In Situ Gene Transfer of Adherent Cells by Electroporation

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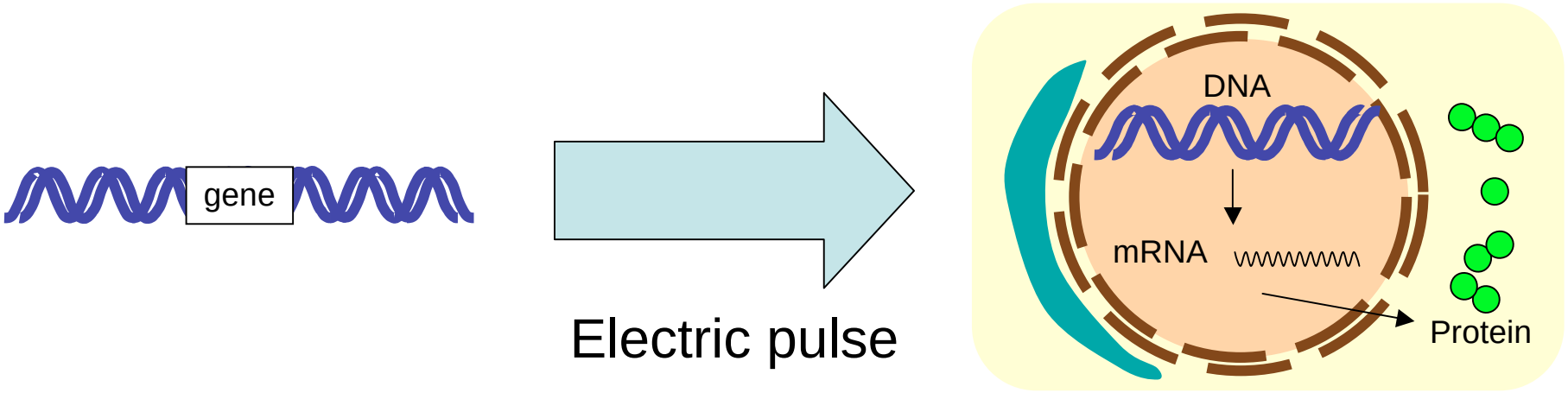
ABSTRACT

Gene transfer is a fundamental technique in molecular cell biology. Common methods use retrovirus particles, liposomes, and electric pulses in cultured cells. Electric pulse is a convenient method, but conventional methods were not satisfactory as regards viability and gene transfer efficiency. This is because perforation of cells was performed by a single or several doses of a high-voltage pulse.

Recently, we developed a new protocol that employs a combination of multiple high- and low-voltage pulses, and attained gene transfer virtually of all cells without loss of viability in both primary strains and established cell lines. In this study, we examined *in situ* gene transfer to adherent cells with a new model, super elctroporator NEPA21 (NEPA GENE CO., LTD).

Methods of Gene Transfer



Electric pulse

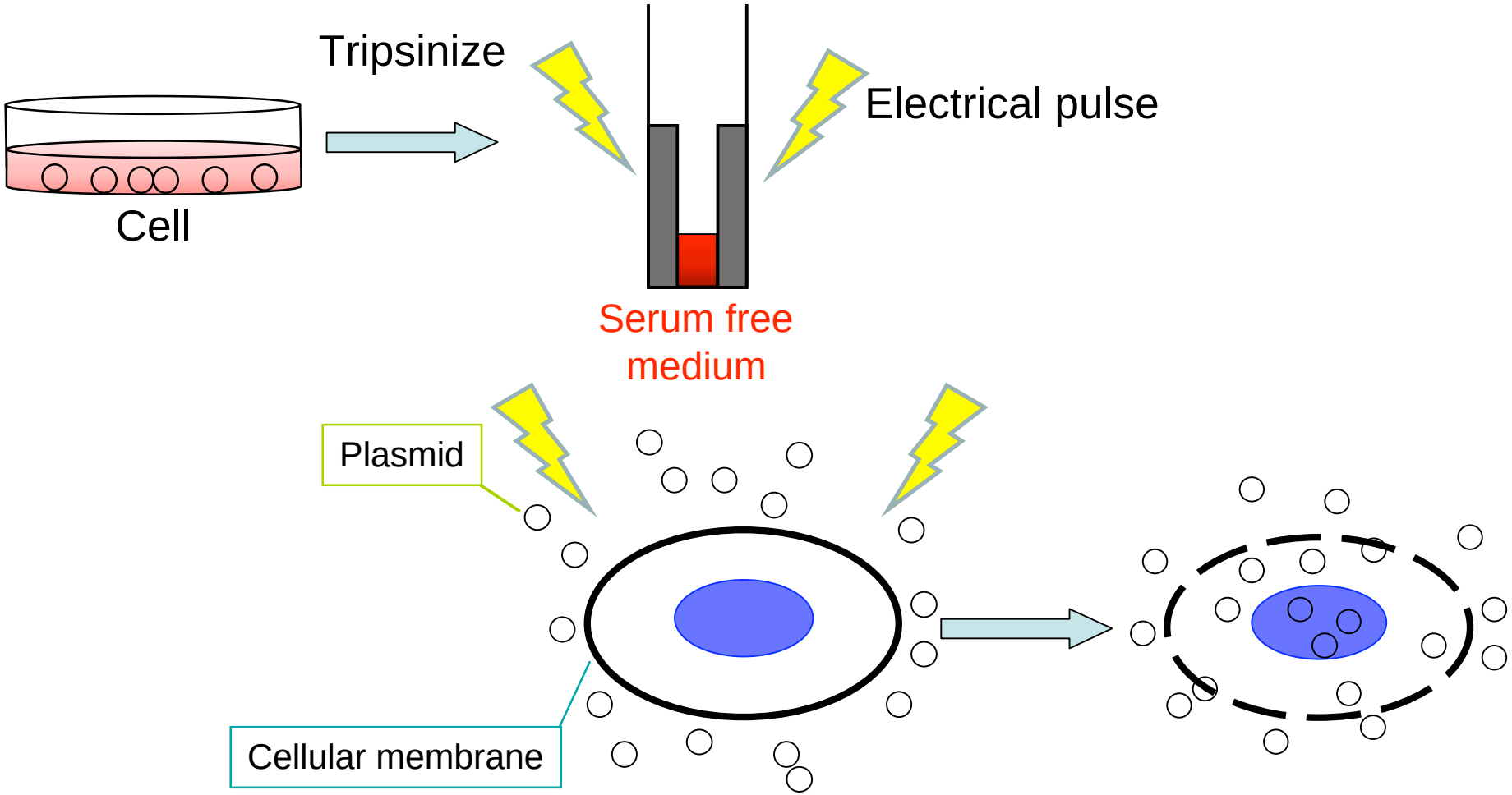
Retrovirus

Liposome

Cell

Gene transfer is the process to introduce DNA/RNA into cells to examine gene functions.

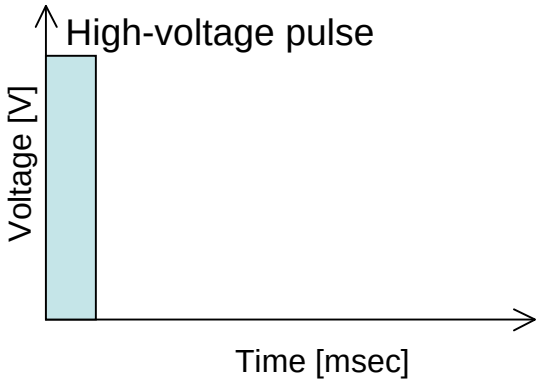
Electroporation



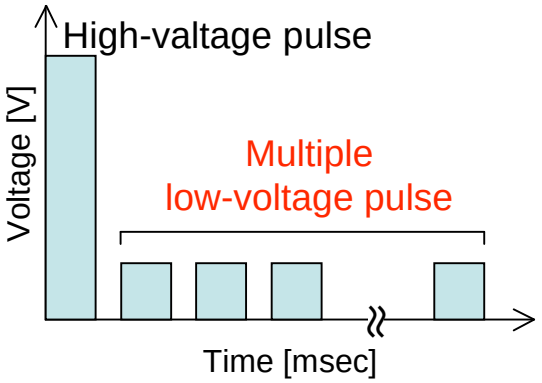
Electroporation tansfers molecules into the nucleus through holes perforated by electric pulses.

Pulsing Protocol

Conventional model

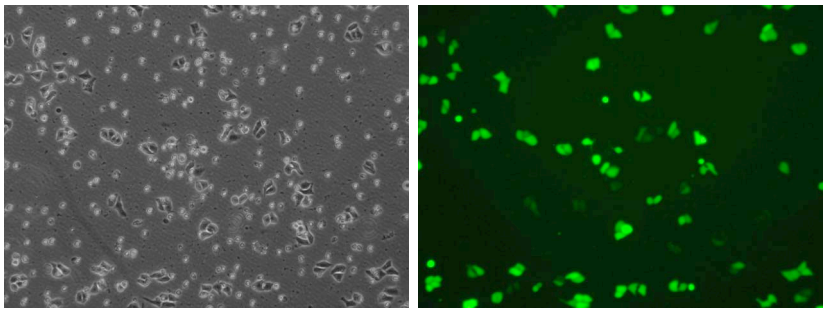


Our unique model



HeLa cells

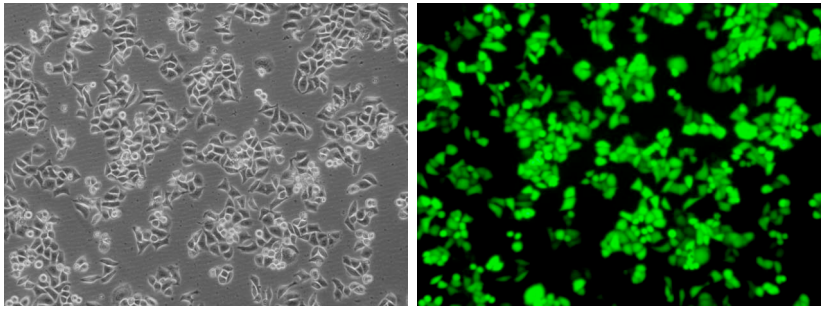
DIC GFP



viability 45%

Transfer efficiency 70%

DIC GFP



viability 95%

Transfer efficiency 98%

(*Transfer efficiency = GFP positive cells / viable cells)

Multiple low-voltage pulses are essential for improvement of viability and transfer efficiency.

Purpose

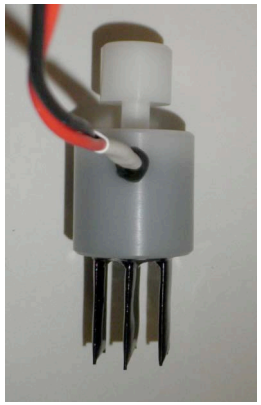
Electroporation usually uses cell suspensions in cuvettes. Adherent cells are detached from the substratum of plastic dishes, which limits experimental designs.

We developed a new electrode that enables gene transfer to adherent cells *in situ*. (*In* situ electroporation).

Machine

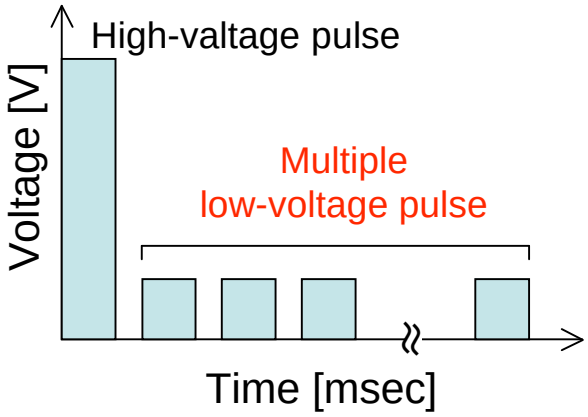
NEPA 21
(NEPA GENE)

Electrode



CUY900-24-3
(NEPA GENE)

Unique pulse model



In Situ Gene Transfer to Adherent Cells

24 well dish

Electrode type

3	5

CUY900-24-3

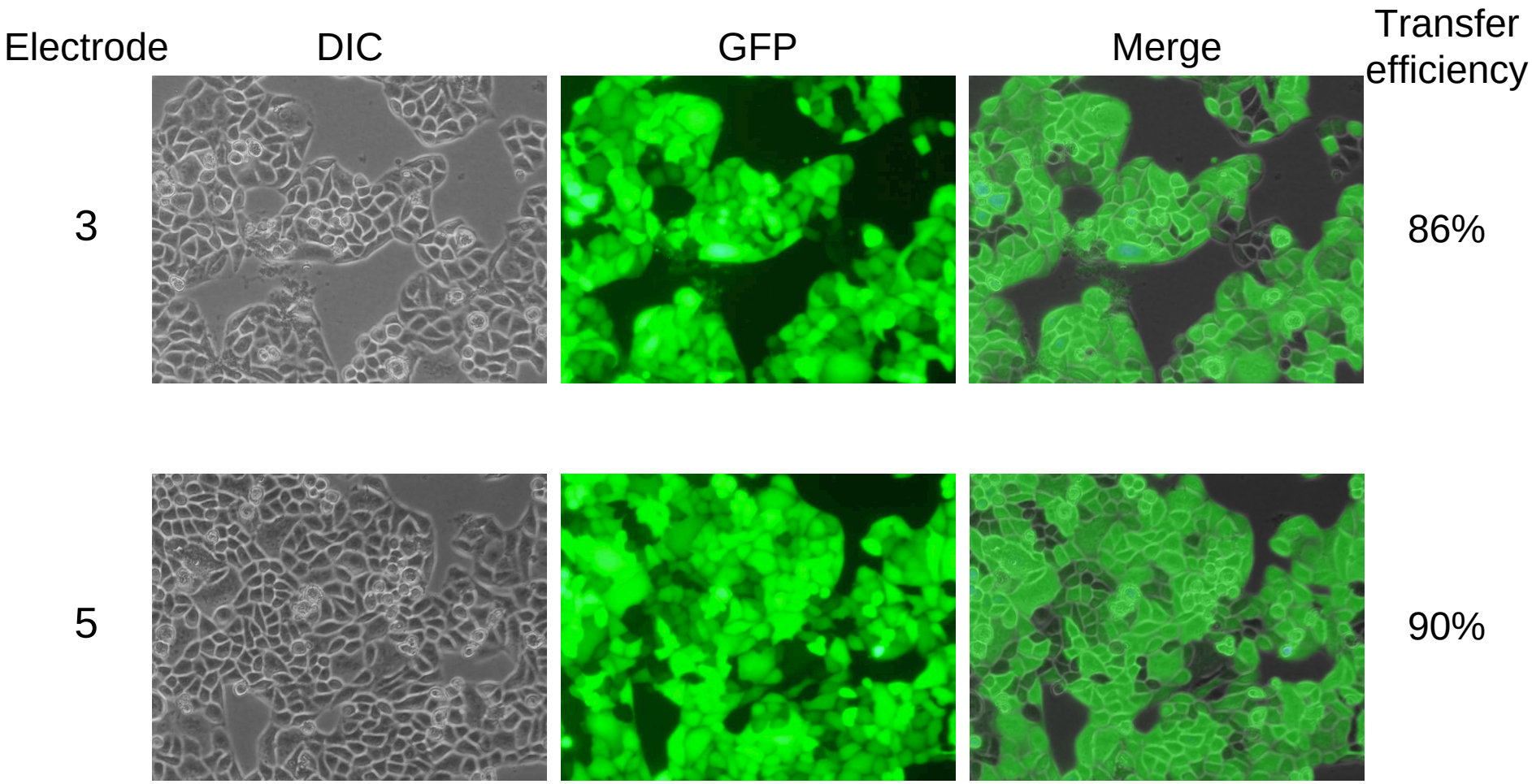
CUY900-24-5

In situ electric pulse

Serum free medium
(without special solution)

In situ electroporation is suitable for cells weak to trypsinization such as neural and senescent cells.

In Situ Electroporation in Adherent HeLa Cells

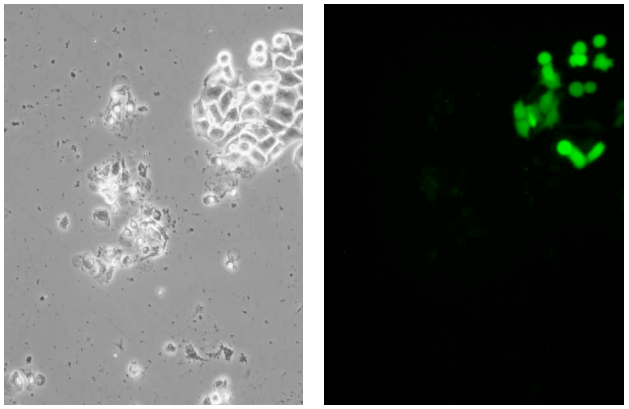


In situ electroporation results in very efficient gene transfer.

Effect of Low-Voltage Pulses on *In Situ* Electroporation

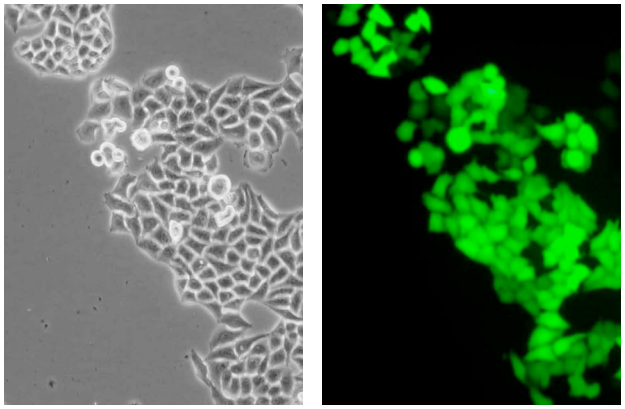
<u>High voltage pulse</u>

DIC GFP



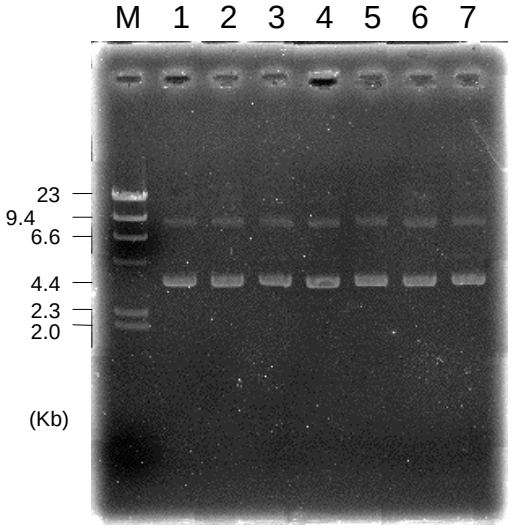
<u>High + Low voltage pulses</u>

DIC GFP



Multiple low-voltage pulses are required for improvement of viability and transfer efficiency

Reuse of Plasmid



Lane M：Marker（λ/HindIII）

Lane 1：New plasmid

Lane 2：Plasmid once used

Lane 3：Plasmid twice used

Lane 4：Plasmid 3-times used

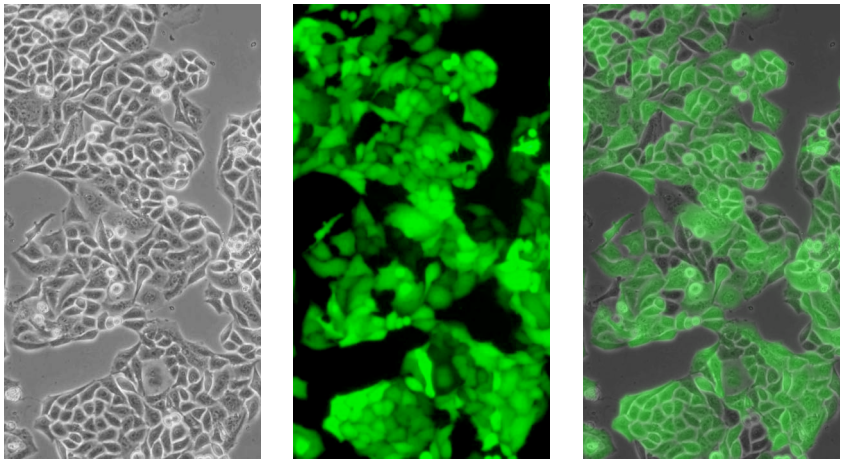
Lane 5：Plasmid 4-times used

Lane 6：Plasmid 5-times used

Lane 7：Plasmid 6-times used

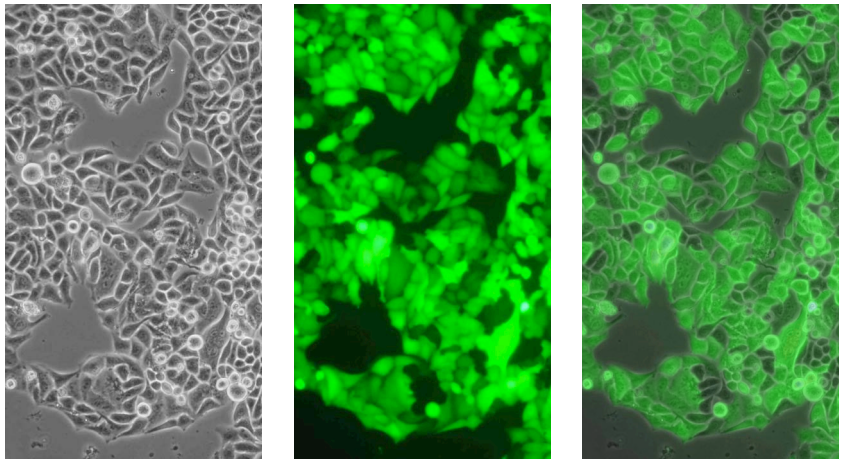
New plasmid

DIC GFP Merge



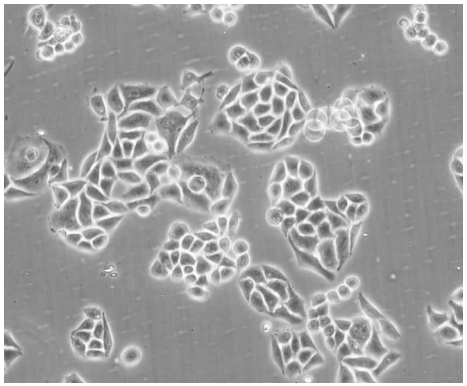
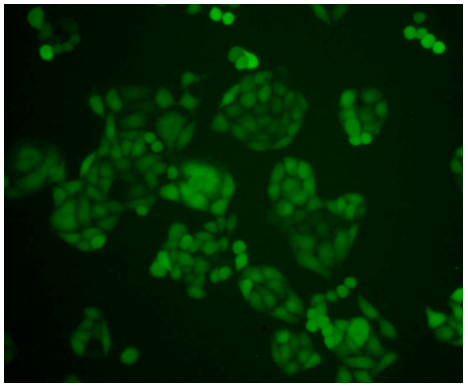
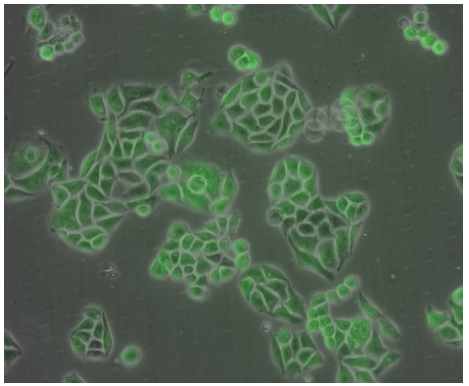
6 times-used plasmid

DIC GFP Merge

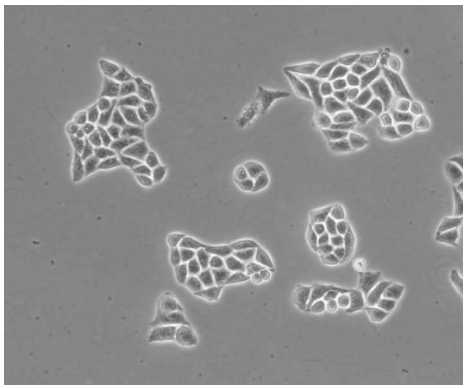
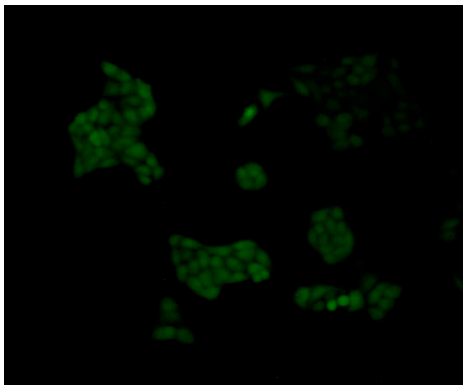
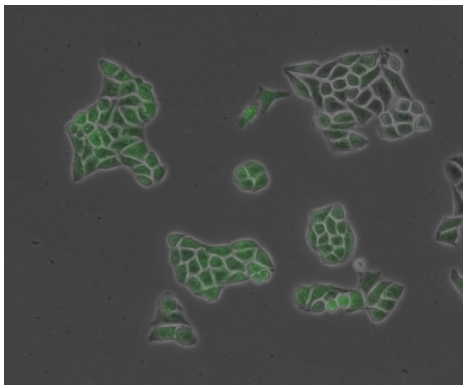


Plasmids can be used repeatedly in *in situ* electroporation

In Situ Transfer of Protein and Peptide

	DIC	GFP	Merge	Transfer efficiency
FITC-Albumin*1 100μg/300μL				> 90%

*1FITC-conjugated human albumin, MW: 66 kDa)

	DIC	GFP	Merge	
FA-peptide*2				> 90%

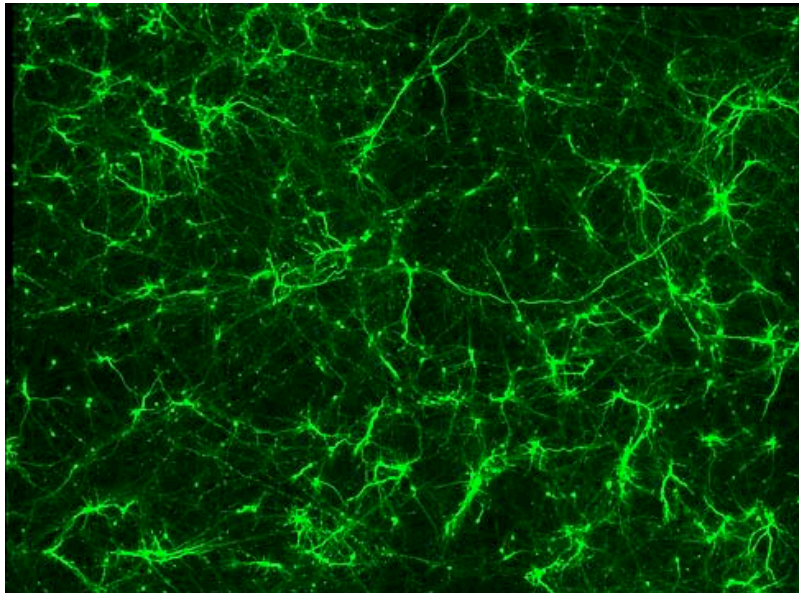
*2Fluorescein-conjugated peptides (octamer), MW: 1~3 kDa

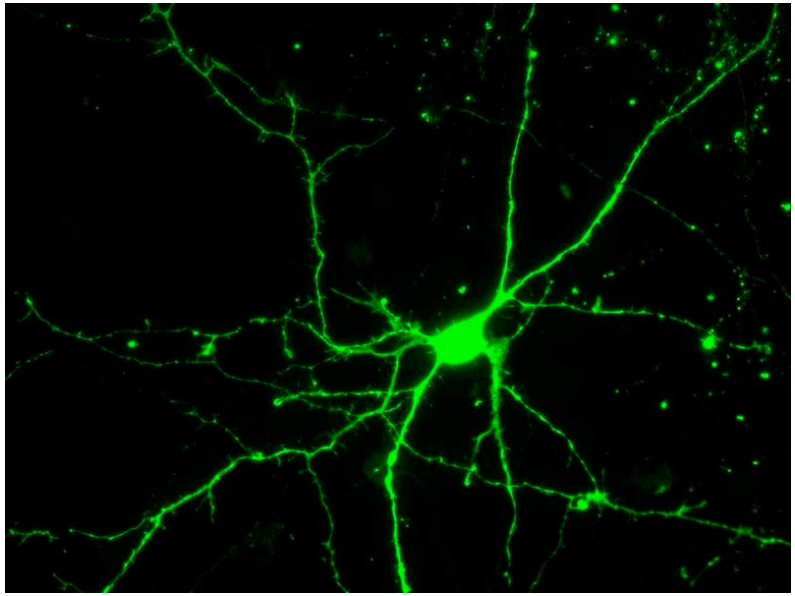
SUMMARY

In our experiments of *in situ* electroporation,

1. High-efficiency gene transfer was observed without loss of viability or special solution.
2. Multiple low-voltage pulses were necessary for maximum efficiencies.
3. Plasmids were used at least 6 times without decrease in gene transfer efficiency.
4. Protein and peptide were also efficiently introduced to cells.

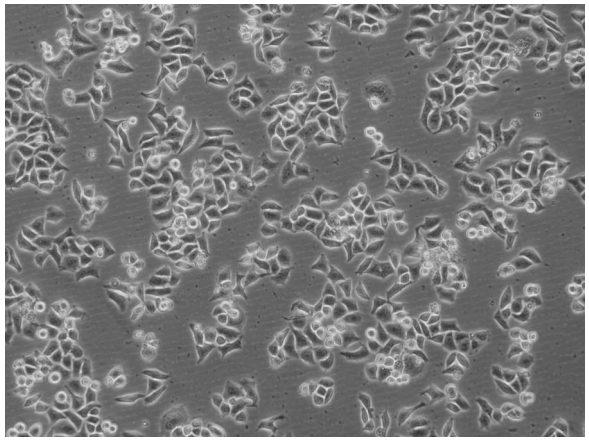
Reference Data



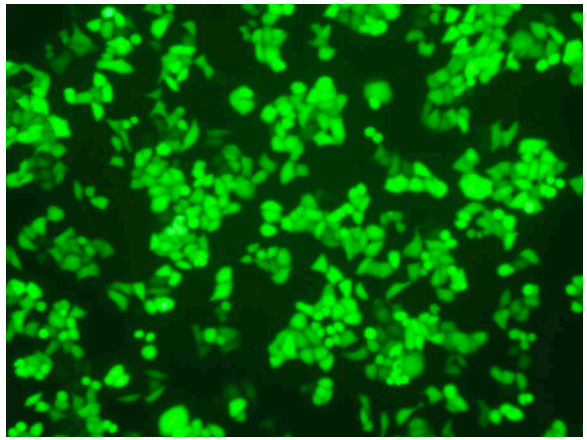


- HeLa cells（human cervical cancer cells）
  Survival ≧95%、 transfection efficiency ≧95%

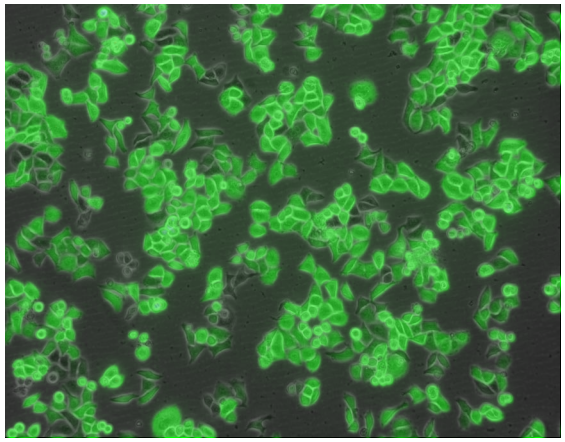
DIC



GFP

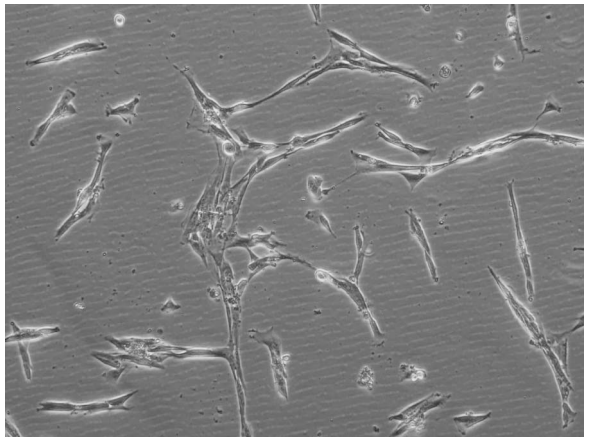


Merge

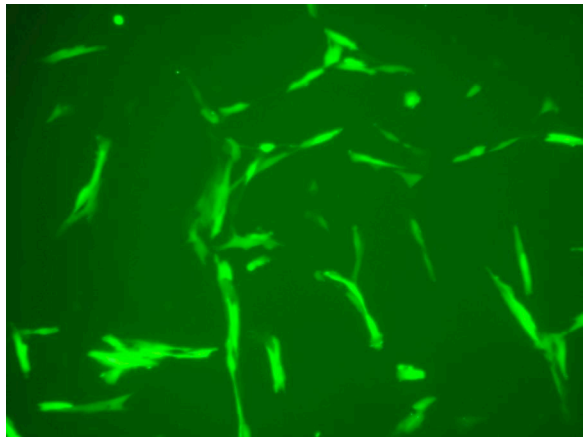


- TIG-7 cells (human normal fibroblasts)
  Survival ≧95%、transfection efficiency ≧70%

DIC



GFP



Merge