

Supplementary Table 1. Putative off-targets found for the sgRNA targeting the four *CiGAS* genes in chicory. The start and end point within the scaffold or contig, on which each off-target has been identified on is given. The annotation of possible genes by gene prediction algorithm using RNA-Seq Data from chicory and annotated genomes of related species is shown together with homologues genes. The PAM is marked in italic and mismatches between off-target side and sgRNA are shown in bold and underlined. Bold and italic off-target names are of special interest due to their annotation or low number of mismatches.

		sequence	mm	superscaffold/ contig	Start/End	position	GENE ID of related genes
WT	3'-	CCTTACATAAGGGATAGAGTACC	- 5'	-	-	-	
OT1	5'-	CCCTACATAAGGAATCGGGTGCC	- 3'	4	100108 100190	3013006-3013028 1090289-1090311	intergenic
OT2	3'-	CCTTATATAAAAGGATAGAGTACC	- 5'	4	100085	5062124-5062146	intergenic
OT3	5'-	CCATACATAAATGGATAACTACA	- 3'	2	100235 100048	937474-937496 3609483-3609505	Exon Lettuce_LSAT_1_V5_GN_7_8140.1.V5_R4 (F-box associated domain (FBA_3))
OT4	3'-	CCATACATAAGGAATTTGAAGACC	- 5'	4	100018	3131294-3131316	intergenic
OT5	3'-	CCCAACATAAAAGGATAGACTACC	- 5'	4	100010	15270129-15270151	intergenic
OT6	5'-	CCCTAATAAGGAATCGGGTACC	- 3'	4	100108 100190	3162820-3162842 940859-940881	intergenic
OT7	5'-	CCCTCCAATAAGATAGAGTACC	- 3'	3	100108	5403976-5403998	intergenic
OT8	3'-	TCCTACATTTAGGGTTAGAGTACC	- 5'	2	100031	1513597-1513619	intergenic
OT9	5'-	CCGAGCATAATGGATAGAGTTCC	- 3'	4	100017	1067652-1067674	Exon Sunflower_HANXRQCHR01G0011831.R1.2.494_R2 Lettuce_LSAT_1_V5_GN_5_96921.1.V5_R0
OT10	3'-	CCCAAGATAAGGAAGAGAGTACC	- 5'	4	100043	9692825-9692847	intergenic
OT11	5'-	CCATCCATAAAAGGAGAGAGTACT	- 3'	2	Contig 20411	3524-3546	Exon Lettuce_LSAT_1_V5_GN_8_93880.1.V5_R0
OT12	5'-	CCACAGATAAGGATAGAGTACC	- 3'	4	100113	177719-177741	intergenic
OT13	3'-	TCATACATAAGGGATTTGAATGCA	- 5'	4	17716	16732712-16732734	Intron Sunflower_HanXRQChr09g0269101.r1.2
OT14	3'-	CCTTTTCATAAGGACAAGAGTACC	- 5'	4	100008	18718286-18718308	intergenic
OT15	3'-	CCTTATATAAGGGTTAGATTAGC	- 5'	4	100240	742431-742453	intergenic
OT16	3'-	CCTTACATAAGGGTTTCAGTAGC	- 5'	4	100066	7238486-7238508	intergenic
OT17	3'-	CCCTACATAAGAGATAAATAAC	- 5'	4	100120	20515-20537	intergenic
OT18	3'-	CCCTAGATAAAAGCTAGAGTACC	- 5'	4	100008	669561-669583	intergenic

Supplementary Table 2. List of primers used to amplify on- and off-target regions in chicory.

Name of primer pair	Sequence	Amplicon length	Description
S1-EX4-F/ S1-EX4-R	GGATTACTCATTGTGTAGCAAA/ TATGTCTTAGCATCATCGCTT	394 bp	<i>CiGAS-S1</i> Exon 4
S2-EX4-F/ S2-EX4-R	GGATTACTCATTGTGTAGCAAA/ CTTCTTAAGGATCATCGCTTAA	391bp	<i>CiGAS-S2</i> Exon 4
S1-KG-F/ S1-KG-R	TGTAAACCGTTGGGCTCGTC/ ACGCATGTGCTATAAACTC	1108 bp	Outer PCR <i>CiGAS-S1</i>
S1/2-E4-Fw/ S1-E4-R	TTGAAGCGAAGTGTAAGGAT/ AGTCGAATCTCTTCAATGGT	254 bp	Inner PCR <i>CiGAS-S1</i> and <i>S2</i>
S2-KG-F/ S2-KG-R	CTATTAATCGGCTTTGGTCAAC/ GTTTGAGCGAGTCCATATGG	1704 bp	Outer PCR <i>CiGAS-S2</i>
S3-KG-F/ S3-E4-R-New-2	GTTATTTTCAGTTCCCCAAGTGT/ CATATGTGTCGTCTAGCAC	272 bp	PCR <i>CiGAS-S3</i>
L1b-E4-FW/ L1b-E4-RV	CGTGTACAGTAAGTTTTTCATTTG/ CAGTGTTTTTGCCTATATATTAC	330 bp	PCR <i>CiGAS-L</i>
OT1_KU_FW/ OT1_KU_REV	AAGAAGACGGAGACCCAGAC/ TCAAATGGTTGGCTATCCTCC	220 bp	PCR OT1
OT2_KU_FW/ OT2_KU_REV	GTGTTCTCCGCCGGTATAAATAA/ CCACCACCAGAAACAATGTAGTT	241 bp	PCR OT2
OT3_KU_FW/ OT3_KU_REV	CGGAGAGAGAAGGGTTCCAA/ TTGGTATTCTACGACGAGGC	191 bp	PCR OT3
OT4_KU_FW/ OT4_KU_REV	GGGATTCACTACTGTATCTAAGG/ ACATCGGTAACAAATTCGCATT	182 bp	PCR OT4
OT5_KU_FW/ OT5_KU_REV	TCGACACCTTATTTACTCAATCC/ CAGTTCTGGTGATGGCTTGT	228 bp	PCR OT5
OT6_KU_FW/ OT6_KU_REV	TATACAAGAAGACGGCGACC/ TGGAAGATCAAAATGGTTGGT	226 bp	PCR OT6
OT7_KU_FW/ OT7_KU_REV	GCTGCTTTACATGTCATTTCGATA/ ACAACTCACTGCATGGATCT	250 bp	PCR OT7
OT8_KU_FW/ OT8_KU_REV	TATTCGAGCATCTTGGGACG/ TGATGGAAGGTGTGGACAAA	240 bp	PCR OT8
OT9_KU_FW/ OT9_KU_REV	AGCTTCGATTGTCACCATTCT/ ACAATTGTTAGACCTCACCA	201 bp	PCR OT9
OT10_KU_FW/ OT10_KU_REV	AGTGTCTTCAAGAAAAGGAAACA/ ACTTCCCAGATGTTGAAAGG	242 bp	PCR OT10
OT11_KU_FW/ OT11_KU_REV	CGTTTGGTGAGACTCCTTCG/ CGCTAAACAGTTCCTCGAGG	202 bp	PCR OT11
OT12_KU_FW/ OT12_KU_REV	ACCAAATCAAAACCAAACATACA/ ATCGAAATGACTTTGCGTGC	229 bp	PCR OT12
OT13_KU_FW/ OT13_KU_REV	AATGAAGTGAGTGGTTACCA/ GAATGTCAAGTTGCCATTTA	219 bp	PCR OT13
OT14_KU_FW/ OT14_KU_REV	TGCCCCAATCTCTGATACCA/ CACCTTTGCGTTGCTACTTG	240 bp	PCR OT14
OT15_KU_FW/ OT15_KU_REV	CGGTTAAGCGGTCGATCC/ ACCCAATCAAACCGAAACCT	226 bp	PCR OT15
OT16_KU_FW/ OT16_KU_REV	CAGGCATCCACATCTATAAGCA/ TGACCTTGGCTTGATCCA	136 bp	PCR OT16
OT17_KU_FW/ OT17_KU_REV	GGGAAGGAAATGAAAAGAACTTT/ TTTGGGTTTTGCTAAATTGC	241 bp	PCR OT17
OT18_KU_FW/ OT18_KU_REV	GCACTTTGGAAGCTTGTCGA/ CTTCTTTCTTTCCTCCCCGC	206 bp	PCR OT18

Supplementary Table 3. Specification of cost vector for respective molecular breeding method.

	<i>Stable transformation</i>		<i>RNP delivery</i>	
	<i>Costs in €</i>	<i>Cost share in %</i>	<i>Costs in €</i>	<i>Cost share in %</i>
<i>Crops</i>	494	7%	152	1%
<i>Chemicals</i>	572	9%	1,289	8%
<i>Rubber and plastic products</i>	765	11%	769	5%
<i>Machinery and equipment</i>	27	0%	22	0%
<i>Office machinery and computers</i>	9	0%	9	0%
<i>Electrical machinery and apparatus</i>	1,293	19%	1,153	7%
<i>Electricity</i>	60	1%	702	4%
<i>Other land transportation services</i>	255	4%	255	2%
<i>Research and development services</i>	240	4%	1,032	6%
<i>Sum of intermediate inputs</i>	3,715	20%	5,382	32%
<i>Value-added</i>	14,726	80%	11,328	68%
<i>Total costs</i>	18,442		16,710	

Supplementary Table 4. CHIC 2.0 genome sequencing and assembly data.

Pacbio Sequence data	data set 1	data set 2	BUSCO	complete single BUSCO's	complete duplicated BUSCO's
Mean Read Length	13.184	11.381			
N50 Read Length	23.266	17.832			
Number of reads	4.242.764	2.673.205			
Total bases	55.936.200.299	30.424.184.294			

Assembly (Flye 2.5)

94,7% 1.418 785

Number of sequences	17.113
Total sequence length	1.548.700.630
Average sequence length	90.498
Maximum sequence length	8.001.753
N50 sequence index	1.081
N50 sequence length	384.198

Polished assembly

Number of sequences	17.113
Total sequence length	1.551.705.774
Average sequence length	90.674
Maximum sequence length	8.737.564
N50 sequence index	1.083
N50 sequence length	384.670

Purged assembly

94,0% 1.823 364

	Haploid assembly	Assigned haplotigs
Number of sequences	8.773	8.340
Total sequence length	1.249.143.101	302.562.673
AV sequence length	142.385	36.278
Max sequence length	8.737.564	1.516.748
N50 sequence length	468.930	153.531
N50 sequence index	741	555

Bionano Genomics dataInput molecule stats
(filtered)

Total number of molecules	639.449
Total length (Mbp)	198.008
Average length (kbp)	309,7
Molecule N50 (kbp)	313,6
Label density (/100kb)	11,7

Genome map De novo assembly

Count = 475	475
Min length (Mbp)	0,24
Median length (Mbp)	3,12
Mean length (Mbp)	5,29
N50 length (Mbp)	9,64
Max length (Mbp)	37,42
Total length (Mbp)	2.514,86

Molecules aligned to the assembly:

Total number of molecules aligned	438.284
Fraction of molecules aligned	0,56
Effective coverage of assembly (X)	51,8

Hybrid scaffold FASTA statistics (CHIC v2.0):

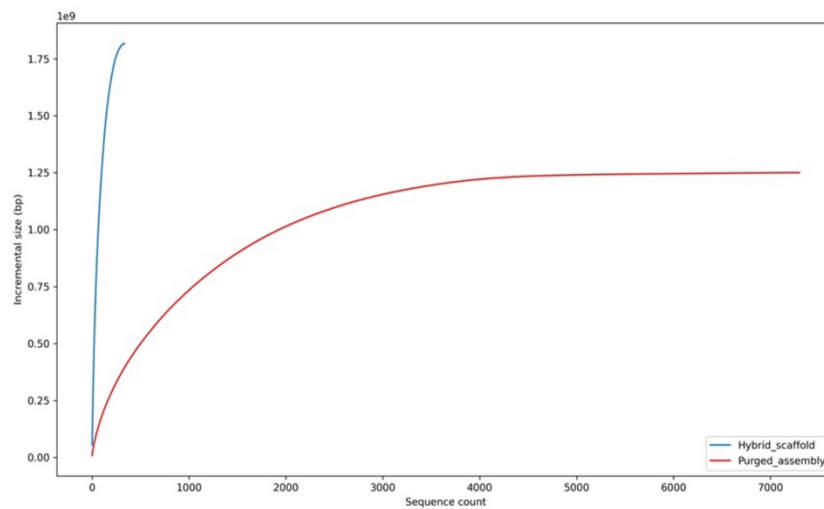
Count	332
Min length (Mbp)	0,10
Median length (Mbp)	3,14
Mean length (Mbp)	5,30
N50 length (Mbp)	9,81
Max length (Mbp)	53,97
Total length (Mbp)	1.761,0

Hybrid scaffold FASTA plus not scaffolded NGS FASTA statistics:

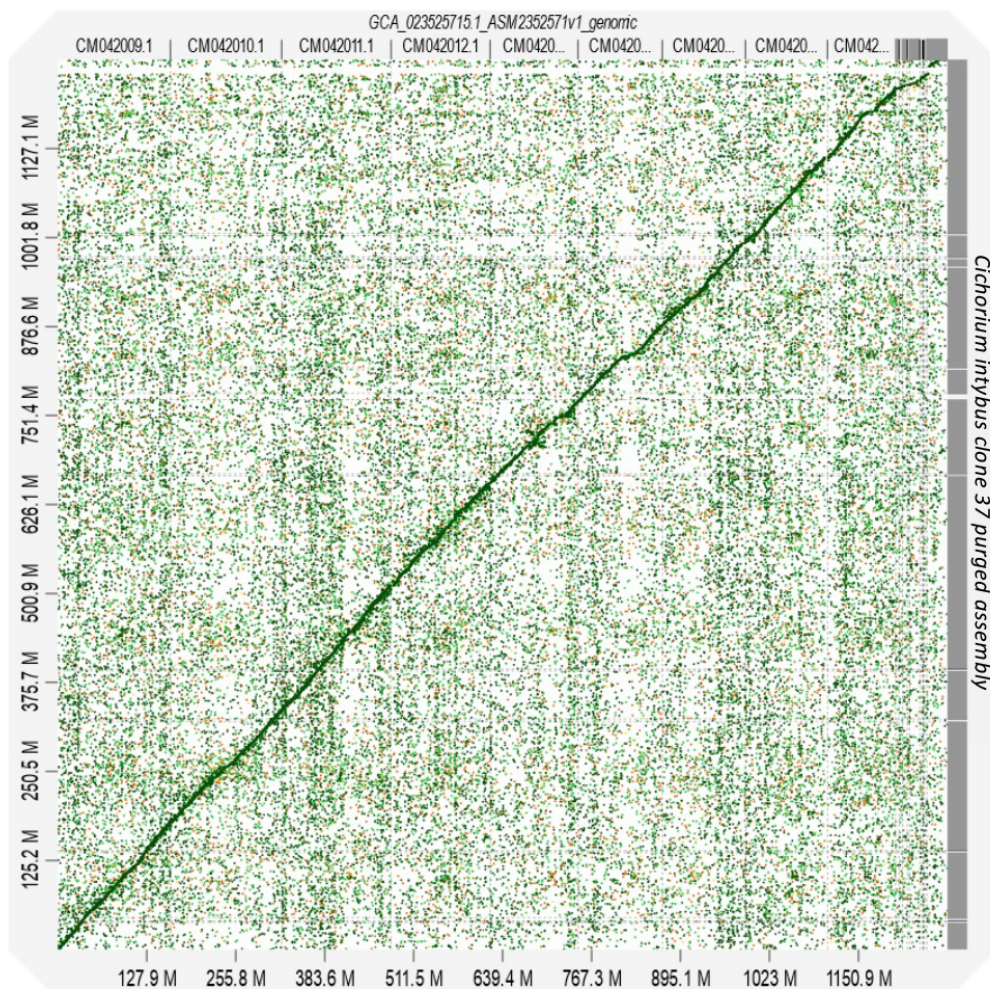
Count	6.649
Min length (Mbp)	0
Median length (Mbp)	0,01
Mean length (Mbp)	0,28

N50 length (Mbp)	8,88
Max length (Mbp)	53,97
Total length (Mbp)	1.872,71

Supplementary Figure 1. A50 plot of *Cichorium intybus* clone 37 purged assembly (red) and hybrid scaffolds of assembly CHIC v2.0 (blue). Graph shown is based on cumulative assembly graph of fragments > 2500 bp.



Supplementary Figure 2. Mummer plot of *Cichorium intybus* clone 37 purged assembly (y-axis) versus public *Cichorium intybus* assembly GCA_023525715.1 (x-axis).



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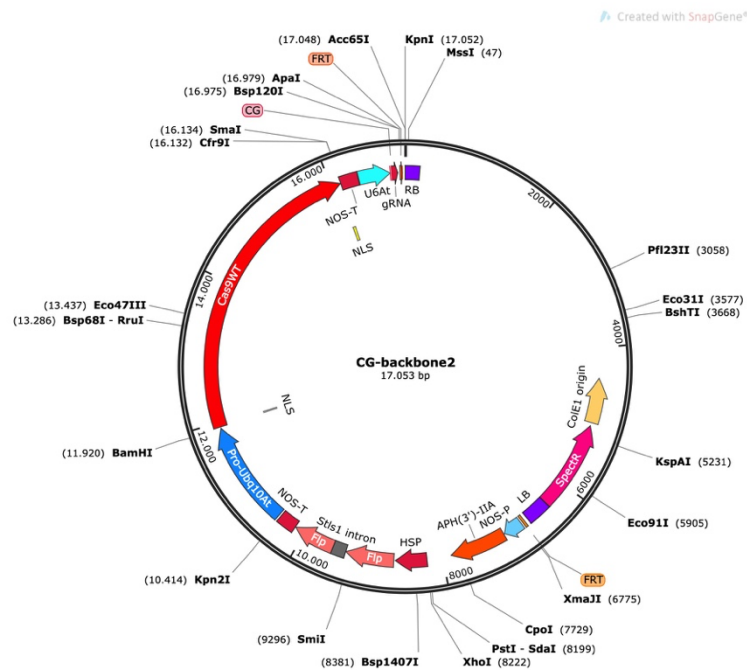
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Supplementary Figure 4. CG-Backbone2 plasmid map and sequence used for Method 2 and 3 (Transient and Stable transformation).



>CG-Backbone2

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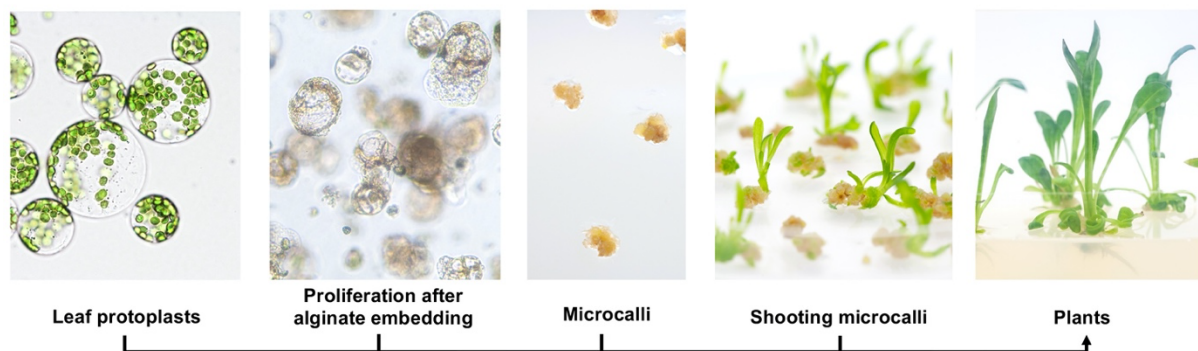
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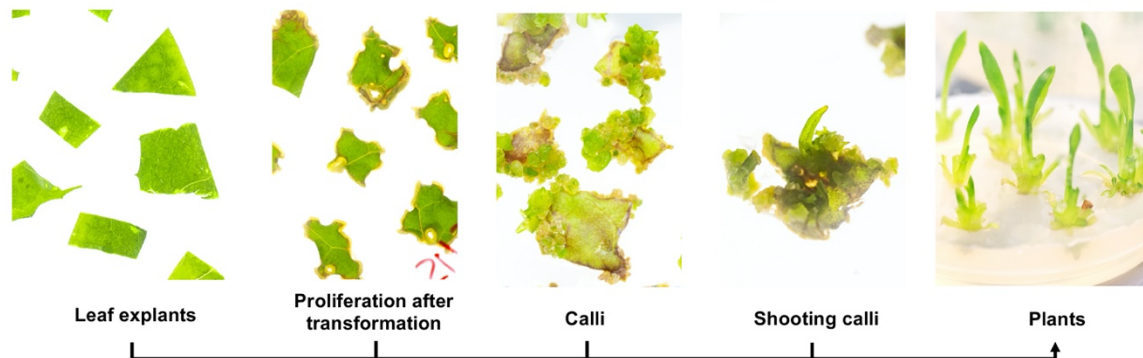
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Supplementary Figure 5. Pictures of the two types of regenerations used in this work. Upper part: protoplast regeneration used in transient delivery (Methods 1 and 2). Lower part: regeneration from leaf explants used in stable delivery (Method 3).

REGENERATION FROM PROTOPLASTS (TRANSIENT DELIVERY)



REGENERATION FROM LEAF EXPLANTS (STABLE DELIVERY)



Supplementary Figure 6. Alignment of CiGAS genes (whole exon 4) where the Common Guide RNA was designed. PAM sequence is highlighted in yellow, and the sgRNA sequence is in red. The single mismatches of CiGAS-S3 and CiGAS-L are in bold. The sequence of the DDxxD motif is depicted in blue.

CiGAS-S1-Ex4	GTGGTGAAGGATATGAGGTTCCAGGAACTACTCCTTACATAAGGGATAGAGTACCAGA
CiGAS-S2-Ex4	GTGGTGAAGGATATGAGGTTCCAGGAACTACTCCTTACATAAGGGATAGAGTACCAGA
CiGAS-S3-Ex4	GTGGTGAAGGATATGAGGTTCCAAGAACTACTCCTTATATAAGGGATAGAGTACCCGA
CiGAS-L-Ex4	GTGGTGAAGGATATGCAATTCCAACAATCCGTCCTTACATAAGAGATAGAGTACCGGA
	***** ***** ***** ** ***** ***** ***** **
CiGAS-S1-Ex4	GATTTACTTATGGATATTGGGATTGTACTTTGAGCCTCGTTACTCCTTGGCACGAATCAT
CiGAS-S2-Ex4	GATTTACTTATGGATATTGGGATTGTACTTTGAGCCTCGTTACTCCTTGGCACGAATCAT

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CiGAS-S3-Ex4      GATCTACTTATGGATATTAGGATTGTACTTTGAGCCTCGTTACTCCTTGGCACGAATCAT
CiGAS-L-Ex4       GATATACCTATGGATTTTGGGGTTATATTTTCGAGCCGTATTACTCTCGGGCACGTATCAT
                  *** ** *  **** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
                  **** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

CiGAS-S1-Ex4      CGCCACAAAAATAACATTGTTTCTGGTGGTGCTAGATGACACATATGATGCGTACGCTAC
CiGAS-S2-Ex4      CGCCACAAAAATTACATTGTTCTGGTGGTGCTAGATGACACATATGATGCATACGCTAC
CiGAS-S3-Ex4      CGCCACAAAAATTACATTGTTTTGGTGGTGCTAGACGACACATATGATGCATATGCTAC
CiGAS-L-Ex4       AGCCACTAAAATCACGTTGTTCTTGGTGGTTTTGGACGATACATATGACGCGTATGCTAC
                  ***** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
                  ***** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

CiGAS-S1-Ex4      CATTGAAGAGATTCGACTTCTAACAGATGCCATAAACAG
CiGAS-S2-Ex4      CATTGAAGAGATTCGACTTCTGACAGATGCCATAAACAG
CiGAS-S3-Ex4      AATTGAAGAAATTCGTCTTTTAACTGATGCCATAAATAG
CiGAS-L-Ex4       AATTGACGAGATCCGATCGATCACAGATGCGATTAATAG
                  ***** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

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Supplementary Figure 7. Number of reads and respective percentage on the total for the protoplast transient assay with RNPs. PAM is highlighted, the target sequence is in uppercase and insertion are in bold.

CiGAS-S1

aaactact <u>cct</u> TAC-ATAAGGGATAGAGTACCaga	WT	3304	47.5%
aaactact <u>cct</u> TAC----AGGGATAGAGTACCaga	-3	868	12.5%
aaactact <u>cct</u> TA-----GAGTACCaga	-11	760	10.9%
aaactact <u>cct</u> TA-----AGAGTACCaga	-10	576	8.3%
aaactact <u>cct</u> TAC---AAGGGATAGAGTACCaga	-2	536	7.7%
aaactact <u>cct</u> TAC- A ATAAGGGATAGAGTACCag	+1	481	6.9%
aaactact <u>cct</u> TAC----GGGATAGAGTACCaga	-4	434	6.2%

CiGAS-S2

aaactact <u>cct</u> TAC-ATAAGGGATAGAGTACCaga	WT	4021	45.9%
aaactact <u>cct</u> TA-----TAGAGTACCaga	-9	872	9.9%
aaactact <u>cct</u> TA-----GTACCaga	-13	867	9.9%
aaactact <u>cct</u> TAC----AGGGATAGAGTACCaga	-3	850	9.7%
aaactact <u>cct</u> TAC-----ATAGAGTACCaga	-7	664	7.6%
aaactact <u>cct</u> TAC-----AGAGTACCaga	-9	570	6.5%
aaactact <u>cct</u> TAC- A ATAAGGGATAGAGTACCag	+1	474	5.4%
aaactact <u>cct</u> TAC---AAGGGATAGAGTACCaga	-2	431	4.9%