Delivery solutions for immune cells

Enabling immune cell research with the Neon Transfection System

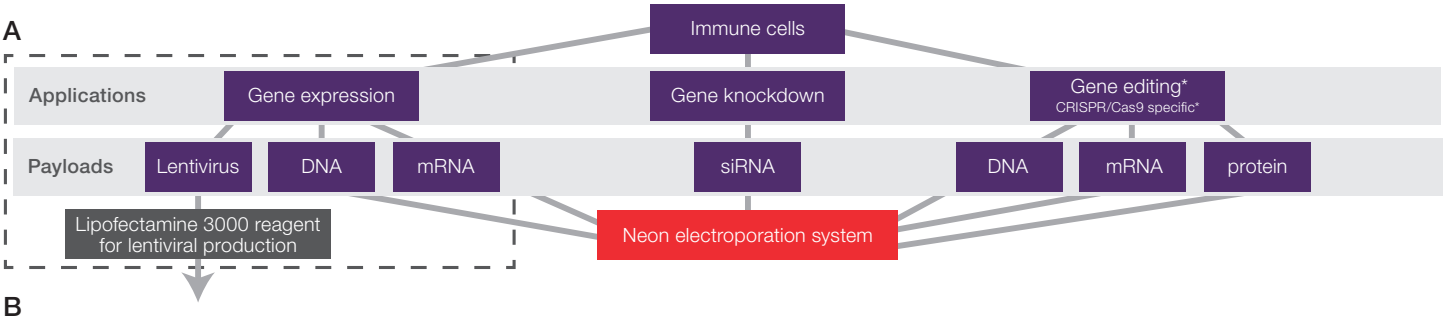
Introduction

Genetic manipulation of blood cells is key to understanding and developing treatments for a broad range of diseases such as leukemia, solid-tumor cancers, and HIV infection. Recent progress in immunotherapy for cancer and infectious diseases, along with advancements in techniques such as genome editing, have stimulated growing interest in experimentation with blood cells. However, the path to discovery is not without challenges. Even as more researchers pursue breakthroughs, the difficulty of delivering molecules into these cells continues to be an impediment to more rapid advancement [1].

Transfection of hematopoietic cells and circulating blood cells is generally difficult, time-consuming, and expensive [1,2]. T lymphocytes, which are particularly interesting because of their therapeutic promise, have proven to be refractory to delivery of DNA and RNA by standard reagent-based methods; consequently, transduction with lentiviruses is now the most widely used approach for introducing nucleic acids into T cells [3–7]. Even though more efficient lentiviral techniques are being developed, safety concerns pertaining to human therapeutic use have yet to be fully addressed. Researchers are exploring alternative physical delivery approaches such as electroporation [8–11].

The Invitrogen™ Neon™ Transfection System delivers DNA, RNA, and protein into cells while avoiding the challenges faced by reagent and viral methods. By using the Neon system to optimize electroporation conditions and cell density, we achieved greater than 80% transfection efficiency with a number of blood cell lines as well as primary T cells (Figure 1). We were also able to successfully utilize the Neon system for genomic engineering with the CRISPR-Cas9 system in multiple blood cell lines, and achieve greater than 90% genomic cleavage efficiency in primary human T cells.

A



B

Gene expression payloads	Platform	Performance	Call viability	Ease of use	Biosafety requirements	Cost per reaction
Lentivirus	Lipofectamine 3000 reagent	++++	+++	+	+++	$$$
DNA	Neon system	+++	+++	+++	+	$
mRNA	Neon system	++++	+++	+++	+	$$

Figure 1. Applications of the Neon Transfection System. (A) Decision diagram based on genetic manipulation experiments on immune cells. *Cell models used: primary T cells, $CD34^+$, Jurkat, K562, NK-92, Raji, SC-1, and THP-1. **(B)** Options available for gene expression payload delivery, and various factors that can influence experiments.

Neon Transfection System

The Neon Transfection System streamlines the electroporation process for greater transfection efficiency and decreased hands-on time. Compared to standard cuvette-based electroporation, the innovative design of the Neon system's tip increases transfection efficiency and cell viability by exposing samples to a more uniform electric field with minimal pH change, less ion formation, and negligible heat generation.

Neon Transfection System features:

- **Small footprint**—benchtop design that fits inside tissue culture hood
- **Flexible**—accommodates 1 x 10^4 to 5 x 10^6 cells per reaction in a sample volume of 10 µL or 100 µL
- **User-friendly**—touch-screen interface for easy programming of electroporation parameters
- **Customizable**—preprogrammed 24-well optimization protocols and open platform for additional protocols
- **Proven in immune cells**—high DNA and RNA delivery efficiency in primary T cells and immune cell lines

Table 1. Results from DNA delivery by the Neon Transfection System.

	Electroporation parameters (10 µL tips)		
Cell line	**Number of cells**	**Transfection efficiency**	**24-well optimization protocol (program #)**
Primary T cells	2 x 10^5	84%	1,600 V/10 ms/3 pulses (#24)
Jurkat	2 x 10^5	86%	1,700 V/20 ms/1 pulse (#5)
NK-92	2 x 10^5	52%	1,300 V/10 ms/3 pulses (#21)
KG-1	2 x 10^5	82%	1,700 V/20 ms/1 pulse (#5)
THP-1	2 x 10^5	42%	1,600 V/10 ms/3 pulses (#24)
SC-1	2 x 10^5	54%	1,700 V/20 ms/1 pulse (#5)
SC	2 x 10^5	70%	1,700 V/20 ms/1 pulse (#5)

Human primary T cells were isolated from LeukoPak™ blood product from healthy donors using Ficoll-Paque™ PLUS medium and the Invitrogen™ Dynabeads™ Untouched™ Human T Cells Kit. The cells were then cultured in Gibco™ OpTmizer™ CTS™ T-Cell Expansion medium with 2% human serum, and activated with Gibco™ Dynabeads™ Human T-Expander CD3/CD28. The transfection experiments with the Neon system were performed 3 days after activation. Each cell line was maintained with Gibco™ medium, serum, and growth factors according to ATCC guidelines. Cell lines were prepared at a density of 2 x 10^5 cells per 10 µL tip, for electroporation in Buffer R (component of Neon Transfection System Kits) with 1–1.5 µg of DNA encoding GFP. The 24-well optimization protocols were performed using the 10 µL Neon tip, and the cells were dispensed into 0.5 mL prewarmed medium in a 24-well plate. Cells were analyzed with the Invitrogen™ Attune™ NxT Flow Cytometer 24 hours post-transfection.

mRNA delivery

The unique properties of mRNA make it preferable to DNA for transfection (Table 1) when working with difficult cell models. Since nuclear entry is not necessary with mRNA, transfection efficiency is generally higher. Additional benefits are that mRNA transfection is transient, and time to protein expression is faster than with DNA. The results below indicate the advantages of using mRNA for gene expression experiments with difficult-to-transfect immune cell models (Table 2).

Table 2. Comparison between mRNA and DNA delivery by the Neon Transfection System.

Electroporation parameters (10 µL tips)				
Cell line	**Number of cells**	**Transfection efficiency with mRNA**	**Transfection efficiency with DNA**	**mRNA 24-well optimization protocol (program #)**
Primary T cells	2 x 10^5	**96%**	84%	1,600 V/10 ms/3 pulses (#24)
Jurkat	2 x 10^5	**95%**	86%	1,400 V/20 ms/2 pulses (#16)
NK-92	2 x 10^5	**98%**	52%	1,300 V/10 ms/3 pulses (#21)
KG-1	2 x 10^5	**95%**	82%	1,600 V/20 ms/1 pulse (#4)
THP-1	1.5 x 10^5	**88%**	42%	1,400 V/20 ms/2 pulses (#16)
SC-1	2 x 10^5	**78%**	54%	1,700 V/20 ms/1 pulse (#5)
SC	2 x 10^5	**89%**	70%	1,600 V/20 ms/1 pulse (#4)
J774A.1	2 x 10^5	**85%**	ND	1,700 V/20 ms/1 pulse (#5)

Human primary T cells were isolated from LeukoPak blood product from healthy donors using Ficoll-Paque PLUS medium and the Invitrogen Dynabeads Untouched Human T Cells Kit. The cells were then cultured in OpTmizer CTS T-Cell Expansion medium with 2% human serum, and activated with Dynabeads Human T-Expander CD3/CD28. The transfection experiments with the Neon system were performed 3 days after activation. Each cell line was maintained with Gibco medium, serum, and growth factors according to ATCC guidelines. Cell lines were prepared at a density of 1.5–2.0 x 10^5 cells per 10 µL tip, for electroporation in Buffer R (component of Neon Transfection System Kits), with 1–1.5 µg of mRNA encoding GFP. The 24-well optimization protocols were performed using the 10 µL Neon tip, and the cells were dispensed into 0.5 mL prewarmed medium in a 24-well plate. Cells were analyzed with the Attune NxT Flow Cytometer 24 hours post-transfection. ND = not determined.

mRNA and DNA delivery in primary T cells

The ability to transfect primary T cells is vital for further understanding of these cells and their functions. Optimized delivery to T cells is also important in new areas of clinical research such as chimeric antigen receptor T cell (CAR-T) cancer therapy. Introduction of mRNA and DNA in primary T cells with the Neon system produced over 90% and 80% transfected cells, respectively (Figure 2).

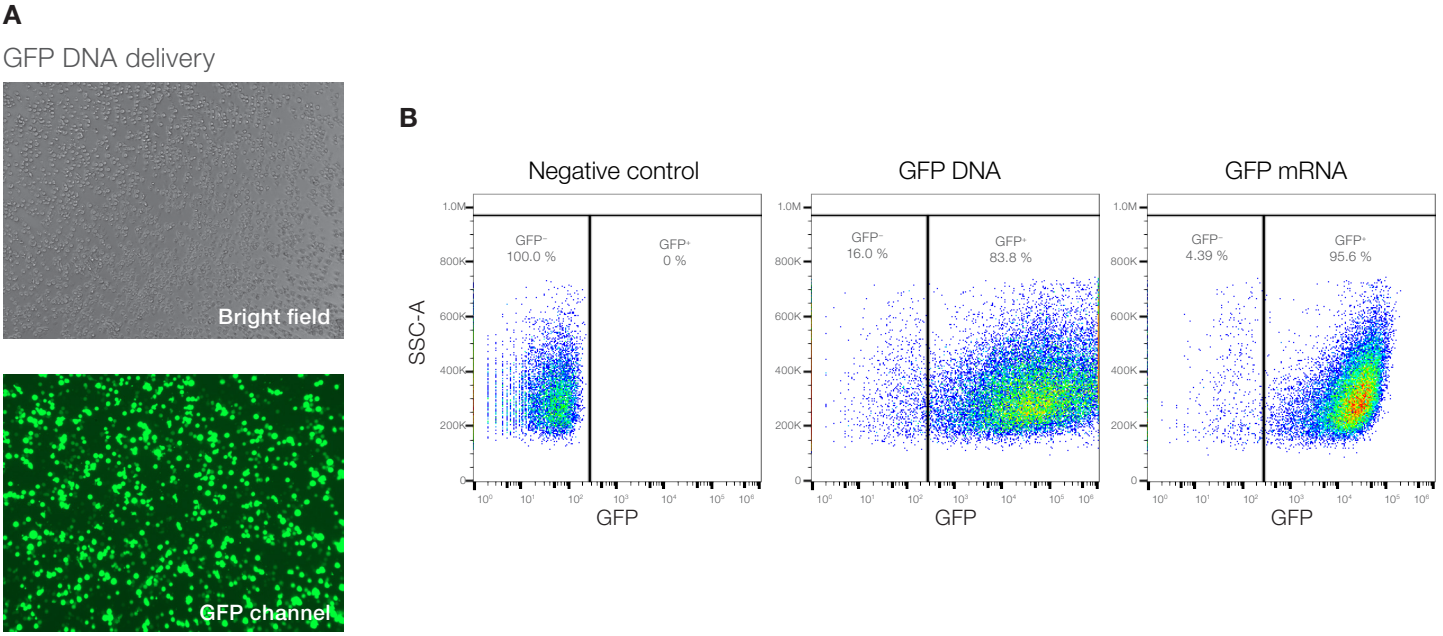


Figure 2. Transfection of human primary T cells by electroporation using the Neon Transfection System. GFP DNA or mRNA (1 µg) was delivered with Neon program #24 (1,600 V/10 ms/3 pulses) to 2 x 10^5 cells per 10 µL tip in Buffer R. Cells were analyzed 24 hours post-electroporation with **(A)** the Invitrogen™ EVOS™ Cell Imaging System and **(B)** the Invitrogen™ Attune™ NxT Flow Cytometer.

Chimeric antigen receptor T cell (CAR-T) therapy focuses on turning the T cells of cancer patients into personalized anti-cancer drugs. CAR-T therapy involves isolating and activating a patient's T cells, delivering a virus, DNA, or mRNA encoding a CAR of interest, and expanding the cells *in vitro*. The CAR-expressing cells are then reinfused into the patient for therapy. Research involving CAR-T is accelerating at an unprecedented rate as positive results from clinical trials are encouraging new collaborations and billions of dollars in investments.

Genomic engineering with the Neon Transfection System and Cas9/gRNA complexes in immune cells

Recently, electroporation was used to facilitate genome editing of primary human T lymphocytes by delivering preformed ribonucleoprotein (RNP) complexes, formed by Cas9 protein and gRNA, into CD4+ T cells, allowing both knockout and knock-in modifications of the HIV-related CXCR4 and PD-1 genes [12]. CRISPR protein streamlines cell engineering by eliminating transcription and translation in the cell (Figure 3). Transfection of Invitrogen™ GeneArt™ Platinum™ Cas9 Nuclease and *in vitro*–transcribed gRNA into cell lines by electroporation resulted in high genomic cleavage efficiency, as shown in Table 3.

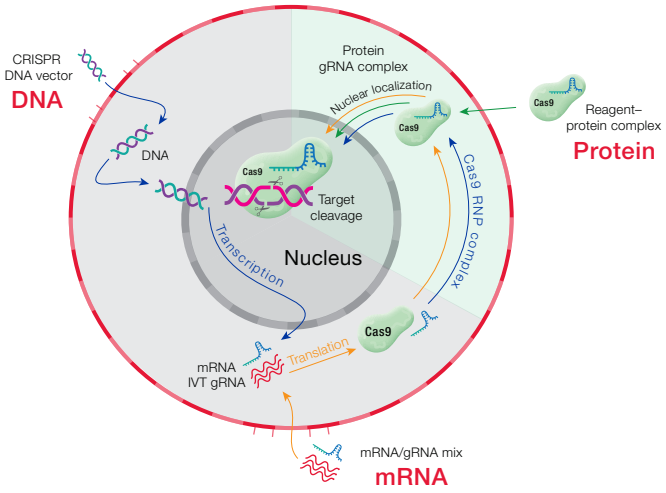


Figure 3. Advantages of the CRISPR-Cas9 system.

Table 3. Improved results from Cas9/gRNA complex delivery by the Neon Transfection System.

	Electroporation parameters (10 µL tips)			
Cell line	**Number of cells**	**24-well optimization protocol (program #)**	**Cas9/gRNA**	**Genomic cleavage efficiency****
Primary T cells	200 x 10^3	1,600 V/10ms/3 pulses (#24)	1,000 ng/240 ng	93% ± 1%
Jurkat	200 x 10^3	1,700 V/20 ms/1 pulse (#5)	1,500 ng/350 ng	94% ± 2%
K562	200 x 10^3	1,400 V/10 ms/3 pulses (#22)	1,000 ng/250 ng	91% ± 1%
THP-1	200 x 10^3	1,600 V/10 ms/3 pulses (#24)	1,000 ng/250 ng	31% ± 3%
SC-1	200 x 10^3	950 V/30 ms/2 pulses (#18)	1,000 ng/250 ng	44% ± 2%
Raji	200 x 10^3	1,600 V/10 ms/3 pulse (#24)	1,000 ng/250 ng	50% ± 5%
NK-92	200 x 10^3	1,400 V/10 ms/3 pulses (#22)	2,000 ng/500 ng	31% ± 5%
CD34+**	200 x 10^3	1,100 V/20 ms/2 pulses (#13)	1,000 ng/250 ng	24% ± 6%

* CD34+ human cord blood cells [13].

** The average and standard deviation were calculated based on the three highest-performing 24-well optimization protocols.

Human primary T cells were isolated from LeukoPak blood product from healthy donors using Ficoll-Paque PLUS medium and the Invitrogen Dynabeads Untouched Human T Cells Kit. The cells were then cultured in OpTmizer CTS T-Cell Expansion medium with 2% human serum, and activated with Dynabeads Human T-Expander CD3/CD28. The transfection experiments with the Neon system were performed three days after activation. Each cell line was maintained with medium, serum, and growth factors according to ATCC guidelines. Cell lines were prepared at a density of 2.0 x 10^5 for electroporation in Buffer R, with the indicated amounts of Cas9/gRNA complex targeting the HPRT-1 locus. The 24-well optimization protocols were performed using the 10 µL Neon tip and the cells dispensed into 0.5 mL prewarmed medium in a 24-well plate. Cells were harvested after 48 hours and prepared according to instructions for the Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit. They were then analyzed for efficiency of genomic cleavage following the kit protocol.

Table 4. Published cleavage efficiencies.

Cell line	Target locus	Neon protocol	Genomic cleavage efficiency	Reference
Primary T cells	CD45	1600 V/10 ms/ 3 pulses (#24)	86% ± 2%	14
CD34+	CD45	1600 V/10 ms/ 3 pulses (#24)	73% ± 16%	14
CD4+ T cells	CXCR4	1600 V/10 ms/ 3 pulses (#24)	55%	12

Please see the references for further information, including details of cell preparation, culture conditions, cell number, and the amount of RNP used in each electroporation.

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Conclusion

Finding ways to meet the challenges of introducing nucleic acids and proteins into blood cells extends the capabilities of scientists working to pioneer many exciting areas of research. The data presented here demonstrate that the Neon system facilitates efficient transfection of a wide range of blood cell lines as well as primary blood cells while providing for uncomplicated optimization of electroporation conditions. When the Neon Transfection System was used to deliver mRNA, 75–98% cells were transfected. In addition, published data show that delivery of DNA, RNA, and protein by electroporation for genomic editing has resulted in high genomic cleavage efficiency in many cell lines as well as primary T lymphocytes. Compared to lentiviral transfection, the Neon system is simpler, faster, and less costly. For primary T cells, the demonstrated effectiveness of the Neon system expands what until recently had been a markedly limited range of available delivery options. For all types of blood cells, transfection with the Neon system is a highly recommended alternative to the currently used standard approaches.

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Additional resources

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Visit the GeneArt Platinum Cas9 Nuclease and Lipofectamine CRISPRMAX website at **thermofisher.com/crisprprotein** for more information and results on genome editing with Cas9

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