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Creation of an Influenza A Virus Neuraminidase consensus vaccine in an Adenoviral vector

An Undergraduate Honors Thesis

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by

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Dedication and Appreciation

I would like to extend my utmost appreciation to Erika Petro-Turnquist and Matt Pekarek, Ph.D. students in the School of Biological Sciences at the University of Nebraska-Lincoln, for guiding and supporting me through my research, analysis, and writing process this the past year. Thank you for taking the time to teach me various concepts, answer my questions, and read over my drafts. Moreover, thank you for being great role models to me. It has been truly an honor to get to know you both.

I would also like to thank my faculty advisors for supporting and advising me—Dr. Eric Weaver and Dr. Peter Angeletti. Dr. Weaver, thank you for welcoming me into your lab and taking time out of your busy schedule to teach me various lab techniques. I have learned so much more in this lab than I could ever learn in a classroom.

Abstract

In the last decade, the estimated annual burden of the flu includes 12,000-52,000 deaths, 140,000-710,000 hospitalizations, and 9 million – 41 million illnesses (Centers for Disease Control and Prevention: *Disease burden of the flu*). Influenza A virus can mutate and infect a variety of different hosts, which results in frequent antigenic changes and even pandemics. This has led to increased attention on the creation of a universal Influenza A vaccine containing broader immunogenic coverage than current vaccines. Our research involved detailing the steps of the cloning process utilizing a consensus neuraminidase antigen. We utilized the pAdEasy Adenoviral Vector System to insert our consensus neuraminidase gene into a human adenovirus type 5 (HAdV-5) to create a more immunogenic vaccine.

Key Words: Immunology, Neuraminidase, Influenza A vaccine, pAdEasy Adenoviral Vector System, Virology

1. Introduction

Influenza viruses are a negative-sense single-stranded RNA virus that can mutate at a rapid rate. This results in yearly strain changes that force updated vaccines to be made each year. The creation of a universal Influenza vaccine has been at the forefront of vaccine research as current vaccine models do not demonstrate broad immunogenic coverage. Every season, circulating strains each season must be predicted months in advance and can sometimes be predicted incorrectly such as in the 2009 H1N1 pandemic (Centers for Disease Control and Prevention*: 2009 H1N1 pandemic*). Recent studies have investigated creating mosaic antigens to produce a more effective Influenza vaccine (Corder 2019 & Weina 2019). Through the creation of an Influenza vaccine with broader immunogenic capabilities, Influenza vaccinations may only need to be given once every 5 years, 10 years, or potentially once in a lifetime.

Here we developed a recombinant adenovirus vaccine utilizing the AdEasy Adenoviral Vector System. The adenoviral vector acts as a delivery system to carry a consensus Influenza A virus (IAV) neuraminidase antigen *in vivo*. Neuraminidase (NA) and hemagglutinin (HA) are two glycoproteins found on the influenza virus membrane (Gamblin 2010) and aid in viral entry and exit from the host cell during infection. The plasticity of these two main proteins serves as a major obstacle in creating a broadly cross-reactive vaccine against IAV. A consensus vaccine is created by determining the average amino acid at each point on a gene and utilizing the most common amino acid to create a vaccine that promotes a broader immune response.

Adenovirus is a common virus that causes cold-like symptoms. By the age of 10, almost every child has at least one adenovirus infection (Centers for Disease Control and Prevention: *Adenovirus*). Adenovirus is also capable of infecting a wide range of cell types just like the Influenza A virus.

For this study, we utilized the human adenovirus serotype 5 (HAdV-5) as a vector. The adenovirus genome is large and is approximately 36kb. This HAdV-5 has had the E1 and E3 genes deleted to render the adenovirus replication defective. With the elimination of the E1 and E3 genes, the recombinant adenovirus will have up to 7.5kb available for the insertion of foreign DNA, and it will not allow the virus to replicate itself. We utilized homologous recombination in *Escherichia coli* through co-transforming pAdEasy-1 and a shuttle vector carrying our gene. The genome of the shuttle vector (pShuttle-CMV) is approximately 7.5kb and its cloning capacity is 6.6kb.

2. Materials & Methods

2.1 N1 Neuraminidase Gene Design

To create the NA gene, all complete human Influenza A N1 sequences isolated between 1930 and 2007 with complete amino acid sequences in human hosts were downloaded from the Influenza Research Database. Duplicate sequences and laboratory strains were excluded. This resulted in 748 sequences. In 2007, Dr. Eric Weaver utilized this process to create the NA-1

consensus sequence. An *in-silico* representation of the NA-1 consensus protein sequence was created with Swiss-Model and visualized in the UCSF Chimera program (Figure I). To visualize the genetic relation of the NA-1 consensus gene to all other natural sequences, a phylogenetic tree was constructed in Geneious using the748 sequences initially used in the immunogen design (Figure II). The gene was then human codon optimized and synthesized by GenScript with the addition of the KpnI and HindIII restriction sites artificially added to the ends for downstream cloning processes. The final gene was created in the plasmid pUC57, containing an ampicillin resistance gene.

2.2 Ligation of pShuttle-CMV and N1 Neuraminidase

To begin, the Genscript-synthesized plasmid was transformed into XL-1 electrocompetent *E coli* cells and selected on Luria Broth (LB)-ampicillin plates. One colony was picked from the pUC57-NA1 consensus transformation and inoculated into a cell culture tube consisting of 4mL LB media (1 liter of LB media contains 10 grams of Tryptone, 10 grams NaCl, 5 grams yeast extract, and the addition of distilled water up to the 1-liter volume) and 4µL Ampicillin (used in a 1:1000 ratio) (Bradburn 2019). The tube was place at 37°C conditions and shook overnight at 225 revolutions per minute (RPM). The next day, a glycerol stock of the pUC57-NA1 was made through the addition of 250µL of 60% glycerol and 750µL of the bacteria to a cryotube. The glycerol stock was vortexed and placed at -80°C for long-term storage.

The remaining culture was miniprepped using the QIAprep spin miniprep kit with the following modifications: bacteria were spun for 1 minute at max speed in step 1, bacteria were spun for 5

minutes at max speed in step 5, and final DNA isolated eluted in 50µl of double distilled water in step 10 (*QIAPrep Miniprep* 2012). The product was vortexed and nanodropped to determine the concentrations. All minipreps performed in the cloning process utilized the same kit and modifications applied as previously described.

The product was then digested using restriction enzymes. A restriction enzyme digest enables the neuraminidase gene to be cut out of the current plasmid so that it can be later introduced into a new vector. In this case, the consensus neuraminidase gene was to be spliced out of the pUC57-NA1 parental plasmid and introduced into pShuttle-CMV. The shuttle vector allows for the insertion of our gene into pAdEasy-1, as outlined in step 2.3. It is challenging to directly ligate our gene of interest into pAdEasy-1 as pAdEasy-1 has very few restriction enzyme sites which results in the need for a shuttle vector (*AdEasy adenoviral vector system*).

KpnI and HindIII were the enzymes used in this procedure. The digests of PUC57-NA1 consensus and pShuttle-CMV were done separately but with the same concentrations of the following components: 10µg DNA, 8µL 10X Cut Smart, 0.5µL KpnI, 0.5µL HindIII, and 21µL double deionized water to create a total volume of 80µL. A 10µg concentration of the DNA was desired, and the 10X Cut Smart used in this procedure consisted of 10% of the total volume. The pShuttle-CMV digest and the pUC57-NA1 consensus digest were both placed at 37°C overnight for complete digestion.

The two restriction digests were run on a 0.8% agarose gel at 130 volts for 35 minutes, and the digested DNA can be seen in Figure III, lanes 2 and 4. The QIAquick Gel Extraction Kit was

used to extract the digested pShuttle-CMV and the neuraminidase gene of interest, and the excise fragements were then purified with a Gel Cleanup Kit (*Qiaquick Gel Extraction Kit and QIAquick PCR & Gel Cleanup Kit*). The products were then nanodropped to determine the 260/280 values and DNA concentration.

The extracted products were then used in a ligation. A ligation utilizes a ligase to join two fragments of DNA. The enzyme used in this procedure was T4 ligase. Three different ligation concentrations were used to join the pUC57-NA1 consensus gene into pShuttle-CMV. Ratios of 3:1, 1:1, and 1:3 were prepared. The ligations were incubated at room temperature for 2 hours and then placed at 4°C overnight.

After the ligation, the three different concentrations were transformed via electroporation into XL-1 electrocompetent *E. coli* bacterial cells. Electroporation uses electricity to create temporary pores in bacterial membranes so that foreign nucleic acid material can enter the cells. 5µL of each ligation, 20µL XL-1 bacterial cells, and 80µL of double deionized water were added to an electrocuvette. A control transformation was also performed which included 20µL XL-1 bacterial cells, and 80µl of double deionized water. A control is used to ensure that there is no contamination or issues with the materials used. If there is a high background on the negative control plate, that could indicate there are problems with the bacterial cells or antibiotics in the agar plates. The samples were placed in the electroporator at 2500 volts. After electroporation, 350µL of Super Optimal broth with Catabolite repression (SOC) media was added to each electrocuvette. SOC media is a nutritionally rich media that enables the damaged cells to recover after the transformation. The products were then transferred to microtubes and shook for 2 hours

at 37°C and 225 RPM. 50µL of each transformation product was plated on a LB-Kanamycin plate and then incubated overnight at 37°C. In this step, kanamycin was used as a selective antibiotic to only allow successfully ligated pShuttle-CMV-NA1 consensus products to grow on the plates.

Colonies from each plate were miniprepped, confirmation digested, and run on a gel. As seen in Figure IV, all six colonies selected contained both pShuttle-CMV and the NA1-consensus insert (lanes 1-6). The same methods were used as explained above for the miniprep, restriction digest, and gel electrophoresis from the prior experiment. We used the QIAspin midiprep kit to isolate the DNA from the XL-1 cells. Performing a midiprep at this step enabled us to collect a higher concentration and amount of pShuttleCMV-NA1-consensus prior to the upcoming co-transformation step. The Qiagen midiprep protocol was followed with the following modifications: spun for 5 minutes at 6000xg in step 6 and eluted with 1mL of 50°C ddH_2O in step 17 (*Qiagen plasmid Mini, MIDI, and Maxi Kits (EN)*)

2.3 Co-transformation into pAdEasy-1

To perform the co-transformation, pShuttle-CMV-NA1 was linearized to insert our gene. 34µL of DNA, 10µL ddH_2O, 5µL Cut Smart, and 1µL of PmeI enzyme were pipetted into a microtube and incubated at 37°C overnight. After linearization of pShuttle-CMV-NA1, Shrimp Alkaline Phosphatase (rSAP) (Biolabs) was added to the digest. rSAP is an enzyme that can catalyze the

removal of phosphate groups of 5´ and 3´ ends of the phosphomonoesters in DNA. 1µL of rSAP was added and incubated at 37°C for 1 hour.

After incubation, the linearized pShuttle-NA1-consensus with removed phosphates was purified using the QIAquick Polymerase Chain Reaction (PCR) purification kit from QIAGEN with the following modification: in step 7, the DNA was eluted with 50µL ddH_2O instead of Buffer EB (*QIAquick PCR purification kit and QIAquick PCR & gel cleanup kit quick-start protocol*).

Then, the purified gene product was co-transformed into BJ5183 *E. coli* cells. BJ5183 cells are used because they contain the *recA* protein and the machinery necessary to perform the homologous recombination between pShuttle-CMV and pAdEasy-1 (*AdEasy adenoviral vector system*).12µL pShuttle-CMV-NA1-consensus, 1µL pAdEasy-1, 20µL BJ5183 cells, and 80µL ddH_2O were added to an electrocuvette and electroporated at 2500 volts. After electroporation, the product was transferred to a 1.5mL microtube and 350µL of SOC media was added and shook for 2 hours at 37°C at 225 RPM. 50µL of each transformation product was plated on a Kanamycin plate and incubated overnight at 37°C. Kanamycin was once again used as a selective antibiotic to only allow the successful transformants to grow.

2.4 Miniprep DNA and confirmation with restriction enzyme digest

20 bacterial colonies were picked after the co-transformation from the LB/Kan plate and miniprepped. After nanodropping, a confirmation digest was performed to see if the gene of interest was successfully inserted into pAdEasy-1. 0.5µL PacI, 2µL 10X Cut Smart, 10µL

pAdEasy-NA1-consensus, and 7.5µL double deionized water were added to a microtube and incubated at 37°C for 30 minutes. The confirmation digest was run on a 0.5% agarose gel for 30 minutes at 130 volts. This successful transformant can be visualized in Figure V (lane 2). At this stage, a successful transformation is seen as no band present. The DNA run in lane 2 was utilized in the subsequent confirmation steps outlined in section 2.5 below.

2.5 Transformation into XL-10-Gold Ultracompetent cells to amplify gene product

To amplify the gene product, the pAdEasy-NA1-consensus was transformed into XL10-Gold (XL-1) ultracompetent cells. XL-1 cells are deficient in *recA* and the endonuclease (*endA1*). The absence of *recA* helps maintain the stability of the insert, and *endA1* improves the quality of the miniprepped DNA (*AdEasy adenoviral vector system*). 20µL pAdEasy-NA1-consensus, 20µl XL-1 cells, and 80µl ddH_2O were added to an electrocuvette and electroporated at 2500 volts. After electroporation, the same end procedure described in the co-transformation step in **2.3** was performed.

To confirm that we had the correct product, a miniprep and confirmation digest were performed. One XL-1 colony was picked from the Kan-LB plate and placed in a cell culture tube with 4mL of LB media and 4µL kanamycin. The tube shook overnight at 37°C and a restriction enzyme digest was performed the next day. The concentrations for the digest were as follows: 30µl pAdEasy-NA1-consensus, 3.3µL 10X Cut Smart, and 0.5µL PacI. The digest was incubated for 1 hour at 37°C and then ran on a 0.8% agarose gel for 30 minutes at 130 volts. Concurrently, an uncut plasmid was run on the agarose gel to better visualize the successful insertion into the

pAdEasy-1 genome. The appropriate DNA band can be seen in Figure VI, lane 2 as ~5.1kB. A glycerol stock of the pAdEasy-NA1-consensus in XL-1 cells was prepared by adding 250µL of 30% glycerol and 750µL of the XL-1 bacteria with our gene of interest to a cryotube. The glycerol stock was vortexed and placed at -80°C.

After confirmation of our gene, we used the QIAspin midiprep kit with the same procedure and exceptions explained in section 2.2 to increase the amount of the pAdEasy-NA1-consensus DNA product.

Results

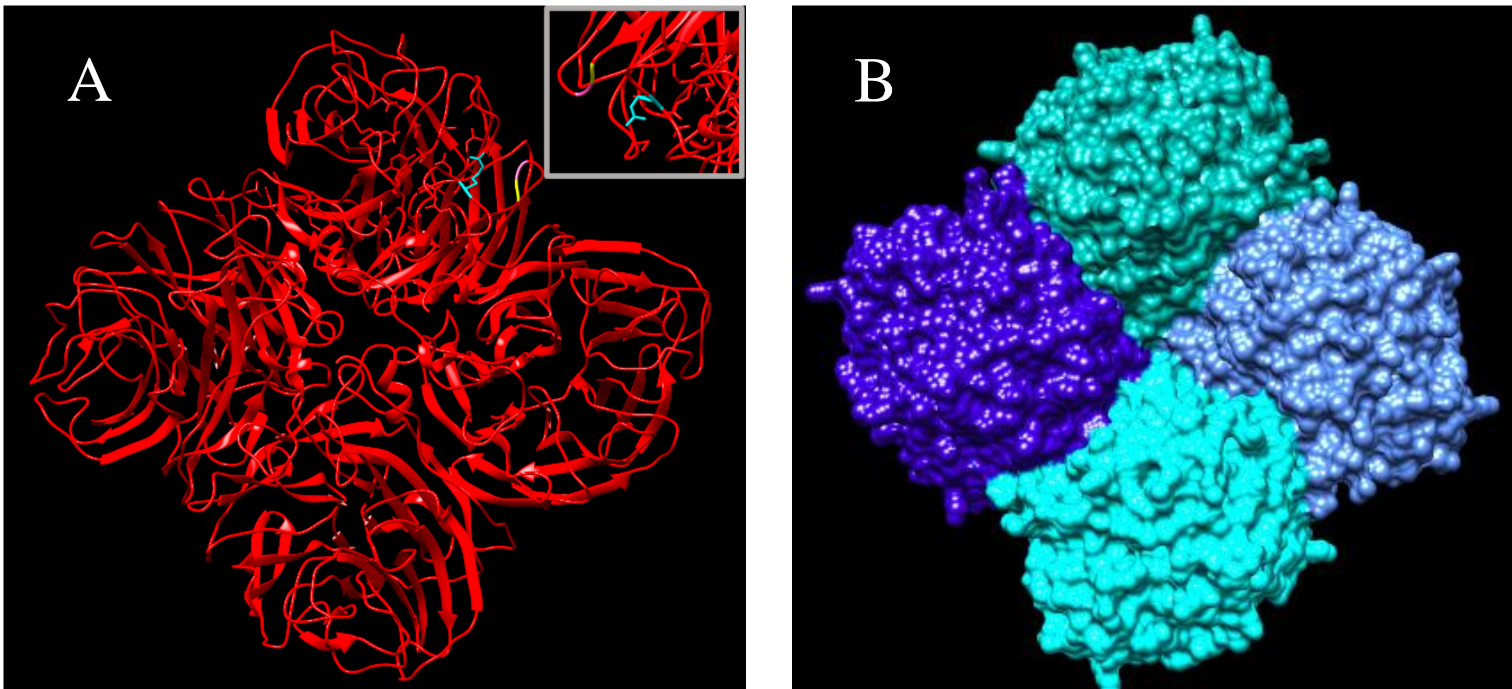

Figure I. Swiss-Model Immunogen reconstructions of NA-1 consensus antigen (GMQE=0.8; QMEAN= 0.93; Template=3b7e.1.A; Sequence Identity= 91.95%) (*Model*). **R152** (highlighted

in blue) has been shown to interact with the light and heavy chain of broadly cross-reactive antibody 1G01 (Stadlbauer 2019).

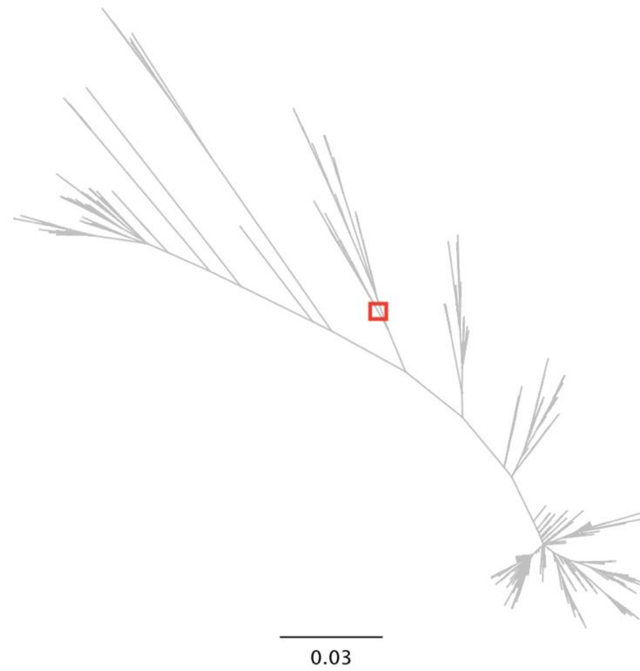

Figure II. Phylogenetic Tree of NA-1 consensus antigen (indicated by the red square) (*Influenza research database*).

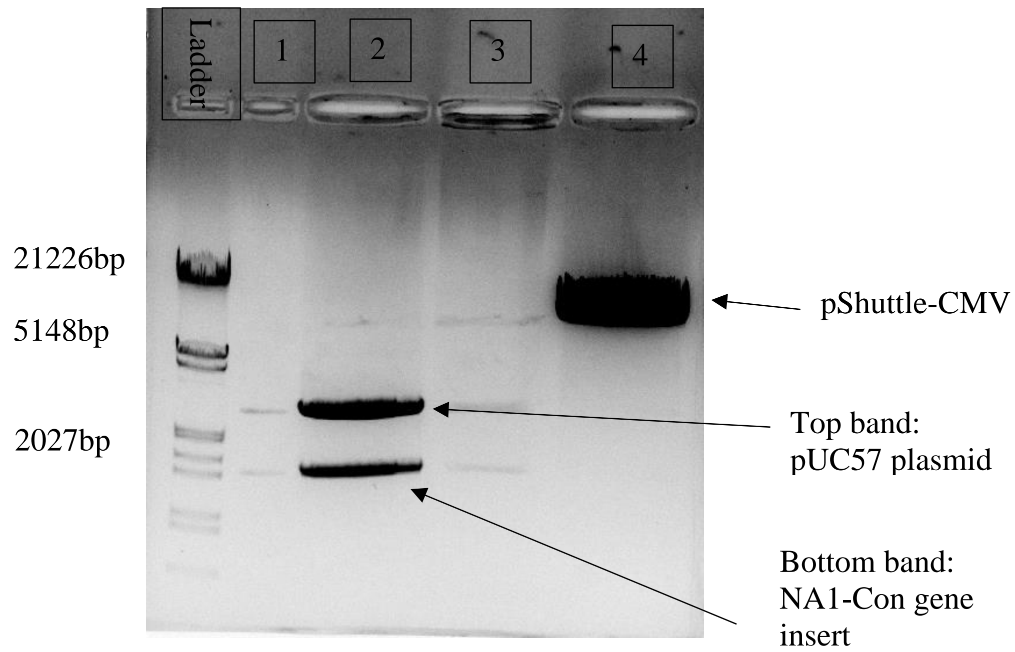

Figure III. KpnI and HindIII digest and gel extraction of pUC57-N1-Con and pShuttle-CMV.

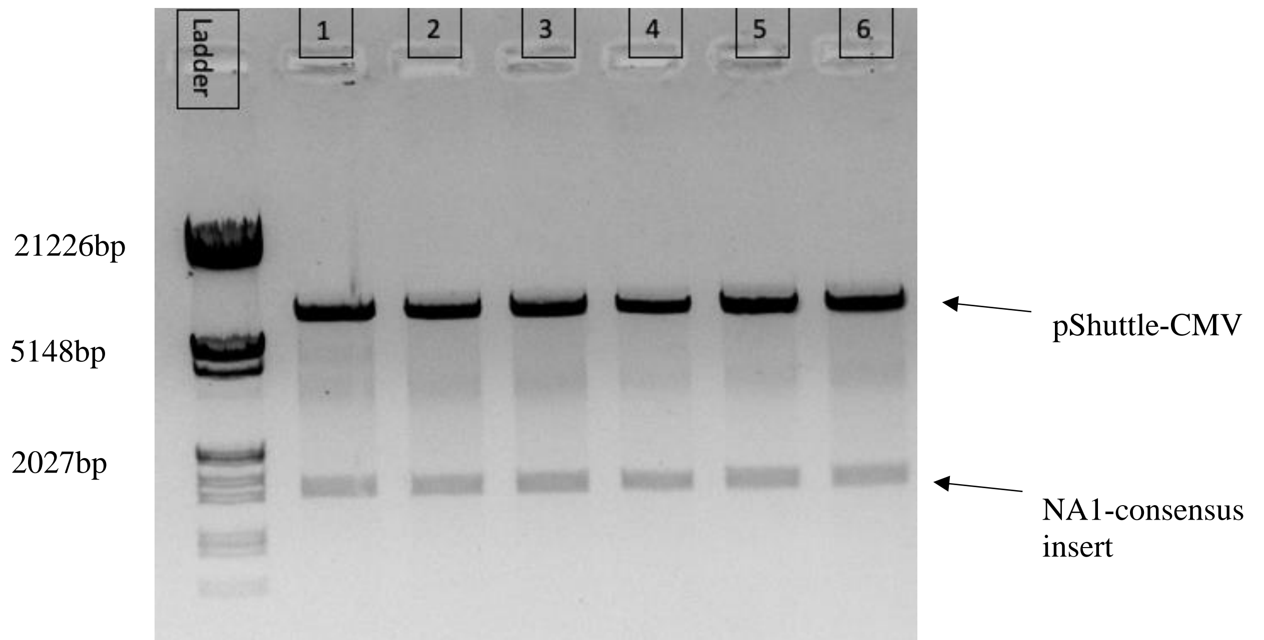

Figure IV. Confirmation of pShut-CMV and NA1-Con ligation for insert.

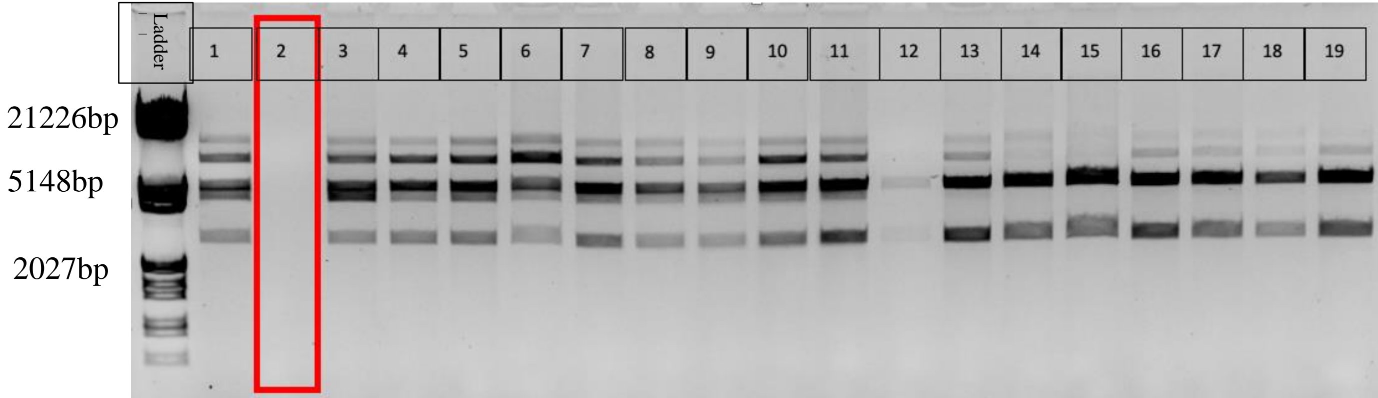

Figure V. Screening colonies for recombinant adenovirus.

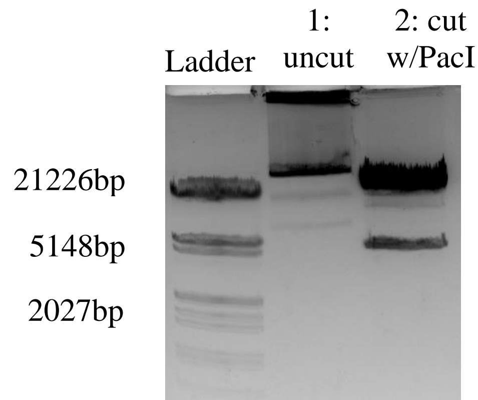

Figure VI. PacI digestion before transfection.

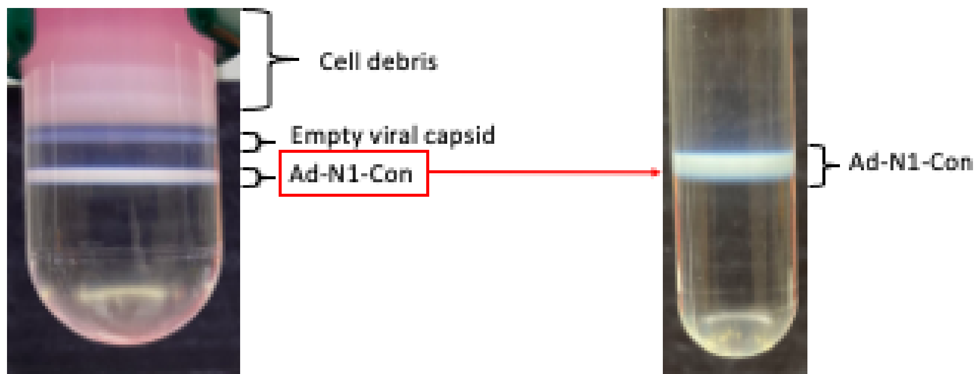

Figure VII. CsCl Prep after amplification.

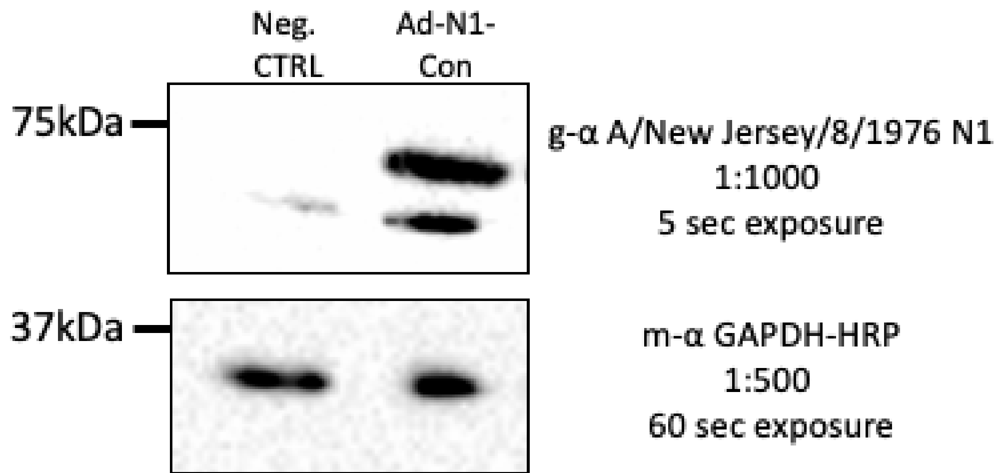

Figure VIII. Western Blot of Ad-NA1-Con.

Discussion

Our gene was successfully cloned into the pAdEasy vector system. In Figure V, no bands appear in lane 2 indicating it was a potential co-transformation success of pAdEasy and pShuttleCMV-NA1-Con. The pAdEasy gene is a large genome consisting of 36 kb. This large genomic material explains why it does not appear in lane 2. Lane 2 also had a very low concentration of 4.8 ng/µL, explaining the lack of band expression. To confirm that it was truly a co-transformation success of pAdEasy and pShuttleCMV-NA1-Con, the colonies were re-screened after a transformation into XL-1 cells. The re-screening of the DNA from lane 2 with a PacI digest indicated a successful co-transformation did occur with our gene of interest and pAdEasy (Figure VI). The uncut DNA is significantly above the 21226bp band which matches the genome of pAdEasy

(~36kb), and the cut DNA shows the presence of our insert. Further demonstration of successful cloning is shown in the CsCl prep and the western blot (Figures VII and VIII).

The implications of this research are vast and could lead to the creation of a universal Influenza A vaccine that utilizes a consensus neuraminidase. Studies have found that antibody 1G01 binds to the neuraminidase antigen and the antigen serves as a target for broadly protective antibodies (Stadlbauer 2019 & Madsen 2020). As neuraminidase is more conserved than hemagglutinin, neuraminidase could serve as a better target in the future for a universal Influenza vaccine than previously thought.

Limitations

Cell culture and further experimentation with animals were not performed by myself as I do not have the credentials to work directly with animals. These steps were completed by graduate student Erika Petro-Turnquist, including the CsCl preparation and the Ad-NA1-Con western blot. The research done prior to cell culture was performed by myself and two other undergraduate students.

References

AdEasy adenoviral vector system - agilent. (n.d.). Retrieved February 19, 2022, from https://www.agilent.com/cs/library/usermanuals/public/240009.pdf.

Biolabs, N. E. (n.d.). *Shrimp alkaline phosphatase (rsap)*. NEB. Retrieved February 19, 2022, from https://www.neb.com/products/m0371-shrimp-alkaline-phosphatase-rsap#Product%20Information.

Bradburn S., (2019, August 9). *How to make LB media*. Top Tip Bio. Retrieved February 19, 2022, from https://toptipbio.com/lb-media-recipe/.

Centers for Disease Control and Prevention. (2019, August 28). *Adenovirus*. Centers for Disease Control and Prevention. Retrieved February 19, 2022, from https://www.cdc.gov/adenovirus/index.html.

Centers for Disease Control and Prevention. (2022, January 7). *Disease burden of flu*. Centers for Disease Control and Prevention. Retrieved February 19, 2022, from https://www.cdc.gov/flu/about/burden/index.html#:~:text=While%20the%20effects%20of%20flu,annually%20between%202010%20and%202020.

Centers for Disease Control and Prevention. (2019, June 11). *2009 H1N1 pandemic (H1N1PDM09 virus)*. Centers for Disease Control and Prevention. Retrieved February 19, 2022, from https://www.cdc.gov/flu/pandemic-resources/2009-h1n1-pandemic.html.

Corder, B. N., Bullard, B. L., DeBeauchamp, J. L., Ilyushina, N. A., Webby, R. J., & Weaver, E. A. (2019). Influenza H1 Mosaic Hemagglutinin Vaccine Induces Broad Immunity and Protection in Mice. *Vaccines*, 7(4), 195. https://doi.org/10.3390/vaccines7040195.

Download - qiagen - sample to insight - qiagen. (n.d.). Retrieved February 19, 2022, from https://www.qiagen.com/cn/resources/download.aspx?id=c164c4ce-3d6a-4d18-91c4-f5763b6d4283&lang=en.

Gamblin, S. J., & Skehel, J. J. (2010). Influenza hemagglutinin and neuraminidase membrane glycoproteins. *The Journal of biological chemistry*, 285(37), 28403–28409. https://doi.org/10.1074/jbc.R110.129809.

Influenza research database -Influenza genome database with visualization and Analysis Tools. Influenza Research Database - Influenza genome database with visualization and analysis tools. (n.d.). Retrieved February 19, 2022, from https://www.fludb.org/.

Madsen A., Dai Y., McMahon M., Schmitz A.J., Turner J.S., Tan J., Lei T., Slsoussi W.B., Storhmeier S., Amor M., Mohammed B.M., Mudd P.A., Simon V., Cox R.J., Fremont D.H., Krammer F., Ellebedy A.H (2020), Human Antibodies Targeting Influenza B Virus Neuraminidase Active Site Are Broadly Protective. *Immunity* 53(4), 852-863. https://doi.org/10.1016/j.immuni.2020.08.015.

Model. SWISS. (n.d.). Retrieved February 19, 2022, from https://swissmodel.expasy.org/.

Qiagen. (n.d.). *Qiagen plasmid Mini, MIDI, and Maxi Kits (EN)*. Retrieved February 23, 2022, from https://www.qiagen.com/cn/resources/resourcedetail?id=c164c4ce-3d6a-4d18-91c4-f5763b6d4283&lang=en

Qiagen. (n.d.). *Qiaquick Gel Extraction Kit and QIAquick PCR & Gel Cleanup Kit - (EN).* QIAGEN. Retrieved February 19, 2022, from

https://www.qiagen.com/gb/resources/resourcedetail?id=a72e2c07-7816-436f-b920-98a0ede5159a&lang=en.

Qiagen. (n.d.). *QIAquick PCR purification kit and QIAquick PCR & gel cleanup kit quick-start protocol - (EN)*. QIAGEN. Retrieved February 19, 2022, from https://www.qiagen.com/cn/resources/resourcedetail?id=e0fab087-ea52-4c16-b79f-c224bf760c39&lang=en.

QIAPrep Miniprep - 2012.igem.org. (n.d.). Retrieved February 19, 2022, from http://2012.igem.org/wiki/images/8/86/QIAprep_miniprep.pdf.

Stadlbauer D., Zhu X., McMahon M.,Turner J. S., Wohlbold T. J, Schmitz A. J., Strohmeier S., Yu W., Nachbagauer R., Mudd P. A., Wilson I. A., Ellebedy A. H., Krammer F. (2019). Broadly protective human antibodies that target the active site of influenza virus neuraminidase. *Science* 366, 499–504. 10.1126/science.aay0678.

Weina, S, Kirkpatrick E., Ermler M., Nachbagauer R., Broecker F., Krammer F., & Palese P. (2019). Development of Influenza B Universal Vaccine Candidates Using the “Mosaic” Hemagglutinin Approach. *The Journal of Virology*, 93(12). https://doi.org/10.1128/JVI.00333-19.