An individually addressable suspending-drop electroporation system for high-throughput cell transfection

Supplementary Methods

Preparation of plasmids, and siRNA

The pEGFP-N1 plasmid, encoding GFP, used in this paper was purchased from Clontech Laboratories Inc (Palo Alto, CA). The plasmid was maintained in Escherichia coli, purified using a Nucleobond Ax Kit (Machery-Nagel Inc, Bethlehem, PA), and dissolved in 10 mM Tris-HCl (pH 8.0) containing 0.1 mM EDTA (ethylenediamine-N,N,N′N′-tetraacetic acid) and stored at -20°C until use. The siRNA against GFP, GAPDH, and a control siRNA targeting an unrelated sequence were custom-synthesized by GenePharma (Shanghai, China).

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from GFP-expressing HeLa cells using TRIzol reagent according to manufacturer's instructions (Invitrogen, Shanghai, China). cDNA was generated from 1 μg RNA using M-MLV reverse transcriptase (Promega, Beijing, China). To evaluate the mRNA levels of GFP, qRT-PCR was performed on an IQ5 Real-Time Detection System (Bio-Rad, Beijing, China) using SYBR Green Master Mix (Tiangen, Beijing, China). The qRT-PCR was performed with the following method: initial denaturation at 94°C for 4 min, amplification and quantification at 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s for 40 cycles with a single fluorescence measurement. The relative expression was quantified using the ΔΔCt method, and the data were normalized to GAPDH or β-actin mRNA expression. The primers used for qRT-PCR are presented in Supplementary Table 1.

Western blotting

Cell lysates were prepared using NP-40 lysis buffer (50 mM Tris-HCl, pH 7.5, 1.0% NP-40, 150 mM NaCl, 2 mM EDTA, 1 mM Na_3VO_4, 10 mM NaF, and 1 mM PMSF). Proteins in

whole cell lysates were separated by 12% SDS-PAGE and transferred to PVDF membranes (Millipore, Beijing, China). The membranes were blocked with 3% bovine serum albumin for 1 h at 37°C and incubated with anti-GFP antibody (Abmart, 1:2000), anti-β-actin, or anti-GAPDH antibody (Abmart, 1:2000) overnight at 4°C. After washing with PBST buffer (phosphate buffered saline solution with 0.1% Tween-20), the membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse antibodies (Abmart, 1:5000) for 1 h at room temperature. Immuno-reactive bands were detected by enhanced chemiluminescence (Thermo, Beijing, China).

Supplementary Table 1

Sequences of PCR primers	
GFP(f)	AGATCCGCCACAACATCGAG
GFP(r)	GTCCATGCCGAGAGTGATCC
GAPDH(f)	AAGGTGAAGGTCGGAGTCAACGG
GAPDH(r)	CCTGGAAGATGGTGATGGGATTT
β-actin (f)	CTCCTGCTTGCTGATCCACAT
β-actin (b)	AACCGCGAGAAGATGACCCAG
Sequences of siRNA against GFP	
Sense	CAAGCUGACCCUGAAGUUCTT
Antisense	GAACUUCAGGGUCAGCUUGTT
Sequences of siRNA against GAPDH	
Sense	GUGGAUAUUGUUGCCAUCATT
Antisense	UGAUGGCAACAAUAUCCACTT

Supplementary Figures

Figure S1. The schematic illustration for the fabrication of the electrodes of PSE device. (a-b) are block diagrams and the schematic top view of the fabricating step for electrodes. After the drilling and copper coating of through-holes were accomplished with the standard fabrication steps of PCB technology, we drill the PCB on the two vertices of the through-holes (position with red ring) using the bore bit with the diameter smaller than the width of through-holes to break the metal inside the through-hole into two separated parts. This drilling step is compatible with the drilling of the through-holes on PCB, and thus the whole fabrication process of PSE can be accomplished in PCB manufacturer.

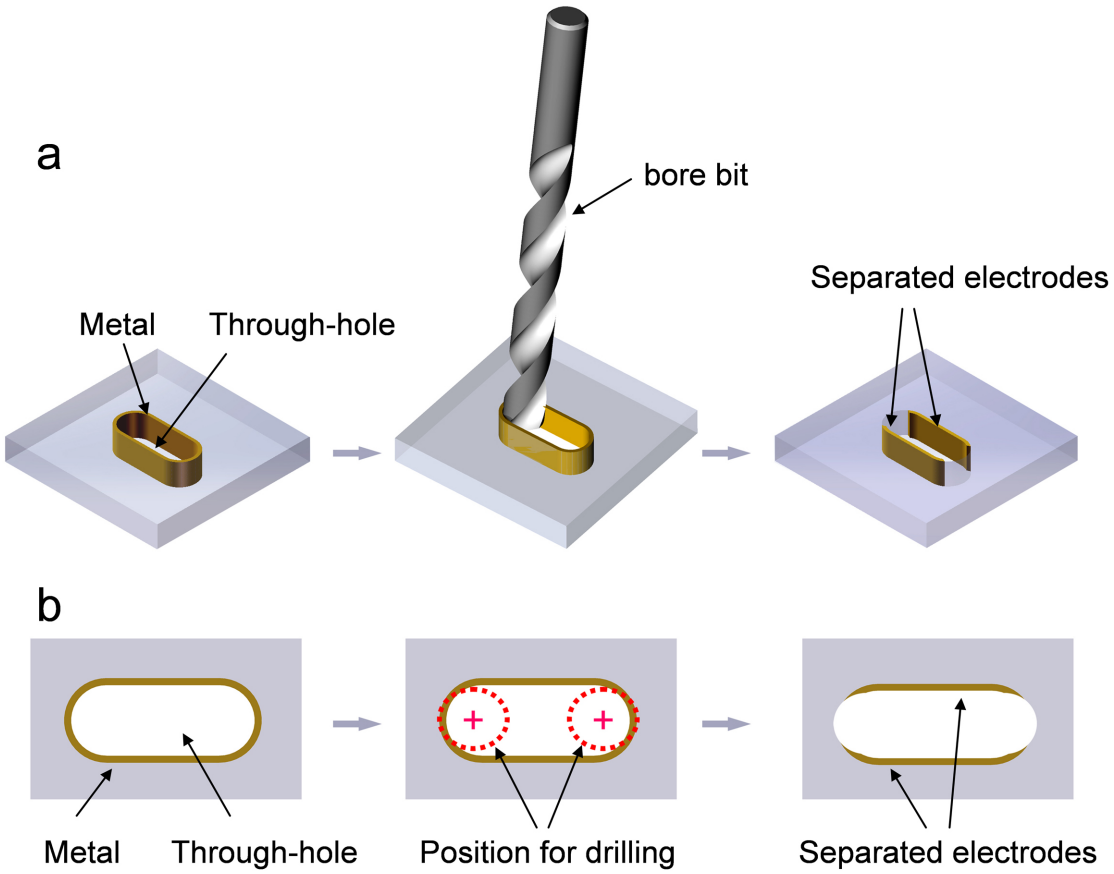

Figure S2. The influence of sample volume to transfection efficiency and cell viability under the same electroporation parameters (140 V, 0.5 ms, 1 pulse). (a) The transfection efficiency and cell viability of HeLa cells with different sample volume. (b) The cross-section of suspending drop in through-hole when the volume of sample is small, medium and large in comparison with the volume of the through-hole.

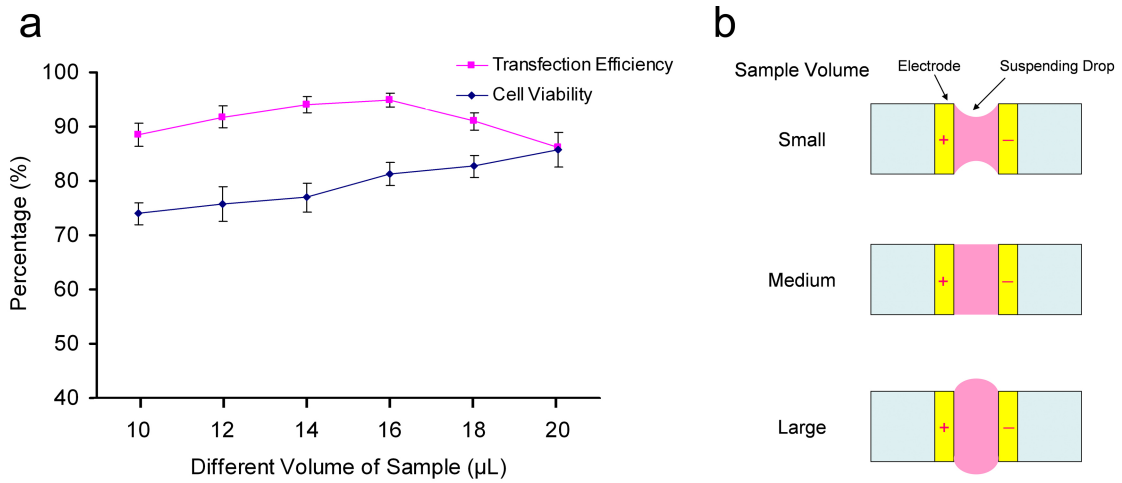

Figure S3. The transfection efficiency and cell viability of HeLa cells between different chambers (a) and different tries (b) under the same electroporation parameters (140 V, 0.5 ms, 1 pulse).

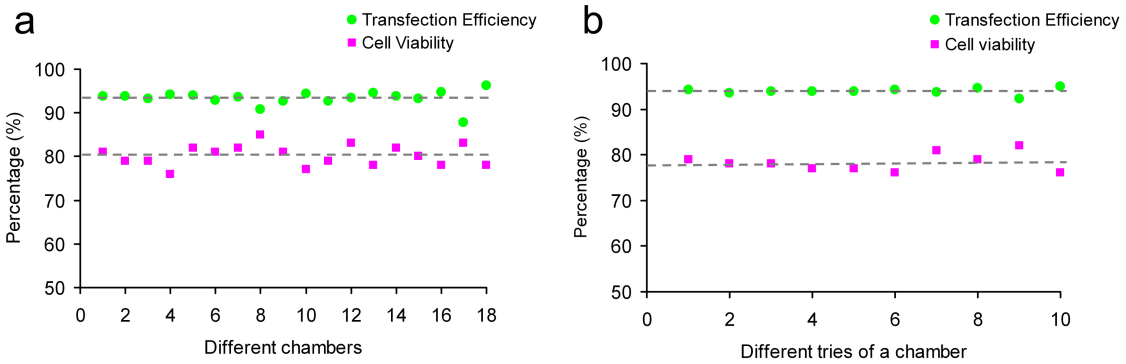

Figure S4. The transfection efficiency and cell viability of various cell types under their corresponding optimized electroporation parameters.

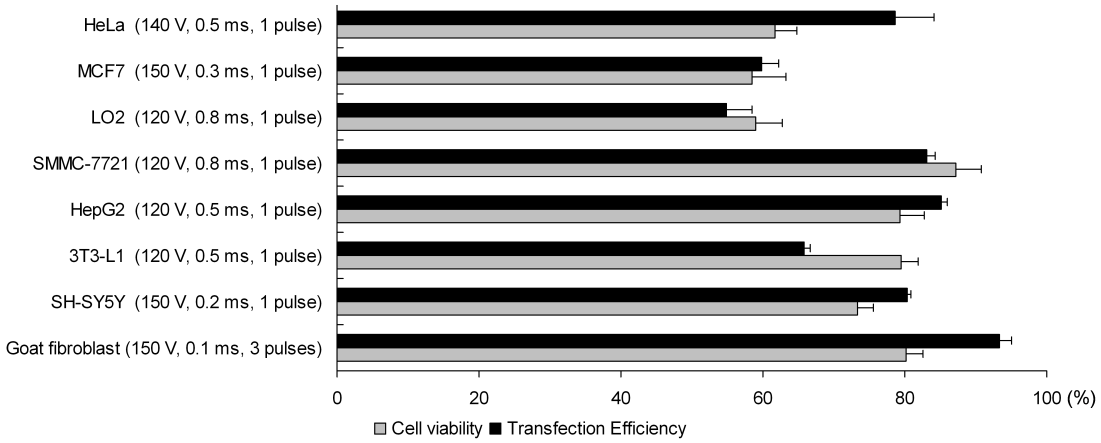

Figure S5. Bright-field images and fluorescent images of SH-SY5Y and 3T3-L1 cells tranfected with pEGFP-N1 plasmid using Lipofectamine 2000. The transfection efficiency of SH-SY5Y and 3T3-L1 cells are 21.3±1.9% and 9.9±2.1%, respectively. Bar = 100 μm.