Analysis of the Catalytic Sites within the Plasmodium BEM46-Like Protein.

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Introduction

The bud emergence (BEM)46 protein, which are highly evolutionarily conserved members of the α/β-hydrolase superfamily, are known to have diverse functions and a wide range of substrates. Here we found a similar protein Plasmodium BEM46-like protein (PBLP), that also shares a structural homology and amino acid identity with BEM46-like proteins in the α/β-hydrolase superfamily. Previous studies have shown that PBLP has an essential role in parasite invasive-stage morphogenesis/growth. However, the precise biochemistry mechanism of action remains unknown. Within this protein, there is a proposed catalytic triad that is important for its function. So, to figure out whether this triad is indeed important for the protein function, we are going to induce mutation in those three amino acids involved in the catalytic triad and figure out whether they impact the biochemical properties of the PBLP protein. The three amino acids we are targeting are Serine 153, aspartic acid 229, and Histidine 258.

B

ggaacgTCTatgggatgtgctgttgctattgaaactgctttaaataattc
gaatagtgtagcgggtttaattgttcaaaacccatttttaagcatgaaaa
aaatggcaaagcttgcaaaaccatttttatttttttattatttttatcttatga
tttattaataagaacaaagatggacaatgaagaaaaaataaagaaga
atcgtgttccagttttgtttaacatttctgaaaagGATaaaatcgttcca
ccagatcatggaaagaaattatatgagatatgcccaagccaaaagttt
atttatactgcaaaagatggagagCATaataat

C

ggaacgAATatgggatgtgctgttgctattgaaactgctttaaataatt
cgaatagtgtagcgggtttaattgttcaaaacccatttttaagcatgaa
aaaaatggcaaagcttgcaaaaccatttttatttttttattatttttatctta
tgatttattaataagaacaaagatggacaatgaagaaaaaataaaga
agaatcgtgttccagttttgtttaacatttctgaaaagAAAaaaatcgt
tccaccagatcatggaaagaaattatatgagatatgcccaagccaaa
agtttatttatactgcaaaagatggagagTTTaataat

Fig 1;
A) PBLP displays a predicted protein structure that is characteristic of the BEM46 family of proteins, which all contain an α/β-hydrolase domain.

B) PBLP wild-type gene sequences. The pink codons indicate the three codons that encode those three catalytic residues (Serine 153, aspartic acid 229, and Histidine 258) that are predicted to be involved in the catalytic function of this protein.

C) Illustrate the three desired mutated codons within the PBLP protein, shown in pink. So, after mutagenesis, the desired amino acids we are looking for to replace those three catalytic residues are asparagine 153, lysine 229, and phenylalanine 258.

References

Miyazaki K. 2011. MEGAWHOP cloning: a method of creating random mutagenesis libraries via megaprimer PCR of whole plasmids. Methods in enzymology. 498:399–406. doi:10.1016/B978-0-12-385120-8.00017-6.

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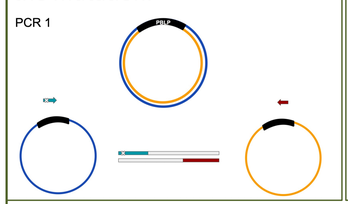
Methods

MEGAprimer PCR

Fig 2: Since we are working on three different codons, three different mutagenesis processes were performed. To generate mutagenesis to those three codons within the PBLP protein, I first amplified, using PCR, three separate DNA fragments that each include one of the three codons that are attempted to be mutated. For each PCR reaction, one of the primers (indicated in green) has mismatches at the targeted codon so to generate the mutation.

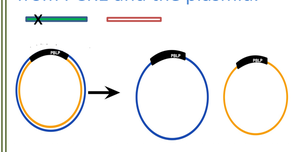


MegaWHOP PCR

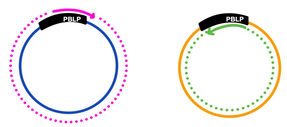
In vitro, to amplify the PBLP gene along with the plasmid that's cloned into, a megaWHOP PCR reaction was performed. In megaWHOP, the mutated DNA fragments from PCR1(megaprimer) were used as a set of complementary primers to make multiple copies of a whole plasmid. Since we are inducing three different and separate mutations, three separate megaWHOP PCR reactions were performed.

megaWHOP PCR

step 1: Denaturation of the mutated megaprimers from PCR1 and the plasmid.



step 2: Annealing and elongation



step 3: The most abundant plasmids are synthesized.

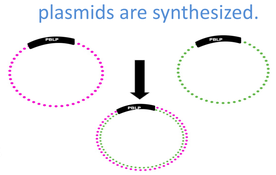
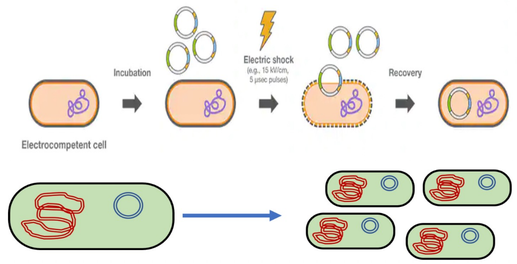


Fig 3: megaWHOP, a laboratory PCR technique used to make multiple copies of a whole plasmid or mutated plasmid using mutated DNA fragments as primers (megaprimers).

Transformation

Fig 4: To replicate the mutated gene of interest, that's now part of a plasmid, in amounts suitable for further analysis and/or manipulation, those mutated PBLP along with the plasmids were transformed into an *E.coli*.



"Bacterial Transformation and Competent Cells–a Brief Introduction." Thermo Fisher Scientific - US.

Purification and Sequencing

After the transformation of *E.coli,* the final step was purifying and sequencing that PBLP protein.

Results

PCR 1 results

Fig 5: Gel electrophoresis was used to test the product of PCR1. Lane one contains a 100bp ladder. Lane two contains the amplified DNA fragments with mutated serine 153 residue's codon. Lane three contains the amplified DNA fragments with mutated aspartic acid 229 residue's codon. And lane four contains the amplified DNA fragments with mutated Histidine 258 residue's codon. Based on this gel, the PCR that amplified the DNA fragments with the mutated serine 153 residue's codon did not work, while the other two PCR worked, and it indicated that those fragments were around 500bp.

MegaWHOP PCR results

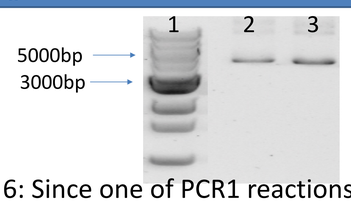


Fig 6: Since one of PCR1 reactions didn't work, we were only left with two fragments/megaprimers to be used for the megaWHOP PCR. After the megaWHOP, the amplified plasmid containing the PBLP gene with a mutated aspartic acid 229 residue's codon was loaded in lane two, while the amplified plasmid containing the PBLP gene with a mutated Histidine 258 residue's codon was loaded in lane three. Lane one contains a 1kb ladder. This gel indicated that both megaWHOP PCR worked and both plasmids were amplified. Based on this gel, those plasmids are about 5000bp

Transformation results

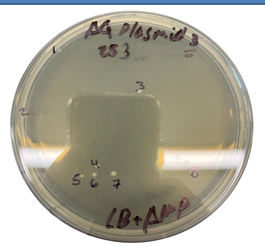


Fig 6: After the transformation of *E. coli*, those *E.coli* were transferred into a 1 LB + amp plate so they can grow. However, only the *E.coli's* that were transformed with the plasmid containing the PBLP gene with a mutated Histidine 258 residue's codon grew, and eight colonies were formed. Based on these results, we can only pursue the next step using those grown *E.coli* since they might have our PBLP gene with a mutated Histidine 258 residue's codon.

Sequencing results

caagccaaaagtttatttactactgca
aaagatggagagTTTaataat

Fig 7: This figure illustrates a portion of the PBLP gene after mutagenesis at a codon that encodes the catalytic residues Histidine 258. These sequencing results indicated that we got our desired mutation (Histidine to phenylalanine) at residue 258.

Future actions

1-Analyzing this purified and mutated Plasmodium BEM46-Like Protein (PBLP)
2- Repeat this experiment to induce mutation at the other two codons that were not successfully mutated.