The TIGR Gene Indices: clustering and assembling EST and known genes and integration with eukaryotic genomes

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Received September 14, 2004; Revised and Accepted October 5, 2004

ABSTRACT

Although the list of completed genome sequencing projects has expanded rapidly, sequencing and analysis of expressed sequence tags (ESTs) remain a primary tool for discovery of novel genes in many eukaryotes and a key element in genome annotation. The TIGR Gene Indices (http://www.tigr.org/tdb/tgi) are a collection of 77 species-specific databases that use a highly refined protocol to analyze gene and EST sequences in an attempt to identify and characterize expressed transcripts and to present them on the Web in a user-friendly, consistent fashion. A Gene Index database is constructed for each selected organism by first clustering, then assembling EST and annotated cDNA and gene sequences from GenBank. This process produces a set of unique, high-fidelity virtual transcripts, or tentative consensus (TC) sequences. The TC sequences can be used to provide putative genes with functional annotation, to link the transcripts to genetic and physical maps, to provide links to orthologous and paralogous genes, and as a resource for comparative and functional genomic analysis.

INTRODUCTION

The TIGR Gene Index databases (TGI) (http://www.tigr.org/tdb/tgi) are constructed using all publicly available expressed sequence tags (EST) and known gene sequence data stored in GenBank for each target species. Sequences are first cleaned to identify and remove contaminating sequences, including vector, adaptor, mitochondrial, ribosomal and chimeric sequences. These sequences are then searched pairwise against each other and grouped into clusters based on shared sequence similarity. The clusters are assembled at high stringency to produce tentative consensus (TC) sequences.

The virtual transcripts represented in the TCs are annotated using a variety of tools for open reading frame (ORF) prediction, single nucleotide polymorphism (SNP) prediction, long oligo prediction for microarrays, putative annotation using a controlled vocabulary, Gene Ontology (GO) and Enzyme Commission (EC) number assignments and maps onto complete or drafted genomes or available genetic maps. The TCs are used to construct a variety of other databases, including the Eukaryotic Gene Orthologs (EGO) database and RESOURCERER, a database that annotates and cross-references microarray resources for plants and animals.

At present, 77 species are represented in the Gene Index databases, including 29 animals, 25 plants, 8 fungi and 15 protists; this includes most species for which public EST projects have released more than 50 000 ESTs. Current release information for each species-specific database is summarized in Table 1. Individual databases are updated and released three times yearly, on February 1, June 1 and October 1, if the number of available ESTs for that species has increased by either 25 000 or >10%, whichever is less.

RECENT DEVELOPMENTS

Construction of the Gene Indices

The process used to assemble each Gene Index is similar to that described previously (1–3), although some modifications have been made to improve the efficiency and accuracy of the process. mgBLAST, a modified version of the Megablast (4) program, is now used for the pairwise sequence comparisons that are the basis for defining the sequence clusters which form the basis for assembly. For large clusters containing hundreds or thousands of sequences (e.g. highly expressed genes such as actin), sequence representation is reduced prior to assembly using a variety of multilayer approaches, including transitive clustering, containment clustering and seeded clustering with known genes. Following clustering, the Paracel Transcript Assembler (PTA), a

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| Species    | Species_name  | TGI   | TC       | sET       | sEST       |
|------------|---------------|-------|----------|-----------|------------|
| Animals (29) |                |       |          |           |            |
| Human      | Homo sapiens  | HGI 15.0 | 22148   | 19740     | 594468     |
| Mouse      | Mus musculus  | MGI 14.0 | 167694  | 7499      | 602312     |
| Rat        | Rattus norvegicus | RGI 13.0 | 56933   | 2131      | 87992      |
| Cattle     | Bos Taurus    | BtGI 10.0 | 38760  | 413       | 56644      |
| Pig        | Sus scrofa    | SsGI 9.0 | 33963   | 519       | 50376      |
| Dog        | Canis familiaris | DogGI 4.0 | 6613   | 684       | 11506      |
| Chicken    | Gallus gallus | GGI 8.0 | 42988   | 848       | 72941      |
| Frog       | Xenopus laevis | XGI 9.0 | 39724   | 626       | 37249      |
| Zebrafish  | Danio rerio   | ZGI 15.0 | 32889   | 395       | 53940      |
| Catfish    | Ictalurus punctatus | Cfi 5.0 | 3254   | 156       | 16694      |
| R.trout    | Oncorhynchus mykiss | RtGI 4.0 | 23135  | 190       | 27448      |
| A.salmon   | Salmo salar   | AeGI 2.1 | 12277   | 93        | 18971      |
| C.intestinalis | Ciona intestinalis | CinGI 3.0 | 20616  | 39       | 30690      |
| Medaka     | Oryzias latipes | OGI 5.0 | 12849   | 171       | 13669      |
| Fugu       | Takifugu rubripes | FGI 1.0 | 3120   | 448       | 7667       |
| A.burtoni  | Astatotilapia burtoni | AbGI 1.0 | 402   | 15        | 2300       |
| H.chilotes | Haplochromis chilotes | HcGI 1.0 | 2147   | 0        | 4030       |
| H.red_tail_sheller | Haplochromis sp. 'ts' | HsGI 1.0 | 1883   | 0        | 4422       |
| Killifish  | Fundulus heteroclitus | FhGI 1.0 | 5540   | 57        | 11941      |
| Honeybee   | Apis mellifera | AMGI 4.0 | 3700   | 53        | 7571       |
| A.aegypti  | Aedes aegypti | AeGI 4.0 | 15888   | 32       | 5075       |
| Drosophila | Drosophila melanogaster | DGI 9.0 | 20693   | 1104      | 6662       |
| A.variegatum | Anopheles gambiae | AgGI 7.0 | 17120  | 6847      | 14940      |
| R.appendic | Rhipicephalus appendiculatus | RatGI 1.0 | 2543   | 19       | 4797       |
| C.elegans  | Caenorhabditis elegans | CeGI 8.0 | 17728  | 5034      | 5678       |
| B.malayi   | Brugia malayi | BmGI 4.0 | 2060   | 44       | 6841       |
| O.volvulus | Onchocerca volvulus | OvGI 3.0 | 1065   | 23        | 2942       |
| S.mansonii | Schistosoma mansoni | SmGI 5.0 | 12912  | 39       | 20753      |
| Plants (25) |                |       |          |           |            |
| Pine       | Pinus         | PGI 4.0 | 13622   | 205       | 17944      |
| Cotton     | Gossypium     | CGI 5.0 | 6812   | 142       | 17396      |
| Arabidopsis | Arabidopsis thaliana | AtGI 11.0 | 28010   | 5188      | 12485      |
| L.japonicus | Lotus japonicus | LiGI 3.0 | 12485  | 56       | 15919      |
| Lettuce    | Lactuca sativa | LiGI 2.0 | 7961   | 56       | 14168      |
| Sunflower  | Helianthus annuus | HaGI 3.0 | 6038   | 110      | 14372      |
| Tomato     | Lycopersicon esculentum | LeGI 9.0 | 20530  | 164      | 14923      |
| Pepper     | Capsicum anuum | CaGI 1.0 | 3203   | 47       | 7462       |
| Potato     | Solanum tuberosum | SGI 9.0 | 19225   | 102      | 13226      |
| Tobacco    | Nicotiana tabacum | NGI 1.0 | 897   | 806      | 8529       |
| N.benthamiana | Nicotiana benthamiana | NbGI 1.1 | 3819   | 44      | 3735       |
| Soybean    | Glycine max   | GmGI 12.0 | 30884  | 141      | 31401      |
| Medicago   | Medicago truncatula | MtGI 7.0 | 17610  | 25       | 19341      |
| Ice_plant  | Mesembryanthemum crystalline | McGI 4.0 | 2851   | 47      | 5557       |
| Grape      | Vitis vinifera | VvGI 3.1 | 13218  | 54       | 9837       |
| Rice       | Oryza sativa  | OsGI 15.0 | 33089  | 17776     | 37900      |
| Maize      | Zea mays      | ZmGI 14.0 | 29414   | 524      | 26426      |
| Wheat      | Triticum aestivum | TaGI 8.0 | 44630  | 169      | 79008      |
| Sorghum    | Sorghum bicolor | SbGI 8.0 | 20029  | 143      | 18976      |
| barley     | Hordeum vulgare | HvGI 9.0 | 21981  | 168      | 27041      |
| S.cereale  | Secale cereale | ReyGI 3.0 | 1391   | 66      | 3890       |
| S.officinarum | Saccharum officinarum | SoGI 1.0 | 23596  | 7      | 72281      |
| A.copa    | Allium cepa   | OnGI 1.0 | 5838   | 18      | 7870       |
| C.reinhardtii | Chlamydomonas reinhardtii | ChiGI 4.0 | 10777 | 96      | 19466      |
| Fungi (8)  |                |       |          |           |            |
| A.flavus   | Aspergillus flavus | AfGI 4.0 | 3749   | 10       | 3459       |
| C.posadasi | Coccidioides posadasi | CpoGI 2.0 | 6275   | 0        | 3037       |
| S.cerevisiae | Saccharomyces cerevisiae | ScGI 3.0 | 4107   | 2005     | 198        |
| S.pombe    | Schizosaccharomyces pombe | SpGI 3.0 | 2449   | 2974     | 510        |
| Cryptococcus | Filobasidiella neoformans | CfGI 7.0 | 2384   | 59       | 3231       |
| N.crassa   | Neurospora crassa | NcrGI 3.0 | 4389  | 6547     | 1586       |
| A.nidulans | Aspergillus nidulans | AnGI 4.0 | 3532   | 6664     | 2904       |
| M.grisea   | Magnaporthe grisea | MgGI 5.0 | 6375   | 6195     | 8320       |
| Prostis (15) |                |       |          |           |            |
| P.berghei  | Plasmodium berghei | PbGI 5.0 | 1168   | 41       | 3980       |
| P.falciparum | Plasmodium falciparum | PfGI 7.0 | 3978   | 2487     | 3142       |
| P.vivax    | Plasmodium vivax | PvGI 0.5 | 158    | 175      | 567        |
TIGR Gene Indices are a collection of species-based databases which assemble the ESTs and the Expressed Transcripts (ETs) into TC sequences. Singletons (sET and sEST) are the ET/EST sequences that are not incorporated into a TC during assembly. TCs, sET and sEST are the unique sequences in TGI. There are 77 gene indices in total (data until September 1, 2004). Each line includes species, species name, gene index name and version, total number of TCs within current release, number of singleton ETs and number of singleton ESTs. For Leishmania, pine and cotton, the ESTs were pooled from dbEST for the genus, not a single species. The table was arranged by grouping the total 77 gene indices into animals (29), plants (25), fungi (8) and protists (15).

| Species | Species name | TGI | TC | sET | sEST |
|---------|--------------|-----|----|-----|------|
| P.yoelii | Plasmodium yoelii | PyGI 5.0 | 3611 | 3784 | 2418 |
| E.tenella | Eimeria tenella | EtGI 4.0 | 2077 | 29 | 3066 |
| T.gondii | Toxoplasma gondii | TgGI 6.0 | 6977 | 31 | 11401 |
| N.caninum | Neospora caninum | NcGI 5.0 | 1980 | 3 | 3715 |
| S.neurona | Sarcozystis neurona | SnGI 4.0 | 665 | 0 | 1644 |
| C.parvum | Cryptosporidium parvum | CpGI 4.0 | 171 | 485 | 254 |
| T.vaginalis | Trichomonas vaginalis | TvGI 1.0 | 87 | 109 | 704 |
| Leishmania | Leishmania | LsGI 4.0 | 600 | 1454 | 1120 |
| T.cruzi | Trypanosoma cruzi | TcGI 4.0 | 2189 | 164 | 4749 |
| T.brucii | Trypanosoma brucei | TbGI 5.0 | 734 | 1287 | 2018 |
| D.discoideum | Dictyostelium discoideum | DdGI 4.0 | 6826 | 172 | 6392 |
| T.thermophila | Tetrahymena thermophila | TtGI 3.0 | 1436 | 165 | 2626 |

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Modified version of CAP3 assembly program (5), is used to assemble each TC. An open source set of software tools that embody this process, TGICL, is available (http://www.tigr.org/tdb/tgi/software) with other open-source utilities for users interested in performing a similar analysis on their own datasets (6).

New features of the TC report

The central element of the TGI databases are the TC sequences and the TC reports that are presented through the project website. Each TC report presents a summary of the assembly and annotation process, including the consensus TC sequence in the FASTA format with a history from previous builds in the header, a map showing component EST and gene sequences, and a table providing links to the primary sequences, putative annotation, an expression summary based on the number of ESTs from various libraries, genomic locations and links to tentative orthologs in EGO. Since the last presentation of the TGI databases in Nucleic Acids Research, several new features have been added to the TC report. Putative polyadenylation signals are identified and shaded in the consensus sequence and putative poly(A/T) trimming sites are shown in sequence map for each of the component ESTs. Potential ORFs are predicted for each TC using a variety of software tools including the NCBI ORF Finder, ESTScan (7) and FrameFinder; predicted ORFs can be searched against a variety of databases using WU-BLAST. Assembly of the TCs can result in incorrect orientations for the consensus and an attempt is now made to determine the proper orientation using the annotated direction of component gene and EST sequences as well as BLAST search results. Putative SNP sites are found by analyzing the multiple sequence alignments that are produced in the assembly stage; putative SNPs are reported only if a variant is found in multiple sequences from independent libraries. Unique 70mers are predicted for each TC using OligoPicker (8). GO terms and metabolic pathway in KEGG are provided for each TC based on protein database searches. Where possible, TCs are aligned with draft genomes and displayed using TGIviewer, gbrowse, EnsEMBL and the UCSC genome viewers.

New databases and tools

The EGO (http://www.tigr.org/tdb/tgi/ego) (9) database, previously known as TIGR Orthologous Gene Alignments (TOGA), uses pairwise sequence similarity searches and a transitive, reciprocal closure process to identify Tentative Ortholog Groups (TOGs) in eukaryotes (9). EGO has expanded its representation to include all 77 species represented in the TGI and TOGs have been cross-referenced to the Online Mendelian in Man (OMIM) database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) database of human disease genes.

RESOURCERER (10) provides annotation based on the TIGR Gene Indices for widely available microarray resources in human, mouse, rat, zebrafish and Xenopus, including widely used clone sets and Affymetrix GeneChips™ as well as a variety of other sequence-based resources such as RefSeq. RESOURCERER provides a wide range of annotation and integration with genomic and other resources, including gene name assignments, GO term and EC number assignments, chromosomal localization, integration with genetic and quantitative trait locus (QTL) maps, ortholog identification, lists of relevant abstracts in PubMed and promoter region identification. Owing to its integration with the TGI and EGO, RESOURCERER also provides links between microarray platforms both within and between species. Users can also submit a list of GenBank accessions corresponding to their microarray databases for annotation and functional analysis. A plant-specific version, Plant RESOURCERER, was released in September 2004 with microarray resources from Arabidopsis, potato, tomato, maize and rice.

Genomic maps align TCs to available complete or draft genomes, including human, mouse, rat, zebrafish, fly, worm, Fugu, mosquito, Arabidopsis, yeast, fission yeast and rice. Also these alignments can be viewed using either TGI-viewer or gbrowse or through a number of distributed annotation system (DAS) viewers (11), including one developed at TIGR. Each Gene Index also includes graphical metabolic pathway maps linked to TCs associated with specific pathways through GO term and EC number annotation. Comparisons between TCs are also used to identify putative alternative splice forms based on shared blocks of sequence similarity.
Using the TIGR Gene Indices

There are many ways in which users can access the TIGR Gene Index databases. Nucleotide or protein sequences can be searched using WU-BLAST against individual TGI databases, EGO or pre-selected classes of species, such as animals or plants. The TGI can be searched using unique identifiers (GB and TC Accessions, EST identifiers and ET numbers from the TIGR PREEGAD database), gene product names, functional classifications based on GO terms, metabolic pathways, library-related expression analysis, map position within various sequenced genomes, TOGs in the EGO database and alternative splice forms. Complete annotations for all of the ESTs and TCs in each TGI database are now also provided through the EST Annotator and TC Annotator features which provide comprehensive lists of sequences within each species-specific database.

All of the TIGR Gene Indices are available for download through the main page for each species. Downloads consist of six files, including a FASTA file for all unique sequences, the TC list, the component ESTs in each TC, GO analysis, predicted oligos and a README file.

Software

Many of the software tools used to create the TGI are available with source code to the research community through the TGI software tools website (http://www.tigr.org/tdb/tgi/software). The TGI Clustering tool (TGICL) (6) is a software system for fast clustering and assembly of large EST datasets. TGICL starts with a large multi-FASTA file (and an optional quality value file) and outputs the assemblies produced by CAP3 (5). Both clustering and assembly phases can be parallelized by distributing the searches and the assembly jobs across multiple CPUs, as TGICL can take advantage of either SMP or PVM (Parallel Virtual Machine) clusters. Other available software includes clview for viewing sequence assemblies in .ace format, SeqClean which is used to remove contaminating sequences from EST and gene sequences and cdbsfasta/cdbyank which index FASTA-formatted files and can be used to rapidly extract sequences from them.

ACKNOWLEDGEMENTS

The authors wish to thank TIGR IT group for their database and computer system support. This work was supported by the US Department of Energy, grant DE-FG02-99ER62852 and the US National Science Foundation, grant DBI-9983070.

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