Non-viral delivery of complex cargos for large-scale 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 the vector free technologies, electroporation is considered the gold standard, with an increasing presence in clinical trials[1].

We developed an improved cartridge for the electroporation of complex cargos into large volumes of T cells, using the 4D-Nucleofector® LV Unit PRO. The optimal cell handling parameters were developed using 100 µL reactions and then adapted for the new large-scale cartridge to transfect up to 1 × 10^9 cells in 10 – 20 mL.

Materials and methods

Cell material: Cryopreserved human PBMC (Lonza) or $CD3^+$ Pan T Cells (Lonza), activated with TransAct™ (Miltenyi) and cultured in optimized conditions. **Transfection:** On the day of transfection, cells were resuspended in P3 Nucleofector® Solution, and the indicated cargo was added prior to electroporation. Cells were then transferred into the required Nucleofector® Vessel and transfected in the 4D-Nucleofector® X Unit, LV Unit or LV Unit PRO (Figure 1). **Analysis:** On the indicated time points, TCR alpha knockout (KO) and GFP knockin (KI), or transient expression were evaluated by flow cytometry (NovoCyte, Agilent). Cells count and cell viability were assessed by either flow cytometry (DAPI staining) or NucleoCounter® NC-202 (Chemometec).

Results

Instrument and cartridge

Figure 1. 4D-Nucleofector® Core Unit with LV Unit PRO, fixed volume 2 mL Nucleocuvette® Cartridge PRO (LV PRO FV) and flow-through LV Nucleocuvette® Cartridge PRO (LV PRO FT). The LV PRO FV enables transfection of fixed volumes of cells, namely 0.5, 1.0, 1.5 or 2.0 mL, while for the first generation LV FV catridge the volume is set at 1 mL. The automated LV PRO FT can process up to 20 mL T-cell supension. Cell densities tested range from 2.5 x 10^7 to 1.0 x 10^8 cells/mL.

Scalablility – Transient expression

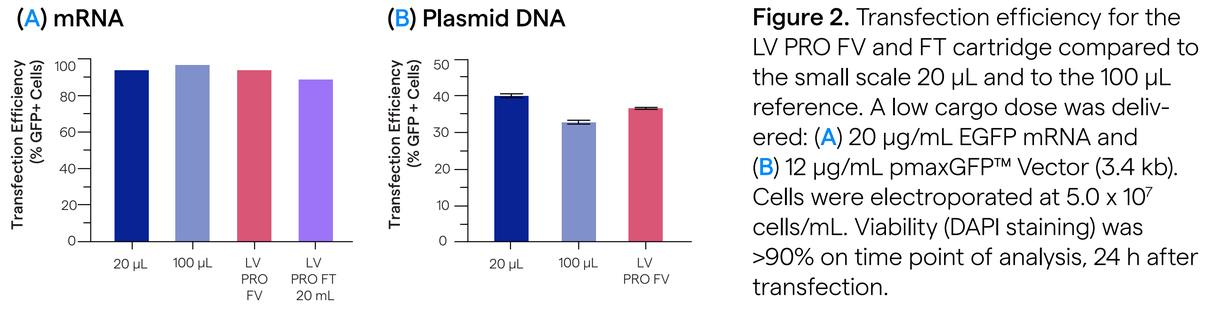
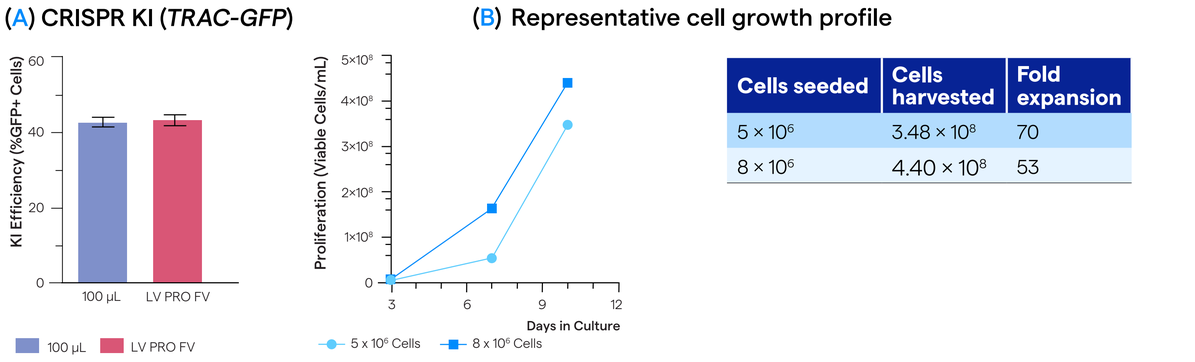


Figure 2. Transfection efficiency for the LV PRO FV and FT cartridge compared to the small scale 20 µL and to the 100 µL reference. A low cargo dose was delivered: (A) 20 µg/mL EGFP mRNA and (B) 12 µg/mL pmaxGFP™ Vector (3.4 kb). Cells were electroporated at 5.0 x 10^7 cells/mL. Viability (DAPI staining) was >90% on time point of analysis, 24 h after transfection.

Scalablility – Genome editing



Cells seeded	Cells harvested	Fold expansion
5 × 10^6	3.48 × 10^8	70
8 × 10^6	4.40 × 10^8	53

Figure 3. (A) Representative KI efficiency on day 7 for the LV PRO FV vs small scale 100 µL reference (1 donor, 8 technical replicates). The cargo system delivered was Cas9 RNP and ds DNA HDR template, 3.5 kb. (B) Representative growth profile in G-Rex® bioreactors (Wilson Wolf). Two different amounts of cells were seeded after electroporation. Cell viability on day 7 was >85%.

Consistency

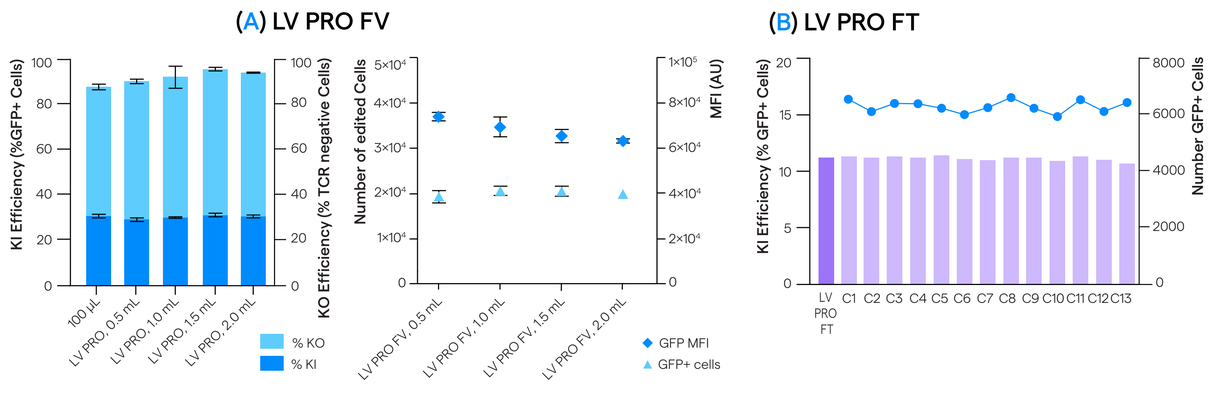


Figure 5. (A) The LV PRO FV enables transfection of fixed volumes of cells, namely 0.5, 1.0, 1.5 or 2.0 mL. Different input volumes result in equivalent performances. Representative data are shown for *TRAC* KO and KI (*TRAC-GFP* 3.5 kb), number of edited cells per unit volume analyzed and MFI (median fluorescence intensity). (B) The LV PRO FT cartridge processes up to 20 mL volume through subsequent filling and emptying cycles, each addressing, in this case, 1.5 mL cell suspension. The figure shows how stable KI Efficiency (*RAB11A-GFP*, 3.5 kb HDRT) is, from the first aliquot (C1) to the last cycle (C13). Equally stable are the volumes recovered (1.54 mL ± 0.02, not shown), and the number of edited cells per unit volume analyzed, suggesting no accumulation of debris or deterioration of the process over time. The total volume recovered is >95% of the input volume.

Conclusion

The new 2 mL Nucleocuvette® LV Cartridge PRO (LV PRO FV) and the LV Nucleocuvette® Cartridge PRO (LV PRO FT) enable

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

Lonza's 4D-Nucleofector® LV Unit PRO can reliably support non-viral manufacturing of cell and gene therapy products.

Scalablility – Genome editing

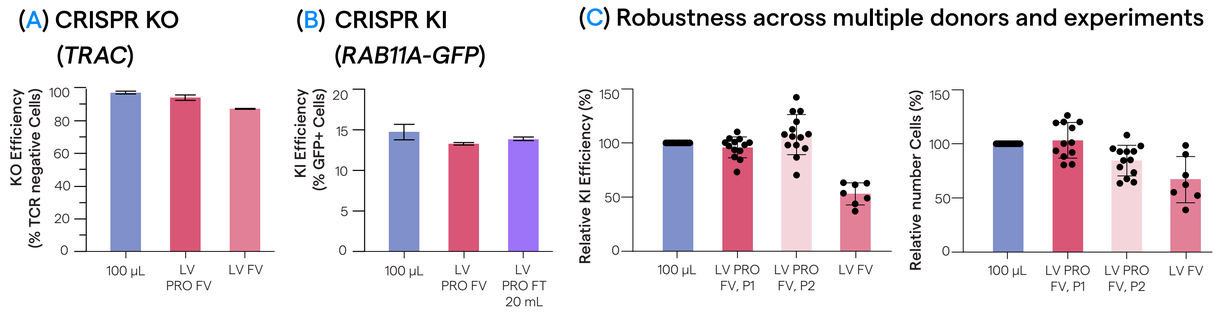
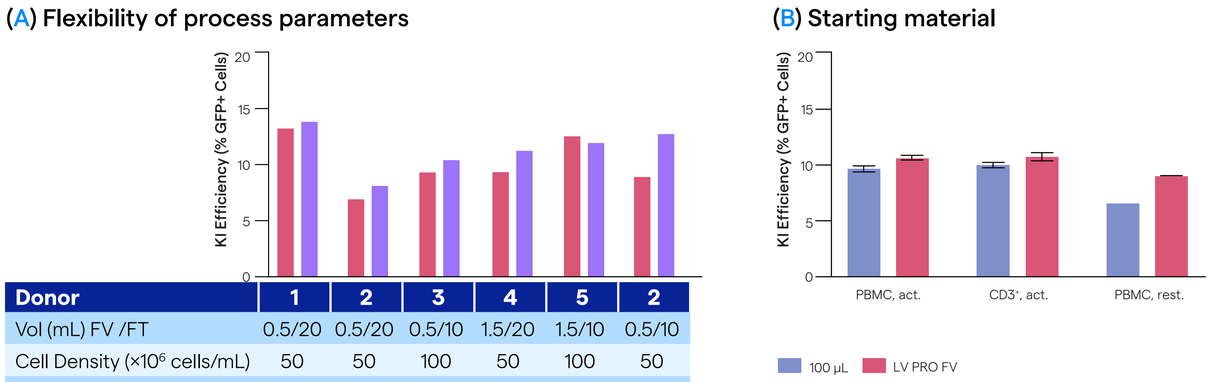


Figure 4. (A) KO efficiency for the LV PRO FV vs small scale 100 µL reference and first-generation LV FV (2 donor, 4 technical replicates). (B) Representative KI efficiency for the LV PRO FV and LV PRO FT cartridges compared to the small scale 100 µL reference (1 donor, 4 technical replicates). (C) KI data from 7 healthy donors and 13 different experiments were normalized versus the 100 µL reference (set at 100%) to account for donor variability. The graphs show KI efficiency and number of viable edited cells with two electric programs. The performances of the first-generation LV FV cartridge are included for comparison. The cargo system delivered was Cas9 RNP and ds DNA HDR template, 1.4 kb (*RAB11A-GFP*).

Versatility of setup



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 6. (A) Data on 5 different healthy donors whereby Cas9 RNP was co-delivered with a dsDNA HDR template 3.5 kb or 1.4 kb. Both templates are designed to introduce a GFP fusion in the housekeeping gene *Rab11A*[2]. Cells were electroporated at 5.0 x 10^7 cells/mL or at 1.0 x 10^8 cells/mL. For all conditions shown, viability (n viable cells × 100/n total cells) was >90% at time point of analysis. (B) KI efficiency with dsDNA-*RAB11A-GFP* 1.4 kb for different starting material.

References:

1. **H. Balke-Want** *et al.*, *IOTECH*, 2023, 18, C, 100375
2. **Roth** *et al.*, *Nature*. 2018 Jul 11;559(7714):405–409

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