Supplementary Information

Microfluidic Electroporation for Cellular Analysis and Delivery

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Table S1 Microfluidic electroporation for delivery of exogenous molecules into cells

Strategy	Electrodes	Cell type		Delivered materials	Optimal delivery results		Throughput	Working mode	Voltage	Ref.
					Efficiency	Viability				
Microchannel	Parallel plate Au electrodes	Huh-7 cells	S	pCMV-LacZ and pEGFP-N1 plasmids	N/A	40%-80%	0.6-2.4 $\times 10^6$ cells/min	Population	dc square pulses	42
Microchannel	Parallel plate Au electrodes	HeLa cells	S	Rhodamine B isothiocyanate-dextran 70S (R-9379)	50-53.42%	78.08-80.56%	N/A	Population	Radio-frequency-oscillating pulses	43
Microchannel	Parallel plate Au electrodes	Primary human T cells	S	CD19-specific chimeric antigen receptor mRNA	80%	50-60%	8.4 $\times 10^7$ cells/min	Population	dc square pulses	44
				EGFP mRNA	91%					
Chamber	Parallel plate Au electrodes	HEK-293 cells	A	pEGFP-C1plasmid	30%	80%	N/A	Population	dc square pulses	48
Chamber and microelectrode array	Parallel plate Au electrodes	HEK-293 cells	A	pEGFP-C1plasmid	80%	79%	5.07 $\times 10^4$ cells/chamber	Population	dc square pulses	49
		Primary hippocampal neurons	A	pEGFP-C1 and pDsRed-C1 plasmids	30%	90%	2.197 $\times 10^5$ cells/chamber			

		d2EGFP-HEK cells	A	GFP siRNA	85% fluorescent reduction compared to control	N/A	N/A			50
Microchamber	Parallel plate Au electrodes	Zebrafish embryos	S	Trypan blue dye	62%	N/A	200 embryos/chip	Single-embryo	dc square pulses	51
				MUA-quantum dots	36%	N/A				
				pEGFP-N1 plasmid	N/A	N/A				
Chamber	Coplanar parallel straight Au strips	Huh-7 and HEK-293T cells	A	pEGFP-N1 plasmid	N/A	N/A	1000 cells/chamber	Population	dc square pulses	53
Microchamber	Coplanar parallel straight Au strips	Plant protoplast	S	PI dye	N/A	60%	N/A	Single-cell	Radio-frequency pulses	54
Electroporation after DEP trapping	Coplanar curved Pt electrodes	U-937 cells	S	pEGFPLuc plasmid	2.7%	75%	3750 cells/chamber	Population	dc square bipolar pulses	60
Chamber	Interdigitated rectangular Au electrodes	Huh-7 and HEK-293T cells	A	pEGFP-N1 plasmid	N/A	N/A	N/A	Population	dc square pulses	61
		Primary HUVECs	A		36%	39%				
Electrophoresis-enhanced electroporation	Interdigitated rectangular Au electrodes	HEK-293T cells, HepG2 cells, Chang liver cells, MC3T3-E1 cells, and primary HUVECs	A	pEGFP-N1 plasmid	N/A	N/A	N/A	Population	dc square pulses	62
Electrophoresis-enhanced electroporation	Interdigitated rectangular Au electrodes	MC3T3-E1 cells	A	pEGFP-N1 plasmid	22.52%	N/A	N/A	Population	dc square pulses	63
Magneto-electroporation	Interdigitated rectangular Au	MC3T3-E1 cells	A	γ-Fe_2O_3 nanoparticles	63.05%	N/A	N/A	Population	dc square pulses	64

	electrodes			γ-Fe_2O_3 nanoparticle-pEGFP-N1 plasmid DNA complex	N/A					
Electrophoresis-enhanced electroporation	Interdigitated rectangular Au electrodes	Basal cell carcinoma cells	A	pEGFP-N1 plasmid	35.89%	N/A	N/A	Population	dc square pulses	65
				Au-DNA(T_{21}) nanoparticles	N/A	N/A				
Electrophoresis-enhanced electroporation	Interdigitated rectangular Au electrodes	NIH 3T3 cells	A	pEGFP-N1 plasmid	40.36%	N/A	N/A	Population	dc square pulses	66
Straight channel	Interdigitated rectangular Au electrodes	HeLa cells	S	Fluorescein labeled dextran	95%	85%	N/A	Population	ac sin wave voltage	71
				Bodipy labeled Phallotoxin	N/A	N/A				
				GFP siRNA	~45% gene knockdown	N/A				
Chamber	Circular Au microelectrode array	T47D cells	A	PI dye	~95%	N/A	N/A	Population	dc square pulses	81
				Bleomycin	N/A	N/A				
Chamber	Square Au microelectrode array	NIH 3T3 cells	A	PI dye	N/A	N/A	6 × 6 array	Population	dc square pulses	84
Chamber	Square Au microelectrode array and a Ag wire as the reference	NIH 3T3 cells	A	Alexa-Fluro-488 labeled siRNA	60%	80%	4 × 4 array	Population	dc square pulses	85
				pCMV-GFP plasmid	N/A	N/A				
Chamber	Square Au microelectrode array and a Ag wire as the reference	Primary rat neurons	A	PI dye	56%	82%	4 × 4 array	Population	dc square pulses	86
				Alexa-Fluro-488 labeled siRNA	N/A	N/A				
Chamber	Individually addressable circular Au	CHO-K1 cells	A	Lucifer Yellow CH dye	100%	N/A	60 electrodes per array	Single-cell	dc square pulses	87
				Trypan Blue dye	100%	~55%				

	microelectrode array and a Ag/AgCl reference electrode			Single-stranded DNA oligonucleotides	80%	N/A				
				ECFP siRNA	>40% gene knockdown					
				pcDNA3.1-EGFP plasmid	46%					
Microfluidic channel	Individually addressable circular Au microelectrode array and a Pt reference electrode	HeLa cells	A	Lucifer Yellow CH dye	N/A	N/A	N/A	Single-cell	dc square pulses	88
				pEGFP-N1 plasmid	50%	N/A				
Serpentine microfluidic channel	Vertical sidewall 3D Al electrodes	K562 cells	S	pWizGFP plasmid	15%	~90%	5×10^3 cell/min	Population	dc unipolar square or exponentially decaying pulses	90
		Mouse embryonic stem cells			20%	90%				
Targeted liposome nanoparticles enhanced electroporation	Vertical sidewall 3D Al electrodes	K562 cells	S	Nanoparticles comprising transferrin-targeted lipoplex encapsulating oligonucleotide G3139	24% increase compared to the case without using targeted liposome nanoparticles	75%	5×10^3 cell/min	Population	dc square pulses	91
Straight microfluidic channel	Vertical sidewall 3D Au electrodes	DC-3F cells	S	PI dye	95%	98%	N/A	Population	Nanosecond dc square pulses	92
Chamber	Vertical sidewall 3D Au electrodes	HeLa cells	S	PI dye	N/A	N/A	N/A	Single-cell	Radio-frequency pulses	93
				Rhodamine B-labeled dextran (D-1824, MW 10 kDa)	~55%	~55%				
				RITC-labeled dextran (R9006, MW 20 kDa)	~55%	~55%				
				FITC-labeled	~25%	<20%				

				dextran (FD-40S, MW 40 kDa)						
				FITC-labeled dextran (FD-70S, MW 70 kDa)	~25%	<20%				
Single-cell and regional electroporation	Vertical sidewall 3D Au electrodes	NIH 3T3 cells	A	EGFP plasmid	N/A	N/A	N/A	Single-cell	N/A	95
		Primary mouse neurons		EGTA (calcium chelator)						
Single-cell electroporation	Vertical sidewall 3D nickel electrodes	N2A cells	A	SYTOX Green dye	82.1%	86.7%	N/A	Single-cell	dc square pulses	96
Regional electroporation	Nanopillar Pt microelectrode array	HL-1 cells	A	Calcein dye	N/A	N/A	N/A	Single-cell	dc biphasic pulses	99
Regional electroporation	Nail-like titanium nitride microelectrode array	N2A cells and primary embryonic cardiomyocytes	A	Fluo-4 AM and PI dyes	N/A	N/A	84 electrodes per array	Single-cell	dc biphasic pulses	102
Regional electroporation	Individually addressable nail-like titanium nitride microelectrode array	NG108-15 cells	A	Fluo-4 AM and PI dyes	N/A	N/A	16,384 electrodes per array	Single-cell	dc biphasic pulses	103
Straight microchannel	Pt wires	SK-OV-3 cells	A	PI dye and pEGFP-N1 plasmid	N/A	N/A	N/A	Population	dc exponentially decaying pulses	108
Straight microchannel	Pt wires	HEK-293 cells	A	pEGFP-N1 plasmid	80%	90%	N/A	Population	dc exponentially decaying pulses	109
		CHO cells	A	pEGFP-N1 plasmid	>90%	>90%				

Alternating wide and narrow microchannels	Pt wires	CHO-K1 cells	S	SYTOX Green dye	56%	50%	3.267×10^3 cells/min	Single-cell	Constant dc voltage	116
Alternating wide and narrow microchannels	Pt wires	CHO-K1 cells	S	SYTOX Green dye	80%	N/A	N/A	Population	Constant dc voltage	121
				pEGFP-C1 plasmid	25%	90%	3.24×10^5 cells/min			
Alternating wide and narrow microchannels	Pt wires	CHO-K1 cells	S	pEGFP-C1 plasmid	75%	65%	3.74×10^6 cells/min	Population	Constant dc voltage	129
Alternating wide and narrow microchannels	Pt wires	CHO-K1 cells	S	pEGFP-C1 plasmid	71%	58%	2.8×10^4 cells/min	Population	Low-frequency ac voltage	132
Alternating wide and narrow microchannels	Stainless steel needles	Murine C3H10T1/2 mesenchymal stem cells	S	pEGFP-N1 plasmid	N/A	N/A	5.1×10^3 cells/min	Single-cell	ac square pulses	134
Microhole constriction	Thin film platinum electrodes and a silicon plate electrode	Human prostate adenocarcinoma ND-1 cells	S	YOYO-1 dye and pEGFP plasmid	N/A	N/A	N/A	Single-cell	dc square pulses	141
Microorifice constriction array	Thin foil electrodes	U-937 cells	S	YO-PRO-1 dye, maleic acid, succinic acid and glutamic acid	100%	100%	10 × 10 array	Single-cell	dc square pulses	143
		Guinea pig ventricular myocytes	S	YO-PRO-1 dye	100%	100%				
Microorifice constriction array	Parallel metal mask and plate electrodes	HeLa cells	S	CFP-importinβ protein	N/A	N/A	200 cells/array	Single-cell	dc square pulses	144

Subcellular-sized microchannel constriction		HeLa cells	S	Calcein and Oregon Green Dextran 514	N/A	N/A	15 cells/array	Single-cell	dc square pulses	149
Subcellular-sized microchannel constriction	Pt film electrodes	C2C12 cells	S	pEGFP-N1 plasmid	75%	N/A	9 cells/array	Single-cell	dc square pulses	150
				Plasmid encoding EGFP-ERK1 fusion protein	65%	N/A				
		Human mesenchymal stem cells	S	pEGFP-N1 plasmid	70%	N/A				
				Plasmid encoding EGFP-ERK1 fusion protein	90%	N/A				
Membrane with nanosized pores	Ag wires	NIH 3T3 cells	S	Plasmid gWiz GFP and SEAP (secreted alkaline phosphatase)	N/A	>90%	10^4 cells per membrane	Population	dc bipolar square pulses	151
Membrane with uniform micronozzle array	Ag wires	Mouse embryonic stem cells	S	Plasmid gWiz SEAP	70 total SEAP activity mU/10,000 intitial input cells	75%	10^4 cells per membrane	Population	dc exponential decay pulses	152
				pmaxGFP plasmid	100%	100%	10^2 cells per membrane			
Membrane with nanosized pores	Parallel plate Pt electrodes	HeLa cells	A	Lucifer Yellow CH dye	N/A	80%	N/A	Population	dc square pulses	154
				pDsRed-C1 plasmid	10%	N/A				
Membrane with protruded alumina nanostraws	An ITO glass and a Pt wire	CHO-K1 cells	A	PI dye	>95%	>95%	N/A	Population	dc square pulses	155
				pmCherry-C1 plasmid encoding RFP	81%	98%				
				pmCherry-C1 plasmid and pEGFP-C1 sequential	74%	N/A				

				delivery						
		HEK-293 cells	A	pmCherry-C1 plasmid	67%	N/A				
Nanochannel	Palladium wires	K562 cells	S	PI dye and Mcl-1 siRNA	N/A	N/A	N/A	Single-cell	dc square pulses	153
		Jurkat cells		Cy3 labeled 18-mer oligodeoxynucleotide (G3139), GAPDH molecular beacon, COOH group conjugated quantum dots, and pMAX-GFP and pCAG-GFP plasmids						
Nanopore	Ag/AgCl electrodes	MDA-MB-231 cells	S	YOYO-1 intercalated dsDNA	N/A	N/A	N/A	Single-cell	dc bias	156
Vortex-assisted electroporation	Pt wires	CHO-K1 cells	S	pEGFP-C1 plasmid	30%	80%	3×10^5 cells/min	Population	Constant dc voltage	26
Hydrodynamic focusing-enhanced electroporation	Ag wires	Yeast cells	S	Fluorescein dye	70%	85%	1.67×10^4 cells/min	Population	Constant dc voltage	157
Hydrodynamic focusing-enhanced electroporation	Parallel Au coplanar electrodes	HEK-293 cells	S	pEGFP-C3 plasmid	90%	60%	2×10^5 cells/min	Population	dc square pulses	158
		HeLa cells			75%	70%				
		Neuro-2a cells			80%	65%				
		C2C 12 cells			85%	75%				
		PC12 cells			70%	55%				
		HEK-293 cells		Lamin A/C siRNA	92% gene knockdown	N/A				
		C2C12 cells			68% gene knockdown	N/A				

		PC12 cells			20% gene knockdown	N/A				
Uniform flow velocity profile	Stainless steel meshes	Primary human dendritic cells	S	GFP mRNA	74%±10%	90%±5%	N/A	Population	dc unipolar square pulses	159
Microdroplet	Parallel Au coplanar electrodes	Yeast cells	S	Fluorescein dye	N/A	N/A	7.2×10^3 cells/min	Single-cell and population	ac square pulses	160
Microdroplet	Parallel Au coplanar electrodes	CHO-K1 cells	S	pEGFP-C1 plasmid	11%	14%-68%	3×10^4 cells/min	Single-cell and population	Constant dc voltage	57
Microdroplet	5 pairs of parallel Au coplanar electrodes	Green microalgae *C. reinhardtii* wall-less mutant strain CC-400 (*cw15 mt*$^+$) cells	S	DNA fragments of pHyg3 with *aph7"* gene conferring hygromycin B resistance	8.14×10^{-4}	81%	4.74×10^3 cells/min	Single-cell and population	Constant dc voltage	161
		Green microalgae *C. reinhardtii* walled strain CC-123 (wild type *mt*$^+$) cells			1.51×10^{-5}	N/A				
Electroporation of cells encapsulated within microdroplets in oil phase	Removable electrodes in plastic cuvette	HeLa cells	S	PI dye	5%	N/A	N/A	Single-cell and population	Radio frequency-oscillating electric pulses	162
Microwell array	Stainless steel electrodes	HEK-293T cells	A	PI dye and Alexa-Fluro-488 labeled siRNA	> 99%	N/A	484 microwells	Population	dc square pulses	163
				GFP plasmid	N/A	N/A				
		Primary mouse macrophages	A	PI dye	91%	93%				

Microwell array	Parallel plate ITO and Al electrodes	HeLa and HEK-293 cells	A	RPS27a, GFP and non-targeting siRNA	N/A	N/A	9 × 9 array	Population	dc square pulses	164
Multi-well	Annular interdigitated Au electrodes	HEK-293 cells	S	pEGFP-C3 plasmid	80%	80%	N/A	Population	dc square pulses	77
		HEK-293 cells	A		90%	80%				
		HeLa cells	A		80%	80%				
		HepG2 cells	A		80%	70%				
		HL-60 cells	S		60%	70%				
		MDCK cells	S		70%	80%				
		Primary HUVECs	S		60%	70%				
		PC12 cells	S		55%	70%				
		Neuro-2A cells	S		75%	80%				
		Rat primary DRG neurons	S		30%	60%				
		HEK-293 cells	A	Lamin A/C siRNA	68% gene knockdown	N/A				
		MDCK cells	A	ANXA2 siRNA	90% gene knockdown	N/A				
		Primary HUVECs	A	Lamin A/C siRNA	84% gene knockdown	N/A				
Microwell array	An individually addressable array of circular electrodes and an ITO electrode	HeLa cells	A	PI dye, pEGFP-N1 plasmid and pDsRed1-1 plasmid	N/A	N/A	N/A	Population	dc square pulses	83
Microwell array	Parallel plate Au and ITO electrodes	Jurkat cells	S	PI dye	100%	N/A	8 × 11 array	Single-cell	dc square pulses	165
Elastomeric microvalves	Pt wires	CHO-K1 cells	A	SYTOX Green dye	51%	78%	N/A	Population	Constant dc voltage	120
			S	SYTOX Green dye	N/A	N/A				

Polyelectrolytic salt bridges	Ag wires	K562 cells	S	pEGFP-C2 plasmid	60%	80%	10^5 cells/min	Population	Constant dc voltage	168
Light-induced electroporation	virtual electrodes created by patterned light	HeLa cells		PI dye	N/A	N/A	N/A	Single-cell	ac bias	169
Electrode-shape-patterned delivery in chamber	A ‘M” shaped Pt planar electrode and a metal wire	Zebrafish embryos	S	Trypan Blue dye	60%	80%	N/A	Single-embryo	dc square pulses	175
				pCS2+EGFP plasmid	38%	91.3%				
				EGFP mRNA	50%	89%				

S: Suspended; A: Adherent; EGFP: enhanced green fluorescence protein; ECFP: enhanced cyan fluorescence protein; RFP: red fluorescence protein; PI: Propidium iodide. **Note**: See references in the main text.