

Materials List for:

Embryo Microinjection and Electroporation in the Chordate *Ciona intestinalis*

Willi Kari¹, Fan Zeng¹, Lena Zitzelsberger¹, Johannes Will¹, Ute Rothbächer¹

¹Department of Evolution and Developmental Biology, Zoological Institute, University Innsbruck

Correspondence to: Ute Rothbächer at Ute.Rothbaecher@uibk.ac.at

URL: <https://www.jove.com/video/54313>

DOI: [doi:10.3791/54313](https://doi.org/10.3791/54313)

Materials

Name	Company	Catalog Number	Comments
Lab with constant temp. (~18 °C)			For embryo work
<i>Ciona intestinalis</i> adults	UPMC - Station Biologique, Roscoff, France		For collecting gametes
NaCl	Roth	3957.1	For ASWH
KCl	Roth	6781.1	For ASWH
CaCl ₂ x2H ₂ O	Roth	A119.1	For ASWH
MgCl ₂ x6H ₂ O	Roth	2189.1	For ASWH
MgSO ₄ x7H ₂ O	Roth	P027.2	For ASWH
NaHCO ₃	Roth	6885.1	For ASWH
Hepes	Roth	HN78.3	For ASWH
Sodium thioglycolate	Sigma	MZ-6	For Dechoriation
Pronase	Sigma	T0632-100G	For Dechoriation
D-Mannitol	Sigma Aldrich	M9546-250G	For electroporation
Plasmid DNA (prep>1µg/µl)	Macherey-Nagel	740410.100	For electroporation or microinjection
Agarose	any supplier		For coating embryo culture dishes
Plastic Petri dishes	VWR	612-2079	Coated with 1% Agarose in ASWH, covered with ASWH
Small scissors	any supplier		For dissecting gametes
NaOH	Roth	6771.1	For activating dechoriation solution
Tris	Roth	5429.3	1 M pH 9.5, for sperm activation
Pasteur pipettes	VWR	612-1701	Treated by immersion in tap water for at least 12 hr
Hand centrifuge	Hettich Zentrifugen	1011	Use glass centrifuge tubes
1.5 ml Eppendorf tubes	Sigma	T3406-250EA	
2 ml Eppendorf tubes	Sigma	T3531-200EA	
Square electroporator	Bio Rad Gene Pulser Xcell		
Electroporation cuvettes 4 mm	Eurogentec	CE-0004-50	
Coverslips	Sto Premium	PR248212600	
Glass slides	VWR	ECN 613-1551	
Glutaraldehyde	Sigma	G6257-10ML	For fixation in LacZ staining
Paraformaldehyde	Sigma	P6148-500G	For fixation of fluorescent samples
Vectashield	Vector	H-1000	For mounting of fluorescent samples
X-Gal (5-Bromo-4-chloro-3-indolyl β-D-Galactopyranoside)	Roth	2315.2	LacZ substrate; stock in 40 mg/ml DMF (Dimethylformamide)

$K_4Fe(CN)_6 \cdot 3H_2O$	Merck	4984	For LacZ staining buffer
$K_3Fe(CN)_6$	Merck	4973	For LacZ staining buffer
Na_2HPO_4	Roth	4984.2	For PBT
KH_2PO_4	Roth	3904.2	For PBT
Tween	Sigma	SLBJ5103V	For PBT
Mineral Oil	Sigma	M8410	Embryo culture tested
Green Vital Dye	Fast Green Sigma	F7251	for microinjection; 10 μ g/ μ l
Micropipette Puller (Flaming/Brown)	Sutter Instruments	Model P-97	For pulling injection needles
Borosilicate glass capillaries GC100TF-10	Harvard Apparatus Ltd. UK	30-0038	1.0 mm O.D. x 0.78 mm I.D., containing filament, for microinjection
Micromanipulator	Narishige	MWS-2	Three-axis Oil Hydraulic System; for 3D fine movement during injection
Injection holder	Narishige	1M-H1	For holding injection needles, entire system filled with mineral oil
All Glass Syringe	Walter Graf & Co GmbH & Co. KG	No. 95199.10427A4	10 ml FortunaOptima; filled with mineral oil, for fine tuning of microinjection pressure
Morpholinos	GeneTools LLC, Pilomath, OR, USA		Injection at 0.25-0.5 mM
Capped mRNA	Ambion mMessage mMachine kit		Injection at 0.05-0.5 μ g/ μ l
Plasmid DNA			Injection at 20 ng/ μ l
Recombination cloning kit	Gateway BP Clonase Enzyme Mix Invitrogen	11789-013	
Recombination cloning kit	Gateway LR Clonase Enzyme mix Invitrogen	11791-019	
anti-HA tag antibody	abcam	ab9110	for Immunofluorescence, use 1:400 in 3% BSA/PBT
TRITC goat anti-rabbit antibody	Jackson	111-025-144	for Immunofluorescence, use 1:300 in 3% BSA/PBT