

Supplementary information

Comparative analysis of genome editors efficiency on a model of mice zygotes microinjection

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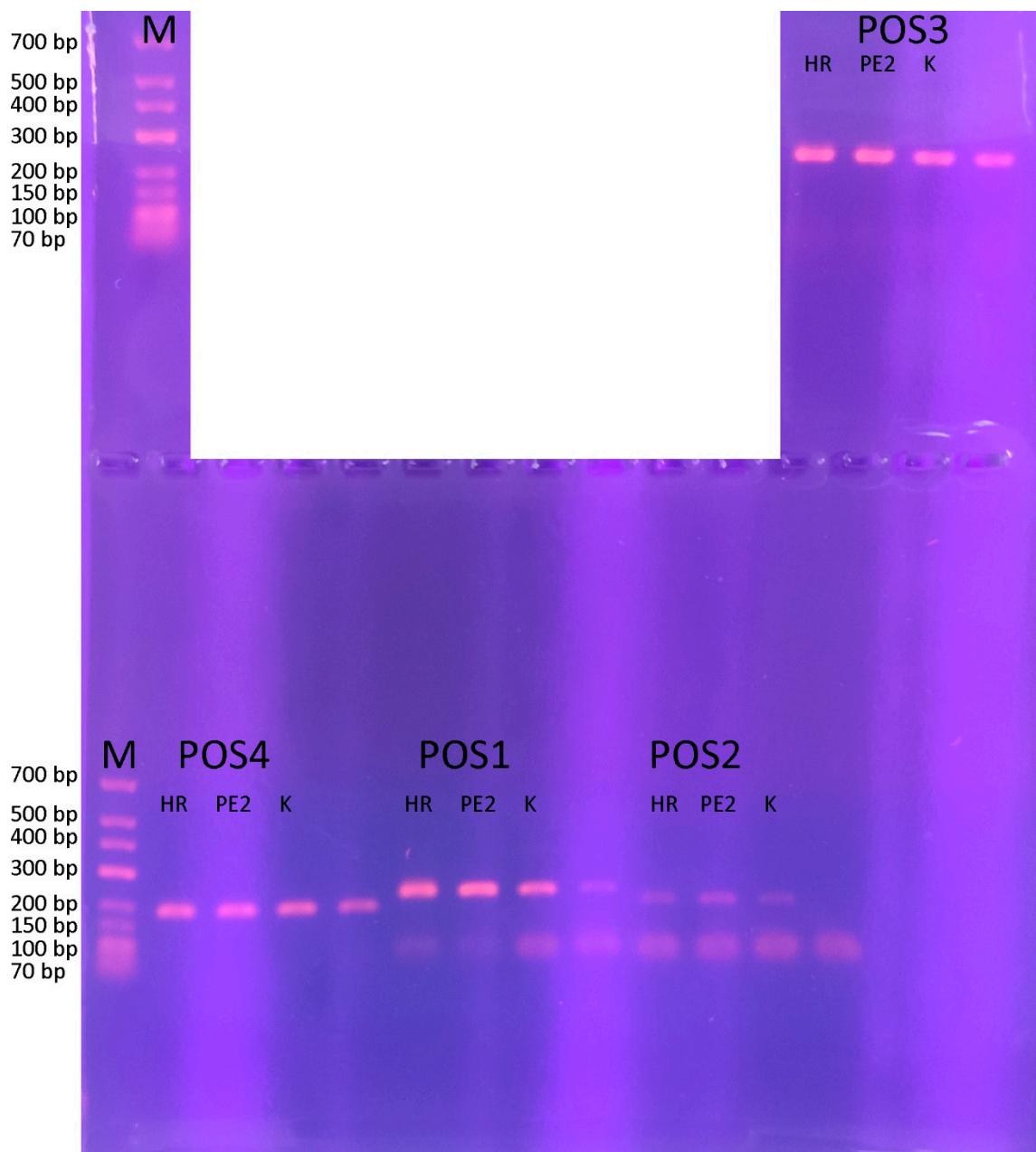
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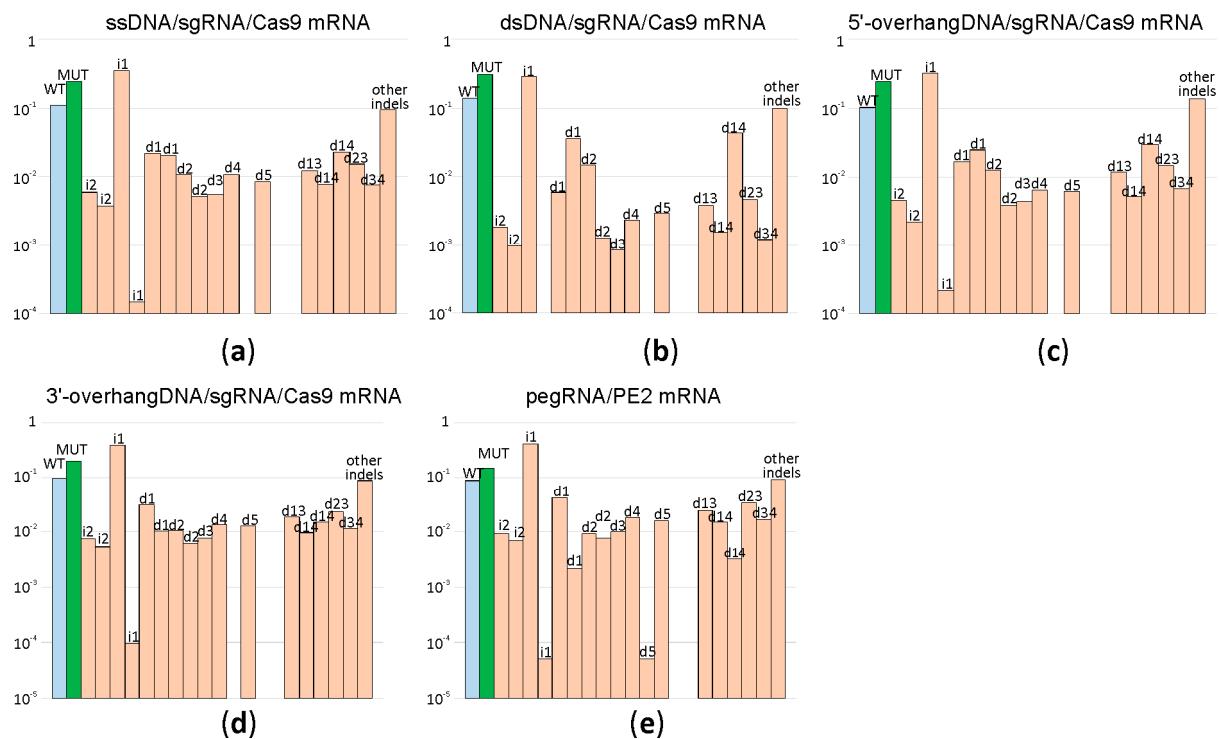
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Supplementary Figure S1. Agarose gel of typical amplicons used for NGS analysis of editing efficiencies. Lanes are marked M (molecular weight markers); POS1-4 (amplicons corresponding to the editing positions 1-4); HR – amplicons derived from blastocysts developed form zygotes injected with Cas9 mRNA, sgRNA and ssDNA template for HR; PE2 – amplicons derived from blastocysts developed form zygotes injected with PE2 mRNA and pegRNA; K-wild type control.



Supplementary Figure S2. Comparison of genome editing efficiencies for HR programmed by different types of templates as well as alternative methods of genome editing (the results of second experiment). Shown are frequencies of allelic variants, in the logarithmic scale, detected in the blastocysts developed from the zygotes after microinjection. Blue bars correspond to the wild type genotype, green to the mutation programmed by a template, while pale red to insertions and deletions. Allelic variants are designated above the bars. (a) Frequency of genome editing products by sgRNA directed cleavage and ssDNA guided HR; Cas9 was provided by injection of Cas9 mRNA. (b) Frequency of genome editing products by sgRNA directed cleavage and dsDNA guided HR; Cas9 was provided by injection of Cas9 mRNA. (c) Frequency of genome editing products by sgRNA directed cleavage and HR guided by DNA template with 5'-overhang; Cas9 was provided by injection of Cas9 mRNA. (d) Frequency of genome editing products by sgRNA directed cleavage and HR guided by DNA template with 3'-overhang; Cas9 was provided by injection of Cas9 mRNA. (e) Frequency of genome editing products by pegRNA directed cleavage and reverse transcription by PE2. For each construct the percentage of zygotes developed to the blastocyst or morula stage is indicated above the graph.

Supplementary Table S1. Sequences of primers.

CasSA_F	TGTAATACGACTCACTATAGGAATACAAGCTACTTGTCTTTTGCA
CasSA_R	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCATTAAAAACCTCCACACC
T7 (PE_F)	TGTAATACGACTCACTATAG
PE_R	TTTTTTTTTTTTTTTTTTTTTTTTTTTTGCATGCAATTCTCATTTATTAGGAAAGG
sgRNA rev	AAAAGCACCGACTCGGTGCC
1_HR	AGGACATCGAGACTGCCCTGGCTCAGTACCATCTCTCCAGCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTGCACCTCTCCAGCTCCCACCCAGAACGACAGCGTCAGCGT
1_PEF	TGTAATACGACTCACTATAGGCTTACCTCTGCACGGCTGCAGTTTAGAGCTAGAAATAGCAAG
1_PER	AAAACCTCTGCACGGCTACATGGAAGAGCACCAGCTCGGTGCCACTTTTC
1_CHK_F1	AGTCTCTCGTGGGCTTCAATGG
1_CHK_F2	CTGCTCTCGTGGGCTTCAATGG
1_CHK_R	GCTGATCACCTAGCTACCTTCC
2_HR	TGTTGGCTGAGGACAGGTAGTGAATGATTGTCATTTCCAGGACTGGACCTACAGAAACATCTGGGAGTTCTGGCAGCTGTTGTCC CATACTGCATCCTATATGACTGAGGGTAAGGTGGTACGGGCTGGGTGAGAGGATGCTGGAAAGAGAGGCCACCCATCACCCACAT CACCCACATGCTCCCTGTCTC
2_PEF	TGTAATACGACTCACTATAGGACTGCATCTATATGACAGAGTTAGAGCTAGAAATAGCAAG
2_PER	AAAACATCTTATGACTGAGGGTAAGGCACCAGCTCGGTGCCACTTTTC
2_CHK_F1	AGTATCTGGGAGTTCTGC CGGC
2_CHK_F2	CTGATCTGGGAGTTCTGC CGGC
2_CHK_R	AGGGATGTGACCTGGAGTGTT
3_HR	TTAAAAAATATTGAACTTTATTTTACCATAGAAAAATTCTTAGAAACAGATATTCTACTGTGAATTTAGCTGATGAAGAATTCT TCCCCTCCAAGAAAACACGTTGACCTGGTGGTAGCAGCTTAAGGTTAGTAATCCATTGTACTTTAAAAAATATGTTACAGAGGCTAGT GAGATACTGACT
3_PEF	TGTAATACGACTCACTATAGGTCAAACGTGTTCTTGAAGTTAGAGCTAGAAATAGCAAG
3_PER	AAAACGTGTTCTGGAGGGGAAGGGCACCAGCTCGGTGCCACTTTTC
3_CHK_F	AGCATTAGGAGTGTCAACAAATGA
3_CHK_R1	AGTGCTGCTAACCAACCAAGGTCAA
3_CHK_R2	CTGGCTGCTAACCAACCAAGGTCAA
4_HR	TGGATCATGACTTGAATCTGAGCTGGGGGTATTGCACTGATCTGAGCTGGGGGTATTGCACTATTCTCTCAAACGTGCCAGTAGTC TATTGTTAGGTATGTAAGAGAGCAACTGTTCTGGAACAGAAAAGCCCTGCTGCACCGAGACACAATGCTGGCAGCTGCAGCGTTACA GAGGTGAGCCGCCACTCC
4_PEF	TGTAATACGACTCACTATAGGAGTAGTCTATTGTTAGGTATGTTAGAGCTAGAAATAGCAAG
4_PER	AAAAGCTTATTGTTAGGTATGTAAGAGAGCAACTGCACCGACTCGGTGCCACTTTTC
4_CHK_F1	AGTATTGCACTTCTCAAACAGTCC
4_CHK_F2	CTGATTGCACTTCTCAAACAGTCC
4_CHK_R	CCATCTGTGATTGCAAGGTAGAA
5_HR	GTTTTCTCTCAGATATTGAAATACTTCAGAAACATTCTAGCATTGAATCCAAGATCACC GTTACAGAATGTGAAGGTACTTGT TGCTACCTCGCGCCTCAGCCTCGGTGTCAGCAACCCAGTGGAAATTCTAAATGAACAGAAGGTACTTGTGTTGATTCTAAATTAA GAAATTCTTGA
5_HR_DS	TAATCAATGAATTCTTAAATTAGAAATCAAACAGTACCTCGTGTTCATTAGAATGAATTCCACTGGGGTCTGACACCGAGGCTGG AGGCAGGCTAGCAACAGAACGTTACATTCTGTAACCGGTGATCTTGATTCAATGCTAAGAAATGTTCTGTGAAGTATTCAAT ATCTGAGAAGAAAACAC
5_HR_5	ATTGTTAATTTGAAATCAATGAGTTCATGATTAACCTCACAGAGTAATTAAATCAATGAATTCTTAAATTAGAAATCAAACAGTACCT TCGTGTTCAATTAGAATGAATTCCACTGGGGTCTGACACCGAGGCTGGAGGCGCAGGTAGCAACAGAACGTTACATTCTGTAACC GGTATCTGGATT
5_HR_3	CATTAGAATGAATTCCACTGGGGTCTGACACCGAGGCTGGAGGCGCAGGTAGCAACAGAACGTTACATTCTGTAACCGGTGAT CTTGAGGATTCAATGCTAAGAAATGTTCTGTGAAGTATTCAATATCTGAGAAGAAAACAGAACGAAACAAACAAAAGAAAACACTCGT GTTAAATATGAAATCTG
5_HR_bio	Biotin- GTTTTCTCTCAGATATTGAAATACTTCAGAAACATTCTAGCATTGAATCCAAGATCACC GTTACAGAATGTGAAGGT ATTCTGGTCTACCTCGCGCCTCAGCCTCGGTGTCAGCAACCCAGTGGAAATTCTAAATGAACACGAAGGTACTTGTGTTGATTCTA AATTAATGAAATTCTTGA
5_PEF	TGTAATACGACTCACTATAGGTGGGGTCTGACACCGAGGCTTTAGAGCTAGAAATAGCAAG
5_PER	C AAAATTGCTGACACCGAGGCTAGAGGCGCAGGTAGCAACAGAACGACCGACTCGGTGCCACTTTTC
5_SGF	TGTAATACGACTCACTATAGGACACCGAGGCTGGAACAGCGGTTTAGAGCTAGAAATAGCAAG
5_CHK_F	GTCAAGAGCTGCACTCACAT
5_CHK_R1	AGTAAATGAATTCCACTGGGGTGC
5_CHK_R2	CTGAGAATGAATTCCACTGGGGTGC
5_CHK_R3	TACAGAATGAATTCCACTGGGGTGC
5_CHK_R4	GCAAGAATGAATTCCACTGGGGTGC
5_CHK_R5	ACGAGAATGAATTCCACTGGGGTGC
5_CHK_R6	CATAGAATGAATTCCACTGGGGTGC
5_CHK_R7	TGAAGAATGAATTCCACTGGGGTGC
5_CHK_R8	GTCAGAATGAATTCCACTGGGGTGC

Supplementary Table S2. Survival rate after microinjection.

Construct	Injected zygotes	Developed to blastocyst stage	Developed to morula stage	Dead	Comments
POS1 HR	58	27 (47%)	4 (7%)	27 (47%)	
POS1 PE2	69	30 (43%)	0 (0%)	39 (57%)	
POS2 HR	182	14 (77%)	9 (5%)	159 (87%)	
POS2 PE2	62	18 (29%)	9 (15%)	35 (56%)	
	64	0 (0%)	0 (0%)	64 (100%)	Not analyzed
POS3 HR	153	26 (17%)	0 (0%)	127 (83%)	
POS3 PE2	132	12 (9%)	4 (3%)	116 (88%)	
POS4 HR	34	21 (62%)	6 (18%)	7 (20%)	
POS4 PE2	64	20 (31%)	0 (0%)	44 (69%)	
POS5 ssDNA	48	4 (8%)	13 (27%)	31 (65%)	
	49	5 (10%)	4 (8%)	40 (82%)	
	286	17 (6%)	28 (10%)	241 (84%)	
	57	11 (19%)	6 (11%)	40 (70%)	
	81	28 (35%)	17 (21%)	36 (44%)	
	134	0 (0%)	0 (0%)	134 (100%)	Not analyzed
	115	25 (22%)	15 (13%)	75 (65%)	Not analyzed
	40	13 (33%)	11 (28%)	16 (40%)	Not analyzed
POS5 dsDNA	55	8 (15%)	12 (22%)	35 (64%)	
	266	0 (0%)	0 (0%)	266 (100%)	Not analyzed
	88	21 (24%)	4 (5%)	63 (72%)	
	64	12 (19%)	20 (31%)	32 (50%)	
POS5 5' overhang	112	0 (0%)	0 (0%)	112 (100%)	
	92	0 (0%)	8 (9%)	84 (91%)	Not analyzed
	29	0 (0%)	0 (0%)	29 (100%)	Not analyzed
	104	21 (20%)	2 (2%)	81 (78%)	
	135	0 (0%)	0 (0%)	135 (100%)	Not analyzed
	135	0 (0%)	0 (0%)	135 (100%)	Not analyzed
	97	4 (4%)	34 (35%)	59 (61%)	
POS5 3' overhang	48	10 (21%)	5 (10%)	33 (69%)	
	38	5 (13%)	2 (5%)	31 (82%)	Not analyzed
	142	0 (0%)	0 (0%)	142 (100%)	
	34	9 (26%)	0 (0%)	25 (74%)	Not analyzed
	63	32 (51%)	0 (0%)	31 (49%)	Not analyzed
POS5 5' bio-ssDNA	63				
		16 (25%)	11 (17%)	26 (41%)	
POS5 HR Cas9 protein	138				
		17 (12%)	4 (3%)	117 (85%)	
POS5 PE2	83	6 (7%)	13 (16%)	64 (77%)	
	58	17 (29%)	3 (5%)	38 (66%)	
	97	48 (49%)	0 (0%)	49 (51%)	
	123	0 (0%)	0 (0%)	123 (100%)	Not analyzed
	76	4 (5%)	4 (5%)	68 (89%)	Not analyzed
No injection	10	10 (100%)	0 (0%)	0 (0%)	Not analyzed
	10	7 (70%)	3 (30%)	0 (0%)	Not analyzed
	10	6 (60%)	1 (10%)	3 (30%)	Not analyzed
	10	4 (40%)	0 (0%)	6 (60%)	Not analyzed
	10	10 (100%)	0 (0%)	0 (0%)	Not analyzed
	10	7 (70%)	0 (0%)	3 (30%)	Not analyzed
	10	7 (70%)	0 (0%)	3 (30%)	Not analyzed
	10	7 (70%)	0 (0%)	3 (30%)	Not analyzed
	14	6 (43%)	0 (0%)	8 (57%)	Not analyzed
	10	4 (40%)	1 (10%)	5 (50%)	Not analyzed
	10	2 (20%)	3 (30%)	5 (50%)	Not analyzed
	10	2 (20%)	3 (30%)	5 (50%)	Not analyzed
	10	3 (30%)	6 (60%)	1 (10%)	Not analyzed
	10	4 (40%)	2 (20%)	4 (40%)	Not analyzed
	10	7 (70%)	0 (0%)	3 (30%)	Not analyzed
	10	0 (0%)	2 (20%)	8 (80%)	Not analyzed
	10	3 (30%)	0 (0%)	7 (70%)	Not analyzed
	10	5 (50%)	0 (0%)	5 (50%)	Not analyzed
	10	5 (50%)	0 (0%)	5 (50%)	Not analyzed
	10	5 (50%)	1 (10%)	4 (40%)	Not analyzed
	10	6 (60%)	0 (0%)	4 (40%)	Not analyzed
	10	5 (50%)	0 (0%)	5 (50%)	Not analyzed
	10	4 (40%)	2 (20%)	4 (40%)	Not analyzed
	10	7 (70%)	3 (30%)	0 (0%)	Not analyzed
	10	4 (40%)	3 (30%)	3 (30%)	Not analyzed
	10	5 (50%)	5 (50%)	0 (0%)	Not analyzed
	10	4 (40%)	2 (20%)	4 (40%)	Not analyzed
	10	5 (50%)	2 (20%)	3 (30%)	Not analyzed
	10	6 (60%)	3 (30%)	1 (10%)	Not analyzed
	10	7 (70%)	0 (0%)	3 (30%)	Not analyzed
	8	1 (13%)	0 (0%)	9 (113%)	Not analyzed
	10	9 (90%)	0 (0%)	1 (10%)	Not analyzed
	48	24 (50%)	20 (42%)	4 (8%)	Not analyzed
	26	12 (46%)	11 (42%)	3 (12%)	Not analyzed

Supplementary Table S3. Sequences and frequencies of allelic variants at position 1.

	Allelic variant	Editing strategy/ frequency of allelic variant		
			HR	PE2
WT	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTCATG	CAGCCGTGAGAGGTAAGC	0,26	0,96
MUT	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTCATG	TAGCCGTGAGAGGTAAGC	0,067	0,002
i1	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTCATG	AAGCCGTGAGAGGTAAGC	0,28	0,006
d3	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTCATG	...CGTGCAGAGGTAAGC	0,076	0,0015
d8	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTCATG	...CAGAGGTAAGC	0,026	0,0005
d3	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTCATG	C CCTGGGAGC...TAAGC	0,019	0,0002
d8	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTC...GTGAGAGGTAAGC	0,017	1E-04	
d1	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTCATG	T GCGGTGAGAGGTAAGC	0,01	0,0002
i2	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTCATG	GCCGTGAGAGGTAAGC	0,01	0,0002
d4	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTCATG	...GTGAGAGGTAAGC	0,007	0,00015
d2	CTCTCGTGGGCTTCATGGGGCAAGGACTGCACCGCCTCTGCACCTCTTCATG	...CCGTGAGAGGTAAGC	0,006	0,0002

d66	CTCTCGCTGGGCTTCAATGGGGCAAGGACTGCACCCACTCTGCACCTCTTCATG	(d66) CAGCTCCCACCCC	0,009	0,0002
i2	CTCTCGCTGGGCTTCAATGGGGCAAGGACTGCACCCACTCTGCACCTCTTCATG	CCATGCCGTGAGAGGTAAGC	0,006	1E-04
i1	CTCTCGCTGGGCTTCAATGGGGCAAGGACTGCACCCACTCTGCACCTCTTCATG	TAAGCCGTGAGAGGTAAGC	0,006	1E-04
d22	CTCTCGCTGGGCTTCAATGGGGCAAGGACTGCACCCACTCTGCACCTCTTCATG	(d22) CCCCTGGGGAGCTAA	0,005	0,0001
d9	CTCTCGCTGGGCTTCAATGGGGCAAGGACTGCACCCACTCTGCACCTCTTC- · · · -	· GTGAGAGGTAAGC	0,005	3E-05
d1	CTCTCGCTGGGCTTCAATGGGGCAAGGACTGCACCCACTCTGCACCTCTTCATG	C · GCGGTGAGAGGTAAGC	0,005	7E-05

Supplementary Table S4. Sequences and frequencies of allelic variants at position 2.

	Allelic variant	Editing strategy/ frequency of allelic variant	
		HR	PE2
WT	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGCATCCTATATGAC	0,68	0,97
MUT	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGCATCCTATATGAC TGAGGGTAAGGTGGTTGACGGGCTGG	0,1	0,005
d4	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGCATCCTATATGAC · · · GGGTAAGGTGGTTGACGGGCTGG	0,05	0,0007
d3	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGCATCCTATATGAC · · · GA · CGGTAAGGTGGTTGACGGGCTGG	0,02	0,0004
d8	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGCATCCTAT · · · · GGGTAAGGTGGTTGACGGGCTGG	0,014	0,00015
i1	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGCATCCTATATGACCAGGGGTAAGGTGGTTGACGGGCTGG	0,013	0,0002
d9	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGCATCCTA · · · · GGGTAAGGTGGTTGACGGGCTGG	0,005	2,5E-05
d3	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGCATCCTATATAGA · · · GGGTAAGGTGGTTGACGGGCTGG	0,011	0,0002
d5	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGCATCCTATA · · · · GAGGGTAAGGTGGTTGACGGGCTGG	0,008	0,0001
d14	ATCTGGGAGTTCTCGCCAGCTGGTGTCC · · · · · TATA · TG AGAGGGTAAGGTGGTTGACGGGCTGG	0,007	1E-04
d1	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGCATCCTATATGAC · · · · · GAGGGTAAGGTGGTTGACGGGCTGG	0,005658	6,24E-05
d12	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTGC · · · · · GAGGGTAAGGTGGTTGACGGGCTGG	0,005606	7,49E-05
d15	ATCTGGGAGTTCTCGCCAGCTGGTGTCCCATACTG · · · · · AGGGTAAGGTGGTTGACGGGCTGG	0,00313	6,24E-05

Supplementary Table S5. Sequences and frequencies of allelic variants at position 3.

	Allelic variant	Editing strategy/ frequency of allelic variant	
WT	GCTGCTAACCCACCAAGGTCAAACGTGTTTCTTGGAAAGGGAAAGGAATTCTTCATCAGCTAAAATATTACAGTAGGAA	HR	PE2
MUT	GCTGCTAACCCACCAAGGTCAAACGTGTTTCTTGGAGGGGAAGGAATTCTTCATCAGCTAAAATATTACAGTAGGAA	0.98	0.99
d1	GCTGCTAACCCACCAAGGTCAAACGTGTTTCTTGGAAAGGGAAAGGAATTCTTCATCAGCTAAAATATTACAGTAGGAA	0.013	0.005
		0.007	0.0001

Supplementary Table S6. Sequences and frequencies of allelic variants at position 4.

	Allelic variant	Editing strategy/ frequency of allelic variant		
		HR	PE2	
WT	ATTTGCAGTATTCTCTCAAACGCCAGTAGTCATTAGGT	ATGGGTGAGAGCAACTGTTCTGGAA	0,97	0,9995
MUT	ATTTGCAGTATTCTCTCAAACGCCAGTAGTCATTAGGT	ATG TAA GAGAGCAACTGTTCTGGAA	0,015	0,0003
d2	ATTTGCAGTATTCTCTCAAACGCCAGTAGTCATTAGGT	ATGGGTGAGAGCAACTGTTCTGGAA	0,005	0,0001
d12	ATTTGCAGTATTCTCTCAAACGCCAGTAG.....	TATGGGTGAGAGCAACTGTTCTGGAA	0,003	4E-05
i11	ATTTGCAGTATTCTCTCAAACGCCAGTAGTCATTAGGT GAGTAGTCTATTATGGGTGAGAGCAACTGTTCTGGAA		0,003	1E-05

Supplementary Table S7. Sequences and frequencies of allelic variants at position 5.