

**Materials List for:****High Resolution Whole Mount *In Situ* Hybridization within Zebrafish Embryos to Study Gene Expression and Function**Babykumari P. Chitramuthu<sup>1,2</sup>, Hugh P. J. Bennett<sup>1,2</sup><sup>1</sup>Endocrine Research Laboratory and Department of Medicine, Royal Victoria Hospital<sup>2</sup>McGill University Health Centre Research InstituteCorrespondence to: Babykumari P. Chitramuthu at [babykumari.chitramuthu@mail.mcgill.ca](mailto:babykumari.chitramuthu@mail.mcgill.ca), Hugh P. J. Bennett at [hugh.bennett@mcgill.ca](mailto:hugh.bennett@mcgill.ca)URL: <https://www.jove.com/video/50644>

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**Materials**

Name	Company	Catalog Number	Comments
Taq Polymerase	Invitrogen	10342053	
TopoTA Cloning Kit- Dual Promoter pCRII-TOPO	Invitrogen	K4650-01	
HQ Mini Plasmid Purification Kit	Invitrogen	K2100-01	
Agarose	Roche	11 685 660 001	
Not1 and HindIII Restriction Enzymes	Invitrogen	15441-025, 15207-012	
Buffer Saturated Phenol, ultrapure	Invitrogen	15513039	Store at 4 °C. Eyes, skin, and respiratory tract irritant and suspected carcinogen. Care should be taken while handling.
Chloroform	Fisher	C607-1	Toxic and suspected carcinogen. Work under fume hood.
RNase-free DNase I	Roche	4716728001	Prepare small aliquots and store at -20°C.
DIG-RNA Labeling Mix with Sp6, T7 and T3 RNA Polymerase	Roche	11277073910	
RNase Inhibitor	Roche	10777-019	
3.0 M Sodium Acetate (pH 5.5)	Fisher	50-751-7355	
100% Ethanol	Commercial alcohols		
Diethyl Pyrocarbonate (DEPC)	Sigma	40718-25ML	DEPC is an eye, skin, and respiratory irritant. Avoid contact with skin and eyes. Use safety glasses, gloves, and mask.
NaOH	Fisher	SS255-1	
EDTA 0.5 M (pH 8.0)	Fisher	50-751-7404	
Instant Ocean Salt	Aquarium Systems	N/A	
Phosphate Buffered Saline (PBS)	Fisher	FL-03-0900	
Paraformaldehyde (PFA)	Sigma	P6148-1KG	Prepare and store at -20°C as 40 ml aliquots for later use. PFA is toxic. Use safety glasses, gloves, and dust mask.
Phenylthiocarbomide (PTC)	Sigma	P7629-25G	PTC is highly toxic. Use safety glasses, gloves, and dust mask.
Methanol	Fisher	A947-4	
Dumont (Watchmaker's) Forceps pattern no. 5	Fine Science Tools		
Six-well Cell Culture Cluster	Sarstedt	83.1839	

Baskets are made of nylon mesh and Eppendorf tubes	In-house preparation		To make the baskets, cut Eppendorf tubes (1.5 ml) with or without rims to remove the conical end. Cut nylon mesh into small pieces corresponding to the size of the cut ends of the Eppendorf tubes. Place tubes with the nylon mesh covering the cut end on an electrical hot plate until both the tube and nylon mesh stick together (carry out in fume hood). Cut off the excess mesh from the baskets and store them in 100% methanol until used.
Polyoxyethylenesorbitan Monolaurate (Tween 20)	Sigma	P1379-500ML	
Proteinase K	Fermentas	EO0491	
Acetic Anhydride	Sigma	242845	
Triethanolamine	Sigma	90279-100ML	
Formamide, high purity grade	Sigma	F9037	
AG501-X8 Resin	Bio Rad	142-6424	
Citric Acid monohydrate	Sigma	C0706-500G	
Heparin Sodium Salt	Sigma	H3393-25KU	
Saline-sodium Citrate Buffer (SSC)	Sigma	S6639	
tRNA from bakers yeast type X	Sigma	R5636-1ML	
NaCl	Fisher	S640-500	
Tris-HCl	Fisher	BP153-1	
MgCl <sub>2</sub>	Fisher	S25403	
Levamisole Hydrochloride	Sigma	31742	
N,N-Dimethylformamide anhydrous (DMF)	Sigma	227056	Irritant, toxic, combustible, and suspected teratogen. Handle with proper safety attire including gloves and goggles.
5-Bromo-4-chloro-3-indolyl phosphate (BCIP)	Sigma	N6639	Protect this solution from light.
Nitroblue tetrazolium (NBT)	Sigma	840W	Protect this solution from light.
Albumin from Bovine Serum, purified fraction V (BSA)	Sigma	A8806	
Sheep Anti-digoxigenin-AP Fab Fragments	Roche	1093274910	
Glycerol	Sigma	G5516-500ML	
96-well cell culture plates	Sarstedt	83.1835	
Tg(mnx1:GFP)ml2/ Tg(hb9:GFP)ml2	ZIRC	Tg(mnx1:GFP)ml2	
Instant Ocean	Aquarium Systems	N/A	