The impact of CPSF6 on the innate immune response to HIV infection

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ABSTRACT

With no current vaccine for Human Immunodeficiency Virus (HIV), it is crucial to study what factors influence how HIV infects host cells to develop new strategies and therapies to combat this pandemic. Cleavage and polyadenylation specificity factor 6 (CPSF6) is an HIV host factor known to influence HIV infectivity and trafficking early in the infection. CPSF6 has been implicated in supporting the innate immune response but its impact in the context of HIV is unclear. We hypothesize that the overexpression of CPSF6 will increase the antiviral response and decrease HIV infection in T cells. To test the hypothesis, we require a way to overexpress CPSF6 in mammalian cells to observe the antiviral response of HIV infection. We are going to implement gene cloning, a process used to replicate DNA for use in later experimentation, to express CPSF6. To make a CPSF6-containing vector, we prepared an empty expression mammalian vector through E. coli transformation and plasmid purification, restriction enzyme digest, and then gel electrophoresis to confirm successful plasmid linearization. Post-purification, the CPSF6 gene was then assembled into the empty vector using Gibson Assembly reactions. Successful isolation of the CPSF6 gene-containing plasmid was confirmed by Sanger sequencing. Further research will consist of expressing the vector in mammalian cells. These cells will be tested for their antiviral gene expression by Western blot and infectivity for HIV by flow cytometry. Ultimately, this experiment will lead to a greater understanding of CPSF6's role in the innate immune response in HIV infection.

INTRODUCTION

An estimated 37.7 million people are currently living with Human Immunodeficiency Virus (HIV), and this infection has led to 680,000 deaths in 2020 alone (World Health Organization, 2020). HIV hijacks host cells and hinders the innate immune response. It is important to study what factors influence how HIV infects host cells to develop new strategies and therapies to combat this endemic (Fanales-Belasio et al., 2010). Cleavage and polyadenylation specificity factor 6 (CPSF6) is a host protein that is known to hijack HIV early in the infection. There is no contention that CPSF6 plays a significant role in why HIV-1 infects cells in an effective manner, making this protein noteworthy (Achuthan et al., 2018). However, there is debate regarding CPSF6 as the depletion or overexpression of CPSF6 in different cell types renders a different impact onto the virus. Preliminary experiments demonstrate that with a knockout of CPSF6 that there is an increase in HIV infection and a decrease in the antiviral response. On the other hand, other labs have found that an overexpression of CPSF6 leads to a decrease in HIV infection, but there has been no research pointing to the effect that an overexpression of CPSF6 has on the antiviral response. Therefore, it is imperative to better understand CPSF6's effect on the antiviral response in regards to HIV infection.

HYPOTHESIS

We hypothesize that the overexpression of CPSF6 will increase the antiviral response and decrease HIV infection in T cells.

METHODOLOGY

- Preparation of the Empty Vector for use in Vector Cloning: E. coli transformation and plasmid purification, restriction enzyme digest, and then gel electrophoresis to confirm successful plasmid linearization.
- Creation of full length CPSF6: Gibson Assembly reactions, E. coli transformation and plasmid purification, restriction enzyme digest, and then gel electrophoresis and Sangar Sequencing to confirm successful plasmid creation.
- Amplification and Expression: Midiprep, Transfection, Western Blot

We ultimately require a method to overexpress CPSF6 in mammalian cells to observe the antiviral response in HIV infection.

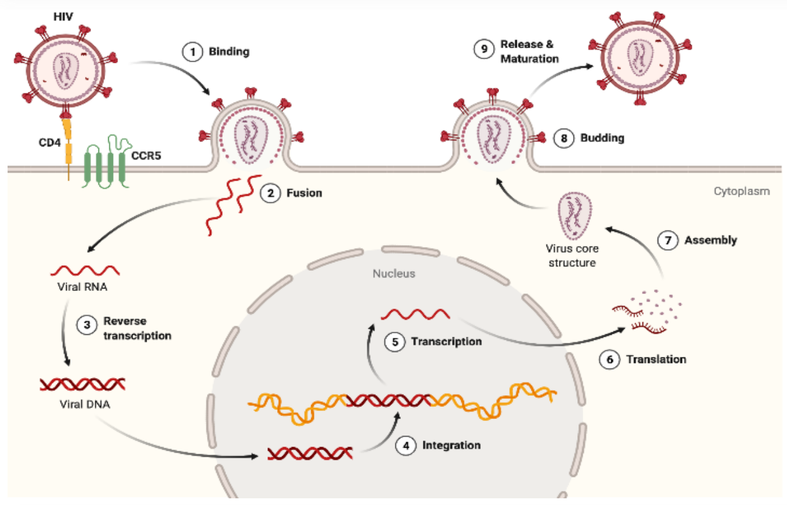

Figure 1: HIV Lifecycle Schematic
A depiction of the process in which Human Immunodeficiency Virus (HIV) hijacks a host cell including the role of CPSF6. HIV inserts itself into the host cell and uses Reverse transcriptase to create more DNA to insert itself into the genome of the host cell to create more copies of itself

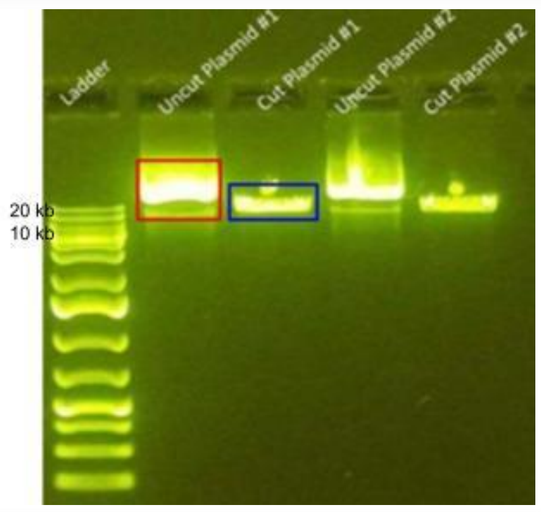

Figure 3: Vector Preparation
An agarose gel showing the results of a restriction enzyme digest using EcoRI, in which the plasmid was correctly cut. The red box identifies the uncut plasmid, and the blue box identifies the linearized plasmid.

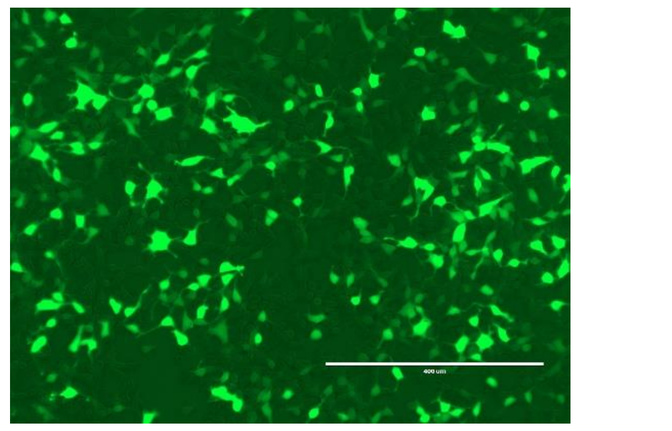

Figure 7: Transfection Control
Transfection of green fluorescent protein (GFP)-containing plasmid in human cell line as a control for CPSF6 transfection

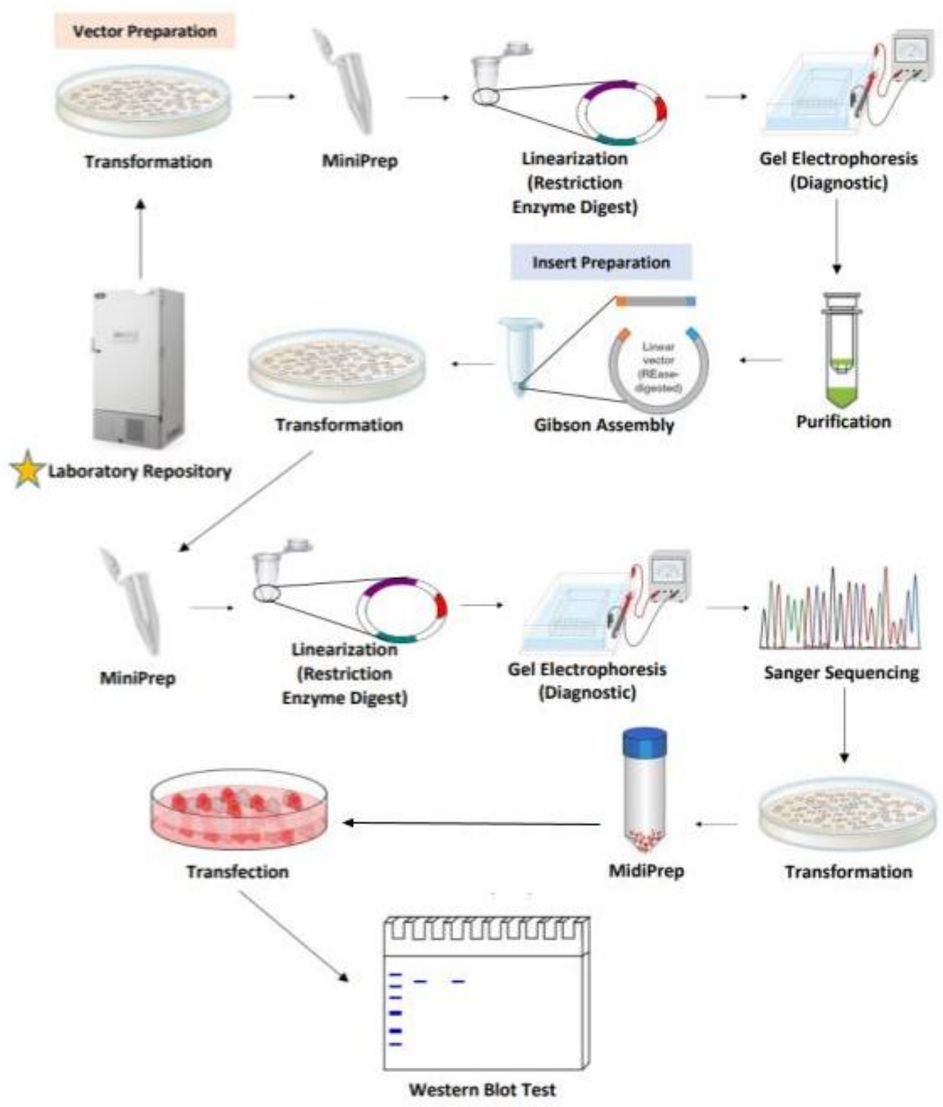

Figure 2: CPSF6 Cloning Pipeline
An illustration of the workflow for this project, starting from the preparation of an empty vector to the detection of CPSF6 in host cells, beginning at the star

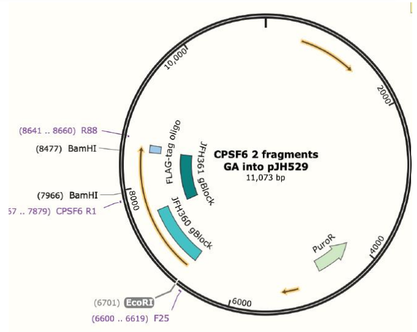

Figure 4: CPSF6 Plasmid Map
Plasmid containing CSPF6 inserts with FLAG tag and puromycin selection marker

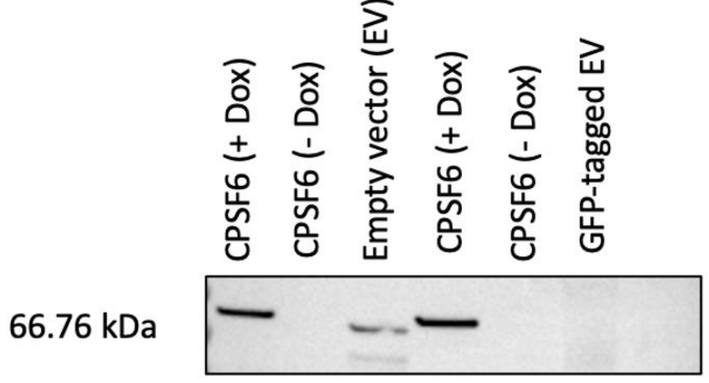

Figure 8: Western Blot
Doxycycline inducible CPSF6 transfection in HEK293T human cell line

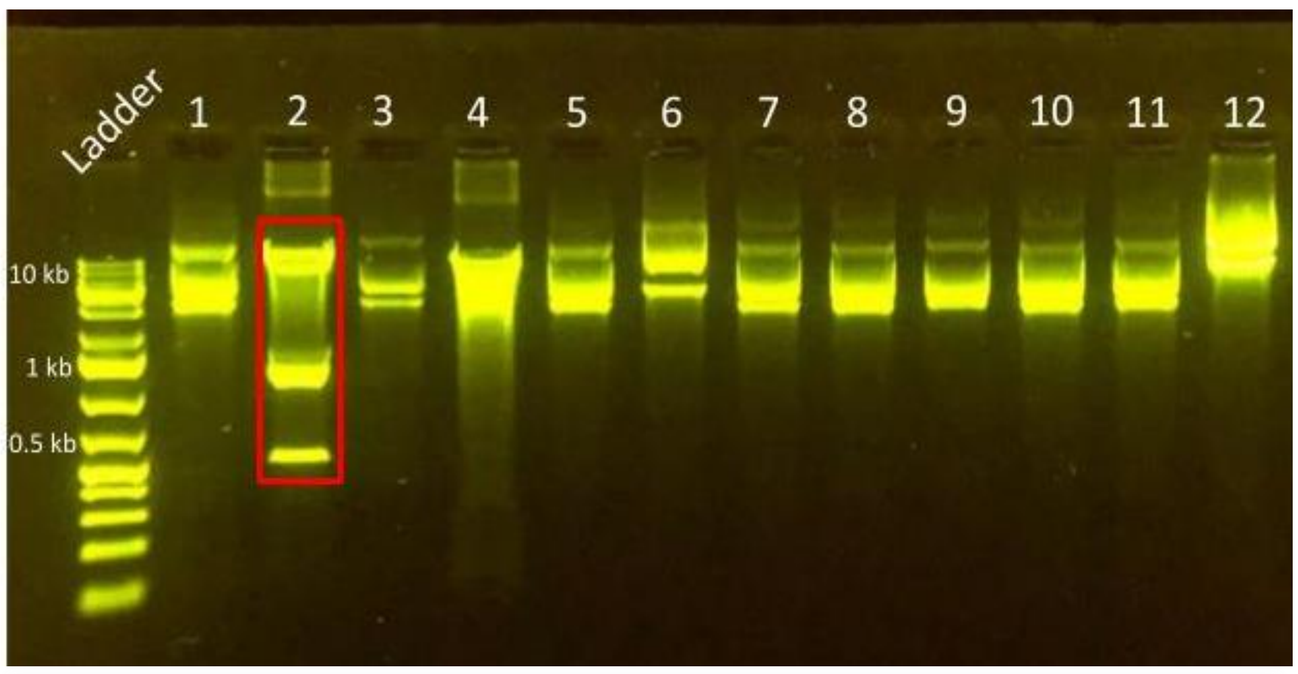

Figure 5: Restriction Enzyme Digest
An agarose gel showing the results of a restriction enzyme digest of the assembled CPSF6-containing plasmid. The Gibson Assembly was successful in Lane 2 as outlined by the red box through bands at the 10kb, 1kb, and 0.5kb marks.

CONCLUSIONS

A successful vector construction was made to overexpress CPSF6 in mammalian cells.

FUTURE DIRECTIONS

Future research will determine the effects of overexpression of CPSF6 on HIV-1 infection through flow cytometry, leading to a greater understanding in CPSF6's role in the innate immune response in HIV infection.

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