Name: Date:

HANDS-ON LAB

Examining Banding Patterns in Polytene Chromosomes

BACKGROUND

Chromosomes are usually very difficult to see under the microscope. However, during metaphase (a phase in the process of cell division), they become more condensed than usual. There is one type of chromosome that is 100 to 200 times the size of an average metaphase chromosome and can be seen easily with a microscope. This is the *polytene chromosome.* These giant chromosomes were observed even 125 years ago. Polytene chromosomes are found in the larval salivary glands of some fly species. They are formed by repeated replication of DNA without separation, so that each chromosome consists of several hundred strands. The giant chromosomes also aggregate together at a central point, called the chromocenter, so the cell's DNA can resemble a sea star with long arms.

When polytene chromosomes are properly stained they show a detailed pattern, alternating between dark and light bands. The dark bands represent areas where the DNA is highly compacted. DNA in the lighter-stained areas is more loosely packed. The banding provides a diagram-like representation of the physical location of genes at specific sites in the cell. In fact, because of their size, polytene chromosomes have become a popular research tool. In this lab, you will observe banding patterns in polytene chromosomes.

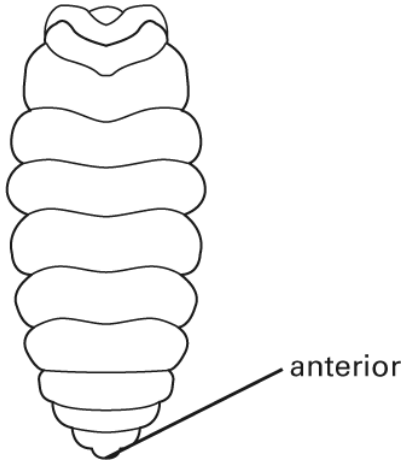
MATERIALS

- acetic acid solution (45%), 20 mL
- aceto-orcein stain, 10 mL
- blowfly larva
- compound microscope
- cover slip (4)
- dissecting pins
- forceps, 2 pairs
- lab wipes
- microscope slide (2)
- pencil with eraser
- pipette, plastic disposable (3)
- saline solution, 20 mL

PROCEDURE

1. Obtain a clean microscope slide and add a drop of saline solution.
2. Select a larva from the culture dish, carefully pick it up with a pair of forceps, and place it in the drop of saline on your slide. Take care not to crush the larva. Take a look at the larva and determine which end is the front (anterior). See the drawing below for comparison. The front end will have black mouthparts, is more pointed than the back end (posterior), and is generally moved forward as the larva crawls. You should be able to see this without magnification, but use a dissecting microscope if you need to.

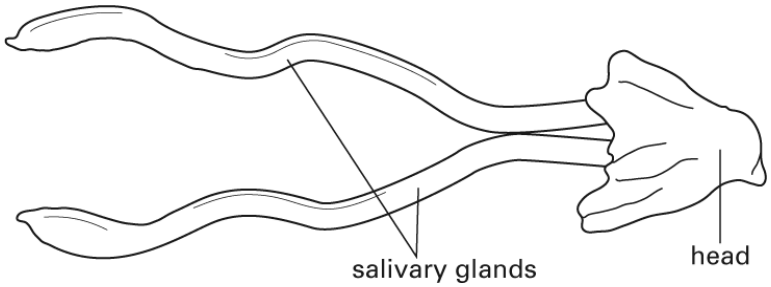
Blowfly larva



3. Gently grasp the back three-quarters of the larva with a forceps and squeeze it slightly so the larval head extends. Be sure to hold the larva still. Then, with another pair of forceps, carefully separate the head from the body.

4. View the larva (still on the slide) with a dissecting microscope. Be sure to keep the larva body and head moist, adding more saline when needed. Locate the salivary glands: they are clear, glistening, long sacs that were probably removed with the head. Be careful not to confuse the salivary glands with body fat, which has a net-like appearance.

Salivary glands and the larval head



5. If you don't find the salivary glands with the head, examine the body. If they are not visible around the body, then firmly hold the larva's body to the side with one dissecting needle and press down on the back end with the side of another dissecting needle and push toward the anterior end. This should push out the salivary glands, as well as the digestive tract and some fat.

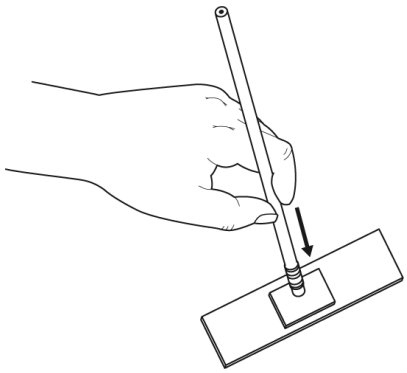
6. Once you have found the salivary glands, move them away from the other tissue and transfer them to a fresh drop of saline on a new slide. Make sure they don't dry out.

7. Wearing gloves and eye protection, cover the salivary glands with acetic acid solution for 1 minute. After 1 minute tilt the slide to drain the acid into the waste container provided by your teacher.

8. Next cover the glands with aceto-orcein stain for 2 minutes. After 2 minutes tilt the slide and let the stain drain to one side (but not off the slide). Look for the darkly stained salivary glands.

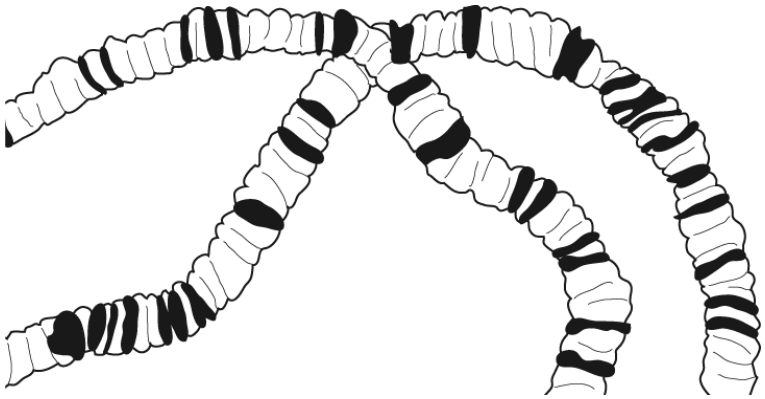
9. Once you have located the glands, rinse the stain from them and the slide with acetic acid solution (and rinse into the appropriate waste container). Keep rinsing until you see no more traces of stain coming from the glands. Be careful not to rinse the glands off the slide.

10. Carefully cover the glands with a cover slip. Put a tissue over the cover slip. With the eraser end of a pencil, gently press down on the cover slip as shown below. If you press too hard the cover slip may move or crack, so press gently. However, you do need to apply enough pressure so that the cells spread into a single layer and release the chromosomes from the nuclei.



11. Observe your preparation with a compound microscope on low power. Search for chromosomes that are spread out. If they are still in the nuclei (and look ball-shaped), press on the cover slip some more until they are released. Once you see them on low power, try observing them under high power. They should look like small ribbons or string with bands on them.

Polytene chromosomes



12. Also observe another student's or group's preparation, and note how it is similar to or different from yours.

13. If your teacher has one available, look at a previously prepared slide of a stained polytene chromosome with the compound microscope.

ANALYZE

1. What is a chromosome? What are the components of chromosomes?

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2. How is a polytene chromosome different from other chromosomes?

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3. Draw a picture in your Evidence Notebook of the isolated and stained chromosomes.

4. Were you able to see the banding patterns of the polytene chromosomes? If not, why do you think you were unable to see the pattern?

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5. Were you able to see the chromocenter? If not, where should it have been?

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6. How did your polytene chromosome preparation compare to the prepared slide you looked at?

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7. Acetic acid is used to break down the plasma membrane and nuclear membrane of the cell. What do you think would happen to your preparation if you did not use acetic acid?

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