Quantitative PCR approach to SNP detection and linkage mapping in *Caenorhabditis elegans*

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I report a method for single nucleotide polymorphism (SNP) detection and linkage mapping in *Caenorhabditis elegans* using automated oligonucleotide design and fluorescence-based quantitative PCR detection. Nine hundred twenty-three oligonucleotide pairs were designed to produce small products of <150 bp for efficient amplification in a PCR, with one oligonucleotide of each pair overlapping a SNP site at the 3′-most nucleotide. A subset of the pairs were tested, and efficient allelic discrimination was obtained for SNPs between N2, the canonical laboratory strain, and CB4856, a strain isolated from Hawaii commonly used for mapping studies. Linkage mapping is demonstrated using the unc-119 locus of *C. elegans*. This quantitative PCR method provides an inexpensive, uniform, and automatatable detection alternative for genetic mapping strategies in *C. elegans* or other organisms.

**INTRODUCTION**

Due to the frequent occurrence of single nucleotide polymorphisms (SNPs) in the individual genomes of heterogeneous populations, SNP mapping is rapidly becoming the method of choice for analyzing genetic variation and performing linkage studies. A variety of quantitative methods have been developed to analyze SNPs rapidly in a high-throughput manner (1). However, many of these methods are limited by high cost and complexity. For example, the 5′ nuclease or TaqMan® assay provides a rapid, specific, and high-throughput method for analyzing SNP variation, but is prohibitively expensive when applied to large-scale association studies (2). Restriction fragment length polymorphisms (RFLPs) generated by SNPs have also been used for mapping. For example, in *Caenorhabditis elegans*, a widely used and effective RFLP mapping method—dubbed Snip-SNP mapping—utilizes a number of RFLPs between N2, the canonical wild-type strain, and CB4856, an isolate from Hawaii (3). However, sequential PCR, restriction digests, and gel-based quantitation reduces the number of SNPs that can be easily analyzed at one time. In addition, different SNPs can require different restriction enzymes, thereby increasing the complexity of mapping experiments using multiple SNPs. Fluorescence polarization template-directed incorporation (FP-TDI) is a viable and cost-effective approach to mapping in *C. elegans* (4). However, the FP-TDI method requires a two-reaction protocol and three oligonucleotides to amplify and detect the SNP variants. A simple alternative detection approach is to design a pair of oligonucleotides that overlay the SNP of interest and provide discrimination in a quantitative PCR platform (5).

This report demonstrates a method for SNP detection and linkage analysis in *C. elegans* using unmodified oligonucleotides in a standard quantitative PCR detection platform. The method utilizes simple rules that can be encoded in batch-processing algorithms for consistent oligonucleotide design. In addition, the method provides an alternative polymorphism detection strategy for described high-throughput mapping methods (4,6). Therefore, the method described here provides a uniform assay that can be tailored to a wide range of mapping resolutions and strategies.

**MATERIALS AND METHODS**

**Reagent Sources**

The *C. elegans* strains N2 (wild-type), CB4856 (Hawaiian variant), and CB4845 [unc-119(e2498) III] were obtained from the Caenorhabditis Genetics Center (CGC; www.cbs.umn.edu/CGC) and were grown under standard *C. elegans* growth conditions (7). Predicted *C. elegans* SNPs were originally obtained from a *C. elegans* SNP database at the Washington University Genome Sequencing Center (genome.wustl.edu). Map locations for the SNP variants were obtained from WormBase (www.wormbase.org). Information on the SNPs is reported in Supplementary Table S1 (available online at www.BioTechniques.com) and is also available at Wormbase. Oligonucleotides were obtained from Integrated DNA Technologies (IDT; Coralville, IA, USA) at 25 nM scale, and were resuspended in water to 900 nM concentration.

**Oligonucleotide Design**

The following constraints were used to design the oligonucleotides: (i) the oligonucleotide pairs were designed to anneal an arbitrary 100 bp apart in the target sequence; (ii) the 3′-most nucleotide of one oligonucleotide was designed to overlap the SNP location; (iii) the oligonucleotides were designed no longer than 30 bp in length; and (iv) the oligonucleotides were designed to have a melting temperature, or $T_m$, not to exceed 76°C. To calculate the $T_m$, 2°C was added for each A or T base and 4°C for each C or G base (8). The 2°/4°C rule was chosen to simplify oligonucleotide design and to provide ease of use in automated design scripts. The standard $T_m$ of 76°C was chosen, balancing maximum sequence specificity (longer oligonucleotides) with performance under standard ABI PRISM® 7900 (Applied Biosystems, Foster City, CA, USA) cycling conditions. These design criteria resulted in a high success rate for specific SNP detection in initial experiments and were applied uniformly to design oligonucleotides for a large number
of SNPs, thereby allowing multiple assays to be run in parallel using the same PCR cycling conditions. For batch processing of SNPs, a Perl-based script was used for automated design of the oligonucleotides (see the supplementary material). For each SNP, the algorithm designed two oligonucleotides, one of which overlies the SNP location at the 3’-most nucleotide. For consistency and simplicity of batch processing and PCRs, all oligonucleotides overlying the SNP position were designed with A at the end. Therefore, only AC and GT SNP variations were targeted for design, with GT SNP variant oligonucleotides designed to the reverse complement of the sequence. The algorithm added base pairs until the \( T_m \) was close to, but not exceeding, 76°C. Oligonucleotide designs were imported into Microsoft® Excel® and sorted to eliminate those that were of low complexity. To provide a normalization control, an oligonucleotide pair was designed by hand using the same design rules as a nonpolymorphic region (Table 1).

Genetics and DNA Preparation

CB4856 (Hawaiian) males were mated to N2 or CB4845 (unc-119 mutant) hermaphrodites as follows: (i) 3–5 CB4856 males were placed with single N2 or CB4845 young adults; (ii) animals were transferred at 24 and again at 48 h; and (iii) working crosses were identified by the presence of males among progeny, and L4 stage F1 progeny were picked from subsequent transfers of the same cross. To obtain an unlinked DNA sample for control reactions, F1 hermaphrodite progeny from a CB4856 mating to N2 were picked to a 60-mm nematode growth medium (NGM) plate and grown until the culture starved out. To map unc-119(e2498), 10 F1 hermaphrodite progeny from a CB4856 mating were placed on an NGM plate and allowed to produce broods. From the broods, 50 Unc F2 hermaphrodites were picked to a 60-mm NGM plate and grown for several generations until the culture was starved out. To prepare DNA, animals were washed from the plates and incubated in lysis buffer [10 mM Tris, pH 8.3, 2.5 mM MgCl₂, 0.45% IGEPAL® CA-630 (Sigma-Aldrich, St. Louis, MO, USA), 0.45% Tween®-20, 200 µg/mL proteinase K, and 100 µg/mL RNase A] for 1 h at 65°C. After incubation, the solution was extracted two times with a 1:1 mixture of phenol: chloroform and ethanol precipitated (9). Precipitated DNA was resuspended in 10 mM Tris buffer, pH 8.3. If the starting concentration of worms was high, and they were grown on agarose-based NGM medium, this treatment was often adequate for efficient PCR. However, the best PCR templates were obtained when the DNA was purified further using a DNeasy® Blood and Tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s recommendation. Single-worm lysates were prepared by dissolving single L4 to adult animals in 5 µL lysis buffer for 40 min at 65°C, followed by 10 min at 95°C. The single-worm preparation was then diluted to a volume of 50 µL with sterile water; 6 µL were used in quantitative PCRs.

Quantitative PCRs

PCRs were performed in an ABI PRISM 7900 Sequence Detection System. Each PCR contained 12 µL 2× SYBR® Green PCR Master Mix (Applied Biosystems), 3 µL each oligonucleotide pair (900 nM in water), and 6 µL C. elegans genomic DNA (10–50 ng total). The cycle at which product is detected, or \( C_T \), was deter-

![Figure 1. Specificity of oligonucleotide pairs in quantitative PCR.](image-url)

(A) Quantitative reactions run using the oligonucleotide pair to the T01A4 clone. Fifty nanograms DNA/reaction were used. The horizontal solid line indicates the threshold at which cycle threshold \( (C_T) \) values were derived. (B) Specificity testing of single nucleotide polymorphism (SNP)-specific oligonucleotide pairs. Each oligonucleotide pair was used in a quantitative reaction with N2, 1:1 N2:CB4856, or CB4856 DNA at 50 ng/reaction. The bars indicate the cycles at which detection of PCR product exceeded the threshold.
**Table 1. Oligonucleotide Pairs Used for Linkage Mapping (CB4856-specific)**

<table>
<thead>
<tr>
<th>Map Location</th>
<th>Physical Location of SNP (bp)</th>
<th>Variation Name</th>
<th>Primer 1</th>
<th>Primer 2</th>
<th>Ave ΔC&lt;sub&gt;T&lt;/sub&gt; (C&lt;sub&gt;T2, N2&lt;/sub&gt; - C&lt;sub&gt;T2, CB4856&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: -15.55</td>
<td>1566709</td>
<td>snp_Y92H12[5]</td>
<td>5′-ATGTTTCAACACGCTTGAATA-3′</td>
<td>5′-GAGCCGGTCCATATTGGCCA-3′</td>
<td>5.0 ± 0.7</td>
</tr>
<tr>
<td>I: -6.04</td>
<td>2839646</td>
<td>snp_W10C8[2]</td>
<td>5′-ATCATGCTCTGATCTTTGCTGT-3′</td>
<td>5′-GTGTCGTTATCTAACGTCCTC-3′</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td>I: -1.05</td>
<td>4464781</td>
<td>snp_T01A4[2]</td>
<td>5′-TGATATGACAGGATTCTTGCA-3′</td>
<td>5′-CTGTCGAGCTGATGAGTGA-3′</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>II: -13.5</td>
<td>1090279</td>
<td>snp_C07D2[1]</td>
<td>5′-TGAGAGTTGAAGATGTTGCA-3′</td>
<td>5′-CAATTCGATGGAAGATCCT-3′</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>II: -6.09</td>
<td>3567932</td>
<td>snp_W10G1[3]</td>
<td>5′-TTAGCTGAACTGAGTGCCTGC-3′</td>
<td>5′-ACCGTGAATAATGGACAATGAGA-3′</td>
<td>6.3 ± 0.3</td>
</tr>
<tr>
<td>II: 11.99</td>
<td>13008794</td>
<td>snp_Y38F1[14]</td>
<td>5′-TTTCTCTGGAGTCTGCTGTCAC-3′</td>
<td>5′-ATATGGGCGCGCAATGTCCTGCT-3′</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>II: 20.15</td>
<td>14249122</td>
<td>Y4866A</td>
<td>5′-AAAATATTTTCCTGTTAAGA-3′</td>
<td>5′-GATAATGACGTTGCAAACATG-3′</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td>III: -23.5</td>
<td>1221469</td>
<td>snp_Y19D3B[7]</td>
<td>5′-TCGTCAACATTCTCCTTCGGGACG-3′</td>
<td>5′-CCTTGTGCTCAGCATCTACTCT-3′</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>III: -8.3</td>
<td>6980475</td>
<td>snp_F31E3[6]</td>
<td>5′-AGCCTGACGGCTGAGGCAAGA-3′</td>
<td>5′-CATTGTCCTGTGATGAGTGA-3′</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>III: 20.75</td>
<td>13070136</td>
<td>snp_Y39E4B[1]</td>
<td>5′-AACGGGAAATAATAGTGTTTATCCTTG-3′</td>
<td>5′-AACCGGAAATAATAGTGTTTATC-3′</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>IV: -2.18</td>
<td>3813652</td>
<td>M02B7</td>
<td>5′-TGTGTCACATTCTTCTTTCGT-3′</td>
<td>5′-CCCATTTTCCGCTGCAAAAGA-3′</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>IV: 3.66</td>
<td>8162278</td>
<td>snp_Y0218[1]</td>
<td>5′-TCCGGTTTTTGTAGTCACCTTGCT-3′</td>
<td>5′-GGCGCAAATAGCTAACCCTTTTGGT-3′</td>
<td>7.4 ± 1.1</td>
</tr>
<tr>
<td>V: 1.92</td>
<td>9384017</td>
<td>snp_E02C12[7]</td>
<td>5′-GCGCAACACTCATTACCTTCGGAATAAA-3′</td>
<td>5′-AAATCATCTTGTGCAAAGAGATCAGTGA-3′</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>V: 10.6</td>
<td>16059975</td>
<td>snp_F14H3[1]</td>
<td>5′-GTCGCTGTCGTCGCTGTCGTTA-3′</td>
<td>5′-GACCAGCAGAATCTAGCTGAGC-3′</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>V: 16.03</td>
<td>17621702</td>
<td>snp_Y60G8[1]</td>
<td>5′-AGCTCTAGTGACCATCCCAAAGGTA-3′</td>
<td>5′-GCTCTCAACACGCGAGAAGATTTTG-3′</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td>X: -19.01</td>
<td>848017</td>
<td>snp_F39H12[4]</td>
<td>5′-GATTTTGCACAACTTATTTGGAAGA-3′</td>
<td>5′-TCTCGAGGACCAGACCGAGATT-3′</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>X: -12.35</td>
<td>2918433</td>
<td>snp_F52B10[2]</td>
<td>5′-CTTATTATCTCTCGGACAGTACGAA-3′</td>
<td>5′-GACGTACGCGGCTGAGACATT-3′</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>X: -5.81</td>
<td>5179694</td>
<td>snp_T03G11[5]</td>
<td>5′-AGCTCTAGTCTGATGAGTTTCCG-3′</td>
<td>5′-TGAATAGTGGACAGTCCTATTCTTTCCG-3′</td>
<td>6.1 ± 0.3</td>
</tr>
<tr>
<td>X: 22.28</td>
<td>15724964</td>
<td>snp_ZK6624[4]</td>
<td>5′-AATCTTCAATATCACGACGTTTGGAGC-3′</td>
<td>5′-ACCCCACTATTTGAGACATATGCGG-3′</td>
<td>8.2 ± 0.1</td>
</tr>
<tr>
<td>Control —</td>
<td>F28C6</td>
<td>5′-AAATTTACATGAGATATCTACATGTCTAC-3′</td>
<td>5′-AAATCTGTGCAACAGGTTTATGCT-3′</td>
<td>N.A.</td>
<td></td>
</tr>
</tbody>
</table>

Bolded bases indicate single nucleotide polymorphism (SNP) location. ΔC<sub>T</sub>, difference in cycle; C<sub>T</sub>, cycle threshold; N.A., not applicable.

T values for DNA from 13008794

Data Analysis

To obtain mapping data in bulk segregation analysis, C<sub>T</sub> values for DNA obtained from an unselected cross between N2 and CB4856 were compared with C<sub>T</sub> values for DNA from the selected progeny from the unc-119 mutant strain crossed to CB4856. Each mapping experiment was composed of two DNA samples to determine the error range. To simplify mapping, PCR was performed with oligonucleotides specific for CB4856 SNP variants. For SNP detection in single-worm PCR, a single reaction was performed, and an allele present or absent determination was made by comparing the C<sub>T</sub> value obtained from control single N2 and CB4856 animals.

mined by the ABI PRISM 7900 as the cycle at which the ratio of SYBR Green signal to an internal ROX dye signal crosses a specified value; for this study, the ratio used was 0.2 (Figure 1A). To determine the specificity of oligonucleotide pairs, PCR was performed using N2 or CB4856 DNA. For mapping reactions, the effective concentration of genomic DNA samples was determined empirically by running PCRs on serial dilutions using the control oligonucleotide pair. A dilution was chosen that resulted in a C<sub>T</sub> value of 28–30 using standard ABI PRISM 7900 cycling conditions (1 cycle of 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, and 60 s at 60°C). For single-worm analysis, 0.6 µL single-worm lysate was used without dilution. Oligonucleotide pairs were validated for single-worm detection using control reactions with single N2 and CB4856 animals.
RESULTS AND DISCUSSION

SNP Detection Using Quantitative PCR

To determine if SNP variants could be specifically detected using unmodified oligonucleotide pairs in a fluorescence-based quantitative PCR assay, oligonucleotide pairs were designed to detect specific SNP variations between the laboratory wild-type strain of C. elegans, N2, and CB4856, a polymorphic isolate from Hawaii (3). To provide SNP detection specificity, the 3' most base pair of one oligonucleotide in each pair was designed to overlie the location of the SNP (5). Nine hundred twenty-three oligonucleotide pairs were designed in an automated fashion, following simple melting temperature and distance guidelines (see Supplementary Table S1). To test for specificity and effectiveness of the oligonucleotide design criteria, fluorometric quantitative PCRs were performed using a subset of the designed oligonucleotide pairs on DNA prepared from the N2 and CB4856 strains of C. elegans. Quantitative PCR was carried out on an ABI Prism 7900 Sequence Detection System using SYBR Green to detect amplified products. Fifteen oligonucleotide pairs were tested on homogeneous N2 DNA, a 1:1 mixture of N2 and CB4856 DNA, and homogeneous CB4856 DNA. Figure 1A shows the amplification plot for one pair of oligonucleotides specific for the CB4856 variant of a SNP located on cosmid T01A4. In this example, the amplification plot of the reaction using a 1:1 mixture of CB4856:N2 DNA crosses the detection threshold one cycle behind the set point of the reaction using an equal concentration of CB4856 DNA, indicating that the oligonucleotide pair can detect a 2-fold difference in the specific SNP variant. Of the other 14 pairs, 12 showed specific amplification to the DNA containing the SNP variant matching the variant present in the oligonucleotide (Figure 1B). Although the T01A4 oligonucleotide pair was able to detect a 2-fold difference in variant concentration, other oligonucleotide pairs were not as sensitive. In addition, although equal concentrations of DNA were used for testing all oligonucleotide pairs, the cycle at which the amplification passed the detection threshold varied between oligonucleotide pairs, reflecting sequence-specific differences in amplification kinetics. Two oligonucleotide pairs did not show discrimination between the DNA samples (Figure 1B, Y49E10 and C18H9), suggesting either that the predicted SNP is not present in the DNA samples or that the oligonucleotides can amplify both variants equally well. This result indicates that the SNP assays should be validated using homozygous DNA samples prior to use in a mapping experiment. Including the oligonucleotide pairs tested for linkage mapping (see the section entitled Linkage Mapping Using Quantitative PCR), 32/38 pairs, or 84%, demonstrated SNP specificity between N2 and CB4856 DNA. In addition, the discriminatory power of the oligo-nucleotide pairs for the different SNP variants averages 41-fold, as indicated by the average difference in C_T values between N2 and CB4856 DNA (Table 1). These results show that a high percentage of oligonucleotide pairs, designed following simple rules, can discriminate SNP variants using this quantitative PCR method.

Linkage Mapping Using Quantitative PCR

To demonstrate the utility of SNP discrimination using quantitative PCR, a simple linkage mapping experiment was carried out. A minimal set of oligonucleotide pairs were picked that provided SNP detection within the six C. elegans chromosomes (Table 1). To maximize the SNP disequilibrium resulting from linkage to an N2 mutant phenotype, oligonucleotides for CB4856-specific SNP variants were chosen. For example, the maximum...
fold-change expected from an N2-specific assay would only be 2-fold for an N2-linked trait (from unlinked to fully linked), whereas the fold-change from a CB4856-specific assay would be limited only by the specificity of the assay itself (on average 41-fold, see the following section). The minimal oligonucleotide set was validated using DNA from homozygous N2 and CB4856 DNA, and the C\textsubscript{T} difference is reported in Table 1.

To test the linkage mapping oligonucleotide set, the recessive unc-119\textit{(e2498)} allele was mapped using quantitative PCR. Homozygous unc-119\textit{(e2498)} animals were crossed to CB4856 animals, and F1 progeny were allowed to self-fertilize and produce broods. Among the F2 progeny, Unc and non-Unc animals were observed. To determine linkage of SNPs to the unc-119 locus, 50 Unc segregants in the F2 generation, which represent homozygous unc-119\textit{(e2498)} animals, were selected and allowed to reproduce en masse. DNA was prepared from this population and subjected to quantitative PCR analysis. The use of a pooled population of \textit{C. elegans} segregants to determine allele frequency has been previously described and is termed bulk segregation analysis, or BSA (3). To provide an unlinked DNA sample for comparison, N2 animals were crossed to CB4856 and DNA was prepared from unselected progeny. Quantitative PCRs were performed on DNA prepared from the Unc segregants and from the unselected N2/CB4856 cross, in quadruplicate, using the linkage oligonucleotide pairs from Table 1. Figure 2 demonstrates the results obtained. SNPs located near the known location of unc-119, III:5.4, demonstrated significant disequilibrium, with smaller error ranges. The reduction in error range compared with unlinked SNPs is due to the nonlinear relationship between the computed allele ratio and the difference in cycle, or \(\Delta C_T\), at which a signal is detected between two DNA samples. The error range in the computed allele ratio diminishes as the \(\Delta C_T\) increases between two samples. The closest SNP to unc-119, III::0.83, had a map ratio <0.2. This reflects an allele ratio of 20% CB4856 allele to 80% N2 allele at this location. Other chromosome III SNPs also show a significant bias in SNP ratio. Therefore, the predominance of the N2 SNP variants on chromosome III due to segregation of the chromosome III-linked unc-119\textit{(e2498)} allele can be detected by quantitative PCR.

To demonstrate the utility of the SNP detection method described here for fine mapping, the location of the unc-119 locus was refined by performing SNP detection in single animals representing potential recombination events. A genetic cross was performed between CB4856 animals and homozygous unc-119\textit{(e2498)}, as described before. However, single Unc F2 segregants were placed in individual wells and lysed for PCR analysis. Each adult animal provided enough material for up to eight SNP reactions. Oligonucleotide pairs were first validated on single adult animals that were either N2 or CB4856 homozygotes. A positive amplification signal for the specific variant detection typically occurred in the 30–35 cycle range (\(C_T\)), depending on the particular oligonucleotide pair, whereas typically no amplification signal was obtained for animals lacking the targeted variant. Because unc-119\textit{(e2498)} is recessive, regions that are positive for CB4856 SNP variants among Unc F2 segregants can be excluded for the unc-119 locus. Among 60 individual animals analyzed for four SNP locations, 20 represented recombinant genotypes (Figure 2B). Analysis of the recombinant genotypes indicate that regions to the right of Y47D3A at genetic position 7.12 and regions to the left of T21C12 at 2.99 can be excluded for the location of the unc-119 locus, thereby mapping unc-119 to a region between these two positions.

As noted previously, the designed oligonucleotide pairs do not consistently detect a 2-fold difference in SNP variant concentration (Figure 1B). Therefore, it is not possible to determine if an animal is heterozygous or homozygous for a particular SNP using a single detection reaction. If detection of both alleles is required in a genetic mapping strategy (e.g., determination of whether an animal is homozygous or heterozygous for a particular SNP), oligonucleotide pairs specific to each variant need be used, and two PCRs need to be carried out for each single-worm DNA preparation. This is not a drawback for mapping recessive alleles, as shown by the unc-119\textit{(e2498)} allele, as it is sufficient to determine whether the Hawaiian SNP variant is present or absent at a particular loci. However, the mapping of dominant alleles would require determination of heterozygosity versus homozygosity.

This report demonstrates SNP detection and linkage mapping in \textit{C. elegans} using automated oligonucleotide design and quantitative PCR methods. The reactions use low-cost, unmodified oligonucleotides in combination with fluorometric detection of PCR product. The utility of this method in linkage mapping was demonstrated by combining quantitative PCR with previously described genetic mapping strategies using the polymorphic strain CB4856 (3,4,6). In mapping experiments where determination of heterozygosity versus homozygosity of single animals is not essential, this method is less complex than previously described SNP mapping methods. Therefore, SNP detection by quantitative PCR provides a uniform, scalable, and automatable alternative applicable to mapping strategies in \textit{C. elegans} and other organisms.

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COMPETING INTERESTS STATEMENT

The author declares no competing interests.

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