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Comparison of DNA isolation methods and storage conditions for successful amplification of *Drosophila* genes using PCR.

Colton, L., and J.B. Clark. Department of Zoology, Weber State University, Ogden UT 84408-2505 USA. Correspondence: jclark1@weber.edu.

The polymerase chain reaction is an invaluable technique for gene characterization in *Drosophila* and other organisms. For routine applications involving amplification of DNA isolated from fresh samples, PCR presents few problems. However, it is sometimes necessary to amplify DNA from older specimens that have been stored under a variety of conditions. This study explores the efficacy of three different DNA isolation methods used on *Drosophila* samples that have been stored under a variety of conditions. These conditions represent those commonly used to collect and store field samples and the results may also be pertinent to PCR amplification from museum specimens of *Drosophila* and other insects.

Storage conditions of fly samples

The Canton S strain of *D. melanogaster* was used for all experiments. In July 1999, 0.2 g (approximately 200 live adults) were collected from laboratory cultures and placed in 15 ml polypropylene centrifuge tubes under one of six storage conditions.

(1) RT: stored dry at room temperature (approximately 22 °C).
(2) 100RT: stored in 100% ethanol at room temperature.
(3) 70RT: stored in 70% ethanol at room temperature.
(4) DMSORT: stored in 20% DMSO, 5% NaCl, 0.25 M EDTA at room temperature.
(5) -20: stored dry at -20°C.
(6) 100/-20: stored in 100% ethanol at -20°C.

After 15 months of storage DNA was isolated from each sample.

DNA isolation methods

Three DNA isolation techniques were used. The first, abbreviated DDE, is a standard method for isolating *Drosophila* DNA using a lysis buffer, proteinase K, phenol and chloroform extractions, and ethanol precipitation (*e.g.*, Ashburner 1989). Reagent volumes can be easily altered depending on sample weight. The second method, abbreviated DNazol, uses a commercial kit based on guanidine-detergent isolation methods (Invitrogen/Life Technologies, Gaithersburg, MD, USA). The manufacturer's instructions were followed and reagent volumes adjusted for the sample weight. The third method, abbreviated DTAB, is a modification of a method originally developed for blood samples that uses a cationic detergent (Gustincich *et al.*, 1991). Its advantage is that it permits isolation of DNA and RNA from a single sample. Because it has been extensively modified, the procedure is described in detail:

DTAB Procedure

1. Select two adult flies and place them in a 1.7 ml microcentrifuge tube.

2. Add 5 µl DTAB solution (approximately two-and-a-half times the flies' weight) and homogenize the tissue thoroughly; incubate at 68 °C for 5 min. (DTAB solution: 8% dodecyltrimethylammoniumbromide, 1.5 M NaCl, 100 mM Tris [pH 8.6], 50 mM EDTA).

3. Add 7.5 µl chloroform (approximately three-and-a-half times the flies' weight) and mix well by inversion; centrifuge 10,000 *g* for 2 min to separate phases and remove the upper aqueous phase to a fresh tube.

4. Add 7.5 µl isopropanol to the aqueous phase, mix gently and centrifuge 10,000 *g* for 15 min to pellet nucleic acids. Discard the supernatant and dry pellet at room temperature.

5. Resuspend pellet in 2.0 µl TE buffer (approximately equal to the flies' weight), add 0.6 µl 8 M LiCl, mix, and incubate on ice for at least 30 min to precipitate the RNA. Centrifuge 10,000 *g* for 10 min. and transfer the supernatant, which contains the DNA, to a clean microcentrifuge tube. The RNA pellet may be saved for further processing if desired.

6. Add 7.5 µl 100% ethanol. If the DNA precipitates immediately, then centrifuge 10,000 *g* for 10 min to pellet the DNA. If immediate precipitation is not visible, incubate the tube at -20°C for at least 15 min and then centrifuge as above.

7. Discard the ethanol, wash the pellet once with 70% ethanol and air dry. Resuspend the DNA in 6 µl of ddH_2O or TE buffer and store at -20°C.

Although there are numerous procedures and commercial reagents available for DNA isolation, it was felt that these three represent techniques commonly employed in *Drosophila* laboratories. For the comparative studies, one adult female and one adult male fly were rinsed in ddH_2O and then homogenized in a 1.7 ml microcentrifuge tube in one of three reagents, according to the isolation method used. Homogenization was accomplished with disposable pestles (Kontes, Vineland NJ USA) matched to the tubes. Following isolation with each method, the DNA pellet was resuspended in 6 µl ddH_2O. DNA quantitation was performed by UV spectrophotometry using 2 µl of the sample volume.

PCR conditions

Once isolated, 1 µl DNA was used as a template in PCR with universal rDNA primers 937 and 938 (Houck *et al*., 1991). These correspond to positions 648-664 and 1444-1459, respectively, of the 18S rRNA gene and amplify an 812 bp fragment. All reactions were carried out in a 25 µl volume with cycling conditions of 94°C, 1 min., 50°C, 1 min., 72°C, 1 min., for a total of 30 cycles. A 10 µl aliquot of the PCR product was removed and examined on a 0.8% agarose-TAE gel stained with ethidium bromide. Purified genomic DNA isolated from fresh flies using the DDE method was used as a positive control for each PCR. Amplification from each isolation method-storage condition combination was repeated at least two times.

Results

These assays simulate the research setting in that a variety of isolation methods and storage conditions are explored to determine if DNA of sufficient quality could be obtained for PCR. DNA yields varied considerably and ranged from 0.150 μg DNA to 2.25 μg DNA per adult fly. The actual amount of template DNA used in each amplification reaction varied from 0.050 μg to 0.750 μg. In each case, the volume of template DNA used for PCR corresponded to 33% of the total DNA isolated from one fly. (Following isolation, DNA from two flies was resuspended in 6 μl and 1 μl of the DNA was used in each amplification reaction.) The variation in DNA yield was due to the different isolation method-storage condition combinations.

PCR was successful when each of the three isolation methods was used with each of the six storage conditions. When the length of time in dry storage at room temperature [condition (1), RT] was extended from 15 to 21 months, only the DNazol method produced positive PCR results, albeit inconsistently. PCR was positive for all three isolation methods on those samples that were stored for 21 months under one of the other five conditions. Spectrophotometry revealed that the DNA yields (using each of the three isolation methods) from the dry samples stored for 21 months were not significantly different from those of the 15-month samples. This suggests that the failure of amplification is due to DNA degradation during the extended storage time at room temperature.

Conclusions

These results indicate that high-quality DNA can be isolated from fly samples stored under a variety of conditions. In addition, successful PCR is possible when the amount of template DNA used for amplification varies widely, here, fifteen-fold. This demonstrates that quantitation of DNA is not necessary, a conclusion with practical implications when the amount of a fly sample is limiting.

This study also addresses the suitability of reagents used to store flies for subsequent DNA isolation. Storage conditions are apparently not critical for fly samples stored for less than a year. However, there was an inability to amplify target DNA from flies stored dry at room temperature for 21 months. This suggests that it is important to either freeze the flies or use a preserving buffer (*e.g.*, DMSO) or ethanol if PCR studies are to be conducted beyond one year. When freezing or storage in a buffer or ethanol is not feasible, it is recommended that dry samples be processed soon after collecting. An alternative is to isolate DNA from dry samples as soon as possible and freeze the purified DNA, which is generally stable indefinitely. There was no difference in the success of PCR with flies that had been stored in 70% or 100% ethanol. This result suggests that, contrary to conventional wisdom, there is no advantage to using 100% ethanol when samples are to be stored for relatively short periods of time. The anecdotal preference for 100% ethanol may be important, however, for storage times exceeding two years.

References: Ashburner, M., 1989, Protocol 47 (pp. 106-107) in *Drosophila: A Laboratory Manual.* Cold Spring Harbor Press, Cold Spring Harbor, NY.; Gustincich, S., G. Manfioletti, G. Del Sal, and C. Schneider 1991, BioTechniques 11: 298-302; Houck, M.A., J.B. Clark, K.R. Peterson, and M.G. Kidwell 1991, Science 253: 1125-1129.