

Transfection into Mouse-Rat: **In Vivo** by Electroporation

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

- The finer control over the delivered energy available with the NEPA21 offers specific and important advantages for BRAIN / Tissue 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).
- For particularly sensitive and delicate targets, identifying and only delivering the required energy (and no more) to porate the membrane is of utmost importance for viability post electroporation.
- 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.

MENUE

- **BRAIN-TISSUE - Neonatal and In Vivo**
- **EMBRYOS - In Utero**
- **CULTURED EMBRYOS - Ex Utero**
- **RETINA / CORNEA / SPINAL CORD / SCIATIC NERVE**
- **LUNG / SPLEEN / LIVER / KIDNEY / STOMACH / INTESTING**
- **PANCREAS / ISLETS OF LANGERHANS**
- **TESTIS / OVARY / PROSTRATE / GONAD / UTERUS**
- **RAT MUSCLE / SKIN / JOINT / CARTILAGR / TUMOR and OTHERS**

OTHERS

- **CHICK RETINA – Ex Vivo**
- **ZEBRAFISH RETINA – In Vivo / Ex Vivo**

Transfection into Mouse-Rat: **BRAIN-TISSUE** by Electroporation

APPLICATIONS

Transfection into Mouse-Rat: **BRAIN-TISSUE (Neonatal and In Vivo)** by Electroporation

Electroporation-mediated gene transfer in the adult rat brain

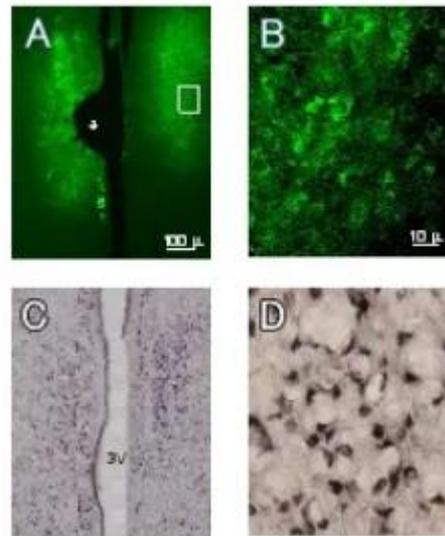
Figure A: EGFP expression in the medial preoptic nuclei of a female rat examined 4 days after bilateral electroporation at 10 weeks of age. (An asterisk indicates the trace of the positioning of the electrode)

Figure B: EGFP-positive cells (high magnification of Fig. A using a 60x objective lens). EGFP fluorescent signals are observed in the perikarya.

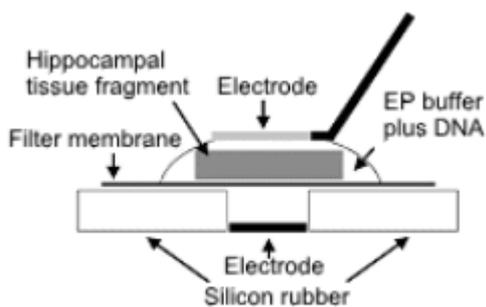
Figure C: Estrogen receptor α immunoreactivity in the medial preoptic nuclei and the periventricular nuclei of an adult female rat. 3V: third ventricle.

Figure D: Estrogen receptor α -positive cells (high magnification of Fig. C using a 60x objective lens). Estrogen receptor immunoreactivity is prominent in the nuclei.

Tetsuo Shirakawa, Center for Advanced Oral Medicine, Hokkaido University Hospital



Electroporation-mediated gene transfer system applied to cultured CNS neurons



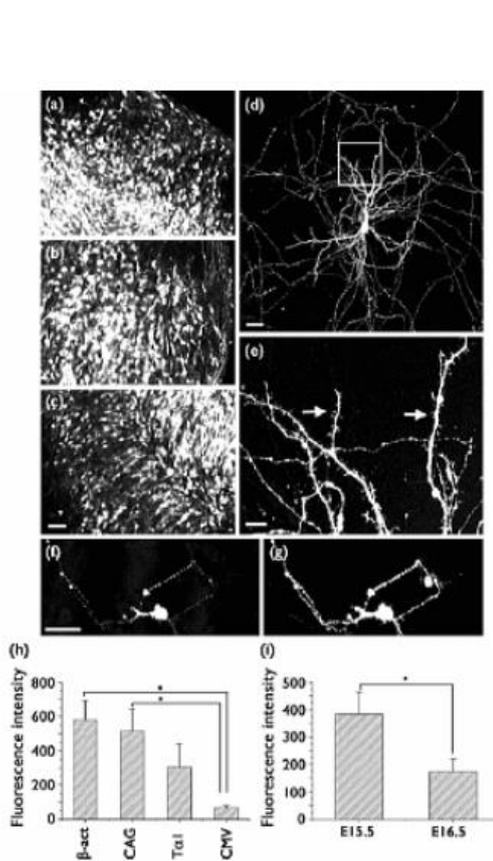
Schematic representation of an electroporation set-up.

A fragment of the mouse embryonic hippocampus was placed on a Millipore membrane filter and 5 μ l EP buffer containing 1mg/ml of plasmid DNA was applied onto the tissue.

A tungsten needle was attached to the surface of a droplet.

After application of square pulses the tissue fragment was returned to a petri dish containing ice-cold HBSS solution.

Electroporation-mediated expression of fluorescent proteins in hippocampal neurons



(a-c) Organ culture of hippocampal tissue fragments three days after electroporation with CAG-eGFP

(a) Ta1X4 -eGFP, (b) and b-actin-eGFP, (c) expression constructs.

(d,e) A mature hippocampal neuron maintained 14 days in dissociated culture after electroporation of a -actin-eGFP expression construct. Higher magnification view of the region marked by a rectangle in (d) reveals dendritic spines on the surface of dendritic shafts (arrows in e).

(f,g) A hippocampal neuron 7 days after electroporation of 1:1 mixture of Ta1X4-eGFP and Ta1X4-mRFP1. Both eGFP fluorescence (f) and mRFP1 fluorescence (g) can be observed in a single cell.

(h) Relative fluorescence intensity of hippocampal tissue fragments after electroporation of eGFP-expression plasmids with four different promoter sequences. The tissue fragments were maintained in culture for 4 days, fixed and observed under a confocal microscope. Fluorescence intensities per unit area of the tissue fragments were determined.

(i) Relative fluorescence intensity of hippocampal tissue fragments isolated at two different developmental stages and electroporated with b-actin-eGFP. Tissue fragments were maintained for 4 days in culture and subsequently fixed. Fluorescence intensities were measured using a confocal microscope.

Shigeo Okabe, Department of Cellular Neurobiology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo
*Neuroreport, Volume 15, Issue 6, Pages 971-975, April 29, 2004

PUBLICATIONS

Transfection into Mouse-Rat: **BRAIN-TISSUE (Neonatal and In Vivo) by Electroporation**

BRAIN - Neonatal and In Vivo

Cerebellum lobe culture / P5_mice

Nesprin-2 coordinates opposing microtubule motors during nuclear migration in neurons

Zhou, C., Wu, Y. K., Ishidate, F., Fujiwara, T. K., Kengaku, M.

J Cell Biol. 2024 Nov 4;223(11):e202405032.

P0_mouse_brains / Forebrain

Genome editing of Nf1, Pten, and Trp53 in neonatal mice induces glioblastomas positive for oligodendrocyte lineage transcription factor 2

Yamamoto H, Yamamura K, Nagasaki H, Suzuki T, Ninomiya F, Matsubara K, Harada N, Ohkubo S.

J Toxicol Pathol. 2021 Oct;34(4):359-365.

P2_mouse_brains / P21_mouse_brains**Primary cilium-dependent cAMP/PKA signaling at the centrosome regulates neuronal migration**

Stoufflet J, Chaulet M, Doulazmi M, Fouquet C, Dubacq C, Métin C, Schneider-Maunoury S, Trembleau A, Vincent P, Caillé I.

Sci Adv. 2020 Sep 2;6(36):eaba3992.

P0_mouse_brains**Rho Family GTPases, Rac and Cdc42, Control the Localization of Neonatal Dentate Granule Cells During Brain Development**

Ito H, Morishita R, Mizuno M, Tabata H, Nagata KI.

Hippocampus. 2019 Jul;29(7):569-578.

P2_Mouse_brains**A dual role for the transcription factor Sp8 in postnatal neurogenesis**

Gaborieau E, Hurtado-Chong A, Fernández M, Azim K, Raineteau O.

Sci Rep. 2018 Sep 28;8(1):14560.

Organotypic brains slices / E15.5**Precise Somatotopic Thalamocortical Axon Guidance Depends on LPA-Mediated PRG-2/Radixin Signaling**

Cheng J, Sahani S, Hausrat TJ, Yang JW, Ji H, Schmarowski N, Endle H, Liu X, Li Y, Böttche R, Radyushkin K, Maric HM, Hoerder-Suabedissen A, Molnár Z, Prouvot PH, Trimbuch T, Ninnemann O, Huai J, Fan W, Visentin B, Sabbadini R, Strømgaard K, Stroh A, Luhmann HJ, Kneussel M, Nitsch R, Vogt J.

Neuron. 2016 Oct 5;92(1):126-142.

Cortical slices / P0_hamster_brains**Cortical excitatory neurons become protected from cell division during neurogenesis in an Rb family-dependent manner.**

Oshikawa M, Okada K, Nakajima K, Ajioka I.

Development. 2013 Jun;140(11):2310-20.

Cortical slices**Wnt/calcium signaling mediates axon growth and guidance in the developing corpus callosum.**

Hutchins BI, Li L, Kalil K.

Dev Neurobiol. 2011 Apr;71(4):269-83.

New born mouse brain**Cellular Mechanisms Underlying Morphine Analgesic Tolerance and Hyperalgesia**

Hiroshi Ueda

Opioid-Induced Hyperalgesia, First, Pages 9-20, October 2009

P0_P4_mouse_brains**Efficient In Vivo Electroporation of the Postnatal Rodent Forebrain**

Boutin C, Diestel S, Desoeuvre A, Tiveron MC, Cremer H.

PLoS One. 2008 Apr 2;3(4):e1883.

New born brains**Plexin-A2 and its ligand, Sema6A, control nucleus-centrosome coupling in migrating granule cells**

Renaud J, Kerjan G, Sumita I, Zagar Y, Georget V, Kim D, Fouquet C, Suda K, Sanbo M, Suto F, Ackerman SL, Mitchell KJ, Fujisawa H, Chédotal A.

Nat Neurosci. 2008 Apr;11(4):440-9.

Adult Mouse brains

Genetic manipulation of adult mouse neurogenic niches by in vivo electroporation

Barnabé-Heider F, Meletis K, Eriksson M, Bergmann O, Sabelström H, Harvey MA, Mikkers H, Frisén J.

Nat Methods. 2008 Feb;5(2):189-96.

P8 mouse brain

Microtubule-based nuclear movement occurs independently of centrosome positioning in migrating neurons

Umeshima H, Hirano T, Kengaku M.

Proc Natl Acad Sci U S A . 2007 Oct 9;104(41):16182-7.

Cerebellar slices

Homer proteins control neuronal differentiation through IP(3) receptor signaling.

Tanaka M, Duncan RS, McClung N, Yannazzo JA, Hwang SY, Marunouchi T, Inokuchi K, Koulen P.

FEBS Lett. 2006 Nov 13;580(26):6145-50.

Cultured telencephalic hemisphere

The Caudal Migratory Stream: A Novel Migratory Stream of Interneurons Derived from the Caudal Ganglionic Eminence in the Developing Mouse Forebrain

Yozu M, Tabata H, Nakajima K.

J Neurosci. 2005 Aug 3;25(31):7268-77.

Embryo / Hippocampus

Electroporation-mediated gene transfer system applied to cultured CNS neurons

Kawabata I, Umeda T, Yamamoto K, Okabe S.

Neuroreport . 2004 Apr 29;15(6):971-5.

Adult mouse brains

Locus-Specific Rescue of GluR1 NMDA Receptors in Mutant Mice Identifies the Brain Regions Important for Morphine Tolerance and Dependence

Inoue M, Mishina M, Ueda H.

J Neurosci. 2003 Jul 23;23(16):6529-36.

Adult rat brains

Up-regulation of Protein-disulfide Isomerase in Response to Hypoxia/Brain Ischemia and Its Protective Effect against Apoptotic Cell Death

Tanaka S, Uehara T, Nomura Y.

J Biol Chem. 2000 Apr 7;275(14):10388-93.

Transfection into Mouse-Rat: **EMBRYOS IN UTERO** by Electroporation

APPLICATIONS

Transfection into Mouse-Rat: **EMBRYOS IN UTERO** by Electroporation

Gene transfer into embryonic brains using in utero electroporation technique

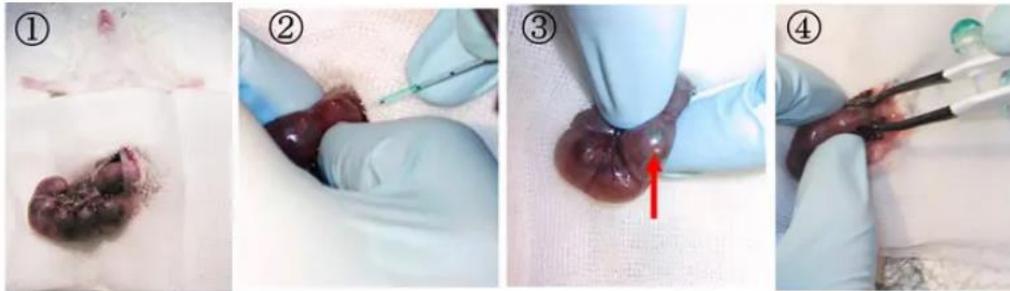
1) Materials



- 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



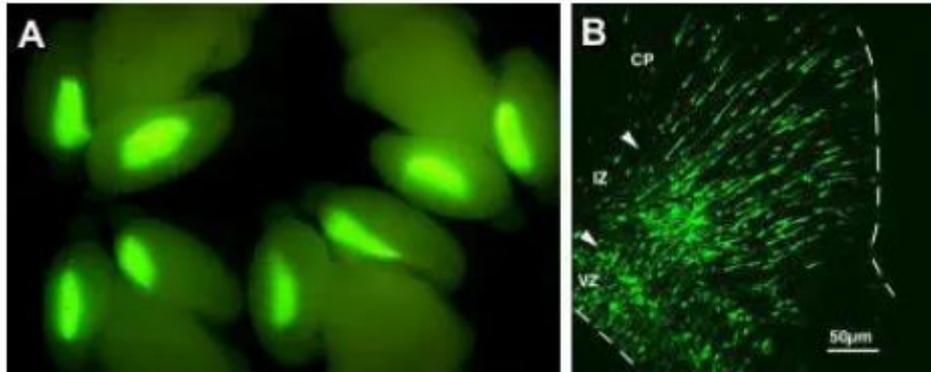
1. A 2 cm midline incision is then made in the abdominal wall along the linea alba using a set of forceps and scissors. A piece of sterile gauze with a hole cut in the center is placed over the incision, and one uterine horn is drawn out through the hole in the gauze.
2. After observing the orientation of the embryos through the wall of the uterine horn, a micropipette is inserted into the lateral ventricle, and 2-5 μ l of plasmid solution is injected by expiratory pressure using the aspirator tube assembly. When CAG-EGFP is used, a concentration of 1 μ g/ μ l 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

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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**p53/p21 pathway activation contributes to the ependymal fate decision downstream of GemC1**

Ortiz-Álvarez G, Fortoul A, Srivastava A, Moreau MX, Bouloudi B, Mailhes-Hamon C, Delgehyr N, Faucourt M, Bahin M, Blugeon C, Breau M, Géli V, Causeret F, Meunier A, Spassky N.

Cell Rep. 2022 Dec 13;41(11):111810.

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**

Crespo I, Pignatelli J, Kinare V, Méndez-Gómez HR, Esgleas M, Román MJ, Canals JM, Tole S, Vicario C.

Mol Neurobiol. 2022 Sep;59(9):5750-5765.

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.

Nat Commun. 2022 Jan 10;13(1):16.

Mouse / E14.0 / Lateral ventricle**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 / E14.0 / Cerebral cortex / Parietal lobe Somatosensory area**Impaired Function of PLEKHG2, a Rho-Guanine Nucleotide-Exchange Factor, Disrupts Corticogenesis in Neurodevelopmental Phenotypes**

Nishikawa M, Ito H, Tabata H, Ueda H, Nagata KI.

Cells. 2022 Feb 16;11(4):696.

Mouse / Lateral ventricle**Simultaneous two-photon imaging of action potentials and subthreshold inputs in vivo**

Bando Y, Wenzel M, Yuste R.

Nat Commun. 2021 Dec 10;12(1):7229

Mouse / E15.0 / Cerebral cortex / Neuroprogenitor cells**IgSF11 homophilic adhesion proteins promote layer-specific synaptic assembly of the cortical interneuron subtype**

Hayano Y, Ishino Y, Hyun JH, Orozco CG, Steinecke A, Potts E, Oisi Y, Thomas CI, Guerrero-Given D, Kim E, Kwon HB, Kamasawa N, Taniguchi H.

Sci Adv. 2021 Jul 14;7(29):eabf1600.

Mouse / E14.5 / Cerebral cortex**A pericellular hyaluronan matrix is required for the morphological maturation of cortical neurons**

Takechi M, Oshima K, Nadano D, Kitagawa H, Matsuda T, Miyata S.

Biochim Biophys Acta Gen Subj. 2020 Oct;1864(10):129679.

Mouse / E13.5 / Cerebral cortex**Huntington's disease alters human neurodevelopment**

Barnat M, Capizzi M, Aparicio E, Boluda S, Wennagel D, Kacher R, Kassem R, Lenoir S, Agasse F, Braz BY, Liu JP, Ighil J, Tessier A, Zeitlin SO, Duyckaerts C, Dommergues M, Durr A, Humbert S.

Science. 2020 Aug 14;369(6505):787-793.

Mouse / Neural tubr**In utero gene transfer system for embryos before neural tube closure reveals a role for Hmga2 in the onset of neurogenesis**

Naohiro Kuwayama, Yusuke Kishi, Yurie Maeda, Yurie Nishiumi, Yutaka Suzuki, Haruhiko Koseki, Yusuke Hirabayashi, Yukiko Gotoh

bioRxiv May 15, 2020

Mouse / E14.5 / Cerebral cortex / Neuroprogenitor cells**Knockdown of Son, a mouse homologue of the ZTTK syndrome gene, causes neuronal migration defects and dendritic spine abnormalities**

Ueda M, Matsuki T, Fukada M, Eda S, Toya A, Iio A, Tabata H, Nakayama A.

Mol Brain. 2020 May 24;13(1):80.

Mouse / E15.5 / Lateral ventricle**DNA Repair Protein RAD51 Enhances the CRISPR/Cas9-mediated Knock-In Efficiency in Brain Neurons**

Taiga Kurihara, Emi Kouyama-Suzuki, Michiru Satoga, Xue Li, Moataz Badawi, Thiha, Deeba Noreen Baig, Toru Yanagawa, Takeshi Uemura, Takuma Mori, Katsuhiko Tabuchi

Biochem Biophys Res Commun. 2020 Apr 9;524(3):621-628.

Mouse / E14.5_E15.5 / Lateral ventricle**In Vivo Imaging of the Coupling Between Neuronal and CREB Activity in the Mouse Brain**

Tal Laviv, Benjamin Scholl, Paula Parra-Bueno, Beth Foote, Chuqiu Zhang, Long Yan, Yuki Hayano, Jun Chu, Ryohei Yasuda

Neuron, 105 (5), 799-812.e5 2020 Mar 4

Mouse / Otocyst**Prenatal Electroporation-Mediated Gene Transfer Restores Slc26a4 Knock-Out Mouse Hearing and Vestibular Function**

Hiroki Takeda, Toru Miwa, Min Young Kim, Byung Yoon Choi, Yori-hisa Orita, Ryosei Minoda

Sci Rep, 9 (1), 17979 2019 Nov 29

Mouse / E15.0 / Postnatal electroporation / P2 / Adult Mouse electroporation / P21**Primary cilium-dependent cAMP/PKA signaling at the centrosome regulates neuronal migration**

Stoufflet J, Chaulet M, Doulazmi M, Fouquet C, Dubacq C, Métin C, Schneider-Maunoury S, Trembleau A, Vincent P, Caillé I.

Sci Adv. 2020 Sep 2;6(36):eaba3992

Mouse / E14.5 / Lateral ventricle**A Novel LGI1 Missense Mutation Causes Dysfunction in Cortical Neuronal Migration and Seizures**

Feng Liu, Chao Du, Xin Tian, Yuanlin Ma, Bei Zhao, Yin Yan, Zijun Lin, Peijia Lin, Ruijiao Zhou, Xuefeng Wang

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**

Toru Miwa, Ryosei Minoda, Yoshihide Ishikawa, Tomohito Kajii, Yori-hisa Orita, Takahiro Ohyama

Biol Open, 8 (8) 2019 Aug 20

Mouse / E14.5 / Lateral ventricle**Estrogen Receptor α Promotes Cav1.2 Ubiquitination and Degradation in Neuronal Cells and in APP/PS1 Mice**

Yu-Jie Lai, Bing-Lin Zhu, Fei Sun, Dong Luo, Yuan-Lin Ma, Bio Luo, Jing Tang, Ming-Jian Xiong, Lu Liu, Yan Long, Xiao-Tong Hu, Ling He, Xiao-Juan Deng, John H Zhang, Jian Yang, Zhen Yan, Guo-Jun Chen

Aging Cell, 18 (4), e12961 Aug 2019

Mouse In Utero / E14.5 / Cerebral ventricles**In Vivo Single-Cell Genotyping of Mouse Cortical Neurons Transfected With CRISPR/Cas9**

Steinecke A, Kurabayashi N, Hayano Y, Ishino Y, Taniguchi H.

Cell Rep, 28 (2), 325-331.e4 2019 Jul 9

Mouse / E14.5 / Lateral ventricle**Both Excitatory and Inhibitory Neurons Transiently Form Clusters at the Outermost Region of the Developing Mammalian Cerebral Neocortex**

Shin M, Kitazawa A, Yoshinaga S, Hayashi K, Hirata Y, Dehay C, Kubo KI, Nakajima K.

J Comp Neurol. 2019 Jul 1;527(10):1577-1597.

Mouse / E13.5 / Cerebral cortex / Somatosensory cortex**Pathological mTOR Mutations Impact Cortical Development**

Tarkowski B, Kuchcinska K, Blazejczyk M, Jaworski J.

Hum Mol Genet. 2019 Jul 1;28(13):2107-2119.

Mouse / E15.0 / Cerebral cortex / Glucocorticoid receptor**Persistence of Learning-Induced Synapses Depends on Neurotrophic Priming of Glucocorticoid Receptors**

Arango-Lievano M, Borie AM, Dromard Y, Murat M, Desarmenien MG, Garabedian MJ, Jeanneteau F.
Proc Natl Acad Sci U S A. 2019 Jun 25;116(26):13097-13106.

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**MicroRNA-129-5p Is Regulated by Choline Availability and Controls EGF Receptor Synthesis and Neurogenesis in the Cerebral Cortex**

Trujillo-Gonzalez I, Wang Y, Friday WB, Vickers KC, Toth CL, Molina-Torres L, Surzenko N, Zeisel SH.
FASEB J. 2019 Mar;33(3):3601-3612.

Mouse / E12.5 / E15.5 / E16.0 / Cortical progenitors**Intersectional Monosynaptic Tracing for Dissecting Subtype-Specific Organization of GABAergic Interneuron Inputs**

Yetman MJ, Washburn E, Hyun JH, Osakada F, Hayano Y, Zeng H, Callaway EM, Kwon HB, Taniguchi H.
Nat Neurosci. 2019 Mar;22(3):492-502.

Single cell profiling of CRISPR/Cas9-induced OTX2 deficient retinas reveals fate switch from restricted progenitors

Miruna G. Ghinia Tegla, Diego F. Buenaventura, Diana Y. Kim, Cassandra Thakurdin, Kevin C. Gonzalez, M. Emerson
bioRxiv February 02, 2019

An Optogenetic Approach to Studies of the Mechanisms of Heterosynaptic Plasticity in Neocortical Neurons

N. A. Simonova, N. V. Bal, P. M. Balaban, M. A. Volgushev, A. Y. Malyshev
Neuroscience and Behavioral Physiology volume 49, pages208–215(2019)

Drebrin-like (Dbnl) Controls Neuronal Migration via Regulating N-Cadherin Expression in the Developing Cerebral Cortex

Seika Inoue, Kanehiro Hayashi, Kyota Fujita, Kazuhiko Tagawa, Hitoshi Okazawa, Ken-Ichiro Kubo, Kazunori Nakajima
J Neurosci, 39 (4), 678-691 2019 Jan 23

Comparative Evaluation of Genetically Encoded Voltage Indicators

Yuki Bando, Masayuki Sakamoto, Samuel Kim, Inbal Ayzenshtat, Rafael Yuste
Cell Rep, 26 (3), 802-813.e4 2019 Jan 15

Niche-derived laminin-511 Promotes Midbrain Dopaminergic Neuron Survival and Differentiation Through YAP

Dawei Zhang, Shanzheng Yang, Enrique M Toledo, Daniel Gyllborg, Carmen Saltó, J Carlos Villaescusa, Ernest Arenas
Sci Signal, 10 (493) 2017 Aug 22

PLGF, a placental marker of fetal brain defects after in utero alcohol exposure.

Lecuyer M, Laquerrière A, Bekri S, Lesueur C, Ramdani Y, Jégou S, Uguen A, Marcocelles P, Marret S, Gonzalez BJ
Acta Neuropathol Commun. 2017 Jun 6;5(1):44.

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Commissural neuron identity is specified by a homeodomain protein, Mbh1, that is directly downstream of Math1

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Hatakeyama et al.

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Mouse / E11.5

Generation of Reelin-Positive Marginal Zone Cells from the Caudomedial Wall of Telencephalic Vesicles

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Mouse / E13.0**Multipolar Migration: The Third Mode of Radial Neuronal Migration in the Developing Cerebral Cortex**

Tabata H, Nakajima K.

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Mouse / E14.0**The in vivo roles of STEF/Tiam1, Rac1 and JNK in cortical neuronal migration**

Kawauchi T, Chihama K, Nabeshima Y, Hoshino M.

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Layering defect in p35 deficiency is linked to improper neuronal-glia interaction in radial migration

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Xie Z, Sanada K, Samuels BA, Shih H, Tsai LH.

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Mouse / E11.5 / Spinal cord**Mammalian BarH1 Confers Commissural Neuron Identity on Dorsal Cells in the Spinal Cord**

Saba R, Nakatsuji N, Saito T.

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Emx2 patterns the neocortex by regulating FGF positional signaling

Fukuchi-Shimogori T, Grove EA.

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Roles of the Basic Helix-Loop-Helix Genes Hes1 and Hes5 in Expansion of Neural Stem Cells of the Developing Brain

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The Journal of Biological Chemistry, Volume 276, Issue 32, Pages 30467-30474, 10 August 2001

Mouse / E12.5_E13 / E14_E15 / E16_E17**Efficient in utero gene transfer system to the developing mouse brain using electroporation: visualization of neuronal migration in the developing cortex**

Tabata H, Nakajima K.

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Transfection into Mouse-Rat: CULTURED EMBRYOS by Ex Utero Electroporation

APPLICATIONS

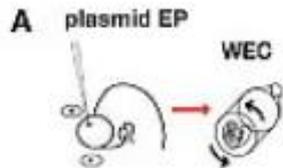
Transfection into Mouse-Rat: CULTURED EMBRYOS by Ex Utero Electroporation

Electroporation for mammalian embryos in the whole embryo culture system

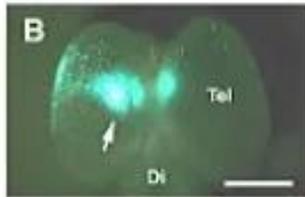
Procedure:

1. Pre-culture embryos in the whole embryo culture system for 1.5-2 hours prior to electroporation.
2. Place the embryo in a Petri dish with Tyrode's solution.
3. Inject 0.1-0.5 µl of plasmid DNA into the brain ventricle with a fine capillary.
4. Apply square pulses using the electroporator (NEPA21/CUY21) and electrodes (chamber-type and forceps-type electrodes are available). 70V, 50 msec at 1-second interval, five pulses are applied for E10.5 mouse embryos.
5. Culture the electroporated embryos for 24-48 hours in the whole embryo culture system. Transfection of a fluorescent protein-expression vector into the developing rat cortex

Transfection of a fluorescent protein-expression vector into the developing rat cortex.



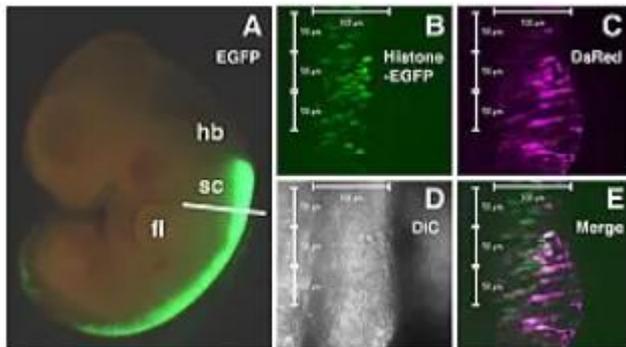
(A) EGFP-expression vector was electroporated into E11.5 rat telencephalon. The electroporated embryo was cultured in the whole embryo culture system (WEC).



(B) 24 hours after electroporation, EGFP-expression was specifically detected at the dorsal part of the telencephalon.

Tel: telencephalon;
Di: diencephalon

Transfection of fluorescent protein-expression vectors into the rat spinal cord.



(A) EGFP-expression vector was transfected into the spinal cord of E12.5 rat embryos by electroporation. The electroporated embryos were cultured for 24 hours in the whole embryo culture system (WEC).

(B-E) Time-lapse analysis of neuroepithelial cells in the slice culture system. Histone-EGFP- and DsRed2-expression vectors were co-electroporated into the E12.0 rat spinal cord. The electroporated embryo was cultured for 24 hours in the WEC, then the spinal cord was sliced and time-lapse recording was performed.

(B-E) Cell nuclei (B, green) and cytoplasm (C, magenta) of neuroepithelial cells in the slice are simultaneously labeled by the electroporation (E). (D) A DIC image.

hb: hindbrain; sc: spinal cord; fl: forelimb; DIC: differential interference contrast.

PUBLICATIONS**Transfection into Mouse-Rat: CULTURED EMBRYOS by Ex Utero Electroporation****Ex utero mouse embryogenesis from pre-gastrulation to late organogenesis**

Aguilera-Castrejon A, Oldak B, Shani T, Ghanem N, Itzkovich C, Slomovich S, Tarazi S, Bayerl J, Chugaeva V, Ayyash M, Ashoukhi S, Sheban D, Livnat N, Lasman L, Viukov S, Zerbib M, Addadi Y, Rais Y, Cheng S, Stelzer Y, Keren-Shaul H, Shlomo R, Massarwa R, Novershtern N, Maza I, Hanna JH.

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IgSF11 homophilic adhesion proteins promote layer-specific synaptic assembly of the cortical interneuron subtype

Hayano Y, Ishino Y, Hyun JH, Orozco CG, Steinecke A, Potts E, Oisi Y, Thomas CI, Guerrero-Given D, Kim E, Kwon HB, Kamasawa N, Taniguchi H.

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FGF8 signaling patterns the telencephalic midline by regulating putative key factors of midline development.

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Isolation and Characterization of Vasohibin-2 as a Homologue of VEGF-Inducible Endothelium-Derived Angiogenesis Inhibitor Vasohibin

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Misrouting of mitral cell progenitors in the Pax6/small eye rat telencephalon

Nomura T, Osumi N.

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Manipulating gene expressions by electroporation in the developing brain of mammalian embryos

Takahashi M, Sato K, Nomura T, Osumi N.

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Pax6 regulates specification of ventral neurone subtypes in the hindbrain by establishing progenitor domains

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Gene Transfer into Cultured Mammalian Embryos by Electroporation

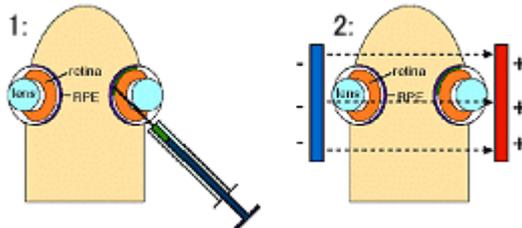
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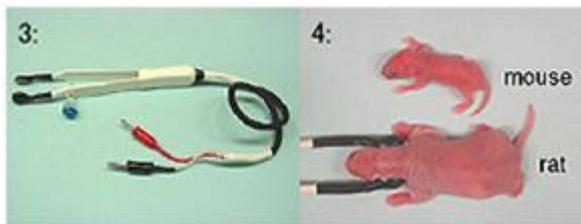
Transfection into Mouse-Rat-Chick-Zebrafish: **RETINA / CORNEA/ SPINAL CORD / SCIATIC NERVE** by Electroporation

APPLICATIONS

Transfection into Mouse-Rat-Chick-Zebrafish: **RETINA / CORNEA/ SPINAL CORD / SCIATIC NERVE** by Electroporation



Schematic illustration showing the in vivo electroporation method.
(1). DNA injection into subretinal space
(2). Electroporation



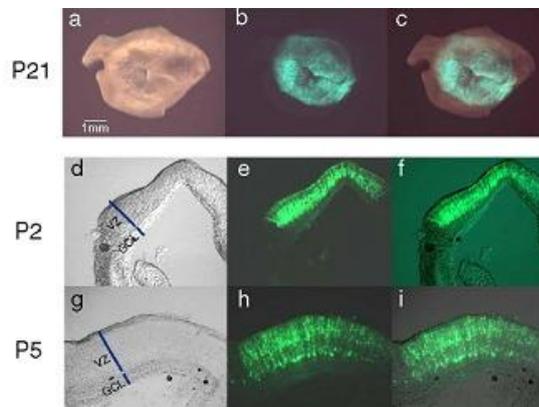
Tweezer-type electrodes (3) are placed to hold the head of newborn (P0) rat or mouse (4).

In vivo electroporated rat retinae harvested at various developmental stages.

(a) Whole-mount preparation of rat retina in vivo electroporated with pCAG-GFP at P0 and harvested at P21. Pictures were taken from the scleral side.

(b) Rat retinae in vivo electroporated with pCAG-GFP at P0 were harvested at P2(d-f), or P5(g-i), and cryosections were prepared.

Takahiko Matsuda, Cepko laboratory
Department of Genetics and Howard Hughes
Medical Institute, Harvard Medical School



PUBLICATIONS

Mouse-Rat-Chick-Zebrafish: **RETINA / CORNEA/ SPINAL CORD / SCIATIC NERVE** by Electroporation

In vivo Retina		Chick Embryos – In Ovo
In vivo/Ex vivo Retina		Chick Embryos – Ex Ovo
In vivo/Ex vivo Cornea		Chick Retina – Ex Vivo
Ex vivo Retina		Zebrafish Retina – In Vivo / Ex Vivo
Ex vivo Spinal Cord		
In vivo Spinal Cord		
In vivo Sciatic Nerve		

In vivo Retina

[dCasMINI-mediated therapy rescues photoreceptors degeneration in a mouse model of retinitis pigmentosa](#)

Wang Q, Xu X, Chen S, Lu R, Li L, Lo CH, Liu Z, Ning K, Li T, Kowal TJ, Wang B, Hartnett ME, Wang S, Qi LS, Sun Y.
Sci Adv. 2024 Dec 20;10(51): eadn7540.

Optimization of HITI-Mediated Gene Insertion for Rhodopsin and Peripherin-2 in Mouse Rod Photoreceptors: Targeting Dominant Retinitis Pigmentosa

Onishi A, Tsunekawa Y, Mandai M, Ishimaru A, Ohigashi Y, Sho J, Yasuda K, Suzuki K, Izpisua Belmonte JC, Matsuzaki F, Takahashi M. Invest Ophthalmol Vis Sci. 2024 Nov 18;65(13):38.

SponGee: A Genetic Tool for Subcellular and Cell-Specific cGMP Manipulation

Ros O, Zagar Y, Ribes S, Baudet S, Loulier K, Couvet S, Ladarre D, Aghaie A, Louail A, Petit C, Mechulam Y, Lenkei Z, Nicol X. Cell Rep. 2019 Jun 25;27(13):4003-4012.e6.

In vivo/Ex vivo Retina**Gene Transfer of Prolyl Hydroxylase Domain 2 Inhibits Hypoxia-inducible Angiogenesis in a Model of Choroidal Neovascularization.**

Takei A, Ekström M, Mammadzada P, Aronsson M, Yu M, Kvanta A, André H. Sci Rep. 2017 Feb 10;7:42546.

Requirement of retinoic acid receptor β for genipin derivative-induced optic nerve regeneration in adult rat retina.

Koriyama Y, Takagi Y, Chiba K, Yamazaki M, Sugitani K, Arai K, Suzuki H, Kato S. PLoS One. 2013 Aug 6;8(8): e71252.

A role for ligand-gated ion channels in rod photoreceptor development

Young TL, Cepko CL. Neuron. 2004 Mar 25;41(6):867-79.

Electroporation and RNA interference in the rodent retina in vivo and in vitro

Matsuda T, Cepko CL. Proc Natl Acad Sci U S A. 2004 Jan 6;101(1):16-22.

Gene transfer into retinal ganglion cells by in vivo electroporation: a new approach

Dezawa M, Takano M, Negishi H, Mo X, Oshitari T, Sawada H.

In vivo/Ex vivo Cornea**Targeted gene transfer to corneal stroma in vivo by electric pulses**

Oshima Y, Sakamoto T, Hisatomi T, Tsutsumi C, Sassa Y, Ishibashi T, Inomata H. Exp Eye Res. 2002 Feb;74(2):191-8.

Ex vivo Retina**Quantitative Analysis of the ThrbCRM1-centered Gene Regulatory Network**

Benjamin Souferi, Mark M Emerson. Biol Open. 2019 Apr 26;8(4): bio039115.

Bulk electroporation and population calcium imaging in the adult mammalian retina.

Briggman KL, Euler T. J Neurophysiol. 2011 May;105(5):2601-9.

Roles of STAT3/SOCS3 pathway in regulating the visual function and ubiquitin-proteasome-dependent degradation of rhodopsin during retinal inflammation.

Ozawa Y et al. J Biol Chem. 2008 Sep 5;283(36):24561-70.

Identification of a Retina-Specific Otx2 Enhancer Element Active in Immature Developing Photoreceptors

Mark M Emerson, Constance L Cepko. Dev Biol, 360 (1), 241-55 2011 Dec 1

Ex vivo Spinal Cord**Explant Culture of the Embryonic Mouse Spinal Cord and Gene Transfer by ex vivo Electroporation**

Kinoshita-Kawada M, Hasegawa H, Hongu T, Yanagi S, Kanaho Y, Masai I, Mishima T, Chen X, Tsuboi Y, Rao Y, Yuasa-Kawada J, Wu JY. Bio Protoc. 2019 Sep 20;9(18): e3373.

A crucial role for Arf6 in the response of commissural axons to Slit

Kinoshita-Kawada M, Hasegawa H, Hongu T, Yanagi S, Kanaho Y, Masai I, Mishima T, Chen X, Tsuboi Y, Rao Y, Yuasa-Kawada J, Wu JY. Development. 2019 Feb 4;146(3)

In vivo Spinal Cord**[Convallatoxin Enhance the Ligand-Induced Mu-Opioid Receptor Endocytosis and Attenuate Morphine Antinociceptive Tolerance in Mice](#)**

Chao PK, Chang HF, Ou LC, Chuang JY, Lee PT, Chang WT, Chen SC, Ueng SH, Hsu JT, Tao PL, Law PY, Loh HH, Yeh SH.
Sci Rep. 2019 Feb 20;9(1):2405.

[Morphine drives internal ribosome entry site-mediated hnRNP K translation in neurons through opioid receptor-dependent signaling.](#)

Lee PT, Chao PK, Ou LC, Chuang JY, Lin YC, Chen SC, Chang HF, Law PY, Loh HH, Chao YS, Su TP, Yeh SH
Nucleic Acids Res. 2014 Dec 1;42(21):13012-25.

[CCL-1 in the spinal cord contributes to neuropathic pain induced by nerve injury.](#)

Akimoto N, Honda K, Uta D, Beppu K, Ushijima Y, Matsuzaki Y, Nakashima S, Kido MA, Imoto K, Takano Y, Noda M.
Cell Death Dis. 2013 Jun 20;4(6): e679

In vivo Sciatic Nerve**[Molecular analysis of axonal-intrinsic and glial-associated co-regulation of axon degeneration](#)**

Catenaccio A, Llaverro Hurtado M, Diaz P, Lamont DJ, Wishart TM, Court FA.
Cell Death Dis. 2017 Nov 9;8(11):e3166

[Schwann cell-derived exosomes enhance axonal regeneration in the peripheral nervous system.](#)

Lopez-Verrilli MA, Picou F, Court FA.
Glia. 2013 Nov;61(11):1795-806

Chick Embryos – In Ovo**[Lineage Tracing Analysis of Cone Photoreceptor Associated Cis-Regulatory Elements in the Developing Chicken Retina](#)**

Estie Schick, Sean D McCaffery, Erin E Keblish, Cassandra Thakurdin, Mark M Emerson
Sci Rep, 9 (1), 9358 2019 June 27

[Single Cell profiling of CRISPR/Cas9-induced OTX2 deficient retinas reveals fate switch from restricted progenitors](#)

Miruna G Ghinia Tegla, Diego F Buenaventura, Diana Y Kim, Cassandra Thakurdin, Kevin G Gonzalez, Mark M Emerson
bioRxiv. 2019 February 2, <https://doi.org/10.1101/538710>

[Irx4-mediated regulation of Slit1 expression contributes to the definition of early axonal paths inside the retina](#)

Jin et al.

Development, Volume 130, Issue 6, Pages 1037-1048, March 2003

Chick Embryos – Ex Ovo**[Single Cell profiling of CRISPR/Cas9-induced OTX2 deficient retinas reveals fate switch from restricted progenitors](#)**

Miruna G Ghinia Tegla, Diego F Buenaventura, Diana Y Kim, Cassandra Thakurdin, Kevin G Gonzalez, Mark M Emerson
bioRxiv. 2019 February 2, <https://doi.org/10.1101/538710>

Chick Retina – Ex Vivo**[Lineage Tracing Analysis of Cone Photoreceptor Associated Cis-Regulatory Elements in the Developing Chicken Retina](#)**

Estie Schick, Sean D McCaffery, Erin E Keblish, Cassandra Thakurdin, Mark M Emerson
Sci Rep, 9 (1), 9358 2019 June 27

Zebrafish Retin – In Vivo / Ex Vivo**[Inhibition of Müller glial cell division blocks regeneration of the light-damaged zebrafish retina](#)**

Thummel et al.,

Developmental Neurobiology, Volume 68, Issue 3, Pages 392-408, 15 February 2008

Cell Cultures (adherent Cells – electroporated directly in the well-plate electrode CUY900-13-35)

Client Laboratory Verified RESULTS

Retinal Ganglion Cells from Human iPSCs

Generation of retinal ganglion cells with functional axons from human induced pluripotent stem cells.

Tanaka T, Yokoi T, Tamalu F, Watanabe S, Nishina S, Azuma N

Sci Rep. 2015 Feb 10; 5: 8344.

Please note these **RETINA** cell line results:

Cell Line		V	TE		Cell Line	V	TE
RPE	Retinal Pigment Epithelium Cells	90%	70%		RPE-1	90%	80%

Mouse-Rat Transfection: LUNG / SPLEEN / LIVER / KIDNEY / STOMACH / INTESTINE**APPLICATIONS****Transfection into Mouse-Rat: LUNG / SPLEEN / LIVER / KIDNEY / STOMACH / INTESTINE by Electroporation**For more specific application information, please email us at: sales@sonidel.com**Stomach****Somatic mouse models of gastric cancer reveal genotype-specific features of metastatic disease**

Leibold J, Tsanov KM, Amor C, Ho YJ, Sánchez-Rivera FJ, Feucht J, Baslan T, Chen HA, Tian S, Simon J, Wuest A, Wilkinson JE, Lowe SW.

Nat Cancer. 2024 Feb;5(2):315-329.

Liver**EP4 receptor-associated protein (EPRAP) regulates gluconeogenesis in the liver and is associated with hyperglycemia in diabetic mice**

Higuchi S, Fujikawa R, Nakatsuji M, Yasui M, Ikeda T, Nagata M, Mishima K, Irie K, Matsumoto M, Yokode M, Minami M

Am J Physiol Endocrinol Metab. 2019 Mar 1;316(3):E410-E417.

Liver**Necroptosis microenvironment directs lineage commitment in liver cancer.**

Seehawer M, Heinzmann F, D'Artista L, Harbig J, Roux PF, Hoenicke L, Dang H, Klotz S, Robinson L, Doré G, Rozenblum N, Kang TW, Chawla R, Buch T, Vucur M, Roth M, Zuber J, Luedde T, Sipos B, Longerich T, Heikenwälder M, Wang XW, Bischof O, Zender L.

Nature. 2018 Oct;562(7725):69-75.

Lung**Stable Somatic Gene Expression in Mouse Lungs Following Electroporation-mediated Tol2 Transposon Delivery.**

Muliawan HS, Nakayama K, Yagi K, Ikeda K, Yagita K, Hirata K, Emoto N

Kobe J Med Sci. 2015 Oct 7;61(2):E47-53.

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Intestine**Estrogen-dependent regulation of sodium/hydrogen exchanger-3 (NHE3) expression via estrogen receptor β in proximal colon of pregnant mice**

Choiyookhuu N, Sato Y, Nishino T, Endo D, Hishikawa Y, Koji T.

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Spleen**Inhibition of nuclear delivery of plasmid DNA and transcription by interferon γ : hurdles to be overcome for sustained gene therapy.**

Zang L, Nishikawa M, Machida K, Ando M, Takahashi Y, Watanabe Y, Takakura Y.

Gene Ther. 2011 Sep;18(9):891-7.

Spleen**Injection site-dependent induction of immune response by DNA vaccine: comparison of skin and spleen as a target for vaccination.**

Guan X et al.

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Liver**Reduction of fibrosis in a rat model of non-alcoholic steatohepatitis cirrhosis by human HGF gene transfection using electroporation.**

Kiyama S, Yamada T, Iwata H, Sekino T, Matsuo H, Yoshida N, Miyahara T, Umeda Y, Matsuno Y, Kimura M, Matsumoto K, Nakamura T, Takemura H.

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Liver**Stabilizing of plasmid DNA in vivo by PEG-modified cationic gold nanoparticles and the gene expression assisted with electrical pulses.**

Kawano T, Yamagata M, Takahashi H, Niidome Y, Yamada S, Katayama Y, Niidome T.

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Liver**Hepatocyte-targeted gene transfer by combination of vascularly delivered plasmid DNA and in vivo electroporation.**

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Gene Therapy, Volume 12, Issue 7, Pages 607-616, April 2005

Kidney**Local actions of endogenous angiotensin II in injured glomeruli**

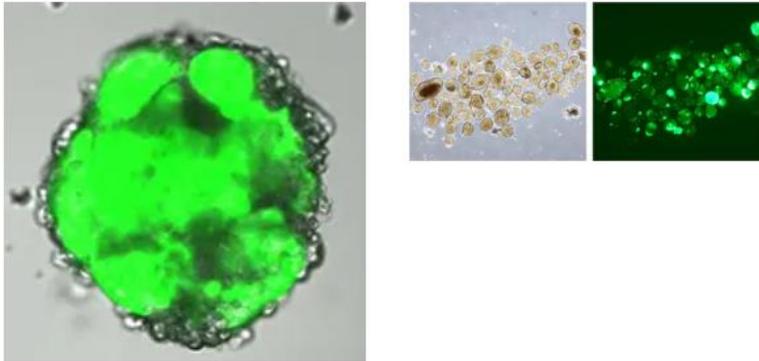
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Kidney**Direct transfer of hepatocyte growth factor gene into kidney suppresses cyclosporin A nephrotoxicity in rats**

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Transfection into Pancreas/Islets of Langerhans by Electroporation**APPLICATIONS****Ex Vivo Transfection Results of GFP Plasmids into Islets of Langerhans****PUBLICATIONS****Pancreas/Islets of Langerhans***Islets of Langerhans – Ex Vivo***ELKS/Voltage-Dependent Ca²⁺ Channel-β Subunit Module Regulates Polarized Ca²⁺ Influx in Pancreatic β Cells**

Mica Ohara-Imaizumi, Kyota Aoyagi, Hajime Yamauchi, Masashi Yoshida, Masayuki X Mori, Yamato Hida, Ha Nam Tran Masamichi Ohkura, Manabu Abe, Yoshihiro Akimoto, Yoko Nakamichi, Chiyono Nishiwaki, Hayato Kawakami, Kazuo Hara, Kenji Sakimura, Shinya Nagamatsu, Yasuo Mori, Junichi Nakai, Masafumi Kakei, Toshihisa Ohtsuka

Cell Rep, 26 (5), 1213-1226.e7 2019 Jan 29

*Islets of Langerhans – Ex Vivo***Imaging of Insulin Exocytosis from Pancreatic Beta Cells.**

Ohara-Imaizumi. et al.

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Aoyagi K. et al.

PLoS One. 2012;7(10):e47381.

*Pancreas – In Vivo***In Vivo Piggy bac-Based Gene Delivery Towards Murine Pancreatic Parenchyma Confers Sustained Expression of Gene of Interest**

Masahiro Sato, Emi Inada, Issei Saitoh, Shingo Nakamura, Satoshi Watanabe

Int J Mol Sci, 20 (13) 2019 Jun 26

Pancreas – In Vivo

Administration of Gemcitabine After Pancreatic Tumor Resection in Mice Induces an Antitumor Immune Response Mediated by Natural Killer Cells

Engin Gürlevik, Bettina Fleischmann-Mundt, Jennifer Brooks, Ihsan Ekin Demir, Katja Steiger, Silvia Ribback, Tetyana Yevsa, Norman Woller, Arnold Kloos, Dmitrij Ostroumov, Nina Armbrrecht, Michael P Manns, Frank Dombrowski, Michael Saborowski, Moritz Kleine, Thomas C Wirth, Helmut Oettle, Güralp O Ceyhan, Irene Esposito, Diego F Calvisi, Stefan Kubicka, Florian Kühnel
Gastroenterology, 151 (2), 338-350.e7 Aug 2016

Pancreas – In Vivo

Multiplexed pancreatic genome engineering and cancer induction by transfection-based CRISPR/Cas9 delivery in mice.

Maresch R, Mueller S, Veltkamp C, Öllinger R, Friedrich M, Heid I, Steiger K, Weber J, Engleitner T, Barenboim M, Klein S, Louzada S, Banerjee R, Strong A, Stauber T, Gross N, Geumann U, Lange S, Ringelhan M, Varela I, Unger K, Yang F, Schmid RM, Vassiliou GS, Braren R, Schneider G, Heikenwalder M, Bradley A, Saur D, Rad R
Nat Commun. 2016 Feb 26;7

Pancreas – In Vivo

Site-targeted non-viral gene delivery by direct DNA injection into the pancreatic parenchyma and subsequent in vivo electroporation in mice.

Sato M, Inada E, Saitoh I, Ohtsuka M, Nakamura S, Sakurai T, Watanabe S
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Pancreas – In Vivo

Injection site-dependent induction of immune response by DNA vaccine: comparison of skin and spleen as a target for vaccination.

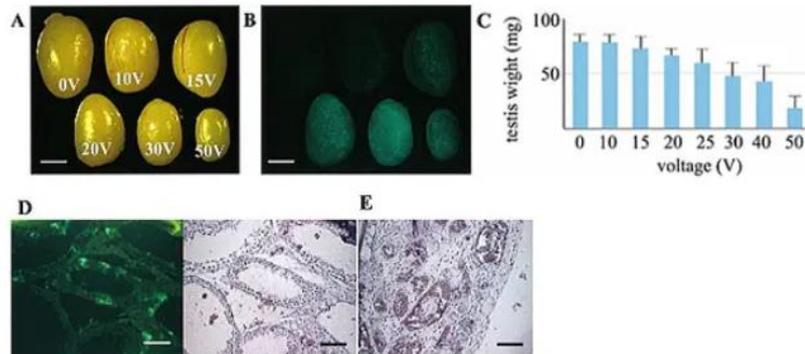
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Mouse-Rat Transfection: TESTIS / OVARY / PROSTATE / GONAD and UTERUS

APPLICATIONS

Transfection into Mouse-Rat: TESTIS, OVARY, PROSTATE, GONAD, and UTERUS by Electroporation

Electroporated Transgene-Rescued Spermatogenesis in Infertile Mutant Mice with a Sertoli Cell Defect



Stereomicroscopic views of transfected testes charged with various voltages and observed under visible (A) or excitation (B) light after 5 wk.

Voltage is indicated on each testis (A).

Loss of testicular weight 5 wk after electric charge in 12-day-old testes (C).

All values are means \pm SD (measured number of testes charged with 0, 10, 15, 20, 25, 30, 40, and 50 V are 4, 4, 4, 12, 12, 15, 10, and 12, respectively).

Cross-sections of a testis at 5 wk after being charged with 50 V, observed with fluorescence microscope under excitation light (D, left), followed by counterstaining of the same section with hematoxylin (D, right) or another section just stained with hematoxylin (E).

In higher voltage charged testes, luminal enlargement of seminiferous tubules (D) or complete degeneration of seminiferous tubules under the capsule (D, left) was observed. In these testes, many fluorescence-positive Sertoli cells were identified easily (E, right), like in 20V charged testes (Day 35).

Bars = 2mm (A, B) or 100 μ m (D, E).

Kentaro Yomogida, School of Human Environmental Sciences, Mukogawa Women's University

*BIOLOGY of REPRODUCTION, Volume 67, Issue 3, Pages 712-717, September 2002

PUBLICATIONS

Transfection into Mouse-Rat: TESTIS, OVARY, PROSTATE, GONAD, and UTERUS by Electroporation

Ovary

High-grade serous ovarian cancer development and anti-PD-1 resistance is driven by IRE1 α activity in neutrophils

Emmanuelli A, Salvagno C, Hwang SM, Awasthi D, Sandoval TA, Chae CS, Cheong JG, Tan C, Iwawaki T, Cubillos-Ruiz JR.

Oncoimmunology. 2024 Oct 2;13(1):2411070.

Testis

Seeding the meiotic DNA break machinery and initiating recombination on chromosome axes

Dereli I, Telychko V, Papanikos F, Raveendran K, Xu J, Boekhout M, Stanzione M, Neuditschko B, Imjeti NS, Selezneva E, Tuncay H, Demir S, Giannattasio T, Gentzel M, Bondarieva A, Stevense M, Barchi M, Schnittger A, Weir JR, Herzog F, Keeney S, Tóth A.

Nat Commun. 2024 Apr 5;15(1):2941.

Endometrium**Multiplexed genome editing by in vivo electroporation of Cas9 ribonucleoproteins effectively induces endometrial carcinoma in mice**

Kobayashi R, Kawabata-Iwakawa R, Sugiyama M, Oyama T, Ohtsuka M, Horii T, Morita S, Nishiyama M, Hatada I. *Int J Cancer*. 2023 Jun 1;152(11):2331-2337.

Ovary**Senescence induction dictates response to chemo- and immunotherapy in preclinical models of ovarian cancer**

Paffenholz SV, Salvagno C, Ho YJ, Limjoco M, Baslan T, Tian S, Kulick A, de Stanchina E, Wilkinson JE, Barriga FM, Zamarin D, Cubillos-Ruiz JR, Leibold J, Lowe SW. *Proc Natl Acad Sci U S A*. 2022 Feb 1;119(5):e2117754119.

Gonads / Ex_Vivo**Divergent Roles of CYP26B1 and Endogenous Retinoic Acid in Mouse Fetal Gonads**

Bellutti L, Abby E, Tourpin S, Messiaen S, Moison D, Trautmann E, Guerquin MJ, Rouiller-Fabre V, Habert R, Livera G. *Biomolecules*. 2019 Sep 26;9(10):536.

Prostate**Somatic Tissue Engineering in Mouse Models Reveals an Actionable Role for WNT Pathway Alterations in Prostate Cancer Metastasis**

Leibold J, Ruscetti M, Cao Z, Ho YJ, Baslan T, Zou M, Abida W, Feucht J, Han T, Barriga FM, Tsanov KM, Zamechek L, Kulick A, Amor C, Tian S, Rybczyk K, Salgado NR, Sánchez-Rivera FJ, Watson PA, de Stanchina E, Wilkinson JE, Dow LE, Abate-Shen C, Sawyers CL, Lowe SW. *Cancer Discov*. 2020 Jul;10(7):1038-1057.

Endometrium**A novel method of gene transduction to the murine endometrium using in vivo electroporation.**

Kobayashi R, Endo K, Ohmori Y, Hondo E. *J Vet Med Sci*. 2017 Sep 29;79(9):1573-1577.

Testis**The Enigmatic Meiotic Dense Body and Its Newly Discovered Component, SCML1, Are Dispensable for Fertility and Gametogenesis in Mice**

Frantzeskos Papanikos, Katrin Daniel, Angelique Goercharn-Ramlal, Ji-Feng Fei, Thomas Kurth, Lukasz Wojtasz, Ihsan Dereli, Jun Fu, Josef Penninger, Bianca Habermann, Azim Surani, A Francis Stewart, Attila Toth *Chromosoma*, 126 (3), 399-415 Jun 2017

Testis**In Vivo Microinjection and Electroporation of Mouse Testis.**

Michaelis M, Sobczak A, Weitzel JM. *J Vis Exp*. 2014 Aug 23;(90).

Testis**cAMP-responsive element in TATA-less core promoter is essential for haploid-specific gene expression in mouse testis**

Somboonthum P, Ohta H, Yamada S, Onishi M, Ike A, Nishimune Y, Nozaki M. *Nucleic Acids Res*. 2005 Jun 10;33(10):3401-11.

Testis

Transient expression analysis of the mouse ornithine decarboxylase antizyme haploid-specific promoter using in vivo electroporation

Ike A, Ohta H, Onishi M, Iguchi N, Nishimune Y, Nozaki M.
FEBS Lett. 2004 Feb 13;559(1-3):159-64.

Testis

Methylation of CpG dinucleotides in the open reading frame of a testicular germ cell-specific intronless gene, *Tact1/Actl7b*, represses its expression in somatic cells

Hisano M, Ohta H, Nishimune Y, Nozaki M.
Nucleic Acids Res. 2003 Aug 15;31(16):4797-804.

Testis

Dramatic Expansion of Germinal Stem Cells by Ectopically Expressed Human Glial Cell Line-Derived Neurotrophic Factor in Mouse Sertoli Cells

Yomogida K, Yagura Y, Tadokoro Y, Nishimune Y.
Biol Reprod. 2003 Oct;69(4):1303-7.

Ovary

Effect of CaCl₂ concentration on the rate of foreign gene transfer and expression by in vivo electroporation in the mouse ovary

Suzuki T, Tsunekawa J, Murai A, Muramatsu T.
Int J Mol Med. 2003 Sep;12(3):365-8.

Testis

Electroporated Transgene-Rescued Spermatogenesis in Infertile Mutant Mice with a Sertoli Cell Defect

Yomogida K, Yagura Y, Nishimune Y.
Biol Reprod. 2002 Sep;67(3):712-7.

Mouse-Rat In Vivo Electroporation: **MUSCLE / SKIN / JOINT / CARTILAGE / TUMOR and OTHERS**

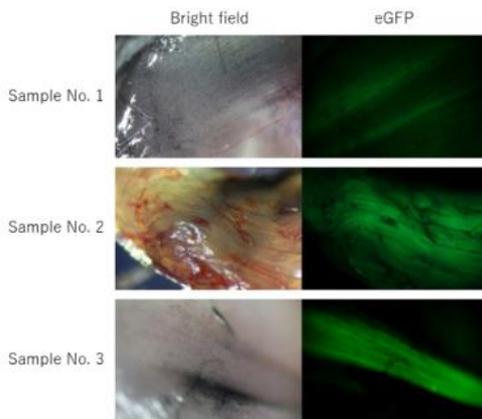
APPLICATIONS

In Vivo Transfection into Mouse-Rat: **MUSCLE / SKIN / JOINT / CARTILAGE / TUMOR and OTHERS**

CRISPR/Cas9 transfection into mouse muscle by in vivo electroporation

Establishment of CRISPR gene repair evaluation system using eGFP KO mice

Figure 1: eGFP gene repair in eGFP KO mouse muscle.



eGFP fluorescence in eGFP KO mouse muscle with attempted eGFP gene repair. Samples No. 1-3 are independent individuals.

To establish an in vivo gene repair evaluation system in muscle, we first investigated transfection conditions using an experimental system in which eGFP expression plasmids were introduced into wild-type mice.

As a result, we found that eGFP fluorescence could be confirmed in muscle with relatively good reproducibility by electroporation with the NEPA21 (Nepa Gene Co, Ltd.), followed by injection of a nucleic acid solution (100 µl) at a depth of 1.5 to 3.5 mm from the epidermis using a CUY568-4-0.5 needle array electrode (Nepa Gene Co., Ltd.), 30 minutes after administration of hyaluronidase.

Next, we examined whether the eGFP gene is repaired in the muscle of eGFP KO mice by direct transfection of CRISPR/Cas9-related nucleic acid.

We found that green fluorescence was observed in the muscles of all individuals, although the extent of fluorescence varied among individuals (Figure 1).

Courtesy:
Dr. Hiromi Miura, Department of Molecular Life Sciences, Basic Medical Science and Molecular Medicine, Tokai University School of Medicine
The Uehara Memorial Foundation Research Report (Volume 31, 2017)

Gene transfer into MUSCLE by In Vivo electroporation



Injection of plasmid DNA into muscle



In Vivo Electroporation

Electric pulses were delivered using an electric pulse generator (NEPA21 or CUY21; Nepa Gene Co.,Ltd.). Electrodes consisted of a pair of stainless-steel needles of 5 mm in length and 0.4 mm in diameter, fixed with a distance (gap) between them of 3 mm or 5 mm (Nepa Gene Co.,Ltd.)

Protocol**Intramuscular DNA Injection**

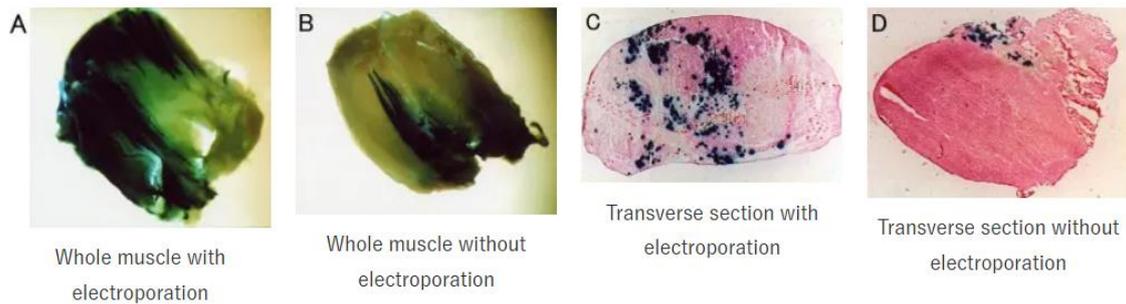
Anesthetize mice by intraperitoneal injection of 0.01 ml/g body weight of 6 mg/ml pentobarbital sodium solution. Inject the tibialis anterior muscles with 50 µg of purified closed circular DNA of pCAGGS-lacZ plasmid at 1.5 µg/µl in PBS using an insulin syringe with a 27-gauge needle.

Electroporation In Vivo

Insert a pair of electrode needles into the muscle to a depth of 5 mm to encompass the DNA injection sites. Deliver electric pulses using an electric pulse generator. Three 50-msec-long pulses of the indicated voltage (50-100 V) followed by three more pulses of the opposite polarity are administered to each injection site at a rate of one pulse per sec.

β-galactosidase Expression

Five days after DNA transfer, the expression of the lacZ gene is visualized by X-gal staining for β-galactosidase activity

**X-gal Staining**

The tibialis anterior muscles were fixed in cold 4% paraformaldehyde in PBS for 3 h, then washed in PBS for 1 h, and stained at 37°C for 18 h in the presence of 1mM X-gal to detect E. coli β-galactosidase activity.

For transverse sections, muscles were embedded in O.C.T. compound and frozen in dry ice-acetone.

Serial sections (15µm thick) were sliced with a cryostat and placed on slide glasses coated with 3-amino-propyltriethoxysilane.

The slices were fixed in 1.5% glutaraldehyde for 10 min at room temperature and then washed three times in PBS.

Samples were then incubated at 37°C for 3 h in the presence of 1 mM X-gal.

The muscle sections were counterstained with eosin. The control muscle (without electropulsation) showed only a small number of stained muscle fibers.

Electroporation increased both the number of positively stained muscle fibers and the density of staining.

Jun-ichi Miyazaki, Division of Stem Cell Regulation Research, G6 Osaka University Medical School

***Nature Biotechnology, Volume 16, Number 9, Pages 867-870, September 1998**

Epidermis-Targeted Gene Transfer Using In Vivo Electroporation



1: Tweezers w/Variable Gap 3 Needle Fork & Stainless-Steel Rectangle Plate Electrode, 5mm x 10mm (CUY663-5X10: NEPA GENE)

2: Pulse generator (CUY21 EDIT Square Wave Electroporator: NEPA GENE).

beta-Galactosidase expression on d 1 (A) and d 7 (B) after the pCAGGS-lacZ transfer with electroporation at 18V.

beta-Galactosidase was expressed in the upper most cell layers (horny, granular, and prickle cell layers) of the epidermis on d 1 (A), and in the subcutaneous muscle layer on d 7 (B).

Magnification: (A) x 250, (B) x 70

Hiroki Maruyama, Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences

*Epidermal Cells Methods and Protocols, Series: Methods in Molecular Biology, Volume 289, Pages 431-436, October 2004

PUBLICATIONS

In Vivo Transfection into Mouse-Rat: **MUSCLE / SKIN / JOINT / CARTILAGE / TUMOR and OTHERS**

Mouse tumor

Evaluating homologous recombination activity in tissues to predict the risk of hereditary breast and ovarian cancer and olaparib sensitivity

Motonari T, Yoshino Y, Haruta M, Endo S, Sasaki S, Miyashita M, Tada H, Watanabe G, Kaneko T, Ishida T, Chiba N. Sci Rep. 2024 Apr 8;14(1):7519.

Muscle

Generation of JC Polyoma Pseudovirus for High-Throughput Measurement of Neutralizing Antibodies

Matsuda M, Li TC, Nakanishi A, Nakamichi K, Saito M, Suzuki T, Matsuura T, Muramatsu M, Suzuki T, Miura Y, Suzuki R.

Diagnostics (Basel). 2024 Jan 31;14(3):311.

Muscle

Identification of neutralizing epitopes in the preS2 domain of the hepatitis B virus

Yato K, Matsuda M, Fukano K, Tanaka T, Moriishi K, Nishitsuji H, Shimotohno K, Tamura K, Wakita T, Muramatsu M, Kato T, Suzuki R.

Virus Res. 2023 Jan 2;323:199014.

Muscle

Active immunization combined with cisplatin confers enhanced therapeutic protection and prevents relapses of HPV-induced tumors at different anatomical sites

Porchia BFMM, Aps LRMM, Moreno ACR, da Silva JR, Silva MO, Sales NS, Alves RPDS, Rocha CRR, Silva MM, Rodrigues KB, Barros TB, Pagni RL, Souza PDC, Diniz MO, Ferreira LCS.

Int J Biol Sci. 2022 Jan 1;18(1):15-29.

Mouse / E13.5 / Cochlea**Stalling interkinetic nuclear migration in curved pseudostratified epithelium of developing cochlea**

Ishii M, Tateya T, Matsuda M, Hirashima T.

R Soc Open Sci 2021 Dec 8;8(12):211024.

Muscle**Enhanced Immune Responses and Protective Immunity to Zika Virus Induced by a DNA Vaccine Encoding a Chimeric NS1 Fused With Type 1 Herpes Virus gD Protein**

Pereira LR, Alves RPDS, Sales NS, Andreato-Santos R, Venceslau-Carvalho AA, Pereira SS, Castro-Amarante MF, Rodrigues-Jesus MJ, Favaro MTP, Chura-Chambi RM, Morganti L, Ferreira LCS.

Front. Med. Technol., 03 December 2020

Muscle**Gene Expression Profile at the Motor Endplate of the Neuromuscular Junction of Fast-Twitch Muscle**

Huang K, Li J, Ito M, Takeda JI, Ohkawara B, Ogi T, Masuda A, Ohno K.

Front Mol Neurosci. 2020 Sep 8;13:154.

Muscle**Myopalladin Promotes Muscle Growth Through Modulation of the Serum Response Factor Pathway**

Filomena MC, Yamamoto DL, Caremani M, Kadarla VK, Mastrototaro G, Serio S, Vydyanath A, Mutarelli M, Garofalo A, Pertici I, Knöll R, Nigro V, Luther PK, Lieber RL, Beck MR, Linari M, Bang ML.

J Cachexia Sarcopenia Muscle, 11 (1), 169-194 Feb 2020

Rat / External auditory canal**Keratinocyte Growth Factor (KGF) Induces Stem/Progenitor Cell Growth in Middle Ear Mucosa**

Yamamoto-Fukuda T, Akiyama N, Kojima H.

Int J Pediatr Otorhinolaryngol, 128, 109699 Jan 2020

Muscle**Bioavailability Study of Recombinant Plasmid DNA Nerve Growth Factor after Intramuscular Injection by Electroporation**

V. V. Shilov, M. A. Yudin, N. G. Vengerovich, T. V. Shcherbakov, A. S. Bogacheva

МЕДИЦИНА, Т. 14. Вып. 2, pp. 89–97 14 Nov 2019

Muscle**Fibroblast Growth Factor 21 Controls Mitophagy and Muscle Mass**

Oost LJ, Kustermann M, Armani A, Blaauw B, Romanello V.

J Cachexia Sarcopenia Muscle, 10 (3), 630-642 Jun 2019

Tumor**Upregulation of Transforming Growth Factor-Beta Type I Receptor by Interferon Consensus Sequence-Binding Protein in Osteosarcoma Cells**

Sung JY, Yoon K, Ye SK, Goh SH, Park SY, Kim JH, Kang HG, Kim YN, Park BK.

Biochim Biophys Acta Mol Cell Res, 1866 (5), 761-772 May 2019

Muscle**Expression of a Soluble IL-10 Receptor Enhances the Therapeutic Effects of a Papillomavirus-Associated Antitumor Vaccine in a Murine Model**

Sung JY, Yoon K, Ye SK, Goh SH, Park SY, Kim JH, Kang HG, Kim YN, Park BK.
Cancer Immunol Immunother, 68 (5), 753-763 May 2019

Muscle**Key Amino Acid Substitution for Infection-Enhancing Activity-Free Designer Dengue Vaccines**

Yamanaka A, Konishi E.
iScience. 2019 Mar 29;13:125-137.

Identification of CD4 and H-2K d-restricted Cytotoxic T Lymphocyte Epitopes on the Human Herpesvirus 6B Glycoprotein Q1 Protein

Nagamata S, Aoshi T, Kawabata A, Yamagishi Y, Nishimura M, Kuwabara S, Murakami K, Yamada H, Mori Y.
Sci Rep, 9 (1), 3911 2019 Mar 7

Tumor**C/EBP β Is a Transcriptional Regulator of Wee1 at the G₂/M Phase of the Cell Cycle**

Lee JH, Sung JY, Choi EK, Yoon HK, Kang BR, Hong EK, Park BK, Kim YN, Rho SB, Yoon K.
Cells, 8 (2) 2019 Feb 11

Muscle**Involvement of the Protein Kinase Akt2 in Insulin-Stimulated Rac1 Activation Leading to Glucose Uptake in Mouse Skeletal Muscle**

Takenaka N, Araki N, Satoh T.
PLoS One, 14 (2), e0212219 2019 Feb 8 eCollection 2019

Muscle**Dendritic Cell Targeting Using a DNA Vaccine Induces Specific Antibodies and CD4 + T Cells to the Dengue Virus Envelope Protein Domain III**

Zaneti AB, Yamamoto MM, Sulczewski FB, Almeida BDS, Souza HFS, Ferreira NS, Maeda DLNF, Sales NS, Rosa DS, Ferreira LCS, Boscardin SB.
Front Immunol, 10, 59 2019 Jan 29 eCollection 2019

Muscle**IGFN1_v1 Is Required for Myoblast Fusion and Differentiation**

Li X, Baker J, Cracknell T, Haynes AR, Blanco G.
PLoS One, 12 (6), e0180217 2017 Jun 30 eCollection 2017

Muscle**Expression of Enhancing-Activity-Free Neutralizing Antibody Against Dengue Type 1 Virus in Plasmid-Inoculated Mice**

Yamanaka A, Pitaksajakul P, Ramasoota P, Konishi E.
Vaccine, 33 (45), 6070-7 2015 Nov 9

Rat / External auditory canal**In Vivo Over-Expression of KGF Mimic Human Middle Ear Cholesteatoma**

Yamamoto-Fukuda T, Akiyama N, Shibata Y, Takahashi H, Ikeda T, Koji T.
Eur Arch Otorhinolaryngol. 2015 Oct;272(10):2689-96.

Skin**Electroporation-mediated intradermal delivery of DNA vaccines in nonhuman primates.**

Adam L, Le Grand R, Martinon F.
Methods Mol Biol. 2014;1121:309-13.

Tumor**Electroporation-mediated siRNA delivery into tumors.**

Takei Y.
Methods Mol Biol. 2014;1121:131-8.

Muscle**Novel polyvalent live vaccine against varicella-zoster and mumps virus infections.**

Matsuura M, Somboonthum P, Murakami K, Ota M, Shoji M, Kawabata K, Mizuguchi H, Gomi Y, Yamanishi K, Mori Y.
Microbiol Immunol. 2013 Oct;57(10):704-14.

Tumor**The endogenous soluble VEGF receptor-2 isoform suppresses lymph node metastasis in a mouse immunocompetent mammary cancer model.**

Shibata MA, Ambati J, Shibata E, Albuquerque RJ, Morimoto J, Ito Y, Otsuki Y.
BMC Med. 2010 Nov 3;8:69.

Skin**Injection site-dependent induction of immune response by DNA vaccine: comparison of skin and spleen as a target for vaccination.**

Guan X, Nishikawa M, Takemoto S, Ohno Y, Yata T, Takakura Y.
J Gene Med. 2010 Mar;12(3):301-9.

Tumor**Visualization of in vivo electroporation-mediated transgene expression in experimental tumors by optical and magnetic resonance imaging.**

Aung W, Hasegawa S, Koshikawa-Yano M, Obata T, Ikehira H, Furukawa T, Aoki I, Saga T.
Gene Ther. 2009 Jul;16(7):830-9.

Tumor**Combination therapy with short interfering RNA vectors against VEGF-C and VEGF-A suppresses lymph node and lung metastasis in a mouse immunocompetent mammary cancer model.**

Shibata MA, Morimoto J, Shibata E, Otsuki Y.
Cancer Gene Ther. 2008 Dec;15(12):776-86.

Tumor**Effective delivery of DNA into tumor cells and tissues by electroporation of polymer-DNA complex**

Kang JH, Toita R, Niidome T, Katayama Y.
Cancer Lett. 2008 Jul 8;265(2):281-8.

Tumor**In vivo silencing of a molecular target by short interfering RNA electroporation: tumor vascularization correlates to delivery efficiency**

Takei Y, Nemoto T, Mu P, Fujishima T, Ishimoto T, Hayakawa Y, Yuzawa Y, Matsuo S, Muramatsu T, Kadomatsu K.
Mol Cancer Ther. 2008 Jan;7(1):211-21.

Skin**Modulation of scratching behavior by silencing an endogenous cyclooxygenase-1 gene in the skin through the administration of siRNA**

Inoue T, Sugimoto M, Sakurai T, Saito R, Futaki N, Hashimoto Y, Honma Y, Arai I, Nakaike S.
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Joint**Sonoporation mediated transduction of pDNA/siRNA into joint synovium in vivo**

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ZEBRAFISH RETINA – In Vivo / Ex Vivo**Survival, excitability, and transfection of retinal neurons in an organotypic culture of mature zebrafish retina**

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Inhibition of Müller glial cell division blocks regeneration of the light-damaged zebrafish retina

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