

Materials List for:

Fast and Efficient Expression of Multiple Proteins in Avian Embryos Using mRNA Electroporation

Martin Tran¹, Mohit Dave^{2,3}, Rusty Lansford^{1,2,3}

Correspondence to: Rusty Lansford at lansford@usc.edu

URL: https://www.jove.com/video/59664

DOI: doi:10.3791/59664

Materials

Name	Company	Catalog Number	Comments
BamHI-HF	New England Biolabs	R3136L	
BgIII	New England Biolabs	R0144S	
BsrG1-HF	New England Biolabs	R3575S	
NotI-HF	New England Biolabs	R3189L	
Sall-HF	New England Biolabs	R3138L	
Phenol:Chloroform:Isoamyl Alcohol	Thermo Fisher	15593031	
SP6 mMessage Machine in vitro transcription kit	Thermo Fisher	AM1340	
Fast Green FCF	Sigma Aldrich	F7252	
Triton X-100	Sigma Aldrich	93443	4-(1,1,3,3-Tetramethylbutyl)phenyl- polyethylene glycol, <i>t</i> - Octylphenoxypolyethoxyethanol, Polyethylene glycol <i>tert</i> -octylphenyl ether
DAPI	Sigma Aldrich	D9542	2-(4-Amidinophenyl)-6- indolecarbamidine dihydrochloride, 4',6-Diamidino-2-phenylindole dihydrochloride, DAPI dihydrochloride
Whatman No.1 filter paper	Sigma Aldrich	WHA1001125	
glycerol	Sigma Aldrich	G9012	
Urea	Sigma Aldrich	51457	
pmTurquoise2-Golgi	Addgene	36205	pmTurquoise2-Golgi was a gift from Dorus Gadella (Addgene plasmid # 36205; http://n2t.net/addgene:36205; RRID:Addgene_36205)
pmEGFP-N1-LifeAct			Nat. Methods 2008;5:605-7. PubMed ID: 18536722
pCS2.Lifeact-mGFP	Addgene		This paper
pCS.H2B-citrine	Addgene	53752	pCS-H2B-citrine was a gift from Sean Megason (Addgene plasmid # 53752; http:// n2t.net/addgene:53752; RRID:Addgene_53752)
pCS.memb-mCherry	Addgene	#53750	pCS-memb-mCherry was a gift from Sean Megason (Addgene plasmid # 53750 ; http://n2t.net/addgene:53750 ; RRID:Addgene_53750)

¹Department of Biological Sciences, University of Southern California, Los Angeles

²Department of Radiology and Developmental Neuroscience Program, Saban Research Institute, Children's Hospital Los Angeles

³Keck School of Medicine, Department of Radiology, University of Southern California, Los Angeles

Zeiss LSM-780 inverted microscope	Carl Zeiss Microscopy GmbH		The LSM-780 is a confocal and multi-photon microscope that offers the sensitivity required for vital imaging work. Equipped with a motorized stage, an autofocus device, and a full stage-top blackout incubator, the 780 is an excellent microscope for highend live cell/embryo imaging. The high-sensitivity 32-channel Quasar detector allows for spectral imaging, linear unmixing, and high
			color count (>4) image acquisition. Excitation can be performed with 6 lines single photon lasers (405, 458, 488, 514, 564 and 633 nm), Chameleon (Coherent) 2- photon laser (range from 690nm to 1000nm), and run with ZEN 2011 SP7 (Black) system software.
CUY-21 EDIT in vivo electroporator	Bex Co., Ltd.		
Platinum flat square electrode, side length 5 mm	Bex Co., Ltd.	LF701P5E	
Olympus MVX10 FL Stereo Microscope	Olympus LifeScience		
XM10 Monochrome camera	Olympus LifeScience		
Phosphate-Buffered Saline (PBS) for HCR (10×, pH 7.4)			To prepare 1 L of a 10× stock solution, combine 80 g of NaCl (Sigma-Aldrich S3014), 2 g of KCl (Sigma-Aldrich P9541), 11.4 g of Na ₂ HPO ₄ (anhydrous; Sigma-Aldrich S3264), and 2.7 g of KH ₂ PO ₄ (anhydrous; Sigma-Aldrich P9791). Adjust the pH to 7.4 with HCl, and bring the final volume to 1 L with ultrapure H2O. Avoid using CaCl2 and MgCl2 in PBS for HCR. It is important that the PBS for HCR is prepared as an RNase-free solution (e.g., via diethylpyrocarbonate [DEPC] treatment).
1.37 M NaCl			
27 mM KCI			
80 mM Na ₂ HPO ₄ 20 mM KH ₂ PO ₄			
PBS/Triton			Add 1 mL of Triton X-100 (Sigma Aldrich 93443) and 100 mL of $10 \times PBS$ to 890 mL of ultrapure distilled H_2O . Filter the solution through a 0.2 μ m filter and store it at 4 ?C until use.
1× phosphate-buffered saline (PBS) (DEPC-treated; pH 7.4)			
0.1% Triton X-100			