Supp.file S3: gRNA/Cas9-mediated mutations in BCTIV genome detected by PCR-RE assay and sanger sequencing. Red arrows indicate non-cut fragments due to the mutation in the RE cleavage site created by gRNA/Cas9 system in the virus genome. WT: fragments of virus obtained from only BCTIV agroinoculated sugar beets; MUT: viral bands formed by RE cutting of PCR fragments obtained from co-agroinoculated sugar beet with gRNA/Cas9 and BCTIV. Sequence of mutated viral genome was analysed with Tracking of Indels by DEcomposition (TIDE) program.

TIDE analysis of viral sequence for the gRNA/Cas9 treated REPA region (C1)
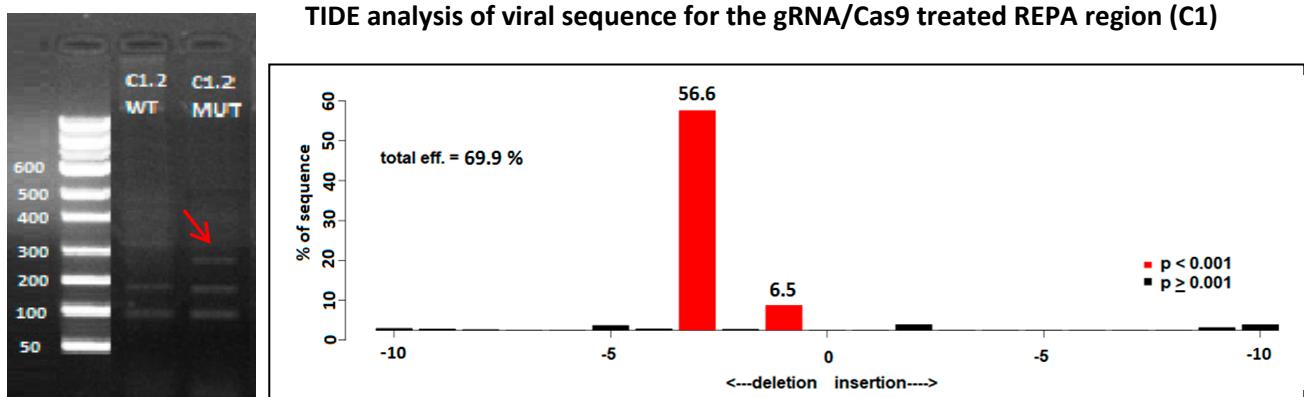
Mutation in gRNA/Cas9 targeted viral REPA (C1) that was not digested with HpyCH4III

```
                                                          HpyCH4III
TCCTTGGTGTTGGATGAATCAAGAATTCGTCTCCATTCCATGTTGCTGTTGCCAATACTGTTTGGAGAGCGCCCTCCCATCG   WT
TCCTTGGTGTTGGATGAATCAAGAATTCGTCTCCATTCCATGTGC<<<<<<<<<<<<<<<<<TTGGAGAGCGCCCTCCCATCG  -17
TCCTTGGTGTTGGATGAATCAAGAATTCGTCTCCATTCCATGTTGCTGTTGCCAA<<<<GTTTGGAGAGCGCCCTCCCATCG   -8
TCCTTGGTGTTGGATGAATCAAGAATTCGTCTCCATTCCATGTTGCTGTTGCCAATACgcTGTTTGGAGAGCGCCCTCCCATCG +2
```

TIDE analysis of viral sequence for the gRNA/Cas9 treated overlapping region between REPA/REPB
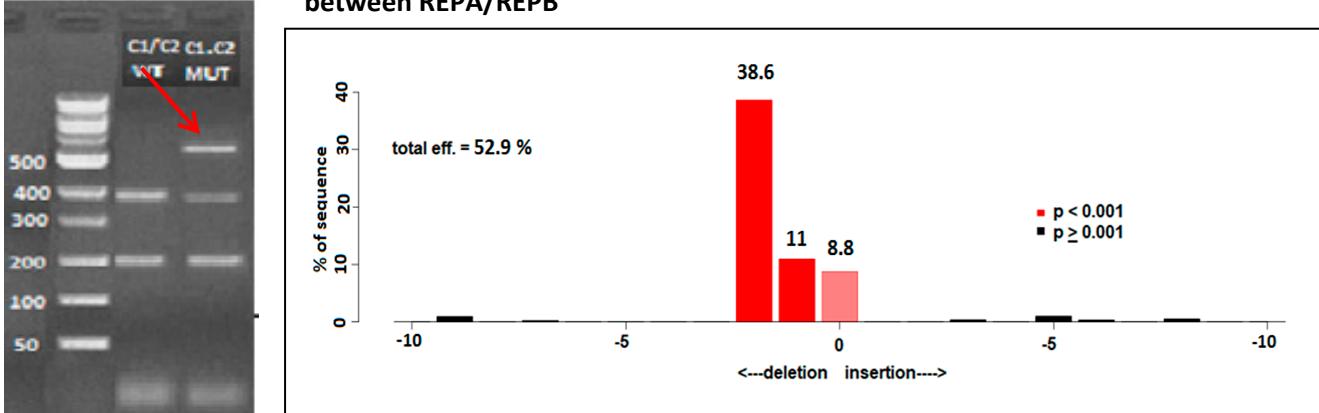
Mutation in gRNA/Cas9 targeted viral REPA/REPB (C1/C2) that was not digested with BtsIMutl

```
                                                    BtsIMutI
GAGACCAATCTAATACCATGCCAGACATATAGTGGTGTCGGCCTAGAGATCTTGCCCACTGGGTCTTTCCTGTTCTGGAT   WT
GAGACCAATCTAATACCATGCCAGACATATAGTGGTGTCGGCC<<<<<<<<<<<<<<<<<<<<<<< CTTTCCTGTTCTGGAT  -23
GAGACCAATCTAATACCATGCCAGACATATAGTGGTGTCGGC<<<<<<<<<<<<<<< CACTGGGTCTTTCCTGTTCTGGAT  -15
GAGACCAATCTAATACCATGCCAGACATATAGTGGTGTCGGCCTAGAGATCTT<<<<< CTGGGTCTTTCCTGTTCTGGAT   -4
GAGACCAATCTAATACCATGCCAGACATATAGTGGTGTCGGCCTAGAGATCTTGtgaCCCACTGGGTCTTTCCTGTTCTGGAT +3
GAGACCAATCTAATACCATGCCAGACATATAGTGGTGTCGGCCTAGAGATCTTGCCGTCTGGGTCTTTCCTGTTCTGGAT  G>C/T>A
```
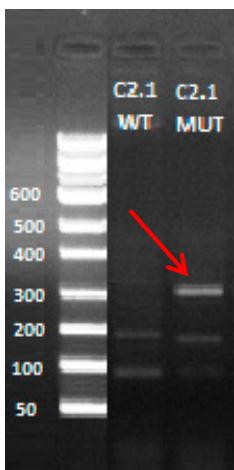
TIDE analysis of viral sequence for the gRNA/Cas9 treated REPB (C2)
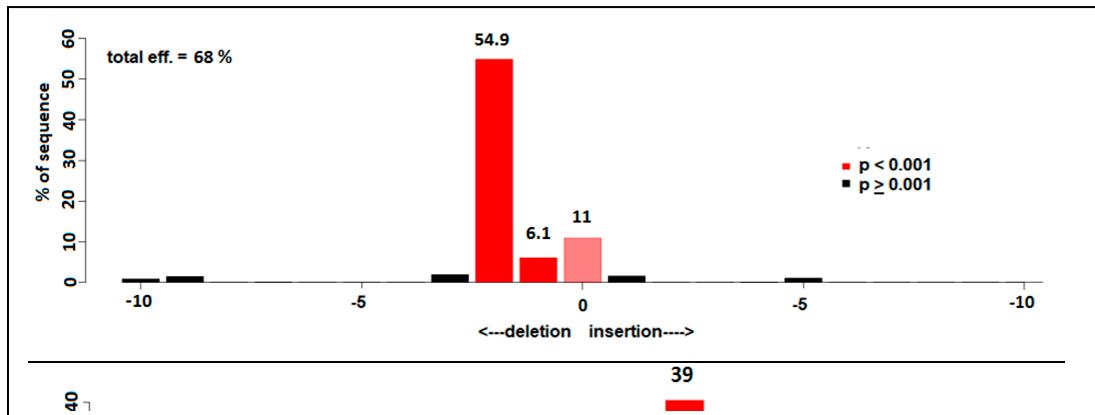
Mutation in gRNA/Cas9 targeted viral REPB (C2) that was not digested with AciI

```
                                                                    AciI
CATAGATTCACTCATACAAGGTATCCAGTCCATATCTGGATTAGTAATAGCAATACAAGGTCTTCCGCCTGGAATGACTAAGT
CATAGATTCACTCATACAAGGTATCCAGTCCATATCTGGAT<<<<<<<<<<<<<<<<<<<<<<<<< GCCTGGAATGACTAAGT   -27
CATAGATTCACTCATACAAGGTATCCAGTCCATATCTGGATTAGTAATAGCAATACAAG<<<<<<<< CTGGAATGACTAAGT   -9
CATAGATTCACTCATACAAGGTATCCAGTCCATATCTGGATTAGTAATAGCAAT<<<<<<<<<<<<<<<<<<<<AATGACTAAGT  -20
CATAGATTCACTCATACAAGGTATCCAGTCCATATCTGGATTAGTAATAGCAATACAAGGTCAACCGCCTGGAATGACTAAGT  A<T
```
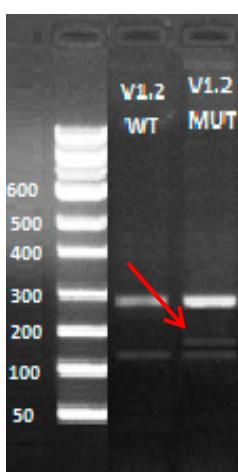
TIDE analysis of viral sequence for the gRNA/Cas9 treated Capsid gene (V1)
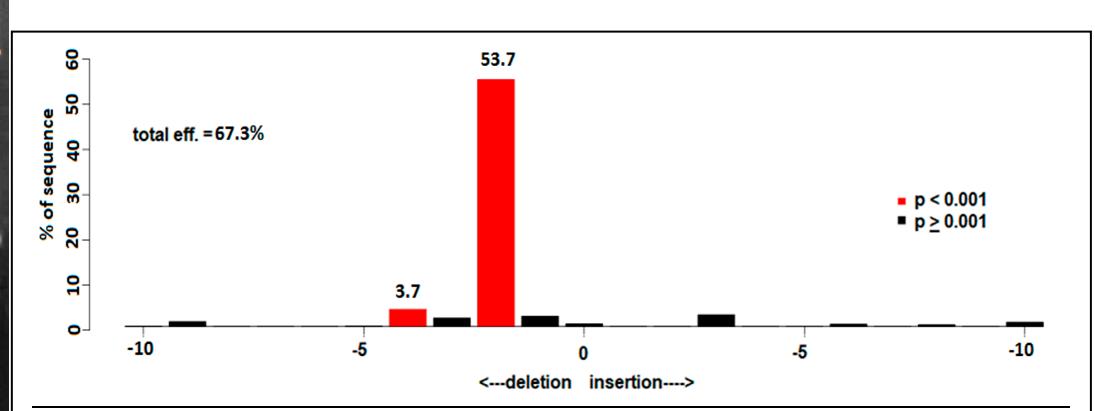
Mutation in gRNA/Cas9 targeted viral CP (V1) that was not digested with BslI

```
                      BslI
GACTGGTGGTCAGGCTATGCCTTCCACTTATCGGATTAGGAGGGATGCGAATGAACGATTTATCGTGAAGAAGAAGTG         WT
GACTGGTGGTCAGGCTATGCCTTCC<<<<<<<<<<<TTAGGAGGGATGCGAATGAACGATTTATCGTGAAGAAGAAGTG       -11
GACTGGTGGTCAGGCTATGCCTT<<<<<<ATCGGATTAGGAGGGATGCGAATGAACGATTTATCGTGAAGAAGAAGTG        -6
GACTGGTGGTCAGGCTATGCCTTCC<<TTATCGGATTAGGAGGGATGCGAATGAACGATTTATCGTGAAGAAGAAGTG        -2
GACTGGTGGTCAGGCTATGCCTTCCtgttACTTATCGGATTAGGAGGGATGCGAATGAACGATTTATCGTGAAGAAGAAGTG   +4
```
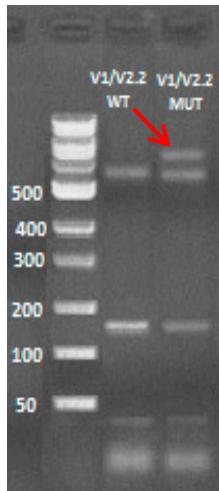
TIDE analysis of viral sequence for the gRNA/Cas9 treated overlapping region between capsid and movement gene (V1/V2)

total eff. = 70.7%

62.8

4.9 5.9

% of sequence

■ p < 0.001
■ p ≥ 0.001

<---deletion   insertion---->
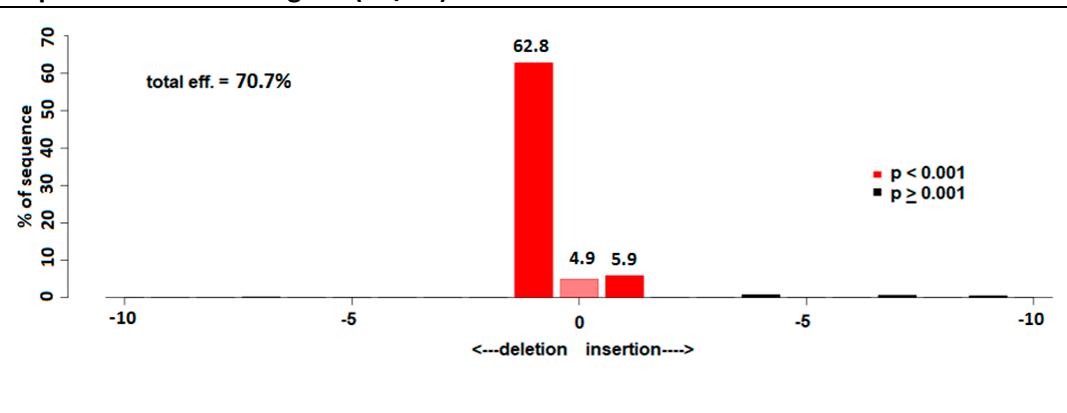
**Sequences of gRNA/Cas9 targeted overlapping CP/MP region (V1/V2.2) that was not cleaved by AgeI**

```
                                                       AgeI
AAAAGAGAAAGTATACTCCTCCGGCCAGTTGGACGAGGAAGAGGAAGACGACCGGTGGGAGGACAGTTTCGAAGAA          WT
AAAAGAGAAAGTATACTCCTCCGGCCAGTTGGACGA<<<<<<<<<<<<<<<<<<<<GGTGGGAGGACAGTTTCGAAGAA      -20
AAAAGAGAAAGTATACTCCTCCGGCCAGTTGGACGAGGAAGAGGA<<<<<<<<< GGTGGGAGGACAGTTTCGAAGAA       -9
AAAAGAGAAAGTATACTCCTCCGGCCAGTTGGACGAGGAAGAGGAAGA<<<<<<<<<< GGAGGACAGTTTCGAAGAA       -10
AAAAGAGAAAGTATACTCCTCCGGCCAGTTGGACGAGGAAGAGGAAGggg<<<<GGTGGGAGGACAGTTTCGAAGAA        -4/+3
```


TIDE analysis of viral sequence for the gRNA/Cas9 treated movement gene (V2)

total eff. = 65.4 %

42.4

6.3 7.4

% of sequence

■ p < 0.001
■ p ≥ 0.001

<---deletion   insertion---->
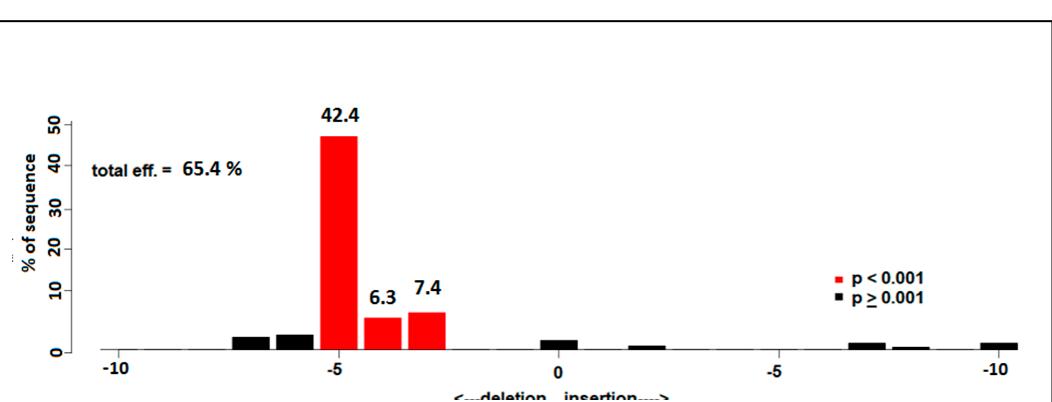
Sequences of gRNA/Cas9 targeted overlapping CP/MP region (V2) that was not cleaved by RsaI

```
                                              RsaI
GGAAGTGAAGAAATTGCTGAAGCTTAAGGTTAGTTTTAAGCGTAAGTGTAGTTTGTACGAGGACATATACAAGAAGTAT
GGAAGTGAAGAAATTGCTGAAGCTTAAGGTTAGTTTTAAGCGTAA<<<<<<<<<<ACGAGGACATATACAAGAAGTAT       -12
GGAAGTGAAGAAATTGCTGAAGCTTAAGGTTAGTTTTAAGCGTAAGTGTAGT<<<<<< AGGACATATACAAGAAGTAT      -7
GGAAGTGAAGAAATTGCTGAAGCTTAAGGTTAGTTTTAAGCGTAAGTGTAGTTTGgtTACGAGGACATATACAAGAAGTAT   +2
```
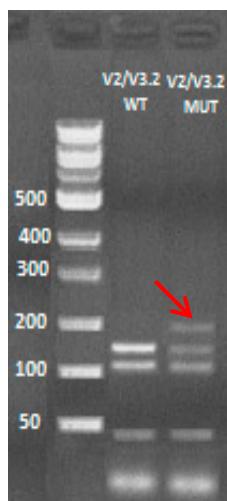
TIDE analysis of viral sequence for the gRNA/Cas9 treated overlapping region between movement and ssDNA regulator gene (V1/V2)
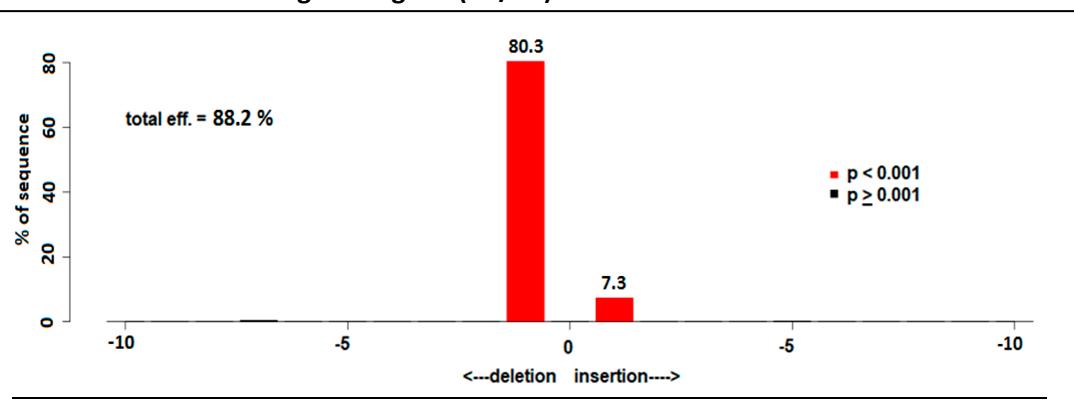
Sequences of gRNA/Cas9 targeted overlapping MP/ssDNA region (V2/V3) that was not cleaved by Hpy188I

```
                                                              Hpy188I
ATTTAGTACGATTCTTCAGTCGGGAATTAATTTCTATGGTACCTTTCAGAGTGAGCGAATTTCCGCGAAGCTATCCAGC
ATTTAGTACGATTCTTCAGTCGGGAATTAATTTCTATGGTAC<<<<<<<<<<<<<<<<< ATTTCCGCGAAGCTATCCAGC        -18
ATTTAGTACGATTCTTCAGTCGGGAATTAATTTCTATGGTACCTTTCAGAG<<<<<<<< ATTTCCGCGAAGCTATCCAGC       -8
ATTTAGTACGATTCTTCAGTCGGGAATTAATTTCTATGGTACCTTTCAGAGTGA<<<gggc TTCCGCGAAGCTATCCAGC      -3/+4
ATTTAGTACGATTCTTCAGTCGGGAATTAATTTCTATGGTACCTTTCAGAGTGAGCGTATTTCCGCGAAGCTATCCAGC       T<A
ATTTAGTACGATTCTTCAGTCGGGAATTAATTTCTATGGTACCTTTCAGAGTGAGCGTaatATTTCCGCGAAGCTATCCAGC    +3
```
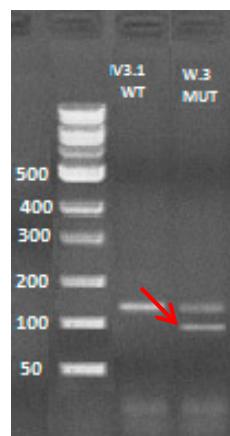

TIDE analysis of viral sequence for the gRNA/Cas9 treated ssDNA regulator gene (V3)
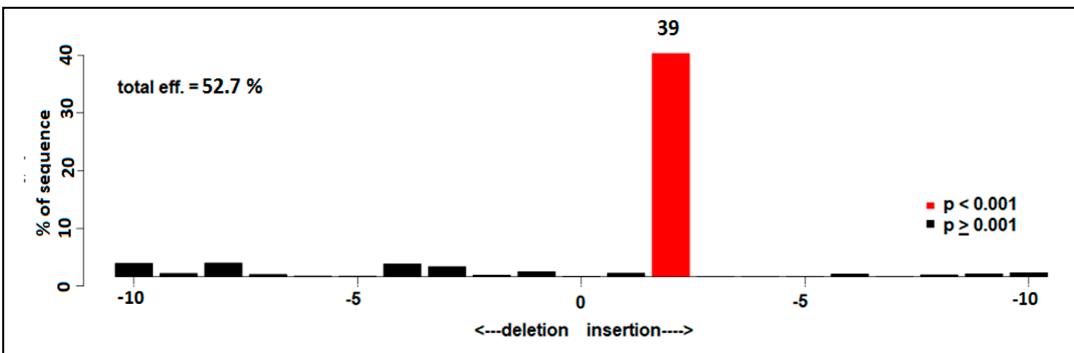
Sequences of gRNA/Cas9 targeted ssDNA regulator region (V3) that was not cleaved by Hpy188III

```
                             Hpy188III
CGTATATTATACGTATAAATGATGGTGTGTATTCCCGACTGGTTATTTCTACTTTTCGTATTTAGTACGATTCTTCAG       WT
CGTATATTATACGTATAAATGATGGTG<<<<<<<<<<<ACTGGTTATTTCTACTTTTCGTATTTAGTACGATTCTTCAG       -11
CGTATATTATACGTATAAATGATGGTGTGTATT<<<<<<< GGTTATTTCTACTTTTCGTATTTAGTACGATTCTTCAG       -7
CGTATATTATACGTATAAATGATGGTGTGTATTC<<< ACTGGTTATTTCTACTTTTCGTATTTAGTACGATTCTTCAG       -3
CGTATATTATACGTATAAATGATGGTGTGTATTC<<gggGACTGGTTATTTCTACTTTTCGTATTTAGTACGATTCTTCAG  -2/C<G
```