Advancing cell therapy manufacturing through scalable electroporation-based gene editing of CD34+ HSCs, NK, and T cells

Lonza
Specialized Modalities

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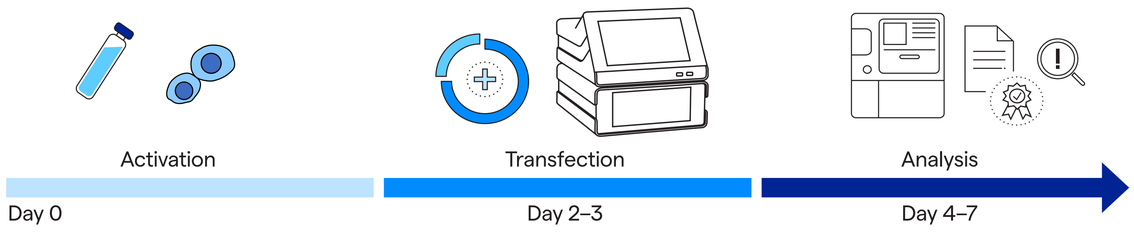
Introduction

Non-viral gene editing technologies have become attractive alternatives to viral transduction for cell therapy manufacturing, offering cargo flexibility and reduced safety concerns. Among these, electroporation is recognized as the gold standard and increasingly adopted in clinical applications, including the first approved CRISPR-based cell therapy product, Casgevy®. However, electroporation approaches have historically faced challenges in scalability and process optimization, often resulting in lengthy development cycles and reduced efficiency at large volumes. To overcome these limitations, we developed an improved cartridge design for our large-volume electroporation platform, enabling efficient delivery of complex cargos into up to 20 mL of cell suspension.

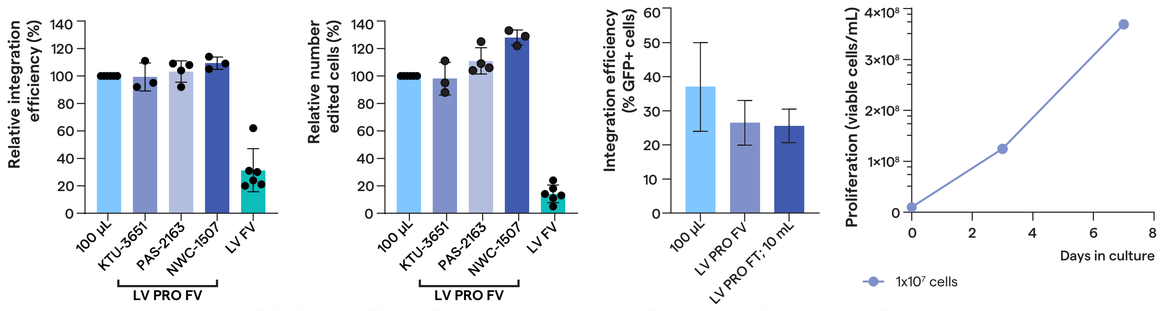
With our **4D-Nucleofector® LV Unit PRO** we provide:

- An optimized cartridge design for improved performance
- Efficient electroporation of complex cargos like CRISPR/Cas9 or Transposon/Transposase
- Easy scale up from 100 µl to up to 20 mL
- Electroporation of large volumes with up to 2.0 x 10^9 cells
- Flexible volumes in fixed volume cartridge (0.5, 1.0, 1.5 or 2.0 mL)
- Easily compatible with cell densities from 2.5x $10e^7$ to $1×10e^8$/mL

T cells

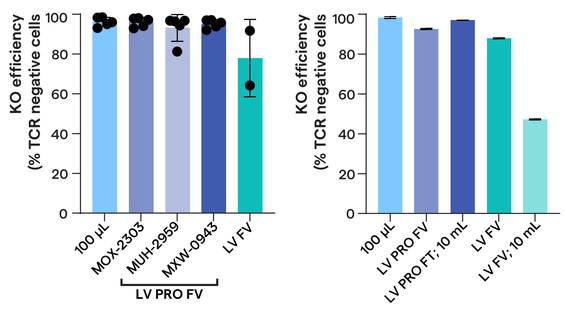


Scalability – Transposon/Transposase (piggyBac®)



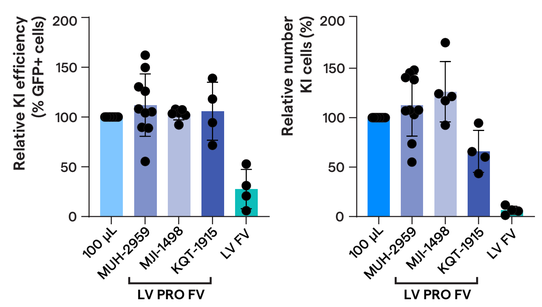
Relative efficiency of LV PRO FV and FT for Transposase mRNA (43 µg/mL) + GenCircle™ Transposon (100 µg/mL; n = 2 donors)

Scalability – *TRAC* KO



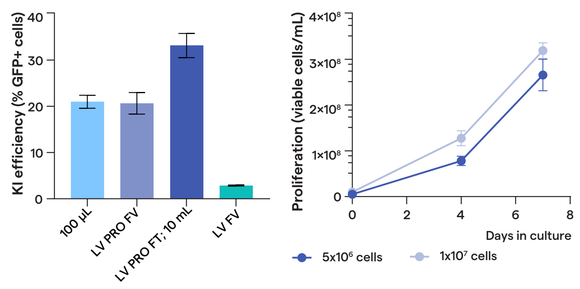
KO in the constant region of TCRα (*TRAC*) with 0.77 µM Cas9 and sgRNA in ratio 1:2 (for FV n = 4 donors; for FT n = 1 donor)

Scalability – *TRAC*-GFP KI

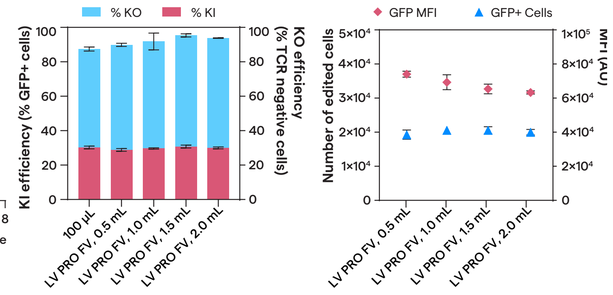


TRAC KI with 0.77 µM Cas9 and sgRNA in ratio 1:2 plus 50 nM dsDNA HDRT (n = 6 donors)

Scalability from 100 µl to 10 mL – *TRAC*-GFP KI

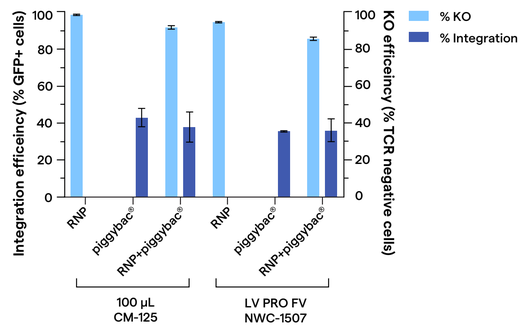


Scalability – Different input volumes



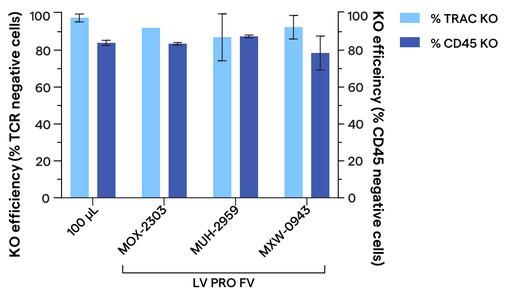
Editing with 0.77 µM Cas9 and sgRNA in ratio 1:2 plus 50 nM dsDNA HDRT (n = 2 donors)

TRAC KO + piggybac® Transposon System



Editing efficiency with Transposase mRNA (86 µg/mL) + GenCircle™ Transposon (200 µg/µL) and *TRAC* KO (0.77 µM RNP) (n = 2)

CRISPR/Cas9 Double KO of *TRAC* and *CD45*



Efficiency with *TRAC* KO + *CD45* KO for 0.77 µM Cas9 + sgRNA in ratio 1:2 each (n = 2 donors)

Material and methods

Cell material: *T cells* – Cryopreserved human PBMC (Lonza) or CD3+ Pan T Cells (Lonza), activated with TransAct™ (Miltenyi; 3 days) or Dynabeads (Thermo Fisher Scientific®; 2 days); *CD34+* – Cells were isolated from a fresh mobilized leukopak and used fresh or cryopreserved; *NK cells* – Isolation from CD34-depleted leukopak using negative isolation (Miltenyi), expansion w/ or w/o LCL-WEI for 12 – 14 days.

Analysis: *T/NK cells* – TCR alpha (*TRAC* KO), *CD45* KO and/or GFP (CRISPR KI) expression was evaluated by flow cytometry (NovoCyte, Agilent). *CD34+* – gDNA was isolated and *BCL11A* locus amplified using PCR, editing detection via Sanger sequencing.

Transfection: Cells were resuspended in P3 Nucleofector® Solution, cargo was added, the mixture transferred into the required Nucleofector® Vessel and transfected.

Abbreviations:

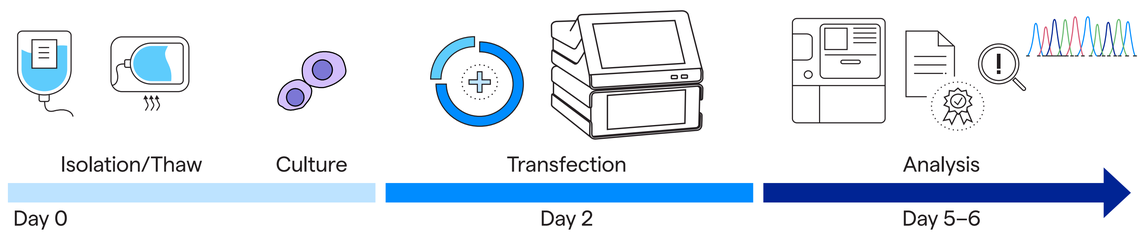
100 µL – 100 µL Nucleocuvette® Vessel

LV PRO FV – Fixed volume 2 mL Nucleocuvette® Cartridge PRO

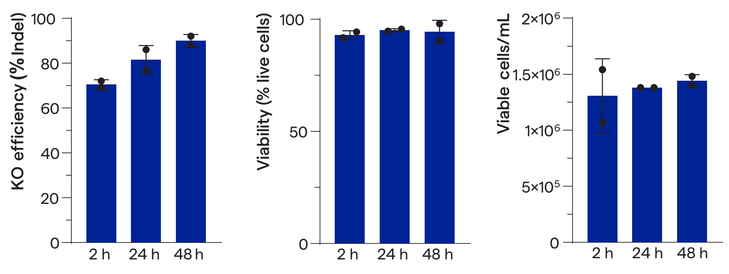
LV PRO FT – Flow-through LV Nucleocuvette® Cartridge PRO

LV FV – Fixed volume 1 mL Nucleocuvette® Cartridge (1st generation)

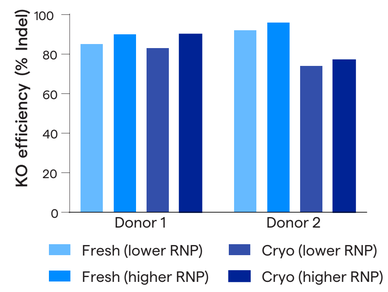
CD34+ HSCs



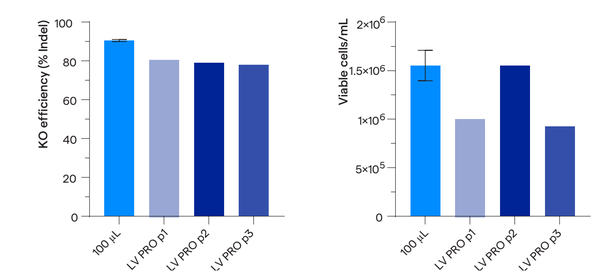
Optimization with 100 µL cuvette



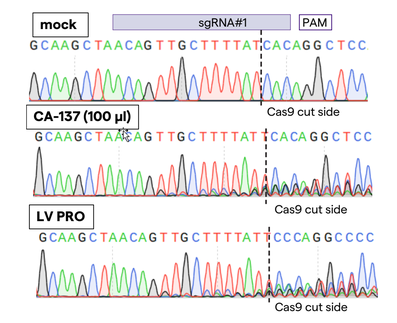
Starting material



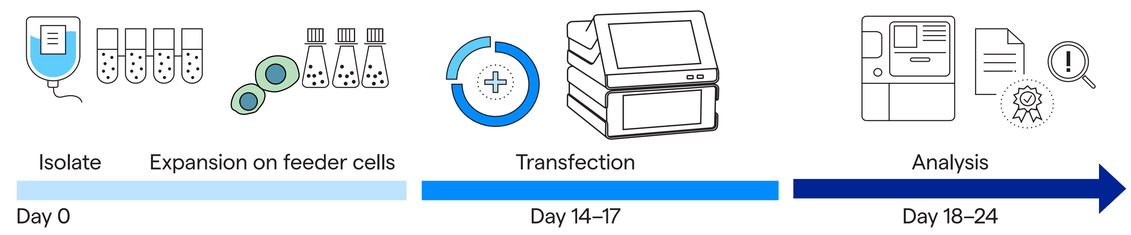
Scalability – *BCL11A* KO



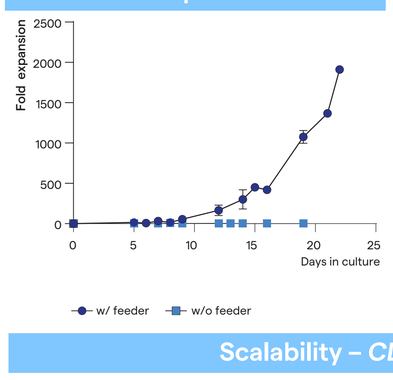
KO of *BCL11A* with 1.25 µM Cas9 and sgRNA in ratio 1:4 (n = 1)



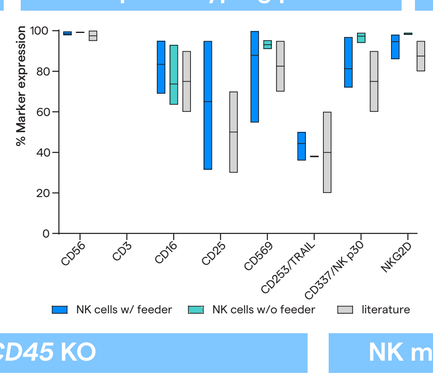
NK cells



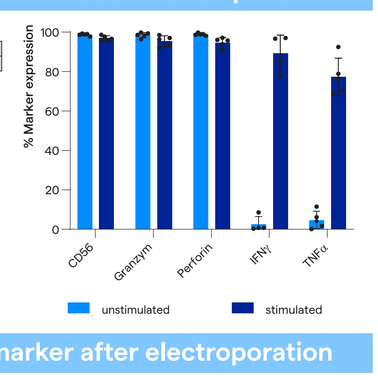
NK expansion



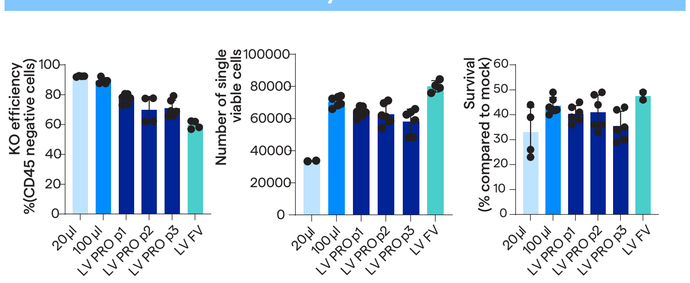
NK phenotyping panel



NK activation panel

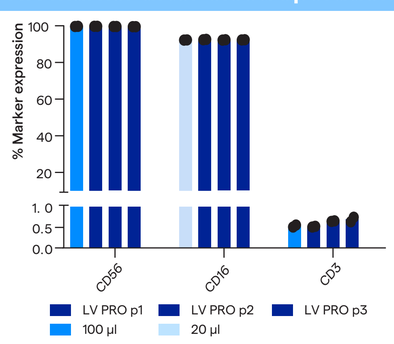


Scalability – *CD45* KO



KO of *CD45* with 1.54 µM Cas9 and sgRNA in ratio 1:2 (n = 3)

NK marker after electroporation



Summary

The next generation 4D-Nucleofector® LV Unit PRO offers

- Reliable, robust and efficient delivery of complex, clinically relevant cargos
- Easy scale up of cell engineering up to 1 billion cells
- Applications protocols for T cells, NK cells and CD34+ HSC

Thus enabling non-viral manufacturing of cell therapy products

Learn more.

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