

Supplemental Information

CRISPR/Cas9-mediated *GJA8* knockout in rabbit recapitulates human congenital cataracts

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Inventory of Supplemental Information

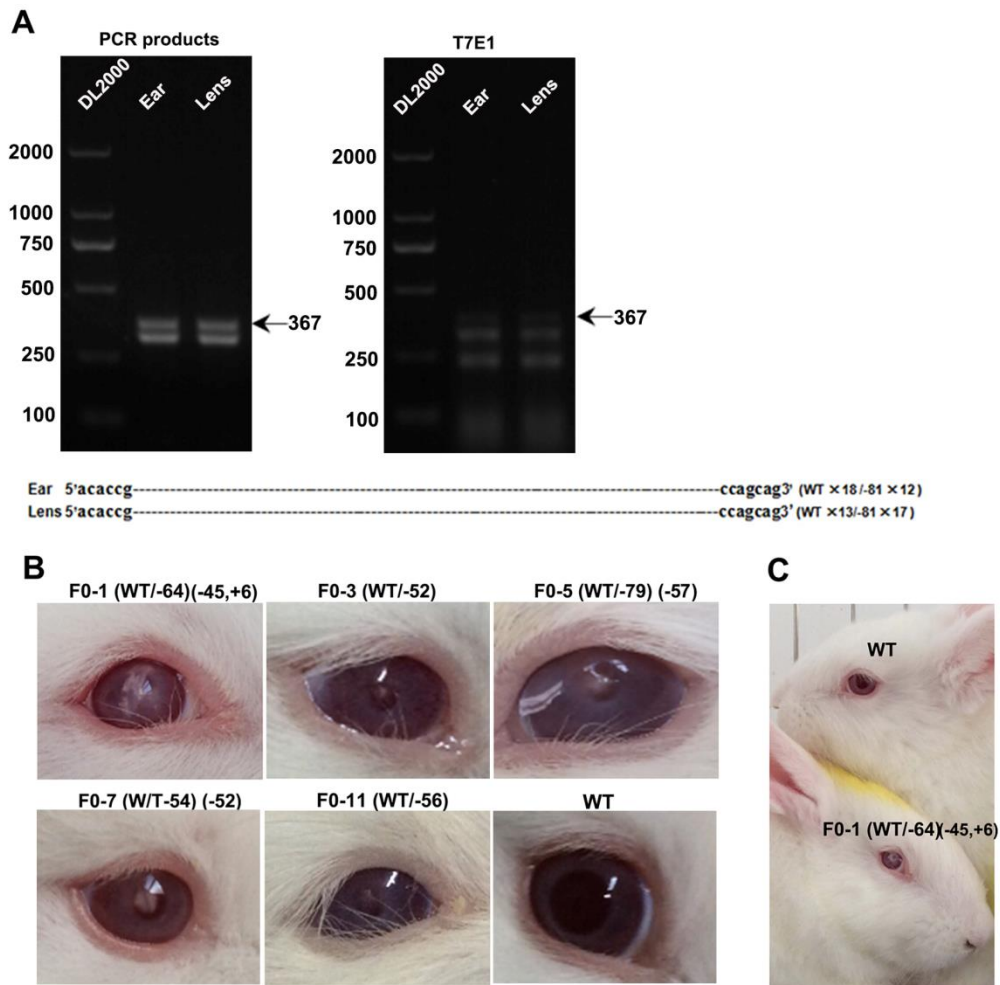
1. Supplemental Figures

2. Supplemental Tables

3. Original file for Figures

1. Supplemental Figures

Fig S1. (A) Chimeras analysis of the ear and lens from F0-8 rabbit. Black arrow indicates the band of WT allele (367 bp). DL2000, marker. (B) Typical phenotypes of cataracts were determined in founder rabbits of F0-1, F0-3, F0-5, F0-7 and F0-11. (C) Photograph of a cataractous rabbit with *GJA8* mutation at the age of 3 months old.



2 . Supplement Tables

Table S1. Two strands of oligonucleotides were used to construct the pUC57-sgRNA vector.

Target gene	Target site	PAM	Oligonucleotide1	Oligonucleotide2
S1	GGCATGGCCACGTACATGA	GGG	TAGGCATGGCCACGTACATGA	AAACTCATGTACGTGGGCCATG
S2	GGAGAAGCGCAAGGACCGCG	AGG	TAGGAGAAGCGCAAGGACCGCG	AAACCGGGTCCTTGCGCTTCT

Table S2. Alleles from the F0 generation rabbits.

Founder No.	Target site mutations	Sequenced clones	Phenotype
F0-1	WT	10	+
	Del 64 bp	12	
	Del 45 bp, insert 6	8	
F0-2	WT	18	+
	Del 52 bp	15	
F0-3	WT	17	+
	Del 53 bp	13	
F0-4	WT	19	-
	Del 57 bp	12	
F0-5	WT	12	+
	Del 79 bp	11	
	Del 57 bp	9	
F0-6	WT	14	-
	Del 51 bp	16	
F0-7	WT	8	+
	Del 52 bp	13	
	Del 54 bp	9	
F0-8	WT	18	+
	Del 81 bp	12	
F0-9	WT	21	+
	Del 56 bp; insert 3 bp	9	
F0-10	WT	11	-
	Del 51 bp	14	
	Del 44 bp; insert 3 bp	7	
F0-11	WT	14	+
	Del 12 bp	10	
	Del 56 bp; insert 3 bp	7	

Note: WT: wild type allele; Del: deletion; insert: insertion.

Table S3. Primer sequences of potential off target sites for *GJA8* gene.

	Gene locus	Potential off target sites	Mismatch	Primer sequences (5'-3')
S1	Chum:+250369	AGGGTAGCGAAAGGACCGCGGAG	[1:3:5:10]	F:AGGCACCTCAAGGCATTT R:GTGTATGCCTTCTCACACCTC
	chr1:+184316759	GGAGCAGCTCTAGGACCGCGCGG	[5:9:11]	F:TAGCGACACAGGTGGGAAA R:GGAGGCTCTACAGGACACTAATC
	chr9:+59430291	GGAGCGGCGCTAGGACCGCGGGG	[5:9:11]	F:GACAATCCAAGTCCACAGAAGT R:AGATGGGAATGGAAAGAAGGC
	chr21-698463	GCAGGAGGTCAAGGACCGCGCAG	[2:5:8:9]	F:CCAAATGCCTGCTTATGTTTCT R:GGGTCCTTATAGGAGGAAGTTAGA
	chr12-142395190	GCAAGAGCTCAAGGACCGCGAAG	[2:4:5:9]	F:CCAAATGCCTGCTTATGTTTCT R:CTTCTGTTTCTCCCTCTGATG
	chrUN0:-10369	TCAGAAGGGCCAGGACCGCGCGG	[1:2:8:11]	F:GGACTAGGAATAGCTCAACAAGAA R:GGCAGCACAATGACGATTTAC
	chr16-20577150	GCAGCAGAGCAAGGACCGCCAG	[2:5:8:20]	F:TCGCTCACCTGCCTCGGCGACG R:GTTCTGGAACCTAAGAGGCCAC
S2	chrUN0:-304499	CCCAGGGCCACGTACATGAGAG	[1:2:5]	F:GCTCTGCACAGTGACCTTTA R:CCTTCCCAGGCAAGAGTTATC
	chr1:+90977471	GGCCTGGCTCACGTACATGTGGG	[4:9:20]	F:CAAGAGTCCAACCACGTCTT R:TTCTGGGAAATGATCCGTGATAG
	chrUN0:+885137	TGTGTGGCCAGGTACATGAGGG	[1:3:4:12]	F:CGAGAGCAGGAAGCTCATAAAG R:CAGACTTGTGCTGTGAGTGTAG
	chr7:+155044047	ATCATGGCCAACGTACATGGCAG	[1:2:10:20]	F:GTAGAAAGCAGTCTGGCCTATG R:GGATGTTGAGGCAGTGAGTATATTA
	chr4:-45197482	GACATGGGTCATGTACATGAGAG	[2:8:9:12]	F:TGCTTCCAGTTATGGGTGTTAT R:TTCATATCCAATCTGCCTGTC
	chr1:+126065974	GTGATGGCTCAAGTACATGAGAG	[2:3:9:12]	F:CACCAAGTGGAAATGTGGTTAGA R:CCATGTGAGATACTGTGCTTCC
	chr15:+38358573	GGCGGAGCCCATGTACATGAAGG	[4:5:6:12]	F:CACCTCTGCTTGTCTCTAACC R:CTTCCCACCCACACTTCTATC

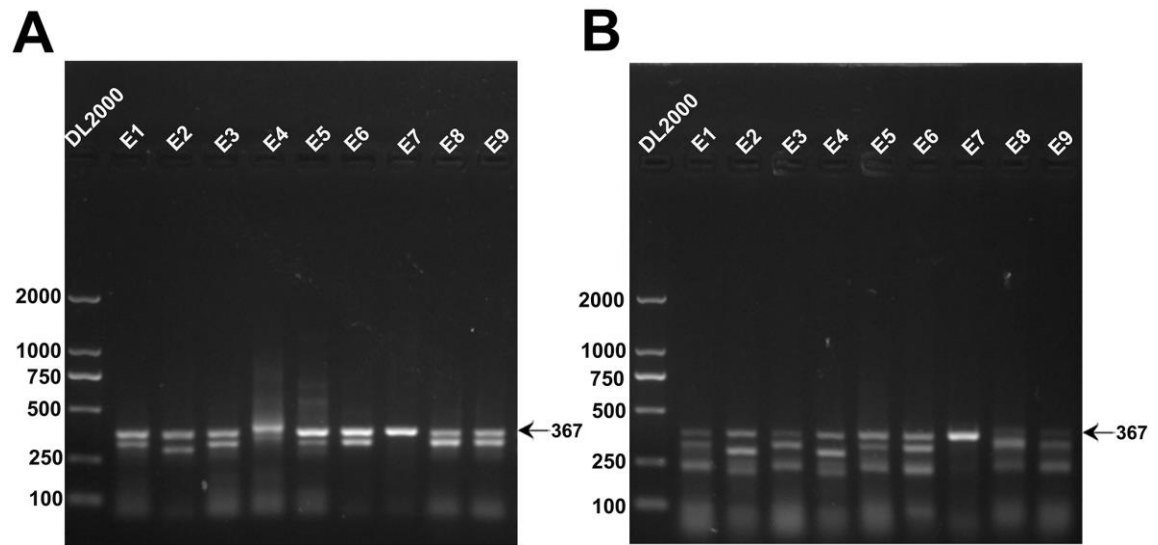
Table S4 . Alleles from the F1 generation rabbits.

Intercross-F0 (Male*Female)	F1 generation No.	Target site mutations	Sequenced cononies	Phenotype	
F0-7* F0-4	F1-1	Del 52 bp	8	+	
		Del 57 bp	12		
	F1-2	WT	15	-	
		Del 54 bp	5		
	F1-3	WT	20	-	
	F1-4	WT	11	+	
		Del 52 bp	9		
	F1-5	WT	20	-	
	F1-6	WT	14	-	
		Del 57 bp	6		
	F1-7	WT	6	-	
		Del 57 bp	14		
	F0-5* F0-8	F1-8	WT	13	+
			Del 79 bp	7	
F1-9		WT	12	+	
		Del 79 bp	8		
F1-10		WT	20	-	
F1-11		WT	7	+	
		Del 79 bp	13		
F1-12		WT	20	-	
F1-13		WT	9	+	
		Del 79 bp	11		
F1-14		WT	9	+	
		Del 79 bp	13		
F1-15		WT	20	-	
F1-16		WT	20	-	
F1-17	WT	12	+		
	Del 79 bp	9			
F0-7* F0-9	F1-18	WT	12	+	
		Del 52 bp	10		
	F1-19	WT	11	+	
		Del 52 bp	9		
	F1-20	WT	13	+	
		Del 52 bp	7		
	F1-21	WT	13	+	
		Del 54 bp	7		
	F1-22	WT	8	+	
		Del 56 bp; insert 3 bp	12		
	F1-23	WT	15	+	
		Del 52 bp	5		
F1-24	WT	13	+		
	Del 56 bp; insert 3 bp	7			

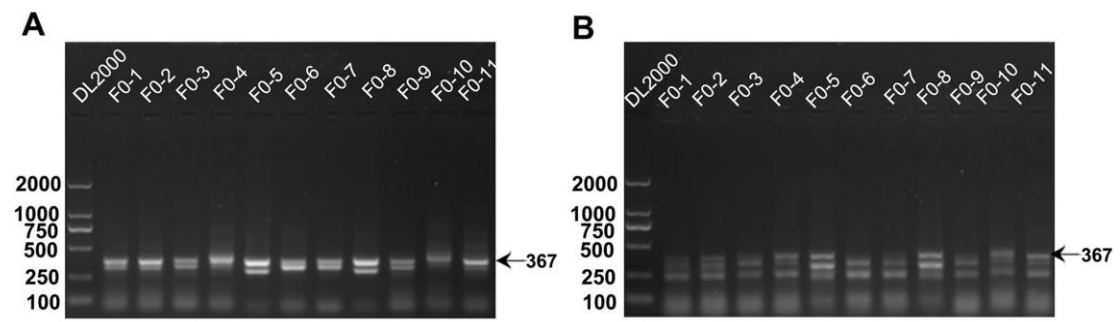
Note: WT: wild type allele; Del: deletion; insert: insertion.

3. Original file for Figures

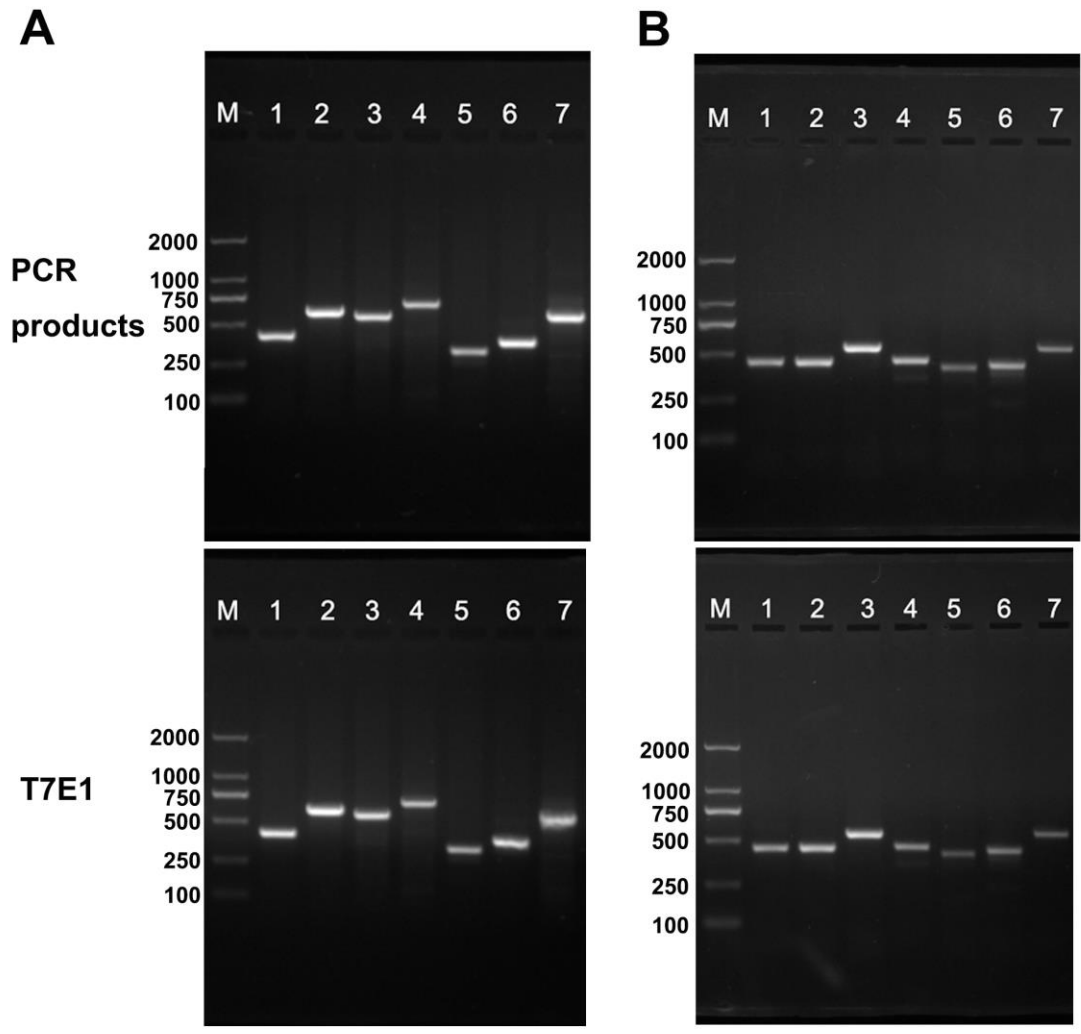
3.1 Authors' original file for figure 1C



3.2 Authors' original file for figure 2B

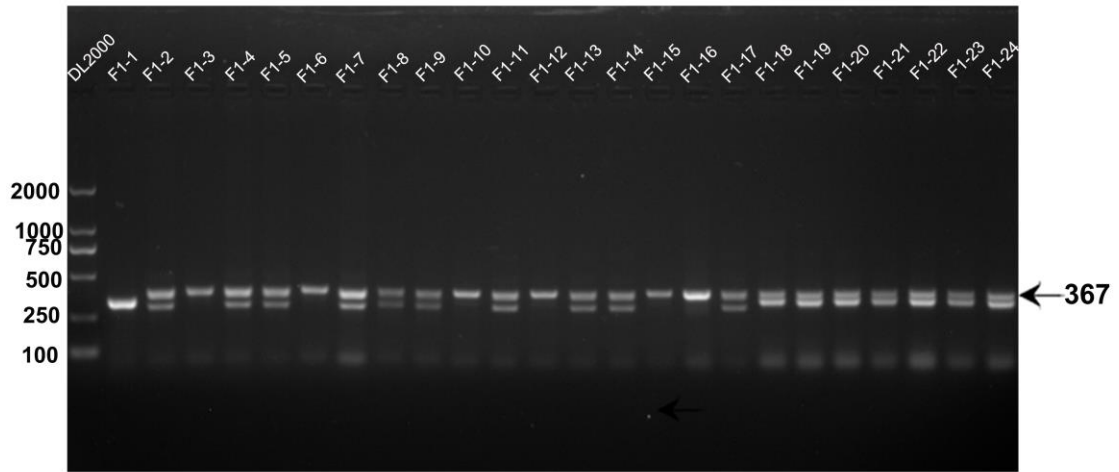


3.3 Authors' original file for figure 3C and 3D



3.4 Authors' original file for figure 5A

A



B

