Blaylock, B. Gordon.[1] Oak Ridge National Laboratory, Tenn. A method for preparing salivary-gland chromosomes.[2]

A modification of a method used by Keyl and Keyl (Arc. F. Hydrobiol 56:43-57, 1959) for Chironomus salivary chromosomes produces good results with Drosophila. There are several advantages in this method over most standard techniques. The staining time is short; only two minutes are required. Because there is good resolution of the fine bands on a clear background and the chromosomes spread easily, this is a good method for studying specific regions of the chromosomes.

The details of this method follow:

1. Dissect large well-fed larvae in a drop of Shen's solution (9 g of NaCl, 0.42 g of KCl, 0.25 g of $CaCl_2$, 1 l of H_2O) on a siliconed slide.
2. Immediately transfer the glands to a drop of 3 parts 95% ethyl alcohol and 1 part 45% acetic acid for 30 seconds. (A stop watch is used for timing).
3. Transfer the glands to a small drop of acetic carmine and acetic orcein on a siliconed cover glass for two minutes. The staining solution is 1 part of 1% carmine boiled in 45% acetic acid mixed with 4 parts of 1% natural orcein dissolved in 50% acetic acid.
4. Absorb the excess stain from the glands with a small strip of bibulous paper making sure that the paper does not come in contact with the glands by using a dissecting needle to hold the glands in place.
5. Immediately place a small drop of 72% lactic acid over the glands.
6. Invert the cover glass on a clean slide and hold it in place while tapping the cover glass several times directly over the glands with the eraser end of a pencil.
7. Fold a sheet of bibulous paper around the slide and press well with the thumb in a rolling motion being careful not to let the cover glass slip. A little experimenting will help determine the amount of pressure to use to obtain the desired amount of spreading.

The slide can be studied immediately using phase contrast. The preparations can be maintained for several weeks without sealing; however, the slides can be made permanent by the dry ice technique (Baker, W. K. DIS 26:129, 1959).

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Doane, W. W. Yale University. Use of disc electrophoresis for analysis of amylase isozymes.

A modification of the acrylamide gel disc electrophoresis method of Ornstein and Davis (1961) has been developed in order to analyze amylase isozymes in various Amy strains of D. melanogaster.

The small pore gel, in which separation occurs, consists of equal parts of "Small-Pore Solutions #1 and #2" but altered to include 0.2% Fisher soluble starch (or Connaught hydrolyzed starch). This is done by heating a 0.4% starch solution in a water bath brought to a boil and, when the solution becomes clear, warming it an additional 3 minutes before cooling. The small-pore solution #2 is then prepared by dissolving 0.14 g. ammonium persulfate in 100 ml. of this starch solution so that the final starch concentration of the gel is 0.2%. This concentration proved best for quantitative estimates of enzyme activity and also for photographic purposes.

Following electrophoresis, gels are removed from their tubes and incubated at 37°C. for given time intervals (from 0 to 45 min.) in either M/20 Tris buffer, pH 7.2, or M/15 phosphate buffer, pH 6.8. With the latter buffer, Cl^- ions should be added in order to enhance amylase activity; p-chloromercuribenzoic acid should also be included in order to inhibit glycogen phosphorylase activity. Immediately after incubation, gels are stained with iodine reagent (Smith & Roe, 1949) diluted to half-strength. This not only stops hydrolysis of starch inside the gel but also stains those regions where enzyme activity does not occur. After 5-10 minutes, gels are rinsed and transferred to 7% acetic acid. Amylase isozymes appear as clear bands in the dark blue gels. These should be photographed immediately as artifacts begin to develop within 15 to 20 minutes.