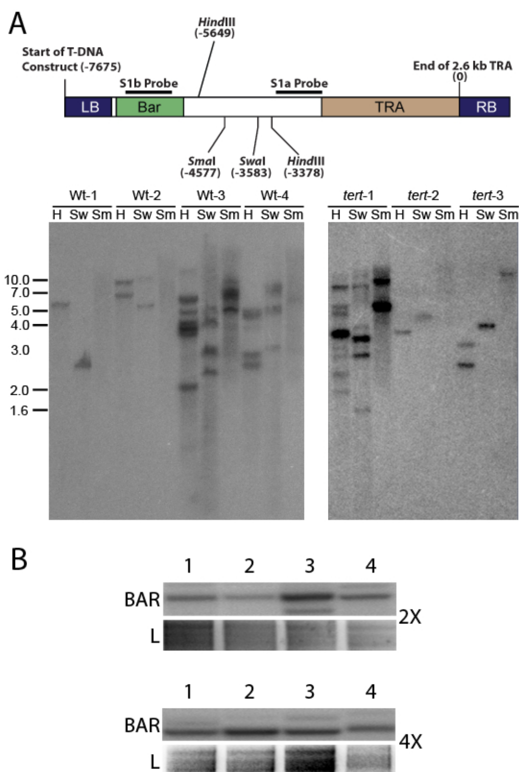
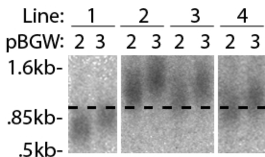
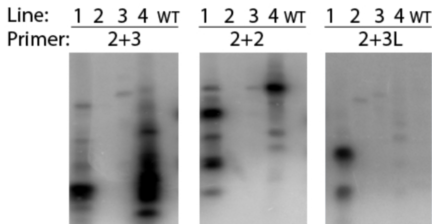


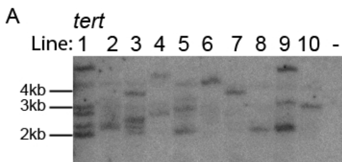
Supplemental Figure 1. DNTF detected by DNA Gel Blot analysis.
A. Detailed schematic diagram of PWY86 (top). Restriction sites for HindIII (H), SwaI (Sw), and SmaI (Sm) are shown along with nucleotide positions. DNA gel blot analysis of A. thaliana transformants (bottom). Results for 4X tert and 4X wild type plants transformed with the 2.6kb TRA using the probe indicated in the top diagram are shown. PETRA revealed DNTF in Wt-3, Wt-4, tert-1, tert-2, and tert-3 transformants, but not in Wt-1 or Wt-2. Hybridization products from DNA digested with HindIII are expected to be ~500bp smaller than SwaI products and 1.5kb smaller than SmaI products. Molecular weight markers are in kilobase pairs. B. DNA gel blot analysis of 2X and 4X wild type lines carrying the 2.6kb transgenic TRA. The blot was hybridized using a probe for the Basta resistance gene (BAR). L (loading) shows EtBr stained gels prior to transfer.

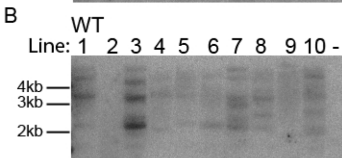


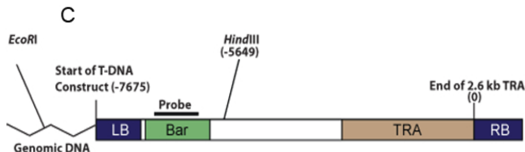


Supplemental Figure 2. TRAs shorter than 1 kb are prone to end-joining reactions.

A. PETRA results are shown for four lines transformed with a 100bp TRA using PBGW-2 and -3 primers, which target a unique sequence in the T-DNA 250 or 420 bp, respectively, upstream of the TRA. At least one telomere in lines #1 and #4 is shorter than 1kb (dashed line). B. TF-PCR results from the lines analyzed in (A). WT indicates a control TF-PCR reaction performed with an untransformed 4X WT control. Primers PBGW-2 and PBGW-3 were used for the reactions on the left, and only primer PBGW-2 was used on the right. A combination of 3L and PBGW-2 primers were used to test for non-sister chromatid fusions.







Supplemental Figure 3. 4X tert acquires the same number of T-DNA insertions as 4X wild type.

DNA gel blot analysis of tert (A) and wild type (B) samples digested with HindIII and EcoRI and hybridized with a probe for the BAR gene (Sb. EcoRI cuts within the adjacent genomic DNA (see map in C), whereas HindIII cuts downstream of the BAR gene. The negative (-) control is 4x WT without a transgene. Numbers indicate nucleotide positions within the PWY86 plasmid.

Supplemental Table 1: DNTF efficiencies in different genetic backgrounds.

Background	Vector	TRA Length	% DNTF
WT 2X	pWY86	2600	10% (12/126)
WT 4X	pBGW	100	16% (13/80)
WT 4X	pBGW	200	25% (24/96)
WT 4X	pWY86	400	35% (28/80)
WT 4X	pWY86	700	37.5% (36/96)
WT 4X	pWY86	800	47% (47/100)
WT 4X	pWY86	900	53.5 (68/127)
WT 4X	pBGW	950	55% (53/96)
WT 4X	pWY86	2600	58% (35/60)
			54% (26/48)
ku70	pWY86	2600	2% (2/88)
lig4	pWY86	2600	35% (26/73)
G4 *tert*	pWY86	2600	71% (68/96)
			74% (53/72)
WT 4X	pBGW	750 (TTAGGG)	19% (15/80)
WT 4X	pBGW	500 (TGGTTGAT)	1.3% (1/80)
WT 4X	pBGW	600 (UAS)	0% (0/100)
WT 4X	pBGW	850 (GGGATTT)	0% (0/80)

Supplemental Table 2: Primers used in this study.

Primers for Gateway cloning of repeat arrays:

Arabidopsis telomere oligo #1	CCCTAAACCCTAAACCCTAAACCCTAAACCCTAAA
Arabidopsis telomere oligo #2	TTTAGGGTTTAGGGTTTAGGGTTTAGGGTTTAGGG
Jumbled Arabidopsis repeat oligo #1	CCATCAACCATCAACCATCAACCATCAACCATCAA
Jumbled Arabidopsis repeat oligo #2	TGGTTGATGGTTGATGGTTGATGGTTGATGG TTGA
UAS oligo #1	CGGAAGACTCTCCTCCGCGGAAGACTCTCCTCCGCGGAAGACTCTCCTCCG
UAS oligo #2	AGTCTTCCGCGGAGGAGAGTCTTCCGCGGAGGAGAGTCTTCCGCGGAGGAG
Human telomere oligo #1	CCCTAACCCTAACCCTAACCCTAACCCTAA
Human telomere oligo #2	GGTTAGGGTTAGGGTTAGGGTTAGGGTTAG

Primers for TAIL-PCR:

TAIL-1	AGC TGC ATT AAT GAA TCG GCC AAC GCG C
TAIL-2	AGC TGC ATT AAT GAA TCG GCC AAC GCG
TAIL-3	AGA GGC GGT TTG CGT ATT GGC TAG AGC
hiTAIL-1	CGT CTA TTG GAC CGA CAG TTG C
hiTAIL-2	TGC ATT TTC CAG GGC ATT TTT
hiTAIL-3	TCC GCT CAC AAT TCC ACA CAA
AD1	NGT CGA SWG ANA WGA A
AD2	TGW GNA GSA NCA SAG A
AD3	AGW GNA GWA NCA WAG G
AD6	WGT GNA GWA NCA NAG A

Primers for PETRA:

PETRA-T	CTCTAGACTGTGAGACTTGGACTACCCTAAACCCT
PETRA-A	CTCTAGACTGTGAGACTTGGACTAC
PWY86#1	TACGTCTAGATCTGGCGCGC
PWY86#3	CATAAAGGATCCCCGATCGTT
PBGW#3	TATGGAACGTCAGTGGAGCATTT
PBGW#4	AGTTGACCGTGCTTGTCTCGAT