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## Examination of germination receptors of *B. subtilis* and *B. megaterium*

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# Examination of Germination Receptors of *B. subtilis* and *B. megaterium*

## Abstract

Many bacterial species including those in the Bacilli group form spores as a mechanism to survive harsh conditions such as extreme temperature, radiation, chemicals, and nutrient starvation. By forming spores, they can remain metabolically dormant for an extended period and revert to their vegetative form when environment becomes favorable. This resumption of metabolism and growth is marked by a process called germination that is triggered by exogenous nutrients such as amino acids, sugars, and nucleotides. The (Ger) germination receptors that are postulated to respond to these germinants, in the case of *B. subtilis* and *B. megaterium*, are a complex of at least three different proteins (the A-, B-, and C- subunits) transcribed from the same operon. While similar in gene arrangement and protein complex formation, these two Bacilli sp. respond to different germinants. This experiment investigates the GerA receptor of *B. subtilis* and the GerU receptor from *B. megaterium*. GerA of *B. subtilis* is activated with L-alanine, while GerU of *B. megaterium* is activated with L-proline. In order to determine the location of the binding site, different fragments of the GerAB gene and the GerUB genes encoding for protein A and B from each operon were amplified and fused together in frame to make a chimeric gene product. recombination. Spores from *B. subtilis* mutant strains expressing chimeric protein complexes will be tested for germination in the presence of L-proline and/or L-alanine. These studies will provide insights into how bacteria sense their environment and possible strategies to control and prevent growth.

## Background

Endospore forming bacteria such as members of the Bacilli group demonstrate remarkable properties of dormancy and resistance that allow them to survive nutrient starvation, chemical stress, radiation and extreme temperatures. Since the spores can survive mild food processing and antiseptic procedures, bacterial spores are major food contaminants and often cause food poisoning.

Despite their exquisite dormancy, spores are capable of resuming metabolism and growth in response to appropriate nutrients. These specific chemical nutrients serve as germinants and trigger the biophysical process, germination, resulting in the loss of spore-specific properties and retrieval of the metabolically active vegetative cell.

Although, the exact molecular pathway of the germination process is unclear, previous studies show that germination requires specific receptor proteins residing in the inner membrane surrounding the spore protoplast. The germination receptor complex is composed of A-, B-, and C- subunit proteins. Based on the amino acid sequence, proteins A and B are most likely transmembrane proteins whereas protein C is a cytosolic protein with a signal peptide. These proteins cooperatively interact to recognize unique germinants. Although, this protein complex is similar in *B. subtilis* and *B. megaterium*, they recognize different germinants. The GerA receptor of *B. subtilis* is activated by L-alanine whereas the GerU receptor of *B. megaterium* responds to L-proline. Although gene knockouts have suggested that the B-subunits of Ger receptors are involved in germinant recognition, no direct evidence of such interaction has been obtained.

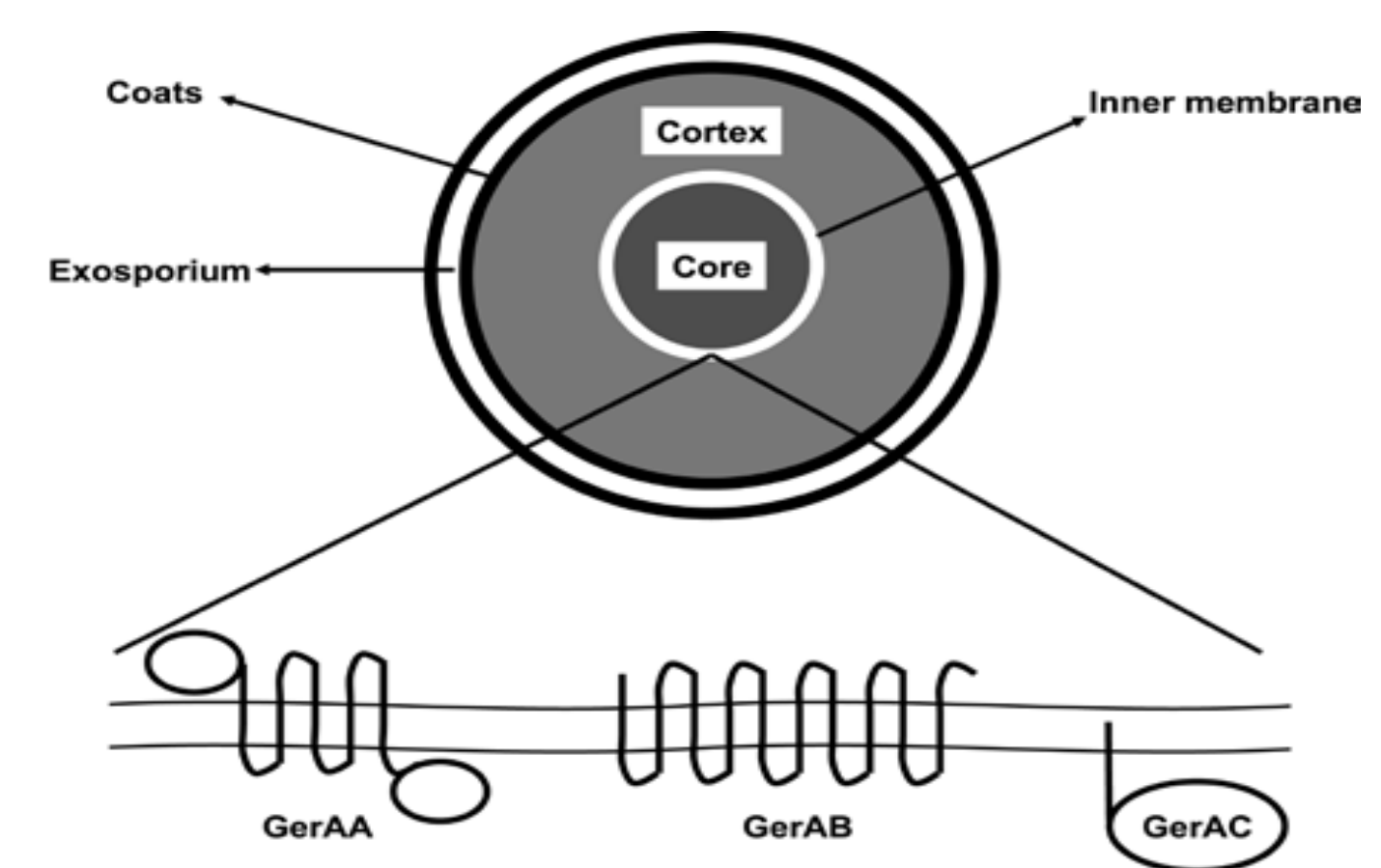
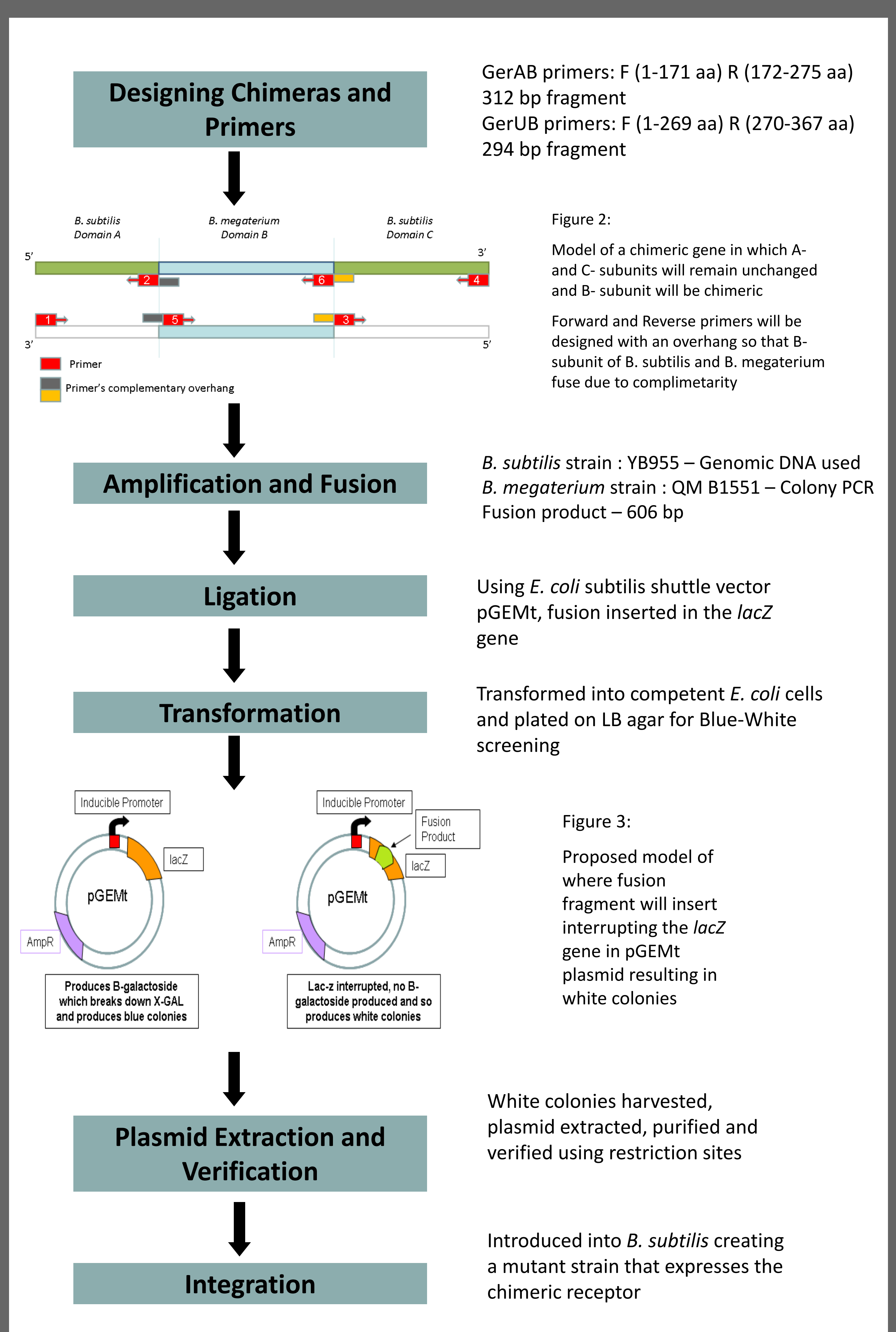


Figure 1:  
Proposed model of the germination receptor GerA of *B. subtilis*. The localization of the subunits is hypothesized to be similar in all Ger receptors  
From (Ross, and Abel-Santos 147-58)

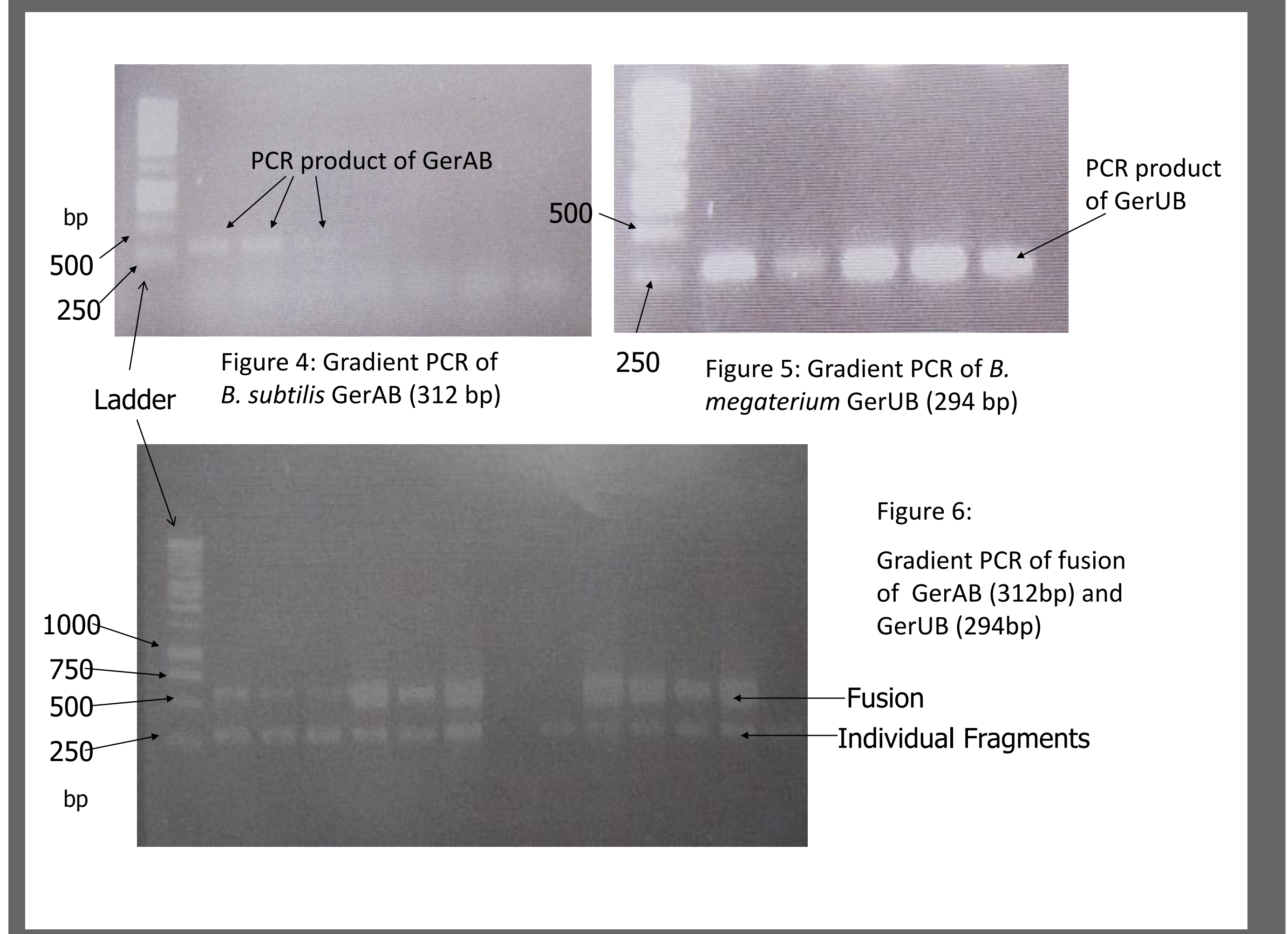
## Hypothesis

Although all three subunits are necessary for the receptor complex to function, we hypothesize that subunit A and C are not involved in germinant recognition and that the unique germinant recognition site is located on the B-subunit. Different chimeric B-subunit receptors from *B. subtilis* and *B. megaterium* will respond differently to germinants and will identify the region that recognizes the germinants.

## Methods



## Results



## Conclusions

The resultant bands from the individual PCR fell between 250 and 500 bp indicating successful amplification of GerAB and GerUB fragments. The fusion product fell between 500 and 750 bp which validates the expected length of 606 bp. We were able to amplify and fuse B-subunit fragments from *B. subtilis* and *B. megaterium*. We have also developed a protocol to test these chimeric receptors in *B. subtilis*.

## Future Directions

- We will design new constructs that fuse together three different fragments and test them similarly
- We will integrate them in *B. subtilis* to create mutant strains producing chimeric receptors
- We will test mutant strains with different germinants.

## References

Ross, Christian, and Ernesto Abel-Santos. "The Ger Receptor Family from Sporulating Bacteria." *Current Issues in Molecular Biology* 12. 147-58. Web. 15 Jun 2010.

## Acknowledgements

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