CONTINUOUS FLOW TRANSFECTION WITH FLOWFECT® TECHNOLOGY ENABLES RAPID OPTIMIZATION AND SCALE-UP FOR EFFICIENT CO-DELIVERY OF mRNA AND PLASMID DNA IN PRIMARY HUMAN T CELLS

Maria Lai, Alyssa Carlson, and Chris Abraham. Kytopen, Cambridge, MA, USA.

INTRODUCTION

Efficient, simultaneous delivery of multiple classes of nucleic acids presents unique challenges for engineered cell therapies. The Flowfect® non-viral cell engineering technology offers increased payload capacities required for delivering multiple transgenes, limited in the viral context, but critical to advancing the advanced cell therapies. Flowfect™ platforms offer a seamless, scalable continuous flow transfection process, from small volumes (50-100 µL), geared towards discovery and optimization experiments on the Flowfect Discover™ 96-well platform to manufacturing volumes (1 mL- >L) for processing up to billions of cells on the Flowfect Tx™ platform.

Here, we highlight the utility of 1) small-scale, rapid and systematic optimization for efficient co-delivery of mRNA and plasmid DNA and 2) direct scale-up of co-delivery with the Flowfect Tx™ platform in primary human T cells. Initial experiments using the Flowfect Discover™ 96-well platform identified optimal parameters for co-delivery of mCherry mRNA and GFP pDNA in primary activated T cells. These conditions were transferred to the large-scale Flowfect Tx™ platform, achieving comparable results without the need for additional optimization.

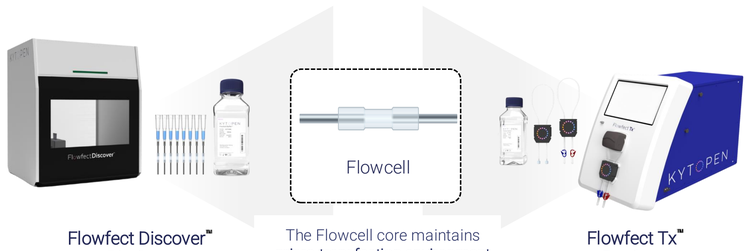
To showcase the performance of Flowfect™ platforms in an immunotherapeutic context, we partnered with Bio-techne, a leading provider of cell and gene therapy reagents. Using Bio-techne's TcBuster™ non-viral transposon system, we demonstrate exceptional efficiencies in transposase mediated insertion of a large 5.1kb multi-gene TcBuster™ Transposon CD19CAR-DHFR-eGFP into primary T cells on both Flowfect™ platforms.

KEY TAKEAWAYS

- Leveraging the Flowfect Discover™ 96-well platform, rapid identification and optimization of parameters driving efficient and simultaneous co-delivery (>60%) of mCherry mRNA and GFP pDNA in primary activated T cells were achieved.
- Optimal parameters identified on the Flowfect Discover™ 96-well platform seamlessly transitioned to large-scale volumes on the Flowfect Tx™ GMP platform with no further optimization, achieving high simultaneous co-transfection efficiencies greater than 60% for both mRNA and pDNA while maintaining high viability.
- Supporting the immunotherapeutic relevance, we partnered with Bio-techne, and merging their TcBuster™ transposon system with the Flowfect Discover™ platform achieved up 50% transposition efficiency of a large 5.1 kb multi-gene TcBuster™ Transposon CD19CAR-DHFR-eGFP with >85% viability in primary activated T cells.
- Scaling towards therapeutic requirements, using the Flowfect GMP Tx™ platform, again with Bio-techne's TcBuster™ Transposon CD19CAR-DHFR-eGFP, we achieved up to 55% transposition and >70% cell viability.

TECHNOLOGY

Flowfect® Technology Drives Optimization and Seamless Translation to Scalable T-Cell Engineering



Flowfect Discover™ Platform
(20-100 µL)
Automated, HTP Screening
96 conditions in < 15 min

The Flowcell core maintains micro-transfection environment across a range of scales

Flowfect Tx™ Platform
(1 - >1000mL)
Closed, Large Scale
Billions of cells in minutes

Rapid Optimization for Improved Co-transfection Efficiency of mRNA and pDNA Using the Flowfect Discover™ 96-well Optimization Platform and Scale-up Using the Flowfect Tx™ GMP Platform

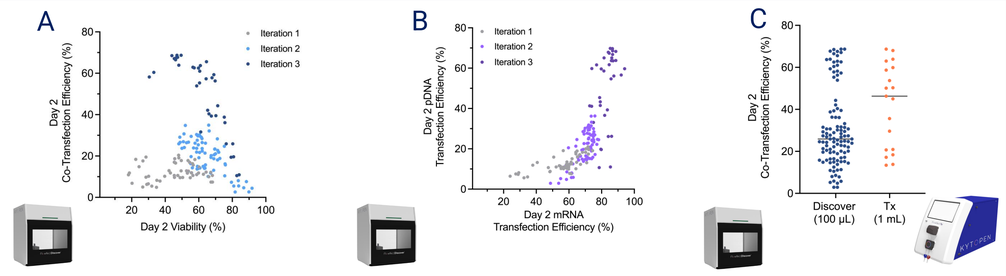
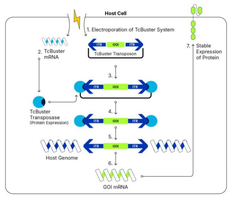


Figure 1. Efficient co-delivery of mRNA and pDNA in primary human T cells using the Flowfect Discover™ (small volume scale, 100 µL) and the Flowfect Tx™ (large volume scale, 1 mL) platforms. Primary activated T cells were co-transfected with mRNA (~1000 bases) and pDNA (~5 kb). Efficiency and viability measurements were taken 2 days post-transfection. A) Co-transfection efficiency compared to viability for samples in the initial experiment (Iteration 1, grey) and in two optimization experiments (Iteration 2 and 3, light blue and dark blue, respectively). B) Co-transfection efficiency compared to mRNA transfection efficiency for samples in the initial experiment (Iteration 1, grey) and in two optimization experiments (Iteration 2 and 3, light purple and dark purple, respectively). C) Comparison of co-transfection efficiency on the Flowfect Discover™ (100 µL scale) and Flowfect Tx™ (1 mL scale) platforms.

TcBuster™ Non-viral Transposon System Enables Insertion of Large, Multi-gene Payloads for Genome Engineering



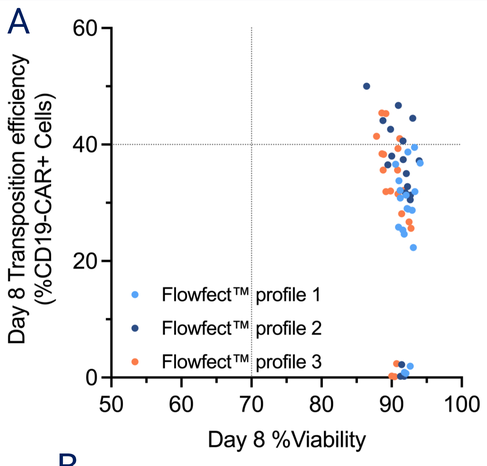
1. TcBuster-M mRNA transposase and plasmid DNA transposon containing gene of interest (GOI) are delivered via electroporation
2. mRNA is translated to TcBuster-M protein
3. TcBuster-M transposase binds ITRs on plasmid DNA
4. TcBuster-M transposase cuts plasmid DNA
5. TcBuster-M randomly inserts DNA into host genome
6. Inserted GOI is transcribed into mRNA
7. GOI mRNA is translated into protein

TcBuster™ Transposon CD19CAR-DHFR-eGFP

+

TcBuster™-M Transposase mRNA

Flowfect Discover™ (Small Volume) Platform Enables Efficient Engineering of CAR-T Cells Using TcBuster™ Non-viral Transposon System



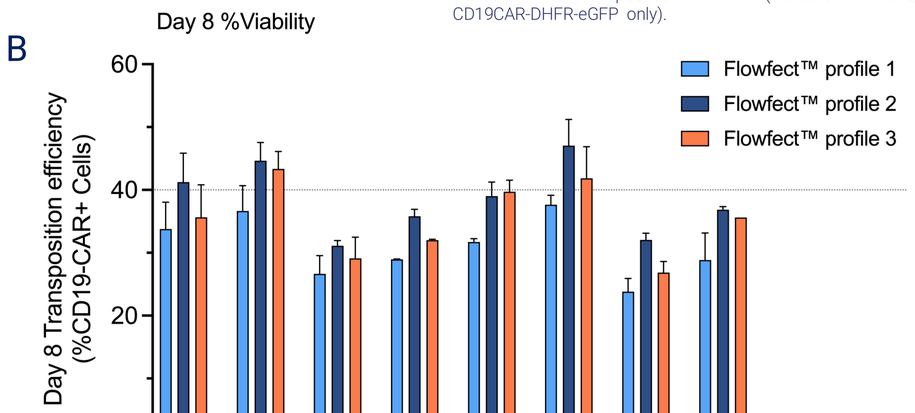
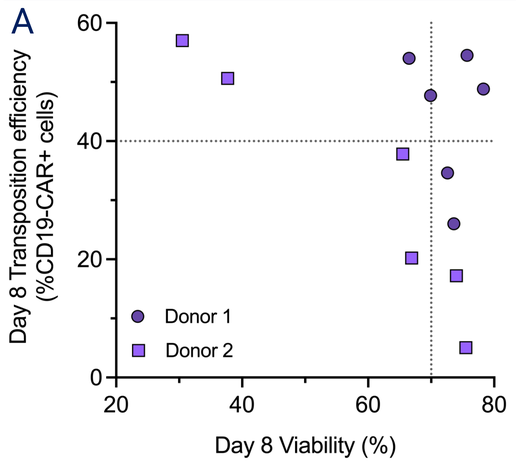
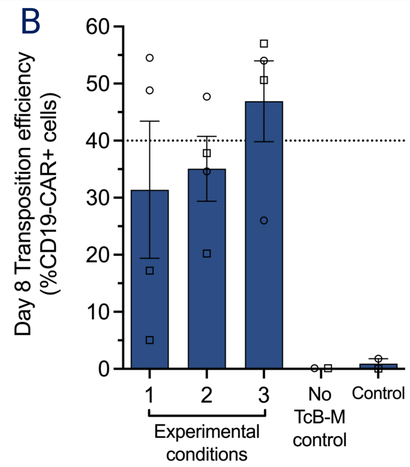
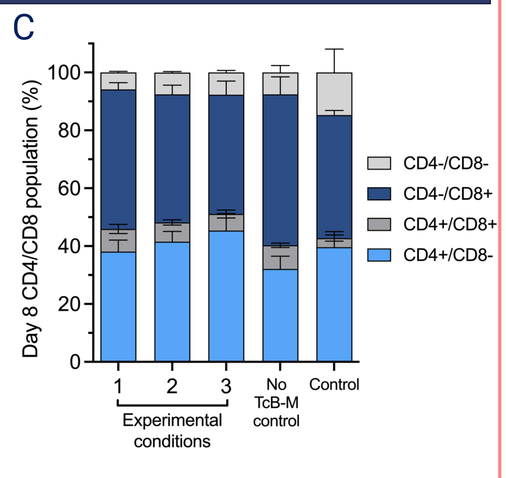


Figure 2. Efficient co-delivery of TcBuster™ non-viral transposon system for genome engineering using the Flowfect Discover™ (small volume scale, 100 µL) platform. Primary human activated T cells were co-transfected with TcBuster™ Transposon CD19CAR-DHFR-eGFP and TcBuster™-M Transposase mRNA to enable transposition of multicistronic payloads. Transposition efficiency measurements were taken 8 days post-transfection via flow cytometry. A) Transposition efficiency, as measured by the percentage of CD19-CAR positive cells, compared to viability, as measured by live/dead staining via plate reader analysis, for samples with varying concentrations of transposon and transposase using three different Flowfect™ profiles for transfection (light blue, dark blue, and orange). Each dot represents a sample technical replicate. Dotted lines indicate 40% transposition efficiency and 70% viability. B) Transposition efficiency, as measured by the percentage of CD19-CAR positive cells, compared to payload concentration of transposon and transposase using three different Flowfect™ profiles for transfection (light blue, dark blue, and orange). The control conditions represent Flowfect™ profile-only (no payload) and no TcBuster™-M Transposase mRNA (TcBuster™ Transposon CD19CAR-DHFR-eGFP only).

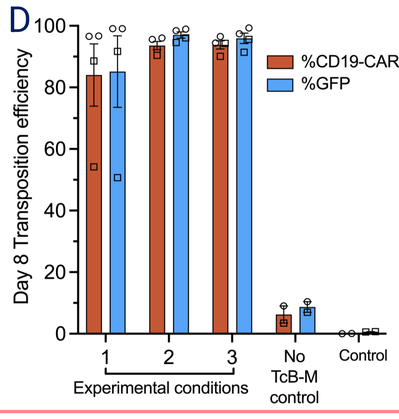
Flowfect Tx™ (Large Volume) Platform Enables Seamless Scale Up for Engineering CAR-T Cells Using TcBuster™ Non-viral Transposon System







Selection For Transposed Cells Using Methotrexate



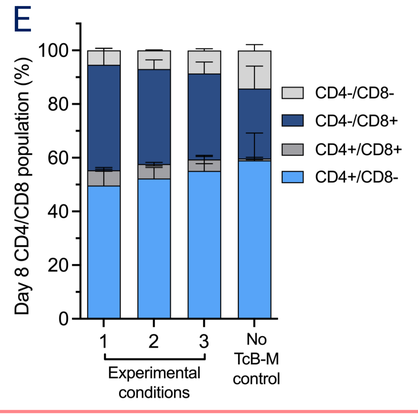


Figure 3. Efficient scale up of TcBuster™ non-viral transposon system for engineering CAR-T cells using the Flowfect Tx™ (large volume scale, 1 mL) platform. Primary human activated T cells were co-transfected with TcBuster™ Transposon CD19CAR-DHFR-eGFP and TcBuster™-M Transposase mRNA to enable transposition of multicistronic payloads at 1 mL volume. Transposition efficiency measurements were taken 8 days post-transfection via flow cytometry. All data presented here for the 1 mL runs are using 2 donors (circles and squares) with 2 technical replicates per donor. The control conditions represent transfection-only (no payload) and no TcBuster™-M Transposase mRNA (TcBuster™ Transposon CD19CAR-DHFR-eGFP only). A) Transposition efficiency, as measured by the percentage of CD19-CAR positive cells, compared to viability, as measured by live/dead staining using plate reader analysis, for samples with varying concentrations of transposon and transposase using Flowfect™ profile 2 for transfection. Dotted lines indicate 40% transposition efficiency and 70% viability. B) Transposition efficiency, as measured by the percentage of CD19-CAR positive cells, compared to payload concentration of transposon and transposase using Flowfect™ profile 2 for transfection (dark blue). C) Percentage of CD4/CD8 population on Day 8 post-transfection. D) Transposition efficiency after methotrexate selection for transposed cells, as measured by the percentage of CD19-CAR positive (orange bars) and GFP positive (light blue bars) cells, compared to payload concentration of transposon and transposase using Flowfect™ profile 2. E) Percentage of CD4/CD8 population on Day 8 post-transfection after methotrexate selection for transposed cells.

CONCLUSIONS

- Harnessing the Flowfect® continuous flow transfection technology drives efficient, simultaneous, co-delivery of multiple nucleic acids. Integrating Bio-techne's TcBuster™ non-viral transposon system, we were able to showcase highly efficient CAR-T engineering while maintaining exceptionally high-viabilities.
- Kytopen's Flowfect® continuous flow transfection technology provides best-in-class performances for both small-scale research utility as well as large-scale manufacturing capability.
- The seamless transition between Kytopen's two Flowfect™ platforms eliminates traditional scale-up bottlenecks while maintaining critical performance attributes, providing a foundation for streamlining cell therapy manufacturing from early development through clinical production.

www.kytopen.com

Contact: hello@kytopen.com