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

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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+/+) 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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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.

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.

REFERENCES

1. Barash, M., Reshef, A. and Brauner, P. 2010. The use of adhesive tape for recovery of DNA from crime scene items. J. Forensic Sci. 55: 1058–1064. [Medline] [CrossRef]

2. Bonaparte, D., Cinelli, P., Douni, E., Hérault, Y., Maas, M., Pakarinen, P., Poutanen, M., Lafuente, M. S., Scavizzi, F. 2013. FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents: a report of the Federation of European Laboratory Animal Science Associations Working Group. Lab. Anim. 47: 134–145. [Medline] [CrossRef]

3. Hayward, J. PCR inhibition by adhesive tape. ZyGEM APPLICATION NOTE NZ107 http://www.zygem.com/images/pdf/15/Z01070_AppNote107_TapeInhib.pdf [accessed March 10, 2017].

4. Maeda, K., Adachi, S., Inoue, K., Ohkura, S. and Tsukamura, H. 2007. Metastin/kisspeptin and control of estrous cycle in rats. Rev. Endocr. Metab. Disord. 8: 21–29. [Medline] [CrossRef]

5. Picazo, M. G. and García-Olmo, D. C. 2015. DNA from tissues of young mice is optimal for genotyping. Electron. J. Biotechnol. 18: 83–87. [CrossRef]

6. Symonds, E. L. and Fenech, M. 2012. A method for non-invasive genotyping of APCmin/+ mice using fecal samples. Biol. Proced. Online 14: 1. [Medline] [CrossRef]

7. Uenoyma, Y., Nakamura, S., Hayakawa, Y., Ikegami, K., Watanabe, Y., Deura, C., Minabe, S., Tomikawa, J., Goto, T., Ieda, N., Inoue, N., Sanbo, M., Tamura, C., Hirabayashi, M., Maeda, K. and Tsukamura, H. 2015. Lack of pulse and surge modes and glutamatergic stimulation of luteinising hormone release in Kiss1 knockout rats. J. Neuroendocrinol. 27: 187–197. [Medline] [CrossRef]

8. Zech, W. D., Malik, N. and Thali, M. 2012. Applicability of DNA analysis on adhesive tape in forensic casework. J. Forensic Sci. 57: 1036–1041. [Medline] [CrossRef]