INTRODUCTION

Several methods for DNA phage purification are available in the literature; “Molecular Cloning” (Maniatis et al., 1982; Sambrook et al., 1989) is the manual of choice for this and other routine techniques. It is particularly applicable for new students introduced to the laboratory and anxious for a bona fide guide. Nevertheless, at least in the case of DNA obtained from lambda bacteriophages by CsCl gradient centrifugation, some methodological simplifications can be useful. Our present modification, requiring less steps and fewer solutions to prepare, produces a DNA suitable for further manipulations like digestions, ligations, labelling, subcloning, etc.

MATERIAL AND METHODS

Phage harvesting

A confluent plaque is covered with 4 ml of 10 mM Tris, pH 7.4, 10 mM MgCl₂, lambda buffer (TM), gently shaken and maintained in the refrigerator for two hours. After adding a few drops of chloroform, the plate is manually shaken, slightly slanted and the buffer withdrawn with a Pasteur pipette. One ml of the phage suspension is mixed with 1 ml of a competent overnight culture resuspended in 10 mM MgCl₂ in 0.4 volumes of the original LB medium (avoiding the use of a single colony from the donor plate), the tube is left for 20 min at 37°C and used to inoculate 400 ml of LB, 10 mM MgCl₂, in an Erlenmeyer flask, following overnight incubation at 37°C, under strong agitation, using only a piece of aluminum foil attached with tape to the mouth of the flask for better aeration; treadlike material is an indication that the material has been obtained in good yield. After adding 2 ml of chloroform, the suspension is vigorously mixed, and then made up to contain 50 g/l of NaCl. After dissolution of the salt, the suspension is centrifuged for 10 min at 8,000 rpm in a type GSA rotor, to pellet cells and debris. The supernatant is transferred to an Erlenmeyer flask containing 40 g (10%) PEG 8000 (Sigma), and kept on ice for at least one hour. The suspension is then agitated, centrifuged for 10 min at 8,000 rpm, and the supernatant discarded.

CsCl centrifugation

The U-shaped precipitate on the tube wall is resuspended in approximately 5 ml of lambda buffer with the help of a serological pipette, and transferred to a polycrystalline centrifuge tube, previously marked to 9 ml, and containing 6.48 g of CsCl (0.72 g/ml). The volume is made up to 9 ml with lambda buffer and the salt dissolved. After filling with mineral oil, the tube is centrifuged overnight in a fixed angle rotor (Beckman) or RP 83 T (Hitachi) centrifuge at 45 K or, alternatively, in an SW 41 type rotor at 35 K, in the 12-ml tube. Quick-seal tubes and VTi rotors can be easily adapted to the above conditions. After the run a bluish white band, easily discerned by contrast against a sheet of used X-ray film, is aspirated after puncturing the tube with a 22-gauge needle fitted in a plastic syringe. The phage suspension is collected in a tube containing 2 ml of 10 mM Tris, pH 8.0, EDTA 1 mM (TE), transferred to a dialysis bag and the suspension dialyzed for two hours against TE (with two half-liter changes of the dialysing medium).

Phenol extraction

Gloves should be worn to prevent severe phenol burning. The content of the dialysis bag is transferred to an ap-
propriate centrifuge tube, the volume is raised to approxi-
matelly 5 ml with TE and extracted for 10 min by inver-
sions with an equal volume of Tris buffer, pH 8.0, satu-
rated phenol, containing 0.1% 8-hydroxyquinoline (Kirby,
1957). If no appropriate plastic tubes are available, a piece
of PVC-based film, firmly attached several times to the
mouth of a 30-ml Corex tube, can be used. After 10-min
centrifugation at 8,000 rpm in an HB-4 type rotor centri-
fuge, the aqueous phase is carefully removed without try-
ing to get the immediate overlaying interface, made up to
0.2 M NaCl and precipitated by gentle addition of cold
absolute ethanol. The tube is maintained on ice with occa-
sional gentle inversion. For a good yield, the precipita-
tion step must be extensive. The phage DNA will then float
and can be easily removed with the help of a glass rod;
alternatively it can be rolled around the rod. Signs of pre-
cipitation still visible in the remaining suspension should
be checked for. The rod with DNA is briefly dipped in a
tube containing ethanol 70% to be free from traces of
phenol, touched in the border of this tube to discard ex-
cess of ethanol and, without drying, the DNA is dissolved
and maintained in 0.5 or 1.0 ml of TE.

RESULTS

Figure 1 shows the digestion pattern of phages ob-
tained using the above described method. The absence of
partial digestion products is a good indication of the qual-
ity of the DNA obtained.

DISCUSSION

After obtaining erratic results using general protein-
ase K/SDS methods for DNA phage purification, we decided
to use the phenol method (Kirby, 1957) adapted by Schleif
and Wensink (1981) for the purification of lambda DNA.
Basically we follow the Maniatis et al. (1989) protocol but
omitting enzymatic treatment (DNAse, RNAse and protein-
ase K/SDS) during the process. It is interesting to notice
that even in the second edition of another much consulted
manual, the “DNA cloning 1 - A practical approach” (1995),
the use of enzymes is still recommended. We also adopted
the suggestion of Latchman and Brickell (1986) to elimi-
nate the chloroform extraction step after the PEG precipi-
tation stage; this step is present in Maniatis’s protocol.

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RESUMO

É proposta uma modificação para o tradicional protocolo
depuração de DNA de fago que utiliza gradiante de cloreto
de cálcio. Este método evita etapas enzimáticas assim como a
necessidade de uma titulação prévia, um processo que consome
tempo e é proposto na maioria dos métodos em uso. A qualidade
do DNA obtido permite manipulações adicionais, tais como
digestões, ligações, marcações, subclonagens, etc.

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