

High-Throughput Gene Mapping in *Caenorhabditis elegans*

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Positional cloning of mutations in model genetic systems is a powerful method for the identification of targets of medical and agricultural importance. To facilitate the high-throughput mapping of mutations in *Caenorhabditis elegans*, we have identified a further 9602 putative new single nucleotide polymorphisms (SNPs) between two *C. elegans* strains, Bristol N2 and the Hawaiian mapping strain CB4856, by sequencing inserts from a CB4856 genomic DNA library and using an informatics pipeline to compare sequences with the canonical N2 genomic sequence. When combined with data from other laboratories, our marker set of 17,189 SNPs provides even coverage of the complete worm genome. To date, we have confirmed >1099 evenly spaced SNPs (one every 91 ± 56 kb) across the six chromosomes and validated the utility of our SNP marker set and new fluorescence polarization-based genotyping methods for systematic and high-throughput identification of genes in *C. elegans* by cloning several proprietary genes. We illustrate our approach by recombination mapping and confirmation of the mutation in the cloned gene, *dpy-18*.

[The sequence data described in this paper have been submitted to the NCBI dbSNP data library under accession nos. 4388625–4389689 and GenBank dbSTS under accession nos. 973810–974874. The following individuals and institutions kindly provided reagents, samples, or unpublished information as indicated in the paper: The *C. elegans* Sequencing Consortium and The *Caenorhabditis* Genetics Center.]

Forward genetic screens in model organisms remain a crucial tool for uncovering new biological information (Matthews and Kocpczynski 2001; Sternberg 2001). These approaches require extensive recombination mapping of a mutation to discover the identity of a gene. Traditional methods in model systems have typically relied on the use of visible phenotypic markers for linkage mapping of mutations. However, single nucleotide polymorphism (SNP) markers are currently favored because of their relative abundance and because they can eliminate confounding interaction with the mutant phenotype, although in some cases outcrossing introduces genetic modifiers.

To date, the only strategy for SNP-based cloning in the nematode *Caenorhabditis elegans* (*C. elegans* Sequencing Consortium 1998) is the snip-SNP approach (Wicks et al. 2001). Here we present an alternative tripartite approach for rapid SNP-based mapping in the worm. We first established a set of finely spaced genome-spanning SNP markers and then combined this resource with a tiered mapping strategy that progressively narrows the region containing the gene of interest. Finally, we used a high-throughput SNP assay that allowed reliable and rapid genotyping with low marker development costs. This strategy afforded rapid gene cloning in *C. elegans* and can be tailored for use in other model organisms with a sequenced genome.

RESULTS

Our initial goal was to create a set of reliable SNP markers at a

density of one marker every 100 kb across the *C. elegans* genome. To achieve this density, a large set of predicted SNPs scattered throughout the *C. elegans* genome was required. We chose to identify polymorphisms between the Hawaiian CB4856 strain of *C. elegans* and the commonly used Bristol N2 laboratory strain, because the CB4856 strain is known to have the most even distribution of SNPs across the chromosomes (Koch et al. 2000; Wicks et al. 2001). A small insert (1.7 kb \pm 0.5 kb) library was constructed from CB4856 genomic DNA. Double-end sequencing of cloned inserts from this library produced 16,941 high quality sequencing reads, which represented 7.3% sequence coverage of the genome. An informatics software pipeline (Vysotskaia et al. 2001) was then used to predict likely polymorphisms between CB4856 sequencing reads and the canonical N2 genomic sequence (WS version 48). The pipeline identified a total of 10,711 predicted polymorphisms; 9602 of these were unique from those previously reported. These unique polymorphisms include 6902 substitutions (SNPs), 1885 deletions (one or more bases removed), and 815 insertions (one or more bases added). We estimate the overall rate of polymorphism between the two strains to be one substitution/insertion/deletion per 840 bases. Transitions accounted for 57% (3906 SNPs) of substitution SNPs, whereas 43% (2996 SNPs) were transversions. These observations agreed with previous findings from a smaller dataset (Wicks et al. 2001). Validated SNP data are available from NCBI dbSNP (accession nos.: 4388625–4389689) and GenBank dbSTS (accession nos.: G73810–G74874).

We combined the publicly available *C. elegans* CB4856 SNP information (http://genome.wustl.edu/gsc/C_elegans/SNP/index.html) with our data to obtain a total of 17,189 predicted polymorphisms throughout the *C. elegans* genome

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and then systematically chose one substitution SNP spaced approximately every 100 kb. Oligonucleotide primers were designed flanking these predicted SNPs for PCR amplification. The presence of a SNP was confirmed by sequencing the PCR product and/or by a fluorescence polarization-template directed incorporation (FP-TDI) SNP genotyping assay (see below). The latter method proved to be faster than sequencing

and had a comparable failure rate of ~10%, which includes mispredictions, primer failure, and assay failure. To date, a set of 1099 markers have been confirmed and formatted for a genotyping assay; 427 (39%) of these 1099 confirmed SNPs were derived from our putative new 9602 SNPs. Our substitution SNP marker set has an average spacing of $91 \text{ kb} \pm 56 \text{ kb}$ across the genome (Fig. 1A). The most telomeric SNPs on av-

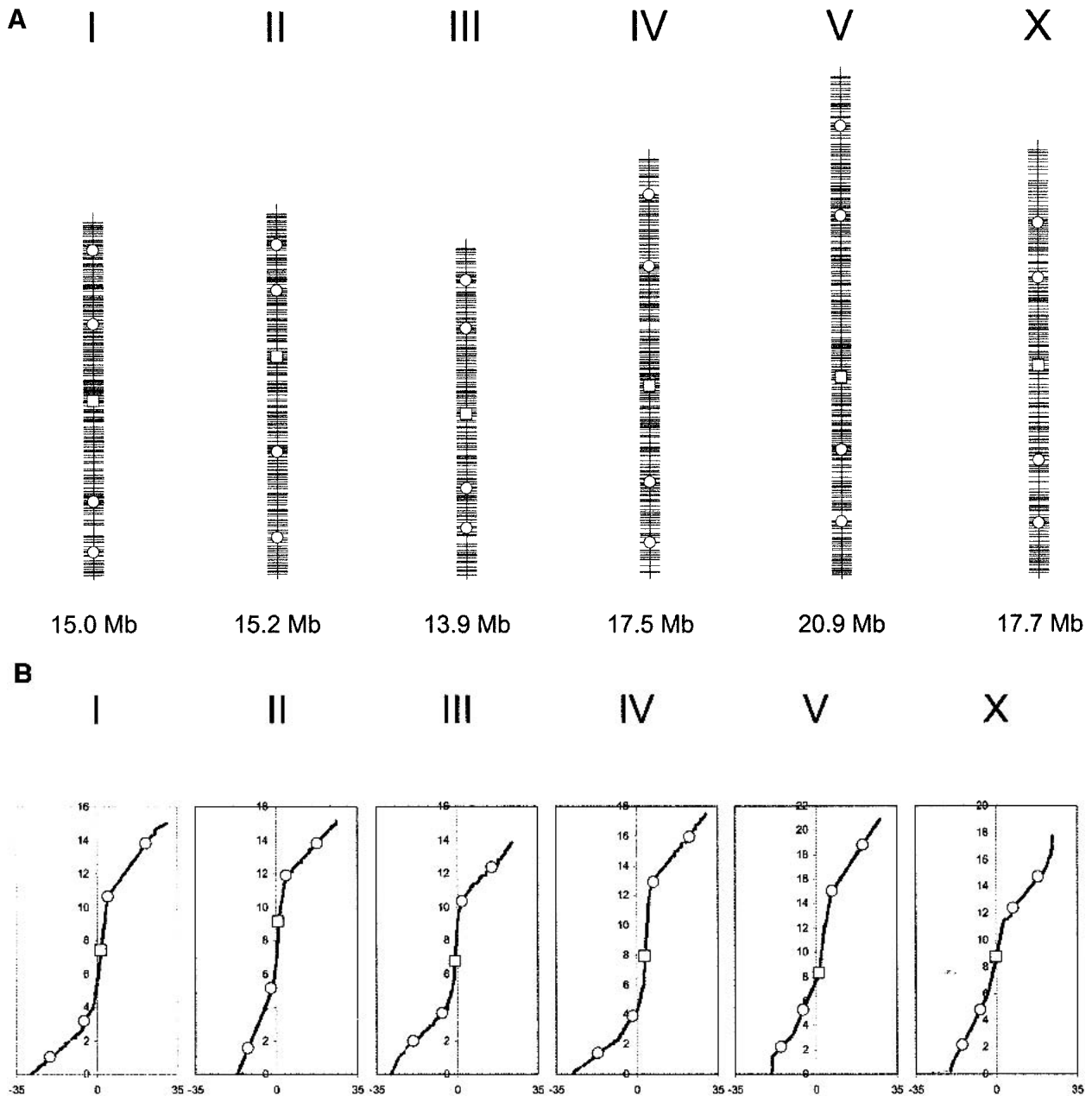


Figure 1 Distribution of single nuclear polymorphism (SNP) markers across the *Caenorhabditis elegans* genome. (A) Marker distribution along the physical map of chromosome. The location of the Tier 1 SNP markers is shown with open squares, and the location of Tier 2 markers are shown by open circles. The location of each of the validated 1099 markers is shown along the chromosomal axis. The largest gap is 449 kb (chromosome I), and the average validated marker spacing is $91 \text{ kb} \pm 56 \text{ kb}$. (B) Recombination rates across the six chromosomes. The physical (Mb) location of all predicted polymorphisms (y-axis) is plotted versus the extrapolated genetic (cM) location (x-axis) of the associated cosmid across the genome (Genetic Map Position from Wormbase; Stein et al. 2001). This illustrates the rate of change in the recombination rate across the chromosome and shows the "gene cluster" effect in the center of the autosomes (Barnes et al. 1995). This information is used during mapping, because the local recombination rate affects the number of putative recombinants that must be genotyped to obtain a 100-kb interval.

erage are located ~72.5 kb from the telomeres of each chromosome, ranging from 16.6 kb (left end of chromosome III) to 178.3 kb (right end of chromosome III).

By use of the comprehensive SNP marker set, we implemented a mapping strategy that uses iterative phases to progressively refine a genomic region of interest (ROI) containing a mutant gene. In *C. elegans* forward genetic screens, mapping often begins by genotyping phenotypically mutant F2 offspring from a cross of a homozygous mutant animal (N2 background) to a wild-type animal (CB4856 background) (see Methods). High-throughput genotyping of 30–60 offspring, using a preselected marker set of 30 SNPs (Table 1; Tier 1, 2), typically localizes the gene of interest to a subchromosomal region that can range in size from 1 to 6.7 Mb (Fig. 1B; Table 1). Alternatively, this same mapping resolution can be achieved by first defining chromosomal linkage for the gene of interest using only the six Tier 1 SNP markers (Fig. 2A; Table 1) and then defining a subchromosomal region by use of the four Tier 2 markers on that particular chromosome (Fig. 2B). The latter method uses the same number of recombinants but requires less genotyping. To further refine the map position to ~100 kb, we first assay any remaining informative recombinants (those that have a recombination event within the ROI) with all markers that lie within the ROI to define the smallest candidate gene region. We then routinely collect an additional 1000–2500 DNA samples (Fig. 1B; Genetic Map Position from Wormbase; Stein et al. 2001) from F2 animals as described above. Genotyping the new F2 DNA samples with SNP markers flanking the ROI identifies additional informative samples. These additional informative samples are then genotyped using the complete set of markers for that region (Fig. 2C). A finer map position than that afforded by the set of 1099 markers may be obtained by validating any predicted SNPs within the mapping interval or by de novo SNP discovery (Fig. 2D, E; Jakubowski and Kornfeld 1999; Koch et al. 2000) coupled with analysis of additional recombinants. In practice, however, fine-scale map data are often not required because candidate genes (or all genes) within the ROI can be identified and sequenced for mutations (Collins 1995). Alternatively, candidate genes can be tested using RNA interference (RNAi; Fire et al. 1998) for gene “knock-down” or cosmid/open reading frame (ORF) rescue for gene complementation (rescue). Large RNAi screens have identified loss-of-function phenotypes for many genes in *C. elegans*, and currently RNAi data are available for >5000 of the 19,000 *C. elegans* genes (Fraser et al. 2000; Gonczy et al. 2000; Maeda 2001).

Our mapping strategy required high-throughput genotyping for maximum speed and efficiency. Of the several available methods for SNP analysis (Kwok 2001), we selected the FP-TDI assay for its consistent and interpretable results, low assay-setup cost, and automated detection. We performed the FP-TDI assay (Chen et al. 1999) using commercial reagents (AcycloPrime-FP SNP Detection Kit, Perkin Elmer Life Sciences, Inc.) in 384-well format with a standard liquid handling robot (Tecan Genesis, Tecan). Figure 3 shows examples of data obtained using this assay. The separation of dyes that is achieved using control N2 and CB4856 genomic DNA is

Table 1. The SNP Markers Used for Tier 1 (Center Markers) and Tier 2 (Left, Left Center, Right Center, and Right Markers) FP-TDI Mapping

Chromosome	Tier	Location	Marker	CB4856	Bristol N2	PhyloLoc ^a	Mb distance ^b	Genamid Inc.	Genamid	PCR Forward Sequence	PCR Reverse Sequence	TDI Forward Sequence	TDI Reverse Sequence
Worm I	Tier2	Left arm	CE1-244	A	T	890151	0.950181	F445	39238	AGTACTGGTTCGGGCGAGACT	GGCTAAGTCCGAGGCTCCDC	AGCATAGGCTGAGCAGTATGATGAGGCTGAT	AAAAAAGTTCGACGATCTGTTTTGGG
Worm I	Tier2	Left cluster	CE1-245	A	T	3144636	2.154485	79234	79234	TCTTCCAAATGAGCTCGATTA	TTCGAAATGGCTCGTAAACGTCGCAAAA	TATTCATTTTAAATCTGAAATATATG	AAAAAAGTTCGACGATCTGTTTTGGG
Worm I	Tier1	Center	CE1-246	A	T	7401787	4.287151	Y84E10A	15145	TTCGAAATGGCTCGATTA	ATTCGAAAGGCGCAAAAGTGT	TCACATTAACGCTGATGAGGATGAT	TCACATTAACGCTGATGAGGATGAT
Worm I	Tier2	Right cluster	CE1-247	A	T	10545051	3.243254	B0205	19175	GA1TCGGATTCGGGATGATCT	ATTCGAAAGGCGCAAAAGTGT	GCCGAGTAAAGCAAGTGAACATGAAAT	TCACATTCGAAATTCATTAAGTTTTT
Worm I	Tier2	Right arm	CE1-248	A	T	1379509	3.133608	W0A48	14138	AGGCTGGAAAGTGAATCCCT	CGAAAGGAGCAAAATCCG	TCCGCACTTTTCCGACACATCCACCTCC	TGGGTTAGAGCCACAAAGCATCCCTT
Worm II	Tier2	Left arm	CE2-234	A	T	1680772	1.680772	T0H6	5536	CAAGGCTGGCTTCAGCAATG	TGGTTCAGATTCACCTCGAA	CAGTCTGTTTATTTTATTAATAATCCAAA	AAACAATCAATRACTCTYATTCCTTTA
Worm II	Tier2	Left cluster	CE2-235	A	T	3190060	3.569266	F0840	10874	CGCCCAATCATCTGAACTTT	TG10TTTTCCACGCTCGAA	TAGAAAGGCTTCACCAAAATTAACATTT	ATGTTTTTCCGAGGTTCAACCAATCGAGC
Worm II	Tier1	Center	CE2-236	T	A	9141824	3.981704	C18D2	10654	CGCAAAATTCGACGCTGAT	GTGGATTTTCCAGCGGCAAT	ACCAAAAAGTTATTAATTAATTAATTTT	GTATTAATTAAGAAATTTGATCGGGAAA
Worm II	Tier2	Right cluster	CE2-237	T	A	11970190	2.769820	ZK080	32574	TGCTGTGAAAGTTCGCTAAT	GGAAATTTGCGCTCATCA	AAATATTCCTTAACATTAATTAAGACGA	TGCTATGCGGCTGCTGGCTCATCTGGCT
Worm II	Tier2	Right arm	CE2-238	T	A	13673009	1.902850	ZK131	19874	GAAAACGAGCAAGTGAAGCC	GCTTGGCAATTTTTCTAGTG	ACAAAATGGGAAATTCACAAATTAAMAAT	AAATAATGGGTTTCCATTAAGCTTTTTT
Worm III	Tier2	Left arm	CE3-216	A	T	1985116	1.985116	B0624	10275	TGAAATTCGAGTTCGCGGT	GAAGAGCACACACCTCGCT	TGTTTAAACTAACCAACTTGTCTCTG	CGGCGAGCAGATAGAAATTTGSAAAATC
Worm III	Tier2	Left cluster	CE3-217	A	T	3824943	1.659927	C54C6	29933	TGCAACCGGGTATACAAACA	TTCGAGAGATGAAGCAATGG	TACCTAAGTTTTTAAATCTGAACTGTGA	AAATCAATTTGTTTTTCACTGTTGAA
Worm III	Tier1	Center	CE3-218	A	T	8750826	3.125685	N07G4	29976	GCCTATGTCGACAGGTTT	TGCAAGCGCCCAAGTAGAT	CACATCTATATATCCAAACTGCTGTA	TTTTTAAACAGTACACACAGTGTATGA
Worm III	Tier2	Right cluster	CE3-219	A	T	1035776	3.565148	Y7G310A	24415	GCCTATGTCGACAGGTTT	GCCTCCGCTGGTCTTGA	CTATGTCGACAGATGCAAGGTTGGGC	AGTTGATCTGGCTGATACCAAAATTA
Worm III	Tier2	Right arm	CE3-220	A	T	12354140	2.038384	Y7688A	213297	AAATGGCTCTAAGGCAACA	C56CAATATGCTGATTTGA	CTATGTCGACAGATGCAAGGTTGGGC	AGTTGATCTGGCTGATACCAAAATTA
Worm IV	Tier2	Left arm	CE4-231	T	A	1380226	1.380226	F68F5	32187	CTGACGCTGGGAGCTGAAT	GTGGGCAATGTGTGTATAGC	AAATTTGAAAATGTGATTTCTGTCACTA	TTACAAMCTGACAAATACTATTTTTT
Worm IV	Tier2	Left cluster	CE4-232	T	A	3898073	2.517847	F37C4	22475	AACTACAGCGGCAATTTACCG	TACCCTATGCTGCTCCG	AAATTTGCTGATTCGAGCTCTCTATCGG	GAGCCACGATGTGTGTGAGATGTGAT
Worm IV	Tier1	Center	CE4-233	A	T	7911864	4.013781	C19H3	3769	GGATCTGCTCTCTCCACA	CGAGAGCAATGAMGGGA	ATAATAAATAAATAAAGCCAGCATACAT	TGTAAATCCGCTAGAAAATTTGTTTTT
Worm IV	Tier2	Right cluster	CE4-234	A	T	1240162	5.008308	K08D8	19889	AGAAACACACTTTCCGCA	TGGCAATCCGAGCCTTAAGT	ATTTTTTAAATTAATTAATTAAGCTTTTTT	GTGGGCAATTCGATGAGTGTGAGGATTTA
Worm IV	Tier2	Right arm	CE4-235	A	T	1507984	2.998022	Y105C8B	43797	GGAGGCTCAGAAATCCGAA	GTTTTCCAGTTCATCTGAGC	CGATTTCTTCAATTTTCAAGGTTTTT	CGAGTTTATCAGCAGCATCGAGGAAATTA
Worm V	Tier2	Left arm	CE5-273	A	T	2225183	2.225183	F4H8	10297	TCCGCTAGTCAAACTGAAA	GGACCGGAGGAAATAAAGG	GTGTTGATTAATTTAAGCTGCCAATAA	GACATAGTCTCAAGTGTCTTTTACAC
Worm V	Tier2	Left cluster	CE5-274	T	A	5210453	2.09425	Y4095B	7569	CTGCGTGTAGTCAAAAGTGT	CAAAAGGCTTTGGCGATC	GGCAATTTGCAAAAATTAATAAAT	GAACCTTTAAAATTTTGGTTTTTAC
Worm V	Tier1	Center	CE5-275	T	A	8027077	3.052064	F21FA	5901	TTTATGCTGAGGCAATC	ATTGSCATCTTGGCGATC	TCACACGCTCGCAAAAGTAACTAAT	CAAGCAACACTGTTGAAATTAATTA
Worm V	Tier2	Right cluster	CE5-276	T	A	15076305	6.742250	F4H8	5017	GTGAGAAATGCAAGGCA	CAAGGTTTACGACGCACTA	TSGAGAAATGCAATTAATTAAGTGTCT	TAACTCAATTAATTAATTAAGTGTCT
Worm V	Tier2	Right arm	CE5-277	A	T	1678824	3.771868	Y17D75	14885	TGTTCCGCAAGTGGCTGTA	AATTCGCAACTCCGACGA	AAATTTTACGCAATATCCAGAAAAT	CTCCAGACGACGATGAGTATTTTTT
Worm X	Tier2	Left arm	CE6-215	T	A	2180782	2.180782	EGAP7	6042	TAGATGTTGGCAGCTGAAA	TGATTTGTTGGGCAATG	AAATTAATTAATTAAGTGTGAAAATTT	TTTTGGGTTGATTAATTTTGGTGGACT
Worm X	Tier2	Left cluster	CE6-216	T	A	4781021	2.589429	C3BE6	4363	AGCCAAATTTGGCAGGCTC	CAAGTCTGGGTAATWGGGA	TTTTTATGGGCAATCCCTCTAGCAATAC	ATATGTTTATGATTAATTTTGGGAGAT
Worm X	Tier1	Center	CE6-217	T	A	8725963	3.945782	F59A9	5091	TTCGCAACTCAGATTTGTC	ATGAACCCCAAAATGTCAG	TAAATGATGAGGAAAGATGCAAGGTTTT	TAAATGATGAGGAAAGATGCAAGGTTTT
Worm X	Tier2	Right cluster	CE6-218	T	A	12389706	3.672723	F19H6	2788	TGCAAAAGCTCCAGTTTTCT	CGAGTTGTTTTTAAAGTGTGTT	AAAAAAGCTTTTAAAGTGTGCAAGTCTT	CGAGTTGTTTTTAAAGTGTGCAAGTCTT
Worm X	Tier2	Right arm	CE6-219	A	T	1473288	2.314582	C26G2	29561	CGGTACATCCCAATTTTTC	CTCCGACCTTGGCCTTATTA	CAATTTTTCCGACCGAAGATTTCAATTA	CGTAAAGGAGGAGTGGTGGATGCTCCAAA

^aPhysical location based on WS18 sequence

SNP, single nucleotide polymorphism; FP-TDI, fluorescence polarization-template directed incorporation.

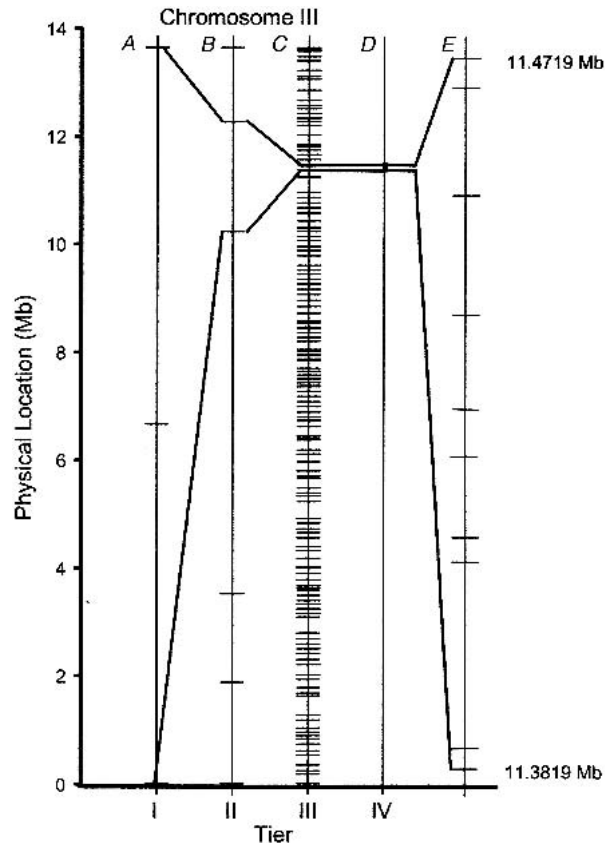


Figure 2 Tiered mapping strategy. Shown is a schematized mapping workflow. (A) Tier 1 mapping localizes the gene of interest to a chromosome by assessing linkage to one centrally located SNP. (B) Tier 2 mapping localizes the gene to a subregion of the chromosome. This region can vary in size between 1 and 6.7 Mb. In the *dpy-18* example shown, the gene falls between two SNP markers that are 2 Mb apart on chromosome III. This resolution is routinely achievable by genotyping 30–60 recombinants. (C) Tier 3 mapping begins by identification of informative animals with recombination breakpoints within the region defined by Tier 2 and then fine mapping with Tier 3 markers to narrow the region of interest. For *dpy-18*, Tier 3 mapping localizes the gene to a region as small as 97 kb and thus narrowed the candidate region to ~0.1% of the worm genome. The number of recombinants required to achieve this mapping resolution will depend on the local recombination rate (Fig. 1B; Genetic Map Position from Wormbase; Stein et al. 2001) along the chromosome in the vicinity of the mutated gene. (D and E) Tier 4 mapping and/or mutation detection. Further refinement of the candidate interval occurs by validation of additional SNP markers and genotyping of informative recombinants. For *dpy-18*, the location of the predicted substitution SNPs located within the 97-kb region of chromosome III are shown. During this fine-mapping process, candidate gene approaches such as cosmid rescue or RNA interference (RNAi) can also be used to help identify the mutation.

illustrated in Figure 3A and B and shows the consistent clarity of base-calling data obtained from six randomly chosen chromosome II SNPs on F2 recombinants (crude worm lysates).

This strategy has been applied extensively for identification of novel genes. To date, we have mapped more than 50 loci and cloned >30 genes from several forward genetic screens. We can routinely identify a gene of interest within a 2- to 4-mo time frame. To illustrate this process, Fig. 3C shows results from Tiers 1 and 2 analysis of just 35 DNA samples in the mapping of the cloned gene *dpy-18* (Hill et al. 2000) to a

2.0-Mb region of chromosome III. This simple analysis narrows the ROI to only 2% of the genome. Figure 3D shows the 97-kb interval bound by the two validated FP-TDI SNP markers flanking *dpy-18*. This panel shows the resolution attainable with the set of markers currently available. In addition, information from the RNAi screen of chromosome III (Gonczy et al. 2000) indicates the sequence *Y47D3B.10* as being a good candidate gene for *dpy-18*. We sequenced this e364 allele and confirmed the published mutation in the third exon (Hill et al. 2000), which introduces a premature stop codon into the coding sequence.

DISCUSSION

We have presented a tripartite, comprehensive strategy for systematic and high-throughput gene identification in *C. elegans*. This strategy required the development of finely spaced, genome-wide SNP markers and combined an iterative mapping approach with the high-throughput FP-TDI SNP marker assay. We optimized the FP-TDI assay for automated reaction setup and nucleotide analog detection. The FP-TDI assay is highly reliable and allows greater flexibility in selecting which SNPs are assayed, as well as how many samples are genotyped. Our strategy effectively speeds mutation detection and gene cloning in *C. elegans*, especially when combined with tools for candidate gene analysis such as cosmid rescue and RNAi. Many aspects of our approach are transportable to other model systems and could allow for rapid and systematic gene identification in these systems.

METHODS

Library Construction and Sequencing

Random, genome-wide DNA sequences from the Hawaiian *C. elegans* strain CB4856 were obtained by constructing a small insert genomic library for shotgun sequencing. Library construction was described previously (Vysotskaia et al. 2001). Double-end sequencing of clones was performed on ABI 3700 (Perkin Elmer) DNA sequencers.

SNP Prediction

The CB4856 sequence traces were aligned against Bristol N2 genomic sequence (*C. elegans* Sequencing Consortium 1998) using a custom script that takes into account the quality of the neighboring sequence as well as that of the potential polymorphic base (Vysotskaia et al. 2001). Polymorphism information can be found in NCBI dbSNP (accession nos. 4388625–4389689) and GenBank dbSTS (accession nos.: G73810–G74874).

SNP Confirmation

We modified primer3 (http://www-genome.wi.mit.edu/genome_software/other/primer3.html) and designed primers for PCR amplicons ranging between 150 and 300 bases that contain the selected putative SNPs. An initial set of ~100 of the predicted polymorphisms between the Bristol N2 and CB4856 strains were confirmed by sequencing the PCR amplicon from each strain. Sequencing was performed using standard protocols, and products were resolved using capillary electrophoresis on ABI 3700 (Perkin Elmer) instruments.

All 1099 SNPs were also confirmed by FP-TDI (Chen et al. 1999). We used the SNP-kit (AcycloPrime-FP SNP Detection Kit, Perkin Elmer Life Sciences, Inc.) and modified the volumes for compatibility with 384-well PCR. Reactions were set up on the Tecan Genesis 150 robot (Tecan). Briefly, a 200- to 300-bp region of the genome containing the SNP was amplified using standard PCR (6 μ L reaction volume). Excess prim-

ground commences by crossing homozygous mutant (N2-background) animals with wild-type CB4856 animals. The F1 progeny are then segregated away from other progeny and allowed to self-fertilize. The resulting F2 animals are then picked onto 6-cm Petri plates and phenotyped for the mutation. Alternatively, after picking of the F2 animals onto plates, the F2 can be allowed to self-fertilize and lay a brood of F3 animals, and these are then phenotyped. Only F2 animals that are homozygous for the recessive mutation (or homozygous without it) are potentially informative and are genotyped.

To map a dominant mutation, essentially the same procedure is followed except the F1 animals are backcrossed to CB4856 animals, and F2 showing the mapping phenotype (mutant/CB4856) are singled from the resulting outcross progeny.

DNA Sample Preparation

DNA samples for PCR were prepared as described previously (Williams et al. 1992). This procedure usually yields DNA at a concentration of 100 ng/μL. A portion of the population not used for the DNA lysate can be saved for reconfirmation of a phenotype.

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