

A Simple and Effective Transplantation Device for Zebrafish Embryos

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Materials

Name	Company	Catalog Number	Comments
1.0 mm glass capillary, ends cut without filament			To make the transplantation needle
1.0 mm glass capillary, ends cut with filament			To make injection needles
200 mL glass beaker			For embryo dechoriation
24-well plastic plate			To be coated with agarose in order to incubate embryos
5 cm diameter glass Petri dish			For embryo dechoriation
6-well plastic plate			To be coated with agarose in order to incubate embryos
Agarose			To coat plastic dishes
<i>dnd1</i> morpholino	Gene Tools		Sequence: GCTGGGCATCCATGTCTCCGAC CAT
Embryo medium			250 mg/L Instant Ocean salt, 1 mg/L methylene blue in reverse osmosis water adjusted to pH 7 with NaHCO ₃
Fluorescence stereomicroscope with GFP/RFP filters and light source			To assess YSL injections and germline transplantations
Glass micropipette puller	Sutter Instrument Company	P-1000	To make the transplantation needle
Glass pasteur pipette	Kimble Chase (via Fisher)	63A53WT	For pipetting embryos; the tips can be flamed to smoothen out the edge
Incubator at 28 °C			For incubating zebrafish embryos
Luer tip 25 µL Hamilton syringe, 1700 series	Hamilton	Ref: 80201	Part of the transplantation device
Manual micromanipulator with 3 axes of movement	Narishige	M-152	For controlling the transplantation device
Manual pipetting pump	Bio-Tek	Cat. # 641	For use with the glass pipettes to transfer embryos
Metal dissecting probe			For moving and rotating zebrafish embryos
Microforge	Narishige	MF2	To make the transplantation needle

Microinjection apparatus			For injection of mRNA and morpholino into embryos
Microinjection molds, triangular grooves	Adaptive Science Tools	TU-1	To prepare microinjection plates with agarose
Microinjection-molds, single wells	Adaptive Science Tools	PT-1	To prepare transplantation plates with agarose
Micropipette holder with Luer fitting for a 1.0 mm glass capillary	World Precision Instruments	MPH6S10	Part of the transplantation device
mMessage mMachin Sp6 transcription kit	Life Technologies	AM1340M	To generate capped mRNA for injection into embryos
Plasmid with <i>GFP-nos1 3'UTR</i>			Plasmid that can be transcribed to produce mRNA encoding GFP with the 3'UTR of <i>nos1</i>
Plastic petri dish 100 mm			To be coated with agarose in order to make injection and transplantation dishes
Protease from <i>Streptomyces griseus</i>	Sigma	P5147	For embryo dechorionation: Make a 5 mg/ml stock and use at 1 mg/ml to dechorionate embryos
Ringer's solution			For 1 L: Add 6.78 g of NaCl, 0.22 g of KCl, 0.26 g of CaCl ₂ and 1.19 g of HEPES; then fill to 1 L; adjust pH to 7.2; sterilize by filtration
Stereomicroscope			For injection and transplantation