Scalable continuous-flow electroporation platform enabling T cell transfection for cellular therapy manufacturing

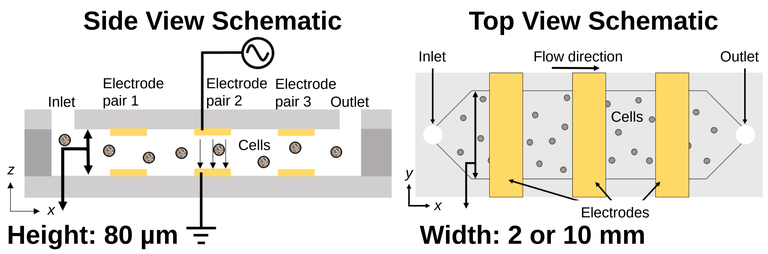
Jacob A. VanderBurgh, Thomas N. Corso, Stephen L. Levy, Harold G. Craighead
CyteQuest, Inc, Ithaca, NY

https://cytequest.com/
jvanderburgh@cytequest.com

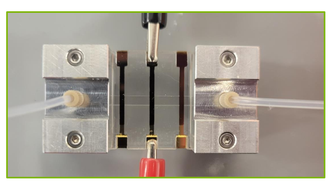
Objective: Scalable transfection of T cells

- Viral vectors have enabled CAR-T cell therapies, but drawbacks include:
  - High cost and complex manufacturing
  - Immunogenicity and potential for insertional mutagenesis
  - Incompatibility with CRISPR/Cas9 mediated gene editing
- Electroporation enables non-viral transfection of primary cells, but:
  - Electroporation typically requires difficult empirical optimization
  - Standard electroporation methods are incompatible with large-scale cell manufacturing methods required for cell therapies
- We developed a microfluidic electroporation platform capable of rapid and reproducible electroporation that can seamlessly scale delivery from the research to clinical scale

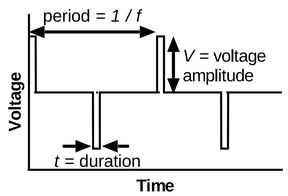
Overview of electroporation platform



- Our platform incorporates a single-use, continuous-flow, microfluidic channel
  - The microfluidic channel has a thin slab geometry for seamless scalability
- The thin channel height (80 µm)
  - Ensures each cell is subjected the same electric field and chemical environment to enable reproducible electroporation
  - Requires relatively low voltage amplitude to achieve electric field strength required to transiently open plasma membrane pores
- The channel width is chosen to achieve the desired experimental throughput
  - Increasing the width (ie. from 2 to 10 mm) increases experimental throughput without changing electric field experienced by the cells
- Flexible electronics permit delivery of any arbitrary electrical waveform



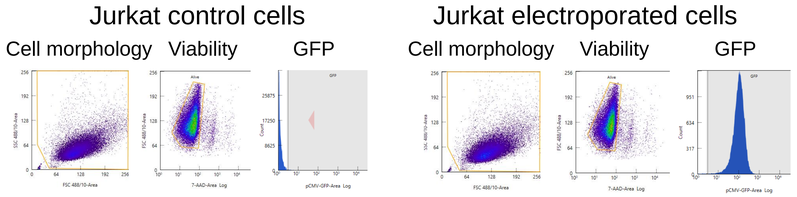
Photograph of a flow cell with three sets of independently addressable electrodes



Plot depicting a bipolar rectangular waveform with frequency *f*, duration *t*, and voltage amplitude *V*

Delivering mRNA, plasmid DNA, & CRISPR RNPs to T cells at the research scale

Representative flow cytometry plots from control and electroporated cells

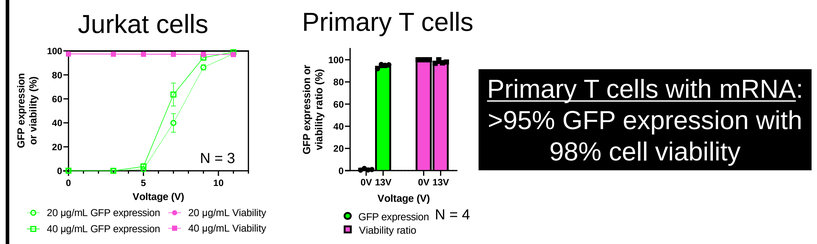


$$Viability = \frac{N_{viable}}{N_{total}}$$

$$GFP\ expression = \frac{N_{expressing}}{N_{viable}}$$

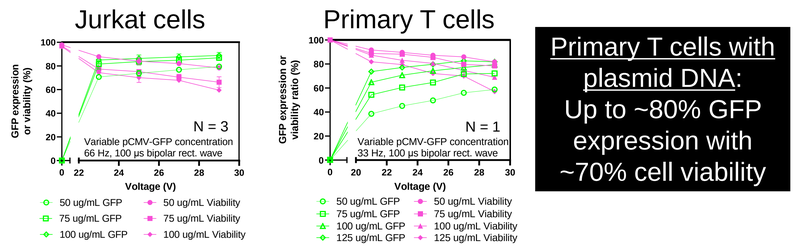
$$Viability\ ratio = \frac{Viability}{Viability_{zero-voltage\ control}}$$

Delivery of mRNA encoding GFP to Jurkat or primary human T cells



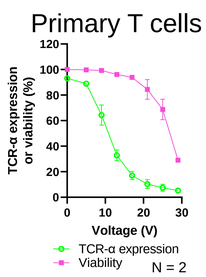
Primary T cells with mRNA:
>95% GFP expression with 98% cell viability

Delivery of plasmid DNA encoding GFP to Jurkat or primary human T cells



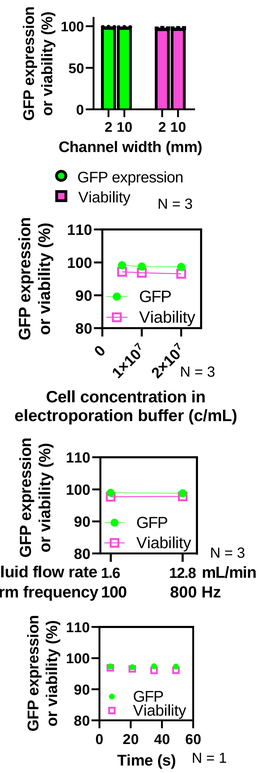
Primary T cells with plasmid DNA:
Up to ~80% GFP expression with ~70% cell viability

Delivery of CRISPR ribonucleoproteins to primary human T cells



Primary T cells with CRISPR RNPs:
~90% knockdown
~80% cell viability

Delivering mRNA encoding GFP at the clinical scale using Jurkat cells



Scaling the channel width by factor of five increases throughput without impacting transfection performance

Increasing the cell concentration up to 20 million cells / mL in electroporation buffer increases throughput without impacting transfection performance

Proportionally increasing the fluid flow rate and waveform frequency increases throughput without impacting transfection performance

Demonstration of mRNA delivery at 256 million cells per minute.
Transfected 240 million cells in 56 s

Conclusions

- Our thin slab geometry enables:
  - Rapid optimization using small volumes of material at the research scale
  - Seamless scaling to clinical scale by scaling the channel width
- We demonstrate:
  - Efficient transfection of primary T cells with plasmid DNA and mRNA that encode GFP and CRISPR RNPs that target T cell receptor α
  - Various approaches to increase experimental throughput
  - Delivery of mRNA encoding GFP to 240 million cells in 56 s

Acknowledgements

This work was performed in part at the Cornell NanoScale Facility, an NNCI member supported by NSF Grant NNCI-2025233.