Supporting Methods

Qi *et al*., Increasing frequencies of site-specific mutagenesis and gene targeting in Arabidopsis by manipulating DNA repair pathways

Arabidopsis Protoplast Transformation

1. Collect 10-15 well-expanded rosette leaves from 4-week-old plants before flowering.
2. Cut the leaves into 0.5–1mm strips from the middle part of a leaf using a new, sharp razor blade.
3. Transfer leaf strips quickly and gently into 10 ml of prepared enzyme solution (0.4 M Mannitol, 20 mM MES, 1.0% Cellulase R10, 0.25% Macerozyme R10, 20 mM KCl, 10 mM CaCl2, 0.1% BSA, pH5.8).
4. Continue digestion by shaking at 30 rpm in the dark for 4 hr at room temperature (RT).
5. Filter the digestion solution with the 70-μm nylon mesh into a 50 ml tube filled with 20 ml W5 solution (2 mM MES, 5 mM KCl, 125 mM CaCl2, 5 mM Glucose, pH5.8).
6. Centrifuge at 200xg to pellet the protoplasts for 2 min at RT.
7. Re-suspend protoplasts with 10 ml W5 solution and centrifuge at 100xg for 1 min at RT.
8. Re-suspend protoplasts in 5ml W5 solution and count the cell number.
9. Incubate the protoplasts on ice for 10 min.
10. Centrifuge at 100xg for 1 min at RT and re-suspend protoplasts at $2\times10^5\,ml^{-1}$ in MMG solution (0.4 M Mannitol, 4 mM MES, 15 mM MgCl2, pH5.8).
11. Add 10-30 μl donor plasmids (15 μg) to a 1.5 ml microfuge tube (15 μg YFP-expressing plasmid, pZHY162, is used as a control in a separate sample for measuring transformation efficiency).
12. Add 200 μl protoplasts and mix gently with donor DNA.
13. Add 210-230ul 40%PEG-Ca transformation buffer (40% PEG, 0.2 M Mannitol, 100 mM CaCl2, pH5.8). Mix gently and incubate at RT for 20 min.
14. Add 900 μl W5 solution and mix well to stop transfection.
15. Centrifuge at 200xg for 2 min at RT and remove supernatant.
16. Add 800 μl W5 solution and centrifuge at 100xg for 2 min at RT and remove supernatant.
17. Re-suspend protoplasts gently with 1 ml PI buffer (0.45 M Mannitol, 1X MS, 1.5%

sucrose, pH5.8) in each well of a 6-well tissue culture plate.

18. Add 20 mM estradiol stock to final concentration of 20 μM. [If necessary]
19. Incubate protoplasts at 25C for 36 hr.
20. Harvest protoplasts for DNA extraction and further analysis.

Sample preparation for 454 sequencing

1. Prepare genomic DNA using a standard CTAB protocol. Adjust DNA concentrations of all samples to 50 ng/μl.
2. Primer design

454 sequencing primers were designed to include specific adaptor sequences according to 454 Life Science's recommendation as shown below:
Forward primer (Primer A-Key):
5'-CGTATCGCCTCCCTCGCGCCATCAG+template-specific-sequence-3'
Reverse primer (Primer B-Key):
5'-CTATGCGCCTTGCCAGCCCGCTCAG+MID+template-specific-sequence-3'

Oligo Name	Sequence
454A-AdhI	CGTATCGCCTCCCTCGCGCCATCAGttctccggtaaagatcggcaacaca
454B-AdhI-01	CTATGCGCCTTGCCAGCCCGCTCAGacgagtgcgtAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-02	CTATGCGCCTTGCCAGCCCGCTCAGacgctcgacaAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-03	CTATGCGCCTTGCCAGCCCGCTCAGagacgcactcAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-04	CTATGCGCCTTGCCAGCCCGCTCAGagcactgtagAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-05	CTATGCGCCTTGCCAGCCCGCTCAGatcagacacgAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-06	CTATGCGCCTTGCCAGCCCGCTCAGatatcgcgagAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-07	CTATGCGCCTTGCCAGCCCGCTCAGcgtgtctctaAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-08	CTATGCGCCTTGCCAGCCCGCTCAGctcgcgtgtcAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-09	CTATGCGCCTTGCCAGCCCGCTCAGtagtatcagcAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-10	CTATGCGCCTTGCCAGCCCGCTCAGtctctatgcgAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-11	CTATGCGCCTTGCCAGCCCGCTCAGtgatacgtctAGATTCTCTTCACTTCTCTCTGTCA
454B-AdhI-12	CTATGCGCCTTGCCAGCCCGCTCAGtactgagctaAGATTCTCTTCACTTCTCTCTGTCA

3. PCR reaction
    a. Prepare a PCR reaction mix as following:

	1X
5X LongTaq PCR buffer (NEB)	10 μl
dNTP (10mM)	1.5 μl

Forward primer (10pmol/µl)	1.5 µl
Reverse primer (10pmol/µl)	1.5 µl
Genomic DNA	2.0 µl
Long Taq (NEB)	1.0 µl
H2O	32.5 µl
	50 µl total

b. PCR program:

Step1	95C	2 min
Step2	94C	20 sec
Step3	56C	20 sec
Step4	65C	30 sec
Step5	go to step 2, 33 times	
Step6	65C	10 min
Step7	10C	30 min

c. Run 5 µl of PCR products on a 1.0% agarose gel to check the result before proceeding further.

4. Clean up of PCR reactions with AMPure XP beads (Agencourt Cat # A63880)

5. Before submitting samples for 454 sequencing:
   a. Run amplicons on a DNA 7500 Bioanalyzer chip to assess the quality
   b. Adjust different amplicons to an equal-molar concentration based on size and DNA amount

454 sequencing data analysis

The 454-derived sequence reads were filtered based on read end quality. Only sequences showing 95% homology to the template sequences in the terminal 20bp of each end were passed for further analyses. These reads were mapped to endogenous and donor template sequences via the Needleman-Wunsch global alignment algorithm. Sequences matching the endogenous sequence perfectly were called as 'Unmodified'. Sequences with fewer than 15 discrepancies with the donor template were called as ‘Homologous recombination (HR)’ events. The remaining reads were passed to a NHEJ calling pipeline. In the NHEJ pipeline a 166 bp window centered on the nuclease binding sites was considered. Reads with at least 2bp of consecutive insertion, deletion or mismatch within the window were called as ‘Modified’; otherwise they were called as ‘Unmodified’. For modified sequences, the largest consecutive region of modification within the window was identified. Other regions of insertion, deletion or mismatch within 5bp were iteratively added to the modification region until no further regions could be added. The resulting modified region, consisting of mismatched,

insertions and deletions, was called as an insertion or deletion based on whichever was more prevalent. If the region consisted only of mismatches then the region was called as a mismatch (nucleotide substitution).

Table. S1. Summary of 454 NHEJ events revealed by 454 sequencing

Treatment	Genotype	Experiment	Valid read	Deletion	Insertion	Mismatch	NHEJ total	NHEJ %
Donor only	*ADH1-ZFN-4*	Exp.1	3727	0	3	3	6	0.16
Donor only	*ADH1-ZFN-4 ku70*	Exp.1	6458	1	4	2	7	0.11
Donor only	*ADH1-ZFN-4 lig4*	Exp.1	6626	0	2	4	6	0.09
Donor only	*ADH1-ZFN-4 smc6b*	Exp.1	5627	0	4	2	6	0.11
Donor only	*ADH1-ZFN-4*	Exp.2	7404	1	1	3	5	0.07
Donor only	*ADH1-ZFN-4 ku70*	Exp.2	7116	0	0	4	4	0.06
Donor only	*ADH1-ZFN-4 lig4*	Exp.2	7208	1	2	2	5	0.07
Donor only	*ADH1-ZFN-4 smc6b*	Exp.2	6844	0	0	4	4	0.06
Estradiol only	*ADH1-ZFN-4*	Exp.1	4427	115	34	6	155	3.50
Estradiol only	*ADH1-ZFN-4 ku70*	Exp.1	4598	353	9	7	369	8.03
Estradiol only	*ADH1-ZFN-4 lig4*	Exp.1	5008	200	5	4	209	4.17
Estradiol only	*ADH1-ZFN-4 smc6b*	Exp.1	3942	1046	268	53	1367	34.68
Estradiol only	*ADH1-ZFN-4*	Exp.2	5144	185	52	12	249	4.84
Estradiol only	*ADH1-ZFN-4 ku70*	Exp.2	4560	404	6	9	419	9.19
Estradiol only	*ADH1-ZFN-4 lig4*	Exp.2	4406	259	11	0	270	6.13
Estradiol only	*ADH1-ZFN-4 smc6b*	Exp.2	3385	1122	321	57	1500	44.31
Estradiol only	*ADH1-ZFN-4*	Exp.3	3850	174	41	8	223	5.79
Estradiol only	*ADH1-ZFN-4 ku70*	Exp.3	5111	382	5	7	394	7.71
Estradiol only	*ADH1-ZFN-4 lig4*	Exp.3	4846	261	7	3	271	5.59
Estradiol only	*ADH1-ZFN-4 smc6b*	Exp.3	4344	1485	190	95	1770	40.75
Donor and Estradiol	*ADH1-ZFN-4*	Exp.1	16043	348	198	15	561	3.50
Donor and Estradiol	*ADH1-ZFN-4 ku70*	Exp.1	15992	502	85	87	674	4.21
Donor and Estradiol	*ADH1-ZFN-4 lig4*	Exp.1	13414	471	38	40	549	4.09
Donor and Estradiol	*ADH1-ZFN-4 smc6b*	Exp.1	13886	3357	883	79	4319	31.10
Donor and Estradiol	*ADH1-ZFN-4*	Exp.2	8506	298	97	23	418	4.91
Donor and Estradiol	*ADH1-ZFN-4 ku70*	Exp.2	7928	636	27	6	669	8.44
Donor and Estradiol	*ADH1-ZFN-4 lig4*	Exp.2	7992	498	19	10	527	6.59
Donor and Estradiol	*ADH1-ZFN-4 smc6b*	Exp.2	5613	1931	564	136	2631	46.87
Donor and Estradiol	*ADH1-ZFN-4*	Exp.3	5613	306	76	18	400	7.13
Donor and Estradiol	*ADH1-ZFN-4 ku70*	Exp.3	6657	515	19	6	540	8.11
Donor and Estradiol	*ADH1-ZFN-4 lig4*	Exp.3	9634	591	28	14	633	6.57
Donor and Estradiol	*ADH1-ZFN-4 smc6b*	Exp.3	5970	2226	621	119	2966	49.68

Table S2. Deletions using no or putative MH of 1 bp or longer

Estradiol only	Exp. 1				Exp. 2				Exp. 3			
	ADH1-ZFN-4				*ADH1-ZFN-4*				*ADH1-ZFN-4*			
Length of MH	*Col*	*ku70*	*lig4*	*smc6b*	*Col*	*ku70*	*lig4*	*smc6b*	*Col*	*ku70*	*lig4*	*smc6b*
0 bp (no putative MH)	47	72	31	406	84	66	33	467	67	52	30	547
1 bp	54	54	22	498	74	56	47	525	86	60	41	768
2 bp	10	59	35	94	18	41	55	86	13	47	37	117
3 bp	1	39	18	13	2	44	41	14	1	56	34	9
4 bp	0	30	9	8	1	50	7	13	4	32	11	3
5 bp	1	22	11	7	1	22	9	4	1	23	15	11
6 bp	1	77	74	19	5	125	67	13	2	112	93	30
7 bp	0	0	0	1	0	0	0	0	0	0	0	0
8 bp or longer	0	0	0	0	0	0	0	0	0	0	0	0
Total deletions	114	353	200	1046	185	404	259	1122	174	382	261	1485

Donor and estradiol	Exp. 1				Exp. 2				Exp. 3			
	ADH1-ZFN-4				*ADH1-ZFN-4*				*ADH1-ZFN-4*			
Length of MH	*Col*	*ku70*	*lig4*	*smc6b*	*Col*	*ku70*	*lig4*	*smc6b*	*Col*	*ku70*	*lig4*	*smc6b*
0 bp (no putative MH)	123	83	69	1120	112	118	85	799	146	79	87	936
1 bp	185	59	71	1755	143	88	91	903	124	48	113	1031
2 bp	33	83	98	364	33	87	78	171	26	71	86	212
3 bp	0	85	76	58	3	73	67	13	2	87	93	18
4 bp	0	39	22	13	2	58	33	5	1	38	25	3
5 bp	0	18	17	0	0	37	19	12	0	36	31	6
6 bp	7	135	118	47	5	175	125	28	7	156	156	20
7 bp	0	0	0	0	0	0	0	0	0	0	0	0
8 bp or longer	0	0	0	0	0	0	0	0	0	0	0	0
Total deletions	348	502	471	3357	298	636	498	1931	306	515	591	2226

Table S3. Analysis of four frequent large deletions (as peaks in Fig. 6C and Fig. S4C)

Estradiol only	**Propotion of each MH-based deletion among total deltions of the same size (%)**				
Deletion size	**Micromhomology**	***ADH1-ZFN-4***	***ADH1-ZFN-4 ku70***	***ADH1-ZFN-4 lig4***	***ADH1-ZFN-4 smc6b***
38 bp	CAC	0	15.33	11.98	21.92
	ATCTTC	100	77.67	86.18	72.60
46 bp	AGGG	33.33	36.51	11.11	21.92
	CTTCG	66.67	47.62	77.78	34.48
50 bp	AAACAC	N/A	98.39	97.62	62.07
142 bp	GA	25	26.79	46.15	20
	GCCA	75	57.14	53.85	80
	ATCTTC	N/A	14.29	N/A	N/A

Donor and estradiol	**Propotion of each MH-based deletion among total deltions of the same size (%)**				
Deletion size	**Micromhomology**	***ADH1-ZFN-4***	***ADH1-ZFN-4 ku70***	***ADH1-ZFN-4 lig4***	***ADH1-ZFN-4 smc6b***
38 bp	CAC	15.38	18.97	20.48	20.49
	ATCTTC	84.62	78.33	76.87	68.85
46 bp	AGGG	N/A	43.24	20.55	13.64
	CTTCG	N/A	41.89	63.01	36.36
50 bp	AAACAC	88.89	97.17	95.95	76.92
142 bp	GA	50	26.83	4.17	41.67
	GCCA	50	57.32	81.25	58.33
	ATCTTC	N/A	14.63	12.5	N/A

Table S4. 454 DNA sequencing reads indicative of imprecise HR among 'GT' events.

Experiments	Position of the imprecise end due to NHEJ repair	Partial 454 sequence reads reflecting imprecise HR
precise HR		**TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG**
Ku70_rep1	left side	TTTCCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCTTCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCATTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCCAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCAAAGTGAGTCGTATTACAGATCTCGGTACCACTGGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCCAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGCCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTGTAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTAGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGATGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGATGAGTCATATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTAGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGATGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGATGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGATGAGTCATATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGATGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTAGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGATGAGTCATATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTGTAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG

Table S4. 454 DNA sequencing reads indicative of imprecise HR among 'GT' events (continued).

Experiments	Position of the imprecise end due to NHEJ repair	Partial 454 sequence reads reflecting imprecise HR
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGATGAGTCATATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCAAAAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGCCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAACCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATGATGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTCGGCCATAAATTGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	left side	TTTCCACGTATCTTAGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGAAGCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTAGCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTACTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTAGCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTAGCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCATGAAGCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGGAAGCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTGAAGCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTAGCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCATGAAGCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTAGCTGGAGGGTAATAG
Ku70_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTAGCTGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGCCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAGAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGTCCTTGGCCGTCGTTTTACGGAGGGTAATTAG
lig4_rep1	left side	TTTCCACGTATCTTCGGCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTTAATAG
lig4_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTAGCTGGAGGGTAATAG
lig4_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTAGCTGGAGGGTAATAG
lig4_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTAGCTGGAGGGTAATAG
smc6b_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
smc6b_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
smc6b_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
smc6b_rep1	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATTAG
smc6b_rep1	right side	TTTCCACGTATCTTCCCCTATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAAGCTGGAGGGTAATAG
ku70_rep2	left side	TTTCCACGTATCTTCGGCCATGAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAG
lig4_rep2	left side	TTTCCACGTATCTTCGGCCATAGTGAGTCGTATTACAGATCTCGGTACCACTAGTCTCGAGGCTAGCCTGGCCGTCGTTTTACGGAGGGTAATAGAA

Table S5. Summary of imprecise HR among 'GT' and 'insertion' events

Genotype	Rep	Valid reads	GT	Imprecise HR among 'GT' events*	Total imprecise HR
Col	1	16043	53	0/0	0
ku70	1	15992	851	78/13	91
lig4	1	13414	127	22/3	25
smc6b	1	13886	188	4\1	5
Col	2	8506	2	0/0	0
ku70	2	7928	9	1\0	1
lig4	2	7992	6	1\0	1
smc6b	2	5613	5	0/0	0
Col	3	5613	0	0/0	0
ku70	3	6657	10	0/0	0
lig4	3	9634	4	0/0	0
smc6b	3	5970	4	0/0	0

** The backslash separates numbers of imprecise repair events on the left and right side of the insertion, respectively.*

Table S6. Oligos used in this study

Name	Sequence	Purpose
XVE_pMDC32_LB_TAIL_1	GCATCAGTTTCATTGCCCACACACCAGAATCCTACTAAGTTTGAG	TAIL-PCR mapping ADH1-ZFN-4 transgene insertion
XVE_pMDC32_LB_TAIL_2	GGGTTGAATTTAAGTAAGAAAGGAACTAACAGTGTGATATTAAGGTGC	TAIL-PCR mapping ADH1-ZFN-4 transgene insertion
XVE_pMDC32_LB_TAIL_3	GTGTCGAGGGGGTACCTTTCTCCATAATAATGTGTGAGTAG	TAIL-PCR mapping ADH1-ZFN-4 transgene insertion
TAIL_DGP_A1	NGTCGASWGANAWGAA	TAIL-PCR mapping ADH1-ZFN-4 transgene insertion
TAIL_DGP_A2	GTNCGASWCANAWGTT	TAIL-PCR mapping ADH1-ZFN-4 transgene insertion
TAIL_DGP_A3	WGTGNAGWANCANAGA	TAIL-PCR mapping ADH1-ZFN-4 transgene insertion
Adh1_ZFN-4_LP	ttcggaaatactgtaatgtgatga	Genotyping for ADH1-ZFN-4 transgene
Adh1_ZFN-4_RP	TGGTCATCAACGGAACAACAGT	Genotyping for ADH1-ZFN-4 transgene
LB_TAIL_4	gttcctatagggtttcgctcatgtg	Genotyping for ADH1-ZFN-4 transgene
ADH1F	TCGAGGAAGTGGAGGTTGCT	Amplify ADH1-ZFN targeting site
ADH1R2	TGGCTGAAGATCAGTCACTCC	Amplify ADH1-ZFN targeting site
TT4-F	tacatggctccttctctggaca	Amplify TT4-ZFN targeting site
TT4-R	agtctggaaggatggtctgagc	Amplify TT4-ZFN targeting site
MPK8-F	AATCCCGATGAAACACATTAGC	Amplify MPK8-ZFN targeting site
MPK8-R	AGGTTGTTGGCAAAGGAAGTTA	Amplify MPK8-ZFN targeting site
At5g61460-F3	CAAGACGCAACAAGCAATGT	RT-PCR
At5g61460-R3	AACATCCACTGCGATCCTTC	RT-PCR
At3g18780 (Actin2)-F	AGTGTCTGGATCGGTGGTTC	RT-PCR
At3g18780 (Actin2)-R	CCCCAGCTTTTTAAGCCTTT	RT-PCR
ZY070-F	AGATTCTCTTCACTTCTCTCTGTCA	Amplify ADH1-ZFN targeting site and detect HR
ZY073-R1	CCTCCGTAAAACGACGGCCAGGCTA	Detection of GT at the ADH1 locus
ZY070-R	TTCTCCGGTAAAGATCGGCAACACA	Detection of GT at the ADH1 locus
SALK_124719-LP	TGGACATTTCTTAATCCGAAGC	Genotyping for *smc6b-3* mutant
SALK_124719-RP	AGGTAATTGACACTGCCATGG	Genotyping for *smc6b-3* mutant
LBb1.3	ATTTTGCCGATTTCGGAAC	Genotyping for *smc6b-3* mutant

(Note: primers used for 454 sequencing were included in "Supporting Methods")