Construction of Cap5M Ortholog Kinases via DNA-to-Protein Expression Pathways

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Introduction

- *Staphylococcus aureus* is a bacteria that is gaining resistance to common antibiotics[1]
- Cap5M is a phosphoglycosyl transferase (PGT) in *S. aureus*
- This study focuses on making the kinases of Cap5M orthologs

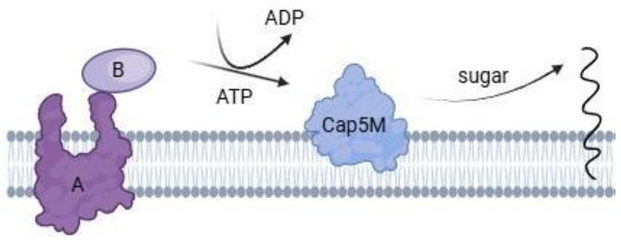


Figure 1
Cap5M is the first membrane-bound step in the peptidoglycan biosynthesis of *Staphylococcus aureus*[2]

Methods

- Three orthologs of Cap5M were found using a sequence similarity network (SSN) and their respective kinases were made
- A polymerase chain reaction (PCR) is used to amplify specific DNA sequences, allowing for the DNA sequence to be studied in greater detail
- A Gibson Assembly was employed to combine multiple DNA fragments, allowing for DNA templates of the kinases of the Cap5M orthologs to be created
- Bacterial transformation allows bacteria takes up foreign DNA, increasing its concentration
- Bacterial expression allows protein to be made from inserted DNA

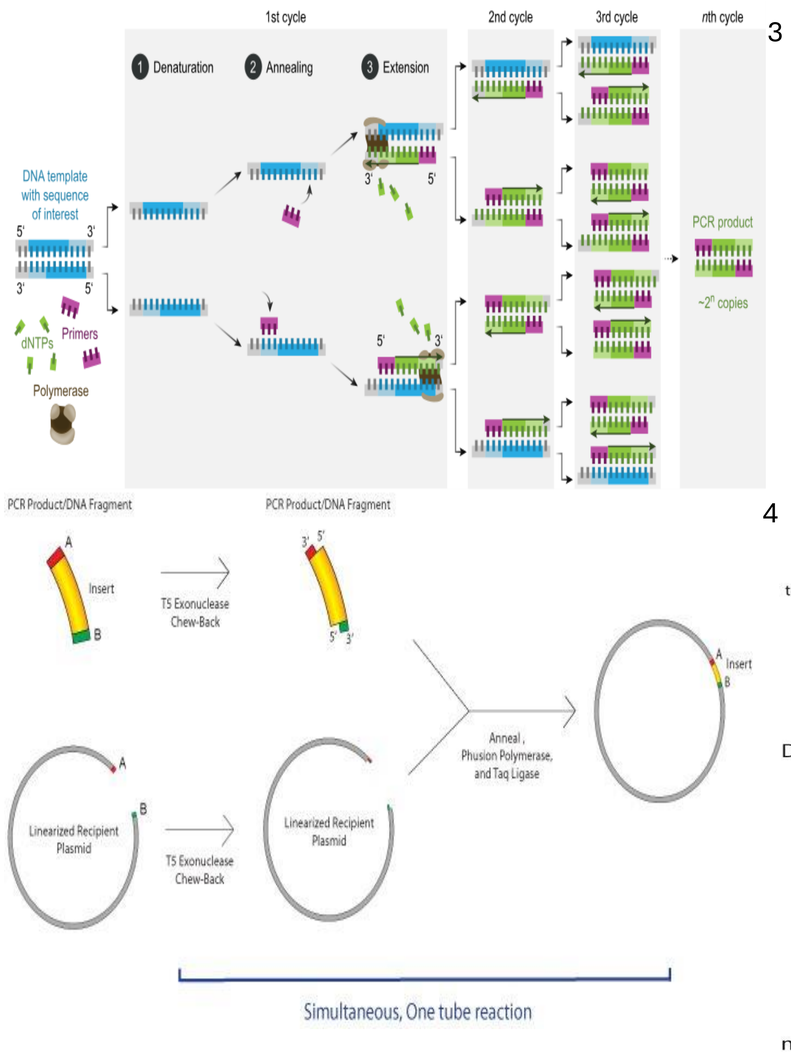
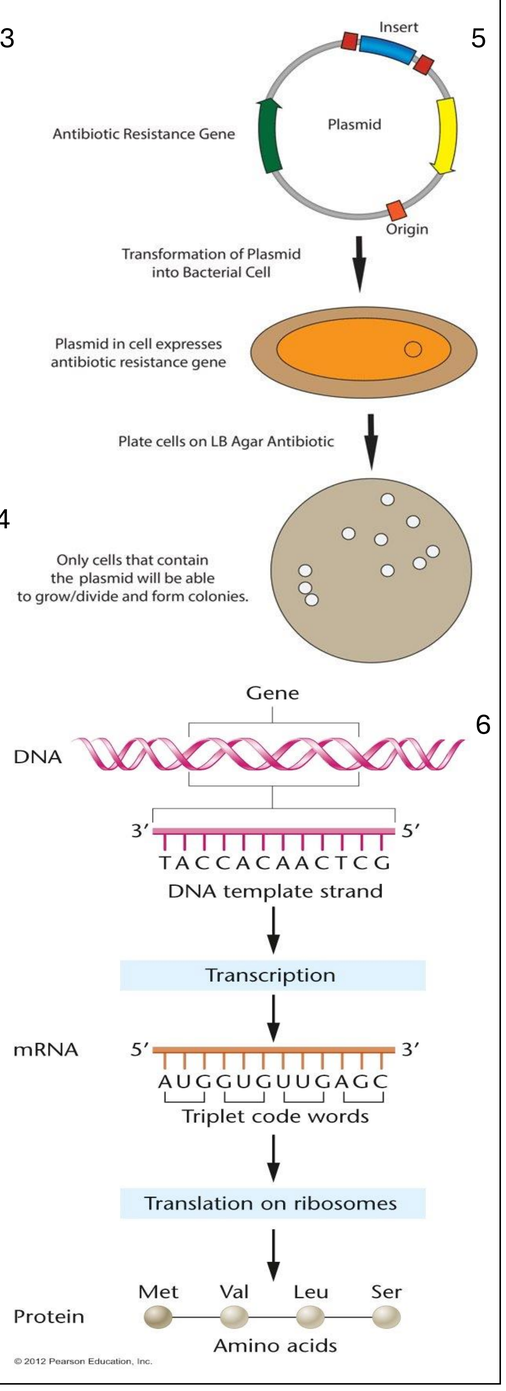


Figure 2 A PCR allows for copies of a DNA sequence to undergo exponential replication

Figure 3 A Gibson Assembly combines DNA fragments together

Figure 4 Bacterial transformation introduces specific DNA sequences into bacteria

Figure 5 Bacterial expression produces protein



Results

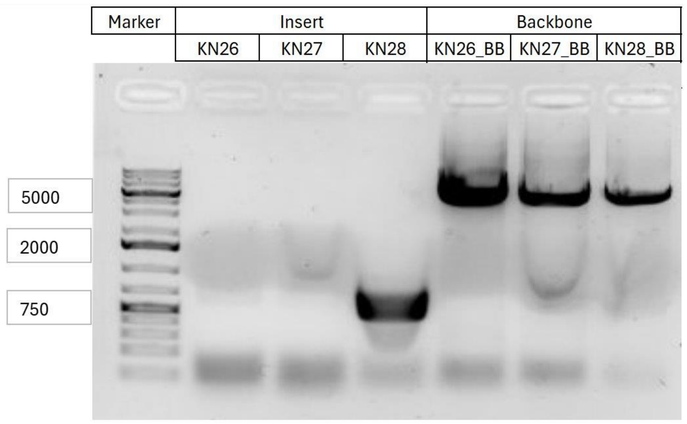


Figure 6
Trial 1 of PCR for kinases of orthologs

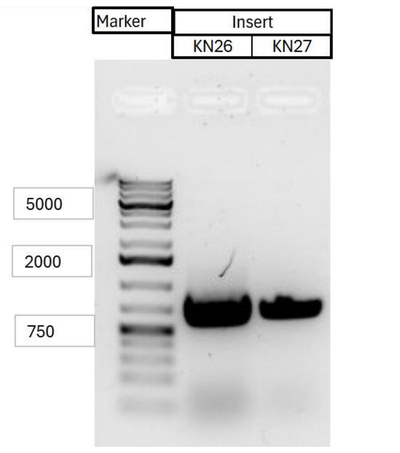


Figure 7
Trial 2 of PCR for kinases of orthologs

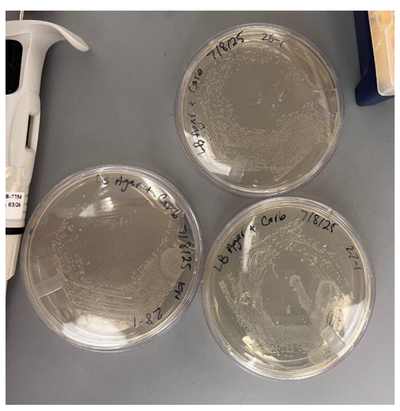


Figure 8
Bacterial transformation results

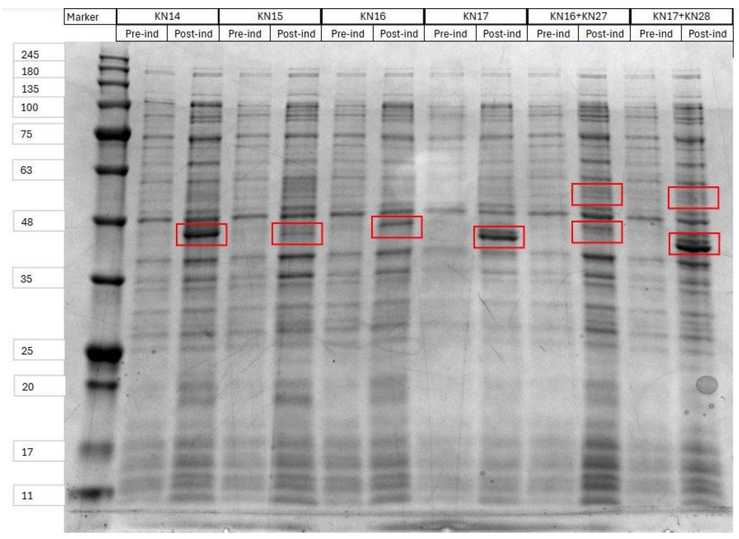


Figure 9
Bacterial expression results

Discussion

- Cap5M and its orthologs require their kinases for phosphorylation, meaning that they are essential to potentially inhibiting *Staphylococcus aureus*'s ability to resist antibiotic drugs.
- By making the kinases of Cap5M orthologs, a more accurate structure of Cap5M and its kinase may be obtained
- This study contributes to antimicrobial research in understanding PGTs

References

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