AD_____

Award Number: DAMD17-99-1-9135

TITLE: Characterization of Two C. Elegans Homologuses of Oncogenic Inhibitor of Apoptosis Proteins (IAPs) and Identification of Interacting Genes

PRINCIPAL INVESTIGATOR: Andrew G. Fraser, Ph.D.
Dr. Julie Ahringer

CONTRACTING ORGANIZATION: University of Cambridge
Cambridge CB2 1TS England

REPORT DATE: September 2000

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2000	Annual Summary (1 Aug 99 - 1 Aug 00)

4. TITLE AND SUBTITLE
Characterization of Two C. elegans Homologuses of Oncogenic Inhibitor of Apoptosis Proteins (IAPs) and Indentification of Interacting Genes

5. FUNDING NUMBERS
DAMD17-99-1-9135

6. AUTHOR(S)
Andrew Fraser, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Cambridge

E-MAIL:
Agflo@hermes.cam

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
This report contains colored photos

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

Inhibitor of Apoptosis Proteins (IAPs) suppress apoptosis both in the fruit fly Drosophila melanogaster and in vertebrates. All IAPs contain at least a single copy of a highly conserved domain, the Baculovirus IAP Repeat (BIR) domain; this is essential for their anti-apoptotic activity. Understanding how a BIR domain regulates apoptosis is thus an important step in furthering our understanding of the molecular mechanisms of apoptosis. I have previously identified two BIR-containing Proteins (BIRPs) in the nematode worm *C. elegans*. One of these, BIR-1, appears to play no role in the regulation of programmed cell death in *C. elegans*; however, BIR-1 is required for the completion of cytokinesis. Cytokinesis is a complex process that is likely to be highly regulated and to involve a large number of proteins. My approach to understanding how BIR-1 functions in cytokinesis has been to try to identify other genes that are also required for cytokinesis in *C. elegans* and subsequently to attempt to understand how BIR-1 relates to this machinery. Rather than examine individual candidate genes to determine whether they have a role in cytokinesis, I wished to carry out a genome-wide screen to identify all genes that are required for cytokinesis; to do this I have made use of RNA-mediated inhibition (RNAi). RNAi is a technique whereby the activity of a particular gene is transiently inhibited following the introduction of dsRNA of sequence specific to the targeted gene. The specificity and potency of RNAi make it an ideal technique to investigate gene function beginning only with genomic sequence. I have screened ~90% of genes on Chromosome I of *C. elegans* for RNAi phenotypes and have identified 5 genes which have a similar RNAi phenotype to BIR-1. I aim to extend this to appraoch over the entire genome in the coming year and thence to spend time characterising genes found to be required for cytokinesis.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
52

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Background

Many tumour cells have lost the ability to undergo apoptosis in response to DNA damage caused either by irradiation or chemical mutagens. This appears to be one of the key reasons that so many tumours are resistant to treatment by either radio- or chemotherapy. Understanding how apoptosis is regulated in normal cells may shed light on the mutations that render tumour cells resistant to apoptotic stimuli and may thus suggest novel therapeutic strategies. The key focus of this project is to understand how a particular conserved family of regulators of apoptosis, the Inhibitor of Apoptosis Proteins (IAPs), suppresses cell death.

IAPs suppress apoptosis both in the fruit fly *Drosophila melanogaster* and in vertebrates. All IAPs contain at least a single copy of a highly conserved domain, the Baculovirus IAP Repeat (BIR) domain; this is essential for their anti-apoptotic activity. Understanding how a BIR domain regulates apoptosis is thus an important step in furthering our understanding of the molecular mechanisms of apoptosis.

The nematode worm *C. elegans* has been a key tool for research into apoptosis since the inception of the field. Genetic analysis of programmed cell death in *C. elegans* led to the identification of the basal machinery of cell death which is conserved between worms and humans. Analysis of the function of IAPs in *C. elegans* might shed light on the function of IAPs in human cells. I have previously identified two BIR-containing Proteins (BIRPs) in the nematode worm *C. elegans*. One of these, BIR-1, appears to play no role in the regulation of programmed cell death in *C. elegans*; however, BIR-1 is required for the completion of cytokinesis. Furthermore, I demonstrated that a human homologue of BIR-1, the BIRP survivin, can partially substitute for BIR-1 in the nematode; this shows that BIRPs have a conserved role in the regulation of cytokinesis. These results have subsequently been extended by other groups who have shown that both fission and budding yeasts contain BIRPs and that in

each case inhibition of these genes leads to profound cell cycle defects and polyploidy.

The BIR domain thus seems to have a role in cytokinesis in eukaryotic cells ranging from single-celled yeasts to human cells. My focus has been to use *C. elegans* to understand the function BIRPs in cytokinesis: how they are regulated, what precise functional role they have, and which proteins do they interact with.

Experimental Approach

Cytokinesis is a complex process that is likely to be highly regulated and to involve a large number of proteins. My approach to understanding how BIR-1 functions in cytokinesis has been to try to identify other genes that are also required for cytokinesis in *C. elegans*. In this way I hope to be able to build up a more comprehensive view of the cytokinesis machinery and subsequently to attempt to understand how BIR-1 relates to this machinery. This approach has recently been used very productively by the lab of Bob Horvitz, who has shown that inhibition of Aurora-like kinase activity leads to a very similar defect in cytokinesis to that seen following inhibition of BIR-1 and, following this observation, that BIR-1 appears required for the localization of Aurora-like kinase to the cytokinesis furrow and mid-body.

Rather than examine individual candidate genes to determine whether they have a role in cytokinesis, I wished to carry out a genome-wide screen to identify all genes that are required for cytokinesis; to do this I have made use of RNA-mediated inhibition (RNAi). RNAi is a technique whereby the activity of a particular gene is transiently inhibited following the introduction of dsRNA of sequence specific to the targeted gene. The specificity and potency of RNAi make it an ideal technique to investigate gene function beginning only with genomic sequence. Ingestion of dsRNA-expressing bacteria results in RNAi of the targeted gene and we have established that this technique is at least as effective as the injection of dsRNA for RNAi. It is thus possible to make a library of bacteria, each expressing dsRNA corresponding to an individual gene, to target each and every predicted gene in the *C. elegans* genome.

I have made such a library for all genes on Chromosome I (~13% of all predicted genes) and have screened these genes for RNAi phenotypes. I have identified 339 genes with RNAi phenotypes of which 221 are embryonic lethal (as would be expected for a gene with a defect in cytokinesis). This analysis of chromosome I is the first systematic reverse genetic analysis of a multicellular organism and has resulted in a Nature Article.

To further characterise the nature of the defect arising from RNAi of each of the 221 genes that are required for embryonic viability, our lab is currently in the process of making time-lapse movies of embryos for all 221 embryonic lethal genes to determine the nature of the defect that gives rise to embryonic lethality. Thus far we have identified several genes that are required for cytokinesis. These include profilin, an actin-binding protein; a gene encoding a protein with high homology to a centromeric protein INCENP; and a homologue of the *S. cerevisiae* SCD6 gene which may be involved in vesicle fusion and trafficking, a process thought to be involved in *C. elegans* cytokinesis.

Future Work

I am in the process of extending the RNAi analysis of the *C. elegans* genome to encompass the remaining five chromosomes. I anticipate that construction of the dsRNA-expressing bacterial library should be complete by early 2001, and that analysis of the RNAi phenotypes of all embryonic lethal genes by time-lapse videomicroscopy should be complete by summer 2001. By the time this screen is completed, I expect to have identified 30-50 genes that are required for cytokinesis. This should have greatly expanded our knowledge of the molecular components of the cytokinesis machinery of *C. elegans*. I will then attempt to elucidate how BIR-1 interacts with the identified gene products and thus to understand the involvement of BIR-1 in cytokinesis.

Achievements

- Cloning of 2496 genes to generate a dsRNA-expressing bacterial library.

- Screening the 2496 gene bacterial library to identify RNAi phenotypes of the cloned genes.

- Identification of 339 genes with RNAi phenotypes, of which 221 are required for embryonic viability. Detailed analysis of these data, culminating in publication as a Nature Article (attached).

- Timelapse videomicroscopic analysis of the RNAi phenotypes of embryos for 150 of the 221 genes required for embryonic viability.

- Identification of 5 new genes required for cytokinesis in *C. elegans*.

Systematic functional genomic analysis of *C. elegans* Chromosome I by RNA interference

Andrew G. Fraser*[1], Ravi S. Kamath*[1], Peder Zipperlen*[1], Maruxa Martinez-Campos*, Marc Sohrmann† and Julie Ahringer*.

**Wellcome/CRC Institute, University of Cambridge, Tennis Court Road, CB2 1QR Cambridge, UK. Phone +44-1223-334144; Fax +44-1223-334089. †The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK.*

[1]These authors contributed equally to this work.

Complete genomic sequence is known for two multicellular eukaryotes, the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, and will be soon for humans. However, biological function has been assigned to only a small proportion of the predicted genes in any animal. We used RNA-mediated interference (RNAi) to target nearly 90% of predicted genes on *C. elegans* Chromosome I by feeding worms with bacteria that express double stranded RNA. We have assigned function to 13.9% of the genes analysed, increasing the number of sequenced genes with known phenotypes on Chromosome I from 70 to 378. While most genes with sterile or embryonic lethal RNAi phenotypes are involved in basal cell metabolism, many genes giving post-embryonic phenotypes have conserved but unknown function. In addition, conserved genes are significantly more likely to have an RNAi phenotype than genes with no conservation. We have constructed a reusable library of bacterial clones that permits unlimited future RNAi screens, which should help develop a more complete view of the relationships between the genome, gene function, and the environment.

The complete genomic sequence of an organism is an invaluable tool in understanding the molecular mechanisms underlying its development and function. The nematode worm *C. elegans* is one of two multicellular eukaryotes for which essentially complete genomic sequence is known[1,2]. 36% of predicted *C. elegans* genes have a significant human match[1,3] including many genes implicated in human diseases[3,4], and functional analysis of the *C. elegans* genome has shed light on many conserved biological processes and molecular pathways. A comprehensive functional analysis of all genes in *C. elegans* would greatly expand our knowledge of conserved gene function. We therefore decided to investigate systematically loss-of-function phenotypes of predicted genes of *C. elegans*, starting with Chromosome I.

RNA-mediated interference (RNAi) is a technique whereby the activity of a gene is transiently inhibited following the introduction of double-stranded RNA (dsRNA) of sequence specific to the targeted gene[5]. The specificity and potency of RNAi make it ideal for investigating gene function beginning only with genomic sequence[6]. Ingestion of dsRNA-expressing bacteria results in RNAi of the targeted gene[7], and we previously established that this technique is at least as effective as the injection of dsRNA for RNAi[8]: embryonic lethal phenotypes are detected with similar efficiency by feeding and injection, but feeding detects over 50% more post-embryonic phenotypes than injection. It is thus possible to make a library of dsRNA-expressing bacteria which could be used for high-throughput genome-wide RNAi screens at very low cost. It is important to note that since RNAi does not efficiently inhibit all genes, an RNAi-based screen will miss some relevant genes. Despite this caveat, RNAi is a useful screening tool to complement classical forward genetics.

Analysis of the function of genes on Chromosome I by RNAi

We constructed a library of bacteria expressing dsRNA corresponding to genes on Chromosome I. Chromosome I is the second smallest chromosome, has few

duplicated gene clusters and has no striking unusual features[1]. Each individual bacterial clone is able to synthesise dsRNA designed to target a single gene; since gene predictions are still changing, a few primer pairs no longer correspond to single genes (see Methods). In total, the resulting library contains 2445 independent clones, corresponding to 2416 predicted genes, a total of 87.3% of the 2769 currently predicted genes of Chromosome I.

We screened the library to identify genes whose inhibition gives a clearly detectable phenotype in wild-type worms as described in Methods. We were able to assign a phenotype to 13.9% of the analysed genes, raising the number of sequenced genes on chromosome I with known phenotypes from 70 to 378 (Table 1). Many genes have more than one associated phenotype, reflecting that genes frequently have multiple functions in the organism. Furthermore, since we examined worms that were only exposed to dsRNA as larvae or adults as well as their progeny, we could assign post-embryonic phenotypes to genes that result in sterility or produce 100% embryonic lethal progeny. A summary of these results and a partial listing of the phenotypes obtained are given in Tables 1 and 3. Full results are in Supplementary Table 1 and arepublicly accessible in WormBase (www.wormbase.org).

Our screen was sufficiently effective to identify 90% of known embryonic lethal genes. In addition, we were able to assign phenotypes to 45% of genes with a known post-embryonic phenotype that should have been detectable in our screen (Table 2 and Supplementary Table 2). However, we failed to find phenotypes for a number of previously characterised genes. In some cases (e. g. *fog-3*), this was not due to an inherent difficulty in inhibiting the genes using RNAi (since we obtained the correct phenotype in a separate experiment), but simply because we overlooked them in the screen. However, only one of eight genes involved in neuronal function gave a detectable RNAi phenotype; this accords well with our finding that neurons appear to be more resistant to RNAi than other cell types[8]. Similarly, we did not detect

phenotypes for several genes involved in sperm development (*fer-1, spe-9*, and *spe-11*).

The largest phenotypic class, comprising over 60% of the genes, are those whose inhibition by RNAi gives rise to embryonic lethality, the Emb genes; these include a large number of components of the basal cellular machinery. More interestingly, we find a homologue of the SMN human disease gene[9], a variety of genes encoding RNA-binding proteins (several such proteins play a role in early polarity; reviewed in [10]), a number of genes involved in chromosome condensation and separation, components of signal transduction pathways and many conserved genes that have no known biochemical function.

The largest class of post-embryonic phenotype is the Uncoordinated (Unc) class. Unc phenotypes arise from defects in the development or function of the neuromuscular system (reviewed in [11]). We find Unc genes encoding proteins involved in vesicle sorting and fusion as well as transcription factors (including a homologue of the zinc finger transcription factor MYT-1 which is only expressed in developing neurons in mammals[12-14]) and components of the cytoskeleton (e. g. a kakapo[15-18] and a talin[19] homologue).

A number of genes showed a high incidence of males (Him) phenotype. *C. elegans* is usually grown as a self-fertilising hermaphrodite with males arising at a low frequency in wild-type cultures due to non-disjunction of the X-chromosome (hermaphrodites have two X chromosomes, males only one). An increased number of males is indicative of either the incorrect segregation and maintenance of chromosomes in the germ line (reviewed in [20]) or defects in sexual specification. The Him genes that we identified include kinesins, a katanin homologue[21,22] and a nuclear hormone receptor.

Conservation of genes with RNAi phenotypes across eukaryotes

We examined the level of cross-species conservation of the genes for which we detected an RNAi phenotype (Fig 1). To find *C. elegans* genes that are conserved in other species, we identified *C. elegans* genes that have hits with BlastP[23] e-values below 1.00E-06 in *Saccharomyces cerevisiae*, *Drosophila melanogaster* or humans; we define these as a "match". Hits with BlastP e-values below 1.00E-10 and in which the conservation extends over at least 80% of the *C. elegans* protein length, we defined as "homologues"; this category includes orthologues. This provides a conservative estimate of the number of genes with regions of conservation (matches) or homologues, respectively.

We found that genes with RNAi phenotypes were much more likely to have a match ($p<0.001$) compared to all genes (Fig 1). Most striking is the similarity that we see between *C. elegans* and *Drosophila*: while 42% of *C. elegans* genes have a match and 19% have a homologue in *Drosophila*, we find that over 72% of genes with an RNAi phenotype have a *Drosophila* match and 43% have a homologue (Fig 1). This analysis shows that genes with a required function in *C. elegans* have been highly conserved across eukaryotic evolution. We also find that highly conserved genes are more likely to have an RNAi phenotype than genes that show no conservation: 26% of *C. elegans* genes that have a homologue in one of the organisms examined give an RNAi phenotype compared to only 5% of genes with no conservation ($p<0.001$).

Physical distribution on chromosome I of genes with RNAi phenotypes

Genes for which we identified an RNAi phenotype are evenly distributed across the chromosome with the exception of two regions (corresponding to segments 2 and 8-9 in Fig 2a) for which there appears to be a drop in number ($p<0.1$). These two regions correspond to the two regions of chromosome I that contain locally duplicated gene clusters[1]. We suggest that the reduction in the number of phenotypes observed by RNAi in these regions may be due to gene duplication and thus redundancy of function. It is worth noting that some of the predicted genes in the

duplicated regions may not be expressed: while genes with RNAi phenotypes are equally likely to have an EST in all regions of the genome (see below), there is a significant drop ($p<0.05$) in the proportion of total genes with ESTs in the second locally duplicated gene cluster region (Fig 2b; 39% of genes in the second cluster have an EST compared with 53% over the entire chromosome). We suggest that a portion of the predicted genes in such regions of duplication may in fact be pseudogenes.

Genes that give RNAi phenotypes are much more likely to have an EST than genes on chromosome I in general (82% versus 53% respectively, $p<0.001$; Fig 2b). The relatively high percentage of genes with RNAi phenotypes that have ESTs may reflect that these genes are expressed at higher levels. It may also be that many genes that currently lack ESTs are only expressed conditionally; we are unlikely to have found phenotypes for such genes.

In *C. elegans*, there is evidence of differences between the chromosome arms and the central regions (the clusters), suggesting that there might be differences in gene type or function across the chromosome[24]. In general, the distribution of genes in any given phenotypic class was similar to that for all genes with an RNAi phenotype (e. g. Emb genes; compare Fig 2c with 2a). However, genes with viable post-embryonic phenotypes (Pep genes) — those that gave a post-embryonic phenotype without any embryonic or post-embryonic lethality, sterility, or developmental delay — show a trend toward enrichment at the arms of chromosome I ($p<0.1$). It has been suggested that the chromosome arms may be more prone to mutation and recombination than the central core portion[24] and, if so, that novel gene functions are more likely to evolve in such regions. Our finding that genes which uniquely affect post-embryonic development cluster at the arms supports this model.

Relationships between the predicted biochemical function of a gene product and its RNAi phenotype

To explore the relationship between the biochemical function of a gene product and its mutant phenotype, we categorised the sterile (Ste), embryonic lethal (Emb), uncoordinated (Unc) and viable post-embryonic phenotype (Pep) genes into the functional classes shown in Fig 3a.

Unsurprisingly, genes involved in basal metabolic processes account for ~50% of Ste and Emb genes (Fig. 3a); this confirms that these basic biochemical processes are indeed essential for viability. In contrast, under 20% of Unc and Pep genes encode components of the basal metabolic machinery, whereas more than twice as many encode proteins with more specialized functions (Figs. 3a, b). There is thus a clear difference between the types of gene required for germline function or embryonic viability (which mainly require basal machinery) and those involved in later developmental processes which appear to require proteins either of more specialized functions or of as yet unknown function (Fig. 3b).

A second clear trend is that the number of genes of unknown function increases greatly in the Unc and Pep genes, making this the largest overall class for those phenotypes (Fig. 3). This shift underlies the fact that while we know a great deal about basic metabolic processes of eukaryotic cells (and thus can readily ascribe function to a large proportion of Ste and Emb genes), much is still to be learnt about the complex processes and the genes that regulate the development and function of a multicellular eukaryote. A significant number (~25%) of genes of unknown function have close homologues in *Drosophila* or humans; further study of these may shed light on conserved processes specific to animals.

Comparison of genes essential for viability of *S. cerevisiae* and *C. elegans*

S. cerevisiae was the first eukaryote to be completely sequenced[25] and reverse genetics has been used extensively to investigate *S. cerevisiae* gene function. In a set of 3680 genes knocked out by targeted disruption, 890 affect viability[26]; we compared

these genes to those that gave different RNAi phenotypes in *C. elegans*. Yeast and worm genes important for viability have a similar distribution within the different functional classes, but are different from the Unc or Pep distributions (Fig 3c; also compare to 3a and 3b). This suggests that similar types of gene are required for viability of yeast and animal cells. A striking difference ($p<0.001$) is that only ~1% of the genes required for viability in yeast are transcription factors, whereas for *C. elegans* it is ~4% (a similar percentage of the genomes of yeast[27] and *C. elegans*[2] encode transcription factors, 3.3% and 2.5% respectively). This suggests that a large fraction of the *C. elegans* transcription factors required for viability may be involved in specific developmental processes.

An estimate of the size of the functionally non-redundant genome

What do our data tell us about the size of the functionally non-redundant genome? We screened 12.7% of the *C. elegans* genome and found that 339 genes gave a clearly discernible phenotype. Taking into account the sensitivity of our screen and scaling up to the entire genome, we estimate that ~5400 genes will be individually required for wild-type *C. elegans* development under standard laboratory conditions (~2300 genes for embryonic viability and ~3100 post-embryonically; see Methods for calculation). This is comparable to previous estimates based on forward genetics[28]. We expect that phenotypes for other genes will be identified under novel conditions (e. g. environmental stress), in other genetic backgrounds, or using more refined and restricted screening conditions.

Discussion

We have taken a systematic approach to identify functions for the predicted genes of *C. elegans* Chromosome I. This is the first large-scale reverse genetic analysis of a multicellular organism and has increased by five-fold the number of sequenced genes with known phenotypes on this chromosome.

While we have identified RNAi phenotypes for many genes, some will have eluded our screen for one of at least two reasons. Firstly, RNAi may have been ineffective against the targeted gene. RNAi does not accurately phenocopy the null phenotype of all genes (e. g. genes involved in neuronal function), and may result in either partial or no loss of function. It should also be noted that if multiple genes have regions of identical or near-identical nucleotide sequence, RNAi could target them simultaneously, so that the observed phenotype may be the result of the inhibition of more than one gene. Secondly, we will not have detected either subtle or conditional phenotypes. However, we anticipate that future RNAi-based screens using specific assays should be able to detect phenotypes for many more genes, thus increasing our understanding of *C. elegans* and hence of metazoan biology in general. Since our library consists of bacterial clones that can be replicated, and the feeding protocol is relatively simple compared with injection, the library can be used repeatedly at low cost and high efficiency for such screens. In addition, we expect that a feeding library and database of associated phenotypes will prove valuable for the positional cloning of genes; currently there are over 300 genes on chromosome I identified by mutation but not yet cloned.

Although the time needed for an RNAi screen using our bacterial library is similar to that for a classical genetic screen, the two approaches have different advantages and will yield different results. Both approaches can be used to screen the entire genome for genes involved in a particular process, and both may identify complete or partial loss-of-function phenotypes. Classical forward genetics generates stable mutant lines that can be maintained indefinitely; furthermore, while some genes are resistant to RNAi, all genes are sensitive to mutagens (albeit to a greater or lesser degree) and could thus be cloned using a classical screen. Also, some mutants isolated by forward genetics are due to gain-of-function mutations, which cannot be generated by RNAi. However, the positional cloning of a gene is often slow and laborious. RNAi, while having the disadvantages mentioned above, has the key advantage of all

reverse genetics: the sequence of the gene is already known, and thus any mutant phenotype observed is automatically connected to a known sequence.

In the future, we aim to extend our library construction and functional analysis to the entire *C. elegans* genome and anticipate that the possibility of genome-wide RNAi screening, in conjunction with other functional genomics approaches such as expression analyses using microarrays[29] and two-hybrid experiments[30] will accelerate *C. elegans* research.

Methods

Generation and cloning of PCR products. PCR products were synthesised using BioTaq polymerase (Bioline) in a reaction containing 25ng of *C. elegans* genomic DNA, 20pmol of *C. elegans* GenePairs primers (Research Genetics) and 100μM dNTPs: 34 cycles of [94°C 30s, 58°C 30s, 72°C 90s] were followed by an extension of 1hr at 72°C to enhance A-tailing of products. Products were ligated into linearized T-tailed L4440 vector[7] and transformed into the HT115(DE3) bacterial strain (L. Timmons and A. Fire, pers. comm.) using standard methods. Colonies containing correct sized insert were identified by PCR using vector specific oligos, and the cloned inserts confirmed by PCR using the original Research Genetics primer pair. Primer sequences are available at http://cmgm.stanford.edu/~kimlab/primers.12-22-99.html.

RNAi screening. RNAi was performed essentially as described in Kamath *et al*[8], where feeding data on 86 of the 2445 genes described here was previously reported. In brief, 4 wells of a 12-well plate containing NGM agar + 1mM IPTG + 25μg/ml carbenicillin were inoculated with bacterial cultures grown 8-18 hours for each targeted gene. 10-15 L3-L4 stage worms were placed in the first of the 4 wells for each gene and left for 72hrs at 15°C. Three worms, now young adults, were removed and individually placed on three remaining wells for each gene and allowed to lay embryos for 24hrs at room temperature; the three worms were then removed (t=0). The phenotypes of adults and progeny remaining in the first well were scored as well as of the progeny in wells 1-3. Our screen was not ideal for detection of phenotypes visible only in adults (e. g. egg-laying defective and progeny sterile); we will have missed some of these. Phenotypic analysis of lethality/sterility was carried out at t=24hr and post-embryonic phenotypes were analysed by two independent observers at t=36hr, t=48hr, t=60hr and t=72hr. Phenotypic classes were defined as follows. Embryonic lethal (Emb) reproducibly has 10-100% embryonic lethality; sterile (Ste) has a brood size of less than or equal to 10 (wild-type worms in these conditions typically give

over 50); progeny sterile (Stp) has a brood size of less than or equal to 10 in the progeny of fed worms. Post-embryonic phenotypes require at least 10% of the analysed worms to display a given phenotype; phenotypic classes are given in Table 1 legend. A full listing of phenotypes obtained is given in Supplementary Table 1; genes that we did not clone, and thus did not analyse, are given in Supplementary Table 3. Thus, any GenePair absent from both Supplementary Tables 1 and 3 was fed and did not give a detectable mutant phenotype.

Bioinformatic analyses and categorisation of genes into functional classes.

Analyses were carried out on GenePairs predictions rather than currently predicted genes since while gene predictions change, phenotypes will always match the GenePair. ~95% of GenePairs genes have a one-to-one match with a currently predicted gene. Current gene predictions that are targeted for RNAi by the primer pairs were identified by comparing electronic PCR (ePCR) fragments (generated using the ePCR program (ftp.ncbi.nlm.nih.gov/pub/schuler/e-PCR)[31] on the whole chromosome DNA files from the WS9 release of ACeDB (ftp.sanger.ac.uk/pub/wormbase)) to gene predictions in ACeDB. To identify additional genes that might be targeted for RNAi by a particular clone we found those with an overlap of 200bp or more with greater than 80% nucleotide identity with the predicted PCR product (asterisks in column 2 of Table 3 denote GenePairs that have such matches); however it is not yet known what level of identity is required for RNAi.

To find *C. elegans* genes with conservation in other organisms, BlastP[23] was carried out for each individual *C. elegans* gene on Chromosome I against *S. cerevisiae, Drosophila melanogaster* and human sequences. The databases used were as follows: *C. elegans* (18337 entries), *S. cerevisae* (6191 entries) and *D. melanogaster* (13743 entries) downloaded on 1 June 2000 from www.ebi.ac.uk/proteome; and *H. sapiens* (35723 entries, confirmed peptides) downloaded on 1 June 2000 from

Systematic functional genomic analysis of *C. elegans* Chromosome I by RNA interference

Andrew G. Fraser*[1], Ravi S. Kamath*[1], Peder Zipperlen*[1], Maruxa Martinez-Campos*, Marc Sohrmann† and Julie Ahringer*.

**Wellcome/CRC Institute, University of Cambridge, Tennis Court Road, CB2 1QR Cambridge, UK. Phone +44-1223-334144; Fax +44-1223-334089. †The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK.*

[1]These authors contributed equally to this work.

Complete genomic sequence is known for two multicellular eukaryotes, the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, and will be soon for humans. However, biological function has been assigned to only a small proportion of the predicted genes in any animal. We used RNA-mediated interference (RNAi) to target nearly 90% of predicted genes on *C. elegans* Chromosome I by feeding worms with bacteria that express double stranded RNA. We have assigned function to 13.9% of the genes analysed, increasing the number of sequenced genes with known phenotypes on Chromosome I from 70 to 378. While most genes with sterile or embryonic lethal RNAi phenotypes are involved in basal cell metabolism, many genes giving post-embryonic phenotypes have conserved but unknown function. In addition, conserved genes are significantly more likely to have an RNAi phenotype than genes with no conservation. We have constructed a reusable library of bacterial clones that permits unlimited future RNAi screens, which should help develop a more complete view of the relationships between the genome, gene function, and the environment.

The complete genomic sequence of an organism is an invaluable tool in understanding the molecular mechanisms underlying its development and function. The nematode worm *C. elegans* is one of two multicellular eukaryotes for which essentially complete genomic sequence is known[1,2]. 36% of predicted *C. elegans* genes have a significant human match[1,3] including many genes implicated in human diseases[3,4], and functional analysis of the *C. elegans* genome has shed light on many conserved biological processes and molecular pathways. A comprehensive functional analysis of all genes in *C. elegans* would greatly expand our knowledge of conserved gene function. We therefore decided to investigate systematically loss-of-function phenotypes of predicted genes of *C. elegans*, starting with Chromosome I.

RNA-mediated interference (RNAi) is a technique whereby the activity of a gene is transiently inhibited following the introduction of double-stranded RNA (dsRNA) of sequence specific to the targeted gene[5]. The specificity and potency of RNAi make it ideal for investigating gene function beginning only with genomic sequence[6]. Ingestion of dsRNA-expressing bacteria results in RNAi of the targeted gene[7], and we previously established that this technique is at least as effective as the injection of dsRNA for RNAi[8]: embryonic lethal phenotypes are detected with similar efficiency by feeding and injection, but feeding detects over 50% more post-embryonic phenotypes than injection. It is thus possible to make a library of dsRNA-expressing bacteria which could be used for high-throughput genome-wide RNAi screens at very low cost. It is important to note that since RNAi does not efficiently inhibit all genes, an RNAi-based screen will miss some relevant genes. Despite this caveat, RNAi is a useful screening tool to complement classical forward genetics.

Analysis of the function of genes on Chromosome I by RNAi

We constructed a library of bacteria expressing dsRNA corresponding to genes on Chromosome I. Chromosome I is the second smallest chromosome, has few

duplicated gene clusters and has no striking unusual features[1]. Each individual bacterial clone is able to synthesise dsRNA designed to target a single gene; since gene predictions are still changing, a few primer pairs no longer correspond to single genes (see Methods). In total, the resulting library contains 2445 independent clones, corresponding to 2416 predicted genes, a total of 87.3% of the 2769 currently predicted genes of Chromosome I.

We screened the library to identify genes whose inhibition gives a clearly detectable phenotype in wild-type worms as described in Methods. We were able to assign a phenotype to 13.9% of the analysed genes, raising the number of sequenced genes on chromosome I with known phenotypes from 70 to 378 (Table 1). Many genes have more than one associated phenotype, reflecting that genes frequently have multiple functions in the organism. Furthermore, since we examined worms that were only exposed to dsRNA as larvae or adults as well as their progeny, we could assign post-embryonic phenotypes to genes that result in sterility or produce 100% embryonic lethal progeny. A summary of these results and a partial listing of the phenotypes obtained are given in Tables 1 and 3. Full results are in Supplementary Table 1 and arepublicly accessible in WormBase (www.wormbase.org).

Our screen was sufficiently effective to identify 90% of known embryonic lethal genes. In addition, we were able to assign phenotypes to 45% of genes with a known post-embryonic phenotype that should have been detectable in our screen (Table 2 and Supplementary Table 2). However, we failed to find phenotypes for a number of previously characterised genes. In some cases (e. g. *fog-3*), this was not due to an inherent difficulty in inhibiting the genes using RNAi (since we obtained the correct phenotype in a separate experiment), but simply because we overlooked them in the screen. However, only one of eight genes involved in neuronal function gave a detectable RNAi phenotype; this accords well with our finding that neurons appear to be more resistant to RNAi than other cell types[8]. Similarly, we did not detect

phenotypes for several genes involved in sperm development (*fer-1, spe-9*, and *spe-11*).

The largest phenotypic class, comprising over 60% of the genes, are those whose inhibition by RNAi gives rise to embryonic lethality, the Emb genes; these include a large number of components of the basal cellular machinery. More interestingly, we find a homologue of the SMN human disease gene[9], a variety of genes encoding RNA-binding proteins (several such proteins play a role in early polarity; reviewed in [10]), a number of genes involved in chromosome condensation and separation, components of signal transduction pathways and many conserved genes that have no known biochemical function.

The largest class of post-embryonic phenotype is the Uncoordinated (Unc) class. Unc phenotypes arise from defects in the development or function of the neuromuscular system (reviewed in [11]). We find Unc genes encoding proteins involved in vesicle sorting and fusion as well as transcription factors (including a homologue of the zinc finger transcription factor MYT-1 which is only expressed in developing neurons in mammals[12-14]) and components of the cytoskeleton (e. g. a kakapo[15-18] and a talin[19] homologue).

A number of genes showed a high incidence of males (Him) phenotype. *C. elegans* is usually grown as a self-fertilising hermaphrodite with males arising at a low frequency in wild-type cultures due to non-disjunction of the X-chromosome (hermaphrodites have two X chromosomes, males only one). An increased number of males is indicative of either the incorrect segregation and maintenance of chromosomes in the germ line (reviewed in [20]) or defects in sexual specification. The Him genes that we identified include kinesins, a katanin homologue[21,22] and a nuclear hormone receptor.

Conservation of genes with RNAi phenotypes across eukaryotes

We examined the level of cross-species conservation of the genes for which we detected an RNAi phenotype (Fig 1). To find *C. elegans* genes that are conserved in other species, we identified *C. elegans* genes that have hits with BlastP[23] e-values below 1.00E-06 in *Saccharomyces cerevisiae*, *Drosophila melanogaster* or humans; we define these as a "match". Hits with BlastP e-values below 1.00E-10 and in which the conservation extends over at least 80% of the *C. elegans* protein length, we defined as "homologues"; this category includes orthologues. This provides a conservative estimate of the number of genes with regions of conservation (matches) or homologues, respectively.

We found that genes with RNAi phenotypes were much more likely to have a match ($p<0.001$) compared to all genes (Fig 1). Most striking is the similarity that we see between *C. elegans* and *Drosophila*: while 42% of *C. elegans* genes have a match and 19% have a homologue in *Drosophila*, we find that over 72% of genes with an RNAi phenotype have a *Drosophila* match and 43% have a homologue (Fig 1). This analysis shows that genes with a required function in *C. elegans* have been highly conserved across eukaryotic evolution. We also find that highly conserved genes are more likely to have an RNAi phenotype than genes that show no conservation: 26% of *C. elegans* genes that have a homologue in one of the organisms examined give an RNAi phenotype compared to only 5% of genes with no conservation ($p<0.001$).

Physical distribution on chromosome I of genes with RNAi phenotypes

Genes for which we identified an RNAi phenotype are evenly distributed across the chromosome with the exception of two regions (corresponding to segments 2 and 8-9 in Fig 2a) for which there appears to be a drop in number ($p<0.1$). These two regions correspond to the two regions of chromosome I that contain locally duplicated gene clusters[1]. We suggest that the reduction in the number of phenotypes observed by RNAi in these regions may be due to gene duplication and thus redundancy of function. It is worth noting that some of the predicted genes in the

duplicated regions may not be expressed: while genes with RNAi phenotypes are equally likely to have an EST in all regions of the genome (see below), there is a significant drop ($p<0.05$) in the proportion of total genes with ESTs in the second locally duplicated gene cluster region (Fig 2b; 39% of genes in the second cluster have an EST compared with 53% over the entire chromosome). We suggest that a portion of the predicted genes in such regions of duplication may in fact be pseudogenes.

Genes that give RNAi phenotypes are much more likely to have an EST than genes on chromosome I in general (82% versus 53% respectively, $p<0.001$; Fig 2b). The relatively high percentage of genes with RNAi phenotypes that have ESTs may reflect that these genes are expressed at higher levels. It may also be that many genes that currently lack ESTs are only expressed conditionally; we are unlikely to have found phenotypes for such genes.

In *C. elegans*, there is evidence of differences between the chromosome arms and the central regions (the clusters), suggesting that there might be differences in gene type or function across the chromosome[24]. In general, the distribution of genes in any given phenotypic class was similar to that for all genes with an RNAi phenotype (e. g. Emb genes; compare Fig 2c with 2a). However, genes with viable post-embryonic phenotypes (Pep genes) — those that gave a post-embryonic phenotype without any embryonic or post-embryonic lethality, sterility, or developmental delay — show a trend toward enrichment at the arms of chromosome I ($p<0.1$). It has been suggested that the chromosome arms may be more prone to mutation and recombination than the central core portion[24] and, if so, that novel gene functions are more likely to evolve in such regions. Our finding that genes which uniquely affect post-embryonic development cluster at the arms supports this model.

Relationships between the predicted biochemical function of a gene product and its RNAi phenotype

To explore the relationship between the biochemical function of a gene product and its mutant phenotype, we categorised the sterile (Ste), embryonic lethal (Emb), uncoordinated (Unc) and viable post-embryonic phenotype (Pep) genes into the functional classes shown in Fig 3a.

Unsurprisingly, genes involved in basal metabolic processes account for ~50% of Ste and Emb genes (Fig. 3a); this confirms that these basic biochemical processes are indeed essential for viability. In contrast, under 20% of Unc and Pep genes encode components of the basal metabolic machinery, whereas more than twice as many encode proteins with more specialized functions (Figs. 3a, b). There is thus a clear difference between the types of gene required for germline function or embryonic viability (which mainly require basal machinery) and those involved in later developmental processes which appear to require proteins either of more specialized functions or of as yet unknown function (Fig. 3b).

A second clear trend is that the number of genes of unknown function increases greatly in the Unc and Pep genes, making this the largest overall class for those phenotypes (Fig. 3). This shift underlies the fact that while we know a great deal about basic metabolic processes of eukaryotic cells (and thus can readily ascribe function to a large proportion of Ste and Emb genes), much is still to be learnt about the complex processes and the genes that regulate the development and function of a multicellular eukaryote. A significant number (~25%) of genes of unknown function have close homologues in *Drosophila* or humans; further study of these may shed light on conserved processes specific to animals.

Comparison of genes essential for viability of *S. cerevisiae* and *C. elegans*

S. cerevisiae was the first eukaryote to be completely sequenced[25] and reverse genetics has been used extensively to investigate *S. cerevisiae* gene function. In a set of 3680 genes knocked out by targeted disruption, 890 affect viability[26]; we compared

these genes to those that gave different RNAi phenotypes in *C. elegans*. Yeast and worm genes important for viability have a similar distribution within the different functional classes, but are different from the Unc or Pep distributions (Fig 3c; also compare to 3a and 3b). This suggests that similar types of gene are required for viability of yeast and animal cells. A striking difference ($p<0.001$) is that only ~1% of the genes required for viability in yeast are transcription factors, whereas for *C. elegans* it is ~4% (a similar percentage of the genomes of yeast[27] and *C. elegans*[2] encode transcription factors, 3.3% and 2.5% respectively). This suggests that a large fraction of the *C. elegans* transcription factors required for viability may be involved in specific developmental processes.

An estimate of the size of the functionally non-redundant genome

What do our data tell us about the size of the functionally non-redundant genome? We screened 12.7% of the *C. elegans* genome and found that 339 genes gave a clearly discernible phenotype. Taking into account the sensitivity of our screen and scaling up to the entire genome, we estimate that ~5400 genes will be individually required for wild-type *C. elegans* development under standard laboratory conditions (~2300 genes for embryonic viability and ~3100 post-embryonically; see Methods for calculation). This is comparable to previous estimates based on forward genetics[28]. We expect that phenotypes for other genes will be identified under novel conditions (e. g. environmental stress), in other genetic backgrounds, or using more refined and restricted screening conditions.

Discussion

We have taken a systematic approach to identify functions for the predicted genes of *C. elegans* Chromosome I. This is the first large-scale reverse genetic analysis of a multicellular organism and has increased by five-fold the number of sequenced genes with known phenotypes on this chromosome.

While we have identified RNAi phenotypes for many genes, some will have eluded our screen for one of at least two reasons. Firstly, RNAi may have been ineffective against the targeted gene. RNAi does not accurately phenocopy the null phenotype of all genes (e. g. genes involved in neuronal function), and may result in either partial or no loss of function. It should also be noted that if multiple genes have regions of identical or near-identical nucleotide sequence, RNAi could target them simultaneously, so that the observed phenotype may be the result of the inhibition of more than one gene. Secondly, we will not have detected either subtle or conditional phenotypes. However, we anticipate that future RNAi-based screens using specific assays should be able to detect phenotypes for many more genes, thus increasing our understanding of *C. elegans* and hence of metazoan biology in general. Since our library consists of bacterial clones that can be replicated, and the feeding protocol is relatively simple compared with injection, the library can be used repeatedly at low cost and high efficiency for such screens. In addition, we expect that a feeding library and database of associated phenotypes will prove valuable for the positional cloning of genes; currently there are over 300 genes on chromosome I identified by mutation but not yet cloned.

Although the time needed for an RNAi screen using our bacterial library is similar to that for a classical genetic screen, the two approaches have different advantages and will yield different results. Both approaches can be used to screen the entire genome for genes involved in a particular process, and both may identify complete or partial loss-of-function phenotypes. Classical forward genetics generates stable mutant lines that can be maintained indefinitely; furthermore, while some genes are resistant to RNAi, all genes are sensitive to mutagens (albeit to a greater or lesser degree) and could thus be cloned using a classical screen. Also, some mutants isolated by forward genetics are due to gain-of-function mutations, which cannot be generated by RNAi. However, the positional cloning of a gene is often slow and laborious. RNAi, while having the disadvantages mentioned above, has the key advantage of all

reverse genetics: the sequence of the gene is already known, and thus any mutant phenotype observed is automatically connected to a known sequence.

In the future, we aim to extend our library construction and functional analysis to the entire *C. elegans* genome and anticipate that the possibility of genome-wide RNAi screening, in conjunction with other functional genomics approaches such as expression analyses using microarrays[29] and two-hybrid experiments[30] will accelerate *C. elegans* research.

Methods

Generation and cloning of PCR products. PCR products were synthesised using BioTaq polymerase (Bioline) in a reaction containing 25ng of *C. elegans* genomic DNA, 20pmol of *C. elegans* GenePairs primers (Research Genetics) and 100μM dNTPs: 34 cycles of [94°C 30s, 58°C 30s, 72°C 90s] were followed by an extension of 1hr at 72°C to enhance A-tailing of products. Products were ligated into linearized T-tailed L4440 vector[7] and transformed into the HT115(DE3) bacterial strain (L. Timmons and A. Fire, pers. comm.) using standard methods. Colonies containing correct sized insert were identified by PCR using vector specific oligos, and the cloned inserts confirmed by PCR using the original Research Genetics primer pair. Primer sequences are available at http://cmgm.stanford.edu/~kimlab/primers.12-22-99.html.

RNAi screening. RNAi was performed essentially as described in Kamath *et al*[8], where feeding data on 86 of the 2445 genes described here was previously reported. In brief, 4 wells of a 12-well plate containing NGM agar + 1mM IPTG + 25μg/ml carbenicillin were inoculated with bacterial cultures grown 8-18 hours for each targeted gene. 10-15 L3-L4 stage worms were placed in the first of the 4 wells for each gene and left for 72hrs at 15°C. Three worms, now young adults, were removed and individually placed on three remaining wells for each gene and allowed to lay embryos for 24hrs at room temperature; the three worms were then removed (t=0). The phenotypes of adults and progeny remaining in the first well were scored as well as of the progeny in wells 1-3. Our screen was not ideal for detection of phenotypes visible only in adults (e. g. egg-laying defective and progeny sterile); we will have missed some of these. Phenotypic analysis of lethality/sterility was carried out at t=24hr and post-embryonic phenotypes were analysed by two independent observers at t=36hr, t=48hr, t=60hr and t=72hr. Phenotypic classes were defined as follows. Embryonic lethal (Emb) reproducibly has 10-100% embryonic lethality; sterile (Ste) has a brood size of less than or equal to 10 (wild-type worms in these conditions typically give

over 50); progeny sterile (Stp) has a brood size of less than or equal to 10 in the progeny of fed worms. Post-embryonic phenotypes require at least 10% of the analysed worms to display a given phenotype; phenotypic classes are given in Table 1 legend. A full listing of phenotypes obtained is given in Supplementary Table 1; genes that we did not clone, and thus did not analyse, are given in Supplementary Table 3. Thus, any GenePair absent from both Supplementary Tables 1 and 3 was fed and did not give a detectable mutant phenotype.

Bioinformatic analyses and categorisation of genes into functional classes.

Analyses were carried out on GenePairs predictions rather than currently predicted genes since while gene predictions change, phenotypes will always match the GenePair. ~95% of GenePairs genes have a one-to-one match with a currently predicted gene. Current gene predictions that are targeted for RNAi by the primer pairs were identified by comparing electronic PCR (ePCR) fragments (generated using the ePCR program (ftp.ncbi.nlm.nih.gov/pub/schuler/e-PCR)[31] on the whole chromosome DNA files from the WS9 release of ACeDB (ftp.sanger.ac.uk/pub/wormbase)) to gene predictions in ACeDB. To identify additional genes that might be targeted for RNAi by a particular clone we found those with an overlap of 200bp or more with greater than 80% nucleotide identity with the predicted PCR product (asterisks in column 2 of Table 3 denote GenePairs that have such matches); however it is not yet known what level of identity is required for RNAi.

To find *C. elegans* genes with conservation in other organisms, BlastP[23] was carried out for each individual *C. elegans* gene on Chromosome I against *S. cerevisiae, Drosophila melanogaster* and human sequences. The databases used were as follows: *C. elegans* (18337 entries), *S. cerevisae* (6191 entries) and *D. melanogaster* (13743 entries) downloaded on 1 June 2000 from www.ebi.ac.uk/proteome; and *H. sapiens* (35723 entries, confirmed peptides) downloaded on 1 June 2000 from

www.ensembl.org. NCBI-Blast2 was used (BLASTP 2.0.6) with the SEG filter, and the search space was set to 7947758.

We defined "sequenced genes with a known phenotype" as being those with a named entry in ACeDB that also have a known phenotype entered in ACeDB, WormBase (www.wormbase.org) or the Proteome database (www.proteome.com). EST data was supplied by the Sanger Centre on 21 June 2000.

Predicted gene products were placed into functional classes by manual inspection, primarily using data from Proteome, InterPro and Blast analysis[23,32]. The functional classes are: (1) DNA synthesis; (2) RNA synthesis and processing including general transcription machinery, splicing/processing, RNA binding and regulation of chromatin; (3) Protein synthesis and proteolysis including translation, degradation and folding; (4) Metabolism including energy production and intermediary metabolism; (5) Cell cycle and chromosome dynamics; (6) Cell biology and cellular structure including cell junction/adhesion, cytoskeleton, ion channels, protein trafficking and vesicle regulation and cell polarity; (7) Gene specific transcription; and (8) Signal transduction including kinases, phosphatases and components of signal transduction pathways.The Unknown functional class contains genes which either have motifs about which there is insufficient information to assign a function, or genes with no significant matches in any organism.

Estimates of non-redundant genome size were done as follows. We detected 90.5% of genes known to give an embryonic lethal phenotype and 32.6% of genes known to give a post-embryonic phenotype. After screening 87.3% of the genes on chromosome I, we identified 226 Emb genes and 113 genes that only gave a post-embryonic RNAi phenotype (including steriles); adjusting for our efficiencies of detection, we estimate that on chromosome I, 286 genes should be required for viability and ~397 for post-embryonic processes. We screened 12.7% of the genome,

and thus for the entire genome we expect 2250 Emb genes and 3130 genes to have a post-embryonic phenotype.

References

1. The *C. elegans* Sequencing Consortium. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**, 2012-2018 (1998).

2. Adams, M. D. *et al.* The genome sequence of *Drosophila melanogaster*. *Science* **287**, 2185-2195 (2000).

3. Rubin, G. M. *et al.* Comparative genomics of the eukaryotes. *Science* **287**, 2204-2215 (2000).

4. Ahringer, J. Turn to the worm! *Curr Opin Genet Dev* **7**, 410-415 (1997).

5. Fire, A. *et al.* Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806-811 (1998).

6. Tabara, H., Grishok, A. & Mello, C. C. RNAi in *C. elegans*: soaking in the genome sequence. *Science* **282**, 430-431 (1998).

7. Timmons, L. & Fire, A. Specific interference by ingested dsRNA. *Nature* **395**, 854 (1998).

8. Kamath, R. S., Martinez-Campos, M., Zipperlen, P., Fraser, A. G. & Ahringer, J. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *C. elegans*. *submitted* (2000).

9. Lefebvre, S. *et al.* Identification and characterization of a spinal muscular atrophy- determining gene. *Cell* **80**, 155-165 (1995).

10. Bowerman, B. & Shelton, C. A. Cell polarity in the early *Caenorhabditis elegans* embryo. *Curr Opin Genet Dev* **9**, 390-395 (1999).

11. Bargmann, C. I. Neurobiology of the *Caenorhabditis elegans* genome. *Science* **282**, 2028-2033 (1998).

12. Kim, J. G. & Hudson, L. D. Novel member of the zinc finger superfamily: A C2-HC finger that recognizes a glia-specific gene. *Mol Cell Biol* **12**, 5632-5639 (1992).

13. Armstrong, R. C., Kim, J. G. & Hudson, L. D. Expression of myelin transcription factor I (MyTI), a "zinc-finger" DNA- binding protein, in developing oligodendrocytes. *Glia* **14**, 303-321 (1995).

14. Kim, J. G. *et al.* Myelin transcription factor 1 (Myt1) of the oligodendrocyte lineage, along with a closely related CCHC zinc finger, is expressed in developing neurons in the mammalian central nervous system. *J Neurosci Res* **50**, 272-290 (1997).

15. Strumpf, D. & Volk, T. Kakapo, a novel cytoskeletal-associated protein is essential for the restricted localization of the neuregulin-like factor, vein, at the muscle-tendon junction site. *J Cell Biol* **143**, 1259-1270 (1998).

16. Gregory, S. L. & Brown, N. H. *kakapo*, a gene required for adhesion between and within cell layers in *Drosophila*, encodes a large cytoskeletal linker protein related to plectin and dystrophin. *J Cell Biol* **143**, 1271-1282 (1998).

17. Lee, S., Harris, K. L., Whitington, P. M. & Kolodziej, P. A. *short stop* is allelic to *kakapo*, and encodes rod-like cytoskeletal- associated proteins required for axon extension. *J Neurosci* **20**, 1096-1108 (2000).

18. Prokop, A., Uhler, J., Roote, J. & Bate, M. The *kakapo* mutation affects terminal arborization and central dendritic sprouting of *Drosophila* motorneurons. *J Cell Biol* **143**, 1283-1294 (1998).

19. Burridge, K. & Connell, L. Talin: a cytoskeletal component concentrated in adhesion plaques and other sites of actin-membrane interaction. *Cell Motil* **3**, 405-417 (1983).

20. Hodgkin, J. Sex, cell death, and the genome of *C. elegans*. *Cell* **98**, 277-280 (1999).

21. McNally, F. J. & Vale, R. D. Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* **75**, 419-429 (1993).

22. Srayko, M., Buster, D. W., Bazirgan, O. A., McNally, F. J. & Mains, P. E. MEI-1/MEI-2 katanin-like microtubule severing activity is required for *Caenorhabditis elegans* meiosis. *Genes Dev* **14**, 1072-1084 (2000).

23. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J Mol Biol* **215**, 403-410 (1990).

24. Barnes, T. M., Kohara, Y., Coulson, A. & Hekimi, S. Meiotic recombination, noncoding DNA and genomic organization in *Caenorhabditis elegans*. *Genetics* **141**, 159-179 (1995).

25. Goffeau, A. *et al.* Life with 6000 genes. *Science* **274**, 546, 563-547 (1996).

26. Mewes, H. W. *et al.* MIPS: a database for genomes and protein sequences. *Nucleic Acids Res* **28**, 37-40 (2000).

27. Svetlov, V. V. & Cooper, T. G. Review: compilation and characteristics of dedicated transcription factors in *Saccharomyces cerevisiae*. *Yeast* **11**, 1439-1484 (1995).

28. Hodgkin, J. & Herman, R. K. Changing styles in *C. elegans* genetics. *Trends Genet* **14**, 352-357 (1998).

29. Chen, J. J. *et al.* Profiling expression patterns and isolating differentially expressed genes by cDNA microarray system with colorimetry detection. *Genomics* **51**, 313-324 (1998).

30. Walhout, A. J. *et al.* Protein interaction mapping in *C. elegans* using proteins involved in vulval development. *Science* **287**, 116-122 (2000).

31. Schuler, G. D. Sequence mapping by electronic PCR. *Genome Res* **7**, 541-550 (1997).

32. Altschul, S. F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389-3402 (1997).

Acknowledgements

We would like to thank Andy Fire and Lisa Timmons for kindly providing protocols, feeding vectors, and the HT115(DE3) bacterial strain and thank Richard Durbin, Daniel St.Johnston and Bjoern Schumacher for critical reading of the manuscript. A. G. F. was supported by a U. S. Army Breast Cancer Research Fellowship, R. S. K. by a Howard Hughes Medical Institute Predoctoral Fellowship, M. M.-C. by an EC-TMR Network Grant, P. Z. by a Wellcome Trust Studentship, M. S. by a Swiss National Science Foundation fellowship and J. A. by a Wellcome Trust Senior Research Fellowship (054523).

Correspondence and requests for materials should be addressed to J.A. (email: jaa@mole.bio.cam.ac.uk).

Figure Legends

Table 1 Summary of phenotypes arising from RNAi of genes on Chromosome I. The number of predicted genes whose targeting via RNAi gave rise to each phenotype is shown. Percentages are given as percentage of total number of clones screened (2445). Phenotypic classes were defined as described in Methods. The phenotypes are Emb (embryonic lethal), Ste (sterile), Stp (sterile progeny), Gro (slow post-embryonic growth), Lva (larval arrest), Lvl (larval lethality), Unc (uncoordinated), Pvl (protruding vulva), Bmd (body morphological defects), Dpy (dumpy), Clr (clear), Him (high incidence of males), Rup (ruptured), Mlt (molt defects), Prz (paralyzed), Sma (small), Egl (egg-laying defective), Sck (sick), Bli (blistering of cuticle), Muv (multivulva), Rol (roller), Adl (adult lethal), Lon (long), and Hya (hyperactive).

Table 2 Detection of forward genetic loci on Chromosome I by RNAi. RNAi phenotypes were compared to those of genes that have known loss-of-function phenotypes. "Genetic loci fed" denotes the number of genes in each category that were analysed by RNAi. "Possible to detect" denotes the number of genes that have a loss-of-function phenotype that would have been detectable in our screen. "RNAi phenotype detected" gives the number of genes for which a phenotype was identified. "Published phenotype detected" gives the number of genes for which the RNAi phenotype matched a published phenotype. Supplementary Table 2 gives full data. RNAi could reduce both maternal gene activity in the P0 and zygotic gene activity in the F1; this could explain some of the differences between RNAi phenotypes and published phenotypes.

Table 3 Partial list of RNAi phenotypes of genes on Chromosome I. RNAi phenotypes are shown for genes in the following functional classes: Chromosome dynamics and cell cycle; Cell structure; Specific transcription; and Signal transduction. For each gene, the following data are shown: the Research Genetics GenePairs name; whether a number of paralogues might be targeted (asterisk in column 2; methods gives criterion); the corresponding genetic locus name if it exists; a short description of gene function; the RNAi phenotype in which embryonic lethality (Emb), fecundity (Ste), post-embryonic phenotypes (P1-3) and developmental delay (Dev) are shown separately. Emb and Ste are classified into weak (white box, black "+") or strong (black box, white "+") phenotypes. For Emb, weak is 10-80% embryonic lethality, strong is 90% embryonic lethal or more; weak Ste denotes a brood size of 1 to 10, whereas strong Ste is totally sterile. Column H shows whether there is a match (white box, black "+") or a homologue (black box, white "+") in *Drosophila melanogaster, Saccharomyces cerevisiae* or humans. Phenotypic abbreviations are given in legend to Table 1. The GenePairs name does not always correspond with the current predicted gene name since gene predictions change.

Figure 1 Conservation of genes with an RNAi phenotype. Matches or homologues of *C. elegans* genes were identified as described in the text. Percentages of all genes (blue bars) or genes with RNAi phenotypes (red bars) with matches or homologues in *S. cerevisiae* (SC), *D. melanogaster* (DM), humans (HS), all three combined (ALL), or with no matches in any organism (NO M) are shown. The significance of the differences between the percentages of genes and the percentages of genes with RNAi phenotypes that have homologues is $p<0.001$ for all cases except for comparison to human homologues for which it is $p<0.1$.

Figure 2 Distribution on Chromosome I of genes with RNAi phenotypes and genes with ESTs. In each panel, chromosome I was analysed in 10 consecutive portions, each containing 10% of predicted genes. **a)** the percentage of all genes with an RNAi phenotype that are in each portion. **b)** the percentage of all predicted genes that have an EST (blue bars) or of genes that gave an RNAi phenotype that have an EST (red bars) in each portion. **c)** the percentage of Emb genes (black bars) or genes with viable post-embryonic phenotypes (pink bars) in each chromosomal portion. The boxes labelled "dup region" show the approximate location of regions containing local duplications.

Figure 3 Functional classes of Emb, Ste, Unc and Pep genes. Predicted products of genes that gave Ste, Emb, Unc or viable post-embryonic (Pep) RNAi phenotypes were placed into functional classes as described in Methods. Genes whose products could not be accurately classified into any of the 8 functional classes were placed into the unknown category (white). Numbers denote the percentage of genes in each functional class; pie charts illustrate these numbers graphically. **b)** Pie charts show distributions of predicted gene products grouped as follows: basal metabolic category (red) comprises the classes of DNA, RNA, protein and intermediate metabolism; specialized functions (blue) comprises cell cycle and chromosome dynamics, cell biology and cellular structure, gene specific transcription factors and signal transduction. Worms show the tissue affected in each phenotypic class shaded in grey. **c)** Distribution of genes giving rise to non-viable RNAi phenotypes in *C. elegans* (worm) or to non-viable phenotypes following disruption in *S. cerevisiae* (yeast).

Supplementary Table 1 Phenotypes arising from RNAi of genes on Chromosome I. Genes that have a detectable RNAi phenotype are grouped

by the functional classes shown in Fig 3. For each gene, the following data are shown: the Research Genetics GenePairs name; whether the sequence might target a number of paralogues (asterisk in column 2; methods gives criterion for this); the corresponding genetic locus name if it exists; a short description of gene function; the RNAi phenotype in which embryonic lethality (Emb), fecundity (Ste), post-embryonic phenotypes (P1-3) and developmental delay (Dev) are shown separately; existence of matches (lower case) or homologues (filled box, white upper-case text) in *C. elegans* (CE), *Drosophila melanogaster* (DM), *Saccharomyces cerevisiae* (SC) or humans (HS); and whether or not the gene has an EST (E). Abbreviations used are Ste (sterile), 1-5 (fed worm had 1-5 progeny), 6-10 (fed worm had 6-10 progeny), Stp (progeny sterile), Lvl (larval lethality), Unc (uncoordinated), Pvl (protruding vulva), Bmd (body morphological defects), Dpy (dumpy), Clr (clear), Him (high incidence of males), Rup (ruptured), Mlt (molt defects), Prz (paralyzed), Sma (small), Egl (egg-laying defective), Sck (sick), Bli (blistering of cuticle), Muv (multivulva), Rol (roller), Adl (adult lethal), Lon (long), Hya (hyperactive), Gro (slow post-embryonic growth) and Lva (larval arrest). "Mult" indicates that the gene has multiple equal-penetrance post-embryonic phenotypes. If the dsRNA overlaps multiple adjacent genes of different function, these appear in the "Multiple genes" category. The GenePairs name does not always correspond with the current predicted gene name since gene predictions change.

Supplementary Table 2 RNAi phenotypes for previously identified loci on Chromosome I. Columns 1 and 2 give genes on chromosome I with previously identified embryonic lethal or post-embryonic phenotypes and the GenePairs primer pair that amplifies a fragment overlapping that gene, respectively . "Mutant Phenotype" gives the published phenotype. RNAi phenotype headings: "Emb" (percentage embryonic lethality); "Ste" (sterility);

"P1", "P2" and "P3" (post-embryonic phenotypes); and "Dev" (slow or arrested growth). "Hit" indicates whether an RNAi phenotype was obtained in the initial screen (tick), whether no mutant phenotype was obtained ("o") or whether a mutant phenotype was obtained in separate feeding experiment ("*"). Phenotype abbreviations are given in the Supplementary Figure 1 legend with the following additions: Slu (sluggish), Vul (vulvaless), Mec (mechanosensory abnormality), Daf (dauer larva formation abnormal), Ttx (thermotaxis abnormal), Che (chemotaxis defective). Also, the following abbrevations are used: phen (phenotype), migr (migration), red (reduced), wk (weak), abnl (abnormal), and dk (dark). Genes with null phenotypes that we would have failed to detect in our screen are shaded in light grey; genes that we failed to clone, and therefore failed to analyse, are shaded in dark grey.

Supplementary Table 3 GenePairs on Chromosome I for which no clone was obtained.

Table 1 Summary of phenotypes arising from RNAi of genes on Chromosome I

PHENOTYPE		NUMBER	PERCENT
All phenotypes	TOTAL	339	13.9
Embryonic lethal	Emb	226	9.2
Sterile	Ste	82	3.4
	Stp	14	0.6
Developmental delay	Gro/Lva	145	5.9
Larval lethal	Lvl	38	1.6
Post-embryonic	Unc	70	2.9
	Pvl	29	1.2
	Bmd	27	1.1
	Dpy	19	0.8
	Clr	14	0.6
	Him	13	0.5
	Rup	9	0.4
	Mlt	8	0.3
	Prz	8	0.3
	Sma	6	0.2
	Egl	5	0.2
	Sck	5	0.2
	Bli	4	0.2
	Muv	2	0.1
	Rol	2	0.1
	Adl	1	< 0.1
	Lon	1	< 0.1
	Hya	1	< 0.1

Table 2 Detection of forward genetic loci on Chromosome I by RNAi.

Phenotype	Genetic loci fed	Possible to detect	RNAi phenotype detected	Published phenotype detected
All phenotypes	62	50	31	25
Embryonic lethal	21	21	19	16
Sterile	3	3	2	2
Sterile progeny	4	4	1	1
Developmental delay	0	0	-	-
Larval lethal	4	4	1	1
Post-embryonic	43	31	14	9

Table 3 Partial list of RNAi phenotypes of genes on Chromosome I

GenePairs		Locus	Description	Emb	Ste	P1	P2	P3	Dev	H
B0511.8			CDC1-like	+	-	-	-	-	Lva	+
C37A2.4		cye-1	cyclin e	+	-	Clr	-	-	-	+
C41G7.2	*		kinesin	+	-	Him	-	-	-	+
C53H9.2			chrom stability	+	-	-	-	-	Gro	+
F57B10.12		mei-2	katanin homol	+	-	-	-	-	-	+
M01E11.6	*		kinesin	+	-	Him	-	-	Gro	+
R06C7.8			Bub1-like	+	-	Pvl	Rup	Lvl	-	+
T01G9.5		mei-1	ATPase	+	-	-	-	-	-	+
W09G3.3	*		RCC1 domains	-	-	Him	-	-	-	+
Y110A7A.d			cdc27 homologue	+	-	-	-	-	Gro	+
Y39G10A_246.e			MCM4-like	+	-	-	-	-	-	+
Y39G10A_246.i			INCENP-like	+	-	-	-	-	-	+
Y47G6A_247.i			pombe Rad2 homol	+	-	-	-	-	-	+
Y52B11A.9			Kin17 homol	+	-	Bmd	Rup	-	-	+
Cell structure										
C01G8.5			Ezrin-like	-	-	Unc	Lvl	-	Gro	+
C10H11.1			villin	-	-	Him	-	-	-	+
C17E4.9			PDZ domain	+	+	Unc	-	-	-	+
C32E8.10		unc-11	vesicle reg	+	-	-	-	-	-	+
C45G3.1			actin-binding	+	-	Unc	-	-	-	+
C47B2.3	*	tba-2	tubulin	+	-	-	-	-	-	+
C53D5.a			nuclear import	+	+	-	-	-	Gro	+
C53D5.i			nuclear import	+	-	-	-	-	Gro	+
DY3.2		lam-1	nuclear lamin	+	+	Lvl	-	-	-	+
E03H4.8	*		beta coatomer-like	-	+	Unc	Stp	Clr	Gro	+
F07A5.7		unc-15	paramyosin	-	-	Unc	Prz	Egl	-	+
F20G4.3		nmy-2	non-muscle myosin	+	+	-	-	-	-	+
F21C3.5			MT nucleation	+	-	Unc	Bmd	-	Gro	+
F26B1.3			karyopherin	+	-	Lvl	-	-	-	+
F26E4.8	*	tba-2	tubulin	+	-	-	-	-	-	+
F26H9.6			ras superfamily	+	+	Lvl	-	-	-	+
F28H1.2			calponin domain	+	-	-	-	-	Lva	+
F30A10.6			transporter	-	-	-	-	-	Gro	+
F36H2.1			cation transporter	+	-	-	-	-	Gro	+
F43G9.10			microfib assoc	+	-	-	-	-	Gro	+
F46F11.5			vacuolar ATPase	+	+	Lvl	-	-	-	+
F53B8.1	*		plectrins	+	-	Unc	Prz	Lvl	-	+
F53F10.5			nucleoporin-like	+	-	-	-	-	-	+
F54C1.7			troponin c	-	+	-	-	-	-	+
F55A12.7			UNC-101 homol	-	-	Unc	-	-	Gro	+
F55F8.5			MT associated	-	-	-	-	-	Lva	+
F56F4.5			transporter	-	-	Him	-	-	-	+
H15N14.1			human NSF-like	+	+	-	-	-	-	+
M01A10.3			ribophorin	+	+	Lvl	Unc	-	-	+
R05D11.3			NTF2 homol	+	+	-	-	-	Gro	+
T03F1.9			UNC-89-like	+	-	-	-	-	-	
T19B4.2			NUP153-like	+	+	-	-	-	-	+
T21E12.4		dhc-1	dynein heavy chain	+	-	-	-	-	-	+
T25G3.2			chitin synthases	+	-	-	-	-	Gro	+
T26E3.3		par-6	PDZ domain	+	-	-	-	-	-	+
W02B9.1		hmr-1	cadherin	+	-	Bmd	Unc	Dpy	-	+
W04C9.1			ABC transporter	+	-	-	-	-	Gro	+
Y105E8C.n			gamma-adaptin	+	-	Unc	Lvl	-	Gro	+
Y18D10A.17			sup of clathrin defic	+	-	-	-	-	-	+
Y18D10A.20		pfn-1	profilin	+	-	-	-	-	-	+

GenePairs		Locus	Description	Emb	Ste	P1	P2	P3	Dev	H
Cell structure										
Y34D9A_152.a			vacuolar sorting	+	-	Unc	Prz	Lvl	Gro	+
Y48G8A_3945.e			adaptin subunit	-	-	Unc	-	-	-	+
Y71A12B.a			gravin-like	+	-	Dpy	Clr	-	-	+
Y71F9A_279.b			NXT1 homol	+	-	Pvl	Unc	-	-	+
Y71F9A_282.b			coatomer subunit	+	+	Unc	-	-	-	+
Y71F9A_290.a			NTF2 homol	-	-	Pvl	Rup	Clr	-	+
Y71G12A_195.e			talin	+	+	Unc	Prz	-	-	+
Y87G2A.s			HuVPS28 homol	-	-	Unc	-	-	-	+
Y87G2A.x			protein trafficking	-	-	Unc	-	-	Gro	+
Y87G2A.y			protein trafficking	-	-	Clr	-	-	-	+
ZK1014.1			vesicle fusion	-	+	Lvl	-	-	-	+
ZK1151.2			spectrin repeats	-	-	Unc	Bmd	Stp	-	+
B0025.3			txnl corepressor	+	-	-	-	-	Gro	+
C01G8.7			eyelid-like	+	+	Bmd	Lvl	-	Gro	+
C01G8.8			eyelid-like	+	-	Bmd	-	-	-	+
C01H6.5		nhr-23	nuc horm recep.	-	-	Unc	Lvl	Dpy	-	+
C12C8.3		lin-41	NHL domains	-	+	-	-	-	-	+
C32F10.7		nhr-2	nuc horm recep.	-	-	Him	-	-	-	+
C48E7.3			bZIP	-	-	-	-	-	Gro	+
D1081.2			SRF homol	-	-	Unc	Prz	-	-	+
F43G9.12			TCF-9-like	+	-	-	-	-	Gro	+
F52F12.6			MYT1 homol	-	-	Unc	-	-	-	+
F55F8.4			txnl repression	+	-	-	-	-	Lva	+
F57B10.1			bZIP	+	+	Sma	Dpy	-	Lva	+
K02B12.1		ceh-6	homeobox	-	-	Unc	Mlt	-	-	+
M05B5.5		hlh-2	bHLH	+	+	Unc	Pvl	-	-	+
W02D3.9		unc-37	groucho family	+	+	Unc	-	-	-	+
W03D8.4		pop-1	HMG box	+	+	-	-	-	-	+
Y40B1A.4			Zn finger	-	-	Unc	Bmd	-	-	+
Y54E5B.3			Mediator complex	+	-	Rup	-	-	-	+
Y65B4A_179.b			txnl activator	-	-	Unc	Dpy	-	-	+
ZC247.3		lin-11	LIM homeodomain	-	-	-	-	-	Gro	+
ZK858.4		mel-26	kruppel-like	+	-	-	-	-	-	+
Signalling										
C09D1.1		unc-89	multiple domains	-	-	Unc	-	-	-	+
C10H11.9		let-502	ROCK	+	-	Dpy	Rol	Lvl	-	+
C26C6.2		goa-1	G-α subunit	+	+	Unc	Pvl	Egl	-	+
C32E8.5			FHA domain	+	+	-	-	-	Gro	+
F26E4.1			PP2A reg subunit	+	-	Lvl	-	-	-	+
F55A12.3			PI-4P 5' kinase	-	+	-	-	-	-	+
F55C7.4		unc-73	GEF	+	-	-	-	-	-	+
F55C7.7		unc-73	GEF	-	-	Egl	-	-	-	+
K04G2.8		apr-1	APC homol	-	-	Unc	Bmd	Lvl	-	+
K05C4.6		hmp-2	B-catenin	+	-	Unc	Dpy	Bmd	-	+
K12C11.2			SUMO-1 like	+	-	Pvl	-	-	-	+
T01G9.6		kin-10	CKIIbeta subunit	+	-	Pvl	-	-	Gro	+
T21E3.1	*		PTPase	+	+	-	-	-	-	+
T23D8.1		mom-5	frizzled-like	-	-	Unc	Bmd	-	-	+
T23H2.5	*		rab family	-	-	Sma	Clr	-	-	+
Y106G6E.6			Casein Kinase 1	+	-	Dpy	-	-	Gro	+
Y18D10A.5		gsk-3	GSK-3	+	-	-	-	-	-	+
ZC581.1			NIMA-like kinase	-	-	Unc	Lvl	Rol	-	+
ZK265.6			G prot coup recep	-	-	-	-	-	Gro	+

Figure 1

a

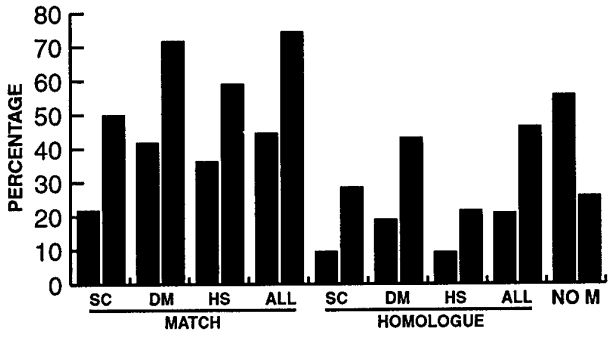
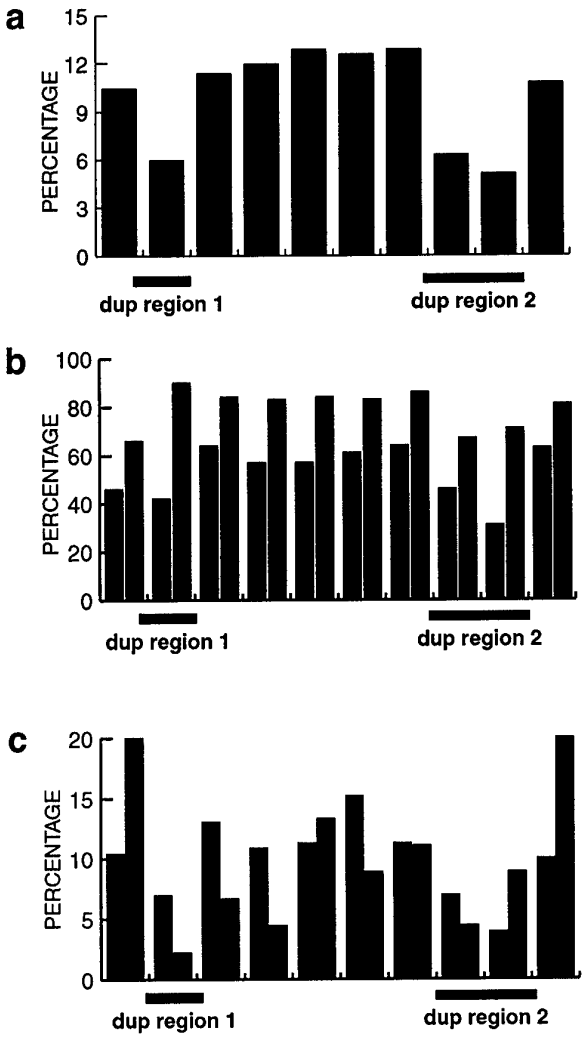
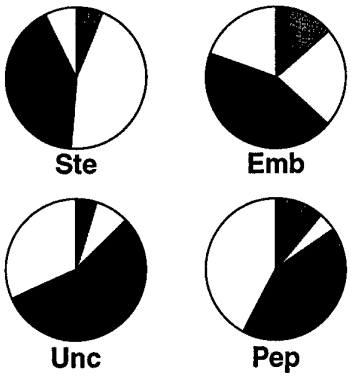

80
70
60
50
40
30
20
10
0
PERCENTAGE
SC
DM
HS
ALL
SC
DM
HS
ALL
NO M
MATCH
HOMOLOGUE

Fig2

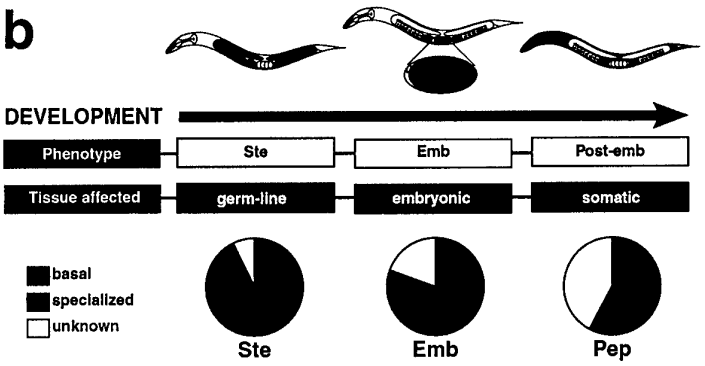

a
15
12
9
6
3
0
PERCENTAGE
dup region 1
dup region 2
b
100
80
60
40
20
0
PERCENTAGE
dup region 1
dup region 2
c
20
15
10
5
0
PERCENTAGE
dup region 1
dup region 2

a

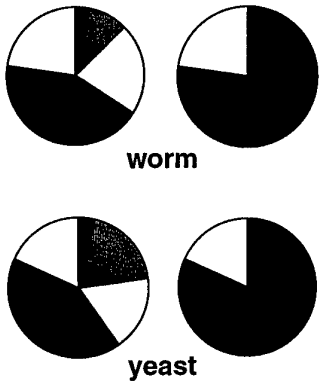
	Ste	Emb	Unc	Pep
DNA synthesis	1.2	1.3	0.0	2.2
RNA metabolism	4.8	11.8	4.7	8.9
Protein metabolism	44.6	22.3	7.8	4.4
Energy/metabolism	10.8	10.9	6.3	2.2
Chrme dynamics/cell cycle	0.0	6.1	0.0	2.2
Cell structure/organisation	18.1	15.7	26.6	15.6
Specific transcription	7.2	4.8	12.5	13.3
Signalling	4.8	4.8	9.4	8.9
Unknown	7.2	18.8	31.3	42.2



b



c



GenePairs		Locus	Description	Emb	Ste	P1	P2	P3	Dev	CE	DM	SC	HS	E
DNA synthesis														
F33H2.5			DNA pol epsilon subunit	100%	-	-	-	-	-		DM	SC	HS	+
M01E5.5			DNA topo I	-	-	Mult	Him	Rup	-		dm	sc	hs	+
W02D9.1			DNA primase (DNA pol alpha subunit)	100%	-	Pvl	-	-	-		DM	SC	hs	+
ZC308.1			DNA topo I-like	100%	Ste	-	-	-	Gro	ce	dm		hs	+
RNA metabolism														
C03D6.3		cel-1	mRNA capping enzyme	-	-	Mult	Bmd	Dpy	-	ce	DM	sc	ha	
C17E4.5			poly-A binding protein	90%	-	Unc	-	-	Lva	ce	dm	sc	hs	+
C26C6.1			HMG protein	-	-	Pvl	-	-	Gro		DM	sc	hs	+
C26C6.5			histone deacetylase complex subunit	-	-	Muv	Pvl	Egl	-		dm		hs	+
C36B1.3			RNA pol II subunit	100%	-	-	-	-	Gro	ce	dm	SC	hs	+
C36B1.5			splicing factor	100%	Ste	Pvl	-	-	Gro	ce	DM	sc	hs	
C37A2.1			RNA-binding protein like T-STAR, GLD-1	-	-	Mult	-	-	-	ce	dm		hs	+
C41G7.1			SMN orthologue	90%	-	Stp	-	-	Gro				HS	+
C48E7.2			RNA pol III subunit		-	-	-	-	Lva		DM		hs	+
C50F2.3			putative splicing factor	100%	-	-	-	-	Gro	ce	DM	SC	hs	+
D1081.8			Myb-like; role in splicing	100%	-	-	-	-	-		DM	sc	hs	+
F10G8.3			mRNA export	-	-	Pvl	Stp	Hya	-	CE	DM	SC	HS	+
F26A3.2			nuclear mRNA cap binding	100%	-	Pvl	-	-	Lva		DM	SC	HS	
F26A3.3		ego-1	RNA-directed RNA polymerase	50-80%	-	-	-	-	Gro	ce				+
F30F8.8			TAF; WD repeat	100%	-	-	-	-	-	ce	DM	sc	hs	+
F32H2.1			SNAPC4-like, snRNA activating complex	50-80%	-	-	-	-	Gro		dm	sc	hs	+
F36F2.3			C-t like RNApol II CTD phosphatase	100%	-	-	-	-	-		dm	sc	hs	+
F37E3.1			mRNA CAP binding	100%	-	Unc	Lvl	-	Gro		DM	sc	hs	+
F48D11.1			putative splicing factor; WD-repeat	100%	-	Unc	-	-	-	CE	DM	SC	HS	+
F53G12.5		mex-3	KH domain	100%	-	-	-	-	-		dm		hs	+
F54C1.3		mes-3	involved in chromatin regulation	-	-	Stp	-	-	-					+
F55A3.3	*		general chromatin factor	100%	-	-	-	-	-	ce	DM	SC	HS	+
F57B10.8			RNA pol II transcriptional activator	-	-	Stp	-	-	Gro		dm	sc		+
H25P06.2a			CDK9 orthologue	90%	-	-	-	-	-	CE	DM	SC	hs	+
K02B12.3			transcriptional repressor	-	Ste	-	-	-	Gro		dm		hs	
K02F2.3			splicing factor	100%	-	Pvl	-	-	-	CE	DM	SC	hs	+
K07A1.11		rba-1	chromatin remodelling	100%	-	Pvl	Unc	-	-	CE	DM	SC	hs	+
K07A1.12		rba-2	chromatin remodelling	100%	-	Pvl	Lvl	-	-	CE	DM	SC	hs	+
T23D8.5	*		histone H4	10%	-	-	-	-	Gro	ce	dm	sc	hs	
T23D8.6	*		histone H2A	100%	-	-	-	-	-	CE	dm	SC	HS	+
T23G11.3		gld-1	RNA-binding; translational regulation	50-80%	Ste	-	-	-	-	ce	dm	sc	ha	+
W01B11.3			snoRNP associated	20-40%	-	-	-	-	Lva	CE	DM	SC	hs	+
W04A8.7			TAF II complex subunit TAF2A	100%	-	-	-	-	-		DM		HS	+
W08E3.1			snRNP polypeptide B; splicing role	100%	1-5	Rup	-	-	-		dm	sc	hs	+
Y110A7A.m			splicing component	100%	-	-	-	-	-	ce	DM	sc	hs	+
Y47G6A_247.h			RNA pol III subunit		-	Clr	-	-	-					+
Y54E10B_159.c			RNA pol II subunit	100%	-	-	-	-	-		dm	SC	hs	+
Y71F9B_286.b			snRNP G	10%	-	Clr	Sck	Unc	-		DM	SC	HS	+
Protein metabolism														
B0041.4			ribosomal protein	100%	6-10	-	-	-	-			SC	HS	+
B0511.10			translational initiation factor	90%	Ste	Pvl	-	-	-		DM		hs	+
C03D6.1	*		ribosomal protein L24	-	-	-	-	-	Gro	CE	DM	sc	HS	
C03D6.8			ribosomal protein L24	-	-	-	-	-	Gro	CE	DM	sc	HS	
C04F12.4			ribosomal protein L14	100%	Ste	-	-	-	Gro		DM	SC	HS	+
C09D4.5			ribosomal protein L19	100%	1-5	-	-	-	Lva		DM	SC	hs	+
C32E8.2			ribosomal protein L13	100%	Ste	-	-	-	-		DM	SC	HS	+
C36B1.4			proteasome subunit	100%	Ste	Lvl	-	-	Gro	CE	DM	SC	HS	+
C37A2.7			ribosomal protein	-	-	-	-	-	Gro				hs	+
C41D11.2			translational initiation factor	100%	-	Lvl	-	-	Gro		dm		hs	+
C43E11.4			elongation factor Tu	-	-	-	-	-	Gro	ce	DM	SC	hs	+
C43E11.9			required for 60S biogenesis	-	-	-	-	-	Gro		DM	SC	HS	+
C47B2.4			proteasome subunit	100%	Ste	Unc	-	-	-	ce	DM	SC	HS	+
C47B2.5			eIF6	-	-	-	-	-	Gro		DM	SC	HS	+
C48B6.2			required for 18S processing		-	Stp	-	-	Gro		DM	SC		
D1007.12			ribosomal protein	100%	1-5	-	-	-	Lva		DM	sc	HS	+
D1007.6			ribosomal protein	90%	Ste	Unc	-	-	-		DM	sc	HS	+
F22D6.3			tRNA synthetase	100%	1-5	-	-	-	Lva	ce	DM	SC	hs	+
F25H2.10			ribosomal protein	-	Ste	-	-	-	-		DM	SC	HS	+
F25H2.9			proteasome subunit	100%	Ste	Lvl	Unc	-	-	CE	DM	SC	HS	+
F25H5.4		eft-2	translational elongation factor	100%	Ste	Pvl	-	-	-	CE	DM	SC	hs	+
F28H1.3			tRNA synthetase	50-80%	1-5	-	-	-	Gro	CE	DM	SC	HS	+
F32A7.6		aex-5	endoprotease	10%	-	-	-	-	Gro	CE	dm	sc	hs	+
F32B5.8			cysteine protease; cathepsin Z homol	10%	-	-	-	-	-	ce	dm		hs	+
F33D11.10	*		translational initiation factor/helicase	100%	-	-	-	-	-	CE	DM	SC	HS	+
F36A2.6			ribosomal protein S15	90%	Ste	-	-	-	Gro		DM	SC	HS	+
F39B2.10			DnaJ domain	50-80%	-	-	-	-	-	CE	DM	SC	HS	+
F39E2.6			ribosomal protein S26	90%	Ste	Unc	Dpy	Lvl	-		dm	sc	hs	+
F39H11.5			proteasome subunit	100%	Ste	Unc	-	-	-		DM	SC	HS	+
F46A9.4			cullin based degradation	90%	-	Unc	Pvl	-	Gro	CE	DM	sc	hs	+
F46A9.5			cullin based degradation	90%	-	-	-	-	Gro	CE	DM	sc	hs	+
F52B5.6			ribosomal protein L25	100%	Ste	-	-	-	-	CE	DM	SC	HS	+
F53G12.10			ribosomal protein	100%	Ste	-	-	-	Gro		DM	SC	HS	+
F56H1.4			proteasome subunit	100%	1-5	-	-	-	-	CE	DM	SC	HS	+
F59A3.3			ribosomal protein	20-40%	1-5	-	-	-	Lva		DM			+
F59C6.4			rRNA processing	-	-	-	-	-	Lva			sc	hs	
K04F10.4		bli-4	subtilase protease		-	Mlt	Dpy	Lvl	-	CE	DM	sc	HS	+
K04G2.1			translational initiation factor	100%	1-5	Pvl	Sma	-	-		dm	sc	HS	+
K04G2.3			AAA ATPase; proteasome subunit	20-40%	-	-	-	-	Gro	ce	DM	sc	hs	+
K05C4.1			proteasome subunit	100%	Ste	-	-	-	-	ce	dm	SC	HS	+
R09B3.4			ubiquitin-conjugating	90%	-	Rup	Sck	-	-	ce	dm	sc	hs	+
R12E2.3			proteasome subunit	100%	Ste	-	-	-	Lva		dm	sc	hs	+
T06G6.9			prefoldin; delivers proteins to chaperones	100%	-	-	-	-	-		DM	SC	HS	+

GenePairs		Locus	Description	Emb	Ste	P1	P2	P3	Dev	CE	DM	SC	HS	E
T06B2.10			ribosomal protein	100%	Ste	Dpy	Sma	-	Gro		DM	SC	HS	+
T06B2.8			ribosomal protein L23	-	-	-	-	-	Lva		DM		hs	+
T06B2.9			tRNA synthetase	90%	1-5	-	-	-	Gro		DM	SC	HS	+
T20F5.2			proteasome subunit	100%	Ste	-	-	-	-		DM	SC	HS	+
T25G3.3			required for stable ribosome 60S formn	100%	-	-	-	-	Gro		DM	SC	HS	+
T27F6.5			tRNA synthetase	-	-	Clr	Sck	-	-		DM	sc	hs	+
W02A11.4			probable ubiquitin activating protein	-	-	Pvl	Egl	-	-	CE	DM	SC	hs	+
W09C5.6			ribosomal protein L31	90%	1-5	Stp	-	-	-		DM	SC	HS	+
Y105E8C.e			ribosomal protein	100%	Ste	-	-	-	-		dm	sc	hs	+
Y110A7A.f			proteasome subunit	100%	Ste	-	-	-	Gro		DM	sc	HS	+
Y110A7A.j			proteasome subunit	100%	Ste	-	-	-	Gro		DM	sc	HS	+
Y23H5A.7			aminoacyl-tRNA synthetase	10%	Ste	Lvl	Mlt	-	Gro	ce	DM	SC	hs	+
Y39G10A_246.c			translation initiation factor eIF2	100%	-	-	-	-	Lva		DM	SC	HS	
Y39G10A_246.h			translation initiation factor eIF2	100%	1-5	Dpy	Sma	Unc	Lva		DM	SC	HS	
Y47H9C.7			eIF2B	10%	-	-	-	-	Gro		DM		HS	+
Y48G8A_3945.c			ribosomal protein L17	50-80%	Ste	-	-	-	Gro		dm	sc	hs	
Y65B4B_10.b			nascent polypeptide associated a-chain	50-80%	-	Mult	Him	Dpy	Gro		dm	sc	hs	+
Y65B4B_10.d			nascent polypeptide associated a-chain	50-80%	-	-	-	-	Gro		DM	sc	HS	+
Y71F9A_294.c			ribosomal protein L10	20-40%	Ste	-	-	-	-		DM	SC	HS	
Y87G2A.l			tRNA synthetase	100%	Ste	-	-	-	-	CE	DM	SC	HS	+
ZC434.2			ribosomal protein S7	50-80%	Ste	-	-	-	-		DM	SC	HS	+
Energy/metabolism														
B0261.4			mitochondrial ribosomal protein	100%	-	Bmd	-	-	Gro		dm			+
C01A2.3			cyt oxidase, F0F1ATPsythase assembly	20-40%	-	-	-	-	Lva		dm	SC	hs	+
C10G11.5			putative pantothenate kinase	50-80%	1-5	-	-	-	Gro	ce	dm	sc	hs	
C25A1.5			cytochrome b family	-	-	-	-	-	Lva		dm	sc	hs	+
C32E8.8			homologous to Niemann-Pick type C	50-80%	Ste	-	-	-	Gro		DM	sc	hs	+
C54G4.8			cytochrome c1	100%	-	-	-	-	Gro		DM	SC	HS	+
C55B7.2			Putative N-acetyl-glucosaminyltransferase	50-80%	-	-	-	-	-				HS	+
D2030.4			NADH dehydrogenase	-	-	-	-	-	Gro		dm		HS	+
F22D6.4			NADH dehydrogenase	50-80%	-	Bmd	-	-	Lva		dm			
F26E4.9			cytochrome c oxidase	50-80%	-	Clr	-	-	Gro		DM		hs	+
F27C1.7			ATP synthase	100%	Ste	-	-	-	Lva		DM	SC		+
F29D11.1		lrp-1	LDL-receptor related		-	Unc	Prz	-	Gro	ce	DM		hs	+
F32H2.5			fatty acid synthase	100%	-	-	-	-	-	ce	dm	sc	hs	+
F32H2.6			fatty acid synthase	100%	-	-	-	-	-	CE	DM		HS	
F37F2.1			mitochondrial ribosomal protein L15	50-80%	-	-	-	-	Gro		DM	sc	HS	+
F43G9.1			NAD+-isocitrate dehydrogenase	100%	-	-	-	-	-	CE	DM	SC	HS	+
F53G12.2	*		NADPH oxidase/ferric reductase		-	Bli	Mlt	Lvl	-	CE	DM		hs	+
F53G12.3	*		NADPH oxidase/ferric reductase	-	-	Bli	Mlt	Lvl	-	CE	DM		hs	+
F54D7.2			CDP-alcohol phosphotransferase		-	-	-	-	Lva	CE	DM	sc		+
F56C11.1	*		NADPH oxidase/ferric reductase	10%	-	Bli	Mlt	Lvl	-	CE	DM		hs	+
F57B10.3			phosphoglycerate mutase	100%	Ste	Unc	Lvl	Bmd	Gro					+
F59C8.5			NADH ubiquinone oxidoreductase	90%	Ste	-	-	-	Lva		dm		hs	+
H28O16.1			ATP sythase	100%	Ste	-	-	-	Gro	ce	DM	SC	HS	+
K02F2.2		dpy-14	S-adenosylhomocysteine hydrolase	20-40%	-	Unc	Lon	-	Lva		DM	SC	HS	+
R06C7.5			Putative adenylosuccinate lyase	50-80%	-	Pvl	Egl	Bmd	-		DM	SC	hs	+
T03F1.8			guanylate kinase	-	-	-	-	-	Gro	CE	DM	SC	HS	
T09B4.9			mitochondrial inner membrane translocase	100%	1-5	-	-	-	Gro		DM	SC	hs	+
T10E9.7			NADH oxidoreductase	50-80%	-	-	-	-	Gro		dm		hs	+
T23G11.2			phosphoglucosamine acetyltransferase	100%	-	-	-	-	-	ce	dm	sc	hs	+
W02D3.7		lbp-5	fatty acid binding protein	-	-	Unc	-	-	-	CE	DM		HS	
W09C5.8			subunit IV of cytochrome c oxidase	-	-	-	-	-	Gro		DM		hs	+
Y47G6A_247.f			ATPase like yeast AFG3	100%	1-5	-	-	-	-	ce	dm	sc	hs	+
Y47G6A_247.g			ATPase like yeast AFG3	100%	-	-	-	-	Lva	ce	dm	sc	hs	+
Y63D3A.7			NADH-ubiquinone oxidoreductase	-	-	-	-	-	Lva		dm		hs	+
ZK973.b			NADH-ubiquinone oxidoreductase	100%	Ste	-	-	-	Lva		dm		hs	+
B0511.8			CDC1-like	100%	-	-	-	-	Lva		dm	sc		+
C37A2.4		cye-1	cyclin e	10%	-	Clr	-	-	-	ce	dm	sc	hs	
C41G7.2	*		claret subfamily kinesin	50-80%	-	Him	-	-	-	CE	DM	sc	HS	+
C53H9.2			role in chromosome stability	90%	-	-	-	-	Gro	ce	DM	SC	hs	+
F57B10.12		mei-2	functional homologue of human katanin	100%	-	-	-	-	-	CE				+
M01E11.6	*		claret subfamily kinesin	90%	-	Him	-	-	Gro	CE	DM	sc	hs	+
R06C7.8			Bub1-like kinase	100%	-	Pvl	Rup	Lvl	-	ce	dm	sc		+
T01G9.5		mei-1	ATPase	100%	-	-	-	-	-	ce	dm	sc	hs	+
W09G3.3	*		regulator of chromosome condensation	-	-	Him	-	-	-	ce	dm		hs	+
Y110A7A.d			cdc27 homologue	50-80%	-	-	-	-	Gro		dm	sc	hs	+
Y39G10A_246.e			CDC21/replication licensing factor MCM4	100%	-	-	-	-	-		dm	sc	hs	
Y39G10A_246.l			INCENP-like; centromeric protein	100%	-	-	-	-	-	ce	dm		hs	
Y47G6A_247.l			S pombe Rad2 homologue	90%	-	-	-	-	-	ce	DM	SC	hs	+
Y52B11A.9			Homologue of Kin17; binds bent DNA	10%	-	Bmd	Rup	-	-		DM	sc	hs	+
Cell structure														
C01G8.5			Ezrin/moesin/merlin-like	-	-	Unc	Lvl	-	Gro	CE	DM		HS	+
C10H11.1			villin	-	-	Him	-	-	-		dm		hs	+
C17E4.9			active Na/K channel	50-80%	Ste	Unc	-	-	-	CE	DM		HS	
C32E8.10		unc-11	regulation of vesicles	10%	-	-	-	-	-		dm	SC	hs	+
C45G3.1			actinin-type actin-binding domain	100%	-	Unc	-	-	-		dm			+
C47B2.3	*	tbe-2	tubulin	100%	-	-	-	-	-	CE	DM	SC	HS	+
C53D5.a			nuclear import complex	100%	1-5	-	-	-	Gro	ce	DM	SC	HS	+
C53D5.l			nuclear import complex	90%	-	-	-	-	Gro	ce	DM	SC	HS	+
DY3.2		lam-1	nuclear lamin	100%	Ste	Lvl	-	-	-	CE	DM	sc	HS	+
E03H4.8	*		beta coatomer-like	-	1-5	Unc	Stp	Clr	Gro	CE	DM	SC	HS	
F07A5.7		unc-15	paramyosin	-	-	Unc	Prz	Egl	-	CE	DM	SC	HS	+
F20G4.3		nmy-2	non-muscle myosin-2	50-80%	Ste	-	-	-	-	CE	DM	SC	HS	+
F21C3.5			probable microtubule nucleation	50-80%	-	Unc	Bmd	-	Gro		DM	sc	hs	+
F28B1.3			karyopherin; nuclear import	100%	-	Lvl	-	-	-	CE	DM	SC	HS	+
F28E4.8	*	tbe-2	tubulin	100%	-	-	-	-	-	CE	DM	SC	HS	+

GenePairs		Locus	Description	emb	Ste	P1	P2	P3	Gro	CE	DM	SC	HS	E
Cell structure														
F26H9.6			ras superfamily GTPase	100%	Ste	Lvl	-	-	-	CE	DM	SC	HS	+
F26H1.2			single calponin domain (CH)	50-80%	-	-	-	-	Lva	CE	DM	SC	hs	+
F30A10.6			small molecule transport	-	-	-	-	-	Gro	CE	DM	SC	hs	
F36H2.1			cation active transporter	100%	-	-	-	-	Gro	CE	DM	SC	hs	+
F43G9.10			Microfibrillar AP homologue	100%	-	-	-	-	Gro		dm		hs	+
F46F11.5			vacuolar ATPase	100%	1-5	Lvl	-	-	-		DM	SC	HS	+
F53B8.1	*		2 genes; both plectrins	100%	-	Unc	Prz	Lvl	-	CE	DM	ac	hs	
F53F10.5			nucleoporin homology	100%	-	-	-	-	-		dm			+
F54C1.7			troponin c	-	Ste	-	-	-	-	CE	DM	SC	HS	+
F55A12.7			clathrin associated; 64%ID UNC-101	-	-	Unc	-	-	Gro	CE	DM	SC	HS	+
F55F8.5			microtubule associated	-	-	-	-	-	Lva		DM	SC	hs	+
F56F4.5			oligopeptide transporter	-	-	Him	-	-	-	ce	DM	ac	hs	+
H15N14.1			vesicular fusion; human NSF-like	100%	Ste	-	-	-	ce	DM	SC	hs		+
M01A10.3			ribophorin; protein trafficking into ER	100%	Ste	Lvl	Unc	-	-		dm		hs	+
R05D11.3			Nuclear Transport Factor 2 homologue	100%	1-5	-	-	-	Gro		DM	SC	HS	+
T03F1.9			like UNC-89/neurofilament	100%	-	-	-	-	-					+
T19B4.2			possible nuclear pore protein	100%	Ste	-	-	-	-		dm		hs	+
T21E12.4		dhc-1	dynein heavy chain	100%	-	-	-	-	-	ce	DM	SC	hs	+
T25G3.2			chitin synthases	20-40%	-	-	-	-	Gro	ce	dm	ac		+
T26E3.3		par-6	PDZ domain	100%	-	-	-	-	-		dm		HS	+
W02B9.1		hmr-1	cadherin	10%	-	Bmd	Unc	Dpy	-	ce	DM		hs	+
W04C9.1			ABC transporter	50-80%	-	-	-	-	Gro	CE	dm	ac	hs	+
Y105E8C.n			gamma-edaptin AP-1	90%	-	Unc	Lvl	-	Gro	ce	dm	ac	hs	+
Y18D10A.17			suppressor of clathrin deficiency	50-80%	-	-	-	-	-		dm	ac	hs	+
Y18D10A.20		pfn-1	profilin	100%	-	-	-	-	-	CE	DM			+
Y34D9A_152.a			vacuolar sorting protein	10%	-	Unc	Prz	Lvl	Gro		DM	ac	HS	
Y48G8A_3945.e			edaptin complex subunit	-	-	Unc	-	-	-		dm	ac	hs	
Y71A12B.a			like Hu gravin domain for localisation of PK	90%	-	Dpy	Clr	-	-		DM	ac	HS	
Y71F9A_279.b			NTF2-related NXT1 orthologue	100%	-	Pvl	Unc	-	-		dm	ac	hs	+
Y71F9A_282.b			coatomer complex alpha subunit	100%	Ste	Unc	-	-	-	ce	DM	ac	hs	+
Y71F9A_290.a			NTF2-related export protein	-	-	Pvl	Rup	Clr	-		dm	ac		+
Y71G12A_195.e			talin	10%	Ste	Unc	Prz	-	-		dm		hs	
Y87G2A.a			46%ID to Hu VPS28	-	-	Unc	-	-	-		DM	SC	HS	+
Y87G2A.x			sec7 domain; non-clathrin protein traffick	-	-	Unc	-	-	Gro	ce	DM	SC	hs	+
Y87G2A.y			sec7 domain; non-clathrin protein traffick	-	-	Clr	-	-	-	ce	DM	SC	hs	+
ZK1014.1			vesicle fusion	-	Ste	Lvl	-	-	-		DM	SC	HS	+
ZK1151.2			spectrin repeats; kakapo-like	-	-	Unc	Bmd	Stp	-	CE	DM	ac	hs	+
B0025.3			corepressor of nuclear hormone receptors	50-80%	-	-	-	-	Gro		dm		hs	+
C01G8.7			eyelid-like	90%	Ste	Bmd	Lvl	-	Gro		dm		hs	+
C01G8.8			eyelid-like	50-80%	-	Bmd	-	-	-		dm		hs	
C01H6.5		nhr-23	nuclear hormone receptor	-	-	Unc	Lvl	Dpy	-	ce	dm		hs	+
C12C8.3		lin-41	NHL domains	-	Ste	-	-	-	-	ce	dm		hs	+
C32F10.7		nhr-2	nuclear hormone receptor	-	-	Him	-	-	-	ce	dm		hs	+
C46E7.3			bZIP transcription factor	-	-	-	-	-	Gro		dm		hs	+
D1081.2			55%ID to SRF	-	-	Unc	Prz	-	-		dm	sc	hs	+
F43G9.12			Hu TCF-9-like	90%	-	-	-	-	Gro				hs	+
F52F12.6			Zn finger; Hu MYT1-like	-	-	Unc	-	-	-		dm		hs	+
F55F8.4			transcriptional repression	100%	-	-	-	-	Lva		dm		hs	+
F57B10.1			bZIP transcription factor	100%	Ste	Sma	Dpy	-	Lva		dm		hs	+
K02B12.1		ceh-6	homeobox transcription factor	-	-	Unc	Mlt	-	-	ce	dm		hs	
M05B5.5		hlh-2	bHLH transcription factor	100%	6-10	Unc	Pvl	-	-		dm		hs	+
W02D3.9		unc-37	groucho family transcription factor	100%	Ste	Unc	-	-	-	ce	dm	ac	hs	+
W03D8.4		pop-1	HMG box protein	100%	1-5	-	-	-	-		dm		hs	
Y40B1A.4			Zn finger transcription factor	-	-	Unc	Bmd	-	-	ce	dm	ac	hs	
Y54E5B.3			Mediator complex subunit	50-80%	-	Rup	-	-	-		dm	ac	hs	+
Y65B4A_179.b			transcriptional activator	-	-	Unc	Dpy	-	-	ce	dm	ac	hs	+
ZC247.3		lin-11	LIM homeodomain transcription factor	-	-	-	-	-	Gro	ce	dm		hs	+
ZK858.4		mel-26	kruppel-like Zn finger transcription factor	100%	-	-	-	-	-	CE	DM		HS	+
Signalling														
C09D1.1		unc-89	multiple signalling domains	-	-	Unc	-	-	-	ce	dm		hs	+
C10H11.9		let-502	ROCK	50-80%	-	Dpy	Rol	Lvl	-	ce	DM	ac	hs	+
C26C6.2		goa-1	G-a subunit	10%	1-5	Unc	Pvl	Egl	-	CE	DM	SC	HS	+
C32E8.5			FHA domain	100%	1-5	-	-	-	Gro	ce	dm		hs	+
F26E4.1			PP2A regulatory subunit	100%	-	Lvl	-	-	-		DM	SC	hs	+
F55A12.3			PI-4P 5' kinase	-	Ste	-	-	-	-	ce	dm	sc	hs	+
F55C7.4		unc-73a	guanine exchange factor	20-40%	-	-	-	-	-	CE	DM		hs	+
F55C7.7		unc-73a	guanine exchange factor	-	-	Egl	-	-	-	ce	DM		hs	+
K04G2.8		apr-1	APC homologue	-	-	Unc	Bmd	Lvl	-		dm		hs	+
K05C4.6		hmp-2	B-catenin	100%	-	Unc	Dpy	Bmd	-	CE	DM	ac	HS	+
K12C11.2			SUMO-1 like	100%	-	Pvl	-	-	-		DM	SC	HS	+
T01G9.6		kin-10	regulatory subunit of CKIIbeta	20-40%	-	Pvl	-	-	Gro		DM	SC	HS	+
T21E3.1	*		protein tyrosine phosphatase	100%	Ste	-	-	-	-	CE	dm		hs	+
T23D8.1		mom-5	frizzled-like	-	-	Unc	Bmd	-	-	CE	DM		HS	+
T23H2.5	*		rab family	-	-	Sma	Clr	-	-	CE	DM	SC	HS	+
Y106G6E.6			Casein Kinase 1	50-80%	-	Dpy	-	-	Gro	CE	DM	ac	HS	+
Y18D10A.5		gsk-3	GSK-3	100%	-	-	-	-	-	CE	DM	SC	HS	+
ZC581.1			NIMA-like kinase	-	-	Unc	Lvl	Rol	-	CE	DM	SC	HS	
ZK265.6			G-protein coupled receptor	-	-	-	-	-	Gro	ce	dm		hs	+
Unknown														
B0261.1				-	-	Stp	-	-	Gro					+
B0414.1				100%	-	-	-	-	-	CE				
B0511.9				100%	-	-	-	-	-					+
C01A2.5				100%	Ste	Bmd	Sck	-	Gro		DM		hs	+
C06A5.1				100%	-	Bmd	Pvl	-	-		dm		hs	
C10H11.8			WD repeat	-	-	-	-	-	Gro		DM	SC	hs	
C25A1.6				-	-	Clr	-	-	Gro					+
C27C7.5				-	-	Unc	-	-	-	CE				
C34B2.4			LIM domains	-	-	Him	-	-	-	CE	dm		hs	

GenePairs		Locus	Description	emb	Ste	P1	P2	P3	Gro	CE	DM	SC	HS	E
C34B2.8				50-80%	-	-	-	-	Gro		DM		HS	+
C34G6.6			repetitive Cys motifs; 4 PAN domains	20-40%	-	Unc	Lvl	Muv	-	CE	dm			+
C41G7.3				-	-	Him	-	-	-		dm			+
C55B7.5				50-80%	Ste	Pvl	Rup	-	-		dm			
D2030.3				-	-	-	-	-	Gro					
E01A2.1			coiled coils	100%	-	Bmd	-	-			dm		hs	+
F18C12.2			DnaJ domain	50-80%	-	Unc	Mlt	Bmd	-				hs	+
F20G4.1				50-80%	Ste	Unc	-	-	Lva					+
F22D6.6				90%	-	-	-	-	-					+
F23C8.6			coiled coils	-	-	Unc	-	-	Gro	CE	DM		hs	+
F25H2.11			TCTP domain	-	-	-	-	-	Gro		DM	SC		+
F25H2.4				50-80%	-	Stp	Unc	Clr	Gro		DM		HS	+
F26E4.4			coiled coils	20-40%	-	-	-	-	Lva					+
F27C1.3				-	-	Stp	-	-	Gro	ce				+
F27D4.2			coiled coils	90%	-	Bmd	Lvl	Unc	-					+
F29D11.2				-	-	Unc	Dpy	Sma	-					+
F30A10.9			PF01942; domain of unknown function	-	-	-	-	-	Gro		dm	sc		+
F31C3.2				-	-	Him	-	-	-	ce	dm			+
F31C3.5				100%	-	Unc	Bmd	-	-		DM	SC		
F32H2.3				100%	-	-	-	-	-					+
F33H2.6				-	-	Him	-	-	-					
F33H2.8				-	-	Unc	-	-	-					
F36A2.7				50-80%	-	-	-	-	Gro	CE				+
F53G12.4				-	-	Bll	Mlt	Lvl	-					
F55A12.8				-	-	Unc	-	-	Gro		DM	SC	hs	+
F55F8.3			WD repeat	-	-	-	-	-	Lva	ce	DM	SC	HS	+
F56A3.2				100%	Ste	-	-	-	Lva		dm	ac	hs	+
F56A3.3			coiled coils	100%	-	-	-	-	-					+
F56A3.4			coiled coils	100%	-	-	-	-	-	CE	DM	SC	HS	+
F56C11.5				100%	-	-	-	-	-					
F57B10.10		dad-1		100%	-	Lvl	-	-	Gro		DM	ac		+
K02A11.1			ankyrin repeat	-	-	Adl	Dpy	Rup	-	ce	dm		hs	+
K02B12.8				-	-	Him	-	-	-					
K05C4.2				-	-	Unc	-	-	-		DM		HS	+
K06A5.4			coiled coils	100%	1-5	-	-	-	-					+
K09H9.6			Dros homologue Peter Pan for larval growt	-	-	-	-	-	Gro	ce	DM	ac	hs	+
K11B4.1				20-40%	-	-	-	-	Lva		dm			+
M01B12.5				-	-	Sck	-	-	-	ce	dm	ac	hs	+
R09B3.5			mago nashi functional orthologue	90%	-	Dpy	-	-	Gro		DM		hs	+
T01G9.4		kup-2		20-40%	-	Clr	-	-	Gro		DM		hs	+
T06G6.8				-	-	Him	-	-	-					
T08G11.4			well-conserved domain at C-terminus	100%	-	Lvl	-	-	Gro			ac	hs	+
T19A6.2			GTPase	-	-	-	-	-	Gro	CE	dm	ac	hs	+
T19B4.5			coiled coils	100%	-	-	-	-	-					+
T21G5.4	*		PDZ domains	-	-	Pvl	Stp	-	Lva	CE				
T23D8.3			coiled coils	50-80%	-	-	-	-	Gro		dm		hs	+
VF36H2L.1				-	-	Unc	-	-	-		DM		hs	+
W01A8.4				50-80%	-	Bmd	-	-	Lva					+
W02A11.8			BTB/POZ domain; large elegans family	-	-	Unc	-	-	-	CE				
W04A4.6				90%	-	Unc	Bmd	-	Gro		dm			+
W09C5.4				20-40%	-	Unc	-	-	Gro					
W10D5.3			DEAD-box binding	-	-	Dpy	Bmd	Pvl	-		dm	ac	hs	+
Y83D3A.5			TrkA-fused gene homol	20-40%	-	Unc	Dpy	Lvl	-	ce			hs	+
Y105E8C.d				50-80%	-	Stp	-	-	Gro		dm	ac		+
Y106G6D.1			coiled coils	50-80%	-	-	-	-	Gro					
Y110A7A.a			PPR domain	50-80%	-	-	-	-	Gro	ce	DM			+
Y110A7A.g			CHORD-containing protein	100%	-	Lvl	-	-	-		DM		hs	
Y23H5A.3				100%	-	-	-	-	-					+
Y39G10A_246.j			coiled coils	100%	-	Lvl	-	-	-					
Y39G10A_246.k				100%	-	-	-	-	-					
Y47G6A_245.b				-	-	Clr	-	-	-		dm	ac	hs	+
Y47G6A_247.a				-	-	Unc	-	-	-		DM	SC	HS	+
Y48G10A.b			coiled coils	100%	-	-	-	-	-					+
Y48G1A_54.d				-	-	-	-	-	Lva		DM	SC	hs	+
Y48G1B_55.c				-	-	Unc	-	-	-	ce				+
Y48G1B_55.d				-	-	Unc	Pvl	-	-	CE				+
Y53C10A.3				-	-	-	-	-	Gro	ce				
Y54E10A_156.a				100%	-	-	-	-	-					+
Y54E10A_159.b				-	-	-	-	-	Gro		dm		hs	+
Y54E10B_159.e				-	-	Stp	-	-	Gro					+
Y65B4A_182.c			coiled coils	-	-	Unc	Prz	-	Gro					+
Y65B4B_10.c				50-80%	-	-	-	-	-					
Y65B4B_12.a				-	-	Unc	-	-	-					+
Y95D11A.a				-	-	Stp	-	-	Gro					
ZC308.3				100%	Ste	-	-	-	-					
ZC328.1				-	-	Unc	Prz	Lvl	-					+
Multiple genes														
F39H11.2				100%	-	-	-	-	-					
K07A12.3				50-80%	-	Bmd	-	-	Gro					
T23D8.4				100%	-	Pvl	Bmd	-	-					
T23D8.9				90%	-	Bmd	-	-	Gro					
Y48G1A_54.b				100%	-	-	-	-	-					
Y48G1A_54.c				100%	-	Pvl	-	-	-					
Y54E10B_152.e				90%	1-5	-	-	-	-					

EMBRYONIC LETHALS

Locus	GenePairs Name	Mutant Phenotype	RNAi phenotype						Hit	Similar to published
			Emb	Ste	P1	P2	P3	Dev		
apr-1	K04G2.8	Emb	-	-	Unc	Bmd	Lvl	-	✓	o
dhc-1	T21E12.4	Emb	100%	-	-	-	-	-	✓	✓
ego-1	F26A3.3	Ste, Emb	50-80%	-	-	-	-	Gro	✓	✓
hlh-2	M05B5.5	Emb	100%	6-10	Unc	Pvl	-	-	✓	✓
hmp-2	K05C4.6	Emb	100%	-	Unc	Dpy	Bmd	-	✓	✓
hmr-1	W02B9.1	Emb	10%	-	Bmd	Unc	Dpy	-	✓	✓
let-502	C10H11.9	Emb	50-80%	-	Dpy	Rol	Lvl	-	✓	✓
mei-1	T01G9.5	Emb	100%	-	-	-	-	-	✓	✓
mel-26	ZK858.4	Emb	100%	-	-	-	-	-	✓	✓
mex-3	F53G12.5	Emb	100%	-	-	-	-	-	✓	✓
mom-5	T23D8.1	Emb	-	-	Unc	Bmd	-	-	✓	o
nhr-2	C32F10.6	Emb	-	-	Him	-	-	-	✓	o
nmy-2	F20G4.3	Emb	50-80%	Ste	-	-	-	-	✓	✓
par-6	T26E3.3	Emb	100%	-	-	-	-	-	✓	✓
pop-1	W03D8.4	Emb	100%	1-5	-	-	-	-	✓	✓
rba-1	K07A1.11	Emb	100%	-	Pvl	Unc	-	-	✓	✓
rba-2	K07A1.12	Emb	100%	-	Pvl	Lvl	-	-	✓	✓
unc-37	W02D3.9	Unc, Emb, Ste	100%	Ste	Unc	-	-	-	✓	✓
unc-73	F55C7.2,3,4,7	Emb, Unc, Egl, Sma, Dpy	20-40%	-	Egl	-	-	-	✓	✓
mom-4	F52F12.3	Emb	-	-	-	-	-	-	o	o
sup-17	DY3.7	Emb, Dpy, Unc	-	-	-	-	-	-	o	o

POST-EMB PHENOTYPES

Locus	GenePairs Name	Mutant Phenotype	RNAi phenotype						Hit	Similar to published
			emb	Ste	P1	P2	P3	Gro		
aex-5	F32A7.6	constipated	10%	-	-	-	-	Gro	✓	o
bli-4	K04F10.4	Bli	-	-	Mlt	Dpy	Lvl	-	✓	✓
dpy-14	K02F2.2	Dpy, Lvl	20-40%	-	Unc	Lon	-	Lva	✓	✓
gld-1	T23G11.3	Ste	50-80%	Ste	-	-	-	-	✓	✓
goa-1	C26C6.2	Hya, Egl-c	10%	1-5	Unc	Pvl	Egl	-	✓	o
lin-11	ZC247.3	Vul, wk Unc	-	-	-	-	-	Gro	✓	o
lrp-1	F29D11.1	Sma, Dpy, Mlt	-	-	Unc	Prz	-	Gro	✓	o
mes-3	F54C1.3	Ste	-	-	Stp	-	-	-	✓	✓
nhr-23	C01H6.5	Mlt, Let, wk Emb	-	-	Unc	Lvl	Dpy	-	✓	✓
unc-11	C32E8.10	Unc, wk thin	10%	-	-	-	-	-	✓	o
unc-15	F07A5.7	Unc, Prz, Egl	-	-	Unc	Prz	Egl	-	✓	✓
unc-37	W02D3.9	Unc, Emb, Ste	100%	Ste	Unc	-	-	-	✓	✓
unc-73	F55C7.2,3,4,7	Emb, Unc, Egl, Sma, Dpy	20-40%	-	Egl	-	-	-	✓	✓
unc-89	C09D1.1	wk Unc, Clr, thin	-	-	Unc	-	-	-	✓	✓
egl-30	M01D7.1,7	Slu, Egl	-	-	-	-	-	-	o	o
fer-1	F43G9.6	Ste, lays oocytes	-	-	-	-	-	-	o	o
fog-3	C03C11.2	Ste, no sperm	-	-	-	-	-	-	*	*
gsa-1	R06A10.2	Lvl	-	-	-	-	-	-	o	o
lin-10	C09H6.2	Vul, Muv	-	-	-	-	-	-	o	o
spe-11	F48C1.7	paternal-effect Emb	-	-	-	-	-	-	o	o
spe-9	C17D12.6	Ste	-	-	-	-	-	-	o	o
sup-17	DY3.7	Emb, Dpy, Unc	-	-	-	-	-	-	o	o
sur-2	F39B2.4	Let, Vul, Egl	-	-	-	-	-	-	*	*
unc-101	K11D2.3	Unc, wk Egl	-	-	-	-	-	-	*	*
unc-13	ZK524.2	Unc, Prz	-	-	-	-	-	-	o	o
unc-14	K10D3.2	Unc, Prz	-	-	-	-	-	-	o	o
unc-29	T08G11.5	Slu L1	-	-	-	-	-	-	o	o
unc-38	F21F3.5	Slu, wk Dpy	-	-	-	-	-	-	o	o
unc-40	T19B4.6,7	wk Unc,wk Dpy	-	-	-	-	-	-	o	o
unc-55	F55D12.4,6	slow Unc	-	-	-	-	-	-	o	o
unc-87	F08B6.4	Unc, Prz	-	-	-	-	-	-	o	o
air-2	not cloned	Emb, Ste								
lin-17	not cloned	wk Unc, Lon, wk Egl, Muv								
lin-28	not cloned	Egl								
mek-2	not cloned	Ste, Vul								
smg-2	not cloned	Pvl								
spe-12	not cloned	Ste								
spe-4	not cloned	Ste								
unc-54	not cloned	Unc								

Supplementary Table 3

GenePair
B0019.3
B0025.2
B0041.7
B0205.10
B0205.6
B0205.7
B0205.9
B0207.1
B0207.11
B0207.4
B0207.5
B0261.2
B0261.5
B0261.7
B0379.5
B0414.6
C01H6.2
C04F12.1
C04F12.2
C04F12.5
C04F12.6
C10H11.4
C15C6.1
C17D12.1a
C17H1.3
C17H1.8
C18E3.2
C18E3.8
C25A1.8
C27C7.4
C27C7.8
C31H5.6
C34B2.5
C36B1.9
C37A5.2
C41G7.4
C48B6.5
C49A1.10
C49A1.4
C53D5.c
C53D5.h
C54C8.6
C54G6.1
C54G6.3
C55C2.1
C55C2.3
D1037.2
D2005.6
DY3.6
E01A2.c

GenePair
E03H4.4
F02E9.2a
F02E9.6
F08B6.3
F11C3.2
F11C3.3
F12B6.3
F14B6.1
F14B6.2
F14B6.5
F15D3.7
F15D3.8
F21A9.1
F21A9.2
F22D6.8
F22G12.2
F26H9.2
F26H9.3
F26H9.5
F27C1.8
F28B3.6
F29D10.5
F31C3.1
F32A7.4
F32A7.7
F32B4.2
F32B4.5
F32B4.8
F32H2.2
F32H2.9
F33D11.4
F33D11.7
F33E2.5
F36F2.4
F37D6.2
F39H2.2
F40E3.6
F43G9.8
F46A8.1
F46A8.10
F46A8.4
F49B2.4
F49D11.5
F49D11.7
F52A8.2
F52F12.7
F53B6.1
F53F10.3
F54A5.3a
F54D7.3

GenePair
F56A6.4
F56G4.5
F57C9.2
H16D19.3
K02B12.4
K02B12.8
K05C4.10
K07A1.10
K07A1.2
K07A1.5
K08C9.4
K10C3.5
K10E9.2
K11B4.2
K12C11.3
M01D7.2
M01D7.4
M01G12.12
M01G12.3
M01G12.5
M04F3.2
R05D7.4
R06A10.4
R06C1.2
R11A5.2
R11A5.5
R12E2.1
R12E2.8
T01A4.2
T01H8.3
T02E1.1
T02E1.5
T02G6.2
T02G6.3
T02G6.4
T03F1.7
T04D1.3
T04D1.4
T05E7.5
T07D10.5
T09E11.3
T09E11.4
T10E9.1
T10E9.5
T15D6.1
T15D6.4
T19A6.1
T22C1.1
T22C1.3
T23D8.2

GenePair
T23H2.6
T27C10.4
T28F2.3
VH15N14R.1
W02D3.1
W03D8.10
W03D8.7
W04C9.6
W05B5.1
W05B5.3
W05F2.1
W05H12.1
W09C5.4
W09C5.5
W09C5.6
W09G3.5
W10C8.2
W10D5.2
Y105E8A.d
Y105E8A.e
Y105E8B.c
Y105E8B.d
Y105E8C.a
Y105E8C.b
Y105E8C.c
Y105E8C.g
Y105E8C.h
Y105E8C.m
Y105E8C.u
Y105E8D.a
Y105E8D.b
Y105E8D.e
Y105E8D.f
Y106G6D.1
Y106G6D.2
Y106G6E.5
Y110A7A.k
Y18D10A.15
Y18D10A.3
Y18H1A_67.a
Y18H1A_67.b
Y18H1A_67.e
Y18H1A_67.h
Y18H1A_67.l
Y18H1A_68.b
Y18H1A_68.c
Y18H1A_68.e
Y23H5A.1
Y26D4A.10
Y26D4A.5

GenePair
Y34D9A_150.a
Y34D9A_150.b
Y34D9A_151.c
Y34D9A_151.f
Y34D9A_152.b
Y34D9A_152.c
Y39G10A_233.a
Y39G10A_241.a
Y39G10A_243.b
Y39G10A_244.a
Y39G10A_244.b
Y39G10A_246.g
Y40B1A.3
Y44E3A.2
Y47G6A_232.a
Y47G6A_232.b
Y47G6A_240.d
Y47G6A_245.e
Y47G6A_246.d
Y47G6A_247.d
Y47G6A_247.j
Y47G6A_247.k
Y47H10A.a
Y47H10A.b
Y47H9C.2
Y47H9C.5
Y48G10A.c
Y48G10B.a
Y48G1A_53.c
Y48G1A_54.a
Y48G1A_54.e
Y48G1C_55.b
Y48G8A_2614.b
Y48G8A_2614.c
Y48G8A_3051.c
Y48G8A_3304.a
Y48G8A_3671.b
Y48G8A_3671.c
Y48G8A_3945.f
Y48G8A_4850.a
Y48G8A_5995.a
Y48G8A_5995.b
Y52B11A.11
Y52B11A.3
Y53H1A.a
Y53H1A.c
Y53H1B.c
Y53H1C.c
Y54E10A_156.b
Y54E10A_156.d

GenePair
Y54E10A_157.a
Y54E10B_151.a
Y54E10B_151.b
Y54E10B_152.c
Y54E10B_154.a
Y54E10B_154.b
Y54E10B_159.d
Y54E5A.3
Y63D3A.4
Y65B4A_165.a
Y65B4A_185.b
Y65B4B_13.a
Y65B4B_15.d
Y65B4B_9.a
Y6B3B.6
Y71F9A_261.a
Y71F9A_269.a
Y71F9A_281.a
Y71F9A_291.b
Y71F9A_294.a
Y71F9A_294.b
Y71F9A_294.g
Y71F9A_295.b
Y71F9B_275.a
Y71F9B_276.b
Y71F9B_287.c
Y71F9B_297.b
Y71F9B_299.b
Y71G12A_187.c
Y71G12A_189.a
Y71G12A_190.a
Y71G12A_190.b
Y71G12A_191.a
Y71G12A_193.a
Y71G12A_195.a
Y71G12A_195.d
Y71G12A_196.a
Y71G12A_197.a
Y71G12A_199.a
Y71G12A_199.b
Y71G12A_199.c
Y71G12A_202.b
Y71G12A_203.b
Y71G12A_203.d
Y71G12A_203.e
Y71G12A_203.f
Y71G12A_205.a
Y71G12A_205.c
Y71G12A_205.d
Y71G12A_205.e

GenePair
Y71G12A_205.f
Y71G12A_205.g
Y74C10A_140.a
Y74C10A_140.b
Y74C10A_148.a
Y74C10A_150.a
Y74C10A_150.b
Y74C10A_151.a
Y74C10A_151.b
Y74C10A_152.a
Y74C10A_152.b
Y74C10A_153.a
Y74C10A_154.a
Y74C10A_154.b
Y74C10A_154.c
Y74C10A_156.a
Y74C10A_156.b
Y74C10A_157.a
Y74C10A_157.b
Y74C10A_158.a
Y74C10A_158.b
Y74C10A_158.c
Y74C10A_158.d
Y74C10A_158.e
Y74C10A_158.f
Y74C10A_158.g
Y87G2A.c
Y87G2A.i
Y87G2A.k
Y87G2A.m
Y87G2A.o
Y87G2A.p
Y87G2A.r
Y87G2A.x
Y95B8A_82.a
Y95B8A_82.b
Y95B8A_84.a
Y95B8A_84.e
Y95B8A_85.b
Y95B8A_85.d
Y95B8A_85.f
Y95B8A_85.g
ZC247.1
ZC247.4
ZC334.1
ZC434.1
ZC434.3
ZC434.4
ZC434.5
ZC434.7

GenePair
ZC434.8
ZK1053.1
ZK1053.2
ZK1053.4
ZK1151.3
ZK265.2
ZK39.4
ZK484.4
ZK524.1
ZK524.3
ZK524.4
ZK849.4
ZK858.3
ZK909.2
ZK909.5
ZK973.l
ZK973.m
ZK993.b