# Unraveling Therapeutic Potential: Targeting Glioblastoma Cells Through Inducible Knockdown of GLUT1 and GCLC Genes

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USC Viterbi School of Engineering

SHINE Summer High School Intensive in Next-Generation Engineering

## Introduction

**Glioblastoma(GBM) is the most common malignancy of the central nervous system in adults**.

- Continued shortfall of many treatments such as temozolomide.
- Stagnant survival rate improvements (<13%)
- Hindrance of Blood Brain Barrier for drug development
- Led to pressing concerns for novel treatment methodologies.

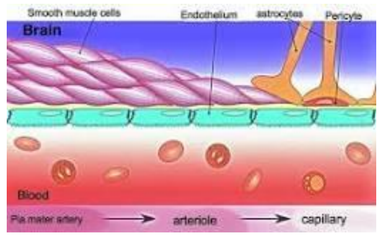



**Figure 1.** Blood Brain Barrier Regulatory Nature - American Society for Microbiology

## Objective

### Project Objective:

1. Treat GBM by inducing glucose deprivation through inducible ShRNA knockdown of GLUT1 & GCLC genes

### Seven Week Objective:

**2.** ShRNA production and cloning into plasmid then transformation to the competent cells

## Biological Specifics

Knockdown Genes:**GLUT1(1) and GCLC(2)**

-Glucose Transporter

-Glutamine cysteine ligase

## Project Procedure

### 1 Plasmid Digestion

Digestion utilizes restriction enzymes in order to cut the plasmid to prepare for the addition of gene of interest.

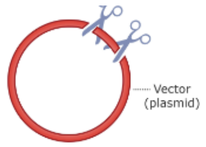



Figure 2. - Addgene

### 2 Annealing Oligos

In order to prepare the gene of interest we must anneal two single stranded nucleotides utilizing a thermocycler

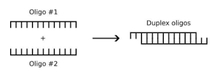



Figure 3. Protocols.io

### 3 Ligation

This step ligates the gene of interest using DNA ligase guided by sticky ends, preparing the recombinant plasmid.

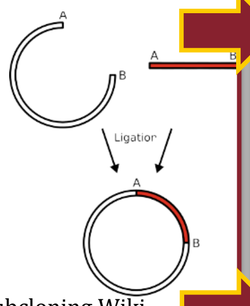



Figure 4. –Subcloning Wiki

### 4 Transformation

Transforming the plasmid introduces the recombinant plasmid into bacteria for gene manipulation and protein production.

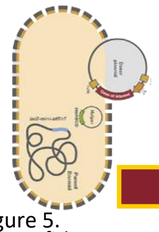


Figure 5. Thermofisher

### 5 Culturing & Sequencing

Bacteria containing recombinant plasmid are plated on LB agar and left to incubate at 37 degrees Celsius for 16-24 hours. Collect incubated bacteria and resuspend in LB broth. Plasmids are then extracted and sent for sequencing to confirm accuracy.

## Skills / Steps Performed

- Omega Plasmid Digestion Kit
- Restriction enzymes Nhel-Hf and EcoRI utilized
- Digested vector is dephosphorylated to avoid religation

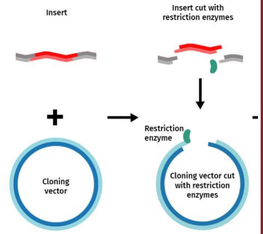



Figure 7. Digestion - Snapgene

- Sense and anti-sense DNA strands annealed
- Thermocycler set t0 start at 95 C° with change when failure is observed
- Drop of 5 C° per minute

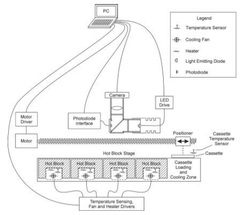


Figure 8. Thermocycler Research Gate

- Thermofisher rapid ligase kit
- Recovery at 37 C° for 30 minutes
- Nanodrop used to measure concentration

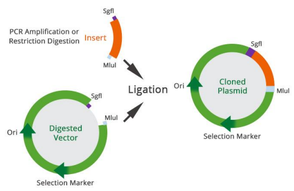



Figure 9. Ligation OriGene

- Heat Shock performed at 42 C° on e-coli to open cell membrane

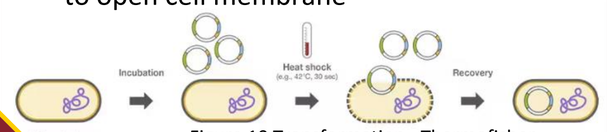
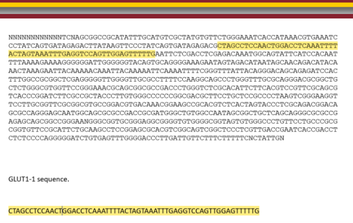



Figure 10 Transformation - Thermofisher

## Results



GLUT1-1 sequence.

CTAGCCTCCAACTGGACCTCAAATTTTACTAGTAAATTTGAGGTCCAGTTGGAGTTTTTG

Gene insert found within transformed plasmid

## Impact Work

The Graham Lab revolutionizes cancer research by employing system biology approaches to develop novel therapies. Through integrating diverse data types, they construct predictive computational models that unlock biological insights. By collaborating with experts across disciplines, they ensure accurate and reliable models. Translating their findings into the clinic, they make a tangible impact on patient outcomes and advance cancer research.

By advancing GBM research we are able to better mitigate current barriers of treatment that keep GBM highly lethal. Furthermore, by utilizing not standard biological the Graham lab widens options for treatments

## My Experience

USC SHINE 2023 was an enriching summer venture that accelerated my biochemistry skills. Engaging in industry-specific tasks utilizing knowledge from my biotechnology class furthered my understanding of current industry standards. This experience also provided valuable insights into the Ph.D. pursuit process; an invaluable step towards achieving my academic aspirations.

## Acknowledgments

I would like to thank Dr. Graham for giving me the opportunity to perform this research as well as my mentor Peyman Nouri for guiding me through my project. Additionally, I would like to thank Jeenisha Shrungare and Carina Curiel Alaniz, my Center Mentor, and Director Monica for guiding me through the program. Their guidance was invaluable.