Genotyping the Mouse Severe Combined Immunodeficiency Mutation Using the Polymerase Chain Reaction with Confronting Two-Pair Primers (PCR-CTPP)

Chika MARUYAMA1, Hiroshi SUEMIZU1, Shojiro TAMAMUSHI2, Shigenobu KIMOTO3, Norikazu TAMAOKI4, and Yasuyuki OHNISHI4

1Central Institute for Experimental Animals, 1430 Nogawa, Miyamae-ku, Kawasaki-shi, 216-0001, and 2CLEA Japan, Inc. NO.2 INARI Bldg., 20-14 Aobadai-2, Meguro-ku, Tokyo 153-8533, Japan

Abstract: An allele specific polymerase chain reaction with confronting two-pair primers (PCR-CTPP) was developed as an assay for genotyping the mouse Prkdc<sup>scid</sup> gene mutation (former name scid). The reverse primer (WR) was designed to include the antisense nucleotide (A) specific for the wild type allele at the 3' end with the counterpart forward primer (F) upstream. The other forward primer (MF) was designed to include the sense nucleotide (A) specific for the Prkdc<sup>scid</sup> mutation at the 3' end with the other counterpart reverse primer (R) downstream. PCR was performed in a single tube with these two pairs of primers. The products specific for each allele extended by F/WR (101 bp) or MF/R (180 bp) were visualized with common PCR products (257 bp) extended by F/R, and three genotypes of mice (Prkdc<sup>scid</sup>/Prkdc<sup>scid</sup>, Prkdc<sup>scid</sup>/+, and +/+ ) were clearly distinguished.

Key words: SCID mice, Prkdc gene, genotyping

Mice homozygous for the severe combined immunodeficiency mutation (SCID mice) are functionally defective in both T and B cells, because the V(D)J recombination reaction cannot occur [2]. The gene responsible for this mutation encodes a DNA-dependent protein catalytic subunit which associates kinase activity with nuclear DNA binding proteins [1, 8], and this mutation is a T to A single nucleotide transversion (Tyr4046) in exon 85 of the Prkdc gene (Prkdc<sup>scid</sup>, formerly called the scid gene) [5, 9].

Genotyping of a causative gene by molecular analytical examination is a useful technique in the breeding and production of mutant laboratory animals [7, 11].

In ordinary breeding and production of SCID mice, both male and female mutant homozygotes can breed, and it is not necessary to examine the zygosity of the gene. However, for certain experiments such as those on the congenic process, or production of mice with multiple complex genes, genotyping at the molecular level is helpful for efficient selection of breeding parents.

For genotyping of a single nucleotide substitution based on the polymerase chain reaction (PCR), PCR-CTPP (PCR with confronting two-pair primers) was recently developed [6] by a modification of PCR-SSP (PCR with sequence specific primers). We developed and report here a PCR-CTPP assay that detects the

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Address corresponding: Y. Ohnishi, Central Institute for Experimental Animals, 1430 Nogawa, Miyamae-ku, Kawasaki-shi, 216-0001, Japan
Prkdc<sup>−/−</sup> mutation for typing the zygosity of SCID mice. This assay carries out simultaneous amplification of three different sizes of DNA segments in a single-tube reaction followed by a single-electrophoresis step.

Mice with three genotypes at the Prkdc locus (Prkdc<sup>−/−</sup>/Prkdc<sup>−/−</sup>, Prkdc<sup>−/−</sup>/+, and +/+) and C.B-17/1cr background were obtained from CLEA Japan Inc. (Tokyo). Tail tips of the mice were severed and digested with proteinase K by ordinary methods [10]. Genomic DNA was extracted using GENEXTRACTOR TA-100 (TaKaRa Inc., Tokyo) according to the manufacturer’s instructions. All animal experimental procedures were performed in accordance with the animal care and use guidelines of the Central Institute for Experimental Animals (Kawasaki, Japan). The reverse primer (WR) was designed to include the antisense nucleotide (A) specific for the wild type allele at the 3’ end with the counterpart forward primer (F) upstream (Fig. 1), and the other forward primer (MF) was designed to include the sense nucleotide (A) specific for the Prkdc<sup>−/−</sup> mutation at the 3’ end with the other reverse primer (R) downstream (Fig. 1). Genomic DNA (100 ng) was subjected to PCR in a total volume of 20 μl of buffer solution, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 1 μM of each primer, 0.2 μM dNTPs, and 0.5 unit of Taq DNA polymerase (TaKaRa Inc.). Thermal cycling conditions were as follows: 94°C, 2 min followed by 30 cycles of 94°C, 30 sec; 60°C, 30 sec; 72°C, 20 sec; and 72°C, 1 min.

The amplification products (5 μl) were subjected to electrophoresis on 16% polyacrylamide gel/1X Tris-glycine, and stained with ethidium bromide (Fig. 2). All samples produced common 257-bp bands extended by F and R primers. In addition, the 180-bp bands specific for the mutant allele extended by MF and R (Fig. 2, lanes 1 to 4) and the 101-bp bands specific for the wild type allele extended by F and WR (lanes 3 to 6) were seen in mice with corresponding genotypes. To verify the specificity of each genotype, we performed sequencing analyses of the PCR-amplified fragments. The junction region between intron 84 and intron 85 containing the mutation site was amplified using F and R primers. The PCR products were directly sequenced using an ABI PRISM 310 Genetic analyzer and ABI PRISM BigDye terminator cycle sequencing ready reaction kits (Applied Biosystems Japan, Tokyo). We tested 6 DNA samples, and all genotypes determined by the PCR-CTPP assay were consistent with the results of DNA sequencing analyses at the Prkdc<sup>−/−</sup> gene mutation site (data not shown). These results suggested that the PCR-CTPP assay developed here accurately distinguishes the Prkdc<sup>−/−</sup> mutation site of the gene.

Since single base substitution in the Prkdc gene has been identified as the molecular basis of the SCID mouse (Prkdc<sup>−/−</sup>) [5,9], molecular analyses such as PCR can be utilized as an assay method for detection and typing of the zygosity of SCID mice. Several PCR-based methods for detecting single base substitution have been developed including PCR-RFLP (restriction fragment length polymorphism), PCR-SSCP (single-strand conformation polymorphism), PCR-SPP, and sequencing [4]. Recently, a modification of DNA
microarray technique has also become applicable [3], but it is relatively more expensive at present. Ordinary PCR-SSP requires two different reaction tubes and separate electrophoresis because the amplified products specific for each allele are almost the same size [6, 11]. The PCR-CTPP assay developed here can detect the mutant Prkdc^wt^ and wild Prkdc^wt^ zygosity in a single one-tube reaction, and does not require enzymatic reaction with restriction endonuclease after PCR. The PCR-CTPP assay has the advantages of simplicity and saving in time, and is cheaper than PCR-RFLP and ordinary PCR-SSP [6]. In addition, the common 257-bp DNA segments in the PCR-CTPP serve as a reaction control, which decreases the risk of spurious results caused by reaction failures. We thank Katsura Ishiware for her excellent technical assistance.

References