In sexually reproducing organisms, the formation of healthy gametes (sperm and eggs) requires the proper establishment and release of meiotic sister chromatid cohesion (SCC). SCC tethers replicated sisters from their formation in premeiotic S phase until the stepwise removal of cohesion in anaphase of meiosis I and II allows the separation of homologs and then sisters. Defects in the establishment or release of meiotic cohesion cause chromosome segregation errors that lead to the formation of aneuploid gametes and inviable embryos. The nematode *C* [illegible] is an attractive model for studies of meiotic sister chromatid cohesion due to its genetic tractability and the excellent cytological properties of the hermaphrodite gonad. Moreover, mutants defective in the establishment or maintenance of meiotic SCC nevertheless produce abundant gametes, allowing analysis of the pattern of chromosome segregation. Here I describe two approaches for analysis of meiotic cohesion in *C.* [illegible]. The first approach relies on cytology to detect and quantify defects in SCC. The second approach relies on PCR and restriction digests to identify embryos that inherited an incorrect complement of chromosomes due to aberrant meiotic chromo-

Kyoko Yokomori and Katsuhiko Shirahige (eds.), *Cohesin and Condensin: Methods and Protocols*, Methods in Molecular Biology, vol. 1515, DOI 10.1007/978-1-4939-6545-8_5, © Springer Science+Business Media New York 2017

be expressed from a transgene that is integrated either together with the *lacO* array or into a different chromosomal site [22–24]. Both methods allow visualization of *lacO*-tagged chromosomes in living and fixed samples. Alternatively, purified LacI or GFP-LacI can be used to stain fixed tissues using standard protocols developed for immunofluorescence [10, 25]. This approach may yield a better signal to noise ratio than can be achieved with in vivo expression of GFP-LacI because there is no background fluorescence from nucleoplasmic GFP-LacI that has not bound the *lacO* array. Additionally, many of the transgenes used for expression of GFP-LacI in nematodes are transcriptionally silenced in the germ line, precluding their use for studies of meiotic cohesion [21]. For these reasons, I prefer to stain *lacO* integrants with bacterially expressed, purified GFP-LacI.

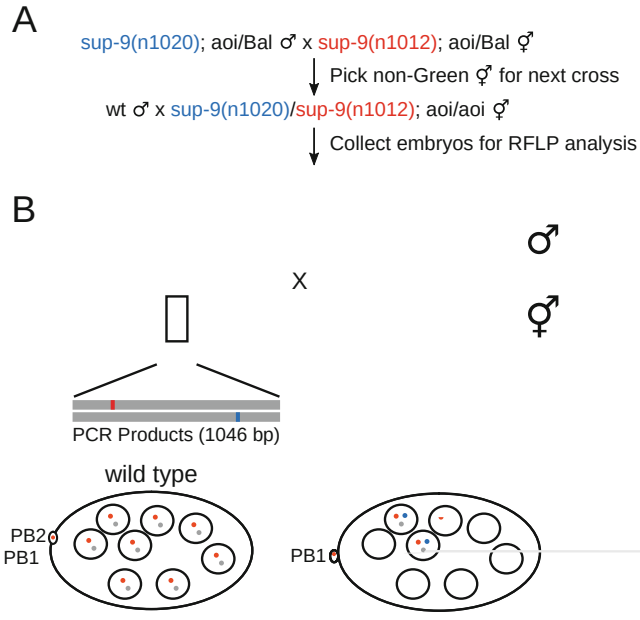
The GFP-LacI/*lacO* system offers significant advantages over other methods used for the analysis of meiotic SCC in *C.* . The simplest and perhaps most commonly used technique for quantifying SCC defects is counting the number of chromosomal structures in meiotic nuclei stained with a DNA dye like DAPI or Hoechst. Counting DAPI-stained bodies requires that chromosomes be far enough apart that they can be resolved. This condition is often met in late diakinesis nuclei of wild-type worms, which have six bivalents, and in mutants defective for meiotic crossover recombination, which have 12 univalents. In contrast, accurate quantification of DAPI-stained bodies is very difficult when 12–24 are present, as occurs in worms with severe SCC defects. Moreover, reliance on this technique limits analysis of SCC to diakinesis and prometaphase, since nuclei are smaller and chromosomes much less compact in earlier stages of meiosis.

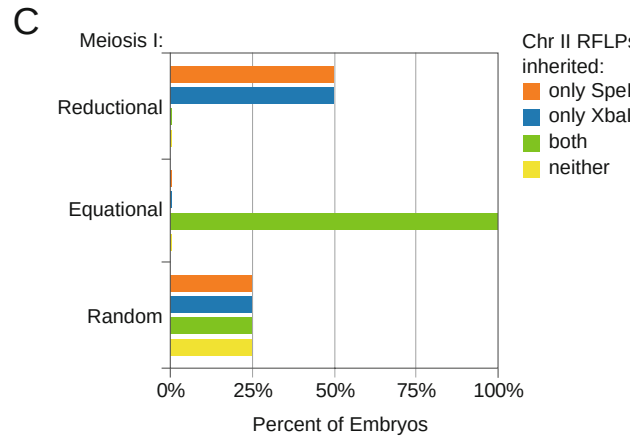
Analysis of SCC by fluorescence in situ hybridization (FISH) allows more accurate quantification of SCC defects than does counting DAPI-stained bodies because FISH utilizes a sequence specific probe to fluorescently mark a single chromosome. Thus, FISH can reliably detect detached sister chromatids in crowded nuclei and in all stages of meiosis and mitosis. However, because FISH relies on a nucleic acid probe, chromosomal DNA must be denatured to allow the probe to bind. Denaturation degrades chromosomal morphology, an effect that is particularly severe in cohesin mutants. Moreover, because FISH probes are usually designed to hybridize to endogenous sequences that are present in both homologs of the targeted chromosome, an increased number of FISH foci can result from defects in synapsis and/or crossover recombination as well as defects in SCC establishment or maintenance. Because cohesin mutations often impair synapsis and crossover formation, relating the number of FISH signals in a nucleus to the frequency of sister separation as a result of defective meiotic cohesion can be challenging.

The GFP-LacI/*lacO* system circumvents many of the shortcomings of FISH and counting DAPI-stained bodies. Like FISH, the GFP-LacI/*lacO* system fluorescently tags a single chromosome, allowing sister separation to be detected even in crowded nuclei. However, unlike FISH probes, binding of GFP-LacI to DNA does not require denaturation. Additionally, because the *lacO* sequence to which GFP-LacI binds is derived from the bacterial *lac* operon rather than an endogenous *C. elegans* chromosomal sequence, SCC can be analyzed in animals heterozygous for the *lacO* array (Note 1). In *lacO* heterozygotes, GFP-LacI binding labels the two sisters of a single homolog; therefore, the presence of two discrete GFP foci is a clear indication of an SCC defect (Note 2). Moreover, because the GFP foci mark the two sisters of a single homolog, the average distance between sisters can be used as a measure of the severity of the SCC defect in animals of a given genotype. A mutation that eliminates meiotic SCC is expected to result in random positioning of the two sisters within the nucleus, allowing their separation by distances as large as the nucleus is wide. In contrast, a mutation that only weakens SCC may slightly increase sister separation but still allow the sisters to maintain their close proximity.

Although the GFP-LacI/*lacO* system has several major advantages over FISH and counts of DAPI-stained bodies for analysis of SCC, it also has some minor disadvantages. The GFP-LacI/*lacO* approach requires the construction of specialized strains that will likely only be used for analysis of SCC. However, the time needed to build the required strains is minimal, so this should not daunt a stalwart *C. elegans* geneticist. Two other potential concerns should be kept in mind. First, the GFP-LacI/*lacO* system allows analysis of only one chromosome in any given experiment. Other chromosomes may behave differently. For example, some mutations that disrupt meiosis have disparate effects on X chromosomes and autosomes [26–28]. Second, the chromosomally integrated *lacO* array could somehow alter the behavior of meiotic chromosomes. However, we have seen no evidence that this occurs to date.

The second approach described here utilizes PCR and restriction digests to identify and characterize embryonic aneuploidy in the progeny of mutant animals in which the two homologs of chromosome II are differentially marked by Snip-SNPs: single nucleotide polymorphisms (SNPs) that create restriction fragment length polymorphisms (RFLPs) (Fig. 1) [9]. Because this method will detect aneuploidy that occurs as a consequence of any defect in chromosome segregation during meiosis or the mitotic divisions of germ line stem cells, it is best used in combination with other methods, such as the GFP-LacI/*lacO* method described above and quantification of the number of polar bodies (see below) [9]. The power of this approach lies in the fact that large numbers of embryos can be analyzed. This allows the identification of rare aneuploidies in mutants with infrequent meiotic errors and reveals patterns of aberrant chromosome segregation that can differentiate between mutations that cause


A
sup-9(n1020); aoi/Bal ♂ x sup-9(n1012); aoi/Bal ♀
Pick non-Green ♀ for next cross
wt ♂ x sup-9(n1020)/sup-9(n1012); aoi/aoi ♀
Collect embryos for RFLP analysis
B
♂
X
♀
PCR Products (1046 bp)
wild type
PB2
PB1
PB1



g. 1 (con 0 T 04 Tw (Percent of 95998 re f q60 l 9w017.c07451 scn -I.2 genetics,sTp3e60 -.0043 8

2 e / –

The protocols in this chapter were written under the assumption that the reader will have access to most equipment typically found in a *C.* , lab, such as dissecting scopes equipped with light sources for transmitted light and epifluorescence, NG agar plates with a large lawn of OP50 *E.* , for strain maintenance, mating plates seeded with a small drop of OP50 for conducting crosses, etc. A basic understanding of the fundamental techniques in nematode genetics, including methods to distinguish between self-progeny and cross-progeny, are also assumed.

Mutations that disrupt meiotic SCC usually result in the production of inviable, aneuploid embryos. Such mutations are therefore typically maintained in a heterozygous state by a dominantly marked, homozygous lethal balancer chromosome, and the protocols described here are written with this expectation in mind. We will use the terms "AOI" to refer to the mutant llele f nterest that is being tested for effects on meiotic SCC and "balanced AOI" to refer to the allele of interest maintained over a balancer chromosome. With minor modifications, the methods described here can be used in RNAi experiments to study the meiotic roles of genes for which no mutation exists or genes that are also required during mitosis.

All reagents should be prepared using Milli-Q / Nanopure water or equivalent.

2.1 Construction of lacO-Tagged Strains

1. Standard equipment and reagents for PCR amplification and agarose gel electrophoresis, including 0.2 mL thin wall PCR tubes (either eight tube strips or multiwell plates), *T* DNA polymerase (5 U / µL) and 10× PCR buffer, dNTP mix (25 mM each dNTP) and sterile, nuclease free water.
2. Access to a compound microscope equipped with epifluorescence optics and at least a 40× oil immersion objective lens.
3. A *C.* strain harboring your mutant allele of interest balanced by a dominantly marked, homozygous lethal balancer chromosome (hereafter referred to as the "balanced AOI").
4. A *C.* strain carrying a chromosomally integrated array of *O* sequences. The *I 44* array is an excellent choice for analysis of meiotic SCC (strain TY5434, full genotype *I 44[-20(+), -16 :: I, O(256)]* V). *I 44* contains multiple, tandem copies of a 256× *O* repeat, a gene encoding a GFP-LacI fusion under control of the heat shock promoter, and a wild-type copy of the *-20* locus. The array is integrated into chromosome V (Note 3) [10, 24, 29]. The large number of *O* sequences within the array yields very robust staining of germ line nuclei with purified LacI-His_6-GFP (Subheadings 3.2 and 3.4) [10]. Other strains with integrated *O* arrays are available [21], and the methods described here can be adapted to analyze meiotic SCC using these arrays (Note 4) [30–32].
5. *C.* lysis buffer: 0.15 mg / mL proteinase K in 1× PCR buffer (Note 5).
6. 100 µM stocks of oligonucleotide primers for following *I 44* by PCR. AFS366 (GCCATGTGTAATCCCAGCA) and AFS369 (GGTGAAACCAGTAACGTTA) amplify an approximately 1100 bp product from homozygous *I 44/ I 44* and heterozygous *I 44/+* worms (Note 6).
7. Oligonucleotide primers for following the AOI. If the allele cannot be followed by PCR, other strategies will be required to demonstrate its presence or absence.
8. A 33 °C incubator or waterbath for heat shock.
9. Multiwell slides (Note 7).
10. Cover glass, 22 mm × 50 mm, #1.
11. Phosphate buffered saline (PBS): In 800 mL of water, dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, and 0.24 g KH_2PO_4. Adjust pH to 7.4 with HCl if necessary. Adjust volume to 1 L with additional water. Sterilize by autoclaving.
12. PBS + 0.2% sodium azide: Add 1 µL 20% (w / v) sodium azide to 100 µL 1× PBS.

1. pLacI-His_6-GFP plasmid

2. Fertile hermaphrodites. This strain should have the same genotype as the males, except that it should lack *I 44*.

1. Glass slides (75 × 25 × 1 mm; Note 9).
2. Subbing solution [35]: Bring 200 mL of water to 60 °C, then add 0.4 g gelatin (from porcine skin). Cool to 40 °C, then add 0.04 g chrome alum (chromium potassium sulfate), 200 mg poly-L-lysine (Note 10), and sodium azide to 1 mM final concentration (65 µL of 20% stock).
3. A slide drying rack.
4. PAP pen (e.g., Electron Microscopy Sciences 71312 or equivalent).
5. A scalpel handle (#3, Stainless Steel) and blades (#15) (Note 11).
6. Cover glass, 18 mm × 18 mm.
7. Cover glass, 22 mm × 22 mm.
8. Humid chamber (Note 12).
9. Liquid nitrogen (Note 13).
10. 10× Dernburg's Modified Egg Buffer (10× EB): 250 mM HEPES-NaOH, pH 7.4, 1.18 M NaCl, 480 mM KCl, 20 mM

at 8.0. Because each addition of base drives more EDTA into solution, it can take a while to reach equilibrium. Add water to bring the total volume to 500 mL. Sterilize by autoclaving.

17. Blocking solution: To 89 mL water, add 10 mL 10× PBS, 1 mL 20% Tween 20, 0.1 mL 20% sodium azide, and 1 g bovine serum albumin (BSA). Rock on a nutator until the BSA has dissolved completely. Store in 5 mL aliquots at −20 °C.
18. PBST wash buffer: To 888 mL water, add 100 mL 10× PBS, 10 mL 20% Tween 20, and 2 mL 0.5 M EDTA.
19. Purified LacI-His_6-GFP (Subheading 3.2).
20. Anti-GFP primary antibody (I use a chicken anti-GFP antibody from Life Technologies (Grand Island, NY).
21.

4. A mouse with a scroll wheel. Although not essential, it is extremely helpful.

1. Standard equipment and reagents for PCR amplification (Subheading 2.1).
2. A 37 °C incubator.
3. An aspirator tube assembly (Sigma-Aldrich, St. Louis, MO).
4. Capillary tubes for mouth pipets (e.g., Kimble-Chase 51 Expansion Borosilicate Glass Melting Point Capillaries, 100 mm length, approximately 1.5 mm outside diameter, 0.25 mm wall thickness, open on both ends).
5. Butane lighter.
6. Worm strains: TY4236 *-8(1489)* IV; *I 10[-2::* V, TY4851 *-9(1012)* II, and TY4852 *-9(1020)* II.
7. Two strains carrying the balanced AOI and a polymorphic allele of *-9*. One strain should have the *-9(1012)* allele, which creates an SpeI cleavage site not present in wild-type, and the other should have the *-9(1020)* allele, which creates an XbaI cleavage site not present in wild-type (Note 17). These strains can be constructed using the strains TY4851 *-9(1012)* II and TY4852 *-9(1020)* II and techniques similar to those outlined for building strains with *I 44* (Subheading 2.1), with the following exceptions: First, the oligonucleotides AFS155 and AFS156 must be substituted for the AFS366 and AFS369 oligonucleotides used to genotype for *I 44*. Second, a small amount of PCR product should be digested with SpeI or XbaI to genotype for the *1012* and *1020* alleles, respectively (Subheading 3.6).
8. *C.* lysis buffer (Subheading 2.1).
9. Oligonucleotide primers for amplifying the *-9* polymorphisms (100 µM each): AFS155 (GACGGAGAATGAGATT CTGCAGG) and AFS156 (CGGCTCGTCTTATGAA ACGGA).
10. *-9* PCR mastermix (without *T* polymerase) sufficient for one hundred 30 µL reactions: To 1.725 mL of water, add 200 µL of 10× PCR buffer (

19

20

21

use 0.1 µL of *T* (5 U/µL) per reaction. Mix well by gently pipetting up and down (Note 22)

8. On ice, add 15 µL of mastermix to each PCR tube. Centrifuge briefly to collect liquid in the bottom of tubes.

5. Perform all subsequent steps at 4 °C. All solutions should be chilled to 4 °C prior to thawing the bacterial pellet.
6. Resuspend pellet in 40 mL Equilibration / Wash buffer.
7. Lyse bacteria by sonicating on ice. The following conditions work well with our sonicator: At maximum microtip power, pulse 1 s on, then 1 s off. After 15 bursts, pause for 1 min. Repeat this cycle three more times.
8. Pellet insoluble debris by centrifugation at 10,000× for 20 min at 4 °C.
9. While centrifuging the bacterial lysate, resuspend the cobalt IMAC resin and transfer a sufficient quantity to yield a 2 mL bed volume to a 50 mL conical tube. Centrifuge at 700× for 2 min, then remove the supernatant. Wash the resin with 20 mL Equilibration / Wash Buffer. Centrifuge, remove the supernatant, and repeat.
10. Mix the lysate supernatant with the resin and rock 20 min. Centrifuge at 700× for 5 min. Remove the supernatant.
11.

3.3 Generating syIs44 Heterozygotes

1. Set up 4–8 crosses, each with 6–10 *I 44* positive males and a single *I 44* negative hermaphrodite. Males and hermaphrodites should both be balanced heterozygotes if your AOI is lethal. Label each mating plate with the date and a unique identifier (cross A, B, etc.).
2. Transfer the males and hermaphrodites to fresh mating plates every day. Label each plate with the date and the unique identifier.
3. When transferring crosses, check plates from previous days for the presence of males. Once a hermaphrodite has mated with a male, most of her offspring will be cross progeny, and approximately 50% will be male. The first plate from each cross to have male progeny should be discarded, because this plate will have a mixture of self progeny produced before the hermaphrodite mated and self-progeny produced after she mated. Worms from the second day of male production or from any subsequent day can be used for staining, provided that there are still approximately 50% male worms on the plate.

3.4 Staining Gonads with LacI-His-GFP

1. Isolate L4 hermaphrodites that are homozygous for the AOI from working crosses (Subheading 3.3). These worms should be heterozygous for *I 44*. Grow the animals for 24 h at 20 °C. Isolating L4s and then allowing them to mature for a defined time ensures that all animals analyzed are similar in age.
2. While waiting for the L4s to mature, prepare adherent "subbed" slides: Immerse slides in a Coplin jar filled with subbing solution for ~30 s, then move slides to a drying rack. Dry slides in a drying oven (approximately 30 min at 80 °C or 3 h at 60 °C should be sufficient) or overnight at room temperature. If drying overnight, cover slides with aluminum foil to protect them from dust and light. Subbed slides can be stored at room temperature in the dark for up to a week, but freshly prepared slides work best.
3. Prepare 1× Egg Buffer + Tween 20 and Levamisole (EBTL): To 885 μL water, add 100 μL 10× EB, 10 μL 250 mg / mL levamisole, and 5 μL 20% Tween 20. Make fresh on day of staining.
4. Prepare 1× Egg Buffer + 2% Paraformaldehyde (EB-PFA): To 775 μL water, add 100 μL 10× EB and 125 μL 16% PFA. Make fresh on day of staining.
5. Using a PAP pen, draw a 1 cm × 1 cm grease square on the front surface of a slide (Fig. 2a).
6. Pipette 6.5 μL of EBTL into the middle of the grease square.
7. Pick ten hermaphrodites and coax them off the end of your pick by swirling it around in the drop of buffer. The levamisole should rapidly anesthetize the worms; if they continue to thrash, it is likely that the anesthetic has gone bad.

15. Immerse the slide in a Coplin jar filled with −20 °C methanol for 1 min, then move the slide to a Coplin jar filled with PBST wash buffer. Wash for 5 min. Repeat 1×.
16. Wipe off the front and back surfaces of the slide (except for the area inside the grease square). Add 20 μL blocking solution and incubate for 30 min in a humid chamber.
17. Wick off the blocking solution with a folded-up Kimwipes or paper towel. Add 5 μL of LacI-His_6-GFP diluted in PBS + 1% BSA + 0.2% Tween 20. Place the slide in a humid chamber. Incubate at least 1 h at room temperature or overnight at 4 °C.
18. Wash 3× in PBST, 10 min each.
19. Wipe off the front and back surfaces of the slide. Add 5 μL of anti-GFP primary antibody diluted in PBS + 1% BSA + 0.2% Tween 20. Place the slide in a humid chamber. Incubate at least 1 h at room temperature or overnight at 4 °C.
20. Wash 3× in PBST, 10 min each.
21. Wipe off the front and back surfaces of the slide. Add 5 μL of fluorescently labeled secondary antibody diluted in PBS + 1% BSA + 0.2% Tween 20. Place the slide in a humid chamber. Incubate at least 1 h at room temperature or overnight at 4 °C.
22. Wash 3× in PBST, 10 min each.
23. Wipe off the front and back surfaces of the slide. Add 7 μL of Prolong Gold or Vectashield mounting medium containing 1 μg/mL DAPI.
24. Apply an 18 mm × 18 mm cover glass to the slide. Seal the edges with fingernail polish. Let nail polish dry for at least 1 h before examining the slide on a microscope.

Because of the wide variety of microscopes and imaging systems available, the specific methods required to collect image datasets are beyond the scope of this paper. However, a few best practices should be followed to ensure the best results. Images should be collected at less than or equal to 0.2 μm axial spacing. Avoid binning, which sacrifices XY resolution to increase the signal to noise ratio. Because images of nuclei on the side of the gonad closest to the coverslip will be of much higher quality than images of nuclei on the opposite side of the rachis, it is advisable to limit the analysis to nuclei adjacent to the coverslip.

Once images have been collected, the number of nuclei with intact sister chromatid cohesion (one spot per nucleus) or defective sister chromatid cohesion (two spots per nucleus) can be quantified [10

Thus, it is informative to measure the distances between LacI-GFP foci in addition to tabulating the number of nuclei in which one or two foci could be detected.

1. Open Fiji. The instructions below have been tested on Fiji 2.0.0-rc-30 / 1.49 t with 64-bit Java 1.6.0_24 installed on Windows 7. Although the exact menu formats and keyboard shortcuts may differ based on operating system or Fiji version, every function that is essential for the analysis described below should be present in all versions and on all platforms.
2. Open your dataset in Fiji. This can be done in several ways. For data types that are supported by the built-in Bio-Formats Image Importer plugin, one can click on File / Open, then navigate through the file system to select your file. Alternatively, one can drag-and-drop the file icon onto the main Fiji window. If your file type is recognized by Fiji, a dialog box entitled "Bio-Formats Import Options" will open. For the analysis of DeltaVision files, I recommend setting the drop-down menu entitled, "Stack viewing / View stack with:" to "Hyperstack," and the drop-down menu entitled, "Color options / Color mode" to "Colorized." Additionally, make sure that only the Autoscale checkbox is selected.
3. Set your preferred display colors (lookup table, or LUT) for each channel. Many investigators prefer to show DNA in red and LacI-GFP in green, although deuteranopes may prefer a different palette. In the Image / Color / menu, click on Channels Tool. Select Channel 2, then click on the "More" button and choose your preferred color for that channel. Repeat for Channel 1. Choose "Composite" from the drop-down menu. You should now see an RGB image with Channels 1 and 2 pseudocolored using the selected LUTs.
4. At the bottom of the image window there are two scroll bars, labeled "c" and "z." The "z" scrollbar changes the focal plane. The focal plane can also be changed with the mouse scroll wheel, although this requires holding down a modifier key—currently the Alt key on Windows. In past versions of Fiji, the Ctrl key was used. Scroll through the image stack to find a focal plane that allows visualization of the entire gonad, if possible.
5. Zoom in on the image: Click the "Magnifying glass" tool in the main Fiji toolbar, then click on the image window. I find that 150–200% zoom works well for point picking using our image acquisition settings. Once the image magnification has been increased, the image region being viewed can be changed by selecting the "Scrolling" tool (hand icon in the main Fiji toolbar) and dragging the image to a new location.
6. Locate the "Point Tool" in the row of buttons in the main Fiji window. The button looks like a cross with a yellow point in

the center. Right-clicking on the button will toggle between the "Point Tool" and the "Multi-point Tool." The "Point Tool" is the best choice for the analysis described here because it allows recording of the *X*, *Y*, and *Z* coordinates of a point, while the "Multi-point Tool" currently assigns the same *Z* coordinate to every point.

7. Double-click on the "Point Tool" icon. Make sure that the "Auto-measure" and "Label points" checkboxes are selected, and the other checkboxes are empty. Click the "OK" button.
8. Scroll through the *Z* stacks and identify nuclei with a single GFP focus (Note 25). For each nucleus in this category, click on the centroid of the LacI-GFP focus. Each mouse click should create a new row in the Results window and log the image name, the *X* and *Y* coordinates of the point selected, and the *Z* section, or slice, that was visible when the point was selected.
9. Once the position of the LacI-GFP focus in each nucleus with a single spot has been recorded, copy the data from the Results window: From the Edit menu, choose "Select All," then "Copy." Paste into a spreadsheet program. There should be six columns of data, corresponding to the Measurement ID Number, the Slice Label, the *X* and *Y* coordinates of the selected point, and the Channel and *Z* Slice that were active when the selection was made. In most spreadsheet programs, this data will be in columns A-F.
10. Paste a second copy of the data immediately to the right of the first copy (i.e., in columns G-L).
11. Clear the data from the Fiji Results window: From the Edit menu, choose "Select All," then "Clear."
12. Scroll through the *Z*-stacks and identify nuclei with two LacI-GFP foci. Work through the dataset systematically, successively clicking on the two foci within each nucleus.
13. Once all the nuclei have been analyzed, copy and paste the data into columns A–F of the spreadsheet, underneath the existing data. Next, edit the spreadsheet such that the measurements for the first focus in a nucleus are in columns A–F and the measurements for the second focus in the nucleus are in columns G–L of the same row. Clean up the spreadsheet by deleting any empty or duplicate rows created while cutting and pasting (Note

and others will be negative; this is expected, and will not affect the calculation of the shortest distance separating the two foci.

15. Ensure that the distance measurements *( )*, *( )*, and *( )* all have the same units (e.g., microns).

approximately 1.5 in. long, narrow tip. Repeat for the other side. The inside diameter of the tip should be approximately 100 µm, or slightly larger than an embryo.

6. Create an approximately 45° bend in the tip of the capillary tube by holding it near the flame of a lit butane lighter. The angled tip will be easier to insert into the wells of a 96-well plate than a straight tip. Place the thick end of the capillary tube into the opening of an aspirator tube assembly.
7. Thaw a 500 µ

12. Briefly centrifuge the 96-well plate to collect the lysate in the bottom of the wells. Place the plate on ice or in a chilled 96-well plate cooling block.
13. Thaw an aliquot of -9 PCR mastermix (or prepare fresh). Add 15 µL *T* polymerase (5 U/µL). Mix well by gently pipetting up and down. Dispense 20 µL into each well of the PCR plate. Seal plate and centrifuge briefly to collect liquid at the bottom of each well. Return plate to ice or 96-well plate cooling block.
14. Run the PCR program on a thermocycler. Amplification with the AFS155 and AFS156 oligonucleotides works well with 35 cycles, a 50 °C annealing temperature, and a 75 s extension time (Note 31).
15. Once the PCR program is complete, visually inspect the wells of the PCR plate. If any wells lost some volume due to evaporation, add a little water to equalize their volume with the other wells.
16. Run 2 µL of the PCR reactions in column 1 of the PCR plate on a 1.5% agarose gel. If the reactions worked, a band of approximately 1 kb in size should be visible. From this gel, estimate the volume of PCR product needed to see bands after restriction digests. Enough PCR product should be cut that a band equivalent to approximately 1/3 of the total digested DNA will be visible. Cutting more DNA than necessary increases the likelihood of incomplete digestion. A maximum of 7 µL of PCR product can be included in each digest.
17. Replicate the PCR plate to three new 96-well plates. Use a multichannel pipet to transfer the volume estimated in the previous step. Once the PCR product has been added, adjust the total volume in each well to 7 µL by addition of water, if necessary.

21. Analyze the digests by gel electrophoresis on a 1.5% agarose gel. The Owl D3-14 Centipede system we use can be configured to have three rows of 50 wells each, which allows the gel to be loaded in such a way that all three digests from a single PCR reaction are aligned in a single column, which greatly simplifies data analysis.
22. Determine whether the control PCR products from the *-9(1012)* and *-9(1020)* homozygous worms were digested to completion. If so, the SpeI and SpeI+XbaI digested DNA from *-9(1012)* and the XbaI and SpeI+XbaI digested DNA from *-9(1020)* mutants should have a prominent band of approximately 800 bp but no detectible 1046 bp band. If a 1046 bp band is visible, the control reactions did not cut to completion and all data from the plate are unreliable.
23. Determine whether any individuals analyzed were self-

28. From the tabulated data, determine the likely pattern of chromosome segregation. If most individuals completed meiosis normally, approximately 50% of individuals should have inherited only the *-9(1012)* allele, and the other 50% should have inherited only the *-9(1020)* allele (Fig. 1c

24. It is also possible to extrude the gonad by nicking the tail of the

30. Avoid sucking the embryo or larva very far into the tip. Ideally, one should stop applying suction as soon as the specimen disappears into the tip. If a large volume of liquid is drawn into the capillary, a large volume of liquid will also need to be expelled from the capillary to eject the embryo. This will

ment of these methods were provided by the CGC, which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440), and the National Bioresource Project. This work was

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