Fristrom, James W. and William Heinze.
University of California, Berkeley, California. The preparative isolation of imaginal discs.

In an earlier paper (Fristrom and Mitchell, 1965) a method was published for the isolation of 2-4,000 discs. It was confidently stated that the limiting factor controlling the yield of discs involved only the number of larvae used. Subsequently, using large numbers of larvae (300-400 gm) it was possible to obtain only 4-6,000 discs indicating that modifications in the method were necessary to obtain a substantial increase in the yield of discs. The following procedure describes a method which normally yields about 40,000 discs, and has given preparations containing as many as 70,000 discs. Only details which differ from the original paper are included. The method starts with ~ 200 gm of larvae.

1. Larval Cultures: The method of Mitchell and Mitchell (1964) is used. Eight boxes of larvae, obtained in one 8 hour and one 16 hour collecting period are used. The larvae are grown at 25° C in 65% relative humidity for five days (after removal of the box from the cage). Only larvae which have crawled to the top of the box or the side of the box are used. Usually some pupae have formed at the time of use.

2. Grinding the Larvae: The larvae are ground in four separate 50 gm batches. The larvae are suspended in cold Ringer's solution (final volume 250 ml) and poured immediately into the running grinder. The grinder is washed with a small volume of Ringer's solution (25 ml).

3. Sieving the Homogenate: The homogenate is poured rapidly through three sieves into beakers; the first two sieves are stainless steel and the last one is made of silk bolting cloth. The sieves have respectively 2 mm, 0.8 mm and 0.2 mm openings. The stainless steel sieves are washed briefly with a stream of Ringer's solution driven through a needle valve at 12 P.S.I. from a 15 liter reservoir. The silk cloth is washed thoroughly until the 1.5 liter glass beaker used to catch the last filtrate is full. The original homogenate and first two washings are caught in 1 liter polyethylene beakers.

4. Reducing the Volume: All steps are carried out in ice baths. The initial 1.5 liter volume is reduced to 50 ml in three steps. The initial suspension is allowed to stand for 10 minutes and the top 1300 ml are removed with an aspirator. The remaining material is transferred with washing to a 250 ml beaker where it is allowed to settle for five minutes. The top 175 ml is removed with an aspirator and the remaining material is transferred with washing to a 100 ml beaker. After settling for three minutes and removing the top 60 ml the material is finally transferred to a 50 ml beaker. After all four concentrations are completed the sediment is combined in two 50 ml beakers, allowed to settle for two minutes and the top 30 ml of fluid is removed.

5. Washing the Material: The material is then run through several washing cycles:

(1) The material is suspended in 50 ml of Ringer's.
(2) The material is allowed to settle 1 to 1.25 min.
(3) The fluid is slowly aspirated from the top down until ~ 15 ml of fluid is left.
(4) The cycle is repeated.

About 20-30 cycles of washing are required. We have designed a machine which will perform all the above washing steps automatically. We will happily provide a description and wiring diagram to anyone desiring to build it.

6. Centifugation: The washed material is then spun on six discontinuous gradients (14 and 20% Ficoll in Ringer's). We have discontinued use of the continuous Ficoll gradient although it may still be used.

7. Manually Removing Debris: Non-disc material can be removed from the preparation with forceps or by using a micro-needle (suitable for disc transplantation) which is attached to an aspirator. Usually about one hour of "picking" is required.

The entire procedure takes about four hours. Because of the increased number of discs, and the decreased efficiency of removing debris the preparations are less free of debris than before, averaging between 90-93% pure. The types of discs are recovered at different frequencies. Leg discs now predominate and increased numbers of genital and eye discs are found. Many variations may be applied to the procedure. The material caught on the first sieve may be reground yielding, at a maximum, an additional 20,000 discs. The material caught on the first sieve may be resuspended and resieved yielding a higher proportion of wing discs.

References:

Fristrom, J. W. and H. K. Mitchell, J. Cell Biol., 1965, v. 27, 445-448.
Mitchell, H. K. and A. Mitchell, D.I.S., 1964, v. 39, 135.