# An improved method for the small scale preparation of bacteriophage DNA based on phage precipitation by zinc chloride

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Procedures which allow the rapid extraction of small amounts of phage DNA in a sufficiently pure form as to be digested with restriction enzymes are an important tool in the analysis of recombinant clones. This communication describes an alternative procedure to the current method of polyethylene glycol (PEG) sedimentation followed by phenol extractions (1), which is based on the precipitating action of zinc chloride ($ZnCl_2$).

Precipitation by $ZnCl_2$ has first been observed in experiments with *B.subtilis* phage SPP1. Suspensions of SPP1 in LB (Tryptone, 10 μg/l; Yeast Extract, 5 g/l and NaCl, 5 g/l) were incubated at 37°C for 30 min in the presence of increasing concentrations of $ZnCl_2$. After centrifugation (8000 rpm, 2 min) phages present in pellets and supernatants were separately titrated. More than 90% of the recovered phages were found in pellets when the $ZnCl_2$ concentration was in excess of 20 mM. This observation was then extended to several *B.subtilis* (SPO1, SPO2, PBS1, SPβ and ϱ 11) and *E.coli* bacteriophages (λ, T4 and M13) indicating that the phenomenon was not phage specific. Total phage recovery (pelleted plus free in supernatant) in different experiments was in the range of 60−80% of the initial titer.

Precipitation by $ZnCl_2$ (40 mM final concentration) was further characterized using SPP1 and λ as model phages. While 90 to 98% of the recovered phages were precipitated at 37°C in media containing less than 6g/l NaCl ( 0. 1 M), these values dropped to about 20% either by incubating the suspensions on ice or by increasing NaCl concentration to 1 M. Variations in pH between 6.0 and 8.0 had no effect on the precipitation level. In all situations an incubation period of 5 min was enough to ensure maximum phage precipitation. In optimal conditions (37°C, low salt), percent precipitation remained constant for phage concentrations in the range of $10^3$ to $10^{11}$ pfu/ml.

For viruses such as λ, sedimentation with PEG requires a high concentration of the polymer (10% w/v) and incubation on ice for at least 1 h (1). Precipitation with $ZnCl_2$ is both faster and more economic.

The use of $ZnCl_2$ proved to be very efficient for the small scale preparation of bacteriophage DNA. The procedure, outlined below, offers the additional advantage of avoiding phenol extractions. DNA molecules isolated by this method can be readily digested with restriction enzymes. Different phages have been submitted to such extraction procedure and in each case good yields of DNA with an acceptable degree of purity were obtained (Figure 1).

The detailed protocol is as follows:
To each ml of a lysate previously treated with DNAaseI (1 μg/ml) and RNAaseA (100 μg/ml) add 20 μl of a filter sterilized, 2 M solution of $ZnCl_2$ (BDH). Incubate for 5 min at 37°C. Centrifuge for 1 min at 10000 rpm and remove supernatant by aspiration. Resuspend pellet in 500 μl TES buffer (0.1 M Tris-HCl, pH 8; 0.1 M EDTA and 0.3% SDS) with the aid of a tip. Incubate at 60°C for 15 min. Add 60 μl of a 3 M potassium acetate solution, pH 5.2, mix thoroughly and leave on ice for 10 to 15 min. A white, dense precipitate will form during this step. Centrifuge for 1 min at 12000 rpm in a refrigerated microfuge and collect supernatant to a new tube. Add an equal volume of isopropanol, mix and leave on ice for 5 min. Centrifuge to pellet the DNA, wash with 70% ethanol, dry on air at room temperature and resuspend in 20−100 μl TE (10 mM Tris-HCl, pH 8; 1 mM EDTA).

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## REFERENCE

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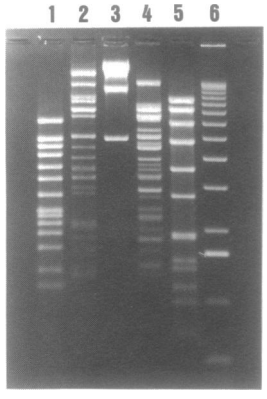


**Figure 1.** EcoRI digested phage DNAs prepared by the $ZnCl_2$ method. DNAs were extracted from 5 ml lysates with approximate titers of $10^{10}$ pfu/ml and recovered in 50 μl of TE as explained in the text. 7 μl of each DNA were digested with 3 units of EcoRI (GIBCO BRL) at 37°C for 2 h and submitted to gel electrophoresis (0.8% agarose). 1-ϱ15 (44 Kb); 2-ϱ11 (120 Kb); 3-λAmJL, a recombinant derivative of λgt10 (48 Kb); 4-SPβ(125 Kb); 5-SPP1 (44 Kb); 6-Molecular size markers (1 Kb DNA Ladder, GIBCO BRL).