CHARACTERIZATION OF PROTEOME OF *PLASMODIUM VIVAX* ERYTHROCYTIC STAGES AND THE IDENTIFICATION OF ASEXUAL STAGE ANTIGENS

WANLAPA ROOBSOONG

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
(MEDICAL TECHNOLOGY)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2011

Thesis
entitled

CHARACTERIZATION OF PROTEOME OF *PLASMODIUM VIVAX* ERYTHROCYTIC STAGES AND THE IDENTIFICATION OF ASEXUAL STAGE ANTIGENS

……..
Miss Wanlapa Roobsoong
Candidate

……..
Prof. Rachanee Udomsangpetch,
Ph.D.
Major advisor

……..
Asst. Prof. Rachada Kiatfuengfoo,
Ph.D.
Co-advisor

……..
Asst. Prof. Wilairat Nuchpramool,
M.D.
Co-advisor

……..
Prof. Banchong Mahaisavariya,
M.D., Dip Thai Board of Orthopedics
Dean
Faculty of Graduate Studies
Mahidol University

……..
Prof. Virapong Prachayasittikul, Ph.D.
Program Director
Doctor of Philosophy Program in
Medical Technology
Faculty of Medical Technology
Mahidol University

Thesis
entitled

CHARACTERIZATION OF PROTEOME OF *PLASMODIUM VIVAX* ERYTHROCYTIC STAGES AND THE IDENTIFICATION OF ASEXUAL STAGE ANTIGENS

was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Doctor of Philosophy (Medical Technology)
on
March 18, 2011

……..
Miss Wanlapa Roobsoong
Candidate

……..
Sittiruk Roytrakul, Ph.D.
Chair

……..
Prof. Rachanee Udomsangpetch, Ph.D.
Member

……
Asst. Prof. Wilairat Nuchpramool, M.D.
Member

……..
Asst. Prof. Rachada Kiatfuengfoo, Ph.D.
Member

……
Prof. Banchong Mahaisavariya, M.D.
M.D., Dip Thai Board of Orthopedics
Dean
Faculty of Graduate Studies
Mahidol University

……
Prof. Virapong Prachayasittikul,
Ph.D. (Microbiology)
Dean
Faculty of Medical Technology
Mahidol University

ACKNOLEDGEMENTS

The success of this thesis can be succeeded by the attentive support from my advisor, Prof.Dr. Rachanee Udomsangpetch, and my co-advisors Asst. Prof. Rachada Kiatfuengfoo and Asst.Prof. Wilairat nuchpramool.

I would like to express my appreciation to Prof.Dr.Liwang Cui for his guidance, invaluable advice supervision, and encouragement throughout this work. I would like to thank all members in Prof.Dr.Liwang's lab, Penn State University, USA, for all the helps and technical assistance.

I would like to express my appreciation to Dr. Sittiruk Roytrakul for his invaluable advice regarding mass spectrometry.

My sincere appreciation is express to Dr. Jetsumon Prachumsri from Department of Entomology, AFRIMs, Thailand, for her guidance. My appreciation extended to all members in Entomology Department of AFRIMS, Thailand, for all help during sample collection in Mae Sot.

I would like to thanks my colleagues at Malaria Unit, Department of Pathobiology, Faculty of Science, Mahidol University, for their support.

This work was supported by Forgarty International Scholar, NIH and Royal Golden Jubilee Ph.D. Program/ Thailand research Fund.

Finally I would like to express my deepest appreciation to my family for their entirely care, love, and support.

Wanlapa Roobsoong

CHARACTERIZATION OF PROTEOME OF *PLASMODIUM VIVAX* ERYTHROCYTIC STAGES AND THE IDENTIFICATION OF ASEXUAL STAGE ANTIGENS

WANLAPA ROOBSOONG 4736822 MTMT/D

Ph.D. (MEDICAL TECHNOLOGY)

THESIS ADVISORY COMMITTEE: RACHANEE UDOMSANGPETCH, Ph.D., RACHADA KIATFUANGFU, Ph.D., WILAIRAT NUCHPRAMOOL, M.D.

ABSTRACT

With the completion of genome sequencing of the malaria parasite *Plasmodium vivax*, it is important to determine the proteome of the parasite in order to assist efforts in identifying novel antigens and drug targets. As the method for continuous *in vitro* culture of *P. vivax* parasite was not available, we tried to study the proteome of the erythrocytic stages using fresh parasite isolates from patients. Three hundred and sixteen proteins were confidently identified by tandem mass spectrometry. Almost 50% of the identified proteins were hypothetical, while other major categories were proteins with a binding function, protein fate, protein synthesis, metabolism, and cellular transport. To identify proteins that are recognized by host humoral immunity, parasite proteins were separated by two-dimensional electrophoresis and confirmed by Western blotting using immune serum from a *P. vivax* patient. Protein spots that were recognized by the serum were identified by mass spectrometry. Among four potential antigens identified, one protein, PV24, was recognized by antibodies from vivax malaria patients.

KEY WORDS: *PLASMODIUM VIVAX*/ ANTIGENS/ ERYTHROCYTIC STAGE/ PROTEOME/ MASS SPECTROMETRY

231 pages

การหาโปรตีนทั้งหมดของเชื้อก่อโรคมาลาเรียชนิด *Plasmodium vivax* ในระยะที่อยู่ในเม็ดเลือดแดงและการหาแอนติเจนของเชื้อระยะไม่มีเพศ

CHARACTERIZATION OF PROTEOME OF *PLASMODIUM VIVAX* ERYTHROCYTIC STAGES AND THE IDENTIFICATION OF ASEXUAL STAGE ANTIGENS

วัลลภา รูปสูง 4736822 MTMT/D

ปร.ด. (เทคนิคการแพทย์)

คณะกรรมการที่ปรึกษาวิทยานิพนธ์: รัชนีย์ อุดมแสงเพ็ชร, Ph.D., รัชฎา เกียรติเฟื่องฟู, Ph.D., วิไลรัตน์ นุชประมูล, M.D.

บทคัดย่อ

จากการที่มีข้อมูลจีโนมของเชื้อก่อโรคมาลาเรียชนิด *Plasmodium vivax* แล้วนั้น การศึกษาProteome ของเชื้อก็มีความสำคัญในแง่ของการหาโปรตีนที่มีคุณสมบัติเป็น antigen รวมถึง drug targets ชนิดใหม่ ในขณะที่วิธีการเลี้ยงเชื้ออย่างต่อเนื่องภายในห้องปฏิบัติการยังไม่ประสบผลสำเร็จ เราได้ทำการศึกษา proteome ในระยะ erythrocytic stage ของเชื้อ *P. vivax* ที่ได้จากผู้ป่วย โดยนำเชื้อที่ได้มาทำการวิเคราะห์หาชนิดของโปรตีนทั้งหมดด้วยเครื่อง Tandem mass spectrometry โดยจากการศึกษาพบโปรตีนทั้งหมด 316 ตัว โดยเกือบร้อยละ 50 ของโปรตีนที่พบนี้เป็น hypothetical protein นอกนั้นเป็นโปรตีนที่ทำหน้าที่ในเรื่องของ binding function, protein fate, protein synthesis, metabolism และ cellular transport

เพื่อค้นหาโปรตีนของเชื้อ *P. vivax* ที่ถูกจดจำด้วยระบบภูมิคุ้มกันของมนุษย์ โปรตีนทั้งหมดของเชื้อได้ถูกนำมาแยกตามคุณสมบัติของค่า pI และน้ำหนักของโปรตีนด้วยวิธี 2D gel electrophoresis แล้วนำโปรตีนที่แยกได้นั้นมาทำปฏิกิริยากับ plasma ผู้ป่วยที่ติดเชื้อ *P. vivax* ด้วยวิธี immunoblotting และทำการวิเคราะห์หาชนิดโปรตีนด้วยเครื่อง mass spectrometry โดยพบว่ามีโปรตีนทั้งหมด 4 ชนิดที่ถูกจดจำโดยแอนติบอดีจาก plasma ผู้ป่วย หนึ่งในนั้นคือ PV24 ซึ่งจากการศึกษาพบว่าโปรตีนชนิดนี้ถูกจดจำโดยระบบภูมิคุ้มกันของผู้ป่วยที่อาศัยอยู่ในแหล่งระบาดของโรค

231 หน้า

CONTENTS

CONTENTS (cont.)

CONTENTS (cont.)

CONTENTS (cont.)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Term
2DE	Two dimensional electrophoresis
ABTS	2, 2'-azino-di-(3-ethylbenzthiaoline sulfonic acid
ACN	Acetronitrile
ACP	Acyl carrier protein
ADA	Adenosine deaminase
ADP	Adenosine diphosphate
AK	Adenosine kinase
Ald	Aldolase
AMA	Apical membrane antigen
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
bp	Base pair
°C	Degree celcious
CCT	Phosphocholine cytidylyltransferase
cDNA	Complementary Deoxyribonucleic acid
CDP	Cytidine diphosphate
CHCA	α-cyanohydroxycinnamic acid
CID	Collision induced dissociation
CK	Choline kinase
clag	Cytoadherence linked asexual gene
CO_2	Carbon dioxide
CRM	Charged-residue-model
CTP	Cytidine triphosphate
dTMP	Deoxythymidine monophosphate
DTT	Dithiothreitol

LIST OF ABBREVIATIONS (cont.)

Abbreviation	Term
dUMP	Deoxyuridine monophosphate
E. coli	*Escherichia* coli
ELISA	Enzyme-linked immunosorbent assay
EMP	Erythrocyte membrane protein
ESI	Electrospray ionization
EXP	Exporting protein
FA	Fatty acids
FDR	False Discovery Rate
GC/MS	Gas chromatography mass spectrometry
GPI	Glycosylphosphatidyl inositol
h	Hour
HEPES	4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid
HGXPRT	Hypoxanthine guanine xanthine phosphoribosyl transferase
His	Histidine
HK	Hexokinase
HRP	Horse radish peroxidase
HSP	Heat shock protein
IEF	Isoelectric focusing
IEM	Ion evaporation model
IMP	Inosine monophosphate
iPRBCs	Packed infected red blood cells
IPTG	Isopropyl-β-D-thiogalactopyranoside
kDa	Kilo dalton
L	Litre
LB	Lulia bertani
LC	Liquid chromatography mass spectrometry

LIST OF ABBREVIATIONS (cont.)

Abbreviation	Term
LC/MS	Liquid chromatography mass spectrometry
LC/MS/MS	Liquid chromatography tandem mass spectrometry
LDH	Lactate dehydrogenase
LIT	Quadrupole linear ion trap
m	Mass
M	Molar
m/z	Mass-to-charge ratio
MALDI	Matrix-assisted laser desorption/ionization
MALDI-TOF/TOF	Matrix assisted laser desorption/ionization time of flight mass spectrometry
MDR	Multidrug resistance protein
min	Minute
ml	Milliliter
mM	Millimolar
mm	Millimeter
mRNA	Messenger Ribonucleic acid
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSP	Merozoite surface protein
N_2	Nitrogen
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NC	Nitrocellulose membrane
nl	Nanolitre
nm	Nanometer
O_2	Oxygen

LIST OF ABBREVIATIONS (cont.)

Abbreviation	Term
OD	Optical density
P	Phosphate
P. falciparum	*Plasmodium falciparum*
P. malariae	*Plasmodium malariae*
P. ovale	*Plasmodium ovale*
P. vivax	*Plasmodium vivax*
P.berghei	*Plasmodium berghei*
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffer saline
PBS	Phosphate buffered saline
PBS-T	Phosphate buffer saline –Tween
PC	Phosphatidylcholine
PCR	Polymerase chain reaction
PE	Phosphatidylethanolamine
Pfs16	*Plasmodium falciparum* surface protein 16
Pfs230	*Plasmodium falciparum* surface protein 230
Pfs48/45	*Plasmodium falciparum* surface protein 48/45
PI	Phosphatidylinositol
PK	Pyruvate kinase
PL	Phospholipids
PMF	Peptide Mass Fingerprinting
PNP	Purine nucleoside phosphorylase
PvtrAg	*Plasmodium vivax* tryptophan rich antigen
QIT	Quadrupole ion trap
QqQ	Triple quadrupole
RAP	Rhoptry-associated protein

LIST OF ABBREVIATIONS (cont.)

Abbreviation	Term
RBC	Red blood cell
RBP	Reticulocyte binding protein
RhopH	High-molecular mass rhoptry protein complex
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT	Room temperature
s16	Sexual stage antigen
SD	Standard deviations
SDS	Sodium dodecylsulfate
SM	Sphingomyelin
SP	Signal peptide
TBS-T	Tween-20 in Tris-buffered saline
TCA	Tricarboxylic acid cycle
TFA	Trifluoroacetic acid
TM	Transmembrane
TOF	Time-of-flight
UDP	Uridine diphosphate
UMP	Uridine monophosphate
UTP	Uridine triphosphate
V	Volt
Vhr	Volt hour
W	Watt
WB	Western blot
z	Charged
µl	Microlittre
µm	Micrometer

CHAPTER I
INTRODUCTION

Malaria is one of the world's most important parasitic diseases which threaten lives across several continents: Africa,Asia, Central and South America and parts of Eastern Europe. It is caused by parasites of the genus *Plasmodium*. At present, an estimated 243 million cases of malaria has been reported globally mostly occurred in African regions (85%), South-East Asia (10%) and Eastern Mediterranean regions (4%). There are four *Plasmodium* species causing malaria in human: *P. falciparum, P. vivax, P. malariae and P. ovalae*.

Plasmodium vivax is responsible for the most wide spread human malaria parasite which effect the human well being and socio-economic problems in endemic countries. Approximately 70-80 million people infected each year and around 56 % of malaria cases outside Africa are due to this species [1]. It has several unique characteristics that distinguish it from other human malaria parasite species. Most notably, *P. vivax* forms hypnozoites in hepatocytes, causing relapses of the disease [2]. *P. vivax* strains from the tropical and temperate zones can vary dramatically in terms of the pattern and frequency of the relapse. Moreover, *P. vivax* requires Duffy receptor on the red cell for invasion, and is thus absent in West Africa where Duffy negativity predominates [3]. It selectively invades reticulocytes [4], thus limiting parasitemias to low levels. Gametocyte production in *P. vivax* takes place much early after infection, even before the manifestation of clinical symptoms. Unlike *Plasmodium falciparum* infection that increases the rigidity of the host cell, *P. vivax* increases the size and deformability of infected red cells [5,6]. *P. vivax* also actively remodels the host cell, producing caveola-vesicle complexes along the plasmalemma in the infected erythrocyte cell, which are visible in Giemsa-stained smears as multiple red dots called "Schüffner's dots". Apart from these characteristics, the ability of *P. vivax* to survive at much lower temperature has allowed this parasite to establish transmission foci in temperate zones. Despite that many of these unique

features of *P. vivax* have been known for a long time, the underlying mechanisms remain poorly understood. Therefore, a better understanding of the fundamental biology of *P. vivax* is needed to effectively control and eventually eradicate this parasite.

The completion of genome [7,8], transcriptome [9], and proteome [10,11] of *P. falciparum* has play significant roles in malaria research. Focused on *P. falciparum* research during the past decades making *P. vivax* research much lagged behide. The unavailable of continuous *in vitro* culture of *P. vivax,* even some experiment has shown the potential [12] but the suitable system still unavailable, is the possibly reason of the delayed in establishment of genome [13] and transcriptome of this parasite.

The available of genome [13], EST [14], and transcriptome [15], of *P. vivax* has played significant roles by the same mean as *P. falciparum* but these information are just the beginning of the understanding of cellular function at the molecular level and may not be able to define all of the proteins in the forms in which they actually exist in cells. The transcription of particular genes at different time point of these 2 species [15] may be the cause of different in the infection mechanism as well as clinical outcomes. As noted, not all transcripts are translated into proteins so the information on *P. vivax* proteome will full fill the information of this complex parasite and provide the new tools for malaria research.

In order to fight against the disease, most of malaria research has been focused on vaccine developments. Several leading vaccines from *P. falciparum* have been tested in clinical trial but seem to be effective for the same strain of infection and could not protect across species. The single subunit vaccine of *P. falciparum* may cause an unexpected outcome in the global epidemiology and may not be able to use in the areas where *P. vivax* and *P. falciparum* are co-existed. In endemic areas where the 2 species are co-existed, multi-subunit or multi-species vaccines could be the better way for vaccine development. Several *P. falciparum* antigens have shown potential as candidates [16,17] but the candidate antigens from *P. vivax* still have to be defined.

The advance in highly sensitive mass spectrometry offers an extraordinary opportunity to determine the proteome of the *P. vivax* parasite in the absence of large

amount of experimental materials from a continuous *in vitro* culture. In this study, we attempted to study the proteome of the erythrocytic stages of *P. vivax* field isolates by highly accurate tandem mass spectrometry (MS/MS). As a way of antigen discovery, we further tried to identify parasite antigens that are recognized by host humoral immunity using immune serum from a *P. vivax* malaria patient. All information reported together is not just full fill the lacking information of *P. vivax* but also facilitate the research on *P. vivax* in order to understand basic biology of this unique parasite and provide the new tools for drug and vaccine developments.

CHAPTER II
OBJECTIVES

1. Characterization of the erythrocytic stages proteome of *P. vivax*.
2. Cloning of *P. vivax'* gene encoding protein which has been recognized by immune serum from *P. vivax* infected patient.
3. Produce the *P. vivax* recombinant protein which has been recognized by immune serum from *P. vivax* infected patient.
4. Study the imunogenicity of *P. vivax'* recombinant protein in malaria endemic area in Thailand.

CHAPTER III
LITERATURE REVIEW

3.1 Life cycle of *Plasmodium vivax*

Malaria is one of the world's most important parasitic diseases which threaten lives across several continents: Asia, Africa, Central and South America and parts of Eastern Europe. It has been estimated that malaria causes 300-500 million infections and 2-4 million deaths each year. Of four species of *Plasmodium* that cause malaria in human (*P. falciparum, P. vivax, P. malariae, P. ovalae*), *P. falciparum* is the most dangerous being associated with cerebral malaria, while *P. vivax* is the most widely distributed human malaria parasite and the most common species observed in temperate regions, representing the major cause of malaria outside Africa. Currently there are 70-80 million *P. vivax* clinical cases each year distributed mainly through the tropical regions; Papua New Guinea, Eastern and Southern Africa, Middle East, Asia and Western Pacific[18] .

Plasmodium vivax causes a form of malaria termed benign tertian malaria. Life cycle of *P. vivax* (figure 3.1) and other human malaria parasites required 2 different hosts, *Anopheles sp* mosquitoes and human. During the blood meal, infected female *Anopheles* mosquito injects sporozoites together with its saliva into blood circulation. Sporozoites go directly to infect liver cells and initiate the exo-erythrocytic stage multiplication to produce schizonts (exo-erythrocytic schizogony). In *P. vivax* and *P. ovalae*, some sporozoites enter the dormant state called hypnozote in hepatocyte which causing the relapse of the disease in months or years later. When hepatocytes rapture, thousands of merozoites are released into blood circulation and invade erythrocytes where they undergo asexually multiplication (erythrocytic schizogony). In *P. vivax*, merozoites selectively invade reticulocytes with the presence of Duffy antigen. The number of reticulocytes in blood circulation is limited to less than 2% so it limits the parasitemia to low level and in some regions of Africa, people with negative Duffy antigen are resistant to *P. vivax* infection [3].

Instead of asexual development some parasites differentiate into sexual stage called gametocyte which will be uptake by female *Anopheles* during blood fed and enter the sporogonic cycle. In *P. vivax*, the gametocytogenesis is occurred by the time of clinical symptoms, earlier than other human malaria parasites, which make the parasites transmitted before treatment. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.

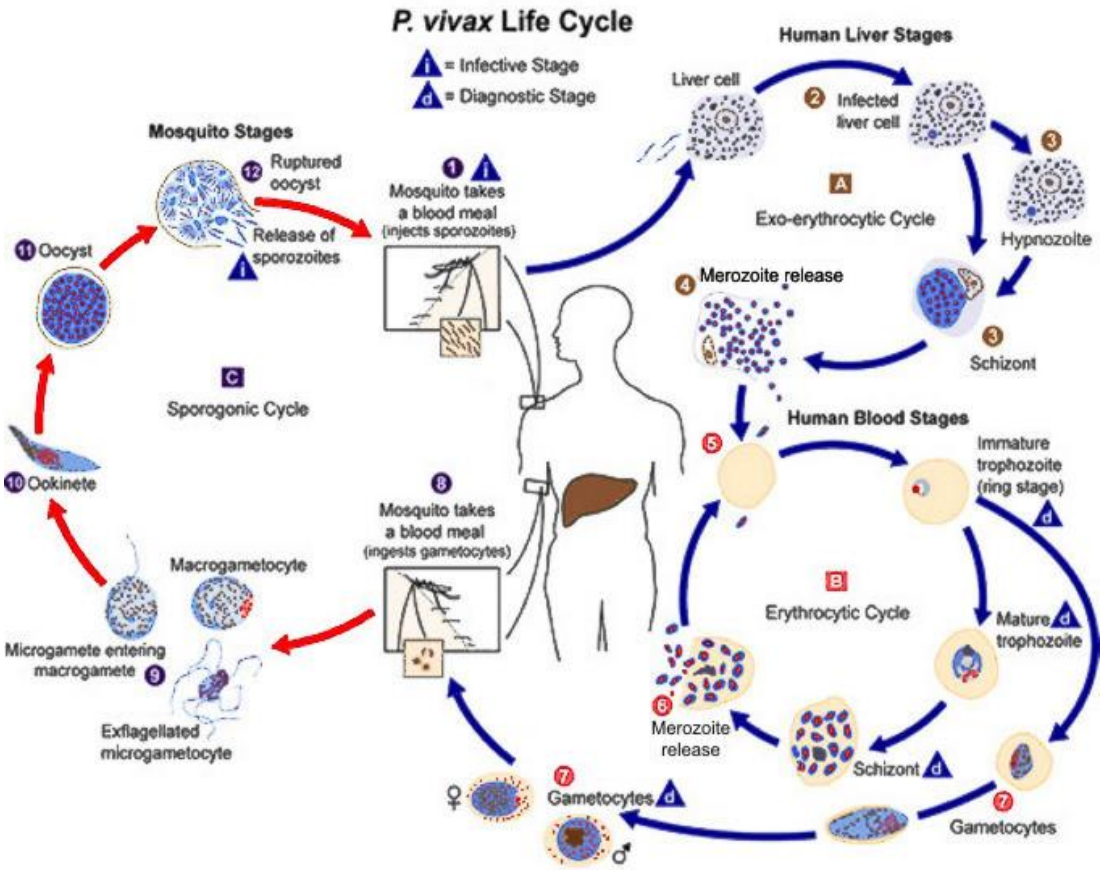


Figure 3.1 Life cycle of *Plasmodium vivax* (http://www.vivaxmalaria.com)

Blood stage parasites are responsible for clinical manifestation of the disease. Asexual stage parasite develops from ring stage to trophozoite and schizont which will rapture and release merozoites to blood circulation. Merozoites invade new erythrocytes and initiate the new cycles of erythrocytic stage. In erythrocytic stage development parasites uptake most of nutrients they need from plasma and red cell cytoplasm. During this period, all of metabolic pathways are running in order to serve parasite growth including Glycolysis, hemoglobin degradation, nucleic acid biosynthesis, and lipid metabolism.

3.2 Metabolic pathways of *Plasmodium*

3.2.1 Glycolysis

The new erythrocytic cycle of parasites begins every 48 h so it needs a lot of energy to fuel this rapid growth. There are no obvious energy stores and enzymes for gluconeogenesis in *Plasmodium* so parasite must continuously import and use glucose in order to meet requirements for rapid growth. Enzymes involve in glycolysis are listed (table3.1).

When intraerythrocytic parasites are transforming into their most metabolically active stage of development, they increase the utilization of glucose by up to 100-fold compared with uninfected red blood cells [19] and the activity of some glycolytic enzymes are greatly up-regulated including hexokinase (HK), aldolase (Ald), enolase, and pyruvate kinase (PK) [20]. Study in *P. falciparum* has shown not only the significantly enhance the glycolytic flux but also down regulation of glucose utilization rate of uninfected erythrocytes induced by parasites. The mechanism of inhibition seem to involve inhibition of regulatory glycolytic enzymes, phosphofructokinase and pyruvate kinase, possibly through some secreted products of parasites [21]. In asexual stages parasite, almost all of glucose used appears to pass through the anaerobic glycolysis and is metabolized almost entirely to lactic acid due to the lack of oxidative phosphorylation and functional TCA cycle (figure 3.2). The increase in anaerobic glycolysis results in the hypoglycemia and hyperlactatemia which often associated with severe malaria.

Due to their importance in *Plasmodium* for energy production, glycolytic enzymes have been targeted for novel anti-malarial development. Lactate

dehydrogenase (LDH) has received the most attention in this context. Lactate dehydrogenase catalyzes the reduction of pyruvate by NADH to lactate and generates NAD^+. Unlike the host enzyme there is no substrate inhibition by pyruvate in which allows fast energy production as required by rapidly growing parasites. The inhibition of LDH prevents regeneration of NAD^+ from NADH and so removes this glycolysis prerequisite, thereby stopping ATP production. The crystal structure of *P. falciparum* LDH shows a large shift in the position of the NADH cofactor binding pocket compared with LDH from other sources, which could be the basis for lack of inhibition by pyruvate of the malarial enzyme. Specific inhibition may be possible, as the plasmodial enzyme differs from its human counterpart by the presence of a 5 amino acid insertion at the pyruvate binding site [22,23]. Although this may represent a potential target, and despite the current screening of compounds, there are no known lead compounds at present. Moreover the distinct antigenic properties of *P. falciparum* LDH have allowed the development of a useful antigen detection assay that has been formulated into a field dipstick [24,25].

Table 3.1 Enzymes involved in glycolysis of *Plasmodium.*

Enzyme	**Substrate**	**product**
Hexokinase	D-glucose, ATP	D-glucose-6P, ADP
Phosphoglucose isomerase	D-glucose-6P	D-fructose-6P
Phosphofructokinase	D-fructose-6P, ATP	D-fructose-1,6P_2, ADP
Aldolase	D-fructose-1,6P_2	Glyceraldehyde-3P or Glycerone-P
Triose phosphate isomerase	Glycerone-P	Glyceraldehyde-3P
Glyceraldehyde -3P dehydrogenase	Glyceraldehyde-3P, NAD^+ +Pi	Glycerate-1,3P_2, NADH + H^+
Phosphoglycerate kinase	Glycerate-1,3P_2, ADP	Glycerate-3P, ATP
Phosphoglycerate mutase	Glycerate-3P	Glycerate-2P
Enolase	Glycerate-2P	Phosphoenol pyruvate
Pyruvate kinase	Phosphoenol pyruvate, ADP	Pyruvate, ATP
Lactate dehydrogenase	Pyruvate, NADH + H^+	Lactate, NAD^+

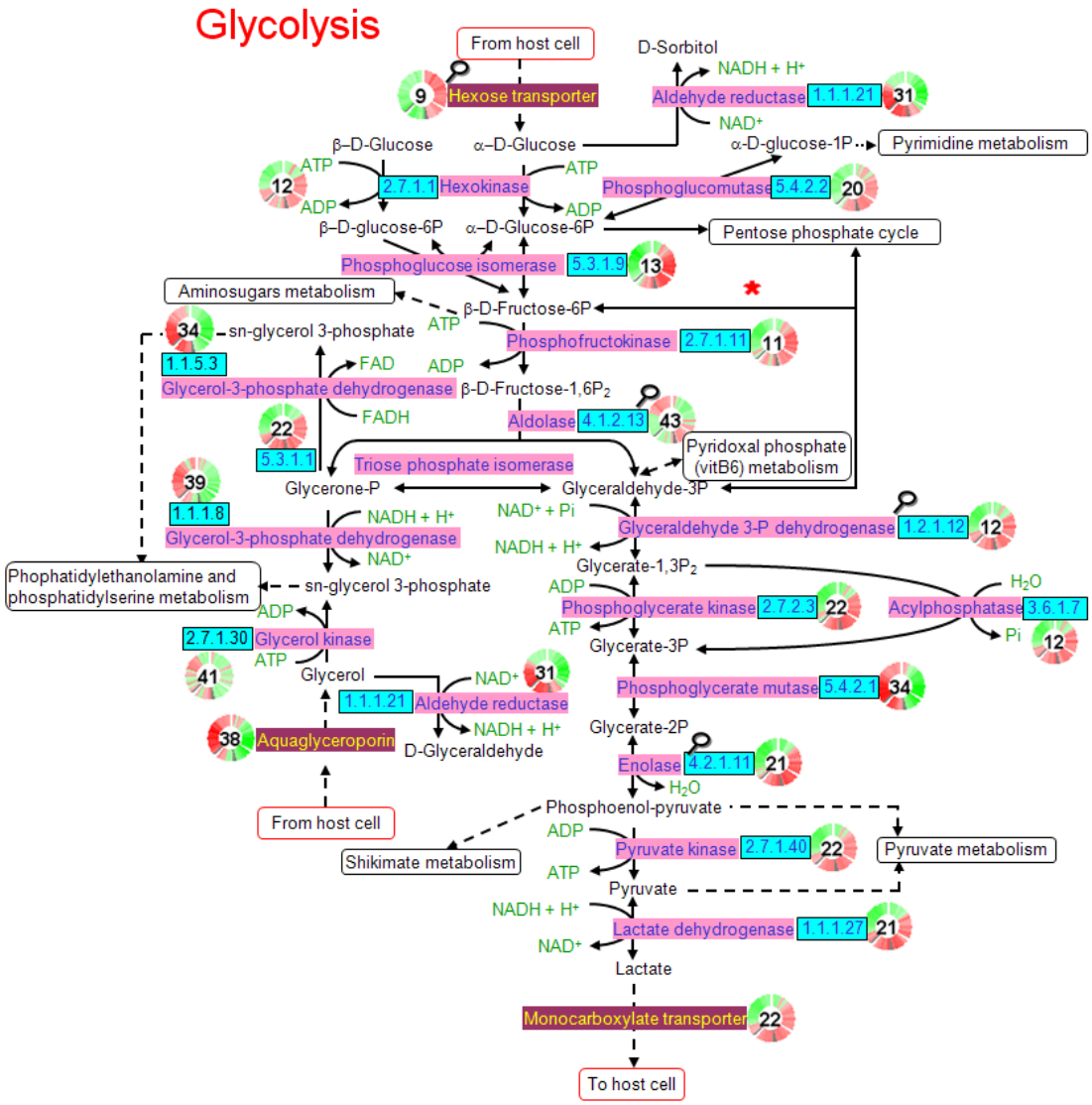


Figure 3.2 Postulated scheme of glycolysis in Plasmodium-infected erythrocyte. Glycolysis is the process of converting glucose into pyruvate and generating small amounts of ATP (energy) and NADH (reducing power). It is the central pathway that produces important precursor metabolites: six carbon compounds of glucose-6P and fructose-6P and three-carbon compounds of glycerone-P, glyceroldehyde-3P, glycerate-3P, phosphoenolpyruvate, and pyruvate. This figure was adapted from http://sites.huji.ac.il/malaria/maps/glycolysispath.html.

3.2.1 Nucleic acid metabolism

During intraerythrocytic schizogony, parasite increases DNA content and multiply rapidly to produce merozoites which varied depend on the species. Replication of DNA and its transcription to the various RNA species necessitate a constant and substantial supply of the constituent nucleotides. The nucleotides and/or their nucleoside and nucleobase precursors can be obtained in several ways: synthesis *de novo*, direct acquisition by salvage or by subsequent interconversions to yield the appropriate repertoire of purines and pyrimidine derivatives found in the nucleic acids and other important molecular species. There are 2 sources of nucleic acid syntheses, purine metabolism and pyrimidine biosynthesis.

3.2.1.1 Purine metabolism and salvage pathway

It's clear that Plasmodium is incapable of de novo purines biosynthesis [26] and absolutely dependent on exogenous purines from environment for synthesis of nucleic acids and other metabolic functions. Host erythrocyte is the abvious source of purine in the form of ATP, 80% of erythrocyte purine are in the form of ATP, as well as purine salvage enzymes. However parasites are unable to direct incorporate nucleotides from its host.

In erythrocyte, adenosine kinase (AK) converts ATP to AMP, which is then converted to inosine monophosphate (IMP) by AMP deaminase. The IMP is converted to inosine and then hypoxanthine which is taken up and used by the parasite, [27] reactions catalized by 5' nucleotidase and purine nucleoside phosphorylase respectively. Moreover plasmodia can also uptake free purines, adenosine, hypoxanthine, xanthine and guanine, from extracellular medium. On the other hands, malaria parasite also has its own enzymes for purine salvage pathway. Parasite purine salvage pathway begins with deamination of adenosine to inosine by ADA, followed by the conversion of inosine to hypoxanthine by PNP. Hypoxanthine is the precursor of all purines and is the central metabolite for nucleic acid synthesis of the parasite. The depletion of hypoxanthine from infected erythrocytes by xanthine oxidase reduced *P. falciparum* growth by ~90% showed clearly that it is the preferred purine source of the parasite [28]. The parasite enzymes convert IMP into adenine and guanine nucleotides, and ultimately these are used for DNA, RNA and nucleotide coenzymes. The purine salvage pathway of plasmodium-infected red cell is shown in

figure 3.3. Since parasite cannot synthesize purines de novo, the utilization of purines by salvage pathway enzymes becomes critical to the development of the parasite. Targeting enzymes in purine salvage pathway could be potential for drugs and vaccine development. Enzyme hypoxanthine guanine xanthine phosphoribosyl transferase (HGXPRT) is an important enzyme for purine nucleotides biosynthesis of parasite and has been targeted as new vaccine candidate [29].

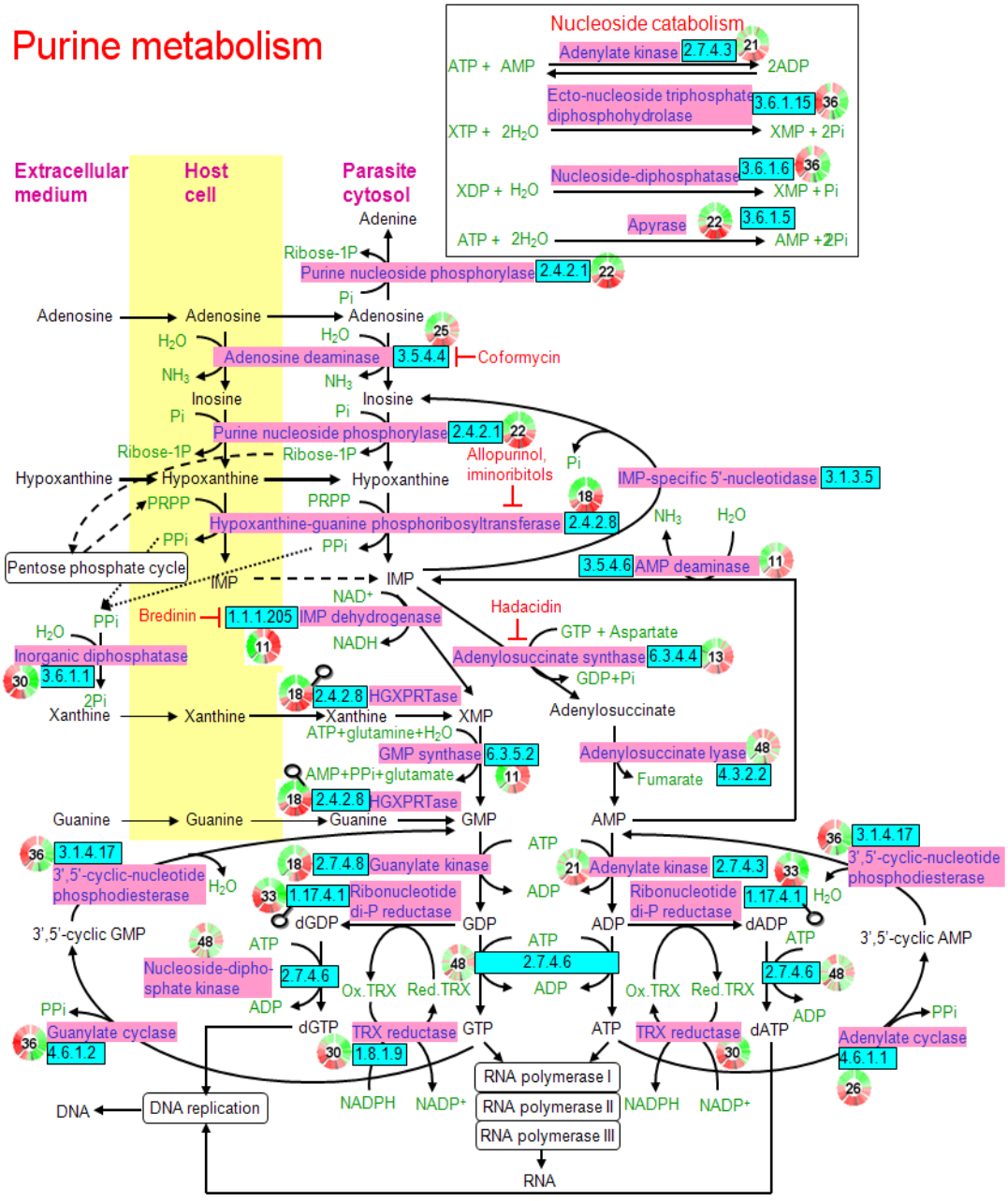


Figure 3.3 Postulated scheme of purine mertabolism in *Plasmodium*-infected erythrocyte.

3.2.1.1 Pyrimidine biosynthesis

By contrast to purines, which have to be uptake from extracellular medium, plasmodia have the capacity to synthesize pyrimidines de novo from simple amino acid precursor's glutamine and aspartic acid. This is a six step process whereby the pyrimidine derivative orotic acid is first assembled, then added to a ribose-5-phosphate moiety to form orotidine 5′-monophosphate. This is subsequently converted *via* decarboxylation to the parent mononucleotide uridine 5′-monophosphate (UMP), which is the precursor of all other pyrimidine nucleotides, figure 3.4. After a double phosphorylation of UMP to UTP, CTP synthase converts the latter to CTP. As well as the above ribonucleotides, deoxyribonucleotides must be made, and in most organisms the conversion to the deoxyribose forms takes place at the level of nucleoside diphosphates, catalysed by ribonucleotide reductase. Formation of thymine nucleotides requires methylation of dUMP to produce dTMP by thymidylate synthase in the folate pathway.

In mature erythrocyte, the importance source of pyrimidines is salvage pathway due to the lost of ability to synthesize pyrimidines *de novo* when mature. By contrast, salvage of pyrimidines is thought to be of little importance in the malaria parasites. The dependence of malaria parasites on pyrimidine biosynthesis and the ability of the host to use salvage pathways makes the *de novo* pathway in the parasite a potentially effective drug target.

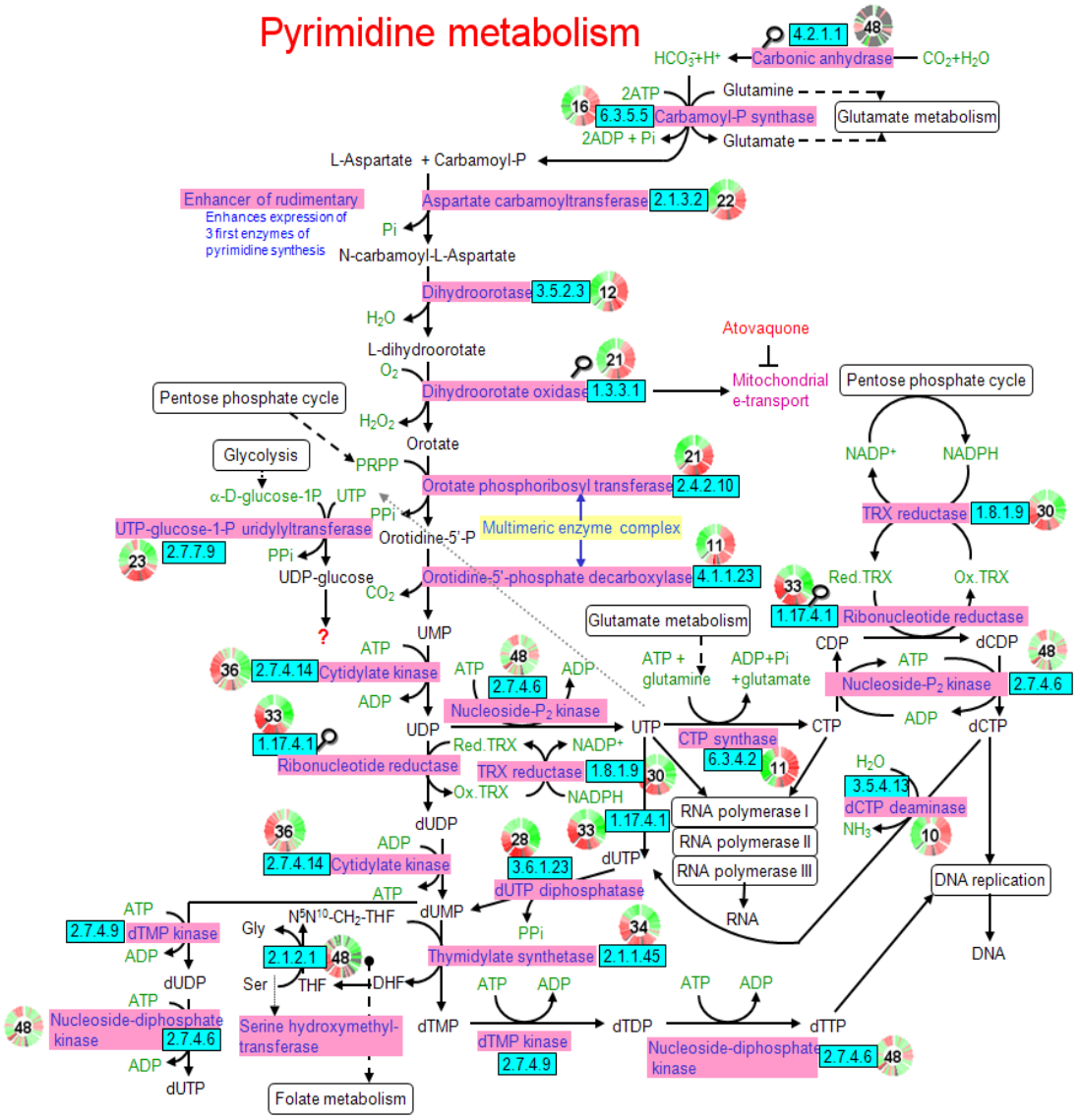


Figure 3.4 Postulated scheme of pyrimidine metabolism in *Plasmodium*-infected erythrocyte.

3.2.2 Protein synthesis

Proteins are important for parasite as their responsibility for structure and cellular function. Several sources of amino acids have been defined; de novo synthesis, uptake free amino acids from host plasma or degradation of hemoglobin.

Hemoglobin digestion is the major catabolic process that occurs in an acidic digestive vacuole which provides free amino acids for parasite growth, up to 65% of total host hemoglobin is digested by the parasite. Different enzymes involved in different level of hemoglobin digestion process; Aspartic proteases (Plasmepsins I) play a role in the initial step of hemoglobinolysis by cleaving the hinge region, the domain responsible for holding the tetramer together when oxygen is bound, of native hemoglobin. Cleavage at this site can be envisioned to unravel hemoglobin and facilitate its proteolysis by other degradative enzymes. Another aspartic proteases (Plasmepsin II) appears to be able to cleave the native hemoglobin but prefers the denatured molecule. The cysteine protease (falcipain) recognizes only the unwound or fragmented molecule; once the aspartic proteases have initiated proteolysis, it can make further attacks and proteolysis can proceed efficiently. At this step heme is released and polymerized into crystalline structure, hemozoin, and the small peptides, up to 20 amino acids in length, are cleaved by metallo-peptidase falcilysin [30]. Small peptides are then transported to the parasite cytosol where the function of amino peptidases takes place in order to release the free amino acids [31].

3.2.3 Lipid metabolism

Lipids are among the most critical components to be duplicated in malaria parasites. Fatty acids, serine, ethanolamine, and choline are of particular importance as they are the major building blocks used by the parasite in the synthesis of its structural and regulatory phospholipids. The metabolic pathways of the synthesis of phospholipids and fatty acids play a crucial role in the growth and proliferation of *Plasmodium* species during the various stages of their life cycle. The intracellular malarial parasites meet their demand for the necessary lipid species by active synthesis through de novo pathways using precursors that are actively transported from the host cytoplasm.

3.2.3.1 Phospholipids

In normal uninfected erythrocyte, cholesterol and phospholipids (PL) constitute the major lipids present at the molar ratio around 1. The major PLs are phosphatidylcholine (PC) (35-40%), phosphatidylethanolamine (PE) (30-35%), sphingomyelin (SM) (15%) and phosphatidylserine (PS) (10%). Phosphatidylinositol (PI) and other PLs, such as PA, account for <3% of total PL, and fatty acids (FA) and neutral lipids are barely detectable. In erythrocyte infected with plasmodium, there is an increase in PC, PE and PA [32]. Analysis of the lipid content of purified parasites identifies PC and PE as the two major PLs in the parasite membranes. PC represents 40-50% of the total PL content, whereas PE represents 35-45% which is unusually high for eukaryotic organism. PI represents 4-11%, whereas AM and PS account for < 5% of the total parasite PL content. In most eukaryotes, including various protozoan parasites, PC is synthesized by two routes. Synthesis can occur from choline via an enzymatic cascade (the de novo cytidine diphosphate (CDP)-choline pathway) involving three enzymes: choline kinase, CTP phosphocholine cytidylyltransferase, and choline/ethanolamine-phosphotransferase. The CDP-choline pathway initiates with the transport of choline from host serum into the infected erythrocyte. Choline is then phosphorylated to phosphocholine by choline kinase (CK), and subsequently coupled to CTP to generate CDP-Cho by a CTP-phosphocholine cytidylyltransferase (CCT) and further converted into PC by a CDP-diacylglycerol-cholinephosphotransferase (CEPT) in the presence of diacylglycerol. The second route is from phosphatidylethanolamine (PE) via three transmethylation reactions that involve one or two phospholipid methyltransferases. The source of PE is via ethanolamine which can be obtained in limited amounts from plasma and in larger quantities from decarboxylation of serine from either transported from the host or obtained from degradation of host hemoglobin. Ethanolamine formed via this reaction is subsequently phosphorylated into PE, which serves as a substrate for PE biosynthesis, or is converted into phosphocholine and incorporated into PC (figure 3.5).

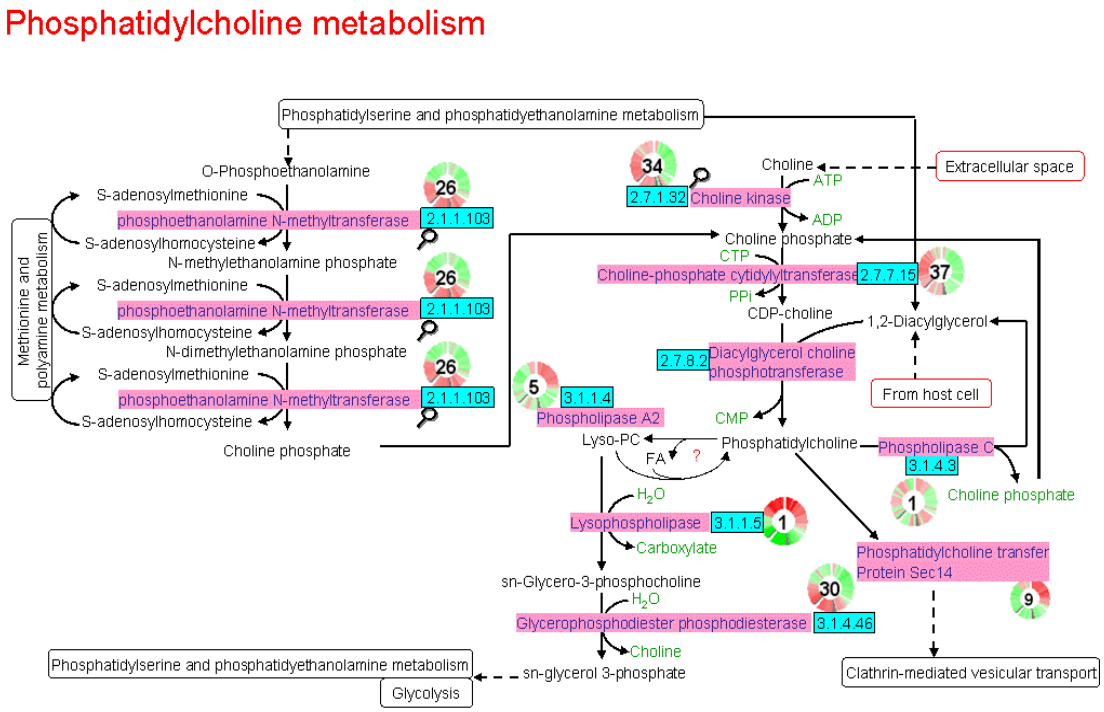


Figure 3.5 Postulated scheme of Phosphatidylcholine metabolism in *Plasmodium*-infected erythrocyte.

3.2.3.2 Fatty acids

Malaria parasites were thought to acquire all of the fatty acids required for blood stage growth through scavenging. By the time the *P. falciparum* genome was completed [7], genes encoding fatty acid biosynthesis enzymes had been identified which suggest the ability to synthesize fatty acids de novo. The main precursor for fatty acid synthesis is acetyl-CoA, which is synthesized from pyruvate by the pyruvate dehydrogenase complex or possibly by acetyl CoA-synthetase. Condensation reactions with acetyl-CoA and acyl carrier protein (ACP) are catalyzed by acetyl-CoA carboxylase, FabD and FabH before the FASII elongation cycle. The central hub of FAS II is the small, soluble protein ACP. In a typical FAS II pathway, [ACP] S-malonyltransferase transfers a malonyl group from malonyl-CoA to ACP. Malonyl-ACP is then the substrate for KASIII, which catalyzes the decarboxylative condensation of the malonyl group with an acetyl group donated by acetyl-CoA. The product of KASIII is then reduced to acyl-ACP by the sequential action of β-ketoacyl-ACP reductase (FabG), β-hydroxyacyl-ACP dehydratase (FabZ), and enoyl-ACP reductase (FabI). Further elongation of the acyl chain requires KASII (FabB/F) followed by FabG, FabZ and FabI. These four enzymes form an elongation cycle that extends the acyl-ACP product by two carbons per cycles and consumes malonyl-ACP. The fatty acid synthesis is illustrated in figure 3.6.

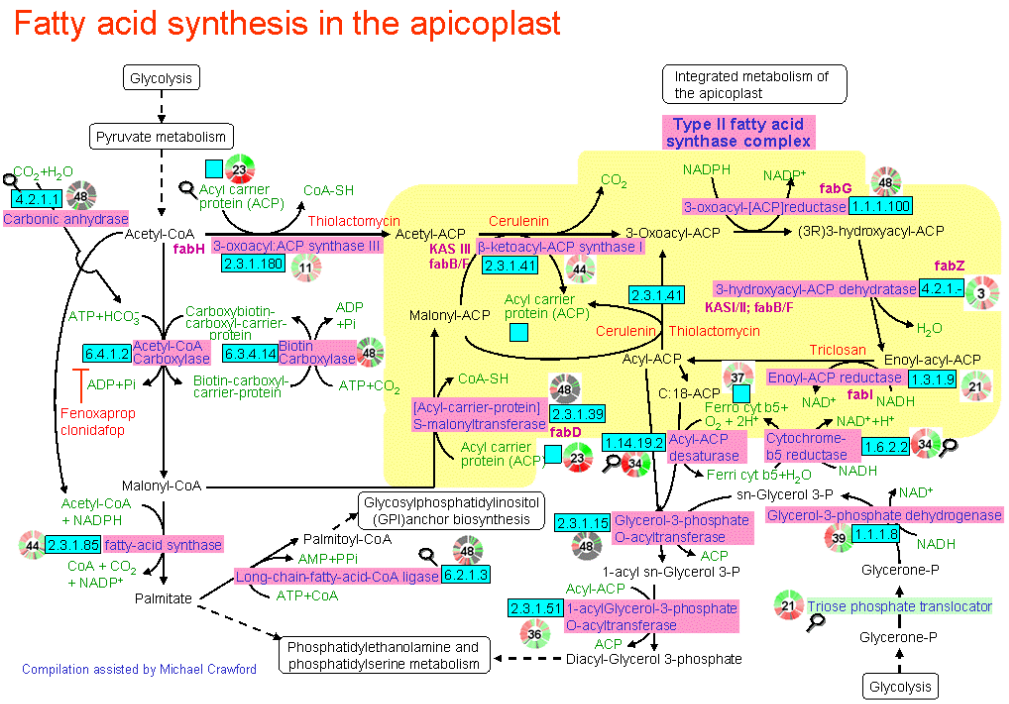


Figure 3.6 Postulated scheme of fatty acid synthesis in the apicoplast of *Plasmodium*.

Plasmodium vivax has several unique characteristics that distinguish it from other human malaria parasite species. Most notably, *P. vivax* forms hypnozoites in hepatocytes [2], causing relapses of the disease. *P. vivax* strains from the tropical and temperate zones can vary dramatically in terms of the pattern and frequency of the relapse. Moreover, *P. vivax* requires Duffy receptor on the red cell for invasion, and is thus absent in West Africa where Duffy negativity predominates [3]. It selectively invades reticulocytes [4], thus limiting parasitemias to low levels. Gametocyte production in *P. vivax* takes place much early after infection, even before the manifestation of clinical symptoms. Unlike *Plasmodium falciparum* infection that increases the rigidity of the host cell, *P. vivax* increases the size and deformability of infected red cells [5,6]. *P. vivax* also actively remodels the host cell, producing caveola-vesicle complexes along the plasmalemma in the infected red cell, which are visible in Giemsa-stained smears as multiple red dots called "Schuffner's dots". Apart from these characteristics, the ability of *P. vivax* to survive at much lower temperature has allowed this parasite to establish transmission foci in temperate zones. Despite that many of these unique features of *P. vivax* have been known for a long time, the underlying mechanisms remain poorly understood mainly due to the lack of a continuous *in vitro* culture method. The completion of *P. vivax* genome sequence has play significant role in understanding the unique biological features of this parasite but it is just a beginning to the understanding of cellular function at the molecular level. In contrast to the static genome, where all information could in principle be obtained from DNA, the proteome is dynamic and highly dependent not only on the type of cell, but also on the state of cell. Proteomics is thus of great importance, allowing the verification of whether the predicted coding sequence is actually translated and present amongst the proteins of an organism.

In the past decade, there have been remarkable advances in proteomic technologies. Mass spectrometry has emerged as the preferred method for in-depth characterization of the protein components of biological systems. Mass spectrometry has become the dominant technique for several reasons, mainly because of its unparalleled ability to acquire high-content quantitative information about biological samples of enormous complexity. Although these technologies will continue to be developed in the quest for improved sensitivity, throughput and proteome coverage,

mass-spectrometry-based proteomics has now developed to the point at which it is routinely applied worldwide to address a large range of biological problems.

3.3 Mass spectrometry

Basic principle of mass spectrometry (MS) is to generate ions from either inorganic or organic compounds, to separate these ions by their mass-to-charge ratio (m/z) and to detect them qualitatively and quantitatively by their respective m/z and abundance. The three essentials' functions of a mass spectrometer are:

1. Ion source
2. Mass analyzer
3. Detector

3.3.1 Ion source

The first step in the mass spectrometric analysis of compounds is the production of gas phase ions of the compounds. In the ion sources, the analyzed samples are ionized prior to analysis by mass spectrometer. The ion sources produce ions mainly by ionizing a neutral molecule through electro ejection, electron capture, protonation, deprotonation, adduct formation or by the transfer of a charged species from a condensed phase to the gas phase. A variety of ionization techniques are used for mass spectrometry. The most important considerations are the internal energy transferred during the ionization process and the physio-chemical properties of the analyte that can be ionized. Some ionization techniques are very energetic and cause extensive fragmentation. Other techniques are softer and only produce ions of the molecular species. Electron ionization, chemical ionization and field ionization are only suitable for gas-phase ionization and thus their use is limited to compounds sufficiently volatile and thermally stable. However, a large number of compounds are thermally labile or do not have sufficient vapor pressure. Molecules of these compounds must be directly extracted from the condensed to the gas phase. These direct ion sources exist under two types:

3.3.1.1 Electrospray ionization (ESI)

Electrospray ionization (ESI) is a soft ionization technique that accomplishes the transfer of ions from solution to the gas phase. The technique is

extremely useful for the analysis of large, non-volatile, chargeable molecules such as proteins and nucleic acid polymers.

Ion formation composed of three steps (figure 3.7):

3.3.1.1.1 Formation of an Electrospray

To understand the formation of continuous spray, consider the surface of an ion-containing liquid at the end of an electrically conducting capillary. At the open end of the capillary this liquid is exposed to an electric field. The electric field causes charge separation in the liquid and finally deformation of the meniscus into a cone. In detail, the surface starts forming an oval under the influence of increasing field strength. When certain field strength is reached, the Taylor cone instantaneously forms and immediately starts ejecting a fine jet of liquid from its apex towards the counter electrode. The jet carries a large excess of ions of one particular charge sign but cannot remain stable for an elongated period so it breaks up into small droplets. Due to their charge, these droplets are driven away from each other.

3.3.1.1.2 Disintegration of Charged droplets

When a micrometer-sized droplet carrying a large excess of ions of one particular charge sign evaporates some solvent, the charge density on its surface is continuously increased. As soon as electrostatic repulsion exceeds the conservative force of surface tension, disintegration of the droplet into smaller sub-units will occur. This process should occur repeatedly to generate increasingly small microdroplets. While the model of a cascading reduction in size holds valid, more recent work has demonstrated that the micro droplets do not explode, but eject a series of much smaller microdroplets from an elongated end. The ejection from an elongated end can be explained by deformation of the flying microdroplets. The charge density on microdroplet is not homogenous, but significantly increased in the region of sharper curvature. The smaller offspring droplets carry off only about 1-2% of the mass, but 10-18% of the charge of the parent droplet.

3.3.1.1.3 Formation of ions from charged droplets.

There is a continuing debate about ion formation by the charged-residue-model (CRM) and the ion evaporation model (IEM). The elder model of ion formation, CRM, assumes the complete desolvation of ions by successive loss of all solvent molecules from droplets that are sufficiently small to contain just one analyte molecule in the end of the cascade of Coulomb fissions. The charges (protons) of this ultimate droplet are then transferred onto the molecule. This would allow that even large protein molecules can form singly charged ions. A later theory, IEM, described the formation of desolvatd ions as directed evaporation from the surface of highly charged microdroplets. The IEM corresponds well to the observation that the number of charges is related to the number of fraction of the microdroplet's surface that a molecule can cover. As the radius diminishes, molecule size and number of droplet charges remain constant but the spacing of the surface charges decreases, thus the increasing charge density brings more charges within the reach of an analytical molecule. Flat and planar molecules therefore exhibit higher average charge states than spherical ones.

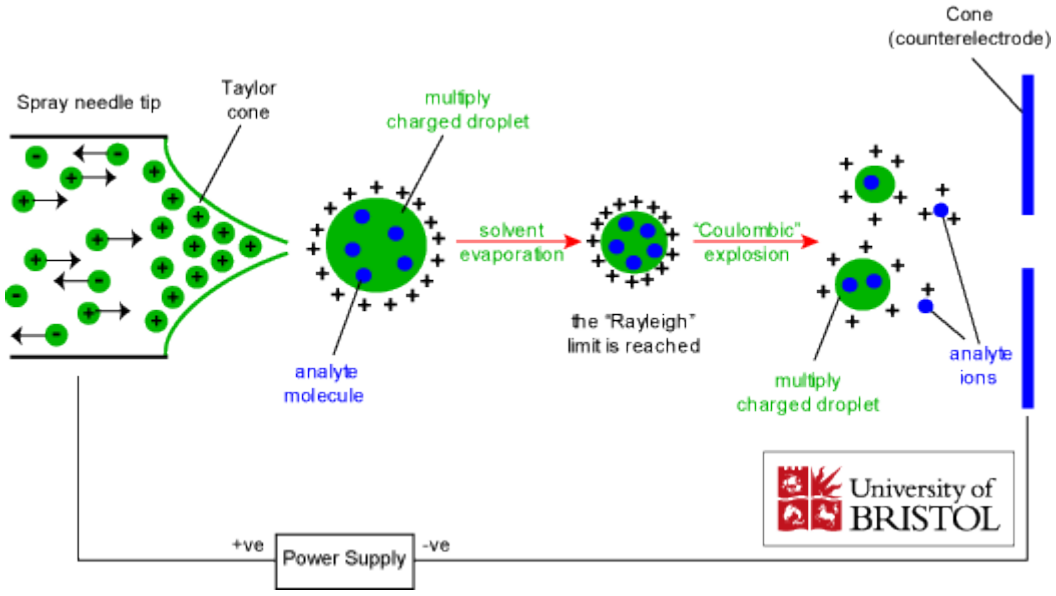


Figure 3.7 Ions formation in ESI (http://www.bris.ac.uk)

3.3.1.2 Matrix-assisted laser desorption/ionization (MALDI)

Matrix-assisted laser desorption/ionization (MALDI) is one of the two “soft” ionization techniques besides ESI. It is being used for rapid identification of proteins using trytic peptide mass mapping of gel-purified proteins. Other applications include visualization of native peptides directly in tissue and even single cells.

In MALDI experiment, the matrix must be added to enhance the ionization efficiency of the analytes. Good matrices generally have high absorption coefficients for the laser wavelength of interest and are usually acidic to be able to donate a proton (positive ion mode) in the plume. The whole trick to success with MALDI is finding the right matrix. Some matrices are extremely good at ionization; others are preferred for proteins, others for DNA, still others for oligosaccharides. The most commonly used matrices are alpha-cyano-hydroxycinnamic acid and sinapinic acid (for peptides on MALDI-TOF instruments), 2, 5-dihydroxy-benzoic acid (for proteins on MALDI-TOF and for proteins/peptides/labile molecules on decoupled instruments), hydroxypicolinic acid (for DNA), and succinic acid (for IR-MALDI). A solution of one of these molecules is made, often in a mixture of highly purified water and an organic solvent.

The mechanism of MALDI is composed of three basic steps (figure 3.8):

1. Formation of a solid solution

It is essential for the matrix to be in access thus leading to the analyte molecules being completely isolated from each other. This eases the formation of the homogenous 'solid solution' required to produce a stable desorption of the analyte.

2. Matrix Excitation

The laser beam is focused onto the surface of the matrix-analyte solid solution. The chromophore of the matrix couples with the laser frequency causing rapid vibrational excitation, bringing about localized disintegration of the solid solution. The clusters ejected from the surface consist of analyte molecules surrounded by matrix and salt ions. The matrix molecules evaporate away from the clusters to leave the free analyte in the gas-phase.

3. Analyte Ionization

The photo-excited matrix molecules are stabilized through proton transfer to the analyte. Cation attachment to the analyte is also encouraged during this process. It is in this way that the characteristic $[M+X]^+$ (X= H, Na, K etc.) analyte ions are formed. These ionization reactions take place in the desorbed matrix-analyte cloud just above the surface. The ions are then extracted into the mass spectrometer for analysis.

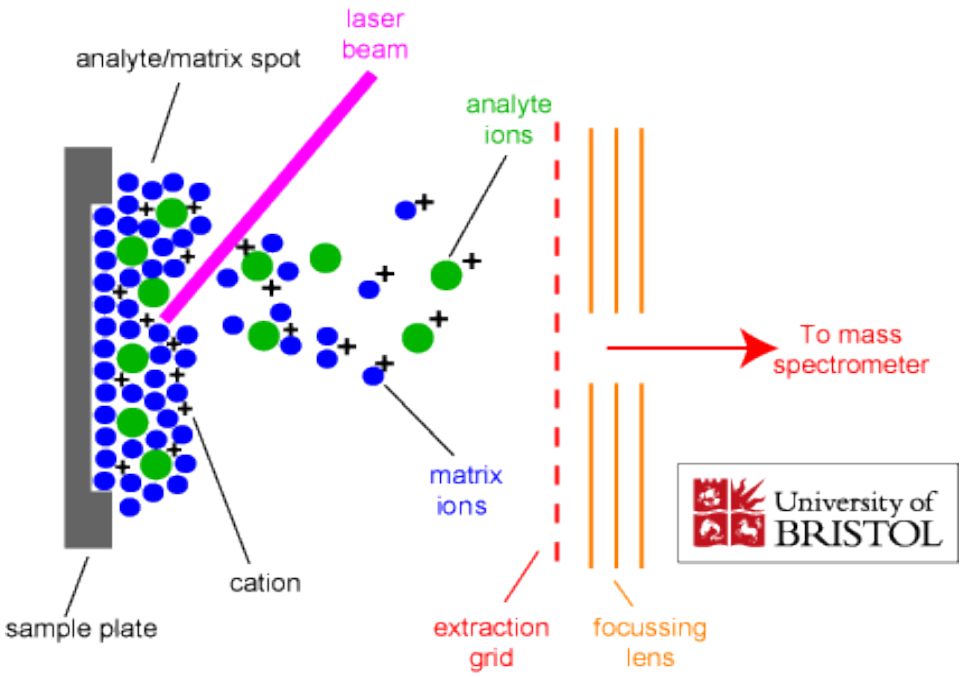


Figure 3.8 Ions formation in MALDI (http://www.bris.ac.uk)

3.3.2 Mass analyzer

Each mass analyzer has its own special characteristics and applications and its own benefits and limitations. The choice of mass analyzer should be based upon the application, cost, and performance desired. There is no ideal mass analyzer that is good for all applications. The basic types of mass analyzers employed for analytical mass spectrometry are time-of-flight (TOF), Quadrupole linear ion trap (LIT), Quadrupole ion trap (QIT), and FT-ICR. This review is focused on TOF and quadrupole instruments.

3.3.2.1 Time-of-Flight instruments

Time-of-flight (TOF) is the simplest measurement method that determines the time required for an ion to move across a set distance. The principle of TOF is quite simple: ions of different m/z are dispersed in time during their flight along a field-free drift path of known length (figure 3.9). Provided all the ions start their journey at the same time or at least within a sufficiently short time interval, the lighter ones will arrive earlier at the detector than the heavier ones. The ions leaving the ion source of a time-of-flight mass spectrometer have neither exactly the same starting times nor exactly the same kinetic energies. Various time-of-flight mass spectrometer designs have been developed to compensate for these differences. A reflectron is an ion optic device in which ions in a time-of-flight mass spectrometer pass through a “mirror” or “reflectron” and their flight is reversed. A linear-field reflectron allows ions with greater kinetic energies to penetrate deeper into the reflectron than ions with smaller kinetics energies. The ions that penetrate deeper will take longer to return to the detector. If a packet of ions of a given mass-to-charge ratio contains ions with varying kinetic energies, then the reflectron will decrease the spread in the ion flight times, and therefore improve the resolution of the time-of-flight mass spectrometer.

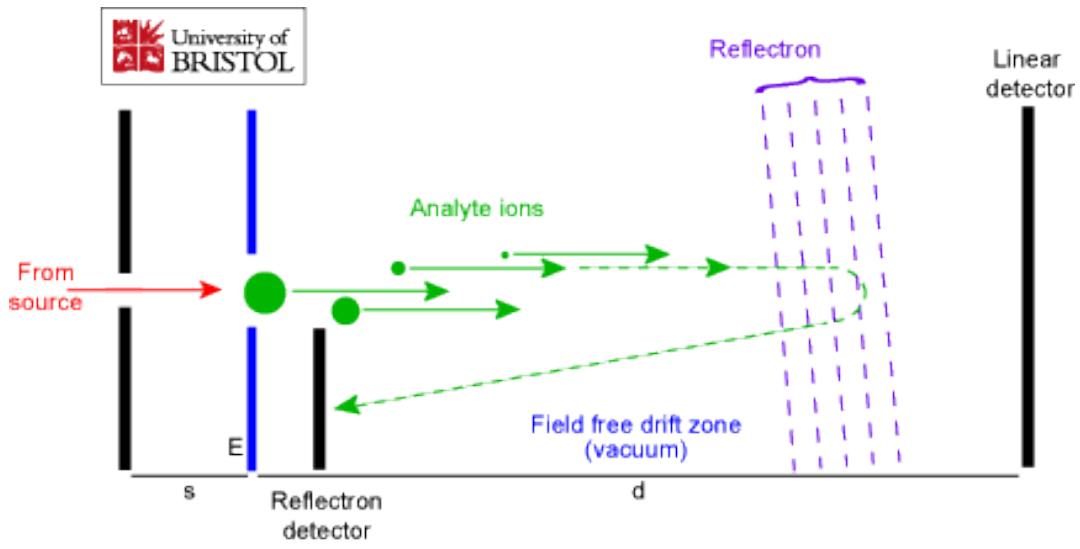


Figure 3.9 Schematic of a Time-of-Flight mass spectrometer operating in Reflectron Mode (http://www.bris.ac.uk).

3.3.2.2 Quadrupole Mass spectrometer

The quadrupole mass analyzer is a "mass filter". It consists of four parallel hyperbolically or cyclindrically shaped rods electrodes extending in the Z direction and mounted in a square configuration (figure 3.10). Each opposing rod pair is connected together at the same potential. Combined DC and RF potentials on the quadrupole rods can be set to pass only a selected mass-to-charge ratio. Ions travel down the quadrupole between the rods in which only ions of a certain *m/z* will reach the detector. All other ions have unstable trajectory through the quadrupole mass analyzer and will collide with the quadrupole rods, never reaching the detector. Quadrupole rods can have other functions besides their use as a mass filter. An RF-only quadrupole will act as an ion guide for ions which is important for transfer ions of low kinetic energy from one region to another without substantial losses, e.g. from an atmospheric pressure ion source (ESI) to the entrance of a mass analyzer.

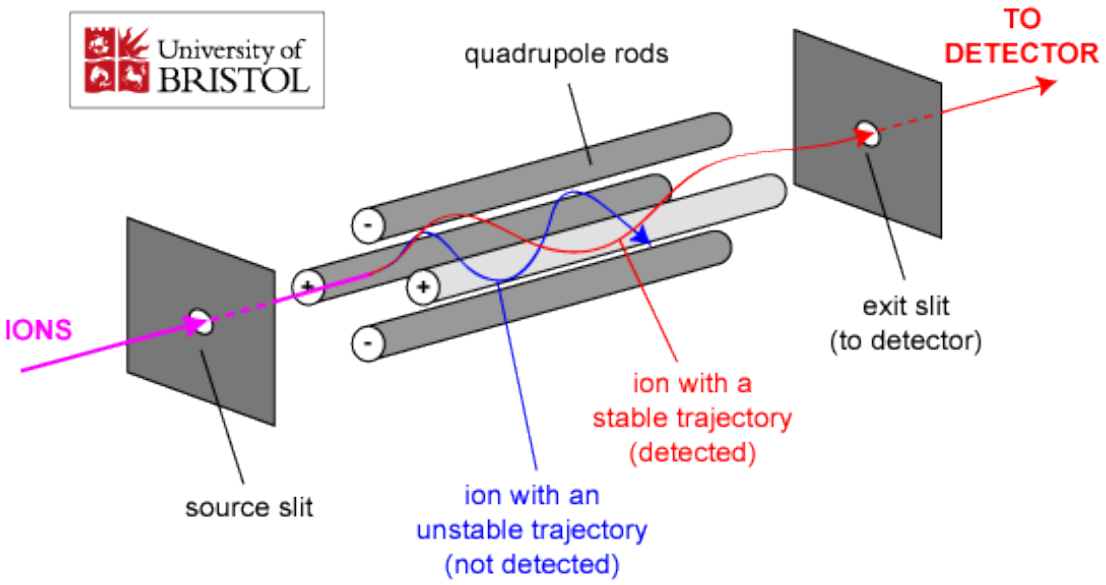


Figure 3.10 Crude schematic of a quadruple mass filter (http://www.bris.ac.uk).

3.3.2.3 Trapped-Ion Mass Analyzers

There are two principal trapped-ion mass analyzers: three-dimensional quadrupole ion traps ("dynamic" traps), and ion cyclotron resonance mass spectrometers ("static" traps). Both operate by storing ions in the trap and manipulating the ions by using DC and RF electric fields in a series of carefully timed events. This provides some unique capabilities, such as extended MS/MS experiments, very high resolution, and high sensitivity. The tradeoff is that trapping the ions for long periods of time (milliseconds to *days*) provides plenty of time for the ions fall apart spontaneously (unimolecular decomposition), to experience undesirable interactions with other ions (space charge effects), neutral molecules (ion-molecule reactions), or perturbations in the ion motion due to imperfect electric fields. This can lead to artifacts and unexpected changes in the mass spectrum (so called "non-classical mass spectra"). Only the Quadrupole Ion Trap is focused in this review.

3.3.2.3.1 Quadrupole Ion Trap

The Quadrupole Ion Trap (QIT) composed of 2 hyperbolic electrodes serving as end caps and a ring electrode that replaces two of the linear quadrupole rods. The ends caps are electrically connected and the DC and RF potentials are applied between them and the ring electrode. The working principle of QIT is based on creating stable trajectories for ions of a certain m/z or m/z range while removing unwanted ions by colliding them with the walls or by axial ejection from the trap due to their unstable trajectories. Ions are formed within the ion trap or injected into an ion trap from an external source (figure 3.11). The RF and DC potentials can be scanned to eject successive mass-to-charge ratios from the trap into the detector (*mass-selective ejection*). The ions are dynamically trapped by the applied RF potentials (a common trap design also makes use of a "bath gas" to help contain the ions in the trap). The trapped ions can be manipulated by RF events analogous to the events in FTICR to perform ion ejection, ion excitation, and mass selective ejection. This provides MS/MS and MS/MS/MS experiments analogous to those performed in FTICR.

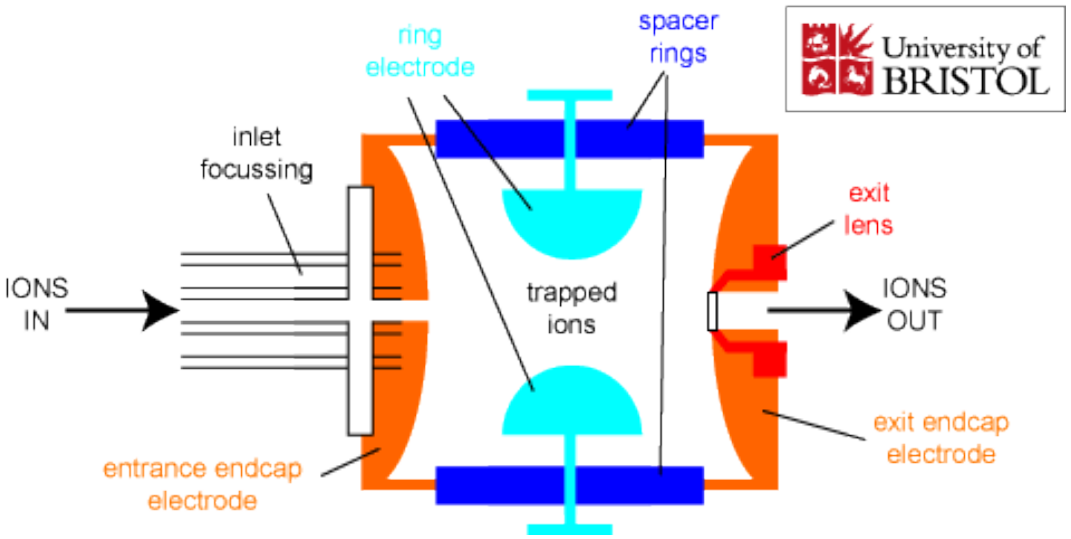


Figure 3.11 Schematic (cutaway view) of a quadrupole ion trap mass analyzer (http://www.bris.ac.uk).

3.3.3 Detector

The final element of the mass spectrometer is the detector. The detector records either the charge induced or the current produced when an ion passes by or hits a surface. In a scanning instrument, the signal produced in the detector during the course of the scan versus where the instrument is in the scan (at what *m/Q*) will produce a mass spectrum a record of ions as a function of *m/Q*. Typically, some type of Electron multiplier is used, though other detectors including Faraday cups and Ion-to-Photon detectors are also used. Because the number of ions leaving the mass analyzer at a particular instant is typically quite small, considerable amplification is often necessary to get a signal. Micro channel plate detectors are commonly used in modern commercial instruments.

3.3.3.1 Electron multiplier

Electron multiplier is a vacuum-tube structure that multiplies incident charges (figure 3.12). In a process called “secondary emission” a single electron can, when bombarded on secondary emissive material, induce emission of roughly 1 to 3 electrons. If an electric potential is applied between these metal plates and yet another, the emitted electrons will accelerate to the next metal plate and induce secondary emission. This can be repeated a number of times, resulting in a large shower of electrons all collected by a metal anode, all having been triggered by just one.

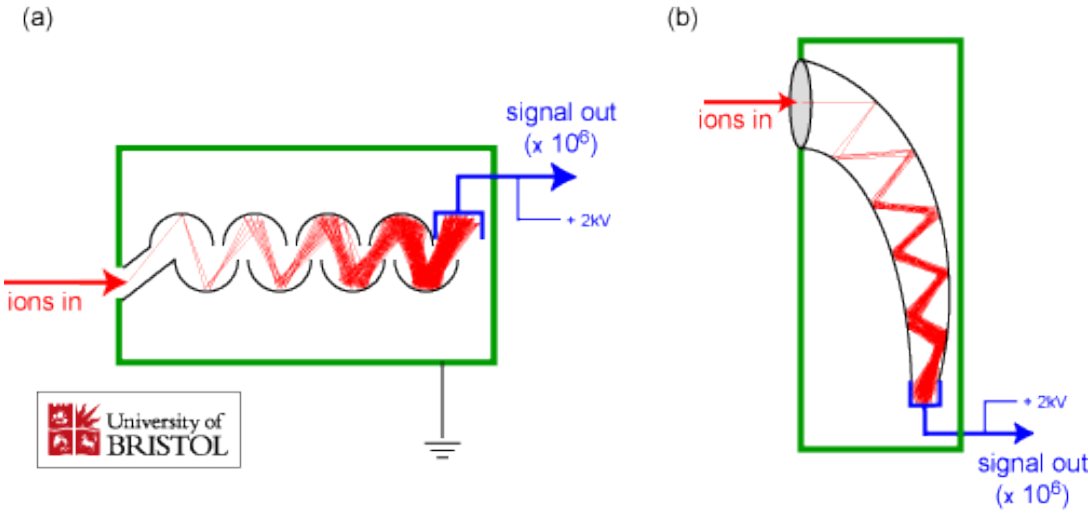


Figure 3.12 Schematics of the two types of electron multiplier, showing the cascade of electrons that result in amplification (http://www.bris.ac.uk).

3.3.3.2 Faraday cup

Faraday cup is a Metal (conductive) cup designed to catch charged particles in vacuum (figure 3.13). The resulting current can be measured and used to determine the number of ions or electron hitting the cup. When a beam or packet of ion hits the metal it gains a small net charge while the ions are neutralized. The metal can then be discharged to measure a small current equivalent to the number of impinging ions. Essentially the faraday cup is part of a electrical circuit where ions are the charge carriers in vacuum and the faraday cup is the interface to the solid metal where electrons act as charge carriers (as in most circuits). By measuring the electrical current (the number of electrons flowing through the circuit per second) in the metal part of the circuit the number of charges being carried by the ions in the vacuum part of the circuit can be determined.

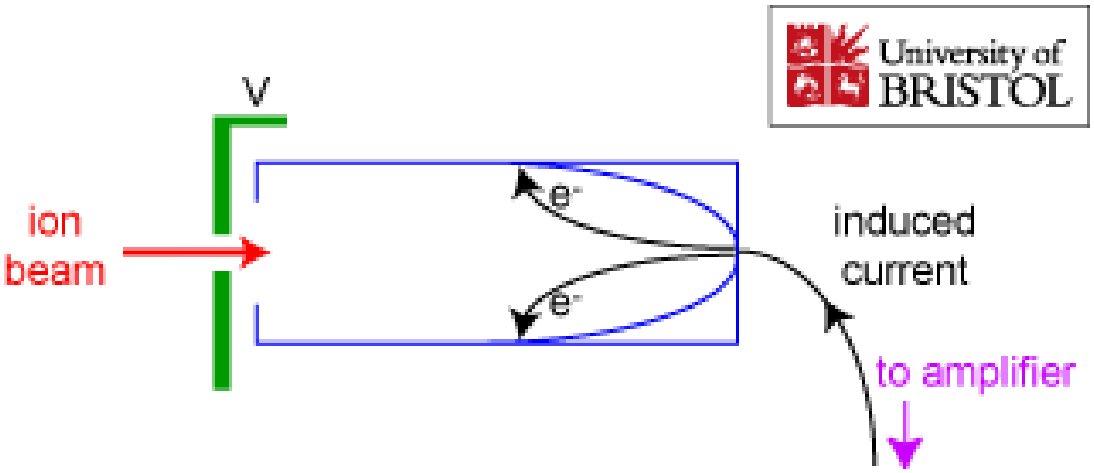


Figure 3.13 Schematic of a Faraday Cup or Cylinder Electrode (http://www.bris.ac.uk).

3.3.3.3 Photomultiplier or scintillation counter

In the photomultiplier (or scintillation counter) the ions initially strike a dynode which results in electron emission (figure 3.14). These electrons then strike a phosphorous screen which in turn releases a burst of photons. The photons then pass into the multiplier where amplification occurs in a cascade fashion - much like with the electron multiplier. The main advantage of using photons is that the multiplier can be kept sealed in a vacuum preventing contamination and greatly extending the lifetime of the detector. Photomultipliers are now probably the most common detectors in modern mass spectrometers.

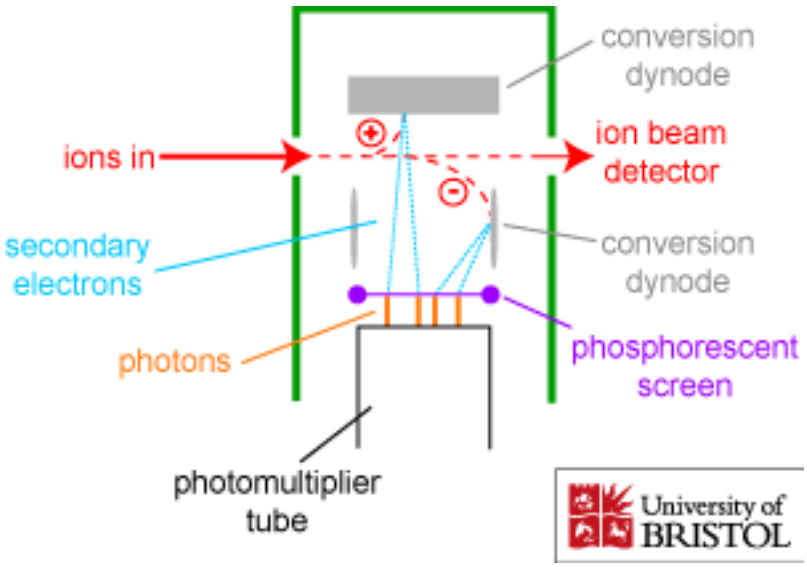


Figure 3.14 Schematic of a photomultiplier, showing the conversion of the ion/electron signal into photon(s) which are then amplified and detected by the photomultiplier (http://www.bris.ac.uk).

Table 3.2 Common types of mass analyzer

Type	Acrynym	Benefits	Limitations	Application
Time-of-Flight	TOF	• Fastest MS analyzer • Well suited for pulsed ionization methods (method of choice for majority of MALDI mass spectrometer systems) • High ion transmission • MS/MS information from post-source decay • Highest practical mass range of all MS analyzers	• Requires pulsed ionization method or ion beam switching (duty cycle is a factor) • Fast digitizers used in TOF can have limited dynamic range • Limited precursor-ion selectivity for most MS/MS experiments	• Almost all MALDI system. • Very fast GC/MS systems
Linear Quadrupole	Q	• Good reproducibility • Relatively small and low-cost systems • Low ion acceleration voltages • High transmission	• Limited resolution • Peak heights variable as a function of mass (mass discrimination). • Peak height vs. mass response must be 'tuned'. • Not well suited for pulsed ionization methods	• Majority of benchtop GC/MS and LC/MS systems • Triple quadrupole MS/MS systems • Sector / quadrupole hybrid MS/MS systems
Quadrupole Ion Trap	QIT	• High sensitivity • Multi-stage mass spectrometry (analogous to FTICR experiments) • Compact mass analyzer	• Poor quantitation • Very poor dynamic range (can sometimes be compensated for by using auto-ranging) • Subject to space charge effects and ion molecule reactions • Collision energy not well-defined in CID MS/MS	• Benchtop GC/MS, LC/MS and MS/MS systems • Target compound screening • Ion chemistry

3.4 Hybrid mass spectrometry

Tandem mass spectrometers (MS/MS) combine two (or more) different types of mass analyzers to enhance determination of molecular structure. The MS/MS involves two stages of MS. In the first stage of MS/MS, ions of a desired *m/z* are isolated from the rest of the ions emanating from the ion source. These isolated ions (termed parent ions or precursor ions) are then induced to undergo a chemical reaction that changes either their mass (*m*) or charge (*z*).Typically; the reactions involve some type of process to increase the internal energy of the ions, leading to dissociation. The ions resulting from the various chemical reactions are termed product ions, and these are analyzed with the second stage of MS/MS. With some instruments, it is possible to repeat this process; leading to what is termed an MS^n experiment, where *n* is equal to the number of stages of MS performed. The MS/MS is particularly useful when analyzing complex mixtures. The early promise of MS/MS led to the development of the triple quadrupole (QqQ) instrument as a cheaper, easier-to use. The triple quadrupole system combines three of the same type of mass analyzers. The first quadrupole is used to separate one ion (m/z value) from all other ions that may be entering at the same time. The middle quadrupole is enclosed in a canister so it can be used as a gas-phase collision cell. When a gas, such as argon, is introduced at low pressure into the cell, gas-phase collisions activate ions to dissociate as they pass through it. Ions produced in this way fragment in a diagnostic manner that reveals information about the covalent structure of the molecule. These ions are then analyzed in the third quadrupole. Following the development of the QqQ instrument, various hybrid instruments were developed. These instruments coupled different types of analyzer together to produce tandem MS instruments. Of the variety of hybrid instruments that were developed, one the quadrupole time-of-flight (Q/TOF), which combines two quadrupole mass filters with a time-of-flight analyzer as the third mass analyzer, has evolved into a mainstay of modern MS/MS instruments. Advantages to this format are the high resolution and mass accuracy capability of the TOF mass analyzer, which yield improved data for both m/z measurement and tandem mass spectrometry analyses.

3.5 Protein identification by mass spectrometry

The advent of large-scale genomic sequencing has greatly simplified the task of determining the primary structures of peptides and proteins in many organisms, because open reading frames in the nucleotide sequence serve as templates for the construction of the corresponding proteins. The masses of peptides produced by proteolytic digestion of an unknown protein can be compared with those predicted to arise from each protein in the database; this 'mass mapping' is often sufficient to identify any protein whose full-length sequence is contained therein. Nevertheless, the genome sequences of most organisms are still unknown. Even for those that are known, modifications such as post-translational events may prevent the identification of all or part of the protein sequence, or at least the definition of the modifications. Thus, complete characterization of the protein primary structure often requires determination of the protein sequence with minimal assistance from genomic data, de novo protein sequencing. Early de novo protein sequencing measurements relied on Edman degradation of the protein, but mass spectrometry (MS) has reduced the need for this technique because it is more sensitive and provides higher sample throughput. It can also cope better with protein mixtures and with modifications to the protein N terminus.

Identification of proteins by mass spectrometry uses peptide masses or the MS/MS fragmentation of a peptide to identify proteins.

3.5.1 Peptide Mass Fingerprinting

Peptide Mass Fingerprinting (PMF) is a technique used to identify proteins by matching their constituent fragment masses (peptide masses) to the theoretical peptide masses generated from a protein or DNA database. The first step in PMF is that an intact, unknown protein is cleaved with a proteolytic enzyme to generate peptides. The premise of peptide mass fingerprinting is that every unique protein will have a unique set of peptides and hence unique peptide masses. Identification is accomplished by matching the observed peptide masses to the theoretical masses derived from a sequence database. PMF identification relies on observing a large number of peptides from the same protein at high mass accuracy. This technique does well with 2D gel spots where the protein purity is high.

3.5.2 MS/MS Peptide Identification:

MS/MS spectral matching uses the un-interpreted peaks in a peptide fragment spectrum to match to a theoretical fragment spectrum in a sequence database. This MS/MS fragment spectrum is the result of a collision induced dissociation, (CID), occurring within a mass spectrometer. The fragmentations are produced either in a collision cell in a tandem mass spectrometer or within an ion trap. In an MS/MS based protein ID experiment multiple peptides are usually found and all of their fragment spectrums are used to correlate to a protein. Hits are ranked by how many of the fragment masses match the theoretical fragment masses in the sequence database. The protein with the greatest number of well correlated peptides is usually the most significant hit. Proteins can be correlated with the fragmentation of a single peptide using this technique.

CHAPTER IV
MATERIALS AND METHODS

4.1. Processing of blood samples

Fresh isolates of *P. vivax* were collected from acute infected patients who were presenting themselves to the malaria clinic in Mae Sot district, Tak province, Thailand. The study protocol was approved by the Ethical Review Committee of Mahidol University under the auspices of the Ethics Committee of the Thai Ministry of Public Health (ethical number MU-IRB2009/300.0112). The written informed consent was obtained from all human adult volunteers and from parents or legal guardians of volunteers who has the age less than 18 years before the blood samples were collected. Twenty milliliters of *P. vivax* infected blood was drawn into sterile tube containing heparin and centrifuged at 2,500 rpm for 10 minutes. The plasma was aliquot to 1.5 ml microcentrifuge tubes and kept at -20°C. Packed infected red blood cells (iPRBCs) were then washed once with 20 ml of RPMI1640 medium. In order to remove white blood cells, iPRBCs were resuspended in 20 ml RPMI1640 and passed through Plasmodipur® filter pre-equilibrated with 5 ml of RPMI1640. The filtrate was centrifuged at 2,500 rpm for 10 min. After discarded supernatant, *P. vivax* parasites were cultured in T75 cm^3 tissue culture flask by resuspended iPRBCs with McCoy's 5A medium supplemented with 25% human AB serum to 5% hematocrit and maintained at 37°C with 5% CO_2. Blood smears were checked regularly to confirm maturation of the parasites.

4.2. Preparation of parasite samples

Mature asexual stages parasites were purified from infected blood by using 60% Percoll® (0.6 volume of 10X RPMI 1640, 5.4 volume of Percoll® and 4 volume of 1X RPMI 1640). Parasite cultures were centrifuged at 2,500 rpm for 10 min to remove culture medium and then washed twice with RPMI 1640. The iPRBCs were diluted with RPMI1640 to 20% hematocrit then gently over-layered onto 60%

Percoll® with the ratio of diluted iPRBCs and 60% Percoll® 2:1 and centrifuged at 2,800 rpm for 20 min at room temperature (RT). The parasite interface was collected and washed twice with Phosphate buffered saline (PBS, pH. 7.4). Parasites were then released by cold PBS containing 0.01% saponin. The suspension was incubated on ice bath for 10 min and centrifuged at 1,200 rpm for 5 min at 4°C. After removed the supernatant, parasite pellet was washed with cold PBS until supernatant was cleared and stored at -70°C until analysis. Every effort was made to minimize enzymatic activity and protein degradation during sampling.

4.3 Separation of parasite proteins

4.3.1 Separation of parasite proteins by SDS-PAGE

The mature asexual stages parasites (10^9 cells) were suspended in 100 mM Tris-Cl pH 7.2 and broken for four 10-sec pulses. Proteins were dissolved in 2X SDS-polyacrylamide gel electrophoresis (PAGE) loading buffer and separated on a linear gradient (4-20%) Tris-glycine mini gel, 1.5 thickness (NOVEX®, invitrogen, USA) at 120 volts for 6 h in Tris-glycine buffer (pH8.3). Gel was stained by Colloidal blue (NOVEX®, invitrogen, USA) for 12 h and destained with dH_2O. Protein bands were horizontally sliced into sections, cut into small pieces (1X1 mm) and kept in separated 1.5 ml siliconized tubes before subjected to in-gel trypsin digestion.

4.3.2 Separation of *P. vivax* proteins by Two-dimensional gel electrophoresis (2DE)

Pooled parasite pellets was solubilized in 2D rehydration buffer [8 M urea, 0.5% CHAPS, 60 mM dithiothreitol (DTT)], and 0.5% ampholyte pH 3–10. Then the sample was vortex followed by centrifugation at 15,000 × g for 10 min and the supernatant was collected. Protein concentration was determined by using Protein assay kit (Biorad) and measured the absorbance (OD) at 595 nm. Forty micrograms of parasite's proteins was subjected to 2D gel electrophoresis. In 2D gel electrophoresis, 2 steps of separation were accompanied. The first step was isoelectric focusing (IEF) which separate proteins according to their pI. The IEF of parasite's proteins was performed with pre-cast Immobiline® Drystrip, 7cm long, pH3-10 using the Ettan IPGphor 3 IEF apparatus (Amersham Bioscience AB, Uppsala, Sweden). The running protocol was as followed; step1, 300 v, 200 Vhr, step2 gradient, 1000 v, 300 Vhr,

step3 gradient, 5000v, 1,400 Vhr and step4, 5000v, 2000 Vhr. The focused strips were equilibrated in 10 ml equilibration solution (50 mM Tris-HCl, pH8.8, 6 M urea, 30% glycerol, 2% SDS, 1% DTT) for 15 min, followed by 10 ml equilibration solution containing 2.5% iodoacetamide instead of DTT for another 15 min. The second step was SDS-PAGE which separate proteins according to their molecular weight. The equilibrated strips were dipped with tris-glycine, pH8.3 running buffer and loaded on 12% gel. The SDS-PAGE was run at 120 volts in Tris-glycine running buffer (pH8.3) for 2 h. One gel was silver stained by using mass spectrometry compatible method according to the manufacturer's protocol (PlusOne Silver staining kit, Amersham) while another gel was used for western blot analysis.

4.4 Visualizations of parasite proteins in protein gel

4.4.1 Colloidal blue stain

After the separation of parasite proteins by 4-20% gradient gel SDS-PAGE, the gel was rinsed with distilled water (dH_2O). Protein bands were visualized by incubated the gel with 200 ml staining solution (Novex®, invitrogen, USA) for 12 h on a rotary shaker. The gel was then destained with at least 200 ml of deionized water for at least 7 hr. Gel can be left in water for up to 3 days without significant changed in band intensity.

4.4.2 Silver stain (mass spectrometry compatible)

The 2D gel was fixed twice for 60 min with 250 ml fixing solution (50% ethanol and 5% acetic acid, glacial); the first fixation may be prolonged up to 3 days. The gel was then sensitized for 60 min with 250 ml sensitizing solution containing 0.2% sodium thiosulfate, glutaraldehyde was omitted in this step. After washed 8 min for five times with distilled water, gel was incubated for 60 min with 250 ml of 0.25% silver nitrate solution without formaldehyde. The gel was washed 1 min for 4 times with distilled water. Protein spots were visualized by adding 250 ml of developing solution. When the desired intensity of protein spots was reached, stopped the reaction by incubated with 250 ml stopping solution for 45 min and washed twice for 30 min with distilled water.

4.5 Immunoblot analysis

Protein spots from unstained 2D gel were transferred to nitrocellulose (NC) membrane (0.45 µ, Pharmacia) by using wet-tanked transfer system (Biorad). Fiber pads, filter papers, and NC membrane were equilibrated for 10 min in cold Transfer buffer (Tris-Glycine containing 20% methanol). Placed pre-equilibrated fiber pad on the cathode (negative, black color) followed by filter paper, protein gel, NC membrane, filter paper and fiber pad. Avoid air bubbles which may cause the localization of non-transferred proteins. Proteins were transferred by using transfer buffer for 4 h at 100 volts in cold condition. The NC membrane was then blocked with 5% skimmed milk Tris-buffered saline (TBS, pH7.2) for overnight in cold condition. The membrane was washed thrice with TBS containing 0.05% Tween-20 TBST) for 5 min at RT. The membrane was then incubated with immune serum from a *P. vivax* patient diluted with blocking buffer at 1:10 dilution for 2 h with rotation at RT. After washed membrane thrice, the HRP-conjugated goat-anti human IgG diluted in blocking buffer at 1:500 dilution was added and incubated for 1 h with rotation at RT. After the last wash, the blot was visualized by incubating with the HRP substrate (4-chloro-1-napthol, methanol and H_2O_2 in TBS). After the positive bands were developed, stop the reaction with dH_2O. The positive spots in immunoblot were picked-up from silver stained-gel and subjected to In-gel trypsin digestion.

4.6 In-gel trypsin digestion

4.6.1 In-gel digestion of proteins from colloidal blue stained gel

Samples were first destained with 50% acetronitrile (ACN), 25 mM NH_4HCO_3 (pH 8.0) until gel pieces became transparent, dehydrated in 100% ACN, and dried completely in a SpeedVac (gel became crystal when it dried). Each sample was reduced with 45 mM DTT, 25mM NH_4HCO_3 (pH 8.0) for 45 min at 55°C and then alkylated with 100 mM iodoacetamide, 25mM NH_4HCO_3 (pH 8.0) for 45 min at RT in the dark. All samples were washed twice with 50% ACN, 25 mM NH_4CO_3 (pH 8.0) and dried in a SpeedVac. Each sample was then in-gel digested with 0.01 ug/ul mass spectrometry grade trypsin (Promega, USA) in 25 mM NH_4HCO_3 (pH 8.0) ,50%ACN for overnight at 37°C. After incubation, the remaining solution from each

sample was collected in separate microcentrifuge tubes. The resultant peptides were extracted twice with 100 µl of 5% trifluoroacetic acid (TFA) in 50% ACN for 15 min with vortexing. Samples were dried down completely by SpeedVac, and then resuspended in 200 µl of deionized water. This procedure was repeated twice, with a third drying down halted when the remaining volume was approximiately 10 µl. One ninth the volume of 1% TFA was added to make the TFA concentration in the sample 0.1%. The peptide samples were cleaned with SCX ZipTips (Millipore, Billerica, Massachusetts, USA) according to manufacturer's instructions. The eluant was dried completely in a SpeedVac and subjected to matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/TOF).

4.6.2 In-gel trypsin digestion of proteins from silver stained 2D gel

Protein spots were excised from the gel, cut into small pieces (1X1 mm) and placed into flat bottom 96-well plate. Samples were washed 2 min for 5 times with milli-Q water followed by 25mM NH_4HCO_3 (pH8.0) and destained with 25mM NH_4HCO_3 containing 1%H_2O_2 until gel pieces became transparent. After washed for 5 min with milli-Q water, 1% formic acid was added and incubated for 5 min. Gel pieces were dehydrated by incubated with 0.1% formic acid, 50% ACN for 5 min followed by pure ACN for 10 min. Each sample was reduced, alkylated and digested with trypsin by the same method mentioned earlier. The resultant peptides were put in 96-well microtiter plate and dried down completely by put the plate in 37°C incubator for overnight. Tryptic peptides were resuspended in 20 ul of 2% ACN, 0.1% TFA and ready for liquid chromatography mass spectrometry (LC/MS/MS) analysis.

4.7 Mass spectrometry analysis and databases search

4.7.1 Matrix-assisted Laser Desorption/Ionization Time of Flight mass spectrometry (MALDI TOF/TOF)

Separation of the peptides were achieved by reverse-phase nanoflow liquid chromatography (LC) using a Chromolith Caprod column (150 x 0.1 mm, Merk) injector loop on a Tempo LC MALDI spotting system (ABI-MDS/Sciex) and eluted with a gradient of 2% ACN/0.1% TFA and 98% ACN/0.1% TFA, respectively. The final eluant of peptides was mixed with MALDI matrix (7 mg/ml recrystallized α-cyanohydroxycinnamic acid (CHCA, Sigma); 2 mg/ml ammonium phosphate; 0.1%

TFA in 80% ACN) post-column and automatically spotted onto a stainless steel MALDI target plate and analyzed on an ABI 4800 MALDI TOF-TOF (Applied Biosystems). The MS spectra were acquired from each sample spot using 500 laser shots from 40 random per spot using Reflectron Positive Ion mode with laser setting of 3200. The highest top ten peaks of each observed m/z value of each MS spectrum (excluding trypsin auto-digestion peaks) were choosing for subsequent MS/MS analysis with CID (collision-induced dissociation) fragmentation. Up to 2500 laser shots at the laser power 4200 were accumulated for each MS/MS spectrum.

The MS and MS/MS data were acquired and searched against the annotated *P. vivax* protein database from PlasmoDB (http://www.plasmodb.org release 5.4 and 5.5) and human protein database from NCBInr. The MS and MS/MS data from MALDI-TOF/TOF were analyzed by Protein Pilot software version 2.01 (Applied Biosystems/MDS Sciex). Search criteria were trypsin-cleved peptides; 200 parts per million mass error tolerances in MS mode; 0.4 Da mass error tolerance for MS/MS fragments; fixed modification of carbamidomethylation of cysteines, and allowed (variable) modification of oxidation of methionine. Protein identification acceptance was ProteinPilot Unused Score > 1.3 (>95% confidence interval) plus False Discovery Rate (FDR) < 5%.

4.7.2 Liquid chromatography tandem mass pectrometry (LC/MS/MS) analysis of parasites proteins.

For identify parasite antigens, peptides from the immune-positive spots of the 2-DE were analyzed by LC/MS/MS in the SYNAPT™ HDMS mass spectrometer (Waters Corp., Manchester, UK). Nanoscale LC separation of tryptic peptides was performed with a NanoAcquity system (Waters Corp., Milford, MA) equipped with a Symmetry C18 5 µm, 180-µm x 200-µm Trap column and a BEH130 C18 1.7 µm, 75-µm x 200-µm analytical reversed phase column (Waters Corp., Milford, MA). The samples were initially transferred with an aqueous 0.1% formic acid solution to the trap column with a flow rate of 3 µl/min for 3 min. Mobile phase A was water with 0.1% formic acid, and mobile phase B was 0.1% formic acid in ACN. The peptides were separated with a gradient of 2–40% mobile phase B over 30 min at a flow rate of 350 nl/min followed by a 10-min rinse with 80% of mobile phase B. The column temperature was maintained at 35 °C. The lock mass was delivered from the auxiliary

pump of the NanoAcquity pump with a constant flow rate of 200 nl/min to the reference sprayer of the NanoLockSpray source of the mass spectrometer. The mass spectrometer was operated in the V-mode of analysis with a resolution of at least 10,000 full-width half-maximum. Analyses were performed using positive nanoelectrospray ion mode. Accurate mass LC-MS data were acquired with expression mode. The spectral acquisition time was 0.6 sec. In MS expression mode, low energy of trap was set at constant collision energy of 6 V. In elevated energy of MS expression mode, the collision energy of trap was ramped from 15 to 40 V during each 0.6-s data collection cycle with one complete cycle of low and elevated energy. In transfer collision energy control, low energy was set at 4 V for low energy and 7 V for high energy.

The MS and MS/MS data were acquired and analyzed by ProteinLynx Global Sever 2.2.5 (Waters Corp., Milford, Massachusetts, USA) using the searching criteria as mentioned earlier.

4.8 Production of recombinant *P. vivax*' protein recognized by hyper immune sera from *P. vivax* infected patient

4.8.1 Generation of PV24 recombinant plasmid

Plasmodium vivax RNA was synthesized into cDNA by using Superscript III Reverse Transcriptase (invitrogen) according to the manufacturer's recommendations. Based on the available *Salvador I* strain genome sequence, the specific primers were designed to amplify the region of PVX_002950, which we termed as PV24, from base pairs 79-618; forward primer contained *BamHI* recognition sequence 5'ATGCGGATCCTACAATGCAAGCGAAAGACAAAATGG 3' and reverse primer contained *SalI* recognition sequence 5'-AGTCGTCGACGAACAAGTAGCCATAATATTTGG-3' (Figure 4.1).

>PVX_002950 | Plasmodium vivax SaI-1 | hypothetical protein, conserved | CDS | length=621

ATGTTGCCCGCTGTGGCCTGTTTAAACGCATTTTTTTTTGTGTGGTTCATTTTTACCTTG
TTGAAAAATAAACTGAGCTACAATGCAAGCGAAAGACAAAATGGTCCTGTCGAAATTGGA
GTGGTCCTGGGCTCCGGAGGGCACACATTCGAGATGTTACAGATATTGAAGCTCATCAAA
GATAGCAACATAAGTTTTCACTTTTTTTACGCAAATAATGACCCATTGAGTAGGGAAAAG
GCAGAAAATGCCTTAGAGGAATACAAAAAGGATTTCTTTGCAATTCCAAGATGCCGAAAT
GTTGGTGAGTCATACGTACGATCGTCAGTGAAACTTTTTTTTGCGTTCATTTATTGTTTA
TTTTTAACATACAGAATGAACATGAGCGTTTTGATGGTGAATGGCCCAGGAACATGTCTG
CCTGTTGCATTTTCCCTGCTATTCAGAAAATATTTTTTTTTTAAAAAAATAAAAATTGTT
TATTTGGAAAGTGTTTGCAGAATATATTCCCTCTCCCTGACTGCAAAAATTTTGTATAAT
TTTTCTGATTTGTTTGTTGTTTTTTCCGAACATTTGCAGAAAAGGTACAAAAAGGCCAAA
TATTATGGCTACTTGTTCTGA

Figure 4.1 DNA sequence of PVX_002950. Forward and reverse primers are indicated in yellow and red bars respectively.

Polymerase chain reactions (PCR) using Advantage® 2 polymerase mix (Clontech) were then performed under the following conditions; 1 cycle at 95°C for 1 min, 25 cycles of 95°C for 15 sec, 60°C for 15 sec, and 72°C for 15 sec and a final extension at 72°C for 10 min. PCR product was mixed with loading buffer containing Sybr green fluorescence dye and loaded to 1% agarose gel together with 1kb DNA ladder. The electrophoresis was performed at 90 volts for 30 min in running buffer (Tris- borate EDTA). The DNA band was visualized under UV light. The DNA band at the expected size was cut and put in 1.5 ml microcentrifuge tube. The PCR product was eluted from agarose gel by using PrepEase® gel extraction kit (USB). The PCR product was ligated to pGEM-T vectors (Promega) to generate recombinant plasmid (pGEM-PV24) using T4 DNA ligase enzyme and incubated at RT for 1h or 16°C overnight. Transformation was performed by using heat shocked protocol. Ligation reaction was mixed with competent cell *Escherichia* coli strain DH5α and incubated on ice for 30 min. Then incubated the tube at 42°C for 90sec and immediately put the tube on ice and wait for 2 min. One milliliter of LB medium was added and the tube was incubated at 37°C for 45 min with shaking. Spread 100 ul of transformed *E. coli* on LB agar containing 50 µg/ml of ampicillin, isopropyl-β-D-thiogalactopyranoside (IPTG) and X-gal, and incubated at 37°C for 12-16 h. The positive clones (colorless colony) were inoculated in 3 ml LB medium containing 50 µg/ml of ampicillin and

incubated at 37°C for overnight with shaking. Plasmids were extracted from bacterial cells by using Zyppy™ Plasmid Miniprep kit (Zymo research) and the open reading frame was confirmed by an automated sequencing in an ABI Hitachi 3730XL DNA analyzer (Applied Biosystems). The PV24 fragment was cut with *BamHI* and *SalI* restriction enzymes for 4 h at 37°C and ligated to pET28 (a+) expression vector which has been cut with the same restriction enzymes. The pET-28a-PV24 recombinant plasmids were transformed to *E. coli* BL21 (DE3) expression host with the same method as mentioned earlier. Transformed bacteria were then spread on LB agar containing 50 µg/ml of kanamycin and incubated at 37°C for 12-16 h. The positive clones were checked by DNA sequencing and the bacterial colonies carrying the pET-28a-PV24 recombinant plasmid were selected for protein expression. The pET28 (a+) expression vector provides the poly-histidine (6 His) tagged for further purification and detection of recombinant protein.

4.8 Expression and purification of PV24 recombinant protein.

Starting culture was made by inoculated a single colony of *E. coli* BL21 (DE3) transformed with the pET-28a-PV24 plasmid in 10 ml of LB medium containing 0.1 mg/ml of Kanamycin for 12-16 h (37°C, 250 rpm). The starting culture was then added to 1 L of culture medium containing 0.1 mg/ml Kanamycin and incubated (37°C, 250 rpm) until the OD600 reached 0.6. Protein expression was induced by adding 1 mM IPTG and incubated at 37°C with 250 rpm shaking for 2 h and cells were harvested by centrifugation at 4,000 g for 20 min at 4°C. Bacterial pellet was resuspended in lysis buffer (8M urea, 10 mM Tris-Cl, 100 mM NaH_2PO_4 and 1 mg/ml lysozyme) supplemented with protease inhibitors and lysed by sonication at 10 sec for 6 cycles at 200-300 W with 10 sec cooling. The solubilized lysate was centrifuged at 8000g for 20 min at RT and supernatant was collected. The PV24 recombinant protein (rPV24) was purified from the soluble cell extract by affinity chromatography using Ni-NTA agarose (Qiagen) according to the manufacturer's recommendations. Briefly, the Ni-NTA slurry was mixed with soluble cell extract for 1 h with gentle agitation then poured into the empty column. The column was washed with washing buffer pH6.3 (8 M urea, 10 mM Tris-Cl, 100 mM NaH_2PO_4) to remove the unbound proteins and the rPV24 was then eluted at least 4

times with 500 µl of the elution buffer pH4.5 (8 M urea, 10 mM Tris-Cl, 100 mM NaH_2PO_4). All fractions were kept at -20°C and the purity of the protein was evaluated by 12% gel SDS-PAGE and western blot using anti his-tagged antibody. The pure recombinant protein fractions were pooled together and dialyzed against PBS pH 7.4.

4.10 Plasma sample collection

Blood samples from 118 *P. vivax* infected patients (20 ml) and 33 *P. falciparum* infected patients (10 ml) were collected from Mae Sot, Tak province and the Hospital of Tropical Diseases, Bangkok during 2000-2009. The plasma was obtained by centrifuging the patient's blood at 2,500 rpm for 10 min. All plasma was aliquot in 1.5 ml microcentrifuge tubes and stored at -20 °C for ELISA.

4.11 Enzyme linked immunosorbent assay (ELISA)

Anti-PV24 antibodies level in patient plasmas were evaluated using and enzyme-linked immunosorbent assay (ELISA). Briefly, Griener flat-bottomed, 96-well microplates were coated with 50 µl per well of 1 µg/ml of purified rPV24 and incubated overnight at 4°C. Microplates were blocked with 100 µl of PBST containing 0.5% BSA for 2 h. After washing twice with PBST, 50 µl of human plasma diluted to 1:50 with blocking buffer were added and incubated for 1 h at RT. Plates were then washed thrice with PBST and 50 µl of secondary rabbit anti-human IgG antibody conjugated with HRP were added with the final dilution of 1:2,000. Plates were incubated for 1 h at RT, washed thrice with PBST, developed with 2, 2'-azino-di-(3-ethylbenzthiaoline sulfonic acid (ABTS) for 30 min and optical density (OD) read at 405 nm. Each sample was tested in duplicate. Mean of OD given by naïve control sera plus three standard deviations was used as the cutoff OD value.

CHAPTER V
RESULTS

5.1 *Plasmodium vivax* erythrocytic stage Proteome

Due to the lack of a suitable continuous *in vitro* culture method for *P. vivax*, we used field isolate parasites obtained from infected patients for proteomic analysis. Our preliminary studies using parasite samples immediately after saponin lysis suggested that host protein contamination was too overwhelming to obtain adequate parasite protein coverage. In order to circumvent this problem, we sought to culture the parasites until they reached schizont stage, purified schizonts on Percoll gradients, and determined the schizont stage proteome.

Approximately 10^9 schizont stage parasites were used for protein separation by SDS-PAGE. The protein bands in the gel were cut into 16 slices (Figure 5.1), in-gel digested with MS-grade trypsin, and subjected to MS/MS analysis by MALDI-TOF/TOF.

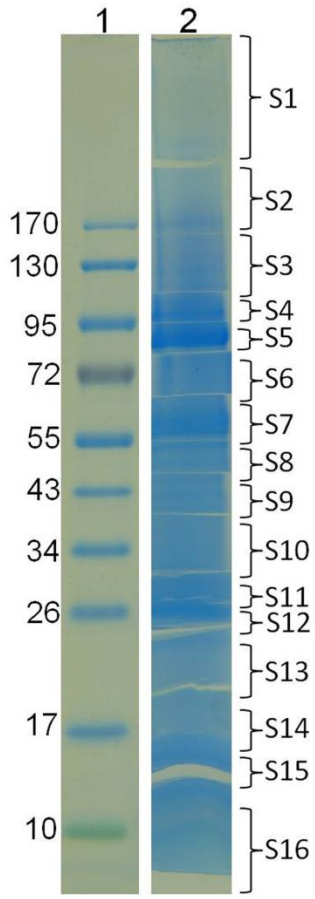


Figure 5.1 SDS-PAGE analysis of *P. vivax* lysate. Approximately 10^9parasites were separated on 12% polyacrylamide gel and then stained with colloidal blue, standard molecular mass is shown in lane 1. Protein bands were horizontally sliced into 16 sections (lane 2) before subjected to in-gel trypsin digestion and mass spectrometry analysis.

By searching databases with MS spectra, 316 proteins were identified with at least 95% confidence (see Appendix A and B), which accounted for 6% of total predicted proteins in the *P. vivax* genome. Almost half of the identified proteins (47%) were hypothetical proteins, among which 15% contained putative signal peptide (SP) and 20% contained at least one transmembrane (TM) domain. These suggested that the identified proteins were either secretory or membrane associated. Further, 15% of the hypothetical proteins contained both SP and TM, suggested that they might play important roles in parasite-host interactions (see Appendix C). The rest 50% of these hypothetical proteins did not contain any SP or TM.

Eleven percent (36 in 316 proteins) of the identified proteins was unique as defined by the absence of orthologs in other human malaria parasites; *P. falciparum, P ovale and P. malariae* (see Appendix D). Half of the unique proteins (18 in 36 proteins) were hypothetical. In addition, 16 in 36 proteins did not have orthologs in other human malaria parasites and *P. knowlesi*.

All identified proteins were classified into functional classes based on the Munich Information Centre for Protein Sequences (MIPS) catalogue [33] (see Appendix E). Proteins were plotted as a function of their broad functional classification; only one class was assigned per protein in order to avoid redundancy (figure 5.2). The predominant class contained hypothetical proteins (47%). Other important functional classes of proteins were protein with binding function (10%), protein fate (5%), protein synthesis (4%), metabolism (4%) and cellular transport (4%). (Table 5.1) These proteins classes are mainly housekeeping proteins which is important for survival of the parasite.

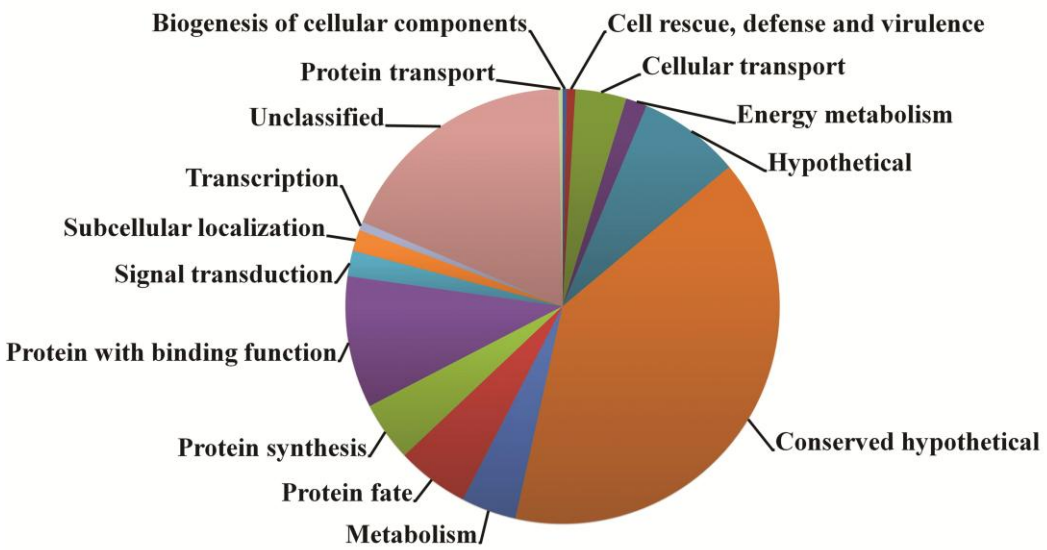


Figure 5.2 Pie chart showing the distribution of functional classes of identified *P. vivax* proteins as defined by the MIPS catalogue.

Table 5.1 Functional categories of all identified protein.

Functional categories	Number of proteins
Biogenesis of cellular components	1
Cell rescue, defense and virulence	2
Cellular transport	12
Conserved hypothetical	125
Energy metabolism	5
Hypothetical	24
Metabolism	13
Protein fate	17
Protein synthesis	14
Protein transport	1
Protein with binding function	31
Signal transduction	6
Subcellular localization	5
Transcription	2
Unclassified	58

Enzymes of major metabolic pathways of the parasite including glycolysis, nucleic acid metabolism, and hemoglobin digestion were identified (Table 5.2). Different transportation mechanisms of parasite have been reported, and several proteins were shown to play significant roles in the transportation machinery including ADP-ribosylation factor, Rab GTPases (Rab2, Rab5c, Rab6 and Rab7) [34-36], translocation protein complex (sec61, sec62 and SEC63), heat shock protein (Hsp70 and HSP101), Cop-coated vesicle membrane protein p24 and EXP2 [37-40]. They were also detected in the schizont stage proteome. Exported proteins potentially involved in immune evasion and host cell invasion including *vir* [41,42], and PvRBP [43] were identified. The PvtrAg or Pv-fam-a is one of the most abundant parasite protein families with 34 predicted members, among which 11 were detected. In addition, both asexual and sexual stage antigens such as *P. vivax* tryptophan-rich antigen (PvtrAg) [44,45] and transmission-blocking target Pfs230 [46] were identified.

Table 5.2 Identified *P. vivax* proteins involved in parasite's metabolic pathways.

Accession No.	Enzyme name	EC	Reaction catalyzed
Glycolysis			
PVX_118255	Fructose 1,6-bisphosphate aldolase	4.1.2.13	Aldolase ↔ Glyceraldehyde-3P
PVX_118495	Triosephosphate isomerase	5.3.1.1	Glyceraldehyde-3P ↔ Glycerone-P
PVX_095015	Enolase, putative	4.2.1.11	Glycerate-2P ↔ Phosphoenol-pyruvate
PVX_114445	Pyruvate kinase, putative	2.7.1.40	Phosphoenol-pyruvate+ADP ↔ Pyruvate+ ATP
PVX_116630	Lactate dehydrogenase	1.1.1.27	Pyruvate + NADH+H^+↔Lactate + NAD^+
Nucleic acid biosynthesis (Purine salvage pathway)			
PVX_111245	Adenosine deaminase, putative	3.5.4.4	Adenosine + H_2O ↔ Inosine + NH_3
PVX_092535	Adenylate and Guanylate cyclase catalytic domain containing protein	4.6.1.2	GTP ↔ 3',5'-cyclic GMP + PPi
PVX_094840	Hypoxanthine phosphoribosyltransferase	2.4.2.8	Hypoxanthine + PRPP ↔ IMP + PPi
Nucleic acid biosynthesis (Pyrimidine metabolism)			
PVX_083135	Aspartate carbamoyltransferase	2.1.3.2	L-Aspatate + Carmoyl-P ↔ N-carbamoyl-L-Aspartate + Pi
TCA			
PVX_084960	ATP-specific succinyl-CoA synthetase beta subunit	6.2.1.5	ATP + Succinate + CoA ↔ ADP + Orthophosphate + Succinyl-CoA
Hemoglobin digestion			
PVX_115000	Falcilysin, putative	3.4.24.-	Acting on peptide bonds
PVX_097935	Subtilisin-like protease precursor	3.4.21.62	Hydrolyzes peptide amides
PVX_086040	Aspartic protease PM4	3.4.23.B14	Cleavage of hemoglobin
PVX_122425	M1-family aminopeptidase	3.4.11.2	Release of an N-terminal amino acid from a peptide
Protein phosphorylation & dephosphorylation			
PVX_118220	serine/threonine kinase-1, putative	2.7.11.1	ATP+ a protein <=> ADP + a phosphoprotein
PVX_095165	protein kinase, putative	2.7.11.17	ATP+ a protein <=> ADP + a phosphoprotein
PVX_089720	Protein kinase domain containing protein	2.7.11.22	ATP+ a protein <=> ADP + a phosphoprotein
PVX_093615	phosphoinositide phosphatase SAC1	3.1.3.56	D-myo-inositol 1,4,5-trisphosphate H_2O <=> myo-inositol 1,4-bisphosphate + phosphate
PVX_099535	phosphoglycerate kinase, putative	2.7.2.3	ATP+ 3-phospho-D-glycerate <=> ADP + 3-phospho-D-glyceroyl phosphate

Plasmodium metabolic pathways can be found at http://sites.huji.ac.il/malaria/.

┼Enzyme Commission (EC) numbers of *P. falciparum* orthologs.

Glycosylphosphatidylinositol (GPI) represents the major carbohydrate modification of the parasite which serves to anchor parasite proteins to the outer leaflet of plasma membrane. Nine predicted GPI-anchored proteins were detected in this schizont stage proteome including well known GPI-anchored protein MSP1, MSP8 and Pf12 (Table 5.3).

Table 5.3 Glycosylphosphatidylinositol (GPI)-anchored proteins identified in this schizont stage proteome.

Accession number	Name
PVX_083135	Aspartate carbamoyltransferase, putative
PVX_088910	Hypothetical protein, conserved
PVX_097625	Merozoite surface protein 8, putative
PVX_099320	Acid phosphatase, putative
PVX_099980	Major blood-stage surface antigen Pv200 (MSP1)
PVX_100835	Hypothetical protein, conserved
PVX_110895	ADP/ATP transporter on adenylate translocase, putative
PVX_113775	Membrane protein pf12 precursor, putative
PVX_122545	Cop-coated vesicle membrane protein p24 precursor, putative

Parasite adhesive molecules are of particular importance due to their roles in parasite invasion, sequestration as well as parasite-host interaction. In *P. vivax*, 137 adhesins were predicted [47] in which 11 were detected in this schizont proteome (table 5.4). Two parasite adhesins, MSP1 and AMA1, have well been characterized and shown to involve in host cell invasion of merozoite [43]. Six of predicted adhesins in this proteome were hypothetical proteins.

Table5.4 *Plasmodium vivax* adhesins predicted by MAAP (Malaria adhesins and adhesion-like proteins predictor) [47]. **P vivax* proteins with P_{maap} score of $\geq$ 07 were predicted as adhesins.

Accession Number	Name	score*	SP	TM
PVX_097950	Hypothetical protein, conserved	0.742	0	0
PVX_096995	Tryptophan-rich antigen (Pv-fam-a)	0.74	0	1
PVX_084720	Hypothetical protein, conserved	0.935	1	0
PVX_092275	Apical merozoite antigen 1	1.001	0	1
PVX_099980	Major blood-stage surface antigen Pv200	1.001	1	1
PVX_096245	Hypothetical protein, conserved	1.008	0	0
PVX_117060	Hypothetical protein, conserved	1.027	0	0
PVX_083560	Hypothetical protein, conserved	1.242	0	0
PVX_112670	Tryptophan-rich antigen (Pv-fam-a)	1.615	1	0
PVX_096070	Early transcribed membrane protein (ETRAMP)	2.113	1	1
PVX_003555	Hypothetical protein, conserved	2.213	0	0

Since protein samples were enriched with schizont-stage parasite, schizont-specific proteins were readily detected. These included proteins associated with merozoite surface and invasive organelles such as GPI-anchored surface protein (MSP1, MSP5, MSP8 and Pf12), merozoite surface protein (MSP7), micronemal protein (AMA1), rhoptry proteins (RAP1, RAP 2, RhopH2, clag), EMP3 and actinomycin motor (actin, myosin). Notably the identification of sexual stage proteins including male fertility protein Pf47 (PVX_083240), sexual stage antigen s16 (PVX_000930) and transmission-blocking target antigen Pfs230 (PVX_000995) is due to the present of sexual stage parasite in biological samples.

5.2 Identification of novel *P. vivax* antigens

In order to identify parasite proteins that are recognized by host humoral immunity, we performed 2-DE of protein extract from schizont stage parasite. Immune serum from an acute *P. vivax* infected patient showing high antibody level to *P. vivax* lysate as determined by ELISA (data not shown) was used in WB analysis. The positive protein spots were excised from the silver stained 2-D gel for proteomic analysis (Fig 5.3). Four proteins were reactive with the immune serum, corresponding to the protein products of PVX_002950, PVX_087070, PVX_095015 and PVX_092835 genes (Table 5.6). Linear B-cell epitope and antigenicity prediction from the BepiPred 1.0 and Kolarskar& Tongaonkar Antigenicity programs (www.immuneepitope.org) suggested that all four proteins contained linear B-cell epitopes and had antiginicity score above the threshold. Since the presence of SP and TM in the protein sequence is an important indicator of secretory and/or cell surface protein, SignalP 3.0 and TMHMM 2.0 (http://www.cbs.dtu.dk) were used to determine the SP and TM of the four proteins. Among them, only PVX_002950 contained predicted SP and TM domains. The PVX_002950 was selected for cloning and protein expression.

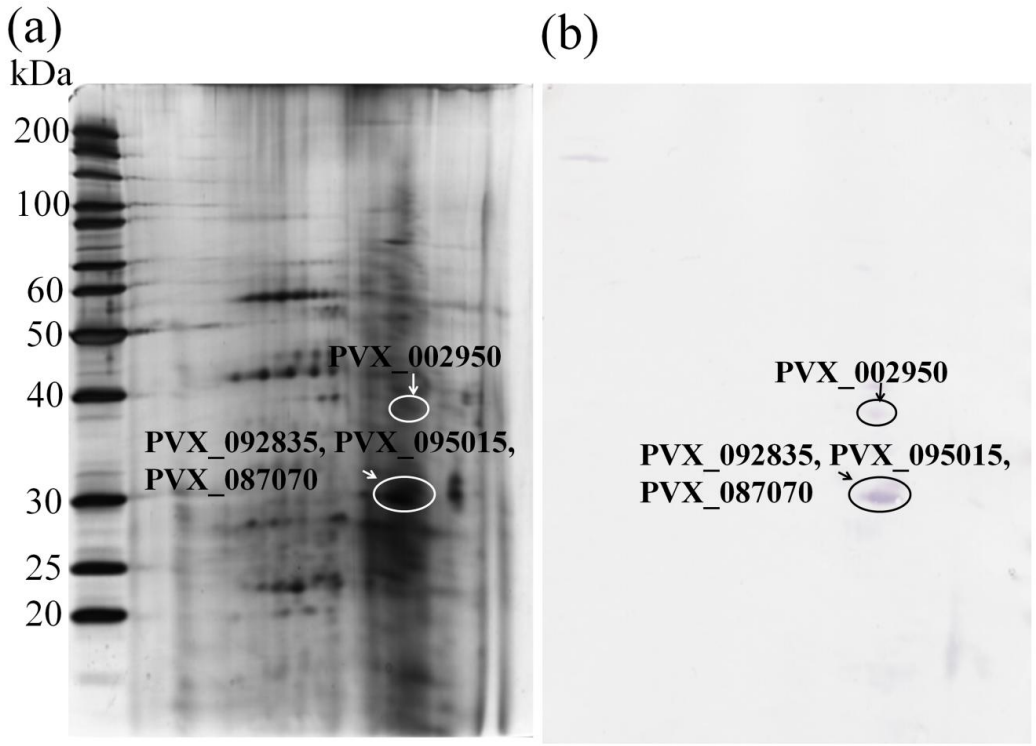


Figure 5.3 Two dimensional gel analysis of blood stage proteins of *P. vivax*. (a) Silver stained gel, and (b) immunoblot with an immune serum of *P. vivax* patient. Circles indicate proteins of *P. vivax* reacted with immune serum.

Table 5.5 *Plasmodium vivax* proteins recognized by immune serum from *P. vivax* patient identified by LC/MS/MS.

Gene ID	Description	Sequence	MW (kDa)	p*I*	SP	TM
PVX_002950	Hypothetical protein	LIKDSNISFHFFYANNDPLSR	24.1	9.81	1	3
PVX_087070	Hypothetical protein	APPTQGEMLLLLVR	110.1	6.53	No	No
PVX_095015	Enolase	AAVPSGASTGIYEALELR	48.8	9.70	No	No
PVX_092835	Hypothetical protein	LGKSNKR, QKAKQVK, ENAERSK, AKISMFK, TNVKKNR, IFEREGK, HYKTNVK, DNKLGKSNK, TSENVNQSK, NILDEIAVK, GSTVNTYILK, QSKVSLKPIK, VKINLNNPVK, MHVFDLDKAK, KNRFTIIETR, KMHVFDLDKAK	41.3	10.13	No	No

5.3 Cloning and expression of PVX_002950

The PVX_002950 is predicted to encode a 24 kDa hypothetical protein with 206 amino acids in lenght, referred to as PV24. Microarray analysis indicates that it is expressed throughout the *P. vivax* erythrocytic cycle. It contains N-terminal signal peptide (SP) and three transmembrane domains in its amino acid sequence (figure 5.4).

>PVX_002950 | Plasmodium vivax SaI-1 | hypothetical protein, conserved | protein | length=206

MLPAVACLNAFFFVWFIFTLLKNKLSYNASERQNGPVEIGVVLGSGGHTFEMLQILKLIK
DSNISFHFFYANNDPLSREKAENALEEYKKDFFAIPRCRNVGESYVRSSVKLFFAFIYCL
FLTYRMNMSVLMVNGPGTCLPVAFSLLFRKYFFFKKIKIVYLESVCRIYSLSLTAKILYN
FSDLFVVFSEHLQKRYKKAKYYGYLF

Figure 5.4 Full amino acid sequence of PVX_002950. Signal peptide (bold italic) and transmembrane domains (red bars) are indicated.

Due to the containing of predicted signal peptide sequence, the cleavage site between amino acid position 26 and 27, we decided to express the 180-residues recombinant protein without the first 26-residues SP sequence. In order to study this protein, the PV24 gene fragment (560 bp) containing additional *BamHI* and *SalI* recognition sequences at the 5' end and 3' end respectively was amplified by PCR. The PCR product was consistent with the expected size of PV24 DNA fragment (figure 5.5). The PCR product was cloned in pGEM and subcloned in pET28 (a+). The open reading frame was confirmed by DNA sequencing (figure 5.6).

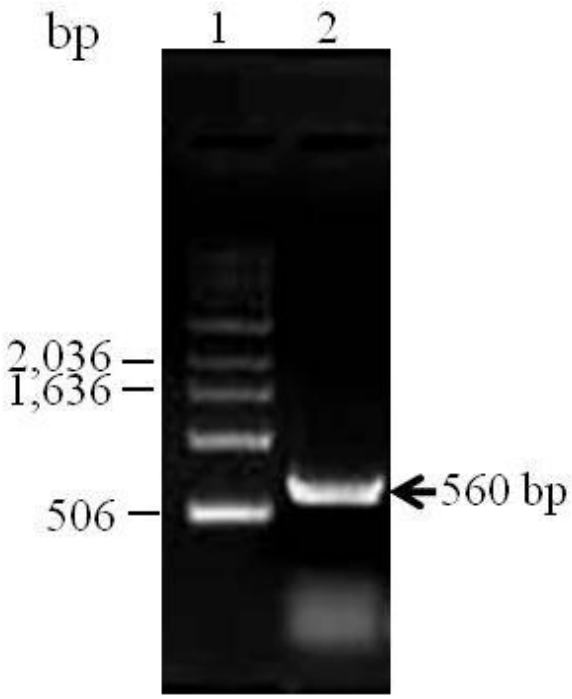


Figure 5.5 PCR amplification of PV24 from cDNA of *P. vivax*. Lane1 = DNA ladder and Lane 2 = PCR product. The PCR product at 560 bp (arrow) is corresponded to the size of PV24.

```
Alignment: C:\BioEdit\Temp\~out.tmp

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          10         20         30         40         50         60
Pv002950  | P    ---------- ---------- ---------- ---------- --TACAATGC AAGCGAAAGA
pET78C_1_1_T7    ATGGCTAGCA TGACTGGTGG ACAGCAAATG GGTCGCGGAT CCTACAATGC AAGCGAAAGA

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         70         80         90        100        110        120
Pv002950  | P    CAAAATGGTC CTGTCGAAAT TGGAGTGGTC CTGGGCTCCG GAGGGCACAC ATTCGAGATG
pET78C_1_1_T7    CAAAATGGTC CTGTCGAAAT TGGAGTGGTC CTGGGCTCCG GAGGGCACAC ATTCGAGATG

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        130        140        150        160        170        180
Pv002950  | P    TTACAGATAT TGAAGCTCAT CAAAGATAGC AACATAAGTT TTCACTTTTT TTACGCAAAT
pET78C_1_1_T7    TTACAGATAT TGAAGCTCAT CAAAGATAGC AACATAAGTT TTCACTTTTT TTACGCAAAT

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        190        200        210        220        230        240
Pv002950  | P    AATGACCCAT TGAGTAGGGA AAAGGCAGAA AATGCCTTAG AGGAATACAA AAAGGATTTC
pET78C_1_1_T7    AATGACCCAT TGAGTAGGGA AAAGGCAGAA AATGCCTTAG AGGAATACAA AAAGGATTTC

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        250        260        270        280        290        300
Pv002950  | P    TTTGCAATTC CAAGATGCCG AAATGTTGGT GAGTCATACG TACGATCGTC AGTGAAACTT
pET78C_1_1_T7    TTTGCAATTC CAAGATGCCG AAATGTTGGT GAGTCATACG TACGATCGTC AGTGAAACTT

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        310        320        330        340        350        360
Pv002950  | P    TTTTTTGCGT TCATTTATTG TTTATTTTTA ACATACAGAA TGAACATGAG CGTTTTGATG
pET78C_1_1_T7    TTTTTTGCGT TCATTTATTG TTTATTTTTA ACATACAGAA TGAACATGAG CGTTTTGATG

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        370        380        390        400        410        420
Pv002950  | P    GTGAATGGCC CAGGAACATG TCTGCCTGTT GCATTTTCCC TGCTATTCAG AAAATATTTT
pET78C_1_1_T7    GTGAATGGCC CAGGAACATG TCTGCCTGTT GCATTTTCCC TGCTATTCAG AAAATATTTT

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        430        440        450        460        470        480
Pv002950  | P    TTTTTTAAAA AAATAAAAAT TGTTTATTTG GAAAGTGTTT GCAGAATATA TTCCCTCTCC
pET78C_1_1_T7    TTTTTTAAAA AAATAAAAAT TGTTTATTTG GAAAGTGTTT GCAGAATATA TTCCCTCTCC

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        490        500        510        520        530        540
Pv002950  | P    CTGACTGCAA AAATTTTGTA TAATTTTTCT GATTTGTTTG TTGTTTTTTC CGAACATTTG
pET78C_1_1_T7    CTGACTGCAA AAATTTTGTA TAATTTTTCT GATTTGTTTG TTGTTTTTTC CGAACATTTG

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        550        560        570        580        590        600
Pv002950  | P    CAGAAAAGGT ACAAAAAGGC CAAATATTAT GGCTACTTGT TC-------- ----------
pET78C_1_1_T7    CAGAAAAGGT ACAAAAAGGC CAAATATTAT GGCTACTTGT TCGTCGACAA GCTTGCGGCC

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        610        620        630        640        650        660
Pv002950  | P    ---------- ---------- ---------- ---------- ---------- ----------
pET78C_1_1_T7    GCACTCGAGC ACCACCACCA CCACCACTGA GATCCGGCTG CTAACAAAGC CCGAAAGGAA
```

Figure 5.6 Sequence alignment of recombinant plasmid containing PV24 DNA fragment with PVX_002950 gene from Salvador 1 strain sequence.

The PV24 protein was expressed in *E. coli* BL21 (DE3) with 6 histidine tag linked to it's both N- and C- terminal. The PV24 recombinant protein (rPV24) was then purified by affinity chromatography using Ni-NTA agarose under denaturing condition. All fractions were analyzed by SDS-PAGE (12%) and western blotting using anti-His monoclonal antibody. Cloning resulted in incorporation of additional 34 (MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGS) and 15 (VDKLAAALE HHHHHH) residues to the N- and C- terminus, respectively, of PV24. This would yield the rPV24 protein of molecular weight 26.4 kDa. As shown on SDS-PAGE, a single band of rPV24 at the molecular weight of ~26 kDa was detected (figure5.7). The pure rPV24 fractions were pooled together and dialyzed against PBS pH 7.4 for overnight at 4°C and kept at -80°C for ELISA.

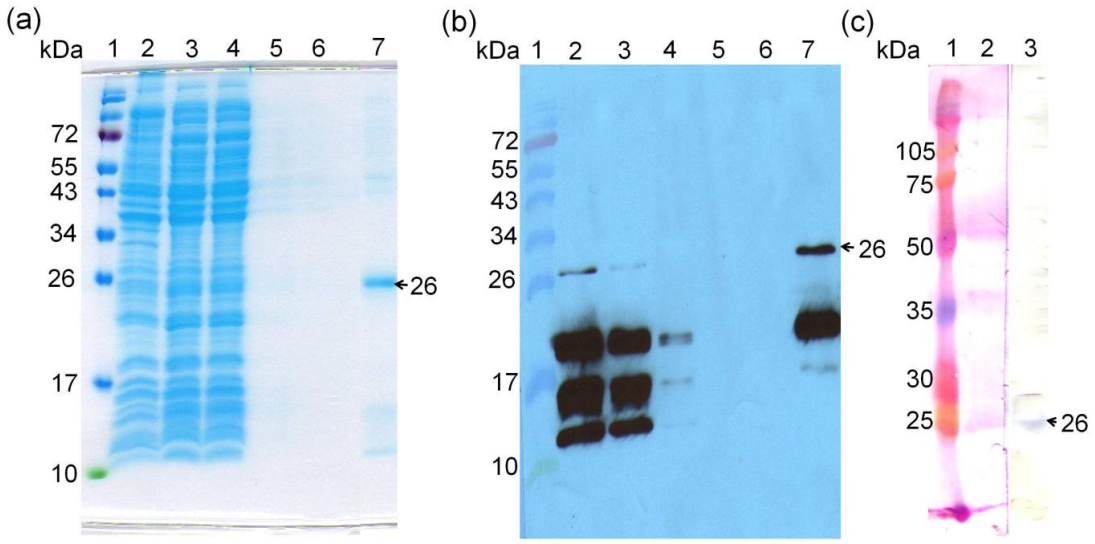


Figure 5.7 Purification of rPV24 using Ni-NTA chromatography. (a) SDS-PAGE analysis and (b) chemiluminescence analysis of immunoblot using anti-his antibody. Lane 1 = standard molecular mass, lane 2 = whole lysate, lane 3 = soluble fraction, lane 4 = flow-through fraction, lane 5 = last washing fraction, and lane 6 and 7 = eluted fractions. (c) Immunoblot analysis of rPV24 reacted with immune serum from a *P. vivax* patient. Lane 1 = standard molecular mass, lane 2 = PonceuS staining of rPV24 protein, and lane 3 = immunoblot of rPV24. ; Arrows indicate purified rPV24.

5.4 Antigenic study of PV24

To determine the antigenicity of rPV24, ELISA was performed by testing rPV24 protein with plasma samples from *P. vivax, P. falciparum* infected patients and malaria naïve donors. Ninety-nine percent of plasma from *P. vivax* infected patients showed reactivity to rPV24 above the cutoff value (mean OD. of naïve control + 3SD) (figure 5.8). The reactivity of *P. vivax* plasma (OD405 = 0.907355±0.172816) was significantly higher than that of naïve group (P<0.001, OD405 = 0.164251±0.185165), and from that of *P. falciparum* plasma (P<0.001, OD405 = 0.116799±0.125510) (Figure 5.9a). Compared with naïve group, the reactivity of plasma sample from *P. falciparum* infected patients with rPV24 was not significant (P>0.05). Plasma from *P. vivax* infected patients collected in different years (2001-2009) were reacted with rPV24 and showed no significant changed (Figure 5.9b). Interestingly, the reactivity of plasma from all follow-up cases (3 and 6 months post treatment) was higher than the cutoff level (Figure 5.9c). In addition, there was a significant inverse correlation between the level of anti-rPV24 antibody and the *P. vivax* parasitemia (correlation coefficient = -0.372, P = 0.039). Altogether, these results indicated that the PV24 was strongly antigenic and elicited long-lasting immunity in *P. vivax* patients.

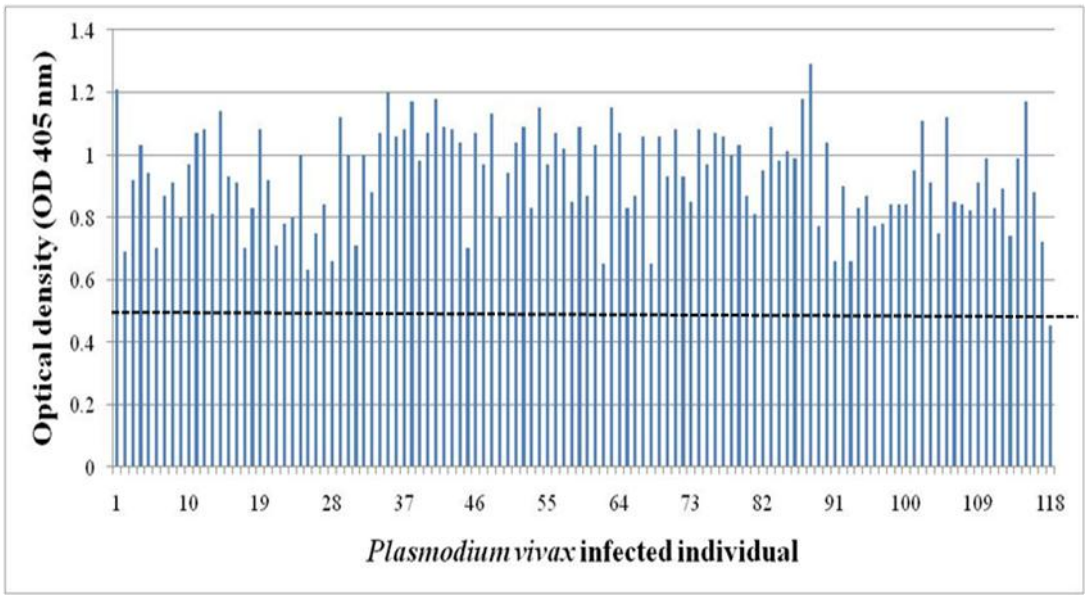


Figure 5.8 Reactivity to rPV24 of sera from *P. vivax* infected individuals

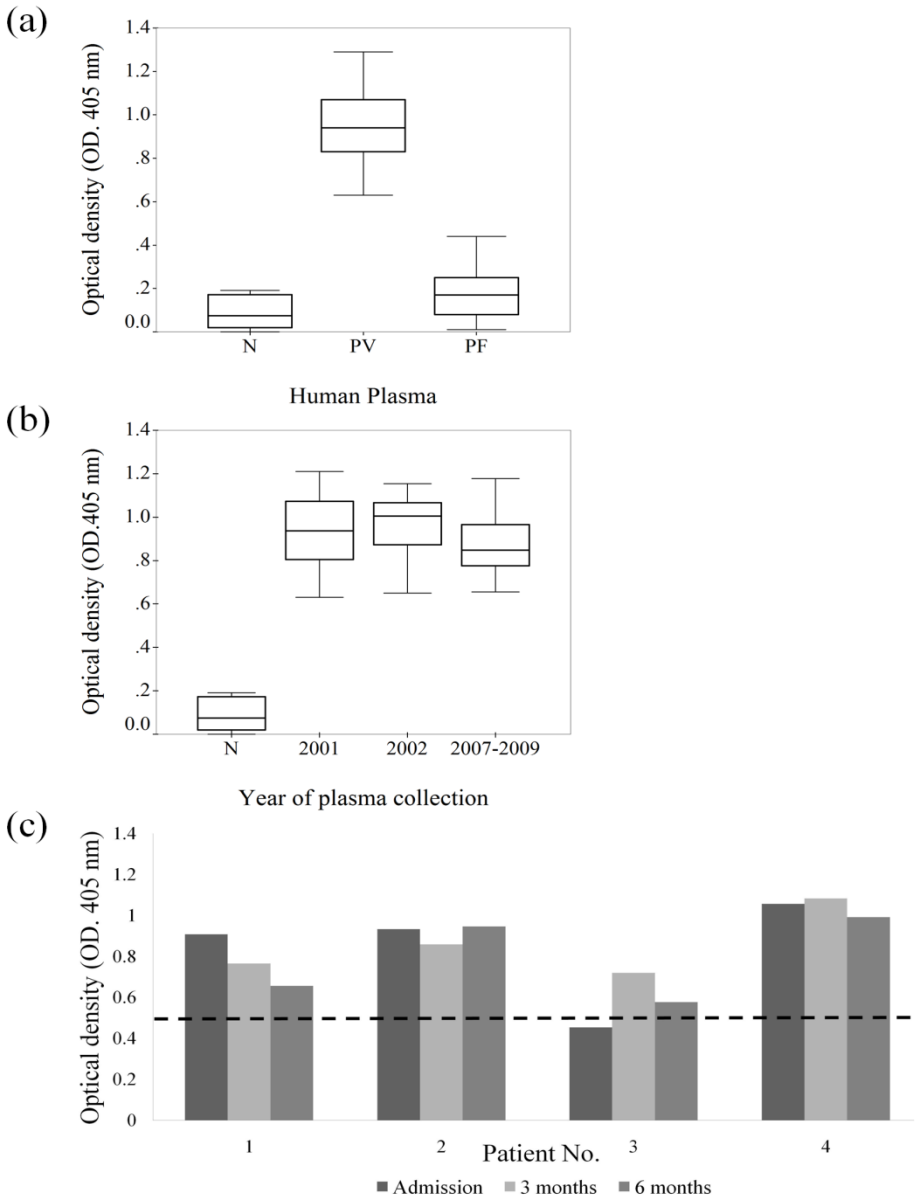


Figure 5.9 (a) Determination of antibody to rPV24 by ELISA. (a) Different groups of human plasma; sera of *P. vivax* infected patients (PV), malaria naïve sera (N), and sera of *P. falciparum* infected patients (PF). (b) plasma from PV collected in different years compared with N. (c) Antibody levels at acute infection and convalescence period collected at 3 and 6 months after treatment (dash line indicates cut-off level at mean + 3SD).

CHAPTER VI
DISCUSSION

This study employed short-term *in vitro* parasite culture and enrichment of mature parasites to reduce the level of host cell proteins in the sample, which was shown to be useful in obtaining the schizont stage proteome. Identification of the proteome from erythrocytic stages should be feasible with this modification of the parasite enrichment procedure.

With the completion of genome sequencing and transcriptome analysis of *P. vivax*, we attempted to determine the proteome of the schizont stage of *P. vivax*. From clinical isolates, 316 *P. vivax* proteins were confidently identified. Of these proteins, 65% were overlapped with those of *P. falciparum* proteins [11]. Over half of the proteins identified here were abundant proteins which might be important in the parasite metabolism, protein synthesis, cellular transport and binding function, etc. The hypothetical proteins accounted for almost 50% of this proteome could be a key to understand basic biology of *P. vivax* parasite.

Abundant proteins in schizont stage were also detected including MSP1, MSP5, MSP7, MSP8, AMA1, RBP2, Pf12, RAP1, RAP2 and RhopH2. Field isolates parasite has undergone genetic diversity due to drug or immune pressure [48]. MSP1, one of the most abundant merozoite proteins and essential for the erythrocyte invasion of merozoite, is one of parasite protein which is highly polymorphic in field isolate parasites. The present study has shown that MSP1 formed complex with other proteins including the detected MSP7, RAP1 and RAP2 [49]. This protein complex was specifically found only in schizont/merozoite stages but not the next ring stage indicating its role in erythrocyte invasion process.

Ribosomal proteins were well represented, 10 ribosomal proteins, in this proteome suggesting the extensive protein synthesis in this blood stage parasite. The big source of amino acids for protein synthesis of the parasite is hemoglobin digestion which occurs in the parasite food vacuole. Enzymes, reside in digestive vacuole,

involved in hemoglobin digestion were detected (falcilysin, subtilisin, aspartic protease PM4 and M1-family amino peptidase). Chloroquine resistance transporter and multidrug resistance protein have been shown to localize on the food vacuole membrane [50].

Protein kinases and phosphatases, involved in phosphorylation and dephosphorylation respectively, are key regulators of many biochemical processes in eukaryotes. Protein phosphorylation, major process in parasitized erythrocyte, has been shown to be an important event in malaria infection [51,52]. Phosphatases play important role in the regulation of protein phosphorylation. Since the inhibitors of enzymes involved in phosphorylation and dephosphorelation can interfere parasite growth, studies in this area may lead to the development of novel therapies.

Plasmodium vivax appears to resist to currently available drugs in several endemic areas [53-55]. Progress research to identify protein involved in the resistance is scanty due to difficulty in the continuous cultivation of the parasite. In this proteome, 52 membrane-associated and 22 soluble proteins were identified. Almost half of integral membrane proteins and secreted proteins were hypothetical. With no homology to known proteins in other organisms, these proteins are of interest as they represent potential *Plasmodium*-specific proteins. Identification of these proteins is one of the progresses leading to such identification therefore providing the new targets for drugs development. Several SP/TM containing proteins have been studied in detail (MSP1, AMA1, Pfs16 and Pfs230) some of which are characterized as vaccine candidates [16,56]. Moreover, SP/TM containing hypothetical proteins may have particular roles in erythrocyte invasion or parasite-host interaction as have been seen in that SP/TM containing proteins, therefore extending the number of potential new targets for vaccine development.

Tryptophan rich antigen (*pvtrag*) and *vir* proteins, parasite antigenic proteins involved in immune evasion, are of the most abundant proteins found in this proteome. Transcriptome analysis of *P. vivax* revealed that members of *vir* and *pvtrag* gene families have two distinct phases of transcription with non-overlapping, immediately after invasion and time to schizogony [15]. Protein members of these gene families (10 *pvtrag* and 4 *vir*) detected in this schizont stage proteome seem to be derived from the first wave of gene expression since all of them shown maximum

transcription in ring stage. The cognate proteins of 52 *vir* and 13 *pvtrag* genes whose have the maximum transcription in schizont stage could not be detected in this proteome possibly due to the delay in protein translation. In contrast all of parasite proteins involved in erythrocyte invasion have the maximal transcription late in the erythrocytic cycle.

Parasite adhesins are of particular importance due to their roles in parasite invasion, sequestration or parasite-host interactions in which several parasite adhesins have been reviewed [42]. Eleven adhsins were detected in this schizont proteome. Merozoite suface protein 1 (MSP1) and AMA1 have been known as adhesive proteins involve in erythrocyte invasion. Out of 11 predicted adhesins, 6 proteins were hypothetical which may involve in parasite-host interaction or host cell invasion as has been seen in other parasite adhesins [57].

Most of merozoite surface proteins are attached to the plasma membrane via GPI anchor which make the GPI-anchored proteins the major carbohydrate modification of the parasite surface proteins. Several GPI-anchored merozoite proteins involved in parasite-host interaction [58-60] and invasion have been identified and emerged as promising vaccine candidates. Well known GPI-anchored proteins were identified, MSP1, MSP8 and Pf12. One micronemal hypothetical protein (PVX_088910) was predicted as novel GPI anchored with the molecular function as host cell surface binding.

The identification of unique *P. vivax* proteins could be meaningful for the development of diagnostic test since miss of *P. vivax* diagnosis in the mixed malaria infection often occurs in endemic areas where *P. vivax* is co-existed. Given that most of the rapid diagnostic tests are based on the pan-*Plasmodium* antigens (e.g. lactate dehydrogenase and aldolase) [24,25,61] which are not specific for *P. vivax* diagnosis, , the unique *P. vivax* proteins reported herein may be of interest for the development of a *P. vivax*-specific test. Moreover, 50% of unique proteins were hypothetical which suggested that these uncharacterized proteins could be the key to understand the unique biology of the parasite.

To date, a number of vaccines have been tested in either preclinical or clinical trials, and most of them focus on *P. falciparum* [16,62-65]. Only one *P. vivax* vaccine candidate, PVRII, has been tested in preclinical trial. Antigen discovery for

vivax malaria vaccine is an important area that needs to be strengthened. We have shown here that the immunoblotting and MS approach could lead to the discovery of novel antigens that are recognized by host immunity. In this proteome, four antigens were recognized by *P. vivax* immune serum. One of which, PV24, showed long lasting reactivity with the convalescence sera collected at 6 months after successful treatment of *P. vivax* infection. In *Plasmodium* infection, T cells play a central role in the regulation of immune responses and the formation of immunologic memory which can control and eliminate the infection [66]. The prolong production of anti-PV24 antibodies implied the role of PV24 in stimulation of T-cells response leading to protective immunity. In the way of antigens discovery, study of humoral and cellular immune response generated against *Plasmodium* antigens during natural infection are required [67]. In this study, PV24 has shown the role in generation of humoral immune response but the immunogenicity and its sero-epidemiological determination in the malaria endemic area as well as the generation of cellular immune response are of interest and await further investigation to confirm its role as a new immunopotentiate malaria protein to prevent malaria infection.

CHAPTER VII
CONCLUDE

In this schizont stage proteome, 316 proteins were confidently identified in which almost 50% were hypothetical. Functional profiles revealed the hypothetical protein, protein with binding function, protein fate, protein synthesis, metabolism and cellular transport as the dominant categories among identified proteins. Four out of 316 proteins were recognized by host humoral immunity. Among four potential antigens identified, one protein, PV24, was recognized by antibodies from vivax malaria patients. Therefore, more reports on the proteins of *P. vivax* parasite provide useful information and availability to facilitate not only basic research on this extraordinary malaria parasite, but also provide the new tools for drug and vaccine developments.

REFERENCES

1.Mendis K, Sina BJ, Marchesini P, Carter R. The neglected burden of *Plasmodium vivax* malaria. Am. J. Trop. Med. Hyg. 2001 Feb;64(1-2 Suppl):97-106.

2.Krotoski WA, Collins WE, Bray RS, Garnham PC, Cogswell FB, Gwadz RW, et al. Demonstration of hypnozoites in sporozoite-transmitted *Plasmodium vivax* infection. Am. J. Trop. Med. Hyg. 1982 Nov;31(6):1291-1293.

3.Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype, FyFy. N. Engl. J. Med. 1976 Aug 5;295(6):302-304.

4.Mons B. Preferential invasion of malarial merozoites into young red blood cells. Blood Cells. 1990;16(2-3):299-312.

5.Suwanarusk R, Cooke BM, Dondorp AM, Silamut K, Sattabongkot J, White NJ, et al. The deformability of red blood cells parasitized by *Plasmodium falciparum* and *P. vivax*. J. Infect. Dis. 2004 Jan 15;189(2):190-194.

6.Handayani S, Chiu DT, Tjitra E, Kuo JS, Lampah D, Kenangalem E, et al. High deformability of *Plasmodium vivax*-infected red blood cells under microfluidic conditions. J. Infect. Dis. 2009 Feb 1;199(3):445-450.

7.Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, et al. Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature. 2002 Oct 3;419(6906):498-511.

8.Mackinnon MJ, Li J, Mok S, Kortok MM, Marsh K, Preiser PR, et al. Comparative transcriptional and genomic analysis of *Plasmodium falciparum* field isolates. PLoS Pathog. 2009 Oct;5(10):e1000644.

9.Bozdech Z, Llinás M, Pulliam BL, Wong ED, Zhu J, DeRisi JL. The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*. PLoS Biol. 2003 Oct;1(1):E5.

10.Florens L, Washburn MP, Raine JD, Anthony RM, Grainger M, Haynes JD, et al. A proteomic view of the *Plasmodium falciparum* life cycle. Nature. 2002 Oct 3;419(6906):520-526.

11.Lasonder E, Ishihama Y, Andersen JS, Vermunt AMW, Pain A, Sauerwein RW, et al. Analysis of the *Plasmodium falciparum* proteome by high-accuracy mass spectrometry. Nature. 2002 Oct 3;419(6906):537-542.

12.Panichakul T, Sattabongkot J, Chotivanich K, Sirichaisinthop J, Cui L, Udomsangpetch R. Production of erythropoietic cells in vitro for continuous culture of *Plasmodium vivax*. Int. J. Parasitol. 2007 Dec;37(14):1551-1557.

13.Carlton JM, Adams JH, Silva JC, Bidwell SL, Lorenzi H, Caler E, et al. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. Nature. 2008 Oct 9;455(7214):757-763.

14.Cui L, Fan Q, Hu Y, Karamycheva SA, Quackenbush J, Khuntirat B, et al. Gene discovery in *Plasmodium vivax* through sequencing of ESTs from mixed blood stages. Mol. Biochem. Parasitol. 2005 Nov;144(1):1-9.

15.Bozdech Z, Mok S, Hu G, Imwong M, Jaidee A, Russell B, et al. The transcriptome of *Plasmodium vivax* reveals divergence and diversity of transcriptional regulation in malaria parasites. Proc. Natl. Acad. Sci. U.S.A. 2008 Oct 21;105(42):16290-16295.

16.Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Kone AK, Guindo AB, et al. Safety and immunogenicity of an AMA1 malaria vaccine in Malian children: results of a phase 1 randomized controlled trial. PLoS ONE. 2010;5(2):e9041.

17.Hu J, Chen Z, Gu J, Wan M, Shen Q, Kieny M, et al. Safety and Immunogenicity of a Malaria Vaccine, *Plasmodium falciparum* AMA-1/MSP-1 Chimeric Protein Formulated in Montanide ISA 720 in Healthy Adults. PLoS ONE. 3(4).

18.Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM. Vivax malaria: neglected and not benign. Am. J. Trop. Med. Hyg. 2007 Dec;77(6 Suppl):79-87.

19.Homewood CA. Transport of carbohydrates by malarial parasites. Bull World Health Organ. 1977;55(2-3):227-227.

20.Roth EF, Calvin MC, Max-Audit I, Rosa J, Rosa R. The enzymes of the glycolytic pathway in erythrocytes infected with *Plasmodium falciparum* malaria parasites. Blood. 1988 Dec;72(6):1922-1925.

21.Mehta M, Sonawat HM, Sharma S. Glycolysis in *Plasmodium falciparum* results in modulation of host enzyme activities. J Vector Borne Dis. 2006 Sep;43(3):95-103.

22.Dunn CR, Banfield MJ, Barker JJ, Higham CW, Moreton KM, Turgut-Balik D, et al. The structure of lactate dehydrogenase from *Plasmodium falciparum* reveals a new target for anti-malarial design. Nat. Struct. Biol. 1996 Nov;3(11):912-915.

23.Wiwanitkit V. *Plasmodium* and host lactate dehydrogenase molecular function and biological pathways: implication for antimalarial drug discovery. Chem Biol Drug Des. 2007 Apr;69(4):280-283.

24.Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, Baum MK, et al. Evaluation of the OptiMAL Test for Rapid Diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* Malaria. J Clin Microbiol. 1998 Jan;36(1):203-206.

25.Van Dijk DPJ, Gillet P, Vlieghe E, Cnops L, Van Esbroeck M, Jacobs J. Evaluation of the Palutop+4 malaria rapid diagnostic test in a non-endemic setting. Malar. J. 2009;8:293.

26.Königk E. Salvage syntheses and their relationship to nucleic acid metabolism. Bull World Health Organ. 1977;55(2-3):249-252.

27.Yamada KA, Sherman IW. Purine metabolism by the avian malarial parasite *Plasmodium lophurae*. Mol. Biochem. Parasitol. 1981 Aug;3(4):253-264.

28.Berman PA, Human L, Freese JA. Xanthine oxidase inhibits growth of *Plasmodium falciparum* in human erythrocytes in vitro. J Clin Invest. 1991 Dec;88(6):1848-1855.

29.Woodberry T, Pinzon-Charry A, Piera KA, Panpisutchai Y, Engwerda CR, Doolan DL, et al. Human T cell recognition of the blood stage antigen *Plasmodium* hypoxanthine guanine xanthine phosphoribosyl transferase

(HGXPRT) in acute malaria. Malar J. 8:122-122.

30.Eggleson KK, Duffin KL, Goldberg DE. Identification and Characterization of Falcilysin, a Metallopeptidase Involved in Hemoglobin Catabolism within the Malaria Parasite *Plasmodium falciparum*. Journal of Biological Chemistry. 1999 Nov 5;274(45):32411 -32417.

31.Gavigan CS, Dalton JP, Bell A. The role of aminopeptidases in haemoglobin degradation in *Plasmodium falciparum*-infected erythrocytes. Mol. Biochem. Parasitol. 2001 Sep 28;117(1):37-48.

32.Ben Mamoun C, Prigge ST, Vial H. Targeting the Lipid Metabolic Pathways for the Treatment of Malaria. Drug Dev Res. 2010 Feb;71(1):44-55.

33.Mewes HW, Frishman D, Güldener U, Mannhaupt G, Mayer K, Mokrejs M, et al. MIPS: a database for genomes and protein sequences. Nucleic Acids Res. 2002 Jan 1;30(1):31-34.

34.Zerial M, McBride H. Rab proteins as membrane organizers. Nat Rev Mol Cell Biol. 2001 Feb;2(2):107-117.

35.Liu P, Bartz R, Zehmer JK, Ying Y, Anderson RGW. Rab-Regulated Membrane Traffic between Adiposomes and Multiple Endomembrane Systems. Methods Enzymol. 2008;439:327-337.

36.Vanlandingham PA, Ceresa BP. Rab7 Regulates Late Endocytic Trafficking Downstream of Multivesicular Body Biogenesis and Cargo Sequestration. Journal of Biological Chemistry. 2009 May 1;284(18):12110 -12124.

37.Ungermann C, Neupert W, Cyr DM. The role of Hsp70 in conferring unidirectionality on protein translocation into mitochondria. Science. 1994 Nov 18;266(5188):1250-1253.

38.Lyman SK, Schekman R. Interaction between BiP and Sec63p is required for the completion of protein translocation into the ER of *Saccharomyces cerevisiae*. J. Cell Biol. 1995 Dec;131(5):1163-1171.

39.Meyer H, Grau H, Kraft R, Kostka S, Prehn S, Kalies K, et al. Mammalian Sec61 Is Associated with Sec62 and Sec63. Journal of Biological Chemistry. 2000 May 12;275(19):14550 -14557.

40.de Koning-Ward TF, Gilson PR, Boddey JA, Rug M, Smith BJ, Papenfuss AT, et al. A newly discovered protein export machine in malaria parasites.

Nature. 2009 Jun 18;459(7249):945-949.

41.del Portillo HA, Lanzer M, Rodriguez-Malaga S, Zavala F, Fernandez-Becerra C. Variant genes and the spleen in *Plasmodium vivax* malaria. Int. J. Parasitol. 2004 Dec;34(13-14):1547-1554.

42.Carvalho BO, Lopes SCP, Nogueira PA, Orlandi PP, Bargieri DY, Blanco YC, et al. On the Cytoadhesion of *Plasmodium vivax*-Infected Erythrocytes. J Infect Dis.2010 Jun 202(4):638–647.

43.Rayner JC, Galinski MR, Ingravallo P, Barnwell JW. Two *Plasmodium falciparum* genes express merozoite proteins that are related to *Plasmodium vivax* and *Plasmodium yoelii* adhesive proteins involved in host cell selection and invasion. Proc. Natl. Acad. Sci. U.S.A. 2000 Aug 15;97(17):9648-9653.

44.Alam MT, Bora H, Mittra P, Singh N, Sharma YD. Cellular immune responses to recombinant *Plasmodium vivax* tryptophan-rich antigen (PvTRAg) among individuals exposed to vivax malaria. Parasite Immunol. 2008 Jul;30(6-7):379-383.

45.Mittra P, Singh N, Sharma YD. *Plasmodium vivax*: Immunological properties of tryptophan rich antigens PvTRAg 35.2 and PvTRAg 80.6. Microbes Infect.2010 Nov;12(12-13):1019-26.

46.Bustamante, Woodruff, Oh, Keister, Muratova, Williamson. Differential ability of specific regions of *Plasmodium falciparum* sexual-stage antigen, Pfs230, to induce malaria transmission-blocking immunity. Parasite Immunol. 2000 8;22(8):373-380.

47.Ansari FA, Kumar N, Bala Subramanyam M, Gnanamani M, Ramachandran S. MAAP: malarial adhesins and adhesin-like proteins predictor. Proteins. 2008 Feb 15;70(3):659-666.

48.Dharia NV, Bright AT, Westenberger SJ, Barnes SW, Batalov S, Kuhen K, et al. Whole-genome sequencing and microarray analysis of ex vivo *Plasmodium vivax* reveal selective pressure on putative drug resistance genes. Proc. Natl. Acad. Sci. U.S.A. 2010 Nov 16;107(46):20045-20050.

49.Ranjan R, Chugh M, Kumar S, Singh S, Kanodia S, Hossain MJ, et al. Proteome Analysis Reveals a Large Merozoite Surface Protein-1 Associated Complex on the *Plasmodium falciparum* Merozoite Surface. Journal of

Proteome Research. 2011 Feb 4;10(2):680-691.

50.Lamarque M, Tastet C, Poncet J, Demettre E, Jouin P, Vial H, et al. Food vacuole proteome of the malarial parasite *Plasmodium falciparum*. Proteomics Clin Appl. 2008 Sep;2(9):1361-1374.

51.Ward GE, Fujioka H, Aikawa M, Miller LH. Staurosporine Inhibits Invasion of Erythrocytes by Malarial Merozoites. Experimental Parasitology. 1994 Nov;79(3):480-487.

52.Leykauf K, Treeck M, Gilson PR, Nebl T, Braulke T, Cowman AF, et al. Protein Kinase A Dependent Phosphorylation of Apical Membrane Antigen 1 Plays an Important Role in Erythrocyte Invasion by the Malaria Parasite. PLoS Pathog. 2010 Jun;6(6).

53.Ketema T, Bacha K, Birhanu T, Petros B. Chloroquine-resistant *Plasmodium vivax* malaria in Serbo town, Jimma zone, south-west Ethiopia. Malar. J. 2009;8:177.

54.Khatoon L, Baliraine FN, Bonizzoni M, Malik SA, Yan G. Prevalence of antimalarial drug resistance mutations in *Plasmodium vivax* and *P. falciparum* from a malaria-endemic area of Pakistan. Am. J. Trop. Med. Hyg. 2009 Sep;81(3):525-528.

55.Sutanto I, Endawati D, Ling LH, Laihad F, Setiabudy R, Baird JK. Evaluation of chloroquine therapy for vivax and falciparum malaria in southern Sumatra, western Indonesia. Malar. J. 2010;9:52.

56.Fernandez-Becerra C, Sanz S, Brucet M, Stanisic DI, Alves FP, Camargo EP, et al. Naturally-acquired humoral immune responses against the N- and C-termini of the *Plasmodium vivax* MSP1 protein in endemic regions of Brazil and Papua New Guinea using a multiplex assay. Malar. J. 2010;9:29.

57.Richard D, MacRaild CA, Riglar DT, Chan J, Foley M, Baum J, et al. Interaction between *Plasmodium falciparum* apical membrane antigen 1 and the rhoptry neck protein complex defines a key step in the erythrocyte invasion process of malaria parasites. J. Biol. Chem. 2010 May 7;285(19):14815-14822.

58.Hoessli DC, Poincelet M, Gupta R, Ilangumaran S, Nasir-ud-Din. *Plasmodium falciparum* merozoite surface protein 1. Eur. J. Biochem. 2003 Jan;270(2):366-375.

59.Sanders PR, Gilson PR, Cantin GT, Greenbaum DC, Nebl T, Carucci DJ, et al. Distinct protein classes including novel merozoite surface antigens in Raft-like membranes of *Plasmodium falciparum*. J. Biol. Chem. 2005 Dec 2;280(48):40169-40176.

60.Gilson PR, Nebl T, Vukcevic D, Moritz RL, Sargeant T, Speed TP, et al. Identification and Stoichiometry of Glycosylphosphatidylinositol-anchored Membrane Proteins of the Human Malaria Parasite *Plasmodium falciparum*. Molecular & Cellular Proteomics. 2006 Jul;5(7):1286 -1299.

61.Cooke AH, Chiodini PL, Doherty T, Moody AH, Ries J, Pinder M. Comparison of a parasite lactate dehydrogenase-based immunochromatographic antigen detection assay (OptiMAL) with microscopy for the detection of malaria parasites in human blood samples. Am. J. Trop. Med. Hyg. 1999 Feb;60(2):173-176.

62.Vincent AA, Fanning S, Caira FC, Williamson KC. Immunogenicity of malaria transmission-blocking vaccine candidate, y230.CA14 following crosslinking in the presence of tetanus toxoid. Parasite Immunol. 1999 Nov;21(11):573-581.

63.Dicko A, Sagara I, Ellis RD, Miura K, Guindo O, Kamate B, et al. Phase 1 study of a combination AMA1 blood stage malaria vaccine in Malian children. PLoS ONE. 2008;3(2):e1563.

64.Wu Y, Ellis RD, Shaffer D, Fontes E, Malkin EM, Mahanty S, et al. Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with montanide ISA 51. PLoS ONE. 2008;3(7):e2636.

65.WHO|Tables of Malaria Vaccine Projects Globally [Internet]. [cited 2010 Jul 26];Availablefrom:http://www.who.int/vaccine_research/links/Rainbow/en/index.html

66.Nardin EH, Nussenzweig RS. T Cell Responses to Pre-Erythrocytic Stages of Malaria: Role in Protection and Vaccine Development Against Pre-Erythrocytic Stages. Annu. Rev. Immunol. 1993 4;11(1):687-727.

67.Joshi SK, Bharadwaj A, Chatterjee S, Chauhan VS. Analysis of Immune Responses against T- and B-Cell Epitopes from *Plasmodium falciparum* Liver-Stage Antigen 1 in Rodent Malaria Models and Malaria-Exposed Human Subjects in India. Infect Immun. 2000 Jan;68(1):141-150.

APPENDICES

APPENDIX A

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS.

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_000930	sexual stage antigen s16, putative	14.29	2	KDGGENNPPPDAENALQELK	1
PVX_000995	transmission-blocking target antigen Pfs230, putative	17.97	6.56	CIYDNQGTVEPHNCFDK	1
				GCDFGNNVVNYFSKPYPVER	1
				IEAKPGEFVGFK	1
				LPHFVQHSYTVQCK	1
				YAFYLK	1
PVX_001780	hypothetical protein, conserved	1.78	1.7	SFGPIVWSEPR	1
PVX_001905	ADP-ribosylation factor, putative	19.34	3.52	NISFTVWDVGGQDK	1
				LGEVVTTIPTIGFNVETVEFR	1
PVX_002785	ATP-dependent acyl-CoA synthetase, putative	7.79	12.73	ALYFTVVPVSK	1
				ETTCTELFQR	1
				FSQDEVVEILNESK	1
				SIAYLSLYR	1
				VAITELEDCEVKR	1
PVX_002790	hypothetical protein, conserved	1.61	3.48	NILESEEILLEPVFTK	1
PVX_002835	T-complex protein 1, theta subunit, putative	2.02	2	VFAESFLVVPR	1
PVX_003545	hypothetical protein, conserved	10.86	3.4	DAYNFLQR	1
				LSEQELIQLLR	1
				MPFDSPEQMLQFLK	1
PVX_003555	hypothetical protein, conserved	6.80	9.4	AAEGEEAAEGEAAEEEAAAESGGLLR	1
				DEFKEILNDVR	1
				QCEEIVDNNFTK	1
				QHEHVNDVFYTFVAK	1
				LRDFEINTK	1
PVX_003640	hypothetical protein, conserved	6.52	3.42	QFHAYENFSK	1
				NEFFNCLR	1
PVX_003770	merozoite surface protein 5	4.39	2	SCSVDNGGCADDQICIR	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_003895	hypothetical protein, conserved	7.41	2	NQIFNTSNAYEQEKDK	1
PVX_079800	T-cell immunomodulatory protein homolog precursor, putative	4.24	6	GVYEENNIDIR	1
				TLGGNAHGPTFK	1
				YGAVGFIR	1
PVX_079955	hypothetical protein, conserved	16.61	7.4	DIQFLNELNIQK	1
				IVEQIRPVIK	1
				TVEKIVEVPVYVDR	1
				IIYQEKIVEVPQIK	1
PVX_080050	karyopherin beta, putative	1.69	2	EGKDDNQELYDIGEESLDR	1
PVX_080100	multidrug resistance protein (mdr1)	4.03	8.17	IGVVSQDPLLFSNSIK	1
				SLEATNNLYEIINR	1
				SVFYQDGQFHDNNPGSK	2
				TYAFVGESGCGK	1
				IGVVSQDPLLFSNSIK	1
PVX_080245	40S ribosomal protein S9, putative	4.76	2	IFQGEALLR	1
PVX_080460	hypothetical protein, conserved	2.37	2.39	LQLPEWNDIER	2
PVX_080555	hypothetical protein, conserved	10.26	13.13	DHDFECQDVNLVR	2
				DSQGYLTIYPK	1
				IENVYEYSNFK	1
				ISGNEIGFQYLASK	1
				VDDYYYIFK	1
				YYCDVNYDDLTVEGEDR	1
PVX_080650	myo-inositol 1-phosphate synthase, putative	2.25	2	ANYLGSVFLSSNVR	1
PVX_081335	hypothetical protein, conserved	7.96	2.46	TKGEGNSEWCNAFVTR	1
PVX_081430	small GTPase Rab5c, putative	5.14	2	TGQNVNELFLR	1
PVX_081455	calcium-transporting ATPase, putative	1.09	2.26	ADDNFNTIVEAIK	1
PVX_081550	hypothetical protein, conserved	15.15	12.01	IRDYENFFCIYPK	2
				SHDTVYEFYQK	2
				SPFFGATVLR	1
				STIDHYLDVCTQIR	2
				SVNEVFSDNSYYTR	1
				YIENQNMLLVANK	1
PVX_082460	ER lumen protein retaining receptor 1, putative	10.92	4	LLSIVPFEGYLPYDK	2
				VAAFTTHFLASQAFSK	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_082595	hypothetical protein, conserved	6.54	2.28	KLPIFETINEHDIK	1
PVX_082665	merozoite surface protein 7 (MSP7)	3.76	2	EFDNFVHGLYGFAK	1
PVX_082675	merozoite surface protein 7 (MSP7)	3.09	2.01	EFDNFVHGLYGFAK	2
PVX_082840	60S ribosomal protein L6, putative	9.52	2	VLTSGLLAVVGPYE VNGVPLKR	1
PVX_082845	elongation factor 1-gamma, putative	2.92	2.28	TQEFLNYSPLGR	1
PVX_082965	60S ribosomal protein L18a, putative	5.98	2	TNIHQYHIVGR	1
PVX_083030	myosin A, putative	3.92	5.73	DLGNTTAEWIR	1
				NGSVVAFLLEK	1
				SYHIFYQFLK	1
PVX_083080	kelch domain-containing protein	3.09	5.8	ALFETEVYDR	1
				AWVEIAPLNTPR	1
PVX_083105	hypothetical protein, conserved	2.16	3.4	KLDNYSAPFFK	1
				LESFIYESR	1
PVX_083135	aspartate carbamoyltransferase, putative	9.68	6.64	FTDLNEYNAYK	1
				GETVQDAFTILAK	1
				VFCSIFLEPSTR	1
PVX_083180	hypothetical protein, conserved	2.03	2	EFEEVYYK	1
PVX_083205	protein transport protein Sec61 alpha subunit (Pfsec61), putative	2.60	2	IIDVDQSLKEDR	1
PVX_083240	male fertility protein pf47, putative	18.01	11.15	GGDYTELETVPANC FTK	1
				VKENETIHFK	1
				YGFLQEHVLNFR	1
				TFNDFVLK	1
				VLVIPGYYK	1
				GIDFTETDELEQTDI VQNGNDK	1
PVX_083270	hypothetical protein, conserved	16.62	10	AILNGLAVGYR	1
				ICLFSGSIYK	1
				IETSYSYFNSNYSYT PGLK	1
				ISPGYVSFHEK	1
				LNFTYDENVFK	1
				AILNGLAVGYR	1
PVX_083480	hypothetical protein, conserved	5.78	2	SIFFAELNEK	1
PVX_083515	protein disulfide-isomerase, putative	15.79	6.64	DMANETFSNIDR	1
				FYAPWCSHCK	1
				IEGFPTIIYFK	2
				DMANETFSNIDR	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_083555	hypothetical protein	16.90	4	FYQTVVHR	1
				LISSIFGFDEEDIDSR	2
PVX_083560	hypothetical protein, conserved	17.95	8	DDEFESTTVENENEEDAEEQKK	1
				EGYLAYEDYSPR	1
				TCQTNCGACTCGFK	1
				TLDLYFEK	1
PVX_084230	nucleosome assembly protein 1, putative	6.90	4	IGTPNLPEFWLR	1
				YHDLYGPIYDKR	1
PVX_084625	P-type ATPase4, putative	0.96	2	GFEPYGGLFDSSK	1
PVX_084640	hypothetical protein	4.13	2.37	LLEPYYANVR	1
PVX_084720	hypothetical protein, conserved	5.95	6	DLSLNETSGVSNEQLNAFLR	1
				KEEEQEDDNFYDAYK	1
				LISEGILTYEDLTEEELKK	2
PVX_084795	mitochondrial glycoprotein domain containing protein	7.09	4	QGGITFYCTTLQNDEKFR	1
PVX_084940	hypothetical protein, conserved	15.57	8.39	FDASGISLLDVK	1
				NLQYSLGGSYTK	1
				SNFDSQYIFSFR	2
				YVHVGSHPK	1
PVX_085645	hypothetical protein, conserved	3.49	4	FESVFNYDDAEQPR	1
				SKLDEISDNLSSIIIQR	1
PVX_085735	60S ribosomal protein L10, putative	8.68	3.7	VDIGQVLLSIR	2
				VHPFHVLR	1
PVX_085915	hypothetical protein, conserved	3.21	2	NVHVNFLSFDK	1
PVX_085930	rhoptry-associated protein 1, putative	18.24	25.7	DYTFLAFK	1
				LDLFTLTNEDLK	2
				LFNEIQKNPEPIFEK	2
				LGNVIGSIGEYHVR	2
				LYEIENDLLK	1
				LYGACFK	1
				LYLLQNGLTYK	1
				NDVNTLENVNFCLLNPK	2
				NFGLFDFELPDNK	2
				NPEPIFEK	2
				TDYVLNDYDPSVK	1
				YQPSLDYMTLADDYK	1
				LFNEIQK	1
PVX_085980	hypothetical protein, conserved	11.16	3.7	GAYIDCYR	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
				YSVADPDHGMQFEEFNR	1
PVX_086040	aspartic protease PM4, putative	18.00	12	ANVIVDSGTTTITAPSEFLNK	1
				DLSIGSIDPIVVELK	2
				FFANLNVIK	1
				VPFLPFYVTTCDNK	1
				YFTVFDYDK	1
PVX_086090	hypothetical protein, conserved	12.68	6	GLFSYDLK	2
				QDGLDDNCHTNR	1
				YTPYPQEQNQNAYYPA	1
PVX_086980	hypothetical protein, conserved	3.80	2	HANFFQGSLSDAIR	1
PVX_087095	hypothetical protein, conserved	4.62	2	YQFINIER	1
PVX_087675	hypothetical protein, conserved	17.27	2.36	LTGGATPYPVGQDDVNAIK	1
PVX_087725	hypothetical protein, conserved	3.43	2	VLFDCAK	1
PVX_087950	heat shock protein 86, putative	5.21	6.01	GVVDSEDLPLNISR	1
				KPEEVTNEEYASFYK	1
PVX_087980	chloroquine resistance transporter, putative	3.30	2	LSAEPEFFIR	1
PVX_088205	vacuolar proton translocating ATPase subunit A, putative	2.65	5.02	QINLPFSEIGTNIK	1
				GLNALHYR	1
				GNTYTYFQSIDENAAPSK	2
PVX_088910	hypothetical protein, conserved	2.20	2	NNVDVAALTADVEQAFK	1
PVX_088960	protein disulfide isomerase, putative	36.72	28.7	ASLGLNEFPGLAYQSSEGR	2
				FYAESPK	2
				HATNTPISVEGVPDLEDGTAEEL	1
				IPLPYEGER	2
				KLEPVYEDLGR	1
				LEPVYEDLGR	1
				LIPEYNEAANMLAEK	2
				SEPIPEDDKAAPVK	2
				SFNEIGDK	1
				SFNEIGDKNR	2
				THFVLLNIPEYADHAR	2
				TIVTFFKDVEEGKVEK	1
				VVVGNSFIDVVLK	1
				VPLSEFVSTESFPLFGEINTENYR	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_089170	hypothetical protein, conserved	7.81	8.22	ENENIDYFLLVNNK	1
				FSNIYSTYALR	1
				VYQQVNNSYASFTSK	1
PVX_089220	hypothetical protein, conserved	1.80	3.21	YNEIEHLTNEPLKK	1
PVX_089425	heat shock 70 kDa protein, putative	13.19	14.85	GVLPDSGGVESDIIYDQQK	1
				ATAGDTHLGGEDFDNR	2
				FHLDGIPPAPR	2
				NAVITVPAYFNDSQR	1
				NSLENYCYGVK	2
				QNHITITNDKGR	1
				SVHEVVLVGGSTR	2
				TTPSYVAFTDTER	1
PVX_089485	hypothetical protein, conserved	5.32	2	AEDHENKPVFLYYLGK	1
PVX_089505	suppressor of Ras1 3-9, putative	25.00	8	AFDDAITEFDNVSEDSYK	1
				ATDIAENELPSTHPIR	2
				EASNFAQEAYQK	2
				TLVEQCVNNDKDELTVEER	2
PVX_089750	60S ribosomal protein L15-1, putative	4.39	2	ALQGFVIYR	1
PVX_090160	40S ribosomal protein S19s, putative	9.88	2	SILQQLENLGYVEQNPK	1
PVX_090215	hypothetical protein, conserved	9.16	4.01	GENHTPFIGYFSSHLLR	1
				HEDEQGGYNEENVYEPY	1
PVX_090240	hypothetical protein, conserved	2.73	2	ILINYEELQK	1
PVX_090250	tryptophan-rich antigen (Pv-fam-a)	4.35	2	LEGEWVDFNNTIEK	2
PVX_090275	tryptophan-rich antigen (Pv-fam-a)	23.30	14.22	KWNNYNPGLDLEYK	1
				LNEFSNWLK	1
				TAAKPNEYVEEWR	1
				WLAAFTNK	1
				WTFFSPNIDDIPVPK	1
				WVANLGDLEK	1
				EFHVVLEDER	1
PVX_090900	hypothetical protein, conserved	11.16	9.43	FLFSEEMNEANLK	1
				GQTLPEFVQDDSDPK	1
				NYPSVVVFNPYK	1
				NYPSVVVFNPYKR	1
				VLVFSNK	1
PVX_090930	histone H4, putative	55.34	20	DSIMYTEHAK	1
				ISGLIYEEIR	1
				ISGLIYEEIRG	1
				RISGLIYEEIR	2
				SGLIYEEIR	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
				TVTAMDIVYSLK	1
				TVTAMDIVYSLKR	1
				VFLENVIK	2
				VFLENVIKDSI	1
				VFLENVIKDSIMYTEHAK	3
				DNIQGITKPAIR	7
				ISGLIYEEIR	3
				ISGLIYEEIRG	1
				LRDNIQGITKPAIR	2
				QGITKPAIR	2
				RDNIQGITKPAIR	6
PVX_090935	histone 2B	55.08	14.42	EIQTAIR	2
				KSMNIMNSFLVDTFEK	2
				QVHPDTGISR	3
				SMNIMNSFLVDTFEK	5
				SRYDSYGLYIFK	2
				VLKQVHPDTGISR	2
				YDSYGLYIFK	4
				LVLPGELAKHAVSEGTK	1
PVX_090945	hypothetical protein, conserved	3.33	4.36	DKDFIISSGR	1
				VFFNESEFNNCYK	1
PVX_090950	40S ribosomal protein S4, putative	7.28	2	LSNVFVIGDNSKPYISLPR	1
PVX_090960	hypothetical protein, conserved	8.13	9.14	ENHFFIISAIK	1
				HTGDLVLTNQLNSLVNAR	1
				LFITENAESVFR	1
				VINFPVNCIPHSAVDYNAGR	1
PVX_091105	membrane-associated calcum-binding protein, putative	25.58	10	ALDDLTNLNDEQVNDILGLDIK	2
				LAVTSLTDYGDILR	2
				QIDADKDGFISLPELNEAF	1
				VITEEELTAWSNYVK	1
				VYFDPSNETGSINLTEVK	1
PVX_091110	DnaJ domain containing protein	1.64	2	SFETFYGNR	1
PVX_091470	heat shock protein 101, putative	7.29	13.01	ILVEPPSVENTIK	1
				LIGATTIAEYR	1
				LIGATTIAEYRK	1
				SGGLYLEQFGSNLNEK	2
				SKYENFYGIHITDK	1
				YYEEYVISGER	1
PVX_091495	hypothetical protein, conserved	7.81	2	FNYLNIIYEK	1
PVX_091515	GTP-binding nuclear protein Ran, putative	10.75	4.56	TQFNVWDTAGQEK	1
				NLQYYDLSAR	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_091700	circumsporozoite-protein related antigen, putative	14.19	4	HPFSLGGGK	2
				IDVHELISEIVR	1
PVX_091990	hypothetical protein, conserved	3.30	2.14	YYEENLELYGTDAR	1
PVX_092065	spermidine synthase, putative	14.95	9.73	NVLIVGGGDGGVIR	1
				SKYQSILVFESR	1
				YQSILVFESR	1
				YYSYENHSAAFR	1
				SISCGFEDKR	1
PVX_092070	hypothetical protein, conserved	13.99	9.89	FDVNFNNSSK	1
				ILSIINEIFTK	1
				KADGGSPFPVEPLNPDDLNR	1
				LNLDNENQEPEEINTITGK	1
PVX_092105	transporter, putative	3.68	4	QSFFNSPSFR	1
				QYEFDNYYSSR	2
PVX_092245	aquaglyceroporin, putative	5.43	2	LLSLAAYGSDAFTK	1
PVX_092275	apical merozoite antigen 1	7.65	6.72	GFNWANFDSVK	1
				SAFLPVGAFNSDNFK	1
				THAASFVMAGDQNSSYR	1
PVX_092310	heat shock protein hsp70 homologue, putative	3.02	1.71	SVNPDEAVALGAAIQGGVLK	1
PVX_092620	myosin heavy chain subunit, putative	0.70	1.7	RLMEHGLGYYNK	2
PVX_092765	hypothetical protein, conserved	6.23	4.01	EIQEEFFEEQR	1
				IGLQHPDFR	1
PVX_092850	small GTPase Rab6, putative	29.55	9.03	DSAAAIVVYDITNR	2
				TLYLDEGPVR	1
				SLIPSYIR	1
				WIQDILNER	1
				LQLWDTAGQER	1
PVX_092990	tryptophan-rich antigen (Pv-fam-a)	0.85	2	EEWNQFVNEIKV	1
PVX_092995	tryptophan-rich antigen (Pv-fam-a)	17.04	13.17	DALKEYTGPEFK	1
				LEQQWDEFMK	2
				NLLQEFGK	1
				QFYEDWCR	2
				QWMQAFAEQWTQDK	1
				QWNTWTEER	1
PVX_093680	Phist protein (Pf-fam-b)	15.49	26.03	AELQEQMTEEELNSK	1
				AHYNMTDELIK	2
				AHYNMTDELIKK	2
				ERGDFQDFYAFVSK	2
				GDFQDFYAFVSK	3

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
				GDFQDFYAFVSKG	1
				GDNEQLPFGCTR	1
				HGPHGDNEQLPFGCTR	3
				MQEYIMQYSQYLQK	5
				NLRPNATVK	1
				SIWMEILTYK	3
				TLLLPTPIR	1
				WDFLYFANAK	3
				AELQEQMTEEELNSK	3
PVX_094345	hypothetical protein, conserved	14.29	1.4	YTVKPMEFEGESGDQNLR	1
PVX_094505	hypothetical protein, conserved	24.30	3.7	SPFDEVIITGLGSATK	1
				IQTAYFSSDR	1
PVX_094535	RNA binding protein, putative	6.80	2	NAIDALNFCNFDGYILK	2
PVX_094755	hypothetical protein, conserved	11.61	4	SGVIITQFNDER	1
				WGSGSGNQLVTAIK	1
PVX_094840	hypoxanthine phosphoribosyltransferase, putative	3.86	2	HLYVEHYVR	2
PVX_094865	hypothetical protein, conserved	3.42	2	IYNTNPFTQTYSR	1
PVX_095000	heat shock protein 60, putative	12.41	10.89	AAVEEGIVPGGGSALLFASK	1
				ELDSVQTDNYDQR	2
				GYISPYFINNSK	1
				NVIIEQSFGSPK	1
				VGGISEVEVNEIKDR	1
PVX_095015	enolase, putative	19.96	11.53	DVQIVGDDLLVTNPTR	2
				IAMDVAASEFYQADTK	1
				IEESLGSNALFAGEK	1
				TGAELVNLYIDMVK	1
				YGAEVYHTLK	1
				AAVPSGASTGIYEALELR	1
PVX_095165	protein kinase, putative	15.25	6	DALQTYSDIAHEEVSLAHLK	1
				DVLYGATFDHLSK	1
				LGSPIFDFIK	1
PVX_095195	ATP-dependent RNA helicase, putative	2.31	2	GITQYYAFVK	1
PVX_095200	hypothetical protein, conserved	4.84	1.7	VAQDYLSER	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_095290	hypothetical protein, conserved	4.47	3.3	DIFLQNFAK	1
				ILNFLNTYDR	1
PVX_095400	hypothetical protein, conserved	4.85	1.4	QYPFLDDLGKK	1
PVX_095405	transporter, putative	12.82	6	AHYPNLSLLAK	1
				NHIFQFLNDIAEK	1
				TACTSEESYIR	1
PVX_096070	early transcribed membrane protein (ETRAMP)	30.77	8	AIDQIDQQIEKK	1
				KAPQVNVENANGAP ATESSETVVQGN	1
				LSGPGTPPPAPTPSP ATPSSK	1
				LSGPGTPPPAPTPSP ATPSSKDDAGK	1
PVX_096245	hypothetical protein, conserved	2.10	2	ATNEENESLYEEK	1
PVX_096295	hypothetical protein, conserved	0.44	2	LNYNFVEER	1
PVX_096340	60S ribosomal protein L11, putative	8.09	2	VLEQLTEQKPIFGK	1
PVX_096970	variable surface protein Vir8-related	2.24	2.83	YGYNPFGIFFK	1
PVX_096975	hypothetical protein	3.78	4	NGSGGFFSSLFR	1
				NYEFSYIAK	1
PVX_096980	variable surface protein Vir, putative	21.97	14.33	DFLDYMCPTEYYDK	2
				DVCTAFFGYVEDVV R	1
				GIYEDFVK	1
				HYPTFTFFR	1
				LFYFHENVGDIK	2
				LTDSTVCNELHVFF DDYDK	1
				YVNDCIDVYR	1
PVX_096985	variable surface protein Vir,	8.79	6.1	GPYGANYR	1
				QTDLNVFTNWNLEK	1
				NHNLDKNYATNY	1
PVX_096995	tryptophan-rich antigen (Pv-fam-a)	5.00	4.01	IKEWVTSEWK	1
				YDDATIQTLTVAER	1
PVX_097575	tryptophan-rich antigen (Pv-fam-a)	0.30	2.38	FIEDFQFK	1
PVX_097577	tryptophan-rich antigen (Pv-fam-a)	2.86	2.05	NEKEEFFYK	1
PVX_097590	rhoptry-associated protein 2, putative	16.75	10.56	AFTIYLIQNYAK	1
				AIVNFHMIK	3
				EDPSISVSEVYAAR	1
				SMSNTDNYETYFK	4

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
				THATYNTNPIPAFAPYDIR	1
PVX_097625	merozoite surface protein 8, putative	8.62	6.03	CPLNSNCYVINGEEVCR	6
				DDTLDCSNNNGGCDVNATCTLIDK	1
				KCPLNSNCYVINGEEVCR	1
PVX_097905	hypothetical protein, conserved	4.38	4	VLYPIYTETVVK	1
				VYNQLGGLNTNYIR	1
PVX_097935	subtilisin-like protease precursor, putative	2.58	2	ICVIDSGIDYNHPDLR	1
PVX_098015	soluble NSF attachment protein (SNAP), putative	5.70	2	NVAEIYEDLYEYGNASK	1
PVX_098605	small GTPase Rab7, putative	11.65	4	FQSLGVAFYR	1
				NAINVDQAFDEIAR	1
PVX_098665	signal peptidase, putative	19.46	4	LPAEYFDNLSANYPLYYPDK	1
				VKNEVIIQDYIITNKK	1
PVX_099035	hypothetical protein, conserved	3.57	2	SFFTNEISVTQPK	1
PVX_099055	hypothetical protein, conserved	5.62	5.22	DPFYIGLK	1
				TQNLHLLHDDIATFTCK	1
PVX_099160	transporter, putative	6.40	4	SGAFSELCGPTDEKQEFK	1
				SYNNEEGFNFSDK	1
PVX_099315	78 kDa glucose-regulated protein precursor (GRP 78), putative	43.71	51.36	AKFEELNDDLFR	1
				DAENWLSNNSNADAEALK	3
				DAENWLSNNSNADAEALKQK	1
				DAGTIAGLNIVR	1
				DNHLLGKFELTGIPPAQR	1
				DVEAVCQPIIVK	1
				EIAQSFLGKPVK	1
				FADEDKNLR	1
				FEELNDDLFR	2
				IDEIVLVGGSTR	2
				IINEPTAAALAYGLDK	2
				ITPSYVSFVDGER	1
				LEATLHPTQTVFDVK	1
				LKDVEAVCQPIIVK	1
				LYGQPGANSPPPSADEDVESDEL	1
				NAVVTVPAYFNDAQR	1
				NGILHVEAEDKGTGK	1
				NNLDNYLQNMK	1
				SKIDEIVLVGGSTR	1
				SQTFSTYQDNQPAVLIQVFEGER	1
				TLLPYEIVNQEGKPNIR	2
				TTFAPEQISAMVLEK	1
				VEILNNELGNR	1
				AKFEELNDDLFR	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
				DAGTIAGLNIVR	1
				FADEDKNLR	1
				FELTGIPPAQR	1
				NAVVTVPAYFNDAQR	2
				SKIDEIVLVGGSTR	1
				SQTFSTYQDNQPAVLIQVFEGER	1
				TTFAPEQISAMVLEK	1
PVX_099320	acid phosphatase, putative	18.99	17.28	DWAGNYNSELLK	1
				FASLGDWGK	1
				IADYIIVVGDQPIYSSGSSR	2
				IADYIIVVGDQPIYSSGSSRG	1
				KIADYIIVVGDQPIYSSGSSR	1
				SLDKVNSLQYFASLPK	1
				VNSLQYFASLPK	3
				VTFIVSPGSNFLDGVK	1
				DWAGNYNSELLK	2
				FASLGDWGK	2
PVX_099370	DNAJ-like molecular chaperone protein, putative	11.90	7.08	ENYYTYLNITPNATK	1
				IYHPDKNPDESADSSFIK	1
				QAYDILTDDVRR	1
PVX_099535	phosphoglycerate kinase, putative	3.61	2	LGDVFINDAFGTAHR	1
PVX_099710	hypothetical protein, conserved	30.33	13.7	DGNLGYILVNFSCDR	2
				IFPGLLCR	2
				NYLFGEQK	2
				QLQGTFYEIATNASDK	4
				TILLETYNK	1
				YEFSGLK	1
				YEFSGLKR	1
PVX_099840	phosphatidylserine decarboxylase, putative	6.78	4.28	FNVNTFLGSNFQK	1
				IINNLFNINER	1
PVX_099930	high molecular weight rhoptry protein-2, putative	9.86	22.23	AYSFFSGTGALTNLPK	1
				EENKNSEYLAFK	1
				FCSDHIHLCQK	1
				FYEQSIIYYR	1
				ITDIYDQDR	1
				NAFQYINVAELLSPR	1
				QAASLLESIILK	1
				TAYFQCK	1
				VIFDNLVTYVDQNSK	1
				YSSETFLSIK	1
				FFHYENDYNDLKEDENKR	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_099980	major blood-stage surface antigen Pv200	6.23	28.2	KLQVSLDHYGK	1
				LDKLEALVVDGYELFHK	1
				LKSEIYDLAQEIR	1
				LQVSLDHYGK	2
				NYTAFFNK	2
				QLTYLEDYVLR	3
				VNEFKPAFNHFYEAR	1
				YLPFLNSLQK	2
				YSSSGEYIIKDPYK	2
				YYIGEPFPLK	2
				KLQVSLDHYGK	1
PVX_100550	chaperonin CPN60, mitochondrial precursor, putative	1.27	2	NHNPIPIQR	1
PVX_100735	ATP synthase beta chain, mitochondrial precursor, putative	5.67	2	FLSQPFAVAEVFTGKPGR	1
				VALTGLTVAEYFR	1
PVX_100835	hypothetical protein, conserved	16.19	11.01	ENLYNELIR	1
				IPIPENAEEAKK	1
				IQKENLYNELIR	1
				LANHNMQLR	2
				RIPIPENAEEAKK	1
PVX_101200	actin	16.49	4.24	AAPEEHPVLLTEAPLNPK	1
				SYELPDGNIITVGNER	2
				IWHHTFYNELR	5
				KYPIEHGIVTNWDDMEK	1
				YPIEHGIVTNWDDMEK	7
PVX_101235	hypothetical protein, conserved	3.83	2	QIPLIIFCNK	1
PVX_101485	hypothetical protein, conserved	0.89	4.01	NHHLNPFINK	1
				NLFDDIAIDR	1
PVX_101515	tryptophan-rich antigen (Pv-fam-a)	6.23	4.36	ESILSQIDAR	1
				YELYENSECK	1
PVX_101520	Pv-fam-d protein	11.85	8.05	FNNEGPIEEHEIK	1
				MYGVYDEQK	1
				QAHHYQQDIEAHQTPY	1
				SALQSMLESFIK	4
PVX_107740	hypothetical protein	2.30	2.27	DLHDYFR	1
PVX_111165	hypothetical protein, conserved	3.79	2.14	EDGSVYAGHFAFNR	1
PVX_111245	adenosine deaminase, putative	2.48	3.1	YSPTFVAFK	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_112110	Phist protein (Pf-fam-b)	4.88	5	LYHLLQHGSCSR	1
				SGYIQFIK	1
				ELFYNEK	1
PVX_112665	tryptophan-rich antigen (Pv-fam-a)	14.24	6.31	KWEHYDEYTFK	1
				LKNEEEEWSNWLDEK	1
				QLNEEGVYSVSEWKK	1
PVX_112670	tryptophan-rich antigen (Pv-fam-a)	3.58	2	STQNWNESQWNK	1
PVX_113465	long chain polyunsaturated fatty acid elongation enzyme, putative	7.90	4	ALNIAFINNLGENILK	1
				YNSYYPR	2
PVX_113775	membrane protein pf12 precursor, putative	5.80	2	LVAHFEFATTPDDQNSVSEPR	4
PVX_113830	myosin-like protein, putative	2.14	6	ANHLFTLDQNDLLK	4
				FSNTSEGSLNEYTVAR	4
				NGFTEYSPELR	4
PVX_114015	histone H2A, putative	36.84	12.8	AGLQFPVGR	2
				FLAGVTFASGGVLPNIHNV	2
				GTSNSAKAGLQFPVGR	1
				HIQLAVR	1
				NDEELNKFLAGVTF	2
				NDEELNKFLAGVTFA	2
				HIQLAVR	3
PVX_114020	histone H3, putative	16.91	3.63	EIAQEYK	1
				EIAQEYKTDLR	1
				VTIMPKDIQLAR	1
PVX_114440	hypothetical protein, conserved	4.07	1.7	FNPNFLESEADADER	1
PVX_114445	pyruvate kinase, putative	4.70	4	IENIEGIINFDK	1
				VGSFQGTDNVLR	1
PVX_114560	DNAJ domain protein, putative	2.63	2	GDFFSVIYTR	1
PVX_114670	ER lumen protein retaining receptor, putative	4.98	2	FKLPISQTYNR	1
PVX_114832	Elongation factor 1 alpha, putative	16.70	11.9	EVLEEARPGDNIGFNVK	2
PVX_114830	elongation factor 1 alpha, putative			FTAQVIILNHPGEIK	1
				IPLQGVYK	2
				YFFTVIDAPGHK	1
				YSEDRYEEIKK	1
				GSFKYAWVLDK	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_115000	falcilysin, putative	3.12	5.53	LAESDFPQFEQILNR	1
				SSEEFVILR	1
				NLLGYFEENDAK	1
PVX_115155	hypothetical protein	7.22	2	ICELAHPYYVDLR	1
PVX_115450	hypothetical protein, conserved	18.37	4.21	ENKEDEEEEEKPLLVQLK	1
				GAAKPVEGAPAGECPCQR	1
PVX_115460	hypothetical protein, conserved	3.24	2	DPSYNFPLDR	1
PVX_116630	lactate dehydrogenase	18.04	12.74	ALDTSHSNVMAYSNCK	1
				IIGLGGVLDTSR	1
				ITDEEVEGIFDR	2
				KITDEEVEGIFDR	1
				YITVGGIPLQEFINNK	1
				YITVGGIPLQEFINNKK	1
PVX_116715	60S ribosomal protein L9, putative	7.37	2	VIIPEGVQVAINSR	1
PVX_116910	sulfate transporter, putative	1.50	2	FLHNNFDSFK	2
PVX_116915	exported protein 2, putative	10.99	7.05	EIVGDNAIER	1
				FWVSEPYLK	1
				LRQDPGLIVAK	1
PVX_117005	phosphoesterase, putative	3.41	2	VLSYDQSINYVSR	1
PVX_117030	RNA helicase-1, putative	8.79	8.89	AQIYEVFK	1
				AQIYEVFKK	1
				GIYSYGFEKPSAIQQR	1
				KDELTLEGIR	1
PVX_117060	hypothetical protein, conserved	0.55	1.42	DANGQSVGKDGGQNGEK	1
PVX_117490	hypothetical protein, conserved	5.57	4	AFIICYGR	1
				LSDEVFSSYNK	1
PVX_117615	signal peptide peptidase domain containing protein	4.12	3.3	EPVIFNTNK	1
				QLEQVDDR	1
PVX_117625	V-type H(+)-translocating pyrophosphatase, putative	4.88	6.07	AADVGADLSGKNEYGIPEDDIR	1
				NEYGIPEDDIR	2
				YIESGALGTEHCK	1
PVX_117890	sortilin, putative	2.45	4	INFWQFHSTK	1
				YESDEVNTFLSR	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_117925	elongation factor 2, putative	2.64	4	ANYLHSNYQWDK	1
				LWGNSFYDAK	1
PVX_118100	multidrug resistance protein 2, putative	0.81	1.48	SFTDISDLTDILR	1
PVX_118162	hypothetical protein	9.52	2	IFNLFDDDKTGAISLK	1
PVX_118255	fructose 1,6-bisphosphate aldolase, putative	15.45	10	FDNINVENTIENR	1
				GKPTDLSIQETAWGLAR	1
				LPAEVAEEIATTAK	1
				RFDNINVENTIENR	1
				TNTQDIGFLTVR	1
PVX_118495	triosephosphate isomerase, putative	18.15	6	FGSGSYTGEISAEIAK	1
				ILYGGSVNTDNCASLIK	1
				VVVCFGESLEQR	1
PVX_118545	2-Cys peroxiredoxin, putative	15.90	6.82	ALDAFHER	1
				DYNVLFDDSVSLR	1
				IIDAIQHHEK	1
PVX_118580	translocation protein sec62, putative	15.69	10	DIEEFSDTFIQK	2
				GDEFVHFLYTR	1
				KFPTLLQNR	1
				SEDLDPDMLALLTCAFDEGVK	1
				TLADLKDIEEFSDTFIQK	1
PVX_118610	hypothetical protein, conserved	6.62	5.7	DINLYYHPIK	1
				STLNDEDYEEFEK	3
				STLNDEDYEEFEKDIK	1
PVX_118620	proteosome subunit alpha type 1, putative	5.58	2	NLYDTDNITYSPEGR	1
PVX_119390	hypothetical protein, conserved	1.31	2.02	QFYELVDNVLR	1
PVX_121885	cytoadherence linked asexual protein, CLAG, putative	2.48	6.05	ALEELNAVFFPK	1
				AYESYFNQR	1
				NEELDKENSYQYSK	1
PVX_121935	hypothetical protein	3.38	2	DLQYEDYK	1
PVX_122425	M1-family aminopeptidase, putative	1.00	1.7	AVYLPFTYNLR	1
PVX_122545	Cop-coated vesicle membrane protein p24 precursor, putative	7.32	2	VTKPGAYSFCYANHK	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_122605	phosphatidylinositol synthase, putative	6.28	2	MFTNISQGIHGAK	2
PVX_122755	translocation protein SEC63, putative	4.03	4.66	DILDFVHQSHESR	1
				SSSLLQIPHFTESIIK	1
PVX_122810	hypothetical protein, conserved	1.83	4.13	LIGIEFEDQEPYR	1
				TFDKYDEEHYK	2
PVX_122910	hypothetical protein, conserved	16.75	6	NADITTTYANIYNK	1
				VFNIFLDTK	1
				YLDEQETLAYTK	1
PVX_123395	hypothetical protein, conserved	0.99	3.06	KVFGDEYIESQK	1
PVX_123435	thioredoxin peroxidase2, putative	5.56	2	QAHDFTAQGLNK	1
PVX_123475	hypothetical protein, conserved	1.53	2	LPVYFLNDR	1
PVX_123745	endoplasmin precursor, putative	3.32	5	VLVADEFVEFLPR	1
				GVVDSDDLPLNVSR	1
PVX_124095	macrophage migration inhibitory factor, putative	10.34	2	FGGSHDGFCFVR	1
PVX_124160	heat shock protein hslv, putative	4.83	2	IDEYPNQLLR	1
PVX_124195	small GTPase Rab2, putative	22.54	2.92	NVEEAFIYTAR	1
				EVSAEEGAQFAR	1
				GAAGALLVYDITR	2
				LQIWDTAGQESFR	1
				YIIIGDTGVGK	1
PVX_215290; PVX_214290; PVX_111580	hypothetical protein; hypothetical protein; phospholipid scramblase 1, putative	2.45	1.3	FSNFFLR	1
PVX_250300;	ADP/ATP transporter on adenylate translocase, putative	5.75	4.09	YFPTQAFNFAFK	2
PVX_110895	ADP/ATP transporter on adenylate translocase, putative			TVVAPIER	1
PVX_251300;	hypothetical protein	15.60	6.68	FKEIEDAEEVIK	1
PVX_193290;	hypothetical protein			NINWEEEYQK	2
PVX_094380	hypothetical protein, conserved			TIIQEKIIHVPK	1

Peptide sequences of *P. vivax* proteins identified by MALDI-TOF/MS/MS (cont.).

Accessions	Names	%Cov	Unused score	Sequence	No. of peptides
PVX_087715	REVERSED hypothetical protein, conserved	0.73	1.52	ANYKTSSARNENLHSFR	1
PVX_119515	REVERSED hypothetical protein, conserved	0.27	1.72	LPASFLKNEK	1
PVX_084985	hypothetical protein, conserved	2.82	2	LVPVNVYVPR	1
PVX_087715	hypothetical protein, conserved	0.73	1.75	ANYKTSSARNENLHSFR	2
PVX_090265	tryptophan-rich antigen (Pv-fam-a)	19.63	14.83	AEQEHWEEFEEK	1
				EEDWNTWIK	1
				KEWSNWLK	1
				RAEQEHWEEFEEK	1
				SGLVPSSNSLQEITLR	1
				SINWTESQWR	1
				WFFENQSR	1
PVX_096335	40S ribosomal protein S10, putative	11.03	1.3	HQYFILNNEGIEYLR	1

Peptide sequences of *P. vivax* proteins identified by LC/MS/MS.

Accessions	Names	%Cov	Sequence
PVX_092045	hypothetical protein, conserved	1.5	GPILNELIK
PVX_081465	vacuolar ATP synthase subunit C, putative	2.4	TKVEAEIIR
PVX_095220	T-complex protein 1, epsilon subunit, putative	3.0	IEGKTGGLLEESTLIK
PVX_101140	hypothetical protein, conserved	1.6	VITKEGGLQLR
PVX_002950	hypothetical protein	10.2	LIKDSNISFHFFYANNDPLSR
PVX_087070	hypothetical protein	1.4	APPTQGEMLLLLVR
PVX_095015	chain enolase putative	4.0	AAVPSGASTGIYEALELR

APPENDIX B

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
PVX_000890	hypothetical protein, conserved	96.73	DILCHR	1
			TFDELAK	1
			SEVDQTR	2
			FYLIER	1
			FTFSSFK	1
			LNLYPNK	1
			QGGSSPHAK	2
			CPLQILK	1
			NNFHVIFK	1
			MFTFSSFK	1
			VTSINKYKK	1
			MKTFDELAK	1
			GIIYNKMIR	1
			GVVLEIGAGSGR	1
			KLNLYPNKR	1
			KNNFHVIFK	1
			SGHLQSEVNDK	1
			NNFHVIFKLVK	1
			TFDELAKSYDEK	1
PVX_000910	hypothetical protein, conserved	96.42	DVLLTNK	1
			LVTFLGR	1
			QQVEAEK	1
			INERER	1
			NRKCLK	1
			KTEQYR	1
			NLMLLPK	3
			ALQRKTK	1
			NSIGTILK	1
			NLSTAIKK	1
			KNECITK	2
			NLHMEIR	1
			KKGNSGWR	1
			LPNCLPPK	1
			QLIDVCQK	1
			NCSEKNKK	1
			SFSLKNVAR	1
			ANFAHLIEK	1
			LQAYLLSCK	1
			EEEINIHKK	1
			IKENEMLHK	1
			VAQLASSLEDK	1
			IKNLHMEIR	1
			NSLATYEHLK	1
			NLMLLPKNSR	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			GSEVLHVDNSR	1
			RSNNQALGELK	1
			RVEDLQNENK	1
			NVEEKYKLVK	1
			VAQLASSLEDKK	1
			IKENEMLHKR	3
			RNPVKWVDGAAK	1
			EIKEQNDILKK	1
			MNVEEKYKLVK	1
			EKNSLATYEHLK	1
			MNVEEKYKLVK	1
			SFGNLQMCKRDK	1
			QLIEAYVCSCIR	1
			DQMPKGREIHFK	1
			VHAQALLRNLSTAIK	1
			NSLATYEHLKNCSEK	1
			KHVEVLESLQNGFSVK	1
PVX_001820	hypothetical protein	95.28	DKLHCK	1
			HFVNCK	1
			MESFKR	2
			RSSQWR	2
			TGRRGER	1
			QMWGAAR	1
			VPPGRGKK	1
			GEKQNGGR	1
			KRQNGER	1
			AVSLKCGR	1
			EGGTNRRR	1
			YNIEFFK	1
			RRSSQWR	1
			MSNLERLK	3
			SEEQFILR	1
			MKIEAMRR	1
			QNGGRQNER	2
			ERVCASAER	1
			QKGEKQNGGR	1
			GDDEMKNKR	1
			EYKREPQR	1
			GSGANHEVEGR	1
			ANGSVEAKAMK	1
			DYHVGRMQK	2
			SNLERLKQR	1
			GNMGGEAEPRK	1
			SEEQFILRK	1
			QNGERQKGEK	1
			SSQWRNNPGR	1
			KANGSVEAKAMK	1
			KIKAFEDEEK	1
			KGSTLVEVPAKK	1
			VLRMKIEAMR	1
			IKAFEDEEKR	1
			EKGNMGGEAEPR	1
			RSSQWRNNPGR	1
			HRGGATHGSEDDR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			QRLEESLEKHK	1
			EKGNMGGEAEPRK	1
			QMWGAARSRSGLSR	1
			QNGGRQNERLPNYSLK	1
PVX_001940	ATP-dependent RNA helicase, putative	99.18	LPFKAPK	2
			SKEERR	1
			NIINIAR	3
			LLLAIQR	1
			QEGERQK	1
			DIVRFVK	1
			QSSSKGKR	1
			KLPFKAPK	1
			GRQSSSKGK	1
			LFEVNFLK	1
			NEDLMSLKR	1
			RPQTGVKAKK	1
			YSYIHRVGR	1
			QLTYGLNLTR	1
			SINCVNKNVK	1
			AHLKRPQTGVK	1
			RLLLAIQRMK	1
			HMRDIVRFVK	1
			SDYQKIINTIK	1
			AITFFMQQDVR	1
			LFEVNFLKHIK	1
			QQEGEKPQEGER	1
			KESVATPSPEEKK	1
			LLLAIQRMKMQK	1
			GWNTICISQTGSGK	1
			LFEVNFLKHIKR	1
			QLTYGLNLTRCLR	2
			EVAKQLTYGLNLTR	1
			AITFFMQQDVRHMR	2
PVX_002685	ATP synthase alpha chain, putative	97.02	QAAGKMR	1
			FLELYK	1
			ELIIGDR	1
			QAAGKMR	1
			QLSLLLR	2
			LLGQQLR	2
			LLERSSK	1
			IGSSAQYK	2
			AFGLNNVK	2
			ISPVEISK	1
			MQAAGKMR	2
			CGKWSDVR	1
			GKILTEILK	1
			NIREGDIIK	1
			MRLLGQQLR	1
			CMKKLASSMK	1
			NIREGDIIKR	1
			SNIAKLVNLLKK	1
			GIIPAINVGLSVSR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			IIDVCVGHELLGR	1
			EAYPGDIFYIHSK	1
PVX_080250	hypothetical protein, conserved	99.97	NIYFKK	1
			GMFVGGQK	1
			IRGHSTR	1
			VSSSVDSR	2
			GMFVGGQK	1
			IQSGWEK	1
			NNKEILK	2
			VGSNVDKR	2
			DGPAQIER	2
			WNFYLNR	1
			RNNKEILK	2
			YITRQLIR	1
			EENRYDDK	1
			RKVELDCR	1
			NIYFKKYK	1
			IQSGWEKDR	1
			EKQNDSHFK	1
			HSSHCDRIR	1
			GVRCYTFAPR	1
			NSNFVTLLVSR	1
			QLIRLASELMR	2
			IVTPPRGAHYNK	1
			LETSLINSSLKR	1
			VGSNVDKRVDAGR	1
			KYKITFDNIYR	1
			LETSLINSSLKRK	1
			QNDSHFKGLHNLK	1
			AEENSKASSSLSGKTNNCVK	1
PVX_080295	hypothetical protein, conserved	95.98	NKSRGLK	1
			NEEIANR	1
			ASFQRNK	1
			NINLTKR	1
			TLRDVVR	1
			GQASQYLR	1
			QDKASFQR	1
			RVDVKSPTR	1
			GQASQYLRR	1
			SSCPGDKMKK	1
			SPTRSSCPGDK	2
			NEEIANRTLR	1
			DALEDSLQSLK	1
			QDKASFQRNK	1
			QKGQASQYLRR	1
			DALEDSLQSLKK	1
			MQDKASFQRNK	3
			LPELSQVEIANLK	2
			INRNLSLSQWNK	1
			NLSLSQWNKEMK	1
			GQASQYLRRVDVK	1
			NINLTKRLISYER	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
PVX_080355	hypothetical protein	99.99	KPGEELK	2
			YFFIVK	1
			MREEKK	2
			GNVIPFGK	1
			WKRMAK	1
			GDNHLER	2
			QKRQER	1
			LERMER	2
			RQLGPCK	1
			LLKVMQK	2
			GKMVGYAK	1
			LNESPPVK	1
			NLQYDNK	1
			VKWVQNK	1
			EAQKDGNR	1
			EQNKFIR	1
			RQERLDK	1
			MKIVAIHK	1
			SWNIEIVK	1
			QMHTCSEK	1
			KEQNKFIR	1
			NVITNGGNFR	1
			GNEQVEEGKK	1
			ARQMAEEYK	1
			QKLLKVMQK	1
			QSQSEEKKIK	2
			SGGKCISNGEGK	1
			LCREEEKEK	1
			LEREQRLER	1
			HLQVDSREGGR	1
			LTSGKPIFSEGK	1
			QLGPCKEPPLR	1
			GNEQVEEGKKGK	1
			RGGPADSSQLGDR	1
			ISRYVPNQLNK	1
			QMAEEYKRER	2
			VMQKKVHIQGR	1
			KLGLLLAIYLNK	1
			SSVRTRGTSHHR	1
			ENAQKGDNHLER	1
			NVITNGGNFRLVK	1
			RQLGPCKEPPLR	1
			TRGTSHHRENAQK	1
			LVKLPNGDHPKSGGK	1
			IKNKNTTEDGTDLK	2
PVX_081770	hypothetical protein, conserved	99.86	QAVVKKR	2
			SIRTPIK	2
			SFVENLK	1
			LFKRMK	1
			VLYENVK	1
			QRVLIVR	1
			EEESKEK	1
			QRVLIVR	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			TESKWNK	1
			KEGAEDYR	1
			QKLVEIGPK	2
			TESKWNKK	1
			TPIKMDIDF	1
			NYVSPQQMR	3
			SEQREEESK	1
			NCKSFVENLK	1
			NVHKKMENLK	1
			YCLWICKNK	1
			KNCKSFVENLK	1
			EVVQDVEAKEEGK	1
			YKNYVSPQQMRMEK	2
			HYQIMPITLADSNNVNKQK	1
PVX_083160	hypothetical protein, conserved	99.79	YMQSQR	2
			DNGGRTAK	1
			LSEIAGNK	1
			QFHGFVK	1
			AMQSMNR	1
			FNMLKVK	1
			FLQLKEK	1
			CLQLQIR	1
			YMQSQRR	2
			MTDRDIIK	1
			RGRFLQLK	1
			FFRFMCR	1
			RMKFNMLK	1
			NSIGGGEKNMK	1
			MKFNMLKVK	1
			RGGPADSSQLGDR	1
			NDGHRLEAQK	2
			YMQSQRRMK	1
			WMNIFSSAQGK	1
			VKNLEAMSRLK	1
			EQLPSRDPPQR	1
			KDHPSSNRGDVK	2
			ISSIKYMQSQR	1
			KISSIKYMQSQR	1
			AMQSMNRKISSIK	1
			RASNHSGESAGNDPK	1
			NDGHRLEAQKGSHK	1
			DPPQRNSIGGGEKNMK	1
			NGNMNYYPFIELSLK	1
PVX_084865	hypothetical protein, conserved	97.16	NLLGKVR	1
			IIVGQMK	1
			LSFGLFK	1
			YYLLLK	1
			KRELLR	1
			NNLKNGR	1
			ISENVTR	1
			KLLYCK	1
			ETGRAHR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			ATSGRKGR	1
			SRSNSKR	1
			IATPDYR	1
			SRSNSKR	1
			CEFEKK	1
			NVLYGKR	1
			DSLMGSNK	1
			EDLTYGR	1
			YILQYR	1
			KNKNNLK	1
			EMNILNK	1
			NAPLEHSK	1
			LEEDKLR	1
			VNDKNGFK	1
			RTKYLNR	1
			NINKATSGR	1
			MDRYGRR	1
			EFPNRMVK	1
			GKEILSYSK	1
			FFDISQLR	1
			SGLSRLSCR	1
			NVKIKFYK	1
			QNLCFNIR	1
			NGGVNTLSGGAK	1
			SRIATPDYR	1
			LRSFLELAR	1
			GHVNLHQGGSK	1
			GADFPPFEQK	1
			QQKVKNLLGK	1
			SPQRSEGEMK	1
			NYLSSATSGGAK	1
			AKSSRGASSQGK	1
			KVTQEGSQRR	1
			QNLCFNIRK	1
			YYLLLKMQK	1
			GGGPKHTSPLNR	1
			VIRENKMPLK	2
			MQKYILQYR	1
			KGHVNLHQGGSK	1
			LNMKSGGGYPSR	1
			REDIKNAWIK	1
			SEMMKSEMMK	1
			MKGTQMNWEK	1
			MPLKISENVTR	1
			DLYPSIYESNK	1
			NEVEEEVNTLR	1
			NVKSNRNLMMR	1
			YKNGGVNTLSGGAK	1
			FYPLSDHGKYR	1
			YKTNAIIDFGDK	1
			TDGDISQLLSDVK	1
			NVKSNRNLMMR	
			SNRNLMMRCGR	1
			GGKPTSNHHHARK	1
			RGSENASQIDNLK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			FLSYYDFVFLK	1
			SVMKNSCSQMNR	1
			SFIQDYNDVLNK	1
			TTNLYYYNFNR	1
			CGVIRIYDEIIK	1
			NNSLFEMLLKKK	1
			KAGAPTVESQLQMR	1
			IAREETSPGSPFPR	1
			HAAGPTASKVRPGRR	1
			KSVMKNSCSQMNR	1
			GNPPDGESALNPSLKK	1
			EMNREMNREMNR	2
			THTFLMHSKQQKVK	1
			GASNPEGDSEHRHRNK	1
			QFANVPNNEELMDEMEK	1
			DASGGNPDKPLFFFSSGNLK	1
			ARGGNPNEGGSHPQEVNNNR	1
PVX_084960	ATP-specific succinyl-CoA synthetase beta subunit, putative	96.58	NTLVTKQTGEEGK	2
			AQILSGGR	1
			CVFCQDMNLAAQK	1
			DLSQENAEEIQAK	1
			EEAAFGKPLIVR	1
			EIFQKR	1
			ERYVALLLDR	1
			ESKFILK	1
			IGFKNTQLDIATDVIANLYK	1
			IINKNPK	1
			INLRRESK	1
			KNPEAIHK	1
			MINLRR	2
			QKEIFQK	2
			QKEIFQKR	1
			SGMANHHALTSK	1
			EFCEKIGFK	1
			IMETAEGAGVR	1
			LNFDDNAEYRQK	1
			MINLRR	2
			NPEAIHK	2
			SGMANHHALTSKR	1
			SMGHWPSLNNGRAK	1
PVX_085070	hypothetical protein, conserved	99.89	AANGEAVVVAGEK	1
			ALHDECVK	1
			ANLDDVR	1
			CGKKVAR	1
			CLKKTQR	1
			CVQNFSVHLLK	1
			DCKRIR	1
			DFQFQK	1
			DYAGETER	2
			EAEGVSQRGDPHK	1
			EDSREIK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			ENKENLLFCRR	1
			EYLFPR	2
			FFILLATHAELK	1
			GDMGWKAVAK	1
			GGKFHFR	1
			HDIASHAGKGSGVATR	1
			HFLVGLHGVR	1
			HSNGTVTR	1
			IVQDVRRCGK	1
			KNYQQK	1
			LGRRTGGK	1
			LIVVASLIDK	1
			LLLQIMNRVSNK	1
			LYARLLLQIMNR	1
			MSQVVAR	1
			NKDCKR	2
			NKKNYQQK	1
			NSTGKVEHR	1
			QSDILIDR	1
			QTGAPYLEGR	1
			QYEVFNYYK	2
			RADPTANHNR	1
			RISNHIASQVEAK	1
			RNQNDKEK	1
			RSDCRLTCR	1
			RSFVNDHYK	1
			RTYHQLAK	1
			RVERWLR	1
			SDCRLTCR	1
			SFVNDHYKMK	1
			SFVNDHYKMKR	1
			TCEFFK	1
			TGGKEAEGVSQR	1
			TYHQLAKIKTNK	1
			VERWLR	1
			VGGCLFGEGVNSTR	1
			VPGGTLQK	1
			VPNLAGLCRTCEFFK	1
			VTDGDEARSPDVGR	1
			WLRWR	1
			WMNIGELKK	1
			YAIVGLEQTK	1
			YFKCLK	1
			YKYAYK	1
			YLMRKK	1
			YVTHFAER	1
PVX_085310	hypothetical protein, conserved	99.04	CTPICKNACWK	1
			DRQGETPIKEVR	2
			EAQQSCINLHPVVK	1
			EELERAVR	1
			EKHTRGK	1
			ENERMKK	1
			EVRFFHENVK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			FFHENVK	1
			GGATPYALLR	1
			GNVPRGGR	1
			GNVPRGGRTCNLLR	1
			HLHRVNQMK	1
			KGNVPRGGR	1
			MPEVVDELK	1
			NDCVVHRLR	2
			NEQAINK	2
			QGETPIKEVR	1
			QGRGGATPYALLRK	1
			QVFLKMCHLSK	1
			RCFHGK	1
			RENERMK	1
			SCASLAEAVK	1
			SKQVFLK	1
			SVLQHIEAK	1
			TKVNHVNR	1
			TKVNHVNRSGQK	1
			VCEYLK	1
			VKSCASLAEAVK	1
			VNHVNRSGQK	1
			VNQMKNEQAINK	1
PVX_085570	hypothetical protein, conserved	95.17	AFISNLQLSAK	1
			ANEAAKRTNEAAK	1
			ASGPENPVQMTVSR	1
			AYLNEIK	1
			CVLQPGIYPVDEVKRAPR	1
			DDHGKGGAKK	1
			DSSASGILSMAR	1
			EDEQKSEAAK	2
			EGGGNTIPEQNMR	1
			ENDYIR	1
			ENISSKLSSK	1
			FKNSGKR	1
			FLSIFPPQSANSLIGSFNR	1
			GDGGHGGKR	1
			GEGGQSSKR	1
			GNLPKNIIQK	1
			GRNEPMEEQNER	1
			HLDSSKK	1
			HMEQLQQK	1
			HNTTIKLEIVK	1
			IDVKDMR	1
			IIQNFLNK	1
			KCPGGSGNK	1
			KHMEQLQQK	2
			KLNNSSESSEIYK	1
			KMSNNLNK	1
			KNVCKR	1
			KQKLVNTK	1
			KRFQCK	2
			KVDAPNR	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			KVDAPNRGDEER	1
			KWLNSICSNKK	1
			LHQLYNMK	1
			LLQQQQELLQQKLQQQK	1
			LQQEQIQK	1
			LSFLLLCECSAYVAK	1
			LVNTKYNGTPSK	1
			MENNKR	1
			MQQELFQK	2
			NANFFSHSSK	1
			NCTSNFGR	1
			NEFFLSNR	1
			NGARSGAKNGGK	1
			NHLGVASR	1
			NIFPSQDLIQQK	1
			NKKFYK	2
			NKNLLKR	1
			NMSCSNLSSVK	1
			NSGKRAHHILK	1
			NSPYVLK	1
			NVNEFLALLK	1
			QFCKKK	2
			QKLVNTK	1
			QMEVAPSGSGK	1
			QQKLLQK	2
			RGDGGQGGK	1
			RLPNLAK	1
			SDHLKNGMIK	1
			SEKGKNR	2
			SGDSGQGGKSGDSGGR	1
			SLLHHPVHSLSNLVNINS	1
			SLLHHPVHSLSNLVNINSNK	1
			SMNISPFHSPTMK	2
			SVFSFNFMNK	1
			SVFSFNFMNKR	1
			TGKKNGASEK	1
			TRPPDLQMVQR	2
			VKFRPNVLMMGK	1
			WLNSICSNKK	1
			YMSEQIEMQR	1
PVX_085765	hypothetical protein, conserved	98.64	AKKTQNK	2
			DISITSHNVSR	1
			EAKDQNNRER	1
			EGNHIKLGEKK	1
			ERANLGKEK	1
			ESNGAVAVGTFSLAK	1
			ETGPIRK	2
			ETSLESIFSK	1
			GAEGAKTYPSK	1
			GAEGAKTYPSKR	1
			GGMNLPRDLR	1
			GKESEKSK	2
			GNSLFNR	3
			GTGSPGSSKETSLESIFSKLK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			GVLQQVNGPLEEEK	1
			IIFQMNANGMLTK	2
			IKLGGNAMTYSKR	1
			IKMNLNGWER	1
			KCIVSFDYASKK	1
			KDLTYKLNNHISK	1
			KERYLK	1
			KGEMDRK	2
			KSSISLGELSK	1
			KTQNKLNR	1
			LGEKKIK	1
			LGGNAMTYSK	1
			LGGNAMTYSKR	1
			LNNHISK	2
			LNNHISKGK	2
			LSIVRNR	1
			LTGKVPPAEGDK	1
			MGSNKLADFFGEPSLNAGSR	1
			MNLNGWER	1
			NALGYLR	1
			NNIFYVGNK	2
			NNIFYVGNKK	1
			NVANEAAVAVR	1
			QSENKNK	1
			QSENKNKK	1
			RGGADAPLRK	1
			RGVFSVR	1
			RLFLDHELNGK	1
			RQQYLSRGFR	1
			RTAPQNMEGGKK	1
			SKMAENTKR	1
			SQNLRRIK	2
			SSISLGELSK	1
			SSKNCVK	2
			SYLLKQRR	1
			TAPQNMEGGK	1
			TAPQNMEGGKK	1
			TPKGNSLFNR	1
			TQNKLNR	1
			VPPAEGDK	1
PVX_086015	hypothetical protein, conserved	96.88	ASHHQMMKQFSR	1
			DLYTEDKKR	1
			DTKNGRR	1
			EIEKGIR	1
			ELNAQEK	1
			ETRKNGK	1
			FENVKSNFESLK	1
			FSYFNDLYLLR	1
			GENTFLYGNLLSR	1
			GGSLLSGMK	1
			GMMSKNK	1
			GMMSKNKHK	1
			GNKTKGNK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			IAVANRR	2
			IDDQKMKK	1
			ILFDFLNIR	1
			ISNKINLKNLMSYHYDK	2
			KDSILSNNK	1
			KKQLTLQEIFDK	1
			KLEDEVLEVLLK	1
			KMNNTVLLQNSK	2
			KQMKMMQK	1
			KRADQTK	1
			KSTDFNK	1
			LEGSNSRLMGTGKGNFLSSR	1
			LFINFR	2
			LHFFNK	3
			LHPAVESK	1
			LMGTGKGNFLSSR	1
			LRSIGMNQK	2
			MELYFSNKNKK	1
			MGIYNKNKMIK	3
			MIKKFK	1
			MKKNYLNFFK	1
			MKMGIYNK	1
			MKMGIYNKNK	1
			MNNTVLLQNSK	2
			MTFSNVK	2
			MTFSNVKK	1
			NEILNEILK	1
			NEYTSGGRLNR	1
			NFIQNMK	2
			NFSLNIVAK	1
			NGNSSLQGSSEK	1
			NIELEMVEKK	1
			NLMSYHYDK	1
			NMNGLGGK	1
			NNLRKYLSR	1
			NNVDIRMGAGER	1
			NSIIYVR	1
			NYEEFLFR	1
			NYLNFFK	1
			NYQNSIK	1
			QAQRQLQR	2
			QIQIVYSVCK	1
			QLDLSVR	2
			QLENQAK	1
			QLENQAKMK	1
			QLENQAKMKK	1
			QMKMMQKR	2
			QSGYDVAEGK	1
			RGMDGGGAAPQGRDTK	1
			RNEEKVTNER	1
			RNNVDIRMGAGER	1
			SEMKKDPNK	1
			SGATHLEAK	1
			SKSSHQK	1
			SNFESLK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			SNIYGRSEMKK	1
			STSRNTPSSSEKR	2
			TRFENVKSNFESLK	1
			VAMALSGR	2
			VGEPSPQVVSNR	1
			VLNIQEEDIEVFK	1
			VTEEDNYIPTK	1
			YFCLHYDISNK	1
			YLKNFR	2
			YMYSKVESR	1
			YSVSISR	2
PVX_086075	fibrillarin, putative	99.01	QPIKYR	2
			VWNPFR	1
			DLTNMSK	2
			KGNKDFK	1
			NFKKDGK	1
			GGGGGGGRGAR	4
			KESCKPK	1
			LTLEPFHR	1
			VWNPFRSK	1
			TGGWFIISIK	1
			GGMWKGNNGGR	3
			SNVVPIVEDAR	1
			SGRDLTNMSKK	1
			EKLTLEPFHR	1
			GNNNNNNNNAVR	1
			FGGGNNNNKKNFK	1
			GNNNNNNNNAVRK	1
			NLVPGESVYGEKR	1
			GGSGGGRFGGGNNNNK	2
			GGGGPRGGGGGGRGGGGPR	1
			MTDAFRGGSGNFKK	1
			SNVVPIVEDARQPIK	1
PVX_086195	hypothetical protein, conserved	98.73	ADLFKDK	2
			DIINLVNYR	2
			DNLTKMK	1
			DNRKQLNQEYK	1
			DTLAKLR	1
			ELNEKKGLLISAYR	1
			ELNKFLYHSR	1
			FDTMKNEKEMK	1
			FFAQLNK	1
			FLDEYENIENLK	1
			FLSIIFTKQEDVVKK	1
			GLLISAYR	2
			ISSTRDELQK	1
			KDIINLVNYR	1
			KFFAQLNK	1
			LFLNKVNLNK	2
			LMFNLRTK	1
			LSNGFHNAAKK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			MISILTQMIK	2
			NDSRADMTNVYSK	1
			NMNPDVFFNLNR	1
			NNNFLNIESIR	1
			NSFDIGANYR	1
			QEDVVKK	3
			QEDVVKKFFAQLNK	1
			QLNQEYKKK	2
			RLINSNR	1
			RTLKELNEK	1
			SEHNIIK	3
			SEHNIIKTIEK	1
			SVIDKFDTMKNEK	2
			TLQLLEK	2
			VLSNVASLKDTLAK	1
			YENLYKELNK	1
			YYISKVR	1
PVX_086245	hypothetical protein, conserved	98.21	AKRTSSEMPLK	2
			AMYEGLFLFKDVER	1
			AVDGNAGGR	2
			CLSANEK	2
			DLLEGQK	2
			DNEMGKNSERK	1
			DVERSFIPSEQNVK	1
			EAVSNLAQK	1
			EAVSNLAQKGEEK	1
			EDALTNR	1
			EGSIPPGDRVNGLDK	1
			ESPNMVAYFHLSYEPLTLK	1
			FHVKDLYDR	2
			GAPNKSVYMR	1
			GEMGTAEFAR	1
			GTNGLERPGGAR	1
			HPKFQGAKK	1
			INSNQLGLSGTSQR	1
			KEDALTNR	1
			KLQGGCSEK	1
			KVEMLHGYLENGR	1
			KVVKNEPHK	1
			LDQENVK	1
			LLSMLNK	3
			MLDQENVK	1
			MLDQENVKLKR	1
			MLLIISR	1
			NAGDVPLK	1
			NAGDVPLKK	1
			NKHDDQFELK	1
			QLFCNILFLCNWLNEPK	1
			QLHQSEFRK	2
			RNAGDVPLKK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			SFCRGSNPFGGR	1
			SFIPSEQNVK	1
			SIYMDR	2
			SIYMDRHPKFQGAK	2
			SLTNTVDYACEVK	1
			SNTLAIGNKKR	1
			SVYVDTK	1
			TQLSNDK	1
			VHQFVEEVYGK	1
			VSTEAEPNYNNAGR	1
			WFQWDTVVRK	2
PVX_086900	hypothetical protein	97.63	AEESKFDDKFK	1
			DESEKSK	1
			DESEKSKAK	1
			DEYVLKQEK	1
			DGSFNLR	1
			DYKNSKNEK	1
			EELSQKFR	1
			EELSQKFRSQDR	1
			EEPARQFAHPNR	1
			EEPARQFSHSNR	1
			EEPARRFPPQDR	1
			ERKMEK	2
			FPPQDREESAR	1
			FPTPNWEELSQK	1
			FSYQDR	1
			FSYQDREESAR	3
			GYFDDKNNYK	1
			HFPPQEREEPVR	1
			HFPSQER	1
			HIPALNWEEAAR	1
			KFSYQDR	1
			KGNSKNQK	1
			LEMKMTK	1
			LPMLEKHSK	1
			MSKRYNFER	1
			MTKDANK	1
			NFPSPKWEEAAR	1
			NSKNEKK	3
			NSSKYKLEMK	1
			QFAHPKR	1
			QFAHPKREEPER	2
			QFAHPNR	2
			QFAHPNRVEPIR	1
			QFAHSNR	1
			QFAHSNREEPAR	1
			QFNQHPFR	3
			QFSHSNR	1
			QFSHSNREGSERK	1
			QNYADGKANGAK	1
			RLNALIHDEK	1
			RYNFERER	1
			SHDLPYLNQPTKPSEQSR	2
			TNKIKTH	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			VEPARQFAHPNR	2
			YKLEMKMTK	1
PVX_087955	O1, putative	99.78	AAEEEEGRSSPQDR	1
			ALAMFNYEER	1
			ARGGSNSTTNRSTSR	2
			ASKRDEK	1
			DEKDRNK	1
			DKHISLNARER	1
			DSWRDK	1
			DSWRDKHISLNAR	1
			EDPIDQMKKK	1
			EEIEEMDEMEEK	1
			EENSVRGVGRASESVR	2
			ENALISDLK	1
			ENQVYSAEEQR	1
			EYGIYDSEKGR	1
			FSSPERAHR	1
			GANNSPPR	2
			GGSNSTTNRSTSR	1
			GKHHKGDSYVDGR	1
			GMRDSPRR	2
			GRSRDGVR	1
			GYPNRNNLKR	1
			HISLNAR	1
			HISLNARER	1
			HKRMNAIR	1
			HRKEEK	1
			KHEEVKR	1
			KKQFEQK	1
			KNDYRFGLPFDENK	1
			KQFEQK	3
			KQFEQKHLELK	1
			MNAIRMRK	1
			MNRKSPNER	1
			NGTSSPSKSLSR	1
			QEKEGSSPNEATPEQAK	1
			QFEQKHLELK	2
			QNEAILNESK	1
			QNRYLYNQR	1
			RARGGSNSTTNR	1
			RDEKDR	1
			RGANNSPPR	1
			RSERER	2
			RSERSGR	1
			SKHEEKK	1
			SPGGEVER	1
			SPGGEVERGR	1
			SRSPGGEVER	1
			VGRGMRDSPR	1
			VVARRGSSSLEER	1
PVX_088250	AAA family ATPase, putative	99.83	ADLEKLCR	1
			AERLRGACR	1
			AKHYRR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			ANPTCEKGPPGR	1
			ASKAWLR	2
			DECRER	1
			EAGEADEVNESNEAGQADKSR	1
			EGSQREK	1
			ERFNHAVR	2
			FKANPTCEK	1
			FLKNER	1
			FNVHADR	1
			GASSNEYQR	1
			GHSDGGHPK	1
			GRPYVEK	1
			GVSGLVESSQLGRR	1
			IMGESEK	1
			KAVNYKWVK	1
			LCFYQHLDR	1
			LRHVFR	1
			LTDLGMPPLR	2
			MVEMVEMAK	1
			MVEMVEMAKAGK	1
			NERRMGEAK	1
			QFVWKK	4
			QYLAHLFK	1
			QYLAHLFKEK	1
			RGEEAPR	1
			RSCIVQVLLK	1
			SEVLRRFK	1
			SGSGKTFLAQR	3
			SPRMVEMVEMAK	1
			VLYPNLSQLTQK	1
			WVMERGTTGK	1
PVX_088935	inner membrane protein oxa1-2, putative	99.01	EAKGVSGGTR	2
			ELTNKLK	3
			GIHMDDFVKGK	1
			GIHMDDFVKGKFT	1
			ILNPLIK	2
			KFFFCGNFFKR	1
			KGIHMDDFVKGK	1
			KMKQVTK	1
			LFYISSLFFFK	1
			LKSNAQDGNIK	1
			MALEFKK	1
			MALEFKKK	1
			MKQVTKLAMR	1
			MSFAHSQIK	1
			NQSDSRR	1
			NQSDSRRR	2
			QVTKLAMR	1
			SFAHSQIK	2
			SSSLSGDDSEFQK	1
PVX_089020	hypothetical protein, conserved	99.20	AYIHKMKK	1
			DGTQLKTSETK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			DGTQLKTSETKQR	1
			DHPVHPFPMPMKVK	1
			EIQEYKLR	1
			EIQEYKLREMNHK	1
			FSNVSMR	1
			GAAQCKAVNNPLK	1
			GASQRSHQR	1
			GGGRASDRVGDPGK	1
			GNHTKSR	2
			GNVSEGNYHR	1
			GPSANNATSGNVGR	1
			HSGWYR	1
			KELEELK	1
			KYTSPYAFK	1
			LREMNHK	3
			LSKLTPR	1
			LYLDQHRK	1
			MGVAIRR	1
			MNVRNFTGR	1
			NEKACEDR	1
			NLSSFKK	1
			QNEENSNAK	1
			QNEVASIKK	2
			QRQNEVASIKK	1
			QSILSGTSQQPFGR	1
			RASLRSQQSGSSFK	1
			RGPDSFR	1
			RMNVRNFTGR	1
			SDYSQVVPSGK	1
			SHLISNSNHMR	1
			SHQQGTSLR	3
			SINASIHNNK	1
			SINASIHNNKR	1
			SNRSCSCTSKFK	1
			SRDGTQLK	1
			TSETKQR	1
			VGDPGKGAAQCK	1
			VNRVATSCGK	1
			VNRVATSCGKATQR	1
PVX_089720	Protein kinase domain containing protein	99.92	AKLRNAK	2
			CGQGIHEMLMSSLK	1
			CNCPGRGGTK	1
			DNPNRNEENQLK	1
			DREKEK	1
			DREKER	1
			DTSRLNYR	1
			EEEKKTEK	1
			EHHANGKER	1
			ERDRER	1
			ERERERWER	1
			ERVTDAK	1
			ERVTDAKGK	1
			ETQGAPNKR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			EVFSRINESAK	1
			FHTDVTPSHMTNR	1
			FINVGNYRR	1
			GGAVKPTGMQGSSK	1
			GNNYKVK	1
			GNNYKVKEQHLEEEHPR	2
			GRESGRDMGR	1
			GSLSGNRAASSK	1
			GVKKEQK	1
			HKLDAEDAK	1
			IANLLIDQNGILK	2
			IMHSRKDGQK	1
			INESAKR	1
			IRDSTSR	1
			LADFGLAR	1
			MGTPSNKEIK	1
			NEENQLKEQK	2
			NEENQLKEQKK	1
			NGNLFTIGEVK	1
			NIVELMGVVYTK	1
			NRDRNR	1
			QEIEFLEGSK	1
			QLKQLNQSQQSQQSR	1
			QLNQSQQSQQSR	1
			QPQQPQQGR	2
			QPQQPQQGRR	2
			QPQQPQQGRRR	1
			QSRQPQQPQQGR	1
			RDYAER	1
			RSRDNPNR	1
			RVQENFR	1
			SIPGVGEVGLDLIK	1
			SRDNPNRNEENQLK	1
			SSRNWAPQK	1
			SSRNWAPQKNPR	1
			TAKEANKQK	1
			TNFFLETNTLELDIKK	1
			VNQNKKSEVENAIR	2
			YKNGNLFTIGEVK	1
PVX_090110	hypothetical protein, conserved	98.67	AAPESGERK	1
			AGAAGGGYSR	1
			AKQMTLNCR	1
			AKQMTLNCRR	1
			ASFEQER	1
			CINRYMIGIR	2
			CNTNNLFFR	1
			CSALGIIHAER	1
			CTRKSIK	1
			EEKNFKMIK	1
			EKTSGVERNGK	1
			ELKTSSLNLNRLK	1
			ERDDVAK	1
			ETNDYNTRK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			EWKNQQK	1
			FDRYVR	1
			FLSNIKIYYLHK	1
			GGGNGGKKK	1
			GLSAGFFQK	1
			GNGNGSGSGNGK	1
			GNGNGSGSGNGKGNAGR	1
			IMEGKPK	1
			ITSVDNKCTRK	1
			IYYLHK	1
			KAKQMTLNCR	1
			KLEKMR	2
			KNTSDNDISLIKK	1
			LEHSDYLLSSDYAAQEGVVK	2
			LRNRNK	2
			MDDMRK	1
			MDDMRKVLPNCDDSTGNK	1
			MRSSPNHDANYK	1
			NANGQVTLEGSNEHAQEEAK	1
			NEAQQDREGGSGK	1
			NELLLHSLMRK	1
			NEMNLEGPK	1
			NFSNLCSSNK	1
			NISFNLAK	1
			NISFNLAKFIAEK	1
			NLAEEMEKK	1
			NTKNEMNLEGPK	2
			NTSDNDISLIKK	1
			QCILYR	1
			QRQQQR	1
			QRQQQRQQQR	1
			RDAYLGLNEIGGGR	1
			RINSPYNK	1
			RMAGNPR	1
			RRINSPYNK	1
			RSVSNTTFGR	1
			SALYGANQVK	1
			SIMVQYEK	1
			SNERLSDERR	1
			SNNLSSVK	1
			SNSANAACRGFPR	1
			SSPNHDANYK	1
			TEWLNPVYVSSSYFYNK	1
			TKGFSVKK	1
			TKSNNLSSVK	1
			TNKGMNGVGEK	1
			TSGVERNGK	1
			TSGVERNGKGFMDGSSK	1
			TSSLNLNR	1
			TSSLNLNRLKEK	1
			VFGFERSRR	1
			VQYIINNSIK	2
			YENEGYLQSKLR	1
			YIQILR	3
			YLSLFSR	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			YSSIPNYERVK	1
			YSYILR	1
			YVEYQSPEK	1
			YYDHYGDQEDK	1
PVX_090325	reticulocyte binding protein 2 precursor (PvRBP-2), putative	95.39	AISKNSAEKTLDK	1
			APQKPATQK	1
			APQKPATQKKPTVQK	1
			ASDYNLAKTLR	1
			ASVNNVREAEK	1
			DEIKSYLSEIKK	1
			DLIVTEK	1
			ECQYVQQEIEK	1
			EEVHTNLQQVK	1
			EISDDTELINTIEK	1
			ELKKTDELLQSVEAK	2
			FTTQKTDLQNKVK	1
			GTSKLSESDK	1
			IENLIKDAPSGK	1
			IESYVYQVELR	1
			IHNSAIDTMK	2
			INDIINEQHEK	1
			INDMIKTEK	1
			IQEFENKVK	1
			IQQNINSLNDMK	1
			IQQNINSLNDMKTK	1
			ISNSSNSATNSK	1
			ITHSMHKNK	1
			ITSDINQCRENIK	1
			KMIEYNK	1
			KSEDIIK	1
			LEEIGKNIK	1
			LIEMGNEIYLK	2
			LNASLLNDNIK	1
			LQEMKSNADK	1
			LSEMNNVLER	1
			LSESDKK	1
			LTAEVNSLR	1
			MFENLSK	1
			MRNGQHK	1
			NAEKNREK	2
			NDEIQKITHSMHK	1
			NHDDDQYMKK	1
			NIQDAYK	1
			NKVNINEIDGNISK	1
			NQINERK	1
			NQKAPQKPATQK	1
			NRLNGKDSTIK	1
			NSKDATIK	1
			NTNTLER	1
			NTSTSFMDHIK	1
			QAFDVVEQNVK	2
			QDAQKEK	1
			QIDAEKASVNNVR	3

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			QIENIIK	2
			QLVENIK	2
			QSEILGVETK	1
			QVSNHEPTNFDKSNK	1
			RFTENLPEK	1
			RKQIHNMK	1
			RNGQHKYNNAK	1
			SFQENLNK	1
			SIEKVYEQMEKTIK	2
			SNIVTNQHSINNVK	1
			SNIVTNQHSINNVKDK	1
			SYLSEIK	1
			TDELLQSVEAK	1
			TETNSDSKLETITK	1
			TLEGEVNALK	1
			TLFYVAAK	1
			VAFDKEK	2
			VEDLNRR	1
			VENTNFEKKK	1
			VKTILGNIDK	1
			VNINEIDGNISK	1
			VVLINQYKNK	1
			VYEQMEKTIK	1
			YKEKCTTEISNSK	1
			YTFIEK	2
			YVLEVNK	1
PVX_091055	hypothetical protein, conserved	97.09	ARQHERSK	1
			DVLKQSR	1
			EEYAQLKKGR	1
			EHLKNDINSLK	1
			EMRNRENK	1
			ENDFLNKK	1
			FNKSLLELNNLR	1
			FNKSLLELNNLRR	1
			GRQSMLK	1
			HDLIESAK	1
			HNEEVKVEIQNER	1
			IINMYTEEIK	1
			ILILENKIDQR	1
			IQHEIAFAK	1
			IQQILESTKIENVQEKVK	1
			KELNYQSDVFSK	1
			KNIKAEQFK	1
			KNVKQAK	1
			KNVQREQK	2
			KPYETLR	1
			LILNQKK	1
			LKKEMR	1
			LNEEFDRKNK	1
			MKNVQR	2
			NDINKNIDK	1
			NDINSLKCEKEK	1
			NELLTNYDQVIK	1
			NGIIRTK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			NHHLIENK	1
			NIKAEQFKR	1
			NIMYIR	6
			NIMYIRLQLEK	1
			NMKDERER	1
			NSDTLSEIGK	1
			NVQREQK	2
			NVQREQKGK	2
			NWFLKQSEMIK	1
			NYNKQMANLKK	1
			QAELKRNYNK	3
			QHERSKLEEDVK	1
			QIRDVLK	1
			QIRDVLKQSR	2
			QKGQAGQAGQTR	1
			QLGKQAELKR	1
			QMANLKK	2
			QQNILLANIK	1
			QRDEAIDEMNTKLMELK	1
			QSRHLEK	1
			RGKEGEK	1
			RNYNKQMANLK	1
			THLLHLSRQR	1
			VNKENDFLNKK	1
			VSENALGR	1
			WSEKKNIK	1
			YLTELTHSYEQLTSK	1
PVX_091210	hypothetical protein, conserved	95.89	ANYEALLK	1
			ANYEALLKR	1
			CPKEVEILVNK	1
			EFIKDYINMK	1
			FYFNKK	1
			GESPGEWFK	1
			GQSSQEEKNLEKILK	1
			HADSLSGVR	1
			IGYPFEEADAISILR	1
			KECFTK	1
			KQTDQLVGNSVGR	1
			LEQAFLNSLR	2
			LEQAFLNSLRGESPGEWFK	1
			LKCVPEDR	1
			LSQDVSYQR	1
			LSQDVSYQRLK	1
			LVDKSGKIFK	1
			MLVENFQSLLCK	1
			NKNLAER	1
			NLEKILKDYK	1
			NWSATRSKLLR	1
			QTDQLVGNSVGR	1
			RLSQDVSYQR	1
			RLSQDVSYQRLK	1
			TRIGELK	2
			TRIGELKEEHK	2
			TWKCPK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			VFSKWKSYK	1
			YDILSFEK	1
PVX_091300	hypothetical protein, conserved	95.28	AFDVQNASLLK	1
			AHQKTKK	2
			ASHPDMKK	2
			AVEPANGPQSGVENR	1
			CIKEELHHSSK	1
			CLTVNKR	1
			DFLKDAEVNLSK	1
			DIPGDQSRKEK	1
			DQWLYK	1
			EDVLNFK	1
			EERFKK	1
			EHNKQR	3
			EKKTNNK	2
			EKTSSSYYK	1
			ELDDSIGKK	1
			ENDTNVR	1
			ENPFNKK	1
			ESNPKESATNR	1
			ESPFWKNGAK	1
			EYEKKK	1
			FFYKSK	1
			FINEESK	1
			FLINHK	1
			FLSTNLDSK	1
			GGFPNVVK	1
			GTLHLKNKK	1
			GVYISGDSLITK	1
			HELENALQLFK	1
			IEEFKIDEK	1
			IHKLVDFMLK	1
			IISNIMK	1
			IIYLSDNMK	1
			IIYMGGAK	1
			IKYNSSGNK	1
			INMASKFRK	1
			INTLPDK	2
			IQVDHFKK	1
			ITQIEMQR	3
			KEKTSSSYYK	1
			KISLLYHAFDIPKEYNEK	1
			KNVLFLNEANLK	1
			KSNVTDMIAQR	1
			LGNTKDDIFK	1
			LLYKKGNYSK	1
			LMDYFISVLNSK	1
			LMNLNSYLR	1
			LYKYQDDSAMK	1
			MERNYALLK	1
			MKDALKYLTEK	1
			MNNMYSKLK	1
			NDLKWK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			NDQVSLK	2
			NDTTDKSAKGR	1
			NELVYFK	1
			NGAKYFTEEVVR	1
			NIEFHQNK	1
			NLISKYITHYAK	1
			NLKESPFWK	1
			NSLYFYRK	1
			NTFNDTLNNIK	1
			NVYYINQNK	1
			NYKMDLK	1
			NYYLIESNR	1
			QSGSLPVDMR	2
			QTLSSDEMNFK	1
			RFINEESKK	1
			RQYVNLESSINR	1
			SAKGRENDTNVR	1
			SNVTDMIAQR	3
			SNVTDMIAQRLK	1
			SQFGIAGK	1
			SVYMYR	1
			SYLSNIK	2
			SYNSHVLPVLGEDFK	1
			TYDVSNYLNK	1
			TYWMCLK	1
			VSNILIR	1
			YKNAIVDFNIFR	1
			YLDDVVCNNKAK	1
			YRTLSCLEK	1
			YSGRNKITK	1
PVX_091585	hypothetical protein, conserved	99.49	ACNAVKVHKGQLK	1
			FIRACNAVKVHK	1
			GVGERVCK	1
			GVGTPTKR	1
			GYLDIVK	1
			LRSLNGK	1
			MLRSLNGK	2
			MSAWGHFR	1
			NSEGLTFNK	1
			SALKSNPYLVNK	1
			SALKSNPYLVNKR	1
			SEDGSFLMDAK	1
			SNPYLVNK	1
			SNPYLVNKR	1
			TPQQLLHK	1
			VHKGQLK	1
			WLDEWRKK	1
			YLTSAGADVNRK	1
PVX_091662	hypothetical protein, conserved	97.02	AEEAVGLTK	1
			CLSILNR	1
			DEHMVEGLK	1
			DMKTNLVEVK	1
			DSNQKFDALIK	1
			DTGGRCAR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			EAMLIMNDTTNR	2
			EEIERNTLIMNK	3
			EMGPPNGRFGK	1
			EMGPPNGRFGKGVSK	1
			ENEAASAK	2
			EPQVTSSR	1
			GSVETALK	1
			HVCECLGEEIK	2
			IEENVKEEIER	1
			INGNINR	1
			INGNINRELK	1
			KANHFYSTVGKR	1
			KKANHFYSTVGK	1
			KRPNQPMDK	1
			LETTFQR	1
			LGDLVSTEMK	1
			LGDLVSTEMKKK	2
			MAQVVCAK	1
			MWIEKDTGGR	1
			NDDGGNERNK	1
			NTLIMNK	3
			NTLIMNKQIK	1
			QGKPAPAARQK	1
			QLNEAEK	1
			QLNEAEKK	2
			QLNNAEK	2
			QMGALLGGMRDEVR	1
			QNVTDQIETNLER	1
			RYLIGGDMMANR	1
			SRKVGDLGGTLYR	1
			STVLTASTR	1
			TEEAINR	1
			TNLVEVKDFFK	2
			TSAATNRNDDGGNER	1
			VYNQVDLKVGK	1
			YLIGGDMMANR	1
PVX_092095	hypothetical protein, conserved	99.89	DGHQATSFTLR	1
			DIFGDHLER	1
			EQAGSSNGK	1
			EQAGSSNGKK	1
			EQAGSSNGKKGR	1
			ERMPNGEANK	1
			GDHPSGEK	1
			GLWSNDK	2
			GLWSNDKQNEK	1
			HIREYER	1
			HKEGAHTDK	1
			ILMYVR	2
			KKMYISNWER	1
			KKNMNR	2
			KKSFFGK	1
			KPGQRGRNDAR	1
			LGQESDSMGGK	1
			LKNYLSCLNLR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			MGNECSCGEVSSAK	1
			MYISNWER	1
			NGDETHR	1
			NGKLQEWIHSSDR	1
			NMNRSGEKGK	2
			NYLSCLNLR	1
			QALRGER	3
			QALRGERGSR	3
			QFYGYHSTNNSK	1
			QPHGEQEETR	1
			RTNKSRPR	1
			SIYIGHWK	1
			SSFAEESSVRK	1
			SYFDHLQRK	1
			TNKSRPR	2
			VKEQAGSSNGK	1
			VKEYFFLNLLK	1
			YIILNYEK	1
PVX_092410	3-oxo-5-alpha-steroid 4-dehydrogenase domain containing	99.33	AIFPFIL	1
			DLGVQISWR	1
			EFESMFVHR	1
			EFPNYPK	1
			EFPNYPKNRK	1
			FSNATMPIR	1
			FSNATMPIRR	1
			HNNYKR	3
			KHNNYK	2
			KHNNYKR	1
			KYHYYPERQK	1
			REFESMFVHR	1
			REFPNYPKNR	1
			SGTFNENGIK	1
			TLKSGTFNENGIK	1
PVX_092835	hypothetical protein, conserved	99.88	AKISMFK	2
			DDTDNVKK	1
			ENAERSK	1
			FTIIETR	1
			HYKTNVK	1
			ISMFKIFEREGK	1
			KNRFTIIETR	1
			MHVFDLDK	1
			MLGGKENEGSSKASVK	1
			NEEHIDSSHTLFK	1
			QKAKQVK	1
			QSKVSLKPIK	1
			QVKKMHVFDLDK	2
			SFSIPSNYIK	1
			TNVKKNR	1
			TSENVNQSKVK	1
			VPKFVSNK	1
			VTDKKPVYFLK	1
			WLQYNNK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
PVX_093615	phosphoinositide phosphatase SAC1, putative	98.76	APYQYMLMSGR	1
			ASYRNFVGK	1
			EFLRGGIIHSR	1
			ELLTSIK	1
			EVQVKRNFQIHR	1
			FFFKNK	2
			GIVMGVLR	1
			GYCPIYFNANMVK	1
			HPCGSDAEAK	1
			IWLVDR	1
			LEDQIGK	1
			LEDQIGKSPR	1
			LIEQIKNR	1
			LIFRVKMK	1
			MKRSESEQR	1
			MQGGSLEPLR	2
			MQGGSLEPLRK	1
			MYMLVHFLKLIFR	1
			NFQIHR	1
			NGQVVVIQR	1
			NHAHLSR	1
			NIFRFR	1
			NIFRFRVWR	1
			NKNIFRFR	2
			NLKFYR	2
			NLYNAHDWEGR	1
			QDFRFFFK	2
			QEAYEVTEVARSR	2
			QGGSLEPLRK	1
			QYAGSTAHKK	2
			QYCNPLSREIFRP	1
			RNFQIHRK	1
			RSESEQR	2
			RTSHLTASR	1
			SREVQVK	1
			THCRPIFK	1
			THCRPIFKKK	1
			TNAAQLFLK	2
			TVGQGNHTVQRR	1
			TYFNQMIDLQKR	1
			VKVYGSR	1
			YGYPIIAINLLSK	2
PVX_094530	hypothetical protein	99.54	AKDTFMQKLDK	1
			CMVTPLIK	1
			CMVTPLIKQK	1
			CMVTPLIKQKHK	1
			DLLQNTEMK	2
			ESQKESTHLSK	1
			ESTHLSK	1
			GEIENLLEVK	1
			GEIENLLEVKQYVK	1
			GSSNVDGKK	2
			GSSNVDGKKR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			KRDLLQNTEMK	1
			MCMVTPLIK	2
			MCMVTPLIKQK	1
			NDVDPYVEDK	1
			NDVDPYVEDKKR	1
			QYVKER	1
			RDLLQNTEMK	2
			SPNLVISK	2
			SQKESQK	1
			SSLLKGTQMR	1
			SSLLKGTQMRSQK	2
			YVEQLGR	1
PVX_094595	hypothetical protein, conserved	98.03	ALKEKNVINK	1
			DVLCFSIHR	1
			DVLCFSIHRFDKK	1
			EELTPSNDSAFRKMQNWR	1
			EKNVINK	2
			EKNVINKMVQIK	1
			EQTIDDGLHEK	1
			GENNKGSSKTFK	1
			GQLSSTSSSNDGEFSANK	1
			HHEVGIK	1
			HKQLQQQR	1
			HKQLQQQRK	1
			HLDSKYK	1
			INHNTHLKR	2
			INHNTHLKRSK	1
			IQKAKSR	1
			IQTLYLP	1
			KDVIYPFDK	1
			KGLENKK	1
			KMQNWR	1
			KMQNWRQVK	1
			KNGIISNQSK	1
			KPNREAKK	1
			LYGDDCDADSIQK	1
			MKNGIISNQSK	4
			MKNGIISNQSKNENK	2
			MVQIKCR	1
			NAMEQKR	2
			NANHLYK	1
			NGGTPPLR	1
			NGIISNQSK	1
			NGKIFPSPK	1
			NKQPSNNTAK	1
			NNMINKNSSK	3
			NNMINKNSSKR	2
			NQWILPAHK	1
			NTNKAHR	1
			NTNKAHRTR	1
			NVINKMVQIK	2
			NVINKMVQIKCR	1
			QAKEFLK	1
			QGLGIRNNMINK	3

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			QGLGIRNNMINKNSSK	1
			QLQQQR	1
			QLQQQRK	1
			RQAKEFLK	2
			RSKSESNLYVMSR	1
			SANVYIIDFAHASLNR	1
			SDINQGDRK	2
			SDINQGDRKK	1
			SKLQKGYSVR	1
			SKSDINQGDRK	1
			SSRNGGTPPLRSAR	1
			TSNKHLK	1
			YNQTYKK	1
PVX_095095	hypothetical protein, conserved	99.70	AHPNRNAPMK	1
			AHPNRNAPMKNNPLNK	1
			CVDLMNK	1
			DAMEWPQLGKWK	2
			DSLPQKDSLPQK	1
			EEMKEEKEVR	1
			EGVGKGKVK	1
			ENNFYIDTETK	1
			ENQKAIK	1
			ESLMSLLDNIK	2
			EVFQLVHFK	1
			FELGKNR	1
			FKNFKK	1
			FMRCLGEDK	1
			FVGHLDATHR	1
			GEVQSFVNSVK	1
			GGGQNVSRENGK	1
			GGRDAMEWPQLGKWK	1
			GKGHNEEASR	1
			GNREGVGK	1
			HFRNFFFPPNLK	1
			HYCRKFVR	1
			ICFFLIYK	1
			IESFSTQKK	1
			ILFQNKK	2
			KEKQQTNR	1
			KKDNYR	1
			KKYHVEPGAR	1
			KNYNPNSK	1
			KYVFQR	1
			LIQNGNFLSMYK	1
			LISFVTHRGK	1
			MNNKRK	1
			MNTNNEKKK	1
			NFFFPPNLK	1
			NFKKENQK	1
			NRILFQNK	2
			NRILFQNKK	1
			QETLLEHILQVR	1
			QGSRSNQGVR	1
			QLGALSRR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			QQTNRMTNRMTNR	1
			RWRHYCR	1
			SNANPSSDPSPHK	1
			SSHALRR	1
			SYCFVNVLLHVLSLK	1
			VEEGMKSNLK	1
			VRRGLYGASER	1
			VVSAIVDANYAK	1
			WFDSGSTNGK	1
			WKDAVAKCSER	1
			YIVKFYR	1
			YNNQQYILIK	1
			YVFQRGPYKK	1
PVX_097950	hypothetical protein, conserved	99.45	AADQTHSR	1
			AKRSGTPK	2
			DAPAESYKR	1
			DDNTLLPSDYFIK	1
			DHRSWK	1
			ELAADGWAASR	1
			EWVWNETRGEKK	1
			FFCKEK	1
			FNEVAER	1
			FVNFKHLHK	1
			GNAAHLVHR	1
			GNPLGSSPHR	1
			HHHGGAANNLTR	1
			IEESVQEK	1
			IEESVQEKK	1
			IIAQRMR	1
			INDANLEK	1
			KFNEVAERR	1
			KFVNFKHLHK	1
			LPQSEEK	1
			MKAVFLHHVK	1
			MKAVFLHHVKGK	1
			NVSLCSER	1
			QAGEMMIR	1
			QPWGGGGPPVER	1
			QTSRRLGGSPPGK	2
			RIIDCK	1
			RLGGSPPGK	1
			RNVSLCSER	1
			RPIEQSQR	1
			RYNSSDEER	1
			SGTPKYTLRK	1
			TKENTQVK	1
			TPASPPIR	1
			VGTSHREREK	1
			VSPSRGTPNR	1
			YNAETVNRTKK	1
			YNSSDEEREK	1
			YVLQQNSLVR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
PVX_098675	ubiquitin carboxyl-terminal hydrolase, putative	99.46	ASGETFNEINQRGSR	1
			DMKSNLK	4
			DMKSNLKEGK	2
			EEAQKGNENVTPK	1
			EEYEKKFNK	2
			EGVWGEVPASK	1
			EKDERK	2
			ENANDSAKETTPSR	1
			FDLNNFTVQNVLKKK	1
			GKVLNVNK	1
			GNENVTPK	2
			GPVGKNENK	1
			GTTDNSVK	1
			GTTDNSVKSATSSPK	1
			IDYCLK	2
			KEEVNSTCEK	1
			KELDDITKNLSFLK	1
			KETPSEK	1
			KKEDAEK	1
			KNGQYIENPKK	1
			LFQLSKMHNKGK	2
			LIYTLQNYVSVK	1
			MHNKGKVLNVNK	1
			MIDLKNK	1
			MIKDNK	1
			MKNMMAPSNPKR	3
			NDAREDPQK	1
			NDAREDPQKDPK	1
			NEENDSAKVK	2
			NGQYIENPKK	1
			NHGVSNRSMYNDK	1
			NIIYLMEPK	1
			NLSDHVK	1
			NMMAPSNPK	1
			QEENRENR	1
			QSANYVNK	1
			RENQTNNATSYFGPK	1
			RHSNGATVWYK	1
			RNSNNIYDR	1
			RQEENR	1
			RQEENRENR	1
			SATSSPKEK	1
			SMNKMIDLKNK	1
			SSANGAIPK	1
			SSANGAIPKKISTER	1
PVX_098985	DNA primase, large subunit, putative	96.42	ANEKKLQNITIK	1
			ATNGASGTGAEK	1
			DHKVCVDK	1
			DSFNAKGR	2
			DTSVGNYNDSKNK	1
			EKQQWFLK	1
			FDKEHRYTIR	1
			FGIQSMK	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			FGIQSMKNYSK	1
			GRANEKK	2
			IFEYKFGIQSMK	1
			ISSFLAALPK	1
			KRSINGTPVEK	1
			KYYKAK	2
			LLLNVHNDSR	1
			LMPQNLYSVYK	1
			MIVRKR	4
			NFDEAHLR	1
			NYEISSNERK	1
			QKIFEYKFGIQSMK	1
			QQLWLFLK	1
			QQWFLK	3
			QSFPPCMR	1
			RIFVNFLK	1
			SIWLQPDKFDK	1
			TDFSPYNCSK	1
			YLEQNEK	1
			YTSFIPNRDTIEK	1
PVX_099060	hypothetical protein, conserved	98.51	AEANKNVSQSNDEEKK	1
			ALRFGIK	1
			CSELKDLLAKR	1
			EEEFKK	1
			FCIVTQK	1
			FGVVSTDEVLESR	1
			KNELLER	1
			LNDSEMK	1
			LNDSEMKRK	1
			MKNYFK	1
			MNYQNLK	2
			MNYQNLKCSELK	3
			MNYQNLKCSELKDLLAK	1
			NDEKAEANK	2
			NILTSNANDEGSK	1
			NVSQSNDEEK	1
			NVSQSNDEEKK	1
			NYQNLKCSELK	2
			RAERFGLVK	1
			RGLPSHGK	1
			SSSSQYIGSQKKK	2
PVX_099280	hypothetical protein, conserved	98.25	FNFFFK	1
			LCEFFVDFIKNK	1
			LLLERMNTLIENK	2
			LMDVWR	2
			MLLLER	2
			MLLLERMNTLIENK	1
			MLLLERMNTLIENKYAK	2
			MNTLIENK	1
			MNTLIENKYAK	1
			NCLLVKNR	1
			NRATGCEIDK	1
			SAPNQACTLKDK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			SNWKRK	2
			TKDSCNDYNILK	1
			YAKSNWK	1
			YNAYGMDALR	1
PVX_100630	hypothetical protein, conserved	99.76	AGKTAKAR	1
			ATARTVRLNR	1
			DLHDQTGR	1
			DLHDQTGRKMQRPR	1
			EMLKMLR	1
			EQCEQRDQPDR	1
			ERSQMVGPPPPK	2
			GALDGCQQR	1
			GNKEMLK	1
			GNKEMLKMLR	1
			KLFTSEK	1
			KMQRPR	3
			KMQRPREDCEK	1
			LLRGMLSVRER	1
			NVNESINGELMK	5
			QRPQQR	3
			RDLHDQTGR	1
			RIYARFVGSGK	1
			RRIYAR	1
			TAKAREK	1
			YCVVEEEQRR	1
			YELVKR	1
			YNLKEVR	1
			YNLKEVRR	1
PVX_101095	hypothetical protein, conserved	95.98	AEMKNAR	2
			ASRNLSTR	1
			EAKSERK	1
			EDTKELK	1
			EECRIQR	2
			EETKRQIK	1
			EIERQR	1
			EIGMCQIPLK	1
			EKEQFENEK	1
			EQVESTQNAGR	1
			EQVESTQNAGRGSKK	1
			ERERQMER	1
			FFNYELLNQRK	1
			FSDMNKLK	1
			FSDMNKLKSLEIK	1
			FWETTTGNVYTLK	1
			GHEGLTLVSMEQK	1
			GIPEGCTDIYK	1
			IEDWKK	1
			IQRGSSMKIYAK	1
			KENTASTSNLSK	1
			KLQEDIEK	1
			KNMNFR	2
			KNMNFRSVFEYR	1
			KRFSDMNK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			MEIVKEK	1
			MEIVKEKAQR	1
			MLDKFK	2
			MMLDKFK	2
			NINIYRASR	1
			NQVNYQNFSKK	1
			NYSHKIEDMELDR	2
			QARNLEK	3
			QIKELFNNR	1
			QKMLREQK	1
			QMLLEEKLK	1
			SGMSTNDVNR	1
			SGMSTNDVNRIK	2
			SGRCHIFPK	2
			SHLRHGFLQK	1
			SKFISDK	1
			SNNYFEK	1
			SPYGAESTNAIFHYVR	1
			SVFEYR	2
			TGRDVEAQPSVREK	1
			THVVSNVAAEQK	2
			TKRFFNYELLNQR	2
			TRFVRK	1
			TSNLDDLNEKK	1
			TSNLDDLNEKKK	1
			VLKSPQEER	1
			VNEWER	1
			VNEWERKEK	1
			VWEYKQFTLNK	1
			YKNDRQAR	1
PVX_101170	hypothetical protein, conserved	96.95	ATESEVESNSTRILK	1
			DNVRRR	1
			GPNHGDKNWNKNR	1
			IENLQDELDQMR	1
			IESNNNNFF	1
			IKIESNNNNFF	1
			IYTEQIKLK	1
			IYTEQIKLKNSK	1
			KIYTEQIKLK	1
			KLMKEIEELNK	1
			KTINLIK	2
			KTINLIKNHYSNK	1
			LMKEIEELNK	1
			LMKEIEELNKSILYLNNK	1
			LREDQIER	1
			MKEGKQK	4
			NHYSNKINK	1
			NNKRPRER	1
			NNRDNVRR	1
			NWNKNRNK	2
			QVDSNKK	4
			QVDSNKKLMK	1
			RYTKNK	2
			TEKRGPNHGDK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			TELDMIDEENK	1
			TELDMIDEENKK	1
			TELDMIDEENKKK	1
			VAVNHGDSKNNK	1
			YKQSFYIIR	1
PVX_111035	aspartyl protease, putative	97.84	DENILTER	1
			DKLPYIVFQIK	2
			DNLQIGFVR	1
			DNLQIGFVRSR	1
			EKGNEKK	1
			EYEPNVNVK	1
			EYEPNVNVKGNQK	1
			FYAASQGVDK	1
			FYAASQGVDKMR	2
			GNEKKGDHFR	1
			GSFLNVRTK	1
			GSGSGNARR	1
			GTNFPDSHKK	1
			GTNFPDSHKKYK	1
			IDQMNNPILIK	1
			KEYEPNVNVK	1
			MRRNSPHSAR	1
			MTTYYGQVAVGEK	1
			NQFGYYLSDR	1
			NSPHSARGDLPSER	1
			RGTYNGPLSA	1
			RPNFVAR	1
			SFRNKFNK	1
PVX_111385	hypothetical protein	99.20	AKAFPPSGK	1
			AQTEEKLNGSKGDK	1
			ARGDANGGEVK	1
			DGAQSGGKPK	1
			DTKEEETADK	1
			EAKSGGGNK	1
			EAKSGGGNKVMASAK	1
			EANAEKANR	1
			EEAQMEERR	1
			EGAAAAPQVSAPPVK	1
			EGMNQILK	1
			EIKMSMK	1
			EISKSEK	1
			EKNAAPGQAK	1
			ESLVERRK	1
			FHGNVVR	1
			GDVGEGERLR	1
			GDVGEGERLRR	1
			GENMIVHAGEEK	1
			GENMIVHAGEEKGGK	1
			GENMIVHAGEEKGGKK	1
			GESKKELPQEGENK	1
			GFIENAVRK	1
			GGETQPPK	1
			GGSNSPPGLSKQEGEPKNVGR	2
			GSASAMGSSPSKGK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			GVRFHGNVVRK	1
			KEANAEKANR	1
			KGSASAMGSSPSK	1
			KKSPNYQQLK	1
			KNENGPPQLDGKK	1
			LFGAIMRNER	1
			LFGAIMRNERVR	1
			LNNMAMK	2
			LQSVKNK	1
			MKSLKLK	1
			MYFNGLFQHIR	1
			NENGPPQLDGK	1
			NGSPCRSDAEK	1
			NIERMLER	1
			NKAIQKK	2
			NKATMKQFPLK	1
			NNFLAKLQSVK	1
			QDGEKKK	3
			QGKCVSRPRNNCPR	1
			QLDLPSTSEK	1
			QLPKGLCKPPSFK	1
			QLTGIMK	2
			QLTGIMKSTSMVLK	1
			QMNATKQMDVTK	2
			QQHGGKESLVER	1
			RISPPLK	2
			RNGSPCR	1
			RREDLR	1
			SEKKLFGAIMR	1
			STSMVLKQQHGGK	1
			SVRDVKSTK	1
			TPMVKPLVK	1
			VPLKLNNMAMK	1
			YSVTLNK	1
PVX_111435	hypothetical protein, conserved	98.03	AIKINGTEFNLSK	1
			AKQKAQLK	1
			ALNGFLER	1
			CILEIFEGVMGK	1
			DVDKYILYQK	1
			EAPLKNATNVTNAK	1
			EAVETHTLQRR	2
			EFFANIKK	1
			EFLAPLK	1
			FFFRQIK	1
			FFHHNIK	1
			FFHHNIKYR	1
			FIILLQKNR	1
			FIKLLMK	1
			FLNYKFVR	1
			FQRTYEGALR	1
			FRQGDGGK	1
			GAERHNAEGR	1
			GEYFNSLLREKNK	1
			GQLREVYDR	1
			GRSSVSSK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			GVDNPNGGLPPEKGK	1
			HINNISIIPR	1
			HIQIRLFK	1
			HLVRMAGSR	1
			HVYQELYK	1
			IQPCYLQLAK	1
			IVNKLRR	1
			KFIKLLMK	1
			KMNEQLER	1
			KMNEQLERLK	1
			KNLLSQMEK	1
			KYLLRK	1
			LDVERVSSEIGAIDLYFKR	2
			LDVSLVNK	1
			LDVSLVNKK	1
			LEVAQQGSYNR	2
			LLMKER	1
			MLINILAIIQK	1
			MNAMKSILHK	1
			MNEQLERLKAGLK	1
			MNKHIQIR	2
			MPLVLMGGSNSAK	1
			MTFMKYQIEK	1
			MTFMKYQIEKSLQLYR	1
			NGIVQGGR	1
			NGVVVNLDFAHHSR	1
			NHFVNKMK	1
			NLLSQMEK	4
			NQGVFKR	1
			NTIVFLLAALINR	1
			NYQLDEFERAERELAAIR	2
			NYSSLKK	1
			QAVNATSTLK	3
			QELNPLLER	1
			QERQERR	1
			QIGNVITQLMVER	2
			QIIERSFATR	2
			QKAQLKNAEK	1
			QLDEELKEK	1
			QQEPLKAHLDER	1
			RMNKHIQIR	2
			RNGIFMK	3
			RNILDVKR	1
			RNQGVFKR	1
			SLNRGTR	1
			STEEEIERR	1
			TDQLSSLVAK	1
			TVIDMIIKK	1
			TYKSAKK	1
			TYLKDTSWDK	1
			VAYQIRDQMR	1
			VEKYIRK	1
			VLDEQTK	1
			VRDITFVER	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			VTSQRDTPPGER	1
			WRELAK	1
			YGGFLKIVFLYFGQR	1
			YILYQK	1
PVX_113620	hypothetical protein, conserved	97.16	CVKGDGAGQDNPR	1
			EDQLLLQEDVK	2
			ETRTGQSR	1
			ETRTGQSRR	1
			EVERLREDQLLLQEDVK	1
			FKFLQDIDVAK	1
			FLQDIDVAKR	1
			FVLGEWKSLSVRR	1
			GEHGESAK	1
			GSARSGCEEEGK	1
			ILNDINER	1
			KEDAELK	1
			KETRTGQSR	1
			KIMEETLVENIR	1
			KLLSQQVAKNEMLER	1
			LLNTINNK	1
			NKILNDINER	1
			NNQTLLERVR	1
			NQLTYAEMR	2
			NQLTYAEMRK	1
			QEGDHPQTK	1
			RKLLNTINNK	1
			RNKILNDINER	1
			SDLATLAK	1
			SLLCVSLWREK	1
			VDAMERQLTQLK	2
			YKFVLGEWK	1
PVX_114090	hypothetical protein, conserved	99.88	AGEEKGEGK	1
			APQICGKHSPR	1
			DGQNVGKNVQNDGK	2
			DMIDESTGGK	2
			DTLSNQK	1
			DTLSNQKGFPHGDKTK	1
			ECAVPSR	1
			EIGHHTK	1
			ETDLYENYNDTTMMVLK	2
			FILGCSYDEIFK	1
			FSGYLSECLPGRYENFKR	2
			FSTMNFTR	1
			GDEIDAAK	1
			GEGKNSNAALSR	1
			GEGKNSNAALSREK	1
			GFSSGVGLQTDK	1
			GGRGGCLLTYRQR	1
			GSHLKAPQICGK	1
			GSTSNWVR	1
			GYMNLIK	2
			GYMNLIKNNVR	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			HLLPCDGVDK	1
			HSPRRR	1
			HSVRRR	1
			IDGLMNSFLK	1
			KKIDGLMNSFLK	1
			KMQQLQR	1
			KNMYNFCSKYK	1
			KRDVINSK	1
			LNGEGNEKKK	1
			LPHVVQHAGNLR	1
			MPVKEEQK	1
			MTYEKNVKR	1
			NILTNIKRIDR	1
			NKVAPKLFPLQR	1
			NLDYGEETPR	1
			NNEDVREDEKK	1
			NRGYMNLIK	1
			NSNAALSR	2
			NVISNIINR	1
			NYFSIGRR	1
			NYNLSLDNRK	1
			QGTQKWGLHK	1
			QNAFTSR	2
			QNAFTSRTTHNR	2
			QQMIRR	2
			QQMIRRYSTAVR	1
			QYVNLASCMK	1
			QYVNLASCMKR	1
			RFSLVSNLEK	1
			RFSTMNFTRVNK	1
			RMTYEK	2
			RNCCVLRR	2
			RNKMVR	3
			RNYNLSLDNRK	1
			SACLDPWHGK	1
			SDASTRADSSPPGR	1
			SDENVSAK	1
			SDKGKSGPPHQR	1
			SGHPDLLR	1
			SKSQEDGFK	1
			SLTTSQFRSDKGK	1
			SRFSLQK	2
			TAPGTTPR	2
			TLIDTIMNPDK	1
			TLNRSGHPDLLR	1
			TSGPDPVDAR	1
			TTHNREK	2
			VSHGDGSR	1
			VVLSLYCLCVK	1
			YTSVALSK	2
PVX_114880	hypothetical protein, conserved	99.12	DMVSTRPEK	1
			EEYKILLK	1
			EKMKNER	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			EREEKK	2
			EYAGKEK	1
			FKIQQLNK	1
			GINPYLK	1
			IEEENYFK	1
			IKAMSKK	1
			IKFKIQQLNK	1
			IQQLNKSSSGR	1
			KKQVGEENAGK	1
			KMKMYSGQHAK	1
			KNKMSNLK	1
			KNQRVSVDTSK	1
			LISDMGADLR	1
			LMNFDEK	1
			LMNFDEKILK	1
			MFRNNINK	1
			MFRNNINKMK	1
			MKMYSGQHAKLK	1
			MKNERNVFLK	1
			MKQMNQMKK	1
			MPKKNQR	1
			MPNKNILQNR	2
			MYSGQHAK	1
			MYSGQHAKLK	1
			NERNVFLK	1
			NERNVFLKEIK	1
			NKMSNLK	5
			NKMSNLKNVGMTSNDLLK	2
			NMLNLKK	2
			NNINKMKQMNQMK	1
			NQRVSVDTSK	1
			NVGMTSNDLLK	1
			PKKNQR	2
			QKEELGK	1
			QKYLKK	1
			QLGEMGSGEARASKR	1
			QMNQMKK	1
			QMNQMKKMK	3
			QVGEENAGKGTTGKEYAGK	1
			RMPNKNILQNR	1
			STDGNARVHNKQK	2
			TNELDNKIK	1
			VHENNYSGEKR	1
			VYNEIKENK	1
			YLKKMK	1
PVX_115085	hypothetical protein, conserved	98.94	ANNCTYVLR	1
			DVLCHKWNDK	1
			ENDIGMEK	1
			ESQDDDDIKTSR	1
			FSSRNKR	1
			ITINTCIQR	1
			ITINTCIQRNK	1
			ITINTCIQRNKK	1
			KESQDDDDIK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			KVSFSNLNNNMR	2
			KVSFSNLNNNMRR	1
			KWLNATK	1
			KWLNATKHMK	1
			LEDEALSKVK	1
			MKNLHFK	1
			NEKVIKMNDR	2
			NVSNKVIK	1
			QEEMLR	3
			QEEMLRR	1
			QEEMLRRMNS	1
			QLAEWSSDMVHK	3
			SRQEEMLRR	1
			TRVLFQSADLKSR	1
			VIKMNDR	2
			VLFQSADLK	1
			VSFSNLNNNMRR	1
			WLNATKHMK	1
			WNDKMK	1
			WNDKMKNLHFK	1
PVX_115345	alanyl-tRNA synthetase, putative	97.94	AEEVEAKFDDLLAK	2
			ALSGRDISGLFK	1
			CMRLSR	1
			ELAQEAVKKYH	1
			ERNSRCMR	1
			GATIKELAQEAVK	1
			GNNALRR	2
			GNTSGGDNLFSGK	1
			GSIKAFLPPR	1
			GSLVDDEK	1
			GSLVDDEKLR	1
			GTSIQADAFMK	1
			GVDQFNK	1
			HIFDDPNEGK	1
			HQLVSDTNNFK	1
			HQLVSDTNNFKINK	1
			IAQSYCK	1
			IESQVNDLIR	1
			KAEEVEAK	1
			KHQLVSDTNNFK	1
			LFDALSGNQK	1
			LNMTEFNELFR	1
			MSGGSENQVEK	1
			MSGGSENQVEKR	1
			NDCVHTVVNFDR	1
			NIIEKTKNMQK	2
			NKAFGSAAK	1
			NMQKELFNK	1
			NQILHNLEK	1
			NSRCMR	1
			NYVIRR	2
			QEEMVFNR	1
			QEEMVFNRTLEK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			QELSVSVR	2
			QELSVSVRTMDLK	3
			RAVDTQKCIR	1
			RGNTSGGDNLFSGK	2
			SSDLGKLK	1
			SVIKQEEMVFNR	1
			TKNMQKELFNK	1
			TLEKGVDQFNK	1
			VLEKIAQSYCK	1
			VLTEYVMHSK	1
			VNYIKSVIK	1
			VNYIKSVIKQEEMVFNR	2
PVX_116515	hypothetical protein, conserved	98.67	AMIKTNFIPTK	1
			ANGLVSNRVNGPTR	1
			ANGVVGNRANGVVGNR	1
			ANGVVSNR	1
			APPKGNIK	1
			ATLSKNSLKDVDAEK	1
			DEKENELLPKEK	1
			EEKEDKVEK	1
			EKTLEEK	1
			ELVKMKSFK	1
			GADQKLSK	1
			GIPKDINR	2
			GIPKDINRQSSK	1
			GPEKPNEAK	1
			GSLLKMEAMK	1
			GVSKGIPKDINR	1
			IKSAMSQMR	1
			KANTFKK	1
			KIKSAMSQMR	1
			KLDTSMSDKNGK	1
			KMSNRANGVVGNR	1
			KNENVPISK	1
			KSTLFLK	1
			KSVKNEK	1
			KYKSTTGNGNSK	1
			LRNASLFNFNR	1
			MAANPSLK	1
			MEAMKRGK	1
			MKSFKDK	1
			NASLFNFNRIK	1
			NEGKDESQNGK	1
			NENVPISK	1
			NGTSKNSPQRK	2
			NIQKGSLLKMEAMK	1
			NMSMESLNLGK	1
			NSLKDVDAEKK	1
			QNAKPELALK	1
			QSSKSSNAAVQK	1
			RAAEVGSK	1
			RNSGETNAGMLK	2
			SDNAPAKK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			SENIYSLLEQK	1
			SKGGEEPPKMAANPSLK	2
			SKQPETTFPPK	1
			SSNAAVQK	1
			STLFLKKANTFK	1
			STTGNGNSKAAK	1
			TDITGNVKEKK	1
			TKFEAKK	1
			TNFIPTK	1
			TSLNVTPK	1
			VKKLDDAAGDNDLDAGK	1
			VLLKSVQEK	1
			VLPFLIKEK	1
			VNNLCSKTPAVGRR	1
			VVHDLTR	1
			YKSTTGNGNSKAAK	2
PVX_116785	splicing factor, putative	95.17	AFQFLNGR	1
			AKRNSSR	1
			DDEFVLK	1
			DDEFVLKKNSHK	1
			DIQCIKDQR	1
			DRERLR	2
			EADLVSGNDRK	1
			ELIGGRR	1
			ELKVSFAQDSK	1
			GFGFIQFFR	1
			GGKSDQQGGK	1
			GHGKEGQK	2
			GRGSGRGSGNGSER	1
			GSGNGSERGGGGAK	1
			GSGRGSGNGSER	1
			ILAQLLAK	1
			IQSSQAEK	2
			IQSSQAEKNR	2
			IQSSQAEKNRAAK	1
			IWLDSKNIDGK	1
			KAKAEEK	1
			KELIGGRR	1
			KSAAERGK	1
			NEAKAKR	1
			NRAAKAAK	2
			REASHSK	1
			RHTGRDSPAK	1
			RRGSQGGR	1
			SAAKRSDNR	1
			SPSSERVSASK	1
			TYSSEESNGGHKGRR	1
			VANGESDNPSENETVGK	1
			YILASEKEAK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
PVX_117305	hypothetical protein	96.07	AERGGGGSR	1
			CIYVNETCR	1
			CQPKNNPK	1
			DAPHNVNK	2
			DAPHNVNKVAK	2
			DMQLLNPEKLK	1
			DNYNLFLNR	1
			DPPIFPSRDAPHNVNK	1
			DRIAANERNMK	1
			DSSLMQYRR	1
			DSSLMQYRRK	1
			DYIYFQK	2
			EGAKTDVDASPR	1
			EHRFCFFSR	1
			ESIPGVAPNGGPTK	1
			FMPSVYEAYFGSK	1
			FNVDISVGR	1
			FRHTHR	1
			FVKIIPK	1
			GHNNYPPVSKNR	1
			GLNLCFNFFNSR	1
			GMTFLYFLMK	2
			GRQTQMGNLK	1
			GTDSHEGDLTRVR	1
			HGKTSER	1
			HLSHAQLNMYEK	1
			HPENYLQVGK	1
			IAANERNMK	1
			IGHHGRMK	1
			IGKSIHR	1
			INMHTNEYIK	1
			KAEYRNLMVMLK	1
			KCHLVR	2
			KIKNVEMYSLHAK	2
			KMSSFGGIIKQK	2
			KNGCTFNVADK	1
			LNQVLPK	2
			LPLEESK	1
			LRCSGHNYVDGR	2
			MDNLVCLKLLR	2
			MDVFFDVMKKK	1
			MKNKFR	1
			MNITLSK	1
			MNITLSKGR	1
			MSSFGGIIK	1
			MSSVKLHLCR	1
			NAHVKGEADK	1
			NDQNKYVK	1
			NGCTFNVADK	1
			NHKGEVQR	1
			NHKGEVQRGK	1
			NIDNAQK	1
			NIKPDISPYGKR	1
			NLMVMLK	1
			NSLYGKK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			NVISIWNYK	1
			NVSSNVVSER	1
			QAAKQAAK	2
			QAAKQTTK	4
			QHVDEYER	1
			QLTHFER	1
			RGDNLQK	2
			RGEITNTMRK	1
			RNDQNKYVK	2
			RSNGDNSR	1
			SAEFNFYERVK	1
			SALNDFPNIQSAVK	1
			SASDKIGK	1
			SIHRGGNKR	1
			SPESHLR	2
			SSGMLAQFGK	1
			SSGMLAQFGKSHK	1
			SSGMLAQFGKSHKGEHPIGK	1
			TFFSDVRRGQK	1
			TKPVSDGGSGNRR	1
			VKMYILCMK	1
			WNDKRGDNLQK	1
			YLYINNFNTK	1
			YTLDDFYKQR	1
PVX_117430	hypothetical protein	97.28	AHNIKDIIDR	1
			ALTKEEESK	1
			CNERCK	1
			CNERCKR	1
			CRQRGK	2
			CTAEMQKPK	1
			DATTQVSLNK	1
			DATTQVSLNKAK	1
			DQERDEVIDEEK	1
			DTLQSDQIK	1
			EFLKKR	1
			ERKIIR	1
			ESGQRQGGANGELR	1
			ETSNHSK	1
			ETSNHSKGKQSK	1
			FESALFK	1
			FPNEKFPNEKNK	1
			GEIKINLNSK	1
			GNYESLK	1
			GVAKRESGQR	1
			KKNNGSHFYLFK	1
			KNFNAPPR	1
			KRTNDILNR	1
			KSCTNGGSVSSQK	3
			KSGKEPPDDDR	1
			LRDLQR	2
			LVQSCAR	2
			MEQNQSEELPNPR	1
			MEQNQSEELPNPRK	2
			NFNAPPR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			NGGHEKGK	2
			NIYTRGKR	1
			NNQLAGKAEK	1
			NQPKCSEKCSEK	1
			NVLTNFIK	1
			NVLTNFIKCVNK	1
			NYIIPYNMYSGSR	1
			NYLNSLNKKK	1
			QGGANGELR	1
			QGKNIYTRGK	1
			QGVDTQNGEIR	1
			QNSGPLGNQK	1
			QYAVNSAK	1
			QYMNGKKNEPAK	1
			RDATTQVSLNK	1
			RTNDILNR	1
			TCKERK	1
			TDQAATKKGDEPK	1
			TNDILNR	1
			VKRCQR	2
			VLPVCTR	1
			VPAEDRNGGHEK	1
			YCVLHILNQQR	1
			YLIENLNR	1
			YSEDGLQRVAQPPAHSASAK	1
			YYNYLK	1
PVX_117550	hypothetical protein, conserved	99.76	AHENNSKGDNGNR	1
			ANTQRENVHLR	2
			CINNYIKR	1
			CWFENKVK	1
			DLYMSKR	1
			DLYMSKRK	1
			EAIRQESTPR	1
			ELEKYVSWMR	1
			ENVHLRNIPK	1
			GDTIYDGSK	1
			GRNNPYDLNHTR	1
			GYSKSSGYPNLNR	1
			IDIVLFLQSWK	1
			ITCKQINKTVK	1
			KGVQDCGEAEEYAK	1
			KIDIVLFLQSWK	1
			KPNVCDALR	1
			KQKEQSSQK	1
			LEMEKLNK	2
			LEMEKLNKMLK	1
			LGELFKANTQR	1
			LIDELTHFLNK	1
			LKMSNVR	1
			LLPEKAHENNSK	1
			LMSRRK	2
			LNNRMSLCK	1
			LSTSLDVK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			LYKNEIR	1
			MLKDLYMSK	1
			MLKDLYMSKR	1
			MQTHSKR	1
			MSNVRLR	2
			NDYLYNYINTK	2
			NNPYDLNHTR	1
			NPPQEEGK	1
			NPPQEEGKLGNFSK	1
			QINKTVK	2
			QQYLQVNGAK	1
			RHIAGTMDLFK	1
			RHIAGTMDLFKYELIFK	2
			RTTKVTGEQNK	1
			SELRKMK	1
			SFIFRMK	1
			SIMSLYNSK	1
			SITHVNYR	1
			VNPNYDDFGMAK	1
			YVSWMR	2
			YYGKQQYLQVNGAK	1
PVX_118215	hypothetical protein	96.95	AYVVKVR	1
			CAEIPKKGR	1
			FEWKAYVVK	1
			GREGERK	1
			HKWPLK	2
			IYDSNIISK	1
			KALALFANC	1
			KQTMRQTMR	3
			MQASALR	3
			MQASALRTIPNVAK	1
			MSTPPNVR	1
			NRKPPALRK	1
			QTMRQTMK	3
			QTMRQTMKQTTK	2
			QTMRQTMR	2
			QTMRQTMRQTMK	2
			QTTEQAK	1
			QTTKQTTEQAK	1
			SQIGVFLK	1
			TKKQTMR	1
			WPLKNR	1
			WVKNNERTNER	1
PVX_118380	hypothetical protein, conserved	98.67	RGQKISK	1
			DLGMITKPPNHK	1
			EEFNESDLNIIDDVIEK	2
			EIARYQLNK	2
			ERNTKIDAK	1
			GEPHTTVR	1
			GHHNKGTTYMQNK	1
			GKNVHKGEPHTTVR	2
			GTTYMQNKQR	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			GTTYMQNKQRK	1
			HPGGETIGNESNK	1
			IDAKIYEQLK	1
			IIISALQEK	1
			IKSDAYK	1
			INDLIEQR	1
			INDLIEQRR	1
			INDLIEQRRLR	2
			INQSCSDQFKR	2
			IQIANEEIACFK	1
			IYEQLKK	1
			KDLGMITKPPNHK	1
			KKDLGMITKPPNHK	1
			KMLGKPMNK	1
			KVDVMVR	1
			LQRKYLNEK	1
			LRAEQVSKNMSHSAR	1
			LSNKTTVYDK	2
			MLGKPMNK	2
			MLGKPMNKLQR	1
			MQLLGQIFR	2
			MQLLGQIFRR	3
			NKLLMRK	1
			QESKYKK	1
			QLLGQIFR	1
			QNLNELRR	1
			REDYSKVK	1
			STLINELCGR	1
			TRSSIIK	1
			WLKKMTR	1
			YAQKQESKYK	1
			YKKGLQLTEQQMEGDR	1
			YNKWLK	1
			YQIVLSK	1
			YRDVQK	2
PVX_118470	hypothetical protein, conserved	99.41	AFISNIKDNK	1
			AIYYCHNDK	1
			DAFILSNQK	1
			DLDEGMAKGDLNR	1
			DLDEGMAKGDLNRK	1
			DLQSVLK	1
			ELWKHLVNYK	1
			GDLNRKR	1
			HQLAVQFLIAK	1
			IPHKDLNDGVR	1
			KKMQYIMALK	2
			KMQYIMALKR	1
			KSLSDINLENRK	1
			LFQWIVK	1
			LNFVSPK	1
			LVKQESEK	1
			MEAAKDER	3
			MLRGRATNEK	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			MQYIMALK	1
			MQYIMALKR	1
			NIDYHTK	1
			NKNLVMNEK	2
			NKNLVMNEKK	3
			NLVMNEK	1
			NLVMNEKK	1
			NNAFAEMLEVSR	1
			NSCGELR	1
			NSCGELRVGR	1
			NTNGVFR	1
			QELSSLK	1
			QELSSLKK	1
			QFGEVAARLR	1
			QSHICR	1
			QSHICRELWK	2
			QVVKNKSANGK	1
			RMPISESPYQFGK	1
			SAPGEVAKGDKNSCGELR	1
			SNMLLSIMDTNK	2
			SVYKQVVKNK	1
			TPLKQSHICR	1
			VHLCNICDIK	1
			YEYLLK	1
PVX_118682	erythrocyte membrane protein 3, putative	99.87	DKSGNNSLGNAK	1
			DTESKTYPLK	1
			DTPQSKPNVSKK	1
			DTPQSKPNVSKKDK	1
			DYQLQNKPHDLK	1
			EKTMQNSLKR	1
			EMAKAGGK	1
			EMAKAGGKK	1
			EPEIKEPK	1
			ESITFQKNK	1
			ESPNNVLK	1
			ESPNNVLKNIPAGEQK	2
			EYNESNISKDYQLK	2
			GDHMGGLK	2
			GNTIKNGSTNSTLK	1
			GTPHNGLK	1
			IYEKPIKDK	1
			KEKTMQNSLK	1
			KGEQTTNSPEK	1
			KGEQTTNSPEKALR	1
			KKPQEGMSK	1
			KPQEGMSK	1
			KPQEGMSKTSELKK	1
			NAPPSGIVGSK	1
			NGTPASVGSQPR	1
			NIPAGEQKENEKNVIK	1
			NIPKSDLTDK	1
			NKPPSGLVGNQK	2
			NNEQKKKPQEGMSK	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			NQLNEKK	1
			NQLNEKKK	1
			NRPPELKK	1
			QRLKEGK	1
			QSASKVNK	2
			QSASKVNKK	2
			RAIPSGLK	1
			RKLPELK	1
			RMNQSSDYK	1
			RMNQSSDYKLK	1
			SAPHTDLNINK	1
			SAPHTDLNINKK	1
			SLAENTGYTQLK	1
			SSPPTGLQANK	1
			TMQNSLK	2
			TSELKKK	1
			TYPLKNIPK	1
			VDKAEVNKNAVK	1
			VDKTEVK	1
			VNKKADVTR	1
			VNNKSIR	1
			VNTETIRPTKQALK	1
PVX_119615	hypothetical protein, conserved	99.83	AAKMIYK	1
			AGEPYRK	2
			AKPVHDEEK	1
			ASDKNAKNK	1
			ATQYVHAIEHK	1
			AVGPPSDSRR	1
			CEDNTMAEMVK	1
			CVGCSIYIHK	1
			DFINLTILK	1
			DLYNNLSEEIGK	1
			DRANRPR	1
			EAVKWKR	1
			EIEQLIYRR	1
			FVSDVDNLK	1
			GASHDLEEGK	1
			GIHRNVLMIR	1
			GNLLEGNK	1
			GSSHSKKK	1
			HKKMASCFFSYR	1
			HLNHTNSGSAKK	1
			IHPDVPIHDK	1
			IQNMKKK	1
			KDGGALKR	1
			KMISSAGREK	1
			KSTANGGNGK	1
			KYLNNVLFDK	1
			LFLQDGEAR	1
			LHLHER	1
			LMQRMAPK	1
			LMQRMAPKMAPK	2
			LNAVNNR	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			LRNGEGGGK	1
			LSEMLLNK	2
			LYSQAAHR	1
			LYSQAAHRSEAHR	2
			MAPKVTPK	1
			MISSAGREKR	3
			NEMNLSVNK	1
			NGKNGKNTK	1
			NKGKNNCQSSK	2
			NQPKNQPKNLPK	1
			NVPSSHVK	1
			NVPSSHVKDQK	1
			NYEKMK	1
			QENGVNICQK	1
			QLKIQNMK	1
			QLKIQNMKK	1
			QNAIKAAPCK	1
			QNELNSFFK	1
			QSDHTHK	1
			QSDHTHKR	1
			RQMEFFYK	1
			RQQKMR	2
			RRPDSGK	1
			RVSQNMGPYCK	1
			RVSQNMGPYCK	1
			RYNSFGGTPR	1
			SEAHRNESLR	1
			SNLNGKAK	1
			SSNAVKGKQPAGK	1
			STANGGNGK	1
			SVMQRSALQR	2
			TNHQSVEAK	1
			VGPYVKDEK	1
			VLNKQIAKR	1
			VSQNMGPYCK	1
			VSQNMGPYCKK	3
			VTPKVTTKMTQK	1
			WNQLAFK	2
			WNQLAFKDCGK	2
			WRSVEEVETK	1
			YHFAYQRK	1
PVX_119810	hypothetical protein, conserved	99.10	AAKQSHLAWSAEK	1
			AIPLGTNR	1
			ANKKLVNYK	1
			DIQEEIFR	1
			DPKTTARGVK	1
			DYRMIR	1
			DYRMIRYFYK	1
			EEVFLLCDGDNGVR	1
			ETPQHGYYFTKEK	1
			FAANKFGANK	1
			FDNLYFLNLVAK	2
			FNLALLK	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			GDQPTLPSELQR	1
			GTNTPFVRLEK	1
			HNIFVCYYGMHAKKANK	2
			INEGTLINCFELFLIK	1
			IVCLKMVNSLK	1
			KFSLNYCRDPK	1
			KKFSLNYCR	1
			KNRDNHLNK	1
			LRHMFQLG	2
			MGQPPSGTGR	1
			MIRYFYK	1
			MVNSLKNER	1
			NEFLTAR	2
			NLTQFISLER	1
			NRPATSR	1
			NVRSVGGLAQLCAQR	1
			QSHLAWSAEK	1
			QSHLAWSAEKYQK	1
			QSVQLSKK	1
			RSDGPSGPRELNR	1
			RSSTPLNSDK	1
			RTPPITK	1
			RVILLGGK	1
			SDGPSGPRELNR	1
			SFIQVIK	3
			SILIKMNK	1
			SRGSWSR	1
			SSTPLNSDKKQK	1
			SVDDVHLGK	1
			SVGGLAQLCAQR	1
			TCLFHELR	1
			VSGKNRPATSRR	1
			VYVGRGR	1
			YFYKMEK	1
			YPAQVSTEGEEVK	1
			YQKMASNFVLR	1
			YVNQLRR	1
PVX_122090	hypothetical protein, conserved	98.94	DGQPFQEGTLK	1
			EGASVDDGASGELK	1
			EGSKLFMTK	1
			EGSKLFMTKK	1
			EQLEMR	2
			EQLEMRKK	1
			EYENIKNIYK	1
			EYENIKNIYKNR	1
			FKEGSKLFMTK	1
			FNQFDYWYSSK	1
			HSAYIMITEK	3
			HSAYIMITEKLSK	1
			HVNFTITENK	1
			IRNEINQK	2
			KDTSTPKK	1
			KLFDIINFKCK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			KNGPIAYDIKNPFMK	1
			LFGSPTSCLLK	1
			LFGSPTSCLLKK	1
			LMQTAVR	3
			LMQTAVRK	1
			NILKEYENIK	1
			NIYKNR	3
			NIYKNRK	1
			NKQERLMQTAVR	2
			NPFMKSK	2
			NPFMKSKMSSK	1
			QERLMQTAVR	1
			QERLMQTAVRK	1
			RDDFTPKK	1
			SEDKTECTK	1
			VQAEKDTSTLKK	2
PVX_122640	hypothetical protein, conserved	96.88	ARLDFIKNVEYK	1
			LDFIKNVEYK	1
			LLALGDESNAK	1
			LMINFSEYLEVLIK	1
			MFNSCVR	1
			NPSLLMQLQKTASK	1
			NSIMYR	2
			NSIMYRR	1
			QMELRK	4
			QMELRKNR	3
			SFVAMQASQK	1
			SKNPSLLMQLQK	2
			SKNSIMYR	1
			SKNSIMYRR	1
			TASKQMELRK	2
			YNAIKSK	1
PVX_122740	structural maintenance of chromosome 2, putative	97.28	CTPYDFENFR	2
			DIDKEIEIHKK	1
			DSQTASETVKYLNK	1
			EEVDGVLLQIENYK	1
			EFSEMNKEK	1
			EIEIHKK	2
			EQVEKKISHLK	2
			EVTEVKRR	1
			FEKEFSEMNKEK	1
			FIDGISTVNR	1
			FISNNEEIEK	2
			FNNEQKPSPLQEPYRDMK	1
			HDVIQKK	1
			HSLDIRQTTNK	1
			IAFNNNWK	2
			IEDAMEEKKVLEK	1
			IEGKEKEK	1
			IEGLEKK	1
			IKLLENEEHK	1
			ITYNANK	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			KDQKLEEINK	1
			KEFLEKDHQLK	1
			KEVTEVK	1
			KIACEKELQK	1
			KIEGLEK	1
			KIQEVIADLDVKK	1
			KKEFLEK	1
			LDNTLIDLQK	1
			LLENEEHKKK	1
			LLTNEVEK	1
			LSVVDGDLSNGIEMK	1
			LSYILVQNK	1
			LYEVKR	1
			MHIEEIILDGFK	1
			NDEKNNKNLK	1
			NINLFLLHYEK	1
			NLINHVK	1
			NRYLLNSHNAKPK	1
			QGQAGITK	2
			QIVLGGRNR	1
			QTTNKKEVTEVK	1
			SQVEEDK	1
			VNRLDELIYK	1
			VTLLPLQDCIVSR	1
			YENKKK	1
			YYVAKNMMEK	1
			YYVAKNMMEKSEGK	1
PVX_123200	hypothetical protein, conserved	96.73	AKEFENISKK	1
			DRDFLR	1
			EEAIKYLSRK	1
			EEEDSLVENK	1
			EEKEEDDEK	1
			GSRSEIQKQIK	1
			IDVFHSGNKSKK	1
			ILTPIVLCLQK	1
			KIIKEIK	1
			KYASNSSK	1
			LDEKDGK	1
			LMDTGQGLNR	1
			LSQIIEK	1
			LSQIIEKEIK	1
			LTHSHNR	1
			LTYEER	1
			MNENERLK	4
			MNENERLKK	1
			MQSGHKVLSVMK	1
			NENERLK	1
			NGLDVLNDNLIK	3
			NSKKNLFR	1
			NSSYSLSIESGK	1
			NYENEQYDTLK	1
			QELMLLIDK	1
			QGSRLTHSHNR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			QIKQKEEAIK	1
			QKEEAIK	2
			SEIQKQIK	1
			TEFNGAQNLK	1
			TVYDLLKGK	1
			VLSVMKK	2
PVX_123340	eukaryotic translation initiation factor 3 subunit 10	99.57	ALIFCKENK	1
			DLEIEQQLLRR	1
			EESRNGDKYYR	1
			EKMTNGK	1
			ELEQEQLKIK	1
			ELEQEQLKIKMEK	1
			ENMNLNKEK	2
			EQHLRK	1
			EQHLRKLK	1
			EREKMTNGK	1
			ESLKNGLK	1
			FQLLSEENSRKR	1
			GKHKPEYQSLLK	1
			GPLKSDSNETSKK	1
			ICMETYKMILEILRATPK	1
			IVEFEKNLKDQK	1
			KEESRNGDK	1
			KKNEDYAK	1
			KKSEAAEQMLK	1
			KLSDLLR	2
			KQKELEQEQLK	1
			KRNFMK	1
			KVDHYFR	1
			LAKENMNLNK	1
			LCTNNTTKILMK	2
			LILQLSK	1
			LLEEKLEK	1
			LMKEEMERK	1
			LRLNER	1
			MILEILR	1
			NEDYAKIFEAHKK	1
			NEEGSDGGGDFTVFKKK	1
			NIPEKEK	1
			NIPEKEKTFMCTK	1
			NLKDQKDR	1
			NWIGTFYQR	1
			QKELEQEQLK	1
			QKELEQEQLKIK	1
			QTFQKPENALK	1
			QTFQKPENALKR	2
			REDDYRR	1
			REEEEER	1
			REQHLR	1
			RLDEAQDSSYR	1
			RREEEEER	1
			SEAAEQMLK	2
			SEAAEQMLKEIK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			VITDFRDK	2
			VRKMEYK	1
			VRLAKENMNLNK	1
			YERKLQNSFR	2
PVX_123585	hypothetical protein, conserved	99.56	DTEKGQTNEQTNAPK	1
			DYYGYK	1
			EILSEQK	1
			ENIFINQIVK	1
			FVITKVGKMYQK	2
			GMYANFLKNDLK	1
			GQTNEQTNAPK	1
			GVTENPYK	1
			GYAYRQELQK	1
			IDNGDAKINCPFR	1
			IKGYAYRQELQK	1
			INCPFR	1
			INCPFREKLLTR	1
			KIWNNK	1
			KNPHRDK	1
			KQGDGAASSEANVVR	1
			LKSEQNVKK	1
			MRSLKNYEFVK	1
			MVQNGKK	4
			MVQNGKKNK	1
			NEGGYRK	1
			NEQVDIMSYLK	3
			NEQVDIMSYLKVK	1
			NEVNNIK	1
			NKKNIFK	1
			NQARGASQQKR	1
			NQNIRAK	1
			NYEFVKEK	1
			NYKGMYANFLK	2
			QELQKSK	1
			QELQKSKFK	1
			QGDGAASSEANVVR	2
			QKEKQNGK	2
			QNGKGEDPHK	1
			RIILNDMMKEK	2
			RMRSLK	1
			RNEVNNIK	1
			RSGTYKIATR	1
			SEQNVKKECIIK	1
			SGNNVCGLSK	1
			SLFHEEFFRILK	1
			SLKNYEFVK	1
			SNLLQKNYK	1
			SQEDYLQLAR	1
			SSTAGLYEAKR	1
			STLLDMEKEK	1
			TILAIYINTLFKSSNEIR	2
			VDLLKCCINK	1
			VGALNAEGK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			VGALNAEGKQKEK	1
			VGKMYQK	1
			VINNVIK	1
			VPVLGENIFIK	1
			VQNGKKNK	1
			YDLIKNLMLILTK	2
			YYNNVK	1
PVX_123725	hypothetical protein, conserved	99.14	DIKRGNWK	1
			EEKIER	1
			EEKIERYK	1
			ENHQFK	1
			FFNPKQFK	2
			FLNRKPDR	1
			FYSVSNK	2
			FYSVSNKYK	1
			IERYKK	1
			KLSDYNK	2
			KREEQK	1
			LSDYNKEEALDQSK	1
			MFFNPK	2
			MFFNPKQFK	2
			NKKPQCGKENHQFK	1
			NLFPEEGKK	1
			NPFKFLNR	1
			QFKNLFPEEGK	1
			QTAVTEDK	1
			QYPKRLGQPIK	1
			REEQKK	2
			RLGQPIK	1
			RWNLKVEK	1
			SNNNTGFGSTDK	2
			WNLKVEKDIK	1
			YRYVPEKNPFK	1
			YVPEKNPFK	1
PVX_123990	hypothetical protein, conserved	99.04	AFHKNLK	1
			AGGRAAGGRAAGVK	1
			ARKSSIYNK	1
			DANINEK	1
			DKPKVWK	1
			EEGQKGQKLQK	1
			EIKSHSK	1
			EKMTNGGVK	1
			FNYMNLVKR	1
			FVANKNK	3
			GQKGQKMQK	2
			GRKSHTGSTPK	1
			HDGGTPNSEK	2
			HTGGSAAEDVVK	1
			IRCINR	1
			ISERFR	1
			KKNFNLSR	1
			KKNQIQMFR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			KSSIYNK	1
			LLMYNR	2
			LNRDKPK	1
			MQKMQTGGTEK	3
			MQTGGTEKEQR	2
			MTNGGVKIHK	1
			NAKAFHK	1
			NEDPFER	1
			NFQITEWNMK	3
			NNMVTQFLK	2
			NPYLICLIK	2
			NREKMTNGGVK	1
			NTFALFSINR	1
			NTFALFSINRNNK	1
			NVFTRAR	1
			QNTAKPSAATAAR	1
			QNTAKQNTAK	1
			QSEGQSERQLER	1
			QSERQSER	1
			RNTFALFSINR	2
			SRFNYMNLVK	1
			SSKSTNNLFLFFR	1
			THVAHTTKGER	1
			TIMGIIPK	1
			TLNIFNINR	1
			VNFDPSGGR	1
			VNTAKQNTAK	1
			YALLAKSVNCK	1
			YMDISPLCRKSR	2
PVX_124100	T-complex protein 1, gamma subunit, putative	98.94	ACTILLRGSTK	1
			CGLFDVK	1
			DIVHPKMR	1
			DIVHPKMRR	2
			GVMLNKDIVHPK	2
			GVSDLAQHFLVK	1
			HETPGGEK	1
			IDDVVSGIGK	1
			IESNVMGRK	1
			KTQLSNIQASR	1
			MVSTLALEAVQCVK	1
			NIIMEGK	4
			NISVIRR	1
			NLHDGMNVAK	1
			RNISVIR	1
			SLIELSR	1
			TDLNRLER	1
			TINELRIR	1
			TQLSNIQASR	1
			TTLGPMAMLK	1
			VEKIPGGDITDSYVLK	1
PVX_083220	hypothetical protein, conserved	99.66	AKLEIGEK	1
			CLTIHK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			CNISLDIHK	1
			DIFLFR	1
			DILNYISVDNLSEEIKKK	1
			DVIFKK	2
			EASNEENGSR	1
			EGDHANDHAPNEVESK	1
			ENRVRGESPK	1
			ENYILK	1
			ENYILKANVR	1
			EPGRKK	1
			ERNFKTNTNMK	1
			EYKKK	1
			FHLGTVK	1
			FKIIRER	1
			GAQNNERVYVHELK	1
			GGGGYVQK	1
			GIDRFQSAQR	1
			GNINLGNLGVRR	1
			GTTAGGGSRSGRSGR	1
			HGESAKR	1
			IIEIMNEANK	1
			IIHGNIGR	1
			IKNKFMR	2
			ILMVSGK	1
			ISALPAEKNMK	1
			KCKIK	1
			KEKDLLK	1
			KENRVR	1
			KIYAVNSK	1
			KLQYGIEK	1
			KVVSSSK	2
			LKNEEELK	1
			LPVGMNPLK	2
			LQCKNTLEK	1
			NALPKLHGQK	1
			NASLAFWQK	1
			NEEELK	1
			NEEEVIDIK	1
			NFSIGFILK	1
			NKLKNEEELK	1
			NNIYFVMR	1
			NPILLTSLENR	1
			NREVLK	1
			NSAFSIK	1
			NSAFSIKNGKNR	1
			NSCTTLTTSLK	1
			NSLDMQEYHK	1
			NYLQKKK	1
			QERITPIGGK	1
			QGLLLFR	2
			QGLLLFRFK	1
			QKERNFK	1
			QLSINLQILQK	1
			QLYIFSLPR	1
			QNDTSPLYFPK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			QQKNKEK	1
			QYKVSK	1
			RALRER	1
			RQDLFVGK	1
			RQKGIK	1
			RVQRLR	1
			RYAVENIR	1
			SENVIIEELR	1
			SGQSGQSGRSGR	2
			SKVFMEFRTNK	1
			SNRQKR	2
			SQNDVPNVGK	1
			SQNDVPNVGKGK	1
			SSSVKNCLVKDK	1
			SVLRRGK	1
			TGNSSNLVYK	1
			TIKQIFKTMK	1
			VFMEFR	1
			VFMEFRTNK	1
			VGQAVSDVTR	1
			VLNGLKK	1
			VLNGLKKK	1
			VRGESPK	1
			VSNSLNVNPNIK	1
			VYKDRR	1
			VYLNFDQK	1
			WERLNAEMERQTNLACK	1
			YAVENIR	1
			YAVENIRIEENVFKSEAK	1
			YNASTVTDER	1
PVX_084285	UGA suppressor tRNA-associated antigenic protein, putative	99.97	ATTSFVKDLIK	2
			AVQGDICSNR	1
			DLIKSFGIK	1
			EENFAWLK	1
			ENTLWNILNYGR	1
			IDHKTCYK	1
			IGERENR	1
			IYHVENPR	1
			IYSALVR	1
			IYSALVRNK	1
			KHYPGR	1
			LDDAIGCFIR	1
			LDDAIGCFIRR	1
			MNTPGVDVR	2
			MNTPGVDVRNGK	1
			NENATHAAILGK	1
			NKYIGFGHGIGR	1
			NSDDIVK	1
			NTPGVDVR	1
			NTPGVDVRNGK	1
			NTPGVDVRNGKAVQGDICSNR	1
			NVKIGER	1
			RASPVER	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			SAGNSVLAK	1
			YLVVDMVYR	1
PVX_088035	hypothetical protein, conserved	100.00	ADANRVSHAR	1
			ADANRVSHARK	1
			AERSLKLQGR	1
			AFAALQQHRQR	1
			AQNCYR	1
			AVATMKR	2
			CRQSRNVK	1
			CTPMKR	1
			CTPMRHLTR	1
			CTPVKR	1
			ECHSLK	1
			EEIKIQSVR	1
			EELLFK	1
			EHKFVR	1
			EKKMK	1
			EKTLQTAK	1
			FQCYGIRNR	1
			FQRHWVSYAR	1
			FRKCK	1
			FVRVNK	1
			GAIFFQIKR	1
			GCLVRSRMQR	2
			GSEENGGSVIR	1
			GSQMLTLNR	1
			HEKTMR	1
			HEKTMRK	1
			HFDCLQLHAHR	1
			HFYEMTRR	1
			HLTRAFLER	1
			HWVSYARK	1
			KAFQSLRDR	1
			KKYFR	1
			KNEQIK	1
			KNYPYR	1
			KRCTQK	1
			KRVFFHQWVK	1
			LCERTIQCR	1
			LFSHVVQK	1
			LLMKKK	1
			LQGRCLSR	1
			MAIYKR	1
			MAIYKR	1
			MEDANDIIK	2
			MEELIQGK	1
			MHIQMGCRGK	1
			MHLHNLLLR	1
			MKYYTFRK	1
			NFSTVMKR	2
			NMSEKYNR	1
			NRRFSFFR	1
			NSLLARRLTEK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			QAKLQIK	2
			QDLKVK	2
			QKRQAR	4
			QKYFSKWK	2
			QLREIHNVITIR	1
			QLSGEILNK	2
			QMCVRR	2
			QMCVRRWR	2
			QMQEEKIK	3
			QMQEEKIKK	1
			QNEKSFMHK	1
			QNSHFYIYQR	1
			QRQATLRR	1
			QYYVRYLK	1
			RAKFGFFSVR	1
			RALSLQK	1
			REQLIR	1
			RKGPAVESAR	1
			RLAATKQK	1
			RNRSNR	1
			RQRANR	2
			RSADPKQNEK	1
			RSNEKR	1
			RSNEKR	1
			SHLKKFFAILR	1
			SPYGPLKMTTLR	1
			TFQQWK	2
			TIQCRTQR	1
			TIQCRTQRR	1
			TLNEYLFRR	1
			TPVKCTPMR	1
			VHTRRMLAK	1
			VIKQSIINSMR	1
			VYPPGGGK	1
			WKSYVEMRR	1
			WTRFKNYSK	1
PVX_089860	RAD protein (Pv-fam-e)	99.88	AMNIGFK	3
			CITQDEYRCLLTEIQVLK	1
			CLLTEIQVLK	1
			ECKDSIAK	1
			ERDKIK	1
			GVEAYQR	1
			KIFYK	1
			KILYKYVK	1
			LINKLFAK	1
			LNEVASGNAIK	1
			MCDSYMVKNR	1
			MKIFYK	1
			RMCDSYMVK	1
			SWEEAIR	1
			SWEEAIRRNEK	1
			VRCITQDEYR	1
			WGDIFLQR	1
			WGDIFLQRTR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
PVX_092535	Adenylate and Guanylate cyclase catalytic domain containing	99.22	AFIVQLIKHR	1
			AHKERR	1
			ANRTIGAVK	1
			DIQLCSRK	1
			DNLFESIVK	2
			EVAMKAR	3
			EVAMKARK	1
			FFKKR	1
			FKHRFSR	1
			FKPTMK	1
			FKPTMKQLHQEDCSTNK	1
			GGGSDGEKYPR	1
			GVGKFEDRQTK	1
			GVSQYMEK	1
			HGSSNRFFK	1
			IGTAVGHQLK	1
			IMKNILKNVYK	1
			INMNTGSVK	1
			INMNTGSVKAR	2
			ITKSCNFFR	1
			KDPENDHLHHRSK	1
			KEADRK	1
			KEDAQK	2
			KETEIK	1
			KGDSMFPNYK	1
			KHGSSNR	2
			KNNIKFK	1
			KYNMSTLK	1
			LILRNK	2
			LQDDTREEDTGR	1
			MCLLINGK	1
			MERKK	2
			MLRNIR	3
			MSVVVKPEFMQSGSVLYVK	1
			NEKKNNIK	2
			NFEEVK	1
			NFLLQQFK	1
			NFSPSNR	1
			NFTKNR	2
			NLHNTGYLLNRK	1
			NLHNTGYLLNRKMR	1
			NSEGGAPGVVK	1
			NSEGGAPGVVKK	1
			NVYEDSRR	1
			NVYKSGNVNR	1
			NYQTYLTQHK	1
			QLRKFSVK	1
			QPNDFTTAEAAWMEKKQK	1
			QQSNDVVKMR	1
			RDQMDASQGK	1
			RKYNMSTLK	2
			RYRQLEK	1
			SCNFFRK	1
			SFIITDKKEIDAMNNSK	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			SHSNTAHNR	1
			SNIDSQINNR	1
			SSKGVSQYMEK	2
			TKHMFKR	1
			TYGGRRQK	1
			VSNLINR	2
			YNMSTLK	1
			YRQLEK	1
			YTFFLNNFSR	1
PVX_099570	NADPH-cytochrome p450 reductase, putative	99.85	DIFKIK	1
			DLVWSLIQK	1
			DVEERK	1
			DVNKTINSLPMHYKQNNK	1
			ELCSRR	1
			ELSTNLR	1
			FFFNHHR	1
			GFKSNDMLPPISEQK	1
			HFNKVAKK	1
			IVIAVEK	1
			KEEISK	2
			KKFFK	2
			KLANYLAK	2
			KMFNHILSNKQR	1
			LANYLAK	1
			LGLDDTDGKKR	1
			LKKEGR	1
			MFNHILSNK	1
			MNPRASR	5
			NVPKSYTISSSPK	1
			QNNKKFTK	1
			QYKHFNK	2
			QYPIHSLR	2
			REIDFLYEREIADAEQGK	1
			RVTSWWLKR	1
			SNDMLPPISEQK	1
			STFFNICK	1
			TINSLPMHYKQNNK	1
			VISNENLLK	1
			VNILKK	2
PVX_099580	hypothetical protein, conserved	98.08	CQREASHR	1
			DLHDKR	1
			DLHDKRNGEK	1
			EDESSSEYR	1
			EDPNLQRR	1
			EEGNRAGAKVK	1
			EGEFPGGARR	1
			ERPLNPHR	1
			ERQDWHK	1
			FLTSSLR	1
			GEADQLAKSKK	1
			GSSRGSSRGR	1
			GSSSFMCHPDGRR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			GSTNKRTYMK	1
			HGKRDK	1
			HPYSRR	1
			INFRNCHLR	1
			KRHYR	1
			KRSVSVCSSDGNAYSHNK	1
			KWKMVAR	1
			LPAEEDSPNIYR	1
			MDLHDKRNGEK	1
			MGKLPPTKK	2
			NDSTIER	1
			NEHPRR	2
			NIMHEEFK	1
			NKIRQR	2
			NPATSPVGNKYCDVPLEKR	1
			NSGTKNDSTIER	1
			QEKNPATSPVGNK	1
			QSEYRQEK	1
			QVPSVEGEANR	1
			QVPSVEGEANRRLSR	1
			RGSHSDR	1
			RLNTYK	1
			RTYHMR	1
			RTYHMRK	1
			RTYMKYHEGEDYSEGEK	1
			SASRRSR	2
			SFLDFLER	1
			STDDDAVER	1
			TAITERDNER	1
			TAITERDNERSDGSSR	1
			TKEYPQSR	1
			WKMVAR	1
			YPTQEAKR	1
PVX_100860	hypothetical protein, conserved	96.34	ANLSPSLR	1
			ASFTSRANK	1
			EWTLVGKK	1
			GKQTSTSTSNGENIGPK	1
			GKTNDQSGDGPK	1
			GRSGASEK	1
			HLVDFIR	1
			HNTPQIEQPNSGK	1
			KASFTSR	1
			KSPSLAK	1
			KWPLSK	2
			LKGRSGASEK	1
			LLTLKGETK	1
			LTEVSTNQMCR	1
			NIHIFLSVEMGK	1
			NNLLHYVDDDR	1
			QEIALCNK	1
			QKSLQLDKR	1
			QKSNKR	2
			QNESAQSIEGEK	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			SHHLKKK	1
			SLQLDKRK	1
			STKEWTLVGK	1
			SVATELGK	1
			VAHAPLGKGK	1
			VSLGNDTLGNR	2
			YTQVTQLNR	1
PVX_113390	hypothetical protein, conserved	99.59	AAAEKSKK	1
			ARSSLEAESLGK	1
			DGRDKDLR	1
			ENKNEK	1
			EPSQQPYR	1
			EQLAMMEK	1
			EQLAMMEKQNILLK	1
			FFRANR	1
			FFRANRYGK	1
			GFGKPFADNMK	1
			GGKGNQKR	1
			GIGSSSGPKK	1
			GNHSIDEVNR	1
			GPMHMKR	1
			GRSSLEGFK	1
			HLHHSATANSGGLEGTHLN MK	1
			HTVGYAPNGK	1
			IENSKK	2
			IKIENSKK	1
			IMNKIK	2
			INLQEEHR	1
			KSDDMGGTVK	1
			MEENIYNK	1
			MKKYK	1
			NGSVVNLASSR	1
			NPNEKK	1
			NPNEKK	1
			NQGGLAAK	3
			NTHRSNYPKDR	1
			NTTSSMELIK	1
			NVVEDDWSNIR	1
			QFNPNQLLGK	1
			QFNPNQLLGKR	1
			QGKAKAGVK	1
			QMKTPGESVK	1
			QNILLKKIMNK	2
			QSEKKK	1
			RKPMSGVHPPNSVSNPNDVR	1
			RSNHLLKNNK	1
			RTINSTSSGSSAR	1
			SNAMGDEEDGGVYNKHHYR	1
			SNYNVVK	2
			SNYNVVKK	1
			SSFVENKK	1
			SSLEAESLGK	1
			SSLMNVSRWLK	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			TAQGTEDHGR	1
			TINSTSSGSSAR	1
			TIRWMPEK	1
			TNSPPER	1
			VEGLMPSR	2
			VEKGEKAEK	1
			VKAATQAYKAK	1
			VRGDVWQYK	1
			YFGSGRGSGQVEGAH ASAKEK	1
			YFPQNLPIR	1
			YLLSDDDRDDR	1
			YYATTDNNR	1
PVX_118220	serine/threonine kinase-1, putative	98.08	CLPLHK	1
			DDGTGKSDVR	1
			DDKSNKSFK	1
			DEIMGSR	1
			DGYHGSIINTR	1
			DSNRNGK	2
			EINDREQR	1
			EKGSQQSLR	1
			FSDMEDNNNR	1
			GKYMGFGNMQR	1
			IEADILKK	1
			IQENYK	2
			IRSFNSTSNSSR	1
			KDSNRNGK	1
			KLYNSDRR	1
			KMICEAVR	1
			KMYYGNKNSFR	1
			LLKGKR	1
			LYCIEMLK	1
			MNNKRK	1
			NAQRRR	3
			NIRKYTK	1
			NSHRNSYR	1
			NYDVDENMIR	1
			NYYSYKK	1
			RATDGKR	1
			RKDSNR	1
			RNLVVCSDKEK	1
			RNNNYYYR	3
			RSYSSMREINDR	1
			RVQIYR	1
			SYSSMREINDR	1
			TKSTGIK	2
			TLVSVRR	1
			TNGAKYINR	1
			TNGAKYINRDGLR	1
			YFNDPR	2
			YKATAVHQK	1
			YMGFGNMQR	1
			YSSKYR	1
			YYAVKVVRNIR	1

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
PVX_122530	hypothetical protein, conserved	97.16	CLGNVRNNNSALFRNTLTK	1
			DHLLQYYR	1
			EDVYRALR	1
			EFGINCIK	1
			EHVVAKRK	1
			EKKNDILNWK	1
			EMKQMQK	1
			ETFQSGK	1
			ETFQSGKNTKR	1
			FLVENRIMR	1
			FYRLVK	1
			GGKQTDR	1
			GIDQKSK	2
			GIDQKSKIYK	1
			ILYQRDQR	1
			IMRNVISK	1
			INIALLK	1
			INWVPK	1
			IREILKNK	1
			IRGGASPSSGDA	1
			ISKQIK	2
			KETFQSGKNTK	1
			KIWGGNINSSK	1
			KKETFQSGK	1
			KKWSK	1
			KMTPDDASKGGK	1
			KNDILNWK	1
			KNKMIYR	2
			KQTVRR	1
			KQVRFFDPEK	1
			LDQLRR	1
			LLGSRHNFK	1
			LPRRNK	1
			LPYNAKQVK	2
			MGSTPQRGR	1
			MGVTPRR	1
			MIYRNK	1
			NEFLKEMK	1
			NHLHLNNVIVEK	1
			NKMIYRNK	1
			NKQSTYFAYKNNFLLLK	1
			NNFLLLK	2
			NNILQHNTTNK	1
			NSPINYMK	1
			NYDFLSFSFK	1
			QANFDQLYSQYFPK	1
			QGYSHIKK	1
			QIKCNSKK	1
			QKSQYVDK	1
			QTEKRVEK	2
			QYKYLFK	1
			RGKLQK	1
			RGTVHMKK	1
			RKINQR	2

Peptide mass fingerprints of *P. vivax* proteins identified by MALDI-TOF/MS (cont.)

ID	Description	Protein Score C.I. %	Sequence	No. of peptides
			RLTGRASFR	1
			RMGIKR	1
			RNDNVAARLK	1
			RNFLLK	1
			SDLLLENVWGER	1
			SVQVEMR	1
			TLKRMFNAFYNDCYNTR	1
			TSAKSACGNLR	1
			VEKRAK	1
			WSKIIINR	1
			YHGERQK	1
			YKNFLLNEK	1
			YLLHGAK	2

APPENDIX C

Predicted SP and TM of identified *P. vivax* proteins (* Signal peptide (SP) predicted by SignalP 3.0 and ** Transmembrane segments TM predicted by TMHMM 2.0)

Accession Number	Names	SP*	TM**
PVX_092410	3-oxo-5-alpha-steroid 4-dehydrogenase domain containing	0	6
PVX_099315	78 kDa glucose-regulated protein precursor (GRP 78), putative	1	0
PVX_099320	acid phosphatase, putative	1	1
PVX_092535	Adenylate and Guanylate cyclase catalytic domain containing	0	21
PVX_110895	ADP/ATP transporter on adenylate translocase, putative	0	3
PVX_250300	ADP/ATP transporter on adenylate translocase, putative	0	3
PVX_115345	alanyl-tRNA synthetase, putative	1	0
PVX_092275	apical merozoite antigen 1	0	1
PVX_092245	aquaglyceroporin, putative	1	6
PVX_083135	aspartate carbamoyltransferase, putative	1	1
PVX_086040	aspartic protease PM4, putative	0	1
PVX_111035	aspartyl protease, putative	1	1
PVX_081455	calcium-transporting ATPase, putative	0	8
PVX_100550	chaperonin CPN60, mitochondrial precursor, putative	1	0
PVX_087980	chloroquine resistance transporter, putative	0	10
PVX_091700	circumsporozoite-protein related antigen, putative	1	0
PVX_122545	Cop-coated vesicle membrane protein p24 precursor, putative	1	3
PVX_121885	cytoadherence linked asexual protein, CLAG, putative	1	1
PVX_091110	DnaJ domain containing protein	1	1
PVX_114560	DNAJ domain protein, putative	1	0
PVX_099370	DNAJ-like molecular chaperone protein, putative	0	6
PVX_096070	early transcribed membrane protein (ETRAMP)	1	1
PVX_123745	endoplasmin precursor, putative	1	0
PVX_082460	ER lumen protein retaining receptor 1, putative	0	7
PVX_114670	ER lumen protein retaining receptor, putative	1	5
PVX_116915	exported protein 2, putative	1	0
PVX_115000	falcilysin, putative	1	0
PVX_091470	heat shock protein 101, putative	1	0
PVX_099930	high molecular weight rhoptry protein-2, putative	1	0
PVX_084640	hypothetical protein	1	0
PVX_096975	hypothetical protein	0	1

Predicted SP and TM of identified *P. vivax* proteins (* Signal peptide (SP) predicted by SignalP 3.0 and ** Transmembrane segments TM predicted by TMHMM 2.0) (cont.)

Accession Number	Names	SP*	TM**
PVX_107740	hypothetical protein	0	1
PVX_086900	hypothetical protein	1	2
PVX_002950	hypothetical protein	1	3
PVX_083555	hypothetical protein	0	1
PVX_121935	hypothetical protein	1	2
PVX_115155	hypothetical protein	0	3
PVX_080555	hypothetical protein, conserved	1	0
PVX_081550	hypothetical protein, conserved	1	0
PVX_084720	hypothetical protein, conserved	1	0
PVX_085915	hypothetical protein, conserved	1	0
PVX_091300	hypothetical protein, conserved	1	0
PVX_122090	hypothetical protein, conserved	1	0
PVX_001780	hypothetical protein, conserved	0	1
PVX_094345	hypothetical protein, conserved	0	1
PVX_096295	hypothetical protein, conserved	0	1
PVX_115450	hypothetical protein, conserved	0	1
PVX_117490	hypothetical protein, conserved	0	1
PVX_000890	hypothetical protein, conserved	1	1
PVX_083105	hypothetical protein, conserved	1	1
PVX_089485	hypothetical protein, conserved	1	1
PVX_091990	hypothetical protein, conserved	1	1
PVX_099055	hypothetical protein, conserved	1	1
PVX_095095	hypothetical protein, conserved	0	2
PVX_095200	hypothetical protein, conserved	0	2
PVX_091495	hypothetical protein, conserved	1	3
PVX_092765	hypothetical protein, conserved	1	3
PVX_101485	hypothetical protein, conserved	1	3
PVX_118610	hypothetical protein, conserved	0	5
PVX_100835	hypothetical protein, conserved	1	5
PVX_086090	hypothetical protein, conserved	0	6
PVX_080460	hypothetical protein, conserved	0	9
PVX_119390	hypothetical protein, conserved	0	11
PVX_123395	hypothetical protein, conserved	1	14
PVX_002790	hypothetical protein, conserved	1	0
PVX_003895	hypothetical protein, conserved	1	0
PVX_083480	hypothetical protein, conserved	1	0
PVX_087725	hypothetical protein, conserved	1	0
PVX_088910	hypothetical protein, conserved	1	0
PVX_090900	hypothetical protein, conserved	1	0
PVX_090960	hypothetical protein, conserved	1	0

Predicted SP and TM of identified *P. vivax* proteins (* Signal peptide (SP) predicted by SignalP 3.0 and ** Transmembrane segments TM predicted by TMHMM 2.0) (cont.)

Accession Number	Names	SP*	TM**
PVX_091585	hypothetical protein, conserved	1	0
PVX_092070	hypothetical protein, conserved	1	0
PVX_094755	hypothetical protein, conserved	1	0
PVX_099035	hypothetical protein, conserved	1	0
PVX_099710	hypothetical protein, conserved	1	0
PVX_122810	hypothetical protein, conserved	1	0
PVX_087675	hypothetical protein, conserved	0	1
PVX_089170	hypothetical protein, conserved	0	1
PVX_101235	hypothetical protein, conserved	0	1
PVX_117550	hypothetical protein, conserved	0	1
PVX_003640	hypothetical protein, conserved	1	1
PVX_080250	hypothetical protein, conserved	1	1
PVX_082595	hypothetical protein, conserved	1	1
PVX_086195	hypothetical protein, conserved	1	1
PVX_122910	hypothetical protein, conserved	1	1
PVX_085645	hypothetical protein, conserved	0	2
PVX_095400	hypothetical protein, conserved	0	2
PVX_115460	hypothetical protein, conserved	0	2
PVX_123585	hypothetical protein, conserved	0	2
PVX_095290	hypothetical protein, conserved	1	2
PVX_119810	hypothetical protein, conserved	1	4
PVX_083160	hypothetical protein, conserved	0	5
PVX_090215	hypothetical protein, conserved	0	5
PVX_099280	hypothetical protein, conserved	0	5
PVX_100630	hypothetical protein, conserved	0	5
PVX_097905	hypothetical protein, conserved	0	7
PVX_123475	hypothetical protein, conserved	1	8
PVX_123990	hypothetical protein, conserved	0	9
PVX_085980	hypothetical protein, conserved	1	9
PVX_090240	hypothetical protein, conserved	1	0
PVX_090945	hypothetical protein, conserved	1	1
PVX_088935	inner membrane protein oxa1-2, putative	0	2
PVX_116630	lactate dehydrogenase	1	1
PVX_113465	long chain polyunsaturated fatty acid elongation enzyme, putative	0	7
PVX_099980	major blood-stage surface antigen Pv200	1	1
PVX_083240	male fertility protein pf47, putative	0	1
PVX_113775	membrane protein pf12 precursor, putative	1	1
PVX_091105	membrane-associated calcum-binding protein, putative	1	0
PVX_003770	merozoite surface protein 5	1	1

Predicted SP and TM of identified *P. vivax* proteins (* Signal peptide (SP) predicted by SignalP 3.0 and ** Transmembrane segments TM predicted by TMHMM 2.0) (cont.)

Accession Number	Names	SP*	TM**
PVX_082675	merozoite surface protein 7 (MSP7)	1	0
PVX_082665	merozoite surface protein 7 (MSP7), putative	1	1
PVX_097625	merozoite surface protein 8, putative	1	1
PVX_080100	multidrug resistance protein (mdr1)	0	11
PVX_118100	multidrug resistance protein 2, putative	0	9
PVX_099570	NADPH-cytochrome p450 reductase, putative	1	0
PVX_093680	Phist protein (Pf-fam-b)	0	1
PVX_112110	Phist protein (Pf-fam-b)	0	3
PVX_122605	phosphatidylinositol synthase, putative	1	3
PVX_099840	phosphatidylserine decarboxylase, putative	0	1
PVX_117005	phosphoesterase, putative	1	1
PVX_088960	protein disulfide isomerase, putative	1	0
PVX_083515	protein disulfide-isomerase, putative	1	1
PVX_095165	protein kinase, putative	0	1
PVX_083205	protein transport protein Sec61 alpha subunit (Pfsec61), putative	0	7
PVX_084625	P-type ATPase4, putative	0	8
PVX_101520	Pv-fam-d protein	0	2
PVX_089860	RAD protein (Pv-fam-e)	1	0
PVX_090325	reticulocyte binding protein 2 precursor (PvRBP-2), putative	0	1
PVX_087715	REVERSED hypothetical protein, conserved	1	0
PVX_085930	rhoptry-associated protein 1, putative	1	0
PVX_097590	rhoptry-associated protein 2, putative	1	0
PVX_000930	sexual stage antigen s16, putative	1	2
PVX_098665	signal peptidase, putative	1	1
PVX_117615	signal peptide peptidase domain containing protein	0	8
PVX_117890	sortilin, putative	0	2
PVX_092065	spermidine synthase, putative	0	1
PVX_097935	subtilisin-like protease precursor, putative	1	0
PVX_116910	sulfate transporter, putative	0	11
PVX_089505	suppressor of Ras1 3-9, putative	0	0
PVX_079800	T-cell immunomodulatory protein homolog precursor, putative	1	2
PVX_118580	translocation protein sec62, putative	0	3
PVX_122755	translocation protein SEC63, putative	0	3
PVX_000995	transmission-blocking target antigen Pfs230, putative	1	0
PVX_099160	transporter, putative	0	11
PVX_095405	transporter, putative	0	5
PVX_092105	transporter, putative	0	11
PVX_090275	tryptophan-rich antigen (Pv-fam-a)	1	0

Predicted SP and TM of identified *P. vivax* proteins (* Signal peptide (SP) predicted by SignalP 3.0 and ** Transmembrane segments TM predicted by TMHMM 2.0) (cont.)

Accession Number	Names	SP*	TM**
PVX_092995	tryptophan-rich antigen (Pv-fam-a)	1	0
PVX_096995	tryptophan-rich antigen (Pv-fam-a)	0	1
PVX_097577	tryptophan-rich antigen (Pv-fam-a)	0	1
PVX_101515	tryptophan-rich antigen (Pv-fam-a)	0	1
PVX_092990	tryptophan-rich antigen (Pv-fam-a)	1	1
PVX_112665	tryptophan-rich antigen (Pv-fam-a)	1	0
PVX_112670	tryptophan-rich antigen (Pv-fam-a)	1	0
PVX_090265	tryptophan-rich antigen (Pv-fam-a)	0	1
PVX_097575	tryptophan-rich antigen (Pv-fam-a)	0	1
PVX_088205	vacuolar proton translocating ATPase subunit A, putative	0	6
PVX_096980	variable surface protein Vir, putative	0	1
PVX_096985	variable surface protein Vir, putative	0	1
PVX_096970	variable surface protein Vir8-related	0	1
PVX_117625	V-type H(+)-translocating pyrophosphatase, putative	1	15
PVX_082655	merozoite surface protein 7 (MSP7), putative	1	1
PVX_122425	M1-family aminopeptidase, putative	1	1

APPENDIX D

Unique *P. vivax* proteins, defined by the absent of orthologs in other human malaria parasites, and their orthologs in *P. knowlesi*.

Gene ID	Description	*P. knowlesi* orthologs
PVX_003545	Hypothetical protein, conserved	PKH_131780
PVX_080355	Hypothetical protein	PKH_101490
PVX_083555	Hypothetical protein	PKH_120080
PVX_084640	Hypothetical protein	PKH_131800
PVX_086900	Hypothetical protein	PKH_010040 PKH_011770 PKH_021590 PKH_031980 PKH_060010 PKH_080030 PKH_081590 PKH_100010 PKH_100011 PKH_110030 PKH_112430
PVX_087070	Hypothetical protein	PKH_073090
PVX_087675	Hypothetical protein, conserved	None
PVX_089860	RAD protein (Pv-fam-e)	None
PVX_090250	Tryptophan-rich antigen (Pv-fam-a)	PKH_052760
PVX_090265	Tryptophan-rich antigen (Pv-fam-a)	PKH_052790
PVX_090275	Tryptophan -rich antigen (Pv-fam-a)	None
PVX_092990	Tryptophan -rich antigen (Pv-fam-a)	PKH_011760 PKH_020030 PKH_131790 PKH_134540
PVX_092995	Tryptophan -rich antigen (Pv-fam-a)	None
PVX_096970	Variable surface protein Vir8-related	None
PVX_096975	Hypothetical protein	PKH_020060

Unique *P. vivax* proteins, defined by the absent of orthologs in other human malaria parasites, and their orthologs in *P. knowlesi* (cont.).

Gene ID	Description	*P. knowlesi* orthologs
PVX_096980	Variable surface protein Vir, putative	None
PVX_096985	Variable surface protein Vir, putative	None
PVX_096995	Tryptophan-rich antigen (Pv-fam-a)	PKH_020080
PVX_097575	Tryptophan-rich antigen (Pv-fam-a)	None
PVX_097577	Tryptophan-rich antigen (Pv-fam-a)	PKH_103210
PVX_101515	Tryptophan-rich antigen (Pv-fam-a)	PKH_147000
PVX_101520	Pv-fam-d protein	None
PVX_107740	Hypothetical protein	None
PVX_111385	Hypothetical protein	PKH_061100
PVX_112110	Phist protein (Pf-fam-b)	PKH_100030
PVX_112665	Tryptophan-rich antigen (Pv-fam-a)	None
PVX_112670	Tryptophan-rich antigen (Pv-fam-a)	None
PVX_115155	Hypothetical protein	PKH_110680
PVX_115460	Hypothetical protein, conserved	None
PVX_117305	Hypothetical protein	PKH_124250
PVX_117430	Hypothetical protein	PKH_124550
PVX_118215	Hypothetical protein	None
PVX_118682	Erythrocyte membrane protein 3, putative	PKH_127040
PVX_121935	Hypothetical protein	PKH_140060
PVX_214290	Hypothetical protein	None
PVX_215290	Hypothetical protein	None

APPENDIX E

Functional classes of identified proteins based on MIPs catalogue

Accession Number	Description	Functional category
PVX_000890	hypothetical protein, conserved	hypothetical, conserved
PVX_000910	hypothetical protein, conserved	hypothetical, conserved
PVX_000930	sexual stage antigen s16, putative	unclassified
PVX_000995	transmission-blocking target antigen Pfs230, putative	unclassified
PVX_001780	hypothetical protein, conserved	hypothetical, conserved
PVX_001820	hypothetical protein	hypothetical
PVX_001905	ADP-ribosylation factor, putative	Signal transduction (cellular signaling)
PVX_001940	ATP-dependent RNA helicase, putative	Protein with binding function
PVX_002685	ATP synthase alpha chain, putative	metabolism (nucleotide)
PVX_002785	ATP-dependent acyl-CoA synthetase, putative	metabolism (nucleotide)
PVX_002790	hypothetical protein, conserved	hypothetical, conserved
PVX_002835	T-complex protein 1, theta subunit, putative	protein fate
PVX_002950	hypothetical protein	hypothetical
PVX_003545	hypothetical protein, conserved	hypothetical, conserved
PVX_003555	hypothetical protein, conserved	hypothetical, conserved
PVX_003640	hypothetical protein, conserved	hypothetical, conserved
PVX_003770	merozoite surface protein 5	unclassified
PVX_003895	hypothetical protein, conserved	hypothetical, conserved
PVX_079800	T-cell immunomodulatory protein homolog precursor, putative	unclassified
PVX_079955	hypothetical protein, conserved	hypothetical, conserved
PVX_080050	karyopherin beta, putative	unclassified
PVX_080100	multidrug resistance protein (mdr1)	Cellular transport
PVX_080245	40S ribosomal protein S9, putative	Protein synthesis
PVX_080250	hypothetical protein, conserved	hypothetical, conserved
PVX_080295	hypothetical protein, conserved	hypothetical, conserved
PVX_080355	hypothetical protein	hypothetical
PVX_080460	hypothetical protein, conserved	hypothetical, conserved
PVX_080555	hypothetical protein, conserved	hypothetical, conserved
PVX_080650	myo-inositol 1-phosphate synthase, putative	metabolism
PVX_081335	hypothetical protein, conserved	hypothetical, conserved
PVX_081430	small GTPase Rab5c, putative	Signal transduction (cellular signaling)
PVX_081455	calcium-transporting ATPase, putative	Cellular transport
PVX_081465	vacuolar ATP synthase subunit C, putative	unclassified
PVX_081550	hypothetical protein, conserved	hypothetical, conserved

Functional classes of identified proteins based on MIPs catalogue (cont.)

Accession Number	Description	Functional category
PVX_081770	hypothetical protein, conserved	hypothetical, conserved
PVX_082460	ER lumen protein retaining receptor 1, putative	unclassifeid
PVX_082595	hypothetical protein, conserved	hypothetical, conserved
PVX_082655	merozoite surface protein 7 (MSP7), putative	unclassified
PVX_082665	merozoite surface protein 7 (MSP7), putative	unclassified
PVX_082675	merozoite surface protein 7 (MSP7)	unclassified
PVX_082840	60S ribosomal protein L6, putative	Protein synthesis
PVX_082845	elongation factor 1-gamma, putative	Protein synthesis
PVX_082965	60S ribosomal protein L18a, putative	Protein synthesis
PVX_083030	myosin A, putative	Protein with binding function
PVX_083080	kelch domain-containing protein	Protein with binding function
PVX_083105	hypothetical protein, conserved	hypothetical, conserved
PVX_083135	aspartate carbamoyltransferase, putative	metabolism (amino acid)
PVX_083160	hypothetical protein, conserved	hypothetical, conserved
PVX_083180	hypothetical protein, conserved	hypothetical, conserved
PVX_083205	protein transport protein Sec61 alpha subunit (Pfsec61), putative	Protein transport
PVX_083220	hypothetical protein, conserved	hypothetical, conserved
PVX_083240	male fertility protein pf47, putative	unclassified
PVX_083270	hypothetical protein, conserved	hypothetical, conserved
PVX_083480	hypothetical protein, conserved	hypothetical, conserved
PVX_083515	protein disulfide-isomerase, putative	Protein fate
PVX_083555	hypothetical protein	hypothetical
PVX_083560	hypothetical protein, conserved	hypothetical, conserved
PVX_084230	nucleosome assembly protein 1, putative	Subcellular localization
PVX_084285	UGA suppressor tRNA-associated antigenic protein, putat	unclassified
PVX_084625	P-type ATPase4, putative	Protein with binding function
PVX_084640	hypothetical protein	hypothetical
PVX_084720	hypothetical protein, conserved	hypothetical, conserved
PVX_084795	mitochondrial glycoprotein domain containing protein	Subcellular localization
PVX_084865	hypothetical protein, conserved	hypothetical, conserved
PVX_084940	hypothetical protein, conserved	hypothetical, conserved
PVX_084960	ATP-specific succinyl-CoA synthetase beta subunit, puta	metabolism
PVX_084985	hypothetical protein, conserved	hypothetical, conserved
PVX_085070	hypothetical protein, conserved	hypothetical, conserved
PVX_085310	hypothetical protein, conserved	hypothetical, conserved
PVX_085570	hypothetical protein, conserved	hypothetical, conserved
PVX_085645	hypothetical protein, conserved	hypothetical, conserved
PVX_085735	60S ribosomal protein L10, putative	Protein synthesis
PVX_085765	hypothetical protein, conserved	hypothetical, conserved
PVX_085915	hypothetical protein, conserved	hypothetical, conserved
PVX_085930	rhoptry-associated protein 1, putative	unclassified
PVX_085980	hypothetical protein, conserved	hypothetical, conserved
PVX_086015	hypothetical protein, conserved	hypothetical, conserved

Functional classes of identified proteins based on MIPs catalogue (cont.)

Accession Number	Description	Functional category
PVX_086040	aspartic protease PM4, putative	metabolism
PVX_086075	fibrillarin, putative	Transcription
PVX_086090	hypothetical protein, conserved	hypothetical, conserved
PVX_086195	hypothetical protein, conserved	hypothetical, conserved
PVX_086245	hypothetical protein, conserved	hypothetical, conserved
PVX_086900	hypothetical protein	hypothetical
PVX_086980	hypothetical protein, conserved	hypothetical, conserved
PVX_087070	hypothetical protein, conserved	hypothetical, conserved
PVX_087095	hypothetical protein, conserved	hypothetical, conserved
PVX_087675	hypothetical protein, conserved	hypothetical, conserved
PVX_087715	REVERSED hypothetical protein, conserved	hypothetical, conserved
PVX_087725	hypothetical protein, conserved	hypothetical, conserved
PVX_087950	heat shock protein 86, putative	Protein with binding function
PVX_087955	O1, putative	Cell rescue, defense and virulence
PVX_087980	chloroquine resistance transporter, putative	Cellular transport
PVX_088035	hypothetical protein, conserved	hypothetical, conserved
PVX_088205	vacuolar proton translocating ATPase subunit A, putative	metabolism
PVX_088250	AAA family ATPase, putative	Protein with binding function
PVX_088910	hypothetical protein, conserved	hypothetical, conserved
PVX_088935	inner membrane protein oxa1-2, putative	Protein fate
PVX_088960	protein disulfide isomerase, putative	Protein fate
PVX_089020	hypothetical protein, conserved	hypothetical, conserved
PVX_089170	hypothetical protein, conserved	hypothetical, conserved
PVX_089220	hypothetical protein, conserved	hypothetical, conserved
PVX_089425	heat shock 70 kDa protein, putative	Protein with binding function
PVX_089485	hypothetical protein, conserved	hypothetical, conserved
PVX_089505	suppressor of Ras1 3-9, putative	unclassified
PVX_089720	Protein kinase domain containing protein	Portein fate
PVX_089750	60S ribosomal protein L15-1, putative	Protein synthesis
PVX_089860	RAD protein (Pv-fam-e)	unclassified
PVX_090110	hypothetical protein, conserved	hypothetical, conserved
PVX_090160	40S ribosomal protein S19s, putative	Protein synthesis
PVX_090215	hypothetical protein, conserved	hypothetical, conserved
PVX_090240	hypothetical protein, conserved	hypothetical, conserved
PVX_090250	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_090265	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_090275	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_090325	reticulocyte binding protein 2 precursor (PvRBP-2), putative	Protein with binding function
PVX_090900	hypothetical protein, conserved	hypothetical, conserved
PVX_090930	histone H4, putative	Protein with binding function
PVX_090935	histone 2B	Protein with binding function
PVX_090945	hypothetical protein, conserved	hypothetical, conserved
PVX_090950	40S ribosomal protein S4, putative	Protein synthesis

Functional classes of identified proteins based on MIPs catalogue (cont.)

Accession Number	Description	Functional category
PVX_090960	hypothetical protein, conserved	hypothetical, conserved
PVX_091055	hypothetical protein, conserved	hypothetical, conserved
PVX_091105	membrane-associated calcum-binding protein, putative	Protein with binding function
PVX_091110	DnaJ domain containing protein	Protein fate
PVX_091210	hypothetical protein, conserved	hypothetical, conserved
PVX_091300	hypothetical protein, conserved	hypothetical, conserved
PVX_091470	heat shock protein 101, putative	Protein with binding function
PVX_091495	hypothetical protein, conserved	hypothetical, conserved
PVX_091515	GTP-binding nuclear protein Ran, putative	Signal transduction
PVX_091585	hypothetical protein, conserved	hypothetical, conserved
PVX_091662	hypothetical protein, conserved	hypothetical, conserved
PVX_091700	circumsporozoite-protein related antigen, putative	unclassified
PVX_091990	hypothetical protein, conserved	hypothetical, conserved
PVX_092045	hypothetical protein, conserved	hypothetical, conserved
PVX_092065	spermidine synthase, putative	Metabolism
PVX_092070	hypothetical protein, conserved	hypothetical, conserved
PVX_092095	hypothetical protein, conserved	hypothetical, conserved
PVX_092105	transporter, putative	Cellular transport
PVX_092245	aquaglyceroporin, putative	Cellular transport
PVX_092275	apical merozoite antigen 1	Subcellular localization
PVX_092310	heat shock protein hsp70 homologue, putative	Protein fate
PVX_092410	3-oxo-5-alpha-steroid 4-dehydrogenase domain containing	Metabolism
PVX_092535	Adenylate and Guanylate cyclase catalytic domain contai	Protein with binding function
PVX_092620	myosin heavy chain subunit, putative	Protein with binding function
PVX_092765	hypothetical protein, conserved	hypothetical, conserved
PVX_092835	hypothetical protein, conserved	hypothetical, conserved
PVX_092850	small GTPase Rab6, putative	Signal transduction
PVX_092990	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_092995	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_093615	phosphoinositide phosphatase SAC1, putative	unclassified
PVX_093680	Phist protein (Pf-fam-b)	unclassified
PVX_094345	hypothetical protein, conserved	hypothetical, conserved
PVX_094380	hypothetical protein	hypothetical
PVX_094505	hypothetical protein, conserved	hypothetical, conserved
PVX_094530	hypothetical protein	hypothetical
PVX_094535	RNA binding protein, putative	Protein with binding function
PVX_094595	hypothetical protein, conserved	hypothetical, conserved
PVX_094755	hypothetical protein, conserved	hypothetical, conserved
PVX_094840	hypoxanthine phosphoribosyltransferase, putative	Metabolism
PVX_094865	hypothetical protein, conserved	hypothetical, conserved
PVX_095000	heat shock protein 60, putative	Protein with binding function
PVX_095015	enolase, putative	Energy
PVX_095095	hypothetical protein, conserved	hypothetical, conserved

Functional classes of identified proteins based on MIPs catalogue (cont.)

Accession Number	Description	Functional category
PVX_095165	protein kinase, putative	Protein fate
PVX_095195	ATP-dependent RNA helicase, putative	Protein with binding function
PVX_095200	hypothetical protein, conserved	hypothetical
PVX_095220	T-complex protein 1, epsilon subunit, putative	Protein fate
PVX_095290	hypothetical protein, conserved	hypothetical, conserved
PVX_095400	hypothetical protein, conserved	hypothetical, conserved
PVX_095405	transporter, putative	Cellular transport
PVX_096070	early transcribed membrane protein (ETRAMP)	unclassified
PVX_096245	hypothetical protein, conserved	hypothetical, conserved
PVX_096295	hypothetical protein, conserved	hypothetical, conserved
PVX_096335	40S ribosomal protein S10, putative	Protein synthesis
PVX_096340	60S ribosomal protein L11, putative	Protein synthesis
PVX_096970	variable surface protein Vir8-related	unclassified
PVX_096975	hypothetical protein	hypothetical
PVX_096980	variable surface protein Vir, putative	unclassified
PVX_096985	variable surface protein Vir, putative	unclassified
PVX_096995	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_097575	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_097577	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_097590	rhoptry-associated protein 2, putative	Protein with binding function
PVX_097625	merozoite surface protein 8, putative	unclassified
PVX_097905	hypothetical protein, conserved	hypothetical
PVX_097935	subtilisin-like protease precursor, putative	Portein fate
PVX_097950	hypothetical protein, conserved	hypothetical
PVX_098015	soluble NSF attachment protein (SNAP), putative	Subcellular localization
PVX_098605	small GTPase Rab7, putative	Signal transduction
PVX_098665	signal peptidase, putative	unclassified
PVX_098675	ubiquitin carboxyl-terminal hydrolase, putative	unclassified
PVX_098985	DNA primase, large subunit, putative	unclassified
PVX_099035	hypothetical protein, conserved	hypothetical, conserved
PVX_099055	hypothetical protein, conserved	hypothetical, conserved
PVX_099060	hypothetical protein, conserved	hypothetical, conserved
PVX_099160	transporter, putative	Cellular transport
PVX_099280	hypothetical protein, conserved	hypothetical, conserved
PVX_099315	78 kDa glucose-regulated protein precursor (GRP 78), putative	Protein with binding function
PVX_099320	acid phosphatase, putative	unclassified
PVX_099370	DNAJ-like molecular chaperone protein, putative	Protien fate
PVX_099535	phosphoglycerate kinase, putative	Energy metabolism
PVX_099570	NADPH-cytochrome p450 reductase, putative	Protein with binding function
PVX_099580	hypothetical protein, conserved	hypothetical, conserved
PVX_099710	hypothetical protein, conserved	hypothetical, conserved
PVX_099840	phosphatidylserine decarboxylase, putative	Metabolism
PVX_099930	high molecular weight rhoptry protein-2, putative	unclassified

Functional classes of identified proteins based on MIPs catalogue (cont.)

Accession Number	Description	Functional category
PVX_099980	major blood-stage surface antigen Pv200	unclassified
PVX_100550	chaperonin CPN60, mitochondrial precursor, putative	Protein with binding function
PVX_100630	hypothetical protein, conserved	hypothetical, conserved
PVX_100735	ATP synthase beta chain, mitochondrial precursor, putative	Protein with binding function
PVX_100835	hypothetical protein, conserved	hypothetical, conserved
PVX_100860	hypothetical protein, conserved	hypothetical, conserved
PVX_101095	hypothetical protein, conserved	hypothetical, conserved
PVX_101140	hypothetical protein, conserved	hypothetical, conserved
PVX_101170	hypothetical protein, conserved	hypothetical, conserved
PVX_101200	actin	Protein with binding function
PVX_101235	hypothetical protein, conserved	hypothetical, conserved
PVX_101485	hypothetical protein, conserved	hypothetical, conserved
PVX_101515	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_101520	Pv-fam-d protein	unclassified
PVX_107740	hypothetical protein	hypothetical
PVX_110895	ADP/ATP transporter on adenylate translocase, putative	Cellular transport
PVX_111035	aspartyl protease, putative	unclassified
PVX_111165	hypothetical protein, conserved	hypothetical, conserved
PVX_111245	adenosine deaminase, putative	unclassified
PVX_111385	hypothetical protein	hypothetical
PVX_111435	hypothetical protein, conserved	hypothetical, conserved
PVX_111580	phospholipid scramblase 1, putative	unclassified
PVX_112110	Phist protein (Pf-fam-b)	unclassified
PVX_112665	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_112670	tryptophan-rich antigen (Pv-fam-a)	unclassified
PVX_113390	hypothetical protein, conserved	hypothetical, conserved
PVX_113465	long chain polyunsaturated fatty acid elongation enzyme, putative	unclassified
PVX_113620	hypothetical protein, conserved	hypothetical, conserved
PVX_113775	membrane protein pf12 precursor, putative	unclassified
PVX_113830	myosin-like protein, putative	Protein with binding function
PVX_114015	histone H2A, putative	Protein with binding function
PVX_114020	histone H3, putative	Protein with binding function
PVX_114090	hypothetical protein, conserved	hypothetical, conserved
PVX_114440	hypothetical protein, conserved	hypothetical, conserved
PVX_114445	pyruvate kinase, putative	Energy metabolism
PVX_114560	DNAJ domain protein, putative	Protein fate
PVX_114670	ER lumen protein retaining receptor, putative	unclassified
PVX_114830	elongation factor 1 alpha putative	Protein synthesis
PVX_114832	elongation factor 1 alpha putative	Protein synthesis
PVX_114880	hypothetical protein, conserved	hypothetical, conserved
PVX_115000	falcilysin, putative	Protein with binding function
PVX_115085	hypothetical protein, conserved	hypothetical, conserved
PVX_115155	hypothetical protein	hypothetical

Functional classes of identified proteins based on MIPs catalogue (cont.)

Accession Number	Description	Functional category
PVX_115345	alanyl-tRNA synthetase, putative	Protein synthesis
PVX_115450	hypothetical protein, conserved	hypothetical, conserved
PVX_115460	hypothetical protein, conserved	hypothetical, conserved
PVX_116515	hypothetical protein, conserved	hypothetical, conserved
PVX_116630	lactate dehydrogenase	Energy metabolism
PVX_116715	60S ribosomal protein L9, putative	Protein synthesis
PVX_116785	splicing factor, putative	Transcription
PVX_116910	sulfate transporter, putative	Cellular transport
PVX_116915	exported protein 2, putative	unclassified
PVX_117005	phosphoesterase, putative	unclassified
PVX_117030	RNA helicase-1, putative	Protein with binding function
PVX_117060	hypothetical protein, conserved	hypothetical, conserved
PVX_117305	hypothetical protein	hypothetical
PVX_117430	hypothetical protein	hypothetical
PVX_117490	hypothetical protein, conserved	hypothetical, conserved
PVX_117550	hypothetical protein, conserved	hypothetical, conserved
PVX_117615	signal peptide peptidase domain containing protein	unclassified
PVX_117625	V-type H(+)-translocating pyrophosphatase, putative	unclassified
PVX_117890	sortilin, putative	unclassified
PVX_117925	elongation factor 2, putative	Protein with binding function
PVX_118100	multidrug resistance protein 2, putative	Cellular transport
PVX_118162	hypothetical protein	hypothetical
PVX_118215	hypothetical protein	hypothetical
PVX_118220	serine/threonine kinase-1, putative	Protein with binding function
PVX_118255	fructose 1,6-bisphosphate aldolase, putative	Energy metabolism
PVX_118380	hypothetical protein, conserved	hypothetical, conserved
PVX_118470	hypothetical protein, conserved	hypothetical, conserved
PVX_118495	triosephosphate isomerase, putative	metabolism
PVX_118545	2-Cys peroxiredoxin, putative	Cell rescue, defense and virulence
PVX_118580	translocation protein sec62, putative	Protein fate
PVX_118610	hypothetical protein, conserved	hypothetical, conserved
PVX_118620	proteosome subunit alpha type 1, putative	unclassified
PVX_118682	erythrocyte membrane protein 3, putative	unclassified
PVX_119390	hypothetical protein, conserved	hypothetical, conserved
PVX_119515	REVERSED hypothetical protein, conserved	hypothetical, conserved
PVX_119615	hypothetical protein, conserved	hypothetical, conserved
PVX_119810	hypothetical protein, conserved	hypothetical, conserved
PVX_121885	cytoadherence linked asexual protein, CLAG, putative	Protein with binding function
PVX_121935	hypothetical protein	hypothetical
PVX_122090	hypothetical protein, conserved	hypothetical, conserved
PVX_122425	M1-family aminopeptidase putative	Subcellular localization
PVX_122530	hypothetical protein, conserved	hypothetical, conserved
PVX_122545	Cop-coated vesicle membrane protein p24 precursor, putative	Cellular transport

Functional classes of identified proteins based on MIPs catalogue (cont.)

Accession Number	Description	Functional category
PVX_122605	phosphatidylinositol synthase, putative	metabolism
PVX_122640	hypothetical protein, conserved	hypothetical, conserved
PVX_122740	structural maintenance of chromosome 2, putative	biogenesis of cellular components
PVX_122755	translocation protein SEC63, putative	Protein fate
PVX_122810	hypothetical protein, conserved	hypothetical, conserved
PVX_122910	hypothetical protein, conserved	hypothetical, conserved
PVX_123200	hypothetical protein, conserved	hypothetical, conserved
PVX_123340	eukaryotic translation initiation factor 3 subunit 10,	unclassified
PVX_123395	hypothetical protein, conserved	hypothetical, conserved
PVX_123435	thioredoxin peroxidase2, putative	unclassified
PVX_123475	hypothetical protein, conserved	hypothetical, conserved
PVX_123585	hypothetical protein, conserved	hypothetical, conserved
PVX_123725	hypothetical protein, conserved	hypothetical, conserved
PVX_123745	endoplasmin precursor, putative	Protein fate
PVX_123990	hypothetical protein, conserved	hypothetical, conserved
PVX_124095	macrophage migration inhibitory factor, putative	unclassified
PVX_124100	T-complex protein 1, gamma subunit, putative	Protein fate
PVX_124160	heat shock protein hslv, putative	Protein fate
PVX_124195	small GTPase Rab2, putative	signal transduction
PVX_193290	hypothetical protein	hypothetical
PVX_214290	hypothetical protein	hypothetical
PVX_215290	hypothetical protein	hypothetical
PVX_250300	ADP/ATP transporter on adenylate translocase, putative	Cellular transport
PVX_251300	hypothetical protein	hypothetical

APPENDIX F
CULTURE MEDIA AND REAGENTS

1. Culture media

1.1 Media for *Plasmodium spp.*

1.1.1 RPMI 1640 incomplete medium 1L

RPMI 1640 powder medium	10.42	g
HEPES (N-2-Hydroxyethylpiperazine-n-2-ethaesulphonic acid)		
$NaHCO_3$	2.00	g
Gentamycin	40.00	mg

Stock medium was made by dissolving RPMI-1640 powder, HEPES, $NaHCO_3$, and gentamicin in DW. The volume was adjusted to 1 L with DW and then mixed the solution with a magnetic stirrer. The medium was sterilized by passing through a 0.22μm Millipore filter (Nalgene®, Nalge Company, and USA) and kept at 4°C. For 10X RPMI 1640 medium, adjusted the volume to 100 ml with DW.

1.1.2 McCoy's 5A incomplete medium 1L

McCoy's 5A powder medium	12.0	g
HEPES (N-2-Hydroxyethylpiperazine-n-2-ethaesulphonic acid)		
$NaHCO_3$	2.00	g
D-glucose	1.80	g
Gentamycin	40.00	mg

Stock medium was made by dissolving McCoy's 5A powder, HEPES, $NaHCO_3$, D-glucose and gentamicin in DW. The volume was adjusted to 1 L with DW and then mixed the solution with a magnetic stirrer. The medium was sterilized by passing through a 0.22μm Millipore filter (Nalgene®, Nalge Company, and USA) and kept at 4°C.

1.1.3 McCoy's 5A complete medium 1L

McCoy's 5A incomplete medium	750	ml
Heat inactivated human AB serum	250	ml

Heat-inactivated serum was thawed and added to 750 ml of McCoy's 5A medium and stored at 4°C.

1.2 Bacterial media

1.2.1 LB medium 1L

To 950 ml of deionized H_2O, add:

Tryptone	10	g
Yeast extract	5	g
NaCl	10	g

All solutes were dissolved in deionized H_2O and adjusted the pH to 7.0 with 5N NaOH. The volume of the solution was adjusted to 1 liter with deionized H_2O. The medium was sterilizeed by autoclaving for 20 minutes at 15 psi on liquid cycle. For LB agar, added Bacto agar 15 g/l before autoclaving.

2 Solutions and reagents

2.1 60% Percoll® 100 ml

Percoll®	60	ml
10X RPMI 1640	7	ml
RPMI 1640 incomplete medium	33	ml

The solution was made by mixed 60 ml of Percoll® with 33 ml of RPMI 1640 and 7 ml of 10X RPMI 1640.

2.2 Phosphate-buffered Saline (PBS), pH 7.4 1L

NaCl	8	g
KH_2PO_4	0.24	g
Na_2HPO_4	1.44	g
KCl	0.2	g
Distilled H_2O	800	ml

All solutes were dissolved in 800 ml of deionized H_2O and mixed with a magnetic stirrer. The pH was adjusted to 7.4 with HCL. The total volume of the solution was adjusted to 1 liter with deionized H_2O and sterilized by autoclaving for 20 minutes at 15 psi on liquid cycle.

2.3 10% (w/v) Saponin 10 ml

Saponin	1	g

1X PBS (pH 7.4)	10	ml

Saponin powder was dissolved in PBS and mixed by a magnetic stirrer. The solution was sterilized by passing through a 0.22μm Millipore filter (Nalgene®, Nalge Company, and USA) and keep at 4°C.

2.4 Washing solution for ELISA (PBS-T) 1L

1X PBS	1.0	L
Tween-20	0.5	ml

Tween 20 (0.5 ml) was added to 1 L of PBS and mixed well with a magnetic stirrer**.** The solution was kept at 4^{o}C.

2.5 Blocking buffer for ELISA 500 ml

1X PBS	500	ml
BSA	2.5	g

BSA was dissolved in 500 ml of PBS and mixed well with a magnetic stirrer**.** The solution was kept at 4^{o}C.

2.6 12% Acrylamide gel 10 ml

H_2O	3.3	ml
30% acrylamide mix	4.0	ml
1.5 M Tris (pH 8.8)	2.5	ml
10% SDS	0.1	ml
10% ammonium persulfate	0.1	ml
TEMED	0.004	ml

All solutions were mixed together. TEMED was added in the last step and immediately poured the gel.

2.7 5% stacking gel 2ml

H2O	1.4	ml
30% acrylamide mix	0.33	ml
1.0 M Tris (pH 6.8)	0.25	ml
10% SDS	0.02	ml
10% ammonium persulfate	0.02	ml
TEMED	0.002	ml

All solutions were mixed together. TEMED was added in the last step and immediately poured the gel.

2.8 2X SDS-PAGE sample buffer

100 mM Tris-Cl pH6.8

200 mM dithiothreitol

4% (w/v) SDS

0.2% (w/v) bromphenol blue

20% (v/v) glycerol

Stored 2X gel-loading buffer lacking dithiothreitol at room temperature. Added dithiothretol from a 1 M stock just before the buffer was used.

2.9 1X Tris-glycine electrophoresis buffer pH 8.3

25 mM Tris

250 mM glycine (electrophoresis grade) pH8.3

0.1% (w/v) SDS

A 5X stock of electrophoresis buffer was prepared by dissolving 15.1 g of Tris-base and 94 g of glycine in 900 ml of deionized H_2O. Then added 50 ml of a 10% (w/v) stock solution of electrophoresis-grade SDS and adjusted volume to 1000 ml with H_2O.

2.10 Transfer buffer

24 mM Tris-base	3	g
192 mM glycine	14.4	g
20% methanol	200	ml

Tris-base and glycine were dissolved in 800 ml distilled H_2O and mixed well with a magnetic stirrer. Methanol was added to the solution and kept at 4 ℃.

2.11 Washing buffer 1L

10 mM Tris (pH7.2)

0.15 M NaCl

0.05% (v/v) Tween-20

Tris-base and NaCl were dissolved in distilled H_2O. Tween-20 was added and mixed with a magnetic stirrer. The solution was stored at 4 ℃.

2.12 Blocking buffer

10 mM Tris (pH7.2)

0.15 M NaCl

5% (w/v) skimmed milk

Tris-base and NaCl were dissolved in distilled H_2O. Skimmed milk was added and mixed with a magnetic stirrer. The solution was stored at 4 °C.

2.13 Substrate for ELISA and Western blot

2.13.1 Horseradish peroxidase staining solutions

4-chloro-1-napthol	18	mg
Methanol	6	ml
1 M Tris-HCl (pH 7.2)	24	ml
30% H_2O_2	60	µl

4-chloro-1-naphthol was dissolved in 6 ml methanol before adding 1 M Tris-HCl (pH 7.2) and 30% hydrogen peroxide (H_2O_2).

2.13.2 Alkaline phosphatase (AP) staining solutions

2.13.2.1 Development buffer

100 mM Tris-Cl, pH 9.5

100 mM NaCl

5 mM $MgCl_2$

2.13.2.2 5%NBT stock solution

5% NBT (nitro blue tetrazolium chloride)

70% Dimethylformamide.

Nitro blue tetrazolium chloride was dissolves in 70% dimethylformamide. The solution was stored at 4°C.

2.13.2.3 5% BCIP stock solution

BCIP (5-bromo-4-chloro-3-indolyl phosphate) was dissolved in 100% dimethylformamide. The solution was stored at -20°C.

2.13.2.4 Staining solution

NBT stock solution	66	µl
BCIP stock solution	33	µl
Development buffer	10	ml

Prepared immediately just before used.

2.14 Reagents for 2D gel

2.14.2 Lysis solution (for 2D gel)

8 M Urea

4% CHAPS

2% Phamalyte 3-10

2.14.3 Rehydration solution

8 M Urea

2% CHAPS

0.002% bromophenol blue

1.5% IPG buffer

0.2% (w/v) DTT

2.14.4 SDS equilibration buffer

1M Tris-HCl (pH8.8)

6M Urea

30% glycerol

2% SDS

0.002% bromophenol blue

2.14.5 Agarose sealing solution

0.5% agarose

0.002% bromophenol blue

2.15 Gel staining solutions

2.15.1 Colloidal coomassie® stain (invitrogen) for 1 gel

Deionized water		55	ml
Methanol (reagent grade)		20	ml
Stainer A		20	ml
Stainer B	(shake well before use)	5	ml

Stainer A and stainer B were added to deionized water and mixed well. Methanol was added just prior to use.

2.15.2 Silver stain for 1 gel

2.15.2.1 Fixing solution

Ethanol	100	ml
Acetic acid, glacial	25	ml

Acetic acid was added to deionized water and then ethanol was added. The total volume was adjusted to 250 ml with distilled water

2.15.2.2 Sensitizing solution

Ethanol	75	ml

Sodium thiosulfate (5% w/v)	10	ml
Sodium acetate	17	g

Sodium thiosulfate and sodium acetate were dissolved in deionized water. Ethanol was added to the solution and the final volume was adjusted to to 250 ml with deionized water

2.15.2.3 Silver reaction solution

Silver nitrate solution (2.5% w/v)	25ml

The solution was made by adding 25 ml of 2.5% silver nitrate solution to 225 ml of deionized water.

2.15.2.4 Developing solution

Sodium carbonate	6.25	g
Formaldehyde (37%)	200	µl

Sodium carbonate was dissolved in 250 ml of deionized water. Formaldehyde solution was added and stirred vigorously to dissolve sodium carbonate.

2.15.2.5 Stopping solution

EDTA-Na_2 x H_2O	3.65	g

EDTA-Na_2 x H_2O was dissolved in 250 ml with distilled water.

2.16 Solutions for gene cloning

2.16.1 Ampicillin stock solution (1 mg/ml)

Ampicillin was dissolved distilled H_2O. The solution was sterilized by passing through a 0.22 um dispossible filtefilter. The solution was dispensed into 1 ml aliquots and stored at -20°C.

2.16.2 IPTG (isopropylthio-β-D-galactoside) (100 mM)

IPTG was dissolved distilled H_2O. The solution was sterilized by passing through a 0.22 um dispossible filter. The solution was dispensed into 1 ml aliquots and stored at -20°C.

2.16.3 X-gal (5-bromo-4-chloro-3-indolyl-β-Dgalactoside) solution

A stock solution was made by dissolving X-gel in dimethylformamide at a concentration 20 mg/ml solution in a glass or polypropylene

tube. The tube containing solution was wrapping in aluminum foil to prevent damage by light and stored at -20°C. It is not necessary to sterilize X-gal solutions by filtration.

2.16.4 6X Gel-loading buffer

0.25% (w/v) bromophenol blue

40% (w/v) sucrose in H_2O

2.16.5 50X Tris-acetate-EDTA (TAE) electrophoresis buffer 1 L

Tris base	242	g
Glacial acetic acid	57.1	ml
0.5 M EDTA (pH8.0)	100	ml

Tris base was dissolve in 800 ml of distilled H_2O and mixed with a magnetic stirrer. Glacial acetic acid and 0.5 M EDTA were added to the solution. The total volume was adjusted to 1 L with distilled H_2O.

2.17 Solution for protein expression and purification

2.17.1 1 M IPTG (isopropylthio-β-D-galactoside) stock solution

IPTG was dissolved in 10 ml of distilled H_2O and sterilized by passing it through a 0.22 um disposable filter. The solution was dispensed into 1 ml aliquots and stored at -20°C.

2.17.2 Kanamycin stock solution (1 mg/ml)

Kanamycin was dissolved in 10 ml of distilled H_2O and sterilized by passing it through a 0.22 um disposable filter. The solution was dispensed into 1 ml aliquots and stored at -20°C.

2.17.3 Lysis buffer

100 mM NaH_2PO_4

10 mM Tris-Cl

8 M Urea

All solutes were dissolved in distilled H_2O and mixed by a magnetic stirrer. The pH of solution was adjusted to 8.0 using HCl and stored at room temperature.

2.17.4 Wash buffer

100 mM NaH_2PO_4
10 mM Tris-Cl
8 M Urea

All solutes were dissolved in distilled H_2O and mixed by a magnetic stirrer. The pH of solution was adjusted to 6.3 using HCl and stored at room temperature.

2.17.5 Elution buffer

100 mM NaH_2PO_4
10 mM Tris-Cl
8 M Urea

All solutes were dissolved in distilled H_2O and mixed by a magnetic stirrer. The pH of solution was adjusted to 4.5 using HCl and stored at room temperature.

2.18 Solutions for In-gel trypsin digestion

2.18.1 25 mM NH4HCO3 100 ml

NH_4HCO_3	0.1977	g
diH_2O	100	ml

NH_4HCO_3 was dissolved in deionized H_2O. The pH of solution was adjusted to 8.0 and filtered through 0.22 um disposable filter.

2.18.2 100 mM Iodoacetamide 10 ml

Iodoacetamide	0.185	g
25 mM NH_4HCO_3	10	ml

Iodoacetamide was dissolved in 10 ml 25 mM NH_4HCO_3 and stored at -20°C.

2.18.3 45 mM DTT 10 ml

DTT	0.0694	g
25 mM NH_4HCO_3	10	ml

DTT was dissolved in 10 ml of 25 mM NH_4HCO_3 and stored at -20 °C.

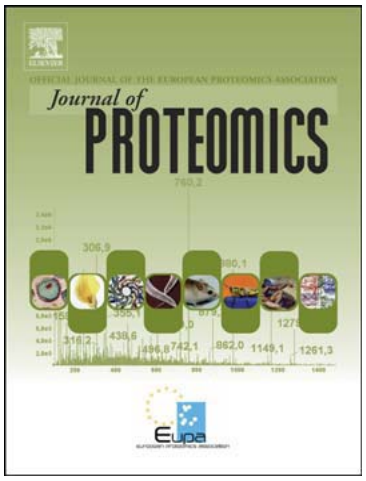
2.18.4 50% acetonitrile (ACN) 50 ml

Pure acetonitrile	25	ml
Deionized H2O	25	ml

2.18.5 5% formic acid in 50% ACN 10 ml

Formic acid	0.5	ml
50% ACN	9.5	ml

APPENDIX G
PUBLICATION



Accepted Manuscript

Determination of the *Plasmodium vivax* schizont stage proteome

Wanlapa Roobsoong, Sittiruk Roytrakul, Jetsumon Sattabongkot, Jianyong, Li, Rachanee Udomsangpetch, Liwang Cui

PII: S1874-3919(11)00142-4
DOI: doi: 10.1016/j.jprot.2011.03.035
Reference: JPROT 497
To appear in: *Journal of Proteomics*
Received date: 22 December 2010
Revised date: 29 March 2011
Accepted date: 30 March 2011

Determination of the *Plasmodium vivax* schizont stage proteome

Wanlapa Roobsoong 1, Sittiruk Roytrakul 2, Jetsumon Sattabongkot 3, Jianyong Li 4, RachaneeUdomsangpetch 5,6* and Liwang Cui 7*

1 Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand,

2 National Center for Genetic Engineering and Biotechnology, Pathumthani 12120, Thailand,

3 Department of Entomology, AFRIMS, Bangkok 10400, Thailand,

4 Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061, USA

5Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok 10400,

Thailand,

6Center for Vectors and Vector-Borne Diseases, SC2 Building, Mahidol University, Nakhon

Pathom 73170, Thailand

7 Department of Entomology, The Pennsylvania State University, 501 ASI Bldg., University

Park, PA 16801 USA

*Corresponding authors:

Liwang Cui

Email: luc2@psu.edu

Tel: 1-8148637663; Fax: 1-8148653048

Rachanee Udomsangpetch

E-mail: scrud@mahidol.ac.th

Tel: +66 220155766; Fax +66 23547158

Abstract

With the genome of the malaria parasite *Plasmodium vivax* sequenced, it is important to determine the proteomes of the parasite in order to assist efforts in antigen and drug target discovery. Since a method for continuous culture of *P. vivax* parasite is not available, we tried to study the proteome of the erythrocytic stages using fresh parasite isolates from patients. In schizont-enriched samples, 316 proteins were confidently identified by tandem mass spectrometry. Almost 50% of the identified proteins were hypothetical, while other major categories include proteins with binding function, protein fate, protein synthesis, metabolism and cellular transport. To identify proteins that are recognized by host humoral immunity, parasite proteins were separated by two-dimensional gel electrophoresis and screened by Western blot using an immune serum from a *P. vivax* patient. Mass spectrometry analysis of protein spots recognized by the serum identified four potential antigens including PV24. The recombinant protein PV24 was recognized by antibodies from vivax malaria patients even during the convalescent period, indicating that PV24 could elicit long-lasting antibody responses in *P. vivax* patients.

Key words: Antigen, Erythrocytic stage, Mass spectrometry, *Plasmodium vivax*, Proteome

1. Introduction

Plasmodium vivax is the most widespread human malaria parasite, which causes significant morbidity and socio-economic problems in endemic countries [1]. It has several unique characteristics that distinguish it from other human malaria parasite species. Most notably, *P. vivax* forms hypnozoites in hepatocytes, causing relapses of the disease [2]. *P. vivax* strains from the tropical and temperate zones can vary dramatically in terms of the pattern and frequency of the relapse. Moreover, *P. vivax* requires Duffy receptor on the red cell for invasion, and is thus absent in West Africa where Duffy negativity predominates [3]. It selectively invades reticulocytes [4], thus limiting parasitemias to low levels. Unlike *Plasmodium falciparum* infection that increases the rigidity of the host cell, *P. vivax* increases the size and deformability of infected red cells [5]. *P. vivax* also actively remodels the host cell, producing caveola-vesicle complexes along the plasmalemma in the infected erythrocyte cell, which are visible in Giemsa-stained smears as multiple red spots called "Schüffner's dots". Apart from these characteristics, the ability of *P. vivax* to survive at much lower temperature has allowed this parasite to establish transmission foci in temperate zones. Despite that many of these unique features of *P. vivax* have been known for a long time, the underlying mechanisms remain poorly understood. Therefore, a better understanding of the fundamental biology of *P. vivax* is needed to effectively control and eventually eradicate this parasite.

The task to eliminate malaria globally requires integrated control measures, one of which is development of vaccines against malaria parasites. Several leading candidate vaccines from *P. falciparum* have been tested in clinical trials but do not offer protection against other *Plasmodium* species [6]. The deployment of such vaccines against *P. falciparum* may cause an unexpected outcome in malaria epidemiology in areas of *P. vivax* and *P. falciparum* coexistence, and therefore, multi-subunit and multi-species vaccines are needed in such endemic areas. Whereas the *P. falciparum* vaccine candidate repertoire is well characterized [7], few *P. vivax* antigens are well defined. Therefore, antigen discovery is a prerequisite for the development of vaccines against *P. vivax*. Completion of the genome [8], transcriptome [9] and proteome [10,11] of *P. falciparum* has played a significant role in advancing research on this parasite.In comparison, research on *P. vivax* malaria has

lagged much behind. One major reason is the unavailability of a continuous *in vitro* culture system for *P. vivax*, although recent work showed promises in this direction [12]. With the recent deciphering of the *P. vivax* genome [13] and transcriptome [14], the parasite proteome remains to be determined. The advance in highly sensitive mass spectrometry (MS) offers an extraordinary opportunity to determine the proteome of the *P. vivax* parasite in the absence of large amount of experimental materials from a continuous *in vitro* culture. In this study, we attempted to study the proteome of the erythrocytic stages of *P. vivax* field isolates by highly accurate tandem mass spectrometry (MS/MS). As a way of antigen discovery, we further tried to identify parasite antigens that are recognized by host humoral immunity using an immune serum from a *P. vivax* malaria patient.

2. Materials and Methods

2. 1. Sample collection

Fresh *P. vivax* isolates were collected from 10 symptomatic malaria patients attending a malaria clinic in Mae Sot district, Tak Province, Thailand. Twenty milliliters of *P. vivax*infected blood were collected from each patient.White blood cells were removed by passing infected blood through a Plasmodipur® filter. Parasites were cultured with McCoy's 5A medium supplemented with 25% human AB serum at 37°C under 5% CO2 until they reached the schizont stage. To reduce contamination of red blood cell proteins, schizont-infected red blood cells were purified on 60% Percoll® and parasites were released by 0.01% saponin treatment. Parasite pellet was washed with phosphate buffered saline (PBS) pH 7.4 until the supernatant was clear and stored at -80°C for proteome analysis.

A total of 118 and 33 plasma samples were collected in 2001, 2002, and 2007-2009 from *P. vivax* and *P. falciparum* patients, respectively, who were attending an outpatient malaria clinic in Mae Sot,or the Hospital of Tropical Diseases, Bangkok, Thailand. Among these patients, follow-up was conducted in four *P. vivax* cases, from whom plasma samples were collected at the time of acute infection, and in three and six months after treatment. During the follow-up, the participants did not experience malaria infections. Control plasma samples from malaria naïve donors were collected from a non-endemic area in Thailand. The study protocol was approved by The

Pennsylvania State University Institutional Review Board and the Ethical Review Committee of Mahidol University. Informed consent or assent was obtained from volunteers before the blood samples were collected.

2.2. Preparation of protein extracts

For proteome analysis, ~109 *P. vivax* schizonts were resuspended in 100 µl of 100 mM Tris-HCl (pH 7.2) and sonicated on ice for four 10-sec pulses. Proteins were dissolved in SDS-PAGE loading buffer and separated on a linear gradient (4-20%, 1.5 mm thick) Trisglycine mini gel (Invitrogen, USA). The gel was stained by Colloidal blue (Invitrogen) and protein bands were horizontally sliced into 16 sections (Fig. 1.) Each gel section was cut into ~1 mm cubes, destained in 50% acetonitrile (ACN) containing 25 mM NH4HCO3 (pH 8.0) until gel pieces became transparent, dehydrated in 100% ACN, and dried completely in a SpeedVac. Afterward, the samples were reduced in 45 mM dithiothreitol (DTT) and 25 mM NH4HCO3(pH 8.0) for 45 min at 55°C and then alkylated in 100 mM iodoacetamide, 25 mM NH4HCO3 (pH 8.0) for 45 min at room temperature in the dark. All samples were washed twice in 50% ACN, 25 mM NH4CO3 (pH 8.0) and dried in a SpeedVac. For in-gel digestion, samples were digested overnight at 37°C with 0.01 µg/µl MS grade trypsin (Promega, Madison, Wisconsin, USA) in 50% ACN, 25 mM NH4HCO3 (pH 8.0).The resultant peptides were extracted twice with 100µl of 5% trifluoroacetic acid (TFA) in 50% ACN for 15 min. Samples were dried down completely by SpeedVac, and then resuspended in 200 µl of deionized water. This procedure was repeated twice, with a third drying down halted when the remaining volume was approximately 10 µl. Then 1% TFA was added to make the final concentration of TFA at 0.1%. The peptide samples were cleaned with SCX ZipTips (Millipore, Billerica, Massachusetts, USA) according to the manufacturer's instructions. The eluant was dried completely in a SpeedVac and resuspended in 15 µl of 2% ACN and 0.1% TFA. Separation of the peptides were achieved by reverse-phase nanoflow liquid chromatography (LC) using a 150 × 0.1mm chromolith caprod column injector loop (Merk, USA) on a Tempo LC matrix assisted laser desorption/ionization (MALDI) spotting system (ABI-MDS/Sciex) and eluted with a gradient of 2% ACN/0.1% TFA and 98% ACN/0.1% TFA, respectively.

2.3. Two-dimensional gel electrophoresis (2-DE) and Western blot

To identify parasite antigens, parasite proteins were subjected to 2-DE, immunoblotting, and LC/MS/MS analysis. Briefly, parasite crude extract (40 μg/gel) was solubilized in 2-D rehydration buffer (8M urea, 0.5% CHAPS, 60 mM DTT), and 0.5% ampholyte (pH 3–10), thoroughly mixed and centrifuged at 15000 × g for 10 min. The resultant supernatant was subjected to 2-DE. Isoelectric focusing was performed with pre-cast 7 cm Immobiline® dry strips (pH 3-10) using the Ettan IPGphor 3 apparatus (Amersham Bioscience AB, Uppsala, Sweden). The running protocol was as follows: step 1, 300 v, 200 Vh; step 2, 1000 v, 300 Vh; step 3, 5000 v, 1,400 Vh; and step 4, 5000 v, 2000 Vh. The focused strips were equilibrated in 10 ml of equilibration solution (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, 1% DTT) for 15 min, followed by incubation in 10 ml equilibration solution containing 2.5% iodoacetamide for another 15 min. The equilibrated strips were loaded on 12% SDS-PAGE gels for the second dimension separation. One gel was silver-stained using a MS compatible PlusOne Silver staining kit (Amersham Bioscience), whereas the other was used for immunoblotting. For Western blot, proteins were transferred to a 0.45 μm nitrocellulose membrane (Pharmacia Biotech, California, USA) under cooling conditions and constant voltage (100 V) for 4 h. The membrane was blocked with 5% skimmed milk in Tris-buffered saline (TBS, pH 7.2) overnight at 4oC. After washing with TBS containing 0.05% Tween20, the membrane was incubated with an immune serum from a *P. vivax* patient at 1:10 dilution for 2 h, and then with HRP-conjugated goat anti-human IgG antibody at 1:500 dilution for1 h. The blot was visualized by incubating with the HRP substrate (4-chloro-1-napthol, methanol and H2O2 in TBS). Positive spots were picked from the silver-stained gel. Each gel spot was destained in 1% H2O2, 25 mM NH4HCO3 (pH 8.0) prior to in-gel trypsin digestion as described above.

2.4. MS analysis and database search

For MS analysis, the final eluant of the peptides separated by reverse phase nanoflow LC was mixed with the MALDI matrix [7 mg/ml recrystallized α-cyanohydroxycinnamic acid (Sigma), 2 mg/ml ammonium phosphate, 0.1% TFA in 50% ACN], and automatically spotted onto a stainless steel MALDI target plate and analyzed on an ABI4800 MALDITOF/ TOF mass spectrometer (Applied Biosystems,

USA). The MS spectra were acquired from each sample spot using 500 laser shots from 40 random per spot using Reflectron Positive Ion mode with laser setting of 3200. The highest top ten peaks of each observed m/z value of each MS spectrum (excluding trypsin auto-digestion peaks) were chosen for subsequent MS/MS analysis with collision-induced dissociation fragmentation. Up to 2500 laser shots at the laser power 4200 were accumulated for each MS/MS spectrum. For identifying parasite antigens, peptides from the immune-positive spots of the 2-DE were analyzed by LC/MS/MS using a SYNAPT™ HDMS mass spectrometer (Waters, Manchester, UK). Nanoscale LC separation of tryptic peptides was performed with a NanoAcquity system (Waters) equipped with a 5 μm, 180 μm x 200 μm symmetry C18 trap column and a 1.7 μm, 75 μm × 200 μm BEH130 C18 analytical reversed phase column (Waters). The samples were initially transferred with an aqueous 0.1% formic acid solution to the trap column with a flow rate of 3 μl/min for 3 min.Mobile phase A was water with 0.1% formic acid, and mobile phase B was 0.1% formic acid in ACN. The peptides were separated with a gradient of 2–40% mobile phase B over 30 min at a flow rate of 350 nl/min followed by a 10 min rinse with 80% of mobile phase B. The lock mass was delivered from the auxiliary pump of the NanoAcquity pump with a constant flow rate of 200nl/min to the reference sprayer of the NanoLockSpray source of the mass spectrometer. After nanoelectrospray ionization, accurate MS/MS data were acquired. The MS and MS/MS data were searched against the annotated *P. vivax* protein database from PlasmoDB (http://www.plasmodb.org) and non-redundant human protein database from National Center for Biotechnology Information.The MS and MS/MS data from MALDI-TOF/TOF and LC/MS/MS were analyzed by Protein Pilot software version 2.01 (Applied Biosystems/MDS Sciex) and ProteinLynx Global Sever 2.2.5 (Waters), respectively. Search criteria were trypsin-cleaved peptides; 200 parts per million mass error tolerance in MS mode; 0.4 Da mass error tolerance for MS/MS fragments; fixed modification of carbamidomethylation of cysteines, and allowed (variable) modification of oxidation of methionine. Protein identification acceptance was ProteinPilot Unused Score of >1.3 (>95% confidence interval) plus <5% false discovery rate. The presence of a signal peptide (SP) and transmembrane domain (TM) in the protein sequence is an important indicator of secretory or cell surface protein. We used SignalP 3.0 and TMHMM 2.0 (http://www.cbs.dtu.dk) to

determine the SP and TM in the proteins. To predict the antigenicity of the proteins, linear B-cell epitope and antigenicity were predicted using the BepiPred 1.0 and Kolarskar & Tongaonkar Antigenicity programs (www.immuneepitope.org).

2.5. Expression of a recombinant protein

To express a putative antigen, PV24 (PVX_002950), identified from the 2-DE/MS analysis, cDNA was synthesized from 1 μg of *P. vivax* total RNA using Superscript®III reverse transcriptase (Invitrogen) [15]. Primers (5'ATGCGGATCCTACAATGCAAGCGAAAGACAAAATGG3' and 5' AGTCGTCGACGAACAAGTAGCCATAATATTTGG 3',*Bam*HI and *Sal*I sites underlined) were designed to amplify a 540 bp fragment (79-618 bp of the open reading frame). The PCR product was cloned in the expression vector pET-28 (a+) (Novagen, Darmstadt, Germany) after digestion with *Bam*HI and *Sal*I. The recombinant PV24 protein (rPV24) was expressed in *Escherichia coli* BL21 after induction by 1 mM isopropyl β-D-1-thiogalactopyranoside for 2 h. The His-tagged recombinant protein was purified by a denaturing method using the Ni-NTA agarose resin (Qiagen, Hilden, Germany). Eluted protein fractions were analyzed by 12% SDS-PAGE and Western blot analysis with mouse anti-His antibody at 1:1000 dilution. Purified recombinant protein was extensively dialyzed against PBS (pH 7.4) at 4oC.

2.6. ELISA

Griener flat-bottomed, 96-well microtiter plates were coated with 50 μl per well of 1 μg/ml of purified rPV24 or crude *P. vivax* parasite protein (10 mg/ml) overnight at 4°C. The plates were blocked for 2 h with 100 μl of PBS with 0.05% Tween 20 (PBST) containing 0.5% bovine serum albumin. After washing twice with PBST, 50 μl of human plasma diluted to1:50 with the blocking buffer were added and incubated for 2 h at room temperature. Plates were washed thrice with PBST and incubated with 50 μl of HRP-conjugated goat anti-human IgG antibody (Caltag,USA) at 1:2,000 for 1 h. Afterwards, the plates were washed thrice with PBST, and developed with 2, 2', azino-diethylbenzothiazolinesulfonic acid (Kirkepaard & Perry Laboratories,USA) for 30 min. The optical density (OD) was read at 405 nm on an ELISA plate reader. Each sample was tested in duplicate. Mean OD given by naïve control sera plus three standard deviations (STD) was used as the cutoff OD value.

2.7. Statistical analysis

Statistical analysis was performed using the SPSS program (Version 11.5, Chicago, USA).The level of anti-rPV24 antibodies among plasma samples from *P. vivax* and *P. falciparum* patients, and naïve control were evaluated by the analysis of variance (ANOVA). The correlation between anti-rPV24 antibody level and parasitemia was determined by Spearman's rank test. The results were considered significant at $P < 0.05$.

3. Results

3.1. The proteome of P. vivax schizons

Due to the lack of a suitable continuous *in vitro* culture method for *P. vivax*, we used field isolates obtained from infected patients for proteomic analysis. Our preliminary studies using parasite samples immediately after saponin lysis suggested that host protein contamination was too overwhelming to obtain an adequate parasite protein coverage. In order to circumvent this problem, parasites were cultured until they reached the schizont stage and purified by Percoll. To determine the schizont stage proteome, protein extracts were obtained from ~109 schizonts, separated by SDS-PAGE and subjected to MS analysis by MALDI-TOF/TOF. Search of the protein databases with the MS/MS spectra identified 316 *P. vivax* proteins with at least 95% confidence (supporting information 1). Almost half of the identified proteins (47%) were hypothetical proteins, among which 29% contained a putative SP and 37% contained at least one TM. Further, 15% of these hypothetical proteins contained both a SP and a TM. This result suggested that the identified proteins were either secretory or/and membrane associated, which might play important roles in parasite-host interactions (supporting information 2). Eleven percent (36 in 316 proteins) of the identified proteins was unique as defined by the absence of orthologs in other human malaria parasites, *P. falciparum, P. ovale and P. malariae* (supporting information 3). The majority of the unique proteins (18 in 36 proteins) were hypothetical. In addition, 16 of 36 proteins did not have orthologs in other human malaria parasites and *P. knowlesi*, which provide potential targets for *P. vivax*-specific diagnosis. The identified proteins were classified into functional classes based on the Munich Information Centre for Protein Sequences (MIPS) catalogue [16] (supporting

information 4). Proteins were plotted as a function of their broad functional classification; only one class was assigned per protein in order to avoid redundancy (Fig. 2). The predominant class contained hypothetical proteins (47%). Other important functional classes of proteins were proteins with binding function (10%), and involved in protein fate (5%), protein synthesis (4%), metabolism (4%) and cellular transport (4%). These protein classes were mainly housekeeping proteins. Enzymes of major metabolic pathways of the parasite including glycolysis, nucleic acid metabolism, and hemoglobin digestion were identified (Table 1). During parasite development, it modifies or exports proteins into red cell membrane which causes dramatic changes in host cell membrane composition,structure and function. Different transportation mechanisms of parasite have been reported, and several proteins shown to play significant roles in the transport machinery including Rab GTPases (Rab2, Rab5c, Rab6 and Rab7) [17,18], translocation protein complex (sec61, sec62 and SEC63),heat shock protein (Hsp70 and HSP101) and EXP2 [19-22] were identified in the schizont stage proteome.Several exported proteins potentially involved in immune evasion and host cell invasion including *vir* and erythrocyte membrane protein 3 were identified [23, 24]. The *P. vivax* tryptophan rich antigen (PvtrAg) or Pv-fam-a is one of the most abundant parasite protein families with 34 predicted members (www.plasmodb.org), among which 11 members were detected. Since our parasite samples were enriched in schizonts, schizont-specific proteins were readily detected. These include proteins associated with merozoite surface and invasive organelles such as merozoite surface proteins (MSP1 [25], MSP5 [26], MSP7 [27], and MSP8 [28]), apical membrane antigen 1 (AMA1) [29], reticulocyte binding protein [30], rhoptry proteins (RAP1 [31], RAP 2 [32], RhopH2, EXP2, and CLAG), and actinomyosin motors (actin, myosin) [33-35]. The parasite samples also contained gametocytes and several sexual stage proteins were detected, including the *P. falciparum* orthologs of male fertility protein Pf47 (PVX_083240), Pfs16 (PVX_000930) and transmission-blocking vaccine candidate Pfs230 (PVX_000995) [36-38]. Parasite adhesins are of particular importance due to their roles in parasite invasion, sequestration or parasite-host interactions [24].The *P. vivax* genome was predicted to encode137 adhesins [39], of which 11 were detected in our schizont stage proteome and six werehypothetical proteins (Table 2). Some of the adhesion proteins such as MSP1 and AMA1 are

known to involve in host cell invasion [40]. Glycosylphosphatidylinositol (GPI) anchor is the major carbohydrate modification of proteins mostly in schizont-stage parasites. Nine putative GPI-anchored proteins were detected in the schizont stage proteome (Table 3), which represent 10% of total GPI-anchored proteins predicted in the *P. vivax* genome. Four of the nine proteins were known GPI-anchored proteins. Some of these GPI-anchored proteins were known to be involved in parasite-host interaction and invasion, and are promising vaccine candidates [41-43]. As a potential novel antigen, PVX_088910 encodes a hypothetical protein with maximum expression in late-stage parasites. Its *P. falciparum* ortholog (PF08_0008) is a GPI-anchored micronemal antigen shown to mediate binding to red blood cell surface, indicating involvement in the host cell invasion process.

3.2. Identification of novel P. vivax antigens

In order to identify parasite proteins that are recognized by host humoral immunity, 2-DE immunoblot of protein extract from schizont stage parasites was performed. Immune serum from an acute *P. vivax* infected patient showing a high antibody level to *P. vivax* lysate as determined by ELISA (data not shown) was used in Western blot analysis. Three immune reactive protein spots were recognized by the antiserum. The positive protein spots were excised from the silver stained 2-DE gel for MS/MS analysis (Fig. 4). Four proteins were detected, corresponding to the protein products of PVX_002950, PVX_087070, PVX_095015 and PVX_092835 genes (Table 4). Antigenicity prediction suggested that all four proteins contained linear B-cell epitopes and had antiginicity score above the threshold. Of the four proteins, only PVX_002950 contained predicted SP and TM.

3.3. Expression and purification of rPV24.

PVX_002950 is predicted to encode a 24 kDa hypothetical protein with 206 amino acids, referred to as PV24, which shared 41% similarities with the *P. falciparum* ortholog (PFB0515w). This gene encodes a putative dolichol-linked oligosaccharide biosynthesis enzyme. Microarray analysis indicates that it is expressed throughout the *P. vivax* erythrocytic cycle [14]. Since the predicted cleavage site of the SP is between amino acids 26 and 27, we decided to express the 180-residue recombinant protein without the first 26-residue SP sequence. The rPv24 expressed in *E. coli* had a molecular size of ~26 kDa on SDS-PAGE gel and reacted with antiserum against 6X

His-tag, which was consistent with the predicted molecular size (26.4 kDa) of rPV24 (Fig. 4a and 4b). Moreover, rPV24 reacted with the immune serum previously used in the 2-DE immunoblot (Fig. 4c).

3.4. Antigenicity of rPV24

To determine the antigenicity of rPV24, ELISA was performed by testing rPV24 protein using plasma samples from *P. vivax* and *P. falciparum* patients, and malaria naïve donors. The reactivity of plasma from *P. vivax* patients was significantly higher than that of the naïve group ($P<0.001$), and from that of plasma from *P. falciparum* patients ($P<0.001$) (Fig. 5a). Compared with the naïve group, the reactivity to rPV24 of plasma from *P. falciparum* infected patients was not significantly different ($P>0.05$). Plasma samples from *P. vivax* patients collected in different years (2001-2009) did not show significant differences in terms of reactivity with rPV24 ($P > 0.05$) (Fig. 5b). Ninety-nine percent of plasma samples from *P. vivax* patients showed reactivity to rPV24 above the cutoff value. Interestingly, the reactivity of plasma samples from all follow-up cases (3 and 6 months post treatment) was also higher than the cutoff level (Fig. 5c). In addition, there was a significant inverse correlation between the level of anti-rPV24 antibody and the *P. vivax* parasitemia (correlation coefficient = -0.372, $P = 0.039$). Altogether, these results indicated that the PV24 was strongly antigenic and elicited long-lasting immunity in *P. vivax* patients.

4. Discussion

With the completion of *P. vivax* genome sequencing and transcriptome projects, it is important to determine the *P. vivax* proteomes. Due to host protein contamination in other erythrocytic stages, we only attempted to purify schizont stage for proteomic analysis. Consequently, our proteome was enriched in schizont stage proteins. In schizonts, 1,212 transcripts were detected (www.plasmodb.org), among which 316 proteins were detected in our proteomic analysis. Of those proteins, 65% overlapped with *P. falciparum* proteins [11]. Over half of the proteins identified were abundant proteins with putative functions in metabolism, protein synthesis, cellular transport and binding, etc. The hypothetical proteins accounting for almost 50% of this proteome could be a key to unravel the unique biology of *P. vivax*. In addition, numerous hypothetical proteins containing predicted SP (45) and/or TM (52) were

discovered, some of which may represent potential antigens. Thirty six (11%) out of 316 proteins were unique in *P. vivax*, as defined by the absence of orthologs in other human malaria parasites. Given that most of the rapid diagnostic tests are based on the pan-*Plasmodium* antigens (e.g., lactate dehydrogenase and aldolase) [44], the unique proteins in *P. vivax* could serve as potential candidates for developing *P. vivax*-specific tests. Transcriptome analysis of *P. vivax* revealed that members of *vir* and *pvtrag* gene families have two distinct phases of transcription, immediately after invasion and during schizogony [14]. Protein members of these gene families (10 *pvtrag* and 4 *vir*) detected in the schizont stage proteome appeared to be derived from the first wave of gene expression since all of them showed maximum transcription at the ring stage. The cognate proteins of 52 *vir* and 13 *pvtrag* genes with maximum transcription in schizont stage could not be detected in our schizont stage proteome possibly due to a delay in protein translation. Antimalarial vaccines are an important component for the integrated approaches to malaria control during the malaria elimination phase [45]. In particular, malaria vaccine development efforts need to target other malaria parasite species, especially *P. vivax*. Currently, there are only two *P.vivax* vaccine candidates being tested in clinical trials, circumsporozoite protein and transmission-blocking vaccine candidate pvs25 [46]. Compared with *P. falciparum*, the number of vaccine candidates in *P. vivax* is very limited, and antigen discovery is essential for *P. vivax* vaccine development. In the schizont stage proteome, we have identified several potential membrane proteins that could serve as vaccine candidates, including those that are predicted adhesions and GPI-anchored proteins. These groups include antigenic proteins such as MSP1 and AMA1 that are already in the vaccine development pipeline. In addition, we used a MS-based method to identify *P. vivax* antigens, which proved to be a viable approach to vivax antigen discovery. In our schizont-stage proteome, four antigens were identified by a *P. vivax* patient serum. We have further characterized one of these proteins, PV24, and showed that *P. vivax* infection induced long-lasting reactivity with this protein, and antibody titers against this protein could still be detected during the convalescence stage six months after successful treatment of the primary infection. During malaria infection, T cells play a central role in the regulation of immune responses and the formation of immunologic memory which helps control and eliminate the infection [47]. For antigen discovery,

we need to understand how the potential antigens elicit humoral and cellular immune responses during natural infection [48]. In this study, the persistence of anti-PV24 antibodies in *P. vivax* patients implies the involvement of T cells in the production of antibodies against this protein [49]. Our study has demonstrated PV24 as a usefulness marker for malaria epidemiology. Whether PV24 could induce protective immunity against malaria infection and serve as a vaccine candidate awaits further investigations.

Acknowledgements

This work was supported by grant R21AI069126 from NIAID, D43-TW006571 from the Fogarty International Center, NIH and The Office of the Higher Education Commission and Mahidol University under the National Research Universities Initiative. WR was a fellow supported by the Fogarty International Center, NIH (D43-TW006571) and by the Royal Golden Jubilee Ph.D. Program (5TMU47H2). We would like to thank the staff at Biotech, Thailand Science Park, and laboratory members at the Department of Entomology and the core facilities of the Hershey Medical Center, Penn State University for technical assistance.

REFERENCES

[1] Price RN, Tjitra E, Guerra CA, Yeung S, et al. Vivax malaria: neglected and not benign. Am J Trop Med Hyg 2007; 77:79-87.

[2] Krotoski WA, Collins WE, Bray RS, Garnham PC, et al. Demonstration of hypnozoites in sporozoite-transmitted *Plasmodium vivax* infection. Am J Trop Med Hyg 1982;31:1291-3.

[3] Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype, FyFy. N Engl J Med 1976;295:302-4.

[4] Mons B. Preferential invasion of malarial merozoites into young red blood cells. Blood Cells 1990;16:299-312.

[5] Suwanarusk R, Cooke BM, Dondorp AM, Silamut K, et al. The deformability of red blood cells parasitized by *Plasmodium falciparum* and *P. vivax*. J Infect Dis 2004;189:190-4.

[6] Ogutu BR, Apollo OJ, McKinney D, Okoth W, et al. Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers no protection to young children in Western Kenya. PLoS ONE 2009;4:e4708.

[7] Hu J, Chen Z, Gu J, Wan M, et al. Safety and Immunogenicity of a Malaria Vaccine, Plasmodium falciparum AMA-1/MSP-1 Chimeric Protein Formulated in Montanide ISA 720 in Healthy Adults. PLoS ONE 2008;3:e1952.

[8] Gardner MJ, Hall N, Fung E, White O, et al. Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 2002;419:498-511.

[9] Bozdech Z, Llinás M, Pulliam BL, Wong ED, et al. The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*. PLoS Biol 2003;1: e5.

[10] Florens L, Washburn MP, Raine JD, Anthony RM, et al. A proteomic view of the *Plasmodium falciparum* life cycle. Nature 2002;419:520-6.

[11] Lasonder E, Ishihama Y, Andersen JS, Vermunt AMW, et al. Analysis of the Plasmodium falciparum proteome by high-accuracy mass spectrometry. Nature 2002; 419:537-42.

[12] Panichakul T, Sattabongkot J, Chotivanich K, Sirichaisinthop J, et al. Production of erythropoietic cells in vitro for continuous culture of *Plasmodium vivax*. Int J Parasitol 2007;37:1551-7.

[13] Carlton JM, Adams JH, Silva JC, Bidwell SL, et al. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. Nature 2008;455:757-63.

[14] Bozdech Z, Mok S, Hu G, Imwong M, et al. The transcriptome of *Plasmodium vivax* reveals divergence and diversity of transcriptional regulation in malaria parasites. Proc Natl Acad Sci USA 2008;105:16290-5.

[15] Cui L, Fan Q, Hu Y, Karamycheva SA, et al. Gene discovery in *Plasmodium vivax* through sequencing of ESTs from mixed blood stages. Mol Biochem Parasitol 2005;144:1-9.

[16] Mewes HW, Frishman D, Güldener U, Mannhaupt G, et al. MIPS: a database for genomes and protein sequences. Nucleic Acids Res 2002;30:31-34.

[17] Zerial M, McBride H, Rab proteins as membrane organizers. Nat Rev Mol Cell Biol 2001; 2:107-17.

[18] Vanlandingham PA, Ceresa BP. Rab7 Regulates Late Endocytic Trafficking Downstream of Multivesicular Body Biogenesis and Cargo Sequestration. J Biol Chem 2009; 284:12110 -24.

[19] Ungermann C, Neupert W, Cyr DM. The role of Hsp70 in conferring unidirectionality on protein translocation into mitochondria.Science 1994;266:1250-3.

[20] Lyman SK, Schekman R. Interaction between BiP and Sec63p is required for the completion of protein translocation into the ER of *Saccharomyces cerevisiae*. J Cell Biol 1995;131:1163-71.

[21] Meyer H, Grau H, Kraft R, Kostka S, et al. Mammalian Sec61 Is Associated with Sec62 and Sec63. J Biol Chem 2000;275:14550 -7.

[22] de Koning-Ward TF, Gilson PR, Boddey JA, Rug M, et al. A newly discovered protein export machine in malaria parasites. Nature 2009;459:945-9.

[23] del Portillo HA, Lanzer M, Rodriguez-Malaga S, Zavala F, Fernandez-Becerra C. Variant genes and the spleen in *Plasmodium vivax* malaria. Int J Parasitol 2004;34:1547-54.

[24] Carvalho BO, Lopes SCP, Nogueira PA, Orlandi PP, et al. On the cytoadhesion of *Plasmodium vivax*-infected erythrocytes. J Infect Dis 2010; 202:638-47.

[25] del Portillo HA, Longacre S, Khouri E, David PH. Primary structure of the merozoite surface antigen 1 of Plasmodium vivax reveals sequences conserved between different Plasmodium species. Proc Natl Acad Sci U S A 1991;88:4030-4.

[26] Black CG, Barnwell JW, Huber CS, Galinski MR, Coppel RL. The *Plasmodium vivax* homologues of merozoite surface proteins 4 and 5 from *Plasmodium falciparum* are expressed at different locations in the merozoite. Mol Biochem Parasitol 2002;120:215-24.

[27] Mongui A, Perez-Leal O, Soto SC, Cortes J, Patarroyo MA. Cloning, expression, and characterisation of a Plasmodium vivax MSP7 family merozoite surface protein. Biochem Biophys Res Commun 2006;351:639-44.

[28] Perez-Leal O, Sierra AY, Barrero CA, Moncada C, Martinez P, Cortes J, et al. Plasmodium vivax merozoite surface protein 8 cloning, expression, and characterisation. Biochem Biophys Res Commun 2004;324:1393-9.

[29] Cheng Q, Saul A. Sequence analysis of the apical membrane antigen I (AMA-1) of *Plasmodium vivax.* Mol Biochem Parasitol 1994;65:183–7.

[30] Rayner JC, Galinski MR, Ingravallo P, Barnwell JW. Two *Plasmodium falciparum* genes express merozoite proteins that are related to *Plasmodium vivax* and *Plasmodium yoelii* adhesive proteins involved in host cell selection and invasion. Proc Natl Acad Sci USA 2000;97:9648-53.

[31] Perez-Leal O, Mongui A, Cortes J, Yepes G, Leiton J, Patarroyo MA. The Plasmodium
vivax rhoptry-associated protein 1. Biochem Biophys Res Commun 2006;341:1053-8.

[32] Patarroyo MA, Perez-Leal O, Lopez Y, Cortes J, Rojas-Caraballo J, Gomez A, et al. Identification and characterisation of the *Plasmodium vivax* rhoptry-associated protein 2. Biochem Biophys Res Commun 2005;337:853-9.

[33] Bannister LH, Hopkins JM, Dluzewski AR, Margos G, et al. *Plasmodium falciparum* apical membrane antigen 1 (PfAMA-1) is translocated within micronemes along subpellicular microtubules during merozoite development. J Cell Sci 2003;116:3825-34.

[34] Meyer EVS, Semenya AA, Okenu DMN, Dluzewski AR, et al. The reticulocyte binding-like proteins of *P.knowlesi* locate to the micronemes of merozoites and define

two new members of this invasion ligand family. Mol Biochem Parasitol 2009;165:111-21.

[35] Singh M, Mukherjee P, Narayanasamy K, Arora R, et al. Proteome analysis of *Plasmodium falciparum* extracellular secretory antigens at asexual blood stages reveals a cohort of proteins with possible roles in immune modulation and signaling. Mol Cell Proteomics 2009;8:2102-18.

[36] Quakyi, IA, Carter, R, Rener, J, Kumar, N, et al. The 230-kDa gamete surface protein of *Plasmodium falciparum* is also a target for transmission-blocking antibodies. J Immunol 1987;139:4213-7.

[37] Lobo CA, Konings RN, Kumar N. Expression of early gametocyte-stage antigens Pfg27 and Pfs16 in synchronized gametocytes and non-gametocyte producing clones of Plasmodium falciparum. *Mol Biochem Parasitol* 1994;68:151-4.

[38] van Schaijk BCL, van Dijk MR, van de Vegte-Bolmer M, van Gemert G, et al. Pfs47, paralog of the male fertility factor Pfs48/45, is a female specific surface protein in *Plasmodium falciparum*. Mol Biochem Parasitol 2006;149:216-22.

[39] Ansari FA, Kumar N, Bala Subramanyam M, Gnanamani M, Ramachandran S. MAAP: malarial adhesins and adhesin-like proteins predictor. Proteins 2008;70:659-66.

[40] Richard D, MacRaild CA, Riglar DT, Chan J, et al. Interaction between *Plasmodium falciparum* apical membrane antigen 1 and the rhoptry neck protein complex defines a key step in the erythrocyte invasion process of malaria parasites. J Biol Chem 2010;285:14815-22.

[41] Hoessli DC, Poincelet M, Gupta R, Ilangumaran S, Nasir-ud-Din. *Plasmodium falciparum* merozoite surface protein 1. Eur J Biochem 2003;270:366-75.

[42] Sanders PR, Gilson PR, Cantin GT, Greenbaum DC, et al. Distinct protein classes including novel merozoite surface antigens in Raft-like membranes of *Plasmodium falciparum.* J Biol Chem 2005;280:40169-76.

[43] Gilson PR, Nebl T, Vukcevic D, Moritz RL, et al. Identification and stoichiometry of glycosylphosphatidylinositol-anchored membrane proteins of the human malaria parasite *Plasmodium falciparum.* Molecular & Cellular Proteomics 2006;5:1286-99.

[44] Lee N, Baker J, Bell D, McCarthy J, Cheng Q. Assessing the genetic diversity of the aldolase genes of *Plasmodium falciparum* and *Plasmodium vivax* and its potential effect on performance of aldolase-detecting rapid diagnostic tests. J Clin Microbiol 2006;44:4547-49.

[45] The malERA Consultative Group on Vaccines. A research agenda for malaria eradication: vaccines. PLoS Med 2011; 8:e1000398.

[46] Arévalo-Herrera M, Chitnis C, Herrera S. Current status of Plasmodium vivax vaccine. Hum Vaccin 2010; 6:124-32.

[47] Nardin EH, Nussenzweig RS. T cell responses to pre-erythrocytic stages of malaria: Role in protection and vaccine development against pre-erythrocytic stages. Annu Rev Immunol 1993;11:687-727.

[48] Joshi SK, Bharadwaj A, Chatterjee S, Chauhan VS. Analysis of immune responses against T- and B-cell epitopes from *Plasmodium falciparum* liver-stage antigen 1 in rodent malaria models and malaria-exposed human subjects in India. Infect Immun 2000;68:141-50.

[49] Jangpatarapongsa K, Sirichaisinthop J, Sattabongkot J, Cui L, Montgomery SM, Looareesuwan S, Troye-Blomberg M, Udomsangpetch U. 2006. Memory T cells protect against *Plasmodium vivax* infection. Microbes Infect 2006; 8:680-6.

[46] Arévalo-Herrera M, Chitnis C, Herrera S. Current status of Plasmodium vivax vaccine.Hum Vaccin 2010; 6:124-32.

[47] Nardin EH, Nussenzweig RS. T cell responses to pre-erythrocytic stages of malaria: Role in protection and vaccine development against pre-erythrocytic stages. Annu Rev Immunol 1993;11:687-727.

[48] Joshi SK, Bharadwaj A, Chatterjee S, Chauhan VS. Analysis of immune responses against T- and B-cell epitopes from *Plasmodium falciparum* liver-stage antigen 1 in rodent malaria models and malaria-exposed human subjects in India. Infect Immun 2000;68:141-50.

[49] Jangpatarapongsa K, Sirichaisinthop J, Sattabongkot J, Cui L, Montgomery SM, Looareesuwan S, Troye-Blomberg M, Udomsangpetch U. 2006. Memory T cells protect against *Plasmodium vivax* infection. Microbes Infect 2006; 8:680-6.

Figure legends

Fig 1 SDS-PAGE analysis of *P vivax* lysate Lysate from ~109 parasites were separated on 12% polyacrylamide gel and stained with colloidal blue. Molecular standards are shown in lane 1. Protein bands were horizontally sliced into 16 sections (lane 2, S1-S16) before subjected to in-gel trypsin digestion and MS analysis.

Fig 2 Pie chart showing the distribution of functional classes of identified *P vivax* proteins as defined by the MIPS catalogue.

Fig 3 2-DE analysis of blood stage proteins of *P vivax* (a) Silver stained gel, and (b) immunoblot with an immune serum of *P vivax* patient. Circles indicate proteins of *P vivax* reacted with the immune serum.

Fig 4 Purification of rPV24 using Ni-NTA chromatography (a) SDS-PAGE analysis and (b) chemiluminescence analysis of immunoblot using anti-His antibody. Lane 1 = molecular weight standards, lane 2 = whole lysate, lane 3 = soluble fraction, lane 4 = flow-through fraction, lane 5 = last washing fraction, and lane 6 and 7 = eluted fractions (c) Immunoblot analysis of rPV24 with the immune serum from a *P vivax* patient. Lane 1 = molecular weight standards, lane 2 = Ponceu S staining of rPV24 protein, and lane 3 = immunoblot of rPV24. Arrows indicate purified rPV24.

Fig 5 - Determination of antibody levels in plasma samples to rPV24 by ELISA. (a) Different groups of human plasmas; sera of *P vivax* infected patients (PV), malaria naïve sera (N), and sera of *P falciparum* infected patients (PF). (b) plasma from PV collected in different years compared with N. (c) Antibody levels during acute infection and convalescence period collected at 3 and 6 months after treatment (dash line indicates cut-off level at mean + 3 standard deviation).

Table1. The detected *P vivax* proteins involved in parasite's metabolic pathways

Gene ID	Enzyme	EC no†	Reaction catalyzed
Glycolysis			
PVX_095015	Enolase	42111	Glycerate-2P ↔ Phosphoenol-pyruvate
PVX_114445	Pyruvate kinase	27140	Phosphoenol-pyruvate + ADP ↔ Pyruvate + ATP
PVX_118495	Triosephosphate isomerase	5311	Glyceraldehyde-3P ↔ Glycerone-P
PVX_118255	Fructose 1,6-bisphosphate aldolase	41213	Aldolase ↔ Glyceraldehyde-3P
PVX_116630	Lactate dehydrogenase	11127	Pyruvate + NADH+H^+↔Lactate + NAD^+
Nucleic acid biosynthesis (Purine salvage pathway)			
PVX_111245	Adenosine deaminase	3544	Adenosine + H_2O ↔ Inosine + NH_3
PVX_092535	Adenylate and Guanylate cyclase catalytic domain containing protein	4612	GTP ↔ 3',5'-cyclic GMP + PPi
PVX_094840	Hypoxanthine phosphoribosyltransferase	2428	Hypoxanthine + PRPP ↔ IMP + PPi
(Pyrimidine metabolism)			
PVX_083135	Aspartate carbamoyltransferase	2132	L-Aspatate + Carmoyl-P ↔ N-carbamoyl-L-Aspartate + Pi
TCA cycle			
PVX_084960	ATP-specific succinyl-CoA synthetase beta subunit	6215	ATP + Succinate + CoA ↔ ADP + Orthophosphate + Succinyl-CoA
Hemoglobin digestion			
PVX_115000	Falcilysin	3424-	Acting on peptide bonds
PVX_097935	Subtilisin-like protease precursor	342162	Hydrolyzes peptide amides
PVX_086040		3423B14	Cleavage of hemoglobin
PVX_122425	Aspartic protease PM4 M1-family aminopeptidase	34112	Release of an N-terminal amino acid from a peptide

†Enzyme Commission (EC) numbers of *P falciparum* orthologs

Plasmodium metabolic pathways can be found at http://siteshujiacil/malaria/

Table2. *Plasmodium vivax* adhesins predicted by MAAP (Malaria adhesins and adhesionlike proteins predictor) [33].**P vivax* proteins with Pmaap score of ≥ 07 were predicted as adhesins.

Accession Number	Name	score*	SP	TM
PVX_097950	Hypothetical protein, conserved	0742	0	0
PVX_096995	Tryptophan-rich antigen (Pv-fam-a)	074	0	1
PVX_084720	Hypothetical protein, conserved	0935	1	0
PVX_092275	Ap cal merozoite antigen 1	1001	0	1
PVX_099980	Major blood-stage surface antigen Pv200	1001	1	1
PVX_096245	Hypothetical protein, conserved	1008	0	0
PVX_117060	Hypothetical protein, conserved	1027	0	0
PVX_083560	Hypothetical protein, conserved	1242	0	0
PVX_112670	Tryptophan-rich antigen (Pv-fam-a)	1615	1	0
PVX_096070	Early transcribed membrane protein (ETRAMP)	2113	1	1
PVX_003555	Hypothetical protein, conserved	2213	0	0

Table 3 GPI-anchored proteins identified in the schizont-stage proteome.

Accession number	Name
PVX_083135	Aspartate carbamoyltransferase, putative
PVX_088910	Hypothetical protein, conserved
PVX_097625	Merozoite surface protein 8, putative
PVX_099320	Acid phosphatase, putative
PVX_099980	Major blood-stage surface antigen Pv200 (MSP1)
PVX_100835	Hypothetical protein, conserved
PVX_110895	ADP/ATP transporter on adenylate translocase, putative
PVX_113775	Membrane protein pf12 precursor, putative
PVX_122545	Cop-coated vesicle membrane protein p24 precursor, putative

Table 4*Plasmodium vivax* proteins recognized by immune serum from *P vivax* patient identified by LC/MS/MS

Gene ID	Description	Sequence	MW (kDa)	*p*I	SP	TM
PVX_002950	hypothetical protein	LIKDSNISFHFFYANNDPLSR	24.1	9.81	1	3
PVX_087070	hypothetical protein	APPTQGEMLLLLVR	110.1	6.53	0	No
PVX_095015	enolase	AAVPSGASTGIYEALELR	48.8	9.70	0	No
PVX_092835	hypothetical protein	LGKSNKR, QKAKQVK, ENAERSK, AKISMFK, TNVKKNR, IFEREGK, HYKTNVK, DNKLGKSNK, TSENVNQSK, NILDEIAVK, GSTVNTYILK, QSKVSLKPIK, VKINLNNPVK, MHVFDLDKAK, KNRFTIIETR, KMHVFDLDKAK	41.3	10.13	0	No

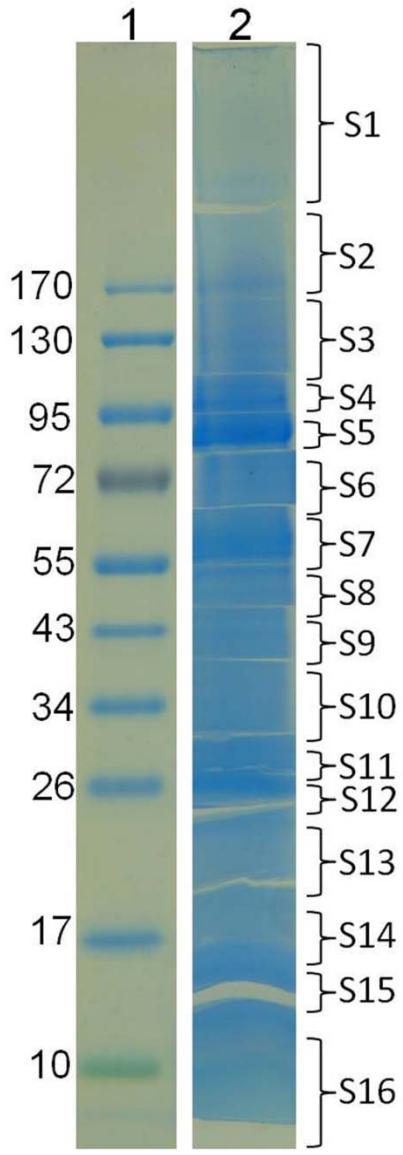

1
2
170
130
95
72
55
43
34
26
17
10
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
S12
S13
S14
S15
S16

Fig. 1

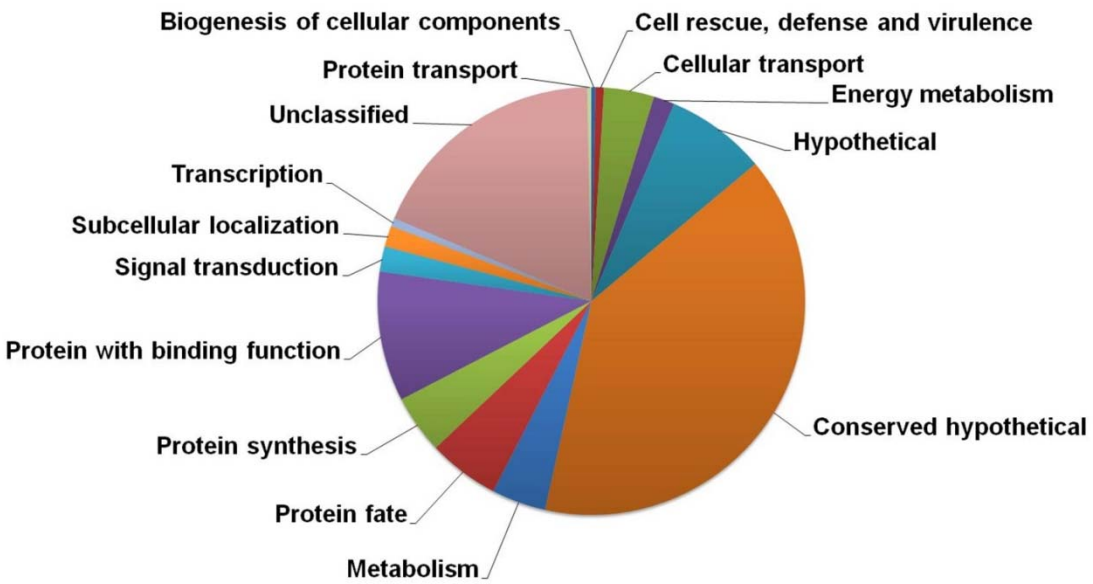

Biogenesis of cellular components
Cell rescue, defense and virulence
Protein transport
Cellular transport
Energy metabolism
Unclassified
Hypothetical
Transcription
Subcellular localization
Signal transduction
Protein with binding function
Conserved hypothetical
Protein synthesis
Protein fate
Metabolism

Fig. 2

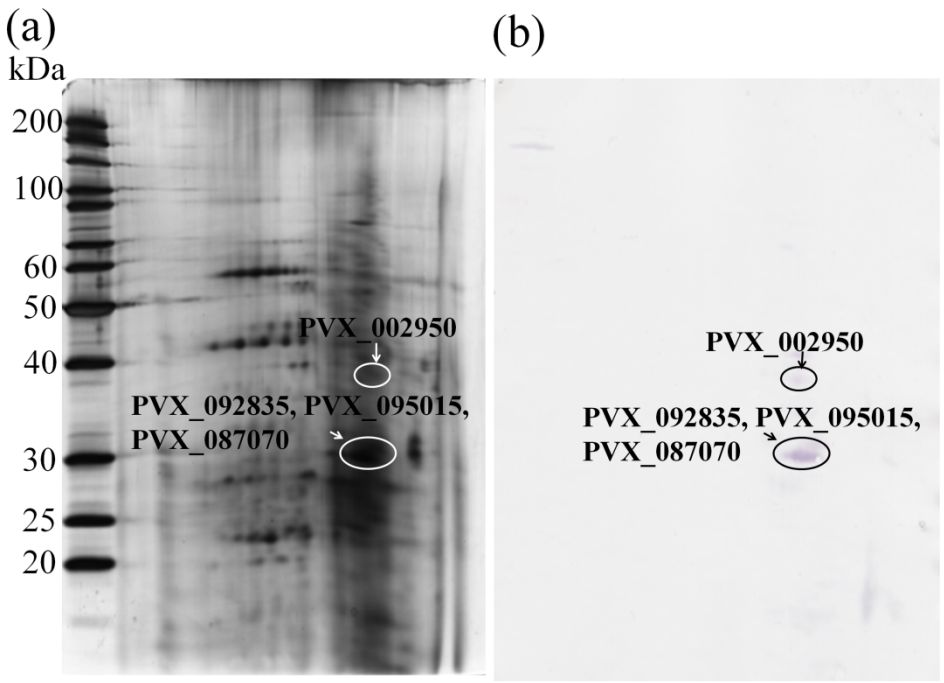

(a)
kDa
200
100
60
50
40
30
25
20
PVX_002950
PVX_092835, PVX_095015,
PVX_087070
(b)
PVX_002950
PVX_092835, PVX_095015,
PVX_087070

Fig. 3

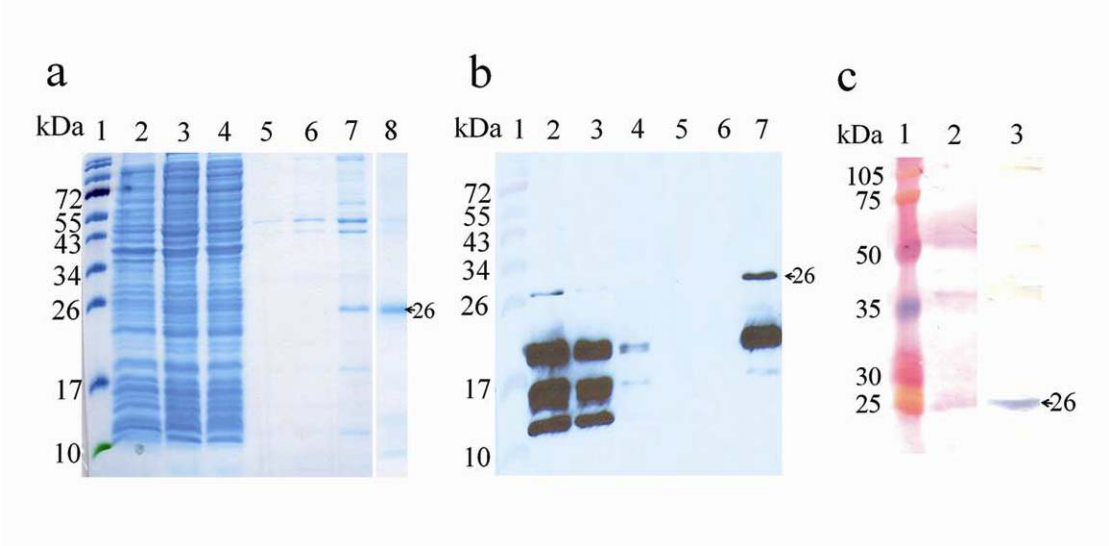

a
kDa 1 2 3 4 5 6 7 8
72
55
43
34
26
17
10
26
b
kDa 1 2 3 4 5 6 7
72
55
43
34
26
17
10
26
c
kDa 1 2 3
105
75
50
35
30
25
26

Fig. 4

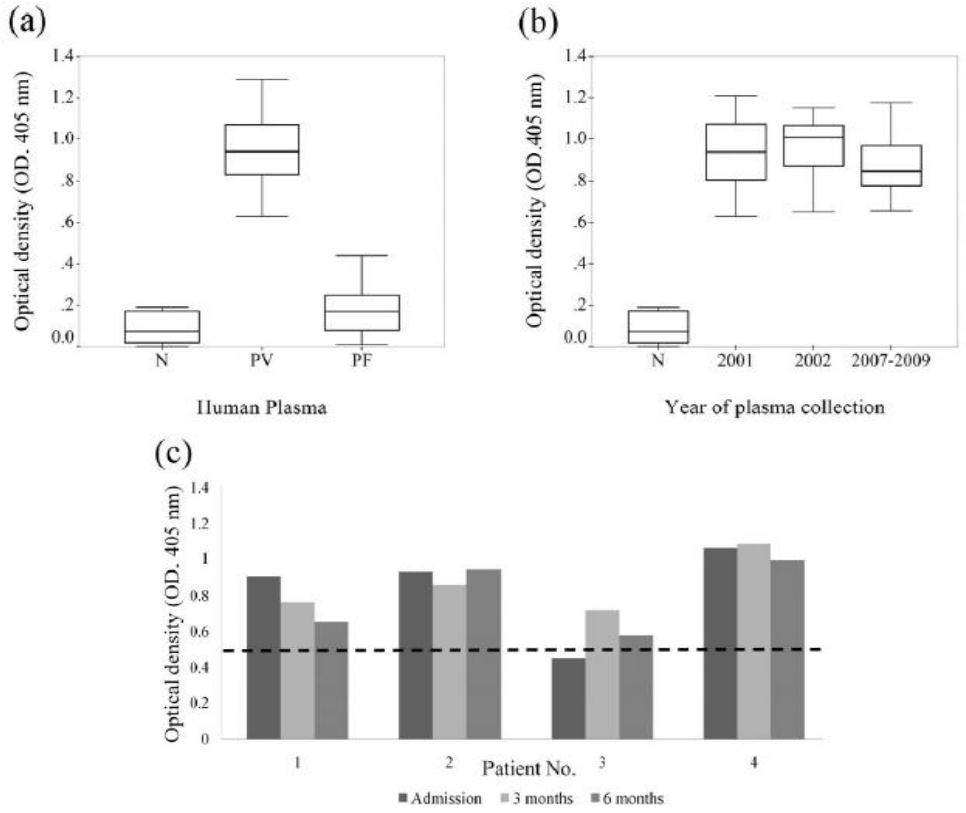
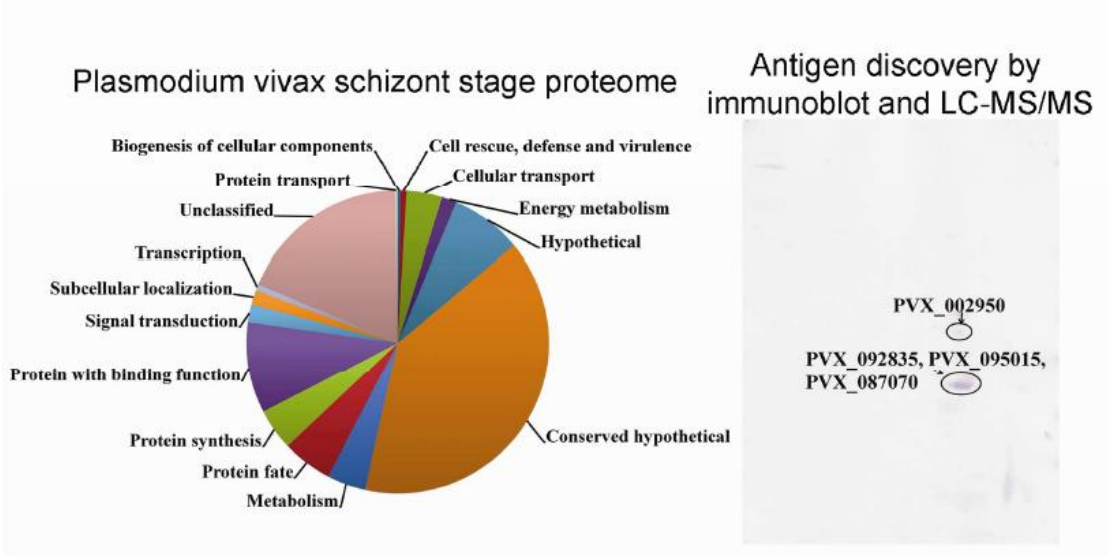

(a)
Optical density (OD. 405 nm)
1.4
1.2
1.0
.8
.6
.4
.2
0.0
N
PV
PF
Human Plasma
(b)
Optical density (OD.405 nm)
N
2001
2002
2007-2009
Year of plasma collection
(c)
Optical density (OD. 405 nm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
Patient No.
Admission
3 months
6 months

Fig. 5



Graphical Abstract

Highlights

- Mass spectrometry analysis identified 316 proteins in *Plasmodium vivax* schizont stage.
- Immunoblot and mass spectrometry identified four potential antigens.
- One novel antigen could elicit strong, long-lasting antibody response in vivax patients.

BIOGRAPHY

NAME	Miss Wanlapa Roobsoong
DATE OF BIRTH	15 July 1981
PLACE OF BIRTH	Nakhonpathom, Thailand
INSTITUTION ATTENDED	Rangsit University, 2000-2004 Bacherlor of Science (Medical Technology) (First Class Honors) Mahidol University, 2004-2011 Doctor of Philosophy (Medical Technology)
SCHOLARSHIP RECEIVED	The Royal Golden Jubilee/ Thailand Research Fund (RGJ/TRF)
RESEARCH GRANTS	The Royal Golden Jubilee/ Thailand Research Fund (RGJ/TRF) Forgarty International Scholar
HOME ADDRESS	61 Moo6, Muang Nakhonpathom, Nakhonpathom, Thailand 73000 Tel.084-919-2908 E-mail: wanlapa152@yahoo.com
PUBLICATION	Determination of the *Plasmodium vivax* schizont stage proteome (Journal of Proteomics, 2011)
PRESENTATION	RJG-Ph.D. Congress IX (2008), Chonburi, Thailand Vivax Malaria Research: 2009 and Beyond (2009), Panama The 5th Annual Symposium of Protein Society of Thailand Protein Research:

From Basic Approaches to Modern Technologies (2010), Bangkok, Thailand (First prize poster presentation award)
Parasite to Prevention: Advances in the Understanding of Malaria (2010), Edinburgh, United Kingdom