Creating a transgenic parasite to express *Plasmodium* BEM46-like protein (PBLP) with a BirA* tag

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**Introduction**

Malaria is a parasitic disease that is transmitted by the female Anopheline mosquito. Those particularly affected by malaria are children under the age of five and people who live in subtropical areas of the world (3). Malaria treatments are available so the disease is preventable, but the rise of antimalarial drug resistance is increasingly problematic.

The life cycle of the malaria parasite within humans begins with the liver-stage, where we see a massive amplification event (4). This stage of development is asymptomatic but the parasite’s surface undergoes many changes (Figure 1). The *Plasmodium* BEM46-like protein (PBLP) is one of the only known proteins that is present on the parasite plasma membrane throughout liver-stage development (4). We want to use PBLP’s consistent expression to our advantage and identify other proteins on the parasite plasma membrane (Figure 2).

In order to characterize the divergent protein population throughout the liver-stage development, we will tag the C-terminus of the PBLP coding sequence with coding sequence for the biotin ligase, BirA* (Figure 4) (2). BirA* is a promiscuous biotin ligase that will biotinylate proximal membrane proteins. Biotinylated proteins can be isolated using existing streptavidin-coded beads and then identified through mass spectrophotometry (Figure 5) (5).

**Methods**

**Figure 2.** We needed to generate our insert using PCR, which allowed us to introduce restriction enzymes (SacII and NotI) to the 5’ and 3’ end of the pblp gene sequence (including the promoter), respectively. When subject to restriction digest, incompatible sticky ends would be generated for ligation into a parasite cloning vector (2).

**Figure 3.** Our parasite cloning vector (pBirA*/Myc) and insert (prmtr-PBLP) can be cut with restriction enzymes (NotI and Saccll) so that they can subsequently be ligated together to create our plasmid (2).

**Figure 4.** The PBLP-BirA* plasmid will be inserted into the p230p locus of the malarial genome via homologous recombination (2).

**Figure 5.** BirA* biotinylates proximal membrane proteins (2), which can then be isolated using streptavidin-coded bead. The extracts can be later analyzed through mass spectrophotometry to identify membrane proteins during liver-stage development (5).

**Results**

**Figure 6.** Agarose gel electrophoresis was used to analyze our insert and vector after restriction digest. Relevant bands were isolated for subsequent cloning steps.

We are currently in the final stages of cloning our PBLP-BirA* plasmid. However, we are troubleshooting these late stage cloning procedures. Once resolved, our modified PBLP-BirA*-coding sequence will be inserted into the p230p locus of the malarial genome (Figure 4).

**Moving Forward**

Creating a transgenic parasite line will allow us to study the proteins on the parasite plasma membrane throughout liver-stage development (2).

We want to identify novel proteins on the parasite’s surface that could allow us to work around the rise in antimalarial drug resistance.

**References**


(2) Groat-Carmona AM. Unpublished


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