Supporting Information

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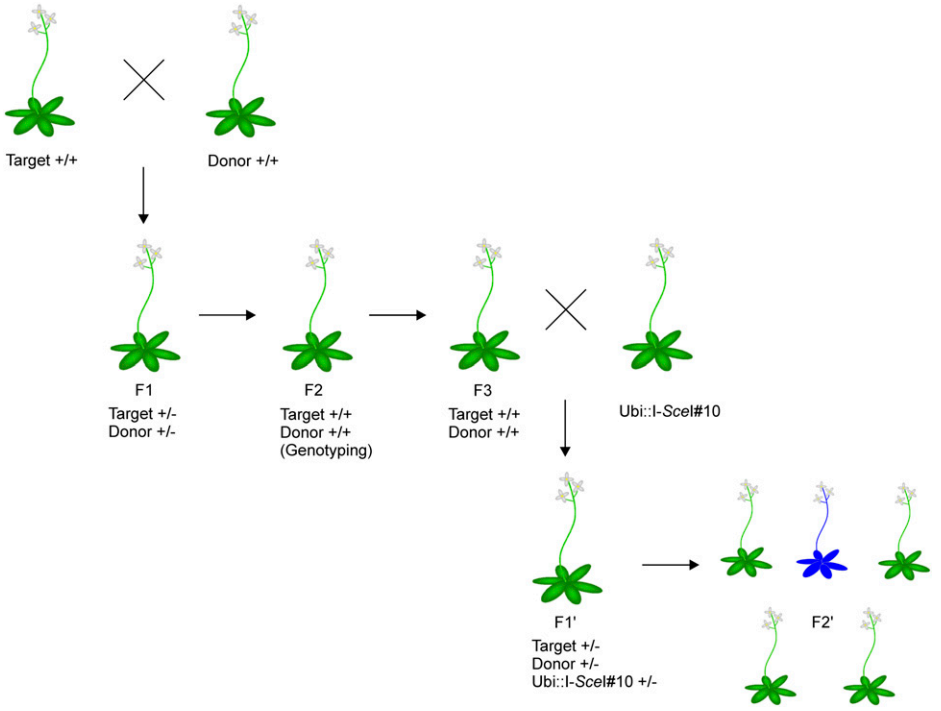

Fig. S1. General crossing schema. Single-copy target and donor lines were crossed, leading to a collection of different target/donor combinations (see Fig. 3). F3 plants, homozygous for both constructs, were crossed with the I-SceI expression line Ubi::I-SceI#10, leading to F1′ plants that show somatic GT events (blue sectors after histochemical staining, see Fig. 2*A*). Progeny of these plants (F2′) was screened for germinal GT events (completely stained blue plants).

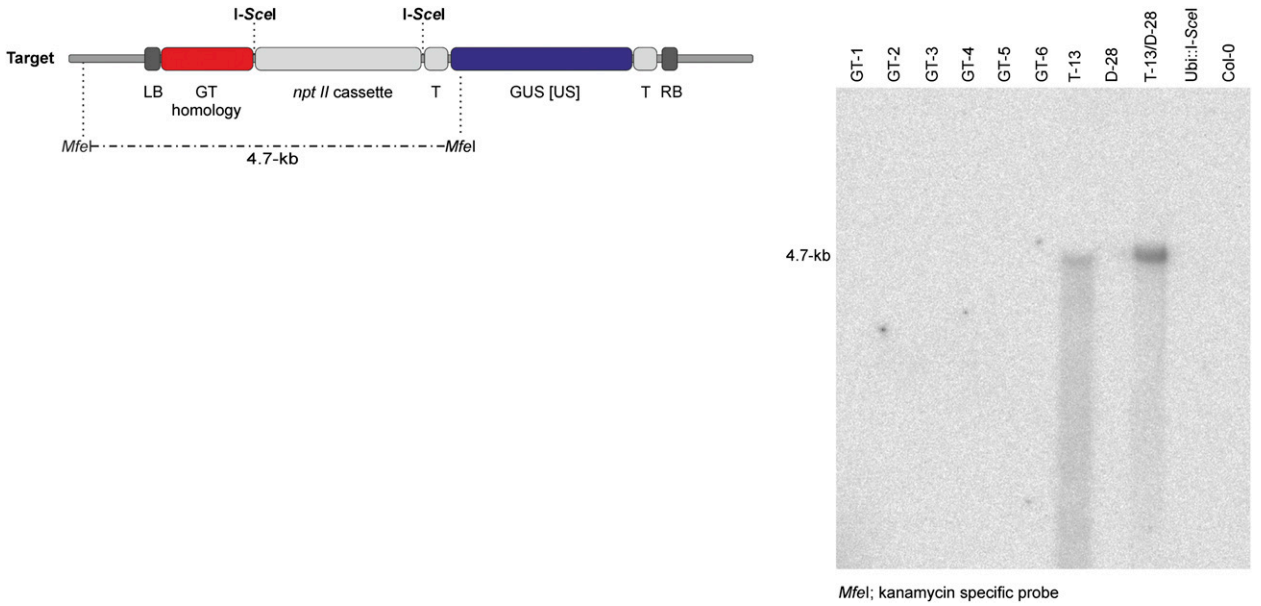

Fig. S2. Reintegration of the kanamycin-resistance cassette. Reintegration of the kanamycin-resistance cassette elsewhere in the genome was checked by Southern blotting. I-SceI expression is leading to an excised kanamycin-resistance cassette, leaving back a DSB within the target construct. To exclude re-integration of the kanamycin-resistance cassette elsewhere in the genome, MfeI digested DNA was transferred to a Hybond N+ membrane and probed with a kanamycin specific probe. The 4.7-kb fragment is indicative for the original T-DNA construct as it has been shown in T-13 and T-13/D-28. No other fragment was detectable in all analyzed plants, respectively, GT-1 to GT-6.

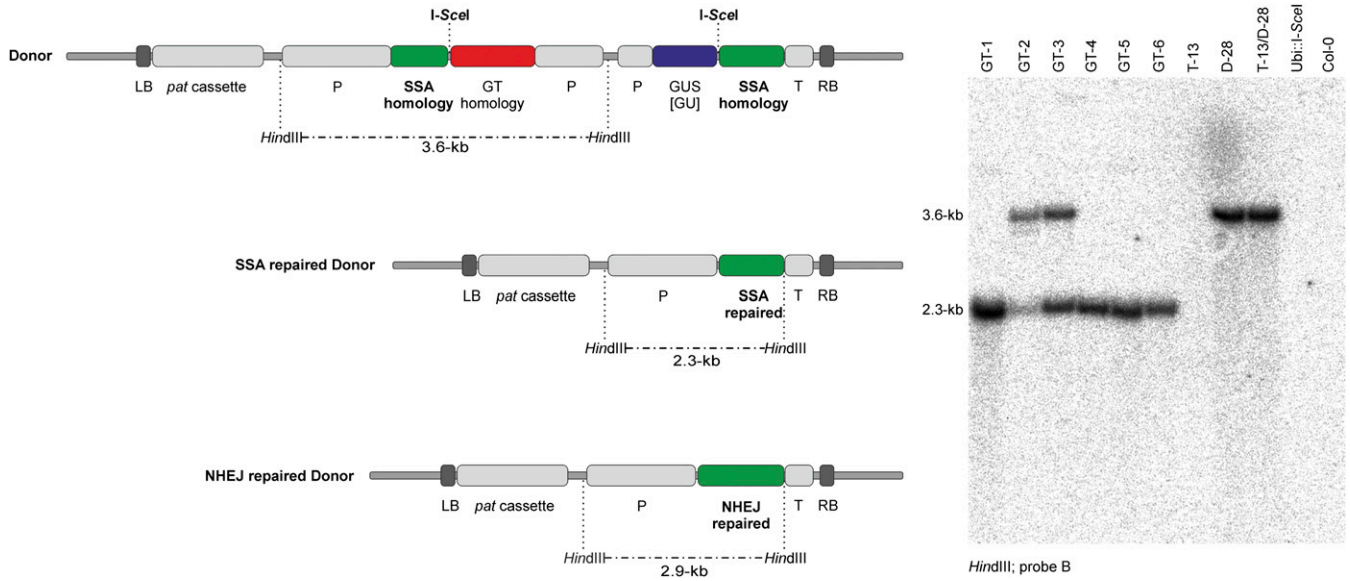

Fig. S3. Donor DSB repair. I-SceI expression is leading to an excised GT vector, leaving back a DSB within the donor construct. This DSB could be repaired either via the SSA pathway or via NHEJ. Genomic DNA of the recombinant lines was digested with HindIII, transferred to a Hybond N+ membrane, and probed with probe B (see Fig. 1). The 2.3-kb fragment is indicative for a SSA-mediated DSBR that has been shown in all analyzed plants, respectively, GT-1 to GT-6.

Table S1. Segregation of the restored GUS ORF in F3′

	Blue seedlings	Uncolored seedlings	$\chi^2_{1;0.95}$	Segregation
GT-1	100	0	0.00	Homozygous
GT-2	68	32	0.65	3:1
GT-3	70	30	0.33	3:1
GT-4	100	0	0.00	Homozygous
GT-5	100	0	0.00	Homozygous
GT-6	79	21	0.21	3:1

Completely stained F2′ plants (see Fig. S1) are either hemi- or homozygous for the GUS gene because of Mendelian segregation of the restored marker after recombination. In the F3′ generation, the recombined locus is acting like an endogenous site during meiosis. We checked 100 7-d-old F3′ seedlings via histochemical staining. We performed analysis of all 20 GT lines for a chi squared test, all of them showing Mendelian segregation of the locus (either 4:0 or 3:1). As example lines, GT-1 to GT-6 are depicted in the table.