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Application News

Microchip electrophoresis system MultiNA™ II MCE-301

Heteroduplex Mobility Assay Using MultiNA II

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User Benefits

- The electrophoresis operation can be performed almost fully automatically.
- Heteroduplex can be detected by differences in mobility.
- Mutations and deletions in multiple genome-edited samples can be efficiently screened.

■ Introduction

The advent of genome editing tools has made it possible to specifically disrupt or insert target genes into target genomic regions.

Genome editing technology is rapidly gaining popularity because it can now be easily applied to microorganisms, animals, plants, and other organisms that have been difficult to genetically modify in the past.

There are two methods for evaluating the presence of mutations at the target site: direct sequence analysis and the use of enzymes that recognize and cleave mismatches in the double strand, both of which are costly and labor-intensive.

HMA (Homoduplex Mobility Assay) is a simple, rapid, and inexpensive method (Fig. 1). In conventional electrophoresis, DNA is fully complementary homoduplex, and its mobility depends on its size (molecular weight). On the other hand, DNA that has a mutation in one part of one of the two strands is heteroduplex DNA, where the mismatch part does not form a complementary strand. The mismatch portion of heteroduplex DNA has a different three-dimensional structure than that of homoduplex DNA. HMA can use this phenomenon to determine the presence or absence of mutations and genotypes by electrophoresis.

In this application, we present an example of HMA with model DNA using the MultiNA II MCE-301 microchip electrophoresis system (Fig. 2).

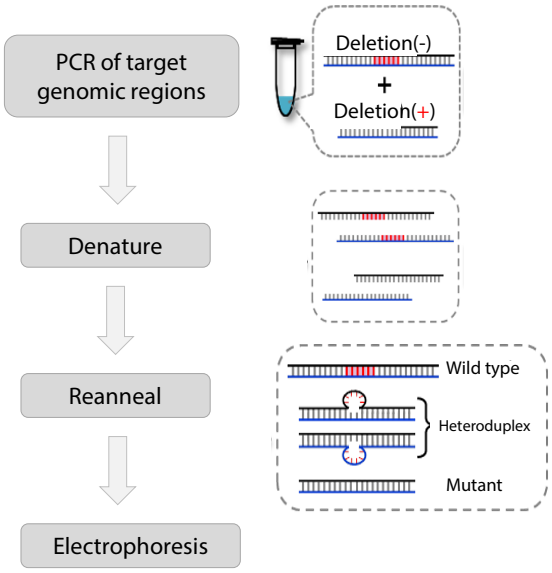

Fig. 1 HMA workflow

Fig. 2 Microchip electrophoresis system MultiNA™ II MCE-301

■ Sample Preparation and Analysis Conditions

Model DNA samples

As a sample, we selected a partial sequence of human β-globin: based on 110 bp, we generated model DNAs of 108 bp with two bases deleted and 105 bp with five bases deleted. Each was inserted into the pEX-A2J1 plasmid vector and used as a template for PCR. The nucleotide sequence of each model DNA is shown in Fig. 3. Red "*" indicates deletion sites.

>Model DNA110 bp
ACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGC
ATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGC
AAGGTGAACGTGGATGAAGTTG

>Model DNA108 bp
ACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGC
ATCTGACTCC**AGGAGAAGTCTGCCGTTACTGCCCTGTGGGGC
AAGGTGAACGTGGATGAAGTTG

>Model DNA105 bp
ACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGC
ATCTGACTC*****GAGAAGTCTGCCGTTACTGCCCTGTGGGGCA
AGGTGAACGTGGATGAAGTTG

Fig.3 Nucleotide sequence of model DNA

PCR conditions

PCR was performed using the above plasmid as a template to amplify the region set as model DNA. The reagent kit for PCR was KOD FX (TOYOBO). The composition of the reaction solution and the cycling conditions of PCR were performed under the conditions shown in Fig. 4.

・Reaction buffer	
Sample	0.5 µL
2 x Buffer	5.0 µL
dNTPs (2 mM)	1.0 µL
Primer F (2 µM)	1.0 µL
Primer R (2 µM)	1.0 µL
Distilled water	1.4 µL
KOD FX	0.1 µL
Total	10.0 µL

・PCR cycle

98 ˚C	1 min	
98 ˚C	10 sec	x 30 cycles
60 ˚C	15 sec	
68 ˚C	15 sec	
68 ˚C	7 min	
4 ˚C	∞	

Fig. 4 PCR conditions

Heteroduplex formation

PCR products of 110 + 108 bp, 110 + 105 bp, and 108 + 105 bp reacted under the conditions shown in Fig. 4 were used to form a heteroduplex using the thermal cycler SimpliAmp (Thermo Fisher Scientific). Reaction conditions were denaturation at 95 ˚C for 5 min, followed by cooling to 5 ˚C at 0.1 ˚C /sec and reannealing.

Electrophoresis

Samples of PCR amplified products with 110 bp, 108 bp, and 105 bp, undenatured PCR amplified products with 110＋108 bp, 110＋105 bp and 108＋105 bp, and denatured and reannealed to form a heteroduplex with 110+108 bp, 110+105 bp, and 108+105 bp were analyzed with the microchip electrophoresis system MultiNA II. The DNA-500 kit was used for the analysis.

■ Results

From the electrophoresis by MultiNA II, clearly separated gel images were obtained as shown in Fig. 5A. The three from the left in the gel image are the results of the analysis of 110 bp, 108 bp, and 105 bp PCR products, followed by the undenatured mixed PCR products of 110+108 bp, 110+105 bp, and 108+105 bp from the left. The three on the right are the results of HMA for 110+108bp, 110+105bp, and 108+105bp.

The 110 bp, 108 bp, and 105 bp PCR products showed a 2 bp difference between 110 bp and 108 bp that was barely discernible, but the difference between 110 bp and 105 bp was confirmed. The 110+108 bp of the undenatured mixed PCR product was detected as a single band. The other two samples (108+105 bp, 110+108 bp) were detected as their respective bands.

The results for the 110+108 bp, 110+105 bp, and 108+105 bp PCR products that formed a heteroduplex showed bands in each sample that were different from the undenatured PCR products. We confirmed the trend that the higher the number of mismatched bases, the more heteroduplex bands were detected on the polymeric side.

■ Conclusions

Heteroduplex could be detected in HMA using MultiNA II. In particular, the 110-108 bp HMA results allowed us to detect a heteroduplex with a 2 bp difference.

When the population of experimental animals that have carried out genome editing or other genetic manipulation is large, it is time-consuming and costly to check for the introduction of mutations or deletions in the animals. Automated analysis using MultiNA II enables screening in a short time and greatly improves the efficiency of mutation introduction and deletion confirmation work.

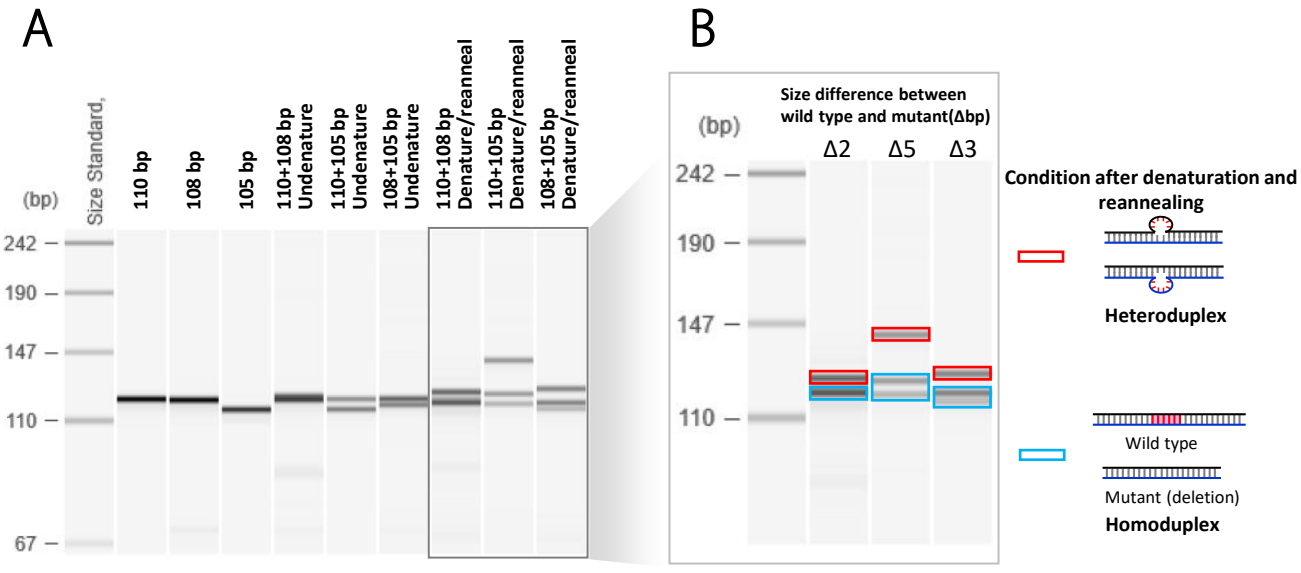

Fig. 5 Electrophoresis results by MultiNA II (gel image)
A: Electrophoresis results of PCR products and heteroduplex samples
B: Details of HMA results

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