

## Nematode Gene Sequences: Update for December 2005<sup>1</sup>

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Molecular characterization of parasitic nematodes now relies heavily on information gained from genomic approaches. The availability of thousands of new nucleotide sequences in searchable public databases accelerates efforts to identify and characterize genes of interest, including those encoding pathogenicity factors, diagnostic markers, targets (for vaccines, drugs, or nematicides), and immunomodulatory molecules. Since 2000, we have provided periodic updates on the status of nematode sequencing projects of interest to the communities of researchers studying parasitic and free-living nematode biology (McCarter et al., 2000, 2002, 2003). Here we report on the availability of sequences from nematodes as of late 2005 and primarily cite literature from 2003–2005. Please see our 2003 update (McCarter et al., 2003) for a thorough listing of the prior literature.

Since the completion of the *Caenorhabditis elegans* genome (The *C. elegans* Sequencing Consortium, 1998), most efforts to extend genomic approaches to parasitic nematodes have relied on sampling from cDNA libraries to generate expressed sequence tags (ESTs). From the time of our December 2003 report, 149,401 new nematode ESTs have been submitted to the Genbank database of expressed sequence tags (dbEST), including 46,490 from parasites (Table 1). As of September 2005, a project at Washington University's Genome Sequencing Center (GSC) in St. Louis sampled a diverse spectrum of the phylum Nematoda, generating 274,794 ESTs from 32 nematode species (Wylie et al., 2004). An additional 32,254 ESTs have been contributed by a sister project at the Sanger Institute (Hinxton, UK) and Edinburgh University (Parkinson et al., 2004a). Sequences from both projects were submitted to dbEST immediately after being obtained and are also available from specialized Web sites (Table 2).

*Caenorhabditis* continues to be the best represented genus. The 347,997 ESTs from the 40 nematode species other than *Caenorhabditis* that have been sampled include sequences from 24 mammalian parasites, 14 plant parasites, and two free-living bacteriovores. From

2003 to 2005, EST sequencing from mammalian parasites has slowed whereas EST generation from plant parasites has continued as the National Science Foundation (NSF)-funded collaboration between the GSC and North Carolina State University has been completed (Fig. 1). Sizable EST collections now exist for six *Meloidogyne* (root-knot nematode) species (Scholl and Bird, 2005), which is more than for any other nematode genus. ESTs have also been generated from three Tylenchida migratory endoparasitic species, the lesion nematodes *Pratylenchus penetrans* (Mitreva et al., 2004a) and *Pratylenchus vulvulus*, and the burrowing nematode *Radopholus similis*. A collection of more than 9,000 ESTs also is available from the dagger nematode *Xiphinema index*. Being a clade I Dorylaimia nematode, this represents the first EST collection from a non-Tylenchida plant-parasitic nematode.

The last 3 years have seen a shift from EST generation to the beginning of projects to complete whole genomes of parasitic nematodes. For some species, sequences that will contribute to an eventual assembled genome already are available, including genome survey sequences (GSS), BAC (bacterial artificial chromosome) end sequences, and whole genome shotgun sequences (WGS). It is presumed that such sequences represent a random sampling of the genome and thus will include non-transcribed regulatory and intergenic regions as well as true genespace. This is an important distinction from ESTs, which are derived solely from transcribed genes (or pseudogenes) and exhibit a bias toward those genes whose steady-state transcripts are present in the nematode at moderate-to-high levels. Emphasis has also moved from generating ESTs to analyzing them, including the completion of the first meta-analysis of the genomic biology of the phylum (reviewed by Mitreva et al., 2005a). This study examined more than 250,000 ESTs from 30 nematode species, which were found to cluster into 93,000 genes defining approximately 60,000 gene families (Parkinson et al., 2004b).

For most nematode species, ESTs dominate all other available sequences, but this is beginning to change as there are now 10 nematode species with full genome projects in various stages of planning, progress, or completion (Bird et al., 2005). Complementing and informing the improving annotation of the *C. elegans* genome (Chen et al., 2005a), a high-quality draft sequence covering more than 98% of the *C. briggsae* genome has been completed at the GSC and Sanger Institute, with primary support from the National Human Genome Research Institute (NHGRI) and the Wellcome Trust (Stein et al., 2003; reviewed in Blaxter, 2003). In 2004, NHGRI announced support for the se-

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TABLE I. Number of sequences obtained from nematode species with more than 50 ESTs registered in the GenBank dbEST database, September 2005.

Nematode species	Type and date of sequence <sup>a</sup>						
	EST 1997 March 1887	EST December 2000	EST June 2002	EST September 2003	EST September 2005	Other September 2003	Other September 2005
<i>Ancylostoma caninum</i> <sup>b</sup>	0	5,546	7,656	9,331	9,331	112	94,581
<i>Ancylostoma ceylanicum</i>	0	0	2,690	10,651	10,651	73	84
<i>Angiostrongylus cantonensis</i>	3	3	3	3	1,279	11	15
<i>Ascaris lumbricoides</i>	0	0	0	1,822	1,822	138	147
<i>Ascaris suum</i> <sup>b</sup>	0	588	24,492	39,242	39,248	426	485
<i>Brugia malayi</i> <sup>c</sup>	7,496	22,392	22,439	26,215	26,215	18,449	1,251,537
<i>Caenorhabditis briggsae</i> <sup>c</sup>	2,424	2,424	2,424	2,424	2,424	1,151	2,378,412
<i>Caenorhabditis elegans</i> <sup>c</sup>	30,196	109,215	191,268	215,200	303,991	96,850	96,590
<i>Caenorhabditis remanei</i> <sup>c</sup>	0	0	0	0	10,766	67	1,944,766
<i>Dirofilaria immitis</i>	0	0	0	4,005	4,005	170	214
<i>Globodera pallida</i>	0	94	1,832	1,832	4,378	66	86
<i>Globodera rostochiensis</i>	0	894	5,934	5,934	5,941	152	194
<i>Haemonchus contortus</i> <sup>c</sup>	0	2,399	4,906	21,967	21,967	552	261,038
<i>Heterodera glycines</i>	0	1,506	4,327	20,114	24,438	366	411
<i>Heterodera schachtii</i>	0	0	0	2,818	2,818	26	35
<i>Litomosoides stigmodontis</i>	0	198	198	873	2,699	33	39
<i>Meloidogyne arenaria</i>	0	0	3,334	5,018	5,108	49	55
<i>Meloidogyne chitwoodi</i>	0	0	0	10,798	12,218	38	79
<i>Meloidogyne hapla</i> <sup>c</sup>	0	0	6,157	15,369	24,452	38	121
<i>Meloidogyne incognita</i>	0	6,626	10,899	14,081	19,934	239	8,585
<i>Meloidogyne javanica</i>	22	1,208	5,600	6,681	7,587	55	84
<i>Meloidogyne paranaensis</i>	0	0	0	0	3,710	0	19
<i>Necator americanus</i>	0	211	961	4,766	4,766	168	172
<i>Nippostrongylus brasiliensis</i>	0	0	734	1,234	1,234	37	34
<i>Onchocerca ochengi</i>	0	60	60	60	60	13	35
<i>Onchocerca volvulus</i>	310	13,802	14,922	14,974	14,974	791	830
<i>Ostertagia ostertagi</i>	0	0	5,591	7,009	7,006	189	215
<i>Parastrongyloides trichosuri</i>	0	0	7,963	7,963	7,963	3	7
<i>Pratylenchus penetrans</i>	0	0	0	1,928	1,928	21	24
<i>Pratylenchus vulvulus</i>	0	0	0	0	2,485	1	4
<i>Pristionchus pacificus</i> <sup>c</sup>	703	4,989	8,818	8,818	12,172	15	229,382
<i>Radopholus similis</i>	0	0	0	0	1,154	49	51
<i>Strongyloides ratti</i>	0	0	8,645	14,761	14,761	23	19,680
<i>Strongyloides stercoralis</i>	57	10,922	11,392	11,392	11,392	55	97
<i>Teladorsagia circumcincta</i>	0	0	315	4,313	4,313	125	218
<i>Toxocara canis</i>	8	519	3,920	4,889	4,889	85	90
<i>Trichinella spiralis</i> <sup>c</sup>	0	0	4,247	10,767	10,767	162	723,620
<i>Trichostrongylus vitrinus</i>	0	9	9	9	368	4	18
<i>Trichuris muris</i>	0	301	2,125	2,716	2,402	315	315
<i>Trichuris vulpis</i>	0	0	0	3,063	3,063	1	0
<i>Wuchereria bancrofti</i>	119	131	131	2,166	4,487	77	89
<i>Xiphinema index</i>	0	0	0	0	9,351	2	26
<i>Zeldia punctata</i>	0	378	391	391	391	5	7
Total sequences	41,338	184,415	364,383	515,777	665,178	121,202	7,012,491
Total non- <i>Caenorhabditis</i>	8,718	72,776	170,691	298,153	347,997	23,134	2,592,723
Total free living	33,323	117,006	202,901	226,833	329,744	98,088	4,649,157
Total mammalian parasites	7,993	57,081	123,399	204,191	210,022	22,012	2,353,560
Total plant parasites	22	10,328	38,083	84,753	125,412	1,102	9,774

<sup>a</sup> EST totals are from dbEST. Totals for other sequences were summed from various sources, including Genbank nucleotide, protein, and genome sequences, which are available from the NCBI taxonomy browser ([www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html](http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html)). For *Caenorhabditis briggsae*, *C. remanei*, *Pristionchus pacificus*, and *Trichinella spiralis*, where full genome projects are under way at GSC, sequences were obtained from the NCBI trace archive at [www.ncbi.nlm.nih.gov/Traces/trace.cgi](http://www.ncbi.nlm.nih.gov/Traces/trace.cgi). For the *B. malayi* genome project, sequences were obtained from [www.tigr.org/tdb/e2k1/bma1](http://www.tigr.org/tdb/e2k1/bma1). For the *H. contortus* genome project, sequences were obtained from [www.sanger.ac.uk/Projects/H\\_contortus](http://www.sanger.ac.uk/Projects/H_contortus).

<sup>b</sup> *Ascaris suum* and *Ancylostoma caninum* are the focus of further EST and GSS sequencing at the GSC. To date, 94,429 GSSs have been generated from *A. caninum*.

<sup>c</sup> Species for which full genome projects are complete or in progress. Genome projects are also beginning at GSC for *Caenorhabditis japonica* and *Caenorhabditis* sp. PB2801.

quencing at GSC of three more *Caenorhabditis* genomes ([www.genome.gov/11007952](http://www.genome.gov/11007952)) and two additional nematodes' genomes, *Pristionchus pacificus* and *Trichinella spiralis* ([www.genome.gov/12511858](http://www.genome.gov/12511858)), as part of a strategy to strategically select genomes based on their

evolutionary position and role in informing the interpretation of human and model organism genomes. The human filarial parasite *Brugia malayi* has been sequenced to 8-fold draft coverage (Ghedini et al., 2004) by The Institute for Genomic Research (TIGR) with

TABLE 2. Web sites for nematode genomics.

Wormbase, <i>C. elegans</i> Genomics	www.wormbase.org
GenBank Sequences	www.ncbi.nlm.gov/Taxonomy/Browser/wwwtax.cgi?id=6231
Blaxter Lab ESTs and Clusters, Nembase	www.nematodes.org
GSC, ESTs and NemaGene Clusters	www.nematode.net
<i>B. malayi</i> Genome Project, TIGR	http://www.tigr.org/tdb/e2k1/bmal1

funding from the National Institute of Allergy and Infectious Diseases (NIAID), and work on the genome of *Haemonchus contortus* (sheep barber pole worm) has begun at the Wellcome Trust Sanger Institute (www.sanger.ac.uk/Projects/H\_contortus). The first project aimed at completing a draft genome of a plant-parasitic nematode has been funded by the joint NSF/U.S. Department of Agriculture Microbial Genome Sequencing Program. North Carolina State University will complete a 5-fold draft genome of *Meloidogyne hapla*. As more genome sequences become available, ESTs will continue to play a crucial role in defining gene exon/intron boundaries and in training gene finder programs to individual species. The EST collection from *Caenorhabditis remanei* was generated and the *M. hapla* EST collection expanded with this purpose in mind.

Recent publications analyzing nematode ESTs include studies of *Nippostrongylus brasiliensis* (Harcus et al.,

2004), *T. spiralis* (Mitreva et al., 2004b), *H. contortus* (Jasmer et al., 2004), *P. penetrans* (Mitreva et al., 2004a), *Strongyloides stercoralis* (Mitreva et al., 2004c), *B. malayi* (Whitton et al., 2004), *Ancylostoma caninum* and *Ancylostoma ceylanicum* (Mitreva et al., 2005b), and *Strongyloides ratti* (Thompson et al., 2005). A number of novel topics have been explored in these papers, such as examining the relative rates of evolution of secreted vs. cytoplasmic gene products, conservation (or lack of conservation) of stage of gene expression between species, and the variation in sequence of a large and abundantly expressed gene family. But ESTs have uses beyond simply the identification of genes. In one study, ESTs were sampled across the Tylenchida to resolve phylogenetic relationships (Scholl and Bird, 2005). ESTs also can be used to fabricate microarrays, and this approach has been used to query the transcriptome of *B. malayi* (Li et al., 2004), *Trichostrongylus vitrinus* (Nisbet and Glasser, 2004), *Ascaris suum* (Morimoto et al., 2003), and *Ancylostoma caninum* (Moser et al., 2005). The information content of ESTs also can be used as the basis for constructing a database of protein sequences against which identified proteins can be screened. Such a strategy has been used in initial proteomic examinations of root-knot nematode (Jaubert et al., 2002), *H. contortus* (Yatsuda et al., 2003), and *A. suum* (Islam et al., 2004). It should be noted, however, that the power of such experiments to identify peptides and proteins is greatly enhanced by having a complete proteome, such as can be deduced only from an entire genome sequence. ESTs can be useful with functional genomic approaches such as RNAi by identifying candidate genes for targeting; this approach has proven successful in *Meloidogyne* (Rosso et al., 2005), *Globodera rostochiensis* (Chen et al., 2005b), and *Onchocerca volvulus* (Lustigman et al., 2004).

In some senses, the actual number of sequences obtained is a little arbitrary but certainly continues to grow. In Table 1, the total number of sequences is given as 7,012,491. However, this figure is a combination of raw data (trace files, which are approximately equivalent to successful sequencing attempts) and more annotated data. The number of trace files is given, as this indicates the amount of "activity" on a particular species even though it excludes the *C. elegans* trace files (as these predated formation of the appropriate NCBI database). Although trace files can be searched, for gene finding it is generally more informative to search data that has been subjected to assembly and annotation. This is especially true for genome sequence data from those species nearing completion (such as *C. briggsae* or *B. malayi*). Further, Table 1 omits species for which there are fewer than 50 entries specifically in the dbEST database of Genbank. Removing that restriction reveals an additional 23 nematode species that have at least 50 total Genbank sequence entries of any type. They are, by species and with the number of sequence entries in

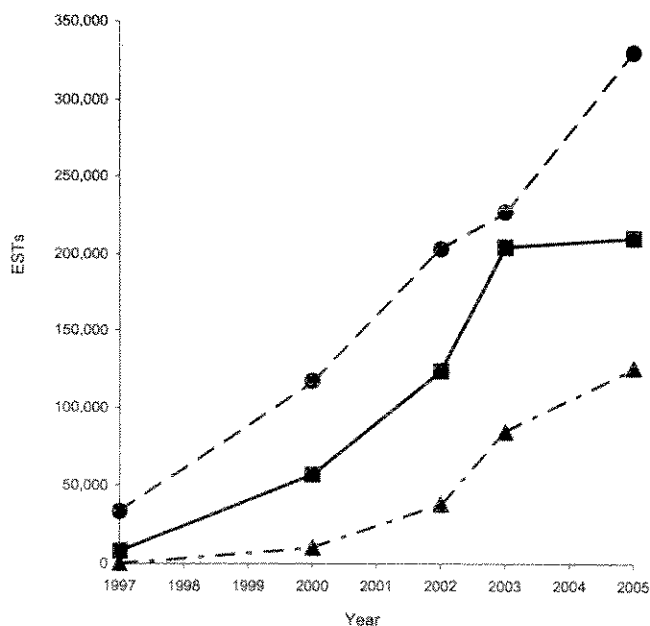


FIG. 1. EST generation from free-living nematodes including *Caenorhabditis elegans* (circles), mammalian parasitic nematodes (squares), and plant-parasitic nematodes (triangles) from 1997 to 2005. During that same time period, total nematode ESTs increased from 41,000 to 665,000 and total ESTs for all species increased from 872,000 to 28,800,000 (www.ncbi.nlm.nih.gov/dbEST/dbEST\_summary.html).

parentheses: *C. japonica* (12,689), *Cooperia oncophora* (1,242), *Haemonchus placei* (276), *Brugia pahangi* (169), *Longistriata caudabullata* (154), *Dictyocaulus viviparus* (141), *Ancrobeloides* sp. *Sourhope farm* (132), *Steinernema feltiae* (120), *Parelaphostrongylus tenuis* (104), *Longistriata blarinae* (96), *Steinernema carpocapsae* (92), *Contracaecum osculatum* (84), *Cooperia punctata* (73), *Ancylostoma duodenale* (65), *Trichinella pseudospiralis* (63), *Mansonella ozzardi* (61), *Bursaphelenchus conicaudatus* (61), *Cyathostomum catinatum* (58), *Anisakis simplex* (56), *Cylicocyclus nassatus* (56), *Trichostrongylus colubriformis* (52), *Acanthocheilonema viteae* (52), and *Rotylenchus reniformis* (50).

Since the late 1990s, we have moved from having one complete nematode genome and very limited sequence information from other nematode species to having partial transcriptomes based on ESTs from more than 30 species. Further, projects to obtain the sequence of 10 nematode genomes are in various stages of progress. These sequencing projects have proved extremely valuable in accelerating the research programs of many nematologists by allowing connections to be made across species. Nematode comparative genomics analyzing sequences from many species is now possible (Parkinson et al., 2004b), and a broad vision for how nematode genomics can mature has been presented (Bird et al., 2005). Additional updates in the years ahead likely will further mark the trend from partial transcriptomes to draft and complete genomes.

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