Delivery of CRISPR in T cell therapeutics

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Introduction

According to the National Cancer Institute 2 millions americans with be diagnosed with cancer this year

The human immune system is under-equipped to successfully combat cancerous cells. Using a viral vector to deliver CRISPR to T-Cells results in T-Cells that are better able to amplify and identify cancer cells.

Transfection protocols are diverse with with a variety of results. There is a great need to improve these protocols to maximize transfection efficiency and amount of viral vectors produced by transfected cells.

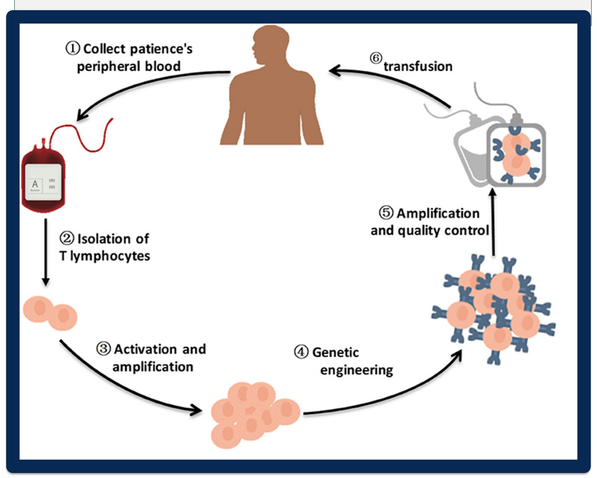

Figure 1. Figure from Frontiersin.org showing cancer T cell therapy treatment.

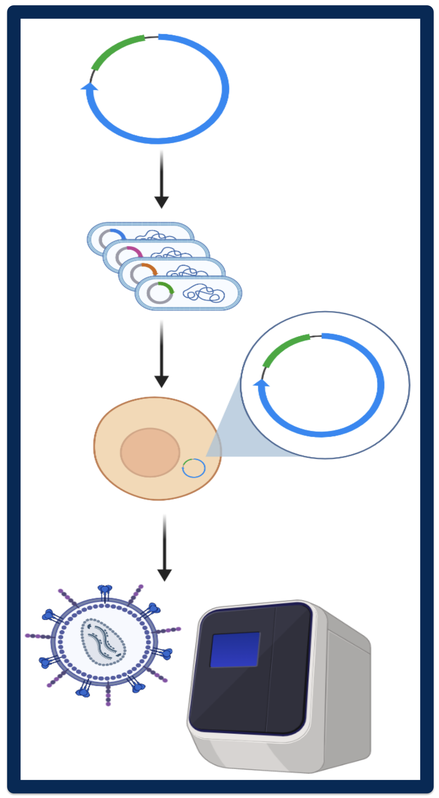

Figure 2. Figure illustrating methods to produce lentivirus capsid containing CRISPR (created in BioRender.com).

Methods

1. Plasmid is created with a GFP and CRISPR backbone
2. Plasmid with backbone is amplified in *E.coli*
3. Plasmid is transfected into HEK cells and analyzed for GFP expression
4. qPCR is then used to analyze lentiviral capsid production

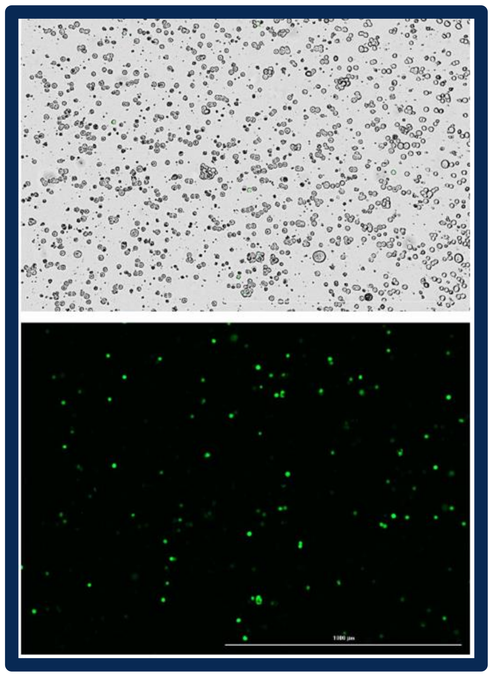

Figure 3. Top image viewed with a brightfield setting, bottom image viewed with GFP setting.

Results

To better understand transfection success, HEK cells were analyzed using a citation machine as seen in Figure 3. Our analysis of GFP expression shown in Figure 4 demonstrates the production effectiveness of current methods and predicted improvements by lowering the cell density upon transfection. Images obtained with Jake Accordino's assistance.

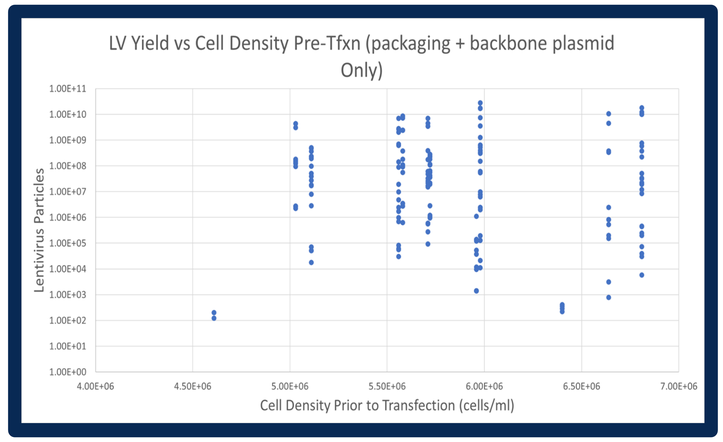

Figure 4. Graph displaying the relationship between Cell density prior to transfection and number of lentiviral particles.

Conclusion

Using standard Thermofisher transfection protocol for HEK-293 cells with a lower cell density at the time of transfection will improve the success rate.

HEK cells shown with GFP expression using the citation machine are predicted to have undergone successful transfection and are capable of building viral caspids housing the CRISPR backbone.

Amplification of viral DNA with qPCR shows that viral vectors containing CRISPR were produced by the HEK cells. These vectors are capable of infecting T cells, using CRISPR to edit their genome.