Non-Viral Cell Engineering and Gene Delivery Optimization From Discovery to Clinical Applications Across Broad Cell Types

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Summary

- **Scalable performance** across the Neon NxT™ and CTS™ Xenon Electroporation Systems enables efficient non-viral payload delivery from small-scale testing to large-volume manufacturing.
- **Flexible optimization** of electroporation settings supports high performance across activated T cells, NK cells, and iPSCs.
- **Volume versatility:** The Neon NxT Electroporation System supports 10 µL and 100 µL formats, while the CTS Xenon Electroporation System enables electroporation up to 25 mL.
- **Cross-platform reproducibility:** Identical or equivalent programs across systems deliver comparable performance with minimal re-optimization.

Thermo Fisher Scientific Electroporation Systems

Neon NxT™ system	
Cell Concentrations	5–100 × 10^6 cells/mL
Electroporation Volume	10µL-100µL
Pulse Voltage	500-2500V
Pulse Duration	1-100ms
Electroporation Pulses	1-10 pulses
Pulse Interval	1ms

CTS™ Xenon system	
Cell Concentrations	20–100 × 10^6 cells/mL
Electroporation Volume	1mL or 5-25mL
Pulse Voltage	500-2500V
Pulse Duration	1-30ms
Electroporation Pulses	1-10 pulses
Pulse Interval	0.5-1ms

Introduction

Cell and gene therapies have emerged as powerful tools for treating cancer, autoimmune disorders, and genetic diseases(1). These approaches often rely on the ability to genetically modify immune or stem cells ex vivo, requiring robust, non-viral delivery platforms that can maintain efficiency and scalability from early-stage research through clinical manufacturing. Electroporation is a key enabling technology in this space, allowing delivery of DNA, RNA, or protein payloads into hard-to-transfect cell types without the complexity and limitations of viral systems(2). Thermo Fisher Scientific has developed two complementary electroporation platforms to address these needs: the research-scale Neon™ NxT Electroporation System and the GMP-compliant CTS™ Xenon™ Electroporation System. Together, these systems support optimization and scale-up of gene editing protocols across a range of clinically relevant cell types.

In this study, we evaluated platform performance in three critical cell types commonly used in immunotherapy and regenerative medicine:

- Activated T cells, where CRISPR-mediated knockout of the TRAC locus (T cell receptor alpha constant gene) eliminates endogenous TCR expression to generate allogeneic "off-the-shelf" CAR T cells and reduce the risk of graft-versus-host disease(3).
- NK cells engineered with CRISPR-mediated disruption of B2M (beta-2 microglobulin) show reduced MHC class I surface expression, which may improve persistence by limiting recognition by host T cells in adoptive immunotherapy (4).
- Edited iPSCs, where CD38 knockout improves persistence and functionality of stem cell–derived immune therapies, and targeted knock-in of a V5-tagged anti-MESO3 CAR transgene enables generation of engineered CAR T or CAR NK products(5).

These gene editing strategies have growing clinical relevance across a broad range of emerging therapies. Here, we evaluated knockout and knock-in performance across activated T cells, NK cells, and CAR-iPSCs to demonstrate that optimized electroporation programs developed on the Neon NxT system can be reliably translated to the CTS Xenon system. Despite differences in individual cell workflows, both platforms supported high editing efficiency, viability, and phenotype preservation, validating a scalable electroporation approach from research to clinical manufacturing.

Methods

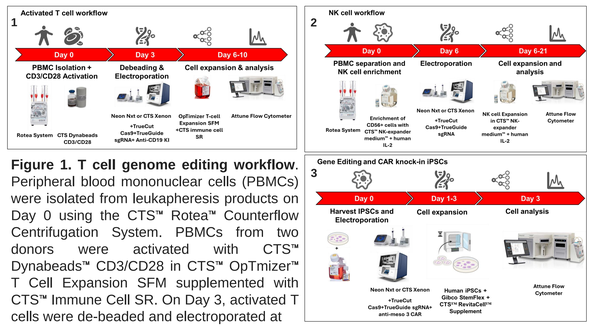


Figure 1. T cell genome editing workflow. Peripheral blood mononuclear cells (PBMCs) were isolated from leukapheresis products on Day 0 using the CTS™ Rotea™ Counterflow Centrifugation System. PBMCs from two donors were activated with CTS™ Dynabeads™ CD3/CD28 in CTS™ OpTmizer™ T Cell Expansion SFM supplemented with CTS™ Immune Cell SR. On Day 3, activated T cells were de-beaded and electroporated at 50 × 10^6 cells/mL using either the Neon™ NxT or CTS™ Xenon™ Electroporation System. Editing was performed with TrueCut™ Cas9 Protein v2 complexed with Invitrogen™ TrueGuide™ Synthetic sgRNA targeting the TRAC locus for TCR knockout, along with a donor dsDNA construct encoding a chimeric antigen receptor (CAR) for knock-in. Electroporation were conducted using a panel of programs spanning a predefined energy range: (1400 V, 10 ms, 3 pulses), (1400 V, 15 ms, 2 pulses), (1600 V, 20 ms, 1 pulse), (1700 V, 20 ms, 1 pulse), (2000 V, 15 ms, 1 pulse), (2300 V, 3 ms, 4 pulses), (2500 V, 2 ms, 5 pulses), and (2500 V, 3 ms, 3 pulses). A non-electroporated control was included. Programs were selected based on prior optimization for high-performance editing of activated T cells across both platforms. Post-electroporation, cells were expanded in culture and analyzed on Day 10 by flow cytometry to assess knockout and knock-in efficiency, cell viability, total viable edited cell number, and fold expansion. **Figure 2. NK cell genome editing workflow** CD56$^+$ NK cells were enriched from PBMCs of three healthy donor leukopaks and expanded in CTS™ NK-Xpander™ Medium supplemented with IL-2 and human serum. On Day 6 post-isolation, genome editing was performed using RNP complexes of CTS™ TrueCut™ Cas9 and a custom sgRNA targeting the B2M locus. Electroporation was conducted at 50 × 10^6 cells/mL using both the Neon™ NxT and CTS™ Xenon™ Electroporation Systems with an identical program: 1700 V, 20 ms, 1 pulse. Following electroporation, NK cells were cultured for 3 days and analyzed by flow cytometry for B2M knockout efficiency, NK cell purity, and viability. A separate experiment was performed using a higher density of 100 × 10^6 cells/mL on the CTS Xenon system to assess editing and viability under high-throughput conditions. **Figure 3. iPSC genome editing workflow.** Human iPSCs were cultured on CTS™ Vitronectin-coated plates in Gibco™ StemFlex™ Medium. At 70–80% confluency, the cells were harvested using CTS™ Versene™ Solution, washed with CTS™ DPBS, and electroporated at 1 × 10^7 cells/mL using either the Neon™ NxT 8-channel system with 10 µL tips or the CTS™ Xenon™ 1 mL SingleShot chamber. Editing was performed using a Cas9 RNP targeting the CD38 locus, along with a linear dsDNA donor encoding a V5-tagged anti-MESO3 CAR transgene driven by the EF1α promoter. Electroporation was conducted under equivalent concentrations and comparable conditions across both systems using the following program: 1200 V, 10 ms, 3 pulses. Post-electroporation, the cells were cultured in CTS™ StemFlex™ Medium supplemented with CTS™ RevitaCell™ Supplement for 24 hours, then returned to standard medium for 72 hours. Flow cytometry was used to assess viability, CAR-V5 knock-in expression, CD38 knockout efficiency, and expression of pluripotency markers (TRA-1-81, SSEA4).

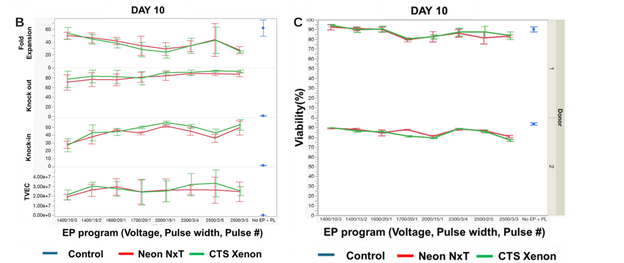
Results

Neon NxT programs seamlessly scale to CTS Xenon for T cell editing.

A

Evaluated Electroporation Programs for Neon NxT™/CTS™ Xenon		
Voltage	Pulse Width (ms)	Pulse #
1400	10	3
1400	15	2
1600	20	1
1700	20	1
2000	15	1
2300	3	4
2500	2	5
2500	3	3

Figure 4. Electroporation performance across Neon NxT and CTS Xenon in activated T cells. (A) Electroporation programs tested with varying voltage, pulse width, and pulse number, selected from prior optimizations. (B) TRAC knockout (%), CD19 CAR knock-in (%), fold expansion, and total viable edited cells (TVEC).(C) Cell Viability% Data from two donors, analyzed on Day 10



Consistent NK cell editing performance across Neon NxT and CTS Xenon platforms

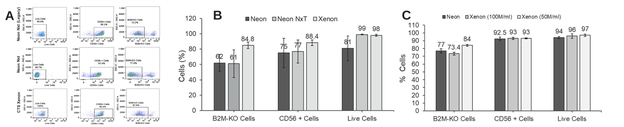
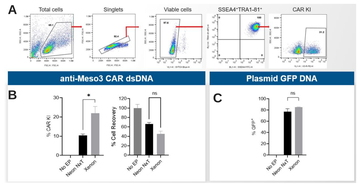


Figure 5. Editing efficiency, phenotype, and viability of NK cells across Neon NxT and CTS Xenon platforms. (A) Representative flow cytometry plots showing gating for live cells, CD56$^+$ cells, and B2M knockout (KO) populations following electroporation with Neon (legacy and NxT) or CTS Xenon. (B) Bar graph summarizing editing efficiency, CD56$^+$ expression, and viability across systems. (C) Editing outcomes at increased electroporation densities (100M and 50M cells/mL) using CTS Xenon. Data represent averages from three independent donors

Efficient genome engineering of iPSCs across Neon NxT and CTS Xenon platforms

Figure 6. Gene editing and transfection of CAR-iPSCs using Neon NxT and CTS Xenon systems. (A) Gating strategy for total, singlet, viable, pluripotent (SSEA4$^+$TRA-1-81$^+$), and CAR knock-in (V5$^+$) iPSCs. (B) CAR knock-in efficiency and cell recovery following electroporation with anti-MESO3 CAR dsDNA. (C) GFP transfection efficiency using a plasmid DNA control. Data from one representative experiment.



Gene-edited iPSC clones maintain pluripotency and stable CAR expression.

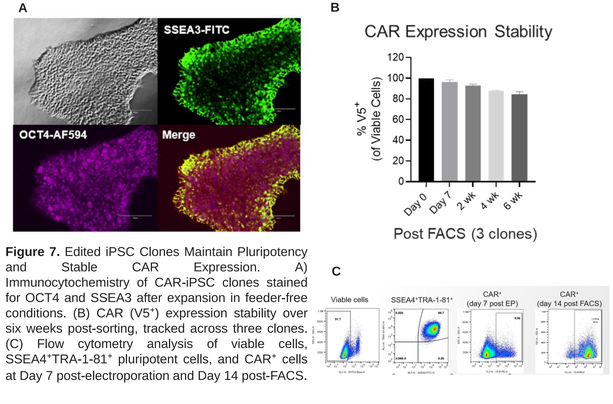


Figure 7. Edited iPSC Clones Maintain Pluripotency and Stable CAR Expression. A) Immunocytochemistry of CAR-iPSC clones stained for OCT4 and SSEA3 after expansion in feeder-free conditions. (B) CAR (V5$^+$) expression stability over six weeks post-sorting, tracked across three clones. (C) Flow cytometry analysis of viable cells, SSEA4$^+$TRA-1-81$^+$ pluripotent cells, and CAR$^+$ cells at Day 7 post-electroporation and Day 14 post-FACS.

Conclusions

Electroporation programs optimized on the Neon NxT system were successfully translated to the CTS Xenon platform, enabling consistent delivery of CRISPR-Cas9 RNP and DNA payloads across multiple cell types with minimal re-optimization. In activated T cells, matched programs achieved robust TRAC knockout and CD19 CAR knock-in, with high viability, reproducible fold expansion, and consistent total viable edited cell (TVEC) output across donors. In NK cells, B2M knockout was efficiently achieved using both Neon NxT and CTS Xenon, with preservation of the CD56$^+$ phenotype and high post-editing viability at both standard and high cell densities (50 million and 100 million cells/mL). In CAR-iPSCs, delivery of a MESO3 CAR dsDNA donor resulted in high knock-in efficiency and CD38 knockout, with maintenance of pluripotency marker expression (SSEA4$^+$/TRA-1-81$^+$) following editing. Long-term CAR expression was stable across iPSC clones for up to six weeks post-sorting. Collectively, these data support a reproducible, non-viral genome editing workflow that is scalable from research optimization to clinical manufacturing, supported by flexible platform compatibility, high-density processing, and consistent biological performance.

References

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