www.sonidel.com

Stem Cell Electroporation

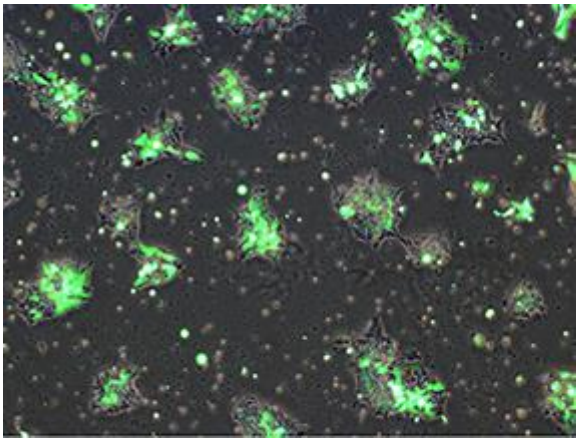
The NEPA21 is the only device on the market to approach Stem Cell Electroporation from the perspective of optimising delivered energy.

- The finer control over the delivered energy available with the NEPA21 offers specific and important advantages for stem cell 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 their viability post electroporation.
- The success of the NEPA21 for cell electroporation is evident by the number of laboratories what have published with the NEPA21 system, and the quantum of client laboratory verified Viability % and Transfection Efficiency %.
- No special buffers required. Cost we electroporation can be as low as E3.50 per cuvette.
- 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

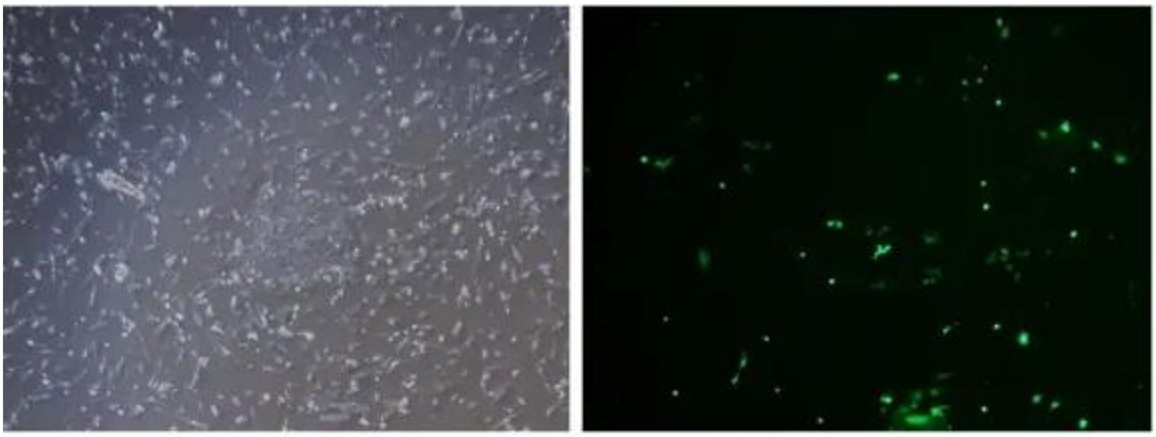
Transfection RESULTS in Client Labs

Human iPS Cells

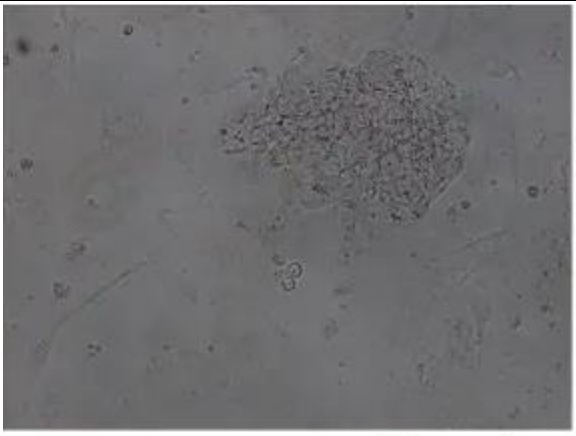


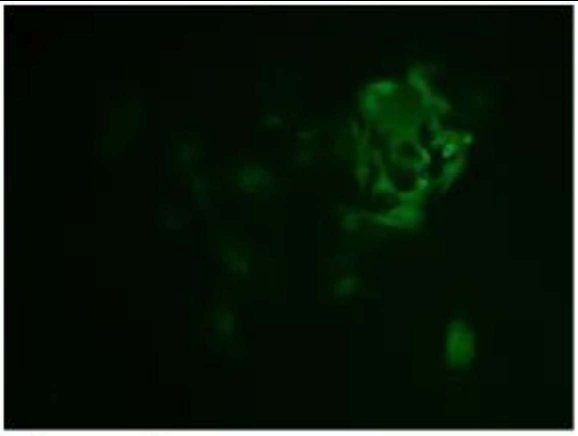
3 days after electroporation

Human iPS Cells



2 days after electroporation

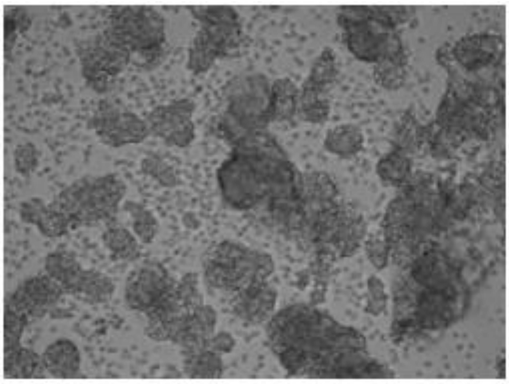




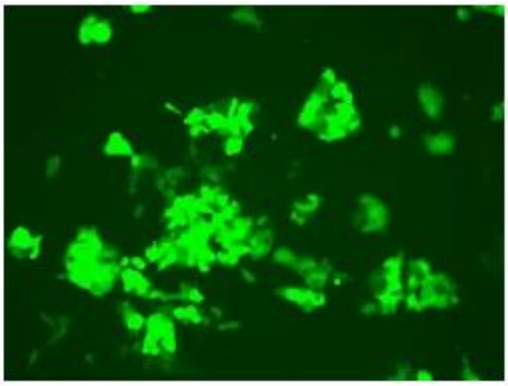
7 days after electroporation

GFP is still expressed in the iPS cell colonies after cell passaging.

Mouse ES Cells

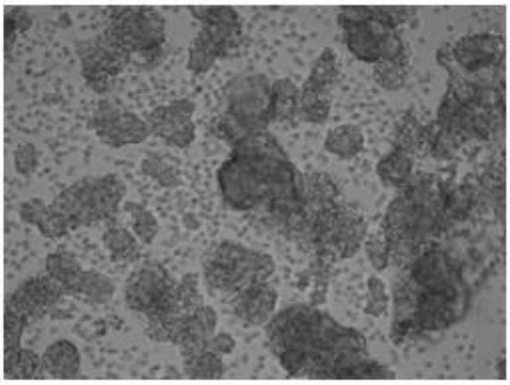


Viability: 74%

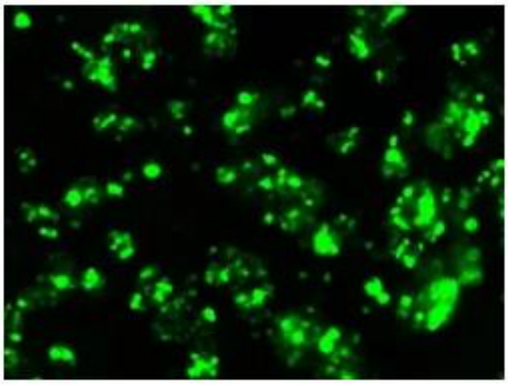


Transfection Efficiency: 88%

Mouse Neurospheres

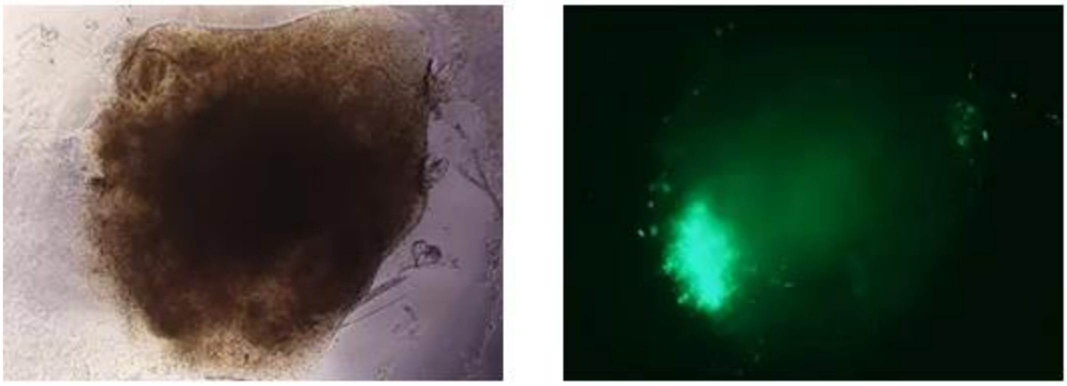


Viability: 90%



Transfection Efficiency: 75%

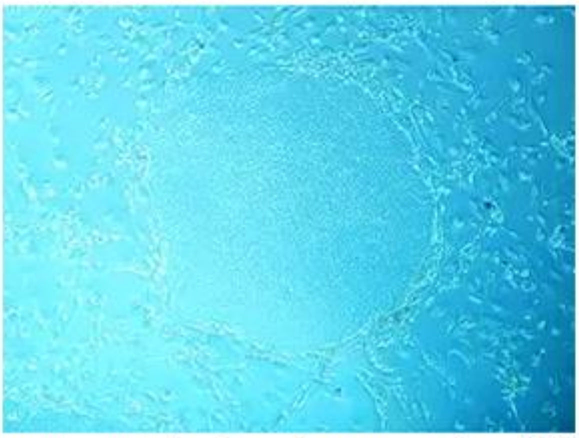
Embryoid Body in Adherence from human iPS Cells



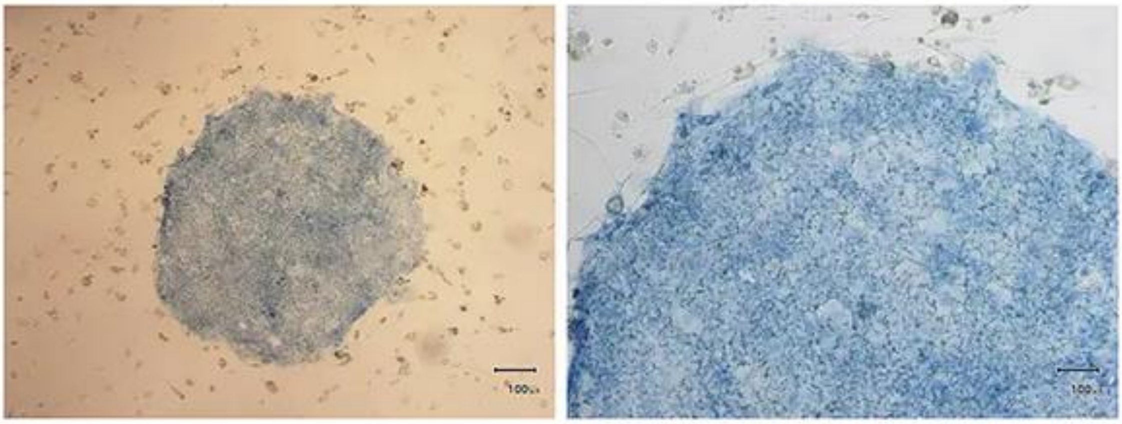
Generation of Induced Pluripotent Stem Cells (iPSCs)

Data of disease-specific iPSC Generation (disease: LQT)

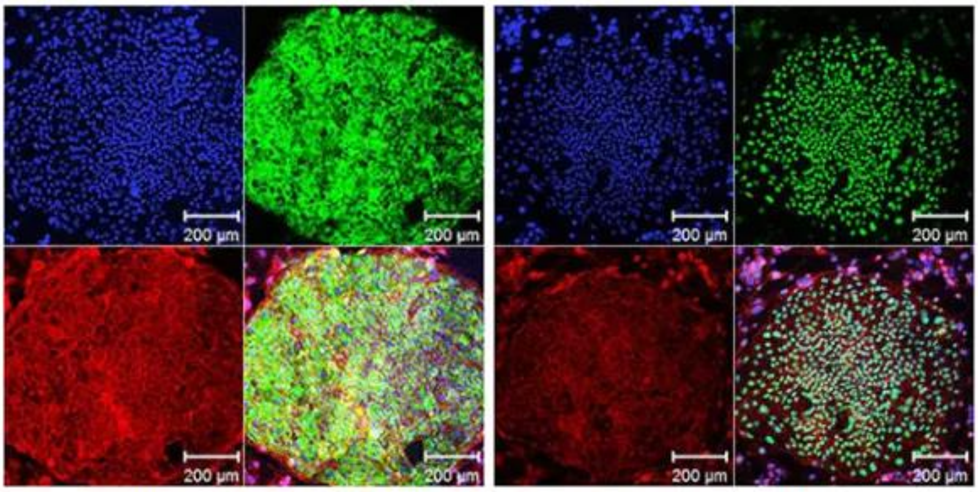
Transfection of multiple episomal plasmids into B cells



iPSC colony one month after electroporation. (5X objective)



Alkaline phosphatase stain (Left: 4X objective Right: 10X objective)



Good expressions of stem cell markers TRA1-60 and OCT4.

(left green: TRA1-60, right green: OCT4, blue: DAPI, red: Phalloidin)

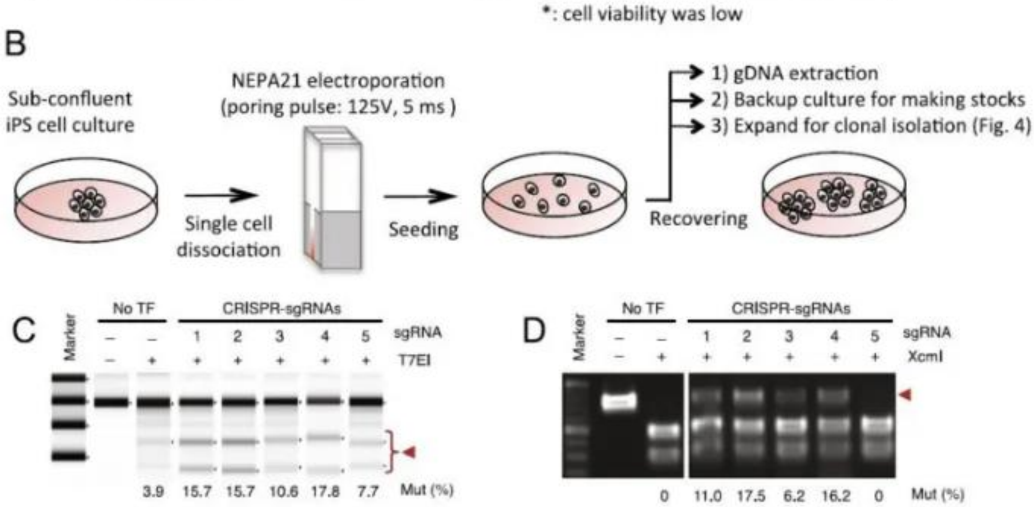
Courtesy of Dr. Toshio Nakanishi laboratory, Department of Pediatric Cardiology, Tokyo Women's Medical University, Japan

Evaluation of CRISPR activity of human iPS cells with NEPA21 transfection

A

	NEPA21 poring pulse		Tested iPSC lines (% of GFP positive cells)		
	Voltage (V)	Pulse (ms)	201B7	404C2	DMD-iPSC
Condition 1	100	5	17.2%	40.6%	39.9%
Condition 2	125	5	20.2%	52.0%	64.4%
Condition 3	150	5	17.0%	37.1%	59.7%
Condition 4	175	5	17.0%	*60.6%	61.9%

*: cell viability was low



A. Optimization of transfection conditions using the NEPA21 super electroporator (Nepa Gene Co., Ltd.) in human iPS cell lines. Voltage and pulse width of Poring Pulse were examined. EGFP expression plasmids were transfected and the percentage of positive cells was analyzed using the LSRFortessa Cell Analyzer (BD).

B. Schematic of transfection procedure.

C. Cas9 expression vector pHL-EF1a-SphcCas9-iP-A (Addgene ID: 60599) and sgRNA expression vector pHL-H1-ccdB-mEF1a-RiH (Addgene ID: 60601) were electroporated into iPS cells and then the endogenous dystrophin gene cleavage activity was evaluated by the T7EI assay. The intensity of the cleaved bands (◀ red arrows) was detected with a TapeStation (Agilent) and the values are shown below the gel electrogram images.

D. The same genomic DNA samples as in (C) were analyzed by RFLP with restriction enzyme XcmI (50-CCANNNNN^NNNTGG-30). The intensity of the uncut bands is indicated by ◀ red arrows. Because gRNA5 cleaves far from the XcmI site, no cleavage activity was detected in the RFLP assay.

Courtesy of Dr. HongMei Li and Dr. Akitsu Hotta, Center for iPS Cell Research and Application (CiRA), Kyoto University

STEM CELL

Client Laboratory Verified RESULTS

Transfection Data: iPS Cells / ES Cells / Other Stem Cells

See the cell images by clicking the cell names.

V: Viability, TE: Transfection Efficiency.

Cells name	V	TE		Cells name	V	TE
Human iPS Cells (201B7)	86%	70%		Human iPS Cells	94%	80%
Human iPS Cells				Human iPS Cells		
Human iPS Cells (201B7)	85%	94%		Human iPS Cells (201B7)		
Human iPS Cells	69%	80%		Human iPS Cells		73%
Human iPS Cells Derived Neural Cells	93%	54%		Human ES Cells		
Human ES Cells (H9 p.51))	55%	55%		Human Mesenchymal Stem Cells	96.2 %	96.7 %
Human Mesenchymal Stem cells (Primary)	78%	75%		Human Mesenchymal Stem Cells	70%	80%
Human Neural Stem Cells	97%	95%		Human Neural Stem Cells	80%	83%
Human Deciduous Teeth Stem Cells (SHED)	90%	92%		Human Nucleated Cells Including Hematopoietic Stem Cells (Before cell isolation)	73%	90%
Mouse iPS Cells	70%	50%		Mouse ES Cells	80%	75%
Mouse ES Cells	80%	68%		Mouse ES Cells	74%	88%
Mouse ES cells (129 strain, R1/E)	80%	90%		Mouse ES Cells	70%	100%
Mouse ES Cells	80%	90%		Mouse iPS cell derived Neural Stem Cells		86%
Mouse Neural Stem Cells	90%	80%		Mouse Neural Stem cells (Primary)	80%	60%
Mouse Neurospheres	90%	75%		Mouse Neurospheres		

Mouse Trophoblast Stem Cells	59%	47%		C3H/10T1/2 Mouse Mesenchymal Stem Cells	70%	85%
Mouse Mesenchymal Stem Cells	99%	89%		Mouse Hematopoietic Stem Cells (c-Kit positive cells)	66%	45%
Rat ES Cells	70%	76%		Rat ES Cells	60%	80%

For clients who have the NEPA21 system, we provide quite a lot of further know-how data for *iPS/ES/Other stem cells transfection* with high efficiency and high viability. Contact sales@sonidel.com for further information or to schedule a TRIAL.

PUBLICATIONS

iPS/ES/OTHER STEM CELLS

hESC	**cyiPSC**	**Human dental pulp stem cells (DPSCs)**
hESCs	**bEDSCs**	**Human adipose mesenchymal stem cells (hAMSCs)**
hiPSCs	**ADSCs**	**Rat hair follicle stem cells**
hiMSC	**Neurosphere**	**Shrew permatogonial stem cells (SSCs)**
Reprogramming of iPSCs	**mESCs**	
iPSCs		

hESC

Perturbed cell fate decision by schizophrenia-associated AS3MTd2d3 isoform during corticogenesis
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hESCs

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Temporal morphogen gradient-driven neural induction shapes single expanded neuroepithelium brain organoids with enhanced cortical identity
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hiPSCs

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Minagawa A, Yoshikawa T, Yasukawa M, Hotta A, Kunitomo M, Iriguchi S, Takiguchi M, Kassai Y, Imai E, Yasui Y, Kawai Y, Zhang R, Uemura Y, Miyoshi H, Nakanishi M, Watanabe A, Hayashi A, Kawana K, Fujii T, Nakatsura T, Kaneko S.
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