

DNA-500

DNA-1000

DNA-2500

RNA

Rat Genotyping by Multiplex 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 rat²⁾ 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 *Attrn* (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.

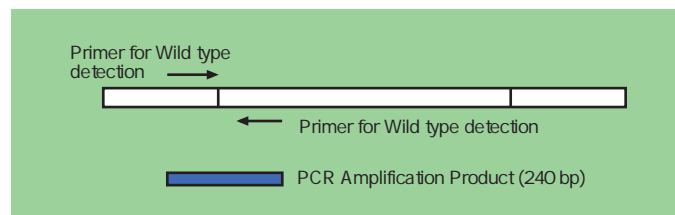
Of the 2 mutant genes, the *tm* mutation presents a long deletion of 200 kb, and in order to detect the presence or absence of that deficiency, multiplex PCR is conducted using 2 pairs of primers. Multiplex PCR is a variant of PCR useful for genotyping applications where simultaneous

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.

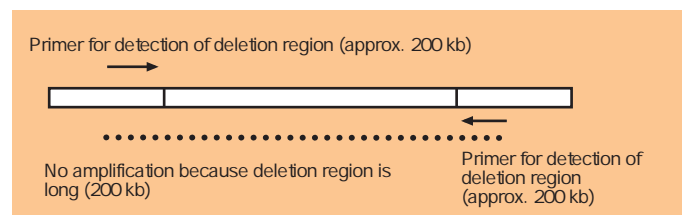
This application demonstrates the use of microchip electrophoresis detection (MultiNA MCE-202) for multiplex PCR genotyping, a technique which can be applied to many areas of DNA testing, including analysis of mutations, deletions and polymorphisms. The combination of multiplex PCR using Ampdirect and 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

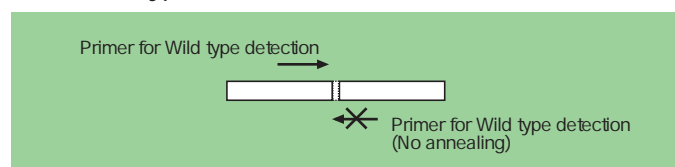


□ *tm* Gene Deletion Detection



2) Rat with Deletion

■ Wild Type Detection



□ *tm* Gene Deletion Detection

