Miklos, G.L.G. The Australian National University, Canberra, Australia. The isolation of high molecular weight DNA from adult heads of D.melanogaster.

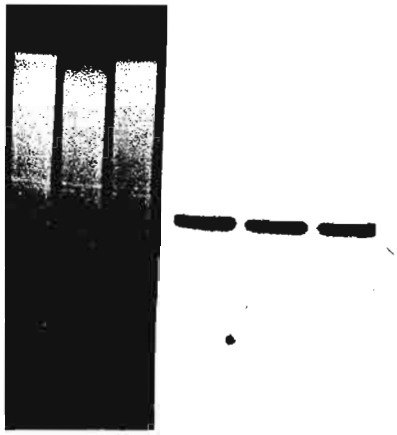
The conventional source of DNA from diploid tissues is early embryos of inbred or homozygous stocks of D.melanogaster. In crosses where chromosomal rearrangements such as deficiencies or duplications are segregating, however, the embryonic pool can contain a mixture of different genotypes and, in general, is an inappropriate starting point for DNA isolations.

Adult heads appear to be an excellent alternative source of DNA since the various genotypes from a given cross can first be identified. Furthermore, the brain contains about half the neural tissue of the adult and is probably near diploid. In brief outline, flies of the appropriate genotype are sorted under the microscope, snap frozen in liquid nitrogen, and stored at -70°C until required. The fractionation of body parts through wire sieves follows the general procedure introduced by Oliver & Phillips (1970). DNA is then extracted and spun to equilibrium in cesium chloride/ethidium bromide gradients. The details of the technique are as follows:

1. Flies of the required genotype are sorted from their sibs, starved for four hours, placed in shell vials and frozen by pouring liquid nitrogen into the vials. When this has evaporated, the vials are stored at -70°C.
2. When required for DNA isolations, liquid nitrogen is again added to a vial containing about one quarter of its volume as adults. As soon as the liquid nitrogen has evaporated, the vial is shaken vigorously half a dozen times. The flies are decapitated by this procedure as well as losing their legs and wings.
3. The shattered debris is quickly poured onto the first of two sieves and quickly moved around with a paint brush. The mesh size of the first sieve (1 mm x 1 mm) allows the heads, legs and wing parts to pass through but retains the bodies. The heads, legs and other small debris are kept cold by immediately pouring more liquid nitrogen into the catchment vessel. The debris is now poured over a second sieve of mesh size (0.5 mm x 0.5 mm) which retains only the heads.
4. The heads are placed in a glass homogeniser, more liquid nitrogen is again added, and upon its evaporation the heads are crushed using a very loose fitting teflon plunger.
5. Approximately 5 mls of ice cold buffer (10 mM TRIS, 20 mM EDTA, pH 8.0) is added and the heads are homogenised at 4°C with half a dozen strokes of a tight fitting teflon homogeniser.
6. The detergent Sarkosyl NL-30 is added to a final concentration of 3% and lysis is allowed to continue for 3 min.
7. 5 mls of the viscous lysate is then added directly to 5 gm of solid cesium chloride in a polyallomer centrifuge tube and allowed to dissolve at room temperature. 0.3 mls of ethidium bromide at 10 mg/ml in TE buffer (10 mM TRIS, 1 mM EDTA, pH 8.0) is added and the solution is overlayed with paraffin oil and spun to equilibrium.
8. Upon completion of the run, the DNA bands (main band and any satellites) are clearly visible with a UV lamp and are withdrawn from the tube by side puncture with a 19 gauge needle.
9. The ethidium bromide is removed by six extractions with isopropanol and the DNA is dialyzed against TE buffer for 16-19 hr.
10. The DNA in TE buffer isethanol precipitated in Eppendorf tubes, resuspended to the desired volume and used as such for restriction digests.

The results illustrated in Figure 1 using this method are from Southern blots of EcoRl genomic digests of Canton S DNA challenged with a radioactive probe made from a cloned Drosophila alcohol dehydrogenase fragment. The same 4.8 kb band is apparent in digests from (a) embryos, (b) ovaries, and (c) male adult heads. No significant DNA amplification or rearrangement events are detectable, as occurs in ovarian follicle cells for the chorion genes (Spradling 1981). We have challenged blots containing DNA from embryos, larval brains, adult ovaries and adult heads with different autosomal and X chromosomal cloned probes and have invariably found that adult heads produce qualitatively and quantitatively similar signals to the above sources.

The DNA from adult heads is of high molecular weight, runs at limiting velocity on agarose gels and cleaves to completion with all restriction endonucleases tested. At least 50 micrograms are available from less than a third of a vial of adults.



a b c a b c

We have found the method to be an easy adjunct to genetic experiments, since instead of discarding the appropriate genotypes at the time of scoring, they are retained at -70°C for use in recombinant DNA experiments. It should also be noted that storage of adults in this way provides an excellent source of undegraded total and polyadenylated RNA from heads as assayed by Northern blots (M.Healy, unpubl.). Since nearly two-thirds of the polysomal RNA complexity of the entire life cycle is present in adult heads (Levy & Manning 1981), adult heads may be a far more useful source of nucleic acids than has previously been imagined.

References: Oliver,D.V. & J.P.Phillips 1970, DIS 45:58; Spradling, A.C. 1981, Cell 27:193-201; Levy, L.S. & J.E. Manning 1981, Developmental Biology 85:141-149.

Rapport, E.[1], J.Kleinjan[2] and R.Dadd[2]. 1-University of Toronto, Ontario, Canada. 2-University of California, Berkeley USNA.

Egg sterilization without dechorionation.

We have adapted a method used for sterilizing mosquito eggs for use with fruit fly eggs. Eggs laid over a 3-5 hr period are loosened with a brush from agar flooded with water. A yeast paste made from sterile killed baker's yeast is used on the agar as an oviposition stimulant. Eggs are collected in a small fine meshed sieve and washed to remove visibly adhering particles. Eggs are then transferred to a sterile 50 ml container with 20 ml of 80% alcohol. Following a suggestion by H.Gordon a vacuum is applied (about 5-10 Hg is adequate) for about a minute. Presumably the alcohol is a wetting agent which is effectively pulled into the interstices of the chorion under vacuum. After 45-60 min in alcohol, using a sterile sieve, the eggs are transferred to a 50 cc sterile snapcap jar with 20 cc aqueous 0.3% Hyamine 10X which has been autoclaved for about 5 min. This compound is available from Sigma as methylbenzethonium chloride. An alcohol sterilized cap is snapped on and the eggs are shaken for 30-40 min. Finally the eggs are placed in sterile water and pipetted into sterile media. In a typical experiment where approximately 10 eggs were transferred to each media vial 85% of the vials remained free of contamination.

Success of this method seems to depend on using only 200-300 eggs per container, using "clean" parents (we do not add live yeast to our cultures) and using "fresh" eggs where there are no hatched larvae. Overnight eggs are less successful.

Rose, V.M. University of Texas, Arlington, Texas USNA.

Method of preparation of Drosophila for scanning electron microscope studies.

Modification of the method of Hodgkin & Bryant (1978) for preparation of Drosophila for SEM studies has resulted in a simple procedure which yields excellent results with minimal distortion of the specimen.

Adult flies were first etherized and submerged in either 70% ethanol or 70% acetone; less damage was observed in the specimens dehydrated with ethanol. In addition, soaking in 70% ethanol for two to three days appeared to reduce damage. The flies were then dehydrated as follows: 70% ethanol for 15 min, 95% for 15 min, and three changes of 100% for 15 min each. Specimens were then placed in sample holders constructed as described by Postek et al. (1980) and slowly critical point fluid. The use of the sample holders necessitates longer diffusion time, but reduces damage due to excessive handling. Whole flies were mounted directly on metal stubs with carbon paint which eliminated charging. Appendages and heads were attached to glass coverslips with double-stick cellophane tape which gives a smooth background; the coverslips were then mounted on metal stubs. Specimens were sputter coated with approximately 200 A of gold-palladium. Specimens were examined with a JEOL 35C scanning electron microscope using an accelerating voltage of 10 kV and a load current of approximately 100 μA. Polaroid 4x5 Land Film Type 55/Positive-Negative produced the best quality prints.