

Efficient PAM-Less Base Editing for Zebrafish Modeling of Human Genetic Disease with zSpRY-ABE8e

 Shaohui Zheng^{1,2}, Fang Liang³, Yu Zhang^{1,2}, Ji-Feng Fei⁴, Wei Qin^{1,2}, Yanmei Liu^{1,2}

¹Key Laboratory of Brain, Cognition and Education Science, Ministry of Education ²Institute for Brain Research and Rehabilitation, Guangdong Key Laboratory of Mental Health and Cognitive Science, South China Normal University ³Institute of Modern Aquaculture Science and Engineering, Guangdong Provincial Engineering Technology Research Center for Environmentally Friendly Aquaculture, Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, School of Life Sciences, South China Normal University ⁴Department of Pathology, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences

Corresponding Authors

Wei Qin

wei-qin@omrf.org

Yanmei Liu

yanmeiliu@m.scnu.edu.cn

Citation

Zheng, S., Liang, F., Zhang, Y., Fei, J.F., Qin, W., Liu, Y. Efficient PAM-Less Base Editing for Zebrafish Modeling of Human Genetic Disease with zSpRY-ABE8e. *J. Vis. Exp.* (192), e64977, doi:10.3791/64977 (2023).

Date Published

February 17, 2023

DOI

10.3791/64977

URL

jove.com/video/64977

Materials

Name	Company	Catalog Number	Comments
Agarose	Sigma-Aldrich	A9539	1.5% Agarose used to make an injection plate
Borosilicate Glass Capillaries	Harvard Apparatus	BS4 30-0016	
Cell culture dishes	Falcon	351029	
ClonExpress Ultra One Step Cloning Kit	Vazyme	C115	Kit for Infusion clone
Codon optimization service	Sangon Biotech		
Drummond Microcaps	Drummond Microcaps	P1299-1PAK	Length:32 mm, capacity:0.5 µL
EasyEdit gRNA service	GenScript		
Fine Forceps	Fine Scientific Instrument	11254-20	Used to break meedle
Flaming/Brown Micropipette Puller	Stutter Instrument	P-97	Used to pull the glass capillaries
HotStart Taq PCR StarMix	Genstar	A033-101	Used for PCR reaction
Intelligent artificial climate box	TENLIN	PRX-1000A	Used to culture zebrafish embryos
Methylcellulose	Sigma-Aldrich	M0512	Used to fix zebrafish when photographing
Microloader pipette tips	Eppendorf	5242956003	
mMACHINE kit			
Mut Express II Fast Mutagenesis Kit V2	Vazyme	C214-01	Kit for site-directed mutagenesis
Pneumatic Microinjector	ZGene Biotech	ZGPCP-1500 PLUS	
pT3TS-zSpCas9	Addgene	46757	
RNeasy FFPE kit	Qiagen	73504	Kit for RNA purification
Sanger Sequencing service	Sangon Biotech		
Sodium hydroxide, granular	Sangon	A100173-0500	NaOH used for genome extraction
Stereo Microscope	Olympus	SZX10	Used for photograph of phenotype
SZ Series Zoom Stereo Microscope	CNOPTec	SZ650	

T3 mMESSAGE	Ambion	AM1348	Kit for in vitro transcription
TIANprep Mini Plasmid Kit	TIANGEN	DP103-03	Kit for plasmid extraction kit
TIANquick Mini Purification Kit	TIANGEN	DP203-02	Kit for purification for linearized plasmid
Tricaine	Sigma-Aldrich	E10521	Used to anesthetize zebrafish
Tris (hydroxymethyl) aminomethane	Sangon	A600194-0500	Component of Tris-HCl used for genome extraction
<i>Xba</i> I	New England Biolabs	R0145S	Restriction endonuclease used for plasmid linearization