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Transfection into Mouse-Rat: EMBRYOS IN UTERO by Electroporation

The NEPA21 is the only device on the market to approach Electroporation from the perspective of optimising delivered energy.

- Compared to devices from other suppliers, the NEPA21 system offers the researcher a level of previously unavailable control over energy delivery to the electroporation target. This control is generated via unique electroporation pulse-output configurations, client-confirmed protocols and application-customised electrodes.
- With this market-leading control and (user-independent) reproducibility of the technique, it is now possible to apply electroporation techniques to applications previously considered too sensitive for electroporation methodologies.

- The finer control over the delivered energy offers specific and important advantages for EMBRYOS IN UTERO electroporation. As the thrust of NEPA21 protocols is to minimise delivered energy, this means that the targets are electroporated with less current (than competing device protocols).
- Only delivering the required energy (and no more) to porate the membrane is of utmost importance for viability post electroporation.
- The success of the NEPA21 for retina electroporation is evident by the Application and Publication information following.
- The NEPA21 system is supported by a suite of over 250 different electrode configurations, which further enhance the applicability of the system and empower researchers with further experimental options and opportunities.

APPLICATIONS

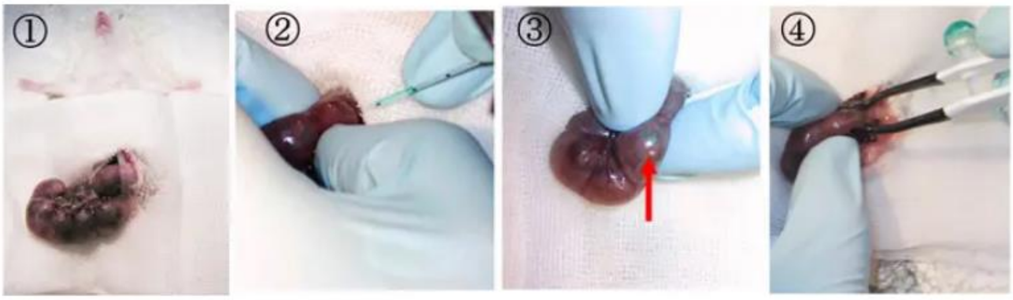
Transfection into Mouse-Rat: EMBRYOS IN UTERO by Electroporation

Gene transfer into embryonic brains using in utero electroporation technique

1) Materials

- In Vivo Electroporator: NEPA21/CUY21 SC/CUY21 EDIT (Nepa Gene Co., Ltd.)
- In Vivo Electrode: CUY650P3/CUY650P5 (Tweezers w/3mm or 5mm diameter platinum disk electrode, Nepa Gene Co., Ltd.)
- Aspirator tube assembly (Drummond)
- Optical fiber light (Technolight, Kenko, #KTS-100RSV)
- Sterile gauze (K-Pine, 7.5cm x 7.5cm)
- Surgical instruments: Fine forceps x 2, Surgery scissors x 2, Ring forceps, Needle holder, Surgical tape
- Nylon suture (Nesco, #HT1605NA75)
- Silk suture (D&G, #112451)

2) In Utero Electroporation Procedure



1. A 2 cm midline incision is then made in the abdominal wall along the linea alba using a set of forceps and scissors. A piece of sterile gauze with a hole cut in the center is placed over the incision, and one uterine horn is drawn out through the hole in the gauze.

2. After observing the orientation of the embryos through the wall of the uterine horn, a micropipette is inserted into the lateral ventricle, and 2-5μl of plasmid solution is injected by expiratory pressure using the aspirator tube assembly. When CAG-EGFP is used, a concentration of 1μg/μl is sufficient to visualize the migrating neurons.

3. After the injection, DNA solution containing 0.01% FastGreen can be seen through the uterine wall (red arrow).

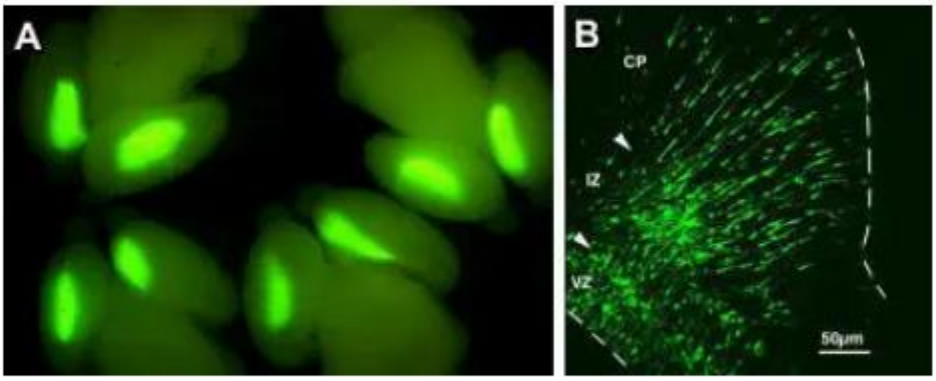
4. After soaking the uterine horn with PBS, the head of embryo is pinched with a tweezers-type electrode, and electronic pulses are applied with the electroporator.

Electroporation settings for ICR mice

Age	Electrode (diameter)	Voltage	Pulse On	Pulse Off	Number of Pulses
E12.5	3mm	33V	30msec	970ms	4
E13.5	5mm	30V	50msec	950ms	4
E14.5	5mm	33V	50msec	950ms	4
E15-	5mm	35V	50msec	950ms	4

If viability was prioritized over transfection efficiency, the number of pulses can be changed to two. The actual current is displayed on the screen of the electroporator (NEPA21/CUY21/CUY21E, Nepa Gene). Make sure that the current would become 30-60mA. The current varies according to how the electrode applied or wetness of the uterine horn. Examine the gap between electrodes and the electrode contact areas to fit the current in the appropriate range. If the current is still above the range after the examination, change the voltage setting.

3) GFP expression



CAG-EGFP was injected into the both lateral ventricles of E14.5 mouse embryos and electronic pulses (33V, 50msec) were charged four times. 3 days later, the embryos (E17.5) were fixed and the brains were removed and examined under a fluorescence stereomicroscope (Fig. A).
Fluorescence was observed in the lateral region of the hemisphere onto which the anode had been placed and in the medial region of the opposite hemisphere.

Brains were frozen and sliced and the fluorescent image was obtained with a confocal laser microscope (Fig. B). GFP positive cells into which DNA was transferred at the ventricular zone (VZ) migrated to the intermediate zone (IZ) and cortical plate (CP). The arrowheads show the border between VZ and IZ and the border between IZ and CP. Dashed line show the border of tissues.
VZ: Ventricular Zone, IZ: Intermediate Zone, CP: Cortical Plate

Hidenori Tabata and Kazunori Nakajima, Department of Anatomy, Keio University School of Medicine

PUBLICATIONS

Transfection into Mouse-Rat: EMBRYOS IN UTERO by Electroporation

E13.5 / E14.5

The microcephaly-associated transcriptional regulator AUTS2 cooperates with Polycomb complex PRC2 to produce upper-layer neurons in mice
Shimaoka K, Hori K, Miyashita S, Inoue YU, Tabe NKN, Sakamoto A, Hasegawa I, Nishitani K, Yamashiro K, Egusa SF, Tatsumoto S, Go Y, Abe M, Sakimura K, Inoue T, Imamura T, Hoshino M.
EMBO J. 2025 Jan 15.

Mouse / E14.0 / E15.5 / Lateral ventricle

Assembly of neuron- and radial glial-cell-derived extracellular matrix molecules promotes radial migration of developing cortical neurons
Mubuchi A, Takechi M, Nishio S, Matsuda T, Itoh Y, Sato C, Kitajima K, Kitagawa H, Miyata S.
Elife. 2024 Mar 21:12:RP92342.

Mouse / E12.5_E13.0 / E14.0_E15.0 / E16.0_E17.0 / Cerebral cortex / Parietal lobe Somatosensory area

Analyses of Conditional Knockout Mice for Pogz, a Gene Responsible for Neurodevelopmental Disorders in Excitatory and Inhibitory Neurons in the Brain
Hamada N, Nishijo T, Iwamoto I, Shifman S, Nagata KI.
Cells. 2024 Mar 19;13(6):540.

Mouse / Lateral_ventricle / E14.5

Lmo4 synergizes with Fezf2 to promote direct in vivo reprogramming of upper layer cortical neurons and cortical glia towards deep-layer neuron identities
Felske T, Tocco C, Péron S, Harb K, Alfano C, Galante C, Berninger B, Studer M.
PLoS Biol. 2023 Aug 8;21(8):e3002237.

Mouse / E14.0 / Lateral ventricle

Histological Analysis of a Mouse Model of the 22q11.2 Microdeletion Syndrome
Tabata H, Mori D, Matsuki T, Yoshizaki K, Asai M, Nakayama A, Ozaki N, Nagata KI.
Biomolecules. 2023 Apr 27;13(5):763.

Mouse / E13.5 / E14.5 / Cerebral ventricles

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Mouse / Cerebral cortex / Parietal lobe Somatosensory area / E14.0

Variant-specific changes in RAC3 function disrupt corticogenesis in neurodevelopmental phenotypes
Scala M, Nishikawa M, Ito H, Tabata H, Khan T, Accogli A, Davids L, Ruiz A, Chiurazzi P, Cericola G, Schulte B, Monaghan KG, Begtrup A, Torella A, Pinelli M, Denommé-Pichon AS, Vitobello A, Racine C, Mancardi MM, Kiss C, Guerin A, Wu W, Gabau Vila E, Mak BC, Martinez-Agosto JA, Gorin MB, Duz B, Bayram Y, Carvalho CMB, Vengoechea JE, Chitayat D, Tan TY, Callewaert B, Kruse B, Bird LM, Faivre L, Zollino M, Biskup S; Undiagnosed Diseases Network; Telethon Undiagnosed Diseases Program; Striano P, Nigro V, Severino M, Capra V, Costain G, Nagata KI.
Brain. 2022 Sep 14;145(9):3308-3327.

Mouse / Cerebral cortex / E14.5

Tbr1 Misexpression Alters Neuronal Development in the Cerebral Cortex
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Mouse

Heterogeneous nuclear ribonucleoprotein U (HNRNPU) safeguards the developing mouse cortex
Sapir T, Kshirsagar A, Gorelik A, Olender T, Porat Z, Scheffer IE, Goldstein DB, Devinsky O, Reiner O.
Nat Commun. 2022 Jul 21;13(1):4209.

Mouse / E13.5 / E14.5 / Lateral ventricle

Endosomal trafficking defects alter neural progenitor proliferation and cause microcephaly
Carpentieri JA, Di Cicco A, Lampic M, Andreau D, Del Maestro L, El Marjou F, Coquand L, Bahi-Buisson N, Brault JB, Baffet AD.
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Mouse / E14.0 / Lateral ventricle

Heterogeneous nuclear ribonucleoprotein U (HNRNPU) safeguards the developing mouse cortex
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Mouse / E14.0 / Cerebral cortex / Parietal lobe Somatosensory area

Impaired Function of PLEKHG2, a Rho-Guanine Nucleotide-Exchange Factor, Disrupts Corticogenesis in Neurodevelopmental Phenotypes
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Mouse / Lateral ventricle

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Mouse / E15.0 / Cerebral cortex / Neuroprogenitor cells

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Mouse / E14.5 / Cerebral cortex

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Mouse / E13.5 / Cerebral cortex

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Mouse / Neural tubr

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Mouse / E14.5 / Cerebral cortex / Neuroprogenitor cells

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Mouse / E15.5 / Lateral ventricle

DNA Repair Protein RAD51 Enhances the CRISPR/Cas9-mediated Knock-In Efficiency in Brain Neurons
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Mouse / E14.5_E15.5 / Lateral ventricle

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Mouse / Otocyst

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Hiroki Takeda, Toru Miwa, Min Young Kim, Byung Yoon Choi, Yorihisa Orita, Ryosei Minoda
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Mouse / E15.0 / Postnatal electroporation / P2 / Adult Mouse electroporation / P21

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Mouse / E14.5 / Lateral ventricle

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Brain Res, 1721, 146332 2019 Oct 15

Mouse / Embryonic inner ear / Otocyst

Role of Dach1 Revealed Using a Novel Inner Ear-Specific Dach1-knockdown Mouse Model
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Mouse / E14.5 / Lateral ventricle

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Mouse In Utero / E14.5 / Cerebral ventricles

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Mouse / E14.5 / Lateral ventricle

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Mouse / E13.5 / Cerebral cortex / Somatosensory cortex

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Mouse / E15.0 / Cerebral cortex / Glucocorticoid receptor

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Mouse / Cerebral cortex /

Role of Per3, a Circadian Clock Gene, in Embryonic Development of Mouse Cerebral Cortex
Mariko Noda, Ikuko Iwamoto, Hidenori Tabata, Takanori Yamagata, Hidenori Ito, Koh-Ichi Nagata Sci Rep, 9 (1), 5874 2019 Apr 10.

Mouse / E14.5 / Lateral ventricles

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Mouse / E12.5 / E15.5 / E16.0 / Cortical progenitors

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Mouse / E11.5

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Mouse / E13.0

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