



The major capsid protein of a bacteriophage is localized close to the infection nucleoid in the host cell.

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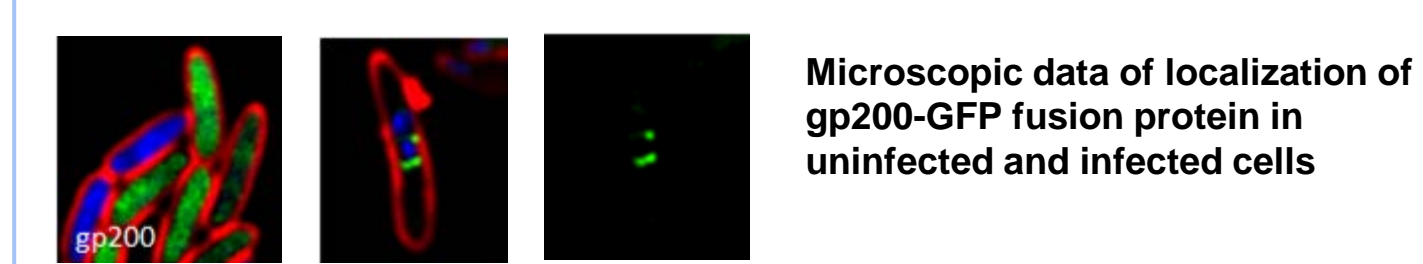
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Abstract

In order for us to better understand the manner in which phages reproduced, it was necessary to be able to view the system *in vivo*. Our objective during this project correlated with this interest -- to attempt to turn a bacteriophage capsid protein, gp200, to present in red, using the mCherry fluorescent protein. Doing so would allow us to view the gp200 capsid protein and give us the insight to understand its functions in the process of phage reproduction. The method employed would require the modification of the DNA of gp200. The process would include the amplification of the DNA through the polymerase chain reaction (PCR), restriction enzyme digestion, and then the ligation step where the gp200 DNA and carrier vector would form into a circular DNA in order to be transformed into the host cell. The colonies of cells would have the opportunity to grow. They were later confirmed the cell viability by re-streaking on a selective agar plate. Microscopy of the transformants was eventually conducted to determine the localization of gp200 capsid protein. As expected, this capsid protein expresses its typical phenotype of multiple foci surrounding the phage DNA in the infected cells. This results demonstrate that we successfully make the construct of gp200 and the red fluorescent mCherry protein that can be expressed in the host cells.

Introduction

Recent studies have found that bacteriophages have the ability to encode their own tubulin-like proteins, also known as PhuZ, into certain prokaryotic cells. The purpose of PhuZ was studied in the Pogliano lab, and was revealed to be vital to phage reproduction in that PhuZ would position the phages DNA in the center of the cell in order to create an optimal 'DNA replication factory'. Further studies uncovered the most abundant virion structural protein, identified as gp200 major capsid protein. In order to understand the roles of this protein in the process of phage reproduction, it was necessary to make the protein visible. Previous microscopic data of gp200 fused with the green fluorescent protein (GFP) revealed that gp200 capsid protein localized close to the infection nucleoid and assembled multiple foci around it with gap, shown below. The project thus is conducted to make a fusion of this protein with a red fluorescent protein, mcherry, in order to confirm the infection phenotype of this protein in the host cells.

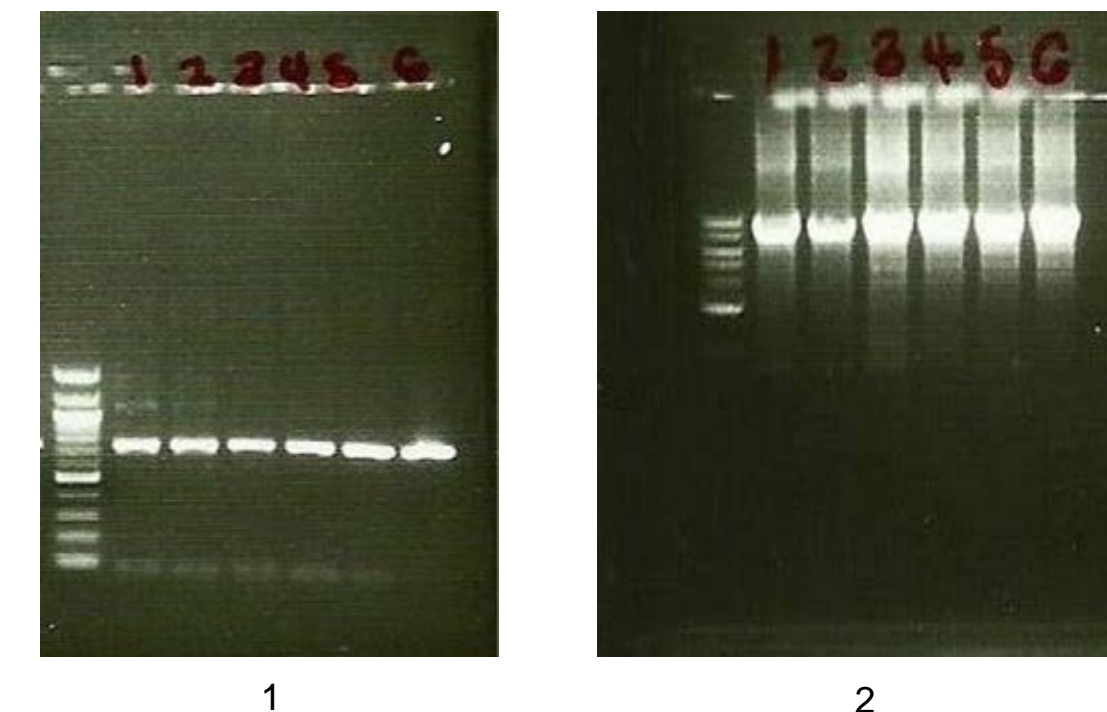


Protocol

1. PCR Amplification (to get DNA amplicon flanked with "Restriction Site") (Running Agarose gel to check the amplicon)
2. PCR Cleanup (to clean up the PCR products)
3. Restriction Enzyme Reaction (to cut the restriction site on DNA amplicon)
4. Gel Purification (to obtain the enzyme - cut DNA amplicon)
5. Ligation Reaction (to ligat the DNA amplicon into DNA vector and to make circular DNA) (25 degrees celcius 15 min, 16 degrees celsius ON)
6. Bacterial Transformation (to put the DNA plasmid into bacteria E.coli DH52 in order to produce more plasmid number) (Re-streak to purify colonies and keep stock)
7. Plasmid Extraction (to extract the DNA plasmid out from the cell)
8. DNA Sequencing (to check if the DNA construct we make contains the correct DNA sequence)
9. Re-transformation (to put the correct construct into the real host for our experiment, pseudomonas chlororaphis) (re-streak to purify colonies and keep stock)
10. Microscopy of the Transformants (to check if our construct works)

PCR Amplification

Optimization of Annealing Temperature for PCR Reaction

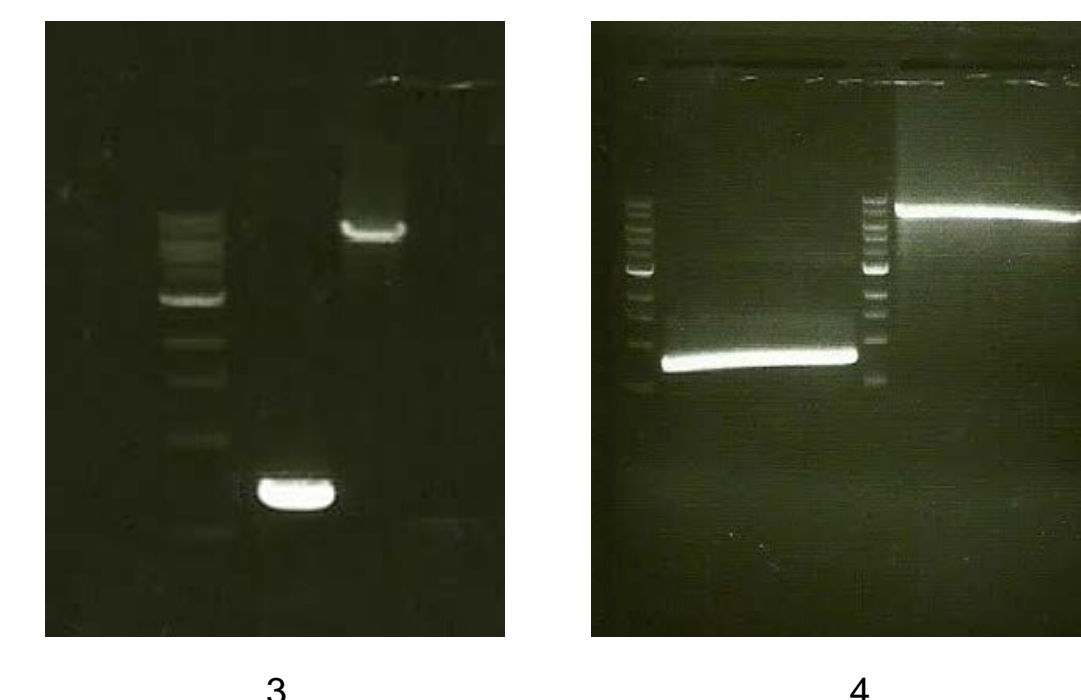


Annealing Temperature per Lane in Celsius	
1 - 49.8°	4 - 58.3°
2 - 51.1°	5 - 62.0°
3 - 54.3°	6 - 64.6°

Varying annealing temperatures were initially utilized in order to determine which would be most effective in the re-amplification process.

Re-amplification of Suitable Annealing Temperature

The 6th lane in the initial amplification demonstrated the most promising results in terms of intensity and specificity, it's temperature of 64.6° celsius was thus used again in the the re-amplification step.

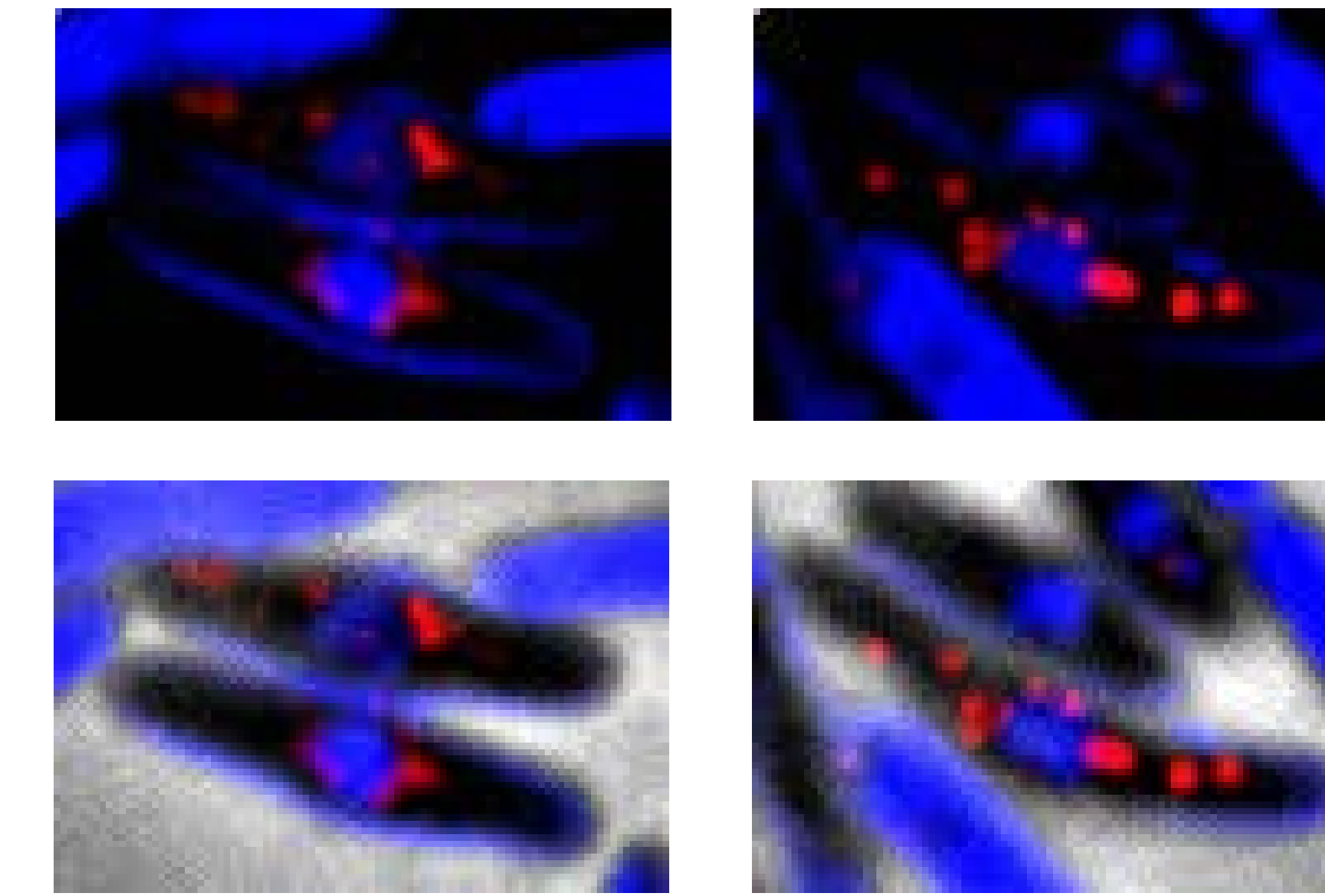


Bacterial Transformation



	Insert	Vector	No. of Colonies
A	0	1	1
B	3	1	185
C	6	1	207

Microscopy of Transformants



As the pictures prove, the results were positive with gp200 major capsid protein expressing the desired phenotype, illuminated with the mCherry fluorescent protein.

- - mCherry
- - DAPI
- - Brightfield

Conclusion

The process to obtain the red fluorescence of gp200 was at times tedious, yet fortunately in these three short weeks the project was completed successfully. We were able to successfully construct the DNA sequence which contained the desired gp200 and the mCherry which would helps us determine the localization of gp200 in the infected cells.

Future Research

To gain better understanding on how the capsid proteins of this bacteriophage are transported into the infection nucleoid, we can perform co-localization experiment of this mcherry-capsid proteins with other phage proteins that are already fused with green fluorescent protein (GFP). This way they will be viewable distinct, bringing scientist closer to understanding the manner in which the two interested proteins work together to aid in the reproduction of the bacteriophage.

Acknowledgments

I would like to extend a warm thank you to my mentor, (Arthur) V. Chaikerasitak, professor Joe Pogliano, and everyone in the lab for allowing me to not only observe the workings of a biology laboratory. And a special thanks to Elizabeth Komives: organizer of Research Scholars, Academic Connections, and Reality Changer for allowing me to take part in this amazing opportunity.