Electroporation

Maximizing large-payload delivery efficiency and cell recovery in iPSCs via the Neon NxT Electroporation System

Introduction

Efficient genetic modification of induced pluripotent stem cells (iPSCs) is a critical enabling step for cell therapy development, disease modeling, and regenerative medicine. Electroporation is widely used for iPSC transfection because it supports nonviral delivery of diverse cargos, including plasmid DNA, ribonucleoprotein complexes, and donor templates, while minimizing risks associated with viral vectors. However, delivery of large genetic constructs (≥10 kb), such as chimeric antigen receptor (CAR) cassettes or complex regulatory elements, remains technically challenging due to reduced transfection efficiency, increased cytotoxicity, and compromised cell recovery. iPSCs are particularly sensitive to membrane perturbation and DNA-induced stress, making careful optimization of electroporation parameters important. Factors including voltage, pulse width, pulse number, buffer composition, cell density, and payload concentration collectively influence membrane permeability, nuclear delivery, and post-electroporation survival. Inadequate optimization can lead to low transfection efficiency, poor clonal recovery, and loss of pluripotency or genomic integrity.

Therefore, systematic optimization of electroporation conditions, including evaluation of post-transfection recovery strategies such as the choice of PSC medium, supporting matrix proteins, or recovery supplements (e.g., Gibco™ RevitaCell™ Supplement or Gibco™ CultureCEPT™ Supplement), is required to achieve robust, reproducible transfection of large constructs in iPSCs while preserving viability and stem cell quality relevant for genome engineering applications.

Materials and methods

The workflow and experimental plan used in this study are summarized in Figure 1. Details of the equipment, media, and reagents used in experimental setups are provided in Table 1. Results are presented according to the experimental plan.

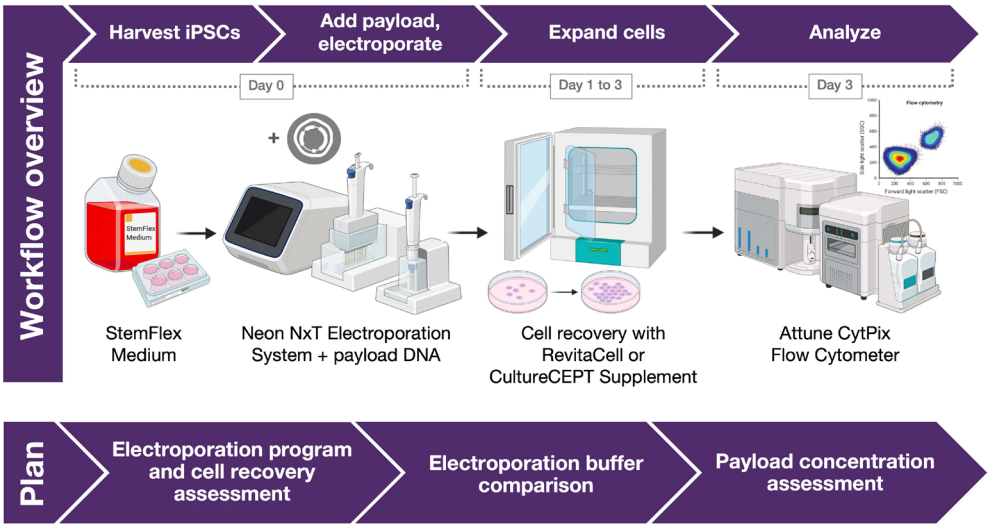

Figure 1. Workflow overview and experimental plan. The top panel shows the overall workflow for transfection of human iPSCs via electroporation, followed by cell recovery, expansion, and analysis. The bottom panel describes the experimental plan to compare and optimize the following key variables: electroporation program, post-electroporation cell recovery supplement (RevitaCell vs. CultureCEPT Supplement), electroporation buffer (R vs. T buffer), and payload concentration.

Table 1. Materials and methods used in this study.

Step	Description
Pre-electroporation cell culture	Human fibroblast–derived iPSCs were grown in Gibco™ StemFlex™ Medium in 6-well tissue culture–treated plates coated with Gibco™ rhLaminin-521. In preparation for electroporation, cells at 70–80% confluency were dissociated with Gibco™ Versene™ Solution. Viability and cell counts were measured using a NucleoCounter™ NC-3000™ cytometer (ChemoMetec). iPSC viability before electroporation should be >80%.
Electroporation	Instrument: Invitrogen™ Neon™ NxT Electroporation System with 1-Channel and 8-Channel Pipettes. Cell density: 1 x 10^7 cells/mL. Buffer: Invitrogen™ Neon™ NxT Resuspension R Buffer or T Buffer were used as specified in the relevant results section. Payload: 50 µg/mL 11 kb green fluorescent protein (GFP) plasmid, unless specified otherwise in the relevant results section. Program: variable (refer to results section). Consumables: Invitrogen™ Neon™ NxT Tips (10 µL), 1-Channel or 8-Channel Tubes, and Electrolytic E10 Buffer.
Post-electroporation cell expansion	Post-electroporation cells were plated in 12-well tissue culture plates coated with rhLaminin-521 at ~1 x 10^5 cells/well. IPSCs were cultured in StemFlex Medium with RevitaCell Supplement (contains ROCK inhibitor) or CultureCEPT Supplement overnight to boost post-transfection cell recovery. On the next day, the medium was replaced with fresh StemFlex Medium without recovery supplement, and cells were kept in culture for two more days prior to analysis.
Characterization and analysis	Transfected and parental iPSC cultures were assessed for cell recovery (total viable cells count), viability, pluripotency, and transfection efficiency (GFP+ out of viable singlets) 72 hours post-electroporation using the Invitrogen™ Attune™ CytPix™ Flow Cytometer. Pluripotency was verified using Invitrogen™ eBioscience™ TRA-1-81 Monoclonal Antibody (APC conjugate), and viability was assessed using Invitrogen™ SYTOX™ Blue Dead Cell Stain. The flow cytometry gating strategy is shown in Figure 2.

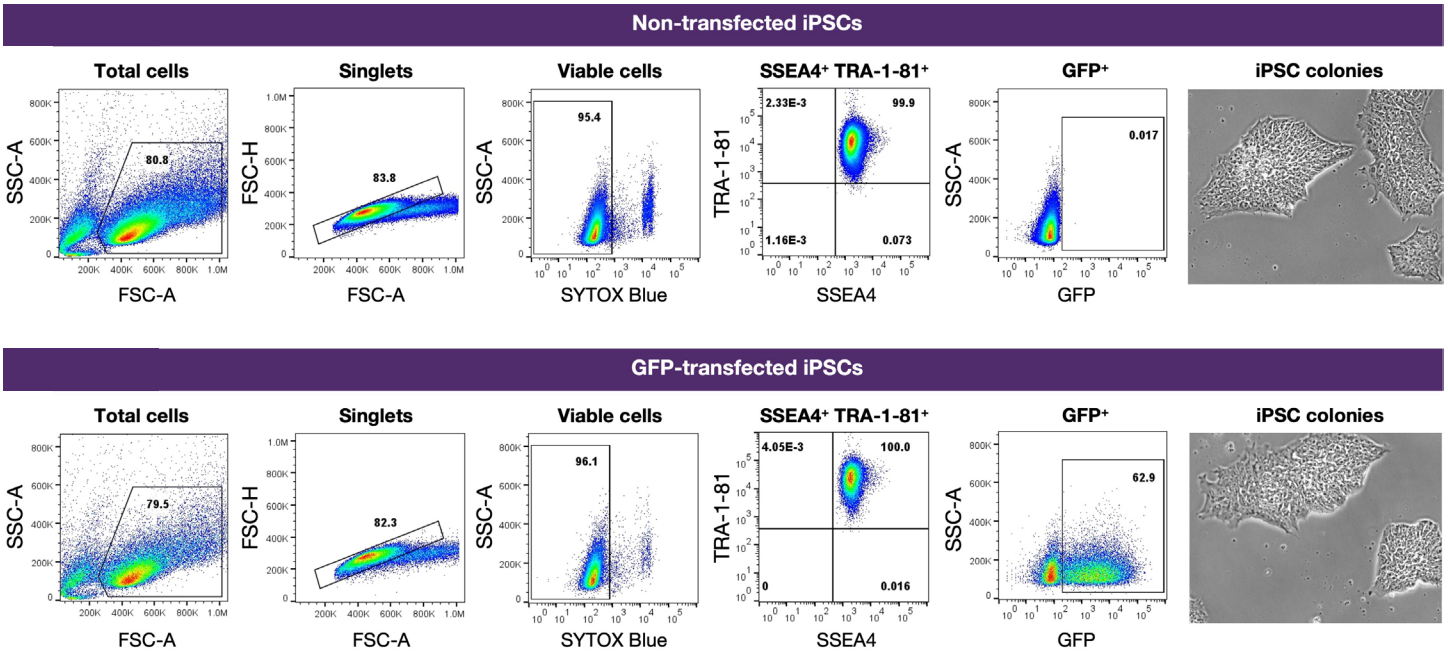

Figure 2. Representative flow cytometry analysis of transfected and non-transfected iPSCs stained with eBioscience TRA-1-81 and SSEA4 antibodies and SYTOX Blue Dead Cell Stain. Transfected and non-transfected iPSCs were recovered in StemFlex Medium supplemented with RevitaCell or CultureCEPT Supplement for 24 hr and harvested for flow cytometric analysis on day 3 post-electroporation.

Results

Electroporation program and cell recovery assessment

Criteria	Conditions
Electroporation buffer	R
Programs	Variable (see Table 2)
Cells	1 x 10^7 cells/mL
Payload	11 kb GFP plasmid
Payload amount	50 µg/mL
Post-electroporation recovery supplement	RevitaCell vs. CultureCEPT Supplement

Screening of 48 electroporation programs revealed substantial variability in transfection performance, as measured by percentage of GFP expression and total viable cell yield obtained per one electroporation reaction 72 hr after transfection (Figure 3). A clear inverse relationship was observed between transfection efficiency and viable cell recovery, with higher GFP expression generally associated with reduced cell yield. Conditions achieving ~70% and higher GFP^+ cells consistently exhibited low recovery (<0.5 x 10^5 cells per electroporation), whereas programs yielding higher viable cell numbers (≥3 x 10^5 cells per electroporation) typically showed low to moderate transfection efficiencies (<40%). Notably, selected electroporation programs (e.g., P14 and P20) achieved balanced performance, combining moderate transfection efficiencies (~40–50% GFP^+) with high recovery (~4 x 10^5 viable cells per electroporation). As expected, the non-electroporated control exhibited high recovery with no detectable GFP expression. Comparison of recovery supplements demonstrated that conditions with CultureCEPT Supplement frequently achieved comparable transfection efficiencies with improved viable cell recovery relative to RevitaCell Supplement. These data indicate that enhanced post-electroporation cell recovery, rather than transfection rate alone, is a key determinant of final GFP^+ cell output.

Table 2. Details of the 48 electroporation parameters used for payload delivery.

Lower-energy optimization programs				Higher-energy optimization programs			
ID	Program (V/ms/pulses)	ID	Program (V/ms/pulses)	ID	Program (V/ms/pulses)	ID	Program (V/ms/pulses)
P1	1,200/10/3	P13	1,100/20/2	P25	1,800/10/1	P37	2,400/5/2
P2	1,400/20/1	P14	1,200/20/2	P26	1,900/10/1	P38	2,500/5/2
P3	1,500/20/1	P15	1,300/20/2	P27	2,000/10/1	P39	2,000/5/3
P4	1,600/20/1	P16	1,400/20/2	P28	2,100/10/1	P40	2,100/5/3
P5	1,700/20/1	P17	850/30/2	P29	2,200/10/1	P41	2,200/5/3
P6	1,100/30/1	P18	950/30/2	P30	2,300/10/1	P42	2,300/5/3
P7	1,200/30/1	P19	1,050/30/2	P31	2,400/10/1	P43	2,400/5/3
P8	1,300/30/1	P20	1,150/30/2	P32	2,500/10/1	P44	2,500/5/3
P9	1,400/30/1	P21	1,300/10/3	P33	2,000/5/2	P45	1,800/15/1
P10	1,000/40/1	P22	1,400/10/3	P34	2,100/5/2	P46	1,900/15/1
P11	1,100/40/1	P23	1,500/10/3	P35	2,200/5/2	P47	2,000/15/1
P12	1,200/40/1	P24	1,600/10/3	P36	2,300/5/2	P48	2,100/15/1

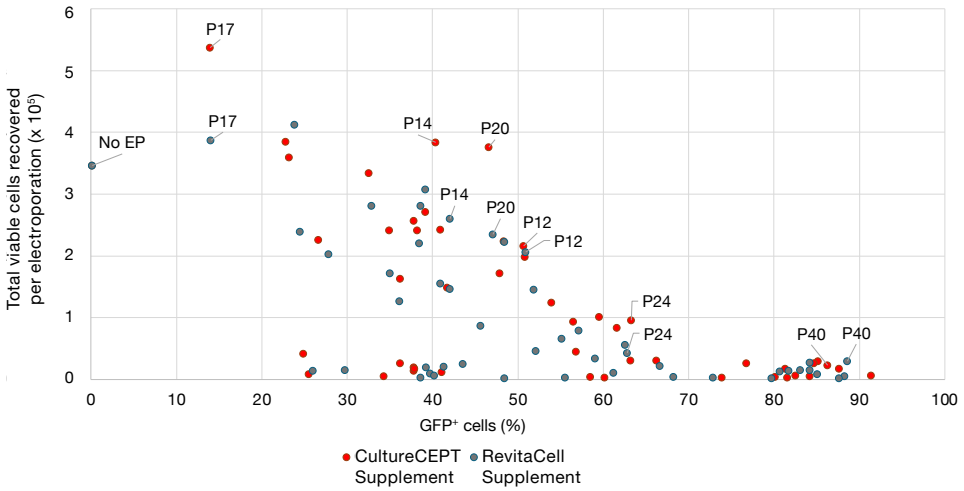

Figure 3. Comparison of electroporation program (P1–P48) performance for large payload (11 kb GFP plasmid) delivery in iPSCs using 10 µL reaction volume tips. The scatter plot shows the relationship between transfection efficiency (percent GFP⁺ cells) and cell recovery (total viable cells per electroporation) under two post-electroporation recovery conditions: CultureCEPT Supplement and RevitaCell Supplement (n = 3).

Electroporation buffer comparison

Plan | Electroporation program and cell recovery assessment | **Electroporation buffer comparison** | Payload concentration assessment

Criteria	Conditions
Electroporation buffer	R vs. T
Programs (V/ms/pulses)	1,200/20/2; 1,150/30/2; 2,000/5/2; 2,200/5/2; 2,100/5/3
Cells	1 x 10^7 cells/mL
Payload	11 kb GFP plasmid
Payload amount	50 µg/mL
Post-electroporation recovery supplement	CultureCEPT Supplement

Buffer composition can strongly affect membrane conductivity, osmotic balance, and intracellular stress during electroporation. As demonstrated in Figure 3, high-energy programs (high voltage/short pulse) can increase delivery into the cell but also trigger cell death. Testing T buffer with high-energy programs on the Neon NxT system is therefore a viable option to maximize delivery, enhance nuclear uptake, and ultimately determine if T buffer can support improved delivery efficiency that outweighs loss in cell viability and cell yields previously reported. Interestingly, comparison of electroporation buffer conditions demonstrated that R buffer consistently outperformed T buffer across all tested electroporation programs, including higher-voltage programs.

While both buffers supported measurable GFP transfection efficiencies (Figure 4A), the choice of electroporation buffer had a strong impact on total GFP⁺ cell yield (Figure 4B). R buffer supported substantially greater recovery of viable transfected cells across all electroporation programs, with total GFP⁺ cell numbers reaching up to ~1.2 x 10^5 cells per electroporation. In contrast, T buffer resulted in markedly reduced cell recovery and negligible GFP⁺ cell yields under several preselected electroporation conditions (Figure 4B). Overall, these results indicate that R buffer provided an effective balance of transfection efficiency and post-electroporation cell recovery and was therefore selected for subsequent large-payload iPSC electroporation studies.

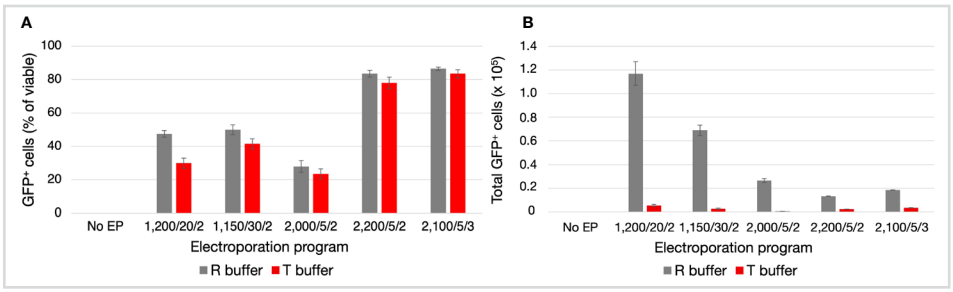

Figure 4. Comparison of R vs. T buffer performance using five selected electroporation programs (among the 48 prescreened in Figure 3) to deliver a large payload (11 kb GFP plasmid) in 10 µL reaction volume tips. (A) Transfection efficiency and **(B)** total GFP⁺ cell yield on day 3 post-electroporation (n = 3). CultureCEPT Supplement was present in the medium for 24 hr to facilitate iPSC recovery. No EP: non-electroporated control condition.

Payload concentration assessment

Plan | Electroporation program and cell recovery assessment | Electroporation buffer comparison | Payload concentration assessment

Criteria	Conditions
Electroporation buffer	R
Program (V/ms/pulses)	1,200/20/2
Cells	1 x 10^7 cells/mL
Payload	11 kb GFP plasmid
Payload amount	0, 25, 50, 75, 100 µg/mL
Post-electroporation recovery supplement	CultureCEPT Supplement

To determine the optimal payload amount to achieve a balanced outcome for both transfection efficiency and total viable GFP⁺ cell yield, a range across DNA concentrations was assessed (Figures 5A, 5B). Transfection efficiency increased in a dose-dependent manner from 25 to 100 µg/mL, rising from approximately 50% GFP⁺ cells at 25 µg/mL to ~75% at 75–100 µg/mL. However, this increase plateaued between 75 and 100 µg/mL, indicating diminishing gains in efficiency at higher concentrations. In contrast, total GFP⁺ cell yield demonstrated a nonlinear relationship with payload concentration. The highest absolute number of GFP⁺ cells was obtained at 50 µg/mL (~1.0 × 10^5 cells), whereas both lower and higher concentrations resulted in reduced total GFP⁺ recovery. Notably, despite high transfection efficiencies at 100 µg/mL, total GFP⁺ cell yield declined markedly, suggesting reduced viable cell recovery at elevated DNA concentrations. Overall, these data indicate that moderate payload concentrations represent an optimal balance between transfection efficiency and cell viability, maximizing recovery of the transfected iPSC population.

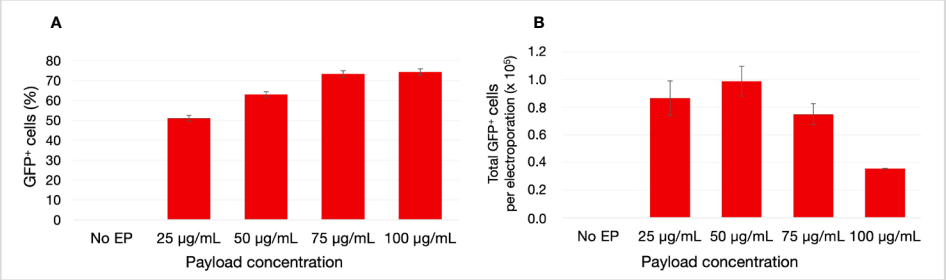

Figure 5. Payload concentration optimization using parameters of 1,200 V/20 ms/2 pulses in 10 µL reaction volumes. **(A)** Transfection efficiency and **(B)** total GFP⁺ cell yield on day 3 post-electroporation (n = 3). Electroporated and non-electroporated iPSCs were recovered in StemFlex Medium with CultureCEPT Supplement for 24 hr. No EP: non-electroporated control condition.

Conclusion

Here, we systematically optimized electroporation conditions for the Neon NxT system for delivery of a large (11 kb) plasmid payload into iPSCs. Screening of 48 electroporation programs revealed a pronounced trade-off between transfection efficiency and post-electroporation cell recovery, demonstrating that maximal GFP expression does not necessarily translate into optimal recoverable transfected cell yield. We identified specific programs that balanced moderate efficiency with high viable cell recovery, resulting in superior total GFP⁺ cell output. Comparative analysis of electroporation buffers showed that R buffer consistently outperformed T buffer, under both low- and high-energy electroporation conditions. Furthermore, titration of payload amount maximized total transfected cell yield, whereas higher concentrations increased efficiency at the expense of viability. Importantly, during high-throughput optimization experiments, use of a Neon NxT 8-channel pipette to electroporate cells minimized variability introduced by prolonged incubation of cells with payload, which can occur when samples are processed sequentially using a single-channel pipette. Collectively, these results demonstrate that optimal program selection combined with supportive experimental conditions such as choice of electroporation buffer and optimization of payload concentration is critical for maximizing functional transfection outcomes, while CultureCEPT Supplement provides a consistent advantage in improving viable, transfected cell yield across a broad range of electroporation conditions.

Ordering information

Product	Quantity	Cat. No.
Neon NxT Electroporation System with 1-Channel and 8-Channel Pipettes	1 system	NEON18S
Neon NxT Electroporation System Starter Kit with 1-Channel and 8-Channel Pipettes	1 kit	NEON18SK
Neon NxT Electroporation System 10 µL Kit with 1-Channel Tubes	25 x 2 reactions	N1096
Neon NxT Electroporation System 10 µL Kit with 8-Channel Tubes	96 x 2 reactions	N1096-8
StemFlex Medium	500 mL	A3349401
SSEA4 Monoclonal Antibody, PE, eBioscience	100 tests	12-8843-42
rhLaminin-521	100 µg	A29248
RevitaCell Supplement	5 mL	A2644501
CultureCEPT Supplement	500 µL	A56799
SYTOX Blue Dead Cell Stain	1 mL	S34857
TRA-1-81 (Podocalyxin) Monoclonal Antibody, APC, eBioscience	100 tests	17-8883-42
CTS PSC Cryomedium	50 mL	A4238801
Versene Solution	100 mL	15040066
Attune CytPix Flow Cytometer	1 system	A51848

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