OMTN, Volume *25*

Supplemental information

Modeling a cataract disorder in mice with prime editing

Jianxiang Lin, Xingchen Liu, Zongyang Lu, Shisheng Huang, Susu Wu, Wenxia Yu, Yao Liu, Xiaoguo Zheng, Xingxu Huang, Qiang Sun, Yunbo Qiao, and Zhen Liu

A

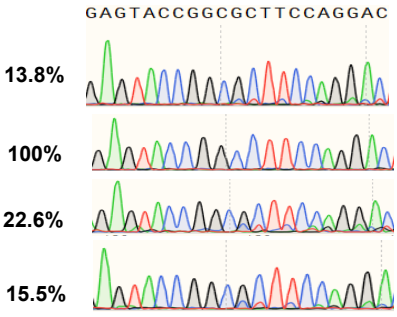
PE2+pegRNA2+nicking sgRNA plasmids

Group	Blastocyst /Injected zygote	Edited/ Sequenced	Editing efficiency (%)
Experiment 1	14/15	0/3	N/A
Experiment 2	13/14	0/13	N/A
Experiment 3	22/23	1/21	Indel

N/A: not available
Edited: number of embryos with editing efficiency > 5%
Sequenced: number of embryos successfully detected

B

Editing efficiency in blastocysts



C

PBS length	Blastocyst /Injected zygote	Edited/ Sequenced	Editing efficiency (%)
10 nt	15/19	0/15	N/A
12 nt	16/16	1/16	10.5%
13 nt	13/17	1/13	15.8%
14 nt	18/18	0/16	N/A
16 nt	13/16	0/13	N/A
17 nt	16/16	0/16	N/A

N/A: not available
Edited: number of embryos with editing efficiency > 5%
Sequenced: number of embryos successfully detected

Figure. S1 Additional data for prime editing *in vivo*.

A. The editing efficiency in blastocysts induced by PE2, pegRNA2, and a nicking sgRNA (three components are all plasmids). Three independent experiments were performed, and the number of blastocysts was presented. Only one blastocyst with indel was identified in experiment 3.

B. Original sanger sequencing data in Figure 2A.

C. The effect of PBS length on prime editing efficiency was examined. pegRNAs with indicated length of PBS were constructed, and pegRNAs were co-injected with PE2 and nicking sgRNA plasmids into zygotes for editing efficiency analysis in blastocysts.

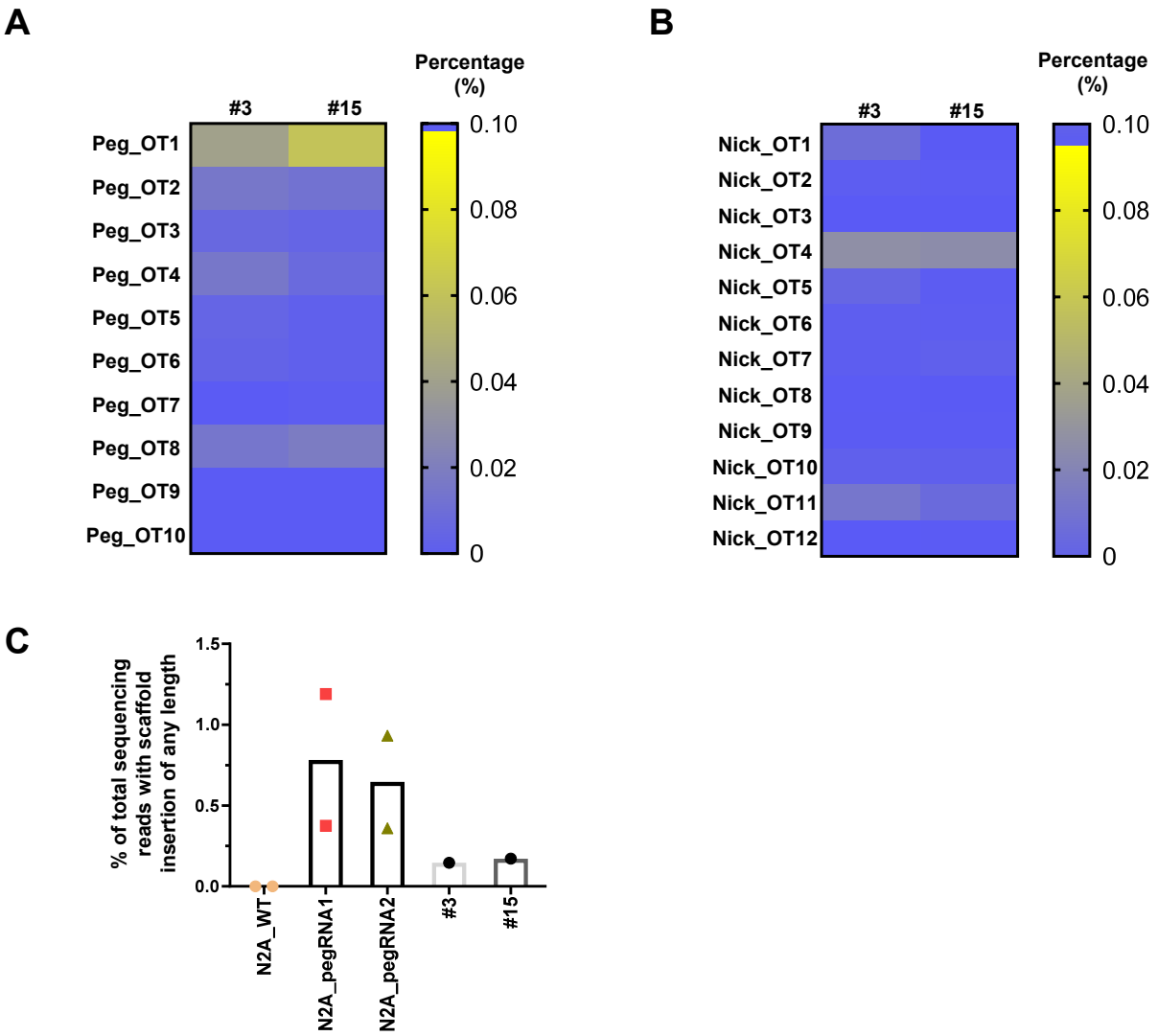


Figure. S2 Off-target analysis.

A. The indel efficiencies (potential off-targeting nucleotide-deletion efficiencies) in two PE3-generated mutant mice. The potential off-target sites for the pegRNA1 were predicted by Cas-OFFinder3, and off-targeting efficiency was measured by targeted deep sequencing.

B. The indel efficiencies induced by the nicking sgRNA in two PE3-generated mutant mice. The potential off-target sites for the nicking sgRNA were predicted by Cas-OFFinder3, and off-targeting efficiency was measured by targeted deep sequencing.

C. The percentage of total sequencing reads containing one or more pegRNA scaffold sequence nucleotides within an insertion adjacent to the RT template was analyzed in PE3 editing experiments in N2A cells (Figure 1F) and edited mice (#3 and #15). Wildtype (WT) N2A cells served as a control.

Table S1. Synthesized DNA oligo sequences for pegRNA construction.

Primer Name	**Primer sequences (5' to 3')**
Crygc-pegRNA-spacer-Forward	accgagccccagtcctggaagcgcgtttt
Crygc-pegRNA-spacer-Reverse	ctctaaaacgcgcttccaggactggggct
Crygc-pegRNA1-3' extension oligo-Forward	gtgcgagtaccggccttccaggactgg
Crygc-pegRNA1-3' extension oligo-Reverse	aaaaccagtcctggaaggccggtactc
Crygc-pegRNA2-3' extension oligo-Forward	gtgccaagagtaccggccttccaggactgg
Crygc-pegRNA2-3' extension oligo-Reverse	aaaaccagtcctggaaggccggtactcttg
Scaffold Forward	agagctagaaatagcaagttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcg
Scaffold Reverse	gcaccgactcggtgccacttttcaagttgataacggactagcctattttaacttgctatttctag
Crygc-pegRNA-spacer-Forward-for correction	accgagccccagtcctggaaggcgtttt
Crygc-pegRNA-spacer-Reverse-for correction	ctctaaaacgccttccaggactggggct
Crygc-pegRNA3-3' extension oligo-Forward-for correction	gtgccaagagtaccggcgcttccaggactgg
Crygc-pegRNA3-3' extension oligo-Reverse-for correction	aaaaccagtcctggaagcgccggtactcttg

Table S2. PCR primer sequences for genotyping.

Primer Name	**Primer sequences (5' to 3')**
Crygc genotyping PCR-Forward	tgatggagctgagcgaggattg
Crygc genotyping PCR-Reverse	cagggtttgccaacaatacagac
Crygc genotyping PCR-Forward-Index1	ATCACGtgatggagctgagcgaggattg
Crygc genotyping PCR-Reverse-Index2	CGATGTTcagggtttgccaacaatacagac
Crygc genotyping PCR-Forward-Index3	TTAGGCAGtgatggagctgagcgaggattg
Crygc genotyping PCR-Reverse-Index4	TGACCAGcagggtttgccaacaatacagac
Crygc genotyping PCR-Forward-Index5	ACAGTGCTtgatggagctgagcgaggattg
Crygc genotyping PCR-Reverse-Index6	GCCAATcagggtttgccaacaatacagac

Table S3. Summary of Off-target sites.

Target sequence	Bulge Type	Bulge size	Mismatch	Number of Found Targets
AGCCCCAGTCCTGGAAGCGCNGG	X	0	0	1
AGCCCCAGTCCTGGAAGCGCNGG	X	0	1	0
AGCCCCAGTCCTGGAAGCGCNGG	X	0	2	1
AGCCCCAGTCCTGGAAGCGCNGG	X	0	3	27
AGCCCCAGTCCTGGAAGCGCNGG	X	0	4	182
AGCCCCAGTCCTGGAAGCGCNGG	X	0	5	1688
TATGAGATGCCTAACTACCGNGG	X	0	0	1
TATGAGATGCCTAACTACCGNGG	X	0	1	1
TATGAGATGCCTAACTACCGNGG	X	0	2	3
TATGAGATGCCTAACTACCGNGG	X	0	3	8
TATGAGATGCCTAACTACCGNGG	X	0	4	46
TATGAGATGCCTAACTACCGNGG	X	0	5	479

Table S4. Potential off-target sites for pegRNA1 and primers for detecting potential off-targeting efficiency.

Name	Sequence (5’ to 3’)
pegRNA off-target site1	AGCCCCTGTCCTGGGAGCCCAGG
pegRNA off-target site1 PCR-Forward	GATACACAGATGATAGGCAG
pegRNA off-target site1 PCR- Reverse	TTGTCTGTCAGTGACTGGTT
pegRNA off-target site2	AGCCCCAGCCCTGGAAGTGGAGG
pegRNA off-target site2 PCR-Forward	CCGTTTCACCACCCATAAGG
pegRNA off-target site2 PCR-Reverse	TTAGTGTAGGCTGTGTACCT
pegRNA off-target site3	AGCCCCACTCTTGGAAACGCTGG
pegRNA off-target site3 PCR-Forward	CAAAGGAAGAATGCTGACC
pegRNA off-target site3 PCR-Reverse	GAAGACACTCCGATTTCAT
pegRNA off-target site4	AGCCCCAGTCCTGGAAAATCGGG
pegRNA off-target site4 PCR-Forward	CAACCAAGAGATGAGTAGCT
pegRNA off-target site4 PCR- Reverse	ATGGAGACAGAGATGACAAG
pegRNA off-target site5	AGCCCCGGGCCAGGAAGCGCTGG
pegRNA off-target site5 PCR-Forward	ATCTGACAGACAGTGTGTG
pegRNA off-target site5 PCR-Reverse	GAGGAGGAATCCTGATCACA
pegRNA off-target site6	AACGCCAGTCCTGGAAGAGCTGG
pegRNA off-target site6 PCR-Forward	TGTTCACCTTGGTAACCTG
pegRNA off-target site6 PCR-Reverse	CCACCACCTGAAATGCAAT
pegRNA off-target site7	AGACCCACTCGTGGAAGCGCAGG
pegRNA off-target site7 PCR-Forward	TTACCTGTAAGGTGTCACAG
pegRNA off-target site7 PCR-everse	TATGTGTCTGTGTGTGTGTC
pegRNA off-target site8	AGCCCCAGCCCTGGCAGCCCTGG
pegRNA off-target site8 PCR-Forward	ACAGATGAAGATGCTGCTT
pegRNA off-target site8 PCR- Reverse	ATGGTTAACCCAGAGAAGG
pegRNA off-target site9	AGGCTCAGTCCAGGAAGCGCTGG
pegRNA off-target site9 PCR-Forward	ACATCATCTCCCTGTGTTC
pegRNA off-target site9 PCR-Reverse	CATGCTAAGCCATTACCTG
pegRNA off-target site10	AGCCTCAGTCCTGGAGGCGATGG
pegRNA off-target site10 PCR-Forward	ATAGTTCTGGACAAGTGGC
pegRNA off-target site10 PCR-Reverse	TGTGACACCCAGTCCTCTT

Table S5. Potential off-target sites for nicking sgRNA and primers for detecting potential off-targeting efficiency.

Name	Sequence (5' to 3')
Nicking off-target site1	TATGAGATGCCCAACTACCGAGG
Nicking off-target site1 PCR-Forward	CTTGTGTCTGAGCTCATGGAC
Nicking off-target site1 PCR- Reverse	GGCTGTAACAAGCAAAAGGAG
Nicking off-target site2	TACGAGATGCCCAACTACCGGGG
Nicking off-target site2 PCR-Forward	CTACAGAGGCCAAATGGTGGA
Nicking off-target site2 PCR- Reverse	CTGTCCAGATGGAGAAAATGG
Nicking off-target site3	TATGAGATGCCTAGCTACAGAGG
Nicking off-target site3 PCR-Forward	GATGACTTCAGAGGACAAATG
Nicking off-target site3 PCR- Reverse	GGCTCTAGAGGAGGAAAGTA
Nicking off-target site4	TACGAGATGCCCAACTACCGGGG
Nicking off-target site4 PCR-Forward	ATCAGGATCTACGAGCGAGA
Nicking off-target site4 PCR- Reverse	CCATGATTCTCCTCAGAGAG
Nicking off-target site5	TATTAGACGCCTAACTACTGAGG
Nicking off-target site5 PCR-Forward	GAGTTAACCCTTGCAGCAAA
Nicking off-target site5 PCR- Reverse	TTAGCGGCTCAGTTACAGTA
Nicking off-target site6	TATGAGGAGCCAAACTACCGCGG
Nicking off-target site6 PCR-Forward	TCAAGGTAGTGAGGAGCTAG
Nicking off-target site6 PCR- Reverse	GTACGCAGTTGGGACTAGAA
Nicking off-target site7	TATAAGACGCCTAACTACTGAGG
Nicking off-target site7 PCR-Forward	TGAGTTAACCCTTGCAGCAA
Nicking off-target site7 PCR- Reverse	GGCTCAGTTACAGTACTCAG
Nicking off-target site8	TGTGAGATGCCTACCTACTGTGG
Nicking off-target site8 PCR-Forward	GAAATGGGCATTCCTATTAG
Nicking off-target site8 PCR- Reverse	TGTCTTGAATACCTCCCTCC
Nicking off-target site9	TATGAGCTACCCAACTACCGTGG
Nicking off-target site9 PCR-Forward	AGATGTACGAAACCACGGAA
Nicking off-target site9 PCR- Reverse	TCAGGGAGAACTCTATGGTC
Nicking off-target site10	TATGAGATGTATAACTACCTAGG
Nicking off-target site10 PCR-Forward	GGGTGAGATGTACTATCATGG
Nicking off-target site10 PCR- Reverse	TCTGTACCAACATAGCCTT
Nicking off-target site11	TATGAGAGCCCTAACTACAGAGG
Nicking off-target site11 PCR-Forward	GGGTCCATGTTTATCAAAGG
Nicking off-target site11 PCR- Reverse	GTTTCTCTGCTCTCAGATC
Nicking off-target site12	TATGAGGTGCCTACCTACAGTGG
Nicking off-target site12 PCR-Forward	TTGGACTGTAGCCAGATTGA
Nicking off-target site12 PCR- Reverse	TAGGTTGAAATAACTTCAA