

Materials List for:

Rapid Genotyping of Animals Followed by Establishing Primary Cultures of Brain Neurons

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URL: <https://www.jove.com/video/51879>

DOI: [doi:10.3791/51879](https://doi.org/10.3791/51879)

Materials

Name	Company	Catalog Number	Comments
REAGENTS - tattooing			
Machine Cleanser	Animal Identification and Marking Systems, Inc.	NMCR3	This is used to clean the needles and the holder after tattooing.
Machine Drying Agent	Animal Identification and Marking Systems, Inc.	NDAR4	This is used to dry the needles and holder after cleaning.
Neonate Tattoo Black Pigment	Animal Identification and Marking Systems, Inc.	NBP01	
Skin Prep Applicator	Animal Identification and Marking Systems, Inc.	NSPA1	Q-tip.
Skin Prep solution	Animal Identification and Marking Systems, Inc.	NSP01	This reagent delivers a thin layer of oil that enhances the efficiency of tattooing and prevents tattoo fading, by (information from vendor): 1) preventing non-tattooed skin from being stained temporarily, thereby allowing the quality of a paw pad tattoo to be easily evaluated before the pup is returned to its home cage – the stained skin surface can be confused with the tattooed skin, 2) reducing skin damage during tattooing – softening the skin and lubricating the needle will help the needle penetrate the skin without causing skin damage, and 3) preventing molecular oxygen from entering the skin, thereby reducing inflammatory responses to reactive oxygen species that can be generated.
REAGENTS - genotyping			
EZ Fast Tissue/Tail PCR Genotyping Kit (Strip Tube Format)	EZ BioResearch LLC	G2001-100	
2X PCR Ready Mix II	EZ BioResearch LLC	G2001-100	A red, loading dye for electrophoresis is included in the 2X PCR Ready Mix solution.
Tissue Lysis Solution A	EZ BioResearch LLC	G2001-100	Prepare DNA Extraction Solution by mixing 20 µl of Tissue Lysis Solution A and 180 µl of Tissue Lysis Solution B per specimen.

Tissue Lysis Solution B	EZ BioResearch LLC	G2001-100	Prepare DNA Extraction Solution by mixing 20 µl of Tissue Lysis Solution A and 180 µl of Tissue Lysis Solution B per specimen.
Acetic acid, glacial	VWR	BDH 3092	
Agarose optimized grade, molecular biology grade	rpi	A20090-500	We use 2% agarose gels in TAE buffer containing the SYBR Safe DNA gel stain (diluted 10,000-fold) or ethidium bromide (0.5 µg/ml gel volume).
Ethidium bromide	Sigma-Aldrich	E7637-1G	
Ethylenediamine tetraacetic acid, disodium salt dihydrate (EDTA)	Fisher	BP120-500	
Filtered Pipet Tips, Aerosol-Free, 0.1-10 µl	Dot Scientific Inc	UG104-96RS	Use pipette tips that are sterile and free of DNA, RNase and DNase. For all steps involving DNA, use filtered pipette tips to avoid cross-contamination.
Filtered Pipet Tips, Premium Fit Filter Tips, 0.5-20 µl	Dot Scientific Inc	UG2020-RS	Use pipette tips that are sterile and free of DNA, RNase and DNase. For all steps involving DNA, use filtered pipette tips to avoid cross-contamination.
Filtered Pipet Tips, Premium Fit Filter Tips, 1-200 µl	Dot Scientific Inc	UG2812-RS	Use pipette tips that are sterile and free of DNA, RNase and DNase. For all steps involving DNA, use filtered pipette tips to avoid cross-contamination.
Molecular weight marker, EZ DNA Even Ladders 100 bp	EZ BioResearch LLC	L1001	We use either of these three molecular weight markers.
Molecular weight marker, EZ DNA Even Ladders 1000 bp	EZ BioResearch LLC	L1010	
Molecular weight marker, TrackIt, 100 bp DNA Ladder	GIBCO-Invitrogen	10488-058	
PCR tubes, 8-tube strips with individually attached dome top caps, natural, 0.2 ml	USA Scientific	1402-2900	Use tubes that are sterile and free of DNA, RNase and DNase. An 8-tube strip is easy to handle and to group the specimens than individual tubes.
PCR tubes, Ultraflux Individual	rpi	145660	Use tubes that are sterile and free of DNA, RNase and DNase.
Seal-Rite 0.5 ml microcentrifuge tube, natural	USA Scientific	1605-0000	Use tubes that are sterile and free of DNA, RNase and DNase.
SYBR Safe DNA gel stain * 10,000x concentration in DMSO	GIBCO-Invitrogen	S33102	
Tris base	rpi	T60040-1000	
Primers for amplifying Tor1a gene in ΔE-torsinA knock-in mice			5'-AGT CTG TGG CTG GCT CTC CC-3' (forward) and 5'-CCT CAG GCT GCT CAC AAC CAC-3' (reverse) (reference 18). These primers were used at a final concentration of 1.0 ng/µl (~0.16 µM) (reference 2).
Primers for amplifying Tfap2a gene in wild-type mice			5'-GAA AGG TGT AGG CAG AAG TTT GTC AGG GC-3' (forward), 5'-CGT GTG GCT GTT GGG GTT GTT GCT GAG GTA-3' (reverse) for the 498-bp amplicon, 5'-CAC CCT ATC AGG GGA GGA CAA CTT TCG-3' (forward), 5'-AGA

			CAC TCG GGC TTT GGA GAT CAT TC-3' (reverse) for the 983-bp amplicon, and 5'-CAC CCT ATC AGG GGA GGA CAA CTT TCG-3' (forward), 5'-ACA GTG TAG TAA GGC AAA GCA AGG AG-3' (reverse) for the 1990-bp amplicon. These primers are used at 0.5 μ M.
REAGENTS - cell culture			
5-Fluoro-2'-deoxyuridine	Sigma-Aldrich	F0503-100MG	See comments section of uridine for more information.
B-27 supplement	GIBCO-Invitrogen	17504-044	
Cell Culture Dishes 35 x 10 mm Dishes, Tissue Culture-treated	BD falcon	353001	
Cell Culture Flasks, T25, Tissue Culture-treated, Canted-neck, plug-seal cap, 25 cm ² Growth Area, 70 ml	BD falcon	353082	
Cell Culture Flasks, T75, Tissue Culture-treated, Canted-neck, vented cap, 75 cm ² Growth Area, 250 ml	BD falcon	353136	
Conical Tube, polypropylene, 15 ml	BD falcon	352095	
Countess (cell number counter) chamber slides	GIBCO-Invitrogen	C10312	
Cytosine β -D-Arabinofuranoside hydrochloride (Ara-C hydrochloride)	Sigma-Aldrich	C6645-100mg	
D-(+)-Glucose (Dextrose) anhydrous, SigmaUltra, 99.5% (GC)	Sigma-Aldrich	G7528-250G	
Dish, Petri glass 100 x 15 mm	Pyrex	3160-101	
Distilled water	GIBCO-Invitrogen	15230-147	
DNase Type II	Sigma-Aldrich	D4527-200KU	Stock solution is prepared at 1,500 units/20 μ l = 75,000 units/ml in distilled water.
Dulbecco's Modified Eagle Medium (DMEM), high glucose, GlutaMAX, pyruvate	GIBCO-Invitrogen	10569-010, 500 ml	
Fast PES Filter Unit, 250 ml, 50 mm diameter membrane, 0.2 μ m Pore Size	Nalgene	568-0020	
Fast PES Filter Unit, 500 ml, 90 mm diameter membrane, 0.2 μ m Pore Size	Nalgene	569-0020	
Fetal bovine serum (FBS)	GIBCO-Invitrogen	26140-079	
Glass coverslip, 12 mm Round, thickness 0.09–0.12 mm, No. 0	Carolina	633017	
GlutaMAX-I	GIBCO-Invitrogen	35050-061	
Hanks' Balanced Salts	Sigma-Aldrich	H2387-10X	
HEPES, \geq 99.5% (titration)	Sigma-Aldrich	H3375-250G	
Hydrochloric acid, 37%, A.C.S reagent	Sigma-Aldrich	258148-100 ML	
Insulin	Sigma-Aldrich	I5500-250 mg	

Magnesium sulfate heptahydrate, MgSO ₄ ·(7H ₂ O), BioUltra, ≥99.5% (Fluka)	Sigma-Aldrich	63138-250G	
Matrigel Basement Membrane Matrix solution, Phenol Red-Free	BD Biosciences	356237	This is the coating material for coverslips and flasks. 1) To prepare it, thaw the Matrigel Basement Membrane Matrix solution on ice, which usually takes ~1 day. Using a pre-cooled pipette, aliquot the thawed solution into pre-cooled T25 flasks on ice, and store the flasks at -20 °C. To prepare the working Matrigel solution, thaw the aliquotted Matrigel in a flask on ice, dilute 50-fold by adding pre-cooled MEM solution and keep the diluted solution at 4 °C. It is important to pre-cool all cultureware and media that come into contact with Matrigel, except during and after the coating of coverslips, to prevent it from prematurely forming a gel. 2) To coat the glass coverslips or culture flasks with Matrigel, apply the Matrigel solution to the surface. Before plating cells, it is important to completely dry up the surface. For this purpose, it might be helpful to aspirate Matrigel during the cellular centrifugation immediately before plating the cells and to allow enough time for drying.
Minimum Essential Medium (MEM)	GIBCO-Invitrogen	51200-038	
MITO+ Serum Extender, 5 ml	BD Biosciences	355006	
Multiwell Plates, Tissue Culture-treated 24-well plate	BD falcon	353047	
Multiwell Plates, Tissue Culture-treated 6-well plate	BD falcon	353046	
Neurobasal-A Medium (1X), liquid	GIBCO-Invitrogen	10888-022	
Nitric Acid	VWR	bdh 3044	
NS (Neuronal Supplement) 21	prepared in the lab		Source: reference 69
Pasteur pipets, 5 ¼"	Fisher	13-678-6A	Use this cotton-plugged 5 ¼" Pasteur pipette for cellular trituration. Fire-polish the tip beforehand to smooth the cut surface and to reduce the internal diameter to 50-80% of the original. Too small a tip will disrupt the cells and reduce cell viability, but too large a tip will decrease the efficiency of trituration.
Pasteur pipets, 9"	Fisher	13-678-6B	
Potassium chloride (KCl), SigmaUltra, ≥99.0%	Sigma-Aldrich	P9333-500G	
Serological pipet, 2 ml	BD falcon	357507	
Serological pipet, 5 ml	BD falcon	357543	
Serological pipet, 10 ml	BD falcon	357551	
Serological pipet, 25 ml	BD falcon	357525	
Serological pipet, 50 ml	BD falcon	357550	

Sodium bicarbonate (NaHCO ₃ , Sodium hydrogen carbonate), SigmaUltra, ≥99.5%	Sigma-Aldrich	S6297-250G	
Sodium chloride (NaCl), SigmaUltra, ≥99.5%	Sigma-Aldrich	S7653-250G	
Sodium hydroxide (NaOH), pellets, 99.998% trace metals basis	Sigma-Aldrich	480878-250G	
Sodium phosphate dibasic heptahydrate (Na ₂ HPO ₄ ·(7H ₂ O)), ≥99.99%, Aldrich	Sigma-Aldrich	431478-250G	
Sucrose, SigmaUltra, ≥99.5% (GC)	Sigma-Aldrich	S7903-250G	
Syringe filter, sterile, 0.2 μm	Corning	431219	
Syringe, 3 ml	BD falcon	309585	
Transferrin, Holo, bovine plasma	Calbiochem	616420	
Trypan Blue stain, 0.4%	GIBCO-Invitrogen	T10282	This is used for counting live/dead cells. Renew an old trypan blue solution if it is re-used many times (e.g. several times a week for several weeks), because it will form precipitates and result in erroneous readouts of cellular density.
Trypsin, type XI	Sigma-Aldrich	T1005-5G	
Trypsin-EDTA solution, 0.25%	GIBCO-Invitrogen	25200-056	
Uridine	Sigma-Aldrich	U3003-5G	Stock solution is prepared at 50-mg 5-fluoro-2'-deoxyuridine and 125 mg uridine in 25 ml DMEM (8.12 and 20.48 mM, respectively).
REAGENTS - immunocytochemistry			
Antibody, rabbit polyclonal anti-MAP2	Merck Millipore	AB5622	
Antibody, mouse monoclonal anti-GFAP cocktail	Merck Millipore	NE1015	
EQUIPMENT - tattooing			
AIMS	Animal Identification and Marking Systems, Inc.	NEO-9	This Neonate Rodent Tattooing System is an electric system that works by rapidly moving 1- or 3-point tattoo needles vertically into the skin. Activate the tattoo machine once for approximately 0.5 sec, while the tattoo needle tips are kept perpendicular to the skin surface. We prefer three-needle tattooing to maximize the tattooed area, but one-needle tattooing is effective on narrower areas, e.g. the toes, or if fine mechanical control is necessary, e.g. when numbers are tattooed. Two rounds of tattooing at the slowest speed (setting "1" out of 3 steps) are typically sufficient to produce a visible and long-lasting tattoo of the paw pads.
EQUIPMENT - genotyping			
Electrophoresis system, horizontal, Wide Mini-Sub Cell GT	BIO-RAD	170-4405	Typical electrophoresis parameters are electrical field strength at 6 V/cm and 25 min duration for a 10 cm gel.

FluorChem 8800	ProteinSimple	FluorChem 8800	
PCR, MJ Mini Thermal Cycler	BIO-RAD	PTC-1148EDU	Our PCR reactions for the Tor1a gene in ΔE -torsinA knock-in mice are as follows: 1 cycle of denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 sec, annealing at 58 °C for 30 sec, extension at 72 °C for 2 min. This is followed by final extension at 72 °C for 10 min, and holding at 4 °C.
Power supply, PowerPac Basic	BIO-RAD	164-5050	
EQUIPMENT - cell culture			
Automated cell counter, Countess	GIBCO-Invitrogen	C10310	This automated cell counter separately measures the densities of live and dead cells (non stained and stained by trypan blue, respectively). It is important to know the optimal range of density measurements: the counter that we use has the highest accuracy in the range from 1×10^5 to 4×10^6 cells/ml. If the measured cell density values fall outside the recommended range, adjust the resuspension volume appropriately.
Biological Safety Cabinet, Class II, Type A2	NUAIRE	NU-425-400	This hood is used for all cell culture procedures, except for brain dissection.
CO ₂ Incubator, AutoFlow, Humidity Control Water Jacket	NUAIRE	NU-4850	
Horizontal Clean Bench	NUAIRE	NU-201-330	This clean bench is used for brain dissection (steps 3.1.1 and 3.1.2 of "Brain Dissection and Cellular Dissociation").
Orbit LS Low Speed Shaker	Labnet	S2030-LS-B	
SORVALL RC-6 Plus Superspeed Centrifuge	Fisher	46910 (centrifuge)/46922 (rotor)	