Non-invasive transgenic mouse genotyping using stool analysis

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Abstract Commonly applied genotyping of transgenic mice involves using tail or ear biopsies which may cause discomfort to the animal. We tested the possibility of polymerase chain reaction (PCR)-based mouse genotyping using stool specimens from three transgenic mouse lines that overexpress 10–18 transgene copies of human keratin polypeptide 18, as compared to genotyping using tail biopsies. Stool specimens were obtained with ease and provided easy detection of the human transgene product. The method was also able to detect endogenous mouse actin and keratin genes which presumably are present at two copies each. Nested PCR was not necessary for genotyping using stool-derived genomic material but did increase the relative magnitude of the signal obtained. The non-invasive genotyping method described herein offers a reproducible, sensitive and effective modality that could replace invasive tissue sampling procedures currently used to test thousands of genetically altered mice.

Key words: Genotyping; Transgenic mouse; Stool analysis

1. Introduction

Studies involving transgenic mice have contributed dramatically to important molecular, genetic and disease-related discoveries that might not otherwise have been possible. Since their introduction in the 1980s, the versatility and unique value of genetically altered mice has led to their production in the hundreds of thousands worldwide (e.g. [1,2]). One important aspect of utilizing transgenic mice is the need to genotype them in order to distinguish mice that carry a disrupted gene or a transgene from mice that do not. This involves using tail or ear biopsies, or collecting blood samples for genomic DNA isolation then polymerase chain reaction (PCR) amplification [3,4]. Other reported sources for DNA isolation from mice include saliva [5] and toes [6]. Toe-clipping is considered invasive and is expressly discouraged by animal welfare regulations [7]. Saliva collection, although non-invasive, may cause moderate handling stress, requires nested PCR in younger animals, and has yet to receive widespread acceptance.

2. Materials and methods

2.1. Animals

The mice used were: non-transgenic FVB/n, and three transgenic lines (FVB/n background) that overexpress human (h) keratin polypeptide 18 (K18). The transgenic lines TG2, F22 and F30 are well described and express 18, 10 and 12 copies of the human transgene [8,9]. Mouse stool was collected after holding the mouse by grasping the skin around the neck and positioning the mouse upright until a stool droplet is excreted (which usually required < 30 s) directly into a DNA extraction tube, or by placing the mouse in a clean cage then collecting the stool droplet. For tail isolation, a 1 cm segment of the distal part of the tail was obtained and used for nucleic acid extraction.

2.2. Genomic DNA isolation and primers

Genomic DNA was purified from mouse tail tissue ($\sim$1 cm) or one stool droplet ($\sim$20 mg) using a Qiagen tissue kit (Valencia, CA) followed by PCR amplification. The DNA extracts were in 200 μl of 10 mM Tris-HCl (pH 8) from which 1 μl and 10 μl were used for PCR amplification of the tail and stool genomic DNA, respectively. PCR amplifications were done in 50 μl of which 1 μl was used (for both tail and stool) for nested PCR. Amplified products were analyzed using 1% agarose gels (10 μl/lane). The PCR primers were: 5′-CAGAAGGCCAGCTTGGAGAAC-3′ and 5′-ATCTCCTGATCCCAGCACGTG-3′ (human K18 transgene), 5′-GTGTTAGACACTGTGGACATGG-3′ and 5′-GAGAGAGCCATACCAAGAATGG-3′ (mouse γ-actin), 5′-ACACAAGAAAAGTCAGAAACCC-3′ and 5′-ACTGTCTTGATGCTCTGGGGTC-3′ (nested mouse γ-actin), 5′-GTCCTCAGCACCCTGTAACCTG-3′ and 5′-CGGTCTGGATTCCACCCATTC-3′ (endogenous mouse-specific K18), and 5′-GACATGCCACAAAATCTTCACC-3′ and 5′-ACCGACACAGGGAGTGGATAAC-3′ (nested mouse K18).

2.3. PCR reactions

The PCR cocktail (50 μl) contained 0.2 mM dNTP mixture, 1.5 mM $MgCl_2$, 1 μM of each of the primer sets and 2.5 units Taq DNA polymerase in 20 mM Tris-HCl, 50 mM KCl (pH 8.4). The PCR conditions were: (i) hK18: 94°C for 3 min followed by 35 cycles at 94°C for 30 s, 58°C for 40 s, and 72°C for 50 s. Cycling was followed by a final extension step at 72°C for 10 min. (ii) mouse (m) K18: 94°C for 10 min followed by 35 cycles at 95°C for 25 s, 65°C for 25 s, and 72°C for 1 min. Cycling was followed by a final extension step at 72°C for 10 min. (iii) Nested mK18: 94°C for 2 min followed by 35 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. Cycling was followed by a final extension step at 72°C for 7 min. (iv) m-actin: 94°C for 2 min followed by 35 cycles at 94°C for 1 min, 57°C for 1 min, and 72°C for 45 s. Cycling was followed by a final extension step at 72°C for 7 min. (v) Nested m-actin: 94°C for 2 min followed by 25 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 20 s. Cycling was followed by a final extension step at 72°C for 10 min.

3. Results

We tested if mouse stool specimens can be used to adequately and reliably detect the presence of transgenes and endogenous genes, given that murine intestinal epithelia re-

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Abbreviations: PCR, polymerase chain reaction; h, human; K18, keratin polypeptide 18; m, mouse

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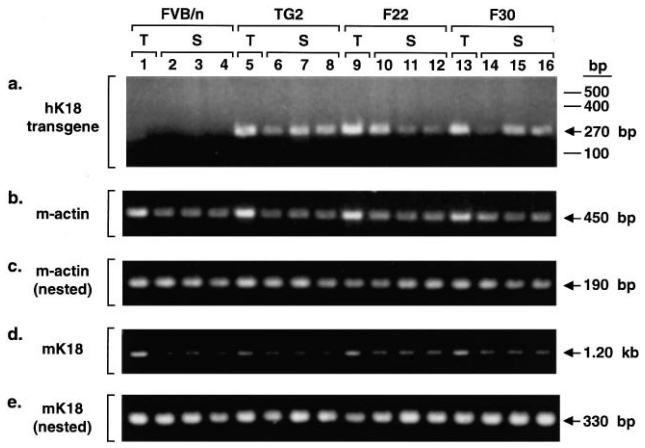

Fig. 1. PCR analysis of DNA from mouse tail tissue and stool. Genomic DNA was purified from mouse tail (T) tissue (~1 cm) or one stool (S) droplet (~20 mg) using a Qiagen tissue kit (Valencia, CA) followed by PCR amplification as described in Section 2. The mice used were: non-transgenic FVB/n, and three transgenic lines (FVB/n background) that overexpress human (h) keratin polypeptide 18 (K18). The transgenic lines (TG2, F22 and F30) express 18, 10 and 12 copies of the human transgene [8,9]. PCR amplifications were done in 50 μl of which 1 μl was used (for both tail and stool) in panels c and e for nested PCR. a: PCR primers were used to amplify a 270 bp fragment of the human K18 transgene. b: PCR primers were used to amplify a 430 bp fragment of mouse (m) γ-actin. c: Nested PCR analysis of m γ-actin was carried out to generate a 190 bp fragment. d: PCR primers were used to amplify a 1.2 kb fragment of the endogenous mouse-specific K18. e: Nested PCR analysis of mK18 was carried out to generate a 330 bp fragment.

generate every 3–4 days [10], sloughing the old cells into the lumen. For this we compared DNA extracted from stool with that extracted from short tail segments for the ability to detect a human keratin polypeptide 18 transgene. As shown in Fig. 1a, analysis of tail DNA from the transgenic lines (so called TG2, F22 and F30) provided a strong signal (lanes 5, 9, 13) but more importantly a single stool pellet contained enough of the transgene to be detected using a single round of PCR (lanes 6–8, 10–12, 14–16). As expected, non-transgenic parental FVB/n mice did not contain the human transgene (Fig. 1a, lanes 1–4). Similar results were obtained using different transgenic mouse lines that overexpress other mutant forms of human K18 (not shown). Since the heterozygous transgenic mice we analyzed contained 10–18 copies of the transgene, we also analyzed the same mice for endogenous mouse γ-actin and mouse K18 which are presumably each represented by two gene copies (excluding any potential pseudogenes). Both mouse γ-actin and mouse K18 were readily detected using stool DNA (Fig. 1b,d). The actin and mK18 signal became significantly magnified with a second round of PCR using nested primers (compare panels b with c; panels d with e).

4. Discussion

Genomic analysis of human stool for mutations [11], and of stool from seals and baboons as part of tracking and assignment of species [12,13], has been reported. In our case we have extended such studies into rodents in a fashion that allows simple, rapid, reliable, non-invasive and efficient genotyping of transgenic mice. Analysis of the stool as described herein has several clear advantages including its non-invasive nature, the possibility of repeated samplings if needed, and the typical need to use only one set of primers as compared with saliva analysis which is somewhat tedious in terms of collection and usually requires nested PCR analysis [5]. In >50% of more than 100 animals examined, stool was collected within 30 s of grasping the mouse and at most required placement in a cage for 3–4 min while stool from the remaining animals was sampled. Notably, similar results were obtained from stool isolated immediately on weaning (i.e. 21 days old, only one stool pellet is needed) or from older mice, and no difference was noted if the stool is fresh or up to 24 h old (not shown). Genotyping using this method offers practical refinement in animal use techniques that could replace invasive tissue sampling procedures currently used to test thousands of genetically altered mice.

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