

Supplementary information for

“Efficient gene transfection by electroporation - *in vitro* and *in silico* study of pulse parameters”

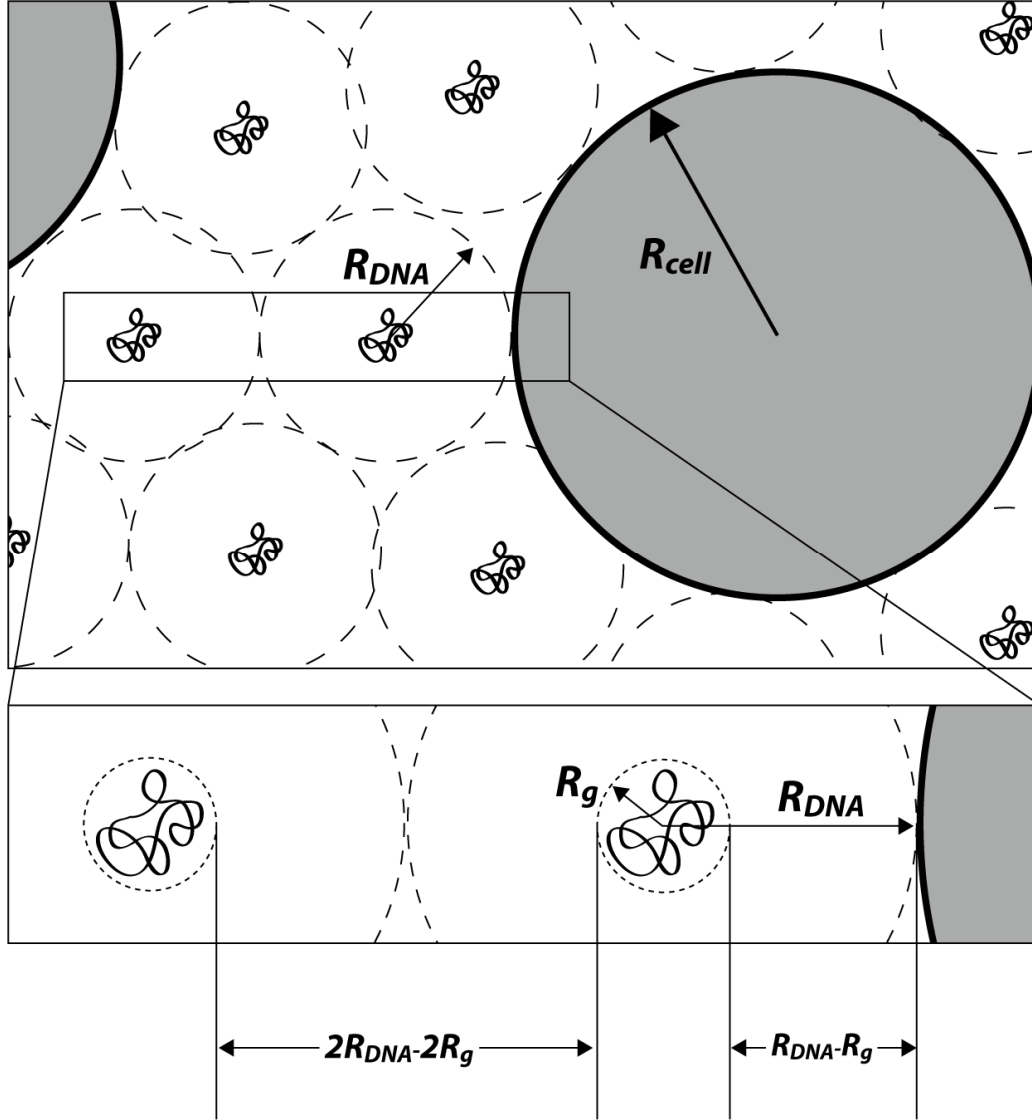
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Various estimates of DNA cell membrane distance as a function of concentration

Supplementary Table S1: Various estimates of the distance between a pDNA molecule and the nearest cell membrane (R_{DNA}) based on equation (3) in the main text. For interpretation of B_L , B_R and R_{DNA} , refer to Figure 1 in the main text.

	B_L, B_R, R_{DNA} (nm)	
pDNA concentration ($\mu\text{g/ml}$)	C2C12	1306
40	312.79	312.98
80	248.26	248.42
100	230.46	230.61
250	169.81	169.91
500	134.78	134.86

In the main text, the model determining the probability that a DNA molecule will come in contact with the cell membrane require a definition of the boundaries within which a DNA molecule is allowed to move. As a first estimate (based on **Figure 1** of main text), the left boundary (B_L) and the right boundary (B_R) were considered as R_{DNA} . However, based on **Supplementary Figure S1**, other estimates of B_L and B_R that can be considered such as: R_{DNA} , $R_{DNA} - R_g$ and $2R_{DNA} - 2R_g$; where R_g is the radius of gyration of the DNA molecule.

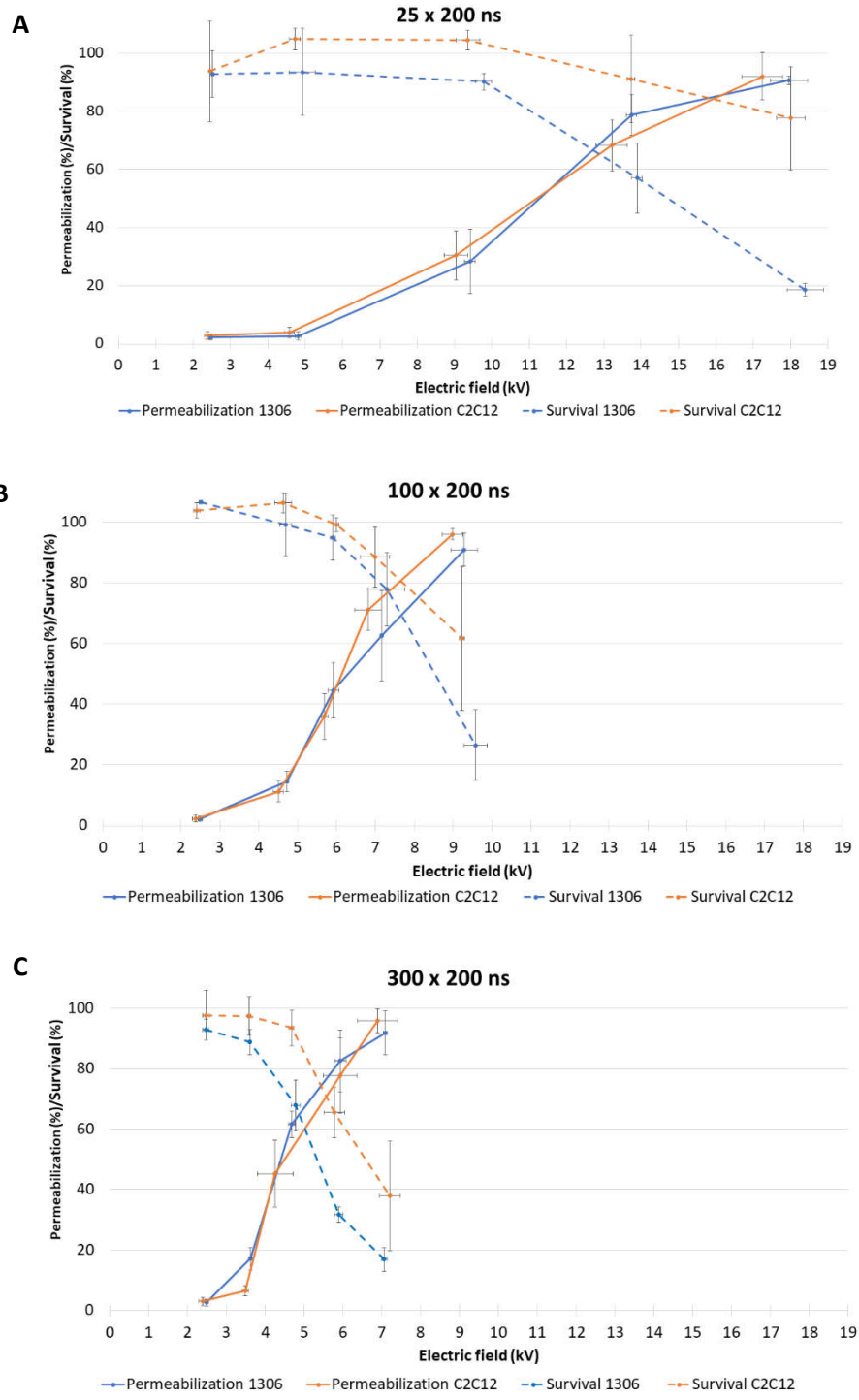


Supplementary Figure S1: Schematic of distribution of pDNA molecules and cells in suspension. R_{cell} represents the radius of the cell, R_{DNA} represents the radius of the (nominal/free) spherical space available to the pDNA molecule and R_g represents the radius of gyration of the pDNA molecule. Subset figure shows various estimates that can be considered as the distance between a DNA molecule and the nearest cell membrane and the distance between 2 DNA molecules.

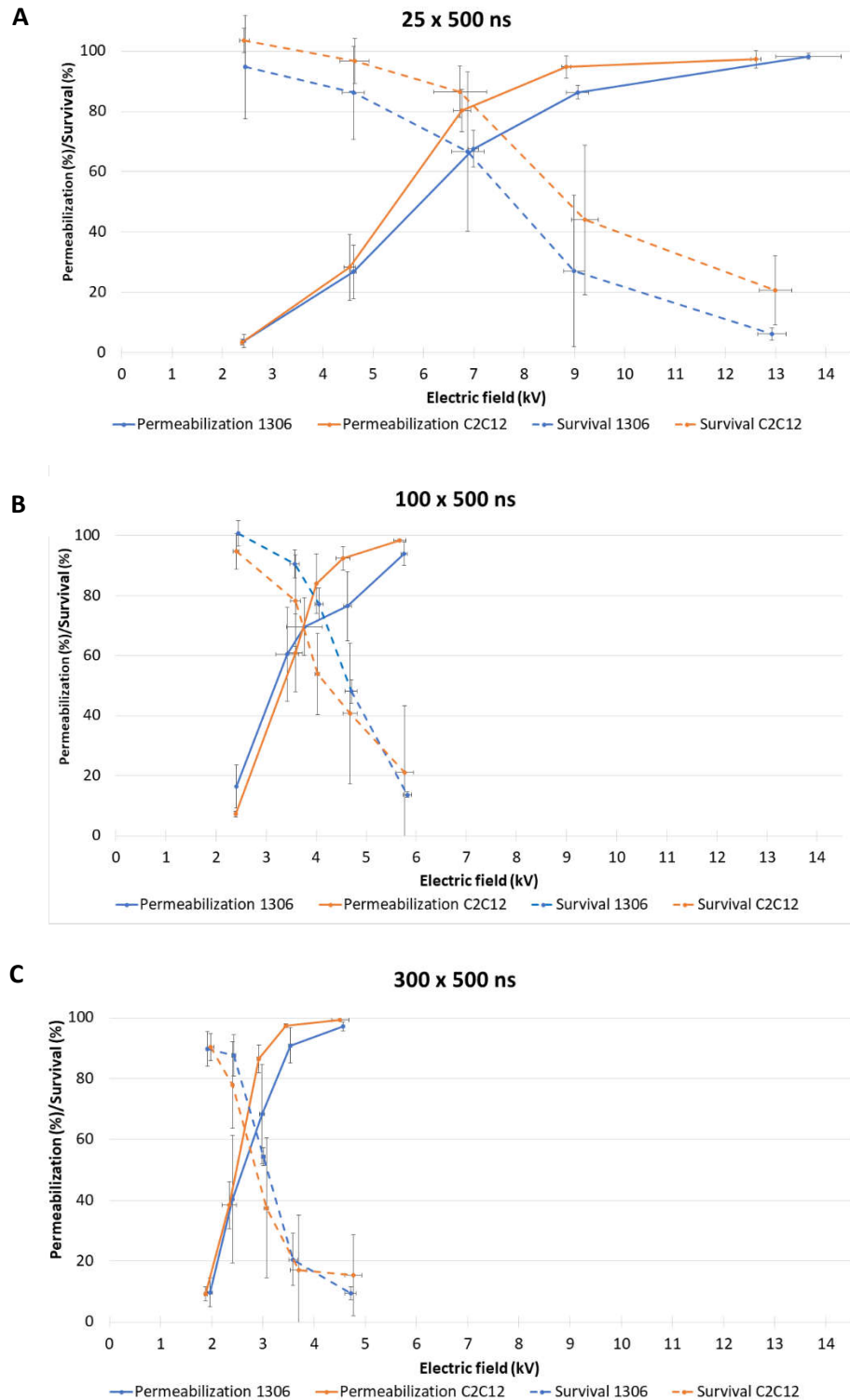
R_g for a stained (YOYO-1) circular 5.9 kbp pDNA molecule is around 131 nm [1]. Since pDNA molecules used in the current study are unstained, the unstained R_g can be obtained as $\frac{R_{g,unstained}}{R_{g,stained}} = \left(\frac{L_{unstained}}{L_{stained}}\right)^{0.58}$ [1,2]. Since YOYO-1 staining increases the contour length of the pDNA molecule by 1.35 times [1], unstained R_g for 5.9 kbp is estimated to be around 110 nm. To calculate the R_g for 4.7 kbp pDNA molecule, the same scaling relation can be used: $\frac{R_{g,4700\text{ bp}}}{R_{g,5900\text{ bp}}} = \left(\frac{L_{4700\text{ bp}}}{L_{5900\text{ bp}}}\right)^{0.58} = \left(\frac{4700}{5900}\right)^{0.58}$. Therefore, R_g for 4700 bp is estimated to be around 96 nm. Various estimates of distance between a DNA molecule and the cell membrane and between DNA molecules are given in **Supplementary Table S2**.

Supplementary Table S2: Various estimates of the distance between a DNA molecule and the nearest cell membrane and between two pDNA molecules.

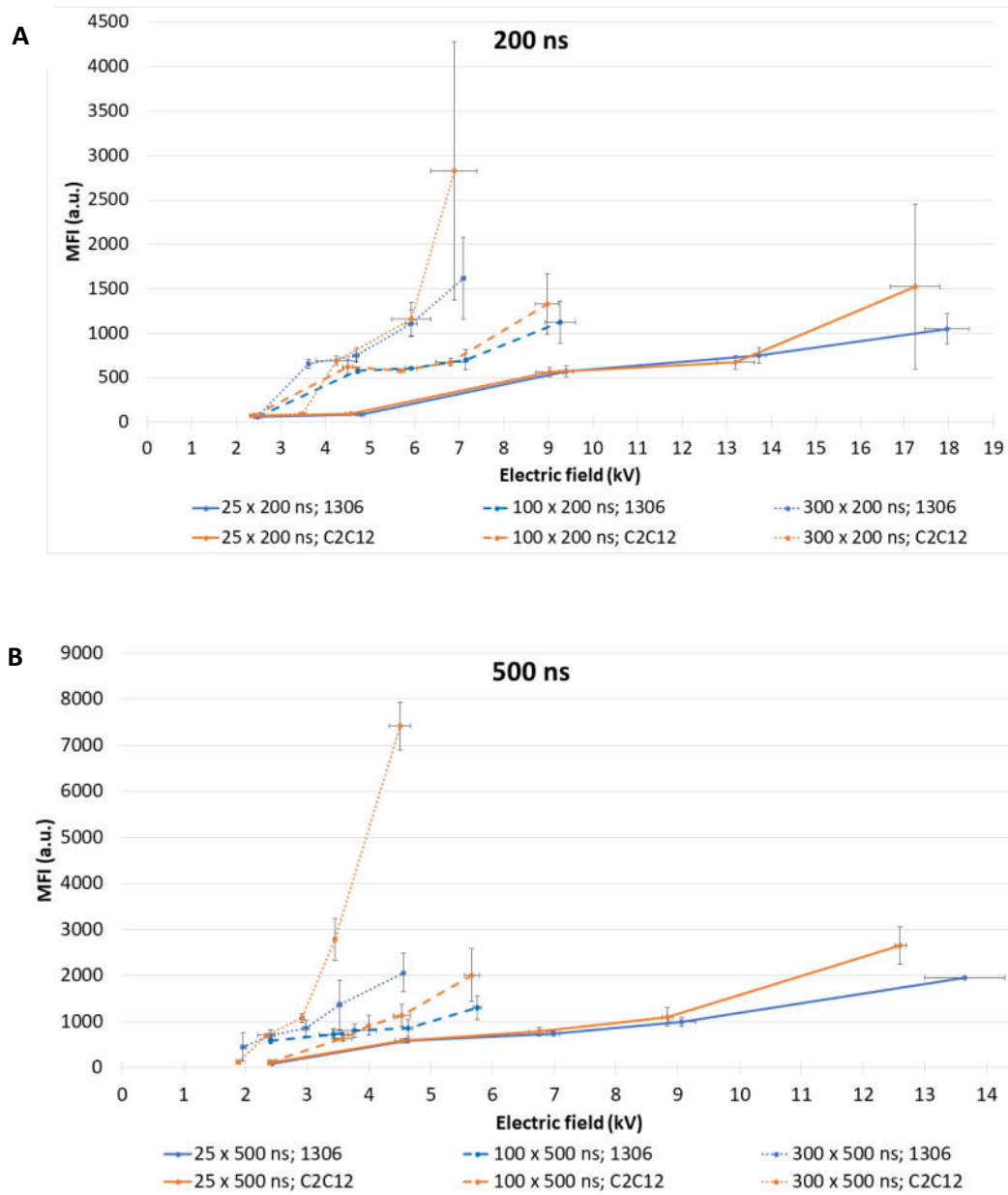
	R_{DNA} (nm)		$2 * R_{DNA} - 2 * R_g$ (nm)		$R_{DNA} - R_g$ (nm)	
Concentration (µg/ml)	C2C12	1306	C2C12	1306	C2C12	1306
40	312.79	312.98	433.57	433.97	216.79	216.98
80	248.26	248.42	304.52	304.83	152.26	152.42
100	230.46	230.61	268.92	269.22	134.46	134.61
250	169.81	169.91	147.61	147.83	73.81	73.91
500	134.78	134.86	77.55	77.72	38.78	38.86



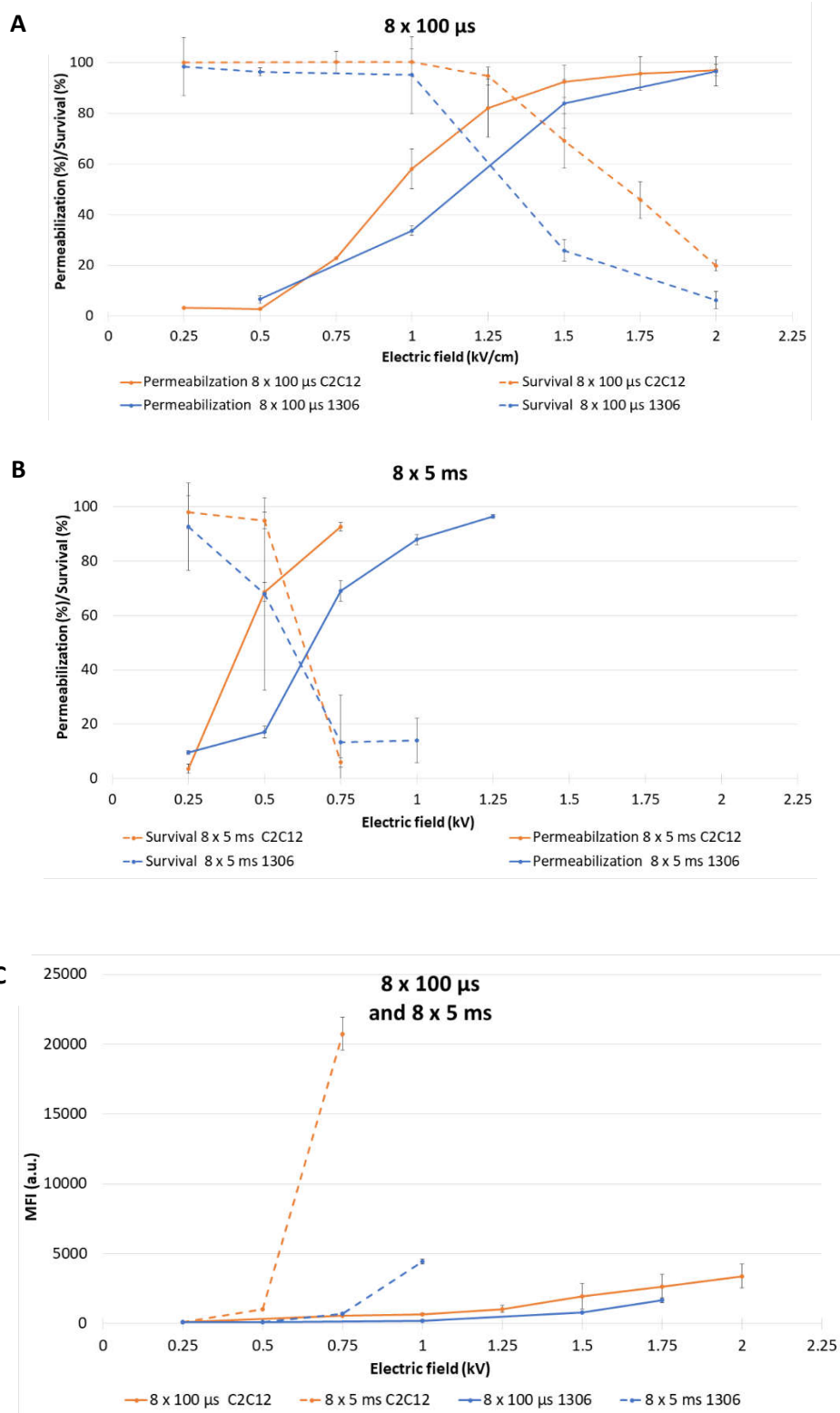
Supplementary figure S2: Comparison of permeabilization and survival curves of C2C12 myoblast and 1306 fibroblasts with 200 ns pulses and 10 Hz pulse repetition rate. **A)** 25 x 200 ns pulses, **B)** 100 x 200 ns pulses, **C)** 300 x 200 ns pulses. Results are presented as average of 3 repetitions. Bars represent standard deviation.



Supplementary figure S3: Comparison of permeabilization and survival curves of C2C12 myoblast and 1306 fibroblasts with 500 ns pulses and 10 Hz pulse repetition rate. **A)** 25 x 500 ns pulses, **B)** 100 x 500 ns pulses, **C)** 300 x 500 ns pulses. Results are presented as average of 3 repetitions. Bars represent standard deviation.

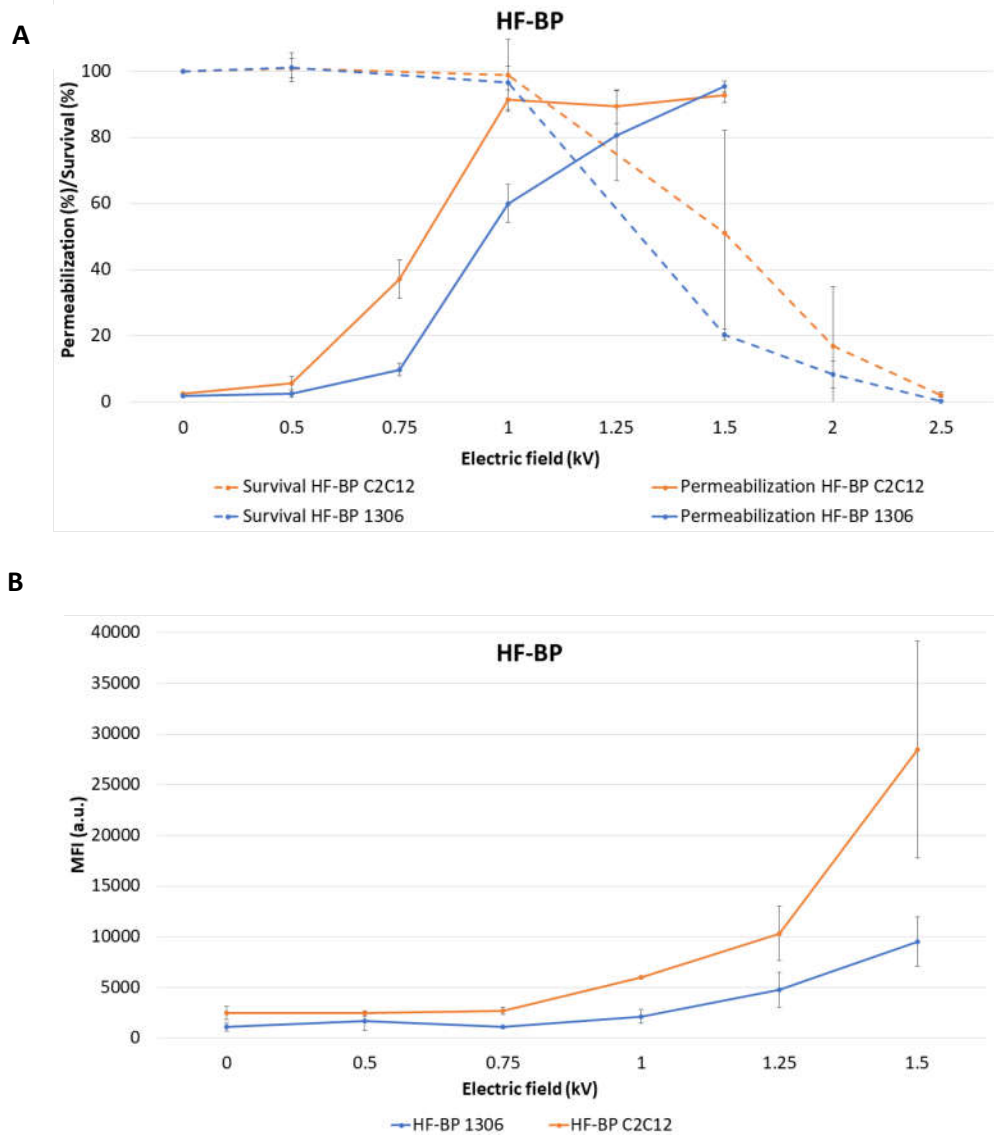


Supplementary figure S4: Comparison of median fluorescence intensity (MFI) of permeabilized C2C12 myoblast and 1306 fibroblasts with **A)** 200 ns pulses and 10 Hz pulse repetition rate and **B)** 500 ns pulses. Results are presented as average of 3 repetitions. Bars represent standard deviation. Please note that the scale for MFI is different between **A** in **B**.



Supplementary figure S5: Comparison of permeabilization and survival curves of C2C12 myoblast and 1306 fibroblasts with **A)** 8 x 100 μ s, 1 Hz pulses and **B)** 8 x 5 ms, 1 Hz pulses. **C)** Median fluorescence

intensity (MFI) of permeabilized cells. Results are presented as average of 3 repetitions. Bars represent standard deviation.



Supplementary figure S6: Comparison of permeabilization and survival curves of C2C12 myoblast and 1306 fibroblasts with **A)** HF-BP pulse protocol and **B)** Median fluorescence intensity (MFI) of permeabilized cells. Results are presented as average of 3 repetitions. Bars represent standard deviation.

Supplementary table S3: Nanosecond, HF-BP, micro and millisecond pulse protocols with which the best overall GET with 500 µg/ml of pEGFP-N1 was achieved in C2C12 myoblasts.

C2C12 myoblasts, 500 µg/ml of pEGFP-N1			
Pulse protocol	GET ± SD (%)	Survival ± SD (%)	Overall GET ± SD (%)
25 x 200 ns, 10 Hz, 15.8 kV/cm	17.71 ± 0.52	66.85 ± 16.09	11.89 ± 4.42
100 x 200 ns, 10 Hz, 6.9 kV/cm	12.28 ± 4.64	75.56 ± 4.82	9.5 ± 4.82
300 x 200 ns, 10 Hz, 6.3 kV/cm	10.35 ± 4.43	33.35 ± 18.01	2.66 ± 0.93
25 x 500 ns, 10 Hz, 6.9 kV/cm	18.20 ± 1.05	57.95 ± 11.83	10.43 ± 1.99
100 x 500 ns, 10 Hz, 4.1 kV/cm	16.98 ± 5.32	63.76 ± 20.06	11.73 ± 7.10
300 x 500 ns, 10 Hz, 2.4 kV/cm	6.36 ± 3.50	64.92 ± 12.60	4.33 ± 3.01
2-2-2-2, 32 pulses, 100 bursts, 1 Hz, 1.25 kV/cm	30.06 ± 1.33	61.45 ± 6.48	18.42 ± 1.17
8 x 100 µs, 1Hz, 1.25 kV/cm	46.02 ± 20.03	71.25 ± 19.31	30.44 ± 5.26
8 x 5 ms, 1Hz, 0.4 kV/cm	39.83 ± 7.16	89.35 ± 13.40	35.39 ± 6.94

Supplementary table S4: Nanosecond, HF-BP, micro and millisecond pulse protocols with which the best overall GET with 500 µg/ml of pEGFP-N1 was achieved in 1306 fibroblasts.

1306 fibroblasts, 500 µg/ml of pEGFP-N1			
Pulse protocol	GET ± SD (%)	Survival ± SD (%)	Overall GET ± SD (%)
25 x 200 ns, 10 Hz, 12.8 kV/cm	35.89 ± 12.33	77.15 ± 7.66	27.45 ± 8.29
100 x 200 ns, 10 Hz, 6.9 kV/cm	29.24 ± 13.70	91.18 ± 12.78	26.49 ± 11.52
300 x 200 ns, 10 Hz, 4.2 kV/cm	25.35 ± 9.87	83.29 ± 6.17	21.28 ± 10.26
25 x 500 ns, 10 Hz, 6.5 kV/cm	42.30 ± 9.18	58.82 ± 15.27	24.89 ± 7.65
100 x 500 ns, 10 Hz, 3.5 kV/cm	30.87 ± 23.40	63.17 ± 17.10	19.16 ± 13.88
300 x 500 ns, 10 Hz, 2.7 kV/cm	24.25 ± 5.66	54.20 ± 16.56	13.30 ± 5.83
2-2-2-2, 32 pulses, 100 bursts, 1 Hz, 1 kV/cm	29.02 ± 4.80	73.87 ± 2.54	21.47 ± 2.49
8 x 100 µs, 1Hz, 1.25 kV/cm	46.38 ± 4.43	68.39 ± 2.63	31.70 ± 2.98
8 x 5 ms, 1Hz, 0.6 kV/cm	25.55 ± 6.16	53.51 ± 17.30	14.37 ± 7.18

Temperature measurement during pulse delivery

For each pulse protocol tested, the temperature increase above the baseline of the cell sample during pulse delivery is given in **Supplementary table S5**. All experiments were performed at room temperature between 24.6 °C and 26.7 °C. The highest increase of 5.4 °C was measured when the 8 x 5 ms pulse protocol was applied to the 1306 fibroblasts sample.

Supplementary table S5. Maximum temperature increases of the cell sample during pulse delivery. For each pulse protocol, the results of two measurements are given.

	200 ns /°C	500 ns /°C	HF-BP/°C	8 x 100 μ s /°C	8 x 5 ms /°C
C2C12 myoblasts	1.4	0.6	5.2	1.0	4.9
	2.0	0.8	5.2	1.0	3.4
1306 fibroblasts	1.3	0.8	2.7	0.9	5.4
	1.4	0.7	3.2	0.8	5.1

References

1. Robertson, R.M.; Smith, D.E. Self-Diffusion of Entangled Linear and Circular DNA Molecules: Dependence on Length and Concentration. *Macromolecules* **2007**, *40*, 3373–3377, doi:10.1021/ma070051h.
2. Robertson, R.M.; Laib, S.; Smith, D.E. Diffusion of Isolated DNA Molecules: Dependence on Length and Topology. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 7310–7314, doi:10.1073/pnas.0601903103.