Bacterial Transformation of Green Fluorescent Protein from pGLO

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Background

- Green fluorescent protein (GFP) is a protein originally found in a jellyfish that emits **fluorescence** (Shimomura, 2009).
- A **PCR** technique is used to code proteins of interest in hopes of **cloning**.
- **Bacterial transformation** allows cells to take in DNA from their environment and **express it.**
- The manipulation of DNA allows for the determination of a gene's role in an organism
- This experiment utilizes the presence of GFP in pGLO plasmid in order to determine the ability of a bacteria (*E. coli)* to glow under the different conditions, +pGLO and -pGLO

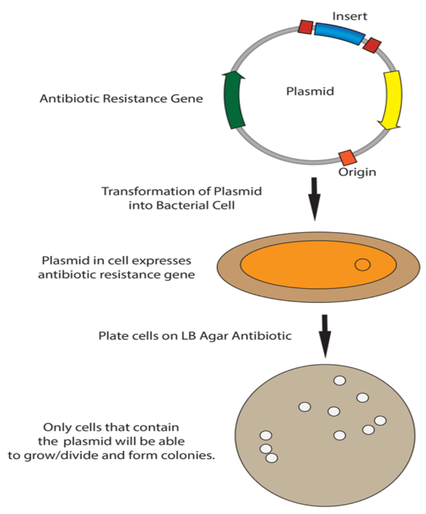

Figure 9. Example of how bacterial transformation occurs (AddGene, 2020)

Specific Aim

Research Question: Can the GFP gene from pGLO plasmid be expressed in *E. coli* bacteria?

Hypothesis: The GFP gene from pGLO plasmid can be expressed in *E. coli* bacteria.

Methods

Determine target gene through a PCR reaction and visualize through gel electrophoresis to determine amplicon size.

Use nanodrop to determine amplicon concentration and calculate the volume for the dilution.

Obtain the contig sequence of the PCR amplicon through CAP3 Sequence Assembly Program.

Use BLAST analysis program to confirm the presence of GFP in the target gene.

Obtain the alignment of the GFP gene through BLAST analysis program.

Determine *E. coli* bacteria growth as well as presence of fluorescence in agar plates

Determine the bacterial growth and fluorescence of pGLO in the presence of different sugars

Results

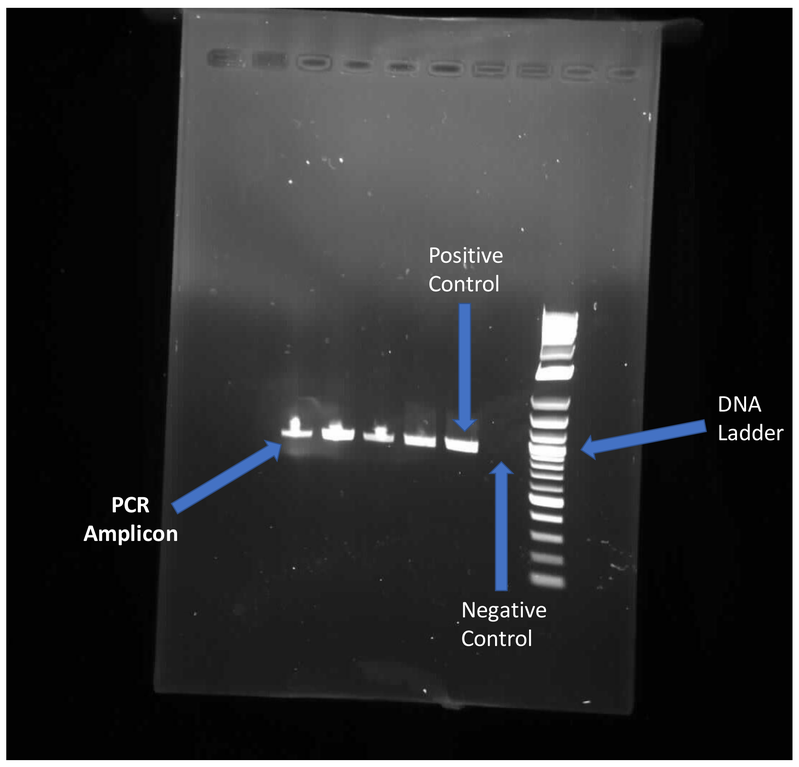

Figure 1. The size of the purified PCR amplicon is shown to be 1000 bp after undergoing gel electrophoresis.

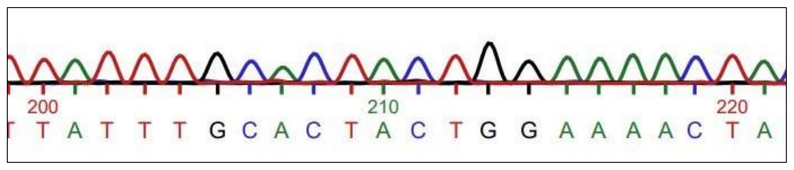

Figure 2. Sequencing data of the pGLO plasmid was collected which iwas then converted into a chromatogram., which is partially displayed in this figure.

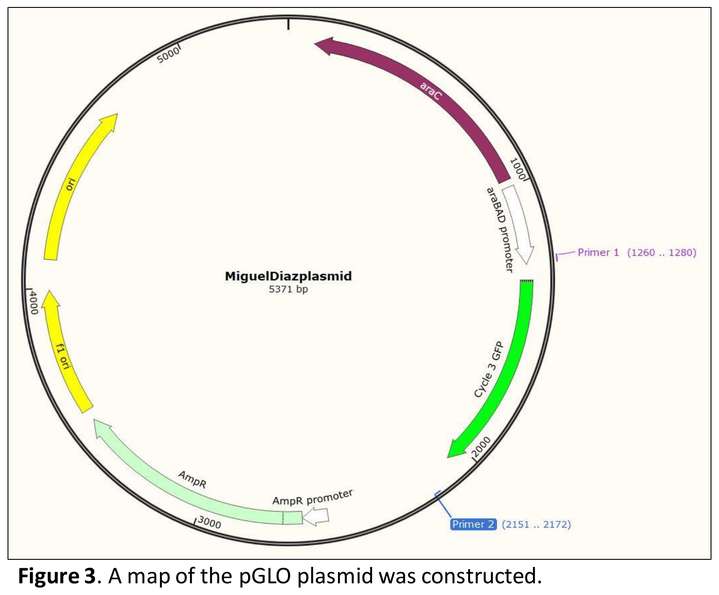

Figure 3. A map of the pGLO plasmid was constructed.

Figure 8. Photo of +/- pGLO transformed bacterial growth in LB/LB+amp/LB+amp+ara plates exposed to UV light.

Plate	Amount of Bacterial Growth
-pGLO (LB)	Infinite growth
-pGLO (LB/ara)	No growth
+pGLO (LB/ara)	5 cells
+pGLO (LB/ara/amp)	4 cells

Table 1. Table of + and -pGLO transformed bacterial growth in LB/LB+amp/LB+amp+ara

Plate	# Colonies	# Glowing Colonies	Relative Brightness
pGLO	36	0	-
pGLO+ara	16	14	+++
pGLO+glu	7	0	-
pGLO+ara +glu	11	10	+

Table 2. Table of pGLO growth and glow in ara or glucose or both sugars plates

Conclusions

- The hypothesis of this research project is supported by the following results:
  - Figure 8 shows plates containing *E. coli* cells that glow because they have been transformed with the GFP gene from the pGLO plasmid
  - Table 1 shows plates with E. coli cells that contain the pGLO plasmid and demonstrate bacterial growth
- A limitation of this study would be that we expressed the GFP gene of the pGLO plasmid only in E. coli cells. As shown in research by Niedenthal et al. (1996), experiments can be conducted on several other different organisms in the future to see if other organisms can also express the GFP gene of the pGLO plasmid.
- A potential experiment that could be done in the future to answer the research question would be to use CRISPR technology to put the pGLO plasmid into the E. coli cells.

References

- Niedenthal, R. K., Riles, L., Johnston, M., & Hegemann, J. H. 1996. Green fluorescent protein as a marker for gene expression and subcellular localization in budding yeast. *Yeast, 12*(8):773-786.
- Shimomura, O. 2009. Discovery of Green Fluorescent Protein (GFP) (Noble Lecture). *Angewandte Chemie.* 48(31): 5590-5602.
- AddGene. 2020. Bacterial Transformation. *AddGene.*