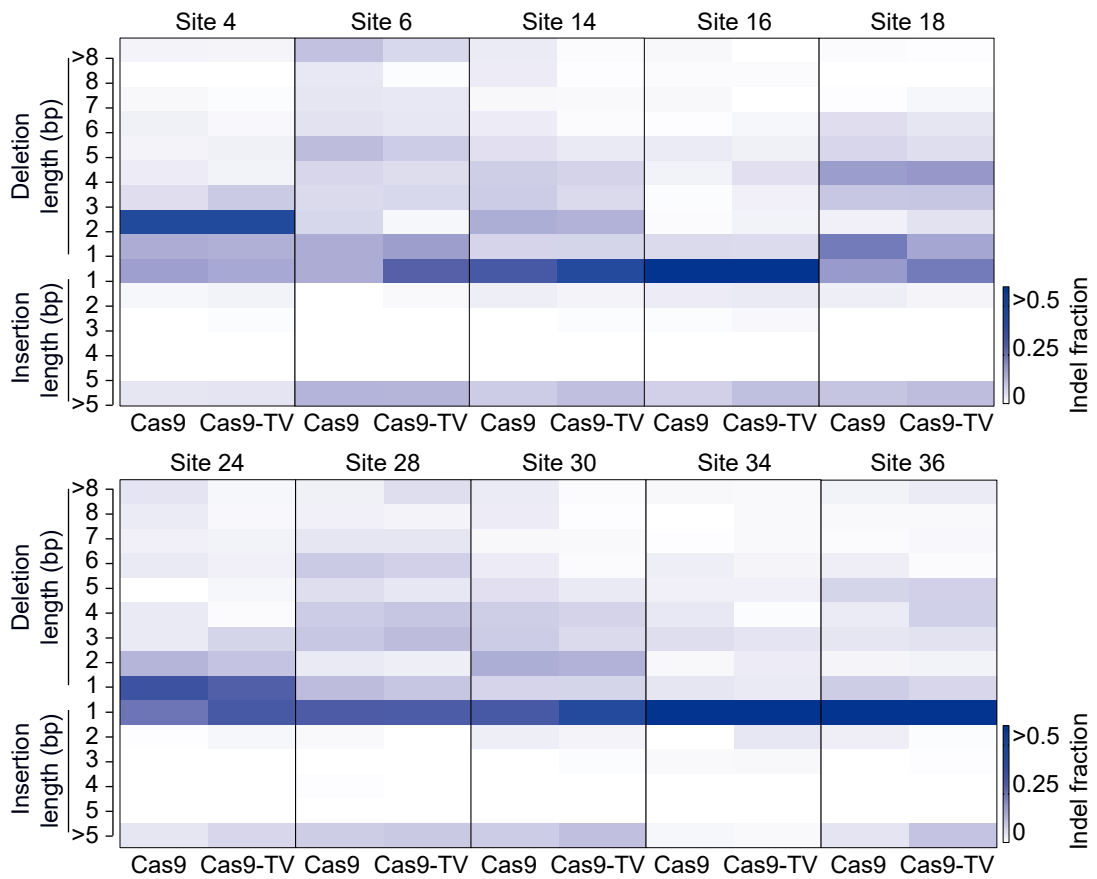


**Fig. S1** Agarose gels of the PCR products containing the 10 target sites shown in Fig. 2 after incubation with Cas9 ribonucleoprotein (RNP) complexes. Each experiment was performed three times (n=3).

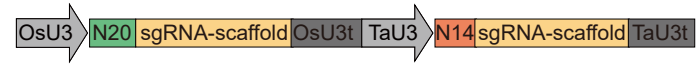


**Fig. S2** Indel patterns generated at the target sites by Cas9 and Cas9-TV. The graphs show representative results of one of three independent experiments. All three experiments gave similar results.

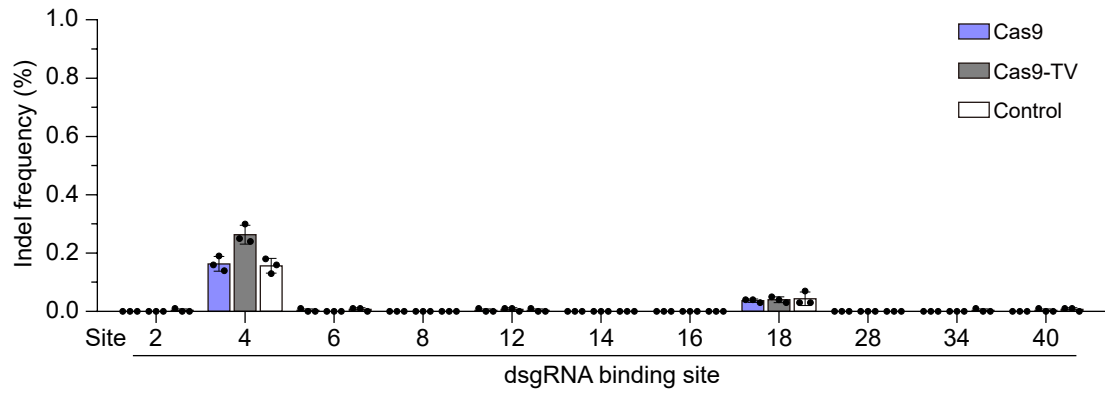
Cas9-TV



sgRNA-dsgRNA

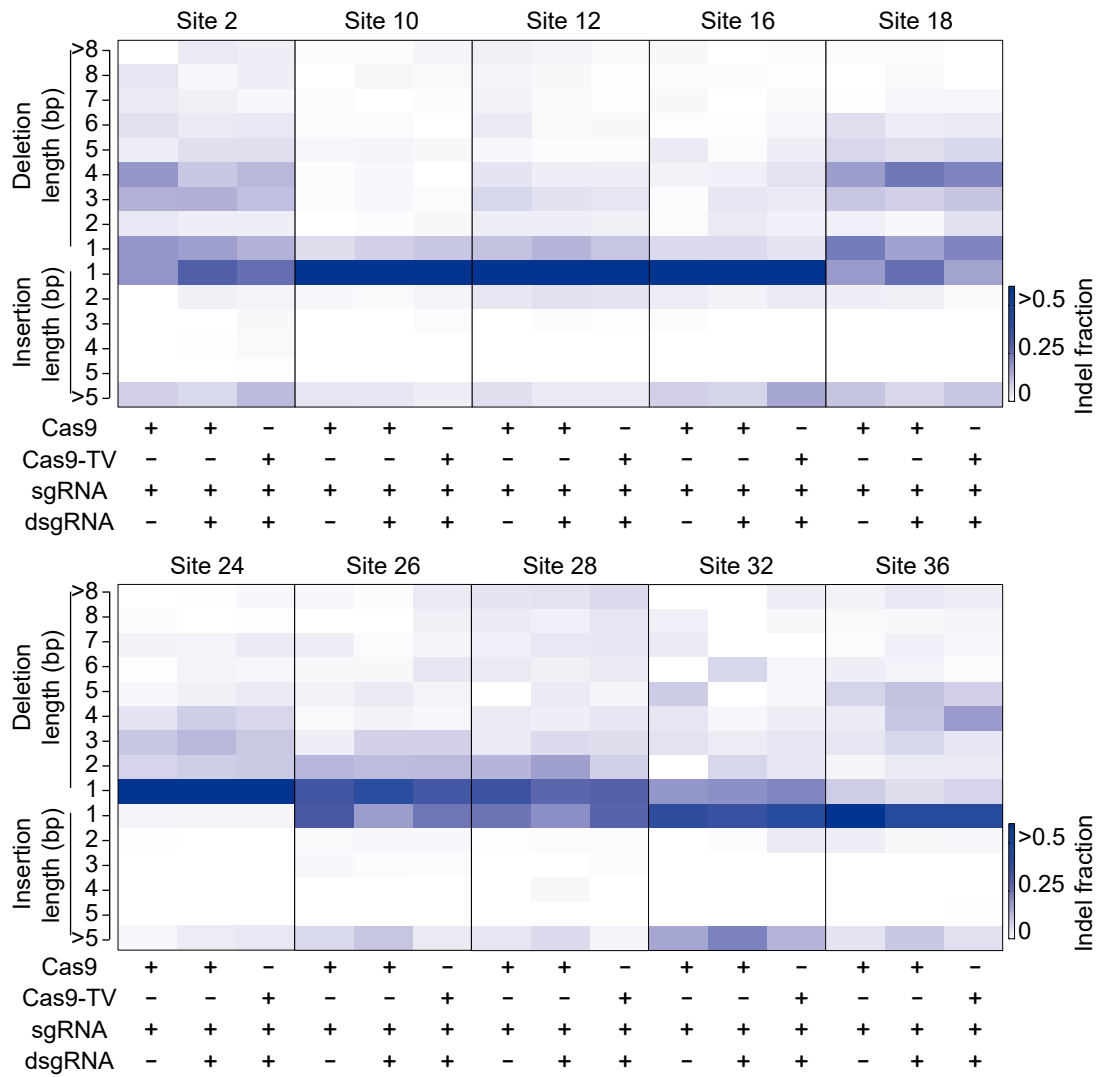


**Fig. S3** Diagrams of the Cas9-TV and sgRNA-dsgRNA constructs.



**Fig. S4** No indels detected at the dsgRNA target sites. The dsgRNAs were co-transformed into rice protoplasts with Cas9 or Cas9-TV, respectively. Indel frequencies were measured by sequencing targeted amplicons. An untreated protoplast sample was used as control. Data are from sets of three independent biological replicates ( $n = 3$ ) and are shown as means  $\pm$  s.e.m.





**Fig. S5** Indel patterns generated at the indicated target sites by Cas9, Cas9/dsgRNA and Cas9-TV/dsgRNA. The graphs show representative results of one of three independent experiments yielding similar results.

5' - GCTCTCCTGATTACTAGCCAGCGATGCATGCAGGGAAAAGAAATTTTCTACATATATAGAGAGAAAGAGAGATATATA-3'  
 |||  
 3' - CGAGAGGACTAATGATCGGGTCGCTACGTACGTCCCTTTCTTTAAAGGATGTATATATCTCTCTTCTCTCATATAT-5'  
**dsgRNA5 target site**

5' - TATCAAACGACATCATCACCATGTGCATATGTAGTTACCCTCAATCCCATCCTAGCAAGCATGGCCGGCTCTTCCCT-3'  
 |||  
 3' - ATAGTTTGCTGTAGTAGTGGTACACGTATACAATCAATGGGAGTTAAGGGTAGGATCGTTCGTACCGCCGAGAAGGA-5'

5' - CTCTTGATCGCTCGCTCACTACAGCTACCACTCCTCCTCTTTCTCCTCGTTGCCGCTGGGATAGGCAGCTACG-3'  
 |||  
 3' - GAGAACGTAGCGGAGCGAGTGATGTCGATGGTGGAGGAGGAAAAGGAGGAGCAACGGCGACGCTATCCGTCGATGC-5'  
**dsgRNA4 target site**

5' - ACCCCAAGGCGTTCTGTTCFAAGACGACAGACGTAGCATCGTGCCTTAAGGTGTTCCCGACACTCCCAGACATCGTCA-3'  
 |||  
 3' - TGGGGTTCCGCAAGACAAGGTTCTGCTGTCTGCATCGTAGCACGGAATCCACAAGGGCTGTGAGGGTCTGTAGCAGT-5'  
**dsgRNA3 target site** **target site 34**

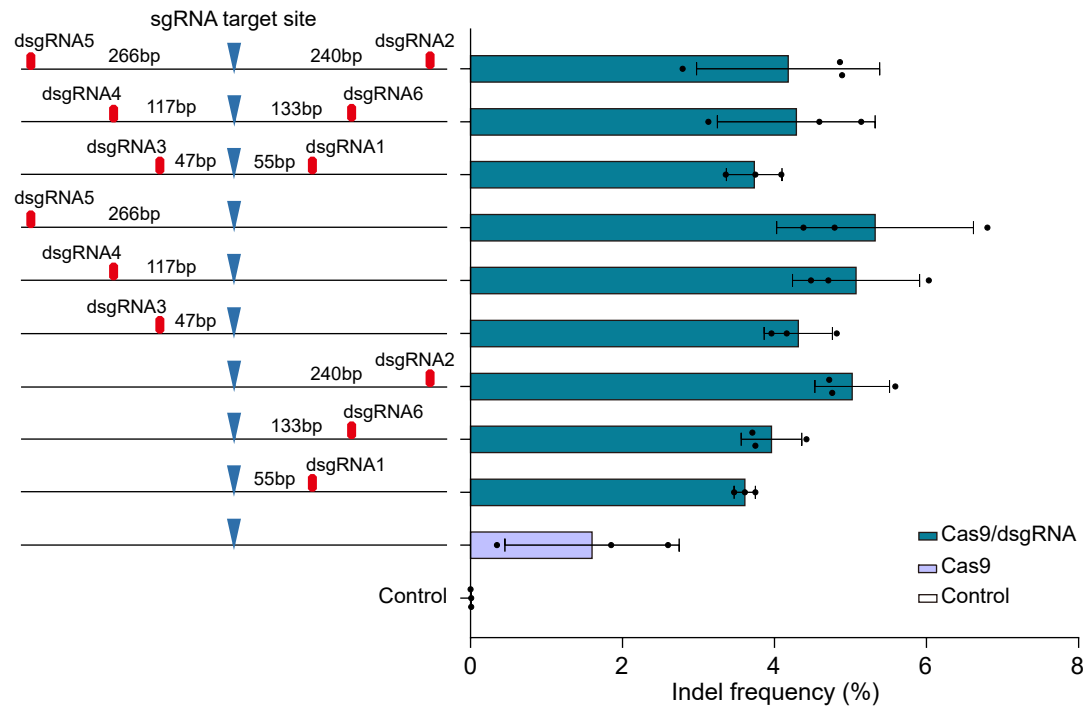
5' - CCAAGGCGCAGGACAACCAAGAGTTGTACAAAACGGCTGGTCCGCTACTGCTCATTCAGACCTACGAGCCACGTCGC-3'  
 |||  
 3' - GGTTCGGCGTCTGTGGTTCTCAACATGTTTGCCGACCAGGCGATGACGAGTAAGTCTGGATGCTCCGGTGCAGCG-5'  
**dsgRNA1 target site**

5' - TCGCGGAATCCATGATTGCCACCACCACCGCCGCAACCCCGGAATATCGCCACCTTCTTCGAGCAGTGGAAGGGG-3'  
 |||  
 3' - AGCGCCTTAGGTACTAACGGTGGTGGCGGGGTTGGGGCGCTTATAGCGGTGGAAGAAGCTCGTCACCTTTCCCC-5'  
**dsgRNA6 target site**

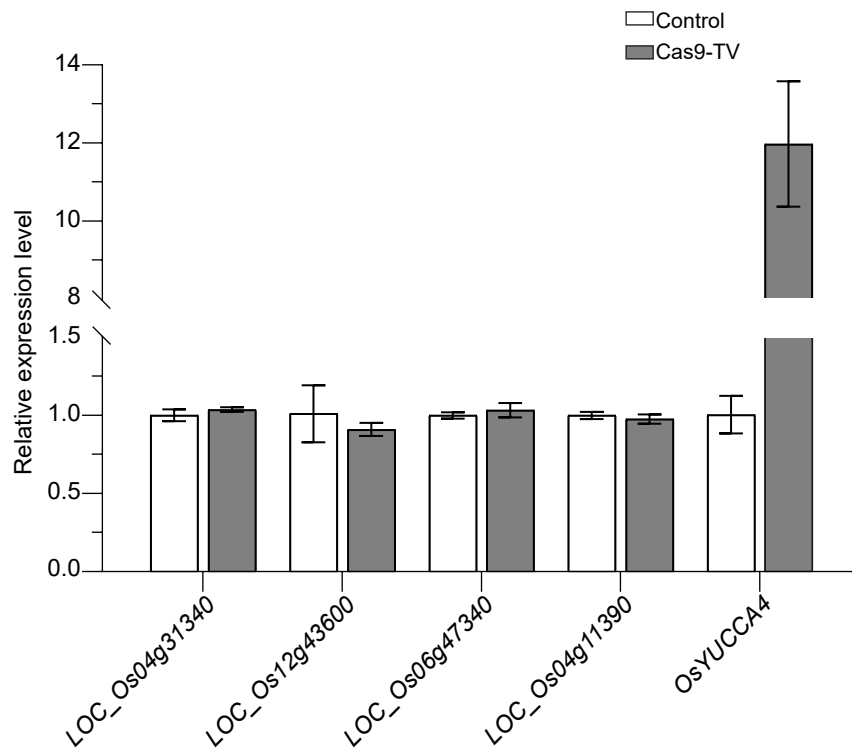
5' - ACGAGGCGATAACGACAAAAACCTCCAGGCAAGTGCTCTTGAGCTGCAACAAGACCATCGGCGAAGTTAACGCCA-3'  
 |||  
 3' - TGCTCCGCTATTGCTGTTTTGTGGAGGTCCGTTACGGAGAACTCGACGTTGTTCTGGTAGCCGCTTCAATTGCGGT-5'

5' - TCCTAACCTGCGGACACACCTACATGGAAGACAGGCCCCCATCATCCACCAGAACCTCACCGTCTTGTTCATGGCG-3'  
 |||  
 3' - AGGATTGGACGCCTGTGTGGATGTACCTTCTGTCCGGGGGTAGTAGGTGGTCTTGGAGTGGCAGAACAAGGTACCGC-5'  
**dsgRNA2 target site**

**Fig. S6** Partial genomic DNA sequence of *LOC\_Os11g08760* showing sgRNA and dsgRNA target sites. The green line indicates the sgRNA target site and the red lines the dsgRNAs target sites.



**Fig. S7** Effects of location of proximal dsgrNA on Cas9 editing activity. dsgrNA target sites and Cas9-TV target sites are separated from each other by a distance in bp indicated with a number respectively. An untreated protoplast sample was used as control. Indel frequencies were measured by sequencing targeted amplicons. Data are from sets of three independent biological replicates ( $n = 3$ ) and are shown as means  $\pm$  s.e.m.



**Fig. S8** Expression of target genes is not affected when Cas9-TV targets coding regions. Quantitative gene expression analysis were performed in rice protoplasts transformed with Cas9-TV and corresponding dsRNA. The dsRNAs for *LOC\_Os04g31340*, *LOC\_Os12g43600*, *LOC\_Os06g47340* and *LOC\_Os04g11390* are same as Fig. 4 and listed in Table S4 and target coding regions, while the dsRNA for *OsYUCCA4* targets its promoter region. An untreated protoplasts sample was used as control. Data were normalized to *Ubiquitin (Ubi)* expression and the expression level of each gene in the control sample was set at one unit. Error bars indicate SD of three replicates.

**Table S1** Summary of mutagenesis efficiency in rice T0 plants induced by CRISPR/Cas9 at different genomic loci

| Locus name     | Target sequence         | Positions                | NO. of transgenic lines | NO. of mutant lines | Mutation rate (%) | Chromatin states |
|----------------|-------------------------|--------------------------|-------------------------|---------------------|-------------------|------------------|
| LOC_Os03g08550 | ATGAGCCAAAACAACCTTGGTGG | chr3:4398956..4398978    | 22                      | 22                  | 100.00            | DH sites         |
| LOC_Os03g17020 | AGTGGGCGAGCGTCTCGTACGGG | chr3:9458718..9458740    | 13                      | 13                  | 100.00            | DH sites         |
| LOC_Os02g54600 | GAGCAGGATCTGGAGCTCGCCGG | chr2:33443338..33443360  | 30                      | 29                  | 96.70             | DH sites         |
| LOC_Os01g55540 | CATGGTGGGGAAAGCTTGGAGGG | chr1:32003246..32003268  | 42                      | 38                  | 90.50             | DH sites         |
| LOC_Os02g54600 | CCGGACGACGACGTCGACGACGG | chr2:33443645..33443667  | 30                      | 25                  | 83.30             | DH sites         |
| LOC_Os02g54600 | GACGTCGGCGAGGAAGGCCTCGG | chr2:33443272..33443294  | 30                      | 24                  | 80.00             | DH sites         |
| LOC_Os01g55540 | CGGACGACGACGCCATGGTGGGG | chr1:32003233..32003255  | 42                      | 33                  | 78.60             | DH sites         |
| LOC_Os05g49140 | GAGGGTGGTGAGGAATCCGATGG | chr5:28189687..28189709  | 21                      | 16                  | 76.20             | DH sites         |
| LOC_Os02g46760 | GCACGGACAGCCGCAGTGCCGG  | chr2:28552759..28552781  | 24                      | 17                  | 70.80             | DH sites         |
| LOC_Os02g48000 | AATTTAACCGTGATCAGAATGG  | chr2:29375178..29375200  | 25                      | 15                  | 60.00             | DH sites         |
| LOC_Os02g46760 | GGCCACGCGGAGGTGCGCGCCGG | chr2:28552992..28553014  | 24                      | 14                  | 58.30             | DH sites         |
| LOC_Os02g53700 | ATCAGCTGCAGTGCCTGCCAGG  | chr2:32864545..32864567  | 16                      | 8                   | 50.00             | DH sites         |
| LOC_Os01g04409 | AGAAGCAAGAGTCACATAATAGG | chr1:1959592..1959614    | 22                      | 9                   | 40.91             | DH sites         |
| LOC_Os03g05200 | AATTCATGGCCGCGCAGTTGGG  | chr3:2525483..2525505    | 44                      | 15                  | 34.09             | DH sites         |
| LOC_Os08g33370 | AACTATCTGTGGCAGGAGGACGG | chr8:20812965..20812987  | 23                      | 6                   | 26.09             | DH sites         |
| LOC_Os02g46760 | ACAGCATCTCGAGCTCCGCCAGG | chr2:28552858..28552880  | 24                      | 6                   | 25.00             | DH sites         |
| LOC_Os12g01200 | ATGATGTAAAATAAATAGCTGG  | chr12:114104..114126     | 24                      | 3                   | 12.50             | Non-DH sites     |
| LOC_Os10g25090 | AATATAGCAAACCACTCAGCTGG | chr10:12924597..12924616 | 27                      | 21                  | 77.78             | Non-DH sites     |
| LOC_Os01g47530 | AGCTTGAGAAAGATCGCATGCGG | chr1:27177431..27177453  | 153                     | 24                  | 13.70             | Non-DH sites     |
| LOC_Os02g53700 | AGACATCCGATGTACATTCCAGG | chr2:32862128..32862150  | 27                      | 3                   | 11.11             | Non-DH sites     |

|                |                         |                          |     |    |       |              |
|----------------|-------------------------|--------------------------|-----|----|-------|--------------|
| LOC_Os05g38950 | AAACAGGATTGCAAGAGTTAAGG | chr5:22838317..22838339  | 22  | 8  | 36.36 | Non-DH sites |
| LOC_Os03g08550 | AACCAATGTGGCACCTATAAAGG | chr3:4400430..4400452    | 16  | 3  | 18.75 | Non-DH sites |
| LOC_Os03g08550 | ATGGACAACCTTGTTGGGGAAGG | chr3:4400503..4400525    | 34  | 32 | 94.12 | Non-DH sites |
| LOC_Os10g25090 | AGAGAAAACGCAAAGGTACAAGG | chr10:12925639..12925661 | 14  | 13 | 92.86 | Non-DH sites |
| LOC_Os11g17080 | TGTGCGAATAGAGTTCCTCAAGG | chr11:9477665..9477687   | 12  | 9  | 75.00 | Non-DH sites |
| LOC_Os07g07194 | AAAGACCTGTTACAGGAATCCGG | chr7:3562066..3562088    | 17  | 7  | 41.18 | Non-DH sites |
| LOC_Os07g07194 | ACTACGAACCAATTGGCTCATGG | chr7:3561913..3561935    | 44  | 20 | 45.45 | Non-DH sites |
| LOC_Os06g26340 | GAAAATATCTGACATGCATGCGG | chr6:15417435..15417457  | 23  | 7  | 30.40 | Non-DH sites |
| LOC_Os02g49070 | AGCAGTATCTCTAGCGCTTCAGG | chr2:30000058..30000080  | 44  | 30 | 68.18 | Non-DH sites |
| LOC_Os02g57080 | ACGCGTCTACCATGGGACCATGG | chr2:34947055..34947077  | 20  | 7  | 60.00 | Non-DH sites |
| LOC_Os02g06930 | TATTGCTTGCTTTGATATCCCGG | chr2:3506669..3506691    | 48  | 27 | 56.30 | Non-DH sites |
| LOC_Os05g05160 | GATATAATTACAGATCTACTAGG | chr5:2515783..2515805    | 109 | 20 | 18.30 | Non-DH sites |
| LOC_Os08g33370 | ATCTCCGAGTTGGATACCCTCGG | chr8:20813145..20813167  | 15  | 9  | 60.00 | Non-DH sites |
| LOC_Os02g06930 | CTATCTTTAGAGTCAAGACAGGG | chr2:3506268..3506290    | 48  | 19 | 39.60 | Non-DH sites |
| LOC_Os02g04230 | AACCACATAATTCAGGAGCTCGG | chr2:1853822..1853844    | 48  | 8  | 16.70 | Non-DH sites |
| LOC_Os10g38950 | AGGGCTAAAATATGTGCACTCGG | chr10:20758294..20758316 | 96  | 14 | 14.60 | Non-DH sites |
| LOC_Os02g53700 | AAAATCCTCTGAATCAAGTGTGG | chr2:32865365..32865387  | 64  | 39 | 60.94 | Non-DH sites |
| LOC_Os04g56160 | AACCTGTTGGTCCTCTTGATCGG | chr4:33461383..33461405  | 48  | 11 | 22.92 | Non-DH sites |
| LOC_Os01g32660 | GTGGTATCGTCCAAGTTCGG    | chr1:17906168..17906190  | 87  | 27 | 31.00 | Non-DH sites |
| LOC_Os04g38600 | CTCATCACCGCCCCGGCAAGGG  | chr4:22937954..22937976  | 42  | 11 | 26.20 | Non-DH sites |
| LOC_Os06g48590 | CTGAATATGTCGTGACCCGGTGG | chr6:29401605..29401627  | 128 | 29 | 22.70 | Non-DH sites |
| LOC_Os11g17080 | ACTGCGACACCCAGATATCGTGG | chr11:9472567..9472589   | 12  | 9  | 75.00 | Non-DH sites |
| LOC_Os05g34070 | GTAGCAGATAGCTTAGTAGATGG | chr5:20128891..20128913  | 36  | 24 | 66.70 | Non-DH sites |
| LOC_Os04g38600 | GCAGCGACGACGAGCTCCTCAGG | chr4:22937368..22937390  | 42  | 21 | 50.00 | Non-DH sites |
| LOC_Os01g45620 | CGTCGAAGGACACTCGAGCAAGG | chr1:25922196..25922218  | 133 | 41 | 30.80 | Non-DH sites |

|                |                          |                          |     |    |       |              |
|----------------|--------------------------|--------------------------|-----|----|-------|--------------|
| LOC_Os08g33370 | ATCTACTGCTGCAGAATCTAAGG  | chr8:20814142..20814164  | 22  | 4  | 18.18 | Non-DH sites |
| LOC_Os04g56160 | AGCTGAAATGTACGCGCGAGGGG  | chr4:33459738..33459760  | 22  | 5  | 22.73 | Non-DH sites |
| LOC_Os10g35450 | AAGCCACGCGATATCGGTGCAGG  | chr10:18971698..18971720 | 40  | 2  | 5.00  | Non-DH sites |
| LOC_Os10g35450 | AAACATTACGAGAAAGATTTCAGG | chr10:18971723..18971745 | 38  | 10 | 26.32 | Non-DH sites |
| LOC_Os10g35450 | AAGGCAGCTCGGGCAATGCTAGG  | chr10:18971856..18971878 | 24  | 7  | 29.17 | Non-DH sites |
| LOC_Os04g59320 | ATGACGTTGTTGGAAGCTGCAGG  | chr4:35271894..35271916  | 44  | 15 | 34.10 | Non-DH sites |
| LOC_Os04g59320 | AAGTGCTTCTTCGGTGTACAAGG  | chr4:35272085..35272107  | 13  | 2  | 15.38 | Non-DH sites |
| LOC_Os06g49430 | GTCGTCGGCGACCTCGAGGTCGG  | chr6:29950247..29950269  | 13  | 9  | 69.20 | Non-DH sites |
| LOC_Os03g11614 | GCAGAGGAGGGAGAGCTCGTAGG  | chr3:6061158..6061180    | 78  | 53 | 67.90 | Non-DH sites |
| LOC_Os03g11614 | CTCGAAGAGGCGGCCGCGGCCGG  | chr3:6061107..6061129    | 78  | 51 | 65.40 | Non-DH sites |
| LOC_Os10g30860 | GAAGAGGACCCTCCCGGCGAAGG  | chr10:16092786..16092808 | 83  | 45 | 54.10 | Non-DH sites |
| LOC_Os07g14700 | GATGGTCTCCCGGTGTCGTAGG   | chr7:8382918..8382940    | 112 | 46 | 40.10 | Non-DH sites |
| LOC_Os07g14700 | CAAGAACACCAAGGACCTGGAGG  | chr7:8382683..8382705    | 112 | 39 | 34.80 | Non-DH sites |
| LOC_Os06g09180 | CCGGAGTTCAGAAGCTTCGTCCG  | chr6:4617592..4617614    | 94  | 16 | 17.00 | Non-DH sites |
| LOC_Os01g04409 | AGGTACCCATTCTGGCTAGGCCG  | chr1:1967198..1967220    | 79  | 1  | 1.30  | Non-DH sites |
| LOC_Os01g62430 | AACGACTTCCTAGGTGAGATAGG  | chr1:36136005..36136027  | 44  | 2  | 4.55  | Non-DH sites |
| LOC_Os03g14260 | ATGTACAAATCTGCTCTTGAGGG  | chr3:7762634..7762656    | 14  | 13 | 92.86 | Non-DH sites |
| LOC_Os03g21540 | CCTCAGGCTCCACTTCTACCCGG  | chr3:12309229..12309251  | 12  | 1  | 8.33  | Non-DH sites |
| LOC_Os03g21540 | ATCTCCCCCTCCAAGGACACGGG  | chr3:12309614..12309636  | 21  | 8  | 38.10 | Non-DH sites |
| LOC_Os01g56330 | CCTCAGGCTCCACTTCTACCCGG  | chr1:32462465..32462487  | 12  | 1  | 8.33  | Non-DH sites |
| LOC_Os01g56330 | ATCTCCCCCTCCAAGGACACGGG  | chr1:32462850..32462872  | 21  | 6  | 28.57 | Non-DH sites |
| LOC_Os06g26340 | TCAGCATCATGTTTCTAGAGAGG  | chr6:15415163..15415185  | 23  | 14 | 60.90 | Non-DH sites |
| LOC_Os02g49070 | ATAGATTCCATGCTTGAGATTGG  | chr2:30000475..30000497  | 28  | 23 | 82.14 | Non-DH sites |
| LOC_Os05g49140 | GATGGATACAGACCTCCACCAGG  | chr5:28192421..28192443  | 21  | 6  | 28.60 | Non-DH sites |
| LOC_Os02g49070 | AAGTCAAACAAGCCTGTTCTAGG  | chr2:30001366..30001388  | 34  | 31 | 91.18 | Non-DH sites |

**Table S2** Information of the 40 target sites chosen

| sgRNA   | Target site | Locus name     | Target sequence          | Region   | Chromatin state  |
|---------|-------------|----------------|--------------------------|----------|------------------|
| sgRNA1  | Site 1      | LOC_Os04g31340 | GCACACATGTCGTCGTACGGTGG  | promoter | open chromatin   |
| sgRNA2  | Site 2      |                | GCACGGCTGGTTCTGCCGTAGGG  | exon     |                  |
| sgRNA3  | Site 3      | LOC_Os04g33830 | TCAGCTAGTAGTTCCTACCGAGG  | promoter | open chromatin   |
| sgRNA4  | Site 4      |                | ACGAGGAACCTGGAGAGGAACGG  | exon     |                  |
| sgRNA5  | Site 5      | LOC_Os04g58720 | TACTATTCAGTCCAGCCGCGCGG  | promoter | open chromatin   |
| sgRNA6  | Site 6      |                | CCTTGTCCCTTGATAGCCGTGCGG | exon     |                  |
| sgRNA7  | Site 7      | LOC_Os04g41560 | CCATCACACAAACCGCCGCGCGG  | promoter | open chromatin   |
| sgRNA8  | Site 8      |                | GGCCACGGTGGTGTGCTGCGCGG  | exon     |                  |
| sgRNA9  | Site 9      | LOC_Os04g42020 | CTACCTACCATGCCTCGCCGCGG  | promoter | open chromatin   |
| sgRNA10 | Site 10     |                | CCCGAGCTCGACATCACCGGCGG  | exon     |                  |
| sgRNA11 | Site 11     | LOC_Os02g02890 | TCCCTAACCCAACCACCCACGG   | promoter | open chromatin   |
| sgRNA12 | Site 12     |                | TGTACGCGAAGGACGTGCCGCGG  | exon     |                  |
| sgRNA13 | Site 13     | LOC_Os05g41060 | GCCCTCACCACAAACCCACGTGG  | promoter | open chromatin   |
| sgRNA14 | Site 14     |                | TCTCCTTCTTGCGAAGAGGCGG   | exon     |                  |
| sgRNA15 | Site 15     | LOC_Os12g43600 | GTGCCTTTGGAATATACGGGGGG  | promoter | open chromatin   |
| sgRNA16 | Site 16     |                | CGAGGCCGCCGACGAAGCAGCGG  | exon     |                  |
| sgRNA17 | Site 17     | LOC_Os06g47340 | TCACCTCACCCGCACGACGACGG  | promoter | open chromatin   |
| sgRNA18 | Site 18     |                | GCAGCACCGTTCTACCAAGGGG   | exon     |                  |
| sgRNA19 | Site 19     | LOC_Os08g03290 | TGTCATTAGCTAGTGACGCGCGG  | promoter | open chromatin   |
| sgRNA20 | Site 20     |                | GAGTATGTCGTGGAGTCCACCGG  | exon     |                  |
| sgRNA21 | Site 21     | LOC_Os04g22730 | GGTGGGGTGGGACTTATGGATGG  | promoter | closed chromatin |
| sgRNA22 | Site 22     |                | AGGGGTGTATCGATGATGCCCGG  | exon     |                  |
| sgRNA23 | Site 23     | LOC_Os04g18650 | GAGATGATGCGTGTGCCGGCCGG  | promoter | closed chromatin |



|         |         |                |                         |          |                  |
|---------|---------|----------------|-------------------------|----------|------------------|
| sgRNA24 | Site 24 |                | ACGGCCGCCTCCGTACGCCGCGG | exon     |                  |
| sgRNA25 | Site 25 | LOC_Os04g11390 | GGAAGGAGGACCTATTACAGG   | promoter | closed chromatin |
| sgRNA26 | Site 26 |                | GGAACAGCCTCATCCCTTGGCGG | exon     |                  |
| sgRNA27 | Site 27 | LOC_Os04g12220 | GGGATACCTGTGACGAGCCGCGG | promoter | closed chromatin |
| sgRNA28 | Site 28 |                | GTCTTTGGACGTAGCCATGGTGG | exon     |                  |
| sgRNA29 | Site 29 | LOC_Os04g13530 | GCTGTGGAGTTGAAGCACAGCGG | promoter | closed chromatin |
| sgRNA30 | Site 30 |                | TGTGCTGCCGAACATCAACCCGG | exon     |                  |
| sgRNA31 | Site 31 | LOC_Os04g13640 | CCAAGCATCTCAGCACCGGCGGG | promoter | closed chromatin |
| sgRNA32 | Site 32 |                | CCATGACATTGTCCGAGCCACGG | exon     |                  |
| sgRNA33 | Site 33 | LOC_Os11g08760 | ATTACTCCGCATGTGACCACCGG | promoter | closed chromatin |
| sgRNA34 | Site 34 |                | AGACATCGTCACCAAGGCGCAGG | exon     |                  |
| sgRNA35 | Site 35 | LOC_Os07g43820 | TCATCCCTCGCCATCGGCGGCGG | promoter | closed chromatin |
| sgRNA36 | Site 36 |                | CTCGTCCCGACCAACCAGTCGG  | exon     |                  |
| sgRNA37 | Site 37 | LOC_Os09g24280 | ACACTCAACCAGATGTTACGTGG | promoter | closed chromatin |
| sgRNA38 | Site 38 |                | TGGGTAATGGTGATATCCCATGG | exon     |                  |
| sgRNA39 | Site 39 | LOC_Os07g43850 | ACGTATCGTCGAACTTGTACCGG | promoter | closed chromatin |
| sgRNA40 | Site 40 |                | CGACGATGACCATGTCCACGTGG | exon     |                  |

**Table S3** Each of the chosen sgRNAs targets two genomic sites with opposite chromatin states

|         | Target site | Target sequence         | Positon                 | Locus name     | Region     | Chromatin states |
|---------|-------------|-------------------------|-------------------------|----------------|------------|------------------|
| sgRNA A | A1          | ACGCAAACGAATCGAGGAGTAGG | chr1:209699..209721     | --             | Intergenic | DH sites         |
|         | A2          | ACGCAAACGAATCGAGGAGTAGG | chr2:33815506..33815528 | --             | Intergenic | Non-DH sites     |
| sgRNA B | B1          | ATCGCCATCGCCATCGCCGCAGG | chr9:20073880..20073858 | LOC_Os09g34000 | CDS        | DH site          |
|         | B2          | ATCGCCATCGCCATCGCCGCCGG | chr1:23313634..23313612 | LOC_Os01g41180 | CDS        | non-DH site      |
| sgRNA C | C1          | CGAAGGCGGCGGCGGCGATCTGG | chr1:1179280..1179299   | LOC_Os01g03070 | UTR        | DH sites         |
|         | C2          | CGAAGGCGGCGGCGGCGATCCGG | chr7:22797802..22797824 | LOC_Os07g37990 | CDS        | Non-DH sites     |
| sgRNA D | D1          | TCTTCTTCTAATCTTGCTGTTGG | chr1:1103803..1103825   | LOC_Os01g02960 | UTR        | DH sites         |
|         | D2          | TCTTCTTCTAATCTTGCTGTTGG | chr1:1319901..1319923   | --             | Intergenic | Non-DH sites     |
| sgRNA E | E1          | GTTGCTGCTGCTGCTGCGATGG  | chr1:1791437..1791458   | LOC_Os01g04110 | UTR        | DH sites         |
|         | E2          | GTTGCTGCTGCTGCTGCGACGG  | chr1:198166..198187     | LOC_Os01g01390 | CDS        | Non-DH sites     |

**Table S4** The chosen sgRNAs and their corresponding proximal dsRNA targeting sites

| sgRNA <sup>a</sup> | dsRNA target sequence <sup>b</sup> | Distance <sup>c</sup> |
|--------------------|------------------------------------|-----------------------|
| sgRNA 2            | GACATCATCTGGCAGGG                  | 50bp                  |
| sgRNA 4            | TGCAGGCTTCACGACGG                  | 32bp                  |
| sgRNA 6            | TGACCTGATGCCCAAGG                  | 55bp                  |
| sgRNA 8            | GCGCTGGTGCTTGCTGG                  | 57bp                  |
| sgRNA 10           | CTTCGCGCGCTCCATGG                  | 35bp                  |
| sgRNA 12           | GGCGTGGGCAAGAGCGG                  | 39bp                  |
| sgRNA 14           | TACAAGCTCAAGCTCGG                  | 50bp                  |
| sgRNA 16           | GGACCTTGGACTCGAGG                  | 55bp                  |
| sgRNA 18           | ACCTGATTGGGTGAAGG                  | 60bp                  |
| sgRNA 20           | TATGGTAGCGAGCGTGG                  | 68bp                  |
| sgRNA 22           | AACAGCTAGGCTCTTGG                  | 39bp                  |
| sgRNA 24           | ACTGCAGGCGCTGCAGG                  | 59bp                  |
| sgRNA 26           | ACTCATCGGTGTGTAGG                  | 92bp                  |
| sgRNA 28           | GTTGATGGACGAGGTGG                  | 61bp                  |
| sgRNA 30           | AGCAGCACGTGCCTCGG                  | 62bp                  |
| sgRNA 32           | GGCCAACCTGAACGACGG                 | 56bp                  |
| sgRNA 34           | GGCCACGTCGCTCGCGG                  | 55bp                  |
| sgRNA 36           | CCGATGCAGCCCACCGG                  | 66bp                  |
| sgRNA 38           | GCGCATTAGACCAAGGG                  | 83bp                  |
| sgRNA 40           | GGCGCGACCAACCACGG                  | 40bp                  |

a, sgRNAs are same to table S2; b, 14nt guide sequence + PAM; c, dsRNA target sites and Cas9-TV target sites are separated from each other by a distance in bp indicated with a number.

**Table S5** dsgrRNA target sequences and their distances to sgRNA34 target site

|                | dsgrRNA    | dsgrRNA target sequence <sup>a</sup> | Distance <sup>b</sup> |
|----------------|------------|--------------------------------------|-----------------------|
| Single dsgrRNA | dsgrRNA1   | GGCCACGTCGCTCGCGG                    | 55                    |
|                | dsgrRNA2   | CCTACATGGAAGACAGG                    | 240                   |
|                | dsgrRNA3   | GGAACAGAACGCCTTGG                    | 47                    |
|                | dsgrRNA4   | AGCTGTAGTGAGCGAGG                    | 117                   |
|                | dsgrRNA5   | CAGCGATGCATGCAGGG                    | 266                   |
|                | dsgrRNA6   | GTGGAAAGGGGACGAGG                    | 133                   |
| Paired dsgrRNA | dsgrRNA1&3 | GGCCACGTCGCTCGCGG                    | 55                    |
|                |            | GGAACAGAACGCCTTGG                    | 47                    |
|                | dsgrRNA2&5 | CCTACATGGAAGACAGG                    | 240                   |
|                |            | CAGCGATGCATGCAGGG                    | 266                   |
|                | dsgrRNA4&6 | GGAACAGAACGCCTTGG                    | 117                   |
|                |            | GTGGAAAGGGGACGAGG                    | 133                   |

a, 14nt guide sequence + PAM; b, dsgrRNA target sites and Cas9-TV target sites are separated from each other by a distance in bp indicated with a number.

**Table S6** Potential off-target sites for the four sgRNAs identified in rice genome

| Target site | Sequence <sup>a</sup>                             | Target gene loci |
|-------------|---|------------------|
| Site 24     | ACGGCCGCCTCCGTACGCCG <b>CGG</b>                   | LOC_Os04g18650   |
| OT24-1      | ACGGCCGC <b>T</b> TCCGCACGCCG <b>CGG</b>          | LOC_Os03g05590   |
| OT24-2      | <b>CCGCT</b> CGCC <b>CC</b> GTACGCCG <b>CGG</b>   | LOC_Os06g11400   |
| OT24-3      | <b>G</b> CGGCCGC <b>GG</b> CCGTACGC <b>TGGG</b>   | LOC_Os01g73410   |
| Site 28     | GTCTTTGGACGTAGCCATGG <b>TGG</b>                   | LOC_Os04g12220   |
| OT28-1      | GTCTTTG <b>CAC</b> ATAGCCATGG <b>CGG</b>          | LOC_Os05g04110   |
| OT28-2      | GTCTTT <b>TGAT</b> GCAGCA <b>ATGGAGG</b>          | LOC_Os01g56140   |
| OT28-3      | GT <b>TTTT</b> GGAC <b>T</b> TAGCCA <b>AGGAGG</b> | LOC_Os04g57390   |
| Site 34     | AGACATCGTCACCAAGGCGC <b>AGG</b>                   | LOC_Os11g08760   |
| OT34-1      | <b>CGACGCCG</b> ACACCAAGGCGC <b>TGG</b>           | LOC_Os04g56110   |
| OT34-2      | <b>GGACG</b> TCCTC <b>G</b> CCAAGGCGC <b>AGG</b>  | LOC_Os09g38050   |
| OT34-3      | <b>GGACATCGTC</b> <b>GTCG</b> AGGCGC <b>TGG</b>   | LOC_Os04g32010   |
| OT34-4      | <b>CGACG</b> TCGT <b>G</b> ACCAAG <b>TGCCGG</b>   | LOC_Os11g04940   |
| OT34-5      | AG <b>TCAT</b> CCTCA <b>ACAAGGCCAGG</b>           | LOC_Os02g14059   |
| Site 38     | TGGGTAATGGTGATATCCCAT <b>TGG</b>                  | LOC_Os09g24280   |
| OT38-1      | <b>TAGGTGATGATGATATACCAAGG</b>                    | LOC_Os12g29220   |
| OT38-2      | <b>TAGGTAGTTGTGATATCACAGGG</b>                    | LOC_Os12g39430   |
| OT38-3      | TGGGT <b>GATGATGATATCCATCGG</b>                   | LOC_Os03g37411   |
| OT38-4      | <b>TATGTGATGGTGATATCCTACGG</b>                    | LOC_Os12g40790   |

a, The mismatch bases are shown in red. The PAM motif is written in bold.

**Table S7** Primers used in this study.

| Primer name | Primer sequence (5'-3')                         | Application                                     |
|-------------|---|---|
| Cas9-F      | GAGGATCCCCATGGCCCCTAAGAAGAAGAGAAAG              | Constructing 163-Cas9-TV                        |
| Cas9-64-R   | TCCAGGGCGTCTGAGCCCCTAGGCTTCTTCTTCGCTTG<br>TCCTG |   |
| 64-Cas9-F   | GCGAAGAAGAAGAAGCCTAGGGGCTCAGACGCCCTG            |   |
| 64-R        | CGCAATTGCTACCTAGCGAGCATGTCGAGGTCGAAG            |   |
| S1-SG-F     | GGCAGCACACATGTCGTCGTACGG                        | Constructing vector expressing sgRNA for Site 1 |
| S1-SG-R     | AAACCCGTACGACGACATGTGTGC                        |   |
| S2-SG-F     | GGCAGCACGGCTGGTTCTGCCGTA                        | Constructing vector expressing sgRNA for Site 2 |
| S2-SG-R     | AAACTACGGCAGAACCAGCCGTGC                        |   |
| S3-SG-F     | GGCATCAGCTAGTAGTTCCTACCG                        | Constructing vector expressing sgRNA for Site 3 |
| S3-SG-R     | AAACCGGTAGGAACTACTAGCTGA                        |   |
| S4-SG-F     | GGCACGAGGAACCTGGAGAGGAA                         | Constructing vector expressing sgRNA for Site 4 |
| S4-SG-R     | AAACTTCCTCTCCAGGTTCTCG                          |   |
| S5-SG-F     | GGCATACTATTCAGTCCAGCCGCG                        | Constructing vector expressing sgRNA for Site 5 |
| S5-SG-R     | AAACCGCGGCTGGACTGAATAGTA                        |   |
| S6-SG-F     | GGCACCTTGTCCTTGATAGCCGTG                        | Constructing vector expressing sgRNA for Site 6 |
| S6-SG-R     | AAACCACGGCTATCAAGGACAAGG                        |   |
| S7-SG-F     | GGCACCATCACACAAACCGCCGCG                        | Constructing vector expressing sgRNA for Site 7 |
| S7-SG-R     | AAACCGCGGCGGTTTGTGTGATGG                        |   |
| S8-SG-F     | GGCAGGCCACGGTGGTGTGCTGCG                        | Constructing vector expressing sgRNA for Site 8 |
| S8-SG-R     | AAACCGCAGCACACCACCGTGGCC                        |   |
| S9-SG-F     | GGCACTACCTACCATGCCTCGCCG                        | Constructing vector expressing sgRNA for Site 9 |
| S9-SG-R     | AAACCGGCGAGGCATGGTAGGTAG                        |   |

|          |                           |  |
|----------|---------------------------|--|
| S10-SG-F | GGCACCCGAGCTCGACATCACCGG  | Constructing vector expressing sgRNA for Site 10 |
| S10-SG-R | AAACCCGGTGATGTCGAGCTCGGG  |  |
| S11-SG-F | GGCATCCCTAACCCAACCACCCCA  | Constructing vector expressing sgRNA for Site 11 |
| S11-SG-R | AAACTGGGGTGGTTGGGTTAGGGA  |  |
| S12-SG-F | GGCATGTACGCGAAGGACGTGCCG  | Constructing vector expressing sgRNA for Site 12 |
| S12-SG-R | AAACCGGCACGTCCTTCGCGTACA  |  |
| S13-SG-F | GGCAGCCCTCACCACAAACCCACG  | Constructing vector expressing sgRNA for Site 13 |
| S13-SG-R | AAACCGTGGGTTTGTGGTGAGGGC  |  |
| S14-SG-F | GGCATCTCCTTCTTGGCGAAGAGG  | Constructing vector expressing sgRNA for Site 14 |
| S14-SG-R | AAACCCTCTTCGCCAAGAAGGAGA  |  |
| S15-SG-F | GGCAGTGCCTTTGGAATATACGGG  | Constructing vector expressing sgRNA for Site 15 |
| S15-SG-R | AAACCCCGTATATTCCAAAGGCAC  |  |
| S16-SG-F | GGCACGAGGCCCGCCGACGAAGCAG | Constructing vector expressing sgRNA for Site 16 |
| S16-SG-R | AAACCTGCTTCGTCGGCGGCCTCG  |  |
| S17-SG-F | GGCATCACCTCACCCGCACGACGA  | Constructing vector expressing sgRNA for Site 17 |
| S17-SG-R | AAACTCGTCGTGCGGGTGAGGTGA  |  |
| S18-SG-F | GGCAGCAGCACCGGTTCTACCAAG  | Constructing vector expressing sgRNA for Site 18 |
| S18-SG-R | AAACCTTGGTAGAACCGGTGCTGC  |  |
| S19-SG-F | GGCATGTCATTAGCTAGTGACGCG  | Constructing vector expressing sgRNA for Site 19 |
| S19-SG-R | AAACCGCGTCACTAGCTAATGACA  |  |
| S20-SG-F | GGCAGAGTATGTCGTGGAGTCCAC  | Constructing vector expressing sgRNA for Site 20 |
| S20-SG-R | AAACGTGGACTCCACGACATACTC  |  |
| S21-SG-F | GGCAGGTGGGGTGGGACTTATGGA  | Constructing vector expressing sgRNA for Site 21 |
| S21-SG-R | AAACTCCATAAGTCCCACCCACC   |  |
| S22-SG-F | GGCAGGGGTGTATCGATGATGCC   | Constructing vector expressing sgRNA for Site 22 |

|          |                          |  |
|----------|--------------------------|--|
| S22-SG-R | AAACGGCATCATCGATACACCCCT |  |
| S23-SG-F | GGCAGAGATGATGCGTGTGCCGGC | Constructing vector expressing sgRNA for Site 23 |
| S23-SG-R | AAACGCCGGCACACGCATCATCTC |  |
| S24-SG-F | GGCACGGCCGCCTCCGTACGCCG  | Constructing vector expressing sgRNA for Site 24 |
| S24-SG-R | AAACCGGCGTACGGAGGCGGCCG  |  |
| S25-SG-F | GGCAGGACTGGAGGACCTATTAC  | Constructing vector expressing sgRNA for Site 25 |
| S25-SG-R | AAACGTGAATAGGTCCTCCAGTCC |  |
| S26-SG-F | GGCAGGAACAGCCTCATCCCTTGG | Constructing vector expressing sgRNA for Site 26 |
| S26-SG-R | AAACCCAAGGGATGAGGCTGTTCC |  |
| S27-SG-F | GGCAGGGATACCTGTGACGAGCCG | Constructing vector expressing sgRNA for Site 27 |
| S27-SG-R | AAACCGGCTCGTCACAGGTATCCC |  |
| S28-SG-F | GGCAGTCTTTGGACGTAGCCATGG | Constructing vector expressing sgRNA for Site 28 |
| S28-SG-R | AAACCCATGGCTACGTCCAAAGAC |  |
| S29-SG-F | GGCAGCTGTGGAGTTGAAGCACAG | Constructing vector expressing sgRNA for Site 29 |
| S29-SG-R | AAACCTGTGCTTCAACTCCACAGC |  |
| S30-SG-F | GGCATGTGCTGCCGAACATCAACC | Constructing vector expressing sgRNA for Site 30 |
| S30-SG-R | AAACGGTTGATGTTCGGCAGCACA |  |
| S31-SG-F | GGCACCAAGCATCTCAGCACCGGC | Constructing vector expressing sgRNA for Site 31 |
| S31-SG-R | AAACGCCGGTGCTGAGATGCTTGG |  |
| S32-SG-F | GGCACCATGACATTGTCCGAGCCA | Constructing vector expressing sgRNA for Site 32 |
| S32-SG-R | AAACTGGCTCGGACAATGTCATGG |  |
| S33-SG-F | GGCATTACTCCGCATGTGACCAC  | Constructing vector expressing sgRNA for Site 33 |
| S33-SG-R | AAACGTGGTCACATGCGGAGTAA  |  |
| S34-SG-F | GGCAGACATCGTCACCAAGGCGC  | Constructing vector expressing sgRNA for Site 34 |
| S34-SG-R | AAACGCGCCTTGGTGACGATGTC  |  |



|          |                          |   |
|----------|--------------------------|---|
| S35-SG-F | GGCATCATCCCTCGCCATCGGCGG | Constructing vector expressing sgRNA for Site 35  |
| S35-SG-R | AAACCCGCCGATGGCGAGGGATGA |   |
| S36-SG-F | GGCACTCGCTCCCGACCAACCAGT | Constructing vector expressing sgRNA for Site 36  |
| S36-SG-R | AAACTGGTTGGTCGGGAGCGAG   |   |
| S37-SG-F | GGCACACTCAACCAGATGTTACG  | Constructing vector expressing sgRNA for Site 37  |
| S37-SG-R | AAACCGTAACATCTGGTTGAGTG  |   |
| S38-SG-F | GGCATGGGTAATGGTGATATCCCA | Constructing vector expressing sgRNA for Site 38  |
| S38-SG-R | AAACTGGGATATCACCATTACCCA |   |
| S39-SG-F | GGCACGTATCGTCGAACTTGAC   | Constructing vector expressing sgRNA for Site 39  |
| S39-SG-R | AAACGTACAAGTTCGACGATACG  |   |
| S40-SG-F | GGCACGACGATGACCATGTCCACG | Constructing vector expressing sgRNA for Site 40  |
| S40-SG-R | AAACCGTGGACATGGTCATCGTCG |   |
| S1-F     | GTGTCTCTAACCTTGCAGCAAG   | 1 <sup>st</sup> PCR for deep sequencing of Site 1 |
| S1-R     | CGAAAGGCGAAATGCTGATCC    |   |
| S2-F     | CCCATGCATGGGAAGTACCTCG   | 1 <sup>st</sup> PCR for deep sequencing of Site 2 |
| S2-R     | CGCCAACCTATGGTGGCACAG    |   |
| S3-F     | CAGTCGTAACCGACTAGAATCCCG | 1 <sup>st</sup> PCR for deep sequencing of Site 3 |
| S3-R     | TTGTGGCAGCGTAGGTTAGGC    |   |
| S4-F     | CGGTGCGTGCGTCACAGTCA     | 1 <sup>st</sup> PCR for deep sequencing of Site 4 |
| S4-R     | CGCGATCCACCCGATCAGCC     |   |
| S5-F     | CCGGGTCATCCTGCCTCTGAG    | 1 <sup>st</sup> PCR for deep sequencing of Site 5 |
| S5-R     | TCGATGGGCCGAAACGCTAGC    |   |
| S6-F     | CCCTTGTTGATGCTACAGATGTTT | 1 <sup>st</sup> PCR for deep sequencing of Site 6 |
| S6-R     | GAACATCCCACGCTTCTCCAG    |   |
| S7-F     | AGTGCGCGTTCTCGTTCTCACG   | 1 <sup>st</sup> PCR for deep sequencing of Site 7 |

|       |                           |  |
|-------|---------------------------|--|
| S7-R  | CGTCGCACTGGATCCTCATGCC    |  |
| S8-F  | GCAGCTAGCCATCCGGTGT       | 1 <sup>st</sup> PCR for deep sequencing of Site 8  |
| S8-R  | ACCAGTGCCTCAGCGACACG      |  |
| S9-F  | GGCATGCCTAAGAAAATAGGCC    | 1 <sup>st</sup> PCR for deep sequencing of Site 9  |
| S9-R  | AATGTGCTCGTCGTTGTCGAG     |  |
| S10-F | GGGCAGAAAGATGGCGCCGTC     | 1 <sup>st</sup> PCR for deep sequencing of Site 10 |
| S10-R | GCTTCTCCCTGTACCGCATCAGC   |  |
| S11-F | GAATATCGCCCACTCGCTCA      | 1 <sup>st</sup> PCR for deep sequencing of Site 11 |
| S11-R | TTCACTTCAGATCGCTGCGT      |  |
| S12-F | CAGGCCACGCAGCGATCTGAA     | 1 <sup>st</sup> PCR for deep sequencing of Site 12 |
| S12-R | CCGTAGATCGACTCCCCTCCCG    |  |
| S13-F | ACCGACGCGTACCGCACAT       | 1 <sup>st</sup> PCR for deep sequencing of Site 13 |
| S13-R | TCGGATCGGAAGCAGACGCAG     |  |
| S14-F | GCGGATCTAGCTGCGGTTGC      | 1 <sup>st</sup> PCR for deep sequencing of Site 14 |
| S14-R | TGGGACAAATGGAGACCGTGCATC  |  |
| S15-F | TGCATACGTGGTGGTGACTCACAT  | 1 <sup>st</sup> PCR for deep sequencing of Site 15 |
| S15-R | AGCGGTACTCAACATCCGGCG     |  |
| S16-F | TCCCACCTGAGGTGCAGCCTTC    | 1 <sup>st</sup> PCR for deep sequencing of Site 16 |
| S16-R | CGCTCGAGAACGTCACGAACCC    |  |
| S17-F | GTCCTCTAGACTCCCGGACCGAC   | 1 <sup>st</sup> PCR for deep sequencing of Site 17 |
| S17-R | GCGTGCGTGGATGGGTGGATAC    |  |
| S18-F | TGGAACAGCAGACCAGCCACGAC   | 1 <sup>st</sup> PCR for deep sequencing of Site 18 |
| S18-R | ACCCCTGAGGAAATTTGCTGTCAGC |  |
| S19-F | CCAAACGACGACACGTAGCCAG    | 1 <sup>st</sup> PCR for deep sequencing of Site 19 |
| S19-R | GAGGTGCGTGGGTGACCTGTC     |  |

|       |                           |  |
|-------|---------------------------|--|
| S20-F | TACCAAACGACGACACGTAGCCAG  | 1 <sup>st</sup> PCR for deep sequencing of Site 20 |
| S20-R | GAGGTGCGTGGGTGACCTGTC     |  |
| S21-F | TGAGGGGGTGGACGGATAGA      | 1 <sup>st</sup> PCR for deep sequencing of Site 21 |
| S21-R | GATTAGTAAGAGCATTATCTGGCGA |  |
| S22-F | AGCTGTTGAATGGGCAAGCGAC    | 1 <sup>st</sup> PCR for deep sequencing of Site 22 |
| S22-R | AAGTCCGTCCCGCTAACACCC     |  |
| S23-F | CCGGGACGGTGCAAGTTGGTTC    | 1 <sup>st</sup> PCR for deep sequencing of Site 23 |
| S23-R | TGCGTGGACGGACGTTCTCCTG    |  |
| S24-F | CGGGCATCATTTCGCGCCAG      | 1 <sup>st</sup> PCR for deep sequencing of Site 24 |
| S24-R | TGACGACGAAGAGCCGGTGC      |  |
| S25-F | TAATGGCCATGGTGTACGCA      | 1 <sup>st</sup> PCR for deep sequencing of Site 25 |
| S25-R | ACGTGTTCCAAACACCCAC       |  |
| S26-F | GCGATGTTCCATTCTGCACTAC    | 1 <sup>st</sup> PCR for deep sequencing of Site 26 |
| S26-R | GTATAAGGACGATGCTGCACCA    |  |
| S27-F | CCTCCAACCGATTTGTGAACAG    | 1 <sup>st</sup> PCR for deep sequencing of Site 27 |
| S27-R | CCATTGGTGGGATGAGTTGTTGC   |  |
| S28-F | ACCAATGGCGCCATTGCGTC      | 1 <sup>st</sup> PCR for deep sequencing of Site 28 |
| S28-R | TAGGTGCCCTCGTAGGCGGT      |  |
| S29-F | TGTTGGGATAGATGACTTGGATTG  | 1 <sup>st</sup> PCR for deep sequencing of Site 29 |
| S29-R | TTGCTATTGTCTGCCGGTGAA     |  |
| S30-F | ACGTGGTAGTGGTCGTATGGTCCG  | 1 <sup>st</sup> PCR for deep sequencing of Site 30 |
| S30-R | CACCTTCTGCGCACCTGCTTC     |  |
| S31-F | CGGGTGTGCAAGTCCTCCCT      | 1 <sup>st</sup> PCR for deep sequencing of Site 31 |
| S31-R | TGGTAGGATCCCTGACTCCTTTGGA |  |
| S32-F | AGTCAGGGATCCTACCAAACAACCG | 1 <sup>st</sup> PCR for deep sequencing of Site 32 |

|        |                             |   |
|--------|-----------------------------|---|
| S32-R  | ACCGTCGTTTCAGTTGGCCCT       |   |
| S33-F  | GAAAGATCGATGGTGTGCGTG       | 1 <sup>st</sup> PCR for deep sequencing of Site 33  |
| S33-R  | TATGCACATGGTGATGATGTCGT     |   |
| S34-F  | GCCGGCTCTTCCTCTCTTGCATC     | 1 <sup>st</sup> PCR for deep sequencing of Site 34  |
| S34-R  | GCTCGAAGAAGGTGGCGATATTTCG   |   |
| S35-F  | CCACGCATGCAACCAATGAG        | 1 <sup>st</sup> PCR for deep sequencing of Site 35  |
| S35-R  | CGAGGAAGAAGAGCTGGAGAAG      |   |
| S36-F  | GGGTCTCTCCGGGAGGCGTG        | 1 <sup>st</sup> PCR for deep sequencing of Site 36  |
| S36-R  | CCGTGGCGAGCACGTCGTAG        |   |
| S37-F  | TCCGGTCCAACATATGCACTCATC    | 1 <sup>st</sup> PCR for deep sequencing of Site 37  |
| S37-R  | TCTAGGCATGGCCCTCGTGC        |   |
| S38-F  | AGACACATATGCTATCATGGCAC     | 1 <sup>st</sup> PCR for deep sequencing of Site 38  |
| S38-R  | GGAACAAATGACCAAAAGGAATTG    |   |
| S39-F  | CACAGTTCACAAGCGAGGGA        | 1 <sup>st</sup> PCR for deep sequencing of Site 39  |
| S39-R  | GGCATATCCGGTCAACGAGA        |   |
| S40-F  | GCGCGTTGCACGCGAATATACG      | 1 <sup>st</sup> PCR for deep sequencing of Site 40  |
| S40-R  | TCGTCGTCGTTTCGTCAGGTGAGG    |   |
| S1-A-F | GACGACCGAGATCGATCAGATCGCACG | 2 <sup>nd</sup> PCR for deep sequencing of Site 1 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S1-A-R | CGATGTGGTGTGGCAGTTGTGTTCTG  |   |
| S1-B-F | TGACCACGAGATCGATCAGATCGCACG | 2 <sup>nd</sup> PCR for deep sequencing of Site 1 in control rice protoplasts (Fig. 1b)   |
| S1-B-R | ACAGTGGGTGTGGCAGTTGTGTTCTG  |   |
| S2-A-F | GACGACCATGAACATCCCATCTTTCTG | 2 <sup>nd</sup> PCR for deep sequencing of Site 2 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S2-A-R | CGATGTCTGATTCTGGGTCAGTGGTG  |   |
| S2-B-F | TGACCACATGAACATCCCATCTTTCTG | 2 <sup>nd</sup> PCR for deep sequencing of Site 2 in control rice protoplasts (Fig.   |

|        |                              |   |
|--------|------------------------------|---|
| S2-B-R | ACAGTGCTGATTCTGGGTCAGTGGTG   | 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)  |
| S3-A-F | GCCAATTGGGGTATCCATAATCTCCAC  | 2 <sup>nd</sup> PCR for deep sequencing of Site 3 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S3-A-R | CAGATCGGATGAAATAGATTCGTCGCC  |   |
| S3-B-F | CTTGTATGGGGTATCCATAATCTCCAC  | 2 <sup>nd</sup> PCR for deep sequencing of Site 3 in control rice protoplasts (Fig. 1b)   |
| S3-B-R | ATCACGGGATGAAATAGATTCGTCGCC  |   |
| S4-A-F | GCCAATCGTGCGTCACAGTCACAATC   | 2 <sup>nd</sup> PCR for deep sequencing of Site 4 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S4-A-R | CAGATCACGCTTGCTGCTCTCAGAAT   |   |
| S4-B-F | CTTGTACGTGCGTCACAGTCACAATC   | 2 <sup>nd</sup> PCR for deep sequencing of Site 4 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                  |
| S4-B-R | ATCACGACGCTTGCTGCTCTCAGAAT   |   |
| S5-A-F | TTAGGCCCGGGTCATCCTGCCTCTGAG  | 2 <sup>nd</sup> PCR for deep sequencing of Site 5 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S5-A-R | ACTTGAGCAGCCAGGGGTTGGTCGT    |   |
| S5-B-F | GATCAGCCGGGTCATCCTGCCTCTGAG  | 2 <sup>nd</sup> PCR for deep sequencing of Site 5 in control rice protoplasts (Fig. 1b)   |
| S5-B-R | TAGCTTGCAGCCAGGGGTTGGTCGT    |   |
| S6-A-F | TTAGGCGGTGTGGAGGTTGCAAGTGCT  | 2 <sup>nd</sup> PCR for deep sequencing of Site 6 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S6-A-R | ACTTGATTCAAGCGCAAGCTCAGGAAGA |   |
| S6-B-F | GATCAGGGTGTGGAGGTTGCAAGTGCT  | 2 <sup>nd</sup> PCR for deep sequencing of Site 6 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                  |
| S6-B-R | TAGCTTTTCAAGCGCAAGCTCAGGAAGA |   |
| S7-A-F | GGCTACTACTCCCCGGTGCTACCACG   | 2 <sup>nd</sup> PCR for deep sequencing of Site 7 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S7-A-R | AGTCAAAAAGGCGCAACGTGCGTCAC   |   |
| S7-B-F | AGTTCCTACTCCCCGGTGCTACCACG   | 2 <sup>nd</sup> PCR for deep sequencing of Site 7 in control rice protoplasts (Fig. 1b)   |
| S7-B-R | ATGTCAAAAGGCGCAACGTGCGTCAC   |   |

|         |                                 |  |
|---------|---------------------------------|--|
| S8-A-F  | GGCTACGAGGGGAGTACATCGGCATG      | 2 <sup>nd</sup> PCR for deep sequencing of Site 8 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts  |
| S8-A-R  | AGTCAACGTAGGAGTACGTTACCTGGC     |  |
| S8-B-F  | AGTTCCGAGGGGAGTACATCGGCATG      | 2 <sup>nd</sup> PCR for deep sequencing of Site 8 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                   |
| S8-B-R  | ATGTCACGTAGGAGTACGTTACCTGGC     |  |
| S9-A-F  | CCGTCCGGGCACACACCAAACCAGCC      | 2 <sup>nd</sup> PCR for deep sequencing of Site 9 treated by Cas9 in rice protoplasts (Fig. 1b)  |
| S9-A-R  | GTAGAGGATGCGACGACGACGGCTGC      |  |
| S9-B-F  | GTCCGCGGGCACACACCAAACCAGCC      | 2 <sup>nd</sup> PCR for deep sequencing of Site 9 in control rice protoplasts (Fig. 1b)  |
| S9-B-R  | GTGAAAGATGCGACGACGACGGCTGC      |  |
| S10-A-F | CCGTCCCTCGACCTCGACTTCGCGC       | 2 <sup>nd</sup> PCR for deep sequencing of Site 10 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S10-A-R | GTAGAGGCATCCATCTCGTCTCAGGACTC   |  |
| S10-B-F | GTCCGCCCTCGACCTCGACTTCGCGC      | 2 <sup>nd</sup> PCR for deep sequencing of Site 10 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                  |
| S10-B-R | GTGAAAGCATCCATCTCGTCTCAGGACTC   |  |
| S11-A-F | GTGGCCCCGATCCGTAAACCTCGCCCA     | 2 <sup>nd</sup> PCR for deep sequencing of Site 11 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S11-A-R | GTTTCGCGGTTGGATCTTTGCGGGGG      |  |
| S11-B-F | CGTACGCCGATCCGTAAACCTCGCCCA     | 2 <sup>nd</sup> PCR for deep sequencing of Site 11 in control rice protoplasts (Fig. 1b)   |
| S11-B-R | GAGTGGCGGTTGGATCTTTGCGGGGG      |  |
| S12-A-F | GTGGCCTTCGACATGACCGTCGGCGGAG    | 2 <sup>nd</sup> PCR for deep sequencing of Site 12 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S12-A-R | GTTTCGTGGAAGGTGCTCCCCTTG TAGTGC |  |
| S12-B-F | CGTACGTTTCGACATGACCGTCGGCGGAG   | 2 <sup>nd</sup> PCR for deep sequencing of Site 12 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                  |
| S12-B-R | GAGTGGTGAAGGTGCTCCCCTTG TAGTGC  |  |

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| S13-A-F | GGTAGCGCGTACCGCACATTTCCGGGG   | 2 <sup>nd</sup> PCR for deep sequencing of Site 13 treated by Cas9 (Fig. 1b) in rice protoplasts   |
| S13-A-R | ACTGATGGGCGGAGAGGGAGAGGAGATAG |  |
| S13-B-F | ATGAGCGCGTACCGCACATTTCCGGGG   | 2 <sup>nd</sup> PCR for deep sequencing of Site 13 in control rice protoplasts (Fig. 1b)   |
| S13-B-R | ATTCCTGGGCGGAGAGGGAGAGGAGATAG |  |
| S14-A-F | GGTAGCGAGCGGAGATGGGGCTCACG    | 2 <sup>nd</sup> PCR for deep sequencing of Site 14 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S14-A-R | ACTGATGTGGATTGGAAACGCACCGATGG |  |
| S14-B-F | ATGAGCGAGCGGAGATGGGGCTCACG    | 2 <sup>nd</sup> PCR for deep sequencing of Site 14 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                  |
| S14-B-R | ATTCCTGTGGATTGGAAACGCACCGATGG |  |
| S15-A-F | CAAAAGCACGTCATGTAGCAAAAGGGCGG | 2 <sup>nd</sup> PCR for deep sequencing of Site 15 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S15-A-R | CAACTAGGGATTTATAGCCGGCTCAGGGG |  |
| S15-B-F | CACCGGCACGTCATGTAGCAAAAGGGCGG | 2 <sup>nd</sup> PCR for deep sequencing of Site 15 in control rice protoplasts (Fig. 1b)   |
| S15-B-R | CACGATGGGATTTATAGCCGGCTCAGGGG |  |
| S16-A-F | CAAAAGTTCGTGGGGTTGGTGGGAAA    | 2 <sup>nd</sup> PCR for deep sequencing of Site 16 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S16-A-R | CAACTAGGAATCAATCCATCGGACCTTGG |  |
| S16-B-F | CACCGGTTTCGTGGGGTTGGTGGGAAA   | 2 <sup>nd</sup> PCR for deep sequencing of Site 16 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                  |
| S16-B-R | CACGATGGAATCAATCCATCGGACCTTGG |  |
| S17-A-F | CACTCAGTCCAACCCAACCCCAACCG    | 2 <sup>nd</sup> PCR for deep sequencing of Site 17 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S17-A-R | CAGGCGAAGGGGAGCGTTGGGTTGGG    |  |
| S17-B-F | CATGGCGTCCAACCCAACCCCAACCG    | 2 <sup>nd</sup> PCR for deep sequencing of Site 17 in control rice protoplasts (Fig. 1b)   |
| S17-B-R | CATTTTAAGGGGAGCGTTGGGTTGGG    |  |
| S18-A-F | CACTCACACCTAGCCGGGTGGCACAC    | 2 <sup>nd</sup> PCR for deep sequencing of Site 18 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or   |
| S18-A-R | CAGGCGCCGTTCTCACCCACAGCATCC   |  |

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|---------|--------------------------------|--|
|         |                                | Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts  |
| S18-B-F | CATGGCCACCTAGCCGGGTGGCACAC     | 2 <sup>nd</sup> PCR for deep sequencing of Site 18 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                  |
| S18-B-R | CATTTTGCCGTTCTCACCCACAGCATCC   |  |
| S19-A-F | CCAACACTTGGGCGACAAACCACAACCTG  | 2 <sup>nd</sup> PCR for deep sequencing of Site 19 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S19-A-R | CGGAATTTTCTCCAGAAGGCGCAGCCG    |  |
| S19-B-F | CTAGCTCTTGGGCGACAAACCACAACCTG  | 2 <sup>nd</sup> PCR for deep sequencing of Site 19 in control rice protoplasts (Fig. 1b)   |
| S19-B-R | CTATACTTTCTCCAGAAGGCGCAGCCG    |  |
| S20-A-F | CCAACAGGTAGCGAGCGTGGAAGTATG    | 2 <sup>nd</sup> PCR for deep sequencing of Site 20 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S20-A-R | CGGAATCTTGCTTGTATGCAATCCCATGGG |  |
| S20-B-F | CTAGCTGGTAGCGAGCGTGGAAGTATG    | 2 <sup>nd</sup> PCR for deep sequencing of Site 20 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                  |
| S20-B-R | CTATACCTTGCTTGTATGCAATCCCATGGG |  |
| S21-A-F | CTCAGAAGACGGACGTAAGTACGTAC     | 2 <sup>nd</sup> PCR for deep sequencing of Site 21 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S21-A-R | GCGCTAAGCAAAACATCCCACCCTTA     |  |
| S21-B-F | TAATCGAGACGGACGTAAGTACGTAC     | 2 <sup>nd</sup> PCR for deep sequencing of Site 21 in control rice protoplasts (Fig. 1b)   |
| S21-B-R | TACAGCAGCAAAACATCCCACCCTTA     |  |
| S22-A-F | CTCAGATGTTGAATGGGCAAGCGAC      | 2 <sup>nd</sup> PCR for deep sequencing of Site 22 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S22-A-R | GCGCTAAATAAAATCTAAAACCGGGGAAC  |  |
| S22-B-F | TAATCGTGTGTTGAATGGGCAAGCGAC    | 2 <sup>nd</sup> PCR for deep sequencing of Site 22 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                  |
| S22-B-R | TACAGCAATAAAATCTAAAACCGGGGAAC  |  |
| S22-C-F | CTGCAGTGTGTTGAATGGGCAAGCGAC    | 2 <sup>nd</sup> PCR for deep sequencing of Site 22 treated by Cas9/sgRNA in rice protoplasts (Fig. 1b)   |
| S22-C-R | GAAGTTAATAAAATCTAAAACCGGGGAAC  |  |



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| S23-A-F | TATAATCCGGTCGATCCATCCATCCATCC    | 2 <sup>nd</sup> PCR for deep sequencing of Site 23 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S23-A-R | TCATTCGCAAGTCGCTGGGAAAGCGG       |  |
| S23-B-F | TCCCGACCGGTCGATCCATCCATCCATCC    | 2 <sup>nd</sup> PCR for deep sequencing of Site 23 in control rice protoplasts (Fig. 1b)   |
| S23-B-R | TCGAAGGCAAGTCGCTGGGAAAGCGG       |  |
| S24-A-F | TATAATTAGTGACCTGCAGCGCCTGCAG     | 2 <sup>nd</sup> PCR for deep sequencing of Site 24 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a; Fig. 7) or Cas9-TV/sgRNA (Fig. 3b; Fig. 4a and Fig. 7) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S24-A-R | TCATTCGGGTGTCGAACGTGCCAAGC       |  |
| S24-B-F | TCCCGATAGTGACCTGCAGCGCCTGCAG     | 2 <sup>nd</sup> PCR for deep sequencing of Site 24 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a and Fig. 7)                       |
| S24-B-R | TCGAAGCGGTGTCGAACGTGCCAAGC       |  |
| S24-C-F | GCACCCTAGTGACCTGCAGCGCCTGCAG     | 2 <sup>nd</sup> PCR for deep sequencing of Site 24 treated by Cas9/sgRNA in rice protoplasts (Fig. 1b)   |
| S24-C-R | GCAGGACGGTGTCGAACGTGCCAAGC       |  |
| S25-A-F | TCGGCAGGTGTTACGCAATGACTAAAGTTG   | 2 <sup>nd</sup> PCR for deep sequencing of Site 25 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S25-A-R | AAACACCTGATCTTTTCTATAGCTAACCTAGC |  |
| S25-B-F | TGAAGGGGTGTTACGCAATGACTAAAGTTG   | 2 <sup>nd</sup> PCR for deep sequencing of Site 25 in control rice protoplasts (Fig. 1b)   |
| S25-B-R | AACATACTGATCTTTTCTATAGCTAACCTAGC |  |
| S26-A-F | TCGGCAGAACAAGCGATCTTTTCTCG       | 2 <sup>nd</sup> PCR for deep sequencing of Site 26 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts                 |
| S26-A-R | AAACACCAGTACTCATCGGTGTGTAG       |  |
| S26-B-F | TGAAGGGAACAAGCGATCTTTTCTCG       | 2 <sup>nd</sup> PCR for deep sequencing of Site 26 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                                  |
| S26-B-R | AACATACAGTACTCATCGGTGTGTAG       |  |
| S26-C-F | GCCGCGGAACAAGCGATCTTTTCTCG       | 2 <sup>nd</sup> PCR for deep sequencing of Site 26 treated by Cas9/sgRNA in rice protoplasts (Fig. 1b)   |
| S26-C-R | GCGGTGTCAGTACTCATCGGTGTGTAG      |  |
| S27-A-F | CGCGTCTGAGTTGCCCGTCACTGGTATTC    | 2 <sup>nd</sup> PCR for deep sequencing of Site 27 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S27-A-R | GATACAGCCAGATCCTCACACCTCTACC     |  |

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| S27-B-F | GGTGTGTGAGTTGCCCGTCACTGGTATTC   | 2 <sup>nd</sup> PCR for deep sequencing of Site 27 in control rice protoplasts (Fig. 1b)   |
| S27-B-R | TAAGAAGCCAGATCCTCACACCTCTACC    |  |
| S28-A-F | CGCGTCCCTGCAGCAAGAGCATCGTCTTC   | 2 <sup>nd</sup> PCR for deep sequencing of Site 28 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a; Fig. 7) or Cas9-TV/sgRNA (Fig. 3b; Fig. 4a and Fig. 7) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S28-A-R | GATACACACGTTGGCCTGGAACGTGC      |  |
| S28-B-F | GGTGTGCCTGCAGCAAGAGCATCGTCTTC   | 2 <sup>nd</sup> PCR for deep sequencing of Site 28 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a and Fig. 7)                       |
| S28-B-R | TAAGAACACGTTGGCCTGGAACGTGC      |  |
| S28-C-F | GTATTACCTGCAGCAAGAGCATCGTCTTC   | 2 <sup>nd</sup> PCR for deep sequencing of Site 29 treated by Cas9/sgRNA in rice protoplasts (Fig. 1b)   |
| S28-C-R | TACGTGCACGTTGGCCTGGAACGTGC      |  |
| S29-A-F | AGCGAGGTAGGGAGAATGAAAAGCACAGTGA | 2 <sup>nd</sup> PCR for deep sequencing of Site 29 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S29-A-R | CGGTTACTGCCACTACTCGTCTTTCCC     |  |
| S29-B-F | AGCTTTGTAGGGAGAATGAAAAGCACAGTGA | 2 <sup>nd</sup> PCR for deep sequencing of Site 29 in control rice protoplasts (Fig. 1b)   |
| S29-B-R | TGGTCTCTGCCACTACTCGTCTTTCCC     |  |
| S30-A-F | AGCGAGTGCTGCTGGCGATCTACAAC      | 2 <sup>nd</sup> PCR for deep sequencing of Site 30 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts                 |
| S30-A-R | CGGTTACTTCTTGGGGGACTTGGTGG      |  |
| S30-B-F | AGCTTTTGCTGCTGGCGATCTACAAC      | 2 <sup>nd</sup> PCR for deep sequencing of Site 30 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                                  |
| S30-B-R | TGGTCTCTTCTTGGGGGACTTGGTGG      |  |
| S30-C-F | TCACATTGCTGCTGGCGATCTACAAC      | 2 <sup>nd</sup> PCR for deep sequencing of Site 30 treated by Cas9/sgRNA in rice protoplasts (Fig. 1b)   |
| S30-C-R | TCTATACTTCTTGGGGGACTTGGTGG      |  |
| S31-A-F | TATCCCGTATGTCGATGCAAAGGGAAGC    | 2 <sup>nd</sup> PCR for deep sequencing of Site 31 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S31-A-R | TGTCGTCACGCAACTCATCTCGTTGG      |  |
| S31-B-F | CCCCACGTATGTCGATGCAAAGGGAAGC    | 2 <sup>nd</sup> PCR for deep sequencing of Site 31 in control rice protoplasts (Fig. 1b)   |
| S31-B-R | ATACGACACGCAACTCATCTCGTTGG      |  |

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| S32-A-F | TATCCCTGCATGTACATGATGATGAGG     | 2 <sup>nd</sup> PCR for deep sequencing of Site 32 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts                                      |
| S32-A-R | TGTCGTGCCCTTGAAAACCTTTTCCAAAG   |   |
| S32-B-F | CCCCACTGCATGTACATGATGATGAGG     | 2 <sup>nd</sup> PCR for deep sequencing of Site 32 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)   |
| S32-B-R | ATACGAGCCCTTGAAAACCTTTTCCAAAG   |   |
| S32-C-F | TGCAAATGCATGTACATGATGATGAGG     | 2 <sup>nd</sup> PCR for deep sequencing of Site 32 treated by Cas9/sgRNA in rice protoplasts (Fig. 1b)  |
| S32-C-R | TGGCACGCCCTTGAAAACCTTTTCCAAAG   |   |
| S33-A-F | CCCTTGCAAATGTGCCACTGGAACAGA     | 2 <sup>nd</sup> PCR for deep sequencing of Site 33 treated by Cas9 in rice protoplasts (Fig. 1b)  |
| S33-A-R | ACCGGCCCGCTAGAACCAAACCTTATTTGCC |   |
| S33-B-F | TTACTGGCAAATGTGCCACTGGAACAGA    | 2 <sup>nd</sup> PCR for deep sequencing of Site 33 in control rice protoplasts (Fig. 1b)  |
| S33-B-R | GGAACTCCGCTAGAACCAAACCTTATTTGCC |   |
| S34-A-F | CCCTTGCCGCTGCGATAGGCAGCTA       | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a; Fig. 7; and Fig. S7) or Cas9-TV/sgRNA (Fig. 3b; Fig. 4a; Fig. 5 and Fig. 7) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S34-A-R | ACCGGCGGTGGCGATATTCGCGGGGT      |   |
| S34-B-F | TTACTGGCCGCTGCGATAGGCAGCTA      | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a; and Fig. 7)   |
| S34-B-R | GGAACTGGTGGCGATATTCGCGGGGT      |   |
| S34-C-F | TGTTAGGCCGCTGCGATAGGCAGCTA      | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA in rice protoplasts (Fig. 1b)  |
| S34-C-R | TTCTATGGTGGCGATATTCGCGGGGT      |   |
| S35-A-F | GTTATTAACCTTTTACATCTCGCAACG     | 2 <sup>nd</sup> PCR for deep sequencing of Site 35 treated by Cas9 in rice protoplasts (Fig. 1b)  |
| S35-A-R | AAAAGTTAAGCGCCATTGATGATCTC      |   |
| S35-B-F | AAGGGAACTTTTACATCTCGCAACG       | 2 <sup>nd</sup> PCR for deep sequencing of Site 35 in control rice protoplasts (Fig. 1b)  |
| S35-B-R | AAGTATTAAGCGCCATTGATGATCTC      |   |
| S36-A-F | GTTATTGACACCCGGTGGGCTGCATC      | 2 <sup>nd</sup> PCR for deep sequencing of Site 36 treated by Cas9/sgRNA (Fig.  |

|         |                                  |  |
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| S36-A-R | AAAAGTTCGAGCACGGCGTCGCCGAAC      | 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts  |
| S36-B-F | AAGGGAGACACCCGGTGGGCTGCATC       | 2 <sup>nd</sup> PCR for deep sequencing of Site 36 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a)                                  |
| S36-B-R | AAGTATTCGAGCACGGCGTCGCCGAAC      |  |
| S36-C-F | GGTACGGACACCCGGTGGGCTGCATC       | 2 <sup>nd</sup> PCR for deep sequencing of Site 36 treated by Cas9/sgRNA in rice protoplasts (Fig. 1b)   |
| S36-C-R | GCTACGTCGAGCACGGCGTCGCCGAAC      |  |
| S37-A-F | ACATCTCCGGTCCAACATATGCACTCATCA   | 2 <sup>nd</sup> PCR for deep sequencing of Site 37 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S37-A-R | ACGATTTTCATGGCCCATAGGTTTTTGACCCT |  |
| S37-B-F | ACGCCGCCGGTCCAACATATGCACTCATCA   | 2 <sup>nd</sup> PCR for deep sequencing of Site 37 in control rice protoplasts (Fig. 1b)   |
| S37-B-R | ACTCTCTCATGGCCCATAGGTTTTTGACCCT  |  |
| S38-A-F | ACATCTGCGTGATGTAAACGGTGATTAG     | 2 <sup>nd</sup> PCR for deep sequencing of Site 38 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a; Fig. 7) or Cas9-TV/sgRNA (Fig. 3b; Fig. 4a and Fig. 7) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts |
| S38-A-R | ACGATTCCAAAGATGCACATCACAAGATG    |  |
| S38-B-F | ACGCCGGCGTGATGTAAACGGTGATTAG     | 2 <sup>nd</sup> PCR for deep sequencing of Site 38 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a and Fig. 7)                       |
| S38-B-R | ACTCTCCCAAAGATGCACATCACAAGATG    |  |
| S38-C-F | TGGAATGCGTGATGTAAACGGTGATTAG     | 2 <sup>nd</sup> PCR for deep sequencing of Site 38 treated by Cas9/sgRNA in rice protoplasts (Fig. 1b)   |
| S38-C-R | CATGTGCCAAAGATGCACATCACAAGATG    |  |
| S39-A-F | AGAATCTGGCTCGTGAGTGC GCGTTG      | 2 <sup>nd</sup> PCR for deep sequencing of Site 39 treated by Cas9 in rice protoplasts (Fig. 1b)   |
| S39-A-R | ATTGGGTGAGCTCGTAACTGAGCAGGCG     |  |
| S39-B-F | CCGCGTTGGCTCGTGAGTGC GCGTTG      | 2 <sup>nd</sup> PCR for deep sequencing of Site 39 in control rice protoplasts (Fig. 1b)   |
| S39-B-R | CGCCCTTGAGCTCGTAACTGAGCAGGCG     |  |
| S40-A-F | AGAATCTACGCACGCATATATCGCCTG      | 2 <sup>nd</sup> PCR for deep sequencing of Site 40 treated by Cas9/sgRNA (Fig. 1b; Fig. 3b; Fig. 4a) or Cas9-TV/sgRNA (Fig. 3b and Fig. 4a) or Cas9/sgRNA-dsgRNA (Fig. 4a) in rice protoplasts                 |
| S40-A-R | ATTGGGTGCGTCGTTTCGTCAGGTGAGG     |  |

|         |                             |   |
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| S40-B-F | CCGCGTTACGCACGCATATATCGCCTG | 2 <sup>nd</sup> PCR for deep sequencing of Site 40 in control rice protoplasts (Fig. 1b; Fig. 3b and Fig.4a) or treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 4a) |
| S40-B-R | CGCCCTTCGTCGTTTCGTCAGGTGAGG |   |
| S40-C-F | GAGATGTACGCACGCATATATCGCCTG | 2 <sup>nd</sup> PCR for deep sequencing of Site 40 treated by Cas9/sgRNA in rice protoplasts (Fig. 1b)  |
| S40-C-R | GATACCTCGTCGTTTCGTCAGGTGAGG |   |
| A-SG-F  | GGCACGCAAACGAATCGAGGAGT     | Constructing vector expressing sgRNA_A  |
| A-SG-R  | AAACACTCCTCGATTCTGTTTGCG    |   |
| B-SG-F  | GGCATCGCCATCGCCATCGCCGC     | Constructing vector expressing sgRNA_B  |
| B-SG-R  | AAACGCGGCGATGGCGATGGCGA     |   |
| C-SG-F  | GGCACGAAGGCGGCGGCGGCGATC    | Constructing vector expressing sgRNA_C  |
| C-SG-R  | AAACGATCGCCGCCGCCCTTCG      |   |
| D-SG-F  | GGCATCTTCTTCTAATCTTGCTGT    | Constructing vector expressing sgRNA_D  |
| D-SG-R  | AAACACAGCAAGATTAGAAGAAGA    |   |
| E-SG-F  | GGCAGTTGCTGCTGCTGCTGCGA     | Constructing vector expressing sgRNA_E  |
| E-SG-R  | AAACTCGCAGCAGCAGCAGCAAC     |   |
| A1-F    | GGAGTAGAGGTGGGAGGAAGCG      | 1 <sup>st</sup> PCR for deep sequencing of A1   |
| A1-R    | GGACGATTCATCCCCGATGCATG     |   |
| A2-F    | GAGTCGGCGGGAATCTGGGTG       | 1 <sup>st</sup> PCR for deep sequencing of A2   |
| A2-R    | AAAAGCGGTGGTGGACAGTCCC      |   |
| B1-F    | GTAGCTCTTGCCGCACGTGTCTG     | 1 <sup>st</sup> PCR for deep sequencing of B1   |
| B1-R    | CTTCCACGCGTGCTTCGACCAG      |   |
| B2-F    | TACGCGGAGCGGATATACCGGTAC    | 1 <sup>st</sup> PCR for deep sequencing of B2   |
| B2-R    | CATGAACTTGGCGGCGACCATGAC    |   |
| C1-F    | TCAGGTTTCGCGAAGGTCCG        | 1 <sup>st</sup> PCR for deep sequencing of C1   |
| C1-R    | GATCGGACGGCCAGAAGAGGC       |   |

|        |                                |   |
|--------|--------------------------------|---|
| C2-F   | GGACCGCAGCATCCGATCGC           | 1 <sup>st</sup> PCR for deep sequencing of C2   |
| C2-R   | GCCGCTGATTCCACCACCGATG         |   |
| D1-F   | AGCGTGGTGAATGGCCTTAGGGT        | 1 <sup>st</sup> PCR for deep sequencing of D1   |
| D1-R   | TGGCCGCAACATCTGGGTCTC          |   |
| D2-F   | CGGAACCCATCATCTGCACTC          | 1 <sup>st</sup> PCR for deep sequencing of D2   |
| D2-R   | CTATAGTGGTGGGAGTGGCGT          |   |
| E1-F   | GGCGGGTCGTTGACCTGAGA           | 1 <sup>st</sup> PCR for deep sequencing of E1   |
| E1-R   | GCGCATTCCGTGGCCCAAGA           |   |
| E2-F   | AGCTCAGCTCGGATCCGTAGGAC        | 1 <sup>st</sup> PCR for deep sequencing of E2   |
| E2-R   | GATGGCCCAATTGCAAGTTGCAACC      |   |
| A1-A-F | GGCTACGGTGGGGTGGGACTACCACC     | 2 <sup>nd</sup> PCR for deep sequencing of A1 treated by Cas9 in rice protoplasts (Fig. 2a) |
| A1-A-R | AGTCAACGAGCGGATGAGAGGGGATCG    |   |
| A1-B-F | AGTTCGGTGGGGTGGGACTACCACC      | 2 <sup>nd</sup> PCR for deep sequencing of A1 in control rice protoplasts (Fig. 2a)         |
| A1-B-R | ATGTCACGAGCGGATGAGAGGGGATCG    |   |
| A2-A-F | CCGTCCGGGATCGATTGATTGCCAACCG   | 2 <sup>nd</sup> PCR for deep sequencing of A2 treated by Cas9 in rice protoplasts (Fig. 2a) |
| A2-A-R | GTAGAGGACTGACTTGTGGTGGGGACTAC  |   |
| A2-B-F | GTCCGCGGGATCGATTGATTGCCAACCG   | 2 <sup>nd</sup> PCR for deep sequencing of A2 in control rice protoplasts (Fig. 2a)         |
| A2-B-R | GTGAAAGACTGACTTGTGGTGGGGACTAC  |   |
| B1-A-F | GACGACGTAGCTCTTGCCGCACGTGTCG   | 2 <sup>nd</sup> PCR for deep sequencing of B1 treated by Cas9 in rice protoplasts (Fig. 2a) |
| B1-A-R | CGATGTCTTCCACGCGTGCTTCGACCAG   |   |
| B1-B-F | TGACCAGTAGCTCTTGCCGCACGTGTCG   | 2 <sup>nd</sup> PCR for deep sequencing of B1 in control rice protoplasts (Fig. 2a)         |
| B1-B-R | ACAGTGCTTCCACGCGTGCTTCGACCAG   |   |
| B2-A-F | GCCAATTACGCGGAGCGGATATACCGGTAC | 2 <sup>nd</sup> PCR for deep sequencing of B2 treated by Cas9 in rice protoplasts (Fig. 2a) |
| B2-A-R | CAGATCCATGAACTTGCGGCGCACCATGAC |   |
| B2-B-F | CTTGTATACGCGGAGCGGATATACCGGTAC | 2 <sup>nd</sup> PCR for deep sequencing of B2 in control rice protoplasts (Fig. 2a)         |

|        |                                 |   |
|--------|---------------------------------|---|
| B2-B-R | ATCACGCATGAACTTGGCGGCGACCATGAC  |   |
| C1-A-F | GGTAGCGTAGATCGCCGGAGACGTGCG     | 2 <sup>nd</sup> PCR for deep sequencing of C1 treated by Cas9 in rice protoplasts (Fig. 2a) |
| C1-A-R | ACTGATGGCCGCGTCGTATTTCACTTGG    |   |
| C1-B-F | ATGAGCGTAGATCGCCGGAGACGTGCG     | 2 <sup>nd</sup> PCR for deep sequencing of C1 in control rice protoplasts (Fig. 2a)         |
| C1-B-R | ATTCTGGCCGCGTCGTATTTCACTTGG     |   |
| C2-A-F | GTGGCCCGATGGACGAGATCTCCACCCG    | 2 <sup>nd</sup> PCR for deep sequencing of C2 treated by Cas9 in rice protoplasts (Fig. 2a) |
| C2-A-R | GTTTCGTCCGCGTCGTCCCTCGTCTG      |   |
| C2-B-F | CGTACGCGATGGACGAGATCTCCACCCG    | 2 <sup>nd</sup> PCR for deep sequencing of C2 in control rice protoplasts (Fig. 2a)         |
| C2-B-R | GAGTGGTCCGCGTCGTCCCTCGTCTG      |   |
| D1-A-F | CTCAGAACCTTGTGCACGGGTGTGTGTG    | 2 <sup>nd</sup> PCR for deep sequencing of D1 treated by Cas9 in rice protoplasts (Fig. 2a) |
| D1-A-R | GCGCTATGGTCTGCCATGATTGCTGCCT    |   |
| D1-B-F | TAATCGACCTTGTGCACGGGTGTGTGTG    | 2 <sup>nd</sup> PCR for deep sequencing of D1 treated by Cas9 in rice protoplasts (Fig. 2a) |
| D1-B-R | TACAGCTGGTCTGCCATGATTGCTGCCT    |   |
| D2-A-F | CCAACATTCCACGAAACGCACCACGC      | 2 <sup>nd</sup> PCR for deep sequencing of D2 treated by Cas9 in rice protoplasts (Fig. 2a) |
| D2-A-R | CGGAATTGAGTGGCATATAAGGAAACTGGCC |   |
| D2-B-F | CTAGCTTTCCACGAAACGCACCACGC      | 2 <sup>nd</sup> PCR for deep sequencing of D2 in control rice protoplasts (Fig. 2a)         |
| D2-B-R | CTATACTGAGTGGCATATAAGGAAACTGGCC |   |
| E1-A-F | TCGGCAGCGTCTTCTGCTTCTGCACCG     | 2 <sup>nd</sup> PCR for deep sequencing of E1 treated by Cas9 in rice protoplasts (Fig. 2a) |
| E1-A-R | AAACACCGTGGTGCTATGAAGTGGGCCTC   |   |
| E1-B-F | TGAAGGGCGTCTTCTGCTTCTGCACCG     | 2 <sup>nd</sup> PCR for deep sequencing of E1 in control rice protoplasts (Fig. 2a)         |
| E1-B-R | AACATACGTGGTGCTATGAAGTGGGCCTC   |   |
| E2-A-F | TATAATGCCTTCTCTTATCACAAATGGCG   | 2 <sup>nd</sup> PCR for deep sequencing of E2 treated by Cas9 in rice protoplasts (Fig. 2a) |
| E2-A-R | TCATTCGATGAAGCAATTGCATGAGCAGC   |   |
| E2-B-F | TCCCGAGCCTTCTCTTATCACAAATGGCG   | 2 <sup>nd</sup> PCR for deep sequencing of E2 in control rice protoplasts (Fig. 2a)         |
| E2-B-R | TCGAAGGATGAAGCAATTGCATGAGCAGC   |   |

|            |  |   |
|------------|--|---|
| T7-A-F     | TAATACGACTCACTATAGGACGCAAACGA              | Transcript sgRNA <i>_A in vitro</i>                   |
| T7-B-F     | TAATACGACTCACTATAGGATCGCCATCG              | Transcript sgRNA <i>_B in vitro</i>                   |
| T7-C-F     | TAATACGACTCACTATAGGCGAAGGCGGC              | Transcript sgRNA <i>_C in vitro</i>                   |
| T7-D-F     | TAATACGACTCACTATAGGTCTTCTTCTA              | Transcript sgRNA <i>_D in vitro</i>                   |
| T7-E-F     | TAATACGACTCACTATAGGTTGCTGCTGC              | Transcript sgRNA <i>_E in vitro</i>                   |
| S2-MT1-BsF | ATATATGGTCTCTGGCAGCACGGCTGGTTCTGCCGTAGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 2 |
| S2-MT1-F0  | CAGCACGGCTGGTTCTGCCGTAGTTTTAGAGCTAGAAATAGC |   |
| S2-MT2-R0  | AACTGCCAGATGATGTCGCTTCTTGGTGCC             |   |
| S2-MT2-BsR | ATTATTGGTCTCTAAACTGCCAGATGATGTCG           |   |
| S4-MT1-BsF | ATATATGGTCTCTGGCACGAGGAACCTGGAGAGGAAGTT    | Constructing vector expressing sgRNA-dsgRNA of Site 4 |
| S4-MT1-F0  | CACGAGGAACCTGGAGAGGAAGTTTTAGAGCTAGAAATAGC  |   |
| S4-MT2-R0  | AACTCGTGAAGCCTGCACGCTTCTTGGTGCC            |   |
| S4-MT2-BsR | ATTATTGGTCTCTAAACTCGTGAAGCCTGCACG          |   |
| S6-MT1-BsF | ATATATGGTCTCTGGCACCTTGTCCTTGATAGCCGTGGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 6 |
| S6-MT1-F0  | CACCTTGTCCTTGATAGCCGTGGTTTTAGAGCTAGAAATAGC |   |
| S6-MT2-R0  | AACTGGGCATCAGGTCACGCTTCTTGGTGCC            |   |
| S6-MT2-BsR | ATTATTGGTCTCTAAACTGGGCATCAGGTCACG          |   |
| S8-MT1-BsF | ATATATGGTCTCTGGCAGGCCACGGTGGTGTGCTGCGGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 8 |
| S8-MT1-F0  | CAGGCCACGGTGGTGTGCTGCGGTTTTAGAGCTAGAAATAGC |   |
| S8-MT2-R0  | AACGCAAGCACCAGCGCGCTTCTTGGTGCC             |   |
| S8-MT2-BsR | ATTATTGGTCTCTAAACGCAAGCACCAGCGCG           |   |



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| S10-MT1-BsF | ATATATGGTCTCTGGCACCCGAGCTCGACATCACCGGGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 10 |
| S10-MT1-F0  | CACCCGAGCTCGACATCACCGGGTTTTAGAGCTAGAAATAGC |  |
| S10-MT2-R0  | AACTGGAGCGCGCGAAGCGCTTCTTGGTGCC            |  |
| S10-MT2-BsR | ATTATTGGTCTCTAAACTGGAGCGCGCGAAGCG          |  |
| S12-MT1-BsF | ATATATGGTCTCTGGCATGTACGCGAAGGACGTGCCGGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 12 |
| S12-MT1-F0  | CATGTACGCGAAGGACGTGCCGGTTTTAGAGCTAGAAATAGC |  |
| S12-MT2-R0  | AACCTCTTGCCACGCCGCTTCTTGGTGCC              |  |
| S12-MT2-BsR | ATTATTGGTCTCTAAACCTCTTGCCACGCCG            |  |
| S14-MT1-BsF | ATATATGGTCTCTGGCATCTCCTTCTTGGCGAAGAGGGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 14 |
| S14-MT1-F0  | CATCTCCTTCTTGGCGAAGAGGGTTTTAGAGCTAGAAATAGC |  |
| S14-MT2-R0  | AACAGCTTGAGCTTGTACGCTTCTTGGTGCC            |  |
| S14-MT2-BsR | ATTATTGGTCTCTAAACAGCTTGAGCTTGTACG          |  |
| S16-MT1-BsF | ATATATGGTCTCTGGCACGAGGCCGCCGACGAAGCAGGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 16 |
| S16-MT1-F0  | CACGAGGCCGCCGACGAAGCAGGTTTTAGAGCTAGAAATAGC |  |
| S16-MT2-R0  | AACCGAGTCCAAGGTCCGCTTCTTGGTGCC             |  |
| S16-MT2-BsR | ATTATTGGTCTCTAAACCGAGTCCAAGGTCCG           |  |
| S18-MT1-BsF | ATATATGGTCTCTGGCAGCAGCACCGGTTCTACCAAGGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 18 |
| S18-MT1-F0  | CAGCAGCACCGGTTCTACCAAGGTTTTAGAGCTAGAAATAGC |  |
| S18-MT2-R0  | AACTCACCAATCAGGTCGCTTCTTGGTGCC             |  |
| S18-MT2-BsR | ATTATTGGTCTCTAAACTCACCAATCAGGTCG           |  |

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|-------------|--|--|
| S20-MT1-BsF | ATATATGGTCTCTGGCAGAGTATGTCGTGGAGTCCACGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 20 |
| S20-MT1-F0  | CAGAGTATGTCGTGGAGTCCACGTTTTAGAGCTAGAAATAGC |  |
| S20-MT2-R0  | AACCGCTCGCTACCATAACGCTTCTTGGTGCC           |  |
| S20-MT2-BsR | ATTATTGGTCTCTAAACCGCTCGCTACCATACG          |  |
| S22-MT1-BsF | ATATATGGTCTCTGGCAGGGGTGTATCGATGATGCCGTT    | Constructing vector expressing sgRNA-dsgRNA of Site 22 |
| S22-MT1-F0  | CAGGGGTGTATCGATGATGCCGTTTTAGAGCTAGAAATAGC  |  |
| S22-MT2-R0  | AACAGAGCCTAGCTGTTTCGCTTCTTGGTGCC           |  |
| S22-MT2-BsR | ATTATTGGTCTCTAAACAGAGCCTAGCTGTTTCG         |  |
| S24-MT1-BsF | ATATATGGTCTCTGGCACGGCCGCCTCCGTACGCCGGTT    | Constructing vector expressing sgRNA-dsgRNA of Site 24 |
| S24-MT1-F0  | CACGGCCGCCTCCGTACGCCGGTTTTAGAGCTAGAAATAGC  |  |
| S24-MT2-R0  | AACGCAGCGCCTGCAGTCGCTTCTTGGTGCC            |  |
| S24-MT2-BsR | ATTATTGGTCTCTAAACGCAGCGCCTGCAGTC           |  |
| S26-MT1-BsF | ATATATGGTCTCTGGCAGGAACAGCCTCATCCCTTGGGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 26 |
| S26-MT1-F0  | CAGGAACAGCCTCATCCCTTGGGTTTTAGAGCTAGAAATAGC |  |
| S26-MT2-R0  | AACACACACCGATGAGTCGCTTCTTGGTGCC            |  |
| S26-MT2-BsR | ATTATTGGTCTCTAAACACACACCGATGAGTCG          |  |
| S28-MT1-BsF | ATATATGGTCTCTGGCAGTCTTTGGACGTAGCCATGGGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 28 |
| S28-MT1-F0  | CAGTCTTTGGACGTAGCCATGGGTTTTAGAGCTAGAAATAGC |  |
| S28-MT2-R0  | AACCCTCGTCCATCAACGCTTCTTGGTGCC             |  |
| S28-MT2-BsR | ATTATTGGTCTCTAAACCCCTCGTCCATCAACG          |  |
| S30-MT1-BsF | ATATATGGTCTCTGGCATGTGCTGCCGAACATCAACCGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 30 |

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| S30-MT1-F0  | CATGTGCTGCCGAACATCAACCGTTTTAGAGCTAGAAATAGC |  |
| S30-MT2-R0  | AACAGGCACGTGCTGCTCGCTTCTTGGTGCC            |  |
| S30-MT2-BsR | ATTATTGGTCTCTAAACAGGCACGTGCTGCTCG          |  |
| S32-MT1-BsF | ATATATGGTCTCTGGCACCATGACATTGTCCGAGCCAGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 32 |
| S32-MT1-F0  | CACCATGACATTGTCCGAGCCAGTTTTAGAGCTAGAAATAGC |  |
| S32-MT2-R0  | AACTCGTTCAGTTGGCCGCTTCTTGGTGCC             |  |
| S32-MT2-BsR | ATTATTGGTCTCTAAACTCGTTCAGTTGGCCG           |  |
| S34-MT1-BsF | ATATATGGTCTCTGGCAGACATCGTCACCAAGGCGCGTT    | Constructing vector expressing sgRNA-dsgRNA of Site 34 |
| S34-MT1-F0  | CAGACATCGTCACCAAGGCGCGTTTTAGAGCTAGAAATAGC  |  |
| S34-MT2-R0  | AACCGAGCGACGTGGCCGCTTCTTGGTGCC             |  |
| S34-MT2-BsR | ATTATTGGTCTCTAAACCGAGCGACGTGGCC            |  |
| S36-MT1-BsF | ATATATGGTCTCTGGCACTCGCTCCCGACCAACCAGTGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 36 |
| S36-MT1-F0  | CACTCGCTCCCGACCAACCAGTGTTTTAGAGCTAGAAATAGC |  |
| S36-MT2-R0  | AACGTGGGCTGCATCGGCGCTTCTTGGTGCC            |  |
| S36-MT2-BsR | ATTATTGGTCTCTAAACGTGGGCTGCATCGGCG          |  |
| S38-MT1-BsF | ATATATGGTCTCTGGCATGGGTAATGGTGATATCCCAGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 38 |
| S38-MT1-F0  | CATGGGTAATGGTGATATCCCAGTTTTAGAGCTAGAAATAGC |  |
| S38-MT2-R0  | AACTTGGTCTAATGCGCGCTTCTTGGTGCC             |  |
| S38-MT2-BsR | ATTATTGGTCTCTAAACTTGGTCTAATGCGC            |  |
| S40-MT1-BsF | ATATATGGTCTCTGGCACGACGATGACCATGTCCACGGTT   | Constructing vector expressing sgRNA-dsgRNA of Site 40 |

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| S40-MT1-F0    | CACGACGATGACCATGTCCACGGTTTTAGAGCTAGAAATAGC |  |
| S40-MT2-R0    | AACTGGTTGGTCGCGCCGCTTCTTGGTGCC             |  |
| S40-MT2-BsR   | ATTATTGGTCTCTAAACTGGTTGGTCGCGCCG           |  |
| S34-2-MT2-R0  | AACGTCTTCCATGTAGGCGCTTCTTGGTGCC            | Constructing vector expressing sgRNA-dsgRNA2 of Site 34  |
| S34-2-MT2-BsR | ATTATTGGTCTCTAAACGTCTTCCATGTAGGCG          |  |
| S34-3-MT2-R0  | AACAGGCGTTCTGTTCCGCTTCTTGGTGCC             | Constructing vector expressing sgRNA-dsgRNA3 of Site 34  |
| S34-3-MT2-BsR | ATTATTGGTCTCTAAACAGGCGTTCTGTTCCG           |  |
| S34-4-MT2-R0  | AACCGCTCACTACAGCTCGCTTCTTGGTGCC            | Constructing vector expressing sgRNA-dsgRNA4 of Site 34  |
| S34-4-MT2-BsR | ATTATTGGTCTCTAAACCGCTCACTACAGCTCG          |  |
| S34-5-MT2-R0  | AACTGCATGCATCGCTGCGCTTCTTGGTGCC            | Constructing vector expressing sgRNA-dsgRNA5 of Site 34  |
| S34-5-MT2-BsR | ATTATTGGTCTCTAAACTGCATGCATCGCTGCG          |  |
| S34-6-MT2-R0  | AACCGTCCCCTTTCCACGCTTCTTGGTGCC             | Constructing vector expressing sgRNA-dsgRNA6 of Site 34  |
| S34-6-MT2-BsR | ATTATTGGTCTCTAAACCGTCCCCTTTCCACG           |  |
| MT0-BsR2      | ATTATTGGTCTCTGCTTCTTGGTGCCGC               | Constructing vector expressing paired dsgRNAs of Site 34   |
| 13-MT2-BsF    | ATATATGGTCTCTAAGCGGCCACGTCGCTCGGTT         | Constructing vector expressing sgRNA-dsgRNA13 of Site 34   |
| 13-MT2-F0     | GGCCACGTCGCTCGGTTTTAGAGCTAGAAATAGC         |  |
| 46-MT2-BsF    | ATATATGGTCTCTAAGCGTGGAAGGGGACGGTT          | Constructing vector expressing sgRNA-dsgRNA46 of Site 34   |
| 46-MT2-F0     | GTGGAAAGGGGACGGTTTTAGAGCTAGAAATAGC         |  |
| 25-MT2-BsF    | ATATATGGTCTCTAAGCGCCTACATGGAAGACGTT        | Constructing vector expressing sgRNA-dsgRNA25 of Site 34   |
| 25-MT2-F0     | GCCTACATGGAAGACGTTTTAGAGCTAGAAATAGC        |  |
| S34-D-F       | GTGGCCCGCGCTGCGATAGGCAGCTA                 | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA-dsgRNA1 in rice protoplasts (Fig. S7) |
| S34-D-R       | GTTTCGGGTGGCGATATTCGCGGGGT                 |  |
| S34-E-F       | CGTACGGCCGCTGCGATAGGCAGCTA                 | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA-dsgRNA2 in rice protoplasts (Fig. S7) |
| S34-E-R       | GAGTGGGGTGGCGATATTCGCGGGGT                 |  |

|         |                            |  |
|---------|----------------------------|--|
| S34-F-F | GGTAGCGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA-dsgRNA3 in rice protoplasts (Fig. S7)     |
| S34-F-R | ACTGATGGTGGCGATATTCGCGGGGT |  |
| S34-G-F | ATGAGCGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA-dsgRNA4 in rice protoplasts (Fig. S7)     |
| S34-G-R | ATCCTGGTGGCGATATTCGCGGGGT  |  |
| S34-H-F | CAAAAGGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA-dsgRNA5 in rice protoplasts (Fig. S7)     |
| S34-H-R | CAACTAGGTGGCGATATTCGCGGGGT |  |
| S34-I-F | CACCGGCCGCTGCGATAGGCAGCTA  | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA-dsgRNA1&3 in rice protoplasts (Fig. S7)   |
| S34-I-R | CACGATGGTGGCGATATTCGCGGGGT |  |
| S34-J-F | CACTCAGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA-dsgRNA4&6 in rice protoplasts (Fig. S7)   |
| S34-J-R | CAGGCGGGTGGCGATATTCGCGGGGT |  |
| S34-K-F | CATGGCGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA-dsgRNA2&5 in rice protoplasts (Fig. S7)   |
| S34-K-R | CATTTTGGTGGCGATATTCGCGGGGT |  |
| S34-L-F | CCAACAGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9-TV/sgRNA-dsgRNA1 in rice protoplasts (Fig. 5)   |
| S34-L-R | CGGAATGGTGGCGATATTCGCGGGGT |  |
| S34-M-F | CTAGCTGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9-TV/sgRNA-dsgRNA2 in rice protoplasts (Fig. 5)   |
| S34-M-R | CTATACGGTGGCGATATTCGCGGGGT |  |
| S34-N-F | TCGGCAGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9-TV/sgRNA-dsgRNA3 in rice protoplasts (Fig. 5)   |
| S34-N-R | AAACACGGTGGCGATATTCGCGGGGT |  |
| S34-O-F | TGAAGGCCGCTGCGATAGGCAGCTA  | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9-TV/sgRNA-dsgRNA4 in rice protoplasts (Fig. 5)   |
| S34-O-R | AACATAGGTGGCGATATTCGCGGGGT |  |
| S34-P-F | CGCGTCGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9-TV/sgRNA-dsgRNA5 in rice protoplasts (Fig. 5)   |
| S34-P-R | GATACAGGTGGCGATATTCGCGGGGT |  |
| S34-Q-F | GGTGTGGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9-TV/sgRNA-dsgRNA1&3 in rice protoplasts (Fig. 5) |
| S34-Q-R | TAAGAAGGTGGCGATATTCGCGGGGT |  |
| S34-R-F | AGCGAGGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9-TV/sgRNA-                                       |

|          |                            |  |
|----------|----------------------------|--|
| S34-R-R  | CGGTTAGGTGGCGATATTCGCGGGGT | dsgRNA4&6 in rice protoplasts (Fig. 5)   |
| S34-S-F  | AGCTTTGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9-TV/sgRNA-dsgRNA2&5 in rice protoplasts (Fig. 5) |
| S34-S-R  | TGGTCTGGTGGCGATATTCGCGGGGT |  |
| S34-T-F  | AGAATCGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA-dsgRNA6 in rice protoplasts (Fig. S7)     |
| S34-T-R  | ATTGGGGGTGGCGATATTCGCGGGGT |  |
| S34-U-F  | CCGCGTGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9-TV/sgRNA-dsgRNA6 in rice protoplasts (Fig. 5)   |
| S34-U-R  | CGCCCTGGTGGCGATATTCGCGGGGT |  |
| S34-V-F  | GTATTAGCCGCTGCGATAGGCAGCTA | 2 <sup>nd</sup> PCR for deep sequencing of Site 34 treated by Cas9/sgRNA in rice protoplasts (Fig. 5)              |
| S34-V-R  | TACGTGGGTGGCGATATTCGCGGGGT |  |
| OT24-1-F | TCCAGCCGCCACATGCACAA       | 1 <sup>st</sup> PCR for deep sequencing of OT24-1  |
| OT24-1-R | GGCGCCCTTCATGGCGAATG       |  |
| OT24-2-F | GCGCAACTCACAGCAGAGGGA      | 1 <sup>st</sup> PCR for deep sequencing of OT24-2  |
| OT24-2-R | TTGCCTTCGAGAACCCGGACC      |  |
| OT24-3-F | CGCACGACGAGCTCCAAGAGA      | 1 <sup>st</sup> PCR for deep sequencing of OT24-3  |
| OT24-3-R | TCACCTACGCTCATTCATCCACA    |  |
| OT28-1-F | TAAACGTCGGTCTCGCGCTCTT     | 1 <sup>st</sup> PCR for deep sequencing of OT28-1  |
| OT28-1-R | GCCGTATCCGCCTGCACAAAG      |  |
| OT28-2-F | TCTGCTGCAGGCCGAGATTGAA     | 1 <sup>st</sup> PCR for deep sequencing of OT28-2  |
| OT28-2-R | TCTGATCTCCCTGCCAACGCC      |  |
| OT28-3-F | CACGCGATGATCGACAGAGA       | 1 <sup>st</sup> PCR for deep sequencing of OT28-3  |
| OT28-3-R | AAGGAAGCCACTGAAGACCG       |  |
| OT34-1-F | CGATGCGCTTCACCTCCGAG       | 1 <sup>st</sup> PCR for deep sequencing of OT34-1  |
| OT34-1-R | AGTCGGCAACCTTCGGGGTG       |  |
| OT34-2-F | ATCATGGCGTACTACGCCTGGAG    | 1 <sup>st</sup> PCR for deep sequencing of OT34-2  |
| OT34-2-R | TGCTAAAGTGGGAGGGTGTGCG     |  |

|           |                             |  |
|-----------|-----------------------------|--|
| OT34-3-F  | ACGCGACAGGGATCGTCCAC        | 1 <sup>st</sup> PCR for deep sequencing of OT34-3  |
| OT34-3-R  | CGGAGGTGGCGACGCAGAC         |  |
| OT34-4-F  | TCACCGCCTACGACATCACGAC      | 1 <sup>st</sup> PCR for deep sequencing of OT34-4  |
| OT34-4-R  | TCAAGGTCATGCGATGCAACCAC     |  |
| OT34-5-F  | GTGCTGCAGGTACGACCCCTC       | 1 <sup>st</sup> PCR for deep sequencing of OT34-5  |
| OT34-5-R  | CTTGGAAGGTGAACTTCTGCCCAAC   |  |
| OT38-1-F  | CCAAGCTAGCAACAACAAGCTC      | 1 <sup>st</sup> PCR for deep sequencing of OT38-1  |
| OT38-1-R  | GTCGTCGCTAGCTATGCGTATC      |  |
| OT38-2-F  | TGTGCTTGTGTTAAAGGGACACACT   | 1 <sup>st</sup> PCR for deep sequencing of OT38-2  |
| OT38-2-R  | GTGTCTCGGTGTCGTCATCTTG      |  |
| OT38-3-F  | CTCGACGACCCTCAGCTCGC        | 1 <sup>st</sup> PCR for deep sequencing of OT38-3  |
| OT38-3-R  | GTGCAACGGTCTCCCCCTCC        |  |
| OT38-4-F  | GCGGATGCCAGCTTTGAGTTC       | 1 <sup>st</sup> PCR for deep sequencing of OT38-4  |
| OT38-4-R  | GGAGCTTTGAGTATGGCACAGC      |  |
| OT24-1-F1 | GACGACCGTCCGCATGGAGGACGACA  | 2 <sup>nd</sup> PCR for deep sequencing of OT24-1 for sgRNA24 treated by Cas9<br>in rice protoplasts (Fig. 7)                  |
| OT24-1-R1 | CGATGTCCTTCATGGCGAATGCGGCG  |  |
| OT24-1-F2 | TGACCACGTCCGCATGGAGGACGACA  | 2 <sup>nd</sup> PCR for deep sequencing of OT24-1 for sgRNA24 treated by Cas9-<br>TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT24-1-R2 | ACAGTGCCTTCATGGCGAATGCGGCG  |  |
| OT24-1-F3 | GCCAATCGTCCGCATGGAGGACGACA  | 2 <sup>nd</sup> PCR for deep sequencing of OT24-1 for sgRNA 24 in rice<br>protoplasts treated by Cas9-TV/sgRNA-dsgRNA (Fig. 7) |
| OT24-1-R3 | CAGATCCCTTCATGGCGAATGCGGCG  |  |
| OT24-1-F4 | CTTGTACGTCCGCATGGAGGACGACA  | 2 <sup>nd</sup> PCR for deep sequencing of OT24-1 for sgRNA 24 in control rice<br>protoplasts (Fig. 7)                         |
| OT24-1-R4 | ATCACGCCTTCATGGCGAATGCGGCG  |  |
| OT24-2-F1 | TTAGGCGCGCAACTCACAGCAGAGGGA | 2 <sup>nd</sup> PCR for deep sequencing of OT24-2 for sgRNA 24 treated by Cas9<br>in rice protoplasts (Fig. 7)                 |
| OT24-2-R1 | ACTTGATTGCCTTCGAGAACCCGGACC |  |
| OT24-2-F2 | GATCAGGCGCAACTCACAGCAGAGGGA | 2 <sup>nd</sup> PCR for deep sequencing of OT24-2 for sgRNA 24 treated by  |

|           |                               |   |
|-----------|-------------------------------|---|
| OT24-2-R2 | TAGCTTTTGCCTTCGAGAACCCGGACC   | Cas9-TV/sgRNA in rice protoplasts (Fig. 7)  |
| OT24-2-F3 | GGCTACGCGCAACTCACAGCAGAGGGA   | 2 <sup>nd</sup> PCR for deep sequencing of OT24-2 for sgRNA 24 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT24-2-R3 | AGTCAATTGCCTTCGAGAACCCGGACC   |   |
| OT24-2-F4 | AGTCCGCGCAACTCACAGCAGAGGGA    | 2 <sup>nd</sup> PCR for deep sequencing of OT24-2 for sgRNA 24 in control rice protoplasts (Fig. 7)                         |
| OT24-2-R4 | ATGTCATTGCCTTCGAGAACCCGGACC   |   |
| OT24-3-F1 | CCGTCCGCACGACGAGCTCCAAGAGAG   | 2 <sup>nd</sup> PCR for deep sequencing of OT24-3 for sgRNA 24 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT24-3-R1 | GTAGAGGCAGCCTCTGCTCCGAGTC     |   |
| OT24-3-F2 | GTCCGCGCACGACGAGCTCCAAGAGAG   | 2 <sup>nd</sup> PCR for deep sequencing of OT24-3 for sgRNA 24 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT24-3-R2 | GTGAAAGCAGCCTCTGCTCCGAGTC     |   |
| OT24-3-F3 | GTGGCCGCACGACGAGCTCCAAGAGAG   | 2 <sup>nd</sup> PCR for deep sequencing of OT24-3 for sgRNA 24 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT24-3-R3 | GTTTCGGCAGCCTCTGCTCCGAGTC     |   |
| OT24-3-F4 | CGTACGGCACGACGAGCTCCAAGAGAG   | 2 <sup>nd</sup> PCR for deep sequencing of OT24-3 for sgRNA 24 in control rice protoplasts (Fig. 7)                         |
| OT24-3-R4 | GAGTGGGCAGCCTCTGCTCCGAGTC     |   |
| OT28-1-F1 | GGTAGCACCGATTATAGCTGACCGGCG   | 2 <sup>nd</sup> PCR for deep sequencing of OT28-1 for sgRNA 28 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT28-1-R1 | ACTGATTCAACGCAAGGGCGTATATCA   |   |
| OT28-1-F2 | ATGAGCACCGATTATAGCTGACCGGCG   | 2 <sup>nd</sup> PCR for deep sequencing of OT28-1 for sgRNA 28 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT28-1-R2 | ATTCCTTCAACGCAAGGGCGTATATCA   |   |
| OT28-1-F3 | CAAAAGACCGATTATAGCTGACCGGCG   | 2 <sup>nd</sup> PCR for deep sequencing of OT28-1 for sgRNA 28 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT28-1-R3 | CAACTATCAACGCAAGGGCGTATATCA   |   |
| OT28-1-F4 | CACCGGACCGATTATAGCTGACCGGCG   | 2 <sup>nd</sup> PCR for deep sequencing of OT28-1 for sgRNA 28 in control rice protoplasts (Fig. 7)                         |
| OT28-1-R4 | CACGATTCAACGCAAGGGCGTATATCA   |   |
| OT28-2-F1 | CACTCACTGCAGGCCGAGATTGAAAGGT  | 2 <sup>nd</sup> PCR for deep sequencing of OT28-2 for sgRNA 28 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT28-2-R1 | CAGGCGTGCCAACGCCTTTTCCTTGCTTG |   |
| OT28-2-F2 | CATGGCCTGCAGGCCGAGATTGAAAGGT  | 2 <sup>nd</sup> PCR for deep sequencing of OT28-2 for sgRNA 28 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT28-2-R2 | CATTTTTGCCAACGCCTTTTCCTTGCTTG |   |



|           |                               |   |
|-----------|-------------------------------|---|
| OT28-2-F3 | CCAACACTGCAGGCCGAGATTGAAAGGT  | 2 <sup>nd</sup> PCR for deep sequencing of OT28-2 for sgRNA 28 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT28-2-R3 | CGGAATTGCCAACGCCTTTTCCTTGCTTG |   |
| OT28-2-F4 | CTAGCTCTGCAGGCCGAGATTGAAAGGT  | 2 <sup>nd</sup> PCR for deep sequencing of OT28-2 for sgRNA 28 in control rice protoplasts (Fig. 7)                         |
| OT28-2-R4 | CTATACTGCCAACGCCTTTTCCTTGCTTG |   |
| OT28-3-F1 | TCGGCACACGCGATGATCGACAGAGA    | 2 <sup>nd</sup> PCR for deep sequencing of OT28-3 for sgRNA 28 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT28-3-R1 | AAACACAAGGAAGCCACTGAAGACCG    |   |
| OT28-3-F2 | TGAAGGCACGCGATGATCGACAGAGA    | 2 <sup>nd</sup> PCR for deep sequencing of OT28-3 for sgRNA 28 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT28-3-R2 | AACATAAAGGAAGCCACTGAAGACCG    |   |
| OT28-3-F3 | CGCGTCCACGCGATGATCGACAGAGA    | 2 <sup>nd</sup> PCR for deep sequencing of OT28-3 for sgRNA 28 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT28-3-R3 | GATACAAAGGAAGCCACTGAAGACCG    |   |
| OT28-3-F4 | GGTGTGCACGCGATGATCGACAGAGA    | 2 <sup>nd</sup> PCR for deep sequencing of OT28-3 for sgRNA 28 in control rice protoplasts (Fig. 7)                         |
| OT28-3-R4 | TAAGAAAAGGAAGCCACTGAAGACCG    |   |
| OT34-1-F1 | AGAATCTGAAGGTGCTCAAGGTGTCC    | 2 <sup>nd</sup> PCR for deep sequencing of OT34-1 for sgRNA 34 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT34-1-R1 | ATTGGGGAGGTACTTCTCCAGCGAGC    |   |
| OT34-1-F2 | CCGCGTTGAAGGTGCTCAAGGTGTCC    | 2 <sup>nd</sup> PCR for deep sequencing of OT34-1 for sgRNA 34 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT34-1-R2 | CGCCCTGAGGTACTTCTCCAGCGAGC    |   |
| OT34-1-F3 | CTGCAGTGAAGGTGCTCAAGGTGTCC    | 2 <sup>nd</sup> PCR for deep sequencing of OT34-1 for sgRNA 34 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT34-1-R3 | GAAGTTGAGGTACTTCTCCAGCGAGC    |   |
| OT34-1-F4 | GCCGCGTGAAGGTGCTCAAGGTGTCC    | 2 <sup>nd</sup> PCR for deep sequencing of OT34-1 for sgRNA 34 in control rice protoplasts (Fig. 7)                         |
| OT34-1-R4 | GGCGGTGAGGTACTTCTCCAGCGAGC    |   |
| OT34-2-F1 | GTATTATACTACGCCTGGAGCCATTA    | 2 <sup>nd</sup> PCR for deep sequencing of OT34-2 for sgRNA 34 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT34-2-R1 | TACGTGGGCTACATAGGAGGTGACTGT   |   |
| OT34-2-F2 | TCACATTACTACGCCTGGAGCCATTA    | 2 <sup>nd</sup> PCR for deep sequencing of OT34-2 for sgRNA 34 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT34-2-R2 | TCTATAGGCTACATAGGAGGTGACTGT   |   |
| OT34-2-F3 | TGCAAATACTACGCCTGGAGCCATTA    | 2 <sup>nd</sup> PCR for deep sequencing of OT34-2 for sgRNA 34 treated by   |

|           |                                |   |
|-----------|--------------------------------|---|
| OT34-2-R3 | TGGCACGGCTACATAGGAGGTGACTGT    | Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7)   |
| OT34-2-F4 | GGTACGTACTACGCCTGGAGCCATTA     | 2 <sup>nd</sup> PCR for deep sequencing of OT34-2 for sgRNA 34 in control rice protoplasts (Fig. 7)                         |
| OT34-2-R4 | GCTACGGGCTACATAGGAGGTGACTGT    |   |
| OT34-3-F1 | GAGATGCGAGAGGTGCTCGACGAC       | 2 <sup>nd</sup> PCR for deep sequencing of OT34-3 for sgRNA 34 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT34-3-R1 | GATACCCCTGGTGGATCTCCATTGAC     |   |
| OT34-3-F2 | TCTAACCGAGAGGTGCTCGACGAC       | 2 <sup>nd</sup> PCR for deep sequencing of OT34-3 for sgRNA 34 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT34-3-R2 | ACTGCACCTGGTGGATCTCCATTGAC     |   |
| OT34-3-F3 | CGGATCCGAGAGGTGCTCGACGAC       | 2 <sup>nd</sup> PCR for deep sequencing of OT34-3 for sgRNA 34 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT34-3-R3 | TAGTGCCCTGGTGGATCTCCATTGAC     |   |
| OT34-3-F4 | ACCAACCGAGAGGTGCTCGACGAC       | 2 <sup>nd</sup> PCR for deep sequencing of OT34-3 for sgRNA 34 in control rice protoplasts (Fig. 7)                         |
| OT34-3-R4 | GACCGGCCTGGTGGATCTCCATTGAC     |   |
| OT34-4-F1 | ATCGATGTGGGCAACGCGGCGTTC       | 2 <sup>nd</sup> PCR for deep sequencing of OT34-4 for sgRNA 34 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT34-4-R1 | CGCTCACAGCCACCTCGGCAACCTC      |   |
| OT34-4-F2 | TCGGCGGTGGGCAACGCGGCGTTC       | 2 <sup>nd</sup> PCR for deep sequencing of OT34-4 for sgRNA 34 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT34-4-R2 | ACTGGCCAGCCACCTCGGCAACCTC      |   |
| OT34-4-F3 | GATTTTGTGGGCAACGCGGCGTTC       | 2 <sup>nd</sup> PCR for deep sequencing of OT34-4 for sgRNA 34 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT34-4-R3 | ACGATTCAGCCACCTCGGCAACCTC      |   |
| OT34-4-F4 | ACGCCGGTGGGCAACGCGGCGTTC       | 2 <sup>nd</sup> PCR for deep sequencing of OT34-4 for sgRNA 34 in control rice protoplasts (Fig. 7)                         |
| OT34-4-R4 | ACTCTCCAGCCACCTCGGCAACCTC      |   |
| OT34-5-F1 | GCACCCCGTCGTCGGAGGTGCAAGT      | 2 <sup>nd</sup> PCR for deep sequencing of OT34-5 for sgRNA 34 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT34-5-R1 | GCAGGATGGAAGGTGAACTTCTGCCCAACA |   |
| OT34-5-F2 | TGGAATCCGTCGTCGGAGGTGCAAGT     | 2 <sup>nd</sup> PCR for deep sequencing of OT34-5 for sgRNA 34 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT34-5-R2 | CATGTGTGGAAGGTGAACTTCTGCCCAACA |   |
| OT34-5-F3 | GTTATCCGTCGTCGGAGGTGCAAGT      | 2 <sup>nd</sup> PCR for deep sequencing of OT34-5 for sgRNA 34 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT34-5-R3 | AAAAGTTGGAAGGTGAACTTCTGCCCAACA |   |

|           |                               |   |
|-----------|-------------------------------|---|
| OT34-5-F4 | AAGGGACCGTCGTCGGAGGTGCAAGT    | 2 <sup>nd</sup> PCR for deep sequencing of OT34-5 for sgRNA 34 in control rice protoplasts (Fig. 7)                         |
| OT34-5-R4 | AAGTATTGGAAGGTGAACCTTGCCCAACA |   |
| OT38-1-F1 | AGCGAGAAAAATGGCTGGCCTGTCCC    | 2 <sup>nd</sup> PCR for deep sequencing of OT38-1 for sgRNA 38 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT38-1-R1 | CGGTTACTATGCGTATCTGCCAGCAAG   |   |
| OT38-1-F2 | AGCTTTAAAAATGGCTGGCCTGTCCC    | 2 <sup>nd</sup> PCR for deep sequencing of OT38-1 for sgRNA 38 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT38-1-R2 | TGGTCTCTATGCGTATCTGCCAGCAAG   |   |
| OT38-1-F3 | TATCCCAAAAATGGCTGGCCTGTCCC    | 2 <sup>nd</sup> PCR for deep sequencing of OT38-1 for sgRNA 38 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT38-1-R3 | TGTCGTCTATGCGTATCTGCCAGCAAG   |   |
| OT38-1-F4 | CCCCACAAAATGGCTGGCCTGTCCC     | 2 <sup>nd</sup> PCR for deep sequencing of OT38-1 for sgRNA 38 in control rice protoplasts (Fig. 7)                         |
| OT38-1-R4 | ATACGACTATGCGTATCTGCCAGCAAG   |   |
| OT38-2-F1 | CCCTTGCTTGTGCACTCTTGCGTGTC    | 2 <sup>nd</sup> PCR for deep sequencing of OT38-2 for sgRNA 38 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT38-2-R1 | ACCGGCTTGATGGTTTAGCCTGGCCC    |   |
| OT38-2-F2 | TTACTGCTTGTGCACTCTTGCGTGTC    | 2 <sup>nd</sup> PCR for deep sequencing of OT38-2 for sgRNA 38 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT38-2-R2 | GGAACCTTGATGGTTTAGCCTGGCCC    |   |
| OT38-2-F3 | GTTATTCCTGTGCACTCTTGCGTGTC    | 2 <sup>nd</sup> PCR for deep sequencing of OT38-2 for sgRNA 38 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT38-2-R3 | AAAAGTTTGATGGTTTAGCCTGGCCC    |   |
| OT38-2-F4 | AAGGGACTTGTGCACTCTTGCGTGTC    | 2 <sup>nd</sup> PCR for deep sequencing of OT38-2 for sgRNA 38 in control rice protoplasts (Fig. 7)                         |
| OT38-2-R4 | AAGTATTTGATGGTTTAGCCTGGCCC    |   |
| OT38-3-F1 | ACATCTCGCTGATTGTGATCGTTGCAT   | 2 <sup>nd</sup> PCR for deep sequencing of OT38-3 for sgRNA 38 treated by Cas9 in rice protoplasts (Fig. 7)                 |
| OT38-3-R1 | ACGATTGTTCCCCACACGAACACTGTA   |   |
| OT38-3-F2 | ACGCCGCGCTGATTGTGATCGTTGCAT   | 2 <sup>nd</sup> PCR for deep sequencing of OT38-3 for sgRNA 38 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |
| OT38-3-R2 | ACTCTCGTTCCCCACACGAACACTGTA   |   |
| OT38-3-F3 | AGAATCCGCTGATTGTGATCGTTGCAT   | 2 <sup>nd</sup> PCR for deep sequencing of OT38-3 for sgRNA 38 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |
| OT38-3-R3 | ATTGGGGTTCCCCACACGAACACTGTA   |   |
| OT38-3-F4 | CCGCGTCGCTGATTGTGATCGTTGCAT   | 2 <sup>nd</sup> PCR for deep sequencing of OT38-3 for sgRNA 38 in control rice  |

|             |                             |   |   |
|-------------|-----------------------------|---|---|
| OT38-3-R4   | CGCCCTGTTCCCCACACGAACACTGTA | protoplasts (Fig. 7)  |   |
| OT38-4-F1   | CTGCAGAGCTCGCACCTTGCAGACTGT | 2 <sup>nd</sup> PCR for deep sequencing of OT38-4 for sgRNA 38 treated by Cas9 in rice protoplasts (Fig. 7)                 |   |
| OT38-4-R1   | GAAGTTTGTTTTCCCGGGTGGCGGA   |   |   |
| OT38-4-F2   | GCACCCAGCTCGCACCTTGCAGACTGT | 2 <sup>nd</sup> PCR for deep sequencing of OT38-4 for sgRNA 38 treated by Cas9-TV/sgRNA in rice protoplasts (Fig. 7)        |   |
| OT38-4-R2   | GCAGGATGTTTTCCCGGGTGGCGGA   |   |   |
| OT38-4-F3   | GCCGCGAGCTCGCACCTTGCAGACTGT | 2 <sup>nd</sup> PCR for deep sequencing of OT38-4 for sgRNA 38 treated by Cas9-TV/sgRNA-dsgRNA in rice protoplasts (Fig. 7) |   |
| OT38-4-R3   | GGCGGTTGTTTTCCCGGGTGGCGGA   |   |   |
| OT38-4-F4   | GTATTAAGCTCGCACCTTGCAGACTGT | 2 <sup>nd</sup> PCR for deep sequencing of OT38-4 for sgRNA 38 in control rice protoplasts (Fig. 7)                         |   |
| OT38-4-R4   | TACGTGTGTTTTCCCGGGTGGCGGA   |   |   |
| S26-DNase-F | TGGAACAGCCTCATCCCTTGGC      | Analyze local chromatin accessibility change of Site 26   |   |
| S26-DNase-R | CATGGTGGGAGTCTGTGGTGGT      |   |   |
| S28-DNase-F | GATACCAACCATGCATCGGCCA      | Analyze local chromatin accessibility change of Site 28   |   |
| S28-DNase-R | CTCTTGCTGCAGGGGCGACAA       |   |   |
| S34-DNase-F | CGACCCCAAGGCGTTCTGTTCCA     | Analyze local chromatin accessibility change of Site 34   |   |
| S34-DNase-R | CTCGTCCCCTTTCCACTGCTCG      |   |   |
| S2-Q-F      | GCAACACCTTCAGGAGCAAC        | Analyze gene expression using real-time PCR   |   |
| S2-Q-R      | GGTGTTCCTTGTTCATCGATC       |   |   |
| S16-Q-F     | GTTCGGGTTCGTGACGTTT         |   |   |
| S16-Q-R     | CCTCGTTGACGGTGATGTTG        |   |   |
| S18-Q-F     | GGGTGAAGGATCTGGATGCTG       |   |   |
| S18-Q-R     | CTGAGGAAATTTGCTGTCAGCTC     |   |   |
| S26-Q-F     | AGGCCTGAACAAAGCGATCT        |   |   |
| S26-Q-R     | GTGCTTGACGATTGCCCTTG        |   |   |
| DSG-yuc-F   | GGCATCGGCGTCGTTGG           |   | Constructing vector expressing dsgRNA for <i>OsYUCCA4</i> promoter region |
| DSG-yuc-R   | AAACCCAACGACGCCGA           |   |   |

|           |                          |   |
|-----------|--------------------------|---|
| DSG-2-F   | GGCAGACATCATCTGGCA       | Constructing vector expressing dsRNA for <i>LOC_Os04g31340</i><br>promoter region |
| DSG-2-R   | AAACTGCCAGATGATGTC       |   |
| DSG-16-F  | GGCAGGACCTTGGACTCG       | Constructing vector expressing dsRNA for <i>LOC_Os12g43600</i><br>promoter region |
| DSG-16-R  | AAACCGAGTCCAAGGTCC       |   |
| DSG-18-F  | GGCACCTGATTGGGTGA        | Constructing vector expressing dsRNA for <i>LOC_Os06g47340</i><br>promoter region |
| DSG-18-R  | AAACTCACCAATCAGG         |   |
| DSG-26-F  | GGCACTCATCGGTGTGT        | Constructing vector expressing dsRNA for <i>LOC_Os04g11390</i><br>promoter region |
| DSG-26-R  | AAACACACACCGATGAG        |   |
| YUCCA-Q-F | GTTGTCATCCTGGCCACAGGATAC | Analyze gene expression using real-time PCR                                       |
| YUCCA-Q-R | CCAACAGCGTACAGGCCATTCTG  |   |
| Ubi-Q-F   | GCTCCGTGGCGGTATCATC      |   |
| Ubi-Q-R   | CGGCAGTTGACAGCCCTAG      |   |

## Sequence of the vectors used in this study

### pOsU3-sgRNA

AAGGAATCTTTAAACATACGAACAGATCACTTAAAGTTCTTCTGAAGCAACTTAAAGTTATCAGGCATGCATGGATCTTGGAGGAATCAGATGTGCAGTCAGGGACCATA  
GCACAAGACAGGCGTCTTCTACTGGTGCTACCAGCAAATGCTGGAAGCCGGGAACACTGGGTACGTCGGAAACCACGTGATGTGAAGAAGTAAGATAAACTGTAGGAGAA  
AAGCATTTCGTAGTGGGCCATGAAGCCTTTCAGGACATGTATTGCAGTATGGGCCGGCCATTACGCAATTGGACGACAACAAAGACTAGTATTAGTACCACCTCGGCTA  
TCCACATAGATCAAAGCTGATTTAAAAGAGTTGTGCAGATGATCCGTGGCATGAGACCAACACAAGGTCTCGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAG  
TCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCCTTTTTT

The rice U3 promoter and sgRNA scaffold are highlighted in green and red. The transcription initiation sites of rice U3 promoter is indicated in yellow. Two *Bsa* I sites are underlined. The guide sequence can be inserted between the two *Bsa* I sites using annealed oligos.

### 2TAL(flanked by *Avr* II and *Nhe* I)

CCTAGGGGCGGAAGCGGCGGCCTCCTGGACCCAGGAACCCCGATGGACGCCGATCTCGTGGCGTCCAGCACAGTGGTCTGGGAGCAGGATGCGGATCCATTCGCGGGAAC  
CGCGGACGATTTCCCAGCCTTCAACGAGGAGGAGCTGGCCTGGCTGATGGAGCTCCTGCCACAGGGCGGCTCAGGCGGCCTCCTGGATCCTGGAACACCGATGGACGCGG  
ATCTCGTGGCCTCATCTACCGTCGTCTGGGAGCAGGACGCCGATCCTTTCGCCGGCACAGCGGACGATTTCCCAGCCTTCAATGAGGAGGAGCTCGCGTGGCTGATGGAG  
CTGCTCCCACAGGCTAGC

### VP64(flanked by *Avr* II and *Nhe* I)

CCTAGGGGCTCAGACGCCCTGGATGATTTTCGACCTCGATATGCTGGGCTCAGATGCCCTGGACGACTTTGACCTCGACATGCTCGGCTCTGACGCGCTCGACGACTTTGA  
CCTCGATATGCTCGGTTCCGATGCCCTGGACGACTTCGACCTCGACATGCTCGCTAGC

### Cas9-6TAL-VP128

ATGGCCCTAAGAAGAAGAGAAAGGTCGGTATTCACGGCGTTCTGCGGCGATGGACAAGAAGTATAGTATTGGTCTGGACATTGGGACGAATTCCGTTGGCTGGGCCGT

GATCACCGATGAGTACAAGGTCCCTTCCAAGAAGTTTAAGGTTCTGGGGAACACCGATCGGCACAGCATCAAGAAGAATCTCATTGGAGCCCTCCTGTTCTGACTCAGGCG  
AGACCGCCGAAGCAACAAGGCTCAAGAGAACCGCAAGGAGACGGTATACAAGAAGGAAGAATAGGATCTGCTACCTGCAGGAGATTTTCAGCAACGAAATGGCGAAGGTG  
GACGATTTCGTTCTTTCATAGATTGGAGGAGAGTTTCCTCGTTCGAGGAAGATAAGAAGCACGAGAGGCATCCTATCTTTGGCAACATTGTGCGACGAGGTTGCCTATCACGA  
AAAGTACCCCAATCTATCATCTGCGGAAGAAGCTTGTGGACTCGACTGATAAGGCGGACCTTAGATTGATCTACCTCGCTCTGGCACACATGATTAAGTTCAGGGGCC  
ATTTTCTGATCGAGGGGGATCTTAACCCGGACAATAGCGATGTGGACAAGTTGTTTCATCCAGCTCGTCCAAACCTACAATCAGCTCTTTGAGGAAAACCCAATTAATGCT  
TCAGGCGTCGACGCCAAGGCGATCCTGTCTGCACGCCTTTCAAAGTCTCGCCGGCTTGAGAACTTGATCGCTCAACTCCCGGGCGAAAAGAAGCGGCTTGTTTCGGGAA  
TCTCATTGCACTTTCGTTGGGGCTCACACCAAACCTCAAGAGTAATTTTGATCTCGCTGAGGACGCAAAGCTGCAGCTTTCCAAGGACACTTATGACGATGACCTGGATA  
ACCTTTTGGCCAAATCGGCGATCAGTACGCGGACTTGTTCCTCGCCGCGAAGAATTTGTTCGGACGCGATCCTCCTGAGTGATATTCTCCGCGTGAACACCGAGATTACA  
AAGGCCCGCTCTCGGCGAGTATGATCAAGCGCTATGACGAGCACCATCAGGATCTGACCCTTTTGAAGGCTTTGGTCCGGCAGCAACTCCCAGAGAAGTACAAGGAAAT  
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The NLS, Cas9, 6TAL and VP128 are highlighted in purple, blue, red and black, respectively. *Avr II* restriction site is underlined.