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METHODOLOGY

A simplified CsCl protocol for lambda DNA purification: no enzymatic treatment/one phenol extraction

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Abstract

A modification of the CsCl gradient centrifugation method for DNA phage purification is presented. It avoids the enzymatic steps as well the need for a preliminary phage titration, a tedious process proposed in the majority of the protocols in use. The quality of the DNA obtained makes it amenable for additional manipulations like digestions, ligations, labelling, subcloning, etc.

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, etc.

Basically, we use polyethyleneglycol (PEG 8000) precipitation and phenol extraction prior to the gradient step. The proposed modifications are detailed in Material and Methods.

MATERIAL AND METHODS

Phage harvesting

A confluent plaque is covered with 4 ml of 10 mM Tris, pH 7.4, 10 mM $MgCl_2$, 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 After adding a few drops of chloroform, the plate is manually shaken, slightly slanted and the buffer withdrawn with a Pasteur pipette.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 $MgCl_2$ 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 $MgCl_2$, 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 polyallomer 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 Ti50 (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-

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propriate centrifuge tube, the volume is raised to aproximatelly 5 ml with TE and extracted for 10 min by inversions with an equal volume of Tris buffer, pH 8.0, saturated 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 centrifuge, the aqueous phase is carefully removed withou trying 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 occasional gentle inversion. For a good yield, the precipitation 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 precipitation 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 excess of ethanol and, without drying, the DNA is dissolved and maintained in 0.5 or 1.0 ml of TE.

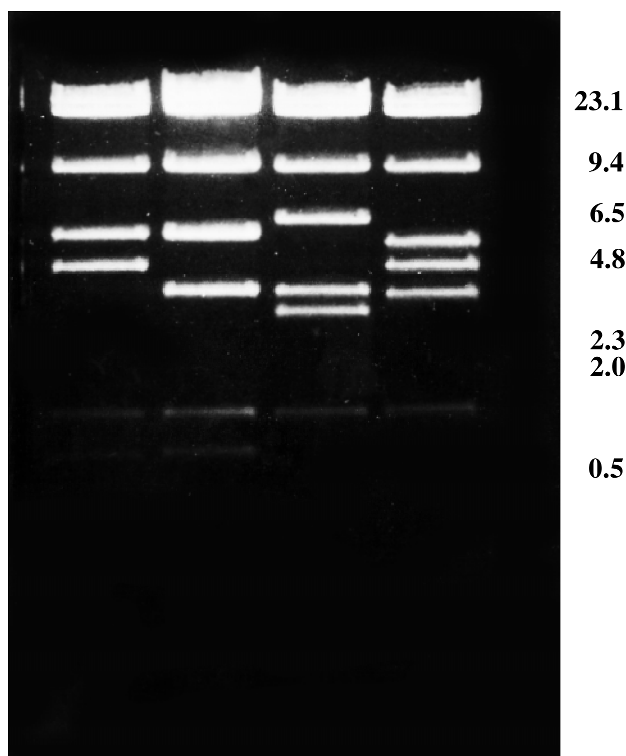

Figure 1 - Four recombinant phages (Dash-Stratagene) were grown using Q359 as the host bacteria and DNAs purified as described in the text. Two µg of each phage was digested with ECOR1 (Boehringer) for one hour and separated in a 0.8% agarose gel using a Horizon 58 (Gibco-BRL) electroforesis apparatus. Bars indicate the positions of lambda/*Hin*dIII markers.

RESULTS

Figure 1 shows the digestion pattern of phages obtained using the above described method. The absence of partial digestion products is a good indication of the quality of the DNA obtained.

DISCUSSION

After obtaining erratic results using general proteinase 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 proteinase 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 eliminate the chloroform extraction step after the PEG precipitation stage; this step is present in Maniatis's protocol.

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RESUMO

É proposta uma modificação para o tradicional protocolo de purificação de DNA de fago que utiliza gradiente de cloreto de césio. 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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