**Table S1.** Mutations detection on five most likely putative off-target sites.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Target** | **NOPO** | **Putative off-target locus** | **Putative off-target sequence** | **MMB** | **NPOM** | **OTS** | **Region** |
| T1 | OT1 | LOC\_Os04G0531400 | GCGACCTCCGCCTTGGACGA  CGG | 5 | 0 | 0.024 | CDS |
| OT2 | LOC\_Os02G0230625 | AGGACGGCCTCCTCGGCACA CGG | 4 | 0 | 0.019 | CDS |
| OT3 | LOC\_Os04G0436100 | CCGACGACCTCCTTGGACAA  GGC | 5 | 0 | 0.008 | CDS |
| OT4 | LOC\_Os05G0581100 | ACGTCGGCCTCCGAGGACCT CGG | 4 | 0 | 0.001 | CDS |
| OT5 | LOC\_Os07G0534400 | CCGACGGCGACGTTGGACGA CGG | 5 | 0 | 0.025 | 5’ UTR |
| T2 | OT6 | LOC\_Os07G0683600 | CGTTCGTCTGCACGCAGGTT  AGG | 4 | 0 | 0.096 | Intron |
| OT7 | LOC\_Os09G0567700 | CGTTCTGCAGGAGACATGAT TGG | 5 | 0 | 0.020 | CDS |
| OT8 | LOC\_Os12G0613250 | TGTTCTTCATCAGACAGGGT  AGC | 4 | 0 | 0.014 | CDS |
| OT9 | LOC\_Os05G0311600 | CGTTGGTCAGCAGGGAGTGT TGG | 4 | 0 | 0.011 | CDS |
| OT10 | LOC\_Os04G0652900 | CGTTCTTCAGCAGCCAATGT  GGT | 4 | 0 | 0.004 | 3’ UTR |

The protospacer adjacent motif (PAM) (NGG) is shown in green background. **Mismatch nucleotides are marked in red**.

NOPO; name of putative off-target, MMB; mis-matching bases, NPOM; number of plants with off-target mutations,

OTS; off-target score. T1 and T2 represent target1 and target2.