A simple and economical procedure for large-scale plasmid DNA isolation

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With the widespread application of recombinant DNA techniques and cloned gene products, preparation of plasmid DNA has become a common laboratory practice. This usually includes growth of plasmid-containing bacteria, extraction of the bacteria and isolation of plasmid DNA from the extract. In large-scale plasmid DNA isolation, removal of bacterial RNAs and other contaminants in the extract may be achieved by cesium chloride isopycnic centrifugation, which requires an expensive ultracentrifuge facility and maintenance, or by digestion with large amounts of ribonuclease, an undesirable procedure in a laboratory also engaged in RNA research. Here we describe an alternative procedure that requires only commonly available laboratory facility and reagents for plasmid DNA isolation from the crude bacterial extract. This is based on the principle that, in concentrated ammonium sulfate solution, large-size RNAs and other contaminants can be precipitated while tRNAs and other small RNA molecules can bind to Sepharose (1). We have found that, in the same ammonium sulfate solution, the plasmid DNA is soluble and does not bind to Sepharose.

In a typical preparative operation, we usually employ the enriched medium method (2) to grow a 250 ml culture of plasmid-containing bacteria and then the alkaline extraction procedure (2, 3) to obtain a crude plasmid DNA extract from the harvested 4–5 ml of wet packed bacteria. Following isopropanol precipitation, ethanol washing and brief lyophilization or vacuum suction (2, 3), the pellet of the crude extract is suspended in 1.8 ml of $T_{10}E_5N_{100}$ solution (10 mM TrisCl/5 mM EDTA/100 mM NaCl, pH 7.6) and transferred to a 10 ml Oak Ridge type centrifuge tube. After adding 0.1 ml of 10% sodium dodecyl sulfate and 0.1 ml of 20 mg/ml self-digested pronase, the sample is incubated at 55°C for 2 hours. This solubilizes the fine particulates in the mixture. At this point, 2 ml of 3 M $(NH_4)_2SO_4$ /5 mM EDTA solution is added and the mixture is kept in an ice-bath for at least 2 hours or overnight. The sample is then centrifuged at $12,000\times g$ (10,000 rpm, Sorvall SS-34 rotor) for 15 min at 0–4°C. If the supernatant is still cloudy, the ice-bath incubation and centrifugation steps should be repeated in another clean centrifuge tube. The 4 ml supernatant is brought to room temperature and applied to a 2 ml Sepharose-4B column equilibrated with 1.5 M $(NH_4)_2SO_4$ /5 mM EDTA/10 mM TrisCl pH 7.6 (in a disposable plastic column with a pre-tested Sepharose-4B lot having tRNA-binding capacity of 15 mg or more per 2 ml in this solution). The column is eluted with the same solution. The first 8 ml of passthrough effluent is collected

and dialyzed at 4°C in 500 ml $T_{10}E_5N_{100}$ solution, which is changed every 6 hours for 4 times to ensure the removal of ammonium sulfate. The isolated plasmid DNA in the dialyzed sample is concentrated by ethanol precipitation.

Figure 1 illustrates the result regularly obtained by the procedure. The isolated plasmid DNA is of high yield and satisfactory for various experimental purposes; these include restriction enzyme digestion and subsequent ligase or phosphatase-kinase reactions, chemical or enzymatic DNA sequencing, polymerase chain reaction, preparations of labeled molecular probes, *in vitro* transcription by RNA polymerases, bacterial transformation and DNA transfection of mammalian

Figure 1. Agarose gel electrophoresis of various fractions in pBOR-ras plasmid DNA isolation by the ammonium sulfate procedure. Lane 1, crude preparation following the alkaline extraction procedure; lanes 2 and 3, the 1.5 M ammonium sulfate precipitated and the supernatant fractions, respectively; lane 4, Sepharose-4B pass-through fraction, representing the isolated plasmid DNA; lane 5, Sepharose-bound fraction subsequently eluted from the column with 10 mM TrisCl/5 mM EDTA (pH 7.6); lanes 6 and 7, isolated plasmid DNA digested with *EcoRI* and *PstI*, respectively; and 'λH', molecular weight marker of a mixture of *HindIII*-cut λ DNA and *HaeIII*-cut φX174 DNA. All sample gel lanes contained amounts of fractions derived from 1/1000 of the nucleic acid extract of a 250-ml bacterial culture, except that about 6 times more of the Sepharose-bound fraction was added in lane 5 to reveal the tRNA staining.

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cells. Further purification is presumably needed for experimental purposes, such as developing transgenic mice, where complete removal of host bacterial DNA is important. The isolated plasmid DNA is mainly in supercoiled closed-circle forms, but we have not carefully examined the behavior of linear and open-circle forms of DNA in this isolation procedure. The ammonium sulfate precipitation step appears to remove some minor DNA forms, including presumably the large-size host bacterial DNA, in addition to the bulk of RNA in the gelatinous precipitate (Figure, lane 2). Also, if rapid plasmid DNA isolation is desirable, this procedure may be modified by using spun-columns instead of the time-consuming dialysis for the desalting step.

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