# Comparison of Electroporation and Peptide-Mediated Engineering of Human Lymphocytes

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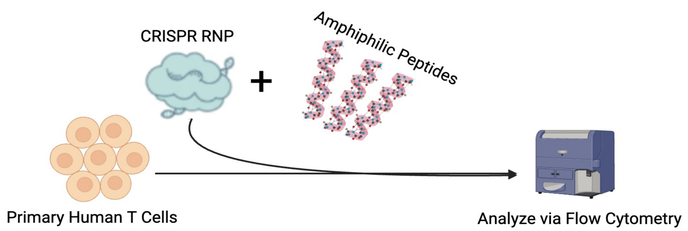
## Background

- Editing of human lymphocytes is often conducted via electroporation, a procedure in which an electrical current increases the permeability of the cell membrane.
- Although very effective for gene editing, electroporation can lead to cytotoxicity and thus less cell viability.
- Peptide-mediated delivery of CRIPSR enzymes also allows for gene editing in a way that causes less cytotoxicity but also lower transduction.
- This project aims to determine if electroporation or peptide-mediated delivery of CRISPR enzymes is a more efficient means of gene editing of human lymphocytes by comparing cell viability, T cell receptor (TCR) knock-out, and chimeric antigen receptor (CAR) knock-in.

## Methods

Peptide-Mediated CRISPR:

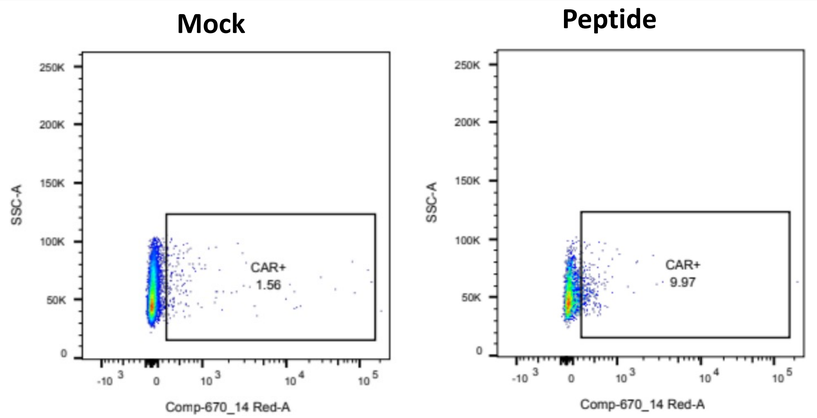
- Human CD4 and CD8 cells are activated and plated with a CRISPR RNP and A5K peptide (1 mM) solution.
- Lentivirus is added to cells for possible CAR knock-in.
- Negative staining is performed to remove cells with TCR present.
- Analysis is performed via flow cytometry.



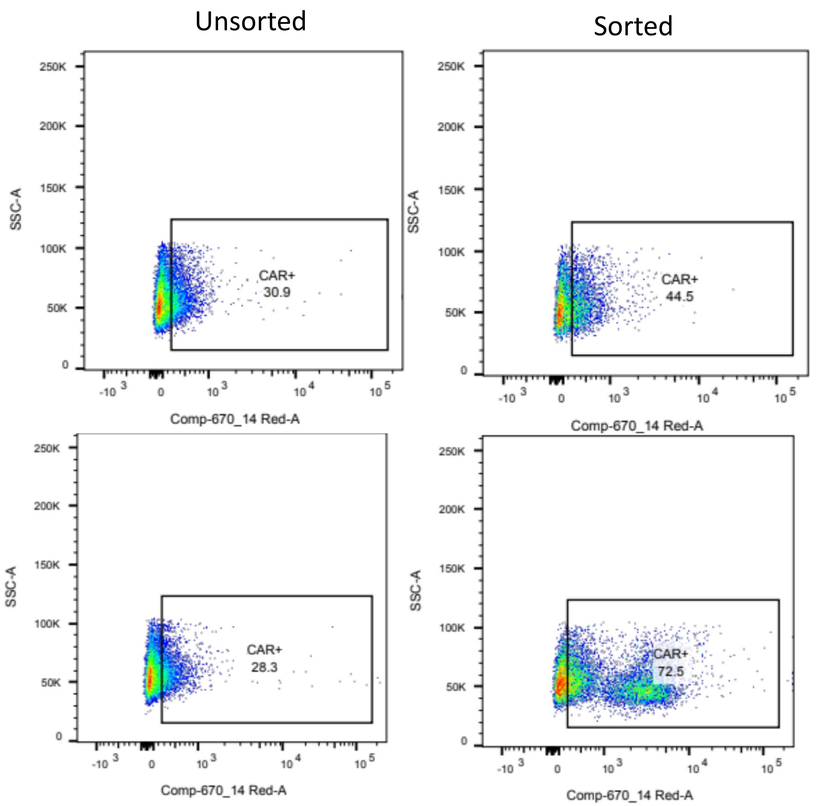

Electroporation:

- Human CD4 and CD8 cells are activated and suspended in electroporation buffer.
- Add CRISPR RNP to the solution for gene editing.
- Apply electric force to generate holes in the cell membrane and allow for influx of gene-editing material into the cell.
- Lentivirus is added to cells for possible CAR knock-in.
- Analysis is performed via flow cytometry.

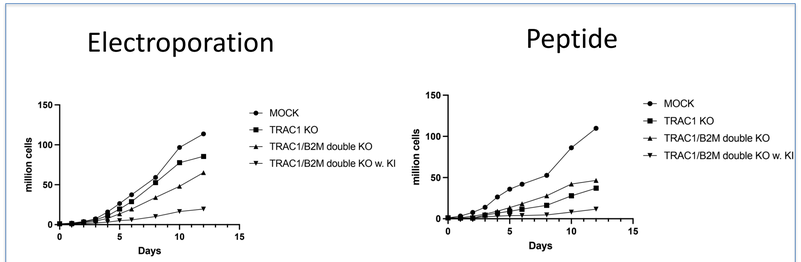
## Results




### Electroporation




## Viability Curves




## Conclusions & Future Steps

- Electroporation shows higher levels of gene-editing efficiency compared to peptide-mediated CRISPR knock-in.
- Peptide-mediated CRISPR knock-in has similar cell viability compared to electroporation.
- Overall, electroporation provides more gene-edited cells compared to peptide-mediated knock-in and is a more efficient method of gene editing of human lymphocytes.
- However, these are preliminary findings and thus this comparison should be retested once the peptide method has been optimized.
- Future projects can test higher concentrations of peptide solution and determine if this correlates with higher levels of CAR+ cells while preserving viability.

## References

Foss, D.V., Muldoon, J.J., Nguyen, D.N. *et al.* Peptide-mediated delivery of CRISPR enzymes for the efficient editing of primary human lymphocytes. *Nat. Biomed. Eng* **7**, 647–660 (2023). https://doi.org/10.1038/s41551-023-01032-2

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