Identification of Vaccine Candidates Against *Falciparum* Malaria Using Genes with Tandem Repeats

By

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BS, Worcester Polytechnic Institute, 2020

Thesis

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Abstract of (Identification of Vaccine Candidates Against Falciparum Malaria Using Genes with Tandem Repeats), by (Annalise Robidoux), ScM, Brown University, May, 2022

Objective: There are 240 million cases of malaria each year, making it one of the most endemic diseases in modern history. However, not substantial preventative measures have been identified. Current research effort focuses on the development of new vaccines. More than 40% of the vaccine candidates identified in the malaria parasite have tandem repeats in their genes. The current research project focuses on identifying vaccine targets utilizing in silico analysis of P. falciparum genes with tandem repeats.

Methods: The P. falciparum genome was scanned for genes that contained tandem repeats. These genes were further down selected using in silico analysis for antigen prediction. Software tools like B-cell & T-cell epitopes, regions of hydrophilicity, low complexity, MHC II binding epitopes, protein 3D structure, low genetic diversity, trans membrane domain and signal peptides. Twenty-nine fragments were identified, and eleven fragments were inserted into a DNA vaccine vector pVR2001, immunized into mice. Polyclonal antibodies were harvested, and the efficacy of each antigen was evaluated using growth inhibition assays (GIAs).

Results: Two of the eleven anti-sera were found to inhibit P. falciparum growth in culture with various degrees (75-98%). These two anti-sera were able to significantly inhibit the parasite in GIA and recognize proteins of the appropriate size from protein extracts from the parasite.

Conclusions: This investigation found that reverse vaccine identification techniques were able to identify potential vaccine targets. Out of the eleven antigens used to vaccinate mice, two were found to have the potential to be used as a vaccine against falciparum malaria.

Chapter 1: Introduction

Malaria is an infectious disease caused by a *Plasmodium* parasite. There are many different *Plasmodium* species, including *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium cynomolgi*. These parasites infect a host by inoculation directly into the bloodstream, a process that occurs when a female *Anopheles* mosquito performs a blood meal on a human host (Bray and Garnhan, 1982). Once infected with the parasite, humans can develop malaria to different degrees. Unspecific illness- fever, chills, and fatigue- characterize mild malaria. Severe malaria is characterized by more specific complications, including anemia, respiratory distress, and neurological distress. These complications typically arise due to a loss of erythrocytes (Mockenhaupt et al, 2004). Patients who suffer from severe malaria can also develop cerebral malaria: when the central nervous system is infected, leading to a deep level of unconsciousness. Cerebral malaria can lead to brain swelling, neurological damage, or seizures in patients. Patients with severe malaria can also develop hypoglycemia, renal failure, circulatory collapse, and pulmonary edema (although its occurrence is rare in children). Pregnant women have particular susceptibility to malaria infections due to weakening the immune system during pregnancy (Rogerson et al, 2007). They tend to be more prone to hypoglycemia and pulmonary edema when severe cases arise.

The Global Impact of Malaria

Malaria infection has been observed globally with 100 endemic countries (Sultana et al, 2017). These countries are located in Africa, North and South America, various regions of Asia, and Europe (Kiszewski et al, 2004). Although many countries may be considered endemic, the frequency of malaria transmission varies from region to region (Miller et al, 1994). Because mosquitos are needed for continued transmission of *Plasmodium* parasites, the transmission rate within a region depends on the regions ability to foster prolonged mosquito activity. Typically, more tropical climates will have a higher degree of endemicity. This is due to their ability to maintain heat and humidity year long- an environment which promotes continuous mosquito growth and reproduction (Kiszewski et al, 2004). Climates that experience cold winters do not support prolonged mosquito life cycles, as mosquitos undergo hibernation and are no longer active. These colder climates therefore are not considered to have stable malaria transmission. Malaria transmission is also affected by both the mosquito and the parasite present in the region. There are many different mosquitoes, but *Anopheles gambiae* is a very efficient mosquito ("CDC - Malaria - Malaria Worldwide - Impact of Malaria", 2020). Its presence is most common in sub-Sahara Africa. In addition to this persistent mosquito, Africa also suffers from the presence of *Plasmodium falciparium*. This *Plasmodium* parasite is most likely to cause sever malaria and eventually lead to death.

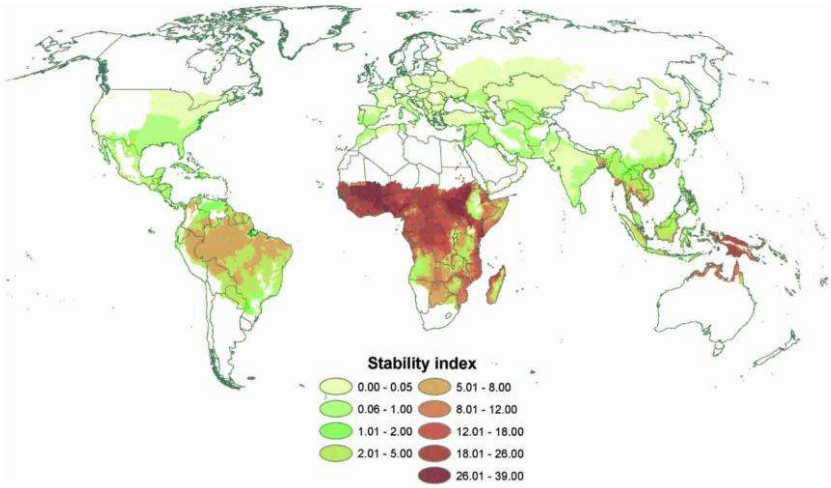

Figure 1: Malaria Transmission is concentrated in tropical regions (Kiszewski et al, 2004).

A combination of the mosquito and the parasite present in the Sub-Saharan African region makes Africa the deadliest continent for malaria transmission, accounting for 80% of the worldwide malaria deaths in 2015. In addition to the deadly carrier/parasite combination, African countries remain poor and rather uneducated. A study in Kenya revealed that malaria infection was more prevalent in poorer communities than in rich communities (Sultana et al, 2017). Additionally, households which had no electricity fostered a higher rate of malaria than households that do. Without access to electricity, the household also do not have access to media, which is why household without radios or television also had higher rates of malaria that those that have these devices (Sultana et al, 2017). This study brings into question the relevance of malaria health education in Sub-Saharan communities where infection is most prevalent.

In more recent years, there has been an effort for African schools to offer education on the preventative measures needed for individual malaria protection. These efforts allow information about malaria prevention and control to reach households, which may not have access to media through radios or TV. It also allows a target education for the most affected age group, as children are more likely to get malaria than their parent. The World Health Organization constructed a set of guidelines for educating children on malaria prevention. Their instruction included information on how to use a insecticide treated net, along with knowledge of what time of day children are most at risk for a mosquito bite.

Spread of information or knowledge may be most effective malaria control mechanism. The Kenyan study found that approximately 70% of subjects found it important to sleep under an insecticide treated net, yet there was still high prevalence of malaria within these communities (Sultana et al, 2017). This suggests that many households in Kenya lack proper knowledge of how to assemble and utilize the preventative measures donated to them.

To address the growing concern for malaria infection in African countries, leaders from 25 African countries gathered at a leadership summit. There they pledge increased research into malaria along with resources for pregnant women and children who may be infected (*The Abuja Declaration and the Plan of Action*, 2000). The leaders created plans of action for each country, laying out how they will budget for malaria prevention equipment and how they will distribute the resources across the country.

In addition to these local efforts, more global efforts have been made. $2.7 million was invested into control and elimination efforts in 2018 through the Global Fund ("The President's

Malaria Initiative and Other U.S. Government Global Malaria Efforts", 2020). The United States is the largest donor to this global fund, with $999 million designated for control efforts and research in 2020. The United States created the Presidents Malaria Initiative Strategy (PMI) in 2005 ("The President's Malaria Initiative and Other U.S. Government Global Malaria Efforts", 2020). Through partnership with the CDC, it works to reduce malaria mortality through expansion of access to malaria control interventions, such as insecticide treated nets, in affected community. The work of this organization is still active today, reaching 24 sub-Saharan countries. This allows communities most affected by malaria transmission to have preventative measures in each household, in hopes of slowing transmission within the region. In addition to extensive effort going towards distribution of malaria control interventions the United States has set aside considerable funding for research into the parasite and preventative agents against its infection ("The President's Malaria Initiative and Other U.S. Government Global Malaria Efforts", 2020).

Plasmodium **life cycle**

The *Plasmodium* life cycle is important to understand in order to develop preventative agents to stop the parasite's infectious spread. *Plasmodium's* life cycle takes place within two hosts- the mosquito and the infected human. The mosquito will infect a human with only one stage of the parasite's life cycle- sporozoites (Bray and Garnhan, 1982). However, once inside the human host, the parasite will undergo multiple different developmental stages – sporozoite, schizonts, merozoites, trophozoites, and gametocytes ("CDC - DPDx - Malaria", 2020 & Bray and Garnhan, 1982). While the mosquito may be immune to the harmful nature of the parasite, it plays an important part in the spread of malaria.

The life cycle begins when an infected *Anopheles* mosquito feeds on the blood of a human (Bray and Garnhan, 1982). The main purpose of this meal is to feed herself and to provide nutrients to her eggs. While taking a blood meal, the female mosquito will inoculate sporozoites into the blood stream of the human host. From there the sporozoites will infect liver cells, beginning the exo-erthrocytic cycle ("CDC - DPDx - Malaria", 2020). Not much is known about the route taken by malaria parasites to get to hepatic cells. However, it is hypothesized that they may travel through the endothelial cells which line the liver. Once a *Plasmodium* parasite reaches a hepatocyte, there are a series of interactions between the parasite and hepatic receptor proteins which take place to allow parasite entry. Not much is known about the exact mechanism behind hepatocyte entry, but it is believed to be quite similar to the entry pattern of erythrocytes (Frevert, 2004) Once inside the hepatocyte, sporozoites will mature into schizonts. These matured schizonts will then rupture from hepatocytes and release merozoites into the blood stream. One schizont has the potential of multiplying into thousands of merozoites from within the hepatocyte (Prudencio, Rodriguez, and Mota, 2006).

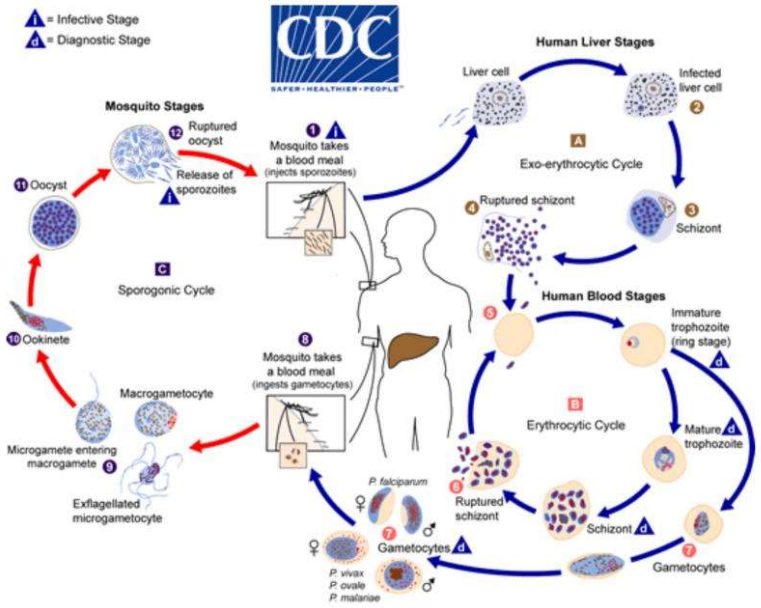

Figure 2: *Plasmodium* Life Cycle ("CDC - DPDx - Malaria", 2020).

Merozoites that have escaped the liver will circle the blood steam and infect erythrocytes. To initiate erythrocyte invasion the malaria parasite will bind to the erythrocyte plasma membrane through ligand-receptor interaction. This is considered to be a reversible attachment of the merozoite to the erythrocyte membrane (Bray and Garnhan, 1982). However, once the merozoite reorients upon the membrane a junction forms between the parasitic and host membranes. The merozoite is then irreversibly attached to the erythrocytic membrane. From there the merozoite will release rhoptry-microneme substances with parasitopherous vacuole formation (Florens et al, 2002). This will stimulate the erythrocyte membrane to surround the merozoite. Once within the erythrocyte, the merozoite will then lose its surface coat so that a parasitophorous vacuole membrane (PVM)- made mostly from erythrocytic lipids- can replace it. The PVM will remain serving as an interface between the parasite and the erythrocyte's cytoplasm (Florens et al, 2002).

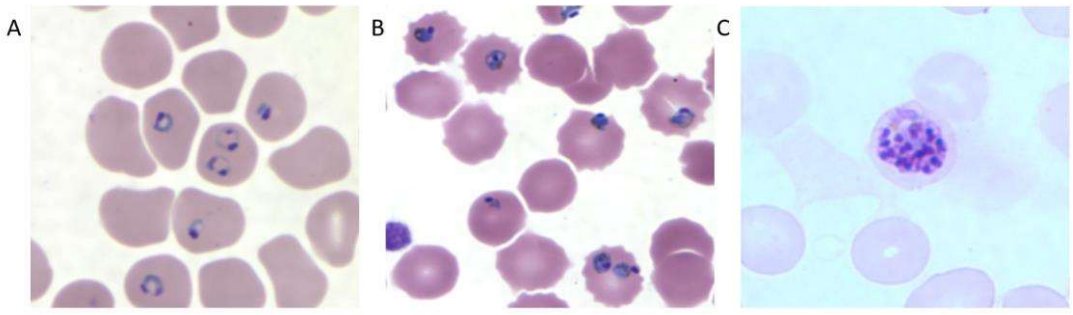

Figure 3: Trophozoite Development. A) Early trophozoite/ ring stage. B) Mature Trophozoite. C) Schizont stage ("CDC - DPDx - Malaria", 2020).

Once a merozoite enters an erythrocyte, two developmental paths can be taken. The first of which continues the erythrocytic cycle. This path consists of the maturation of the merozoite into immature trophozoites (ring stage) (Florens et al, 2002). This is mostly done through the degradation of the parasite's inner membrane complex. The parasite will gain nutrients by

feeding off the erythrocyte's cytoplasm, using hemoglobin as a source of amino acids for its own protein formation. After some time, the parasite will develop into mature trophozoites within the erythrocyte (Florens et al, 2002). Upon multiplication/division of the mature trophozoite, they will then return back into their previous developmental stage of schizont. Schizonts will continue to multiply within the erythrocyte leading to erythrocytic rupture and schizont release into the blood stream (Bray and Garnhan, 1982). These schizonts can then infect new erythrocytes leading to increased parasitemia and a more ill patient. This is an extremely important aspect of the parasitic nature of malaria. Without this endless erythrocytic cycle, the parasite would not be able to expand within the human host.

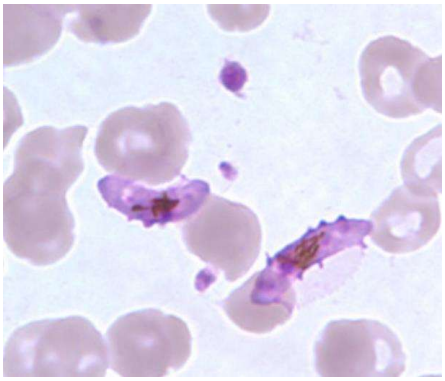

Figure 4: Gametocyte Presence in Erythrocytes ("CDC - DPDx - Malaria", 2020).

The second developmental path that the parasite could follow involves sexual maturation. Once an erythrocyte is infected with an immature trophozoite, it can develop into gametocytes (Josling and Llinas, 2015). These gametocytes will differentiate into two sexes. Both male (micro-) and female (macro-) gametocytes will be ingested by an *Anopheles* mosquito if the host is fed on again. Within the mosquito's stomach, the microgametocyte will fertilize the macrogametocyte, fusing to generate a zygote (Josling and Llinas, 2015). The zygotes will invade the midgut wall of the mosquito where they develop into oocysts. Once the oocysts mature, they will rupture and release sporozoites. These sporozoites will make their way to the salivary glands of the mosquito, where they can infect another human host upon the mosquito's next blood meal (Bray and Garnhan, 1982).

Pathogenesis and Disease Manifestation

Once within the human host *Plasmodium* parasites can reck havoc on the body. Malaria can result in flu-like symptoms including fever and chills. In more severe cases, patients can develop anemia and cerebral malaria. Cerebral malaria is the result of infected erythrocytes adhering to each other, uninfected erythrocytes, and the endothelium. This is believed to be a direct result of knob like protrusions on the erythrocyte's surface. These knob protrusions are developed on the host cell's membrane as the parasite matures from within and will bind to molecules such as intercellular adhesion molecule (ICAM-1) on uninfected erythrocytes (Rasti, Wahlgren and Chen, 2004). The adherence of the erythrocytes leads to blockage of blood vessels. If this occurs within the brain, brain swelling, or other traumas could result. Injury to the brain can result in seizures, stroke, or death (Mockenhaupt et al, 2004).

Typically, first signs of illness are detected when merozoites ruptures from erythrocytes. The free parasite brings about many immune responses, including an increased presence of cytokines. These include interferons, interleukins, and proinflammatory cytokines. Tumor necrosis factor (TNF)-α is one cytokine that is associated with severe malaria (Clark and Schofield, 2000). Initially the release of TNF-α is beneficial as high levels of the cytokine have antiparasitic effects. Because of its involvement in acute immune response, high levels during early malaria infection can inhibit parasite multiplication within the blood phase of its life cycle. However, prolonged elevation of TNF-α can be harmful. TNF-α has been correlated with severe malaria and cerebral malaria in African children (Kinra & Dutta, 2013). TNF-α is believed to upregulate ICAM-1 in uninfected murine erythrocytes, making infected erythrocyte adherence easier (Rudin et al, 1997). This theory is also supported by immunohistochemistry data on patient cerebral malaria patient serums. It was shown that an increase of TNF- α was accompanied by an increase in ICAM-1 (Armah et al, 2005). The correlation between the increased cell adhesion and blockage of cerebral capillaries brings about the belief that prolonged presence of TNF-α in severe malaria cases leads to complications involving brain damage, seizures, heart attack or stroke (Clark and Schofield, 2000).

In addition to erythrocyte adherence through ICAM-1 binding, red blood cell deformability is believed to lead to blood vessel blockage (Rasti, Wahlgren and Chen, 2004). Deformability is defined as the characteristic moldability of erythrocytes. It is what allows them to alter their shape when shifting through capillaries or blood vessels of smaller diameter. Erythrocyte deformability is significantly reduced in healthy, uninfected red blood cells when a patient is infected by a *Plasmodium* parasite. This can lead to blockage of capillaries in the heart or brain, causing serious complication. This physiological change of heathy erythrocyte is suspected to be the result of parasitic heme metabolites (Nuchsongsin et al, 2007). When a *Plasmodium* parasite matures from within an erythrocytic host, it will metabolize hemoglobin. This results in the harmful biproduct of β-hematin (heme), which is released into the blood stream upon infected erythrocyte rupture. Circulating heme has harmful oxidative properties (Nuchsongsin et al, 2007).

There is a negative correlation between the of β-hematin and erythrocyte deformability (Nuchsongsin et al, 2007). Heme released from erythrocytes during schizont rupture, increases the concentration of oxidative species in the blood stream. This is believed to lead to lipid peroxidation on healthy erythrocytic membranes. Lipid peroxidation in the cerebral spinal fluid can lead to complications such as stroke and brain injury as oxidative stress can affect nucleic acids, proteins, and other biological molecules. Additionally, lipid peroxidation is believed to cause hemolysis (rupture of erythrocytes) (Erel et al, 1997) or increased erythrocyte clearance (Griffiths et al, 2001). Since lipid peroxidation occurs on healthy cells, the patient will lose healthy erythrocytes in addition to the parasitized ones. This is one explanation behind the development of anemia in severe malaria patients. Anemia is one of the most common complications amongst malaria patients with roughly 43% of cases resulting in an anemia of some sort. Oxidative stress, erythrocyte deformability, schizont rupture, and immune mechanism are all believed to lead to an overall loss in erythrocytes. In addition to the loss of erythrocytes in serum, TNF and other cytokines may suppress erythrogenesis in bone marrow, leaving the patient unable to replenish erythrocyte supply.

Treatment of Malaria

Treatment of malaria has proven to be difficult as the *Plasmodium* parasite is physiologically different at its different stages of development. Because of this, pharmacological agents have been developed to target many physiological pathways, some of which are heme metabolism, electron transport, and protein translation. As mentioned before, trophozoite stage malaria parasites will metabolize hemoglobin from within erythrocytes for their own source of nutrients. This leads to a hypoxic environment and the release of toxic heme and ROS into the blood stream. Many antimalaria agents target the parasite's breakdown of hemoglobin with the intent of reducing the number of ROS in the patient and creating a toxic environment for the parasite. Hemoglobin is first broken down into ferriprotoporphyrin IX (Golan, Armstrong & Armstrong, 2017). This molecule is destructive to *Plasmodium* parasites. Therefore, the parasite will quickly polymerize the ferriprotoporphyrin IX into hemozoin, which is the metabolite released upon erythrocyte rupture. Therapeutic agents aim to block ferriprotoporphyrin IX's polymerization. Chloroquine, quinin, mefloquine, and artemisinin are some of the drugs that do this (Golan, Armstrong & Armstrong, 2017). Chloroquine works by entering the parasite's food vacuole, where heme metabolism is localized. Since the food vacuole is highly acidic, chloroquine is rapidly phosphorylated, turning it into its active form and preventing it from exiting the vacuole (Golan, Armstrong & Armstrong, 2017). In its active form it prevents the polymerization of ferriprotoporphyrin IX. Quinine, mefloquine and artemisinin all act with the similar pharmacologic actions (Golan, Armstrong & Armstrong, 2017).

Many anti-malaria therapeutics target the *Plasmodium* electron transport chain. Like many primitive eukaryotic cells, *Plasmodium* parasites have an early version of the mitochondrial electron transport chain, consisting of multiple cytochromes and ubiquinone (Golan, Armstrong & Armstrong, 2017). Primaquine is a drug, which when metabolized by the parasite, can disrupt normal function of ubiquinone. This drug has a unique use, as it clears hepatic stage parasites (Golan, Armstrong & Armstrong, 2017). Although symptoms arise when merozoites rupture from hepatocytes and enter the blood stream, anti-schizont drugs will clear any remain parasites from the liver and prevent a surge in symptoms after the patient overcomes a first round of symptoms. This is due to the dormant nature of some parasites and their ability to remain in the liver for extended periods of time, only to be released upon random into the blood stream (Golan, Armstrong & Armstrong, 2017).

In normal physiological conditions, the electron transport chain will consist of multiple proteins, which undergo reduction, and oxidation reactions in order to successfully transport electrons down the chain (Ke et al, 2011). This is almost identical in mitochondrial electron transport. Ubiquinone acts as a shuttle between two larger membrane bound cytochrome molecules. Atovaquone is a structural analogue of ubiquinone and therefore will inhibit this "shuttle service" (Golan, Armstrong & Armstrong, 2017). It will do so by inhibiting the interaction between reduced ubiquinone and the cytochrome which usually accepts its two electrons. This prevents the oxidation of ubiquinone and disrupts the electron transport chain, as the electrons are not passed on (Golan, Armstrong & Armstrong, 2017).

Plasmodium parasites rely on the electron transport chain for pyrimidine production (Golan, Armstrong & Armstrong, 2017). Both primaquine and atovaquone prevent the synthesis of pyrimidine and therefore also prevent *Plasmodium* DNA replication. In this sense drugs that target the electron transport chain target inhibit *Plasmodium* growth. When the electron transport chain is halted within schizont, *Plasmodium* will not be able to mature into merozoites and infect

the blood. When the electron transport chain is halted within trophozoite, *Plasmodium* will be unable to mature into erythrocytic schizonts and prolong/exacerbate the infection. Targeting both of these stages in the *Plasmodium* life cycle may be key to clearing a patient of malaria.

In addition to targeting the heme metabolic pathway and electron transport chain, anti-malarial therapeutics have also been designed to target translation of malarial proteins. These pharmacological drugs were first used to fight off bacterial infections, but since there is similarity between the ribosomal subunits of *Plasmodium* parasites and most bacterial, the drugs will effectively target translation in both. These pharmacological agents (doxycycline, tetracycline and clindamycin) bind to either the 30S or 50S subunits of *Plasmodium* ribosomes in order to prevent translation and kill the parasite (Golan, Armstrong & Armstrong, 2017).

Despite the success of these pharmacological agents against parasitic malaria infections, they are no longer as effective as they once were. Quinine was first used to treat malaria in the 1800s. Chloroquine was first developed in the 1930s. Primaquine was approved for use in 1952. These agents have been around for a long time. As decades went on *Plasmodium* parasites became resistant to many of these agents. Almost all of sub-Sahara Africa, which is severely endemic, is chloroquine resistant (Golan, Armstrong & Armstrong, 2017). Chloroquine resistance extends into southern Asia and northern South America. All of these areas are resistant to many other drugs. For instance, southern Asia is also resistant to mefloquine and artemisinin. Increased resistance of *Plasmodium* parasites called for the development of new drugs. Much of the PMI funding goes towards novel malaria research. Modern day scientists have developed drugs such as Salirasib, which was originally intended to be an anti-cancer agent. However, Salirasib has shown promising chemical properties which may have anti-malarial effects (Porta et al, 2019). Although it has promising preliminary data, there is still a lot of work to do before Salirasib becomes readily available for malaria treatment.

Vaccine Development

Of course, with the promise of new drugs comes the fear of increased resistance. This is why many modern scientists are focusing their efforts on creating vaccines for malaria. However, there has been little progress. The RTS,S vaccine, targeting a repeat sequence on the circumsporozoite protein is the only vaccine endorsed by the World Health Organization. However, those administered the vaccine do not hold high levels of antibodies for an extended period of time (Laurens, 2020). Additionally the RTS,S vaccine was only able to prevent severe malaria in 36% of patients (Laurens, 2020).

Due to little success, focus has shifted away from traditional vaccine development methods. A recent study by Raj, et al utilized the creation of a whole proteome cDNA phage library generated from sera of resistant 2-year-old patients to identify potential antigens. This led to the discovery of PfGARP. Polyclonal antibodies against this protein were present in resistant individuals, but not in non-resistant individuals (Raj et al, 2020). This protein was found to have a functional *Plasmodium falciparum* export element (PEXEL) motif which would allow for the protein's exportation to the erythrocytic membrane. In vitro analysis revealed that anti-sera against pfGARP was able to inhibit *Plasmodium* parasite growth in red blood cell culture by 94-99% (Raj, et al). This suggests that the protein has antigenic properties which would make it a suitable vaccine target in the future.

Despite the discovery of pfGARP, there is still a need for additional vaccine targets. The phage library generated by Raj et al was novel in that it started with the serum of resistant individuals, but it had one major limitation. *Plasmodium* parasites are eukaryotic, which means their proteins are assembled with the help of different chaperone proteins. However, the phage expression system is prokaryotic and lacks chaperone proteins to assist with the proper folding expressed proteins. This eliminates the possibility to analyze the secondary and tertiary epitopes present on these proteins. This is why this study aims to create a novel method of vaccine discovery which uses mammalian expression to aid in antigen discovery.

In addition to the inclusion of secondary and tertiary epitopes, this screen aims to include tandem repeats, B-cell epitopes and MHC II binding sites. This study utilized XSTREAM to analyze the *Plasmodium falciparum* genome for tandem repeats. Tandem repeats are a suggested characteristic of antigens. In a similar study, the *P. vivax* merozoite surface protein-9 (MSP-9) was analyzed for immunogenicity. A specific region of MSP-9, rich in tandem repeats of the following sequence EAAPENAEPVHENA, was identified as an immunogenic linear B cell epitope (Nunes Rodrigues-da-Silva et al, 2016). Additionally, it was found that PfGARP contained regions of tandem repeats (Raj et al, 2020), furthering the argument that tandem repeats may hold antigenic properties.

B-cell epitopes are a region of the antigen that can bind antibodies. Many B-cells are coated with membrane-bound surface antibodies, to which an antigen can bind. If the antigen binds a membrane-bound antibody, it acts as a receptor, activating the B cell and stimulating production of more antibodies specific to the bound antigen. Since B-cell binding elicits antibody production, the identification of B-cell binding epitopes is beneficial when antigen discovery efforts are made. The *P. vivax* MSP-9 region mentioned above also contained linear B cell epitopes which aided in activation of the immune system during in vivo studies (Nunes Rodrigues-da-Silva et al, 2016). This is why this study included B-cell epitopes in the screening process.

Although B-cell binding stimulates the immune system, an antigen with only B-cell binding epitopes may not be sufficient. Membrane-bound antibodies are present in memory B cells, which are created through helper T cell stimulation. This is why this study also aimed to identify MHC-II binding sites in potential antigens. MHC-II is a molecule which will bind a foreign substance, process it, and present it to CD4(+) T cells. When a CD4 binds to an antigen-MHC II complex the cell is activated and will differentiate into effector T cells, stimulating the initial immune response (Goldsby et al, 2003).

This study combined the unique targeting of the blood stage of *Plasmodium falciparum* with in silico antigen discovery techniques. Proteins, which combined regions of tandem repeats, B cell epitopes, and MHC II binding sites were identified. In addition, the proteins were analyzed for other characteristics of antigens. These included signal peptide domains, which would allow for secretion to the erythrocytic membrane, and hydrophilic domains, which ensures the region is expresses outside the membrane rather than within. The proteins were also analyzed for single nucleotide polymorphism to ensure that the region of the protein was conserved, and variability was minimal. Lastly, the proteins were analyzed for predicted antigenicity scores. Only those with high antigenicity scores were included. If the protein had all of the above, it was considered a potential antigen. After in silico screening, the antigens identified were expressed in a mammalian expression system to keep secondary and tertiary epitopes intact. Antigen expression was used to elicit polyclonal antibody production These polyclonal antibodies were used in in vitro analysis.

Chapter 2: Methods

In silico Antigen Identification and Down Selection of the *Plasmodium* Genome

The *Plasmodium falciparum* genome (all 14 chromosomes, mitochondrial DNA and apoplast DNA) was scanned for tandem repeats using XSTREAM. Genes without tandem repeats or with a percent error of 20% or greater were excluded. PlasmoDB was used for further down selection of the genome included identifying signal peptide, transmembrane domains, regions of hydrophilicity, regions of low complexity, alpha helix presence, single nucleotide polymorphisms, predicted PEXEL regions, and lastly, predicted function and localization. The genes are further analyzed for B cell & T cell epitopes using IEDB Bepipred Linear Epitope Prediction. MHC II binding sites using IEDB TepiTool (selection of predicted peptides identified by "7-allele method" as described by (Paul et al, 2015). Antigenicity was evaluated using Vaxijen software tool and a final list of gene fragments were selected.

Codon Optimization and DNA Vaccine Design

Following *in silico* antigen identification, 29 gene fragments were identified from 22 genes. These fragments were codon optimized for expression in mouse model using GenScript Codon Optimization software. They were then ordered from GeneWiz in a modified pUC57 plasmid with ampicillin resistance selection marker. The fragments were digested out of pUC57 using BamH1 and then ligated into the vaccine vector VR2001 using T4 ligase. VR2001 is a vaccine vector with a CMV promoter for higher levels of mammalian expression, perfect for the mouse model. VR2001 also contains a kanamycin resistant gene for selection and a signal peptide region (GCA GTC TTC GTT CCC AGC GGT ACC GGA TCC CTT) for protein secretion which can also be used to evaluate if the fragment has been inserted in the correct orientation to the CMV promoter.

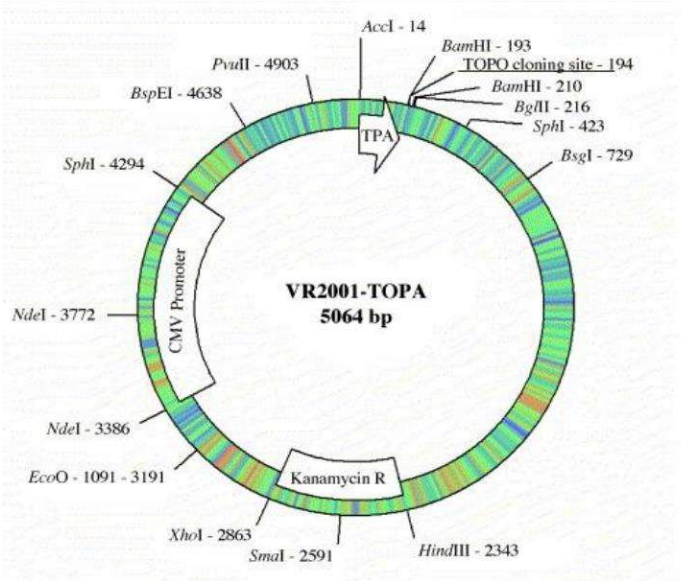

Figure 5: VR2001 Plasmid Map

Construction of DNA Vaccine

The modified pUC57 vectors with codon optimized *P. falciparum* fragments were obtained from Gene script as 4 µg of lyophilized powder. 20 µL of TE buffer was added to each tube to dissolve DNA to a concentration of 0.2 µg/uL. The pUC57 vectors were transformed into JM109 competent cells for DNA amplification and then extracted using Invitrogen Miniprep Kit. To remove the fragment from pUC57, 1000-3000 ng of the vector was digested with BamH1 restriction enzyme. Restriction digest was run on 1% agarose gel and fragment was extracted. The fragment was then ligated into BamH1-digested and Shrimp-Alkaline-Phosphatase-treated VR2001 using T4 ligase. After ligation the assembled vector was transformed into Top10 cloning cells. Insertion of vector was confirmed by growing colonies in LB + Kanamycin and extracting plasmid from the liquid culture. Liquid culture was stored in glycerol stock (700 µL of culture mixed with 300 µL of 50% glycerol) at -80 °C for future use. Extracted plasmid was then digested with BamH1 restriction digest and run on 1 % agarose gel to confirm fragment insertion. Fragment was gel extracted and set for sequencing. Sequencing was analyzed using Benchling and signal peptide was used to determine if orientation of fragment was correct. If the sequence was correct, 1 uL of glycerol stock was added to starter culture (LB + Kanamycin) and grown overnight. The starter culture was then added to 500 – 1000 mL of LB + Kanamycin and incubated overnight. The vaccination vector was then extracted at mega prep level and stored at -20 °C until mice immunization.

Mouse Inoculation and Serum Harvest

Female BALB/cJ mice of 6-8 weeks of age were ordered from Jackson laboratories and acclimatized in our animal facilities for 72 hours. Mice were divided into groups of 6. Each group was injected with one of the 29 pVR2001 vaccine construct. Four doses of the vaccine were delivered to each mouse subcutaneously. Dose one was delivered through tail and footpad injection. Doses two, three and four were delivered though tail injections. Three-week intervals separated the doses.

Three weeks after the final dose was administered, mice from all of the groups were sacrificed and blood was harvested. Blood was collected from ventricular cavity using a 3 ml syringe and 21-gauge needles. Blood was allowed to clot at 4 ◦C overnight. Blood was then centrifuged at 4°C and 4,000 rpm for 15 minutes and the supernatant (serum) was collected in laminar hood under sterile condition and stored in sterile 1.5 mL centrifuge tubes. Serum was stored at -20 ◦C until further use.

Parasite Culture Maintenance

P. falciparum strains (3D7, Dd2, and D10) were obtained from MR4. Two parasites from adult and children each were isolated from the field site and adapted in our collaborator lab at NIH. The parasites were cultured *in vitro* according to the methods of Trager and Jensen with minor modifications. Briefly, parasites were maintained in RPMI 1640 medium containing 25 mm HEPES, 5% human O+ erythrocytes, 0.5% Albumax II (Invitrogen) or 10% heat-inactivated

human AB+ serum, 24 mm sodium bicarbonate, and 10 μg/ml gentamycin at 37 °C with 5% CO2, 1% O2, and 94% N2. Parasites were synchronized using 5% sorbitol using published method (Raj et al science, 2014)

Growth Inhibition Assay

Growth inhibition assays (GIA) were carried out with anti-PfGARP mouse sera or controls as described (Raj et al Science 2014) with minor modifications. Briefly, sera were heat inactivated at 56°C for 30 min and pre-incubated with human RBC for 1 hour before use in GIA assays. GIA assays were carried out using W2, 3D7 and D10 strains of *P. falciparum*. Parasites were synchronized to the ring stage by treatment with 5% sorbitol for three successive replication cycles and cultured to the ring stage. Parasites at 0.3-0.4% parasitemia and 2% hematocrit were incubated with anti-sera at a final concentration of 10%, in a final volume of 100 μl in microtiter wells. Cultures were performed in triplicate with three replicates (comprising a total of 9 individual wells) prepared for each treatment condition. After 72 hr, blood films were prepared from each replicate, stained with Giemsa, a microscopist blinded to the treatment conditions enumerated RBCs infected with ring stage parasites, and the results from the three wells were averaged. The relationship between the treatment group and parasitemia outcome of the five replicates was analyzed by Mann-Whitney U test.

Western Blot

Plasmodium falciparum 3D7 blood culture was grown to approximately 5% parasitemia. Both mixed and synchronized culture was used. It was then collected in 1.5 mL centrifuge tubes and centrifuged a maximum speed for 5 minutes. Supernatant was removed and culture was stored at -20 ◦C until further use. To harvest parasite protein from culture, the culture was incubated on ice with 800 uL of 1X PBS and 20 uL of 3% saponin for 5 minutes. After incubation the culture was centrifuged at 10,000 rpm for 5 minutes and supernatant was removed. This was repeated until pellet was black in color, then 200 μL of Y-PER yeast was added.

15 μL of harvested 3D7 protein was boiled at 100 ◦C with 5 μL of SDS buffer. Protein was then loaded into SDS protein gel and ran at 250 V for approximately 20 minutes. Gel was then use for protein transfer onto nitrocellulose membrane by running at 100 V for one hour.

The nitrocellulose membrane was incubated in 2% BSA in 1X TBS-T for one hour, then washed with 1X TBS-T 3 times with 5-minute intervals between washes. Nitrocellulose membrane was then incubated in 10 uL of 2% BSA buffer with 30 uL of serum harvested from mice as a primary antibody. The membrane was incubated in primary antibody at 4 ◦C overnight. After incubation in primary antibody the nitrocellulose membrane was washed with 1X TBS-T 3 times with 5-minute intervals between washes. It was then incubated in the secondary antibody by adding 15 ul of anti-mouse secondary antibodies (Li-COR) to 15 mL of 1 X TBS-T (1:1000 dilution). It was then imaged using Li_CORsystem.

Chapter 3: Results

Identification of Fragments with Tandem Repeats

5,597 *Plasmodium falciparum* genes were analyzed, and it was found that 1,993 contained regions of tandem repeats. Genes with tandem repeats were then selected for the presence of a signal peptide sequence and transmembrane domains. This led to 158 genes with possible antigenicity. The 158 genes were then analyzed for hydrophilic domains, low complexity regions, presence of B cell epitopes and MCH II binding epitopes, fragments of low polymorphism, and lastly, a high antigenicity prediction score. The down selection led to the isolation of 29 fragments in 22 different genes which are believed to be possible antigens with vaccine potential (Supplement A).

Examples of down selection is shown below on one successful gene (PF3D7_1401200). Down selection for the eleven fragments successfully clones into the vaccination vector are in Supplement B.

- Protein Sequence
  MFPSYIRKFSFTLLLCIIALSCNNNTDIYYLTKYKNFPIVKSPHIRSLAESYKQYKIN
  SKYDELRTLGASSPQKRKPSKYDDIRCYDQPKQKQKKPSKYDDVRGFGEPAQKK
  KKTSKYDDLRRFGVPTQKKKMPSKYDYLRTLKEQNVNNKWKPTTNDDLKLLSD
  NYEKEKTEKYKLLKFIKKKDKENSERQKHGLPPDMSFKGLSSKKETEEYVSSDV
  GYTIKKGILKALKFTWRSISFFIKLIFFGLISLLFWTCRCISCLF

- Protein Analysis

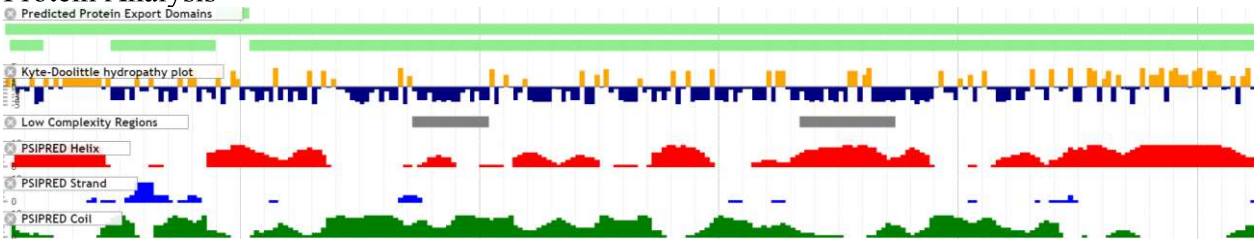

Figure 6: PF3D7_1401200 Protein Features and Properties (PlasmoDB)

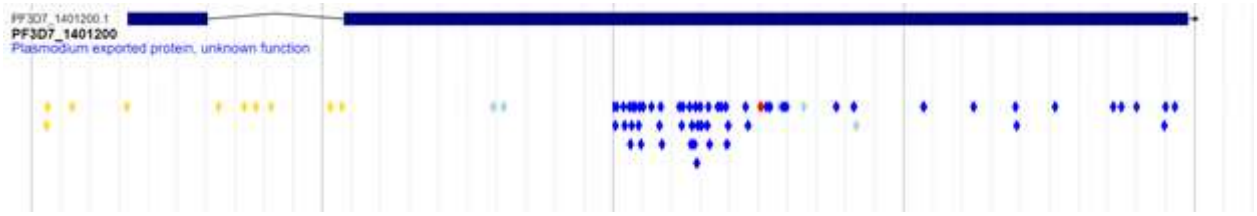

Figure 7: PF3D7_1401200 SNP Map (PlasmoDB)

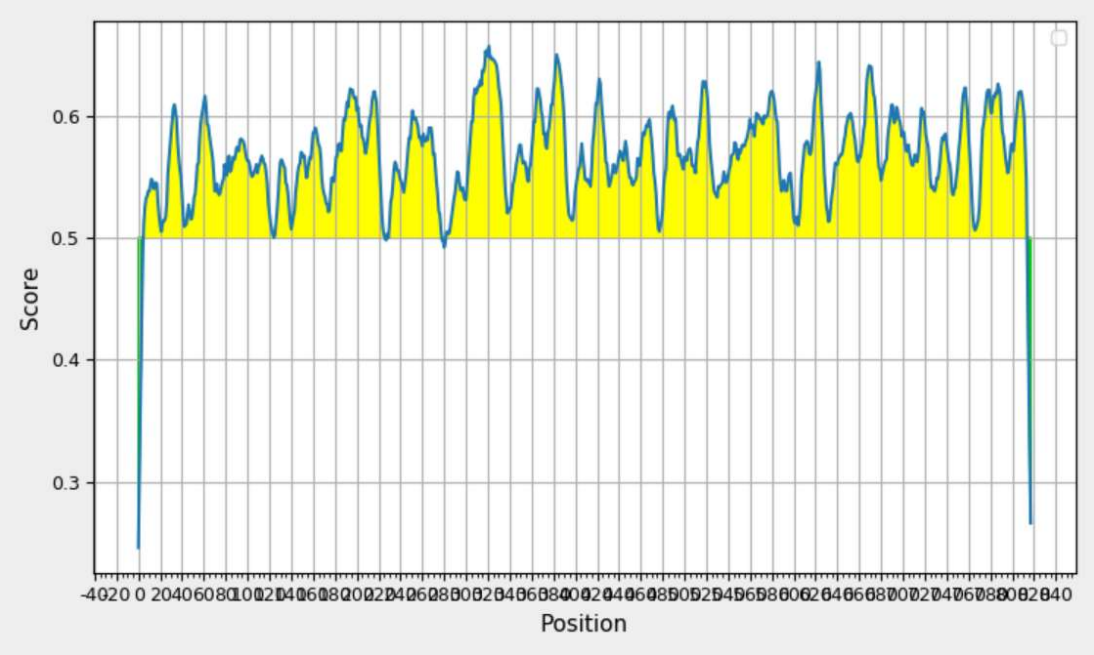

Figure 8: PF3D7_1401200 B-cell Epitope Prediction Map (Tepitool)

Seq #	Peptide start	Peptide end	Peptide	Median consensus percentile
1	236	250	RSISFFIKLIFFGLI	17.0
1	231	245	LKFTWRSISFFIKLI	14.0
1	226	240	GILKALKFTWRSISF	18.0
1	31	45	LTKYKNFPIVKSPHI	12.0
1	1	15	MFPSYIRKFSFTLLL	13.0
1	241	255	FIKLIFFGLISLLFW	12.0
1	36	50	NFPIVKSPHIRSLAE	6.0
1	26	40	TDIYYLTKYKNFPIV	14.0
1	221	235	YTIKKGILKALKFTW	16.0

Figure 9: PF3D7_1401200 MHC II Binding Epitope List (TepiTool)

- Analysis:
  PF3D7_1401200 contains multiple hydrophilic domains, areas of low complexity, predicted protein export domains, alpha helixes and minimal SNPs with only 10% of the population containing the minor allele. The fragment selected for PF3D7_1401200 also contains predicted liner B-cell epitopes throughout the fragment. There are nine MHC II binding sites predicted by TepiTool using the "7-allele method". Literature has also predicted this protein to be expressed during the erythrocytic cycle and to be exported to the RBC membrane.

Construction of DNA vaccine for mice immunization

The 29 fragments identified from *in silico* vaccine design techniques were then codon optimized for the mouse model and ordered in a modified pUC57 vector. Of the 29 fragments, 11 were successfully digested out of pUC57 and ligated into VR2001, a vaccination vector engineered for expression in mouse models. Insertion into VR2001 was confirmed by restriction digestion and sequencing. Sequencing results were also analyzed for correct orientation of fragment through location of signal peptide. After the DNA vaccines were constructed, mice were inoculated four times to elicit antibody production. Anti-sera were harvested two weeks after the fourth injection. These sera were used for in vitro and protein assays. Figure 10 represents an example of this process. In Figure 10A, PF3D7_0526700F1 is represented in well E at around 1000 base pairs. In Figure 10B, individual colonies transformed with PF3D7_0526700F1-VR2001 were digested and ran on a 1% agarose gel. Colony 8 is the only colony of the correct size. This colony was sent for sequencing and came back fully matched and in the correct orientation.

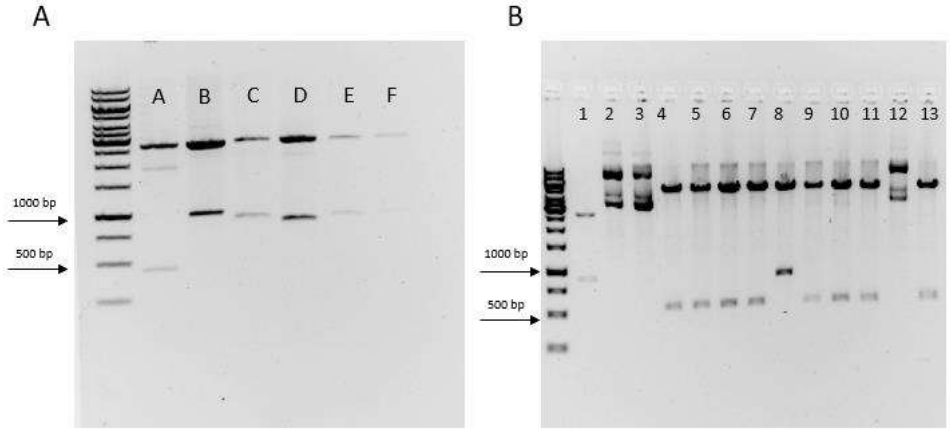

Figure 10: Restriction Digest with BamHI. A) Digest out of pUC57 (A-F denote specific gene fragments - A: PF3D7_1411800, B: PF3D7_0405900, C: PF3D7_0102500, D: PF3D7_0820300F1, E: PF3D7_0526700F1, F: PF3D7_0704300F2). B) Digest out of VR2001 (1-13 denote individual colonies of PF3D7_0526700F1).

Polyclonal Antibodies Produced Against the Selected Antigens Inhibit Parasite Growth

Once the fragments were successfully inserted into the vaccination vector, they were used to immunize mice. After immunization, serum was harvested from the mice and used for in vitro analysis. 3D7 culture was set up in a 96 well plate with 20% of the total volume being harvested serum. Parasite growth was determined using trophozoite percentages determined by microscopic evaluation for six of the sera (Figure 11) and by LDH activity for eleven of the sera (Figure 12). Based on microscopic trophozoite counts, one of the six sera (PF3D7_1401200) significantly inhibited parasite growth ($P<0.001$) (Figure 11). When analyzed by LDH activity, anti-PF3D7_14012200 sera maintained its significance (Figure 12). One anti-sera (anti-PF3D7_0102500) displayed significant inhibition of parasite growth when compared to another

anti-sera. However, it was not significant when compared to pre-immune sera. Anti-PF3D7_1401200 serum inhibited the growth of 3D7 parasites by 95-99%.

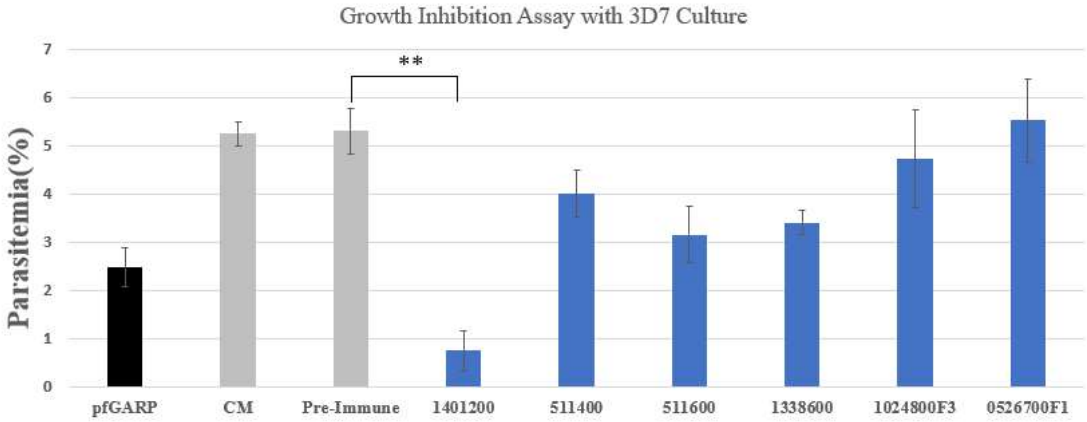

Figure 11: Growth Inhibition Assay (GIA) Analyzed by Parasitemia Counts; Polyclonal antibodies generated by codon optimized DNA vaccination constructs against selected gene fragments in mice significantly inhabits parasite growth against homologues parasite. Ring stage 3D7 parasites were cultured in the presence of corresponding vaccinated mouse sera at 1:10 dilution. Pre-immune and no serum complete media taken as negative control and polyclonal serum against malaria antigen PfGARP with significant growth inhibitory effect in GIA was taken as positive control. Bars represent the mean of 3 independent replicates. Error bars represent SEMs. P values were calculated by non-parametric Mann-Whitney U test are indicated (**=P <0.001).

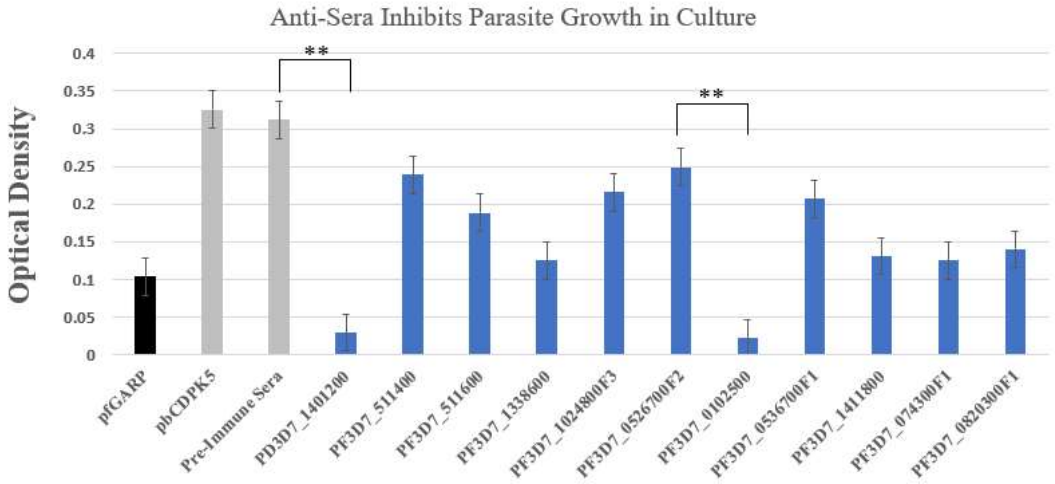

Figure 12: GIA Analyzed by LDH Activity; Polyclonal antibodies generated by codon optimized DNA vaccination constructs against selected gene fragments in mice significantly inhabits parasite growth against homologues parasite. Ring stage 3D7 parasites were cultured in the presence of corresponding vaccinated mouse sera at 1:10 dilution. Pre-immune and no serum complete media taken as negative control and polyclonal serum against malaria antigen PfGARP with significant growth inhibitory effect in GIA was taken as positive control. Bars represent the mean of 3 independent replicates. Error bars represent SEMs. P values were calculated by non-parametric Mann-Whitney U test are indicated (*=P <0.001).

Since Anti-PF3D7_1401200 serum inhibited 3D7 parasites greater than 90%, we wished to see if the antiserum could inhibit heterologous *P. falciparum* parasites. W2 is a *P. falciparum* parasite that displays drug resistance. Inhibiting the growth of this parasite would prove to be impactful in effected regions. 3D7 and W2 cultures were set up with 20% anti-PF3D7_1401200 serum. It was found that the inhibition displayed in 3D7 culture was also displayed in the W2 culture ($P<0.05$) (Figure 13). This suggests that the antigen is expressed by both parasites and that inhibition of heterologous strains is possible.

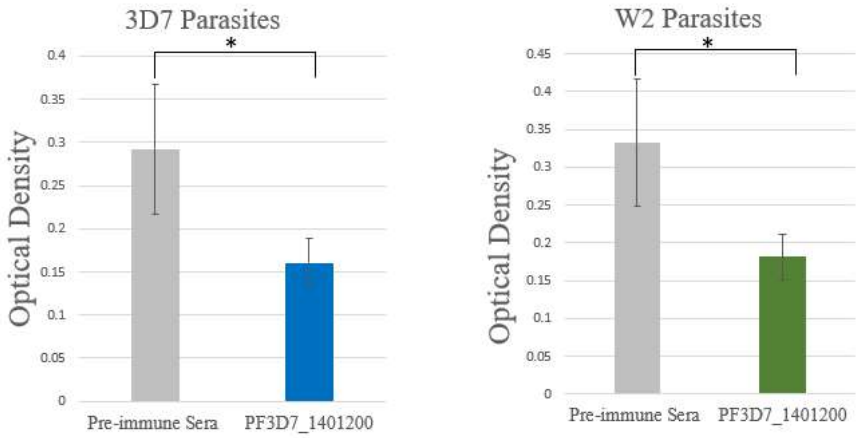

Figure 13: GIA with Heterologous Parasite; Polyclonal antibodies generated by codon optimized DNA vaccination constructs against PF3D7_1401200 in mice significantly inhabits parasite growth against heterologous parasite. Ring stage 3D7 and W2 parasites were cultured in the presence of PF3D7_1401200 vaccinated mouse sera at 1:10 dilution. Pre-immune taken as negative control. Bars represent the mean of 3 independent replicates. Error bars represent SEMs. P values were calculated by non-parametric Mann-Whitney U test are indicated (*=$P<0.05$).

Polyclonal antibodies generated against the selected fragments interact with the parasite protein in western blot

Since anti-PF3D7-1401200 serum was able to prevent parasite growth in 3D7 culture, we performed western blots to determine if this anti-serum can bind to protein of the appropriate size. This was also done for an additional anti-serum (anti-PF3D7_1338600) which displayed insignificant parasite inhibition to see if polyclonal antibodies which do not inhibit can still bind to their targeted antigens. PF3D7_1401200 and PF3D7_1338600 are 31 kDa and 112 kDa in size respectively. If the anti-sera interact with proteins in culture, bands of these specific sized should appear when 3D7 protein extracts are run on an SDS gel and transferred to nitrocellulose paper. Western blot analysis revealed that anti-PF3D7_1401200 serum recognized a protein slightly larger that 20 kDa (Figure 14A). Anti-PF3D7_1338600 serum recognized protein between 100 and 150 kDa (Figure 14B). Pre-immune serum did not interact with protein of these sizes. This suggests the ability of our immunization to generate specific polyclonal antibodies within mouse serum.

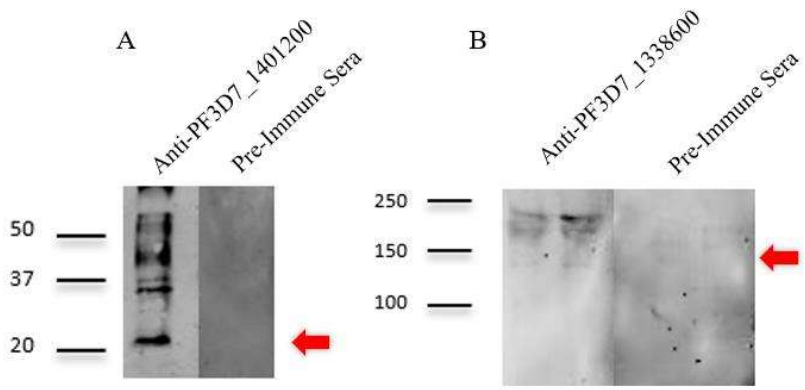

Figure 14: Western Blot with mixed stage 3D7 Culture.

In order to determine the stage of erythrocytic development the protein is expressed in, western blot analysis was performed on synchronized 3D7 culture. Protein extracts from ring, trophozoite and schizonts stages were collected. Western blot analysis revealed that anti-PF3D7_1401200 serum recognized protein of the appropriate size in schizont protein extract (Figure 15). However, protein of this size was not recognized in protein extracts from trophozoite and ring culture. Pre-immune serum failed to recognize protein of any size. Analysis revealed that anti-PF3D7-1338600 serum was able to recognize protein of an appropriate size in protein extract from ring culture (Figure 16). This protein was not recognized in schizont or trophozoite culture.

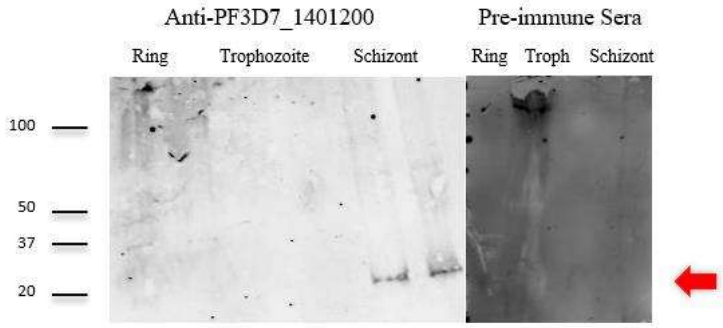

Figure 15: Western Blot with synchronized stages of 3D7 Culture.

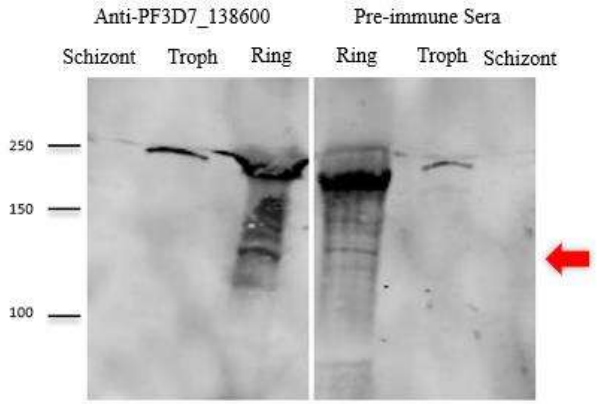

Figure 16: Western Blot with synchronized stages of 3D7 Culture.

Chapter 4: Discussion

Malaria is one of the deadliest endemic diseases of the modern era (Rogerson et al, 2007). With the development of parasite resistant to antimalarial drug and insecticide resistant mosquitoes the prevention and control of the disease becoming increasingly difficult (Golan, Armstrong & Armstrong, 2017). An approach to control malaria using insecticide treated mosquito net failed due to the lack of knowledge to proper use by people in the endemic regions of world (Sultana et al, 2017). The novel RTS,S vaccine is a promising step in the correct direction. However, with only preventing severe malaria in 29% of patients, the efficacy of the vaccine is still too low to significantly effect malarial transmission (Laurens, 2020).

This study combined two unique vaccine design strategies. Most vaccine research has targeted either the sporozoite or merozoite stage of the *Plasmodium* life cycle. This is when the parasite is exposed to the patients' blood antibodies. However, these stages are short (few seconds) making immune response difficult (Frevert, 2004). This study sought to target the erythrocytic cycle, when the parasite is embedded within red blood cells. This will extend the time in which antigens can be identified by the immune system.

Additionally, this study utilized reverse vaccinology and in silico vaccine design techniques to identify potential vaccine targets within the 3D7 *Plasmodium falciparum* genome. These reverse vaccinology efforts included tandem repeats, a known characteristic of antigenicity (Raj et al, 2020), and eukaryotic expression to keep secondary and tertiary epitopes intact.

This study suggests that reverse vaccinology is a powerful methodological tool, which should be used in vaccine design in the future. Our screen identified 29 potential fragments which could hold immunological effects. Eleven were successfully used to immunize mouse models. Of the 11, one was antigenic in nature and were able to generate polyclonal antibodies which inhibited 3D7 parasite growth in culture. Anti-PF3D7_1401200 was also able to inhibit the growth of W2, suggesting conservation of the antigen across heterologous *P. falciparum* strains. Additionally, we were able to show that anti-PF3D7_1401200 and anti-PF3D7_1338600 were able to recognize protein of the appropriate size in specific stages of the parasite. This suggests stage specific targeting.

Future experimentation should include identification of antigen localization using immunofluorescence and confocal microscopy. This screen included the identification of PEXEL

motifs, which should ensure that the antigen is excreted to the erythrocytic membrane. Confocal microscopy would verify this analysis by visualizing the exact location of the antigen in the infected red blood cell. This would ensure that antigens can be targeted by the immune system.

It was shown that anti-PF3D7_1401200 serum could inhibit W2. However, additional heterologous strains of the parasite should be investigated to ensure the efficacy of the vaccine and its ability to inhibit the growth of a large proportion of *P. falciparum* strains. Additionally, polymorphisms across these heterologous strains should be analyzed to ensure genetic conservation and the efficacy of the vaccine.

In addition to the experiments above, the remaining 18 fragments identified should be cloned into VR2001 to identify additional vaccine targets against *falciparum* malaria. The use of reverse vaccinology could be an instrumental tool for scientists to use in the future. It allows for direct and accurate identification of vaccine targets in a short amount of time and removes experimental guess work, allowing scientists to test the most probable candidates. This study supports the use of reverse vaccinology and has highlighted potential vaccine candidates which could be targeted to prevent the spread of malaria. In the future reverse vaccinology could be utilized to identify vaccine targets for other diseases.

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Appendix A: Selected Genes and Fragments

- **PF3D7_1401200 cDNA Sequence:**

ATGTTCCCTTCTTATATTAGAAAATTTTCATTTACACTTTTATTATGTATAATA
GCATTATCATGCAATAATAATACAGATATTTATTATCTGACAAAATATAAAA
ACTTCCCAATAGTAAAATCACCGCATATAAGATCATTAGCAGAAAGTTACAA
ACAATATAAAATAAATAGTAAGTATGATGAGCTTAGAACTTTAGGTGCGTCT
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AAGGTTTGGTGTACCCACACAAAAAAAGAAAATGCCTAGTAAGTATGATTAC
CTTAGAACATTAAAAGAACAAAACGTTAATAATAAATGGAAACCAACAACTA
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ACAAAAACATGGACTACCTCCAGATATGTCATTTAAGGGATTATCCTCAAAA
AAAGAAACAGAAGAATATGTATCGTCAGATGTAGGATATACAATAAAAAAA
GGAATATTAAAGGCTTTAAAATTTACTTGGAGATCTATCAGCTTTTTTATAAA
ACTTATATTCTTTGGATTAATATCACTCCTGTTTTGGACATGTAGATGTATATC
CTGTTTATTTTAA

Fragment Selected:
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GCATTATCATGCAATAATAATACAGATATTTATTATCTGACAAAATATAAAA
ACTTCCCAATAGTAAAATCACCGCATATAAGATCATTAGCAGAAAGTTACAA
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CTGTTTATTTTAA

- **PF3D7_1411800 cDNA Sequence:**
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TATTTTACATGA

Fragment Selected:
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- **PF3D7_1476500 cDNA Sequence:**
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TATGGACAATGCTGGTACACTATATCCAAACTCTAGATAA

Selected Fragment:
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TATGGACAATGCTGGTACACTATATCCAAACTCTAGATAA

- **PF3D7_0405900 cDNA Sequence:**
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Selected Fragment:
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C

- **PF3D7_0102500 cDNA Sequence:**
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Fragment Selected:
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- **PF3D7_0401900 cDNA Sequence:**
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TTTGTTAAGAGTGATTCTATGTTTAGTGGTTACTTTTTAGAAAGAGAATATAC

AAAGAATGCATTTACTGATGATGGTTATTTTAAAACAGGTGATGTTTTTCAAA
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GATTCTTTTTGTAAATTTTTGTGTTGCATATGGTGATGATTCTATGGATGGTCC
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AATTGACGAAATATTAAATCAATCTATTTATGTTGATTATGTAAAGGGGAAG
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ATATCAAAACCTGAACAAAGAAATGTTTTAAAATGTGAACAAAATAATGCTG
TATGTAATTTTCCGACAAATAAGAAAATAGTTAAAGAAACAAACTTAAAACA
ATTAAGAGTTCCAAATGTACCCGAATTACAAGTCAATGCATAA

Fragment Selected:
TTCAAGGAGAATACATAGAGACCGATATGTTAAATAATCTTTATTCTGTGATT
CTTTTTGTAAATTTTTGTGTTGCATATGGTGATGATTCTATGGATGGTCCTCTG
GCCATTATATCTGTTGATAAAAGTTTATTGTTCAAATGTTTAAAAGAAGATAA
TATGTTAGAAGGTACTGGAGTTAATGAAAAGAATTATTCAGAAAAATTAATT
GACGAAATATTAAATCAATCTATTTATGTTGATTATGTAAAGGGGAAGATGA
TGGAAGTATATAAGAAAACCAATTTAAACAGATATAATATTATTAATGACAT
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AGTGTAAAGAGTGGTGGCAAGAAGGAAGAAAAGGAAAATAAAAAGAACGA
TATAGGGGAAAATAAAGTAAAAAGTGAGGTAAAAAATGAGGTAAAAAATGA
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AAATGAGGTAAAAAGTGAGGTAAAAAGTGAGGTAAAAAGTGAGGTAAAAAG
TGAGGTAAAAAGTGAGGTAAAAAATGAGGTAAAAAGTGAGGTAAAAAGTGA
AGAAAAAAATGAAAAGAAAGCAAAAGAACGATTAACAAAATCAAAAATATC
AAAACCTGAACAAAGAGGGATCC

- **PF3D7_0318900 cDNA Sequence:**
  ATGCAAAAATTTATATCAGTCATATTCCTTTTTATAATATATCTTTCTTTTCAT
  TTGATCTATACAAAGAAAAATGATAATATAGATGATATACTAATTGTGGATG
  ATTCTAATGATGTAGATTACAACAATGATTTCTTAAATAAAGCGAAAAGTGG
  AGATGACTTAGGTGGAAATAACTATAACATGGGCTTCAACGATAATGTGTAT

TCTTGGATGGGAGAAAATGAGAAATTATTTTACATAAAGATTGGTGTTTTTTT
CGTAATTTTAATTATTGTTATACTTAACGGAGTTATAGGAAGAAAGACGAAT
AAAATGATTGCTCTTTATTGGCTTAGGTCTTGTAAAGAAATTTTTATTGAAAA
TTTTGCAAAGGTAGGAAATGATAAATCTTTTCTTTTAGAAAAATCTTATGATA
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ACAATAATAAAAAAAAAGAAGAGAATGTTAAAACAGAAAAACAAAAATATT
GCTATCTAAATTTAATTATTCCAAAAGATGTTGAAGAATTAAAAAAATTTATT
CATTTTTCTATATATATGATAGATGCTTGTTCTTCCATCGAATTACCAGAAAA
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GAAATATGAAGAAAAAAAACAAAAAAAAAGCTTAAGAAAAATGAAAAAAA
TTAAAATTATTAAAATGTAA

Fragment Selected:
TTAATTGTAATTATTATTTTGTGAATTTATATTTGAAAAGAAGACAATGTCTA
TGGAGATATTATATATTTAATTATTTTATTAATGAAAATGATACTATGCTTAT
CGTTATAAATTTTGAAAGATTGGATAAAAATGTTTTATGTGTTTATAAAAAAA
GTCAAAAAAACCAAGTTGAAAGGAAATTTCCAAACTTATATAAGTACACTAA
ATTAATTAAAAAAAAAGAATTAAAAGAAATGTATGAAATTAAAGGAGACTCT
TCAGAAGTTACCGACTTAGTCCTTAGCGGGAAAATTCTTAACTTTTTTAATAC
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ATGAAGACAAATCAAATGATCATAATAAAAAAAAAGATGAAAATATTAAAA
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ATGACAATAATAAAAATATTGATGACAATAATAAAAAAAAAGAAGAGAATG
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ATATATGGTTGAAAAAGATGATATCAAAAGAAAACAAGAATTGAGAGAATT
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AGAAAATGTCTGCTGAACAACAAAGGAAATATGAAGAAAAAAAACAAAAAA
AAAGCTTAAGAAAAATGAAAAAAATTAAAATTATTAAAATGGGGATCC

- **PF3D7_0511400 cDNA Sequence:**
  ATGAACATGATTAATATTGGATATTTGCTTTTAGTTTTATCTTTTTTGTTATTG
  GAAAAGACATTTTATGGTAGTGATACCTTTAGAGTATTTGAAGATTTTGATGT
  TAGAAGAAAAAACAAAATATGGTGTTGTAATTTAAATCATGAAAAAAGTTAT

TTTCATAACAAAACTATAAATACGTCATACAAAAATAGAGTTTTAAAAGAAA
GCATTTTACTTAATTTAGATTTAGATATGAAGAAATATAAGGATAATATCATA
ACCAGAAAGAAAACACCTGAAAATATTTATAAAGAAATATATGAAAATAATT
ATGAAATGAAATATGATGAAGATATTCCTAATAATATGAGTGAAGAAAAAAG
AGATGAAAAGGAAGTGATTGAACATCTAGAAATAGACGAAAAAAACGGAAA
ACAATACAAAAGTGATATAAATAAACCAGTTAGTTTATCACATCTTAAACAA
TATAAAAATATTTATGTTAATAACAATAATAAAATAAATAAAAAAAAAAGTA
TAGACAAACATTTACCTTCATATAATTTAGAAAGGAAAAATAATAAGTATCT
TAACTTTTTACTCGTAGATAATAGGAATGAATCTTATACCTTTATGGTGCCTA
TGAAATTTTATATAAATCATGAAATGTATAATATATCAGATGAGGAATATAA
TAAATTAATGGAAGATAATAGTGTAGATGTTTATTTAAATAATATATTGGTTG
AATACAAATATGAAAATTTCGAAATAAAAGAAGGAGAAGTTGATGGAGAAG
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AACACTGAATTTCAAGAAAATGATAACAATGATAGTGTAATTATGAAATATA
CCATTATTATTTCAGGATTAGTTCTCTTATTTTGTATCAGTTTTATTATTTATTA
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AATGCAACCATGGCAATAAATAGAGACAAAATTCAAGAGGAATTTATGTGA

Fragment Selected:
ACCAGAAAGAAAACACCTGAAAATATTTATAAAGAAATATATGAAAATAATT
ATGAAATGAAATATGATGAAGATATTCCTAATAATATGAGTGAAGAAAAAAG
AGATGAAAAGGAAGTGATTGAACATCTAGAAATAGACGAAAAAAACGGAAA
ACAATACAAAAGTGATATAAATAAACCAGTTAGTTTATCACATCTTAAACAA
TATAAAAATATTTATGTTAATAACAATAATAAAATAAATAAAAAAAAAAGTA
TAGACAAACATTTACCTTCATATAATTTAGAAAGGAAAAATAATAAGTATCT
TAACTTTTTACTCGTAGATAATAGGAATGAATCTTATACCTTTATGGTGCCTA
TGAAATTTTATATAAATCATGAAATGTATAATATATCAGATGAGGAATATAA
TAAATTAATGGAAGATAATAGTGTAGATGTTTATTTAAATAATATATTGGTTG
AATACAAATATGAAAATTTCGAAATAAAAGAAGGAGAAGTTGATGGAGAAG
TTGAAGGAGAAGGAGAAGTAGAAGGAGAAGTAGAAGGAGAAGTAAAAGGA
AAAGTGGTAGAAGGAATAGAAAATAACATGAATGAGGAAGAAAAATATAAT
AAAGATAATAAAGATAAGGAAAATCAAATAAATTCAAACGGACAAGATGAA
AACACTGAATTTCAAGAAAATGATAACAATGATAGTGTAATTATGAAATATA
CCATTATTATTTCAGGATTAGTTCTCTTATTTTGTATCAGTTTTATTATTTATTA
TTTTGATATTATACAAAAGGTAAAAATGAAGCTAAATAAAAAAAGAAAATCT
AATGCAACCATGGCAATAAATAGAGACAAAATTCAAGAGGAATTTATG

- **PF3D7_0511600 cDNA Sequence:**
ATGAAGAAAATATATTTCATTTTGTTAATCCTATTTCATTTAAATTTTATGGAA
TGTTTCAGAAAATATGATAAAAATAAGAATAAGATTTTAATAAGTCATTCAA
TAAATAATAATAATAATAGTATCAAAAATAATAACAATAATAATAATAGTAT
CAAAAATAATAACAATAATAATAATAGTATCAAAAATAATAACAATAATAAT
AATAGTTTTAGTGCTACTTCTTTTTCAAGTGAAAAAAATAAAAACAAAAGTTA
TACAAATGTGTTAAAAAAAAAGAATATATATATACGAGAGGAATCAAATAA

AACAACAAACATTAAAGAAGACGAAGAAAAAAATAAAAAAATAAATAATAA
TGATAAGGAAACGAATTATTCATTTTTATCATTGAAGTTTTTTCCATTCATTTT
AACTTCTTTACTCCATACAGGAATTAATCAAATACCACGTAATACTGAAATTG
AATTATATGAATTTGAAAAGAGTCCGATGATAAGACATATGTTAGTAGCAGA
AGAGAGGAAAAATGCATATACCTATATGTTTTTTATTGTTATATCTTTTGTTGT
TGTTGTACTTATAGCTCTTTTTATTTTTAAATTTTTTTTCAATCTTTAA

Fragment Selected:
GATAAAAATAAGAATAAGATTTTAATAAGTCATTCAATAAATAATAATAATA
ATAGTATCAAAAATAATAACAATAATAATAATAGTATCAAAAATAATAACAA
TAATAATAATAGTATCAAAAATAATAACAATAATAATAATAGTTTTAGTGCT
ACTTCTTTTTCAAGTGAAAAAAATAAAAACAAAAGTTATACAAATGTGTTAA
AAAAAAAGAATATATATATACGAGAGGAATCAAATAAAACAACAAACATTA
AAGAAGACGAAGAAAAAAATAAAAAAATAAATAATAATGATAAGGAAACG
AATTATTCATTTTTATCATTGAAGTTTTTTCCATTCATTTTAACTTCTTTACTCC
ATACAGGAATTAATCAAATACCACGTAATACTGAAATTGAATTATATGAATT
TGAAAAGAGTCCGATGATAAGACATATGTTAGTAGCAGAAGAGAGGAAAAA
TGCA

- **PF3D7_1338600 cDNA Sequence:**
ATGTTTTTTTTTATGTACTCAAAAGAGAAAAAAAAATATAAGAATGTATTGTT
GATTTTTTTTTTATATATAATTTTTTTTGTCTCTAAATCTTATAGTGGAAAAAC
ACTAGCATGTAATTATAGCTTAATTCCTTTTCACATTAAAACAAAAGAAAGGT
TGCCTTTGAATTTTATTAAAAATGATTTGTATGAGATAAAGAGGAAGCAAAG
AAGAAAGGAAATATATTCATCTAATATAACAAAAACGTTTGAAAATACGATA
CCTGAAGAGTATATTAAATATAACATTCCCGAAGAGCCAATCTATCCGTATAT
ATCAGTACCTAACAATCTTTACACAAAAACATATAATGAGAATAATAACGAT
AATGTGAGCGATAAACAAGAGAAGGATAAAAATTGTCCTAAATTAACACATG
GTATAAAGAAAATGTCTTATGAAGAATATAAAAAATGGAATGAAGAGAAAA
TGAAAGAGAGCAAAGATTTACCTGAACCAACGATAGATGATTATATTGAAGA
TGTGGAATATGAGAAATATATACACCCTGCTTTAATTAAAAATGTGGATGTA
AATAATCCAAGATATTCACTTTATTTTTTAAAAGATAAAATAAAAAATGATAC
GGATCAATGTGCTACGTTAAATTGTTTTGATAAAAATGATTTAGAAGATGATC
TTACAGAATATGATGCTGCATTTAATGGTATAGGCCCATGGCCATCGACAGA
AGAATTAATAAAACATCAAAATAATATAGAATATGATAAAACAGATATGGA
AATGGAATATAATATAACATATGATAAAACAGGTTATTATCAATATAAAGAA
AAATTAATCAACGAACAAATGAAAGGCATAATAAACAAGAAAGATATTAAC
AAAAATGTTTCAGAAACATTACATAAGCGTGCTACTACTATACAAAAGGATC
CAAATAATGATAAAGAAGAAAATAATAAAAATTGTATGTATAAAACAGAAA
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TTATTACATAAGGAAAAAAATATCACTACAGATGATATTAGAAAATTATACT
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GCAGGTAGCCAAATGTTTATGGATATGAAAGATGCATATGATAATAAAGAAA

ATTTAACCTCATGGCCAAGTGATCATATGAACGACAAAATGAACAATATAAG
TGATATTCAAATTCCGAGTGTTAAGGTTGGTCGTTTAAATGAATTAACCAAAT
TGTATGGAAATAATCCATCATCAGACGATATTATAGATGATATGAGAAATAA
TATAAATAATAAGTCCATAAATAATATGATACATAATATAAGTGATCACATG
AAGAACGATCCAACATTTGGACAAGAAATTAATTTAAAAGAGTCACAAAATA
AATACACGAATTTTCGATTATTAGAAGATAATGTTTTATATACTAGAGCCAAA
TCTCCAATAAACGAAATATTATATGAATGGGATGATCCATTAAATTGTTTATG
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TTTAAAGAATTAAGAAGACCATCTAATTTAGATCTATATGATGTTACATGGTA
TGCTGGGAAAATCGATATCTTCGTTAAAGTAGAAGAAGGAAAAAATTATAAA
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AAGTACTTGAAATTGATGATGAAATAGTTATTTTGCCATTCTTTGAATTAGTT
GTATCGTCTTTACCTAAAAAAAATATATTAATATGTAGAAGAGACTGGAATA
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GTCGAAGGAATATTACTAGGATCCCCAAGTGTTTTCCATGTAATTATTAATCT
TAATAATCAAAAAATATTAAACCTTGTAATAAATAACATTGACAAAATTATTT
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AAGTCAAAATGAAAATGCAAACATAAATGAACAAAATCGTAAAAACATAAA
TGAAAACGCAAATTTAGAAAATGATATTAAAAAAATTGATGACCAAGATATA
TATGATCAAGGAGATAATACATTTGATAATAAATATGATAAGCAAAAAACGA
AAAATGAATTTAAAGATATCGAATTTGATGAATTAGACAAATTAAGAAATGC
TAACAAAACATCCAAGGACAAACGGGTTAATGTAATGAAAAATATTAATAAT
ATAGATCAGGTCCAGGATGACATAACTAATGTTATAAATGAAGAAGATGATC
AAGATGAAGAAGAGGCAGAAGATAATGACGAATATGATGATGACGATATGG
ATAATGATGTGGATAGTGACGTGGATAATGATGTGGATAATGATGTGGATAA
TGATGTGGATAATGATGTGGATAATGACATTGATAGTGACATTGATAGTGAC
ATTGATAGTGACATGGATAGTGACATGGATAGTGACATGGATAGTGACATGG
ATAGTGACATGGATAACGATATGGATAACGATATTGATTCGTATGACAATGA
TTATAACGATTATTCATCAGGTGAATAA

Fragment Selected:
GAAGAAGGAAAAAATTATAAAATTACACTCTTCGATTTAAAACAACTCGTCA
AAAAAATTGCTGAAAGATTAAAAGTACTTGAAATTGATGATGAAATAGTTAT
TTTGCCATTCTTTGAATTAGTTGTATCGTCTTTACCTAAAAAAAATATATTAAT
ATGTAGAAGAGACTGGAATAATAATATAGGTAAAGAAGTTGTTGTCTTTTTT
AAAGATAACATCCTTCAACCTGTCGAAGGAATATTACTAGGATCCCCAAGTG
TTTTCCATGTAATTATTAATCTTAATAATCAAAAAATATTAAACCTTGTAATA
AATAACATTGACAAAATTATTTTGAAAAATACGGAGGATGAGTTAAAAGATA
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AATTAGACAAATTAAGAAATGCTAACAAAACATCCAAGGACAAACGGGTTA
ATGTAATGAAAAATATTAATAATATAGATCAGGTCCAGGATGACATAACTAA
TGTTATAAATGAAGAAGATGATCAAGATGAAGAAGAGGCAGAAGATAATGA
CGAATATGATGATGACGATATGGATAATGATGTGGAT

- **PF3D7_1301400 cDNA Sequence:**
  ATGTTTTCTTCTACAACAAATATATTTTTTTCATTAGTATTTCTAGTTCTATTAT
  TATTATTATCAATTAAAACTAAAAATCATCTGAAATTCAAGGAAGAATATAA
  ATGGATTATATTCGAAGCATTTTAGATTGTTAGCAGAACCATCTTCACATGGC
  TCTTCAAAAAAGACCATGAAAGAAAATGAAAACGAAGAAGAAGATGAAGAA
  GTAAATGAAGATCAAAATGAAGATCAAAATGATGATGAAAATGAAGATCAA
  AATGATGATGAAAATGAAGATCAAAATGAAGATGAATATGAAGAAGAAGAT
  GATGATGAAAAAGAAGATGAAGAAGAAGATGAGGATGAAGAAAACGA
  AGATGAAGAAGATAATGATAAAGAAGACGAAGATGATGAAGATAATGATGA
  TGAAGAAGTAAATGAAGACGAAGAAAATGAGGAACAATATTGTGAATTAAT
  ACCTATTCTACCAAATAGCTCGGAAGATACATTGAAAAAATCAAAAAATATG
  AATATACCAAAACATAATGCTAATAGCGAATATTTATTAGATAAAAATTTAT
  ATGATCTAAAGACATTTAAGAATTTAAATGCTGCAAAAAAAAATTACGCAGA
  TAGTATAATAGACAATATGGATCTTACGGAAAACGTGAAAAATATATTTAAA
  GAATTTTTATATTATTATATAAATAAAAAAGACCCCAATTATCAACTTAAATT
  ATATAAGCAAATTGAGCCTGATATAGAAAAATACAAAAAAAACCACGTAATT
  TGTAGTATTATAGATTTTAGAAATCTAAATAGTGACCTTCAATATTTAAGGAA
  CCCTAAAGGAGACTTTCATGTACTAAGTAATGAAGAATATGAAAACAGAGAA
  AAAAAAAAGAAAGAAAAACAACAAAAAAAAAAAAGATAAATTACAAAAAAA
  AATAAAGAAGGAACAAGATAAAATTAAAAAAGAACGAATAAAAGAATTGGA
  AAAACAAGAAAAAATGAAATACGAAAAACAAGAACAAGAAAAAGTGTATTT
  GGAAAAAAAAGAACTACAGGAAAAACATGAACAAAGTCAAAAGCAAAAAG
  AAAAGGAAATAAAGGATAGAAGAAATAAGCTTCTTAGCTTAAGACACTAA
  Fragment Selected:
  AGCAGAACCATCTTCACATGGCTCTTCAAAAAAGACCATGAAAGAAAATGAA
  AACGAAGAAGAAGATGAAGAAGTAAATGAAGATCAAAATGAAGATCAAAAT
  GATGATGAAAATGAAGATCAAAATGATGATGAAAATGAAGATCAAAATGAA
  GATGAATATGAAGAAGAAGATGATGATGAAAAAGAAGATGAAGAAGAAGA
  AGATGAGGATGAAGAAAACGAAGATGAAGAAGATAATGATAAAGAAGACG
  AAGATGATGAAGATAATGATGATGAAGAAGTAAATGAAGACGAAGAAAATG
  AGGAACAATATTGTGAATTAATACCTATTCTACCAAATAGCTCGGAAGATAC
  ATTGAAAAAATCAAAAAATATGAATATACCAAAACATAATGCTAATAGCGAA
  TATTTATTAGATAAAAATTTATATGATCTAAAGACATTTAAGAATTTAAATGC
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  AACGTGAAAAATATATTTAAAGAATTTTTATATTATTATATAAATAAAAAAG
  ACCCCAATTATCAACTTAAATTATATAAGCAAATTGAGCCTGATATAGAAAA
  ATACAAAAAAAAACCACGTAATTTGTAGTATTATAGATTTTAGAAATCTAAAT
  AGTGACCTTCAATATTTAAGGAACCCTAAAGGAGACTTTCATGTACTAAGTA
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  AAAAAAGATAAATTACAAAAAAAAATAAAGAAGGAACAAGATAAAATTAAA
  AAAGAACGAATAAAAGAATTGGAAAAACAAGAAAAAATGAAATACGAAAA
  ACAAGAACAAGAAAAAGTGTATTTGGAAAAAAAAGAACTACAGGAAAAACA
  TGAACAAAGTCAAAAGCAAAAAGAAAAGGAAATAAAGGGGATCC

- **PF3D7_1102300 cDNA Sequence:**
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  TCTTGGGACATTATCTGTCGTGTCATTGTATTTATATATACCTAATGAGAATA
  TTAATTTAAGTTTTCATAAATATGATTCATATATAAGAATATTATGCGAGAAA
  CATCCAGATGATAATCAATTTTCGGGAAGTCTTTGTAGGTCGGATTGTAATAG
  TAATTACAATAAAGATATAGAAAGAGAAAGTGATAAAAAGAAAAATATAAT
  ATTGGAAAATATGGGACAAAATGGAATGAATAAGACTAAAGAGCATCATAT
  GAATTTAACGACACCTTTAGTTAAATATGAAGAAATACAAAACCAAGATAGA
  GAAAAAGATGATCAAAACGAACGTCAGAAAAGGGAAAGGAAAGAAATGGA
  GGAAAGAGAAGAACGTGAAAGGAACGAAAGGAAGAAAAGAGAAGAACATG
  AAAGGAACGAAAGGGAAAAACAGGAAAATAAAGAAAGGAAAGAAAGGGAG
  AAACAAGAAAGGAAGGAAAGAGAAGAACGTGAAAATAAAGAAAGGAAAGA
  AAGGGAGAAACAAGAAAGGAAGGAAAGAGAAGAACGTGAAAAAAAGGAAA
  GGAAAGAAAGAGAAGAACGTGAAAAGAAAGAAAGGAAGAAAAGAGAAGAA
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  ACAAAAACGTGACAAAGAAGAAAGGAAAGAAAGGGAAAGAAGGGAAAGAC
  AACTAAAAAAGGAAAGAGAAAAACAAGAAAAAAGAGAAAGTAAACAACGT
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  AGAAAGGAAACAGCGTGATAAACGAGAAAAGAAAGAAAAGGAGGAGCGTG
  AGAAACGAGAAAAGAAAGAAAAGGAGGAGCGTGAGAAACGAGAAAAGAAA
  GAAAAGGAGGAGCGTGAGAAACGAGAAAAGAAAGAAAAGGAGGAGCGTGA
  GAAACGAGAAAAAAAAGAAAAGGAGGAGCGTGAGAAACGAGAAAAGAAAG
  AAAAGGAGGAGCGTGAGAAACGAGAAAAAAAAGAAAAGGAGGAGCGTGAG
  AAACGAGAAAAAAAAGAAAAGGAGGAGCGTGATAAACGAGAAAAGAAAGA
  AAAGGAGGAGCGTGAGAAACAAGAAAAAAAAGAAAAGGAGGAGCGTGAGA
  AACAAGAAAAAATGAAAATGGAAAATGTCTAA

  Fragment Selected:

  TTAGTTAAATATGAAGAAATACAAAACCAAGATAGAGAAAAAGATGATCAA
  AACGAACGTCAGAAAAGGGAAAGGAAAGAAATGGAGGAAAGAGAAGAACG
  TGAAAGGAACGAAAGGAAGAAAAGAGAAGAACATGAAAGGAACGAAAGGG
  AAAAACAGGAAAATAAAGAAAGGAAAGAAAGGGAGAAACAAGAAAGGAAG
  GAAAGAGAAGAACGTGAAAATAAAGAAAGGAAAGAAAGGGAGAAACAAGA
  AAGGAAGGAAAGAGAAGAACGTGAAAAAAAGGAAAGGAAAGAAAGAGAAG
  AACGTGAAAAGAAAGAAAGGAAGAAAAGAGAAGAACGTGAAAAAAAAGAA
  TGCGAACAAAGGGAAAAACGATTAAAAAAGGAAAAACAAAAACGTGACAA
  AGAAGAAAGGAAAGAAAGGGAAAGAAGGGAAAGACAACTAAAAAAGGAAA
  GAGAAAAACAAGAAAAAAGAGAAAGTAAACAACGTGAAAAACAAGAAAAG
  CAAGAAAGACAAAAACGTGAAAAAGAGGAAAGGAAAGAAAGGAAACAGCG
  TGATAAACGAGAAAAGAAAGAAAAGGAGGAGCGTGAGAAACGAGAAAAGA
  AAGAAAAGGAGGAGCGTGAGAAACGAGAAAAGAAAGAAAAGGAGGAGCGT
  GAGAAACGAGAAAAGAAAGAAAAGGAGGAGCGTGAGAAACGAGAAAAAAA
  AGAAAAGGAGGAGCGTGAGAAACGAGAAAAGAAAGAAAAGGAGGAGCGTG
  AGAAACGAGAAAAAAAAGAAAAGGAGGAGCGTGAGAAACGAGAAAAAAAA
  GAAAAGGAGGAGCGTGATAAACGAGAAAAGAAAGAAAAGGAGGAGCGTGA

GAAACAAGAAAAAAAAGAAAAGGAGGAGCGTGAGAAACAAGAAAAAATGA
AAATGGAAAATGTCGGGATCC

- **PF3D7_1201400 cDNA Sequence:**
ATGGAATTATTTGTATTAGTACGGATAGTTATATTCATTACTTTTTTCTATATA
TTACTAAATATTTTACATGACAATCTTTTTATGATAAAATTAGTGAATAACTA
TTCTTATTCTTATTTGTGGAATATTGTTAAATTAAAAAATAGAAAAATTCTGA
GCGAATTATCAGACGTACAATTAGAGGACAATGATATAGAAGATTTTATTGT
TAATAATAATGTATTATATTCGAATGATTTTTTAAATATAATTGATCCTATACT
TTTTGAAAATTACGACAATATAAATTTGGATGAATATATTCAAAATTTTGATA
ATATAAAGAAAGAAAGTTCTTCAGTTAGAAATTTTTCAATAGATAAATGCGA
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Fragment Selected:
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AATAATGACGAAAGTAATGAAGTGAATAATGAAGAGAGTAATGAAGTGAAT
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TGATGAATCTAGTGAGGAAGAAAGTGATGAAGAAATGCATGATTATGGGATC
C

- **PF3D7_1032000 cDNA Sequence:**

ATGATTGTACTCTCAAGTTATAAAAAATGCTTAGTTTTCTCATATTATATTCTT
CTTTTATGGGTCCTTAAATATTATATGTTAACTTGCCAAGCTATCAATTTTAAA
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GTGATGCTTTAAAACTAAAAAATTGTTTAATTTGTGATGATAAAAGAACTTTT
CCAGATTTAGGAGAAAATCAATATATATCTACAGATCTCCTAAATTTTGATAT
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GATAATTTTGATGGATATTCTTATAAAAAAATTTTTAAATGTGATTATTGTGA
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AGAATATTTTTTAGTGCCAATCATAAAGGAAAAAACAATAAGGTCCGTTCAT
TATGAAGACAAAAAAATATACCTAGATATAAGTACCATTTTTTTGATCGACG
ATAATAAATGA

Fragment Selected:
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GGGATAAGGAAAATACGATTAAATGAAAAAAAAAAAGATAATATATATGAT
CATAACCATCAATCTCAACATAGTTTAACAAATAATAATATGCCGGAGTATCT
TGAAAAAGAAGAATCTGAAGAAAACACTGCGAAACATAAGAAAGAAGAAGA
AATAAATCAAGGAAACAGACAATTTTTGACAGAGACTAAATATTATAAGCGA
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GTAGTGATGGTAATAGTAGTGATGGTAGTAGTAGTAGTAGTAATTATAA
GAATACCCAATCGTATAGTAAACATACCGAATTTATAAATAACTATAATGGG
ATCC

- **PF3D7_1024800 cDNA Sequence:**

ATGCAAATCCCAACAAAATTTTTAGTTTTTTTTAATTTGGTTATATTATTTGTA
TTTTTAAATTTTGAGCGTGTAGAGTTAAAGTTAAACAATATAATATATGACAA
GAATAATTTTAGTAACAATAATTATAATTATTATGTAAATAATAATAATGATG
AAAATAACGAAGGAAAGAATTCTGCCGATAATATAGACATAATAAAGAAGG
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AATCTCCATAACGAAGAAAAGGATGATGATGATGATAATAATAATATCG
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Fragments Selected:

Fragment 1:

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Fragment 2:

TGATGAAGAAGATAATATGTCGAATGAAAATAATACTACCTATGCTTACCTA
AATGATTACCATAGTGATAGTACCCATGTTCCCGATGAAGATAATGAAGCAC
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GAAGACGATGAGGAAGAAAAAGAAGAAGAGACAACTTCACAACCAAAAGAT
GTTGATGATAATGGACCAAATGATCCATTTTACGATGGAACATTCAACGTTTT
AAATAAAGAAAATGTACAAACAACTTCACAAGGGATCC

Fragment 3

CAAGGTTCAAGTGGTCAAACACAAGAATATATAAAAGACGTAGAAAATAAT
GTAGAACGAAATAATGAAAACAATATTAAAAAAAAGAATATTGATAATAAT
GATAATAATGATAATAATGACGACGACGATGATGATGATGATGATGATA
GTACAAATAAAAATGGAAATTTATTAAAAGGTACAATATCGGATAATCAAGT
AACAAGCAATTTTAGGGAGAAATTATTTATATTTATAATACTTTGTTTAATAT
CTGTATGCCTTGCTTTATTAGTAAGTATTGCTATAAAGGTATAT

- **PF3D8_1028700 cDNA Sequence:**
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  TGACGTAAAAGGTATATCAACACATGATACATGCGATGAATGGTCAGAATGG
  TCTGCATGTACTCATGGAATCAGTACCAGGAAATGTTTAAGTGATTCTTCTAT
  TAAGGATGAGACACTTGTATGTACAAAATGTGATAAATGGGGAGAATGGTCA
  GAATGTAAAGATGGGAGAATGCATAGAAAAGTTTTGAATTGTCCTTTTATTA
  AAGAAGAACAAGAATGTGATGTAAATAATGAAATGGCGGAGGACACACATA
  TGAATAATAGCTATATATATTTCAACGCAGATGATGGTGATAATGAATATGA
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  GATGATAAAAATGATGATGATAAAAATGATGATGATAAAAATGATGATGATA
  AAAATGATGATGATAAAAATGATGATGAGGAAAATTATAATGATACTGAAG
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  ACAAATATACATCCAGTGAATTTTATACAAGAAAAATATACAAGAAATAATA
  AATATAGATCTGATAATTTTTCTAAAATATTAAATAACATGAATCATATAAAT
  AATAATAATTATAATAGTAGAAGTAGTAGTACTTCTTCGAAAAATGCTAGAG
  GTTATAGAGGAGGAAGCAGTAATATGTATCCACATGTACCAAATTACACGAG
  TTCTTCTGTACATAATAGTACAAATAATGAAAGAAAAAGTGATGAAGACTTG
  GATAATATAGAGGGTGATAATATAACTAAAGAAGAAAGGATTGTTCCAATAA
  ATAACAAAAATTATGATAATCATGATGAACATAGTAATATACACGAGCATGA
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  TATTGCTAGTGGTGTAGCTACTCTTGTACTCTTGGGAGGAAGTATAACTTTCT
  ATTTCTTACGTAAAGAAAAAACAGAAAAAGTTGTACAAGAAGAAACAAAAG
  AGGAAAACTTTGAAGTCATGTTTAATGATGATGCTCTCAAGGGAAAGGATAA
  CAAAGCTATGGATGAAGAAGAATTCTGGGCACTCGAATGA

  Fragment Selected:
  TGGTGATAATGAATATGAAGATCATGATGATAAAAATGATGATGATAAAAAT
  TATGATAATGAAAATGATGATGATAAAAATGATGATGATAAAAATGATGATG
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  ATTATAATGATACTGAAGAAAAGGTAAAAAATAATGATATACATAATTCTAG
  TGCTAATAGTAATAATGAGGTTACTAATTTCATACAGATAAAAGATAAAATT
  ATGATTAAACATAAAACAACAAATATACATCCAGTGAATTTTATACAAGAAA
  AATATACAAGAAATAATAAATATAGATCTGATAATTTTTCTAAAATATTAGG
  GATCC

- **PF3D7_0820300 cDNA Sequence:**
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  TAATAATAAAAATAATGATAATATTATGAATAATGATAATATTATGAATAAT
  AATAATTATATTGATGATGGATATATGAATAGCGATATAAGTAGTGATACGT
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  ATTCTTAATAATGAGAGTAATAAAAGAATAGAAATTAAAAGTATTAATCTAA
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  TAGTATTATAGATCCAAGAAGAACTTTTTATAAAATCCTTGAAGCTTACAATA
  TAACCAACACAGAAATTTTTAGTTATTGTAATATGCGTCATAATTTAAATGTA
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  AAAGTTTTAA

  Fragments Selected
  Fragment 1:
  AAAATATTTAGATTATTATTAAGGAATAATGTAAATTTTGAGTGTATTAAAAA
  TCATATAAGATTTAATAAGAGTAGGAAAAAAGTAAATTACAAAGATGAAAA
  AAAATTGATAACAAATGTTGTTTATAATAAATCTATAAACAGTGATAATAAA
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  CAGGAAGGAAGAGATTTTACCTTTTCATTAATAAAAATGCAGATCAGCAAAA
  TAATGCTATATTTAAAAAAAGTAATCCTGATGATTTAATAACCACGTCCATAA

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AATACATCGAAT

Fragment 2:
TATAATAATACATCGAATTGTTCAAATGTAAAGAAAAAAAAAGATGGTGTTT
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ACGTTTAATGATTTAGAAAGCGATAACAACAGCGAATCAATAACTAGTGATG
AGCATTTAGCTCATGCTCAAAATAAGTTTAGGAATTGTAAAAATAATATGTTG
GATAATCACAATAAATCAAAAAGTAATAATAATTATAATAGTTTTAGTAATT
ATAGTAGTGATAGTATTTATAGTAGTGATAGCATTTATAGTAGTGATAGTAAT
TATAATAATGAAAATCCTAGTAGTGTGATCAAAAAAATGAATCCATCCAATA
ATGTTATTCTTAATAATGAGAGTAATAAAAGAATAGAAATTAAAAGTATTAA
TCTAAATACAAATCAAGAAACAAAACCATTAAAATCTATTTTAAAAAAATGT
AATAATAATACAACTTCTGATAAAAATAAAAATAATAATAGACATAT

- **PF3D7_0526700 cDNA Sequence:**
ATGATTAGATTGATATTTTTGTTTATCATCGATTTCTTTATTGGTGTTATTATT
ATTAATGTTTTTTTTTTTGAATATGTAAAATGCCAGGGAAAGTTCGTTAATAC
TAAGAAAGAGTTCCCCAAAATAAAAAATATAAGTAATACTTTTAAACAAATT
AAGTTCTTTGCTCCATCCAAAAAAAAAAAAAGAAATTACCTGTTTTTATCAAA
ACATAACTTTAAAAAGAACAATGATAATGGTATCTCTTCGTCAAACAAAAAA
AAAAACTTTCTTTTAAAATACCAAACTAGGAATAATGTTATGTATCGAATTAT
ATGTAATCACAATTTGTTAATTCTTAACAACACCTCTTTTTTGAAGCCTACAA
AATTAACCATTTATAATTATGACGCTCCTAAAAATGACCTTTCAGCAAATGAT
CCAGAAAAAGGTGGAAGAAAAAGTAAAGCATTACCAACAAACAATTTGAAT
GACAAAAAAGAAGAAAACGTACTACCAACAAACAATTTGAATGACAAAAAA
GAAGAAAACGTACTACCAACAAACAATTTGAATGACAAAAAAGAAGAAAAC
GTACTACCAATCGACAAAGCAAACGATCAAAATGATAATGTAACCTTACCAG
TTAATAATTTGAACGAAGATCAAACTAATAATTTGTCTTTAAAAAAAAATGA
AGAAACTAAGAATAAGGATATTTCTGAAAATAATGATACATGGAAAAAGTAT
TTAGAAGATAAAAGAAAGAAGGAAATATTAAAGGATCTGGAAAACAAAAGG
AACAAGAATAATAATTGTAATAATAATAATATTAAGAAGAAGAAGAAGAAT
TATAATAATGAATATATGAAAAATATAAAAAAAATATACATATAATAATAATA
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CATAATAATGATTATTATGAAGATGATGATAATGATTATTATGAAGATGATG

ATGATGATTATGATGATGATTATGATAATGATTATGATAACGATAATGATAAT
GATTTTATTGATGAATATAGCGATTTAGAAAAACAAGAGAGACAATATGATA
ATTTACAAAATGGAAAATTTCCATATTTTAATAATATAAATGATACAAATAA
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AACAATAAATATTTTGATAAAATCTGTAGTATATCTCCAAACGAATTAATAA
ATAGATTTTTTGAAAATACTTCAGAAAGAGTAAAAGAAGCTGTGAAAAATAT
TATATTTAATATTATAGGAAATATACAAAAATATACAATTGAGACATCTATAT
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TATATGATAAAAAATGCAGACTATAGATTAACTTTAAATGAATCCTTATATGA
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GAAGATGTAAATAAACAAAAACAACAGAATAATCAATATCAAGAAGACGAT
TTATTTAATTTAAAAAAATCTTTTCATACCTTATTTTCTGATAATAATATAAAT
AATATATCAGAAGAACAATTAAATAAGGGCGAAATGTTAAATTCAAAACATA
TAATTAATCCAAATGGTACTACTACTACTAATAATAATAATAATATAGATAAT
AATAATATAGATAATTATCAGAGAAATGGGGAATCAGATGATACAACAAATG
ATATAACAAATGATACAACAAATGAGACAGCAAACGGTACAACAAATAATA
TAACAAATAATATAACAAATGATGATTACCCTATTATTAATACGAAAAATTA
TATCCTCTTTTTAAGGAAAAAAATTAACTCTTTAGAAAAACAACTCAATATAT
TAAAAGAAAGTAAAACATTTTTAAATGATGACTTACTTTCTTACATAAAATCA
TTAACAGAAATACAACTACGTTCTTTGACAGATAACATTGGACCACTTGTATT
AGATTCTACCAAAAAAATTGTCGAACTAGTAATTCAAGGTATGACTCTAAAT
ATAAATAAAAATATGTCCAATGAACTTATATATGTAAGTGGTTCAGTTCTGAC
ATATATATGTTTCTGGCAATTAATTATTGGATATACCCTCAGAGAAATGGAAA
TAAGAGACGAGCTTTCAGATTATCTTAAGGGAAGTTGA

Fragments Selected:
Fragment 1:
TTGAATGACAAAAAAGAAGAAAACGTACTACCAATCGACAAAGCAAACGAT
CAAAATGATAATGTAACCTTACCAGTTAATAATTTGAACGAAGATCAAACTA
ATAATTTGTCTTTAAAAAAAAATGAAGAAACTAAGAATAAGGATATTTCTGA
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ATTAAAGGATCTGGAAAACAAAAGGAACAAGAATAATAATTGTAATAATAA
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CATGTGACATAATTCAAAAAAACGAATTAAGTGGAAACGATGAAATTATTAA
GGACCAAAGTAATTATTCCAATGACCATAATAATGATTATTATGAAGATGAT
GATAATGATTATTATGAAGATGATGATGATGATTATGATGATGATTATGATA
ATGATTATGATAACGATAATGATAATGATTTTATTGATGAATATAGCGATTTA
GAAAAACAAGAGAGACAATATGATAATTTACAAAATGGAAAATTTCCATATT
TTAATAATATAAATGATACAAATAAAAATTTAAGAAATCAGAAAACGTATAA
TGAAGAAAAAAAAAATAATAATAATAATTCTTTCTCATTAAACGATAGTATA
AATAATATTTTTAGTTCAAATAAAAAAAATGATTTACCTTTATTCTTTTCATTA
AATAATAAATCATTCGAAAATAGTAACAATAAATATTTTGATAAAATCTGTA

GTATATCTCCAAACGAATTAATAAATAGATTTTTTGAAAATACTTCAGAAAG
AGTAAAAGAAGCT

Fragment 2:
TTCTTATTACAAATTATATTAACAGGGTATATGATAAAAAATGCAGACTATAG
ATTAACTTTAAATGAATCCTTATATGATCAAAATAACATCTTGAATAAAAAA
GATTATGATCAACAAGAAGATGAACAAGAAGATGTAAATAAACAAAAACAA
CAGAATAATCAATATCAAGAAGACGATTTATTTAATTTAAAAAAATCTTTTCA
TACCTTATTTTCTGATAATAATATAAATAATATATCAGAAGAACAATTAAATA
AGGGCGAAATGTTAAATTCAAAACATATAATTAATCCAAATGGTACTACTAC
TACTAATAATAATAATAATATAGATAATAATAATATAGATAATTATCAGAGA
AATGGGGAATCAGATGATACAACAAATGATATAACAAATGATACAACAAAT
GAGACAGCAAACGGTACAACAAATAATATAACAAATAATATAACAAATGAT
GATTACCC

- **PF3D7_0704300 cDNA Sequence:**
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TTAACAAACAAAGAGATTGGCCTAGAATAACTTTTACCTTTTTAGTATTCGTG
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ATTATTTGTGGTGTTTTTAATATTATGTGTGGTTTCTTTTTATGGTCTTATAGTT
AATACAATTAAATCTCTCGTATTATATATATTAATATTAAGCTTAGTATTAAC
AAGTATTAGCCTGTCTTTTTATAATATCATATATATAGAATATATATCTAAAG
TTTTTGAGCATGCATTTCTTTCGACAGTATTATATTCTACGTTCCCTTTATTGG
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CAGCAATACTAATGACAAAAGTTATAACATCACTCAAAATGATCCTAGAAAA
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TATATAAAGCAAGATACTGAAATCATCGAACCATTCTATGAATATAAAAAAC
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AGAATAAAAGCAACAAAAAAAATAACAGAAAAAAAAATAAAAAAAAATAA

Fragments Selected:
Fragment 1:
GATAATACAAATGAAGAAAAGGACAAAAAAAATGAAGGAAAACTTTTATCA
TTACAAATCGATTTAGAAAAGAATCAGCTAAACACATGTAATAAAGAATACA
AATATTATTTAACTCATTATAAAAATAAAAAATATATATGCATAGAAAAAAA
AACAGACTATTCTAGTGAAGATGAAATATATGCTAAATATATACAAGACAAA
AGTAGCGACAACAGCTATCAAGGATATGATAAGTCAAAATTAATTAACACAA
GTAATATTAATATGTTAAATGTAAAAACAAATAAAAAAAACGTAAATCATTC
CATGTCATCAAATACTATTCAACAAGATTTGAGTTTTATACACTCCAGTATAA
ACAAATATGAAAAAAAAAAAGAAAAAGAAAATAAAAATTATGACAAAAATA
AGAAAAGCAGCAATACTAATGACAAAAGTTATAACATCACTCAAAATGATCC
TAGAAAAAACAATCAGAACAAAGAATTTGTTGATAATAATAACAAAAGAAA
CGATCATAATAAAAATAATGAACTTGAACAAGTATATTATAACAATCCAAAT
GTTCATCAGAACAATTATCAGCTTAGCAAGAACAAAATGAATACCACAGAAT

TACAACATGATAATTTATTTAATAAAATAAATCCATTATCATCAGATAATACA
TCGTCAATTATATTAAATAGTAATAATATGAATAAATCTATTAATAAAGATAC
GTATGTTAATATGTACGAAAAACACGAAAAACCTCTCATGGTCATAACACAA
AAAGAAGAAAATCTTAAGAAAGATAATGTCCTGAACACATCCTTATCCAGTA
ATAATGAACAAAATTGTATTATTGAAAATTTTATAGAAAAAAATATAAATAT
TCAAAGGAAAGAC

Fragment 2:
ATAACGTCTAATAAAGATAACAAAGAACAACAAAATAAAAATTACAACAAC
GATAATAATAATGATAATAATGATAATAATAATAATGATAATAATAATGATA
AAAATAATAACAAAAGTAATACATATGACCATTTAACTAGTTCCCCTTCAAC
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ACGAAGTTCATCAAAAAAATGAACAGAAAGAAAAAAATATATTGTCTAATTC
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GAATAATTTAACAAAGGACTCTTTTAAGGATAACCTTAATATGGATAAAACA
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ATGTATCC

Fragment 3:
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AAATCAAGATATAAATACATTTCGAAATAACGAAATTAAAGTTTTAGAAAAT
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- **PF3D7_0719400 cDNA Sequence:**

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Selected Fragments:

Fragment 1:
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Fragment 2:
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- **PF3D7_0832500 cDNA Sequence:**
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Selected Fragment:
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- **PF3D7_0808800 cDNA Sequence:**
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  GACTTATATACGTCCATTTATGACAACGATCCAGAAATGAATATGGTAATGC
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  ACAAGAAAAACGAAAAAAATGTAAAGAACAATGCGAAAGGGATATACAAAA
  AATTATTTTAAAAGATAAAATAGAAAAGGAATTAACAGAAAAGTTGGATGCA
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  TGATATCATAA

  Selected Fragment:
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  GATCCAGAAATGAATATGGTAATGCAACAATTTGATGAACGGACATCGGAAC
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  AACAATGCGAAAGGGATATACAAAAAATTATTTTAAAAGATAAAATAGAAA
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  CGGGTATTGCAACAGCCATTGACGAATTATATAATAATTTTTTTCTAACAATA
  TTAGGTCGTAAATCAGTGCAAGATGTTATTACTGCAG

Appendix B: Down Selected Fragments

PF3D7_1401200

- Protein Sequence
  MFPSYIRKFSFTLLLCIIALSCNNNTDIYYLTKYKNFPIVKSPHIRSLAESYKQYKIN
  SKYDELRTLGASSPQKRKPSKYDDIRCYDQPKQKQKKPSKYDDVRGFGEPAQKK

KKTSKYDDLRRFGVPTQKKKMPSKYDYLRTLKEQNVNNKWKPTTNDDLKLLSD
NYEKEKTEKYKLLKFIKKKDKENSERQKHGLPPDMSFKGLSSKKETEEYVSSDV
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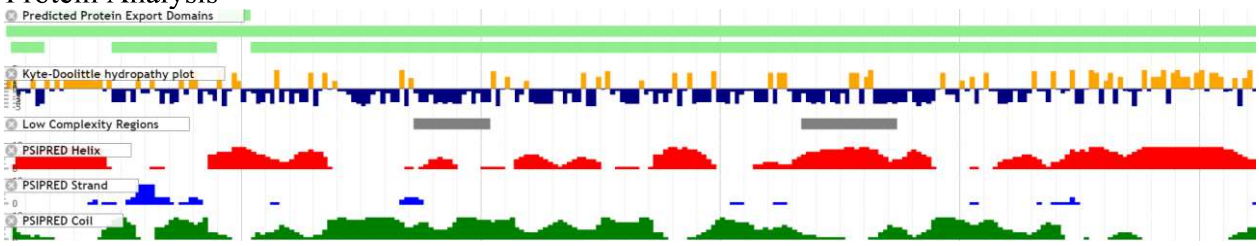
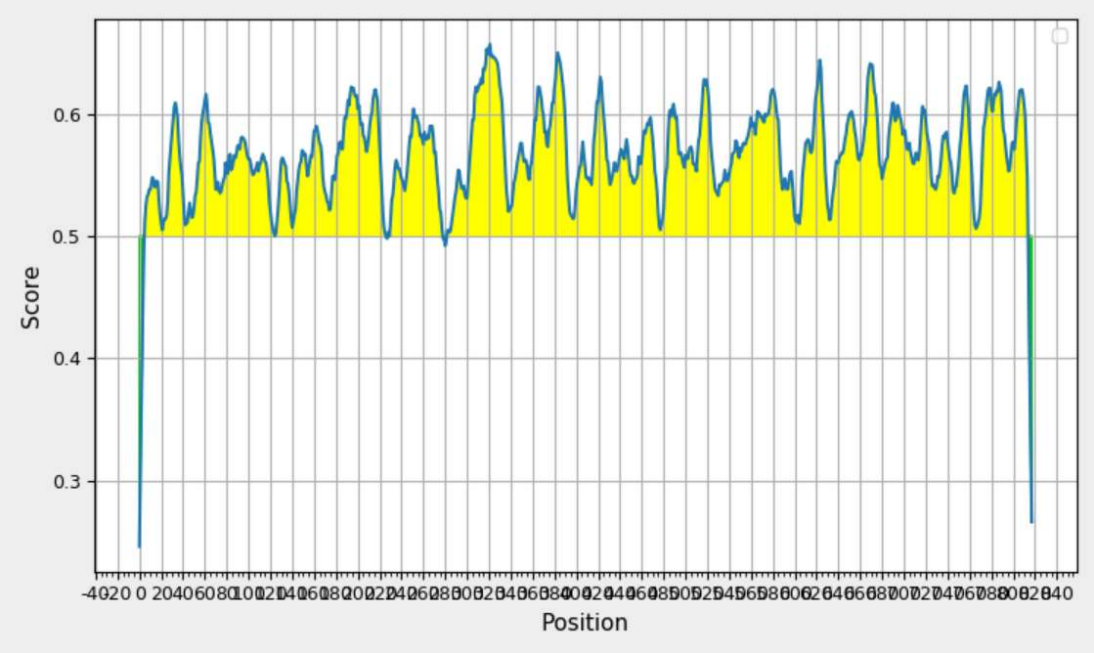
- Protein Analysis



- Polymorphisms

- B-cell epitope Prediction



- MHC II Binding Sites

Seq #	Peptide start	Peptide end	Peptide	Median consensus percentile
1	236	250	RSISFFIKLIFFGLI	17.0
1	231	245	LKFTWRSISFFIKLI	14.0
1	226	240	GILKALKFTWRSISF	18.0
1	31	45	LTKYKNFPIVKSPHI	12.0
1	1	15	MFPSYIRKFSFTLLL	13.0
1	241	255	FIKLIFFGLISLLFW	12.0
1	36	50	NFPIVKSPHIRSLAE	6.0
1	26	40	TDIYYLTKYKNFPIV	14.0
1	221	235	YTIKKGILKALKFTW	16.0

PF3D7_0511400

- Protein Sequence
  TRKKTPENIYKEIYENNYEMKYDEDIPNNMSEEKRDEKEVIEHLEIDEKNGKQYK
  SDINKPVSLSHLKQYKNIYVNNNNKINKKKSIDKHLPSYNLERKNNKYLNFLLVD
  NRNESYTFMVPMKFYINHEMYNISDEEYNKLMEDNSVDVYLNNILVEYKYENFE
  IKEGEVDGEVEGEGEVEGEVEGEVKGKVVEGIENNMNEEEKYNKDNKDKENQI
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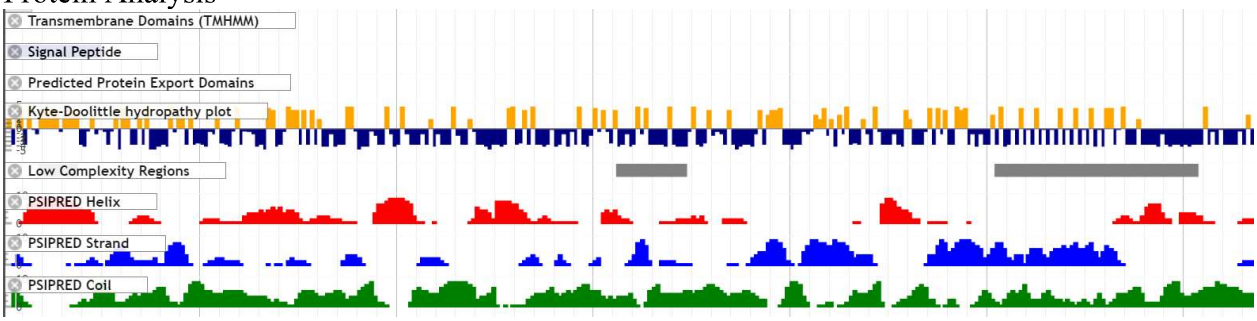
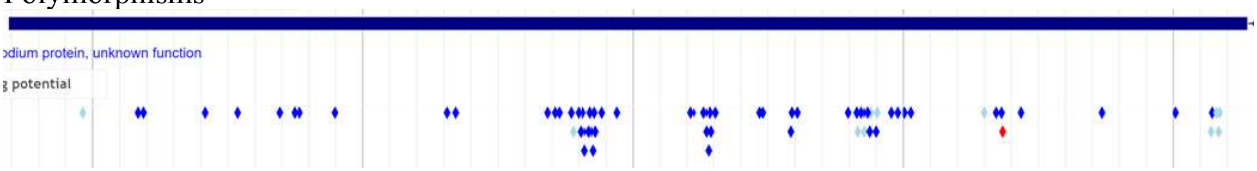
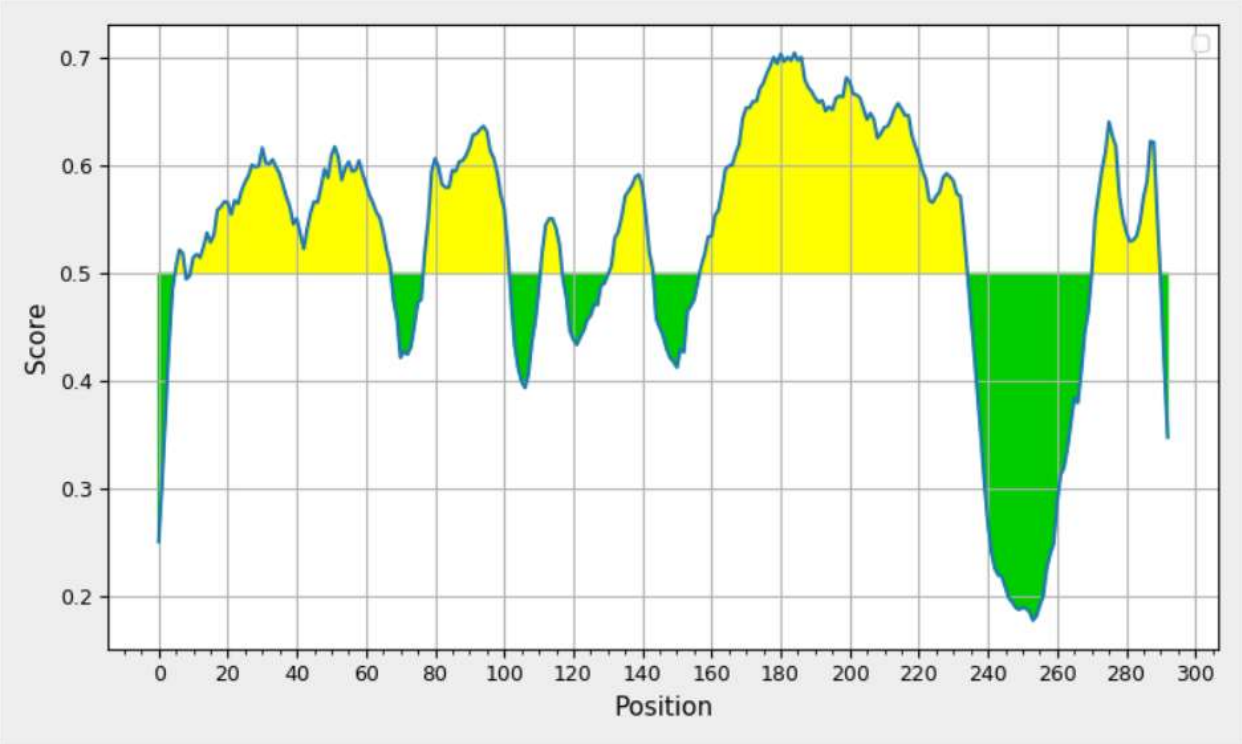
  RKSNATMAINR
- Protein Analysis



- Polymorphisms



- B-Cell Epitope Prediction



- MHC II Binding Sites

Seq #	Peptide start	Peptide end	Peptide	Median consensus percentile
1	71	85	KNIYVNNNNKINKKK	19.0
1	256	270	FIIYYFDIIQKVKMK	8.8
1	246	260	SGLVLLFCISFIIYY	16.0
1	261	275	FDIIQKVKMKLNKKR	16.0
1	111	125	NRNESYTFMVPMKFY	18.0
1	236	250	SVIMKYTIIISGLVL	14.0
1	241	255	YTIIISGLVLLFCIS	16.0

PF3D7_0511600

- Protein Sequence
  MKKIYFILLILFHLNFMECFRKYDKNKNKILISHSINNNNNSIKNNNNNNNNSIKNN
  NNNNNSIKNNNNNNNSFSATSFSSEKNKNKSYTNVLKKKNIYIREESNKTTNIKE
  DEEKNKKINNNDKETNYSFLSLKFFPFILTSLLHTGINQIPRNTEIELYEFEKSPMIR
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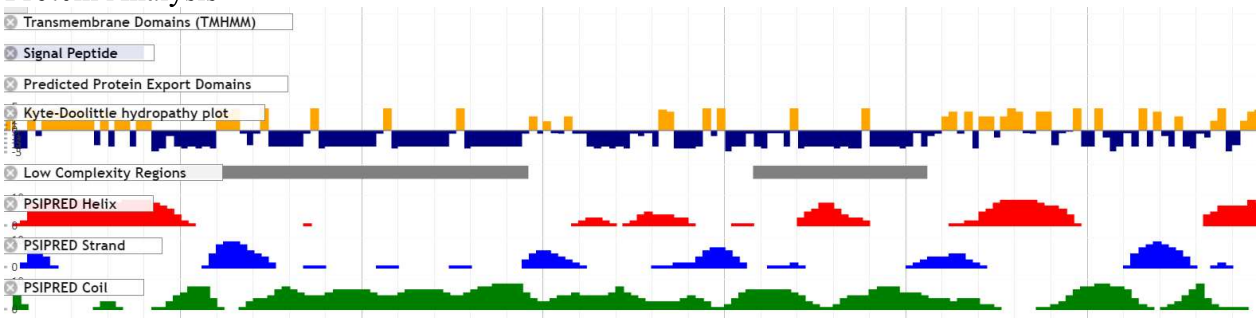
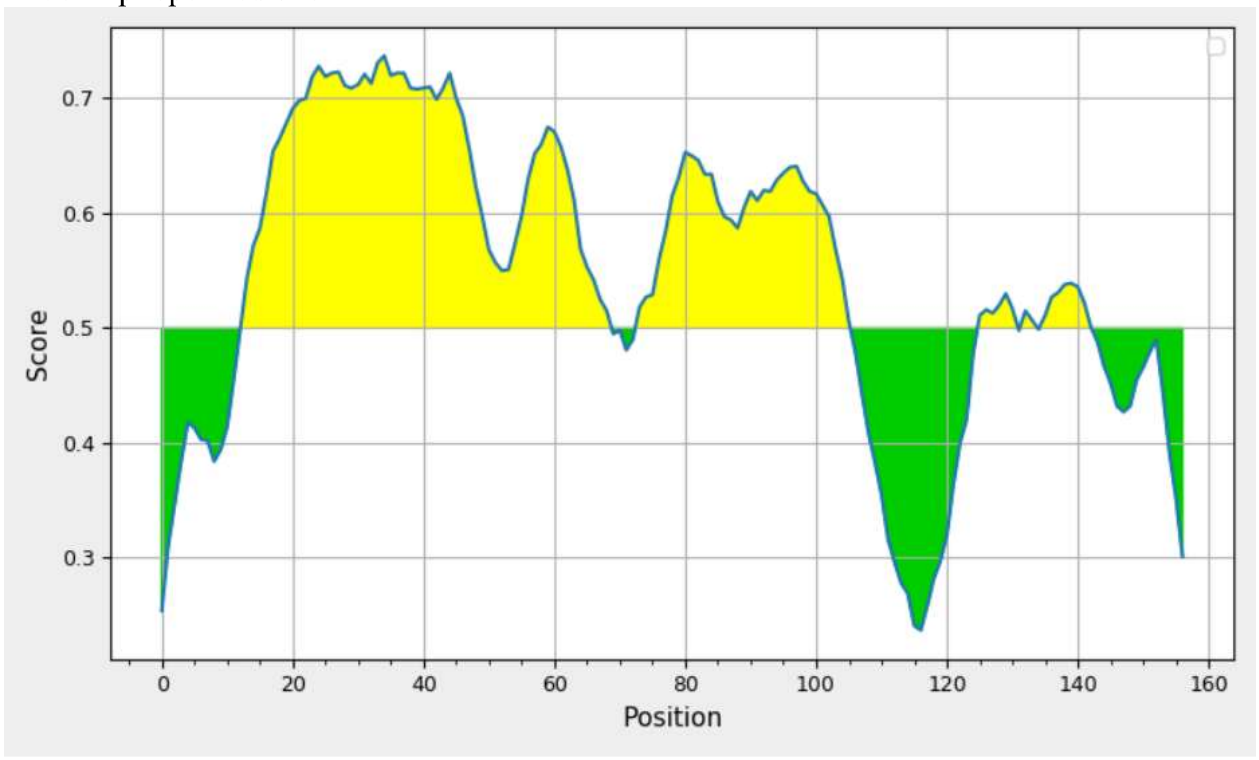
- Protein Analysis



- Polymorphisms

- B-Cell Epitope Prediction



- MHC II Binding Sites

Seq #	Peptide start	Peptide end	Peptide	Median consensus percentile
1	126	140	TNYSFLSLKFFPFIL	13.0
1	156	170	EIELYEFEKSPMIRH	15.0
1	181	195	YTYMFFIVISFVVVV	5.4
1	26	40	NKNKILISHSINNNN	13.0
1	1	15	MKKIYFILLILFHLN	18.0
1	6	20	FILLILFHLNFMECF	13.0
1	186	200	FIVISFVVVVLIALF	15.0
1	191	205	FVVVVLIALFIFKFF	7.7

PF3D7_1338600

- Protein Sequence
  EEGKNYKITLFDLKQLVKKIAERLKVLEIDDEIVILPFFELVVSSLPKKNILICRRD
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  TFDNKYDKQKTKNEFKDIEFDELDKLRNANKTSKDKRVNVMKNINNIDQVQDDI
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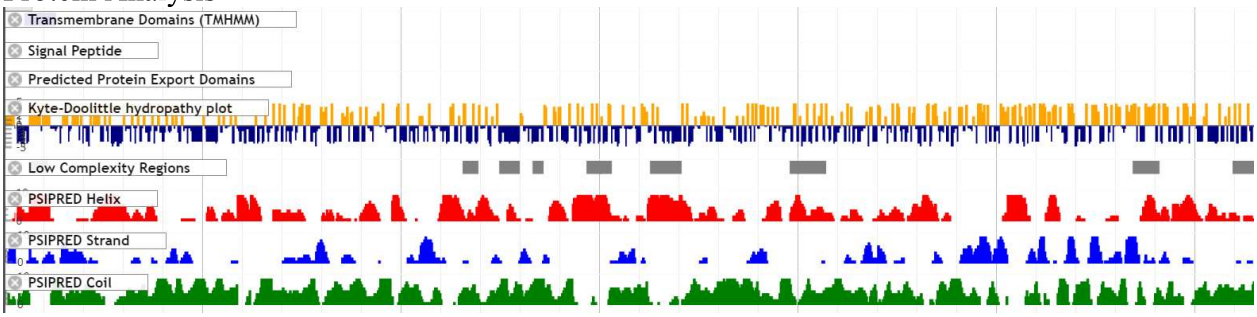
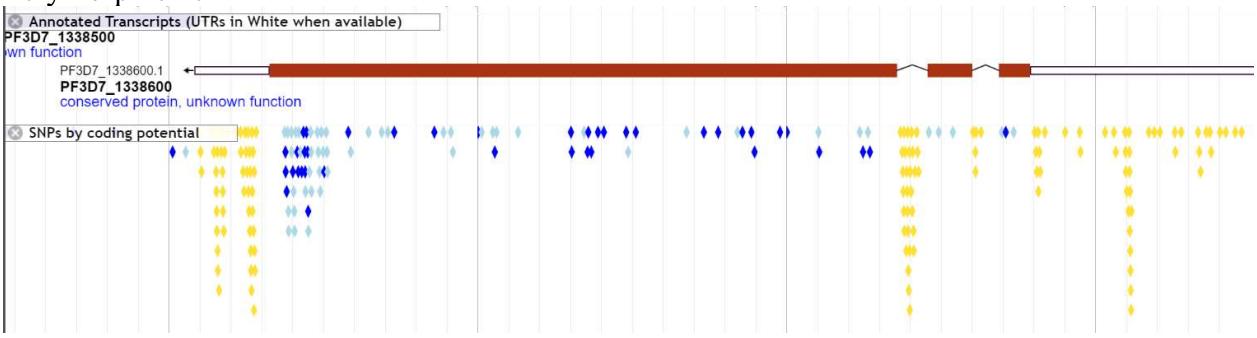
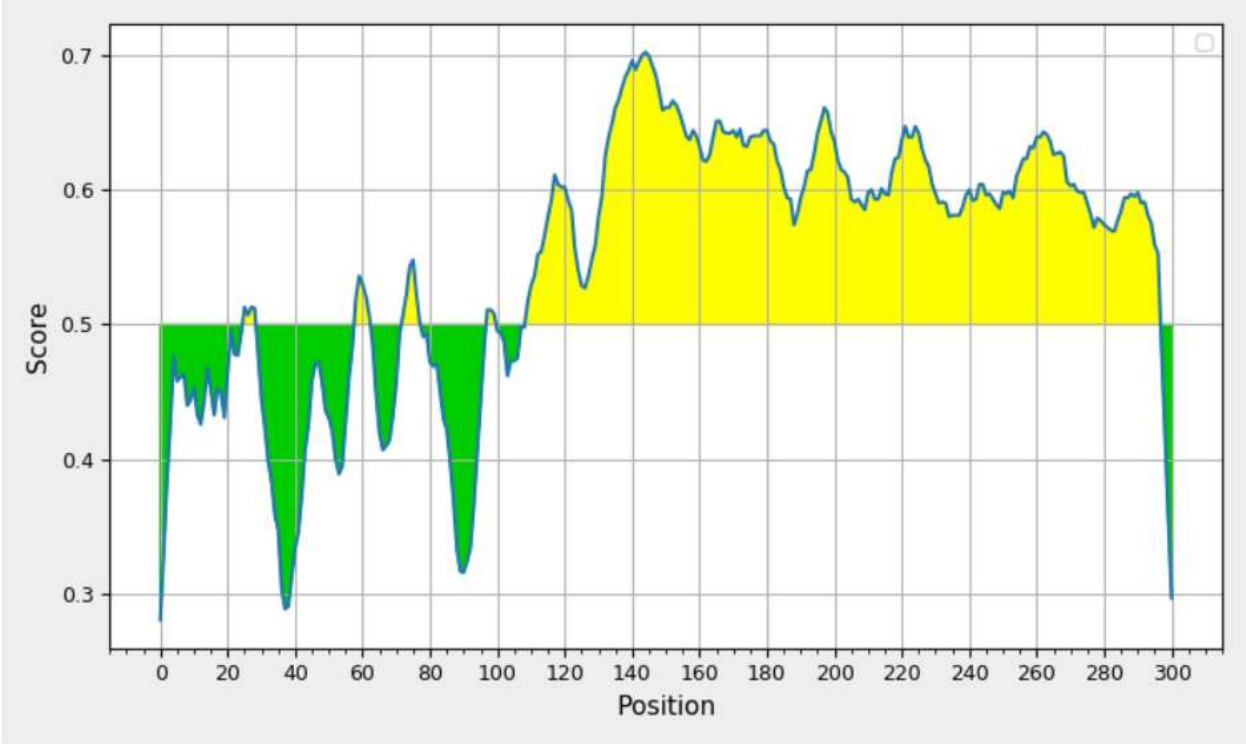
- Protein Analysis



- Polymorphisms



- B-Cell Epitope Prediction



- MHC II Binding Sites

Seq #	Peptide start	Peptide end	Peptide	Median consensus percentile
1	36	50	LPFFELVVSSLPKKN	9.4
1	86	100	PSVFHVIINLNNQKI	14.0
1	66	80	VVVFFKDNILQPVEG	16.0
1	101	115	LNLVINNIDKIILKN	5.6
1	91	105	VIINLNNQKILNLVI	11.0

PF3D7_1024800F3

- Protein Sequence
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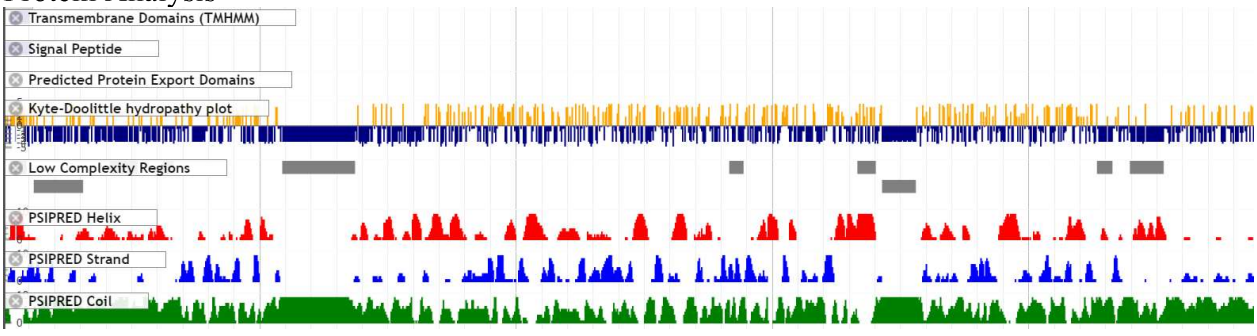
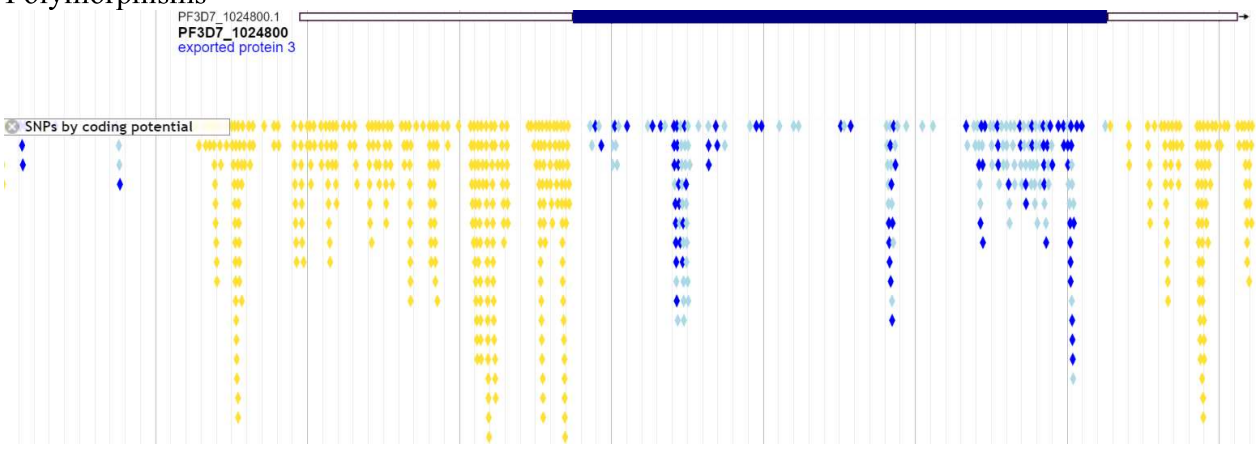
- Protein Analysis



- Polymorphisms



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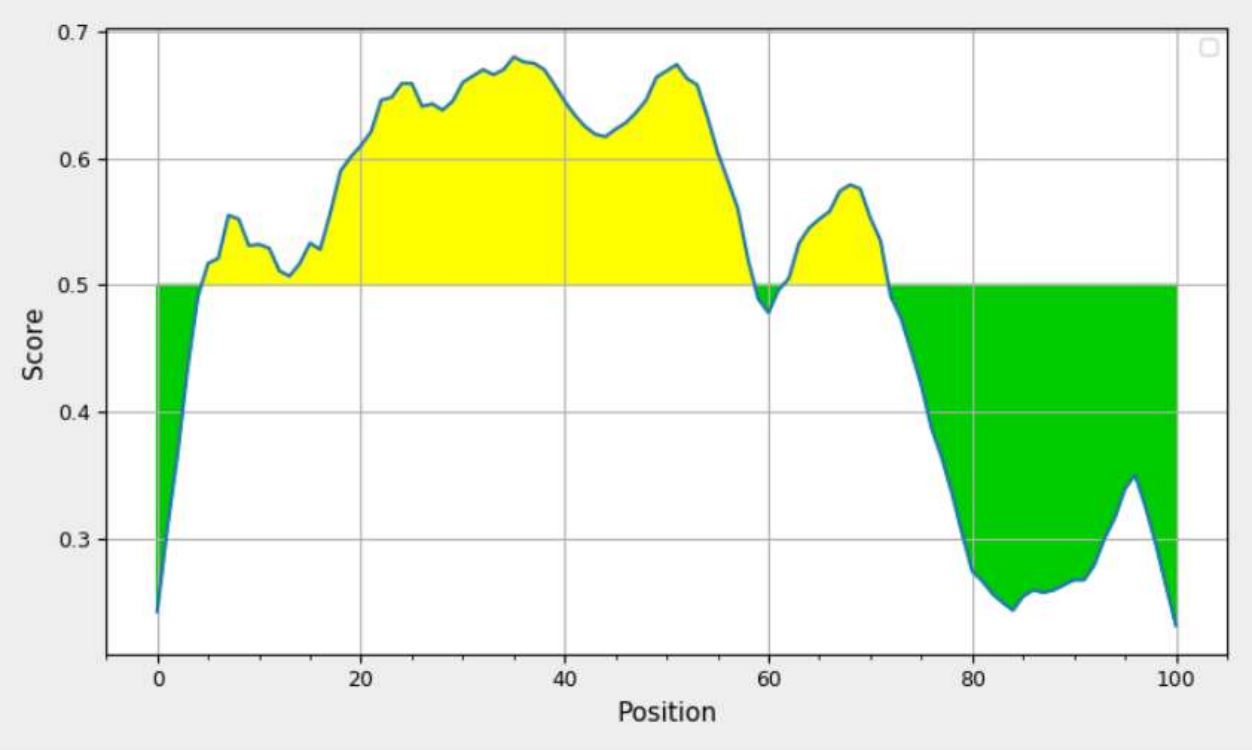

- MHC II Binding Sites

Seq #	Peptide start	Peptide end	Peptide	Median consensus percentile
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1	76	90	KLFIFIILCLISVCL	19.0
1	86	100	ISVCLALLVSIAIKV	19.0
1	81	95	IILCLISVCLALLVS	20.0

PF3D7_0526700F2

- Protein Sequence
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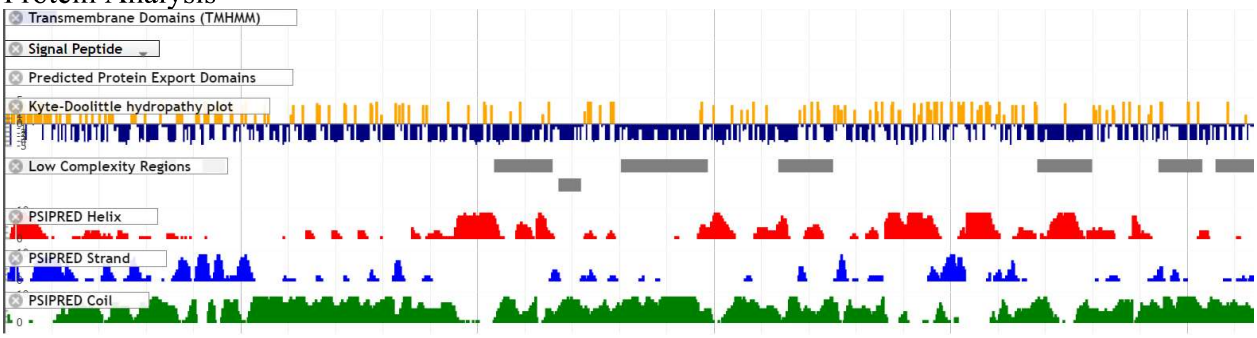
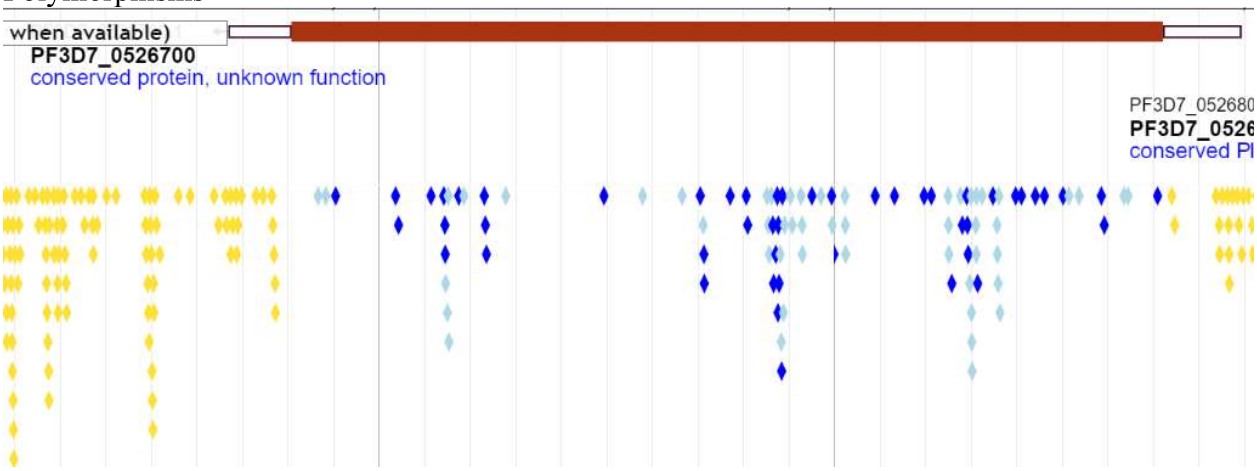
- Protein Analysis



- Polymorphisms



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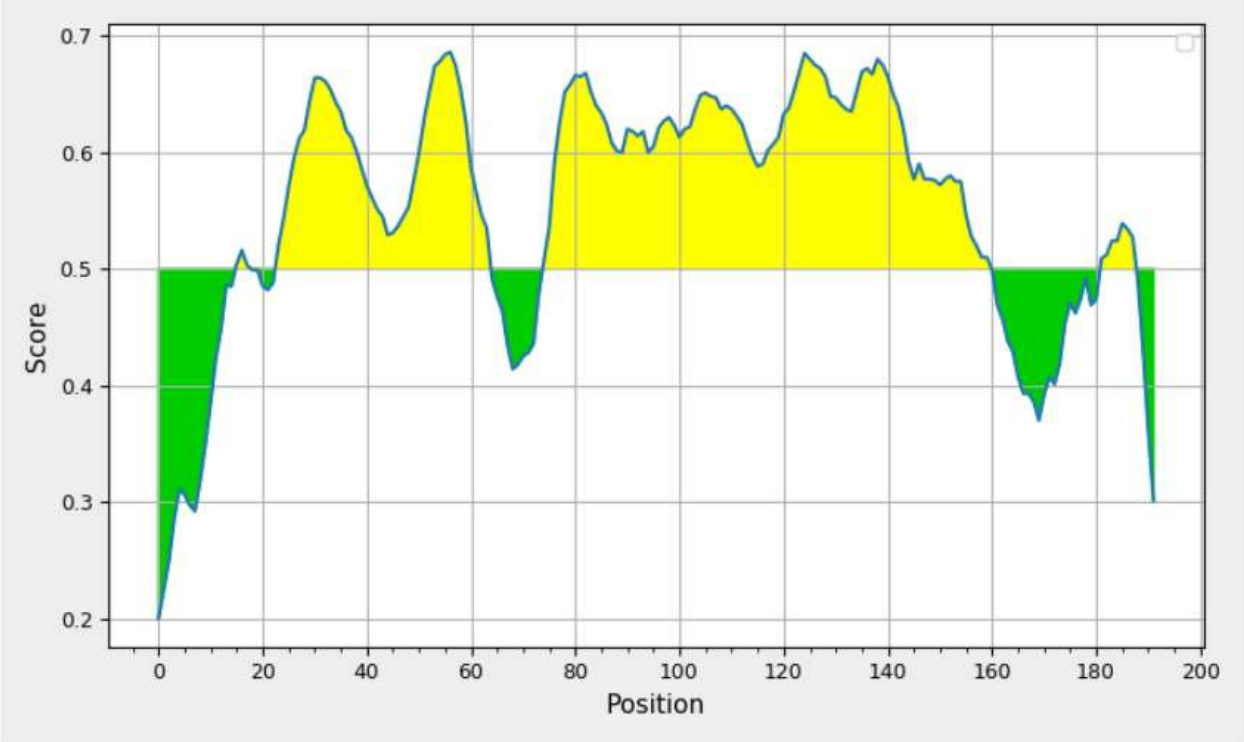

- MHC II Binding Sites

Seq #	Peptide start	Peptide end	Peptide	Median consensus percentile
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1	166	180	YILFLRKKINSLEKQ	12.0
1	61	75	DLFNLKKSFHTLFSD	14.0
1	178	192	EKQLNILKESKTFLN	20.0
1	1	15	FLLQIILTGYMIKNA	14.0
1	11	25	MIKNADYRLTLNESL	16.0

PF3D7_0820300F1

- Protein Sequence
  KIFRLLLRNNVNFECIKNHIRFNKSRKKVNYKDEKKLITNVVYNKSINSDNKNNS
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  DDHEGNENDDDNNNNNNNNIYMEENKNVNKNNLKCKKHANSSHENYTETLCE
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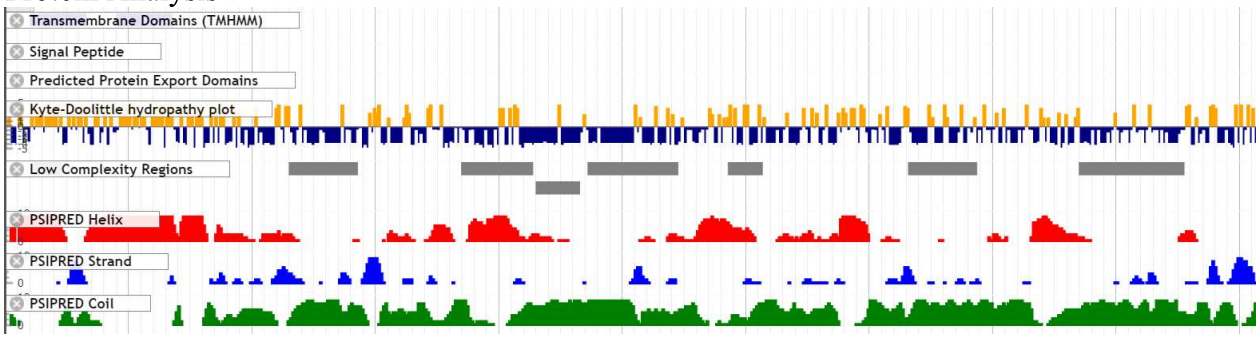
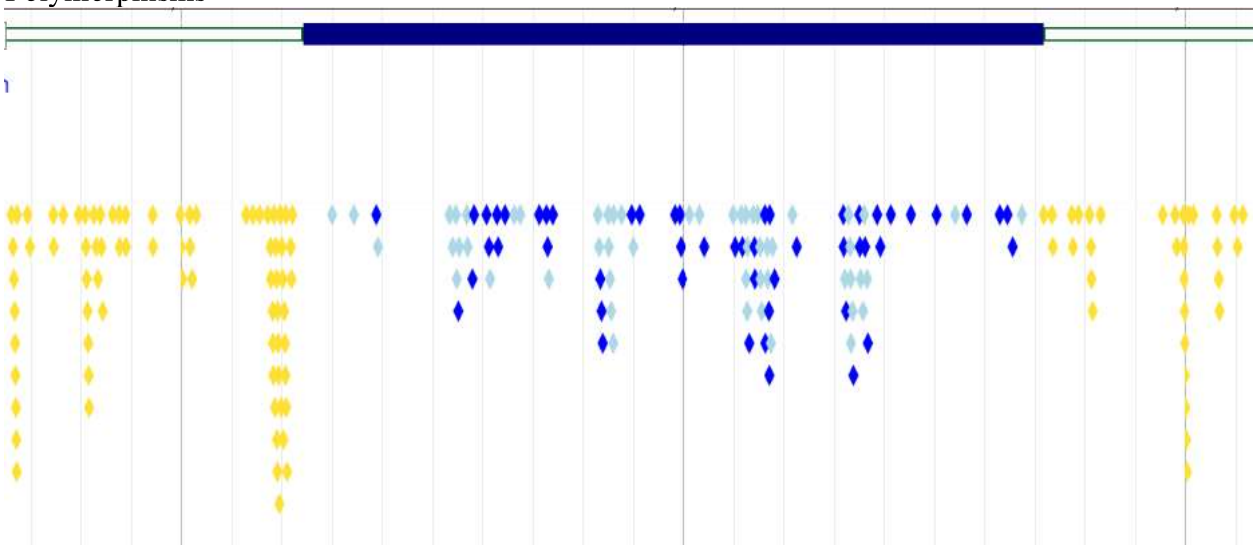
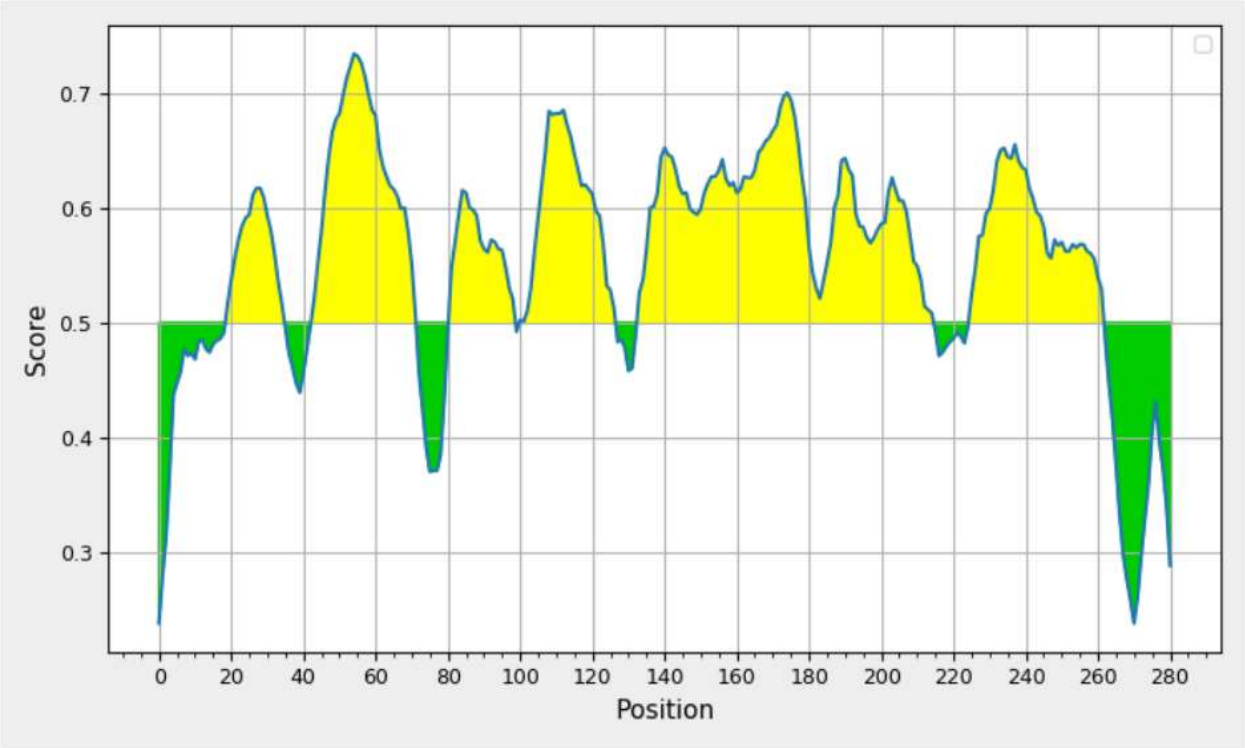
- Protein Analysis



- Polymorphisms



- B-Cell Epitope Prediction



- MHC II Binding Sites

Seq #	Peptide start	Peptide end	Peptide	Median consensus percentile
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1	121	135	RSKKRTKLLKKLKIL	19.0
1	261	275	NEKNFYLFSLLSNIL	18.0
1	1	15	KIFRLLLRNNVNFEC	13.0
1	266	280	YLFSLLSNILYNNTS	16.0
1	216	230	ECDAMINLKNKIIKK	20.0

PF3D7_0102500

- Protein Sequence
  EELYVNHNSVSVASGNKEIEKSKDEKQPEKEAKQTNGTLTVRTDKDSDRNKGK
  DTATDTKNSPENLKVQEHGTNGETIKEEPPKLPESSETLQSQEQLEAEAQKQKQE
  EEPKKKQEEEPKKKQEEEQKREQEQKQEQEEEEQKQEEEQQIQDQSQSGLDQSS
  KVGVASEQNEISSGQEQNVKSSSPEVVPQETTSENGSSQDTKISSTEPNENSVVDR
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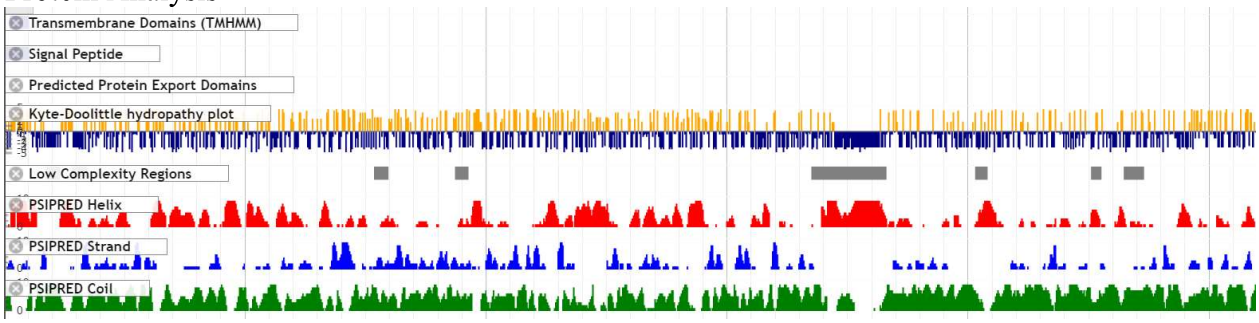
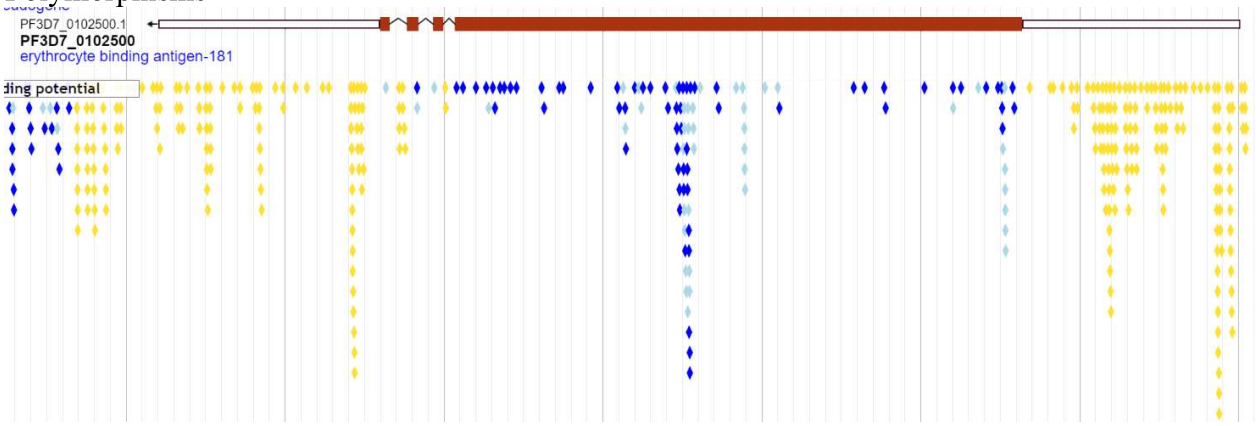
  EQDVQSTPPEDTPTVEGKVGDKA

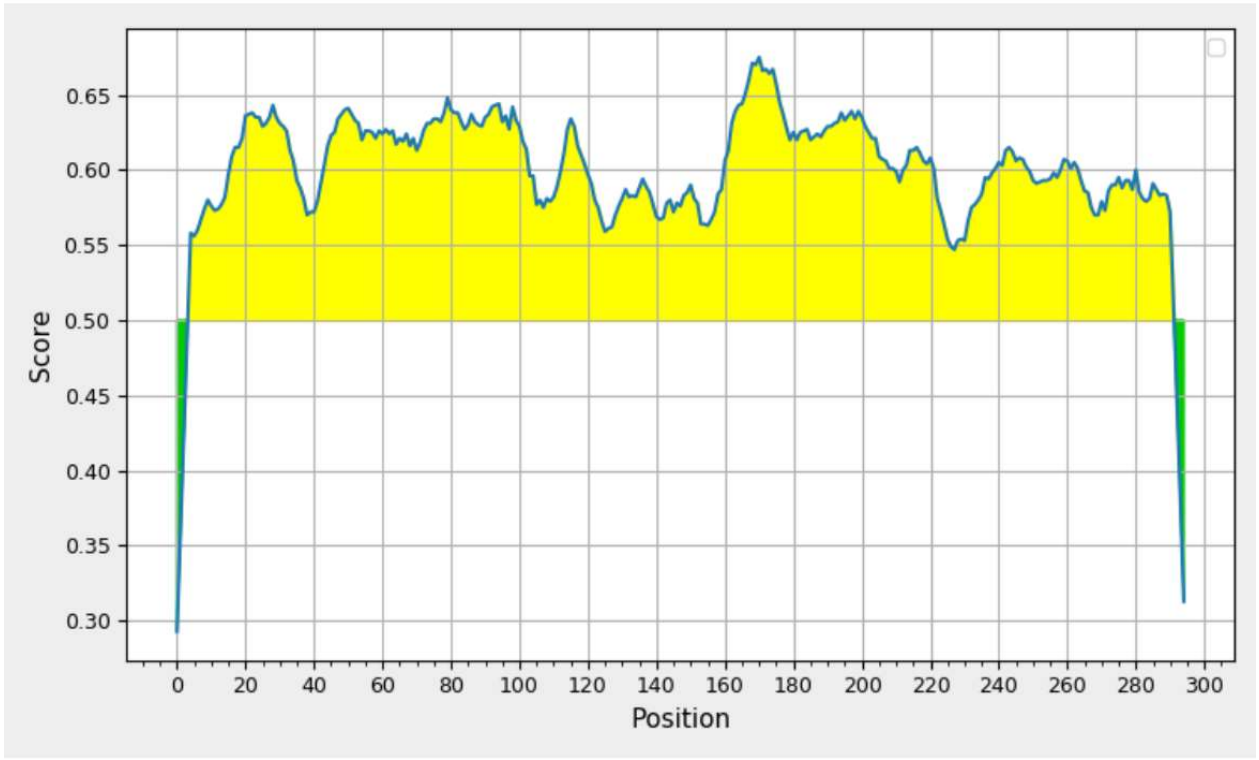
- Protein Analysis



- Polymorphisms



- B-Cell Epitope Prediction



PF3D7_0526700F1

- Protein Sequence
  LNDKKEENVLPIDKANDQNDNVTLPVNNLNEDQTNNLSLKKNEETKNKDISENN
  DTWKKYLEDKRKKEILKDLENKRNKNNNCNNNNIKKKKKNYNNEYMKNIKKY
  TYNNNNNNNNSNSNTCDIIQKNELSGNDEIIKDQSNYSNDHNNDYYEDDDNDYY
  EDDDDDYDDDYDNDYDNDNDNDFIDEYSDLEKQERQYDNLQNGKFPYFNNIND
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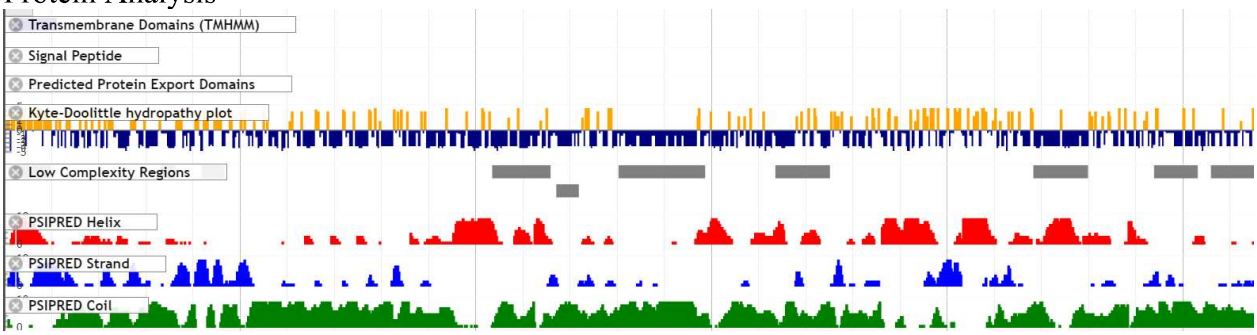
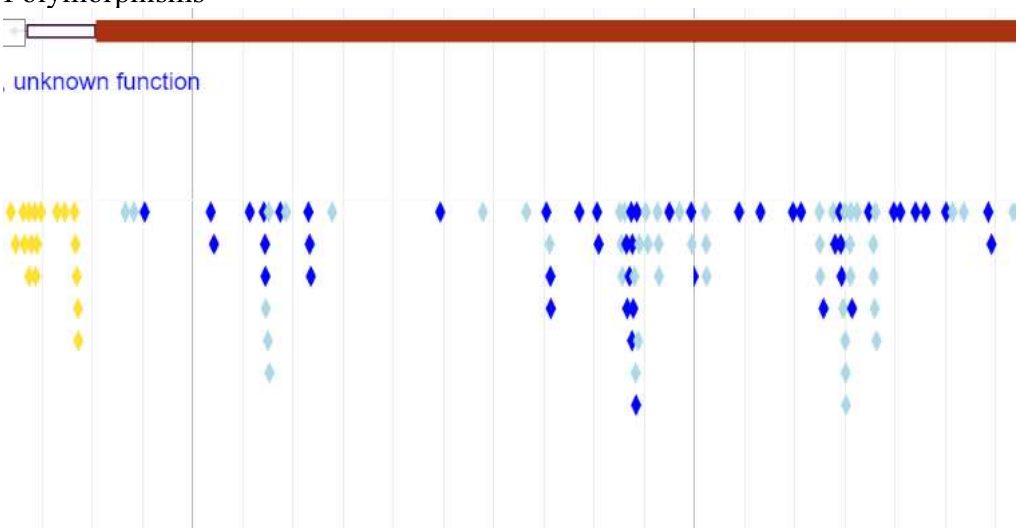
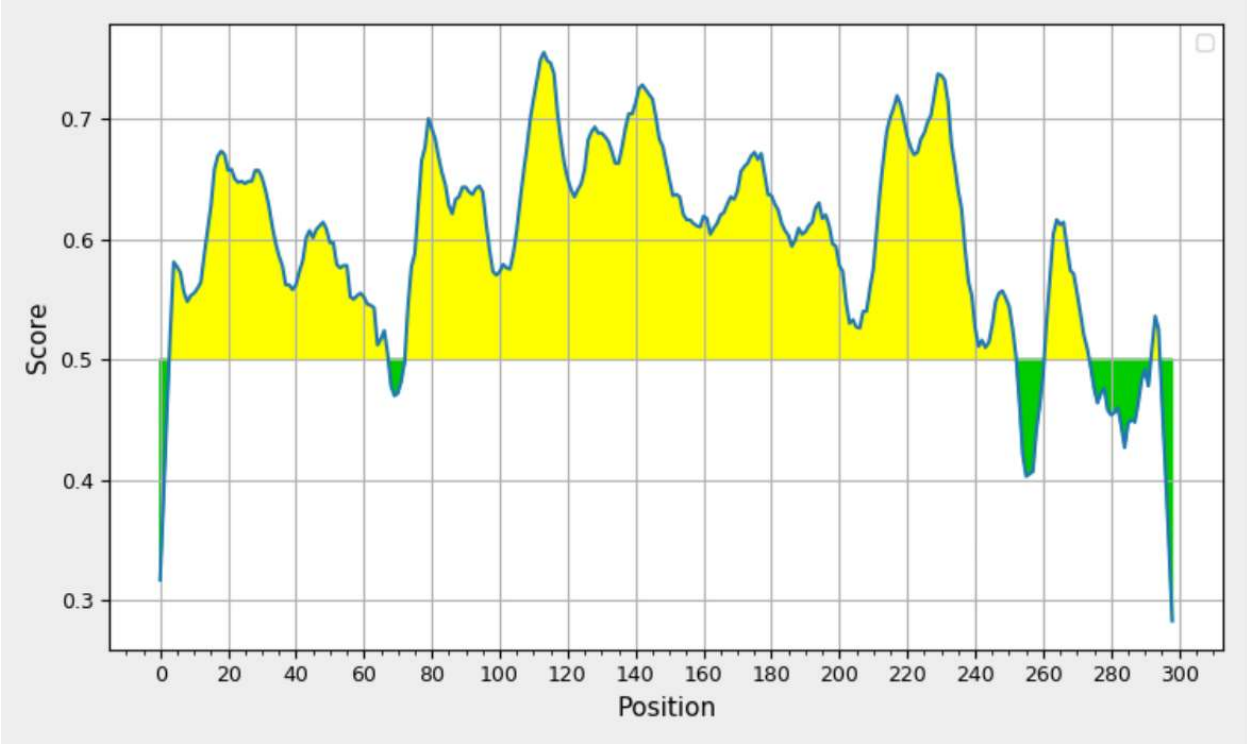
  NNKYFDKICSISPNELINRFFENTSERVKEA
- Protein Analysis



- Polymorphisms



- B-Cell Epitope Prediction

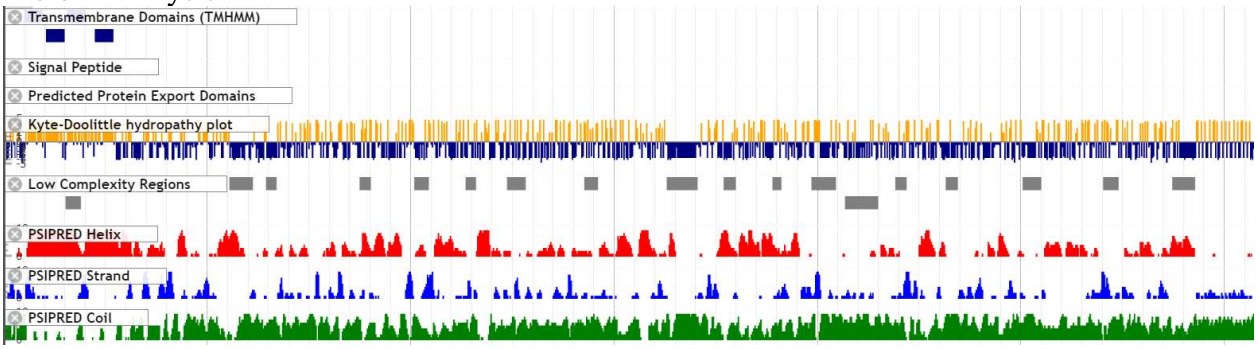


- MHC II Binding Sites

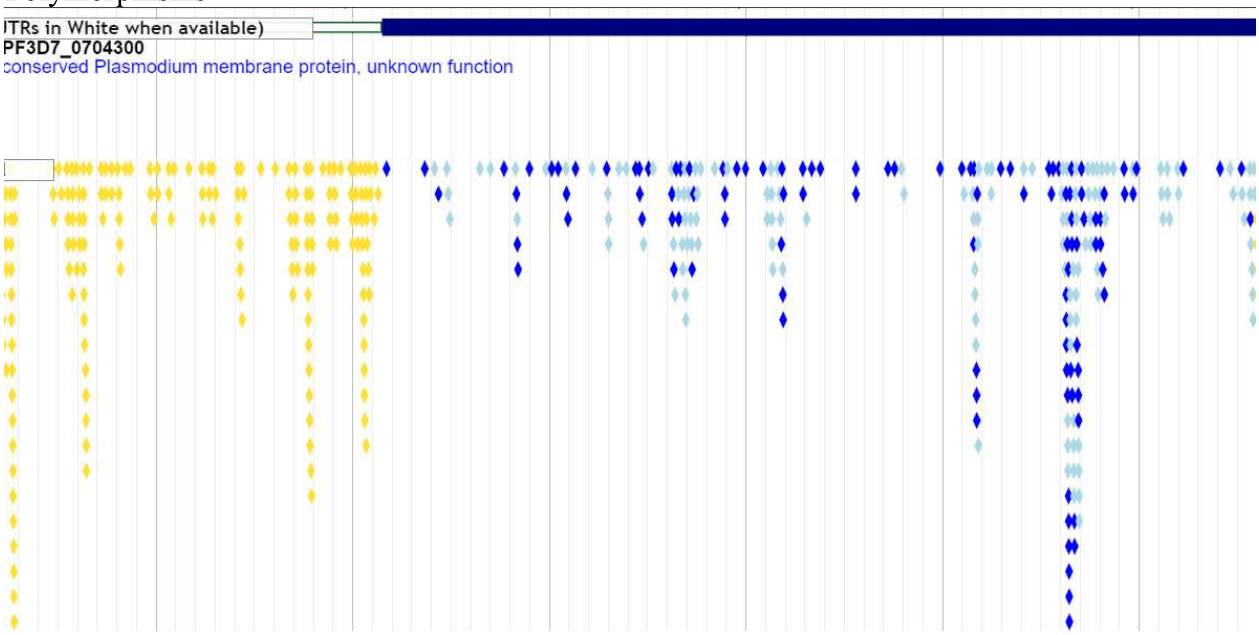
Seq #	Peptide start	Peptide end	Peptide	Median consensus percentile
1	251	265	KNDLPLFFSLNNKSF	11.0
1	256	270	LFFSLNNKSFENSNN	17.0

PF3D7_0704300F1

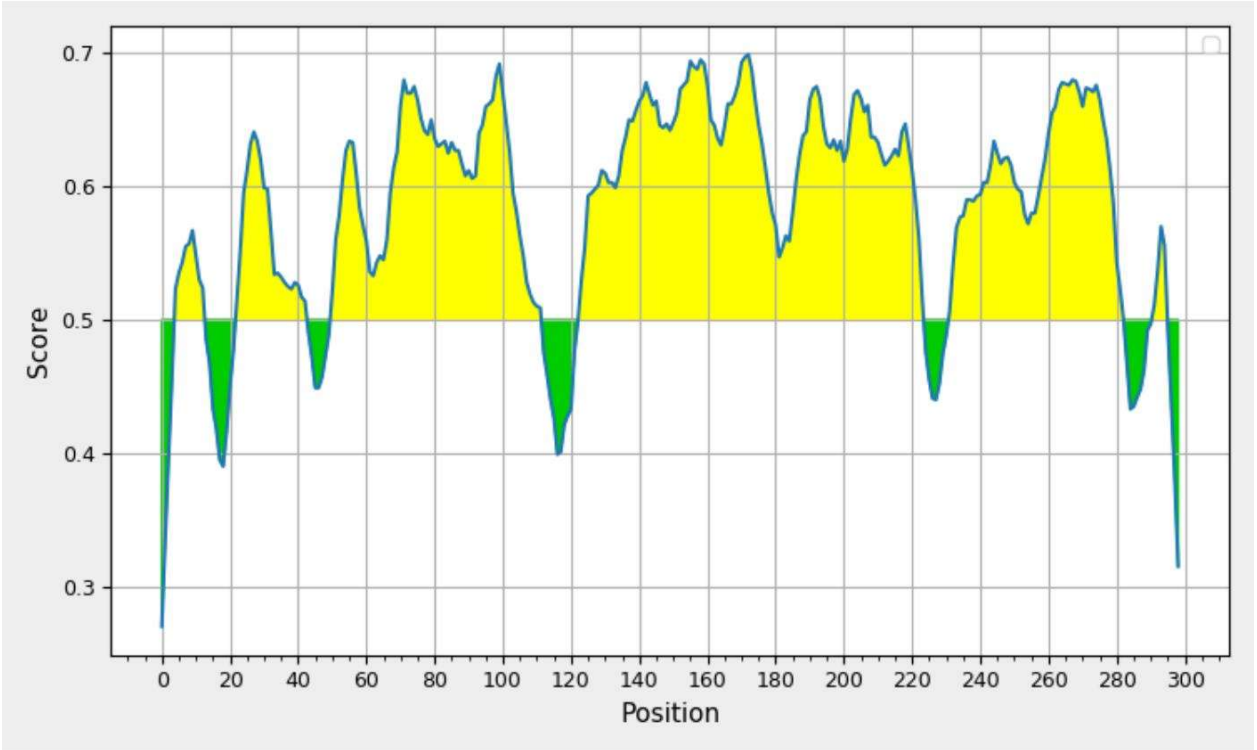
- Protein Sequence
  DNTNEEKDKKNEGKLLSLQIDLEKNQLNTCNKEYKYYLTHYKNKKYICIEKKTD
  YSSEDEIYAKYIQDKSSDNSYQGYDKSKLINTSNINMLNVKTNKKNVNHSMSSN
  TIQQDLSFIHSSINKYEKKKEKENKNYDKNKKSSNTNDKSYNITQNDPRKNNQNK
  EFVDNNNKRNDHNKNNELEQVYYNNPNVHQNNYQLSKNKMNTTELQHDNLFN
  KINPLSSDNTSSIILNSNNMNKSINKDTYVNMYEKHEKPLMVITQKEENLKKDNV
  LNTSLSSNNEQNCIIENFIEKNINIQRKD
- Protein Analysis



- Polymorphisms



- B-Cell Epitope Prediction

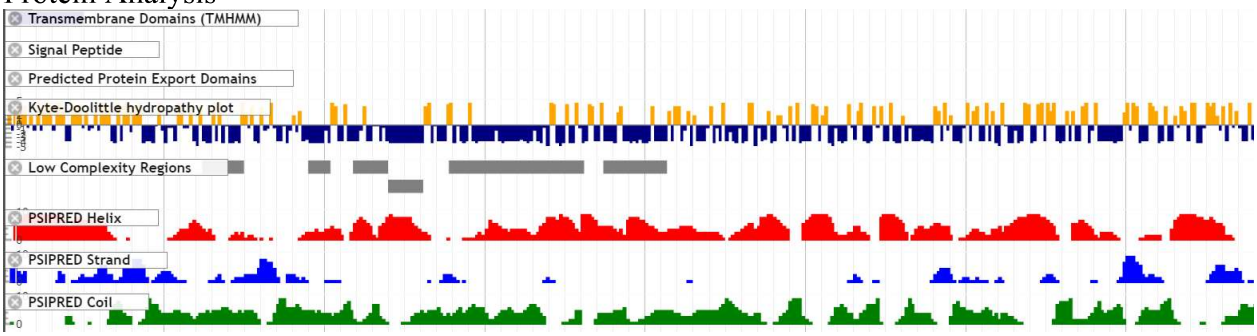


- MHC II Binding Sites

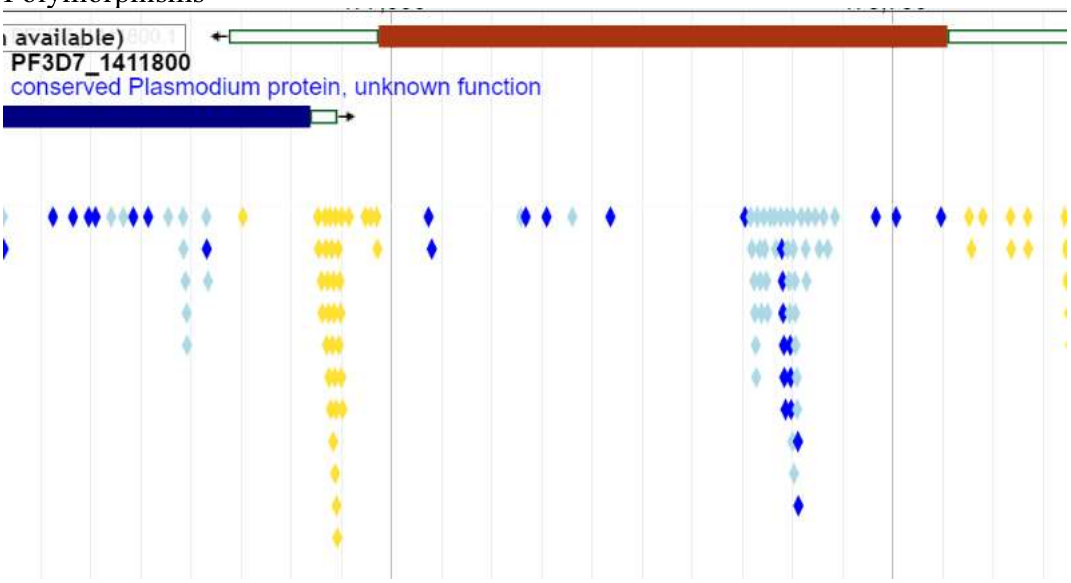
Seq #	Peptide start	Peptide end	Peptide	Median consensus percentile
1	81	95	SKLINTSNINMLNVK	17.0
1	111	125	QQDLSFIHSSINKYE	15.0
1	216	230	KINPLSSDNTSSIIL	20.0

PF3D7_1411800

- Protein Sequence
  NKKINIKGKKEKICSLQKLYWISNEGKYMPFQHKKKKKYKILQNVNNEEYLEEG
  GETEKKKKKKKKKKKLIKGNNEVIKGNKDINKENNEEYNKENNEEHNKDYNKT
  RIVKRKVKKISKDVLQNIEENKCLNEKEKHKKELENEE
- Protein Analysis



- Polymorphisms



- B-Cell Epitope Prediction