A rapid biochemical method for purifying lambda DNA from phage lysates

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Purification of phage DNA from liquid lysates is a frequent step in genomic and cDNA cloning. Many rapid methods for phage DNA isolation have been developed - almost all require time-consuming or labor-intensive steps to separate phage from bacterial DNA. Usually some physical means such as chromatography or CsCl ultracentrifugation is used to enrich for phage DNA. I modified a standard method for phage preparation* by substituting a biochemical step for ultracentrifugation and eliminating a chloroform extraction and dialysis step. The strategy is to digest the unprotected host nucleic acids with nucleases while the phage DNA remains protected in a protein capsule. The high MW phage DNA is separated from the degraded host DNA and RNA by polyethylene glycol (PEG) precipitation. In practice, the PEG precipitation is necessary only when one anticipates isolating low MW (<500 bp) fragments from the recombinant phage. DNA has been purified from charon 4, λ gtWESB, EMBL 3 and 4 vectors. Our method is rapid, and yields highly purified phage DNA in amounts equal to if not greater than the standard method.

To an overnight 500 ml liquid lysate of phage, chloroform is added to 2%, DNase I and RNase A to 1 μ g/ml, and solid NaCl to 1M final concentration. After incubation at 37°C for 15-30 minutes, the aqueous phase is clarified by centrifugation at 6000 xg at 4°C for 10 minutes. Solid PEG 8000 is added to 10% w/v and the cloudy mixture stored at 4°C for at least one hour. The intact phage are recovered by centrifugation at 6000 xg at 4°C for 20 minutes and resuspended in 3 ml of SM buffer (20 mM Tris-HCl, pH 7.6; 10 mM MgSO₄; 100 mM NaCl, 0.01% gelatin). DNase I and RNase A are added to 5 μ g/ml and 100 μ g/ml, respectively. After a 30-minute incubation at 37°C, the phage are lysed by adding 10% SDS to 0.5%, 0.5M EDTA (pH 8.0) to 20 mM, and proteinase K to 100 μ g/ml, and heating to 68°C for 30 minutes. The phage DNA is extracted with equal volumes of phenol, phenol/chloroform, and then chloroform, and precipitated by adding 1/2 volume of 5M ammonium acetate and two volumes of ethanol. After storing on ice for 15 minutes, the precipitate is recovered by centrifugation at 10,000 xg at 4°C for 15 minutes. To the dry pellet 1.6 ml of distilled H₂O, 0.4 ml of 4M NaCl and 2.0 ml of 13% PEG w/v are added. The resulting precipitate collected after an hour incubation on ice followed by a 15-minute centrifugation at 10,000 xg is rinsed with 70% ethanol, dried and resuspended in 1X TE (10 mM Tris, pH 8.0; and 1 mM EDTA, pH 8.0) to a final concentration of 1 mg/ml. Yields are usually 0.5-2.0 μ g/ml of liquid lysate, and the method takes less than eight hours to complete.

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*Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982), "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory.