Using CRISPR-Cas9 Applications for ACE2 Knockout in Liver Epithelial Stem Cells and Impact on SARS-CoV-2

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ABSTRACT

The emerging Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) poses a major threat to public health. COVID-19 is a viral respiratory illness caused by SARS-CoV-2 and can be contracted between individuals who are in close contact with one another or by touching a contaminated object. SARS-CoV-2 entry depends on the host cell factors, ACE2 and TMPRSS2. Angiotensin I Converting Enzyme 2 (ACE2) is a functional receptor for the spike glycoprotein SARS-CoV-2 and TMPRSS2 is a transmembrane protease that serves as a primer for SARS-CoV-2 entry into the cell. ACE2 is expressed in the human airway epithelium, gastrointestinal cells, and some organs of the digestive system such as the liver. My objective is to knock out ACE2 in liver epithelial stem cells using CRISPR Cas9 technology as a means for preventing the entry of SARS-CoV-2. This process involves using chemical-based transfection in order to insert plasmids with the target gRNA and Cas9 enzyme as well as a GFP reporter that would serve as a marker for cells that have been edited. After transfection, positive selection with GFP reporting signal will be done and it will be followed by DNA analysis through sequencing. From this, we can infect the edited cells with SARS-CoV-2 and assess the effectiveness of the gene knockout on the prevention of COVID-19.

INTRODUCTION

What is CRISPR?

CRISPR (Clustered Regularly Interspaced Palindromic Repeats) is short regions of DNA found in bacterial and archaeal genomes, which serves as a defense mechanism, allowing for immunity against phages[3]. The CRISPR/Cas9 system is a gene-editing technology adapted from this natural form of defense and allows us to correct errors in the genome or efficiently turn genes on and off in eukaryotic genomes.

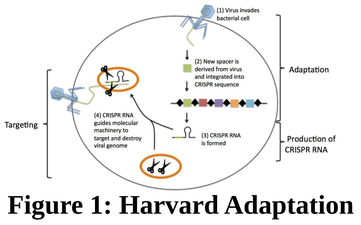


Figure 1: Harvard Adaptation from Barrangou, R. (2014)

How Does CRISPR/Cas9 Work?

This process initiates when the enzyme, Cas9, and a guide RNA recognize and bind to a short segment of DNA adjacent to the target site, which initiates the unwinding of the DNA helix. The guide RNA then pairs with a specific target sequence in the DNA, and Cas9 cleaves the DNA, forming a double strand break. In addition, Protospacer Adjacent Motifs (PAM) sites are short DNA sequences that are a safety mechanism to ensure that Cas9 doesn't make random cuts in the genome[2]. Once the DNA is cut, the cell tries to repair the cut through nonhomologous end joining and in doing so, it introduces additions or deletions in that part of the repair.

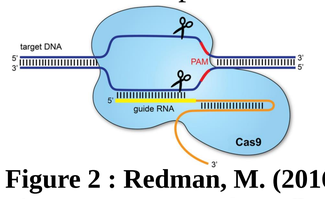
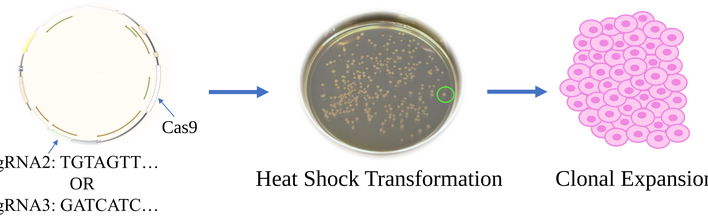


Figure 2 : Redman, M. (2016)

METHODOLOGY

- Bacterial Transformation and Plasmid Amplification:



- Chemical-Based Transfection: A plasmid with the Cas9 enzyme and gRNA sequence is used to introduce a DSB for genome editing. A GFP reporter is also used in transfection to screen cells that have successfully received the plasmid.
- FACS and DNA Sequencing: GFP positive cells are sorted out, grown in a plate and sent out for sequencing.

RESULTS

1. Bacterial Transformation and Plasmid Amplification

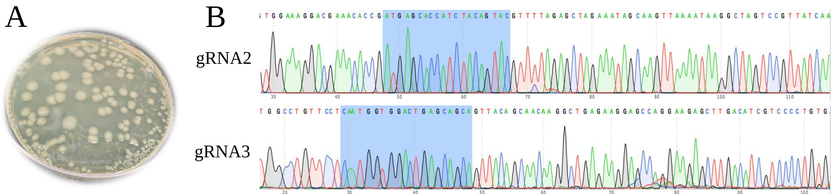


Figure 3: Amplification of plasmid using bacteria
A. Bacterial colony growing in antibiotic plate after heat shock transformation with plasmids with antibiotic resistance gene B. Sanger sequencing on gRNA carrying plasmids for validation

2. Liver ACE2 KO gRNA Transfection and FACS

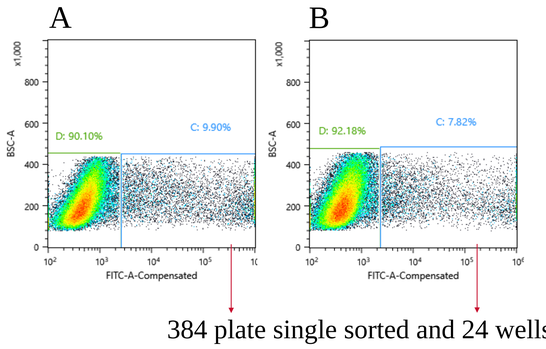


Figure 4: FACS Results After Transfection
A. FACS analysis for 3p plasmid to measure transfection efficiency B. FACS analysis for 2p plasmid

384 plate single sorted and 24 wells

3. GFP+ Cell Selection and Expansion of a Single Cell Colony

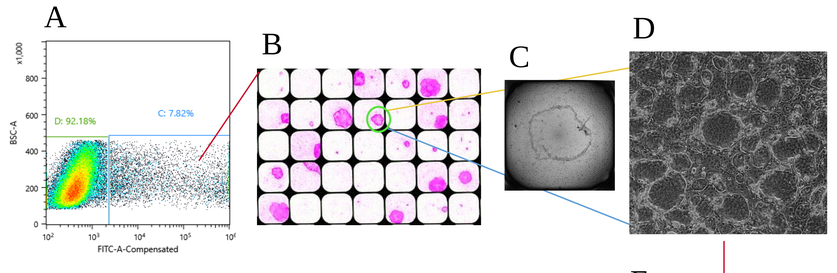


Figure 5: FACS Results After Transfection
A. Selection of GFP+ cells after FACS B. Single cell sorting in a well plate C. Single cell derived ISC colony D. Single cell derived pedigree after expansion E. Sanger Sequencing on pedigrees

DISCUSSION

- Sequencing results confirm effective knockout of the ACE2 gene in human liver epithelia using CRISPR/Cas9 applications
- While current research efforts are focused on studying the correlation between ACE2 and airway epithelia, future research should also focus on studying intestinal epithelia and other organs of the digestive tract

CONCLUSIONS

- Future goals for this project include infecting the edited cells with SARS-CoV-2 and analyzing whether there is any impact on the entry of the virus into the cell
- We will also be performing this process on other cells where ACE2 is expressed such as intestinal stem cells and the human airway epithelium

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