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NOTE

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Noninvasive sample collection for the genotyping of neonatal rats using adhesive tape

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ABSTRACT. To develop a noninvasive sample collection method for genotyping, we compared PCR products from samples collected from neonates using five different brands of adhesive tape. Next, the youngest application age to distinguish genotypes was established. The tapes were applied on the backs of rats on postnatal day (PND) 10. DNA extracts from two brands provided clear PCR products that enabled genotype identification. The youngest age for distinguishing genotypes was PND 5; however, the youngest age that provided accurate results was PND 7. Thus, the present method allows for genotyping during the neonatal period without invasive burden and may improve animal welfare by refining.

KEY WORDS: adhesive tape, genotyping, neonatal rat, PCR, refinement

Genotyping is an essential process for studies using genetically modified animals. Genotyping at younger ages provides significant advantages, as the early selection of suitable animals for experiments extends the time available to plan experiments and allows researchers to maintain manageable colony sizes. To perform genotyping, living tissues must be collected from animals to extract DNA for PCR. As summarized by the Federation of European Laboratory Animal Science (FELSA), DNA samples for genotyping are commonly collected via invasive methods, such as cutting a small piece of the tip of the tail, phalanx removal or removal of ear tissue from pinna at individual identification [2]. Among these methods, removal of ear tissue is unable to apply to the neonatal rats and mice, since the pinna unfolding occurs during the neonatal period. Furthermore, FELSA recommends tail tip collection between postnatal days (PNDs) 14 and 17 in their guidelines. Fecal and hair samples can be collected less invasively; however, these methods are not applicable for neonatal rats and mice [5, 6]. Therefore, the present study intended to develop a new noninvasive method for sample collection from neonatal rats.

Adhesive tape is frequently used in forensic evaluations, and the application of tape samples for DNA profiling has been evaluated [1, 8]. Application of these methods for genotyping may allow for noninvasive sample collection; however, adhesive tape may contain inhibitors of DNA extraction and PCR. Hayward [3] examined PCR inhibition by comparing the PCR products of the *E. coli* 16s rRNA gene extracted with 15 brands of adhesive tapes. Several available brands were compatible with a one-step DNA extraction for PCR. Therefore, we determined the most suitable tapes among commercially available brands for genotyping by PCR and established the youngest age to distinguish genotype.

In the present study, *kiss1* gene knockout rats (*Kiss1*−/−) as well as heterozygotes (*Kiss1*+/−) and homozygotes (*Kiss1*+/+), which were generated by the homologous recombination of the *kiss1* gene with the *tandem dimer Tomato* (*tdTomato*) reporter gene [7], were used. *Kiss1* encodes Kisspeptin, a neuropeptide regulating gonadotropin releasing hormone (GnRH) secretion [4], and both sexes of *Kiss1*−/− are infertile [7]. Therefore, these animals were obtained by mating heterozygotes. The animals were maintained in an animal husbandry facility with a controlled temperature and humidity of 21 ± 1°C and 50%–60%, respectively. The animals were housed in plastic cages with bedding materials (Sunflake; Oriental Kobo, Tokyo, Japan). Pellet chow (CE-2; Clea Japan, Tokyo, Japan) and tap water (Kanagawa Prefectural Government, Yokohama, Japan) were provided *ad libitum*.

First, we determined the most suitable tapes among several commercially available adhesives, including Scotch® Mending Tape (3M Japan, Tokyo, Japan), Scotch® Toumei-Nenchaku Tape Toumei-Bishoku® (3M Japan, Tokyo, Japan), Post-it (3M Japan), Scotch® Removable Tape (3M Japan) and adhesive cloth tape (Hitachi Maxell, Osaka, Japan). The tapes were cut into

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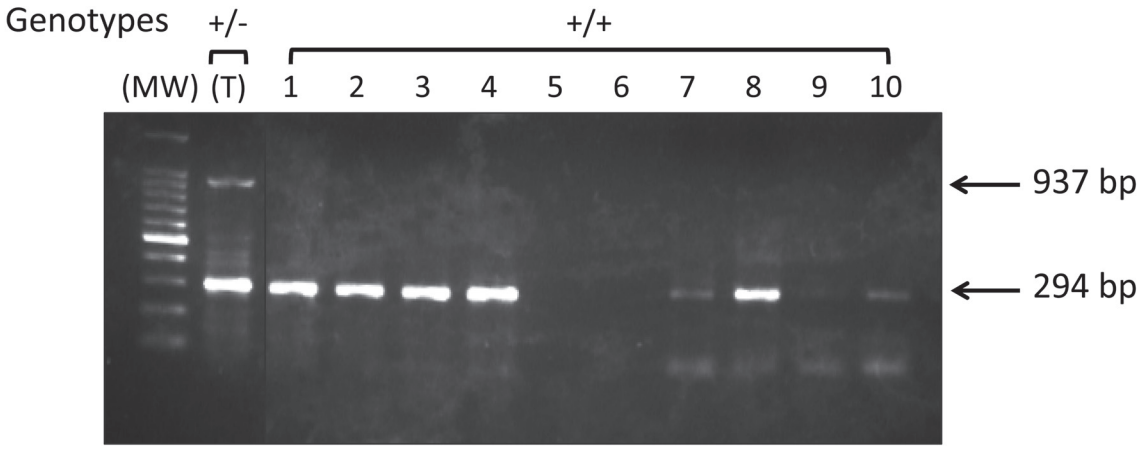

Fig. 1. Amplification products of *Kiss1* gene from specimens collected from the back of neonatal rats on postnatal day 10 with several brands of commercially available adhesive tapes. (MW), 100-bp molecular weight marker; (T), amplification product from tail chip DNA of the heterozygotes (there are two bands of 937 and 294 bp, amplified from the *tandem dimer Tomato* (*tdTomato*) reporter gene and *kiss1* gene, respectively). Lanes 1 and 2: specimens collected with Scotch® Mending Tape (3M Japan); lanes 3 and 4: specimens collected with Scotch® Toumei-Nenchaku Tape Toumei-Bishoku® (3M Japan); lanes 5 and 6: specimens collected with Scotch® Removable Tape (3M Japan); lanes 7 and 8: specimens collected with adhesive cloth tape (Hitachi Maxell); and lanes 9 and 10: specimens collected with Post-it (3M Japan)

approximately 1 × 4 cm rectangles and applied as a skin patch to the back of *Kiss1*+/+ rats on PND 10. Next, the tape was removed with skin tissue. Approximately 1 × 0.5 cm of tape with the greatest extent of adhered skin tissue was cut in small pieces with sterilized scissors. DNA extraction and PCR were performed using a direct PCR kit (KAPA Mouse Genotyping Kit, Kapa Biosystems, Wilmington, MA, U.S.A.) according to the manufacturer's instructions. Since the kit includes extraction reagents and PCR-master mix, extract from the pieces of tape was directly used for PCR. After initial denaturation at 95°C for 3 min, PCR was performed for 35 cycles consisting of denaturation at 95°C for 15 sec, annealing at 55°C for 15 sec and primer extension at 72°C for 15 sec. Primers used to amplify either the 937-bp fragment of *tdTomato* (*Kiss1*−/−), 294-bp fragment of *kiss1* gene (*Kiss1*+/+) or both (*Kiss1*+/−) were 5′-CCTTGTTTGGGGCTTATCCT (forward), 5′-GATGACGGCCATGTTGTTGT (revers for *tdTomato*) and 5′-CTTTTCCGGGATGGTGTGTA (revers for *Kiss1*). The PCR products were loaded on a 2% agarose gel, and the images were captured. DNA was also extracted and amplified similarly from the tail of *Kiss1*+/− rats on PND 21 as a positive control. Clear amplification products with a size of 294 bp were observed from samples collected with Scotch® Mending Tape or Scotch® Toumei-Nenchaku Tape Toumei-Bishoku® (Fig. 1, lanes 1–4). The adhered tissue was more visible on the former tape when compared with the latter one; thus, Scotch® Mending Tape was used to establish the youngest age possible for the identification of genotypes with this method. In contrast, no amplification product was observed from samples collected with Scotch® Removable Tape (Fig. 1, lanes 5 and 6), and those collected with adhesive cloth tape and Post-it did not provide clear products (Fig. 1, lanes 7–10). These tapes may inhibit some reaction either at extraction or at amplification.

In this experiment, the tape was applied to the same pups at different ages: PNDs 1, 3, 5 and 7. The pups were born from *Kiss1*+/− parents and were identified individually by tattoo on limbs with India ink on PND 1. Sample collection, DNA extraction and PCR were performed as described above. As shown in Fig. 2, the genotypes of the pups were identified at PNDs 7 and 5 (A and B), and the amplification from the PND 7 sample (A) allowed accurate genotyping for all of the samples. More DNA was yielded when more tape was used for the extraction; however, this modification also caused nonspecific amplification, likely due to the inhibitory effects of adhesive tape on PCR (data not shown).

We conclude that the application of Scotch® Mending Tape to the back of neonatal rats beginning at PND 7 can distinguish genotypes reliably without invasive burden. The 3Rs are strongly recommended in animal experiments, and genetically modified animals have been applied in various fields of life sciences. Thus, the present methods may contribute not only to refinement but also to the improvement of life science research.

The animal experiments described above were approved by the Committee of Animal Experiments at Azabu University.

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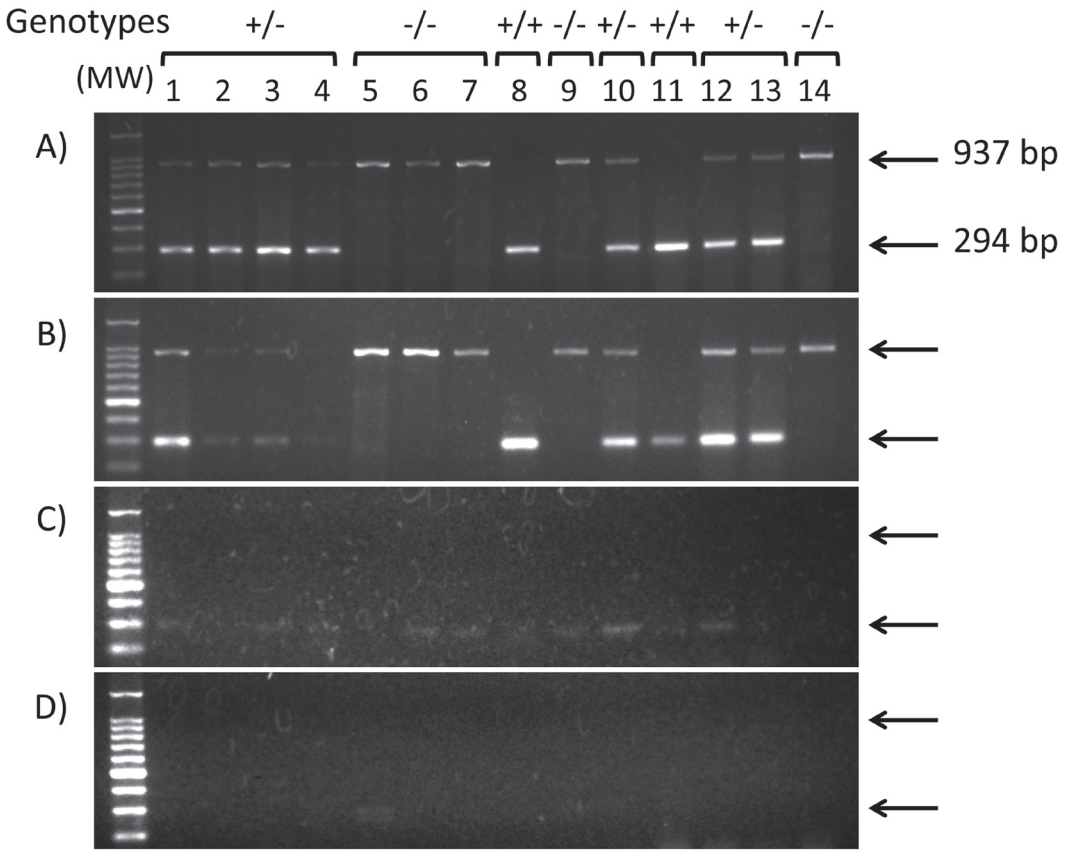

Fig. 2. Amplification products of the *Kiss1* gene from specimens collected from the back of neonates in the same litter with adhesive tape on postnatal days (PNDs) 7 (A), 5 (B), 3 (C) and 1 (D). The neonates were identified individually on PND 1, and samples were loaded according to age on 2% agarose gels in numerical order of the neonate identification numbers. A 937-bp product was amplified from the *tandem dimer Tomato* (*tdTomato*) reporter gene generated by homologous recombination with the *kiss1* gene. A 294-bp product was amplified from the *kiss1* gene. Six heterozygotes, 5 knockouts and 3 homozygotes were identified at PNDs 7 and 5, but not at PND 3 or 1. MW, 100-bp molecular weight marker. Numbers on the top panel represent the identification number of each neonate.

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