Non-viral and large-scale delivery of gene editing tools for T-cell manufacturing

Lonza
Specialized Modalities

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Introduction

In recent years, non-viral methods for cell engineering have emerged as promising alternatives to viral transduction. Amongst virus-free technologies, electroporation is considered the gold standard, with an increasing presence in clinical trials. Historically, non-viral cell transfection methods, including electroporation, have been reported to suffer with poor scalability and lengthy optimization, sometimes linked to unsatisfactory transfection efficiency at large scale. An ideal technology platform should enable process development and optimization at small scale, with minimal effort required for scaling up and translation to clinical settings.

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 up to 20 mL
- Electroporation of large volumes with up to 2.0 x 10^9 T cells
- Flexible volumes in fixed-volume cartridge (0.5, 1.0, 1.5 or 2.0 mL)
- Compatibility with cell densities from 2.5x 10^7 to 1×10^8/mL

Material and methods

Cell material: Cryopreserved human PBMC (Lonza) or CD3+ Pan T Cells (Lonza), activated with TransAct™ (Miltenyi; 3 days) or Dynabeads (Thermo Fisher Scientific®; 2 days)
Transfection: Cells were resuspended in P3 Nucleofector® Solution, cargo was added, the mixture transferred into the required Nucleofector® Vessel and transfected.
Analysis: On day of analysis, TCR alpha (*TRAC* KO) and/or *GFP* (KI or transient) expression was evaluated by flow cytometry (NovoCyte, Agilent). Cell count and cell viability were assessed with NucleoCounter® NC-202 (Chemometec).

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)
LV - Large volume

Transient cargos

Scalability – mRNA

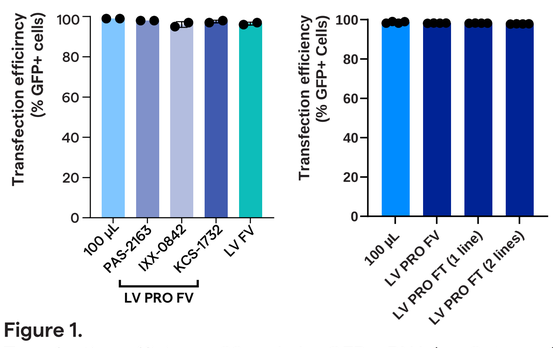


Figure 1.
Transfection efficiency 20 µg/mL *eGFP* mRNA (n = 2 donors)

Scalability – plasmid

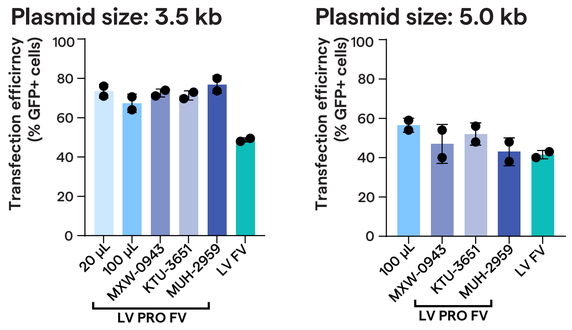
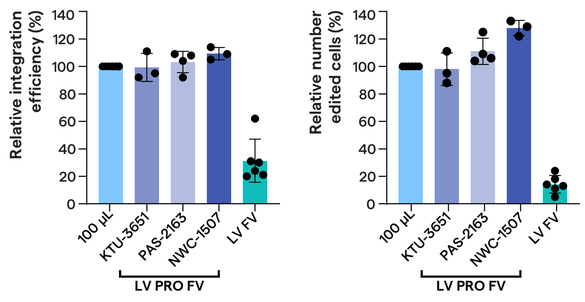


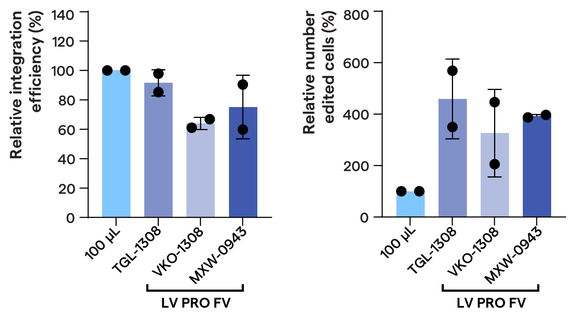
Figure 2.
Transfection efficiency 20 µg/mL plasmid (3.5 kB) or 37.5 µg/mL plasmid (5.0 kB) (n = 2 donors)

piggybac® Transposon System

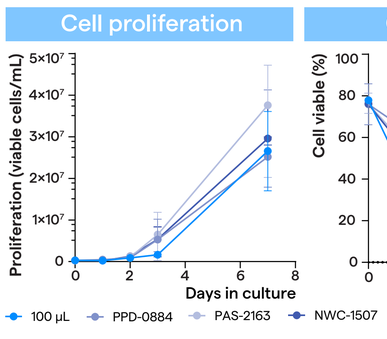
Scalability – activated T cells



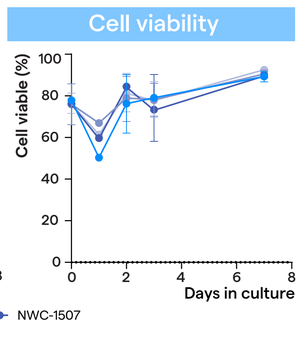
Scalability – resting T cells



Cell proliferation



Cell viability



Scalability from 100 µL to 10 mL – activated T cells

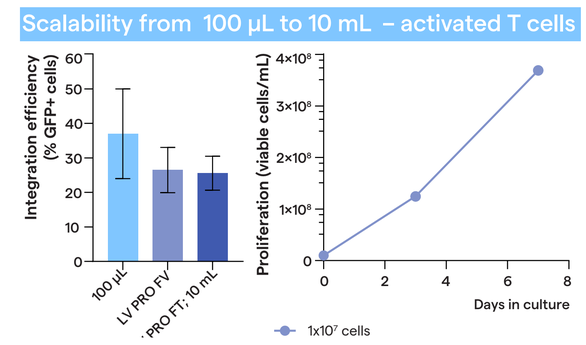
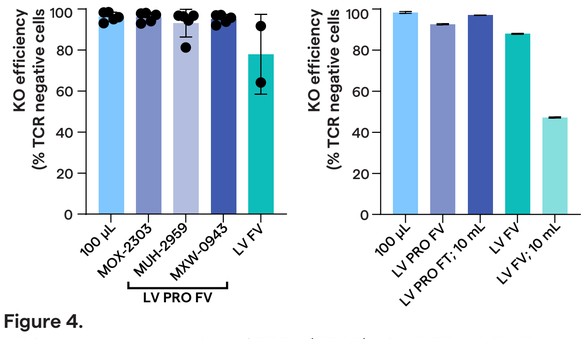


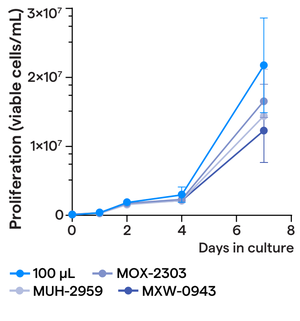
Figure 3.
Transfection of Transposase mRNA (43 µg/mL) + GenCircle™ Transposon (100 µg/mL; n = 2 donors)

CRISPR/Cas9 Knock-out

Scalability – *TRAC* KO



Cell proliferation



Cell viability

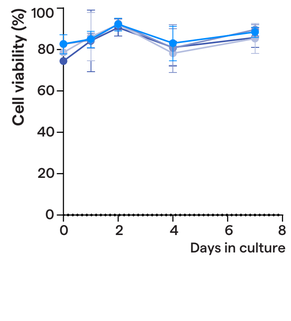
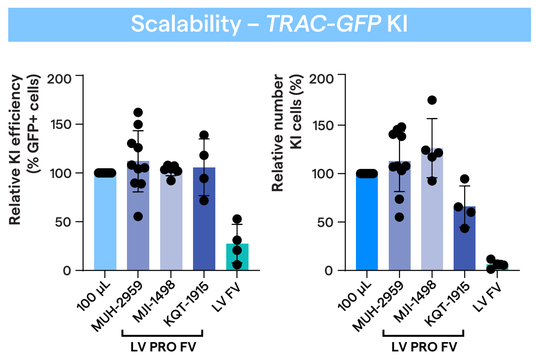


Figure 4.
KO in the constant region of TCRα (*TRAC*) with 0.77 µM Cas9 and sgRNA in ratio 1:2 (for FV n = 4 donors; for FT n = 1 donor)

CRISPR/Cas Knock-in

Scalability – *TRAC-GFP* KI



Scalability – Different input volumes

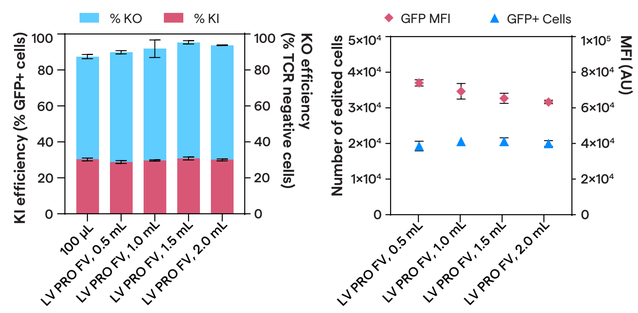


Figure 5.
TRAC KI with 0.77 µM Cas9 and sgRNA in ratio 1:2 plus 50 nM dsDNA HDRT (n = 6 donors)

Scalability - Different HDRT sizes

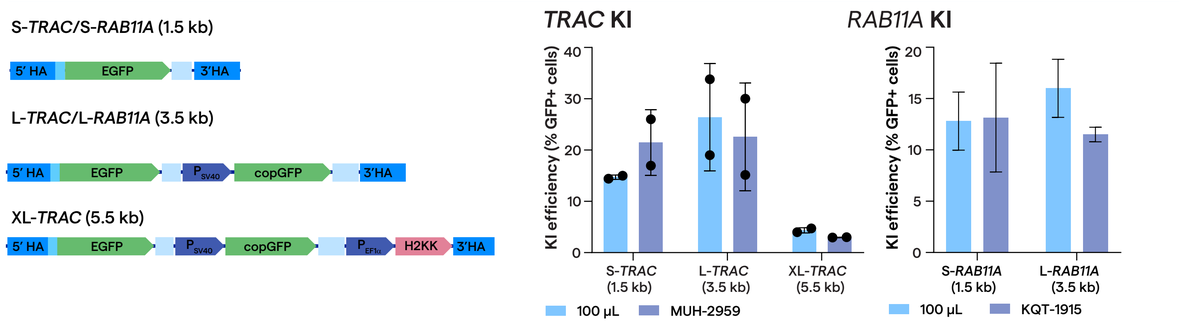
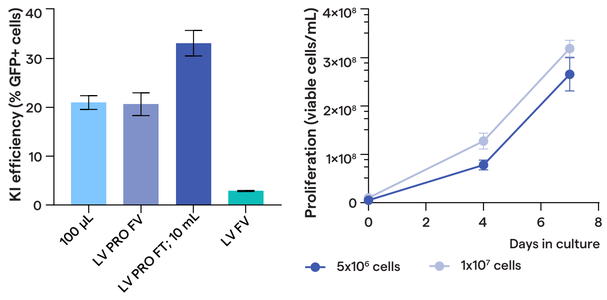


Figure 6.
KI efficiency with 0.77 µM (*TRAC*) or 0,93 µM (*RAB11A*) Cas9 and sgRNA in ratio 1:2 plus dsDNA HDRT (n = 2 donors)

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



Scalability from 100 µL to 20 mL – *RAB11A-GFP* KI

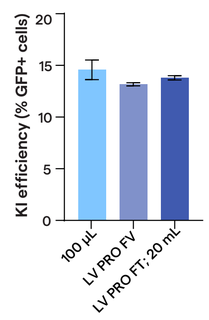
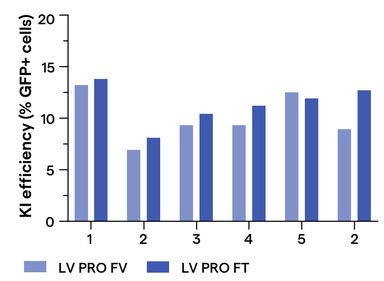


Figure 7.
Editing with 0.77 µM Cas9 and sgRNA in ratio 1:2 plus 50 nM dsDNA HDRT (n = 2 donors)

Figure 8.
Editing with 0.93 µM Cas9 and sgRNA in ratio 1:2 plus 8.4 µM dsDNA HDRT (n = 1 donor)

Robustness – *RAB11A-GFP* KI



Donor	1	2	3	4	5	2
Vol (mL) FV /FT	0.5/20	0.5/20	0.5/10	1.5/20	1.5/10	0.5/10
Cell Density (×10^6 cells/mL)	50	50	100	50	100	50
HDRT (Kb)	3.5	3.5	3.5	3.5	1.4	1.4

Figure 9.
KI efficiency with 0.93 µM Cas9 and sgRNA in ratio 1:2 plus dsDNA HDRT

Double editing

TRAC-KO + piggybac® Transposon System

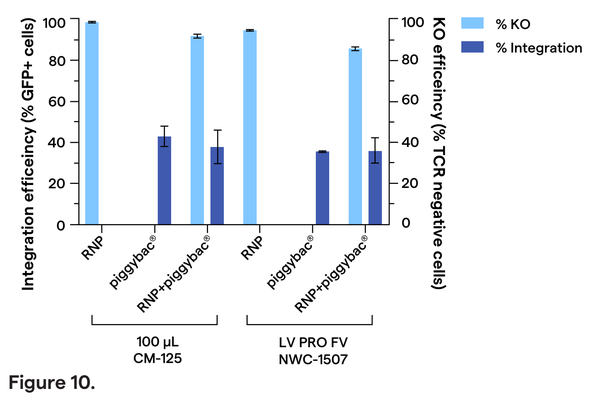


Figure 10.
Editing efficiency with Transposase mRNA (86 µg/mL) + GenCircle™ Transposon (200 µg/µL) and *TRAC* KO (0.77 µM RNP)

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

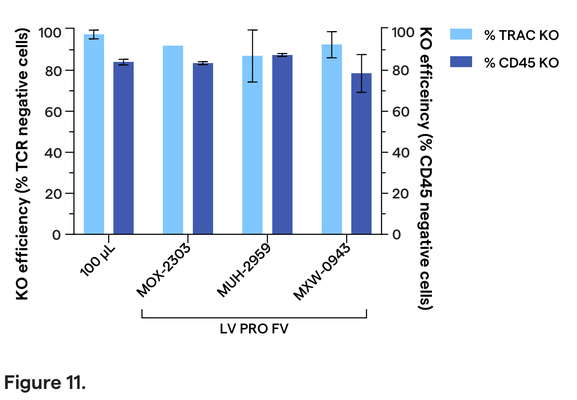


Figure 11.
Efficiency with *TRAC* KO + *CD45* KO for 0.77 µM Cas9 + sgRNA in ratio 1:2 each (n = 2 donors)

Summary

The next generation 4D-Nucleofector® LV Unit PRO can reliably support non-viral manufacturing of genetically modified T cells. It helps enable:

- Reliable, robust and efficient delivery of complex, clinically relevant cargos
- Easy scale up of the cell engineering process for up to 1 billion cells

Learn more.

Lonza Cologne GmbH – 50829 Cologne, Germany

This research was conducted under a non-exclusive sublicense granted by Arsenal Biosciences, Inc. to U.S. and ex-US patents and pending applications directed to targeted nonviral DNA insertion.

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