High voltage electroporation *in vitro*

The ELEPO21 *In Vitro* High-Voltage Electroporator developed by Nepa Gene Co., Ltd. has a unique pulsing system composed of 4-step multiple pulses with decaying, and it can achieve transformation efficiency markedly higher than traditional methods that use a single-step exponential pulse wave in bacteria, yeasts, and fungi.

Bacteria (*E. coli*)

● High-effciency gene transfer in bacteria by multi-step electroporation

We measured gene transfer efficiency using the ELEPO21 in Gram-negative bacteria. Competent cells were prepared as usual from *E. coli* DH5 α The competent cells were mixed with pUC19 DNA, and a 20 μl aliquot (containing 10^9-10^{11} cells and 10 pg DNA in 10% glycerol solution) was transferred to the 1 mm gap electrode cuvette (EC-001, Nepa Gene). The cuvette was set in the chamber connected to the-ELEPO21, and delivered 3-step pulses as described below. All steps were done on ice. After electroporation, the cells were plated on LB agarose medium containing ampicillin, and colonies formed were counted. Transformation efficiency was expressed as a number of colonies per μg plasmid DNA used.

[ELEPO21 pulsing conditions, Fig. 1]
Poring Pulse (voltage: 2,000 V, pulse length : 2.5 msec, pulse interval : 50 ms, number of pulse : 1, polarity : +)
Transfer Pulse (voltage: 150 V, pulse length : 50 msec, pulse interval : 50 ms, number of pulse : 5, polarity : +/−)

To evaluate the above results, we measured gene transfer efficiencies using a conventional electroporator (ECM630, BTX) that deliver a single exponential pulse as described below.

[ECM630 pulsing conditions, Fig. 2]
Single pulse (voltage : 2,000 V, resistance : 200 Ω, capacitance : 25 μF, number of pulse : 1)

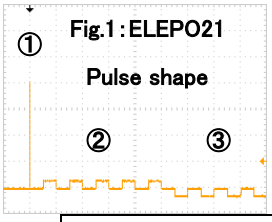


Fig.1 : ELEPO21

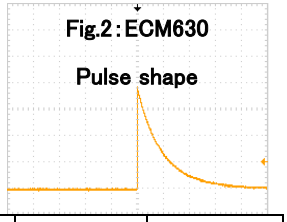


Fig.2 : ECM630

Fig.3

E. coli DH5 α		ELEPO21	ECM630
Efficiency	x 10^{10} cfu	1.40 ±0.07	0.29 ±0.11
	Relative value	4.9	1.0

〈Experimental results〉
The above cell suspensions (sample resistance value: 7.7 KΩ) were used for electroporation. The tranformation efficiency obtained by the ELEPO21 electroporator was approximately 5 times higher than that by the ECM630 electroporator (Fig. 3).

Fig. 1 : Multi-step pulses by the ELEPO21
①Poring pulse ②Transfer pulse (+) ③Transfer pulse (−)

※ The values are averages of repeated experiments.
※ The optimum pulsing conditions were used for ELEPO21 and ECM630.
※ cfu: colony forming unit.

Yeast (*S. cerevisiae*)

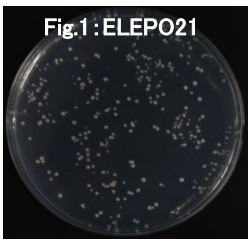
● High-effciency gene transfer in Yeast by multi-step electroporation

We measured gene transfer efficiency using the-ELEPO21 in yeast. Competent cells were prepared as usual from budding yeast S. cerevisiae. The competent cells were mixed with pAS2 DNA, and a 20 μl aliquot (containing 10^8-10^{10} cells and 50 ng DNA in 1M sorbitol solution) was transferred to the 1 mm gap electrode cuvette (EC-001, Nepa Gene). The cuvette was set in the chamber connected to the ELEPO21, and delivered 3-step pulses as described below. All steps were done on ice. After electroporation, the cells were plated on selective agarose medium devoid of nutrients, and the colonies formed were counted. Transformation efficiency was expessed as a number of colonieis per μg plasmid DNA.

[ELEPO21 pulsing conditions]
Poring pulse (voltage : 500 V, pulse width : 1.5 msec, pulse interval : 50 ms, number of pulse : 5, polarity : +)
Transfer pulse (voltage : 50 V, pulse width : 50 msec, pulse interval : 50 ms, number of pulse : 5, polarity : +/−)

To evaluate the above results, we measured gene transfer efficiency using a conventional electroporator (ECM630, BTX) that can deliver a single exponential pulse only as described below.

[ECM630 pulsing conditions]
Single pulse (voltage : 750 V, Resistance : 200 Ω, Capacitance : 25 μF, number of pulse : 1)



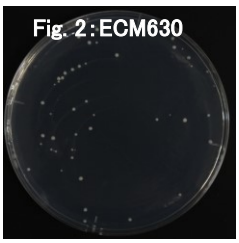


Fig.3

S. cerevisiae FY24		ELEPO21	ECM630
Efficiency	x 10^4 cfu	2.49±0.24	0.40±0.11
	Relative value	6.3	1.0

◆ Experimental results ◆
The above cell suspensions (sample resistance value : 12.36 KΩ) were used for electroporation. The tranformation efficiency obtained by the ELEPO21 was approximately 6 times higher than that by the ECM630 electroporator (Fig. 1-3).

※ The values are averages of repeated experiments.
※ The optimum pulsing conditions were used for ELEPO21 and ECM630.
※ cfu: colony forming unit.