SONIDEL LIMITED

www.sonidel.com

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 In Vivo - i-GONAD (Mouse) and r-GONAD (RAT) Methods for Transfection into Zygotes in Oviducts.

In Vivo - i-GONAD (Mouse) and r-GONAD (RAT) Methods for Transfection into Zygotes in Oviducts

The most widely used methodology to create genome-edited animals employs 3 major *ex vivo* embryo handling steps:

- Isolation of the zygotes from a pregnant female (previously mated with a male)
- Surgical transfer of electroporated zygotes back into the oviduct of a pseudo pregnant female
- Each of the above step requires a high level of technical expertise and proficiency, expensive apparatus (micromanipulator) and relevant animal licences.

Each step requires a high level of technical expertise and proficiency, expensive apparatus (micromanipulator) and relevant animal licences.

The improved *i-GONAD* (**Mouse**) and *r-GONAD* (**Rat**) techniques are a step-by-step methodology for a novel and improved *in vivo* genome editing system that **does not require *ex vivo* handling of embryos**. The ex vivo handling steps are replaced by an efficient and faster in situ electroporation methodology. This new methodology is enabled by **proprietary custom manufactured concave electrodes** specially optimised for use with the NEPA21 device. Electrodes have been designed for both **MOUSE** and **RAT** models. The electrodes match and fit the shape of the target oviduct, thus enabling in situ zygote oviduct electroporation

The methodology is called GONAD *(**G**enome-editing via **O**viductal **N**ucleic **A**cids* **D***elivery)*:

- *i-GONAD* (improved GONAD) for **MICE**, and
- *r-GONAD* for **RATS**

The very first *in vivo GONAD* applications (Ohtsuka: using Cas9 mRNA and sgRNA) report *genome editing efficiency of approximately 25%.*

- The new improved NEPA21 *i-GONAD* and *r-GONAD* application (Matsuyama: using Cas9 protein and crRNA/tracrRNA complex) report:
- **Knock-out**: 50-100% and
- **Knock-in:** 15-40%

It is particularly applicable for laboratories:

- where traditional gene targeting using ES cells is not well established in mammals such as guinea pig, hamster, cow, pig and other animals
- keen to minimise their 3R footprint – as i*GONAD* does not require the euthanasia of pregnant females unlike in traditional methodologies where females need to be sacrificed for isolating zygotes (for introduction of genome editing components *ex vivo*). In addition, some females who undergo *GONAD* electroporation can deliver the pups.

Video:
Regarding the i-GONAD Technique, this page also references: "a High-Definition Video of the i-GONAD Protocol and Technique."
This page is protected by a password.
Please note that the password is: 11022021

www.sonidel.com

Application-Customised Electrode Options
i-GONAD and r-GONAD Method
There is a choice of two custom manufactured electrodes:

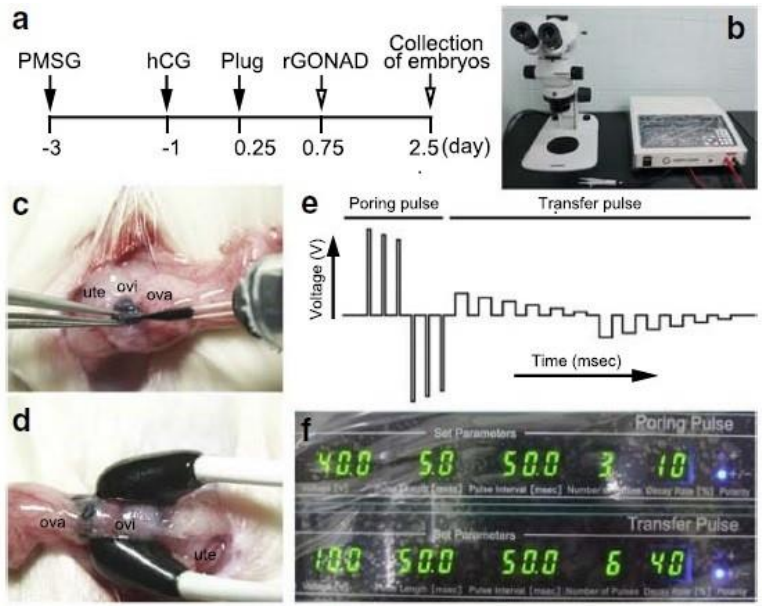
- The **CUY652P2.5x4** is concave in shape and optimised to fit a **MOUSE** oviduct.
- The **CUY652P3x4.5** is concave in shape and optimised to fit a **RAT** oviduct

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/

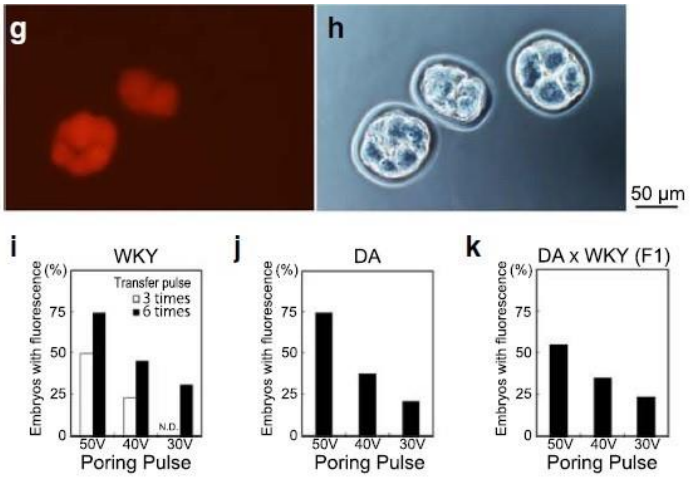
APPLICATIONS

Generation of genome-edited rats by the r-GONAD method

Optimization of conditions for electroporation in the r-GONAD method

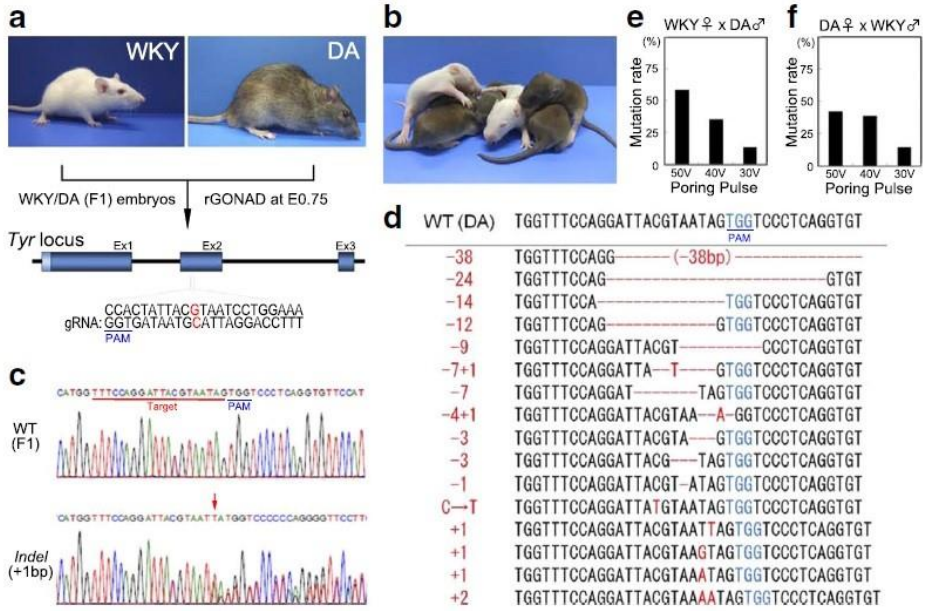


a. Experimental procedure for evaluating electroporation efficiency in the GONAD method
b. Stereo microscope SZX7 (Olympus) and super electroporator NEPA21 (Nepa Gene Co., Ltd.)
c. Inject Tetramethylrhodamine-labelled dextran into the oviductal lumen through the wall of the oviduct near the tubal fimbriae using a micropipette.
d. After injection, cover the fallopian tube region with a Kimwipe soaked in PBS and cut to the appropriate size and electroporate using CUY652P2.5X4 (Tweezers w/2.5mm x 4mm concave platinum electrodes, Nepa gene Co., Ltd.) Ova: ovary, Ovi: oviduct, Ute: uterus
e. Pulse waveform of electroporation
f. NEPA21 electrical conditions
    - Poring Pulse Voltage: 40V, Pulse width: 5msec, Pulse interval: 50msec, Number of pulse: 3 times, Decay rate: 10%, Polarity: +/- (with switching)
    - Transfer Pulse Voltage: 10V, Pulse Width: 50msec, Pulse Interval: 50msec, Number of pulse: 6 times, Decay rate: 40%, Polarity: +/- (with switching)



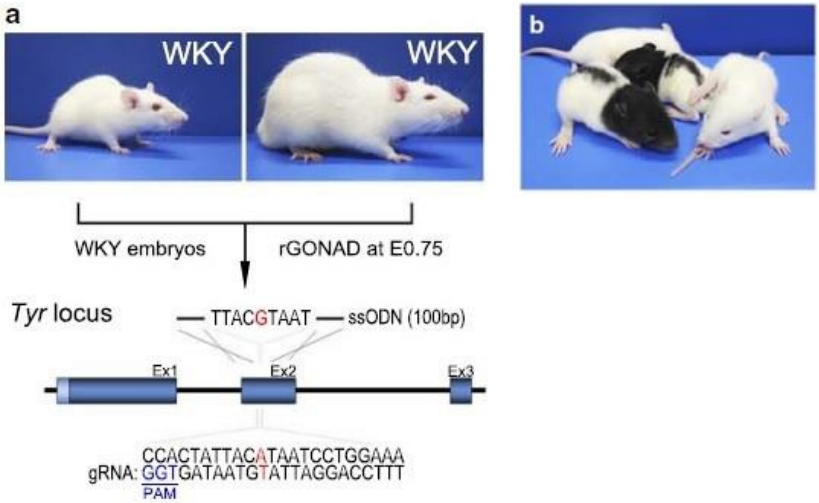
g. h. Fluorescence analysis of tetramethylrhodamine-labelled dextran Scale bar: 50 μm
i. Fluorescence analysis graph of electroporation efficiency in rat WKY
j. Fluorescence analysis graph of electroporation efficiency in rat DA
k. Fluorescence analysis graph of electroporation efficiency in rat DA×WKY

Generation of Try gene knockout (KO) rats by r-GONAD method



a. Scheme of allele-specific genome editing for knock-out rats
   - The r-GONAD method was performed in 0.75 day pregnant *albino* WKY crossed to *agouti* DA strains.
   - The fertilized eggs are (WKY x DA) F1 hybrid. The target sequence and PAM at *Tyr* locus are shown.
b. Some the editing rats had *albino* coloured coats.
c. Direct sequencing results of wild-type F1 (upper; WT) or the editing (below; *indel*) rats. Red arrow indicates *indel* mutation.
d. Sequence analysis of the pups showed a variety of *indel* mutation at the *Tyr* locus, as shown in red.
e. Graph shows analysis of the percentage of genome edited efficiency in *Tyr* gene in WKY female x DA male
f. Graph shows analysis of the percentage of genome edited efficiency in *Tyr* gene in WKY female x male

Recovery of coat-colour mutation in albino WKY rats using KI approach



a. Scheme of hair colour mutation repair
   - The r-GONAD method was performed on WKY female rats at 0.75 days gestation mated with WKY male rats.
   - Diagram of target sequences, PAM sequences, and ssODNs at the Tyr locus.

b. Non-agouti, hooded-spotted albino KI rats.

Mutation of F1 (WKY x DA) rats via Tyr

Stain	Poring Pulse Voltage (V)	Injected	Pregnant	Pups (A)	KO (B)	% (B/A)
WKY♀ x DA♂	50	10	9	46	27	58.7
	40	10	9	68	24	35.3
	30	9	8	67	9	13.4
DA♀ x WKY♂	50	9	6	19	8	42.1
	40	9	5	26	10	38.5
	30	7	6	28	4	14.3

Courtesy of Dr. Makoto Matsuyama, Division of Molecular Genetics, Shigei Medical Research Institute
Kobayashi et al., BMC Biotechnology (2018)

SONIDEL LIMITED

www.sonidel.com

PUBLICATIONS

Transfection into Mouse/Rat Zygotes in Oviducts by Electroporation (i-GONAD/rGONAD)

KI mice	**KI rats**
KO mice	**KO rats**

KI mice

Mitochondrial complexity is regulated at ER-mitochondria contact sites via PDZD8-FKBP8 tethering
Nakamura K, Aoyama-Ishiwatari S, Nagao T, Paaran M, Obara CJ, Sakurai-Saito Y, Johnston J, Du Y, Suga S, Tsuboi M, Nakakido M, Tsumoto K, Kishi Y, Gotoh Y, Kwak C, Rhee HW, Seo JK, Kosako H, Potter C, Carragher B, Lippincott-Schwartz J, Polleux F, Hirabayashi Y.
Nat Commun. 2025 Apr 17;16(1):3401.

Spatial organizations of heterochromatin underpin nuclear structural integrity of ventricular cardiomyocytes against mechanical stress
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.
Cell Rep. 2024 Dec 24;43(12):115048.

Phosphorylation of phase-separated p62 bodies by ULK1 activates a redox-independent stress response
Ikeda R, Noshiro D, Morishita H, Takada S, Kageyama S, Fujioka Y, Funakoshi T, Komatsu-Hirota S, Arai R, Ryzhii E, Abe M, Koga T, Motohashi H, Nakao M, Sakimura K, Horii A, Waguri S, Ichimura Y, Noda NN, Komatsu M.
EMBO J. 2023 Jul 17;42(14):e113349.

A novel technique for large-fragment knock-in animal production without ex vivo handling of zygotes
Abe M, Nakatsukasa E, Natsume R, Hamada S, Sakimura K, Watabe AM, Ohtsuka
Sci Rep. 2023 Feb 8;13(1):2245.

Functional validation of epitope-tagged ATF5 knock-in mice generated by improved genome editing of oviductal nucleic acid delivery (i-GONAD)
Haruo Nakano, Shiori Kawai, Yusaku Ooki, Tomoki Chiba, Chiharu Ishii, Takumi Nozawa, Hisako Utsuki, Mariko Umemura, Shigeru Takahashi, Yuji Takahashi
Cell Tissue Res. 2021 Jul;385(1):239-249.

Sequential i-GONAD: An Improved In Vivo Technique for CRISPR/Cas9-Based Genetic Manipulations in Mice
Masahiro Sato, Rico Miyagasako, Shuji Takabayashi, Masato Ohtsuka, Izuho Hatada, Takuro Horii
Cells. 2020 Feb 26;9(3):546.

KO mice

Structural insights into how DEK nucleosome binding facilitates H3K27 trimethylation in chromati
Kujirai T, Echigoya K, Kishi Y, Saeki M, Ito T, Kato J, Negishi L, Kimura H, Masumoto H, Takizawa Y, Gotoh Y, Kurumizaka H.
Nat Struct Mol Biol. 2025 Feb 21.

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.

Maternal immunoglobulins are distributed in the offspring's brain to support the maintenance of cortical interneurons in the postnatal period
Morimoto K, Takahashi R, Takahashi G, Miyajima M, Nakajima K.
Inflamm Regen. 2024 May 15;44(1):24.

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.

Generation of a recessive dystrophic epidermolysis bullosa mouse model with patient-derived compound heterozygous mutations
Takaki S, Shimbo T, Ikegami K, Kitayama T, Yamamoto Y, Yamazaki S, Mori S, Tamai K.
Lab Invest. 2022 Jun;102(6):574-580.

Loss of Atg2b and Gskip Impairs the Maintenance of the Hematopoietic Stem Cell Pool Size
Shun-Suke Sakai, Atsushi Hasegawa, Ryosuke Ishimura, Naoki Tamura, Shun Kageyama, Satoko Komatsu-Hirota, Manabu Abe, Yiwei Ling, Shujiro Okuda, Manabu Funayama, Mika Kikkawa, Yoshiki Miura, Kenji Sakimura, Ichiei Narita, Satoshi Waguri, Ritsuko Shimizu, Masaaki Komatsu
Mol Cell Biol. 2022 Jan 20;42(1): e0002421.

SONIDEL LIMITED

www.sonidel.com

An efficient i-GONAD method for creating and maintaining lethal mutant mice using an inversion balancer identified from the C3H/HeJJcl strain
Satoru Iwata, Takahisa Sasaki, Miki Nagahara, Takashi Iwamoto
G3 (Bethesda). 2021 Aug 7;11(8):jkab194.

Improvement of genome editing by electroporation using embryos artificially removed cumulus cells in the oviducts
Takehito Kaneko, Shungo Tanaka
Biochem Biophys Res Commun. 2020 Jul 5;527(4):1039-1042.

In utero gene transfer system for embryos before neural tube closure reveals a role for Hmga2 in the onset of neurogenesis
Kuwayama N, Kishi Y, Maeda Y, Nishiumi Y, Suzuki Y, Koseki H, Hirabayashi Y, Gotoh Y.
bioRxiv May 15, 2020

Simple and large-scale chromosomal engineering of mouse zygotes via in vitro and in vivo electroporation.
Iwata S, Nakadai H, Fukushi D, Jose M, Nagahara M, Iwamoto T
Sci Rep. 2019 Oct 11;9(1):14713.

The p21 dependent G2 arrest of the cell cycle in epithelial tubular cells links to the early stage of renal fibrosis.
Koyano T, Namba M, Kobayashi T, Nakakuni K, Nakano D, Fukushima M, Nishiyama A, Matsuyama M.
Sci Rep. 2019 Aug 19;9(1):12059.

KO mice
KI mice

Efficient genome editing in wild strains of mice using the i-GONAD method
Imai Y, Tanave A, Matsuyama M, Koide T.
Sci Rep. 2022 Aug 15;12(1):13821.

KI rats

A missense mutation in the Hspa8 gene encoding heat shock cognate protein 70 causes neuroaxonal dystrophy in rats
Tanaka M, Fujikawa R, Sekiguchi T, Hernandez J, Johnson OT, Tanaka D, Kumafuji K, Serikawa T, Hoang Trung H, Hattori K, Mashimo T, Kuwamura M, Gestwicki JE, Kuramoto T.
Front Neurosci. 2024 Feb 6:18:1263724.

KO rats

Neuromedin U-deficient Rats do not Lose Body Weight or Food Intake
Yokogi K, Goto Y, Otsuka M, Ojima F, Kobayashi T, Tsuchiba Y, Takeuchi Y, Namba M, Kohno M, Tetsuka M, Takeuchi S, Matsuyama M, Aizawa S.
Sci Rep. 2022 Oct 27;12(1):17472.

Creation of X-linked Alport Syndrome Rat Model with Col4a5 Deficiency
Masumi Namba, Tomoe Kobayashi, Mayumi Kohno, Takayuki Koyano, Takuo Hirose, Masaki Fukushima, Makoto Matsuyama
Sci Rep. 2021 Oct 21;11(1):20836

KO mice
KI mice
KO rats
KI rats

GONAD: A new method for germline genome editing in mice and rats
Masumi Namba, Tomoe Kobayashi, Takayuki Koyano, Mayumi Kohno, Masato Ohtsuka, Makoto Matsuyama
Dev Growth Differ. 2021 Aug 25.

KO rats
KI rats

i-GONAD (improved genome-editing via oviductal nucleic acids delivery), a convenient in vivo tool to produce genome-edited rats.
Takabayashi S, Aoshima T, Kabashima K, Aoto K, Ohtsuka M, Sato M
Sci Rep. 2018 Aug 13;8(1):12059.

KO rats
KI rats

Successful production of genome-edited rats by the rGONAD method.
Kobayashi T, Namba M, Koyano T, Fukushima M, Sato M, Ohtsuka M, Matsuyama M
BMC Biotechnol. 2018 Apr 2;18(1):19