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A simple method for the preparation of plasmid and chromosomal *E.coli* DNA

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Methods described for the isolation of DNA from E. coli usually involve organic solvent extractions or include the addition of other enzymatic inhibitors and a subsequent alcohol or salt precipitation step is always necessary (1,2). We describe a simple and efficient method for the preparation of chromosomal or plasmid DNA (or both) which obviates all of the above by proteinase K treatment of the bacterial lysate. Proteinase K is inactivated after 2 hours incubation at 65° (3). The DNA in the lysate can be used directly in enzymatic reactions or for transformation assays. A mini prep is made using 1 ml of an overnight grown culture. Cells are pelleted in a microfuge, washed with 1 ml TNE (10mM Tris pH 8, 10 mM NaCl, 10 mM EDTA) and then resuspended in 270 µl TNE containing 1% Triton X-100. 30 µl of a 5 mg/ml freshly prepared lysozyme solution are added and the suspension is incubated 30 minutes at 37°. At this point total DNA (chromosomal and plasmid if present) is obtained by incubation for 2 hours at 65° in the presence of 1mg/ml proteinase K For plasmid DNA isolation the lysate is centrifuged for 30 minutes in a microfuge at 4°, the plasmid containing supernatant is carefully collected and then incubated with proteinase K as above. The high molecular weight chromosomal DNA and the plasmid DNA can be restricted or modified by enzymes after the addition of 10mM $MgCl_2$ and the plasmid DNA can also be used for transformation. Fig 1. shows the restriction of the plasmid DNA with various enzymes as well as the ligation of the Bam H-1 cut DNA. When a large number of samples has to be processed this method requires very little labor and is completed in less than 3 hours with a high yield. This method can be used for DNA purification from any microorganism including viruses as long as their lysis does not require the addition of enzyme inhibitors.

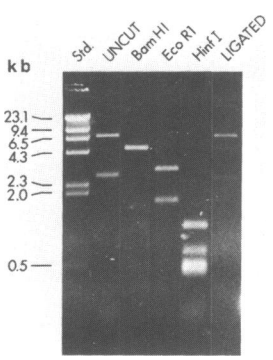

Fig.1 - Plasmid DNA prepared from E. coli HB 101 carrying a Bluescript sk^+ plasmid with a 1.8 Kb insert. The plasmid containing supernatant (see text) is electrophoresed in a 1% agarose gel; uncut, linearized with Bam H-1, the 1.8 kb insert is excised with Eco R1, and Hinf I has multiple restriction sites, the Bam H-1 plasmid was recircularized with T4-DNA ligase. Ø x Hae III standards are shown on the left with their respective molecular weight.

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