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Rapid isolation of *Drosophila* high molecular weight DNA to obtain genomic libraries

J.Piñol, O.Francino, A.Fontdevila and O.Cabré

Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
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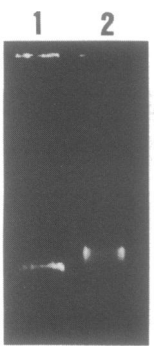
Isolation of high molecular weight (hmw) DNA from *Drosophila* is usually accomplished by protease digestion of crude adult homogenates, several phenol extractions, long dialysis and RNAase treatment (1,2). The total process is 2-3 days long. We report a different strategy that allows to isolate hmw Drosophila DNA in 2-3 hours. This method is based on the observation that a brief nuclei purification from crude adult homogenates and two chloroform extractions renders DNA virtually free of RNA and cutable, in a reproducible way, with restriction nucleases.

A Dounce homogenizer (clearance: 0.2-0.5 mm) is filled up to 2 ml with anaesthesized flies and the volume is adjusted to 5 ml with ice-cold buffer A (100 mM NaCl, 10 mM EDTA, 0.5% Triton X-100 and 20 mM Tris.HCl pH 7.5). Ten strokes are made with the pestle at 2000 rpm. The final volume of homogenate is adjusted to 40 ml per gram of flies, passed through 150 mesh nylon sieve and 10 ml aliquots dispensed in plastic centrifuge tubes. Nuclei are pelleted during 5 minutes at 7500xg, washed three times with ice-cold buffer B (buffer A without Triton) and finally resuspended in 1.5 ml of buffer B. Then, 1 ml of 4% SDS and 1 ml of 4 M Sodium Perchlorate are added and the viscous lisates are gently extracted twice with Chloroform-Isoamylalcohol (24:1). DNA is precipitated by adding 7 ml of absolute Ethanol. Precipitated DNAs are collected in one tube, washed twice with 70% Ethanol and dried. We do not recommend to disolve DNA at concentrations greater than 0.1 mg/ml (the disolution process can be accelerated if the DNA containing tube is placed in a rotating platform overnight). The yield of this procedure is 0.5-0.7 mg of DNA per gram of adult flies. Electrophoresis shows that DNA is longer than 50 Kb.



To construct genomic libraries, hmwDNA (30 μg) is digested 15 minutes with 1.5 units of Mbo I to obtain 15-20 Kb fragments. These digestion conditions are similar to those used when DNA is isolated by conventional methods (1,3). DNA is extracted twice with Phenol-Chloroform (1:1) precipitated with Ethanol and dried. At this point DNA is ready to be dephosphorilated, ligated and in vitro packaged. Using EMBL3 arms (3) and Promega packaging extracts we usually obtain 10^6-10^7 pfu per μg of *Drosophila* DNA isolated and processed according to this method.

Figure: 0.4% Agarose gel electrophoresis; 1: λ DNA, 2: Drosophila DNA isolated according to this method.

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