The first phage protein detected in the infected host assembles a ring structure surrounding the infected nucleoid

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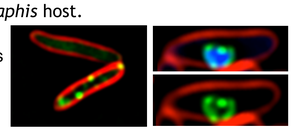
ABSTRACT

PhuZ has been recently identified as a tubulin-like protein encoded in bacteriophages that can infect *Pseudomonas Chlororaphis*. It is believed that PhuZ works along with other proteins to function as a cytoskeletal element that places the phage's DNA at mid cell, a crucial step for bacterial reproduction. The phage protein, gp105, was previously identified as the first phage protein expressed in the host cell. Tagging the gp105 with green fluorescent protein (GFP) showed the ring phenotype of gp105 surrounding the infection nucleoid in an infected cell, shown below. In order to confirm the phenotype, we decided to make a construct of protein gp105 with the mCherry, a red fluorescent protein. This will allow us to determine the location of the protein in the phage infected cell under microscopy. Here, we successfully create the plasmid construct which can be expressed under arabinose promotor in the *P. Chlororaphis* host.

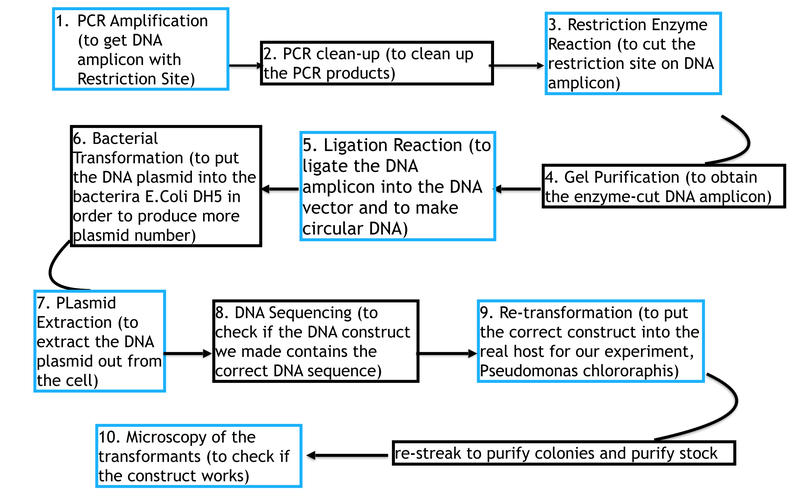
Introduction



Microscopic pictures of gp105 in uninfected and infected cells

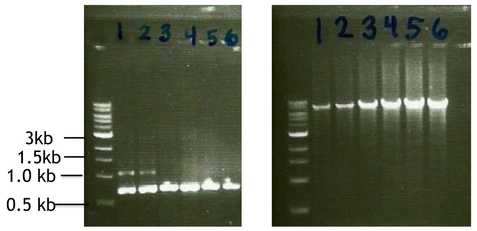
Long thought to be only part of the eukaryotic anatomy, the cytoskeleton has been discovered in the prokaryotic cell just twenty years ago. This discovery has riveted researches because it is now known to have evolved first in prokaryotes; therefore an urge to discover the various functions that they perform will provide insight into how the eukaryotic cytoskeleton developed. PhuZ is a tubulin-like protein encoded in the bacteriophage's DNA and when it is injected into the host, it is required to position the DNA in the middle of the cell. Bacteria nor phages were known to posses the necessary spindles to get the positioning done. Imaging of phage infected cells depicts the GFP-gp105 proteins encapsulating the genetic material of phage in the middle of the cell. One goal in Dr. Pogliano's lab, is to "identify and characterize the major families of dynamic cytoskeletal proteins that exist in bacteria and bacteriophages, and to determine their biological functions." Our objectives are to make construct of protein 105 with mCherry and to determine its location in the phage infected cell.

METHODS



GEL RESULTS

Optimization of Annealing Temperature for PCR

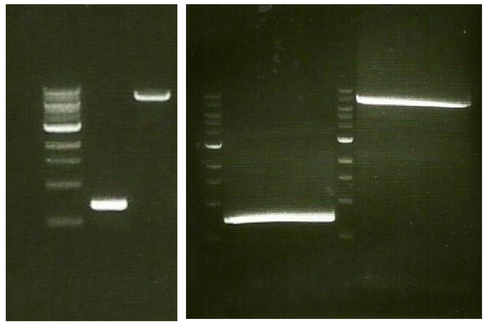


Annealing Temperatures

1. 49.8°C
2. 51.1°C
3. 54.3°C
4. 58.3°C
5. 62.0°C
6. 64.6°C

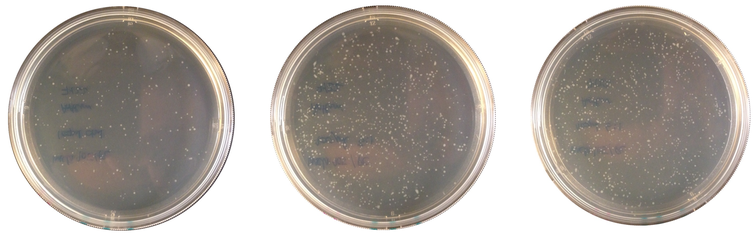
The different annealing temperatures are helpful in determining the ideal re-amplification temperature. Lane 6 was chosen for its strong band.

Re-amplification of suitable annealing temperature (64°C) to obtain the desired PCR amplicons



After confirming the annealing temperature (64°C) that favored the PCR amplicons, the restriction enzyme digestion would allow the fusion of the vector and insert, followed by the gel purification which would allow us to extract the desired DNA.

TRANSFORMATION *PSEUDOMONAS CHLORORAPHIS*

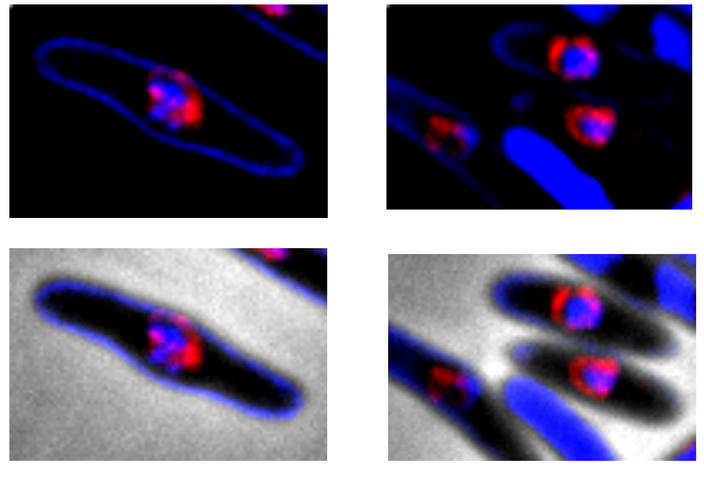


A B C

	Insert	Vector	Number of Colonies
A	0	1	188
B	3	1	625
c	6	1	716

The ideal DNA construct containing protein 105 was grown on plates supplemented with a selective drug. After overnight of incubation the control dish (A) containing only the vector produced unexpected colonies. Dishes B and C demonstrated promising results outgrowing the number of colonies in the control.

MICROSCOPY



Tagging the gp105 with mcherry, red fluorescent protein showed the ring phenotype of gp105 surrounding the infection nucleoid in an infected cell. Together with previous result of gp105 localization, it can confirm that the ring phenotype is not an artifact.

Bright Field

DAPI

mCherry

CONCLUSION

We were able to successfully construct the DNA sequence which contained the desired gp105 protein and the mCherry (red fluorescent protein) which would allow us to investigate the localization of this protein in infected cells under the microscope.

FUTURE RESEARCH

This experiment will help in the understanding of bacteriophage reproduction and the biological functions of gp105.

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