

Materials List for:

Microinjection of CRISPR/Cas9 Protein into Channel Catfish, *Ictalurus Punctatus*, Embryos for Gene Editing

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Materials

Name	Company	Catalog Number	Comments
Reproboost implant	Center of Marine Biotechnology		Luteinizing hormone releasing hormone analog (LHRHa) for induction of ovulation in channel catfish females
TRICAINE-S	Western Chemical. Inc.		For sedation of brood stock fish during hormone injection and egg stripping.
Phenol red	Sigma-Aldrich	P0290	0.5%, sterile filtered
Stereo microscope	Olympus	213709	For visualizing the eggs during microinjection
Microinjector	ASI-Applied Scientific Instrumentation	Model MPPI-3	For the delivery of the injection material into the embryos
Micromanipulator	ASI-Applied Scientific Instrumentation	Model MM33	For holding and controlling the movement of the injection needle.
Eppendorf Microloader	Eppendorf	5242956.003	For loading injection solution into microinjection needles.
Vertical needle puller	David Kopf Instruments	Model 720	For pulling microinjection needles
Cas9 protein	PNA Bio Inc.	CP01	Recombinant Cas9 protein from <i>Streptococcus pyogenes</i> .
Expand High FidelityPLUS PCR System	Roche Diagnostics, USA		For PCR and amplification of DNA templates to be used in gRNA preparation
Borosilicate glass capillaries	Fisher Scientific		1 mm outer diameter (OD), for making microinjection needles.
Petri dish	VWR	25384-302	For holding the embryos during the microinjection.
Crisco	The J.M. Smucker Company		Vegetable shortening for coating spawning pans and petri dishes.
Holtfreter's solution	Home Made		59 mM NaCl, 0.67 mM KCl, 2.4 mM NaHCO ₃ , 0.76 mM CaCl ₂ , 1.67 mM MgSO ₄ to incubate the microinjected embryos till hatch.
Doxycycline hyclate USP (monohydrate)	Letco Medical	690904	Antibiotic added to Holtfreter's solution to 10 ppm to prevent bacterial infections.