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Application News

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MCE

Detection of Circular Plasmid DNA by MultiNA™

Various plasmids are used in gene recombination experiments, and in recent years, plasmids have also been used in storage and distribution of bioresources due to their convenience. Conventionally, agarose gel electrophoresis has been used to measure the purity and size of plasmids, but plasmids can exist in conformations with different 3-dimensional structures, even in the same nucleotide sequence, and this affects the mobility of plasmids when passed through a molecular sieve in electrophoresis. The different 3-dimensional structures are the three conformations called supercoiled, open-circular, and linear, as illustrated in Fig. 1. The supercoiled conformation contains no nicks in the double-stranded DNA and has a shape like a tightly-twisted rubber band. The open-circular conformation has a circular shape in which the twisting is relaxed (i.e., the supercoils are released) by a nick in one strand of the double-stranded DNA. The linear type is a straight chain-like conformation resulting from complete cutting of both strands in any part of the double-stranded DNA. Since these three conformations have different apparent sizes, the sizes of plasmids detected by agarose gel electrophoresis may differ, depending on the three conformations, even when the size of plasmids is identical.

At present, microchip electrophoresis (MCE) systems have gained wide acceptance as an alternative technique to conventional agarose gel electrophoresis. Among these systems, the Shimadzu MCE-202 MultiNA microchip electrophoresis system can perform all of the work processes of agarose gel electrophoresis automatically.

This article introduces an actual example of an analysis of three types of plasmids using the MultiNA microchip electrophoresis system and an agarose gel electrophoresis system.

Y. Sogabe

MultiNA™ Microchip Electrophoresis System

Samples and Methods

The analysis samples used here were plasmid DNA of three different sizes, pGEM®-3zf(+) Vector (3,197 bp) (P227A, Promega Corporation), M13mp18RF (7,249 bp) (3118, Takara Bio Inc.), and pUC19 (2,686 bp) (3219, Takara Bio Inc.).

Linear plasmid samples were prepared by using the restriction enzyme cut site (*Eco*R I) which is common on the multicloning sites of the three types of plasmid DNA.

Analyses of the resulting circular and linear plasmids were conducted using the MultiNA microchip electrophoresis system and an agarose gel electrophoresis system (Fig. 2).

A DNA-12000 kit was used in the analysis by the MultiNA. For agarose gel electrophoresis, 0.7 % agarose gel was prepared, and staining with ethidium bromide (EthBr) was used in detection of the nucleic acid.

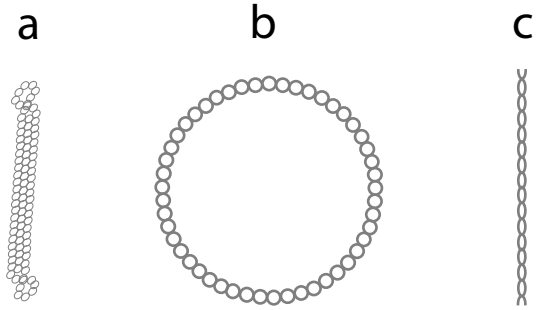

Fig. 1 Three Conformations of Plasmids
(a: Supercoiled, b: Open-Circular, c: Linear)

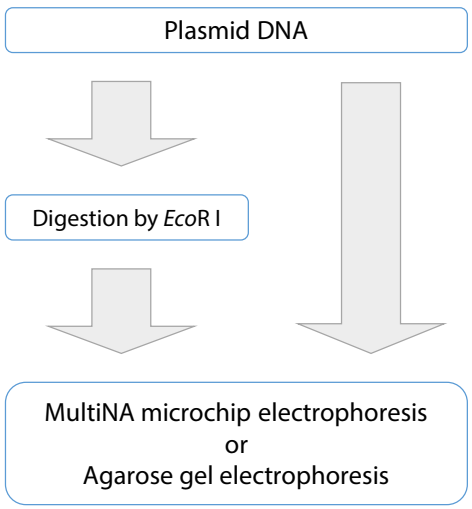

Fig. 2 Analysis Protocol

Results

Clear analysis results (gel images) could be obtained by electrophoresis using the MultiNA, as shown in Fig. 3(a1) and (a2).

In the analysis of the circular plasmids, the three types of plasmids were detected at pGEM®-3zf(+) (approx. 1,100 bp), M13mp18RF (approx. 1,750 bp), and pUC19 (approx. 950 bp) (Fig. 3 (a1)), respectively. All three samples were detected at a size about 1/3 smaller than the theoretical value. In these results, it is conjectured that the plasmids were detected in the supercoiled state. When restriction enzyme treatment was carried out and the plasmids were analyzed in the linear state, they were detected at the respective positions of their theoretical sizes (Fig. 3(a2)).

Fig. 3(b1) and (b2) show that gel images could also be obtained by analyzing the same samples by agarose gel electrophoresis. Considering the difference in sensitivity with the MultiNA system, a 10 times larger amount was applied in the analysis by agarose gel electrophoresis.

In the analysis in the circular state without further plasmid preparation, sizes approximately 1,000 bp to 2,000 bp smaller than the theoretical size and sizes close to the theoretical values were detected (Fig. 3(b1)). However, the images of the sizes close to the theoretical values were quite weak.

In the agarose gel electrophoresis analysis of the plasmids in the linear state obtained by restriction enzyme treatment, sizes close to the theoretical size were detected, as in the analysis by the MultiNA (Fig. 3(b2)). Unfortunately, with agarose gel electrophoresis, only quite large sizes could be estimated from the ladder markers, and it would also be difficult to say that the electrophoresis images are clear results.

Conclusion

As distinctive features of plasmid analysis by the MultiNA system, (1) sizes approximately 1/3 smaller than normal are detected in the supercoiled state if the analysis is performed without adequate plasmid preparation, and (2) detection of the intrinsic size is possible if samples in the linear state are prepared by restriction enzyme treatment. It is conjectured that the difference in the analysis of plasmids in the circular state by the MultiNA and agarose gel electrophoresis methods is due to the difference in the molecular sieve structure of the separation media used in the two methods.

As shown in this example, in size analysis of plasmids by the MultiNA system, the results of the original molecular size can be obtained if samples in the linear state are prepared by restriction enzyme treatment. Moreover, because analysis by MultiNA provides sensitivity approximately one order of magnitude higher than that of the EthBr staining method used in agarose gel electrophoresis, sample consumption for verification can be minimized.

Automatic analysis by the MultiNA microchip electrophoresis system does not require additional labor during the analysis, as the manual procedures are limited to only setting the sample and reagent. As a result, work efficiency can be greatly improved, particularly in cases treatment of many samples is necessary.

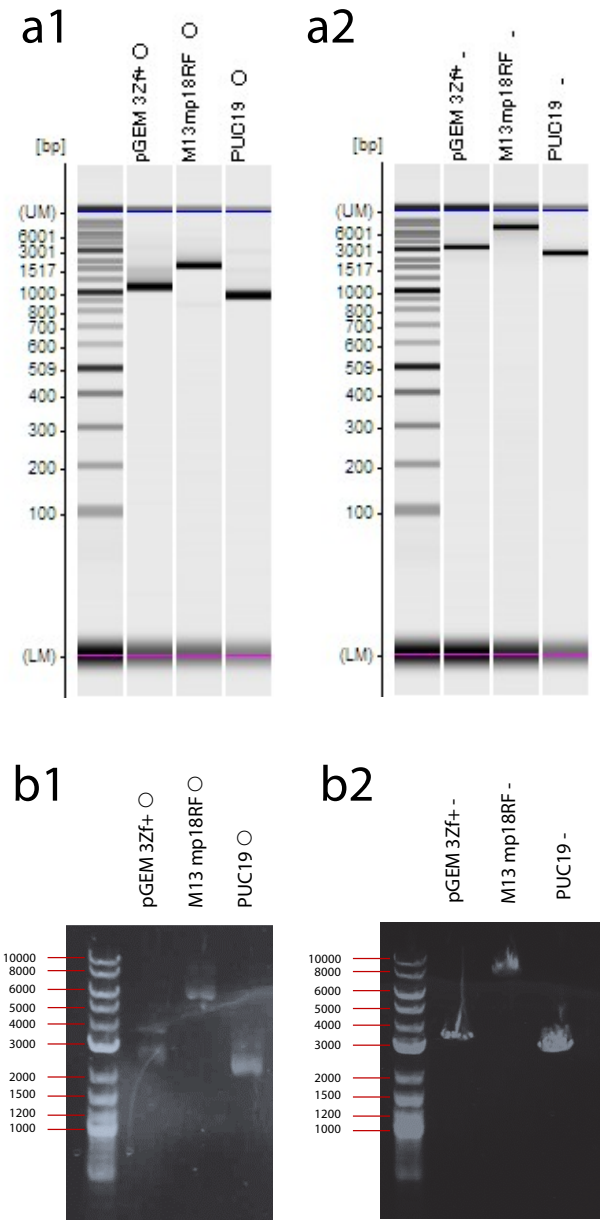

Fig. 3 Results of Electrophoresis Analysis
a: Gel Images Obtained by MultiNA
(a1: Analysis of Circular Plasmids, a2: Analysis of Linear Plasmids)
b: 0.7 % Agarose Gel Electrophoresis
(b1: Analysis of Circular Plasmids, b2: Analysis of Linear Plasmids)

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