FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68183

Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20795863

Title: Frequency and Distribution of Crossovers in *Caenorhabditis elegans* Meiosis by SNP Genotyping Using Real-Time PCR

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   No

   Leika S9, Nikon SMZ745, Olympus SZ61

   SCOPE shots: 2.1.2, 2.3.1, 2.4.2., 2.5.2, 2.7.1

   Videographer: Please film the above-mentioned shots using the scope kit

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, done**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 18
Number of Shots: 44 (10 SC, 5 Scope)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Akihide Onishi:** Our research focuses on the regulation of meiotic recombination in *Caenorhabditis elegans*. The question we are trying to answer is how the frequency and distribution of crossovers are regulated at the molecular level during meiosis.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

Videographer's NOTE: Use Take 2 (shot last)

What technologies are currently used to advance research in your field?

1.2. **Miku Koishihara:** SNP genotyping is one of the primary methods for measuring crossover frequency in *C. elegans*. It is commonly referred to as snip-SNP, including PCR, restriction digestion, and gel electrophoresis.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*
Videographer's NOTE: Use Take 4

What are the current experimental challenges?

1.3. **Akari Hirose:** This kind of multi-step approach to SNP genotyping is labor intensive and sometimes inaccurate. Therefore, our current experimental challenge is to modify it into simple and accurate method.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*
Videographer's NOTE: Use Take 1

What advantage does your protocol offer compared to other techniques?

1.4. **Takamune Saito:** Our method is a qPCR-based approach for detecting meiotic crossover recombination. It can be widely applied in genetic mutants, different sexes, and various growth conditions as well.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1.1*

Videographer's NOTE: Use Take 2

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Facilitating Crosses and Sampling the Worms

Demonstrator: Miku Koishihara

2.1. To begin, obtain the required hermaphrodites for the procedure **[1]**. Place three L4-stage hermaphrodites from the Bristol N2 strain, or from mutants on a Bristol background **[2]**, along with nine young adult Hawaiian CB4856 *(C-B-48-56)* males onto a mating plate **[3]**.

2.1.1. WIDE: Talent placing the dish with the hermaphrodites on the work bench. **Videographer's NOTE:** Use Take 1

2.1.2. SCOPE: using a worm pick to place three Bristol hermaphrodites onto the surface of a mating plate. ***Videographer: Please film the SCOPE shots using the scope kit***

2.1.3. Close up of the plate after adding all worms.
Videographer's NOTE: Use Take 1

2.2. Incubate the mating plate for 1.5 days at 20 degrees Celsius to allow mating between the two strains **[1]**. After incubation, transfer each hermaphrodite to a new individual nematode growth medium plate seeded with OP-50 bacteria **[2-TXT]**.

2.2.1. Talent placing the mating plate into a 20 degree Celsius incubator.
Videographer's NOTE: Use Take 3

2.2.2. Talent transferring individual hermaphrodites from the mating plate to separate NGM plates using a worm pick. **TXT: Incubate again for laying eggs and growth of progeny**

Videographer's NOTE: Use Take 1

2.3. For the second cross, pick six L4-stage crossed hermaphrodites that are Bristol-Hawaiian hybrids **[1]** and transfer them to a new mating plate **[2]** along with 12 to 15 TTS24 *(T-T-S-24)* Bristol males carrying the ccIs4251 *(C-C-I-S-42-51)* transgene array **[3]**.
NOTE: VO added for the extra shot

2.3.1. SCOPE: picking hybrid hermaphrodite using a worm picker.

Videographer's NOTE: shot twice, **Take 1 is end slate, misslated as 2.3.2**

Take 2 started off zoomed in to show the inside of the worm, then zoomed out in order to show moving to another plate

2.3.2. Talent placing the worm on a fresh mating plate
Videographer's NOTE: 2.3.2 filmed together with 2.3.1

Added shot: 2.3.3 scope: picking ccIs4251 male using a worm picker, and transfer it to the meeting plate

Videographer's NOTE: Use Take one - a little blurry – slated, but author likes this one best
Take two - less blurry - no slate

2.4. Incubate the plate for 1.5 days at 20 degrees Celsius to enable mating **[1]**. After incubation, transfer each hermaphrodite to an individual nematode growth medium plate containing OP50 **[2]**.

2.4.1. Talent placing the second mating plate into the 20 degree Celsius incubator.

Videographer's NOTE: Use Take 1

2.4.2. SCOPE: transferring hermaphrodite to fresh NGM plates using a worm pick.

Videographer's NOTE: Use Take 1

2.5. Incubate the new plate at 20 degrees Celsius for 1 day to allow the hermaphrodites to lay fertilized eggs **[1]**. After 1 day, transfer each hermaphrodite again to a new individual plate with OP50 **[2-TXT]**.

2.5.1. Talent placing the plates into the incubator.
Videographer's NOTE: Use Take 1

2.5.2. SCOPE: transferring hermaphrodites to a fresh round of NGM plates. **TXT: Incubate again for laying eggs and growth of progeny**
Videographer's NOTE: Use Take 1, slated

2.6. Check the genotypes of the parental worms as Bristol or Hawaiian hybrids using PCR in a region of chromosome 5 **[1]**. Separate the amplified bands by performing electrophoresis on a 2.5 percent agarose gel **[2]**.

2.6.1. Talent loading PCR tubes into a thermal cycler.
Videographer's NOTE: Use Take 1

2.6.2. Talent loading the samples on a 2.5 percent agarose gel.

Videographer's NOTE: Use Take 1 wide, Take 2 close up

2.7. After checking GFP expression, pick a single GFP-positive worm and place it in a PCR tube preloaded with 3 microliters of worm lysis buffer **[1]**. Then, place the tube in a minus 80-degree Celsius freezer for 5 minutes before lysis **[2-TXT]**.

2.7.1. SCOPE: transferring a single worm into a PCR tube containing worm lysis buffer.

Videographer's NOTE: Use 2.7.1 take 1 on scope (long video, good part around 1.48 timestamp)

2.7.1a on camera to show PCR

2.7.1b scope close up of the inside of the tube (shaky)

2.7.2. Talent placing labeled PCR tubes containing worms into the negative 80 degree Celsius freezer. **TXT: Lysis: 60 °C (1 h), followed by 95 °C (15 min)**

Videographer's NOTE: Use take 2

2.8. For lysis, set the PCR tube in the thermal cycler and run the program at 60 degrees Celsius for 1 hour, followed by 95 degrees Celsius for 15 minutes **[1]**. After the thermal cycling, add 100 microliters of nuclease-free water to each tube and mix thoroughly **[2]**.

2.8.1. Talent placing PCR tubes into the thermal cycler and initiating the temperature program. **Videographer's NOTE**: Use Take 1

2.8.2. Talent adding nuclease-free water to each PCR tube and mixing by pipetting. **Videographer's NOTE:** Use Take 2

3. Single Nucleotide Polymorphism (SNP) Genotyping

Demonstrator: Akihide Onishi

3.1. Now, prepare the mixture containing the buffer, probes and the primers **[1]**.

3.1.1. TEXT ON PLAIN BACKGROUND:

- 125 µL of Master mix
- 6 µL of SNP genotyping assay mix (40x)
  - Forward primer (450 nM) and reverse primer (450 nM)
  - Probe 1 (100 nM) and probe 2 (100 nM)
- 280 µL of Distilled water (DW)

3.2. Mix the solution thoroughly by pipetting several times **[1]**. Using a 12-channel micropipette, dispense 4 microliters of the mixture into each well of a 96-well PCR plate placed on the support base **[2]**.

3.2.1. Talent pipetting the reaction mixture up and down to mix thoroughly.

3.2.2. Talent using a 12-channel micropipette to dispense the solution into a 96-well plate.

Videographer's NOTE: Use shot together take 1

3.3. Next, add 1 microliter of lysate containing genomic DNA to each well **[1]** and pipette the contents several times to mix thoroughly **[2]**.

3.3.1. Talent adding lysate into the wells using a micropipette.

3.3.2. Talent mixing the contents in each well by pipetting up and down.

Videographer's NOTE: Use Shot together take 1

3.4. Seal the plate with an optical adhesive film **[1]**. Use an applicator to press the film down firmly, especially around the rims of each well **[2]**.

3.4.1. Talent placing an adhesive film over the 96-well plate.

3.4.2. Talent pressing the film over each well using an applicator.

Videographer's NOTE: Use Shot together take 1

3.5. Centrifuge the sealed plate briefly to collect the liquid at the bottom and eliminate any air bubbles **[1]**.

3.5.1. Talent placing the sealed 96-well plate in a centrifuge.

Videographer's NOTE: Use Take 1

3.6. Now, load the plate onto the real-time PCR instrument **[1]**. On the software screen, click on the **Create New Experiment** button **[2]**.

3.6.1. Talent loading the plate into the real-time PCR machine.

Videographer's NOTE: Use Take 1

3.6.2. SCREEN: 3.6.2.-02-05.mp4 00:00-00:06

3.7. After adjusting the settings, click on the **Run** button to initiate the protocol **[1]**.

3.7.1. Shot of **Run** button being clicked on touchscreen.

4. Real-Time PCR Data Analysis

Demonstrator: Akari Hirose

4.1. Review the allelic discrimination plot generated from the data to visualize genotype groupings **[1-TXT]**.

4.1.1. SCREEN: 4.1.1.mp4 00:07-00:20. **TXT: Confirm that the clusters are distinctly separated on the plot**

4.2. Identify the genotypes based on their position for Bristol homozygotes **[1]**, Bristol-Hawaiian hybrids **[2]**, Hawaiian homozygotes **[3]**, and negative controls **[4]**. Note the applied color codes of Red for Bristol, Blue for Hawaiian, Green for heterozygous, and Black for negative control **[5]**.

4.2.1. SCREEN: 4.2.1.,-4.2.2.,-4.2.3.,-4.2.4.,-4.2.5..mp4 00:06-00:09.

4.2.2. SCREEN: 4.2.1.,-4.2.2.,-4.2.3.,-4.2.4.,-4.2.5..mp4 00:13-00:17.

4.2.3. SCREEN: 4.2.1.,-4.2.2.,-4.2.3.,-4.2.4.,-4.2.5..mp4 00:19-00:23.

4.2.4. SCREEN: 4.2.1.,-4.2.2.,-4.2.3.,-4.2.4.,-4.2.5..mp4 00:28-00:30.

4.2.5. SCREEN: 4.2.1.,-4.2.2.,-4.2.3.,-4.2.4.,-4.2.5..mp4 00:37-00:42.

4.3. Finally, examine the SNP patterns to identify the crossovers **[1]**. For instance, [B/H]-[B/B]-[B/B]-[B/B] *(Bristol Hawaiian heterozygote, Bristol homozygote, Bristol homozygote, Bristol homozygote)* indicates a single crossover between positions 1 and 2 **[2]**.

4.3.1. SCREEN: 4.3.1.mp4 00:08-00:13.

4.3.2. SCREEN: 4.3.2..mp4 00:18-00:26

Results

5. Results

5.1. Chromosome II *(2)* was divided into three regions, the left arm, center, and right arm using four selected single nucleotide polymorphism markers **[1]**.

5.1.1. LAB MEDIA: Figure 4A. *Video editor: Sequentially Highlight the three labeled sections—"Left arm", "Center", and "Right arm".*

5.2. The crossover frequency in the left arm of chromosome II was significantly higher at 23.9 centimorgans **[1]** compared to the center region at 5.8 centimorgans **[2]**.

5.2.1. LAB MEDIA: Figure 4B*. Video editor: Highlight the tall blue bar labeled "Left*".

5.2.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the bar labeled "Center*".

5.3. The crossover frequency in the right arm was also significantly higher at 19.8 centimorgans **[1]** compared to the center region **[2]**.

5.3.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the blue bar labeled "Right*" .

5.3.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the bar labeled "Center*".

5.4. No significant difference was observed between the crossover frequencies of the left and right arms **[1]**.

5.4.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the "NS" annotation at the top and the bars "Left" and "Right".*

1. hermaphrodite

- Pronunciation link: https://www.merriam-webster.com/dictionary/hermaphrodite
- IPA: /hər-ˈmaf-rə-ˌdīt/
- Phonetic: hur-MAF-ruh-dite

2. nematode

- Pronunciation link: https://www.merriam-webster.com/dictionary/nematode
- IPA: /ˈnə-mə-ˌtōd/
- Phonetic: NEM-uh-tohd

3. agarose

- Pronunciation link: https://www.merriam-webster.com/dictionary/agarose
- IPA: /ə-ˈge-rōs/
- Phonetic: uh-GA-rose

4. electrophoresis

- Pronunciation link: https://www.merriam-webster.com/dictionary/electrophoresis
- IPA: /ə-ˌlek-trō-fə-ˈrē-səs/
- Phonetic: uh-LEK-troh-fuh-REE-sis

5. ccIs4251

- Pronunciation link: No confirmed link found
- IPA: /si si aɪ es fɔr tu faɪv wʌn/
- Phonetic: see-see-I-es-four-two-five-one

6. SNP

- Pronunciation link: No confirmed link found (acronym)
- IPA: /ɛs-ɛn-pi/
- Phonetic: S-N-P

7. genotyping

- Pronunciation link: https://www.merriam-webster.com/dictionary/genotype (for base)
- IPA: /ˈdʒē-noʊ-ˌtī-pɪŋ/
- Phonetic: JEE-no-type-ing

8. transgene

- Pronunciation link: https://www.merriam-webster.com/dictionary/transgene
- IPA: /ˈtranz-ˌjēn/
- Phonetic: TRANZ-jeen