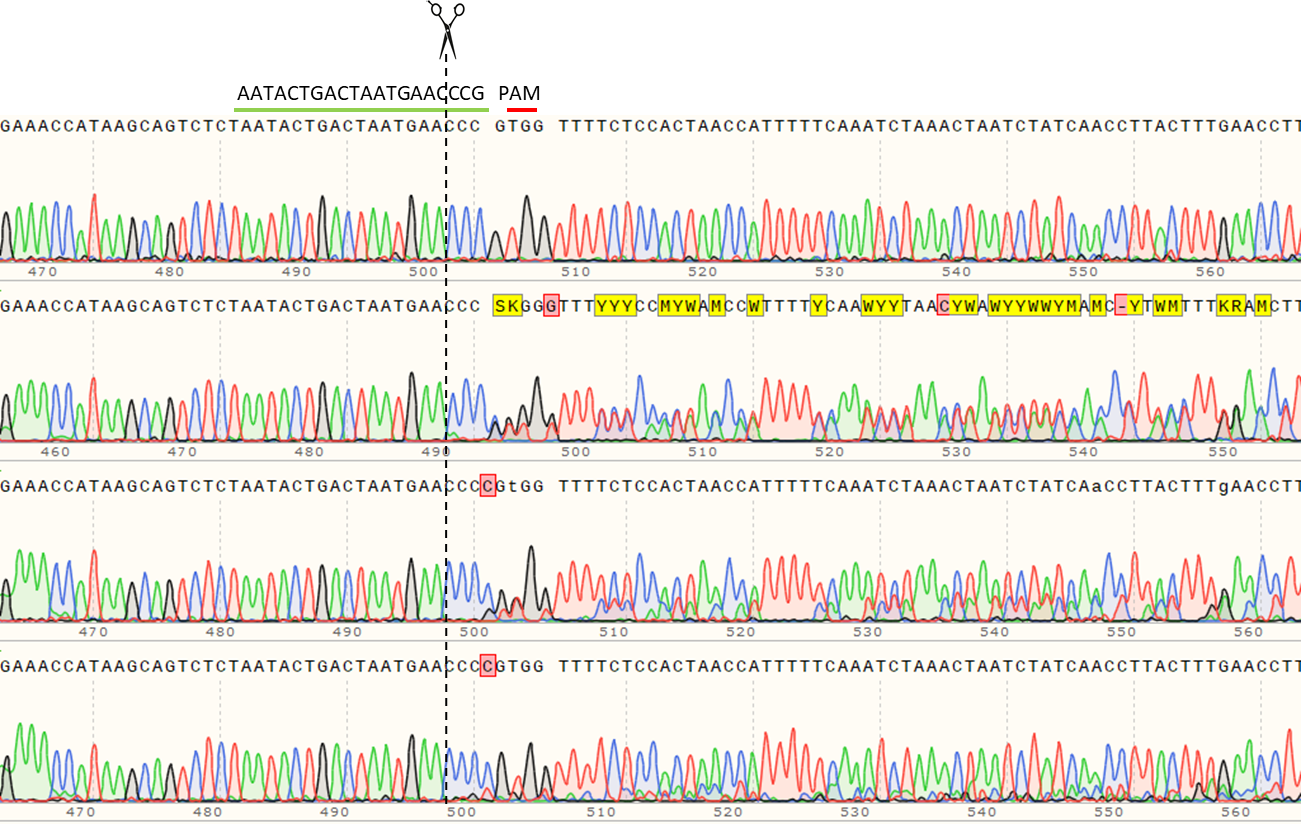
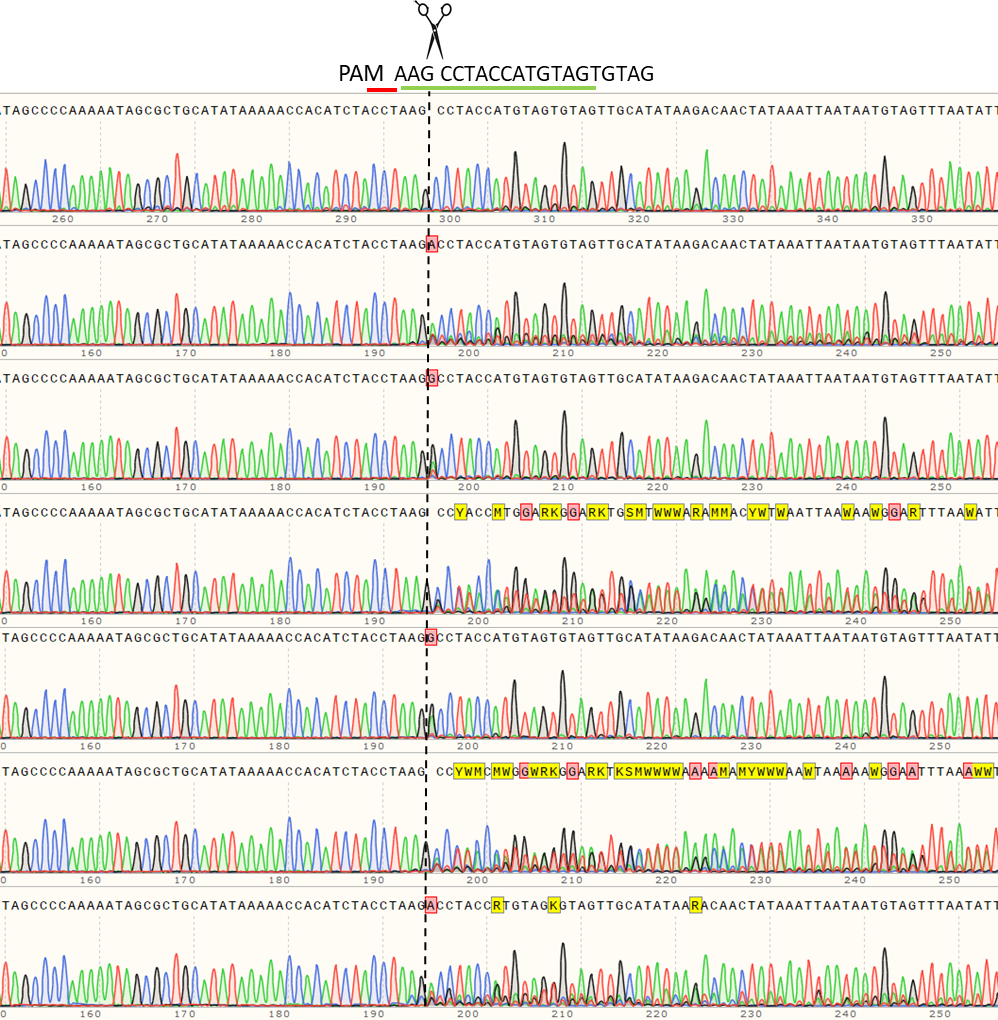
**Table S1.** List of primers used in this study.

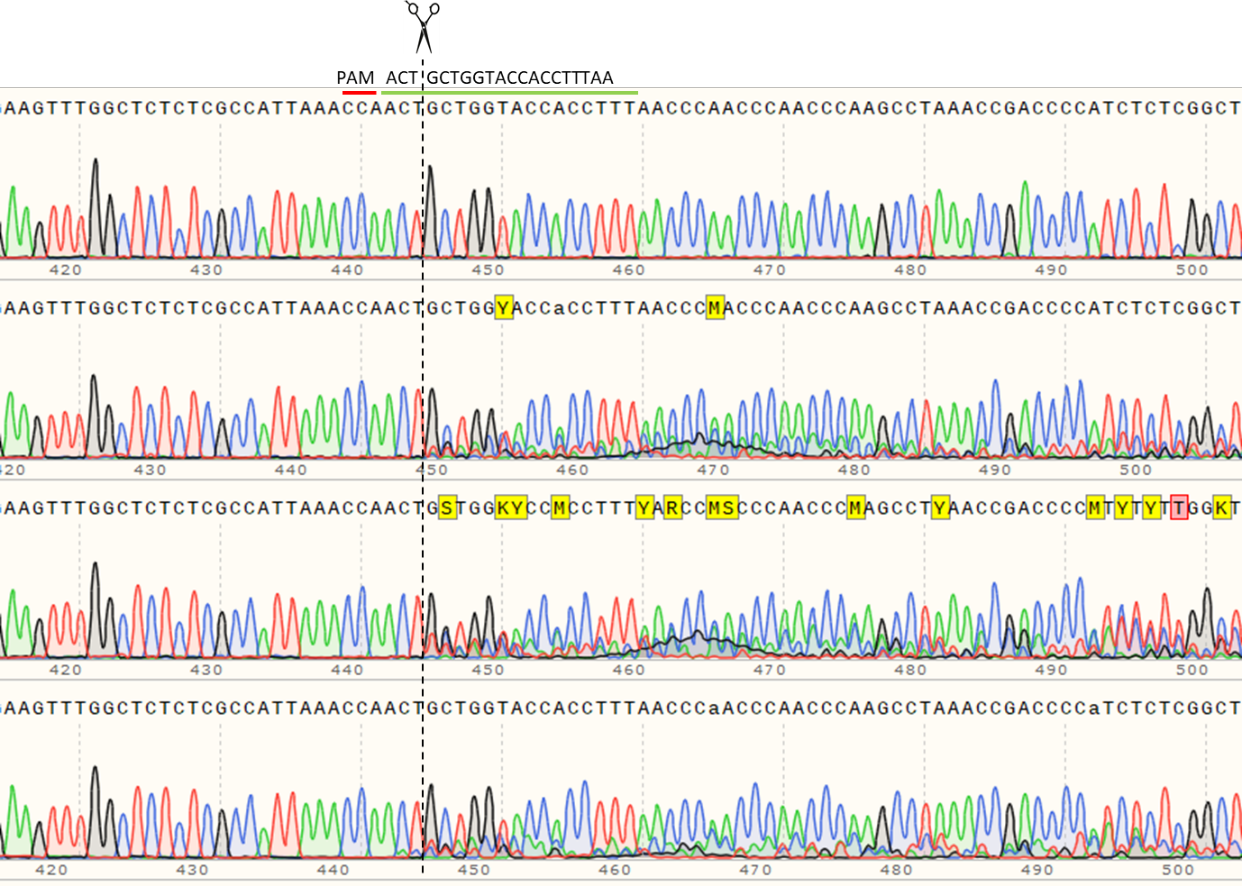
|  |  |  |
| --- | --- | --- |
| **Primer name** | **Primer sequence (5’ – 3’)** |  |
| **Primers to confirm the presence of Cas9 gene in transgenic lines** | | | | |
| zCas9-F | CGGCCTCGATATTGGGACTAACTCT | | |
| zCas9-F | CTTATCTGTGGAGTCCACGAGCTTC | | |
| **Primers to amplify the fragment flanking target site for AT1G72350** | | | | |  |
| MP5-F | CTTCCAAACCCGTCCTGTAA | | |
| MP5-R | TGGTATTAAATGCGCGTCTC | | |
| **Primers to amplify the fragment flanking target site for AT1G09970** | | | | |
| MP8-F | ACGAGTTTGGCAGGACGTTA | | |
| MP8-R | CTTAAGCTTTGCCGCTTTGA | | |
| **Primers to amplify the fragment flanking target site for AT3G17320** | | | | |
| MP14-F | CCGAGACACACAAGAGGTGA | | |
| MP14-R | TTCGATCAAAAGACGGAACC | | |
| **Primers to amplify the fragment flanking target site for AT5G28770** | | | | |
| MP18-F | CCCCTTTCAAACGTGGAATA | | |
| MP18-R | GATTGGGCAGTCTTGCATTT | | |
| **Primers to make double strand gRNA fragment targeting AT1G72350** | | | | |
| gRNA1-F | ATTGAATACTGACTAATGAACCCG | | |
| gRNA1-R | AAACCGGGTTCATTAGTCAGTATT | | |
| **Primers to make double strand gRNA fragment targeting AT1G09970** | | | | |
| gRNA2-F | ATTGCTACACTACATGGTAGGCTT | | |
| gRNA2-R | AAACAAGCCTACCATGTAGTGTAG | | |
| **Primers to make double strand gRNA fragment targeting AT3G17320** | | | | |
| gRNA3-F | ATTGTTAAAGGTGGTACCAGCAGT | | |
| gRNA3-R | AAACACTGCTGGTACCACCTTTAA | | |
| **Primers to make double strand gRNA fragment targeting AT5G28770** | | | | |
| gRNA4-R | ATTGCCCTTTATGGTAGAGGACGT | | |
| gRNA4-R | AAACACGTCCTCTACCATAAAGGG | | |
| **Primers to amplify the template for in vitro sgRNAs synthesis** | | | | |
| T7\_sgRNA-R | GCACCGACTCGGTGCCACTT | | |
| T7\_MP5 (AT1G72350)-F | TAATACGACTCACTATAGGGAATACTGACTAATGAACCCG | | |
| T7\_MP8 (AT1G09970)-F | TAATACGACTCACTATAGGGCTACACTACATGGTAGGCTT | | |
| T7\_MP14 (AT3G17320)-F | TAATACGACTCACTATAGGGTTAAAGGTGGTACCAGCAGT | | |
| T7\_MP18 (AT5G28770)-F | TAATACGACTCACTATAGGGCCCTTTATGGTAGAGGACGT | | |
| **Primers to amplify the fragment flanking potential off-target sites for AT1G72350** | | | | |
| MP5-off-F1 | AGCTGACCACTGCAACACAT | | |
| MP5-off-R1 | ATGGCGTTTGGTCTCATTTC | | |
| MP5-off-F2 | CCACTTTGGATTCCTTTTGC | | |
| MP5-off-R2 | ACCGCCTTAAAATTGAATCGAA | | |
| MP5-off-F3 | AGGAAGGCAGGTTGAAATCC | | |
| MP5-off-R3 | GGAACCACGTACCTCAGCAT | | |
| MP5-off-F4 | TCGTTTCTGTTCCGGTTTTC | | |
| MP5-off-R4 | CGCCTTAAAATTGAATCGAA | | |
| MP5-off-F5 | TGATTGAGGCCAATGTTTTG | | |
| MP5-off-R5&6 | CGGGTGTGGGTTTTAAAAAGT | | |
| MP5-off-F6 | TTGAGGGTTTTCACCAATCC | | |
| MP5-off-F7 | CAGTGTTAGACATTTAGTTGGATTTCA | | |
| MP5-off-R7 | GCCCATCAAAATTTGCCATA | | |
| MP5-off-F8 | GGTGTTAGACATTTAGTTGGATTTCA | | |
| MP5-off-R8 | TCCTCCCACCAACAACATTC | | |
| **Primers to amplify the fragment flanking potential off-target sites for AT1G09970** | | | | |
| MP8-off-F1 | CCAAGGAGATGACCCAGCTA | | |
| MP8-off-R1 | GGTCTCACAAGATGGAACTGG | | |
| MP8-off-F2 | ACTTGCTTTTCAGCCAAGGA | | |
| MP8-off-R2 | CCACGCCTGTACAAGAACAA | | |
| **Primers to amplify the fragment flanking potential off-target sites for AT3G17320** | | | | |
| MP14-off-F1 | ATCAATCGGGATTGGATCTG | | |
| MP14-off-R1 | AGTGCCATTGCCTTTGAAAC | | |
| MP14-off-F2 | GACGAACTTTGAGCCTCTGG | | |
| MP14-off-R2 | CATCCTCTTCCCAAGATCCA | | |
| MP14-off-F3 | AACGCTTTACGAAATTCCAG | | |
| MP14-off-R3 | AACCAAAACCAGCCAATATGA | | |
| MP14-off-F4 | ACCAGTTTTGGTCCAGGAAA | | |
| MP14-off-R4 | TGAGAACACCATCAGGAGCA | | |
| MP14-off-F5 | AGACACTGCTTTCACCACACA | | |
| MP14-off-R5 | GCCAAACATAGCTGTGATGC | | |
| MP14-off-F6 | ACCAAGATTGGCGAGAGATG | | |
| MP14-off-R6 | CTGAGAGTTCGCTGTTGTCG | | |
| **Primers for bisulfite genomic sequencing AT1G72350** | | | | |
| MP5\_BS-F1 | AGATTTTGGGAAGATTGTTAAATGT | | |
| MP5\_BS-R | TCTTTAATTAATTAACCACTCCACAAA | | |
| **Primers for bisulfite genomic sequencing AT1G09970** | | | | |
| MP8\_BS-F | TATAAAATAAAAATATTTTATAGATAGATATAATATATAAATTTTTATATATATTAA | | |
| MP8\_BS-R2 | CTTAAACAAAACTTAACTAAAATCATTATACCCCCAA | | |
| **Primers for bisulfite genomic sequencing AT3G17320** | | | | |
| MP14\_BS-F | AAGGATGTYGTTAAGAAG | | |
| MP14\_BS-R1 | AATTCTTTARATCCCTTTACTTTTAT | | |
| **Primers for bisulfite genomic sequencing AT5G28770** | | | | |
| MP18\_BS-F | TTAATAATTTTTGTTTTYTTAATAATATTATTTATTTTTTTTATAAAATGGT | | |
| MP18\_BS-R | CTTAACCCACCTTATCTCAATATCTTTCTTC | | |
| **Primers for bisulfite genomic sequencing lambda phage DNA** | | | | |
| Lambda\_BS-F2 | TACAGAAAGACGGACGAAGG | | |
| Lambda\_BS-R2 | TGGTGGGCGTTTTCATACAT | | |



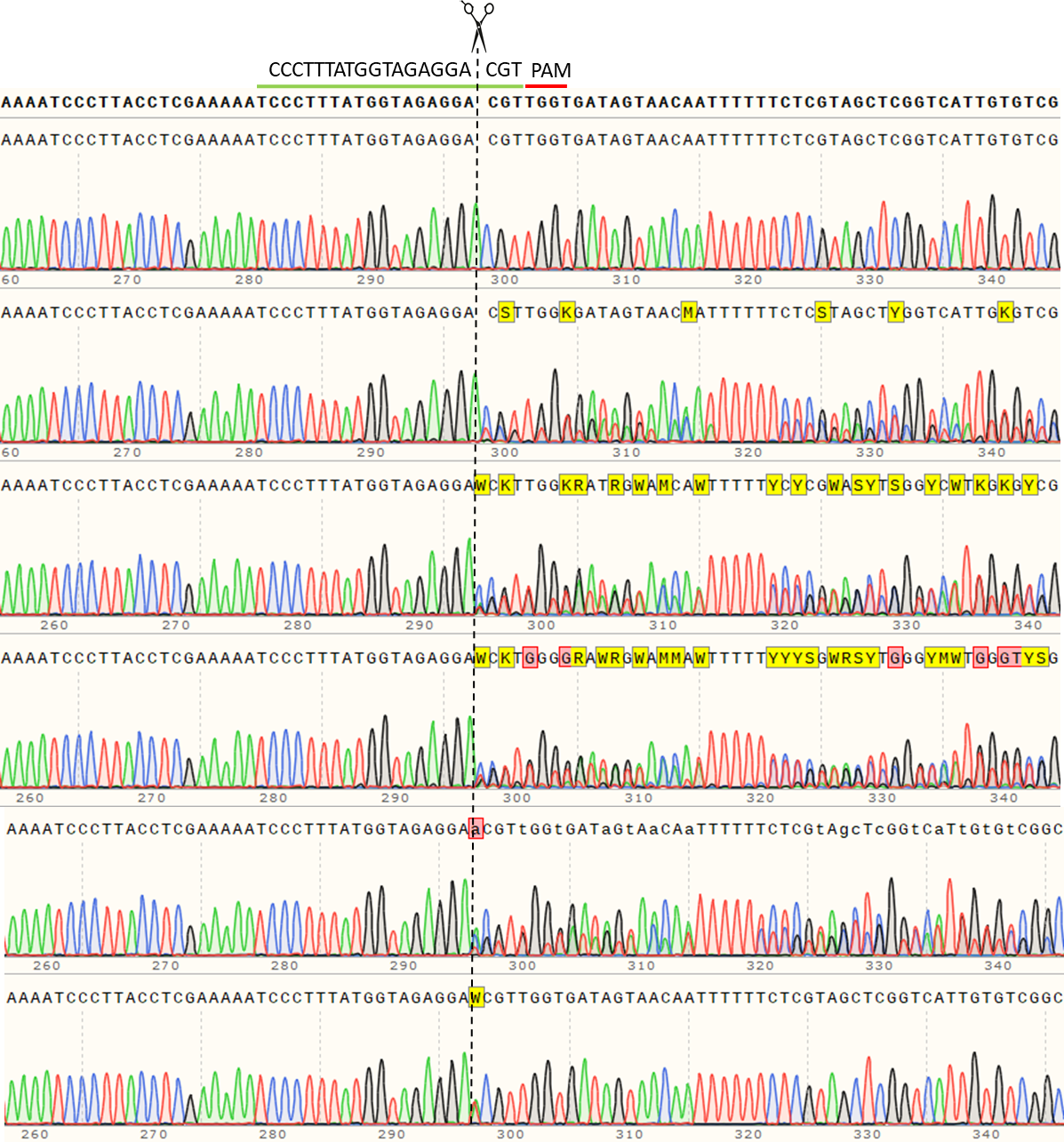
**Figure S1.** Genotyping of T1 plants targeting AT1G72350 gene. PCR amplicons flanking the target site were directly sequenced and then aligned. Top panel showed the sequencing chromatogram of wild-type and the below showed those of independent edited T1 plants. Multiple peaks of sequencing chromatogram in downstream from expected Cas9 cleavage site (3-nt upstream from PAM, marked with scissors and dot line) indicated InDel mutation was created.



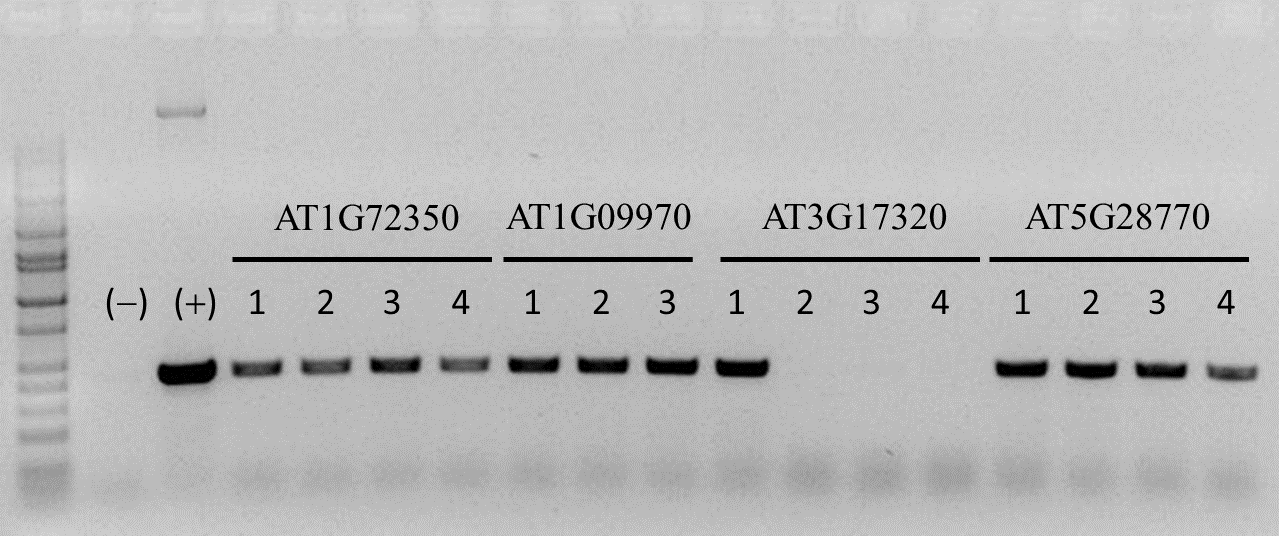
**Figure S2.** Genotyping of T1 plants targeting AT1G09970 gene. PCR amplicons flanking the target site were directly sequenced and then aligned. Top panel showed the sequencing chromatogram of wild-type and the below showed those of independent edited T1 plants. Multiple peaks of sequencing chromatogram in downstream from expected Cas9 cleavage site (3-nt upstream from PAM, marked with scissors and dot line) indicated InDel mutation was created.



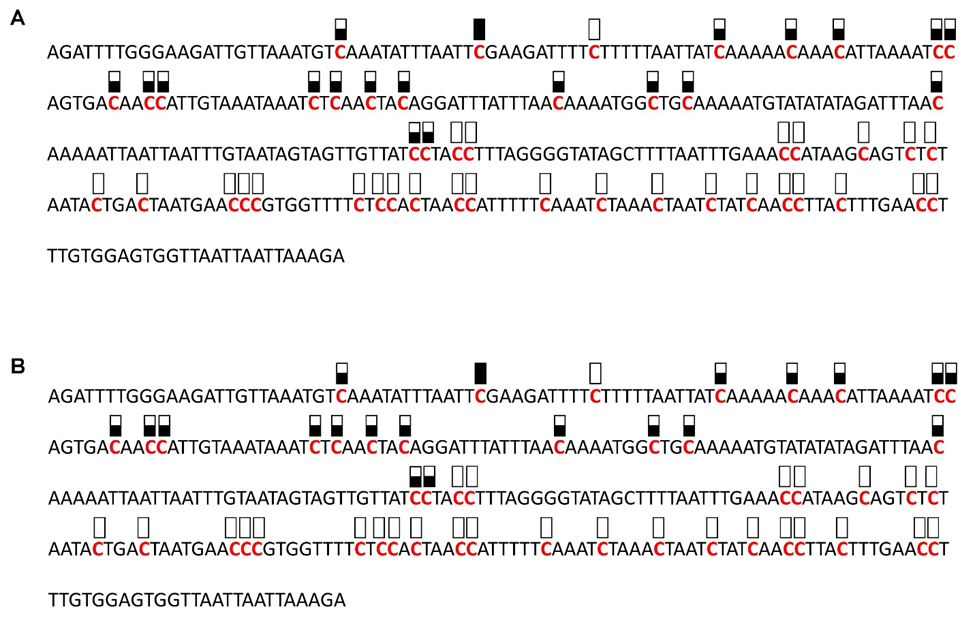
**Figure S3.** Genotyping of T1 plants targeting AT3G17320 gene. PCR amplicons flanking the target site were directly sequenced and then aligned. Top panel showed the sequencing chromatogram of wild-type and the below showed those of independent edited T1 plants. Multiple peaks of sequencing chromatogram in downstream from expected Cas9 cleavage site (3-nt upstream from PAM, marked with scissors and dot line) indicated InDel mutation was created.

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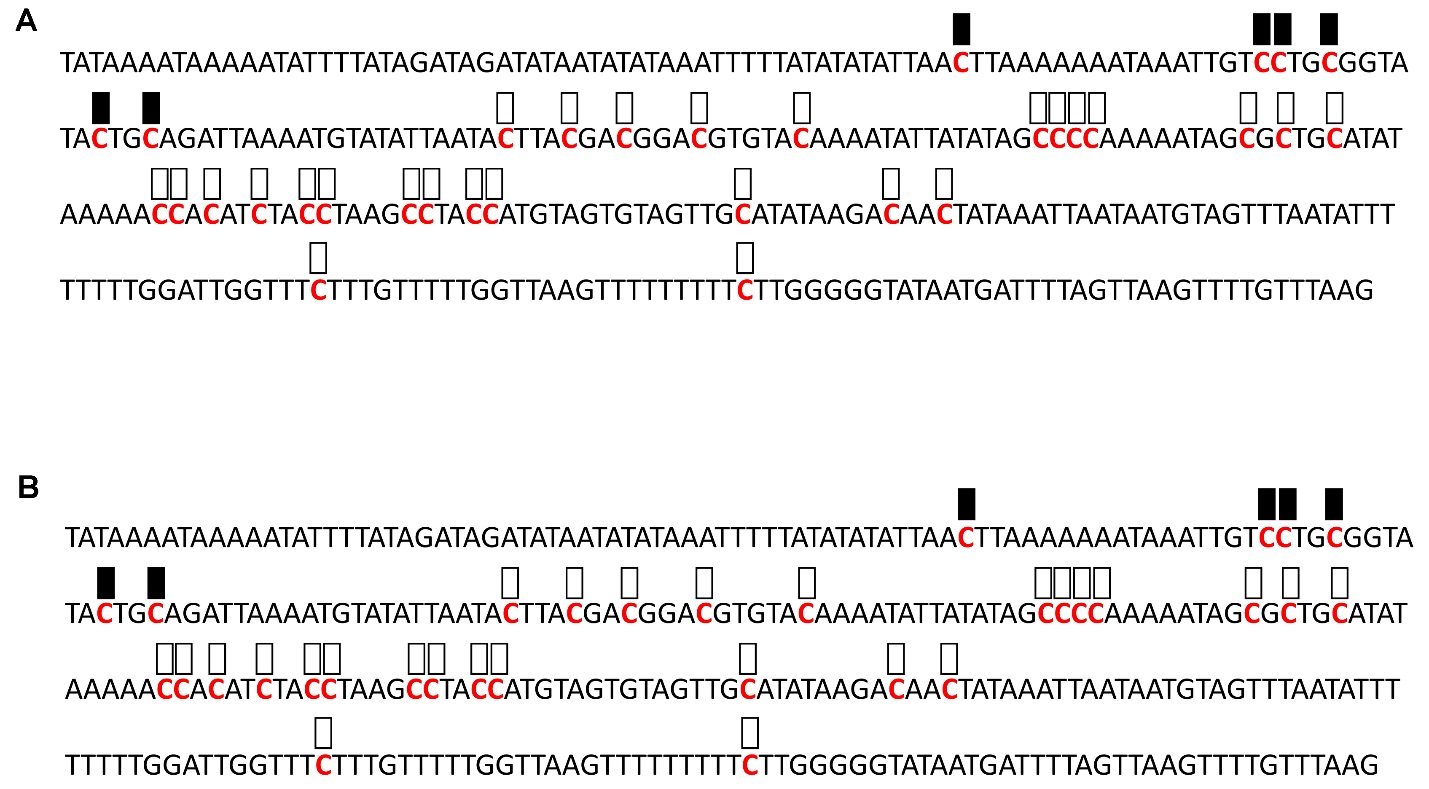
**Figure S4.** Genotyping of T1 plants targeting AT5G28770 gene. PCR amplicons flanking the target site were directly sequenced and then aligned. Top panel showed the sequencing chromatogram of wild-type and the below showed those of independent edited T1 plants. Multiple peaks of sequencing chromatogram in downstream from expected Cas9 cleavage site (3-nt upstream from PAM, marked with scissors and dot line) indicated InDel mutation was created.



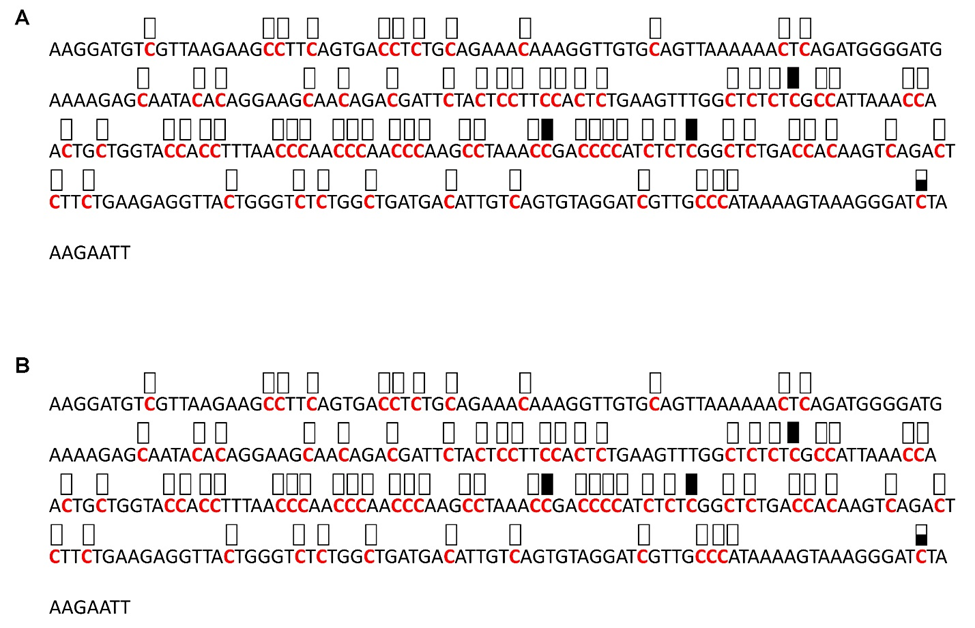
**Figure S5.** PCR confirmation of the presence of *Cas9* gene in independent edited T2 plants targeting AT1G72350, AT1G09970, AT3G17320, and AT5G28770. Most of the tested plants still contained *Cas9* gene, although escape of the gene was detected from three independent plants targeting AT3G17320 gene. (), wild-type negative control; (+), pKSE401 plasmid positive control; 3-4 independent edited T2 lines of each target gene.

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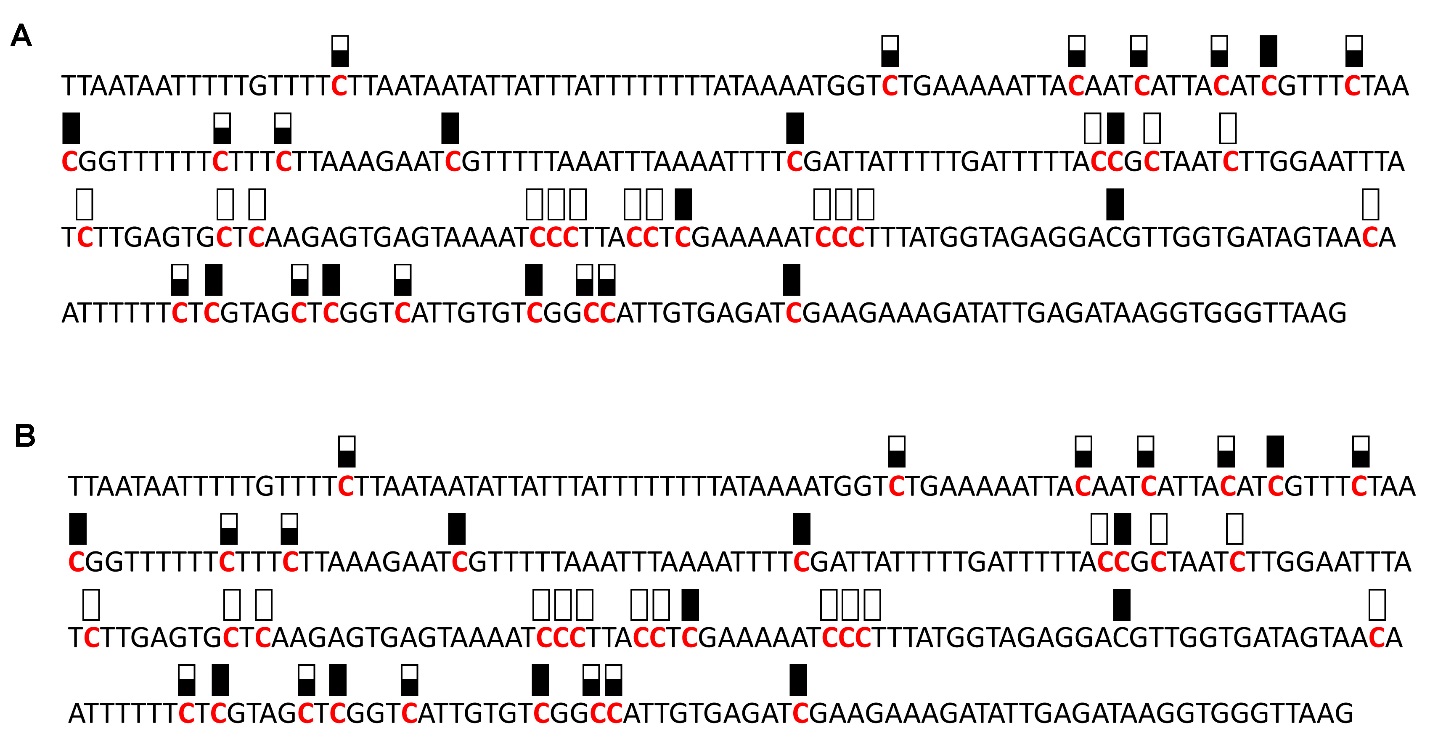
**Figure S6.** Pattern of methylation of the (AT1G72350) region in wild-type (A) and in the transgenic AT1G09970-edited (B) plants. Because the data were obtained by sequencing of independent pGEM-T colonies, an average level of methylation was determined for each cytosine. Solid boxes indicate that the cytosine at this position were methylated (>90 %), open boxes indicate that cytosine methylation was not detected (<10 %), and half-shaded boxes indicate that the cytosine was methylated at a range of 40-60 %. This figure presents results of 4 independent biological replicates.



**Figure S7.** Pattern of methylation of the (AT1G09970) region in wild-type (A) and in the transgenic AT1G72350-edited (B) plants. Because the data were obtained by sequencing of independent pGEM-T colonies, an average level of methylation was determined for each cytosine. Solid boxes indicate that the cytosine at this position were methylated (>90 %), open boxes indicate that cytosine methylation was not detected (<10 %), and half-shaded boxes indicate that the cytosine was methylated at a range of 40-60 %. This figure presents results of 4 independent biological replicates.



**Figure S8.** Pattern of methylation of the (AT3G17320) region in wild-type (A) and in the transgenic AT5G28770-edited (B) plants. Because the data were obtained by sequencing of independent pGEM-T colonies, an average level of methylation was determined for each cytosine. Solid boxes indicate that the cytosine at this position were methylated (>90 %), open boxes indicate that cytosine methylation was not detected (<10 %), and half-shaded boxes indicate that the cytosine was methylated at a range of 40-60 %. This figure presents results of 4 independent biological replicates.



**Figure S9.** Pattern of methylation of the (AT5G28770) region in wild-type (A) and in the transgenic AT3G17320-edited (B) plants. Because the data were obtained by sequencing of independent pGEM-T colonies, an average level of methylation was determined for each cytosine. Solid boxes indicate that the cytosine at this position were methylated (>90 %), open boxes indicate that cytosine methylation was not detected (<10 %), and half-shaded boxes indicate that the cytosine was methylated at a range of 40-60 %. This figure presents results of 4 independent biological replicates.