Characterization of the ACE2 protein/SARS-CoV-2 spike protein interaction in the nasal passageway

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Introduction

Due to the current pandemic, I was inclined to research Covid-19. After hearing that many of my peers had lost their sense of smell in response to the virus, I decided to research how the Covid affects the nasal cavity. I chose Dr. Wolyniak to be my adviser and we began to make progress on this project May 2021. This research is intriguing because it could help propose and test inhibitors for Covid and other viruses. The research as it stands now is incomplete but I hope to finish it before the year ends. Because Angiotensin Converting Enzyme 2, ACE2, is the primary receptor for ACE2 is the focus of my research. Blume et al. discovered the new short ACE2 isoform and suggests that this short isoform plays a role in the Covid infection. In comparison, the new short ACE2 isoform’s open reading frame is 1441 base pairs while the long isoform is 2415 base pairs. Being about half the size of the long isoform, the short isoform lacks a few important portions. The start codon, TATTA box and Covid spike binding site are not included in the short isoform. While Blume et al. provides some possible alternatives for these things, it is surrounded by a surplus of uncertainty. The goal of this project is to first construct the short ACE2 isoform and the covid spike protein cDNA. Then induce the spike protein, short, and long ACE2 isoforms to express their proteins. If successful, the binding affinity between the spike protein and both ACE2 isoforms is to be assessed and compared using fluorescents.

Materials & Methods

First, ACE2 cDNA was ordered from Origene and Sars-Covid 2 spike cDNA was ordered from R&D systems. Both cDNA’s were bacterially transformed into E. coli cells then the plasmid DNA was isolated from the E. coli cells in order to replicate the original cDNAs.

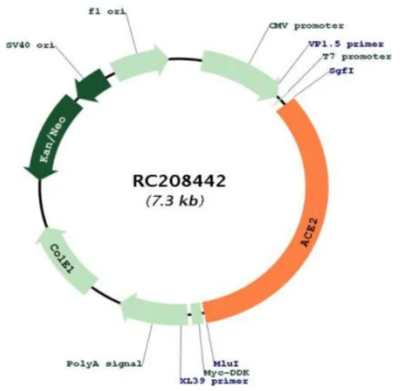

Figure 1: Origene ACE2 Plasmid Map

This plasmid map reveals some key components.

The orange portion is the ACE2 open reading frame

Kanamycin resistance which allows for screening (Dark Green)

The T7 Promoter indicates that the plasmid can be induced by IPTG

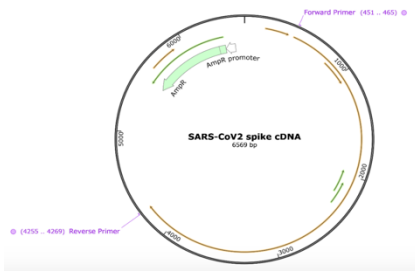

Figure 2a: R&D Systems Spike Plasmid Map

This plasmid map reveals some key components.

The large yellow line is the Covid spike open reading frame

The short green arrow represents ampicillin resistance for screening

Both primers can be seen on this graphic

The Covid spike vector is only compatible with human cells so the open reading frame was amplified out using a forward and reverse primer and ligated into a PQE70 vector.

In order to move the spike open reading frame into the pQE70 vector the restriction enzyme Sph1 cut both vectors then the open reading frame was ligated into the cut pQE70 vector.

The ACE2 short isoform is created by cutting both the Long ACE2 isoform and a constructed oligonucleotide using BamH1 enzymes then ligating the two together. The cut ACE2 and oligo fragments were purified via gel purification.

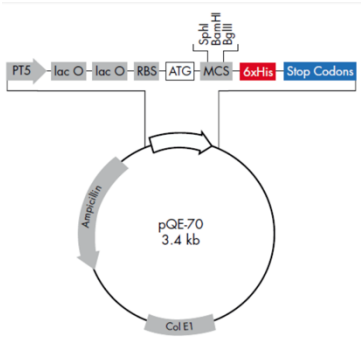

Figure 2b: pQE-70 vector

This vector is designed to allow easy protein expression.

The graphic shows the targeted restriction site: SphI

The vector is screened with ampicillin.

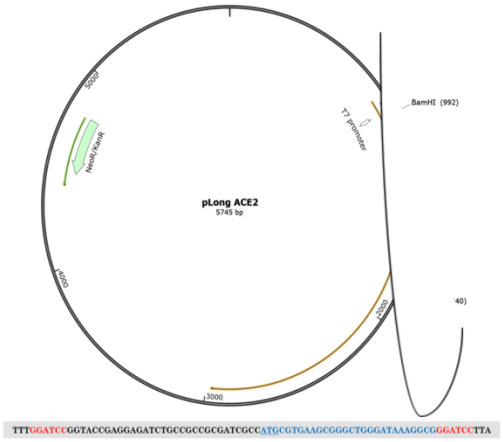

Figure 3: Long Ace2 vector & ordered Oligonucleotide

The graphic shows the targeted restriction site: BamH1

The vector is screened with Kanamycin

The red portion of the oligo represents the BamH1 restriction sites

The blue portion represent the predicted transcription site (Blume et all)

The arrow shows where the oligo would be ligated

Once synthesized a sample of both, short ACE2 and spike vectors are mailed out to Eurofins Genomics to be sequenced. After determining that the short isoform and the spike DNA have been successfully synthesized, their proteins must be expressed. Then the two ACE isoforms and their interaction with the spike protein can be easily compared.

The long ACE2 isoform was used to optimize the protein expression conditions. Variables that were altered include the IPTG concentration and the bacterial density and induction duration. Optimization is purely based on experimentation and directed by trial and error.

Results

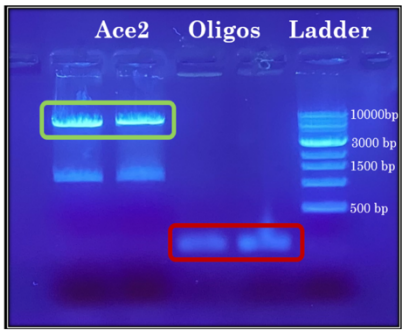

Figure 4: Gel Purification

This picture shows my ACE cDNA and oligo after gel purification

The portions in the green square are the cut ACE2 fragments that are included in the short isoform

The ladder allows size measurement thus allowing selection of only desired dna fragments

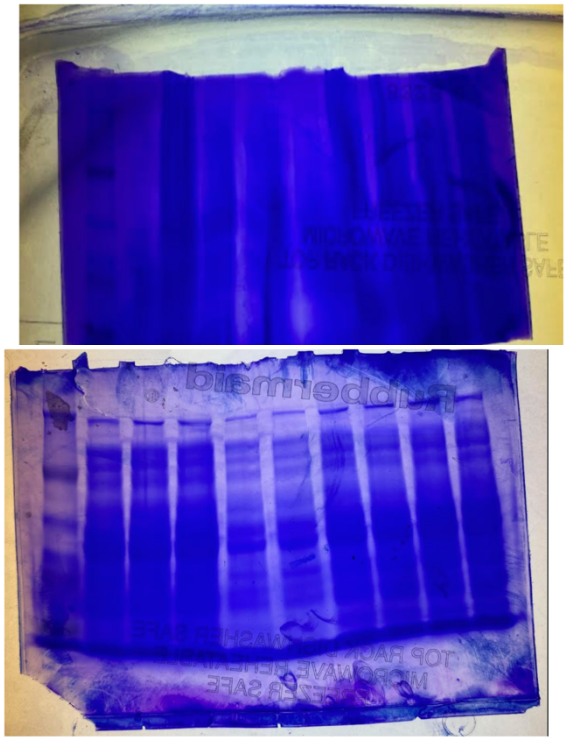

Figure 5 a&b: Protein Optimization (Attempt 1 & 2)

The first gel is the first run protein gel for the IPGTG induced long ACE2 isoform.

The second gel is the second run protein gel for the IPTG induced long ACE2 isoform.

Discussion

Throughout this project I have learned about and improved various methods of experimentation including Bacterial Transformation, Plasmid DNA isolation, Digestions, Ligations and Protein Expression.

As I was conducting Bacterial Transformation, I managed to improve the H-SC's standard method for bacterial transformation. Instead of creating one large pellet, as the old method called for, I created as many pellets as I could. This slight change allowed me to retrieve 3 times as much yield as the old method.

After Bacterial Transformation, samples of ACE2 in E. coli cells were frozen and stored for future use. I also conducted Bacterial Transformation on and freeze stored 3 other vectors: pQE16, pQE60, pQE70. I only used pQE70 so I prepared the other two to freeze and for the lab's future use.

With help from Dr. Wolyniak, I used snap genes to create the blueprint for the synthesized vectors. Blume et al. calls for the exon MREAGWDKGG to be included in the ACE2 isoform for it to function properly. I translated the protein code into DNA favorable to E. coli. The ordered ACE2 oligo

was constructed in hope of creating clean cuts at the BamH1 restriction sites. I ensured this would happen by adding 3 t's to the beginning and end which allows the enzyme to create a clean cut with a higher success rate.

In my first attempt at protein optimization, I used the procedure suggested by Dr. Wolyniak's colleague. In my second attempt, I spun down the lysed cells in order to separate the cell debris from the supernatant. The supernatant contains most of the DNA so I believe this is why the second protein gel came out cleaner than the first. I began a 3rd attempt at gel optimization where I added less IPTG and induced it for less time. Hopefully the results from this attempt will also improve.

Although not a lot of successful results were retrieved, I have created a solid foundation for this research to grow from.

REFERENCES

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