

One step 'miniprep' method for the isolation of plasmid DNA

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All 'miniprep' methods reported so far for the isolation of plasmid DNA involve multiple pipetting, extraction, centrifugation and changes of minifuge tubes. For screening large number of samples, they are therefore cumbersome, time consuming and not economical.

Here, I report a very fast, simple and one step 'miniprep' procedure. The quality and quantity of DNA obtained by using this procedure is similar to those obtained by the other commonly used procedures of Serghini *et al.* (1) or Birnboim and Doly (2). According to this procedure, the bacterial culture is directly extracted with a mixture of phenol-chloroform-isoamylalcohol and the liberated DNA is precipitated with isopropanol. This method is now being used routinely in our laboratory for isolating plasmids upto 12 kb in size. A detailed description of the method is presented below:

1. Take 0.5 ml of overnight *E. coli* culture in a microfuge tube. We routinely grow our cells in 'standard 1' bacteriological media supplied by Merck, Germany.
2. Add 0.5 ml of phenol:chloroform:isoamylalcohol (25:24:1). The phenol was saturated with TE (10 mM Tris, 7.5, 1 mM EDTA) prior to mixing with chloroform and isoamylalcohol.
3. Mix by vortexing at the maximum speed for 1 minute. Alternatively, vortex for 10 seconds and then transfer to eppendorf mixer (No. 5432) for 5 minutes.
4. Spin at 12000 g for 5 minutes. During the spin, prepare microfuge tubes with 0.5 ml of isopropanol. After the spin, remove carefully about 0.45 ml of the upper aqueous phase leaving the interphase undisturbed and add it to the isopropanol. Mix well and spin immediately at 12000 g for 5 minutes. Addition of salt and cooling is unnecessary.
5. Pour off the supernatant, add carefully 0.5 ml of 70% ethanol to the side of the tube, pour off. Repeat the washing once more. Vacuum dry the pellet and suspend in 100 microliters of TER (10 mM Tris, 7.5, 1 mM EDTA, 20 microgram/ml RNAse). About 5–10 microliter of this DNA can now be cleaved with appropriate restriction enzyme(s) for analysis.

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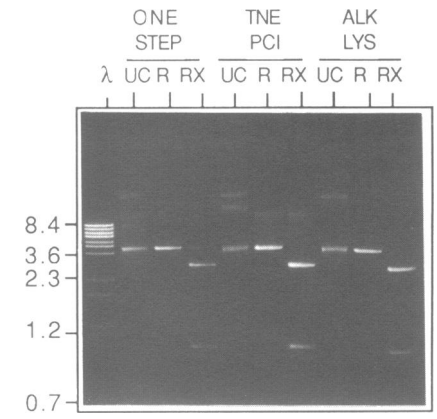

Figure 1. Comparison of the DNA isolated by the one step 'miniprep' method with the widely used TNE-phenol (1) and the alkaline lysis (2) procedures. From an overnight bacterial culture 0.5 ml aliquots were taken and the plasmid DNAs were prepared according to the procedure described here or as described previously (1, 2). The final DNA pellets were suspended in 100 microliter each of TER (10 mM Tris, 7.5, 1 mM EDTA, 20 microgram/ml RNAse). Eight microliter each of the DNAs were electrophoresed in 0.8% agarose gel either uncleaved (UC) or after cleaving with EcoRI (R) or EcoRI plus XhoI (RX). The plasmid described here contained an EcoRI-XhoI insert of about 1150 base pairs in bluescript vector.