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Application News

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MCE

Detection of Multi-Base Mutation by Genome Editing Using MultiNA™

With the advent of genome editing technology, specific genes can now be knocked out and inserted in target genes.

However, in confirming the results of genome editing, it is necessary to check whether the mutation is on-target or not. Although direct sequence analysis is the most reliable method, assays involving large numbers of samples are costly. The heteroduplex mobility assay (HMA) is a simple method in which wild- and mutant-type duplexes are analyzed by electrophoresis and on-target editing is confirmed by the difference in mobility between the homoduplex and heteroduplex strands. The HMA method has gained wide acceptance as a tool that enables easy confirmation of genome editing results by using a fully-automatic electrophoresis system.

However, due to differences in multiple bases (from 1 to 5 base pairs), it is sometimes impossible to ascertain on-target editing by HMA because the difference in mobility is cancelled out by the 3-dimensional structures of the homoduplexes and heteroduplexes. In such cases, confirmation is possible by the enzyme mismatch cleavage method using the enzymes called T7 endonuclease I (T7EI) and Cel 1. These enzymes possess activity that recognizes and cleaves mismatches in double-stranded DNA. Because the enzymes can also recognize mismatches of multiple bases, this method is effective for detecting mutations that cannot be detected by HMA. This article introduces an example of an analysis in which quick and simple detection of mismatch cleavage by T7EI was possible by using a Shimadzu microchip electrophoresis system.

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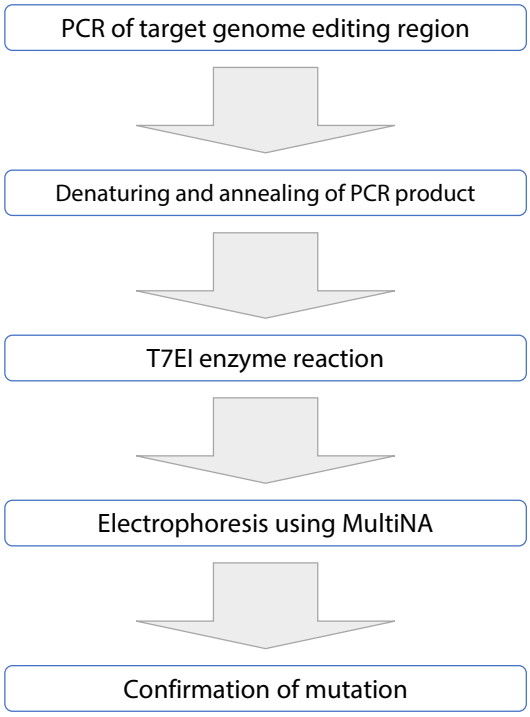

Fig. 1 Analysis Protocol

Fig. 2 MultiNA™ Microchip Electrophoresis System for DNA/RNA Analysis

Preparation of Standard Samples

In this analysis, 110 base pairs (bp) were selected arbitrarily from the Homo sapiens beta globin region (HBB@) and beta globin locus transcript 3 (non-protein coding) (BGLT3) and were indicated as a wild type model. Five types of mutant model DNA were prepared by insertion/deletion (indel) of from 1 base to 5 bases, respectively, from a wild type model sequence. Samples were also synthesized by inserting these into plasmid. In the PCR (polymerase chain reaction), 2×10^8 copies of each of the 5 mutant type sequences and the wild type sequence were mixed at a ratio of 1 : 1, and were used as a wild type-mutant type hetero model template.

Method

• PCR

Table 1 shows the conditions used in the PCR.

Table 1 PCR Analysis Conditions

PCR reaction solution	
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

PCR cycle		
98 °C	1 min	
98 °C	10 sec	x35 cycles
60 °C	15 sec	
68 °C	15 sec	
68 °C	7 min	
4 °C	∞	

• Formation of heteroduplexes

The PCR products were denatured and annealed using a thermal cycler. The conditions followed the protocol in the attachment to T7 Endonuclease I reaction Mix (Nippon Gene Co., Ltd.).

95 °C	5 min
95 °C → 85 °C	2 °C/sec
85 °C → 25 °C	0.1 °C/sec

• Enzyme reaction (T7EI)

Using 9 µL of the PCR products in which the heteroduplexes were formed, 1 µL of the above-mentioned T7 Endonuclease I reaction Mix was added, and the reaction was conducted at 37 °C for 60 min.

• Electrophoresis

After enzyme digestion with T7EI, electrophoresis of the PCR products was carried out with a Shimadzu MCE-202 MultiNA microchip electrophoresis system, and the size was confirmed. For comparison, electrophoresis of PCR products without T7EI digestion was also conducted. The Shimadzu DNA-500 dedicated reagent kit for MultiNA was used in the analysis with the MultiNA.

Analysis Results

It was possible to obtain clear analysis results (gel images), as shown in Fig. 3, by electrophoresis using the MultiNA. The symbols Δ1 to Δ5 in the sample names indicate the number of insertions/deletions.

Fig. 3(a) shows the gel image of a PCR product before digestion with T7EI. Although these results are prior to thermal denaturing and annealing treatment, it was possible to detect the heteroduplex for Δ5 with indel of 5 bases. Thus, mutation could be confirmed even without T7EI.

Fig. 3(b) shows the gel image of a PCR product after digestion with T7EI. Here, fragments cleaved by T7EI were detected for all of Δ1 to Δ5. The difference in the sizes of the digested fragments is due to differences in the indel positions. Although a heteroduplex was detected in Δ5, extinction of that heteroduplex by the T7EI could be confirmed.

Conclusion

In cases where separation of the size difference between a wild type homozygous and mutant type homozygous with the difference of multiple bases is difficult, as in this analysis example, the presence or absence of mutation can be judged in a simple manner by detecting the fragments obtained by T7EI digestion.

Assessment of genome editing requires considerable trouble and expense when a large number of experimental animals must be examined. A substantial reduction in that work is possible by automatic analysis using the MCE-202 MultiNA.

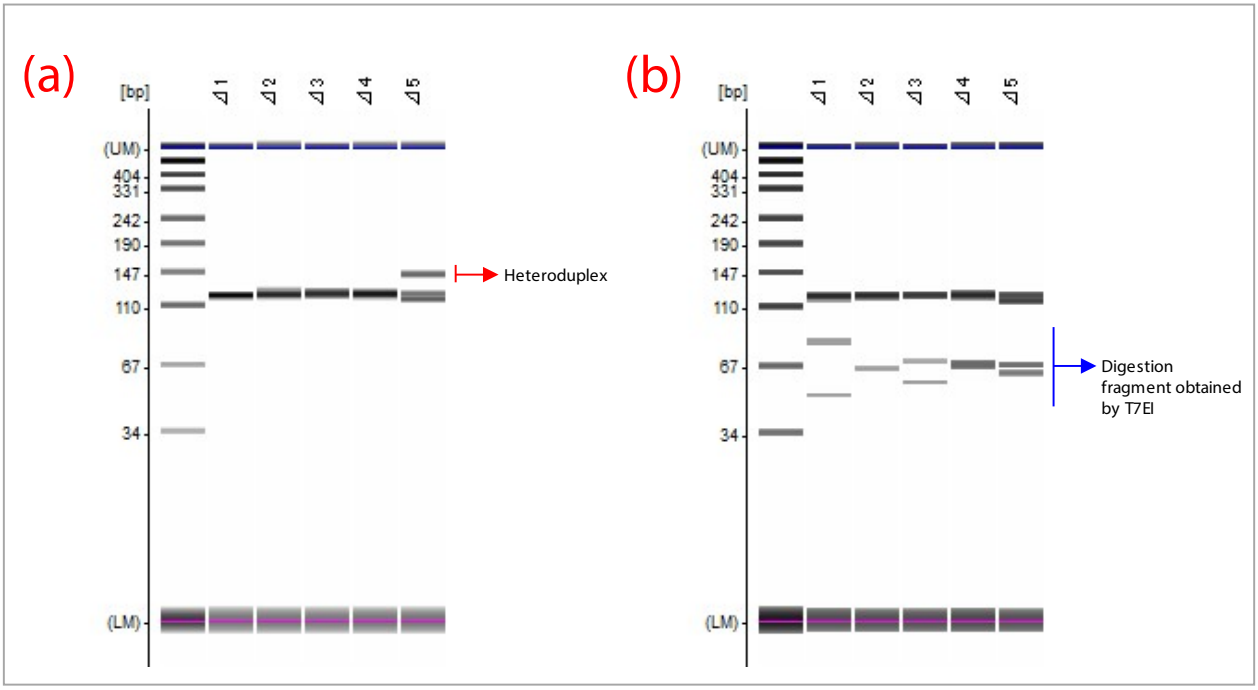

Fig. 3 Results of Electrophoresis by MultiNA (Gel Images)
(a) Gel Image After Completion of PCR, (b) Gel Image of PCR Products After T7EI Digestion

* The analysis results of this method may differ depending on the target sequence and its neighboring sequences.

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