

Materials List for:

Efficient Production and Identification of CRISPR/Cas9-generated Gene Knockouts in the Model System *Danio rerio*

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Materials

Name	Company	Catalog Number	Comments
CRISPOR			sgRNA analysis software. http://crispor.tefor.net/
Breaking-Cas			sgRNA analysis software. https://omictools.com/breaking-cas-tool
ChopChop			sgRNA analysis software. https://chopchop.rc.fas.harvard.edu/
Primer 3			PCR primer design. http://bioinfo.ut.ee/primer3-0.4.0/
Rnase free technique			http://genomics.no/oslo/uploads/PDFs/workingwithrna.pdf
RNase-free water	Any brand		Synthesis of guide RNAs. Thermo Fisher and Qiagen both carry suitable water.
RNase-free ethanol	Any brand		Molecular biology grade ethanol is RNase free. Fischer, VWR sell suitable ethanol.
Cas9 Protein	PNA Bio	CP01	Cas9 protein with nuclear localization signal.
Target specific oligos	Integrated DNA Technologies (IDT)		Synthesize guide RNAs.
PCR primers flanking guide RNA cut site	Integrated DNA Technologies (IDT)		Use for heteroduplex analysis, 100-300 bp product.
PCR primers flanking guide RNA cut site	Integrated DNA Technologies (IDT)		Use for sequencing CRISPR-alleles, 300-600 bp product recommended.
EnGen sgRNA Synthesis Kit	New England BioLabs	E3322	In vitro transcribe guide RNAs.
10X TBE	Thermo Fisher	B52	RNase-free buffer for electrophoresis of nucleic acids.
6X Load Dye	New England BioLabs	B7025S	Loading DNA for electrophoresis.
5M Ammonium acetate	Thermo Fisher	AM9071	Clean up reagent for purification of guide RNAs.
Ethanol (200 proof, nuclease-free)	Any brand		Clean up reagent for purification of guide RNAs. Molecular grade materials are RNase free.
Nuclease-free Water	Any brand		For synthesis and purification of guide RNAs.
RNase away	Thermo Fisher	10328011	Eliminates RNase contamination from surfaces, equipment, glassware.
DNA Ladder, 100 bp	New England BioLabs	N0467S	Molecular weight standards for gel electrophoresis of DNA.

DNA Ladder, 1 kb	New England BioLabs	N0552S	Molecular weight standards for gel electrophoresis of DNA.
RNA Ladder	New England BioLabs	N0364S	Single strand RNA marker to verify that full length sgRNA has been synthesized
TEMED	Thermo Fisher	17919	Casting polyacrylamide gel.
Ammonium persulfate (APS)	Sigma-Aldrich	A3678	Casting polyacrylamide gel.
40% Polyacrylamide (19:1)	BioRad	161-0154	Casting denaturing polyacrylamide gel.
Urea	Sigma-Aldrich	U5378-100G	Casting denaturing polyacrylamide gel.
RNA Gel Loading Dye (2x)	Thermo Fisher	R0641	Loading guide RNA onto denaturing gel for electrophoresis.
Ethidium bromide	Sigma-Aldrich	E1510-10ML	Visualization of nucleic acids.
SYBER Green	Thermo Fisher	S7563	Alternate method to ethidium bromide for detection of dsDNA in agarose or polyacrylamide gels.
Microcentrifuge	Eppendorf	5424	RNA clean up, PCR purification.
MyTaq Polymerase	Bioline	BIO-21105	For amplification of DNA using PCR.
Thermocycler	Any brand		PCR amplification.
Spectrophotometer	Any brand		NanoDrop or related product to quantify the amount of DNA/RNA.
E3 embryo media	Made in house		Media for raising embryos and making injection plate. Recipe: http://cshprotocols.cshlp.org/content/2011/10/pdb.rec66449
Methylene Blue	Sigma-Aldrich	M9140	Added to 1x E3 media to prevent fungal growth on embryos.
Petri dish	Thermo Fisher	FB0875711Z	Store embryos, cast injection plate.
Agarose	Denville	CA3510-8	Casting injection plate, agarose gels.
Microinjection mold	Adaptive Science Tools	TU-1	To create wells to hold embryos during injection.
Phenol Red	Sigma-Aldrich	P0290	Dye for visualization of injection.
Mineral Oil	Sigma-Aldrich	M5904-5ML	To calibrate needle injection volume.
Transfer pipettes	Any brand		Moving embryos.
Razor blade	Thermo Fisher	11295-10	Cutting injection needle, tail clipping adult fish.
Incubator	Any brand		Maintaining embryos at 28.5 °C.
Verticle pipette puller	David Kopf Instruments	700C	Geneate needles for injection. Additional needle pulling instructions: http://cshprotocols.cshlp.org/content/2006/7/pdb.prot4651.long
Capillary tubes	Sutter Instruments	BF100-58-10	Geneate needles for injection.
Microloader tips	Eppendorf	930001007	Load solution into injection needles.
Microinjector	World Precision Instruments	PV 820	Injecting embryos.
Disecting microscope	Leica		Injecting embryos.
30% Polyacrylamide (29:1)	BioRad	161-0156	Heteroduplex gel casting.
MS-222 (Tricaine)	Sigma-Aldrich	A-5040	Anesthetize zebrafish for tail clipping and gDNA extraction

			from embryos. Recipe: https://wiki.zfin.org/display/prot/TRICAINE
Microwave	Any brand		Casting injection plate, agarose gels.
Scale	Any brand		Casting injection plate, agarose gels.
Gloves	Any brand		For all aspects of the protocol.
N2	Any brand		To expell liquid from the capillary for embryo injection.
1,000 µL tips	Any brand		For all aspects of the protocol.
200 µL tips	Any brand		For all aspects of the protocol.
10 µL tips	Any brand		For all aspects of the protocol.
PCR strip tubes	Any brand		For all aspects of the protocol.
Micropipettes	Any brand		For all aspects of the protocol.
NGS Sequencing platform: Wideseq			If your institution does not offer this service visit: https://www.purdue.edu/hla/sites/genomics/wideseq-2/ (External cost: \$35).
Software for sequence analysis			For Sanger sequencing of heterozygous fish. Visit: http://yosttools.genetics.utah.edu/PolyPeakParser/
Software for sequence analysis			SnapGene Viewer, Sequence Scanner for analysis of NGS sequencing.