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Application News

No. B65

Microchip Electrophoresis

Heteroduplex Mobility Assay (HMA) Using MultiNA

Introduction

The arrival of genome editing tools that make use of TAL (transcription activator-like) effector nuclease and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins 9) has enabled genes to be specifically destroyed and introduced with respect to target genes.

These tools are rapidly becoming popular because they can now be applied to organisms, such as microorganisms, animals, and plants, for which genetic modification has proved difficult.

The evaluation of whether or not there are mutations at target regions can be done by either directly analyzing genetic sequences or by utilizing enzymes that recognize and cut mismatches in duplexes. However, these methods are expensive and laborious.

Heteroduplex mobility assay (HMA), on the other hand, is a method that can be performed simply, rapidly, and inexpensively (Fig. 1). In normal electrophoresis, DNA is a homoduplex of perfectly complementary strands and mobility depends on molecular weight (size). However, in DNA where either of the two strands has a mutation, the mismatched section does not form a complementary strand and as such becomes heteroduplex DNA. Mismatched sections of heteroduplex DNA have a different spatial structure to that of homoduplex DNA. For this reason, the mobility of heteroduplex DNA in electrophoresis has a tendency to be slower. By utilizing this phenomenon, HMA can determine the presence or absence of mutations and judge genotypes through electrophoresis.

This article introduces an example of analysis where HMA detection with model DNA is performed using the MCE-202 MultiNA microchip electrophoresis system for DNA/RNA analysis.

Sogabe

Preparation of Standard Sample

Model DNA for analysis were prepared by setting 110 bp as the reference and creating 108 bp with two base pairs deletion and 105 bp with five base pairs deletion. Each model DNA was then inserted into plasmids and used as PCR templates. The sequence of each model DNA is as follows. An asterisk (*) denotes deletion.

> Model DNA 110 bp
ACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCATCT
GACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA
ACGTGGATGAAGTTG

> Model DNA 108 bp
ACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCATCT
GACTCC**AGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAA
CGTGGATGAAGTTG

> Model DNA 105 bp
ACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCATCT
GACTC*****GAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAAC
GTGGATGAAGTTG

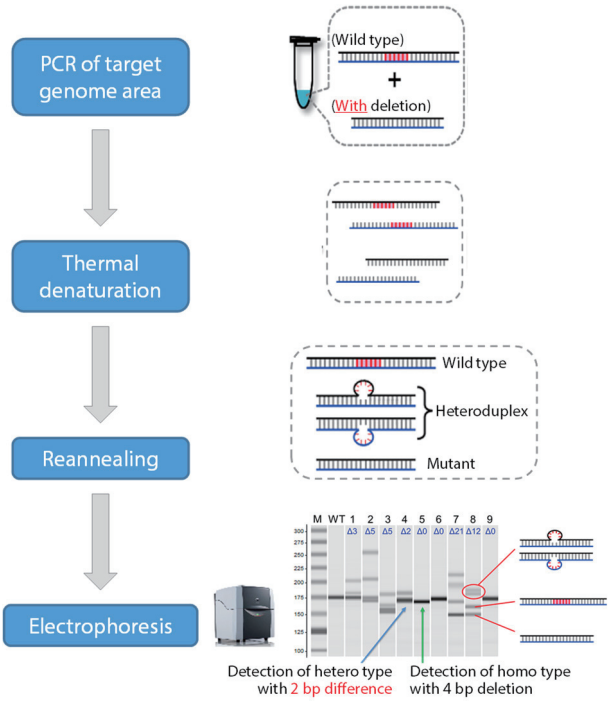

Fig. 1 Sequence of HMA

Method

• Components of Reaction Mixture

Sample	0.5 µL
2xBuffer	5.0 µL
dNTPs (2 mM)	1.0 µL
Primer F (2 µM)	1.0 µL
Primer R (2 µM)	1.0 µL
DW	1.4 µL
KOD FX	0.1 µL
Total	10.0 µL

• Cycling Conditions

98 °C	1 min	
98 °C	10 sec	×30 cycles
60 °C	15 sec	
68 °C	15 sec	
68 °C	7 min	
4 °C	∞	

- **Heteroduplex Formation**

The PCR products which were reacted under the conditions of the previous section were mixed at a 1:1 ratio in the combinations of 110 bp - 108 bp, 110 bp - 105 bp, and 108 bp - 105 bp. After denaturation of the mixtures for five minutes at 95 °C, cooling to 5 °C at 0.1 °C per second was performed to form heteroduplexes.

- **Electrophoresis**

The PCR products and samples of formed heteroduplexes were analyzed using the MultiNA microchip electrophoresis system. Analysis used SYBR Gold for the fluorescent dye and the DNA-500 kit for the separation buffer.

Analysis Results

We were able to obtain the clear analysis result (gel image) shown in Fig. 2 from electrophoresis using MultiNA. The three bands from the left of the gel image correspond to the PCR product analysis results of 110 bp, 108 bp, and 105 bp. The fourth and subsequent bands from the left correspond to the HMA results of 110 - 108 bp, 110 -105 bp, and 108 - 105 bp.

Multiple bands were detected for each HMA of 110 - 108 bp, 110 - 105 bp, and 108 - 105 bp in which heteroduplexes were formed.

The bands on the high molecular side (enclosed in red in Fig. 2) correspond to the heteroduplex bands. We confirmed that heteroduplex bands tend to be detected on the high molecular side as the number of base mismatches increases.

In particular, the result of the 110 - 108 bp HMA shows that the band on the low molecular side results from the mixture of 108 bp and 110 bp and the band on the high molecular side corresponds to the heteroduplex band. In this way, we succeeded in separating heteroduplexes that differ by 2 bp using MultiNA.

MultiNA obtains the results of electrophoresis as electropherogram data (waveform data). Functions in the data browsing software (MultiNA Viewer) allow the electropherogram of each sample to be selected and overlaid for display. Fig. 3 shows electropherograms that compare the analysis results of HMA and each of the two PCR products used in HMA combinations.

MultiNA excels in terms of separation and repeatability compared to agarose gel electrophoresis and the peaks obtained for homoduplexes in the HMA samples match with the peaks of the respective PCR product.

In addition, the mol concentration can be calculated automatically from the peak area in the electropherograms. By utilizing this function to compare the mol concentrations of heteroduplexes and homoduplexes, individual mutation rates in genome editing can be easily obtained.

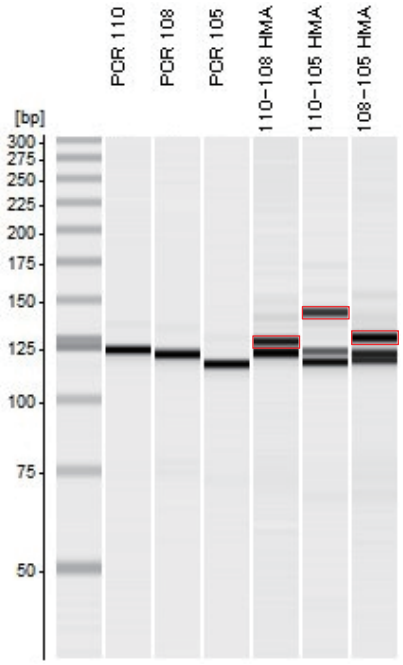

Fig. 2 Gel Image of PCR Products and HMA Obtained Using MultiNA

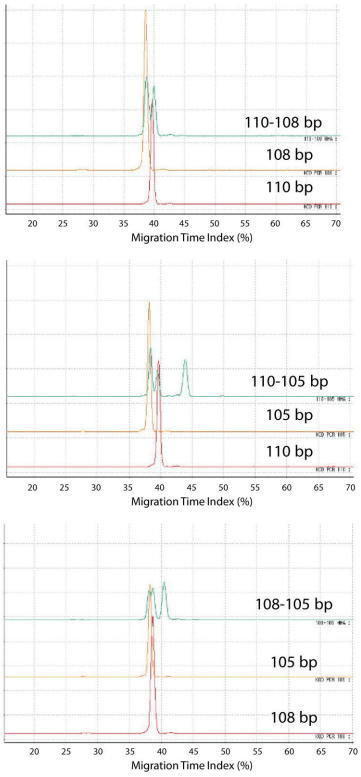

Fig. 3 Comparison of Mobility of PCR Products and HMA (Electropherograms)

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