Benchmarks

Improved Transfection Efficiency of 293 Cells by Radio Frequency Electroporation

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Electroporation, a widely used technique allowing the transfer of biological molecules into a variety of cell types (13), has been used with success for introducing plasmids and short oligonucleotides into mammalian, yeast and a variety of other cell types (1,2,9,10). A modification of the square-wave pulse technique that uses radio frequency (RF) electrical bursts is now being used effectively for transient transfections (5). RF electroporation uses bursts of sinusoidal waves, similar to alternating current (3,4) that reportedly result in better transient permeation of the cell membrane (6). This type of electroporation uses protocols and equipment already familiar to users of previously available types of electroporation. Consequently, the RF procedure can be useful to incorporate in a variety of investigations for creation of stable cell lines and in transient transfections. We describe here optimized RF electroporation parameters for the 293 human embryonic kidney cell line.

Plasmid EGFP (Clontech Laboratories, Palo Alto, CA, USA) was prepared from stock solution (4.9 µg/µL) in quantities of 10 µg by cold ethanol/sodium acetate precipitation and resuspended in 400 µL aliquots of phosphate-buffered sucrose electroporation medium: 150 mM sucrose, 27 mM Na₂HPO₄, pH 7.5 (3). The 293 cells obtained from Elliott Kieff (Harvard Medical School) were maintained in DMEM (Life Technologies, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 25 U/mL penicillin, 25 µg/mL streptomycin and 10 µg/mL gentamicin, allowed to reach 70% confluency, then harvested by trypsin/PBS and vigorous pipetting for 30 s. Trypsinization was stopped by addition of complete media supplemented with fetal bovine serum, and cell counts were determined by trypan blue staining. Approximately 5 × 10⁶ cells were washed with PBS, resus-

Table 1. Transfection Efficiency at Various Radio Frequency Electroporation Settings, Presented as the Average of Independent Transfections

	Voltage	RF Burst Duration (ms) or Standard Capacitance (µF)	Transfection Efficiency (Percent Transfected Cells)
RF	200	3 ms	40% ± 2.0%
RF	200	4 ms	39% ± 2.5%
RF	200	5 ms	33% ± 5.5%
STANDARD	200	975 µF	18% ± 3.5%
RF	210	3 ms	40% ± 2.5%
RF	210	4 ms	48% ± 6.0% ^a
RF	210	5 ms	43% ± 3.5%
STANDARD	210	975 µF	20% ± 3.5%
RF	220	3 ms	39% ± 2.5%
RF	220	4 ms	41% ± 4.0% ^a
RF	220	5 ms	40% ± 7.5%
STANDARD	220	975 µF	30% ± 2.5%
RF	230	3 ms	48% ± 2.0%
RF	230	4 ms	56% ± 16% ^a
RF	230	5 ms	45% ± 4.5%
STANDARD	230	975 µF	20% ± 0.5%
RF	240	3 ms	40% ± 8.0%
RF	240	4 ms	34% ± 4.5%
RF	240	5 ms	25% ± 11%
STANDARD	240	975 µF	24% ± 3.0%

^aElectroporation conditions where maximum transfection efficiencies were consistently obtained.

Percent transfected cells is defined as the percentage of cells present exhibiting GFP fluorescence 24 h after transfection. A manual count of three separate 100-cell fields was made for each independent trial. Standard electroporation was performed at similar voltages as those for the RF transfections at a capacitance of 975 µF with delivery of one pulse.

ended in the previously prepared aliquots of electroporation medium containing EGFP plasmid. Pellets were then pipetted vigorously to ensure uniform suspension of single cells. This suspension was then immediately transferred to a 0.4 cm electroporation cuvette (Eppendorf Scientific, Westbury, NY, USA). Cells were pulsed at various voltages (200–240 V) and burst duration combinations (3–5 ms) using the Gene Pulser II® RF Module for the Gene Pulser II Electroporation System (BioRad Laboratories, Hercules, CA, USA). The remaining electroporation parameters were held constant for all experiments: 100% modulation, 40 kHz radio frequency, 5 bursts and 1 s interval between bursts. The cell sus-

pension was immediately transferred to 10 mL of complete culture medium (as described above) and placed in a 37°C, 5% CO₂ incubator overnight. After 24 h, cells were again harvested by trypsin/PBS, washed in media and resuspended in 500 µL PBS. Transfection efficiency was detected by fluorescence microscopy using Model IX70 microscope and 1X-FLA Fluorescence Observation® attachment (both from Olympus America, Melville, NY, USA). A 405, 490, 570 nm triple filter was used for optimum excitation of GFP. Percent transfection was measured as the average of three 100-cell fields counted manually under low power and is defined as the percent of cells exhibiting GFP fluorescence.

Benchmarks

Results are presented as the average of multiple independent transfection experiments (Table 1). Equivalent transfections were carried out at each voltage setting using both radio frequency and standard pulse electroporation. Cells transfected at voltages in the range of 210–240 V with burst durations ranging from 3–5 ms had relatively high transfection efficiencies. Specifically, we obtained an average of 40%, 48% and 43% transfected cells at 210 V and 3, 4 and 5 ms burst duration, respectively, with standard deviation within 2%–5.5%. At 220 V and 3, 4 and 5 ms burst duration, respectively, we obtained an average of 39%, 41% and 40% transfected cells. Finally, at 230 V and 3, 4 and 5 ms, we obtained an average of 48%, 56% and 45% transfected cells. The best results were obtained at 210 V and 4 ms (48% average), as well as at 230 V and 4 ms burst duration (56% average). In the most effective range of settings (210 V and 4 ms, or 230 V and 4 ms) approximately 15%–25% of cells were killed by the radio frequency electroporation as measured by cell counts and trypan blue staining 24 h after shock (data not shown). Cell viability following standard pulse electroporation was consistently lower, with cell killing ranging from 20%–35% at voltages ranging from 200–240 V.

In preliminary trials, two electroporation media were used with little success. Poor transfection efficiency was achieved with D10 complete culture medium alone (DMEM supplemented with 10% fetal bovine serum; 10 μ g pEGFP/400 μ L complete medium), as well as 360 μ L D10 plus 40 μ L 150 mM sucrose (10 μ g pEGFP/400 μ L medium). In fact, these media tended to electrically overload the RF module when pulses higher than 200 V were used. Consistent results were achieved with phosphate-buffered sucrose medium, pH 7.5 (as described previously) across a large range of voltage settings (190–260 V) and burst durations (1–15 ms) without the problem of electrically overloading the device. Optimal results were obtained at settings of 230 V and 4 ms burst duration using approximately 5 million 293 cells and 10 μ g pEGFP suspended in 400 μ L phosphate-buffered sucrose medium. High per-

centages of transfected cells, generally greater than 50% efficiency (and even up to 73%) were also obtained at settings of 210 V/4 ms and 220 V/4 ms with this electroporation buffer. Additionally, it should be stressed that this resulted in much greater cell viability compared with standard electroporation.

In conclusion, this protocol for RF electroporation of 293 cells represents an improved method for transfecting 293 cells with plasmid DNA. It can potentially be applied to a variety of other cells types with some adjustment of electroporation parameters. A greater than 50% transfection efficiency was seen in the B cell line, BJAB, on multiple occasions using similar parameters (210 V/4–5 ms or 230 V/4 ms) to those used with 293 cells (data not shown). The 293 cell line is commonly used in laboratories where transient transfections are used routinely. Thus, radio frequency electroporation provides a simple and familiar method for increasing transfection efficiency with wide applicability.

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