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

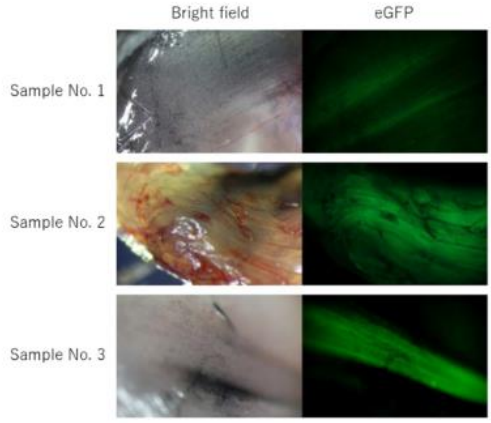
APPLICATIONS

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)

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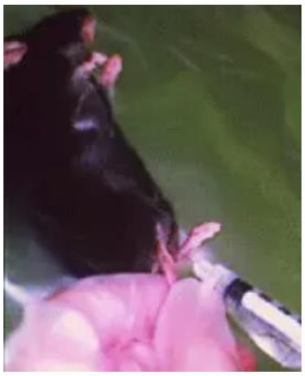
Gene transfer into MUSCLE by In Vivo electroporation



Injection of plasmid DNA into muscle

In Vivo Eletroporation

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

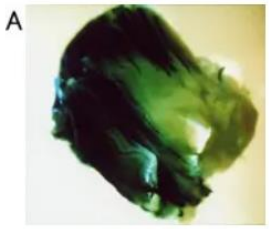
Anesthetize mice by intraperitoneal injection of 0.01 ml/g body weight of 6 mg/ml pentobarbital sodium solution. Inject the tibialis anterior muscles with 50 µg of purified closed circular DNA of pCAGGS-lacZ plasmid at 1.5 µg/µl in PBS using an insulin syringe with a 27-gauge needle.

Electroporation In Vivo

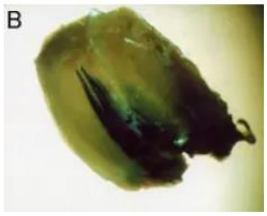
Insert a pair of electrode needles into the muscle to a depth of 5 mm to encompass the DNA injection sites. Deliver electric pulses using an electric pulse generator. Three 50-msec-long pulses of the indicated voltage (50-100 V) followed by three more pulses of the opposite polarity are administered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

β-galactosidase Expression

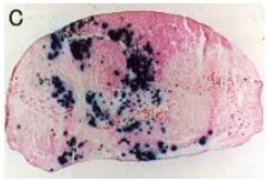
Five days after DNA transfer, the expression of the lacZ gene is visualized by X-gal staining for -galactosidase activity



Whole muscle with electroporation



Whole muscle without electroporation



Transverse section with electroporation

Transverse section without electroporation

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.

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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, Andreata-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.

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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.

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Rat / External auditory canal

Keratinocyte Growth Factor (KGF) Induces Stem/Progenitor Cell Growth in Middle Ear Mucosa

Yamamoto-Fukuda T, Akiyama N, Kojima H.

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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

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Fibroblast Growth Factor 21 Controls Mitophagy and Muscle Mass

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

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Tumor

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

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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.

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Muscle

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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/EBPβ Is a Transcriptional Regulator of Wee1 at the G_2/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.

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Takenaka N, Araki N, Satoh T.

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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.

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Li X, Baker J, Cracknell T, Haynes AR, Blanco G.

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Muscle

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Yamanaka A, Pitaksajjakul P, Ramasoota P, Konishi E.

Vaccine, 33 (45), 6070-7 2015 Nov 9

Rat / External auditory canal

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Yamamoto-Fukuda T, Akiyama N, Shibata Y, Takahashi H, Ikeda T, Koji T.

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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.

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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.

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Shibata MA, Ambati J, Shibata E, Albuquerque RJ, Morimoto J, Ito Y, Otsuki Y.

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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.

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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.

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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.

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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.

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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.

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Joint

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Multivalent DNA vaccine protects mice against pulmonary infection caused by Pseudomonas aeruginosa

Saha S, Takeshita F, Sasaki S, Matsuda T, Tanaka T, Tozuka M, Takase K, Matsumoto T, Okuda K, Ishii N, Yamaguchi K, Klinman DM, Xin KQ, Okuda K.

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Simultaneous gene transfer of bone morphogenetic protein (BMP) -2 and BMP-7 by in vivo electroporation induces rapid bone formation and BMP-4 expression.

Kawai M, Bessho K, Maruyama H, Miyazaki J, Yamamoto T.

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Toll-Like Receptor Adaptor Molecules Enhance DNA-Raised Adaptive Immune Responses against Influenza and Tumors through Activation of Innate Immunity

Takeshita F, Tanaka T, Matsuda T, Tozuka M, Kobiyama K, Saha S, Matsui K, Ishii KJ, Coban C, Akira S, Ishii N, Suzuki K, Klinman DM, Okuda K, Sasaki S.

J Virol. 2006 Jul;80(13):6218-24.

Muscle

Immune deficiency enhances expression of recombinant human antibody in mice after nonviral in vivo gene transfer

Kitaguchi K, Toda M, Takekoshi M, Maeda F, Muramatsu T, Murai A.

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Rabbits / Muscle

Production of research-grade antibody by in vivo electroporation of DNA-encoding target protein

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Joint

Electro-transfer of small interfering RNA ameliorated arthritis in rats

Inoue A, Takahashi KA, Mazda O, Terauchi R, Arai Y, Kishida T, Shin-Ya M, Asada H, Morihara T, Tonomura H, Ohashi S, Kajikawa Y, Kawahito Y, Imanishi J, Kawata M, Kubo T.

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Maruyama H, Miyazaki J, Gejyo F.

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Muscle

Skeletal muscle targeting in vivo electroporation-mediated HGF gene therapy of bleomycin-induced pulmonary fibrosis in mice

Umeda Y, Marui T, Matsuno Y, Shirahashi K, Iwata H, Takagi H, Matsumoto K, Nakamura T, Kosugi A, Mori Y, Takemura H.

Lab Invest. 2004 Jul;84(7):836-44.

Muscle

Gene Therapy for Central Diabetes Insipidus: Effective Antidiuresis by Muscle-Targeted Gene Transfer

Yoshida M, Iwasaki Y, Asai M, Nigawara T, Oiso Y.

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Efficient gene delivery to articular cartilage using electroporation

Katayama R, Kimura T, Tomita T, Matsuno H, Morita Y, Matsushita I, Gejo R.

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Muscle

Ectopic Bone Formation by Human Bone Morphogenetic Protein-2 Gene Transfer to Skeletal Muscle Using Transcutaneous Electroporation

Kawai M, Bessho K, Kaihara S, Sonobe J, Oda K, Iizuka T, Maruyama H.

Hum Gene Ther. 2003 Nov 1;14(16):1547-56.

Muscle

Hepatocyte growth factor gene therapy accelerates regeneration in cirrhotic mouse livers after hepatectomy
Xue F, Takahara T, Yata Y, Kuwabara Y, Shinno E, Nonome K, Minemura M, Takahara S, Li X, Yamato E, Watanabe A.
Gut. 2003 May;52(5):694-700.

Muscle

Anti-monocyte chemoattractant protein-1 gene therapy limits progression and destabilization of established atherosclerosis in apolipoprotein E-knockout mice
Inoue S, Egashira K, Ni W, Kitamoto S, Usui M, Otani K, Ishibashi M, Hiasa K, Nishida K, Takeshita A.
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Attenuated acute liver injury in mice by naked hepatocyte growth factor gene transfer into skeletal muscle with electroporation
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Muscle

Gene Therapy for Mitochondrial Disease by Delivering Restriction Endonuclease SmaI into Mitochondria
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Elevated gastrin secretion by in vivo gene electroporation in skeletal muscle
Yasui A, Oda K, Usunomiya H, Kakudo K, Suzuki T, Yoshida T, Park HM, Fukazawa K, Muramatsu T.
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Protection Against Autoimmune Myocarditis by Gene Transfer of Interleukin-10 by Electroporation
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Muscle

Infectivity-Enhancing Antibodies to Ebola Virus Glycoprotein
Takada A, Watanabe S, Okazaki K, Kida H, Kawaoka Y.
J Virol. 2001 Mar;75(5):2324-30.

OTHERS

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 Jun 27

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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

Kustermann et al.

Cell and Tissue Research, Volume 332, Number 2, Pages 195-209, May 2008

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