

Nematode Gene Sequences: Update for December 2003

JAMES P. McCARTER,^{1,3} MAKEDONKA MITREVA,¹ SANDRA W. CLIFTON,¹ DAVID MCK. BIRD,² AND ROBERT H. WATERSTON^{1,4}

The molecular characterization of parasitic nematodes and development of novel control strategies can benefit from genomic approaches. The high-throughput generation of expressed sequence tags (ESTs) from numerous nematode cDNA libraries is now providing thousands of new gene sequences, and their availability in public databases will facilitate broad characterization of their function. A project at Washington University's Genome Sequencing Center is nearing the completion of 235,000 5' ESTs from 28 nematode species (227,272 ESTs as of October 2003). Another 32,078 ESTs from seven species have been contributed by a sister project at the Sanger Institute and Edinburgh University. Sequences are immediately submitted to the database of expressed sequence tags (dbEST) at GenBank and are also available from specialized Web sites (Table 1A). Nematode ESTs have diverse applications (e.g., Blaxter et al., 2002; Jasmer et al., 2001; Murray et al., 2001; Pleasance et al., 2003; Scholl et al., 2003; Srinivasan et al., 2002), and strategies for their use have been reviewed (Blaxter et al., 1999; Grant and Viney, 2001; Marra et al., 1998; McCarter et al., 2000; Parkinson et al., 2001; Parkinson et al., 2003).

Here we provide an update on the availability of nematode ESTs and genomes. Since our June 2002 report (McCarter et al., 2002) 153,750 new nematode ESTs have been submitted to dbEST including 129,818 from parasites. A review by Parkinson et al. (2003), focusing on NEMBASE and NemaGene clustering also provides EST totals from November 2002. *Caenorhabditis elegans* has long been a focus of genome studies (The *C. elegans* Sequencing Consortium, 1998), and 42% of all nematode ESTs are from *C. elegans* (Kohara, 1996; McCombie et al., 1992; Waterston et al., 1992). The 300,497 ESTs from 34 nematode species beyond *Caenorhabditis* include sequences from 22 mammalian para-

sites, 10 plant parasites, and two free-living bacteriovores (Table 2). For most species, ESTs dominate the conventionally submitted nucleotide and protein sequences in GenBank. ESTs are redundant, with common mRNAs being highly represented. Extrapolating from initial clustering of ESTs in 12 species, the 300,497 ESTs likely represent no more than 100,000 genes. For example, clustering of 12,269 ESTs from *Onchocerca volvulus* formed 4,208 groups (Williams et al., 2002), and 5,713 ESTs from *Meloidogyne incognita* formed 1,625 clusters (McCarter et al., 2003). New gene discovery can be maximized by sampling multiple cDNA libraries produced from different stages and tissues. In *Strongyloides stercoralis*, for example, several thousand ESTs were generated from both first (L1) and third (L3) larva stages. Despite redundancy within each library, only 12% of clusters contained ESTs from both stages (Mitreva et al., 2003). Generating two distinct libraries per source can also substantially increase the pool of identified transcripts. For example, *Ancylostoma caninum* L3 libraries produced by an SL1 splice leader PCR protocol vs. a modified SMARTTM protocol and sequenced to a depth of ~800 and ~2,800, respectively, found only 3.5% overlap (Mitreva, unpubl.). *Ancylostoma caninum* clusters, along with those from seven other species, are searchable on www.nematode.net, a Web-accessible resource for investigating nematode gene sequences (Fig. 1). Among its features, the site provides NemaGene EST cluster consensus sequences, enhanced online BLAST search tools, functional classifications of cluster sequences, and instructions for clone requests (Wylie et al., 2003). Hundreds of cDNA clones have been provided to 37 nematologists in 12 countries.

Plant-parasitic nematode ESTs have been generated from root-knot nematodes (five species, 54,483 ESTs) (Dautova et al., 2001; McCarter et al., 2003), cyst nematodes (four species, 30,698 ESTs) (Popeijus et al., 2000), and one migratory endoparasite, *Pratylenchus penetrans* (1,928 ESTs, in preparation). Representation of multiple life-stages is improving with 20,114 ESTs now available from five life stages for *Heterodera glycines* (egg, J2, J3, J4, virgin female) and sequencing from five life stages of *Meloidogyne* species in progress. However, plant-parasitic nematode genomics is still a nascent field. Within clade IVB (Blaxter et al., 1998), sampling has yet to occur from most migratory *Tylenchida* species. Virtually no sequence information is available from the distant clade II nematodes including the *Dorylaimida* and *Triplonchida* parasites. Perhaps the most visible gap hindering the field is the lack of a draft or complete plant-parasitic nematode genome. The recent announced opening of the National Science Foundation/

Received for publication 14 October 2003.

¹ Genome Sequencing Center, Department of Genetics, Box 8501, Washington University School of Medicine, St. Louis, MO 63108.

² Plant Nematode Genetics Group, Box 7253, North Carolina State University, Raleigh, NC 27695.

³ Divergence Inc., 892 North Warson Road, St. Louis, MO 63141.

⁴ Department of Genome Sciences, Box 357730, University of Washington, Seattle, WA 98195.

Nematode EST sequencing at Washington University was supported in part by NIH grant AI 46593 to R.H.W., NSF grant 0077503 to D.M.B. and S.W.C., and a Helen Hay Whitney/Merck Fellowship to J.P.M. The authors thank members of our EST lab, especially Deana Pape, John Martin, Todd Wylie, Mike Dante, Brandi Chiapelli, and Claire Murphy, and the many collaborators who have generously provided materials (www.nematode.net/Collaborators/), especially Thomas Baum for supplying staged libraries of *Heterodera glycines*. JPM and DMB are equity holders of Divergence Inc., and JPM is a Divergence employee; this research was not company funded.

E-mail: mccarter@genetics.wustl.edu

This paper was edited by J. L. Starr.

TABLE 1. Web sites for nematode genomics

1A. Nematode ESTs Resources	
GenBank dbEST	www.ncbi.nlm.nih.gov/dbEST
Genome Sequencing Center ESTs and NemaGene Clusters	www.nematode.net
Blaxter Lab ESTs and Clusters, Nembase	www.nematodes.org
EMBL Parasite Genome Server	www.ebi.ac.uk/parasites/parasite-genome.html
The Filarial Genome Network	http://circuit.neb.com/fgn/ests.html
1B. <i>Caenorhabditis</i> Genome Resources	
Wormbase	www.wormbase.org
<i>C. elegans</i> WWW Server	http://elegans.swmed.edu
Sanger Centre—Wormpep, <i>C. elegans</i> and <i>C. briggsae</i> Projects	www.sanger.ac.uk
Genome Sequencing Center— <i>C. elegans</i> and <i>C. briggsae</i> Projects	http://genome.wustl.edu

TABLE 2. Nematode species with more than 50 ESTs registered in the GenBank dbEST database, October 2003.

Nematode species	ESTs 3/97	ESTs 12/00	ESTs 6/02	ESTs 10/03	Other GenBank entries 10/03	Major EST sources
<i>Caenorhabditis elegans</i>	30,196	109,215	191,268	215,200	96,850	1, 2, 11
<i>Ascaris suum</i>	0	588	24,492	39,242	426	2, 3, 6
<i>Brugia malayi</i>	7,496	22,392	22,439	26,215	18,449	3, 4, 5
<i>Haemonchus contortus</i>	0	2,399	4,906	21,967	552	3, 6, 9, 10
<i>Heterodera glycines</i>	0	1,506	4,327	20,114	366	2
<i>Meloidogyne hapla</i>	0	0	6,157	16,305	38	2
<i>Onchocerca volvulus</i>	310	13,802	14,922	14,974	791	5, 2
<i>Strongyloides ratti</i>	0	0	8,645	14,761	23	2
<i>Meloidogyne incognita</i>	0	6,626	10,899	14,081	239	2, 7
<i>Meloidogyne chitwoodi</i>	0	0	0	12,218	38	2
<i>Strongyloides stercoralis</i>	57	10,922	11,392	11,392	55	2
<i>Trichinella spiralis</i>	0	0	4,247	10,767	162	2
<i>Ancylostoma ceylanicum</i>	0	0	2,690	10,651	73	2
<i>Ancylostoma caninum</i>	0	5,546	7,656	9,331	112	2
<i>Pristionchus pacificus</i>	703	4,989	8,818	8,818	15	2
<i>Parastrongyloides trichosuri</i>	0	0	7,963	7,963	3	2
<i>Ostertagia ostertagi</i>	0	0	5,591	7,009	189	2, 3, 6
<i>Meloidogyne javanica</i>	22	1,208	5,600	6,861	55	2
<i>Globodera rostochiensis</i>	0	894	5,934	5,934	152	2, 7, 8
<i>Meloidogyne arenaria</i>	0	0	3,334	5,018	49	2
<i>Toxocara canis</i>	8	519	3,920	4,889	85	2, 3
<i>Necator americanus</i>	0	211	961	4,766	168	3, 6
<i>Teladorsagia circumcincta</i>	0	0	315	4,313	125	3, 6
<i>Dirofilaria immitis</i>	0	0	0	4,005	170	2
<i>Trichuris vulpis</i>	0	0	0	3,063	1	2
<i>Trichuris muris</i>	0	301	2,125	2,716	315	3, 6
<i>Heterodera schachtii</i>	0	0	0	2,818	26	2
<i>Caenorhabditis briggsae</i>	2,424	2,424	2,424	2,424	1,151	2
<i>Wuchereria bancrofti</i>	119	131	131	2,166	77	5
<i>Pratylenchus penetrans</i>	0	0	0	1,928	21	2
<i>Globodera pallida</i>	0	94	1,832	1,832	66	12
<i>Ascaris lumbricoides</i>	0	0	0	1,822	138	3
<i>Nippostrongylus brasiliensis</i>	0	0	734	1,234	37	3
<i>Litomosoides sigmodontis</i>	0	198	198	873	33	3
<i>Zeldia punctata</i>	0	378	391	391	5	2
<i>Onchocerca ochengi</i>	0	60	60	60	13	5
<i>Meloidogyne paranaensis</i>	0	0	0	Pending	0	2
Total sequences	41,335	184,403	364,371	518,121	121,068	
Total non- <i>Caenorhabditis</i>	8,715	72,764	170,679	300,497	23,067	

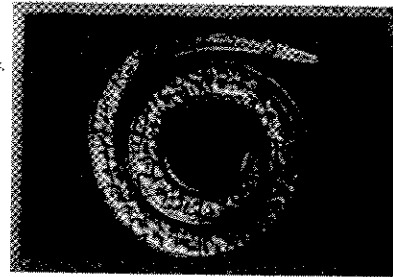
- National Institute of Genetics, Mishima, Japan.
- Genome Sequencing Center, Washington University School of Medicine, St. Louis, MO.
- Institute of Cell, Animal, and Population Biology, University of Edinburgh, Edinburgh, UK.
- World Health Organization Filarial Genome Network.
- Department of Biology, Smith College, Northampton, MA.
- The Wellcome Trust Sanger Institute, Hinxton, UK.
- Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands.
- Nematology Department, Scottish Crop Research Institute, Dundee, UK.
- Institute for Animal Science and Health, Lelystad, The Netherlands.
- Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA.
- The Institute for Genomic Research, Rockville, MD.
- Collaboration: Rothamsted Research, Harpenden, UK, and Center for the Biology of Nematode Parasitism, North Carolina State University, Raleigh, NC.

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

Parasitic Nematode Sequencing Project

Projects are currently underway to generate 315,000 ESTs (Expressed Sequence Tags) from ~20 parasitic nematode species, including 235,000 ESTs at the GSC, St. Louis and 80,000 at the Sanger Centre & Edinburgh University. We have established Nematode Net to allow easy access to this new gene sequence information including expressed sequence tags (ESTs), NemaGene EST clusters, and sequence trace files. Please provide us feedback on how this site can serve as a better resource for you.



Please note that our lab is a sequencing facility; we are not clinicians, and as such we are not qualified to provide medical advice regarding parasitic nematode afflictions. If you are in need of such assistance, please instead contact a qualified physician, preferably one with a specialty in parasitic diseases.

Species Quick Links

Ancylostoma caninum	Ancylostoma ceylanicum	Ancylostoma duodenale	Ascaris lumbricoides	Ascaris suum
Brugia malayi	Caenorhabditis briggsae	Caenorhabditis elegans	Dirofilaria immitis	Glossodora pallida
Glossodora rotachianalis	Haemonchus contortus	Heterodera glabra	Litomosoides sigmodontis	Meloidogyne arenaria
Meloidogyne actia	Meloidogyne chitwoodi	Meloidogyne hapla	Meloidogyne incognita	Meloidogyne javanica
Necator americanus	Onchocerca ochengi	Onchocerca volvulus	Ostertagia ostertagi	Parastongylodes trichosus
Prionchus pacificus	Strongyloides ratti	Strongyloides stercoralis	Teladorsagia circumcincta	Toxocara canis
Trichinella spiralis	Trichuris muris	Trichuris trichiura	Wuchereria bancrofti	Zeldia punctata

NEWS: March 2003

► AmiGO and Kegg data sets have been added here.

FIG. 1. Nematode.net Homepage.

U.S. Department of Agriculture Microbial Genome Sequencing Program to proposals for nematode genomes may mark a significant step toward this goal (www.nsf.gov/pubsys/ods/getpub.cfm?ods_key=nsf03603).

There are 204,179 ESTs available from human- and animal-parasitic nematodes, and analyses of many species have been completed (Allen et al., 2000; Blaxter 2000; Blaxter et al., 1996, 2002; Daub et al., 2000; Hoekstra et al., 2000; Lizotte-Waniewski et al., 2000; Maizels et al., 2000; Moore et al., 1996; Tetteh et al., 1999; Unnasch and Williams, 2000). Marking the first generation of a draft genome sequence from a parasitic nematode, the human-filarial parasite *Brugia malayi* has now been sequenced to 8X draft coverage by the Institute for Genomic Research with funding from the National Institute of Allergy and Infectious Diseases (www.tigr.org/tdb/e2k1/bma1/). Publication and data release are expected in 2004.

For most parasitic nematodes, comparison to the complete *C. elegans* genome is more informative than comparison to any species' sequence (Bird et al., 1999; The *C. elegans* Sequencing Consortium, 1998). Analysis of the 2,826 *S. stercoralis* clusters with significant BLAST

homologies in other species ($<10^{-5}$) (Altschul et al., 1990), for example, found *C. elegans* homologs for 90%, and these homologs were typically the top-ranking match available (Mitreva et al., 2003). Among the 1,280 *M. incognita* clusters with homologs in other species, 85% had matches in *C. elegans* (McCarter et al., 2003). *Caenorhabditis elegans* information is easily accessible from several sources (Table 1B) and includes the complete cell lineage, a map of its neuroanatomy, an extensive genetic map, predicted exon/intron structure and translation products for all of its genes, and expression information for most genes. Mapped genetic mutations and publications on various aspects of *C. elegans* molecular biology number in the thousands. The discovery of RNA interference in *C. elegans* (Fire et al., 1998) has allowed systematic knockout of most of the worm's genes, defining mutant phenotypes for more than 1,700 genes (Fraser et al., 2000; Gonczy et al., 2000; Kamath et al., 2003; Maeda et al., 2001; Piano et al., 2002). RNA interference is also effective in some parasitic nematodes (Aboobaker and Blaxter, 2003; Hussein et al., 2002; Urwin et al., 2002). The *C. briggsae* genome was sequenced to 10X draft coverage and

made available in July 2002 by the groups at Washington University and the Wellcome Trust Sanger Institute that completed the *C. elegans* genome (<ftp://ftp.sanger.ac.uk/pub/wormbase/cbriggsae/>). Whole genome comparisons between *C. elegans* and *C. briggsae* (Kent and Zahler, 2000; Le et al., 2003; Webb et al., 2002; Witherspoon and Robertson, 2003) as well as *C. elegans* and *B. malayi* (Guiliano et al., 2002) have begun.

DNA sequence information, along with the tools of comparative and functional genomics, has the potential to further accelerate discovery in molecular nematology and parasitology in the years ahead. We will continue to provide periodic updates on the status of nematode genomics as the sequence data sets continue their rapid expansion.

LITERATURE CITED

- Aboobaker, A. A., and M. L. Blaxter. 2003. Use of RNA interference to investigate gene function in the human filarial nematode parasite *Brugia malayi*. *Molecular and Biochemical Parasitology* 129: 41–51.
- Allen, J. E., J. Daub, D. Guiliano, A. McDonnell, M. Lizotte-Waniewski, D. W. Taylor, and M. Blaxter. 2000. Analysis of genes expressed at the infective larval stage validates utility of *Litomosoides sigmodontis* as a murine model for filarial vaccine development. *Infection and Immunity* 68:5454–5458.
- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215:403–410.
- Bird, D. M., C. H. Opperman, S. J. M. Jones, and D. L. Baillie. 1999. The *Caenorhabditis elegans* genome: A guide in the post genomics age. *Annual Review of Phytopathology* 37:247–265.
- Blaxter, M. 2000. Genes and genomes of *Necator americanus* and related hookworms. *International Journal of Parasitology* 30:347–355.
- Blaxter, M., M. Aslett, D. Guiliano, and J. Daub. 1999. Parasitic helminth genomics. *Filarial Genome Project. Parasitology* 118:S39–51.
- Blaxter, M. L., J. Daub, D. Guiliano, J. Parkinson, C. Whitton, and F. G. Project. 2002. The *Brugia malayi* genome project: Expressed sequence tags and gene discovery. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 96:7–17.
- Blaxter, M. L., P. De Ley, J. R. Garey, L. X. Liu, P. Scheldeman, A. Vierstraete, J. R. Vanfleteren, L. Y. Mackey, M. Dorris, L. M. Frisse, J. T. Vida, and W. K. Thomas. 1998. A molecular evolutionary framework for the phylum *Nematoda*. *Nature* 392:71–75.
- Blaxter, M. L., N. Raghavan, I. Ghosh, D. Guiliano, W. Lu, S. A. Williams, B. Slatko, and A. L. Scott. 1996. Genes expressed in *Brugia malayi* infective third stage larvae. *Molecular and Biochemical Parasitology* 77:77–93.
- Daub, J., A. Loukas, D. I. Pritchard, and M. Blaxter. 2000. A survey of genes expressed in adults of the human hookworm, *Necator americanus*. *Parasitology* 120:171–184.
- Dautova, M., M. N. Rosso, P. Abad, F. J. Gommers, J. Bakker, and G. Smant. 2001. Single-pass cDNA sequencing—a powerful tool to analyse gene expression in preparasitic juveniles of the southern root-knot nematode *Meloidogyne incognita*. *Nematology* 3:129–139.
- Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391:806–811.
- Fraser, A. G., R. S. Kamath, P. Zipperlen, M. Martinez-Campos, M. Sohrmann, and J. Ahringer. 2000. Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. *Nature* 408: 325–330.
- Gonczy, P., C. Echeverri, K. Oegema, A. Coulson, S. J. Jones, R. R. Copley, J. Duperon, J. Oegema, M. Brehm, E. Cassin, E. Hannak, M. Kirkham, S. Pichler, K. Flohrs, A. Goessen, S. Leidel, A. M. Alleaume, C. Martin, N. Ozlu, P. Bork, and A. A. Hyman. 2000. Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. *Nature* 408:331–336.
- Grant, W. N., and M. E. Viney. 2001. Post-genomic nematode parasitology. *International Journal of Parasitology* 31:879–888.
- Guiliano, D. B., N. Hall, S. J. Jones, L. N. Clark, C. H. Corton, B. G. Barrell, and M. L. Blaxter. 2002. Conservation of long-range synteny and microsynteny between the genomes of two distantly related nematodes. *Genome Biology* 3:RESEARCH0057.
- Hoekstra R., A. Visser, M. Otsen, J. Tibben, J. A. Lenstra, and M. H. Roos. 2000. EST sequencing of the parasitic nematode *Haemonchus contortus* suggests a shift in gene expression during transition to the parasitic stages. *Molecular and Biochemical Parasitology* 110:53–68.
- Hussein, A. S., K. Kichenin, and P. M. Selkzer. 2002. Suppression of selected acetylcholinesterase expression in *Nippostrongylus brasiliensis* by RNA interference. *Molecular and Biochemical Parasitology* 122: 91–94.
- Jasmer, D. P., J. Roth, and P. J. Myler. 2001. Cathepsin B-like cysteine proteases and *Caenorhabditis elegans* homologues dominate gene products expressed in adult *Haemonchus contortus* intestine. *Molecular and Biochemical Parasitology* 116:159–169.
- Kamath, R. S., A. G. Fraser, Y. Dong, G. Poulin, R. Durbin, M. Gotta, A. Kanapin, N. Le Bot, S. Moreno, M. Sohrmann, D. P. Welchman, P. Zipperlen, and J. Ahringer. 2003. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* 421:231–237.
- Kent, W. J., and A. M. Zahler. 2000. The intronator: Exploring introns and alternative splicing in *Caenorhabditis elegans*. *Nucleic Acids Research* 28:91–93.
- Kohara, Y. 1996. Large-scale analysis of *C. elegans* cDNA. *Tanpakushitsu Kakusan Koso* 41:715–720.
- Le, S. Y., J. H. Chen, D. Konings, and J. V. J. Maizel. 2003. Discovering well-ordered folding patterns in nucleotide sequences. *Bioinformatics* 19:354–361.
- Lizotte-Waniewski, M., W. Tawe, D. B. Guiliano, W. Lu, J. Liu, S. A. Williams, and S. Lustigman. 2000. Identification of potential vaccine and drug target candidates by expressed sequence tag analysis and immunoscreening of *Onchocerca volvulus* larval cDNA libraries. *Infection and Immunity* 68:3491–3501.
- Maeda, I., Y. Kohara, M. Yamamoto, and A. Sugimoto. 2001. Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. *Current Biology* 11:171–176.
- Maizels, R. M., K. K. Tetteh, and A. Loukas. 2000. *Toxocara canis*: Genes expressed by the arrested infective larval stage of a parasitic nematode. *International Journal of Parasitology* 30:495–508.
- Marra, M. A., L. Hillier, and R. H. Waterston. 1998. Expressed sequence tags—ESTablishing bridges between genomes. *Trends in Genetics* 14:4–7.
- McCarter, J., P. Abad, J. T. Jones, and D. Bird. 2000. Rapid gene discovery in plant-parasitic nematodes via expressed sequence tags. *Nematology* 2:719–731.
- McCarter, J. P., S. Clifton, D. M. Bird, and R. H. Waterston. 2002. Nematode gene sequences, Update for June 2002. *Journal of Nematology* 34:71–74.
- McCarter, J., M. Dautova Mitreva, J. Martin, M. Dante, T. Wylie, U. Rao, D. Pape, Y. Bowers, B. Theising, C. V. Murphy, A. P. Kloek, B. J. Chiappelli, S. W. Clifton, M. D. Bird, and R. Waterston. 2003. Analysis and functional classification of transcripts from the nematode *Meloidogyne incognita*. *Genome Biology* 4:R26:1–19.
- McCombie, W. R., M. D. Adams, J. M. Kelley, M. G. FitzGerald, T. R. Utterback, M. Khan, M. Dubnick, A. R. Kerlavage, J. C. Venter, and C. Fields. 1992. *Caenorhabditis elegans* expressed sequence tags identify gene families and potential disease gene homologues. *Nature Genetics* 2:124–131.
- Mitreva, M., J. P. McCarter, J. Martin, M. Dante, T. Wylie, B. Chiappelli, D. Pape, S. W. Clifton, T. B. Nutman, and R. H. Waterston. 2003. Comparative genomics of gene expression in the parasitic and free-living nematodes *Strongyloides stercoralis* and *Caenorhabditis elegans*. *Genome Research*, in press.
- Moore, T. A., S. Ramachandran, A. A. Gam, F. A. Neva, W. Lu, L. Saunders, S. A. Williams, and T. B. Nutman. 1996. Identification of

- novel sequences and codon usage in *Strongyloides stercoralis*. *Molecular and Biochemical Parasitology* 79:243–248.
- Murray, J., W. F. Gregory, N. Gomez-Escobar, A. K. Atmadja, and R. M. Maizels. 2001. Expression and immune recognition of *Brugia malayi* VAL-1, a homologue of vespid venom allergens and Ancylostoma secreted proteins. *Molecular and Biochemical Parasitology* 118:89–96.
- Parkinson, J., M. Mitreva, N. Hall, M. Blaxter, and J. P. McCarter. 2003. 400,000 nematode ESTs on the Net. *Trends in Parasitology* 4922:132–136.
- Parkinson, J., C. Whitton, D. Guiliano, J. Daub, and M. Blaxter. 2001. 200,000 nematode expressed sequence tags on the Net. *Trends in Parasitology* 17:394–396.
- Piano, F., A. J. Schetter, D. G. Morton, K. C. Gunsalus, V. Reinke, S. K. Kim, and K. J. Kempthues. 2002. Gene clustering based on RNAi phenotypes of ovary-enriched genes in *C. elegans*. *Current Biology* 12:1959–1964.
- Pleasance, E. D., M. A. Marra, and S. J. Jones. 2003. Assessment of SAGE in transcript identification. *Genome Research* 13:1203–1215.
- Popeijus, H., V. C. Blok, L. Cardle, E. Bakker, M. S. Phillips, J. Helder, G. Smant, and J. T. Jones. 2000. Analysis of genes expressed in second-stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* using the expressed sequence tag approach. *Nematology* 2:567–574.
- Scholl, E. H., J. L. Thorne, J. P. McCarter, and D. M. Bird. 2003. Horizontally transferred genes in plant-parasitic nematodes: A high-throughput genomic approach. *Genome Biology* 4:R39.
- Srinivasan, J., W. Sinz, C. Lanz, A. Brand, R. Nandakumar, G. Radatz, H. Witte, H. Keller, I. Kipping, A. Pires-daSilva, T. Jesse, J. Millare, M. de Both, S. C. Schuster, and R. J. Sommer. 2002. A bacterial artificial chromosome-based genetic linkage map of the nematode *Pristionchus pacificus*. *Genetics* 162:129–134.
- Tetteh, K. K., A. Loukas, C. Tripp, and R. M. Maizels. 1999. Identification of abundantly expressed novel and conserved genes from the infective larval stage of *Toxocara canis* by an expressed sequence tag strategy. *Infection and Immunity* 67:4771–4779.
- The *C. elegans* Sequencing Consortium. 1998. Genome sequence of the nematode *C. elegans*: A platform for investigating biology. *Science* 282:2012–2018.
- Unnasch, T. R., and S. A. Williams. 2000. The genomes of *Onchocerca volvulus*. *International Journal of Parasitology* 30:543–552.
- Urwin, P. E., C. J. Lilley, and H. J. Atkinson. 2002. Ingestion of double-stranded RNA by preparasitic juvenile cyst nematodes leads to RNA interference. *Molecular and Plant-Microbe Interactions* 15:747–752.
- Waterston, R., C. Martin, M. Craxton, C. Huynh, A. Coulson, L. Hillier, R. Durbin, P. Green, R. Shownkeen, N. Halloran, M. Metzstein, T. Hawkins, R. Wilson, M. Berks, Z. Du, K. Thomas, J. Thierry-Mieg, and J. Sulston. 1992. A survey of expressed genes in *Caenorhabditis elegans*. *Nature Genetics* 1:114–123.
- Webb, C. T., S. A. Shabalina, A. Y. Ogurtsov, and A. S. Kondrashov. 2002. Analysis of similarity within 142 pairs of orthologous intergenic regions of *Caenorhabditis elegans* and *Caenorhabditis briggsae*. *Nucleic Acids Research* 30:1233–1239.
- Williams, S. A., S. J. Laney, M. Lizotte-Waniewski, and L. A. Bierwert. 2002. The riverblindness genome project. *Trends in Parasitology* 18:86–90.
- Witherspoon, D. J., and H. M. Robertson. 2003. Neutral evolution of ten types of mariner transposons in the genomes of *Caenorhabditis elegans* and *Caenorhabditis briggsae*. *Journal of Molecular Evolution* 56:751–769.
- Wylie, T., J. Martin, M. Dante, M. Mitreva, S. W. Clifton, A. Chinwalla, R. H. Waterston, R. K. Wilson, and J. P. McCarter. 2003. Nematode.net: A tool for navigating sequences from parasitic and free-living nematodes. *Nucleic Acids Research*, in press.