Rapid single nucleotide polymorphism mapping in *C. elegans*

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Abstract

**Background:** In *C. elegans*, single nucleotide polymorphisms (SNPs) can function as silent genetic markers, with applications ranging from classical two- and three-factor mapping to measuring recombination across whole chromosomes.

**Results:** Here, we describe a set of 48 primer pairs that flank SNPs evenly spaced across the *C. elegans* genome and that work under identical PCR conditions. Each SNP in this set alters a Dral site, enabling rapid and parallel scoring. We describe a procedure using these reagents to quickly and reliably map mutations. We show that these techniques correctly map a known gene, *dpy-5*. We then use these techniques to map mutations in an uncharacterized strain, and show that its behavioral phenotype can be simultaneously mapped to three loci.

**Conclusion:** Together, the reagents and methods described represent a significant advance in the accurate, rapid and inexpensive mapping of genes in *C. elegans*.

Background

Single Nucleotide Polymorphism (SNP) mapping has transformed studies of genetic linkage in *C. elegans* since its introduction in 2001[1]. In SNP mapping, DNA sequence polymorphisms between the wild-type *C. elegans* strain (N2 Bristol) and a closely related strain (CB4856 Hawaiian) are used as genetic markers. Compared to other markers that have been used for genetic mapping, SNPs have two distinct advantages. First, unlike conventional marker mutations that cause visible phenotypes, SNPs in general have no associated phenotype. Thus, mutant phenotypes that are masked by conventional marker mutations, such as those with subtle behavioral defects, can be mapped using SNPs. Second, SNPs are far denser than other markers, including both visible markers and DNA polymorphisms such as Tc1 insertions. Because SNPs are approximately as dense as genes, SNP mapping can in theory provide single-gene resolution [2]. Together, these two advantages have made SNP mapping the technique of choice for many *C. elegans* researchers.

SNP mapping is usually done in two phases. The first phase, chromosome mapping, is similar to traditional two-factor mapping and seeks to identify the relevant chromosome and rough position of the gene of interest. The second phase, interval mapping, seeks to place the gene of interest in an interval between two SNPs, and can be used iteratively to fine map the gene. SNP detection in both phases is typically performed by using only SNPs that alter a restriction site, which are also known as snip-
SNPs [1]. Although other SNP detection methods have been used for mapping in
C. elegans, such as fluorescence polarimetry and indel detection [3,4], snip-SNPs are
attractive because they require low initial investment and do not require specialized
equipment. However, snip-SNP detection requires PCR amplification of the SNP region,
digestion with the appropriate restriction enzyme, and gel electrophoresis. These multiple steps can be daunting,
particularly during chromosome mapping when many
SNPs need to be assayed simultaneously.

The method described here streamlines the procedure for
detecting snip-SNPs, making faster, more efficient mapping possible. First, we identified 48 SNPs that met our
criteria (8 per chromosome). This means that every part of
the genome is linked to multiple SNPs, so that adjacent
SNPs serve as internal controls, and also that sub-chromo-
some position can be determined. Second, we simplified
the PCR step by identifying primer sets that all work at
identical amplification conditions, and that are tolerant of
being added to reactions by pin-replication. This enabled
us to quickly amplify across 48 snip-SNPs in a single 96-
well PCR plate. Third, we streamlined restriction digestion
by using only snip-SNPs that can be distinguished by a
single restriction enzyme, DraI. This enzyme is relatively
inexpensive and tolerant of PCR buffer salts. This allowed
us to perform digestion in the original PCR plate, by adding
to each well an identical digestion cocktail. Finally,
primer locations were designed so that the informative
digestion products can be resolved on an agarose gel. The
accessibility of this mapping procedure, together with its
speed, low cost, robustness, and accuracy, should make it
a preferred option for most C. elegans labs.

Results and discussion
Chromosome mapping
To facilitate mapping a mutation onto a chromosome, we
designed a set of PCR reagents based on modifications of
the principle of bulk segregant analysis described by
Wicks et al. [1]. Our primary goal was to simplify the pro-
cedure by performing all steps in a 96-well format PCR
plate, and designing each SNP reaction to be performed
under identical conditions. To design a set of primers that
then can be used for SNP mapping in a 96-well format, two
conditions must be met: the primers must all use the same
conditions for polymorphism detection, and the primers
must all use the same conditions for amplification. First,
we simplified SNP detection by using only SNPs that
could be detected using a single restriction enzyme. Since
SNPs are concentrated in non-coding A/T-rich regions of
the genome, we reasoned that good coverage would be
obtained from the enzyme DraI, which recognizes the
sequence TTT^AAA. We identified all DraI SNPs in a cus-
tom database (available as supplementary material) that
incorporated all SNPs identified by the Genome Sequenc-
ing Center, Washington U, St. Louis, MO (6,333 total
SNPs, 248 DraI SNPs in our database) [5] and by Exelixis,
South San Francisco, CA (9,295 total SNPs, 257 DraI SNPs
in our database) [3]. From among these we selected eight
candidate DraI SNPs on each chromosome that were far
enough from nearby DraI sites (typically >200 bp on one
side, >50 bp on the other) to enable detection of cleavage
at the SNP DraI site. The genetic positions of the resulting
48 DraI SNPs are shown in Figure 1.

Next, to enable simultaneous amplification of all selected
SNPs in a 96-well format, we chose primer pairs with sim-
ilar annealing temperatures and product length. The pro-
gram Primer3 [6] was used to design primers to amplify
short sequences (typically 300–500 bp) containing each
selected DraI SNP. Optimum Tm was set to 60°C. Primer
pairs were tested, and unsatisfactory pairs were redesigned
until all 48 primer pairs amplified robustly in simultane-
ous PCR reactions in a single plate. The resulting primer
sequences are shown in Table 1.

Finally, we devised a set of procedures that maximize
speed and minimize the potential for error during reac-
tion set up and gel loading (Figure 2). Our chromosome
mapping procedure begins with the same genetic

| Chromosome | SNP Positions |
|------------|--------------|
| I          | -19 -12 -6 -1 5 13 14 26 |
| II         | -18 -14 -6 1 4 11 16 22 |
| III        | -25 -19 -12 -7 -1 4 12 21 |
| IV         | -24 -16 -7 5 1 8 12 14 |
| V          | -17 -13 -3 1 6 10 13 18 |
| X          | -17 -8 -4 2 8 11 17 23 |
Table 1: DraI SNP primers, locations and band sizes. In each pair of primers, the left primer is listed first; all primer sequences are given 5’ to 3’. Interpolated genetic positions are from [9] release WS143.

| Genetic Location | Physical Location | Clone | N2 digest | CB4856 digest | Primers | Wormbase Identifier |
|------------------|------------------|-------|-----------|----------------|---------|---------------------|
| I, -19           | 169, 017         | F56C11 | 354, 146  | 500            | ATGCCAGTGATAAGGAACGGTCACCACATCCCTTGTGGAATGAA | snp_F56C11 [4] |
| I, -12           | 1,905,969        | Y71G12A | 503, 72   | 377, 126, 72  | TCGAAATCAGGGAAGTTAGTGACGTTATTTGGGGAGTGTGTTT | snp_Y71G12 [3] |
| I, -6            | 2,818,973        | W03D8  | 395, 144  | 538            | GCTTTTCACTTTTGCCTGTTGTTAAGGCGCATATACA | pkP1052 |
| I, -1            | 4,594,014        | D1007  | 325, 134, 41 | 459, 41       | AAAATATCGGAAATGTTAGGCGTTTAAATAGGTTTTG | snp_D1007 [7] |
| I, 5             | 10,722,146       | B0205  | 494       | 365, 129      | ATCTGACAAATATAGTACGTTGTAAGGGGACGTTTACG | CE1-247 |
| I, 13            | 12,047,594       | F58DS  | 445       | 295, 151      | TCCCTGGAATCCCTCAGTTGTTTACCGAGAACAG | snp_F58DS [4] |
| I, 14            | 12,729,812       | T06G6  | 236, 99, 78 | 335, 78       | TTGAAATCTTCCCTTTTAAATCTTCTGGAAGTTTTGAGT | uCE1-1361 |
| I, 26            | 14,682,016       | Y105E8B | 360, 114, 27 | 474, 27      | ATTTAATGGCAGGCTAGGTTTACCCACACATCTACCTTAC | snp_Y105E8B [3] |
| II, -18          | 176,720          | T01D1  | 263, 112  | 375            | AGTGCACCGTGCGGAGTTCTG | pkP2101 |
| II, -14          | 2,121,018        | R52    | 345       | 236, 109      | CTGTCTGCTATGAGATTTGAGTTTTGATA | snp_R52 [5] |
| II, -6           | 3,828,599        | F54D10 | 516       | 387, 129      | TGGTGAAGTCCTATCTCATCTGTTGACGGTTTATACG | pkP2103 |
| II, 1            | 9,052,466        | T24B8  | 373, 121  | 494            | TCGAAATCCCTGAACTAATCCTG | snp_T24B8 [1] |
| II, 4            | 11,827,835       | Y6D1A  | 224, 117, 124, 44 | 340, 124, 44 | TTCTCCTAAAGTCATGTTCAGCAAAGGAGGAAAC | snp_Y6D1 [1] |
| II, 11           | 12,605,350       | Y38E10A | 483       | 352, 132      | CCGAATTTCTGACTGATGCTGTAACGGAAATG | uCE2-2131 |
| II, 16           | 13,235,564       | F15D4  | 500       | 368, 132      | TCCAGGATATACATAACATAACTCTG | pkP2116 |
| II, 22           | 14,132,466       | K09E4  | 365, 119  | 484            | CACCTGCGCTTATAAGGTTTTTCTAGGTAAGGAAATGTC | CE2-215 |
| III, -25         | 939,698          | T12B5  | 206, 189  | 395            | TATCAGTAAATCCGCGTTTGAAGTTTTGATG | uCE3-637 |
| III, -19         | 1,827,732        | Y39A3CL | 342, 78, 76 | 272, 78, 76, 70 | TCCATATCTTACCTAAAAACG | uCE3-735 |
| III, -12         | 2,599,699        | Y71H2B | 368, 105  | 473            | GAGGAAATCTACACTTGGCAGTACCC | snp_Y71H2B [2] |
| III, -7          | 3,559,033        | F45H7  | 239, 85, 27 | 196, 85, 43, 27 | TTTCTGCACATCTTTTTTTCTTT | CE3-127 |
| III, -1          | 7,320,107        | F56C9  | 486       | 354, 132      | AAAACATATGCTACACACTCACC | snp_F56C9 [1] |
| III, 4           | 10,652,476       | Y39A1A | 355, 142, 30 | 497, 30      | AGGGTTAAGTACGGTTATTTGCTAATTTTATTCTTTC | snp_Y39A1 [9] |
| III, 12          | 11,656,188       | Y41C4A | 339, 156  | 495            | ATCAACCGCGACTTGCACGTACCGGTAC | snp_Y41C4 [2] |
| III, 4+2         | 13,715,622       | W06F12 | 273, 137, 78 | 200, 137, 78, 73 | AACAGATCGCAGGTTTTCCTTGTCA | uCE3-1426 |
| IV, -24          | 795,461          | F56B3  | 301, 128, 71 | 429, 71      | TGATGCTGCTGCTGCTGCTGCTG | uCE4-515 |
| IV, -16          | 1,799,032        | Y38C1BA | 187, 304  | 491            | AAGCCTGGAGGCGCGTAGA | snp_Y38C1BA [2] |
| IV, -7           | 2,761,525        | Y54G2A | 498       | 250, 248      | ACTGGCAGTACCTACGC | snp_Y54G2 [5] |
| IV, -5           | 3,347,952        | F42A6  | 295, 124  | 419            | GCTGAGTTTTTTTAGTGTGTTGTTTAT | pkP4055 |
| IV, 1            | 4,991,851        | E03H12 | 376       | 300, 76       | TGAATGGAAGCGTACAAAAAAGCCTGATTAC | pkP4071 |
manipulations as other SNP mapping protocols. Hawaiian males are crossed into the mutant strain to produce heterozygous F1 animals. Homozygous F2 animals from the heterozygous F1 animals are identified based on their mutant phenotype. At the same time, animals with a non-mutant phenotype, which are enriched for Hawaiian sequences at the locus of interest, are also isolated (Figure 2). Thirty to fifty animals of each class are combined into two tubes and lysed using detergent and proteinase K (Figure 2). These three simple steps generate a set of 96 PCR master mixes ready for the final addition of primers.

Because there are 96 separate reactions, each requiring addition of a specific primer pair, we generated a pre-arrayed set of primers, which are then added to the PCR master mixes by pin replication. Primer pairs described above are arrayed in pairs into a microtiter plate at 10 µM each primer (primers' Figure 2A), with each row containing the primer pairs for the eight SNPs along a single chromosome. Adjacent rows contain a duplicate set of primers for a particular chromosome, and the plate of primers is pin-replicated into the master PCR mix. After

Table 1: DraI SNP primers, locations and band sizes. In each pair of primers, the left primer is listed first; all primer sequences are given 5’ to 3’. Interpolated genetic positions are from [9] release WS143. (Continued)

| Chromosome | Position | Primer Name | Location | Band Size |
|------------|----------|-------------|----------|-----------|
| IV, 8      | 13,049,020 | Y57G11B    | 313, 77  | 390       |
| IV, 12     | 14,566,396 | Y57G11B    | 284, 162 | 52*       |
| IV, 14     | 16,085,085 | Y105C5B    | 241, 108 | 78, 48    |
| V, -17     | 1,773,464  | F36H9      | 307, 87  | 79        |
| V, -13     | 2,726,662  | C24B9      | 288, 167 | 455       |
| V, -5      | 4,550,757  | Y61A9LA    | 454      | 307, 147  |
| V, 1       | 7,089,411  | VC5        | 435, 70  | 300, 135  |
| V, 6       | 13,951,850 | R10D12     | 500      | 348, 152  |
| V, 10      | 16,321,481 | F57G8      | 475      | 288, 187  |
| V, 13      | 17,610,508 | Y6G8       | 282, 205 | 487       |
| V, 18      | 18,782,547 | Y17D7B     | 324, 164 | 488       |
| X, -17     | 2,065,464  | F49H12     | 540      | 321, 219  |
| X, -8      | 4,161,493  | ZK470      | 422, 72  | 40        |
| X, -4      | 5,934,688  | C46F4      | 169, 54  | 51, 35, 22|
| X, 2       | 10,637,922 | F11A1      | 409, 133 | 542       |
| X, 8       | 12,750,713 | F22E10     | 341, 126 | 467       |
| X, 11      | 13,339,566 | F46G10     | 318, 191 | 37        |
| X, 17      | 14,547,382 | T24C2      | 409, 34  | 302, 107  |
| X, 23      | 15,500,013 | H13N06     | 358, 134 | 492       |

*Y57G11B has two DraI SNPs within a single PCR product.
**dbSNP IDs are given where the SNP has been submitted to the NCBI dbSNP database [10].
***The SNP on R10D12 has not been added to wormbase. It can be found at [11].

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Procedure for chromosome mapping. (A), Method. Typically 30 mutant animals (homozygous Bristol DNA surrounding the mutation) and 30 wild-type animals (heterozygous Bristol/Hawaiian or homozygous Hawaiian DNA) are lysed in 20 µL lysis buffer. The lysate is then added to a PCR mix lacking primers, and the mix is aliquoted into every other row of a 96 well plate. Primers are added by pin replication from a master plate. Because the 8-channel pipette loads every other lane of the gel, each mutant reaction is placed next to its control. DNA ladder is typically placed in lanes 17 and 34. (B), Results from homozygous N2 Bristol and CB4856 Hawaiian genotypes. 50 Bristol adults and 50 Hawaiian adults were lysed in 20 µL lysis buffer, and used for the DNA template for the 48 PCR reactions covering all six chromosomes. Note that pure Bristol and Hawaiian DNA was used for each PCR reaction in the gel shown. When mapping a recessive mutant in the Bristol background against the Hawaiian strain, unlinked SNPs will display a 50-50 mix of Bristol bands and Hawaiian bands in both mutant and non-mutant lanes. Linked SNPs will display an enrichment of Bristol bands in the mutant lane, approaching 100% Bristol for tight linkage. The non-mutant lane will display a 2/3 to 1/3 enrichment of Hawaiian compared to Bristol DNA.
amplification, PCR products are digested in the plate with DraI in a final volume of 15 µL and loaded onto a 2.5% agarose gel using an 8-channel pipette. Because we use a gel comb with wells spaced half the distance between pipette tips of the multi-channel pipette, we can automatically load the mutant samples from the upper row and wild-type samples from the lower row for each SNP pair in adjacent wells. The resulting gel displays all 48 SNP markers, from left to right and from chromosome I to X (Figure 2B). Each mutant SNP is next to its non-mutant control, so that the whole genome can be quickly scanned for linkage.

To validate the final set of primers for chromosome mapping experiments, we used them to map dpy-5, a mutation with a well known genetic position. We crossed CB4856 Hawaiian males to a triply-marked mapping strain, EG1000 dpy-5(e61) I; rol-6(e187) II; lon-1(e1820) III. We allowed the heterozygous F1s to self, and from the F2 generation we picked 50 Dpy and 50 non-Dpy animals into separate lysis reactions. We performed chromosome mapping PCR on these lysates using primer sets from LGI and LGII (Figure 3A). As expected, we found linkage to the center of LGI and no linkage to LGII.

Interval mapping

After determining the rough position of a mutation on a chromosome using chromosome mapping, mutations can be quickly mapped to a genetic interval using the same efficiencies of the 96-well format employed in chromosome mapping. Interval mapping differs from chromosome mapping in that the genotype of individual mutant animals, rather than the genotype of pooled animals, must be determined. Also, it is necessary to assay these mutant DNAs for many SNPs within the interval for which linkage has been established. Therefore, it is most convenient to pin replicate the DNA templates, rather than the primers (Figure 4).

Briefly, we crossed Hawaiian males into our mutant strain (isolated from Bristol N2) to generate a heterozygous strain. From the progeny of these heterozygotes, we singled 96 mutant animals onto worm growth plates and allowed them to lay self-fertilized embryos. After the F3 progeny had grown to the adult stage, we washed about a quarter of the F3 progeny from the plate into individual wells of the 96-well plate (see Methods). Deriving templates from the self progeny of a homozygous mutant has the advantage of allowing each recombinant mutant to be scored as a population, rather than a single animal. Also, having additional template is convenient for additional rounds of PCR if higher resolution is desired. We found that 96-well plates containing the lysed worms can be frozen at -80°C and reused successfully after many rounds of thawing and re-freezing. If more rapid mapping was required, we found it possible to remove the single F2 animal (after it had laid sufficient embryos to ensure propagation of the strain) and to lyse it for analysis of its SNPs. Specifically, a drawn out and sealed Pasteur pipette was used as a pick to place each F2 adult individually into 5 µL of lysis buffer in a 96-well plate. The embryos on the worm plate could later be used as a source of DNA for additional mapping experiments.

Lysed DNA from each well was pin replicated into 96-well PCR plates containing complete PCR cocktail minus template. Each plate included the primer set for a single SNP. Typically we use plates representing four adjacent SNPs from the section of a particular chromosome that had shown linkage in the chromosome mapping experiments. For each plate, PCR, digestion and gel electrophoresis were performed as for chromosome mapping.

To validate the technique for mapping a mutation to an interval, we once again used the previously mapped gene dpy-5. We crossed Hawaiian males into the strain EG1000 as described above, and allowed the heterozygous progeny to self-fertilize. From the F2 progeny, we singled 48 Dpy animals onto individual plates. When these plates had starved, we washed each population of progeny into a well containing lysis buffer in a 96-well plate. The lysed DNA was assayed using three SNP primer pairs from the center of LGI (Figure 3B). Since each well contained progeny of a single F2 animal, it was possible to determine whether that animal was homozygous Bristol, homozygous Hawaiian, or heterozygous Bristol/Hawaiian at each SNP. From these data we could identify dpy-5-containing Bristol chromosomes that have recombined with Hawaiian DNA to the left of dpy-5 (recombinant types ‘a’ and ‘b’, Figure 3B and 3C), and to the right of dpy-5 (recombinant type ‘c’, Figure 3B and 3C). Keep in mind that each worm contains two dpy-5-containing chromosomes, but at regions near the mutation, usually only one is recombinant as illustrated in Figure 3C. We found, as expected, that our map data placed dpy-5 between -1 and 5 on LGI at approximately 0.3, very close to the known map position of 0.0 for dpy-5 (Figure 3C). Interestingly, one of the 48 Dpy F2 animals showed no linkage to LGI (see * in Figure 3B). The plate of worms that had been used to generate that lysate was chunked onto a new plate to verify the Dpy phenotype. Surprisingly, they were Dpy, but less so than dpy-5 homozygotes. We have observed that this Dpy phenotype segregates at a low frequency from several unrelated crosses between Hawaiian CB4856 and Bristol N2. Indeed, the plate segregated Rol-6 animals that were also Dpy. This confirms that the phenotype is not due to a dpy-5 mutation, since dpy-5 is epistatic to rol-6, but apparently the synthetic Dpy phenotype is not.
Mapping unknown mutations

To illustrate the utility of these methods we mapped a suppressor mutation of a behavioral phenotype. The map data demonstrate that these methods were able to map the original uncoordinated mutation and two other loci that synthetically suppress it in a single experiment. This strain, KY5029, was isolated in a screen for suppressors of unc-31(e928) (gift of Liakot Khan and Kouichi Iwasaki).
unc-31 encodes the *C. elegans* homolog of CAPS, a protein required for dense-core vesicle release [7]. *unc-31(e928)* mutants are very inactive – almost paralyzed – on food. The suppressor strain KY5029 moves well, in fact it is slightly hyperactive. The strain KY5029 was crossed to Hawaiian males. Heterozygous F1 progeny were singed to plates. 85 Unc-31 animals were singed from among the F2 progeny. Most of these plates of Unc-31 animals segregated active suppressed animals, demonstrating that the suppressors are recessive. Two relatively active animals from each of the 85 plates were singed. From among the 170 plates, 30 plates were found to have the hyperactive phenotype of KY5029. Animals from these 30 plates were combined and used for chromosome mapping. From the chromosome mapping experiment we found that the suppressed *unc-31* phenotype of KY5029 animals was linked to three genetic regions, on chromosomes I, II, and IV (data not shown). The region on chromosome IV contains *unc-31*, and linkage to IV is expected since Unc-31 animals were selected from among the F2 generation. Thus, the regions on chromosomes I and II must contain mutations suppressing the Unc-31 phenotype.

We found that the individual suppressor loci are weak *unc-31* suppressors on their own. From the *unc-31(e928)* plates we singed animals that were less strongly suppressed; specifically, they were slightly sluggish rather than hyperactive. Linkage to the Bristol N2 genotype in these animals was observed on chromosomes I and IV, while linkage to the Hawaiian genotype was observed on chromosome II (data not shown). Thus, chromosome I contains a novel suppressor of *unc-31* that partially suppresses the *unc-31(e928)* phenotype. Chromosome II contains a mutation that, in combination with the mutation on chromosome I, suppresses the *unc-31(e928)* phenotype to produce hyperactive worms. Further analysis determined that the suppressor on II could also partially suppress *unc-31(e928)* on its own. To simplify future mapping experiments we mapped the independent suppressing activities of the mutations on chromosomes I and II in the *unc-31(e928)* background. Both suppressors were then fine mapped using primer sets on I and II independently to the genetic intervals depicted in Figure 5A and 5B. Together, these data suggest that KY5029 contains two mutant loci in addition to *unc-31(e928)*. These complex interactions were deciphered with a minimum of time, effort, and confusion.

**Conclusion**

In summary, these methods comprise an accurate and fast technique for mapping that has advantages over both traditional mapping experiments and over other SNP mapping approaches that have been previously described. Compared to traditional mapping, this technique offers standardized, efficient mapping to small intervals for...
large numbers of mutations, such as might result from a genetic screen. In addition, it allows the mapping of subtle phenotypes (such as behavior) and complex genotypes (such as suppressor or synthetic mutations).

Compared to other SNP mapping methods, the technique described here occupies a comfortable midpoint between the simple but less efficient method described by Wicks et al. [1], and the high-throughput but complex techniques published by Swan et al. [3] and Zipperlen et al. [4]. Although the SNP detection technique described by Wicks is simple and inexpensive, it requires setting up a number of individual PCR reactions and matching the correct PCR product with the correct restriction enzyme. In the methods described here, once the primers (in chromosome mapping) or templates (in interval mapping) are arrayed into a plate, reaction components are accurately dispensed automatically and repeatably. Errors in matching restriction enzymes and buffers to primer sets have been eliminated by using DraI for all reactions. Further, we have found that assaying 8 SNPs on each chromosome means that every mutation is linked to multiple SNPs, giving a high level of redundancy. In fact, because of its cost and accuracy advantages, our technique has been successfully applied in an undergraduate teaching lab setting (M. Peters, personal communication).

The fluorescent polarimetry technique described by Swan et al., and the indel detection technique described by Zipperlen et al., enable high throughpout SNP detection in C. elegans. However, these techniques require specialized equipment (a fluorescence polarimeter or capillary sequencer) that are not accessible to every laboratory and that require significant operator knowledge. Compared to those SNP mapping approaches, the technique described here is cheaper and more accessible, since it relies on methods that most labs already use.

In this paper, we present a happy medium between previous approaches to SNP mapping in worms. We build upon the simple, inexpensive, accessible and robust restriction digestion SNP detection technique of Wicks. However our primer sets, equipment and techniques substantially reduce user effort relative to the Wicks method, and so provide the efficiency and low error rate of the Swan or Zipperlen technologies.

**Methods**

**Chromosome mapping**

Hawaiian CB4856 males were crossed into EG1000 *dpy-5(e61) I; rol-6(e187) II; lon-1(e1820) III.* Fifty Dpy animals and fifty non-Dpy animals from among the self-progeny of EG1000/CB4856 heterozygote hermaphrodites were picked into separate tubes, each containing 20 µL single-worm lysis buffer (50 mM KCl, 10 mM Tris pH 8.3, 2.5
Dra I (10 units/µL, New England Biolabs)). This digestion in the plate with the restriction enzyme 60°C, 1' 72°C, 5' 72°C. After amplification, PCR cycling conditions: 2' 94°C, 35 cycles of (15" 94°C, 45" into the master mix. PCR reactions were done using the enzyme buffer mix to each well using a multi-channel pipette. The resulting gel displays all 48 SNP reactions were incubated at 37°C at least 4 hours. Samples were then loaded onto a 2.5% agarose gel using an 8-channel pipette. The resulting gel displays all 48 SNP markers, from left to right and from chromosome I to X. Each Mutant SNP is next to its non-Mutant control, so that markers, from left to right and from chromosome I to X. The Dpy lysate DNA templates were then added to a PCR master mix containing 424 µL water, 52 µL 10X PCR buffer (10X: 22.5 mM MgCl₂, 500 mM Tris-HCl, 140 mM (NH₄)₂SO₄, pH 9.2 at 25°C), 10.4 µL 10 mM dNTPs, and 3.12 µL Taq (5 units/µL). A similar mix was made with the non-Mutant animals. 9.8 µL of the mutant mix or the non-mutant mix was aliquoted into alternate rows of a 96-well PCR plate (Figure 1A). Primer pairs were arrayed into a microtiter plate at 10 µM each primer, so that neighboring rows contain duplicate pairs, and pin-replicated into the master mix. PCR reactions were done using the cycling conditions: 2' 94°C, 35 cycles of (15° 94°C, 45° 60°C, 1° 72°C), 5° 72°C. After amplification, PCR products were digested in the plate with the restriction enzyme Dral in a final volume of 16 µL (10 µL PCR product, 4.15 µL H2O 1.6 µL 10X Dral buffer (New England Biolabs), 0.25 µL Dral (10 units/µL, New England Biolabs)). This was accomplished by adding 6 µL of the enzyme plus enzyme buffer mix to each well using a multi-channel pipette followed by brief centrifugation in a Sorval RT6000D centrifuge with an H1000B rotor. Digestion reactions were incubated at 37°C at least 4 hours. Samples were then loaded onto a 2.5% agarose gel using an 8-channel pipette. The resulting gel displays all 48 SNP markers, from left to right and from chromosome I to X. Each Mutant SNP is next to its non-Mutant control, so that the whole genome can be quickly scanned for linkage.

**Interval mapping**

PCR templates were generated by cloning mutant animals from among the self-progeny of EG1000/CB4586 F₁ hermaphrodites (described above) onto individual seeded plates. After 5 days, self progeny were washed from each plate using water (>100 worms / plate) and placed in a single well of a 96-well plate. Worms were allowed to settle to the bottom of the wells for 15° at 4°C then excess water was pipetted off to leave 45 µL in each well. The plates were frozen and stored at -80°C. The plates were thawed and 15 µL of 4X lysis buffer (200 mM KCl, 40 mM Tris pH 8.3, 10 mM MgCl₂, 1.8% IGEPA CA-630, 1.8% Tween 20, 0.04% (w/v) gelatin, 240 µg/ml proteinase K) was then added to each well to give 1X lysis buffer. The plates were covered with sealing tape and briefly vortexed to break up the worm pellet. The worms were lysed by incubation at 65°C 1 hour and 95°C 15 minutes. These PCR templates were stored frozen at -80°C and thawed prior to each use. For each PCR, each well of the 96-well plate received 9.8 µL of a PCR mix containing 8.5 µL water, 1 µL 10X buffer, 0.2 µL 10 mM dNTP, 0.02 µL each primer (100 µM), and 0.06 µL Taq (5 units/µL). Templates were then pin-replicated from the lysis plate. PCR conditions and Dral digests were the same as in chromosome some mapping.

**unc-31(e928) suppression mapping**

We cloned 85 animals with an unc-31(e928)-like phenotype from the self progeny of KY5029/CB4586 hermaphrodites. unc-31(e928) animals are lethargic and uncoordinated; however, most animals exhibit periodic moments of coordinated movement making it difficult to distinguish between plates with no suppressed progeny and plates with weakly suppressed progeny. Therefore, two animals exhibiting coordinated movement were cloned from each plate. We scored the progeny of these 170 animals and divided them into five classes: uncoordinated, sluggish yet coordinated, coordinated, hyperactive, and mixed. For the sluggish and hyperactive phenotypes, we collected animals for chromosome mapping by combining two animals from each plate. Chromosome mapping was performed as described above, suggesting the presence of two suppressors located on chromosomes I and II (data not shown). To confirm the mapping results, individual recombinants were assayed for SNPs on chromosomes I, II, and IV (data not shown). To simplify further interval mapping experiments these suppressors were crossed away form each other to generate two partially suppressed strains, EG5296 ox300; unc-31(e928) and EG5297 dpy-5(e61); ox305; unc-31(e928). For EG5297, we verified the loss of the ox300 chromosome by homoyogosing a dpy-5(e61) marker. Interval mapping of ox300 was carried out by cloning 11 unc-31(e928)-like self progeny from EG5296/CB4586 hermaphrodites. From these self-progeny 35 sluggish yet coordinated animals were cloned and assayed at SNPs flanking the suppressor (Figure 5A). ox300 is located on Chromosome I between W03D8 and B0205. ox305 was mapped by cloning 8 unc-31(e928)-like self progeny from EG5297/CB4586 hermaphrodites. From these self-progeny 21 partially suppressed animals were cloned and assayed at SNPs flanking the suppressor (Figure 5B). The suppressor in ox305 is located on Chromosome II between R52 and T10D4. Four new SNPs that are not part of the chromosome mapping set were used. Wormbase allele snp_F12B6[1] was amplified with primers 5'-caggttgttttttgcagagt-3' 5'-tttggatatcactccggcagc -3' and detected with Mf31. Wormbase allele snp_F28H1[1] was amplified with primers 5'-caggttgttttttgcagagt-3' 5'-tttggatatcactccggcagc -3' and 5'-ttttcagcactttgacagctg -3' and was detected with HpyCH4V. pkP2135 was amplified with primers 5'-tttggatatcactccggcagc -3' and detected with HpyCH4V. pkP2135 was amplified with primers 5'-tttggatatcactccggcagc -3' and detected with HpyCH4V. pkP2135 was amplified with primers 5'-tttggatatcactccggcagc -3' and detected with HpyCH4V. pkP2135 was amplified with primers 5'-ttagggactgtggcagcagt-3' and 5'-ttttcagcactttgacagctg -3' and was detected with Dra I. A polymorphism on T10D4, referenced at the web page [8] was amplified with primers 5'-gtagccctaaaaatggag -3' and 5'-accaccaacaataacttcctg -3' and was detected with Mfsel.
Abbreviations used
SNP: Single nucleotide polymorphism.

Authors’ contributions
MWD MH conceived and designed the SNP methods, carried out and supervised the experiments and drafted the manuscript. MWD wrote the scripts that extracted and formatted the SNP data. TH and SO tested SNP primers PH carried out the unc-31 mapping experiments. EMJ helped coordinate the experiments and draft the manuscript. All authors read and approved the final manuscript.

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