Supplementary Information for

Nuclear shuttling of donor DNA via sgRNA recognition sequences drives CRISPR/Cas9 gene targeting efficiency

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Supplementary Information

Supplementary Figure Legends

Supplementary Figure S1. Primer design for detecting GT events and details of the constructs.

A, Primer design for the detection of GT events. External and full-length primers are capable of amplifying both endogenous and knock-in GT alleles. External and full-length primers are capable of amplifying both endogenous and knock-in GT alleles. The primers are specific for GT alleles. **B, C,** Details of the *GFP*-KI constructs for *SOS1* (**B**) and *ROS1*(**C**). The diagrams present detailed information regarding the locations of the donor excision sites for the *GFP*-KI constructs. The lowercase red letters indicate the PAM sequence.

Supplementary Figure S2. Correlation analysis of GT events.

A correlation analysis of precise GT, 5' arm GT, and 3' arm GT ratio was performed for all *SOS1-GFP* KI constructs (Table 1). The analysis included the following comparisons: precise GT and 5' arm GT (**A**), precise GT and 3' arm GT (**B**), and 5' arm and 3' arm GT efficiency (**C**).

Supplementary Figure S3. Imprecise knock-in event in T1 generation.

A, PCR genotyping of precise and imprecise *SOS1-GFP* KI. The use of specific primer sets in genotyping PCR revealed the presence of an imprecise GT event at the 5' arm in an *SG-ex* T1 plant. **B,** Schematic diagram of 5' homology arm imprecise knock-in for *SOS1-GFP*. The 5' specific PCR product depicted in Supplementary Figure 3A was subjected to Sanger sequencing, which facilitated the identification of the precise details of the 5' arm imprecise GT event.

Supplementary Figure S4. Schematic diagram of target site recognition by Cas9.

A, Target site at dsDNA recognition by Cas9. **B,** Target site at the ssT-DNA recognition by Cas9. The protospacer sequence of Cas9 is indicated by "20 N", while the PAM sequence is shown in lowercase red letters.

Supplementary Figure S5. Nuclear T-DNA copy number in tobacco and Arabidopsis.

A, The copy number of *RbsL*. The copy number of the *RbsL* gene, which is encoded in the chloroplast genome, was examined in both the total and nuclear fractions of Agrobacterium-infiltrated tobacco leaves. **B,** The copy number of *GFP*. The copy number of *GFP*,

which represents the copy number of T-DNA, was examined in the enriched nuclear fractions of Agrobacterium-infected Arabidopsis flower tissue.

Supplementary Figure S6. Original electrophoresis gel pictures.

53 **Supplementary Table S1. Sequences of primers used in this study.**

Primer name and use	Primer sequence (5' – 3')
GT detection & genotyping	
SOS1-sgRNA	GTTAGAAGGTGATAATGCGG cgg
SOS1-GFP 5' specific	CAGGTGTGCAAGCAACTTAC
	GACACGCTGAACTTGTGGC
SOS1-GFP 3' specific	CCGGTTGGTATCAAAGAAGAGC
	CGAACCGTGGCAATATAAGCTTAG
SOS1-GFP 5' external	CCTCAGGTTGAGAGTCTTGTC
	GAATTATGGAAGCTTTGATTCCCG
SOS1-GFP 3' external	CCGGTTGGTATCAAAGAAGAGC
	AAGATAACAATTGATCGGTTGAGTG
SOS1-GFP Full-length	CCTCAGGTTGAGAGTCTTGTC
	AAGATAACAATTGATCGGTTGAGTG
SG-es-S/AS Full-length	CAGGTGTGCAAGCAACTTAC
	CGAACCGTGGCAATATAAGCTTAG
SG/SG-ex donor excision	GGCTAGTCCGTTATCAACTTGA
	AGCTCACTCATTAGGCACCC
SG-2sg/SG-ex-2sg donor excision	GTTAGAAGGTGATAATGCGG
	CACTACTTCGACTCTAGCTG
SG-es-S/AS donor excision	CTTCCGGCTCGTATGTTGTG
	CTCTTCGCTATTACGCCAGC
ROS1-GFP Full-length	TGACCAAGCATGAACTCGTAAG
	GAGTTGAAAGTGAGCTCAGCC
ROS1-GFP donor excision	CTTCCGGCTCGTATGTTGTG
	CTCTTCGCTATTACGCCAGC

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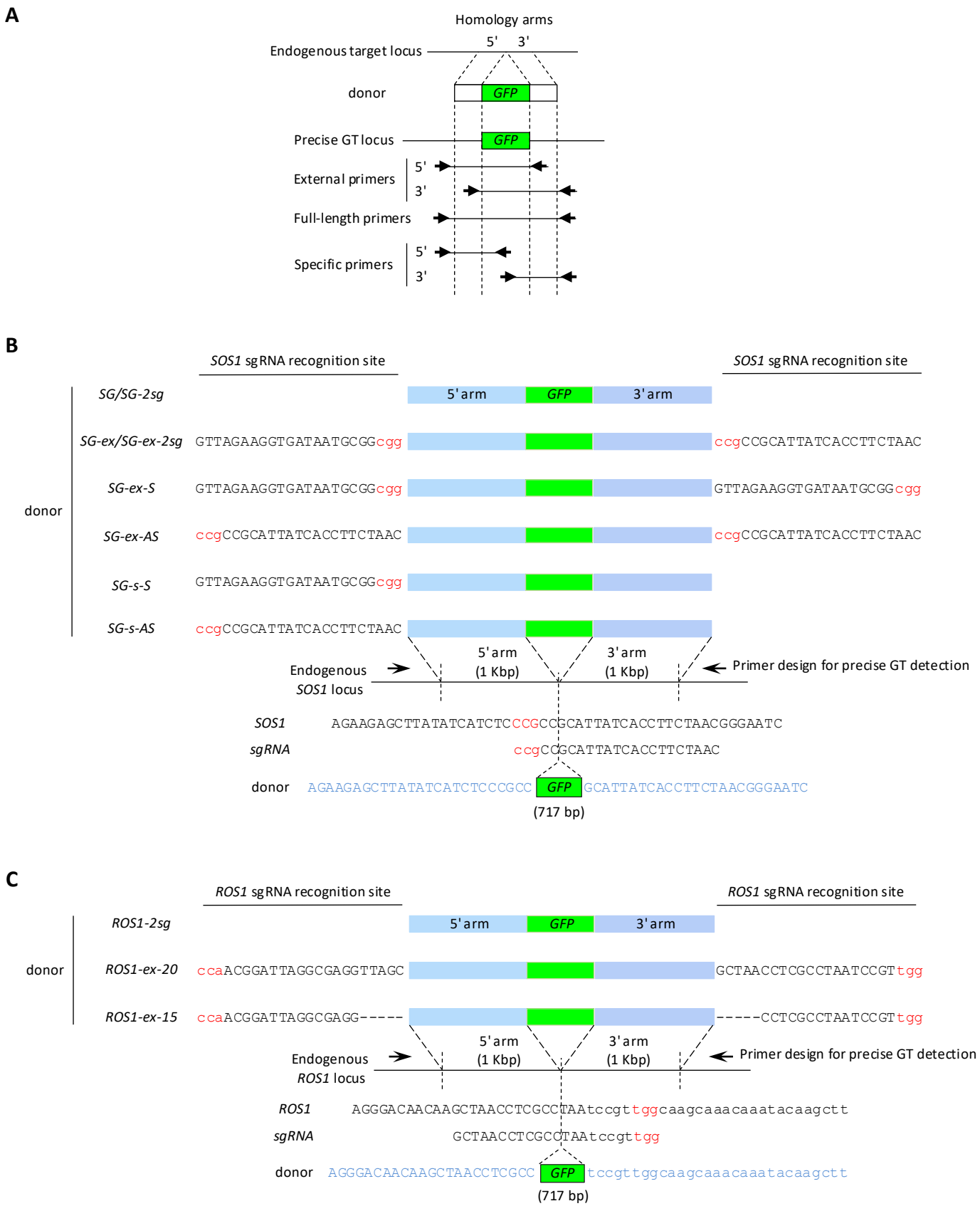
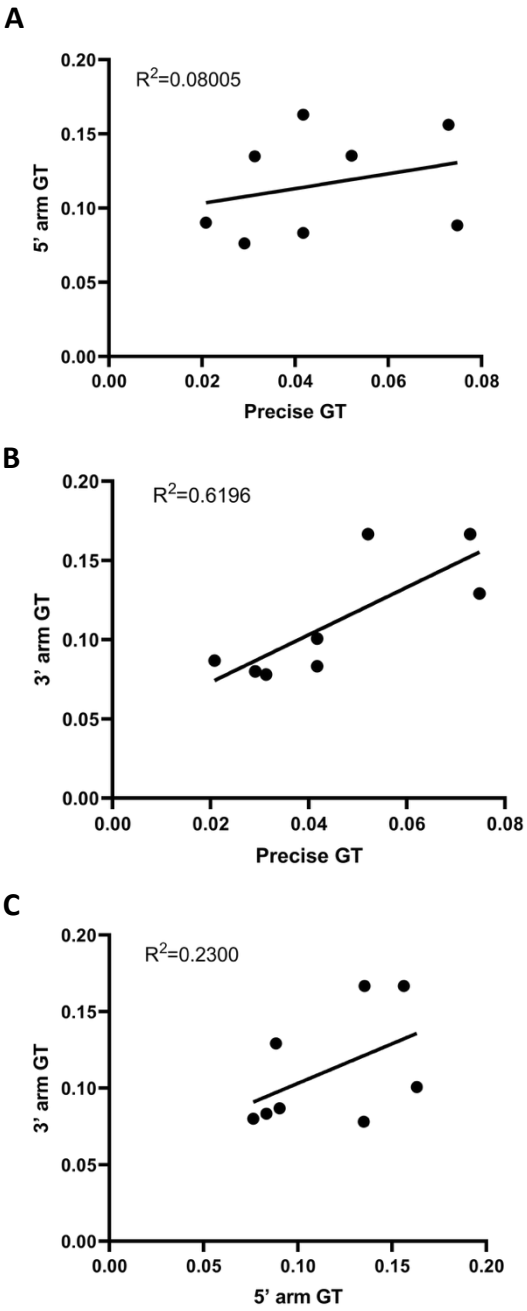
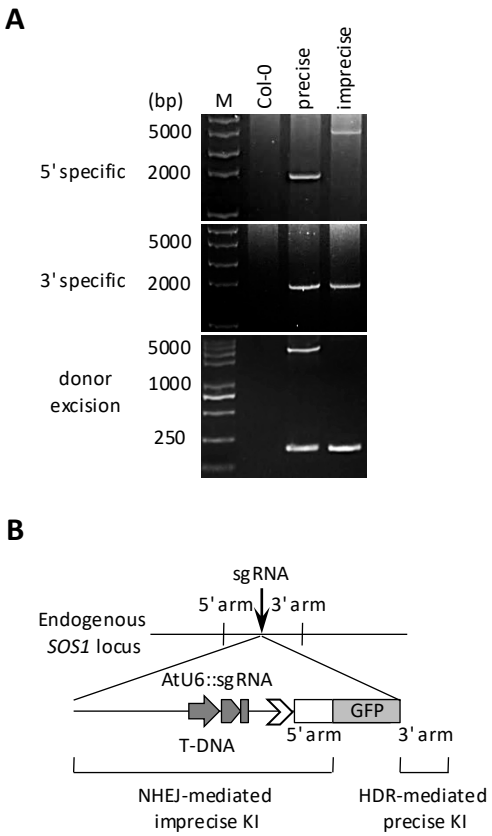


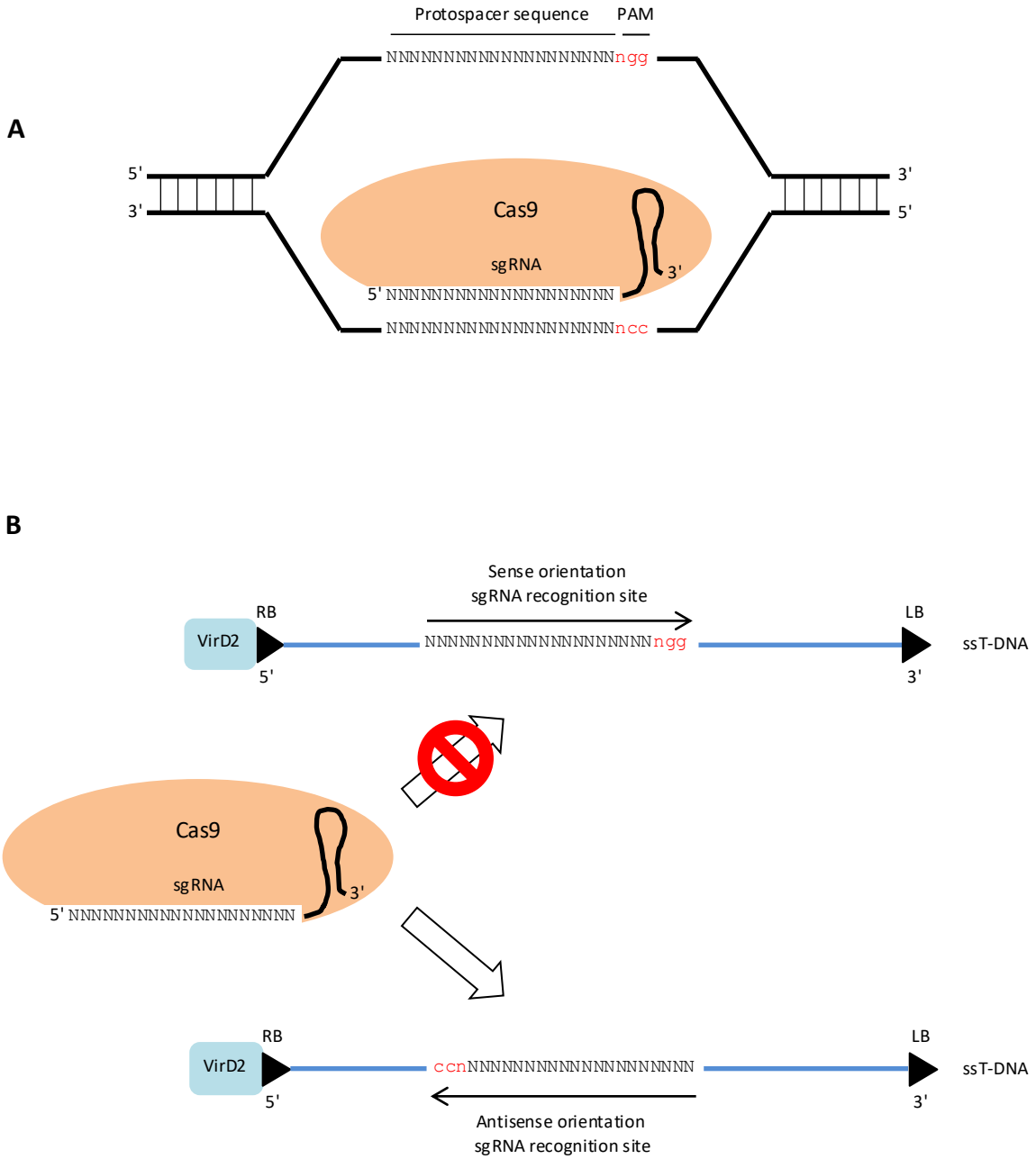
Supplementary Figure S1. Primer design for detecting GT events and details of the constructs.



Supplementary Figure S2. Correlation analysis of GT events.

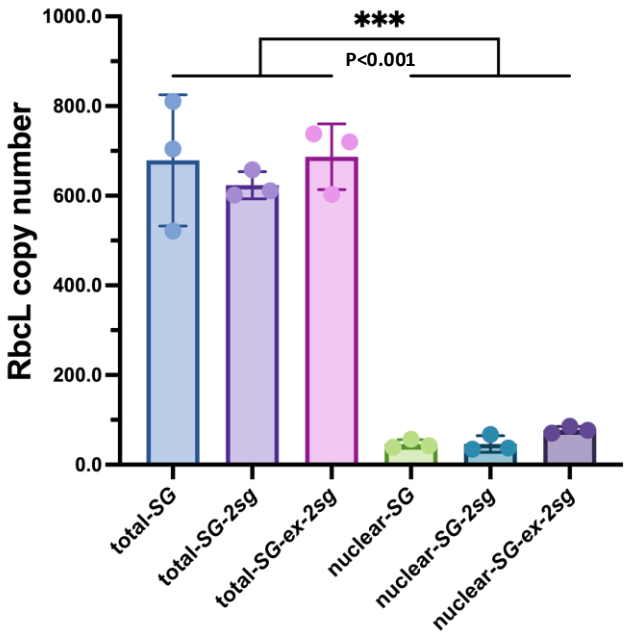


Supplementary Figure S3. Imprecise knock-in event in T1 generation.

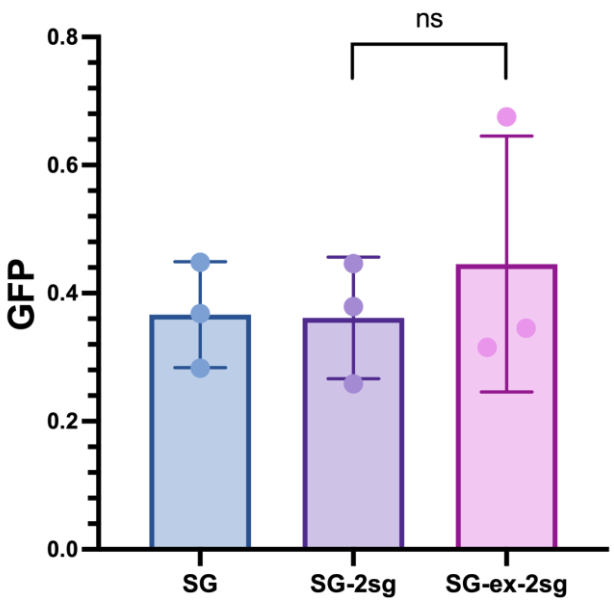


Supplementary Figure S4. Schematic diagram of target site recognition by Cas9.

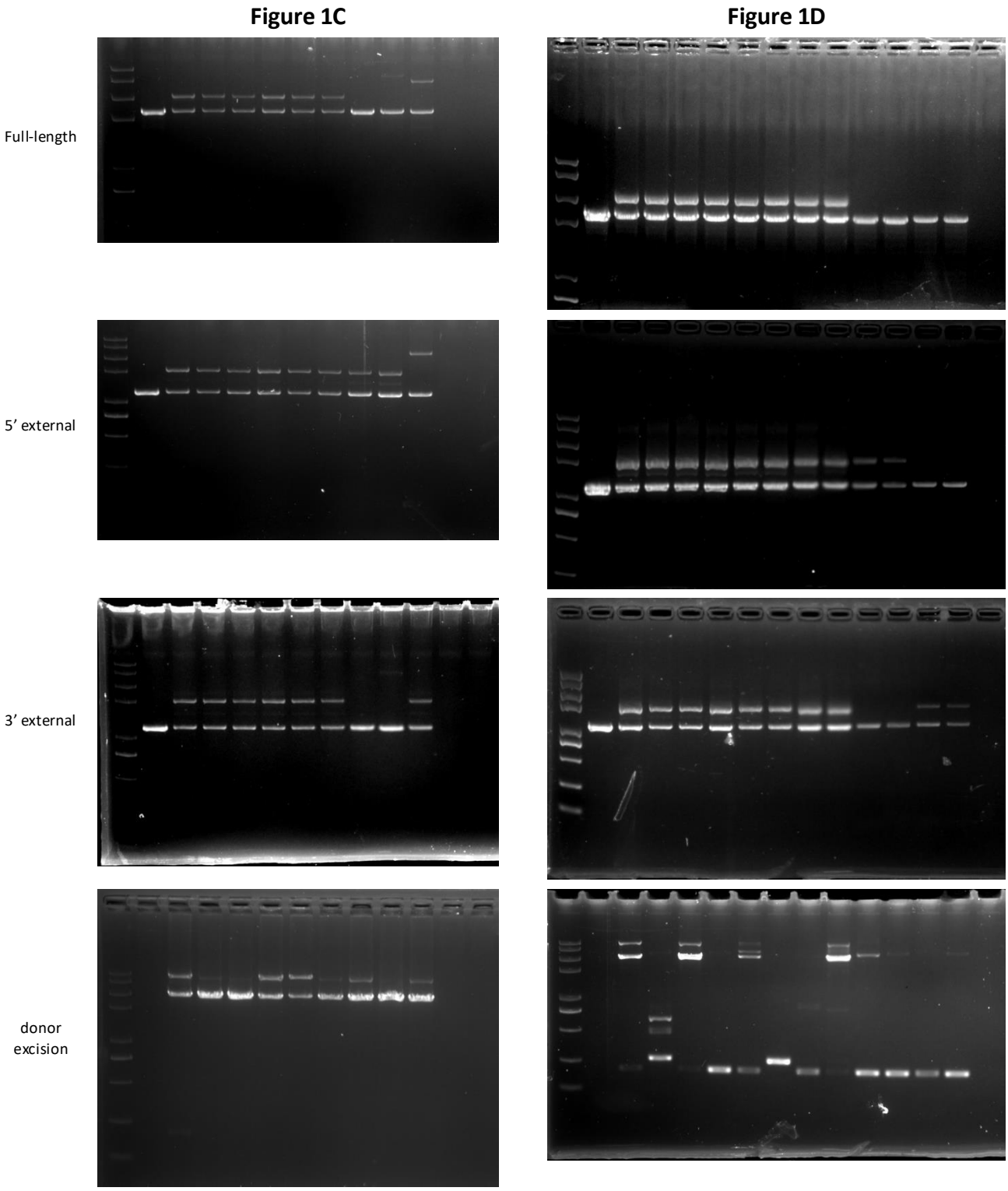
A



B



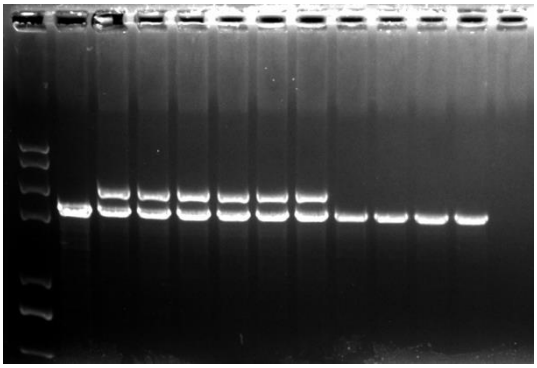
Supplementary Figure S5. Nuclear T-DNA copy number in tobacco and Arabidopsis.

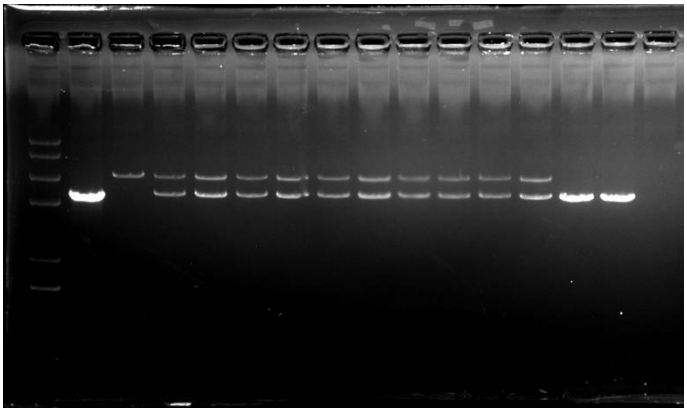


Supplementary Figure S6. Original electrophoresis gel pictures (Figure 1).

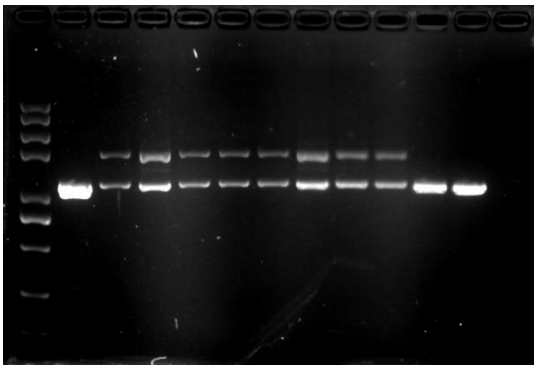
Figure 2B **Figure 2C**

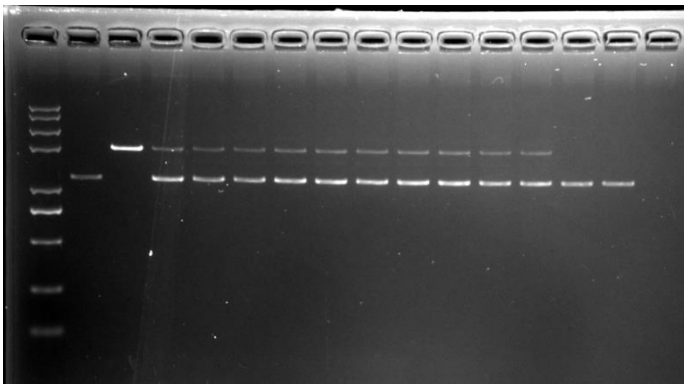
Full-length



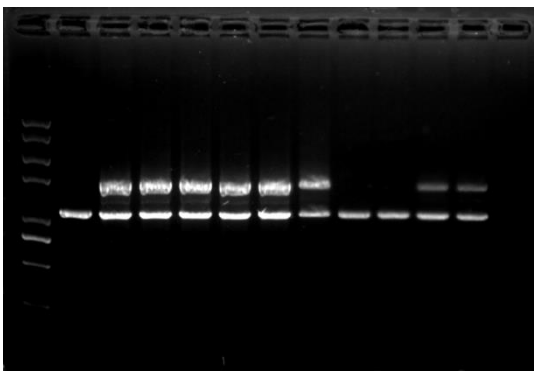


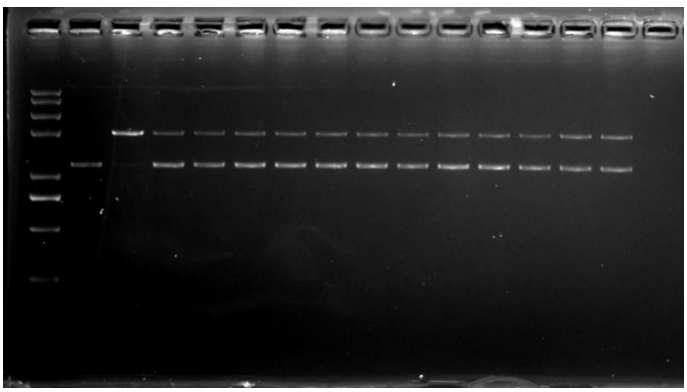
5’ external



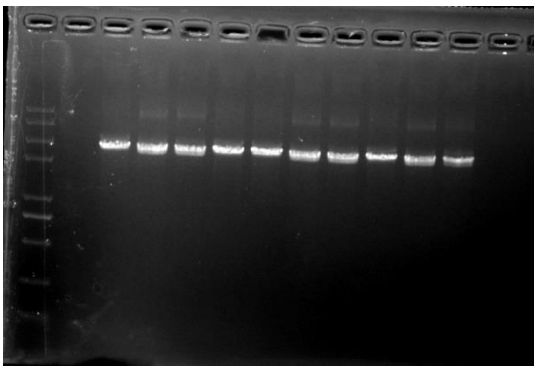


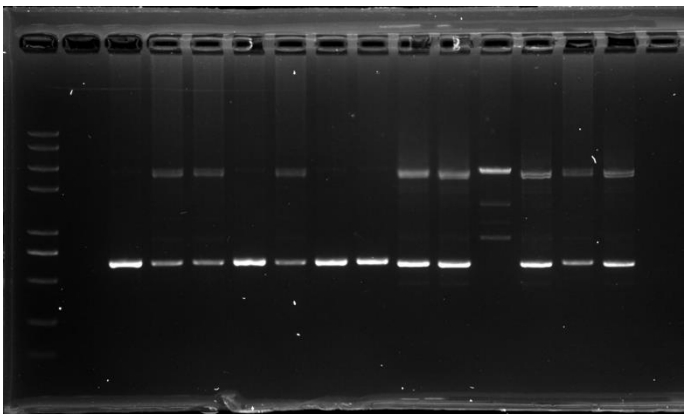
3’ external



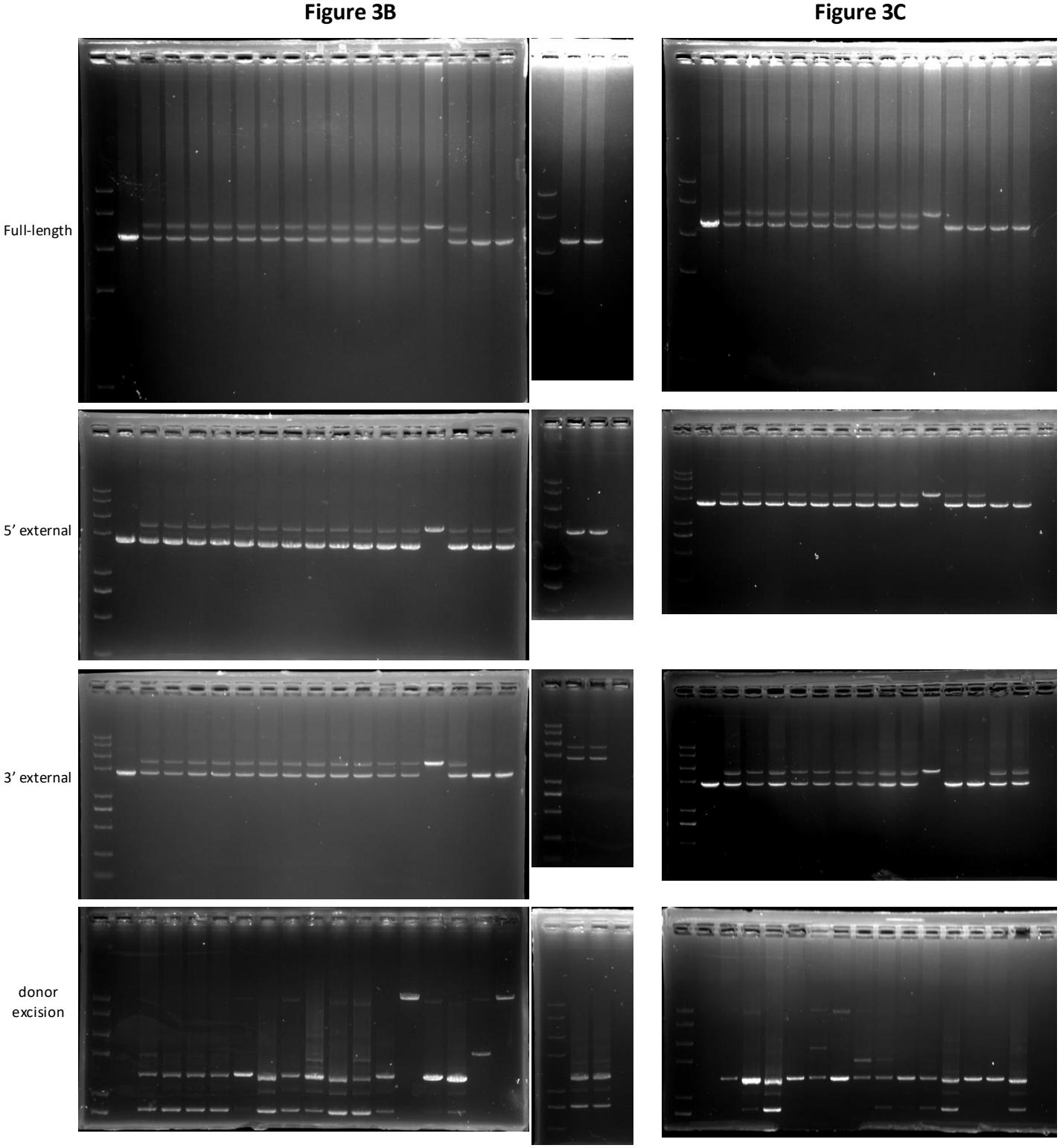


donor excision

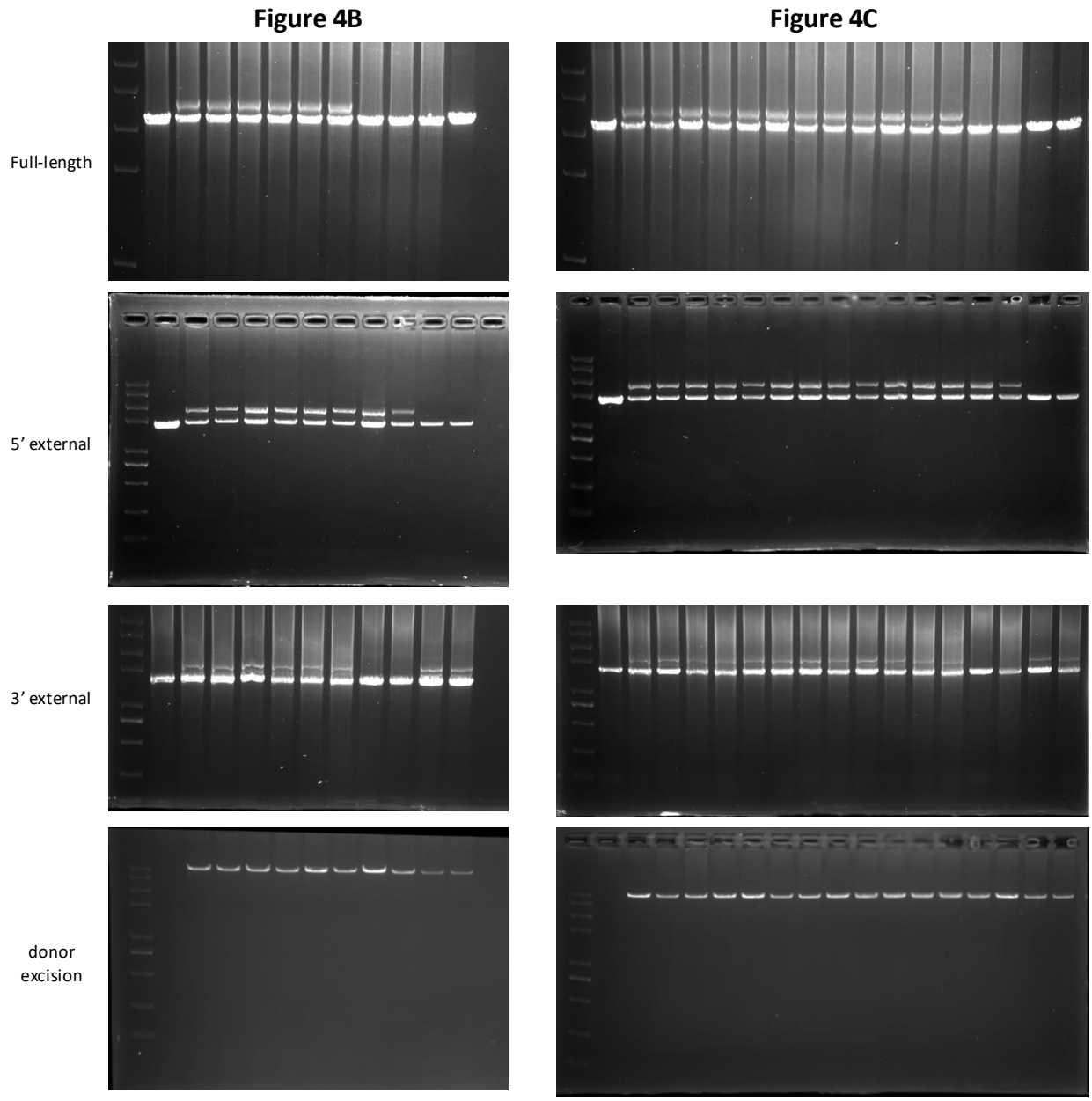




Supplementary Figure S6. Original electrophoresis gel pictures (Figure 2).

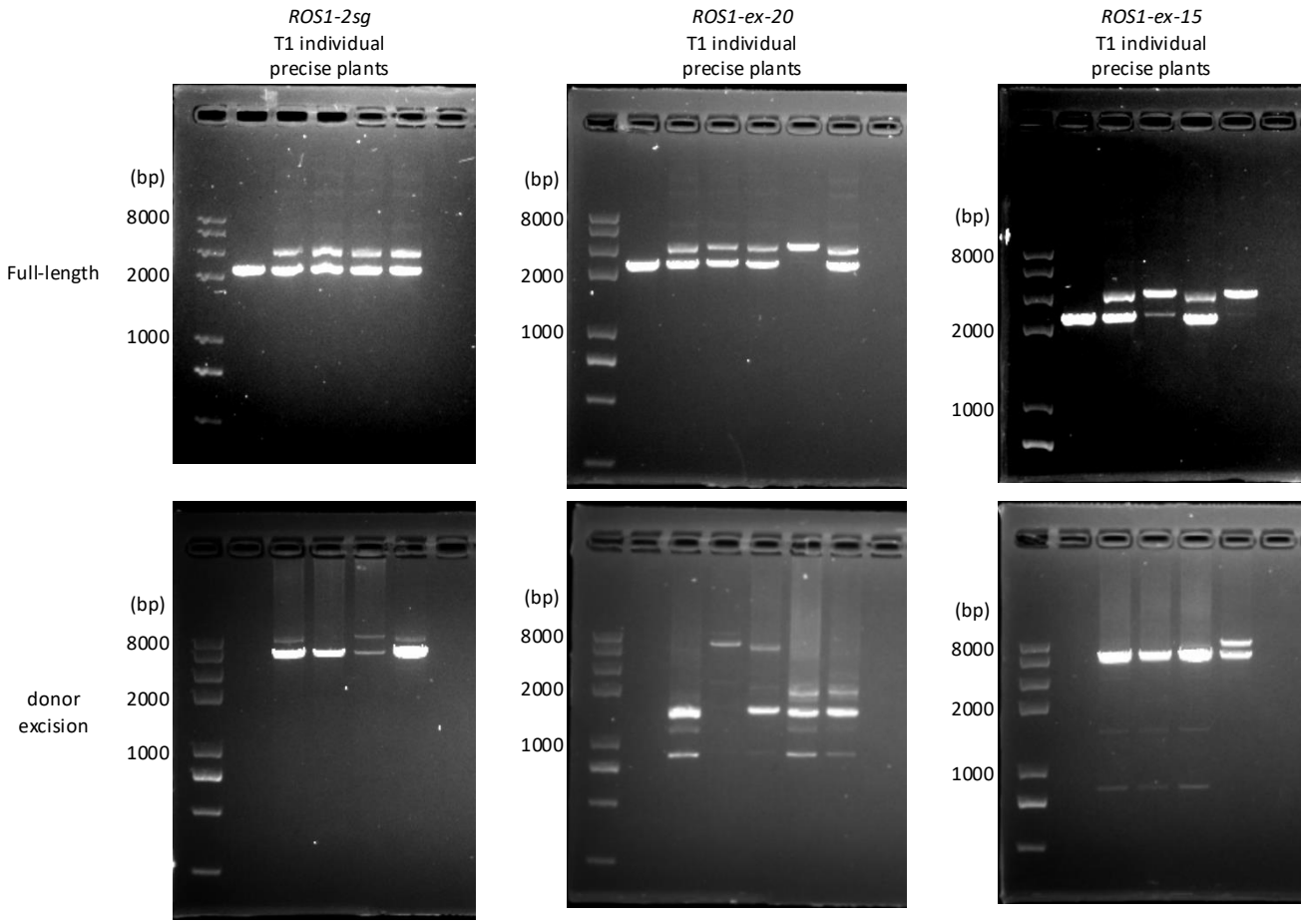


Supplementary Figure S6. Original electrophoresis gel pictures (Figure 3).



Supplementary Figure S6. Original electrophoresis gel pictures (Figure 4).

Figure 6



Supplementary Figure S6. Original electrophoresis gel pictures (Figure 6).