Acta Biochimica Polonica

Vol. 53 No. 3/2006, 613–616

on-line at: www.actabp.pl

Technical note

Rapid and simple screening of transgenic mice: novel extraction-free, filter-based PCR genotyping from blood samples

Naoya Suematsu and Fumihide Isohashi✉

Department of Biochemistry, St. Marianna University School of Medicine, Kanagawa, Japan;
✉e-mail: n2sue@marianna-u.ac.jp

Received: 10 April, 2006; revised: 01 June, 2006; accepted: 27 June, 2006
available on-line: 02 September, 2006

We evaluated the effectiveness of using Flinders Technology Associates (FTA) filter paper for the polymerase chain reaction (PCR) genotyping of transgenic mice. Tail prick blood sample dried on an FTA filter disc was processed for genomic PCR. It is easy and rapid to prepare DNA templates because the protocol is extraction-free and only requires minimal handling of wash briefly blood-stained FTA filter discs. Progeny of a transgene-positive founder mated with wild-type mice was screened for the presence of the transgene by the filter-based PCR using transgene-specific primers. The resulting amplicons with expected sizes of 3134 bp, 1152 bp, 877 bp and 688 bp were robust and reproducible, allowing a distinction between transgenic (n=44) and wild-type (n=47) mice showing no signal. The filter-based PCR screening took only half a day. The present study confirmed the validity and usefulness of the novel rapid extraction-free genotyping method.

Keywords: PCR genotyping, transgenic mice, FTA filter paper, blood samples, cytosolic acetyl-CoA hydrolase

INTRODUCTION

To date, genomic DNA extraction from tail biopsies has been routinely employed in genotyping transgenic mice (Conner, 2005). However, it is time-consuming (2 days) and troublesome to digest biopsy fragments overnight followed by hours of DNA extraction.

We describe here a much simpler and useful method of extraction-free PCR genotyping from small volume of blood instead of biopsy samples. The protocol takes only half a day using commercially available FTA filter paper (Whatman Japan KK). The filter is recently used for collection, distribution and long-term storage of a wide variety of wildlife samples for DNA analysis, including animals (Smith & Burgoyne, 2004) and plants (Tsukaya *et al*., 2005). It is also used for surveillance and detection of a variety of pathogens, including protozoa (Gonzales *et al*., 2006), bacteria (Liu *et al*., 2005) and viruses (Ndunguru *et al*., 2005). Further, in genetic studies on human population and identification, it is used for sampling human materials such as blood (Seah *et al*., 2003), buccal epithelial cells (Salvador *et al*., 2004), and casualty remains (Fujita & Kubo, 2006).

FTA filter is a cellulose membrane containing lyophilized chemicals that lyse cell membranes and denature proteins. In the case of blood samples, the nuclear DNA released from blood cells is physically entrapped within the matrix of the paper, where the DNA remains stable at room temperature for more than 14 years (unpublished observations in Whatman data sheet) against attacking agents such as nucleases, oxidants, UV and microorganisms (Seah & Burgoyne, 2000). It has been established that mouse blood samples spotted on the FTA filter can be directly used as a template for PCR (Smith & Burgoyne, 2004) as well as PCR-based Southern blotting (Hsiao *et al*., 1999). RNA species on FTA filter were also used as templates for real-time RT-PCR (Ndunguru *et al*., 2005). FTA filter now offers the most reliable choice for extraction-free PCR genotyping of

Abbreviations: CACH, cytosolic acetyl-CoA hydrolase; FTA, Flinders Technology Associates; PCR, polymerase chain reaction; SAP, serum amyloid P component. **Enzyme**: acetyl-CoA hydrolase (EC 3.1.2.1).

transgenic mice from only a small volume of blood samples instead of conventional tail biopsies.

MATERIALS AND METHODS

Mice. A CACH-transgenic mouse C57BL/6N-*TgN(SAP-hCACH)* was generated (Macrogen Inc., Seoul, Korea) by zygote microinjection of the transgene presented in Fig. 1A. Wild-type C57BL/6N inbred mice were from Clea Japan Inc. (Tokyo, Japan) and our own colony. The transgenic progeny was maintained as heterozygotes by crossing transgene carriers with the same C57BL/6N strain wild-type mice. The F1–F4 generations were genetically screened for the transgene at 3–4 weeks of age. Mice were fed food and water ad libitum. Animal care was in accordance with the national guidelines for animal experimentation.

Blood samples. Tail prick whole blood samples (< 5 μL each) obtained under ether anesthesia were spotted onto FTA filter papers (Whatman Japan KK). The bloodstained filters were allowed to dry completely for 1 h at room temp. before being punched cleanly. For sample processing, two discs were manually punched from each bloodstained filter with a Harris Micro-Punch fitted with a 1.2-mm steel tip, and were placed one-by-one in sterile 200-μL PCR microcentrifuge tubes. To prevent cross-contamination between samples, the puncher was cleaned by taking one punch from an unspotted area of the filter paper. Each bloodstained disc 1.2 mm in diameter was subsequently subjected to a brief series of washes carried out in the PCR tube according to the manufacturer's instructions (Whatman Japan KK), with impurities being washed away and the DNA being entangled within the discs. Briefly, the sample discs were washed twice in 200 μL of mild buffer (Whatman FTA purification reagent) and then rinsed once in 200 μL of DNase-free water by 1-min incubation at room temp. The washes were discarded after each centrifugation at 12 000 × g for 10 s. The resultant genomic DNA-loaded discs were white and now ready for downstream PCR analysis.

PCR genotyping. The processed sample disc was then placed directly in 20-μL genomic PCR mixture containing 300 pM of each primer, 2.5 mM of each dNTP, 2.5 mM magnesium chloride, 20 mM Tris/HCl buffer (pH 8.0) and 1 U *LA Taq* polymerase (TaKaRa). Transgene-specific primers used were listed in Table 1, and the targeted bands are represented in Fig. 1B. PCR conditions were as follows: denaturation at 94°C for 5 min followed by 35 cycles of 98°C for 10 s, annealing at 58°C for 1 min and extension at 72°C for 3 min 10 s, followed by a final extension at 72°C for 5 min. PCR products were separated by agarose gel electrophoresis using 1.5% SeaKem® GTG agarose (TaKaRa) and then visualized by ethidium bromide staining for typing analysis. High DNA Mass™ Ladder (Gibco BRL) and 100 bp DNA Ladder (Invitrogen) were used.

RESULTS AND DISCUSSION

PCR products with expected sizes were successfully amplified from the sample discs as well as from a plasmid containing the transgene (not shown). The filter-based PCR using a primer pair of S1/A3 (Fig. 1B) amplified a large product with the expected size of 3134 bp (Fig. 2), while the PCR using three pairs of S1/A1, S2/A2 and S3/A3 (Fig. 1B) resulted in the expected 688-bp, 877-bp and 1152-bp products (Fig. 2). Although genotyping primers are conventionally designed for amplifying relatively small products (from about 150 to 500 bp) to ensure that the reaction is robust (Conner, 2005), all of the four larger products were robustly amplified (Fig.

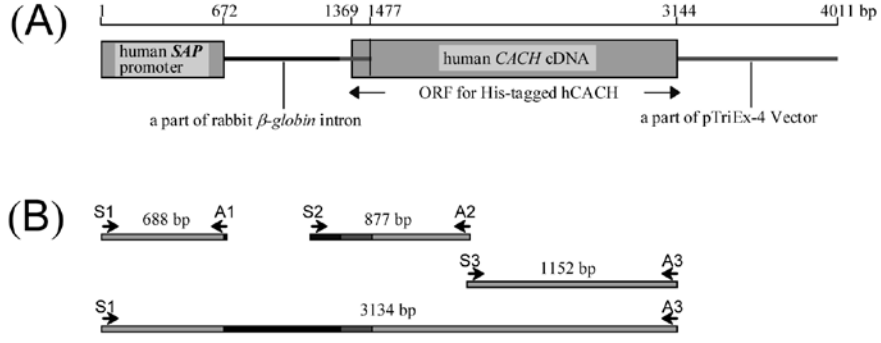

Figure 1. Schematic depiction of PCR strategy for detection of a transgene.
(A) The transgene construct used for generation of CACH-transgenic mice. Human serum amyloid P component (SAP) promoter was ligated to the ORF of His-tagged human cytosolic acetyl-CoA hydrolase (CACH) (Isohashi *et al.*, 1984; Suematsu *et al.*, 2002) *via* a part of rabbit β-globin intron. Human SAP promoter can direct liver-specific expression after birth. Numbers mark nucleotide positions. (B) Lines show four DNA fragments targeted for PCR amplification. Arrows indicate the location and orientation of four genotyping primer pairs used (Table 1).

Table 1. Genotyping primers used.

Nucleotide positions are numbered as in Fig. 1A. Human specific coding sequences distinct from those of mouse are highlighted in bold type. S, sense; A, antisense; CDS, coding sequence.

Primer	Sequence (5′– 3′)	Length (mer)	*Tm* (°C)	Position	Location
S1	TAGAGCTTCATGGGGACAGAAAG	23	58.7	2/24	promoter
S2	CATCCTGGTCATCATCCTGCC	21	60.4	1131/1151	intron
S3	**G**ATGAAGA**G**GAAGGA**G**CGG**TTT**C	23	60.5	1984/2006	CDS
A1	CTGAAGTTCTCAGGATCCTCTAGG	24	60.5	689/666	intron
A2	GG**AAA**CCG**C**TCCTTC**C**TCTTC	21	60.4	2007/1987	CDS
A3	**G**CTT**AC**AA**AC**CCATCA**TC**AG**G**AG**G**	24	60.5	3135/3112	CDS

2), showing high effectiveness of the filter-based PCR genotyping. Further, the amplified 3134-bp product (Fig. 2), which covers extensively the promoter and the full-length ORF (Fig. 1), demonstrated that the transgene was stably integrated without rearrangement or partial deletion observed in some cases of transferring genes. Of all the progeny (n = 91) analyzed, the FTA filter-based PCR genotyping provided successful distinction between the transgenic carrier (n = 44) showing a strong signal and wild type (n = 47) showing no signal (Fig. 2). The proportion of the confirmed transgenic carrier (48%) appears well with the predicted Mendelian frequency, revealing that the transgene has been stably integrated and transmitted. Although not only PCR but also Southern blotting is widely used for screening transgenic mice, PCR is generally accepted as a method of choice because of its simplicity and sensitivity (Conner, 2005). Therefore, genetic screening strategy using the filter-based PCR described here is probably the best method to use at this stage.

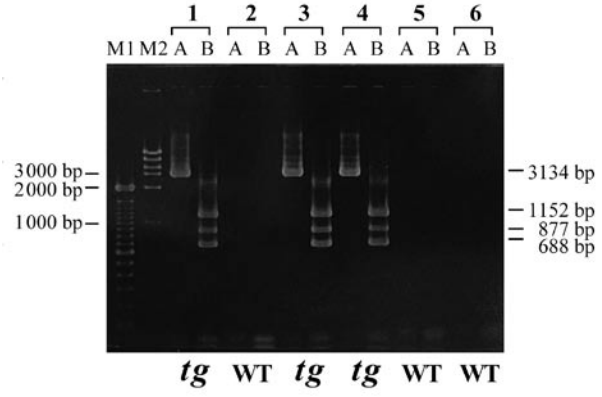

Figure 2. Typical examples of PCR genotyping for detection of a transgene from mouse whole blood samples spotted on FTA filter paper.
Genomic DNA retained on a 1.2-mm FTA disc was used as a template for a 20-μL PCR reaction. Two microliters of amplified sample was loaded and electrophoresed on a 1.5% SeaKem GTG agarose gel followed by ethidium bromide staining. Samples 1–6 were from six F3 offspring. Lanes denoted A contain PCR products using the primer pair S1/A3, while lanes denoted B contain those using three primer pairs S1/A1, S2/A2 and S3/A3 (Fig. 1B). Expected sizes of amplicons obtained with primer pairs S1/A1, S2/A2, S3/A3 or S1/A3 are 688 bp, 877 bp, 1152 bp or 3134 bp, respectively, as indicated on the right. The lane denoted M1 contains a 100-bp DNA ladder composed of 15 blunt-ended fragments between 100 and 1500 bp in multiples of 100 bp and an additional fragment at 2072 bp, and M2 contains High DNA Mass™ composed of equimolar mixture of six DNA fragments of 10000, 6000, 4000, 3000, 2000 and 1000 bp. Samples 1, 3 and 4 were judged to represent transgenic genotype (*tg*), while samples 2, 5 and 6 were judged to represent wild type (WT).

In the present paper, we extend the utility of FTA filter to routine PCR genotyping of transgenic mice. We have demonstrated here the effectiveness of the rapid PCR genotyping using FTA filter from minute intravenous blood samples. The novel method eases the animals' pain by circumventing conventional tail biopsy, and saves a lot of labor for genomic DNA extraction inevitable in conventional genetic screening employed to date in labs worldwide. Thus, the extraction-free, filter-based genotyping from very small amounts of blood is recommended as a standard tool for genetic identification in routine studies using transgenic mice as well as in other genotyping or mutation analyses.

Acknowledgements

This research was supported by a grant from the Vitamin Society of Japan.

REFERENCES

Conner DA (2005) In *Current Protocols in Molecular Biology* (Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, eds) pp 23.10.1–23.10.8. John Wiley & Sons, Inc., USA.

Fujita Y, Kubo S (2006) *Leg Med (Tokyo)* **8**: 43–47.

Gonzales JL, Loza A, Chacon E (2006) *Vet Parasitol* **136**: 119–126.

Hsiao KM, Lin HM, Pan H, Li TC, Chen SS, Jou SB, Chiu YL, Wu MF, Lin CC, Li SY (1999) *J Clin Lab Anal* **13**: 188–193.

Isohashi F, Nakanishi Y, Matsunaga T, Sakamoto Y (1984) *Eur J Biochem* **142**: 177–181.

Liu JW, Zhang W, He JP, Zhou W, Yuan YW (2005) *Sheng Wu Gong Cheng Xue Bao* **21**: 1009–1013.

Ndunguru J, Taylor NJ, Yadav J, Aly H, Legg JP, Aveling T, Thompson G, Fauquet CM (2005) *Virol J* **2**: 45–56.

Salvador JM, Corazon M, De Ungria A (2004) *Oral Oncol* **40**: 231–232.
Seah LH, Burgoyne LA (2000) In *Progress in Forensic Genetics* (Sensabaugh GF, Lincoln PJ, Olaisen B, eds) **8**, pp 74–77. Elsevier, Amsterdam.
Seah LH, Jeevan NH, Othman MI, Jaya P, Ooi YS, Wong PC, Kee SS (2003) *Forensic Sci Int* **138**: 134–137.
Smith LM, Burgoyne LA (2004) *BMC Ecol* **4**: 4–14.
Suematsu N, Okamoto K, Isohashi F (2002) *Acta Biochim Polon* **49**: 937–945.
Tsukaya H, Iokawa Y, Kondo M, Ohba H (2005) *J Plant Res* **118**: 57–60.