

Supplementary information

Efficient gene editing in a medaka (*Oryzias latipes*) cell line and embryos by SpCas9/tRNA-gRNA

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Table S1 List of the primers used for amplification

Primer name	Gene ID	Sequence (5' to 3')	Annealing temperature (°C)	Usage
<i>tyr</i> gRNA F	100049427	TGTAATACGACTCACTATAGGGGACAAACCTCTGACCTG TGGTTTTAGAGCTAGAAAT	60	Synthesize <i>tyr</i> sgRNA
<i>tyr</i> gRNA R	100049427	AAAAGCACCGACTCGGTGCC	60	
<i>tyr</i> check F	100049427	CGAGTACGCCTACCTGTT	60	Amplify <i>tyr</i>
<i>tyr</i> check R	100049427	CTAGATGTGGTCGGTGAGA	60	
<i>pax6.1</i> check F	100049356	TTTGCTCATTGATACTGTTGTGGGT	60	Amplify <i>pax6.1</i>
<i>pax6.1</i> check R	100049356	ATGTCATGCCTTTGTTGCCC	60	
PT-F		TTCCCGGCTGGTGCATGCCTGGTGAATCCGGCAGGTTT TAGAGCTAGAAATAGC	60	Construct pCS2-tRNA-PT plasmid
PT-R		TTCTAGCTCTAAAACATCTGTGGCTCTGGACTGTGCA CCAGCCGGGAATCGAAC	60	
all-F		AAAGCTAGCGTCGACCATAGCCAATTCAATATG	60	Construct pCMV-tRNA-PT-zCas9-puro plasmid
all-R		AAAGCTAGCAAAAAAGCACCGACTCGGTG	60	
zCas9 Afe I F		CGCATGCTGGCAAGCGCTGGAGAGCTGCAGAAAGG	60	Construct pCV-zCas9-puro plasmid
zCas9 Afe I R		GCCCGCTCCGAATCCACCTTTCTCTTCTCTTAGG	60	
zCas9 puro F		GAATTCGGAAGCGGGCAGTGCACCAACTACGCCCTG	60	
zCas9 puro R		ATTGGGTACCTCTAGACATGCCTGCTATTCTCTTCC	60	
CMV-T7 F		CCCAAGCTTGGGCTCGAGCGGGCCAGATATACGCGTTG	58	Construct pCV-zCas9 plasmid
CMV-T7 R		CCCAAGCTTGGGCTCGACGTGGCGGCTCTCCCTATAGTG	58	

Sequence S1>tRNA sequence

AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCGGCT
GGTGCA

Sequence S2>scaffold sequence

GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGAGTCG
GTGC

Sequence S3>*tyr* gRNA sequence

GGACAAACCTCTGACCTGTG

Sequence S4> tRNA: *BbsI-BbsI*: scaffold-tRNA: *tyr* gRNA scaffold sequence

AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCGGCT
GGTGCAAGGTTCTTCGAGAAGACCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA
ACTTGAAAAAGTGGCACCAGTCCGGTGCAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCAC
GGTACAGACCCGGGTTTCGATTCCCGGCTGGTGCAAGACAAACCTCTGACCTGTGTTTTAGAGCTAGAAAT
AGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCCGGTGCTTTTTT

Sequence S5>*pax6.1* gRNA sequence

TGCCTGGTGAATCCGGCAG

Sequence S6> CMV-tRNA-*pax6.1* gRNA-scaffold-tRNA-*tyr* gRNA-scaffold sequence

GTCGACCATAGCCAATTCAATATGGCGTATATGGACTCATGCCAATTCAATATGGTGGATCTGGACCTGTGCCA
ATCAATATGGCGTATATGGACTCGTGCCAATTCAATATGGTGGATCTGGACCCAGCCAATTCAATATGGCG
GACTTGGCACCATGCCAATTCAATATGGCGGACTTGGCACTGTGCCAACTGGGGAGGGGTCTACTTGGCAC
GGTGCCAAGTTTGAGGAGGGGTCTTGGCCCTGTGCCAAGTCCGCCATATTGAATTGGCATGGTGCCAATAAT
GGCGCCATATTGGCTATATGCCAGGATCAATATATAGGCAATATCCAATATGGCCCTATGCCAATATGGCTATT
GGCCAGGTTCAATACTATGTATTGGCCCTATGCCATATAGTATTCCATATATGGGTTTTCTATTGACGTAGATA
GCCCCTCCAATGGGCGGTCCCATATACCATATATGGGGCTTCTAATACCGCCCATAGCCACTCCCCATTGA
CGTCAATGGTCTCTATATATGGTCTTCTCTATTGACGTCAATATGGGCGGTCTATTGACGTATATGGCGCCTCCC
CCATTGACGTCAATTACGGTAAATGGCCCGCTGGCTCAATGCCATTGACGTCAATAGGACCACCCACCATT
GACGTCAATGGGATGGCTCATTGCCATTCAATATCCGTTCTCACGCCCCCTATTGACGTCAATGACGGTAAAT
GGCCCACTTGGCAGTACATCAATATCTATTAATAGTAACTTGGAAGTACATTACTATTGGAAGGACGCCAGG
GTACATTGGCAGTACTCCATTGACGTCAATGGCGGTAAATGGCCCGGATGGCTGCCAAGTACATCCCATT
GACGTCAATGGGGAGGGGCAATGACGCAAATGGCGTTCATTGACGTAAATGGGCGGTAGGCGTGCCTA
ATGGGAGGTCTATATAAGCAATGCTCGTTAGGGAACCGCCATTCTGCCTGGGGACGTCCGAGCAAGCTTG
ATTTAGGTGACACTATAGAATACAAGTACTTGTCTTTTTGAGGATCCCATCGATTTCGAATTAACAAAGC
ACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCGGCTGGTGCA
CTGGTGAATCCGGCAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAA
AAAGTGGCACCAGTCCGGTGCAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAG
ACCCGGGTTTCGATTCCCGGCTGGTGCAAGACAAACCTCTGACCTGTGTTTTAGAGCTAGAAATAGCAAGT
TAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCCGGTGCTTTTTT

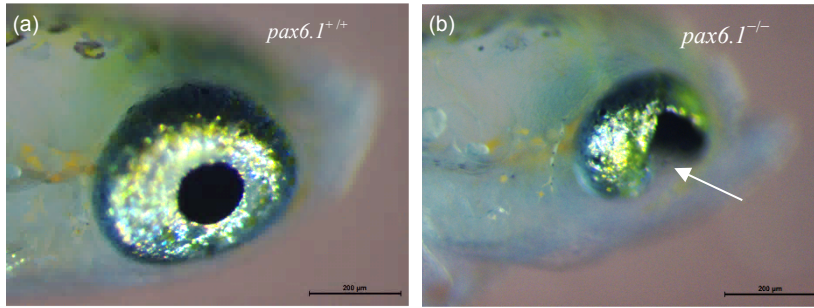


Fig. S1 Medaka eye deformities induced by *pax6.1* mutation. (a) *pax6.1^{+/+}*; (b) *pax6.1^{-/-}*. The white arrow shows eye hypoplasia. Scale bar: 200 µm.