

PlasmoDB: a functional genomic database for malaria parasites

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ABSTRACT

PlasmoDB (<http://PlasmoDB.org>) is a functional genomic database for *Plasmodium* spp. that provides a resource for data analysis and visualization in a gene-by-gene or genome-wide scale. PlasmoDB belongs to a family of genomic resources that are housed under the EuPathDB (<http://EuPathDB.org>) Bioinformatics Resource Center (BRC) umbrella. The latest release, PlasmoDB 5.5, contains numerous new data types from several broad categories—annotated genomes, evidence of transcription, proteomics evidence, protein function evidence, population biology and evolution. Data in PlasmoDB can be queried by selecting the data of interest from a query grid or drop down menus. Various results can then be combined with each other on the query history page. Search results can be downloaded with associated functional data and registered users can store their query history for future retrieval or analysis.

INTRODUCTION

Plasmodium spp. are obligate intracellular protozoan parasites of humans and animals, and are the causative agents of malaria. Transmission of these parasites to humans occurs via the *Anopheles* mosquito vector and the

geographic distribution of endemic regions puts almost half of the world's population at risk to contracting malaria. This disease is a major source of morbidity and mortality worldwide, which results in 300–500 million clinical cases and 1–2 million deaths annually (1,2). While several species of *Plasmodium* cause disease in humans (including *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*), *P. falciparum* is by far the deadliest (1,3). The life cycle of the *Plasmodium* parasite takes it through multiple cell types (in the vertebrate host and arthropod vector) during which the parasite undergoes multiple developmental changes (both sexual and asexual). The different life-cycle stages are marked by specific genomic, transcriptomic, proteomic and metabolomic states. Understanding how these changes are triggered and orchestrated requires mechanisms to view and interrogate genomic and functional genomic data in a powerful and intuitive manner. Over the past 10 years, PlasmoDB has evolved into a venue that integrates such data and allows the user to perform complex queries tailored to their specific needs and interests.

UPDATED DATA CONTENT

The data available in PlasmoDB has expanded to include genomic and functional data from eight *Plasmodium* species and is summarized in Table 1 (4). The current release (PlasmoDB 5.5) contains fully sequenced and annotated genomes of *P. falciparum*, *P. vivax*, *P. yoelii*, *P. berghei*,

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Table 1. Types of data available in PlasmoDB and example queries

Type of Data	Species for which this data is available	Example query
Genomic data		
Full sequence and annotation	<i>P. falciparum</i> , <i>P. vivax</i> , <i>P. yoelii</i> , <i>P. berghei</i> , <i>P. chabaudi</i> , <i>P. knowlesi</i>	Search annotations for specific keyword (see Figure 1C).
Sequence only	<i>P. reichenowi</i> , <i>P. gallinaceum</i>	Find sequence similarity using BLAST.
Transcript expression data		
Microarray	<i>P. falciparum</i> , <i>P. berghei</i> , <i>P. yoelii</i>	Identify genes expressed at specific life-cycle stages.
EST	<i>P. falciparum</i> , <i>P. vivax</i> , <i>P. berghei</i> , <i>P. yoelii</i>	Confirm gene models and alternative gene models.
SAGE	<i>P. falciparum</i>	Identify genes with transcript evidence.
Protein expression data	<i>P. falciparum</i> , <i>P. berghei</i> , <i>P. yoelii</i>	Identify genes with protein expression evidence at specific life-cycle stages.
Population biology		
SNP	<i>P. falciparum</i>	Find highly polymorphic genes or distinguish isolates based on their SNP profile.
Microsatellite		
Isolate data		
Protein interaction		
Yeast two hybrid	<i>P. falciparum</i>	Identify possible interaction partners of a gene of interest.
Interactome map		
Putative function		
GO annotation	<i>P. falciparum</i> , <i>P. vivax</i> , <i>P. yoelii</i> , <i>P. berghei</i> , <i>P. chabaudi</i> , <i>P. knowlesi</i>	Identify genes that have GO annotations.
EC numbers	<i>P. falciparum</i> , <i>P. yoelii</i> , <i>P. knowlesi</i>	Identify genes with enzymatic annotations.
Metabolic pathways	<i>P. falciparum</i>	Identify parasite-specific or missing metabolic pathways.
Evolutionary		
Orthology based	<i>P. falciparum</i> , <i>P. vivax</i> , <i>P. yoelii</i> , <i>P. berghei</i> , <i>P. chabaudi</i> , <i>P. knowlesi</i>	Identify genes specific to apicomplexa.
Homology based	<i>P. falciparum</i> and <i>P. yoelii</i>	Identify homologs of a gene or list of genes of interest.
Protein features		
Protein motifs	<i>P. falciparum</i> , <i>P. vivax</i> , <i>P. yoelii</i> , <i>P. berghei</i> , <i>P. chabaudi</i> , <i>P. knowlesi</i>	Identify genes with specific protein attributes.
Interpro/pfam domains		
Molecular weight		
Isoelectric point		
Protein structure		
Immune epitopes		
Protein localization		
Signal peptide	<i>P. falciparum</i> , <i>P. vivax</i> , <i>P. yoelii</i> , <i>P. berghei</i> , <i>P. chabaudi</i> , <i>P. knowlesi</i>	Identify genes targeted to the host cell.
Transmembrane domains		
Targeting to the RBC		
Apicoplast targeting	<i>P. falciparum</i>	Identify genes targeted to the apicoplast.

P. chabaudi and *P. knowlesi*. Importantly, PlasmoDB 5.5 contains results of annotation efforts from multiple sources including the recent systematic effort to update the *P. falciparum* genome that is an ongoing project started at a workshop in late 2007 co-organized by the Wellcome Trust Sanger Institute (WTSI) and EuPathDB (formerly ApiDB) teams. Reannotation data have been released in incremental steps (snapshots) in order to provide timely information to users of PlasmoDB and to solicit user comments regarding the reannotations.

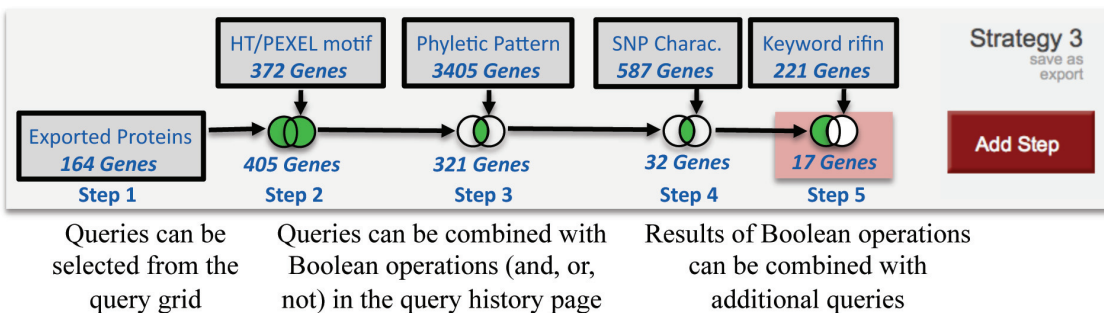
Transcript expression data [microarray, expressed sequence tags (ESTs) and serial analysis of gene expression (SAGE)] available through PlasmoDB has expanded dramatically over the past few releases to include microarray data from multiple life-cycle stages, gene knock-out mutants of *P. falciparum* and *P. berghei* (5–12) and multiple stages of *P. yoelii* (mosquito, erythrocytic and liver stages) (13). Also included are EST data from over 130

libraries (*P. falciparum*, *P. vivax*, *P. berghei* and *P. yoelii*) (14,15) [dbEST (<http://www.ncbi.nlm.nih.gov/dbEST/>)] and SAGE data (*P. falciparum* only) (16–18). Protein expression evidence includes data from various life-cycle stages (*P. falciparum*, *P. berghei* and *P. yoelii*) (11,13,19–21; Leiden Malaria Group, unpublished data).

Population biology evidence (*P. falciparum* only) includes mapping of microsatellite data (22) onto the genome (available as a genome browser track), single nucleotide polymorphism (SNP) data from resequencing efforts of more than 20 *P. falciparum* strains (*P. reichenowi* is included as an out-group for comparison purposes) and data from nearly 100 *P. falciparum* isolates (23–25). OrthoMCL analyses provide ortholog determinations between the different species facilitating discovery of shared genes between lineages (26). Protein function assignments are aided by a number of additional functional data types available through PlasmoDB 5.5 including

A

B



C

Figure 1. Screenshots from PlasmoDB 5.5 and query workflow. (A) The top of the screenshot shows the PlasmoDB logo. On the left side are links to various sections of PlasmoDB and a point for logging in or registering as a user (not required for using the site but useful for storing search histories). The query grid is in the center and provides an access point to all searchable data in PlasmoDB. (B) This is a scheme of a workflow that a user may follow when building a set of queries. Beginning at the left, queries can be performed starting from the query grid and the results can be joined using operations available through the query history page. (C) Screen shots of a 'key word' search page, an example gene query history and a gene results page. Note the add column feature in the results page that allows the addition of columns with additional data and the ability to sort results.

evidence of protein–protein interaction (yeast two hybrid and predicted interactome) (27,28), Genome Ontology (GO) (29) and InterPro domain (30) annotations for *P. falciparum*, *P. vivax*, *P. berghei*, *P. yoelii*, *P. knowlesi* and *P. chabaudi*, Enzyme Commission (EC) number (29) annotation for *P. falciparum*, *P. yoelii* and *P. knowlesi* (31) and metabolic pathway assignments for *P. falciparum* (31). In addition, subcellular localization of proteins is available through signal peptide (32) and transmembrane domain predictions (33) for *P. falciparum*, *P. vivax*, *P. berghei*, *P. yoelii*, *P. knowlesi* and *P. chabaudi*, and parasite-specific predictions (*P. falciparum* only) for apicoplast localization (34) and export to the host cell (35–37).

HOW TO USE PLASMODB

A visitor to PlasmoDB can use the database in two general ways: (i) To retrieve all available information associated with a particular gene of interest using a search for an exact gene ID, gene name or gene product name. (ii) To ask single questions (Table 1) and/or conduct a series of searches followed by refining the results by combining them or subtracting them from one another. Starting with the PlasmoDB home page (Figure 1A), a user can perform a quick search by entering an identifier or test term, or select a specific query from a number of drop-down menus (data not shown). Alternatively, queries may be accessed by visiting the ‘Queries and Tools’ section of PlasmoDB (Figure 1A), which includes a grid displaying all available queries/searches. By using the queries and tools, a user can interrogate data in PlasmoDB—the third column of Table 1 includes example data-specific questions that are available.

When conducting queries with the purpose of combining results it may be useful to visualize the searches in a workflow environment where nodes are connected using different criteria (‘and’, ‘or’, ‘not’) (Figure 1B). In PlasmoDB this would be accomplished by performing a number of queries and subsequently combining the results in the ‘query history’ section (Figure 1C, middle screen shot). For example, one may be interested in identifying a short list of possible vaccine candidates. One possible way of accomplishing this would be by identifying all proteins predicted to be exported to the host cell in *P. falciparum*. There are three exported protein datasets in PlasmoDB and a union (‘or’ function) of all three results retrieves 405 genes (Figure 1B, steps 1 and 2). To restrict this list further, intersecting (‘and’ function) these results with genes that have no orthologs in mammals reduces the results to 321 genes (Figure 1B, Step 3). Next a user may further prune this list by intersecting the results with other queries, such as genes that are nonpolymorphic between a chloroquine sensitive (3D7) and resistant strain (Dd2). This cuts the number of candidates to 32 genes (Figure 1B, Step 4 and Figure 1C, right screen shot). Alternatively, one may be interested in the genes that have protein expression evidence in a particular stage in the parasite’s life cycle (the results of an intersection with genes that have proteomic evidence in gametocyte yields 27 genes). Finally, examination of the list reveals several genes

encoding for rifins (a family of clonally variant proteins expressed on the surface of infected red blood cells) (38), and a user may wish to investigate genes other than rifins—this can be accomplished by excluding (‘not’ operation) results of a keyword query using the term ‘rifin’ (Figure 1B, Step 5 and Figure 1C, left most panel). A user may examine the specific gene pages for more gene-specific details, download results with their associated data or log in (if they have not done so already) to ensure that their search strategy is saved for future examination.

FUTURE DIRECTIONS

It is expected that PlasmoDB will continue its data content and tool expansion as user needs require. We anticipate the incorporation of multiple new data sets including microarray, proteomic and specific parasite isolate data. Additionally, over the next few years we look forward to incorporating sequence data from a dramatically expanded *Plasmodium spp.* sequencing effort (<http://www.genome.gov/26525388>). In the coming year, we will also release a new user interface that will include a workflow-based search strategy page, similar to what is shown in Figure 1B, which we anticipate will provide a more biologically intuitive and dynamic experience for scientists accessing PlasmoDB and other EuPathDB sites.

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