

Screening Sperm for the Rapid Isolation of Germline Edits in Zebrafish

Brittney Voigt¹, Ryoko Minowa², Ryan S. Gray^{1,2}

¹Department of Molecular Biosciences, The University of Texas at Austin ²Department of Nutritional Sciences, The University of Texas at Austin

Corresponding Author

Ryan S. Gray

ryan.gray@austin.utexas.edu

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jove.com/video/64686

Materials

Name	Company	Catalog Number	Comments
Agarose powder	Fisher BioReagents	BP1356-100	
Breeding tanks	Carolina Biological	161937	
BstNI Restriction Enzyme	NEB	R0168S	
Cas9 Endonuclease	IDT	1081060	
DNA Ladder, 100 bp	Thermo Scientific	FERSM0241	
dnah10 donor construct	Sigma-Aldrich	DNA Oligo in Tube; 0.025 nM, standard desalt purification, dry. Phosphorothioate bond on the donor at the first three phosphate bonds on both the 5' and 3' ends (5'-CCTCTCTCCCTTTCAGAAGCTTC TGCTCATCCGCTGCTTCTGCCT GGACCGAGTGTACCGTGCCGTC AGTGATTACGTCACGC-3')	
dnah10 forward primer	Sigma-Aldrich	DNA Oligo in Tube; 0.025 nM, standard desalt purification, dry (5'-CATGGAAGTCTTCTCTAATGAGT TTGGC-3')	
dnah10 reverse primer	Sigma-Aldrich	DNA Oligo in Tube; 0.025 nM, standard desalt purification, dry (5'-AGTAGAGATCACACATCAACAGA ATACAGC-3')	
dnah10 synthetic sgRNA	Synthego	Synthetic sgRNA, target sequence: 5'-GCTCATCCGCTGCTTCAGGC-3'	
Electrophoresis power supply	Thermo Scientific	105ECA-115	
Filter forceps	Millipore	XX6200006P	
Fish (system) water	Generic	n/a	
Gel electrophoresis system (including casting frame, comb, and electrophoresis chamber)	Thermo Scientific	B2	
Gel imaging light box	Azure Biosystems	AZI200-01	
Gel stain, 10000X	Invitrogen	S33102	
Glass bowl, 250 mL	Generic	n/a	
Isolation tanks, 0.8 L	Aquaneering	ZT080	

Microcapillary tube with bulb, 20 μ L	Drummond	1-000-0020/CA	
Minicentrifuge	Bio-Rad	12011919EDU	
Micropipettes, various with appropriate tips	Generic	n/a	
Microwave	Generic	n/a	
Nuclease free water	Promega	P119-C	
Paper towels	Generic	n/a	
PCR tubes, 0.2 mL	Bioexpress	T-3196-1	
Plastic spoon, with drilled holes/slots	Generic	n/a	
KCl solution, 0.2 M RNase Free	Sigma-Aldrich	P9333	
p2ry12 forward primer	Sigma-Aldrich	DNA Oligo in Tube; 0.025 nM, standard desalt purification, dry (5'-CCCAAATGTAATCCTGACCAGT-3')	
p2ry12 reverse primer	Sigma-Aldrich	DNA Oligo in Tube; 0.025 nM, standard desalt purification, dry (5'-CCAGGAACACATTAACCTGGAT-3')	
p2ry12 synthetic sgRNA	Synthego	Synthetic sgRNA, target sequence: 5'-GGCCGCACGAGGTCTCCGCG-3'	
Restriction Enzyme 10X Buffer	NEB	B6003SVIAL	
NaOH solution, 50 mM	Thermo Scientific	S318; 424330010	
Sponge, 1-inch x 1-inch cut with small oval divot	Generic	n/a	
Stereomicroscope	Zeiss	Stemi 508	
Taq polymerase master mix, 2X	Promega	M7122	
TBE Buffer Concentrate, 10X	VWR	E442	
Thermal Cycler	Bio-Rad	1861096	
Tissue paper	Fisher Scientific	06-666	
Tricaine-methanesulfonate solution (Syncline, MS-222), 0.016% in fish water (pH 7.0 $\pm$ 0.2)	Syndel	200-266	
Tris Base, 1M (Buffered with HCl to pH 8.0)	Promega	H5131	