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Zygote Electroporation for Transgenic Animal Production

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

- Compared to other devices on the market, 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. One such application is **Zygote Electroporation for Transgenic Animal Production**.
- The finer control over the delivered energy available with the NEPA21 offers specific and important 3R advantages. 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 living organisms, this means less **pain** experienced during the electroporation event and time to recovery is minimised and improved.
- the enhanced reproducibility from one electroporation event to the next also has significant 3R advantages. Where previously one had to experiment with 10 animal models to be sure of a successful outcome, with the NEPA21, once one has optimised an EP protocol, one only needs one animal to ensure the expected results. Ancillary animal welfare (feeding, housing and husbandry) costs are also significantly reduced as less animals are required. In addition, less personnel time is required to manage the animals as less animals are required.
- With the NEPA21 device, Zygote Electroporation, can be performed both ex vivo (TAKE Method) and in vivo (i-GONAD and r-GONAD Methods). Choice of methodology is dictated by the level of micro-manipulation skill accessible in the laboratory and access to animal license certification.
- If a laboratory has minimal previous experience of Zygote Electroporation, we recommend the Ex Vivo (TAKE) Method. Alternatively, for a laboratory with access to relevant animal licenses and the required manipulation skills, we recommend the In Vivo (i-GONAD/r-GONAD) Method
- With the **Ex Vivo TAKE Method**, it only takes 5 minutes to electroporate up to 150 embryos
- With the **In Vivo i-GONAD/r-GONAD Method**, embryos can be electroporated in-situ in the Oviduct obviating the need for the ex vivo handling steps and stages of the Ex Vivo TAKE Method
- For KNOCK-OUT and KNOCK-IN applications, results are more reproducible and, in many cases, better than with microinjection

This document details the Ex Vivo TAKE Method.

Ex Vivo - Take Method

Traditionally, researchers have used **microinjection** techniques for transgenic animal production. While a robustly successful technique, it has intrinsic disadvantages. The NEPA21 ex vivo zygote electroporation methodology improves upon microinjection as follows:

- **No** requirement for microinjection
- **No** requirement to remove the Zona Pellucida
- **No** need to weaken the zona pellucida by pre-treatment with Tyrode solution
- **No** need for specialist training – all team members can perform the technique
- It takes 5 minutes to electroporate 150 embryos
- For KNOCK-OUT and KNOCK-IN applications, results are more reproducible and, in many cases, better than with microinjection

Application-Customised Electrode Options

TAKE Method

There is a choice of two custom manufactured electrodes:

- the **CUY501P1-1.5** electrode is 1mm in gap width and is a small volume solution. It accommodates 5ul of CRISPR solution in its chamber and electroporates from 5-50 embryos at a time.
- the **CUY505P5** electrode is 5mm in gap width and is a larger volume solution. It accommodates 45ul of CRISPR solution in its chamber and electroporates from 20-150 embryos at a time.

A full know-how resource for how to use the **NEPA21 system** for *Zygote EP/Transgenic Animal Production* is summarised in this link to our website:

https://articles.sonidel.com/free-nepa21-demo-and-trial-zygote-electroporation-for-transgenic-animal-production/

APPLICATIONS

Introduction of ZFN, TALEN, and CRISPR-Cas into mammalian fertilized eggs by electroporation

Key Points of this Research Results

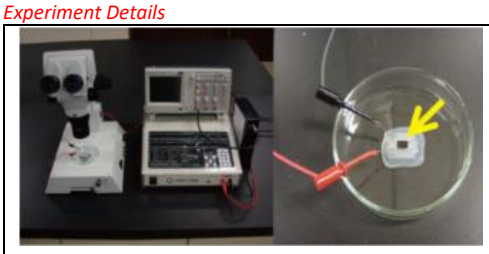
- Our NEPA21 system was the first in the world to produce genetically modified animals (RATS) using electroporation.
- We also successfully introduced ZFN, TALEN, and CRISPR-Cas into zygotes. Illustrative publications have attracted attention in recent years from researchers worldwide. This method can be used as a technology to accelerate the genome editing technology.
- The electroporation method is easier to operate than the microinjection method, and animals with the target gene modified can be easily produced at research institutes and laboratories where skilled workers are not available.
- The method can be applied to the introduction of genes into zygotes of many animal species, allowing the preparation of genetically modified animals in a short period of time using animal species suitable for research, thereby improving research efficiency (knockout and knock-in of mice and rats have also been successfully performed).

- This technique is named Technique for Animal Knockout system by Electroporation (TAKE method).

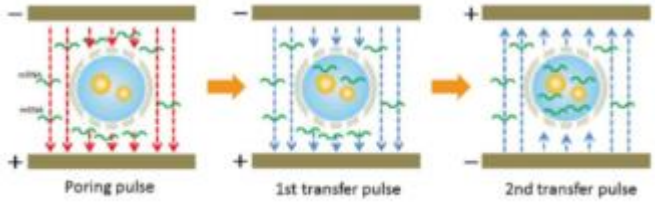
Results

The target gene was the interleukin 2 receptor gamma chain (Il2rg) gene, the causative gene of X-linked severe combined immunodeficiency (X-SCID). mRNA was prepared from ZFN, TALEN, and CRISPR-Cas. A CUY520P5 petri dish electrode connected to NEPA21 was filled with a solution of mRNA mixture, and the zygotes were placed in it for mRNA introduction by electroporation using the TAKE method. The zygotes were then transplanted into the female parents and raised to litter. As a result, we succeeded in obtaining offspring in which the target genes were disrupted in all cases of ZFN, TALEN, and CRISPR-Cas.

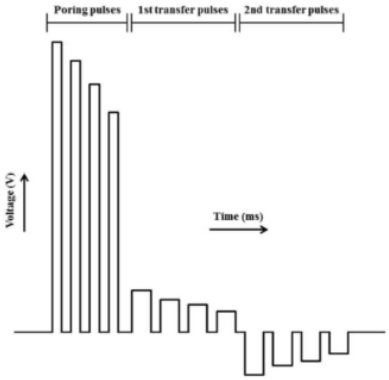
Experiment Details



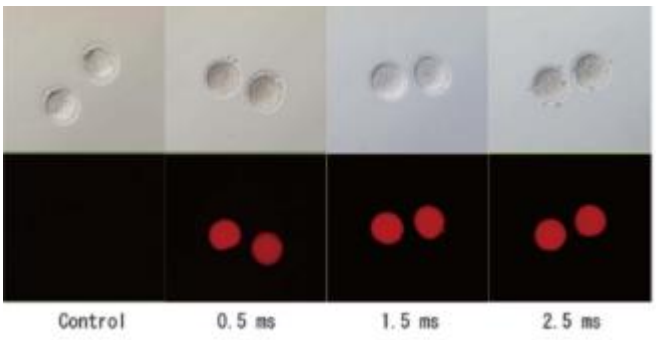
A B



C



D



E

In the study, the NEPA21 Super Electroporator (Picture A, SONIDEL Limited) and CUY520P5 Bath w/platinum plate electrodes on petridish, 5mm gap, (Picture B, SONIDEL Limited.) were used to set up the 3-step electroporation (Figure C), where cells were punctured by the first-step electric pulse, and the second and the third step of the electrical pulse to introduce the gene into the zygotes in a stepwise manner. (Figure D)

Tetramethylrhodamine-labelled dextran (3 kDa, easy to visualize and non-cytotoxic) was used to examine the conditions for introducing foreign material into zygotes by electroporation. Zygotes in the pronuclear phase were collected from superovulated female rats one day after mating. Tetramethylrhodamine-labelled dextran was electroporated into rat zygotes with pulse widths of: 0 ms (control), 0.5 ms, 1.5 ms, and 2.5 ms. Dextran was introduced throughout the cytoplasm of the zygotes. (Picture E)

Prospects

Until the NEPA21 system, the generation of genetically modified animals has required delicate and skilled techniques, and this has hindered the progress of research. The TAKE method developed in this study has made it possible to create animals with the desired gene modification in a short period of time with ease and **totally independent** of the skill of the technician. The results of this research will greatly contribute to the acceleration of research requiring genetically modified animals, and it is expected that the method will be successfully applied to other animal species in the future.

- **Courtesy of Dr. Takehito Kaneko and Dr. Tomoji Mashimo, Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University**
- **Courtesy of Dr. Tetsushi Sakuma and Dr. Takashi Yamamoto, Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University**

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PUBLICATIONS

TAKE METHOD

Knockdown mice	**Mutant mice**
KI mice	**KO rats**
KO mice	**KI rats**

Knockdown mice

Atf3 controls transitioning in female mitochondrial cardiomyopathy as identified by spatial and single-cell transcriptomics
Qaqorh T, Takahashi Y, Sameshima K, Otani K, Yazawa I, Nishida Y, Tonai K, Fujihara Y, Honda M, Oki S, Ohkawa Y, Thorburn DR, Frazier AE, Takeda A, Ikeda Y, Sakaguchi H, Watanabe T, Fukushima N, Tsukamoto Y, Makita N, Yamaguchi O, Murayama K, Ohtake A, Okazaki Y, Kimura T, Kato H, Inoue H, Matsuoka K, Takashima S, Shintani Y.
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KI mice

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Fujiwara K, Inoue T, Kimoto A, Zixian J, Tokuhiro K, Yasukochi Y, Akama TO, Cai CL, Shiojima I, Kimura H, Yoshimura SH, Nakamura T, Hirai M.
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Neuron. 2024 Jul 9:S0896-6273(24)00452-5.

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Kessler S, Minoux M, Joshi O, Ben Zouari Y, Ducret S, Ross F, Vilain N, Salvi A, Wolff J, Kohler H, Stadler MB, Rijli FM.
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KO mice

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KO rats

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KI rats

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KI mice
KO rats
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KO rats
KI rats

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