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C297-E084

MCE-202 MultiNA

APPLICATION 13

DNA-500
DNA-1000
DNA-2500
RNA

Rat Genotyping by Multplex PCR

- PCR from Rat Blood Coated on FTA® Card using Ampdirect® -

Introduction

The SER (Spontaneously Epileptic Rat) [1] is an animal model for severe absence-like seizure and tonic seizure in human epilepsy. The SER, a double mutant rat which is homozygous for both the tremor (*tm*) and zitter (*zi*) mutations, was developed by the Kyoto University Institute of Laboratory Animals Graduate School of Medicine by crossing two different mutant rat strains, the tremor rat [2] and the zitter rat. The *tm* mutation is a 200-kb genomic deletion in the *Aspa* (aspartoacylase) gene, and *zi* is an 8-bp deletion in the *Atrn* (attractin) gene.

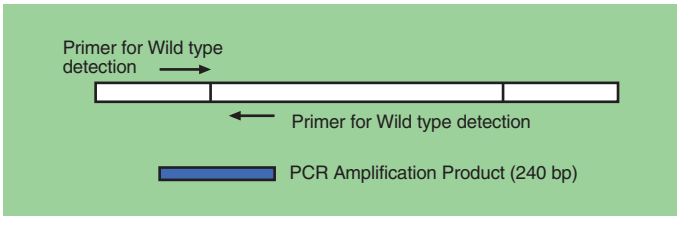
The SER strain is maintained by sibling mating of a zitter homozygote with a tremor heterozygote. These deficiencies must be detected in order to select the parent rats for creating the next generation.

Of the 2 mutant genes, the *tm* mutation presents a long deletion of 200 kb, and in order to detect the presence or absence of that deficiency, multiplex PCR is conducted using 2 pairs of primers. Multiplex PCR is a variant of PCR useful for genotyping applications where simultaneous analyses of multiple markers is necessary. This technique produces amplicons of differing sizes specific to varying DNA sequences. When a deletion is present, as in the case of the *tm* mutation, a unique PCR product (at **179** bp for the *tm* mutant) is obtained. When there is no deletion, this PCR product is not obtained.

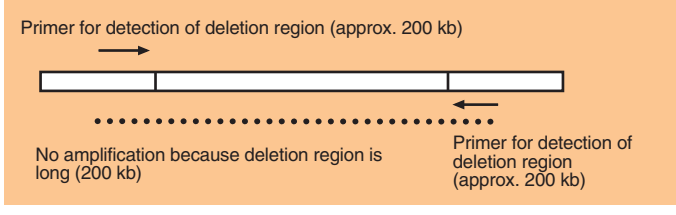
This application demonstrates the use of microchip electrophoresis detection (MultiNA MCE-202) for multiplex PCR genotyping, a technique which can be applied to many areas of DNA testing, including analysis of mutations, deletions and polymorphisms. The combination of multiplex PCR using Ampdirect and MultiNA detection provides a low cost method for genotyping with many research and commercial applications including gene expression, whole-genome sequencing, forensic analysis research, SNP genotyping and infectious disease research. MultiNA is a low-cost, automated electrophoresis platform ideal for the design, testing, and optimization of multiplex PCR primer mixtures.

1) Rat without Deletion

■ Wild Type Detection

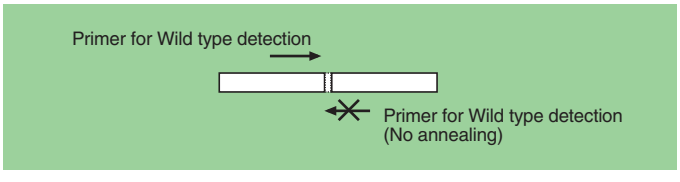


□ *tm* Gene Deletion Detection

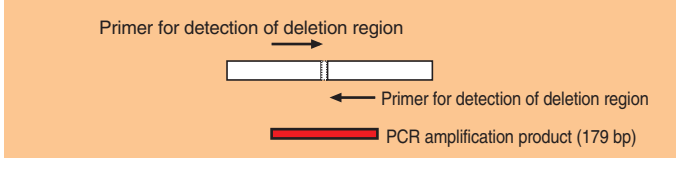


2) Rat with Deletion

■ Wild Type Detection



□ *tm* Gene Deletion Detection

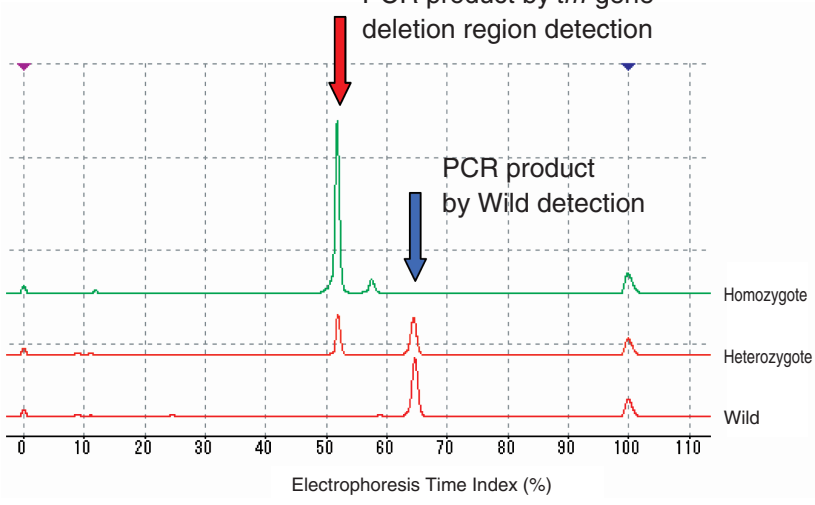


Summary Table

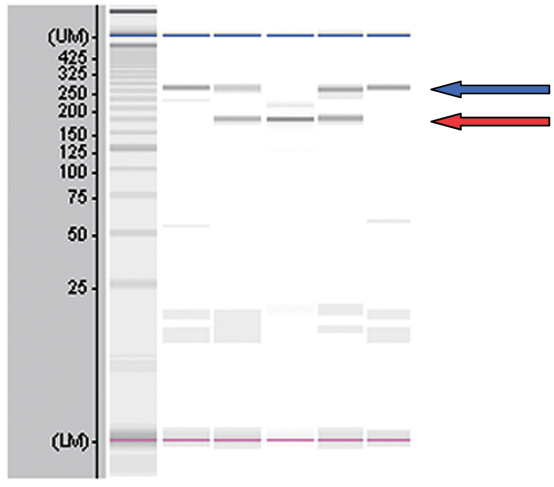
	Wild Type Detection PCR Amplification Products	*tm* Gene Deletion Detection PCR Products
Wild	Detected	Not detected
Homozygote	Not detected	Detected
Heterozygote	Detected	Detected

Results

1. Analysis by MultiNA (Kit used: DNA-500 Pre-mix mode)



Gel Image



From left: ladder, homozygote, heterozygote Wild, heterozygote, homozygote

Analytical Procedure

Instrument: MCE-202 “MultiNA”

Analysis mode: DNA-500 Pre-mix

PCR primers: Refer to following URL link.

http://www.anim.med.kyoto-u.ac.jp/NBR/strains/Strains_d.aspx?StrainID=28&s_Strainname=SER&s_SpontMutant=1

Sample: After coating FTA® card with rat blood, Ampdirect® reagent was used obtain PCR amplification products

Reagents:

- DNA-500 Reagent Kit for MultiNA (Shimadzu Corp.) P/N 292- 27910-91
- SYBR® Gold nucleic acid gel stain (Invitrogen) S-11494
- 25bp DNA ladder (Invitrogen) 10597-011

Sample provided by:

Associate Professor Takashi Kuramoto, Ph.D., Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University

References

1. Serikawa, T. *et al*. Epileptic seizures in rats homozygous for two mutations, zitter and tremor. Journal of Heredity. 77, 441-444 (1986).
2. Kitada, K. *et al*. Accumulation of N-acetyl-L-aspartate in the brain of the tremor rat, a mutant exhibiting absence-like seizure and spongiform degeneration in the central nervous system. Journal of Neurochemistry. 74, 2512-2519 (2000).

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