

Arenavirus glycoprotein-host cell receptor interactions

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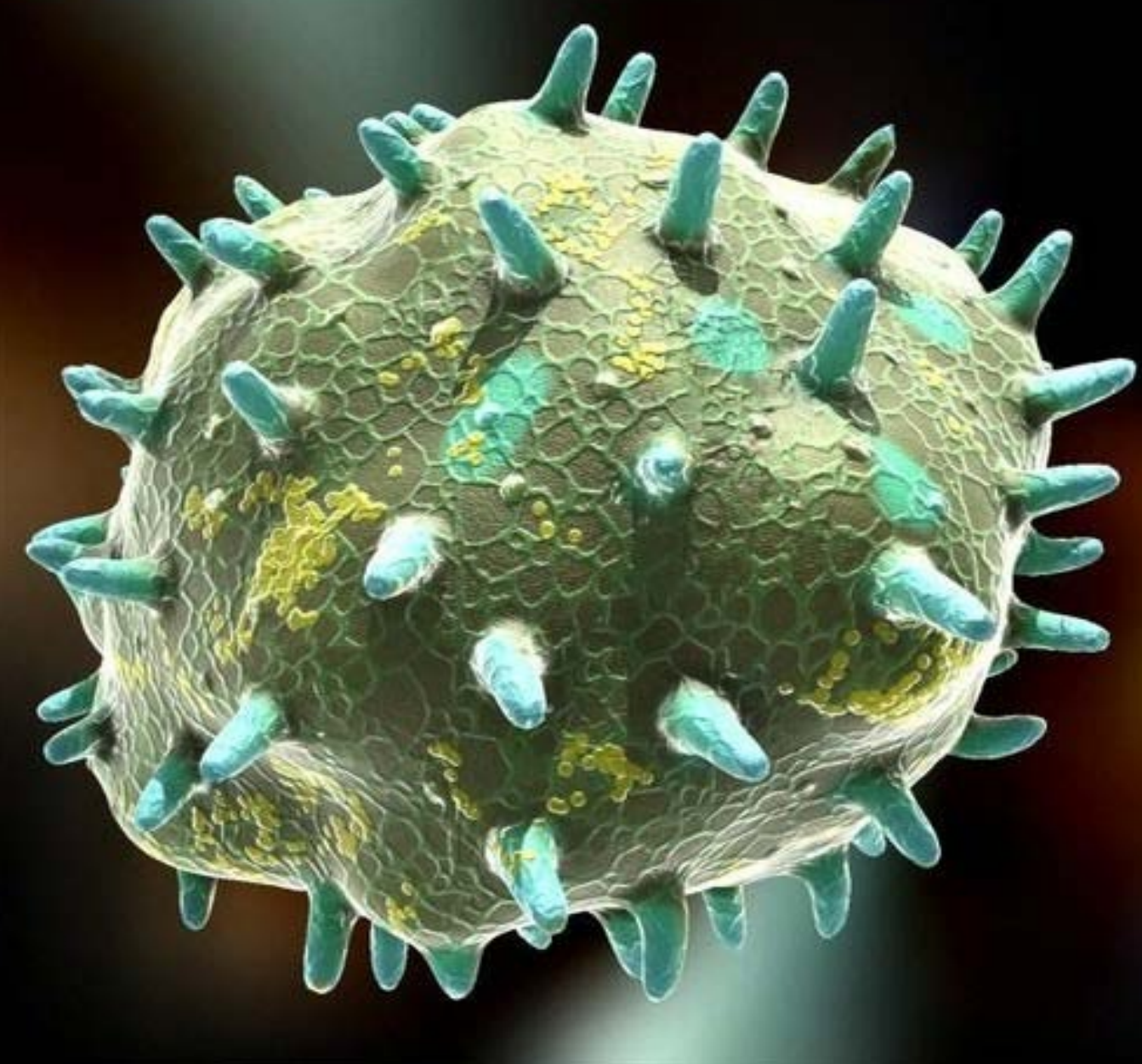
Abstract

Pichinde virus (PICV) is an enveloped single-stranded RNA New World arenavirus. The new world arenaviruses are divided into three separate clades designated as A, B, and C. The clade B arenaviruses are known to cause severe viral hemorrhagic fever. The clade A PICV is prevalent in the rodent population of Colombia. PICV is currently designated as a biosafety level-2 (BSL 2) pathogen due to its low pathogenicity in humans. Efforts have been made to identify the cellular receptors utilized by the virus glycoproteins to enter the host cell. Clade B arenaviruses utilize various species-specific forms of Transferrin Receptor 1 to gain access to the inside of a host cell where they replicate. The cellular receptor that mediates entry by clade A arenaviruses has not been identified and is the basis of my research. The goal of this study is to purify PICV glycoprotein variants containing multiple truncations in order to study the binding of the glycoprotein to cells from highly susceptible host species. We have designed and purified multiple protein truncations of the PICV virus glycoprotein. The binding interactions of these protein truncations requires further study.

Introduction

- PICV is prevalent in the rodent populations of the northeast region of South America.
- The cell surface protein receptor for the PICV virus has yet to be identified.
- New World arenaviruses have been separated into 3 distinct clades: A, B, & C. PICV is a clade A arenavirus.
- Arenaviruses are negative sense single strand RNA enveloped viruses
- PICV is less pathogenic than other arenaviruses such as the Junin, Clade B, arenavirus.
- This study is modeled after former studies used in identifying the protein receptor for the Clade B Machupo arenavirus.¹
- Due to its non-pathogenicity in humans, PICV is commonly used to evaluate treatments for arenaviral hemorrhagic fever.
- The PICV glycoprotein exists as a trimer.

Engineered Pichinde virus²



Objective

- Transfect and amplify various truncations of the PICV viral glycoprotein in HEK293 cells.
- Extract glycoprotein and determine concentration via a Bradford Assay.
- Purify protein using affinity chromatography. Measure the binding affinity of the PICV glycoprotein to baby hamster kidney cells (BHK) surface receptors. Attempt to identify the cell surface protein receptor for PICV.

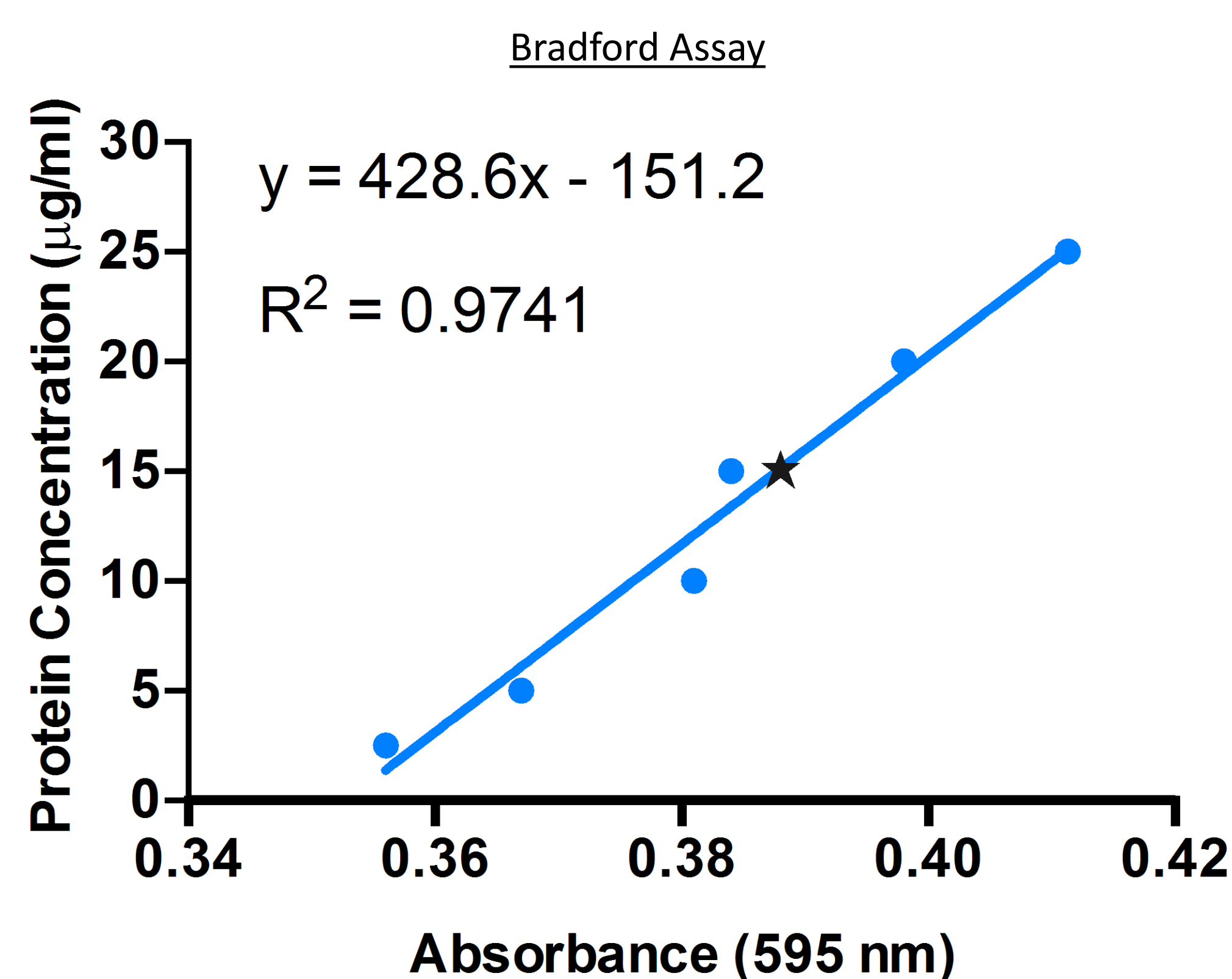
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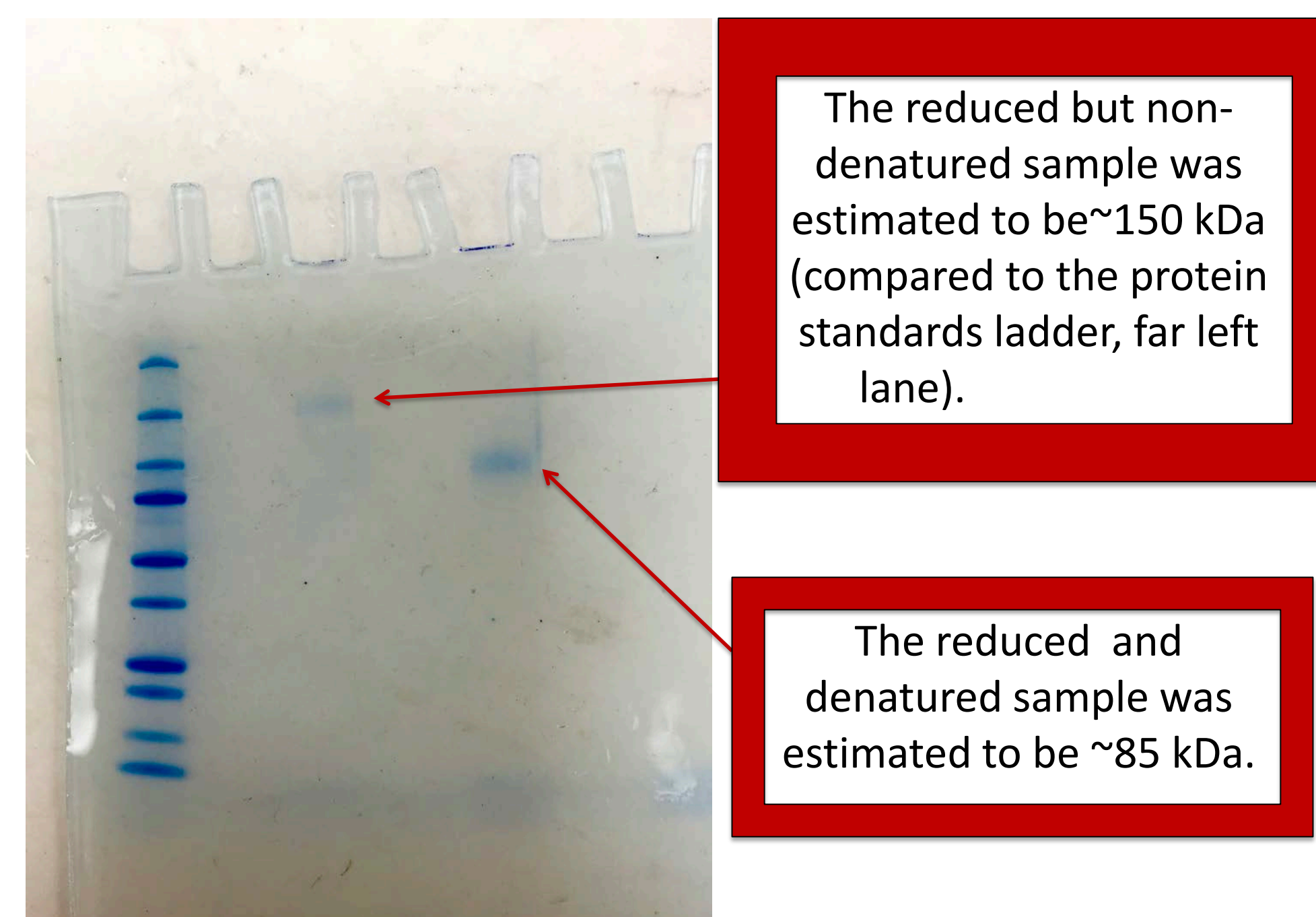
Materials and Methods

- **Cells.** Human Embryonic Kidney 293 cells (HEK-293) were purchased from American Type Culture Collection (ATCC) and grown in DMEM media.
- **Plasmid.** Multiple plasmids were constructed to express different truncations of the PICV glycoprotein. The PICV plasmids contain the human Fc region which allows for purification with affinity chromatography.
- Plasmids were amplified using MC1061 *E. coli* cells, and purified using a Qiagen Maxi Kit (cat. #12162).
- HEK-293 cells were grown in T150 flask at 37 °C and 5% CO₂ in DMEM media with 10% FBS at a density of 1.6x10⁷ cells/flask.
- At 18 hours post seeding the cells were transfected with 80 µg of an expression plasmid coding for the PICV viral glycoprotein.
- The cells were transfected with the plasmids via the calcium phosphate method.
- After a 6 hour incubation period, the cells were washed using warm PBS and SFMII media that contained 1x L-glutamine, non-essential amino acids, amino acids, and sodium butyrate.
- Protein A – Sepharose 4B beads (Invitrogen, cat. #101041) were used to bind to the Fc region of the plasmid for affinity chromatography.
- After purification, dialysis was performed overnight at 4 °C in 1X PBS.
- Once the protein was purified it was concentrated using a 50 kDa centrifugal filter (Amicon, cat. #UFC505024).
- Protein concentration was determined by performing a Bradford Assay.
- Protein identification was performed using an SDS-Page gel to verify the size and purity of the protein sample.

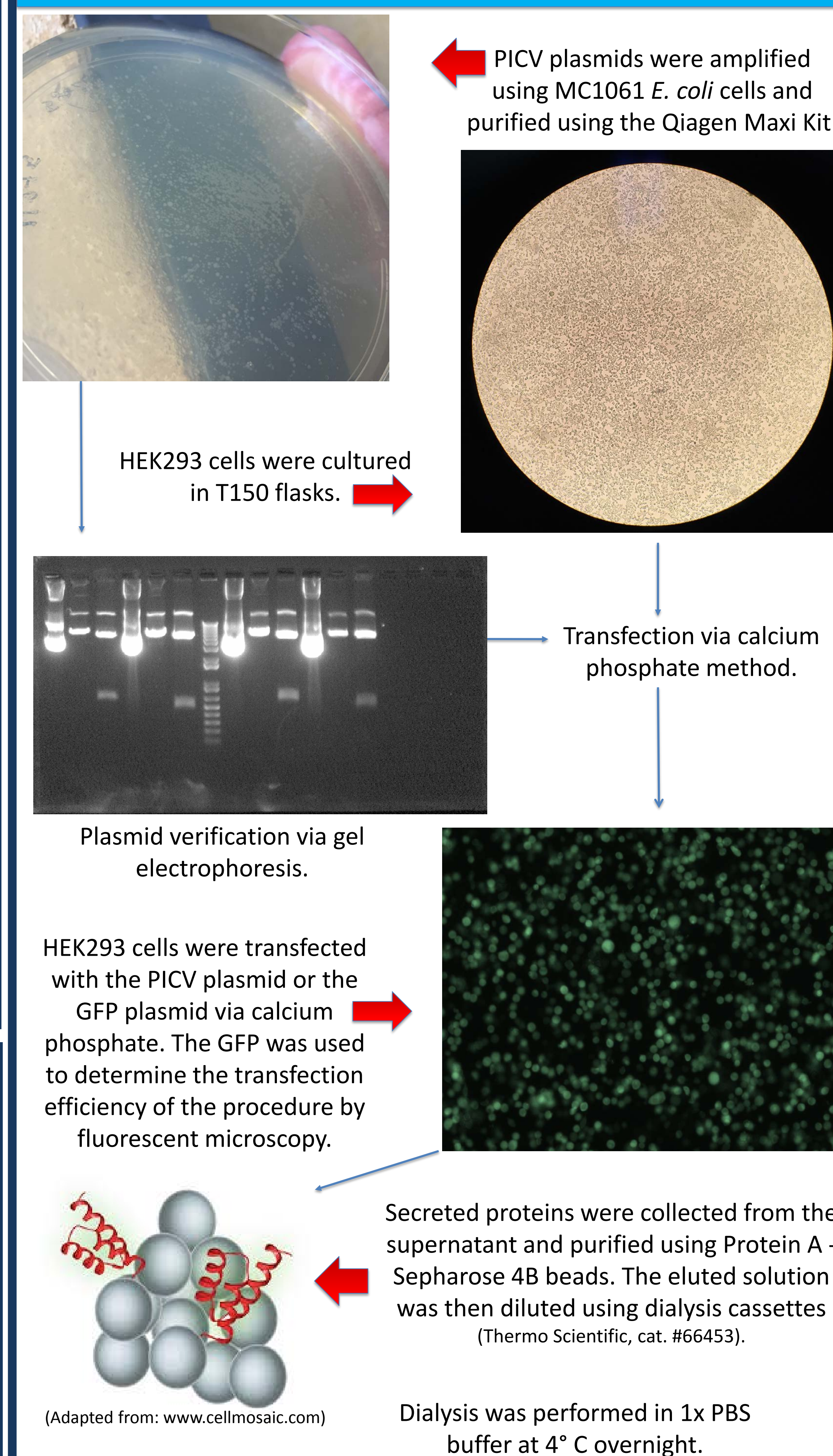
Results



SDS Page gel electrophoresis



Experiment Flowchart



Discussion

- The concentration of 14.7 µg/ml was within the range of the expected yield.
- The PICV glycoprotein and the denatured band is larger than calculated, likely due to glycosylation by the HEK293 cells.
- The next step is to study the binding affinities of the purified PICV glycoproteins with the baby hamster kidney (BHK) cell surface receptors.

References:

1. Radoshitzky, Sheli R. et al. "Receptor Determinants of Zoonotic Transmission of New World Hemorrhagic Fever Arenaviruses." *Proceedings of the National Academy of Sciences of the United States of America* 105.7 (2008): 2664–2669. *PMC*. Web. 13 Apr. 2016.
2. Liang, Y., & Ly, H. (2016). Arenavirus Pichinde Virus Engineered Virus. Retrieved April 12, 2016, from http://license.umn.edu/technologies/20130251_engineered-pichinde-arenavirus

Conclusion

- The protein amplification and purification protocol proved to be a successful and effective method.
- Due to time restrictions, the binding affinity assays have yet to be performed.
- Once the affinity assays are completed additional studies will be conducted to identify the specific cell surface receptor utilized by PICV.