

Heritable transgene-free genome editing in plants by grafting of wild-type shoots to transgenic donor rootstocks

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Supplementary Information

Heritable transgene-free genome editing in plants by grafting of wild-type shoots to transgenic donor rootstock

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Supplementary Table 1 | Number of *NIA1* Genome Edits Detected in the Transgene-free Offspring.

Note that each tested pool consisted of genomes isolated from ~70 to ~100 seedlings from a grafted parent plant indicated by the line #. Presence of edits (deletions) was confirmed by additional PCR assays and by Sanger sequencing of positive PCR amplicons (see Fig. 4c and Extended Data Fig. 3).

Graft Combination (scion/rootstock)	Grafted line # from which seedlings originated	Pools of seedlings tested	Edit (deletion) detected in a pool	Total number of seedlings tested	Minimal editing frequency per 1000 seedlings
Col-0 / <i>Cas9</i> x <i>gNIA1</i>	1	2	0	198	0
	2	1	0	255	0
	3	1	0	119	0
	4	2	0	119	0
	5	2	0	255	0
	6	1	0	153	0
	7	1	0	323	0
	8	1	0	221	0
	9	2	0	220	0
	10	1	0	238	0
	11	2	0	178	0
Total	11	16	0	2280	0
Col-0 / <i>Cas9-TLS1</i> x <i>gNIA1-TLS1</i>	1	2	1	143	7.0
	2	2	2	154	13.0
	3	3	1	242	4.1
	4	3	3	199	15.1
	5	2	0	110	0
	6	3	1	220	4.6
	7	3	2	264	7.6
	8	2	1	132	7.6
	9	2	1	198	5.1
	10	2	2	198	10.1
	11	1	1	77	13.0
	12	1	0	308	0

	13	2	0	242	0
	14	4	2	286	7.0
	15	1	0	198	0
Total	15	33	17	2971	5.7
Col-0 / <i>Cas9-TLS2</i> x <i>gNIA1-TLS2</i>	1	2	1	352	2.8
	2	2	0	270	0
	3	3	0	299	0
	4	3	2	396	5.1
	5	3	1	330	3.0
	6	5	0	462	0
	7	4	1	440	2.3
	8	5	3	385	7.8
	9	6	5	529	9.5
	10	3	3	484	6.2
	11	2	2	294	6.8
	12	5	3	360	8.3
	13	5	3	252	11.9
	14	4	3	360	8.3
	15	4	2	360	5.6
	16	4	3	378	7.9
	17	3	3	410	7.3
	18	5	4	504	7.9
	19	4	3	418	7.2
	20	2	0	520	0
	21	2	2	526	3.8
	22	3	0	437	0
Total	22	79	44	8766	5.0

Supplementary Table 2 | Number of *Venus* Genome Edits Detected in the Transgene-free Offspring.

Note that each tested pool consisted of genomes isolated from ~ 40 seedlings from a grafted parent plant indicated by the line #. Presence of edits (deletions) was confirmed by additional PCR assays and by Sanger sequencing of positive PCR amplicons (see Extended Data Fig. 4d and Extended Data Fig. 5).

Graft Combination (scion/rootstock)	Grafted line # from which seedlings originated	Pools of seedlings tested	Edit (deletion) detected in a pool	Total number of seedlings tested	Minimal editing frequency per 1000 seedlings
Col-0 / <i>Cas9</i> x <i>gVenus</i>	1	4	0	167	0
	2	4	0	138	0
	3	4	0	156	0
	4	4	0	145	0
	5	3	0	116	0
	6	4	0	157	0
	7	3	0	125	0
	8	4	0	177	0
	9	4	0	184	0
	10	4	0	158	0
	11	3	0	123	0
	12	4	0	139	0
	13	4	0	173	0
	14	4	0	164	0
	15	4	0	137	0
	16	4	0	173	0
	17	4	0	166	0
	18	4	0	184	0
	19	4	0	156	0
Total	19	76	0	2938	0
Col-0 / <i>Cas9-TLS1</i> x <i>gVenus-TLS1</i>	1	6	4	234	17.1
	2	5	3	205	14.6
	3	5	4	212	18.9
	4	6	6	243	24.7
	5	6	3	233	12.9

	6	5	4	198	20.2
	7	5	3	203	14.8
	8	5	2	189	10.6
	9	5	4	198	20.2
	10	6	0	226	0
	11	6	4	246	16.3
	12	5	2	208	9.6
	13	5	3	202	14.9
	14	6	1	226	4.4
	15	6	1	238	4.2
	16	5	4	199	20.1
	17	5	3	188	16.0
	18	6	4	247	16.2
	19	6	6	238	25.2
	20	6	4	234	17.1
Total	20	110	65	4367	14.9
Col-0 / Cas9-TLS2 x gVenus-TLS2	1	5	2	205	9.8
	2	5	4	212	18.9
	3	6	4	224	17.9
	4	3	2	116	17.2
	5	5	5	185	27.0
	6	5	3	193	15.5
	7	4	2	147	13.6
	8	5	3	237	12.7
	9	6	4	247	16.2
	10	5	4	233	17.2
	11	5	2	199	10.1
	12	6	4	226	17.7
	13	6	2	244	8.2
	14	7	0	276	0
	15	6	5	243	20.6
	16	6	5	238	21.0
	17	7	5	277	18.1
	18	7	7	268	26.1
Total	18	99	63	3970	15.9

Supplementary Table 3 | Primer Sequences Used in the Study.

Primer	Sequence	Expected Product Size (bp)
<i>rbcsT</i> _FP	GCGGGTCTCGTCGAGAGAGCTTTCGTTTCGTATCATCGGTTTCGAC	
<i>rbcsT</i> _RP	GCGGGTCTCCTCGACGTTGTCAATCAATTGGCAAGTCATAAAATGCATTAA AAAATATTTTCATA	665
<i>Cas9</i> FP	TCGATGAGACCATGGATTACAAGGACCACGACGGGATTACAAGG	
<i>Cas9</i> RP	GCGTCGATGAGACCTCACTTCTTCTTCTCGCCTGC	4276
<i>Cas9-tRNA^{Met}</i> RP	GCGGGTCTCCTCGATATCAGAGCCAGGTTTCGATCCTGGGACCTGTGGGTT ATGGGCCCACCACGTTCCGCTGCGCCACTCTGATAATTCACCTTCTTCTCT TCGCCTGCCCCGCCTTCTT	4351
<i>Cas9-tRNA^{Met} ΔDT</i> RP	GCGGGTCTCCTCGATATCAGAGCGGACCTGTGGGTTATGGGCCCACCACCA CTCTGATAATTCACCTTCTTCTTCTCGCCTGCCCCGCCTTCTT	4323
<i>NIA1</i> Fragment2 FP /	CGCGGTCTCCATTGACAACACTGCTGACTCTGCAGTTTTAGAGCTAGAAAT	<i>gRNA</i> Null: 621
<i>Venus</i> Fragment2 FP	AGC	<i>gRNA tRNAMet</i> : 704
<i>NIA1</i> Fragment2 RP /	GCGGGTCTCTAAACCCCTTCACGTTGTAACCCATCAATCTCTTAGTCGACTCT	<i>gRNA tRNAMetDT</i> : 676
<i>Venus</i> Fragment2 RP	ACC	
<i>NIA1</i> Edit Detection FP	AGGAGCGTCAGCTTGAGATT	Wild-type <i>NIA1</i> : 1469
<i>NIA1</i> Edit Detection RP	GTTGTGACATGAGTCTGACATGC	Edited <i>NIA1</i> : ~430
<i>Cas9</i> RT-FP	AAGTCCAAGAAGCTCAAGAGC	
<i>Cas9</i> RT-RP	GAAGTTCACATACTTGGACGGC	249
<i>gRNA</i> RT-FP	GTTTTAGAGCTAGAAATAGCAAG	
<i>gRNA</i> RT-RP	GCACCGACTCGGTGCCAC	76
<i>Hyg</i> RT-FP	ATGAAAAAGCCTGAACTCACC	
<i>Hyg</i> RT-RP	GCTGAAAGCACGAGATTCTTC	120
<i>Kan</i> RT-FP	AGAGGCTATTCGGCTATGACTGG	
<i>Kan</i> RT-RP	ATCGCCATGGGTCACGACGAGAT	450
<i>ACTIN2</i> -FP	GGAAGGATCTGTACGGTAAC	
<i>ACTIN2</i> -RP	TGTGAACGATTCTGGACCT	245
<i>UBQ10 qRT-FP</i>	CACACTTCACTTGGTCTTGCGT	
<i>UBQ10 qRT-RP</i>	TAGTCTTTCGGTGAGAGTCTTCA	71
<i>Cas9 qRT-FP</i>	AAGTCCAAGAAGCTCAAGAGC	
<i>Cas9 qRT-RP</i>	GAAGTTCACATACTTGGACGGC	249
<i>Venus</i> Edit Detection FP	TATCCTTCGCAAGACCCTTCTCTC	Wild-type <i>Venus</i> : 1719
<i>Venus</i> Edit Detection RP	CGAGGTCGTCCGTCCTCACTC	Edited <i>Venus</i> : ~250

FP: Forward primer; RP: Reverse primer

Supplementary Table 4 | Binary *Cas9* and *gNIA1* Plasmids Created in the Study.

Plasmid	Function	Resistance Bacteria	Resistance Plants
pMDC7_ <i>Cas9</i>	Estradiol-inducible expression of <i>Cas9</i>	Spectinomycin	Hygromycin
pMDC7_ <i>Cas9-tRNA^{Met}</i>	Estradiol-inducible expression of <i>Cas9-TLS1</i>	Spectinomycin	Hygromycin
pMDC7_ <i>Cas9-tRNA^{Met} ΔDT</i>	Estradiol-inducible expression of <i>Cas9-TLS2</i>	Spectinomycin	Hygromycin
pMDC100_ <i>gNIA1</i>	Constitutive expression of 2 <i>gRNAs</i> targeting <i>NIA1</i>	Kanamycin	Kanamycin
pMDC100_ <i>gNIA1-tRNA^{Met}</i>	Constitutive expression of 2 <i>gRNA-TLS1</i> targeting <i>NIA1</i>	Kanamycin	Kanamycin
pMDC100_ <i>gNIA1-tRNA^{Met} ΔDT</i>	Constitutive expression of 2 <i>gRNA-TLS2</i> targeting <i>NIA1</i>	Kanamycin	Kanamycin
pMDC100_ <i>gVenus</i>	Constitutive expression of 2 <i>gRNAs</i> targeting <i>Venus</i>	Kanamycin	Kanamycin
pMDC100_ <i>gVenus-tRNA^{Met}</i>	Constitutive expression of 2 <i>gRNA-TLS1</i> targeting <i>Venus</i>	Kanamycin	Kanamycin
pMDC100_ <i>gVenus-tRNA^{Met} ΔDT</i>	Constitutive expression of 2 <i>gRNA-TLS2</i> targeting <i>Venus</i>	Kanamycin	Kanamycin
<i>pRI909 35S::H2B-Venus_Basta</i>			

Supplementary Table 5 | Nutrient Composition of the MS Growth Medium.

Component	+NH ₄ Medium	-NH ₄ Medium
NH ₄ NO ₃	2.5mM	0
KNO ₃	2.5mM	5 mM
MgSO ₄	2 mM	2 mM
CaCl ₂	1 mM	1 mM
MES (pH 5.8)	1.25 mM	1.25 mM
H ₃ BO ₃	50 µM	50 µM
MnSO ₄	12 µM	12 µM
ZnCl ₂	1 µM	1 µM
CuSO ₄	1 µM	1 µM
Na ₂ MoO ₄	0.2 µM	0.2 µM
Sucrose	0.5%	0.5%
Micro-agar	0.5%	0.5%

Supplementary Data 1

a, Sequences of the *Cas9*, *Cas9-tRNA^{Met}* (TLS1) and *Cas9-tRNA^{Met} Δ DT* (TLS2) constructs in pMDC binary vector used in the study.

Light Grey colour background indicates *pG10-90* promoter; Light blue colour background indicates *XVE* gene sequence; Green colour background indicates *Hygromycin* resistance gene sequence. Dark Grey colour background indicates *LexA-35S* minimal promoter. Yellow colour background indicates *Cas9* gene sequence. Pink colour background indicates *tRNA^{Met}* (*TLS1*) or *tRNA^{Met} ΔDT* (*TLS2*) sequences fused to *Cas9*.

1. pMDC_ *Cas9*

GTTATACCCGCCAATATATCTCTGTCAAACACTGATAGTTTAAACTGAAGGCGGGAAACGACAATCTGATCCAAAGCTCAAGCTAAGCTTGCATGCCTGCAGGATA
 TCGTGGATCCAAAGCTTGGCAGCTGCCCGCAGTGGCCGACCTGCCCGCAGCTGCCCTAGAGAGTCCATCTCCACTGACGTAAAGGATGACGCCAATCCCA
 CTATCCTTCGCAAGACCCCTTCTCTATATAAAGAAAGTTCATTTCATTTGGAGAGGACACCTGGGATCCCCAATCCCGGGCGGAATGAAGCGCTTAACGGCCA
 GGCAACAAGAGGTTGTTTGATCTCATCCGTGATCACAATCAGCCAGACAGGTATGCCGCCGACGCGTGCGGAATCGCGCAGCGTTTGGGGTTCCGTTCCCAAA
 CGCGGCTGAAGAACTCTGAAGCGGCTGGCAGCGCAAGAGCGCTTATTGAAATGTTTTCGGCGCATCAGCGGGGATTCGCTGTTTGCAGGAAGAGGAAGAGGG
 TTGCGCGCTGGTAGTCTGTGTGCTGCGGTGAACCGTGCAGCGCCCCCGCCACAGTATGTCAGCTGGGGGACGAGCTCCACTAGACGGCGAGACGCTGGCA
 TGGCCGACGCGCAGCGCTAGACGATTTTCGATCTGGACATGTTGGGGGACGGGGAATCCCGGGTCCGGGATTTACCCCCACGACCTCCGCGCCCTACGCGCG
 TCTGATATGGCCGCACTTCGAGTTTTCGAGCAGATGTTTACCGATGCCCTTGGAAATGACGAGTACGGTGGGATCCGCTCTGCTGGAGACATGAGAGCTGCCAAC
 CTTTGGCCAAAGCCGCTCATGATCAAACGCTTAAAGAAGAACAGCTGGCTTGTCCCTGACGCGCAGCATGAGTGCATGCGCTTGTGGATGCTGAGCC
 CACATACCTTATTCGAGATGATCTCAGACACCTTCAGTGAAGCTTCGATGATGGCTTACTGACCAACTGGCAGCAGGAGGAGTGGTTACATGATCAZ
 CTGGGCGAAGAGGGTGCAGGCTTTGTGGATTTGACCCTCACTGATCAGGTCACCTTCTTGAATGTGCCTGGCTAGAGATCCTGATGATTTGGTCTCGTCTGG
 CGCTCCATGGAGGACCCGAGTGAAGCTACTGTTTGTCTCTAATTTGCTTTCGACAGGAACAGGGAAATGTGATAGGGCATGGTGGAGATTTTCGACATGC
 TGCTGGCTACATCATCTCGGTTCCGATGATGAATCTGCAGGAGAGGAGTTTGTGTGCTCAATCTATTATTTTGGCTTAATCTGGAGGTACACATTTCT
 GTCCAGCACCCCTGAAGTCTCTGGAAGAGAAGGACCATATCCACGAGTCTGTGGACAAGATCAGACACTTTGATCCACTGATGCCAAAGCAGGCTTGACC
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 AGAAGCTGTGTGCCCTCTATGACTGCTGCTGGAGATGCTGGAGGCCACCGCTACATCGGCCCATAGCGCTGGAGGGGATCCGTGGAGGAGACGGACCA
 AAGCCACTTGGCCACTGCGGGCTCTACTTTCATCGCATTCCTTGCAAAAGTATTACATCACGGGGAGGCGAGAGGGTTTCCCTGCCACAGTCTGAGAGCTCCCT
 GCGGAATCCCGAGAGATGTTAGCTGAATATCATCATCAATCAGATACCAAATATCAAATGGAATATCAAAGAGCTCTGTTTTCATCAAAGATGACTCGACC
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 CGCTATCGAACTGTGAAATGGAAATGGATGGAGAAGAGTTAATGAATGATATGGTCTCTTTGTTTCATTCTCAAATTAATATTTATTTGTTTTTCTCTATTG
 TTTGTGTTGTTGAAATTTGAAATTAAGAGATGCAACAACATTTGTTTGTGATGAAATATGTGTCAACTGCGTGCCCTTAATGACCGAAGTTAATATGAGGATA
 AAACATCCCAAGCAAGCTTGAAACTGAAGCGGGAACGACAACTCTGATCATGACGGAGAAATTAAGGAGTCACTTATGCCCCCGCGCATGACGCGGGA
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110 CATCTCGGGGAGCTGCACGCTATCCTGAGGAGGCAGGAGGACTTCTACCCTTTCTCTCAAGGATAACCGCGAGAAGATCGAGAAGATTCTGACTTTCAGGATCC
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CAAGGGCGCTAGCGCTCAGTCGTTTCATCGAGAGGATGACGAATTTCGACAAGAACCTGCCAATGAGAAGGTGCTCCCTAAGCAC'TCGC'FCTGTACGAGTAC
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120 GGTACACCGGCTGGGGAGGCTGAGCAGGAAGCTCATCAACGGCATTTCGGGACAAGCAGTCCGGGAAGACGATCCTCGACTTCTGAAGAGCGATGGCTTCGC
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125 GTCCCAGATCCTCAAGGAGCACCCGGTGGAGAACACGAGCTGCAGAATGAGAAGCTCTACCTGTACTACCTCCAGAATGGCCGCGATATGTATGTGGACCAG
GAGCTGGATATTAACAGGCTCAGCGATTACGACGTCGATCATATCGTTCACAGTCATTCTGAAGGATGACTCCATTGACAACAAGGTCCTCACCAGGTCGG
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130 CCGACTTCAGGAAGGATTTCAGGTTCTACAGGTTTCGGGAGATCAACAATTACCACCATGCCCATGACCGGTACCTGAACGCGGTGGTCGGCAGAGCTTGAT
CAAGAAGTACCCAAAGCTCGAGAGCGAGTTTCGTGTACGGGGACTACAAGGTTTACGATGTGAGGAAGATGATCGCCAAGTCGGAGCAGGAGATTGGCAAGGCT
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135 GCGAGACGGGGGAGATCGTGTGGGACAAGGCGAGGATTTCGCGACCGCTCAGGAAGGTTTCTTCCATGCCACAAGTGAATATCGTCAAGAAGACAGAGGTCCA
GACTGGCGGGTCTCTAAGGAGTCAATTCTGCCTAAGCGGAACAGCGACAAGCTCATCGCCCGCAAGAAGGACTGGGATCCGAAGAAGTACGGCGGGTTCGAC
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140 AGCGCTCCAGCTTCGAGAAGAACCAGATCGATTTCCTGGAGGCGAAGGGCTGAAGAAGGACCTGATCATTAAAGCTCCCAAGTACTCCTACTCTT
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145 GAATATCTTACCTCTCTTCACTTACCACTCGGCCCTCGCTGCTTTCAGTACTTTCACACAACCTATCGATCGCAAGGATACAGGACATAAGGAG
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AGGCGGGGCGAGGCGAAGAAGAAGTGA

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