of indole are substituted with the three substituents (2Br, CH_3). Irradiation of the methyl signal at δ2.60 sharpened the signal at δ6.40, indicating that the methyl group was located in the 2-position. Thus, compound **2** is 4,6-dibromo-2-methylindole.

Bromine substitution at the 4- and 6-positions without any substituents at the 3-position has never been reported before from natural sources. To date more than 70 indolic compounds have been isolated from marine organisms[9]. Many of them bear halogens, most probably one of the halogens at the 3-position unless the site is occupied by other substituents. This is consistent with a biosynthetic mechanism involving the attack of peroxidase-mediated cationic species on an indolic substrate, since the 3-position of indole is the site most susceptible to the attack of electrophilic halogenating reagents[10]. Biosynthesis of 3-unsubstituted haloindoles may therefore involve a different mechanistic scheme from that of 3-halogenated indoles. In the biosynthesis of the present compounds **1** and **2** and of the previously-known 5,7-dibromo-6-methoxyindole (**5**)[11] halogenation of an aromatic amino acid such as phenylalanine (tyrosine in case of **5**) may be one of the first steps, and be followed by cyclization to an indole-2-carboxylic acid as proposed earlier[4]. This, however, is contrary to the known biosynthetic scheme for some brominated phenols from aromatic amino acids in algae, in that bromination occurs only at the later stages of the catabolism[12].

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An economical large scale procedure to purify *E. coli* amplifiable plasmids for DNA sequencing, in vitro transcription and in vitro mutagenesis[1]

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Summary. A reproducible and economical procedure for obtaining a large and quantitative yield of highly purified covalently closed circular plasmid DNA is described. The procedure departs in several ways from more commonly used methods. These are a) avoidance of the use of CsCl, ethidium bromide and ultracentrifuge, b) enrichment of the plasmid DNA by selective denaturation of chromosomal DNA with an alkaline-SDS solution, c) enrichment of covalently closed circular plasmid DNA by extraction with acid-phenol, and d) removal of small degraded RNA fragements by molecular sieve chromatography after digestion with RNase A. The plasmid DNA prepared by this new procedure is free of contaminants and has been used for DNA sequencing, in vitro transcription, transformation and in vitro mutagenesis.
Key words. Economy large scale purification; CCC plasmid DNA; no EtBr; no ultracentrifugation.

The importance of plasmid DNA to recombinant DNA technology has led to the development of various methods for isolating plasmid DNA. Most of the small scale methods are useful for rapid screening of a large number of colonies, but are not practical for preparation of large quantities of pure covalently closed circular plasmid DNA. Several large scale procedures[3–8] have been developed. Each has its advantages, but all suffer from at least one major disadvantage, such as reproducibility, ability to achieve truly large quantities of plasmids, and/or the need for expensive chemicals and equipment. For these reasons, we have developed an economical and useful large scale method for the preparation of pure covalently closed circular plasmid DNA which avoids most of the disavantages of presently available procedures. The method avoids exposure of DNA to ethidium bromide and does not require the use of expensive CsCl and ultracentrifuge. Plasmid DNAs were recovered in good yield with high purity and biological activities.

Procedure and results. The *E. coli* K12 strain HB101 (pro$^-$, leu$^-$, thi$^-$, lacy$^-$, hsdR$^-$, endA$^-$, recA$^-$, rpsL20, ara-14, galK2, Xyl-5, mtl-1, supE44) was used as the host bacterial strain for all plasmids. The recombinant plasmid used in this study was pAd123 which contained an insert of 1.65 Kb of an adenovirus type 2 DNA fragment (31.5 to 36.9% map unit). This fragment was derived by a double digestion of adenovirus type 2 DNA with restriction enzymes Hind III and Sal 1. Fifty other pBR322 derived recombinant plasmids with inserts ranging from 0.6 to 8 Kb were also used.

All procedures are expressed per liter of harvested bacterial cells and can be easily scaled up to any desired volume. Bacteria with plasmids were grown in 1 l of M9CA medium[9] containing 50 μg/ml of ampicillin (Sigma) until an absorbancy of 0.7 to 1.0 at 550 nm was obtained. Chloramphenicol (Sigma) was then added to a final concentration of 140 μg/ml and the culture was continued for another 16 h to allow amplification of the plasmid[10]. For preparation of plasmids alkaline-SDS-denaturation[11] was chosen as the first step to effectively remove most of the chromosomal DNA and cellular RNA; the enriched plasmid DNA was recovered in the supernatant. To accomplish this, the cells were harvested by centrifugation at 4000 × g for 10 min at 4 °C. The cell pellet was gently suspended in 20 ml of a freshly prepared lysozyme solution [2 mg/ml lysozyme (Sigma), 9 mg/ml D-glucose, 10 mM Na·EDTA (pH 7.9) and 25 mM Tris·HCl (pH

7.9)], and the cell walls were partially digested by incubation at 0 °C for 30 to 60 min. At the end of incubation, a 40 ml aliquot of freshly prepared alkaline-SDS solution (1.8% SDS and 0.2 N NaOH) was added to the mixture (final pH 12.2). The mixture was thoroughly stirred and allowed to stand for 5 min on ice. Next, 30 ml of 3 M sodium acetate (pH 4.0) was added, and the mixture was swirled several times. During the next 60 min, most of the proteins, high molecular weight RNAs and chromosomal DNA precipitated. The precipitate was removed by centrifugation at 12,000 × g for 10 min. The supernatant was collected and filtered through one layer of Miracloth (Calbiochem) to remove the residual floating white precipitate. Plasmid DNA was precipitated by addition of two volumes of cold 95% (v/v) ethanol. After standing for 10 min at 70 °C, the precipitate was collected by centrifugation at 6500 × g for 10 min. The pellet was suspended in 25 ml of TE [10 mM Tris·HCl (pH 7.9) and 0.1 mM Na·EDTA], and reprecipitated by ethanol after addition of NaCl to a final concentration of 0.1 M. The pellet collected by centrifugation was dissolved in 25 ml of TE and treated with 0.1 mg/ml heat-treated RNaseA (Sigma) for 60 min at 37 °C. This mild RNaseA digestion step resulted in degradation of RNA to a size of 4S or smaller and was mandatory for the successful chromatographic separation of plasmid DNA from the cellular RNAs (see fig.). Subsequently, the RNaseA was digested with 0.4 mg/ml of pronase (Calbiochem.) after addition of SDS and $CaCl_2$ to final concentrations of 1% and 1 mM, respectively. Pronase digestion was carried out at 37 °C for 60 min and stopped by addition of Na·EDTA to a final concentration of 10 mM. The mixture was deproteinized by extracting three times with an equal volume of 88% phenol (redistilled). Sodium chloride was added to the upper phase to a final concentration of 0.1 M followed by addition of two volumes of cold 95% ethanol. The precipitate was collected by centrifugation after standing at −70 °C for 10 min and dissolved in 20 ml of 50 ml sodium acetate (pH 3.8). Next, the acid-phenol extraction method[12] was used for removing fragmented chromosomal DNA, and linear and nicked circular plasmid DNA. NaCl was added to the DNA solution to a final concentration of 0.075 M. The DNA solution was extracted three times by vigorously shaking with an equal volume of phenol saturated with 50 mM sodium acetate (pH 3.8) at 4 °C. The final upper phase was neutralized by addition of a Tris·HCl (pH 7.9) solution to a final concentration of 0.075 M. DNA in the upper phase was precipitated by ethanol, collected by centrifugation and dissolved in 3 ml of TE. Essentially, all of the plasmid DNA purified up to this step was in a covalently closed circular form as shown in figure 1b (T lane in inset). Final purification was achieved by molecular sieve chromatography using a column (2.5 × 35 cm) packed with Sephacryl-S300[13] (Pharmacia), which was used to remove RNA contaminants from DNA. Elution was carried out at 4 °C with a flow rate of 75 ml/h. The DNA pooled from the fractions under peak I in figure 1b was concentrated by ethanol precipitation and stored at −20 °C.

In summary, this large scale procedure has been routinely used in this and other laboratories to purify pBR322 derived recombinant plasmids with inserts ranging from 0.6 to 8 Kb. The yield of plasmid DNA was about 1 mg/l of amplified culture. The plasmid DNA was spectrophotometrically pure, with an A260/A280 ratio of 1.61, and it was also biochemically pure as characterized by restriction enzyme cleavages and DNA sequencing analysis (data not shown). At least 98% of the plasmid DNA was in convalently closed circular form, as shown in figure 1b. It was free of proteins, RNAase and RNA which is inhibitory to transcription. Furthermore, based on the following observations, it has high biological activity[14]: The DNA was compatible to all restriction enzyme cleavages and transformed[15] *E. coli* K12 strain HB101 with an efficiency of 11.3×10^5 Amp^R-transformants per μg of DNA. Plasmids containing intact adenovirus type 2 VARNA genes could direct faithful transcription of these genes in vitro[16]. The plasmid DNA could also be used for rapid DNA sequencing using either an enzymatic[17] or a chemical method[18]. It has also been used for generation of deletion mutations in vitro. In conclusion, we have found this method to be a highly useful and economical large scale procedure for obtaining mg quantities of pure, biologically active plasmid DNA.

Removal of RNA contaminants from plasmid DNA by Molecular Sieve Chromatography using a Sephacryl S-300 column. Plasmid DNA, pAd123, was isolated as described in the text without a) or with b) ribonuclease treatment. The photograph insets show agarose gel electrophoresis patterns of the DNA in fractions 15 to 27 as indicated in a) and in the pooled fractions I, II and III as indicted in b), respectively. T stands for the plasmid DNA preparation before molecular sieve chromatography.

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Announcements

Correction

O. Ghisalba and H.-P. Schär: New directions in methanotrophy, Experientia *41* (1985) 553–554. Please note the following corrections: page 553, line 15: ATCC 27886; page 554, line 1: At the 4th C_1-Symposium it was reported (J.A. Duine et al., TH Delft) that *Methylococcus capsulatus* contains two MDH **activities, the classical MDH (c-MDH, PQQ-dependent) and a novel** MDH (N-MDH, PQQ- *and* NAD-dependent).

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