A rapid and efficient 'miniprep' for isolation of plasmid DNA

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Several methods have been reported to isolate plasmid DNA from small cultures inoculated with single colonies of bacteria. For example : alkaline SDS lysis method (1,2) and modified boiling Triton lysis method (3,4).

We report here an efficient and much faster method for minipreparation of either plasmid DNA or RF M13 phage DNA which are pure enough to be digested with commonly used restriction enzymes or to be used to transform other bacteria cells. The method developed needs less than one hour and consists of two steps : phenol/chloroform treatment and EtOH precipitation with ammonium acetate of the DNA. Selected colonies are grown overnight in selective medium at 37°C (cells infected with M13 phage are grown for 6-8 hours). 1.5 ml culture is transferred to an Eppendorf tube. Cells are pelleted by 3 min. centrifugation at 12000 g, resuspended in 50 µl TNE (Tris-HCl 10 mM pH8, NaCl 100 mM, EDTA 1mM) and 50 µl of mixed (v/v/v) phenol/chloroform isoamyl alcohol (25/24/1) are added. The mixture is vigourously vortexed and centrifuged for 5 min. at 12000 g to yield an almost clear supernatant. 50 µl of aqueous phase are transferred to a second centrifuge tube and precipitated with 2M ammonium acetate (final concentration) and 2 vol. of cold 95° EtOH for 15 min. on ice. The precipitated DNA is collected by 10 min. centrifugation at 12000 g, washed with 75° EtOH, dried and dissolved in 25 µl H_2O. Portions of 2 µl (containing 0.2 - 0.3 µg of DNA) can be digested with restriction enzymes. The RNAs of low molecular weight present in the samples which can mask small fragments of DNA in agarose gels may be hydrolysed by addition of DNase-free RNase A (50 µg/ml) to restriction buffer. Finally, the samples were analyzed in agarose gel or by PAGE.

We have tested different combinations of plasmids and bacteria. The method was reliable and suitable in all cases tested as shown in Fig.1.

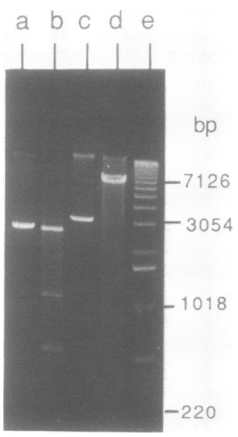

Fig.1 : Analysis of digested plasmids by electrophoresis in 1% agarose gel. **a-** pUC9 in JM 103 (5). **b-** pUC9 with insert in C_{600}5K. **c-** Bluescribe (+) in JM 109 (Stratagene). **d-** RF of M13 mp19 in JM 103 (6). **e-** Ladder 1Kb (BRL). pUC9, Bluescribe (+) and M13 are digested with Hind III. pUC9 with insert is double digested with EcoRI-Hind III.

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