

## Supplementary Materials:

**Table S1: Targets Description.** Targets names described the chromosome number and position. The epigenetic selection of the targets was based on CpG methylation and Nucleosome occupancy data from Zemach et al. 2013 [71], H3K4me3 data was based on Roudier et al. 2011 [36]. Set#1 yellow and blue are highlighting euchromatic and heterochromatic targets embedded within euchromatin respectively. Set #2 yellow and blue highlighting are euchromatic targets on chromosome arms and heterochromatic pericentric targets respectively.

Cas9 DSB site	Cas9 Target Seq. (PAM in red)	Col vs Ler	Gene	Cpg methylation (0.0-1.0)	Nucleosomes occupancy (0 -296)	H3k4me3 (-4.85-5.05)	Heterochromatin/ Euchromatin	%NHEJ
<b>Set#1</b>								
Chr3:1228466	aactgctttgaatgtccata <b>gg</b>	tgg→tag	at3g04560.1	0	1.95	0	Euchromatin	4.4
Chr3:1222177	gctggagaaccgctttaac <b>gg</b>	similar	at3g04530.1	0	7.82	2.77	Euchromatin	19.8
Chr3:1261146	cgcttgatgatgaccactg <b>gg</b>	similar	at3g04640.1	0	74.13-96.53	2.25	Euchromatin	0
Chr3:1352618	atattgttttcatatttt <b>gg</b>	tgg→ttg	—	0	1.02	0	Euchromatin	0
Chr3:1352127	ccaaaaaataacagtcgt	similar	—	0	5.49	0	Euchromatin	0
Chr3:1854159	gtttccgccaccgcctc <b>gg</b>	similar	at3g06130.2	0	27.25	3.27	Euchromatin	11.6
Chr3:1843852	tctcaaatgattgaaggt <b>gg</b>	ggg→agg	at3g06110.3	0	4.3	0	Euchromatin	0
Chr3:1858597	agagttgatctgtgctgtg <b>gg</b>	similar	at3g06140.1	0	13.95	3.04	Euchromatin	73.6
Chr3:4684724	tgactgcaggtgagcttaca <b>gg</b>	similar	at3g14120.3	0.81	2573	-2.15	Heterochromatin	37.7
Chr3:1565357	<b>cct</b> tggaatttctctccaa	cct→ctt	at3g05420.1	1	36.04	-0.78	Heterochromatin	0
Chr3:1559196	<b>ccc</b> gacatttaattgatttt	5 kb del in Ler	at3g05415.1	0.85	138.175	0	Heterochromatin	0
Chr3:4639826	agaagttcagaagtcgcc <b>gg</b>	similar	at3g14010.4	1	65.74	-2.47	Heterochromatin	0.8
<b>Set#2</b>								
Chr3:1231856	ttgttgaggagcgtggtg <b>gg</b>	ggc→ggg	at3g04570.1	0	13.1	1.23	Euchromatin	1.3
Chr3:1265118	<b>cca</b> gatctacgtactatttc	tca→cca	at3g04660.1	0	21.26	0.79	Euchromatin	4.8
Chr3:1797873	<b>cct</b> cacaatgattccagccgtt	tct→cct	at3g05990.1	0	9.42	0.76	Euchromatin	35.8
Chr3:2036584	atcaacgccccagtgctta <b>gg</b>	aga→agg	at3g06540.1	0	20.88	3.1	Euchromatin	6.8
Chr3:4064382	<b>ccc</b> agcagcggttaacaagaa	ggc→ccc	at3g12800.1	0	11.83	2.96	Euchromatin	0.4
Chr5:18158030	gtagcgtatacgaataata <b>gg</b>	gga→ggg	at5g44973.1	0	3.02	-1.2	Euchromatin	9.1
Chr5:19187725	<b>ccc</b> gagcactgggatcctattg	acc→ccc	at5g47250.1	0	6.34	1.825	Euchromatin	0
Chr1:12765381	ccttaaggaccatgtaaggct	tct→cct	at1g34790.1	0.6	30.16	-1.03	Heterochromatin	0
Chr1:13325352	cccattccaacagattagaagg	tcc→ccc	at1g35840.1	0.933	39.82	-2.94	Heterochromatin	0
Chr1:13394390	ccaaccacgtgaacgatcccc	tca→cca	at1g35960.1	0.6875	36.45	-3.4	Heterochromatin	0
Chr1:16855554	agaagactgctctgttca <b>gg</b>	gga→ggg	at1g44510.1	0.833	42	-1.61	Heterochromatin	0
Chr5:13564651	gtgagcattccgtgaggac <b>gg</b>	cga→cgg	at5g35340	0.81	19.5	-0.629	Heterochromatin	13.9
Chr5:15815729	<b>ccct</b> gcctcagaggaagtgttt	tcc→ccc	at5g39500.1	1	19.52	1.8	Heterochromatin	0
Chr5:16450556	<b>cca</b> gagcggactaattagaggtta	tca→cca	at5g41100.2	0.56	22.72	-0.88	Heterochromatin	11.7

**Table S2: Germinally transmitted DNA DSBs events in F2 Arabidopsis plant populations, Set #1: Cold and Hot spots.** DSB signatures documented in thirteen F2 plant populations by whole genome sequencing are summarized in the table.

F1 plant	F2 seed sorting (GFP only/RFP only/ no sorting)	Total F2 plants sequenced	Total Cas9+, gRNA+ plants (% in the population)	Plants with NHEJ signature (% of plants in F2 population, % alleles in average of PacBio and Illumina data)	Plants with visible Induced crossover (% of plants in F2 population)	Plants with visible Induced non-crossover (% of homozygote plants in F2 population)	Homozygote plants at DSB area	Heterochromatin/Euchromatin
Chr3:1228466-#1	No sorting	35	18 (51%)	0 (0%, 0%)	0 (0%)	4 (20%)	20	Euchromatin
Chr3:1228466-#2	RFP only	17	9 (53%)	0 (0%, 0%)	1 (5.88%)	2 (22.22%)	9	Euchromatin
Chr3:1228466-#2	GFP only	10	1 (10%)	0 (0%, 0%)	0 (0%)	0 (0%)	5	Euchromatin
Chr3:1228466-#5	RFP only	6	1 (17%)	0 (0%, 0%)	0 (0%)	3 (60%)	5	Euchromatin
Chr3:1228466-#5	GFP only	4	4 (100%)	0 (0%, 0%)	0 (0%)	0 (0%)	1	Euchromatin
Chr3:1228466-#7	No sorting	34	10 (29%)	0 (0%, 0%)	0 (0%)	4 (22.2%)	18	Euchromatin
Chr3:1854159-#2	No sorting	39	17 (44%)	1 (3%, 0%)	0 (0%)	0 (0%)	19	Euchromatin
Chr3:1854159-#6	No sorting	25	8 (32%)	12 (48%, 50%)	0 (0%)	0 (0%)	10	Euchromatin
Chr3:1854159-#9	GFP only	14	9 (64%)	0 (0%, 48%)	0 (0%)	0 (0%)	4	Euchromatin
Chr3:1854159-#11	GFP only	5	0 (0%)	0 (0%, 0%)	0 (0%)	0 (0%)	3	Euchromatin
Chr3:1854159-#12	GFP only	10	2 (20%)	4 (40%, 50%)	0 (0%)	0 (0%)	3	Euchromatin
Chr3:1854159-#13	GFP only	10	2 (20%)	2 (20%, 47%)	0 (0%)	0 (0%)	3	Euchromatin
Chr3:4639826-#4	No sorting	27	4 (15%)	0 (0%, 0%)	0 (0%)	2 (12.5%)	16	Heterochromatin
Total		236	85 (36%)	19	1 (0.42%)	15 (12.9%)	115	

**Table S3: Germinal transmitted DNA DSBs events in F2 Arabidopsis plant populations. Set#2: Euchromatic and Heterochromatic plants.** DSB signatures documented in eight F2 plant populations by whole genome sequencing are summarized in the table.

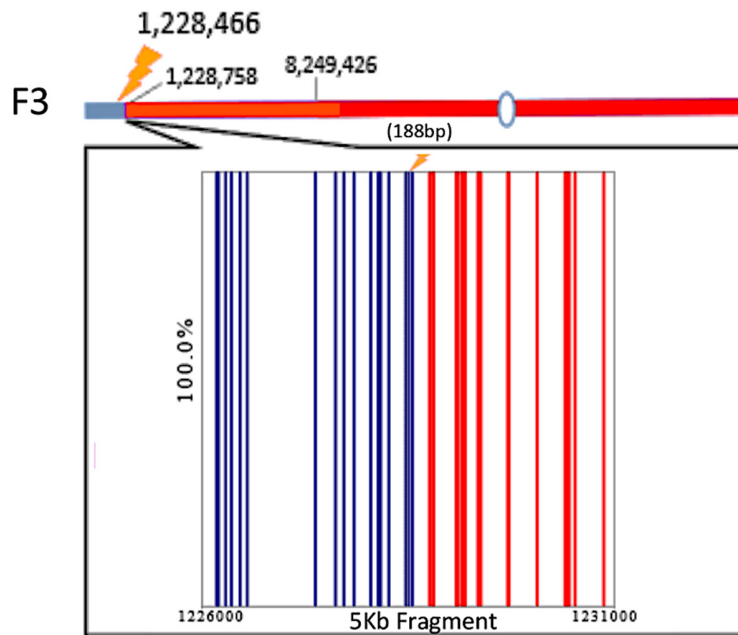
F1 plant	Total F2 plants sequenced	Plants with NHEJ signature, based on F2 WGS (% of plants in F2 population)	Plants with visible Induced non-crossover (% of plants in F2)	Heterochromatin/Euchromatin
Chr5:13564651-#1A	13	2 (15.4%)	0 (0%)	Heterochromatin
Chr5:13564651-#1C	19	4 (21.1%)	0 (0%)	Heterochromatin
Chr5:13564651-#1D	45	11 (24.4%)	1 (2.2%)	Heterochromatin
Chr3:1797873-#4A	20	4 (20%)	0 (0%)	Euchromatin
Chr3:1797873-#4E	19	8 (42.1%)	1 (5.2%)	Euchromatin
Chr3:1797873-#4F	19	3 (15.8%)	0 (0%)	Euchromatin
Chr3:1797873-#5E	46	2 (4.3%)	0 (0%)	Euchromatin
Chr3:1797873-#5F	36	2 (5.6%)	0 (0%)	Euchromatin
Total	217	36 (16.6%)	2 (1%)	

Table S4: List of Primers for NHEJ experiments.

Primers for NHEJ sequencing-Set#1	
4639826_HT_F	NNNNNNAATCTGAAAGCTCTTCGGG
4639826_HT_R	NNNNNNCGATTGGAGATTGAGGTGGT
4684724_HT_F	NNNNNNAACAAGATGTTACAATGAAAGC/
4684724_HT_R	NNNNNNTCGAGATACATATGTCTGCAATGTT
1858597_HT_F	NNNNNNCTGAGGGTGATAATGGTAATGG
1858597_HT_R	NNNNNNTTGCTTTCTGTCTTGTCTCTG
1565357_HT_F	NNNNNNTGGCGGTGATAACAAGAGTG
1565357_HT_R	NNNNNNTGAAAAGGCTTCTACCGTGTT
1559196_HT_F	NNNNNNGTTAAAACCGCGCACATTC
1559196_HT_R	NNNNNNTGAGTTCCCCACAATCTCCT
1228466_HT_F	NNNNNNAAGCTGTCTCAGTGAAGAGGT
1228466_HT_R	NNNNNNCCTCGAAGCTTGCTATACGG
1222177_HT_F	NNNNNNCGCTGGTGTAGTGATATACAG
1222177_HT_R	NNNNNNTTCCAACGTTTCATCATCCA
1843852_HT_F	NNNNNNTTGGCTCCTCTTATCCTGA
1843852_HT_R	NNNNNNCCATGAAGCAATGAACCAA
1261146_HT_F	NNNNNNAACCAACCTAAACCGGCTA
1261146_HT_R	NNNNNNTCGAAGCCAATTTACCGAAA
1352127_HT_F	NNNNNTTACATATTTTAATTAGATTGGTAGAACAA
1352127_HT_R	NNNNNNTGCCTCGTTGTCACTTAACG
1352618_HT_F	NNNNNNAGTCTTTTGGCAATCACGTT
1352618_HT_R	NNNNNNTTGGCCTTTTCTCTCACAC
1854159_HT_F	NNNNNNCGTTTTTCTGGCCTTCTTG
1854159_HT_R	NNNNNNTCAGGTGTTTTACGACCAA
Primers for NHEJ sequencing-Set#2	
il3_1856F	TAACCGTTCTTTCTGCCAGG
il1856R	CCGTCGCCTCCACCAGCAC
il5118F	AGTCACCGCCGAAATCCATAG
il5118R	ATCATAGCTTTAGTATTGTTTTGAC
il7873F	GATTAGCTTATGAGTCTCTGTTT
il7873R	GGGAGTTTCAGGTTTCGCTAGC
il6584F	TACATAGATACATCCTTTGACAGC
il6584R	CTCAAGACCAAGAGGGGATAG
il4382F	ATGAGAAAACGAAAAGGAACCTG
il4382R	TGCTCTTCGATCTCTTGAATC
il8030F	CTCTGATCTGTAGTTTATTCAAG
il8030R	ATCATCCAACATTCAAATACAGAC
il7725F	TGTCCTCATTTGCCCTCATAAC
il7725R	GGTTGAATCGTCTAAAGATGCAG
il5352F	AGAAATCTCAACAAATCTCCAACC
il5352R	TCACAGTCTTGATTCTTCTAGC
il4390F	AGACCAAGCTGAAATAATGAAGG
il4390R	ACAGAGCGTTATCATTATGGGA
il5729F	TTACTCTGAAACTGTGAATGTGG
il5729R	CATCTAATCAACTCATGCATTGTG
il0556F	CGCCTCTCCGCTGACTCCA
il0556R	CTTCTATTTACTTATGGGTCTC
il5381F	TTGACCATCCTCGTTCCAAGC
il5381R	AGACAGATGAATTAGAAGCCTGC
il5554F	CAGGAGAAGACGAGGAGGATG
il5554R	TCAGCGTCTTCGTGTTTGG
il4651F	TCGAAAGGTGATGCAAAGATAGAG
il4651R	ATCTACACATTCACAATCACGCAC

**Table S5: List of Primers for PacBio® experiments.**

Primers for PacBio sequencing	
pb_1222177_f	TCCATCGTACCGTCTTCCTC
pb_1222177_r	CGATTGCGGATTTCGACCTA
pb_1228466_f	CTACCAACCGTTCCGACCAC
pb_1228466_r	GGAACCAGCAGGACGTCCAC
pb_1261146_f	TTTGTTCTGGATAAAAAGACAATTC
pb_1261146_r	TCCACACCTTGAAGCATCCCG
pb_1261146_f	ACAGACCAAAATCAGACCCTTC
pb_1261146_r	TTATTGTTTTTGTCTTCTCTGACA
pb_1565357_f	CAAACCATGGGAATATTACAC
pb_1565357_r	CCTTCTACCTACTTCATTCATAG
pb_1843852_f	ATCCTCCTGAAATCGGTCTAAC
pb_1843852_r	TATCTGGGATCCTATTAAATAAGC
pb_1854159_f	GTAATAATTGCATGGTTGTACGT
pb_1854159_r	GAGAGAAAAAGAGGAGAAGTTGA
pb_1858597_f	ATCTTTGGCGCATTACTGTTAC
pb_1858597_r	TGTAGAGTTGACTTTTGAGTAGCAT
pb_4639826_f	CCCGTTCCGAATTTGTCAGGA
pb_4639826_r	TAAAATAGAAAGTAAAGATTCAGAC
pb_4684724_f	TGGTGGTAGGGGTCTCGAAAT
pb_4684724_r	CTATCTATGTTAGGAGAAATCGC

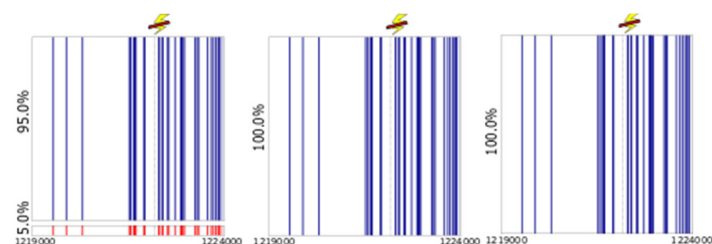


**Figure S1: Confirmation of the crossover site with PacBio® sequencing.**

A 5Kb fragment flanking the DSB site was amplified from the homozygous F3 offspring Chr3:1228466\_RFP only plant# 14 and sequenced with PacBio® Sequel system. The allelic distribution shown in the square. Red vertical lines represent Columbia SNPs, blue vertical lines represent Landsberg SNPs. The height of the rectangle represents the frequency of this consensus sequence/allele (percentage of subreads that aligned to calculate the consensus sequence); >2000 single molecules were sequenced. The distance between the induced DNA DSB site (yellow lightning) and the crossover site was 188bp.

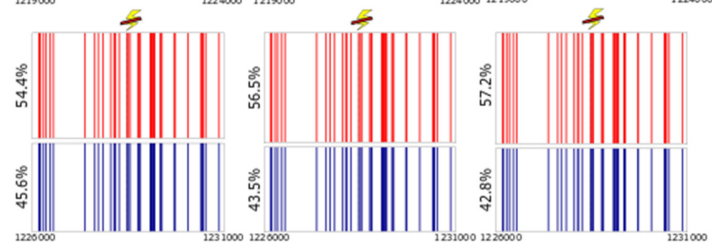
### Chr3:1222177

GCTGAGAACGCGCTTTAACGG



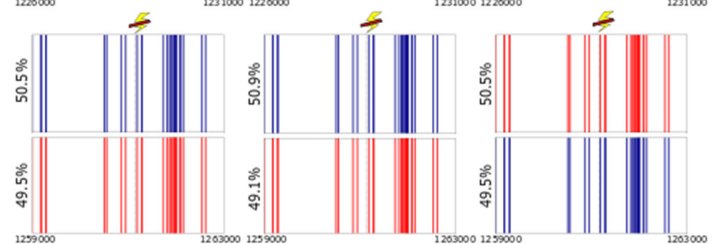
### Chr3:1228466

AACTGCTTTGAATGTCCATAGG/  
AACTGCTTTGAATGTCCATAGG



### Chr3:1261146

CGCTTGAAATGATGACCACTGCG

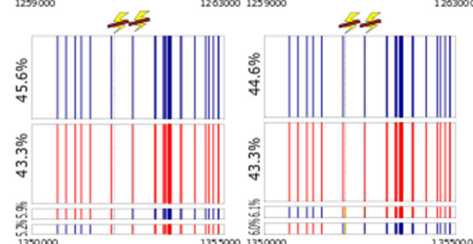


### Chr3:1352127

ACGACTGATTTTTTTTTTTTGG

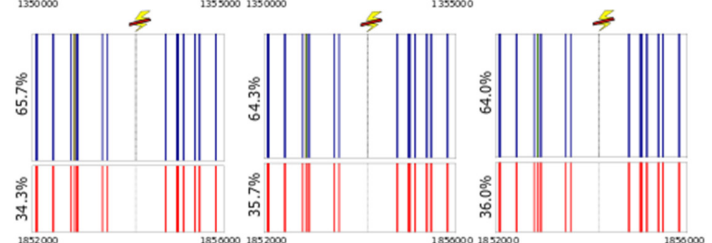
### Chr3:1352618

ATATTGTTTTTTCATATTTTGG/  
ATATTGTTTTTTCATATTTTGG



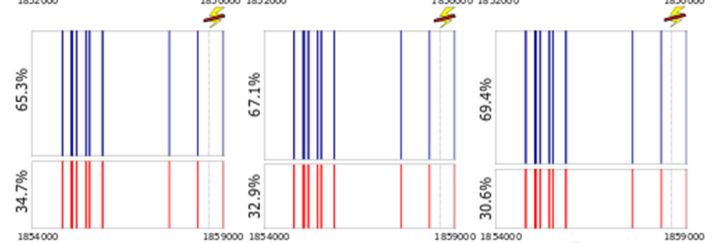
### Chr3:1854159

TTCCGCCACCAACGCCCTCCGG



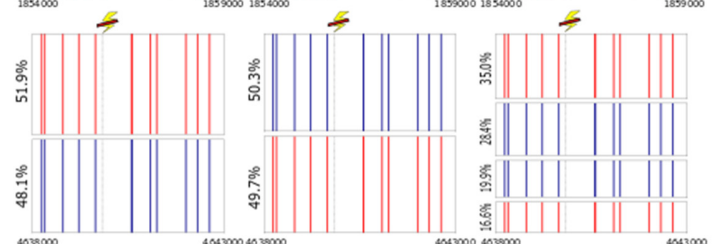
### Chr3:1858597

AGTTGATCTGTGGCTGTGGCGG



### Chr3:4639826

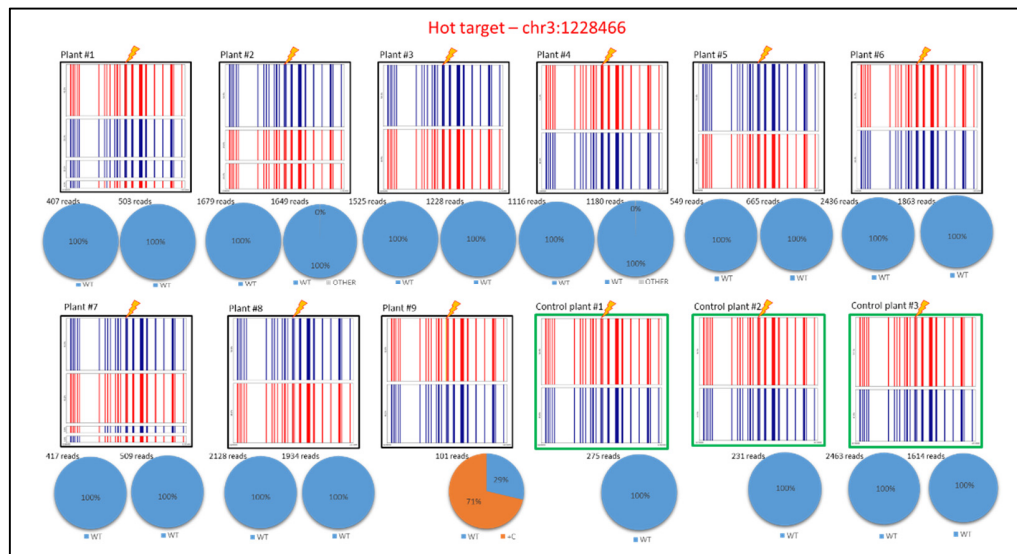
AGAAGTTCAAGAAATGCCCCAGG



**Figure S2: Allelic distribution of Ler and Col alleles in Control plants (with No DSB induction) as shown by long-range sequencing.** Distribution of parental alleles in 3 control plants of F1 Ler x Col tester line at different targets using PacBio® sequencing (target# and sequences are shown on the left of each row of squares PAM sequences are green). Within every square we show the allelic distribution for one plant in a ~5Kb window flanking the target. The red and blue lines correspond to the Columbia and Landsberg SNPs respectively, dashed black line with lightning represent the DNA DSB site (the sites are signed by X because there is no DSB induction in the control plants). In each square, each rectangle represents one consensus sequence. In some cases, (for example in cold target – chr3:4639826), there are more than two consensus sequences of each allele, differing in indels that are not located at the DSB point and correspond to neither Col/Ler SNPs. The height of the rectangle represents the frequency of this consensus sequence/allele. In case of the presence of a SNP in the target or PAM site (e.g., target chr3:1352618) the Columbia allele sequence is before the slash and Landsberg allele is after the slash.

Targets Chr3:1261146, Chr3:1228466 and Chr3:4639826 show balanced (~1:1) allelic distribution of Columbia vs. Landsberg (~ 50% of amplification of the Col allele and ~50% of the Ler allele). For targets 1854159 and 1858597 the allelic distribution is biased toward the Ler allele with 31-36% of molecules coming from Col allele and 64-71% coming from Ler allele, possibly due to differences in PCR efficiencies for each allele. Target 1222177 also shows biased allelic distribution, with 0-5% of amplification of the Col allele and 95-100% of the Ler allele. For all the above targets, in the control plants without DSB induction no recombinant molecules were obtained among the amplification products. For targets 1352127 & 1352618 (shown in the same sequence window due to targets proximity), the allelic distribution is balanced, both control plants showed alleles with recombinant consensus sequences. For this target we also observed a deletion of -A at the DNA DSB site 1352127 in control plant #2. The deletion was observed only in the recombinant alleles and thus may reflect authentic recombination, or might be caused by polymerase slippage during poly-A PCR amplification or might be due to PacBio®'s tendency for generating indels at homopolymers [69]. In conclusion, the method described here of PacBio® sequencing and analysis is appropriate for allele distribution and crossover rate only under PCR and analysis conditions that have been tested and shown to give relatively balanced allelic distribution for control plants and no crossover or indels patterns. Plants Chr3:1261146, Chr3:1228466, Chr3:1854159, Chr3:1858597 and Chr3:4639823, where selected for the DSB experiment.





**Figure S3: Allelic distribution of the F1 plants (Ler WT x Col (Ubi:Cas9 x U6-26:1228466 gRNA)) and control plants-** (same legend for Supplementary Figures 8-12). Every square represents the allelic distribution for one plant in ~5Kb window flanking the DNA double strand break site as measured using PacBio® sequencing. F1 plants are in black squares, Control F1 Ler x Columbia tester plants in green squares. In each square, each rectangle represents one consensus sequence calculated by LAA2 analysis. Red vertical lines represent Columbia SNPs, blue vertical lines represent Landsberg SNPs, green vertical lines represent SNPs that are different from both Columbia and Landsberg SNPs, yellow line represents deletion / insertion at the break site and lightning represents the DNA DSB site. The height of the rectangle represents the frequency of this consensus sequence/allele (percentage of subreads that aligned to calculate the consensus sequence) presented always from higher to lower percentage. Pie charts under each square represent the total and distribution of Illumina-Hiseq reads for a single plant. The right pie under each square was analyzed with CRISPResso and the left analyzed with Cas-analyzer.



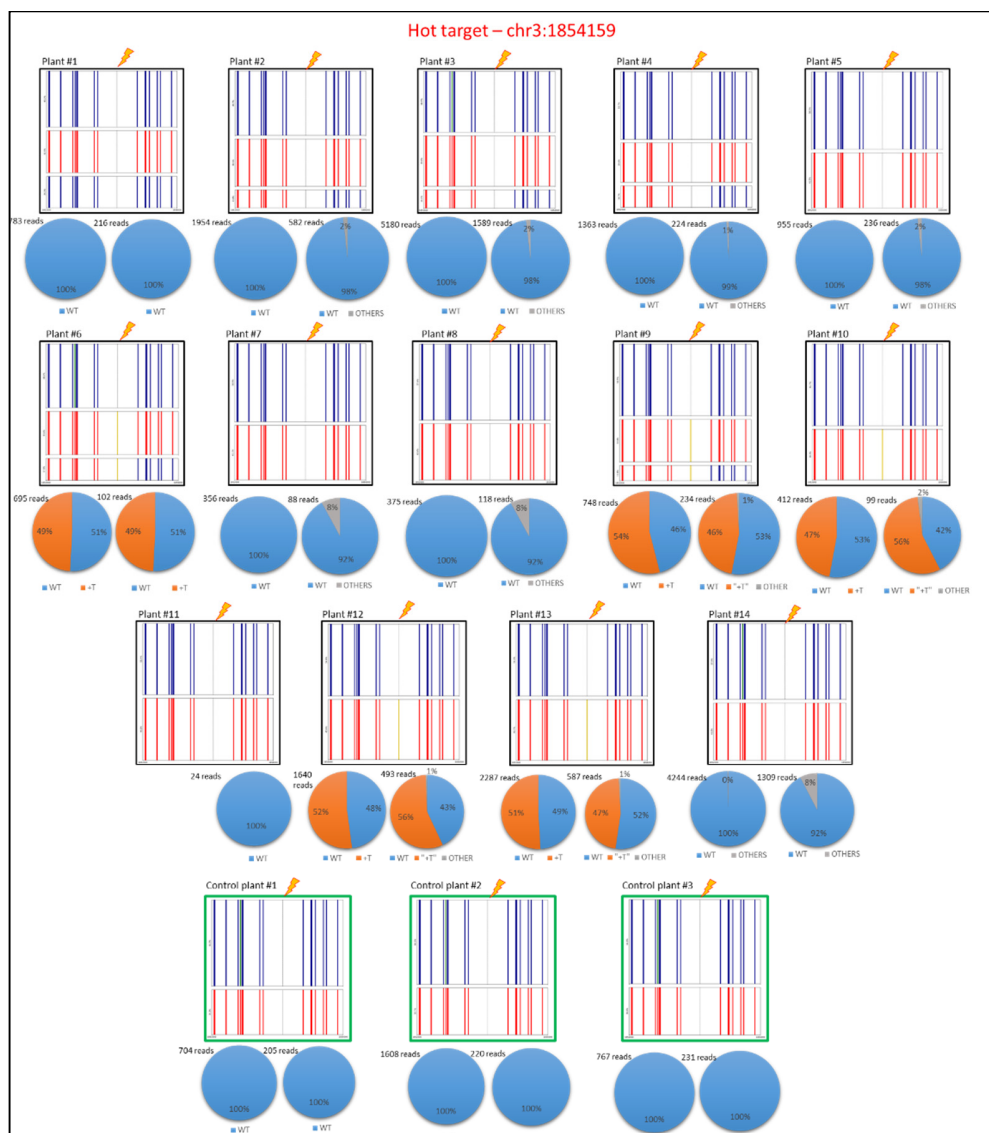


Figure S4: Allelic distribution of the F2 plants (Ler x Ubi:Cas9 and U6-26:1854159 gRNA) and control plants.

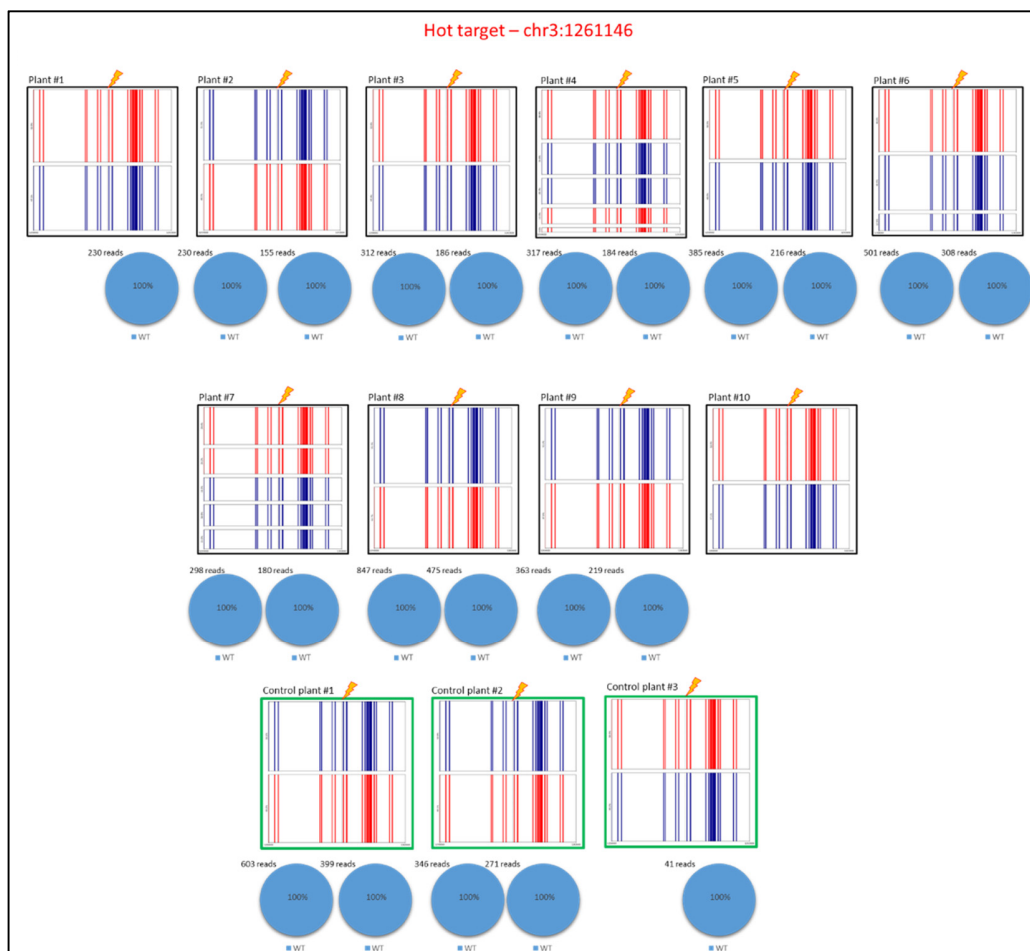


Figure S5: Allelic distribution of the F2 plants (Ler x Ubi:Cas9 and U6-26:1261146 gRNA) and control plants.

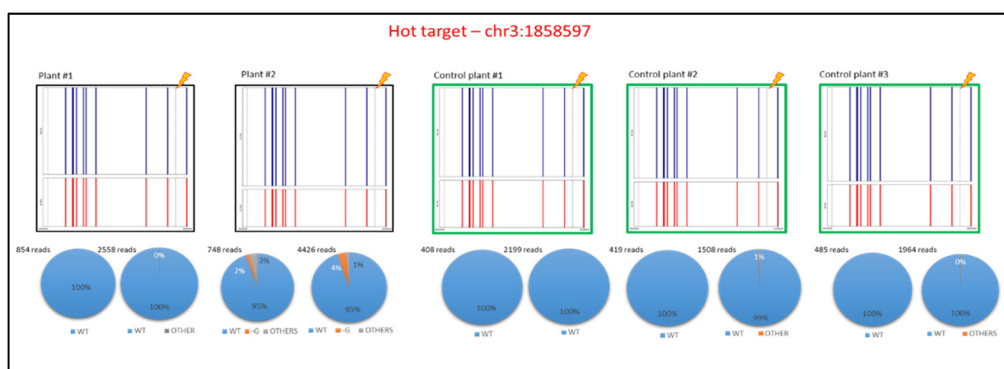


Figure S6: Allelic distribution of the F2 plants (Ler x Ubi:Cas9 and U6-26:1858597 gRNA) and control plants.