Optimizing non-viral genome editing workflows for induced pluripotent stem cells with advanced electroporation technology

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Key Takeaways

- **The development of versatile and efficient non-viral based cell engineering workflows using electroporation (EP) delivery method has significant implications in cell and gene therapy field.**
- **The Neon NxT Electroporation System with 8-Channel Pipette enables rapid and efficient assessment of electroporation condition to achieve high genome editing efficiency of cells of interest.**
- **This study highlights the importance of optimizing electroporation conditions to achieve optimal knock-in efficiency balanced with superior CAR-iPSC recovery with CultureCEPT™ supplement.**
- **Our findings demonstrate the potential of CultureCEPT™ supplement in cell engineering applications.**

Abstract & Introduction

Induced pluripotent stem cells (iPSCs) have become an essential research platform to study various human diseases and hold significant potential in disease modelling, drug discovery and cell therapy applications. The CRISPR/Cas9 system represents another breakthrough in biomedical research, enabling precise genome editing in mammalian cells. In our current workflow, we utilized the Neon™ NxT Electroporation System with the 8-Channel Pipette for rapid optimization of electroporation parameters for editing iPSCs. This system is a powerful tool for optimization of electroporation programs and cell engineering conditions for primary cells and cell lines.

We demonstrated the system's performance in efficiently optimizing CRISPR/Cas9-based iPSCs cell engineering. Using the Neon™ NxT Electroporation System, we generated chimeric antigen receptor (CAR)-iPSCs by efficiently delivering CD38-loci targeted anti-Meso3 donor DNA and CTS™ HiFi™ Cas9 and TrueGuide™ Synthetic gRNA to iPSCs. Editing efficiency and cell viability were measured using flow cytometry and Next Generation Sequencing.

Our results highlight the ability to simultaneously test multiple electroporation conditions, significantly reducing the time required for optimization experiments. Furthermore, we evaluated different electroporation buffers and achieved up to 30% CAR knock-in efficiency in iPSCs. Through systematic screening of voltage, pulse duration, and pulse number, we optimized the payload delivery while maintaining high cell viability in presence of CultureCEPT™ supplement. Utilization of CultureCEPT™ supplement for post genome editing iPSC recovery resulted in improved cell viability and higher total CAR-iPSC number.

In summary, the Neon™ NxT Electroporation System with the 8-channel pipette has proven to be an effective tool not only for the rapid and efficient optimization of non-viral based genome editing, but also for engineered iPSC manufacturing workflows. This capability significantly advances iPSC-based research and cell therapy applications, addressing scalability challenges and improving patient outcomes.

Material & Methods

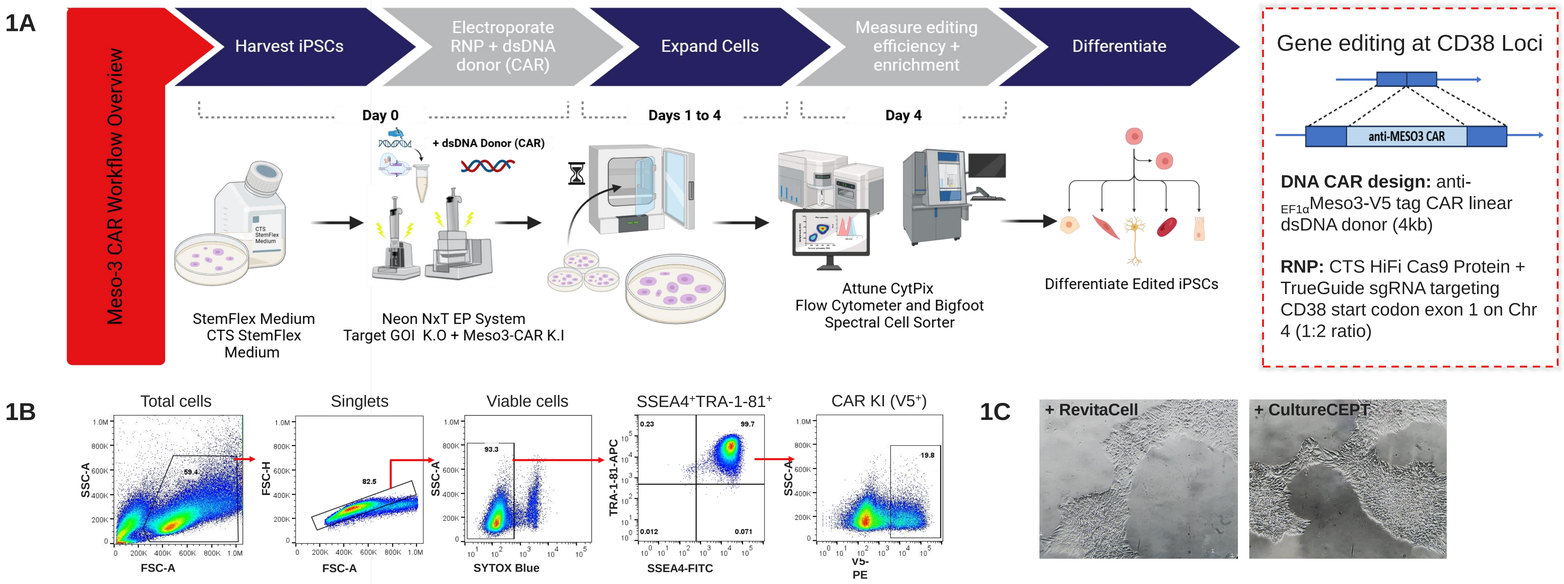
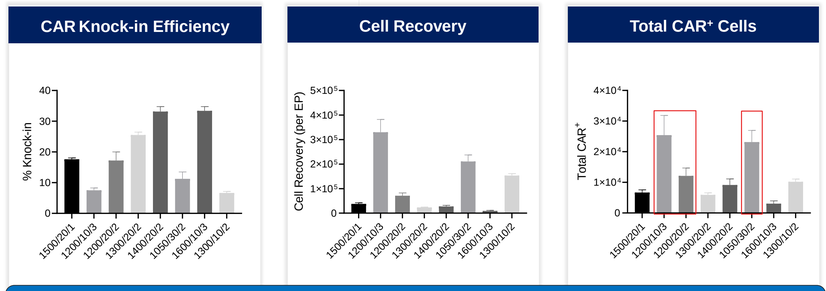


Figure 1. iPSC cell engineering protocol workflow diagram

A. Human iPSCs (TFSi001-A) were grown in Gibco™ CTS™ StemFlex™ Medium (cat#A5465001) in Gibco™ CTS™ Vitronectin (cat#CTS279S3)-coated culture dishes. At the confluency of 70-80%, the cells were harvested using CTS™ Versene™ Solution (cat#A4239101), washed once with Gibco CTS DPBS (cat#A1285801) to prepare for electroporation. Genome editing was performed using CTS HiFi Cas9 , TrueGuide™ Synthetic Guide RNA target CD38 loci and anti-$_{EF1\alpha}$Meso3-CAR (V5-tagged) dsDNA payloads. Neon™ NxT 8 channel EP system and 10 µl tips were used to deliver gene editing payloads to 10e6 cells/mL in R buffer. After electroporation, cells were cultured in the same condition medium supplemented with Gibco™ CTS™ RevitaCell™ Supplement (cat#A4238401) for 24 hours. Next day the medium was replaced without RevitaCell™ Supplement and cells were maintained in culture for three more days. **B.** 4 days post EP cells were analyzed for viability, recovery and anti-Meso3-CAR knock-in efficiency (%V5 positive cells) using Attune™ CytPix™ Flow Cytometer. SYTOX™ Blue Dead Cell Stain (cat#S34857) was used to measure cell viability. The iPSCs were stained with anti-V5 tag-PE (cat#12-6796-42) to quantify CAR knock-in efficiency and with anti-TRA-1-81-APC (cat#17-8883-42) and anti-SSEA4-FITC (cat#53-8843-42) antibodies to confirm pluripotency. The cells were stained in eBioscience™ Flow Cytometry Staining Buffer (cat#00-4222-26) at 4ºC for 40 minutes prior to flow cytometry. **C.** Representative images of CAR-iPSC recovered in presence of CultureCEPT or RevitaCell. Images were taken on day 4 post EP using EVOS™ M5000 Imaging System.

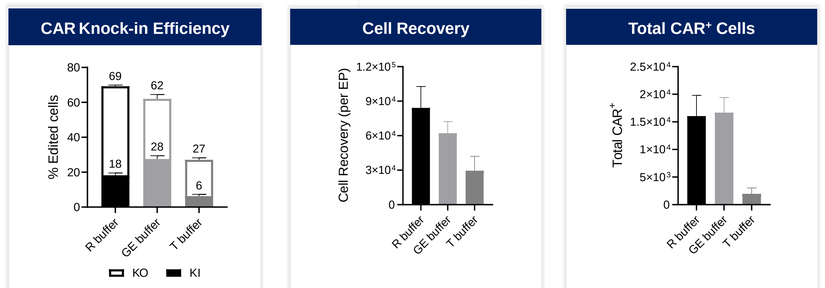
Results

2A. Neon NxT electroporation program optimization



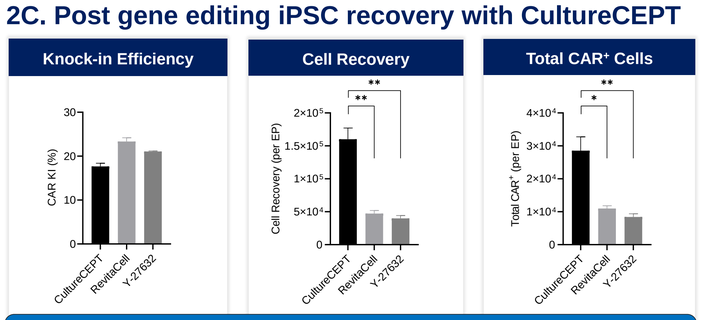
Optimal electroporation parameters to knock in CAR in hiPSC: 1200V 10ms 3pls; 1200V 20ms 2pls; 1050V 30ms 2pls

2B. Neon NxT electroporation buffer comparison



Genome Editing resuspension buffer could improve CAR knock-in in iPSC compared to R buffer

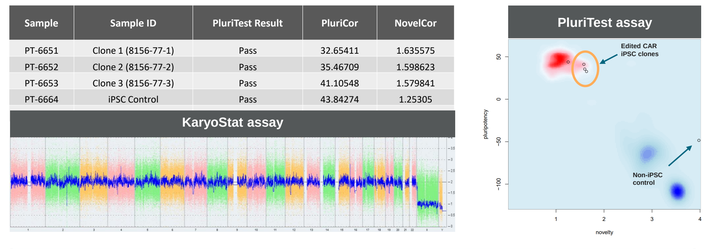
2C. Post gene editing iPSC recovery with CultureCEPT



CultureCEPT™ improves post genome editing iPSC recovery

2D. Characterization of CAR-iPSCs

Sample	Sample ID	PluriTest Result	PluriCor	NovelCor
PT-6651	Clone 1 (8156-77-1)	Pass	32.65411	1.635575
PT-6652	Clone 2 (8156-77-2)	Pass	35.46709	1.598623
PT-6653	Clone 3 (8156-77-3)	Pass	41.10548	1.579841
PT-6664	iPSC Control	Pass	43.84274	1.25305



All CAR-iPSC monoclonal lines maintained pluripotency with identical genetic background and no chromosomal aberrations

Figure 2. Optimization of electroporation conditions for CAR knock-in in hiPSC

A. Several preselected electroporation parameters were assessed to evaluate CAR knock-in efficiency, total viable cell number and total CAR-iPSC positive cells recovered four days post electroporation. 10e6/mL cells were electroporated with Cas9 RNP + 50 µg/mL anti-Meso3 CAR payload using 10 µL tips and R buffer. **B.** Different Neon NxT buffers (R, GE and T) were evaluated at selected EP condition (1200V 20ms 2pls) and post EP, % editing efficiency, total viable cell number and total CAR-iPSC positive cells were analyzed (n≥3). **C.** Post genome editing cell recovery in presence of CultureCEPT™ vs Y-27632 or RevitaCell. The analysis represents CAR knock-in efficiency, total viable cell number and total CAR-iPSC positive cells recovered on day 4 post electroporation. CAR knock-in was performed using 1200V 20ms 2pls electroporation parameters in GE buffer. Electroporated cells were cultured in Gibco™ CTS™ StemFlex™ Medium supplemented with either Gibco™ CultureCEPT™, CTS™ RevitaCell™ Supplement or ROCK inhibitor Y-27632 from another vendor for 24 hours (n≥3). Cells recovered using media supplemented with CultureCEPT showed better recovery and Total CAR positive cells. **D.** Characterization of CAR-iPSCs after clonal selection with KaryoStat assay to assess genomic stability and by PluriTest assay to confirm CAR-iPSC pluripotency and genetic background.

Conclusions

The Neon NxT Electroporation System with 8-channel pipette demonstrates remarkable versatility in rapidly optimizing electroporation conditions ensuring consistent and efficient workflows. The ability of Neon NxT Electroporation System to achieve high editing efficiency along with efficient cell recovery in presence of CultureCEPT™ supplement underscores the system's robustness and reliability for non-viral CAR-iPSC engineering applications.

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