Optimizing iPSC genome editing

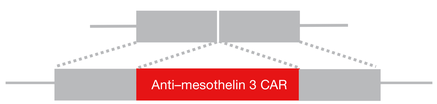
with advanced electroporation technology

Olga Cohen, Ranga Somasagara, Rateb Mir, Greg Bonello, Sung Lee, Vivek Chandra, Namritha Ravinder
Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, CA 92008, USA

Abstract

Induced pluripotent stem cells (iPSCs) are powerful tools for disease modeling and cell therapy, but efficient nonviral genome editing remains challenging. Using the Neon NxT Electroporation System with 8-Channel Pipette, we rapidly optimized electroporation parameters for delivering anti–mesothelin 3 CAR donor DNA plus RNP payload into iPSCs, achieving up to 30% stable knock-in efficiency with high cell viability. Systematic testing of voltage and pulse settings, along with CultureCEPT Supplement for post-editing recovery, enabled reproducible and scalable CAR-iPSC generation. Overall, this workflow supports a fast, reliable, and efficient platform for nonviral iPSC engineering and therapeutic cell manufacturing.

Gene editing at the CD38 locus



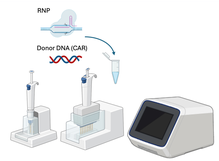
Donor DNA (4 kb): Encodes anti–mesothelin 3 CAR (V5-tagged), which targets a specific epitope present on solid tumors

Ribonucleoprotein (RNP): Cas9 protein plus synthetic guide RNA (gRNA) targeting the CD38 start codon (1:2 ratio)

Workflow overview for CAR-iPSC generation

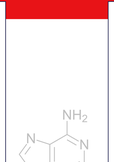
Harvest iPSCs

- Human iPSCs grown in Gibco™ StemFlex™ or CTS™ StemFlex™ Medium on dishes coated with Gibco™ rhLaminin-521 or CTS™ Vitronectin
- iPSCs harvested manually or with the closed Gibco™ CTS™ Rotea™ Counterflow Centrifugation System at 70–80% confluency



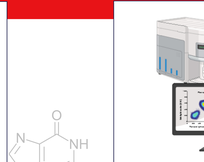
Electroporate cells

- RNP prepared with Gibco™ CTS™ HiFi Cas9 Protein and Invitrogen™ TrueGuide™ Synthetic gRNA
- RNP plus donor DNA encoding anti–mesothelin 3 CAR delivered to 10^5 cells with the Invitrogen™ Neon™ NxT Electroporation System with 1-Channel or 8-Channel Pipette



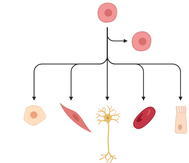
Expand cells

- iPSCs cultured in original medium with Gibco™ RevitaCell™ Supplement or CultureCEPT™ Supplement for 24 hours
- Medium replaced without a supplement and iPSCs maintained for 3 more days



Measure editing efficiency; enrich or sort cells

- CAR knock-in efficiency, cell recovery, and total CAR⁺ iPSCs analyzed with the Invitrogen™ Attune™ CytPix™ Flow Cytometer
- Cell enrichment or single-cell clonal sorting performed using the Invitrogen™ Bigfoot™ Spectral Cell Sorter



Differentiate

- Differentiation of CAR-iPSCs into functional cells such as iPSC-derived natural killer (iNK) cells using Gibco™ PeproGMP™ growth factors and cytokines, Gibco™ CTS™ StemPro™-34 Medium, and Gibco™ CTS™ NK-Xpander™ Medium

Day 0 — **Days 1–4** — **Day 4**

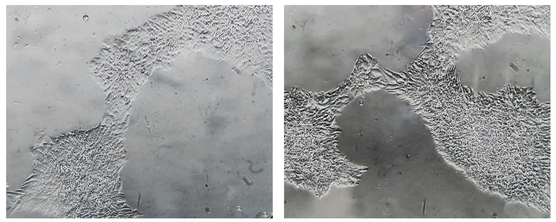
Key findings

The Neon NxT Electroporation System with 8-Channel Pipette enables rapid and efficient optimization of electroporation parameters for genome editing of induced pluripotent stem cells (iPSCs)

Efficient knock-in of chimeric antigen receptor (CAR) donor DNA is achieved at the CD38 locus with the CRISPR-Cas9 system

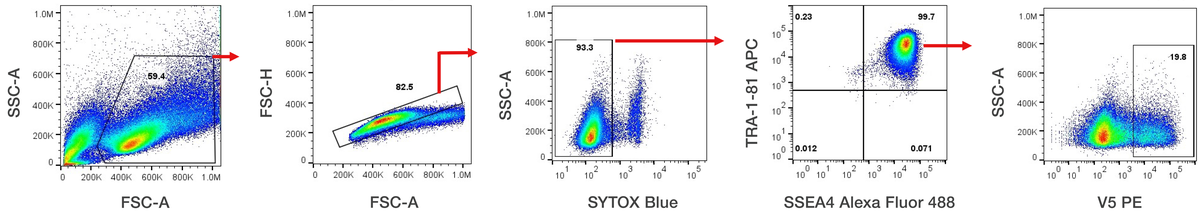
Improved cell viability and higher total CAR-iPSC number are observed after recovery from electroporation with CultureCEPT Supplement

Verification of CAR-iPSC morphology



- Representative images of recovered CAR-iPSCs were taken using the Invitrogen™ EVOS™ M5000 Imaging System on day 4 post-electroporation

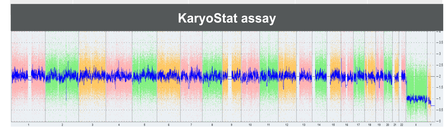
Representative flow cytometry analysis of CAR-iPSCs

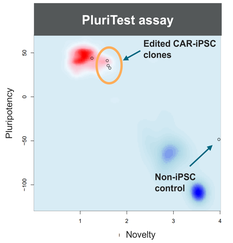


- Cell viability was measured with Invitrogen™ SYTOX™ Blue Dead Cell Stain
- Pluripotency was verified with Invitrogen™ eBioscience™ TRA-1-81 Monoclonal Antibody (APC conjugate) and SSEA4 Monoclonal Antibody (Invitrogen™ Alexa Fluor™ 488 conjugate)
- CAR knock-in efficiency was measured with Invitrogen™ eBioscience™ V5 Tag Monoclonal Antibody (PE conjugate)

Genomic stability and pluripotency of iPSCs are maintained

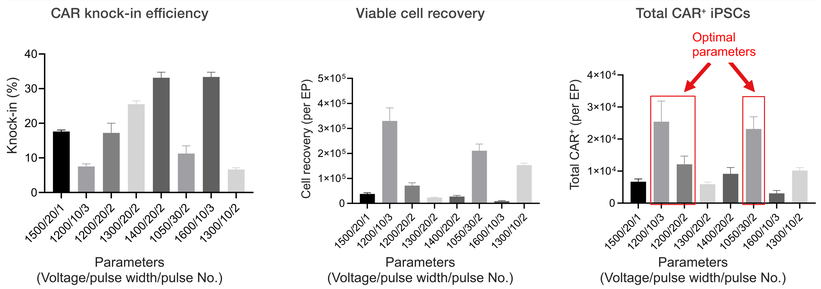
Sample	Sample ID	PluriTest assay	Pluripotency score	Novelty score
PT-6651	Clone 1 (8156-77-1)	Pass	32.65411	1.635575
PT-6652	Clone 2 (8156-77-2)	Pass	35.46709	1.598623
PT-6653	Clone 3 (8156-77-3)	Pass	41.10548	1.579841
PT-6664	iPSC control	Pass	43.84274	1.25305





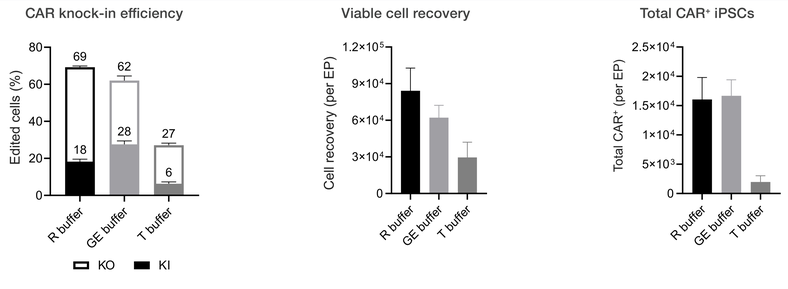
- Genomic stability of CAR-iPSC monoclonal lines was verified with the Applied Biosystems™ KaryoStat™ assay
- Pluripotency and genetic background were confirmed with the PluriTest™ assay

Rapid determination of optimal parameters



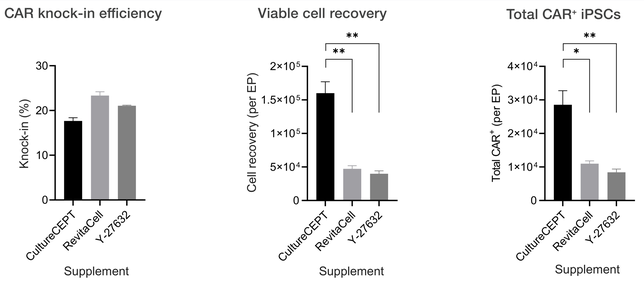
- Preselected electroporation parameters were assessed to evaluate CAR knock-in efficiency, total viable cell recovery, and total CAR⁺ iPSCs
- Data represent iPSCs recovered on day 4 post-electroporation in R buffer (n ≥3)

Enhanced KI efficiency with genome editing (GE) buffer



- Different Neon NxT buffers (R, GE, and T) were evaluated for knockout (KO) and knock-in (KI) efficiency, total viable cell recovery, and total CAR⁺ iPSCs
- The optimal parameters of 1,200 V, 20 ms, 2 pulses were used for electroporation (n ≥3)

Improved iPSC recovery with CultureCEPT Supplement



- After electroporation using the optimal conditions of 1,200 V, 20 ms, and 2 pulses in GE buffer, iPSCs were cultured with the indicated supplements for 24 hours
- Data represent iPSCs recovered on day 4 post-electroporation (n ≥3); differences compared to CultureCEPT Supplement are indicated as * p <0.05, ** p <0.01 (t-test)

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Acknowledgements: We would like to acknowledge Shahan Mistla, Arseny Smirnov, Mark Kennedy, and Jacquelyn McGlenny, members of the cell and gene therapy team at Thermo Fisher Scientific, for their support in acquiring and processing the data for this poster. Workflow images were created with BioRender.com.

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