



Creating Vectors for the Testing of Bartonella Effector Proteins

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ABSTRACT

The purpose of this research was to create plasmids to examine the interaction of *Bartonella* effector proteins (Bep) with their host, specifically the interaction between BepC and the host's proteins. The importance of this project is seen in the possibility of understanding how the disease is able to manipulate the host cell and spread throughout the body. To produce these plasmids, DNA extraction, PCR, *E. coli* plating, and gel electrophoresis were used to build the vectors by inserting the desired fragments of BepC and BepE into mammalian expression plasmids with different epitope tags. Through gel electrophoresis, it has been proved that pcDNA3-4 holds BepE. However, BepC had negative results and was not placed in the vector. This was due to the primers having another site for the enzyme to cut, eliminating the reinsertion process. These errors were corrected and the correct plasmid with BepC was created.

BACKGROUND

- *Bartonella* is an infectious bacteria with influencing abilities through effector proteins.
- This pathogen is transmitted via scratches from infected cats or feces from insects of the Ectoparasite species. These methods of transmission can be common and hard to detect.
- The different types of *Bartonella* effector proteins (Beps) are released to affect the host cell's proteins to manipulate the environment, allowing the bacteria to remain and spread throughout the body.



Enlarged lymph nodes, a symptom of Bartonella



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GOALS

- To create the vectors necessary for the experiment. This was done by:
 - Creating a mammalian expression vector with a Tandem Affinity Purification Tag (TAP-tag)
 - Insert BepC into the newly created vector
 - Insert BepE containing a His-tag into the mammalian expression vector

METHODS

- To compare the interactions between BepC and the host cell, along with BepE and BepC by creating the necessary vectors for the experiment, purification of plasmid DNA, PCR, gel electrophoresis, and the Gibson Assembly were utilized.
 - Isolate the plasmids for the vectors
 - PCR to have enough DNA for gel electrophoresis
 - Gel electrophoresis to identify and select the fragments, pcDNA3-4, TAP, BepC, and BepE
 - Cutting the fragments from the gel electrophoresis
 - Gibson Assembly for TAP and BepE into pcDNA3-4
 - Transformation of Gibson Assembly into *E. coli*
 - Gibson Assembly for BepC into pcDNA-TAP

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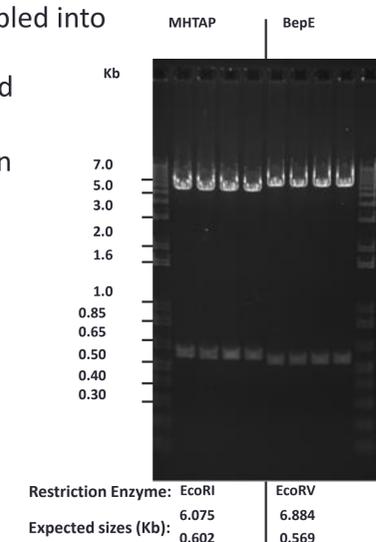
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RESULTS

- pcDNA-TAP
 - This was first cut with SacII, however the plasmids did not cut to completion.
 - This was fixed by cutting TAP with EcoRI that confirmed the desired plasmid had been made.
- pcDNA-BepE-His
 - This was first cut with BglII, however the plasmid did not cut to completion.
 - This was fixed by digesting with EcoRV
- pcDNA-BepC-TAP
 - The vector pcDNA-Tap was first cut with KpnI and NheI.
 - Gibson Assembly was used to place BepC into the digested plasmid and then transformed into *E. coli*. No colonies were isolated with the correct insert.
 - Reanalysis of cloning strategy revealed an NheI restriction site within the TAP-tag resulting in the loss of homology of the 3' end of BepC and the vector.
 - pcDNA-TAP was digested with KpnI and XhoI
 - BepC was re-amplified with the correct flanking sequences and Gibson assembled into digested vector.
 - Digest of plasmid DNA isolated from the colonies with BglII confirmed that BepC had been inserted into the vector.

Restriction digest of pcDNA-TAP and pcDNA-BepE-His showing that the plasmid DNA isolated from the colonies contained the correct insert



CONCLUSIONS

- TAP, BepE, and BepC were successfully put into the vector.
 - These plasmids will be put into mammalian cells to determine what host proteins they interact with.
 - The expressed proteins will be affinity purified and the interacting host proteins identified by mass spectrometry.
- The importance of this is to address what the Beps use to manipulate the cell into allowing the disease to thrive and spread throughout the body.