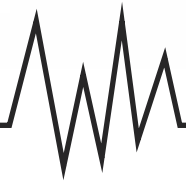


The BTX Current

Holliston, MA USA

In Vivo Electroporation Edition



“Electroporation safer and more effective than Chemical Transfections”

Viral and non-viral vectors have been developed for gene therapy, but their use is associated with unresolved problems of efficacy and safety. Efficient and safe methods of DNA delivery need to be found for medical application. Here we report a new monopolar system for non-viral electro-gene transfer into the thymus in vivo of electrical pulses after the introduction of the DNA.

We assessed the proof of concept of this approach by correcting ZAP-70 deficient severe combined immunodeficiency (SCID) in mice. The thymic electro-gene transfer of the pCMV-ZAP-70-IRESGFP vector in these mice resulted in rapid T cell differentiation in the thymus with mature lymphocytes detected by three weeks in secondary lymphoid organs. Moreover, this system resulted in the generation of long-term functional T lymphocytes. Peripheral reconstituted T cells displayed a diversified T cell receptor (TCR) repertoire and were responsive to alloantigens in vivo.

This process applied to the thymus could represent a simplified and effective alternative for gene therapy of T cell immunodeficiencies.

Used ECM830 and homemade electrode.

Reference;
Iria, M., Saade, M., Kissenpfening, A., Franz Poulin, L., Leserman, L., Marche, P., Jouvin-Marche, E., Merger, F., Nguyen, C., 2008. ZAP-70 Restoration in Mice by In Vivo Thymic Electroporation. PLoS One. April; Vol 3 (4):e2059

In Vivo Electrodes & Applications



L Shaped Genetrodes

- In Ovo
- Embryo
- Ex Plant
- Organ

Genetrodes

- Transdermal
- Muscle
- Organ

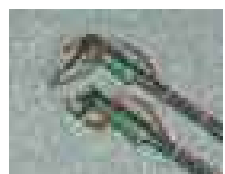


Genepaddles

- Muscle
- Organ
- Ex Plant

Tweezertrodes

- In Ovo
- In Utero
- Transdermal
- Muscle
- Organ

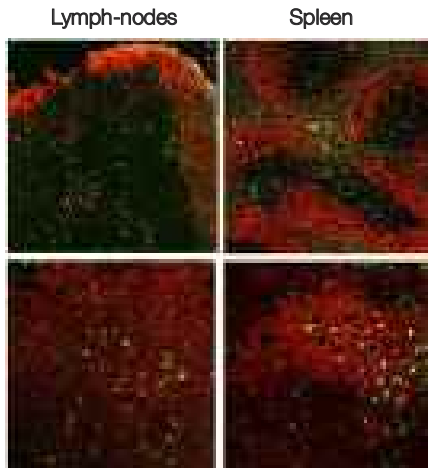


Calipers

- Transdermal
- Muscle
- Organ

2-Needle Array

- Transdermal
- Muscle



Sections of lymph-nodes and the spleen were analyzed by confocal microscopy after staining with a B cell specific antibody (B220) and an anti-CD3 antibody that specifically reacts with T lymphocytes. Multiple representative clusters of EGFP transfected cells were found mainly in T zones of different lymph-nodes and spleen

BTX Ranked Highest in In Vivo Electroporation Applications

Gene and drug delivery into living tissue had significant implications in gene therapy and cancer research. Electroporation mediated gene and drug delivery has been shown to significantly increase intracellular uptake and expression of DNA, siRNA, miRNA and many other molecules into various tissues. The use of the BTX ECM 830 square wave electroporator provides users with the flexibility they need. The ECM 830 can process in vitro cuvettes experiments, electrode based in vivo and in ovo applications, High Throughput electroporation and is capable of experiment monitoring.



ECM 830 Generator

BTX Item No.	Description
45-0052	ECM 830 Generator ONLY
45-0002	ECM 830 Electroporation System Includes: ECM 830 Generator, 30 Cuvettes (10 of each size), Cuvette Safety Stand and Cuvette Rack
45-0160	Genetrode Kit, Straight, Gold Tip, 5 mm
45-0161	Genetrode Kit, Straight, Gold Tip, 10 mm
45-0162	Genetrode L-Shape Kit, Bent, Gold Tip, 5 mm
45-0163	Genetrode L-Shape Kit, Bent, Gold Tip, 3 mm
45-0164	Genetrode L-Shape Kit, Bent, Gold Tip, 1 mm
45-0165	Tweezertrode Kit, 7 mm Stainelss Steel
45-0166	Tweezertrode Kit, 10 mm Stainelss Steel
45-0186	Tweezertrode Kit, 7 mm Platinum
45-0187	Tweezertrode Kit, 3 mm Platinum
45-0188	Tweezertrode Kit, 1 mm Platinum
45-0167	2-Needle Array Kit, 10 mm
45-0168	2-Needle Array Kit, 5 mm
45-0169	Genepaddle Kit, 3x5 mm
45-0170	Genepaddle Kit, 5x7 mm



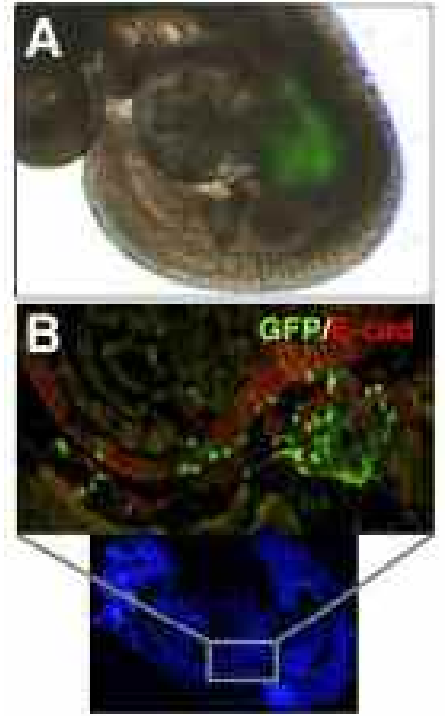
Gene Transfer into Mouse Prepancreatic Endoderm by Whole Embryo Electroporation

CONTEXT: Understand gene function in developing pancreas is a major issue for pancreatic cell therapy. The in vivo analysis of gene function has essentially been performed by modulating gene expression in transgenesis. A faster and easier method is electroporation of mouse embryos. This technique, coupled with whole embryo culture, enables one to deliver genes and analyze their effects in a spatially and temporally regulated manner.

OBJECTIVE: We wanted to adapt the electroporation technique for gene transfer of e8.5 mouse embryos into the endoderm to allow expression to transgenes in pancreas or liver.

RESULTS: Using two platinum plate electrodes, low voltage and a precise positioning of the embryo in the electroporation cuvette we could target and express DNA constructs in the prepancreatic or prehepatic territories, identified with cell markers. We also demonstrated that this technique is a valuable tool in the study of transcriptional regulation in the developing endoderm.

CONCLUSIONS: Targeted electroporation of whole embryos is a useful method of characterizing the gene network which controls pancreatic development. **Used ECM 830 & Platinum Tweezertrodes**



Gene targeting of a restricted region of the mouse endoderm by whole embryo electroporation. A. Overlay of a bright field and fluorescence images of an embryo electroporated at the 8-somite stage with a GFP expression vector and cultured for 24h, showing GFP activity (green) only in the midgut region. B. Immunolocalization of GFP (green) in the E-cadherin (red)-positive epithelium of the primitive gut. The GFP positive region is boxed in the lower panel, which shows Hoechst staining of a section of the whole embryo.

Reference;
Pierreux, C., Poll, A., Jacquemin, P., Iemalgre, F., Rouseau, G., 2005. gene Transfer into mouse Prepancreatic Endoderm by Whole Embryo Electroporation. JOP. J Pancreas (online) 2005; 6 (2): 128-135 Reference

Trivia:

What is the worlds fastest bird?

Peregrine Falcon

BTX Offers the broadest range of product to solve your transfection needs.

- Electrode based In Vivo Applications
- Standard Cuvette
- High Throughput
- Adherent Cells
- ElectroFUSION
- Experiment Monitoring

Call BTX or your local dealer today to arrange a **FREE 30 DAY TRIAL** in your lab

Many Challenges,
One **Solution**

www.btxonline.com

A Novel DNA Vaccine Protects Joints From Inflammation and Destruction in Murine Models of Arthritis.

OBJECTIVE: Previous studies have demonstrated that neutralization of macrophage migration inhibitory factor (MIF) by anti-MIF antibodies decreases joint inflammation and destruction in a type II collagen-induced arthritis model in mice. The aim of this study was to develop and describe a simple and effective method of active immunization that induces anti-MIF autoantibodies, which may neutralize MIF bioactivity.

METHODS: We developed a MIF DNA vaccine by introducing oligonucleotides encoding a tetanus toxoid (TTX) Th cell epitope into the complementary DNA sequence of murine MIF. Mice were injected with this construct in conjunction with electroporation. The ability of this immunization to inhibit the development of collagen antibody-induced arthritis CAIA in BALB/c mice and spontaneous autoimmune arthritis in interleukin-1 receptor antagonist (IL-1Ra)-deficient mice was then evaluated.

RESULTS: Mice that received the MIF/TTX DNA vaccine developed high titers of autoantibodies that reacted to native MIF. Compared with unvaccinated mice, vaccinated mice also produced less serum tumor necrosis factor alpha after receiving an intravenous injection of lipopolysaccharide. In addition, vaccination with MIF/TTX DNA resulted in significant amelioration of both CAIA in BALB/c mice and symptoms of autoimmune arthritis in IL-1Ra-knockout mice.

CONCLUSION: These results suggest that MIF/TTX DNA vaccination may be useful for ameliorating the symptoms of rheumatoid arthritis. (1) **USED ECM 830 & 2-Needle Array**

Reference; Onodera, S., Ohshima, S., Tohyama, H., Yasuda K., Nishihira, J., Iwakura, Y., Matsuda, I., Minami, A., and Koyama, Y. 2007. A Novel DNA Vaccine Targeting Macrophage Migration Inhibitory Factor Protects Joints From Inflammation and Destruction in Murine Models of Arthritis. *Arthritis and Rheumatism*, 56(2): 521-530

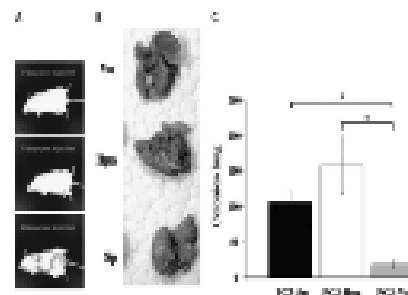
Function of Heparanase in Prostate Tumorigenesis: Potential for Therapy

PURPOSE: Heparanase is the predominant enzyme that cleaves heparan sulfate, main polysaccharide in the extracellular matrix. Whereas the role of heparanase sustaining the pathology of human cancer is well documented, its association with prostate carcinoma remains uncertain. Our research was undertaken to elucidate the significance of heparanase in prostate tumorigenesis and bone metastasis.

EXPERIMENTAL DESIGN: We applied immunohistochemical analysis of tissue microarray, in vitro adhesion and invasion assays, as well as mouse models of intraosseous growth and spontaneous metastasis of prostate cancer, monitored by wholebody bioluminescent imaging. Electroporation-assisted administration of anti-heparanase small interfering RNA in vivo was applied as a therapeutic approach.

RESULTS: We report a highly statistically significant ($P < 0.0001$) prevalence of heparanase overexpression in prostate carcinomas versus noncancerous tissue, as well as strong correlation between tumor grade and the extent of heparanase expression. We observed >5-fold increase in the metastatic potential of PC-3 prostate carcinoma cells engineered to overexpress heparanase. Notably, overexpression of a secreted form of the enzyme also led to a dramatic increase in intraosseous prostate tumor growth. Local in vivo silencing of heparanase resulted in a 4-fold inhibition of prostate tumor growth, representing the first successful application of anticancer therapy based on heparanase small interfering RNA and validating the potential of heparanase as a target for prostate cancer treatment.

CONCLUSIONS: Heparanase directly contributes to prostate tumor growth in bone and its ability to metastasize to distant organs. Thus, anti-heparanase strategy may become an important modality in treatment of prostate cancer patients, particularly those with bone metastases. (1) **USED ECM 830 & Calipers**



Overexpression of heparanase increases pulmonary metastasis in SCID mice. PC3-Vo, PC3-Hpa, and PC3-Sp cells, stably cotransfected with LUC expressing vector, were injected into the right tibia of SCID mice. A, at 46 d postinjection, when the presence of lung metastases was detected by real-time in vivo bioluminescence imaging in mice injected with either PC3-Hpa or PC3-Sp cells, but not PC3-Vo cells, all mice were euthanized and their lungs were fixed and examined for the number of carcinoma colonies on the lung surface. B, gross appearance of lungs of mice injected with PC3-Vo (top), PC3-Hpa (middle), or PC3-Sp (bottom) cells. C, columns, represent the mean number of colonies per lung ($n = 5$ mice); bars, SE. A statistically significant difference in the number of colonies per lung was observed between PC3-Vo

Reference; Lerner I, Baraz L, Plikarsky E, Melrovitz A, Edovitsky E, Peretz T, Vodavsky I, Elkin M., *Clin Cancer Res*, 2008 Feb
Function of Heparanase in Prostate Tumorigenesis: Potential for Therapy 1;14(3):668-76

Recent Reference Articles for BTX In-Vivo Electroporation Applications:

Efficient Gene Transfer into the Embryonic Mouse Brain Using in Vivo Electroporation
Tetsuhiro Saito¹ and Norio Nakatsuji Department of Development and Differentiation, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, 606-8507, Japan

Intraoviductal introduction of plasmid DNA and subsequent electroporation for efficient in vivo gene transfer to murine oviductal epithelium. Sato M. The Institute of Medical Sciences, Tokai University, Bohseidai, Isehara, Kanagawa, Japan. masasato@is.icc.u-tokai.ac.jp

A critical function for beta-amyloid precursor protein in neuronal migration revealed by in utero RNA interference. Young-Pearse TL, Bai J, Chang R, Zheng JB, LoTurco JJ, Selkoe DJ. Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA

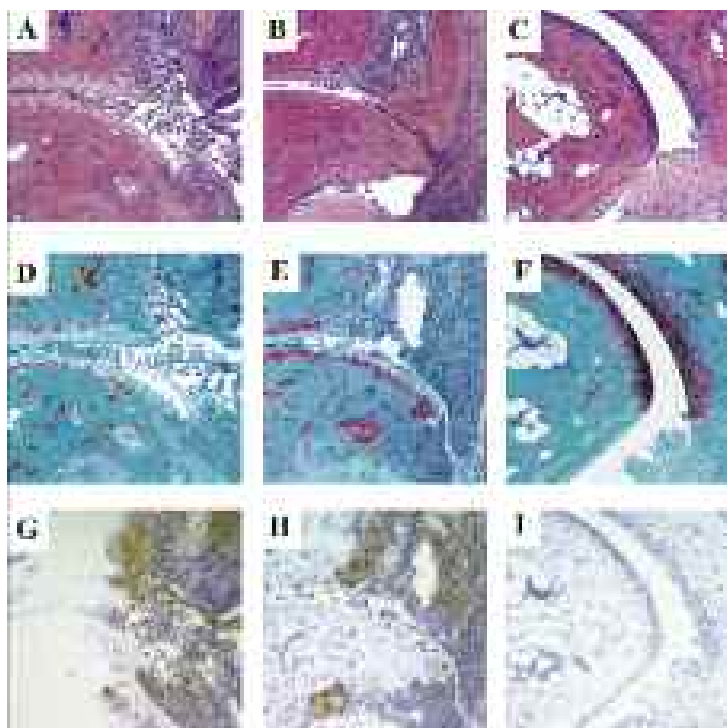
Activity-dependent development of callosal projections in the somatosensory cortex. Wang CL, Zhang L, Zhou Y, Zhou J, Yang XJ, Duan SM, Xiong ZQ, Ding YQ. Institute of Neuroscience and Key Laboratory of Neurobiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China.

Validating in utero electroporation for the rapid analysis of gene regulatory elements in the murine telencephalon. Langevin LM, Mattar P, Scardigli R, Roussigné M, Logan C, Blader P, Schuurmans C. Institute of Maternal and Child Health, HBI, University of Calgary, Calgary, Canada.

Direct visualization of nucleogenesis by precerebellar neurons: involvement of ventricledirected, radial fibre-associated migration Daisuke Kawauchi^{1,2,*}, Hiroki Taniguchi³, Haruyasu Watanabe¹, Tetsuhiro Saito^{4,*} and Fujio Murakami¹, 1 Laboratory of Neuroscience, Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka 565-0871, Japan. 2 SORST, Japan Science and Technology, Kawaguchi, Saitama 332-0012, Japan. 3 Division of Behavior and Neurobiology, National Institute for Basic Biology, Myodaiji-cho, Okazaki 444-8585, Japan. 4 Department of Development and Differentiation, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan.

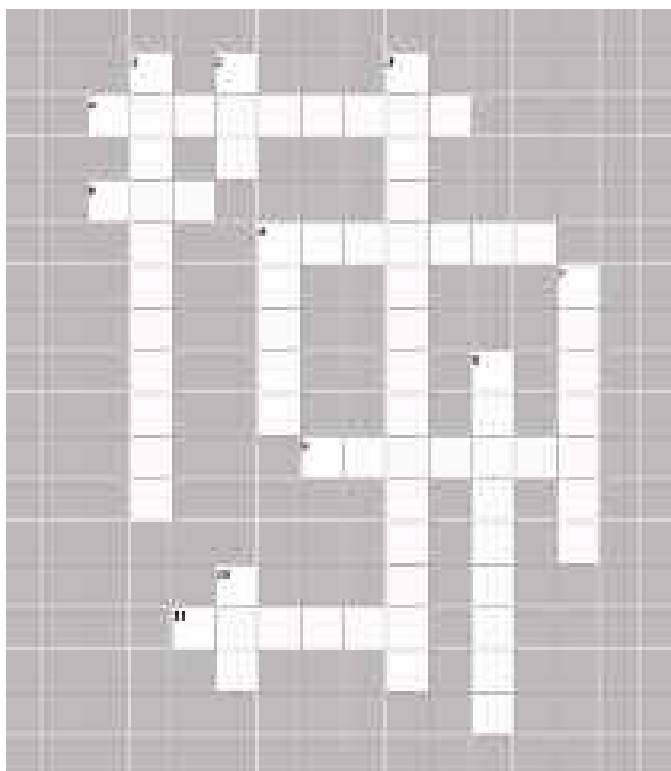
Molecular and Morphological Heterogeneity of Neural Precursors in the Mouse Neocortical Proliferative Zones Jonathan S. Gal,¹ Yury M. Morozov,² Albert E. Ayoub,² Mitali Chatterjee,¹ Pasko Rakic,^{2,3} and Tarik F. Haydar¹ 1 Center for Neuroscience Research, Children's Research Institute, Children's National Medical Center, Washington, DC 20010, and 2 Department of Neurobiology and 3 Kavli Institute for Neuroscience, Yale Medical School, New Haven, Connecticut 06510

Ephrin-As Guide the Formation of Functional Maps in the Visual Cortex Jianhua Cang,^{1,3} Megumi Kaneko,^{1,3} Jena Yamada,² Georgia Woods,² Michael P. Stryker,^{1*} and David A. Feldheim^{2*} 1 W. M. Keck Foundation Center for Integrative Neuroscience, Department of Physiology, University of California, San Francisco, San Francisco, California 94143 2 Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, Santa Cruz, California 95064



Representative results of histologic analysis of the effects of vaccination with saline (A, D, and G), pCAGGS (B, E, and H), or macrophage migration inhibitory factor/tetanus toxoid (C, F, and I). Ankle joints from interleukin-1 receptor antagonist (IL-1Ra)-knockout mice were collected 16 weeks after vaccination, and specimens were stained with hematoxylin and eosin (A-C) or Safranin O (D-F), or were immunohistochemically stained with polyclonal anti-MIF antibodies (G-I). (Original magnification 200.)

BTX In Vivo Crossword



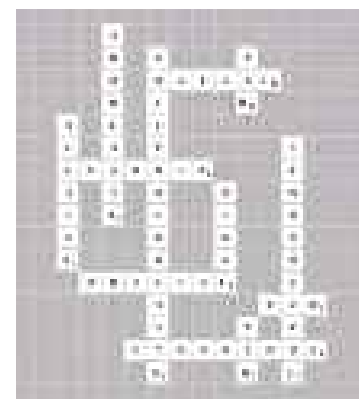
Down

- Absorption through
- The highest ranked company
- Application of electric surface
- In the egg (2 words)
- Small laboratory vessel
- A conductor used contact
- Ribonucleic acid

Across

- Official accounting
- deoxyribonucleic
- In the uterus (2 words)
- A flow of electric
- In the living body

(Answers below)



BTX
HARVARD APPARATUS
Molecular Delivery Systems

BTX Harvard Apparatus
84 October Hill Rd
Holliston, MA 01746
Toll Free: 800-272-2775
Phone: 508-893-8999
Fax: 508-429-5732

Many Challenges,
One Solution

www.btxonline.com