Quick method for high yields of λ bacteriophage DNA

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This protocol describes the purification of λ DNA utilizing DEAE chromatography. The procedure allows the isolation of λ DNA at a much higher yield than similar methods (1). Starting from a single plate lysate (100×15 mm) the procedure yields on average 35 μg of high quality DNA within only 6 hours. In contrast to similar procedures, the phages are not precipitated by IPTG or isopropanol, but by Ethanol which is most important for the high yield (2, 3). Furthermore, the protocol eliminates the need for a CsCl gradient, precipitation by ultracentrifugation and proteinase K digestion (4, 5). Unlike other protocols, the procedure does not depend upon liquid lysate which yields inconsistent results (5). As demonstrated in the figure the DNA was in all cases free of host chromosomal DNA, undegraded and cleavable by restriction enzymes. The protocol is ideal for the quick isolation of workable amounts of DNA from a large number of samples.

DEAE-cellulose (DE52 Whatman) is prepared according to Benson and Taylor (2). In brief, HCl (0.05N) is slowly added to DE52 resin until the pH is <4.5. With constant stirring the pH is brought up to 6.8 by concentrated NaOH. After the resin has settled, the supernatant is discarded and the ionexchanger is washed several times with Luria-Bertani (LB) medium (pH 7.4). Na-azide is added to a final concentration of 0.1% and the resin is stored as a 60% slurry at 4°C. Resin columns are conveniently prepared in Poly-Prep chromatography columns (Biorad). We found that resin equilibrated with LB medium produces consistently higher yields of λ DNA than resin equilibrated with Tris hydrochloride buffer (1). The columns are loaded with ~6 ml slurry until approximately 2 ml resin is packed. Up to 7 ml plate lysate is applied to the columns and allowed to run through. The eluent is collected, the column is washed with 2 ml LB medium and the runthrough is combined. NaCl is added to the eluent to a final concentration of 0.07 M and the phages are precipitated by 2 volumes of 100% Ethanol (20 min at −20°C). Ethanol precipitation was found to be superior to the commonly used isopropanol precipitation (2). Following centrifugation at 12,000 G for 15 minutes in 15 ml glass Corex tubes, the supernatant is discarded and the pellet is washed with 2 volumes of 70% Ethanol. The phages are carefully resuspended in 2 ml TE (10 mM Tris-HCl, 1 mM EDTA [pH 7.6]) containing 0.2% SDS and lysed by the addition of 2 ml phenol. The samples are then vortexed for 10 seconds and centrifuged at 12,000 G for 5 minutes. The aqueous layer is collected and extracted with phenol again. Bacteriophage DNA is precipitated with 2 volumes 100% Ethanol and washed twice with 70% and 100% Ethanol. The isolated DNA is routinely used for subcloning and Southern blotting.

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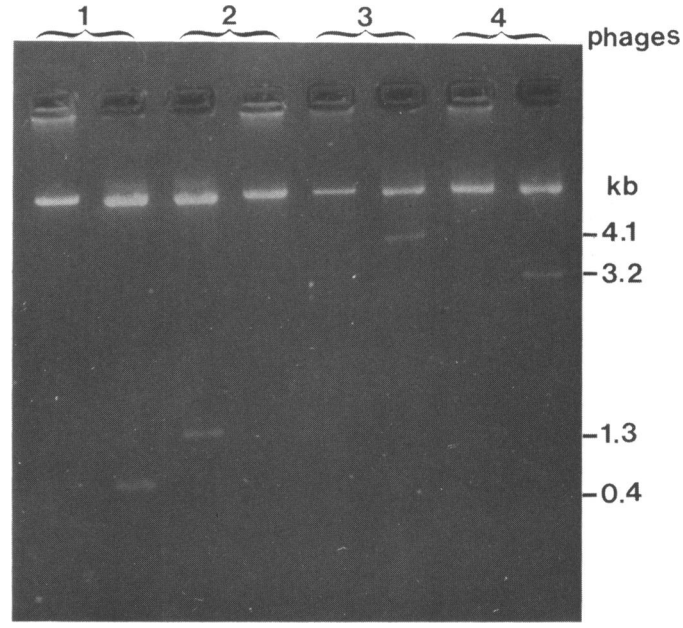

Ethidium bromide stained 1% agarose gel. DNA from four recombinant λ clones was isolated according to the protocol, cleaved by EcoRI and separated along with undigested DNA. The molecular weight of the released inserts is shown on the right.

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