

Targeted Microinjection and Electroporation of Primate Cerebral Organoids for Genetic Modification

Lidiia Tynianskaia¹, Nesil Eşiyok¹, Wieland B. Huttner², Michael Heide^{1,2}

¹German Primate Center, Leibniz Institute for Primate Research ²Max Planck Institute of Molecular Cell Biology and Genetics

Corresponding Author

Michael Heide
mheide@dpz.eu

Citation

Tynianskaia, L., Eşiyok, N., Huttner, W.B., Heide, M. Targeted Microinjection and Electroporation of Primate Cerebral Organoids for Genetic Modification. *J. Vis. Exp.* (193), e65176, doi:10.3791/65176 (2023).

Date Published

March 24, 2023

DOI

10.3791/65176

URL

jove.com/video/65176

Materials

Name	Company	Catalog Number	Comments
20 µL Microloader	Eppendorf	5242956003	
2-Mercaptoethanol	Merck	8.05740.0005	
35 mm cell culture dishes	Sarstedt	83.3900	
60 mm cell culture dishes	CytoOne	CC7682-3359	
Activin A	Sigma-Aldrich	SRP3003	
AOC1	Selleckchem	S7217	
Axio Observer.Z1 Inverted Fluorescence Microscope	Zeiss		replacable by comparable fluorescent microscopes
AZD0530	Selleckchem	S1006	
B-27 Supplement with Vitamin A (retinoic acid, RA) (50x)	Gibco	17504-044	
B-27 Supplement without Vitamin A (50x)	Gibco	12587-010	
BTX ECM 830 Square Wave Electroporation System	BTX	45-2052	
CGP77675	Sigma-Aldrich	SML0314	
Chimpanzee induced pluripotent stem cell line Sandra A			doi: 10.7554/elife.18683
Common marmoset induced pluripotent stem cell line cj_160419_5			doi: 10.3390/cells9112422
Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12 (DMEM/F12)	Gibco	11320-033	
Dulbecco's phosphate-buffered saline (DPBS)	Gibco	14190-094	pH 7.0–7.3; warm to room temperature before use
Fast Green	Sigma-Aldrich	F7252-5G	
Forskolin	Selleckchem	2449	
GlutaMAX Supplement (100x)	Gibco	35050-061	glutamine substitute supplement
Heparin (1 mg/mL stock)	Sigma-Aldrich	H3149	
Human induced pluripotent stem cell line iLanza2.2			doi: 10.3390/cells9061349

Human Neurotrophin-3 (NT-3)	PeproTech	450-03	
Insulin	Sigma-Aldrich	19278	
IWR1	Sigma-Aldrich	I0161	
Leica MS5 stereomicroscope (MDG 17 transmitted-light base)	Leica	10473849	replacable by comparable stereomicroscopes
Matrigel	Corning	354277/354234	basement membrane matrix; alternatively, Geltrex (ThermoFisher Scientific, A1413302) can be used
MEM Non-Essential Amino Acids Solution (100x)	Sigma-Aldrich	M7145	
N-2 Supplement (100x)	Gibco	17502-048	
Neurobasal medium	Gibco	21103-049	
Parafilm	Sigma-Aldrich	P7793	
Paraformaldehyde	Merck	818715	handle with caution due to cancerogenicity
Penicillin/Streptomycin (10,000 U/mL)	PanBiotech	P06-07100	
Petri dish electrode chamber	self-produced (see Supplemental File 1)		also commercially available
Pre-Pulled Glass Pipettes	WPI	TIP10LT	borosilicate glass pipettes with long taper, 10 μ m tip diameter
Pro-Survival Compound	MerckMillipore	529659	
Recombinant Human/Murine/RatBrain-Derived Neurotrophic Factor (BDNF)	PeproTech	AF-450-02	
Rhesus macaque induced pluripotent stem cell line iRh33.1			doi: 10.3390/cells9061349
StemMACS iPS-Brew XF	Miltenyi Biotech	130-104-368	
StemPro Accutase Cell Dissociation Reagent	Gibco	A1110501	proteolytic and collagenolytic enzyme mixture
TrypLE	Gibco	12604-013	recombinant trypsin substitute; warm to room temperature before use
Ultra-Low Attachment 96-well plates	Costar	7007	
Y27632	Stemcell Technologies	72305	