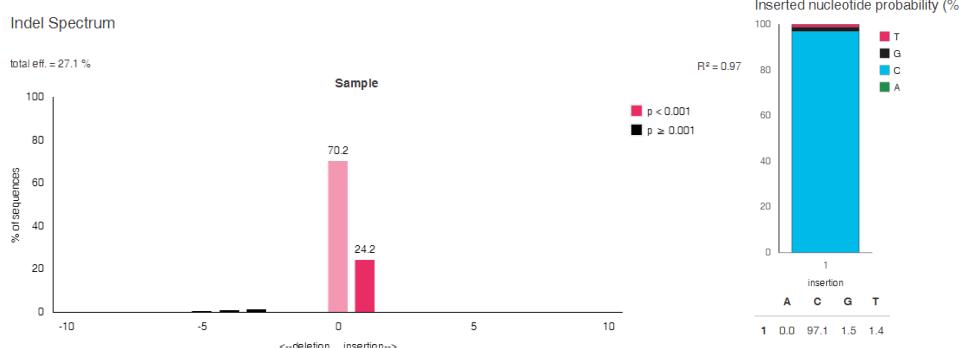
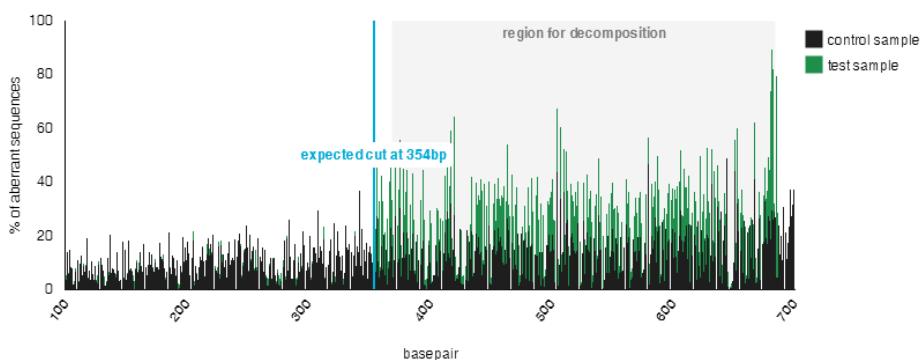


Supplementary Data:

gGGTA1 F2/R2



Quality control - Aberrant sequence signal



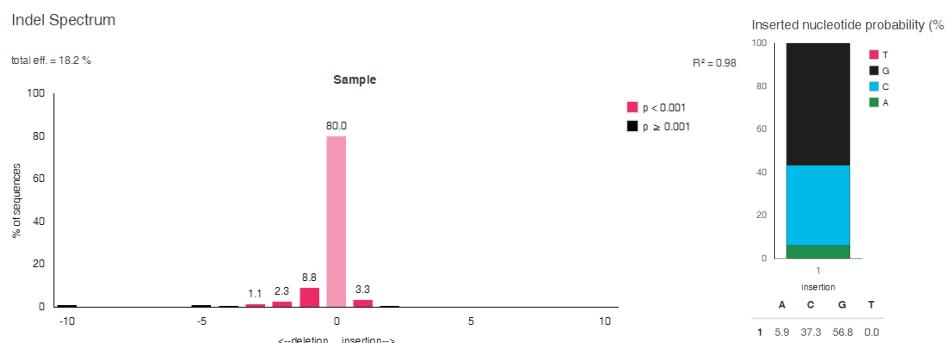
Alignment - local pairwise alignment score: 233.0

```
ctrl align: -----ATAGAGCTGGGTCTCTGCCTTAAAGTGGTTGAGATCAAGTCCGAGAAGAGGTGGCAAGACATCAGCATGATGCGATGAAGACCATC
GGGGAGCACATCCTGGCCCACATCCAGCAGGAGGTGGACTTCCTTCTGCATGGACGTGGATCAGGTCTCCAAAACAACTTGGGGTGGAGACCCTGGCCAGTCGGTGGCTCAGC
TACAGGCCTGGTACAAGGCACATCCGACNAG

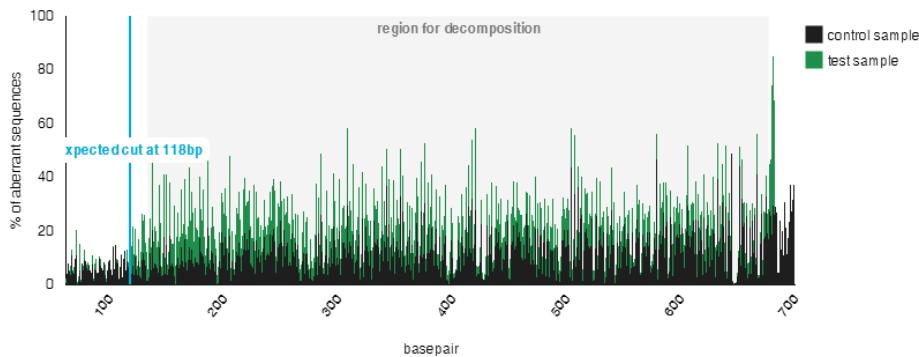
edit align: CNGGATGCCCTTGATAGAGCTGGTCTCTGCCTTAAAGTGGTTGAGATCAAGTCCGAGAAGAGGTGGCAAGACATCAGCATGATGCGATGAAGACCAT
CGGGGAGCACATCCTGGCCCACATCCAGCAGCAGGAGGTGGACTTCCTTCTGCATGGACGTGGATCAGGTCTCCAAAACAACTTGGGGTGGAGACCCTGGCCAGTCGGTGGCTCAG
CTACAGGCCTGGTGGTACAAGGC-----
```

(a)

gGGTA1 F3/R3



Quality control - Aberrant sequence signal

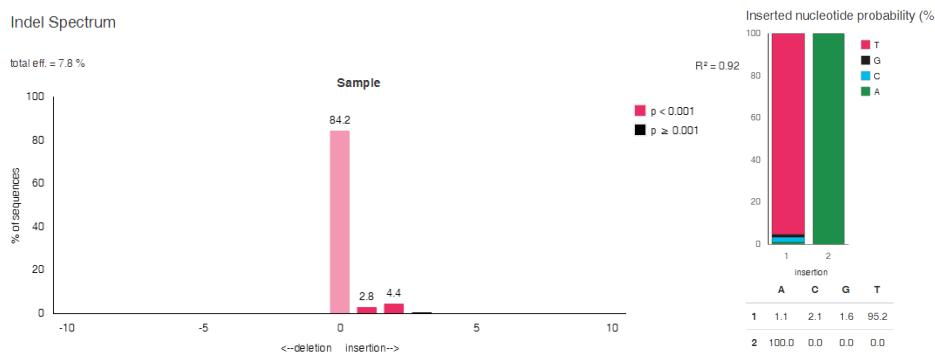


Alignment - local pairwise alignment score: 33.0

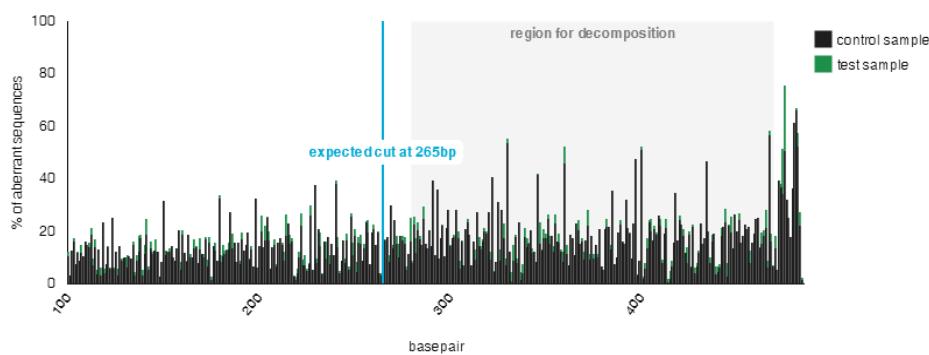
```
ctrl align: -----TTTACATCATGGTGGATGATATCTCAGGATGCCTTGATAGAGCTGG
edit align: TGGCCACAAAGTCATCTTACATCATGGTGGATGATATCTCAGGATG-----
```

(b)

gCMAH F1/R1



Quality control - Aberrant sequence signal

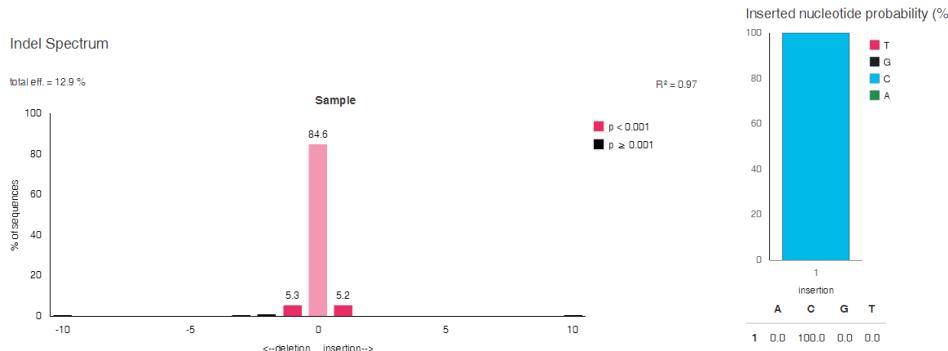


Alignment - local pairwise alignment score: 151.0

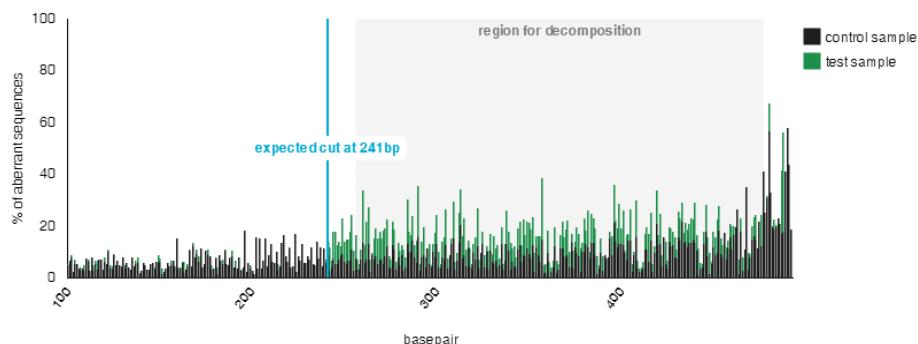
```
ctrl align: -GGCTTGTCTGCTAACCGCCCAGTTCTTNTCTGGCTGGTAATGATCCGATCTGAATCTCACTGTCTCCAACAGATCACGTACCTACTCACGCCNGC
ATGGACCTCAAGCTGGNNACAAGAGNATGGTGGTCAACAGCCCAGTTCTTNTCTGGCTGGTAATGATCCGATCTGAATCTCACTGTCTCCAACANATCACGTACCTACTCACGCCCTG
edit align: TGGCTTGTCTGCTAACCGCCCAGTTCTTNTCTGGCTGGTAATGATCCGATCTGAATCTCACTGTCTCCAACANATCACGTACCTACTCACGCCCTG
CATGGACCTCAAGCTGGNNACAAGAGNATGGTGGTCAACAGCCCAGTTCTTNTCTGGCTGGTAATGATCCGATCTGAATCTCACTGTCTCCAACANATCACGTACCTACTCACGCCCTG
```

(c)

gCMAH F2/R2



Quality control - Aberrant sequence signal

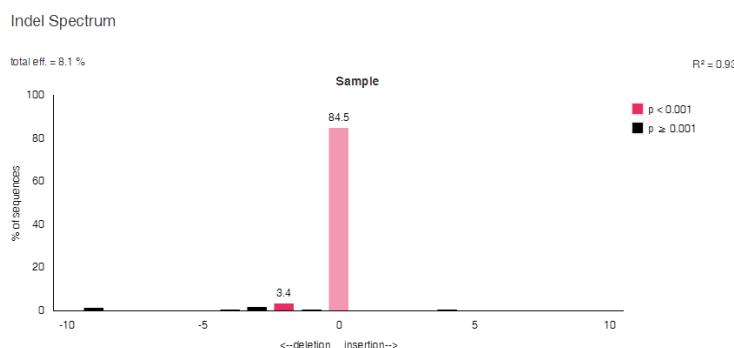


Alignment - local pairwise alignment score: 132.0

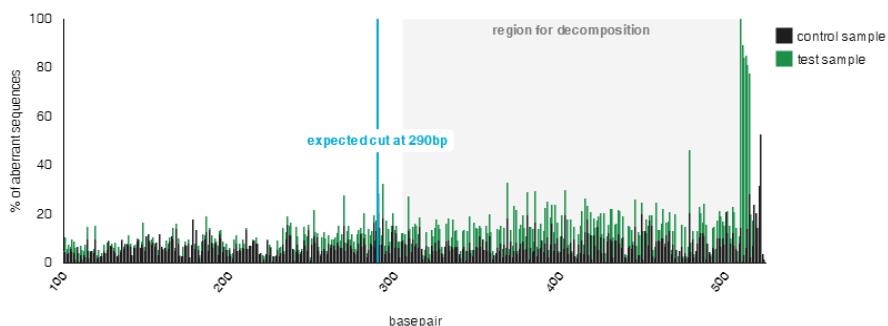
```
ctrl align: -TCCTGTTGTGCCCTCACCTGCCGAAGCTGCCAATCTCAAGGAAGGAATCAATTGTTGAAATAAGAGCACTGGCAAGGATTACATCTTATTAAGAATAA
GAGCCGCCTGAAGGCATGTAAGAACATGTG-
edit align: ATCCTGTTGTGCCCTCACCTGCCGAAGCTGCCAATCTCAAGGAAGGAATCAATTGTTGAAATAAGAGCACTGGCAAGGATTACATCTTATTAAGAATAA
AGAGCCGCCTGAAGGCATGTAAGAACATGT-
```

(d)

gβ4GalNT2 F1/R1



Quality control - Aberrant sequence signal



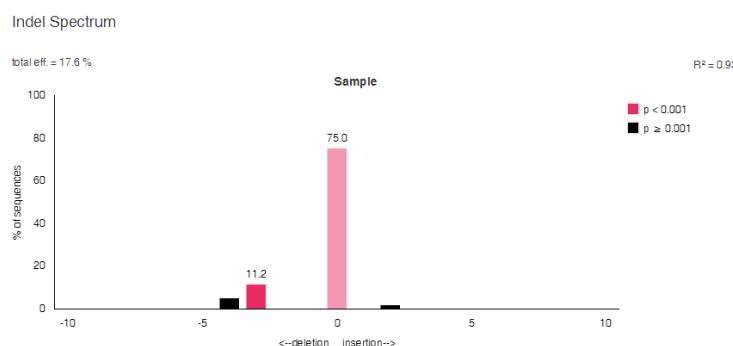
Alignment - local pairwise alignment score: 209.0

```
ctrl align: --ATCAATTGATACTGAGTTTACAGTCATCCCATTGACTTTGCTACACCACAAAGTATGCATCTGAGATTAATCTCAAACCTTAGGCC
AGAAAAGATTTAACAGCGTTGCTCTTGCTCCAACAGCCCTAGATGTCTGTCGATCCTCAAGATATNGATGGTCTTTGGCCTGAGCGTTGACTCTTATG
```

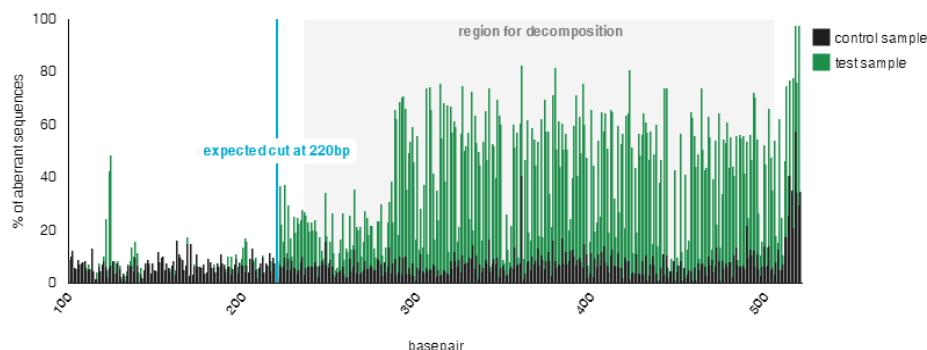
```
edit align: AAATCAATTGATACTGAGTTTACAGTCATCCCATTGACTTTGCTCAAGTTGCTATTCCATCTATGTCGACAATGAAATCTTTAACATAC
CAGAAAAGATTTAACAGCGTTGCTCTTGCTCCAACAGCCCTAGATGTCTGTCGATCCTCAAGATATNGATGGTCTTTGGCCTGAGCGTTGACTCTTATG--
```

(e)

gβ4GalNT2 F2/R2



Quality control - Aberrant sequence signal



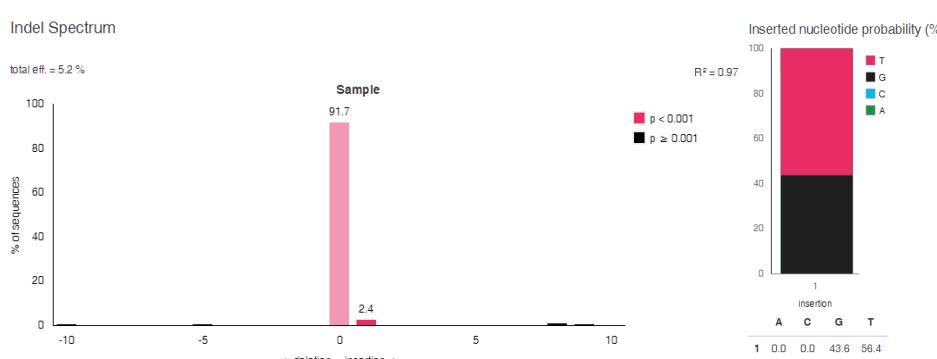
Alignment - local pairwise alignment score: 111.0

```
ctrl align: CACGGATCTACTGAGTTTACAGTCATCCCATTGACTTTGCTACACCACAAAGTATGCATCTGAGATTAATCTCAAACCTTAGGCC
TCACTTGC-
```

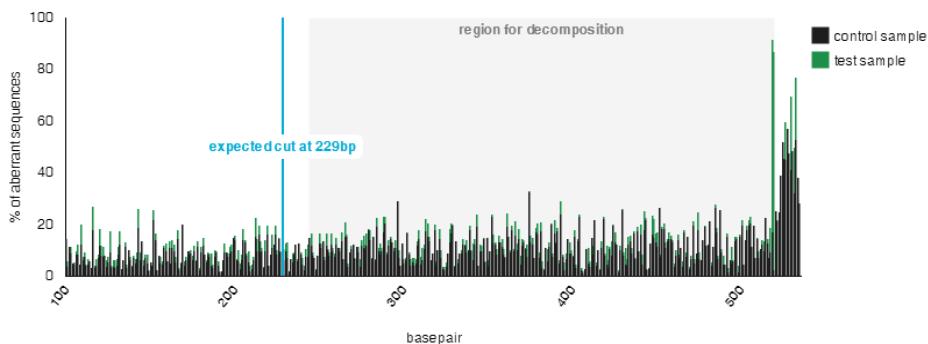
```
edit align: -ACGGATCTACTGAGTTTACAGTCATCCCATTGACTTTGCTACACCACAAAGTATGCATCTGAGATTAATCTCAAACCTTAGGCC
CTCACTTGCA
```

(f)

gvWF F1/R1



Quality control - Aberrant sequence signal



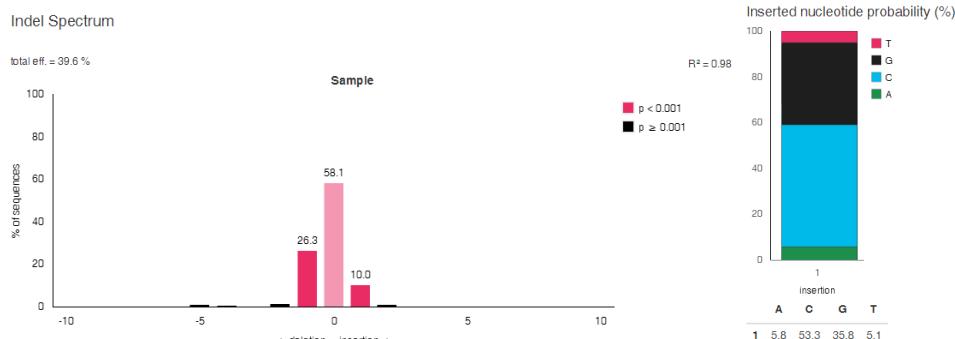
Alignment - local pairwise alignment score: 120.0

```
ctrl align: -AGAGTGCCTCGGTGTTGCCCGCTGCCACCCCTGGTGGACCCCGAGCCTTCGTGGCCCTGTGTGAGAAGATGCTGTCCATGTGCCAGGGGCTGCAGTG
CCCGTCCCCCGCGCTCT
```

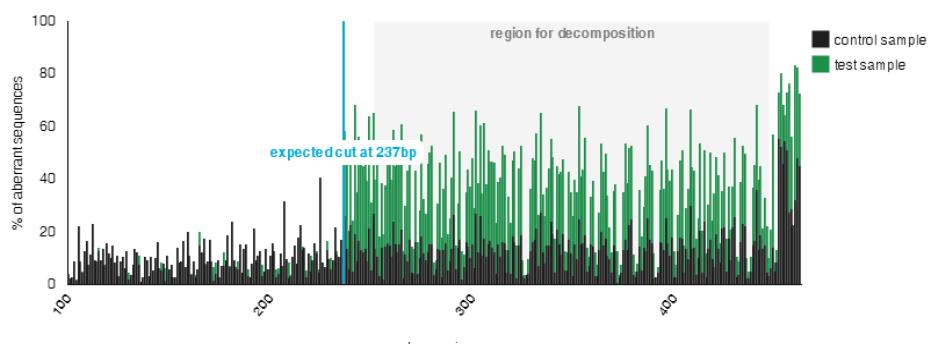
```
edit align: AAGAGTGCCTCGGTGTTGCCCGCTGCCACCCCTGGTGGACCCCGAGCCTTCGTGGCCCTGTGTGAGAAGATGCTGTCCATGTGCCAGGGGCTGCAGT
GCCCGTCCCCCGCGCTCC-
```

(g)

gvWF F3/R3



Quality control - Aberrant sequence signal



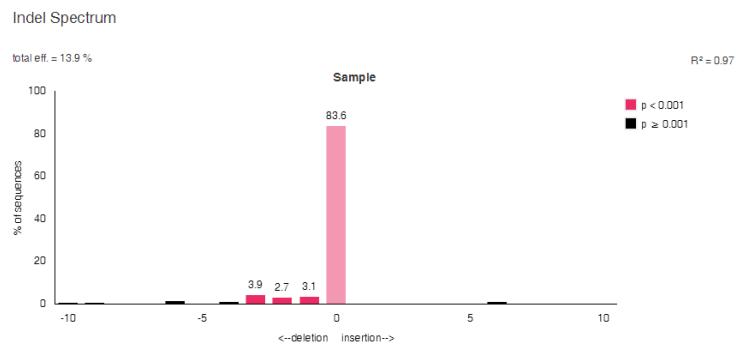
Alignment- local pairwise alignment score: 123.0

```
ctrl align: CTTTCGCAAGACGTGCTGAGCTGATGGTCACCCACGGGGATTCTGAGGTTATGGGATGAGTAAGGCTGATGACAAGGTTGAGTCTGGTTGATGCCCTGA
CCCANAGGGACAGCTCCTTGACNAC----
```

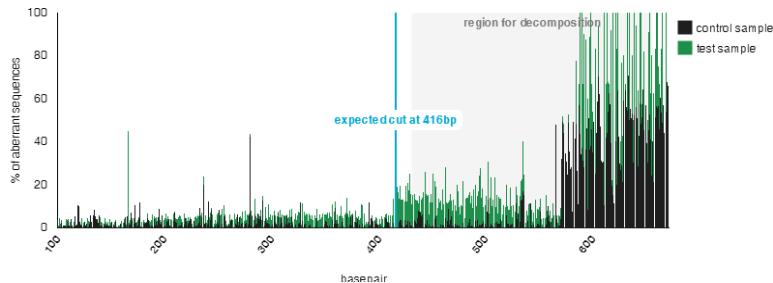
```
edit align: ----TCGCAAGACGTGCTGAGCTGATGGTCACCCACGGGGATTCTGAGGTTATGGGATGAGTAAGGCTGATGACAAGGTTGAGTCTGGTTGATGCCCTG
ACCCAGAGGGACAGCTCCTTGACGACGGCC
```

(h)

gASGR1 F1/R1



Quality control - Aberrant sequence signal

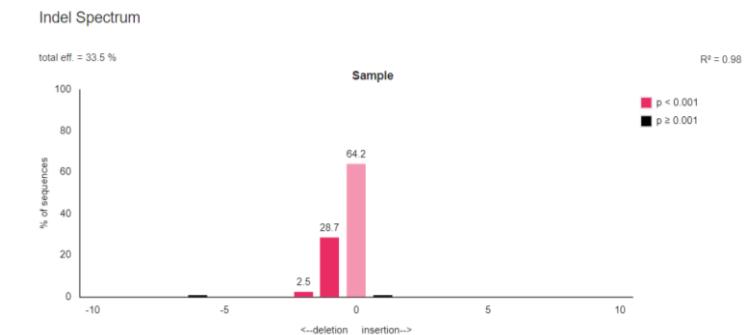


Alignment - local pairwise alignment score: 306.0

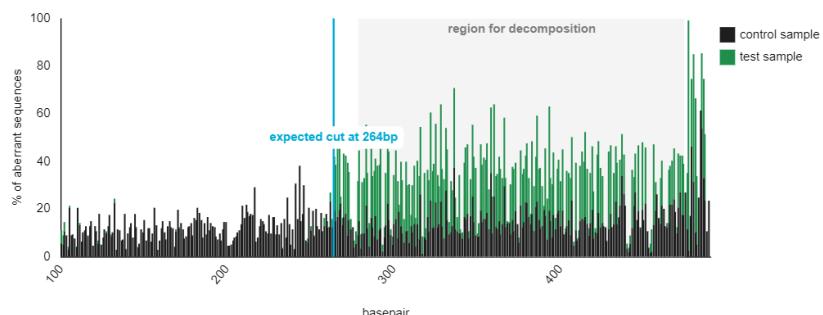
ctrl align: --GTGCTTTCTCTTCTTCCCTTCCCTTCCCTTCCCTTCTTCTTGGCTGTCTACATGCAGAAAATTCTGGGCTAGGGATTGAA
CCCTTGCCATGGCAATGCTGGCTTAAACCCACCGAGCCACCAAGGGAACTCCCATGTTCTTAATCTATAAGGTTCTGTCGTCCTACAAGAGGAGGCCAGGATGCTCCCTCTA
GGACCCCCAGAGCTGCCCTGCCAGGTCCCCCTGTCCCTGAGGGCGTGACGCCCTCCCTTGCAGGGCCACCTCTAACAGTCA

(i)

gASGR1 F2/R2



Quality control - Aberrant sequence signal



Alignment - local pairwise alignment score: 154.0

```
ctrl align: --TGCAGCGCCCCGTGGCTCTGCCCTCAAATGCGCCCCCTCCCACTGGCAGGGTCCAGGGGTGCCAGGCTGCTCCCTGGCTGAGGCTCTGGCCCCCTGGGGCCCCGTCGTCTGGCTCTTTAGGAACCTGGAGACCCGANCAGCCGGATGA
```

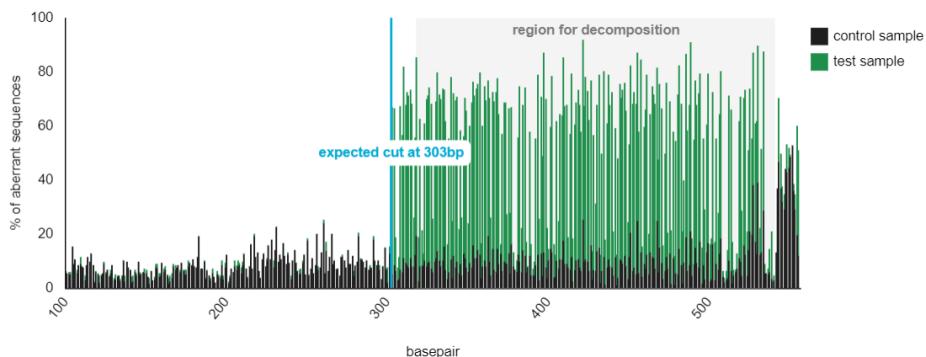
```
edit align: TGTGCAGCGCCCCGTGGCTCTGCCCTCAAATGCGCCCCCTCCCACTGGCAGGGTCCAGGGGTGCCAGGCTGCTCCCTGGCTGAGGCTCTGGCCCCCTGGGGCCCCGTCGTCTGGCTCTTTAGGAACCTGGAGACCCGANCAGCCGGAT--
```

(j)

Figure S1. The indel spectrum and inserted nucleotide probability results, alignment and decomposition quality controls for the other CRISPR/Cas9 genetic constructs containing gRNA tested for disruption of the porcine genes. Results obtained after use the plasmids with: (a) gGGTA1 F2/R2; (b) gGGTA1 F3/R3; (c) gCMAH F1/R1; (d) gCMAH F2/R2; (e) g β 4GalNT2 F1/R1; (f) g β 4GalNT2 F2/R2; (g) gvWF F1/R1; (h) gvWF F3/R3; (i) gASGR1 F1/R1; (j) gASGR1 F2/R2.

gGGTA1 F1/R1

Quality control - Aberrant sequence signal



Alignment - local pairwise alignment score: 195.0

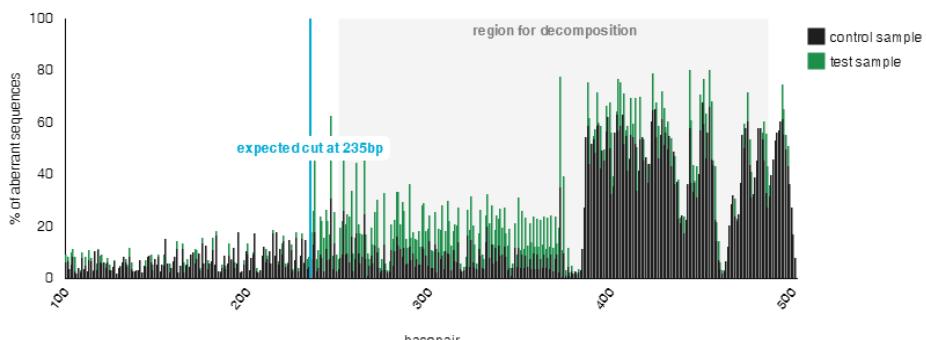
```
ctrl align: GATACATTGAGCATTACTTGGAGGAGTTCTAACATCTGCAAATACATACTTCATGGTGGCCACAAAGTCATCTTTACATCATGGTGGATGATATCTCCAGGATGCCTTGATAGAGCTGGGTCTCTGCGTCTAAAGTGGAGATCAAGTCCGAGAAGAGGTGGCAAGACATCAGCATGATGCGCA
```

```
edit align: GATACATTGAGCATTACTTGGAGGAGTTCTAACATCTGCAAATACATACTTCATGGTGGCCACAAAGTCATCTTTACATCATGGTGGATGATATCTCCAGGATGCCTTGATAGAGCTGGGTCTCTGCGTCTAAAGTGGAGATCAAGTCCGAGAAGAGGTGGCAAGACATCAGCATGATGCGCA
```

(a)

gCMAH F3/R3

Quality control - Aberrant sequence signal



Alignment - local pairwise alignment score: 123.0

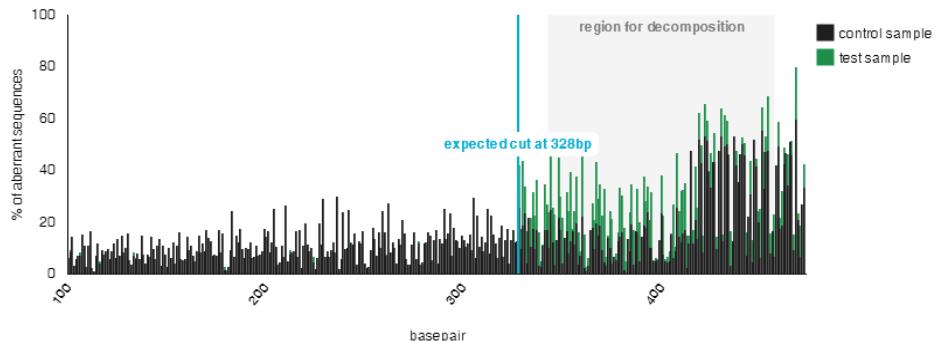
```
ctrl align: ----CATGCATTCTGTCTTAGCGATGCTTTAAAGTCATTTGGTTGATTATCCAGATTGTCACCTTGCTTAGTTGAGAAAAGGATGAAGAAAATGGAGTTTGCTTAGAACTAAATC
```

```
edit align: GAGCCATGCATTCTGTCTTAGCAGTGTGTTAAAAGTCATTTGGTTGATTATCCAGATTGTCCACCTTGCTTAGTTGAGAAAAGGATGAAGAA
AATGGAGTTTGCTCTAGAACTA---
```

(b)

g β 4GalNT2 F3/R3

Quality control - Aberrant sequence signal



Alignment - local pairwise alignment score: 212.0

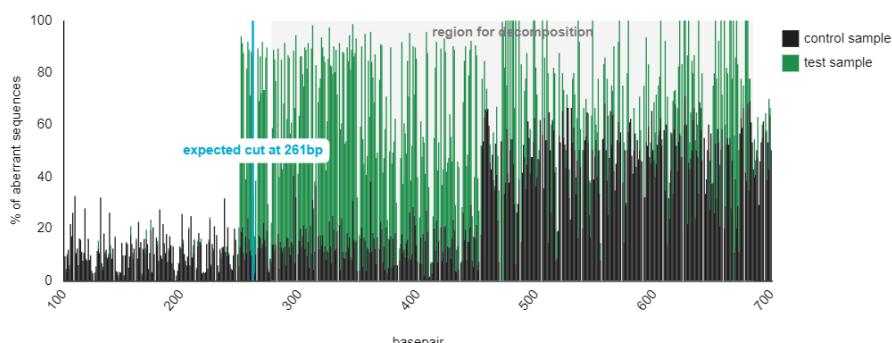
```
ctrl align: TGCTGGCGTCCTGGGTATGTGCCCTCTGCATTTAGGCTTCTACCTCCTTATCCTAGGTACCCCTGACAGCTTCTGGGGACACTGAACACCCTGCTGA
CGTCCCAGACAATGTGGTGAAGGGCANAGGCCAGAACAGCTAACATTTGACCACTAGCCGGAGCTTTGAATTTCATCCTCCAGCATGTGACATACGAGCACAGAGTACC --
-----
```

```
edit align: -----GTCCTGGGTATGTGCCCTCTGCATTTAGGCTTCTACCTCCTTATCCTAGGTACCCCTGACAGCTTCTGGGGACACTGAACACCCTGCTG
ACGTCCCAGACAATGTGGTGAAGGGCANAGGCCAGAACAGCTAACATTTGACCACTAGCCGGAGCTTTGAATTTCATCCTCCAGCATGTGACATACGAGCACAGAGTACCA
CCTCCAC
```

(c)

gvWF F2/R2

Quality control - Aberrant sequence signal



Alignment - local pairwise alignment score: 150.0

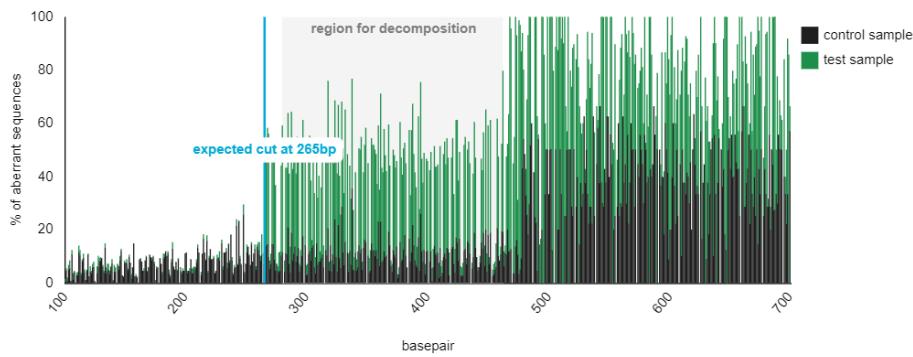
```
ctrl align: -TGGCCTCTGGGAACTGAAGATGAGGGGGGCAGACACTCAGCGGTGGGGGGAGGCAGGCTCTGAGCACCATGGCTGTGAGATGCTGCCTGAAGGGGGCGC
TGGGGAGACTCCTGATTTCCATTGCAGGCCANACTGCCCGCGGGCA
```

```
edit align: ATGGCCTCTGGGAACTGAAGATGAGGGGGGCAGACACTCAGCGGTGGGGGGAGGCAGGCTCTGAGCACCATGGCTGTGAGATGCTGCCTGAAGGGGGCG
CTGGGGAGACTCCTGATTTCCATTGCAGGCCANACTGCCCGCGGGN-
```

(d)

gASGR1 F3/R3

Quality control - Aberrant sequence signal



Alignment - local pairwise alignment score: 155.0

```
ctrl align: GTGCGGGCCCCGTGGCTCTGCCCTCCAAGTGCAGGGCTGCCAGGGGTGCCCAGGCTGCTCCCTCGGCTGAGGCTCTGGCCCCCTGG
GGCCCCCGTCCGTCTGGCTCTTTAGGAACGTGGAGACCCGAGCAGCCGGATGA --
edit align: --GCGGCGCCCCGTGGCTCTGCCCTCCAAGTGCAGGGCTGCCAGGGCTGCTCCCTCGGCTGAGGCTCTGGCCCCCTGG
GGCCCCCGTCCGTCTGGCTCTTTAGGAACGTGGAGACCCGAGCAGCCGGATGACT
```

(e)

Figure S2. The alignment and decomposition quality controls for the CRISPR/Cas9 genetic constructs containing gRNA chosen as the best for the disruption of the porcine genes. Results obtained after use the plasmids with: (a) gGGTA1 F1/R1; (b) gCMAH F3/R3; (c) g β 4GalNT2 F3/R3; (d) gvWF F2/R2; (e) gASGR1 F3/R3.

Detailed results of the insertions and deletions obtained at potential off-target sites. Figure S3 shows the results for the genetic construct containing gGGTA1 F1/R1 excluding site No. 1 are shown in Figure 3.

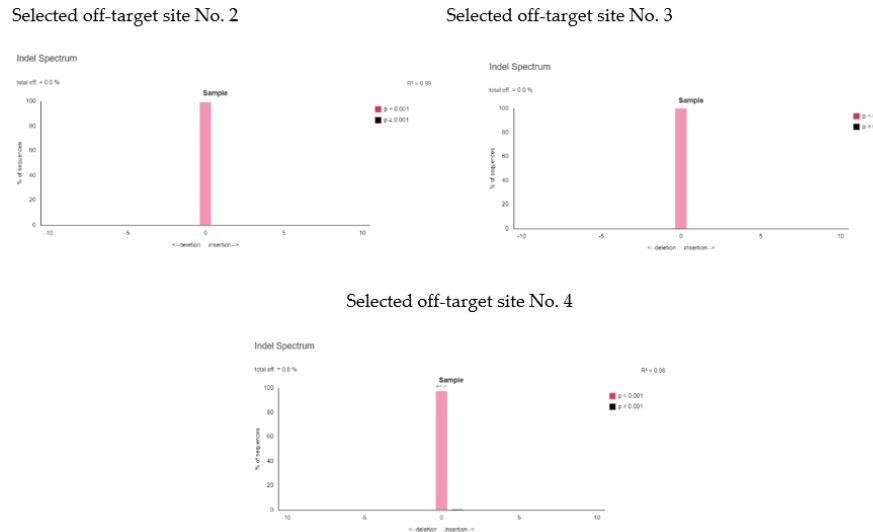
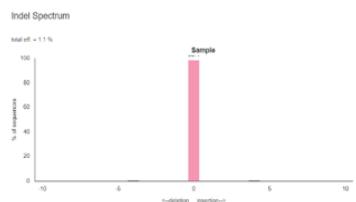


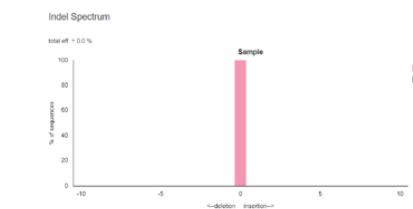
Figure S3. The indel spectrum for the numbers 2–4 off-target loci after the use of CRISPR/Cas9 genetic construct containing gGGTA1 F1/R1 chosen as the best for disruption of porcine GGTA1 gene.

Figure S4 shows the indel spectrum results for the potential off-target sites for genetic construct containing gCMAH F3/R3.

Selected off-target site No. 5



Selected off-target site No. 6



Selected off-target site No. 7

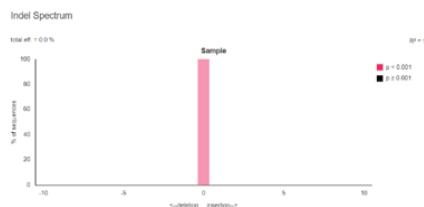
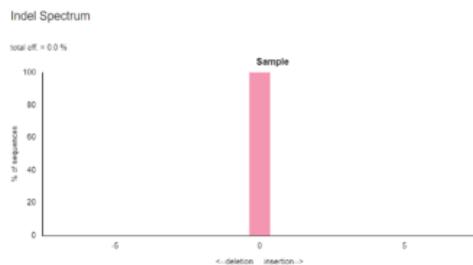


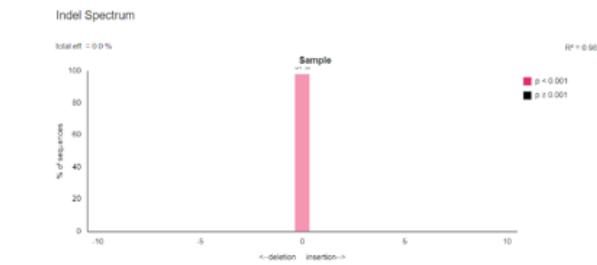
Figure S4. The indel spectrum for the numbers 5–7 off-target loci after the use of CRISPR/Cas9 genetic construct containing gCMAH F3/R3 chosen as the best for disruption of porcine *CMAH* gene.

Figure S5 shows the indel spectrum results for the potential off-target sites for genetic construct containing β 4GalNT2 F3/R3.

Selected off-target site No. 8



Selected off-target site No. 9



Selected off-target site No. 10

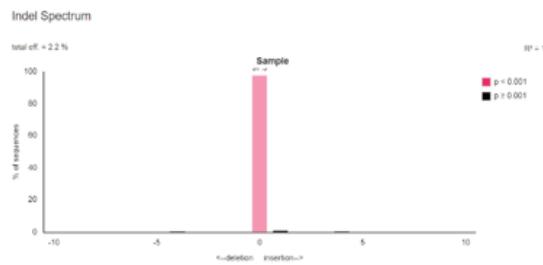
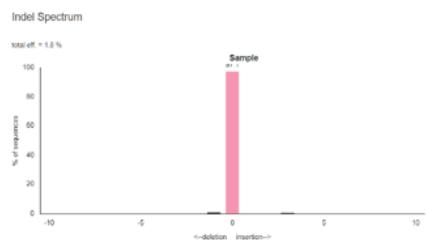


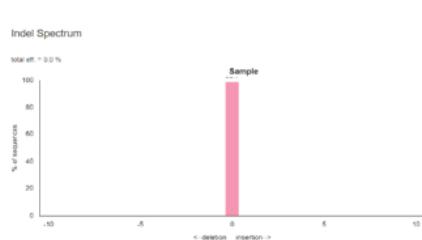
Figure S5. The indel spectrum for the number 8–10 off-target loci after the use of CRISPR/Cas9 genetic construct containing β 4GalNT2 F3/R3 chosen as the best for disruption of porcine β 4GalNT2 gene.

Figure S6 shows the indel spectrum results for the potential off-target sites for genetic construct containing gvWF F2/R2.

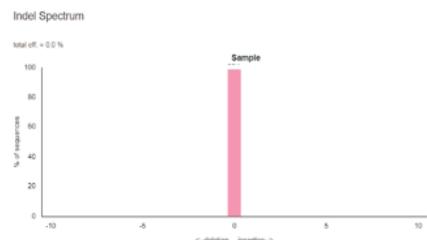
Selected off-target site No. 11



Selected off-target site No. 12



Selected off-target site No. 13



Selected off-target site No. 14

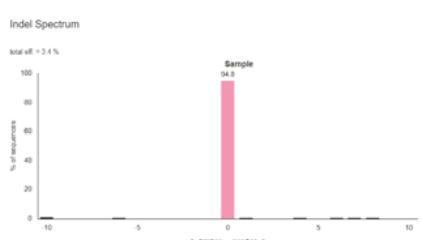
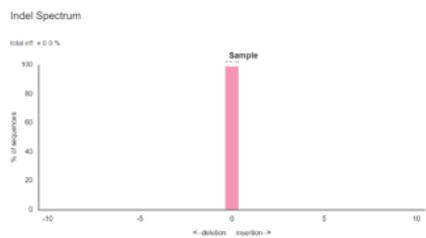


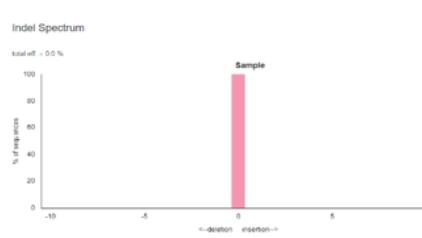
Figure S6. The indel spectrum for the numbers 11–14 off-target loci after the use of CRISPR/Cas9 genetic construct containing gvWF F2/R2 chosen as the best for disruption of porcine *vWF* gene.

Figure S7 shows the indel spectrum results for the potential off-target sites for genetic construct containing gASGR1 F3/R3.

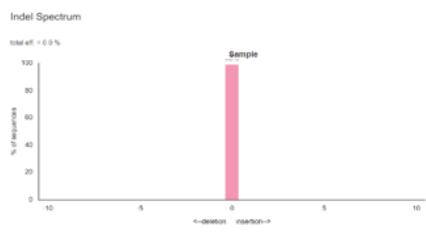
Selected off-target site No. 15



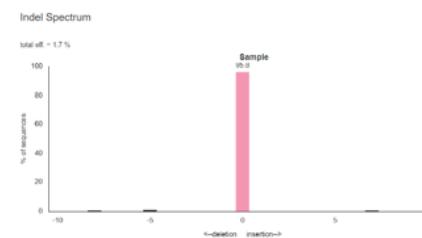
Selected off-target site No. 16



Selected off-target site No. 17



Selected off-target site No. 18



Selected off-target site No. 18

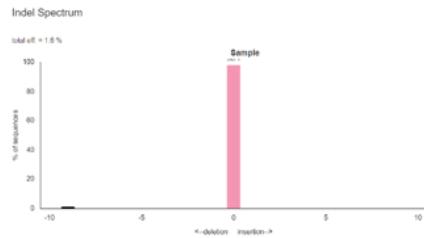


Figure S7. The indel spectrum for the numbers 15–19 off-target loci after the use of CRISPR/Cas9 genetic construct containing gASGR1 F3/R3 chosen as the best for disruption of porcine *ASGR1* gene.