The Red Flags of *Plasmodium yoelii*:

Expressing *Plasmodium* BEM46-like Protein (PBLP)-BirA to Characterize Parasite Surface Proteins

Jamie Anne Dahan, Ayat Alkadban, Jack Vincent, and Anna Groat Carmona*

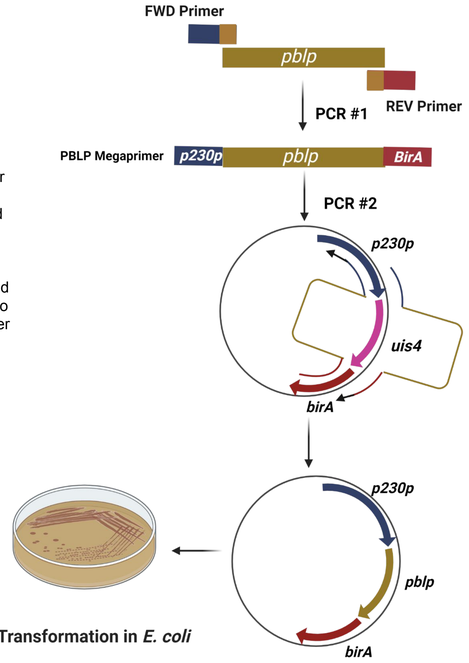


Figure 6. Using MegaWHOP PCR to create a parasite cloning plasmid that expresses PBLP-BirA. Mutagenesis involves the creation of a PBLP megaprimer through standard PCR. The PBLP megaprimer is then used to replace *uis4* on the existing plasmid with the desired *pblp* coding sequence during MEGAWHOP PCR. The desired plasmid is then transformed into *E. coli* to ligate the nicks leftover from MEGAWHOP PCR and replicate the plasmid to high numbers. Image created with BioRender.

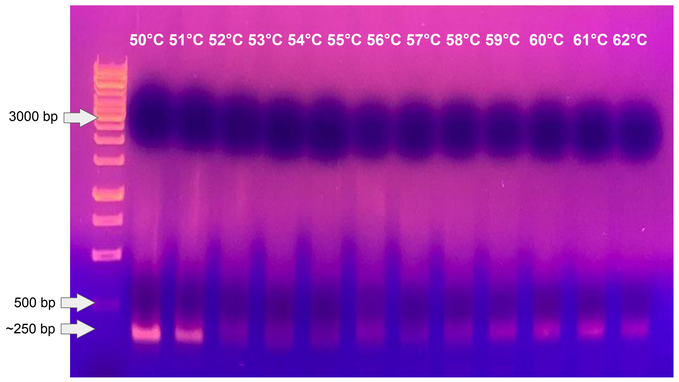


Figure 7. Agarose gel from gradient PCR to generate PBLP megaprimer (PCR #1). Each well contains the same PCR mastermix; the only variation being the annealing temperature, which ranged from 50°C (left) to 62°C (right). These reactions produced mainly primer dimers (~250 bp; bottom arrow). No desired bands (3013 bp) were observed (top arrow), indicating no amplification of the PBLP megaprimer.

Forward Primers \ Reverse Primers	2	3*	4	5*	6*	7***	8*
3							
4***		D		E	F	A	
5***							
6							
7*							
8***		G		H	I	B	
9							
10***		J		K	L	C	

Table 1. MEGAWHOP primer combinations with a focus on generating optimal GC caps. New MEGAWHOP primers were designed using the target PBLP-BirA plasmid. Forward primers amplify the 5' end of PBLP and part of the p230p targeting sequence and the reverse primers amplify the 3' end of PBLP and the 5' end of the BirA sequence. The purple and green boxes are being prioritized over the white boxes, and they're listed in order of optimal GC caps (e.g. A = best, L = suboptimal).

FUTURE DIRECTIONS

We will continue to work with MegaWHOP PCR by troubleshooting the next set of primer combinations while considering other PCR additives to enhance annealing and create the PBLP megaprimer.

INTRODUCTION

Malaria is a dangerous disease caused by parasites in the *Plasmodium* genus and is transmitted by Anopheline mosquitoes. Much of the protein content on the parasite's plasma membrane remains unknown, which complicates our understanding of its pathogenesis. PBLP, the *Plasmodium* BEM46-like protein, is expressed throughout the parasite's invasive and developmental stages, remaining membrane-bound when the parasites are found in the liver and blood. Due to the protein's consistent expression throughout those stages, it is the best candidate for biotin-tagging membrane proteins. BirA is a promiscuous biotin ligase that indiscriminately biotinylates neighboring proteins. Our goal is to tag proteins on the parasite surface with biotin whenever PBLP-BirA is expressed on the parasite's membrane. This will enable surface proteins to be pulled down using streptavidin based assays for analysis and identification.

Figure 1. Life cycle of *Plasmodium* parasites. Sporozoites introduced from female mosquitoes after a blood meal will enter the bloodstream to access liver cells (hepatocytes). In liver-stage development, the sporozoites will differentiate into trophozoites. After nuclear division, it becomes a schizont, and its membrane will eventually invaginate to create 10,000s merozoites. These merozoites are then released into the bloodstream to infect red blood cells and cause symptomatic disease. Their replication is cyclic within red blood cells and a few dozen merozoites are made during each cycle. This research focuses on the morphological changes that the *P. yoelii* membrane undergoes during liver-stage development, when PBLP is expressed on the membrane. Image created with BioRender.

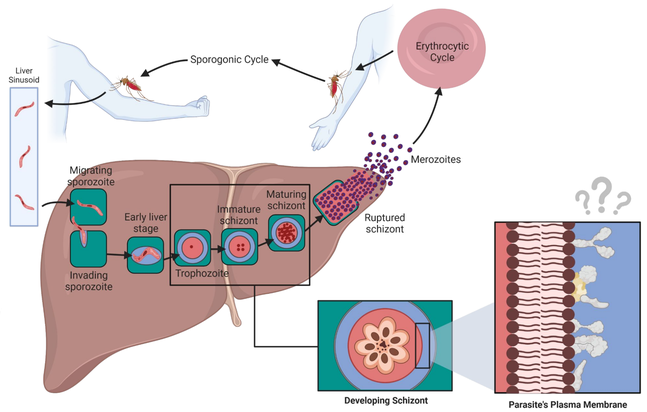
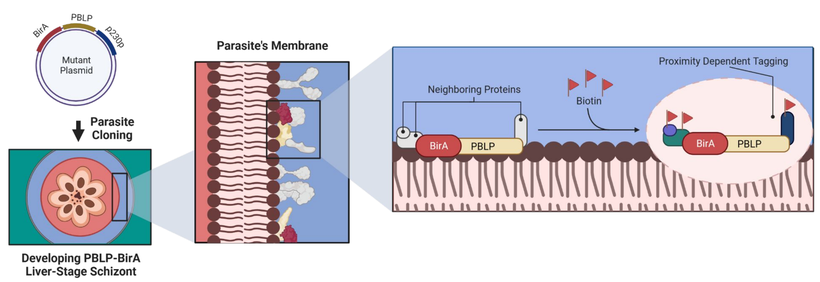


Figure 2. Characterizing the parasite's membrane surface through biotinylation. Introduction of the PBLP-BirA coding sequence in *Plasmodium yoelii* genome will enable biotin attachment to neighboring surface proteins when PBLP-BirA+ parasites are exposed to biotin during liver-stage development. This will enable isolation of those neighboring proteins for identification and analysis. Image created with BioRender.



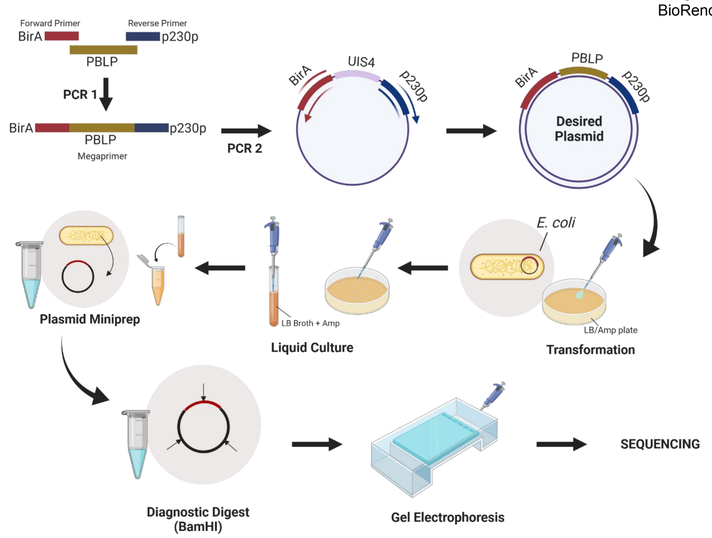


Figure 3. Methodology for the Creation of a PBLP-BirA Parasite Cloning Plasmid. These steps ensure successful replacement of the BirA-tagged *uis4* gene with the *pblp* gene. The desired parasite cloning plasmid is composed of the target vector with the p230p targeting sequence and the PBLP-BirA-tag coding sequence under control of the PBLP endogenous promoter. Once formed, the desired plasmid will be transformed into *E. coli* cells and isolated plasmids will be confirmed with restriction digests and gel electrophoresis. Sequenced plasmids will then be linearized and transfected into *Plasmodium yoelii* parasites for further characterization. Image created with BioRender.

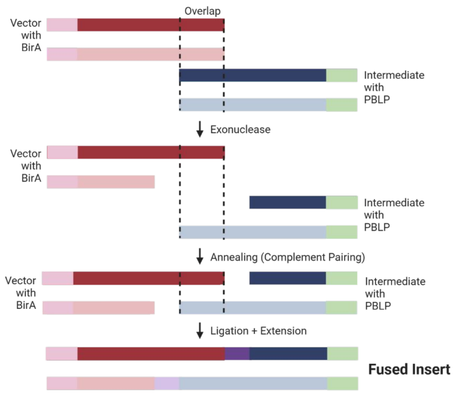


Figure 4. Mutagenesis of existing UIS4-BirA plasmid using Gibson Assembly. Primer design is based off the target PBLP-BirA plasmid sequence, where the ends of the individual inserts create an overlap region between each other. During annealing, the overlapping ends align with the target vector, facilitating fusion. The inserts consist of the target gene (*pblp*) from an intermediate vector and the BirA-tag from the target vector. Once the annealed insert and the target vector fuse, they will be transformed into *E. coli* to be screened and ensure successful mutagenesis. Image created with BioRender.

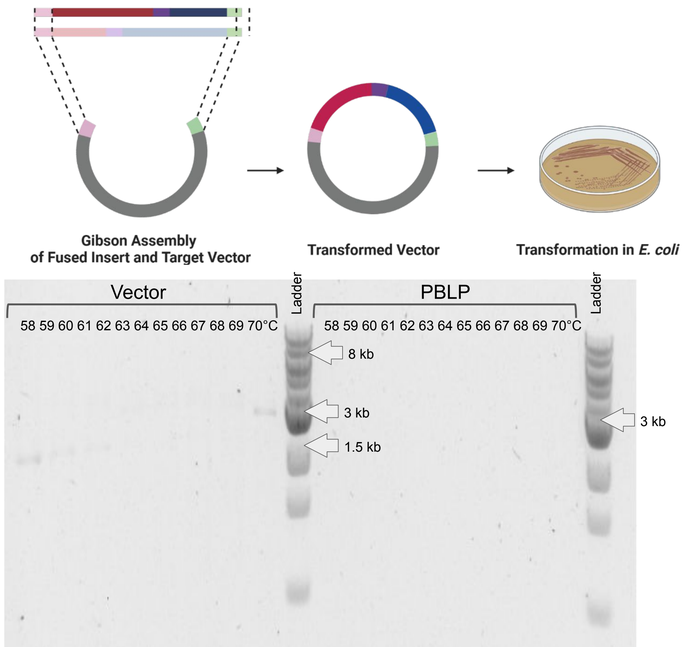


Figure 5. Gel image of gradient PCR to amplify desired inserts with respective primers. The left side of the gel consists of the amplification for the target vector (BirA), the right side consists of the amplification for the target gene (*pblp*). The expected sizes for the vector and the gene is 8 kb and 3 kb, respectively. There were incorrectly sized bands generated for the BirA sequence and no bands generated for the *pblp* gene.

CONCLUSIONS

Our experiments have not produced the desired PBLP megaprimer due to unsuccessful amplification. So far we either produce primer dimers, generate incorrect bands (based on size), or yield no bands. Further troubleshooting efforts will consist of changing thermocycler conditions (e.g. adjusting annealing temperature range), and amending the PCR 1 protocol to include a new additive (Betaine) to aid primer annealing. We also ordered new cloning primers to generate the PBLP megaprimer.

SIGNIFICANCE

Analysis and characterization of the surface proteins on the parasite's membrane during liver-stage development will help us better understand the physiology and morphological changes that govern infectivity. This research could potentially introduce new antimalarial drug targets.

ACKNOWLEDGMENTS

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