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ToxoDB: an integrated *Toxoplasma gondii* database resource

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ABSTRACT

ToxoDB (http://ToxoDB.org) is a genome and functional genomic database for the protozoan parasite *Toxoplasma gondii*. It incorporates the sequence and annotation of the *T. gondii* ME49 strain, as well as genome sequences for the GT1, VEG and RH (Chr Ia, Chr Ib) strains. Sequence information is integrated with various other genomic-scale data, including community annotation, ESTs, gene expression and proteomics data. ToxoDB has matured significantly since its initial release. Here we outline the numerous updates with respect to the data and increased functionality available on the website.

INTRODUCTION

Toxoplasma gondii is an intracellular apicomplexan parasite capable of infecting humans. Infection is typically asymptomatic in healthy individuals, but may lead to congenital birth defects and encephalitis in immunosuppressed individuals (1,2). ToxoDB, initially released in May 2001, has been substantially updated in both content and functionality since last described in January 2003 (3). ToxoDB provides access to the genome sequence and annotation of the *T. gondii* ME49 strain. It also incorporates the genomic sequence of multiple other strains. The parasite genome is ~63 Mb in size and consists of 14 chromosomes (4).

The initial ToxoDB release was not supported by a relational database and thus the site had restricted functionality and little capability to integrate diverse data types such as gene expression data and single nucleotide polymorphism data (SNPs) with genomic sequence. Since initial publication, ToxoDB has been completely rebuilt using a common architecture similar to another apicomplexan database project, PlasmoDB (5). Both sites, along with CryptoDB, are component sites of ApiDB, the Apicomplexan Bioinformatics Resource Center (6). Many of the new methods of data loading, querying and presentation that are mentioned here have been applied to all of the ApiDB sites to provide a common research platform and facilitate data access among this group of related organisms. ApiDB (http://apidb.org/) serves as an 'umbrella' site for cross-species comparisons. Researchers can mine for *Toxoplasma* genes at ApiDB directly or via their orthologous relationship(s) to genes in other apicomplexan species.

CONTENT OF THE CURRENT RELEASE

Data

ToxoDB provides access to the genome sequence and annotation of *T. gondii* (ME49 strain) and the genomic sequence of the GT1, VEG and RH (Chr Ia and Chr Ib) strains. Annotation is also available for the apicoplast genome. The current database version (Release 4.2) also contains manual annotation (solicited in the initial genome annotation and entered by users as user comments), ESTs, TIGR Gene Indices clustered ESTs, SAGE tags, SNPs, cosmid and BAC ends, microarray and proteomics studies, all of which have been mapped to the genome (7,8). The database contains the results of automated analyses including gene predictions (using various algorithms), open reading frames (ORFs) greater than 50 aa and protein feature predictions

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Table 1. Data and analyses that have been integrated into ToxoDB and the number of genes that are impacted

Data type	Data source	Number of genes
Genes	TIGR	8032
Community annotation	Various contributors	1610
Orthologs	Generated from OrthoMCL	4616
GO terms	TIGR; InterPro	3136
EC numbers	TIGR	800
SNPs	John Boothroyd Laboratory; David Roos Laboratory	7322
Microarray	David Roos Laboratory	7664
ESTs	dbEST, TIGR Gene Indices	6080
SAGE tags	TgSAGEDB (14)	6284
Proteomics	Johnathan Wastling Laboratory; John Murray Laboratory	2435
Epitopes	IEDB	10
Metabolic pathways	KEGG Pathway	614

[signal peptides, transmembrane domains, hydrophobicity plots, AA content and InterPro domains (9)], Gene Ontology function predictions, and BLAST similarities to the NCBI non-redundant protein database (Table 1).

In addition, we have used the OrthoMCL algorithm to group genes from *T. gondii* with orthologous genes from 86 other eukaryotic and prokaryotic genomes (10). A mapping of immune epitopes identified in *Toxoplasma* provided by the Immune Epitope Database and Analysis Resource (IEDB) (11) has been integrated. Affymetrix probes mapped to the genome are visible in GBrowse, as are SNPs generated from nucmer alignments of sequences from the GT1, VEG and RH (Chr Ia and Ib) strains against the reference ME49 sequence. Two expression experiments utilizing a *Toxoplasma* Affymetrix array have also been deposited in ToxoDB. Users gain access to these new data types in record pages and by queries using the powerful query interface (see Data-Mining section).

Database architecture

As a part of the complete restructure of the ToxoDB resource, the practice of using flat files as a means of data storage was abandoned in early 2006. We now use GUS 3.5, and load data into an underlying Oracle database in a systematic fashion. GUS (Genomics Unified Schema) is an open source project (www.gusdb.org) with a rich relational schema including sequence annotation, expression data and proteomics using controlled vocabularies and ontologies (12).

ToxoDB also employs the GUS WDK (Web Development Kit, www.gusdb.org/wdk), to access the database from the internet dramatically improving the way the website operates. This transformation has added considerable increased functionality for database users and conforms to the model used by all ApiDB projects, making it possible for us to generate future database releases in short cycles.

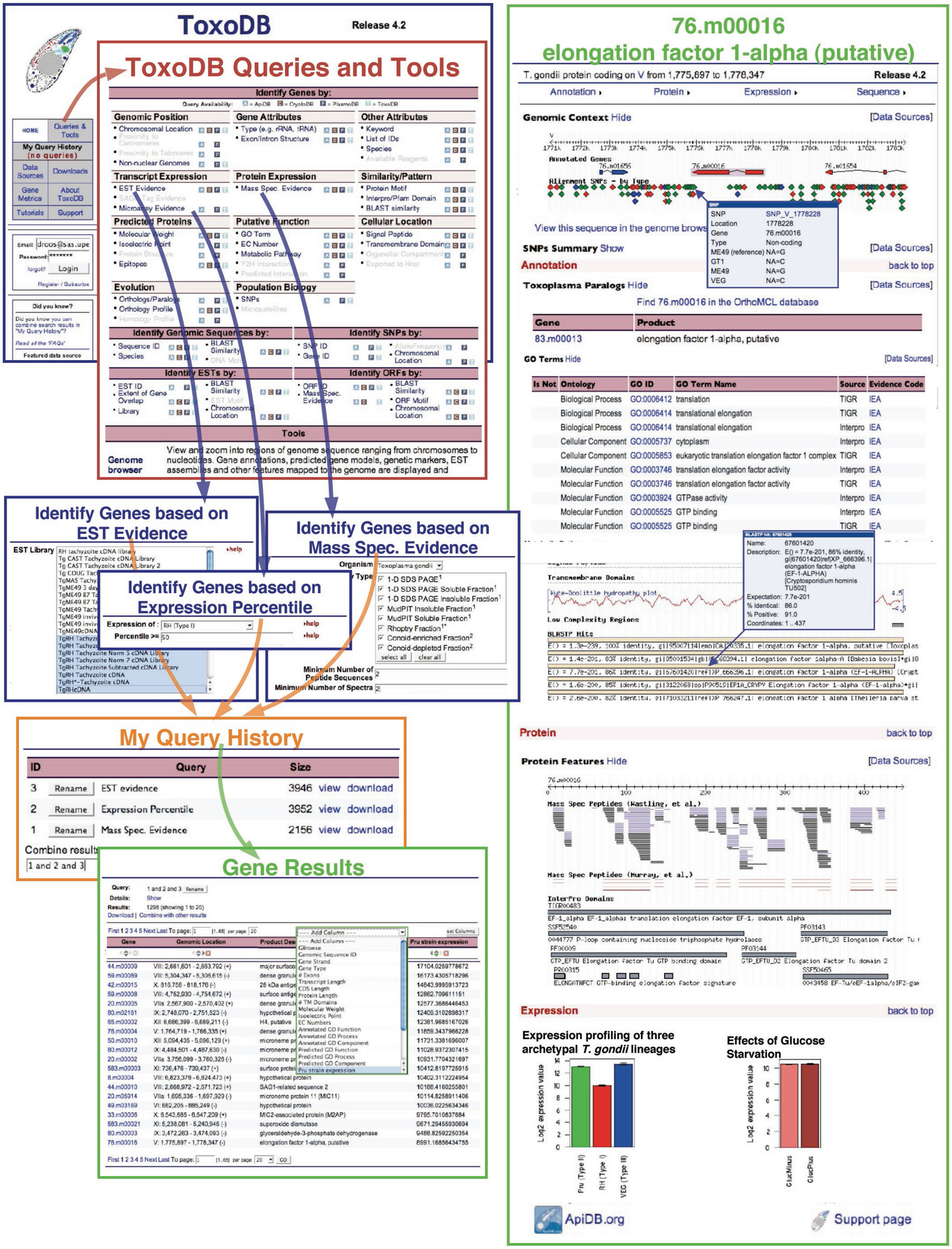
DATA-MINING TOOLS

ToxoDB currently provides 40 different queries of the data and several ancillary tools for analyzing, retrieving or viewing the data such as BLAST, Pathway Tools and an installation of the GMOD project Genome Browser (13). The ToxoDB 'Query & Tools' page has been restructured to make all queries available at a glance. Most of the individual queries have been reorganized into categories such as 'Position', 'Expression' and 'Function' to make them more intuitive to the average researcher. Enhanced functionality for the queries has also been added. For example, the ToxoDB keyword search has been significantly improved, offering the user control over which fields in the database are searched, including the official annotation, synonyms, user-supplied comments, domain names, BLAST similarities, etc. Many queries, such as 'Find SNPs based on Gene ID', now allow a gene ID list as input [either typed (or copied) by hand or uploaded from a file] facilitating analyses on large groups of genes. The results from all queries can be sorted based on various criteria (columns in the returned data set) and users can also add additional criteria for display (e.g. add columns to display protein features, GO annotation, expression characteristics for gene results, etc.) and sort on them as well. Once the appropriate selection of data types to display has been achieved, users can integrate these search results with other search results using the 'Query History' page, or the data can be downloaded in multiple formats for further analysis by the researcher (Figure 1).

ToxoDB uses the GBrowse genome browser (www.gmod.org) (13) to display gene models, EST alignments, SNPs, SAGE tags, etc. GBrowse enables visualization of the parasite genome and gene models, custom restriction-site identification, open reading frame identification, and facilitates download of data in various formats. Different data sets or analyses are displayed as individual tracks within the genome browser. There are approximately 50 GBrowse tracks available in the current version of ToxoDB. All genome sequences [ME49, GT1, VEG and RH (Chr Ia, Ib)] are also available in BLAST-searchable databases and for download in FASTA, GenBank and EMBL formats.

ToxoDB users may now register and log in to the site. Doing so enables a researcher to add comments to genes and genomic sequences. It also lets users save query results permanently. Queries in the Query History page can be organized (re-named or deleted) as well as combined with other results (Figure 1). This is a very powerful feature that allows users to refine their results so that precise sets of genes can be discovered.

The results may be downloaded using ToxoDB's improved reporting facility. It supports summary reports (Excel compatible tab delimited text), GFF, FASTA and a detailed report that includes almost all available data for each gene in the users result table. Use of this facility as well as many others on the site are now described in short video tutorials that are accessible from the database home page.



ToxoDB Release 4.2

HOME | Queries & Tools
My Query History (no queries)
Data Sources | Downloads
Gene Metrics | About ToxoDB
Tutorials | Support

Email: droos@sas.upe
Password: *******
forgot? Login
Register / Subscribe

Did you know?
Did you know you can combine search results in "My Query History"?
Read all the 'FAQs'
Featured data source

ToxoDB Queries and Tools

Identify Genes by:

Query Availability: A = ApiDB C = CryptoDB P = PlasmoDB T = ToxoDB

Genomic Position
- Chromosomal Location A C P T
- Proximity to Centromeres A P
- Proximity to Telomeres A P
- Non-nuclear Genomes A P T

Gene Attributes
- Type (e.g. rRNA, tRNA) A C P T
- Exon/Intron Structure A C P T

Other Attributes
- Keyword A C P T
- List of IDs A C P T
- Species A C P T
- Available Reagents A P

Transcript Expression
- EST Evidence A C P T
- SAGE Tag Evidence
- Microarray Evidence A P T

Protein Expression
- Mass Spec. Evidence A C P T

Similarity/Pattern
- Protein Motif A C P T
- Interpro/Pfam Domain A C P T
- BLAST similarity A C P T

Predicted Proteins
- Molecular Weight A C P T
- Isoelectric Point A P T
- Protein Structure A P
- Epitopes A C P T

Putative Function
- GO Term A C P T
- EC Number A C P T
- Metabolic Pathway A C P T
- Y2H Interaction A P
- Predicted Interaction A P

Cellular Location
- Signal Peptide A C P T
- Transmembrane Domain A C P T
- Organellar Compartment A P
- Exported to Host A P

Evolution
- Orthologs/Paralogs A P T
- Orthology Profile A C P T
- Homology Profile A P

Population Biology
- SNPs A P T
- Microsatellites

Identify Genomic Sequences by:
- Sequence ID A C P T
- Species A C P T
- BLAST Similarity A C P T
- DNA Motif

Identify SNPs by:
- SNP ID A P T
- Gene ID A P T
- AlleleFrequency A P
- Chromosomal Location A P T

Identify ESTs by:
- EST ID A P
- Extent of Gene Overlap A C P
- Library A C P
- BLAST Similarity A C P T
- EST Motif
- Chromosomal Location A C P T

Identify ORFs by:
- ORF ID A C P T
- Mass Spec. Evidence A C T
- BLAST Similarity A C P T
- ORF Motif A C P T
- Chromosomal Location A C P T

Tools

Genome browser View and zoom into regions of genome sequence ranging from chromosomes to nucleotides. Gene annotations, predicted gene models, genetic markers, EST assemblies and other features mapped to the genome are displayed and

Identify Genes based on EST Evidence

EST Library ▸help

RH tachyzoite cDNA library
Tg CAST Tachyzoite cDNA Library
Tg CAST Tachyzoite cDNA Library 2
Tg COUG Tach
TgMAS Tachy
TgME49 3 day
TgME49 B7 Ta
TgME49 B7 Ta
TgME49 Tach
TgME49 invivo
TgME49 invivo
TgME49cDNA
TgRH Tachyzo
TgRH Tachyzo
TgRH Tachyzoite Norm 5 cDNA Library
TgRH Tachyzoite Norm 7 cDNA Library
TgRH Tachyzoite Subtracted cDNA Library
TgRH Tachyzoite cDNA
TgRH*-Tachyzoite cDNA
TgRHcDNA

Identify Genes based on Expression Percentile

Expression of : RH (Type I) ▸help
Percentile >= 50 ▸help

Identify Genes based on Mass Spec. Evidence

Organism: Toxoplasma gondii
Type:
☑ 1-D SDS PAGE[1]
☑ 1-D SDS PAGE Soluble Fraction[1]
☑ 1-D SDS PAGE Insoluble Fraction[1]
☑ MudPIT Insoluble Fraction[1]
☑ MudPIT Soluble Fraction[1]
☑ Rhoptry Fraction[1*]
☑ Conoid-enriched Fraction[2]
☑ Conoid-depleted Fraction[2]
select all | clear all

Minimum Number of Peptide Sequences: 2
Minimum Number of Spectra: 2

My Query History

ID		Query	Size		
3	Rename	EST evidence	3946	view	download
2	Rename	Expression Percentile	3952	view	download
1	Rename	Mass Spec. Evidence	2156	view	download

Combine results
1 and 2 and 3

Gene Results

Query: 1 and 2 and 3 Rename
Details: Show
Results: 1298 (showing 1 to 20)
Download | Combine with other results

First 1 2 3 4 5 Next Last To page: 1 [1..65] per page: 20

--- Add Column --- | Reset Columns

Gene	Genomic Location	Product Des	Pru strain expression
44.m00009	VIII: 2,661,601 - 2,663,792 (+)	major surface	17104.0259778672
59.m00089	VIII: 5,304,347 - 5,306,615 (-)	dense granule	16173.4305718296
42.m00015	X: 816,758 - 818,176 (-)	28 kDa antige	14843.9995913723
59.m00008	VIII: 4,752,930 - 4,754,672 (+)	surface antige	12862.709611151
20.m00005	VIIa: 2,567,900 - 2,570,402 (+)	dense granule	12577.3686446453
80.m02161	IX: 2,748,070 - 2,751,523 (-)	hypothetical p	12409.3102898317
65.m00002	XII: 6,686,399 - 6,689,211 (-)	H4, putative	12381.9685167026
76.m00004	V: 1,764,719 - 1,766,335 (+)	dense granule	11859.3437966228
50.m00010	XII: 5,094,435 - 5,096,129 (+)	microneme pr	11731.3381696007
80.m00012	IX: 4,484,501 - 4,487,630 (-)	microneme pr	11028.9372307415
20.m00002	VIIa: 3,756,099 - 3,760,326 (-)	microneme pr	10931.7704321697
583.m00003	XI: 736,476 - 739,437 (+)	surface protei	10412.8197725915
8.m00004	VIII: 6,823,378 - 6,824,473 (+)	hypothetical protein	10402.3112224954
44.m00010	VIII: 2,668,972 - 2,671,723 (+)	SAG1-related sequence 2	10188.4160255801
20.m05914	VIIa: 1,695,336 - 1,697,929 (-)	microneme protein 11 (MIC11)	10114.8258911406
49.m03169	VI: 882,205 - 885,249 (-)	hypothetical protein	10036.0225634346
33.m00006	X: 6,543,685 - 6,547,209 (+)	MIC2-associated protein (M2AP)	9795.7010837684
583.m00021	XI: 5,238,081 - 5,240,945 (-)	superoxide dismutase	9671.29455930694
80.m00003	IX: 3,472,263 - 3,474,093 (-)	glyceraldehyde-3-phosphate dehydrogenase	9488.82592250354
76.m00016	V: 1,775,897 - 1,778,347 (-)	elongation factor 1-alpha, putative	8991.16856434755

First 1 2 3 4 5 Next Last To page: 1 [1..65] per page: 20 GO

76.m00016 elongation factor 1-alpha (putative)

T. gondii protein coding on V from 1,775,897 to 1,778,347 Release 4.2

Annotation ▸ Protein ▸ Expression ▸ Sequence ▸

Genomic Context Hide [Data Sources]

V
1771k 1772k 1773k 1774k 1775k 1776k 1777k 1778k 1779k 1780k 1781k 1782k 1783k
Annotated Genes
76.m01656
76.m00016
76.m01654
Alignment SNPs - by Type

SNP

SNP	SNP_V_1778228
Location	1778228
Gene	76.m00016
Type	Non-coding
ME49 (reference)	NA=G
GT1	NA=C
ME49	NA=G
VEG	NA=C

View this sequence in the genome brows

SNPs Summary Show [Data Sources]

Annotation back to top

Toxoplasma Paralogs Hide [Data Sources]

Find 76.m00016 in the OrthoMCL database

Gene	Product
83.m00013	elongation factor 1-alpha, putative

GO Terms Hide [Data Sources]

Is Not	Ontology	GO ID	GO Term Name	Source	Evidence Code
	Biological Process	GO:0006412	translation	TIGR	IEA
	Biological Process	GO:0006414	translational elongation	TIGR	IEA
	Biological Process	GO:0006414	translational elongation	Interpro	IEA
	Cellular Component	GO:0005737	cytoplasm	Interpro	IEA
	Cellular Component	GO:0005853	eukaryotic translation elongation factor 1 complex	TIGR	IEA
	Molecular Function	GO:0003746	translation elongation factor activity	Interpro	IEA
	Molecular Function	GO:0003746	translation elongation factor activity	TIGR	IEA
	Molecular Function	GO:0003924	GTPase activity	Interpro	IEA
	Molecular Function	GO:0005525	GTP binding	Interpro	IEA
	Molecular Function	GO:0005525	GTP binding	TIGR	IEA

BLASTP hit: 67601420

Name:	67601420
Description:	E() = 7.7e-201, 86% identity, gi|67601420|ref|XP_666396.1| elongation factor 1-alpha (EF-1-ALPHA) [Cryptosporidium hominis TU502]
Expectation:	7.7e-201
% Identical:	86.0
% Positive:	91.0
Coordinates:	1 .. 437

```
E() = 1.3e-239, 100% identity, gi|95007114|emb|CAJ20335.1| elongation factor 1-alpha, putative [Toxoplas
E() = 1.4e-201, 83% identity, gi|85001534|gb|AF68394.1| elongation factor 1alpha-A [Babesia bovis]•gi|8
E() = 7.7e-201, 86% identity, gi|67601420|ref|XP_666396.1| elongation factor 1-alpha (EF-1-ALPHA) [Crypt
E() = 1.6e-200, 85% identity, gi|3122068|sp|P90519|EF1A_CRYPV Elongation factor 1-alpha (EF-1-alpha)•gi|
E() = 2.6e-200, 82% identity, gi|71033211|ref|XP 766247.1| elongation factor 1 alpha [Theileria parva st
```

Protein back to top

Protein Features Hide [Data Sources]

76.m00016
0
100
200
300
400
Mass Spec Peptides (Wastling, et al.)
Mass Spec Peptides (Murray, et al.)
InterPro Domains
TIGR00483
EF-1_alpha EF-1_alpha: translation elongation factor EF-1, subunit alpha
SSF52540
0044777 P-loop containing nucleoside triphosphate hydrolases
PF03143
GTP_EFTU_D3 Elongation factor Tu
PF00009
GTP_EFTU Elongation factor Tu GTP binding domain
PF03144
GTP_EFTU_D2 Elongation factor Tu domain 2
PR00315
ELONGATNFCT GTP-binding elongation factor signature
SSF50465
0043458 EF-Tu/eEF-1alpha/eIF2-gam

Expression back to top

Figure 1. Screenshots showing the flow of a query in ToxoDB. From the Query & Tools page, users can go to particular queries for expression evidence (EST or Mass Spec Evidence), to the Results page where they can sort, manage (add or delete) columns of data and open gene pages. The Query History page permits users to manipulate previous queries including combining them and/or downloading the resulting data. Individual genes are listed on the Gene Results page and each gene has its own gene page, illustrated here by the gene encoding elongation factor 1-alpha. The gene page summarizes all information that is available for a gene including gene model predictions, SNPs, BLAST similarities, protein domains, ESTs, proteomic evidence of expression and microarray expression analyses.

FUTURE DIRECTIONS

The last two years were spent on major infrastructure and design elements for ToxoDB. Our future growth will be in the area of increased data acquisition and integration with existing and future data sets. Specifically, we are planning to load and integrate many expression data sets (RNA expression and protein expression) that are just becoming available. We also expect to load and integrate other array-based data sets such as ChIP on Chip and array CGH. As new data are added, we will be adding additional queries and tools to view these data. An area of significant future development will be improving the ability of users to compare the various different sequenced parasite strains visually and download sequence alignments between them.

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