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Frequency and Distribution of Crossovers in *Caenorhabditis elegans* Meiosis by SNP Genotyping using Real-time PCR

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Abstract

Caenorhabditis elegans is an excellent model organism for studying meiosis. In addition to general advantages, such as a short reproductive cycle, many progenies, and a transparent body for imaging, there are six pairs of homologous holocentric chromosomes. There is strong crossover interference and regulation of crossover distribution. To measure the crossover frequency, visible marker mapping, snip-SNP mapping, and quantification of cytological markers as precursors of crossover formation have been developed. Here, we introduce a modified SNP genotyping method to measure the crossover frequency and distribution in *C. elegans* oogenesis. This method can omit the laborious steps of restriction digestion and gel electrophoresis and avoid the ambiguous judgment of uncut DNA bands. Single-nucleotide polymorphism (SNP) genotyping is performed using the Bristol/Hawaiian hybrid strain. Crossing hermaphrodites with myo-3p::GFP-expressing males enables us to focus on oogenesis. A single worm lysate produces sufficient DNA template for all six chromosomes. The 5' exonuclease-based TaqMan chemistry and SNP-specific minor groove binder (MGB) probes allow the precise detection of SNP at a genotyping rate of approximately 97.7%. We utilize the accurate detection of SNPs to measure crossover frequency in *C. elegans*. This method can also be applied to determine the crossover frequency in male or crossover-defective mutants, as well as for more specific chromosomal intervals.

Introduction

Crossover formation between homologous chromosomes is essential for the proper segregation of chromosomes and also for the generation of genetic diversity[1]. Errors in crossover formation cause aneuploidy, which can lead

to infertility, miscarriage, and birth defects[2]. Crossover frequency varies by chromosome region, mutant type, age, and sex, underscoring the need to accurately measure crossover frequency under each of these conditions[3,4,5]. The overall goal of this method is to accurately measure the crossover frequency of each chromosomal domain and thereby accurately determine the distribution of crossovers over the entire length of the *C. elegans* chromosome.

Traditionally, two-point mapping using morphological markers has been used to measure crossover frequency[6,7,8,9,10]. In *C. elegans*, dumpy, short, fat body (Dpy) and uncoordinated movement (Unc) phenotypes are often used to create chromosome maps[6]. The advantages of this method are its low cost and ability to analyze many progenies. The disadvantages of these methods include the limitation of choosing the loci of interest and the inability to use lethal progenies because their phenotypes are not visible. In order to overcome the inconvenience of site choice, Tc1 transposons have been used as markers for polymorphic sequence-tagged sites (STS)[11,12]. Tc1s can be detected using PCR and electrophoresis. While the standard Bristol N2 strain had 30 copies of the Tc1 transposon, the *C. elegans* isolates from Bergerac in France (RW7000 and DP13) had 500 copies of Tc1. Thus, the number and distribution of Tc1 transposons vary among isolates, and this is used to analyze the chromosomes of the progeny of hybrid worms between isolates by PCR. Subsequently, SNPs were also used to measure crossover frequency and distribution[13,14]. The SNP between the Bristol N2 and Hawaiian CB4856 strains appeared every 1,000 bp[15,16]. SNPs are detected as restriction fragment length polymorphisms (RFLP) using PCR, restriction digestion, and electrophoresis. Recently, small insertion/deletion (InDel) sites as well as SNPs between Bristol N2 and Hawaiian CB4856 have been proposed as more efficient markers[17]. However, measuring crossover frequency using Tc1, RFLP, and InDel requires labor-intensive processes such as PCR, restriction enzyme treatment, and electrophoresis. If each process is not optimized in a large-scale analysis, the results may be misinterpreted due to factors such as the accuracy of PCR, the type of restriction enzyme used, the efficiency of DNA cleavage, and the clarity and separation of bands during electrophoresis. In contrast, the proposed method utilizes SNP genotyping by real-time PCR to facilitate accurate and precise quantification.

Crossovers are not randomly distributed along chromosomes. In *C. elegans*, single crossovers tend to form more frequently in the arms and are suppressed in the center[3,5]. Morphological two-point mapping cannot identify where crossovers occur outside of marker genes. Conversely, SNP mapping allows for an increase in markers and has been shown to facilitate the determination of the exact region of a crossover along the entire chromosome[5,14,18]. In this study, we employed the chromosomal domain boundaries proposed by Rockman and Kruyglyak[5] and selected four SNPs for analysis: at both ends of the chromosome, and at both ends of the center/arm boundaries.

By taking advantage of the previous methods, we proposed an accurate and rapid SNP detection method by real-time PCR using TaqMan, 5' nuclease[19] to measure the crossover among the left arm, center, and right arm of each chromosome. The advantages of our method over alternative technologies, as compared to prior studies[20,21,22], are accuracy and simplicity. This method is a qPCR-applied crossover analysis method for *C. elegans* and is widely suitable for researchers studying meiosis.

Protocol

1. First genetic cross

1. Prepare mating plates that comprise normal Nematode Growth Medium (NGM) plates with a small OP50 spot (~5 mm diameter) in the 35 mm dish.
2. Add three L4 hermaphrodites of the Bristol N2 strain (or mutants with a Bristol background) and nine young adult males of the Hawaiian CB4856 strain on a mating plate (**Figure 1A**).
   NOTE: To analyze mutants with high embryonic lethality, it is necessary to use balanced mutants with both Bristol and Hawaiian backgrounds in this cross. To obtain mutants with a Hawaiian background, the Bristol mutants must be outcrossed with the Hawaiian strain at least six times until all the SNP sites of interest have a Hawaiian background. As a result, the Hawaiian mutant contains Bristol background only in the mutated region and the balancer chromosome.
3. Incubate the plate for 1.5 days at 20 °C to mate the two strains. Transfer each hermaphrodite to a new individual NGM plate with OP50.
4. Place the plates in a 20 °C incubator for 1 day to allow them to lay fertilized eggs. Transfer each hermaphrodite to a new individual NGM plate with OP50.
5. Place the plates in a 20 °C incubator for 1 day to allow fertilized eggs to be laid again. Remove the hermaphrodites. Keep the plates for 0.5 - 1 day in a 20 °C incubator to grow the progenies to L4.

2. Second cross

1. Pick six crossed-L4-hermaphrodites (Bristol/ Hawaiian hybrids) and 12-15 *ccIs4251[(pSAK2) myo-3p::GFP::LacZ::NLS + (pSAK4) myo-3p::mitochondrial GFP + dpy-20(+)] I* (TTS24) Bristol males onto a new mating plate (**Figure 1B**).
   NOTE: The ccIs4251 inserted strain is obtained from the Caenorhabditis Genetics Center, and it must be outcrossed with wild-type N2 strain more than six times.
2. Keep the plate for 1.5 days at 20 °C in an incubator to mate. Transfer each hermaphrodite to a new NGM plate with OP50.
3. Place the plates in a 20 °C incubator for 1 day to allow them to lay fertilized eggs. Transfer each hermaphrodite to a new individual NGM plate with OP50.
4. Keep the plates for 1 day in a 20 °C incubator to let them lay fertilized eggs again. Transfer each parental hermaphrodite to a PCR tube with 3 µL of lysis buffer to check if the first cross was successful.
5. Check the genotypes of the parents as a Bristol/Hawaiian hybrid by PCR in a region of chromosome V. Separate the bands by 2.5% agarose gel electrophoresis.
   1. In the SNP, WBVar01973670, the length of the PCR amplicon from the Bristol genome is 220 bp, and from the Hawaiian genome is 196 bp. If the PCR results indicate that the parents are Bristol homozygotes, this is due to self-fertilization of the first cross. Do not use the progeny of these worms for further analysis.

3. Sampling

1. Prepare Worm lysis buffer as follows: 50 mM KCL, 10 mM Tris pH 8.2, 2.5 mM $MgCl_2$, 0.45% IGEPAL CA-630 (NP-40), 0.45% Tween 20, and 0.01% gelatin. Sterilize by filtration and store at room temperature. Immediately before use, add 3 µL of 20 mg/mL proteinase K (in H_2O) to 1 mL of lysis buffer and store in aliquots at -20 °C.
2. Check the expression of the GFP at the body wall muscle of the progenies of the second cross by fluorescent dissecting microscope before adding the worms to individual PCR tubes. The magnification is 6.1- 55x, and the wavelength used is 440-460 nm.
3. Pick a GFP-positive single worm and add to each PCR tube containing 3 µL of worm lysis buffer (**Figure 1C**). Collect a total of 360 (90 x 4 biological replicates) adult male crossed progenies in each of 360 PCR tubes.
   NOTE: To avoid picking self-fertilized offspring, GFP-positive worms should be selected for the sampling. Unmated hermaphrodites younger than L4 are also available for analysis of autosomes, but because they have different numbers of X chromosomes, males and hermaphrodites must be identified after growing to L4 and sampled when analyzing the X chromosome.
4. Place the samples in a -80 °C freezer for 5 min. Samples can be stored at -80 °C until lysis can begin.
5. When analyzing mutants with high embryonic lethality, collect the dead embryos instead of live larvae to obtain unbiased results. To collect embryos, use a pipette tip with 0.5 µL of chitinase solution (20 mg/mL chitinase, 50 mM NaCl, 70 mM KCl, 2.5 mM $MgCl_2$, 2.5 mM $CaCl_2$). Collect embryos within 4 h of laying.
   NOTE: Dead embryos laid more than 4 h ago are unsuitable for PCR analysis, probably due to the degradation of genomic DNA.

4. Sample lysis

1. Set the PCR tubes in the thermal cycler at 60 °C for 1 h, followed by 95 °C for 15 min. Add 100 µL of nuclease-free water to each tube and mix well.
2. Store samples at -20 °C until starting SNP genotyping by real-time PCR.

5. SNP genotyping

1. Make a mixture as follows (**Figure 2**). In a 1.5 mL tube, add 125 µL of Master Mix, 6 µL of SNP Genotyping Assay (40x), 280 µL of Distilled water (DW), Forward primer (450 nM), Reverse primer (450 nM), Probe 1 (100 nM), Probe 2 (100 nM).
   NOTE: The probes and primers are listed in **Table 1**.
2. Mix thoroughly by pipetting several times to ensure a uniform concentration. Add 4 µL of the mixture to each well of a 96-well PCR plate on the support base with a 12-channel micropipette.
3. Add 1 µL of lysate containing each genomic DNA to each well and mix by pipetting several times.
4. Prepare positive controls, i.e., Bristol homozygotes, Hawaiian homozygotes, and Bristol/Hawaiian hybrids, and three no-template controls as negative controls (**Figure 3A**).
5. Seal the plate with an optical adhesive film. Press the film focusing on the rims of each well using an applicator.
6. Centrifuge the plate briefly to collect the liquid at the bottom and remove air bubbles from the liquid.

7. Load the plate onto the real-time PCR instrument. Click the **Create New Experiment** button in the Software.
8. In the Properties setting, set Instrument type to QuantStudio 1 System, Block type to 96-well 0.2 mL Block, Experimental type to Genotyping, Chemistry to TaqMan Reagents, and Run mode to Fast.
9. In the Method setting, set Volume to 5 µL (ensuring that the correct reaction volume matches the setup), Cycle Count to 40 cycles (default). Set the temperature and time settings as follows: Pre-Read Stage at 25 °C for 30 s, Hold Stage (enzyme activation, hot start) at 95 °C for 20 s, PCR Stage at 95 °C for 1 s, 60 °C for 20 s, and Post-Read Stage at 25 °C for 30 s.
10. In the Plate setting, match the positions of the samples and controls to each well on the software screen.
11. Save the Experiment in a USB device, load it into the real-time PCR instrument, and click the **Run** button.

6. Data analysis

1. Save the results to a USB and transfer them to a computer for data analysis.
2. Analyze Real-Time delta Rn (dRn) Data using the formula below, where Rn (normalized reporter) is the fluorescence signal from the reporter dye, VIC and FAM, normalized by dividing by the fluorescence signal of the passive reference dye, ROX.
   dRn = Rn - baseline
3. Review the allelic discrimination plot.
4. Confirm the separation of clusters (**Figure 3B**). In the allele discrimination plot, Bristol homozygotes are grouped in the lower right corner, Bristol/Hawaiian hybrids are grouped in the upper right corner, Hawaiian homozygotes are grouped in the upper left corner, and negative controls are grouped in the lower left corner. Use the following color codes:
   Red: Bristol allele
   Blue: Hawaiian allele
   Green: Heterozygous
   Black: Negative control
5. Export the results to a spreadsheet file and assemble the genotyping data from all four SNP positions. If there are undetermined calls in four consecutive positions, exclude the data from the N-value. Use 90 samples per plate for analysis.
   1. Identify the crossovers as follows: If the SNP at position #1 is a Bristol/Hawaiian hybrid, and positions #2-#4 are all Bristol homozygotes [B/H]-[B/B]-[B/B]-[B/B] or position #1 is a Bristol homozygote and position #2-#4 are all Bristol/Hawaiian hybrids [B/B]-[B/H]-[B/H]-[B/H], these results indicate a single crossover between positions #1 and #2 (somewhere on the left arm of the chromosome). Similarly, in the case of [B/H]-[B/H]-[B/B]-[B/B] or [B/B]-[B/B]-[B/H]-[B/H], a single crossover occurs between #2 and #3 (somewhere in the central region of the chromosome), and in the case of [B/H]-[B/H]-[B/H]-[B/B] or [B/B]-[B/B]-[B/B]-[B/H], a single crossover occurs between #3 and #4 (somewhere on the right arm of the chromosome).
      NOTE: There are six patterns of double crossovers. Left and right: [B/B]-[B/H]-[B/H]-[B/B] and [B/H]-[B/B]-[B/B]-[B/H], left and center: [B/B]-[B/H]-[B/B]-[B/B] and [B/H]-[B/B]-[B/H]-[B/H], center and right: [B/B]-[B/B]-[B/H]-[B/B] and [B/H]-[B/H]-[B/B]-[B/H].

7. Statistical analysis

1. Insert the values into the SPSS Statistics software (IBM). For the comparison of the crossover distribution at the left arm, the central region, and the right arm of the chromosomes, insert 82 chromosomes with a crossover at the left arm, 20 chromosomes with a crossover at the central region, and 68 chromosomes with a crossover at the right arm.
2. Perform ANOVA and Bonferroni correction and determine statistical significance using a two-tailed p-value. Perform as many comparisons as required, such as left arm versus center or WT versus mutant.

Representative Results

We detected the four SNP sites on chromosome II in *C. elegans* by SNP genotyping assay to quantify crossover formation per full length of the chromosome, in the left arm, in the central region, and in the right arm. The detection of SNPs is performed according to the manufacturer's instructions. The master mix contains Taq DNA Polymerase, Heat-labile uracil-N-glycosylase (UNG), dNTP with dUTP, and Passive reference ROX dye. Taq DNA polymerase is activated at 95 °C, enabling the inhibition of non-specific amplification at low temperatures. UNG and dUTP allow for the degradation of previously amplified PCR products, preventing carryover contamination and potential false positives[23].

SNP-specific probes and PCR primer sets were generated by Thermo Fisher Scientific based on 1 kb of genomic DNA sequence containing the SNP site (**Table 1**). The 5' end of the Bristol probe is labeled with VIC dye and the 3' end is conjugated by non-fluorescent quencher (NFQ) and minor groove binder (MGB). The MGB allows the genome and probe to be annealed at a high melting temperature, reducing the distance between the fluorescent reporter and the NFQ and increasing the efficiency of FRET quenching[24].

When DNA synthesis from the forward primer reaches the VIC dye, the 5' exonuclease activity of the DNA polymerase removes the VIC dye from the probe, causing fluorescence emission (**Figure 2B**). On the other hand, the Hawaiian probe with FAM dye conjugated to the 5' end cannot completely anneal to the Bristol SNP due to a base mismatch, and the Hawaiian probe itself is removed when DNA polymerase encounters the probe (**Figure 2C**). FAM fluorescence is quenched by an NFQ. As a result, only VIC emits fluorescence, and the Bristol allele is determined. Similarly, FAM fluorescence is detected in the Hawaiian genome samples, and both VIC and FAM fluorescence are detected in the Bristol/Hawaiian hybrid samples.

According to Rockman and Kruglyak[5] and Barnes et al.[3], in a constant recombination frequency region, the recombination frequency between any two points in the region is constant. It can be roughly divided into three chromosomal domains: left arm, center, and right arm. For example, the crossover frequency of the left arm of chromosome II is 4.92 cM/Mb[5]. The crossover frequency of the central region is 1.33 cM/Mb[5]. The crossover frequency of the right arm is 8.47 cM/Mb[5]. We selected four SNP sites to divide chromosome II, depending on the constant recombination rate within each of the domains that can divide the chromosomal locus into the left arm, center, and right arm[5] (**Figure 4A**). SNPs were selected using the Wormbase, and the left-most SNP with specificity to the adjacent sequence is WBVar00095914 (T/C), located at the base 943 (**Figure 4A**). The SNP at the threshold of the left arm/center region is WBVar00171785 (C/G), located at the base 4,876,037. The SNP at the threshold of the center region/right arm is WBVar00176517 (C/T), located

at the base 12,019,914. The right-most SNP with specificity to the adjacent sequence is WBVar00095914 (A/T), located at the base 15,278,485. SNP detection was performed using real-time PCR. VIC-conjugated SNP probes are comprised of nearly 10 bases flanking the sequence of the SNP. It hybridizes the SNP site[19].

We collected a total of 360 F2 crossed worms for analysis (90 progenies x four plates). The genotyping ratios of the four probes used in this study are as follows: Probe 1; 98.3% (354/360), probe 2; 97.5% (351/360), probe 3; 97.5% (351/360), probe 4; 97.2% (350/360), total 97.7% (1406/1440). This genotyping rate was higher than that of our snip-SNP genotyping before[21]. The regional crossover frequencies in the left, center, and right arms were 23.9, 5.8, and 19.8 cM, respectively (**Table 2**; **Figure 4B**). The results are reproducible as data estimated from past papers (22.5, 9.5, 21.9 cM[5]; 23.9, 4.6, 22.0 cM[3]). The total map units of chromosome II were 49.6 cM, and double crossovers were not detected in this analysis (**Table 2**), thus confirming that single crossover formation in the bivalent occurs during *C. elegans* oocyte meiosis[3,6,25,26,27,28]. The crossover frequency is higher in the arm regions and lower in the central region of all chromosomes in *C. elegans*[3]. This tendency is weaker in X chromosomes than in autosomes[5]. We calculated the p-value of the crossover frequency for each chromosomal region using ANOVA with Bonferroni correction. As expected, we detected a significant difference between the crossover frequencies of the arms and center ($p < 0.001$; **Figure 4B**). No significant differences were detected between the left and right arms (**Figure 4B**). Although the pairing center is located in the subtelomeric region of the left arm of chromosome II, where ZIM-1 binds and promotes tethering of the pairing center to the nuclear membrane[29], crossovers are also formed in a similar ratio on the other side of the pairing center.

This measurement is used to compare the crossover frequency between the wild type and recombination-defective mutants. To date, it has been difficult to measure crossover frequency in recombination-defective mutants because living progenies are rare in these mutants. This precise detection of SNPs will allow us to determine abnormalities in the number and distribution of crossover formations in various kinds of mutants and various physiological conditions.

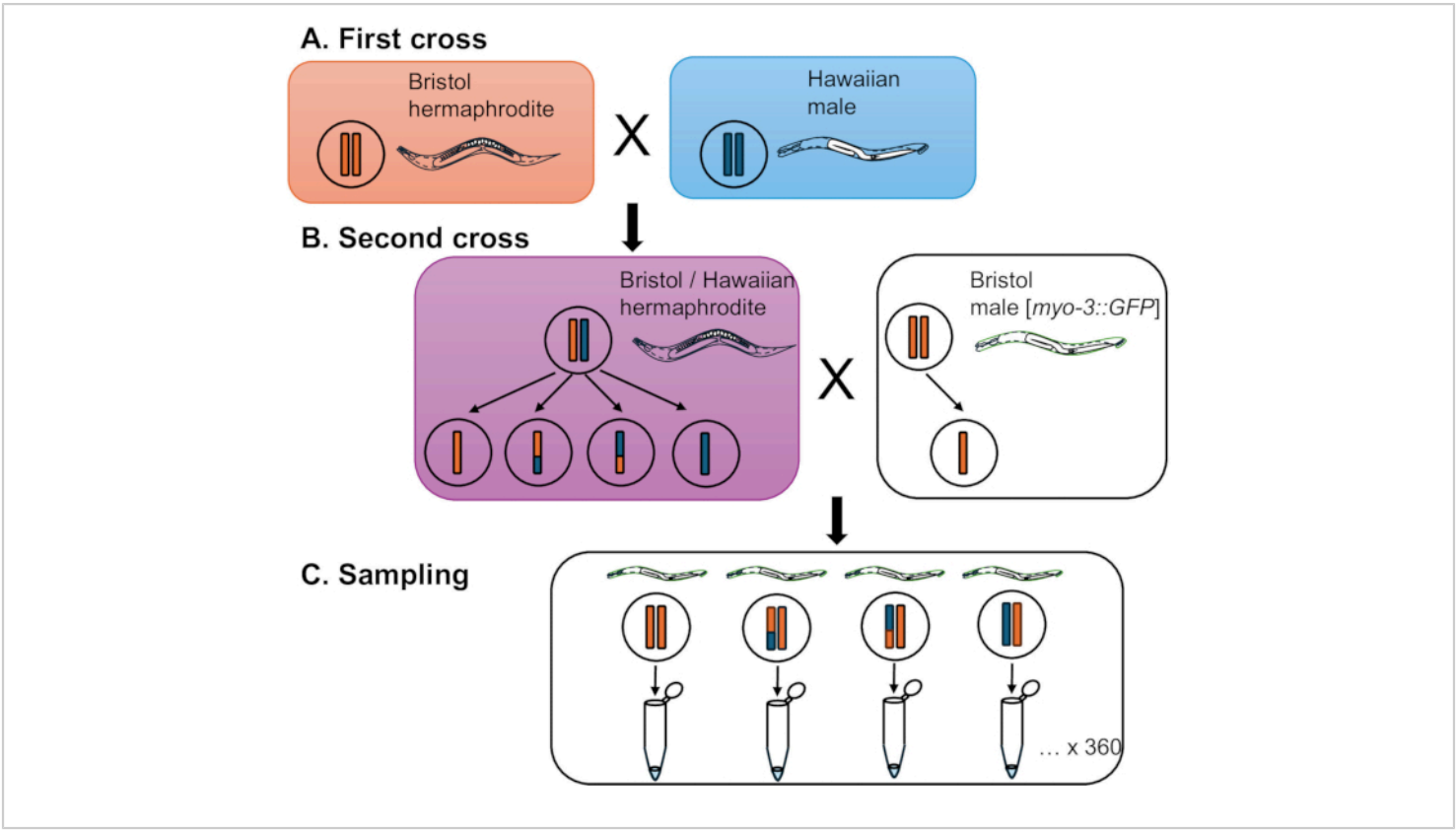

Figure 1: Procedure for genetic crosses to obtain genomic DNA samples for SNP genotyping. (**A**) First genetic cross. Bristol hermaphrodites cross with Hawaiian males. The origin of the chromosomes is indicated in a circle. Orange rectangles are chromosomes from the Bristol strain. Blue rectangles are chromosomes from the Hawaiian strain. (**B**) Second genetic cross. Bristol/Hawaiian hybrid hermaphrodites cross with *myo-3p::GFP* Bristol males. Bristol/Hawaiian hybrid hermaphrodites produce oocytes containing recombinant chromosomes during meiosis. (**C**) Sampling of crossed progeny (GFP-positive) in an individual PCR tube. Each PCR tube contains lysis buffer. Please click here to view a larger version of this figure.

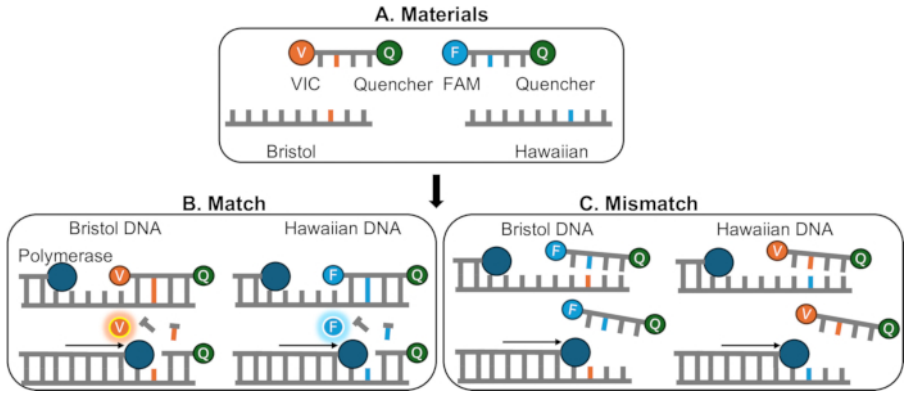

Figure 2: Molecular mechanisms of SNP genotyping chemistry. (**A**) Materials used for the 5' nuclease assay. The probe for detecting Bristol SNP is designed as 5' VIC fluorescence dye conjugation and 3' Quencher conjugation. The Hawaiian probe is conjugated to the FAM fluorescence dye at the 5' end. A minor groove binder (MGB) is added at the 3' end of the probe to increase the annealing temperature, allowing the probe length to be shortened (not shown). (**B**) Probes matched the SNP sites. Fluorescence is inhibited when the probes anneal to the SNP site, and Taq DNA polymerase can release the fluorescence from the probes through its 5' exonuclease activity. (**C**) Probes mismatched to the SNP sites. DNA polymerase cannot detach fluorescence from the probe. The quencher inhibits fluorescence in the probe. Please click here to view a larger version of this figure.

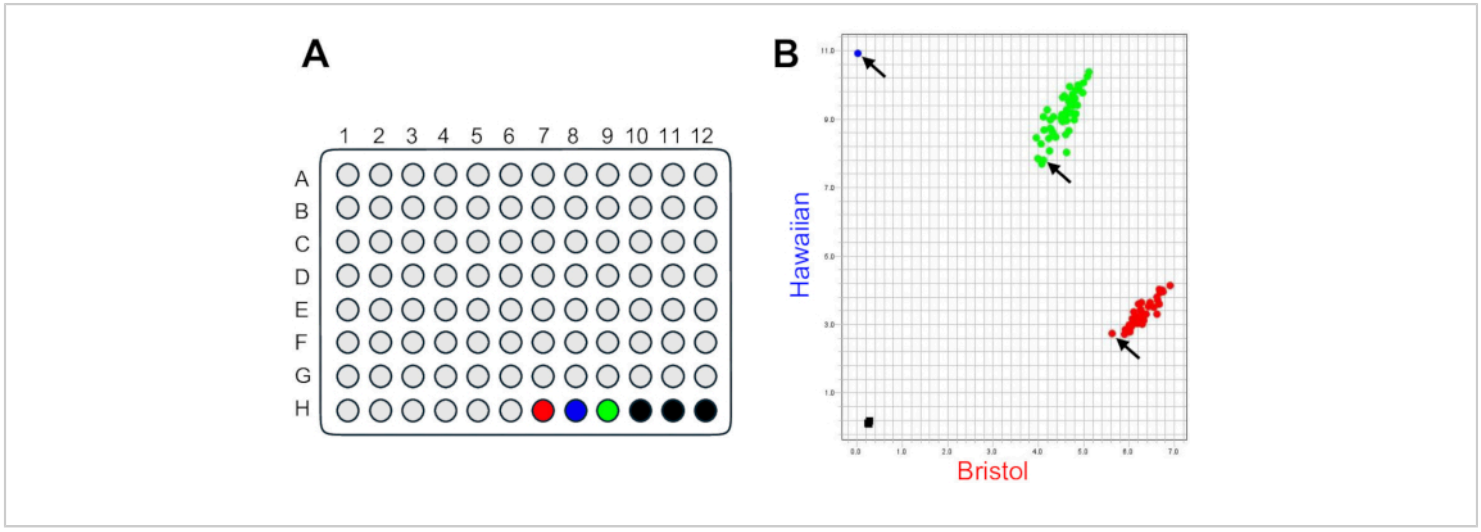

Figure 3: Example of a PCR plate and allele discrimination plot. (**A**) PCR plate format. A total of 90 progenies are analyzed in PCR tubes (gray circles). Red, Bristol homozygote control. Blue, Hawaiian homozygote control. Green, Bristol/Hawaiian heterozygote control. Black, non-template negative controls. (**B**) Allelic discrimination plot for the SNP (WBVar00171785) on chromosome II. Red, Bristol homozygotes. Blue, Hawaiian homozygote. Green, Bristol/Hawaiian heterozygotes. Black, negative controls. Arrows indicate the plots of each control. Please click here to view a larger version of this figure.

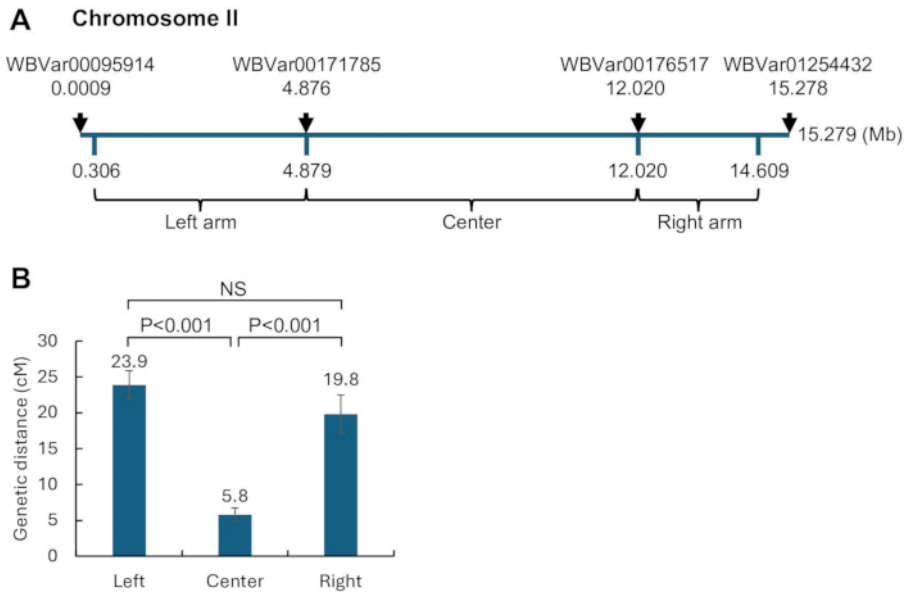

Figure 4: Crossover frequency and distributions on chromosome II in *C. elegans* oogenesis. (**A**) Boundaries of chromosome II and the sites of SNP analyzed in the study. Chromosome boundaries were determined according to Rockman and Kruglyak[5]. (**B**) The Crossover distribution on chromosome II. A total of 343 chromosomes were analyzed. Statistical analyses were performed using ANOVA with Bonferroni correction. Error bars indicate standard error. Please click here to view a larger version of this figure.

Table 1: List of SNPs, primers, and probes used in this study. Please click here to download this Table.

Table 2: The entire dataset of crossover analysis. Please click here to download this Table.

Discussion

Here, we have introduced a method for measuring the crossover frequency and distribution in *C. elegans* meiosis based on SNP genotyping of crossed progenies from the Bristol/Hawaiian hybrid species. While the conventional snip-SNP method (RFLP) requires gel electrophoresis after PCR, and the detection of DNA bands requires a gel documentation system[14,18], SNP genotyping by the 5' nuclease assay using real-time PCR does not require a laborious electrophoresis procedure.

Critical steps in the method

This protocol involves several critical steps. If the mutants to be analyzed are created in the Bristol strain, their SNPs should be replaced with Hawaiian backgrounds. Normally, outcrossing the Bristol mutants with the Hawaiian wild type more than six times helps to completely replace the background. Precise genetic crosses are essential for the detection of crossovers. The genotype of the Bristol/Hawaiian hybrids is confirmed using PCR. We use a small deletion in chromosome V. After the second cross, the crossed progenies are judged based on the expression of GFP in the body wall muscles. For the double check, picking the

male progeny allows us to ensure that the sperm is derived from males. The construction of primers and probes is also important for the precise detection of target SNPs. To select the SNP marker, we check the homology among the genomes. A 50-100 bp amplicon should be unique.

Modifications and troubleshooting of the method

As the number of SNP positions investigated increases, it should be possible to identify the presence of multiple crossovers and a more detailed distribution under strains or conditions that exhibit the hyper-recombination phenotypes. In cases where the number of progenies is small, it was difficult to analyze using the conventional method of analyzing live worms. However, with the method we propose here, dead embryos would also be useful for genome analysis. In such a case, the DNA sequence of GFP or the expression of GFP in the progeny should be checked using real-time PCR.

Limitations of the method

With this method, it is not possible to detect the breakpoint of the crossover at the nucleotide level. High-density SNP genotyping[5], microarray analysis of *S. cerevisiae*[30], whole-genome sequencing of *S. cerevisiae*[31], and whole-chromosome SNP sequencing of *Drosophila*[32] overcome this problem. However, high-throughput methods are still expensive to implement. Therefore, improvements in low-cost sequencing technologies are required.

Significance of the method with respect to existing/ alternative methods

We employed real-time PCR to detect SNPs as an alternative to RFLP. The latter have been utilized generally for the mapping of crossovers in *C. elegans*[13,22,28,33,34]. The significant point regarding this method is that it skips restriction digestion and electrophoresis. Determination of the origin of the amplicons is more precise than that of the DNA bands in the gel.

Quantification of the crossover designation factor (COSA-1, MSH-4/5, and ZHP-3) also allows for estimating the number of crossovers in late prophase of meiosis I[35,36]. It is easy to quantify the crossover designation using GFP conjugation and immunofluorescence of the crossover designation factors. However, poor antibody quality and background staining lead to erroneous counts of actual foci. Importantly, the designation itself does not imply a crossover outcome. For example, Holliday junction resolvase-defective mutants showed six ZHP-3 foci but reduced crossover frequency due to unresolved Holliday junctions after crossover designation[21,22]. Furthermore, although not normally formed in wild-type, class II crossovers are not marked by crossover designation factors[10,37,38,39,40]. Consequently, studies analyzing class II crossovers should not use the quantification of the crossover designation factors as the total number of crossovers. Therefore, crossover mappings of progenies or gametes are essential for quantifying all crossover events during meiosis. The method proposed in this study can be useful for any type of model organism that is isolated with SNPs other than the standard strain.

Disclosures

The authors declare no conflicts of interest.

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