

**Table S1** Primers used in this study.

Locus	Primer name	orientation	Sequence 5'-3'
<i>XPF</i>	PpXPF#1	Forward	CTGGACGACAATTCCGATTA
	PpXPF#2	Reverse	TTTCTCCTGGTCATCTTCTG
	PpXPF#12	Reverse	AGCGCTGACCCTGTCAACCTT
	PpXPF#13	Forward	AGGTCCTTTCATGCTAGAGTG
<i>ERCC1</i>	PpERCC1#1	Forward	GTAGCACAGAAGGACAACAC
	PpERCC1#2	Reverse	GCTTGCCATGTCTGCTATTC
	PpERCC1#17	Forward	GATGTGATCAAACCGCTAC
	PpERCC1#18	Reverse	GCTTGCCATGTCTGCTATTC
<i>APT</i>	PpAPT#2	Forward	TTTTTGCGCTCGCTGTTTCTG
	PpAPT#14	Forward	AGATGTCGGCCTCCAAGGATG
	PpAPT#19	Reverse	CCCGACAACCTTCTCACGACCC
	PpAPT#20	Reverse	TAAATAATTCTGACCCAAAGT
HygR	ProRev	Reverse	GTCTTGCGAAGGATAGTGGG
	TerFwd	Forward	CGCTGAAATCACCAGTCTCTCT

**Table S2** Comparison of transformation and gene targeting efficiencies using an ends-out type targeting construct containing no heterologous selectable marker.

Type of transforming DNA	Genotypes	Regenerants <sup>a</sup>	2FA <sup>R</sup> Plants <sup>b</sup>	Gene targeting frequency (%) <sup>c</sup>
Ends-out	Wild type	359848	2584	0.72 ± 0.13 <sup>d</sup>
	<i>xpf</i>	464812	2026*	0.43 ± 0.08
Homologous ends	<i>ercc1</i>	200385	1042*	0.52 ± 0.07
	Wild type	54667	82	0.15 ± 0.03
Ends-out	<i>xpf</i>	62500	75	0.12 ± 0.04
	<i>ercc1</i>	56364	62	0.11 ± 0.07
Heterologous ends	Wild type	30682	183	0.59 ± 0.03
	<i>xpf</i>	36667	176**	0.48 ± 0.03
Ends-in	<i>ercc1</i>	49524	208*	0.42 ± 0.04
	Wild type	72857	102	0.14 ± 0.01 <sup>d</sup>
Ends-in	<i>xpf</i>	54286	38*	0.07 ± 0.02
	<i>ercc1</i>	87013	87**	0.1 ± 0.02
Heterologous ends				

<sup>a</sup> Protoplasts were transformed with the different vectors (see Fig. 3 and Fig.4) and regenerants were selected on 2-fluoroadenine.

<sup>b</sup> 2-FA<sup>R</sup> clones are the clones that experienced a gene targeting event and thus survived after subculture on 2-FA medium. Differences between wild type and the mutants were compared using Fisher's exact test. \* Correspond to p value <0.01, \*\* Correspond to p value <0.05.

<sup>c</sup> GT frequency (in %) express the frequency of 2-FA resistant among the population of regenerants.

<sup>d</sup> Average and standard deviation were determined from at least 3 independent experiments.