

David B. Fankhauser, PhD

16Jan92, rvsn 16Jan96, 6Jan97, 21Jan97, 5Jan98, 4Jan02, 2Jan03, 18Jan05, 30Dec08, 19Jan10, 18Jan11

http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Drosophila_chromosomes/Drosophila_Chromosomes.htmNOTE: **Five points are awarded** when you show the instructor your banded chromosomes!**Equipment & Supplies:**

paint brush
 microscope slides
 cover slips
 dissecting scope w/ transilluminator
 two *sharp* dissecting needles

Per desk in a test tube rack:

three Pasteur pipets with bulbs
 three 13x100 mm t.t. labeled: Ringers
 HOAc
 Waste

Drosophila melanogaster third instar larvae
 (Start the culture one week earlier than lab.)

In dropper bottles:

1) Ringer's solution (or other isotonic solution)
 2) 45% acetic acid (HOAc) (take 1 mL in test tube
 and rack to your desk)
 3) aceto-orcein stain (filtered and *without crystals*)
 Bunsen burner (two at the side to warm staining slides)
 microscope
 immersion oil
 clear nail polish

- Pick a third instar larvae** from a culture flask by picking up with a dissecting needle (or a paint brush with rolling motion), place it in a small drop of Ringer's solution on a microscope slide. If you have too much liquid, it jumps when touched by needles. Too little, the glands dry out.
- Isolate a pair of salivary glands** by dissecting out with two needles under a dissecting scope as demonstrated. (The glands are attached close to mouth, transparent, and paired). Push all debris to edge of drop and remove with Pasteur pipet. Clean glands of extra material (fat bodies, etc) using needle tips as "scissors." Keep wet but not too much liquid.
- Fix in 45% HOAc:** Remove most of the Ringer's solution to the waste tube with a Pasteur pipette, replace with 45% HOAc to fix the tissue. Make sure glands are surrounded with/and immersed in HOAc. Trim off any superfluous material which may still be present. Let sit for at least 10-15 minutes. Meanwhile, illustrate dissected-out glands.
- Remove HOAc** with Pasteur pipette, discard in "waste" test tube. Wash again with 45% HOAc if floating particles are still visible. Always keep glands in view. *Never let the glands dry out.*
- Stain with aceto-orcein stain**, covering the fixed glands. Make sure glands are surrounded by and immersed in stain. Warm by placing on a 37 C hot block. (Do not let dry out!) Check periodically under dissecting scope. It may take 20 minutes for the nuclei to become *dark*.
- Remove stain, wash with 45% HOAc:** When gland nuclei look quite dark, carefully remove excess stain with a Pasteur pipette. (Keep a close eye on where the glands are--you don't want to suck them up...) Position glands in center of slide. Wash 2x with HOAc, remove and discard each successive wash. There should be no visible stain or other material except in the glands following the second wash. Leave a *small amount* of colorless HOAc in which the glands are immersed.
- Cover with coverslip:** With glands in center of slide, lie slide on white paper, lower coverslip carefully over the glands as if hinged. Note the red smudge formed by the flattened gland.
- Squash the chromosomes:** Holding the coverslip in place from the edges to prevent movement, pound firmly numerous times *straight down* with a pencil eraser over the flattened gland to squash it out. If it spreads well, examine with 40x objective to see chromosomes. If they look like balls of yarn, they need more squashing. Repeat tapping. *Do not smear* by moving the cover slip, it will shear the chromosomes.
- Examine under the microscope:** After adequate spreading is obtained, scan field with 10x objective, looking for well-spread and banded chromosomes. Switch to 40x objective, pick best banding. At 1000x, illustrate in your book. Take a photograph if possible. **CLEAN UP YOUR MICROSCOPE.** Carefully prepare the dissecting scope and lamp for storage, return to storage.
- To preserve your slide**, seal edges of the coverslip with a bead of fingernail polish, let completely dry, label slide: *Drosophila salivary chromosomes*, your name, and date.

cuthere:.....

REAGENTS AND STAINS FOR DROSOPHILA CHROMOSOME VISUALIZATION**Ringers:**

860 mg NaCl
 30 mg KCl
 35 mg CaCl₂

Dissolve in 100 mL d H₂O. Store at 4°C.**Aceto-orcein stain (freshly filtered):**

1 g orcein stain
 50 mL 45% HOAc

Heat to dissolve, let cool, freshly filter through
 Whatman #1 filter paper.

Observations 2005:

I let my glands fix for about 15 (or more) minutes. The chromosomes were well formed, bands very distinct.

Students may have smeared the chromosomes, they look stretched and slightly melted.

STILL needed to spread out chromosomes more, perhaps by many pounds to flex the chromosomes out away from the ball?

Problems 1997:

Students did not watch carefully the dissection of the glands, had trouble locating glands.

Critical point is just after pulling the head off--if you dig around blindly and lose the glands then you may not find them again.

Use the fat bodies as indicators of the glands--be sure that you have glands before cleaning them up

Aceto-orcein was FULL of crap. Filtered, and it seemed to work.

Don't forget to warm during the staining.

Problems 1996:

Offered 10 points for well banded chromosomes. Need to offer at beginning of class. (should be 5 points?)

Students could not find glands (many)

Some needles were dull.

students lost glands during staining (some)

Some students quit before 4:00, did not have chromosomes

squashed glands looked flattened, distorted.