Moree, R., C.Z. Pakonen and N.E. Balkin. Washington State University, Pullman, Washington. Attempts to improve triple balancer strains.

In attempting to improve the chromosomal integrity of triple balancers we have tried the following combinations: 1) Basc/Basc;SM1/Pm;P88/TM1
2) Ext/FM6;SM1/Pm;P88/Sb
3) Ext/FM6;SM1/Pm;P88/TM2
4) FM7a/FM7a;SM1/Tg;TM6/Sb

P88 designates Ins(3LR)P88,In(3R)C,D^3 bx^{34e} e, an intermediate stage in the development of an improved third chromosome balancer by E.B. Lewis. TM6 designates E.B. Lewis's original TM6, to which he has added the excellent marker Ubx^{P15}. In combination 1) Basc, not unexpectedly, broke down too frequently to be useful. Combination 2) had extremely low productivity and some breakdown of P88, and so was discarded. Combination 3) was lost because of extremely low fecundity and viability; many pupae in the final crosses and in the stock failed to emerge. Combination 4), though somewhat better, was lost after several generations because of generally poor productivity. The schedules we used are available for anyone who would like to have them. Special thanks are due to Prof. E.B. Lewis for providing us with the intermediate stage and the improved form of TM6. (Aided by funds from the State of Washington Initiative Measure No. 171 for the Support of Biological and Medical Research.)

Selker, H.P.* and J.H. Postlethwait. University of Oregon, Eugene. Electrophoretic separation and detection of non-enzymatic proteins from single flies.

Although enzymes can be readily separated and detected in homogenates of single flies by standard electrophoretic techniques (e.g. Dickinson, 1971), detection of proteins from single flies using standard disc gel electrophoresis has not been feasible. So to detect non-enzymatic proteins, gels have been made in capillaries (Grossbach, 1965; Ward et al., 1970). These methods have required the use of a pair of expensive micromanipulators to form the stacking gel (Grossbach, 1965), or have involved a specially made apparatus to remove the gel from the tube (Ward et al., 1970). We have developed a simple procedure for capillary disc gel electrophoresis that avoids these problems and provides protein bands from a single ovary from a single fly.

Twenty-microliter Wiretrol^r (Drummond Scientific Co.) capillaries are filled completely with the desired resolving gel (such as used by Jovin et al., 1964) and are allowed to polymerize. Since de-gassing seems to help prevent bubbles from occurring in the gels, the gel solution without catalyst solution is aspirated five to ten minutes in a 25-milliliter filter flask. The catalyst solution is then added and the solution is allowed to aspirate a few more minutes. The gel solution is then introduced into the tube by capillary action.

After the resolving gel has polymerized, the gel is pushed from the bottom of the capillary by the Wiretrol^r plunger until a small length (ca. 3mm) of gel protrudes from the top. The protruding end is then cut off flat with a razor blade, and then pushed back into the tube by air pressure supplied by a 100 cc syringe fitted with an 18 gauge needle and connected to the top of the capillary by a length of polyethylene tubing (i.d. 0.047 in Intramedic^r by Clay Adams). If the gel is pushed back down 5 mm, this allows about three microliters total for the stacking gel and sample.

The stacking gel is applied immediately with a small pipette made by drawing out a 100-microliter Microcap^r (Drummond) until it is about 100 μ O.D.). In our experience, a third to a half of the remaining space should be filled with stacking gel (one to one and one half microliters). After the stacking gel has polymerized, the sample and tracking dye can be applied using a micropipette.

The gels can then be run on a conventional disc electrophoresis apparatus (e.g. Canalco Model 1200), the only adaptation needed being to use the gum rubber plugs from the bulb dispenser that comes in a Microcap^r package as an insert within the usual grommets in order to accomodate the small diameter of the capillaries.

The gels are run at 0.2 ma per gel and are removed from the tube by use of the Wiretrol plunger. They can then be stained with Coomassie Blue stain in 5:5:1 methanol:water:acetic acid, and destained in the same solution without the dye.

This method seems to work well with Acrylamide gels of about 4-5% with SDS, but successful runs have been done with 7.5% gels and without SDS. Higher percentage gels may form bubbles and adhere more strongly to the capillary wall, as do gels that have been allowed to stand for more than 3 hours. Both these problems are lessened by treating the capillaries

with photoflow and chilling them before putting in the resolving gel.

This research was supported by grant GM 19307 from NIH.

References: Dickinson, W.J. 1971, Develop. Biol. 26:77-88; Grossbach, U. 1965, Biochem. Biophys. Acta 107:180-182; Jovin, T., A. Chrambach and M.A. Naughton 1964, Anal. Biochem. 9: 351-369; Ward, S., D.O. Wilson and J.J. Gilliam 1970, Anal. Biochem. 38:90-97.

* Present address: Department of Biology, Reed College, Portland, Oregon 97202.

Guest, W.C. and T.C. Hsu. University of Arkansas, Fayetteville, and University of Texas M.D. Anderson Hospital, Houston.

A new technique for preparing Drosophila neuroblast chromosomes.

The value of D. melanogaster larval neuroblast cells for studying somatic metaphase chromosomes has long been recognized and a variety of squash techniques have been developed. Hsu (1971) used brain squashes to obtain metaphases for demonstrating the similarity of Drosophila heterochromatin to the heterochromatin in mammalian chromosomes. However, it is frequently difficult to obtain numerous division figures with well spread chromosomes using conventional squash techniques.

We have recently applied some of the Giemsa banding procedures widely used in studying mammalian chromosomes to Drosophila using a modification of the technique developed by Stock, Burnham and Hsu (1972) for obtaining well spread metaphase figures from solid tissues. This procedure, in addition to giving large numbers of metaphase and late prophase figures with well spread chromosomes, permits the slides produced to be handled as air dried preparations.

The procedure is as follows:

1. Twenty-five to thirty larval brains are dissected out in physiological saline or insect Ringer's solution. The brains should be washed several times in saline to remove debris. The physiological saline is replaced with a hypotonic solution. Both 1% sodium citrate and physiological saline diluted with equal parts of distilled water were used with ten to fifteen minutes giving good results. The hypotonic saline is removed with 1 ml tuberculin syringe or with a microcapillary pipette.

2. The ganglia are fixed in methanol acetic acid (3:1) with the fixative being changed several times to insure complete removal of water. The fixed material may be used immediately or may be stored in the fixative. Fixed material stored in absolute alcohol is much more difficult to work with than ganglia stored in the fixative.

3. Slides which have been cleaned thoroughly are warmed to 40-45^{o}C on a hot plate or slide warmer.

4. The fixed ganglia are transferred to a well slide. We used a standard depression slide with a cylindrical well 3 mm deep. Remove as much of the fixative as possible with a syringe or microcapillary pipette. It is helpful to perform these transfers under the low magnification of a stereoscopic microscope. Add approximately 0.2 ml of 60% acetic acid to the well. Under the dissecting microscope the tissue will begin to swell and become translucent. Using a Drummond micropipette (see Stock, et al. for description) agitate the material by drawing the ganglia into the pipette several times. As this is done the ganglia will begin to disintegrate. This is a critical step. If the material remains in the strong acetic acid too long the cells will be distorted or destroyed. Best results have been obtained by adding the acetic acid, agitating briefly, then applying the material to warmed slides while some of the brains are still intact but swollen.

5. The cell suspension and intact tissues are drawn into the Drummond pipette and a small drop placed on the heated slide. Immediately the drop is drawn back into the pipette leaving a thin circular film on the slide containing a monolayer of cells. This process is repeated many times over the entire slide very quickly while the slide is warm. An area that can be covered by a 22 x 40 or 22 x 50 mm cover slip can be spotted on each slide. It is important that the slide be kept warm to accelerate evaporation of the acetic acid.

6. The slides are dried thoroughly before using. While we have used slides made in this manner for banding chromosomes or for staining heterochromatin, the slides can be used in a number of techniques that require a monolayer of air dried cells.

References: Hsu, T.C. 1971, J. Heredity 62:285-287; Stock, A.D., D.B. Burnham and T.C. Hsu 1972, Cytogenetics 11:534-539.