Transfection

Unlocking optimal transfection: harnessing the power and flexibility of the Neon NxT Electroporation System with the 8-channel pipette for large plasmid delivery

Keywords
Neon Nxt Electroporation System, electroporation, transfection optimization, large plasmid delivery

Introduction

Electroporation (EP) is a well-established technique that uses short electric pulses to create transient pores in a cell's membrane, allowing efficient delivery of exogenous molecules into the cell. It has revolutionized transfection and genetic manipulation, finding applications in cell engineering, gene expression studies, protein production, and functional analysis.

The Invitrogen™ Neon™ Transfection System is a trusted instrument for electroporation. The Invitrogen™ Neon™ NxT Electroporation System is an enhanced version of the Neon Transfection System, building upon its success. It combines the trusted electroporation tip design with Thermo Scientific™ ClipTip™ technology and improved usability. The system allows precise control of electrical parameters and offers the Invitrogen™ TransfectionLab™ cloud application for experimental design.

The Invitrogen™ Neon™ NxT 8-Channel Pipette is an exciting addition to the Neon NxT system that enables processing of up to eight samples at once. This capability reduces experimental time and effort, providing a more efficient workflow.

The Neon NxT system seamlessly integrates into both upstream and downstream workflow optimization processes. For upstream applications like gene editing or protein expression, it enables efficient delivery of nucleic acids into target cells, for high transfection efficiency with minimal variability. In downstream applications like drug discovery or functional genomics, the system's multi-sample capability accelerates the screening process and enhances the reliability of results.

The Neon NxT Electroporation System with the Neon NxT 8-Channel Pipette has an advantage over other electroporation systems. Electroporation with the Neon NxT system requires less experimental time and effort, due to fewer required pipetting steps. The system is also compatible with design of experiments (DOE) methodologies, allowing researchers to explore and optimize multiple variables simultaneously. This approach not only streamlines parameter screening and reduces variability but also predicts optimal conditions that may not have been directly tested, enabling customers to more fully explore their design space.

Here we aim to provide a comprehensive guideline for optimizing various electroporation conditions using the Neon NxT Electroporation System with the Neon NxT 8-Channel Pipette. The intent was to successfully transfect Jurkat cells with plasmid DNA by optimizing the electroporation programs, resuspension buffer type, cell concentration, payload concentration, and post-electroporation recovery time. Detailed optimization was conducted for 2 different plasmid sizes, specifically 5 kb and 11.5 kb, with an emphasis on optimizing conditions for the 11.5 kb plasmid. The objective was to establish a standardized approach that researchers can follow to attain optimal transfection results using the Neon NxT system.

Materials and methods

Jurkat cells were cultured in Gibco™ RPMI 1640 Medium with GlutaMAX™ Supplement, and additional supplementation with 10% Gibco™ Fetal Bovine Serum (FBS), Premium Plus. Cells were reseeded at a concentration of 8 x 10^5 cells/mL 24 hours prior to electroporation. Electroporation was performed using Neon NxT 10 µL tips, and electroporated cells were seeded in a 96-well plate using the same culture conditions. Table 1 provides the conditions of the studies described in sections 1–5 of the Results, including the tested factors, constant factors, and the plasmid size.

At 24 hours post-electroporation, cells were analyzed for viability and transfection efficiency of the GFP plasmid, using the Invitrogen™ Attune™ NxT Flow Cytometer. A schematic description of the workflow is shown in Figure 1.

Table 1. Constant and variable electroporation conditions of the studies herein.

Results section	Tested and variable factors	Constant factors	Plasmid size (kb)
1	Resuspension buffer, EP program, payload size	Cell concentration, payload concentration, post-EP recovery time	5 and 11.5
2	Cell concentration	Resuspension buffer, EP program, payload concentration, post-EP recovery time	11.5
3	Payload concentration	Resuspension buffer, EP program, cell concentration, post-EP recovery time	11.5
4	Post-EP recovery time	Resuspension buffer, EP program, cell concentration, payload concentration	11.5
5	Resuspension buffer, EP program, cell concentration, payload concentration (DOE study)	Post-EP recovery time	11.5

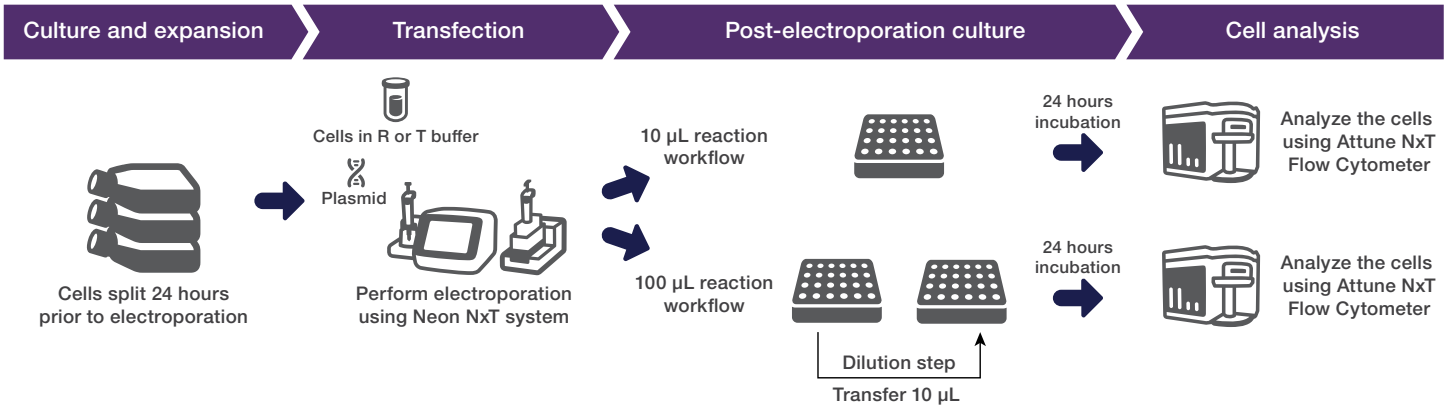

Figure 1. Schematic of the transfection and cell analysis workflow with the Neon NxT Electroporation System and the Attune NxT Flow Cytometer.

Invitrogen™ SYTOX™ Red Dead Cell Stain was used to assess viability, and event counts from flow cytometry analysis were used to quantify the total number of viable transfected cells (TVTC). The flow cytometry gating strategy is shown in Figure 2. The experimental design was facilitated by use of the TransfectionLab app, and the DOE and subsequent model analysis for the study described in section 5 of the Results were performed using JMP™ statistical software.

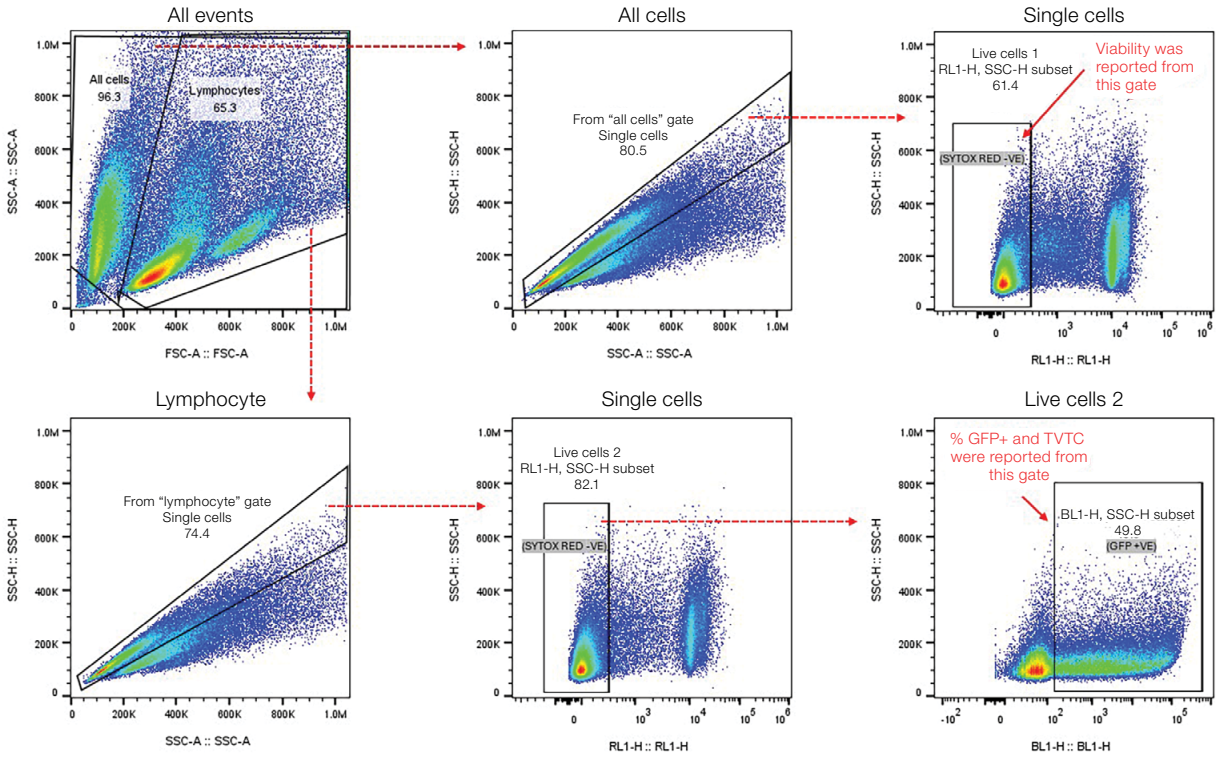

Figure 2. Gating strategy for the Attune NxT Flow Cytometer used for cell analysis of the studies herein.

TransfectionLab cloud-based app

The TransfectionLab app is a cloud-based application developed to support researchers in designing and optimizing their transfection experiments. It provides a user-friendly interface that allows researchers to easily create experimental designs for the Neon NxT Electroporation System. Additionally, the TransfectionLab app enables users to seamlessly import their experimental plate design into the Neon NxT system.

The TransfectionLab app was used to design the experiments reported in this application note. Figure 3 shows an example of the plate map and electroporation guide generated by the TransfectionLab app for the experiments described in section 1 of the Results.

Each well is color-coded with its respective electroporation program, and the green outline around each well shows the cell density of 5 x 10^6 cells/mL. The payload is indicated by the number in each well: 2 represents a 5 kb plasmid, and 3 represents an 11.5 kb plasmid.

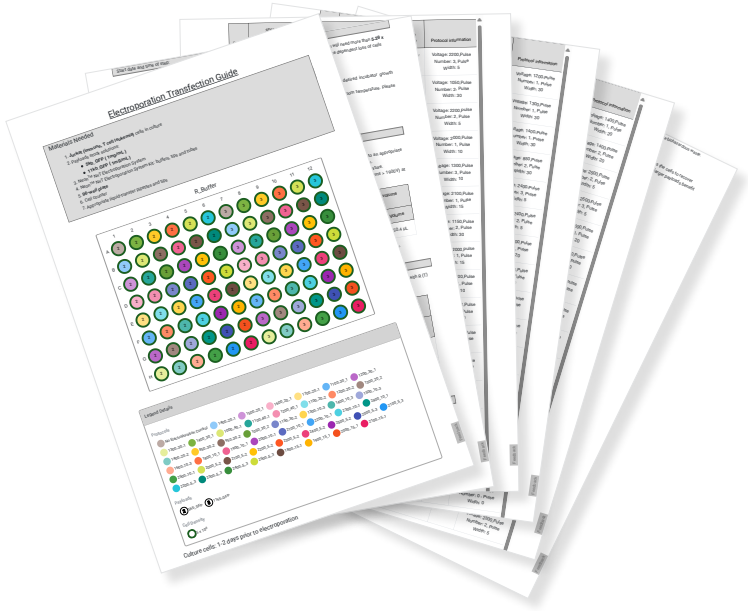

Figure 3. Step-by-step guide generated with the TransfectionLab app.

Results and discussion

The findings of the studies are elaborated and discussed in the following sections. In these sections, we provide a comprehensive analysis and interpretation of the results obtained from the experiments.

Section 1: Optimization of electroporation program and resuspension buffer

In this study, we aimed to evaluate the optimal electroporation program and resuspension buffer for payloads of different sizes. To accomplish this, we electroporated cells with either a 5 kb or an 11.5 kb GFP plasmid. A total of 48 different electroporation programs was used, which are listed in Table 2. Out of these 48 programs, 24 are standard Neon system optimization programs. To explore a broader range of conditions, we added 24 higher-voltage programs to cover a wider range of energy settings on the Neon NxT system. The flexibility of the Neon NxT 8-Channel Pipette made it easy to test various programs across different channels, streamlining optimization. Since higher voltages could increase energy and risk cell damage, we managed this by adjusting pulse number and width to stay within safe energy levels and protect cell viability. Table 2 includes 24 such programs, referred to as "24 higher- voltage programs."

To determine the optimal resuspension buffer for different sizes of payload, we conducted electroporation with Neon NxT Resuspension R Buffer and Neon NxT Resuspension T Buffer. We excluded Neon NxT Resuspension Genome Editing Buffer from this study, as it is specifically designed for CRISPR-Cas9 knock-in genome editing.

To maintain consistency, we kept the cell and payload concentrations constant throughout the study. We used concentrations of 5 x 10^6 cells/mL and 0.1 mg/mL for both payloads. Cells were transferred to culture medium immediately after electroporation with no post-EP recovery time.

Table 2. The 48 electroporation programs used for optimization.

Neon 24 optimization programs				24 higher-voltage programs			
ID	Program*	ID	Program*	ID	Program*	ID	Program*
P1	No EP	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

* Voltage (V)/pulse width (ms)/number of pulses

The results obtained for the 5 kb and 11.5 kb plasmids are shown in Figures 4A and 4B, respectively.

In our analysis, we aimed to identify optimal conditions that would result in a balanced higher percentage of GFP+ cells and higher TVTC. We observed that certain programs, such as P44 (2,500 V; 5 ms; 3 pulses) with both R and T buffers, achieved more than 90% transfection efficiency with both buffers tested (Figure 4A). However, it was noted that some of these programs also resulted in lower TVTC, indicating a higher rate of cell death during electroporation.

The circled data points located at the top-right corners of Figures 4A and 4B demonstrate a notable balance between higher transfection percentage and higher TVTC. The data clearly indicated that the use of R buffer resulted in the best outcomes for transfection of the 5 kb plasmid (Figure 4A), while the use of T buffer resulted in the best outcomes for transfection of the 11.5 kb plasmid (Figure 4B). We observed comparable transfection efficiencies with the 5 kb plasmid in both buffers using program P41 (2,200 V; 5 ms; 3 pulses) (Figure 4A). However, the TVTC with R buffer was more than twice the TVTC with T buffer. With the 11.5 kb plasmid (Figure 4B), T buffer significantly improved TVTC (programs P41 and P42). This observation strongly suggests that R buffer plays a significant role in preventing cell death during transfection with smaller (e.g., 5 kb) plasmids, while T buffer improves cell viability during transfection with larger (e.g., 11.5 kb) plasmids.

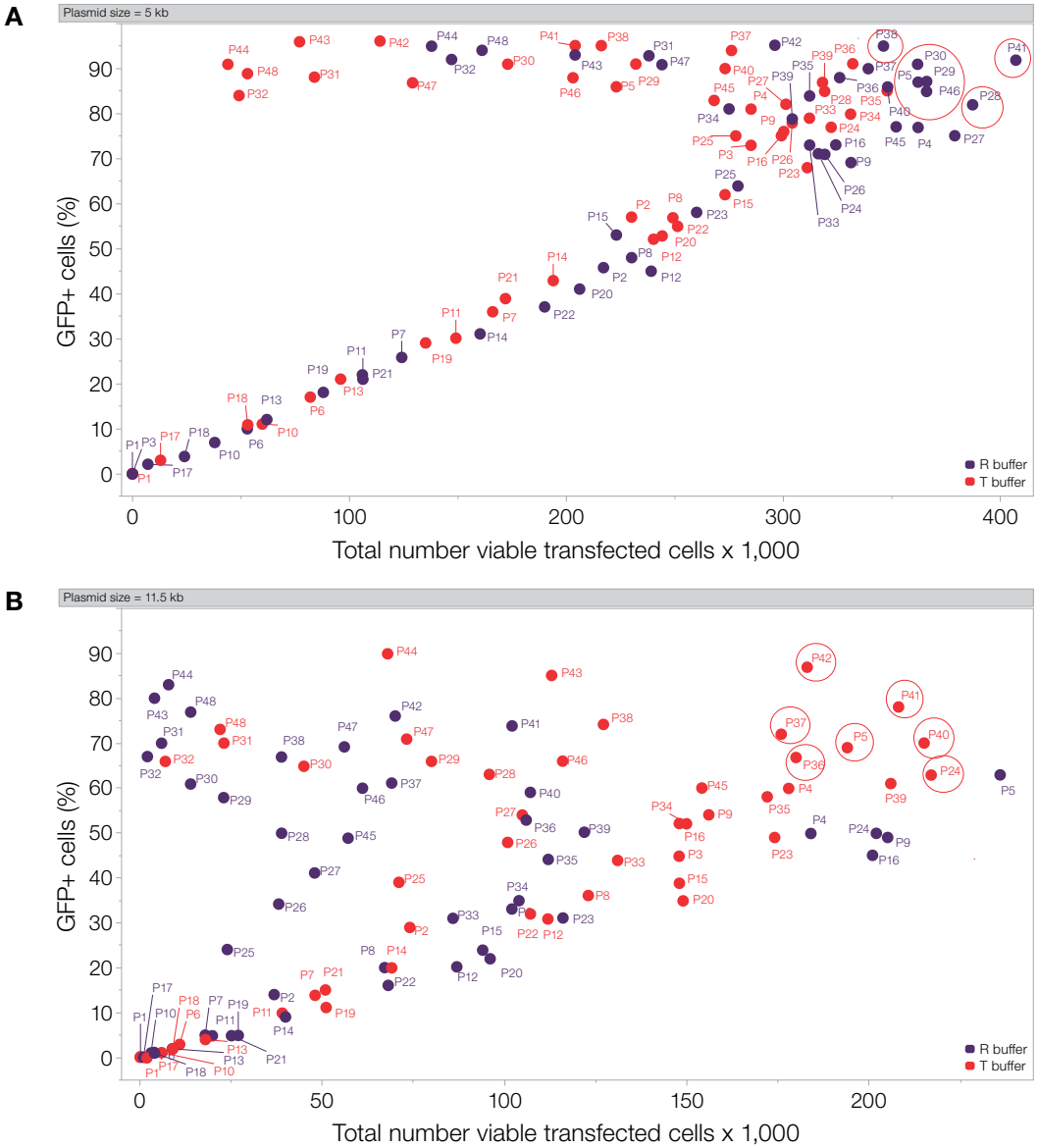

Figure 4. Electroporation performance with Resuspension R Buffer vs. Resuspension T Buffer and 48 programs for (A) 5 kb GFP plasmid and (B) 11.5 kb GFP plasmid. Percentage of GFP-positive cells was plotted against TVTC. Purple data points represent results obtained with R buffer, and red data points represent results obtained with T buffer. Each data point (n = 1) in the graph represents a specific electroporation program, which is listed in Table 2. The red-circled data points represent the best-performing conditions, characterized by a balance between higher percentages of GFP+ cells and higher TVTC.

We identified the 7 best-performing programs for the 5 kb and 11.5 kb plasmids separately, highlighted with red circles in the figure. The performance of these programs is summarized in Table 3.

Table 3. Summary of results from best-performing programs.

Plasmid size (kb)	EP program*	Program ID	GFP+ (%)	Viability (%)	TVTC x 1,000	Buffer
5	1,700/20/1	P5	87	83	362	R
	2,100/10/1	P28	82	83	387	
	2,200/10/1	P29	87	81	366	
	2,300/10/1	P30	91	78	362	
	2,500/5/2	P38	95	82	346	
	2,200/5/3	P41	92	81	407	
	1,900/15/1	P46	85	82	366	
11.5	1,700/20/1	P5	69	68	194	T
	1,600/10/3	P24	63	75	217	
	2,300/5/2	P36	67	56	180	
	2,400/5/2	P37	72	56	176	
	2,100/5/3	P40	70	66	215	
	2,200/5/3	P41	78	59	208	
	2,300/5/3	P42	87	54	183	

* Voltage (V)/pulse width (ms)/number of pulses

Section 2: Optimization of electroporation cell concentration

In this study, we investigated the impact of cell concentration on transfection efficiency with the 11.5 kb plasmid. Cells were resuspended in T buffer at three different concentrations: 5, 20, and 50 x 10^6 cells/mL. We used the seven best-performing programs for the 11.5 kb plasmid, as listed in Table 3. The payload concentration was kept constant at 0.1 mg/mL, and cells were transferred into culture medium immediately after EP. The findings are presented in Figure 5.

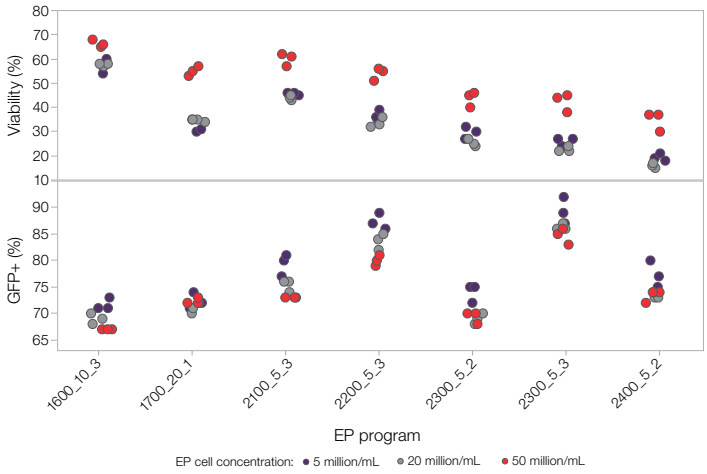

Figure 5. Impact of cell concentration on electroporation efficiency. The graph plots the percentages of GFP-positive cells and viable cells obtained with the respective electroporation programs used. Data points are color-coded to represent different cell concentrations: purple (5 x 10^6 cells/mL), gray (20 x 10^6 cells/mL), and red (50 x 10^6 cells/mL). Each cell concentration and program combination was performed in triplicate (n = 3).

The data provided clear evidence that different cell concentrations affect electroporation performance. While the impact on percentage of transfected cells was minimal, 5 x 10^6 cells/mL slightly outperformed other concentrations across most of the programs. In terms of cell viability, electroporation at 50 x 10^6 cells/mL yielded significantly better results than electroporation at other concentrations across all 7 programs. Based on these findings, we identified 50 x 10^6 cells/mL as the best cell concentration for further optimization of electroporation conditions for transfection of an 11.5 kb plasmid.

Section 3: Optimization of payload concentration

For optimization of payload concentration, we performed an experiment similar to that described in section 2 of the Results. The 11.5 kb GFP plasmid was added at three different final concentrations (0.05, 0.10, and 0.20 mg/mL) for transfection of cells resuspended in T buffer. The same 7 best-performing EP programs were used, and cell concentration was maintained at a constant value of 50 x 10^6 cells/mL. The findings are presented in Figure 6.

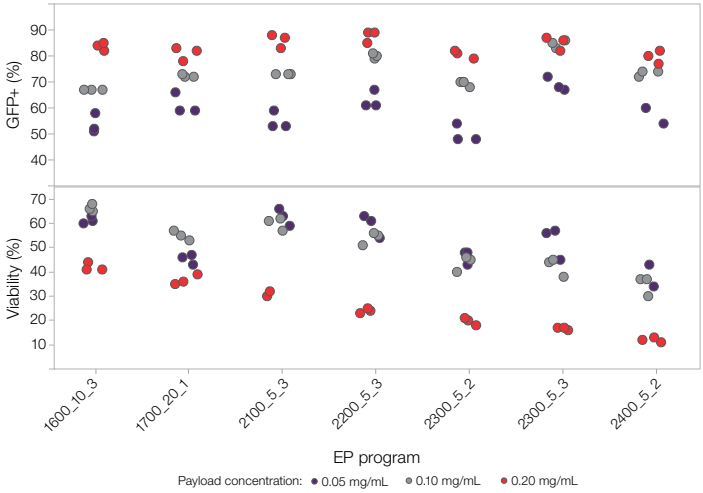

Figure 6. Impact of payload concentration on electroporation efficiency. The graph plots the percentages of GFP-positive cells and viable cells against the respective EP programs. Data points are color-coded to represent different payload concentrations: purple (0.05 mg/mL), gray (0.10 mg/mL), and red (0.20 mg/mL). Each EP payload concentration and program combination was tested in triplicate (n = 3).

The data provided compelling evidence of the impact of different payload concentrations on electroporation performance. For the plasmid concentration of 0.20 mg/mL, the percentage of GFP+ cells was significantly higher than with other concentrations, while cell viability was poorer with most of the programs.

While the difference in cell viability between electroporation with 0.05 mg/mL and 0.10 mg/mL payloads was minimal, transfection efficiency with the 0.10 mg/mL plasmid concentration was significantly higher than it was with 0.05 mg/mL. Based on these findings, we identified 0.10 mg/mL as the optimal payload concentration for the 11.5 kb plasmid.

Section 4: Optimization of post-electroporation recovery time

Results of a previous study suggest that incorporating a recovery period following the delivery of electric pulses can significantly enhance the electroporation performance, particularly with large plasmids ranging from 6 to 16 kb [1]. In this study, we aimed to optimize the post-electroporation recovery time and evaluate its impact on transfection efficiency with the 11.5 kb plasmid. Following electroporation, cells were seeded into the culture vessel and incubated for a specific duration before the addition of the culture medium. The same electroporation conditions described in section 3 of the Results were used while maintaining a constant payload concentration of 0.1 mg/mL. The results are presented in Figure 7.

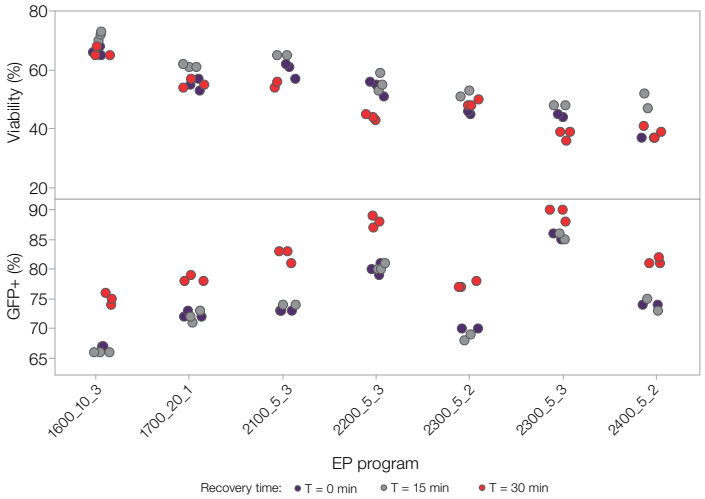

Figure 7. Impact of post-electroporation recovery time on transfection efficiency. The percentages of GFP-positive cells and viable cells are plotted against the respective electroporation programs. Data points are color-coded to represent different post-EP recovery periods: purple (T = 0 min), gray (T = 15 min), and red (T = 30 min). Each recovery period and program combination was tested in triplicate (n = 3).

The data presented in Figure 7 clearly demonstrate the impact of the post-electroporation recovery period on electroporation performance. A 30-minute recovery period gave significantly better results in terms of transfection efficiency, while cell viability was slightly poorer with most of the programs compared to with a 15-minute recovery period and no recovery period.

While there was no substantial difference in the total percentage of GFP+ cells after a 15-minute recovery period or no recovery period, the 15-minute recovery time slightly improved cell viability. Based on these findings, we recognize that a recovery period of 15 to 30 minutes may enhance electroporation performance for transfecting the 11.5 kb plasmid.

Section 5: DOE to find optimal conditions for electroporation

For the experiments described in sections 1–4 of the Results, we implemented a sequential optimization approach for refining various electroporation conditions. However, this method does not allow the evaluation of the interdependencies among different input factors. To address this limitation, a DOE study can be employed to optimize the electroporation conditions and identify any relationships and dependencies among the various electroporation factors. By conducting a DOE study, we can simultaneously optimize multiple electroporation parameters for a more comprehensive and efficient optimization process.

We performed a DOE study to evaluate the impact of different electroporation parameters on transfection performance with the 11.5 kb plasmid. The JMP software was employed to design an experiment with 192 electroporation conditions using the main effects model. Electroporation was conducted with two different resuspension buffers, cell concentrations in the range of 5–50 x 10^6 cells/mL, payload concentrations in the range of 0.05–0.2 mg/mL, and 48 different electroporation programs listed in Table 2. These electroporation conditions were used as input factors for the model. Electroporation performance was evaluated in terms of transfection efficiency and transfection viability. Figure 8, which was generated using the JMP prediction profiler, visually represents the main effects model derived from the DOE.

The use of the Neon NxT 8-Channel Pipette allowed for higher-throughput electroporation, making it a powerful tool for the DOE-based biological studies. Additionally, the TransfectionLab design tool provided detailed experiment guidance and calculations, enabling seamless execution of this complex DOE study. The Neon NxT 8-Channel Pipette enables a larger number of electroporations to be performed within a shorter period of time. For instance, a full 96-well plate of EP reactions could be completed in approximately 15 minutes, a fourth of the time compared to utilizing the 1-channel pipette.

The optimal predictive plot is shown in Figure 8. Based on the DOE study, optimal conditions for transfection of the 11.5 kb plasmid are: T buffer, 50 x 10^6 cells/mL, 0.1833 mg/mL payload, and electroporation program P39 (2,000 V; 5 ms; 3 pulses). These conditions are expected to yield 77.6% transfection efficiency and 74.5% transfection viability.

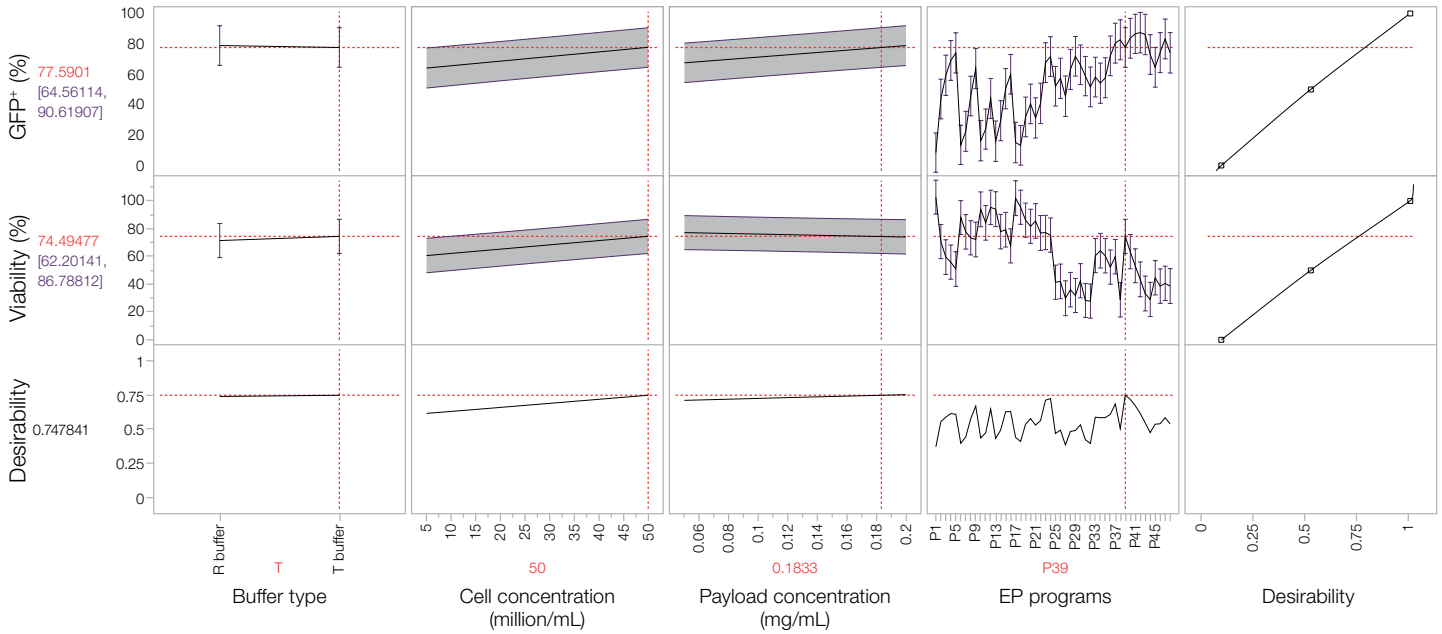

Figure 8. DOE predictive plot. The input factors are displayed on the x-axis, and the y-axis represents the responses. The vertical red lines indicate the current values of the factors, which are highlighted in red below the horizontal axis. The red values on the vertical axis represent the predicted responses based on the current values of the factors. These current values were generated by the predictive model as the optimal values to achieve maximum desirability for each response with equal importance.

Conclusion

Electroporation is a robust and safe nonviral technique widely employed for delivering exogenous molecules of different sizes into cells. While small reporter plasmids (3.5 kb) can be transfected with high efficiency and viability, larger plasmids (6–16 kb) are more challenging in terms of maintaining cell viability and transfection efficacy under the same conditions.

Here we provide a comprehensive guideline for optimizing electroporation conditions specifically for larger plasmids. The data presented highlight the significant impact of factors such as the EP program, resuspension buffer, payload and cell concentrations, and post-EP recovery time on overall electroporation performance. Among these factors, the electroporation program stands out as the most crucial determinant of performance.

Our findings indicate that Neon NxT Resuspension T Buffer is more suitable for delivering larger plasmids (11.5 kb), while Neon NxT Resuspension R Buffer is better for delivering smaller plasmids (5 kb). Furthermore, we have determined that certain higher-voltage programs with lower pulse numbers and pulse widths can greatly enhance electroporation performance.

By applying the optimal electroporation conditions, we successfully delivered the 11.5 kb plasmid into Jurkat cells with up to 80% transfection efficiency and the 5 kb plasmid with up to 95% transfection efficiency, while ensuring minimal impact on cell viability.

The insights gained from our study can serve as a guide for future experiments, ultimately leading to enhanced efficiency in electroporation. However, it is essential to recognize the importance of customizing the electroporation approach to meet specific needs. Whether the focus is on optimizing transfection efficiency, preserving cell viability, striking a balance between the two, or maximizing the total number of transfected cells, tailoring the electroporation conditions accordingly is critical to achieving desired outcomes.

The Neon NxT 8-Channel Pipette greatly facilitates the optimization process by enabling higher throughput, optimization of individual variables, and DOE approaches to identify optimal conditions, ultimately saving a considerable amount of valuable research time and resources.

Reference

1. Lesueur L, Mir L, André F (2016) Overcoming the specific toxicity of large plasmids electrotransfer in primary cells *in vitro*. *Mol Ther Nucleic Acids* 5:e291.

Ordering information

Description	Cat. No.
Neon NxT Electroporation System Starter Kit with 1-Channel and 8-Channel Pipettes	NEON18SK
Neon NxT Electroporation System with 1-Channel and 8-Channel Pipettes	NEON18S
Neon NxT Electroporation System 10 µL Kit, 8-Channel Tubes	N1096-8
Neon NxT Electroporation System 100 µL Kit, 8-Channel Tubes	N10096-8
RPMI 1640 Medium, GlutaMAX Supplement	61870-036
Fetal Bovine Serum, Premium Plus	A5669701
CTS DPBS, without calcium chloride, without magnesium chloride	A1285602
Attune CytPix Flow Cytometer	A51849
CytKick Max Autosampler	A42973
Attune Wash Solution	A24974
Attune Shutdown Solution (1X)	A24975
Attune Performance Tracking Beads, 3 mL	4449754
Attune Focusing Fluid (1X)	4449791
SYTOX Red Dead Cell Stain, for 633 or 635 nm excitation	S34859
Nunc MicroWell 96-Well, Nunclon Delta Treated, Flat-Bottom Microplate	167008
Nunc EasYFlask Cell Culture Flasks	159910
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450

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