Mutagenesis and Isolation of the *Plasmodium* BEM46-like Protein (PBLP)

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Introduction

Malaria is caused by eukaryotic parasites from the genus *Plasmodium*. These obligate, intracellular pathogens are transmitted to a vertebrate host through their *Anopheles* mosquito vector. People with the disease may experience fever, nausea, and fatigue [1]. Throughout the life cycle of malaria, the parasite expresses various proteins that aid in its infection (Figure 1).

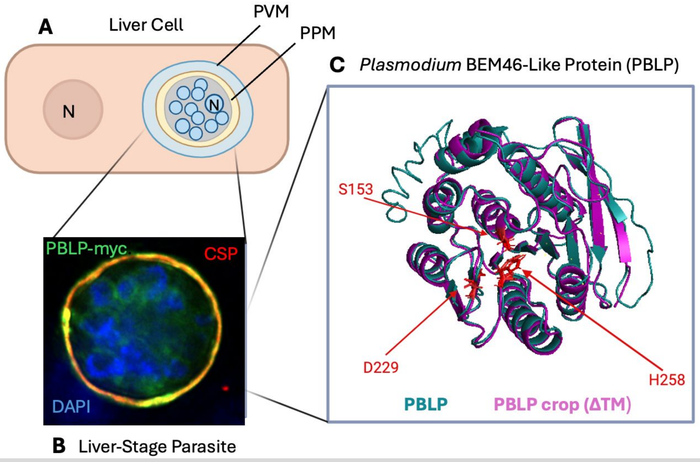

Figure 1. The *Plasmodium* infection begins with an infected *Anopheles* mosquito transmitting infectious sporozoites to the host. (A) Liver cells become infected with sporozoites, which develop asymptomatically into liver-stage parasites. Cellular structures in liver-stage parasites indicated, including the nucleus (N), parasitophorous vacuole membrane (PVM) and parasite plasma membrane (PPM). (B) PBLP localization was done using *P. yoelii* infected liver cells stained with mouse monoclonal anti-PyCSP IgG (red) as CSP is the most abundant PPM marker, mouse anti-c-Myc IgG (green) for PBLP, and DAPI (blue) to indicate dsDNA in nuclei before being observed using immunofluorescence microscopy [2]. (C) 3D rendering of wild type (WT) PBLP (cyan) predicted structure (I-TASSER) aligned with the cropped version of the WT PBLP (purple) which lacks its transmembrane domain. Proposed active site residues are indicted (red, COFACTOR). Image created using PYMOL and BioRender.

This research aims to characterize the catalytic function of the *Plasmodium* BEM46-like protein (PBLP), which was shown to be expressed throughout liver- and blood-stage development (Figure 1) [2]. There is a highlighted importance on understanding PBLP's impact on malaria infectivity because it has the potentially to limit transmission of the disease if it were to become a therapeutic target. While PBLP is predicted to contain an α/β-hydrolase domain at its C-terminus, the predictive catalytic domain includes a catalytic triad consisting of Serine (S153), Aspartic Acid (D229) and Histidine (H258) (Figures 1 and 2). Its exact biochemical structure and function in conferring infectivity remains unknown.

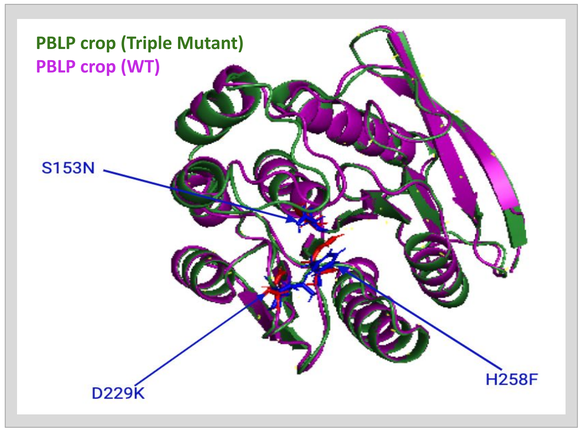

Figure 2. 3D rendering of cropped PBLP triple mutant (green) predicted structure (I-TASSER) with proposed active site mutations indicated (blue), aligned with a cropped structure of WT PBLP (purple, active site residues shown in red). Both WT and triple mutant PBLP lack the predicted transmembrane domain. Image created using PYMOL.

Mutagenesis

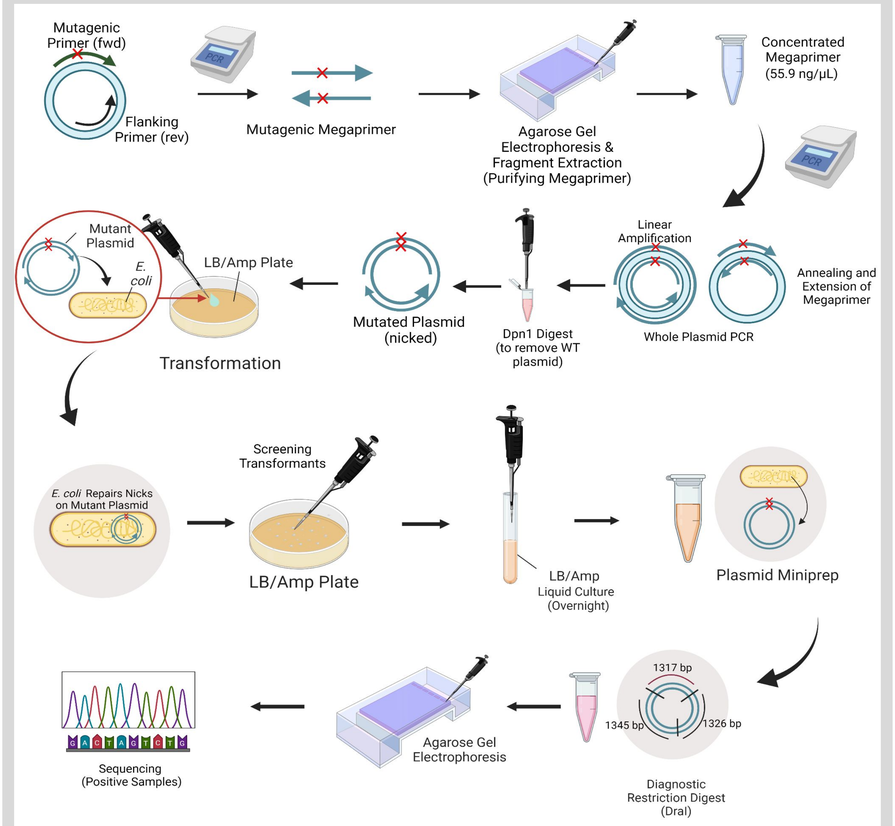

Figure 3. Schematic for the mutagenesis and subsequent production of a catalytically inactive (cropped) PBLP triple mutant (S153N, D229K, and H258F) within a protein expression vector. PCR #1 was carried out to develop a mutant megaprimer, which underwent gel extraction and concentration until it reached the desired concentration (>50 ng/μL). Whole plasmid PCR (PCR #2) was performed to linearly amplify the plasmid using the mutant megaprimer. Dpn1 digest was used to cleave the methylated WT plasmid, and retain only the mutated (nicked) plasmid. Mutated plasmid was transformed into *Escherichia coli* to repair the nicks and a miniprep was utilized to isolate the plasmid DNA for diagnostic restriction digest. Gel electrophoresis of digested plasmids (Dra1) confirmed products of expected size (3x bands of ~1300 bp), and positive plasmid samples were sent to an outside laboratory (Azenta) for sequencing to confirm the successful creation of the (cropped) PBLP triple mutant. Image created using BioRender.

Future Work

Through this research, we were able to successfully subclone a triple mutant version of the cropped PBLP coding region (Figure 2) into a protein expression vector to be used in further biochemical assays (Figure 3). Future plans include optimizing our protein isolation conditions and determining the dimerization potential of PBLP under native conditions (Figures 4 and 5). Malaria is a vector-borne parasite transmitted by the *Anopheles* mosquito [1]. This parasite is endemic in many underdeveloped countries where it frequently persists as one of the top ten causes of death worldwide [3]. As climate change creates an environment that allows the mosquito vector to thrive, malaria rates are likely to increase in areas where it has not been historically endemic [4]. Current vaccination strategies are limited and preventative pharmaceuticals for malaria are marginally effective given the global rise in antimalarial drug resistance so understanding the function of PBLP will allow us to determine its potential as a novel antimalarial drug target.

Acknowledgments

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Protein Isolation

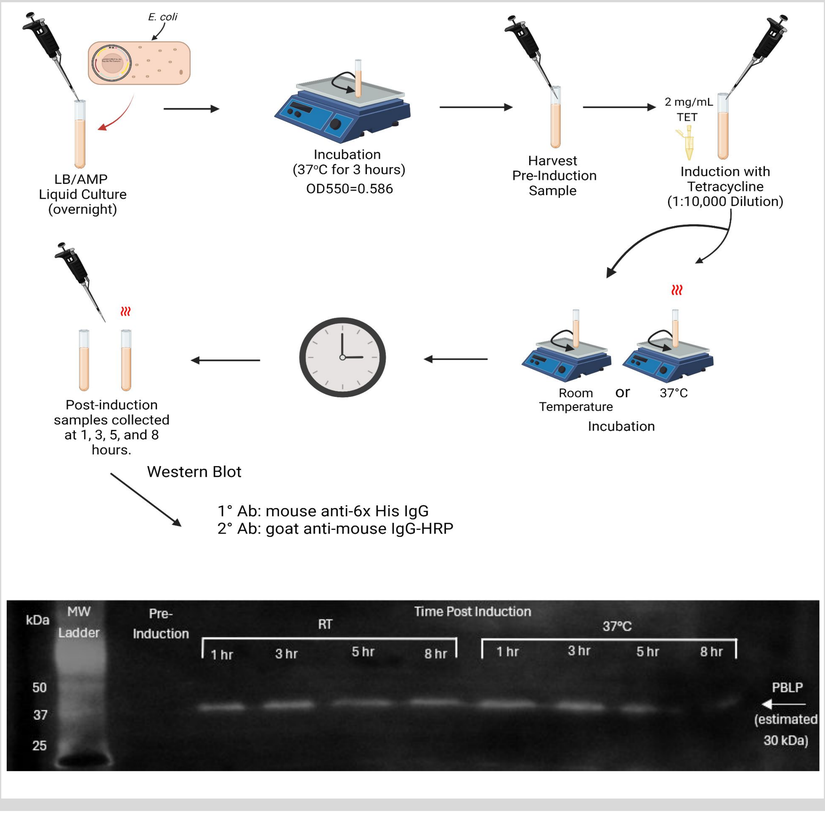
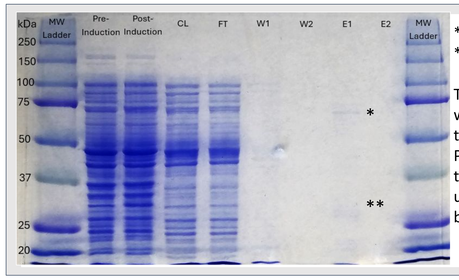


Figure 4. A time course was performed utilizing WT PBLP expression constructs to monitor for gene expression changes in order to optimize conditions for protein induction and isolation. Bacterial cultures with WT PBLP plasmid were grown in LB broth with 100 μg/mL ampicillin and incubated at 37°C for three hours (OD_{550} 0.5-0.6). Pre-induction sample was collected for evaluation and remainder of solution was induced with 1:10,000 dilution of tetracycline (2 mg/mL). Induced cultures were separated into two sample tubes and subjected to different temperature conditions; one tube was incubated at 37°C and the other at room temperature. Post-induction samples were collected from each condition at one, three, five, and eight hours. The samples were evaluated using polyacrylamide gel electrophoresis (PAGE) under native conditions and Western blot (mouse anti-6x His IgG [primary antibody] and goat anti-mouse IgG conjugated with horseradish peroxidase (HRP) enzyme [secondary antibody]). Preliminary results indicate that WT PBLP is expressed best at room temperature and is about 30-37 kDa in size, which implies it is a monomeric protein. Image created using BioRender.



* ~70 kDa, suggestive of a multimeric protein.
** ~30 kDa, suggestive of a monomeric protein.

The presence of multiple bands in first elution (E1) was unexpected and indicates a need for further troubleshooting of the protein isolation protocol. Proposed protocol modifications include adjusting tetracycline concentration used for induction or utilization of a different vector backbone (and/or bacterial strain) for protein expression.

Figure 5. PAGE gel electrophoresis (stained with coomassie blue) results of PBLP isolation using a modified induction protocol conducted at room temperature. The protein bands seen at ~70 kDa and ~30 kDa in elution 1 (E1) suggesting PBLP has a multimeric and monomeric conformation, respectively. Figure abbreviations: cleared lysate (CL), flow through (FT), wash 1 (W1), wash 2 (W2), elution 1 (E1), and elution 2 (E2).

References

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