

Materials List for:

In Vivo Direct Reprogramming of Resident Glial Cells into Interneurons by Intracerebral Injection of Viral Vectors

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URL: <https://www.jove.com/video/59465>

DOI: [doi:10.3791/59465](https://doi.org/10.3791/59465)

Materials

Name	Company	Catalog Number	Comments
REAGENTS FOR AAV5 CLONING AND VIRAL VECTOR PREPARATION			
pAAV-CA-FLEX	AddGene	38042	
Ascl1	AddGene	67291	NM_008553.4
Lmx1a	AddGene	33013	NM_0033652.5
Nurr1	AddGene	35000	NM_013613.2
GFP-syn	AddGene	30456	
LoxP (FLEX) sequence 1			GATCTccataactcgtataaagtacctatacgaagttatatcaaaataggaagaccaatgctcaccatcgaccgaaatgccaagcatcaccatcgaccataactcgtataatgatgtatatacgaagtatactagtcgggaaggcgaagacgcggaagaggctctaga
LoxP (FLEX) sequences 2			tactagtataacttctgtataggatactttatacgaagttatcattgggattctctatttgatccaagcatcaccatcgaccctctagtccacatctcaccatcgaccataactcgtatagcatacatatacgaagttatgtccctcgaagaggttcgaaatcgttaaacGGTACCCTCGAC
pDP5	Plasmid Factory	PF435	
pDP6	Plasmid Factory	PF436	
Phosphate-Buffered Saline (PBS)	Thermo Fisher Scientific	10010023	
FBS (Fetal bovine serum)	Thermo Fisher Scientific	10500064	
Penicillin streptomycin	Thermo Fisher Scientific	15140122	
DMEM (Dulbecco's Modified Eagle Medium)+ Glutamax	Thermo Fisher Scientific	61965026	
DPBS (Dulbecco's Phosphate Buffer Saline)	Thermo Fisher Scientific	14190094	
HEK293 cells	Thermo Fisher Scientific	85120602-1VL	
Flasks	BD Falcon	10078780	175 cm2
Tris H-CL	Sigma Aldrich	10812846001	For TE buffer use 10 mM, pH 8.0; for lysis buffer use 50mM, pH 8.5; for IE buffer use 20 mM, pH 8.0; for elution buffer use 20 mM, pH 8.0
EDTA	EDTA: Invitrogen	EDTA: AM9260G	For TE buffer use 1 mM EDTA
Ultrapure water			see Ultrapure water system
CaCl2	SigmaAldrich	C5080	2.5 M
Dulbecco's phosphate-buffered saline (DPBS)	Thermo Fisher Scientific	14190136	

NaCl	Sigma-Aldrich	S3014	FOR HBS use 140 mM; for Lysis Buffer use 150 mM; for IE buffer use 15 mM; for elution buffer use 250 mM
MgCl ₂	Sigma-Aldrich	M8266-100G	For lysis Buffer: 1 mM
Ultracentrifuge sealing tubes	Beckman Coulter	Quick-Seal® Polypropylene Tube	
OptiPrep™ Density Gradient Medium	Sigma Aldrich	D1556-250ML	
10-mL syringe-18G needle	BD	305064	
Laboratory glass bottles	VWR	?	
Anion exchange filter	PALL laboratory	MSTG25Q6	Acrodisc unit with Mustang Q membrane
Centrifugal filter unit	Merck	Z740210-24EA	Amicon Ultra-4 device
Endotoxin-Free Plasmid DNA Isolation Kits	Thermo Fisher Scientific	A33073	
Na ₂ PO ₄	Sigma-Aldrich	S7907	
HEPES	Sigma-Aldrich	H7523	15 mL
Falcon tube	Thermo Fisher Scientific	Corning 352196	
Falcon tube	Thermo Fisher Scientific	Corning 352070	
Glass Vials	Novatech	30209-1232	CGGCCTCAGFGAGCGA
Forward Primer for Inverted Terminal Repeat (ITR) sequence			GGAACCCCTAGTGATGGAGTT
Reverse Primer for Inverted Terminal Repeat (ITR) sequence			CACTCCCTCTCTGCGGCTCG
5'FAM / 3'BHQ1 probe	Jena Bioscience		
0.22 mm filter	Merck	SLGV004SL	Millex-GV filter
EQUIPMENT AAV5 VIRAL VECTOR PREPARATION			
Freezer -20 °C			
Freezer -80 °C			
Fridge +4 °C			
AAV viral room			
Ultrapure Water system	Merck	Milli-Q® IQ 7000	
Vortex mixer	VWR	444-0004	
Ultracentrifuge	Beckman Coulter	Beckman Optima LE-80K Ultracentrifuge	
Autoclave	Tuttnauer	2540 EL	
Polymerase Chain Reaction (PCR)	BioRad	C1000 Touch Thermal Cycler	
Quantitative PCR (qPCR)	Roche	LightCycler® 480 System	
Centrifuge	Thermo Fisher Scientific	Sorvall ST16	
Water Bath	Thermo Fisher Scientific	TSGP02	
ANIMAL MODEL			
NG2-Cre mice	Jackson	NG2-CrexB6129, Stock #008533	
REAGENTS FOR INJECTION OF REPROGRAMMING FACTORS INTO THE BRAIN			
Water			
Saline	Apoteket AB		70%
Ethanol	Solveco		

Isoflurane	Apoteket AB		Dilute to 1% solution with warm water.
Virkon	Viroderm	7511	
Pentobarbital	Apoteket AB	P0500000	i.p. for terminal procedure at the dose of 60 mg/ml.
Sucrose	Merck	S0389-500G	
Trypan Blu	Thermo Fisher Scientific	15250061	
Retrobeads	Lumafluor	R170	
Buprenorphine	Apoteket AB		
EQUIPMENT FOR INJECTION OF REPROGRAMMING FACTORS INTO THE BRAIN			From RSG Solingen.
Scissors	VWR	233-1552	From Biochem.
Tweezers	VWR	232-0007	
Forcep	VWR	232-0120	
Scalpel holder	VWR	RSGA106.621	Number 20.
Scalpel	VWR	RSGA106.200	
Stereotaxic frame	Stoelting Europe	51500D	
Mouse ear bars	Stoelting Europe	51648	5 ul
Syringe with Removable needle	Hamilton Company	65	0,75 inner diameter and 1.5 outer diameter.
Glass capillaries	Stoelting	50811	
Glass capillary puller	Sutter company	P-1000	
Dental drill	Agthos AB	1464	
Shaver	Agthos AB	GT420	
Isoflurane Chamber and pump	Agthos AB	8323101	2 mL
Syringes	Merck	Z118400-30EA	25G
Needles	Merck	Z192414 Aldrich	
Mouse and neonatal rat adaptor for stereotaxic fram	Stoelting	51625	
Heating pad	Braintree scientific, inc	53800M	From Covidien 2187.
Cotton gauze	Fisher Scientific	22-037-902	
Rubber tube	Elfa Distrelec	HFT-A-9.5/4.8 PO-X BK 150	
Cotton swabs	Fisher Scientific	18-366-473	
REAGENTS FOR WHOLE-CELL PATCH CLAMP RECORDINGS			
NaCl	Sigma-Aldrich	S3014	
KCl	Sigma-Aldrich	P9333	
NaH ₂ PO ₄ ·H ₂ O	Sigma-Aldrich	S9638	
MgCl ₂ ·6 H ₂ O	Sigma-Aldrich	M2670	
CaCl ₂ ·6 H ₂ O	Sigma-Aldrich	C8106	
MgSO ₄ ·7 H ₂ O	Sigma-Aldrich	230391	
NaHCO ₃	Sigma-Aldrich	S5761	
Glucose	Sigma-Aldrich	G7021	see Ultrapure Water system
Ultrapure Water			Prepare 1 or 2M.
KOH	Sigma-Aldrich	P5958-500G	
K-D-gluconate	Sigma-Aldrich	G4500 Sigma	
KCl	Sigma-Aldrich	P9541	

KOH-EGTA (Etilene glycol-bis-N-tetracetic acid)	Sigma-Aldrich	E3889 Sigma	
KOH- Hepes acid (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)	Sigma-Aldrich	H7523	
NaCl	Sigma-Aldrich	S3014	
Mg2ATP	Sigma-Aldrich	A9187	
Na3GTP	Sigma-Aldrich	G8877	
Biocytin	Sigma-Aldrich	B4261	
Picrotoxin	Merck	P1675 Sigma	
CNQX	Merck	C239 Sigma	
Ice			
EQUIPMENT FOR WHOLE-CELL PATCH CLAMP RECORDINGS			
Borosilicate glass pipette	Sutter Company	B150-86-10	
Glass capillary puller	Sutter company	P-1000	
Vibratome	Leica	Leica VT1000 S	
WaterBath	Thermo Fisher Scientific	TSGP02	
Clampfit software	Molecular Devices		
Multiclamp software	Molecular Devices		
REAGENTS FOR IMMUNOHISTOCHEMISTRY			Use at a concentration of 4%. CAUTION: PFA is a potent fixative. Avoid ingestion and contact with skin.
Paraformaldehyde (PFA)	Merck Millipore	1040051000	Use at a concentration of 0.1%.
Triton X-100	Fisher Scientific	10254640	Donkey. Use at a concentration of 1 : 400.
Serum	Merck Millipore	S30-100ML	Reconstitute the powder in Milli-Q water to 1 mg/mL. Aliquot and store at -20°C, light sensitive. Use at a concentration of 1 : 500.
4',6-Diamidino-2'-phenylindole dihydrochloride (DAPI)	Sigma Aldrich	D9542	1: 600 in KPBS-T.
streptavidin- Cy3	Thermo Fisher Scientific	434315	1:5000, rabbit.
Anti-GAD65/67	Abcam		1:2000, mouse.
Anti-PV	Sigma	P3088	1:200, goat.
Anti-Chat	Merk	AB143	1:5000, rabbit.
Anti-NPY	Immunostar	22940	
K ₂ HPO ₄	Sigma-Aldrich	P3786	
NaCl	Sigma-Aldrich	S3014	
KH ₂ PO ₄	Sigma-Aldrich	NIST200B	1:1000, chicken.
Anti-GFP	Abcam	ab13970	
Mounting solution	Merck	10981	Polyvinyl alcohol mounting medium with DABCO®, antifading
OCT	Agar Scientific		
Glycerol	Sigma-Aldrich	G9012-100ML	
Distilled water			
Anti-freeze solution	Tissue Pro Technology	AFS05-1000N, 1000 mL/ea.	
EQUIPMENT FOR IMMUNOHISTOCHEMISTRY			

Inverted fluorescence microscope	Leica	DMI6000 B	
Confocal microscope	Leica	TCS SP8 laser scanning confocal microscope.	
Prism	GraphPad		
Microtome	Leica	SM2010R	