

Curation of the *Plasmodium falciparum* genome

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The malaria genome has proved invaluable to researchers worldwide in the continuing fight against malaria by stimulating and underpinning molecular approaches in gene expression studies, vaccine and drug discovery research, and by providing data to facilitate hypothesis-driven research. The combination of *in silico* and experimental investigations has already yielded dividends by strengthening our understanding of the many facets of the malaria parasite *Plasmodium falciparum*. The recently initiated curation of the genome resource is a vital investment for maintaining and enhancing the use of this genomic information in the post-genomic era.

Since the publication of the *Plasmodium falciparum* genome [1–3], efforts have continued to close the few remaining gaps in the sequence assemblies (Table 1). Chromosomes one to six, nine to 12, and 14 are now complete from telomere to telomere. Chromosomes seven and eight are both in two contigs, whereas chromosome 13 is currently in five contigs.

Sequence analysis has aided molecular investigations into many key areas of the parasite's biology by providing holistic, yet detailed, information, thus allowing the identification of putative regulatory elements, for example, those involved in the regulation of *var* gene expression. The *var* genes encode variant antigens, expressed on the host erythrocyte, which are known as *P. falciparum* erythrocyte membrane proteins (PfEMP). These proteins help the parasite to evade the host immune response. There are three conserved promoters, *upsA*, *upsB* and *upsC*, and their associated sub-structural sequence motifs, SPE1, SPE2 and CPE, defined to date [4] which correlate strongly with the chromosomal location of the associated *var* gene. The internal *var* genes are transcribed by *upsC*, the most subtelomerically located by *upsB*, and a third set by *upsA*. The *upsA* type is differentiated from the *upsB* type by being located closer to the centromere and is transcribed towards the centromere. There is evidence that the *var* intron has a key role in both silencing and activating transcription via interaction with a conserved regulatory sequence that is located upstream of *var* exon 1 [5]. This sequence is similar to the metazoan initiator element, Inr, a

pyrimidine-rich motif that is involved in initiation of transcription of many eukaryotic genes. Sequence alignments of *var* gene introns revealed three conserved regions with characteristic base-pair compositions, each with different effects on *var* gene silencing. The centrally located AT-rich element is sufficient in itself to silence transcription, but is also required for transcriptional activation [5]. Curation and re-annotation activities also assist in the goal of complete reconstruction of the parasite's metabolic pathways and their compartmentalization to define its metabolic capacity, and to identify enzymes and transporters for exploitation as potential drug targets. The completion of the genome sequence of *P. falciparum*, the addition of gene ontology annotation and enzyme commission (EC) numbers, and the attribution of function to hypothetical genes all contribute towards this important goal.

Accessing the *Plasmodium falciparum* genome

The malaria genome project consortium has a policy to release data to the research community as quickly as possible. Access to the genome and associated resources is available from several sources (Table 2). In addition to the public databases [e.g. GenBank (<http://www.ncbi.nih.gov/Genbank/>), European Molecular Biology Laboratory (<http://www.embl.org>) and DNA Data Bank of Japan (<http://www.ddbj.nig.ac.jp/>)], many malaria researchers are familiar with the *Plasmodium* genome database PlasmoDB [8–10]. This database integrates the DNA sequence with automated and curated annotation, RNA and protein expression profiling data [6–9], and cross-genome comparisons [10], in addition to other tools and emerging genomic- and proteomic-scale datasets. The relational database architecture of PlasmoDB permits queries based on: (i) DNA and protein sequences; (ii) gene and/or protein annotations; (iii) RNA and protein expression data [11–13]; (iii) taxonomic relationships with related species [14]; and (vi) comparisons with GenBank and other databases. These queries provide a powerful means of exploring the *Plasmodium* genome.

In 2003, another database option in the form of GeneDB [15] became available to malaria researchers. GeneDB is a multi-organism genome database for both eukaryotes and prokaryotes containing data produced by the Wellcome Trust Sanger Institute (WTSI; <http://www.sanger.ac.uk>)

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Table 1. *Plasmodium falciparum* genome sequencing status^a

Chromosome no. and sequencing centre (accession no.)	Chromosome size (Kb)	Protein encoding genes (those with introns)	Status at publication by October 2002	Current status or no. of contigs	Interesting genes and/or links	Gene identifier	Refs
1 WTSI (AL844501)	643 292	142 (43)	Finished	1	Calcium-transporting ATPase	MAL1P1.54	[1–3]
2 TIGR and NMRC (AE001362)	947 102	223 (127)	Finished	1	Transmission-blocking target antigen s230 precursor	PFB0405w	[21]
3 WTSI (AL844502)	1 060 087	240 (141)	Finished	1	Cyclophilin	PFC0975c	[22]
4 WTSI (AL844503)	1 204 112	242 (138)	Finished	1	Blood-stage antigen (ag-1 homologue)	PFD0255w	[1–3]
5 WTSI (AL844504)	1 343 552	313 (164)	Finished	1	Rab1 protein	PFE0690c	[1–3]
6 WTSI (AL844505)	1 418 244	312 (165)	Gap closure (gaps 8)	1	PK4 protein kinase	PFF1370w	
7 WTSI (AL844506)	1 351 552	275 (153)	Gap closure (gaps 13)	2	Putative chloroquine transporter	MAL7P1.27	
8 WTSI (AL844507)	1 325 595	296 (169)	Gap closure (gaps 24)	2	Rifin	PF08_0104	
9 WTSI (AL844508)	1 541 723	365 (216)	Finished	1	Cytoadherence-linked sexual protein	CLAG-9	[1–3]
10 TIGR and NMRC (AE014185)	1 694 445	403 (207)	Gap closure (gaps 4)	5	MSP-3	PF10_0345	
11 TIGR and NMRC (AE014186)	2 035 250	504 (250)	Gap closure (gaps 3)	4	AMA-1; apical membrane precursor 1	PF11_0344	
12 Stanford (AE014188)	2 271 477	526 (269)	Finished	1	HMG	PFL0145c	[23]
13 WTSI (AL844509)	2 732 359	672 (354)	Gap closure (gaps 37)	5	RNA polymerase α subunit	PF13_0150	
14 TIGR and NMRC (AL014187)	3 291 006	769 (384)	Gap closure (gaps 3)	4	Trophozoite cysteine proteinase precursor,	PF14_0553	

^aAbbreviations: AMA, apical membrane antigen; HMG, high mobility group protein; MSP, merozoite surface protein; NMRC, Centre for Molecular and Biomolecular Informatics at the University of Nijmegen, (<http://www.cmbi.kun.nl/>); Stanford, Stanford University (<http://www.stanford.edu/>); TIGR, The Institute for Genomic Research (<http://www.tigr.org/>); WTSI, Wellcome Trust Sanger Institute.

and other collaborating sequencing centres. Although both finished and ongoing projects are represented in GeneDB, the emphasis is on the manual curation of genomes. GeneDB focuses on representing the most up-to-date view of the manual annotation of the *P. falciparum* genome. The *Plasmodium berghei*, *Plasmodium chabaudi* and *Plasmodium knowlesi* genomes are also represented although, for the most part, these species have not yet been manually annotated. Manual annotation of the *P. knowlesi* genome recently commenced in collaboration with the Centre for Molecular and Biomolecular Informatics at the University of Nijmegen, The Netherlands (<http://www.cmbi.kun.nl/>). GeneDB also houses the genomes of many other parasitic organisms, including manually curated genomes of *Leishmania major*, *Trypanosoma brucei* and *Trypanosoma cruzi*. PlasmoDB and GeneDB are both based on a common underlying database architecture (the genomics unified schema; GUS) [16], which helps in the exchange of data and tools between databases. For instance, the Boolean query interface of GeneDB should be familiar to users of PlasmoDB.

The role of curation

A coordinated and effective genome curation effort is important for maximizing the benefits from the investment

made to obtain the *P. falciparum* genome sequence. This effort must evolve to ensure the databases provide new data and analysis methods as they become available. The benefits of such a long-term commitment have been evident in the curation of other genomes, such as *Saccharomyces cerevisiae*, which supports and stimulates experimental research by making *in silico* investigations as thorough, incisive and convenient as possible. The *P. falciparum* curation process is in its early stages and has been a core activity at the WTSI since October 2003. The Institute for Genomic Research (TIGR; <http://www.tigr.org/>) has recently initiated curation activities for *P. falciparum*, supported by funds remaining from the chromosome 14 sequencing project (supported by the Burroughs Wellcome Fund). The initial phase will be concomitant with provision of the finished sequence, standardization of gene names and resubmission to the public databases. Although these resources lack many of the sophisticated and organism-specific query tools found in more specialized databases, major public databases such as GenBank, EMBL and DDBJ are still the primary references for many researchers, particularly those outside the malaria field. The curators will work together along with the PlasmoDB and GeneDB teams to provide annotation and curation updates to support these and other appropriate databases.

Table 2. Plasmodium web resources^a

Resource	URL	Description
PlasmoDB	http://plasmodb.org	PlasmoDB home page
Release 4.2	http://plasmodb.org/restricted/ddspecies.shtml	Web downloads
GeneDB	http://www.genedb.org/	GeneDB home page
WTSI	http://www.sanger.ac.uk/Projects/P_falciparum/ ftp://ftp.sanger.ac.uk/pub/pathogens/malaria2/	WTSI sequencing project details FTP download
TIGR	http://www.tigr.org/tdb/e2k1/pfa1/ ftp://ftp.tigr.org/pub/data/Eukaryotic_Projects/p_falciparum	TIGR home page Data download
SGTC	http://sequence-www.stanford.edu/group/malaria/	Useful links to publications and the malaria research community
PlasmoCyc	http://biocyc.org/PFA/server.html	Metabolic pathway reconstruction and mapping
Plasmodium metabolic pathways	http://sites.huji.ac.il/malaria/	Knowledge base for <i>P. falciparum</i> biology, biochemistry and physiology
MR4	http://www.malaria.mr4.org/	Malaria Research and Reference Reagent Resource Center
NCBI	Http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=36329	Chromosome maps (STS, contigs, genes) with sequence viewer. Extensive links and download facility.
ACT	http://www.sanger.ac.uk/Software/ACT/	DNA sequence comparison viewer based on Artemis. Comparison usually based on Blastn or Tblastx search. Available as freeware (GNU general public licence). Full documentation and download installation help.

^aAbbreviations: ACT, Artemis Comparison Tool; NCBI, National Center for Biotechnology Information; SGTC, Stanford Genome Technology Center; TIGR, The Institute for Genomic Research; WTSI, Wellcome Trust Sanger Institute.

Curation combines several activities intended to facilitate querying and retrieval of data in a form that is accurate, comprehensive and relevant. Standard procedures are being developed to ensure the accuracy and consistency of annotation across the entire genome, and that data are synchronized and unified across each of the centres involved. It is important that genome sequences and annotation are updated regularly, and ensures that the different views of the genome annotation remain consistent as new sequence data, publications and feedback from the research community become available. Curation will also support query strategies and methodologies to more rapidly identify sets of proteins or genes of interest to the researcher. This is particularly true for gene ontology annotation (classifying gene products according to their molecular function, subcellular localization and metabolic processes [17]) and EC number annotation.

The roles of PlasmoDB and GeneDB

The curation activities at TIGR and WTSI are required for providing consistent genome-wide annotation to PlasmoDB and GeneDB, which enables them to generate complementary views of the genome. PlasmoDB gene pages differ from those of GeneDB in that they offer the user a comprehensive unfiltered view of alternative automated predictions with supporting evidence from many data sources. This allows the user to weigh the evidence and to make their own conclusion as to the correct gene model, and also permits annotation oversights or the need for revision to be detected. GeneDB, however, aims to show a single manually annotated view of the genome as a result of a careful review of predictions and their supporting evidence, which is updated on a weekly basis. This difference can be harnessed. Gene annotations are refined by capturing new information as it emerges, thus facilitating a more accurate description of the gene and its product. These modifications will be

submitted back to PlasmoDB, thus improving the depth, accuracy and quality of the annotation, and making querying and analysis more accurate and insightful. The presentation of GeneDB, especially the Artemis applet [18,19], allows the user to view the genome and annotation equivalent to that of an annotator's view. This enables convenient visualization of fine structural features, such as intron–exon boundaries or regulatory elements in the context of the surrounding genome sequence. Members of the research community are invited to have an active role in accelerating this process by donating their expertise and knowledge into the manual review of gene annotation, and by providing evidence to ensure that their experimental results are represented and interpreted properly. Feedback can be directed via several routes: (i) e-mail to help@PlasmoDB.org is entered into a tracking system, which is accessible to curators at TIGR and GeneDB. PlasmoDB has also implemented a 'user comments' form enabling researchers to add comments to any gene. These comments are fully searchable and can be reviewed by other users, providing an opportunity for dissemination of ideas and discussion threads to develop. (ii) Feedback can also be made directly to the curators at WTSI and TIGR by following the hyperlink at the bottom of GeneDB gene pages, thus allowing users to make structured comments for specific aspects of a gene's annotation or to make more general points. Such comments can be viewed by all users, and will be used by curators at WTSI and TIGR to inform them in the curation process.

Initial work

The published *P. falciparum* genome sequence comprised seven finished chromosomes and seven unfinished chromosomes. Genes encoded by unfinished chromosomes were assigned temporary identifiers. As chromosomes are finished and re-annotated, an obvious difference that will be seen is that the temporary identifiers will be replaced with final identifiers according to an agreed format

[e.g. MAL6P1.1 became PFF1595c, where PF denotes *Plasmodium falciparum*, and F denotes chromosome six; the suffix w or c is used to denote the forward (Watson) or reverse (Crick) strand]. Because the old gene names are already in widespread use and have been used in the literature, a mapping between the new and old names is provided (<ftp://ftp.sanger.ac.uk/pub/pathogens/Plasmodium/falciparum/>), and old names have been retained as fully searchable synonyms. Since the publication of the *P. falciparum* genome, the sequence of chromosome six has been completed adding ~39 Kb to the previous version. This and revision of the existing sequence have resulted in the addition of eight new gene predictions: (i) PFF0040c, *rif* pseudogene; (ii) PFF0045c, *rif* pseudogene; (iii) PFF0050c, conserved hypothetical; (iv) PFF0060w, conserved hypothetical; (v) PFF0065c, conserved hypothetical; (vi) PFF0070w, *var* pseudogene; (vii) PFF0847w, *stevor* pseudogene; and (viii) PFF1510w, conserved hypothetical). Upon re-mapping, MAL6P1.310 was removed because part of its underlying sequence was incorporated into PFF0040c. MALP1.297 was also removed because of a lack of supporting evidence for a convincing gene model. Interestingly, the new gene predictions are located in a region of new sequence in the left subtelomere that is inverted with respect to a similar region in the right subtelomere. These differences can be viewed using the Artemis comparison tool (ACT), a DNA sequence comparison viewer based on the Artemis viewer and using either Blastn or Tblastx to make comparison (see Figure 1 for an example view). The regions of similarity are joined by red bars between the DNA sequences under comparison. ACT is available as freeware (Table 2) and will run on any system with a recent version of Java installed. An ACT comparison of the finished assembly of MAL6 to the previous version is also available (<ftp://ftp.sanger.ac.uk/pathogens/Plasmodium/falciparum/ACT/>).

Recently, glycosylphosphatidylinositol (GPI) anchor sequences have been added to the existing annotation. UTRs have crucial roles in controlling stability of the

mRNA transcript, its cellular and subcellular localization, and its efficiency of translation. UTRs can also harbor nucleotide sequence motifs that can be recognized by regulatory proteins. Curation can make the resource more easily to navigate by providing cross-links to external databases for primary sequence, for example: Uniprot, formerly the separate Swiss-Prot and TrEMBL (<http://www.ebi.uniprot.org/index.shtml>); (ii) metabolic pathways (KEGG; <http://www.genome.jp/kegg/>); (iii) protein families [SCOP (<http://scop.mrc-lmb.cam.ac.uk/scop/>), Pfam (<http://www.sanger.ac.uk/Software/Pfam/>) and Interpro (<http://www.ebi.ac.uk/interpro/>); (iv) Gene Ontology (GO; <http://www.geneontology.org/>) and (v) publications (PubMed; <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>). Part of the long-term commitment to curate the genome is to revise the annotation based on new publications appearing in the literature, and to revise and add gene ontology annotations. Although there is still much work to be done and a fully annotated genome will always be a 'moving target', some initial steps to ensure consistency and capture literature-sourced information have been implemented for *var*, *rifin*, *stevor* gene families and merozoite-surface proteins (MSP).

***Plasmodium falciparum* as a reference genome**

The regions of conserved synteny that are observed when comparing the *P. falciparum* genome with those of other *Plasmodium* spp. (*P. knowlesi*, *P. berghei* and *P. chabaudi*) will probably provide additional evidence to increase the accuracy of gene prediction and annotation [20]. An initial comparison between a whole-genome shotgun sequence assembly of *P. knowlesi* and chromosome six of *P. falciparum* show considerable regions of conserved synteny (Figure 1 shows a view within ACT). This approach has been extended to a comparison of synteny between *P. falciparum*, *P. knowlesi* and *P. yoelii*, and is being used to improve the accuracy of gene prediction and speed manual annotation in *P. knowlesi* where possible. The available contigs of *P. knowlesi* and *P. yoelii* were ordered into metachromosomes (artificial constructs) by

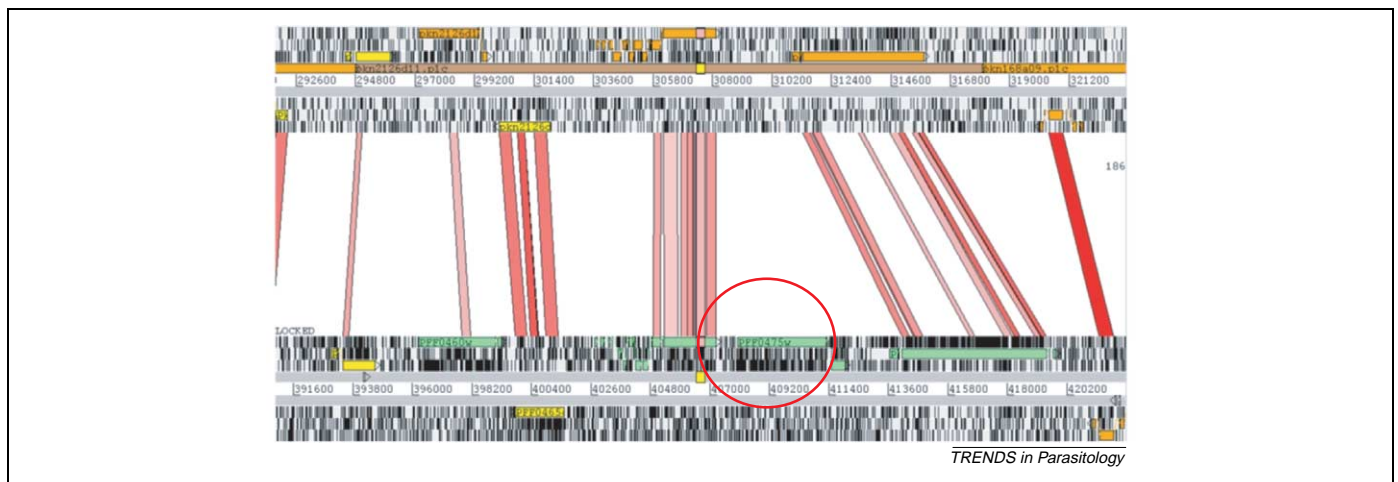


Figure 1. A view of an interruption of synteny between *Plasmodium knowlesi* and *Plasmodium falciparum*. An Artemis comparison tool (ACT; <http://www.sanger.ac.uk/Software/ACT>) view of a metachromosome from *P. knowlesi* (upper sequence) and *P. falciparum* chromosome six (lower sequence), showing conservation of gene order between the two sequences. This conservation is interrupted by the presence of PFF0475w in *P. falciparum* (circled red). Gene predictions for both sequences are displayed. BLAST hits are represented by bars (different shades of red) linking the locations of conservation between top and bottom sequences. Stop codons are represented by black vertical bars, as shown in the six frames of each sequence.

Tblastx comparison with *P. falciparum* chromosomes. Currently, gaps are present in the metachromosomes because the methodology does not process small contigs. This approach is not designed to replace the analysis that will come with a finished sequence, but does accelerate the annotation of *P. knowlesi* while sequencing is being completed. Comparison of the *P. knowlesi* and *P. yoelii* metachromosomes with their equivalent *P. falciparum* chromosome in Artemis Comparison Tool aids gene prediction, in particular, the definition of intron–exon boundaries, which can be difficult to predict computationally. This strategy has the advantage of highlighting where regions of conserved synteny are interrupted. Such breaks probably include species-specific genes, which will provide an interesting perspective for the investigation of the molecular mechanisms underlying key biological differences between the *Plasmodium* spp.

Conclusion

Cooperative and coordinated curation activities are needed to ensure that the *P. falciparum* genome sequence remains a potent stimulus to molecular research as we approach the completion of the sequence, and look forward to the accumulation of additional data derived from functional genomics, proteomics and other high-throughput approaches. A coordinated curation effort to maintain consistent and accurate genome-wide annotation, in addition to one that incorporates new datasets and analysis tools as they become available, is required. This effort will indirectly support the analysis of the genomes of other *Plasmodium* species. PlasmoDB and GeneDB provide different perspectives on the genome, with different characteristics that allow them to have mutually supportive roles in the process of incorporating new annotation as new experimental evidence emerges. Feedback from members of the research community can help improve accuracy, quality and depth of the annotation, in addition to database presentation and querying approaches.

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