Characterization of Artemisinin Resistance in
Plasmodium falciparum

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LIST OF ABBREVIATIONS

ACT	Artemisinin Combination Therapy
AE	Arteether
AL	Artelinic Acid
AM	Artemether
ART	Artemisinin
AS	Artesunate
BSD	Blasticidin
BWA	Burrows-Wheeler Aligner
cDNA	Complementay ribonucleic acid
CGH	Comparative Genomic Hyrbridization
CNV	Copy number variation
CQ	Chloroquine
DHA	Dihydroartemisinin
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthetase
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
dUTP	Deoxyuridine triphosphate
DV	Digestive vacuole
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene Glycol Tetraacetic Acid
EPX	Epoxomicin
FBS	Fetal Bovine Serum
GADA	Genome Alteration Detection Algorithm
GWAS	Genome-wide association studies
HA	Hemagglutinin

HBSS	Hanks' Balanced Salt Solution
HPI	Hours post invasion
HRP	Horseradish peroxidase
IDC	Intraerythrocytic Developmental Cycle
IRS	Indoor Residual Sprays
LB	Luria-Bertani
MEF	Mefloquine
MT	MitoTracker Deep Red
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate Buffered Saline
PCR	Polymerase chain reaction
PI3P	Phosphatidylinositol 3-phosphate
PV	Parasitophorous vacuole
PYR	Pyrimethamine
QN	Quinine
RBC	Red blood cells
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RSA	Ring-stage survival assay
SDS	Sodium dodecyl sulfate
SERCA	Sarco/endoplasmic reticulum Ca-ATPase
SG	Sybr Green I
SMART	Switching Mechanism At the 5' end of RNA Template
SNP	Single nucleotide polymorphism
TBS-T	Tris-buffered saline with Tween 20
UPR	Unfolded protein response
WWARN	Worldwide Antimalarial Resistance Network

ABSTRACT

Artemisinins are an important class of antimalarials because of their remarkable antimalarial activity and exceptional safety profile. However, the decline in efficacy of artemisinin-based drugs jeopardizes global efforts that aim to control and ultimately eradicate malaria. In order to better understand the Artemisinin resistance phenotype, we developed Artemisinin-resistant parasite lines derived from two isogenic clones (6A and 11C) of the 3D7 strain of *Plasmodium falciparum* using a selection regimen that mimics how parasites interact with the drug in infected patients. Long term *in vitro* selection produced two parasite lines that displayed profound stage-specific resistance to artemisinin and its relative compounds. Chemosensitivity and transcriptional profiling of ART-resistant parasites indicate that enhanced adaptive responses against oxidative stress and protein damage are associated with the artemisinin resistance phenotype. Genomic characterization of both parasite lines also identified a spectrum of mutated and copy number-variable genes that could play a role in mediating artemisinin sensitivity. Collectively, this work represents a useful resource for investigating artemisinin response and resistance in *P. falciparum*.

Chapter 1. Introduction

1.1 Malaria Burden and Pathogenesis

1.1.1 Malaria

Malaria is the most prevalent vector-borne disease in the world—with an estimated two hundred million cases and four hundred thousand deaths recorded in 2015 alone². Much of the global burden of malaria is found in Africa (88%), followed by Southeast Asia (10%) and the Eastern Mediterranean (2%)². It is caused by the protozoan parasite belonging to the genus *Plasmodium*. In humans, the disease is caused by five species namely *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. While *P. vivax* and *P. knowlesi* are also able to cause severe malaria in humans, majority of the severe and often fatal cases reported have been attributed to *P. falciparum*³. The disease is transmitted by female mosquitoes belonging to the genus *Anopheles*, with approximately 70 species recorded to be able to transmit parasites between human hosts⁴. Although less fatal than *P. falciparum*, *P. vivax* is known to exhibit a larger geographic coverage, due to its ability to survive in a wide variety of climates and conditions². The classical clinical manifestations of uncomplicated malaria include periodic fever, malaise, chills and nausea. However, severe cases of the disease can also be characterized by complications in the respiratory and central nervous systems, hepatic dysfunction, hypoglycemia and severe anemia⁵.

1.1.2 The life cycle of the *Plasmodium* Parasite

The life cycle of the *Plasmodium* parasite is highly complex, involving a human and mosquito host and multiple morphological stages. The parasite is introduced into the human host following an infective blood meal by a female Anophelene mosquito. *Plasmodium* sporozoites that reside in the mosquito's saliva are injected into the bloodstream and will then travel towards the liver where they will invade hepatocytes. Once inside liver

cells, the sporozoites will undergo multiple rounds of division producing merozoites, which will then be released into the bloodstream and invade red blood cells upon hepatocyte rupture. This marks the beginning of the asexual blood stage or intraerythrocytic development cycle (IDC) of the *Plasmodium* infection which is responsible for the clinical or symptomatic phase of the disease.

Figure 1.1 Life Cycle of the Malaria Parasite

In its entirety, the life cycle of the *Plasmodium* parasite has 3 main stages: the asexual liver and blood stages in the vertebrate host, and the sexual stage within the mosquito vector

Source: Global Health Division of Parasitic Diseases and Malaria, Centers for Disease Control & Prevention, 2010. Retrieved from: <http://www.cdc.gov/malaria/about/biology/>

During the IDC, which lasts ~48 hrs for *P. falciparum*, *P. vivax* and *P. ovale*, ~72 hrs for *P. Malariae* and ~24 hrs for *P. knowlesi*, the parasite takes on different morphologies, each characterized by unique transcriptomic and

metabolic features^{6,7}; These distinct morphologies are: the early ring or immature trophozoite stage, the mature trophozoite stage—which is also the most metabolically active, and the schizont stage wherein the parasite once again undergoes division to produce more merozoites in preparation for the re-invasion of red blood cells thereby initiating another round of the IDC.

Figure 1.2 Different stages of the *Plasmodium* Intraerythrocytic Development Cycle (IDC)

In *P. falciparum*, the asexual blood stage, also known as the IDC, lasts ~48 hrs. It is typically divided into three metabolically and transcriptomically distinct phases: the quiescent ring stage which constitutes the period right after invasion, the highly metabolic trophozoite stage, and the mitotically active schizont stage.

Instead of developing into mature schizonts, some parasites may also opt to exit the IDC, and differentiate into sexual forms becoming either male (microgametocytes) or female (macrogametocytes) gametocytes. During a blood meal, these gametocytes are ingested by the female Anopheles mosquito. In the mosquito gut, microgametes will fertilize macrogametes generating zygotes which will then form ookinetes that will then mature

into oocysts which—upon rupture—will release sporozoites. These sporozoites will then make their way into the mosquito's salivary glands from which they shall be introduced into another human host when the mosquito takes a blood meal, marking the beginning of a new infection.

1.2 Malaria Control

Given the fact that the each stage of the parasite's highly complex life cycle is defined by its own distinct biological properties at the transcriptional and metabolic levels, it is not surprising that the activity of antimalarial control strategies also demonstrate different target specificities. Control, and ultimately eradication, of malaria requires a multi-faceted approach that takes into consideration the parasite, the vector and the host. The World Health Organization implements a 3-tier intervention plan towards global malaria control. They include vector control, which focuses on blocking or minimizing transmission; case prevention, which aims to prevent infections in human hosts; and finally, case management, which involves the rapid diagnosis and treatment of new and existing malaria infections².

1.2.1 Vector Control

Malaria vector control programs rely primarily on the use of insecticides to kill mosquitoes and block transmission. This includes the use of insecticidal compounds that are either sprayed on interior surfaces (Indoor Residual Sprays, IRS) or formulated into a "space spray" or "fog" that is sprayed into areas that cannot be reached by IRS, and typically administered at dusk when adult mosquitoes are known to be most active. Alternatively, the use of insecticide-coated materials such as bednets, screens, canvas tents, curtains and wall linings have also been shown to reduce the incidence of malaria infections. Besides killing the adult vectors via insecticides, another possible approach is to kill the mosquito larvae by lacing known mosquito breeding grounds with larvicidal chemicals. Apart

from chemical methods, insect-specific bacterial, viral or fungal pathogens, as well as natural predators of adult and larval stages of the mosquito vector are also being utilized as effective biocontrol agents to target the malaria vector population. Both modes of vector control have been shown to be inexpensive and effective methods in diminishing malaria burden⁸.

Besides restricting the disease transmission through vector control, the other two tiers in malaria control are geared towards the human host, rather than the mosquito, and involve either the a) preclusion of new infections; or b) the management of malaria cases that are already underway.

1.2.2 Case Prevention

Preventive therapies against malaria can be either causal or suppressive, depending on which stage of the *Plasmodium* life cycle they are aimed towards. Causal prophylaxis completely blocks infection in the human host and acts against the pre-hepatic stage. Causal prophylactics can involve vaccination against pre-hepatic forms of the parasite, or the administration of drugs that block the establishment of infection in the liver, while suppressive prophylaxis refers to preventive strategies that act against the asexual blood stage of the parasite. Although suppressive prophylactics do not prevent infection with the malaria parasite itself, it does preclude the development of actual clinical illness^{9,10}.

Commonly employed causal chemoprophylactics include atovaquone-proguanil¹¹ (packaged together as Malarone), and primaquine¹². Atovaquone and proguanil each have distinct mechanisms of action against the parasite—the former antagonizes mitochondrial electron transport¹³ while the latter inhibits with the parasite's folate biosynthesis pathway¹⁴—but display a synergistic activity when administered together¹⁵. Both drugs

are also active against the pre-erythrocytic as well as the erythrocytic stages of the *Plasmodium* life cycle. Primaquine also displays both liver stage and blood schizonticidal activity in the case of *P. vivax*^{16,17}. However, it is active only against the pre-erythrocytic stage of *Plasmodium falciparum*, and has no apparent effect on the blood stages¹⁸. And although it has long been used as a prophylactic drug against malaria, its specific mode of action is still poorly understood, although the parasite mitochondrial membrane has been documented as a target^{12,19}.

On the other hand, examples of widely used suppressive prophylactics that only act against the parasite during the asexual blood stage include mefloquine, chloroquine and doxycycline^{9,10}. Apart from doxycycline, both mefloquine and chloroquine have also long been utilized to treat clinical cases of malaria rather than just as a preventive measure to prevent disease onset. Both belong to the quinoline class of compounds and are discussed in more detail in the succeeding sections. Doxycycline on the other hand is a broad-spectrum antibiotic that is used to treat bacterial, in addition to protozoal infections²⁰. While it is effective against the blood stages, doxycycline demonstrates a delayed parasitocidal activity, only exerting its effects after at least one 48-hr erythrocytic cycle^{21,22}. It has been shown to target the apicoplast and mitochondrial organelles within the *Plasmodium* parasite, possibly due to the inhibition of mitochondrial translation²³ and the expression and replication of the apicoplast genome²².

Although current prophylactic strategies involve chemical intervention, the use of vaccines also provides a viable alternative approach for disease prevention. The most advanced malaria vaccine to date is the RTS,S/AS01 which has just been put through Phase 3 clinical trials. This subunit vaccine candidate, which targets the pre-erythrocytic stage, utilizes a combination of epitopes derived from the circumsporozoite protein which is secreted by

Plasmodium liver stage sporozoites^{24,25}. Although the RTS,S vaccine is currently the most promising candidate and exhibits satisfactory tolerability and safety, it only exhibits a modest vaccine efficacy of 25-36% against clinical malaria over a period of 3-4 years^{26,27}. Although the implementation of the RTS,S vaccine in its current state can substantially benefit the state of malaria public health, the sheer magnitude of the global burden of the disease also means that much of the intervention strategies will remain reliant on the management of present clinical cases using chemotherapeutics.

1.2.3 Case Management

The final and perhaps most important aspect of the malaria intervention is the use of antimalarial drugs, with several classes of compounds that have been widely employed for decades. Currently however, the cornerstone of global malaria control programs is artemisinin (ART) combination therapy or ACT. ACT combines the highly potent, rapidly acting artemisinin-based drugs with less active, but longer-lasting partner drugs that have a different mode of action and pharmacodynamics to artemisinin. The rationale behind this strategy is to delay or avert the emergence of drug resistance—a prevalent issue that has greatly hindered the advancement of malaria control²⁸. In addition, the use of combination therapy to combat malaria has also led to better cure rates and reduced transmissibility in the field^{28,29}.

1.3 Non-artemisinin Antimalarials

1.3.1 Quinolines and Related Compounds

Quinolines are nitrogen-containing heterocyclic, aromatic compounds. Quinoline-based antimalarials, by extension, are typified by the central quinoline ring structure, but also contain various functional side groups that in turn contribute to drug action and solubility. Historically, the earliest iterations of quinoline drugs were crude extracts from the Cinchona tree,

which in turn led to the isolation of the active parasitocidal component quinine³⁰. However, limitations with regard to accessibility of the drug, concerns regarding toxicity even at therapeutic concentrations, as well as its demanding dosing regimen, led to the development of synthetic derivatives that would prove easier to obtain and are more effective^{31,32}. It's also been observed that this particular class of antimalarial drugs exert the most activity during the mid to late trophozoite stage of the IDC²⁸.

Among the commonly used quinoline type drugs are chloroquine, mefloquine, halofantrine, lumefantrine and piperazine. The antimalarial action of these compounds has been shown to be based on the disruption of hemozoin formation^{33,34}. Heme, which is found in hemoglobin, is toxic to *Plasmodium* parasites and is therefore detoxified and crystallized into insoluble hemozoin to keep it from killing the parasite. Quinine, chloroquine and related drugs prevent the conversion of free heme to hemozoin leading to the accumulation of chemical products that are harmful to the cell. However, It is likely that each specific quinoline compound hits a different spectrum of targets albeit within in the same pathway³¹.

Resistance to this particular class of drugs is well-documented in Malaria-endemic areas. Resistance to quinine was first reported in 1910, with areas of diminished drug sensitivity in Asia and South America³⁵. Chloroquine resistance emerged in the 1950s along the Thai-Cambodian border spreading from Southeast Asia to South America, India and Africa through the 1960s and 1970s^{33,36,37}. As for mefloquine—which was originally developed as an alternative antimalarial due to chloroquine's reduced efficacy—resistance was detected in the late 1980s within six years of introducing the drug. Owing to the similar structure and mode of action of these drugs, cross resistance is not uncommon. Cross resistance between

chloroquine and amodiaquine, mefloquine and lumefantrine, and between mefloquine and halofantrine have also been reported^{34,38}.

Two of the genes most commonly associated with resistance to members of this class of compounds include transporter-encoding *Pfcr*t and *Pfmdr*1. Linkage mapping of the chloroquine-resistance locus revealed a region on chromosome 7 of the *P. falciparum* genome to be responsible for phenotype³⁹. Further analyses of this region identified the *Pfcr*t gene to be strongly associated with chloroquine resistance^{40,41}. This gene codes for a transmembrane transport protein in the parasite's digestive vacuole, which is the site of hemoglobin digestion and chloroquine activity^{40,42}. Polymorphisms in *Pfcr*t have also been linked to an altered susceptibility to quinine and its dextroisomer quinidine^{43,44}. Another marker that is associated with resistance—though to a lesser extent—to chloroquine in addition to related drugs such as mefloquine, amodiaquine, halofantrine, lumefantrine and quinine is *Pfmdr*1⁴⁵⁻⁴⁷, which is located in chromosome 5 and encodes a P-glycoprotein homologue that is also localized in the digestive vacuole⁴⁸. Amplification of *Pfmdr*1⁴⁷, as well as the presence of specific polymorphisms⁴⁹ have been associated with resistance in clinical isolates, and have also been functionally validated to modulate parasite sensitivity to a variety of quinoline drugs. Interestingly, increase of *Pfmdr*1 copy number has been associated with elevated susceptibility to a bisquinoline compound, piperazine^{50,51}. *Pfnhe-1*, which encodes a Na⁺/H⁺ exchanger, has also been found to influence sensitivity to quinine⁵²⁻⁵⁴.

More recently, a the amplification of gene cluster on chromosome 14 containing *Plasmepsin 2* and *Plasmepsin 3* has been identified by several genome-wide association studies as a marker of resistance against piperazine⁵⁵⁻⁵⁷. Both genes encode aspartic proteases that are essential to the parasite's hemoglobin degradation pathway. Given that the quinoline

class of antimalarials is believed to target this particular pathway, it plausible that increase in the copy number of these two genes is indeed able to mediate sensitivity to piperazine.

Although the use of these drugs as monotherapy against *P. falciparum* malaria has been discontinued due to their reduced efficacy because of the emergence of resistance, quinoline drugs are still currently deployed in combination with artemisinin-based drugs, such as artemether-lumefantrine, artesunate-amodiaquine, dihydroartemisinin-piperazine, and artesunate-mefloquine².

1.3.2 Antifolates

Introduced as a first line alternative to chloroquine upon emergence of chloroquine-resistance, antifolate drugs target the folate biosynthesis pathway of the *Plasmodium* parasite. Principal antifolates include pyrimethamine and proguanil—both of which inhibit dihydrofolate reductase (DHFR), as well as the sulfa drugs sulfadoxine and dapsone, which target dihydropteroate synthetase (DHPS). All of these drugs act as competitive inhibitors of the substrates of their enzyme targets^{14,33,58}. As a result of the disruption to the folate biosynthesis pathway, there is a marked reduction in amino acid and DNA synthesis within the parasite⁵⁹. And because they target DNA synthesis and replication, antifolate drugs have been shown to have affect the stages of the IDC during which these processes are actively taking place, which is from late trophozoite to early schizont stages²⁰.

Drug resistance to antifolate antimalarials was quick to emerge upon introduction. Treatment failures for proguanil were reported within two years of use as a therapeutic and prophylactic in the late 1940s. As for pyrimethamine, parasitological resistance emerged within months of

clinical use when it was first introduced in the early 1950s⁵⁸. While resistance develops rapidly when such drugs are employed as monotherapy, it was found that administering them in combination produces a synergistic effect that can still be therapeutically efficacious even in the event of parasite resistance to the individual components; Examples of such combinations include sulfadoxine-pyrimethamine and chlorproguanil-dapsone, although diminished parasite sensitivity to these combinations have also been detected in Southeast asia, South America and Africa^{14,58,60}.

Resistance to antifolate drugs has been attributed to mutations in the genes *Pfdhfr* and *Pfdhps*, which encode the antifolate enzyme targets. Studies have identified a well-documented set of amino-acid alterations that are strong predictors of treatment failure using antifolate antimalarials^{14,58,61}. Currently, sulfadoxine-pyrimethamine is typically administered in combination with artesunate⁶².

1.3.3 Napthoquinones

First used as an antimalarial in the late 1990s, atovaquone is a 2-hydroxynaphthoquinone derivative exerts its antimalarial effect by collapsing the parasite's mitochondrial membrane potential through electron transport inhibition, as well as by disrupting pyrimidine biosynthesis³³. Specifically, atovaquone blocks the binding of cytochrome b of the cytochrome bc1 complex thereby preventing electron transfer from cytochrome b to c1⁶³.

However, reduced susceptibility to this antimalarial arose rapidly—within months of initial clinical use as a monotherapy. Genetic surveillance of resistant field isolates as well as resistant parasite lines derived *in vitro* revealed atovaquone resistance to be due to mutations that confer amino

acid substitutions in the atovaquone binding site of the Cyt b protein which is encoded by the mitochondrial gene *Pfcytb*⁶³⁻⁶⁵. Although naphthoquinone type antimalarials are no longer utilized as monotherapy, atovaquone is still used in the field in combination with artesunate and proguanil⁶⁶. Unfortunately the high cost of atovaquone production makes it unaffordable in many malaria-endemic regions, which limits its therapeutic utility⁶².

1.4 Artemisinin antimalarials

1.4.1 History and clinical use

Artemisinin (ART) is originally derived from the plant known as sweet wormwood (scientific name *Artemisia annua*). This plant, also referred to as “qinghao”, has long been used—at least 2000 years—in traditional Chinese medicine to treat patients with fever⁶⁷. It was only in the early 1970s that the active compound was identified and purified and then referred to as “qinghaosu” which translates to essence of qinghao. The name was later changed to artemisinin⁶⁷⁻⁶⁹. Artemisinin type compounds were widely used in China in the 1980s and use of these drugs as an antimalarial spread to South East Asia by the early 1990s⁶⁸.

Artemisinin-based drugs are currently being utilized as the first-line treatment for uncomplicated malaria and are administered in combination with non-artemisinin drugs to prevent the development of resistance. While artemisinin by itself is a potent antimalarial, it exhibited poor bioavailability upon administration. This, in turn, necessitated the development of semisynthetic artemisinin derivatives that were more bioavailable and also more potent⁷⁰. Clinically available artemisinin-based drugs include the parent extract artemisinin, as well as its derivatives: the water-soluble dihydroartemisinin (DHA), artesunate (AS) and artelinic acid (AL), and the oil-soluble artemether (AM), and arteether (AE)⁷¹.

Figure 1.3 Artemisinin (ART) and its semisynthetic derivatives

(A) ART was originally isolated from the plant *Artemisia annua* or “Qing Hao”—which had long been used in traditional Chinese medicine for the treatment of fever. (B) ART and its semisynthetic derivatives (shown in the figure) are now routinely used all over the world for the treatment of uncomplicated and severe malaria.

In addition to having a better bioavailability profile, semisynthetic artemisinin derivatives are rapidly metabolized into DHA, which is the active metabolite that is responsible for parasite killing⁷². Artemisinin itself however, is not actually metabolized into DHA, but acts as the primary antimalarial before being metabolized into a number of compounds that have no antimalarial activity⁷³. Artemisinin-based compounds are especially effective therapeutics compared to other antimalarials because of their safety profile, wide therapeutic index, very high kill rates and potency against both sexual and asexual parasites^{69,74,75}. This particular class of compounds is also typified by their rapid elimination half-life which ranges from 1-6 hrs post administration⁷⁰. In contrast, majority of non-artemisinin antimalarials are longer acting and display half-lives lasting over

several days to several weeks—with a few exceptions⁷⁶. Another key advantage of artemisinin drugs is their broad range of activity. Where the quinolines, antifolates and naphthoquinones are only effective against select stages of the IDC, artemisinin and its derivatives have been shown to be active throughout the IDC. Artemisinin drugs are capable of killing the earliest (rings) to the most mature forms (schizonts) of blood-stage parasites, with Artemisinin sensitivity being highest at the trophozoite stage and, lowest during the early ring stage⁷⁷.

However, apart from their parasitocidal effect, artemisinin-type drugs have also been documented *in vitro* to induce growth arrest in exposed parasites. This induction of parasite dormancy that typically occurs in the ring stage has been hypothesized to allow the parasites to evade killing by the drug and possibly lead to recrudescence of infection^{78,79}.

1.4.2 Mechanism of Action

The mode of action by which artemisinin-based compounds kills parasites is still very much the subject of scientific study, with several different mechanisms and biological targets under investigation. Artemisinins belong to the class of sesquiterpene lactones that bear an endoperoxide bridge that is essential for its antimalarial activity^{68,80}. It is widely accepted that parasite killing by artemisinin is due to the bioactivation of the peroxide structure within the drug which then leads to an increase in oxidative stress within the parasite. However, the specific biochemical mechanism which causes the formation of radical oxygen species (ROS) from the endoperoxide bridge is still under debate. One hypothesis posits that the iron within the parasite reacts with the drug to liberate O-centered radicals which then rearrange to form C-centered radicals that are free to alkylate essential parasite proteins^{81,82}. Another hypothesis suggests that free radicals are generated by the opening of the peroxide ring due to direct

protonation or complexation with iron resulting in the generation of hydroperoxide and hydroxy radicals. The ROS generation in this case is not completely dependent on activation of the drug by metal ions^{69,83}. The formation of these artemisinin -derived free radicals can then cause damage to biomolecules such as proteins and lipids, in the vicinity. It's also been documented that the parasite's response to an artemisinin challenge involves the engagement of the ubiquitin-proteasome system⁸⁴, which is one of the primary adaptive responses against oxidative stress and protein damage⁸⁵. However, the exceptionally high therapeutic activity of artemisinins suggests that they act not just by indiscriminately damaging biomolecules, but through the selective binding of specific cellular targets^{68,69,75,80,86-88}. Furthermore, the bioactivation of artemisinin drugs is also largely driven by heme-derived iron that is sourced from the host hemoglobin, which explains why artemisinin displays peak activity during the later stages of the IDC after hemoglobin digestion has taken place^{77,89}.

Multiple reports on the interaction of artemisinin with *Plasmodium*-specific proteins suggest that the drug is able to hit a number of different targets. artemisinin -derived radicals are able to alkylate heme, which could lead to disruption of hemozoin synthesis⁹⁰ or the formation of heme-artemisinin adducts that interact with sulfur ligands in other proteins^{68,69}. On the other hand, several earlier studies that utilized radiolabeled artemisinin identified a translationally controlled tumor protein homolog (PfTCTP)—which is located in both the cytoplasm and the food vacuole—as the specific target of the drug^{68,69,80,88}.

Scientists have also identified the ER-resident PfATP6 protein—the parasite ortholog of SERCA (sarco/endoplasmic reticulum membrane calcium ATPase) as the specific target of artemisinin. This is based on the similarity between artemisinin and thapsigargin, a highly specific inhibitor of SERCA

and another sesquiterpene lactone. It was found that artemisinin inhibited PfATP6 when it was exogenously expressed in *Xenopus* oocytes and that artemisinin activity correlates well with parasite killing and is also iron-dependent.⁹¹ Mutations in *Patp6* gene have also been found to modulate sensitivity to artemisinin.^{92,93} Since the emergence of the SERCA-artemisinin association, however, there have also been reports that contradict the SERCA hypothesis. One study reported that yeast-expressed PfATP6 is insensitive to artemisinin⁹⁴, while another study detected no competitive antagonism between thapsigargin and artemisinin in parasites⁹⁵. Results of genetic association studies between *Pfatzp6* and artemisinin sensitivity remain inconsistent⁹⁶.

A recent study has also identified the *Plasmodium* phosphatidylinositol-3-kinase (Pfpi3k) enzyme as a target of artemisinin -based antimalarials—demonstrating that DHA is able to inhibit purified Pfpi3k⁹⁷. They propose that Pfpi3k is targeted by the drug specifically in the early ring stage of the IDC, during which it functions in the phosphorylation of phosphatidylinositol to produce phosphatidylinositol-3-phosphate (PI3P)—a signaling molecule which appears to play a role in mediating response and resistance to artemisinin⁹⁷.

Artemisinin-based drugs have also been found to act upon parasite membranes. It has been shown that artemisinin is also distributed to the parasite's mitochondria and causes membrane depolarization via ROS⁹⁸. Furthermore, this class of drugs can also cause an ROS-dependent depolarization of the parasite's plasma membrane as well, suggesting another mechanism of parasite killing⁹⁹.

The most recent high throughput proteomics analyses that utilized tagged artemisinin analogues to probe interacting partners of the drug within

parasitized erythrocytes revealed that the artemisinin is capable of alkylating many different parasite proteins across the various time points in the asexual blood stage. These putative targets also encompass a wide spectrum of functionalities including essential parasite processes involved in glycolysis, hemoglobin digestion, antioxidant defense and protein synthesis^{89,100}.

Taken together, these studies strongly suggest that artemisinin—and by extension, endoperoxide-based drugs—demonstrate promiscuous targeting and are able to specifically engage multiple targets in various cellular compartments. However, the true impact of these drug-target interactions on parasite killing is still ambiguous and requires further investigation.

1.4.3 Artemisinin Resistance

From the late 2000s pockets of decreased drug sensitivity to artemisinin-based drugs have been found in Southeast Asia—originating in Western Cambodia, and then spreading to its neighboring regions in Southern Thailand, Southern Myanmar, and Southern Vietnam¹⁰¹⁻¹⁰⁴. What was originally characterized by delayed parasite clearance among patients treated with ACTs¹⁰¹—i.e. patients taking longer than the typical 72 hr-period it takes to be clear of infection—has now escalated to an alarming surge in treatment failure rates^{105,106} marking the beginning of true clinical resistance to ACT. Interestingly, standard *ex vivo* 72-hr drug assays that are used to measure artemisinin sensitivity are not able to differentiate between the slow-clearing (artemisinin-resistant) vs fast-clearing (artemisinin-sensitive) parasites¹⁰⁷.

Figure 1.4 Sites of artemisinin resistance across Southeast Asia

Source: World Health Organization, 2013. Emergency Response to Artemisinin Resistance in the Greater Mekong Subregion¹

Over the years, molecular and cellular characterizations of artemisinin resistance based on field isolates have distinguished certain features of the artemisinin resistance phenotype. Studies on Southeast Asian field isolates have revealed that the decreased susceptibility to artemisinin drugs is specific only to the early ring stage of the IDC, and that the mature forms of the parasite remain susceptible to the drug¹⁰⁷. Furthermore, transcriptional and morphological analyses of slow-clearing clinical isolates showed that they exhibit an altered growth phenotype marked by a delayed progression during the early stages of the life cycle during which they are least susceptible to the effects of the drug¹⁰⁸⁻¹¹⁰. It's also been observed that the decrease in artemisinin sensitivity is mediated by a state of quiescence

whereby ring-stage parasites are able to enter a period of developmental arrest upon drug exposure, and then resume normal growth after the removal of drug pressure^{111,112}. On the other hand, the assumption of quiescence in response to artemisinin is not unique to resistant parasites, but is a phenotype that is conserved across different drug-sensitive strains of *Plasmodium falciparum*⁷⁹. In addition to alterations in the parasite's cell cycle, another proposed mechanism through which the parasites are able to overcome the effects of artemisinin is through an enhanced cellular stress response against cytotoxic effect of the drug. Large-scale population transcriptomics of over 1000 field isolates identified a significant upregulation of pathways related to antioxidant defense and the unfolded protein response¹⁰⁹.

Apart from characterizing decreased artemisinin sensitivity based on *in vivo* studies, a number of lab-adapted *P. falciparum* parasite lines have been previously developed by different groups to exhibit a robust artemisinin-resistant phenotype *in vitro* by placing previously drug-sensitive parasites under drug pressure over an extended period of time. These *in vitro* models utilized varied drug selection strategies using different artemisinin derivatives for selection, as well as made use of different starting parasite lines¹¹³⁻¹¹⁶. Interestingly, the stage-specificity of drug tolerance varied among *in vitro* resistant lines in that some exhibit decreased sensitivity at ring stages^{112,114}, while for others, the resistance extends to mature stages as well^{115,117}. It can be said, however, that there are similarities between the artemisinin resistance phenotypes in Southeast Asia and those exhibited by the *in vitro* models. One *in vitro* resistant parasite line that was generated from an F32 strain through long-term DHA pressure also pointed to a temporary growth arrest at early stages as the potential mechanism for resistance^{79,114,117}. On the other hand, the findings of a separate group that subjected D6 and W2 strains of *P. falciparum* through selection with

artemisinin and AL suggest that it is not the induction of dormancy that enables the parasites to tolerate artemisinin-based drugs, and instead, it is the heightened ability to exit dormancy and resume a normal growth rate that facilitates resistance to artemisinin¹¹⁶. Meanwhile, transcriptomic and biochemical analysis of the DHA-resistant parasites generated by Cui et al. also reported an upregulation of genes pertaining to antioxidant defense and protein damage as possible contributors to mediating artemisinin resistance¹¹⁵. Genotyping of *in vitro*-derived artemisinin-resistant parasites have also identified specific genetic alterations that are associated with the artemisinin resistance phenotype. Among them, are copy number amplifications in the transporter gene *Pfmdr1*¹¹⁵ and RNA helicase-encoding *PfPrp22*¹¹⁸, as well as a nonsense mutation in the gene *Pffp2a* which encodes cysteine proteinase falcipain 2a—a crucial hemoglobin digestion enzyme^{114,119,120}. Additionally, downregulation of *Pffp2a* has been shown to result in a decrease in artemisinin sensitivity at the early ring stage¹²¹. While these genetic changes could indeed be capable of influencing artemisinin sensitivity, none of them have yet been observed to be linked to the delayed clearance in field isolates.

A great deal of research has also been dedicated to identifying biomarkers of clinical artemisinin resistance in *P. falciparum*. Genetic surveillance of clinical isolates have found an association between artemisinin sensitivity and the presence of mutations in certain candidate genes. Early studies that conducted targeted investigation of specific candidates among Southeast Asian isolates identified mutations in genes encoding transport proteins such as *Pfmdr1*¹²², *Pfmrp*¹²³ and *PfG7*¹²⁴ to be associated with decreased sensitivity to artemisinin. Subsequent genome-wide association studies (GWAS) also identified two mutations in chromosome 10 (PF3D7_1017000) and chromosome 13 (PF3D7_1343400, *Pfrad5*) to be associated with delayed parasite clearance¹²⁵. Interestingly, both of the

genes identified are involved in DNA replication, DNA repair and cell cycle regulation¹²⁶. While there is a plausible role for such functions as metabolite transport, DNA damage tolerance and cell-cycle regulation in mediating artemisinin resistance, none of these gene hits have yet been identified in other GWAS, nor subjected to functional validation.

It was not until 2014 that Arieu et al were able to identify a biomarker of clinical artemisinin resistance with high confidence using both *in vitro* and *in vivo* datasets¹¹⁹. After sequencing over 150 Cambodian isolates, they found mutations in one gene on chromosome 13 that was similarly mutated in a separate artemisinin-resistant parasite line that was subjected to DHA exposure for over five years^{114,119}. Interestingly, the chromosome 13 region around which the gene sits has been previously identified by multiple reports as under strong selection pressure particularly among Thai and Cambodian isolates that exhibit slow clearance rates^{125,127}. The gene identified, PF3D7_1343700 or *Pfk13*, encodes a protein belonging to the Kelch superfamily of proteins on account of the presence of the Kelch motif—which is known to be responsible for facilitating protein-protein interactions¹²⁸. Among different organisms, Kelch proteins can be found in various localizations within the cell and are able to participate in multiple aspects of cellular function such as maintaining structural integrity, signaling and even the regulation of gene expression¹²⁸. Clinical studies have identified a significant association between the presence of mutations specifically in the C-terminal propeller domain of *Pfk13* and the delayed parasite clearance among Southeast Asian isolates¹⁰⁵. Functional studies have also validated that specifically mutating the *Pfk13* propeller locus significantly increases the rate of parasite survival after ring-stage treatment with DHA¹²⁹. Furthermore, this *Pfk13*-mediated decrease in artemisinin sensitivity has been found to encompass different genetic backgrounds of *P. falciparum*^{130,131}.

Although the actual function of Pfk13, as well as the specific mechanism through which it is able to confer artemisinin resistance in parasites remains to be elucidated, it has been shown to be essential to parasite survival during the blood stage¹³². It was also found to be localized in specific foci within the parasite cytoplasm—with at least one of the foci being in close proximity to the digestive vacuole¹³². Pfk13 has been shown to be homologous to the mammalian Kelch proteins KLHL12, KLHL2 and KEAP1¹¹⁹; KLHL12, and KLHL2 are involved in ubiquitin-mediated protein degradation^{133,134}, while KEAP1 is a key regulator of cytoprotective responses against oxidative stress¹³⁵. Given that the activity of artemisinin and its derivatives is linked to cellular oxidative damage, it's been proposed that Pfk13 might be acting in a similar way—as a regulator of cellular stress responses in the parasite, thereby enhancing their ability to cope with drug pressure. Pfk13 has also been shown to directly interact with Pfp3k—which is a proposed target of artemisinin—and is able to regulate its level of ubiquitination⁹⁷. *Pfk13* mutants have been found to exhibit reduced binding of Pfp3k, which leads to lower levels of ubiquitination of the protein and subsequently, its degradation. This in turn leads to higher levels of the PI3P signaling molecule which may induce the downstream activation of cellular processes that lead to artemisinin resistance⁹⁷. Currently, much investigation is still under way to better understand the role of Pfk13 in parasite biology and how it relates to the artemisinin resistance phenotype.

Since the identification of *Pfk13* as a strong biomarker for artemisinin resistance in Southeast Asia, the prevalence of mutations in this gene have also been monitored in other malaria-endemic territories, particularly in Africa, which shoulders the largest disease burden. Unlike Southeast Asia, however, ACT strategies for the treatment of malaria remain efficacious in

the region^{105,136,137}. Genetic surveillance studies have shown that *Pfk13* mutations are naturally occurring in African isolates but exhibit a different spectrum of SNPs compared to Southeast Asia—none of which have been correlated with artemisinin resistance^{136,138,139}. And despite the heterogeneity of mutations detected, the *Pfk13* gene does not appear to be under selection pressure in Africa¹³⁸. The fact that *Pfk13* is important for parasite survival and is highly conserved across *Plasmodium* species suggests that the presence of *Pfk13* mutations comes with a fitness cost. It is possible that the specific Southeast Asian genetic backgrounds on which *Pfk13* mutations arise are more permissive of the relevant resistance-conferring changes in *Pfk13* due to the presence of pre-existing compensatory mutations. Not only does this allude to the involvement of other genetic loci in mediating artemisinin resistance, but it could also account for the different spectrum of *Pfk13* mutations that we observe outside Southeast Asia. Further research is required to determine the clinical impact of these alternative *Pfk13* genotypes on African isolates.

While reports of longer parasite clearance times after treatment with ACT remain geographically confined to a number of regions in Southeast Asia, the possibility of artemisinin resistance spreading to other regions is a looming threat that needs to be promptly addressed. The gravity of the situation is underscored by the fact that there are currently no immediately available, alternative antimalarials that are as safe and potent as artemisinin-based drugs that can be used to treat the disease should ACT fail. Furthermore, the variety of artemisinin resistance phenotypes and genotypes that have been observed *in vitro* strongly suggest many other biological and genetic factors may be able to confer decreased sensitivity to artemisinin and its cognate drugs. And although the current landscape of artemisinin resistance that we observe in Southeast Asia appears to be largely mediated by the mutant alleles in *Pfk13*, it is possible that there are

alternative or secondary determinants that can modulate artemisinin response and sensitivity among *Plasmodium* parasites in other regions and under a different set of epidemiological conditions. It is therefore imperative that we try to understand the molecular processes that govern the acquisition of artemisinin resistance so that we might be able to aid in the development of strategies to prevent and contain it. With this in mind, we endeavored to derive several artemisinin -resistant parasite lines *in vitro* in order to fully characterize the artemisinin resistance phenotype at the cellular and molecular levels, as well as to try and identify possible mechanisms through which it arises.

Chapter 2. Materials and Methods

2.1 *In vitro* culture of *Plasmodium falciparum*

Two clonal parasite lines, named 6A and 11C, were grown in parallel and used as parents for drug selection. Each clone was previously derived from the *P. falciparum* 3D7 strain using limiting dilution. The 3D7 strain is considered to be artemisinin-sensitive as well as chloroquine-sensitive, and is a commonly utilized reference strain. Parasite cultures were maintained in purified human red blood cells at 1-2 % hematocrit, in RPMI 1640 medium supplemented with 0.25 % Albumax I (Gibco), 2 g/L sodium bicarbonate (NaHCO₃) (Sigma), 0.1 mM hypoxanthine (Sigma), and 50 µg/L gentamicin (Gibco). Parasite cultures were kept at 37 °C with 5 % carbon dioxide (CO₂), 3 % oxygen (O₂), and 92 % nitrogen (N₂) and treated twice with 5 % (v/v) sorbitol every cycle to maintain stage synchronicity. Culture medium was replenished every 12-24 hrs and freshly washed uninfected red blood cells (RBC) was added to the culture as needed. Monitoring of parasitemia and parasite morphology was performed using microscopic evaluation of thin blood smears stained with Giemsa (Sigma).

2.2 *In vitro* Selection of artemisinin-resistant *P. falciparum*

6A and 11C parents were each divided into two parasite lines: one selection line (6A-R, 11C-R), which would be subjected to artemisinin selection, and one control line (6A and 11C), which would undergo mock treatment with dimethyl sulfoxide (DMSO). All parasite lines were synchronized at 4-6 hpi and diluted to a parasitemia (percentage of parasitized erythrocytes) of 2-5 %. Each selection line was then pulse treated with a 900 nM artemisinin (Sigma) diluted in DMSO (Sigma) for 4 hrs from 10-14 hpi. Control lines were also pulse treated in parallel with pure DMSO, for 4 hrs at 10-14 hpi. During treatment, all parasites were kept at 2 % hematocrit with 1 mL of parasitized blood. After 4 hrs, cultures were centrifuged and media containing artemisinin and DMSO were removed. Cultures were then

washed twice with 30 mL fresh media to ensure removal of artemisinin or DMSO. After washing, cells were resuspended in fresh media. Blood smears fixed with methanol (Merck) and then stained with Giemsa (Sigma) were prepared for each parasite line 20-24 hrs after washing to obtain post-treatment parasitemia, as well as observe any morphological effects of drug treatment. Cultures were not kept away from drug/DMSO treatment for more than three consecutive generations. Both sets of parasite lines were subjected to the same number of artemisinin and DMSO treatments throughout the course of drug selection, and at the same generations.

2.3 Evaluation of chemosensitivity

2.3.1 4-hr pulse drug assay

Ring-stage parasites growing in a tightly synchronous culture were synchronized by treatment with 5 % (v/v) sorbitol (Sigma) at 2-6 hpi, and diluted down to 1 % parasitemia, 2 % hematocrit. They were then dispensed into 24-well plates containing varying concentrations of the drug being tested—prepared via serial dilution—to make for a final parasitemia of 1 %, at 1 % hematocrit. Parasites were incubated in drug for 4 hrs at 10-14 hpi for the ring stage, 20-24 hpi for the trophozoite stage and 30-34 hpi at the schizont stage, after which each plate was centrifuged and drug-containing media was removed. Each plate was then washed twice with fresh media and parasites were finally resuspended at 1 % hematocrit and then allowed to reinvade. Number of new, viable parasites in each well on the subsequent cycle after invasion was then evaluated by flow cytometry using a double staining method that utilizes Hoechst 33342 and dihydroethidium (see section 2.3.4.1). All assays involved technical duplicates per dose and was always done in parallel for 6A-R and 6A, and 11C-R and 11C at the same generations. Dose-response curves were plotted and fitted using the 'drc' package in R; IC50_{4hr} values were obtained using the same package.

2.3.2 Standard 72-hr drug assay

Ring-stage parasites growing in a tightly synchronous culture were synchronized by treatment with 5 % (v/v) sorbitol (Sigma) at 2-4 hpi and diluted down to 1 % parasitemia, 2 % hematocrit. They were then dispensed into 24-well plates containing varying concentrations of the drug being tested—prepared via serial dilution—to make for a final parasitemia of 1 %, at 1 % hematocrit. The parasites were incubated in drug starting from 6 hpi all the way into the next invasion cycle for 66-72 hrs. Number of new, viable parasites after reinvasion was then evaluated by flow cytometry using a double staining method that utilizes Hoechst 33342 and dihydroethidium (see section 2.3.4.1). All assays were carried out in biological triplicates, where each experiment involved technical duplicates per dose, and was always done in parallel for 6A-R and 6A, and 11C-R and 11C at the same generations. Dose-response curves were plotted and fitted using the ‘drc’ package in R; IC50 values were obtained using the same package.

2.3.3 Ring-stage survival assay (RSA)

The ring-stage survival assay (RSA) was developed as an alternative to the 72-hr drug assay for phenotyping artemisinin sensitivity in clinical isolates, and has now been adapted as a standard technique for evaluating *in vitro* artemisinin sensitivity¹⁴⁰.

For RSA, tightly synchronous parasites are synchronized at 1-2 hpi and then normalized to 0.5-1 % parasitemia, and resuspended at 2 % hematocrit. The parasite culture was then aliquoted into a 48-well plate, where the wells contained either pure DMSO or 700 nM DHA (dissolved in DMSO), to a final volume of 1 mL. Parasites were then incubated for 6 hrs at 37 °C, after which the culture media containing DMSO or DHA was promptly removed.

The parasitized RBC fraction was then washed twice, and then resuspended in fresh culture medium to a hematocrit of 2 %, and transferred to a fresh 48-well plate. The parasite cultures were then incubated at 37 °C for 66-72 hrs and allowed to grow into the next invasion cycle, after which the amount of remaining viable parasites was measured with flow cytometry using Sybr Green I and MitoTracker Deep Red FM (see section 2.3.4.2). Percent survival was then calculated by dividing the viable parasitemia after DHA treatment, over the viable parasitemia in DMSO-treated wells. All assays were carried out in biological triplicates, where each experiment involved technical duplicates per dose, and was always done in parallel for 6A-R and 6A, and 11C-R and 11C at the same generations.

2.3.4 Detection of viable parasites using flow cytometry

2.3.4.1 Double staining using Hoechst 33324 and dihydroethidium

Viable parasites were quantified using a double-staining protocol as previously described¹⁴¹. Briefly, 1 µL of parasitized blood was incubated with a mixture of 8 µM Hoechst 33324 (SIGMA) and 5 µg/mL dihydroethidium (Sigma) in 1x phosphate buffered saline (PBS) (137 mM sodium chloride (NaCl), 2.7 mM potassium chloride (KCl), 10 mM disodium hydrogen phosphate (Na₂HPO₄), 1.8 mM potassium dihydrogen phosphate (KH₂PO₄)) at 1 % hematocrit for 20 mins. 400 µL cold 1 x PBS was then added, and the parasites were then detected with the LSR II or Fortessa X20 Flow Cytometer (BD Biosciences) using the Indo-1 blue and PE channels for Hoechst 33324 and dihydroethidium, respectively. The viable parasitemia was measured from a total of 100,000 events (cells).

2.3.4.2 Double staining using Sybr Green and MitoTrackera

Alternatively, viable parasites were also quantified using double staining with Sybr Green I (SG) (Invitrogen) and MitoTracker Deep Red FM (MT) (Molecular Probes). This flow cytometry-based detection protocol was

developed in conjunction with the RSA¹⁴². Parasitized RBC was first washed with a flow cytometry wash solution (1 x Hank's Balanced Salt Solution (HBSS) (Gibco), 2% Fetal Bovine Serum (FBS) (Sigma), and then stained at 0.5 % hematocrit with a mixture of 0.2 x SG and 0.3 μ M MT by incubating at 37 ° C for 30 mins. Afterwards the mixture was centrifuged, washed twice and then resuspended in the flow cytometry wash solution to 0.7 % hematocrit, and then read out using the Fortessa X20 Flow Cytometer (BD Biosciences) at the FITC and APC channels for SG and MT, respectively. The viable parasitemia was measured from a total of 300,000 events (cells).

2.4 Genomic profiling of *Plasmodium falciparum*

2.4.1 Genomic DNA Isolation

Parasite genomic DNA was extracted using a phenol-chloroform extraction protocol described previously¹⁴³. RBC-free parasites were first prepared by lysing parasitized blood (200-300 μ L) with freshly prepared 0.05 % saponin dissolved in 1 x PBS. The resultant RBC-free parasite pellet was then incubated in a lysis buffer containing 40 mM tris hydrochloride (tris-HCl), 80 mM ethylenediaminetetraacetic acid (EDTA) and 2 % sodium dodecyl sulfate (SDS) for 2 hrs at 37 ° C. An equivalent volume of phenol solution (equilibrated with 10 mM Tris-HCl, pH 8.0 and 1 mM EDTA) (Sigma) was then added to the resulting lysate, and the sample was centrifuged to separate the aqueous and organic phases. Further purification of the aqueous phase was performed by the addition of an equivalent volume of chloroform (Fisher Scientific) and subsequent centrifugation—after which the aqueous phase was transferred to a fresh tube and once again subjected to another chloroform extraction step. Absolute ethanol (2.5 volumes) (Merck), and 3 M sodium acetate ($C_2H_3NaO_2$), pH 5.2 (0.1 volume) was then added to the resulting aqueous fraction, and the mixture was incubated at -20 ° C overnight to precipitate the DNA. After centrifugation

of the sample, the resulting DNA pellet was then washed with 70 % ethanol, and allowed to air-dry under a vacuum at room temperature. The DNA pellet was then resuspended in 50-200 µL nuclease-free water.

Alternatively, genomic DNA that was specifically sent for whole genome sequencing was purified from parasitized RBCs using the Easy DNA kit (Invitrogen) according to the manufacturer's specifications. All DNA samples were stored at -20 °C.

2.4.2 Copy Number Variation (CNV) detection using Comparative Genomic Hybridization

Comparative Genomic Hybridization (CGH) was performed as previously described in detail^{144,145}. Genomic DNA (3 µg) first amplified and coupled with aminoallyl-dUTP (aa-dUTP) (Biotium) using Klenow Fragment (3'→5'exo⁻) enzyme (New England Biolabs) and random (N9) primers (1 mM); the Klenow synthesis reaction was carried out for 6 hrs at 37 °C. The reaction products were then purified using the MinElute PCR purification kit (Qiagen) following the manufacturer's instructions. 2-3 µg of Klenow reaction product was then labelled with either Cy5 (GE Healthcare) or Cy3 (GE Healthcare) in the presence of 0.1 M NaHCO₃, pH 9.0 for 4 hrs in the dark. After incubation, all labelled products were purified using the MinElute PCR purification kit (Qiagen). Equal amounts of Cy5-labelled sample DNA and Cy3-labelled reference DNA were then mixed with 2 x Hybridization Buffer (Agilent Technologies) and deposited onto a microarray chip containing 10,367 probes covering over 5,000 genes in the *Plasmodium falciparum* genome. Microarrays were incubated at 65 °C in rotator oven (Agilent Technologies) for 20 hrs and then scanned using the PowerScanner (Tecan). Background subtraction, normalization and filtering of raw signal intensities for each array was carried out using the R package

'limma'¹⁴⁶, and detection of copy number alterations was carried out using the R package 'GADA' (Genomic Alteration Detection Analysis)¹⁴⁷.

2.4.3 Whole Genome Sequencing and SNP identification

Genomic DNA was subjected to high throughput sequencing using the Illumina MiSeq platform. Raw reads were mapped back to the *Plasmodium falciparum* 3D7 reference genome using the Burrows-Wheeler Alignment tool (BWA)¹⁴⁸, and reads having a mapping quality score < 30 were discarded. The sequence alignment data was then post-processed using SAMtools¹⁴⁹ and SNPs were called using SAMtools:mpileup. Only high quality intragenic SNPs where the non-reference allele has position coverage ≥ 75 % were included in the analysis.

2.4.4 Genotyping of SNPs in the artemisinin resistance gene *Pfk13*

Currently, PF3D7_1343700 (*Pfk13*) is the only gene that has been strongly associated with artemisinin resistance both *in vitro* and *in vivo*. Detection of resistance-related mutations in the *Pfk13* gene is therefore an integral part of monitoring artemisinin resistance. The protocol for *Pfk13* genotyping via PCR and sequencing was performed according to the standard procedure developed by the Institut Pasteur du Cambodge and endorsed by the Worldwide Antimalarial Resistance Network (WWARN)¹⁵⁰.

Pfk13 was first PCR-amplified using gene-specific forward and reverse primers and *Taq* DNA Polymerase (New England Biolabs) at the following cycling conditions: initial denaturation at 95 °C for 15 mins, 30 cycles of the following steps—95 °C for 30 s, 58 °C for 2 mins, and 72 °C for 2 mins—and final elongation at 72 °C for 10 mins. The PCR products were then purified using the MinElute PCR purification kit (Qiagen) and sent for capillary sequencing both the forward and reverse PCR primers.

Table 2.1 Primers used for Genotyping *Pfk13*

GENE	NAME	SEQUENCE (5'-3')
PF3D7_1343700, <i>Pfk13</i>	Kelch13_F	CGGAGTGACCAAATCTGGGA
	Kelch13_R	GGGAATCTGGTGGTAACAGC

2.5 Transcriptional profiling

2.5.1 Total RNA Isolation using TRIzol

Total RNA was extracted using the TRIzol-Chloroform method as described previously¹⁴⁵. Parasitized RBCs were resuspended in TRIzol reagent (Invitrogen) at a ratio of 1:10 (RBC:TRIzol). Chloroform (Fisher Scientific) was then added in a 5:1 (TRIzol: chloroform) ratio, and the resulting mixture was centrifuged to separate the aqueous and organic phases. The aqueous phase was then recovered and mixed with an equal volume of Isopropanol (Fisher Scientific) and kept at -20 °C overnight to precipitate the RNA. The resulting RNA pellet was then washed with ice-cold 70 % ethanol, and then air-dried under vacuum at room temperature. The RNA was then resuspended in nuclease-free water and stored in aliquots at -80 °C.

2.5.2 cDNA synthesis and SMART amplification

The protocol for first strand cDNA synthesis and subsequent SMART amplification of double-stranded cDNA was carried out using the protocol previously published by Bozdech et al¹⁴⁵. The SMART (Switching Mechanism at 5' End of RNA Template) method takes advantage of the template switching activity of the reverse transcriptase as well as its propensity to add a polyC track to the 3' of the resulting single-stranded cDNA product. First-strand cDNA synthesis is performed using the SMART-N9 and SMART-oligo-dT primers. The SMART TS-oligo primers containing a polyG sequence will then base-pair with the polyC track and the enzyme switches templates to form an extended single stranded cDNA. Because the resulting products contain the SMART adapter sequence, these can

then be amplified via PCR using primers that are specific to the SMART sequence (Primer IIa).

Table 2.2 Primers Used for Reverse Transcription and SMART Amplification

NAME	SEQUENCE (5'-3')
SMART-oligo-dT	AAGCAGTGGTATCAACGCAGAGTAC(T)30VN (N = A, C, G, T; V = A, G, C)
SMART-N9	AAGCAGTGGTATCAACGCAGAGT(N)9
SMART-TS-oligo	AAGCAGTGGTATCAACGCAGAGTACGCrGrGrG
Primer IIa	AAGCAGTGGTATCAACGCAGAGT

For first-strand cDNA synthesis, 500 ng of total RNA was mixed with SMART-N9, SMART-oligo-dT and SMART TS-oligo primers and reverse transcription was carried out using SuperScriptII (Invitrogen) reverse transcriptase at 42 °C for 50 min. The cDNA product—which represents the total cDNA pool—was then amplified by PCR with Primer IIa, *Taq* DNA Polymerase (New England Biolabs) and using a mixture of aa-dUTP (Biotium) and dNTPs (Fermentas). All aa-dUTP-containing amplification products were then purified using the Minelute PCR Purification Kit (Qiagen).

2.5.3 Microarray Hybridization

After purification, 4 µg of SMART-amplified products were then labelled with either Cy3 (GE Healthcare) or Cy5 (GE Healthcare) in the presence of 0.1M NaHCO₃, pH 9.0 for 4 hrs in the dark. By convention, sample cDNA is labelled with Cy5 and reference cDNA is labelled with Cy3. All Cy-dye labelled products were subsequently purified using the MinElute PCR purification kit (Qiagen). Equal amounts of Cy5-labelled sample cDNA and Cy3-labelled reference cDNA were then mixed with 2 x Hybridization Buffer (Agilent Technologies) and deposited onto a microarray chip containing

10,265 probes covering over 5,000 genes in the *Plasmodium falciparum* genome. Microarrays were incubated at 65 ° C in rotator oven (Agilent Technologies) for 20 hrs and then scanned using the PowerScanner (Tecan). Background subtraction, normalization and filtering of raw signal intensities for each array was done using the R package 'limma'¹⁴⁶. The resulting log2ratio expression values of redundant probe sets representing a single gene were then averaged to obtain the mean expression for that particular gene. These gene expression datasets was then used for downstream analyses.

2.6 Generation of Overexpression Parasite Lines

Four candidate genes (PF3D7_1454700, 6-phosphogluconate dehydrogenase (*Pf6pgd*), PF3D7_1457200, thioredoxin (*Pftrx1*), PF3D7_1457000, signal peptide peptidase (*Pfspp*), and PF3D7_1115600, cyclophilin B (*Pfcyp19b*) were chosen for functional validation using a stable, episomal overexpression system. Each gene was inserted into pBcamR_3xHA vector¹⁵¹, transfected into 3D7-6A parasites and maintained via positive selection with blasticidin (BSD). Expression of each candidate gene is driven by the camI modulin (cam) promoter located upstream of the vector's multiple cloning site. The resulting overexpression parasite lines were then phenotyped for drug sensitivity.

Figure 2.1 Map of pBcamR_3xHA vector

A pBcamR_3xHA vector was used as a backbone for recombinant episome construction. Inserts contain the full-length transcript sequence of the respective candidate gene, and are conjugated to a triplet haemagglutinin (HA) sequence at the 3' end. All recombinant proteins are therefore conjugated to a 3xHA tag at the C terminus which allows for detection using a monoclonal anti-HA antibody.

2.6.1 Recombinant plasmid construction

Full-length cDNA of candidate genes was first derived using the total RNA from isolated from the 6A subclone of 3D7. Single-stranded cDNA of each gene was first generated via reverse transcription using oligo-dT and RevertAid H- Reverse Transcriptase (Thermo Scientific), and then PCR-amplified using gene-specific primers having the *NheI* restriction site on the 5' end forward primer and the *BamHI* restriction site on the 5' end of the reverse primer. Using this first-strand template, PCR amplification of full-length cDNA was performed using the KAPA Hifi DNA polymerase (KAPA Biosystems) using the following cycling profile: initial denaturation at 95 °C

for 5 mins, 30 cycles of the following steps—98 ° C for 2 mins, 58 ° C for 30 s, and 60 ° C for 30 to 90 s depending on the transcript length—and final elongation at 60 ° C for 5 mins. Both the pBcamR_3xHA vector (1 µg) and the resulting PCR amplicons (1 µg) were then subjected to simultaneous double restriction enzyme digestion with *NheI* (New England Biolabs) and *BamHI* (New England Biolabs). Double digestion products were then purified using the MinElute PCR Purification kit (Qiagen) according to the manufacturer's instructions. After purification, each double-digested PCR product was then ligated into double-digested pBcamR_3xHA vector at a 1:5 (mass) vector to insert ratio using T4 DNA ligase (New England Biolabs).

Table 2.3 List of Primers used for Cloning

GENE	NAME	SEQUENCE (5'-3')
PF3D7_1454700 (<i>Pf6pgd</i>)	6pgd_BamHI	ATGCGGATCCATGTGTGATATTGGTTTGATAGGT
	6pgd_NheI	ATGCGCTAGCCCACAAGGTGTGATAATTTC
PF3D7_1457200 (<i>Pftrx1</i>)	Trx_BamHI	ATCGGGATCCATGGTAAAAATTGTAAGTAGTCAA
	Trx_NheI	ATCGGCTAGCAGCTGCGTATTTTTCGATAAG
PF3D7_1457000 (<i>Pfspp</i>)	Spp_BamHI	ATCGGGATCCATGAATTTATTAATAATTGGA
	Spp_NheI	ATCGGCTAGCTTTATTGGTAATTCTTTTTTTC
PF3D7_1115600 (<i>Pfcyp19b</i>)	Cyp19b_BamHI	ATCGGGATCCATGAATAAATTAGTGCAATCATT
	Cyp19b_NheI	ATCGGCTAGCCAATGGCAATTCTCTGATTC

The oligos listed above were designed to specifically amplify the full-length cDNA of each candidate gene. The 5' and 3' ends of each forward and reverse primer sets were appended with restriction sites for *BamHI* and *NheI*, respectively, to allow for unidirectional cloning into the pBcamR_3xHA vector.

2.6.2 Bacterial transformation with recombinant plasmids

Each ligation product (10 µL) was then mixed with competent DH5α (100 µL) and subjected to heat shock transformation at 42 ° C for 1 min, after which the mixture was topped up with 1 mL LB broth and allowed to

recover for 45 mins at 37 ° C, with shaking at 220 rpm. The bacterial cells were then centrifuged, and the pellet was resuspended in 150 µL LB broth and spread onto LB plates containing 100 µg/mL of ampicillin (Gold Biotechnology). Plates were then incubated at 37 ° C for 16 hrs, and 10 colonies from each set of transformants were screened for the present of inserts. Cells from each each colony were used to inoculate 3 mL of LB-ampicillin broth and allowed to grow at 37 ° C for 12-16 hrs with shaking. Plasmids were then extracted using the QIAprep Spin Miniprep kit (Qiagen) according the manufacturer's instructions, and subsequently subjected to double digestion with *Bam*HI (New England Biolabs) and *Nhe*I (New England Biolabs). All insert-positive plasmids were then sent for capillary sequencing using primers that flank the multiple cloning site of the pBcamR_3xHA to verify the insert sequences were indeed identical to the wild-type, full-length transcripts. After insert verification via sequencing, a single colony for each set of transformants was chosen for large-scale cultivation for downstream plasmid purification using the QIAprep Spin Maxiprep protocol following the manufacturer's instructions, and subsequent transfection in parasites from a 3D7-6A background.

2.6.3 Stable Episomal Transfection of *P. falciparum*

Five independent transfection experiments were carried out, with four involving the recombinant plasmids containing each candidate gene, and one using the empty pBcamR_3xHA to serve as a control. Tightly synchronized ring-stage parasites (4-6 hpi) from a 3D7-6A background, and having a parasitemia of 15 % were used for transfection via electroporation.

The purified recombinant plasmid DNA (100 µg) was first topped up with 300 µL Cytomix (120 mM Potassium Chloride (KCl), 0.15 mM Calcium Chloride (CaCl₂), 10 mM Potassium Phosphate buffer (K₂HPO₄/KH₂PO₄), 25

mM HEPES/2 mM Ethylene Glycol tetra-Acetic Acid (EGTA), pH 7.6), and then mixed with 200 μ L parasitized RBC in a pre-chilled 0.2 cm cuvette and electroporated at 320 V, 950 μ F and 0 Ω using the Gene Pulser Excell Total Electroporation system (Bio-Rad). Time constants range from 13-15 ms. Right after transfection, transfected parasites were first grown for 8 hrs in regular culture medium without antibiotic, and then subsequently grown under positive selection in culture medium with 2.5 μ g/mL BSD (Sigma). BSD-resistant parasites were detected after 2-3 weeks of continuous culture under BSD selection. Total (genomic and episomal) DNA was isolated from each transfected parasite line using the Easy DNA Purification kit (Invitrogen) according to the manufacturer's instructions, and was verified for the presence of the each episome containing the gene of interest via PCR amplification of the insert using primers flanking the relevant multiple cloning site.

Table 2.4 List of Primers used to Validate the Presence of the Episome

NAME	SEQUENCE (5'-3')
Cam3HA_mcsF	GTATATTTTAACTAGAAAAGGAATAAC
Cam3HA_mcsR	CATATGTATTTTTTTTGTAAATTCTGTG

In order to validate that the parasites were successfully transfected, the presence of the recombinant episomes was validated via PCR using primers that flank the relevant multiple cloning site.

2.6.4 Verification of Overexpression using Quantitative PCR

To verify that the overexpression lines were indeed producing a surplus of each candidate gene, real-time quantitative PCR (relative quantification) was performed using gene-specific primers that are able to interrogate both the endogenous and episomal transcript, and using PF3D7_1218600 (Arginyl-tRNA synthetase) as the reference gene. The parasite line transfected with the empty pBcamR_3xHA vector was used as the control sample for $\Delta\Delta$ Ct.

First strand cDNA synthesis was first carried out using oligo-dT-primed reverse transcription using SuperScript II Reverse Transcriptase (Invitrogen) and 50 ng of RNA extracted from each transfectant parasite line using TRIzol (see section 2.5.1). The resulting first-strand product was then diluted 10-fold, and 1 μ L of the dilution mixture was then used as the starting template for real-time qPCR. The qPCR reaction consisted of the The KAPA SYBR® FAST qPCR Universal Master Mix (KAPA Biosystems), the first-strand cDNA template, and 0.2 μ M of forward and reverse primers specifically designed for each candidate gene. Cycling was carried out using the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) using the manufacturer's recommended cycling profile. The $\Delta\Delta$ Ct method was used to analyze the relative changes in expression level for each of the four candidate genes, where $\Delta\Delta$ Ct = [(Ct of Sample Target Gene – Ct of Sample Reference Gene) – (Ct of Control Target Gene – Ct of Control Reference Gene)], and $2^{-\Delta\Delta$ Ct is taken as the fold-change of the relative gene expression.

Table 2.5 List of Primers used for Real-time Quantitative PCR

GENE	NAME	SEQUENCE (5'-3')
PF3D7_1454700 (<i>Pf6pgd</i>)	6pgd_F	TGATGGTGGGAATGAATGG
	6pgd_R	ATTCCCTGAGGAACCTGGAC
PF3D7_1457200 (<i>Pftrx1</i>)	Trx_F	TTTTGCTGAATGGTGTGGAC
	Trx_R	TTAATGCTGAGTCGTTTGCTC
PF3D7_1457000 (<i>Pfspp</i>)	Spp_F	TCCCCAGTTTCGAGTGATCC
	Spp_R	GAAGAGCTGGTTGAGGATGTTT
PF3D7_1115600 (<i>Pfcyp19b</i>)	Cyp19b_F	AAAGGGACGGTTGTAGATGG
	Cyp19b_R	CCATCAAGCCATGGAGTAGG
PF3D7_1218600	R-tRNA_synth_F	GAGTACCCCAATCACCTACA
	R-tRNA_synth_R	AAGAGATGCATGTTGCTCATTT

The oligos listed above were used to for real-time qPCR to evaluate the level of overexpression in the transfected parasite lines. The primers for each gene were designed such that both the endogenous and episomally-derived transcripts were amplified and quantified. PF3D7_1218600 serves as the reference gene.

2.6.5 Verification of HA-tagged protein production

In order to confirm that each overexpression parasite line were indeed producing their respective proteins, western blot analysis was carried out using a monoclonal antibody that is specific for the HA tag that can be found on episomally derived protein products.

2.6.5.1 Total Protein Extraction

The parasitized RBC fraction infected with late-stage (36 hpi) parasites at 5 % parasitemia was first lysed with freshly-made 0.1 % Saponin dissolved in 1 x PBS, and then washed three times with 1 x PBS. The resulting RBC-free parasite pellet was then subsequently resuspended in 2 x Laemmli Buffer (100 mM tris-HCl, pH 6.8, 4 % SDS) 20 % glycerol, and 0.2 % bromophenol blue) mixed with 1 x complete, EDTA-free rotease inhibitor (Roche), and then boiled at 100 ° C for 10-15 mins. The mixture was then

centrifuged at 13,200 rpm for 10 mins, and the supernatant containing the total soluble protein lysate was collected and used for polyacrylamide gel electrophoresis (PAGE) and Western blot analysis. Protein samples were stored at -20 °C.

2.6.5.2 Western blot Analysis

Identical amounts of protein lysate (100 µg) from each transfectant line were first mixed at a 1:1 ratio with 2 x Laemmli Buffer that had been freshly supplemented with β-mercaptoethanol to a final concentration of 5 %. Each sample was then loaded onto and separated using a 12 % SDS-PAGE gel, and then transferred onto 0.2 µm Nitrocellulose membrane (Bio-Rad) for 2 hrs at 100 V using a wet-transfer system (Bio-Rad). The membrane was then blocked with 5 % skim milk (Bio-Rad) for 1 hr at room temperature, and then incubated with the primary antibodies at 1:2000 dilution at 4 °C, overnight. To probe the protein of interest, an anti-HA mouse monoclonal antibody (Santa Cruz Biotechnology) at 1:2000 dilution was used, while an anti-β-actin mouse monoclonal antibody (Sigma) at 1:2000 was used as a loading control. The membranes were then incubated with the secondary antibodies for 1.5 hrs at room temperature. An horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody (Santa Cruz Biotechnology) at 1:2000 dilution was used to probe the anti-HA primary antibody, and an HRP-conjugated sheep anti-mouse antibody (GE Healthcare) at 1:2000 dilution was used against the anti-actin antibody. The membranes were washed thoroughly with 1 x tris-buffered saline with tween-20 (TBS-T) between each blocking and probing step. Detection was performed using the Immunocruz Western Blotting Luminol Reagent (Santa Cruz Biotechnology), and the chemiluminescent image was acquired using the Luminescent Image Analyzer LAS4000 System (Fujifilm).

Chapter 3. Baseline phenotypic and genotypic characterization of *Plasmodium falciparum* prior to *in vitro* artemisinin selection

BACKGROUND

To investigate the development of artemisinin (ART) resistance in *Plasmodium falciparum*, resistant parasite lines were generated by *in vitro* selection from two isogenic clones, 6A and 11C. Both clones were previously derived by subcloning the artemisinin-sensitive 3D7 strain¹⁵². In order to gain more insight into artemisinin response, particularly during the asexual stage of the *Plasmodium* life cycle, sensitivity profiles from each clone were obtained prior to artemisinin selection. Baseline genotypic information regarding the copy number variation (CNV) profiles of each clone was also obtained, and then utilized to serve as a baseline reference so that we can track the genetic changes acquired by the parasites as they become resistant to the drug. Using this approach, we hope to fully characterize the spectrum of phenotypic and genotypic differences between artemisinin-sensitive and artemisinin-resistant parasites.

3.1 Evaluation of baseline artemisinin sensitivity of parent 3D7 clones prior to ART selection

3.1.1 Baseline artemisinin sensitivity across the IDC

Baseline sensitivities of 6A and 11C to artemisinin were first measured before each clone was subjected to *in vitro* selection to determine whether or not there was significant difference in artemisinin sensitivity between the clones. Instead of performing the standard 72-hr drug assay where parasites are exposed to varying concentrations of the drug over the course of the entire parasite life cycle, our modified assay employed a short 4-hr pulse exposure to varying concentrations of the drug, taking into account the very short elimination half-life of artemisinin. In addition, artemisinin sensitivity was measured at different stages (10-14 hpi at ring, 20-24 hpi at trophozoite, and 30-34 hpi at schizont) from at least five independent

experiments to discern any stage-specific variations in artemisinin response across the IDC.

ART IC₅₀_{4hr} values between the two clones at ring (Student's t-test, p-value = 0.77), trophozoite (Student's t-test, p-value = 0.76) and schizont (Student's t-test, p-value = 0.43) stages appear to be similar, indicating an equivalent baseline sensitivity to artemisinin between 6A and 11C (Figure 3.1B; Table 3.1). However, there does seem to be stage-dependent differences in ART sensitivity across the IDC for both of the clones with respect to their IC₅₀_{4hr} (ANOVA, p-value 6A = 0.01, p-value 11C = 0.003) and IC₉₀_{4hr} (ANOVA p-value 6A = 0.02, p-value 11C = 0.01) (Figure 3.1B). In terms of IC₅₀_{4hr}, values obtained for the trophozoite-stage are the lowest compared to the IC₅₀_{4hr} at ring- and schizont-stages, suggesting that this stage is the most susceptible to the antimalarial effects of artemisinin. And while the IC₅₀_{4hr} values for the ring and schizont stages for both clones are comparable, the ring-stage IC₉₀_{4hr} is higher than that of the schizont-stage, indicating that although the same dose is able to inhibit half the parasite population at both ring and schizont stages, it takes a higher concentration of drug—four times higher for 6A, and three times higher for 11C—to kill 90% of ring-stage parasites compared to late stage schizonts. Comparing the steepness of the dose-response curves of both clones at the three stages of the IDC, it can also be observed that the ring-stage dose-response curves exhibit the mildest slope, the trophozoite-stage curves—the steepest, while the slope for the schizont-stage dose-response curve is sharper than that of the ring-stage but slightly flatter than the trophozoite-stage curve (Figure 3.1A; Table 3.1). A flat dose-response curve having a smaller slope suggests that an increase in dose will result in a milder effect, so that the effects of the drug is observed over a wider dose range; while a steeper curve indicates that even a small increase in dose can produce a more pronounced effect, resulting in a narrower dose-range across which we can

observe variations in drug response¹⁵³. It is this slope difference between ring-stage and schizont-stage curves that accounts for the discrepancy in $IC_{90_{4hr}}$ between two stages, despite their similar $IC_{50_{4hr}}$ values. Overall, these findings suggest that the ring stage is the least sensitive to artemisinin, while the trophozoite stage is the most sensitive.

Figure 3.1 Measurement of baseline ART response of unselected clones across the IDC

(A) Shown are the mean baseline dose-response curves for a 4-hr ART pulse at 10-14 hpi, 20-24 hpi and 30-34 hpi for 6A and 11C. (B) Histogram shows the mean ART $IC_{50_{4hr}}$ and ART $IC_{90_{4hr}}$ values at 10-14 hpi, 20-24 hpi and 30-34 hpi for 6A and 11C prior to ART selection. Error bars represent the standard deviation. Data shown are derived from at least five biological replicates. Pairwise comparison of stage-specific $IC_{50_{4hr}}$ values between 6A and 11C was performed using Student's t-test. Comparison of $IC_{50_{4hr}}$ and $IC_{90_{4hr}}$ across the three stages of the IDC for each clone was also performed using ANOVA.

Table 3.1 Baseline artemisinin IC₅₀_{4hr} values of unselected clones across the IDC

Clone	Stage	IC ₅₀ _{4hr} (nM)	IC ₉₀ _{4hr} (nM)	γ
6A	Ring (IC₅₀_{10hpi/4hr})	74 ± 144	553 ± 2739	1.09
	Trophozoite (IC₅₀_{20hpi/4hr})	45 ± 11	76 ± 16	4.17
	Schizont (IC₅₀_{30hpi/4hr})	75 ± 18	139 ± 28	3.52
11C	Ring (IC₅₀_{10hpi/4hr})	72 ± 131	420 ± 1285	1.25
	Trophozoite (IC₅₀_{20hpi/4hr})	47 ± 9	79 ± 11	4.20
	Schizont (IC₅₀_{30hpi/4hr})	79 ± 19	142 ± 33	3.79

Numbers represent the IC₅₀_{4hr} and IC₉₀_{4hr} values (mean ± standard deviation), while γ represents the slope of the dose-response curve.

In addition to detecting a differential sensitivity to artemisinin across the three stages of the IDC, it was also observed from looking at the individual dose-response curves generated from biological replicates, that drug-response in the ring stage was more highly variable when compared to drug response at the trophozoite and schizont stages, thereby resulting in a larger range and higher variance in IC₅₀_{4hr} values, as well as highly variable dose-response curves (Table 3.1, Appendix A). We hypothesized that even within the ring stage of the IDC (which accounts for ~1-18 hpi), there might also exist an even more refined stage-dependency in artemisinin response, and that that high level of variability that was observed might be due to the presence of asynchronicity in the population of parasites being exposed to the drug. To further investigate this, a higher resolution evaluation of drug sensitivity at ring stage was performed.

3.1.2 Baseline artemisinin sensitivity within the Ring stage of the IDC

In order to further investigate the intra-stage variability of artemisinin response at the ring stage, sensitivity to a 4-hr drug pulse was measured in

wild-type 3D7 parasites at 2-hr intervals across the ring stage from 6-10 hpi, 8-12 hpi, 10-14 hpi, 12-16 hpi, 14-18 hpi and 16-20 hpi.

We observed that even within the ring stage of the IDC, which lasts until ~20 hpi, parasites appear to slightly differ in their susceptibility to artemisinin with respect to their age (Figure 3.2A, B), although the gradient observed in $IC_{50_{4hr}}$ was not statistically significant (ANOVA, p-value = 0.11). From the data, it appears that drug susceptibility is highest in mid-rings (10-14 hpi) at which the $IC_{50_{4hr}}$ and $IC_{90_{4hr}}$ is lowest, while early rings and late rings appear to be less sensitive to the drug—as demonstrated by the peaks in $IC_{50_{4hr}}$ at the 6-10 hpi and 14-18 hpi timepoints. Although the $IC_{50_{4hr}}$ values at 6-10 hpi and 14-18 hpi timepoints are comparable, it is the early ring stage (~6 hpi) which exhibits the flattest dose-response curve and the highest $IC_{90_{4hr}}$, indicating that it is the least sensitive to artemisinin.

Looking at the gradient of artemisinin susceptibility throughout the ring stage, it seems that from a state of low artemisinin sensitivity in the early stages, susceptibility to the drug peaks as it reaches the mid-ring stage, and then once again decreases as it approaches the later ring stages. Finally, as the parasites transition from the ring to the trophozoite stage, the $IC_{50_{4hr}}$ starts to decline once again as the parasites become more susceptible to the drug. It can also be observed that as the parasites mature from the ring stage to the trophozoite stage, there is a gradual increase in the slope (steepness) of the dose-response curve, which echoes the previously observed stage-dependence of artemisinin response in *Plasmodium*, wherein the trophozoite-stage curve is steeper compared to that of the ring-stage.

Figure 3.2 Measurement of artemisinin response within the ring stage (6-20hpi) of the IDC

(A) Mean ART dose-response curves for a 4-hr ART pulse at 6-10, 8-12, 10-14, 12-16, 14-18 and 16-20 hpi for wild-type *P. falciparum*, 3D7 strain. (B) Histogram shows mean ART IC₅₀_{4hr} and ART IC₉₀_{4hr} values across the ring stage at 2-hr intervals. Error bars represent the standard deviation. Assays were carried out in biological triplicates. Comparison of IC₅₀_{4hr} across different timepoints within the ring stage was performed using ANOVA.

Table 3.2 Baseline artemisinin IC_{4hr} values across the ring stage

Stage	IC50 _{4hr} (nM)	IC90 _{4hr} (nM)	γ
6-10 hpi	101 ± 28	879 ± 339	1.02
8-10 hpi	51 ± 14	293 ± 190	1.26
10-12 hpi	55 ± 10	235 ± 69	1.52
12-16 hpi	83 ± 11	337 ± 66	1.56
14-18 hpi	102 ± 14	295 ± 73	2.06
16-20 hpi	76 ± 23	221 ± 74	2.07

Numbers represent the IC50_{4hr} and IC90_{4hr} values (mean ± standard deviation), while γ represents the slope of the dose-response curve.

3.2 Baseline Copy Number Variation (CNV) profiling of parent clones prior to *in vitro* artemisinin selection

In order to get a copy number profile of each clone prior to selection, comparative genomic hybridization (CGH) was performed on 6A and 11C using genomic DNA from the 3D7 strain of *P. falciparum* as a reference genome. In this context, the CNVs we are able to detect represent amplified or deleted regions in the genome of either clone relative to the 3D7 parent. This copy number information will serve as a baseline against which the DNA of drug-resistant progeny will be compared in order to track any copy number changes that have occurred in each parasite line throughout the course of selection. CGH was also performed to scan for any preexisting CNVs in each of the clones that might suggest a predisposition toward a lower or higher sensitivity to artemisinin as well as other antimalarial drugs. DNA from each clone was co-hybridized with reference DNA onto a microarray containing 10,367 probes covering over 5,000 genes.

Using CGH, we were able to detect a distinct spectrum of CNVs in both 6A and 11C. In 6A, we identified two amplifications in chromosomes 4 and 7, deletions in chromosomes 8, 11 and 14. On other hand, 11C was found to house 7 amplifications in chromosomes 2, 10, 11, 12, 13 and 14 as well as 6

deletions in chromosomes 4, 10, 11, 12 and 14 (Figure 3.3, Table 3.3). Unsurprisingly, a number of large-scale CNVs in were detected in the subtelomeric regions of chromosome 2 (PF3D7_0201500 - PF3D7_0202200), chromosome 4 (PF3D7_0421100 - PF3D7_0421300), and chromosome 12 (PF3D7_1200100 - PF3D7_1201300). Many of the genes comprising these long amplifications/deletions belong to the highly variable *Plasmodium* multigene families such as *Pfemp1*, *rifin*, *hyp* and *phist*, that encode variant surface antigens and exported proteins, and are known to possess high sequence diversity¹⁵⁴⁻¹⁵⁶.

Among the CNVs in 6A, none have been previously associated with any alterations in drug sensitivity. For 11C, however, the copy number alteration in chromosome 10 (PF3D7_1028600 – PF3D7_1030300) and chromosome 12 (PF3D7_122400) have been linked to altered sensitivity to artemisinin-based antimalarials¹¹⁸ and antifolate drugs¹⁵⁷, respectively. Besides these two aforementioned CNVs, we were unable to detect any changes in other copy number variable genes—such as PF3D7_0709000 (*pfprt*), PF3D7_0523000 (*pfmdr1*), and PF3D7_0112200 (*pfmrp1*)—that have been previously reported to be significantly associated with multidrug resistance in *Plasmodium*^{40-43,122,158-160}.

Figure 3.3 CNVs detected in unselected clones 6A and 11C relative to their 3D7 parent

Chromosome plots reflect the subtracted log2ratio of the clone-specific gDNA relative to that of the parental 3D7 strain. Red lines mark the statistically significant copy number variations detected, where $\log_2\text{ratios} < 0$ represent copy losses in the clone relative to the 3D7 parent, and $\log_2\text{ratios} > 0$ represent copy gains in the clone relative to the 3D7 parent.

Table 3.3 List of copy number variable genes in unselected clones 6A and 11C

CLONE	CHR	CLASS	GENE	DESCRIPTION
6A	Chr4	loss	PF3D7_0409000	conserved Plasmodium protein, unknown function
		gain	PF3D7_0419000	conserved Plasmodium protein, unknown function
	Chr7	gain	PF3D7_0703300	conserved Plasmodium protein, unknown function
	Chr8	loss	PF3D7_0831000	Plasmodium exported protein (PHISTb), unknown function
	Chr11	loss	PF3D7_1121800	petidase, M16 family
	Chr14	loss	PF3D7_1477900	acyl-CoA synthetase, pseudogene
11C	Chr2	gain	PF3D7_0201500	Plasmodium exported protein (hyp9), unknown function
			PF3D7_0201600	Plasmodium exported protein (PHISTb), unknown function
			PF3D7_0201700	DnaJ protein, putative
			PF3D7_0201800	knob associated heat shock protein 40
			PF3D7_0201900	erythrocyte membrane protein 3
			PF3D7_0202000	knob-associated histidine-rich protein
			PF3D7_0202100	Plasmodium exported protein (PHISTc), unknown function liver stage associated protein 2
			PF3D7_0202200	EMP1-trafficking protein
	Chr4	loss	PF3D7_0421100	erythrocyte membrane protein 1, PfEMP1
			PF3D7_0421200	rifin PIR protein
			PF3D7_0421300	erythrocyte membrane protein 1, PfEMP1
	Chr10	loss	PF3D7_1016500	Plasmodium exported protein (PHISTc), unknown function
		gain	PF3D7_1028600	conserved Plasmodium protein, unknown function
			PF3D7_1028700	merozoite TRAP-like protein
			PF3D7_1028800	conserved Plasmodium protein, unknown function
			PF3D7_1028900	conserved Plasmodium protein, unknown function
			PF3D7_1029000	conserved Plasmodium protein, unknown function, pseudogene
			PF3D7_1029100	conserved Plasmodium protein, unknown function
			PF3D7_1029200	conserved Plasmodium protein, unknown function
			PF3D7_1029300	conserved Plasmodium protein, unknown function
			PF3D7_1029400	conserved Plasmodium protein, unknown function
			PF3D7_1029500	conserved Plasmodium protein, unknown function
			PF3D7_1029600	adenosine deaminase
			PF3D7_1029700	conserved Plasmodium protein, unknown function
			PF3D7_1029800	RAP protein, putative
			PF3D7_1029900	conserved Plasmodium protein, unknown function
			PF3D7_1030000	transcription factor, putative
				pre-mRNA-splicing factor ATP-dependent RNA helicase PRP22, putative
			PF3D7_1030100	
			PF3D7_1030200	conserved Plasmodium protein, unknown function
			PF3D7_1030300	conserved Plasmodium protein, unknown function
		gain	PF3D7_1034800	conserved Plasmodium protein, unknown function
	Chr11	gain	PF3D7_1131100	serpentine receptor, putative
		loss	PF3D7_1147000	sporozoite asparagine-rich protein
	Chr12	gain	PF3D7_1200100	erythrocyte membrane protein 1, PfEMP1
			PF3D7_1200200	rifin PIR protein
			PF3D7_1200300	rifin PIR protein
			PF3D7_1200400	erythrocyte membrane protein 1, PfEMP1
			PF3D7_1200500	rifin PIR protein
			PF3D7_1200600	erythrocyte membrane protein 1, PfEMP1
			PF3D7_1200700	acyl-CoA synthetase
			PF3D7_1200800	serine/threonine protein kinase, FIKK family
			PF3D7_1200900	Plasmodium exported protein (PHISTc), unknown function
			PF3D7_1201000	Plasmodium exported protein (PHISTb), unknown function
			PF3D7_1201100	RESA-like protein with PHIST and DnaJ domains
			PF3D7_1201200	Plasmodium exported protein (PHISTa-like), unknown function
			PF3D7_1201300	liver stage associated protein 1
		loss	PF3D7_1224000	GTP cyclohydrolase I
	Chr13	gain	PF3D7_1355500	serine/threonine protein phosphatase
	Chr14	gain	PF3D7_1412700	AAA family ATPase, putative
		loss	PF3D7_1436800	ATP-dependent Clp protease proteolytic subunit
		loss	PF3D7_1474200	conserved Plasmodium membrane protein, unknown function

3.3 Discussion

With the increasing number of reports of treatment failure in the field and the apparent emergence of artemisinin-resistant *Plasmodium falciparum* in Southeast Asia, scientific investigation into the cellular and molecular mechanisms that govern artemisinin resistance has intensified in order to develop more effective measures to control the spread of resistance. By inducing artemisinin resistance *in vitro*, we hope to be able to gain a deeper understanding of how parasites respond to artemisinin-type drugs as well as better understand the process through which they acquire the resistance phenotype. A number of groups have previously reported the derivation of parasite lines that have been induced to tolerate markedly higher concentrations of artemisinin and its derivatives, using different parental *P. falciparum* strains and through different selection methods¹¹³⁻¹¹⁷. In this study, we sought to derive ART-resistant parasite lines from two isogenic populations of *Plasmodium falciparum* that were obtained by subcloning the artemisinin-sensitive 3D7 reference strain of *P. falciparum*¹⁵². Instead of a multiclonal population of parasites, isogenic clones were chosen as parents for drug selection because of their more uniform genomic background which should allow for better detection of the unique genetic features of each clone, and tracking of genomic alterations that may arise as a result of drug selection. Accordingly, baseline information about the 6A and 11C subclones—such as such as artemisinin sensitivity, copy number and SNP profiles—were first evaluated, in order to identify any distinct phenotypic or genotypic differences among the two parasite lines that might inform our understanding of any future phenotypes that could arise as a result of *in vitro* artemisinin selection.

First, we evaluated artemisinin sensitivity for both clones at the ring (early), trophozoite (middle), and schizont (late) stages of the IDC. Artemisinin sensitivity across the asexual blood stage was found to be stage-dependent

for both clones, with trophozoites being the most sensitive to the drug, while rings and schizonts are less sensitive. This is in agreement with a previous report documenting maximal drug effects from artemisinin between 18-24 hrs of parasite development¹⁶¹. This heightened susceptibility of trophozoites to artemisinin could be attributed to the fact that hemoglobin digestion, which has been shown to greatly enhance the effects of artemisinin^{77,162}, is most active at this stage of the IDC, thereby leading to more drug-induced killing. In contrast with trophozoites, ring-stage parasites appear to be the most resistant to artemisinin—a finding that is corroborated by other *in vitro* studies on the stage-specificity of artemisinin response in *Plasmodium falciparum*^{77,114}. Values of artemisinin IC₅₀_{4hr} obtained from our drug pulse sensitivity assays were noted to be higher than those typically obtained from standard *in vitro* drug assays^{95,115} likely due to the much shorter incubation time (4 hrs) compared to the 72 hrs it takes for standard drug sensitivity tests. However, in comparison with a recent study by Klonis et al.⁷⁷ that also evaluated the stage-specific artemisinin sensitivity of 3D7 to a 4-hr artemisinin pulse, IC₅₀_{4hr} values from our own experiments were markedly (>100-fold) lower. It is possible that this variation may be a result of differences in assay methodology and parameters such as initial parasite density and hematocrit. Differences in variance was also observed in the IC₅₀ values obtained at different stages of the IDC, with ring-stage IC₅₀_{4hr} values exhibiting more variability relative to the other two stages. Interestingly, this property was observed from a previous intrahost modeling study on the stage-specific interactions of the parasite with the artemisinin¹¹¹. Further evaluation of artemisinin sensitivity across the ring stage also showed intra-stage differences to a short artemisinin pulse, with early rings (6 hpi) appearing to be the most resistant to the drug, and mid-rings (8-10 hpi) and very late (16hpi) being more sensitive. Although this difference was deemed not statistically significant, the observed trend in artemisinin response could provide clues

as to how parasite development is related to the action of artemisinin drugs. Artemisinins require iron for activation¹⁶². Iron, in turn, is largely sourced from host-derived heme obtained via hemoglobin digestion which occurs in the mid ring to trophozoite stages. The low artemisinin sensitivity of early rings might be explained by the fact that this stage precedes the uptake and digestion of hemoglobin—a process which begins later into IDC¹⁶³. Rather, the lower amounts of heme produced from the parasite's own heme biosynthesis pathways at the early ring stages is likely what accounts for its activity at this point in the asexual blood stage⁸⁹. Similarly, the increase in artemisinin susceptibility we observed as the parasites mature is coincident with the increase of hemoglobin uptake and digestion which starts from the mid to late ring stages, and peaks at the mid trophozoite stage¹⁶³. Alternatively, other hemoglobin-independent processes and targets could also account for the observed artemisinin sensitivity profile of ring-stage parasites. The spike in IC₅₀_{4hr} observed at 14 hpi may be also attributed to increased expression of antioxidant defense genes in anticipation of the oxidative stress that accompanies hemoglobin digestion which is at its maximum during very late ring and early trophozoite stages^{164,165}; though it is also probable that the variations in IC₅₀_{4hr} observed from 8-20 hpi may just be described as random fluctuations and not necessarily biologically significant. Ultimately, comparison of the artemisinin sensitivity profiles of 6A and 11C across the IDC also demonstrated that there is no significant difference in baseline sensitivity between the two clones.

In addition to obtaining baseline artemisinin sensitivities for each clone, we also sought to identify distinct, baseline genetic alterations between 6A and 11C. Historically, variations in drug sensitivity in *Plasmodium* have been ascribed to specific changes in the parasite genome both at the SNP and CNV levels. By identifying naturally occurring gene amplifications, gene

deletions and mutations in each clone prior to drug selection, we will be able to better account for possible differences in drug resistance phenotypes that may arise in 6A and 11C as a result of artemisinin selection. Moreover, not only will this information serve as a baseline reference that we can use to monitor genetic changes in each clone over increasing cycles of drug treatment, but it also revealed that neither clone demonstrates any genetic predisposition towards antimalarial drug resistance.

Using CGH we were able to detect 6 and 13 CNVs in 6A and 11C, respectively, relative to the parental 3D7 strain from which they were both derived. Unsurprisingly, copy number polymorphisms were observed in the subtelomeric regions of the chromosomes—particularly in chromosomes 2, 4 and 12 of 11C. Such regions on the ends of the chromosome are known for their high degree of sequence and copy number variability, and typically contain the gene families that encode antigens that are exposed on the surface of the infected red blood cells, as well as proteins that get exported into the host red blood cell during the asexual stage of the life cycle¹⁵⁴⁻¹⁵⁶. Previous genomic studies on 6A by Mok et al. revealed a ~4kb deletion in the promoter of PF3D7_1229100 which encodes *Pfmrp2*—an ATP-binding cassette transporter that resides in the plasma membrane of the parasite^{152,166}. This deletion, in turn, resulted in an increased resistance to chloroquine, mefloquine and quinine. However, apart from this genetic alteration in *Pfmrp2*, none of the CNVs in 6A detected from this experiment have been associated with any prior drug resistance phenotypes. On the other hand, we identified two genes in 11C whose copy number have been associated with resistance to antimalarial drugs. One of those genes, PF3D7_1030100 (*Pfprp22*), which codes for a pre-mRNA-splicing factor ATP-dependent RNA helicase, has been found to be duplicated in an artemisinin-resistant parasite line that was derived through *in vitro* drug

selection^{113,118}; moreover they found that the increase in gene copy number contributes ascribes increased fitness to the parasite against treatment with DHA¹¹⁸. However, the presence of this amplification did not appear to confer a decreased sensitivity to artemisinin in 11C relative to 6A. Furthermore, the large-scale amplification on chromosome 10 spanning 18 genes (PF3D7_1028600 - PF3D7_1030100) has also been found to be a naturally occurring CNV in the 3D7 strain of *P. falciparum* even in the absence of prior artemisinin exposure¹⁶⁷. Apart from PF3D7_1030100, 11C also bore a copy loss in PF3D7_1224000 which encodes the GTP cyclohydrolase enzyme (*Pfgch1*). That *Pfgch1* is copy number variable in 3D7 is unsurprising, as it has been previously shown that the 3D7 parental strain has multiple copies of the gene¹⁵⁷, and could very likely contain multiple subpopulations of parasites with varying copy numbers of the gene. An increase in *Pfgch1* gene dosage has been observed in clinical isolates from regions that have experienced long term antifolate drug pressure¹⁶⁸, and Heinberg et al. have also demonstrated that alterations in *Pfgch1* copy number and gene expression is also able to change the parasite's sensitivity to pyrimethamine, a commonly used antifolate antimalarial¹⁵⁷. There is no evidence, however, that it has any significant effect on artemisinin sensitivity.

High throughput whole genome sequencing has also been previously performed on both 6A and 11C in order to further identify genetic differences between the two, and it was found that none of the genes previously implicated in artemisinin resistance contained any relevant SNPs¹⁵². Overall, our results suggest that given the distinct spectrum of genetic polymorphisms in 6A and 11C, the baseline artemisinin sensitivity between the two subclones remains comparable and neither appears more or less susceptible to artemisinin than the other.

Chapter 4. *In vitro* selection of artemisinin-resistant *P. falciparum* from a parental 3D7 strain using repeated ring-stage pulse exposure.

BACKGROUND

It has been previously shown that resistance to artemisinin can be induced in lab-adapted *P. falciparum* strains through discontinuous, long-term exposure to artemisinin or artemisinin -based derivatives¹¹³⁻¹¹⁶. Similarly, we also set out to derive artemisinin resistance in two subclones of a lab-adapted reference strain (3D7). The selection protocol involved periodic exposure of each clone to 900 nM artemisinin over a period of 4 hrs (Figure 4.1). We chose an artemisinin dose that is similar to the maximum recorded plasma concentration of the drug^{169,170}, and the 4-hr pulse is meant to mimic the rapid elimination half-life of artemisinin. Both clones were also treated during the ring stage of the IDC, as it is this morphological phase of parasite asexual development that has been previously implicated in artemisinin resistance *in vitro*¹¹⁴ as well as *in vivo*^{107-109,114}. Moreover, the majority of the *P. falciparum* parasites in the natural infections are present in the peripheral blood at the mid ring stage^{108,109}. Compared to previously published reports characterizing *in vitro*-derived resistant *P. falciparum* parasites, what makes this study unique is that it takes into account the *in vivo* conditions that characterize drug-parasite interactions within the infected host.

In order to monitor changes in artemisinin response with increasing cycles of artemisinin exposure over time, phenotypes with respect to parasite survival and gross morphology were observed throughout the early stages of artemisinin selection. To measure changes in artemisinin sensitivity between the drug-treated and control parasites, the 4-hr drug pulse assay at the ring stage (IC₅₀_{10hpi/4hr}) was performed periodically throughout the course of drug selection.

Figure 4.1 Workflow for *in vitro* selection of ART resistance using ring-stage pulse exposure

The *in vitro* ART selection protocol involved repeated 4-hr pulse treatments of synchronized mid-ring stage parasites (6A and 11C) to 900 nM ART. DMSO-treated 6A and 11C parasites were grown alongside the ART-treated parasites (renamed as 6A-R and 11C-R) to serve as controls. Both sets of parasites were subjected to the same number of ART and DMSO treatments throughout drug selection, and at the same generations.

4.1 Surveillance of parasite response to artemisinin throughout *in vitro* selection

4.1.1 Effects of artemisinin pulse exposure on *Plasmodium falciparum* parasites

To get a better picture of how 6A and 11C respond to a pulse treatment of ART during the ring stage (10hpi), synchronized parasites were incubated with 900 nM artemisinin for 4 hrs, after which parasite morphology and viability were observed 14 hrs and 26 hrs after treatment.

We found that in addition to killing parasites, artemisinin treatment also resulted in delaying the growth of surviving parasites (Figure 4.2). Microscopy images reveal the presence of dead parasites—demonstrated by the presence of pyknotic bodies (blue and purple dots) in the smears across all timepoints. Moreover, some of the parasites exhibiting a viable morphology appeared to be progressing at a slower rate across the IDC. Comparing the morphology of control and artemisinin-treated parasites, DMSO-treated parasites appear to progress through the IDC normally; they move on from the early ring stage into the trophozoite stage, and then mature into the actively dividing schizont stage. On the other hand, images of the artemisinin-treated cultures show the persistence of earlier-stage parasites, such as rings and trophozoites, even many hours post-treatment when the parasites were expected to have fully matured into schizonts. These observations indicate that while a pharmacologically relevant dose of artemisinin (900 nM) is indeed parasitocidal even in the early stages of the IDC, a subpopulation of ring-stage parasites also have the ability to tolerate drug, although their growth and subsequent progression throughout the IDC is retarded.

Figure 4.2 Effects of a 4-hr pulse of 900 nM ART on *Plasmodium* parasites at 10-14hpi

Following a 4-hr pulse treatment of unselected 6A and 11C parasites from 10-14 hpi, Giemsa-stained smears were prepared at three different timepoints: right after treatment, 14 hrs after treatment and 26 hrs after treatment. In parallel, smears of DMSO-treated parasites were also monitored at the same timepoints for comparison.

4.1.2 Monitoring parasite survival and morphology throughout *in vitro* selection

During the initial stages of *in vitro* drug selection, the level of survival and morphology during every round of pulse treatment of synchronous parasites was also monitored. Giemsa-stained smears were prepared right before treatment at ring stage, and then 20-24 hrs after artemisinin was removed when cultures were expected to have progressed to the mature schizont stage.

Similar to the abovementioned findings, death and retardation in IDC progression was observed following artemisinin treatment. In addition, a small fraction of parasites was also able to survive exposure as well as progress through the IDC normally. Figure 4.3 summarizes the proportion of parasites that were able to survive the treatment, as well the different morphological stages observed through microscopy. During the first few rounds of pulse treatment, parasitemia levels dropped post-treatment, demonstrating that a large fraction of susceptible parasites in the culture were being killed by the drug. Stalling of the IDC progression was also observed in treated cultures, where even 20-24 hrs after treatment, ring and trophozoite forms were still present in the smear even though they were all expected to be undergoing schizogony. As the cycles of treatment increased, however, a greater proportion of parasites were able to survive exposure to the drug, with later treatment cycles resulting in at least 85% parasite survival. In addition to increased survival, more surviving parasites were also able to progress through IDC at a normal rate resulting in a larger fraction of mature, schizont-stage parasites with fewer stallers.

Figure 4.3 Monitoring parasite viability and morphology in 6A-R and 11C-R

At the start of ART selection, parasite viability and morphology after 4-hr ART treatment was monitored in both 6A-R and 11C-R using microscopic evaluation of Giemsa-stained blood smears. The solid gray line depicts the proportion of surviving parasites 24 hrs post treatment normalized to the starting parasitemia, while stacked bars depict proportions of ring, trophozoite and schizont-stage morphologies observed among the remaining parasites that appeared to be viable.

4.2 Evaluation of ring-stage artemisinin sensitivity throughout *in vitro* selection

To more quantitatively assess changes in ring-stage artemisinin response in 6A-R and 11C-R over increasing cycles of drug selection, chemosensitivity was measured at different generations throughout the course of artemisinin selection using ART pulse drug assays. Dose-response curves and $IC_{50_{10\text{hpi}/4\text{hr}}}$ values from each parasite line were then obtained and compared.

Monitoring of artemisinin sensitivity throughout artemisinin selection demonstrated an increase in the $IC_{50_{10\text{hpi}/4\text{hr}}}$ of clones that were subjected to multiple rounds of artemisinin treatment compared to control clones that were mock-treated in parallel with DMSO. Within 1.5 months and after 8 rounds of ring-stage pulse exposure, 6A-R had a 3-fold higher $IC_{50_{10\text{hpi}/4\text{hr}}}$ than 6A, and 11C-R demonstrated a 16-fold higher $IC_{50_{10\text{hpi}/4\text{hr}}}$ than 11C (Figure 4.4A, Table 4.1). This steady increase in $IC_{50_{10\text{hpi}/4\text{hr}}}$ of the artemisinin-selected clones compared to their controls was also accompanied by a rightward shift in their dose-response curves against a 4-hr treatment with artemisinin at the ring stage of the IDC (Figure 4.4B).

We also observed differences between 6A-R and 11C-R in the rate of emergence and level of artemisinin resistance acquired. 11C-R was able to rapidly acquire a high level of resistance within fewer cycles of selection compared to 6A-R, but subsequently plateaued after 12 months; whereas 6A-R demonstrated only a modest decrease in artemisinin susceptibility for the first 12 months of *in vitro* selection, but was ultimately able to reach a higher fold-difference in artemisinin $IC_{50_{10\text{hpi}/4\text{hr}}}$ compared to its control in the succeeding months of periodic drug treatment. The range of acquired change in artemisinin sensitivity for 6A-R and 11C-R also appeared to be variable, with 6A-R peaking at 398 times higher $IC_{50_{10\text{hpi}/4\text{hr}}}$ compared to its

parental clone after 20 months of selection, while 11C-R maximally reached a 70-fold higher $IC_{50_{10hpi/4hr}}$ compared to 11C after only 12 months of selection.

Figure 4.4 Monitoring the ring-stage ART sensitivity ($IC_{50_{10hpi/4hr}}$) of 6A-R and 11C-R

(Cont'd) **Figure 4.4 Monitoring the ring-stage ART sensitivity ($IC_{50_{10hpi/4hr}}$) of 6A-R and 11C-R**

(A) Line graphs illustrate the mean ART $IC_{50_{10hpi/4hr}}$ values of 6A-R and 11C-R relative to their nonselected counterparts, at different points in time throughout the course of ART selection, with the fold-difference between the ART-treated clones and their controls indicated by the grey bars. Error bars represent the standard deviation. Mean values of ART $IC_{50_{10hpi/4hr}}$ for all clones were calculated from ART dose-response curves depicted in (B). All drug assays were carried out in duplicates.

Table 4.1 IC₅₀_{10hpi/4hr} values of 6A-R, 6A, 11C-R and 11C throughout the course of ART selection

SELECTION CYCLE	6A-R IC ₅₀ _{10hpi/4hr} (nM)	6A IC ₅₀ _{10hpi/4hr} (nM)	FOLD CHANGE IN IC ₅₀ _{10hpi/4hr}
6	55 ± 17	18 ± 4	3
8	130 ± 15	42 ± 6	3
28	3,619 ± 92	1,606 ± 533	2
30	13,211 ± 5,798	2,780 ± 1,684	5
37	626 ± 140	235 ± 22	3
38	275 ± 15	86 ± 4	3
40	421 ± 23	64 ± 7	7
100	6,304 ± 591	93 ± 3	68
112	3,880 ± 193	137 ± 34	28
140	281 ± 53	27 ± 0.4	10
175	33,726 ± 8,540	85 ± 7	398
237	19,097 ± 5,331	97 ± 4	196

SELECTION CYCLE	11C-R IC ₅₀ _{10hpi/4hr} (nM)	11C IC ₅₀ _{10hpi/4hr} (nM)	FOLD CHANGE IN IC ₅₀ _{10hpi/4hr}
6	36 ± 1	29 ± 0.4	1
8	522 ± 160	32 ± 4	17
28	11,914 ± 2,106	781 ± 159	15
30	14,217 ± 1,409	1,354 ± 272	11
37	3,052 ± 53	155 ± 8	20
38	2,060 ± 289	59 ± 3	35
40	2,459 ± 525	70 ± 4	35
100	3,135 ± 224	45 ± 13	69
112	2,145 ± 399	159 ± 20	14
140	395 ± 31	29 ± 1	14
175	1,587 ± 145	56 ± 6	28
237	1,983 ± 117	63 ± 3	31

Numbers represent the IC₅₀_{10hpi/4hr} values (mean ± standard deviation).

4.3 Validating artemisinin resistance using alternative chemosensitivity assays

In addition to comparing the ring-stage artemisinin sensitivity in between *in vitro* selected and control parasite lines, artemisinin resistance was also validated in the later stages of *in vitro* selection using a standard 72-hr drug

assay test and a novel phenotypic test, the Ring Stage Survival assay (RSA). The RSA was specifically developed to interrogate artemisinin resistance in slow clearing isolates¹⁴⁰. In contrast with the ring stage pulse assay that we typically employ to quantify artemisinin response, standard chemosensitivity tests for *Plasmodium* involve incubation of the parasites over a range of drug concentrations for 72 hrs—lasting all throughout the entire IDC and well into the next invasion cycle. On the other hand, the RSA involves only a 6-hr pulse exposure of the parasites during the very early ring stage (0-3 hpi) of the IDC, and to a single, physiologically relevant dose of DHA (700 nM). Rather than calculating for the IC50, this assay measures the proportion of surviving parasites post DHA treatment. The RSA was originally developed to specifically identify artemisinin-resistant parasites *ex vivo*, since typical drug susceptibility assays that measure IC50 are unable to differentiate between slow-clearing (artemisinin-resistant) and fast-clearing (artemisinin-sensitive) isolates^{107,140,171}. The RSA outcomes, however, correlate very well with the rate of parasite clearance among clinical isolates in Southeast Asia—likely due to the fact that resistance mechanism observed in this region is linked only to the early stage of the IDC¹⁰⁷.

Measuring the drug sensitivity using a standard 72-hr assay, we found that 6A-R and 11C-R both have significantly elevated (~2-fold-higher) artemisinin IC50 values compared to their non-selected controls (Figure 4.5A). Similarly, we also found using RSA, that the percentage of surviving parasitemia after DHA treatment was also higher for 6A-R and 11C-R compared to their controls, although this observation was only borderline significant for 11C-R (p-value = 0.06) (Figure 4.5B). Nevertheless, these findings reinforce the conclusion that both 6A-R and 11C-R have indeed acquired artemisinin resistance as a result of periodic ring-stage pulse treatment with the drug.

Figure 4.5 Evaluating ART sensitivity of 6A-R and 11C-R using standard and novel phenotypic assays

Differential susceptibility to ART between ART-selected and control parasites was evaluated using a standard 72-hr drug assay (IC₅₀) where parasites are exposed to the drug throughout the entire IDC (A), and via the RSA—a method that has been shown to differentiate slow-clearing vs. fast clearing isolates (B). Mean values of ART IC₅₀ each parasite line was calculated from their respective mean dose-response curves as depicted in the figure. Error bars represent the standard deviation. Data shown are derived from at least three biological replicates. Pairwise comparison of IC₅₀ and RSA values between resistant and sensitive parasites was performed using Student's t-test.

Table 4.2 Artemisinin IC₅₀ values of 6A-R, 6A, 11C-R and 11C based on a standard drug sensitivity assay

ART IC (nM)	6A-R	6A	11C-R	11C
IC ₅₀	50 ± 7	23 ± 3	35 ± 1	20 ± 3
IC ₉₀	97 ± 11	43 ± 6	62 ± 8	34 ± 7

Numbers represent the 72-hr IC₅₀ and IC₉₀ values (mean ± standard deviation).

4.4 Evaluating the stage specificity of artemisinin resistance

Because clinical artemisinin resistance has been exclusively attributed to the early stages of the IDC, phenotyping of drug sensitivity has been mostly targeted toward the ring stage. Likewise, to match our own temporal window of *in vitro* selection (10-14 hpi) we have employed a ring stage-specific drug pulse assay to quantify artemisinin resistance in 6A-R and 11C-R. However, to further address how the artemisinin resistance phenotype induced in our *in vitro*-selected parasites affects the other stages of the asexual life cycle, we also obtained the IC₅₀_{4hr} of our resistant and control parasite lines at the trophozoite (20-24hpi) and schizont (30-34hpi) stages of the IDC.

We found that for 6A-R, the artemisinin-resistance phenotype is specific only to the ring stage and that its artemisinin sensitivity remains comparable with 6A at the trophozoite and schizont stages (Figure 4.6A, B). In the case of 11C-R, however, the window of decreased artemisinin susceptibility extends further into the IDC up until the initial trophozoite stage (~20hpi) (Fig 4.6A, B). Overall, these observations suggest that the artemisinin resistance phenotype exhibited by 6A-R and 11C-R are specific to the earlier stages of the asexual life cycle of the parasite during which drug selection was carried out.

Figure 4.6 ART sensitivity of 6A-R and 11C-R in the later stages of the IDC

ty at later timepoints in the IDC was evaluated in all parasite lines. Mean IC₅₀_{4hr} values were obtained for the trophozoite stage (20 hpi) (A), as well as the schizont stage (30 hpi) (B). Calculated values and mean dose-response curves shown are derived from three biological replicates. Error bars represent the standard deviation. Pairwise comparison of stage-specific IC₅₀_{4hr} values between resistant and sensitive parasites was performed using Student's t-test.

Table 4.3 ART IC_{4hr} values of 6A-R, 6A, 11C-R and 11C in the later stages of the IDC

ART IC	6A-R	6A	11C-R	11C
IC ₅₀ _{20hpi/4hr} (nM)	34 ± 4	35 ± 9	59 ± 9	38 ± 2
IC ₉₀ _{20hpi/4hr} (nM)	60 ± 8	58 ± 13	156 ± 65	55 ± 8
IC ₅₀ _{30hpi/4hr} (nM)	53 ± 7	58 ± 22	54 ± 6	67 ± 8
IC ₉₀ _{30hpi/4hr} (nM)	85 ± 15	122 ± 41	85 ± 13	127 ± 21

Numbers represent the IC₅₀_{4hr} and IC₉₀_{4hr} values (mean ± standard deviation).

4.5 Discussion

In vitro models are valuable resources for elucidating the biological underpinnings of specific phenotypes. Likewise, we also aimed to generate our own models of artemisinin resistance that would allow us to investigate the emerging artemisinin resistance that has been observed in Southeast Asian clinical isolates. Accordingly, we employed a selection regimen that was designed in a way that approximates how the malaria parasites interact with the drug *in vivo* where artemisinin is rapidly cleared from the host's system in a matter of a few hours^{70,76}, and a large proportion of the circulating parasite population are still in the early (ring) stages of the IDC¹⁷². To select for artemisinin resistance *in vitro*, two clones of the reference strain 3D7 having a comparable baseline artemisinin sensitivity were periodically exposed to a pharmacologically relevant dose of artemisinin (900 nM)^{169,170}, from 10-14hpi. And although other groups have previously generated artemisinin-resistant parasites through *in vitro* selection¹¹³⁻¹¹⁶, these previous studies were carried out using a different genetic background of lab-adapted strains which were more than likely to be polyclonal, as a result of continuous culture over countless generations. Moreover these artemisinin-resistant lines were also derived through putting the parasites under long-term, continuous drug pressure with artemisinin or its synthetic derivatives a manner that is expressly different from how they are exposed to artemisinin within infected patients.

It must be noted however, that despite our best efforts to recreate the physiological conditions that typify artemisinin exposure of *Plasmodium* parasites, we are unable to completely recapitulate all of the clinical conditions. Since artemisinin has been globally endorsed as first-line therapy for malaria, it has been administered combinatorially with other less potent, but longer acting partner drugs; this is referred to as artemisinin combination therapy or ACT. However, to eliminate the

confounding effect of an additional drug pressure to our *in vitro* models, resistance was raised using a drug selection protocol that utilized artemisinin exclusively. In addition, our *in vitro* drug selection method also varies slightly from clinical conditions in terms of dosing frequency. Under a typical 3-day course of ACT, the parasites are exposed to artemisinin for two consecutive asexual cycles. Accordingly, the drug selection protocol was originally designed to have a dosing frequency of every generation. In this case, however, consecutive rounds of treatment could not be performed during the initial stages of drug selection because 6A and 11C were allowed to first reconstitute a suitable level of viable parasitemia ($\geq 2\%$) before pulse-treatment was resumed; this meant a recovery period of one or two asexual cycles where the parasites were not exposed to the drug. Once we observed that a substantial proportion of parasites in either clone were able to consistently survive pulse treatment, the dosing frequency was increased to every generation. Over time, however, this dosing regimen proved to be too logistically demanding to maintain. Ultimately, the frequency of exposure was set to a more pragmatic rate of every other asexual cycle—with a few rare exceptions. Parasite lines were never kept away from drug/mock treatment for longer than three consecutive generations and treatment was always done in parallel the same number of times and at the same generations.

Observing the effect of artemisinin on each 6A-R and 11C-R during the initial rounds of treatment, both parasite lines appeared better able to survive a short pulse of artemisinin, as well as developed the ability to bypass the growth retardation effect that artemisinin-based drugs have been known to induce in ring-stage parasites^{79,114,162,173}. Such resistance characteristics were also observed in by Teuscher et al.¹¹⁷ and Tucker et al.¹¹⁶ in other resistant parasite lines that were generated *in vitro*; these resistant parasites were able to mature and multiply normally even under

drug exposure, either by tolerating the drug without undergoing growth arrest or recovering from dormancy faster than sensitive parasites. Witkowski et al.¹¹⁴ and Cui et al.¹¹⁵ similarly reported early recovery following exposure among resistant parasites compared to the sensitive parental line. It must be noted that the resistant parasites studied by other groups were derived from different strains (D6, W2, F32 and Dd2) of *P. falciparum*, as well as selected through cycles of continuous drug pressure, rather than the short pulse treatments utilized in this study. That this tolerance/recovery phenotype is observed in all *in vitro* selected parasite lines suggests a related, if not identical, mechanism is responsible for causing a lower sensitivity to artemisinin-type drugs. *In vivo*, however, it has been established that ring-stage resistance is attributed to the parasite's ability to remain in a quiescent or "dormant ring" state^{107,110}, rather than an enhanced ability to recover and subsequently exit dormancy—which is the phenotype we observed in both 6A-R and 11C-R. This marked difference in artemisinin response hints at potential differences between the artemisinin resistance phenotype that we have so far observed in Southeast Asia versus what we have selected for via *in vitro* drug selection.

Through monitoring the artemisinin chemosensitivity of artemisinin-treated and control parasite lines, we also found that both 6A-R and 11C-R displayed lower susceptibility to artemisinin based on short pulse assays (IC₅₀_{10hpi/4hr}) within as little as 1.5 months of initiating drug selection. This is in stark contrast with a previous report by Witkowski et al.¹¹⁴ where they observed that the chemosensitivity of artemisinin-treated *P. falciparum* parasites from an F32 background remained unaltered until after 3 years, and 100 cycles of drug pressure. Furthermore, Cui et al. reported to be unable to raise drug resistance in a 3D7 strain at all, after subjecting several lab-adapted strains—7G8, Dd2, HB3, D10 and 3D7—to drug

selection using DHA¹¹⁵. While both 6A-R and 11C-R remained less susceptible to artemisinin throughout the course of *in vitro* selection, the fold increase in IC50_{10hpi/4hr} relative to their controls did not appear to be consistently unidirectional—possibly due to the presence of a fluctuating population of resistant and sensitive parasites in the culture. This may also be attributed to slight differences in parasite age and synchronicity between the resistant and sensitive parasites during the experiment; It has been shown previously that ring-stage parasites exhibit markedly different sensitivities to short pulse of artemisinin⁷⁷.

Comparing the rate of acquisition of artemisinin-resistance between each clone, we also observed differences in the selection dynamics of each clone. 11C-R acquired a high (>10-fold higher IC50_{10hpi/4hr}) level of resistance much earlier (~1.5 months), and after fewer cycles of treatment, compared to 6A-R. On the other hand, while 6A-R only exhibited only up to a 6.5-fold difference in IC50_{10hpi/4hr} after 40 rounds of artemisinin selection, it ultimately acquired a greater level of resistance next to 11C-R, after over 12 months and 100 cycles of artemisinin pulse exposure. That we observed marked differences in the dynamics and degree of artemisinin resistance acquisition between the two clones is interesting, considering the fact that both parasite lines were derived from the same strain and had similar drug sensitivities and comparable IC50_{10hpi/4hr} values prior to selection. This could be due to a pre-existing difference in their predisposition to develop a high level of artemisinin resistance, or a result of divergent molecular events through which each clone developed resistance—both of which are likely to be genetically determined. On the other hand, it is also possible that these perceived differences in selection dynamics between the two clones may have also just arisen by chance and do not strictly represent biologically distinct phenotypes. Multiple, independent, rounds of artemisinin selection on different batches of 6A and 11C will have to be performed in order to

validate these observations. In addition, the fact that resistance was induced in previously artemisinin-sensitive isogenic clones suggests that the resistance phenotype observed here emerged *de novo* and was not through the enrichment of a pre-existing subpopulation of resistant parasites.

After over a year of drug selection, we further validated artemisinin resistance in both 6A-R and 11C-R using alternative chemosensitivity tests such as the standard 72-hr growth inhibition assay (IC₅₀) for *Plasmodium*, as well as the RSA. When artemisinin sensitivity was evaluated using a standard 72-hr drug assay, we found that both artemisinin-treated parasite lines became significantly less susceptible to artemisinin and exhibited ~2-fold higher IC₅₀ values compared to their controls. A similar phenotype has also been identified in previously *in vitro*-derived parasite lines, with groups reporting significantly elevated artemisinin IC₅₀ values for their artemisinin-resistant parasite lines that were selected from Dd2, W2 and D6 parental backgrounds^{113,115,116}. Interestingly, this observation deviates from previously documented artemisinin resistance in clinical isolates, whereby the slow-clearing (artemisinin-resistant) and fast-clearing parasites (artemisinin-sensitive) had comparable artemisinin IC₅₀ values^{102,107,174}. This lack of change in IC₅₀ was likewise observed by Witkowski et al¹¹⁴ when their group detected no difference between IC₅₀ of their lab-adapted artemisinin-resistant strain relative to the control. Upon quantifying artemisinin sensitivity based on RSA, both 6A-R and 11C-R exhibited a modest but significant increase in their RSA values. However, the range of RSA values we obtained were much lower than what has been previously reported in artemisinin-resistant clinical isolates (typically >10% survival); and the difference between our control and artemisinin-resistant parasites appeared to be less pronounced^{129,140}. These observations further reinforce the idea that there might be fundamental differences in the artemisinin resistance phenotype between our *in vitro*-derived parasites

compared to artemisinin-resistant parasites in the field. While 6A-R and 11C-R exhibit a high level of resistance in the midring timepoints and demonstrate a recovery-mediated artemisinin resistance phenotype, slow-clearing Southeast Asian isolates are highly resistant only in the very early ring stages of the IDC, and appear to exhibit a resistance phenotype that is driven by an ability to remain in a protracted state of quiescence or dormancy. What remains similar between them, however, is that artemisinin resistance is specific only to the ring stages of the IDC, and that the parasites remain as sensitive to the drug as their nonselected controls if initial artemisinin exposure occurs during the later (>20hpi) stages of the asexual blood stage.

Taken together, these results demonstrate that the repeated short exposure of ring-stage *P. falciparum* parasites to artemisinin in the context of clinically relevant conditions can readily produce parasite populations with substantial stage-specific resistance to the drug, and that this resistance phenotype is characterized by a heightened tolerance against artemisinin and an ability to circumvent artemisinin-induced dormancy. Moreover, based on our observations of 6A-R and 11C-R, we infer that the ability to acquire artemisinin-resistance is not necessarily a one-track process, and might even be driven by different genetic backgrounds and even different biological processes.

Chapter 5. Chemosensitivity phenotypes of artemisinin-resistant *P. falciparum*

BACKGROUND

Based on the current clinical framework, artemisinin-combination therapy (ACT) is used as a first-line treatment for uncomplicated malaria in the majority of malaria-endemic territories. With ACT, artemisinin-based drugs are administered in conjunction with other slow-acting non-artemisinin antimalarial drugs that have very different mechanisms of action such as pyrimethamine (PYR)—an antifolate, and the quinoline type antimalarials such as mefloquine (MEF), quinine (QN) and chloroquine (CQ)^{28,69,76}. Artemisinin, along with its synthetic derivatives are classified as endoperoxide antimalarials due to the endoperoxide bridge within their structures that appear to be essential to their parasite-killing activity^{68,72,88}. Its mode of action is largely attributed to the alkylation of particular intracellular targets by artemisinin-derived free radicals that are released as a result of iron-mediated activation of the drug inside the parasite^{68,72,88}. In contrast, PYR is a competitive inhibitor of the dihydrofolate reductase (DHFR) enzyme, and its parasitocidal effect is due to the disruption of folate metabolism and the subsequent reduction of cofactors that are essential for the synthesis of DNA and select amino acids^{14,58,175}. As ACT component, PYR is always administered combinatorially with a sulfonamide drug, sulfadoxine. On the other hand, quinoline drugs MEF, QN and CQ are thought to disrupt heme detoxification and hemozoin formation in parasites, leading to death³¹. With the emergence and spread of artemisinin resistance in the field, other classes of antimalarials provide possible alternatives to ACTs. It is therefore important to address how the development of artemisinin resistance in clinical isolates affects response to non-artemisinin antimalarials. Accordingly, we also sought to investigate how our *in vitro*-derived artemisinin-resistant parasite lines respond against non-artemisinin compounds, as well as similarly-acting synthetic

artemisinin derivatives such as artesunate (ATS) and dihydroartemisinin (DHA).

We also attempted to specifically identify probable biochemical pathways that could be mediating artemisinin resistance by challenging our *in vitro*-derived parasites with small molecular compounds that have a defined mode of action, and that are also related to the killing mechanism of artemisinin-based drugs—which is largely thought to be profound oxidative damage^{68,88}. It is therefore unsurprising that stress responses against cellular damage such as antioxidant defense^{109,115} and the unfolded protein response^{84,109} have been implicated in resistance to artemisinin *in vitro* as well as *in vivo*. To test for this, we challenged our parasites with Hydrogen Peroxide (H₂O₂)—a powerful oxidizer, as well as two agents that are known disruptors of protein homeostasis^{176,177}: dithiothreitol (DTT)—a reducing agent, and epoxomicin (EPX)—a proteasome inhibitor.

5.1 Evaluating the chemosensitivity artemisinin-resistant *P. falciparum* against other antimalarial drugs

After inducing artemisinin resistance in 6A-R and 11C-R, their sensitivity to other antimalarial drugs was also measured a standard 72-hr growth inhibition assay. We found that the resistance induced in both parasite lines as a result of artemisinin selection did not extend to other classes of antimalarials such as PYR, QN, CQ and MEF. Interestingly, both artemisinin-resistant parasite lines appeared to be significantly more sensitive (~2-fold) to MEF compared to the nonselected controls (Figure 5.1B, Table 5.1). It is worth noting, however, that this significant alteration in MEF sensitivity was not observed for an artemisinin-resistant T996-derived parasite line that was previously subjected to *in vitro* drug selection in our lab using the same regimen (Appendix B).

On the other hand, both 6A-R and 11C-R demonstrated significantly elevated $IC_{50_{10\text{hpi}/4\text{hr}}}$ values to other widely used synthetic artemisinin derivatives ATS and DHA, suggesting that the ring-stage resistance phenotype we obtained through long-term *in vitro* selection also applies to other artemisinin-based drugs and similarly acting endoperoxides (Figure 5.1A, Table 5.1). As previously demonstrated by the incremental tracking of artemisinin $IC_{50_{10\text{hpi}/4\text{hr}}}$ for all parasite lines over time, 6A-R and 11C-R once again display varying levels of resistance to ATS and DHA—with the former appearing to have acquired a greater degree of resistance to artemisinin and its derivatives, compared to the latter. Compared to their nonselected controls, 6A-R exhibits 5- and 8.6-fold higher $IC_{50_{10\text{hpi}/4\text{hr}}}$ against DHA and ATS, respectively, while 11C-R only appears to be 2.4- and 2.7-fold more resistant to DHA and ATS, respectively. Moreover, we also observed that while 6A-R and 11C-R were highly resistant to artemisinin—reaching several hundred- or ten-folds higher than their parental controls—the decrease in sensitivity against DHA and ATS were substantially more modest.

Figure 5.1 Evaluating the chemosensitivity of *in vitro*-selected *P. falciparum* against other antimalarial drugs

(Cont'd) Figure 5.1 Evaluating the chemosensitivity of *in vitro*-selected *P. falciparum* against other antimalarial drugs

Ring-stage chemosensitivity (IC₅₀_{10hpi/4hr}) of *in vitro*-selected parasites against pulse exposure to artemisinin derivatives artesunate (ATS) and dihydroartemisinin (DHA) was monitored (A), while susceptibility to non-artemisinin antimalarials quinine (QN), chloroquine (CQ), mefloquine (MEF) and pyrimethamine (PYR), was evaluated using a standard 72-hr drug assay (IC₅₀) (B). Mean values of IC₅₀/IC₅₀_{10hpi/4hr} for each antimalarial drug were calculated from their respective mean dose-response curves as depicted in the figure. Error bars represent the standard deviation. All drug assays were

performed in biological triplicates. Pairwise comparison of IC₅₀_{10hpi/4hr} and IC₅₀ values between resistant and sensitive parasites was performed using Student's t-test.

Table 5.1 IC values of 6A-R, 6A, 11C-R and 11C against ART derivatives and other classes of antimalarials

DRUG	IC (nM)	6A-R	6A	11C-R	11C
DHA	IC ₅₀ _{10hpi/4hr}	67 ± 31	13 ± 4	21 ± 3	9 ± 3
	IC ₉₀ _{10hpi/4hr}	430 ± 278	47 ± 13	83 ± 18	28 ± 10
ATS	IC ₅₀ _{10hpi/4hr}	1,693 ± 2,043	195 ± 20	327 ± 109	120 ± 14
	IC ₉₀ _{10hpi/4hr}	27,492 ± 85,095	808 ± 159	1,696 ± 882	404 ± 44
QN	IC ₅₀	30 ± 4	32 ± 3	28 ± 2	32 ± 31
	IC ₉₀	50 ± 4	46 ± 2	46 ± 3	47 ± 2
CQ	IC ₅₀	9 ± 0.2	12 ± 3	10 ± 0.4	12 ± 2
	IC ₉₀	12 ± 0.2	20 ± 7	12 ± 0.8	17 ± 4
MEF	IC ₅₀	8 ± 1	16 ± 1	7 ± 0.4	17 ± 3
	IC ₉₀	12 ± 0.7	24 ± 0.5	11 ± 2	28 ± 5
PYR	IC ₅₀	25 ± 0.6	23 ± 2	24 ± 2	20 ± 2
	IC ₉₀	46 ± 4	39 ± 3	41 ± 2	36 ± 3

Numbers represent the IC₅₀_{4hr} and IC₉₀_{4hr} values (mean ± standard deviation) for ART derivatives (DHA and ATS) and the 72-hr IC₅₀ and IC₉₀ values (mean ± standard deviation) against non-ART derivatives (QN, CQ, MEF, PYR).

DHA = dihydroartemisinin; ATS = artesunate; QN = quinine; CQ = chloroquine; MEF = mefloquine; PYR = pyrimethamine

5.2 Evaluating the chemosensitivity of in artemisinin-resistant *P. falciparum* against artemisinin-related compounds

To gain further insight into the stage-specific resistance mechanisms acquired by 6A-R and 11C-R, we also assessed the susceptibility of resistant and control parasite lines against H₂O₂, DTT and EPX; H₂O₂ is known to

induce widespread oxidative damage, while DTT and EPX are known inducers of ER stress—causing an accumulation of damaged/misfolded proteins inside the cell^{176,177}. These compounds were chosen to specifically interrogate the oxidative stress response and unfolded protein response pathways as possible mediators of artemisinin resistance in our *in vitro*-derived resistant parasite lines.

Surprisingly, we found that 6A-R and 11C-R demonstrated different chemosensitivity phenotypes against these three drugs. 6A-R became significantly more resistant to all three compounds, and demonstrated 4-, 2- and 1.5-fold higher IC₅₀_{10hpi/4hr} values compared to 6A for H₂O₂, DTT and EPX, respectively. In contrast, no difference in ring-stage sensitivity against H₂O₂, DTT and EPX were observed between 11C-R and 11C.

Figure 5.2 Evaluating the chemosensitivity of in *vitro*-selected *P. falciparum*

Ring-stage chemosensitivity ($IC_{50_{10hpi/4hr}}$) against pulse exposure of oxidizing agent—hydrogen peroxide (H_2O_2), and ER-stress inducers—dithiothreitol (DTT) and epoxomicin (EPX), was evaluated for are parasite lines. Mean $IC_{50}/IC_{50_{10hpi/4hr}}$ for each compound were calculated from their respective dose-response curves as depicted in the figure. Error bars represent the standard deviation. All drug assays were performed in biological triplicates. Pairwise comparison of $IC_{50_{10hpi/4hr}}$ values between resistant and sensitive parasites was performed using Student's t-test.

Table 5.2 IC_{4hr} values of 6A-R, 6A, 11C-R and 11C against ART-related compounds

DRUG	IC	6A-R	6A	11C-R	11C
H₂O₂	IC50_{10hpi/4hr}mM	23 ± 6	5 ± 2	5 ± 0.9	6 ± 2
	IC90_{10hpi/4hr}mM	184 ± 59	52 ± 4	38 ± 13	64 ± 18
DTT	IC50_{10hpi/4hr}uM	1,471 ± 134	665 ± 81	898 ± 155	754 ± 107
	IC90_{10hpi/4hr}uM	4,784 ± 705	1,157 ± 216	1,777 ± 121	1,342 ± 218
EPX	IC50_{10hpi/4hr}nM	16 ± 0.1	10 ± 0.2	12 ± 0.6	10 ± 0.5
	IC90_{10hpi/4hr}nM	27 ± 2	17 ± 2	19 ± 0.8	17 ± 2

Numbers represent the IC50_{4hr} and IC90_{4hr} values (mean ± standard deviation).

H₂O₂ = hydrogen peroxide; DTT = dithiothreitol; EPX = epoxomicin

5.3 Discussion

After validating artemisinin resistance in both our 3D7-derived parasite lines 6A-R and 11C-R, we then went on to phenotype their chemosensitivity to other clinically relevant drugs that are still widely used for treating malaria-infected patients. We profiled antimalarials that were biochemically unrelated to artemisinin such as the quinoline drugs QN, CQ and MEF, as well as the antifolate compound PYR, in addition to two commonly used synthetic artemisinin derivatives, DHA and ATS.

Our findings demonstrate that the artemisinin resistance phenotype we selected for does not readily result in cross-resistance to the other classes of antimalarials tested in this study. Moreover, both artemisinin-resistant parasite lines also appeared to be significantly more sensitive to MEF, while remaining comparably susceptible to its cognate drugs QN and CQ. On the other hand, we were unable to observe any significant alteration in MEF susceptibility in an artemisinin-resistant T996-derived parasite line that was previously generated in our lab using the selfsame method implemented for 6A-R and 11C-R. Interestingly, previously published findings regarding

the chemosensitivity profiles of *in vitro*-derived artemisinin-resistant parasites also vary widely. The artemisinin-resistant parasites selected by Cui et al. from a Dd2-parent via continuous DHA exposure for became significantly more resistant not only against artemisinin-based drugs, but also to those belonging to the quinoline class of antimalarials—including QN, CQ and MEF¹¹⁵. In contrast, artemisinin-resistant lines that were derived using long term drug pressure with artelinic acid (AL) from D6 and W2 backgrounds showed cross-resistance against MEF, while developing increased susceptibility to CQ^{113,116}. Furthermore, a lab-adapted clinical isolate from Thailand named TM91C235 that was subjected in parallel to the exact same selection protocol with AL showed no significant change in sensitivity to other non-artemisinin antimalarials^{113,116}. Our observations in this regard, in addition to the previous reports on *in vitro* models of artemisinin resistance, demonstrates the complex dynamics that underlie antimalarial drug resistance and highlights the difficulty in evaluating the impact of artemisinin resistance on non-artemisinin-based medicines. And while we can make no claims over the translatability of our *in vitro* results with respect to the artemisinin resistance in clinical isolates, our observations allude to the possibility that resistance to artemisinin-based drugs could also affect the clinical efficacy of its partner drugs, current alternative non-ACT therapies, and perhaps even potential antimalarial drugs that are currently in the pipeline. These results highlight the importance of testing for cross-resistance as an integral part of drug development, and also demonstrate a key use for *in vitro* drug resistance models that can be utilized as a platform with which to perform such extensive and rigorous studies.

While no cross-resistance was detected in 6A-R and 11C-R, both artemisinin-resistant parasite lines also appeared to be significantly less sensitive to ATS and DHA. Both of these drugs are synthetic derivatives of

artemisinin and are have been more commonly deployed in the field for malaria treatment via ACTs since their development¹⁷⁸. All three compounds are classified as sesquiterpene endoperoxide drugs—and as such, are thought to possess a shared mechanism through they exert their parasitocidal effects on *Plasmodium* parasites⁷⁰; It is therefore unsurprising that the significantly decreased artemisinin sensitivity acquired by 6A-R and 11C-R also extends to other artemisinin derivatives. Interestingly, we observed varying degrees of decreased sensitivity among these three examples of artemisinin class antimalarials. Where 6A-R and 11C-R reached up to almost 70 and 400 times higher artemisinin IC₅₀_{10hpi/4hr} values than their controls, respectively, both parasite lines exhibited an increase of less than 5- and 10-fold in their DHA and ATS IC₅₀_{10hpi/4hr} values relative to their nonselected counterparts, respectively. This difference in degrees of resistance might be linked to the fact that although artemisinin, DHA and ATS are grouped under a single class of drugs, there are key differences in their pharmacokinetic and pharmacodynamic profiles—particularly their absorption and how they are metabolized. Compared to the plant-derived parent drug, both ATS and DHA are more water-soluble synthetic formulations of artemisinin and are therefore more readily absorbed^{70,115}. They are also considered to be more potent antimalarials as DHA is itself the active metabolite that is responsible for parasite killing, while ATS is immediately converted into dihydroartemisinin upon administration^{68,72}. In contrast, DHA is not a principal metabolite of artemisinin. Artemisinin itself acts as the primary antimalarial agent, and is subsequently primarily transformed into inactive metabolites deoxyartemisinin and dihydrodeoxyartemisinin^{75,86,179}. This variance in resistance level could be attributed to the differential levels of cytopathic activity of each drug, with DHA and ATS being remarkably more potent than artemisinin. It might also be explained by distinct mechanistic differences between how artemisinin and DHA biochemically behave inside the malaria-infected erythrocytes, be

it in their mode of activation or even the actual protein targets each compound is specifically able to engage. This observation underscores the possibility of having different resistance mechanisms that are specific to the compound.

Besides evaluating the chemosensitivity of 6A-R and 11C-R against other antimalarials, we also attempted to interrogate the ER stress and oxidative stress pathways as putative mechanisms of resistance. To do that, our artemisinin-resistant parasites were challenged with known compounds that trigger these selfsame pathways: H₂O₂, DTT, and EPX. Like artemisinin, H₂O₂ is a free radical generator and is therefore a potent inducer of oxidative stress in the parasite. On the other hand, DTT¹⁷⁷ and EPX¹⁷⁶ are both known disruptors of protein homeostasis. DTT is a reducing agent and an inhibitor of disulfide bond formation, while EPX is a proteasome inhibitor; both compounds therefore lead to the accumulation of misfolded proteins within the cell resulting in ER stress¹⁷⁷. Likewise, artemisinin-based compounds have also been shown to cause the ROS-mediated induction of ER stress *in vitro*^{180,181}. It is therefore unsurprising that *in vitro* and *in vivo* artemisinin resistance in *P. falciparum* have been linked to an upregulation of antioxidant defense pathways^{109,115}, as well as the unfolded protein response (UPR)^{84,109}. We hypothesize that if ER stress and/or oxidative stress responses are truly mediating the resistance phenotype in either or both of our artemisinin-selected parasites, they would manifest cross-resistance against H₂O₂ and/or DTT and EPX. Interestingly, this is exactly what we observed in the case of 6A-R, but not 11C-R. 6A-R showed significantly elevated H₂O₂, DTT and EPX IC₅₀_{10hpi/4hr} values relative to 6A. In contrast, 11C-R displayed comparable values relative to its control. That 6A-R became less susceptible to all three compounds suggests that it has developed an enhanced ability to cope with oxidative damage and ER stress as a result of artemisinin selection, and that this enhanced coping

mechanism might in fact be responsible for the resistance phenotype we are observing. For 11C-R, on the other hand, not developing cross-resistance could indicate that other biological processes might be at play in mediating its artemisinin resistance. Alternatively, it is also possible that although the same pathways are at play—they might just be induced to a lesser degree, thus leading to a weaker, less pronounced phenotype that is not easily validated.

Taken together, the heterogeneity of chemosensitivity phenotypes we have identified in our 3D7-derived artemisinin-resistant parasite lines—and even those reported from literature—allude to the complexity of artemisinin resistance. Our findings in this matter suggest that the acquisition of resistance against artemisinin and its derivatives is unlikely to be unilateral and could involve different processes and multiple genetic determinants. Furthermore, the cross-resistance displayed by 6A-R against H₂O₂, DTT and EPX also highlights the possibility of antioxidant defense and protein damage response pathways as playing a central role in artemisinin resistance.

Chapter 6. Functional Genomics of artemisinin-resistant *P. falciparum*

BACKGROUND

Over the years, functional genomics has proven to be invaluable in deepening our understanding of artemisinin resistance—with genome-wide and transcriptomic studies on *in vitro* and *in vivo* populations of resistant parasites paving the way to the discovery of key genetic and functional markers of artemisinin resistance.

High resolution GWAS and *in vitro* evolution studies have led to the identification of the sole gene marker for artemisinin slow parasite clearance in Southeast Asia. Currently, F3D7_1343700, also known as the “Kelch 13” or *Pfk13* gene, which resides in chromosome 13, has been found to be consistently associated with clinical artemisinin resistance in Southeast Asia^{105,119}. Specifically, point mutations in its 6-blade propeller domain on the C terminal end (after codon 440) have been correlated with a significant increase in parasite clearance half-life. Parasites having single *Pfk13* mutations in codon 441 onwards display prolonged (> 5hrs) clearance half-life, while clinical isolates that were *pfk13*-wild type or had SNPs outside the propeller domain were relatively faster clearing and therefore more artemisinin-sensitive¹⁰⁵. Gene editing studies done on a number of *in vitro* reference lines as well as lab-adapted clinical isolates have demonstrated that such *Pfk13* gene mutations are indeed resistance-conferring and result in a significantly increased survival rate based on the RSA¹²⁹. Interestingly, the same mutations in *Pfk13* were also detected in a previously *in vitro*-selected parasite line derived from a lab-adapted African (Tanzania) clone named F32-ART^{114,119}—thus providing a concrete example of the translatability of *in vitro* models for studying *in vivo* phenotypes.

Genome-wide transcriptional studies on slow-clearing parasites have also revealed a link between decreased sensitivity to artemisinin and alterations

in the parasite's cell cycle^{108,109} as well as an upregulation of adaptive responses to stress¹⁰⁹. A number of studies that utilize the transcriptome information of slow clearing clinical isolates to estimate parasite age indicated that artemisinin-resistant parasites demonstrate a markedly slower progression throughout the first half of the IDC compared to their sensitive counterparts¹⁰⁸⁻¹¹⁰. In addition, large-scale population transcriptomics of Southeast Asian isolates have revealed that genes involved in oxidative stress response and the UPR are significantly associated with artemisinin resistance¹⁰⁹.

Likewise, using functional genomics to investigate the genomic and transcriptional characteristics of our *in vitro*-selected parasites, we hope to identify and investigate molecular markers in 6A-R and 11C-R that could be associated with artemisinin resistance.

6.1 Genomic Profiling of artemisinin-resistant *P. falciparum*

6.1.1 SNP Identification using whole genome sequencing

Whole genome sequencing was performed for both resistant parasite lines and their respective controls to identify mutations associated with resistance. The scope of our analysis only covered intragenic SNPs that passed stringent quality filtering and had a high position coverage ($\geq 75\%$), and that were unique to the artemisinin-resistant lines. We were able to identify five SNPs in five genes in 6A-R and six SNPs in six genes in 11C-R; these included 3 and 5 missense mutations in 6A-R and 11C-R, respectively, one nonsense mutation in each parasite line and an additional intronic SNP in 6A-R (Table 6.1). We observed no overlap in the spectrum of mutated genes in the two parasite lines despite having come from the same parental strain; and perhaps most notably, we were also unable to detect any SNPs in *Pfk13*. This result was also validated using PCR-based genotyping method prescribed by the WorldWide Antimalarial Resistance Network

(WWARN)^{119,150}. Among the SNP-containing genes in either resistant parasite line, only the nonsense mutation in PF3D7_1115700 (*Pffp2a*)—which encodes a key enzyme in hemoglobin digestion—falcipain 2a—has been previously linked with artemisinin resistance *in vitro*^{114,119}. Whole genome sequencing also revealed no SNPs in genes that have been previously associated with altered susceptibility to artemisinin and other antimalarials, such as *pfcr1*^{182,183}, *pfmrp1*^{184,185}, *pfmdr1*¹⁸⁶, *pfnhe-1*¹⁸⁷, *pfdhps*¹⁸⁸, *pfdhfr*^{189,190}, *pfatp6*⁹², and *pfubp-1*¹⁹¹.

Table 6.1 Single nucleotide polymorphisms identified in 6A-R and 11C-R

	GENE ID	DESCRIPTION	SNP	EFFECT
6A-R	PF3D7_0704800	conserved Plasmodium protein, unknown	T241086C	T322V
	PF3D7_0730300	transcription factor with AP2 domain(s)	T1301352G	H1298Q
	PF3D7_1115700	cysteine proteinase falcipain 2a	G593481T	S35stop
	PF3D7_1141800	phd finger protein, putative	A1673564G	intron
	PF3D7_1427100	lipase, putative	G1059278T	W1039C
11C-R	PF3D7_0420300	transcription factor with AP2 domain(s)	A923966G	T1992A
	PF3D7_0528300	conserved Plasmodium protein, unknown	G1167265T	P248H
	PF3D7_0617100	alpha adaptin-like protein, putative	A712508C	H816P
	PF3D7_0810600	RNA helicase, putative	G543210T	A413S
	PF3D7_1230000	conserved Plasmodium protein, unknown	C1237510A	V815stop
	PF3D7_1368400	conserved Plasmodium protein, unknown	A2720248T	F201Y

All ART-resistant and ART-sensitive lines were subjected to high throughput sequencing using the Illumina MiSeq platform, and nonsynonymous intragenic single nucleotide polymorphisms (SNPs) were called using SAMtools. Only high quality SNPs with at least 75% position coverage, and not present in control parasite lines are presented in this table. Mutations in the hypervariable multigene families were excluded from the analysis.

6.1.2 CNV Profiling using CGH

Besides SNP identification, CNV profiling using comparative genomic hybridization (CGH) was also performed for all resistant and control parasite lines.

Comparing the CGH profiles of our artemisinin-resistant parasites against their respective controls, we were able to detect a number of CNVs in both lines. In 6A-R, we detected a distinct copy number gain on chromosome 14 spanning 40 genes (PF3D7_1454000 - PF3D7_1458000), and another on 11C-R on chromosome 12 covering 9 genes (PF3D7_1228000 - PF3D7_1228800); both artemisinin-resistant lines also contained a common large-scale amplification in chromosome 10 spanning 17 genes (PF3D7_1028700 - PF3D7_1030300) (Fig 6.1, Table 6.2). These three CNVs on chromosomes 10, 12 and 14 were detected during the later stages of drug selection and subsequent culturing, and were found to be stable—having been consistently detected from three rounds of CNV profiling via CGH over a period of five months and over 89 generations (Fig 6.1A, B). Interestingly, the chromosome 10 CNV has been previously identified as a naturally occurring amplification in 3D7¹⁶⁷, but was also found to be duplicated in an artemisinin-resistant parasite line derived from a D6 strain of *P. falciparum*^{116,118}—thus its role in drug resistance is unclear. On the other hand, the two unique amplifications we identified on chromosomes 12 and 14 have not yet been reported previously from either *in vitro* or *in vivo* studies. We did, however, detect a 28-gene amplification in chromosome 14 (PF3D7_1453700 - PF3D7_1456500) in an *in vitro*-derived artemisinin resistant line that was previously selected for in our lab using a T996 background, and this chromosome 14 CNV displayed a 25-gene overlap with that of 6A-R (Appendices D, E).

Figure 6.1 CNV Profiling of *in vitro*-selected *P. falciparum*

R and (B)11C-R were identified using CGH. CNV profiles were assessed three times over multiple generations and continuous cultivation under ART selection. Chromosome plots reflect the subtracted $\log_2$ ratio of the ART-resistant parasite lines relative to their control counterparts.

Table 6.2 List of copy number-amplified genes detected in 6A-R and 11C-R

	GENE ID	DESCRIPTION
CHROOMOSME 10 (6A-R and 11C-R)	PF3D7_1028700	merozoite TRAP-like protein
	PF3D7_1028800	conserved Plasmodium protein, unknown function
	PF3D7_1028900	conserved Plasmodium protein, unknown function
	PF3D7_1029000	conserved Plasmodium protein, unknown function, pseudogene
	PF3D7_1029100	conserved Plasmodium protein, unknown function
	PF3D7_1029200	conserved Plasmodium protein, unknown function
	PF3D7_1029300	conserved Plasmodium protein, unknown function
	PF3D7_1029400	conserved Plasmodium protein, unknown function
	PF3D7_1029500	conserved Plasmodium protein, unknown function
	PF3D7_1029600	adenosine deaminase
	PF3D7_1029700	conserved Plasmodium protein, unknown function
	PF3D7_1029800	RAP protein, putative
	PF3D7_1029900	conserved Plasmodium protein, unknown function
	PF3D7_1030000	transcription factor, putative
	PF3D7_1030100	pre-mRNA-splicing factor ATP-dependent RNA helicase PRP22, putative
	PF3D7_1030200	conserved Plasmodium protein, unknown function
	PF3D7_1030300	conserved Plasmodium protein, unknown function
CHROOMOSME 12 (11C-R)	PF3D7_1228000	conserved Plasmodium protein, unknown function
	PF3D7_1228100	leucine-rich repeat protein
	PF3D7_1228200	conserved Plasmodium protein, unknown function
	PF3D7_1228300	NIMA related kinase 1
	PF3D7_1228400	conserved Plasmodium protein, unknown function
	PF3D7_1228500	RNA pseudouridylate synthase, putative
	PF3D7_1228600	merozoite surface protein 9
	PF3D7_1228700	conserved Plasmodium protein, unknown function
	PF3D7_1228800	conserved Plasmodium protein, unknown function
CHROOMOSME 14 (6A-R)	PF3D7_1454000	RNA-binding protein, putative
	PF3D7_1454100	tRNA intron endonuclease, putative
	PF3D7_1454200	conserved Plasmodium protein, unknown function
	PF3D7_1454300	serine/threonine protein kinase, putative
	PF3D7_1454400	aminopeptidase P
	PF3D7_1454500	nifU protein, putative
	PF3D7_1454600	mitochondrial ribosomal protein S11 precursor, putative
	PF3D7_1454700	6-phosphogluconate dehydrogenase, decarboxylating, putative
	PF3D7_1454800	conserved Plasmodium protein, unknown function
	PF3D7_1454900	conserved Plasmodium protein, unknown function
	PF3D7_1455000	protein phosphatase, putative
	PF3D7_1455100	protein phosphatase, putative
	PF3D7_1455200	methyltransferase, putative
	PF3D7_1455300	conserved Plasmodium protein, unknown function
	PF3D7_1455400	hemolysin, putative
	PF3D7_1455500	gamma-adaptin, putative
	PF3D7_1455600	ferlin, putative
	PF3D7_1455700	conserved Plasmodium protein, unknown function
	PF3D7_1455800	LCCL domain-containing protein
	PF3D7_1456000	transcription factor with AP2 domain(s)
	PF3D7_1456100	serine hydroxymethyltransferase, putative
	PF3D7_1456200	SNARE protein, putative
	PF3D7_1456300	conserved Plasmodium protein, unknown function
	PF3D7_1456400	conserved Plasmodium protein, unknown function

PF3D7_1456500	conserved Plasmodium protein, unknown function
PF3D7_1456600	mitochondrial ribosomal protein L28 precursor, putative
PF3D7_1456700	conserved Plasmodium protein, unknown function
PF3D7_1456800	V-type H(+)-translocating pyrophosphatase, putative
PF3D7_1456900	conserved protein, unknown function
PF3D7_1457000	signal peptide peptidase
PF3D7_1457100	conserved Plasmodium protein, unknown function
PF3D7_1457200	thioredoxin 1
PF3D7_1457300	conserved Plasmodium protein, unknown function
PF3D7_1457400	conserved Plasmodium protein, unknown function
PF3D7_1457500	vacuolar protein sorting-associated protein 4
PF3D7_1457600	conserved Plasmodium protein, unknown function
PF3D7_1457700	large ribosomal subunit nuclear export factor, putative
PF3D7_1457800	conserved Plasmodium protein, unknown function
PF3D7_1457900	conserved Plasmodium protein, unknown function
PF3D7_1458000	cysteine proteinase falcipain 1

6.2 Transcriptional Profiling of artemisinin-resistant *P. falciparum*

6.2.1 Transcriptional profiling of artemisinin-resistant and artemisinin-sensitive parasites across the IDC

Comparative whole transcriptome profiling of the artemisinin-resistant parasites and their controls across the IDC was performed to identify global transcriptional changes that may have arisen as a result of drug selection, and also particularly to investigate any changes in their progression throughout the asexual erythrocytic cycle.

One of the most striking characteristics of slow clearing parasites is their delayed progression throughout the IDC—with artemisinin-resistant parasites exhibiting “a ring-stage arrest” phenotype, and appearing to progress through the earlier phases of the IDC slower than artemisinin-susceptible parasites¹⁰⁸⁻¹¹⁰. To assess whether our own *in vitro* derived lines also exhibited a similar phenotype, we performed transcriptional profiling of the resistant parasites and their controls at 8-hr intervals throughout the asexual blood stage and estimated the “best fit” parasite age based on its correlation with the reference IDC transcriptome¹⁰⁸. As expected, the transcriptomes of all parasite lines—be it resistant or sensitive—depict the monophasic, “just-in-time” gene cascade that is distinctively conserved

among *Plasmodium* species^{6,192} (Figure 6.2A). Comparing our findings to what has been observed in artemisinin-resistant clinical isolates, however, neither 6A-R nor 11C-R appears to be growing markedly slower than their nonselected counterparts (Fig 6.2B). In fact, transcriptome-based age projection estimates both resistant parasite lines to be older (10hpi) than their sensitive counterparts at the first sampling interval where they were judged to be early ring (~4-6hpi) by both microscopy and Spearman rank correlation with the IDC reference. Except for this timepoint, the estimated age and rate of progression between artemisinin resistant and sensitive parasites throughout the rest of the asexual stage are comparable, indicating no significant developmental differences between the resistant and sensitive parasite lines.

We then evaluated the global expression levels across the IDC of the copy number-amplified genes in chromosomes 10, 12, and 14 that were identified, and found that there was indeed a significant upregulation of genes in the regions covered in those CNV segments: PF3D7_1028700 - PF3D7_1030300 for both 6A-R and 11C-R, PF3D7_1028700 - PF3D7_1030300 for 11C-R, and PF3D7_1454000 - PF3D7_1458000 for 6A-R. This finding confirms the increase in gene dosage that was detected using CGH is also driving transcript levels and in turn, could be responsible for modulating response in our artemisinin-selected parasites (Figure 6.2C).

Figure 6.2 Transcriptional profiling of ART-resistant and ART-sensitive *P. falciparum* across the IDC

C

(Cont'd) Figure 6.2 Transcriptional profiling of ART-resistant and ART-sensitive *P. falciparum* across the IDC

Genome-wide transcriptional profiling throughout the asexual erythrocytic stage was performed for all parasite lines. (A) Heatmaps represent the IDC transcriptomes of 6A-R, 6A, 11C-R and 11C across the IDC, over 6 timepoints and sampled at 8-hr intervals; Genes have been ordered according to the phase and frequency of expression using Fourier analysis. (B) Parasite age in hpi was estimated by calculating the Spearman rank correlation of the transcriptomic information at each timepoint with a reference IDC transcriptome. (C) Differential gene expression in the copy number amplified genes we identified in chromosomes 10, 12 and 14 was also evaluated between ART-resistant parasite lines and their corresponding controls. Boxplots show average log₂ratio values of all copy number amplified genes for six timepoints collected across the IDC.

6.2.2 Steady state transcriptional profiling of artemisinin-resistant and artemisinin-sensitive parasites during the ring stage (10-20HPI)

We also characterized the transcriptome of the mid to late ring stage (10-20 hpi) at 2-hr intervals for both 6A-R and 11C-R growing in the absence of artemisinin. This was performed in order to understand baseline transcriptional changes that might be associated with the stage-specific artemisinin resistance the parasites acquired via *in vitro* selection.

Ring-stage (10-20hpi) transcriptomic analysis for both 6A-R and 11C-R revealed a variable spectrum of transcriptional variations between the two resistant parasite lines (Figure 6.3A, B). Most notably, in 6A-R we observed the upregulation of pathways related to adaptive responses to stressors of redox and protein homeostasis. Protein turnover-associated genes that were significantly upregulated include: heat shock and chaperone proteins, and a number of enzymes involved in proteolysis. Similarly, redox-associated genes that were significantly overexpressed include: antioxidant defense proteins thioredoxin peroxidase and 1-cys peroxiredoxin; targets of S-glutathionylation, as well as several enzymes that play roles in NADPH-generating processes such pentose phosphate pathway genes, and enzymes involved in nitrogen and isoprenoid metabolism—specifically, glutamate dehydrogenase¹⁹³ and ferredoxin--NADP reductase¹⁹⁴. This baseline upregulation of such pathways in 6A-R suggests an enhanced ability to cope with cellular stressors and or agents that inflict oxidative or protein damage to the parasite. We also observed an upregulation of genes involved fatty acid metabolism, as well as protein transport, particularly vesicular traffic between the ER and Golgi complex¹⁹⁵. On the other hand, significantly downregulated pathways notably include processes which are related to host-parasite interactions, as well as those related to regulation of translational initiation and gene expression, such as: PK4, which a known regulator of protein synthesis¹⁹⁶, a number of AP2-containing DNA-binding

proteins, genes involved in chromatin remodelling, and an abundance of RNA-binding proteins. Chromatin modification-related genes we found to be downregulated include several histone methyl- and acetyltransferases, a number of SET domain proteins, as well as a cyclin-dependent kinase-related kinase (*Pfcrk-1*) which has been shown to play a role in histone modification¹⁹⁷. We also observed many downregulated genes encoding RNA helicases, mRNA splicing factors, and RNA-binding proteins that were likely to play a role in mRNA splicing, RNA metabolism and ribosomal maturation (Figure 6.3A).

For 11C-R—as with 6A-R—we identified an enrichment of genes that were associated with defense against oxidative stress, such as S-glutathionylated proteins, and glutaredoxin 1, as well as a number of pathways involved with coping with protein damage—including chaperones, genes involved in proteasome-mediated degradation, and ER translocation. In contrast with 6A-R, however, 11C-R exhibited an upregulation of processes related to early translation events, and quite a number of pathways pertaining to transcriptional and post-transcriptional mechanisms of gene regulation such as chromatin modification, mRNA splicing, and particularly—genes that appear to be involved in the CCR4-Not complex as well as the formation of P-bodies (Fig 6.3B). Interestingly, the presence of p-bodies has been observed to be elevated under stress, or under conditions that repress translation initiation¹⁹⁸⁻²⁰⁰. Notably, two genes that have also been associated with artemisinin resistance in clinical isolates—*Pfk13* and *Pfcyp19b*—also exhibit a significant increase in expression during the mid-late ring stage in 11C-R. *Pfcyp19b* in particular—which encodes a prolyl-peptidyl isomerase that acts as a molecular chaperone and plays a role in protein folding^{201,202}—was found to be overexpressed among slow-clearing Southeast Asian parasites, and was the most significantly associated with clinical artemisinin resistance; and because our findings in our *in vitro*

system reinforce its correlation with artemisinin resistance, we also selected this gene for subsequent functional validation experiments. As for the significantly downregulated pathways in 11C-R relative to 11C, we observed those that pertain to host-parasite interactions, vesicular transport, RNA processing—such as the Lsm proteins—and translational elongation factors (Figure 6.3B).

All differentially expressed genes for steady-state ring-stage transcriptional profiling of 6A-R vs 6A, and 11C-R vs 11C are listed in Appendices F and G, respectively.

Figure 6.3 Transcriptional Profiling of ART-resistant and ART-sensitive *P. falciparum* during the ring stage (10-20HPI)

(Cont'd) Figure 6.3 Transcriptional Profiling of ART-resistant and ART-sensitive *P. falciparum* during the ring stage (10-20HPI)

Steady-state comparative transcriptomic analysis was done on 6A-R vs 6A (A) and 11C-R vs 11C (B) across the mid-ring stage, which matches the window of drug selection. Heatmaps depict significantly up- and downregulated genes ($p \leq 0.05$, student's T-test) in the ART-resistant parasites in comparison with their ART-sensitive controls. Noted on the right are the differentially expressed pathways between ART-resistant and ART-resistant parasites ($p < 0.05$, Hypergeometric test).

6.2.3 Transcriptional profiling of artemisinin-resistant and artemisinin-sensitive parasites under artemisinin pressure at the Ring stage of the IDC

In addition to steady-state transcriptional profiling, we also performed comparative transcriptomic analysis on artemisinin-treated resistant and sensitive parasites (Fig 6.4A, B); drug exposure conditions used were identical to the one implemented for artemisinin selection.

In the presence of artemisinin pressure, 6A-R was found to have a markedly higher expression of components related to vesicular as well as transmembrane transport, particularly those on the mitochondrion, the digestive vacuole (DV) and the parasitophorous vacuole (PV). Notable examples of this include the DV-resident chloroquine resistance transporter, *Pfcr1* (PF3D7_0709000)—which has been consistently associated with resistance to the quinoline drug CQ^{42,182,183,203}, as well as PV-resident glutathione-transporter *Pfexp1* (PF3D7_1121600) that has also been shown to be inhibited by ATS, and has also been associated with artesunate sensitivity and metabolism²⁰⁴. We also observed key genes involved in the ubiquitin-proteasome system, antioxidant defense, DNA repair, cell cycle control, as well as RNA-related processes are in turn associated with the regulation of gene expression and translation.

On the other hand, significantly downregulated pathways include those that are related to: invasion and pathogenesis, apoptosis and cell cycle arrest, vesicular transport, the mitochondrial electron transport chain, nucleotide metabolism, initiation of translation, as well as a few genes related to protein folding. Interestingly, downregulation of apoptosis and cell cycle arrest genes might be related to the ability of 6A-R to tolerate artemisinin, and be able to overcome the drug induced quiescence (Figure 6.4A).

For 11C-R, on the other hand, we observe an upregulation of pathways related to ATP synthesis—both in the DV and the mitochondrion, fatty acid metabolism, and events that are related to translation—particularly an abundance of processes related to rRNA and tRNA modification. Interestingly, downregulated pathways also include those related to early translation events, DNA repair, as well as protein turnover—which is in contrast with 6A-R. Significantly downregulated genes also appear to be enriched for processes related to host-parasite interactions and nucleosome assembly (Figure 6.4B).

All differentially expressed genes for the comparative transcriptional profiling of ring-stage artemisinin response between 6A-R vs 6A, and 11C-R vs 11C are listed in Appendices H and I, respectively.

UPREGULATED PATHWAYS		p val
Protein Turnover	GO:endorpeptidase Clp complex	0.04
	GO:ubiquitin ligase complex	0.05
	GO:proteasome activator complex	0.04
Transport	Rab and other proteins involved in intracellular traffic	0.02
	Transporters of the ER/Golgi and digestive vacuole membranes	0.03
	GO:nucleocytoplasmic transporter activity	0.04
	GO:trans-Golgi network transport vesicle	0.04
	GO:recycling endosome	0.04
	GO:negative regulation of nucleocytoplasmic transport	0.04
	GO:endoplasmic reticulum-Golgi intermediate compartment membrane	0.04
	GO:dicarboxylic acid transport	0.04
	GO:mitochondrial transport	0.04
	GO:monovalent inorganic cation transmembrane transporter activity	0.04
	GO:malate transport	0.04
	GO:oxoglutarate:malate antiporter activity	0.04
	GO:alpha-ketoglutarate transport	0.04
	GO:glutathione transport	0.04
Translation	GO:rRNA (adenosine-2'-O-)-methyltransferase activity	0.04
	GO:rRNA methylation	0.00
	GO:organellar small ribosomal subunit	0.00
	GO:mitochondrial small ribosomal subunit	0.00
	GO:tRNA 3'-trailer cleavage	0.04
Oxidative stress	GO:antioxidant activity	0.05
	GO:peroxidase activity	0.04
Cell development	BP:regulation of cell development	0.04
	BP:regulation of cell division	0.04
DNA repair	MF:cyclin-dependent protein kinase regulator activity	0.00
	GO:PCNA complex	0.00
Others	Catabolism of polyphosphates	0.05
	Proteins of detergent-resistant membranes	0.05
	BP:tubulin complex assembly	0.04
DOWNREGULATED PATHWAYS		p val
Invasion, pathogenesis, cytoadherence	MPM:PFEMP1 domain architectures	0.02
	BP:pathogenesis	0.00
	BP:modulation by symbiont of host erythrocyte aggregation	0.01
	BP:cytoadherence to microvasculature, mediated by symbiont protein	0.01
	BP:entry into host cell	0.01
Mt ATP synthesis	BP:cell-cell adhesion	0.00
	MPM:Mitochondrial antioxidant system	0.05
	Biogenesis of cytochrome oxidase	0.00
	BP:respiratory chain complex IV assembly	0.00
Transport	BP:mitochondrial ATP synthesis coupled proton transport	0.03
	MPM: Classical clathrin-mediated vesicular transport	0.02
	MPM: Phosphoinositides and membrane traffic	0.02
	BP:intracellular protein transport	0.04
Cell development	BP:microtubule-based process	0.01
	BP:cell cycle arrest	0.03
Signaling	BP:apoptotic process	0.03
	MPM: Signaling machinery in malaria parasites	0.03
Nucleotide metabolism	BP:peptidyl-serine phosphorylation	0.03
	BP:cyclic nucleotide metabolic process	0.03
Translation	BP:nucleobase-containing compound metabolic process	0.04
	BP:regulation of translational initiation	0.02
Protein Turnover	BP:chaperone cofactor-dependent protein refolding	0.03
	MPM: Shikimate Biosynthesis	0.00
others	BP:glutamine biosynthetic process	0.03
	BP:macromolecule biosynthetic process	0.03
	BP:N-terminal protein myristoylation	0.03

Figure 6.4 Transcriptional Profiling of ART-resistant and ART-sensitive *P. falciparum* under ART pressure

(Cont'd) **Figure 6.4 Transcriptional Profiling of ART-resistant and ART-sensitive *P. falciparum* under ART pressure**

Comparative transcriptomic analysis was done on 6A-R vs 6A (A) and 11C-R vs 11C (B) under ART pressure, using the exact same conditions used for *in vitro* drug selection (900 nM ART, 4 hrs at 10-14 hpi). RNA was harvested at three timepoints over the duration of drug treatment. Heatmaps depict significantly up- and downregulated genes ($p \leq 0.05$, student's T-test) in the ART-resistant parasites in comparison with their ART-sensitive controls, while the tables show the differentially expressed pathways between ART-resistant and ART-resistant parasites ($p < 0.05$, Hypergeometric test).

6.3 Discussion

Through the genomic and transcriptomic profiling of our *in vitro*-derived artemisinin-resistant parasites 6A-R and 11C-R, we hoped to better understand the molecular underpinnings of their respective artemisinin resistance phenotypes

Whole genome sequencing of both 6A-R and 11C-R revealed a unique spectrum of mutations for each resistant parasite line. Interestingly, no overlap was observed IN SNP-containing genes between the two parasite lines despite having come from the same parental strain. This stark difference in the spectrum of mutated genes between 6A-R and 11C-R further supports the possibility of distinct artemisinin resistance mechanisms between the two parasite lines that was also suggested by the differences we previously observed with regard to their selection dynamics, as well as their chemosensitivity profiles. Furthermore, we also found no overlap with any previously associated SNP or gene markers that has been identified in large-scale GWAS of field isolates. These genes include: PF3D7_1017000¹²⁵, which encodes a DNA polymerase delta catalytic subunit; PF3D7_1343400 (*Pfrad5*)²⁰⁵, which encodes a RAD5 DNA repair protein homolog; PF3D7_1417400²⁰⁵, which encodes a rap guanine nucleotide exchange factor, and finally, PF3D7_1343700 (*Pfk13*)¹¹⁹, which encodes a Kelch propeller domain containing protein. What is unique about *Pfk13* is that it has consistently been linked to the artemisinin resistance phenotype both *in vivo* and *in vitro*. It is located in chromosome 13 locus that has been shown to be under recent positive selection in a slow-clearing populations of field isolates, specifically in Cambodia^{125,127,206}, and was then subsequently identified as significantly associated with clinical artemisinin resistance through a large multicenter GWAS¹¹⁹. Additionally, it was found to be similarly mutated in an *in vitro*-derived artemisinin-resistant parasite line that was subjected to long term drug selection using

DHA¹¹⁹. Notably, neither 6A-R nor 11C-R harbored any mutations in the relevant kelch propeller domain—or any other region—in the *Pfk13* gene. This absence of SNPs in the *Pfk13* gene, which is the principal genetic correlate of artemisinin resistance in Southeast Asia, strongly indicates that the phenotypes we have induced in both parasite lines is independent of *Pfk13* mutation status. Similarly, the *in vitro* artemisinin resistance models developed by Tucker et al. also did not contain any of the documented resistant-conferring *Pfk13* SNPs^{116,118}. Together, these results strongly indicate that there could other multiple genetic factors that could be responsible for artemisinin resistance in *P. falciparum*. These observations also reinforce that the phenotype we have induced through our *in vitro* selection protocol is likely driven by a divergent genetic provenance compared to what has been observed and reported in Southeast asia, and might even be driven by different molecular events.

Although we were unable to see any overlap with reported *in vivo* candidate genes, one SNP-containing gene in 6A-R has been definitively linked to modulating artemisinin sensitivity *in vitro*. This gene is *Pffp2a* (PF3D7_1115700), which encodes cysteine proteinase falcipain 2a—one of the main proteases involved in hemoglobin digestion, which is central to both the survival of the parasite as well as well as to artemisinin action¹⁶². *Pffp2a* harbors a premature stop codon in 6A-R, and was also found to be similarly mutated in another *in vitro*-derived parasite line F32-ART^{114,119}. Moreover, it has been demonstrated that knocking down *Pffp2a* also significantly reduces artemisinin susceptibility in *P. falciparum* parasites in the early ring and trophozoite stages^{121,162}. Given that artemisinin is predominantly activated by haemoglobin-derived iron, it is probable that the presence of this nonsense mutation the *Pffp2a* does indeed contribute to the artemisinin resistance exhibited by 6A-R.

We also observed that none of the SNP-containing genes that we found in either 6A-R and 11C-R match with any previously reported putative targets and interacting partners of artemisinin drugs, with some notable examples being *Pfatp6*⁹¹, *Pfpi3k*⁹⁷ and *Pfttcp*²⁰⁷, among many others^{89,100}. Interestingly, we see a number of mutated genes that might play a role in the regulation of gene expression such as: several AP2 domain-containing transcription factors, a putative phd (plant homeodomain) finger, and an RNA helicase. Alterations in any of these genes could lead to significant changes to the expression of one or multiple genes within the tightly regulated transcriptional cascade in the asexual erythrocytic stage of the *Plasmodium* parasite, and subsequently contribute to the distinct artemisinin resistance phenotypes that we observe in both parasite lines. However, further experimental validation via gene-editing studies is needed to truly verify whether the SNPs we identified in 6A-R and 11C-R are indeed resistance-conferring.

In addition to SNP identification, we also conducted CNV profiling of both 6A-R and 11C-R after long term drug selection to seek out any structural variations that may have arisen as a result of drug selection. Although most large-scale GWAS in recent years have focused solely on identifying point mutations that correlate with changes in artemisinin sensitivity^{125,127,130,206}, resistance phenotypes against different antimalarials have also been attributed to structural variations in the *Plasmodium* genome, which makes CNV profiling just as important as SNP identification. And while clinical artemisinin resistance in field isolates has not yet been correlated to any copy number changes in the *P. falciparum* genome, previous *In vitro* evolution studies have linked CNVs—specifically, the well-documented copy number amplification in *Pfmdr1*—with artemisinin resistance^{113,115}. Cui and colleagues¹¹⁵ observed an increase in gene copy number after DHA selection of a Dd2 parent, but ultimately concluded that while the resulting

amplification contributed to resistance, it was not solely responsible for the phenotype. Chavchich et al¹¹³ also observed an increase in *Pfmdr1* copy number upon drug selection using artelinic acid in a W2-derived line, but not in a D6-derived progeny. Similarly, functional studies have shown that downregulation of *Pfmdr1* significantly increases the sensitivity against multiple antimalarial drugs—including artemisinin⁴⁷. In our case, neither 6A-R nor 11C-R harbored CNVs in *Pfmdr1*. They did however exhibit several large-scale amplifications on chromosomes 10, 12 and 14. Of these copy number variable genes, only the *Pfprp22* gene on the chromosome 10 amplification has been linked to artemisinin resistance *in vitro*¹¹⁸; while the amplifications in chromosome 12 and 14 are being reported for the first time in this study. All CNVs were found to be stable over multiple generations, and were accompanied by a concomitant increase in expression in the genes encompassed by the amplified regions. Considering what is known so far about artemisinin resistance, we can identify a few potential candidates that are worth pursuing. One such candidate in the chromosome 12 CNV is *Pfnek1* (PF3D7_1228300)—which encodes a protein that appears to be essential for the completion of the asexual stage of the parasite²⁰⁸. This gene is also related to NIMA-like kinases²⁰⁹ which are, in turn, important players in cell development²¹⁰. Interestingly, decreased sensitivity to artemisinin among clinical isolates has also been linked to a dysregulation of the parasite's progression through the IDC^{108,109,114}; However, whether or not the amplification of *Pfnek1* is in fact able to contribute to alterations in artemisinin response in *Plasmodium* requires further rigorous investigation. In addition to *PfNek1* on chromosome 12, the chromosome 14 CNV also contains several genes that are involved in cellular stress responses. Among them are: *PfTrx1* (PF3D7_1457200), a key contributor to the NADPH-dependent antioxidant thioredoxin system^{211,212}, *Pf6pgd* (PF3D7_1454700), an enzyme in the pentose phosphate pathway which acts as the main NADPH source for the parasite^{164,213-215}, and *PfSpp*

(PF3D7_1457000) a signal peptide peptidase that is a likely component of the *Plasmodium* ER-associated degradation pathway which is stimulated by protein misfolding and ER stress²¹⁶. Relating the biological roles of these genes with the chemosensitivity phenotypes observed in 6A-R, *Pftrx1*, *Pf6pgd* and *Pfspp* were chosen as suitable candidates for functional validation.

Taken together, the range of variations we observe in our *in vitro* selected artemisinin-resistant strains—on top of what has been reported in literature—hints at the genomic heterogeneity at both SNP and CNV levels that underpins artemisinin resistance. Although the fact that the artemisinin resistance phenotypes we have selected for are independent of *Pfk13* limits the translatability of our *in vitro* models to the current clinical situation, it also highlights the possibility of the emergence of artemisinin resistance that is independent of mutations in the K13 propeller gene—particularly in the malaria-endemic territories beyond Southeast Asia. Moreover, not only do our findings suggest that other SNP-containing genes could be driving the phenotype, it also underscores the possibility that structural variations—and not just point mutations—are involved in the process; and this is something should also be considered in designing future genome scanning studies related to drug resistance.

Finally, we carried out transcriptomic profiling of all resistant and sensitive parasite lines under several conditions: at several timepoints across the IDC, and during the ring stage in the presence and absence of artemisinin drug pressure. IDC transcriptional profiling was done to specifically evaluate the developmental progression of the all parasite lines across the asexual blood stage. Artemisinin resistance has been associated with an altered growth pattern both *in vitro* and *in vivo* where the resistant parasites appear to grow slower than their sensitive counterparts, specifically during

the earlier stages of the IDC—akin to having a “prolonged ring” state^{108-110,116}. As discussed earlier, the initial segment of the IDC or the ring stage is the least sensitive to artemisinin. It is probable that the parasites that remain at the ring stage longer gain a resistance advantage by having an extended period of reduced drug susceptibility compared to the parasites that progress at the normal rate. In the case of 6A-R and 11C-R however, we observed no dramatic slowing down in the cell cycle progression, which is yet another discrepancy with our artemisinin-resistant parasites compared to the slow-clearing Asian clinical isolates.

On the other hand, both ring-stage profiling of 6A-R and 11C-R reveals transcriptional similarities with previously documented *in vivo* and *in vitro* populations of resistant *P. falciparum*, wherein they demonstrate an upregulation of pathways related to antioxidant defense, as well as the unfolded protein response^{109,115}—which could very well account for the cross-resistance demonstrated by 6A-R against H₂O₂, and the ER stressors DTT and EPX. Furthermore, we also see an enrichment of processes that are related to translational control and the regulation of gene expression, including early translational events (initiation), binding and processing of ribosomal and messenger RNA, as well as transcriptional regulation via transcription factors and chromatin modification. Interestingly, it has been shown that that *Plasmodium* is also able to cope with cellular stresses via translational repression—a process that has involves the eif2 α -mediated attenuation of global protein synthesis²¹⁷⁻²¹⁹ and the association of mRNA with RNA-binding proteins that facilitate their stability (stress granules) or degradation (P-bodies)^{217,218,220}. Overall, our transcriptome data supports the hypothesis that these pathways are indeed playing a role in enhancing the ability of the parasite to respond to artemisinin-induced stress.

What's striking about these results is that regardless of *Pfk13* mutation status, artemisinin-resistant parasites exhibit a consistent transcriptional dysregulation in processes that are related to the unfolded protein response—which involves trafficking across cellular compartments, enzymatic processing of proteins to mediate their folding and degradation, and attenuation of translation to mitigate the ER workload^{177,219,221}. These findings reinforce that the UPR is indeed central to mediating the ART resistance in *Plasmodium*. And given that the killing action of artemisinin is largely due to the generation of free radicals that are able to inflict widespread damage to a range of cellular components and parasite proteins, it is not surprising that these stress response pathways could indeed be responsible for the enhanced ability of artemisinin-resistant parasites to survive drug treatment.

Chapter 7. Functional validation of candidate genes

BACKGROUND

Four candidate genes were selected for overexpression studies to determine whether they are able to modulate artemisinin sensitivity. Among them are three genes on the chromosome 14 amplification identified in 6A-R and that were also found to be significantly upregulated across the IDC (PF3D7_1454700, PF3D7_1457000 and PF3D7_1457200) (Figure 7.1A), and the other one is PF3D7_1115600, which we found to be upregulated in 11C-R at the mid-ring stage in addition to being identified as the most strongly associated overexpression marker of artemisinin resistance in a recent population transcriptomics study on Southeast Asian isolates¹⁰⁹ (Figure 7.1B). These four genes were selected for functional studies because of their roles in the cellular stress responses that have been closely linked to artemisinin response. Two of these genes, PF3D7_1454700 and PF3D7_1457200, are key players in the antioxidant defense machinery inside the parasite. PF3D7_1457200 (*Pftrx1*) encodes thioredoxin 1, a key enzyme in the *Plasmodium* NADPH-dependent thioredoxin system which functions for the detoxification of reactive oxygen metabolites, redox regulation of transcription factors, and control of protein folding^{193,211,212}; while PF3D7_1454700 (*Pf6pgd*) encodes 6-phosphogluconate dehydrogenase, which catalyzes an NADPH-generating step in the pentose phosphate pathway—which also acts as the main source of NADPH/reducing power within the parasite^{193,214}. The other two, PF3D7_1457000 (*Pfspp*) and PF3D7_1115600 (*Pfcyp19b*), are involved in maintaining protein homeostasis within the parasite. The *Pfcyp19b* gene encodes a cyclophilin-like molecular chaperone that is likely involved in protein folding and repair within the parasite^{201,222}, and *Pfspp* encodes an transmembrane protease component of the ER-associated degradation pathway, which is utilized by the parasite to cope with damaged or misfolded proteins²¹⁶.

Overexpression parasite lines expressing each gene were generated and then phenotyped for artemisinin sensitivity to validate whether or not these candidate genes are able to confer resistance.

Figure 7.1 Candidate genes selected for overexpression

The four genes depicted above were found to be significantly overexpressed ($p < 0.05$) in our ART-resistant parasite lines and were subsequently chosen to be episomally overexpressed in wild-type 3D7-6A parasites in order to evaluate their capacity to modulate ART response in *Plasmodium*. Three of the four genes were copy number-amplified and significantly upregulated in 6A-R (PF3D7_1454700, *Pf6Pg*d; PF3D7_1457000, *Pfspp*; and, PF3D7_1457200, *Pftrx1*) (A), while PF3D7_1115600 (*Pfcyp19b*) was found to be significantly upregulated in 11C-R (B). Heatmaps show the subtracted log₂ratio values between ART-resistant parasites and their controls across multiple timepoints collected throughout the IDC for 6A-R, and throughout the mid-late ring stage for 11C-R. Differential expression between the ART-resistant vs control transcriptome datasets was evaluated using paired student's T-test.

7.1 Validation of Recombinant Expression

In order to determine whether or not upregulation of these stress-response genes influence artemisinin sensitivity, each gene was stably and episomally overexpressed in a wild-type 3D7-6A strain using blasticidin (BSD) selection. Expression of mRNA is driven by the constitutive cam promoter located upstream of the multiple cloning site into which the full-length cDNA of candidate each gene was inserted. A control parasite line

from the same background was also generated by transfection with the empty vector. The transgene products were also tagged with hemagglutinin (HA) to facilitate detection using western blot analysis.

Before phenotyping the transfected parasite lines, we first validated the presence of each respective episome, as well gene overexpression and production of the HA-tagged gene product. Using PCR we were able to confirm that all five (overexpression and control) transfectants did indeed contain the correct episome as shown by the correctly-sized PCR amplicons generated using forward and reverse primers that flank the multiple cloning site of the transfection vector (Figure 7.2A). The levels of mRNA expression of each candidate was also evaluated using relative quantification via qPCR with the transfectant containing the empty vector serving as the baseline control; the primers used for PCR are able to target both the endogenous and transgenic species of mRNA, and arginyl-tRNA synthetase (PF3D7_1218600) was used as the endogenous reference. Our results confirm that each transfectant is indeed overexpressing their respective transgene, albeit to varying degrees—with the *Pf6pgd* and *Pfcyp19b* transfectants demonstrating over 7- and 2-fold higher expression levels of their respective genes, while the *Pftrx1* and *Pfspp* transfectants exhibit more modest (<2-fold) levels of overexpression (Figure 7.2B). For comparison, the amplified genes *Pf6pgd*, *Pfspp* and *Pftrx1* in 6A-R display a 1.5-fold increase in expression across the IDC, while *Pfcyp19b* shows a 1.1-fold increased expression during the ring stages in 11C-R. This indicates that the transfectant parasite lines are able to overexpress their respective candidate genes to a higher extent than their untransfected, resistant counterparts.

Western blot analysis using an anti-HA antibody also confirmed the production of the HA-tagged transgene products for each parasite line with

each lane showing a signal corresponding to each tagged protein sitting at their expected molecular weights (Figure 7.2C). Similar to the qPCR results, the western blot likewise shows higher levels of protein for the transfected lines overexpressing *Pf6pgd* and *Pfcyp19b* compared to those overexpressing *Pftrx1* and *Pfspp* (Figure 7.2C). In the case of *Pf6pgd*—although the intact, tagged protein (~56kDa) gives off the strongest signal—additional bands at lower molecular weights can be seen on the blot, suggesting the presence of protein degradation products. Nevertheless, these findings confirm that overexpression lines were successfully generated for each candidate gene and are suitable for downstream phenotyping.

Figure 7.2 Validation of episomal gene expression

Prior to phenotyping, levels of gene overexpression and translation of the transgene product in each transfected parasite line was first verified. (A) Presence of the transgene-containing episome was verified via PCR using primers that flank the vector MCS. Lanes 4-8 depict amplicons where the total DNA from the transfected parasite lines was used as the PCR template, and Lane 3 depicts the PCR product using the empty plasmid DNA as a template. (B) Real-time qPCR was then used to determine the relative overexpression of *Pftrx1*, *Pf6pgd*, *Pfspp* and *Pfcyp19b* from their respective overexpression parasite lines—expressed as the fold-change derived using the $\Delta\Delta Ct$ method—compared to the vector control. (C) Western blot analysis was also carried out on all overexpression and control parasite lines using a monoclonal mouse anti-HA antibody; Actin was used as a loading control. Bands marked with red arrows indicate the tagged proteins sitting at their expected molecular weights. All parasite lines were grown under positive selection at 2.5 $\mu\text{g/mL}$ BSD.

7.2 Evaluating artemisinin sensitivity among overexpression parasite lines

We then assessed artemisinin sensitivity among the transfectants using a 4-hr artemisinin pulse drug assay and compared their artemisinin $IC_{50_{10\text{hpi}/4\text{hr}}}$ relative to the vector control. While all overexpression and vector control lines were grown under constant BSD pressure (2.5 $\mu\text{g/mL}$) to maintain the episome, the drug was removed during the cycles when ART drug assay was performed.

We observed that artemisinin $IC_{50_{10\text{hpi}/4\text{hr}}}$ for the *Pftrx1*-, *Pfspp*-, and *Pfcyp19b*-overexpression lines were significant higher—1.7-fold, 2.9-fold and 2.8-fold respectively—compared to the vector control. On the other hand, no significant difference in artemisinin sensitivity could be observed in the parasites overexpressing *pf6Pg*d (Figure 7.3, Table 7.1). These results indicate that the specific upregulation of *Pftrx1*, *Pfspp* and *Pfcyp19b* can indeed modulate sensitivity to artemisinin.

Figure 7.3 Evaluating ART sensitivity of parasite lines overexpressing *Pftrx1*, *Pf6pgd*, *Pf5pp* and *Pfcyp19b*

Ring-stage chemosensitivity (IC₅₀_{10hpi/4hr}) against a pulse exposure of ART was evaluated for all overexpression parasite lines and compared against the vector control. Mean IC₅₀_{10hpi/4hr} for all transfectants were calculated from their respective mean dose-response curves as depicted in the figure. All drug assays were performed in biological triplicates.

Table 7.1 ART IC50_{10hpi/4hr} values for *Pftrx1*-, *Pf6pgd*-, *Pfspp*- and *Pfcyp19b*-overexpression and vector control parasite lines

Overexpression Parasite Line	ART IC50 _{10hpi/4hr} (nM)
Vector ctrl	63.48 ± 16.95
<i>Pftrx1</i>	105.68 ± 28.39
<i>Pf6pgd</i>	64.79 ± 39.54
<i>Pfspp</i>	186.08 ± 60.03
<i>Pfcyp19b</i>	179.79 ± 65.48

Numbers represent the IC50_{4hr} values (mean ± standard deviation).

7.3 Discussion

Over the course of characterizing of our 3D7-derived artemisinin-resistant parasites 6A-R and 11C-R, we were able to gather evidence suggesting that—similar to what has been observed in both *in vitro* and *in vivo* studies—the cellular stress responses pertaining to antioxidant defense, as well as the unfolded protein response were potential drivers of artemisinin resistance. Furthermore, we also identified several candidate genes, namely *Pftrx1*, *Pf6pgd*, *Pfspp* and *Pfcyp19b*, from our genomic and transcriptomic datasets that were significantly being upregulated in our artemisinin-resistant parasite lines, and could likely influence artemisinin sensitivity because of their key involvement in the aforementioned stress responses. To test for this, each of the candidate genes was episomally overexpressed using a 3D7-6A background and then phenotyped for artemisinin sensitivity. In addition to overexpressing *Pftrx1*, *Pf6pgd*, *Pfspp* and *Pfcyp19b*, a control was also generated by transfecting the same parasite background with the empty vector—the same plasmid backbone that was used for the four candidate genes—to serve as a control. Like the overexpression transfectants, the control was also grown under constant BSD pressure to maintain the episome, but did not produce any exogenous mRNA/protein species. This was done so that the vector control could correct for the interference that growth under continuous BSD pressure might have on the artemisinin sensitivity of the transfectants.

Prior to profiling the artemisinin sensitivity of each transfectant parasite line, overexpression of mRNA and protein products were first validated using qPCR and western blot analysis, respectively. Interestingly, even though all four candidate genes are under the control of the same cam promoter and grown under identical doses of BSD to normalize for plasmid copy number, we observed variability in mRNA levels for each respective gene; these variations were likewise reflected in the levels of their corresponding tagged proteins. One possible reason for this is that there might be differences in the episome copy number being maintained within each parasite line—possibly due to differences in episome size or even gene composition of the insert. However, contrary to expectation, the largest episome containing the longest insert (*Pf6pgd*) appears to be the most abundant. Unfortunately, because the episome copy number was not specifically quantified in each overexpression line, it cannot be confirmed if variations in mRNA levels are indeed due to discrepancies in the amount of recombinant episome present in each transfectant line. On the other hand, the differences in mRNA levels can also be attributed to variations in the post-transcriptional regulation of each candidate gene within the parasite—with the overexpression of certain genes being more stably maintained and tolerated compared to others.

Upon measuring artemisinin sensitivity, our results demonstrated that upregulation of three candidate genes *Pftrx1*, *Pfspp* and *Pfcyp19b* led to a significant increase in artemisinin IC₅₀_{10hpi/4hr} relative to the vector control. On the other hand, the *Pf6pgd*-overexpressing line showed no significant change in artemisinin susceptibility despite exhibiting the highest level of mRNA overexpression and tagged-protein production. This lack of phenotypic change in the *Pf6pgd* transfectant also further demonstrates that the observed decrease in artemisinin susceptibility in the other three

parasite lines is indeed specific to the genes and not just a generic effect brought about by the presence of the episome. It also suggests that this particular enzyme and its function—the generation of NADPH-supplied reducing power through the pentose-phosphate pathway—is not, by itself, sufficient to significantly enhance the parasite’s ability to cope with oxidative damage. The same observation was previously made regarding another NADPH-generating, pentose phosphate pathway enzyme *Pfglupho*—where it was found that this enzyme does not appear to be dynamically regulated in response to oxidative stress²¹⁴. Interestingly, we see a range of artemisinin resistance levels among the three parasite lines, with the genes directly involved in the UPR, such as *Pfspp* and *Pfcyp19b*, appearing to have a more pronounced effect on decreasing artemisinin susceptibility compared to redox-related gene *Pftrx1*. This dynamic variation in their chemosensitivity hints at the different levels at which these two processes—oxidative stress and protein stress responses—contribute to mediating artemisinin response and sensitivity, with the UPR appearing to be the more significant factor. Furthermore, our findings provide direct proof that the observed increase in copy number of *Pftrx1* and *Pfspp* in 6A-R are indeed contributing to its artemisinin resistance.

Collectively, our functional studies of the four candidate reinforces the possibility that in addition to point mutations, resistance to artemisinin in *Plasmodium* might also be attributed to changes in the level of expression of certain genes—whether as a result of alterations in gene dosage, or as a downstream effect of variations in gene regulation. Moreover, it also reiterates the role of cellular stress response pathways as key mediators of artemisinin response and artemisinin resistance in *Plasmodium*.

Chapter 8. Summary and conclusion

This thesis presents the work we have done towards generating and characterizing *in vitro* models of artemisinin resistance in *P. falciparum*. Through the periodic exposure of ring-stage (10hpi) parasites to a 4-hr pulse of a pharmacologically relevant dose of artemisinin, we were able to select for resistance in two previously artemisinin-sensitive parasite lines derived from the 3D7 strain of *P. falciparum*. Both of the resistant lines, named 6A-R and 11C-R, were found to be able to survive artemisinin pressure at the ring-stage without entering a state of developmental arrest that is typically observed with parasites exposed to artemisinin during the early stages of the IDC. Quantitative assessment of artemisinin response revealed that both drug-selected lines were significantly less sensitive to the drug compared to their respective controls at the ring stage, and exhibited markedly higher values with regard to the 4-hr pulse assay ($IC_{50_{10hpi/4hr}}$), the standard 72-hr drug assay (IC_{50}), as well as based on the RSA.

Chemosensitivity profiling of 6A-R and 11C-R revealed that the resistance induced through selection using the parent drug artemisinin also extends to its derivatives ATS and DHA, albeit to a lesser extent. On the other hand, this resistance to artemisinin-based drugs did not translate to the other classes of antimalarials including QN, CQ, MEF and PYR. 6A-R also demonstrated cross-resistance against oxidative stress agent H_2O_2 and UPR inducers DTT and EPX. Interestingly, transcriptomic analysis of our artemisinin resistant parasites suggests that, like artemisinin-resistant clinical isolates, they also exhibit an upregulation of pathways related to parasite antioxidant defense and the UPR.

Genomic characterization of our *in vitro* artemisinin-resistant parasite lines revealed a distinct spectrum of SNPs and CNVs between 6A-R and 11C-R

that notably did not include *Pfk13* mutations. Of the genes that were found to be mutated in either parasite line, only two have been previously linked to artemisinin resistance: *Pffp2a* and *Pfprp22*. *Pffp2a* has been shown to modulate artemisinin sensitivity in the ring stages¹²¹, and was also found to contain a nonsense mutation in another *in vitro*-derived DHA-resistant parasite line^{114,119}. While *Pfprp22*—which lies on a copy number amplification found in both 6A-R and 11C-R, was found to show an increased copy number under DHA challenge¹¹⁸. On the other hand, we were also able to identify a set of genetic alterations that have not been previously associated with any drug resistance phenotypes in *Plasmodium*. These include intragenic SNPs in the ff. genes: PF3D7_0420300, PF3D7_0528300, PF3D7_0617100, PF3D7_0730300, PF3D7_0704800, PF3D7_0810600, PF3D7_1141800, PF3D7_1230000, PF3D7_1368400 and PF3D7_1427100, as well as large scale amplifications in chromosome 12 (PF3D7_1228000 - PF3D7_1228800) and chromosome 14 (PF3D7_1454000 - PF3D7_1458000).

Finally, four stress response-related genes (*Pftrx1*, *Pfspp*, *Pf6pgd* and *Pfcyp19B*) from our genomic and transcriptomic datasets were chosen for functional validation via episomal overexpression. All four were found to be significantly upregulated in our artemisinin-resistant parasites, and three (*Pftrx1*, *Pfspp*, *Pf6pgd*) are additionally located in the chromosome 14 amplification found in 6A-R. We found that parasites overexpressing *Pftrx1*, *Pfspp*, and *Pfcyp19b*—but not *Pf6pgd*—experienced a significant reduction in artemisinin sensitivity compared to the transfectant that only carried the empty vector. These results indicate that the specific upregulation these genes are indeed able to alter artemisinin sensitivity in *P. falciparum*.

Taken together, phenotypic and genotypic investigation of our own *in vitro*-generated artemisinin resistant lines provided additional insight into

artemisinin resistance in *Plasmodium*, as well as reinforced previous observations based on work done on other *in vitro* models and actual clinical samples. Similar to what has been documented in slow-clearing, Southeast Asian isolates, our findings support the fundamental involvement of cellular stress responses in mediating artemisinin resistance in *P. falciparum*. On the other hand, the lack of *Pfk13* mutations in both 6A-R and 11C-R indicates that the artemisinin resistance phenotype we have selected is independent of Pfk13, which appears to be the principal driver of artemisinin resistance in Southeast Asia. Furthermore, the heterogeneity of SNPs and CNVs that we were able to identify between 6A-R and 11C-R demonstrates that multiple, alternative genetic events that we have yet to observe in the field could also be responsible for artemisinin resistance. These findings represent a valuable resource for the discovery and validation of novel biomarkers for drug resistance phenotypes in *P. falciparum*, and perhaps even shed light on alternative mechanisms for artemisinin resistance that might emerge in the future, beyond the context of the Southeast Asian paradigm.

Further work can be directed toward evaluating whether or not other gene candidates from our dataset are capable of influencing artemisinin susceptibility, as well as validating the role of specific pathways highlighted in this study in relation to drug resistance. Overall, the artemisinin-resistant parasites we have derived represent a unique and valuable resource for further studies that are geared toward understanding mechanisms of artemisinin resistance, identifying and validating potential biomarkers, and even unravelling the mode of action of artemisinin and its derivatives.

Chapter 9. Appendices

Appendix A Baseline ART dose-response curves at 10-14 hpi, 20-24hpi and 30-34 hpi for 6A and 11C

The figure shows ART dose-response curves of nonselected 6A and 11C at the ring stage (10-14 hpi), trophozoite stage (20-24 hpi), and schizont stage (30-34 hpi); Each line in the graph illustrates data from an individual biological replicate.

Appendix B Evaluating the chemosensitivity of T996-derived ART-resistant *P. falciparum* against MEF

The histogram depicts the mean IC₅₀ for T996-R and its nonselected parent, while the adjacent plot shows their corresponding mean dose-response curves. Drug assays were performed in biological triplicates.

Appendix C IC values of T996-R and T996 against MEF

DRUG	IC (nM)	T996-R	T996
MEF	IC ₅₀	21 ± 5	17 ± 6
	IC ₉₀	39 ± 9	33 ± 9

Appendix D CNV profiling of T996-derived ART-resistant *P. falciparum*

Copy number variations in T996-R were identified using CGH. Chromosome plot shown above depicts the subtracted log₂ratio T996-R relative to nonselected T996.

Appendix E List of copy number-amplified genes detected in T996-R

	GENE ID	DESCRIPTION
CHROMOSOME 14 (T996-R)	PF3D7_1453700	co-chaperone p23
	PF3D7_1453800	glucose-6-phosphate dehydrogenase-6-phosphogluconolactonase
	PF3D7_1453900	conserved Plasmodium protein, unknown function
	PF3D7_1454000	RNA-binding protein, putative
	PF3D7_1454100	tRNA intron endonuclease, putative
	PF3D7_1454200	conserved Plasmodium protein, unknown function
	PF3D7_1454300	serine/threonine protein kinase, putative
	PF3D7_1454400	aminopeptidase P
	PF3D7_1454500	nifU protein, putative
	PF3D7_1454600	mitochondrial ribosomal protein S11 precursor, putative
	PF3D7_1454700	6-phosphogluconate dehydrogenase, decarboxylating, putative
	PF3D7_1454800	conserved Plasmodium protein, unknown function
	PF3D7_1454900	conserved Plasmodium protein, unknown function
	PF3D7_1455000	protein phosphatase, putative
	PF3D7_1455100	protein phosphatase, putative
	PF3D7_1455200	methyltransferase, putative
	PF3D7_1455300	conserved Plasmodium protein, unknown function
	PF3D7_1455400	hemolysin, putative
	PF3D7_1455500	gamma-adaptin, putative
	PF3D7_1455600	ferlin, putative
	PF3D7_1455700	conserved Plasmodium protein, unknown function
	PF3D7_1455800	LCCL domain-containing protein
	PF3D7_1456000	transcription factor with AP2 domain(s)
	PF3D7_1456100	serine hydroxymethyltransferase, putative
	PF3D7_1456200	SNARE protein, putative
	PF3D7_1456300	conserved Plasmodium protein, unknown function
	PF3D7_1456400	conserved Plasmodium protein, unknown function
	PF3D7_1456500	conserved Plasmodium protein, unknown function

Appendix F: List of significantly up- and downregulated genes 6A-R and 6A based on steady-state transcriptional profiling at 10-20hpi ($p < 0.05$). Differential expression was measured using paired student's T-test.

UPREGULATED IN 6A-R vs 6A at 10-20HPI		
Gene ID	Gene Description	<i>p value</i>
PF3D7_1454700	6-phosphogluconate dehydrogenase, decarboxylating, putative	1.45E-05
PF3D7_0607400	G-protein associated signal transduction protein, putative	3.75E-05
PF3D7_0814600	conserved Plasmodium protein, unknown function	7.69E-05
PF3D7_1456700	conserved Plasmodium protein, unknown function	8.94E-05
PF3D7_0522100	conserved Plasmodium protein, unknown function	1.00E-04
PF3D7_0825400	conserved Plasmodium protein, unknown function	1.03E-04
PF3D7_1456800	V-type H(+)-translocating pyrophosphatase, putative	1.20E-04
PF3D7_0315400	conserved Plasmodium protein, unknown function	1.44E-04
PF3D7_1466300	26S proteasome regulatory subunit, putative	1.55E-04
PF3D7_1329900	conserved Plasmodium protein, unknown function	1.96E-04
PF3D7_0802400	conserved Plasmodium protein, unknown function	2.06E-04
PF3D7_0922200	S-adenosylmethionine synthetase	2.40E-04
PF3D7_1455300	conserved Plasmodium protein, unknown function	2.48E-04
PF3D7_1457900	conserved Plasmodium protein, unknown function	2.62E-04
PF3D7_1457200	thioredoxin 1	2.70E-04
PF3D7_1133600	conserved Plasmodium protein, unknown function	2.77E-04
PF3D7_0830600	Plasmodium exported protein (PHISTc), unknown function	3.49E-04
PF3D7_1016500	Plasmodium exported protein (PHISTc), unknown function	4.09E-04
PF3D7_1321000	conserved Plasmodium protein, unknown function	5.15E-04
PF3D7_1243900	double C2-like domain-containing protein	5.15E-04
PF3D7_0629000	conserved protein, unknown function	5.27E-04
PF3D7_1140100	vacuolar ATP synthase subunit f, putative	5.58E-04
PF3D7_0709100	Cg1 protein	6.25E-04
PF3D7_1343600	UDP-N-acetylglucosamine pyrophosphorylase, putative	6.69E-04
PF3D7_0216400	vacuolar protein sorting-associated protein 45, putative	6.85E-04
PF3D7_1419800	glutathione reductase	7.22E-04
PF3D7_1454600	mitochondrial ribosomal protein S11 precursor, putative	7.43E-04
PF3D7_1466600	conserved Plasmodium protein, unknown function	7.51E-04
PF3D7_1454200	conserved Plasmodium protein, unknown function	7.56E-04
PF3D7_1036600	conserved Plasmodium protein, unknown function	7.59E-04
PF3D7_1361400	actin-depolymerizing factor 2	7.95E-04
PF3D7_1306400	26S proteasome regulatory subunit, putative	8.06E-04
PF3D7_1447000	40S ribosomal protein S2, putative	8.07E-04
PF3D7_0409100	U4/U6 small nuclear ribonucleoprotein PRP31, putative	9.24E-04
PF3D7_1454900	conserved Plasmodium protein, unknown function	9.28E-04
PF3D7_0624300	conserved Plasmodium protein, unknown function	9.34E-04
PF3D7_0816600	chaperone protein ClpB1	9.48E-04
PF3D7_0605600	nucleoside diphosphate kinase, putative	9.49E-04
PF3D7_0934500	vacuolar ATP synthase subunit e, putative	9.78E-04
PF3D7_0616100	conserved Plasmodium protein, unknown function	9.85E-04
PF3D7_0816700	conserved Plasmodium protein, unknown function	1.01E-03
PF3D7_1104700	RNA polymerase subunit, putative	1.02E-03
PF3D7_1367500	NADH-cytochrome b5 reductase, putative	1.12E-03
PF3D7_1356000	conserved Plasmodium protein, unknown function	1.16E-03
PF3D7_1029700	conserved Plasmodium protein, unknown function	1.22E-03
PF3D7_1012300	ubiquinol-cytochrome c reductase complex subunit, putative	1.36E-03
PF3D7_0727200	cysteine desulfurase, putative	1.38E-03
PF3D7_0113400	Plasmodium exported protein, unknown function	1.42E-03
PF3D7_1322900	conserved Plasmodium protein, unknown function	1.42E-03
PF3D7_0720600	conserved Plasmodium protein, unknown function	1.43E-03

PF3D7_1226800	ataxin-3, putative	1.52E-03
PF3D7_1120600	conserved Plasmodium protein, unknown function	1.53E-03
PF3D7_1478100	Plasmodium exported protein (hyp13), unknown function	1.60E-03
PF3D7_1311300	ATP synthase subunit gamma, mitochondrial	1.61E-03
PF3D7_0814700	conserved Plasmodium protein, unknown function	1.63E-03
PF3D7_1117500	tyrosine--tRNA ligase	1.65E-03
PF3D7_1457500	vacuolar protein sorting-associated protein 4	1.70E-03
PF3D7_1366600	signal recognition particle receptor alpha subunit	1.70E-03
PF3D7_1106000	RuvB-like helicase 2	1.75E-03
PF3D7_0604600	DNA helicase, putative	1.75E-03
PF3D7_0501000	Plasmodium exported protein, unknown function	1.84E-03
PF3D7_0936700	lysophospholipase, putative	1.86E-03
PF3D7_1400400	rifin PIR protein	1.87E-03
PF3D7_1329200	conserved Plasmodium protein, unknown function	1.87E-03
PF3D7_1457000	signal peptide peptidase	1.94E-03
PF3D7_1335300	reticulocyte binding protein 2 homologue b	1.97E-03
PF3D7_1454400	aminopeptidase P	1.98E-03
PF3D7_0710300	conserved Plasmodium membrane protein, unknown function	2.03E-03
PF3D7_1415300	RNA-binding protein Nova-1, putative	2.04E-03
PF3D7_0310700	sybindin-like protein, putative	2.05E-03
PF3D7_0505100	conserved Plasmodium protein, unknown function	2.18E-03
PF3D7_0624200	conserved Plasmodium protein, unknown function	2.29E-03
PF3D7_1108500	succinyl-CoA synthetase alpha subunit, putative	2.29E-03
PF3D7_1364500	exosome complex exonuclease, putative	2.35E-03
PF3D7_0723100	conserved Plasmodium protein, unknown function	2.38E-03
PF3D7_1454500	nifU protein, putative	2.46E-03
PF3D7_1247100	conserved Plasmodium protein, unknown function	2.46E-03
PF3D7_1213200	conserved Plasmodium protein, unknown function	2.49E-03
PF3D7_1364700	conserved Plasmodium protein, unknown function, pseudogene	2.53E-03
PF3D7_1367800	secreted ookinete protein, putative	2.75E-03
PF3D7_1455400	hemolysin, putative	2.78E-03
PF3D7_1115800	conserved Plasmodium protein, unknown function	2.81E-03
PF3D7_1472400	M1-family alanyl aminopeptidase, putative	2.85E-03
PF3D7_0107900	conserved Plasmodium protein, unknown function	2.89E-03
PF3D7_0611600	conserved Plasmodium protein, unknown function	2.89E-03
PF3D7_1455500	gamma-adaptin, putative	2.90E-03
PF3D7_1211500	mitochondrial ribosomal protein S18 precursor, putative	2.91E-03
PF3D7_1230600	sun-family protein, putative	2.92E-03
PF3D7_1234700	CPW-WPC family protein	2.96E-03
PF3D7_1329400	AMP deaminase, putative	3.09E-03
PF3D7_1365600	DNA topoisomerase VI, b subunit, putative	3.10E-03
PF3D7_1366200	conserved Plasmodium protein, unknown function	3.18E-03
PF3D7_0918300	eukaryotic translation initiation factor 3 subunit 5, putative	3.20E-03
PF3D7_0827900	protein disulfide isomerase	3.22E-03
PF3D7_1224700	conserved Plasmodium protein, unknown function	3.26E-03
PF3D7_0623000	chorismate synthase	3.33E-03
PF3D7_1445900	DEAD/DEAH box ATP-dependent RNA helicase, putative	3.37E-03
PF3D7_0820100	conserved Plasmodium protein, unknown function	3.48E-03
PF3D7_1134800	coatamer delta subunit, putative	3.50E-03
PF3D7_0517400	FACT complex subunit SPT16, putative	3.50E-03
PF3D7_1021600	deoxyribose-phosphate aldolase, putative	3.52E-03
PF3D7_1453100	dynactin subunit 4, putative	3.52E-03
PF3D7_1303100	methyltransferase-like protein, putative	3.52E-03
PF3D7_1455200	methyltransferase, putative	3.56E-03
PF3D7_0630200	secreted ookinete protein, putative	3.58E-03
PF3D7_0731300	Plasmodium exported protein (PHISTb), unknown function	3.61E-03
PF3D7_0419500	conserved Plasmodium membrane protein, unknown function	3.68E-03
PF3D7_0107100	conserved Plasmodium protein, unknown function	3.71E-03
PF3D7_0522300	S-adenosylmethionine-dependent methyltransferase, putative	3.73E-03
PF3D7_1363400	polyubiquitin binding protein, putative	3.73E-03
PF3D7_1129500	A/G-specific adenine glycosylase, putative	3.75E-03

PF3D7_1354500	adenylosuccinate synthetase	3.86E-03
PF3D7_1141900	inner membrane complex protein 1b, putative	3.91E-03
PF3D7_1216200	glycerol-3-phosphate dehydrogenase, putative	4.06E-03
PF3D7_1438900	thioredoxin peroxidase 1	4.14E-03
PF3D7_1352500	thioredoxin-related protein, putative	4.19E-03
PF3D7_1106400	conserved Plasmodium protein, unknown function	4.20E-03
PF3D7_1406700	phosphatase, putative	4.28E-03
PF3D7_1405300	conserved Plasmodium protein, unknown function	4.31E-03
PF3D7_1245500	conserved Plasmodium protein, unknown function	4.33E-03
PF3D7_1475000	mediator complex subunit 31, putative	4.43E-03
PF3D7_1215300	10 kDa chaperonin	4.46E-03
PF3D7_1106300	exonuclease, putative	4.48E-03
PF3D7_1117800	DNA mismatch repair protein MLH	4.50E-03
PF3D7_1213600	dynein light chain 1, putative	4.55E-03
PF3D7_1011900	heme oxygenase	4.56E-03
PF3D7_0818300	dynactin subunit 6, putative	4.57E-03
PF3D7_1470800	conserved Plasmodium protein, unknown function	4.59E-03
PF3D7_0903000	conserved protein, unknown function	4.59E-03
PF3D7_0906400	dynein light intermediate chain 2, cytosolic	4.65E-03
PF3D7_0212000	GDP-fructose:GMP antiporter, putative	4.66E-03
PF3D7_0211200	ras-related protein Rab-5A	4.71E-03
PF3D7_0801500	conserved Plasmodium protein, unknown function	4.74E-03
PF3D7_0933500	gamma-tubulin complex component, putative	4.80E-03
PF3D7_1457600	conserved Plasmodium protein, unknown function	4.81E-03
PF3D7_0315000	zinc finger protein, putative	4.81E-03
PF3D7_1368600	mitochondrial import inner membrane translocase subunit TIM9, putative	4.91E-03
PF3D7_1469500	conserved Plasmodium protein, unknown function	4.94E-03
PF3D7_0907100	conserved Plasmodium protein, unknown function	4.98E-03
PF3D7_0812900	probable protein, unknown function	5.09E-03
PF3D7_1355600	conserved Plasmodium protein, unknown function	5.09E-03
PF3D7_1351600	glycerol kinase	5.10E-03
PF3D7_1455700	conserved Plasmodium protein, unknown function	5.13E-03
PF3D7_1403000	conserved Plasmodium protein, unknown function	5.16E-03
PF3D7_0321300	conserved Plasmodium protein, unknown function	5.17E-03
PF3D7_0902700	Plasmodium exported protein (PHISTb), unknown function, pseudogene	5.30E-03
PF3D7_0727500	conserved Plasmodium protein, unknown function	5.31E-03
PF3D7_1226000	conserved Plasmodium protein, unknown function	5.31E-03
PF3D7_0210700	syntaxin, Qa-SNARE family	5.33E-03
PF3D7_1037100	pyruvate kinase 2	5.35E-03
PF3D7_0726700	conserved Plasmodium protein, unknown function	5.38E-03
PF3D7_0608800	ornithine aminotransferase	5.47E-03
PF3D7_0526200	ADP-ribosylation factor GTPase-activating protein, putative	5.51E-03
PF3D7_0317900	conserved Plasmodium protein, unknown function	5.52E-03
PF3D7_0531200	mitochondrial ribosomal protein S16 precursor, putative	5.56E-03
PF3D7_1018100	conserved Plasmodium protein, unknown function	5.78E-03
PF3D7_0313100	ubiquitin-protein ligase, putative	5.83E-03
PF3D7_0502800	DnaJ protein, putative	5.91E-03
PF3D7_1127900	conserved Plasmodium protein, unknown function	6.09E-03
PF3D7_1336600	conserved Plasmodium protein, unknown function	6.26E-03
PF3D7_0717400	queuine tRNA-ribosyltransferase, putative	6.29E-03
PF3D7_0504100	AN1-like zinc finger family protein	6.45E-03
PF3D7_1010700	dolichyl-phosphate-mannose protein mannosyltransferase, putative	6.52E-03
PF3D7_1002000	Plasmodium exported protein (hyp2), unknown function	6.75E-03
PF3D7_0223400	rifin PIR protein	6.83E-03
PF3D7_0730000	conserved Plasmodium protein, unknown function	6.85E-03
PF3D7_1458000	cysteine proteinase falcipain 1	6.87E-03
PF3D7_0907900	peptide deformylase	6.95E-03
PF3D7_1224300	polyadenylate-binding protein, putative	7.15E-03
PF3D7_1416500	NADP-specific glutamate dehydrogenase	7.15E-03
PF3D7_1234000	conserved Plasmodium protein, unknown function	7.17E-03
PF3D7_0624500	anaphase promoting complex subunit, putative	7.29E-03

PF3D7_1124100	neutral-sphingomyelinase activation factor protein, putative	7.44E-03
PF3D7_1327800	ribose-phosphate pyrophosphokinase, putative	7.46E-03
PF3D7_0205500	DNA-directed RNA polymerase II 16 kDa subunit, putative	7.47E-03
PF3D7_1002500	conserved Plasmodium protein, unknown function	7.60E-03
PF3D7_0212700	conserved Plasmodium protein, unknown function	7.70E-03
PF3D7_1233000	elongation factor G	7.75E-03
PF3D7_1462800	glyceraldehyde-3-phosphate dehydrogenase	7.83E-03
PF3D7_1328400	conserved Plasmodium protein, unknown function	8.08E-03
PF3D7_1320200	TBC domain protein, putative	8.09E-03
PF3D7_0417500	memo-like protein	8.12E-03
PF3D7_1363100	conserved Plasmodium protein, unknown function	8.13E-03
PF3D7_1455100	protein phosphatase, putative	8.28E-03
PF3D7_0611400	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin	8.30E-03
PF3D7_1009900	conserved Plasmodium protein, unknown function	8.31E-03
PF3D7_1431800	apyrase, putative	8.37E-03
PF3D7_0918800	dihydrouridine synthase, putative	8.38E-03
PF3D7_0301400	Plasmodium exported protein, unknown function	8.48E-03
PF3D7_1356700	DnaJ protein, putative	8.49E-03
PF3D7_1456300	conserved Plasmodium protein, unknown function	8.60E-03
PF3D7_0731100	EMP1-trafficking protein	8.65E-03
PF3D7_1011000	inner membrane complex protein, putative	8.69E-03
PF3D7_0708600	heat shock protein 86 family protein	8.78E-03
PF3D7_0820500	C-13 antigen	8.82E-03
PF3D7_0424000	Plasmodium exported protein (PHISTc), unknown function	8.95E-03
PF3D7_0609400	mitochondrial cardiolipin synthase, putative	9.08E-03
PF3D7_1466400	transcription factor with AP2 domain(s)	9.11E-03
PF3D7_1304400	conserved Plasmodium protein, unknown function	9.21E-03
PF3D7_0213800	conserved Plasmodium protein, unknown function	9.27E-03
PF3D7_1007000	conserved membrane protein, unknown function	9.27E-03
PF3D7_1469000	translation initiation factor IF-1, putative	9.27E-03
PF3D7_0729200	1-cys peroxiredoxin	9.57E-03
PF3D7_1116500	folate transporter 2	9.58E-03
PF3D7_0412400	erythrocyte membrane protein 1, PfEMP1	9.62E-03
PF3D7_1208700	conserved Plasmodium protein, unknown function	9.63E-03
PF3D7_0830400	conserved Plasmodium protein, unknown function	9.85E-03
PF3D7_1144900	ras-related protein Rab-6	9.85E-03
PF3D7_0425200	Plasmodium exported protein (hyp15), unknown function	1.01E-02
PF3D7_1224400	conserved Plasmodium protein, unknown function	1.01E-02
PF3D7_0915000	type II NADH:ubiquinone oxidoreductase	1.01E-02
PF3D7_1253000	gametocyte erythrocyte cytosolic protein	1.02E-02
PF3D7_1229400	macrophage migration inhibitory factor	1.03E-02
PF3D7_0918200	50S ribosomal protein L3, apicoplast, putative	1.04E-02
PF3D7_0109400	tubulin-specific chaperone a, putative	1.07E-02
PF3D7_1311200	alternative splicing regulator, putative	1.07E-02
PF3D7_0728000	eukaryotic translation initiation factor 2 alpha subunit, putative	1.08E-02
PF3D7_1438500	cleavage and polyadenylation specificity factor, putative	1.08E-02
PF3D7_0820400	conserved Plasmodium protein, unknown function	1.08E-02
PF3D7_1028200	zinc finger, C3HC4 type, putative	1.09E-02
PF3D7_1307300	DEAD box helicase, putative	1.10E-02
PF3D7_1110600	mitochondrial ribosomal protein L11 precursor, putative	1.11E-02
PF3D7_0217300	clathrin coat assembly protein, putative	1.12E-02
PF3D7_1443500	poly(A)-specific ribonuclease PARN, putative	1.13E-02
PF3D7_0829300	U6 snRNA-associated Sm-like protein LSm8, putative	1.14E-02
PF3D7_1401800	choline kinase	1.15E-02
PF3D7_1315400	zinc finger (CCCH type) protein, putative	1.15E-02
PF3D7_0622700	conserved Plasmodium membrane protein, unknown function	1.19E-02
PF3D7_1428800	transcription initiation TFIID-like, putative	1.20E-02
PF3D7_1027900	palmitoyltransferase, putative	1.21E-02
PF3D7_0610600	calcium-dependent protein kinase 2	1.21E-02
PF3D7_1478800	Plasmodium exported protein, unknown function	1.22E-02
PF3D7_1358000	patatin-like phospholipase, putative	1.22E-02

PF3D7_0103400	zinc-carboxypeptidase, putative	1.23E-02
PF3D7_1463800	mitochondrial ribosomal protein S6-2 precursor, putative	1.23E-02
PF3D7_1227000	conserved Plasmodium protein, unknown function	1.24E-02
PF3D7_1150400	erythrocyte membrane protein 1, PfEMP1	1.24E-02
PF3D7_0416300	DNA helicase MCM9, putative	1.26E-02
PF3D7_0109700	conserved protein, unknown function	1.27E-02
PF3D7_0929200	RNA-binding protein, putative	1.27E-02
PF3D7_1419200	conserved Plasmodium protein, unknown function	1.27E-02
PF3D7_1437500	conserved Plasmodium membrane protein, unknown function	1.29E-02
PF3D7_1008800	nucleolar protein 5, putative	1.30E-02
PF3D7_1368100	proteasome regulatory subunit, putative	1.31E-02
PF3D7_0825100	conserved Plasmodium protein, unknown function	1.31E-02
PF3D7_1115300	cysteine proteinase falcipain 2b	1.32E-02
PF3D7_1005300	conserved Plasmodium protein, unknown function	1.32E-02
PF3D7_1002100	EMP1-trafficking protein	1.33E-02
PF3D7_0305400	conserved Plasmodium protein, unknown function	1.34E-02
PF3D7_1338500	conserved Plasmodium protein, unknown function	1.34E-02
PF3D7_0628500	conserved Plasmodium protein, unknown function	1.35E-02
PF3D7_1447300	mitochondrial ribosomal protein S14 precursor, putative	1.37E-02
PF3D7_0105900	DNA binding protein, putative	1.37E-02
PF3D7_1314700	conserved Plasmodium protein, unknown function	1.38E-02
PF3D7_0104400	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	1.38E-02
PF3D7_1147900	conserved Plasmodium protein, unknown function	1.39E-02
PF3D7_0613200	conserved Plasmodium protein, unknown function	1.40E-02
PF3D7_1460600	inner membrane complex protein, putative	1.40E-02
PF3D7_1214300	geranylgeranyl transferase type2 beta subunit, putative	1.42E-02
PF3D7_0208700	conserved Plasmodium protein, unknown function	1.42E-02
PF3D7_1345000	conserved Plasmodium protein, unknown function	1.43E-02
PF3D7_1434100	queueine tRNA-ribosyltransferase, putative	1.44E-02
PF3D7_0511200	stearoyl-CoA desaturase	1.51E-02
PF3D7_1033700	bromodomain protein, putative	1.51E-02
PF3D7_1440200	stromal-processing peptidase, putative	1.52E-02
PF3D7_1337100	protein kinase 6	1.53E-02
PF3D7_0202400	conserved Plasmodium protein, unknown function	1.59E-02
PF3D7_1033000	conserved Plasmodium protein, unknown function	1.59E-02
PF3D7_1134000	heat shock protein 70	1.60E-02
PF3D7_1245300	ubiquitin conjugating enzyme E2, putative	1.62E-02
PF3D7_1361700	cytochrome c oxidase subunit 2, putative	1.62E-02
PF3D7_1430100	phosphotyrosyl phosphatase activator, putative	1.63E-02
PF3D7_0914200	phospholipid or glycerol acyltransferase, putative	1.63E-02
PF3D7_1235500	mRNA methyltransferase, putative	1.65E-02
PF3D7_1205600	conserved Plasmodium protein, unknown function	1.65E-02
PF3D7_0104500	conserved protein, unknown function	1.66E-02
PF3D7_1409900	cytidine diphosphate-diacylglycerol synthase	1.66E-02
PF3D7_1411400	plastid replication-repair enzyme	1.67E-02
PF3D7_1303300	conserved Plasmodium protein, unknown function	1.68E-02
PF3D7_1251400	conserved Plasmodium protein, unknown function	1.69E-02
PF3D7_0532200	Plasmodium exported protein (PHISTc), unknown function	1.69E-02
PF3D7_1363600	conserved Plasmodium protein, unknown function	1.70E-02
PF3D7_1457800	conserved Plasmodium protein, unknown function	1.71E-02
PF3D7_0707400	AAA family ATPase, putative	1.72E-02
PF3D7_1334700	MSP7-like protein	1.73E-02
PF3D7_1012000	E3 ubiquitin-protein ligase, putative	1.73E-02
PF3D7_1357400	conserved Plasmodium protein, unknown function	1.73E-02
PF3D7_1347100	DNA topoisomerase III, putative	1.74E-02
PF3D7_0322900	40S ribosomal protein S3A, putative	1.74E-02
PF3D7_0915400	6-phosphofructokinase	1.74E-02
PF3D7_1407900	plasmepsin I	1.78E-02
PF3D7_1471300	conserved Plasmodium protein, unknown function	1.79E-02
PF3D7_0904100	adaptor-related protein, putative	1.79E-02
PF3D7_1031400	OTU-like cysteine protease, putative	1.79E-02

PF3D7_1206000	shewanella-like protein phosphatase 2	1.79E-02
PF3D7_0726200	serine/threonine protein kinase, FIKK family	1.80E-02
PF3D7_1476800	lysophospholipase, putative	1.81E-02
PF3D7_1456600	mitochondrial ribosomal protein L28 precursor, putative	1.82E-02
PF3D7_0521500	ribosomal large subunit pseudouridylate synthase, putative	1.83E-02
PF3D7_0522000	conserved Plasmodium protein, unknown function	1.83E-02
PF3D7_0517700	eukaryotic translation initiation factor, putative	1.86E-02
PF3D7_1018700	conserved Plasmodium protein, unknown function	1.87E-02
PF3D7_0623700	ATP dependent DEAD-box helicase, putative	1.89E-02
PF3D7_0814400	phospholipase DDHD1, putative	1.91E-02
PF3D7_1322000	adenosine-diphosphatase, putative	1.93E-02
PF3D7_1414300	60S ribosomal protein L10, putative	1.94E-02
PF3D7_0823000	protein kinase, putative	1.94E-02
PF3D7_1465700	plasmepsin VIII	1.95E-02
PF3D7_0912600	conserved Plasmodium protein, unknown function	1.95E-02
PF3D7_0912700	conserved Plasmodium protein, unknown function	1.96E-02
PF3D7_0602400	elongation factor G	1.96E-02
PF3D7_0106100	vacuolar ATP synthase subunit c, putative	1.99E-02
PF3D7_1015200	cysteine--tRNA ligase, putative	1.99E-02
PF3D7_0923700	conserved protein, unknown function	2.00E-02
PF3D7_1316600	choline-phosphate cytidyltransferase	2.01E-02
PF3D7_0931400	conserved Plasmodium protein, unknown function	2.01E-02
PF3D7_1354700	conserved Plasmodium protein, unknown function	2.02E-02
PF3D7_1308700	conserved Plasmodium protein, unknown function	2.02E-02
PF3D7_1339000	probable protein, unknown function	2.03E-02
PF3D7_1357600	conserved Plasmodium protein, unknown function	2.04E-02
PF3D7_0513000	conserved Plasmodium protein, unknown function	2.10E-02
PF3D7_0929100	conserved Plasmodium protein, unknown function	2.10E-02
PF3D7_0312800	60S ribosomal protein L26, putative	2.11E-02
PF3D7_0318100	stomatin-like protein	2.11E-02
PF3D7_1017400	phosphomannomutase, putative	2.12E-02
PF3D7_1216000	serine--tRNA ligase, putative	2.13E-02
PF3D7_1126800	RNA-binding protein, putative	2.14E-02
PF3D7_0716600	cysteine desulfurase	2.16E-02
PF3D7_1457300	conserved Plasmodium protein, unknown function	2.16E-02
PF3D7_1449500	transcription factor with AP2 domain(s), putative	2.16E-02
PF3D7_1010400	conserved Plasmodium protein, unknown function	2.17E-02
PF3D7_1237700	conserved Plasmodium membrane protein, unknown function	2.18E-02
PF3D7_1219900	D-ribulose-5-phosphate 3-epimerase, putative	2.19E-02
PF3D7_1124500	pyruvate dehydrogenase E1 alpha subunit	2.19E-02
PF3D7_0109900	phosphatidate cytidyltransferase, putative	2.19E-02
PF3D7_1418200	conserved Plasmodium protein, unknown function	2.20E-02
PF3D7_1143300	DNA-directed RNA polymerase I, putative	2.22E-02
PF3D7_0501300	skeleton-binding protein 1	2.23E-02
PF3D7_0906910	tubulin-specific chaperone, putative	2.24E-02
PF3D7_0905700	autophagy-related protein 3, putative	2.24E-02
PF3D7_0406800	conserved Plasmodium protein, unknown function	2.24E-02
PF3D7_0912200	conserved Plasmodium membrane protein, unknown function	2.25E-02
PF3D7_1401900	conserved Plasmodium protein, unknown function	2.26E-02
PF3D7_0709800	conserved Plasmodium protein, unknown function	2.27E-02
PF3D7_1208900	conserved Plasmodium protein, unknown function	2.31E-02
PF3D7_0809100	erythrocyte membrane protein 1, PfEMP1	2.31E-02
PF3D7_1111100	replication factor C subunit 5, putative	2.32E-02
PF3D7_1230700	protein transport protein SEC13	2.32E-02
PF3D7_1335800	conserved Plasmodium protein, unknown function	2.33E-02
PF3D7_0713000	rifin PIR protein	2.34E-02
PF3D7_1212200	conserved Plasmodium protein, unknown function	2.37E-02
PF3D7_0401600	rifin PIR protein	2.38E-02
PF3D7_0419200	CGI-141 protein homolog, putative	2.39E-02
PF3D7_1368500	conserved Plasmodium protein, unknown function	2.39E-02
PF3D7_1345500	ubiquitin-conjugating enzyme E2, putative	2.39E-02

PF3D7_1137700	conserved Plasmodium protein, unknown function	2.41E-02
PF3D7_0414400	conserved Plasmodium protein, unknown function	2.41E-02
PF3D7_0623900	ribonuclease H2 subunit A, putative	2.43E-02
PF3D7_1210900	GPI mannosyltransferase I	2.44E-02
PF3D7_1335600	conserved Plasmodium protein, unknown function	2.46E-02
PF3D7_1218900	conserved Plasmodium protein, unknown function	2.46E-02
PF3D7_1135800	conserved Plasmodium protein, unknown function	2.48E-02
PF3D7_1001100	acyl-CoA binding protein, isoform 1, ACBP1	2.48E-02
PF3D7_0626300	3-oxoacyl-acyl-carrier protein synthase I/II	2.51E-02
PF3D7_1226900	conserved Plasmodium protein, unknown function	2.51E-02
PF3D7_1338100	proteasome regulatory subunit, putative	2.52E-02
PF3D7_0606800	probable protein, unknown function	2.53E-02
PF3D7_0725300	conserved Plasmodium protein, unknown function	2.54E-02
PF3D7_1119500	clathrin coat assembly protein, putative	2.59E-02
PF3D7_1455600	ferlin, putative	2.59E-02
PF3D7_0204000	conserved Plasmodium protein, unknown function	2.64E-02
PF3D7_0517300	pre-mRNA-splicing factor	2.65E-02
PF3D7_1310800	conserved Plasmodium protein, unknown function	2.65E-02
PF3D7_0314100	vesicle transport v-SNARE protein, putative	2.65E-02
PF3D7_1012400	hypoxanthine-guanine phosphoribosyltransferase	2.65E-02
PF3D7_1332600	apurinic/aprimidinic endonuclease Apn1, putative	2.66E-02
PF3D7_0104200	StAR-related lipid transfer protein	2.67E-02
PF3D7_1408100	plasmepsin III histo-aspartic protease	2.70E-02
PF3D7_1440400	conserved Plasmodium protein, unknown function	2.71E-02
PF3D7_1123400	translation elongation factor EF-1, subunit alpha, putative	2.71E-02
PF3D7_0503100	4-diphosphocytidyl-2c-methyl-D-erythritol kinase (CMK), putative	2.71E-02
PF3D7_0205300	conserved Plasmodium protein, unknown function	2.71E-02
PF3D7_0523100	mitochondrial processing peptidase alpha subunit, putative	2.72E-02
PF3D7_1115000	conserved Plasmodium protein, unknown function	2.73E-02
PF3D7_0809800	conserved Plasmodium protein, unknown function	2.76E-02
PF3D7_1232900	nucleotidyltransferase, putative	2.78E-02
PF3D7_0410700	GTPase, putative	2.79E-02
PF3D7_0109300	fatty acid elongation protein, GNS1/SUR4 family, putative	2.79E-02
PF3D7_0408800	conserved Plasmodium protein, unknown function	2.79E-02
PF3D7_0523300	conserved Plasmodium protein, unknown function	2.79E-02
PF3D7_0503900	conserved Plasmodium protein, unknown function	2.79E-02
PF3D7_1115500	transcription factor with AP2 domain(s), putative	2.79E-02
PF3D7_0409700	peptide chain release factor 2, putative	2.81E-02
PF3D7_1346100	protein transport protein SEC61 subunit alpha	2.82E-02
PF3D7_1367900	conserved Plasmodium protein, unknown function	2.83E-02
PF3D7_0606200	ubiquitin conjugating enzyme E2, putative	2.84E-02
PF3D7_1454300	serine/threonine protein kinase, putative	2.84E-02
PF3D7_1435700	ataxin-2 like protein, putative	2.85E-02
PF3D7_1023400	HORMA domain protein, putative	2.85E-02
PF3D7_0519900	conserved Plasmodium protein, unknown function	2.85E-02
PF3D7_1321500	3',5'-cyclic nucleotide phosphodiesterase, putative	2.85E-02
PF3D7_0908800	transporter, putative	2.85E-02
PF3D7_1112000	conserved Plasmodium protein, unknown function	2.85E-02
PF3D7_1134400	conserved Plasmodium protein, unknown function	2.86E-02
PF3D7_1421500	mitochondrial import inner membrane translocase subunit TIM8, putative	2.86E-02
PF3D7_1021000	conserved Plasmodium protein, unknown function	2.87E-02
PF3D7_1220900	heterochromatin protein 1	2.87E-02
PF3D7_1221100	conserved Plasmodium protein, unknown function	2.88E-02
PF3D7_0922600	glutamine synthetase, putative	2.91E-02
PF3D7_1134600	zinc finger protein, putative	2.93E-02
PF3D7_0517100	conserved Plasmodium protein, unknown function	2.93E-02
PF3D7_1332300	sedlin, putative	2.95E-02
PF3D7_0208800	conserved Plasmodium protein, unknown function	2.98E-02
PF3D7_0830500	tryptophan/threonine-rich antigen	2.98E-02
PF3D7_0310600	SAC3/GNAP family-related protein, putative	3.04E-02
PF3D7_0819200	perforin-like protein 5	3.11E-02

PF3D7_1336400	RanBPM and CLTH-like protein, putative	3.12E-02
PF3D7_1401100	DnaJ protein, putative	3.16E-02
PF3D7_0315900	conserved Plasmodium protein, unknown function	3.17E-02
PF3D7_1456900	conserved protein, unknown function	3.18E-02
PF3D7_1457400	conserved Plasmodium protein, unknown function	3.20E-02
PF3D7_0623200	ferredoxin-NADP reductase	3.21E-02
PF3D7_0113900	Plasmodium exported protein (hyp8), unknown function	3.21E-02
PF3D7_1003300	N-acetyltransferase, putative	3.25E-02
PF3D7_1318700	conserved Plasmodium protein, unknown function	3.29E-02
PF3D7_0812220	GTPase, putative	3.30E-02
PF3D7_0520800	conserved Plasmodium protein, unknown function	3.30E-02
PF3D7_0810900	conserved Plasmodium protein, unknown function	3.31E-02
PF3D7_1331200	conserved Plasmodium protein, unknown function	3.34E-02
PF3D7_0818800	U3 small nucleolar ribonucleoprotein protein, putative	3.36E-02
PF3D7_0616000	pyridoxal kinase	3.37E-02
PF3D7_1218700	conserved Plasmodium protein, unknown function	3.37E-02
PF3D7_0801600	conserved Plasmodium protein, unknown function	3.37E-02
PF3D7_0919600	dolichyl-diphosphooligosaccharide--protein glycosyltransferase, putative	3.38E-02
PF3D7_1223200	nucleus export protein BRR6, putative	3.39E-02
PF3D7_1025100	glutamine--fructose-6-phosphate aminotransferase [isomerizing], putative	3.41E-02
PF3D7_1312200	conserved Plasmodium protein, unknown function	3.41E-02
PF3D7_0409300	conserved Plasmodium protein, unknown function	3.45E-02
PF3D7_0513800	ras-related protein Rab-1A	3.46E-02
PF3D7_0823600	lipoate-protein ligase B	3.47E-02
PF3D7_0206700	adenylosuccinate lyase	3.48E-02
PF3D7_0308700	conserved Plasmodium protein, unknown function	3.48E-02
PF3D7_1030300	conserved Plasmodium protein, unknown function	3.49E-02
PF3D7_1320500	SNARE protein, putative	3.49E-02
PF3D7_0731600	acyl-CoA synthetase	3.50E-02
PF3D7_0624700	N-acetylglucosaminylphosphatidylinositol deacetylase, putative	3.53E-02
PF3D7_1141700	conserved Plasmodium protein, unknown function	3.54E-02
PF3D7_1242000	conserved Plasmodium protein, unknown function	3.54E-02
PF3D7_1459500	conserved Plasmodium protein, unknown function	3.54E-02
PF3D7_1349300	tyrosine kinase-like protein	3.55E-02
PF3D7_1304500	small heat shock protein, putative	3.55E-02
PF3D7_0520500	phosphomethylpyrimidine kinase, putative	3.55E-02
PF3D7_1121400	WD domain, G-beta repeat-containing protein	3.56E-02
PF3D7_1364000	conserved Plasmodium protein, unknown function	3.56E-02
PF3D7_1467600	conserved Plasmodium protein, unknown function	3.57E-02
PF3D7_0106000	conserved Plasmodium protein, unknown function	3.59E-02
PF3D7_0824400	nucleoside transporter 2	3.61E-02
PF3D7_0602500	geranylgeranyltransferase, putative	3.62E-02
PF3D7_0305700	ubiquitin conjugating enzyme E2, putative	3.63E-02
PF3D7_0924000	patatin-like phospholipase, putative	3.64E-02
PF3D7_0609300	conserved Plasmodium protein, unknown function	3.64E-02
PF3D7_0731200	Plasmodium exported protein, unknown function	3.64E-02
PF3D7_0307200	60S ribosomal protein L7, putative	3.65E-02
PF3D7_0810400	aquaporin, putative	3.68E-02
PF3D7_1107200	conserved Plasmodium protein, unknown function	3.69E-02
PF3D7_1366700	conserved Plasmodium protein, unknown function	3.69E-02
PF3D7_1131900	conserved Plasmodium protein, unknown function	3.70E-02
PF3D7_1359500	conserved Plasmodium protein, unknown function	3.71E-02
PF3D7_0532600	Plasmodium exported protein, unknown function	3.71E-02
PF3D7_1216400	conserved Plasmodium membrane protein, unknown function	3.72E-02
PF3D7_0414700	GTP-binding protein, putative	3.73E-02
PF3D7_1447500	conserved Plasmodium protein, unknown function	3.73E-02
PF3D7_0915200	ribonuclease H2 subunit C, putative	3.75E-02
PF3D7_0932300	M18 aspartyl aminopeptidase	3.79E-02
PF3D7_0410600	conserved Plasmodium protein, unknown function	3.80E-02
PF3D7_1318200	apicoplast glycerol-3-phosphate acyltransferase, putative	3.80E-02
PF3D7_1215100	conserved Plasmodium protein, unknown function	3.80E-02

PF3D7_0819400	perforin-like protein 4	3.81E-02
PF3D7_1338800	CPW-WPC family protein	3.81E-02
PF3D7_0423000	conserved Plasmodium protein, unknown function	3.83E-02
PF3D7_1230100	thioredoxin-like associated protein 1, putative	3.86E-02
PF3D7_0710400	nucleotide excision repair protein, putative	3.87E-02
PF3D7_1347700	ethanolamine-phosphate cytidyltransferase	3.87E-02
PF3D7_1128200	multiprotein bridging factor type 1, putative	3.89E-02
PF3D7_0218500	small nuclear ribonucleoprotein Sm D2, putative	3.91E-02
PF3D7_0630500	microtubule-associated protein ytm1 homologue, putative	3.92E-02
PF3D7_0530000	conserved Plasmodium protein, unknown function	3.94E-02
PF3D7_1453000	conserved Plasmodium protein, unknown function	3.95E-02
PF3D7_1006000	conserved Plasmodium protein, unknown function	3.97E-02
PF3D7_0627700	transportin	3.97E-02
PF3D7_1141500	conserved Plasmodium protein, unknown function	4.02E-02
PF3D7_0932900	conserved Plasmodium protein, unknown function	4.03E-02
PF3D7_0617900	histone H3 variant, putative	4.04E-02
PF3D7_0615500	cdc2-related protein kinase 5	4.06E-02
PF3D7_0719100	conserved Plasmodium protein, unknown function	4.08E-02
PF3D7_0627900	conserved Plasmodium protein, unknown function	4.12E-02
PF3D7_1242800	rab specific GDP dissociation inhibitor	4.12E-02
PF3D7_1343300	conserved Plasmodium protein, unknown function	4.15E-02
PF3D7_1215700	conserved Plasmodium protein, unknown function	4.22E-02
PF3D7_1454100	tRNA intron endonuclease, putative	4.23E-02
PF3D7_0305000	elongation factor Ts	4.24E-02
PF3D7_1252500	Plasmodium exported protein, unknown function	4.26E-02
PF3D7_1117700	GTP-binding nuclear protein RAN/TC4	4.26E-02
PF3D7_1031100	conserved Plasmodium protein, unknown function	4.28E-02
PF3D7_1245200	conserved Plasmodium membrane protein, unknown function	4.29E-02
PF3D7_0611300	conserved Plasmodium protein, unknown function	4.29E-02
PF3D7_0919200	PPPDE peptidase, putative	4.30E-02
PF3D7_0214900	rhopty neck protein 6	4.32E-02
PF3D7_0407400	conserved Plasmodium protein, unknown function	4.34E-02
PF3D7_1225200	conserved Plasmodium protein, unknown function	4.35E-02
PF3D7_1225600	conserved Plasmodium protein, unknown function	4.37E-02
PF3D7_1106100	apicoplast ribosomal protein S15 precursor, putative	4.37E-02
PF3D7_1337400	conserved Plasmodium protein, unknown function	4.39E-02
PF3D7_1441000	conserved Plasmodium protein, unknown function	4.40E-02
PF3D7_0814300	ATPase, putative	4.40E-02
PF3D7_1404300	secreted ookinete adhesive protein	4.40E-02
PF3D7_1305900	conserved Plasmodium protein, unknown function	4.40E-02
PF3D7_0625200	conserved Plasmodium protein, unknown function	4.41E-02
PF3D7_0710000	conserved Plasmodium protein, unknown function	4.42E-02
PF3D7_1469300	small subunit rRNA processing KH domain protein, putative	4.44E-02
PF3D7_1401300	aminopeptidase, putative	4.44E-02
PF3D7_0624800	conserved Plasmodium protein, unknown function	4.47E-02
PF3D7_1337800	calcium-dependent protein kinase 5	4.48E-02
PF3D7_1141800	phd finger protein, putative	4.49E-02
PF3D7_1209200	U6 snRNA-associated Sm-like protein LSm7, putative	4.51E-02
PF3D7_0935400	gametocyte development protein 1	4.54E-02
PF3D7_1127100	deoxyuridine 5'-triphosphate nucleotidohydrolase	4.55E-02
PF3D7_0113600	surface-associated interspersed protein 1.2 (SURFIN 1.2), pseudogene	4.57E-02
PF3D7_1314500	cop-coated vesicle membrane protein p24 precursor, putative	4.57E-02
PF3D7_1366400	rhopty protein	4.66E-02
PF3D7_1013500	phosphoinositide-specific phospholipase C	4.67E-02
PF3D7_1443200	conserved Plasmodium protein, unknown function	4.71E-02
PF3D7_1430000	conserved Plasmodium protein, unknown function	4.71E-02
PF3D7_0218600	conserved Plasmodium protein, unknown function	4.72E-02
PF3D7_0409800	zinc finger protein, putative	4.73E-02
PF3D7_1006900	PPPDE peptidase, putative	4.74E-02
PF3D7_1207800	conserved Plasmodium protein, unknown function	4.75E-02
PF3D7_0313800	conserved Plasmodium protein, unknown function	4.78E-02

PF3D7_0935000	small nuclear ribonucleoprotein (snRNP), putative	4.79E-02
PF3D7_0302900	exportin-1, putative	4.79E-02
PF3D7_1467700	conserved Plasmodium protein, unknown function	4.80E-02
PF3D7_0423800	cysteine-rich protective antigen	4.84E-02
PF3D7_0108600	conserved Plasmodium protein, unknown function	4.84E-02
PF3D7_1117900	conserved Plasmodium protein, unknown function	4.88E-02
PF3D7_0114500	Plasmodium exported protein (hyp10), unknown function	4.89E-02
PF3D7_1128000	conserved Plasmodium protein, unknown function	4.90E-02
PF3D7_1321600	phosphodiesterase gamma, putative	4.90E-02
PF3D7_1002600	conserved Plasmodium protein, unknown function	4.91E-02
PF3D7_0210300	monocarboxylate transporter, putative	4.92E-02
PF3D7_0820600	conserved Plasmodium membrane protein, unknown function	4.94E-02
PF3D7_1359800	ADP-ribosylation factor, putative	4.95E-02
PF3D7_1245800	clathrin coat assembly protein AP180, putative	4.95E-02
PF3D7_0933400	conserved Plasmodium protein, unknown function	4.96E-02
PF3D7_1131100	serpentine receptor, putative	4.97E-02
PF3D7_1026800	40S ribosomal protein S2B, putative	4.99E-02
PF3D7_0214800	conserved Plasmodium membrane protein, unknown function	4.99E-02
PF3D7_1209800	ATP synthase mitochondrial F1 complex assembly factor 1, putative	4.99E-02
PF3D7_0722800	conserved Plasmodium protein, unknown function	5.00E-02
PF3D7_1402700	pre-mRNA splicing factor, putative	5.00E-02

DOWNREGULATED IN 6A-R vs 6A at 10-20HPI

Gene ID	Gene Description	<i>p value</i>
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1	1.01E-06
PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1	6.44E-06
PF3D7_0400400	erythrocyte membrane protein 1, PfEMP1	4.40E-05
PF3D7_1417200	NOT family protein, putative	6.42E-05
PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1	1.00E-04
PF3D7_1300400	rifin PIR protein	1.53E-04
PF3D7_1301300	Plasmodium exported protein (PHISTa-like), unknown function, pseudogene	1.53E-04
PF3D7_1366300	conserved Plasmodium protein, unknown function	1.90E-04
PF3D7_0712900	erythrocyte membrane protein 1, PfEMP1	2.11E-04
PF3D7_1252100	roptry neck protein 3	2.19E-04
PF3D7_1445200	RNA helicase, putative	2.31E-04
PF3D7_0821100	protein kinase 1	2.97E-04
PF3D7_0600200	erythrocyte membrane protein 1, PfEMP1	3.15E-04
PF3D7_1118600	histone acetyltransferase	4.45E-04
PF3D7_1030100	pre-mRNA-splicing factor ATP-dependent RNA helicase PRP22, putative	4.52E-04
PF3D7_1005000	methionine--tRNA ligase, putative	4.62E-04
PF3D7_0614700	conserved Plasmodium protein, unknown function	5.33E-04
PF3D7_0207600	serine repeat antigen 5	5.36E-04
PF3D7_1322100	variant-silencing SET protein	5.49E-04
PF3D7_0702000	Plasmodium exported protein (hyp12), unknown function	5.74E-04
PF3D7_0302300	erythrocyte membrane protein 1 (PfEMP1), pseudogene	6.18E-04
PF3D7_1351200	conserved Plasmodium protein, unknown function	6.30E-04
PF3D7_1108000	IWS1-like protein, putative	6.38E-04
PF3D7_1344200	heat shock protein 110, putative	7.71E-04
PF3D7_1000100	erythrocyte membrane protein 1, PfEMP1	8.00E-04
PF3D7_1405900	RNA-binding protein, putative	8.88E-04
PF3D7_1238300	cell cycle control protein, putative	9.11E-04
PF3D7_0303000	N-ethylmaleimide-sensitive fusion protein	9.70E-04
PF3D7_0404600	conserved Plasmodium membrane protein, unknown function	1.01E-03
PF3D7_1008100	conserved Plasmodium protein, unknown function	1.03E-03
PF3D7_1408200	transcription factor with AP2 domain(s)	1.05E-03
PF3D7_1372600	rifin PIR protein	1.09E-03
PF3D7_0926500	conserved Plasmodium protein, unknown function	1.14E-03
PF3D7_0401900	acyl-CoA synthetase	1.15E-03
PF3D7_0519400	40S ribosomal protein S24, putative	1.21E-03
PF3D7_1235300	CCR4-NOT transcription complex subunit 4, putative	1.23E-03
PF3D7_1244800	cytoplasmic translation machinery associated protein, putative	1.25E-03

PF3D7_0617500	rifin, pseudogene PIR protein, pseudogene	1.26E-03
PF3D7_1351000	phosphatidylinositol transfer protein, putative	1.30E-03
PF3D7_1441100	conserved Plasmodium protein, unknown function	1.36E-03
PF3D7_1206500	Tat binding protein 1 (TBP-1)-interacting protein, putative	1.36E-03
PF3D7_1220300	cell cycle associated protein, putative	1.41E-03
PF3D7_0600400	erythrocyte membrane protein 1, PfEMP1	1.52E-03
PF3D7_1035800	probable protein, unknown function	1.61E-03
PF3D7_1251600	conserved Plasmodium protein, unknown function	1.61E-03
PF3D7_0733000	erythrocyte membrane protein 1, PfEMP1	1.62E-03
PF3D7_0220800	cytoadherence linked asexual protein 2	1.64E-03
PF3D7_0722600	nucleolar rRNA processing protein, putative	1.71E-03
PF3D7_0506800	transcription factor 25, putative	1.78E-03
PF3D7_0105300	cyclase-associated protein, putative	1.81E-03
PF3D7_1116800	heat shock protein 101 chaperone protein ClpB2	1.87E-03
PF3D7_0321000	conserved Plasmodium protein, unknown function	2.01E-03
PF3D7_1102900	Plasmodium exported protein (hyp11), unknown function	2.01E-03
PF3D7_0704600	ubiquitin transferase, putative	2.01E-03
PF3D7_1122600	conserved Plasmodium protein, unknown function	2.02E-03
PF3D7_1241700	replication factor C subunit 4, putative	2.06E-03
PF3D7_1326500	conserved Plasmodium protein, unknown function	2.11E-03
PF3D7_0300100	erythrocyte membrane protein 1, PfEMP1	2.13E-03
PF3D7_1139100	RNA-binding protein, putative	2.14E-03
PF3D7_1014600	transcriptional coactivator ADA2	2.26E-03
PF3D7_1040300	rifin PIR protein	2.41E-03
PF3D7_0518200	SWIB/MDM2 domain-containing protein, putative	2.54E-03
PF3D7_0702300	sporozoite threonine and asparagine-rich protein	2.79E-03
PF3D7_1302100	gamete antigen 27/25	2.87E-03
PF3D7_0617400	erythrocyte membrane protein 1, PfEMP1	2.97E-03
PF3D7_0512200	glutathione synthetase	3.00E-03
PF3D7_1479000	acyl-CoA synthetase	3.07E-03
PF3D7_1425500	conserved Plasmodium protein, unknown function	3.15E-03
PF3D7_1420100	conserved Plasmodium protein, unknown function	3.39E-03
PF3D7_1305800	conserved Plasmodium protein, unknown function	3.51E-03
PF3D7_1241900	tetratricopeptide repeat protein, putative	3.64E-03
PF3D7_1313800	conserved Plasmodium membrane protein, unknown function	3.76E-03
PF3D7_0319500	RNA-binding protein, putative	3.77E-03
PF3D7_1026600	conserved Plasmodium protein, unknown function	3.83E-03
PF3D7_0712400	erythrocyte membrane protein 1, PfEMP1	4.03E-03
PF3D7_1356800	serine/threonine protein kinase, putative	4.13E-03
PF3D7_1208400	amino acid transporter, putative	4.25E-03
PF3D7_0607600	conserved Plasmodium protein, unknown function	4.43E-03
PF3D7_1406200	conserved Plasmodium protein, unknown function	4.49E-03
PF3D7_1110400	asparagine-rich antigen	4.54E-03
PF3D7_1133100	conserved membrane protein, unknown function	4.74E-03
PF3D7_0300900	stevor-like PIR protein	5.36E-03
PF3D7_1345600	inner membrane complex protein	5.40E-03
PF3D7_0323700	U4/U6.U5 tri-snRNP-associated protein 1, putative	5.49E-03
PF3D7_0315600	conserved Plasmodium protein, unknown function	5.58E-03
PF3D7_1239300	conserved Plasmodium protein, unknown function	5.60E-03
PF3D7_1027100	small subunit rRNA processing stabilizing factor, putative	5.60E-03
PF3D7_0922800	conserved Plasmodium protein, unknown function	5.66E-03
PF3D7_1230200	vacuolar transporter chaperone, putative, pseudogene	5.83E-03
PF3D7_1354000	conserved Plasmodium protein, unknown function	5.94E-03
PF3D7_1200300	rifin PIR protein	5.96E-03
PF3D7_0831400	Plasmodium exported protein, unknown function	6.03E-03
PF3D7_0201600	Plasmodium exported protein (PHISTb), unknown function	6.11E-03
PF3D7_1447700	conserved Plasmodium protein, unknown function	6.25E-03
PF3D7_0829100	conserved protein, unknown function	6.29E-03
PF3D7_1112800	conserved Plasmodium protein, unknown function	6.42E-03
PF3D7_0717200	conserved Plasmodium protein, unknown function	6.61E-03
PF3D7_0417400	conserved Plasmodium protein, unknown function	6.67E-03

PF3D7_1436400	conserved Plasmodium protein, unknown function	6.69E-03
PF3D7_1207200	conserved Plasmodium protein, unknown function	6.75E-03
PF3D7_0700100	erythrocyte membrane protein 1, PfEMP1	6.79E-03
PF3D7_0723400	conserved Plasmodium protein, unknown function	6.82E-03
PF3D7_0532400	Plasmodium exported protein (PHISTb), unknown function	6.95E-03
PF3D7_1147300	conserved Plasmodium protein, unknown function	6.98E-03
PF3D7_0920700	conserved Plasmodium protein, unknown function	7.40E-03
PF3D7_1200800	serine/threonine protein kinase, FIKK family	7.41E-03
PF3D7_1241800	DEAD/DEAH box ATP-dependent RNA helicase, putative	7.59E-03
PF3D7_0420400	ribosome-recycling factor	7.61E-03
PF3D7_0831600	cytoadherence linked asexual protein 8	7.63E-03
PF3D7_0910300	conserved Plasmodium protein, unknown function	7.72E-03
PF3D7_0720100	small subunit rRNA processing protein, putative	7.76E-03
PF3D7_1330800	conserved Plasmodium protein, unknown function	7.85E-03
PF3D7_1467100	DNA-3-methyladenine glycosylase, putative	7.85E-03
PF3D7_0812500	RNA-binding protein, putative	7.97E-03
PF3D7_1334200	chaperone binding protein, putative	8.00E-03
PF3D7_0609900	conserved Plasmodium protein, unknown function	8.28E-03
PF3D7_0400500	rifin PIR protein	8.45E-03
PF3D7_1471900	conserved Plasmodium protein, unknown function	8.56E-03
PF3D7_0104300	ubiquitin carboxyl-terminal hydrolase 1, putative	8.82E-03
PF3D7_1233200	conserved Plasmodium protein, unknown function	8.91E-03
PF3D7_1319900	conserved Plasmodium protein, unknown function	8.98E-03
PF3D7_0529600	tRNA Arginine	9.24E-03
PF3D7_1308300	40S ribosomal protein S27, putative	9.37E-03
PF3D7_0630400	conserved Plasmodium protein, unknown function	9.45E-03
PF3D7_0918900	gamma-glutamylcysteine synthetase	9.49E-03
PF3D7_0507900	conserved Plasmodium protein, unknown function	9.50E-03
PF3D7_1029900	conserved Plasmodium protein, unknown function	9.57E-03
PF3D7_1431900	conserved Plasmodium membrane protein, unknown function	9.89E-03
PF3D7_1034700	ADP-ribosylation factor, putative	9.92E-03
PF3D7_1120000	conserved Plasmodium protein, unknown function	1.01E-02
PF3D7_1017600	conserved Plasmodium protein, unknown function	1.01E-02
PF3D7_1452700	U1 snRNA associated protein, putative	1.02E-02
PF3D7_0102500	erythrocyte binding antigen-181	1.02E-02
PF3D7_1122800	calcium-dependent protein kinase 6	1.03E-02
PF3D7_0417800	cdc2-related protein kinase 1	1.03E-02
PF3D7_0707300	rho-try-associated membrane antigen	1.05E-02
PF3D7_0421300	erythrocyte membrane protein 1, PfEMP1	1.05E-02
PF3D7_1446500	conserved Plasmodium protein, unknown function	1.06E-02
PF3D7_1305300	conserved Plasmodium protein, unknown function	1.07E-02
PF3D7_0317300	conserved Plasmodium protein, unknown function	1.09E-02
PF3D7_1414200	conserved Plasmodium protein, unknown function	1.11E-02
PF3D7_1139300	transcription factor with AP2 domain(s)	1.12E-02
PF3D7_0207100	conserved Plasmodium protein, unknown function	1.13E-02
PF3D7_0731500	erythrocyte binding antigen-175	1.13E-02
PF3D7_0223500	erythrocyte membrane protein 1, PfEMP1	1.14E-02
PF3D7_1000200	rifin PIR protein	1.15E-02
PF3D7_0701900	Plasmodium exported protein, unknown function	1.16E-02
PF3D7_1143100	transcription factor with AP2 domain(s)	1.16E-02
PF3D7_1353000	tryptophan-rich antigen, pseudogene	1.17E-02
PF3D7_1234300	DNA polymerase epsilon subunit b, putative	1.18E-02
PF3D7_0509400	RNA polymerase I	1.19E-02
PF3D7_0722100	PelOta protein homologue, putative	1.20E-02
PF3D7_0311300	phosphatidylinositol 3- and 4-kinase, putative	1.20E-02
PF3D7_1364400	conserved Plasmodium protein, unknown function	1.20E-02
PF3D7_1302000	EMP1-trafficking protein	1.22E-02
PF3D7_0704700	phosphopantetheine adenyltransferase, putative	1.22E-02
PF3D7_0509800	phosphatidylinositol 4-kinase	1.22E-02
PF3D7_1028800	conserved Plasmodium protein, unknown function	1.24E-02
PF3D7_1368300	conserved Plasmodium protein, unknown function	1.25E-02

PF3D7_1140700	conserved Plasmodium protein, unknown function	1.25E-02
PF3D7_1347400	conserved Plasmodium protein, unknown function	1.26E-02
PF3D7_1013100	U3 snoRNA-associated small subunit rRNA processing protein, putative	1.26E-02
PF3D7_0402100	Plasmodium exported protein (PHISTb), unknown function	1.31E-02
PF3D7_0415800	RING zinc finger protein, putative	1.32E-02
PF3D7_0504000	cation transporting P-ATPase	1.34E-02
PF3D7_0216700	conserved Plasmodium protein, unknown function	1.35E-02
PF3D7_1423800	conserved Plasmodium protein, unknown function	1.35E-02
PF3D7_0727900	conserved Plasmodium protein, unknown function	1.36E-02
PF3D7_0416400	histone acetyltransferase, putative	1.37E-02
PF3D7_1346400	conserved Plasmodium protein, unknown function	1.37E-02
PF3D7_0724800	kelch protein, putative	1.39E-02
PF3D7_1233900	senrin-specific protease 1	1.40E-02
PF3D7_1302700	DEAD/DEAH box ATP-dependent RNA helicase, putative	1.41E-02
PF3D7_0102400	lysophospholipase, putative, pseudogene	1.42E-02
PF3D7_1450500	conserved Plasmodium protein, unknown function	1.42E-02
PF3D7_1430200	plasmepsin IX	1.43E-02
PF3D7_0702900	centrin, putative	1.43E-02
PF3D7_0914100	conserved Plasmodium protein, unknown function	1.43E-02
PF3D7_0818500	zinc finger protein, putative	1.46E-02
PF3D7_1348800	E1-E2 ATPase, putative	1.47E-02
PF3D7_1344300	conserved protein, unknown function	1.47E-02
PF3D7_0604100	transcription factor with AP2 domain(s) SPE2-interacting protein	1.47E-02
PF3D7_1359700	conserved Plasmodium protein, unknown function	1.49E-02
PF3D7_0902300	serine/threonine protein kinase, FIKK family	1.50E-02
PF3D7_1123100	calcium-dependent protein kinase 7	1.53E-02
PF3D7_0926100	protein kinase, putative	1.53E-02
PF3D7_1464200	zinc finger protein, putative	1.57E-02
PF3D7_1343500	conserved Plasmodium protein, unknown function	1.58E-02
PF3D7_1105300	conserved Plasmodium protein, unknown function	1.59E-02
PF3D7_1417600	conserved Plasmodium protein, unknown function	1.59E-02
PF3D7_0115700	erythrocyte membrane protein 1, PfEMP1	1.61E-02
PF3D7_0728100	conserved Plasmodium membrane protein, unknown function	1.62E-02
PF3D7_0511500	RNA pseudouridylate synthase, putative	1.63E-02
PF3D7_0911800	conserved Plasmodium protein, unknown function	1.68E-02
PF3D7_0825500	conserved protein, unknown function	1.69E-02
PF3D7_1223000	conserved Plasmodium protein, unknown function	1.73E-02
PF3D7_1001900	Plasmodium exported protein (hyp16), unknown function	1.73E-02
PF3D7_1033500	WD-repeat protein, putative	1.74E-02
PF3D7_1442900	protein transport protein SEC7, putative	1.75E-02
PF3D7_1460700	60S ribosomal protein L27, putative	1.77E-02
PF3D7_0402000	Plasmodium exported protein (PHISTa), unknown function	1.79E-02
PF3D7_0413900	ubiquitin carboxyl-terminal hydrolase 13, putative	1.79E-02
PF3D7_1011500	conserved Plasmodium membrane protein, unknown function	1.81E-02
PF3D7_1324200	micro-fibrillar-associated protein, putative	1.84E-02
PF3D7_1419400	conserved Plasmodium membrane protein, unknown function	1.85E-02
PF3D7_1351900	conserved Plasmodium protein, unknown function	1.87E-02
PF3D7_0310300	phosphoglycerate mutase, putative	1.88E-02
PF3D7_0601900	conserved Plasmodium protein, unknown function	1.89E-02
PF3D7_0102300	Plasmodium exported protein, unknown function	1.90E-02
PF3D7_0932100	protein MAM3, putative	1.91E-02
PF3D7_1227500	cyclin	1.93E-02
PF3D7_0508700	pre-mRNA-processing ATP-dependent RNA helicase PRP5, putative	1.94E-02
PF3D7_1325000	U6 snRNA-associated Sm-like protein LSM6, putative	1.94E-02
PF3D7_0315800	zinc finger protein, putative	1.94E-02
PF3D7_0423700	early transcribed membrane protein 4	1.94E-02
PF3D7_0914900	BSD-domain protein, putative	1.95E-02
PF3D7_0732000	stevor PIR protein	1.96E-02
PF3D7_0408200	conserved Plasmodium protein, unknown function	1.98E-02
PF3D7_0623600	transcription or splicing factor-like protein, putative	1.99E-02
PF3D7_0927300	fumarate hydratase, putative	2.00E-02

PF3D7_0417000	conserved Plasmodium protein, unknown function	2.02E-02
PF3D7_1420700	surface protein, Pf113	2.04E-02
PF3D7_0716100	large ribosomal subunit assembling factor, putative	2.05E-02
PF3D7_1477700	Plasmodium exported protein (PHISTa), unknown function	2.08E-02
PF3D7_1426100	basic transcription factor 3b, putative	2.09E-02
PF3D7_1252600	lysophospholipase, putative	2.10E-02
PF3D7_1477500	Plasmodium exported protein (PHISTb), unknown function	2.10E-02
PF3D7_0624600	SNF2 helicase, putative	2.11E-02
PF3D7_0322100	RNA triphosphatase	2.12E-02
PF3D7_0309500	asparagine synthetase, putative	2.15E-02
PF3D7_1408700	conserved Plasmodium protein, unknown function	2.16E-02
PF3D7_1215900	serpentine receptor, putative	2.20E-02
PF3D7_0931000	elongation factor Tu, putative	2.22E-02
PF3D7_1024700	conserved Plasmodium protein, unknown function	2.23E-02
PF3D7_1419700	conserved Plasmodium protein, unknown function	2.24E-02
PF3D7_0832700	Plasmodium exported protein (PHISTa-like), unknown function, pseudogene	2.25E-02
PF3D7_1112900	conserved Plasmodium protein, unknown function	2.25E-02
PF3D7_0114900	Plasmodium exported protein, unknown function, pseudogene	2.25E-02
PF3D7_0308100	conserved Plasmodium protein, unknown function	2.28E-02
PF3D7_1224200	conserved Plasmodium protein, unknown function	2.29E-02
PF3D7_1300500	rifin PIR protein	2.32E-02
PF3D7_0713900	conserved Plasmodium protein, unknown function	2.32E-02
PF3D7_1037600	DNA repair helicase RAD25, putative	2.32E-02
PF3D7_1410300	conserved Plasmodium protein, unknown function	2.33E-02
PF3D7_1209600	porphobilinogen deaminase	2.34E-02
PF3D7_0113000	glutamic acid-rich protein	2.36E-02
PF3D7_0321800	conserved Plasmodium protein, unknown function	2.39E-02
PF3D7_0505700	conserved Plasmodium membrane protein, unknown function	2.40E-02
PF3D7_0827800	SET domain protein, putative	2.41E-02
PF3D7_1133400	apical membrane antigen 1	2.41E-02
PF3D7_1456100	serine hydroxymethyltransferase, putative	2.42E-02
PF3D7_0213000	conserved Plasmodium protein, unknown function	2.42E-02
PF3D7_0602000	conserved Plasmodium protein, unknown function	2.44E-02
PF3D7_1107300	polyadenylate-binding protein-interacting protein 1, putative	2.46E-02
PF3D7_1035100	probable protein, unknown function	2.50E-02
PF3D7_0422500	pre-mRNA-splicing helicase BRR2, putative	2.53E-02
PF3D7_1030900	28 kDa ookinete surface protein	2.53E-02
PF3D7_0806700	conserved Plasmodium membrane protein, unknown function	2.54E-02
PF3D7_0503500	protein kinase, putative	2.56E-02
PF3D7_0701600	Pfmc-2TM Maurer's cleft two transmembrane protein	2.57E-02
PF3D7_0832900	stevor PIR protein	2.57E-02
PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1	2.58E-02
PF3D7_0719200	NIMA related kinase 4	2.63E-02
PF3D7_1303800	conserved Plasmodium protein, unknown function	2.66E-02
PF3D7_0314500	conserved Plasmodium protein, unknown function	2.66E-02
PF3D7_1350500	conserved Plasmodium protein, unknown function	2.68E-02
PF3D7_0617200	conserved Plasmodium protein, unknown function	2.69E-02
PF3D7_0204300	conserved Plasmodium protein, unknown function	2.69E-02
PF3D7_1229600	conserved Plasmodium protein, unknown function	2.72E-02
PF3D7_1435600	conserved Plasmodium protein, unknown function	2.74E-02
PF3D7_1366500	nucleoside diphosphate kinase	2.74E-02
PF3D7_0613900	myosin E, putative	2.75E-02
PF3D7_1006700	conserved Plasmodium protein, unknown function	2.76E-02
PF3D7_1445300	mitochondrial ribosomal protein S29 precursor, putative	2.77E-02
PF3D7_0705600	RNA helicase, putative	2.78E-02
PF3D7_0814000	60S ribosomal protein L13-2, putative	2.79E-02
PF3D7_1451900	small subunit rRNA processing factor, putative	2.79E-02
PF3D7_0530100	SNARE protein, putative	2.86E-02
PF3D7_0930400	conserved Plasmodium protein, unknown function	2.94E-02
PF3D7_0500800	mature parasite-infected erythrocyte surface antigen erythrocyte membrane protein 2	2.94E-02

PF3D7_1113700	glyoxalase I	2.94E-02
PF3D7_1127800	conserved Plasmodium protein, unknown function	2.95E-02
PF3D7_1458300	conserved Plasmodium protein, unknown function	2.97E-02
PF3D7_1446400	pyruvate dehydrogenase E1 beta subunit	2.98E-02
PF3D7_1349400	cytidine and deoxycytidylate deaminase, putative	3.02E-02
PF3D7_0906300	Maf-like protein, putative	3.05E-02
PF3D7_1241400	RNA-binding protein, putative	3.05E-02
PF3D7_1358100	small subunit rRNA processing factor, putative	3.11E-02
PF3D7_0811500	histone-arginine methyltransferase, putative	3.14E-02
PF3D7_0903600	conserved Plasmodium protein, unknown function	3.14E-02
PF3D7_1035200	S-antigen	3.15E-02
PF3D7_0313600	conserved Plasmodium protein, unknown function	3.16E-02
PF3D7_1036900	conserved Plasmodium protein, unknown function	3.20E-02
PF3D7_1102800	early transcribed membrane protein 11.2	3.21E-02
PF3D7_0108300	conserved Plasmodium protein, unknown function	3.23E-02
PF3D7_0311100	pre-mRNA splicing factor, putative	3.23E-02
PF3D7_0514400	tRNA Phenylalanine	3.28E-02
PF3D7_1307800	fork head domain protein, putative	3.30E-02
PF3D7_1412700	AAA family ATPase, putative	3.31E-02
PF3D7_0603600	conserved Plasmodium protein, unknown function	3.32E-02
PF3D7_0528500	f-actin capping protein alpha subunit, putative	3.33E-02
PF3D7_1325400	conserved Plasmodium protein, unknown function	3.35E-02
PF3D7_1236200	conserved Plasmodium protein, unknown function	3.37E-02
PF3D7_1472800	conserved Plasmodium protein, unknown function	3.39E-02
PF3D7_1311600	conserved Plasmodium protein, unknown function	3.44E-02
PF3D7_1028900	conserved Plasmodium protein, unknown function	3.47E-02
PF3D7_1138000	conserved Plasmodium protein, unknown function	3.49E-02
PF3D7_1448700	conserved Plasmodium protein, unknown function	3.49E-02
PF3D7_1318300	conserved Plasmodium protein, unknown function	3.53E-02
PF3D7_1353800	proteasome subunit, putative	3.55E-02
PF3D7_1404900	conserved Plasmodium protein, unknown function	3.57E-02
PF3D7_1343000	phosphoethanolamine N-methyltransferase	3.58E-02
PF3D7_1436100	conserved Plasmodium membrane protein, unknown function	3.62E-02
PF3D7_0811700	conserved Plasmodium protein, unknown function	3.68E-02
PF3D7_0420000	zinc finger protein, putative	3.75E-02
PF3D7_0827000	ATP-dependent RNA helicase DBP10, putative	3.77E-02
PF3D7_1013600	conserved Plasmodium protein, unknown function	3.79E-02
PF3D7_1439400	ubiquinol-cytochrome c reductase iron-sulfur subunit, putative	3.81E-02
PF3D7_0629700	SET domain protein, putative	3.81E-02
PF3D7_0708800	heat shock protein 110	3.81E-02
PF3D7_1402400	U1 small nuclear ribonucleoprotein C, putative	3.82E-02
PF3D7_1122900	dynein heavy chain, putative	3.85E-02
PF3D7_0628200	protein kinase PK4	3.85E-02
PF3D7_1229800	myosin D	3.93E-02
PF3D7_0917100	N-glycosylase/DNA lyase, putative	3.98E-02
PF3D7_0937800	erythrocyte membrane protein 1, PfEMP1	3.98E-02
PF3D7_1301600	erythrocyte binding antigen-140	4.01E-02
PF3D7_1019600	conserved Plasmodium protein, unknown function	4.04E-02
PF3D7_1431600	ATP-specific succinyl-CoA synthetase beta subunit, putative	4.05E-02
PF3D7_1217500	conserved Plasmodium protein, unknown function	4.09E-02
PF3D7_0802600	adenylyl cyclase beta	4.11E-02
PF3D7_1226200	conserved Plasmodium protein, unknown function	4.11E-02
PF3D7_1031700	protein phosphatase inhibitor 3	4.13E-02
PF3D7_1033800	plasmepsin VII	4.16E-02
PF3D7_0613800	transcription factor with AP2 domain(s)	4.17E-02
PF3D7_0201900	erythrocyte membrane protein 3	4.22E-02
PF3D7_1474000	probable protein, unknown function	4.22E-02
PF3D7_0632800	erythrocyte membrane protein 1, PfEMP1	4.24E-02
PF3D7_0610100	pre-mRNA-splicing factor SLU7, putative	4.27E-02
PF3D7_0626600	conserved Plasmodium protein, unknown function	4.28E-02
PF3D7_1029600	adenosine deaminase	4.29E-02

PF3D7_0712300	erythrocyte membrane protein 1, PfEMP1	4.29E-02
PF3D7_0623800	tyrosine kinase-like protein, putative	4.30E-02
PF3D7_1442600	TRAP-like protein sporozoite-specific transmembrane protein S6	4.32E-02
PF3D7_0818600	conserved Plasmodium protein, unknown function	4.34E-02
PF3D7_1011700	DNA repair protein RAD23, putative	4.37E-02
PF3D7_0423500	glideosome associated protein with multiple membrane spans 2	4.38E-02
PF3D7_0933300	conserved Plasmodium protein, unknown function	4.40E-02
PF3D7_0519800	conserved protein, unknown function	4.43E-02
PF3D7_0816300	conserved Plasmodium protein, unknown function	4.44E-02
PF3D7_0908400	conserved Plasmodium protein, unknown function	4.44E-02
PF3D7_0913200	elongation factor 1-beta	4.44E-02
PF3D7_1013900	initiation factor 2 subunit family, putative	4.47E-02
PF3D7_0710700	conserved Plasmodium protein, unknown function	4.49E-02
PF3D7_0818100	zinc finger protein, putative	4.50E-02
PF3D7_1445400	protein serine/threonine kinase-1	4.52E-02
PF3D7_1305500	mitogen-activated protein kinase phosphatase 1, putative	4.53E-02
PF3D7_0936200	Plasmodium exported protein (hyp11), unknown function	4.58E-02
PF3D7_1443600	conserved Plasmodium protein, unknown function	4.62E-02
PF3D7_0600300	rifin PIR protein	4.64E-02
PF3D7_1349000	conserved Plasmodium protein, unknown function	4.70E-02
PF3D7_1138700	conserved Plasmodium protein, unknown function	4.74E-02
PF3D7_1300100	erythrocyte membrane protein 1, PfEMP1	4.78E-02
PF3D7_0803100	conserved Plasmodium protein, unknown function	4.79E-02
PF3D7_1476300	Plasmodium exported protein (PHISTb), unknown function	4.82E-02
PF3D7_1476500	probable protein, unknown function	4.83E-02
PF3D7_1340300	nucleolar complex protein 2, putative	4.83E-02
PF3D7_1358200	conserved Plasmodium protein, unknown function	4.83E-02
PF3D7_0935900	ring-exported protein 1	4.85E-02
PF3D7_1021300	conserved Plasmodium membrane protein, unknown function	4.88E-02
PF3D7_1002200	tryptophan-rich antigen 3	4.89E-02
PF3D7_0813600	translation initiation factor SU11, putative	4.89E-02
PF3D7_0519300	cytochrome c oxidase assembly protein (heme A: farnesyltransferase), putative	4.90E-02
PF3D7_0930900	NifU-like protein, putative	4.91E-02
PF3D7_0421800	conserved Plasmodium protein, unknown function	4.94E-02
PF3D7_0528300	conserved Plasmodium protein, unknown function	4.95E-02
PF3D7_1466100	protein phosphatase containing kelch-like domains	4.96E-02
PF3D7_0318800	triosephosphate isomerase, putative	4.99E-02

Appendix G: List of significantly up- and downregulated genes 11C-R and 11C based on steady-state transcriptional profiling at 10-20hpi ($p < 0.05$). Differential expression was measured using paired student's T-test.

UPREGULATED IN 11C-R vs 11C at 10-20HPI		
Gene ID	Gene Description	<i>p value</i>
PF3D7_1476000	conserved Plasmodium protein, unknown function	1.49E-06
PF3D7_1476400	serine/threonine protein kinase, FIKK family, pseudogene	2.05E-06
PF3D7_0830400	conserved Plasmodium protein, unknown function	2.18E-06
PF3D7_0703900	conserved Plasmodium membrane protein, unknown function	6.32E-06
PF3D7_1476800	lysophospholipase, putative	7.43E-06
PF3D7_1200800	serine/threonine protein kinase, FIKK family	1.68E-05
PF3D7_0208400	conserved Plasmodium protein, unknown function	2.15E-05
PF3D7_1019900	autophagy-related protein 8	3.65E-05
PF3D7_1434700	mitochondrial import inner membrane translocase subunit TIM17, putative	4.48E-05
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1	4.62E-05
PF3D7_0523000	multidrug resistance protein	4.92E-05
PF3D7_1200900	Plasmodium exported protein (PHISTc), unknown function	5.67E-05
PF3D7_1423000	GTPase, putative	7.63E-05
PF3D7_1149100	Plasmodium exported protein, unknown function	1.06E-04
PF3D7_1104400	conserved protein, unknown function	1.15E-04
PF3D7_1477500	Plasmodium exported protein (PHISTb), unknown function	1.32E-04
PF3D7_1478600	EMP1-trafficking protein	1.37E-04
PF3D7_1427900	conserved protein, unknown function	1.53E-04
PF3D7_1405100	GTPase-activating protein, putative	1.76E-04
PF3D7_1442900	protein transport protein SEC7, putative	1.78E-04
PF3D7_1250800	DNA repair protein rhp16, putative	2.02E-04
PF3D7_0905300	dynein heavy chain, putative	2.05E-04
PF3D7_0830300	sporozoite invasion-associated protein 2	2.45E-04
PF3D7_1030100	pre-mRNA-splicing factor ATP-dependent RNA helicase PRP22, putative	2.57E-04
PF3D7_0726500	ubiquitin carboxyl-terminal hydrolase, putative	2.75E-04
PF3D7_0614800	endonuclease III homologue, putative	3.10E-04
PF3D7_1008400	26S proteasome regulatory subunit 4, putative	4.14E-04
PF3D7_1008000	inositol polyphosphate kinase, putative	4.33E-04
PF3D7_0424900	Plasmodium exported protein (PHISTa), unknown function	4.78E-04
PF3D7_0517600	f-actin capping protein beta subunit, putative	4.87E-04
PF3D7_1237200	conserved Plasmodium protein, unknown function	5.34E-04
PF3D7_0301300	alpha/beta hydrolase, putative	5.45E-04
PF3D7_0212500	conserved Plasmodium protein, unknown function	6.06E-04
PF3D7_0702300	sporozoite threonine and asparagine-rich protein	6.06E-04
PF3D7_1245400	mitochondrial ribosomal protein L3 precursor, putative	6.40E-04
PF3D7_1233600	asparagine and aspartate rich protein 1	6.89E-04
PF3D7_1134700	DNA-directed RNA polymerase I subunit RPA2, putative	6.95E-04
PF3D7_1477000	Plasmodium exported protein (hyp17), unknown function, pseudogene	7.08E-04
PF3D7_0814200	DNA/RNA-binding protein Alba 1	7.22E-04
PF3D7_0110500	bromodomain protein, putative	7.69E-04
PF3D7_1359000	conserved Plasmodium protein, unknown function	7.77E-04
PF3D7_0705500	inositol-phosphate phosphatase, putative	7.86E-04
PF3D7_1013900	initiation factor 2 subunit family, putative	8.11E-04
PF3D7_0323100	conserved Plasmodium protein, unknown function	8.18E-04
PF3D7_1219000	formin 2	8.73E-04
PF3D7_0910200	conserved Plasmodium protein, unknown function	8.77E-04
PF3D7_1201000	Plasmodium exported protein (PHISTb), unknown function	9.08E-04
PF3D7_0603400	trophozoite exported protein 1	9.11E-04
PF3D7_1476200	Plasmodium exported protein (PHISTb), unknown function	9.36E-04
PF3D7_0826500	ubiquitin conjugation factor E4 B, putative	9.69E-04
PF3D7_0710200	conserved Plasmodium protein, unknown function	1.06E-03
PF3D7_1328500	alpha/beta-hydrolase, putative	1.06E-03
PF3D7_0619900	splicing factor 3A subunit 2, putative	1.09E-03
PF3D7_0621800	nascent polypeptide associated complex alpha chain, putative	1.09E-03

PF3D7_0716200	conserved Plasmodium protein, unknown function	1.10E-03
PF3D7_0906300	Maf-like protein, putative	1.15E-03
PF3D7_1458000	cysteine proteinase falcipain 1	1.16E-03
PF3D7_1415400	conserved Plasmodium protein, unknown function	1.17E-03
PF3D7_1462400	conserved Plasmodium protein, unknown function	1.19E-03
PF3D7_1201200	Plasmodium exported protein (PHISTa-like), unknown function	1.23E-03
PF3D7_0602600	conserved Plasmodium protein, unknown function	1.36E-03
PF3D7_0310200	phd finger protein, putative	1.36E-03
PF3D7_1408700	conserved Plasmodium protein, unknown function	1.36E-03
PF3D7_1133900	conserved Plasmodium protein, unknown function	1.39E-03
PF3D7_1478800	Plasmodium exported protein, unknown function	1.39E-03
PF3D7_0806700	conserved Plasmodium membrane protein, unknown function	1.39E-03
PF3D7_0931100	nucleolar protein Nop52, putative	1.40E-03
PF3D7_0108300	conserved Plasmodium protein, unknown function	1.54E-03
PF3D7_0914500	conserved Plasmodium protein, unknown function	1.56E-03
PF3D7_0727900	conserved Plasmodium protein, unknown function	1.57E-03
PF3D7_0607800	GTP-binding protein, putative	1.61E-03
PF3D7_1453700	co-chaperone p23	1.62E-03
PF3D7_0422500	pre-mRNA-splicing helicase BRR2, putative	1.71E-03
PF3D7_0913300	conserved Plasmodium protein, unknown function	1.75E-03
PF3D7_0930500	diacylglycerol kinase, putative	1.89E-03
PF3D7_1478100	Plasmodium exported protein (hyp13), unknown function	1.90E-03
PF3D7_1300100	erythrocyte membrane protein 1, PfEMP1	1.97E-03
PF3D7_0705600	RNA helicase, putative	1.97E-03
PF3D7_1201300	liver stage associated protein 1	2.00E-03
PF3D7_0913200	elongation factor 1-beta	2.08E-03
PF3D7_0607000	translation initiation factor IF-2, putative	2.12E-03
PF3D7_0104300	ubiquitin carboxyl-terminal hydrolase 1, putative	2.12E-03
PF3D7_1302700	DEAD/DEAH box ATP-dependent RNA helicase, putative	2.17E-03
PF3D7_0416400	histone acetyltransferase, putative	2.21E-03
PF3D7_1308200	carbamoyl phosphate synthetase	2.25E-03
PF3D7_1237000	SUMO-activating enzyme subunit 2	2.34E-03
PF3D7_1032700	conserved Plasmodium protein, unknown function	2.38E-03
PF3D7_1224300	polyadenylate-binding protein, putative	2.39E-03
PF3D7_0303100	conserved Plasmodium protein, unknown function	2.39E-03
PF3D7_1305300	conserved Plasmodium protein, unknown function	2.43E-03
PF3D7_1476600	Plasmodium exported protein, unknown function	2.46E-03
PF3D7_1203700	nucleosome assembly protein	2.70E-03
PF3D7_0103400	zinc-carboxypeptidase, putative	2.73E-03
PF3D7_0210800	conserved Plasmodium protein, unknown function	2.76E-03
PF3D7_0609500	conserved Plasmodium protein, unknown function	2.94E-03
PF3D7_1477600	surface-associated interspersed protein 14.1 (SURFIN 14.1)	3.01E-03
PF3D7_0709200	Cg6 protein glutaredoxin-like protein	3.07E-03
PF3D7_0629700	SET domain protein, putative	3.19E-03
PF3D7_1200700	acyl-CoA synthetase	3.32E-03
PF3D7_0523200	conserved Plasmodium protein, unknown function	3.35E-03
PF3D7_1032100	mRNA-decapping enzyme subunit 1, putative	3.39E-03
PF3D7_0423700	early transcribed membrane protein 4	3.45E-03
PF3D7_0406500	conserved Plasmodium protein, unknown function	3.46E-03
PF3D7_0933500	gamma-tubulin complex component, putative	3.48E-03
PF3D7_0815900	lipoamide dehydrogenase	3.50E-03
PF3D7_1250200	conserved Plasmodium membrane protein, unknown function	3.51E-03
PF3D7_1472200	histone deacetylase, putative	3.54E-03
PF3D7_1005500	regulator of nonsense transcripts, putative	3.56E-03
PF3D7_1223500	conserved Plasmodium protein, unknown function	3.59E-03
PF3D7_0319700	ABC transporter, putative	3.77E-03
PF3D7_1128000	conserved Plasmodium protein, unknown function	3.82E-03
PF3D7_1476900	Plasmodium exported protein, unknown function, pseudogene	3.84E-03
PF3D7_0418200	golgi organization and biogenesis factor, putative	3.95E-03
PF3D7_1018300	conserved Plasmodium protein, unknown function	4.06E-03
PF3D7_0812500	RNA-binding protein, putative	4.08E-03
PF3D7_1464900	ATP-dependent zinc metalloprotease FTSH, putative	4.09E-03
PF3D7_1477700	Plasmodium exported protein (PHISTa), unknown function	4.15E-03
PF3D7_0832000	stevor PIR protein	4.18E-03

PF3D7_0726400	conserved Plasmodium membrane protein, unknown function	4.19E-03
PF3D7_1351000	phosphatidylinositol transfer protein, putative	4.26E-03
PF3D7_1366900	conserved Plasmodium protein, unknown function	4.26E-03
PF3D7_0320800	ATP-dependent RNA helicase DDX6	4.28E-03
PF3D7_0503300	Ser/Arg-rich splicing factor, putative	4.29E-03
PF3D7_1351400	60S ribosomal protein L17, putative	4.30E-03
PF3D7_1247900	nucleolar rRNA processing protein, putative	4.32E-03
PF3D7_1137400	UVB-resistance protein UVR8 homologue	4.33E-03
PF3D7_1407200	conserved Plasmodium protein, unknown function	4.33E-03
PF3D7_1110400	asparagine-rich antigen	4.43E-03
PF3D7_0608500	proteasome subunit alpha type 2, putative	4.50E-03
PF3D7_0502500	conserved Plasmodium protein, unknown function	4.64E-03
PF3D7_0311100	pre-mRNA splicing factor, putative	4.66E-03
PF3D7_1426100	basic transcription factor 3b, putative	4.69E-03
PF3D7_1120400	alpha/beta hydrolase fold domain containing protein, putative	4.71E-03
PF3D7_1120000	conserved Plasmodium protein, unknown function	4.87E-03
PF3D7_1001900	Plasmodium exported protein (hyp16), unknown function	4.89E-03
PF3D7_1235300	CCR4-NOT transcription complex subunit 4, putative	4.97E-03
PF3D7_1123000	conserved Plasmodium protein, unknown function	4.97E-03
PF3D7_0602000	conserved Plasmodium protein, unknown function	5.04E-03
PF3D7_0629200	DnaJ protein, putative	5.10E-03
PF3D7_1302500	conserved Plasmodium protein, unknown function	5.14E-03
PF3D7_1121100	conserved Plasmodium protein, unknown function	5.16E-03
PF3D7_1030300	conserved Plasmodium protein, unknown function	5.26E-03
PF3D7_0113400	Plasmodium exported protein, unknown function	5.30E-03
PF3D7_1479000	acyl-CoA synthetase	5.42E-03
PF3D7_1471200	inorganic anion exchanger, inorganic anion antiporter	5.43E-03
PF3D7_0609900	conserved Plasmodium protein, unknown function	5.44E-03
PF3D7_1142300	conserved Plasmodium membrane protein, unknown function	5.61E-03
PF3D7_0300700	rifin PIR protein	5.71E-03
PF3D7_0418300	conserved Plasmodium protein, unknown function	5.77E-03
PF3D7_0606600	conserved Plasmodium protein, unknown function	5.88E-03
PF3D7_1136300	tudor staphylococcal nuclease	5.90E-03
PF3D7_0112900	Plasmodium exported protein, unknown function	5.90E-03
PF3D7_0420000	zinc finger protein, putative	6.01E-03
PF3D7_0906500	arginase	6.06E-03
PF3D7_0721000	conserved Plasmodium membrane protein, unknown function	6.10E-03
PF3D7_0115600	PIR protein rifin	6.13E-03
PF3D7_1134000	heat shock protein 70	6.15E-03
PF3D7_0632500	erythrocyte membrane protein 1, PfEMP1	6.16E-03
PF3D7_1200100	erythrocyte membrane protein 1, PfEMP1	6.33E-03
PF3D7_1373500	erythrocyte membrane protein 1, PfEMP1	6.36E-03
PF3D7_1348300	elongation factor Tu, putative	6.38E-03
PF3D7_1369600	conserved Plasmodium protein, unknown function	6.42E-03
PF3D7_0220000	liver stage antigen 3	6.44E-03
PF3D7_1121600	circumsporozoite-related antigen exported protein 1	6.53E-03
PF3D7_0622900	transcription factor with AP2 domain(s), putative	6.54E-03
PF3D7_0529400	conserved Plasmodium protein, unknown function	6.57E-03
PF3D7_0215000	acyl-CoA synthetase	6.72E-03
PF3D7_1019700	conserved Plasmodium protein, unknown function	6.79E-03
PF3D7_1006800	RNA-binding protein, putative	6.80E-03
PF3D7_0606000	conserved Plasmodium protein, unknown function	6.90E-03
PF3D7_0622800	leucine--tRNA ligase, putative	6.93E-03
PF3D7_1113100	protein tyrosine phosphatase	7.02E-03
PF3D7_0308200	TCP-1/cpn60 chaperonin family, putative	7.06E-03
PF3D7_1217500	conserved Plasmodium protein, unknown function	7.21E-03
PF3D7_1228800	conserved Plasmodium protein, unknown function	7.27E-03
PF3D7_0309900	conserved Plasmodium protein, unknown function	7.39E-03
PF3D7_1116800	heat shock protein 101 chaperone protein ClpB2	7.41E-03
PF3D7_1015900	enolase	7.44E-03
PF3D7_1434200	calmodulin	7.50E-03
PF3D7_1234100	conserved Plasmodium protein, unknown function	7.52E-03
PF3D7_0302100	serine/threonine protein kinase	7.55E-03
PF3D7_1317000	U4/U6.U5 tri-snRNP-associated protein 2, putative	7.66E-03

PF3D7_0306300	glutaredoxin 1	7.69E-03
PF3D7_1406200	conserved Plasmodium protein, unknown function	7.74E-03
PF3D7_0503100	4-diphosphocytidyl-2c-methyl-D-erythritol kinase (CMK), putative	7.76E-03
PF3D7_0416100	glutamyl-tRNA(Gln) amidotransferase subunit A, putative	7.84E-03
PF3D7_1110900	ES2 protein, putative	7.85E-03
PF3D7_1206200	eukaryotic translation initiation factor 3 subunit 8, putative	7.90E-03
PF3D7_1349600	conserved Plasmodium protein, unknown function	7.96E-03
PF3D7_0706400	ribosomal protein, L37e, putative	8.00E-03
PF3D7_0424400	surface-associated interspersed protein 4.2 (SURFIN 4.2)	8.05E-03
PF3D7_0801800	mannose-6-phosphate isomerase, putative	8.06E-03
PF3D7_1108000	IWS1-like protein, putative	8.10E-03
PF3D7_1368300	conserved Plasmodium protein, unknown function	8.26E-03
PF3D7_0807600	conserved Plasmodium protein, unknown function	8.30E-03
PF3D7_1302000	EMP1-trafficking protein	8.33E-03
PF3D7_0703200	conserved Plasmodium protein, unknown function	8.51E-03
PF3D7_1133100	conserved membrane protein, unknown function	8.55E-03
PF3D7_0420300	transcription factor with AP2 domain(s)	8.71E-03
PF3D7_0405100	protein transport protein Sec24B	8.74E-03
PF3D7_0603600	conserved Plasmodium protein, unknown function	8.76E-03
PF3D7_1231800	asparagine-rich protein, putative	8.78E-03
PF3D7_0817300	asparagine-rich antigen	8.79E-03
PF3D7_0100300	erythrocyte membrane protein 1, PfEMP1	8.91E-03
PF3D7_1226200	conserved Plasmodium protein, unknown function	8.96E-03
PF3D7_1402400	U1 small nuclear ribonucleoprotein C, putative	9.02E-03
PF3D7_1426800	conserved Plasmodium protein, unknown function	9.06E-03
PF3D7_1464600	phosphatase, putative	9.06E-03
PF3D7_0609700	conserved Plasmodium protein, unknown function	9.12E-03
PF3D7_0810600	RNA helicase, putative	9.22E-03
PF3D7_0419800	60S ribosomal protein L7ae/L30e, putative	9.26E-03
PF3D7_1323200	vacuolar ATP synthase subunit g, putative	9.27E-03
PF3D7_0509400	RNA polymerase I	9.28E-03
PF3D7_1107800	transcription factor with AP2 domain(s)	9.36E-03
PF3D7_1311600	conserved Plasmodium protein, unknown function	9.39E-03
PF3D7_0916200	conserved Plasmodium protein, unknown function	9.48E-03
PF3D7_0324900	erythrocyte membrane protein 1, PfEMP1	9.64E-03
PF3D7_0318900	conserved protein, unknown function	9.69E-03
PF3D7_0402100	Plasmodium exported protein (PHISTb), unknown function	9.70E-03
PF3D7_0317300	conserved Plasmodium protein, unknown function	9.83E-03
PF3D7_1424100	60S ribosomal protein L5, putative	9.85E-03
PF3D7_1315700	tRNA (adenine(58)-N(1))-methyltransferase catalytic subunit TRM61, putative	9.94E-03
PF3D7_0826100	E3 ubiquitin-protein ligase, putative	9.97E-03
PF3D7_0512800	conserved Plasmodium protein, unknown function	9.99E-03
PF3D7_0311800	conserved protein, unknown function	1.00E-02
PF3D7_1023900	chromodomain-helicase-DNA-binding protein 1 homolog, putative	1.01E-02
PF3D7_1303400	conserved Plasmodium protein, unknown function	1.01E-02
PF3D7_1131000	RNA-binding protein s1, putative	1.02E-02
PF3D7_1430300	acid phosphatase, putative	1.02E-02
PF3D7_0300100	erythrocyte membrane protein 1, PfEMP1	1.03E-02
PF3D7_1452700	U1 snRNA associated protein, putative	1.04E-02
PF3D7_0511500	RNA pseudouridylate synthase, putative	1.06E-02
PF3D7_1024800	conserved Plasmodium protein, unknown function	1.06E-02
PF3D7_1410900	ribosome maturation protein SBDS, putative	1.07E-02
PF3D7_1342400	casein kinase II beta chain	1.07E-02
PF3D7_0728700	alpha/beta hydrolase, putative	1.09E-02
PF3D7_0705000	methyltransferase, putative	1.10E-02
PF3D7_0110600	phosphatidylinositol-4-phosphate 5-kinase	1.12E-02
PF3D7_0501900	conserved protein, unknown function	1.12E-02
PF3D7_0905600	conserved Plasmodium protein, unknown function	1.12E-02
PF3D7_0828200	leucine--tRNA ligase, putative	1.12E-02
PF3D7_1011800	PRE-binding protein	1.13E-02
PF3D7_1033600	myb2 transcription factor, putative	1.13E-02
PF3D7_1318800	secretory complex protein 63	1.15E-02
PF3D7_0909700	FHA domain protein, putative	1.16E-02
PF3D7_0422200	erythrocyte membrane-associated antigen	1.16E-02

PF3D7_1123100	calcium-dependent protein kinase 7	1.19E-02
PF3D7_0422400	40S ribosomal protein S19, putative	1.19E-02
PF3D7_0814500	conserved Plasmodium protein, unknown function	1.21E-02
PF3D7_1248700	conserved Plasmodium protein, unknown function	1.21E-02
PF3D7_1115600	peptidyl-prolyl cis-trans isomerase	1.21E-02
PF3D7_1457700	large ribosomal subunit nuclear export factor, putative	1.21E-02
PF3D7_1212700	eukaryotic translation initiation factor 3 subunit 10, putative	1.23E-02
PF3D7_0827000	ATP-dependent RNA helicase DBP10, putative	1.24E-02
PF3D7_0617200	conserved Plasmodium protein, unknown function	1.24E-02
PF3D7_1001700	Plasmodium exported protein (PHISTc), unknown function	1.25E-02
PF3D7_0818000	conserved protein, unknown function	1.25E-02
PF3D7_0624600	SNF2 helicase, putative	1.27E-02
PF3D7_0905200	mitochondrial carrier protein, putative	1.27E-02
PF3D7_1314100	conserved Plasmodium protein, unknown function	1.29E-02
PF3D7_0409600	replication protein A1, large subunit	1.29E-02
PF3D7_1369200	conserved Plasmodium protein, unknown function	1.30E-02
PF3D7_1418900	DEAD/DEAH box ATP-dependent RNA helicase, putative	1.30E-02
PF3D7_1452600	conserved Plasmodium protein, unknown function	1.32E-02
PF3D7_1410600	eukaryotic translation initiation factor 2 gamma subunit, putative	1.33E-02
PF3D7_0509000	SNAP protein (soluble N-ethylmaleimide-sensitive factor attachment protein), putative	1.34E-02
PF3D7_0320900	histone H2A variant, putative	1.36E-02
PF3D7_1130100	60S ribosomal protein L38e, putative	1.39E-02
PF3D7_0627100	ankyrin-repeat protein, putative	1.40E-02
PF3D7_1353000	tryptophan-rich antigen, pseudogene	1.41E-02
PF3D7_0907200	GTPase-activating protein, putative	1.42E-02
PF3D7_1335700	conserved Plasmodium protein, unknown function	1.43E-02
PF3D7_0512600	ras-related protein Rab-1B	1.43E-02
PF3D7_0614500	60S ribosomal protein L19, putative	1.44E-02
PF3D7_0829100	conserved protein, unknown function	1.45E-02
PF3D7_0519400	40S ribosomal protein S24, putative	1.46E-02
PF3D7_1409300	DNA damage-inducible protein 1, putative	1.47E-02
PF3D7_0729100	conserved Plasmodium protein, unknown function	1.47E-02
PF3D7_1434600	methionine aminopeptidase 2	1.48E-02
PF3D7_1459400	conserved Plasmodium protein, unknown function	1.49E-02
PF3D7_1136600	conserved Plasmodium protein, unknown function	1.49E-02
PF3D7_0819000	conserved Plasmodium protein, unknown function	1.50E-02
PF3D7_0716300	conserved Plasmodium protein, unknown function	1.50E-02
PF3D7_1441100	conserved Plasmodium protein, unknown function	1.52E-02
PF3D7_1010600	eukaryotic translation initiation factor 2 beta subunit, putative	1.53E-02
PF3D7_1419700	conserved Plasmodium protein, unknown function	1.53E-02
PF3D7_1119900	conserved Plasmodium protein, unknown function	1.55E-02
PF3D7_0615400	ribonuclease, putative	1.55E-02
PF3D7_0701900	Plasmodium exported protein, unknown function	1.55E-02
PF3D7_0704600	ubiquitin transferase, putative	1.56E-02
PF3D7_0317500	kinesin-5, putative	1.56E-02
PF3D7_1471600	conserved Plasmodium protein, unknown function	1.58E-02
PF3D7_0415900	60S ribosomal protein L15, putative	1.60E-02
PF3D7_0202400	conserved Plasmodium protein, unknown function	1.62E-02
PF3D7_1307800	fork head domain protein, putative	1.69E-02
PF3D7_0621200	pyridoxine biosynthesis protein PDX1	1.70E-02
PF3D7_1014600	transcriptional coactivator ADA2	1.71E-02
PF3D7_1231300	conserved Plasmodium protein, unknown function	1.72E-02
PF3D7_1007400	conserved Plasmodium protein, unknown function	1.72E-02
PF3D7_0917100	N-glycosylase/DNA lyase, putative	1.72E-02
PF3D7_1102500	Plasmodium exported protein (PHISTb), unknown function	1.74E-02
PF3D7_1326400	translation initiation factor EIF-2B gamma subunit, putative	1.74E-02
PF3D7_1438000	eukaryotic translation initiation factor eIF2A, putative	1.75E-02
PF3D7_1343700	kelch protein, putative	1.77E-02
PF3D7_1364400	conserved Plasmodium protein, unknown function	1.79E-02
PF3D7_0623800	tyrosine kinase-like protein, putative	1.79E-02
PF3D7_0610900	transcription factor, putative	1.79E-02
PF3D7_0411300	conserved Plasmodium protein, unknown function	1.80E-02
PF3D7_1251600	conserved Plasmodium protein, unknown function	1.80E-02

PF3D7_1252700	Plasmodium exported protein (PHISTb), unknown function	1.83E-02
PF3D7_1443800	zinc finger protein, putative	1.83E-02
PF3D7_1440500	allantoicase, putative	1.83E-02
PF3D7_1129000	spermidine synthase	1.84E-02
PF3D7_1107700	pescadillo-like protein	1.84E-02
PF3D7_1149600	DnaJ protein, putative	1.85E-02
PF3D7_1205600	conserved Plasmodium protein, unknown function	1.86E-02
PF3D7_0828300	conserved Plasmodium protein, unknown function	1.88E-02
PF3D7_0813700	ABC transporter, putative	1.89E-02
PF3D7_0804700	conserved Plasmodium protein, unknown function	1.89E-02
PF3D7_1422400	conserved Plasmodium protein, unknown function	1.89E-02
PF3D7_0806500	DnaJ protein, putative	1.90E-02
PF3D7_1363400	polyubiquitin binding protein, putative	1.90E-02
PF3D7_1343800	conserved Plasmodium protein, unknown function	1.90E-02
PF3D7_1358200	conserved Plasmodium protein, unknown function	1.91E-02
PF3D7_0814000	60S ribosomal protein L13-2, putative	1.91E-02
PF3D7_1429200	transcription factor with AP2 domain(s), putative	1.92E-02
PF3D7_1336800	nuclear movement protein, putative	1.93E-02
PF3D7_1325400	conserved Plasmodium protein, unknown function	1.93E-02
PF3D7_0322500	iron-sulfur assembly protein, putative	1.96E-02
PF3D7_0917900	heat shock protein 70	1.96E-02
PF3D7_1437900	HSP40, subfamily A, putative	1.96E-02
PF3D7_1440400	conserved Plasmodium protein, unknown function	1.99E-02
PF3D7_0707500	conserved Plasmodium protein, unknown function	1.99E-02
PF3D7_1358700	HVA22-like protein, putative	2.00E-02
PF3D7_1225700	conserved Plasmodium protein, unknown function	2.00E-02
PF3D7_0322100	RNA triphosphatase	2.02E-02
PF3D7_0524400	cytosolic preribosomal GTP-binding protein, putative	2.03E-02
PF3D7_0720700	phosphoinositide-binding protein, putative	2.04E-02
PF3D7_0903900	60S ribosomal protein L32, putative	2.05E-02
PF3D7_0707700	E3 ubiquitin-protein ligase, putative	2.05E-02
PF3D7_1467600	conserved Plasmodium protein, unknown function	2.06E-02
PF3D7_1116600	oligosaccharyl transferase STT3 subunit, putative	2.07E-02
PF3D7_1126500	conserved Plasmodium protein, unknown function	2.08E-02
PF3D7_0525100	acyl-CoA synthetase	2.08E-02
PF3D7_0828700	conserved Plasmodium protein, unknown function	2.09E-02
PF3D7_0520200	conserved Plasmodium protein, unknown function	2.09E-02
PF3D7_1354300	large subunit rRNA methyltransferase, putative	2.10E-02
PF3D7_0313500	GTP-binding protein EngA, putative	2.12E-02
PF3D7_1303500	sodium/hydrogen exchanger, Na ⁺ , H ⁺ antiporter	2.12E-02
PF3D7_0112800	Plasmodium exported protein (hyp11), unknown function	2.13E-02
PF3D7_0904100	adapter-related protein, putative	2.15E-02
PF3D7_0514000	tubulin-tyrosine ligase, putative	2.17E-02
PF3D7_0710600	60S ribosomal protein L34a, putative	2.17E-02
PF3D7_1146800	conserved Plasmodium protein, unknown function	2.18E-02
PF3D7_1137300	conserved Plasmodium membrane protein, unknown function	2.19E-02
PF3D7_1038800	RESA-like protein with PHIST and DnaJ domains	2.24E-02
PF3D7_0501800	chromosome assembly factor 1	2.25E-02
PF3D7_1414500	atypical protein kinase, ABC-1 family, putative	2.27E-02
PF3D7_1108600	endoplasmic reticulum-resident calcium binding protein	2.29E-02
PF3D7_0220200	Plasmodium exported protein, unknown function	2.30E-02
PF3D7_1308400	conserved Plasmodium protein, unknown function	2.32E-02
PF3D7_0320400	oocyst capsule protein	2.32E-02
PF3D7_1412800	N-myristoyltransferase	2.33E-02
PF3D7_1125400	mitochondrial import inner membrane translocase subunit TIM44, putative	2.33E-02
PF3D7_0623300	EGF-like membrane protein, putative	2.34E-02
PF3D7_0719600	60S ribosomal protein L11a, putative	2.34E-02
PF3D7_1004400	RNA-binding protein, putative	2.36E-02
PF3D7_0935700	Plasmodium exported protein, unknown function	2.36E-02
PF3D7_0306400	FAD-dependent glycerol-3-phosphate dehydrogenase, putative	2.38E-02
PF3D7_0631900	stevor PIR protein	2.38E-02
PF3D7_1464500	conserved Plasmodium membrane protein, unknown function	2.42E-02
PF3D7_1478300	Plasmodium exported protein, unknown function, pseudogene	2.42E-02
PF3D7_0716100	large ribosomal subunit assembling factor, putative	2.43E-02

PF3D7_1342800	phosphoenolpyruvate carboxykinase	2.43E-02
PF3D7_1239700	ATP-dependent zinc metalloprotease FTSH 1	2.44E-02
PF3D7_1409000	conserved Plasmodium protein, unknown function	2.44E-02
PF3D7_1205800	high mobility group protein B3, putative	2.44E-02
PF3D7_1343300	conserved Plasmodium protein, unknown function	2.45E-02
PF3D7_0524100	conserved Plasmodium protein, unknown function	2.47E-02
PF3D7_1359100	riboflavin kinase / FAD synthase family protein, putative	2.50E-02
PF3D7_0827800	SET domain protein, putative	2.50E-02
PF3D7_1245300	ubiquitin conjugating enzyme E2, putative	2.50E-02
PF3D7_1322100	variant-silencing SET protein	2.51E-02
PF3D7_1450800	conserved Plasmodium protein, unknown function	2.53E-02
PF3D7_1367400	conserved Plasmodium protein, unknown function	2.54E-02
PF3D7_1301700	Plasmodium exported protein (hyp8), unknown function	2.56E-02
PF3D7_0918900	gamma-glutamylcysteine synthetase	2.57E-02
PF3D7_0926100	protein kinase, putative	2.58E-02
PF3D7_0928900	guanylate kinase	2.58E-02
PF3D7_1235100	conserved Plasmodium protein, unknown function	2.60E-02
PF3D7_1417200	NOT family protein, putative	2.61E-02
PF3D7_0420100	RIO-like serine/threonine kinase	2.62E-02
PF3D7_0811300	CCR4-associated factor 1	2.63E-02
PF3D7_0922100	ubiquitin-like protein, putative	2.64E-02
PF3D7_1104300	conserved Plasmodium protein, unknown function	2.67E-02
PF3D7_1200600	erythrocyte membrane protein 1, PfEMP1	2.68E-02
PF3D7_1339500	mitochondrial ribosomal protein L49 precursor, putative	2.70E-02
PF3D7_1030700	RNA methyltransferase, putative	2.70E-02
PF3D7_1133700	conserved Plasmodium protein, unknown function	2.71E-02
PF3D7_0903400	DEAD/DEAH box helicase, putative	2.73E-02
PF3D7_1212900	bromodomain protein, putative	2.76E-02
PF3D7_1316800	protein transport protein SEC20, putative	2.77E-02
PF3D7_1343000	phosphoethanolamine N-methyltransferase	2.79E-02
PF3D7_0419600	ran binding protein 1, putative	2.82E-02
PF3D7_1147000	sporozoite asparagine-rich protein	2.82E-02
PF3D7_0524700	mitochondrial import receptor subunit TOM22, putative	2.86E-02
PF3D7_1327500	conserved Plasmodium protein, unknown function	2.92E-02
PF3D7_1237900	conserved Plasmodium protein, unknown function	2.92E-02
PF3D7_1405400	DNA mismatch repair protein, putative	2.95E-02
PF3D7_1353400	Ran-binding protein, putative	2.95E-02
PF3D7_0611600	conserved Plasmodium protein, unknown function	2.95E-02
PF3D7_1312100	conserved Plasmodium protein, unknown function	2.96E-02
PF3D7_0607100	MYND finger protein, putative	2.99E-02
PF3D7_0319500	RNA-binding protein, putative	3.00E-02
PF3D7_0300400	stevor PIR protein	3.01E-02
PF3D7_1236000	vesicle transport v-SNARE protein VTI1, putative	3.02E-02
PF3D7_1017600	conserved Plasmodium protein, unknown function	3.02E-02
PF3D7_0505100	conserved Plasmodium protein, unknown function	3.04E-02
PF3D7_1029900	conserved Plasmodium protein, unknown function	3.05E-02
PF3D7_0618300	60S ribosomal protein L27a, putative	3.06E-02
PF3D7_1020000	large subunit rRNA processing RRM protein, putative	3.08E-02
PF3D7_0321800	conserved Plasmodium protein, unknown function	3.08E-02
PF3D7_1451200	conserved Plasmodium protein, unknown function	3.08E-02
PF3D7_1227100	DNA helicase 60	3.13E-02
PF3D7_0820300	conserved Plasmodium protein, unknown function	3.14E-02
PF3D7_1367000	suppressor of kinetochore protein 1, putative	3.15E-02
PF3D7_0315800	zinc finger protein, putative	3.15E-02
PF3D7_0207200	iron-sulfur assembly protein, putative	3.17E-02
PF3D7_1031900	conserved Plasmodium protein, unknown function	3.17E-02
PF3D7_0821400	conserved Plasmodium protein, unknown function	3.17E-02
PF3D7_0316000	microneme associated antigen	3.23E-02
PF3D7_1325600	mitochondrial fission 1 protein, putative	3.23E-02
PF3D7_0201000	rifin PIR protein	3.29E-02
PF3D7_0804500	conserved Plasmodium membrane protein, unknown function	3.29E-02
PF3D7_0922200	S-adenosylmethionine synthetase	3.29E-02
PF3D7_0314500	conserved Plasmodium protein, unknown function	3.32E-02
PF3D7_1369400	conserved Plasmodium protein, unknown function	3.32E-02

PF3D7_0402400	Plasmodium exported protein, unknown function	3.33E-02
PF3D7_1422700	conserved Plasmodium protein, unknown function	3.33E-02
PF3D7_0306600	conserved Plasmodium protein, unknown function	3.34E-02
PF3D7_0405400	pre-mRNA-processing-splicing factor 8, putative	3.35E-02
PF3D7_1405800	large subunit rRNA processing protein, putative	3.35E-02
PF3D7_1420400	glycine--tRNA ligase	3.38E-02
PF3D7_1029500	conserved Plasmodium protein, unknown function	3.41E-02
PF3D7_0312200	TPR domain containing protein	3.41E-02
PF3D7_0307700	conserved Plasmodium protein, unknown function	3.42E-02
PF3D7_0931800	proteasome precursor, putative	3.42E-02
PF3D7_0932200	profilin, putative	3.44E-02
PF3D7_1021100	conserved Plasmodium protein, unknown function	3.47E-02
PF3D7_1348400	conserved Plasmodium membrane protein, unknown function	3.47E-02
PF3D7_0914300	met-10+ like protein, putative	3.48E-02
PF3D7_1457900	conserved Plasmodium protein, unknown function	3.50E-02
PF3D7_0825500	conserved protein, unknown function	3.51E-02
PF3D7_1242700	40S ribosomal protein S17, putative	3.51E-02
PF3D7_1473700	nucleoporin NUP116/NSP116, putative	3.51E-02
PF3D7_0108700	secreted ookinete protein, putative	3.57E-02
PF3D7_1409100	aldo-keto reductase, putative	3.58E-02
PF3D7_0730300	transcription factor with AP2 domain(s)	3.60E-02
PF3D7_0717800	conserved Plasmodium protein, unknown function	3.60E-02
PF3D7_0706500	conserved Plasmodium protein, unknown function	3.60E-02
PF3D7_1123900	splicing factor, putative	3.62E-02
PF3D7_1409800	RNA binding protein Bruno, putative	3.66E-02
PF3D7_1323400	60S ribosomal protein L23a, putative	3.67E-02
PF3D7_1417800	DNA replication licensing factor MCM2	3.67E-02
PF3D7_1118600	histone acetyltransferase	3.68E-02
PF3D7_1415600	conserved Plasmodium protein, unknown function	3.76E-02
PF3D7_1440800	major facilitator superfamily, putative	3.78E-02
PF3D7_0322000	peptidyl-prolyl cis-trans isomerase	3.79E-02
PF3D7_1457100	conserved Plasmodium protein, unknown function	3.79E-02
PF3D7_1369100	conserved Plasmodium protein, unknown function	3.83E-02
PF3D7_1219100	clathrin heavy chain, putative	3.84E-02
PF3D7_1211200	conserved Plasmodium protein, unknown function	3.85E-02
PF3D7_0112000	TatD-like deoxyribonuclease, putative	3.87E-02
PF3D7_1148800	Plasmodium exported protein (hyp11), unknown function	3.88E-02
PF3D7_0614900	conserved Plasmodium membrane protein, unknown function	3.91E-02
PF3D7_0527500	Hsc70-interacting protein	3.93E-02
PF3D7_0820000	Snf2-related CBP activator, putative	3.97E-02
PF3D7_1233000	elongation factor G	4.00E-02
PF3D7_0907700	subunit of proteasome activator complex, putative	4.04E-02
PF3D7_1411600	GTP-binding protein, putative	4.07E-02
PF3D7_0936700	lysophospholipase, putative	4.07E-02
PF3D7_1013000	zinc finger protein, putative	4.12E-02
PF3D7_1431500	mitogen-activated protein kinase 1	4.12E-02
PF3D7_0924400	conserved Plasmodium protein, unknown function	4.15E-02
PF3D7_0608200	conserved Plasmodium protein, unknown function	4.16E-02
PF3D7_1005600	DnaJ protein, putative	4.16E-02
PF3D7_1243600	translation initiation factor SUI1, putative	4.18E-02
PF3D7_1340000	secreted ookinete protein, putative	4.18E-02
PF3D7_1002700	conserved Plasmodium protein, unknown function	4.21E-02
PF3D7_0508500	guanidine nucleotide exchange factor	4.21E-02
PF3D7_0905500	conserved Plasmodium protein, unknown function	4.23E-02
PF3D7_0614100	conserved Plasmodium protein, unknown function	4.26E-02
PF3D7_0318200	DNA-directed RNA polymerase II, putative	4.27E-02
PF3D7_1443100	conserved Plasmodium protein, unknown function	4.27E-02
PF3D7_0930300	merozoite surface protein 1	4.28E-02
PF3D7_0514200	conserved Plasmodium protein, unknown function	4.29E-02
PF3D7_1230800	conserved Plasmodium protein, unknown function	4.29E-02
PF3D7_1149000	antigen 332, DBL-like protein	4.32E-02
PF3D7_1352100	ABC transporter, (heavy metal transporter family), putative	4.35E-02
PF3D7_1321700	splicing factor, putative	4.35E-02
PF3D7_1126000	threonine--tRNA ligase	4.36E-02

PF3D7_1313800	conserved Plasmodium membrane protein, unknown function	4.37E-02
PF3D7_1220100	pre-mRNA splicing factor, putative	4.39E-02
PF3D7_0315600	conserved Plasmodium protein, unknown function	4.39E-02
PF3D7_0317000	proteasome component C8, putative	4.40E-02
PF3D7_0206500	conserved Plasmodium protein, unknown function	4.42E-02
PF3D7_1321800	SFT2-like protein, putative	4.43E-02
PF3D7_1329000	DNA-directed RNA polymerase 3 largest subunit	4.45E-02
PF3D7_1013100	U3 snoRNA-associated small subunit rRNA processing protein, putative	4.46E-02
PF3D7_0619600	conserved Plasmodium protein, unknown function	4.46E-02
PF3D7_1216900	DNA-binding chaperone, putative	4.51E-02
PF3D7_0912400	conserved Plasmodium protein, unknown function	4.52E-02
PF3D7_0809800	conserved Plasmodium protein, unknown function	4.55E-02
PF3D7_1354700	conserved Plasmodium protein, unknown function	4.58E-02
PF3D7_1331400	CPW-WPC family protein	4.58E-02
PF3D7_1016900	early transcribed membrane protein 10.3	4.61E-02
PF3D7_0928100	conserved Plasmodium protein, unknown function	4.63E-02
PF3D7_0930100	conserved Plasmodium protein, unknown function	4.65E-02
PF3D7_1247500	serine/threonine protein kinase, putative	4.69E-02
PF3D7_0216700	conserved Plasmodium protein, unknown function	4.70E-02
PF3D7_0505000	conserved Plasmodium membrane protein, unknown function	4.75E-02
PF3D7_1002000	Plasmodium exported protein (hyp2), unknown function	4.77E-02
PF3D7_1131900	conserved Plasmodium protein, unknown function	4.78E-02
PF3D7_0520000	40S ribosomal protein S9, putative	4.80E-02
PF3D7_0602400	elongation factor G	4.81E-02
PF3D7_0513200	conserved Plasmodium protein, unknown function	4.82E-02
PF3D7_0214800	conserved Plasmodium membrane protein, unknown function	4.83E-02
PF3D7_1210000	mitochondrial ribosomal protein L1-2 precursor, putative	4.84E-02
PF3D7_0805600	PAP2-like protein, putative	4.85E-02
PF3D7_1329500	conserved Plasmodium protein, unknown function	4.87E-02
PF3D7_1420700	surface protein, Pf113	4.88E-02
PF3D7_1428900	conserved Plasmodium protein, unknown function	4.90E-02
PF3D7_0714600	conserved Plasmodium protein, unknown function	4.92E-02
PF3D7_0923200	flavodoxin-like protein	4.93E-02
PF3D7_1241800	DEAD/DEAH box ATP-dependent RNA helicase, putative	4.95E-02
PF3D7_1324900	L-lactate dehydrogenase	4.97E-02

DOWNREGULATED IN 11C-R vs 11C at 10-20HPI

Gene ID	Gene Description	<i>p</i> value
PF3D7_0425800	erythrocyte membrane protein 1, PfEMP1	5.55E-07
PF3D7_1150400	erythrocyte membrane protein 1, PfEMP1	1.08E-05
PF3D7_0712900	erythrocyte membrane protein 1, PfEMP1	2.24E-05
PF3D7_0201800	knob associated heat shock protein 40	3.61E-05
PF3D7_1028600	conserved Plasmodium protein, unknown function	3.84E-05
PF3D7_0400400	erythrocyte membrane protein 1, PfEMP1	5.49E-05
PF3D7_0302300	erythrocyte membrane protein 1 (PfEMP1), pseudogene	5.71E-05
PF3D7_1323900	conserved Plasmodium protein, unknown function	9.42E-05
PF3D7_0600400	erythrocyte membrane protein 1, PfEMP1	9.96E-05
PF3D7_0321400	protein kinase, putative	1.10E-04
PF3D7_0907300	conserved Plasmodium protein, unknown function	1.17E-04
PF3D7_0202000	knob-associated histidine-rich protein	1.30E-04
PF3D7_1100200	erythrocyte membrane protein 1, PfEMP1	1.33E-04
PF3D7_0807200	conserved Plasmodium membrane protein, unknown function	1.39E-04
PF3D7_0826700	receptor for activated c kinase	1.76E-04
PF3D7_1248800	conserved Plasmodium protein, unknown function	1.77E-04
PF3D7_0215300	acyl-CoA synthetase	1.78E-04
PF3D7_0600300	rifin PIR protein	1.83E-04
PF3D7_0400500	rifin PIR protein	1.86E-04
PF3D7_1432900	SF-assemblin, putative	2.49E-04
PF3D7_1231000	conserved Plasmodium protein, unknown function	2.82E-04
PF3D7_1202800	conserved Plasmodium protein, unknown function	2.97E-04
PF3D7_0600200	erythrocyte membrane protein 1, PfEMP1	3.04E-04
PF3D7_1124100	neutral-sphingomyelinase activation factor protein, putative	3.11E-04

PF3D7_0305000	elongation factor Ts	3.41E-04
PF3D7_1463900	conserved Plasmodium membrane protein, unknown function	3.46E-04
PF3D7_1473100	GTPase-activating protein, putative	3.48E-04
PF3D7_1141100	conserved Plasmodium protein, unknown function	4.08E-04
PF3D7_1003300	N-acetyltransferase, putative	4.40E-04
PF3D7_1314900	TFIIH basal transcription factor subunit, putative	4.91E-04
PF3D7_1320900	RNA-binding protein, putative	5.26E-04
PF3D7_1406000	RNA-binding protein, putative	5.35E-04
PF3D7_1240600	erythrocyte membrane protein 1, PfEMP1	5.76E-04
PF3D7_1232300	cytochrome b5, putative	5.90E-04
PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1	6.09E-04
PF3D7_0201600	Plasmodium exported protein (PHISTb), unknown function	6.36E-04
PF3D7_1444200	calmodulin-like protein	7.47E-04
PF3D7_1404900	conserved Plasmodium protein, unknown function	7.83E-04
PF3D7_0222100	Pfmc-2TM Maurer's cleft two transmembrane protein	8.15E-04
PF3D7_0201900	erythrocyte membrane protein 3	8.20E-04
PF3D7_1434400	conserved Plasmodium membrane protein, unknown function	8.48E-04
PF3D7_1139100	RNA-binding protein, putative	8.97E-04
PF3D7_1214600	adrenodoxin-type ferredoxin, putative	9.36E-04
PF3D7_0723900	conserved Plasmodium protein, unknown function	9.56E-04
PF3D7_1114100	rhomboid protease ROM1	1.02E-03
PF3D7_0810500	protein phosphatase, putative	1.06E-03
PF3D7_0424000	Plasmodium exported protein (PHISTc), unknown function	1.08E-03
PF3D7_1464700	ATP synthase (C/AC39) subunit, putative	1.10E-03
PF3D7_0114500	Plasmodium exported protein (hyp10), unknown function	1.15E-03
PF3D7_1122000	conserved Plasmodium protein, unknown function	1.16E-03
PF3D7_1219400	erythrocyte membrane protein 1 (PfEMP1), pseudogene	1.18E-03
PF3D7_1301800	surface-associated interspersed protein 13.1 (SURFIN 13.1), pseudogene	1.22E-03
PF3D7_1131400	conserved Plasmodium protein, unknown function	1.26E-03
PF3D7_1002900	conserved Plasmodium protein, unknown function	1.34E-03
PF3D7_0211900	conserved Plasmodium protein, unknown function	1.35E-03
PF3D7_1349200	glutamate-tRNA ligase, putative	1.43E-03
PF3D7_0833500	erythrocyte membrane protein 1, PfEMP1	1.43E-03
PF3D7_1425700	conserved Plasmodium protein, unknown function	1.44E-03
PF3D7_1366800	phosphatidylserine synthase, putative	1.48E-03
PF3D7_1001200	acyl-CoA binding protein, isoform 2, ACBP2	1.50E-03
PF3D7_0425200	Plasmodium exported protein (hyp15), unknown function	1.50E-03
PF3D7_1234300	DNA polymerase epsilon subunit b, putative	1.62E-03
PF3D7_0825600	cytochrome c oxidase assembly protein, putative	1.63E-03
PF3D7_0931900	adenylate kinase-like protein 2	1.69E-03
PF3D7_1314200	telomerase reverse transcriptase, putative	1.73E-03
PF3D7_1127300	methyltransferase, putative	1.79E-03
PF3D7_0919300	thioredoxin-like protein 1, putative	1.85E-03
PF3D7_0515400	conserved Plasmodium protein, unknown function	1.93E-03
PF3D7_0308500	activator of Hsp90 ATPase, putative	1.93E-03
PF3D7_0319600	elongation factor 1 (EF-1), putative	2.00E-03
PF3D7_0528700	peptidyl-prolyl cis-trans isomerase	2.00E-03
PF3D7_0617400	erythrocyte membrane protein 1, PfEMP1	2.02E-03
PF3D7_0602800	JmjC domain containing protein	2.03E-03
PF3D7_1223200	nucleus export protein BRR6, putative	2.03E-03
PF3D7_1249400	conserved Plasmodium protein, unknown function	2.09E-03
PF3D7_0109500	N-acetyltransferase, putative	2.10E-03
PF3D7_1208400	amino acid transporter, putative	2.16E-03
PF3D7_0531300	conserved Plasmodium protein, unknown function	2.17E-03
PF3D7_0409100	U4/U6 small nuclear ribonucleoprotein PRP31, putative	2.29E-03
PF3D7_0418400	conserved Plasmodium protein, unknown function	2.33E-03
PF3D7_1304300	conserved Plasmodium protein, unknown function	2.48E-03
PF3D7_0412700	erythrocyte membrane protein 1, PfEMP1	2.48E-03
PF3D7_0934200	conserved Plasmodium protein, unknown function	2.49E-03
PF3D7_1317600	conserved Plasmodium protein, unknown function	2.52E-03
PF3D7_1311700	cytochrome c2 precursor, putative	2.56E-03

PF3D7_1226100	hydrolase/phosphatase, putative	2.57E-03
PF3D7_1338300	elongation factor 1-gamma, putative	2.58E-03
PF3D7_0623400	RNA-binding protein mei2 homologue, putative	2.64E-03
PF3D7_0906400	dynein light intermediate chain 2, cytosolic	2.70E-03
PF3D7_1473600	conserved Plasmodium protein, unknown function	2.74E-03
PF3D7_1327000	conserved Plasmodium protein, unknown function	2.74E-03
PF3D7_1360200	conserved protein, unknown function	2.74E-03
PF3D7_1368900	conserved Plasmodium protein, unknown function	2.81E-03
PF3D7_0823500	membrane skeletal protein IMC1-related	2.83E-03
PF3D7_1240100	early transcribed membrane protein 12	2.88E-03
PF3D7_0107900	conserved Plasmodium protein, unknown function	2.95E-03
PF3D7_1445100	histidine--tRNA ligase, putative	2.96E-03
PF3D7_1332500	SAM-dependent RNA methyltransferase, putative	2.99E-03
PF3D7_1436200	conserved Plasmodium protein, unknown function	3.06E-03
PF3D7_1132200	TCP-1/cpn60 chaperonin family, putative	3.09E-03
PF3D7_1114500	conserved Plasmodium protein, unknown function	3.10E-03
PF3D7_0720300	conserved Plasmodium protein, unknown function	3.20E-03
PF3D7_1106200	conserved Plasmodium protein, unknown function	3.22E-03
PF3D7_1231500	DIM1 protein homolog, putative	3.26E-03
PF3D7_0906100	developmental protein, putative	3.29E-03
PF3D7_0827500	apicoplast ribosomal protein L21 precursor, putative	3.32E-03
PF3D7_1251700	tryptophan--tRNA ligase, putative	3.47E-03
PF3D7_1117400	conserved Plasmodium protein, unknown function	3.48E-03
PF3D7_0104400	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	3.59E-03
PF3D7_0110100	selenocysteine-specific elongation factor selB homologue, putative	3.67E-03
PF3D7_0218400	DEAD/DEAH box ATP-dependent RNA helicase, putative	3.70E-03
PF3D7_0808800	rifin PIR protein	3.73E-03
PF3D7_1245500	conserved Plasmodium protein, unknown function	3.91E-03
PF3D7_0732900	rifin PIR protein	3.97E-03
PF3D7_1131500	conserved Plasmodium protein, unknown function	4.02E-03
PF3D7_1224600	cytochrome c heme lyase, putative	4.06E-03
PF3D7_0936400	ring-exported protein 4	4.23E-03
PF3D7_1343200	tRNA guanosine-2'-O-methyltransferase, putative	4.25E-03
PF3D7_1312200	conserved Plasmodium protein, unknown function	4.25E-03
PF3D7_0932300	M18 aspartyl aminopeptidase	4.42E-03
PF3D7_1470800	conserved Plasmodium protein, unknown function	4.62E-03
PF3D7_1370000	tRNA Arginine	4.65E-03
PF3D7_1243500	vacuolar-sorting protein SNF7, putative	4.69E-03
PF3D7_0918300	eukaryotic translation initiation factor 3 subunit 5, putative	4.75E-03
PF3D7_0201500	Plasmodium exported protein (hyp9), unknown function	4.85E-03
PF3D7_0910700	actin-like protein, putative	4.86E-03
PF3D7_0721800	conserved Plasmodium protein, unknown function	4.95E-03
PF3D7_1235600	serine hydroxymethyltransferase	5.01E-03
PF3D7_1147800	merozoite adhesive erythrocytic binding protein	5.02E-03
PF3D7_1204200	conserved Plasmodium protein, unknown function	5.02E-03
PF3D7_0911400	conserved Plasmodium protein, unknown function	5.21E-03
PF3D7_0715600	GTP-binding translation elongation factor tu family protein, putative	5.31E-03
PF3D7_1355900	conserved protein, unknown function	5.34E-03
PF3D7_0915100	SUMO-conjugating enzyme UBC9	5.41E-03
PF3D7_0729300	60S ribosomal subunit export protein, putative	5.43E-03
PF3D7_0419400	conserved Plasmodium protein, unknown function	5.45E-03
PF3D7_0805400	acetyltransferase, putative	5.48E-03
PF3D7_0806900	conserved Plasmodium protein, unknown function	5.56E-03
PF3D7_0407100	methyltransferase, putative	5.60E-03
PF3D7_0700200	rifin PIR protein	5.65E-03
PF3D7_1141600	dolichol phosphate mannose synthase	5.70E-03
PF3D7_0830700	Plasmodium exported protein (hyp9), unknown function	5.71E-03
PF3D7_0308600	pre-mRNA-processing factor 19, putative	5.75E-03
PF3D7_0926000	protein kinase, putative	5.76E-03
PF3D7_0816300	conserved Plasmodium protein, unknown function	5.76E-03
PF3D7_0423800	cysteine-rich protective antigen	5.88E-03

PF3D7_0522800	G10 protein, putative	5.90E-03
PF3D7_1361500	conserved Plasmodium protein, unknown function	5.93E-03
PF3D7_1343400	DNA repair protein RAD5, putative	6.01E-03
PF3D7_1309000	conserved Plasmodium protein, unknown function	6.09E-03
PF3D7_0102800	conserved Plasmodium protein, unknown function	6.12E-03
PF3D7_0714500	transcription elongation factor s-II, putative	6.18E-03
PF3D7_0406200	sexual stage-specific protein precursor	6.45E-03
PF3D7_1337700	conserved Plasmodium protein, unknown function	6.47E-03
PF3D7_1468200	conserved protein, unknown function	6.48E-03
PF3D7_1018400	conserved Plasmodium protein, unknown function	6.51E-03
PF3D7_1352200	conserved Plasmodium protein, unknown function	6.53E-03
PF3D7_0315000	zinc finger protein, putative	6.58E-03
PF3D7_0811200	conserved protein, unknown function	6.64E-03
PF3D7_1407400	conserved Plasmodium protein, unknown function	6.73E-03
PF3D7_0210700	syntaxin, Qa-SNARE family	6.82E-03
PF3D7_1438300	tRNA Methionine	6.99E-03
PF3D7_1419900	YTH domain-containing protein, putative	7.07E-03
PF3D7_1413900	DnaJ protein, putative	7.15E-03
PF3D7_0914400	conserved Plasmodium protein, unknown function	7.38E-03
PF3D7_1358800	40S ribosomal protein S13, putative	7.38E-03
PF3D7_1354500	adenylosuccinate synthetase	7.48E-03
PF3D7_1240400	erythrocyte membrane protein 1, PfEMP1	7.51E-03
PF3D7_1332300	sedlin, putative	7.55E-03
PF3D7_1136200	conserved Plasmodium protein, unknown function	7.58E-03
PF3D7_0523100	mitochondrial processing peptidase alpha subunit, putative	7.66E-03
PF3D7_0724200	type 2A phosphatase-associated protein 42, putative	7.66E-03
PF3D7_0923100	OTU-like cysteine protease, putative	7.66E-03
PF3D7_1108100	conserved Plasmodium protein, unknown function	7.70E-03
PF3D7_0804400	methionine aminopeptidase 1c, putative	7.73E-03
PF3D7_1146200	conserved Plasmodium protein, unknown function	7.75E-03
PF3D7_0217600	conserved Plasmodium protein, unknown function	7.84E-03
PF3D7_1113200	conserved Plasmodium protein, unknown function	7.88E-03
PF3D7_1454600	mitochondrial ribosomal protein S11 precursor, putative	7.93E-03
PF3D7_0206600	transcription factor, putative	8.02E-03
PF3D7_0606500	polypyrimidine tract binding protein, putative	8.18E-03
PF3D7_1458900	conserved protein, unknown function	8.21E-03
PF3D7_1238400	conserved Plasmodium protein, unknown function	8.21E-03
PF3D7_1465100	conserved Plasmodium protein, unknown function	8.34E-03
PF3D7_1222400	transcription factor with AP2 domain(s)	8.35E-03
PF3D7_0936100	early transcribed membrane protein	8.50E-03
PF3D7_1357800	TCP-1/cpn60 chaperonin family, putative	8.63E-03
PF3D7_0710900	mitochondrial ribosomal protein L1 precursor, putative	8.83E-03
PF3D7_0305600	AP endonuclease (DNA-[apurinic or apyrimidinic site] lyase), putative	9.01E-03
PF3D7_1307000	PNAS-3 related protein, putative	9.05E-03
PF3D7_1426200	arginine methyltransferase 1	9.09E-03
PF3D7_1030200	conserved Plasmodium protein, unknown function	9.33E-03
PF3D7_1446900	glutaminy-peptide cyclotransferase, putative	9.52E-03
PF3D7_1142600	60S ribosomal protein L35ae, putative	9.52E-03
PF3D7_0816200	vacuolar protein-sorting protein VPS2, putative	9.57E-03
PF3D7_0624300	conserved Plasmodium protein, unknown function	9.84E-03
PF3D7_1205100	O-phosphoseryl-tRNA(Sec) selenium transferase, putative	9.86E-03
PF3D7_1427800	exosome complex exonuclease RRP41, putative	9.88E-03
PF3D7_0903700	alpha tubulin 1	1.01E-02
PF3D7_1421000	conserved Plasmodium protein, unknown function	1.01E-02
PF3D7_1108700	heat shock protein DnaJ homologue Pfj2	1.02E-02
PF3D7_0305200	conserved Plasmodium protein, unknown function	1.04E-02
PF3D7_1036800	acetyl-CoA transporter, putative	1.08E-02
PF3D7_1362600	conserved Plasmodium protein, unknown function	1.09E-02
PF3D7_1032500	DER1-like protein, putative	1.11E-02
PF3D7_1127500	protein disulfide isomerase, putative	1.11E-02
PF3D7_1103000	conserved Plasmodium protein, unknown function	1.11E-02

PF3D7_0702700	tRNA Alanine	1.13E-02
PF3D7_0724800	kelch protein, putative	1.14E-02
PF3D7_1035800	probable protein, unknown function	1.18E-02
PF3D7_1456700	conserved Plasmodium protein, unknown function	1.18E-02
PF3D7_0810000	acyl-CoA binding protein, putative	1.18E-02
PF3D7_1213300	conserved Plasmodium protein, unknown function	1.21E-02
PF3D7_1332200	conserved Plasmodium protein, unknown function	1.22E-02
PF3D7_1422800	actin-related protein, putative	1.23E-02
PF3D7_0910300	conserved Plasmodium protein, unknown function	1.24E-02
PF3D7_0605500	cyclin dependent kinase binding protein, putative	1.25E-02
PF3D7_1420000	splicing factor 3B subunit 4, putative	1.25E-02
PF3D7_0107700	conserved Plasmodium membrane protein, unknown function	1.26E-02
PF3D7_1244700	conserved Plasmodium protein, unknown function	1.26E-02
PF3D7_1408000	plasmepsin II	1.26E-02
PF3D7_0917000	conserved Plasmodium protein, unknown function	1.26E-02
PF3D7_0805200	conserved Plasmodium protein, unknown function	1.29E-02
PF3D7_1402300	26S proteasome regulatory subunit	1.29E-02
PF3D7_1415200	DNA-directed RNA polymerase I/III subunit, putative	1.30E-02
PF3D7_0220900	Plasmodium exported protein, unknown function, pseudogene	1.30E-02
PF3D7_0310000	conserved Plasmodium protein, unknown function	1.31E-02
PF3D7_0821600	clp1-related protein, putative	1.32E-02
PF3D7_0713400	serpentine receptor, putative	1.33E-02
PF3D7_1333700	histone H3 variant	1.33E-02
PF3D7_1131800	oxysterol-binding protein-related protein 2	1.33E-02
PF3D7_1018100	conserved Plasmodium protein, unknown function	1.34E-02
PF3D7_1116300	peptidyl-prolyl cis-trans isomerase	1.35E-02
PF3D7_1363600	conserved Plasmodium protein, unknown function	1.36E-02
PF3D7_1032900	RNA polymerase II-associated protein 1, putative	1.36E-02
PF3D7_1040300	rifin PIR protein	1.37E-02
PF3D7_0716400	conserved Plasmodium protein, unknown function	1.38E-02
PF3D7_1031400	OTU-like cysteine protease, putative	1.38E-02
PF3D7_0514900	conserved Plasmodium protein, unknown function	1.39E-02
PF3D7_1007000	conserved membrane protein, unknown function	1.40E-02
PF3D7_1446100	conserved Plasmodium protein, unknown function	1.40E-02
PF3D7_1403100	conserved Plasmodium protein, unknown function	1.43E-02
PF3D7_1107500	prefoldin, putative	1.43E-02
PF3D7_0110900	adenylate kinase-like protein 1	1.44E-02
PF3D7_0726700	conserved Plasmodium protein, unknown function	1.44E-02
PF3D7_0423000	conserved Plasmodium protein, unknown function	1.46E-02
PF3D7_0722700	conserved Plasmodium protein, unknown function	1.46E-02
PF3D7_0324800	rifin PIR protein	1.48E-02
PF3D7_1402100	conserved Plasmodium protein, unknown function	1.49E-02
PF3D7_1208000	apicoplast ribosomal protein L29 precursor, putative	1.49E-02
PF3D7_1229400	macrophage migration inhibitory factor	1.50E-02
PF3D7_1231600	pre-mRNA-splicing factor ATP-dependent RNA helicase PRP2, putative pre-mRNA-splicing factor ATP-dependent RNA helicase PRP2, putative	1.51E-02
PF3D7_0932900	conserved Plasmodium protein, unknown function	1.51E-02
PF3D7_0522500	50S ribosomal protein L17, apicoplast, putative	1.52E-02
PF3D7_1252800	Plasmodium exported protein (PHISTb), unknown function	1.52E-02
PF3D7_0412400	erythrocyte membrane protein 1, PfEMP1	1.52E-02
PF3D7_1028100	protoporphyrinogen oxidase	1.52E-02
PF3D7_1408100	plasmepsin III histo-aspartic protease	1.53E-02
PF3D7_1317900	small ribosomal subunit nuclear export protein, putative	1.54E-02
PF3D7_1235500	mRNA methyltransferase, putative	1.54E-02
PF3D7_1328600	conserved Plasmodium protein, unknown function	1.54E-02
PF3D7_0523700	conserved Plasmodium membrane protein, unknown function	1.55E-02
PF3D7_1132000	ubiquitin-like protein, putative	1.55E-02
PF3D7_1408500	conserved Plasmodium protein, unknown function	1.55E-02
PF3D7_1035300	glutamate-rich protein	1.58E-02
PF3D7_0312400	glycogen synthase kinase 3	1.59E-02
PF3D7_1014700	prohibitin, putative	1.60E-02

PF3D7_0604600	DNA helicase, putative	1.61E-02
PF3D7_0729200	1-cys peroxiredoxin	1.61E-02
PF3D7_0309800	YTH domain-containing protein, putative	1.61E-02
PF3D7_0422300	alpha tubulin 2	1.62E-02
PF3D7_1366100	conserved Plasmodium protein, unknown function	1.62E-02
PF3D7_0506000	conserved Plasmodium protein, unknown function	1.62E-02
PF3D7_0921800	nucleolar preribosomal assembly protein, putative	1.63E-02
PF3D7_0104200	StAR-related lipid transfer protein	1.65E-02
PF3D7_0315900	conserved Plasmodium protein, unknown function	1.66E-02
PF3D7_1314500	cop-coated vesicle membrane protein p24 precursor, putative	1.66E-02
PF3D7_0216200	conserved Plasmodium protein, unknown function	1.67E-02
PF3D7_0407600	conserved Plasmodium protein, unknown function	1.68E-02
PF3D7_0623900	ribonuclease H2 subunit A, putative	1.68E-02
PF3D7_1126300	DnaJ protein, putative	1.68E-02
PF3D7_0213700	conserved protein, unknown function	1.68E-02
PF3D7_0824500	conserved Plasmodium protein, unknown function	1.68E-02
PF3D7_1119500	clathrin coat assembly protein, putative	1.69E-02
PF3D7_1133500	conserved Plasmodium protein, unknown function	1.70E-02
PF3D7_1426600	conserved Plasmodium protein, unknown function	1.70E-02
PF3D7_1251200	coronin	1.71E-02
PF3D7_1451000	conserved Plasmodium protein, unknown function	1.72E-02
PF3D7_1358400	conserved Plasmodium protein, unknown function	1.73E-02
PF3D7_1362900	conserved Plasmodium protein, unknown function	1.73E-02
PF3D7_1448100	conserved Plasmodium protein, unknown function	1.73E-02
PF3D7_0702600	chitinase precursor fragment, truncated	1.74E-02
PF3D7_1404000	DNA-directed RNA polymerase II subunit RPB4, putative	1.74E-02
PF3D7_0824200	conserved Plasmodium protein, unknown function	1.75E-02
PF3D7_1106100	apicoplast ribosomal protein S15 precursor, putative	1.76E-02
PF3D7_1363200	bifunctional polynucleotide phosphatase/kinase	1.76E-02
PF3D7_1402000	conserved Plasmodium protein, unknown function	1.77E-02
PF3D7_1446800	heme detoxification protein	1.77E-02
PF3D7_0424500	serine/threonine protein kinase, FIKK family	1.78E-02
PF3D7_0402000	Plasmodium exported protein (PHISTa), unknown function	1.78E-02
PF3D7_1244600	ADP-ribosylation factor GTPase-activating protein, putative	1.78E-02
PF3D7_0418500	Bet3 transport protein, putative	1.79E-02
PF3D7_0403600	conserved Plasmodium protein, unknown function	1.80E-02
PF3D7_0618800	conserved Plasmodium protein, unknown function	1.80E-02
PF3D7_1311200	alternative splicing regulator, putative	1.81E-02
PF3D7_1414000	26S proteasome regulatory subunit RPN13, putative	1.82E-02
PF3D7_1008700	tubulin beta chain	1.82E-02
PF3D7_0910800	nucleotide binding protein, putative	1.85E-02
PF3D7_1009100	conserved Plasmodium membrane protein, unknown function	1.85E-02
PF3D7_0604700	glyoxalase I	1.85E-02
PF3D7_0313800	conserved Plasmodium protein, unknown function	1.86E-02
PF3D7_1135500	conserved Plasmodium protein, unknown function	1.89E-02
PF3D7_1341900	vacuolar ATP synthase subunit d, putative	1.89E-02
PF3D7_0906700	leucine-rich repeat protein	1.91E-02
PF3D7_0729000	signal recognition particle SRP9	1.94E-02
PF3D7_1038100	GDP dissociation inhibitor, putative	1.94E-02
PF3D7_0514600	ribose 5-phosphate epimerase, putative	1.95E-02
PF3D7_0927400	conserved Plasmodium protein, unknown function	1.96E-02
PF3D7_1358900	GTP-binding protein, putative	1.96E-02
PF3D7_1217000	conserved Plasmodium protein, unknown function	1.97E-02
PF3D7_1209000	conserved Plasmodium protein, unknown function	1.97E-02
PF3D7_1460400	ubiquitin carboxyl-terminal hydrolase isozyme L3	1.99E-02
PF3D7_1467000	conserved Plasmodium membrane protein, unknown function	1.99E-02
PF3D7_0104100	conserved Plasmodium membrane protein, unknown function	1.99E-02
PF3D7_1455300	conserved Plasmodium protein, unknown function	2.00E-02
PF3D7_0302600	ABC transporter, (TAP family), putative	2.00E-02
PF3D7_1124400	U6 snRNA-associated Sm-like protein LSM1, putative	2.01E-02
PF3D7_1131100	serpentine receptor, putative	2.02E-02

PF3D7_1361000	protein arginine N-methyltransferase 5, putative	2.03E-02
PF3D7_0727700	conserved Plasmodium protein, unknown function	2.04E-02
PF3D7_1364700	conserved Plasmodium protein, unknown function, pseudogene	2.04E-02
PF3D7_0919600	dolichyl-diphosphooligosaccharide--protein glycosyltransferase, putative	2.05E-02
PF3D7_0408000	conserved Plasmodium protein, unknown function	2.05E-02
PF3D7_0630300	DNA polymerase epsilon, catalytic subunit a, putative	2.05E-02
PF3D7_0925300	proline--tRNA ligase, putative	2.05E-02
PF3D7_0223400	rifin PIR protein	2.06E-02
PF3D7_1222900	conserved Plasmodium protein, unknown function	2.06E-02
PF3D7_1341000	tRNA Arginine	2.07E-02
PF3D7_1480000	rifin PIR protein	2.07E-02
PF3D7_1315300	conserved Plasmodium protein, unknown function	2.07E-02
PF3D7_0711000	AAA family ATPase, CDC48 subfamily	2.08E-02
PF3D7_0506300	conserved Plasmodium protein, unknown function	2.09E-02
PF3D7_0927800	cytochrome c oxidase subunit 5B, putative	2.09E-02
PF3D7_0909800	small nuclear ribonucleoprotein Sm D3, putative	2.14E-02
PF3D7_1419000	conserved Plasmodium protein, unknown function	2.14E-02
PF3D7_0401800	Plasmodium exported protein (PHISTb), unknown function	2.14E-02
PF3D7_0914800	GINS complex subunit Psf3, putative	2.15E-02
PF3D7_0719100	conserved Plasmodium protein, unknown function	2.15E-02
PF3D7_0630500	microtubule-associated protein ytm1 homologue, putative	2.15E-02
PF3D7_0928500	conserved Plasmodium protein, unknown function	2.16E-02
PF3D7_1127600	CRAL/TRIO domain-containing protein, putative	2.16E-02
PF3D7_1403000	conserved Plasmodium protein, unknown function	2.18E-02
PF3D7_1009400	zinc finger protein, putative	2.18E-02
PF3D7_1244000	glucose inhibited division protein a homologue, putative	2.19E-02
PF3D7_1314300	conserved Plasmodium protein, unknown function	2.20E-02
PF3D7_1351800	conserved Plasmodium protein, unknown function	2.22E-02
PF3D7_1444400	conserved Plasmodium protein, unknown function	2.22E-02
PF3D7_0528100	beta adaptin protein, putative	2.24E-02
PF3D7_0217900	conserved Plasmodium protein, unknown function	2.24E-02
PF3D7_0902300	serine/threonine protein kinase, FIKK family	2.25E-02
PF3D7_0520300	U6 snRNA-associated Sm-like protein LSM2, putative	2.27E-02
PF3D7_0219900	Plasmodium exported protein, unknown function	2.27E-02
PF3D7_1245800	clathrin coat assembly protein AP180, putative	2.27E-02
PF3D7_1439900	triosephosphate isomerase	2.28E-02
PF3D7_0526700	conserved Plasmodium protein, unknown function	2.30E-02
PF3D7_0408800	conserved Plasmodium protein, unknown function	2.30E-02
PF3D7_0319200	endonuclease/exonuclease/phosphatase family protein, putative	2.31E-02
PF3D7_1124300	conserved Plasmodium protein, unknown function	2.31E-02
PF3D7_1432200	conserved Plasmodium protein, unknown function	2.32E-02
PF3D7_1435100	ribonuclease H2 subunit B, putative	2.32E-02
PF3D7_0911900	falstatin	2.32E-02
PF3D7_1143900	carbohydrate kinase, putative	2.33E-02
PF3D7_1239300	conserved Plasmodium protein, unknown function	2.34E-02
PF3D7_1400200	rifin PIR protein	2.35E-02
PF3D7_1421400	DNA-directed RNA polymerase III subunit C, putative	2.36E-02
PF3D7_1317500	conserved Plasmodium protein, unknown function	2.36E-02
PF3D7_1325000	U6 snRNA-associated Sm-like protein LSM6, putative	2.37E-02
PF3D7_1472100	Yip1 protein, putative	2.37E-02
PF3D7_0833200	rifin PIR protein	2.38E-02
PF3D7_1330300	DnaJ protein, putative	2.39E-02
PF3D7_1021000	conserved Plasmodium protein, unknown function	2.39E-02
PF3D7_1130700	structural maintenance of chromosome protein, putative	2.41E-02
PF3D7_1208600	mitochondrial import inner membrane translocase subunit TIM10, putative	2.42E-02
PF3D7_1310500	conserved protein, unknown function	2.43E-02
PF3D7_0505900	conserved Plasmodium protein, unknown function	2.46E-02
PF3D7_0414200	calmodulin-like protein	2.46E-02
PF3D7_0304600	circumsporozoite (CS) protein	2.47E-02
PF3D7_0920600	conserved Plasmodium protein, unknown function	2.50E-02
PF3D7_1352900	conserved Plasmodium protein, unknown function	2.50E-02

PF3D7_1450600	conserved Plasmodium protein, unknown function	2.51E-02
PF3D7_0422800	serpentine receptor, putative	2.52E-02
PF3D7_0812600	ubiquitin conjugating enzyme, putative	2.52E-02
PF3D7_1333800	conserved Plasmodium protein, unknown function	2.57E-02
PF3D7_1205300	conserved Plasmodium protein, unknown function	2.57E-02
PF3D7_1246000	conserved Plasmodium protein, unknown function	2.57E-02
PF3D7_0600100	erythrocyte membrane protein 1 (PfEMP1), pseudogene	2.58E-02
PF3D7_1466800	conserved Plasmodium protein, unknown function	2.59E-02
PF3D7_1234600	conserved Plasmodium protein, unknown function	2.60E-02
PF3D7_1033700	bromodomain protein, putative	2.61E-02
PF3D7_1453100	dynactin subunit 4, putative	2.62E-02
PF3D7_0113800	DBL containing protein, unknown function	2.63E-02
PF3D7_0612700	6-cysteine protein	2.64E-02
PF3D7_0712800	erythrocyte membrane protein 1, PfEMP1	2.64E-02
PF3D7_0728800	conserved Plasmodium protein, unknown function	2.66E-02
PF3D7_0108400	mitochondrial carrier protein, putative	2.67E-02
PF3D7_1137200	conserved Plasmodium protein, unknown function	2.67E-02
PF3D7_0208200	RNA-binding protein, putative	2.68E-02
PF3D7_1226300	cof-like hydrolase, had-superfamily, subfamily iib	2.69E-02
PF3D7_1000400	rifin PIR protein	2.71E-02
PF3D7_0319800	conserved Plasmodium protein, unknown function	2.71E-02
PF3D7_0414700	GTP-binding protein, putative	2.74E-02
PF3D7_1428800	transcription initiation TFIID-like, putative	2.77E-02
PF3D7_0409500	conserved Plasmodium protein, unknown function	2.77E-02
PF3D7_1316200	ADP-ribosylation factor, putative	2.79E-02
PF3D7_0404400	6-cysteine protein	2.80E-02
PF3D7_0821000	conserved Plasmodium protein, unknown function	2.80E-02
PF3D7_1365000	conserved Plasmodium protein, unknown function	2.81E-02
PF3D7_1011700	DNA repair protein RAD23, putative	2.83E-02
PF3D7_1032400	phosphatidylinositol N-acetylglucosaminyltransferase subunit A, putative	2.83E-02
PF3D7_1470400	mitochondrial pyruvate carrier protein 2, putative	2.84E-02
PF3D7_0603500	cation/H+ antiporter	2.84E-02
PF3D7_0905900	beta subunit of coatomer complex, putative	2.86E-02
PF3D7_1104100	syntaxin, Qa-SNARE family	2.86E-02
PF3D7_0112100	conserved Plasmodium protein, unknown function	2.87E-02
PF3D7_0612500	conserved Plasmodium protein, unknown function	2.89E-02
PF3D7_1018200	serine/threonine protein phosphatase, putative	2.89E-02
PF3D7_0616200	Ndc80 homologue, putative	2.89E-02
PF3D7_0719500	conserved membrane protein, unknown function	2.90E-02
PF3D7_1347400	conserved Plasmodium protein, unknown function	2.90E-02
PF3D7_0205700	conserved Plasmodium protein, unknown function	2.90E-02
PF3D7_1141900	inner membrane complex protein 1b, putative	2.91E-02
PF3D7_0804800	peptidyl-prolyl cis-trans isomerase	2.93E-02
PF3D7_1111900	conserved Plasmodium protein, unknown function	2.93E-02
PF3D7_1248200	RNA-binding protein, putative	2.96E-02
PF3D7_1370100	tRNA Cysteine	2.98E-02
PF3D7_0619100	conserved Plasmodium protein, unknown function	3.01E-02
PF3D7_1204500	conserved Plasmodium protein, unknown function	3.01E-02
PF3D7_0112700	28S ribosomal RNA	3.02E-02
PF3D7_1022500	citrate synthase, mitochondrial precursor, putative	3.03E-02
PF3D7_1208700	conserved Plasmodium protein, unknown function	3.05E-02
PF3D7_1433600	microsomal signal peptidase protein, putative	3.05E-02
PF3D7_0421100	erythrocyte membrane protein 1, PfEMP1	3.08E-02
PF3D7_1129300	conserved Plasmodium protein, unknown function	3.11E-02
PF3D7_0725300	conserved Plasmodium protein, unknown function	3.14E-02
PF3D7_0308800	conserved protein, unknown function	3.14E-02
PF3D7_0418000	conserved Plasmodium protein, unknown function	3.15E-02
PF3D7_0813000	conserved Plasmodium protein, unknown function	3.17E-02
PF3D7_1466600	conserved Plasmodium protein, unknown function	3.17E-02
PF3D7_1350200	small nuclear ribonucleoprotein E, putative	3.17E-02
PF3D7_0623000	chorismate synthase	3.22E-02

PF3D7_0712400	erythrocyte membrane protein 1, PfEMP1	3.23E-02
PF3D7_1365100	mitochondrial ribosomal protein S17 precursor, putative	3.24E-02
PF3D7_0906910	tubulin-specific chaperone, putative	3.24E-02
PF3D7_0617700	rifin PIR protein	3.24E-02
PF3D7_1331100	DEAD box helicase, putative	3.26E-02
PF3D7_1112200	ubiquinone biosynthesis protein COQ4, putative	3.27E-02
PF3D7_0626600	conserved Plasmodium protein, unknown function	3.27E-02
PF3D7_0316500	conserved Plasmodium protein, unknown function	3.28E-02
PF3D7_0720800	Ham1-like protein, putative	3.28E-02
PF3D7_1373000	rifin PIR protein	3.30E-02
PF3D7_0810800	dihydropteroate synthetase	3.30E-02
PF3D7_0828100	conserved Plasmodium protein, unknown function	3.31E-02
PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1	3.31E-02
PF3D7_1448600	SNARE protein, putative	3.32E-02
PF3D7_0301900	conserved Plasmodium protein, unknown function	3.32E-02
PF3D7_1111200	conserved Plasmodium protein, unknown function	3.32E-02
PF3D7_1339100	tRNA Methionine	3.33E-02
PF3D7_0713500	conserved Plasmodium protein, unknown function	3.33E-02
PF3D7_0816600	chaperone protein ClpB1	3.35E-02
PF3D7_1359400	rRNA associated RNA binding protein, putative	3.35E-02
PF3D7_0404100	transcription factor with AP2 domain(s), putative	3.36E-02
PF3D7_0625400	conserved Plasmodium protein, unknown function	3.37E-02
PF3D7_0407200	peptidyl-tRNA hydrolase 2, putative	3.39E-02
PF3D7_0616000	pyridoxal kinase	3.39E-02
PF3D7_0528400	palmitoyltransferase	3.43E-02
PF3D7_1036600	conserved Plasmodium protein, unknown function	3.46E-02
PF3D7_1300200	rifin PIR protein	3.47E-02
PF3D7_0517100	conserved Plasmodium protein, unknown function	3.48E-02
PF3D7_1002500	conserved Plasmodium protein, unknown function	3.48E-02
PF3D7_1401900	conserved Plasmodium protein, unknown function	3.48E-02
PF3D7_1443400	conserved Plasmodium protein, unknown function	3.49E-02
PF3D7_1330600	elongation factor Tu, putative	3.49E-02
PF3D7_0909600	conserved Plasmodium protein, unknown function	3.50E-02
PF3D7_0206700	adenylosuccinate lyase	3.51E-02
PF3D7_0810700	conserved Plasmodium protein, unknown function	3.55E-02
PF3D7_1336900	tryptophan--tRNA ligase	3.56E-02
PF3D7_0408900	peptidase, M22 family, putative	3.57E-02
PF3D7_1335600	conserved Plasmodium protein, unknown function	3.58E-02
PF3D7_1001000	Plasmodium exported protein (hyp12), unknown function	3.58E-02
PF3D7_0320700	signal peptidase complex subunit 2, putative	3.59E-02
PF3D7_0900100	erythrocyte membrane protein 1, PfEMP1	3.61E-02
PF3D7_1121700	protein GCN20	3.62E-02
PF3D7_0513100	conserved Plasmodium protein, unknown function	3.63E-02
PF3D7_0718400	mitochondrial ribosomal protein S8 precursor, putative	3.64E-02
PF3D7_0409200	40S ribosomal processing protein, putative	3.64E-02
PF3D7_1441500	conserved Plasmodium protein, unknown function	3.65E-02
PF3D7_1136500	casein kinase 1	3.66E-02
PF3D7_0513500	mitochondrial import inner membrane translocase subunit TIM16, putative	3.66E-02
PF3D7_1135400	conserved Plasmodium protein, unknown function	3.67E-02
PF3D7_1248600	conserved Plasmodium protein, unknown function	3.67E-02
PF3D7_1404100	cytochrome c, putative	3.67E-02
PF3D7_1124500	pyruvate dehydrogenase E1 alpha subunit	3.68E-02
PF3D7_0322900	40S ribosomal protein S3A, putative	3.71E-02
PF3D7_1216600	cell traversal protein for ookinetes and sporozoites	3.71E-02
PF3D7_1141400	phosphatidylinositol N-acetylglucosaminyltransferase subunit H, putative	3.71E-02
PF3D7_0615800	conserved Plasmodium protein, unknown function	3.71E-02
PF3D7_1339000	probable protein, unknown function	3.71E-02
PF3D7_1031000	25 kDa ookinete surface antigen precursor	3.75E-02
PF3D7_1346100	protein transport protein SEC61 subunit alpha	3.76E-02
PF3D7_1332700	DEAD/DEAH box ATP-dependent RNA helicase, putative	3.76E-02
PF3D7_0522300	S-adenosylmethionine-dependent methyltransferase, putative	3.77E-02

PF3D7_1347300	conserved Plasmodium membrane protein, unknown function	3.78E-02
PF3D7_0627500	4-methyl-5(B-hydroxyethyl)-thiazol monophosphate biosynthesis enzyme	3.80E-02
PF3D7_1015100	conserved protein, unknown function	3.81E-02
PF3D7_1136000	conserved Plasmodium protein, unknown function	3.82E-02
PF3D7_1338500	conserved Plasmodium protein, unknown function	3.82E-02
PF3D7_1024300	conserved Plasmodium protein, unknown function	3.84E-02
PF3D7_1465600	conserved Plasmodium protein, unknown function	3.88E-02
PF3D7_1356200	mitochondrial import inner membrane translocase subunit TIM23, putative	3.90E-02
PF3D7_0716500	conserved Plasmodium protein, unknown function	3.90E-02
PF3D7_1463800	mitochondrial ribosomal protein S6-2 precursor, putative	3.91E-02
PF3D7_0105500	conserved Plasmodium protein, unknown function	3.93E-02
PF3D7_1402900	conserved Plasmodium protein, unknown function	3.94E-02
PF3D7_0613700	syntaxin binding protein, putative	3.95E-02
PF3D7_1416400	conserved Plasmodium protein, unknown function	3.99E-02
PF3D7_1408300	conserved Plasmodium protein, unknown function	3.99E-02
PF3D7_0820400	conserved Plasmodium protein, unknown function	4.00E-02
PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1	4.00E-02
PF3D7_1469400	nucleoside transporter 3, putative	4.06E-02
PF3D7_0901900	probable protein, unknown function	4.07E-02
PF3D7_0627800	acetyl-CoA synthetase, putative	4.08E-02
PF3D7_0406000	conserved Plasmodium protein, unknown function	4.08E-02
PF3D7_1210300	conserved Plasmodium protein, unknown function	4.09E-02
PF3D7_0811900	RNA-binding protein, putative	4.10E-02
PF3D7_1126900	small nuclear ribonucleoprotein F, putative	4.15E-02
PF3D7_0214500	conserved Plasmodium protein, unknown function	4.18E-02
PF3D7_1346500	conserved Plasmodium protein, unknown function	4.19E-02
PF3D7_0624400	conserved Plasmodium protein, unknown function	4.24E-02
PF3D7_0105900	DNA binding protein, putative	4.24E-02
PF3D7_1308100	CCAAT-binding transcription factor, putative	4.25E-02
PF3D7_0106100	vacuolar ATP synthase subunit c, putative	4.26E-02
PF3D7_1342300	tetratricopeptide repeat family protein, putative	4.27E-02
PF3D7_1330100	conserved Plasmodium protein, unknown function	4.28E-02
PF3D7_0731600	acyl-CoA synthetase	4.29E-02
PF3D7_1442600	TRAP-like protein sporozoite-specific transmembrane protein S6	4.30E-02
PF3D7_1252900	Plasmodium exported protein, unknown function	4.31E-02
PF3D7_0710800	conserved Plasmodium protein, unknown function	4.33E-02
PF3D7_0525400	G-protein coupled receptor, putative	4.34E-02
PF3D7_1354600	60S ribosomal protein L7-2, putative	4.34E-02
PF3D7_1234900	conserved Plasmodium protein, unknown function	4.35E-02
PF3D7_1147900	conserved Plasmodium protein, unknown function	4.38E-02
PF3D7_0304700	conserved Plasmodium protein, unknown function	4.38E-02
PF3D7_1303300	conserved Plasmodium protein, unknown function	4.39E-02
PF3D7_1037300	ADP/ATP transporter on adenylate translocase	4.39E-02
PF3D7_0106000	conserved Plasmodium protein, unknown function	4.41E-02
PF3D7_0515200	conserved Plasmodium protein, unknown function	4.42E-02
PF3D7_0713300	erythrocyte membrane protein 1 (PfEMP1), pseudogene	4.43E-02
PF3D7_0805500	conserved Plasmodium protein, unknown function	4.44E-02
PF3D7_0525800	membrane skeletal protein IMC1-related	4.44E-02
PF3D7_1456200	SNARE protein, putative	4.44E-02
PF3D7_0931500	conserved Plasmodium protein, unknown function	4.47E-02
PF3D7_1013200	conserved Plasmodium protein, unknown function	4.52E-02
PF3D7_1226900	conserved Plasmodium protein, unknown function	4.57E-02
PF3D7_1004900	conserved Plasmodium protein, unknown function	4.60E-02
PF3D7_1334900	MSP7-like protein, fragment, pseudogene	4.61E-02
PF3D7_1034900	methionine--tRNA ligase, putative	4.64E-02
PF3D7_1225100	isoleucine--tRNA ligase, putative	4.66E-02
PF3D7_0936000	ring-exported protein 2	4.67E-02
PF3D7_1246500	conserved Plasmodium protein, unknown function	4.69E-02
PF3D7_1034800	conserved Plasmodium protein, unknown function	4.69E-02
PF3D7_1322200	conserved Plasmodium protein, unknown function	4.70E-02
PF3D7_1350000	conserved Plasmodium protein, unknown function	4.71E-02

PF3D7_0807400	conserved Plasmodium protein, unknown function	4.72E-02
PF3D7_1445500	conserved Plasmodium protein, unknown function	4.74E-02
PF3D7_1315400	zinc finger (CCCH type) protein, putative	4.74E-02
PF3D7_0417300	LETM1-like protein, putative	4.74E-02
PF3D7_0217100	ATP synthase F1, alpha subunit	4.77E-02
PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1	4.78E-02
PF3D7_1116200	pyridoxine biosynthesis protein PDX2	4.79E-02
PF3D7_1433300	chromatin assembly factor 1 P55 subunit, putative	4.79E-02
PF3D7_1466700	large ribosomal subunit assembling protein, putative	4.80E-02
PF3D7_0511100	conserved Plasmodium protein, unknown function	4.81E-02
PF3D7_0813800	GDP-mannose 4,6-dehydratase	4.83E-02
PF3D7_0931200	selenoprotein	4.85E-02
PF3D7_0201700	DnaJ protein, putative	4.85E-02
PF3D7_0321700	microtubule and actin binding protein, putative	4.85E-02
PF3D7_1111400	conserved Plasmodium protein, unknown function	4.86E-02
PF3D7_0703600	conserved Plasmodium protein, unknown function	4.87E-02
PF3D7_0702900	centrin, putative	4.87E-02
PF3D7_1444300	apicoplast 1-acyl-sn-glycerol-3-phosphate acyltransferase, putative	4.90E-02
PF3D7_1317700	conserved Plasmodium protein, unknown function	4.90E-02
PF3D7_0712300	erythrocyte membrane protein 1, PfEMP1	4.92E-02
PF3D7_0629000	conserved protein, unknown function	4.93E-02
PF3D7_1348500	TBC domain protein, putative	4.93E-02
PF3D7_1124800	nuclear preribosomal assembly protein, putative	4.95E-02
PF3D7_0102400	lysophospholipase, putative, pseudogene	4.95E-02
PF3D7_0819900	U6 snRNA-associated Sm-like protein LSm3, putative	4.98E-02
PF3D7_1412600	deoxyhypusine synthase	4.99E-02

Appendix H: List of significantly up- and downregulated genes between 6A-R and 6A under ART pressure (900nM ART at 10-14hpi) ($p < 0.05$). Differential expression was measured using paired student's T-test.

UPREGULATED IN 6A-R vs 6A UNDER ART PRESSURE		
GENE ID	Gene Description	<i>p value</i>
PF3D7_0630100	conserved Plasmodium protein, unknown function	7.57E-05
PF3D7_1429400	rRNA (adenosine-2'-O-)-methyltransferase, putative	1.03E-04
PF3D7_1373400	rifin PIR protein	1.95E-04
PF3D7_1309600	ribosomal RNA methyltransferase, putative	4.87E-04
PF3D7_0921700	conserved Plasmodium protein, unknown function	7.12E-04
PF3D7_1460600	inner membrane complex protein, putative	7.95E-04
PF3D7_0926100	protein kinase, putative	8.21E-04
PF3D7_1242100	conserved Plasmodium protein, unknown function	1.04E-03
PF3D7_0717000	conserved Plasmodium membrane protein, unknown function	1.04E-03
PF3D7_1128500	conserved protein, unknown function	1.16E-03
PF3D7_1361000	protein arginine N-methyltransferase 5, putative	1.25E-03
PF3D7_0824600	anamorsin related protein, putative	1.40E-03
PF3D7_1012100	conserved Plasmodium protein, unknown function	1.49E-03
PF3D7_0625700	conserved Plasmodium protein, unknown function	1.59E-03
PF3D7_0928000	cytochrome c oxidase subunit 6B, putative	1.60E-03
PF3D7_0509600	asparagine--tRNA ligase, putative	1.76E-03
PF3D7_0929400	high molecular weight rhoptry protein 2	1.81E-03
PF3D7_1465600	conserved Plasmodium protein, unknown function	1.85E-03
PF3D7_0106200	conserved Plasmodium protein, unknown function	1.99E-03
PF3D7_0520600	bis(5'-nucleosyl)-tetraphosphatase [asymmetrical] Ap4A hydrolase	2.24E-03
PF3D7_0408900	peptidase, M22 family, putative	2.28E-03
PF3D7_0713100	Pfmc-2TM Maurer's cleft two transmembrane protein	2.47E-03
PF3D7_0514800	kinase, putative	2.79E-03
PF3D7_1442300	tRNA binding protein, putative	2.80E-03
PF3D7_0721400	conserved Plasmodium protein, unknown function	3.03E-03
PF3D7_0409900	actin-like protein, putative	3.09E-03
PF3D7_1313700	septum formation protein MAF homologue, putative	3.38E-03
PF3D7_0605500	cyclin dependent kinase binding protein, putative	3.59E-03
PF3D7_1035000	U2 snRNA/tRNA pseudouridine synthase, putative	3.90E-03
PF3D7_1368100	proteasome regulatory subunit, putative	3.93E-03
PF3D7_1232500	CG2-related protein, putative	4.05E-03
PF3D7_0723700	metallo-hydrolase/oxidoreductase, putative	4.09E-03
PF3D7_0512000	prefoldin subunit, putative	4.24E-03
PF3D7_0219800	Plasmodium exported protein (PHISTc), unknown function	4.28E-03
PF3D7_0624300	conserved Plasmodium protein, unknown function	4.43E-03
PF3D7_1102800	early transcribed membrane protein 11.2	4.90E-03
PF3D7_1475000	mediator complex subunit 31, putative	5.03E-03
PF3D7_1247500	serine/threonine protein kinase, putative	6.36E-03
PF3D7_1321400	palmitoyltransferase, putative	6.55E-03
PF3D7_1414300	60S ribosomal protein L10, putative	7.27E-03
PF3D7_1450000	serine/threonine protein kinase, putative	7.30E-03
PF3D7_1331000	protein kinase, putative	7.46E-03
PF3D7_1338600	conserved Plasmodium protein, unknown function	7.50E-03
PF3D7_0217800	40S ribosomal protein S26e, putative	7.55E-03
PF3D7_1435200	conserved Plasmodium protein, unknown function	7.62E-03
PF3D7_0824500	conserved Plasmodium protein, unknown function	7.98E-03
PF3D7_1012400	hypoxanthine-guanine phosphoribosyltransferase	8.02E-03
PF3D7_1006900	PPPDE peptidase, putative	8.11E-03
PF3D7_0718400	mitochondrial ribosomal protein S8 precursor, putative	8.26E-03
PF3D7_1225900	conserved Plasmodium protein, unknown function	8.44E-03
PF3D7_1013400	conserved Plasmodium protein, unknown function	8.56E-03
PF3D7_1148900	probable protein, unknown function	8.69E-03

PF3D7_1463200	replication factor C3	8.69E-03
PF3D7_1030300	conserved Plasmodium protein, unknown function	8.90E-03
PF3D7_0105100	conserved Plasmodium protein, unknown function	9.56E-03
PF3D7_0525400	G-protein coupled receptor, putative	9.59E-03
PF3D7_1444200	calmodulin-like protein	9.66E-03
PF3D7_1033400	haloacid dehalogenase-like hydrolase, putative	9.77E-03
PF3D7_1352400	conserved Plasmodium protein, unknown function	9.79E-03
PF3D7_0611400	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin	1.01E-02
PF3D7_0900600	rifin PIR protein	1.01E-02
PF3D7_0709000	chloroquine resistance transporter	1.04E-02
PF3D7_1311100	conserved Plasmodium protein, unknown function	1.09E-02
PF3D7_0320800	ATP-dependent RNA helicase DDX6	1.11E-02
PF3D7_1010100	conserved Plasmodium protein, unknown function	1.13E-02
PF3D7_1038000	antigen UB05	1.13E-02
PF3D7_1142000	conserved Plasmodium protein, unknown function	1.22E-02
PF3D7_0922700	pre-mRNA splicing factor, putative	1.23E-02
PF3D7_1009900	conserved Plasmodium protein, unknown function	1.24E-02
PF3D7_0304900	conserved Plasmodium protein, unknown function	1.25E-02
PF3D7_0523200	conserved Plasmodium protein, unknown function	1.29E-02
PF3D7_1122700	conserved Plasmodium protein, unknown function	1.33E-02
PF3D7_0210000	secretory complex protein 61 gamma subunit	1.35E-02
PF3D7_0531200	mitochondrial ribosomal protein S16 precursor, putative	1.36E-02
PF3D7_1369800	tRNA Arginine	1.38E-02
PF3D7_1135400	conserved Plasmodium protein, unknown function	1.38E-02
PF3D7_0314700	zinc finger protein, putative	1.40E-02
PF3D7_1248900	26S protease regulatory subunit 8, putative	1.44E-02
PF3D7_0823900	dicarboxylate/tricarboxylate carrier	1.45E-02
PF3D7_1454600	mitochondrial ribosomal protein S11 precursor, putative	1.46E-02
PF3D7_0921300	conserved Plasmodium protein, unknown function	1.52E-02
PF3D7_1324300	conserved Plasmodium membrane protein, unknown function	1.54E-02
PF3D7_1103300	tRNA Leucine	1.67E-02
PF3D7_1020700	ribosomal processing ATPase, putative	1.67E-02
PF3D7_0724500	conserved Plasmodium protein, unknown function	1.75E-02
PF3D7_1215800	conserved Plasmodium protein, unknown function	1.76E-02
PF3D7_0801400	probable protein, unknown function	1.77E-02
PF3D7_0501600	rho-try-associated protein 2	1.80E-02
PF3D7_0304200	EH (Eps15 homology) protein	1.85E-02
PF3D7_0903100	retrieval receptor for endoplasmic reticulum membrane proteins, putative	1.85E-02
PF3D7_1012500	phosphoglucomutase, putative	1.85E-02
PF3D7_1355900	conserved protein, unknown function	1.85E-02
PF3D7_1232100	60 kDa chaperonin	1.88E-02
PF3D7_0814400	phospholipase DDHD1, putative	1.90E-02
PF3D7_0629600	conserved Plasmodium protein, unknown function	1.91E-02
PF3D7_1226600	proliferating cell nuclear antigen 2	1.94E-02
PF3D7_1238100	calcyclin binding protein, putative	1.99E-02
PF3D7_1032300	conserved Plasmodium protein, unknown function	2.04E-02
PF3D7_1439900	triosephosphate isomerase	2.08E-02
PF3D7_1348800	E1-E2 ATPase, putative	2.13E-02
PF3D7_1231200	conserved Plasmodium protein, unknown function	2.14E-02
PF3D7_1022000	RNA-binding protein, putative	2.17E-02
PF3D7_1031700	protein phosphatase inhibitor 3	2.31E-02
PF3D7_1348300	elongation factor Tu, putative	2.36E-02
PF3D7_0903200	ras-related protein RAB7	2.38E-02
PF3D7_1136400	conserved Plasmodium protein, unknown function	2.39E-02
PF3D7_1106300	exonuclease, putative	2.40E-02
PF3D7_1354300	large subunit rRNA methyltransferase, putative	2.46E-02
PF3D7_1321900	conserved Plasmodium protein, unknown function	2.47E-02
PF3D7_1466300	26S proteasome regulatory subunit, putative	2.61E-02
PF3D7_1024300	conserved Plasmodium protein, unknown function	2.61E-02
PF3D7_1346800	6-cysteine protein	2.62E-02
PF3D7_1416300	conserved protein, unknown function	2.65E-02

PF3D7_0819300	conserved Plasmodium protein, unknown function	2.65E-02
PF3D7_1364700	conserved Plasmodium protein, unknown function, pseudogene	2.67E-02
PF3D7_1205500	zinc finger protein, putative	2.67E-02
PF3D7_0105200	conserved Plasmodium protein, unknown function	2.69E-02
PF3D7_0814100	conserved Plasmodium protein, unknown function	2.78E-02
PF3D7_1352500	thioredoxin-related protein, putative	2.78E-02
PF3D7_1421500	mitochondrial import inner membrane translocase subunit TIM8, putative	2.81E-02
PF3D7_0716500	conserved Plasmodium protein, unknown function	2.83E-02
PF3D7_0828500	translation initiation factor EIF-2b alpha subunit, putative	2.92E-02
PF3D7_0208700	conserved Plasmodium protein, unknown function	2.93E-02
PF3D7_1447300	mitochondrial ribosomal protein S14 precursor, putative	2.93E-02
PF3D7_1358700	HVA22-like protein, putative	2.94E-02
PF3D7_1351800	conserved Plasmodium protein, unknown function	2.95E-02
PF3D7_0203300	ERCC1 nucleotide excision repair protein, putative	2.97E-02
PF3D7_0934300	conserved Plasmodium protein, unknown function	3.00E-02
PF3D7_0514200	conserved Plasmodium protein, unknown function	3.02E-02
PF3D7_0802200	1-cys peroxiredoxin	3.02E-02
PF3D7_1241500	conserved Plasmodium protein, unknown function	3.06E-02
PF3D7_1311000	protein ISD11, putative	3.11E-02
PF3D7_1430500	conserved Plasmodium protein, unknown function	3.12E-02
PF3D7_0309000	dual specificity protein phosphatase	3.15E-02
PF3D7_1027800	60S ribosomal protein L3, putative	3.17E-02
PF3D7_0833200	rifin PIR protein	3.20E-02
PF3D7_1115200	SET domain protein, putative	3.21E-02
PF3D7_0205500	DNA-directed RNA polymerase II 16 kDa subunit, putative	3.22E-02
PF3D7_1412700	AAA family ATPase, putative	3.25E-02
PF3D7_1316100	inositol polyphosphate kinase, putative	3.26E-02
PF3D7_0715000	4-nitrophenylphosphatase	3.31E-02
PF3D7_0816300	conserved Plasmodium protein, unknown function	3.32E-02
PF3D7_1113500	GTP-binding protein, putative	3.33E-02
PF3D7_1411400	plastid replication-repair enzyme	3.36E-02
PF3D7_0205700	conserved Plasmodium protein, unknown function	3.36E-02
PF3D7_0802500	inositol phosphatase, putative	3.37E-02
PF3D7_0928200	conserved Plasmodium protein, unknown function	3.41E-02
PF3D7_1423200	peptidyl-prolyl cis-trans isomerase	3.42E-02
PF3D7_1128200	multiprotein bridging factor type 1, putative	3.42E-02
PF3D7_1408100	plasmepsin III histo-aspartic protease	3.44E-02
PF3D7_0707700	E3 ubiquitin-protein ligase, putative	3.44E-02
PF3D7_0517500	UTP--glucose-1-phosphate uridylyltransferase, putative	3.44E-02
PF3D7_0625400	conserved Plasmodium protein, unknown function	3.45E-02
PF3D7_0910500	DNA repair protein REV1, putative	3.46E-02
PF3D7_1100100	erythrocyte membrane protein 1, PfEMP1	3.46E-02
PF3D7_1365100	mitochondrial ribosomal protein S17 precursor, putative	3.47E-02
PF3D7_0302700	CDGSH iron-sulfur domain-containing protein, putative	3.48E-02
PF3D7_0906000	RNB-like protein, putative	3.51E-02
PF3D7_0307400	ATP-dependent Clp protease proteolytic subunit	3.51E-02
PF3D7_0423900	probable protein, unknown function	3.52E-02
PF3D7_1014400	MO15-related protein kinase	3.56E-02
PF3D7_1344700	conserved Plasmodium protein, unknown function	3.56E-02
PF3D7_1430000	conserved Plasmodium protein, unknown function	3.60E-02
PF3D7_1473200	DnaJ protein, putative	3.63E-02
PF3D7_1032000	mitochondrial preribosomal assembly protein rimM precursor, putative	3.63E-02
PF3D7_0913600	conserved Plasmodium protein, unknown function	3.65E-02
PF3D7_1224400	conserved Plasmodium protein, unknown function	3.70E-02
PF3D7_1110100	conserved Plasmodium protein, unknown function	3.70E-02
PF3D7_1419400	conserved Plasmodium membrane protein, unknown function	3.71E-02
PF3D7_1014700	prohibitin, putative	3.74E-02
PF3D7_0519200	V-type proton ATPase 16 kDa proteolipid subunit	3.75E-02
PF3D7_1303000	conserved Plasmodium protein, unknown function	3.78E-02
PF3D7_0407000	conserved Plasmodium protein, unknown function	3.83E-02
PF3D7_0109400	tubulin-specific chaperone a, putative	3.85E-02

PF3D7_0608310	sorting assembly machinery 50 kDa subunit, putative	3.87E-02
PF3D7_1320600	ras-related protein Rab-11A	3.94E-02
PF3D7_1142500	60S ribosomal protein L28, putative	3.96E-02
PF3D7_0210900	conserved Plasmodium protein, unknown function	3.99E-02
PF3D7_1037100	pyruvate kinase 2	3.99E-02
PF3D7_1410100	alpha/beta hydrolase, putative	4.03E-02
PF3D7_1440100	conserved Plasmodium protein, unknown function	4.03E-02
PF3D7_0800900	Plasmodium exported protein (hyp7), unknown function, pseudogene	4.05E-02
PF3D7_0402100	Plasmodium exported protein (PHISTb), unknown function	4.08E-02
PF3D7_1331200	conserved Plasmodium protein, unknown function	4.09E-02
PF3D7_0515400	conserved Plasmodium protein, unknown function	4.12E-02
PF3D7_1131500	conserved Plasmodium protein, unknown function	4.13E-02
PF3D7_0827700	conserved Plasmodium membrane protein, unknown function	4.16E-02
PF3D7_0926400	monocarboxylate transporter, putative	4.16E-02
PF3D7_0503600	myosin B	4.17E-02
PF3D7_0729700	conserved Plasmodium protein, unknown function	4.21E-02
PF3D7_1102400	flavoprotein, putative	4.22E-02
PF3D7_0716900	drug metabolite transporter, putative	4.30E-02
PF3D7_1466900	rhopty protein, putative	4.30E-02
PF3D7_0711200	conserved Plasmodium protein, unknown function	4.33E-02
PF3D7_1120900	heat shock factor binding protein 1, putative	4.38E-02
PF3D7_1121600	circumsporozoite-related antigen exported protein 1	4.40E-02
PF3D7_1348900	conserved Plasmodium protein, unknown function	4.41E-02
PF3D7_1132900	glycine cleavage H protein	4.43E-02
PF3D7_0907700	subunit of proteasome activator complex, putative	4.43E-02
PF3D7_1232900	nucleotidyltransferase, putative	4.47E-02
PF3D7_1248200	RNA-binding protein, putative	4.55E-02
PF3D7_0627000	conserved Plasmodium protein, unknown function	4.56E-02
PF3D7_0911800	conserved Plasmodium protein, unknown function	4.57E-02
PF3D7_0214500	conserved Plasmodium protein, unknown function	4.63E-02
PF3D7_0820900	conserved Plasmodium protein, unknown function	4.65E-02
PF3D7_0702100	Plasmodium exported protein (PHISTb), unknown function, pseudogene	4.65E-02
PF3D7_0526400	conserved Plasmodium protein, unknown function	4.68E-02
PF3D7_1451100	elongation factor 2	4.69E-02
PF3D7_1419200	conserved Plasmodium protein, unknown function	4.76E-02
PF3D7_0921900	conserved Plasmodium protein, unknown function	4.77E-02
PF3D7_0114300	exported protein family 4, pseudogene Plasmodium exported protein, unknown function, pseudogene	4.77E-02
PF3D7_1124200	conserved Plasmodium protein, unknown function	4.77E-02
PF3D7_1428100	WW domain-binding protein 11, putative	4.79E-02
PF3D7_1445100	histidine--tRNA ligase, putative	4.80E-02
PF3D7_1036800	acetyl-CoA transporter, putative	4.83E-02
PF3D7_0923500	cyclin-dependent kinases regulatory subunit, putative	4.83E-02
PF3D7_1436800	ATP-dependent Clp protease proteolytic subunit	4.84E-02
PF3D7_1408500	conserved Plasmodium protein, unknown function	4.85E-02
PF3D7_0319100	ubiquitin-protein ligase, putative	4.90E-02
PF3D7_1446300	conserved Plasmodium membrane protein, unknown function	4.92E-02
PF3D7_1476900	Plasmodium exported protein, unknown function, pseudogene	4.92E-02
PF3D7_0315300	conserved Plasmodium protein, unknown function	4.93E-02
PF3D7_1431900	conserved Plasmodium membrane protein, unknown function	4.98E-02
PF3D7_0819800	conserved Plasmodium protein, unknown function	4.98E-02
PF3D7_0911900	falstatin	4.99E-02
DOWNREGULATED IN 6A-R vs 6A UNDER ART PRESSURE		
GENE ID	Gene Description	p value
PF3D7_1303700	conserved Plasmodium protein, unknown function	7.63E-05
PF3D7_1225000	conserved Plasmodium protein, unknown function	5.83E-04
PF3D7_1035500	merozoite surface protein 6	5.86E-04
PF3D7_1146800	conserved Plasmodium protein, unknown function	9.94E-04
PF3D7_0902700	Plasmodium exported protein (PHISTb), unknown function, pseudogene	1.49E-03

PF3D7_1342600	myosin A	1.57E-03
PF3D7_0513700	secreted ookinete protein, putative	2.13E-03
PF3D7_0214300	conserved Plasmodium protein, unknown function	2.16E-03
PF3D7_1473500	conserved Plasmodium protein, unknown function	2.28E-03
PF3D7_0911100	conserved Plasmodium protein, unknown function	2.34E-03
PF3D7_1329000	DNA-directed RNA polymerase 3 largest subunit	2.37E-03
PF3D7_1354800	metacaspase 1	2.67E-03
PF3D7_0507300	conserved Plasmodium protein, unknown function	2.72E-03
PF3D7_0424900	Plasmodium exported protein (PHISTa), unknown function	2.75E-03
PF3D7_1202300	dynein heavy chain, putative	2.99E-03
PF3D7_0807800	proteasome subunit alpha type 5, putative	4.44E-03
PF3D7_1345900	conserved Plasmodium protein, unknown function	4.90E-03
PF3D7_1450800	conserved Plasmodium protein, unknown function	4.90E-03
PF3D7_1412000	p1/s1 nuclease, putative	5.10E-03
PF3D7_0832600	stevor PIR protein	5.69E-03
PF3D7_0623000	chorismate synthase	5.99E-03
PF3D7_0916000	sugar transporter, putative	6.22E-03
PF3D7_1415400	conserved Plasmodium protein, unknown function	6.23E-03
PF3D7_1000800	stevor, pseudogene PIR protein, pseudogene	6.54E-03
PF3D7_1346000	dynactin subunit 2, putative	6.84E-03
PF3D7_0922600	glutamine synthetase, putative	8.14E-03
PF3D7_1464000	YL1 nuclear protein, putative	8.33E-03
PF3D7_0308200	TCP-1/cpn60 chaperonin family, putative	8.60E-03
PF3D7_1459600	conserved Plasmodium protein, unknown function	9.04E-03
PF3D7_1301300	Plasmodium exported protein (PHISTa-like), unknown function, pseudogene	9.12E-03
PF3D7_0418800	conserved Plasmodium protein, unknown function	9.17E-03
PF3D7_1105700	conserved protein, unknown function	9.51E-03
PF3D7_1104100	syntaxin, Qa-SNARE family	9.65E-03
PF3D7_1147700	mitochondrial ATP synthase delta subunit, putative	1.18E-02
PF3D7_1320800	dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, putative	1.20E-02
PF3D7_1471600	conserved Plasmodium protein, unknown function	1.24E-02
PF3D7_0614000	thiamin-phosphate pyrophosphorylase, putative	1.25E-02
PF3D7_1201400	Plasmodium exported protein, unknown function	1.26E-02
PF3D7_0526900	transmembrane emp24 domain-containing protein, putative	1.27E-02
PF3D7_1335000	MSP7-like protein	1.28E-02
PF3D7_1366900	conserved Plasmodium protein, unknown function	1.33E-02
PF3D7_0913100	conserved Plasmodium protein, unknown function	1.34E-02
PF3D7_0620600	conserved Plasmodium protein, unknown function	1.35E-02
PF3D7_1301200	glycophorin binding protein	1.35E-02
PF3D7_0930300	merozoite surface protein 1	1.42E-02
PF3D7_1347000	G-beta repeat protein, putative	1.47E-02
PF3D7_0422700	eukaryotic initiation factor, putative	1.47E-02
PF3D7_1407500	tRNA m2G10 methyltransferase subunit, putative	1.50E-02
PF3D7_1218100	conserved Plasmodium protein, unknown function	1.56E-02
PF3D7_0811600	conserved Plasmodium protein, unknown function	1.65E-02
PF3D7_1310400	conserved Plasmodium protein, unknown function	1.69E-02
PF3D7_1037500	dynammin-like protein	1.73E-02
PF3D7_0806000	AAA family ATPase, putative	1.75E-02
PF3D7_0525000	zinc finger protein, putative	1.78E-02
PF3D7_0718700	conserved Plasmodium membrane protein, unknown function	1.86E-02
PF3D7_1118100	clathrin assembly protein AP19, putative	1.93E-02
PF3D7_1218500	conserved Plasmodium protein, unknown function	1.95E-02
PF3D7_0712900	erythrocyte membrane protein 1, PfEMP1	1.97E-02
PF3D7_1240900	erythrocyte membrane protein 1, PfEMP1	2.00E-02
PF3D7_1121100	conserved Plasmodium protein, unknown function	2.02E-02
PF3D7_1412800	N-myristoyltransferase	2.04E-02
PF3D7_1327400	conserved Plasmodium protein, unknown function	2.07E-02
PF3D7_0826300	SPRY domain, putative	2.09E-02
PF3D7_1117300	conserved Plasmodium protein, unknown function	2.10E-02
PF3D7_0106300	calcium-transporting ATPase	2.17E-02

PF3D7_1150400	erythrocyte membrane protein 1, PfEMP1	2.21E-02
PF3D7_0322300	diacylglycerol O-acyltransferase	2.24E-02
PF3D7_0832000	stevor PIR protein	2.30E-02
PF3D7_1472800	conserved Plasmodium protein, unknown function	2.33E-02
PF3D7_1470500	phosphodiesterase delta, putative	2.34E-02
PF3D7_1418100	liver specific protein 1, putative	2.35E-02
PF3D7_1123900	splicing factor, putative	2.36E-02
PF3D7_1025700	conserved Plasmodium protein, unknown function	2.42E-02
PF3D7_0403800	alpha/beta hydrolase, putative	2.43E-02
PF3D7_1338400	conserved Plasmodium protein, unknown function	2.50E-02
PF3D7_0923700	conserved protein, unknown function	2.62E-02
PF3D7_1127800	conserved Plasmodium protein, unknown function	2.68E-02
PF3D7_1445800	conserved Plasmodium membrane protein, unknown function	2.69E-02
PF3D7_1451200	conserved Plasmodium protein, unknown function	2.71E-02
PF3D7_0522900	zinc finger protein, putative	2.72E-02
PF3D7_1419300	glutathione S-transferase	2.74E-02
PF3D7_1236000	vesicle transport v-SNARE protein VT11, putative	2.75E-02
PF3D7_0613300	rhopty protein	2.81E-02
PF3D7_0708900	Cg3 protein	2.92E-02
PF3D7_1215900	serpentine receptor, putative	2.95E-02
PF3D7_1220400	debranching enzyme-associated ribonuclease, putative	2.99E-02
PF3D7_1239100	mitochondrial ribosomal protein L23 precursor, putative	3.03E-02
PF3D7_1354700	conserved Plasmodium protein, unknown function	3.10E-02
PF3D7_1302000	EMP1-trafficking protein	3.10E-02
PF3D7_0502600	conserved Plasmodium protein, unknown function	3.22E-02
PF3D7_1113000	conserved Plasmodium protein, unknown function	3.24E-02
PF3D7_0812300	conserved Plasmodium protein, unknown function	3.28E-02
PF3D7_1469300	small subunit rRNA processing KH domain protein, putative	3.33E-02
PF3D7_0602400	elongation factor G	3.36E-02
PF3D7_0313600	conserved Plasmodium protein, unknown function	3.47E-02
PF3D7_1324500	DEAD box helicase, putative	3.47E-02
PF3D7_1363700	conserved Plasmodium protein, unknown function	3.49E-02
PF3D7_1307600	DNA-directed RNA polymerase alpha chain, putative	3.57E-02
PF3D7_0629300	phosphatidylcholine-sterol acyltransferase, putative	3.58E-02
PF3D7_0901500	rifin PIR protein	3.64E-02
PF3D7_1422400	conserved Plasmodium protein, unknown function	3.68E-02
PF3D7_0519300	cytochrome c oxidase assembly protein (heme A: farnesyltransferase), putative	3.69E-02
PF3D7_1408900	tRNA-dihydrouridine synthase, putative	3.70E-02
PF3D7_1019600	conserved Plasmodium protein, unknown function	3.74E-02
PF3D7_1247000	DNA replication origin binding protein, putative	3.77E-02
PF3D7_0414900	armadillo-domain containing rhopty protein	3.84E-02
PF3D7_1212700	eukaryotic translation initiation factor 3 subunit 10, putative	3.84E-02
PF3D7_0612900	nucleolar GTP-binding protein 1, putative	3.86E-02
PF3D7_0828700	conserved Plasmodium protein, unknown function	3.87E-02
PF3D7_0815900	lipoamide dehydrogenase	3.90E-02
PF3D7_1211700	DNA replication licensing factor MCM5, putative	4.00E-02
PF3D7_0712000	erythrocyte membrane protein 1, PfEMP1	4.06E-02
PF3D7_0415600	GTP:AMP phosphotransferase	4.07E-02
PF3D7_0703300	conserved Plasmodium protein, unknown function	4.08E-02
PF3D7_0620900	tRNA Leucine	4.17E-02
PF3D7_1300200	rifin PIR protein	4.17E-02
PF3D7_0932400	peptide release factor, putative	4.18E-02
PF3D7_0405100	protein transport protein Sec24B	4.18E-02
PF3D7_1100400	rifin PIR protein	4.23E-02
PF3D7_1144000	40S ribosomal protein S21e, putative	4.27E-02
PF3D7_0936500	Plasmodium exported protein, unknown function	4.28E-02
PF3D7_1365600	DNA topoisomerase VI, b subunit, putative	4.31E-02
PF3D7_0206200	pantothenate transporter	4.31E-02
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1	4.33E-02
PF3D7_0102600	serine/threonine protein kinase, FIKK family	4.34E-02
PF3D7_1201000	Plasmodium exported protein (PHISTb), unknown function	4.39E-02

PF3D7_1324700	SNARE protein, putative	4.40E-02
PF3D7_0312400	glycogen synthase kinase 3	4.44E-02
PF3D7_1406500	conserved Plasmodium protein, unknown function	4.53E-02
PF3D7_0935600	gametocytogenesis-implicated protein	4.58E-02
PF3D7_0527500	Hsc70-interacting protein	4.59E-02
PF3D7_1351600	glycerol kinase	4.62E-02
PF3D7_1201800	cytochrome c oxidase assembly protein COX19, putative	4.62E-02
PF3D7_0216000	DEAD/DEAH box helicase, putative	4.63E-02
PF3D7_1455200	methyltransferase, putative	4.63E-02
PF3D7_0420200	holo-(acyl-carrier protein) synthase, putative	4.65E-02
PF3D7_0102500	erythrocyte binding antigen-181	4.71E-02
PF3D7_0519600	zinc finger protein, putative	4.72E-02
PF3D7_0617900	histone H3 variant, putative	4.75E-02
PF3D7_1475500	LCCL domain-containing protein	4.81E-02
PF3D7_1472700	DNA-directed RNA polymerase, alpha subunit, putative	4.85E-02
PF3D7_1237600	rRNA processing WD-repeat protein, putative	4.90E-02
PF3D7_0421300	erythrocyte membrane protein 1, PfEMP1	4.90E-02
PF3D7_1031200	MORN repeat-containing protein 1	4.91E-02
PF3D7_1428300	proliferation-associated protein 2g4, putative	4.94E-02
PF3D7_1435000	cytochrome c oxidase assembly protein, putative	4.99E-02

Appendix I: List of significantly up- and downregulated genes between 11C-R and 11C under ART pressure (900nM ART at 10-14hpi) ($p < 0.05$). Differential expression was measured using paired student's T-test.

UPREGULATED IN 11C-R vs 11C UNDER ART PRESSURE		
GENE ID	Gene Description	<i>p value</i>
PF3D7_1349600	conserved Plasmodium protein, unknown function	5.64E-05
PF3D7_0833000	rifin PIR protein	2.06E-04
PF3D7_1018800	conserved protein, unknown function	3.89E-04
PF3D7_1017400	phosphomannomutase, putative	4.32E-04
PF3D7_0103000	vacuolar protein sorting-associated protein VTA1, putative	6.75E-04
PF3D7_1231800	asparagine-rich protein, putative	6.97E-04
PF3D7_1127000	protein phosphatase, putative	8.88E-04
PF3D7_0931900	adenylate kinase-like protein 2	8.97E-04
PF3D7_1236200	conserved Plasmodium protein, unknown function	9.66E-04
PF3D7_0611900	lsm12, putative	1.00E-03
PF3D7_1254700	rifin PIR protein	1.10E-03
PF3D7_1252700	Plasmodium exported protein (PHISTb), unknown function	1.20E-03
PF3D7_0903000	conserved protein, unknown function	1.28E-03
PF3D7_1421200	40S ribosomal protein S25, putative	1.47E-03
PF3D7_1104300	conserved Plasmodium protein, unknown function	1.61E-03
PF3D7_1436900	histidine triad protein, putative	1.65E-03
PF3D7_1444200	calmodulin-like protein	1.69E-03
PF3D7_1433200	conserved Plasmodium protein, unknown function	1.70E-03
PF3D7_0404600	conserved Plasmodium membrane protein, unknown function	2.17E-03
PF3D7_1034800	conserved Plasmodium protein, unknown function	2.38E-03
PF3D7_0316100	mitochondrial ribosomal protein L27 precursor, putative	2.47E-03
PF3D7_1111900	conserved Plasmodium protein, unknown function	2.51E-03
PF3D7_1412600	deoxyhypusine synthase	2.52E-03
PF3D7_0513900	conserved Plasmodium protein, unknown function	2.68E-03
PF3D7_0623100	coronin binding protein, putative	3.18E-03
PF3D7_1445400	protein serine/threonine kinase-1	3.33E-03
PF3D7_1405300	conserved Plasmodium protein, unknown function	3.44E-03
PF3D7_1450000	serine/threonine protein kinase, putative	3.48E-03
PF3D7_0108400	mitochondrial carrier protein, putative	3.56E-03
PF3D7_0521500	ribosomal large subunit pseudouridylate synthase, putative	3.64E-03
PF3D7_0724000	Rab GTPase activator and protein kinase, putative	3.65E-03
PF3D7_0911300	cysteine repeat modular protein 1	3.67E-03
PF3D7_1246100	delta-aminolevulinic acid synthetase	3.97E-03
PF3D7_0529500	cell cycle regulator protein, putative	4.13E-03
PF3D7_1224100	conserved Plasmodium protein, unknown function	4.17E-03
PF3D7_0416100	glutamyl-tRNA(Gln) amidotransferase subunit A, putative	4.33E-03
PF3D7_1308300	40S ribosomal protein S27, putative	4.39E-03
PF3D7_0519200	V-type proton ATPase 16 kDa proteolipid subunit	4.46E-03
PF3D7_1206000	shewanella-like protein phosphatase 2	4.71E-03
PF3D7_0718900	conserved Plasmodium protein, unknown function	5.04E-03
PF3D7_1316400	conserved Plasmodium protein, unknown function	5.12E-03
PF3D7_1454600	mitochondrial ribosomal protein S11 precursor, putative	5.17E-03
PF3D7_0900600	rifin PIR protein	5.40E-03
PF3D7_0934600	conserved Plasmodium protein, unknown function	5.97E-03
PF3D7_0715600	GTP-binding translation elongation factor tu family protein, putative	6.08E-03
PF3D7_1021400	endomembrane protein 70, putative	6.30E-03
PF3D7_0313800	conserved Plasmodium protein, unknown function	6.48E-03
PF3D7_1322800	conserved Plasmodium protein, unknown function	6.54E-03
PF3D7_0509000	SNAP protein (soluble N-ethylmaleimide-sensitive factor attachment protein), putative	6.64E-03
PF3D7_1440800	major facilitator superfamily, putative	6.69E-03
PF3D7_1230300	subpellicular microtubule protein 2, putative	6.72E-03

PF3D7_1019500	conserved Plasmodium membrane protein, unknown function	7.11E-03
PF3D7_1311300	ATP synthase subunit gamma, mitochondrial	7.14E-03
PF3D7_0906200	conserved Plasmodium protein, unknown function	7.18E-03
PF3D7_1423100	ankyrin, putative	7.19E-03
PF3D7_1311900	vacuolar ATP synthase subunit a	7.31E-03
PF3D7_0400500	rifin PIR protein	7.37E-03
PF3D7_1328900	conserved Plasmodium protein, unknown function	7.55E-03
PF3D7_0504100	AN1-like zinc finger family protein	7.66E-03
PF3D7_0912200	conserved Plasmodium membrane protein, unknown function	7.70E-03
PF3D7_1425700	conserved Plasmodium protein, unknown function	7.76E-03
PF3D7_1207900	conserved Plasmodium protein, unknown function	7.97E-03
PF3D7_0511800	myo-inositol 1-phosphate synthase, putative	8.17E-03
PF3D7_0203300	ERCC1 nucleotide excision repair protein, putative	8.30E-03
PF3D7_1037800	dynein intermediate chain, putative	8.38E-03
PF3D7_1126900	small nuclear ribonucleoprotein F, putative	8.55E-03
PF3D7_0619100	conserved Plasmodium protein, unknown function	8.96E-03
PF3D7_1140200	conserved Plasmodium protein, unknown function	8.97E-03
PF3D7_1452500	SNARE protein, putative	9.16E-03
PF3D7_1314500	cop-coated vesicle membrane protein p24 precursor, putative	9.31E-03
PF3D7_1443900	heat shock protein 90, putative	9.44E-03
PF3D7_1339400	conserved Plasmodium protein, unknown function	9.83E-03
PF3D7_0203900	5'-3' exonuclease, N-terminal resolvase-like domain, putative	1.01E-02
PF3D7_1314700	conserved Plasmodium protein, unknown function	1.02E-02
PF3D7_1354400	V-type proton ATPase 21 kDa proteolipid subunit, putative	1.05E-02
PF3D7_1131100	serpentine receptor, putative	1.10E-02
PF3D7_1472900	dihydroorotase, putative	1.17E-02
PF3D7_1234000	conserved Plasmodium protein, unknown function	1.19E-02
PF3D7_0906400	dynein light intermediate chain 2, cytosolic	1.19E-02
PF3D7_1323600	conserved Plasmodium protein, unknown function	1.23E-02
PF3D7_1428000	conserved Plasmodium membrane protein, unknown function	1.24E-02
PF3D7_0911200	conserved Plasmodium protein, unknown function	1.25E-02
PF3D7_0914200	phospholipid or glycerol acyltransferase, putative	1.26E-02
PF3D7_1421300	conserved Plasmodium protein, unknown function	1.29E-02
PF3D7_0512700	orotate phosphoribosyltransferase	1.29E-02
PF3D7_1105900	conserved Plasmodium membrane protein, unknown function	1.29E-02
PF3D7_1477900	acyl-CoA synthetase, pseudogene	1.31E-02
PF3D7_1418300	calmodulin, putative	1.32E-02
PF3D7_0720600	conserved Plasmodium protein, unknown function	1.32E-02
PF3D7_1130900	mitochondrial rpoD precursor, putative	1.33E-02
PF3D7_0510300	conserved Plasmodium protein, unknown function	1.34E-02
PF3D7_0512900	conserved Plasmodium protein, unknown function	1.36E-02
PF3D7_0727500	conserved Plasmodium protein, unknown function	1.37E-02
PF3D7_0306000	conserved Plasmodium protein, unknown function	1.37E-02
PF3D7_1230800	conserved Plasmodium protein, unknown function	1.38E-02
PF3D7_1408300	conserved Plasmodium protein, unknown function	1.39E-02
PF3D7_0303500	spindle pole body protein, putative	1.42E-02
PF3D7_0106000	conserved Plasmodium protein, unknown function	1.42E-02
PF3D7_1308800	tyrosine recombinase	1.43E-02
PF3D7_0807000	gas41 homologue, putative	1.43E-02
PF3D7_1005500	regulator of nonsense transcripts, putative	1.44E-02
PF3D7_0411400	DEAD box ATP-dependent RNA helicase, putative	1.48E-02
PF3D7_0623900	ribonuclease H2 subunit A, putative	1.49E-02
PF3D7_0522100	conserved Plasmodium protein, unknown function	1.57E-02
PF3D7_1004500	conserved Plasmodium protein, unknown function	1.58E-02
PF3D7_0208700	conserved Plasmodium protein, unknown function	1.61E-02
PF3D7_1322200	conserved Plasmodium protein, unknown function	1.64E-02
PF3D7_1343300	conserved Plasmodium protein, unknown function	1.64E-02
PF3D7_0934700	UBX domain, putative	1.64E-02
PF3D7_1438400	metacaspase-like protein	1.65E-02
PF3D7_0927900	phosphatidylserine decarboxylase	1.66E-02
PF3D7_0816400	conserved Plasmodium protein, unknown function	1.68E-02

PF3D7_0934000	histidine--tRNA ligase, putative	1.72E-02
PF3D7_0402700	rifin, pseudogene PIR protein, pseudogene	1.74E-02
PF3D7_0508200	longevity-assurance (LAG1) protein, putative	1.77E-02
PF3D7_1010600	eukaryotic translation initiation factor 2 beta subunit, putative	1.77E-02
PF3D7_1004200	conserved Plasmodium membrane protein, unknown function	1.79E-02
PF3D7_1464500	conserved Plasmodium membrane protein, unknown function	1.79E-02
PF3D7_0215100	RING zinc finger protein, putative	1.80E-02
PF3D7_1236900	conserved Plasmodium protein, unknown function	1.83E-02
PF3D7_0727700	conserved Plasmodium protein, unknown function	1.84E-02
PF3D7_1002500	conserved Plasmodium protein, unknown function	1.87E-02
PF3D7_0403100	regulator of chromosome condensation, putative	1.91E-02
PF3D7_1365500	glycine cleavage T protein, putative	1.96E-02
PF3D7_1032000	mitochondrial preribosomal assembly protein rimM precursor, putative	1.97E-02
PF3D7_0811800	conserved Plasmodium protein, unknown function	1.98E-02
PF3D7_0925100	conserved Plasmodium protein, unknown function	1.98E-02
PF3D7_1143400	translation initiation factor eIF-1A, putative	1.98E-02
PF3D7_0927400	conserved Plasmodium protein, unknown function	2.05E-02
PF3D7_0526700	conserved Plasmodium protein, unknown function	2.05E-02
PF3D7_1352300	conserved Plasmodium protein, unknown function	2.08E-02
PF3D7_1449400	DNA replication related protein, putative	2.10E-02
PF3D7_1368900	conserved Plasmodium protein, unknown function	2.11E-02
PF3D7_1445100	histidine--tRNA ligase, putative	2.14E-02
PF3D7_0816700	conserved Plasmodium protein, unknown function	2.14E-02
PF3D7_1239800	conserved Plasmodium protein, unknown function	2.16E-02
PF3D7_1443500	poly(A)-specific ribonuclease PARN, putative	2.18E-02
PF3D7_0416000	RNA-binding protein, putative	2.21E-02
PF3D7_0408600	sporozoite invasion-associated protein 1	2.21E-02
PF3D7_1436500	GTP-binding protein, putative	2.24E-02
PF3D7_0105100	conserved Plasmodium protein, unknown function	2.25E-02
PF3D7_0912500	conserved Plasmodium protein, unknown function	2.25E-02
PF3D7_1454400	aminopeptidase P	2.29E-02
PF3D7_0711400	sin3 associated polypeptide p18-like protein	2.30E-02
PF3D7_1230600	sun-family protein, putative	2.31E-02
PF3D7_0623400	RNA-binding protein mei2 homologue, putative	2.31E-02
PF3D7_0528600	conserved Plasmodium protein, unknown function	2.32E-02
PF3D7_1349300	tyrosine kinase-like protein	2.32E-02
PF3D7_1447300	mitochondrial ribosomal protein S14 precursor, putative	2.41E-02
PF3D7_1330500	conserved Plasmodium protein, unknown function	2.44E-02
PF3D7_1432000	syntaxin, Qa-SNARE family	2.46E-02
PF3D7_0411200	conserved Plasmodium protein, unknown function	2.47E-02
PF3D7_1352000	conserved Plasmodium protein, unknown function	2.50E-02
PF3D7_1315100	serine/threonine protein kinase	2.51E-02
PF3D7_0622800	leucine--tRNA ligase, putative	2.52E-02
PF3D7_1417700	conserved Plasmodium protein, unknown function	2.52E-02
PF3D7_1125300	DNA-directed RNA polymerase	2.54E-02
PF3D7_0416500	repressor of RNA polymerase III transcription MAF1, putative	2.58E-02
PF3D7_1334300	MSP7-like protein	2.61E-02
PF3D7_0703200	conserved Plasmodium protein, unknown function	2.62E-02
PF3D7_1124200	conserved Plasmodium protein, unknown function	2.63E-02
PF3D7_1308400	conserved Plasmodium protein, unknown function	2.64E-02
PF3D7_0100800	rifin PIR protein	2.66E-02
PF3D7_1468100	conserved Plasmodium protein, unknown function	2.66E-02
PF3D7_0819400	perforin-like protein 4	2.66E-02
PF3D7_0424500	serine/threonine protein kinase, FIKK family	2.68E-02
PF3D7_1474700	protein kinase, putative	2.68E-02
PF3D7_1132900	glycine cleavage H protein	2.70E-02
PF3D7_0718100	exported serine/threonine protein kinase	2.72E-02
PF3D7_1469000	translation initiation factor IF-1, putative	2.74E-02
PF3D7_1146200	conserved Plasmodium protein, unknown function	2.80E-02
PF3D7_0710300	conserved Plasmodium membrane protein, unknown function	2.80E-02
PF3D7_1422700	conserved Plasmodium protein, unknown function	2.81E-02

PF3D7_0910100	Ran-binding protein, putative	2.82E-02
PF3D7_0610600	calcium-dependent protein kinase 2	2.88E-02
PF3D7_1126300	DnaJ protein, putative	2.88E-02
PF3D7_0928700	conserved Plasmodium protein, unknown function	2.91E-02
PF3D7_1201000	Plasmodium exported protein (PHISTb), unknown function	2.91E-02
PF3D7_1106100	apicoplast ribosomal protein S15 precursor, putative	2.92E-02
PF3D7_1309400	HORMA domain protein, putative	2.94E-02
PF3D7_0916200	conserved Plasmodium protein, unknown function	2.95E-02
PF3D7_1480100	erythrocyte membrane protein 1 (PfEMP1), truncated, pseudogene	2.96E-02
PF3D7_1217000	conserved Plasmodium protein, unknown function	2.97E-02
PF3D7_1119600	ATP-dependent zinc metalloprotease FTSH, putative	2.98E-02
PF3D7_0826100	E3 ubiquitin-protein ligase, putative	3.01E-02
PF3D7_0305500	conserved Plasmodium protein, unknown function	3.02E-02
PF3D7_0504300	conserved Plasmodium protein, unknown function	3.07E-02
PF3D7_0315900	conserved Plasmodium protein, unknown function	3.09E-02
PF3D7_1133500	conserved Plasmodium protein, unknown function	3.11E-02
PF3D7_1349700	conserved Plasmodium membrane protein, unknown function	3.12E-02
PF3D7_0623700	ATP dependent DEAD-box helicase, putative	3.13E-02
PF3D7_1003900	conserved Plasmodium protein, unknown function	3.17E-02
PF3D7_1028100	protoporphyrinogen oxidase	3.21E-02
PF3D7_1445000	conserved Plasmodium protein, unknown function	3.25E-02
PF3D7_1342200	conserved Plasmodium membrane protein, unknown function	3.29E-02
PF3D7_0404400	6-cysteine protein	3.29E-02
PF3D7_1412500	actin II	3.29E-02
PF3D7_1111400	conserved Plasmodium protein, unknown function	3.29E-02
PF3D7_1249400	conserved Plasmodium protein, unknown function	3.31E-02
PF3D7_1313900	conserved Plasmodium protein, unknown function	3.33E-02
PF3D7_1405600	ribonucleotide reductase small subunit	3.45E-02
PF3D7_0526200	ADP-ribosylation factor GTPase-activating protein, putative	3.45E-02
PF3D7_1477100	Plasmodium exported protein (hyp6), unknown function	3.47E-02
PF3D7_0323200	conserved Plasmodium protein, unknown function	3.50E-02
PF3D7_1444400	conserved Plasmodium protein, unknown function	3.50E-02
PF3D7_1208900	conserved Plasmodium protein, unknown function	3.54E-02
PF3D7_1328600	conserved Plasmodium protein, unknown function	3.56E-02
PF3D7_1466300	26S proteasome regulatory subunit, putative	3.60E-02
PF3D7_0512000	prefoldin subunit, putative	3.60E-02
PF3D7_1446900	glutaminyl-peptide cyclotransferase, putative	3.61E-02
PF3D7_1366500	nucleoside diphosphate kinase	3.61E-02
PF3D7_0211200	ras-related protein Rab-5A	3.62E-02
PF3D7_1351800	conserved Plasmodium protein, unknown function	3.62E-02
PF3D7_1308100	CCAAT-binding transcription factor, putative	3.62E-02
PF3D7_1110100	conserved Plasmodium protein, unknown function	3.67E-02
PF3D7_1136000	conserved Plasmodium protein, unknown function	3.68E-02
PF3D7_1450300	DNA polymerase delta interacting protein, putative	3.68E-02
PF3D7_1339600	conserved Plasmodium protein, unknown function	3.69E-02
PF3D7_1457400	conserved Plasmodium protein, unknown function	3.71E-02
PF3D7_1221000	histone-lysine N-methyltransferase, H3 lysine-4 specific	3.73E-02
PF3D7_1244400	conserved Plasmodium protein, unknown function	3.76E-02
PF3D7_1413200	conserved Plasmodium protein, unknown function	3.77E-02
PF3D7_1215300	10 kDa chaperonin	3.78E-02
PF3D7_0706600	conserved Plasmodium protein, unknown function	3.80E-02
PF3D7_0920000	long chain fatty acid elongation enzyme, putative	3.83E-02
PF3D7_1303900	conserved Plasmodium protein, unknown function	3.86E-02
PF3D7_1368100	proteasome regulatory subunit, putative	3.87E-02
PF3D7_0109300	fatty acid elongation protein, GNS1/SUR4 family, putative	3.89E-02
PF3D7_1317500	conserved Plasmodium protein, unknown function	3.89E-02
PF3D7_0720300	conserved Plasmodium protein, unknown function	3.90E-02
PF3D7_0624300	conserved Plasmodium protein, unknown function	3.91E-02
PF3D7_0908300	conserved Plasmodium membrane protein, unknown function	3.91E-02
PF3D7_0723600	conserved Plasmodium protein, unknown function	3.94E-02
PF3D7_1205100	O-phosphoseryl-tRNA(Sec) selenium transferase, putative	3.95E-02

PF3D7_1134500	alpha/beta hydrolase, putative	3.95E-02
PF3D7_0218600	conserved Plasmodium protein, unknown function	3.96E-02
PF3D7_1457100	conserved Plasmodium protein, unknown function	3.97E-02
PF3D7_0729000	signal recognition particle SRP9	3.99E-02
PF3D7_0815400	conserved Plasmodium protein, unknown function	4.01E-02
PF3D7_1211300	DNA helicase MCM8, putative	4.04E-02
PF3D7_1450700	conserved Plasmodium protein, unknown function	4.04E-02
PF3D7_0621700	conserved Plasmodium protein, unknown function	4.07E-02
PF3D7_1106500	conserved Plasmodium protein, unknown function	4.07E-02
PF3D7_0709800	conserved Plasmodium protein, unknown function	4.12E-02
PF3D7_0815000	selenoprotein	4.13E-02
PF3D7_1332000	syntaxin, Qa-SNARE family	4.16E-02
PF3D7_0802600	adenylyl cyclase beta	4.18E-02
PF3D7_1407400	conserved Plasmodium protein, unknown function	4.21E-02
PF3D7_1127900	conserved Plasmodium protein, unknown function	4.22E-02
PF3D7_0502700	conserved Plasmodium protein, unknown function	4.26E-02
PF3D7_1209400	conserved Plasmodium protein, unknown function	4.29E-02
PF3D7_0731600	acyl-CoA synthetase	4.29E-02
PF3D7_1476800	lysophospholipase, putative	4.30E-02
PF3D7_1225400	conserved Plasmodium protein, unknown function	4.32E-02
PF3D7_0811100	mitochondrial carrier protein, putative	4.32E-02
PF3D7_1105200	conserved Plasmodium protein, unknown function	4.33E-02
PF3D7_1471300	conserved Plasmodium protein, unknown function	4.34E-02
PF3D7_1474000	probable protein, unknown function	4.35E-02
PF3D7_0915800	glycolipid transfer protein, putative	4.36E-02
PF3D7_1032300	conserved Plasmodium protein, unknown function	4.41E-02
PF3D7_0832700	Plasmodium exported protein (PHISTA-like), unknown function, pseudogene	4.47E-02
PF3D7_0113800	DBL containing protein, unknown function	4.50E-02
PF3D7_1017800	conserved Plasmodium protein, unknown function	4.56E-02
PF3D7_1330100	conserved Plasmodium protein, unknown function	4.56E-02
PF3D7_1027500	conserved Plasmodium protein, unknown function	4.56E-02
PF3D7_0105900	DNA binding protein, putative	4.59E-02
PF3D7_1338500	conserved Plasmodium protein, unknown function	4.61E-02
PF3D7_1037100	pyruvate kinase 2	4.65E-02
PF3D7_1307200	DnaJ protein, putative	4.65E-02
PF3D7_1135800	conserved Plasmodium protein, unknown function	4.65E-02
PF3D7_0805100	conserved Plasmodium protein, unknown function	4.68E-02
PF3D7_0408900	peptidase, M22 family, putative	4.76E-02
PF3D7_0810400	aquaporin, putative	4.77E-02
PF3D7_1129500	A/G-specific adenine glycosylase, putative	4.79E-02
PF3D7_0725000	exonuclease I, putative	4.83E-02
PF3D7_1401800	choline kinase	4.84E-02
PF3D7_0318100	stomatin-like protein	4.87E-02
PF3D7_0213200	conserved Plasmodium protein, unknown function	4.88E-02
PF3D7_0214500	conserved Plasmodium protein, unknown function	4.88E-02
PF3D7_1144500	ubiquitin activating enzyme (E1) subunit Aos1, putative	4.88E-02
PF3D7_0102800	conserved Plasmodium protein, unknown function	4.90E-02
PF3D7_1116500	folate transporter 2	4.92E-02
PF3D7_1105800	conserved Plasmodium protein, unknown function	4.93E-02
PF3D7_0933400	conserved Plasmodium protein, unknown function	4.93E-02
PF3D7_1229000	conserved Plasmodium membrane protein, unknown function	4.93E-02
PF3D7_1419200	conserved Plasmodium protein, unknown function	4.97E-02

DOWNREGULATED IN 11C-R vs 11C UNDER ART PRESSURE

GENE ID	Gene Description	<i>p value</i>
PF3D7_0221200	Plasmodium exported protein (hyp15), unknown function	3.16E-05
PF3D7_0319700	ABC transporter, putative	2.28E-04
PF3D7_0802300	rRNA processing WD-repeat protein, putative	9.92E-04
PF3D7_1457700	large ribosomal subunit nuclear export factor, putative	1.30E-03
PF3D7_1147300	conserved Plasmodium protein, unknown function	1.33E-03

PF3D7_1203900	ubiquitin conjugating enzyme E2	1.40E-03
PF3D7_0318300	conserved Plasmodium protein, unknown function	1.78E-03
PF3D7_1411600	GTP-binding protein, putative	2.22E-03
PF3D7_1340900	sodium-dependent phosphate transporter	2.28E-03
PF3D7_0522400	conserved Plasmodium protein, unknown function	2.46E-03
PF3D7_1037000	DNA polymerase zeta catalytic subunit, putative	2.62E-03
PF3D7_1025500	conserved Plasmodium protein, unknown function	2.74E-03
PF3D7_0819000	conserved Plasmodium protein, unknown function	2.89E-03
PF3D7_1221200	conserved Plasmodium protein, unknown function	3.02E-03
PF3D7_0517700	eukaryotic translation initiation factor, putative	3.06E-03
PF3D7_0923200	flavodoxin-like protein	3.15E-03
PF3D7_1019600	conserved Plasmodium protein, unknown function	3.18E-03
PF3D7_0908600	ribosomal RNA methyltransferase, putative	3.86E-03
PF3D7_0825900	conserved Plasmodium protein, unknown function	3.97E-03
PF3D7_1463900	conserved Plasmodium membrane protein, unknown function	4.48E-03
PF3D7_1227500	cyclin	4.65E-03
PF3D7_0832200	Plasmodium exported protein (PHISTa-like), unknown function	4.78E-03
PF3D7_0407800	conserved Plasmodium protein, unknown function	4.92E-03
PF3D7_0617900	histone H3 variant, putative	5.32E-03
PF3D7_0208100	conserved Plasmodium protein, unknown function	5.41E-03
PF3D7_1464200	zinc finger protein, putative	5.92E-03
PF3D7_1446400	pyruvate dehydrogenase E1 beta subunit	6.54E-03
PF3D7_1123900	splicing factor, putative	7.27E-03
PF3D7_0617500	rifin, pseudogene PIR protein, pseudogene	7.49E-03
PF3D7_0617600	stevor PIR protein	7.72E-03
PF3D7_1121600	circumsporozoite-related antigen exported protein 1	8.11E-03
PF3D7_0722200	rhoptry-associated leucine zipper-like protein 1	8.20E-03
PF3D7_1131000	RNA-binding protein s1, putative	8.21E-03
PF3D7_1204400	conserved Plasmodium membrane protein, unknown function	8.41E-03
PF3D7_1331400	CPW-WPC family protein	8.91E-03
PF3D7_1228600	merozoite surface protein 9	8.98E-03
PF3D7_1022400	pre-mRNA splicing factor, putative	9.20E-03
PF3D7_1469300	small subunit rRNA processing KH domain protein, putative	9.62E-03
PF3D7_0206000	DNA repair endonuclease, putative	1.04E-02
PF3D7_0415500	nuclear cap-binding protein, putative	1.04E-02
PF3D7_0710100	conserved Plasmodium protein, unknown function	1.07E-02
PF3D7_1034700	ADP-ribosylation factor, putative	1.13E-02
PF3D7_1416100	root hair defective 3 GTP-binding protein (RHD3) homolog, putative	1.15E-02
PF3D7_1008200	endonuclease, putative	1.17E-02
PF3D7_0300900	stevor-like PIR protein	1.18E-02
PF3D7_0408400	conserved Plasmodium protein, unknown function	1.18E-02
PF3D7_1017200	enoyl-CoA hydratase-related protein, putative	1.22E-02
PF3D7_1372500	stevor, pseudogene PIR protein, pseudogene	1.22E-02
PF3D7_0205400	conserved Plasmodium protein, unknown function	1.26E-02
PF3D7_0114600	stevor, pseudogene PIR protein, pseudogene	1.29E-02
PF3D7_1335100	merozoite surface protein 7	1.31E-02
PF3D7_1240600	erythrocyte membrane protein 1, PfEMP1	1.34E-02
PF3D7_0908500	conserved Plasmodium protein, unknown function	1.35E-02
PF3D7_0614100	conserved Plasmodium protein, unknown function	1.43E-02
PF3D7_1212700	eukaryotic translation initiation factor 3 subunit 10, putative	1.44E-02
PF3D7_1347100	DNA topoisomerase III, putative	1.44E-02
PF3D7_0410200	tRNA Isoleucine	1.45E-02
PF3D7_0705000	methyltransferase, putative	1.46E-02
PF3D7_0721300	DEAD/DEAH box ATP-dependent RNA helicase, putative	1.48E-02
PF3D7_1475800	conserved Plasmodium protein, unknown function	1.50E-02
PF3D7_0619900	splicing factor 3A subunit 2, putative	1.50E-02
PF3D7_0314300	DER1-like protein	1.51E-02
PF3D7_0425800	erythrocyte membrane protein 1, PfEMP1	1.52E-02
PF3D7_0624600	SNF2 helicase, putative	1.59E-02
PF3D7_0100100	erythrocyte membrane protein 1, PfEMP1	1.60E-02
PF3D7_0309500	asparagine synthetase, putative	1.64E-02

PF3D7_0313600	conserved Plasmodium protein, unknown function	1.68E-02
PF3D7_0829400	prolyl 4-hydroxylase alpha subunit 1, putative	1.72E-02
PF3D7_0324900	erythrocyte membrane protein 1, PfEMP1	1.74E-02
PF3D7_0516900	60S ribosomal protein L8, putative	1.90E-02
PF3D7_0413200	rifin PIR protein	1.93E-02
PF3D7_1354300	large subunit rRNA methyltransferase, putative	1.94E-02
PF3D7_1425500	conserved Plasmodium protein, unknown function	1.95E-02
PF3D7_0607100	MYND finger protein, putative	1.96E-02
PF3D7_1027100	small subunit rRNA processing stabilizing factor, putative	2.04E-02
PF3D7_1339100	tRNA Methionine	2.05E-02
PF3D7_0510200	peptidyl-prolyl cis-trans isomerase	2.09E-02
PF3D7_1353800	proteasome subunit, putative	2.10E-02
PF3D7_0935900	ring-exported protein 1	2.12E-02
PF3D7_0515700	glideosome-associated protein 40, putative	2.13E-02
PF3D7_1239900	vesicle fusion and protein sorting subunit 16, putative	2.23E-02
PF3D7_0617700	rifin PIR protein	2.24E-02
PF3D7_1035200	S-antigen	2.28E-02
PF3D7_1000600	rifin PIR protein	2.35E-02
PF3D7_0419800	60S ribosomal protein L7ae/L30e, putative	2.42E-02
PF3D7_0813300	conserved Plasmodium protein, unknown function	2.47E-02
PF3D7_0204600	conserved Plasmodium protein, unknown function	2.51E-02
PF3D7_1150400	erythrocyte membrane protein 1, PfEMP1	2.55E-02
PF3D7_1243700	ubiquitin conjugating enzyme E2, putative	2.60E-02
PF3D7_1406200	conserved Plasmodium protein, unknown function	2.61E-02
PF3D7_1354900	conserved Plasmodium protein, unknown function	2.67E-02
PF3D7_1214000	conserved Plasmodium protein, unknown function	2.67E-02
PF3D7_1033800	plasmepsin VII	2.70E-02
PF3D7_0219900	Plasmodium exported protein, unknown function	2.70E-02
PF3D7_0422800	serpentine receptor, putative	2.77E-02
PF3D7_0202500	early transcribed membrane protein 2	2.84E-02
PF3D7_1143100	transcription factor with AP2 domain(s)	2.90E-02
PF3D7_1339300	conserved Plasmodium protein, unknown function	2.91E-02
PF3D7_1250000	rRNA-processing protein, putative	3.07E-02
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1	3.09E-02
PF3D7_1478300	Plasmodium exported protein, unknown function, pseudogene	3.11E-02
PF3D7_1476900	Plasmodium exported protein, unknown function, pseudogene	3.15E-02
PF3D7_1133700	conserved Plasmodium protein, unknown function	3.16E-02
PF3D7_1036300	merozoite surface protein	3.19E-02
PF3D7_0900100	erythrocyte membrane protein 1, PfEMP1	3.19E-02
PF3D7_1215400	conserved Plasmodium protein, unknown function	3.19E-02
PF3D7_1469200	shewanella-like protein phosphatase 1, putative	3.19E-02
PF3D7_1463300	DNA polymerase alpha subunit, putative	3.20E-02
PF3D7_0505800	small ubiquitin-related modifier	3.24E-02
PF3D7_1205800	high mobility group protein B3, putative	3.27E-02
PF3D7_1364400	conserved Plasmodium protein, unknown function	3.27E-02
PF3D7_0528000	conserved Plasmodium protein, unknown function	3.28E-02
PF3D7_0104300	ubiquitin carboxyl-terminal hydrolase 1, putative	3.31E-02
PF3D7_1132600	pre-mRNA splicing factor, putative	3.31E-02
PF3D7_1426800	conserved Plasmodium protein, unknown function	3.32E-02
PF3D7_0522700	iron-sulfur assembly protein, putative	3.33E-02
PF3D7_0814500	conserved Plasmodium protein, unknown function	3.35E-02
PF3D7_0603700	phenylalanine--tRNA ligase, putative	3.36E-02
PF3D7_1369600	conserved Plasmodium protein, unknown function	3.46E-02
PF3D7_1305000	conserved Plasmodium protein, unknown function	3.50E-02
PF3D7_0502500	conserved Plasmodium protein, unknown function	3.52E-02
PF3D7_0803400	DNA repair protein RAD54, putative	3.54E-02
PF3D7_1439500	CCAAT-binding transcription factor, putative	3.58E-02
PF3D7_0930000	procollagen lysine 5-dioxygenase, putative	3.59E-02
PF3D7_1114900	conserved Plasmodium protein, unknown function	3.61E-02
PF3D7_1433800	conserved Plasmodium protein, unknown function	3.61E-02
PF3D7_0104000	thrombospondin-related sporozoite protein	3.63E-02

PF3D7_1307800	fork head domain protein, putative	3.66E-02
PF3D7_1301700	Plasmodium exported protein (hyp8), unknown function	3.69E-02
PF3D7_0931100	nucleolar protein Nop52, putative	3.71E-02
PF3D7_1246400	myosin light chain 1 myosin A tail domain interacting protein	3.73E-02
PF3D7_0501600	rho-try-associated protein 2	3.78E-02
PF3D7_0219600	replication factor C subunit 1, putative	3.80E-02
PF3D7_0704700	phosphopantetheine adenylyltransferase, putative	3.85E-02
PF3D7_0519600	zinc finger protein, putative	3.89E-02
PF3D7_0723400	conserved Plasmodium protein, unknown function	3.96E-02
PF3D7_1454000	RNA-binding protein, putative	3.97E-02
PF3D7_1204100	conserved Plasmodium protein, unknown function	3.97E-02
PF3D7_1333600	U3 snoRNA-associated small subunit rRNA processing associated protein, putative	3.99E-02
PF3D7_0530600	XAP-5 DNA binding protein, putative	4.02E-02
PF3D7_0220800	cytoadherence linked asexual protein 2	4.08E-02
PF3D7_0614500	60S ribosomal protein L19, putative	4.11E-02
PF3D7_1038800	RESA-like protein with PHIST and DnaJ domains	4.11E-02
PF3D7_1354700	conserved Plasmodium protein, unknown function	4.13E-02
PF3D7_1334000	conserved Plasmodium protein, unknown function	4.19E-02
PF3D7_0218400	DEAD/DEAH box ATP-dependent RNA helicase, putative	4.26E-02
PF3D7_0417200	bifunctional dihydrofolate reductase-thymidylate synthase	4.27E-02
PF3D7_0905400	high molecular weight rho-try protein 3	4.30E-02
PF3D7_0815900	lipoamide dehydrogenase	4.31E-02
PF3D7_0410100	tRNA Serine	4.33E-02
PF3D7_1221700	FbpA domain protein, putative	4.35E-02
PF3D7_0411500	tRNA Alanine	4.39E-02
PF3D7_1405900	RNA-binding protein, putative	4.43E-02
PF3D7_1331300	signal peptidase 21 kDa subunit	4.57E-02
PF3D7_1148800	Plasmodium exported protein (hyp11), unknown function	4.59E-02
PF3D7_1001500	early transcribed membrane protein 10.1	4.62E-02
PF3D7_0525900	NIMA related kinase 2	4.64E-02
PF3D7_0509100	chromosome condensation protein, putative	4.79E-02
PF3D7_0812600	ubiquitin conjugating enzyme, putative	4.83E-02
PF3D7_1441100	conserved Plasmodium protein, unknown function	4.86E-02
PF3D7_0910200	conserved Plasmodium protein, unknown function	4.88E-02
PF3D7_1335600	conserved Plasmodium protein, unknown function	4.89E-02
PF3D7_0706900	tRNA Histidine	4.92E-02
PF3D7_1206500	Tat binding protein 1(TBP-1)-interacting protein, putative	4.94E-02
PF3D7_1033600	myb2 transcription factor, putative	4.95E-02
PF3D7_1139100	RNA-binding protein, putative	4.95E-02

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