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Heme Expression from Caulobacter Crescentus in *E. coli*

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Abstract:

The focus of my research is to create a clone of a Heme transporter from Caulobacter crescentus and transformed into *E. coli* OKN359 and fluorescently label it so that it can detect Heme in the environment. This sensor will be combined with others in a fluorescence assay to analyze pathogenic bacteria and identify drugs that are the most effective in inhibiting their iron transport. To do so, I used Gibson cloning and made a hybrid gene, pITS27, that contains a small initial portion of an *E.coli* gene *fepA* followed by the full Caulobacter crescentus gene *hutA*. The initial portion of the *E.coli* gene was necessary to ensure proper insertion of the Caulobacter crescentus HutA protein in the outer membrane.

Introduction:

There is a growing need to create novel agents to fight against Gram-negative, antibiotic resistant bacteria also known as ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp*). A novel agent could target the inhibition of iron acquisition systems in bacteria, as iron is a key component in energy production, DNA synthesis, metabolism, nitrogen fixation, and oxygen detoxification. Gram-negative bacteria are unicellular organisms with a double membrane surrounding the cell. Iron must cross both membranes to enter the cell, and the process involved outer membrane and inner membrane proteins. One classic example is seen in the outer membrane protein FepA which actively transports an iron complex called ferric enterobactin (FeEnt). The inner membrane protein TonB provides the energy for this reaction.

Methods:

- Identification of insert in Caulobacter and vector in pITS23
- Mapping genome
- Creating DNA Primers
- Polymerase Chain Reactions (PCR)
- Purification of insert and vector
- Gibson Cloning
- Transformation of *E. Coli*
- Verification of protein expression

Results:

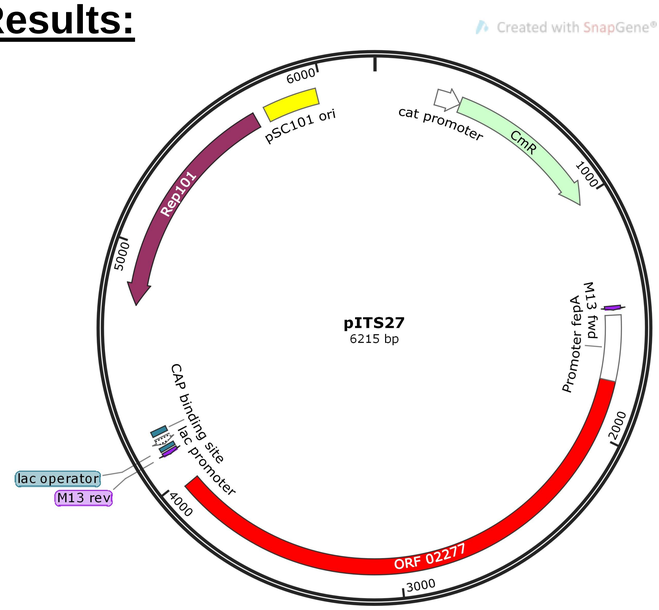

Figure 1: Map of pITS27 clone containing the *hutA* gene, *E. coli* promoter region, and *E. coli* signal sequence.

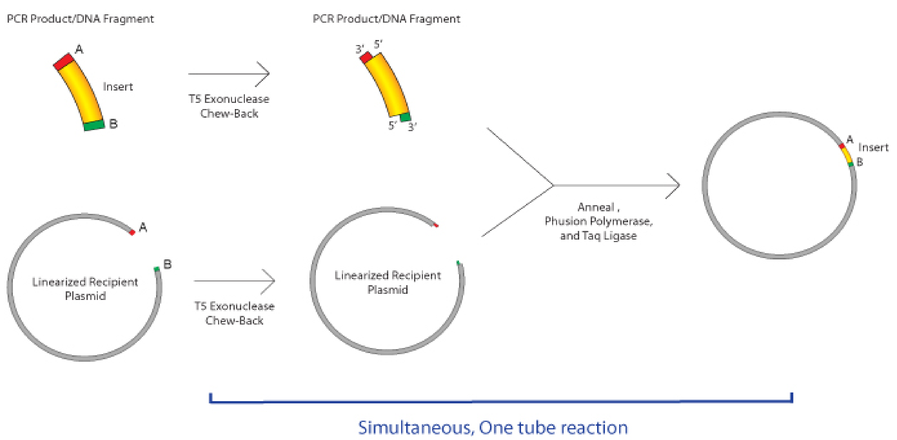

Figure 2: Gibson cloning construct.

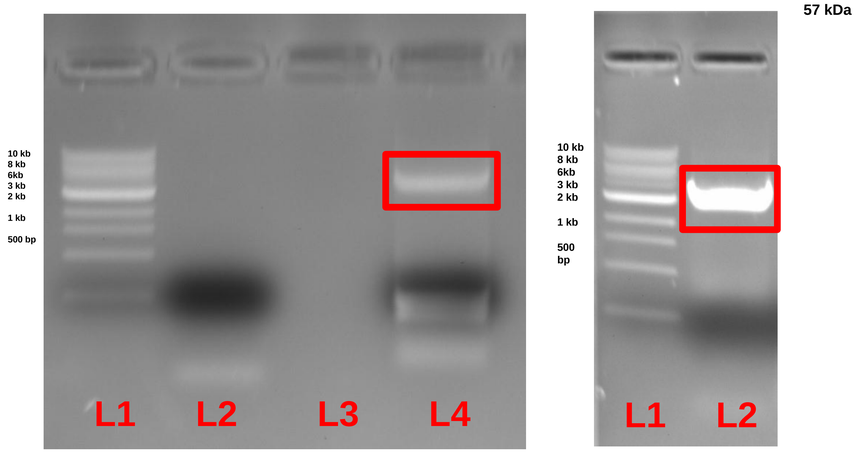

Figure 3: On the left is an image of the verification of the amplification of the pITS23 vector and on the right is the hutA insert.

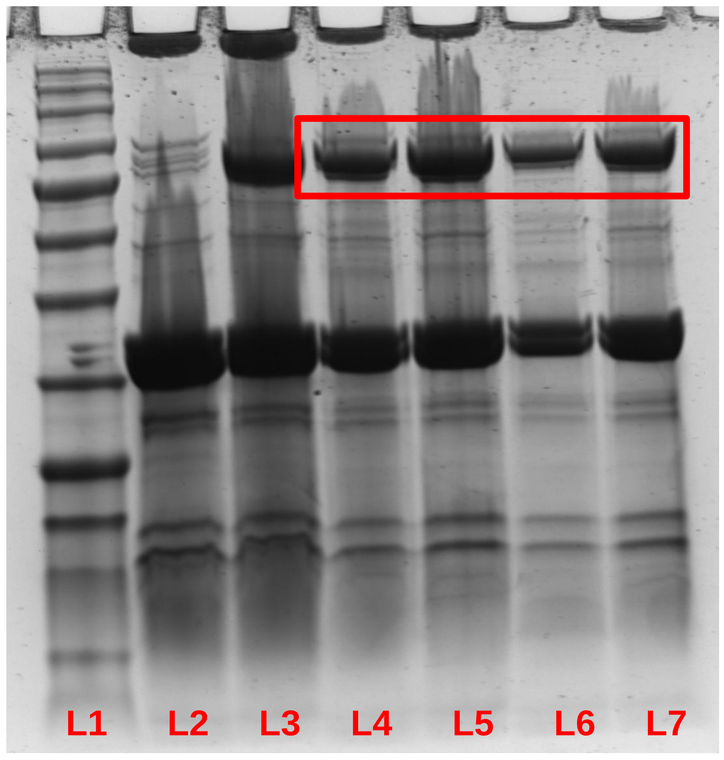

Figure 4: The highlighted region in lanes 4-7 (L4-L7) shows the verification of Heme expression in OKN359 on a protein electrophoresis gel. The approximate molecular weight of the protein is 75 kDa.

Results:

- Designed PCR primers and using PCR successfully amplified the vector (pITS23) and the desired insert (Caulobacter crescentus HutA) structure gene (Figure 3).
- Verified the correct clone by DNA sequencing off site.
- Transformed the pITS27 clone which contains Caulobacter crescentus HutA under the control of the *E. coli fepA* promoter region and signal sequence into *E. coli* OKN359.
- Grew transformed clones and isolated outer membrane portions and ran them on an SDS gel and visualized the expression of the HutA protein.

References:

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Balhesteros et al., 2017. Bacteriol. 199: pii: e00723-16