2. Proteinase K digestion. Immediately add 5 microliters of ice cold Proteinase K (10 mg/ml) and place at 65°C for 30-60 min.

3. Phenol:chloroform extraction (2X). (a) Add 200 microliters of1:1 phenol:chloroform solution. Phenol is buffered with TE (10 mM Tris HCl, pH 8.0; 1 mM EDTA, pH 8.0). (b) Mix phases thoroughly by gently inverting tube 5 or 6 times. Microfuge for 3 min (first extraction), 2 min (second extraction). (c) Take top layer using a cut pipet tip. Use cut tips whenever handling genomic DNA to prevent shearing of DNA. (d) Repeat (a) - (c).

4. Isoamyl alcohol extraction. (a) Add 200 microliters of isoamyl alcohol. (b) Microfuge briefly (2-3 seconds). (c) Discard top layer, which is isoamyl alcohol.

5. Dialysis. (a) We use spectrapor membrane tubing 1.0 cm, molecular weight cutoff: 12,000-14,000. We boil tubing in approx. 5mM EDTA, pH 8.0 twice, and then store tubing in 50% ethanol. (b) Take sample (with cut pipet tip) and place in dialysis bag--seal bag. (c) Dialyze against TE. We use roughly 1500-2000 fold volume for 36-48 hr, changing solution four times. (d) Remove DNA samples.

Procedure can work just as well for ten flies using the same procedure, but raising volumes as follows: homogenization buffer -SDS: 200 microliters; homogenization buffer +SDS: 200 microliters; Proteinase K (10 mg/ml): 20 microliters; Phenol:chloroform: 800 microliters; Isoamyl alcohol: 800 microliters. Note: for multiple extractions a single homogenizer can be used. After each homogenization wipe homogenizer, dip homogenizer in pure ethanol, wipe, dip in distilled water, wipe.

McRobert, S.P. and L. Tompkins. Temple University, Philadelphia, Pennsylvania USNA.
A method for observing the behavior of groups of flies.

We have developed a simple procedure for observing the behavior of groups of flies. This technique has been used to study courtship, although it could be used to study any behavior that flies in a group perform.

The observation chamber is a square plastic petri dish (Falcon 1012, 100 x 100 x 15 mm) into which a thin layer of cornmeal-molasses-agar medium has been poured. After hardening, a small section of medium is removed from two opposing corners of the dish so that flies can be introduced without their sticking to the food. The flies are transferred to the chamber by aspirating them through small holes in the lid, which have been made with a soldering iron, that are over the corners from which medium has been removed. In our study the flies were anesthetized with CO_2, although it would be easy to introduce un-anesthetized flies into the chamber. The entrance holes are then covered with clear tape to prevent flies from escaping. A light is positioned over the chamber and the behavior of the flies is monitored by observation through the lid.

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Milner, M.J.. University of St. Andrews, Fife, Scotland. Culture medium parameters for the eversion and differentiation of **Drosophila melanogaster** imaginal discs in vitro.

We use Shields and Sang's M3 (Shields & Sang 1977) for the culture of imaginal discs in vitro. Originally, the 1977 formulation supplemented with 10% non-heat inactivated foetal bovine serum (FBS) was used, but more recently we have reduced the amount of FBS to 2%, as this yields better differentiation. This necessitates a non-serum formulation of the medium (Table 1) to compensate for the absence of various ions previously supplied via the higher level of serum (Shields & Sang's M3(NS) - Shields & Sang, pers. comm.). It should be noted that this formulation is also bicarbonate-free. The medium is made up as before except that the pH is raised directly to 6.8 by addition of 1% NaOH. A batch of medium may be used for up to 6 weeks after preparation, and a dilution series of 20-hydroxy ecdysone is best used within 10 days of preparation. As found for embryonic cell culture, optimal medium conditions are reached between the first and second week after serum addition (Shields & Sang 1977).

It may be desirable to culture discs in the absence of serum, either because of difficulty in obtaining a suitable batch of serum, or in order to culture discs in more rigorously defined medium conditions. To this end, I have tested a number of serum substitutes used in other tissue culture systems, at a range of concentrations, adding them directly to M3(NS) and assessing their ability to support eversion, differentiation and pigmentation of wing discs on a scale of 1 to 3. One represents poor differentiation, 3 good development and 2 an intermediate level. All additives were purchased from Sigma. The results

Table 1. Shields and Sang's medium M3(NS). (amounts in mg/100 mls). * = optional

Kcl	260	Threonine	50	Tyrosine	25
$MgSO_4 \cdot 7H_2O$	400	Serine	35	Phenylalanine	25
$CaCl_2 \cdot 6H_2O$	140	Asparagine	30	beta-Alanine	25
Na·glutamate	786	Glutamine	60	Histidine	55
$NaH_2PO_4 \cdot 2H_2O$	88	Proline	40	Tryptophan	10
glucose	1000	Glycine	50	Arginine	50
oxaloacetic acid	25	alpha-Alanine	150	Lysine HCl	85
BIS-TRIS	105	Valine	40	Cysteine HCl	20
T.C. Yeastolate (Difco)	100	Methionine	25	Choline HCl	5
Aspartic acid	30	Iso-leucine	25	Penicillin G.Na*	3
		Leucine	40	Streptomycin sulphate*	10

Table 2.

Additive to G3(NS)	Concentration mg/ml	Differentiation level	Additive to G3(NS)	Concentration mg/ml	Differentiation level
No additive		2-3	Polyvinyl Alcohol type 11	1	3*
2% Foetal Bovine Serum (average batch)		3	(B.D.Bavister 1981, J.Exp. Zool. 217:45-51)	0.1	2
	5	1	gamma-Globulins (bovine Cohn Fraction 11)	1	2
Bovine Albumin (fatty acid free)	1	3*			
	0.1	2	Chicken egg white globulins	5+	2
10	3*		Insulin (crystalline, from bovine pancreas)	0.1+	1
Bovine Albumin fraction V	1	3*			
	0.1	3*	(G.Mosna 1981, Experim. 37:466-467)	0.001	2

* indicates acceptable substitutes for 2% FBS. + used as saturated solution.

of these experiments are given in Table 2. A control with no additive gave a reasonable level of differentiation, compared to the control of 2% FBS. However, the discs are difficult to handle in medium alone as they stick to glass surfaces and cannot easily be moved through surface tension layers, so this cannot be recommended. Acceptable substitutes to FBS are indicated in Table 2; however, I consider that a good batch of FBS can give qualitatively better results than these. It is interesting to note that under these conditions insulin inhibited differentiation at a range of concentrations.

Reference: Shields, G. & J.H. Sang 1977, DIS 52:161.

Nichols, R. and W.L. Pak. Purdue University, West Lafayette, Indiana USNA.

A simple medium for vitamin A deprivation of **Drosophila melanogaster.**

One method of studying proteins that contain a vitamin A-derived group, such as rhodopsin, is to observe the effect of reducing vitamin A in the diet of the organism being studied. In studies today, vitamin A deprivation of Drosophila is accomlished by raising flies on one of several synthetic diets (Sang 1956; Falk & Nash 1974). These synthetic media have numerous disadvantages: (1) it is laborious to prepare the medium; (2) mold and bacterial growths are difficult to control; (3) the antibiotics and mold inhibitors included in the medium could have undesirable side effects on flies; (4) eggs or larvae often need to be sterilized before introducing them onto fresh medium at each generation; (5) the deprived flies are often unhealthy; (6) the degree of deprivation is inconsistent. We describe in this report a procedure for producing vitamin A deprived flies that circumvents many of these difficulties.