Rapid large-scale purification of plasmid DNA by medium or low pressure gel filtration. Application: construction of thermoamplifiable expression vectors

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This paper describes a new method of plasmid DNA purification which is fast and reliable enough for most purposes in recombinant DNA technology. The present method does not require the use of toxic chemicals such as phenol or ethidium bromide, costly ultracentrifugation procedures or other processes which can modify the supercoiled structure of the plasmids, such as adsorption on glass fiber. This method is based on the principle of gel filtration chromatography, at low pressure (1 bar) or medium pressure (between 5 and 10 bars), using Sephacryl S1000 or Superose 6B. It permits recovery of plasmids: (I) in preparative quantities (from 300 μg to 4 mg), (II) exempt from RNA, DNA and protein contamination, and (III) suitable for various common genetic engineering procedures immediately after purification. To test the reliability of the technique as well as the degree of purification, the plasmids were used to construct thermoamplifiable vectors, carrying the lacUV5 promoter and the 5' end of the β-galactosidase gene with a single EcoR1 site in each of the three possible translational phases. This set of vectors is designed for the expression of foreign genes as hybrid proteins in Escherichia coli.

During the last decade, with the introduction and continuous expansion of recombinant DNA technology, the interest of most research workers has been focused on the problem of nucleic acid purification of both eucaryotic and procaryotic origin, especially plasmid vectors, which nowadays play an important role in molecular biology. To date, satisfactory purification of supercoiled plasmids is achieved by several methods including isopycnic gradient of caesium chloride (Clewell, 1972), the rapid extraction procedure based on
alkaline hydrolysis (Birnboim & Doly, 1979), chromatography on hydroxyapatite (Colman et al., 1978), RPC-5 chromatography (Best et al., 1981) or adsorption on glass powder (Marko et al., 1982). All these methods are of limited capacity and are either expensive and time-consuming if one requires a high level of purity, or rapid and inexpensive but resulting in only mild purification as far as usual contamination by RNA, protein and chromosomal DNA are concerned.

We describe here a fast and reliable two-step purification procedure which does not depend on CsCl centrifugation, and which is rapid, inexpensive and yields DNA of high purity.

Our method takes advantage of selective precipitation of proteins and chromosomal DNA at high salt concentration after alkaline denaturation. The plasmid is then separated from RNA and further purified by gel filtration chromatography.

The technique described below in three variations permits work in different situations. All three are simple to carry out, differing only in the equipment used, the time taken in the process of purification being inversely proportional to the cost in equipment. The first variation (the least expensive) makes use of a standard low pressure column, and yields pure material after 250 min of gel filtration. The second takes 45 min on a fast protein liquid column (FPLC) system. The third, using the same equipment as the second, purifies the plasmids in 20 min, but necessitates the use of RNase.

To test the reliability of the method, we undertook the construction of a vector easy to use (thermoamplifiable) and suitable for the expression of genes lacking promoters. We therefore constructed a plasmid containing the duplication origin of pKN402 (Uhlin et al., 1979) and the ampicillinase gene of pBR322 (Bolivar et al., 1977). Subsequently, in the resulting plasmid termed pVT46, we have inserted the lac regions from the previously described plasmids pPCφ1, pPCφ2 and pPCφ3 (Charnay et al., 1978). The vectors thus obtained called pVT46φ1, pVT46φ2 and pVT46φ3 are stable, thermoamplifiable, resistant to ampicillin and enable the expression of foreign genes as hybrid proteins in Escherichia coli.

Materials and Methods

The FPLC system was from Pharmacia.

Chemicals

The following chemicals were used: Xgal: 5-bromo-4-chloro-3-indolyl-beta-D galactoside, agarose, lysozyme (Sigma). Low melting agarose (BRL). Sephacryl S1000 and Superose 6B (Pharmacia).

Enzymes

Restriction endonucleases, T4 DNA ligase and DNA polymerase (Klenow fragment) were obtained from New England Biolabs and were used as recommended by the supplier.

Bacterial and phage strains and plasmids

MC1061 rec A, r- m- was a gift from M. Zakin (Institut Pasteur, Paris); pBR322 was a gift from F. Bolivar; pKN402 was a gift from K. Nordström; pPCφ1, pPCφ2, pPCφ3 were gifts from P. Charnay;