The Red Flags of *Plasmodium yoelii:*

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

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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 development, remaining membrane-bound when the parasites are found in the liver. This research requires generating a mutant parasite cloning plasmid to create a *P. yoelii* clone that expresses a PBLP-BirA fusion protein, which will promiscuously tag membrane surface proteins with biotin during liver-stage development. In order to create the desired plasmid, a megaprimer is created through standard PCR and then used to replace unnecessary sequences within the desired vector during MEGAWHOP PCR. Due to cloning difficulties, a few troubleshooting experiments have been conducted to obtain the desired megaprimers. Potential sources of error include the A-T rich nature of *pblp* as well as the need to swap out large gene sequences and regulatory elements, which necessitates the creation of longer primers with optimal G-C caps. We have generated a new list of primer combinations and plan to utilize new PCR additives to generate the intended plasmid. Once the mutant parasite cloning plasmid is made and introduced into *P. yoelii* parasites, expression of PBLP-BirA will aid the

characterization of cell membrane proteins throughout liver-stage development. Identifying unknown proteins on the parasite's surface will help further our understanding of its basic biology, and potentially, identify novel drug targets or vaccine candidates for malaria.