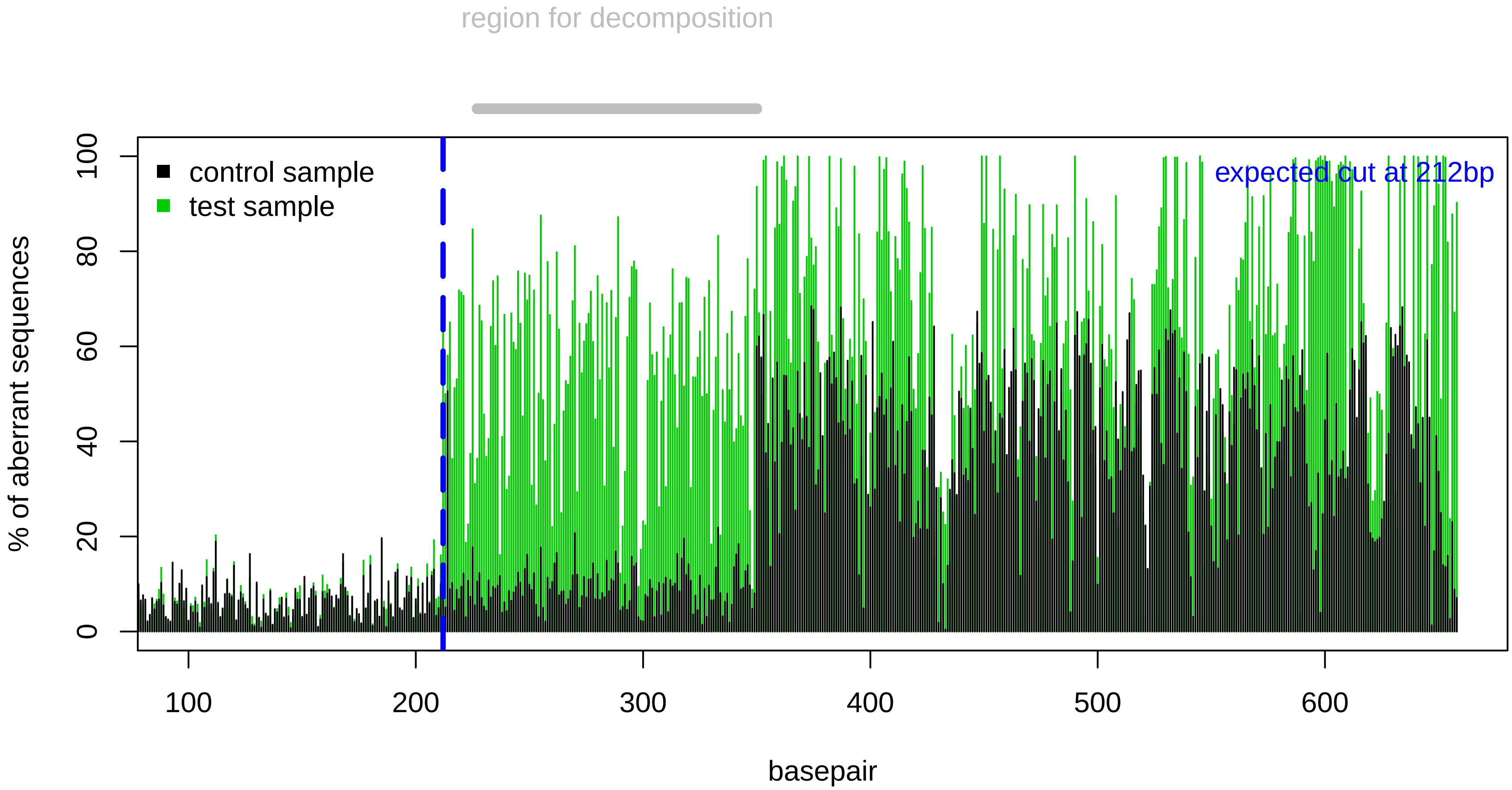
sample: Bth/WT cell line, gRNA 1.1, forward
guide: GGGTGGGACAGAACTTCCCC



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

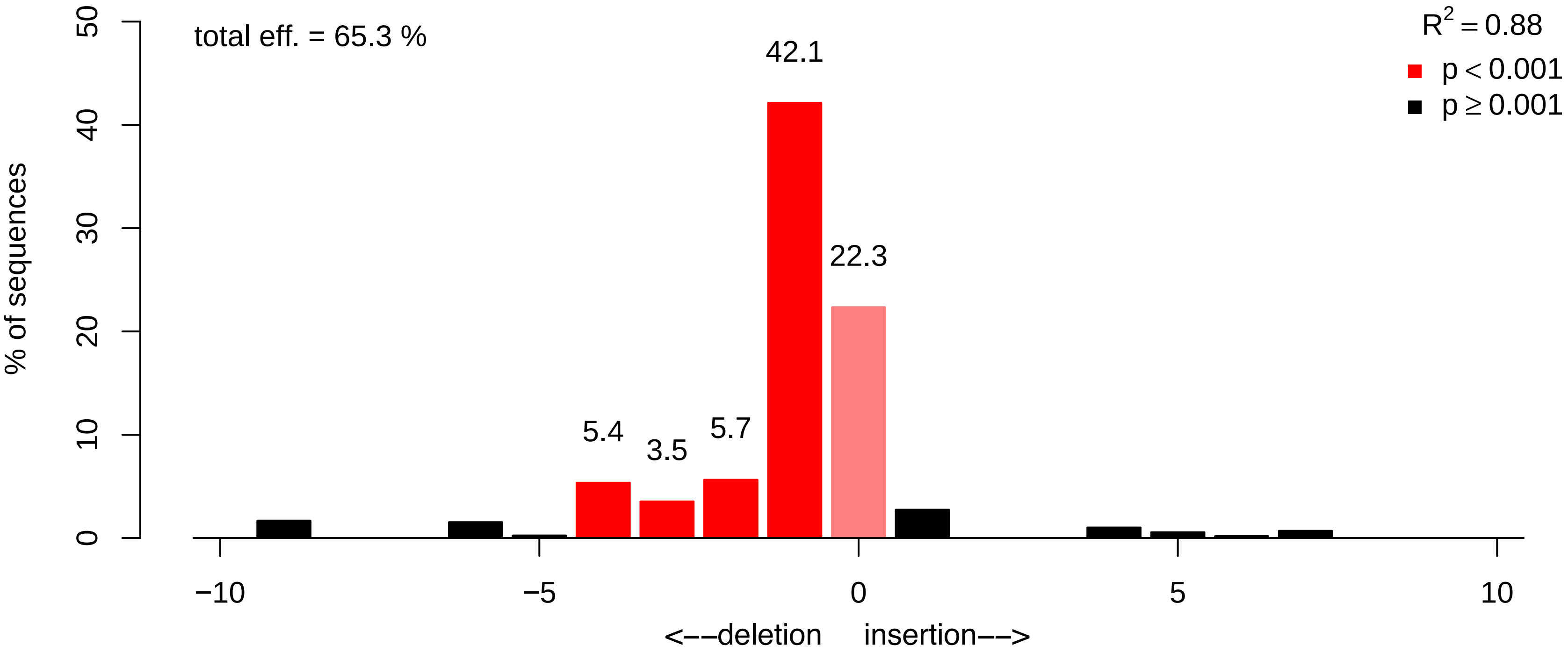
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 1.1, forward
guide: GGGTGGGACAGAACTTCCCC



Warning: left boundary of decomposition window was adjusted 227 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

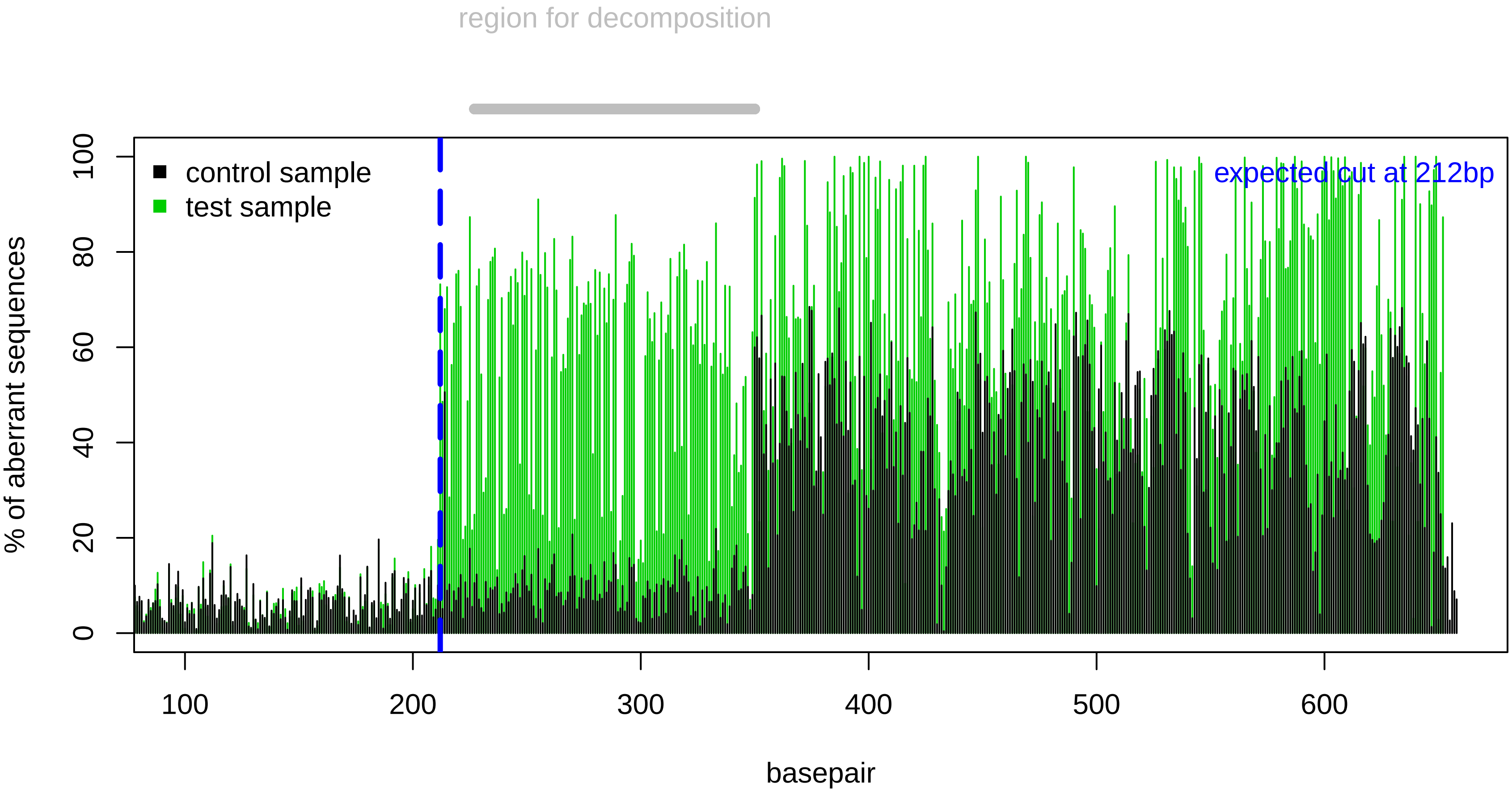
sample: Bth/WT cell line, gRNA 1.2, forward
guide: GGGTGGGACAGAACTTCCCC



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

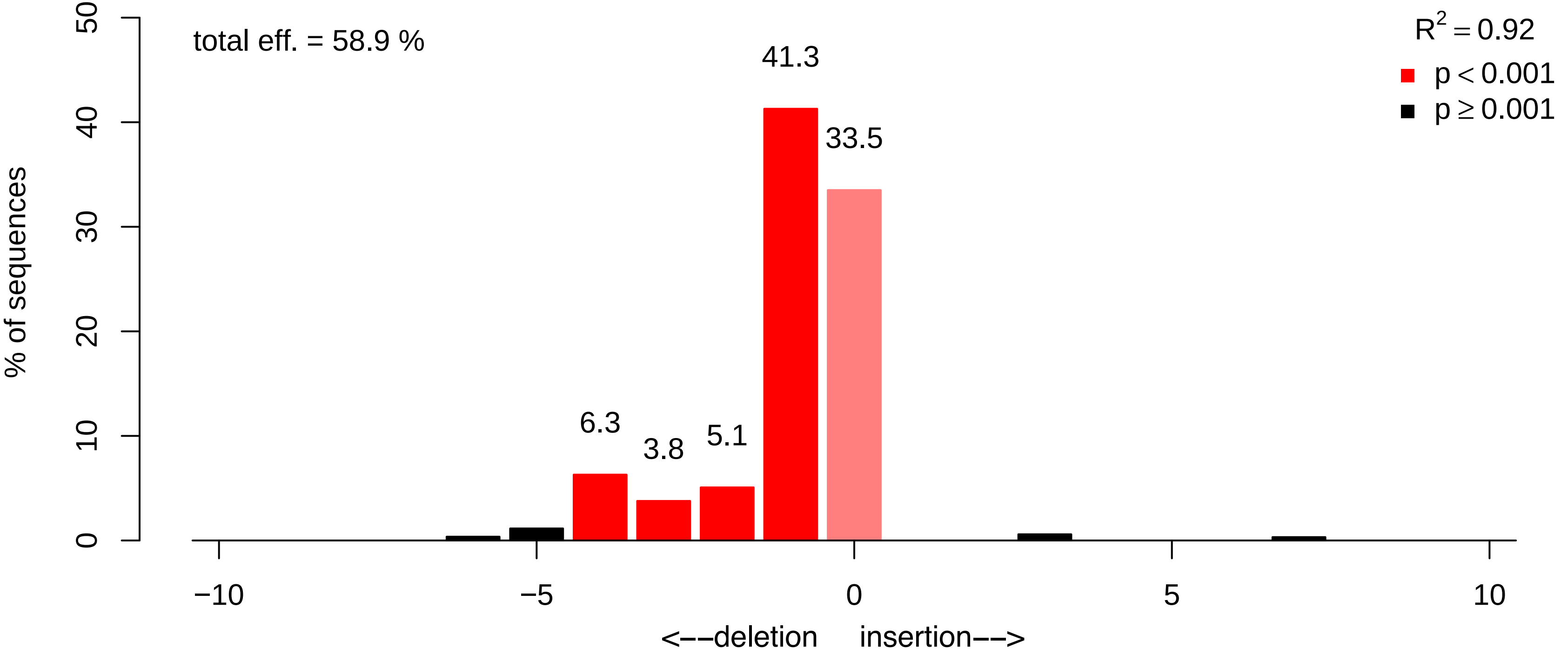
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 1.2, forward
guide: GGGTGGGACAGAACTTCCCC



Warning: left boundary of decomposition window was adjusted 227 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

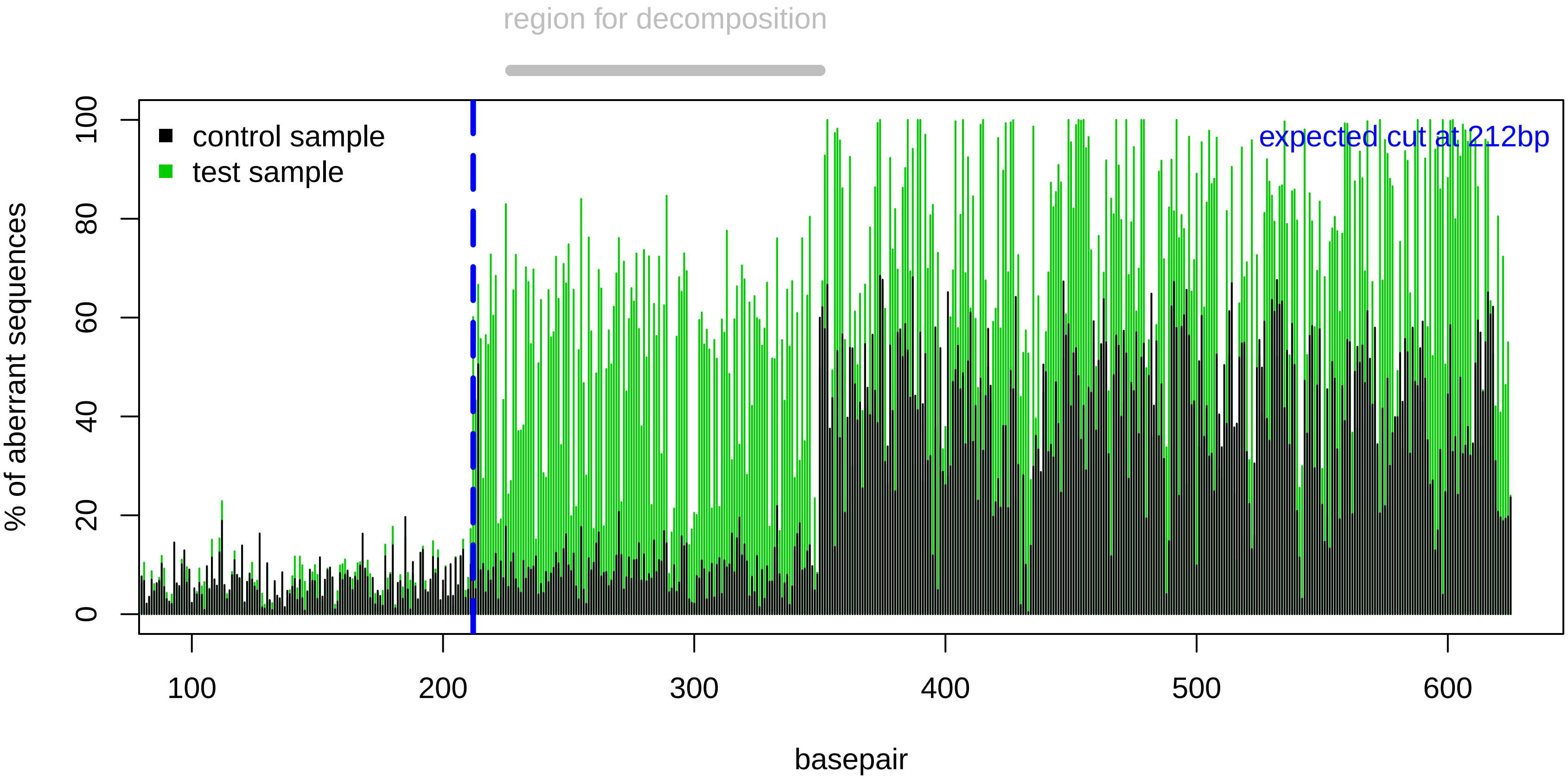
sample: Bth/WT cell line, gRNA 1.3, forward
guide: GGGTGGGACAGAACTTCCCC



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

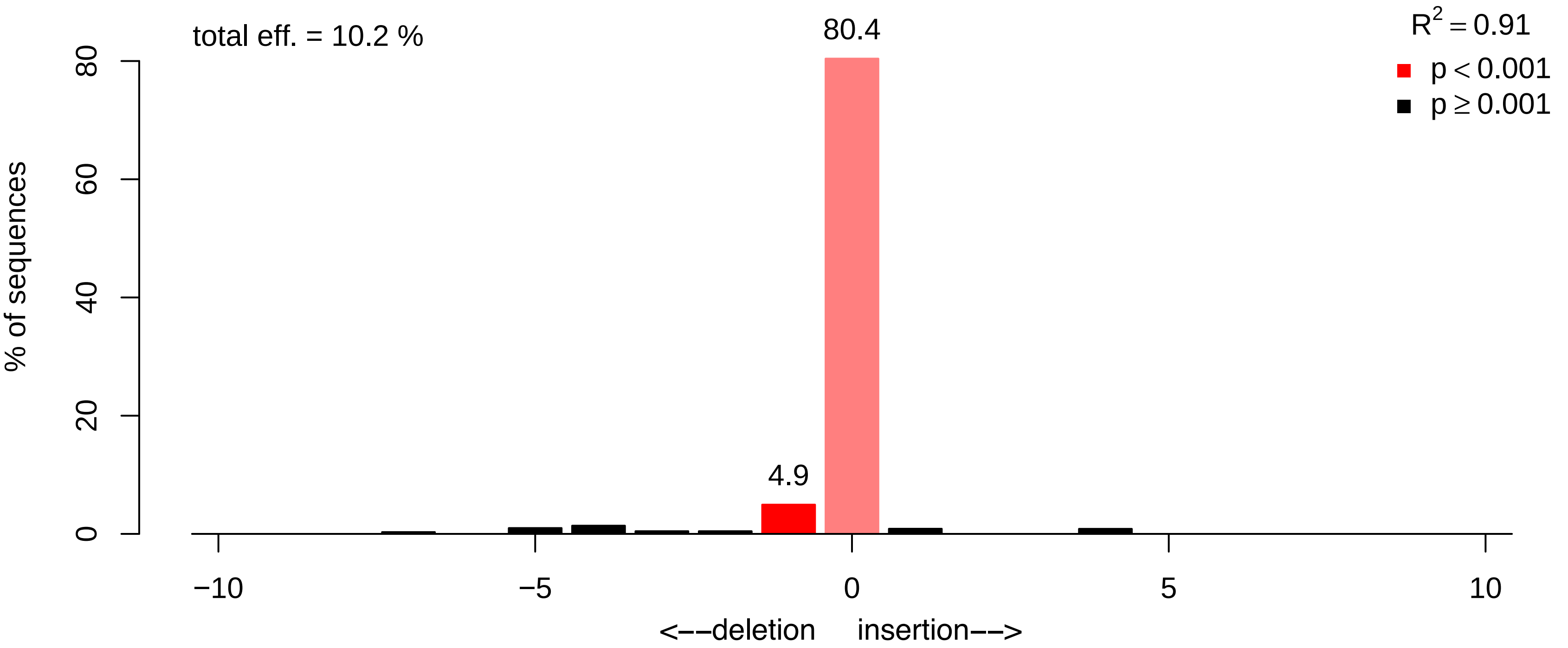
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 1.3, forward
guide: GGGTGGGACAGAACTTCCCC



Warning: left boundary of decomposition window was adjusted 227 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

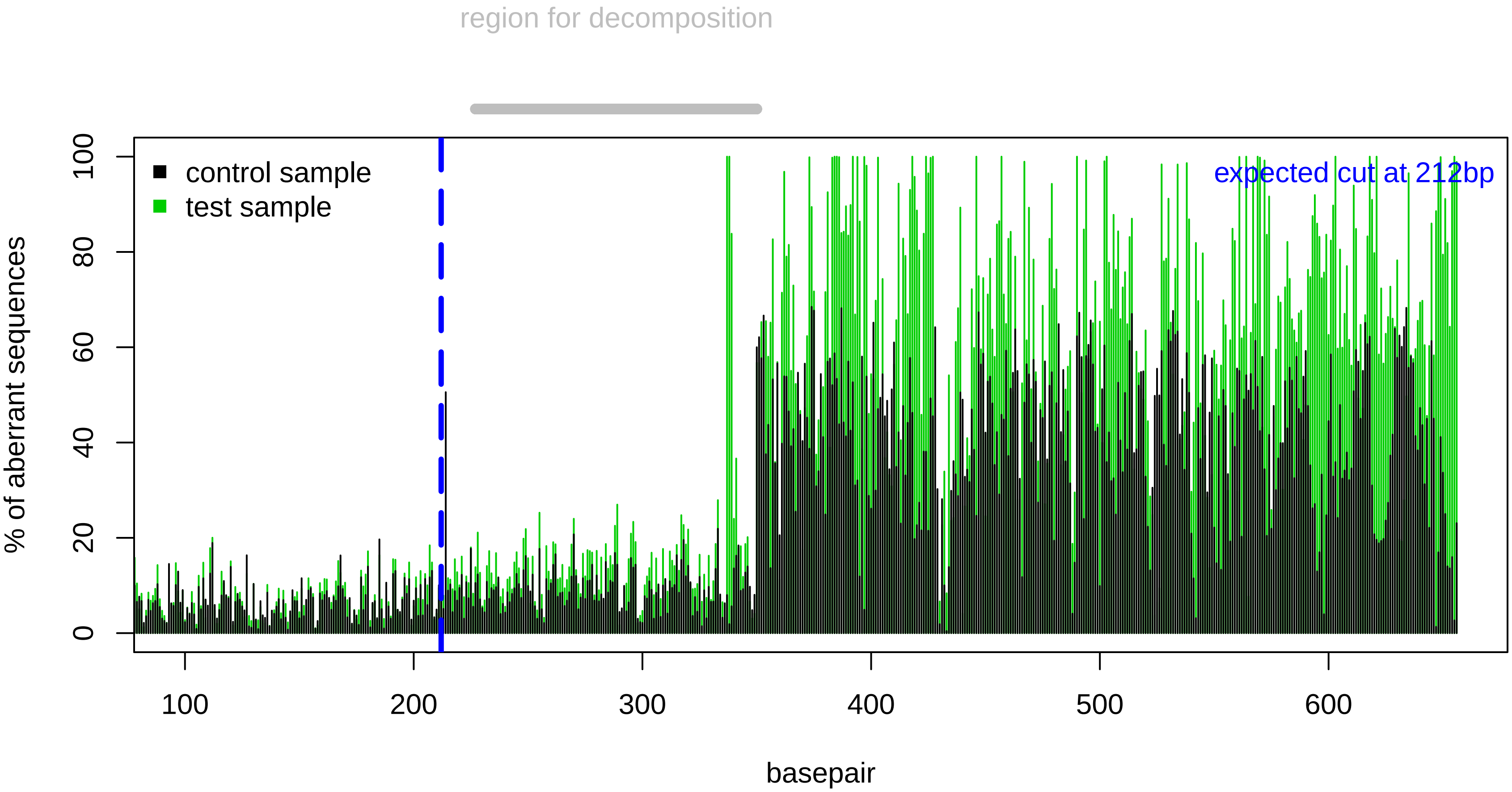
sample: Bth/WT cell line, gRNA 1.4, forward
guide: GGGTGGGACAGAACTTCCCC



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

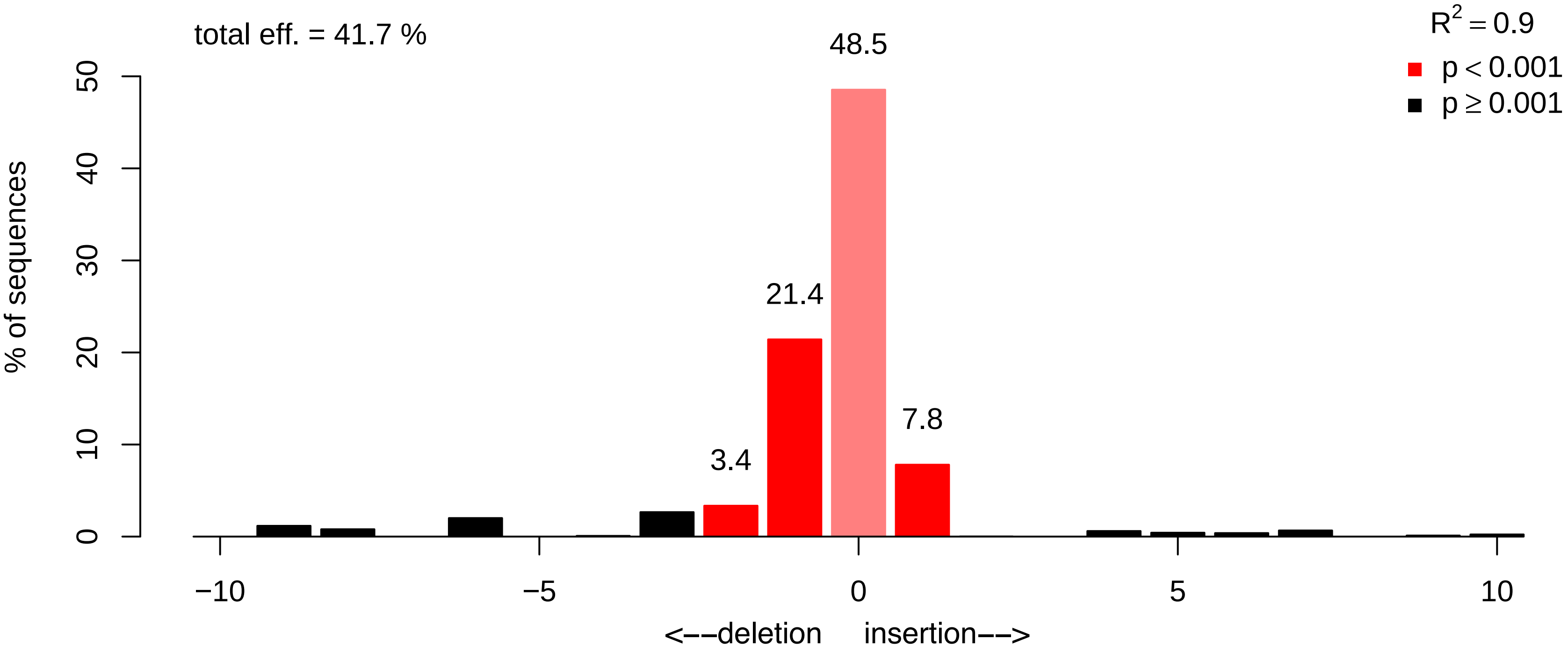
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 1.4, forward
guide: GGGTGGGACAGAACTTCCCC



Warning: left boundary of decomposition window was adjusted 227 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

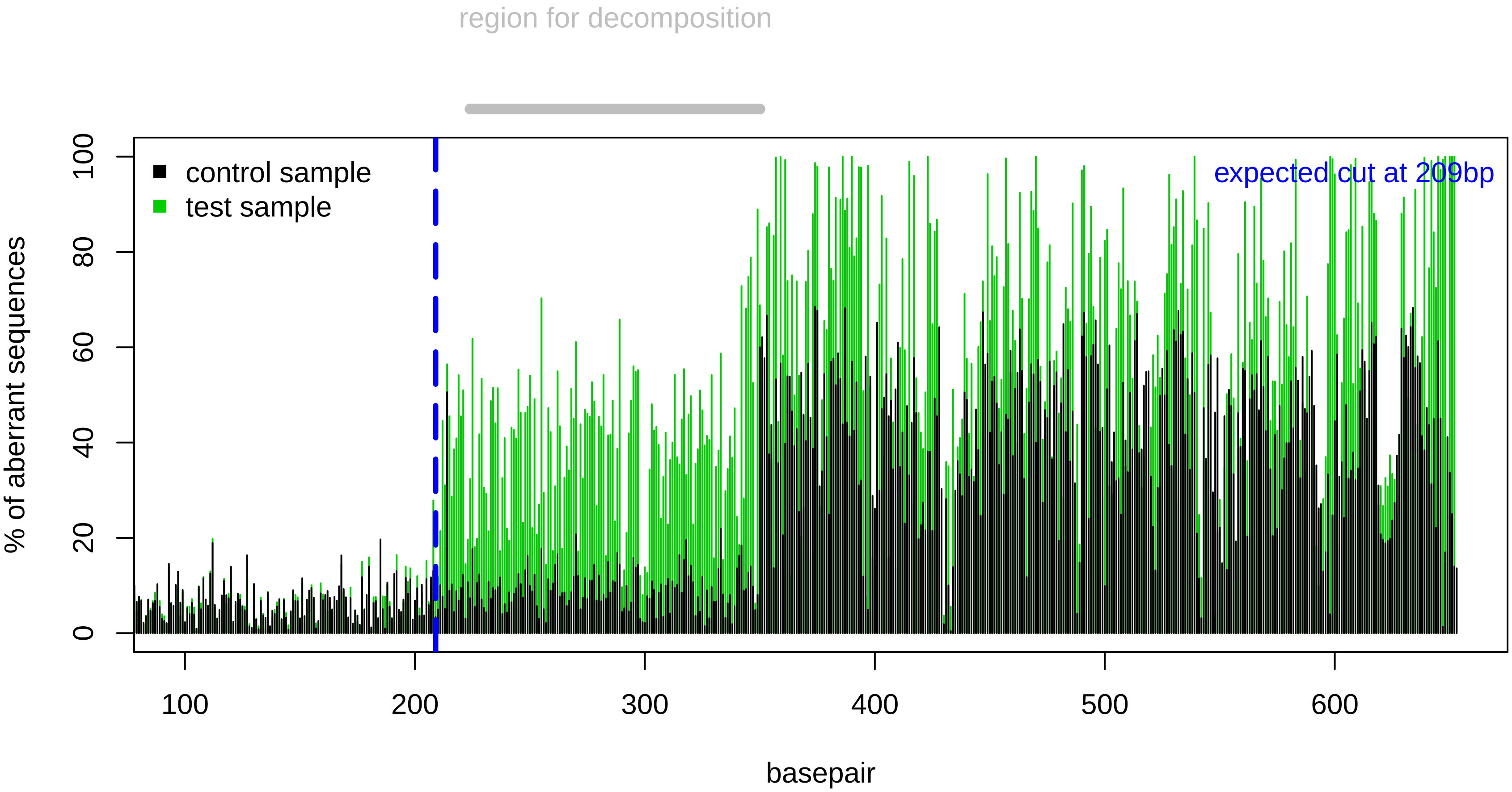
sample: Bth/WT cell line, gRNA 2.1, forward
guide: TGGGACAGAACTTCCCCAGG



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

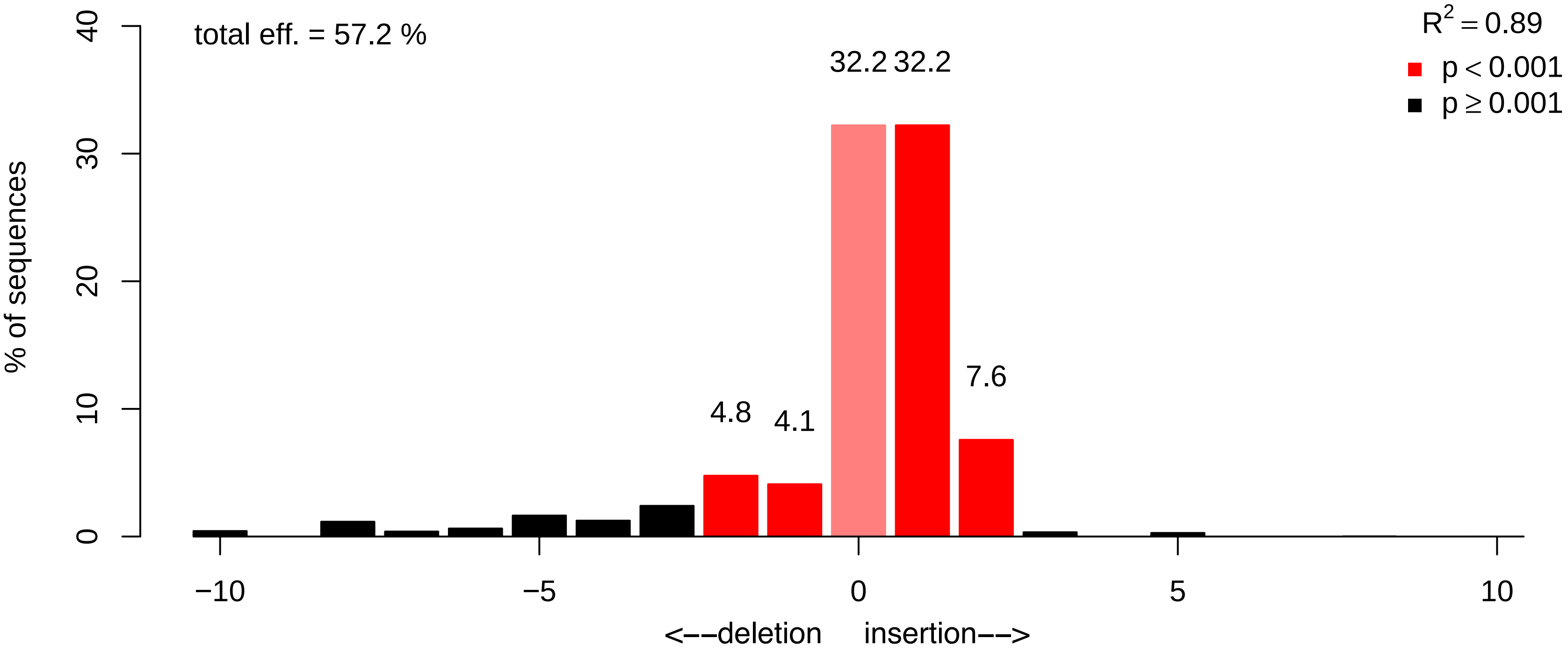
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 2.1, forward
guide: TGGGACAGAACTTCCCCAGG



Warning: left boundary of decomposition window was adjusted 224 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

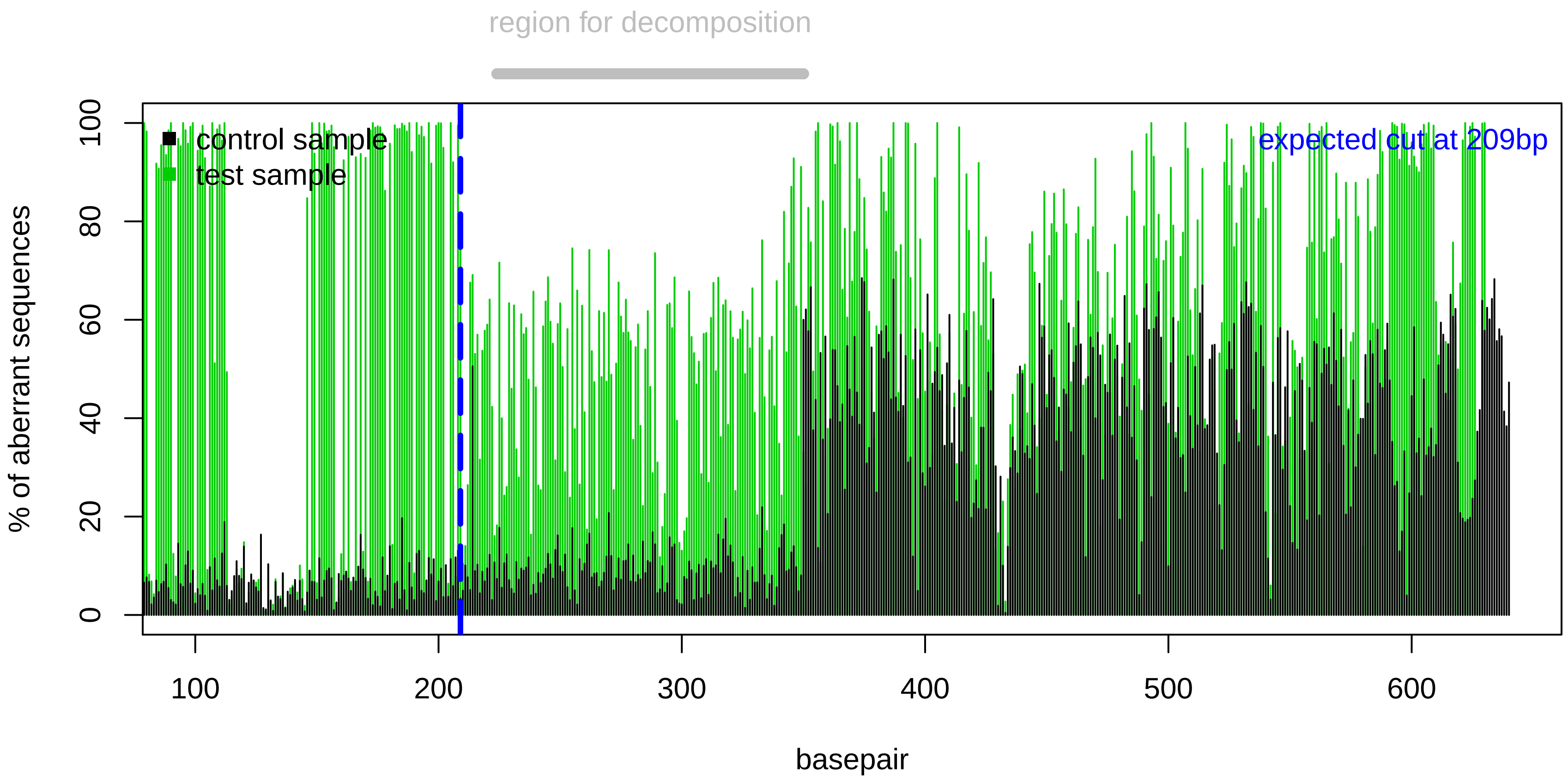
sample: Bth/WT cell line, gRNA 2.2, forward
6 guide: TGGGACAGAACTTCCCCAGG



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

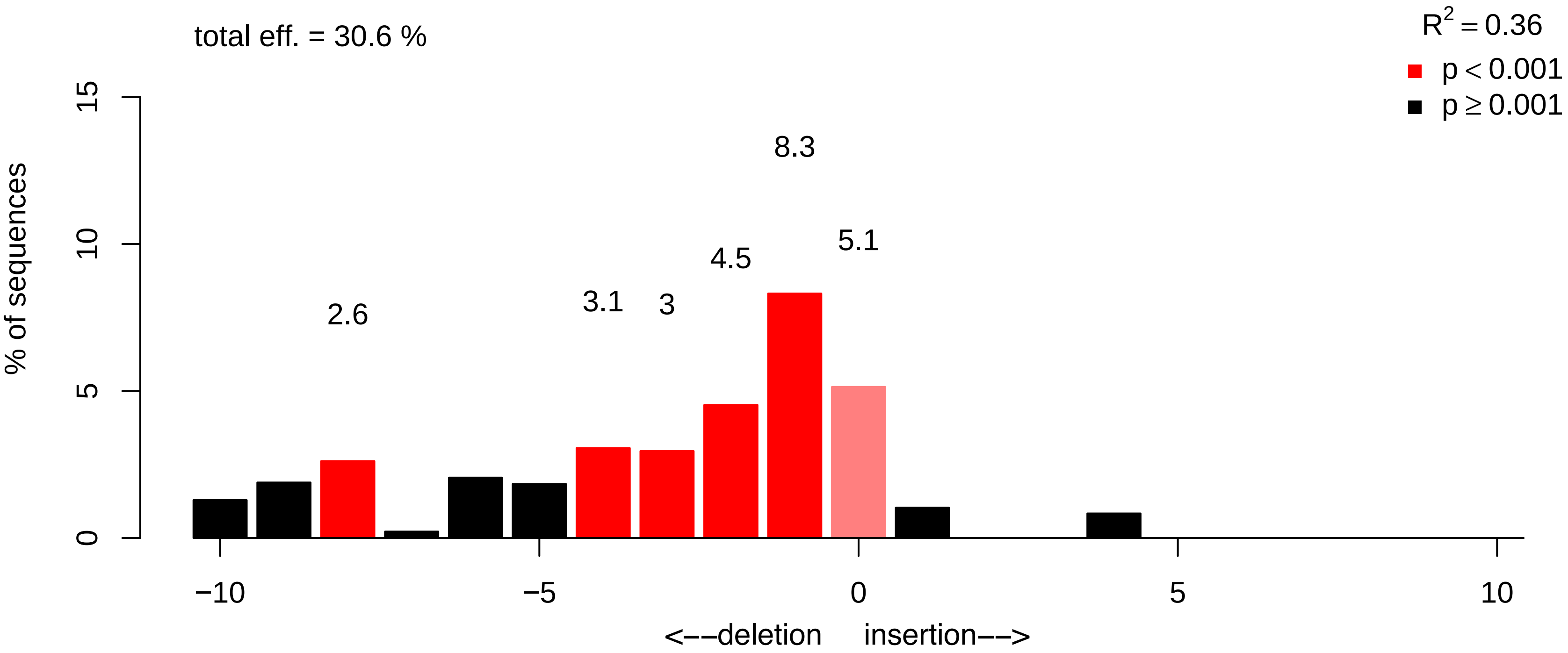
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 2.2, forward
guide: TGGGACAGAACTTCCCCAGG



Warning: left boundary of decomposition window was adjusted 224 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

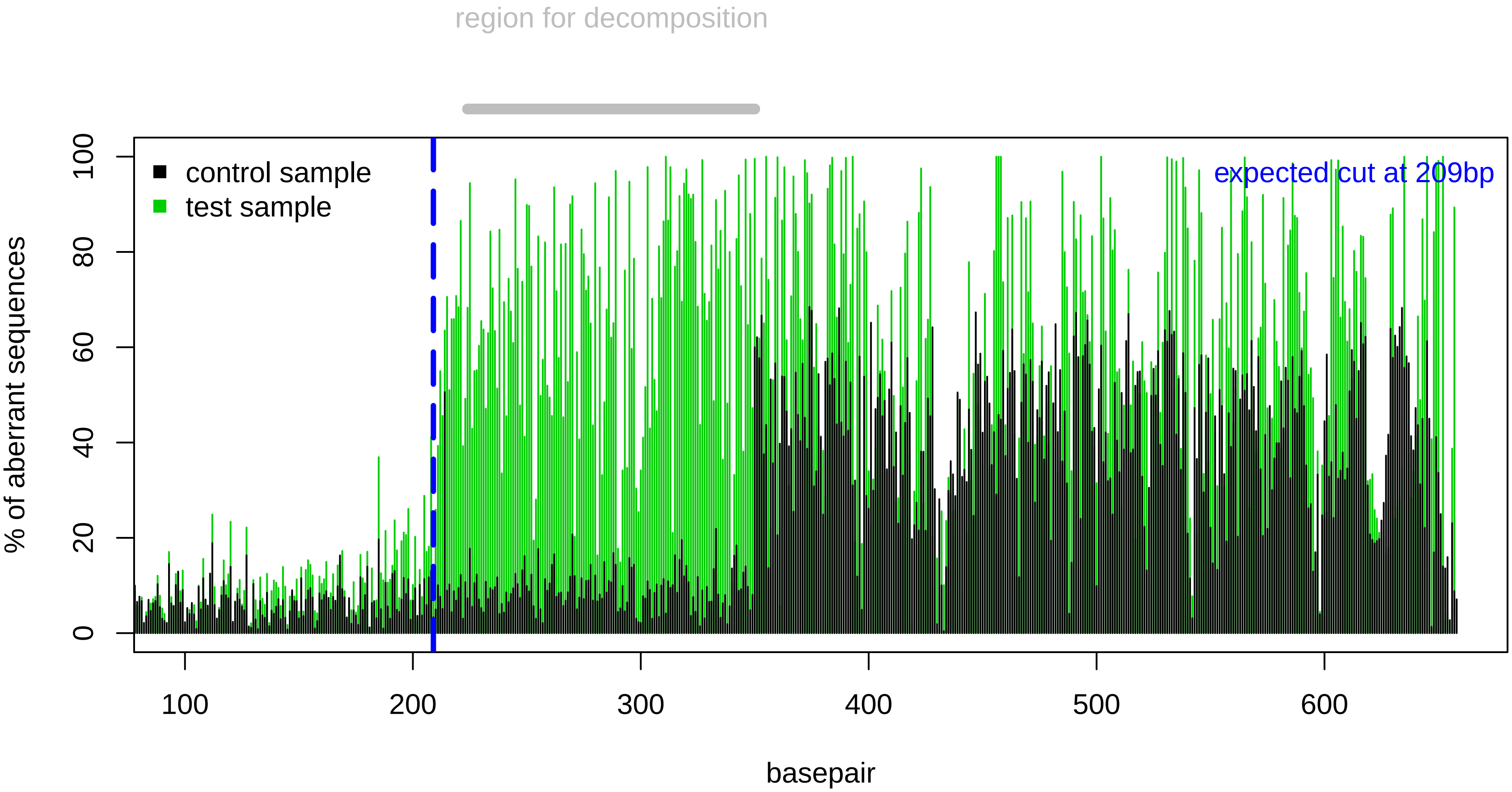
sample: Bth/WT cell line, gRNA 2.3, forward
guide: TGGGACAGAACTTCCCCAGG



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

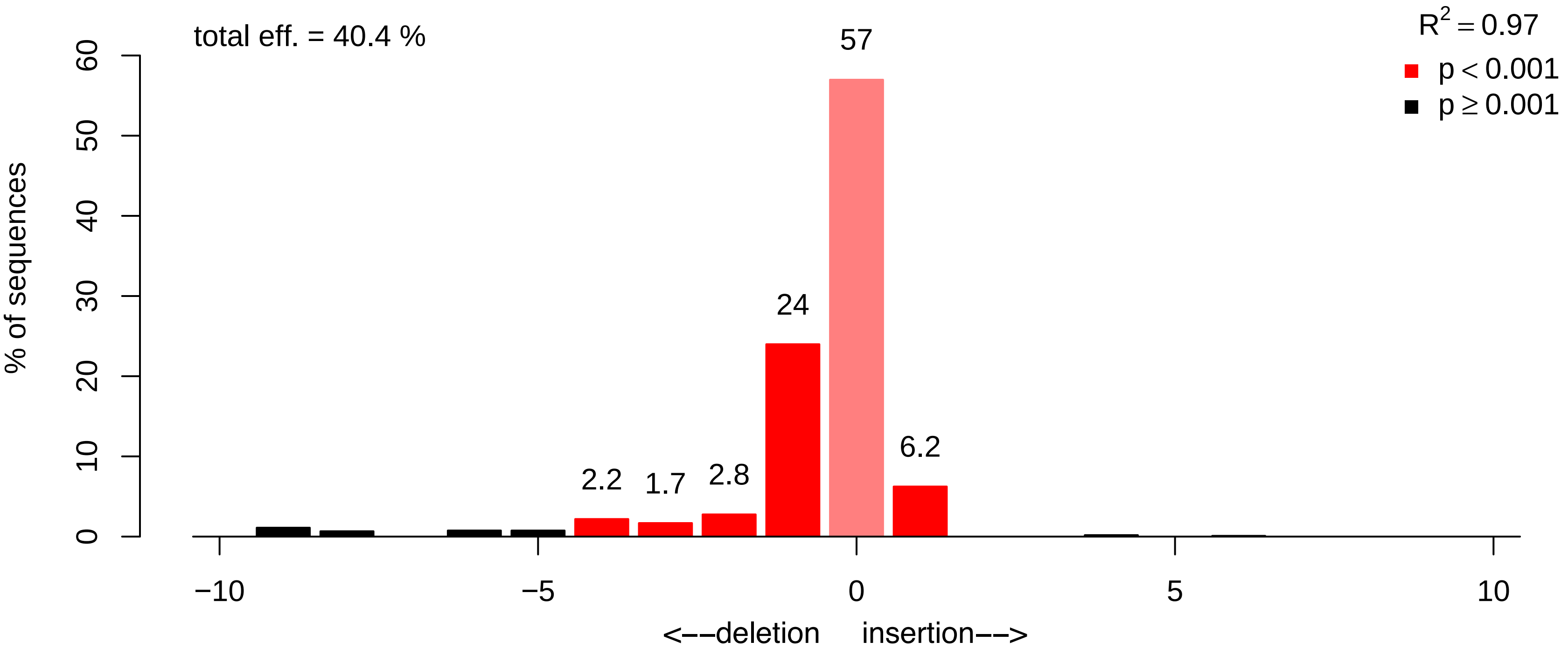
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 2.3, forward
guide: TGGGACAGAACTTCCCCAGG



Warning: left boundary of decomposition window was adjusted 224 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

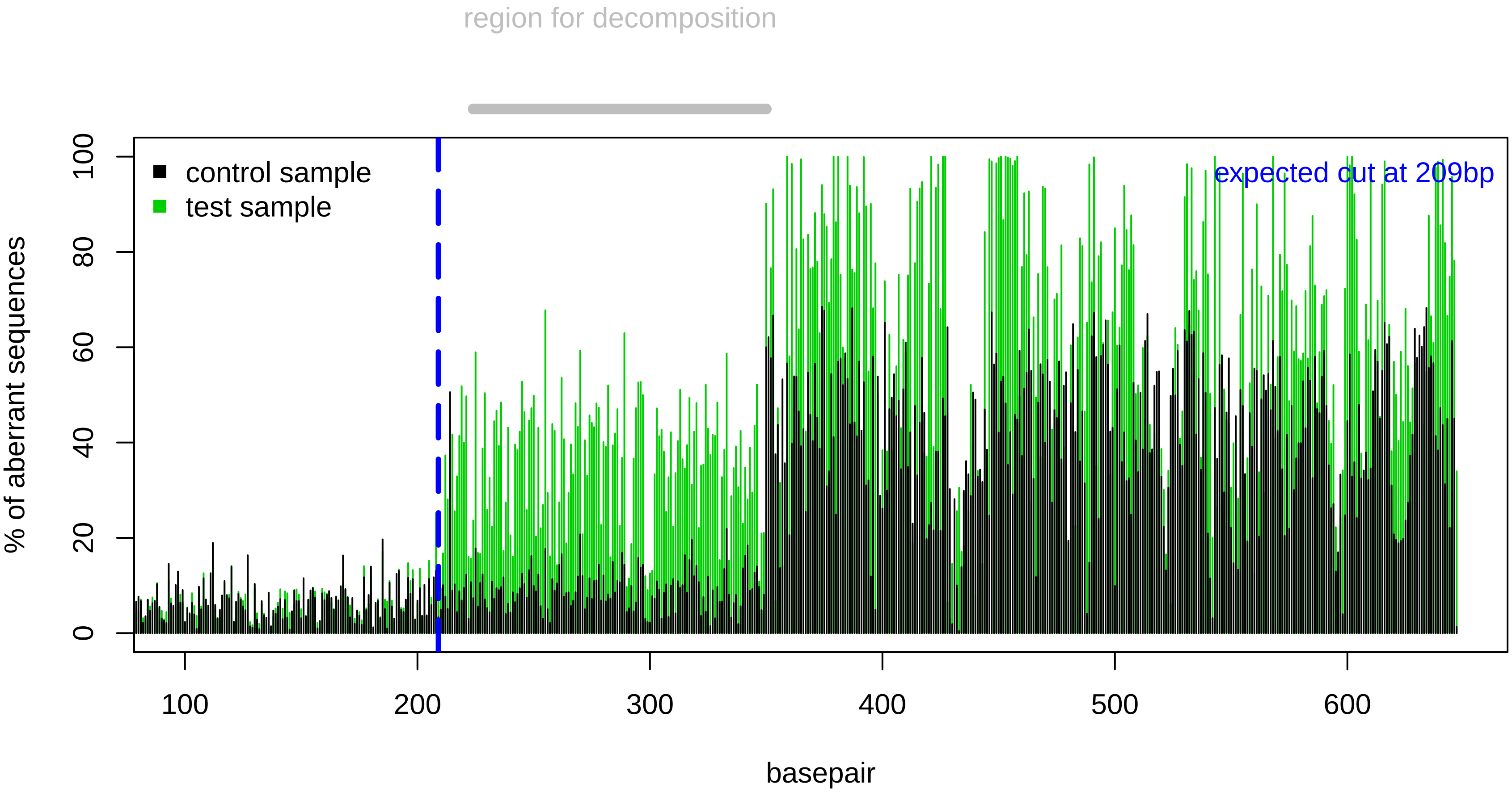
sample: Bth/WT cell line, gRNA 2.4, forward
guide: TGGGACAGAACTTCCCCAGG



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

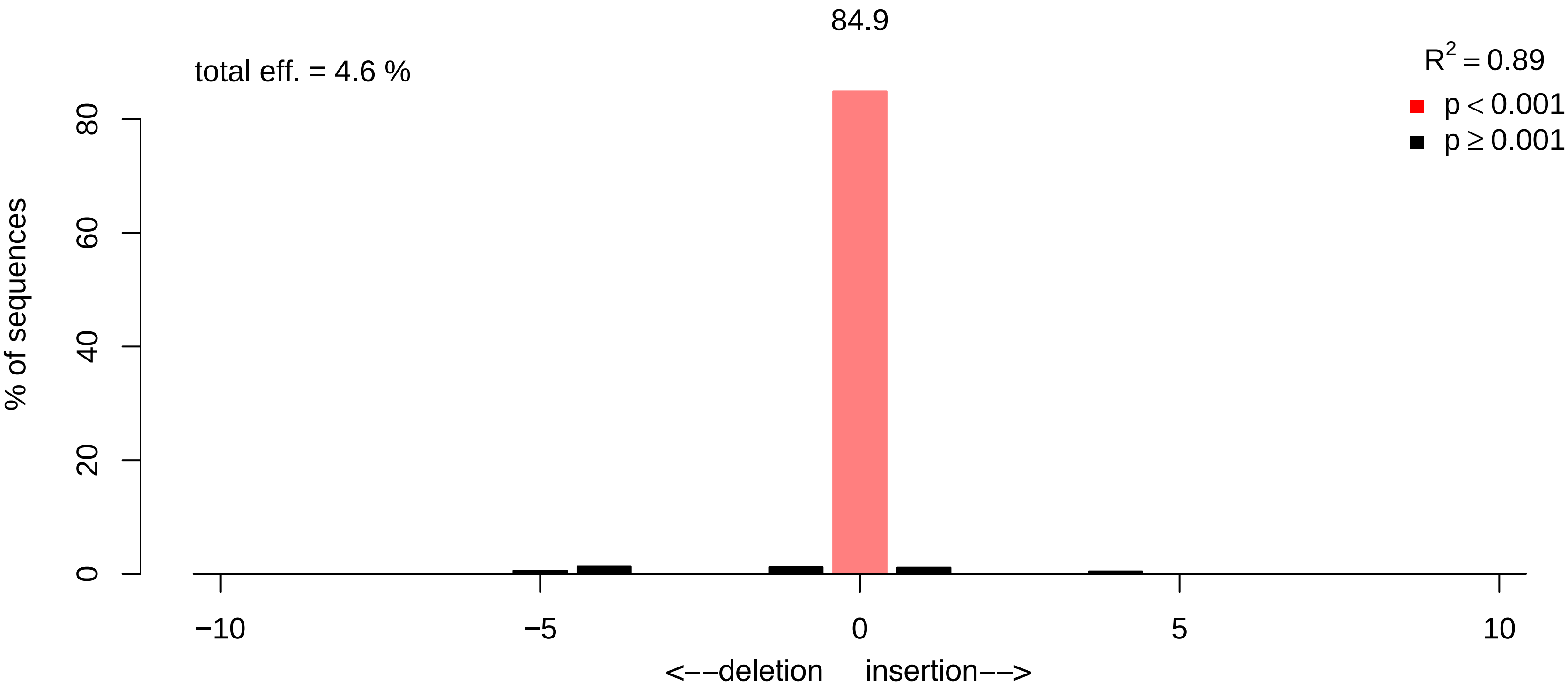
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 2.4, forward
guide: TGGGACAGAACTTCCCCAGG



Warning: left boundary of decomposition window was adjusted 224 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

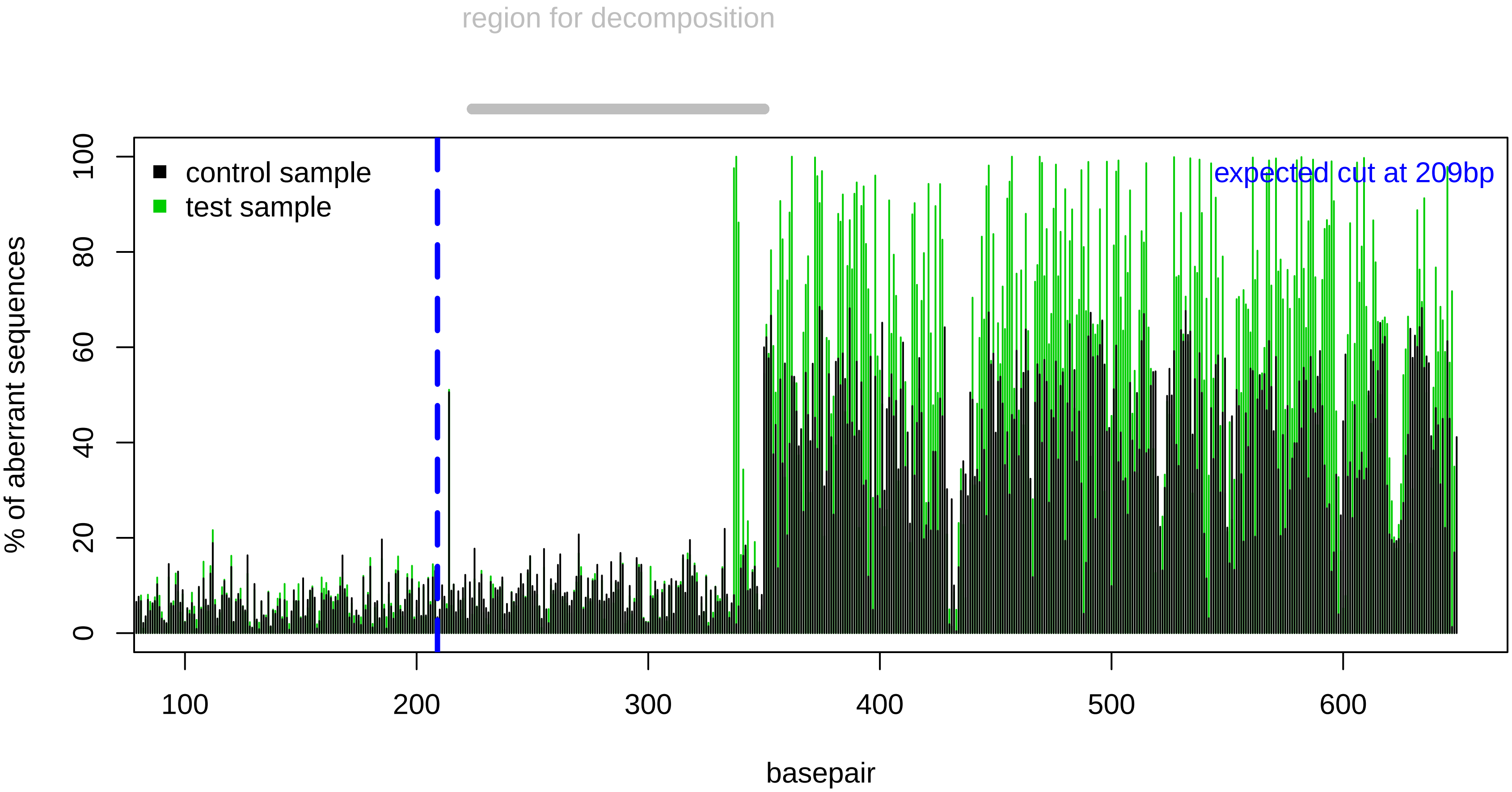
sample: Bth/WT cell line, gRNA 3.1, forward
guide: TGGTAATGTCCCTCCTGGGG



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

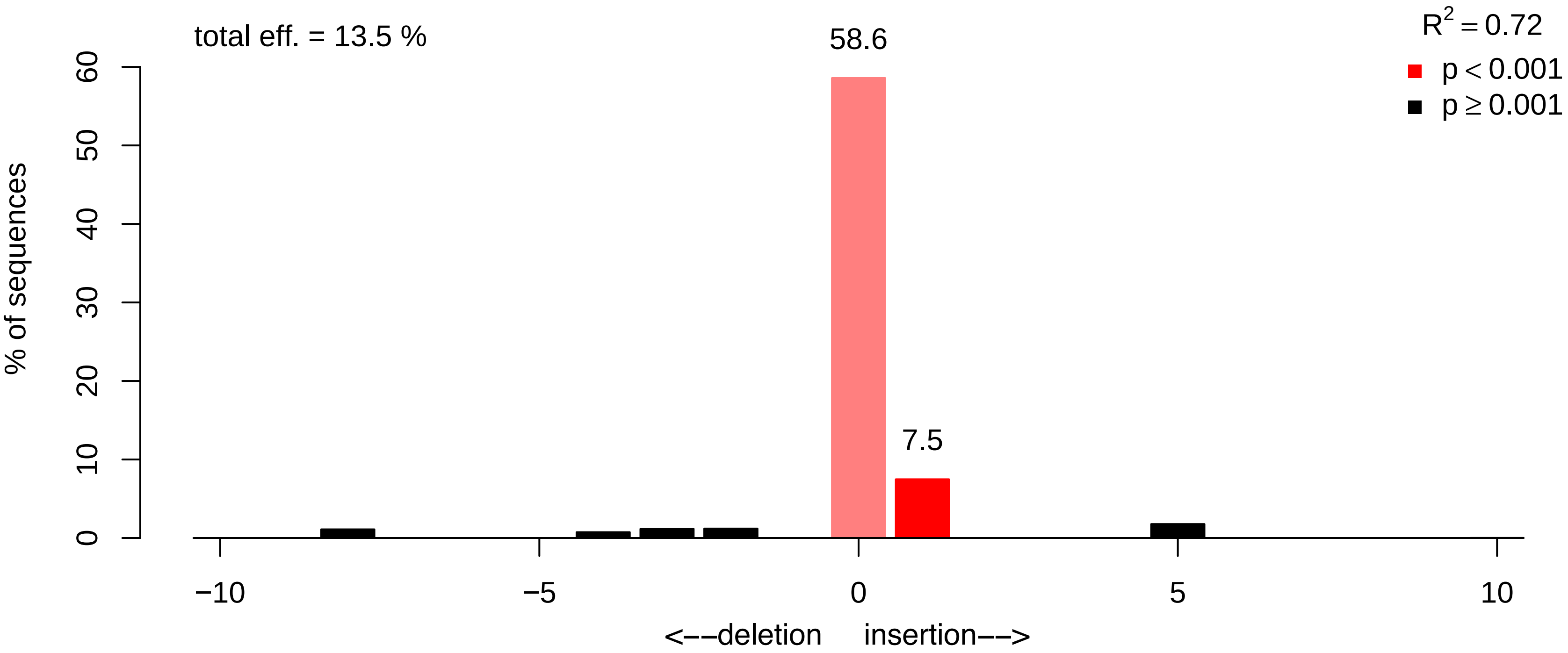
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 3.1, forward
guide: TGGTAATGTCCCTCCTGGGG



Warning: left boundary of decomposition window was adjusted 224 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

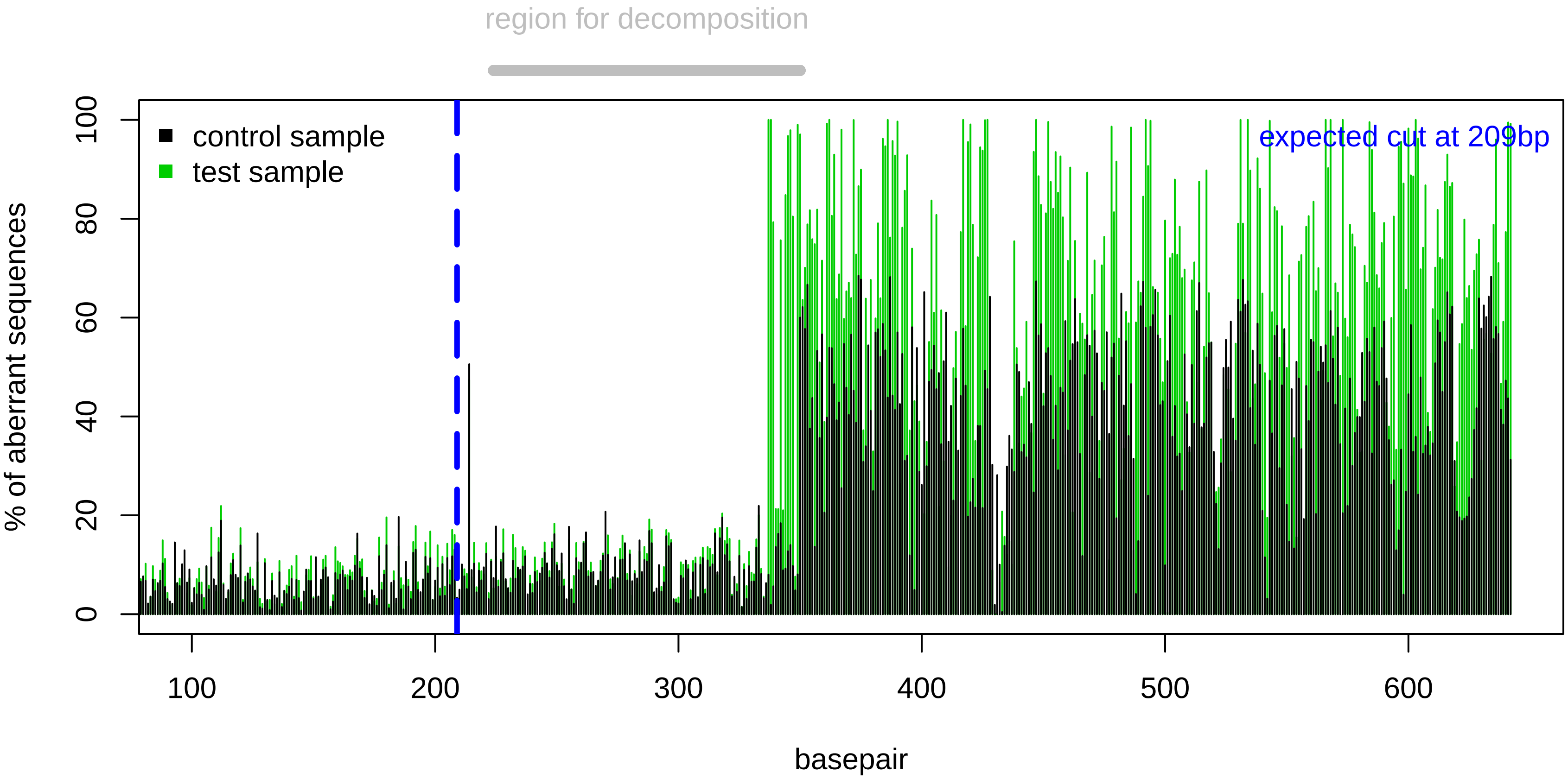
sample: Bth/WT cell line, gRNA 3.2, forward
guide: TGGTAATGTCCCTCCTGGGG



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

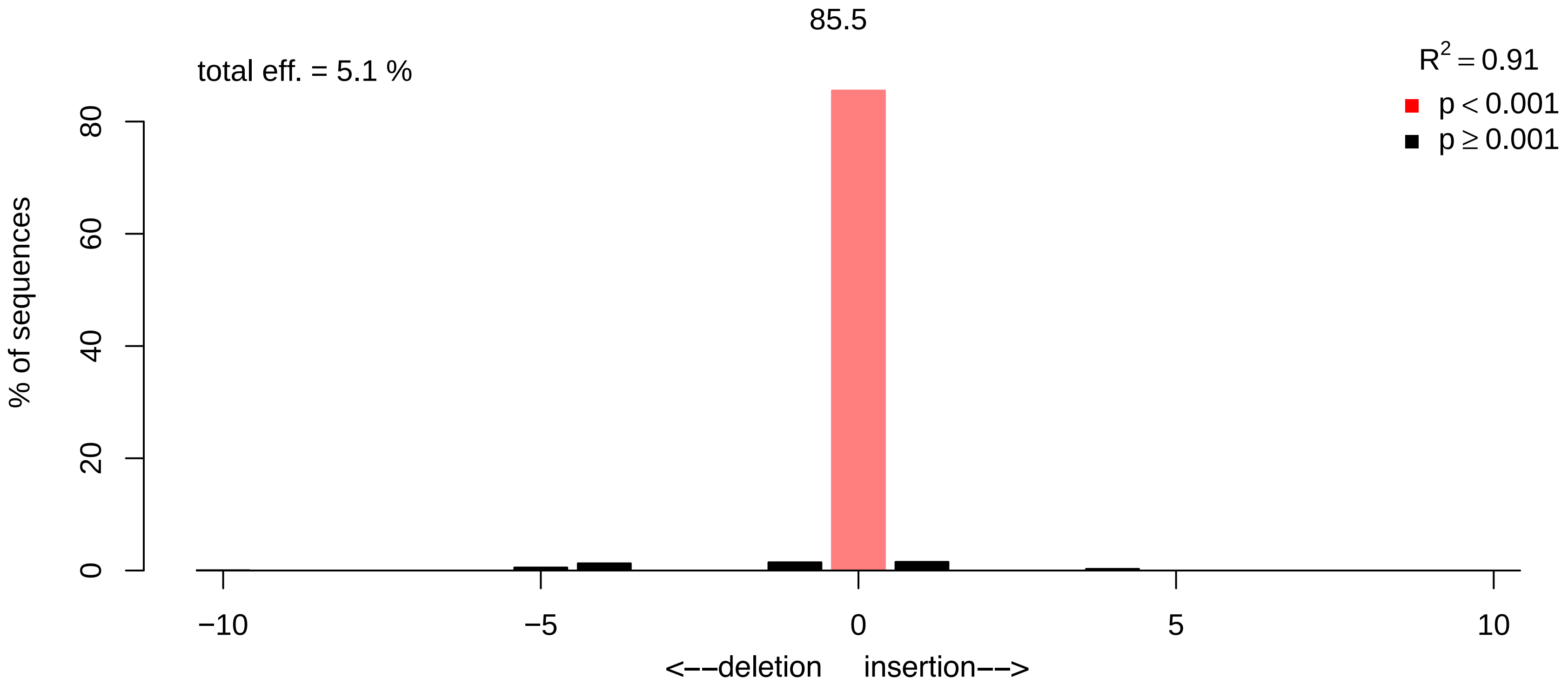
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 3.2, forward
guide: TGGTAATGTCCCTCCTGGGG



Warning: left boundary of decomposition window was adjusted 224 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

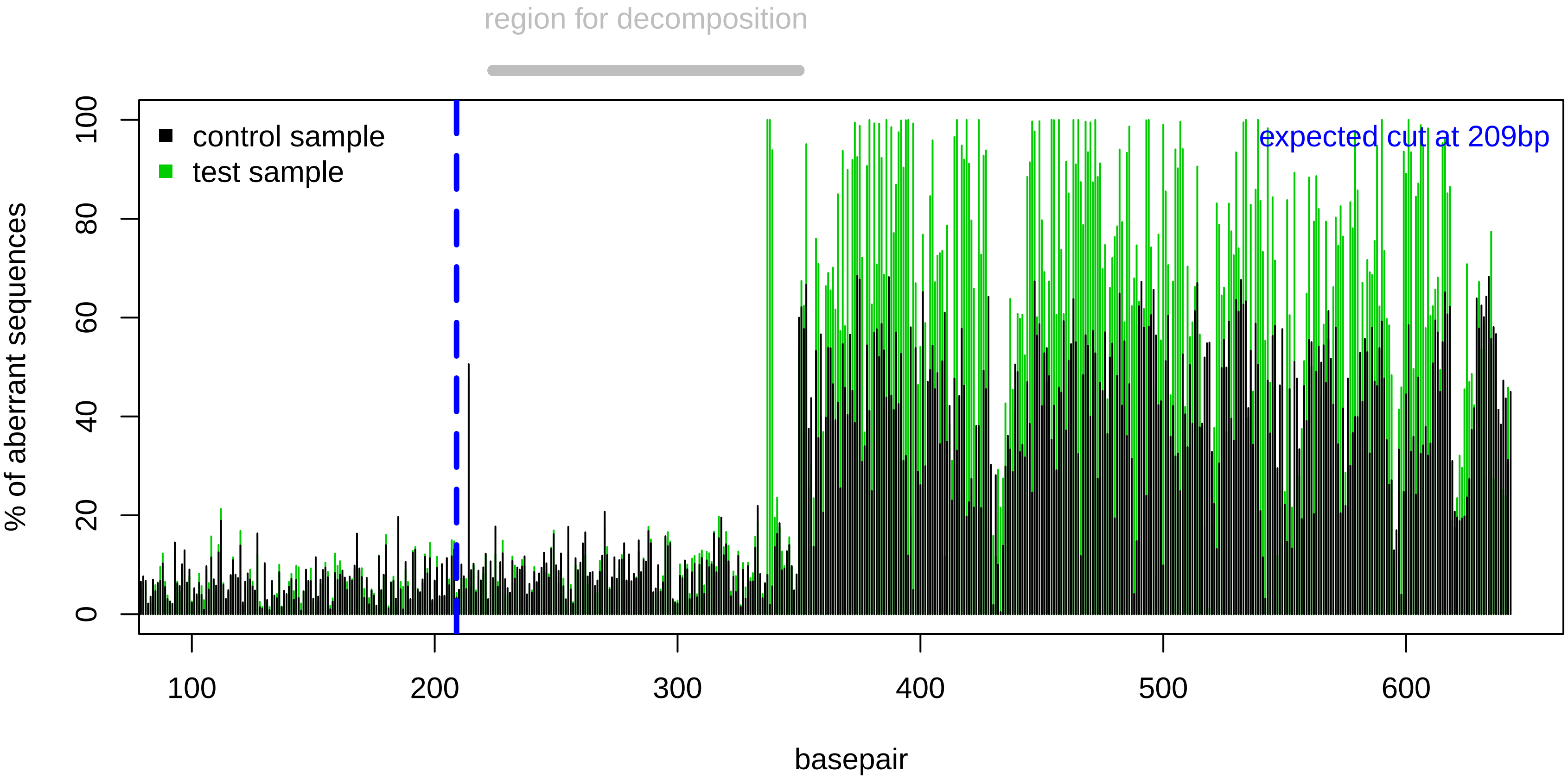
sample: Bth/WT cell line, gRNA 3.3, forward
guide: TGGTAATGTCCCTCCTGGGG



Warning: the spacing between the nucleotides in (one of) sanger sequence file(s)is not constant.

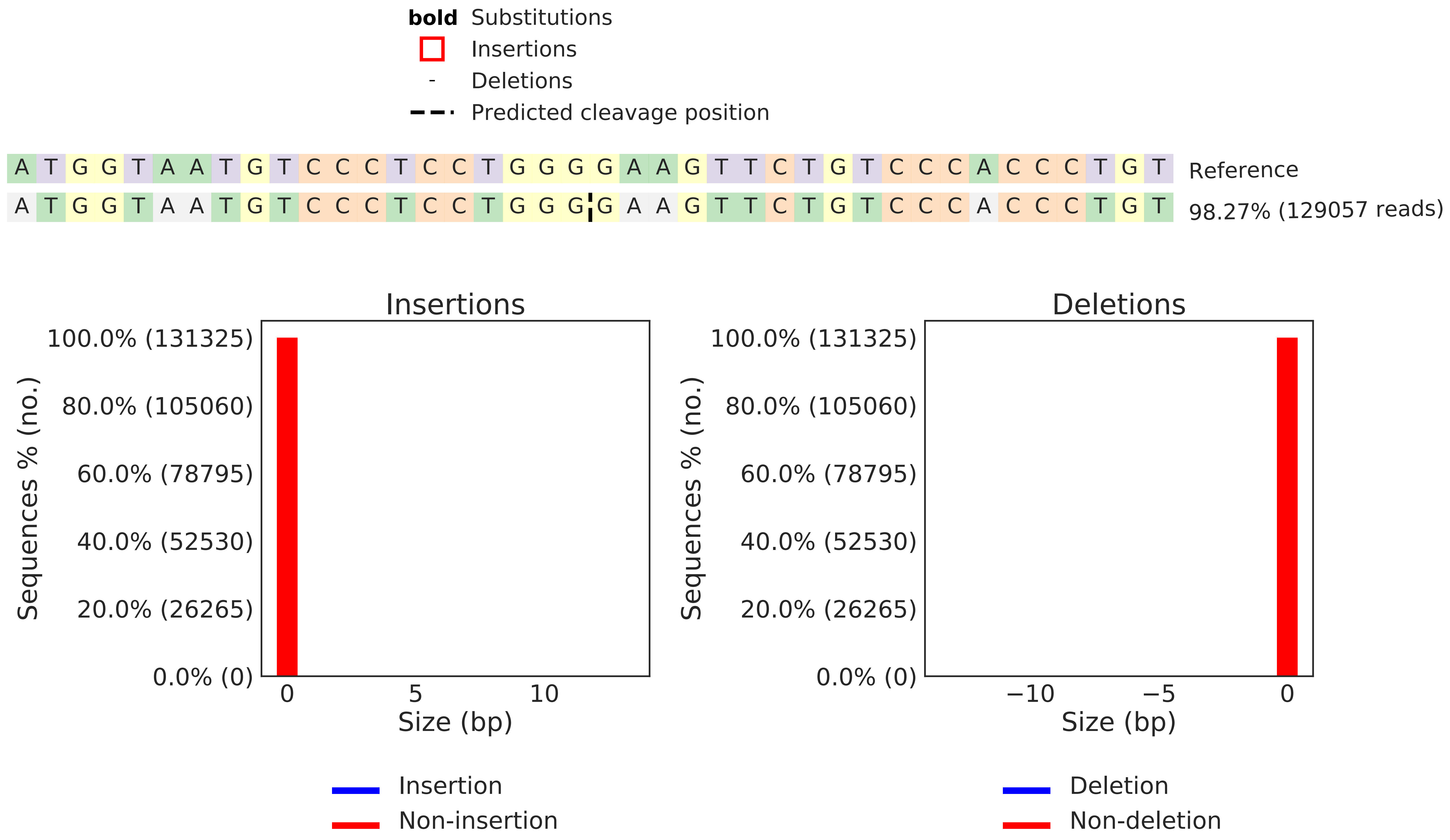
This might indicate for wrongly unannotated or wrongly additional annotated nucleotides.
Decomposition window lower and check the chromotogram for abnormalities.

sample: Bth/WT cell line, gRNA 3.3, forward
guide: TGGTAATGTCCCTCCTGGGG

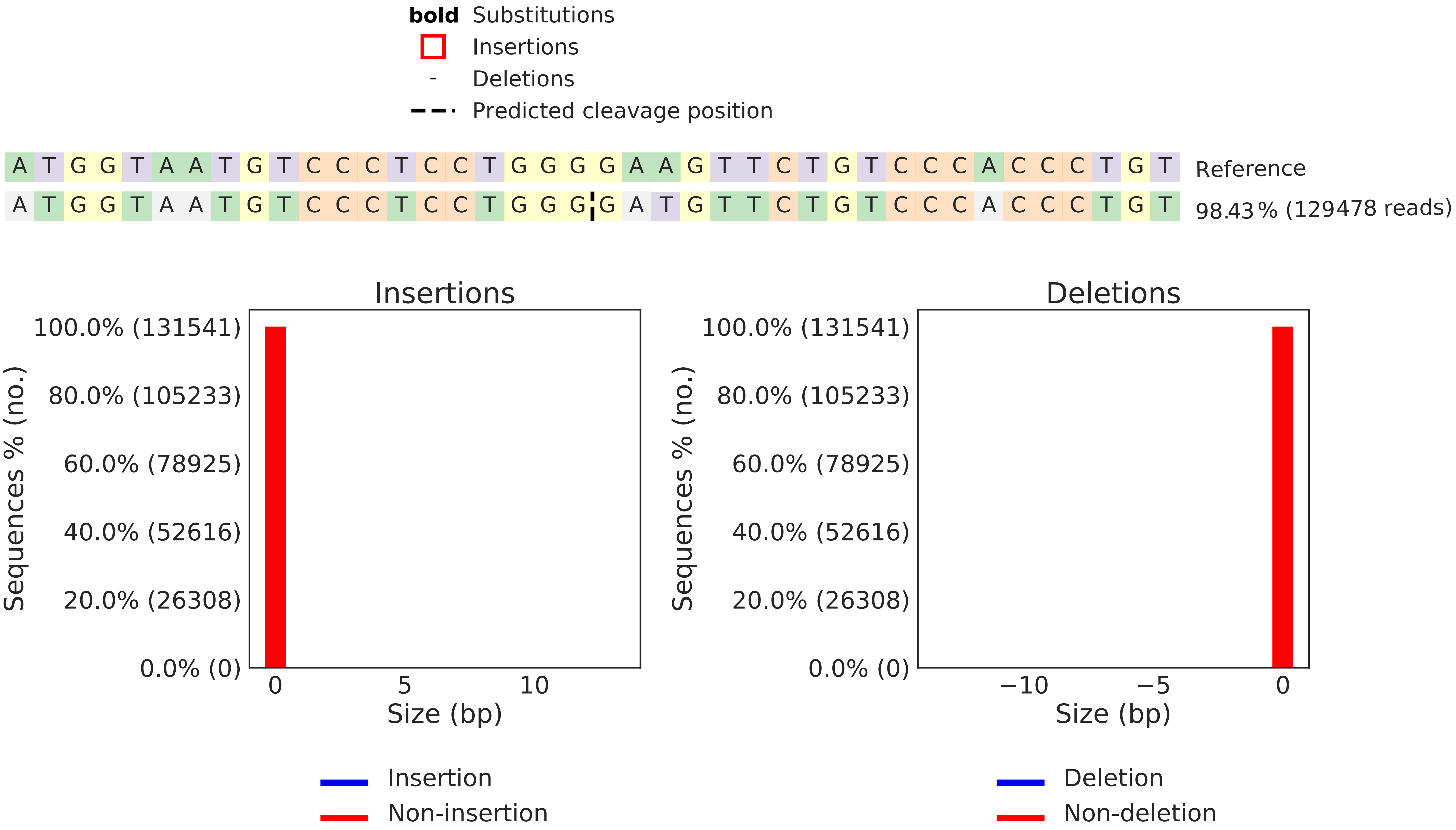


Warning: left boundary of decomposition window was adjusted 224 . It must be at least 5bp plus the maximum indel size downstream of the expected break site

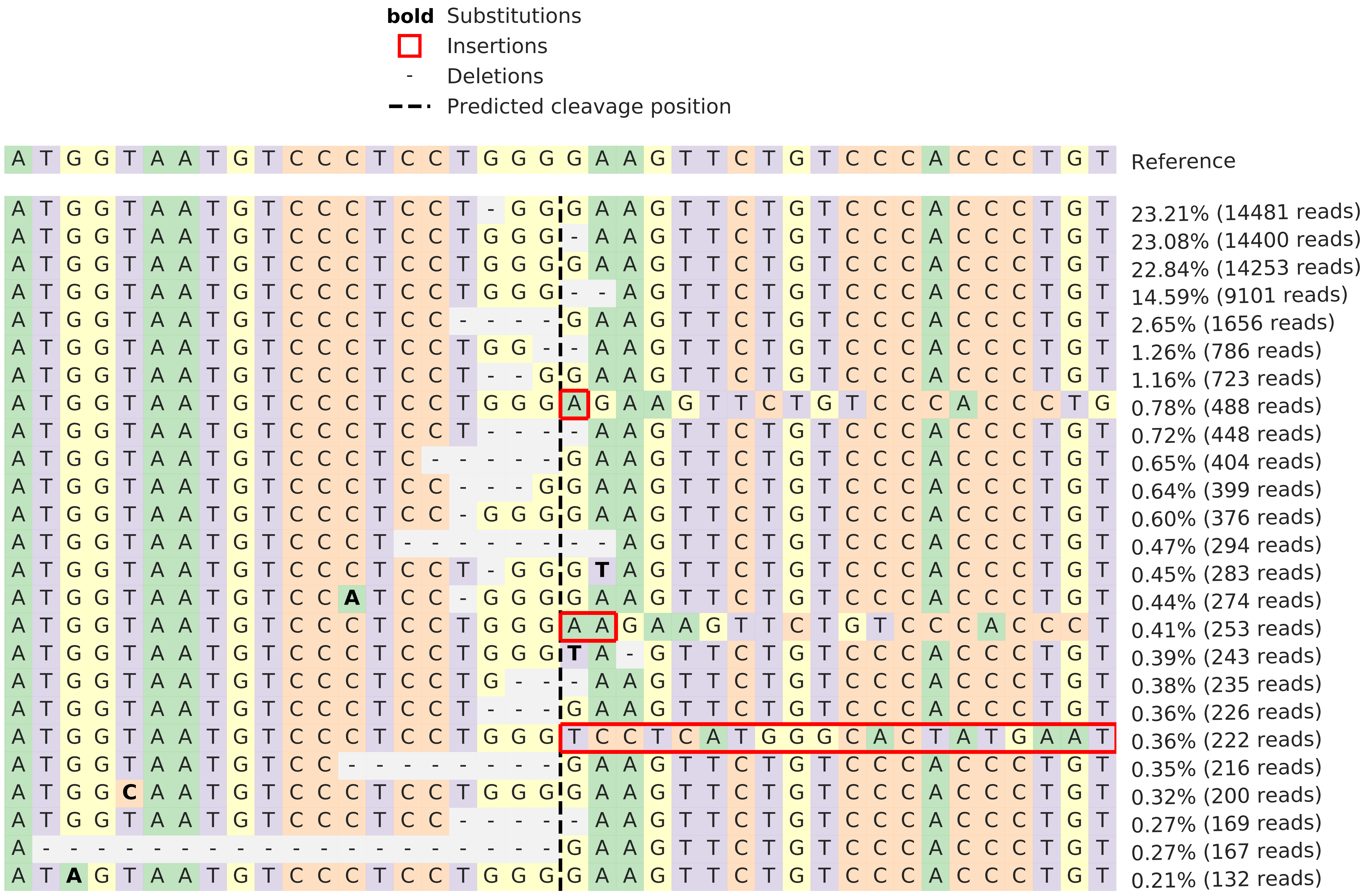
Tmc1$^{Bth/WT}$, SpCas9 only, Bth allele only



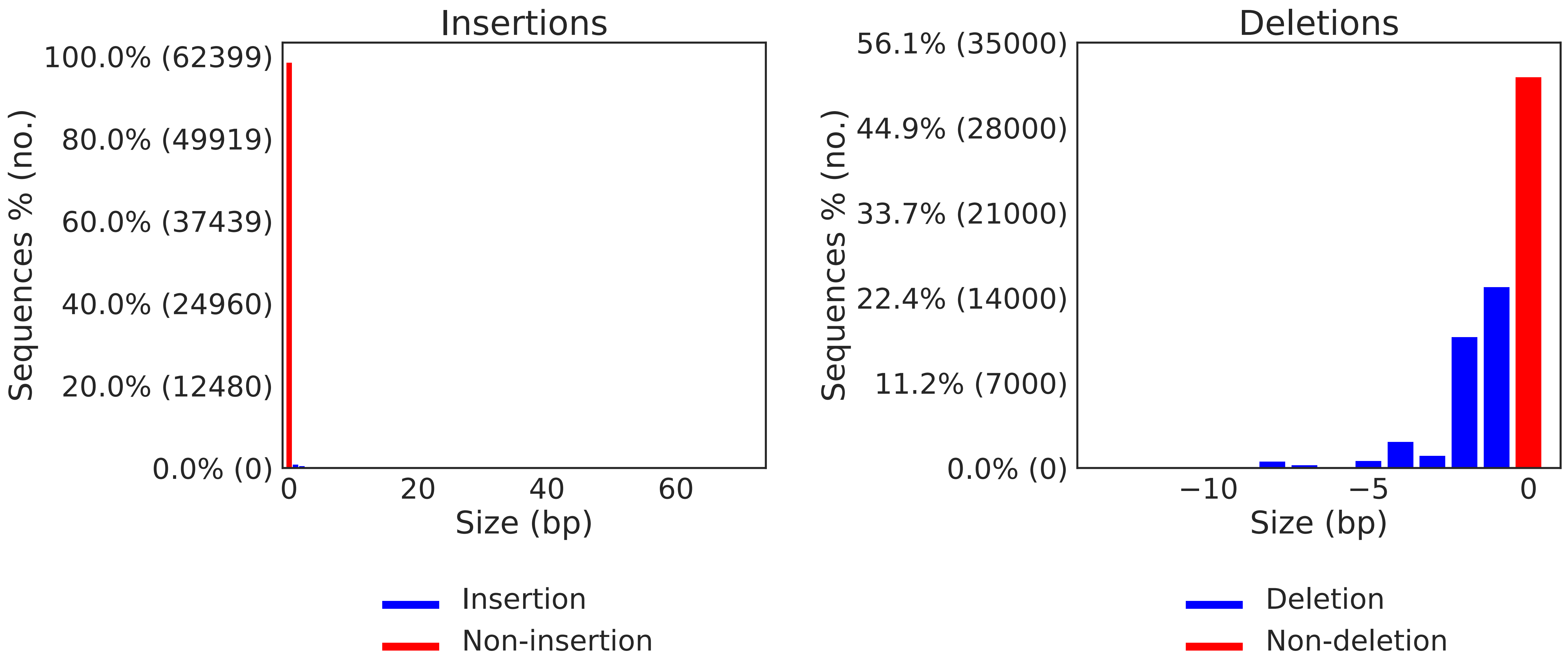
Tmc1$^{Bth/WT}$, SpCas9 only, WT allele only



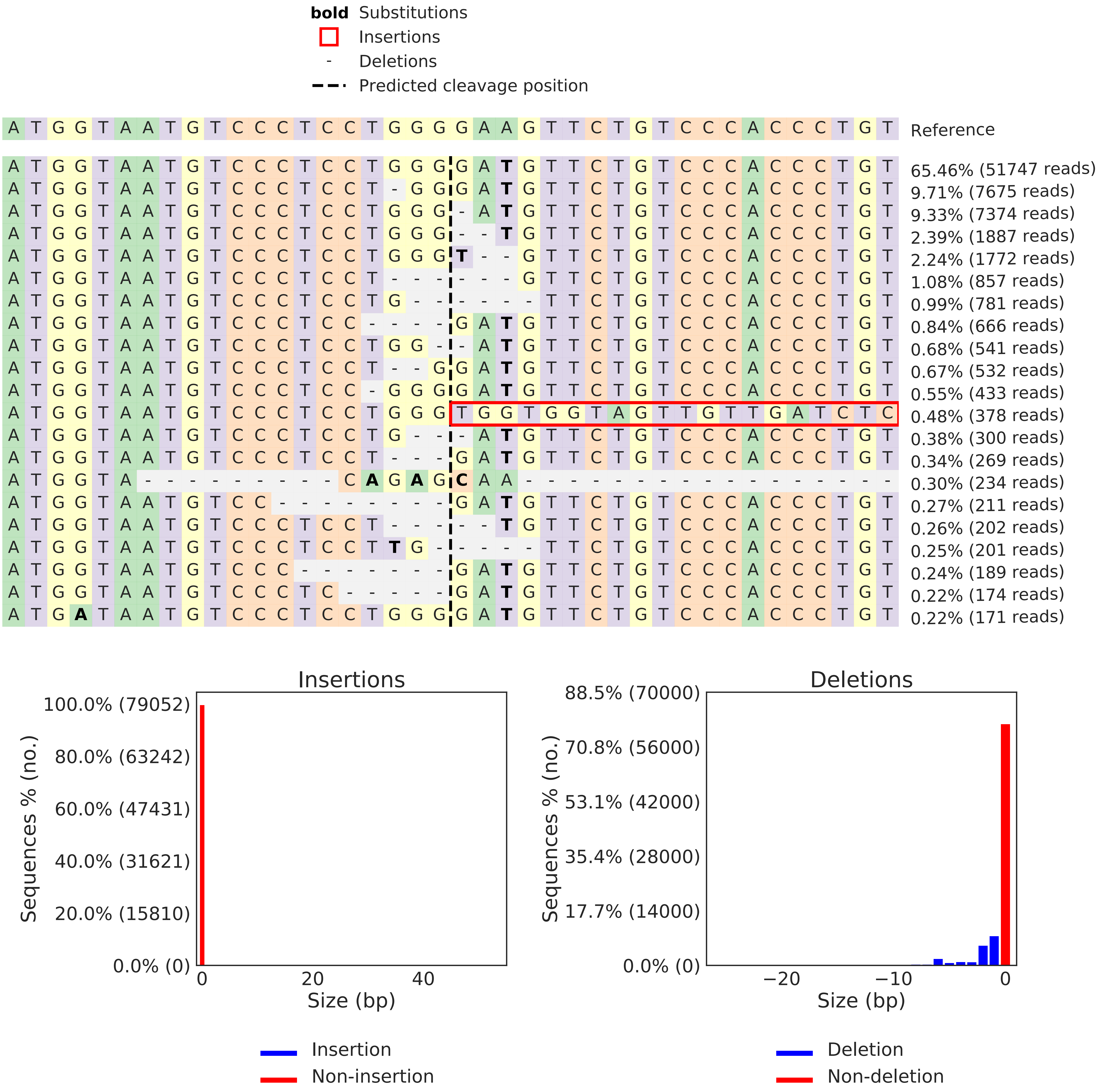
Tmc1$^{Bth/WT}$, SpCas9 + gRNA 1.1, Bth allele only



Sequence	
ATGGTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCCTGT	Reference
ATGGTAATGTCCCTCCT-GGGAAGTTCTGTCCCACCCTGT	23.21% (14481 reads)
ATGGTAATGTCCCTCCTGGG-AAGTTCTGTCCCACCCTGT	23.08% (14400 reads)
ATGGTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCCTGT	22.84% (14253 reads)
ATGGTAATGTCCCTCCTGGG--AGTTCTGTCCCACCCTGT	14.59% (9101 reads)
ATGGTAATGTCCCTCC----GAAGTTCTGTCCCACCCTGT	2.65% (1656 reads)
ATGGTAATGTCCCTCCTGG--AAGTTCTGTCCCACCCTGT	1.26% (786 reads)
ATGGTAATGTCCCTCCT--GGAAGTTCTGTCCCACCCTGT	1.16% (723 reads)
ATGGTAATGTCCCTCCTGGGAGAAGTTCTGTCCCACCCTG	0.78% (488 reads)
ATGGTAATGTCCCTCCT----AAGTTCTGTCCCACCCTGT	0.72% (448 reads)
ATGGTAATGTCCCTC-----GAAGTTCTGTCCCACCCTGT	0.65% (404 reads)
ATGGTAATGTCCCTCC---GGAAGTTCTGTCCCACCCTGT	0.64% (399 reads)
ATGGTAATGTCCCTCC-GGGGAAGTTCTGTCCCACCCTGT	0.60% (376 reads)
ATGGTAATGTCCCT--------AGTTCTGTCCCACCCTGT	0.47% (294 reads)
ATGGTAATGTCCCTCCT-GGG**T**AGTTCTGTCCCACCCTGT	0.45% (283 reads)
ATGGTAATGTCC**A**TCC-GGGGAAGTTCTGTCCCACCCTGT	0.44% (274 reads)
ATGGTAATGTCCCTCCTGGGAAGAAGTTCTGTCCCACCCT	0.41% (253 reads)
ATGGTAATGTCCCTCCTGGG**T**A-GTTCTGTCCCACCCTGT	0.39% (243 reads)
ATGGTAATGTCCCTCCTG---AAGTTCTGTCCCACCCTGT	0.38% (235 reads)
ATGGTAATGTCCCTCCT---GAAGTTCTGTCCCACCCTGT	0.36% (226 reads)
ATGGTAATGTCCCTCCTGGGTCCTCATGGGCACTATGAAT	0.36% (222 reads)
ATGGTAATGTCC--------GAAGTTCTGTCCCACCCTGT	0.35% (216 reads)
ATGG**C**AATGTCCCTCCTGGGGAAGTTCTGTCCCACCCTGT	0.32% (200 reads)
ATGGTAATGTCCCTCC-----AAGTTCTGTCCCACCCTGT	0.27% (169 reads)
A-------------------GAAGTTCTGTCCCACCCTGT	0.27% (167 reads)
AT**A**GTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCCTGT	0.21% (132 reads)



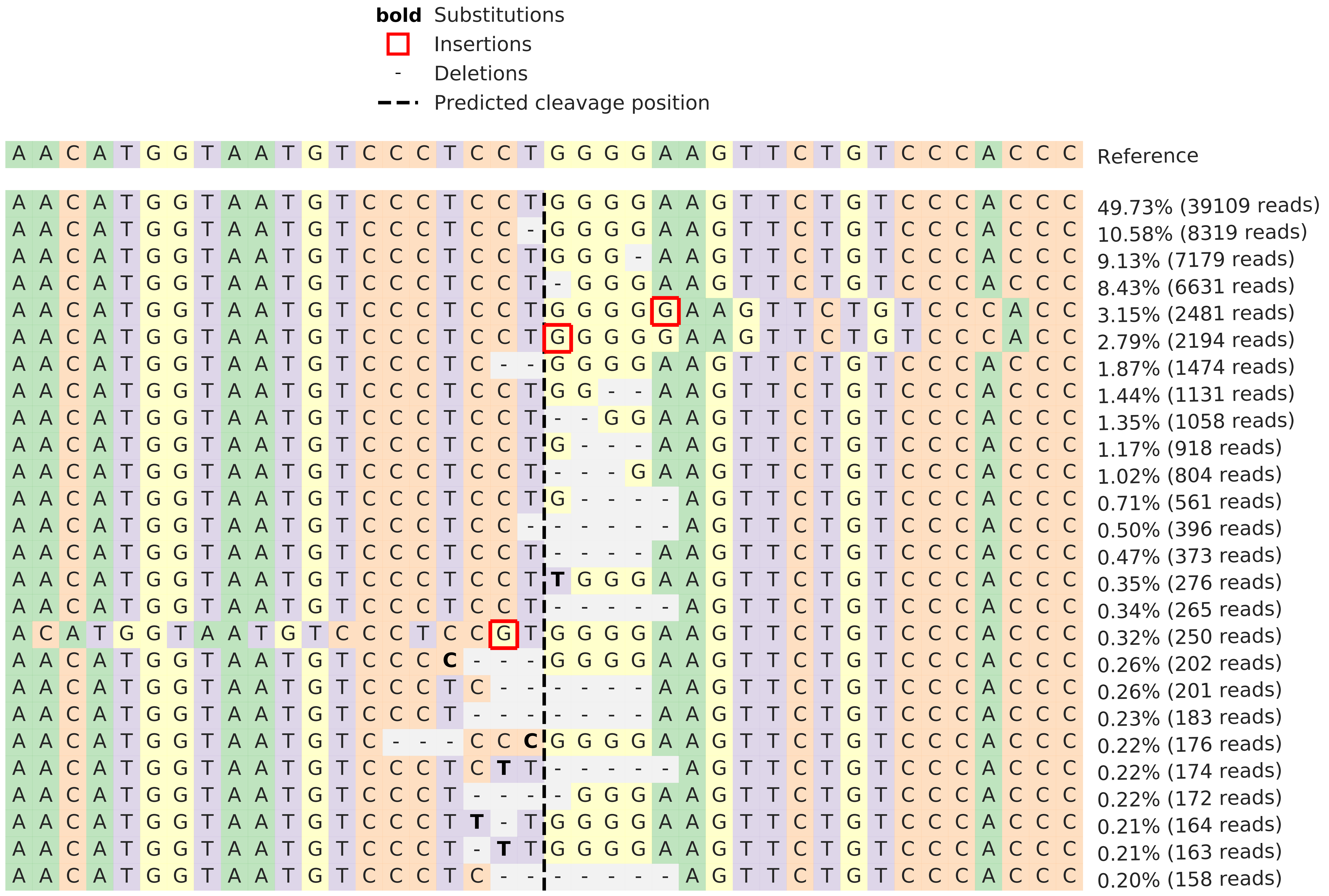
Tmc1$^{Bth/WT}$, SpCas9 + gRNA 1.1, WT allele only



bold Substitutions
☐ Insertions
\- Deletions
\- - - Predicted cleavage position

ATGGTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCCTGT	Reference
ATGGTAATGTCCCTCCTGGGGA**T**GTTCTGTCCCACCCTGT	65.46% (51747 reads)
ATGGTAATGTCCCTCCT-GGGA**T**GTTCTGTCCCACCCTGT	9.71% (7675 reads)
ATGGTAATGTCCCTCCTGGG-A**T**GTTCTGTCCCACCCTGT	9.33% (7374 reads)
ATGGTAATGTCCCTCCTGGG--**T**GTTCTGTCCCACCCTGT	2.39% (1887 reads)
ATGGTAATGTCCCTCCTGGG**T**--GTTCTGTCCCACCCTGT	2.24% (1772 reads)
ATGGTAATGTCCCTCCT------GTTCTGTCCCACCCTGT	1.08% (857 reads)
ATGGTAATGTCCCTCCTG------TTCTGTCCCACCCTGT	0.99% (781 reads)
ATGGTAATGTCCCTCC----GA**T**GTTCTGTCCCACCCTGT	0.84% (666 reads)
ATGGTAATGTCCCTCCTGG--A**T**GTTCTGTCCCACCCTGT	0.68% (541 reads)
ATGGTAATGTCCCTCCT--GGA**T**GTTCTGTCCCACCCTGT	0.67% (532 reads)
ATGGTAATGTCCCTCC-GGGGA**T**GTTCTGTCCCACCCTGT	0.55% (433 reads)
ATGGTAATGTCCCTCCTGGGTGGTGGTAGTTGTTGATCTC	0.48% (378 reads)
ATGGTAATGTCCCTCCTG---A**T**GTTCTGTCCCACCCTGT	0.38% (300 reads)
ATGGTAATGTCCCTCCT---GA**T**GTTCTGTCCCACCCTGT	0.34% (269 reads)
ATGGTA---------C**A**G**A**G**C**AA-----------------	0.30% (234 reads)
ATGGTAATGTCC--------GA**T**GTTCTGTCCCACCCTGT	0.27% (211 reads)
ATGGTAATGTCCCTCCT-----**T**GTTCTGTCCCACCCTGT	0.26% (202 reads)
ATGGTAATGTCCCTCCT**T**G-----TTCTGTCCCACCCTGT	0.25% (201 reads)
ATGGTAATGTCCC-------GA**T**GTTCTGTCCCACCCTGT	0.24% (189 reads)
ATGGTAATGTCCCTC-----GA**T**GTTCTGTCCCACCCTGT	0.22% (174 reads)
ATG**A**TAATGTCCCTCCTGGGGA**T**GTTCTGTCCCACCCTGT	0.22% (171 reads)

Tmc1$^{Bth/WT}$, SpCas9 + gRNA 2.1, Bth allele only



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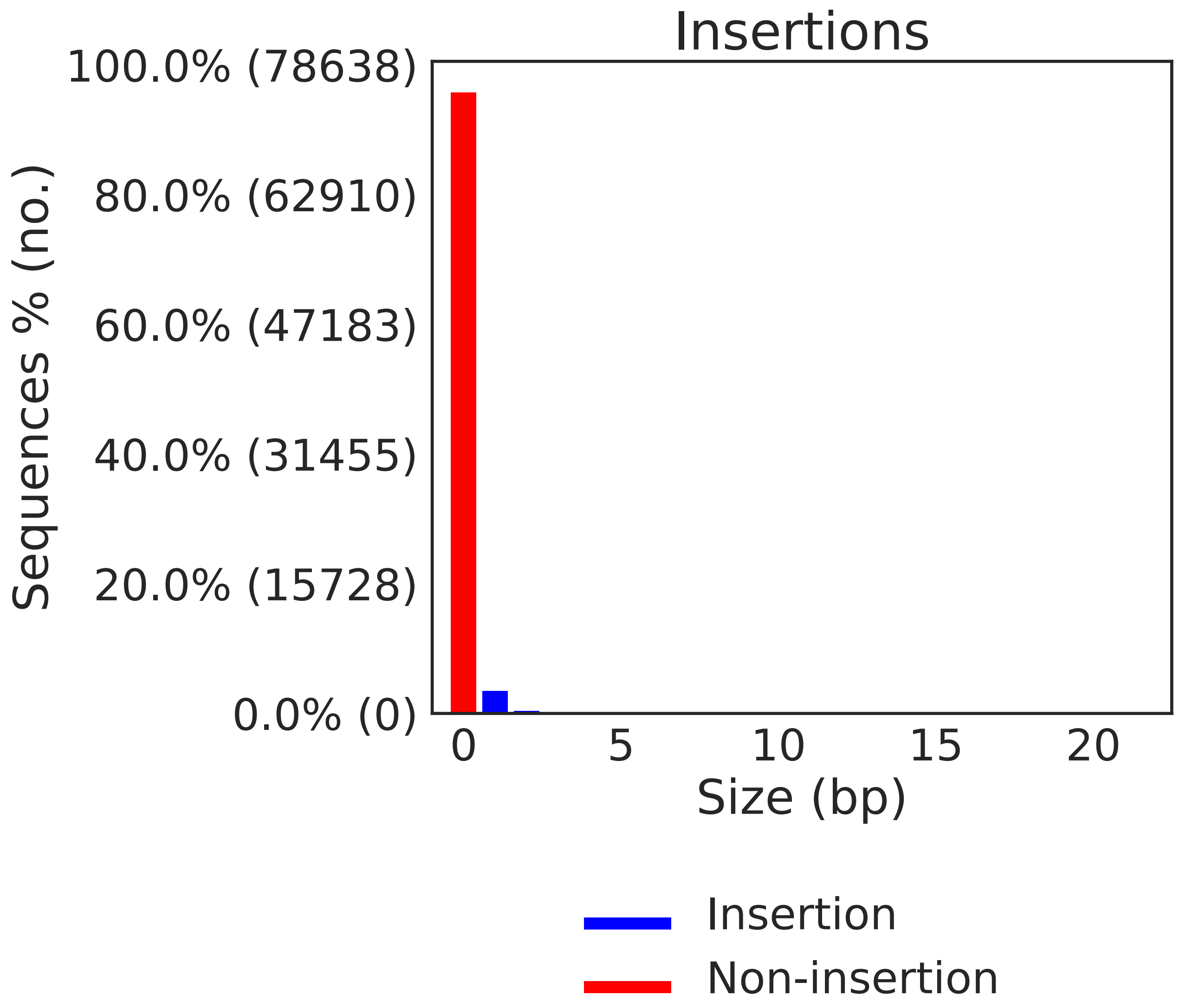
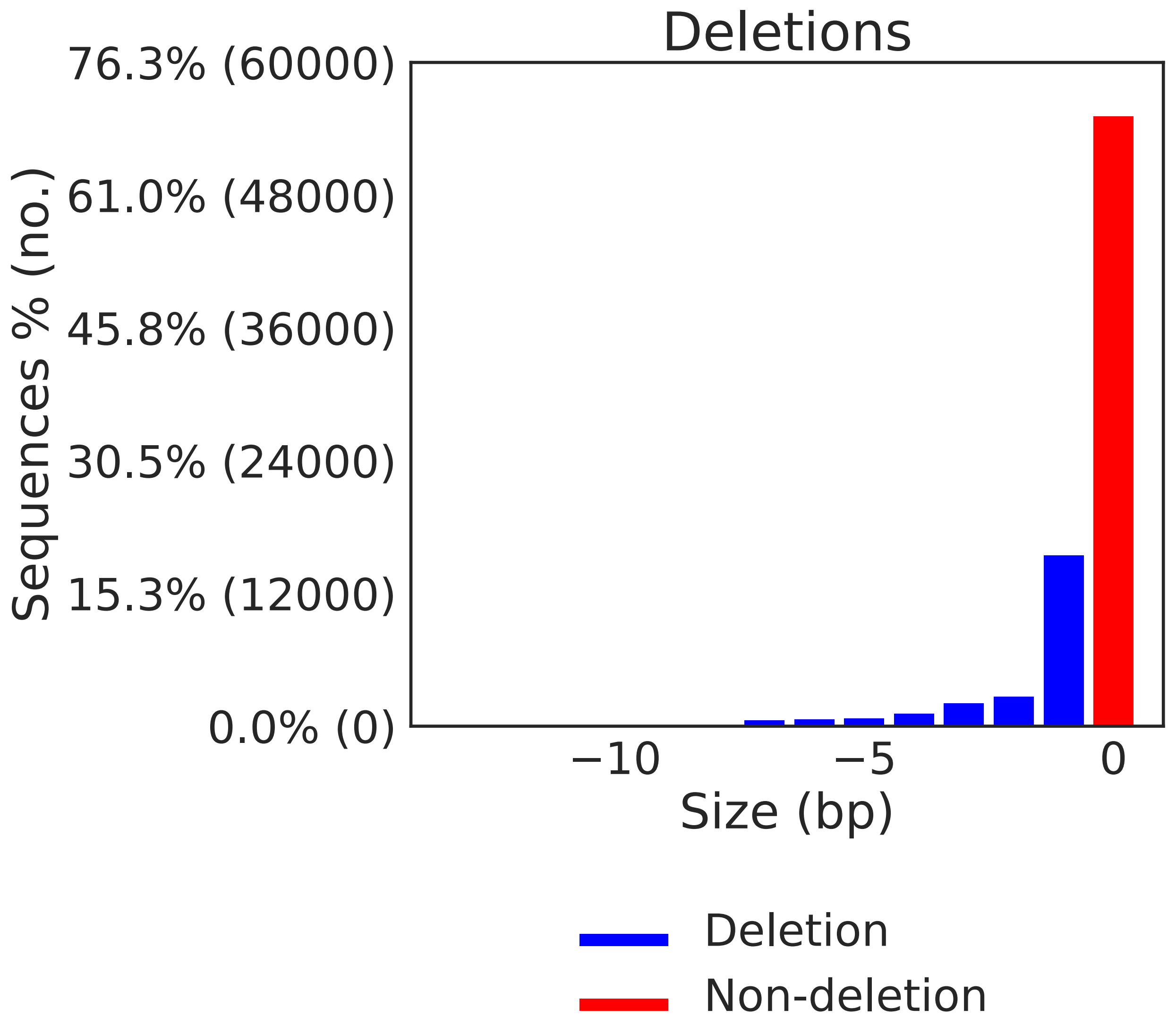
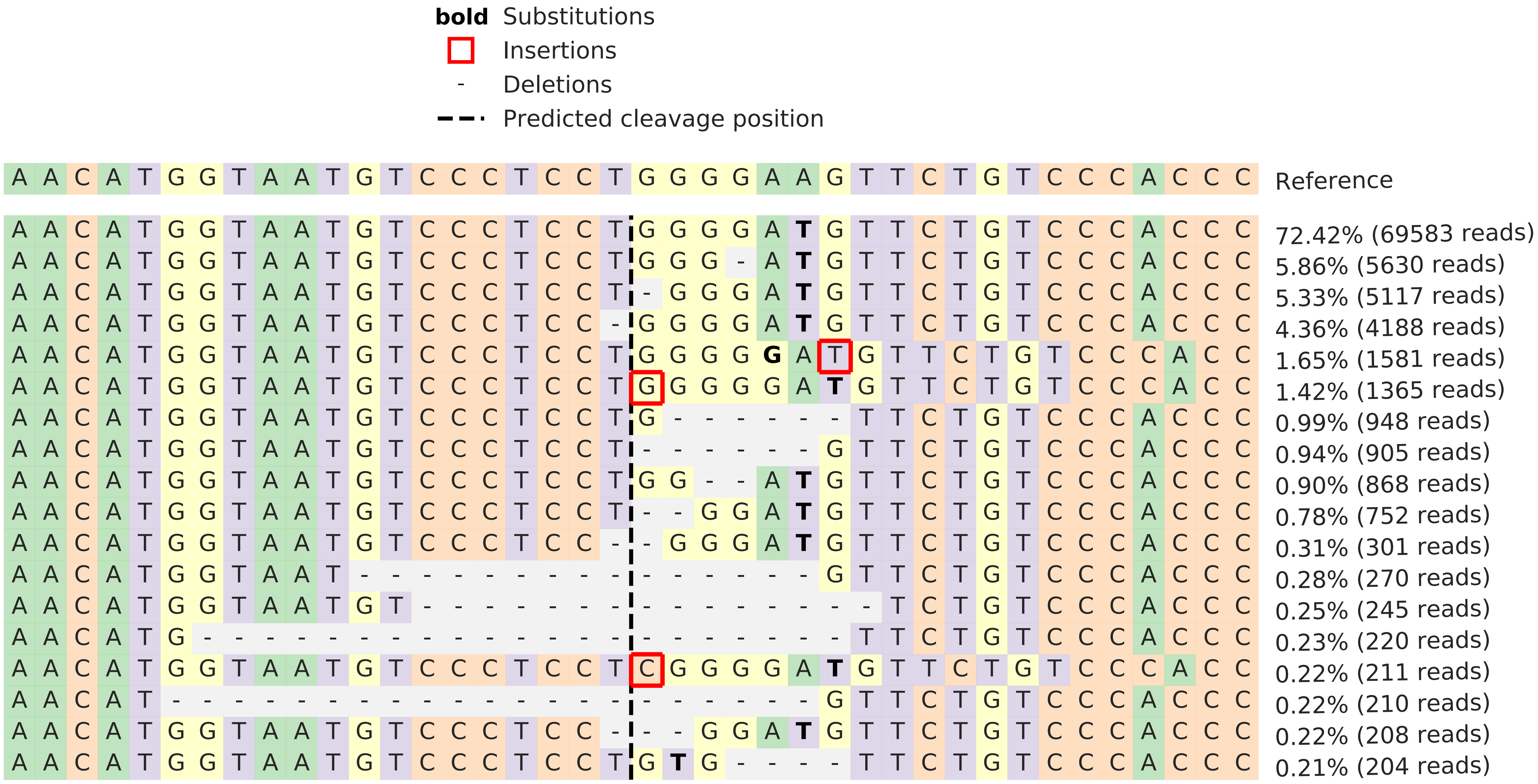
A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C   Reference

A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C   49.73% (39109 reads)
A A C A T G G T A A T G T C C C T C C - G G G G A A G T T C T G T C C C A C C C   10.58% (8319 reads)
A A C A T G G T A A T G T C C C T C C T G G G - A A G T T C T G T C C C A C C C   9.13% (7179 reads)
A A C A T G G T A A T G T C C C T C C T - G G G A A G T T C T G T C C C A C C C   8.43% (6631 reads)
A A C A T G G T A A T G T C C C T C C T G G G G G A A G T T C T G T C C C A C C   3.15% (2481 reads)
A A C A T G G T A A T G T C C C T C C T G G G G G A A G T T C T G T C C C A C C   2.79% (2194 reads)
A A C A T G G T A A T G T C C C T C - - G G G G A A G T T C T G T C C C A C C C   1.87% (1474 reads)
A A C A T G G T A A T G T C C C T C C T G G - - A A G T T C T G T C C C A C C C   1.44% (1131 reads)
A A C A T G G T A A T G T C C C T C C T - - G G A A G T T C T G T C C C A C C C   1.35% (1058 reads)
A A C A T G G T A A T G T C C C T C C T G - - - A A G T T C T G T C C C A C C C   1.17% (918 reads)
A A C A T G G T A A T G T C C C T C C T - - - G A A G T T C T G T C C C A C C C   1.02% (804 reads)
A A C A T G G T A A T G T C C C T C C T G - - - - A G T T C T G T C C C A C C C   0.71% (561 reads)
A A C A T G G T A A T G T C C C T C C - - - - - - A G T T C T G T C C C A C C C   0.50% (396 reads)
A A C A T G G T A A T G T C C C T C C T - - - - A A G T T C T G T C C C A C C C   0.47% (373 reads)
A A C A T G G T A A T G T C C C T C C T T G G G A A G T T C T G T C C C A C C C   0.35% (276 reads)
A A C A T G G T A A T G T C C C T C C T - - - - - A G T T C T G T C C C A C C C   0.34% (265 reads)
A C A T G G T A A T G T C C C T C C G T G G G G A A G T T C T G T C C C A C C C   0.32% (250 reads)
A A C A T G G T A A T G T C C C C - - - G G G G A A G T T C T G T C C C A C C C   0.26% (202 reads)
A A C A T G G T A A T G T C C C T C - - - - - - A A G T T C T G T C C C A C C C   0.26% (201 reads)
A A C A T G G T A A T G T C C C T - - - - - - - A A G T T C T G T C C C A C C C   0.23% (183 reads)
A A C A T G G T A A T G T C - - - C C C G G G G A A G T T C T G T C C C A C C C   0.22% (176 reads)
A A C A T G G T A A T G T C C C T C T T - - - - - A G T T C T G T C C C A C C C   0.22% (174 reads)
A A C A T G G T A A T G T C C C T - - - - G G G A A G T T C T G T C C C A C C C   0.22% (172 reads)
A A C A T G G T A A T G T C C C T T - T G G G G A A G T T C T G T C C C A C C C   0.21% (164 reads)
A A C A T G G T A A T G T C C C T - T T G G G G A A G T T C T G T C C C A C C C   0.21% (163 reads)
A A C A T G G T A A T G T C C C T C - - - - - - - A G T T C T G T C C C A C C C   0.20% (158 reads)
```

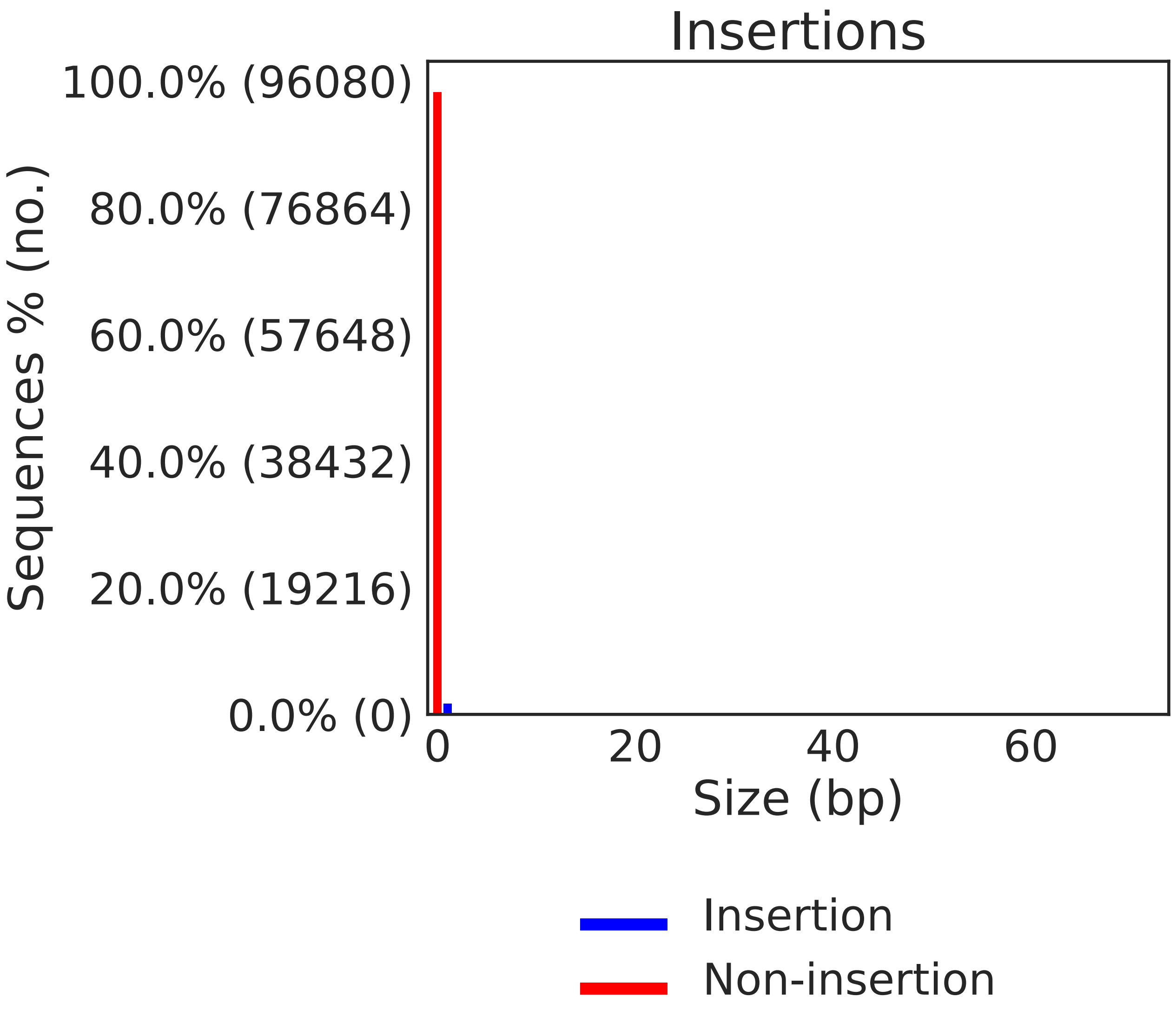


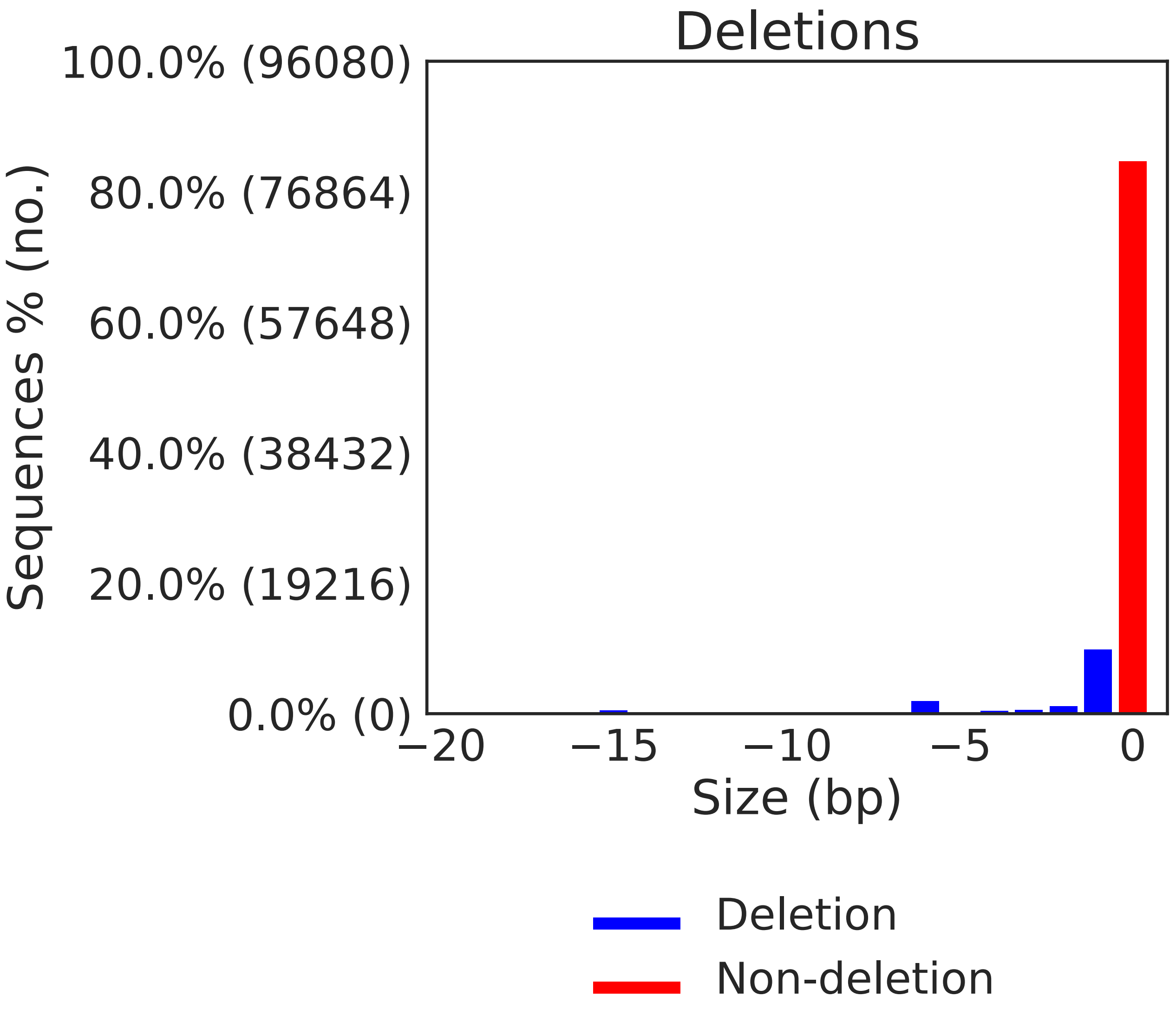


Tmc1$^{Bth/WT}$, SpCas9 + gRNA 2.1, WT allele only

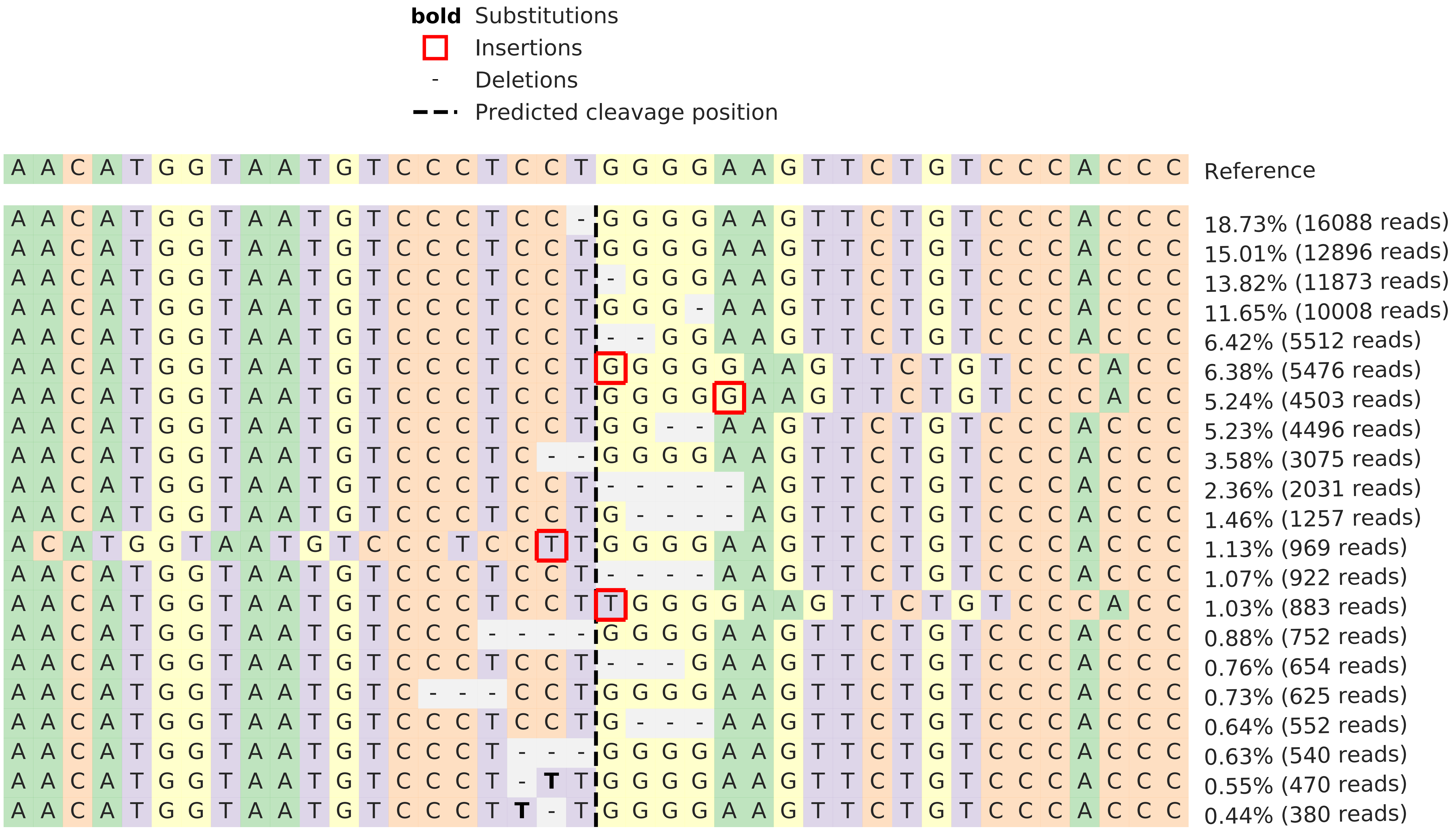


A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C	Reference
A A C A T G G T A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C	72.42% (69583 reads)
A A C A T G G T A A T G T C C C T C C T G G G - A **T** G T T C T G T C C C A C C C	5.86% (5630 reads)
A A C A T G G T A A T G T C C C T C C T - G G G A **T** G T T C T G T C C C A C C C	5.33% (5117 reads)
A A C A T G G T A A T G T C C C T C C - G G G G A **T** G T T C T G T C C C A C C C	4.36% (4188 reads)
A A C A T G G T A A T G T C C C T C C T G G G G **G** A T G T T C T G T C C C A C C	1.65% (1581 reads)
A A C A T G G T A A T G T C C C T C C T G G G G G A **T** G T T C T G T C C C A C C	1.42% (1365 reads)
A A C A T G G T A A T G T C C C T C C T G - - - - - - T T C T G T C C C A C C C	0.99% (948 reads)
A A C A T G G T A A T G T C C C T C C T - - - - - - G T T C T G T C C C A C C C	0.94% (905 reads)
A A C A T G G T A A T G T C C C T C C T G G - - A **T** G T T C T G T C C C A C C C	0.90% (868 reads)
A A C A T G G T A A T G T C C C T C C T - - G G A **T** G T T C T G T C C C A C C C	0.78% (752 reads)
A A C A T G G T A A T G T C C C T C C - - G G G A **T** G T T C T G T C C C A C C C	0.31% (301 reads)
A A C A T G G T A A T - - - - - - - - - - - - - - - G T T C T G T C C C A C C C	0.28% (270 reads)
A A C A T G G T A A T G T - - - - - - - - - - - - - - - T C T G T C C C A C C C	0.25% (245 reads)
A A C A T G - T T C T G T C C C A C C C	0.23% (220 reads)
A A C A T G G T A A T G T C C C T C C T C G G G G A **T** G T T C T G T C C C A C C	0.22% (211 reads)
A A C A T - G T T C T G T C C C A C C C	0.22% (210 reads)
A A C A T G G T A A T G T C C C T C C - - - G G A **T** G T T C T G T C C C A C C C	0.22% (208 reads)
A A C A T G G T A A T G T C C C T C C T G **T** G - - - - T T C T G T C C C A C C C	0.21% (204 reads)

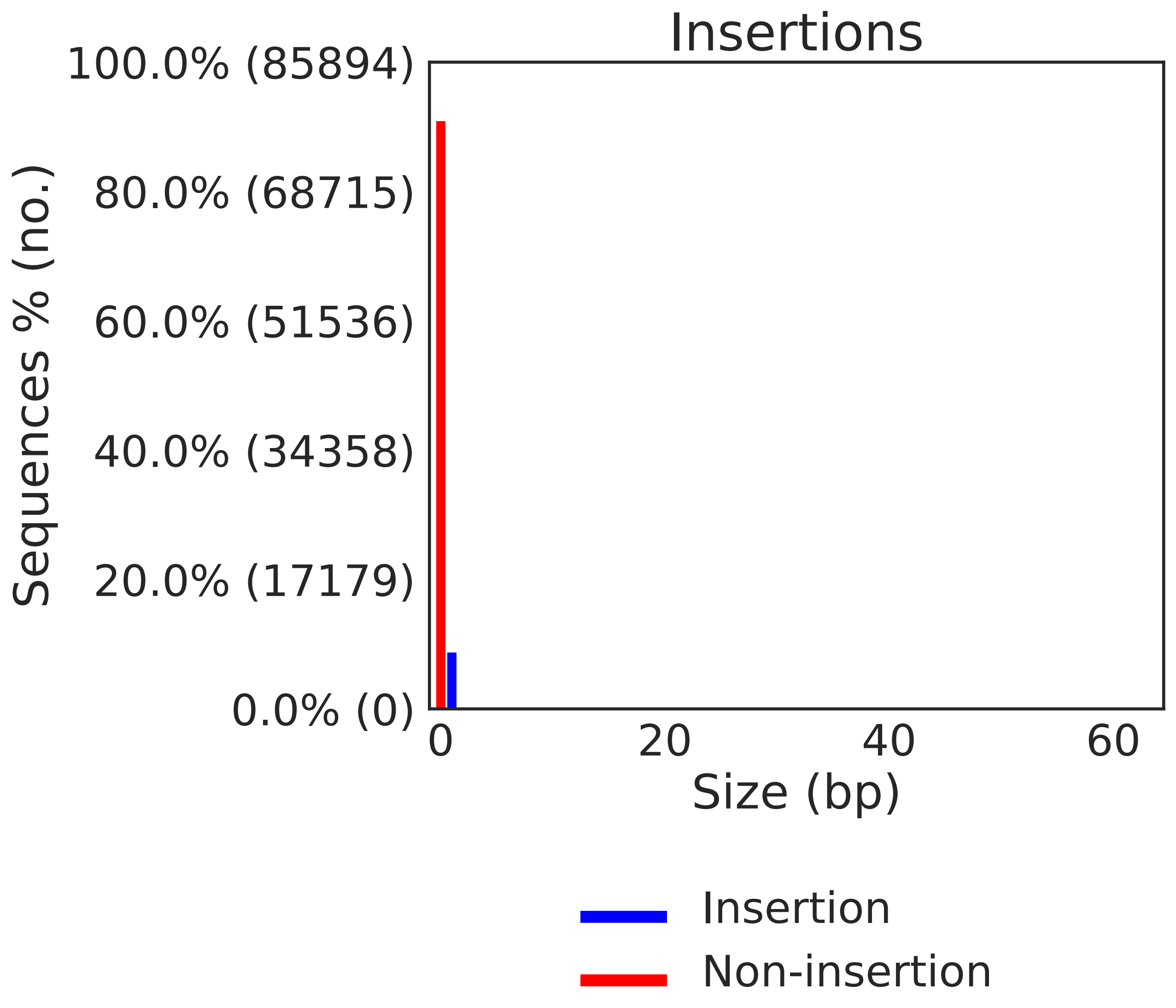


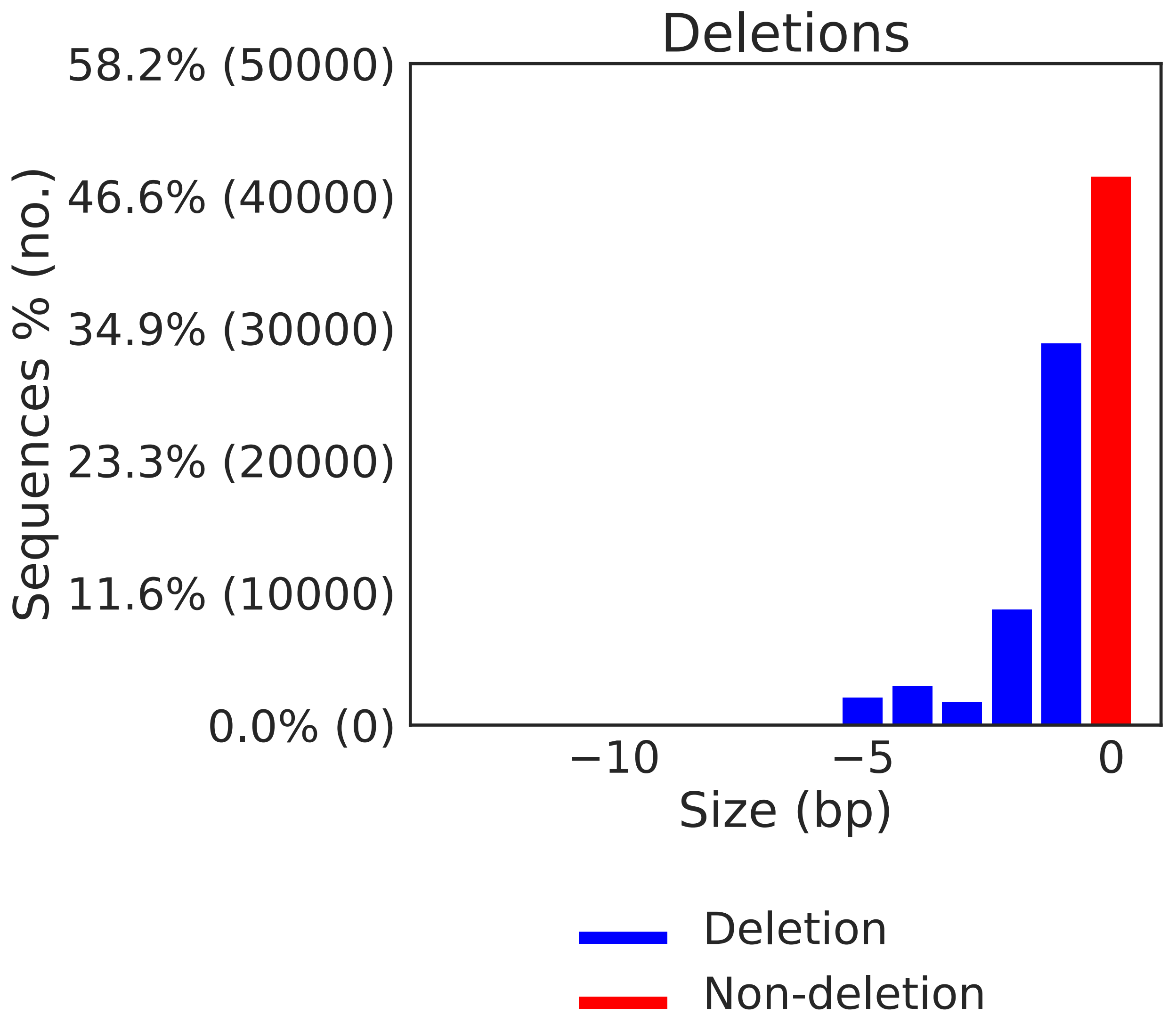


Tmc1$^{Bth/WT}$, SpCas9 + gRNA 2.4, Bth allele only

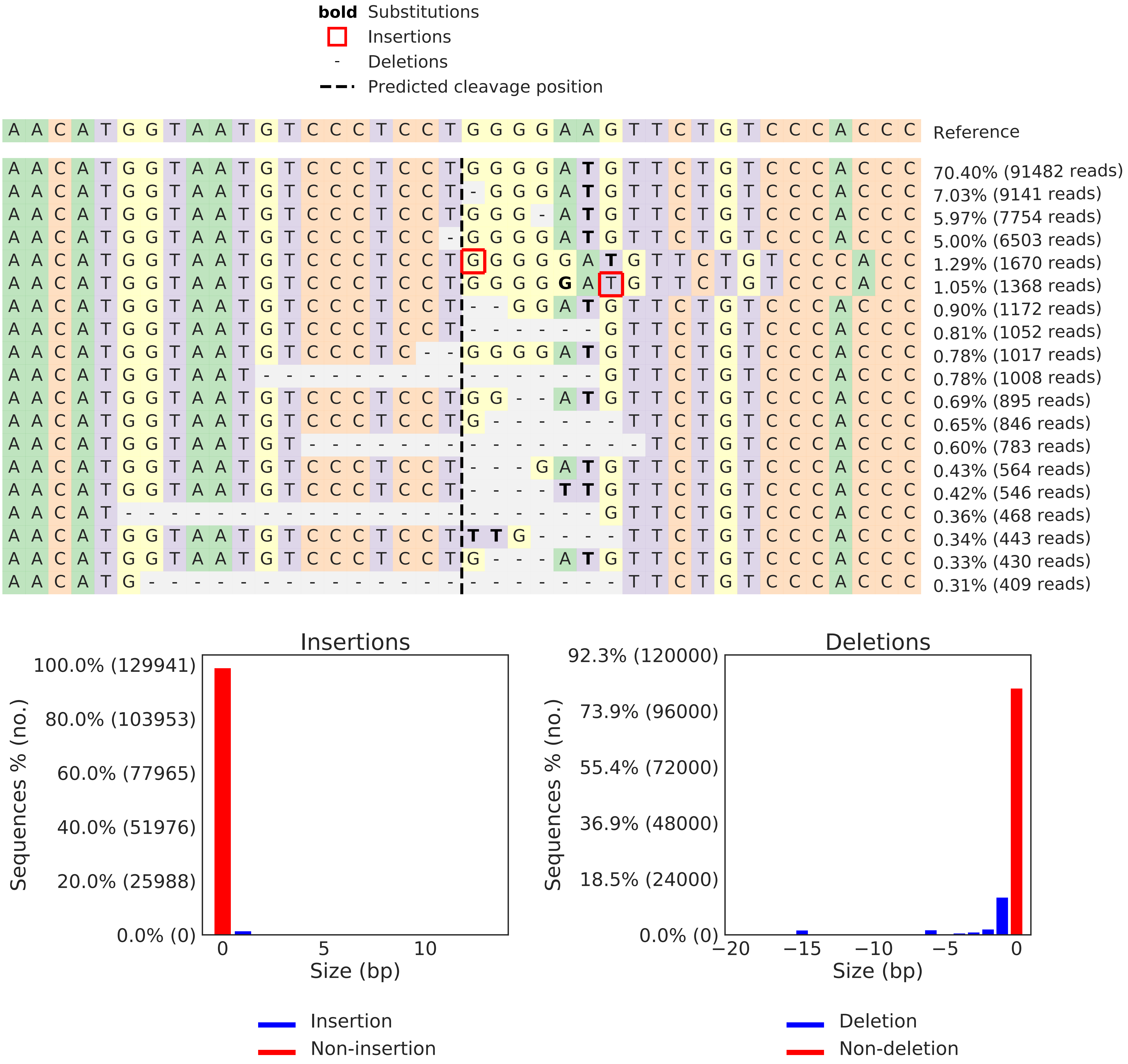


AACATGGTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCC	Reference
AACATGGTAATGTCCCTCC-GGGGAAGTTCTGTCCCACCC	18.73% (16088 reads)
AACATGGTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCC	15.01% (12896 reads)
AACATGGTAATGTCCCTCCT-GGGAAGTTCTGTCCCACCC	13.82% (11873 reads)
AACATGGTAATGTCCCTCCTGGG-AAGTTCTGTCCCACCC	11.65% (10008 reads)
AACATGGTAATGTCCCTCCT--GGAAGTTCTGTCCCACCC	6.42% (5512 reads)
AACATGGTAATGTCCCTCCTGGGGGAAGTTCTGTCCCACC	6.38% (5476 reads)
AACATGGTAATGTCCCTCCTGGGGGAAGTTCTGTCCCACC	5.24% (4503 reads)
AACATGGTAATGTCCCTCCTGG--AAGTTCTGTCCCACCC	5.23% (4496 reads)
AACATGGTAATGTCCCTC--GGGGAAGTTCTGTCCCACCC	3.58% (3075 reads)
AACATGGTAATGTCCCTCCT-----AGTTCTGTCCCACCC	2.36% (2031 reads)
AACATGGTAATGTCCCTCCTG----AGTTCTGTCCCACCC	1.46% (1257 reads)
ACATGGTAATGTCCCTCCTTGGGGAAGTTCTGTCCCACCC	1.13% (969 reads)
AACATGGTAATGTCCCTCCT----AAGTTCTGTCCCACCC	1.07% (922 reads)
AACATGGTAATGTCCCTCCTTGGGGAAGTTCTGTCCCACC	1.03% (883 reads)
AACATGGTAATGTCCC----GGGGAAGTTCTGTCCCACCC	0.88% (752 reads)
AACATGGTAATGTCCCTCCT---GAAGTTCTGTCCCACCC	0.76% (654 reads)
AACATGGTAATGTC---CCTGGGGAAGTTCTGTCCCACCC	0.73% (625 reads)
AACATGGTAATGTCCCTCCTG---AAGTTCTGTCCCACCC	0.64% (552 reads)
AACATGGTAATGTCCCT---GGGGAAGTTCTGTCCCACCC	0.63% (540 reads)
AACATGGTAATGTCCCT-**T**TGGGGAAGTTCTGTCCCACCC	0.55% (470 reads)
AACATGGTAATGTCCCT**T**-TGGGGAAGTTCTGTCCCACCC	0.44% (380 reads)





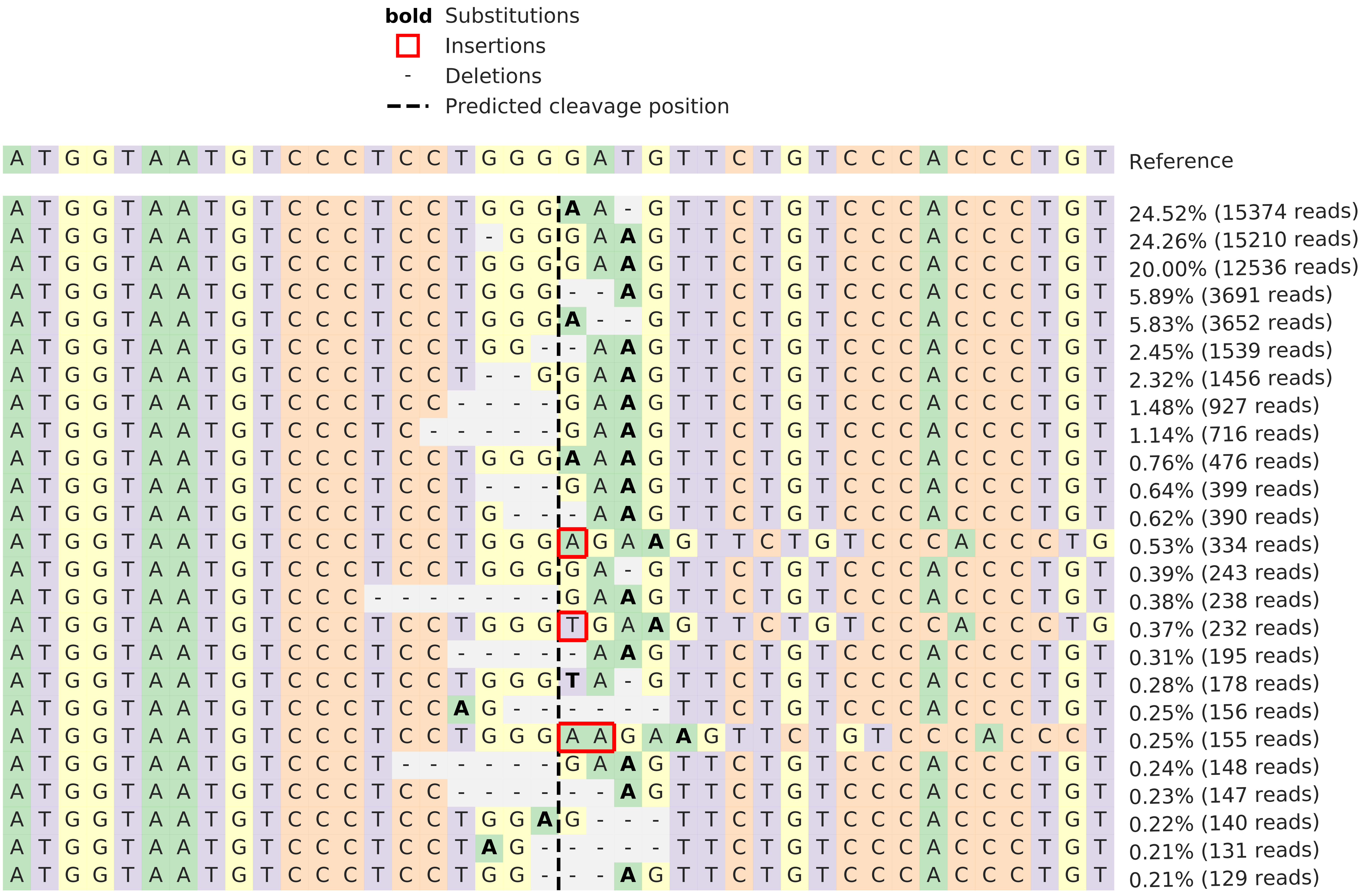
Tmc1$^{Bth/WT}$, SpCas9 + gRNA 2.4, WT allele only



A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C Reference

A A C A T G G T A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C 70.40% (91482 reads)
A A C A T G G T A A T G T C C C T C C T - G G G A **T** G T T C T G T C C C A C C C 7.03% (9141 reads)
A A C A T G G T A A T G T C C C T C C T G G G - A **T** G T T C T G T C C C A C C C 5.97% (7754 reads)
A A C A T G G T A A T G T C C C T C C - G G G G A **T** G T T C T G T C C C A C C C 5.00% (6503 reads)
A A C A T G G T A A T G T C C C T C C T G G G G G A **T** G T T C T G T C C C A C C 1.29% (1670 reads)
A A C A T G G T A A T G T C C C T C C T G G G G **G** A T G T T C T G T C C C A C C 1.05% (1368 reads)
A A C A T G G T A A T G T C C C T C C T - - G G A **T** G T T C T G T C C C A C C C 0.90% (1172 reads)
A A C A T G G T A A T G T C C C T C C T - - - - - - G T T C T G T C C C A C C C 0.81% (1052 reads)
A A C A T G G T A A T G T C C C T C - - G G G G A **T** G T T C T G T C C C A C C C 0.78% (1017 reads)
A A C A T G G T A A T - - - - - - - - - - - - - - - G T T C T G T C C C A C C C 0.78% (1008 reads)
A A C A T G G T A A T G T C C C T C C T G G - - A **T** G T T C T G T C C C A C C C 0.69% (895 reads)
A A C A T G G T A A T G T C C C T C C T G - - - - - - T T C T G T C C C A C C C 0.65% (846 reads)
A A C A T G G T A A T G T - - - - - - - - - - - - - - - T C T G T C C C A C C C 0.60% (783 reads)
A A C A T G G T A A T G T C C C T C C T - - - G A **T** G T T C T G T C C C A C C C 0.43% (564 reads)
A A C A T G G T A A T G T C C C T C C T - - - - **T** **T** G T T C T G T C C C A C C C 0.42% (546 reads)
A A C A T - G T T C T G T C C C A C C C 0.36% (468 reads)
A A C A T G G T A A T G T C C C T C C T **T** **T** G - - - - T T C T G T C C C A C C C 0.34% (443 reads)
A A C A T G G T A A T G T C C C T C C T G - - - A **T** G T T C T G T C C C A C C C 0.33% (430 reads)
A A C A T G - T T C T G T C C C A C C C 0.31% (409 reads)

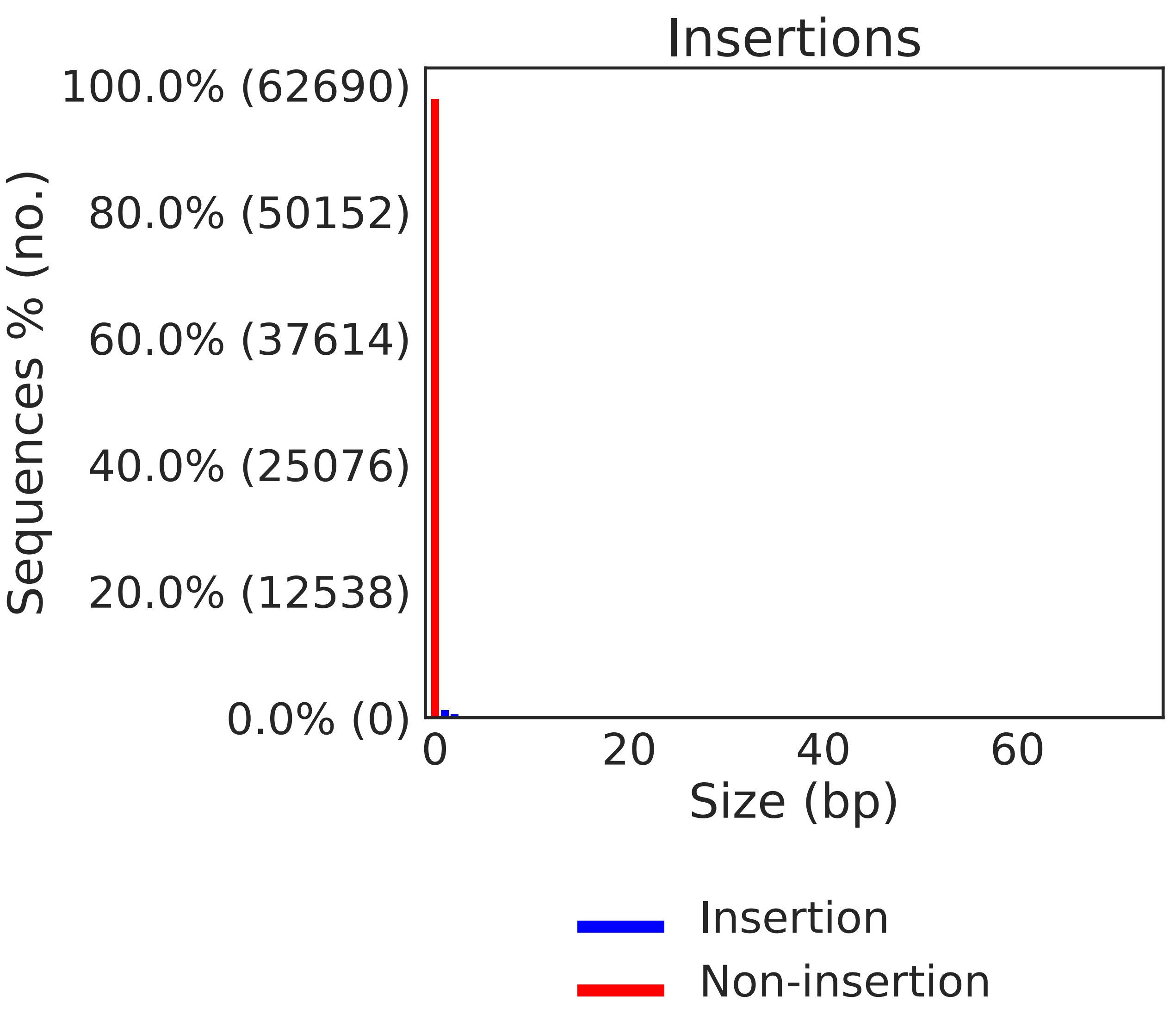
Tmc1$^{Bth/WT}$, SpCas9 + gRNA WT, Bth allele only

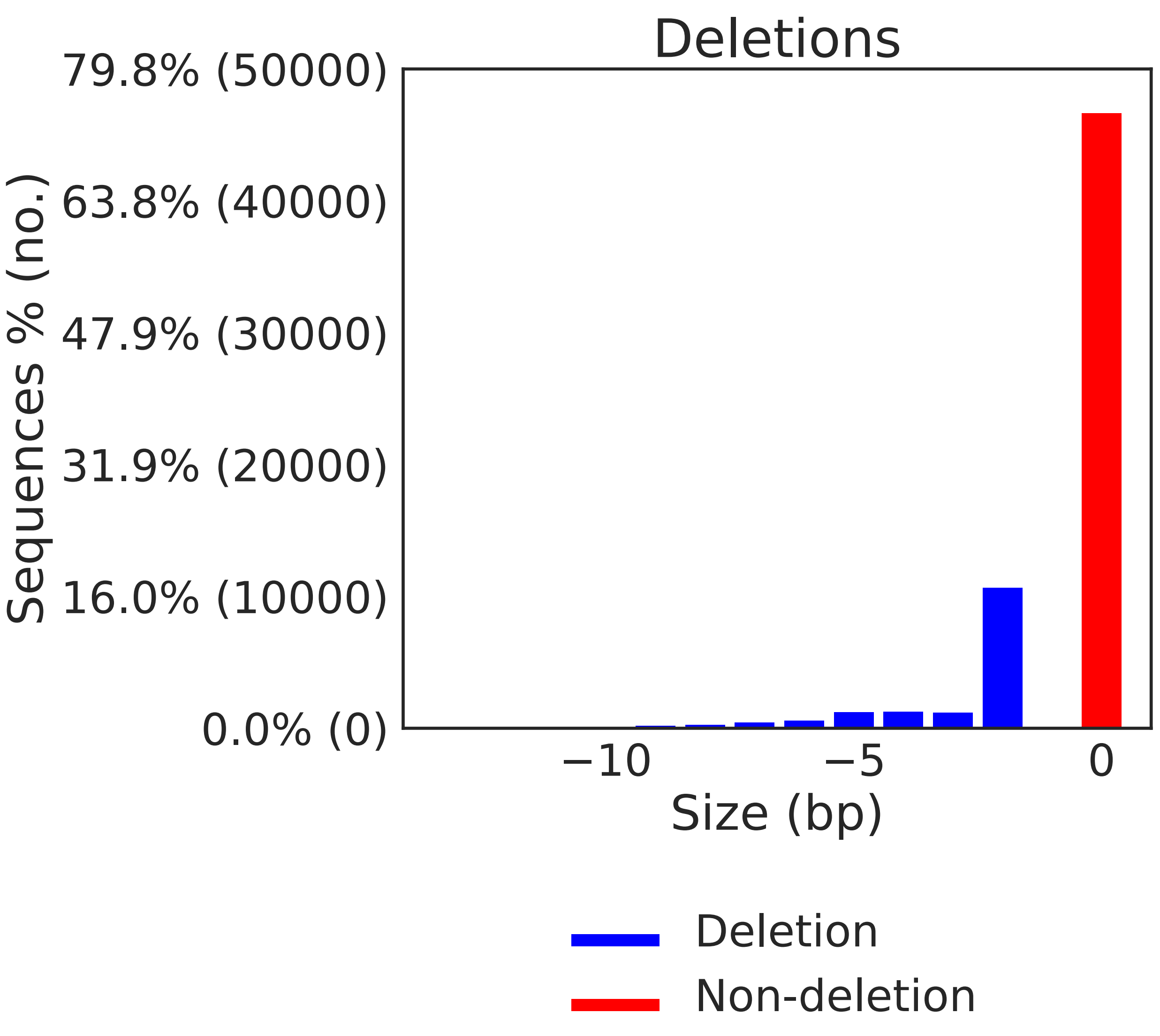


bold Substitutions
☐ Insertions
\- Deletions
\- - - Predicted cleavage position

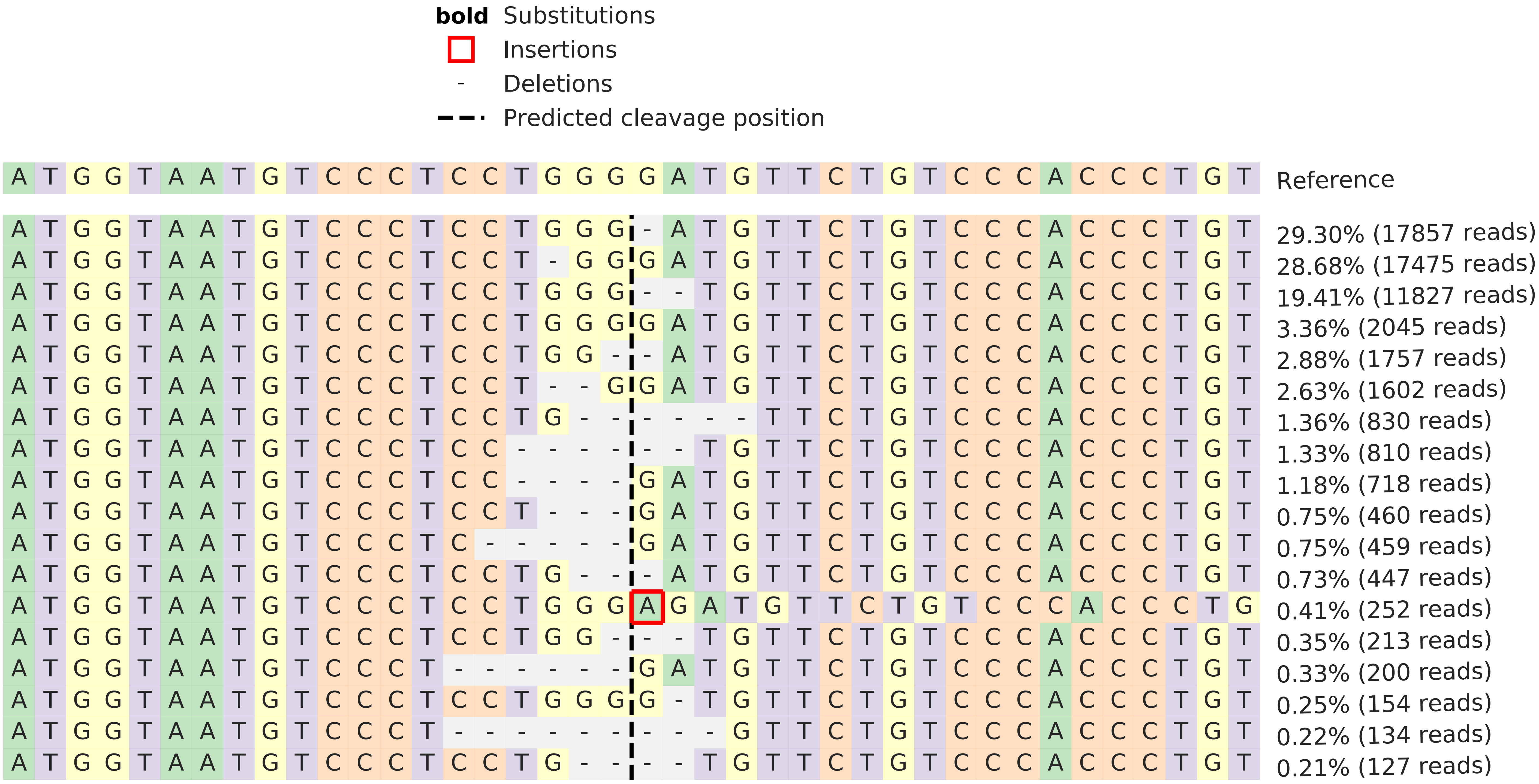
ATGGTAATGTCCCTCCTGGGGATGTTCTGTCCCACCCTGT Reference

ATGGTAATGTCCCTCCTGGG**A**A-GTTCTGTCCCACCCTGT 24.52% (15374 reads)
ATGGTAATGTCCCTCCT-GGGA**A**GTTCTGTCCCACCCTGT 24.26% (15210 reads)
ATGGTAATGTCCCTCCTGGGGA**A**GTTCTGTCCCACCCTGT 20.00% (12536 reads)
ATGGTAATGTCCCTCCTGGG--**A**GTTCTGTCCCACCCTGT 5.89% (3691 reads)
ATGGTAATGTCCCTCCTGGG**A**--GTTCTGTCCCACCCTGT 5.83% (3652 reads)
ATGGTAATGTCCCTCCTGG--A**A**GTTCTGTCCCACCCTGT 2.45% (1539 reads)
ATGGTAATGTCCCTCCT--GGA**A**GTTCTGTCCCACCCTGT 2.32% (1456 reads)
ATGGTAATGTCCCTCC----GA**A**GTTCTGTCCCACCCTGT 1.48% (927 reads)
ATGGTAATGTCCCTC-----GA**A**GTTCTGTCCCACCCTGT 1.14% (716 reads)
ATGGTAATGTCCCTCCTGGG**A**A**A**GTTCTGTCCCACCCTGT 0.76% (476 reads)
ATGGTAATGTCCCTCCT---GA**A**GTTCTGTCCCACCCTGT 0.64% (399 reads)
ATGGTAATGTCCCTCCTG---A**A**GTTCTGTCCCACCCTGT 0.62% (390 reads)
ATGGTAATGTCCCTCCTGGGAGA**A**GTTCTGTCCCACCCTG 0.53% (334 reads)
ATGGTAATGTCCCTCCTGGGGA-GTTCTGTCCCACCCTGT 0.39% (243 reads)
ATGGTAATGTCCC-------GA**A**GTTCTGTCCCACCCTGT 0.38% (238 reads)
ATGGTAATGTCCCTCCTGGGTGA**A**GTTCTGTCCCACCCTG 0.37% (232 reads)
ATGGTAATGTCCCTCC-----A**A**GTTCTGTCCCACCCTGT 0.31% (195 reads)
ATGGTAATGTCCCTCCTGGG**T**A-GTTCTGTCCCACCCTGT 0.28% (178 reads)
ATGGTAATGTCCCTCC**A**G------TTCTGTCCCACCCTGT 0.25% (156 reads)
ATGGTAATGTCCCTCCTGGGAAGA**A**GTTCTGTCCCACCCT 0.25% (155 reads)
ATGGTAATGTCCCT------GA**A**GTTCTGTCCCACCCTGT 0.24% (148 reads)
ATGGTAATGTCCCTCC------**A**GTTCTGTCCCACCCTGT 0.23% (147 reads)
ATGGTAATGTCCCTCCTGG**A**G---TTCTGTCCCACCCTGT 0.22% (140 reads)
ATGGTAATGTCCCTCCT**A**G-----TTCTGTCCCACCCTGT 0.21% (131 reads)
ATGGTAATGTCCCTCCTGG---**A**GTTCTGTCCCACCCTGT 0.21% (129 reads)





Tmc1$^{Bth/WT}$, SpCas9 + gRNA WT, WT allele only



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A T G G T A A T G T C C C T C C T G G G A T G T T C T G T C C C A C C C T G T   Reference

A T G G T A A T G T C C C T C C T G G G - A T G T T C T G T C C C A C C C T G T   29.30% (17857 reads)
A T G G T A A T G T C C C T C C T - G G G A T G T T C T G T C C C A C C C T G T   28.68% (17475 reads)
A T G G T A A T G T C C C T C C T G G G - - T G T T C T G T C C C A C C C T G T   19.41% (11827 reads)
A T G G T A A T G T C C C T C C T G G G G A T G T T C T G T C C C A C C C T G T   3.36% (2045 reads)
A T G G T A A T G T C C C T C C T G G - - A T G T T C T G T C C C A C C C T G T   2.88% (1757 reads)
A T G G T A A T G T C C C T C C T - - G G A T G T T C T G T C C C A C C C T G T   2.63% (1602 reads)
A T G G T A A T G T C C C T C C T G - - - - - - T T C T G T C C C A C C C T G T   1.36% (830 reads)
A T G G T A A T G T C C C T C C - - - - - - T G T T C T G T C C C A C C C T G T   1.33% (810 reads)
A T G G T A A T G T C C C T C C - - - - G A T G T T C T G T C C C A C C C T G T   1.18% (718 reads)
A T G G T A A T G T C C C T C C T - - - G A T G T T C T G T C C C A C C C T G T   0.75% (460 reads)
A T G G T A A T G T C C C T C - - - - - G A T G T T C T G T C C C A C C C T G T   0.75% (459 reads)
A T G G T A A T G T C C C T C C T G - - - A T G T T C T G T C C C A C C C T G T   0.73% (447 reads)
A T G G T A A T G T C C C T C C T G G G A G A T G T T C T G T C C C A C C C T G   0.41% (252 reads)
A T G G T A A T G T C C C T C C T G G - - - T G T T C T G T C C C A C C C T G T   0.35% (213 reads)
A T G G T A A T G T C C C T - - - - - - G A T G T T C T G T C C C A C C C T G T   0.33% (200 reads)
A T G G T A A T G T C C C T C C T G G G G - T G T T C T G T C C C A C C C T G T   0.25% (154 reads)
A T G G T A A T G T C C C T - - - - - - - - - G T T C T G T C C C A C C C T G T   0.22% (134 reads)
A T G G T A A T G T C C C T C C T G - - - - T G T T C T G T C C C A C C C T G T   0.21% (127 reads)
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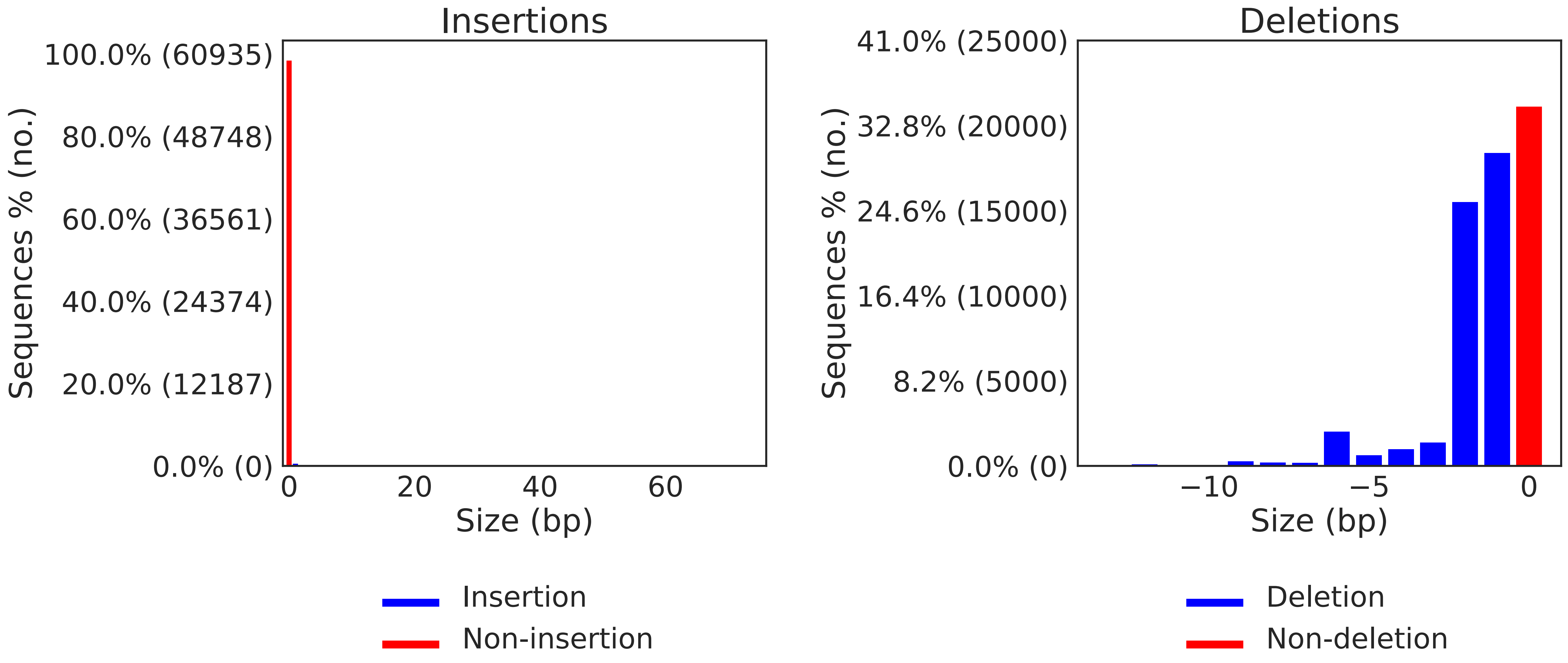
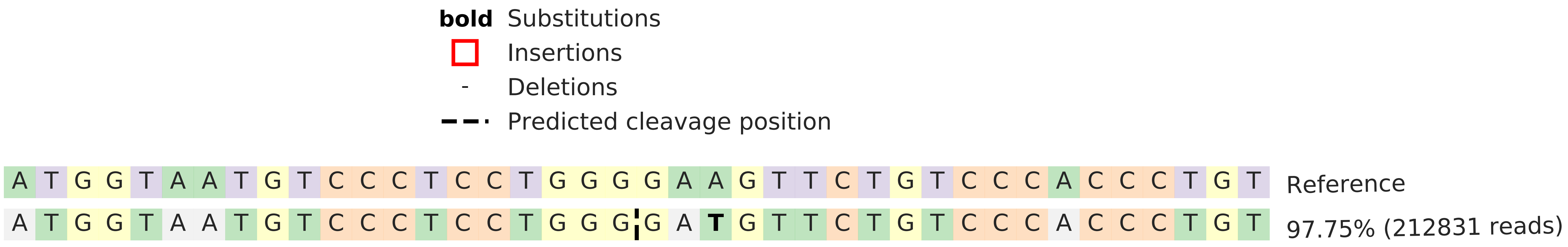
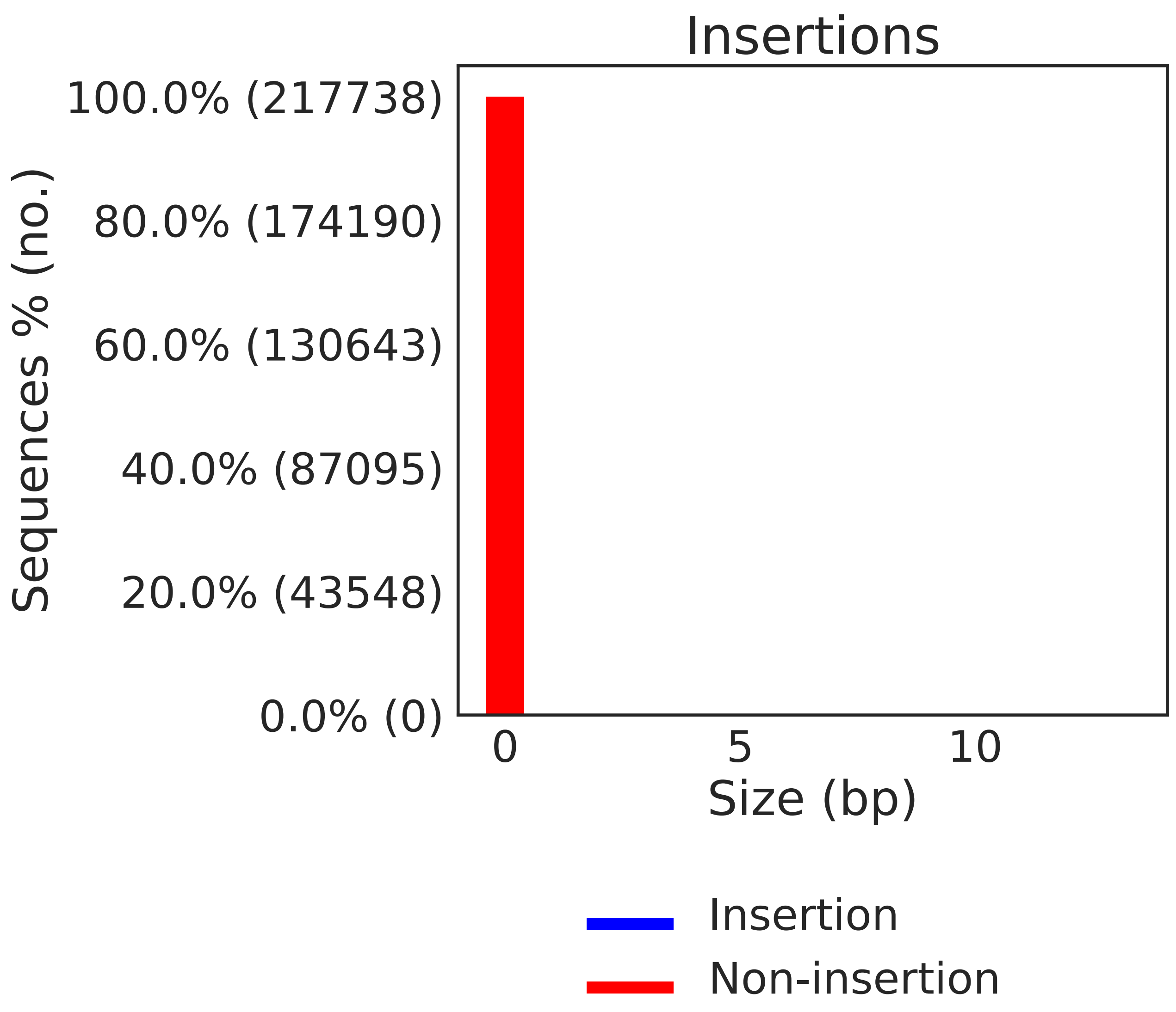


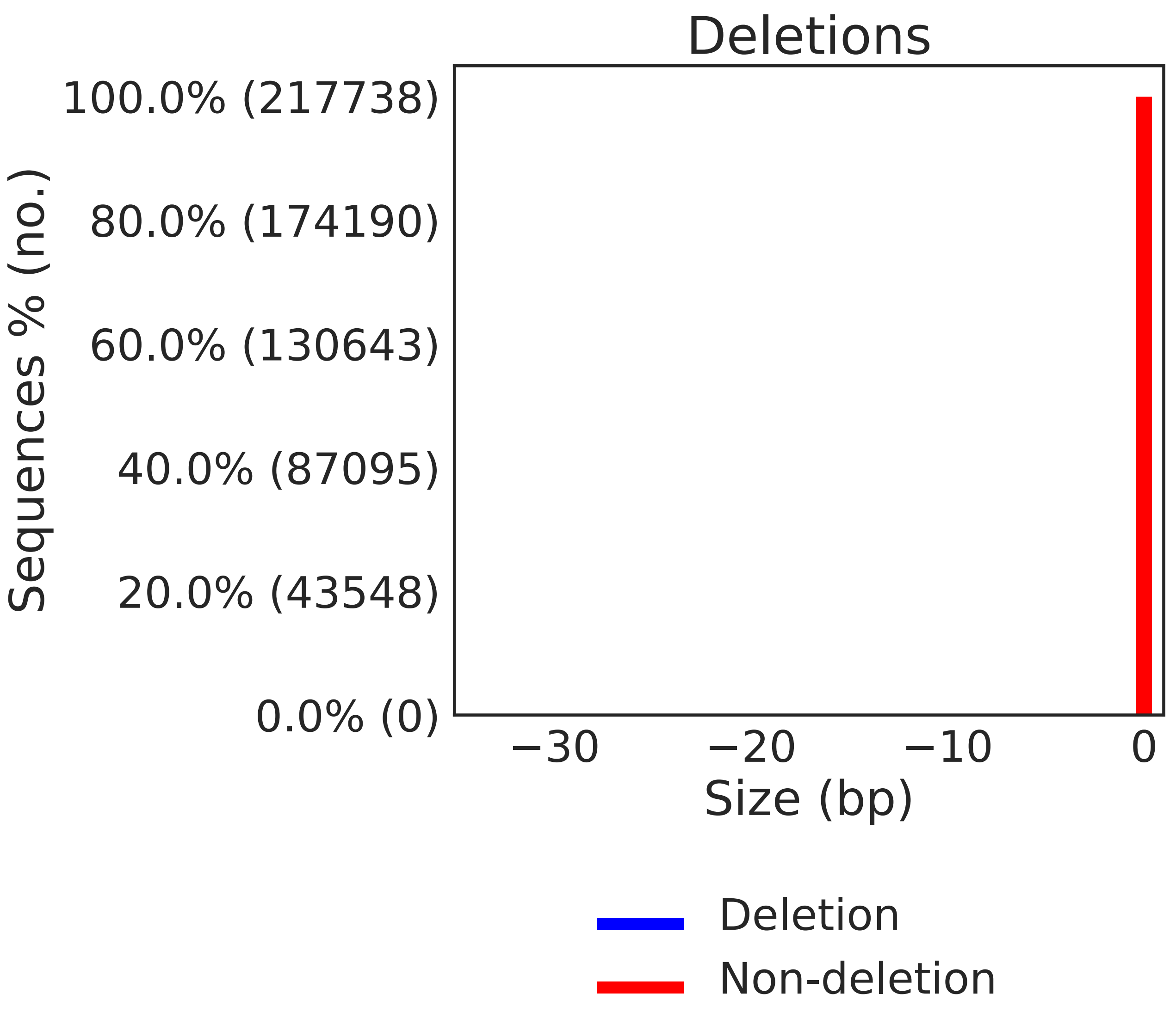
Tmc1$^{WT/WT}$, SpCas9 only



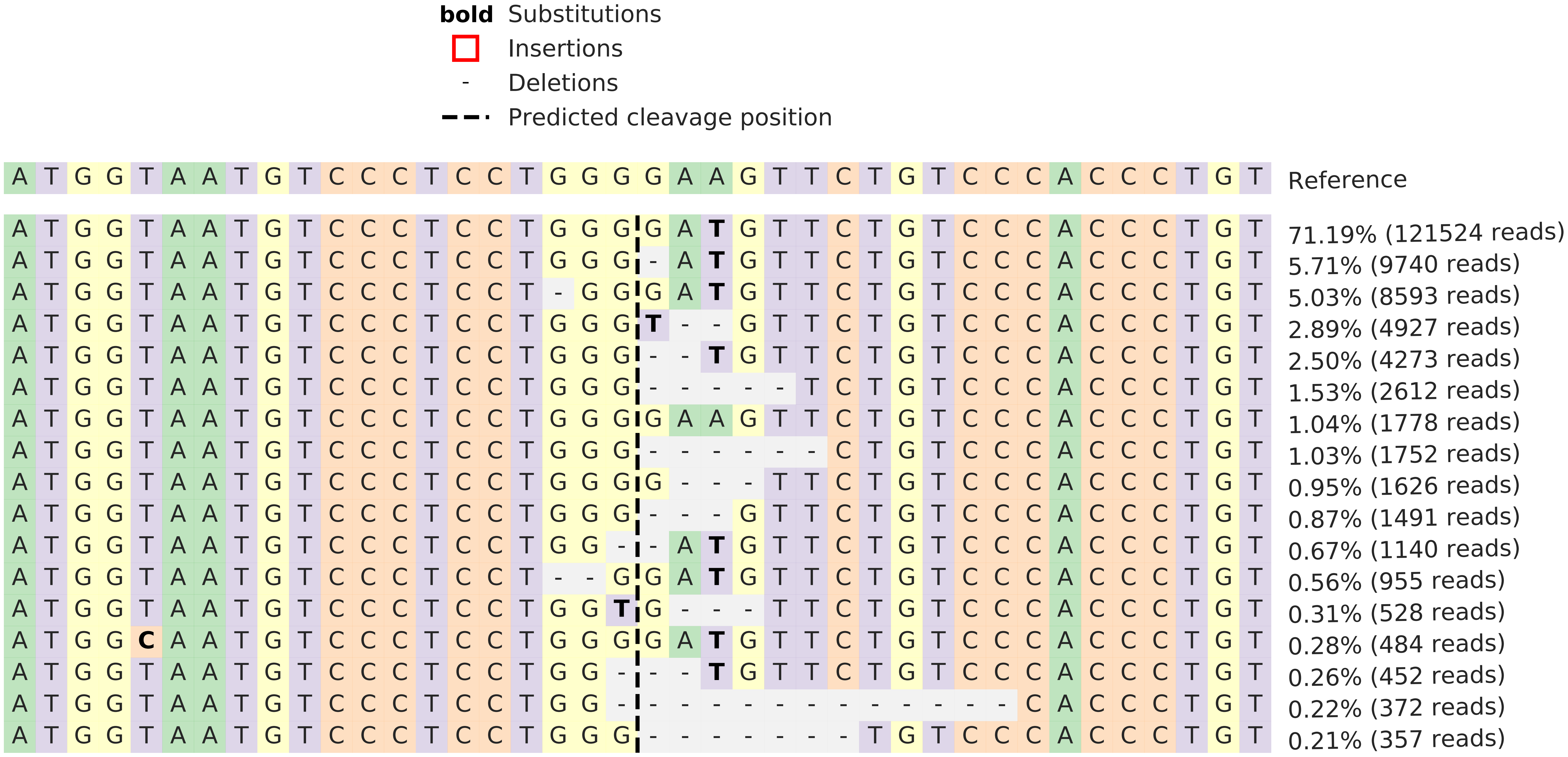
A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T Reference

A T G G T A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C T G T 97.75% (212831 reads)

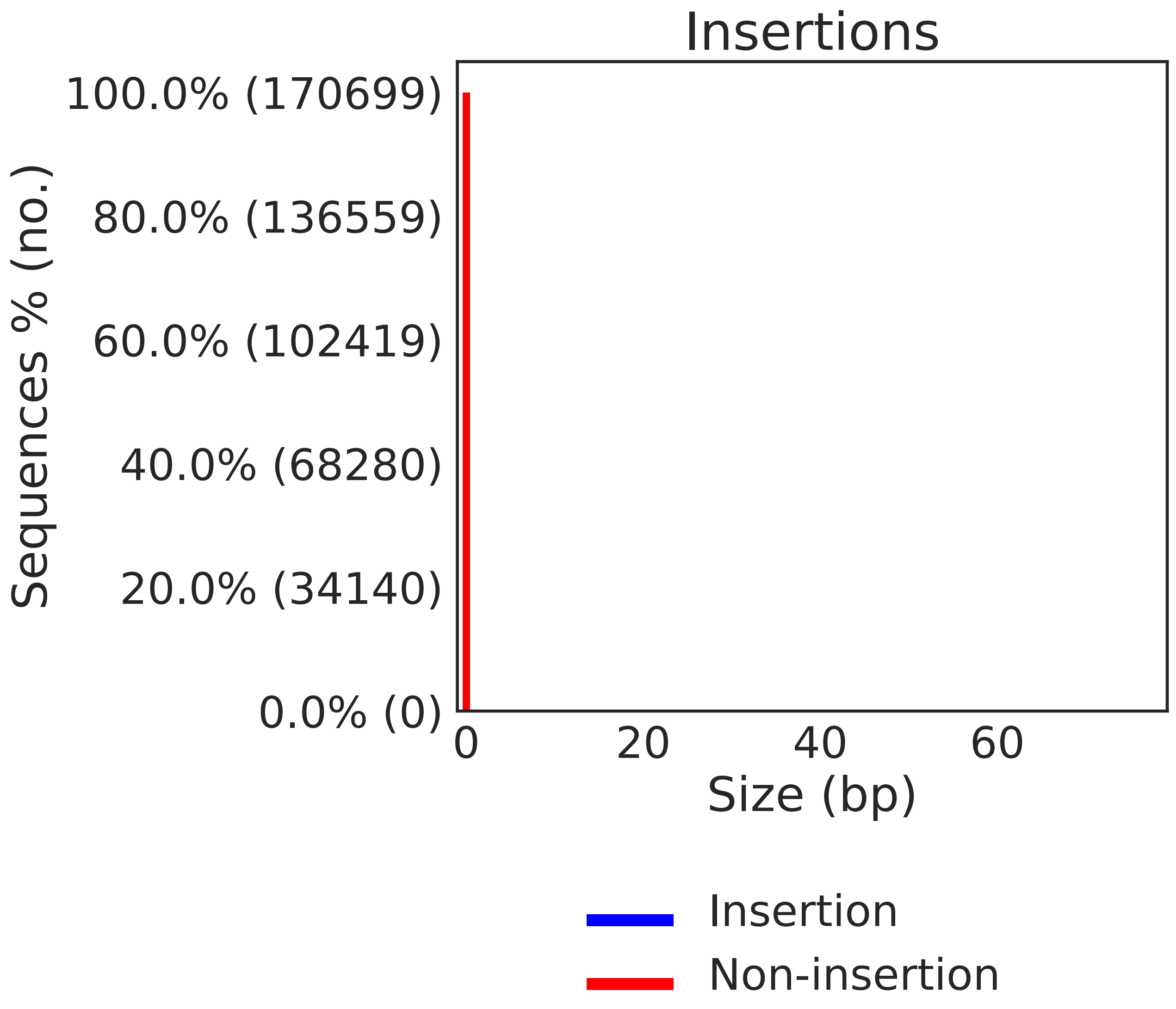


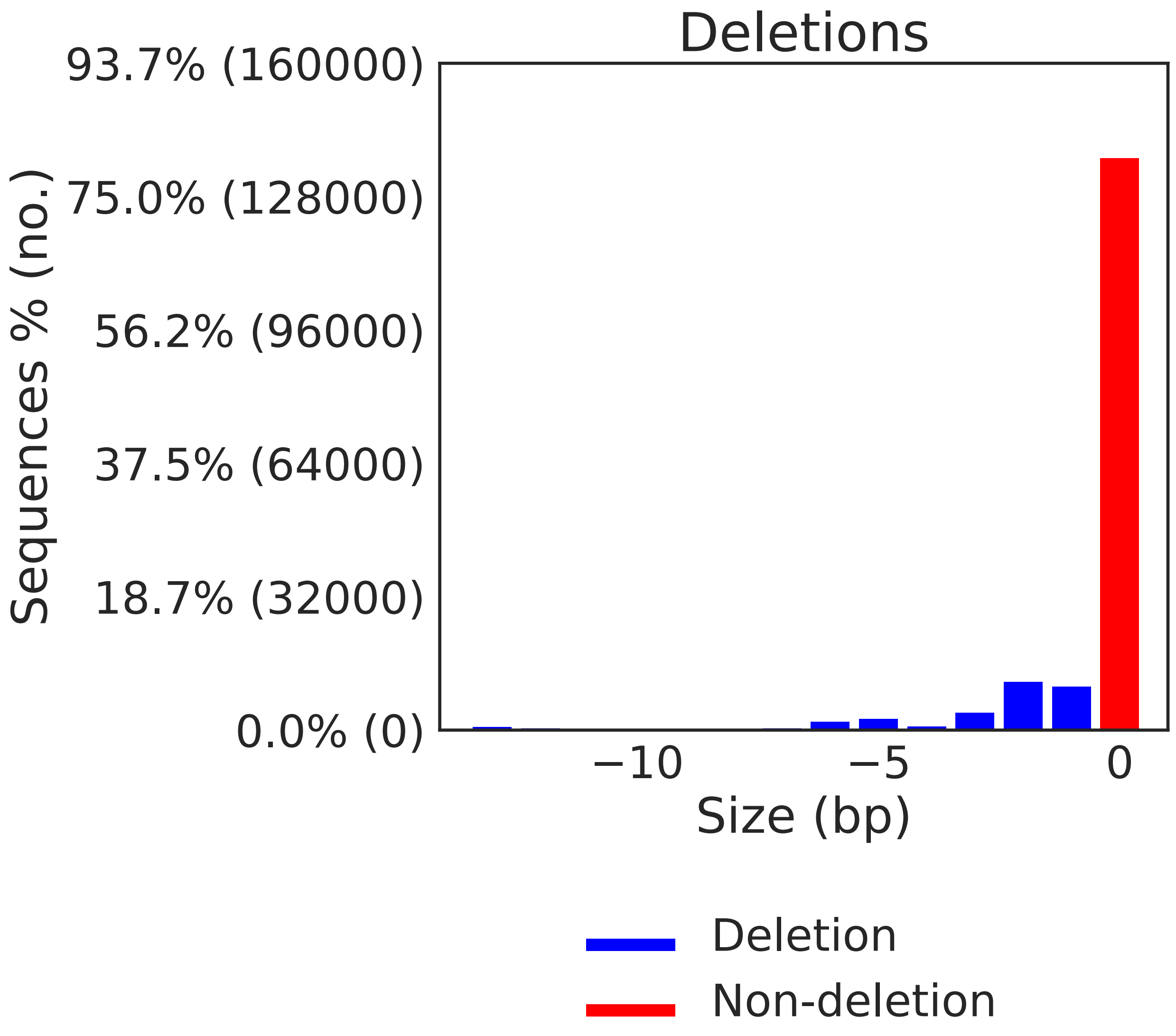


Tmc1$^{WT/WT}$, SpCas9 + gRNA 1.1

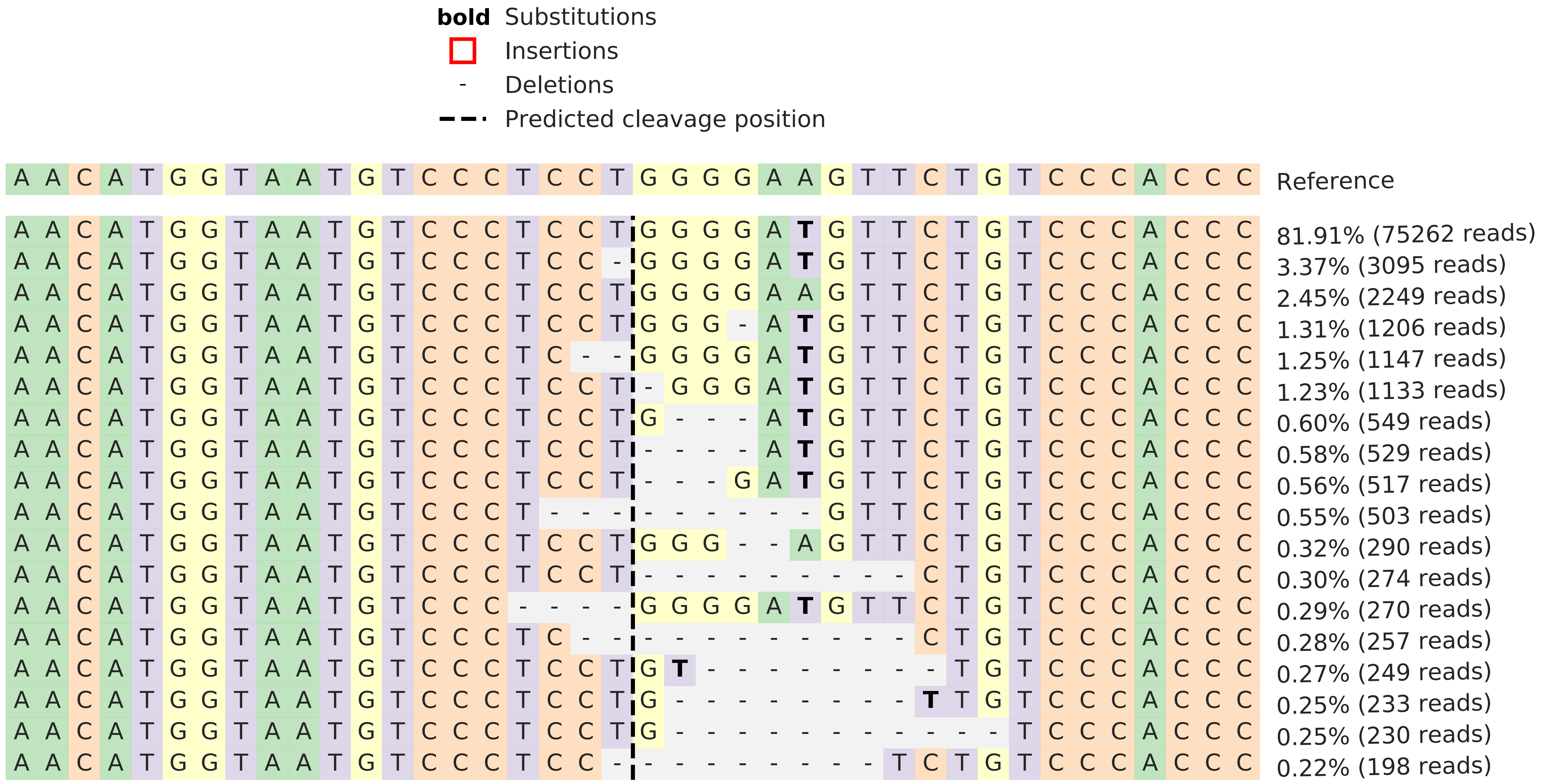


A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T	Reference
A T G G T A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C T G T	71.19% (121524 reads)
A T G G T A A T G T C C C T C C T G G G - A **T** G T T C T G T C C C A C C C T G T	5.71% (9740 reads)
A T G G T A A T G T C C C T C C T - G G G A **T** G T T C T G T C C C A C C C T G T	5.03% (8593 reads)
A T G G T A A T G T C C C T C C T G G G **T** - - G T T C T G T C C C A C C C T G T	2.89% (4927 reads)
A T G G T A A T G T C C C T C C T G G G - - **T** G T T C T G T C C C A C C C T G T	2.50% (4273 reads)
A T G G T A A T G T C C C T C C T G G G - - - - - T C T G T C C C A C C C T G T	1.53% (2612 reads)
A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T	1.04% (1778 reads)
A T G G T A A T G T C C C T C C T G G G - - - - - - C T G T C C C A C C C T G T	1.03% (1752 reads)
A T G G T A A T G T C C C T C C T G G G G - - - T T C T G T C C C A C C C T G T	0.95% (1626 reads)
A T G G T A A T G T C C C T C C T G G G - - - G T T C T G T C C C A C C C T G T	0.87% (1491 reads)
A T G G T A A T G T C C C T C C T G G - - A **T** G T T C T G T C C C A C C C T G T	0.67% (1140 reads)
A T G G T A A T G T C C C T C C T - - G G A **T** G T T C T G T C C C A C C C T G T	0.56% (955 reads)
A T G G T A A T G T C C C T C C T G G **T** G - - - T T C T G T C C C A C C C T G T	0.31% (528 reads)
A T G G **C** A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C T G T	0.28% (484 reads)
A T G G T A A T G T C C C T C C T G G - - - **T** G T T C T G T C C C A C C C T G T	0.26% (452 reads)
A T G G T A A T G T C C C T C C T G G - - - - - - - - - - - - - C A C C C T G T	0.22% (372 reads)
A T G G T A A T G T C C C T C C T G G G - - - - - - - T G T C C C A C C C T G T	0.21% (357 reads)



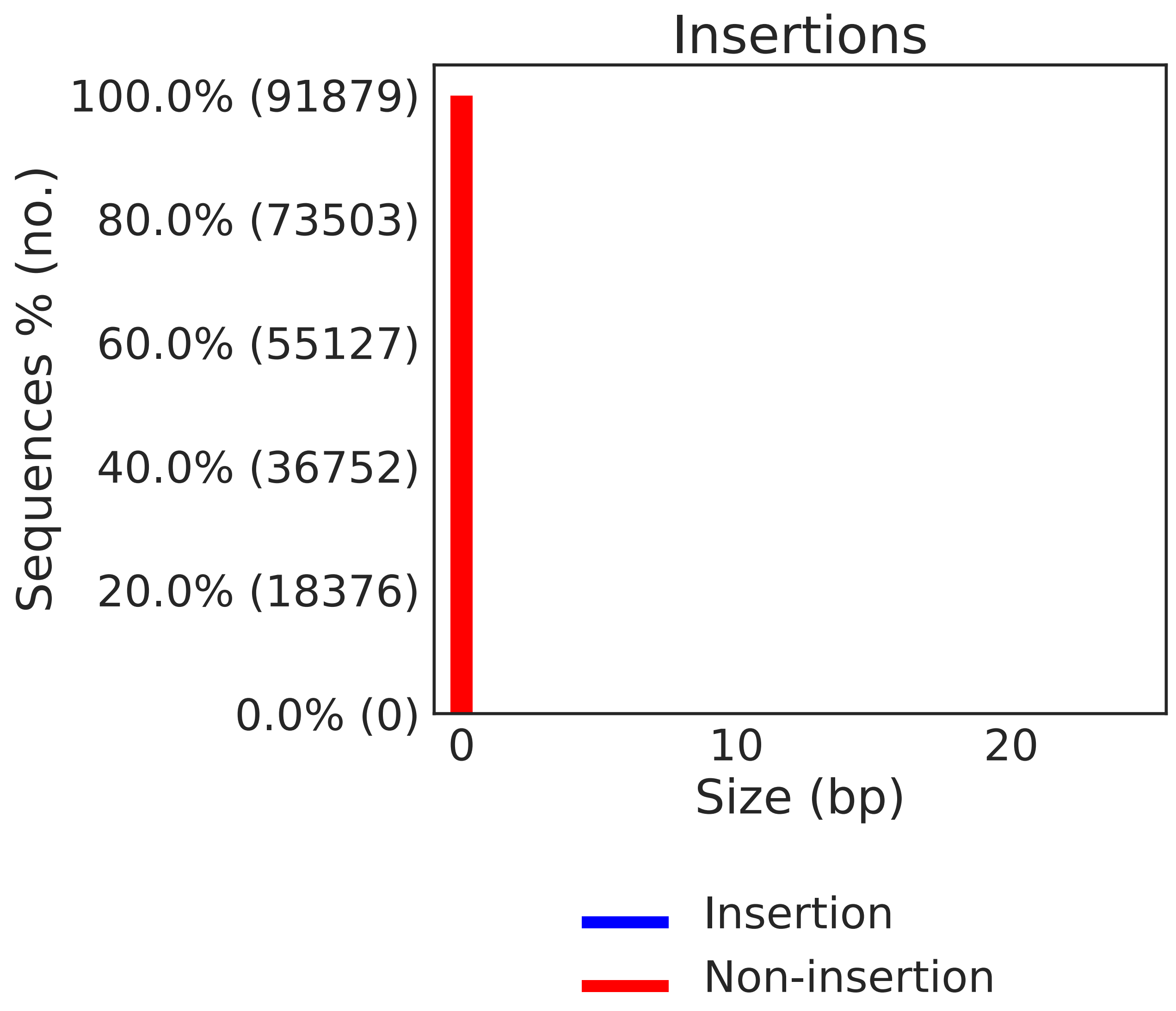


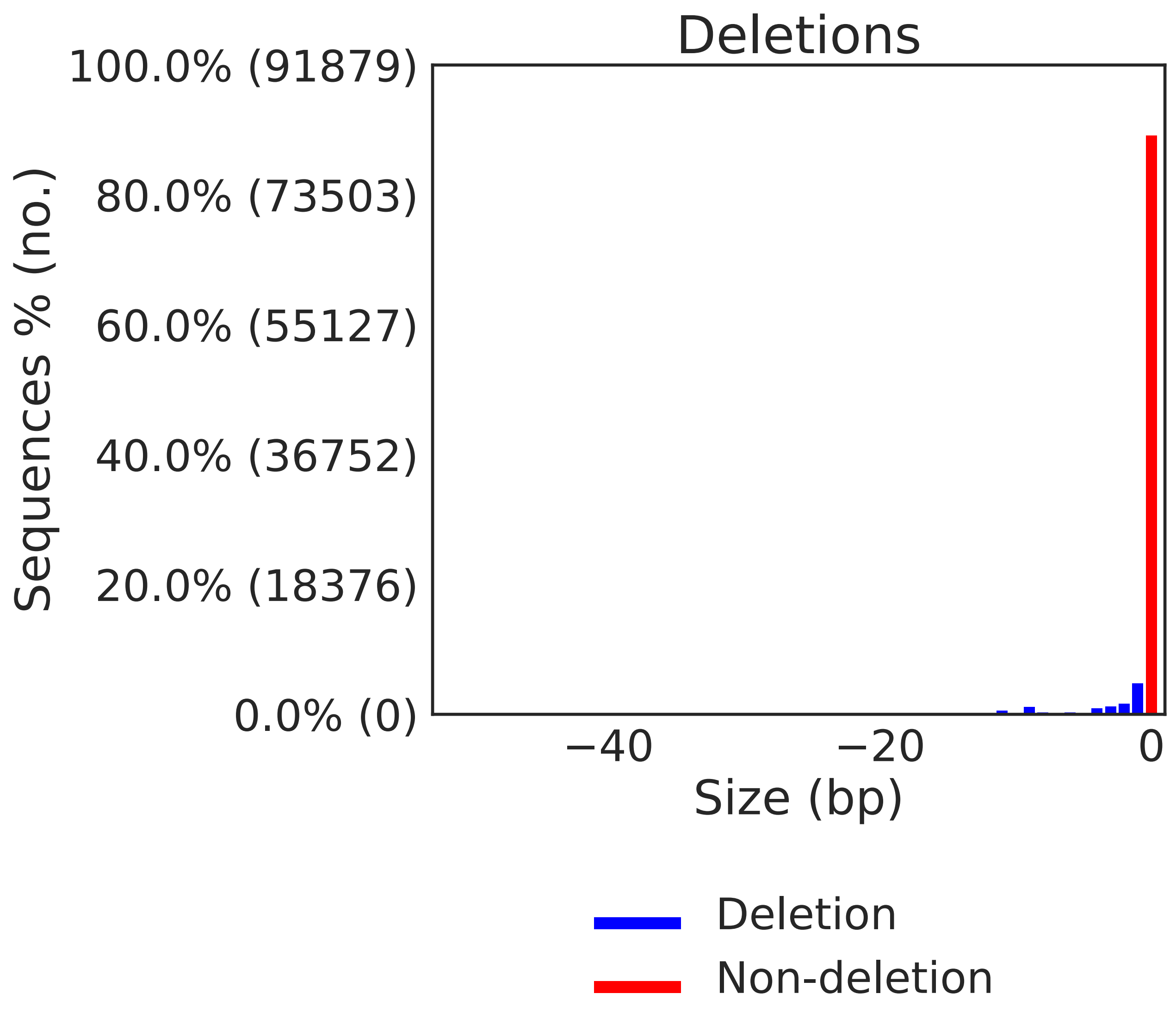
Tmc1$^{WT/WT}$, SpCas9 + gRNA 2.1



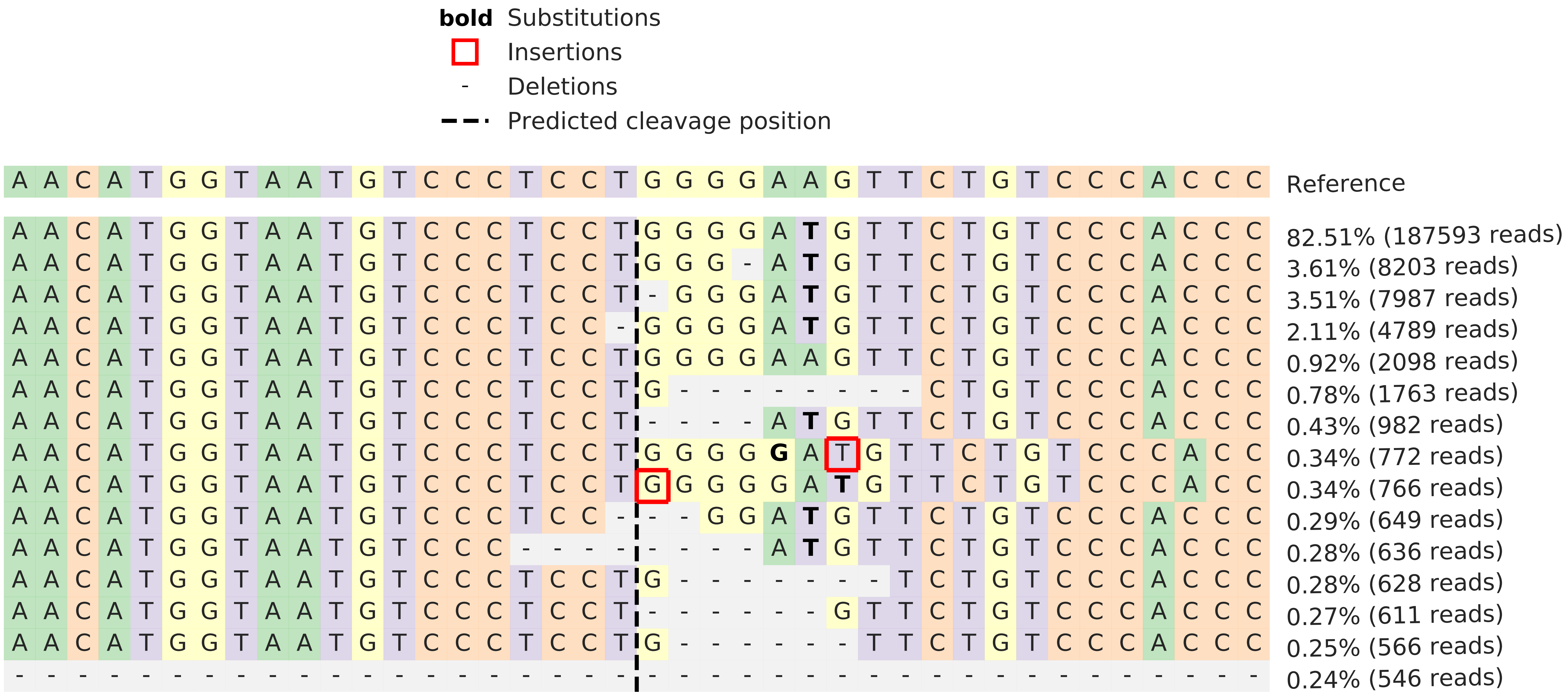
bold Substitutions
☐ Insertions
\- Deletions
\- - Predicted cleavage position

AACATGGTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCC	Reference
AACATGGTAATGTCCCTCCTGGGGA**T**GTTCTGTCCCACCC	81.91% (75262 reads)
AACATGGTAATGTCCCTCC-GGGGA**T**GTTCTGTCCCACCC	3.37% (3095 reads)
AACATGGTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCC	2.45% (2249 reads)
AACATGGTAATGTCCCTCCTGGG-A**T**GTTCTGTCCCACCC	1.31% (1206 reads)
AACATGGTAATGTCCCTC--GGGGA**T**GTTCTGTCCCACCC	1.25% (1147 reads)
AACATGGTAATGTCCCTCCT-GGGA**T**GTTCTGTCCCACCC	1.23% (1133 reads)
AACATGGTAATGTCCCTCCTG---A**T**GTTCTGTCCCACCC	0.60% (549 reads)
AACATGGTAATGTCCCTCCT----A**T**GTTCTGTCCCACCC	0.58% (529 reads)
AACATGGTAATGTCCCTCCT---GA**T**GTTCTGTCCCACCC	0.56% (517 reads)
AACATGGTAATGTCCCT---------GTTCTGTCCCACCC	0.55% (503 reads)
AACATGGTAATGTCCCTCCTGGG--AGTTCTGTCCCACCC	0.32% (290 reads)
AACATGGTAATGTCCCTCCT---------CTGTCCCACCC	0.30% (274 reads)
AACATGGTAATGTCCC----GGGGA**T**GTTCTGTCCCACCC	0.29% (270 reads)
AACATGGTAATGTCCCTC-----------CTGTCCCACCC	0.28% (257 reads)
AACATGGTAATGTCCCTCCTG**T**--------TGTCCCACCC	0.27% (249 reads)
AACATGGTAATGTCCCTCCTG--------**T**TGTCCCACCC	0.25% (233 reads)
AACATGGTAATGTCCCTCCTG-----------TCCCACCC	0.25% (230 reads)
AACATGGTAATGTCCCTCC---------TCTGTCCCACCC	0.22% (198 reads)



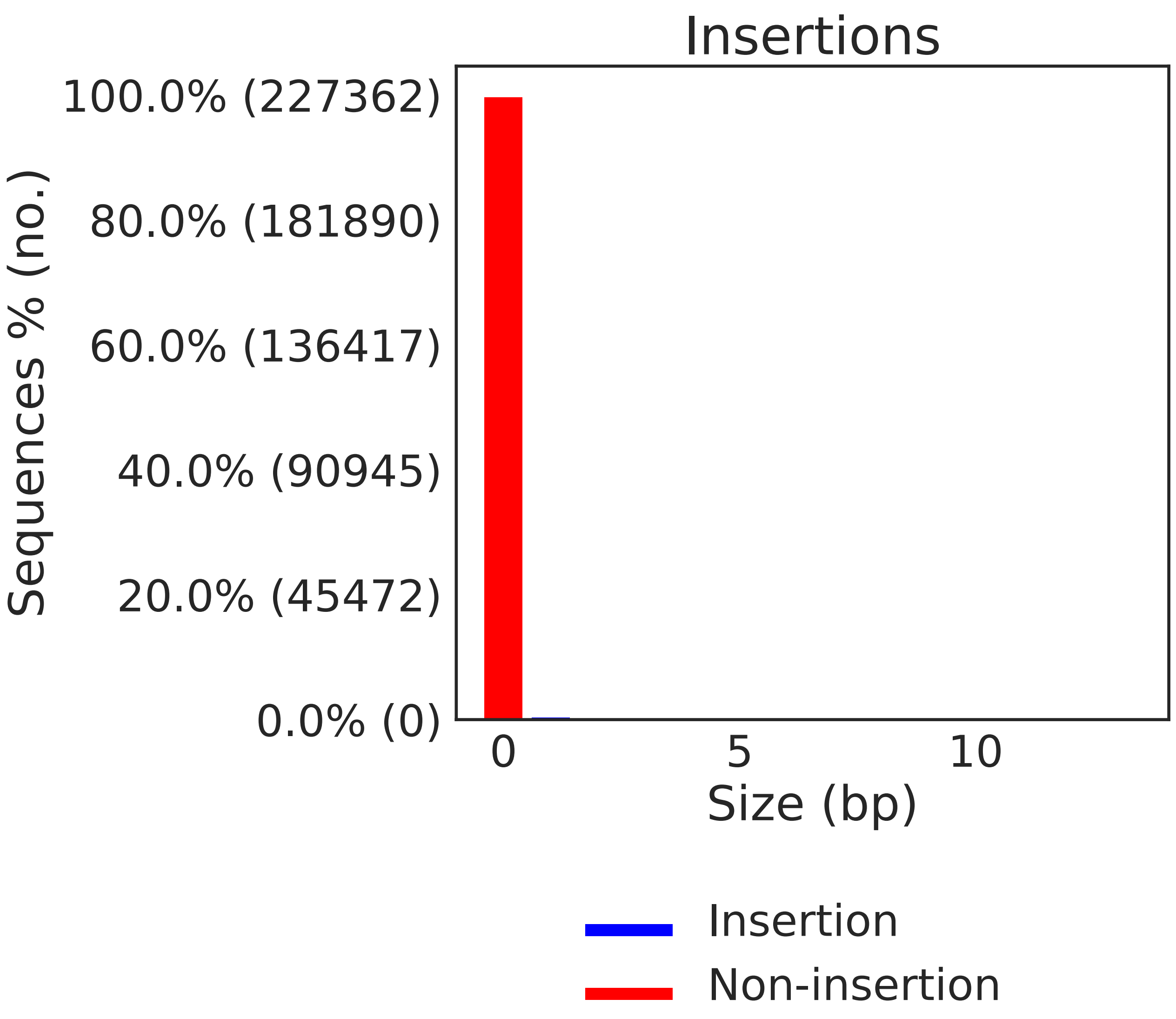


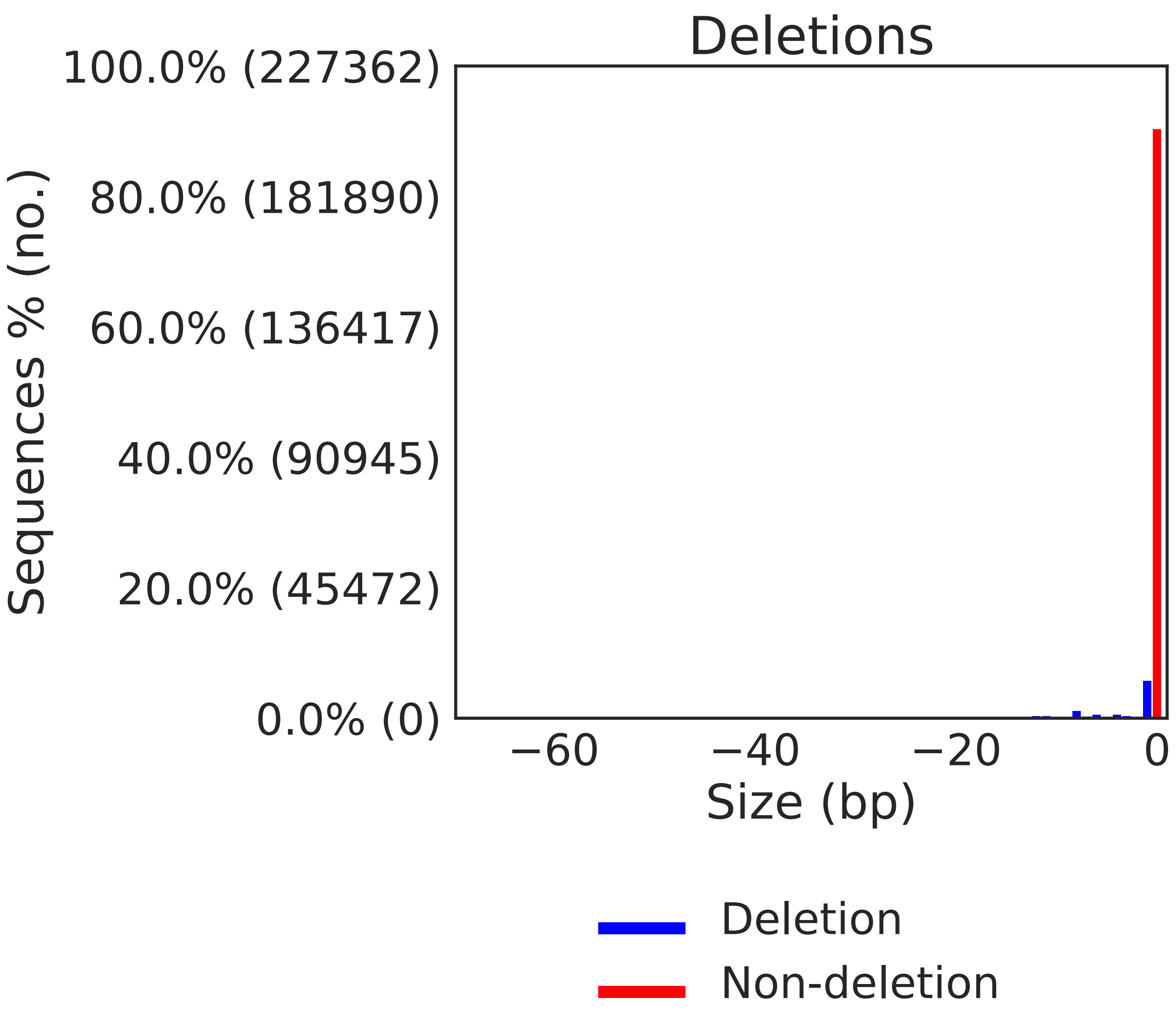
Tmc1$^{WT/WT}$, SpCas9 + gRNA 2.4



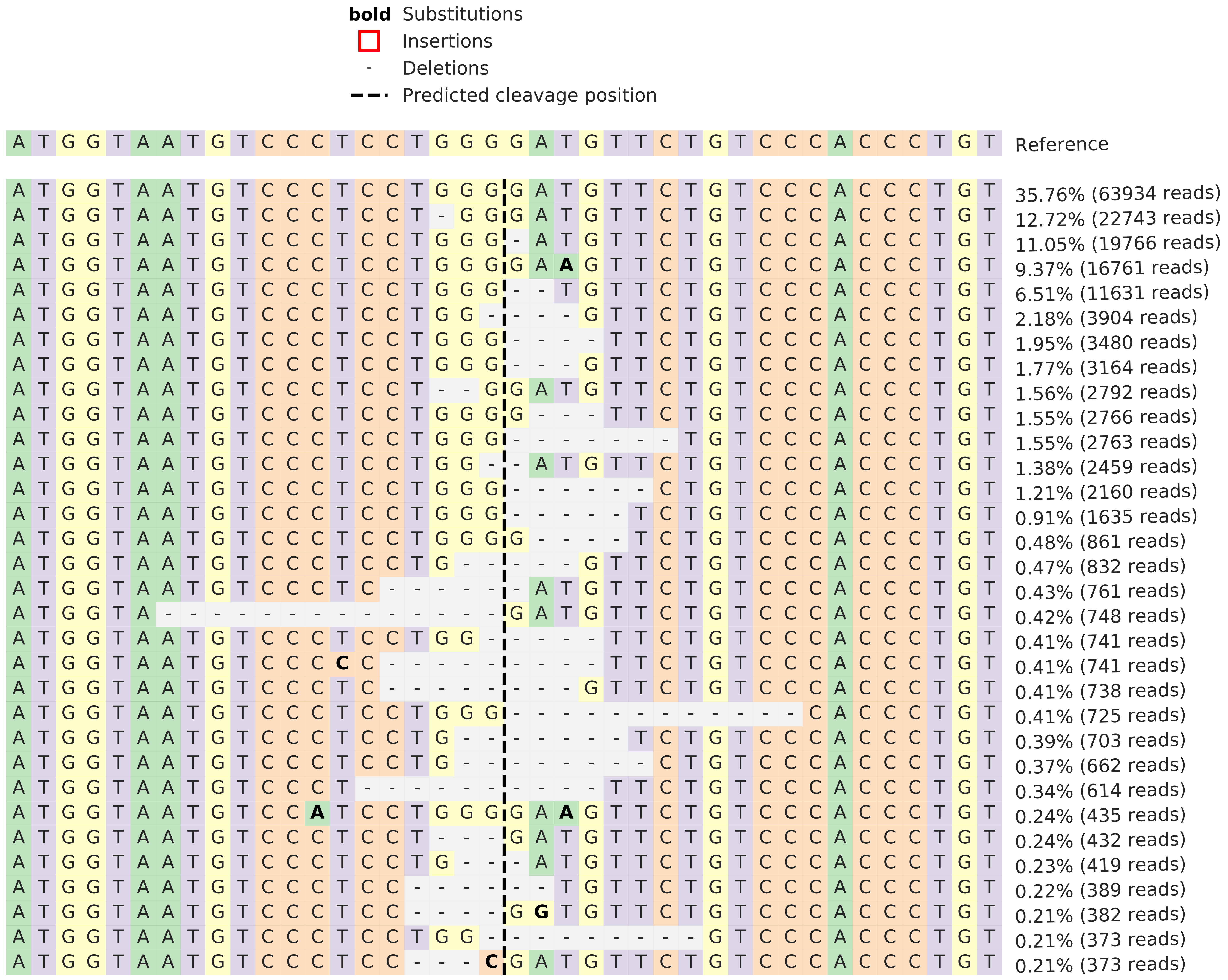
A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C Reference

A A C A T G G T A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C 82.51% (187593 reads)
A A C A T G G T A A T G T C C C T C C T G G G - A **T** G T T C T G T C C C A C C C 3.61% (8203 reads)
A A C A T G G T A A T G T C C C T C C T - G G G A **T** G T T C T G T C C C A C C C 3.51% (7987 reads)
A A C A T G G T A A T G T C C C T C C - G G G G A **T** G T T C T G T C C C A C C C 2.11% (4789 reads)
A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C 0.92% (2098 reads)
A A C A T G G T A A T G T C C C T C C T G - - - - - - - - C T G T C C C A C C C 0.78% (1763 reads)
A A C A T G G T A A T G T C C C T C C T - - - - A **T** G T T C T G T C C C A C C C 0.43% (982 reads)
A A C A T G G T A A T G T C C C T C C T G G G G **G** A T G T T C T G T C C C A C C C 0.34% (772 reads)
A A C A T G G T A A T G T C C C T C C T G G G G G A **T** G T T C T G T C C C A C C C 0.34% (766 reads)
A A C A T G G T A A T G T C C C T C C - - - G G A **T** G T T C T G T C C C A C C C 0.29% (649 reads)
A A C A T G G T A A T G T C C C - - - - - - - - A **T** G T T C T G T C C C A C C C 0.28% (636 reads)
A A C A T G G T A A T G T C C C T C C T G - - - - - - - T C T G T C C C A C C C 0.28% (628 reads)
A A C A T G G T A A T G T C C C T C C T - - - - - - G T T C T G T C C C A C C C 0.27% (611 reads)
A A C A T G G T A A T G T C C C T C C T G - - - - - - T T C T G T C C C A C C C 0.25% (566 reads)
- 0.24% (546 reads)

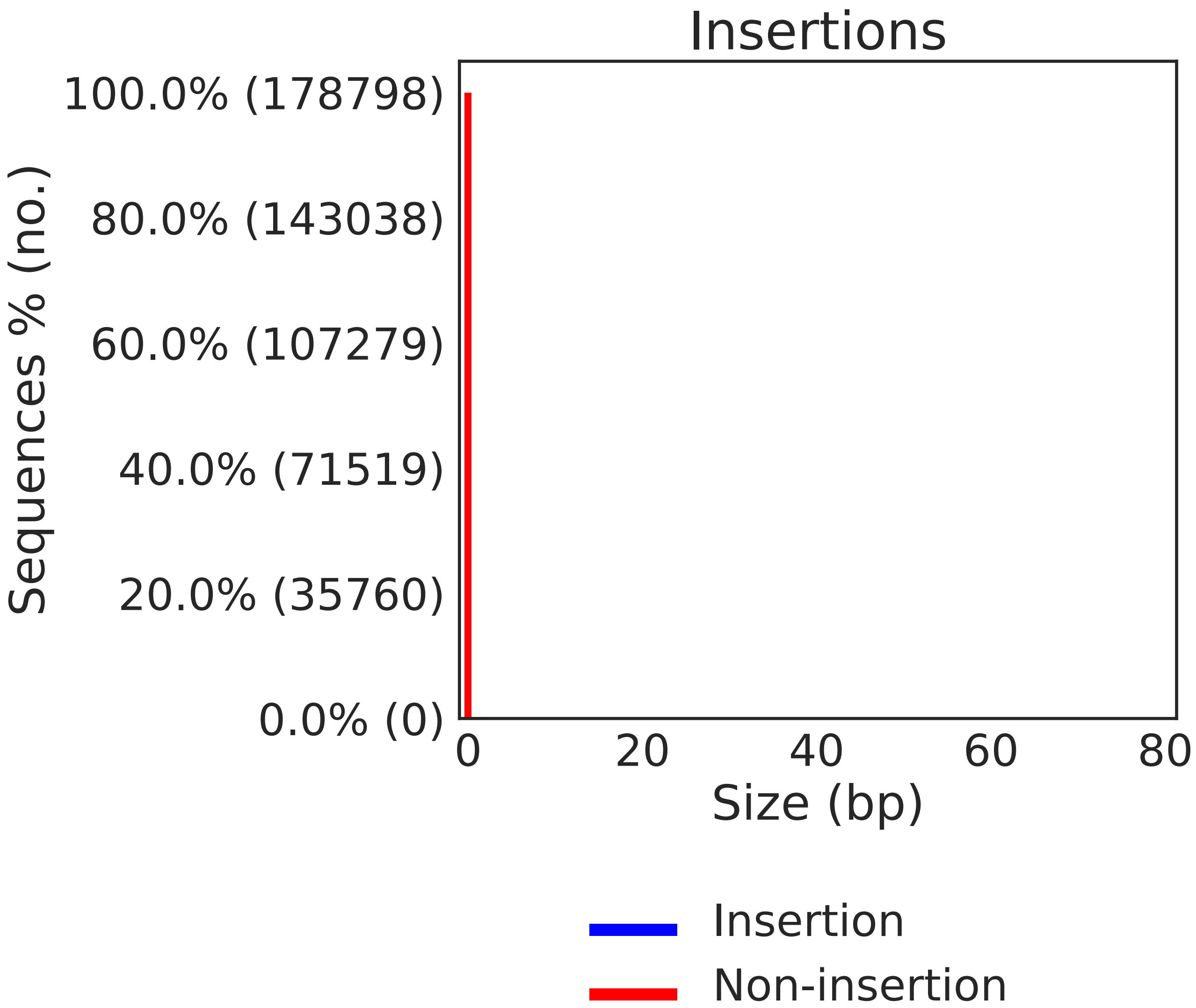


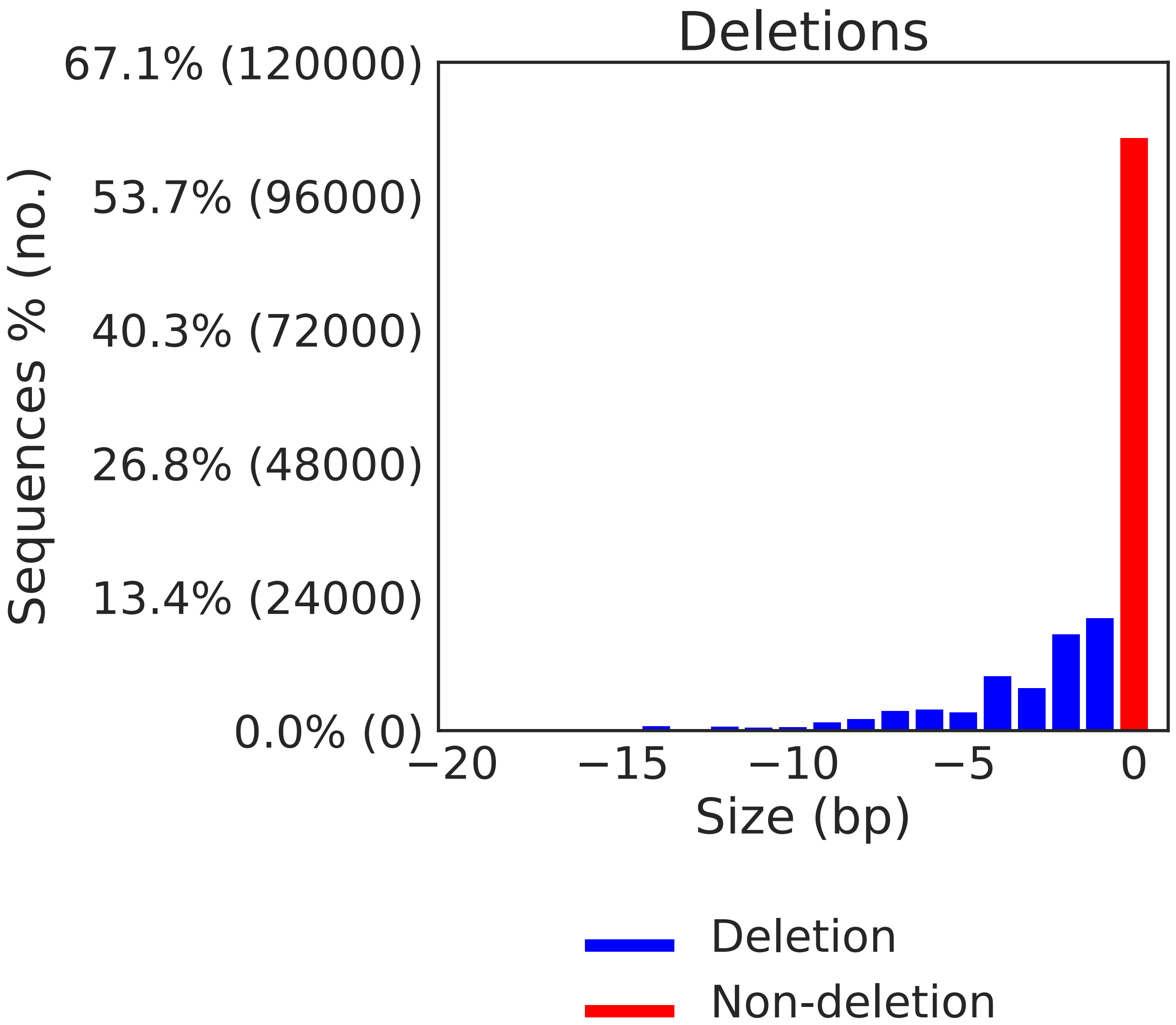


Tmc1$^{WT/WT}$, SpCas9 + gRNA WT

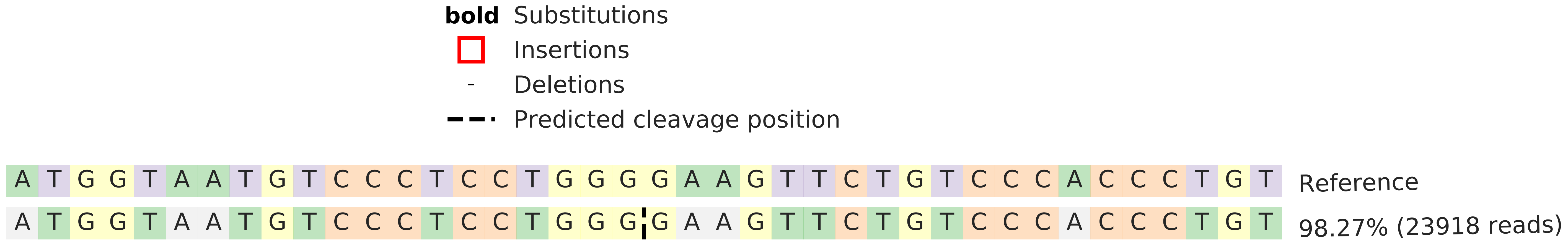


ATGGTAATGTCCCTCCTGGGGATGTTCTGTCCCACCCTGT	Reference
ATGGTAATGTCCCTCCTGGGGATGTTCTGTCCCACCCTGT	35.76% (63934 reads)
ATGGTAATGTCCCTCCT-GGGATGTTCTGTCCCACCCTGT	12.72% (22743 reads)
ATGGTAATGTCCCTCCTGGG-ATGTTCTGTCCCACCCTGT	11.05% (19766 reads)
ATGGTAATGTCCCTCCTGGGGA**A**GTTCTGTCCCACCCTGT	9.37% (16761 reads)
ATGGTAATGTCCCTCCTGGG--TGTTCTGTCCCACCCTGT	6.51% (11631 reads)
ATGGTAATGTCCCTCCTGG----GTTCTGTCCCACCCTGT	2.18% (3904 reads)
ATGGTAATGTCCCTCCTGGG----TTCTGTCCCACCCTGT	1.95% (3480 reads)
ATGGTAATGTCCCTCCTGGG---GTTCTGTCCCACCCTGT	1.77% (3164 reads)
ATGGTAATGTCCCTCCT--GGATGTTCTGTCCCACCCTGT	1.56% (2792 reads)
ATGGTAATGTCCCTCCTGGGG---TTCTGTCCCACCCTGT	1.55% (2766 reads)
ATGGTAATGTCCCTCCTGGG-------TGTCCCACCCTGT	1.55% (2763 reads)
ATGGTAATGTCCCTCCTGG--ATGTTCTGTCCCACCCTGT	1.38% (2459 reads)
ATGGTAATGTCCCTCCTGGG------CTGTCCCACCCTGT	1.21% (2160 reads)
ATGGTAATGTCCCTCCTGGG-----TCTGTCCCACCCTGT	0.91% (1635 reads)
ATGGTAATGTCCCTCCTGGGG----TCTGTCCCACCCTGT	0.48% (861 reads)
ATGGTAATGTCCCTCCTG-----GTTCTGTCCCACCCTGT	0.47% (832 reads)
ATGGTAATGTCCCTC------ATGTTCTGTCCCACCCTGT	0.43% (761 reads)
ATGGTA--------------GATGTTCTGTCCCACCCTGT	0.42% (748 reads)
ATGGTAATGTCCCTCCTGG-----TTCTGTCCCACCCTGT	0.41% (741 reads)
ATGGTAATGTCCC**C**C---------TTCTGTCCCACCCTGT	0.41% (741 reads)
ATGGTAATGTCCCTC--------GTTCTGTCCCACCCTGT	0.41% (738 reads)
ATGGTAATGTCCCTCCTGGG------------CACCCTGT	0.41% (725 reads)
ATGGTAATGTCCCTCCTG-------TCTGTCCCACCCTGT	0.39% (703 reads)
ATGGTAATGTCCCTCCTG--------CTGTCCCACCCTGT	0.37% (662 reads)
ATGGTAATGTCCCT----------TTCTGTCCCACCCTGT	0.34% (614 reads)
ATGGTAATGTCC**A**TCCTGGGGA**A**GTTCTGTCCCACCCTGT	0.24% (435 reads)
ATGGTAATGTCCCTCCT---GATGTTCTGTCCCACCCTGT	0.24% (432 reads)
ATGGTAATGTCCCTCCTG---ATGTTCTGTCCCACCCTGT	0.23% (419 reads)
ATGGTAATGTCCCTCC------TGTTCTGTCCCACCCTGT	0.22% (389 reads)
ATGGTAATGTCCCTCC----G**G**TGTTCTGTCCCACCCTGT	0.21% (382 reads)
ATGGTAATGTCCCTCCTGG---------GTCCCACCCTGT	0.21% (373 reads)
ATGGTAATGTCCCTCC---**C**GATGTTCTGTCCCACCCTGT	0.21% (373 reads)



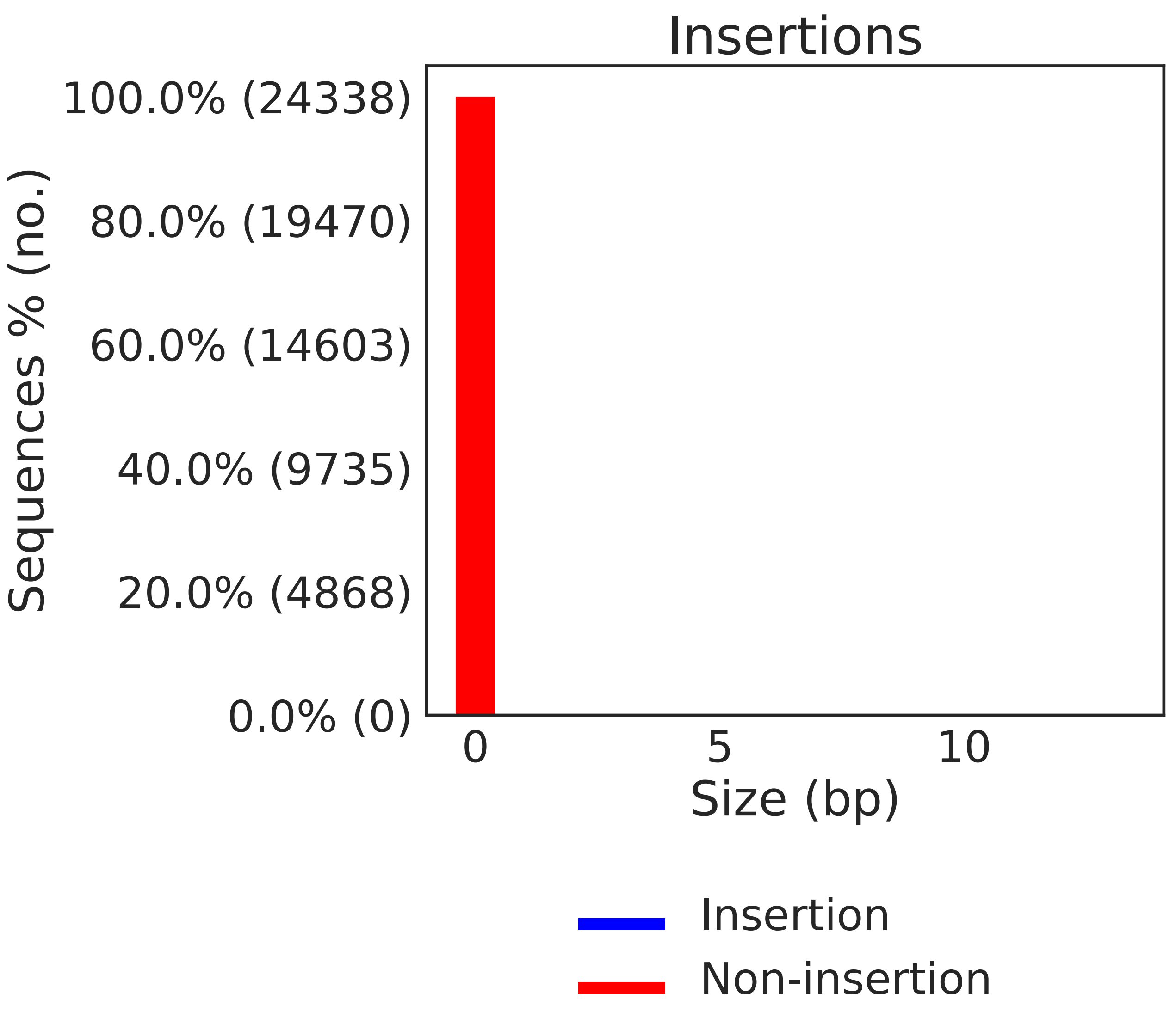


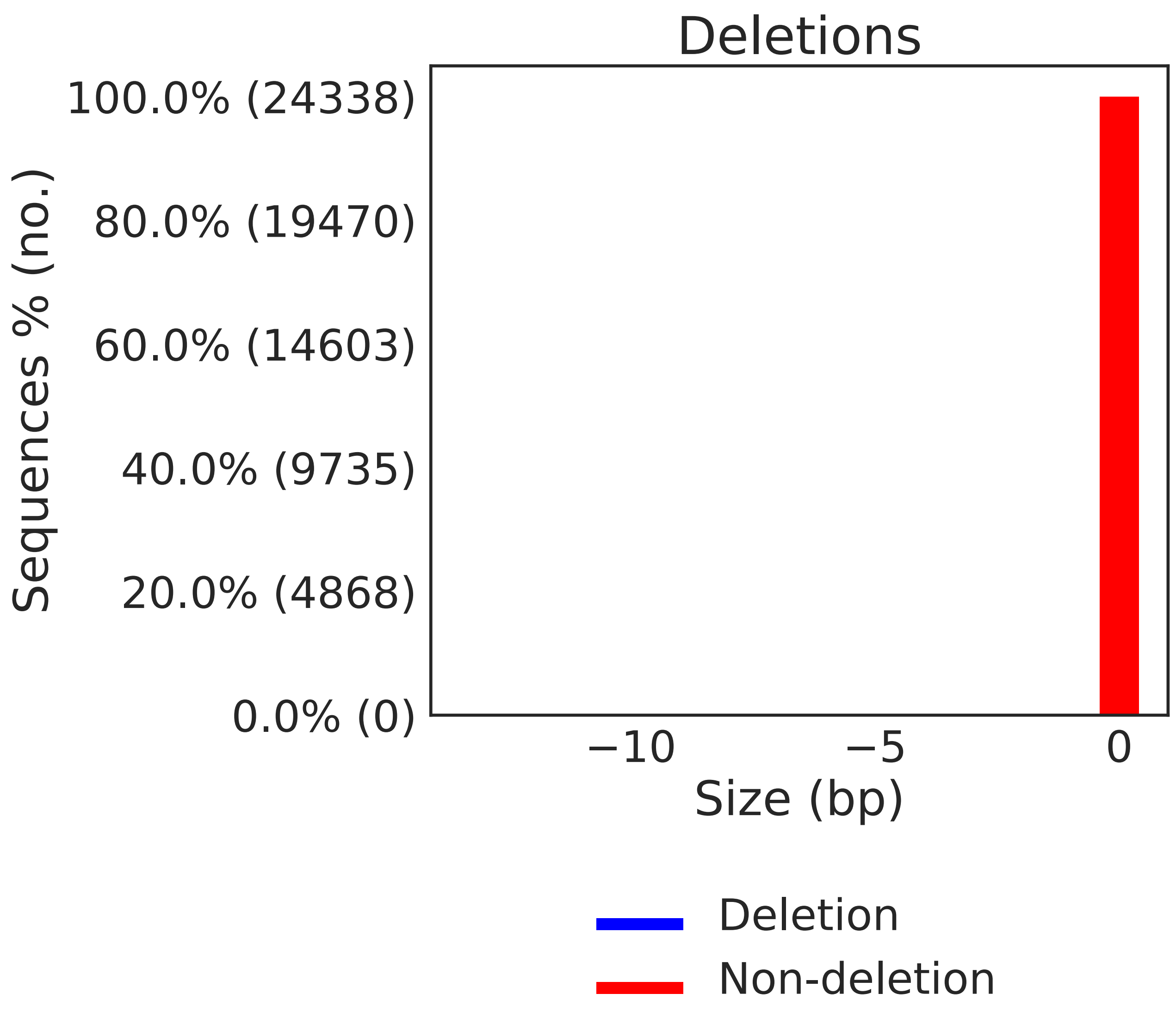
Tmc$^{Bth/WT}$, SpCas9 only, Bth allele



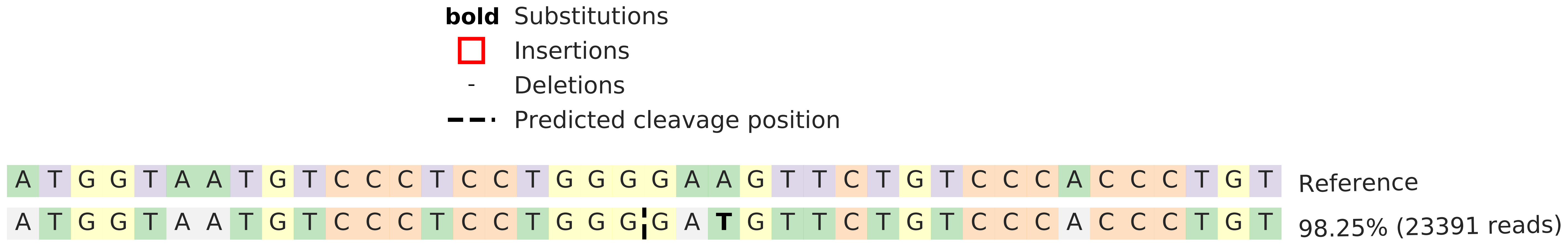
A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T Reference

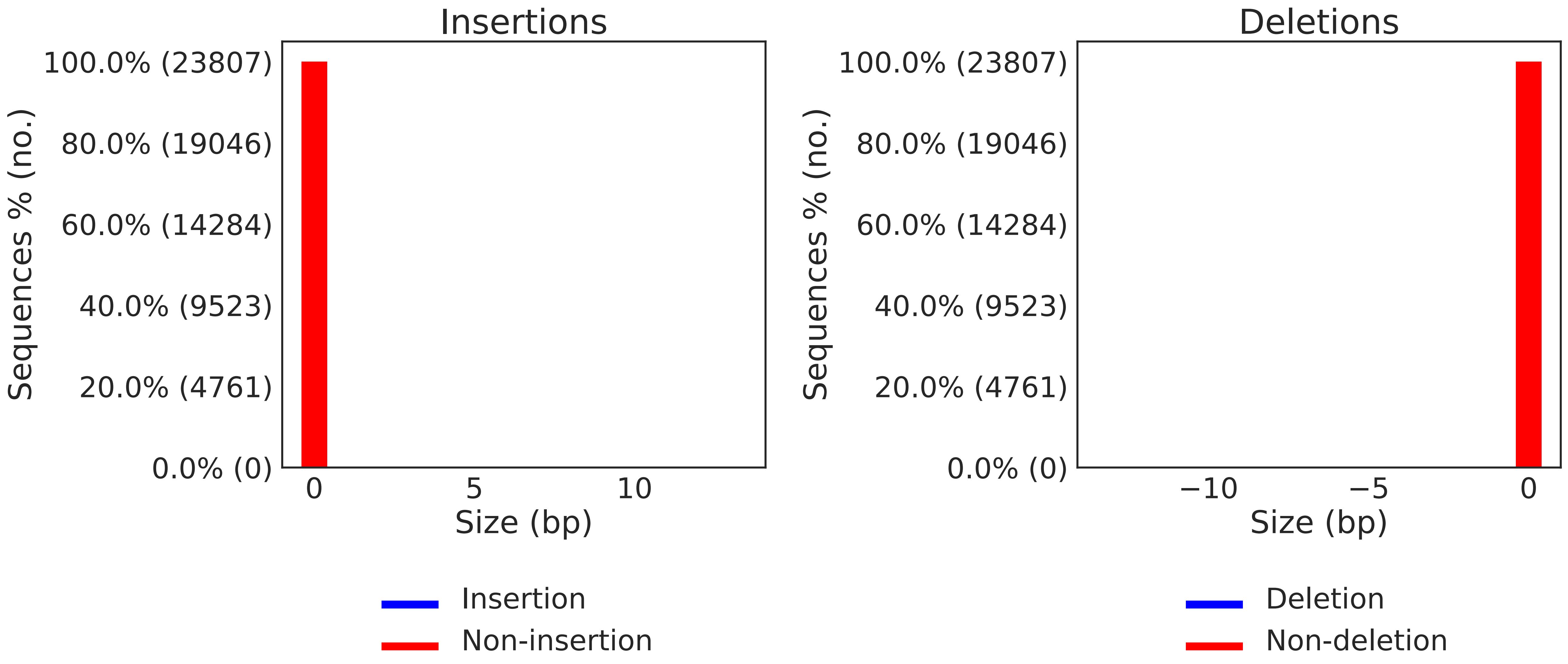
A T G G T A A T G T C C C T C C T G G G | G A A G T T C T G T C C C A C C C T G T 98.27% (23918 reads)



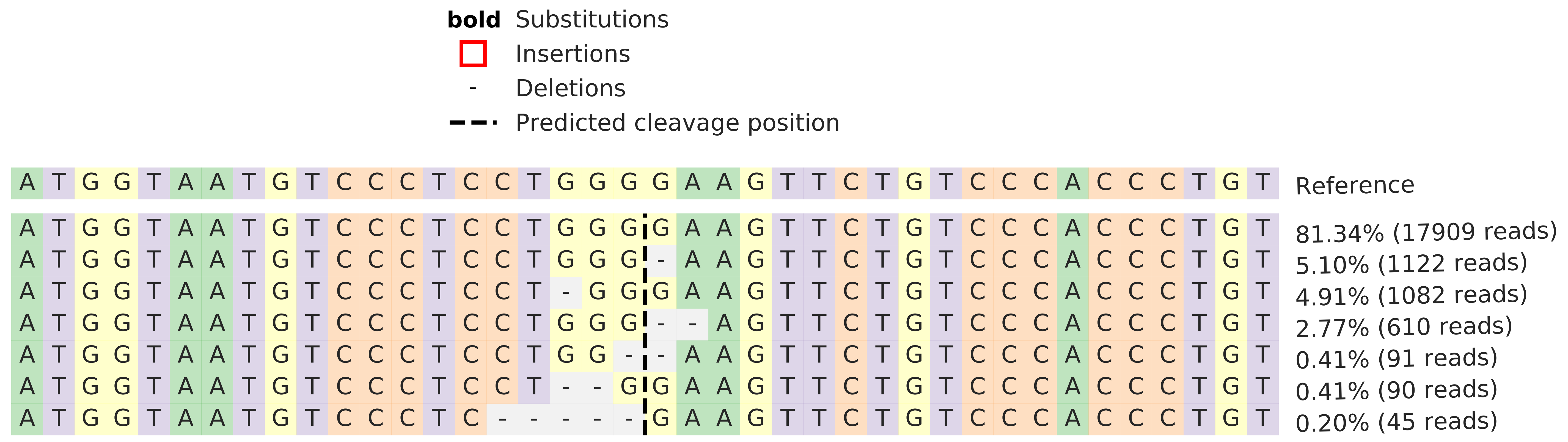


$Tmc^{Bth/WT}$, SpCas9 only, WT allele



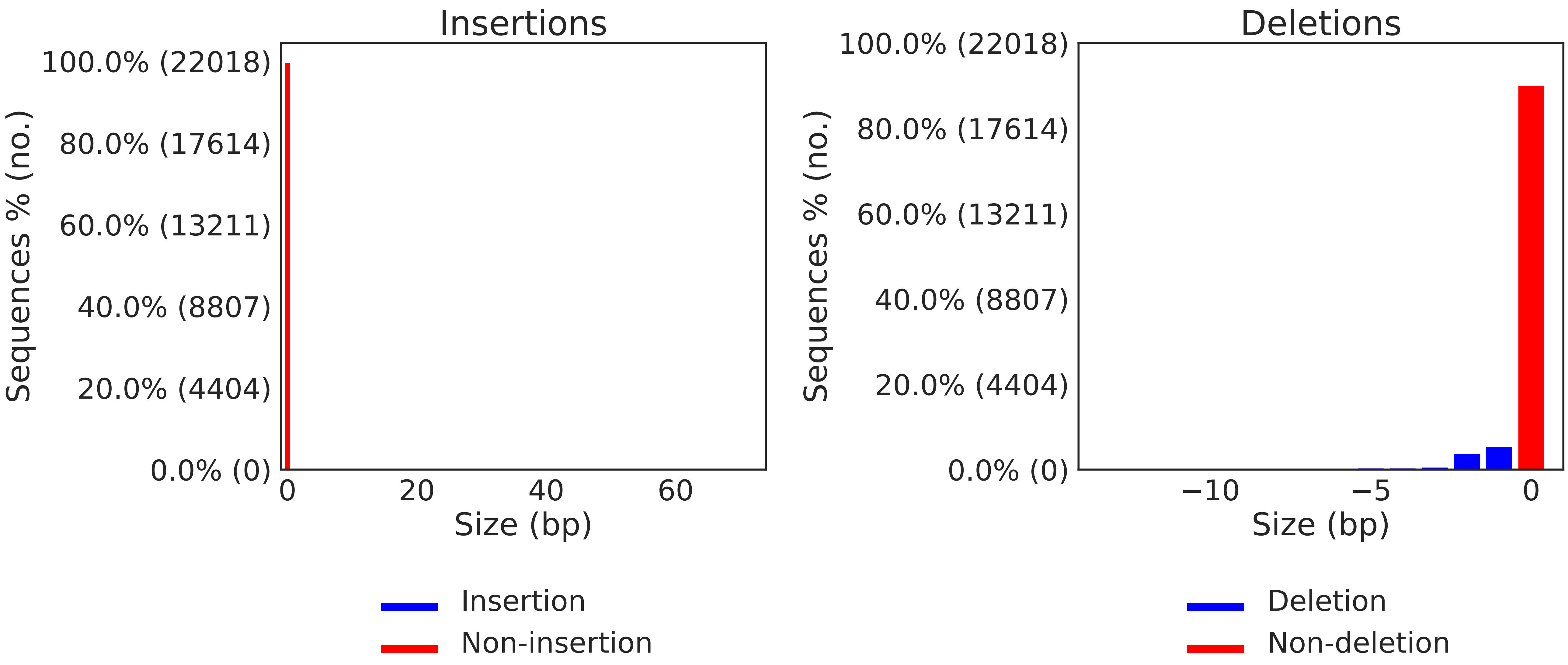


Tmc$^{Bth/WT}$, SpCas9 + gRNA 1.1, Bth allele

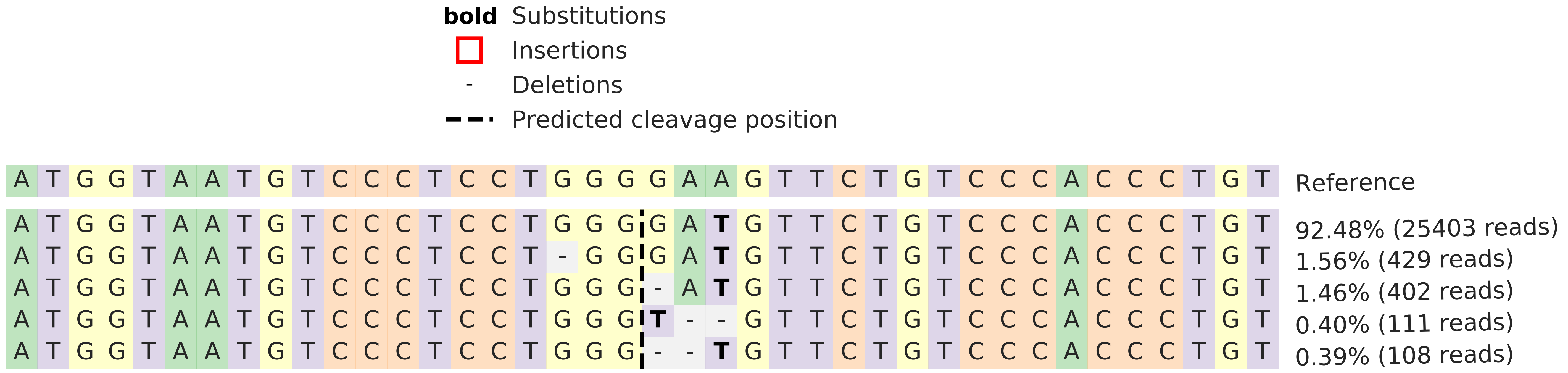


bold Substitutions
☐ Insertions
\- Deletions
-- - Predicted cleavage position

A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T	Reference
A T G G T A A T G T C C C T C C T G G G ¦ G A A G T T C T G T C C C A C C C T G T	81.34% (17909 reads)
A T G G T A A T G T C C C T C C T G G G ¦ - A A G T T C T G T C C C A C C C T G T	5.10% (1122 reads)
A T G G T A A T G T C C C T C C T - G G ¦ G A A G T T C T G T C C C A C C C T G T	4.91% (1082 reads)
A T G G T A A T G T C C C T C C T G G G ¦ - - A G T T C T G T C C C A C C C T G T	2.77% (610 reads)
A T G G T A A T G T C C C T C C T G G - ¦ - A A G T T C T G T C C C A C C C T G T	0.41% (91 reads)
A T G G T A A T G T C C C T C C T - - G ¦ G A A G T T C T G T C C C A C C C T G T	0.41% (90 reads)
A T G G T A A T G T C C C T C - - - - - ¦ G A A G T T C T G T C C C A C C C T G T	0.20% (45 reads)



Tmc$^{Bth/WT}$, SpCas9 + gRNA 1.1, WT allele



bold Substitutions

☐ Insertions

\- Deletions

-- Predicted cleavage position

A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T Reference

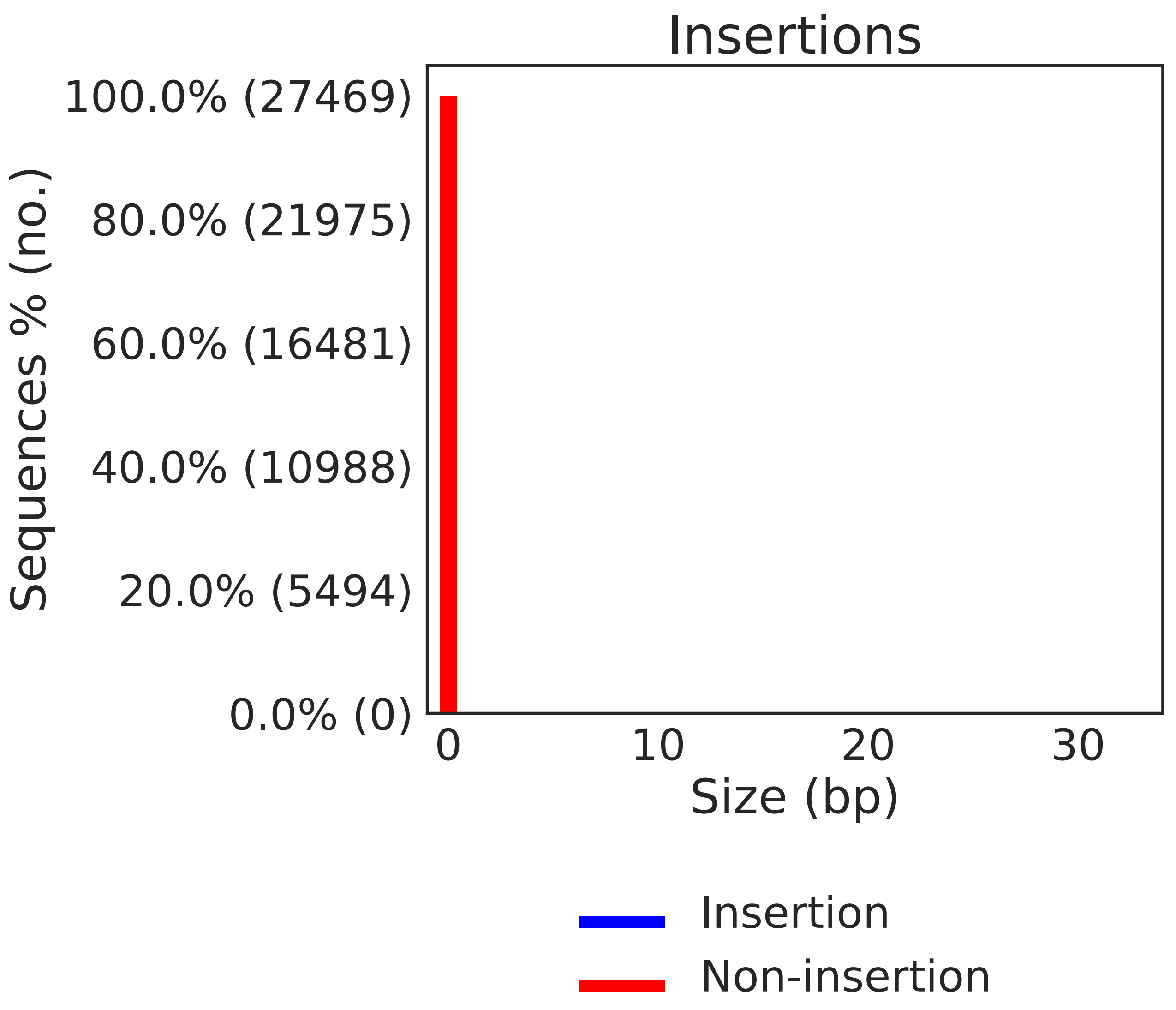
A T G G T A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C T G T 92.48% (25403 reads)

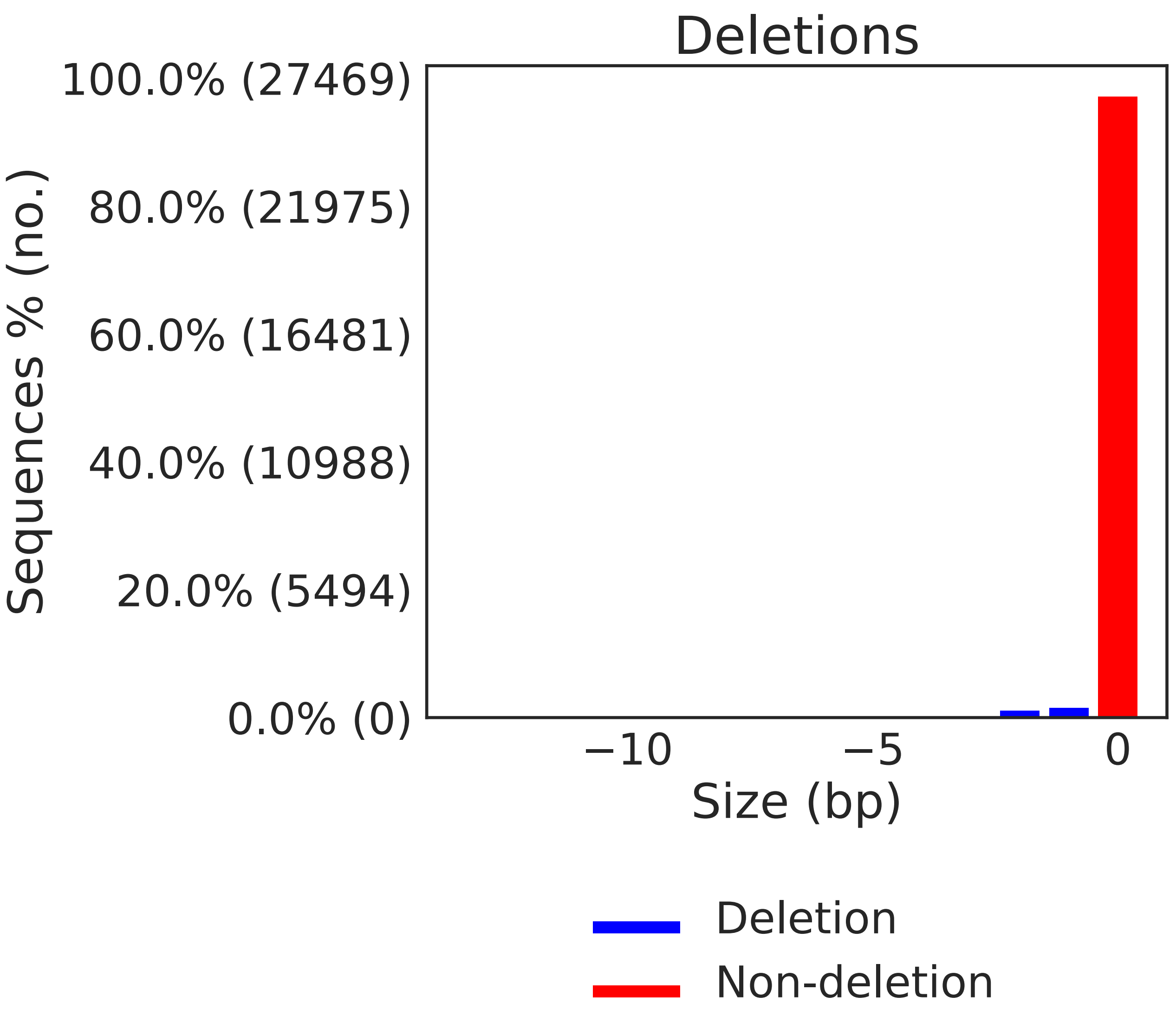
A T G G T A A T G T C C C T C C T - G G G A **T** G T T C T G T C C C A C C C T G T 1.56% (429 reads)

A T G G T A A T G T C C C T C C T G G G - A **T** G T T C T G T C C C A C C C T G T 1.46% (402 reads)

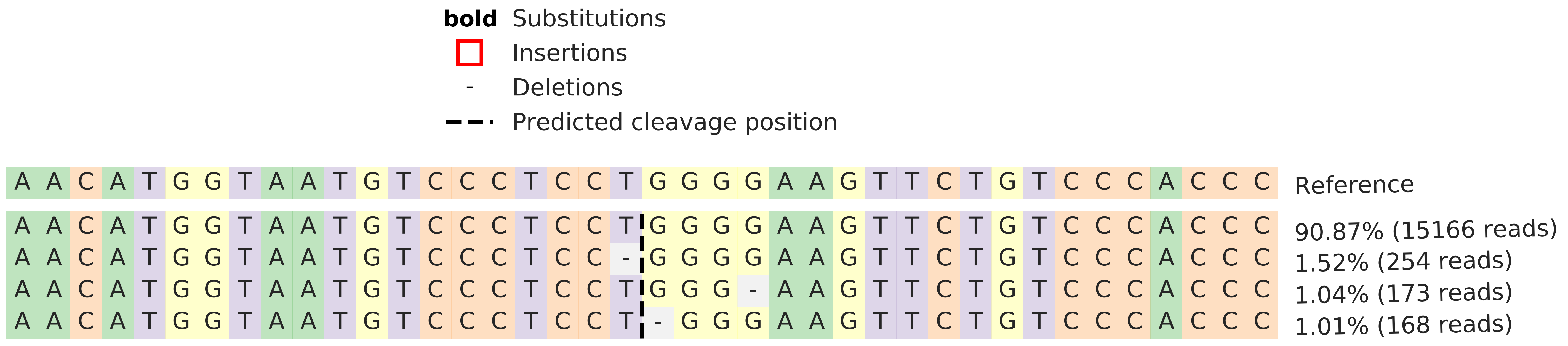
A T G G T A A T G T C C C T C C T G G G **T** - - G T T C T G T C C C A C C C T G T 0.40% (111 reads)

A T G G T A A T G T C C C T C C T G G G - - **T** G T T C T G T C C C A C C C T G T 0.39% (108 reads)

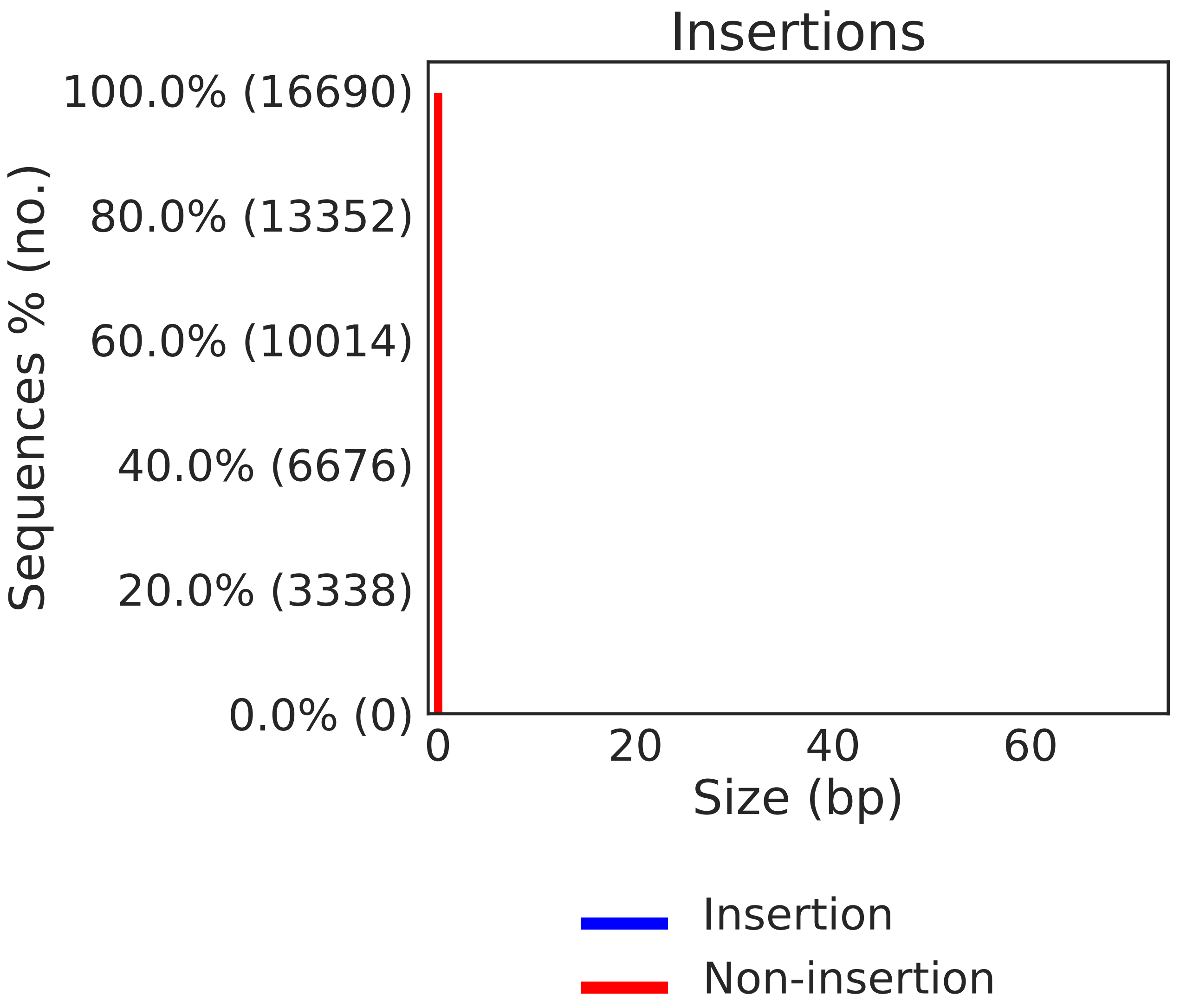


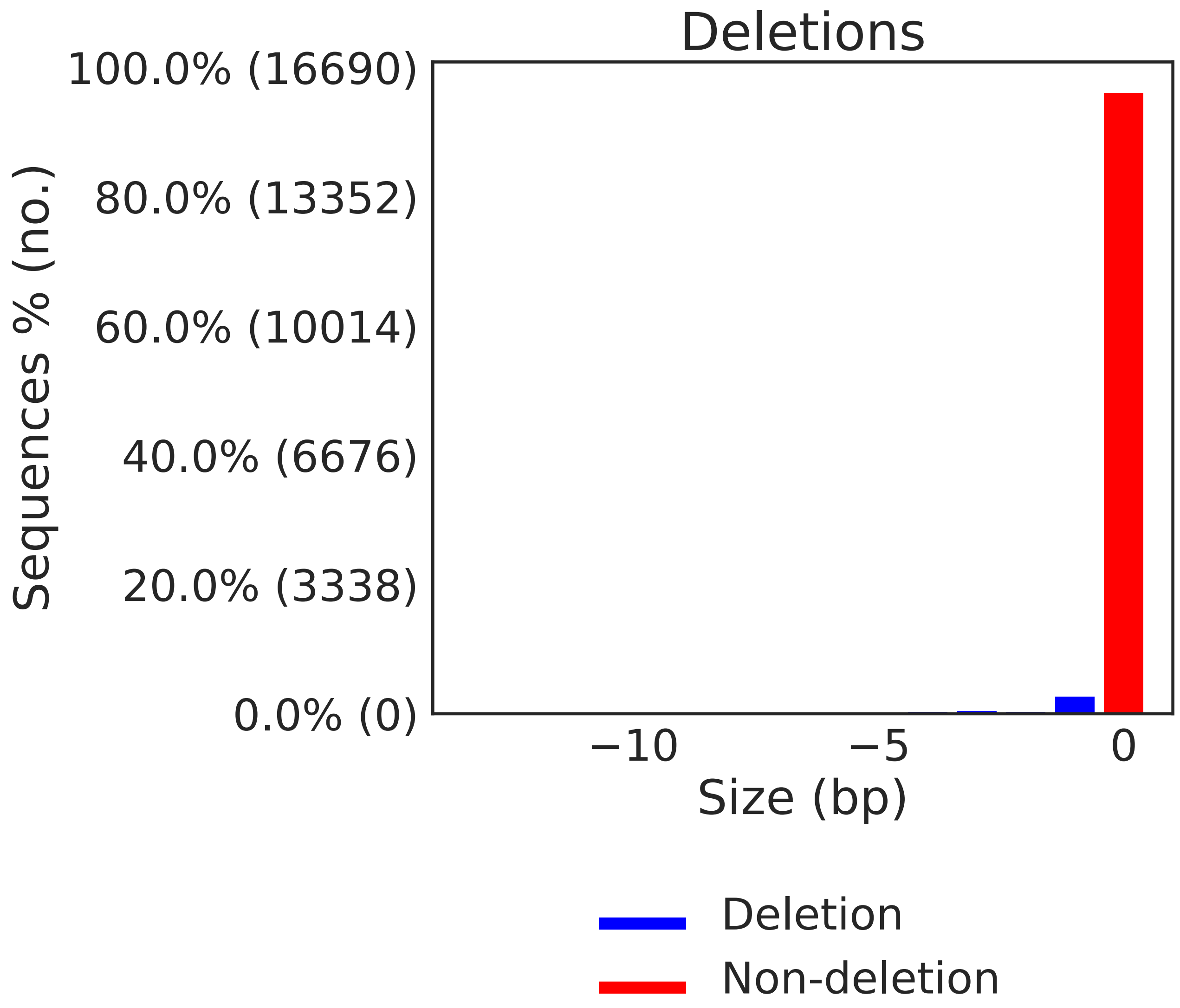


Tmc$^{Bth/WT}$, SpCas9 + gRNA 2.1, Bth allele

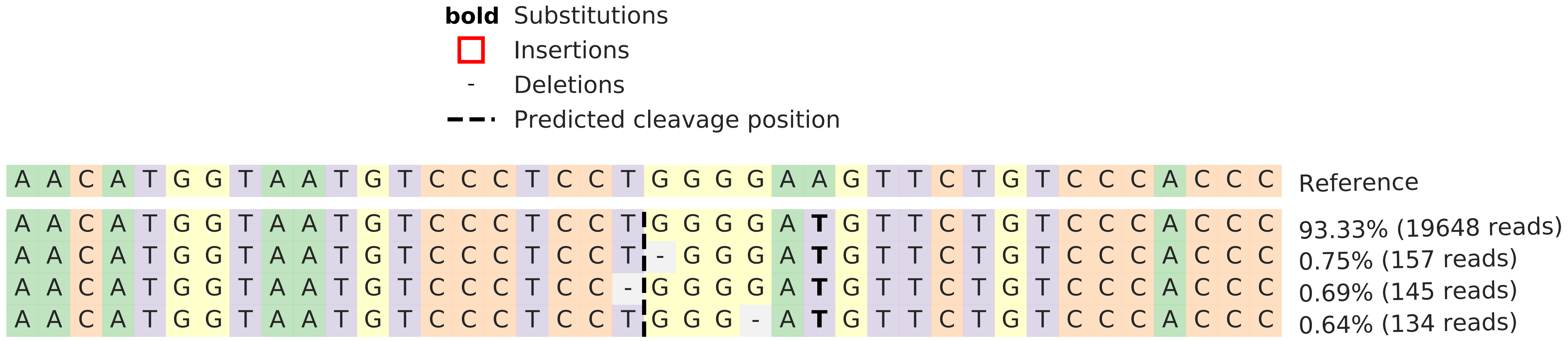


A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C	Reference
A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C	90.87% (15166 reads)
A A C A T G G T A A T G T C C C T C C - G G G G A A G T T C T G T C C C A C C C	1.52% (254 reads)
A A C A T G G T A A T G T C C C T C C T G G G - A A G T T C T G T C C C A C C C	1.04% (173 reads)
A A C A T G G T A A T G T C C C T C C T - G G G A A G T T C T G T C C C A C C C	1.01% (168 reads)





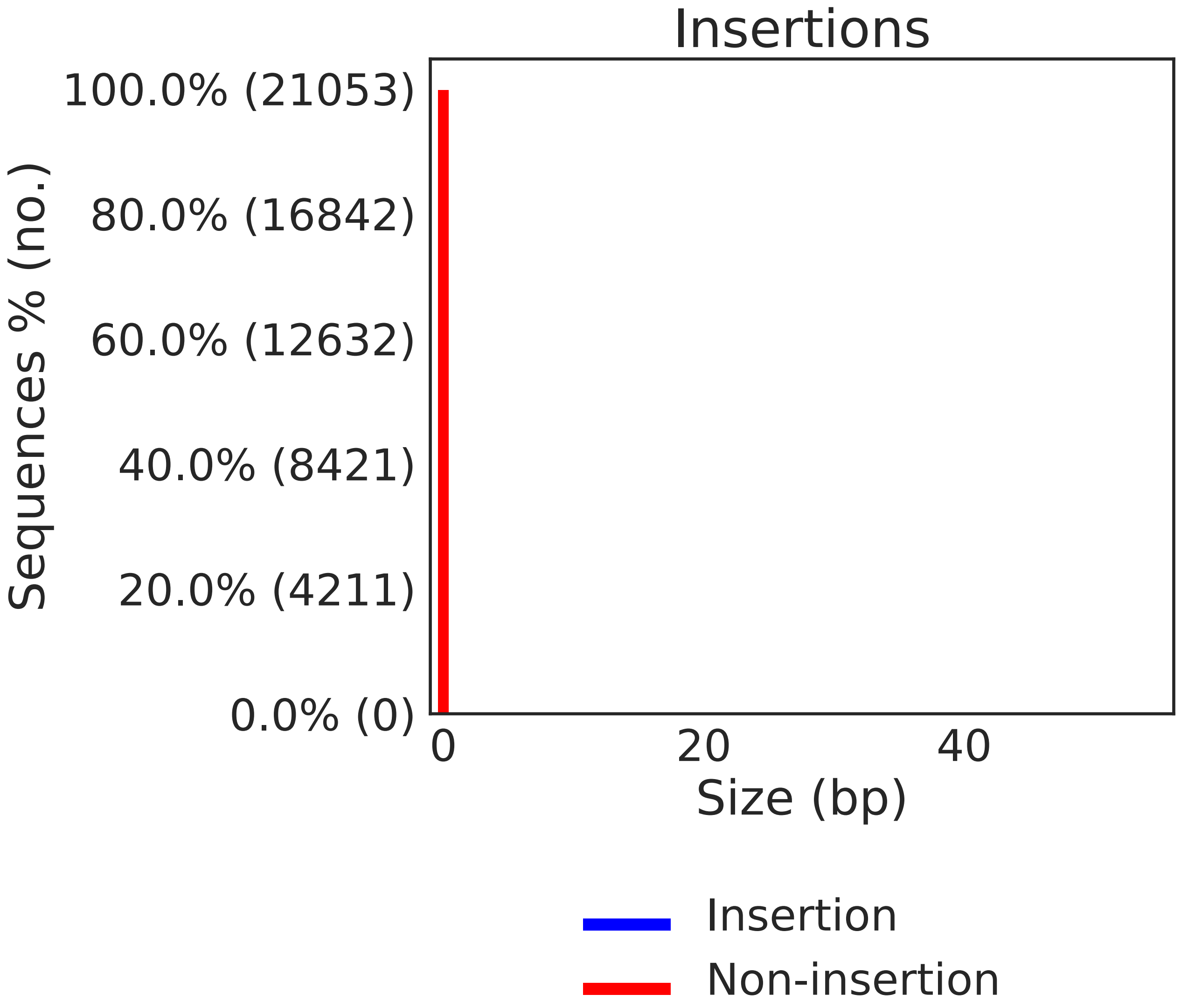
Tmc$^{Bth/WT}$, SpCas9 + gRNA 2.1, WT allele

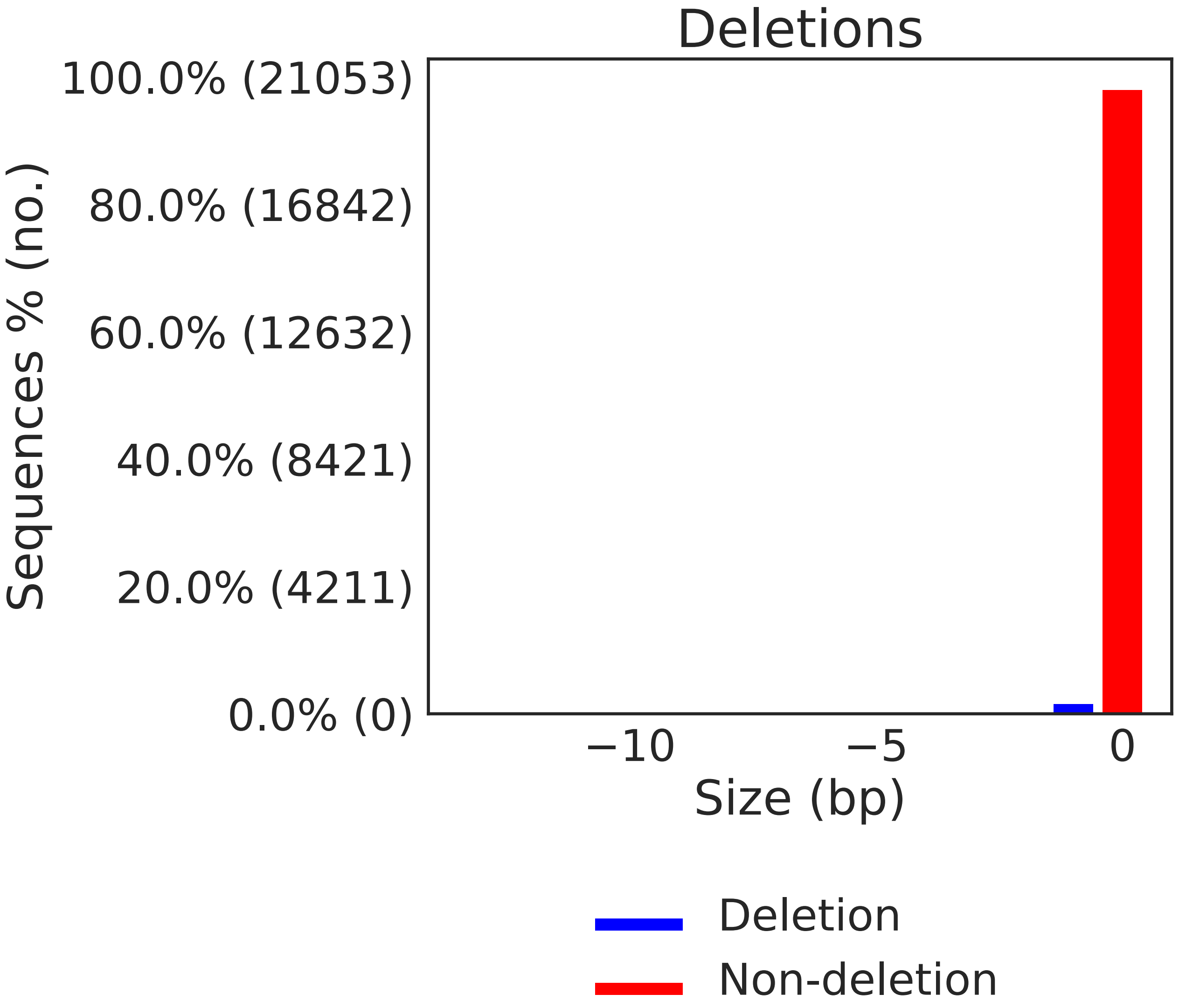


bold Substitutions
☐ Insertions
\- Deletions
\- - Predicted cleavage position

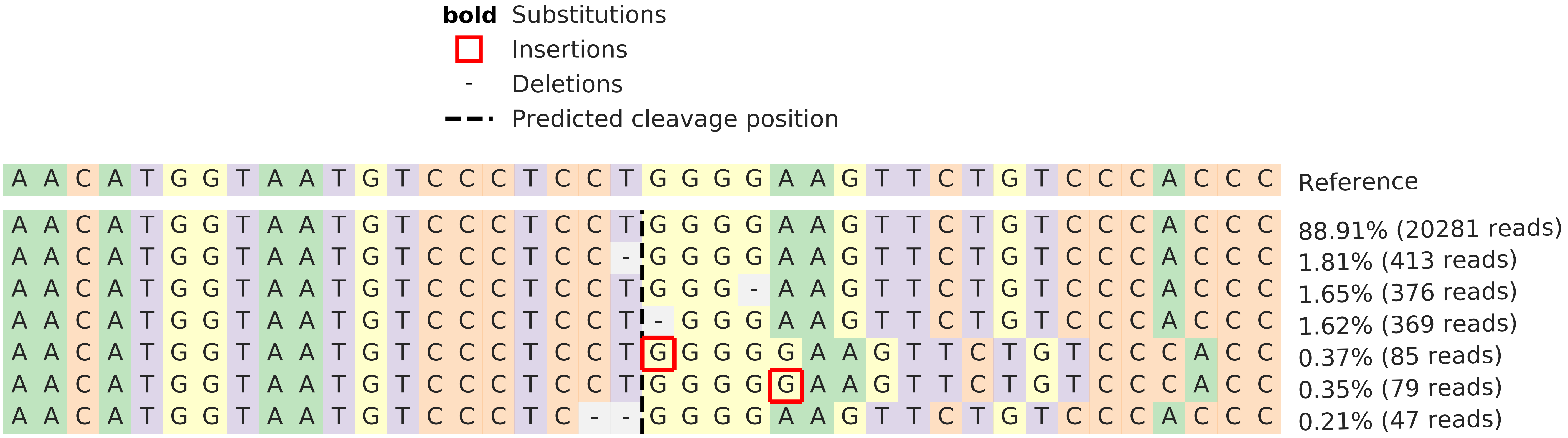
A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C Reference

A A C A T G G T A A T G T C C C T C C T ¦ G G G G A **T** G T T C T G T C C C A C C C 93.33% (19648 reads)
A A C A T G G T A A T G T C C C T C C T ¦ - G G G A **T** G T T C T G T C C C A C C C 0.75% (157 reads)
A A C A T G G T A A T G T C C C T C C - ¦ G G G G A **T** G T T C T G T C C C A C C C 0.69% (145 reads)
A A C A T G G T A A T G T C C C T C C T ¦ G G G - A **T** G T T C T G T C C C A C C C 0.64% (134 reads)

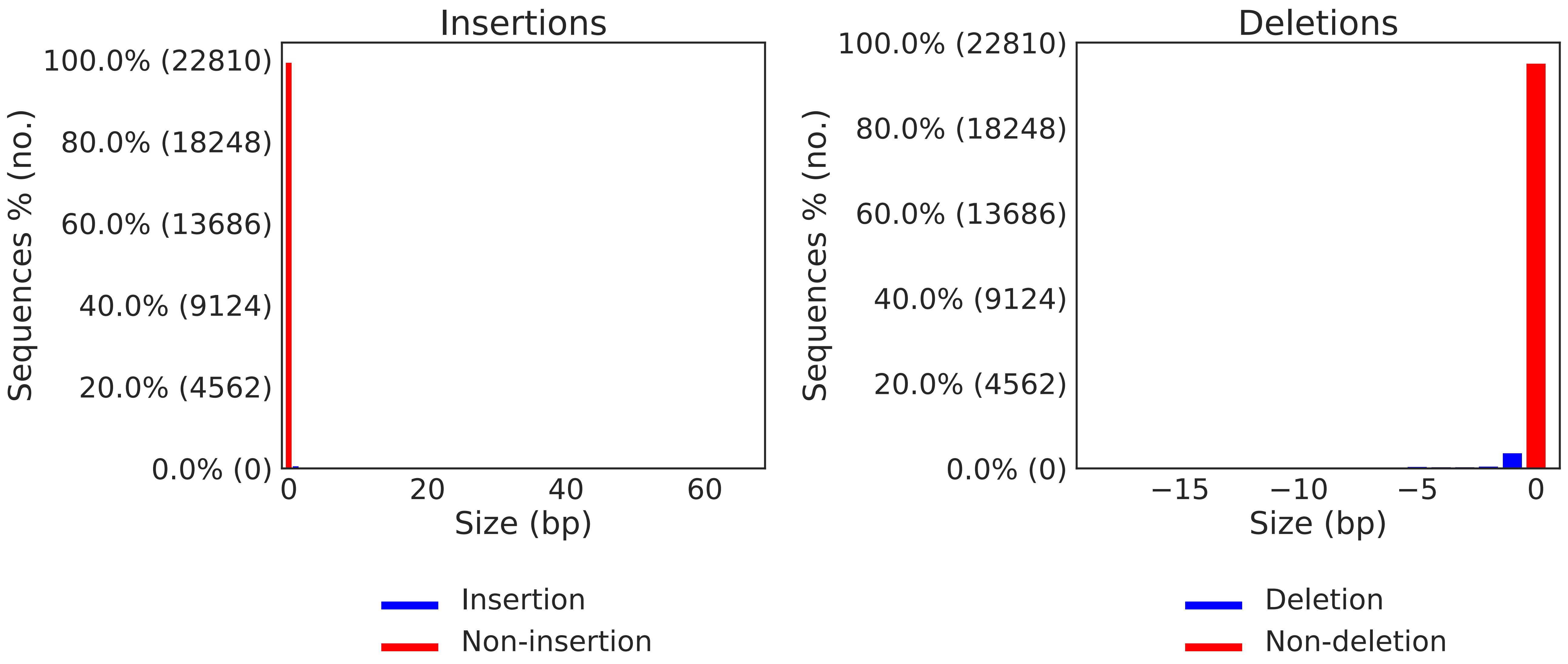




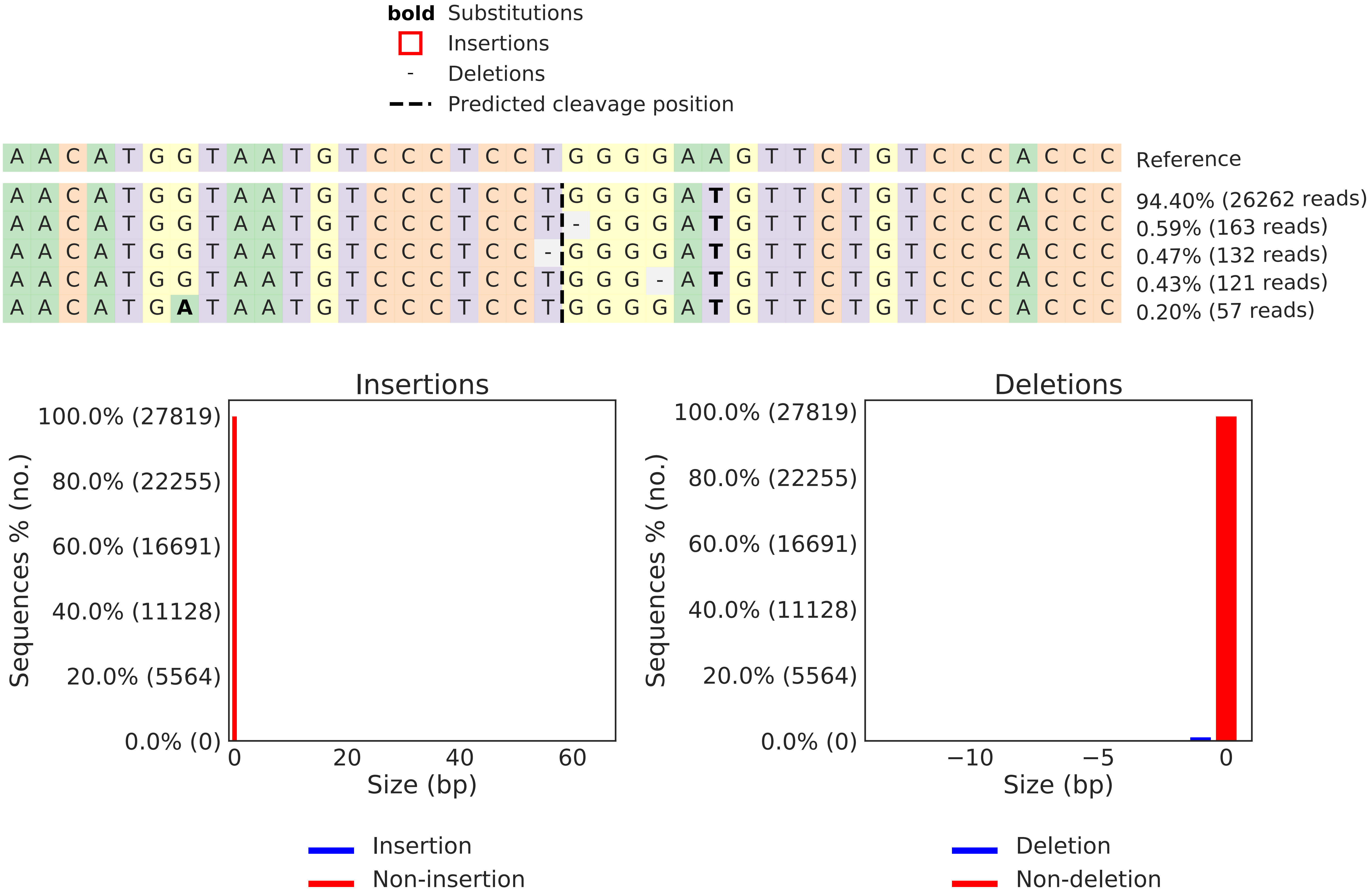
Tmc$^{Bth/WT}$, SpCas9 + gRNA 2.4, Bth allele



Sequence	
A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C	Reference
A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C	88.91% (20281 reads)
A A C A T G G T A A T G T C C C T C C - G G G G A A G T T C T G T C C C A C C C	1.81% (413 reads)
A A C A T G G T A A T G T C C C T C C T G G G - A A G T T C T G T C C C A C C C	1.65% (376 reads)
A A C A T G G T A A T G T C C C T C C T - G G G A A G T T C T G T C C C A C C C	1.62% (369 reads)
A A C A T G G T A A T G T C C C T C C T G G G G G A A G T T C T G T C C C A C C	0.37% (85 reads)
A A C A T G G T A A T G T C C C T C C T G G G G G A A G T T C T G T C C C A C C	0.35% (79 reads)
A A C A T G G T A A T G T C C C T C - - G G G G A A G T T C T G T C C C A C C C	0.21% (47 reads)

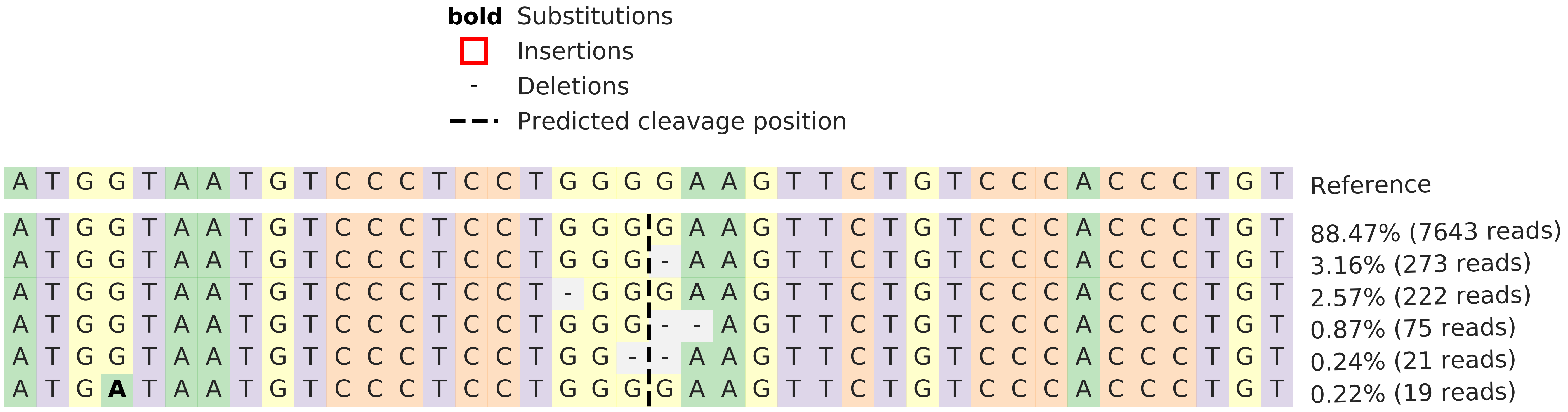


Tmc$^{Bth/WT}$, SpCas9 + gRNA 2.4, WT allele



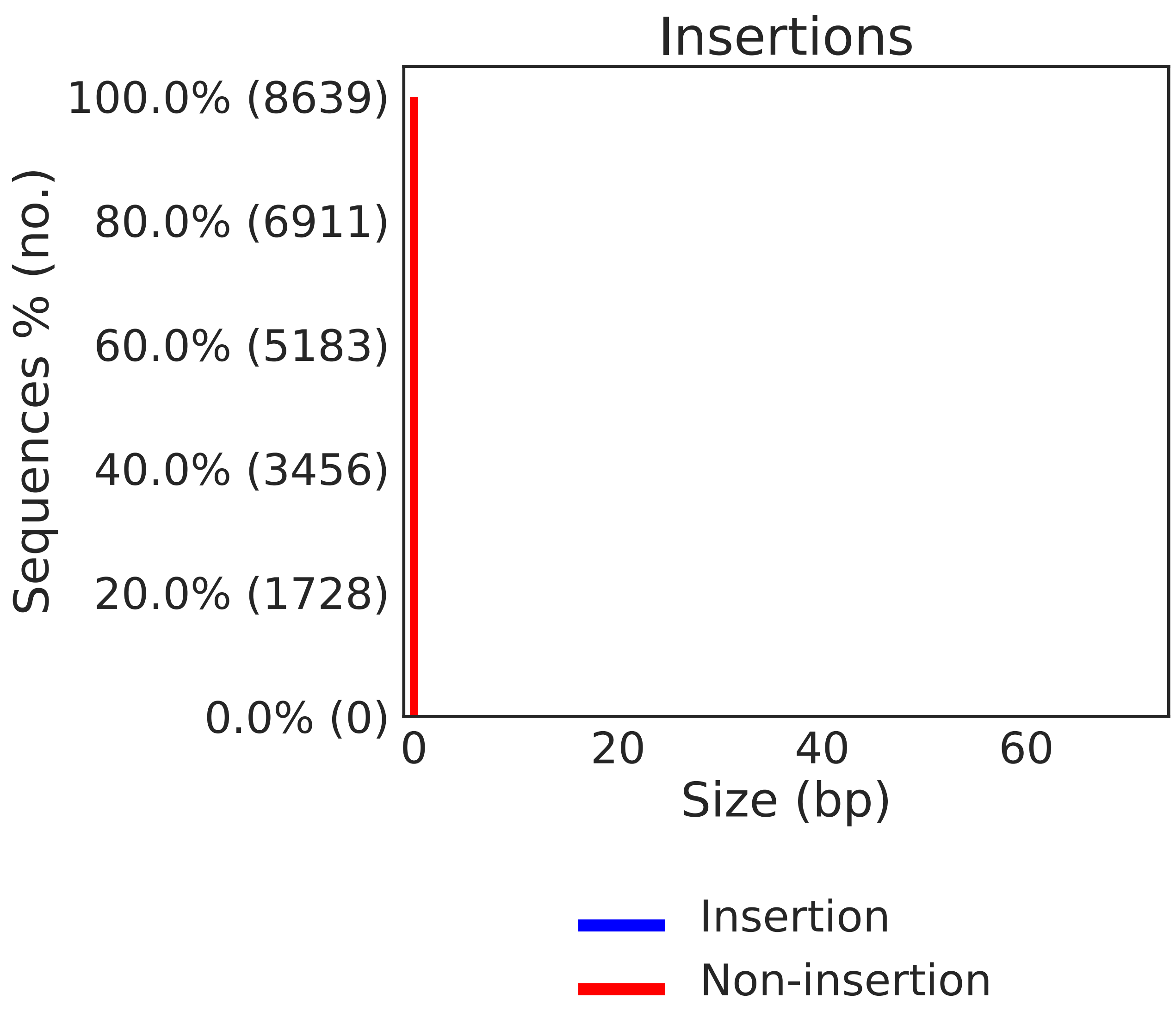
AACATGGTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCC	Reference
AACATGGTAATGTCCCTCCTGGGGA**T**GTTCTGTCCCACCC	94.40% (26262 reads)
AACATGGTAATGTCCCTCCT-GGGA**T**GTTCTGTCCCACCC	0.59% (163 reads)
AACATGGTAATGTCCCTCC-GGGGA**T**GTTCTGTCCCACCC	0.47% (132 reads)
AACATGGTAATGTCCCTCCTGGG-A**T**GTTCTGTCCCACCC	0.43% (121 reads)
AACATG**A**TAATGTCCCTCCTGGGGA**T**GTTCTGTCCCACCC	0.20% (57 reads)

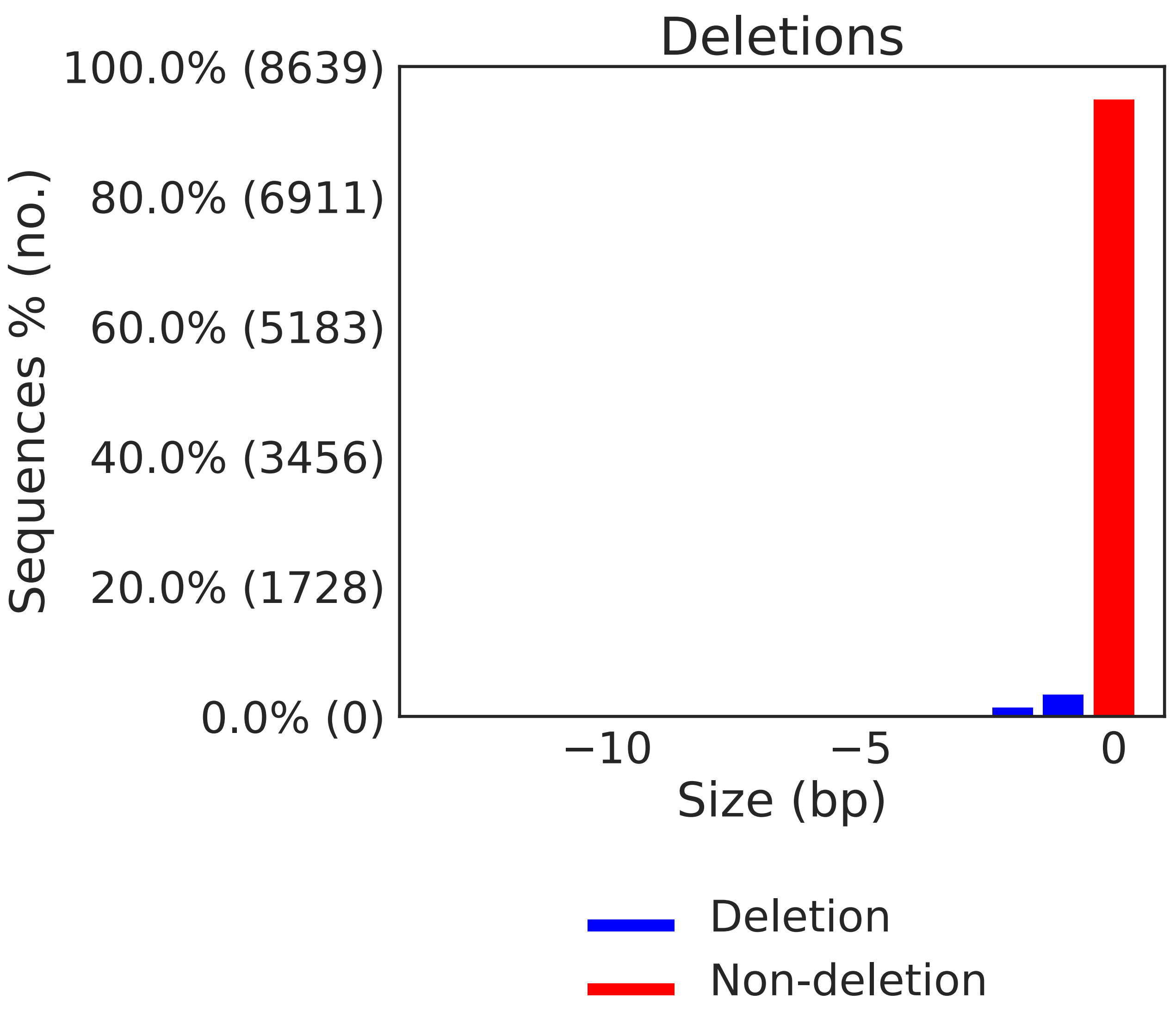
Tmc$^{Bth/WT}$, eSpCas9 + gRNA 1.1, Bth allele



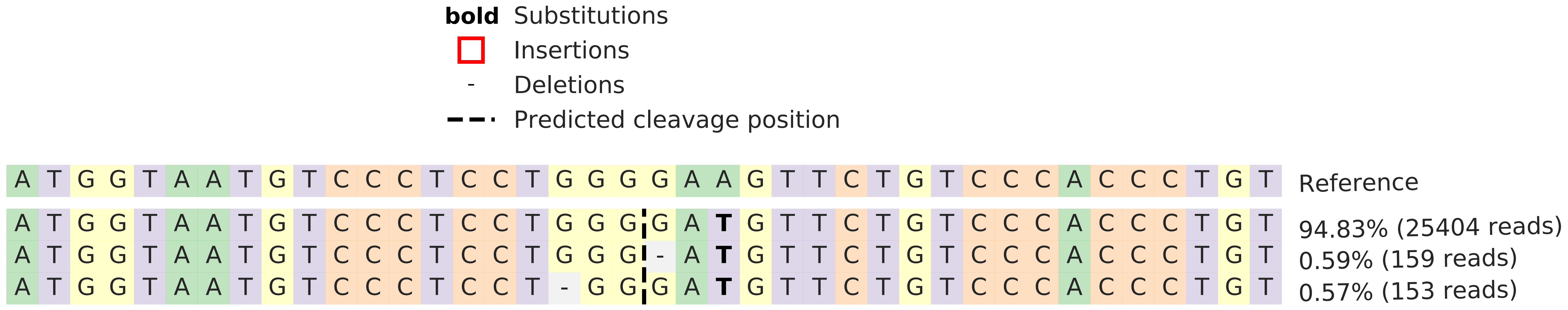
bold Substitutions
☐ Insertions
\- Deletions
\-\-\- Predicted cleavage position

A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T	Reference
A T G G T A A T G T C C C T C C T G G G ¦ G A A G T T C T G T C C C A C C C T G T	88.47% (7643 reads)
A T G G T A A T G T C C C T C C T G G G ¦ - A A G T T C T G T C C C A C C C T G T	3.16% (273 reads)
A T G G T A A T G T C C C T C C T - G G ¦ G A A G T T C T G T C C C A C C C T G T	2.57% (222 reads)
A T G G T A A T G T C C C T C C T G G G ¦ - - A G T T C T G T C C C A C C C T G T	0.87% (75 reads)
A T G G T A A T G T C C C T C C T G G - ¦ - A A G T T C T G T C C C A C C C T G T	0.24% (21 reads)
A T G **A** T A A T G T C C C T C C T G G G ¦ G A A G T T C T G T C C C A C C C T G T	0.22% (19 reads)





Tmc$^{Bth/WT}$, eSpCas9 + gRNA 1.1, WT allele



bold Substitutions

☐ Insertions

\- Deletions

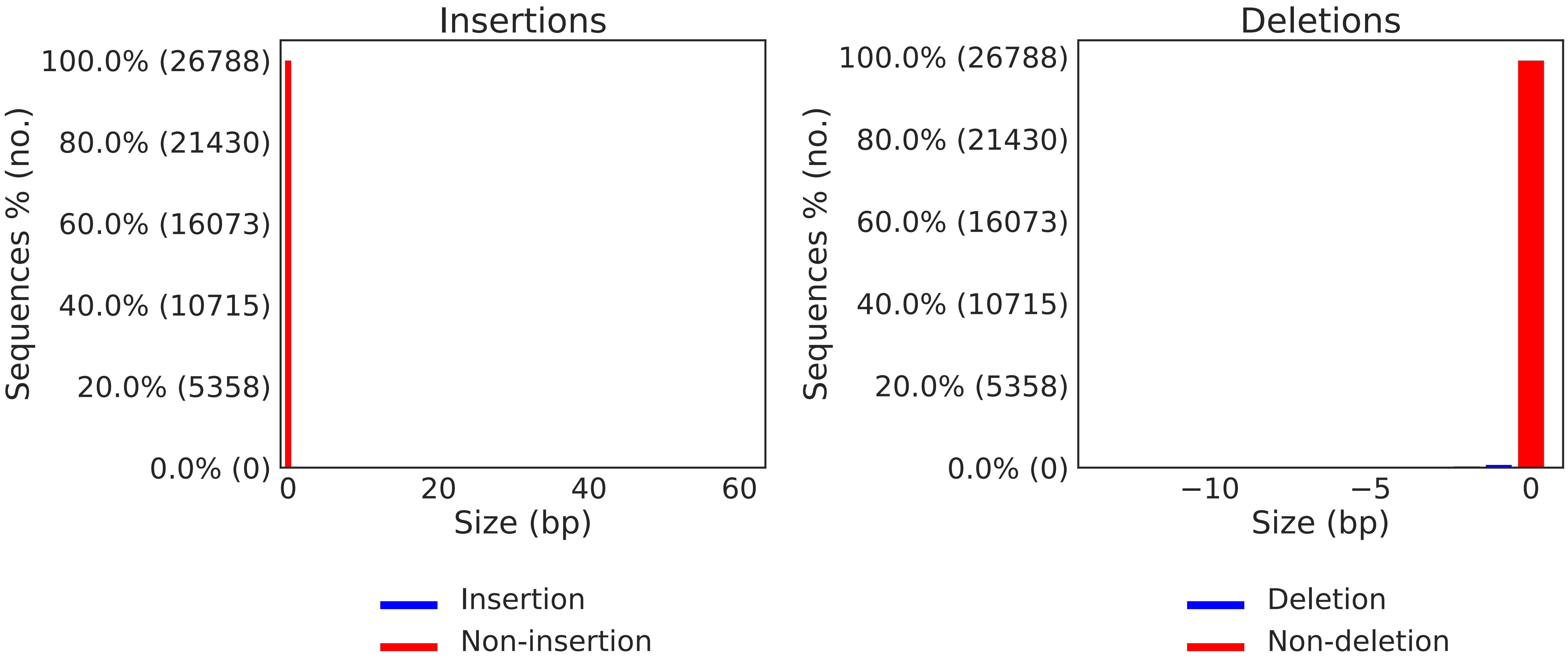
-- Predicted cleavage position

A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T Reference

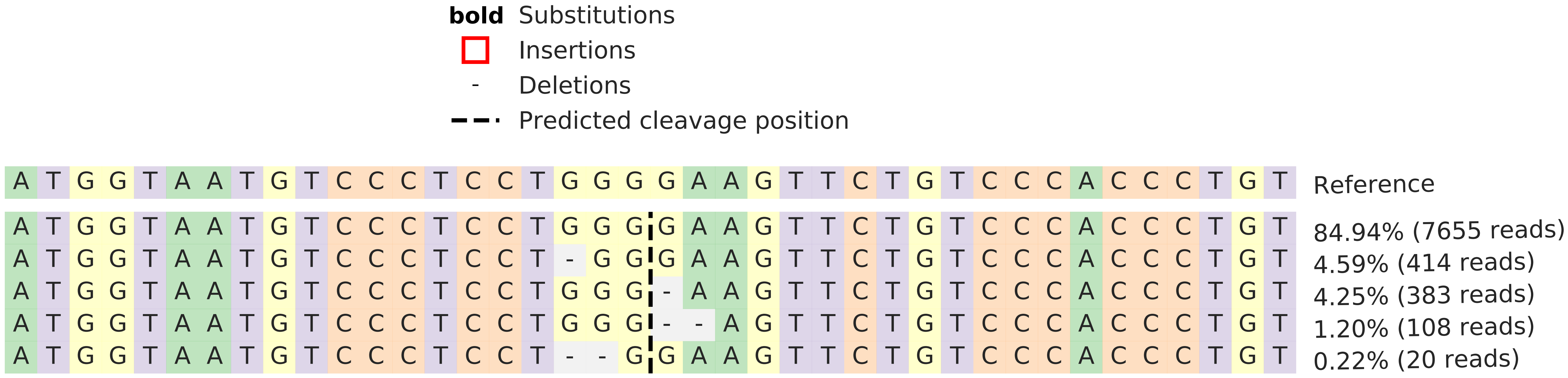
A T G G T A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C T G T 94.83% (25404 reads)

A T G G T A A T G T C C C T C C T G G G - A **T** G T T C T G T C C C A C C C T G T 0.59% (159 reads)

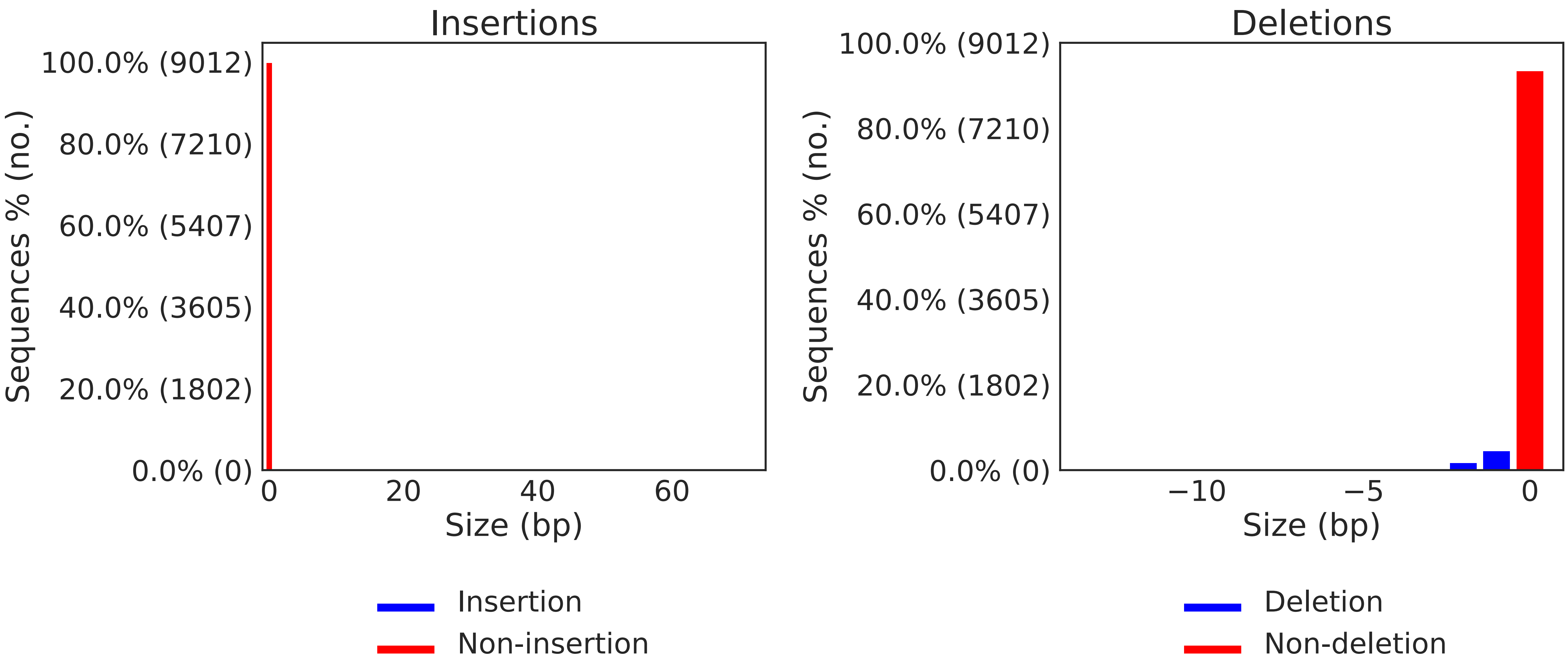
A T G G T A A T G T C C C T C C T - G G G A **T** G T T C T G T C C C A C C C T G T 0.57% (153 reads)



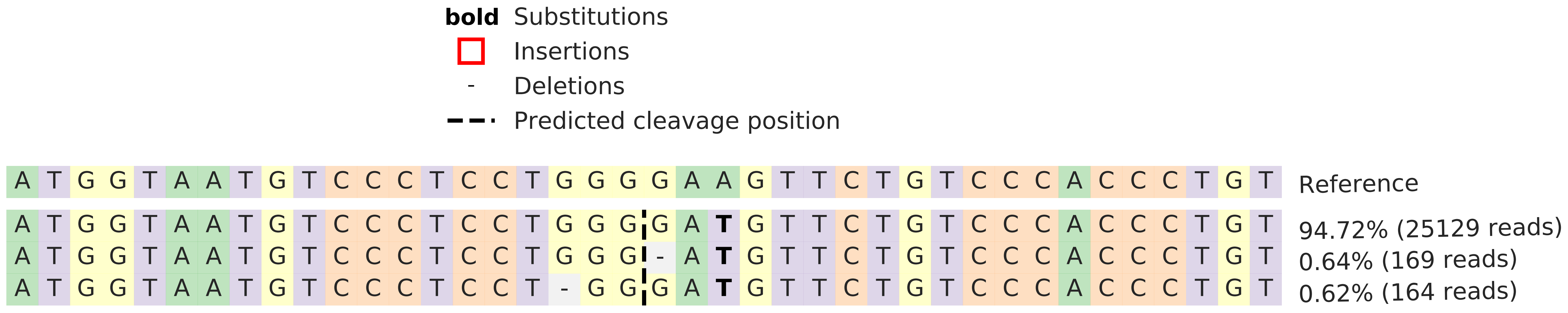
Tmc$^{Bth/WT}$, HypaCas9 + gRNA 1.1, Bth allele



A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T	Reference
A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T	84.94% (7655 reads)
A T G G T A A T G T C C C T C C T - G G G A A G T T C T G T C C C A C C C T G T	4.59% (414 reads)
A T G G T A A T G T C C C T C C T G G G - A A G T T C T G T C C C A C C C T G T	4.25% (383 reads)
A T G G T A A T G T C C C T C C T G G G - - A G T T C T G T C C C A C C C T G T	1.20% (108 reads)
A T G G T A A T G T C C C T C C T - - G G A A G T T C T G T C C C A C C C T G T	0.22% (20 reads)

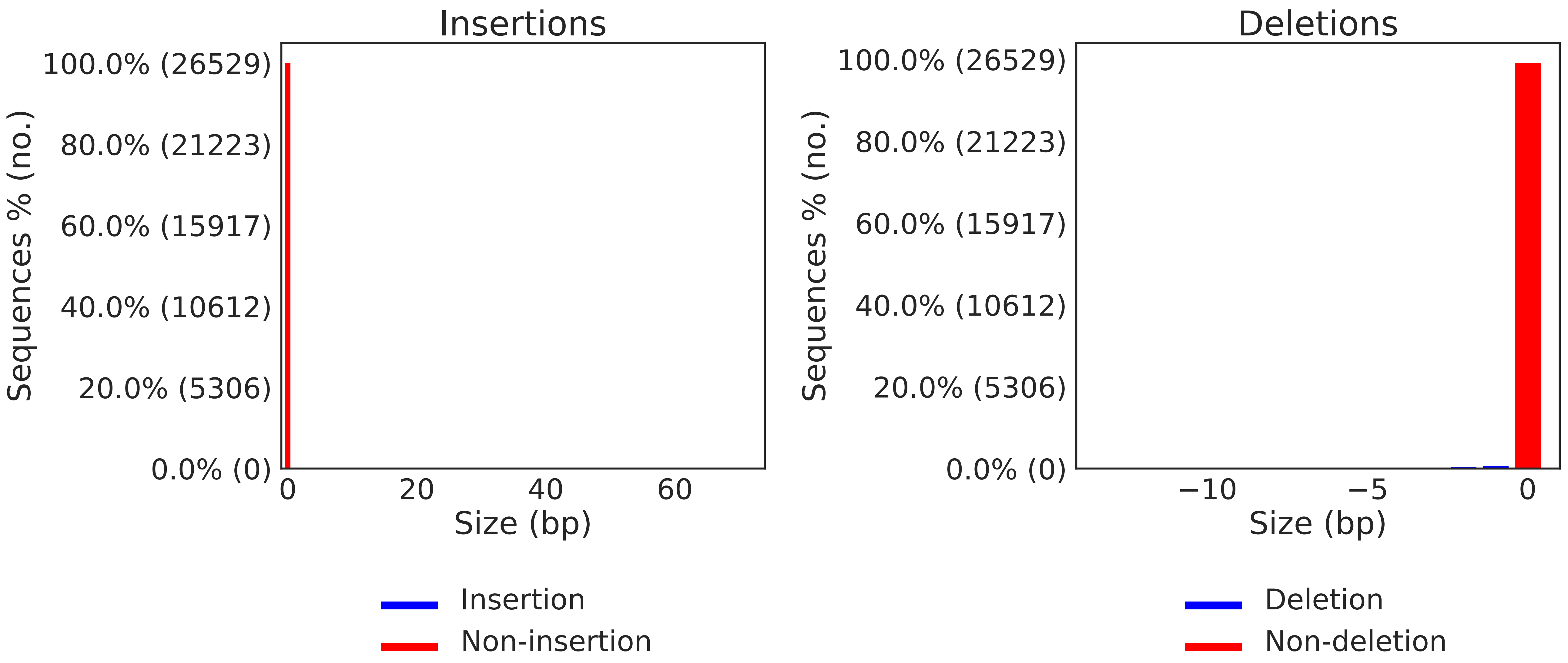


Tmc$^{Bth/WT}$, HypaCas9 + gRNA 1.1, WT allele

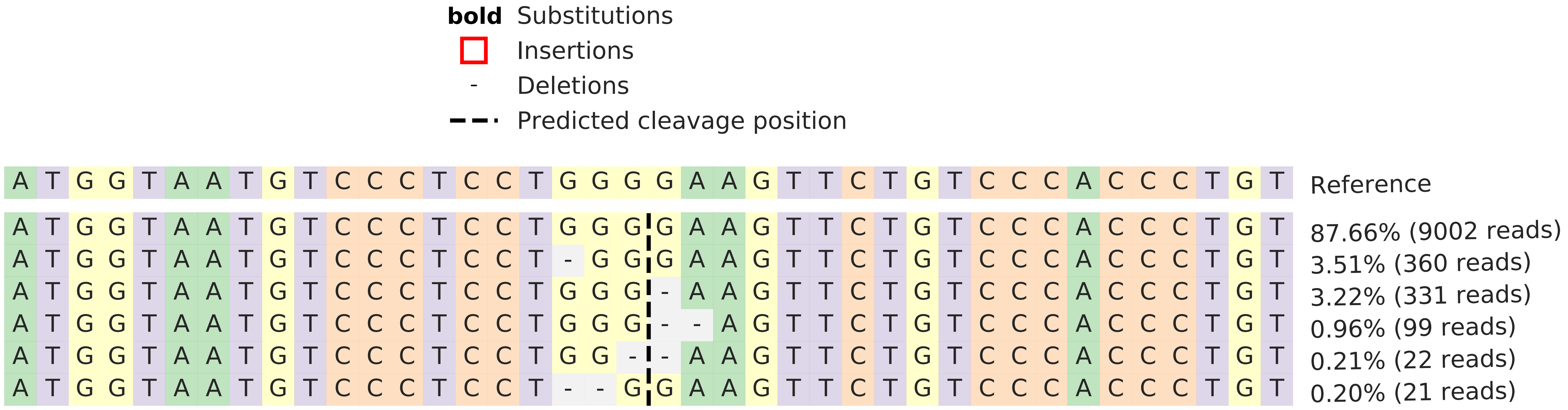


bold Substitutions
☐ Insertions
\- Deletions
-- Predicted cleavage position

ATGGTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCCTGT	Reference
ATGGTAATGTCCCTCCTGGGGA**T**GTTCTGTCCCACCCTGT	94.72% (25129 reads)
ATGGTAATGTCCCTCCTGGG-A**T**GTTCTGTCCCACCCTGT	0.64% (169 reads)
ATGGTAATGTCCCTCCT-GGGA**T**GTTCTGTCCCACCCTGT	0.62% (164 reads)

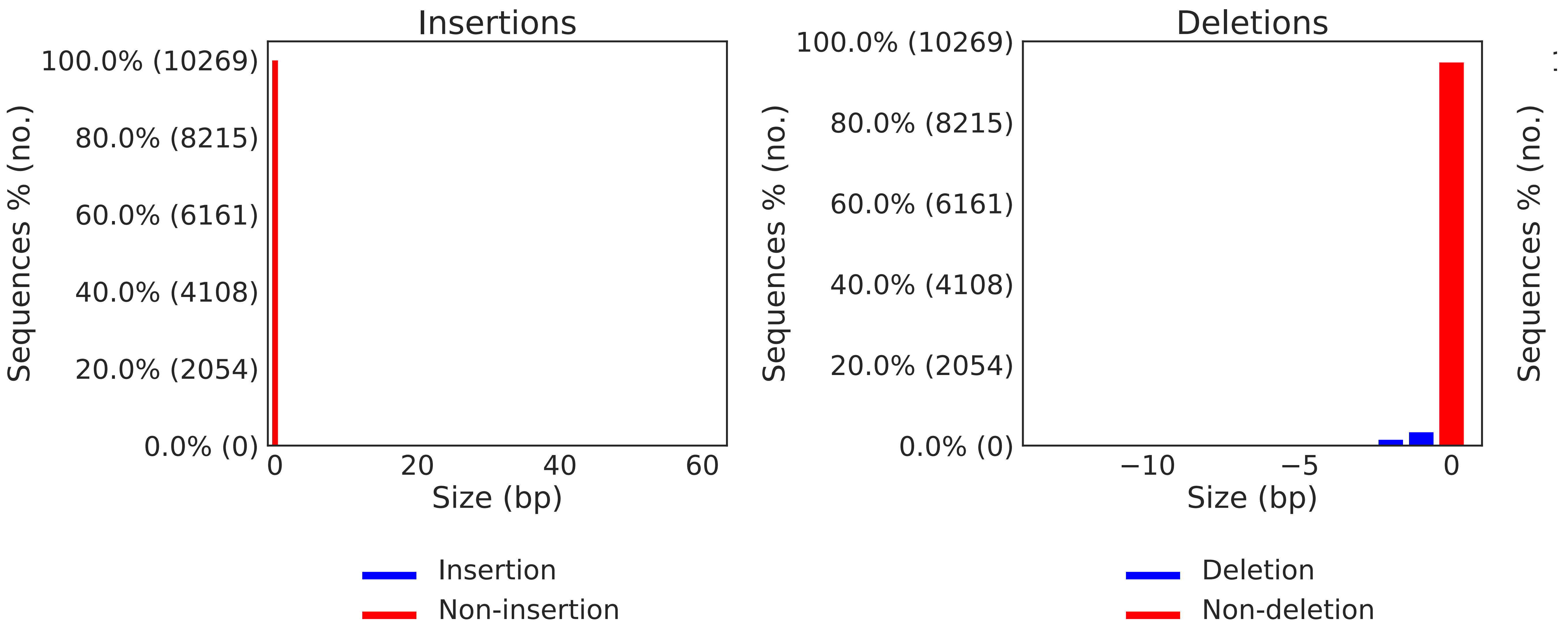


Tmc$^{Bth/WT}$, SpCas9-HF1 + gRNA 1.1, Bth allele

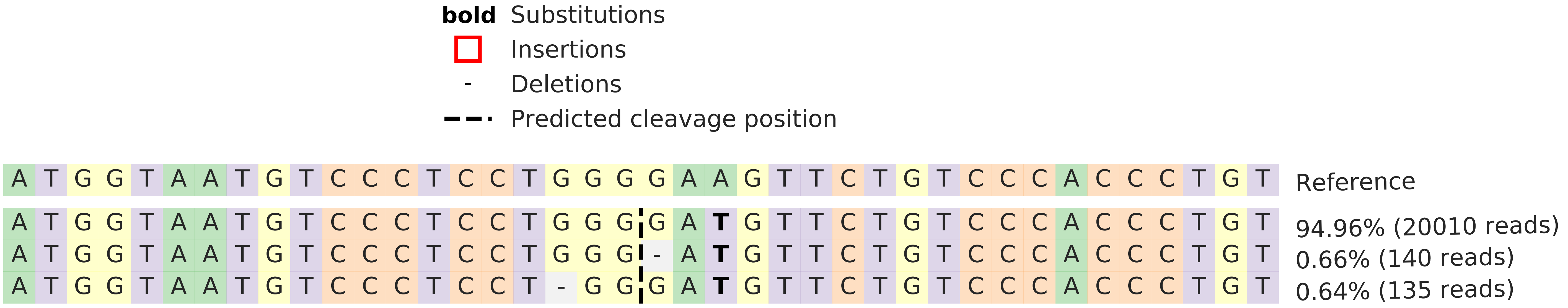


A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T Reference

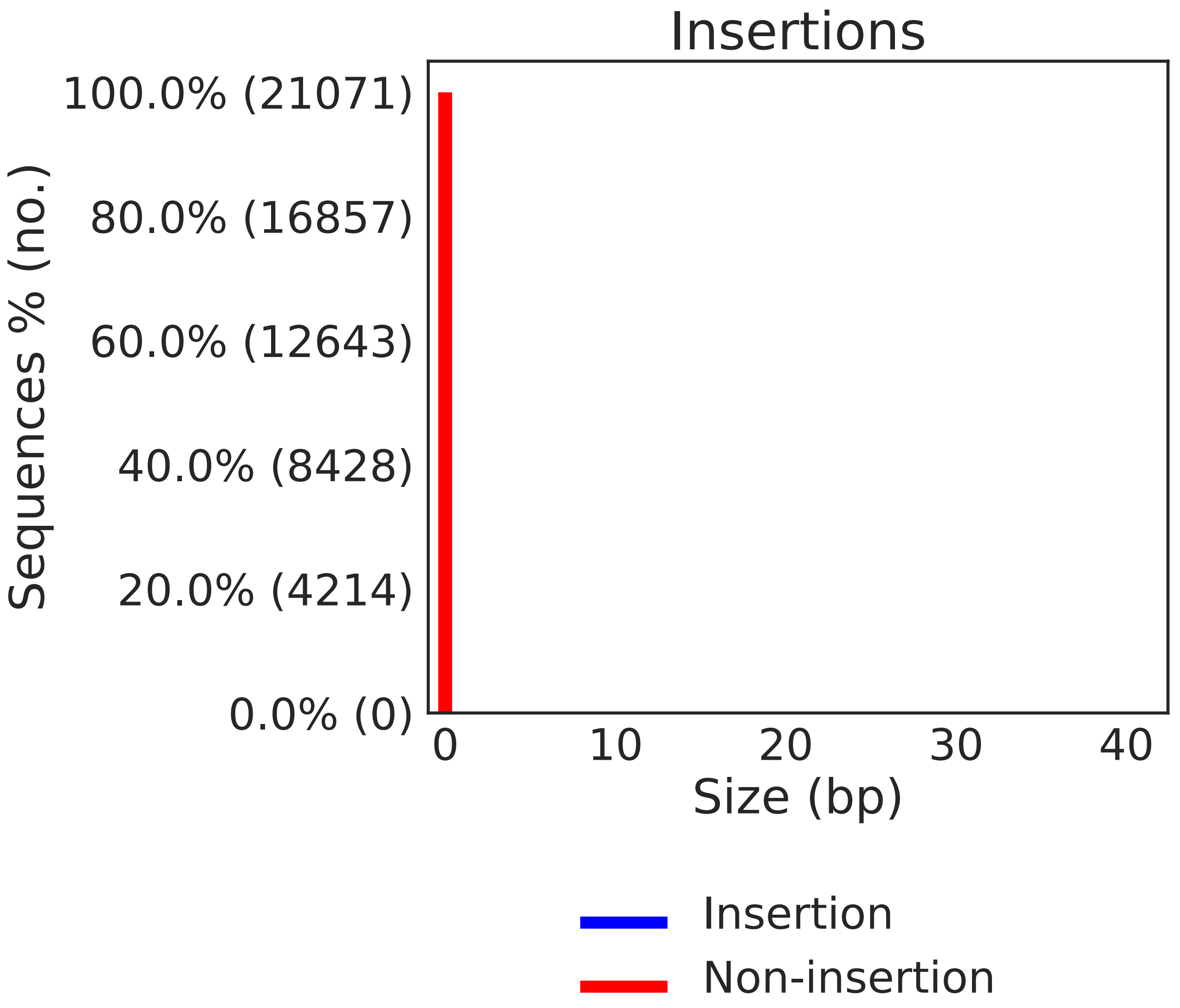
A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T 87.66% (9002 reads)
A T G G T A A T G T C C C T C C T - G G G A A G T T C T G T C C C A C C C T G T 3.51% (360 reads)
A T G G T A A T G T C C C T C C T G G G - A A G T T C T G T C C C A C C C T G T 3.22% (331 reads)
A T G G T A A T G T C C C T C C T G G G - - A G T T C T G T C C C A C C C T G T 0.96% (99 reads)
A T G G T A A T G T C C C T C C T G G - - A A G T T C T G T C C C A C C C T G T 0.21% (22 reads)
A T G G T A A T G T C C C T C C T - - G G A A G T T C T G T C C C A C C C T G T 0.20% (21 reads)

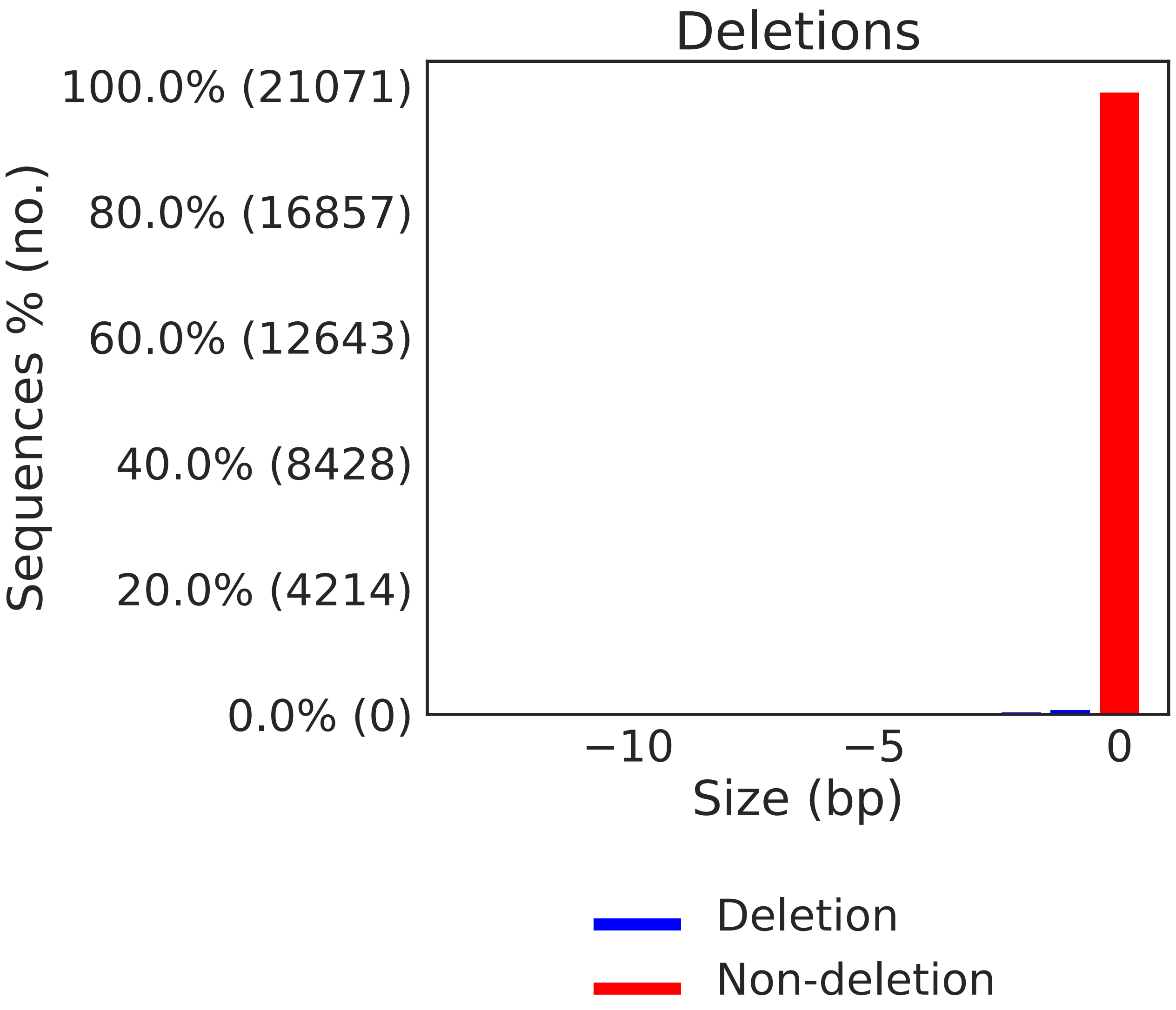


Tmc$^{Bth/WT}$, SpCas9-HF1 + gRNA 1.1, WT allele

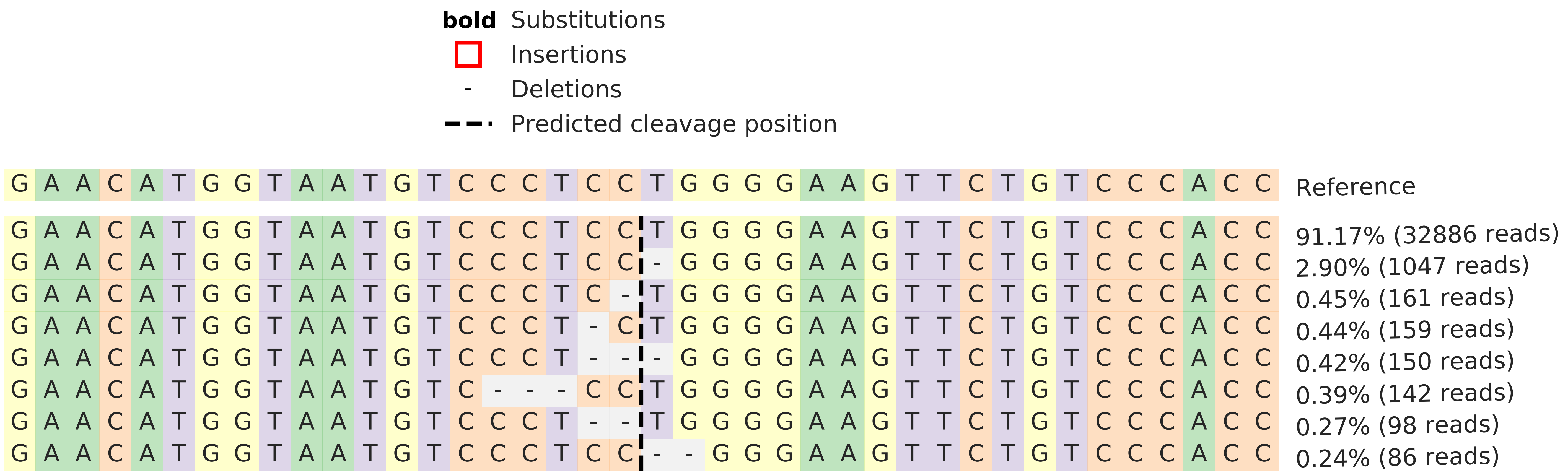


A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T	Reference
A T G G T A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C T G T	94.96% (20010 reads)
A T G G T A A T G T C C C T C C T G G G - A **T** G T T C T G T C C C A C C C T G T	0.66% (140 reads)
A T G G T A A T G T C C C T C C T - G G G A **T** G T T C T G T C C C A C C C T G T	0.64% (135 reads)





Tmc$^{Bth/WT}$, SaCas9^{KKH} + gRNA 4.1, Bth allele



bold Substitutions
☐ Insertions
\- Deletions
\- - Predicted cleavage position

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G A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C   Reference

G A A C A T G G T A A T G T C C C T C C¦T G G G G A A G T T C T G T C C C A C C   91.17% (32886 reads)
G A A C A T G G T A A T G T C C C T C C¦- G G G G A A G T T C T G T C C C A C C   2.90% (1047 reads)
G A A C A T G G T A A T G T C C C T C -¦T G G G G A A G T T C T G T C C C A C C   0.45% (161 reads)
G A A C A T G G T A A T G T C C C T - C¦T G G G G A A G T T C T G T C C C A C C   0.44% (159 reads)
G A A C A T G G T A A T G T C C C T - -¦- G G G G A A G T T C T G T C C C A C C   0.42% (150 reads)
G A A C A T G G T A A T G T C - - - C C¦T G G G G A A G T T C T G T C C C A C C   0.39% (142 reads)
G A A C A T G G T A A T G T C C C T - -¦T G G G G A A G T T C T G T C C C A C C   0.27% (98 reads)
G A A C A T G G T A A T G T C C C T C C¦- - G G G A A G T T C T G T C C C A C C   0.24% (86 reads)
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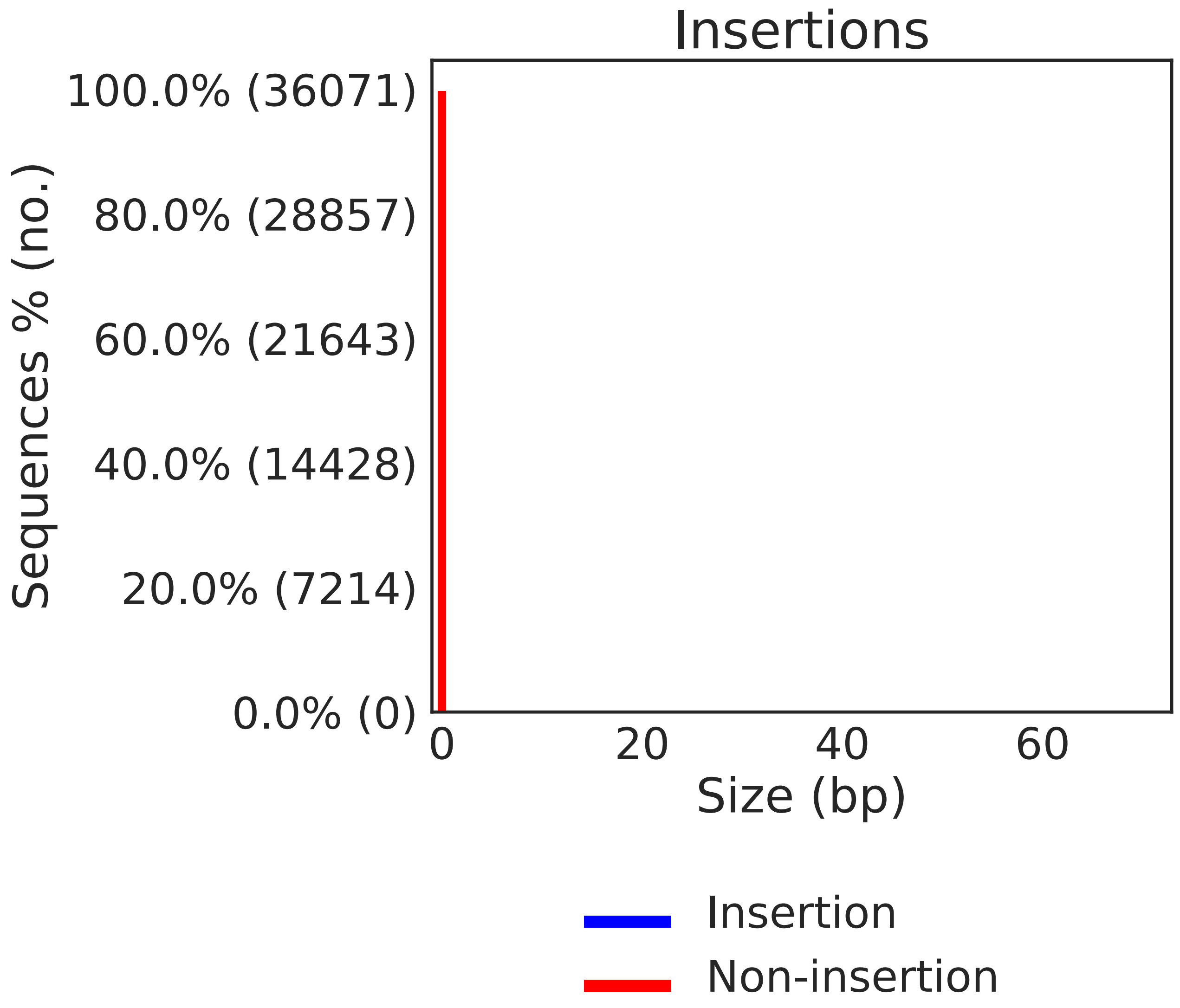
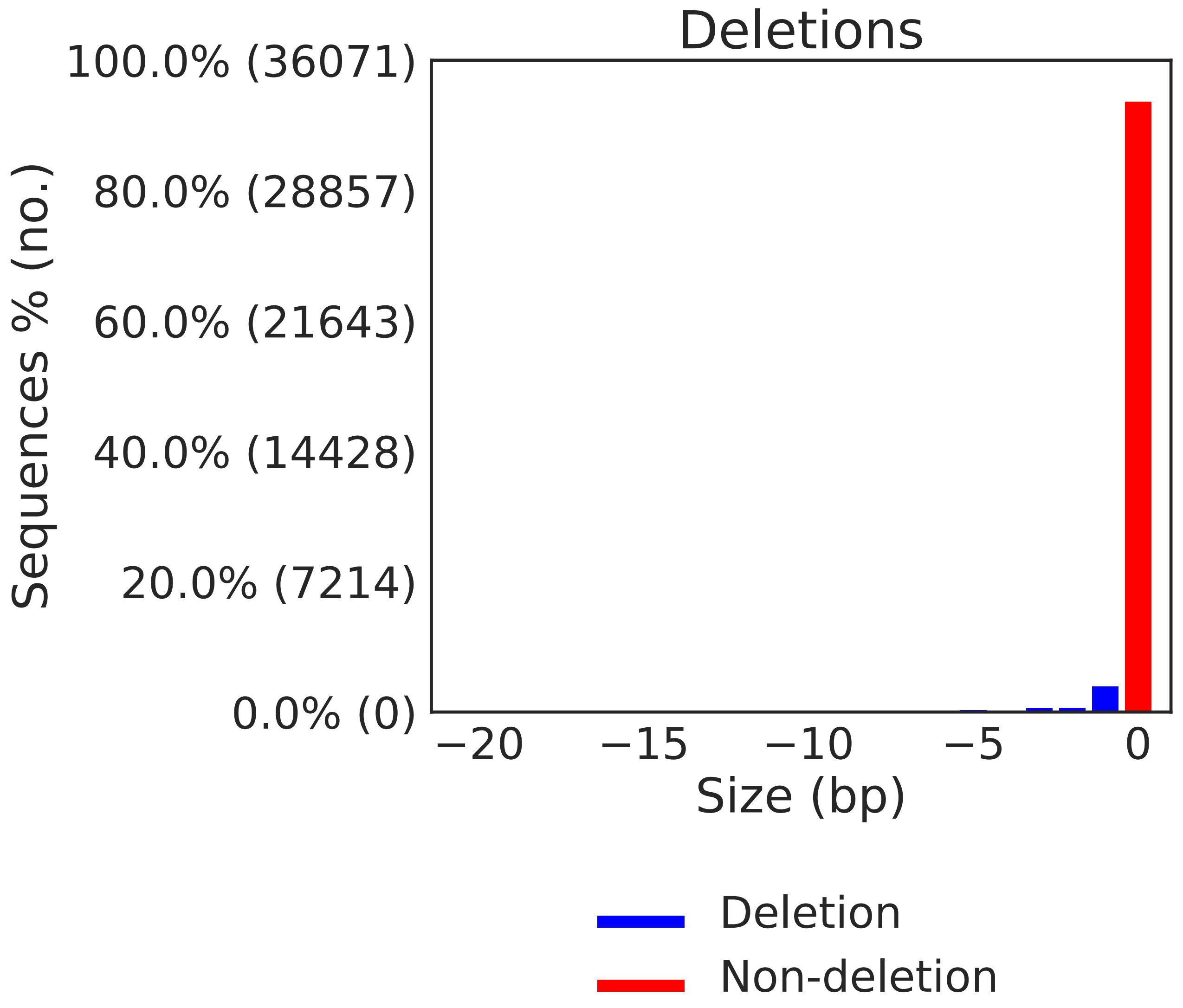
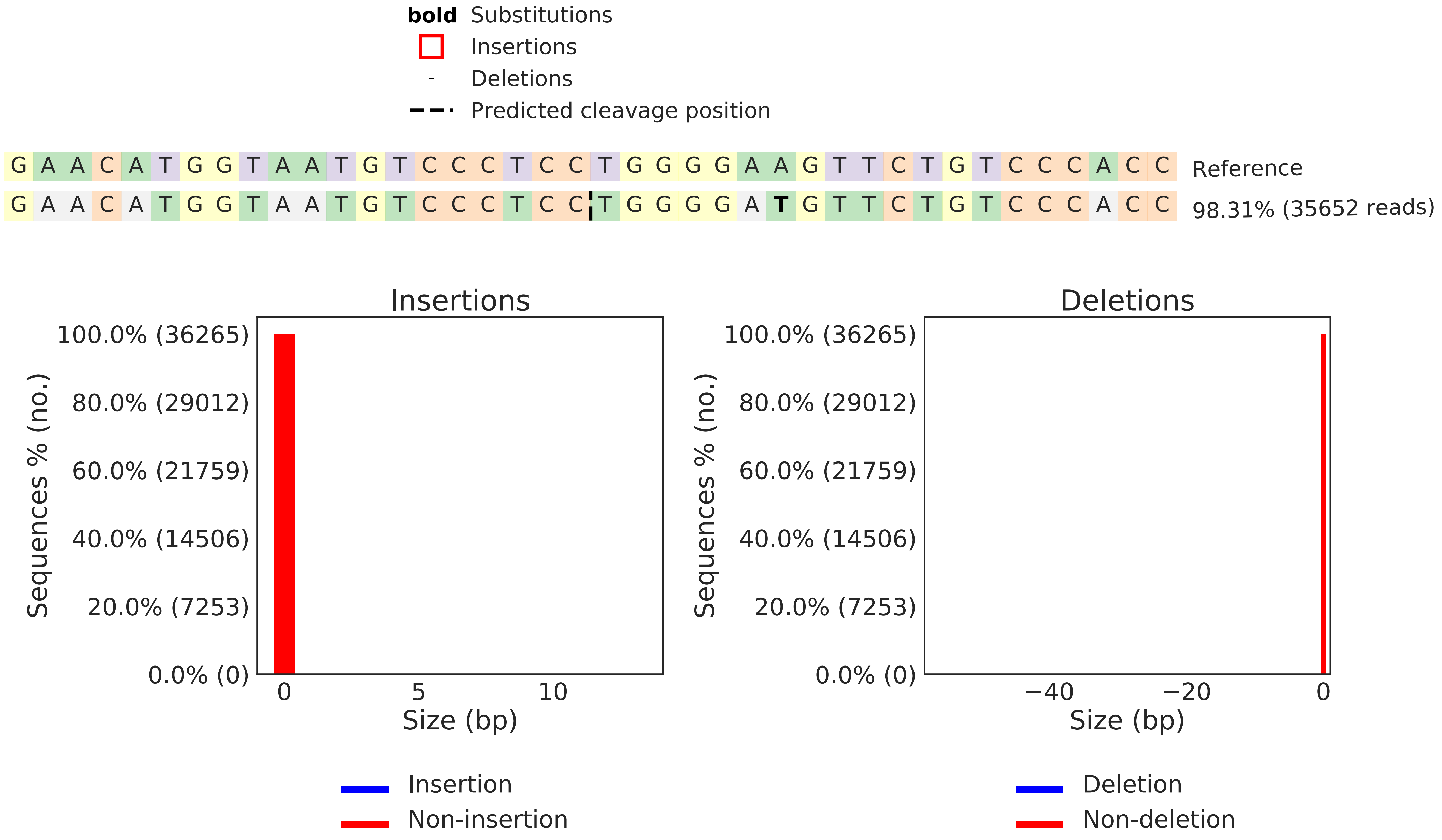
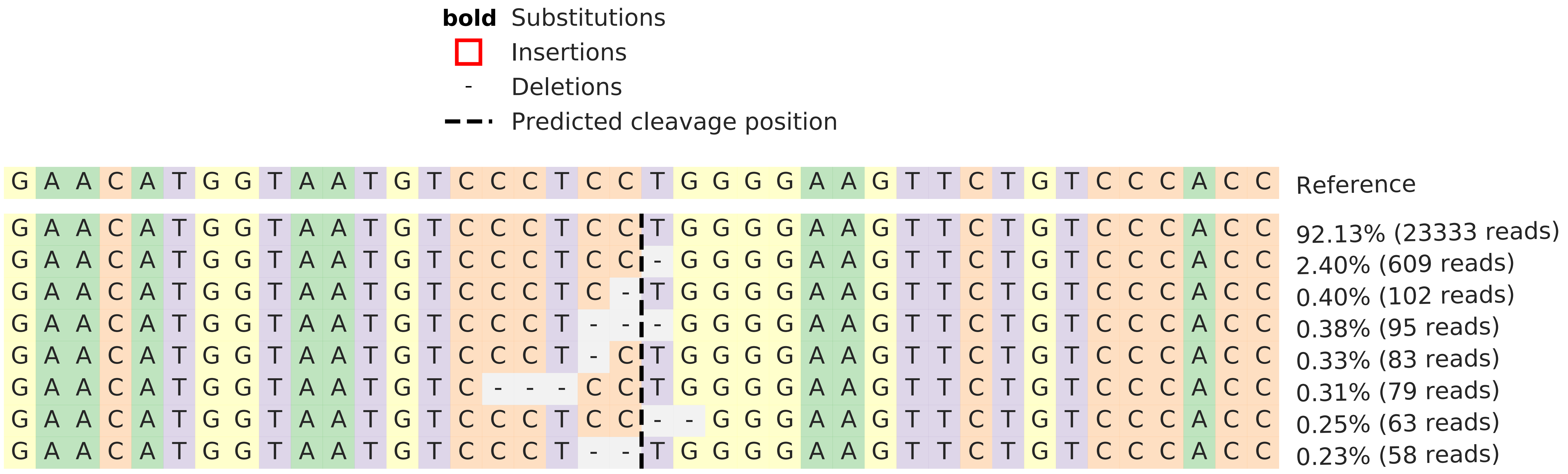




Tmc$^{Bth/WT}$, SaCas9^{KKH} + gRNA 4.1, WT allele



Tmc$^{Bth/WT}$, SpCas9^{KKH} + gRNA 4.2, Bth allele



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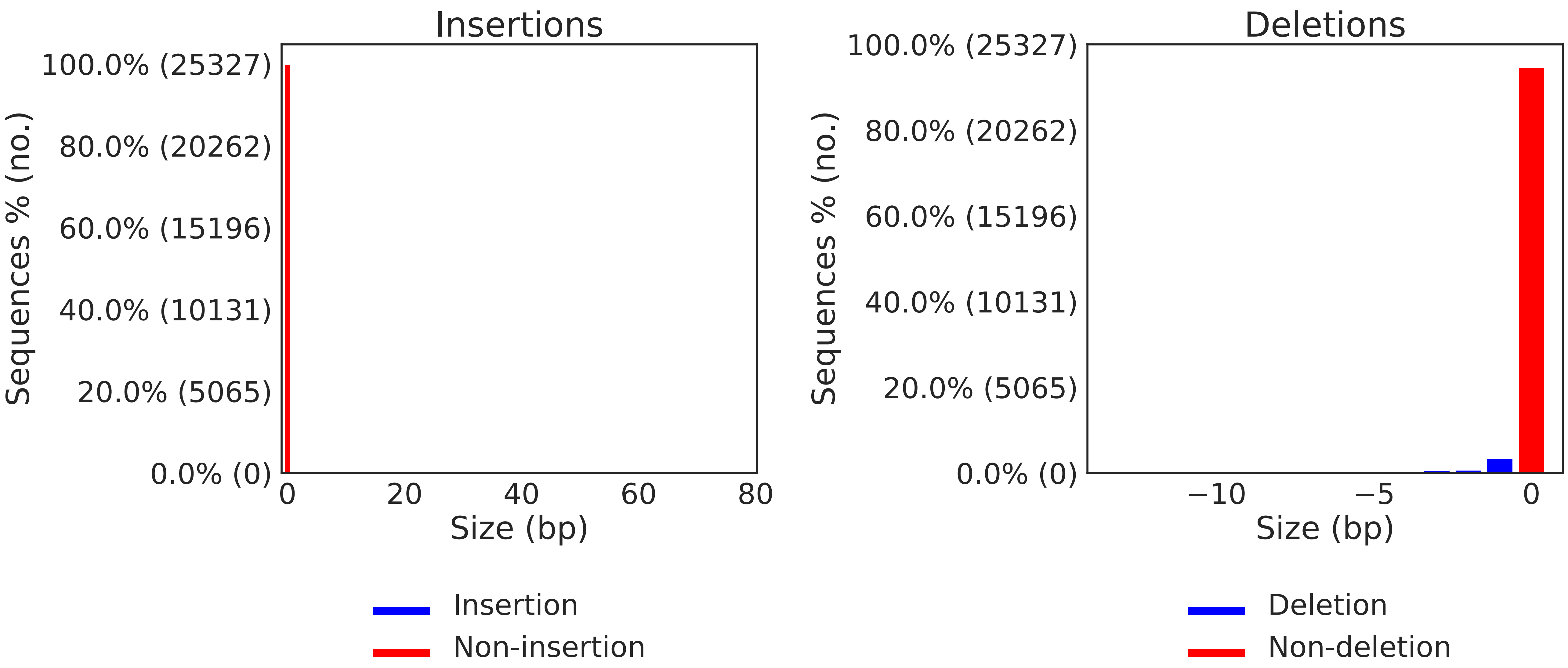
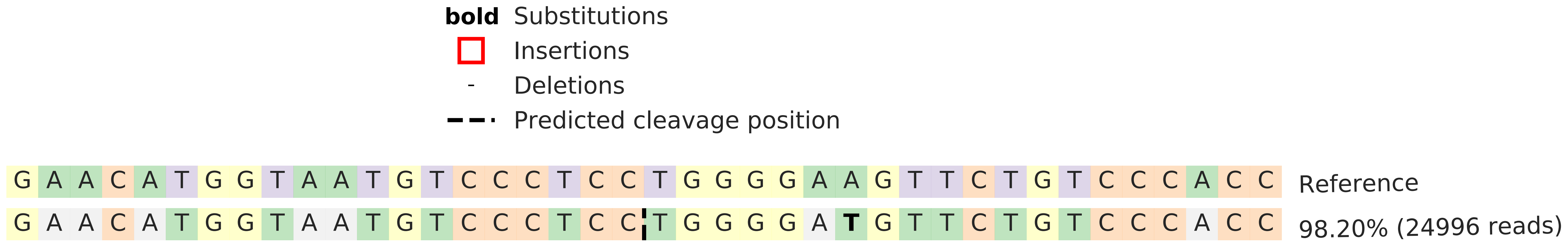
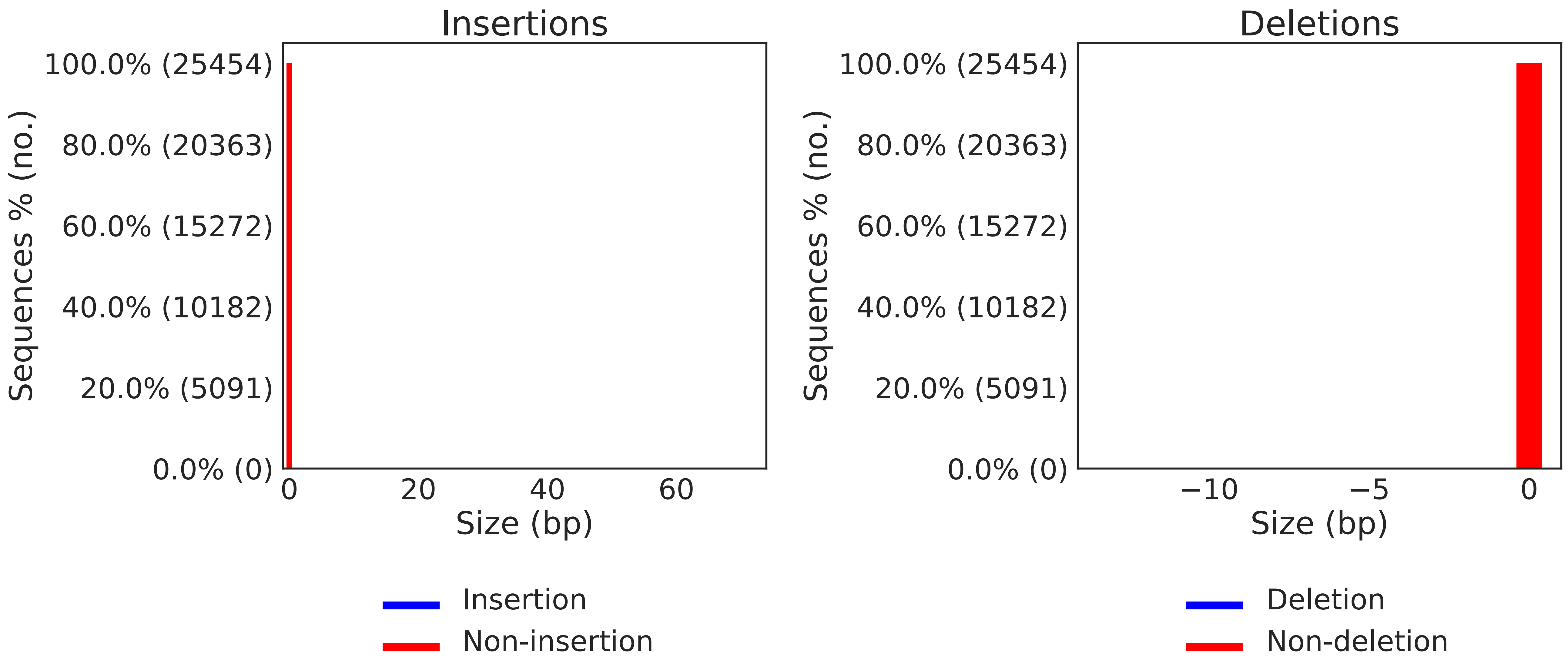
G A A C A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C 92.13% (23333 reads)
G A A C A T G G T A A T G T C C C T C C - G G G G A A G T T C T G T C C C A C C 2.40% (609 reads)
G A A C A T G G T A A T G T C C C T C - T G G G G A A G T T C T G T C C C A C C 0.40% (102 reads)
G A A C A T G G T A A T G T C C C T - - - G G G G A A G T T C T G T C C C A C C 0.38% (95 reads)
G A A C A T G G T A A T G T C C C T - C T G G G G A A G T T C T G T C C C A C C 0.33% (83 reads)
G A A C A T G G T A A T G T C - - - C C T G G G G A A G T T C T G T C C C A C C 0.31% (79 reads)
G A A C A T G G T A A T G T C C C T C C - - G G G A A G T T C T G T C C C A C C 0.25% (63 reads)
G A A C A T G G T A A T G T C C C T - - T G G G G A A G T T C T G T C C C A C C 0.23% (58 reads)

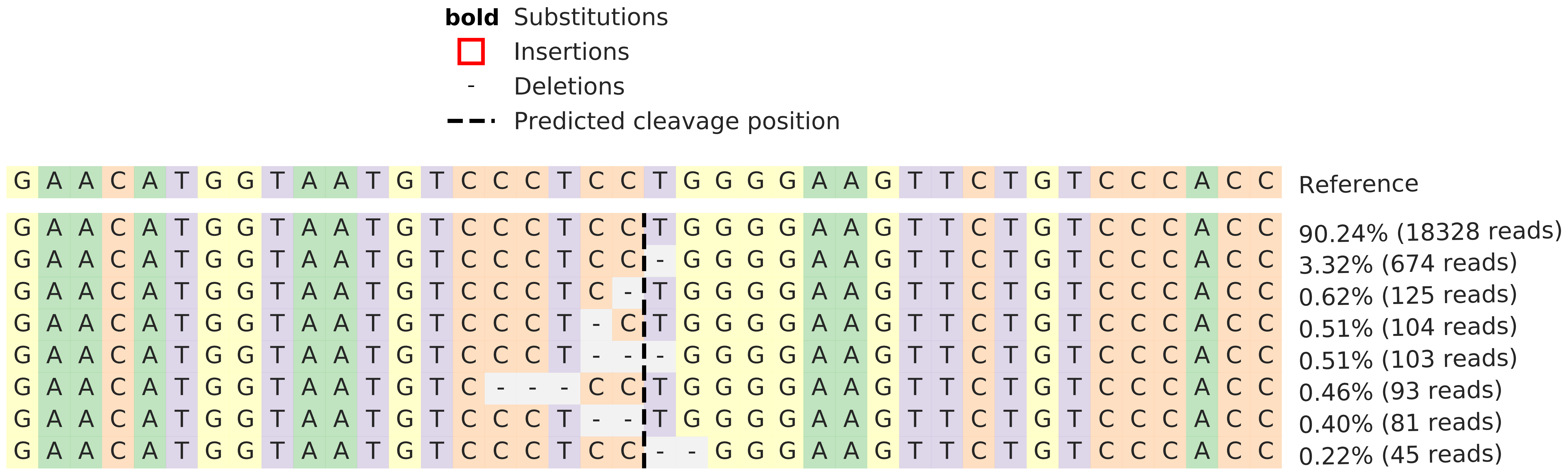


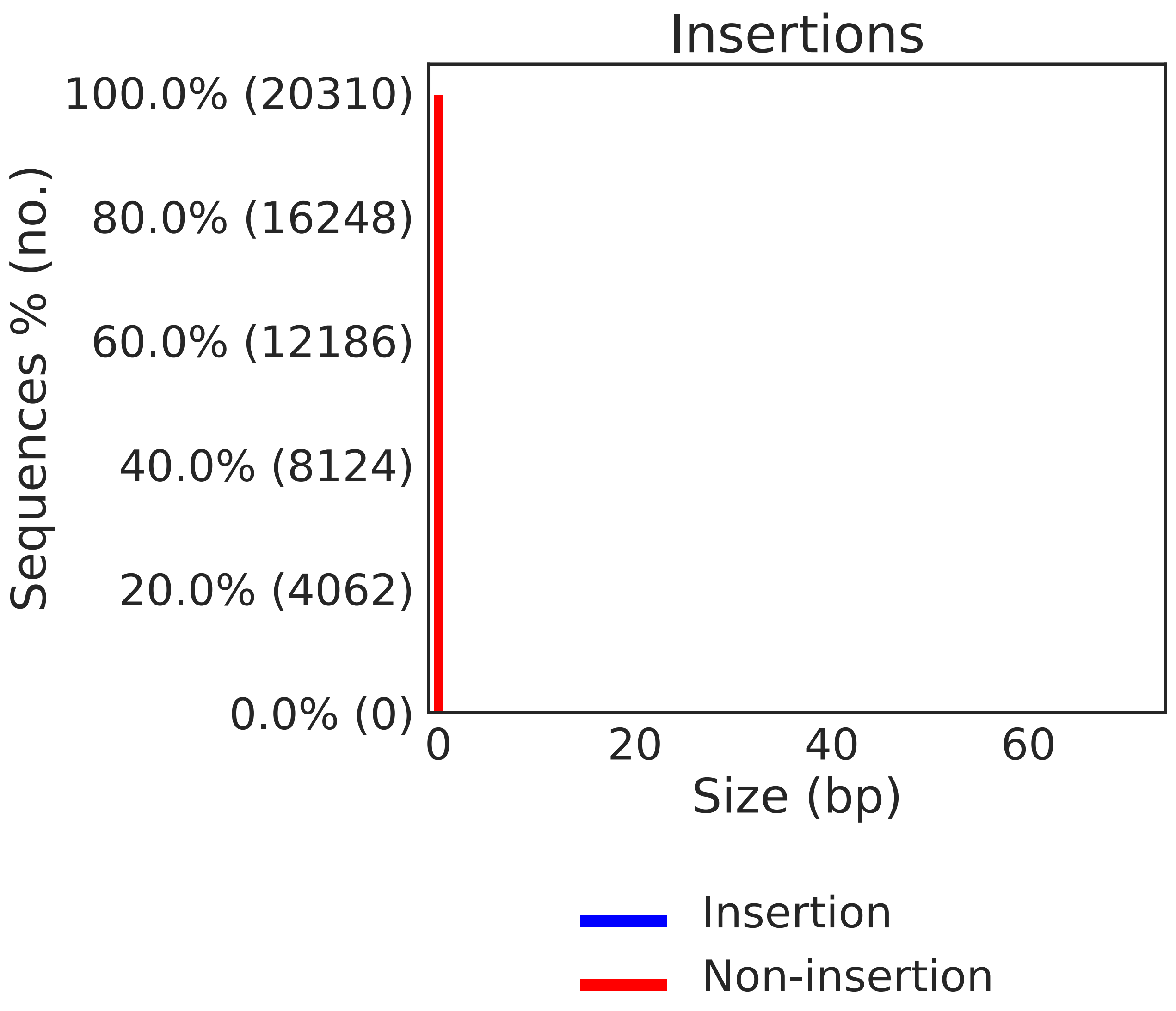
Tmc$^{Bth/WT}$, SaCas9^{KKH} + gRNA 4.2, WT allele

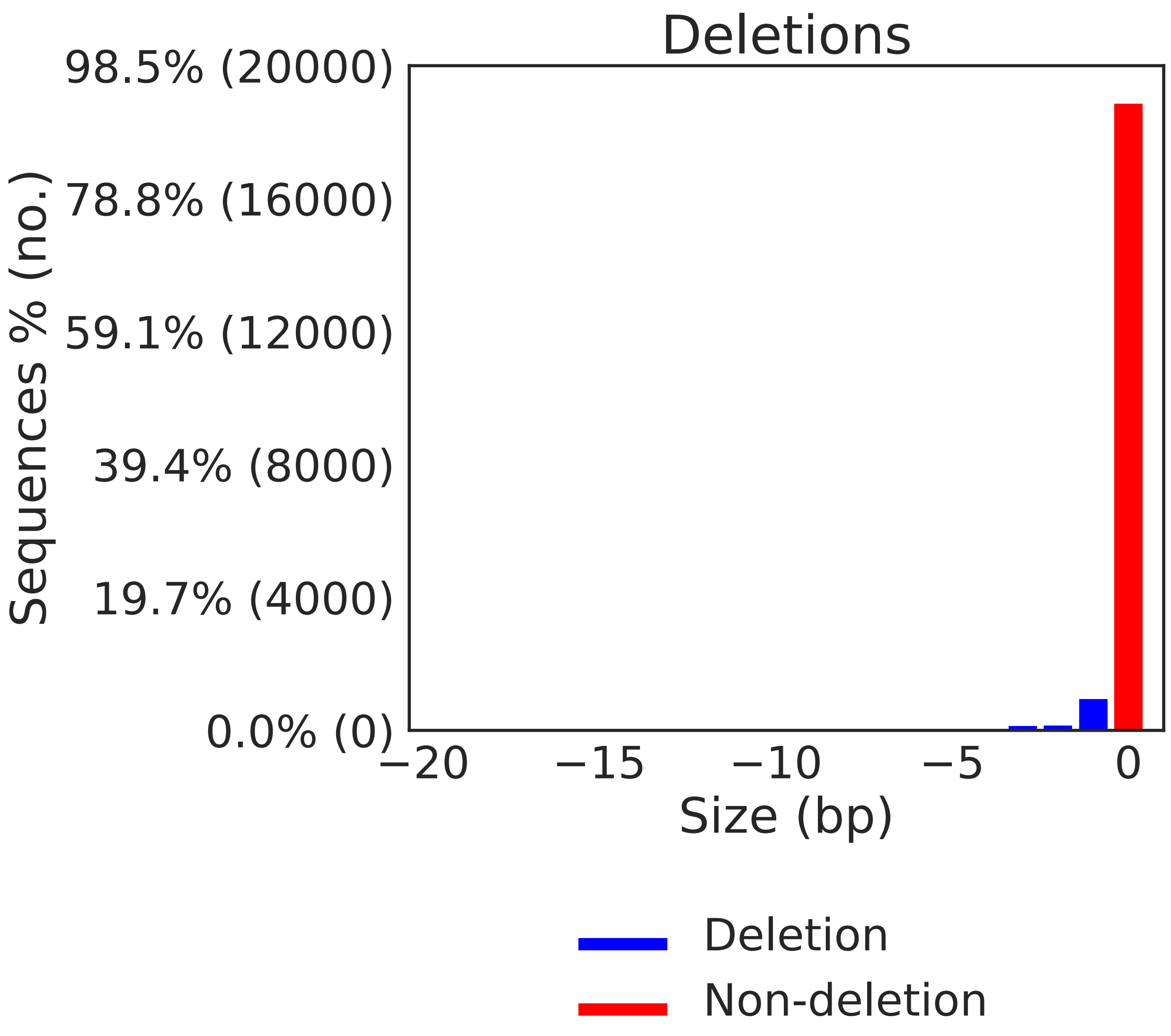




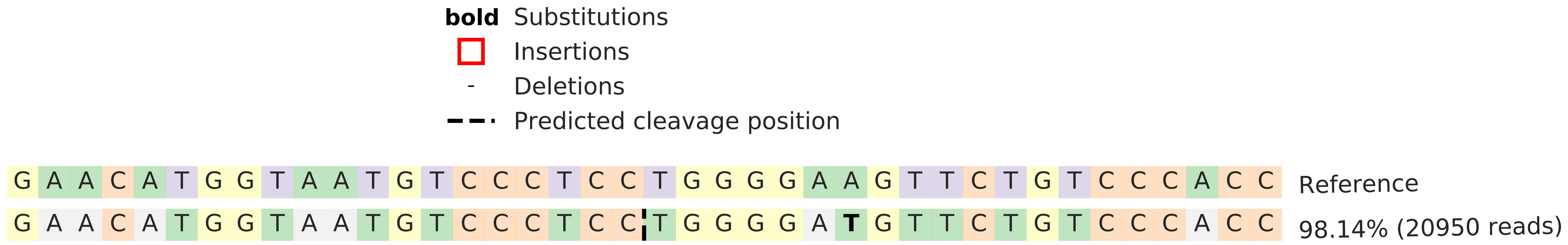
Tmc$^{Bth/WT}$, SaCas9^{KKH} + gRNA 4.3, Bth allele

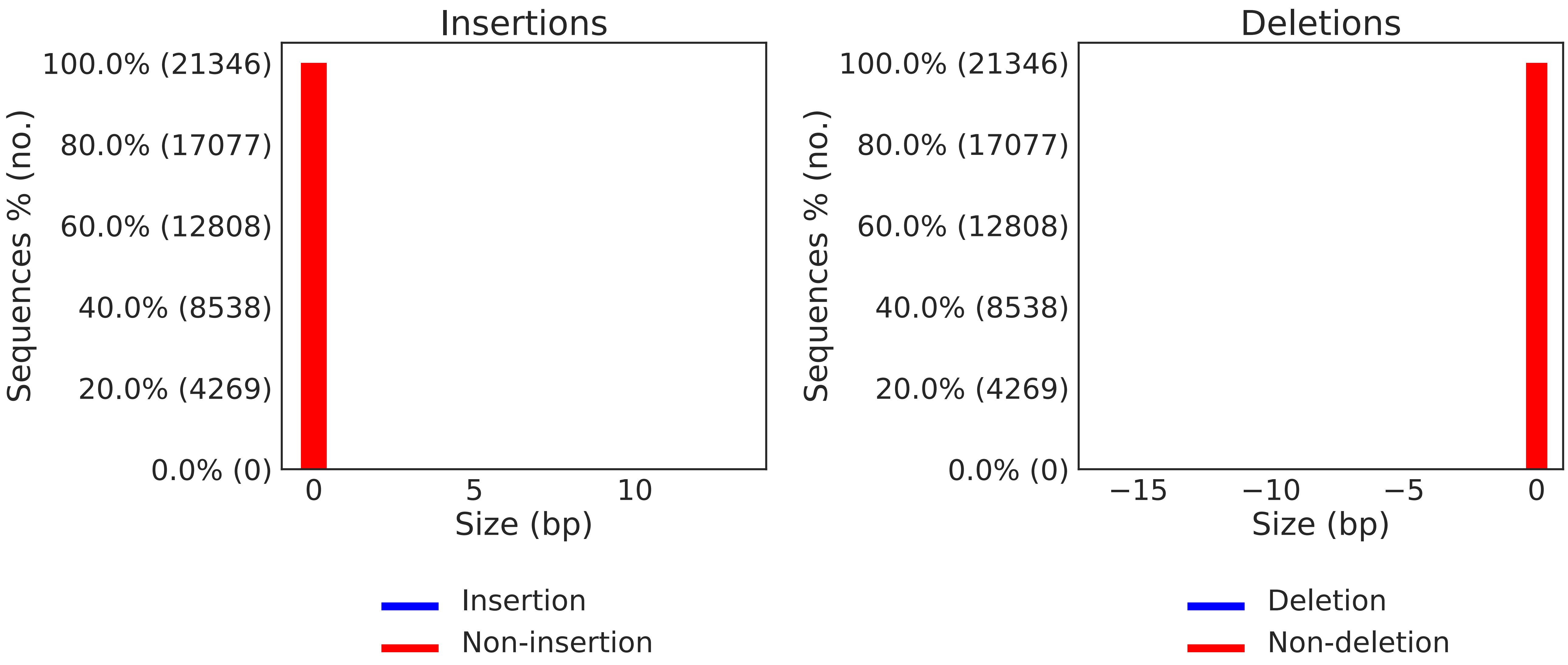




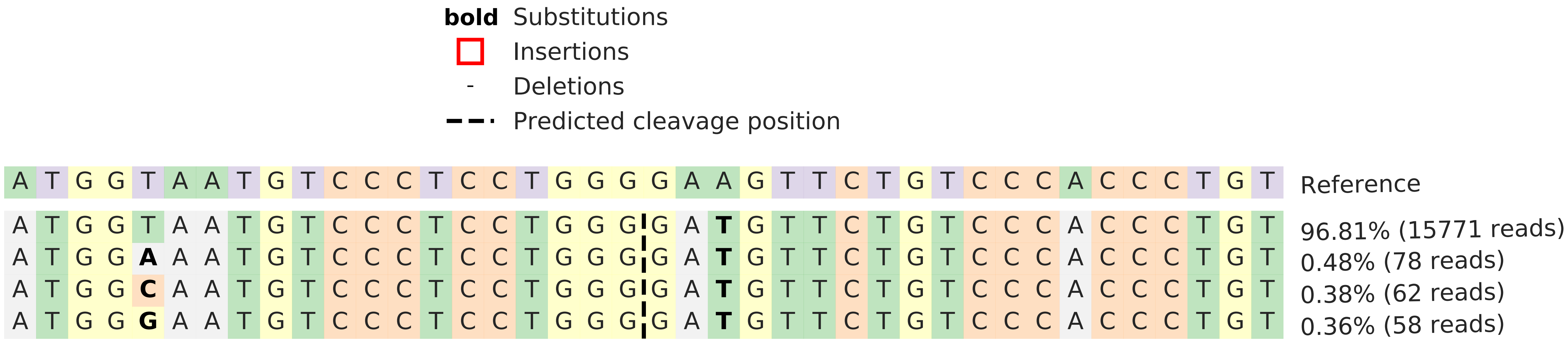


Tmc$^{Bth/WT}$, SaCas9^{KKH} + gRNA 4.3, WT allele





Tmc$^{WT/WT}$, SpCas9 only



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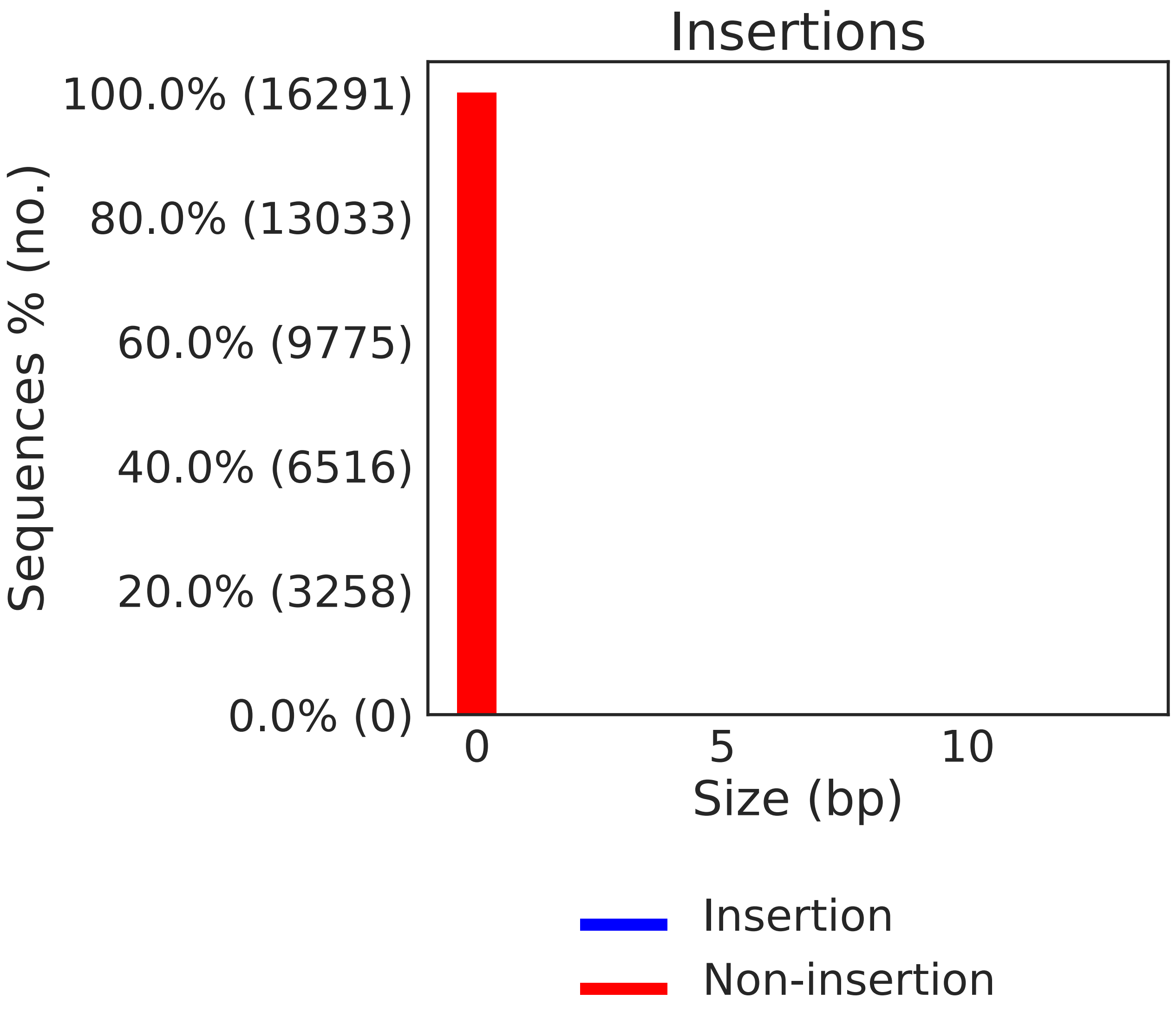
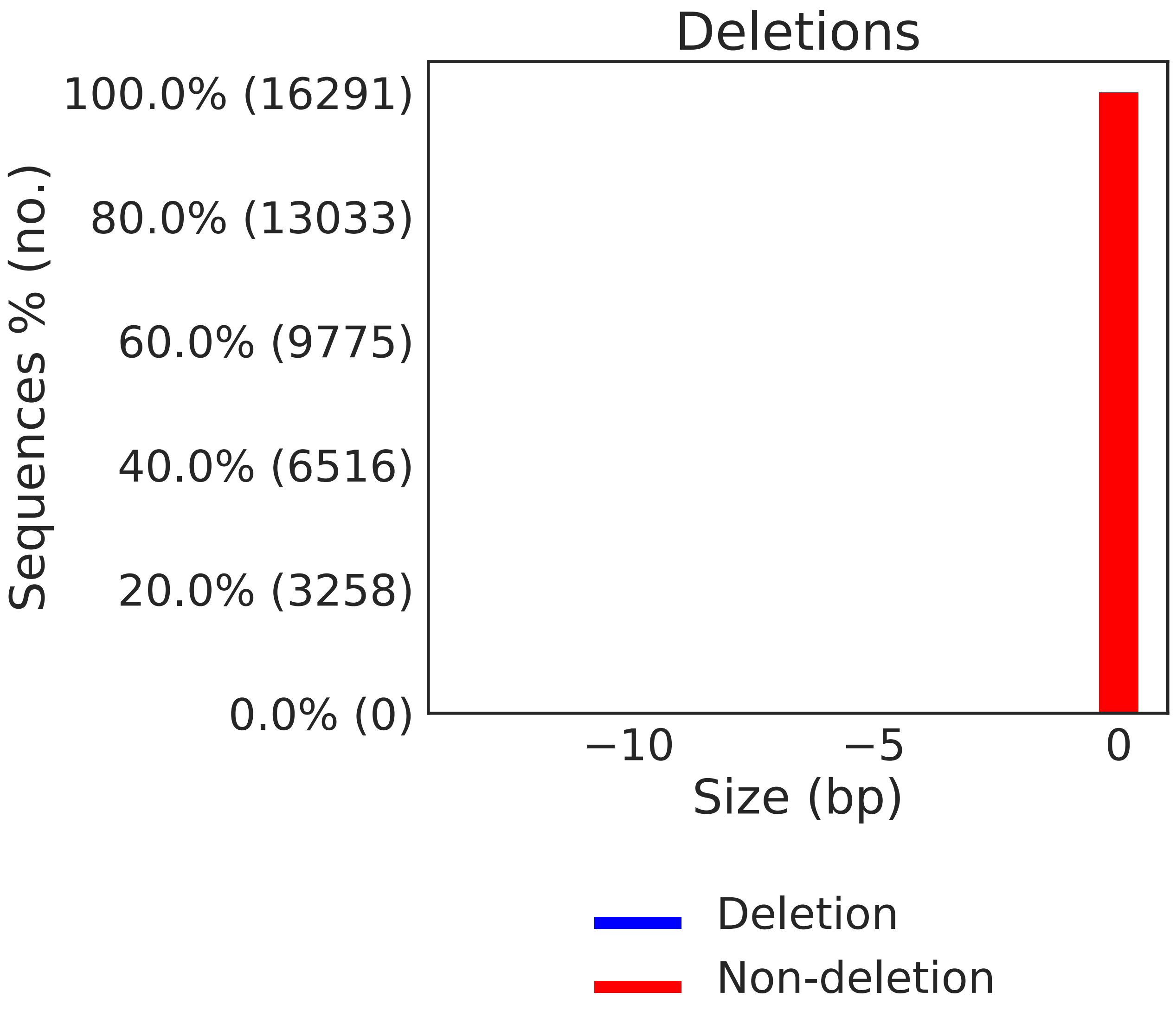
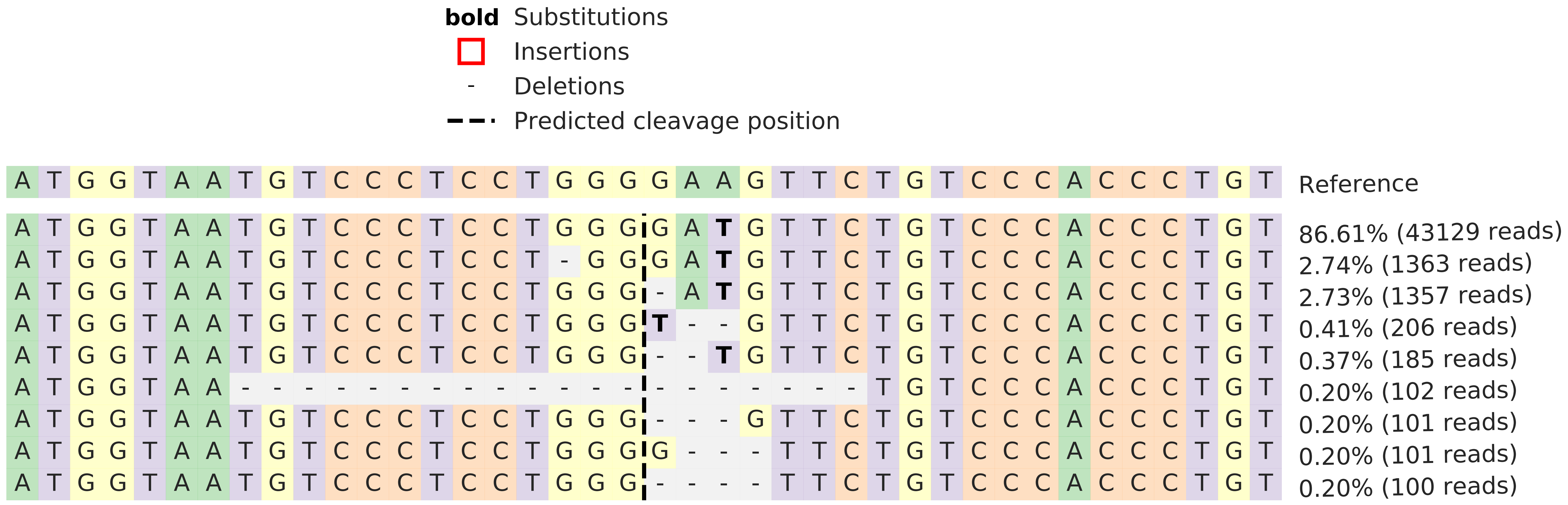
A T G G T A A T G T C C C T C C T G G G | G A **T** G T T C T G T C C C A C C C T G T	96.81% (15771 reads)
A T G G **A** A A T G T C C C T C C T G G G | G A **T** G T T C T G T C C C A C C C T G T	0.48% (78 reads)
A T G G **C** A A T G T C C C T C C T G G G | G A **T** G T T C T G T C C C A C C C T G T	0.38% (62 reads)
A T G G **G** A A T G T C C C T C C T G G G | G A **T** G T T C T G T C C C A C C C T G T	0.36% (58 reads)





Tmc$^{WT/WT}$, SpCas9 + gRNA 1.1



A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T	Reference
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A T G G T A A T G T C C C T C C T G G G - A **T** G T T C T G T C C C A C C C T G T	2.73% (1357 reads)
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A T G G T A A T G T C C C T C C T G G G - - **T** G T T C T G T C C C A C C C T G T	0.37% (185 reads)
A T G G T A A - T G T C C C A C C C T G T	0.20% (102 reads)
A T G G T A A T G T C C C T C C T G G G - - - G T T C T G T C C C A C C C T G T	0.20% (101 reads)
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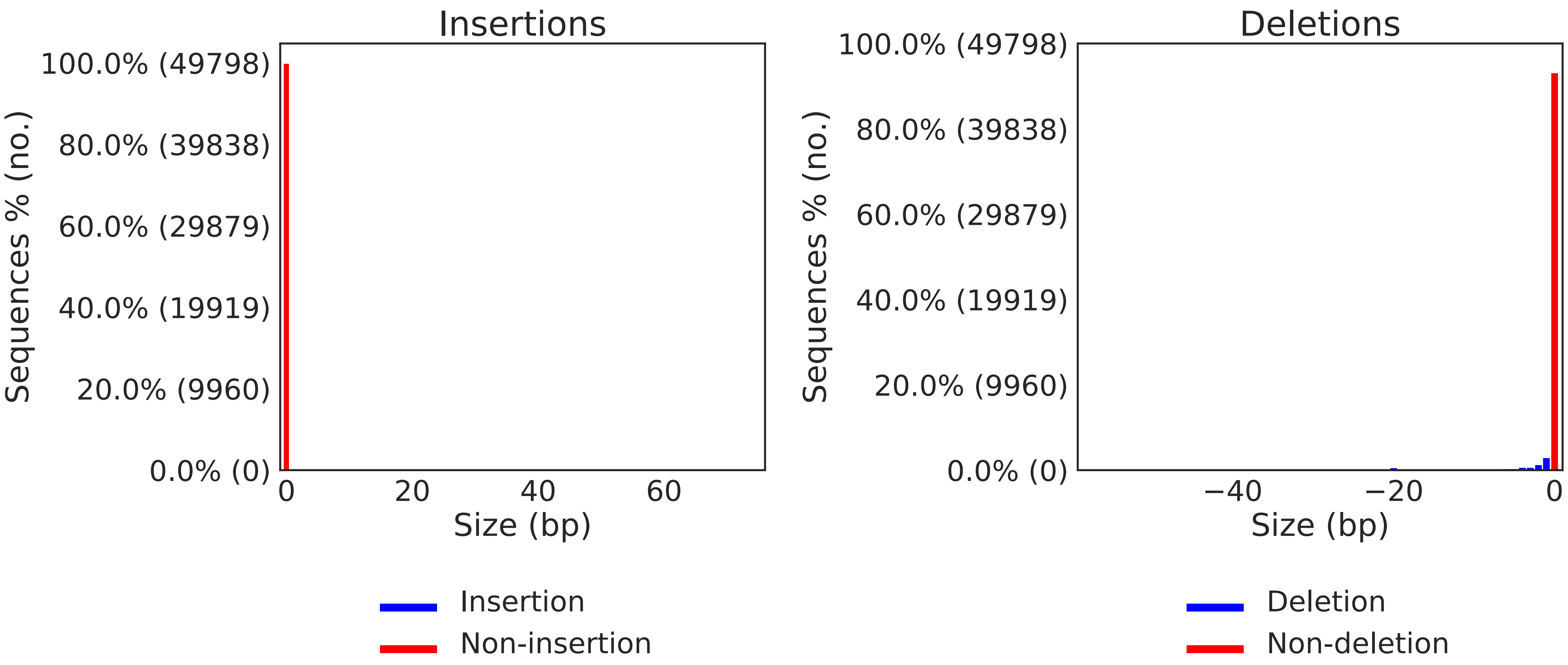
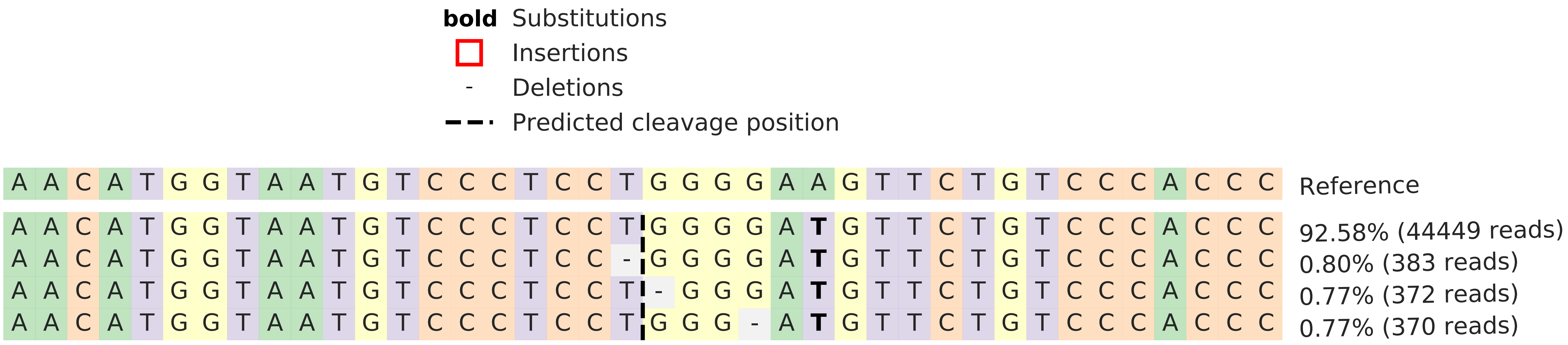


Tmc$^{WT/WT}$, SpCas9 + gRNA 2.1



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A A C A T G G T A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C	92.58% (44449 reads)
A A C A T G G T A A T G T C C C T C C - G G G G A **T** G T T C T G T C C C A C C C	0.80% (383 reads)
A A C A T G G T A A T G T C C C T C C T - G G G A **T** G T T C T G T C C C A C C C	0.77% (372 reads)
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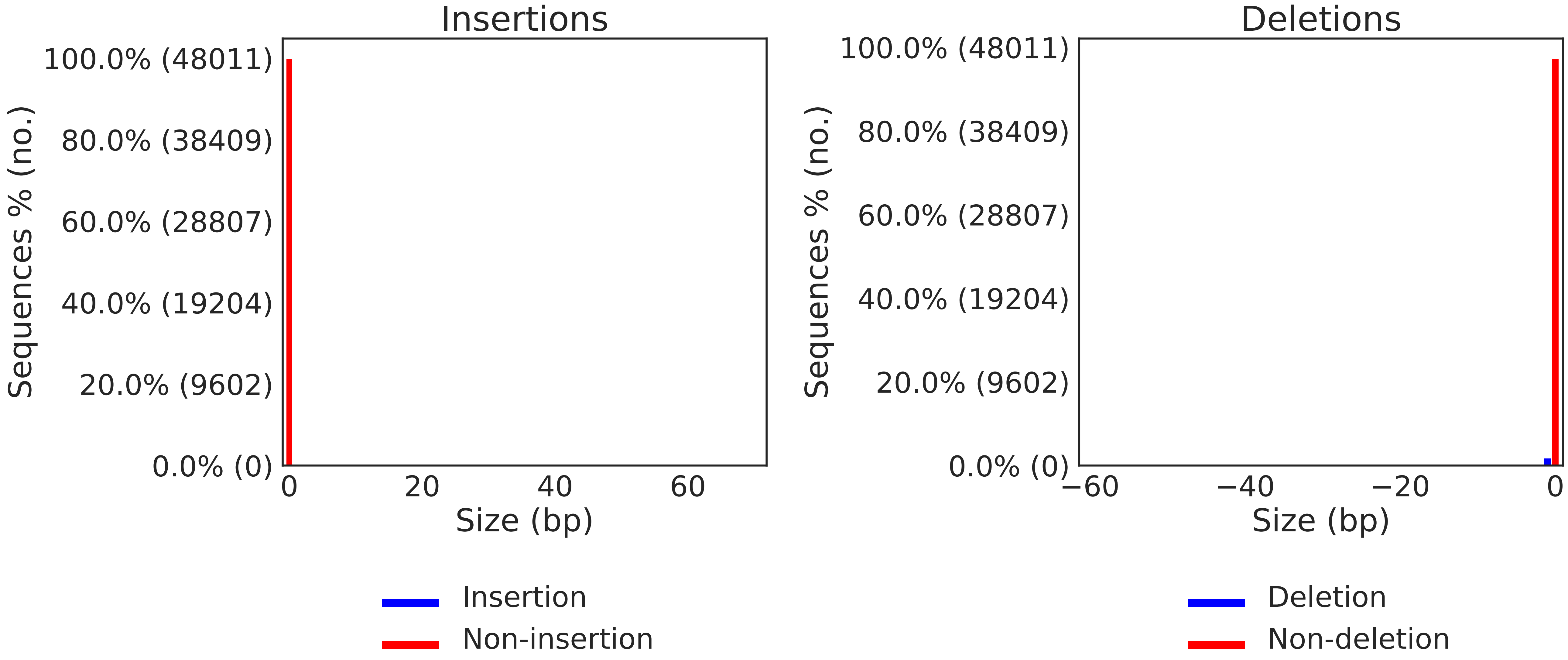
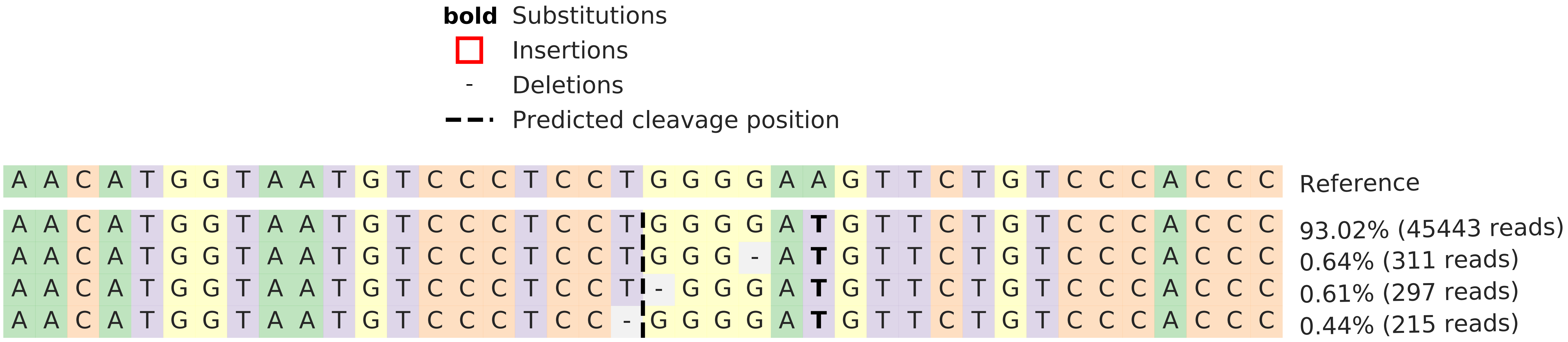
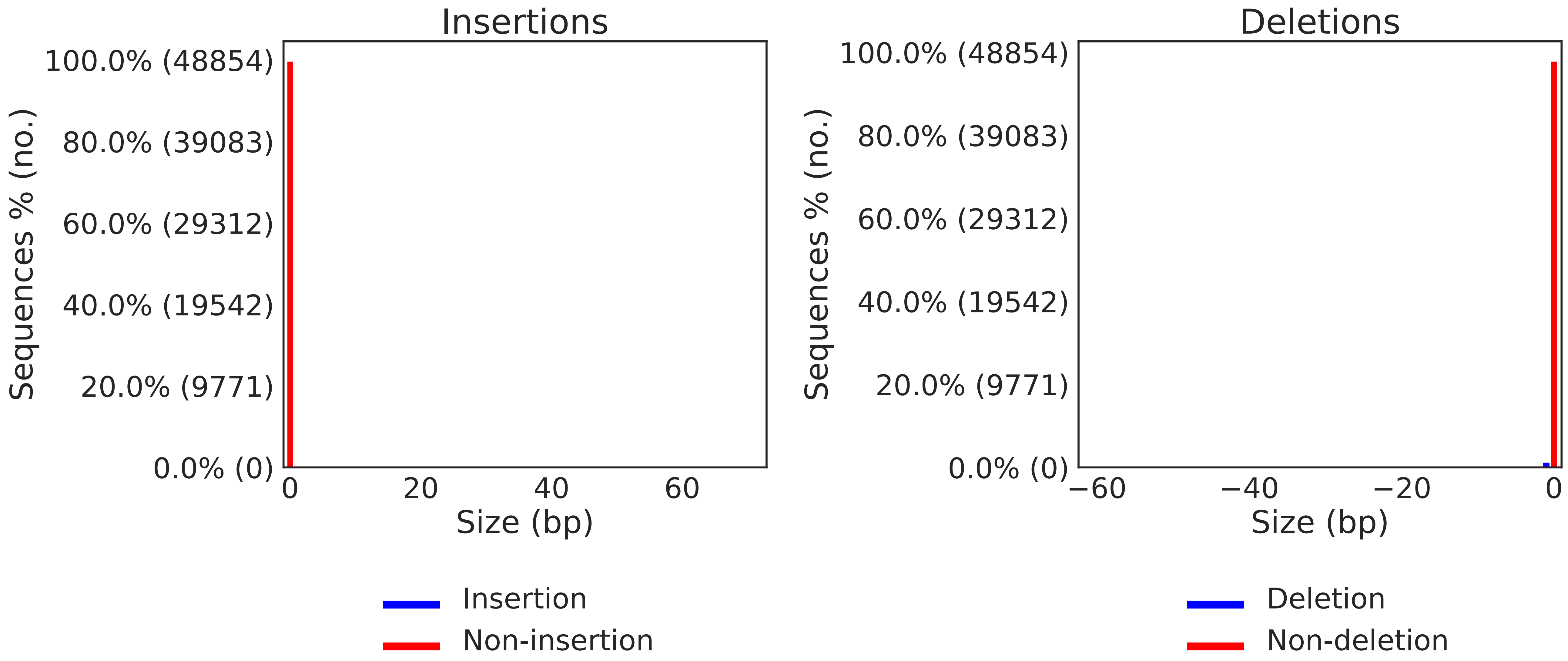


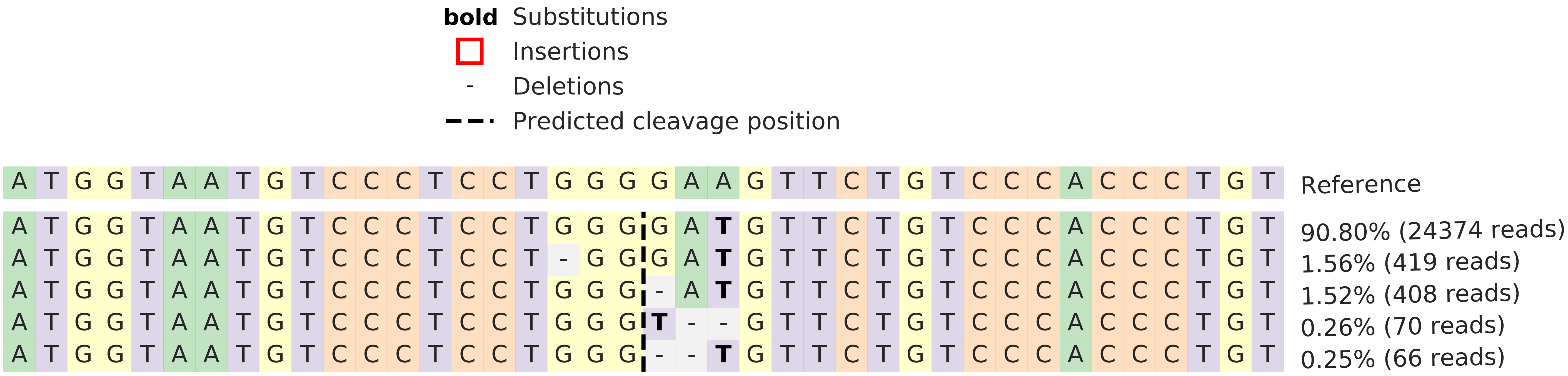
$Tmc^{WT/WT}$, SpCas9 + gRNA 2.4



AACATGGTAATGTCCCTCCTGGGGAAGTTCTGTCCCACCC	Reference
AACATGGTAATGTCCCTCCTGGGGA**T**GTTCTGTCCCACCC	93.02% (45443 reads)
AACATGGTAATGTCCCTCCTGGG-A**T**GTTCTGTCCCACCC	0.64% (311 reads)
AACATGGTAATGTCCCTCCT-GGGA**T**GTTCTGTCCCACCC	0.61% (297 reads)
AACATGGTAATGTCCCTCC-GGGGA**T**GTTCTGTCCCACCC	0.44% (215 reads)



Tmc$^{WT/WT}$, eSpCas9 + gRNA 1.1


bold Substitutions
☐ Insertions
\- Deletions
\- - - Predicted cleavage position

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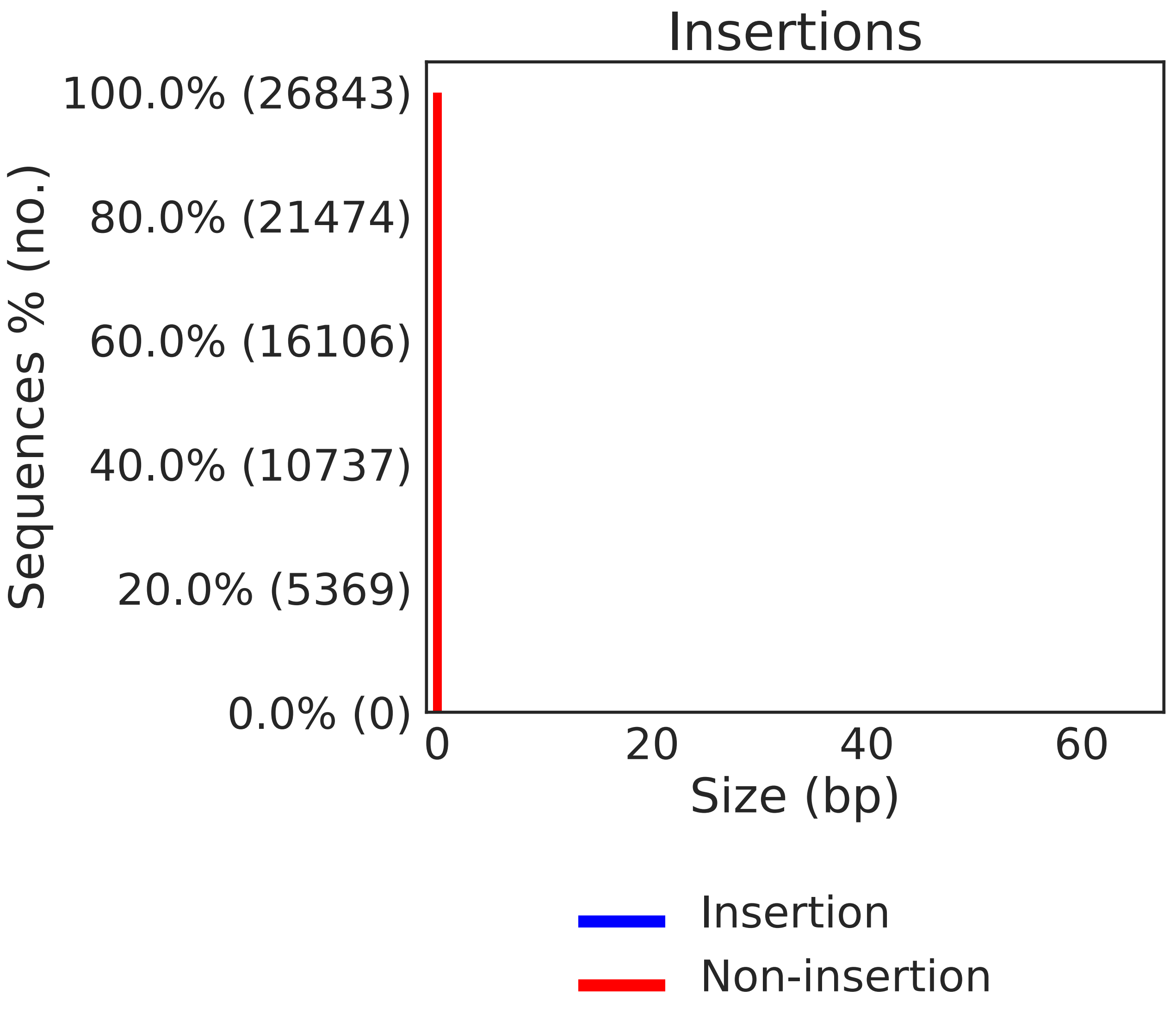
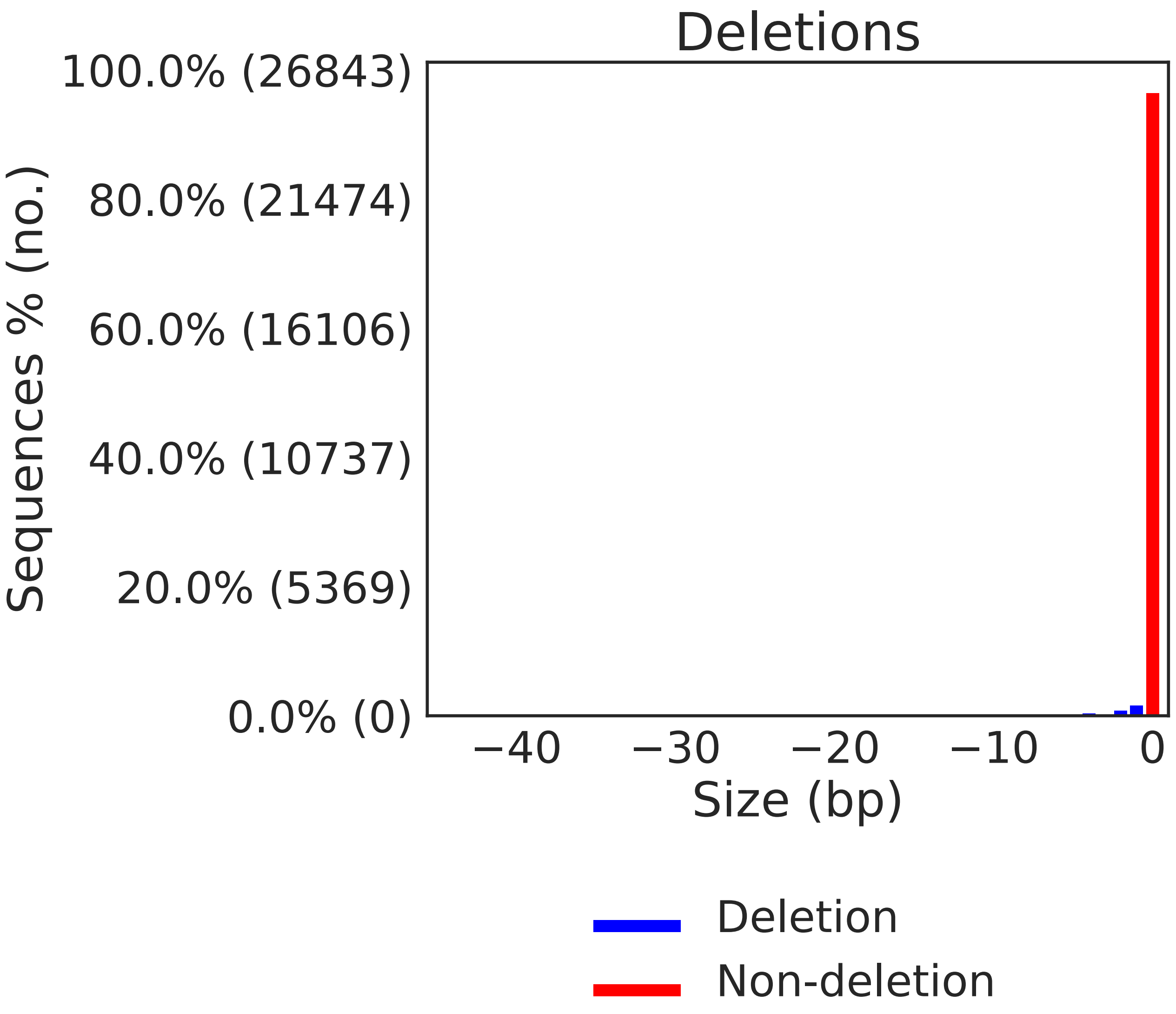
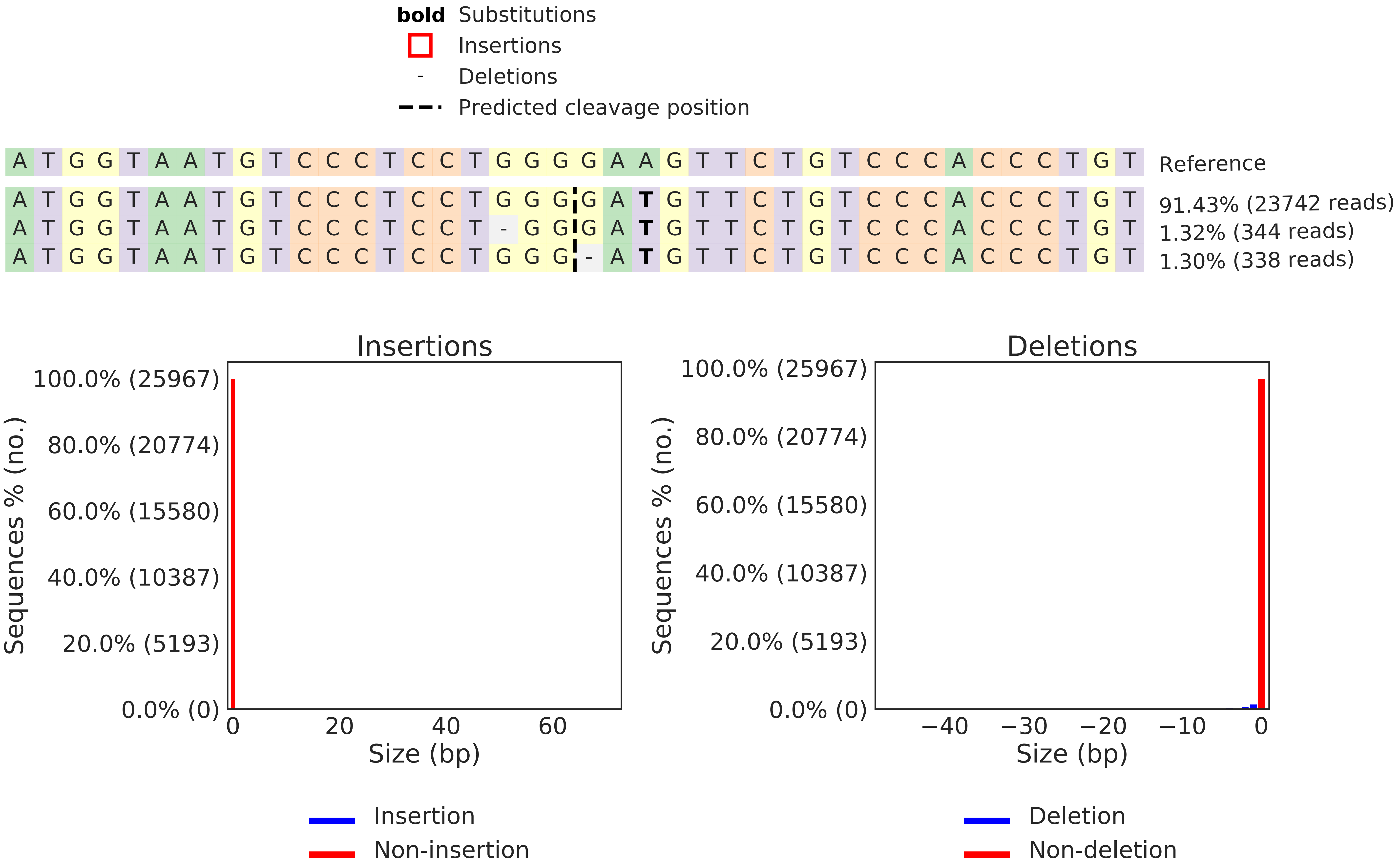
A T G G T A A T G T C C C T C C T G G G ¦ G A **T** G T T C T G T C C C A C C C T G T 90.80% (24374 reads)
A T G G T A A T G T C C C T C C T - G G ¦ G A **T** G T T C T G T C C C A C C C T G T 1.56% (419 reads)
A T G G T A A T G T C C C T C C T G G G ¦ - A **T** G T T C T G T C C C A C C C T G T 1.52% (408 reads)
A T G G T A A T G T C C C T C C T G G G ¦ **T** - - G T T C T G T C C C A C C C T G T 0.26% (70 reads)
A T G G T A A T G T C C C T C C T G G G ¦ - - **T** G T T C T G T C C C A C C C T G T 0.25% (66 reads)





$Tmc^{WT/WT}$, HypaCas9 + gRNA 1.1



bold Substitutions

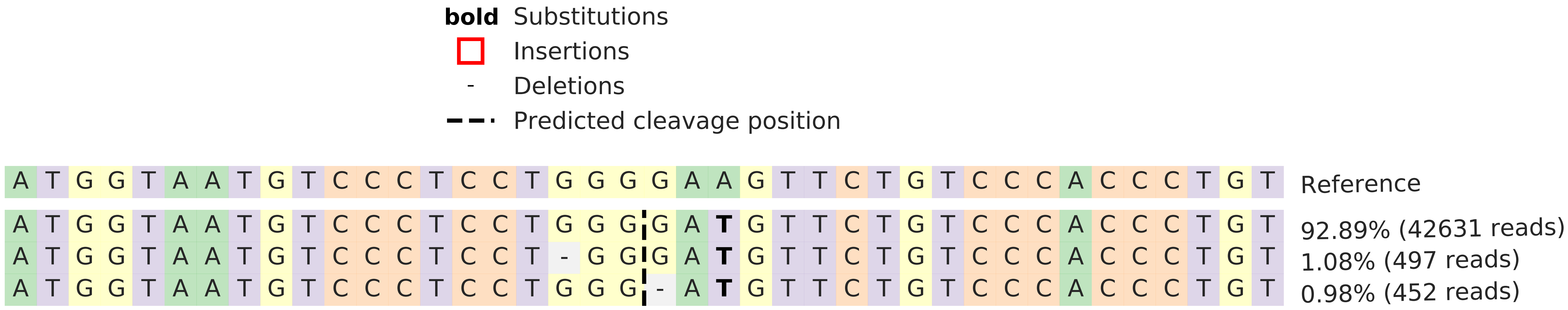
☐ Insertions

\- Deletions

╍ Predicted cleavage position

Sequence	
A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T	Reference
A T G G T A A T G T C C C T C C T G G G ¦ G A **T** G T T C T G T C C C A C C C T G T	91.43% (23742 reads)
A T G G T A A T G T C C C T C C T - G G ¦ G A **T** G T T C T G T C C C A C C C T G T	1.32% (344 reads)
A T G G T A A T G T C C C T C C T G G G ¦ - A **T** G T T C T G T C C C A C C C T G T	1.30% (338 reads)

Tmc$^{WT/WT}$, SpCas9-HF1 + gRNA 1.1



A T G G T A A T G T C C C T C C T G G G G A A G T T C T G T C C C A C C C T G T Reference

A T G G T A A T G T C C C T C C T G G G G A **T** G T T C T G T C C C A C C C T G T 92.89% (42631 reads)

A T G G T A A T G T C C C T C C T - G G G A **T** G T T C T G T C C C A C C C T G T 1.08% (497 reads)

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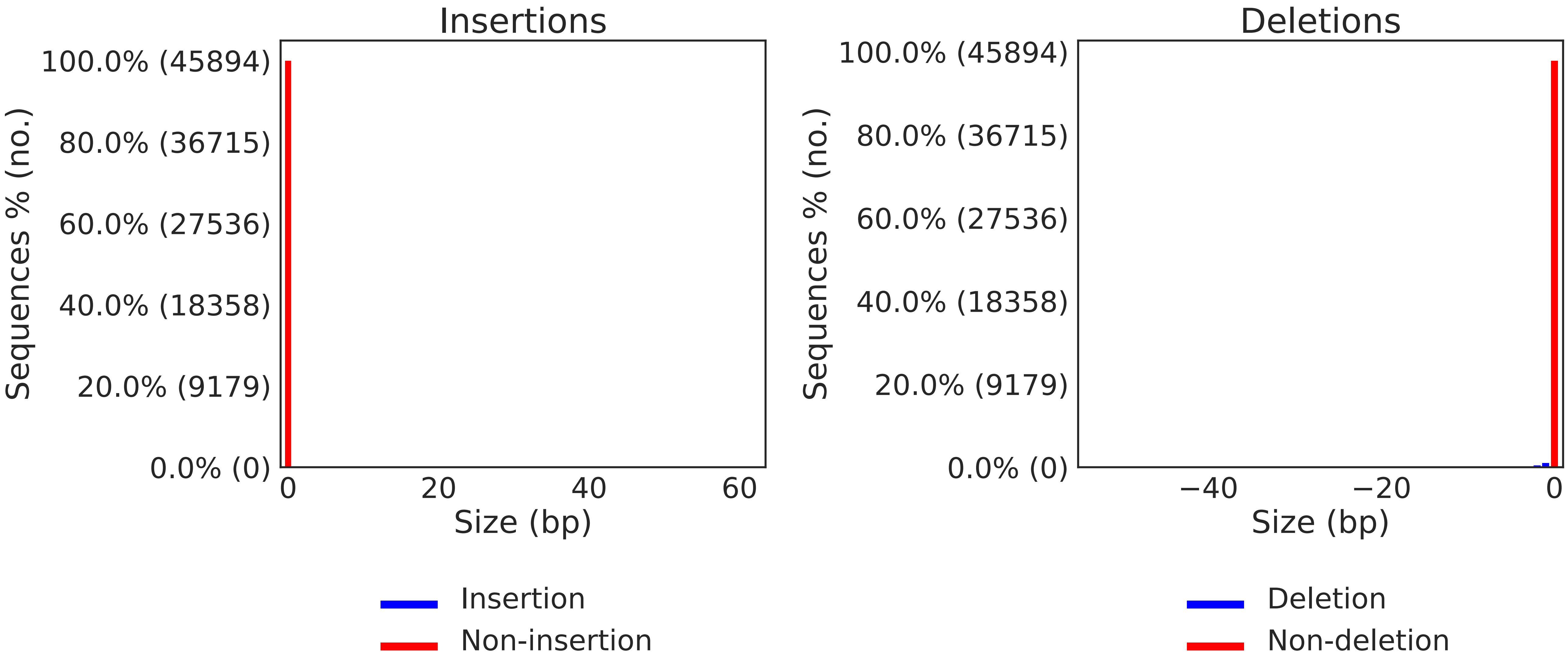
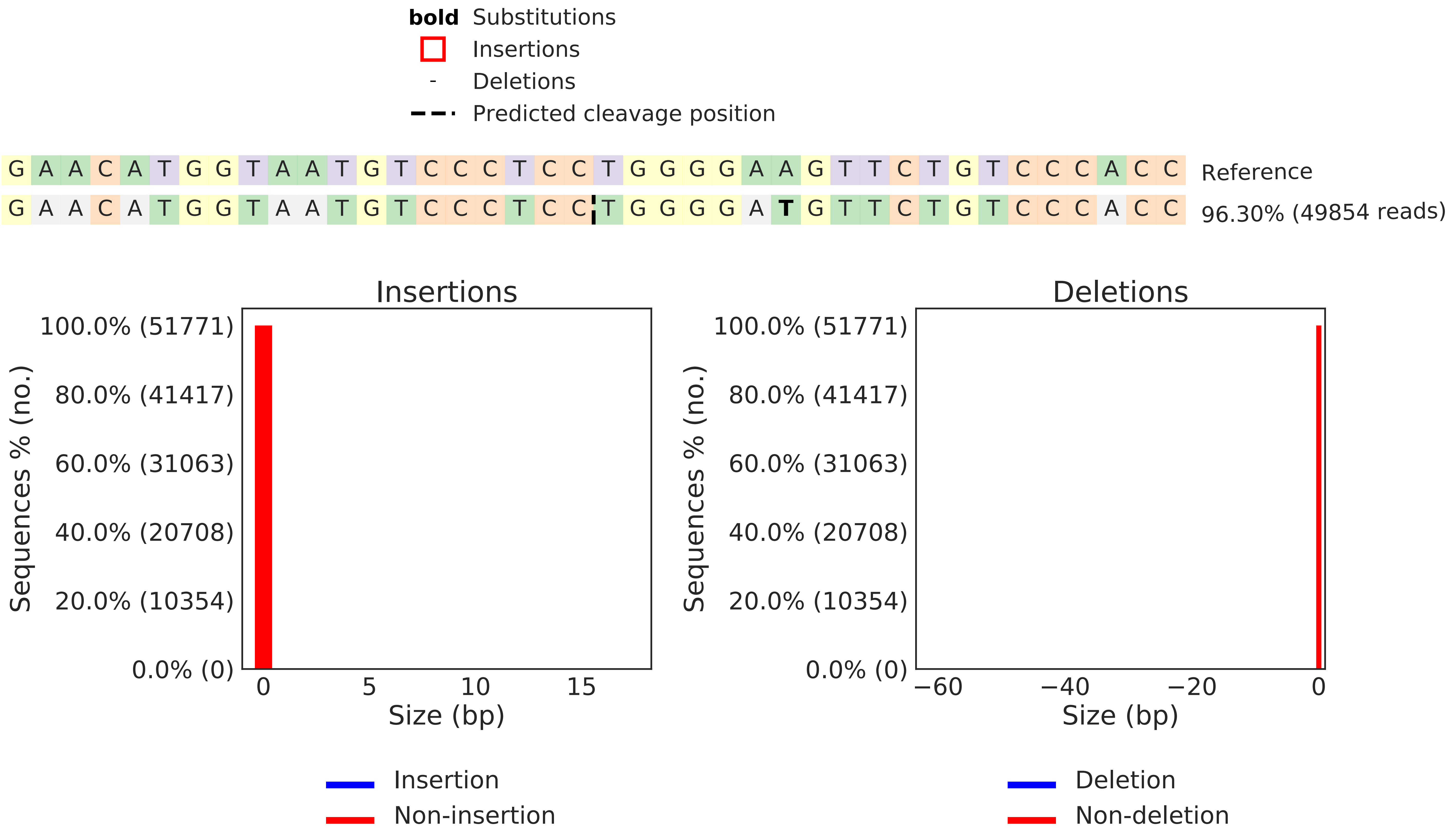
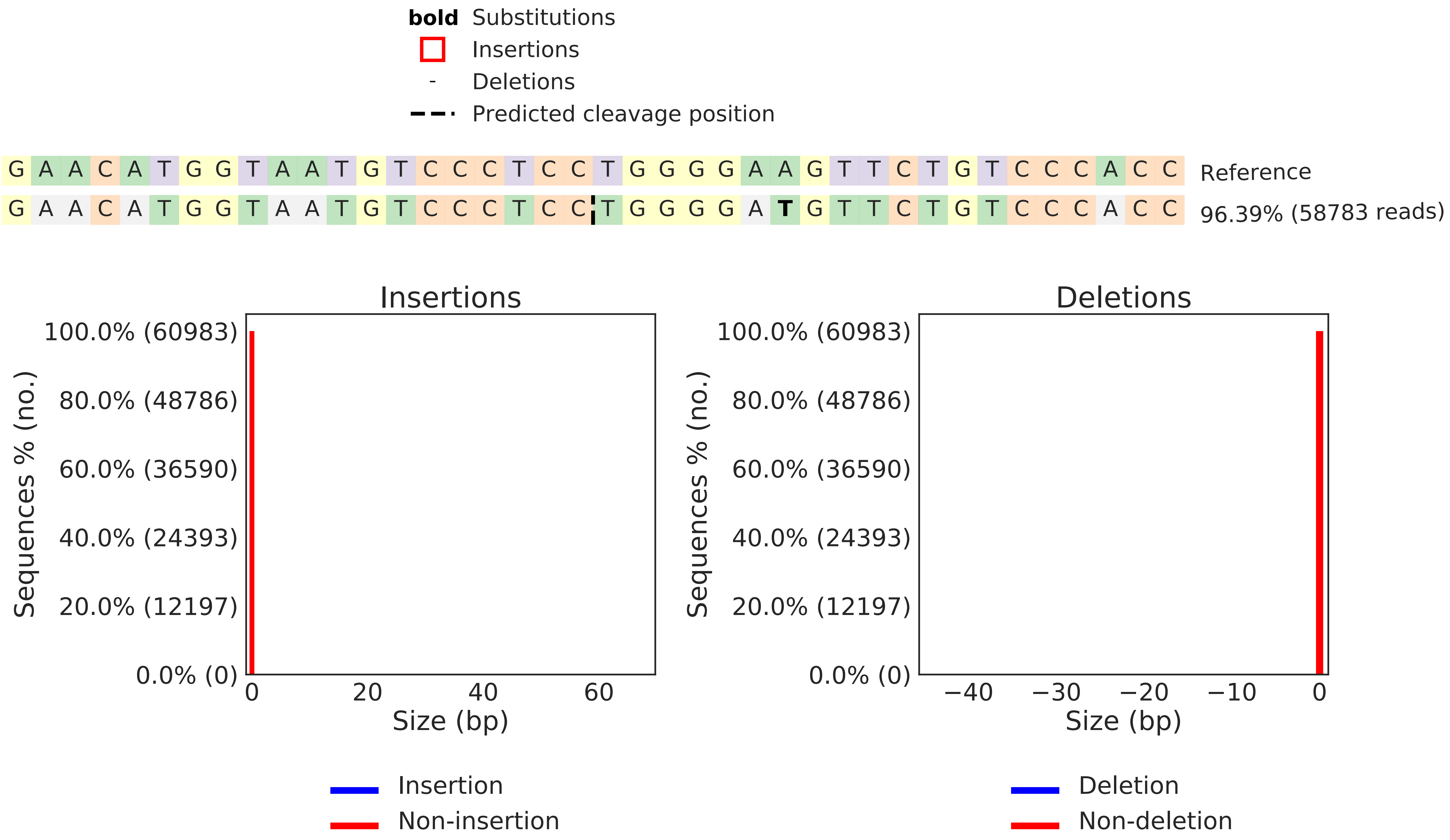


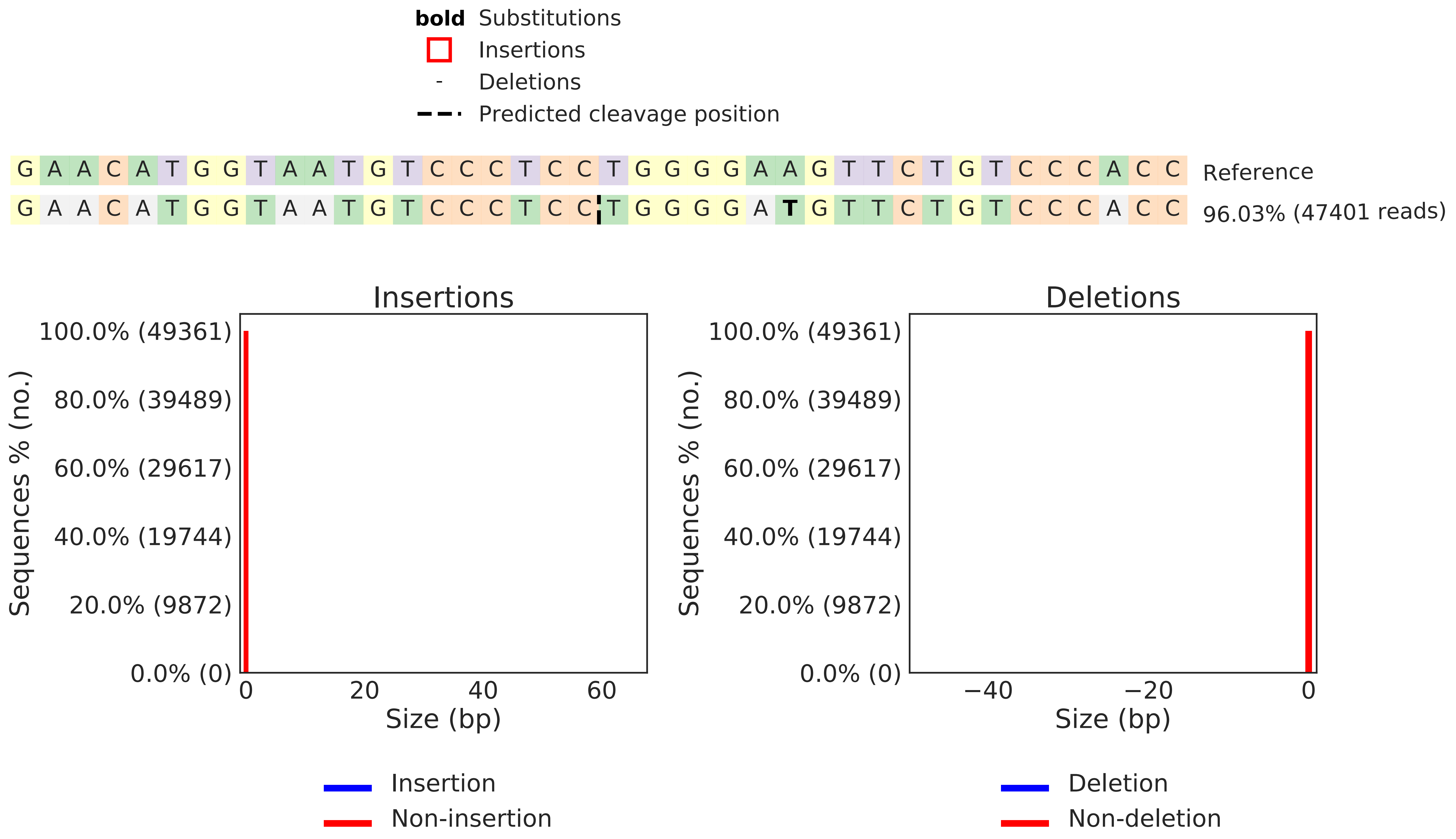
Tmc$^{WT/WT}$, SaCas9^{KKH} + gRNA 4.1



Tmc$^{WT/WT}$, SaCas9^{KKH} + gRNA 4.2



Tmc$^{WT/WT}$, SaCas9^{KKH} + gRNA 4.3



Supplementary Dataset 4. Sequencing result from pBG201 (pAAV-CMV-NLS(SV40)-SaCas9(E782K/N968K/R1015H)-NLS(nucleoplasmin)-3xHA-bGHpA0-U6-BsaI-sgRNA)

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