Note

Rapid and Simple DNA Isolation from *Escherichia coli* (*E. coli*) by a Modified Boiling Method Using Bivalent Metal Ion Chelate

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Phenol-chloroform extraction,¹⁾ alkaline extraction²⁾ and boiling³⁾ methods have been commonly used for isolating plasmids from *Escherichia coli*. However, these methods require multiple centrifugation and, therefore, are not adequate for the application to an automatic nucleic acid extractor.

The facts that copper complex was used for preparing glycine⁴⁾ and that metal chelate affinity chromatography has been useful for fractionating proteins^{5,6)} led us to investigate whether the chelating activities of various metal ions could be used for the purification of plasmids from *E. coli* carrying a given plasmid. In this manuscript, we describe a modified and convenient method that uses bivalent metal ions to rapidly separate plasmids from *E. coli*. This method, named the metal chelating method, is simpler than the usual methods of phenol-chloroform extraction or alkaline extraction, the recovery of plasmids from *E. coli* being almost the same as that by the previous methods, and the digestion of plasmids with *Hind*III was complete.

An overnight culture (1 ml) of *E. coli* MC1061 carrying pBS M13⁻ from Stratagene of San Diego was centrifuged at 12,000 rpm for 2 min, before the pellet was suspended in 700 μ l of STET buffer (8% sucrose, 0.5% Triton X-100, 50 mM EDTA and 10 mM Tris-Cl, pH 8.0) and 50 μ l of lysozyme (10 mg/ml). Then, 70 μ l of either 1% (w/v) CuCl₂ (pH 4.1), 1% (w/v) ZnCl₂ (pH 5.3), 1% (w/v) MnCl₂ (pH 5.4), 1% (w/v) CoCl₂ (pH 5.6), 1% (w/v) NiCl₂ (pH 5.4) or 1% (w/v) MgCl₂ (pH 6.4) was added to each tube. After vortexing, the mixture was heated for 30 seconds at 90°C. The sample was then centrifuged for 10 min at 15,000 rpm, and the supernatant was transferred to a new tube. Three μ l of ribonuclease solution (10 mg/ml) was added to the supernatant, and the mixture was incubated for 20 min at 37°C. After the addition of 80 μ l of 3 M sodium acetate (pH 5.2) and 800 μ l of isopropanol, the sample mixture

was chilled at -20°C for 1 hr, centrifuged for 10 min at 12,000 rpm, rinsed with 80% ethanol and finally dried *in vacuo*. The dried sample was dissolved in 50 μ l of TE buffer (10 mM Tris-Cl (pH 8.0) and 1 mM EDTA) and used for analysis.

Ultraviolet absorption of the plasmids isolated by the metal chelating method showed that the ratios of $A_{260\text{ nm}}$ to $A_{280\text{ nm}}$ varied between 1.8 and 2.0. We measured the protein content in plasmid preparations before and after treating with phenol and chloroform, and with Zn²⁺. The Zn²⁺-chelating method could eliminate almost 98% of protein in the sample, while the phenol and chloroform treatment could remove 87% of protein, as determined by the Lowry method.⁷⁾ These data indicate that the Zn²⁺-chelating method was almost the same as the phenol-chloroform extraction method with respect to its effectiveness for deproteinization.

When the plasmids prepared by the metal chelating method were digested with a restriction enzyme such as *Hind*III, and electrophoresed with agarose gel, similar patterns to those by the usual method were invariably obtained, as shown in Fig. 1. It was found that Zn²⁺, Cu²⁺ and Co²⁺ were the best metal ions among those tested (lanes A, B and F), Ni²⁺, Mg²⁺, and Mn²⁺ not giving satisfactory results (lanes C~E). The comparison between the metal chelating method in this study and the alkaline-extraction method, the boiling method and the boiling method with phenol-chloroform treatment was done under the same experimental conditions. The figure shows that the electrophoretic pattern of the samples digested with *Hind*III prepared by the Zn²⁺-chelating method was the same as that of the alkaline method and better than that of the original boiling method, since it was recognized that plasmids isolated by the original boiling method were not digested by treating with *Hind*III, as shown in lane J. However, when these plasmids were further treated with phenol and chloroform, the digestion with *Hind*III was completed (lane K). Thus, the present method can eliminate the treatment of plasmids with phenol and chloroform. The addition of 70 or 140 μ l of Zn²⁺ solution to 700 μ l of the lysate from 1 ml of bacterial culture was shown to be the optimal condition for isolating plasmids (lanes F~H). It was also found that the plasmid obtained from *E. coli* by the Zn²⁺-chelating method could be labeled with ³²P-dCTP by nick translation. This Zn²⁺-chelating method was also useful for extracting pUC18 and pBR322 (data not shown). Since it has been previously shown by Sin and Eichhörn⁸⁾ that Zn²⁺ as well as Cu²⁺ binds strongly enough to the phosphate moiety of DNA molecules to stabilize the DNA double helix, the present method of using Zn²⁺ may thus be more suitable than the boiling method, the phenol-extraction method and the alkaline-extraction method in terms of the stability of the extracted plasmids. Furthermore, the present method has the advantages that the reagents used are not expensive and corrosive, that it is easy to apply for an automatic nucleic acid extractor, and that it is not time-consuming as

Fig. 1. Electrophoretic Patterns of *Hind*III-digested pBS M13—Isolated by the Metal Chelating Method, the Alkaline Method²⁾ and the Boiling Method.³⁾

Plasmids were prepared from 1 ml of bacterial culture, and then digested with *Hind*III.

(A) Cu^{2+} , 70 μl ; (B) Co^{2+} , 70 μl ; (C) Ni^{2+} , 70 μl ; (D) Mg^{2+} , 70 μl ; (E) Mn^{2+} , 70 μl ; (F) Zn^{2+} , 35 μl ; (G) Zn^{2+} , 70 μl ; (H) Zn^{2+} , 140 μl ; (I) alkaline method; (J) boiling method; (K) boiling method with phenol-chloroform extraction; and (L) *Hind*III-digest of λ .

compared to the other methods. Thus, we can conclude that the method using metal ions is very useful for the rapid separation of plasmids from *E. coli*, and has potential value for future application to an automatic nucleic acid extractor.

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