

Nematode.net: a tool for navigating sequences from parasitic and free-living nematodes

Todd Wylie^{1,*}, John C. Martin¹, Michael Dante¹, Makedonka Dautova Mitreva¹, Sandra W. Clifton¹, Asif Chinwalla¹, Robert H. Waterston^{1,2}, Richard K. Wilson¹ and James P. McCarter^{1,3}

¹The Genome Sequencing Center, Washington University School of Medicine, St Louis, MO 63108, USA,

²Department of Genome Sciences, University of Washington, 1705 NE Pacific Street, Seattle, WA 98195, USA

and ³Divergence Inc., 893 North Warson Road, St Louis, MO 63141, USA

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ABSTRACT

Nematode.net (www.nematode.net) is a web-accessible resource for investigating gene sequences from nematode genomes. The database is an outgrowth of the parasitic nematode EST project at Washington University's Genome Sequencing Center (GSC), St Louis. A sister project at the University of Edinburgh and the Sanger Institute is also underway. More than 295 000 ESTs have been generated from >30 nematodes other than *Caenorhabditis elegans* including key parasites of humans, animals and plants. Nematode.net currently provides NemaGene EST cluster consensus sequence, enhanced online BLAST search tools, functional classifications of cluster sequences and comprehensive information concerning the ongoing generation of nematode genome data. The long-term goal of nematode.net is to provide the scientific community with the highest quality sequence information and tools for studying these diverse species.

INTRODUCTION

Nematodes or roundworms are members of an ancient phylum that accounts for perhaps four out of every five individual animals in the world (1). Parasitic nematodes infect nearly half the world's human population, resulting in significant morbidity and mortality. Nematodes also parasitize livestock and companion animals and cause over 80 billion dollars in crop damage annually (2,3). Nematode.net is a specialty database that makes accessible the rapidly expanding nucleotide sequence data and related resources from species across this phylum to target audiences including human/mammalian parasitologists, plant nematologists, *Caenorhabditis elegans* biologists and other scientists.

SEQUENCES FROM PARASITIC NEMATODES

Following the completion of the first fully sequenced animal genome, the nematode *C.elegans* (4), increasing efforts have

been made to rapidly generate and make public gene sequences from parasitic nematodes of medical and economic importance as a route toward research on new anthelmintic drugs, vaccines, safe pesticides and resistant plants. Initiatives have primarily utilized expressed sequence tags (ESTs), focusing first on the filarial worms responsible for elephantiasis and river blindness (5,6). A collaboration is currently underway involving the Genome Sequencing Center (GSC) at Washington University in St Louis, the Wellcome Trust Sanger Institute, the University of Edinburgh and dozens of participating parasitologists to extend EST-based gene discovery to more than 30 nematode species (7,8). To date, over 295 000 ESTs have been generated from nematodes beyond *C.elegans*, with nearly 220 000 of these sequences provided by the GSC (Table 1).

NemaGene CLUSTERS AND NemaBLAST SEARCHES

While GSC-generated ESTs are immediately deposited in GenBank's database of ESTs (dbEST), no such repository exists for nematode EST cluster consensus sequences, nor are tailored BLAST searches easily performed. Nematode.net began in 2000 by providing these services. NemaGene clustering improves upon EST data by reducing data redundancy, increasing transcript length and improving base accuracy. The NemaGene method uses the Phred/Phrap/Consed suite of analysis programs (10), together with internal supplemental scripts, and has the advantage that clusters can be edited when necessary and tracked by name through multiple builds (11). Clusters can be searched on the nematode.net website by EST name, putative identity and individual contig or cluster name (Fig. 1). Cluster entries provide EST membership with NCBI links, as well as SWIR non-redundant protein database, Sanger Centre and *C.elegans* (Wormpep) homology. Cluster information and sequences can also be downloaded by FTP. NemaGene clusters have so far been generated for 15 species (Table 1). Both NemaGene clusters and individual ESTs can be searched for sequence identity using the online NemaBLAST tool, which utilizes a local WU-BLAST server (12) (<http://blast.wustl.edu>). Searches can be performed on ESTs from specific species, clades, stages and libraries, in any combination desired by the user.

*To whom correspondence should be addressed. Tel: +1 314 286 1114; Fax: +1 314 286 1810; Email: twylie@watson.wustl.edu

Table 1. Nematode EST projects by species

Clade	Nematode species	Host	Total ESTs	GSC ESTs	ESTs clustered	Clusters	Database
V	<i>Ancylostoma caninum</i>	Mammal	9331	9331	9286	4020	NemaGene
	<i>Ancylostoma ceylanicum</i>	Mammal	10651	10590	10590	3369	NemaGene
	<i>Caenorhabditis briggsae</i>	Free-living	2424	2424			
	<i>Caenorhabditis elegans</i>	Free-living	215202	388			Wormbase
	<i>Haemonchus contortus</i>	Mammal	21967	14014	5181	1970	NEMBASE
	<i>Necator americanus</i>	Mammal	4766		4766	2298	NEMBASE
	<i>Nippostrongylus brasiliensis</i>	Mammal	1234		1234	750	NEMBASE
	<i>Ostertagia ostertagi</i>	Mammal	7009	6558			
	<i>Pristionchus pacificus</i>	Free-living	8818	8818	4979	2603	NemaGene
	<i>Teladorsagia circumcincta</i>	Mammal	4313				
IVA	<i>Strongyloides stercoralis</i>	Mammal	11392	11335	10908	3311	NemaGene
	<i>Strongyloides ratti</i>	Mammal	14822	14822	8618	2941	NemaGene
	<i>Parastrongyloides trichosuri</i>	Mammal	7963	7963	4528	2155	NemaGene
IVB	<i>Globodera rostochiensis</i>	Plant	5934	5040	5039	2375	NemaGene
	<i>Globodera pallida</i>	Plant	1832				
	<i>Heterodera glycines</i>	Plant	20114	20109	4307	1790	NemaGene
	<i>Heterodera schachtii</i>	Plant	2662	2662			
	<i>Meloidogyne arenaria</i>	Plant	3519	3519	3321	1866	NemaGene
	<i>Meloidogyne chitwoodi</i>	Plant	10789	10789			
	<i>Meloidogyne hapla</i>	Plant	13869	13869			
	<i>Meloidogyne incognita</i>	Plant	13452	13168	5661	1625	NemaGene
	<i>Meloidogyne javanica</i>	Plant	5600	5578	5574	2598	NemaGene
	<i>Pratylenchus penetrans</i>	Plant	1928	1928	1926	420	NemaGene
<i>Zeldia punctata</i>	Free-living	391	391	378	195	NemaGene	
III	<i>Ascaris lumbricoides</i>	Mammal	1822				
	<i>Ascaris suum</i>	Mammal	39242	29960	19280	4262	NemaGene
	<i>Brugia malayi</i>	Mammal	26212	3773	18741	8392	NEMBASE
	<i>Dirofilaria immitis</i>	Mammal	4005	4005			
	<i>Litomosoides sigmodontis</i>	Mammal	873				
	<i>Onchocerca volvulus</i>	Mammal	14971	1230	7911	3504	NEMBASE
	<i>Toxocara canis</i>	Mammal	4889	4370			
<i>Wuchereria bancrofti</i>	Mammal	2166					
I	<i>Trichinella spiralis</i>	Mammal	10 767	10548	10130	3454	NemaGene
	<i>Trichuris muris</i>	Mammal	3063		2125	1322	NEMBASE
	<i>Trichuris vulpis</i>	Mammal	2402	2402			
	Totals		510394	219584	144483	55220	

Nematodes with >100 ESTs are shown. NEMBASE clusters are available at www.nematodes.org. Clades are based upon (9).

FUNCTIONAL CLASSIFICATIONS AND OTHER FEATURES

Nematode.net provides the user with two avenues to explore the putative function of NemaGene clusters. Both are based on extrapolation from homology and must be regarded as providing only a starting hypothesis in studying function. Cluster sequences were used to search the Interpro protein domain database (13) (www.ebi.ac.uk/interpro) with InterProScan. Based on the presence of conserved domains, clusters were then mapped onto the Gene Ontology (GO) classification scheme (14) (www.geneontology.org). GO biological, molecular and cellular classifications are provided at nematode.net with the AmiGO interface. NemaGene clusters have also been mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database of biochemical pathways using enzyme commission (EC) numbers as the basis for putative assignment (15) (www.genome.ad.jp/kegg). Additional useful features of nematode.net include summaries of sequence status for all nematode species, cDNA library

descriptions, project specifics, >300 organized nematology links and a trace viewer that allows users to examine raw sequence data. Nematode.net is also used to manage requests for clones generated by the project. Since 1999, 377 clones and dozens of plates have been provided to 37 investigators in 14 countries.

SITE AND DATABASE DESIGN

The Nematode.net interface was constructed using the Dreamweaver MX web development application in combination with a Perl CGI/DBI database interface. The GUI-based Dreamweaver MX editor was chosen for HTML design due to ease of use, ability to make rapid site-wide modifications and project tracking features. HTML pages written under Dreamweaver MX are sourced by a GSC Perl module, which has proved to be fast, extensible, and useful for recycling previously written code. Relational databases were initially built in MySQL and are now being replaced by a single, more efficient Oracle database.

Nematode.net

Genome Sequencing Center

Links: dbEST | PubMed | WormBase | C. elegans Server | SWISS-PROT
BaNG | Washington University | GSC | Comprehensive Links

NemaGene Cluster Search

Project
Home
Species Summaries
Sequencing Totals
Reference
Staff
Collaborators
Grant
Phyla
Library Descriptions
Data FTP
Accession IDs
Sequencing Spreadsheets
Trace Viewer
Software
Clone Requests
FAQ

NemaGene
Functional Classifications
NemaBLAST
NemaGene Cluster Search
NemaGene Cluster BLAST
Cluster Data FTP
NemaGene FAQ

Links
dbEST
PubMed

WashU clustergroup name => AC00300.cl
 NemaGene contig ID => AC00300

Contig Consensus: AC00300

```

GATCAGCAGAGGCTCATCTTTGCCGGCAAACAACCTCGAAGATGGCCGCTACTCTTTCCGAT
TACAACATCCAGAAGGAATCCACTCTCCATCTTGTGCTCCGCCTTCGAGGAGGAATGCAG
ATTTTCGTGAAGACCTTGACCGGGAAGACCATCACCTTGAAGTCGAGGCTTCTGATACG
ATTGAGAATGTGAAGGCTAAGATCCAGGACCAAGGAAGGTATTCCCTCCAGACCAGCAGAGG
CTCATCTTCGCCGGCAAACAACCTCGAGGACGGTCGCTACTCTTTCCGACTACAATATCCAG
AAAGAAATCCACACTCCACTTGGTGCTTCGCCTTCGTGGAGGCTGCAACTGAACCTGACTTT
GTGAACATGTTCTGTGCGATTGTGTAATAAACCTTGTGAATCAAAAAAAAAAAAAAAAAAAAA
      
```

Contig contains the following EST members
(Putative id of that EST shown if one exists)

```

=====
pa29e12.y1 PUT_ID: gb:M26880 UBIQUITIN (HUMAN);
pa64d10.y1 PUT_ID: gb:M26880 UBIQUITIN (HUMAN);
      
```

Contig consensus has the following WORMPEP hits

```

=====
F25B5.4c CE31915 locus:ubq-1 status:Confirmed TR:Q8MYQ4
F25B5.4c CE31915 locus:ubq-1 status:Confirmed TR:Q8MYQ4
F25B5.4c CE31915 locus:ubq-1 status:Confirmed TR:Q8MYQ4
F25B5.4c CE31915 locus:ubq-1 status:Confirmed TR:Q8MYQ4
F25B5.4c CE31915 locus:ubq-1 status:Confirmed TR:Q8MYQ4
F25B5.4c CE31915 locus:ubq-1 status:Confirmed TR:Q8MYQ4
F25B5.4c CE31915 locus:ubq-1 status:Confirmed TR:Q8MYQ4
F25B5.4a CE01921 locus:ubq-1 status:Confirmed SW:P14792
F25B5.4a CE01921 locus:ubq-1 status:Confirmed SW:P14792
F25B5.4a CE01921 locus:ubq-1 status:Confirmed SW:P14792
      
```

Contig consensus has the following SWIR hits

```

=====
gb|AAB92373.1| polyubiquitin [Ovis aries]
gb|AAB92373.1| polyubiquitin [Ovis aries]
gb|AAB92373.1| polyubiquitin [Ovis aries]
gb|AAB92373.1| polyubiquitin [Ovis aries]
ref|NP_776558.1| polyubiquitin [Bos taurus]
ref|NP_776558.1| polyubiquitin [Bos taurus]
ref|NP_776558.1| polyubiquitin [Bos taurus]
ref|NP_776558.1| polyubiquitin [Bos taurus]
prf|1908225A ubiquitin
prf|1908225A ubiquitin
      
```

End of contig entry: AC00300

-- SEARCH FINISHED --

Figure 1. A NemaGene Cluster Search query response showing constituents of consensus sequence by contig.

FUTURE DIRECTIONS

Nematode.net is a work in progress with the long-term goal of providing the nematology community with useful, consistent and lasting integrated databases and tools. With over 29 000 unique users in the past year, nematode.net is already providing a useful service, but improvements are envisioned in three areas. First, the site's current databases will be extended to include almost all available nematode species and sequences, expedited by further automation of clustering algorithms. Second, nematode.net will become more closely integrated with the *C.elegans* database Wormbase (16) (www.wormbase.org) and Nembase (www.nematodes.org), a

site maintained by our collaborators at the University of Edinburgh that also provides tools for investigating nematode sequences (8). Plans for Wormbase integration include the layering of non-*C.elegans* nematode gene sequences over *C.elegans* homologs using the Distributed Annotation System (DAS) method (17). Currently, 9894 *C.elegans* genes have strong homologs in other nematodes (BLAST score of $<1e-20$). *C.elegans* information will continue to reside only at Wormbase. Third, in collaboration with Nembase, additional features for navigating nematode sequences will be made available. Databases covering all nematodes will include: postulated amino acid translations of EST clusters; protein domains connected to Pfam (18) and Interpro

including new nematode-specific domains; genes with homologs in *C.elegans* where RNA interference phenotype information is available (19); proteins with predicted signal peptide sequences; and codon usage tables for each species. Other possible additions include the integration of whole-genome information for parasitic nematode species (e.g. *Brugia malayi*) as such data become available.

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