Analyzing the Biochemistry of the *Plasmodium* BEM46-like Protein (PBLP)

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Malaria Life Cycle

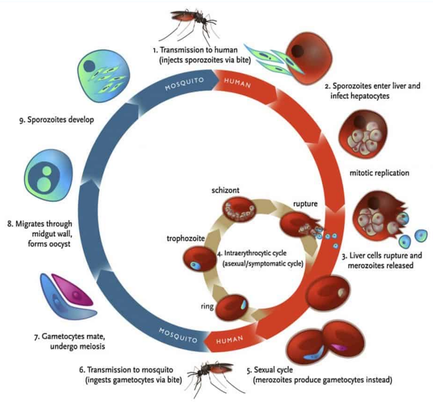


Figure 1. The *Plasmodium* life cycle involves cycling between a mammalian host and its Anopheline mosquito vector.
Image credit: https://cddep.org/tool/life_cycle_malaria_parasite/

What is PBLP?

- The *Plasmodium* BEM46-like protein (PBLP) is an α/β hydrolase that is expressed at all stages of the malarial life cycle (Groat Carmona *et al*. 2015).
- Uncommon for malaria proteins to be constantly expressed throughout life cycle.
- Membrane localized protein during liver-stages and blood-stages of malaria life cycle (Groat Carmona *et al*. 2015).
- Has a role in invasive-stage morphogenesis (Groat Carmona *et al*. 2015).

Objective

This study aims to understand the biochemistry of PBLP in order to further discern its role in modulating malarial infectivity.

Conclusions/Future Directions

- Understanding PBLP and its function could ultimately aid in the development of antimalarial drug therapies.
- Determining the source of cloning issues will help us generate our mutant PBLP panel for future biochemical analysis.
- Troubleshooting the protein isolation process to increase protein yields will allow for in depth analysis of PBLP function.

Cloning

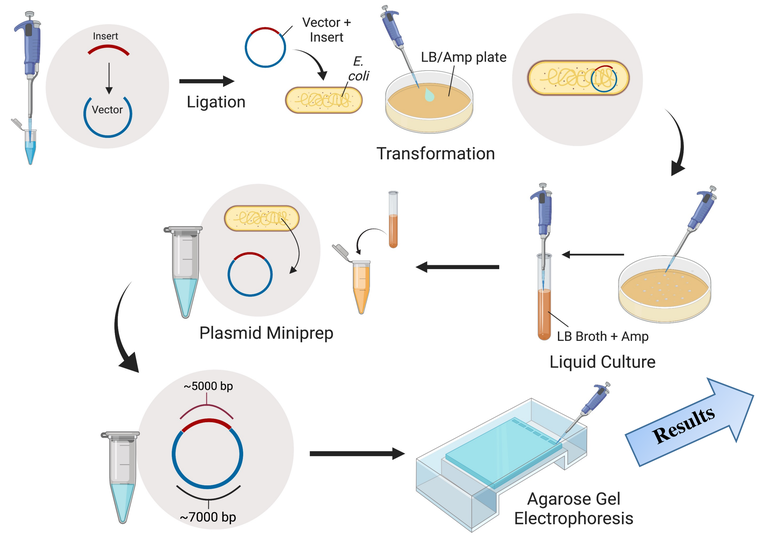


Figure 2. Schematic of molecular cloning methods to characterize transformants via restriction digest (BamHI). Created with BioRender.

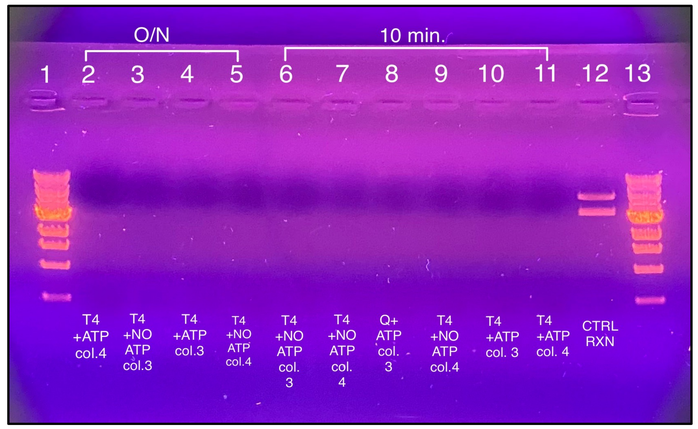


Figure 3. Agarose gel electrophoresis of restriction digests (BamHI) of transformants. Wells 1 and 13 contain the 500 bp DNA ladder (BioRad). Wells 2-11 are the sample plasmids (from transformants), and well 10 contains the control plasmid (pSL404).

Table 1. Variables tested for ligation step of cloning procedure.

	T4 Ligase	Quick Ligase
+ATP	O/N or 10 min	
-ATP		

Protein Isolation

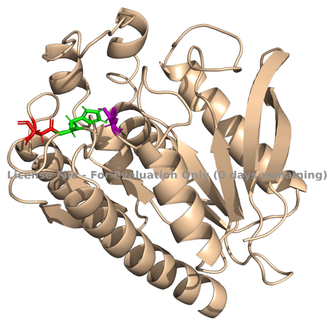


Figure 4. 3D prediction of WT cropped PBLP (tan, I-TASSER). Active site (S153, D229, H258) shown in red, green, and purple, respectively.

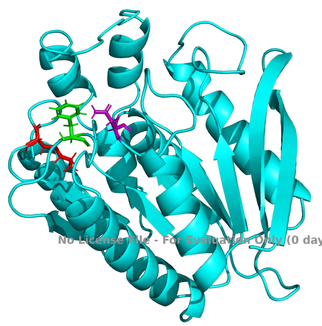


Figure 5. 3D prediction of cropped triple mutant PBLP (blue, I-TASSER). Active site mutations (S153N, D229K, H258F) shown in red, green, and purple, respectively.

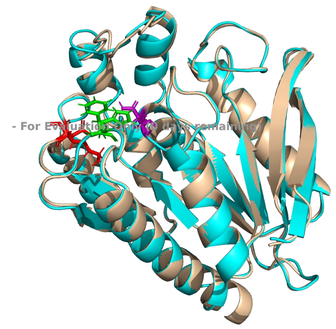
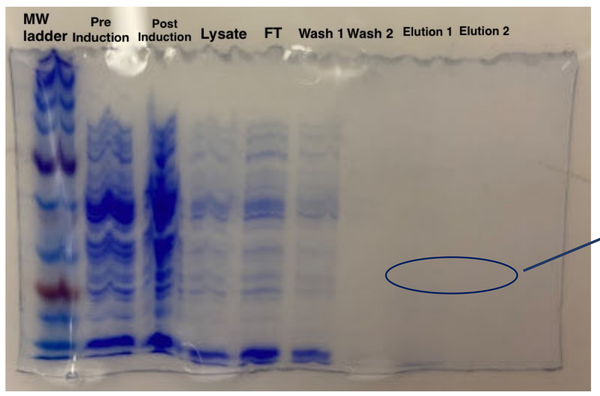


Figure 6. Overlapped 3D prediction of cropped WT (tan) and triple mutant (blue) PBLP (I-TASSER). Active site (S153, D229, H258) and mutations (S153N, D229K, H258F) shown in red, green, and purple, respectively.



Bands around 30 kilodaltons (KDa) would indicate PBLP is a monomer.

Figure 7. PAGE gel of PBLP samples after initial protein purification.

References

CDC-Centers for Disease Control and Prevention. 2020. CDC - Malaria - About Malaria - Biology. [accessed 2022 Jun 3]. https://www.cdc.gov/malaria/about/biology/index.html.

Groat-Carmona AM, Kain H, Brownell J, Douglass AN, Aly AS, Kappe SH. 2015. A Plasmodium α/β-hydrolase modulates the development of invasive stages. Cell Microbiol. (12):1848-67.

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Acknowledgements

Special thanks to mentor Dr. Anna Groat Carmona and lab partner, Jillian Sheppard.

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CONTACT INFO