

Materials List for

# Microinjection of Recombinant RCAS(A) Retrovirus into Embryonic Chicken Lens

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jove.com/video/65727

## Materials

Name	Company	Catalog Number	Comments
0.22 µm Filter	Corning	431118	For removing cellular debris from media
35 mm x 10 mm Culture Dish	FisherScientific	50-202-030	For using during microinjection
Centrifuge	Fisherbrand	13-100-676	Spinning down solution
Constructs	GENEWIZ	-	For generation of constructs
Dissecting microscope	AmScope	SM-4TZ-144A	Visualization of lens for microinjection
DNA PCR primers	Integrated DNA Technologies	-	Generation of primers: Intracellular loop (IL)-deleted Cx50 (residues 1–97 and 149–400) as well as the Cla12NCO vector were obtained with the following pair of primers: sense, CTCCTGAGAACCTACATCCT; antisense, CACCGCATGCCCAAAGTACAC ILs of Cx43 (residues 98–150) and Cx46 (residues 98–166) were obtained with the following pairs of primers: sense, TACGTGATGAGGAAAGAAGAG; antisense, TCCTCCACGCATCTTACCTTG; sense, CACATTGTACGCATGGAAGAG; antisense, AGCACCTCCC AT ACGGATTC, respectively Cla12NCO-Cx43 construct template was obtained with the following pair of primers: sense, CTGCTTCGTA CTTACATCATC; antisense, GAACAC GTGCGCCAGGTAC ILs of Cx50 (residues 98–148) or Cx46 (residues 98–166) were cloned by using Cla12NCO-Cx50 and Cla12NCO-Cx46 constructs as the templates with the following pair of primers: sense, CACCATGTCCGCATGGAGGAGA;

			antisense, GGTCCTC TC CAGGCGAAAC; sense, CACATTGTACGCATGGAAGAG; antisense, AGCACCTCCCATACGGATTC, respectively
Drummond Nanoject II Automatic Nanoliter Injector	Drummond Scientific	3-000-204	Microinjection Pipet
Dual Gooseneck Lights Microscope Illuminator	AmScope	LED-50WY	Lighting for visualization
Dulbecco's Modified Eagle Medium (DMEM)	Invitrogen		For cell culture
Egg Holder	-	-	Homemade styrofoam rings with 2-inch diameter and one-half inch height
Egg Incubator	GQF Manufacturing Company Inc.	1502	For incubation of fertilized eggs
Fast Green	Fisher scientific	F99-10	For visualization of viral stock injection
Fertilized white leghorn chicken eggs	Texas A&M University	N/A	Animal model of choice for microinjection ( <a href="https://posc.tamu.edu/fertile-egg-orders/">https://posc.tamu.edu/fertile-egg-orders/</a> )
Fetal Bovine Serum (FBS)	Hyclone Laboratories		For cell culture
Fluorescein-conjugated anti-mouse IgG	Jackson ImmunoResearch	115-095-003	For anti-FLAG 1:500
Forceps	FisherScientific	22-327379	For moving things around and isolation
Glass capillaries	Sutter Instruments	B100-75-10	Glass micropipette for microinjection (O.D. 1.0 mm, I.D. 0.75 mm, 10 cm length)
Lipofectamine	Invitrogen	L3000001	For transfection
Manual vertical micropipette puller	Sutter Instruments	P-30	To obtain glass micropipette of the correct size
Microcentrifuge Tubes	FisherScientific	02-682-004	Dissolving solution
Microscope	Keyence	BZ-X710	For imaging staining
Parafilm	FisherScientific	03-448-254	Placing solution
Penicillin/Streptomycin	Invitrogen		For cell culture
Pico-Injector	Harvard Apparatus	PLI-100	For delivering small liquid volumes precisely through micropipettes by applying a regulated pressure for a digitally set period of time
rabbit anti-chick AQP0	Self generated	-	Jiang JX, White TW, Goodenough DA, Paul DL. Molecular cloning and functional characterization of chick lens fiber connexin 45.6. Mol Biol Cell. 1994 Mar;5(3):363-73. doi: 10.1091/mbc.5.3.363.
rabbit anti-FLAG antibody	Rockland Immunichemicals	600-401-383	For staining FLAG
Rhodamine-conjugated anti-rabbit IgG	Jackson ImmunoResearch	111-295-003	For anti-AQP0 1:500
Sponge clamping pad	Sutter Instruments	BX10	For storage of glass micropipette