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Analysis of meiotic sister chromatid cohesion in *Caenorhabditis elegans*

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Abstract

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 *Caenorhabditis elegans* is an excellent 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 will describe two approaches for analysis of meiotic cohesion in *C. elegans*. 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 chromosome segregation. Both approaches are sensitive enough to identify rare errors and precise enough to reveal distinctive phenotypes resulting from mutations that perturb meiotic SCC in different ways. The robust, quantitative nature of these assays should strengthen phenotypic comparisons of different meiotic mutants and enhance the reproducibility of data generated by different investigators.

Keywords

cohesin; meiosis; gametogenesis; kleisin; chromosome segregation; aneuploidy; rec8; coh-3; coh-4; Rad21L

1. Introduction

During mitosis and meiosis, sister chromatid cohesion (SCC) tethers replicated sister chromatids and is critical for accurate chromosome segregation. Sister chromatid cohesion is mediated by a widely conserved protein complex called cohesin. Pioneering work in yeast demonstrated that mitotic and meiotic cohesins differ in a key subunit, the α -kleisin, and

that changing this one subunit is critical to establishing the distinct mitotic and meiotic patterns of chromosome segregation (1). Meiotic cohesin complexes associate with the α -kleisin Rec8, and Rec8 is cleaved in two steps by the cysteine protease separase to allow the separation of homologous chromosomes in anaphase of meiosis I and of sister chromatids in anaphase of meiosis II (2–4). Mitotic cohesin complexes associate with the α -kleisin Scc1 (also called Mcd1 and Rad21) (5–7). During mitosis, a single round of separase-dependent cleavage removes Scc1 cohesin, allowing sisters to separate (8).

Our work in *C. elegans* demonstrated that kleisins other than REC-8 are essential for normal gametogenesis. Two nearly identical and functionally redundant α -kleisins, called COH-3 and COH-4 (hereafter, COH-3/4) act together with REC-8 to mediate meiotic SCC (9). Remarkably, the kleisin subunit influences nearly every facet of meiotic cohesin function (10). The kleisin specifies the mechanisms that promote the stable binding of cohesin to chromosomes and that trigger cohesin to establish SCC. The kleisin determines whether a complex promotes sister chromatid co-orientation, the process that ensures the attachment of sister chromatids to microtubules from the same spindle pole in meiosis I. Finally, the kleisin determines whether a cohesin complex may persist on chromosomes after anaphase of meiosis I to hold sisters together until anaphase of meiosis II (10). It has since been shown that multiple, functionally-specialized kleisins are required for meiosis in mammals and plants, indicating that the involvement of multiple kleisins in gametogenesis is widely conserved (11–16).

Given the complex nature of meiotic sister chromatid cohesion in higher eukaryotes, it is critical to develop reliable and quantitative methods to characterize defects in the establishment or maintenance of SCC and to determine the consequences of cohesin misregulation on the reduction of ploidy. Here, I describe two such methods. The first is a cytological method that allows direct visualization of SCC defects. The second is a molecular approach to identify embryos with abnormal chromosomal content formed as a consequence of errors in meiotic chromosome segregation. Because this technique allows the analysis of a large number of embryos, the nature of the chromosome segregation defect can often be inferred from the population averaged pattern of chromosomal inheritance. In the remainder of this section, I will introduce these methods and discuss their utility for studies of meiotic SCC.

The ability to uniquely mark a single chromosome with a fluorescently labeled, sequence-specific DNA binding protein revolutionized studies of nuclear architecture and chromosome segregation (17). The most widely used implementation of this technique relies on the binding of a Green Fluorescent Protein - Lac Repressor fusion protein (GFP-LacI) to a chromosomally integrated array of 256 tandem repeats of *lac operator* (*lacO*) DNA (18–20). The high affinity of GFP-LacI for *lacO* DNA results in the specific accumulation of GFP fluorescence at the *lacO* array. A number of researchers have implemented the GFP-LacI/*lacO* system in *C. elegans* (see Ref. 21 for a thorough review). GFP-LacI can be expressed from a transgene that is either integrated 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 germline, 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. elegans*. 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 (see 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 (see Note 2). Moreover, because the

¹Because *lacO* sequences are common in plasmids used for routine cloning, many transgenic worm strains have *lacO* sequences in their genome. While GFP-LacI binding to a single *lacO* site is usually not detectable, GFP-LacI binding to simple extrachromosomal and integrated arrays made with plasmids harboring single copy *lacO* sequences often is (21).

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 germline 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 premature, equational separation of sister chromatids at anaphase I (as occurs in *rec-8* single mutants) and those that cause random segregation of homologs at anaphase I (as occurs in *coh-4 coh-3* double mutants) (9). As with the GFP-LacI/*lacO* analysis described above, this technique requires building specialized strains, and it only analyzes the segregation of a single autosome. However, the sensitivity and robustness of this approach offer advantages that far outweigh these minor shortcomings.

2. Materials

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

²The presence two GFP-LacI foci in meiotic nuclei of a *lacO* heterozygote could also result from mitotic chromosome segregation defects in germline progenitor cells. However, such defects would also lead to the formation of nuclei with zero foci and more than two foci. Such nuclei cannot result from a defect in meiotic SCC.

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 allele of interest 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 8 tube strips or multiwell plates), *Taq* DNA polymerase (5 U/ μ L) and 10x 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 40x oil immersion objective lens.
3. A *C. elegans* 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. elegans* strain carrying a chromosomally-integrated array of *lacO* sequences. The *syIs44* array is an excellent choice for analysis of meiotic SCC (strain TY5434, full genotype *syIs44[dpy-20(+), hsp-16^{promoter}::gfp-lacI, lacO(256)]V*). *syIs44* contains multiple, tandem copies of a 256x *lacO* repeat, a gene encoding a GFP-LacI fusion under control of the heat shock promoter, and a wild-type copy of the *dpy-20* locus. The array is integrated into chromosome V (see Note 3) (10, 24, 30). The large number of *lacO* sequences within the array yields very robust staining of germline nuclei with purified LacI-His₆-GFP (see Sections 3.2 and 3.4) (10). Other strains with integrated *lacO* arrays are available (21), and the methods described here can be adapted to analyze meiotic SCC using these arrays (see Note 4) (31–33).

³I mapped *syIs44* to chromosome V using a panel of 48 single nucleotide polymorphisms that introduce or remove cleavage sites for the restriction endonuclease *DraI* (29). The exact map position of *syIs44* is unknown. However, analysis of recombinant strains generated while linking *syIs44* to *coh-3(gk112)*V and *coh-4(tm1857)*V positioned *syIs44* between *coh-4* (LG V:–6.34) and *coh-3* (LG V:6.41), perhaps somewhere around –4 cM.

⁴Many of the available strains with *lacO*-tagged chromosomes created by the miniMos method (31) also carry *syIs46*, an integrated extrachromosomal array that contains a GFP-LacI transgene under control of the heat shock promoter. Because the plasmids in this array also harbor *lacO* sequences (21), it is advisable to outcross these strains to eliminate *syIs46* before utilizing them to analyze meiotic SCC.

5. *C. elegans* lysis buffer: 0.15 mg/mL proteinase K in 1x PCR buffer (*see* Note 5).
6. 100 μ M stocks of oligonucleotide primers for following *syIs44* by PCR. AFS366 (GCCATGTGTAATCCCAGCA) and AFS369 (GGTGAAACCAGTAACGTTA) amplify an approximately 1100 base pair product from homozygous *syIs44/syIs44* and heterozygous *syIs44/+* worms (*see* 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.
9. Multiwell slides (*see* Note 7).
10. Coverglass, 22 mm $\times$ 50 mm, #1.
11. Phosphate Buffered Saline (PBS): In 800 mL of water, dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄, and 0.24 g KH₂PO₄. 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 μ L 20% (w/v) sodium azide to 100 μ L 1x PBS.

2.2. Expression and purification of LacI-His₆-GFP

1. pLacI-His₆-GFP plasmid (34), available from the author on request.
2. Competent BL21 bacteria (transformation efficiencies obtained with the CaCl₂ method (35) should be sufficient).
3. LB Agar plates containing 50 μ g/mL ampicillin.
4. LB medium, Miller formulation: to 800 mL water, add 10 g tryptone, 5 g yeast extract, and 10 g NaCl. Adjust volume to 1 L with additional water. Sterilize by autoclaving.
5. 50 mg/ml (1000x) ampicillin stock, dissolved in water and sterile filtered.
6. Sonicator equipped with a microtip.
7. Cobalt IMAC resin (*e.g.*, HisPur Resin, Thermo Scientific, Waltham, MA).

⁵I typically make lysis buffer in 10 mL batches, then freeze 50 μ L and 500 μ L aliquots in liquid nitrogen. These aliquots are sufficient to set up 5 μ L lysis reactions in 8 tube strips and 96-well plates, respectively. Aliquots can be stored at -80° C for up to one year.

⁶Because *syIs44* is a repetitive, integrated array and the exact structure and chromosomal location of the array are unknown, designing primers to follow the array is challenging. Primers AFS366 and AFS369, which bind to the GFP sequences and LacI sequences in the array, respectively, reliably amplify a product of approximately 1100 base pairs from *syIs44* and have not given false positive results in strains carrying other integrated GFP transgenes, such as those used to dominantly mark many balancers.

⁷The multi-well slides that we use are manufactured by Thermo Fisher Scientific Slides & Specialty Glass (Portsmouth, NH) and can be ordered through Fisher Scientific (Pittsburgh, PA) as a non-catalog/special-order item. Reference part number 30-943A, Epoxy Blue Slide with 18 wells. Similar products from other manufacturers will likely work equally well.

8. Disposable polypropylene column (*e.g.*, Poly-Prep Chromatography Columns, Bio-Rad, Inc., Hercules, CA).
9. Equilibration/Wash Buffer (pH 7.0): 50 mM sodium phosphate, 300 mM NaCl.
10. Elution Buffer (pH 7.0): 50 mM sodium phosphate, 300 mM NaCl, 150 mM imidazole.
11. Wash Buffer with 5 mM imidazole: Add 0.3 mL Elution Buffer to 9.7 mL Equilibration/Wash Buffer.
12. PBS (*see* **Section 2.1**).
13. 80% glycerol: Add 20 mL water to a 100 mL graduated cylinder. Slowly add glycerol until the total volume equals 100 mL. Mix thoroughly by sealing the graduated cylinder with parafilm and inverting periodically over the course of several hours or by adding a stir bar and mixing on a stir plate. Sterilize by autoclaving.

2.3. Generating *syIs44* heterozygotes

It is often desirable to examine LacI-His₆-GFP in meiotic nuclei of animals heterozygous for *syIs44*. For example, pachytene nuclei of *syIs44* homozygotes could have two LacI-His₆-GFP foci as a consequence of asynapsis or defective sister chromatid cohesion, but the presence of two foci in *syIs44* heterozygotes is strong evidence of an SCC defect (*see* Note 2). Moreover, when two LacI-His₆-GFP foci are detected in meiotic nuclei of *syIs44/+* heterozygotes, measuring the average distance between foci can provide information about the severity of the SCC defect (*see* **Section 3.5**) (10). This is not true when analyzing *syIs44* homozygotes, because it is not possible to know which foci mark sister chromatids and which mark homologous chromosomes.

1. Fertile males carrying the balanced AOI and the *syIs44* array (*see* **Section 3.1 and** Note 8). Depending on the genotype of the strain being tested, *syIs44* may be heterozygous (if covered by the balancer) or homozygous.
2. Fertile hermaphrodites. This strain should have the same genotype as the males, except that it should lack *syIs44*.

2.4. Staining gonads with LacI-His₆-GFP

1. Glass slides (75 × 25 × 1 mm; *see* Note 9).

⁸If the *syIs44*-tagged chromosome is contributed by the male, then any worms with *lacO* arrays must be cross progeny and therefore heterozygous for *syIs44*. Any hermaphrodite self-progeny would not inherit *syIs44*. In contrast, if *syIs44* were contributed by the hermaphrodite, both cross-progeny and self-progeny would inherit *syIs44*. Because self-progeny would be homozygous for *syIs44*, their inclusion could confound the analysis.

⁹*C. elegans* gonads do not adhere well to standard glass microscope slides. To increase the adhesion of gonadal tissues, one can use commercially available charged slides like Fisherbrand SuperFrost Plus slides. Alternatively, one can coat standard slides with “subbing solution.” In our experience, each method has distinct advantages and disadvantages. Worm gonads adhere very strongly to subbed slides, making tissue loss less likely but also decreasing the chances that the gonad will spread out completely and lay flat on the slide. Worm gonads adhere less strongly to charged slides, making it easier to get a nicely spread gonad at the expense of increased likelihood of tissue loss.

2. Subbing solution (36): Bring 200 mL of water to 60°C, then add 0.4 g gelatin (Sigma G-2500, from porcine skin). Cool to 40°C, then add 0.04 g chrome alum (Sigma C-5926, Chromium Potassium Sulfate), 200 mg poly-L-lysine (Sigma P-1524; *see* 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) (*see* Note 11).
6. Coverglass, No. 1, 18 mm × 18 mm.
7. Coverglass, No. 1.5, 22 mm × 22 mm.
8. Humid chamber (*see* Note 12).
9. Liquid nitrogen (*see* Note 13).
10. 10x Dernburg's Modified Egg Buffer (10x EB): 250 mM HEPES-NaOH, pH 7.4, 1.18 M NaCl, 480 mM KCl, 20mM EDTA, 5mM EGTA (*see* Note 14).
11. 250 mg/mL levamisole: Add 0.5 g levamisole (also called tetramisole hydrochloride) to 2 mL of water. Store in 100 µL aliquots at −20° C for up to a year (*see* Note 15).
12. 20% Tween-20. To prepare 200 mL, add 160 mL of water to a 250 mL graduated cylinder. Slowly add Tween-20 until the total volume is 200 mL. Mix thoroughly by sealing the graduated cylinder with parafilm and

¹⁰Making 200 mL of subbing solution requires 200 mg of poly-L-lysine. For convenience, I order 2x100 mg vials of poly-L-lysine (Sigma P1524-100MG). Alternatively, one can order larger vials to minimize costs; however, due to the sticky, cotton candy-like nature of poly-L-lysine, it is difficult to weigh out small amounts. When larger volumes are ordered, I recommend dissolving the entire volume to a 100 mg/mL concentration in water. The aqueous solution can be stored at −20° C for up to a year.

¹¹Other investigators prefer #11 blades, while still others prefer to use a pair of 25 GA syringe needles. The best tool for the job is the one that gets the head off the worm most quickly in your hands!

¹²The humid chamber prevents evaporation of the small amounts of solution applied to slides during prolonged incubations. Any airtight container lined with a layer of an absorbent material soaked with water can serve as a humid chamber. I prefer Snapware brand 4.5 cup food storage containers, which have a tightly sealing, gasketed lid. In each Snapware container, we place a single layer of open-weave tool drawer liner (*e.g.* Cleverbrand liner, ASIN B003XNWD1U, or Craftsman 65514), several layers of paper towels, and then another layer of drawer liner. The top layer of drawer liner elevates the slides slightly above the damp paper towels and prevents them from shifting when the chamber is moved, while the layer of paper towels and the bottom layer of drawer liner retain enough water to ensure that the box remains humid for the duration of the experiment.

¹³The freeze-crack permeabilization protocol we use relies on rapid freezing of the fixed sample, which is immobilized under a coverglass, followed by abrupt removal of the coverglass. To freeze slides in liquid nitrogen, I place a 250 mL Nalgene beaker in a Styrofoam shipping box, then add liquid nitrogen until the beaker is full and the bottom of the box is covered by about an inch. After each slide is fixed, it is lowered into the beaker of liquid nitrogen. The beaker keeps the slides in an upright position, which simplifies their later retrieval. Others prefer to freeze slides by pressing them against a flat aluminum block in a bed of dry ice (37). In my hands, the two methods produce equivalent results.

¹⁴The original *C. elegans* egg buffer was developed by Lois Edgar to sustain development of embryonic blastomeres that have been removed from their eggshells (38). This formulation was adopted and subsequently modified by Abby Dernburg and colleagues to preserve chromosomal morphology during fixation of gonadal tissues (37). The revised formulation has a higher concentration of HEPES buffer and replaces the sources of divalent cations (CaCl₂ and MgCl₂) with chelators (EDTA and EGTA), which may reduce nuclease activity. I have used both formulations for gonadal staining with equivalent results. I usually make 100 mL batches and store 5 mL aliquots at −20° C.

¹⁵Levamisole is an acetylcholine receptor agonist and triggers muscle contraction and paralysis in *C. elegans*. Paralysis aids in the dissection of gonadal tissues, while the contraction of body wall muscles may facilitate extrusion of the gonad. Once thawed, aliquots of levamisole can be stored at 4° C for 1–2 months, although the effectiveness will diminish over time. Other investigators prefer to immobilize worms with sodium azide (15–30 mM final concentration) (37, 39).

inverting periodically over the course of several hours or by adding a stir bar and mixing on a stir plate.

13. Paraformaldehyde, 16% solution, electron microscopy grade (Electron Microscopy Sciences, Hatfield, PA). After opening, ampules should be sealed with parafilm and stored at 4° C for two weeks to one month.
14. Methanol, chilled to –20° C in a Coplin jar.
15. 10x PBS: To 800 mL of water, add 80 g NaCl, 2 g KCl, 14.4 g Na₂HPO₄, and 2.4 g KH₂PO₄. Adjust pH to 7.4 with HCl if necessary. Bring volume to 1 L by addition of additional water. Sterilize by autoclaving.
16. 0.5 M EDTA, pH 8.0: Add 93.06 g disodium EDTA dihydrate (fw 372.24) to 400 ml water. Gradually add approximately 9 g of NaOH pellets. As the pH approaches 8.0, the solution will begin to clear. At this point, add 5 N NaOH (approximately 5–10 mL) very patiently until the pH stabilizes 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 10x 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 10x PBS, 10 mL 20% Tween-20, and 2 mL 0.5 M EDTA.
19. Purified LacI-His₆-GFP (*see Section 3.2*).
20. Anti-GFP primary antibody (I use a chicken anti-GFP antibody from Life Technologies (Grand Island, NY).
21. Fluorescently-conjugated secondary antibody that recognizes the anti-GFP primary antibody (I typically use an Alexa 488 conjugated donkey anti-Chicken antibody from Jackson Immunochemicals (West Grove, PA) (*see Note 16*).
22. DAPI (4',6-diamidino-2-phenylindole dihydrochloride): Resuspend at a 1 mg/mL concentration in water and store at –20° C.
23. Mounting medium: I typically use Prolong Gold anti-fade reagent (Life Technologies, Grand Island, NY). Prolong must cure for 24 hours before imaging to acquire the best optical properties. For faster results, I use Vectashield (Vector Labs, Burlingame, CA; original formulation, not

¹⁶Staining with LacI-His₆-GFP is compatible with most standard immunofluorescence protocols used for analysis of *C. elegans* meiosis. GFP foci are usually bright enough to image without use of additional antibodies, at least when analyzing *syIs44* strains. Nevertheless, I typically stain with an anti-GFP primary antibody and a secondary labeled with Alexa 488 or an equivalent fluor. I have not determined whether LacI-His₆-GFP is compatible with fluorescent *in situ* hybridization (FISH). However, others have shown that 256x *lacO* repeats can be reliably detected with a fluorescently labeled oligonucleotide FISH probe (32, 33).

hardset). To label chromosomes, DAPI is added directly to the mounting medium (1 µg/mL final concentration).

24. Clear fingernail polish for sealing coverslips.

2.5. Analysis of LacI-GFP focus number and distribution

1. A high quality system for collection of digital microscope images. Either a confocal or widefield microscope will work, but because of the large number of images that must be acquired, high speed acquisition is desirable. A wide variety of suitable microscopes and imaging systems are available, and I make no specific recommendations here. However, some minimal requirements must be met for the method described here to give reliable results. Most importantly, the microscope must have an accurate and precise computer-controlled focusing mechanism and a high numerical aperture, oil immersion 60x or 100x objective. My lab uses a DeltaVision microscope (GE Life Sciences, Pittsburgh, PA) equipped with a 100x/1.4 NA objective lens.
2. Fiji image processing and analysis software (40). Fiji is based on ImageJ (41), but Fiji is installed with plugins and addins that enable it to handle a large number of image formats out of the box. It is therefore recommended for users without significant experience with ImageJ. However, the methods described here can also be performed with ImageJ.
3. Microsoft Excel, Apple Numbers, LibreOffice Calc, or equivalent spreadsheet software.
4. A mouse with a scroll wheel. Although not essential, it is extremely helpful.

2.6. Analysis of meiotic chromosome segregation using chromosome II RFLPs

1. Standard equipment and reagents for PCR amplification (*see Section 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 *him-8(e1489)* IV; *mIs10[myo-2::gfp]* V, TY4851 *sup-9(n1012)* II, and TY4852 *sup-9(n1020)* II.
7. Two strains carrying the balanced AOI and a polymorphic allele of *sup-9*. One strain should have the *sup-9(n1012)* allele, which creates a SpeI cleavage site not present in wild-type, and the other should have the

sup-9(n1020) allele, which creates an XbaI cleavage site not present in wild-type (see Note 17). These strains can be constructed using the strains TY4851 *sup-9(n1012)* II and TY4852 *sup-9(n1020)* II and techniques similar to those outlined for building strains with *syIs44* (see Section 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 *syIs44*. Second, a small amount of PCR product should be digested with SpeI or XbaI to genotype for the *n1012* and *n1020* alleles, respectively (see Section 3.6).

8. *C. elegans* lysis buffer (see Section 2.1).
9. Oligonucleotide primers for amplifying the *sup-9* polymorphisms (100 μ M each): AFS155 (GACGGAGAATGAGATTCTGCAGG) and AFS156 (CGGCTCGTCTTATGAAACGGA).
10. *sup-9* PCR mastermix (without *Taq* polymerase) sufficient for 100 30 μ L reactions: To 1.725 ml of water, add 200 μ L of 10x PCR buffer (see Note 18), 30 μ L of dNTP mix (25 mM each dNTP), and 15 μ L each of AFS155 and AFS156 oligos (100 μ M stock). I typically scale this mastermix up 10-fold, then freeze 100 reaction mixes at -20° C until needed.
11. Restriction endonucleases: SpeI (10 U/ μ L) and XbaI (20 U/ μ L), along with stocks of the recommended buffers and BSA if not included in the buffer mix.
12. A large gel box for agarose gel electrophoresis; for example, the Owl D3-14 “Centipede” system.

3. Methods

3.1. Construction of lacO-tagged strains

1. Using standard genetic methods, perform crosses to introduce *syIs44* or another integrated *lacO* array into your mutant background. Crossing *syIs44* into a strain with a balanced AOI requires two sequential crosses. In the first cross, *syIs44* homozygotes are mated with a strain carrying the balanced AOI (P0 generation). Next, a cross is set up between male cross progeny that inherited the balancer and their hermaphrodite siblings that did not (F1 generation; see Note 19). In most cases, either 1/4 or 1/8 of the F2 progeny that inherit the balancer will be the desired genotype, depending on whether *syIs44* is covered by the balancer (for example, *nTI*) or needs to be maintained in a homozygous state (see Note 20).

¹⁷The *sup-9* alleles used here were identified in screens for suppressors of a semidominant, gain-of-function *unc-93* allele (42). Neither allele causes a detectable phenotype other than suppression of *unc-93*.

¹⁸Although the total volume of each PCR reaction will be 30 μ L, only 2 μ L of 10x PCR buffer per reaction is needed because the 10 μ L of worm lysate used as template already includes 1x PCR buffer.

¹⁹In principle, the sexes can be reversed and the cross can be set up with hermaphrodites that inherited the balancer and males that did not. However, since many balancers are dominantly marked by GFP expression, passing the balancer through the male allows the identification of cross progeny in the next generation.

2. Pick a sufficient number of L4 hermaphrodites carrying the balancer to ensure a high probability of finding at least one worm of the desired genotype. If the fraction of worms expected to have the correct genotype is 1/4, 16 L4s should be sufficient to ensure that one will obtain the desired strain 99% of the time. If the fraction is 1/8, pick 32 L4s. Every worm should be picked onto a separate plate.
3. Allow hermaphrodites to self-fertilize overnight at 25° C. When the singled worms have laid at least 40–50 eggs, genotype them by single-worm PCR. Thaw aliquots of *C. elegans* lysis buffer and dispense into PCR tubes, 10 µL per tube (if genotyping for *syIs44* and a single mutant allele; see Note 21). It is often convenient to use 8-well strips or multiwell plates. Briefly centrifuge to collect the buffer in the bottom of the tube. Pick the mom from each plate into the drop of lysis buffer.
4. Incubate at 60° C for 60 minutes, then 95° C for 20 minutes. Visually examine the tubes on a dissecting scope to confirm that the lysis reactions worked. Some embryos and a bit of cuticle might remain, but most of the worm should have been digested.
5. Vortex the PCR tubes to break up any remaining tissues, then briefly centrifuge the PCR tubes to collect the buffer in the bottom of the tube.
6. Move 5 µL of lysate into a new tube or well for each genotyping PCR. If available, a multichannel pipettor greatly speeds up this process.
7. On ice, make a PCR mastermix sufficient to genotype each worm for *syIs44*. For each reaction, use 13 µL water, 1.5 µL of 10x PCR buffer, 0.2 µL of dNTP mix (25 mM each dNTP), 0.1 µL each of AFS366 and AFS369 oligos (100 µM stock). Also make a PCR mastermix to genotype for your AOI. When both mixes are ready, add *Taq* polymerase. For the *syIs44* PCR, use 0.1 µL of *Taq* (5 U/µL) per reaction. Mix well by gently pipetting up and down (see Note 22).
8. On ice, add 15 µL of mastermix to each PCR tube. Centrifuge briefly to collect liquid in the bottom of tubes.
9. Perform PCR. AFS366 and AFS369 work well with 35 cycles, a 54° C annealing temperature, and a 1 minute extension time.
10. Separate your PCR products on a 1–2% agarose gel. A band of approximately 1100 base pairs indicates that the lysed worm was either heterozygous or homozygous for *syIs44*. No band will be detected if the lysed worm had not inherited the *syIs44* array.

²⁰Other strategies are necessary when working with unmarked or homozygous viable balancers or when using meiotic recombination to link *syIs44* to a mutation on the same chromosome. These approaches are beyond the scope of this review.

²¹If genotyping for more than two loci, use 5 µL of lysis buffer per locus. With well-designed oligos, the lysate from a single adult worm is sufficient for at least five PCR reactions.

²²In my experience teaching newbies to PCR genotype worms, inconsistent amplification (*i.e.*, a few reactions work very well but most fail miserably) is almost always a consequence of insufficient mixing, which leaves most of the *Taq* polymerase on the bottom of the tube of mastermix.

11. If the balancer used to maintain the AOI also covers *syIs44* (for example, the *nT1* balancer), PCR genotyping for *syIs44* is sufficient to determine whether the lysed worm was *syIs44/Balancer* or *+/Balancer*. However, if *syIs44* is not covered by the balancer, it will be necessary to identify *syIs44* homozygous strains by fluorescence microscopy. Once the progeny of the lysed worms are young adults, heat shock the *syIs44* positive plates at 33° C for 1 hour. Two hours later, pipet 2 µL of PBS + 0.2% sodium azide into four wells in the middle of a gasketed slide. Pick 20 L4 or young adult hermaphrodites from a heat-shocked plate into the first well, then repeat for three additional plates. Work quickly to add the worms before the drops evaporate. If necessary, the slide can be stored in a humid chamber while picking worms (see **Section 2.4**). Gently lower a 22 mm × 50 mm coverslip onto the slide. Examine the worms in each well for expression of LacI-GFP. The sodium azide will paralyze the worms, simplifying the visualization of GFP foci, but GFP will bleach very quickly. If the mom was homozygous for *syIs44*, GFP should be visible in somatic nuclei of every worm, and most nuclei should have two bright GFP foci (see Note 23). In contrast, if the mom was heterozygous for *syIs44*, only 75% of the progeny will express LacI-GFP, and 2/3 of these will have a single GFP focus in each somatic nucleus.

3.2. Expression and purification of LacI-His₆-GFP

1. Transform the pLacI-His₆-GFP plasmid into competent BL21 bacteria. Plate transformed bacteria on LB plates containing 50 µg/mL ampicillin. Grow overnight at 37° C.
2. Inoculate a 5 mL LB culture containing 50 µg/mL ampicillin with a single colony. Shake the culture in a 30° C incubator overnight.
3. Inoculate a 500 mL LB culture containing 50 µg/mL ampicillin with 50 µL of the overnight culture. Shake the culture in a 30° C incubator overnight.
4. Pellet bacteria by centrifugation at 3000 × g for 10 min. Discard the supernatant and freeze the pellet at –80° C.
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 1s on, then 1s off. After 15 bursts, pause for 1 minute. Repeat this cycle three more times.

²³The GFP signal is often strongest in intestinal nuclei, but since these nuclei are polyploid, the number of GFP foci cannot be used to determine homozygosity. Be careful to analyze only healthy adult worms, since heat shock often does not induce robust GFP-LacI expression in elderly worms.

8. Pellet insoluble debris by centrifugation at $10,000 \times g$ for 20 minutes 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 $700 \times g$ for 2 minutes, 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 minutes. Centrifuge at $700 \times g$ for 5 minutes. Remove the supernatant.
11. Add 20 mL 1x Equilibration/Wash Buffer to resin. Rock 10 minutes, then centrifuge and remove the supernatant. Repeat 2x.
12. Add 2 mL Equilibration/Wash Buffer to resin and vortex to resuspend. Pour into column, let settle, then drain until liquid is at level of top of bed. Wash with 10 mL Equilibration/Wash Buffer.
13. Wash with 10 mL Wash Buffer with 5 mM imidazole.
14. Elute in 10 mL Elution Buffer. Collect 0.5 mL fractions.
15. Fractions containing LacI-His₆-GFP will be noticeably yellow. Pool these fractions and concentrate in an Amicon Ultra-15 Centrifugal Filter Unit (30 kDa MWCO) by centrifuging at $4000 \times g$ for 15 minutes. This should be sufficient to reduce the volume to approximately 250–500 μ L.
16. Discard the flowthrough, then add 10 mL of 1x PBS to the filter unit. Centrifuge at $4000 \times g$ for 15 minutes or until retentate volume is less than 500 μ L. Repeat 2x.
17. Gently pipet retentate up and down to resuspend any LacI-His₆-GFP precipitate that may have collected on the membrane. Transfer to a microcentrifuge tube and adjust the total volume to 500 μ L by addition of 1x PBS.
18. Add 835 μ L of 80% glycerol and mix thoroughly by pipetting gently up and down. Centrifuge at top speed in a refrigerated microcentrifuge to pellet any aggregates.
19. Freeze 100 μ L aliquots in liquid N₂. Store one working aliquot at -20° C. The remaining tubes can be stored for several years at -80° C.
20. Determine ideal concentration for staining: Dissect gonads, then fix and stain with LacI-His₆-GFP diluted 1:250, 1:500, 1:1000, and 1:2000 in block (see Section 3.4).

3.3. Generating *syIs44* heterozygotes

1. Set up 4–8 crosses, each with 6–10 *syIs44* positive males and a single *syIs44* 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 (*e.g.*, 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-His₆-GFP

1. Isolate L4 hermaphrodites that are homozygous for the AOI from working crosses (*see Section 3.3*). These worms should be heterozygous for *syIs44*. Grow the animals for 24 hours 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 ~30s, then move slides to a drying rack. Dry slides in a drying oven (approximately 30 minutes at 80° C or three hours 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 1x Egg Buffer + Tween-20 and Levamisole (EBTL): To 885 µL water, add 100 µL 10x EB, 10 µL 250 mg/mL levamisole, and 5 µL 20% Tween-20. Make fresh on day of staining.
4. Prepare 1x Egg Buffer + 2% Paraformaldehyde (EB-PFA): To 775 µL water, add 100 µL 10x 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 10 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.

8. Using your scalpel, lop off the head of each worm between the pharynx and the gonad (*see* Note 24).
9. Once all worms have been dissected, add 6.5 μ L of EB-PFA.
10. Gently lower a coverslip at a 45° angle to the slide, such that the coverslip entirely covers the grease square but still hangs over one edge of the slide (Fig. 2B).
11. Examine the slide. It should be clear whether the dissected worms stuck to the slide and if the coverslip has compressed the gonadal tissue. Ideally, the coverslip should provide sufficient pressure to slightly flatten the gonad. If this is not the case, press the torn edge of a paper towel gently against the side of the coverslip where it meets the slide to wick out a small amount of liquid. Continue until the coverslip is lightly pressing down on the gonad.
12. Incubate the slide in a humid chamber for 5 minutes, then gently lower the slide into a beaker filled with liquid nitrogen.
13. Slides should be left in liquid nitrogen for at least two minutes, but may be kept in liquid nitrogen for much longer. If preparing more than one slide, repeat steps 5–12, collecting slides in liquid nitrogen until all animals have been dissected and fixed.
14. Remove the slide from liquid nitrogen with a pair of forceps. Hold the slide with the coverslipped side pointed away from your body, and flick the overhanging corner of the coverslip to remove the coverslip from the slide.
15. Immerse the slide in a Coplin jar filled with –20° C methanol for 1 minute, then move the slide to a Coplin jar filled with PBST wash buffer. Wash for 5 minutes. Repeat 1x.
16. Wipe off the front and back surfaces of the slide (except for the area inside the grease square). Add 20 μ L blocking solution and incubate for 30 minutes in a humid chamber.
17. Wick off the blocking solution with a folded-up Kimwipe or paper towel. Add 5 μ L of LacI-His₆-GFP diluted in PBS + 1% BSA + 0.2% Tween 20. Place the slide in a humid chamber. Incubate at least 1 hour at room temperature or overnight at 4° C.
18. Wash 3x in PBST, 10 minutes each.
19. Wipe off the front and back surfaces of the slide. Add 5 μ L of anti-GFP primary antibody diluted in PBS + 1% BSA + 0.2% Tween 20. Place the

²⁴It is also possible to extrude the gonad by nicking the tail of the worm. However, in my hands the best extrusion occurs when the incision is made just posterior to the pharyngeal bulb.

slide in a humid chamber. Incubate at least 1 hour at room temperature or overnight at 4°C.

20. Wash 3x in PBST, 10 minutes each.
21. Wipe off the front and back surfaces of the slide. Add 5 µL of fluorescently-labelled secondary antibody diluted in PBS + 1% BSA + 0.2% Tween 20. Place the slide in a humid chamber. Incubate at least 1 hour at room temperature or overnight at 4°C.
22. Wash 3x in PBST, 10 minutes 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 one hour before examining the slide on a microscope.

3.5. Analysis of LacI-GFP focus number and distribution

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 micron 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). With this information, one can determine whether SCC is normal or defective. However, not all SCC defects are equal, and measuring the distance between LacI-GFP foci can reveal differences in the severity of SCC disruption in different mutants (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-30/1.49t 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 dropdown menu entitled, “Stack viewing/View stack with:” to “Hyperstack,” and the dropdown 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 dropdown 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, labelled “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 (see 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

²⁵In regions where two nuclei contact one another, it may not be possible to determine which nucleus a LacI-GFP focus belongs to. In such cases, both nuclei should be excluded from the analysis.

- 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 (*see* Note 26).
 14. For each row, subtract the X, Y, and Z coordinates in columns in G–L from the X, Y, and Z coordinates in columns A–F, respectively, to calculate the distance between the two LacI-GFP spots in each dimension. I will refer to these distances as $d(x)$, $d(y)$ and $d(z)$. For nuclei in which two foci could not be resolved, all three distances should equal zero. For nuclei in which two foci could be resolved, some values will be positive 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 $d(x)$, $d(y)$, and $d(z)$ all have the same units (*e.g.*, pixels or microns). $d(x)$ and $d(y)$ may be converted from pixels to microns by multiplying by the XY pixel size, in microns. $d(z)$ may be converted from pixels to microns by multiplying $d(z)$ by the step size, in microns, used during image acquisition. If the XY pixel dimensions are not known, they can be measured using a stage

²⁶The following process simplifies pasting the coordinates of two subnuclear foci into one row of the spreadsheet: After copying the data from the Fiji Results window, paste the data into columns A–F of the spreadsheet, leaving a single empty row between any existing data and the data you are pasting. Next, paste the same data into columns G–L, but this time, do not leave an empty row above the pasted data. Delete the first row (the row with empty cells in columns A–F and the coordinates of the first focus analyzed in columns G–L) and every second row after that.

micrometer; however, the methods used to determine pixel size are beyond the scope of this work.

16. Once $d(x)$, $d(y)$ and $d(z)$ have been calculated and converted to the same units, the shortest distance between each pair of foci can be calculated using the equation $D = [d(x)^2 + d(y)^2 + d(z)^2]^{1/2}$.

3.6. Analysis of meiotic chromosome segregation using chromosome II RFLPs

1. Cross 1: To generate heteroallelic *sup-9(1020)/sup-9(1012)* hermaphrodites that are homozygous for your AOI, cross *sup-9(n1020)* males with *sup-9(n1012)* hermaphrodites. The males and hermaphrodites should both carry the balanced AOI. Ensuring that the same *sup-9* allele is always contributed by the male simplifies a data validation step that will be performed later.
2. Cross 2: When Cross 1 is working, pick 8–12 L4 hermaphrodites that are homozygous for your AOI individually onto mating plates. Add 6–10 young adult *him-8(e1489)IV*; *mIs10[myo-2::GFP]V* males to each plate (Cross 2) (*see* Notes 27 and 28).
3. Collect embryos for analysis: When Cross 2 is working, transfer the mated hermaphrodites and males to fresh mating plates. Check the plates frequently to observe the number of eggs laid. When most worms have laid approximately 12–15 embryos, remove the worms from the plates (*see* Note 29).
4. Allow the collected embryos to develop on the plate for approximately 16 hours at 20° C. This amount of time should be sufficient to allow all embryos to develop until they have either hatched or ceased divisions. However, it should be short enough that the embryos will not decay to the point that their DNA is unsuitable for PCR analysis.
5. Prepare a mouth pipet: Hold a capillary tube by both ends and heat the middle over a gas burner until the glass begins to melt. Remove the capillary from the flame and quickly pull the ends in opposite directions. Ideally, this will draw out the molten glass such that a thin, relatively

²⁷Many meiotic mutants produce aneuploid embryos that die during embryogenesis. Thus, one cannot use the presence of male progeny to determine when a cross has started working. For this reason, it is helpful to use males that are homozygous for a transgene that drives robust GFP expression, such as *mIs10[Pmyo-2::gfp]*. Because many aneuploid embryos undergo cellular differentiation before arresting, the pharyngeal GFP expressed from the *mIs10* transgene can be detected in arrested embryos once the cross is working.

²⁸This cross will allow analysis of defects in chromosome segregation during oocyte meiosis. To analyze defects during spermatogenesis, mate mutant males produced in Cross 1 with wild-type hermaphrodites (9). If the mutation of interest results in high levels of aneuploidy, successful crosses can be identified by the presence of dead embryos.

²⁹We collect 12–15 embryos from each of 8–12 crosses for three reasons. First, if some embryos produced by a hermaphrodite hatch and others arrest during embryonic development, it is easy to introduce experimental bias by selecting a disproportionate number of dead embryos or hatched larvae for analysis. This can be avoided by collecting a relatively small number of progeny from each hermaphrodite and genotyping them all for the *sup-9* alleles. Second, at least 6 embryos or larvae should be analyzed from each worm. If 6 or more embryos all have the *sup-9(n1012)* allele derived from the grandmother but lack the *sup-9(n1020)* allele derived from the grandfather, the grandmother was almost certainly a *sup-9(n1012)* homozygote rather than the desired *sup-9(n1012)/sup-9(n1020)* transheterozygote. Third, embryos from at least six worms should be included in the analysis in case there is brood-to-brood variability.

flexible central region connects the two ends. Carefully bend the thin central region near one end to break the glass, creating a mouth pipet with an approximately 1.5 inch long, narrow tip. Repeat for the other side. The inside diameter of the tip should be slightly larger than an embryo (approximately 100 μm).

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 μL aliquot of *C. elegans* lysis buffer and dispense 5 μL into each well of a 96-well plate. Centrifuge the plate briefly to collect the liquid in the bottom of the wells. Keep the plate on ice or in a 96-well plate cooling block between embryo additions.
8. Flood one plate of embryos with 1–2 ml of water. Place the tip of the mouth pipette next to an embryo or larva and apply gentle suction to the aspirator tube assembly to draw the specimen into the capillary tube (see Note 30). It may be necessary to gently dislodge embryos from the surface of the plate using the tip of the capillary tube. Place the 96-well plate of lysis buffer on the dissecting scope and focus on the first well. If possible, use low enough magnification that both the top of the well and the drop of lysis buffer are in focus. Carefully insert the tip of the capillary into the tube until it is immersed in lysis buffer. It may be necessary to adjust the focus or magnification during this process. Once the capillary has broken the surface of the lysis buffer, increase the magnification and focus on the tip of the capillary. Gently blow the embryo or larva out of the mouth pipet. As long as no air bubbles are created, the specimen should be visible as it exits the tip. Repeat this process with the remaining embryos and larvae on the mating plate, then repeat for the remaining mating plates until samples have been added to 90 wells. Make sure that each well receives only one embryo or larva. If there is any doubt that a well contains a single specimen, mark the questionable well for exclusion from subsequent analysis. Keep track of which mating plate each specimen came from and whether it was a larva or arrested embryo. To the remaining 6 wells, add three L1 larvae from *sup-9(1012)* homozygous worms and three from *sup-9(n1020)* homozygous worms as controls.
9. Seal the plate and freeze at -80°C for one hour to overnight.

³⁰Avoid 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 change the volume and composition of the lysis reaction.

10. Thaw the 96-well plate and gently remove the seal. Thaw a 500 μ L aliquot of *C. elegans* lysis buffer and dispense 5 μ L into each well. Centrifuge the plate briefly to collect the liquid in the bottom of the wells.
11. Seal the plate and incubate at 60° C for 60 minutes, then 95° C for 20 minutes to lyse the embryos.
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 *sup-9* PCR mastermix (or prepare fresh). Add 15 μ L *Taq* polymerase (5 U/ μ L). Mix well by gently pipetting up and down. Dispense 20 μ L 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 second extension time (*see* 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 μ L 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 μ L of PCR product can be included in each digest.
17. Replicate the PCR plate to 3 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 μ L by addition of water, if necessary.
18. Prepare three 2x restriction digestion mastermixes (*see* Note 32). Keep the mastermixes on ice and add the enzymes just before dispensing into 96-well plates. After adding the enzymes, mix the reaction well by pipetting up and down. *SpeI* single digest: To 540 μ L water, add 140 μ L 10x CutSmart buffer and 20 μ L of *SpeI*. *XbaI* single digest: To 520 μ L water, add 140 μ L 10x CutSmart buffer and 40 μ L of *XbaI*. *SpeI*+*XbaI* double

³¹Commercial hot-start products are not necessary for successful amplification of the *sup-9* amplicon. However, this single-embryo PCR is somewhat touchy, and I recommend keeping the PCR plate on ice or in a 96-well plate cooling block until the thermocycler has reached the denaturation temperature. The program can then be paused temporarily while the PCR plate is placed in the machine.

³²The recipes described here were developed using enzymes and buffers from New England Biolabs, but they should work equally well with enzymes from other manufacturers with minimal changes.

digest: To 500 μ L water, add 140 μ L 10x CutSmart buffer, 20 μ L of SpeI and 40 μ L of XbaI.

19. Add 7 μ L of mastermix to each well of a 96 well plate. If available, a repeat pipettor greatly simplifies this process.
20. Seal each plate and centrifuge briefly to collect liquid in the bottom of the wells. Incubate plates overnight in a 37° C incubator (*see* Note 33).
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 *sup-9(n1012)* and *sup-9(n1020)* homozygous worms were digested to completion. If so, the SpeI and SpeI+XbaI digested DNA from *sup-9(n1012)* and the XbaI and SpeI+XbaI digested DNA from *sup-9(n1020)* mutants should have a prominent band of approximately 800 base pair but no detectible 1046 base pair 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-progeny of the *sup-9(n1020)/sup-9(n1012)* hermaphrodite used in Cross 2 rather than cross progeny of the hermaphrodite and the *him-8; mIs10* males. All cross progeny will have a *sup-9(+)* allele inherited from the father, resulting in an uncut 1046 base pair band in the SpeI+XbaI double digest. Individuals lacking this band should be excluded from the analysis.
24. Identify lanes with non-specific PCR or digestion products. All digests other than those in the six control lanes should have either one band of 1046 base pair or two bands, one of 1046 base pairs and one of approximately 800 base pairs. An approximately 200 base pair digestion product may also be visible, but this band is often faint or undetectable. If additional bands are present in a digest, data from that individual should be excluded from the analysis.
25. Determine whether each specimen remaining in the analysis inherited only the *sup-9(n1012)* allele, only the *sup-9(n1020)* allele, both *sup-9* alleles, or neither *sup-9* allele. Detection of an approximately 800 base pair band in the SpeI single digest indicates that the embryo inherited the *sup-9(n1012)* allele, while detection of an approximately 800 base pair band in the XbaI single digest indicates that the embryo inherited the *sup-9(n1020)* allele.

³³I usually place the three 96-well plates in covered 96-well plate racks, then place these in a single layer on the bottom of a covered plastic bin that I have pre-warmed to 37° C. I put several layers of wet paper towels both underneath and on top of the plates to minimize any chance of evaporation. However, this may not be necessary.

26. Compare the data for all embryos and larvae collected from the same mother. Did any inherit the *sup-9(n1020)* allele? If not, the mother was likely a *sup-9(n1012)* homozygote rather than a *sup-9(n1020)/sup-9(n1012)* heterozygote, and the data from all of the siblings should be excluded from the analysis.
27. For all individuals that passed the three quality control checks (control PCR products from the *sup-9(n1012)* and *sup-9(n1020)* homozygotes were digested to completion, an uncut band was present in the SpeI+XbaI double digest, and the *sup-9(n1020)* allele was detected in at least one sibling), tabulate how many inherited only the *sup-9(n1012)* allele, only the *sup-9(n1020)* allele, both *sup-9* alleles, and neither *sup-9* allele.
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 *sup-9(n1012)* allele, and the other 50% should have inherited only the *sup-9(n1020)* allele (Fig. 1C, Reductional pattern). If crossover formation failed but sisters remained together during meiosis I, as occurs in *coh-4 coh-3* double mutants (9), it is expected that 25% of individuals would inherit only the *sup-9(n1012)* allele, 25% would inherit only the *sup-9(n1020)* allele, 25% would inherit both *sup-9* alleles, and 25% would not inherit either *sup-9* allele (Fig. 1C, Random pattern). If meiotic recombination failed and premature, equational sister separation occurred in meiosis I (as occurs in *rec-8* mutants) (9), most individuals analyzed should test positive for both *sup-9(n1012)* and *sup-9(n1020)* (Fig. 1C, Equational pattern) (see Note 34).

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References

1. Nasmyth K, Haering CH. Cohesin: its roles and mechanisms. *Annu Rev Genet.* 2009; 43:525–558. DOI: 10.1146/annurev-genet-102108-134233 [PubMed: 19886810]

³⁴It is formally possible that patterns of chromosome segregation other than those described here could give rise to similar patterns of RFLP inheritance. For example, the reason that the progeny of *rec-8* mutant hermaphrodites test positive for both RFLPs is that premature, equational sister separation occurs during meiosis I, then polar body extrusion fails during meiosis II. The zygote therefore inherits one sister from each homolog. Consistent with this, DAPI staining of fixed 1 and 2 cell-stage embryos shows that most embryos have a single polar body (9). The same pattern of RFLP inheritance would be observed if polar body extrusion failed during both rounds of meiosis and the zygote inherited every chromatid from every homolog. However, in such cases, zygotes would not have any polar bodies. Thus, it is desirable to examine DAPI stained early embryos (1–4 cell stage) and also LacI-GFP/*lacO* in pronuclear migration-stage embryos to validate the conclusions drawn through RFLP analysis (9).

2. Klein F, Mahr P, Galova M, et al. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell*. 1999; 98:91–103. [PubMed: 10412984]
3. Buonomo SB, Clyne RK, Fuchs J, et al. Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. *Cell*. 2000; 103:387–98. [PubMed: 11081626]
4. Kitajima TS, Miyazaki Y, Yamamoto M, Watanabe Y. Rec8 cleavage by separase is required for meiotic nuclear divisions in fission yeast. *EMBO J*. 2003; 22:5643–53. [PubMed: 14532136]
5. Guacci V, Koshland D, Strunnikov A. A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in *S. cerevisiae*. *Cell*. 1997; 91:47–57. [PubMed: 9335334]
6. Michaelis C, Ciosk R, Nasmyth K. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell*. 1997; 91:35–45. [PubMed: 9335333]
7. Birkenbihl RP, Subramani S. Cloning and characterization of rad21 an essential gene of *Schizosaccharomyces pombe* involved in DNA double-strand-break repair. *Nucleic Acids Res*. 1992; 20:6605–6611. [PubMed: 1480481]
8. Uhlmann F, Wernic D, Poupert MA, et al. Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. *Cell*. 2000; 103:375–386. [PubMed: 11081625]
9. Severson AF, Ling L, van Zuylen V, Meyer BJ. The axial element protein HTP-3 promotes cohesin loading and meiotic axis assembly in *C. elegans* to implement the meiotic program of chromosome segregation. *Genes Dev*. 2009; 23:1763–78. DOI: 10.1101/gad.1808809 [PubMed: 19574299]
10. Severson AF, Meyer BJ. Divergent kleisin subunits of cohesin specify mechanisms to tether and release meiotic chromosomes. *eLife*. 2014; 3:e03467.doi: 10.7554/eLife.03467 [PubMed: 25171895]
11. Lee J, Hirano T. RAD21L, a novel cohesin subunit implicated in linking homologous chromosomes in mammalian meiosis. *J Cell Biol*. 2011; 192:263–276. DOI: 10.1083/jcb.201008005 [PubMed: 21242291]
12. Ishiguro K, Kim J, Fujiyama-Nakamura S, et al. A new meiosis-specific cohesin complex implicated in the cohesin code for homologous pairing. *EMBO Rep*. 2011; 12:267–275. DOI: 10.1038/embor.2011.2 [PubMed: 21274006]
13. Herran Y, Gutierrez-Caballero C, Sanchez-Martin M, et al. The cohesin subunit RAD21L functions in meiotic synapsis and exhibits sexual dimorphism in fertility. *EMBO J*. 2011; 30:3091–3105. DOI: 10.1038/emboj.2011.222 [PubMed: 21743440]
14. Llano E, Herran Y, Garcia-Tunon I, et al. Meiotic cohesin complexes are essential for the formation of the axial element in mice. *J Cell Biol*. 2012; 197:877–885. DOI: 10.1083/jcb.201201100 [PubMed: 22711701]
15. Yuan L, Yang X, Ellis JL, et al. The *Arabidopsis* SYN3 cohesin protein is important for early meiotic events. *Plant J*. 2012; 71:147–160. DOI: 10.1111/j.1365-313X.2012.04979.x [PubMed: 22381039]
16. Ishiguro KI, Kim J, Shibuya H, et al. Meiosis-specific cohesin mediates homolog recognition in mouse spermatocytes. *Genes Dev*. 2014; 28:594–607. DOI: 10.1101/gad.237313.113 [PubMed: 24589552]
17. Belmont AS. Visualizing chromosome dynamics with GFP. *Trends Cell Biol*. 2001; 11:250–257. [PubMed: 11356361]
18. Belmont AS, Straight AF. In vivo visualization of chromosomes using lac operator-repressor binding. *Trends Cell Biol*. 1998; 8:121–124. [PubMed: 9695822]
19. Robinett CC, Straight A, Li G, et al. In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. *J Cell Biol*. 1996; 135:1685–1700. [PubMed: 8991083]
20. Straight AF, Belmont AS, Robinett CC, Murray AW. GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. *Curr Biol*. 1996; 6:1599–1608. [PubMed: 8994824]
21. Askjaer P, Ercan S, Meister P. Modern techniques for the analysis of chromatin and nuclear organization in *C. elegans*. *WormBook*.

22. Yuen K W Y, Nabeshima K, Oegema K, Desai A. Rapid de novo centromere formation occurs independently of heterochromatin protein 1 in *C. elegans* embryos. *Curr Biol.* 2011; 21:1800–1807. DOI: 10.1016/j.cub.2011.09.016 [PubMed: 22018540]
23. Bilgir C, Dombecki CR, Chen PF, et al. Assembly of the Synaptonemal Complex is a Highly Temperature-Sensitive Process that is Supported by PGL-1 during *Caenorhabditis elegans* Meiosis. *G3 (Bethesda).* 2013; doi: 10.1534/g3.112.005165
24. Gonzalez-Serricchio A, Sternberg P. Visualization of *C. elegans* transgenic arrays by GFP. *BMC Genet.* 2006; 7:36–44. [PubMed: 16759392]
25. Monen J, Maddox PS, Hyndman F, et al. Differential role of CENP-A in the segregation of holocentric *C. elegans* chromosomes during meiosis and mitosis. *Nat Cell Biol.* 2005; 7:1148–1155. DOI: 10.1038/ncb1331
26. Martinez-Perez E, Villeneuve AM. HTP-1-dependent constraints coordinate homolog pairing and synapsis and promote chiasma formation during *C. elegans* meiosis. *Genes Dev.* 2005; 19:2727–43. [PubMed: 16291646]
27. Reddy KC, Villeneuve AM. *C. elegans* HIM-17 links chromatin modification and competence for initiation of meiotic recombination. *Cell.* 2004; 118:439–52. [PubMed: 15315757]
28. Nabeshima K, Villeneuve AM, Hillers KJ. Chromosome-wide regulation of meiotic crossover formation in *Caenorhabditis elegans* requires properly assembled chromosome axes. *Genetics.* 2004; 168:1275–92. [PubMed: 15579685]
29. Davis MW, Hammarlund M, Harrach T, et al. Rapid single nucleotide polymorphism mapping in *C. elegans*. *BMC Genomics.* 2005; 6:118.doi: 10.1186/1471-2164-6-118 [PubMed: 16156901]
30. Schvarzstein M, Pattabiraman D, Libuda DE, et al. DNA helicase HIM-6/BLM both promotes MutSγ-dependent crossovers and antagonizes MutSγ-independent interhomolog associations during *Caenorhabditis elegans* meiosis. *Genetics.* 2014; 198:193–207. DOI: 10.1534/genetics.114.161513 [PubMed: 25053665]
31. Frøkjær-Jensen C, Davis MW, Sarov M, et al. Random and targeted transgene insertion in *Caenorhabditis elegans* using a modified Mos1 transposon. *Nat Methods.* 2014; 11:529–534. DOI: 10.1038/nmeth.2889 [PubMed: 24820376]
32. Checchi PM, Lawrence KS, Van MV, et al. Pseudosynapsis and Decreased Stringency of Meiotic Repair Pathway Choice on the Hemizygous Sex Chromosome of *Caenorhabditis elegans* Males. *Genetics.* 2014; 197:543–560. DOI: 10.1534/genetics.114.164152 [PubMed: 24939994]
33. Cortes DB, McNally KL, Mains PE, McNally FJ. The asymmetry of female meiosis reduces the frequency of inheritance of unpaired chromosomes. *Elife.* 2015; doi: 10.7554/eLife.06056
34. Darby RAJ, Hine AV. LacI-mediated sequence-specific affinity purification of plasmid DNA for therapeutic applications. *FASEB J.* 2005; 19:801–803. DOI: 10.1096/fj.04-2812fje [PubMed: 15760969]
35. Sambrook, J.; Russel, DW. *Molecular cloning: a laboratory manual.* Cold Spring Harbor Laboratory Press; New York: 2001.
36. Skop AR, White JG. The dynactin complex is required for cleavage plane specification in early *Caenorhabditis elegans* embryos. *Curr Biol.* 1998; 8:1110–6. [PubMed: 9778526]
37. Phillips CM, McDonald KL, Dernburg AF. Cytological analysis of meiosis in *Caenorhabditis elegans*. *Methods Mol Biol.* 2009; 558:171–195. DOI: 10.1007/978-1-60761-103-5_11 [PubMed: 19685325]
38. Edgar LG. Blastomere culture and analysis. *Methods Cell Biol.* 1995; 48:303–321. [PubMed: 8531731]
39. Nabeshima K. Chromosome structure and homologous chromosome association during meiotic prophase in *Caenorhabditis elegans*. *Methods Mol Biol.* 2011; 745:549–562. DOI: 10.1007/978-1-61779-129-1_32 [PubMed: 21660716]
40. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods.* 2012; 9:676–682. DOI: 10.1038/nmeth.2019 [PubMed: 22743772]
41. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* 2012; 9:671–675. [PubMed: 22930834]
42. Greenwald I, Horvitz HR. A visible allele of the muscle gene sup-10X of *C. elegans*. *Genetics.* 1986; 113:63–72. [PubMed: 3710144]

Figure 1.

A method to detect meiotic chromosome segregation errors using restriction fragment length polymorphisms. (A) A series of two crosses is used to generate embryos in which the patroclinous copy of chromosome II and both potential matroclinous copies can be molecularly identified by restriction fragment length polymorphisms (RFLPs). The RFLPs result from single nucleotide polymorphisms (SNPs) in the *sup-9* gene. *aoi* = the mutant allele of interest being tested for effects on meiotic chromosome segregation. *Bal* = the balancer chromosome used to allow the stable propagation of *aoi* heterozygotes. (B) In the second cross of the series, male worms that are homozygous for the wild-type *sup-9* allele are mated with heteroallelic *sup-9(n1020)/sup-9(n1012)* hermaphrodites that were

generated in the first cross. The mutation in *sup-9(n1012)* creates a SpeI restriction site, while the mutation in *sup-9(n1020)* creates an XbaI site. To genotype embryos and larvae for the wild-type, *n1012*, and *n1020* alleles of *sup-9*, a PCR product that spans both SNPs is cut in three restriction digests: a SpeI single digest, an XbaI single digest, and a SpeI+XbaI double digest. Analysis of the restriction fragments by agarose gel electrophoresis can yield information regarding the pattern of meiotic chromosome segregation. (B,C) In meiosis I of wild-type animals, homologs segregate away from one another (reductional division). One set of homologs is inherited by the zygote, and the other is extruded into the first polar body (PB1). Sisters separate in meiosis II. One set of sisters is inherited by the zygote, and the other is extruded into the second polar body (PB2). The zygote is equally likely to inherit the SpeI RFLP or the XbaI RFLP. In meiosis I of *rec-8* mutants, sister chromatids segregate prematurely away from one another (equational division). The zygote therefore inherits both the SpeI and the XbaI RFLPs. Polar body extrusion fails in meiosis II. In *coh-4 coh-3* double mutants, homologs segregate randomly in meiosis I, and the zygote is equally likely to inherit the SpeI RFLP, the XbaI RFLP, neither RFLP, or both RFLPs.

Figure 2.

Freeze-crack fixation of dissected gonads for LacI-GFP staining. (A) An approximately 1 cm square grease barrier is drawn on the slide surface using a PAP pen. The hydrophobic barrier allows staining with as little as 5 μ L of antibody solution. (B) After gonads have been microsurgically extruded from the body of the worm and fixed in PFA, a coverslip is lowered onto the slide at a 45° angle. The overhanging lip allows easy removal of the coverslip by flicking it off with the tip of a finger.