

Supplementary Information

Ongoing gene transfer from chloroplasts to mitochondria in tobacco

Masaki Odahara, Maai Mori and Keiji Numata

Supplementary Table 1

Supplementary Figure 1-9

Supplementary Table 1. Deep sequencing analysis of *sul* integration efficiency.

	CP-AS S#49			CP-AS SR#51			CP-AS SR#65			CP-AS control		
	Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3
Total mapped reads	864,800	524,420	549,223	647,091	595,573	427,601	488,454	571,020	480,581	1,001,636	237,168	577,102
Number of reads mapped to <i>sul</i>	2	6	2	8	1	2	6	3	2	0	1	0
<i>sul</i> /total mtDNA (%)	0.00067	0.0033	0.0011	0.0036	0.00049	0.0014	0.0036	0.0015	0.0012	0	0.0012	0

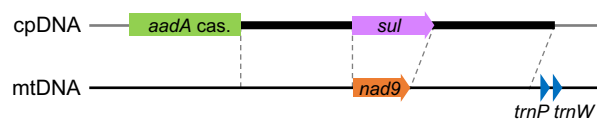


Figure S1. Targeting of the mitochondrial *nad9* locus via homologous recombination. A DNA segment harboring *sul* gene is transferred from chloroplast DNA and then integrated into the *nad9* via homologous recombination.

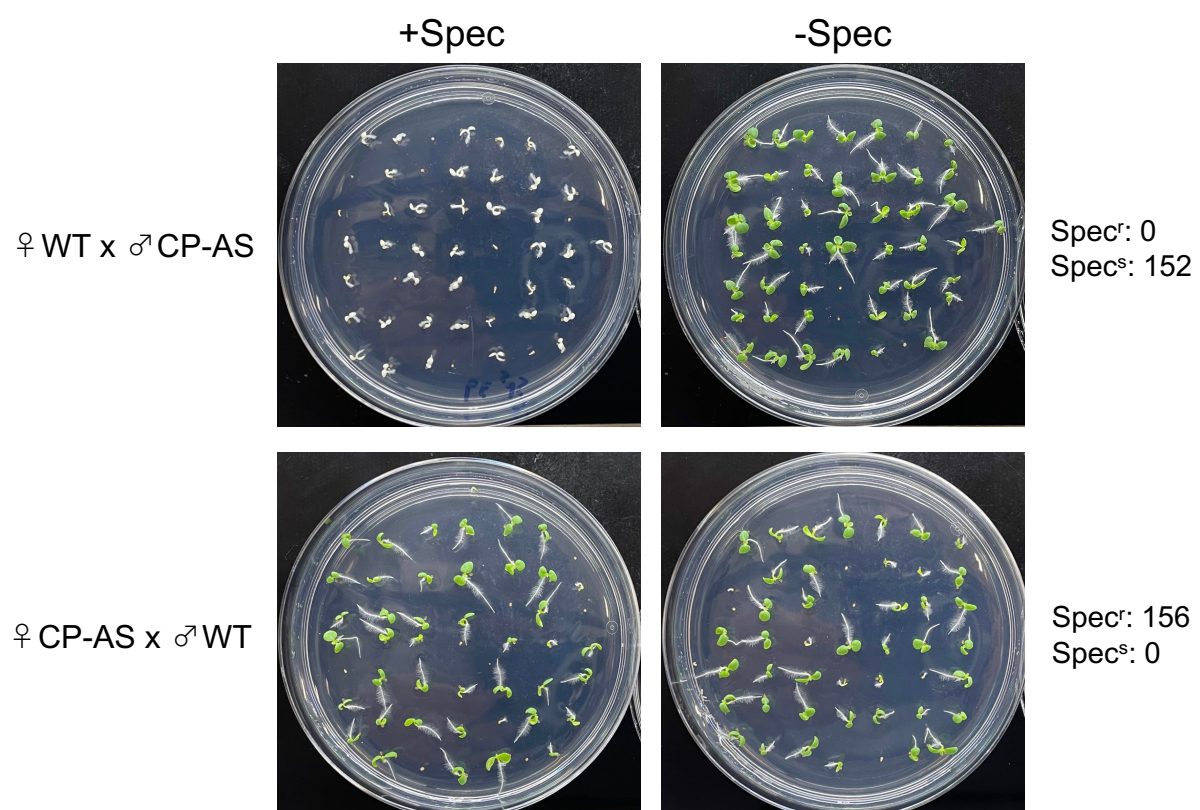


Figure S2. Confirmation of marker gene integration into CP-AS chloroplast DNA.
Inheritance of chloroplast marker gene of CP-AS plants was analyzed by crossing with WT to test nuclear insertion of the marker gene. Number of spectinomycin resistant or sensitive-seedlings are shown in the right of the figure.

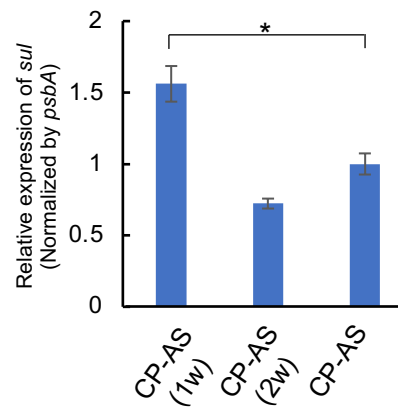


Figure S3. Expression of *sul* after heat-shock treatment.

Relative transcript levels of *sul* in CP-AS plants with or without heat-shock treatment were analyzed by quantitative RT-PCR. Relative amount of *sul* transcript was normalized by that of chloroplast gene *psbA*. * $p < 0.05$ (one-way ANOVA), $n=3$

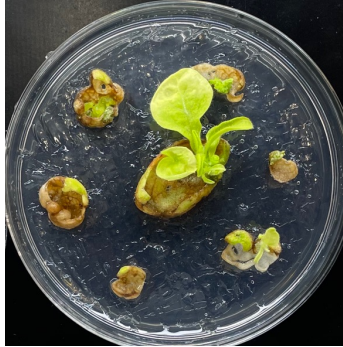


Figure S4. Generation of sulfadiazine-resistant shoots using heat-shocked-CP-AS leaves.

Shoots were regenerated from heat-shocked CP-AS leaf explants on a shoot regeneration medium containing 15 mg/L sulfadiazine.

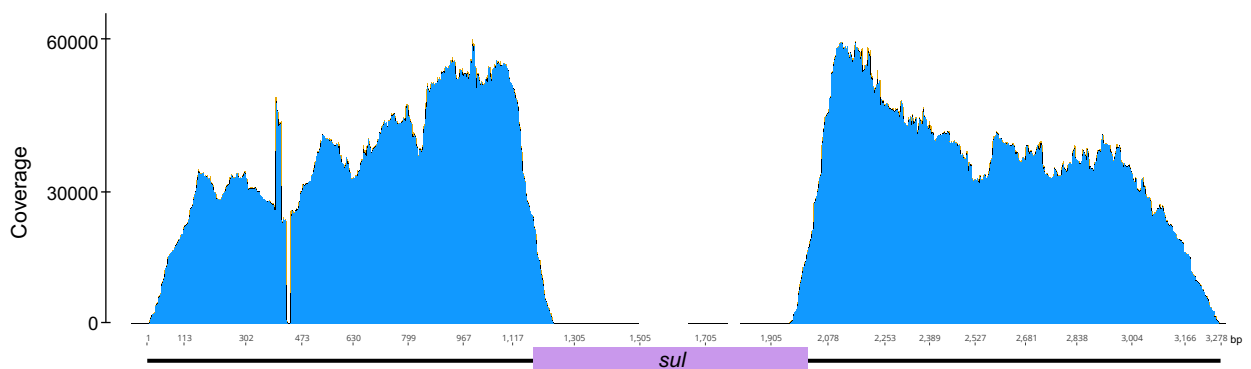


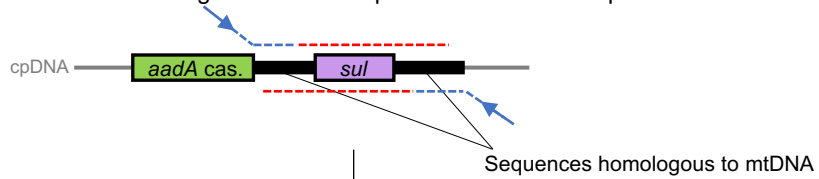
Figure S5. Mapping of reads to *sul* and surrounding sequences.

An example of mapping of reads from SR-51 to the *sul* and surrounding mitochondrial DNA sequences. Reads were mapped to the mtDNA target locus, in which *nad9* is replaced with *sul*.

1. Incomplete synthesis of the mitochondrial DNA segments from the primers.



2. Hybridization of the mitochondrial DNA segments to chloroplast DNA and subsequent extension from the segments.



Mitochondrial DNA segments and chloroplast *sul* gene are fused.

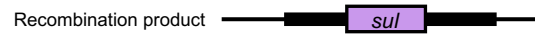


Figure S6. Diagram of PCR jumping to a create recombination product.

PCR jumping can occur between mitochondrial *nad9* locus and chloroplast exogenous marker genes via sequences homologous to mitochondrial DNA.

SR49-1 Read-2 AGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCAAGCAATGCCAAAAGTCCCATGCCTTTCTTGGTCGGACCAACCCAACCGGCGATTTCCGACA.
 mtDNA MH2 AGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCAAGCAATGCCAAAAGTCCCATGCCTTTCTTGGTCGGACCAACCCAACCGGCGATTTCCGACA.
 NuMT MH2 like-1 AGGGTGCTTTGTAATCACTAGGTAGCCATAAGCAACTCGGCCAAGCAATGCTAAAAAGTCCCATGCCTTTCTTGGTCGGACCAACCCAACCGGCGATTTCCGCA.
 NuMT MH2 like-2 AGGGTACTTTGTAATCACTAGGTAGCCATACAACACTAGCCCAAGCAATGCCAAAAGTCCCATGCCTTTCTTAGTGGACCAACCAACCGGCGATTTCCGACA.
 NuMT MH2 like-3 AGGGTGTTTGTAATCACTAGGTAGACATACAACACTATCCCAAGAAATACCCAAAAGTCCCATGCCTTTCTTGGTCGGACCAACCCAATGCGATTTTGACACA.

SR49-2 Read-2 CCCTAGTCTCGTTTTGCCGAGCCTGATTCTCTATGGCATGGACGTCTTTGATTGGATGGAGACAGATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCA
 mtDNA MH2 CCCTAGTCTCGTTTTGCCGAGCCTGATTCTCTATGGCATGGACGTCTTTGATTGGATGGAGACAGATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCA
 NuMT MH2 like-1 CCCTAGTCTTAGTTTTGCCGAGCCTGATTCTCTATGGCATGGACGTCTTTGATTCTATGGAGACAGATATAGAAAATGTGCGGTGAGGGTGCTTTGTAATCA
 NuMT MH2 like-2 CCCTAGTCTCGTTTTACCGAACCAGATTCTCTATGGCATGGAGTGTCTTTGATTGGATGGAGACAGATAATAGAAAAGTGTGCAGTGAGGGTACTTTGTAATCA
 NuMT MH2 like-3 CCCTAGTCTCGTTTTACCGAGACTGATTCTCTATGGCATGGACGTCTTTGATTGGATGGAGAAGGTATATAGAAAAGTGTAGTGAGGGTGTTTGTAATCA

SR51-1 Read-2 ACAGGAAGAAAGCGGGGAGCATGGTCCCTAGTCTCGTTTTGCCGAGCCTGATTCTCTATGGCATGGACGTCTTTGATTGGATGGAGACAGATATAGAAAAGTG
 mtDNA MH2 ACAGGAAGAAAGCGGGGAGCATGGTCCCTAGTCTCGTTTTGCCGAGCCTGATTCTCTATGGCATGGACGTCTTTGATTGGATGGAGACAGATATAGAAAAGTG
 NuMT MH2 like-1 ATAGGAAGAAAGCGGGGAGCATGGTCCCTAGTCTCGTTTTGCCGAGCCTGATTCTCTATGGCATGGACGTCTTTGATTGGATGGAGACAGATATAGAAAATG
 NuMT MH2 like-2 ACCGGAAGAAAGCGGGGAGCATGGTCCCTAGTCTCGTTTTACCGAACCAGATTCTCTATGGCATGGAGTGTCTTTGATTGGATGGAGACAGATAATAGAAAAGTG
 NuMT MH2 like-3 ATAGGAAGAAAGCGGGGAGATGGTCCCTAGTCTCGTTTTACCGAGACTGATTCTCTATGGCATGGACGTCTTTGATTGGATGGAGAAGGTATATAGAAAAGTG

SR51-2 Read-2 TCTATGGCATGGACGTCTTTGATTGGATGGAGACAGATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCCAAGCA
 mtDNA MH2 TCTATGGCATGGACGTCTTTGATTGGATGGAGACAGATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCCAAGCA
 NuMT MH2 like-1 TCTATGGCATGGACGTCTTTGATTCTATGGAGACAGATATAGAAAATGTGCGGTGAGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCCAAGCA
 NuMT MH2 like-2 TCTATGGCATGGAGTCTTTGATTGGATGGAGACAGATAATAGAAAAGTGTGCAGTGAGGGTACTTTGTAATCACTAGGTAGCCATACAACACTAGCCCAAGCA
 NuMT MH2 like-3 TCTATGGCATGGACGTCTTTGATTGGATGGAGAAGGTATATAGAAAAGTGTAGTGAGGGTGTTGTAATCACTAGGTAGACATACAACACTACCCCAAGCA

SR51-3 Read-2 GACGTCTTTGATTGGATGGAGACAGATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCAAGCAATGCCAAAAG
 mtDNA MH2 GACGTCTTTGATTGGATGGAGACAGATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCAAGCAATGCCAAAAG
 NuMT MH2 like-1 GACGTCTTTGATTCTATGGAGACAGATATAGAAAATGTGCGGTGAGGGTGCTTTGTAATCACTAGGTAGCCATAGAACACTCGGCCCAAGCAATGCCAAAAG
 NuMT MH2 like-2 GAGTCTTTGATTGGATGGAGACAGATAATAGAAAAGTGTGCAGTGAGGGTACTTTGTAATCACTAGGTAGCCATACAACACTAGCCCAAGCAATGCCAAAAG
 NuMT MH2 like-3 GACGTCTTTGATTGGATGGAGAAGGTATATAGAAAAGTGTAGTGAGGGTGTTGTAATCACTAGGTAGACATACAACACTATCCCAAGAAATACCCAAAAG

SR51-4 Read-2 AACGTAATAGGCTGCTACTCTAGGCTCGCTTCGCTTCTTCAAGCTCGCCCTCTTTTCAAAAATCAAAAAACAGCGCTAACGAGCAAGAAAACGGATGCGCTAA
 mtDNA MH2 AACGTAATAGGCTGCTACTCTAGGCTCGCTTCGCTTCTTCAAGCTCGCCCTCTTTTCAAAAATCAAAAAACAGCGCTAACGAGCAAGAAAACGGATGCGCTAA
 NuMT MH2 like-1 AACGTAATGG-----CTCGCTTCTTCTTCAAGCTAACACTTATTTCAAAATCAAAAAACAGCTAACGAGCAAGAAAACGGATGCGCTAA
 NuMT MH2 like-2 AAGTAAGAGGTTGCTACTCTAGGCTCGCTTCGCTTCTTCAAGCTCGCCCTCTTTTCAAA--AATCAAAAAAAGCACTAACGCAAGAAAACGGATGCGCTAA
 NuMT MH2 like-3 AACGTAAGACTGCTACTACTAGGCAAGTCTCGCTTCTTCAAGCTCGCCACTTTTCAAAAATCAAAAAAAGCGCTAACGAGCAAGAAAACGGATGCGCTAA

SR51-5 Read-2 TACGAAACGACTGGTTTTTTTTCTTTCTTTCGGTTTCATTGATAACAGAAAAGACTTACTCTAAAGGGCGCGTAGCGCTTGACTAATAACATATCAAAGGGGCC
 mtDNA MH2 TACGAAACGACTGGTTTTTTTTCTTTCTTTCGGTTTCATTGATAACAGAAAAGACTTACTCTAAAGGGGGCGTAGCGCTTGACTAATAACATATCAAAGGGGCC
 NuMT MH2 like-1 TACGAACGACTGGTTTT-TTCTTTCTTTCGGTTTCATTGATAACAAAAAAGCTTACTCTAAAGGGCGTAGCGCTTGACTAATAACATATCAAAGGGGCC
 NuMT MH2 like-2 TACGAAACGACTAGTTTTTTTCT---ATTCGGTTTCATTGATAAGAAAAGACTTACTCTAA--AGGCGTAGCGCTTGACTAATAACATATCAAAGGGGCC
 NuMT MH2 like-3 TACGAAAAGACTATTT-----TTTTCGGTTTCATTGATAAGAAAAGACTTACTCTAAAGGGGGCGTAGCGCTTGACTAATAACATATCAAAGGGGCC

SR51-6 Read-2 ATTGGATGGAGACAGATATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCAAGCAATGCCAAAAGTCCCATGCCT
 mtDNA MH2 ATTGGATGGAGACAGATATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCAAGCAATGCCAAAAGTCCCATGCCT
 NuMT MH2 like-1 ATTCTATGGAGACAGATATATAGAAAATGTGCGGTGAGGGTGCTTTGTAATCACTAGGTAGCCATAGAACACTCGGCCAAGCAATGCCAAAAGTCCCATGCCT
 NuMT MH2 like-2 ATTGGATGGAGACAGATAATAGAAAAGTGTGCAGTGAGGGTACTTTGTAATCACTAGGTAGCCATACAACACTAGCCCAAGCAATGCCAAAAGTCCCATGCCT
 NuMT MH2 like-3 ATTGGATGGAGAAGGTATATAGAAAAGTGTAGTGAGGGTGTTGTAATCACTAGGTAGACATACAACACTATCCCAAGAAATACCCAAAAGTCCCATGCCT

SR51-7 Read-2 ACAGAAAAAGCACCAGCTGGGCTCTCTCTTTGTTTCGATGAAAGCCTCAACGCTCGCGTTCTACGGGGAACTTTTTGTACTATTTACCTTCATCAAAGCCGGA
 mtDNA MH2 ACAGAAAAAGCACCAGCTGGGCTCTCTCTTTGTTTCGATGAAAGCCTCAACGCTCGCGTTCTACGGGGAACTTTTTGTACTATTTACCTTCATCAAAGCCGGA
 NuMT MH2 like-1 ACAGAAAAAGCACCAGCTGGCTCTCTCTATTGTTTCGATGAAAGCCTCAACGCTCGCATTCACGGGGAACATTTTGTATTTACCTTCATCAAAGCCAGA
 NuMT MH2 like-2 ACAAAAAAGCACCAGCTGCACCTCTCTCTTTGTTTCGATGAAAGCCTCAACGCTCGGTCTACGGGAACAACTTTTTG-ACTATTTACCTTCATCAAACCAA
 NuMT MH2 like-3 ACAGAAAAAGCACCAGCTGGGCTTCTCTTTGTTTCGATGAAAGCCTCAACGCTCGCATTCACGGGAAGCGGTTTTG-ATATTTACCTTCATCAAACCGGA

SR65-1 Read-2 TAGGCTGCTACTCTAGGCTCGCTTCGCTTCTTCAAGCTCGCCCTCTTTTCAAAAATCAAAAAACAGCGCTAACAGCAAGAAAACGGATGCGCTAAGGCGCAA
 mtDNA MH2 TAGGCTGCTACTCTAGGCTCGCTTCGCTTCTTCAAGCTCGCCCTCTTTTCAAAAATCAAAAAACAGCGCTAACAGCAAGAAAACGGATGCGCTAAGGCGCAA
 NuMT MH2 like-1 TTGG-----CTCGCTTCTTCTTCAAGCTAGCAACTTATTTCAAAATCAAAAAACAGCTAACAGCAAGAAAACGGATGCGCTAAGGCGCAA
 NuMT MH2 like-2 GAGCTGCTACTCTAGGCTCGCTTCGCTTCTTCAAGCTCGCCCTCTTTTCAAA--AATCAAAAAAAGCACTAACGCAAGAAAACGGATGCGCTAAGGCGCAA
 NuMT MH2 like-3 GAGCTGCTACTACTAGGCTCGCTTCTTCAAGCTCGCCACTTTTCAAAAATCAAAAAAAGCGCTAACAGCAAGAAAACGGATGCGCTAAGGCGCAA

SR65-2 Read-2 TAGTCTCGTTTTGCCGAGCCTGATTCTCTATGGCATGGACGTCTTTGATTGGATGGAGACAGATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCAC
 mtDNA MH2 TAGTCTCGTTTTGCCGAGCCTGATTCTCTATGGCATGGACGTCTTTGATTGGATGGAGACAGATATAGAAAAGTGTGCGGTGAGGGTGCTTTGTAATCAC
 NuMT MH2 like-1 TAGTCTAGTTTTGCCGAGCCTGATTCTCTATGGCATGGACGTCTTTGATTCTATGGAGACAGATATAGAAAATGTGCGGTGAGGGTGCTTTGTAATCAC
 NuMT MH2 like-2 TAGTCTCGTTTTACCGAACCAGATTCTCTATGGCATGGAGTGTCTTTGATTGGATGGAGACAGATAATAGAAAAGTGTGCAGTGAGGGTACTTTGTAATCAC
 NuMT MH2 like-3 TAGTCTCGTTTTACCGAGACTGATTCTCTATGGCATGGACGTCTTTGATTGGATGGAGAAGGTATATAGAAAAGTGTAGTGAGGGTGTTTGTAATCAC

SR65-3 Read-2 TTGGATGGAGACAGATATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCAAGCAATGCCAAAAGTCCCATGCCTT
 mtDNA MH2 TTGGATGGAGACAGATATATAGAAAAGTGTGCAGTGAGGGTGCTTTGTAATCACTAGGTAGCCATACAACACTCGGCCAAGCAATGCCAAAAGTCCCATGCCTT
 NuMT MH2 like-1 TTCTATGGAGACAGATATAGAAAATGTGCGGTGAGGGTGCTTTGTAATCACTAGGTAGCCATAGAACACTCGGCCAAGCAATGCCAAAAGTCCCATGCCTT
 NuMT MH2 like-2 TTGGATGGAGACAGATAATAGAAAAGTGTGCAGTGAGGGTACTTTGTAATCACTAGGTAGCCATACAACACTAGCCCAAGCAATGCCAAAAGTCCCATGCCTT
 NuMT MH2 like-3 TTGGATGGAGAAGGTATATAGAAAAGTGTAGTGAGGGTGTTGTAATCACTAGGTAGACATACAACACTATCCCAAGAAATACCCAAAAGTCCCATGCCTT

SR65-4 Read-2 TGCTTGTGTTGTGGTTTTGAGTTCCTTGGCTTGTGTTCTAATAGTAACCAAGGAAGAAAGCGGGGAGCATGGTCCCTAGTCTCGTTTTGCCGAGCCTGATTCT
 mtDNA MH2 TGCTTGTGTTGTGGTTTTGAGTTCCTTGGCTTGTGTTCTAATAGTAACCAAGGAAGAAAGCGGGGAGCATGGTCCCTAGTCTCGTTTTGCCGAGCCTGATTCT
 NuMT MH2 like-1 TGCTTGTGTTGTGGTTTTGAGTTCCTTGGCTTGTGTTCTAATAGTAACCAAGGAAGAAAGCGGGGAGCATGGTCCCTAGTCTTAGTTTTGCCGAGCCTGATTCT
 NuMT MH2 like-2 TGCT-----TGGATTGTGTTCTAAAGTAACCAAGGAAGAAAGCGGGGAGCATGGTCCCTAGTCTCGTTTTACCGAACCAGGATTCT
 NuMT MH2 like-3 TGCTTGTGTTGTGGTTTTGAGTTCCTTGGCTTAGTGTCTAATAGTAACCAAGGAAGAAAGCGGGGAGATGGTCCCTAGTCTCGTTTTACCGAGACTGATTCT

Figure S7. Multiple alignment of reads from deep sequencing and NuMT sequences.
 Nucleotide sequence alignment of the paired read (read-2), mitochondrial sequence (MH2), and NuMTs. Colored sequences show nonidentical sequences.

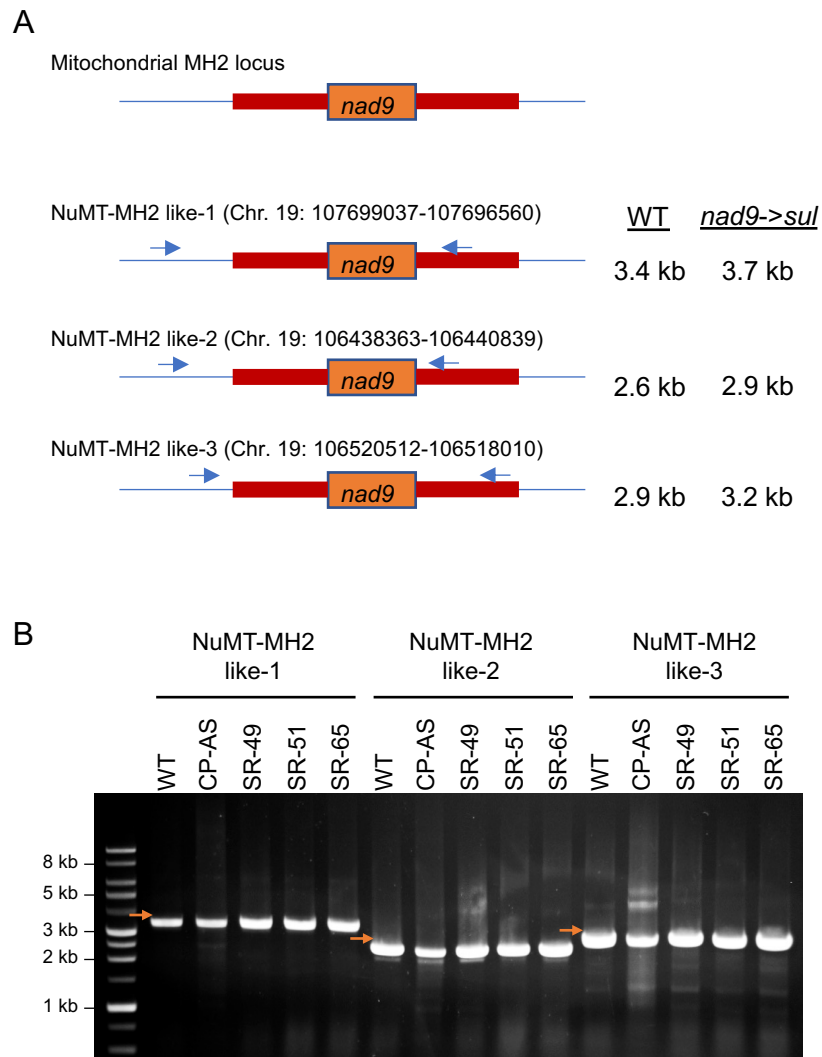


Figure S8. Analysis of NuMTs in SR plants.

A. Positions of primers for amplification of NuMT sequences are shown by arrows on the maps of the NuMTs. The expected sizes of the products from WT and *sul* integration at *nad9* locus are shown in the right.

B. Analysis of marker integration into NuMT sequences. Segments of three NuMT sequences homologous to the mitochondrial target region were amplified by PCR to test integration of the marker gene *sul*. Orange arrows denote positions of *sul* integration into the NuMT sequences.

Fig. 1B

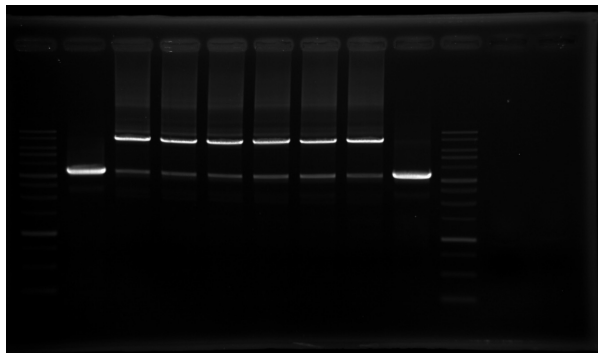


Fig. 3C

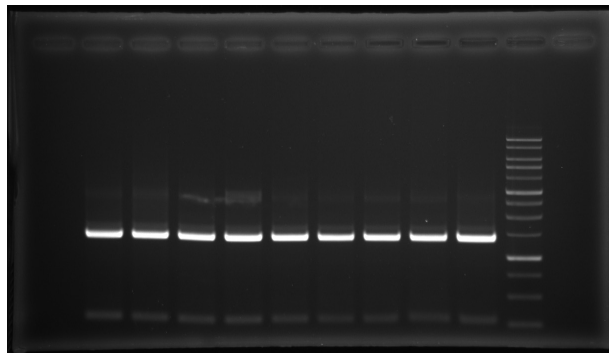


Fig. 4A

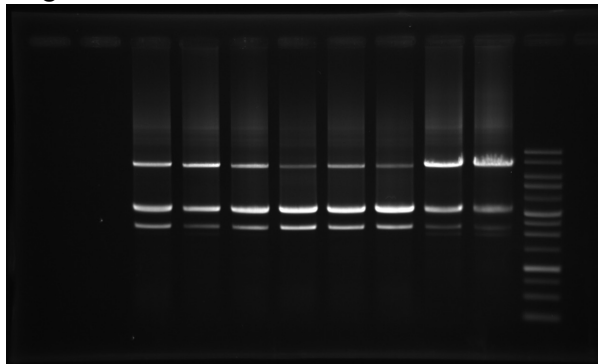


Fig. S8B

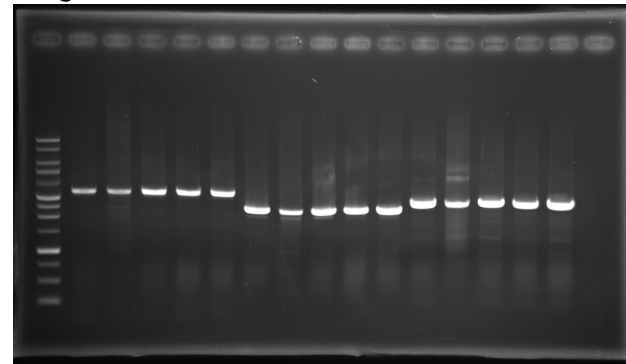


Figure S9. Original gel images used for the figures.