Supplemental information

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SUPPLEMENTAL INFORMATION

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Supplemental Materials and Methods

Vector construction

Maize codon optimized (z) zCas12j2 in attL1-attR5 Gateway compatible entry clone pYPCas12j2 (Addgene # 189780) was prepared using two synthetic DNA fragments (IDT gBlocks), Cas12j2-gBk1 and Cas12j2-gBk2 (Supplemental Table 1). Based on the synthesized Cas12j2-gBk1 and PCR amplified pYPC230 (Addgene # 86210) backbone with primers 230_BB_F and 230_BB_R (Supplemental Table 1), NEBuilder® HiFi DNA Assembly kit was used. Second round of cloning was needed to introduce Cas12j2-gBk2 into pYPQ230-Cas12j2-gBk1 with restriction digestion and ligation cloning using SphI-HF (NEB, catalog # R3182*) and SpeI-HF (NEB, catalog # R3133*) and T4 ligase (NEB, catalog # M0202*). The DNA sequence of zCas12j2 in pYPQCas12j2 vector was confirmed with Sanger sequencing with primers Attl1-APB-F, Cas12j2-Seq1, Cas12j2-Seq2, Cas12j2-Seq3, Nos-term-R2 (Supplemental Table 1). pYPQ141-ZmUBI-RZ-Cas12j2 (Addgene #189781) sgRNA attL5-attL2 Gateway compatible entry clone was prepared by restriction digestion of pYPQ141-ZmUbi-RZ-Lb (Addgene #86197) and Cas12j2-crRNA-gBk (Supplemental Table 1) synthetic DNA fragment (IDT, gBlock) with BamHI-HF (NEB, catalog #R3136*) and EcoRI-HF (NEB, catalog # R3101*) followed by ligation. Golden Gate compatible vectors pYPQ131-RZ-Cas12j2 (Addgene # 189782), pYPQ132-RZ-Cas12j2 (Addgene # 189783), pYPQ133-RZ-Cas12j2 (Addgene # 189784), and pYPQ134-RZ-Cas12j2 (Addgene # 189785) were prepared by restriction digestion and ligation cloning of PCR amplicons and pYPQ131-STU-Lb (Addgene # 138096), pYPQ132-STU-Lb (Addgene # 138099), pYPQ133-STU-Lb (Addgene # 138102), and pYPQ134-STU-Lb (Addgene # 138105), respectively, with BsaI-HFv2 (NEB, catalog # R3733*). PCR amplicons were prepared from pYPQ141-ZmUbi-RZ-Cas12j2 vector using primers pYPQ131-F-STU-Cas12j2 and pYPQ131-R-STU (Supplemental Table 1) in case of pYPQ131-RZ-Cas12j2, pYPQ132-F-STU-Cas12j2 and pYPQ132-R-STU in case of pYPQ132-RZ-Cas12j2, pYPQ133-F-STU-Cas12j2 and pYPQ133-R-STU in case of pYPQ133-RZ-Cas12j2, and pYPQ134-F-STU-Cas12j2 and pYPQ134-R-STU in case of pYPQ134-RZ-Cas12j2.

T-DNA vectors with pLR prefix (Supplemental Table 2) for CRISPR-Cas12j2 mediated editing were prepared using Gateway LR assembly reactions based on the protocols described previously (Lowder et al., 2015). Briefly, forward and reverse primers (Supplemental Table 1) were phosphorylated with T4 polynucleotide kinase (NEB, catalog #M0201*), annealed, and ligated with T4 DNA ligase (NEB, catalog #M0202*) into BsmBI (ThermoFisher scientific, catalog #ER045*) restriction digested pYPQ141-ZmUBI-RZ-12j2 sgRNA entry clones. In case of multiplexing four target sites, phosphorylated and annealed oligos were each ligated into BsmBI digested pYPQ131-RZ-Cas12j2 to pYPQ134-RZ-Cas12j2. While pYPQ141-ZmUBI-RZ-12j2 can be directly used in Gateway LR reaction, four target sites in vectors pYPQ131-RZ-Cas12j2 to pYPQ134-RZ-Cas12j2 need to be introduced using Golden gate reaction with BsaI-HFv2 and T4 ligase into pYPQ144-ZmUbi-pT (Addgene
vector. Individual Gateway LR reactions each consisted of an assembled attL5-attL2 sgRNA entry clone, the attL1-attR5 Cas12j2 entry clone, and the attR1-attR2 destination vector pYPQ203 (Addgene # 86207) containing a ZmUbi1 promoter for Cas12j2 expression in case of rice. The attR1-attR2 destination vector pYPQ202 (Addgene # 86198) containing a AtUBQ10 promoter for Cas12j2 expression was used in case of poplar and pCGS710 containing 2x35S promoter was used for Cas12j2 expression in case of tomato. gRNAs were in all cases expressed with a ZmUbi1 promoter. Both sgRNA and Cas12j2 entry clone recombination regions were confirmed by Sanger sequencing. Final T-DNA vectors were confirmed by restriction digestion with EcoRI-HF (NEB, catalog # R3101*).

pGEL637 (Addgene # 189832) with vCas12j2 was prepared from 2 overlapping PCR amplicons and assembled into the HindIII and XmaI digested T-DNA vector pGEL635 using Gibson assembly. To prepare pGEL638 (Addgene # 189831) with nCas12j2, 5 mutations (E159A, S160A, S164A, D167A, E168A) were introduced into Cas12j2 with mutagenesis PCR. nCas12j2 was introduced into the HindIII and XmaI digested T-DNA vector pGEL635 (Addgene # 189829) using Gibson assembly. To prepare the vector for gene activation pGEL636 (Addgene # 189830), the activator 6 TAL_VP128, which was a gift from Li lab (Li et al., 2017), was PCR amplified. Afterwards, the PCR amplified 6TAL_VP128 activator was introduced in the HindIII and XmaI digested T-DNA vector pGEL635 using Gibson assembly. To prepare the vector for methylation pGEL639 (Addgene # 189833), the rice codon optimized Dnmt3A and Dnmt3L catalytic domains were synthesized and cloned in pMOD_A using Golden Gate assembly to construct vector pLSS514. The mature crRNA repeat was synthesized by overlapping extension PCR, then assembled into the vector containing pOsUbi1-HH-ccdb-HDV-AtNOS-T using Gibson assembly to construct entry vector pLSS551. The entry vector pLSS514, pLSS551 and pMOD_C0000a were assembled into the T-DNA backbone vector pTRANS_210d (Addgene Plasmid #91109) using Golden Gate assembly to generate the DNA methylation vector pGEL639.

The target genes and protospacer sequences used in this study are summarized in Supplemental Table 3.

Plants transformation

The Japonica cultivar *Nipponbare* rice was used in this study. Transgenic rice lines were prepared using *Agrobacterium*-mediated transformation which was conducted as published previously (Zhong et al., 2019). Succinctly, the rice calli were induced from seeds which grew on N6-D medium for 7 days at 32°C under light. The T-DNA vectors were transformed into *Agrobacterium tumefaciens* strain EHA105. Each transformed *Agrobacterium* EHA105 was cultured at 28°C and re-suspended in AAM-AS medium (OD600 = 0.1) containing 100 mM acetosyringone. After co-cultivation with the *Agrobacterium* for 3 days, the calli were washed with sterile water and transferred to N6-S medium containing 400 mg/l timentin and 50 mg/l hygromycin and incubated for 2 weeks. Resistant calli were transferred to REIII medium with 400 mg/l timentin and 50 mg/l hygromycin to obtain regenerated plants.
For rice protoplast transformation, the rice seedlings were grown for 11 days in the dark at 28 °C. The leaves were cut to about 1.0 mm long strips and transferred into the enzyme solution followed by vacuum-infiltration for 30 minutes. Afterwards, strips in enzyme solution were incubated at 70-80 rpm for 6-8 hours at 25°C in dark. Each digestion mixture was filtered using a 40 μm cell strainer. The protoplasts were centrifuged at 100 g for 5 min. Supernatant was removed and the protoplasts were re-suspended in W5 solution. Then, the W5 solution was decanted, and protoplasts were re-suspended at 2×10^6 ml^-1 in MMG solution. 30 μg of T-DNA plasmid and MMG were added to make up 30 μl, and then transformed into 200 μl protoplasts (4×10^5 protoplasts). The mixture was incubated in the same volume PEG solution for 30 min. The transfection mixture was diluted with 1 ml W5 solution and centrifuged at 250 g for 5 min to remove the supernatant. The protoplasts were then gently resuspended with the W5 solution in each well of a 12-well tissue culture plate. Protoplast cells were collected for genome editing analysis after 48 hours or 72 hours incubation at 32°C in dark.

For tomato protoplast transformation, the Micro Tom Tomato cultivar was used. The tomato protoplast transformation was performed according to a recent publication (Randall et al., 2021). Briefly, the tomatoes were grown at 25°C (12 h light/12 h dark). 10-14-day-old cotyledons were isolated by cutting the petiole where it meets the leaf and incubated in the enzyme solution at 28°C in the dark for 8-10 hours. Then, the digested cells were filtered by a 75-μm Nylon cell strainer wetted by W5 solution and centrifuged for 10 min at 200 g. The supernatant was pipetted off and cells were resuspended by 0.55M sucrose. After centrifugation at 200 g for 30 min, a band of protoplasts was visible at the interface, which were transferred to new 10 mL tubes and washed with 10 mL W5 buffer twice. The cells were resuspended in MMG and adjusted the final concentration to 5 × 10^5/mL. 20 μL (1000 ng/μL) plasmid DNA, 200 μL protoplasts and 220 μL of filter sterilized 40% PEG solution were mixed gently in a 2 mL round tube. After 20 min incubation at room temperature, the reactions were stopped by adding 900 μL W5 buffer. Protoplasts were collected by centrifugation and transferred into 12-well culture plates. Plates were incubated at 30°C in the dark for 60 h.

For poplar stable transformation, Populus alba x tremula clone 717-1B4 was used as previously described (Leple et al., 1992). Transformed shoots were selected by regenerating on media containing 20 mg/l hygromycin. Regenerated shoots of 1-2 cm-long were transferred to rooting media containing 20 mg/l hygromycin. The rooted plants were used for further genotyping. Tissue culture plants were propagated in 23 °C with 24h of light condition.

**NGS sequencing and data analysis**

Next-generation sequencing (NGS) of PCR amplicons was used for the detection and quantification of mutations at target sites. For rice, genomic DNA was extracted from protoplasts and stable lines using the CTAB method (Stewart and Via, 1993). Target sites were amplified with barcoded primers according to our previously published protocols(Zhong et al., 2019). The PCR products were gel-purified, and then sequenced by Novogene (Tianjin,
China) with NovaSeq 6000-PE150 sequencing strategy. The data were analyzed with CRISPRMatch software (You et al., 2018).

For tomato, the method was performed according to a recent publication (Randall et al., 2021). Transformed tomato protoplasts were directly mixed with dilution buffer for PCR amplification of the target sites using the Phire Plant Direct PCR Kit (Thermo Fisher, USA). PCR products were pooled together for next-generation sequencing (Genewiz, USA).

For poplar, leaf samples were mixed with dilution buffer (ThermoFisher) by following the manufacturer’s manuals and two rounds of Hi-Tom PCR (Liu et al., 2019) were preceded using Phire Plant Direct PCR Master Mix (ThermoFisher). PCR products were pooled together for next-generation sequencing (Genewiz, USA).

**RNA extraction and qPCR analysis**

1 μg of leaf or 8 × 10^5 protoplasts of rice were used for RNA extraction. Total RNA extraction and DNA removal were achieved using Steady Pure Plant RNA Extraction Kit (AG21019, ACCURATE BIOLOGY) according to the manufacturer’s instructions. Then, 400 ng of total RNA was used for cDNA synthesis using HiScript III RT SuperMix for qPCR Kit (R323-01, Vazyme). The qPCR analysis was performed using the ChamQ Universal SYBR qPCR Master Mix (Q711-02, Vazyme) coupled with the Real-Time PCR Detection System (QTOWER3G, Analytikjena) to detect transcript expression levels. OsActin was used as the endogenous control genes for rice. Fold changes were calculated by the 2^−ΔΔCt method. All primers used in this study are listed in Supplemental Table 1.

**BS-PCR for methylation analysis**

The Japonica cultivar Nipponbare rice were used. Genomic DNA was extracted from the rice protoplast by the CTAB method. Bisulfite treatment was conducted by using the EpiTect Bisulfite Kit (59104; QIANGEN) according to the manufacturer’s instructions. Bisulfite-treated DNA was then used to amplify the target regions using barcoded primers listed in Supplemental Table 1. PCR products were pooled together and purified by agarose gel electrophoresis. Then PCR amplicons were sequenced by Novogene (Tianjin, China) with NovaSeq 6000-PE250 sequencing strategy. The sequencing results were analyzed with Bismark software (Krueger and Andrews, 2011) and the methylation percentages of each cytosine site were calculated.

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## Supplemental Table 1. Oligos and gBlocks used in this study

| Name  | Sequence 5'-3' | Description                                      |
|-------|----------------|--------------------------------------------------|
| 3945-HTS-F1 | ATCACGGCAATCAATGCTACCGCGT | Forward primer of OsAA01_TTT for NGS            |
| 3945-HTS-R1 | CAAAAGAGGCTGCTGTTATGTTGGA | Reverse primer of OsAA01_TTT for NGS            |
| 3945-HTS-F5 | ACAGTGTCACTGCTGTGACTGGCATGT | Forward primer of OsAA01_TT for NGS             |
| 3945-HTS-R5 | CAACTAGGCTAGCTGAGACACCTGC | Reverse primer of OsAA01_TT for NGS             |
| 3946-HTS-F1 | GTCCGCTTCCTGCACCGCCACATACC | Forward primer of OsAA02_TTT for NGS            |
| 3946-HTS-R1 | CTAGCTTAATGATGCGCCCTACACGA | Reverse primer of OsAA02_TTT for NGS            |
| 3946-HTS-F5 | GTAGAGGCCACCCTAAGTTCCTCG | Forward primer of OsAA02_TT for NGS             |
| 3946-HTS-R5 | CAAAAGAATGCCGCGCGACAGAAA | Reverse primer of OsAA02_TT for NGS             |
| 3947-HTS-F1 | ACTTGGAAGATCCGTTCTCTACACGGCTT | Forward primer of OsAC01_TTT for NGS             |
| 3947-HTS-R1 | CACCGGAGCCGGATGAGAGCAGAGGAG | Reverse primer of OsAC01_TTT for NGS             |
| 3947-HTS-F7 | GTCCGCTTCCTACACCGCGTTGATC | Forward primer of OsAC01_TT for NGS             |
| 3947-HTS-R7 | CACTACAGAGCGCGTTACACGTGACCAC | Reverse primer of OsAC01_TT for NGS             |
| 3948-HTS-F1 | GTGAACCTGCTGCCAGCTGTGCTTGCT | Forward primer of OsAC02_TTT for NGS             |
| 3948-HTS-R1 | TACAGGCCCAACCGACCAACATTGTC | Reverse primer of OsAC02_TTT for NGS             |
| 3948-HTS-F7 | AGTCAATGCTTGGCATCGTGTCTAT | Forward primer of OsAC02_TT for NGS             |
| 3948-HTS-R7 | CTATACGGGAGACCCGCGATACCTCC | Reverse primer of OsAC02_TT for NGS             |
| 3949-HTS-F1 | TTAGGCTCGGCAACCGCGCCCTCATTG | Forward primer of OsAG01_TTT for NGS             |
| 3949-HTS-R1 | CATTTTGCTGCGAAGACATGCCTG | Reverse primer of OsAG01_TTT for NGS             |
| 3949-HTS-F6 | GTGGCCGTCGCGACACCGGCCCTCTTGG | Forward primer of OsAG01_TT for NGS             |
| 3949-HTS-R6 | TATAATCGAAACACAAATTGCTGCAG | Reverse primer of OsAG01_TT for NGS             |
| 3950-HTS-F3 | GATCAGCGATCAAATTTGAGCAGAGGAG | Forward primer of OsAG02_TTT for NGS             |
| 3950-HTS-R3 | TATAATACATGCACTACAGCATCAGCTCGGCTC | Reverse primer of OsAG02_TT for NGS             |
| 3950-HTS-F5 | GTAGAGCGAGATCGACTCCTACCTCCTG | Forward primer of OsAG02_TT for NGS             |
| 3950-HTS-R5 | CGGAATTCTAAGCTTCCCTTGGAGCC | Reverse primer of OsAG02_TT for NGS             |
| 3951-HTS-F3 | GTGAAGCGAGAGAGAGCTGCACTCCTG | Forward primer of OsCA01_TTT for NGS             |
| 3951-HTS-R3 | CTATACGATGACTGTTAGTGCCAAAAC | Reverse primer of OsCA01_TTT for NGS             |
| 3951-HTS-F5 | ACAGTGGAAGAAGCAGAGGAGAGCTGCG | Forward primer of OsCA01_TT for NGS |
| 3951-HTS-R5 | CAACTAGAGGGACTGATTAGTGCCCCAA | Reverse primer of OsCA01_TT for NGS |
| 3952-HTS-F3 | ACAGTGCGTGTATGTGGTATGGTGCGCC | Forward primer of OsCA02_TTT for NGS |
| 3952-HTS-R3 | GACGACGAGTGTACCTTGTGATACATC | Reverse primer of OsCA02_TTT for NGS |
| 3952-HTS-F5 | CGATGTGGTCTCTCAACTCTTTGGATG | Forward primer of OsCA02_TT for NGS |
| 3952-HTS-R5 | ACTGATTAGGTCATATAGCAGCGTGCG | Reverse primer of OsCA02_TT for NGS |
| 3953-HTS-F3 | CTATACGGATACATAATAACTTTAGAAGTC | Reverse primer of OsCC01_TTT for NGS |
| 3953-HTS-R3 | TTAGGCACATGTACCTTCTTCTCCG | Forward primer of OsCC01_TT for NGS |
| 3953-HTS-R4 | CTAGCTGGATACATAATAACTTTAGAAGTC | Reverse primer of OsCC01_TT for NGS |
| 3954-HTS-R3 | GATCGGCGCTCTGTTCTTCTGATCCATCACA | Forward primer of OsCC02_TTT for NGS |
| 3954-HTS-R5 | GACGACCTCAGCTCGTTGAGGAACACGCAGC | Reverse primer of OsCC02_TTT for NGS |
| 3955-HTS-F3 | GTAGAGGCCACACCTTGATAGTACCA | Forward primer of OsCG01_TTT for NGS |
| 3955-HTS-R3 | TAATCGCTCTTAGCATTCCCCATTAGC | Reverse primer of OsCG01_TTT for NGS |
| 3955-HTS-F5 | TGACCACATCCAAGGAGACTGACGT | Forward primer of OsCG01_TT for NGS |
| 3955-HTS-R5 | CCAACAGCATCCACAGGACGCTTCTC | Reverse primer of OsCG01_TT for NGS |
| 3956-HTS-F3 | ATACGCTTGAGGATTGGAAGGCA | Forward primer of OsCG02_TTT for NGS |
| 3956-HTS-R3 | CTATACGAGATGTCCTGCAATTGAG | Reverse primer of OsCG02_TTT for NGS |
| 3956-HTS-R5 | GTAGAGCATGATGACCTGCTTGGAGGT | Forward primer of OsCG02_TT for NGS |
| 3956-HTS-R6 | CGGAATACAGATGCTCTGCAATTGATAC | Reverse primer of OsCG02_TT for NGS |
| 3956-HTS-F6 | GTGGAAGAGGAGCTGCTTGGAGGAT | Forward primer of OsCG02_TT for NGS |
| 3957-HTS-F3 | GATCGACGACATTTTATGAGGCTGACTTAC | Forward primer of OsCA01_TTT for NGS |
| 3957-HTS-R3 | TATAATAGTAGGCTGCTTGGAGG | Reverse primer of OsCA01_TTT for NGS |
| 3957-HTS-F5 | GTAGAGGCTGAGCTGCTTGGAGG | Forward primer of OsCA01_TT for NGS |
| 3957-HTS-R5 | CAAAAGGCCAAGCATCGTTGCTCTGCTTAC | Reverse primer of OsCA01_TT for NGS |
| 3958-HTS-F3 | ACAGTGGAAGAAGCAGAGGAGAGCTGCG | Forward primer of OsGA02_TTT for NGS |
| 3958-HTS-R3 | GACGACGAGTGTACCTTGTGATACATC | Reverse primer of OsGA02_TTT for NGS |
| 3958-HTS-F5 | ACAGTGGAAGAAGCAGAGGAGAGCTGCG | Forward primer of OsGA02_TT for NGS |
3958-HTS-R5  CAACTAggccgaagctactacgaaag  Reverse primer of OsGA02_TT for NGS
3959-HTS-F3  GTTTCGGGTTGACCATCTTTTGGGAC  Forward primer of OsGC01_TTT for NGS
3959-HTS-R3  TCCGCAGTCACTACAGGATCACC  Reverse primer of OsGC01_TTT for NGS
3959-HTS-F5  CGATGTCTCCTTACGGGCCACTTCTA  Forward primer of OsGC01_TT for NGS
3959-HTS-R5  ACTGATCCTAGTAGTGATAGAGGAAACCCAC  Reverse primer of OsGC01_TT for NGS
3960-HTS-F3  GTAGAGctgcaaggatcagttacctttagg  Forward primer of OsGC02_TTT for NGS
3960-HTS-R3  TAATCGcgcctttatcactcttgac  Reverse primer of OsGC02_TTT for NGS
3960-HTS-F5  CTTGTACTGCAAGACATTTCTGTAGCC  Forward primer of OsGC02_TT for NGS
3960-HTS-R5  ATTCCTGCCAAGCATGGTTACTGTTA  Reverse primer of OsGC02_TT for NGS
3961-HTS-F3  ATACAGGTTTACTCCAGTAGGAGAC  Forward primer of OsGG01_TTT for NGS
3961-HTS-R3  CTATAACCAAAATTAGGAAAGGGAC  Reverse primer of OsGG01_TTT for NGS
3961-HTS-F5  TGACCACAATCTTTAGCCAGGATC  Forward primer of OsGG01_TT for NGS
3961-HTS-R5  CCAACACGGTAAGCAACTGACCAA  Reverse primer of OsGG01_TT for NGS
3968-HTS-F5  TGACCACGATAAAGTTGAGCAAGAGGAG  Forward primer of multiplex sites of OsTG02 for NGS
3968-HTS-R5  CTATAACCGATGCGACTACATCGCCGTC  Reverse primer of multiplex sites of OsTG02 for NGS
3968-HTS-F17  CGATGTCAGGATCGACTCCTACCTCCTG  Forward primer of multiplex sites of OsAG02 for NGS
3968-HTS-R17  ACTGATCTAAGGTTCCCTTTGGAGGC  Reverse primer of multiplex sites of OsAG02 for NGS
3968-HTS-F22  GTTTGACATGGTACCTTCTTCTCCG  Forward primer of multiplex sites of OsTC01 for NGS
3968-HTS-R22  TAATCGGATACATATAAACTTAAGTTC  Reverse primer of multiplex sites of OsTC01 for NGS
3968-HTS-F23  CTTGTACCCATCTTTCTCTTCCG  Forward primer of multiplex sites of OsCC01 for NGS
3968-HTS-R23  ATTCCTGAATCAGGTCCATAC  Reverse primer of multiplex sites of OsCC01 for NGS
458-TTC-HTS-F3  TTAGGCAGAGGTAGGAAGGTTGAGGATTG  Forward primer of OsGC05 for NGS
| 458-TTC-HTS-R3  | CATTTTcttacaaaaataaaactacca            | Reverse primer of OsGC05 for NGS |
| 458-TTC-HTS-F5  | ACAGTGgacccactaatcacaacgtagtggc       | Forward primer of OsCC06 for NGS |
| 458-TTC-HTS-R5  | TACAGGaatgttttgccctcagaacc            | Reverse primer of OsCC06 for NGS |
| 468-TTC-HTS-F13 | CCGTCCCTTCGCAAGTGACAGCATCCA           | Forward primer of OsCG05 for NGS |
| 468-TTC-HTS-R13 | CTATACcaagagatggtgaggcaat             | Reverse primer of OsCG05 for NGS |
| 468-TTC-HTS-F23 | TCGAAAggctttctataacggaacac            | Forward primer of OsTG04 for NGS |
| 468-TTC-HTS-R23 | GTGACGCCTGCCTTGATTTGCAACGAGCA         | Reverse primer of OsTG04 for NGS |
| AA-Cas12j-F1    | ATCACG tgtgcctcacaacacagcatatg        | Forward primer of OsAA03 for NGS |
| AA-Cas12j-R1    | CAACTAAAGGGTGCAAGCTAGTCTTTC           | Reverse primer of OsAA03 for NGS |
| AT-Cas12j-F3    | GGCTACGCAAGGAAGATGTTGATTTGTCG        | Forward primer of OsAG03 for NGS |
| AT-Cas12j-R3    | CCAACGctactcataacagaagctcctc         | Reverse primer of OsAG03 for NGS |
| CA-Cas12j-F1    | CCGTCCTAGGCAAGGCCATCTCCCTC           | Forward primer of OsAT03 for NGS |
| CA-Cas12j-R1    | CTATACAAGGACCTGCTAGTCTACC            | Reverse primer of OsAT03 for NGS |
| CC-Cas12j-F3    | CTCAGAGTCAGTAGGCGACAGATGGA            | Forward primer of OsCA03 for NGS |
| CC-Cas12j-R3    | CCTCAGGAGACATTAGCCTAGGAC              | Reverse primer of OsCA03 for NGS |
| CG-Cas12j-F1    | ACTGATcctgaggcttttatgtgct            | Forward primer of OsCC03 for NGS |
| CG-Cas12j-R1    | GTTTCGCCACTCAGGAACAGTTAAACC          | Reverse primer of OsCC03 for NGS |
| CT-Cas12j-F3    | TTAGGGCTCAAGTAGGCGACAGATGGA           | Forward primer of OsCT03 for NGS |
| CT-Cas12j-R3    | CATTTTGGCTCTCTACAAGGGCAAGGC          | Reverse primer of OsCT03 for NGS |
| GA-Cas12j-F1    | ACAGTGcccacattggcacattc              | Forward primer of OsGA03 for NGS |
| GA-Cas12j-R1    | TACAGGcacaagaaacaataacacagag         | Reverse primer of OsGA03 for NGS |
| GG-Cas12j-F3    | CGTACGcatgtgctcactgaaggg             | Forward primer of OsGG03 for NGS |
| GG-Cas12j-R3    | TCACTTCCTCTGATCAGCACCAGTCTCCA        | Forward primer of OsGG03 for NGS |
| TA-Cas12j-F1    | CAAAAAgatacgctcactgctgtgct           | Forward primer of OsTA03 for NGS |
| TA-Cas12j-R1    | CTTGTAttaaatgcaacatgaacataag         | Reverse primer of OsTA03 for NGS |
| TG-Cas12j-F3    | TTAGGGCTAAGTGACAGCTAGCTACC           | Forward primer of OsTG03 for NGS |
| TG-Cas12j-R3    | CATTTTCCGCAAATTTGAGCTCGTTTTAG        | Reverse primer of OsTG03 for NGS |
| TT-Cas12j-F1    | ACAGTGCCCAAAACACTACAACTGT            | Forward primer of OsTT01 for NGS |
| TT-Cas12j-R1    | TACAGGctgtgctgagcgaggg              | Reverse primer of OsTT01 for NGS |
| SLCC02-Cas12j-F3| GGCTACgcatcagcagcagccccacac          | Forward primer of OsCC04 for NGS |
| SLCC02-Cas12j-R3 | CCAACActccacagagaacagctag | Reverse primer of OsCC04 for NGS |
| SLCA05-Cas12j-F1 | CCGTCTCTCGCAAGTAGCAGCATCCA | Forward primer of OsCA04 for NGS |
| SLCA05-Cas12j-R1 | CTATAcaagagatggaatgtgagcaat | Reverse primer of OsCA04 for NGS |
| SLCA06-Cas12j-F3 | CTCGACATGCCCATCTCCCTGTCTAC | Forward primer of OsCA05 for NGS |
| SLCA06-Cas12j-R3 | GTCCGCGAATGCATGGACTAAGAGC | Reverse primer of OsCA05 for NGS |
| SLGA08-Cas12j-F1 | ATCACGgtgtgcctctctaatccaggg | Forward primer of OsGA04 for NGS |
| SLGA08-Cas12j-R1 | CAACTAGTGCCCTTTGATTGAAACAGCA | Reverse primer of OsGA04 for NGS |
| SLGG09-Cas12j-F3 | TGCACCGCGTTGACAGGGGGAATTTGC | Forward primer of OsGG04 for NGS |
| SLGG09-Cas12j-R3 | GTCCGCGCTACGTCTTACACTATGAAGC | Reverse primer of OsGG04 for NGS |
| SLGG10-Cas12j-F1 | ACTTTGACTACACAGCTCCTTGGCCC | Forward primer of OsGG05 for NGS |
| SLGG10-Cas12j-R1 | CACGATaggagaacagaagaaaagacaag | Reverse primer of OsGG05 for NGS |
| SLGC12-Cas12j-F3 | CTCAGACTTATGTTCCGTTCCAATTC | Forward primer of OsGC04 for NGS |
| SLGC12-Cas12j-R3 | GTCCGCGCTACGTCTTACACTATGATGAAGC | Reverse primer of OsGC04 for NGS |
| SLCT13-Cas12j-F1 | ACTGATTTCGCAAGTAGCAGCATCCA | Forward primer of OsCT04 for NGS |
| SLCT13-Cas12j-R1 | GTTTCGcaagagatggaatgtgagcaat | Reverse primer of OsCT04 for NGS |
| AG-Cas12j-F3 | AGTTCCCTTACATACTTGTAAGACGTAGC | Forward primer of OsAG03 for NGS |
| AG-Cas12j-R3 | CTTGTACAAATGGACACATCAACTACTGC | Reverse primer of OsAG03 for NGS |
| GT-Cas12j-F1 | CATGGGctgtTATctgtTATCCATCTTGC | Forward primer of OsGT03 for NGS |
| GT-Cas12j-R1 | GTCCGCGCGAGCAGCTCCTGACTACAGCA | Reverse primer of OsGT03 for NGS |
| SLGC01-Cas12j-F3 | ACTTGATGGGGGAGGACGAGCGGCTGGTG | Forward primer of OsGC04 for NGS |
| SLGC01-Cas12j-R3 | CACTCAGggccccgaatttctgcaag | Reverse primer of OsGC04 for NGS |
| SLCC04-Cas12j-F1 | GGCTACAAGGAGCACCATTCCCTGCCCAT | Forward primer of OsCC05 for NGS |
| SLCC04-Cas12j-R1 | TACAGCCAGCTACACACGGACGCCG | Reverse primer of OsCC05 for NGS |
| AG02-TT-F3 | TTAGGCCCAGGATGACACTCTCTACCTTCCTG | Forward primer of OsAG02_TT for NGS |
| AG02-TT-R3 | CTAGCTTCTAAGGTCTCCCTTTGAGACC | Reverse primer of OsAG02_TT for NGS |
| AG02-TTT-F1 | ATACGCGAAAAATAAGCGAGGACGATAAAG | Forward primer of OsAG02_TTT for NGS |
| AG02-TTT-R1 | CACTCACTCTCTCTCCAGTTAGCCAC | Reverse primer of OsAG02_TTT for NGS |
| AG02-TTT-F2 | CGATGTCAAATAAGACGAGGACGATAAAG | Forward primer of OsAG02_TTT for NGS |
| AG02-TTT-R2 | CATTTTCTCCCTCCACGTTTAGCCAC | Reverse primer of OsAG02_TTT for NGS |
| AG02-TTT-F3 | TTAGGCCCAGGATGACACTCTCTACCTTCCTG | Forward primer of OsAG02_TTT for NGS |
AG02-TTT-R3  CTAGCTCTCCTTTTCCAGTTATAGCCAC  Reverse primer of OsAG02_TTT for NGS
AG02-TTT-F4  GCCAATCAATAAAGACGGAGCATCAAAG  Forward primer of OsAG02_TTT for NGS
AG02-TTT-R4  TACAGCCTCTCCTTTCCAGTTATTAGCCAC  Reverse primer of OsAG02_TTT for NGS
AG02-TTT-F5  ACAGTGCAATAAGACGGACGTAACAG  Forward primer of OsAG02_TTT for NGS
AG02-TTT-R5  CACCGGCTCCTCCTTTCCAGTTATTAGCCAC  Reverse primer of OsAG02_TTT for NGS
AG04-TT-F3  TGACCAgtccctgttgatccagag  Forward primer of OsAG04_TT for NGS
AG03-TT-R3  CATTTTgcatctataagatcagctgtc  Forward primer of OsAG04_TT for NGS
AG05-TT-F1  ACTTGAatctgttcttcagcctgaag  Forward primer of OsAG05_TT for NGS
AG05-TT-R1  CACGATtgatgaagatgcgaccatttctg  Reverse primer of OsAG05_TT for NGS
AG06-TT-F3  CGTACGgagatgttgggcgatgttc  Forward primer of OsAG06_TT for NGS
AG06-TT-R3  TCATTCTgtcctgtgtaatcttggag  Reverse primer of OsAG06_TT for NGS
AG07-TT-F1  CAAAAAGgttggaagaaagagaatgtc  Forward primer of OsAG07_TT for NGS
AG07-TT-R1  CTTGTAgtctgtaaccaaaagctacatt  Reverse primer of OsAG07_TT for NGS
AG09-TT-F3  TAGCTTAgcgcctgttcctgcag  Forward primer of OsAG09_TT for NGS
AG09-TT-R3  CATTCTatcactgcaggaacgggc  Reverse primer of OsAG09_TT for NGS
AG04-TTT-F1  ACAGTGccatgcacactcagacac  Forward primer of OsAG04_TTT for NGS
AG04-TTT-R1  TACAGCcttgctcttcagcctgaag  Reverse primer of OsAG04_TTT for NGS
AG05-TTT-F3  GGCTACccatgctttttcatccctgag  Forward primer of OsAG05_TTT for NGS
AG05-TTT-R3  CCAACAattgctgactgatgacatgg  Reverse primer of OsAG05_TTT for NGS
AG06-TTT-F1  CCGTCCctgccctggtaaatttggagg  Forward primer of OsAG06_TTT for NGS
AG06-TTT-R1  CTATACgagatgttgggcattgttc  Reverse primer of OsAG06_TTT for NGS
AG07-TTT-R3  GTCCGCggcttcgacttctgcttgtg  Reverse primer of OsAG07_TTT for NGS
AG09-TTT-R3  ACAGTGatcgacggcagcaacaggtc  Forward primer of OsAG09_TTT for NGS
AG09-TTT-F2  ACTTTAatcgacggcagcaacaggtc  Reverse primer of OsAG09_TTT for NGS
AG09-TTT-R2  CACTCAatctgcaggaacgggc  Forward primer of OsAG09_TTT for NGS
AG09-TTT-F3  TAGCTTAatcgacggcagcaacaggtc  Reverse primer of OsAG09_TTT for NGS
GBSS1-HTS-F3  CATGGCAagcgcagagaaaaacacggg  Forward primer of OsGBSS1-act for NGS
GBSS1-HTS-R3  GTAGAGttctgtgctccctgttgct  Reverse primer of OsGBSS1-act for NGS
ER1-HTS-F1  TTAGGCaactgagatgcacatcagtgctg  Forward primer of OsER1-act for NGS
| Gene     | Forward Primer | Reverse Primer |
|----------|----------------|----------------|
| ER1-HTS-R1 | CATTTTctactgtaagctacacttatgag | Reverse primer of OsER1 -act for NGS |
| NRT1.1-HTS-F3 | ATGTCAttgctccaatctctgtgag | Forward primer of OsNRT1.1A -act for NGS |
| NRT1.1-HTS-R3 | GTAGAGcttaagatgacaaagttatatc | Reverse primer of OsNRT1.1A -act for NGS |
| CHS-HTS-F1   | TAAATCGagtcggctccgggtgagaaaacgg | Forward primer of OsCHS -act for NGS |
| CHS-HTS-R1   | CAGGCGaagagagatccacttcagggag | Reverse primer of OsCHS -act for NGS |
| NRT1.1A-Act-F | GGACaaggttatctctagt | Forward primer of OsNRT1.1A active for T-DNA construct |
| NRT1.1A-Act-R | GGCCactaggatataaatctt | Reverse primer of OsNRT1.1A active for T-DNA construct |
| ER1-Act-F    | GGACGCCTAGAGTTGGTAGGA | Forward primer of OsER1 active for T-DNA construct |
| ER1-Act-R    | GGCTCCTCTACCAACTCTAGGC | Reverse primer of OsER1 active for T-DNA construct |
| CHS-Act-F    | GGACgccacgagagcagaatcA | Forward primer of OsCHS active for T-DNA construct |
| CHS-Act-R    | GGCTAgttggtcgcgttgc | Reverse primer of OsCHS active for T-DNA construct |
| GBSS1-Act-F  | GGACcaccgcacggtgtGGc | Forward primer of OsGBSS1 active for T-DNA construct |
| GBSS1-Act-R  | GGCCgCCaccgcgtggtg | Reverse primer of OsGBSS1 active for T-DNA construct |
| GBSS1-Methy-F1 | GGTCTCCGGAGtctctgtattacagccggGCGGCGCATGGTCCCAAGCCTCC | Forward primer of OsGBSS1-crR01 methylation for T-DNA construct |
| GBSS1-Methy-R1 | GGTCTCCcaggGGCCTCTGAGGGGCAATCGTGG | Reverse primer of OsGBSS1-crR01 methylation for T-DNA construct |
| GBSS1-Methy-F2 | GGTCTCccctgattagacgtagtaaatGGCCGCATGGTCCAGCCCTC | Forward primer of OsGBSS1-crR02 methylation for T-DNA construct |
| GBSS1-Methy-R2 | GGTCTCTcattcGTCCCTCCTGAGGGGCAATCGTGG | Reverse primer of OsGBSS1-crR02 methylation for T-DNA construct |
| Primer Name  | Sequence                                                                 | Description                                                                 |
|-------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| GBSS1-Methy-F3 | GGTCTCCgataaatgctgtgacctgcaGGCCGGCATGGTCCC AGCCTCC | Forward primer of OsGBSS1-crR03 methylation for T-DNA construct |
| GBSS1-Methy-R3  | GGTCTCCGGCCtatttgcacaggcctaatttGTCCCCTCGTGAGGGGCAATCGTTGG | Reverse primer of OsGBSS1-crR03 methylation for T-DNA construct |
| GBSS1-Methy-R4  | GGTCTCCGGCCtatttgcacaggcctaatttGTCCC | Reverse primer of OsGBSS1-crR04 methylation for T-DNA construct |
| OsER1-qF      | CTGTAGCCACGGGATAATAACAC | Forward primer of OsER1 for qPCR |
| OsER1-qR      | gccatagctgtgcacgcacagc | Reverse primer of OsER1 for qPCR |
| OsActin-qF     | CCACTATGTTCCCTGGCATT | Forward primer of OsActin for qPCR |
| OsActin-qR     | GTACTCAGCCTTGGCAATCC | Reverse primer of OsActin for qPCR |
| OsNRT1.1-qF   | CCCACACCAAGCAATTCCAGG | Forward primer of OsNRT1.1A for qPCR |
| OsNRT1.1-qR   | GTCTTCACCTCCCTCCACGTC | Reverse primer of OsNRT1.1A for qPCR |
| OsGBSS1-qF    | ggctcgtgacagcgcgtcat | Forward primer of OsGBSS1 for qPCR |
| OsGBSS1-qR    | GTCTCTGTGTTATGCAATTCGTTCC | Reverse primer of OsGBSS1 for qPCR |
| OsCHS-qF      | gcgagcatggtgcagctc | Forward primer of OsCHS for qPCR |
| OsCHS-qR      | tctctcctgctgctgctag | Reverse primer of OsCHS for qPCR |
| Cas12j-qF     | ACCACCGAACCTTCAACCTCC | Forward primer of Cas12j2 for qPCR |
| Cas12j-qR     | TTTCACTAGTGCTAACGGCGTAG | Reverse primer of Cas12j2 for qPCR |
| GBSS1-BS-F1   | TTAGGCCTGGGTYYATGAGGTTTATTAT | Forward primer of OsGBSS1-R1 range of BS-PCR for NGS |
| GBSS1-BS-R1   | CTAGCTCATTCTTACRTCTAATCAAACA | Reverse primer of OsGBSS1-R1 range of BS-PCR for NGS |
| GBSS1-BS-F2   | GGCTACATTGTTTAGAYGGTAAGAATG | Forward primer of OsGBSS1-R2 range of BS-PCR for NGS |
| GBSS1-BS-R2   | CGGAATACCTACCAACTAACAACCTCAACAG | Reverse primer of OsGBSS1-R2 range of BS-PCR for NGS |
| GBSS1-BS-F3   | CTCAGAGAAAYAGYTGGAAGYAAAG | Forward primer of OsGBSS1-R3 range of BS-PCR for NGS |
| GBSS1-BS-R3   | GTGAAATCCCCRRACRATATCAAC | Reverse primer of OsGBSS1-R3 range of BS-PCR for NGS |

GBSS1-Methy-F3

GBSS1-Methy-R3

GBSS1-Methy-R4

OsER1-qF

OsER1-qR

OsActin-qF

OsActin-qR

OsNRT1.1-qF

OsNRT1.1-qR

OsGBSS1-qF

OsGBSS1-qR

OsCHS-qF

OsCHS-qR

Cas12j-qF

Cas12j-qR

GBSS1-BS-F1

GBSS1-BS-R1

GBSS1-BS-F2

GBSS1-BS-R2

GBSS1-BS-F3

GBSS1-BS-R3
| Primer Set | Forward Primer | Reverse Primer |
|------------|----------------|----------------|
| Blc-CasF-F1 | tctagctgtggttgtggtgtg | Forward primer of SIBlc_crR01 for NGS |
| Blc-CasF-R1 | gctgttacctattaaccttcca | Reverse primer of SIBlc_crR01 for NGS |
| Blc-CasF-F2 | cagggcaacagtgtgtgcttgga | Forward primer of SIBlc_crR02 for NGS |
| Blc-CasF-R2 | catgctagagaaaagttggaac | Reverse primer of SIBlc_crR02 for NGS |
| Blc-CasF-F3 | gaattggtgtgcttggagt | Forward primer of SIBlc_crR03 for NGS |
| Blc-CasF-R3 | ttcattatgtggcttggagt | Reverse primer of SIBlc_crR03 for NGS |
| Blc-CasF-F4 | gaacctgttattggcatgg | Forward primer of SIBlc_crR04 for NGS |
| Blc-CasF-R4 | ttggcaacaaatatgagcag | Reverse primer of SIBlc_crR04 for NGS |
| SGR1-CasF-F1 | ccagtaatattggaactccaag | Forward primer of SISGR_crR01 for NGS |
| SGR1-CasF-R1 | tccaaataaccttgccacct | Reverse primer of SISGR_crR01 for NGS |
| SGR1-CasF-F2 | agggtggcaaggttatttgga | Forward primer of SISGR_crR02 for NGS |
| SGR1-CasF-R2 | gagttgtttacgcaacctt | Reverse primer of SISGR_crR02 for NGS |
| SGR1-CasF-F3 | ttgcagttgcaaggttggta | Forward primer of SISGR_crR03 for NGS |
| SGR1-CasF-R3 | ttgggaagttcgaacgacat | Reverse primer of SISGR_crR03 for NGS |
| SGR1-CasF-F4 | atccagagttacaagaagct | Forward primer of SISGR_crR04 for NGS |
| SGR1-CasF-R4 | cttgttgatggtcaccatt | Reverse primer of SISGR_crR04 for NGS |
| SAg07-CasF-F1 | CTCATGACAAAAACCTTTC | Forward primer of SIAgo7_crR01 for NGS |
| SAg07-CasF-R1 | AAATCAGAAGGGGTGCAA | Reverse primer of SIAgo7_crR01 for NGS |
| SAg07-CasF-F2 | GAGCCCAACGGAGAAGTGTA | Forward primer of SIAgo7_crR02 for NGS |
| SAg07-CasF-R2 | CGTCAAGCTCTTGGTTTCCC | Reverse primer of SIAgo7_crR02 for NGS |
| SAg07-CasF-F3 | acatgcacccttggtatgta | Forward primer of SIAgo7_crR03 for NGS |
| SAg07-CasF-R3 | CTTCGATGGATAGGTGAAGAA | Reverse primer of SIAgo7_crR03 for NGS |
| SAg07-CasF-F4 | ACATCAGGCTCTTGTTTGC | Forward primer of SIAgo7_crR04 for NGS |
| SAg07-CasF-R4 | GCCCTTTCTTACCTGGTCT | Reverse primer of SIAgo7_crR04 for NGS |
| SIALS-CasF-F1 | TGAAAGGGAAGGTGTTACGG | Forward primer of SIALS_crR01 for NGS |
| SIALS-CasF-R1 | ATCATCCTCTTGGCACAATC | Reverse primer of SIALS_crR01 for NGS |
| SIALS-CasF-F2 | GGTTGTCTCTGTAAGGCATT | Forward primer of SIALS_crR02 for NGS |
| SIALS-CasF-R2 | AAGCTCAAGACACCATCAA | Reverse primer of SIALS_crR02 for NGS |
| SIALS-CasF-F3 | CGCTCACAATTCTTATGACC | Forward primer of SIALS_crR03 for NGS |
| SIALS-CasF-R3 | TCACAAACCTTCTGGGTTC | Reverse primer of SIALS_crR03 for NGS |
| Sequence Name | Primers |
|---------------|---------|
| 230_BB_F      | TGGTTGACATTGACGGTGAT CAAGTATGGCCAGAGGTGT | Sanger sequencing primer for zCas12j2 | Forward primer of SIALS_crR04 for NGS | Reverse primer of SIALS_crR04 for NGS | Forward primer for preparing pYPQCas12j2 | Reverse primer for preparing pYPQCas12j2 |
| 230_BB_R      | gaacatacgctctAGCACTAGTGCCGCGCCGCC | Sanger sequencing primer for zCas12j2 | Reverse primer of SIALS_crR04 for NGS |
| AttL1-APB-F    | ttagagccatgGTGAAGGGCGAATTCCGGAGCC | Sanger sequencing primer for zCas12j2 | Reverse primer of SIALS_crR04 for NGS |
| Cas12j2-Seq1   | ccaagtcttaagcgcggccccca | Sanger sequencing primer for zCas12j2 | Sanger sequencing primer for zCas12j2 |
| Cas12j2-Seq2   | GATGGGGTGTTGAAGAAGG | Sanger sequencing primer for zCas12j2 | Sanger sequencing primer for zCas12j2 |
| Cas12j2-Seq3   | CGCTTACAAACCGAGACTG | Sanger sequencing primer for zCas12j2 | Sanger sequencing primer for zCas12j2 |
| Nos-term-R2    | aatcatgcaagacggcaacagg | Sanger sequencing primer for zCas12j2 | Sanger sequencing primer for zCas12j2 |
| pYPQ131-F-STU-Cas12j2 | gccGGTCTCgtattcttgCTGATGAGTCCGTGAGGACGA | Forward primer for preparing pYPQ131-RZ-Cas12j2 | Forward primer for preparing pYPQ131-RZ-Cas12j2 |
| pYPQ131-R-STU  | tccGGTCTCcctagAAAAAGCACCCTCGGTGCCAAN | Reverse primer for preparing pYPQ131-RZ-Cas12j2 | Reverse primer for preparing pYPQ131-RZ-Cas12j2 |
| pYPQ132-F-STU-Cas12j2 | gccGGTCTCccatgtcttgCTGATGAGTCCGTGAGGACG | Forward primer for preparing pYPQ132-RZ-Cas12j2 | Forward primer for preparing pYPQ132-RZ-Cas12j2 |
| pYPQ132-R-STU  | tccGGTCTCgtccAAAACGGCCACCTCGGTGCCACTTT | Reverse primer for preparing pYPQ132-RZ-Cas12j2 | Reverse primer for preparing pYPQ132-RZ-Cas12j2 |
| pYPQ133-F-STU-Cas12j2 | gccGGTCTCagactcttgctgATGAGTCCGTGAGGACGA | Forward primer for preparing pYPQ133-RZ-Cas12j2 | Forward primer for preparing pYPQ133-RZ-Cas12j2 |
| pYPQ133-R-STU  | tccGGTCTCcctggAAAAAGCACCCTCGGTGCCAATTT | Reverse primer for preparing pYPQ133-RZ-Cas12j2 | Reverse primer for preparing pYPQ133-RZ-Cas12j2 |
| pYPQ134-F-STU-Cas12j2 | gccGGTCTCgccagcttggtgCTGATGAGTCCGTGAGGACG | Forward primer for preparing pYPQ134-RZ-Cas12j2 | Forward primer for preparing pYPQ134-RZ-Cas12j2 |
| pYPQ134-R-STU  | tccGGTCTCcaacaAAAAAGCACCCTCGGTGCCAATT | Reverse primer for preparing pYPQ134-RZ-Cas12j2 | Reverse primer for preparing pYPQ134-RZ-Cas12j2 |
| Pt4CL1-Hitom-F5 | ggagtgaagtacgggtgtgcTGAGATGATCTACACCTATGCTG | Hitom Primers for Pt4CL1 target sites in poplar | 17 |
Hitom Primers for Pt4CL1 target sites in poplar

Pt4CL1-Hitom-R5
gagttggatgctgatggTAGCTCTGCAGGGGTGGA

Hitom Primers for Pt4CL1 target sites in poplar

Pt4CL1-Hitom-F6
gagttggatgctgatggTCCACCTGTGATGATGTCAATTG

Hitom Primers for PtPDS target sites in poplar

PtPDS-Hitom-F4
gagttggatgctgatggAAAGATTTTCATCACCCTGCAACCAG

Hitom Primers for PtPDS target sites in poplar

PtPDS-Hitom-R4
gagttggatgctgatggAGAGTTGGTTCTGTATGG

Hitom Primers for PtPDS target sites in poplar

PtPDS-Hitom-F5
gagttggatgctgatggGAGTTGGTTCTGTATGG

Hitom Primers for PtPDS target sites in poplar

PtPDS-Hitom-R5
gagttggatgctgatggGGCAACACACAAGTTGCATACCAT

Hitom Primers for PtPDS target sites in poplar

PtPDS-Hitom-F6
gagttggatgctgatggGTGAATGCTGACTTGGCCAG

Hitom Primers for PtPDS target sites in poplar

PtPDS-Hitom-R6
gagttggatgctgatggTTGTGCATAAGCTGCTTACGCA

Hitom Primers for PtPDS target sites in poplar

PtMYB182-Hitom-F1
gagttggatgctgatggACGCTATGAGAAAACCTTGCTGC

Hitom Primers for PtMYB182 target sites in poplar

PtMYB182-Hitom-R1
gagttggatgctgatggTTCGATATTGCAAGGCGGAAT

Hitom Primers for PtMYB182 target sites in poplar

PtMYB182-Hitom-F2
gagttggatgctgatggACGCTATGAGAAAACCTTGCTGC

Hitom Primers for PtMYB182 target sites in poplar

PtMYB182-Hitom-R2
gagttggatgctgatggTTCGATATTGCAAGGCGGAAT

Hitom Primers for PtMYB182 target sites in poplar

PtMYB182-Hitom-F3
gagttggatgctgatggAGTTGGTTCTGTATGG

Hitom Primers for PtMYB182 target sites in poplar

PtMYB182-Hitom-R3
gagttggatgctgatggAGTTGGTTCTGTATGG

Hitom Primers for PtMYB182 target sites in poplar

Cas12j2-qPCR-F1
gagttggatgctgatggAGTTGGTTCTGTATGG

qPCR primers for Cas12j2

Cas12j2- qPCR-R1
gagttggatgctgatggAGTTGGTTCTGTATGG

qPCR primers for Cas12j2
Hi-TOM-F-1  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttG  
CGTtgaggtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-2  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttG  
TAGtggaggtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-3  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttA  
CGCtggagtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-4  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttC  
TCGtggagtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-5  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttG  
CTCtggagtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-6  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttC  
GACtggagtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-7  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttA  
GACtggagtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-8  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttC  
ATGtggagtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-9  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttA  
TACtggagtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-10  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttC  
ACAtggagtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-11  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttA  
TGtggagtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-F-12  
ACACTCTTTCCCTACACGACGCTCTTCCGATCTgcttA  
CTAtggagtgagtacggtgtgc  
HiTom barcoding primers

Hi-TOM-R-A  
ACACTGGAAGTTTCAGACGTGTGCTCTTCCGATCTcgtG  
GTtgagttgagctgtgttg  
HiTom barcoding primers

Hi-TOM-R-B  
ACACTGGAAGTTTCAGACGTGTGCTCTTCCGATCTcgtG  
AGtgaaggagttgagctgtgttg  
HiTom barcoding primers

Hi-TOM-R-C  
ACACTGGAAGTTTCAGACGTGTGCTCTTCCGATCTcgtG  
GCtgaaggagttgagctgtgttg  
HiTom barcoding primers
| Hi-TOM-R-D | GACTGGAGTTTCAGACGTGTGCTCTTCCGATCTctgtCTCGtgtGC | HiTom barcoding primers |
| Hi-TOM-R-E | TCtgagtttgatgtctgattg | HiTom barcoding primers |
| Hi-TOM-R-F | TCtgagtttgatctgattg | HiTom barcoding primers |
| Hi-TOM-R-G | ACtgagtttgatctgattg | HiTom barcoding primers |
| Hi-TOM-R-H | TGtgagtttgatctgattg | HiTom barcoding primers |
| Cas12j2-gBK1 | GAGATCGTCTTTCCGCTGAGTACGCTGGCTACGTT | First gBlock to prepare pYPQCas12j2 |
AGGGACCCAAACGCGCCTATACCGTTGGGCGTCGT
TAGGAATAGATGCGATATACAGAAAGGCTGCCCAGG
GTATATACCCGAGTGGCAGCGCGAGGCTGGGACTG
CGATATCACCTAAACCAGCAAGCGGTATACGTAGTG
CGGGATTGAGTCCTAAAAAAAATAAGAGAATGAGGA
GGTATTGGCGTCAGAGAAGGAGAAGGCCCAAGAT
GCACTCCTGTCAACCTGAGGATAGGCACGGGATTG
GTGCTCATTGACGTTAGGGCTTTTGAAGGACAGCA
CGGTGAGAGCTATTGGCCGGAAGAGACATTAGTCGT
AACGCCTTCTGATCTTCACTGGGCAACCTGATT
ATTGATGTGAGGGGGAACATAGTCACCTTTACTTATA
CTCTGGACGCATGCGGAACATACGCTCTAGCAGCTAG
TGCC
CTCTGGACGCATGCGGAACATACGCTCGCAAGTGG
ACCCTGAAAGGAAAGCAGACTAA
GGCCACACTGGAT
AAGTTGACAGCCACGCAAGCAGTCGGCTTTTGAAGGCC
ATAGACCTTGGGCAAACCAACTTGAAGTGCCGGG
ATCGACGGGTATCTCAGGAAATGTTGCACTTACG
TGCGAGCCCTCTCGATCGCTTTACCTCCCGATGAT
CTTCTAAAGCATCAGTGCCTACGCTCTTGGCTGG
GACCGGAACGAGGAACATCCTCGGCTCAGTGGT
TGAAGCCTTGGCCAGAGAGCAGAGCTTGGGTTA
GGGCACCTGGAGTGGGCTTTAAGAGACTGCCAGAA
CTCAATTGTGCTGTATTTTGGGCTTGATCCTAAGAG
ACTCCCTTGGGATAAAATGTCGTCGAATACTACCTTC
ATCTCCAGGCGCCCTGCTTAGTAACAGCGTTAGTCGC
GACCAAGTCTTTTTTCACCCCGCCCGAAAACAGG
GCGAAAAAGAAGCTCCGGTGGAAATGTTGCACTGAGGGA
GACCGCAACATGGGCACGCGCTTACAAACCGA
GACT
GTCAGTTGAAGGCAGAAATGGAAGACAGCAGCT

Second gBlock to prepare pYPQCas12j2

Cas12j2-gBK2
gBlock to prepare Cas12j2-crRNA-gBk

Cas12j2-crRNA-gBK

GGCGCCCGCTGGGCAACATCGTGCATGGCGACA
TGTTTGGTGTTACTTCTGCAGGATCCtcgttgCTGATG
AGTCCCGTGAGGACGAAACGAGTAAGCTCGTCCAAC
GATTGCCCTCACGAGGGGACgagacggagctcagtctgc
cgcggcgtctctGGCGCCGATGGTCCCAGCCTCCTGCTG
TGGGACggtaccggccgaattcgaccagcgttct
| Construct | Backbone | Target gene | Purpose |
|-----------|----------|-------------|---------|
| pLR3945   | pYPQ203  | OsAA01      | For Cas12j2 genome editing in rice |
| pLR3946   | pYPQ203  | OsAA02      | For Cas12j2 genome editing in rice |
| pLR3947   | pYPQ203  | OsAC01      | For Cas12j2 genome editing in rice |
| pLR3948   | pYPQ203  | OsAC02      | For Cas12j2 genome editing in rice |
| pLR3949   | pYPQ203  | OsAG01      | For Cas12j2 genome editing in rice |
| pLR3950   | pYPQ203  | OsAG02      | For Cas12j2 genome editing in rice |
| pLR3951   | pYPQ203  | OsCA01      | For Cas12j2 genome editing in rice |
| pLR3952   | pYPQ203  | OsCA02      | For Cas12j2 genome editing in rice |
| pLR3953   | pYPQ203  | OsCC01      | For Cas12j2 genome editing in rice |
| pLR3954   | pYPQ203  | OsCC02      | For Cas12j2 genome editing in rice |
| pLR3955   | pYPQ203  | OsCG01      | For Cas12j2 genome editing in rice |
| pLR3956   | pYPQ203  | OsCG02      | For Cas12j2 genome editing in rice |
| pLR3957   | pYPQ203  | OsGA01      | For Cas12j2 genome editing in rice |
| pLR3958   | pYPQ203  | OsGA02      | For Cas12j2 genome editing in rice |
| pLR3959   | pYPQ203  | OsGC01      | For Cas12j2 genome editing in rice |
| pLR3960   | pYPQ203  | OsGC02      | For Cas12j2 genome editing in rice |
| pLR3961   | pYPQ203  | OsGG01      | For Cas12j2 genome editing in rice |
| pLR3963   | pYPQ203  | OsTCA01     | For Cas12j2 genome editing in rice |
| pLR3964   | pYPQ203  | OsTCA02     | For Cas12j2 genome editing in rice |
| pLR3965   | pYPQ203  | OsTGA01     | For Cas12j2 genome editing in rice |
| pLR3966   | pYPQ203  | OsTGC01     | For Cas12j2 genome editing in rice |
| pLR3968   | pYPQ203  | OsCC01, OsAG02, OsTC01, OsTG02 | For Cas12j2 genome editing in rice |
| pLR4365   | pYPQ203  | OsAA03      | For Cas12j2 genome editing in rice |
| pLR4366   | pYPQ203  | OsAC03      | For Cas12j2 genome editing in rice |
| pLR4367  | pYPQ203 | OsAG03        | For Cas12j2 genome editing in rice |
|----------|---------|---------------|----------------------------------|
| pLR4368  | pYPQ203 | OsAT03        | For Cas12j2 genome editing in rice |
| pLR4369  | pYPQ203 | OsCA03        | For Cas12j2 genome editing in rice |
| pLR4370  | pYPQ203 | OsCC03        | For Cas12j2 genome editing in rice |
| pLR4371  | pYPQ203 | OsCG03        | For Cas12j2 genome editing in rice |
| pLR4372  | pYPQ203 | OsCT03        | For Cas12j2 genome editing in rice |
| pLR4373  | pYPQ203 | OsCA03        | For Cas12j2 genome editing in rice |
| pLR4374  | pYPQ203 | OsGC03        | For Cas12j2 genome editing in rice |
| pLR4375  | pYPQ203 | OsGT03        | For Cas12j2 genome editing in rice |
| pLR4376  | pYPQ203 | OsTA03        | For Cas12j2 genome editing in rice |
| pLR4377  | pYPQ203 | OsTC03        | For Cas12j2 genome editing in rice |
| pLR4378  | pYPQ203 | OsTG03        | For Cas12j2 genome editing in rice |
| pLR4379  | pYPQ203 | OsTT01        | For Cas12j2 genome editing in rice |
| pLR4380  | pYPQ203 | OsGC04        | For Cas12j2 genome editing in rice |
| pLR4381  | pYPQ203 | OsCC04        | For Cas12j2 genome editing in rice |
| pLR4382  | pYPQ203 | OsCA05        | For Cas12j2 genome editing in rice |
| pLR4383  | pYPQ203 | OsGA04        | For Cas12j2 genome editing in rice |
| pLR4384  | pYPQ203 | OsGA05        | For Cas12j2 genome editing in rice |
| pLR4385  | pYPQ203 | OsGG04        | For Cas12j2 genome editing in rice |
| pLR4386  | pYPQ203 | OsGG05        | For Cas12j2 genome editing in rice |
| pLR4387  | pYPQ203 | OsCG04        | For Cas12j2 genome editing in rice |
| pLR4388  | pYPQ203 | OsCT04        | For Cas12j2 genome editing in rice |
| pLR4389  | pYPQ203 | OsTT02        | For Cas12j2 genome editing in rice |
| pLR4390  | pYPQ203 | OsCT05        | For Cas12j2 genome editing in rice |
| pLR4391  | pYPQ203 | OsTT03        | For Cas12j2 genome editing in rice |
| pCasΦK_OsAG02-24bp | pGEL635 | OsAG02 | For Cas12j2 genome editing with 24bp spacer length in rice |
|---------------------|---------|--------|-------------------------------------------------------------|
| pCasΦK_OsAG02-22bp | pGEL635 | OsAG02 | For Cas12j2 genome editing with 22bp spacer length in rice |
| pCasΦK_OsAG02-20bp | pGEL635 | OsAG02 | For Cas12j2 genome editing with 20bp spacer length in rice |
| pCasΦK_OsAG02-18bp | pGEL635 | OsAG02 | For Cas12j2 genome editing with 18bp spacer length in rice |
| pCasΦK_OsAG02-16bp | pGEL635 | OsAG02 | For Cas12j2 genome editing with 16bp spacer length in rice |
| pCasΦK_OsAG02-14bp | pGEL635 | OsAG02 | For Cas12j2 genome editing with 14bp spacer length in rice |
| pLR4419           | pYPQ203 | OsAG02 | For Cas12j2 genome editing with 2bp mismatch in rice         |
| pLR4420           | pYPQ203 | OsAG02 | For Cas12j2 genome editing with 2bp mismatch in rice         |
| pLR4421           | pYPQ203 | OsAG02 | For Cas12j2 genome editing with 2bp mismatch in rice         |
| pLR4422           | pYPQ203 | OsAG02 | For Cas12j2 genome editing with 2bp mismatch in rice         |
| pLR4423           | pYPQ203 | OsAG02 | For Cas12j2 genome editing with 2bp mismatch in rice         |
| pLR4424           | pYPQ203 | OsAG02 | For Cas12j2 genome editing with 2bp mismatch in rice         |
| pLR4425           | pYPQ203 | OsAG02 | For Cas12j2 genome editing with 2bp mismatch in rice         |
| pLR4426           | pYPQ203 | OsAG02 | For Cas12j2 genome editing with 2bp mismatch in rice         |
| pLR4427           | pYPQ203 | OsAG02 | For Cas12j2 genome editing with 2bp mismatch in rice         |
| Construct                     | Vector | RNA ID | Function                              |
|------------------------------|--------|--------|---------------------------------------|
| pLR4428                      | pYPQ203| OsAG02 | For Cas12j2 genome editing with 2bp mismatch in rice |
| pCasФK_OsGC05                | pGEL635| OsGC05 | For Cas12j2 genome editing in rice     |
| pCasФK_OsCC06                | pGEL635| OsCC06 | For Cas12j2 genome editing in rice     |
| pCasФK_OsGC06                | pGEL635| OsGC06 | For Cas12j2 genome editing in rice     |
| pCasФK_OsTG04                | pGEL635| OsTG04 | For Cas12j2 genome editing in rice     |
| pCasФK_OsCG05                | pGEL635| OsCG05 | For Cas12j2 genome editing in rice     |
| pCasФK_OsGA05                | pGEL635| OsGA05 | For Cas12j2 genome editing in rice     |
| pCasФK_OsCC17                | pGEL635| OsCC17 | For Cas12j2 genome editing in rice     |
| pCasФK_OsCC21                | pGEL635| OsCC21 | For Cas12j2 genome editing in rice     |
| pCasФK_OsAG04                | pGEL635| OsAG04 | For Cas12j2 genome editing in rice     |
| pCasФK_OsAG05                | pGEL635| OsAG05 | For Cas12j2 genome editing in rice     |
| pCasФK_OsAG06                | pGEL635| OsAG06 | For Cas12j2 genome editing in rice     |
| pCasФK_OsAG07                | pGEL635| OsAG07 | For Cas12j2 genome editing in rice     |
| pvCasФK_OsCC17               | pGEL637| OsCC17 | For vCas12j2 genome editing in rice    |
| pvCasФK_OsCC21               | pGEL637| OsCC21 | For vCas12j2 genome editing in rice    |
| pvCasФK_OsAG04               | pGEL637| OsAG04 | For vCas12j2 genome editing in rice    |
| pvCasФK_OsAG05               | pGEL637| OsAG05 | For vCas12j2 genome editing in rice    |
| pvCasФK_OsAG06               | pGEL637| OsAG06 | For vCas12j2 genome editing in rice    |
| pvCasФK_OsAG07               | pGEL637| OsAG07 | For vCas12j2 genome editing in rice    |
| pvCasФK_OsAG09               | pGEL637| OsAG09 | For vCas12j2 genome editing in rice    |
| pnCasФK_OsCC17               | pGEL638| OsCC17 | For nCas12j2 genome editing in rice    |
| pnCasФK_OsCC21               | pGEL638| OsCC21 | For nCas12j2 genome editing in rice    |
| pnCasФK_OsAG04               | pGEL638| OsAG04 | For nCas12j2 genome editing in rice    |
| pnCasФK_OsAG05               | pGEL638| OsAG05 | For nCas12j2 genome editing in rice    |
| pnCasФK_OsAG06               | pGEL638| OsAG06 | For nCas12j2 genome editing in rice    |
| pnCasФK_OsAG07               | pGEL638| OsAG07 | For nCas12j2 genome editing in rice    |
| pCasΦK_OsAG09  | pGEL638  | OsAG09  | For nCas12j2 genome editing in rice |
|----------------|----------|---------|-----------------------------------|
| pGEL635        | pLSS424  | None    | For Cas12j2 based genome editing in rice |
| pGEL637        | pGEL635  | None    | For vCas12j2 based genome editing in rice |
| pGEL638        | pGEL635  | None    | For nCas12j2 based genome editing in rice |
| pGEL636        | pLSS635  | None    | For Cas12j2 based gene activation backbone in rice |
| pCasΦA_OsER1   | pGEL636  | OsER1   | For Cas12j2 based gene active in OsER1 |
| pCasΦA_OsNRT1.1A | pGEL636 | OsNRT1.1A | For Cas12j2 based gene active in OsNRT1.1A |
| pCasΦA_OsCHS   | pGEL636  | OsCHS   | For Cas12j2 based gene active in OsCHS |
| pCasΦA_OsGBSS1 | pGEL636  | OsGBSS1 | For Cas12j2 based gene active in OsGBSS1 |
| pGEL639        | pLSS550  | None    | For DNA methylation-based gene silencing backbone in rice |
| pCasΦM_OsGBSS1 | pGEL639  | OsGBSS1 | For DNA methylation-based gene silencing in OsGBSS1 |
| pLR4503        | pYPQ202  | Pt4CL1-crR1-4 | For Cas12j2 genome editing in poplar |
| pLR4504        | pYPQ202  | PtPDS-crR1-4  | For Cas12j2 genome editing in poplar |
| pLR4505        | pYPQ202  | PtMYB182-crR1-4 | For Cas12j2 genome editing in poplar |
| pLR4506        | pYPQ202  | PtSVP-crR1-4  | For Cas12j2 genome editing in poplar |
| pLR4507        | pCGS710  | SIALS-crR1, SIALS-crR2, SIALS-crR3, SIALS-crR4 | For Cas12j2 genome editing in tomato |
| pLR4508        | pCGS710  | SIALS-crR1, SIALS-crR2, SIALS-crR3, SIALS-crR4 | For Cas12j2 genome editing in tomato |
| pLR4509        | pCGS710  | SISGR1-crR1, SISGR1-crR2, SISGR1-crR3, SISGR1-crR4 | For Cas12j2 genome editing in tomato |
| pLR4510        | pCGS710  | SIBlc-crR1, SIBlc-crR2, SIBlc-crR3, SIBlc-crR4 | For Cas12j2 genome editing in tomato |
Supplemental Table 3. Target genes and protospacer sequences in this study

| Targeted gene   | Targeted site | Spacer sequence                     |
|-----------------|---------------|-------------------------------------|
| LOC_Os03g52594  | OsAA01_TTT    | TCAGGTTCAGAATGCTGGTC                |
| LOC_Os02g54120  | OsAA01_TT     | TCAGGTTCAGAATGCTGGTC                |
| LOC_Os02g43194  | OsAA02_TTT    | CCGGTGAAAAGGACCTTTGTC               |
| LOC_Os04g45720  | OsAA02_TT     | CCGGTGAAAAGGACCTTTGTC               |
| LOC_Os01g02690  | OsAC01_TTT    | TGGGGCCTTGCAAGGTCACC                |
| LOC_Os01g02420  | OsAC01_TT     | TGGGGCCTTGCAAGGTCACC                |
| LOC_Os03g48170  | OsAC02_TTT    | GTCTCCCTGCAAAACCACGC                |
| LOC_Os06g22970  | OsAC02_TT     | GTCTCCCTGCAAAACCACGC                |
| LOC_Os11g01450  | OsAG01_TTT    | CCGGCAGCTAATAGGGATCT                |
| LOC_Os12g01480  | OsAG01_TT     | CCGGCAGCTAATAGGGATCT                |
| LOC_Os11g44430  | OsAG02_TTT    | GGGCATGGAGACAGGAGAGA                |
| LOC_Os11g44260  | OsAG02_TT     | GGGCATGGAGACAGGAGAGA                |
| LOC_Os07g10860  | OsCA01_TTT    | TACGTGGAACAATGACAGT                 |
| LOC_Os07g47284  | OsCA01_TT     | TACGTGGAACAATGACAGT                 |
| LOC_Os10g40824  | OsCA02_TTT    | ACCCTGTGTAATGGTCAGT                 |
| LOC_Os05g36070  | OsCA02_TT     | ACCCTGTGTAATGGTCAGT                 |
| LOC_Os02g49270  | OsCC01_TTT    | TCCTGAGGACAGAGCCCAT                 |
| LOC_Os09g37860  | OsCC01_TT     | TCCTGAGGACAGAGCCCAT                 |
| LOC_Os04g50120  | OsCC02_TTT    | TGATATCCGACACCAGGAT                 |
| LOC_Os02g46610  | OsCC02_TT     | TGATATCCGACACCAGGAT                 |
| LOC_Os06g18810  | OsCG01_TTT    | GATGATGACACTGGTACTAG                |
| LOC_Os12g16490  | OsCG01_TT     | GATGATGACACTGGTACTAG                |
| LOC_Os07g40404  | OsCG02_TTT    | GCACCATATGCTTGCTGATC                |
| LOC_Os07g40790  | OsCG02_TT     | GCACCATATGCTTGCTGATC                |
| LOC_Os04g0375400| OsGA01_TTT    | TAATATGGAATCCACCGATT                |
| LOC_Os12g12600 | OsGA01_TT | TAATATTGGATCCACCGATT  |
| LOC_Os07g0696000 | OsGA02_TTT | GTATTTCTGAATTTTCATGCC  |
| LOC_Os08g01020 | OsGA02_TT | GTATTTCTGAATTTTCATGCC  |
| LOC_Os11g20160 | OsGC01_TTT | TTCACAATCTTTATAGTCACT  |
| LOC_Os11g19880 | OsGC01_TT | TTCACAATCTTTATAGTCACT  |
| LOC_Os01g09810 | OsGC02_TTT | TGAATGCTCCGGAGCGCTTA  |
| LOC_Os03g52910 | OsGC02_TT | TGAATGCTCCGGAGCGCTTA  |
| LOC_Os01g23900 | OsGG01_TTT | AGCATATGTTGTAACCTCA  |
| LOC_Os12g24050 | OsGG01_TT | AGCATATGTTGTAACCTCA  |
| OsEPFL9 | OsTCA01 | atctgtgcaaaaggggtacC  |
| OsROC5 | OsTCA02 | cacagccggaaggtaccctc  |
| OsEPFL9 | OsTGA01 | agaaaggttATGGGCAATGC  |
| OsROC5 | OsTGC01 | ttcttgcaatgccggtac  |
| OsPDS | OsAA03 | ATACCAAGCTCGGCAAAC  |
| OsROC5 | OsAC03 | ATACACCGGCGTAGTACC  |
| OsROC5 | OsAG03 | GTACTAGAGTGAGAGACC  |
| OsEPFL9 | OsAT03 | GCATCCACTGGAGGCTGCT  |
| OsROC5 | OsCA03 | GGGTCTCTCGTGACTACC  |
| OsROC5 | OsCC03 | CTACGTCGATTGGCATGAA  |
| OsPDS | OsCG03 | GCCAGAGAAGGTGAGTGTTG  |
| OsROC5 | OsCT03 | GAGGAGATCCTCTCCTCCA  |
| OsPDS | OsGA03 | CTGACATGTCGGGATAACA  |
| OsROC5 | OsGC03 | CTAGGGAAGCCCATCTCCCT  |
| OsPDS | OsGG03 | GACGGAGTGACACTGAAATC  |
| OsDEP1 | OsGT03 | GGCTTACAGCATGGCTGTC  |
| OsPDS | OsTA03 | TGACCAAGCATCTGCGAGATA  |
| OsPDS | OsTC03 | AGTGTCCTCCGTCGACC  |
| OsROC5 | OsTG03 | CTGTCATCAGCAGAGGAC  |
| OsGS3 | OsTT01 | CTCAACATGTTGTCGAGA  |
| OsDEP1 | OsGC04 | CTCAAGGCTAGCGGCGCCCG  |
OsmiR528  OsCC04  aaactgaatctCCTgCTCCT
OsROC5  OsCC05  GAGCTTGGATCTGCTCGG
OsPDS  OsCA04  AGACAAGAGTTTCACTCCCA
OsGS3  OsCA05  CTCTTGCAGCATCTGGAGG
OsEPFL9  OsGA04  TGGCCAATGCTTGCCCACA
OsROC5  OsGG04  CTGGGCTCGGCGTCTCGAC
OsPDS  OsGG05  CAAATCGGAGGACCATGGCA
OsDEP1  OsCT04  AGGAGGTGTCTACCAGCATTT
OsROC5  OsCT05  GGAGTGAATCTTTGTTCTTT
OsDEP1  OsTT02  CCAagaaagagaaggagcagc
OsROC5  OsTT03  GCctctctctCCTgtgcttg
LOC_Os02g49270  OsTC01  TCCTGAGGAGCAAGGAGCCAT
LOC_Os11g44430  OsTG02  GGGCATGGAGACAGGAGACA
OsPDS  OsGC05  aaaaaccccttagatatacta
OsDEP1  OsCC06  tcttctGGAAaaggaaaaaa
OsROC5  OsGC06  CTTTTCCAGTGCTCTCGC
OsEPFL9  OsTG04  aagaaggtATGGCCAATG
OsPDS  OsCG05  TCTTAAGGAAATAAGGAAAA
OsDEP1  OsGA05  caagtgCTCACCAGATGCA
LOC_Os11g44430  OsAG02_TTT-MM1  GGGCATGGAGACAGGAGATc
LOC_Os11g44430  OsAG02_TTT-MM2  GGGCATGGAGACAGGAgCA
LOC_Os11g44430  OsAG02_TTT-MM3  GGGCATGGAGACAGGAgG
LOC_Os11g44430  OsAG02_TTT-MM4  GGGCATGGAGACACAGGACAg
LOC_Os11g44430  OsAG02_TTT-MM5  GGGCATGGAGAGGaAGAGACA
LOC_Os11g44430  OsAG02_TTT-MM6  GGGCATGGGtACAGGAGACA
LOC_Os11g44430  OsAG02_TTT-MM7  GGGCAtAGACAGGAGACA
LOC_Os11g44430  OsAG02_TTT-MM8  GGGCgcGGAGACAGGAGACA
LOC_Os11g44430  OsAG02_TTT-MM9  GGttATGGAGACAGGAGACA
LOC_Os11g44430  OsAG02_TTT-MM10  atGCATGGAGACAGGAGACA
| Location          | Transposon Name       | Sequence  |
|-------------------|-----------------------|-----------|
| LOC_Os11g44260    | OsAG02_TT-MM1         | GGGCATGGAGACAGGAGACTc |
| LOC_Os11g44260    | OsAG02_TT-MM2         | GGGCATGGAGACAGGAGAtgCA |
| LOC_Os11g44260    | OsAG02_TT-MM3         | GGGCATGGAGACAGGAGAtcGACA |
| LOC_Os11g44260    | OsAG02_TT-MM4         | GGGCATGGAGACAGGAGAcaGAGACA |
| LOC_Os11g44260    | OsAG02_TT-MM5         | GGGCATGGAGGtgAGGAGACA |
| LOC_Os11g44260    | OsAG02_TT-MM6         | GGGCATGgcGAGACAGGAGACA |
| LOC_Os11g44260    | OsAG02_TT-MM7         | GGttATGGAGACAGGAGACA |
| LOC_Os11g44260    | OsAG02_TT-MM10        | atGCATGGAGACAGGAGACA |
| LOC_Os11g44430    | OsAG02_TTT-22bp       | GGGCATGGAGACAGGAGACATA |
| LOC_Os11g44430    | OsAG02_TTT-20bp       | GGGCATGGAGACAGGAGACA |
| LOC_Os11g44430    | OsAG02_TTT-18bp       | GGGCATGGAGACAGGAGA |
| LOC_Os11g44430    | OsAG02_TTT-16bp       | GGGCATGGAGACAGGAGA |
| LOC_Os11g44430    | OsAG02_TTT-14bp       | GGGCATGGAGACAG |
| LOC_Os11g44260    | OsAG02_TT-24bp        | GGGCATGGAGACAGGAGACATAAGc |
| LOC_Os11g44260    | OsAG02_TT-22bp        | GGGCATGGAGACAGGAGACATA |
| LOC_Os11g44260    | OsAG02_TT-20bp        | GGGCATGGAGACAGGAGACA |
| LOC_Os11g44260    | OsAG02_TT-18bp        | GGGCATGGAGACAGGAGA |
| LOC_Os11g44260    | OsAG02_TT-16bp        | GGGCATGGAGACAG |
| LOC_Os11g44260    | OsAG02_TT-14bp        | GGGCATGGAGACAG |
| LOC_Os04g01130    | OsAG04                | AATACCTTACCCAGGCTGCGT |
| LOC_Os08g03620    | OsAG05                | TTGATGGAGACCTGTTCT |
| LOC_Os02g04670    | OsAG06                | GAGCAGAAAATGCAGAATCA |
| LOC_Os03g32700    | OsAG07                | GTTCTCCCTCCCTAAAGGAG |
| LOC_Os08g41940    | OsAG09                | TAGTTGATCTTCATTGCTATT |
| LOC_Os02g44970    | OsCC17 TT             | ATCATGGCTGTCCATGAAAC |
| LOC_Os02g46610    | OsCC21 TT             | TGTATCCGCACCCGGAT |
| LOC_Os11g0545600  | OsTG04                | AATACCTTACCAGGCTGCGT |
| LOC_Os03g40840    | OsTG05                | TTGATGGAGACTTTCATT |
| LOC_Os08g29640 | OsTG06   | GAGCAGAAAATGCAGAATCA |
| LOC_Os04g31420 | OsTG07   | GTTCTCCTCCCTAAAGAAGG |
| LOC_Os02g24720 | OsTG09   | TAGTTCATCTCATTGTCATT |
| OsER1       | OsER1-act | GCCTAGAGTTGGTAGGA |
| OsNRT1.1A   | OsNRT1.1A-act | aaggttatatccctagt |
| OsCHS       | OsCHS-act | gccacgcgagccaactA |
| OsGBSS1     | OsGBSS1-act | caccgcacgcgtggtGGc |
| OsGBSS1     | OsGBSS1-crR01 | aaattaggccgtgcaaat |
| OsGBSS1     | OsGBSS1-crR02 | tcctagcttattacgcc |
| OsGBSS1     | OsGBSS1-crR03 | cctgattagaggttaagaat |
| OsGBSS1     | OsGBSS1-crR04 | gataaatgtgtgatcctga |
| SIALS       | SIALS-crR01 | CTACCTCTGGTCCCCGGAGCT |
| SIALS       | SIALS-crR02 | TTAGGCTGATTTCCGAGTGC |
| SIALS       | SIALS-crR03 | CTCAAATCTTCCACCATTTC |
| SIALS       | SIALS-crR04 | CCTAATATGTGAAATTTGC |
| SISGR1      | SISGR1-crR01 | TTGAGCTTAGATGGAAACCAC |
| SISGR1      | SISGR1-crR02 | ATGGTTTGGAGATAGCCAA |
| SISGR1      | SISGR1-crR03 | ATGTCCATTGCCACATTAGT |
| SISGR1      | SISGR1-crR04 | ACCCCACCTACCCACCAGA |
| SIAgo7      | SIAgo7-crR01 | ATTTATATTAGTATTTGAT |
| SIAgo7      | SIAgo7-crR02 | AGAGCAAGTCTTTGTTTGT |
| SIAgo7      | SIAgo7-crR03 | TTGCCACCACAACAGATTCT |
| SIAgo7      | SIAgo7-crR04 | AAGGATCATTACAATATGA |
| SIBlc       | SIBlc-crR01 | ATATGGGCTAAACAATATGG |
| SIBlc       | SIBlc-crR02 | GCACAAGTGGAAGGACATCC |
| SIBlc       | SIBlc-crR03 | CAGTAATAACCAGGAGGTA |
| SIBlc       | SIBlc-crR04 | TAGATTAGAGATAATGACCA |
| Pt4CL1      | Pt4CL1-crR01 | GTATTCAAACAAGGAGCAGT |
| Pt4CL1      | Pt4CL1-crR02 | TCTCCATCTACCTGTAGGC |
| Pt4CL1      | Pt4CL1-crR03 | TTTGAGGATGATAAAATCTG |
| Gene   | Primer Pair  | Sequence                  |
|--------|--------------|---------------------------|
| Pt4CL1 | Pt4CL1-crR04 | ATCTCTGCATTCCTGACTAC     |
| PtPDS  | PtPDS-crR01  | AACTTGAGCTGGCATAGTAA      |
| PtPDS  | PtPDS-crR02  | TTGGTGGACAGGCTTATGTT      |
| PtPDS  | PtPDS-crR03  | TCCGCGTCCAGCTAAACCAT      |
| PtPDS  | PtPDS-crR04  | CATTGCAACCTATCATTGAT      |
| PtSVP  | PtSVP-crR01  | CTGCAGCTAGAGAAGTCTCT      |
| PtSVP  | PtSVP-crR02  | ATGGAAGAGAATGAGAGACT      |
| PtSVP  | PtSVP-crR03  | CTTACTCAACCTGGAGCAGG      |
| PtSVP  | PtSVP-crR04  | TCAAGATTTCTCGAGTGCAA      |
| PtMYB182 | PtMYB182-crR01 | ATTATATTCAAACTCACGGT     |
| PtMYB182 | PtMYB182-crR02 | CCAAGGAGAGCATGCAGCTT     |
| PtMYB182 | PtMYB182-crR03 | AAGCTGAGCCTGCTGTCTCCT    |
| PtMYB182 | PtMYB182-crR04 | GAGACAAGTTGCTGTGCAG     |
Supplemental Figure 1. Assessment of a CRISPR-Cas12j2 system for genome editing in rice cells with TSN PAM. Four TSN (TCN/TGN) PAM sites were tested by Cas12j2 in rice protoplasts. The editing efficiency was measured by NGS of PCR amplicons. The error bars denote standard deviations of three biological replicates.
Supplemental Figure 2. Assessment of Cas12j2 for genome editing in tomato cells.
Three multiplexed constructs were tested in tomato protoplasts, with each simultaneous
targeting four sites in SlAgo7 (A), SIALS (B), and SlBlc (C). The editing efficiency was
measured by NGS of PCR amplicons. The error bars denote standard deviations of three
biological replicates.
Supplemental Figure 3. Genome editing profiles by Cas12j2 in rice and tomato. Deletion positions were analyzed at three target sites in rice and one target site in tomato. The PAM sites are in red and the protospacer sequences are in blue. The error bars denote standard deviations of three biological replicates.
Supplemental Figure 4. Deletion size analysis of Cas12j2 in rice and tomato. Deletion sizes were analyzed at three target sites in rice and one target site in tomato. The PAM sites are in red and the protospacer sequences are in blue. The error bars denote standard deviations of three biological replicates.
Supplemental Figure 5. Improved genome editing efficiency with Cas12j2 variants. Genome editing efficiency of Cas12j2, vCas12j2 and nCas12j2 were compared at five target sites with VTTV PAMs. The efficiency was quantified by NGS of PCR amplicons. The error bars denote standard deviations of three biological replicates. Asterisks are used to denote statistical significance by student t test (*P < 0.05; **P < 0.01; ns means 'not significant').
Supplemental Figure 6. nCas12j2 based genome editing in stable T0 rice. (A-D) Genome editing efficiency at four target sites among individual T0 transgenic rice lines. (E) Relative Cas12j2 expression in four T0 rice lines quantified by qRT-PCR. For rice, genome editing efficiency was quantify by NGS of PCR amplicons.
Supplemental Figure 7. Cas12j2 based genome editing in T0 poplar lines. (A-D) Genome editing efficiency at sixteen target sites among individual T0 transgenic poplar lines (N.D means Not Detected). (E) Relative Cas12j2 expression in eight T0 poplar lines quantified by qRT-PCR. For poplar, genome editing efficiency was quantified by NGS of PCR amplicons.
Supplemental Figure 8. nCas12j2 based genome editing in T0 transgene rice tissue and protoplasts sourced from the same tissue. (A) Sanger sequencing-based genotyping of three CRISPR-Cas12j2 T0 lines targeting the AG17-TT site in rice. (B, C) Sanger sequencing-based genotyping of protoplasts sourced from the same tissue after 48 hours or 72 hours resting of the isolated protoplasts.
Supplemental Figure 9. Cas12j2 based gene activation in rice cell. (A) Pairing Cas12j2 with a 16 bp protospacer generated undetectable genome editing activity at the promoter target sites of two genes, OsCHS and OsGBSS1. (B) Transcriptional activation of OsCHS and OsGBSS1 by Cas12j2 based transcriptional activator. mRNA levels of target gene were measured by qRT-PCR. The error bars denote standard deviations of three biological replicates. Asterisks are used to denote statistical significance by student t test (*P < 0.05; **P < 0.01; ns means 'not significant').