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PREPARATION OF DNA MARKERS BASED ON *E. coli* PLASMID DNA

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DNA fragments of various length were produced by enzymatic restriction of plasmid DNA from various strains of E. coli. *A molecular DNA marker was constructed based on the DNA restricts.*

Key words: marker, DNA, restriction enzymes, restriction mixture, pDNA precipitation, DNA analysis.

Modern research in molecular biology involves mainly the analysis of nucleic acids in DNA and RNA. Nucleic acids relay genetic information and changes occuring in the organism before they become macroscopically apparent [1].

Our goal was to prepare DNA markers for analysis that are based on plasmid DNA from *E. coli*. Many various commercial markers are available, e.g., λ/Hind III, pBR322/Hae III, and pUC-18/Hpa I. They are considered type I markers and are prepared from DNA (less often RNA) by hydrolysis by a certain endonuclease with several restriction sites in the DNA sequence. The reaction is performed in a certain buffer, the composition of which depends on the type of endonuclease. Type II markers are prepared by ultrasonic disintegration of DNA, which disrupts any high-molecular-weight DNA into fragments of a rigidly defined size. The size of these fragments depends on the ultrasound frequency and the treatment time. The first type of markers combines both high-molecular-weight different fragments, including DNA fragments less than 1,000 nucleotide pairs (Knp) (λ/Hind III) and low-molecular-weight fragments with DNA fragments <1 Knp (pBR322/Hae III, pUC-18/Hpa I) [1, 2]. The second type of markers, LADDERS, includes exclusively high-molecular-weight DNA markers [2].

Various plasmid DNAs from *E. coli* are under study in the IGEBP. These can be restricted at certain sites to produce low-molecular-weight and high-molecular-weight markers with fragments of a certain length and a high-molecular-weight marker as a mixture of highly purified linearized plasmids of known length [3].

Plasmid DNA from *E. coli* was prepared by alkaline lysis with subsequent extraction by phenol and precipitation with absolute ethanol. Plasmid DNA occurs in three conformations: super-spiral, with a single-strand break, and linear [1]. In performing electrophoresis of plasmid DNA, we found three closely spaced DNA fragments. Each of them corresponds to one of the three DNA conformations [4].

Plasmid DNA in the linear conformation is necessary for markers. We used plasmid DNA from *E. coli* such as pUC-18, pYI, preS1-S, and p-15. Using linear DNA from plasmids of pUC-18, preS1-S, p-15, and pYI produced plasmid marker M1. Linear pDNA molecules have the following molecular dimensions:

1) pUC-18 was split by specific endonuclease E.coR I to give a DNA fragment of 3.2 Knp;

2) pYI was split by specific endonuclease Knp I to give a DNA fragment of 7.1 Knp;

3) preS1-S was split by specific endonuclease Sal I to give a DNA fragment of 8.0 Knp;

4) p-15 was split by specific endonuclease Kpn I to give a DNA fragment of 11.5 Knp.

Mixing the pDNAs resulted in a marker with fragments corresponding to molecular weights 3.2, 7.1, 8.0 and 11.5 Knp.

Figure 1 shows gel electrophoresis of marker (M1) compared with commercial marker λ/Hind III. Our marker consists of fragments with molecular weights 11.5, 8.0, 7.1, and 3.2 Knp.

Thus, we isolated a marker containing fragments of a defined length. The purity and concentration of the markers were determined spectrometrically and compared with standard markers (Sigma).

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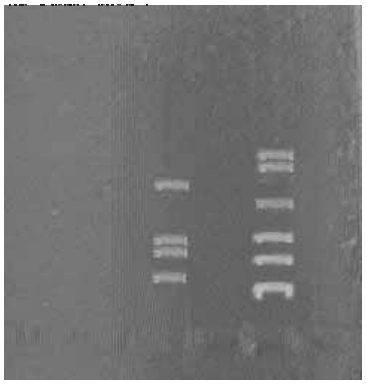

Fig. 1. Electrophoresis of restriction marker. M1, restriction marker containing fragments 11.5, 8.0, 7.1, and 3.2; M2, commercial marker λ/Hind III.

EXPERIMENTAL

Bacteria were cultivated by the standard method [5].

Isolation and Purification of pDNA. Plasmid DNA was isolated by alkaline lysis with subsequent deproteinization of the mixture by phenol:chloroform and precipitation with absolute ethanol [5]. The precipitate of pDNA was dissolved in TE-buffer (1.0 mL) and deproteinized again with proteinase K solution at a final concentration of 20 μg/mL at 37°C for 1 h. RNA-ase A was added to a final concentration of 10 μg/mL to remove RNA impurities. The solution was incubated at 50°C for 30 min. The mixture was extracted with phenol:chloroform. The supernatant was collected and treated with NH_4OAc at a final concentration of 0.3 M. pDNA was precipitated with 0.6 volumes of absolute isopropanol at -20°C for 2 h. The solution was centrifuged for 10 min at 14,000 rpm. The precipitate was washed with ethanol (70%) and dissolved in TE-buffer (0.5 mL). The purity of the DNA samples was determined from the ratio of the optical density coefficients at 260 and 280 nm. The coefficient for the DNA samples was 1.7-1.8. The pDNA concentration was determined spectrometrically from a calibration curve constructed using measurements of standard pDNA solutions of pUC-18 (Sigma) at A_{260}. The concentration of all pDNA samples was equilibrated to 1 μg/mL by dilution with TE-buffer. DNA samples were stored at -20°C [6].

The size of pDNA from strains pUC-18, pYI, preS1-S, and p-15 of *E. coli* was calculated by performing gel electrophoresis with standard marker λ/Hind III and comparing with the plasmid molecular map. The sizes of the prepared pDNA corresponded with those of the molecular maps and was for the plasmids: pUC-18, 3.1; pYI, 7.1; preS1-S, 8.0, and p-15, 11.5 Knp.

Preparation of Plasmid Marker. Plasmid DNA marker was prepared by fragmenting DNA of each plasmid using hydrolysis with specific endonuclease at a unique site of the molecular map of the corresponding plasmid (data are given in the text).

The linearization was performed by the standard method in enzyme-specific solutions. Linear pDNA molecules were purified of enzyme by phenol extraction, precipitated by ethanol, and dissolved in TE-buffer to a concentration of 1 μg/mL. DNA was stored at -20°C [7].

Marker was prepared by mixing solution (300 mL) of each plasmid and thoroughly peptizing. The mixture was left at 4°C for 3 h, divided into aliquots (50 μL each), and frozen at -20°C. The quality of the resulting marker was determined by electrophoresis with standard marker λ/Hind III (Sigma).

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