Optimizing non-viral genome editing workflow for primary activated T cells with advanced electroporation technology

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Key Takeaways

- The Neon NxT 8-Channel Pipette allows for rapid and efficient optimization of electroporation parameters, crucial for high genome editing efficiency and reproducibility in T cells.
- The scalability of electroporation parameters from the Neon NxT to the CTS Xenon Electroporation Systems ensures reliable translation from small-scale experiments to larger-scale manufacturing processes.
- The study highlights the importance of donor material, showing variability in editing efficiency across different donors. This underscores the need for consistent optimization to achieve high editing efficiency in diverse donor samples.

Introduction

Electroporation is a key technique in molecular biology and genetic engineering for introducing genetic material into cells by applying short, high-voltage electrical pulses to create temporary pores in the cell membrane, allowing efficient delivery of DNA, RNA, or other molecules. It is highly effective for gene modifications, including knockouts (KO), knock-ins (KI), and gene editing.

The Invitrogen™ Neon™ Transfection System is a trusted tool for electroporation, with the enhanced Neon™ NxT Electroporation System offering improved usability and precise control, along with the TransfectionLab™ Cloud Application for experimental design. The Neon NxT 8-Channel Pipette further enhances efficiency by allowing the processing of up to eight samples at once with different electroporation parameters, significantly increasing throughput and optimizing workflows.

We demonstrate the optimization of electroporation parameters for activated primary T cells using the Neon NxT 8-Channel Pipette. This process is crucial for reliable and reproducible genome editing results and is facilitated by the pipette's user-friendly interface.

Optimization results from the Neon NxT System are valuable for CAR-T cell therapies. Thermo Fisher Scientific's Gibco™ CTS™ Xenon™ Electroporation System integrates seamlessly into existing workflows, enabling closed-system, nonviral genetic material delivery, reducing manufacturing risk, time, and complexity. This system ensures consistent genome editing workflows, scalable from research to large-scale production, highlighting the utility of the 8-Channel Pipette for T cell editing and its application to clinical CAR-T therapy development.

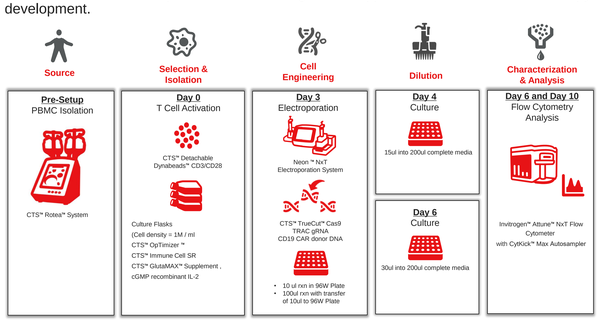

Figure 1. T cell Editing Workflow for the Neon ™ NxT Electroporation System

Materials and methods

PBMCs were isolated from fresh leukapheresis products from healthy donors using the peripheral blood mononuclear cells (PBMCs) were isolated from fresh leukapheresis products obtained from healthy human donors using the CTS™ Rotea™ System and were subsequently cryopreserved for later use. When needed, the cryopreserved PBMCs were thawed and activated using CTS™ Detachable Dynabeads™ CD3/CD28 at a ratio of 1:3 (cells:beads). Following activation, the cells were de-beaded using a Dynamag-50 magnet before proceeding with electroporation.

For electroporation, a cell density of 50 million cells per milliliter was maintained. The Resuspension Genome Editing Buffer was used for all experiments unless specified otherwise. The payload included 120 µg/mL Gibco™ CTS™ HiFi Cas9 Protein, 32 µg/mL TRAC TrueGuide™ gRNA, and 240 µg/mL Anti-CD19 CAR. Prior to electroporation, the activated PBMCs were cultured in T-175 flasks at a density of 1 million cells per milliliter for three days.Post-electroporation, the cells were cultured in 96-well plates at an approximate density of 2.5 million cells per milliliter. The cell density was adjusted with fresh complete media on days 4 and 6, which correspond to 24 hours and 72 hours post-electroporation. The complete media composition included 89.4% OpTmizer™ T-Cell Expansion Basal Medium , 2.6% OpTmizer™ T-Cell Expansion Supplement, 5% CTS™ Immune Cell Serum Replacement, 3% GlutaMAX™ Supplement (6mM), and 100 UI/mL Gibco PeproGMP® Human IL-2 Recombinant Protein.

Characterization and analysis of the cells were performed using flow cytometry on days 6 and 10. The cells were analyzed for viability, recovery, and knockout/knock-in efficiency using the Attune™ NxT Flow Cytometer.

Results

Enhanced genome editing with Resuspension Genome Editing Buffer

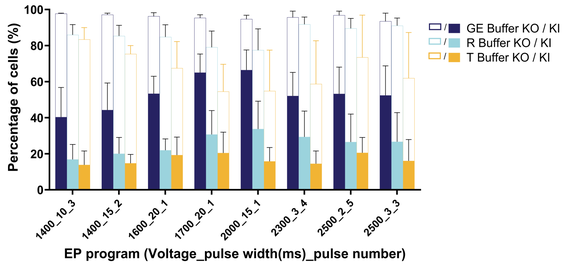

Figure 2. Comparison of electroporation buffer types on activated T cell editing across different electroporation programs. Cells were resuspended in either R, T, or Genome Editing (GE) buffer prior to performing electroporation and efficiency was measured.

Neon NxT 1-Channel and 8-Channel Pipettes: Same Platform, Same Performance

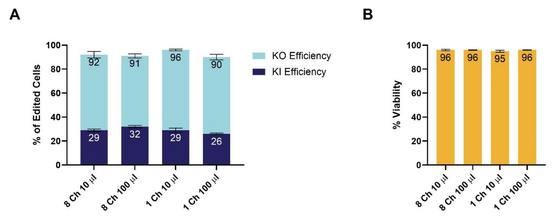

Figure 3. Genome editing performance of Neon NxT 1-Channel and 8-Channel Pipettes A. KO/KI efficiency B. Viability.

Results

Reproducibility of electroporation program performance across multiple donors

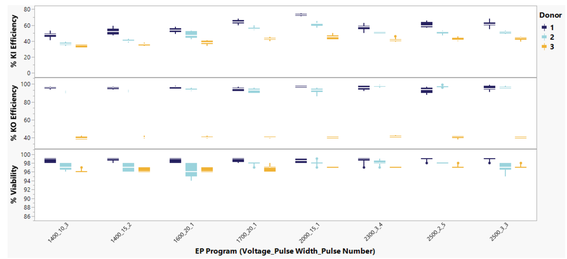

Figure 4. Editing efficiency across three different donors for 7 different programs. The program 2000V/15ms/1 pulse achieved the highest knock-in efficiency and optimal cell viability across all donors, though significant variability was noted, with Donor 3 showing much lower knockout efficiency. These findings underscore the critical impact of the PBMC material source on editing efficiency.

Neon NxT to Xenon Scalability assessment

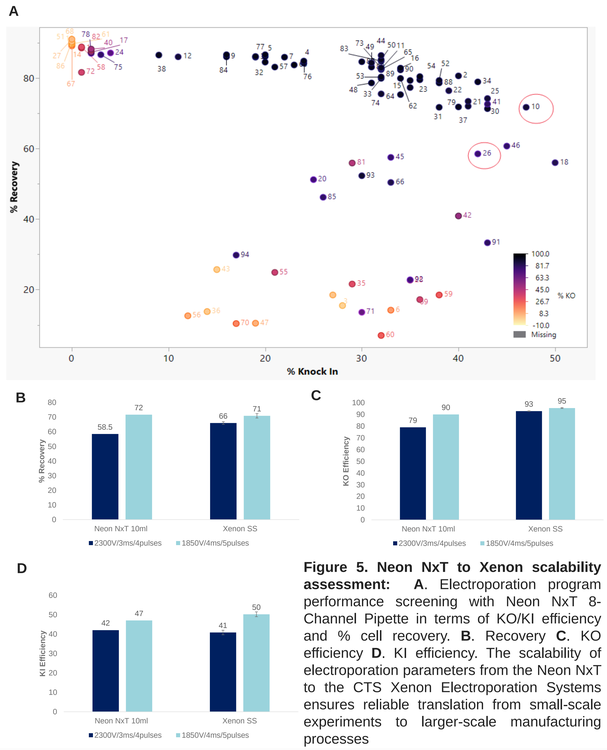

Figure 5. Neon NxT to Xenon scalability assessment: A. Electroporation program performance screening with Neon NxT 8-Channel Pipette in terms of KO/KI efficiency and % cell recovery. **B**. Recovery **C**. KO efficiency **D**. KI efficiency. The scalability of electroporation parameters from the Neon NxT to the CTS Xenon Electroporation Systems ensures reliable translation from small-scale experiments to larger-scale manufacturing processes

Results

Day 6 vs Day 10 characterization:

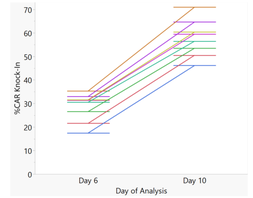

Figure 6: Knock-in efficiency in Day 6 vs Day 10 analysis. The observed increase in CAR+ T cell knock-in efficiency from day 6 to day 10 could be due to several factors. By day 10, more successfully edited cells may be present due to ongoing expansion and proliferation, and CAR expression might take time to reach optimal levels, becoming more detectable. Additionally, non-edited cells may die off or be outcompeted, increasing the proportion of CAR+ cells.

No toxicity from payload and Resuspension Genome Editing Buffer

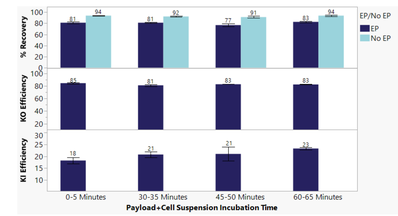

Figure 7. Editing efficiency with different payload and Genome Editing buffer incubation time. The Neon NxT 8-Channel Pipette efficiently processes large sample sizes, and incubation of cells in GE buffer with payload for up to an hour before electroporation does not harm cell recovery or editing performance. Extended incubation may even enhance knock-in efficiency.

Conclusions

The Neon NxT Electroporation System demonstrates remarkable versatility for fast optimization of electroporation parameters. The scalability of the system from small-scale to larger-scale applications with the CTS Xenon Electroporation System, ensures consistent and efficient workflows. The ability to achieve high editing efficiency, particularly with the GE buffer, and the reproducibility of results across multiple donors underscores the system's robustness and reliability. This positions the Neon NxT System and CTS Xenon System as a powerful family of tools for both scaled-out research and scaled-up clinical process development in cell therapy workflows.

Acknowledgements

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