D334–D338 *Nucleic Acids Research, 2004, Vol. 32, Database issue*
DOI: 10.1093/nar/gkh115

Full-malaria 2004: an enlarged database for comparative studies of full-length cDNAs of malaria parasites, *Plasmodium* species

Junichi Watanabe*, Yutaka Suzuki[1], Masahide Sasaki[1] and Sumio Sugano[1]

Department of Parasitology and [1]Laboratory of Genome Structure Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minatoku, Tokyo 108-8639, Japan

Received September 15, 2003; Revised and Accepted October 15, 2003

ABSTRACT

Full-malaria (http://fullmal.ims.u-tokyo.ac.jp), a database for full-length cDNAs from the human malaria parasite, *Plasmodium falciparum* has been updated in at least three points. (i) We added 8934 sequences generated from the addition of new libraries, so that our collection of 11 424 full-length cDNAs covers 1375 (25%) of the estimated number of the entire 5409 parasite genes. (ii) All of our full-length cDNAs and GenBank EST sequences were mapped to genomic sequences together with publicly available annotated genes and other predictions. This precisely determined the gene structures and positions of the transcriptional start sites, which are indispensable for the identification of the promoter regions. (iii) A total of 4257 cDNA sequences were newly generated from murine malaria parasites, *Plasmodium yoelii yoelii*. The genome/cDNA sequences were compared at both nucleotide and amino acid levels, with those of *P.falciparum*, and the sequence alignment for each gene is presented graphically. This part of the database serves as a versatile platform to elucidate the function(s) of malaria genes by a comparative genomic approach. It should also be noted that all of the cDNAs represented in this database are supported by physical cDNA clones, which are publicly and freely available, and should serve as indispensable resources to explore functional analyses of malaria genomes.

INTRODUCTION

Malaria is the most devastating parasitic disease in the world; it kills more than a million people every year. *Plasmodium falciparum* is the causative agent of the lethal form of malaria in humans. Thus, the recent completion of the genome sequencing for *P.falciparum*, ~23 Mb on 14 chromosomes (seven finished and seven unfinished) has been a great milestone, which provides invaluable information about this organism (1–5). Mass spectrometry and oligonucleotide array techniques have been utilized to characterize ~5000 candidate genes (6,7). However, these techniques depend upon the correct annotation of the gene structure. Furthermore, to understand the mechanism(s) by which the parasite controls expression of genes throughout its complicated life cycle, the elucidation of transcription factors and binding motifs are mandatory.

Full-malaria started as a database for full-length cDNA clones produced from the erythrocyte-stage parasite of *P.falciparum* using the oligo-capping method, while the genome sequencing efforts were concurrently underway (8,9). It consisted of 5′ one-pass information, supported by corresponding physical plasmid clones, which are deposited at MR4 (http://www.malaria.mr4.org/).

NEW FEATURES

In this update, we made two additional libraries from *P.falciparum* and determined 8934 sequences. Originally we used a full-length enriched library from erythrocyte-stage parasites of *P.falciparum* and reported 5′ end one-pass sequence of 2490 random clones (8). Since then, we have produced two additional libraries from parasites, which were grown under different condition(s), and determined a total of 11 424 clones. Determined sequences were compared with genome nucleotide sequences and displayed on the graphical map along with annotated and predicted genes with three different software packages (PlasmoDB). In total, 1375 genes were represented by full-length clones. Their physical plasmids are available for various experiments (Table 1).

As the genome sequences became publicly available, all the cDNA sequences were mapped on 14 chromosomes using BLAT and sim4 programs (10,11) and the exact alignment was graphically presented.

The chromosome map is viewed by choosing the chromosome number and the positions of both ends of the region of interest, or by searching for the Full-malaria clone name or the annotated gene name (Fig. 1). The magnification level can easily be changed. Alternatively, BLASTN will search for similar sequences within the database, enabling the location of the gene to be determined. Regarding each of the genes, hydropathy plot analysis and motif searches (Pfam: http://www.ebi.ac.uk/interpro/) were performed based on the deduced amino acid sequences and the results are represented graphically. Predictions of protein subcellular localization is

*To whom correspondence should be addressed. Tel/Fax: +81 3 5689 3979; Email: jwatanab@ims.u-tokyo.ac.jp

Table 1. The numbers of predicted annotated genes and genes represented by full-length clones are shown for *Plasmodium falciparum* and *Plasmodium yoelii*

Chromosome	*P.falciparum*				*P.yoelii* orthologues			
	Annotated genes	Genes represented by full cDNAs	Genes represented by ESTs	Total represented genes	Annotated genes	Genes represented by full cDNAs	Genes represented by ESTs	Total represented genes
Chr1	155	28	18	44	81	10	53	53
Chr2	224	50	43	80	136	26	83	87
Chr3	245	61	59	114	195	35	120	129
Chr4	249	63	42	91	163	40	107	114
Chr5	330	69	84	146	261	59	157	172
Chr6	319	99	71	153	253	46	155	163
Chr7	297	59	53	104	214	58	120	137
Chr8	299	82	73	150	233	50	136	144
Chr9	366	97	114	176	281	49	142	157
Chr10	404	105	106	191	295	65	173	184
Chr11	514	132	127	230	380	61	217	225
Chr12	533	132	120	230	425	88	243	259
Chr13_1	682	194	166	327	573	116	312	344
Chr13_2	5	0	0	0	3	1	2	2
Chr14	776	204	171	327	636	122	326	353
Unmapped1	2	0	0	0	0	0	0	0
Unmapped2	1	0	0	0	0	0	0	0
Unmapped3	1	0	0	0	0	0	0	0
Unmapped4	7	0	0	0	7	1	1	1
Total	5409	1375	1247	2363	4136	827	2347	2524

also possible, using PSORT, PSORTII (http://psort.ims.u-tokyo.ac.jp) and SubLoc (http://www.bioinfo.tsinghua.edu.cn/SubLoc/eu_batchpredict.htm) (Fig. 1).

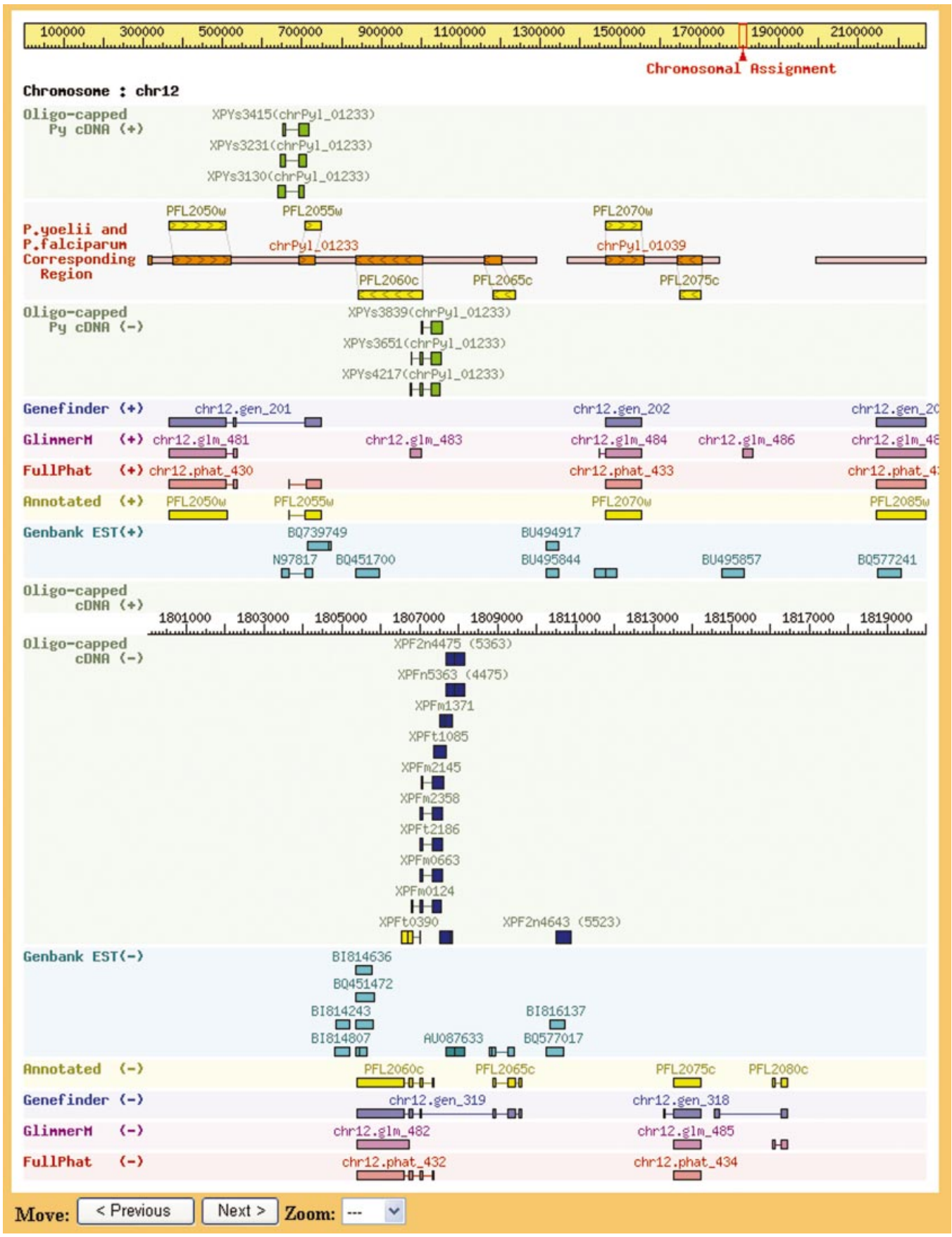
We incorporated EST sequence data downloaded from GenBank and mapped on the chromosomes. Interestingly, some Full-malaria clones and ESTs represent different sets of genes. Using both Full-malaria cDNAs and ESTs, numerous modifications in gene structures were identified, including the existence of non-coding exon(s), alternative splicing events, correction of splicing and even the identification of hitherto unknown genes. A summary of the statistics from the current Full-malaria database is shown in Table 1.

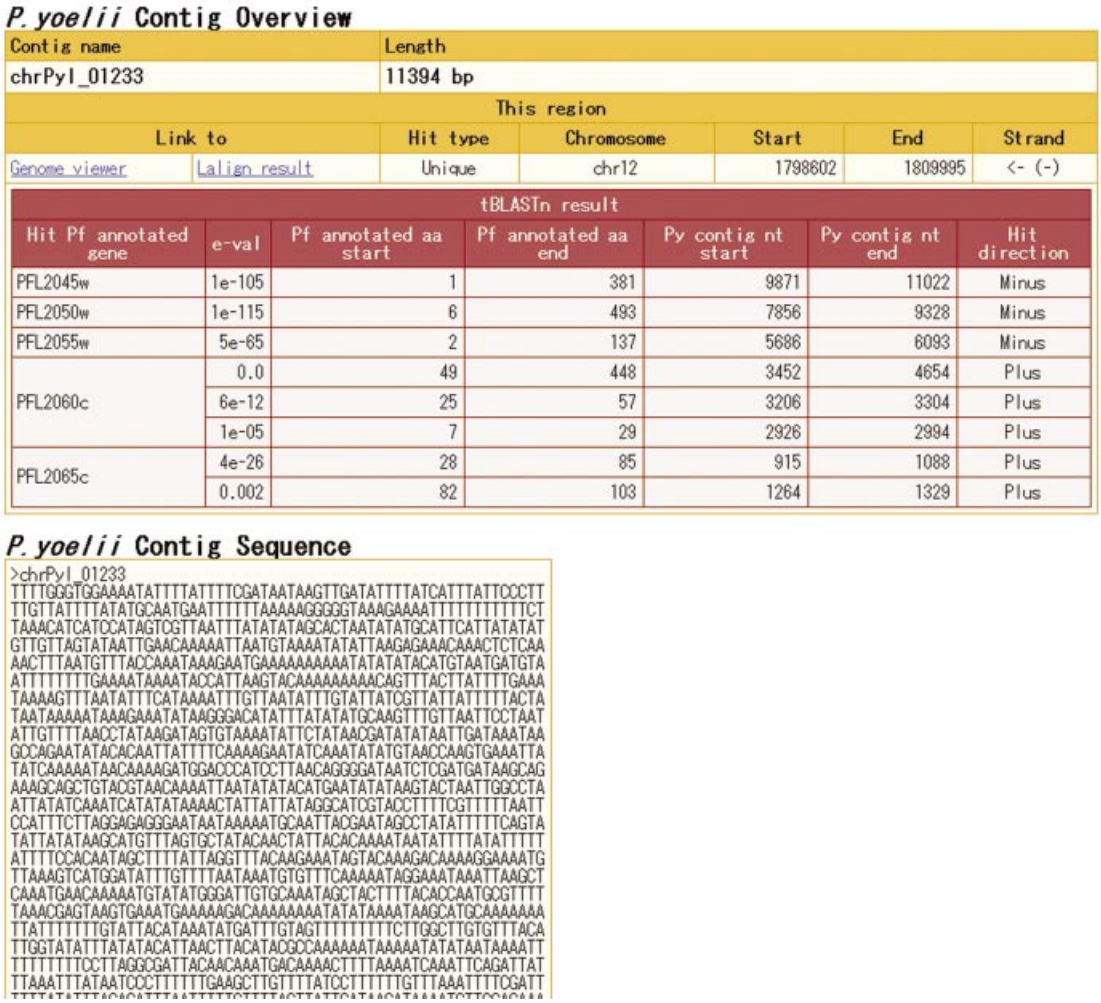
Furthermore, in order to provide a useful platform for the comparative genomics of *Plasmodium* species, we constructed a full-length cDNA library from murine malaria parasite *Plasmodium yoelii*, which was propagated *in vivo*. As a result of random sequencing analysis, we determined 4257 5′end one-pass sequences. We also mapped those cDNA sequences along with 5×-coverage draft genome sequences of this organism (12) (Fig. 1 upper part). Comparisons of contig nucleotide sequences of *P.yoelii* with the amino acid sequences of annotated genes of *P.falciparum* using TBLASTN, successfully aligned 1740 contigs with 4136 genes (Figs 1 and 2). Synteny is conserved in all *P.yoelii* genes at the genomic level, except for one contig in which the gene order is reversed.

The sequence alignments were further analyzed at the nucleotide level using Lalign (13). These results are shown in the *P.falciparum* chromosome map and a click on the *P.yoelii* contig box will display the details of these comparisons (Fig. 3). Furthermore, at the nucleotide level synteny is quite well preserved between these two species. The locations of full-length clones are mostly in accordance with the predicted gene structures. Comparison of the promoter regions of both species is of great interest.

Comparative analysis of full-length cDNA of *P.falciparum* and conservation of amino acid sequences with *P.yoelii* revealed that the start sites of some of the annotated genes are predicted falsely. The actual gene may start from a position further downstream. Some very large annotated genes seem to represent two or more genes. Indeed, exact information on full-length cDNAs supported by physical full-length cDNA clones is indispensable for precise annotation of the correct gene structures. For further information regarding genes for which revision of the annotation should be necessary, please refer to our database (http://fullmal.ims.u-tokyo.ac.jp/annotation); the details of this issue will be described elsewhere (J. Watanabe, M. Sasaki, Y. Suzuki and S. Sugano, in preparation). Expansion of comparative analysis to genome sequences along with full-length cDNA of other apicomplexan organisms will be also useful for investigations of evolution and for analysis of the pathogenicity of respective parasites.

Figure 1. (Next page) A view of the map showing a region of chromosome 12 (1800001–182000). The scale in the center shows the position within the *P.falciparum* genome sequence. Structures of the annotated genes and genes predicted by Genefinder, GlimmerM and FullPhat are shown as colored boxes. Boxes above the scale indicate that the genes are in the positive direction and those below are in the negative direction. Full-malaria clones are shown in the boxes nearest to the scale. Blue box, full-length clone; dark blue, probably full-length clone; light blue, possibly full-length clone; yellow, non-full clone. GenBank ESTs are shown in turquoise. In the upper part of the map, *P.yoelii* contigs are aligned with the *P.falciparum* genome, as described in the text. Red line, unique alignment; blue line, alignment with multiple sites; purple line, chimeric contig. Brown boxes represent the aligned *P.yoelii* predicted genes. Yellow boxes next to the contig line are the *P.falciparum* annotated genes. Boxes above the line are plus direction and those below the line are minus direction. Arrows in boxes also show the forward direction of the genes. A click on the contig line will open the alignment table.


100000
300000
500000
700000
900000
1100000
1300000
1500000
1700000
1900000
2100000
Chromosomal Assignment
Chromosome : chr12
Oligo-capped Py cDNA (+)
XPYs3415(chrPyl_01233)
XPYs3231(chrPyl_01233)
XPYs3130(chrPyl_01233)
P.yoelii and P.falciparum Corresponding Region
PFL2050w
PFL2055w
PFL2070w
chrPyl_01233
chrPyl_01039
PFL2060c
PFL2065c
PFL2075c
Oligo-capped Py cDNA (-)
XPYs3839(chrPyl_01233)
XPYs3651(chrPyl_01233)
XPYs4217(chrPyl_01233)
Genefinder (+)
chr12.gen_201
chr12.gen_202
GlimmerM (+)
chr12.glm_481
chr12.glm_483
chr12.glm_484
chr12.glm_486
FullPhat (+)
chr12.phat_430
chr12.phat_433
Annotated (+)
PFL2050w
PFL2055w
PFL2070w
PFL2085w
Genbank EST(+)
BQ739749
BU494917
N97817
BQ451700
BU495844
BU495857
BQ577241
Oligo-capped cDNA (+)
1801000
1803000
1805000
1807000
1809000
1811000
1813000
1815000
1817000
1819000
Oligo-capped cDNA (-)
XPF2n4475 (5363)
XPFn5363 (4475)
XPFm1371
XPFt1085
XPFm2145
XPFm2358
XPFt2186
XPFm0663
XPFm0124
XPFt0390
XPF2n4643 (5523)
Genbank EST(-)
BI814636
BQ451472
BI814243
BI816137
BI814807
AU087633
BQ577017
Annotated (-)
PFL2060c
PFL2065c
PFL2075c
PFL2080c
Genefinder (-)
chr12.gen_319
chr12.gen_318
GlimmerM (-)
chr12.glm_482
chr12.glm_485
FullPhat (-)
chr12.phat_432
chr12.phat_434
Move:
< Previous
Next >
Zoom:



***P. yoelii* Contig Overview**

Contig name	Length
chrPyI_01233	11394 bp

This region

Link to		Hit type	Chromosome	Start	End	Strand
Genome viewer	Lalign result	Unique	chr12	1798602	1809995	<- (-)

tBLASTn result

Hit Pf annotated gene	e-val	Pf annotated aa start	Pf annotated aa end	Py contig nt start	Py contig nt end	Hit direction
PFL2045w	1e-105	1	381	9871	11022	Minus
PFL2050w	1e-115	6	493	7856	9328	Minus
PFL2055w	5e-65	2	137	5686	6093	Minus
PFL2060c	0.0	49	448	3452	4654	Plus
	6e-12	25	57	3206	3304	Plus
	1e-05	7	29	2926	2994	Plus
PFL2065c	4e-26	28	85	915	1088	Plus
	0.002	82	103	1264	1329	Plus

***P. yoelii* Contig Sequence**

>chrPyI_01233
TTTTGGGTGGAAAATATTTTATTTTCGATAATAAGTTGATATTTTATCATTTATTCCCTT
TTGTTATTTTATATGCAATGAATTTTTTAAAAAGGGGGTAAAGAAAATTTTTTTTTTCT
TAAACATCATCCATAGTCGTTAATTTATATATAGCACTAATATATGCATTCATTATATAT
GTTGTTAGTATAATTGAACAAAAATTAATGTAAAATATATTAAGAGAAACAAACTCTCAA
AACTTTAATGTTTACCAAATAAAGAATGAAAAAAAAAATATATATACATGTAATGATGTA
ATTTTTTTTGAAAATAAAATACCATTAAGTACAAAAAAAAACAGTTTACTTATTTTGAAA
TAAAAGTTTAATATTTCATAAAATTTGTTAATATTTGTATTATCGTTATTATTTTTACTA
TAATAAAAATAAAGAAATATAAGGGACATATTTATATATGCAAGTTTGTTAATTCCTAAT
ATTGTTTTAACCTATAAGATAGTGTAAAATATTCTATAACGATATATAATTGATAAATAA
GCCAGAATATACACAATTATTTTCAAAAGAATATCAAATATATGTAACCAAGTGAAATTA
TATCAAAAATAACAAAAGATGGACCCATCCTTAACAGGGGATAATCTCGATGATAAGCAG
AAAGCAGCTGTACGTAACAAAATTAATATATACATGAATATATAAGTACTAATTGGCCTA
ATTATATCAAATCATATATAAAACTATTATTATAGGCATCGTACCTTTTCGTTTTTAATT
CCATTTCTTAGGAGAGGGAATAATAAAAATGCAATTACGAATAGCCTATATTTTTCAGTA
TATTATATAAGCATGTTTAGTGCTATACAACTATTACACAAAATAATATTTTATATTTTT
ATTTTCCACAATAGCTTTTATTAGGTTTACAAGAAATAGTACAAAGACAAAAGGAAAATG
TTAAAGTCATGGATATTTGTTTTAATAAATGTGTTTCAAAAATAGGAAATAAATTAAGCT
CAAATGAACAAAAATGTATATGGGATTGTGCAAATAGCTACTTTTACACCAATGCGTTTT
TAAACGAGTAAGTGAAATGAAAAAGACAAAAAAAATATATAAAATAAGCATGCAAAAAAA
TTATTTTTTTGTATTACATAAATATGATTTGTAGTTTTTTTTTCTTGGCTTGTGTTTACA
TTGGTATATTTATATACATTAACTTACATACGCCAAAAAATAAAAATATATAATAAAATT
TTTTTTTTCCTTAGGCGATTACAACAAATGACAAAACTTTTAAAATCAAATTCAGATTAT
TTAAATTTATAATCCCTTTTTTGAAGCTTGTTTTATCCTTTTTTGTTTAAATTTTCGATT

Figure 2. The results of TBLASTN are shown in table and graphic view. A click of the Lalign button will show the results of Lalign (as in Fig. 3).

ACKNOWLEDGEMENTS

We thank DYNACOM Co., Ltd for providing experienced technical assistance. Nucleotide sequences and gene predictions were downloaded from PlasmoDB (http://plasmoDB.org). This database has been constructed and maintained by a Grant-in-Aid for Publication of Scientific Research Results from the Japan Society for the Promotion of Science.

REFERENCES

1. Gardner,M.J., Hall,N., Fung,E., White,O., Berriman,M., Hyman,R.W., Carlton,J.M., Pain,A., Nelson,K.E., Bowman,S. *et al.* (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature*, **419**, 498–511.
2. Florens,L., Washburn,M.P., Raine,J.D., Anthony,R.M., Grainger,M., Haynes,J.D., Moch,J.K., Muster,N., Sacci,J.B., Tabb,D.L. *et al.* (2002) A proteomic view of the *Plasmodium falciparum* life cycle. *Nature*, **419**, 520–526.
3. Hall,N., Pain,A., Berriman,M., Churcher,C., Harris,B., Harris,D., Mungall,K., Bowman,S., Atkin,R., Baker,S. *et al.* (2002) Sequence of *Plasmodium falciparum* chromosomes 1, 3–9 and 13. *Nature*, **419**, 527–531.
4. Gardner,M.J., Shallom,S.J., Carlton,J.M., Salzberg,S.L., Nene,V., Shoaibi,A., Ciecko,A., Lynn,J., Rizzo,M., Weaver,B. *et al.* (2002) Sequence of *Plasmodium falciparum* chromosomes 2, 10, 11 and 14. *Nature*, **419**, 531–534.
5. Hyman,R.W., Fung,E., Conway,A., Kurdi,O., Mao,J., Miranda,M., Nakao,B., Rowley,D., Tamaki,T., Wang,F. *et al.* (2002) Sequence of of *Plasmodium falciparum* chromosome 12. *Nature*, **419**, 534–537.
6. Lasonder,E., Ishihama,Y., Andersen,J.S., Vermunt,A.M., Pain,A., Sauerwein,R.W., Eling,W.M., Hall,N., Waters,A.P., Stunnenberg,H.G. *et al.* (2002) Analysis of the *Plasmodium falciparum* proteome by high-accuracy mass spectrometry. *Nature*, **419**, 537–542.
7. Le Roch,K.G., Zhou,Y., Blair,P.L., Grainger,M., Moch,J.K., Haynes,J.D., De la Vega,P., Holder,A.A., Batalov,S., Carucci,D.J. *et al.* (2003) Discovery of gene function by expression profiling of the malaria parasite life cycle. *Science*, **301**, 1503–1508.
8. Watanabe,J., Sasaki,M., Suzuki,Y. and Sugano,S. (2001) FULL-malaria: a database for a full-length enriched cDNA library from human malaria parasites, *Plasmodium falciparum*. *Nucleic Acids Res.*, **29**, 70–71.
9. Suzuki,Y. and Sugano,S. (2003) Construction of a full-length enriched and a 5′-end enriched cDNA library using the oligo-capping method. *Methods Mol. Biol.*, **221**, 73–91.
10. Florea,L., Hartzell,G., Zhang,Z., Rubin,G.M. and Miller,W. (1998) A computer program for aligning a cDNA sequence with a genomic DNA sequence. *Genome Res.*, **8**, 967–974.
11. Kent,W.J. (2002) BLAT—the BLAST-like alignment tool. *Genome Res.*, **12**, 656–664.

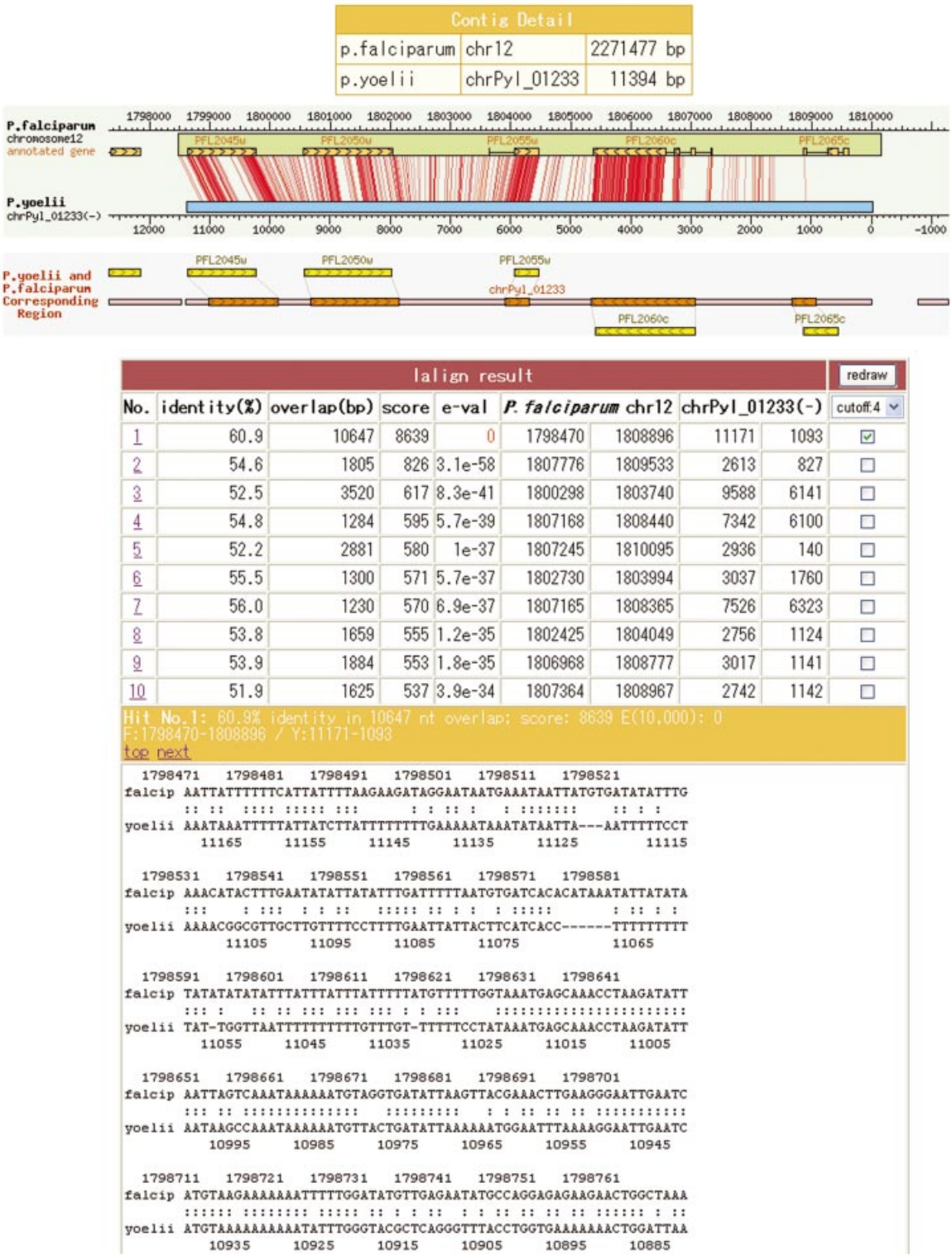


Figure 3. Similarity of the local nucleotide sequences is shown as red lines. A click on the Redraw button will show a new picture of the alignment at a different level.

12. Carlton,J.M., Angiuoli,S.V., Suh,B.B., Kooij,T.W., Pertea,M., Silva,J.C., Ermolaeva,M.D., Allen,J.E., Selengut,J.D., Koo,H.L. *et al*. (2002) Genome sequence and comparative analysis of the model rodent malaria parasite *Plasmodium yoelii yoelii*. *Nature*, **419**, 512–519.

13. Huang,X., Miller,W., Schwartz,S. and Hardison,R.C. (1992) Parallelization of a local similarity algorithm. *Comput. Appl. Biosci.*, **8**, 155–165.