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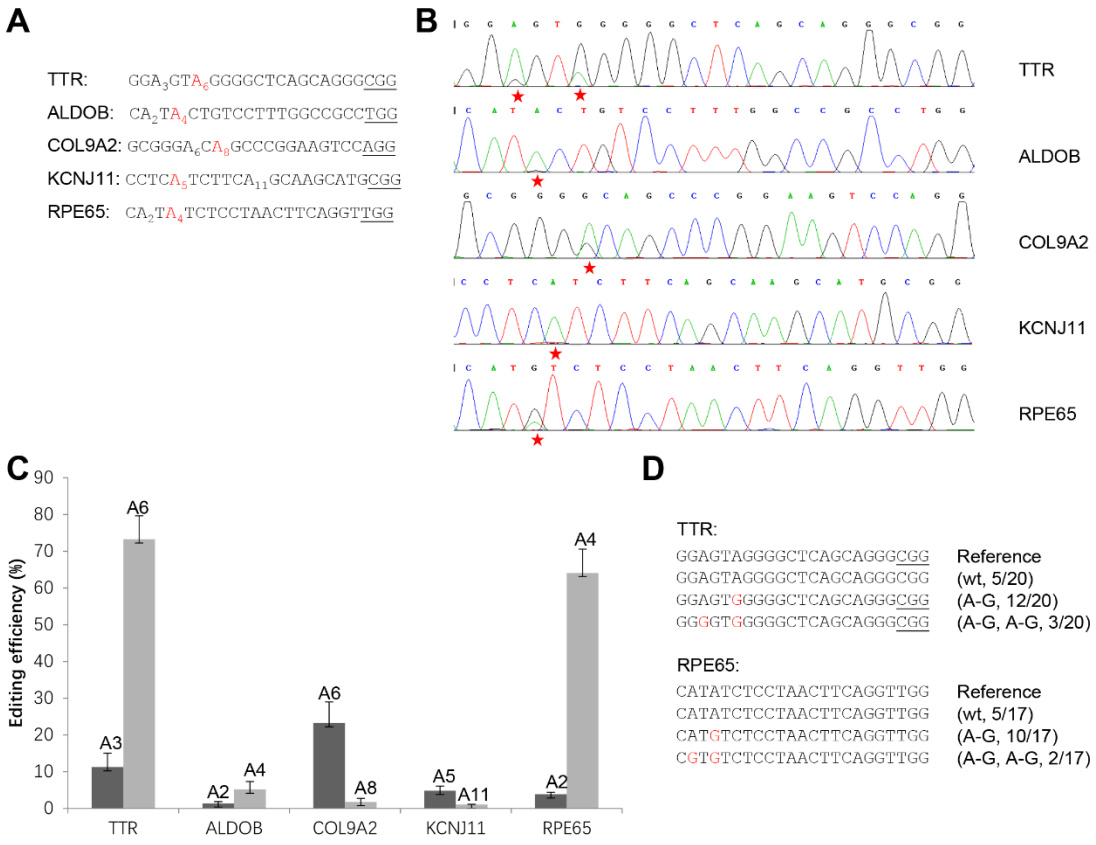
Supplemental Information

Efficient Generation of Pathogenic A-to-G Mutations in Human Tripronuclear Embryos via ABE-Mediated Base Editing

Guanglei Li, Xinyi Liu, Shisheng Huang, Yanting Zeng, Guang Yang, Zongyang Lu, Yu Zhang, Xu Ma, Lisheng Wang, Xingxu Huang, and Jianqiao Liu

SUPPLEMENTARY INFORMATION



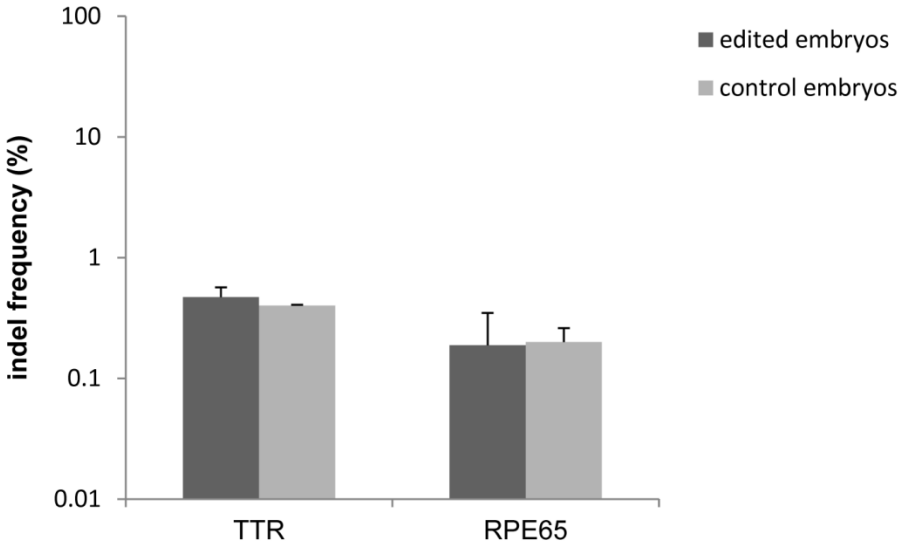
Supplementary Figure 1 Detection of pathogenic A-to-G substitution induced by ABE in cells.

A. The representative pathogenic mutations. Five human genes with reported pathogenic mutation were selected, and the related pathogenic points are highlighted in red. The PAM sequences are underlined.

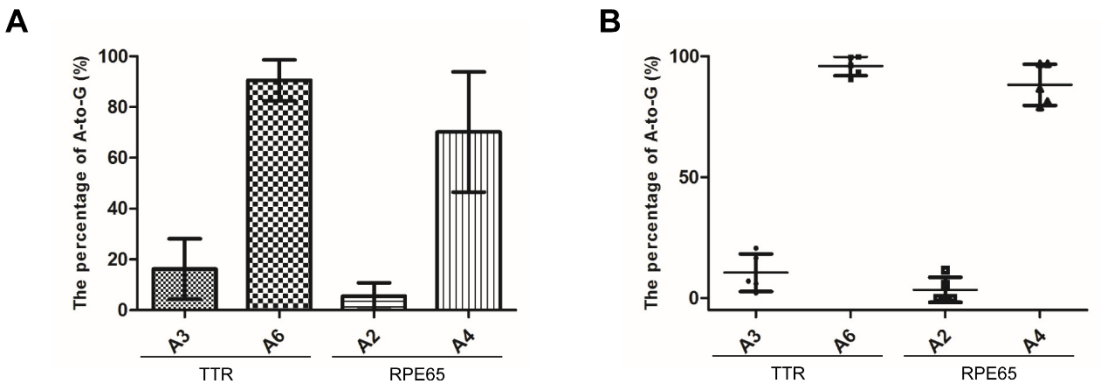
B. The representative chromatogram of the Sanger sequencing of target sites from genomic DNA of HEK293T cells transfected with ABE and related sgRNAs. The red stars indicate the conversion of A-to-G.

C. The editing efficiency of A-to-G within the target sites. Data from three independent experiments were shown as means ± s.d.

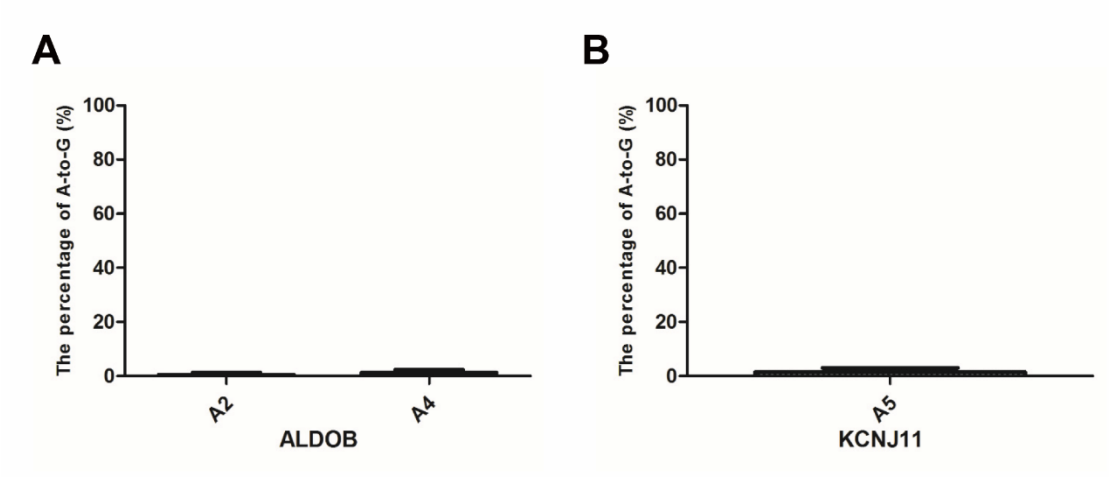
D. Sequences of the PCR product after TA cloning for *TTR* and *RPE65*. The PAM sequences are underlined; the modified bases highlighted in red. N/N indicates bacterial colonies with base editing out of total number of the sequenced bacterial colonies.



Supplementary Figure 2 Detection of indels for *TTR* and *RPE65*



Supplementary Figure 3 Simultaneous editing of multiple sites. A. The editing efficiency was calculated depending on the chromatogram of the Sanger sequencing of target sites. B. The ediitng efficiency was calculated depending on the deep sequencing.



Supplementary Figure 4 The editing efficiency for *ALDOB* and *KCNJ11* in human embryos.

Supplementary Table 1 Summary of the used embryos and editing efficiency

Used sgRNAs	Embryo No.	Editing efficiency（A-to-G）		Used sgRNAs	Embryo No.	Editing efficiency（A-to-G）		Used sgRNAs	Embryo No.	Editing efficiency（A-to-G）	
1. ABE+ SITE6	Position	A3	A7	4. ABE+ COL9A2	Position	A2	A4	8. ABE+ TTR	#68	22%	86%
	#1	7%	41%		#35	8%	55%		#69	14%	89%
	#2	0%	39%		#36	3%	90%		#70	22%	95%
	#3	0%	49%		#37	78%	91%		#71	35%	89%
	#4	0%	60%		#38	37%	100%		#72	2%	94%
	#5[a]	0%	88%	5. ABE+ KCNJ11	Position	A5			#73	7%	98%
	#6	25%	70%		#39	0%			#74	8%	90%
	#7	5%	48%		#40	2%			#75	35%	71%
	#8	45%	65%		#41	0%			#76	5%	98%
	#9	44%	93%		#42	4%			#77	12%	96%
	#10	0%	45%		#43	0%		8. ABE+ RPE65	Positon	A2	A4
	#11	6%	55%		#44	3%			#68	12%	50%
	#12	0%	100%		#45	1%			#69	0%	50%
2. ABE+ SITE2	Position	A2	A5 A8	6. ABE+ TTR	Position	A3	A6		#70	4%	87%
	#13	4%	99% 1%		#46	0%	78%		#71	3%	70%
	#14	4%	100% 3%		#47	80%	90%		#72	1%	98%
	#15	3%	89% 10%		#48	18%	80%		#73	16%	25%
	#16	4%	96% 2%		#49	18%	92%		#74	2%	97%
	#17	3%	99% 8%		#50	13%	88%		#75	3%	60%
	#18	2%	99% 1%		#51	6%	90%		#76	10%	85%
	#19	2%	86% 2%		#52	1%	82%		#77	4%	80%
	#20	6%	91% 3%		#53	2%	95%	9. ABE+ TTR	Position	A3	A6
	#21	3%	50% 16%		#54	35%	86%		#78	14%	95%
	#22	8%	99% 14%		#55	14%	74%		#79	0	96%
	#23	0%	100% 0%		#56	9%	70%		#80	0	80%
	#24	1%	83% 2%		#57	9%	87%		#81	33%	100%
3. ABE+ ALDOB	Position	A2	A4	7. ABE+ RPE65	Position	A2	A4		#82	10%	87%
	#25	0%	2%		#58	12%	60%		#83	30%	89%
	#26	0%	0%		#59	0%	38%	10. ABE-NG+ BCS1L-1	Position	A4	A7
	#27	1%	3%		#60	8%	64%		#85	19%	11%
	#28	2%	1%		#61	1%	42%		#86	26%	15%
	#29	0%	0%		#62	0%	50%		#87	21%	14%
	#30	1%	1%		#63	0%	26%	11. ABE-NG+ BCS1L-2	Position	A5	A7
	#31	0%	2%		#64	0%	70%		#88	42%	24%
4. ABE+ COL9A2	Position	A6	A8		#65	0%	53%		#89	33%	0
	#32	29%	87%		#66	2%	60%		#90	32%	0
	#33	52%	30%		#67	5%	41%				
	#34	36%	48%		Position	A3	A6				

[a] The embryos marked in red were subjected for deep sequencing.

Supplementary Table 2 Summary of the off-target sites for TTR

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	N	G	G	Location			
Target site	G	G	A	G	T	A	G	G	G	G	C	T	C	A	G	C	A	G	G	G	C	G	G	Chr.	Strand	Start	End
TTR-OF1	A	G	A	G	T	G	G	G	G	G	C	T	C	A	G	C	A	G	G	G	T	G	G	7	-	101886959	101886981
TTR-OF2	G	G	A	G	C	A	C	A	G	G	C	T	C	A	G	C	A	G	G	G	T	G	G	9	-	134834935	134834957
TTR-OF3	C	G	G	G	C	A	G	G	C	G	C	T	C	A	G	C	A	G	G	G	T	G	G	1	-	54459729	54459751
TTR-OF4	G	G	G	G	G	G	C	G	G	G	C	T	C	A	G	C	A	G	G	G	A	G	G	1	-	233055471	233055493
TTR-OF5	A	G	C	C	T	A	G	G	G	C	C	T	C	A	G	C	A	G	G	G	A	G	G	16	-	3144125	3144147
TTR-OF6	G	C	C	G	T	C	G	G	C	G	C	T	C	A	G	C	A	G	G	G	A	G	G	1	-	217186869	217186891
TTR-OF7	G	C	C	G	G	A	G	G	G	G	C	T	C	A	G	C	A	G	G	G	C	A	G	19	-	35925685	35925707
TTR-OF8	G	G	C	C	A	A	G	G	G	G	C	T	C	A	G	C	A	G	G	G	G	A	G	2	-	16397188	16397210
TTR-OF9	A	G	A	G	C	T	G	G	G	G	C	T	C	A	G	C	A	G	G	G	G	A	G	11	+	119792041	119792063
TTR-OF10	G	G	T	G	T	T	G	G	G	G	C	T	C	A	G	C	A	G	G	G	T	G	G	12	+	68329077	68329099
TTR-OF11	G	G	C	T	C	A	G	G	G	G	C	T	C	A	G	C	A	G	G	G	T	G	G	3	+	129512561	129512583
TTR-OF12	A	G	A	G	T	C	A	G	G	G	C	T	C	A	G	C	A	G	G	G	A	G	G	17	+	18256612	18256634
TTR-OF13	T	G	G	G	C	T	G	G	G	G	C	T	C	A	G	C	A	G	G	G	A	G	G	7	-	1057874	1057896
TTR-OF14	G	T	T	G	A	T	G	G	G	G	C	T	C	A	G	C	A	G	G	G	T	G	G	11	-	2894294	2894316
TTR-OF15	T	T	A	G	G	A	A	G	G	G	C	T	C	A	G	C	A	G	G	G	T	G	G	9	-	136104150	136104172
TTR-OF16	G	G	A	A	T	G	G	A	G	G	C	T	C	A	G	C	A	G	G	G	T	G	G	17	-	17627042	17627064
TTR-OF17	A	C	A	G	C	A	G	T	G	G	C	T	C	A	G	C	A	G	G	G	A	G	G	2	-	199141685	199141707
TTR-OF18	C	C	T	G	T	A	G	G	C	G	C	T	C	A	G	C	A	G	G	G	T	G	G	9	+	137070161	137070183
TTR-OF19	G	C	T	A	T	A	G	A	G	G	C	T	C	A	G	C	A	G	G	G	A	G	G	12	+	12331390	12331412
TTR-OF20	G	T	G	G	T	C	G	A	G	G	C	T	C	A	G	C	A	G	G	G	C	G	G	2	+	120794406	120794428
TTR-OF21	G	T	A	G	A	T	A	G	G	G	C	T	C	A	G	C	A	G	G	G	G	G	G	10	+	13995879	13995901

Supplementary Table 3 Summary of the off-target sites for RPE65

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	N	G	G	Location			
target site	C	A	T	A	T	C	T	C	C	T	A	A	C	T	T	C	A	G	G	T	T	G	G	Chr.	Strand	Start	End
RPE65-OF1	C	T	T	G	T	C	T	T	C	A	A	A	C	T	T	C	A	G	G	T	T	A	G	9	-	117426716	117426738
RPE65-OF2	G	G	T	A	T	C	T	T	C	T	A	A	A	T	T	C	A	G	G	T	G	G	G	6	-	106501910	106501932
RPE65-OF3	C	A	G	A	C	C	A	C	C	C	A	A	C	T	T	C	A	G	G	T	G	G	G	3	+	28844733	28844755
RPE65-OF4	G	A	T	C	A	C	T	C	C	T	A	C	C	T	T	C	A	G	G	T	G	G	G	10	+	60419751	60419773
RPE65-OF5	A	A	T	G	G	C	T	C	C	T	A	G	C	T	T	C	A	G	G	T	A	G	G	7	+	119799948	119799970
RPE65-OF6	C	A	T	C	T	G	T	C	C	A	A	G	C	T	T	C	A	G	G	T	G	G	G	15	+	80808417	80808439
RPE65-OF7	C	A	T	C	T	G	T	C	C	T	G	T	C	T	T	C	A	G	G	T	T	G	G	21	-	21012624	21012646
RPE65-OF8	C	A	T	G	A	C	T	A	C	T	A	A	C	C	T	C	A	G	G	T	T	G	G	2	-	20195444	20195466
RPE65-OF9	C	A	T	T	T	C	T	A	C	T	T	A	G	T	T	C	A	G	G	T	A	G	G	14	+	81537161	81537183
RPE65-OF10	G	A	G	A	C	C	T	C	A	T	A	A	C	T	T	C	A	G	G	T	C	A	G	2	-	24436599	24436621
RPE65-OF11	C	A	T	A	G	C	G	A	C	A	A	A	C	T	T	C	A	G	G	T	T	G	G	2	-	173678502	173678524
RPE65-OF12	A	A	T	G	A	A	T	C	C	T	A	A	C	T	T	C	A	G	G	T	G	A	G	14	+	20442316	20442338
RPE65-OF13	G	A	T	A	T	C	T	T	A	A	A	A	C	T	T	C	A	G	G	T	T	G	G	17	-	11634934	11634956
RPE65-OF14	C	C	T	C	C	A	T	C	C	T	A	A	C	T	T	C	A	G	G	T	G	G	G	2	+	218577991	218578013
RPE65-OF15	T	T	T	A	T	T	T	C	C	C	A	A	C	T	T	C	A	G	G	T	A	A	G	20	-	41219765	41219787
RPE65-OF16	C	C	T	G	T	G	T	C	C	A	A	A	C	T	T	C	A	G	G	T	G	G	G	12	-	76712189	76712211
RPE65-OF17	T	A	T	C	C	C	T	C	C	T	A	A	C	T	T	C	A	G	G	A	C	A	G	7	+	82392310	82392332
RPE65-OF18	A	A	T	A	C	C	T	A	C	T	A	A	C	T	T	C	A	G	G	A	T	G	G	3	-	128451040	128451062
RPE65-OF19	C	A	G	A	T	C	T	C	C	T	C	A	A	T	T	C	A	G	G	T	G	G	G	6	+	167581892	167581914
RPE65-OF20	C	C	T	A	C	C	T	C	C	T	T	A	T	T	T	C	A	G	G	T	T	G	G	10	+	69872858	69872880
RPE65-OF21	C	A	C	A	T	A	C	C	C	T	A	T	C	T	T	C	A	G	G	T	A	G	G	16	-	52342604	52342626
RPE65-OF22	G	G	T	T	T	C	A	C	C	T	A	A	C	T	T	C	A	G	G	T	A	G	G	10	-	57853408	57853430
RPE65-OF23	A	A	T	A	A	C	T	A	T	T	A	A	C	T	T	C	A	G	G	T	G	G	G	20	-	32437696	32437718

Supplementary Table 4 Primers used in the study

Primer name	Sequence (5'-3')	Product length (bp)	Usage
TTR-ON-249-F	TTTTTCGGGCTCTGGTG	249	On-target detection
TTR-ON-249-R	TATGAGGTGAAAACACTGCTT		
RPE65-ON-248-F	TGTCATTGCCTGTGCTCA	248	
RPE65-ON-248-R	ACATGAGGCAGGAGGACAA		
COL9A2-ON-269-F	GCCTCTGGATCTCAGTTTC	269	
COL9A2-ON-269-R	ACAGAGTTGGTAACAAGGCA		
KCNJ11-ON-237-F	TCCTGATCCTCATCGTGC	237	
KCNJ11-ON-237-R	TGGTGGTCTTGCGTACCA		
ALDOB-ON-238-F	AGGCAGACAGGGTCAAGG	238	
ALDOB-ON-238-R	GGATTGGAGGAAAAGTTGC		
SITE6-ON-230-F	GGGAAACGCCCATGCAATTA	230	
SITE6-ON-230-R	GTCAACCAGTATCCCGGTGC		
SITE2-ON-236-F	AGCTCCTGAGATACAGTCACGAG	236	
SITE2-ON-236-R	AGCTTCCTGAAATGCTGTGCGTGT		
RPE65-OF1-257-F	CCAGAGCCCACTGATGTTGAT	257	Off-target for RPE65
RPE65-OF1-257-R	AAAGCAGGCTGGGGGGA		
RPE65-OF2-252-F	TGGGCAGTGTATATTAATTGG	252	
RPE65-OF2-252-R	ACAGCTACAGCCAAGTCAGA		
RPE65-OF3-241-F	GACGGTTACCAGAGTGCG	241	
RPE65-OF3-241-R	ATCCCTGTGGCTCTCAATA		
RPE65-OF4-238-F	AGTCCCTTCTCCTGCCTAC	238	
RPE65-OF4-238-R	GAGAAAAGAAAAGCAAGGC		
RPE65-OF5-250-F	TCAACTAATTACTCAAAGAGAAA	250	
RPE65-OF5-250-R	GGACATAAATAAATGCCCTA		
RPE65-OF6-243-F	TTCCACTGCTGAGACCCT	243	
RPE65-OF6-243-R	CCACTGTATCCTGGCTTG		
RPE65-OF7-262-F	ATTTCTACTCCTGGTTTTGC	262	
RPE65-OF7-262-R	CAGAGACCCAAGGAGAGC		
RPE65-OF8-274-F	AAACATCTGAAATGATTCCTAAC	274	
RPE65-OF8-274-R	AGCCATTATCAGTAAACACCTC		
RPE65-OF9-275-F	GTAAATTAAAGTTTTCATGCATA	275	
RPE65-OF9-275-R	CAAACATACTCCTCCACAATC		
RPE65-OF10-250-F	AATGAACAGAAACAACACCTAAG	250	
RPE65-OF10-250-R	TGGCAACAGAGCGAGACT		
RPE65-OF11-246-F	AGACCACTTTTCCTAAGTCACTA	246	
RPE65-OF11-246-R	GAGGAAGAGTCAATAAAATGCT		
RPE65-OF12-267-F	AAGACAGTCTGGGAGGCAA	267	
RPE65-OF12-267-R	TTGGGGTAGTGCCAGAAA		
RPE65-OF13-273-F	CCATACCTGGTCATTCTGC	273	
RPE65-OF13-273-R	CCCTTGCTTTAAGTCAACG		

RPE65-OF14-248-F	CCCCTTTGGTTACTGGATTGT	248	
RPE65-OF14-248-R	GCTTTTTTCCTCCTTTCCCA		
RPE65-OF15-249-F	AGCAAGCCCTATAACTCAAGA	249	
RPE65-OF15-249-R	CTGATAGAGTAGCCGCCAT		
RPE65-OF16-245-F	TTTTAATAGAGACGGGGTTTC	245	
RPE65-OF16-245-R	CCTTCTTGCTATTTGCTGATT		
RPE65-OF17-239-F	CCAACCTTTGAATGATGCC	239	
RPE65-OF17-239-R	AAAAATGAGGTTACTCCGACA		
RPE65-OF18-237-F	TCTTCCCTTCTGCCTCCTGT	237	
RPE65-OF18-237-R	CTGCCATCGCCATCACG		
RPE65-OF19-274-F	CGTGGCTAACTTGACCTCTG	274	
RPE65-OF19-274-R	CTGGGACTGCTACCAATGTG		
RPE65-OF20-234-F	TTGAAACCGTAAGAAGAGCC	234	
RPE65-OF20-234-R	GGGTTTTGAAGGTGGAGC		
RPE65-OF21-244-F	AAATAAATAAATAGCATCCTTCA	244	
RPE65-OF21-244-R	TTCACCTCAGACCAGCCT		
RPE65-OF22-250-F	AAGTATTAGAAGTTTGAGAGAAG	250	
RPE65-OF22-250-R	AAAGTTTTAGCCCTGGTT		
RPE65-OF23-236-F	CCTGAGCTCTCCTGCAAG	236	
RPE65-OF23-236-R	CCCTGTGCTGGCTTCTTT		
TTR-OF1-236-F	ACCTAAATGGGAGGCTTGC	236	Off-target for TTR
TTR-OF1-236-R	AGGACTCAACAACGCCCA		
TTR-OF2-235-F	CAGTGCGTTTCCAGGTAGT	235	
TTR-OF2-235-R	TGGTAGCAGTGGTAGGTGA		
TTR-OF3-242-F	AAATGTGTTTGAAGGAGCGAG	242	
TTR-OF3-242-R	GGGCTGGGACAGACCTCA		
TTR-OF4-236-F	GGAGGAAGCAGCAAAGAAG	236	
TTR-OF4-236-R	CCCACAGGACCACAGACC		
TTR-OF5-265-F	GCCCCGTCTCGCCCTAT	265	
TTR-OF5-265-R	CAGGGCAGTGACTACAGCGG		
TTR-OF6-243-F	TGCCAGGTGACAGTCAGAAC	243	
TTR-OF6-243-R	TATTTAGGGCATCTTGAGTCTCT		
TTR-OF7-235-F	ATCAGCCACCTTGGACAT	235	
TTR-OF7-235-R	TTAGAGTGAGGGTTGAGTTTG		
TTR-OF8-239-F	CAGATGAAGATGGGAGAAAG	239	
TTR-OF8-239-R	GCTACTTCAAAAATACCAGGA		
TTR-OF9-250-F	CAGGACTAGGAGCAAGATTG	250	
TTR-OF9-250-R	GGGGACCTAGCACATTTG		
TTR-OF10-255-F	TTCATCACCTTCCCCTCAA	255	
TTR-OF10-255-R	GGGTGTCCCTGCTTCTCC		
TTR-OF11-242-F	TTCCTTCCAGAGCACTTTC	242	
TTR-OF11-242-R	TCTTCCATTTCAGTCACACC		
TTR-OF12-247-F	GAAGCGTGGTCAGGTTGT	247	

TTR-OF12-247-R	CTATGAGCAGAGCTGGAAGA		
TTR-OF13-265-F	GGAGTGTGGGCGGCGAA	265	
TTR-OF13-265-R	TTCTGCATCTTGGCGCACTC		
TTR-OF14-243-F	CTCAAGAGTTCCAGACCCA	243	
TTR-OF14-243-R	TACAAATAAGACCCCACATAA		
TTR-OF15-258-F	AGTGCCTCTGTGCAGTGGA	258	
TTR-OF15-258-R	TGGGACACCAGTGCTCTCT		
TTR-OF16-246-F	ATAATCCCACTACAGTCCCA	246	
TTR-OF16-246-R	GCTGGTGAGAGCATCCC		
TTR-OF17-258-F	GCCAGGGAAAGCTTGAAG	258	
TTR-OF17-258-R	CCTCTCTACTGGCAGGTCAT		
TTR-OF18-237-F	GGAGCGAACACCAGGCG	237	
TTR-OF18-237-R	CCTGCGCGAGATCGAGTC		
TTR-OF19-257-F	TGATAACGCCGCCTCTCTA	257	
TTR-OF19-257-R	ATTCTCCCTGCCAACCTTT		
TTR-OF20-272-F	AAGGTGAAGGGTTTCCAGT	272	
TTR-OF20-272-R	CTCTGGGTCTTGGCACTT		
TTR-OF21-267-F	TGACATAAGCACACCATTCT	267	
TTR-OF21-267-R	AACTATGAGCAATAAACTTCTGT		