Short Communication

Rapid Preparation of Plasmid by Gel Permeation Chromatography on Toyopearl HW75S

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Several isolation methods for plasmid DNA were established.¹⁾ The most commonly used procedure is CsCl-ethidium bromide equilibrium density gradient centrifugation,²⁾ but this procedure is time-consuming and cannot completely remove low molecular weight RNA from the plasmid preparations.

Gel permeation chromatography has recently been employed to remove low molecular weight RNA.^{3,4)} The material prepared by CsCl centrifugation was passed through a Sephacryl S-300 column.³⁾ Without the use of CsCl centrifugation, the material freed from bacterial DNA with acid-phenol extraction and that freed from high molecular weight ribosomal RNA with ammonium sulfate precipitation was applied to gel filtration through Sepharose CL 4B.⁴⁾

Our method is simpler, the cleared lysate was directly applied to gel filtration through Toyopearl HW75S. Plasmid DNA which was virtually free from chromosomal DNA and RNA was obtained by overnight elution.

The cleared lysate was prepared according to the method of Clewell *et al.*⁵⁾ with the modification of Zahn *et al.*⁶⁾ from a culture of *E. coli* HB101 containing plasmid pBR322. One ml of the cleared lysate together with 0.02 ml of marker dye (0.1% bromophenol blue) was applied to a Toyopearl HW75S column (1.0 × 54 cm). Toyopearl HW75S was supplied by Toyosoda Co. (Akasaka 1-7-7, Minato-ku, Tokyo) which is also available from Merck Co. (Darmstadt, Germany) as Fractogel TSK75S. This material, a hydro-

philic semi-rigid spherical gel manufactured from vinyl polymers, is mechanically stronger than dextran-type gels and allows a higher speed elution. The column was eluted with an elution buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5) at a flow rate of 5 ml/hr. The eluate was monitored with a Uvicord (LKB, Stockholm) and fractionated into 50 drops/tube. Chromatography was carried out at room temperature, each fraction being concentrated by precipitating with ethanol was analyzed by agarose gel electrophoresis. The same column was repeatedly used after replacing the upper 1 cm portion of Toyopearl with new material.

The elution profile (Fig. 1) and agarose gel electrophoresis (Fig. 2) show that chromosomal DNA was eluted mostly in fractions 14~16 and plasmid DNA in fractions 17~19. RNA and protein were mostly in peak 1 and peak 2, respectively. Bromophenol blue, a small molecular marker, was found in fractions 49~51.

The plasmid DNA isolated by this method was virtually free from RNA and susceptible

FIG. 1: Gel Permeation Chromatography.

One ml of the cleared lysate was prepared from *E. coli* HB101 containing pBR322, applied to a Toyopearl HW75S column (1 × 54 cm), and the column was eluted as described in the text. The elution profile recorded on Uvicord is presented. Each fraction contained 50 drops (about 1.5 ml).

FIG. 2. Agarose Gel Electrophoresis.

Each fraction from 14 to 21 of Figure 1 was precipitated and dissolved in 100 μ l of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). 20 μ l each was applied to agarose gel electrophoresis (14~21). Another plasmid preparation, which was isolated from the same cleared lysate by CsCl-ethidium bromide centrifugation, was also analyzed (M). (Chr), chromosomal DNA; (OC), open circular form; (L), linear form; (CCC), covalently closed circular form.

to digestion by several restriction enzymes including *Bam*HI, *Bgl*I, *Eco*RI, *Hind*III, *Pst*I, *Pvu*II, and *Sal*I. It was also used successfully for long term storage and transformation.

An amount of 0.81 A_{260} unit (0.60 ml of $A_{260}=0.135 \times 10$) of pBR322, purified by the column, was applied again to the column and the amount of 0.85 A_{260} unit (7.6 ml of $A_{260}=0.112$) of DNA was recovered in the eluate.

This indicates that the recovery from our procedure was almost complete.

The intactness of the plasmid was compared before and after chromatography by analyzing the samples with agarose gel electrophoresis. Purified pBR322 containing the covalently closed circular form (CCC) and open circular form (OC), CCC/OC=90.7/9.3, was applied to the column. Two forms, CCC/OC=90.9/9.1, were recovered in the eluate. It can be concluded that plasmid DNA did not suffer any damage during the chromatography.

In conclusion we found that this rapid method was useful for pilot experiments of plasmid DNA ranging in molecular weight from 2.6 to 8.7 mega-daltons and for long term storage of plasmids. When a larger amount of plasmid DNA was required for a detailed analysis, this column was used only to remove low molecular weight RNA after CsCl-ethidium bromide centrifugation, because we could not get clear separation of more than 20 μ g of plasmid by this direct rapid method using this size of column.

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