

Materials List for:

Stable and Efficient Genetic Modification of Cells in the Adult Mouse V-SVZ for the Analysis of Neural Stem Cell Autonomous and Non-autonomous Effects

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Materials

Name	Company	Catalog Number	Comments
Part 1: Generation of LV for in vivo delivery.			
Equipment:			
Ultracentrifuge	Beckman Coulter	Optima XL-100K	
Ultracentrifuge rotor	Beckman Coulter	SW-28	
Ultracentrifuge rotor	Beckman Coulter	SW-55	
Ultracentrifuge tubes	Beckman Coulter	358126	25X89 mm
Ultracentrifuge tubes	Beckman Coulter	326819	13X51 mm
Ultracentrifuge adapters	Beckman Coulter	358156	
6-well plate	SPL	PLC-30006	
24-well plate	SPL	PLC-30024	
10 cm dish	SPL	PLC-20101	100x20 style
FACS tubes	Afora	DE400800	12x75 mm, 5 ml
Cup sterile FACS filter	BD	340626	30 µm
Nitrocellulose filter	Millipore	SCGPU05RE	0.22 µm
Flow cytometer	BD	LSR Fortessa	Blue laser 488 nm
Steritop filter	Biofil	FPE-204-500	0.22 µm
Reagents:			
pMDLg/pRRE plasmid	Addgene	#12251	Core packaging plasmid
pRSV.REV plasmid	Addgene	#12253	Core packaging plasmid
pMD ₂ G plasmid	Addgene	#12259	Envelope plasmid
pRRL-SIN-PPT.PGK.EGFP.Wpre plasmid	Addgene	#12252	Transfer vector plasmid
Dulbecco's Modified Eagle's Medium	Biowest	L0101-500	For HeLa cell culture
Iscove's Modified Dulbecco's Medium	Life technologies	12440-053	For 293T cell culture
Tris-EDTA (TE)			Tris-HCl (sigma, T5941), 0.1 mM EDTA (sigma, E5134), pH 7.6, DNase/RNase-free, 0.2 µm sterile-filtered
2x HBS			0.28 M NaCl (Sigma, S7653), 0.05 M HEPES (Sigma, H7523), 1.5 mM anhydrous Na ₂ HPO ₄ (Sigma,

			S7907) in dH ₂ O (preferably not MilliQ). Adjust pH to 7.0 with NaOH solution (Calbiochem, 567530).
Fetal bovine serum (FBS)	Biowest	S181B-500	Stock solution at 100X, used to prepare HeLa and 293T culture medium at a final concentration of 10X.
Glutamine	Sigma-Aldrich	G7513-100	Stock solution at 200 mM, used to prepare HeLa and 293T culture medium at a final concentration of 6 mM.
Sodium pyruvate	Life technologies	11360-039	Stock solution at 100 mM, used to prepare HeLa and 293T culture medium at a final concentration of 1 mM.
GlutaMAX Supplement	Life technologies	35050-061	Used to prepare 293T culture medium at a final concentration of 1%.
Penicillin/streptomycin	Sigma-Aldrich	P4458	Stock solution contains 5,000 units/ml penicillin and 5 mg/ml streptomycin. Used to prepare HeLa and 293T culture medium at a final concentration of 1%.
Trypsin-EDTA	Life Technologies	25200-056	With phenol red, contains 2.5 g porcine trypsin and 0.2 g EDTA 4Na/L HBSS.
Phosphate buffered saline (PBS)	Sigma-Aldrich	D1408	Without calcium chloride and magnesium chloride, 10X, liquid, sterile-filtered, suitable for cell culture. Stock solution used to prepare 1X PBS in cell culture grade water.
Polybrene (hexadimethrine bromide)	Sigma-Aldrich	H9268	Powder. Prepare a 1000X stock solution at 8 mg/ml in dH ₂ O
Paraformaldehyde EM grade 16%	EM Sciences	15710	
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Part 2: Stereotaxic injection of LV into the SEZ proper or the lateral ventricle.			
Equipment:			
Vernier stereotaxic instrument	NeuroLab, Leica	39463001	http://www.leicabiosystems.com/
Cunningham mouse and neonatal rat adaptor	NeuroLab, Leica	39462950	
Syringe holder	KD Scientific	KDS-311-CE	
33 gauge syringe	Hamilton	P/N 84851/00	#85RN
Electric drill	Fine Science Tool	98096	
Thermal blanket	Ufesa	AL5512/01	230-240 V, 100-110 W, type C_AL01
Shaver	Jata	MP373N	Model: beauty, 3 V, 300 mA, type HT-03.
Reagents:			
Medetomidine	Esteve	DOMTOR	Comercial solution at 1 mg/ml.
Ketamine	Merial	Imalgene 500	Comercial solution at 50 mg/ml
Medetomidina/ketamine mixture			Prepare a working mixture of medetomidine at a final concentration of 0.2 mg/ml dilution and ketamine at a final

			concentration of 15 mg/ml in saline solution. Use as anesthesia injecting a volume to get a final concentration of 0.5-1 mg medetomidina per kg body weight and 50-75 mg ketamine per kg body weight
Butorphanol	Pfizer	Torbugesic	Stock solution at 10 mg/ml. Used as analgesia at 1 mg/ml in saline solution.
Atipamezole	Esteve	Antisedan	Stock solution at 5 mg/ml, used in a final concentration of 0.5 mg/ml in saline solution to exit from anesthesia.
0.9% saline solution	Braun	13465412	
Histoacryl	Braun	1050052	Topical skin adhesive
HydroGel	Clear H ₂ O	70-01-5022	
Kimwipes	Kimberly-Clark	34120	11x21 cm
Bleach/Virkon	Dupont		
Surgical marker pen	Staedler	313-9	Permanent lumocolor
Ophthalmic lubricant		SICCAFLUID	0.5 g/dosis, carbomer 974P
Povidone-iodine	Betadine	694109.6	10% povidone-iodine
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Part 3: Histological analysis.			
Equipment:			
Automatic peristaltic pump	Cole-Parmer Inst. Co.	HV-07524-55	Masterflex L/S variable-speed economy drive, 1.6-100 rpm, 230 V
Pump head	Cole-Parmer Inst. Co.	HV-07518-00	Masterflex L/S Easy-Load pump head for precision tubing; PSF housing, CRS rotor
Silicone tube	Cole-Parmer Inst. Co.	HV-96410-16	Platinum L/S 16
Scalp vein set	Vygon V-green	70246.05T	25G, 30 cm tube length
Vibratome	Leica	VT1000	
Confocal microscope	Olympus	FluoView FV10i	
Hot plate	Tehcnica	SHP-10	
Reagents:			
Phosphate buffer (PB)			0.2 M PB: 0.2 M Na ₂ HPO ₄ (Sigma, S7907) and 0.2 M NaH ₂ PO ₄ (Panreac, 141965.1211) in dH ₂ O, adjust pH to 7.2-7.4
Paraformaldehyde (PFA)	Panreac	141451.1211	Prepare fresh every time. Heat dH ₂ O up to 55–60 °C using a hot plate placed in a fume hood and pour PFA powder while stirring to obtain an 8% solution. The solution is cloudy white as PFA does not dissolve easily. Add 1N NaOH drop by drop just until the solution clears. Cool down, filter through Whatman paper and add an equivalent volume of 0.2 M PB.
Saline solution			0.9% NaCl in dH ₂ O
Superglue	LOCTITE	767547	
Sodium azide	Panreac	122712.1608	
Glycine	Sigma-Aldrich	G7126-100	

Normal goat serum	Millipore	S30-100	
Triton X-100	Sigma-Aldrich	T9284	Detergent
Anti-GFP rabbit antibody	ROCKLAND	600-401-215	Use at a 1:500 dilution
Alexa Fluor 488 Donkey Anti-Rabbit IgG (H+L) Antibody	Molecular probes	A-21206	Use at a 1:750 dilution
6-Diamindino-2-phenylindole dihydrochloride hydrate (DAPI)	Sigma-Aldrich	D9542	Fluorescent nuclear staining. Use at 2 mg/ml in ddH ₂ O. Keep in the dark at 4 °C.
Fluoromount-G	EM Sciences	17984-25	Mounting medium for fluorescent preparations