Animal-friendly mouse genotyping using direct PCR

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Introduction

Transgenic mice are widely used models in basic research. Such models require long-term identification of the animals and screening for the presence or absence of transgenes. Both identification and genotyping are usually achieved using invasive methods. Microchip transponders, tattoos, and ear notching are popular approaches for identifying mice, whereas tail and ear samples are still widely used as DNA sources.

The European Union Directive 2010/63/EU on the protection of animals used for scientific purposes says that the choice of methods should provide the most satisfactory results and likely cause the minimum pain, suffering, or distress [1]. Although the methods undertaken for identification are not dictated by this directive, the Federation of European Laboratory Animal Science Associations (FELASA) proposed guidelines to refine rodent identification [2] and genotyping [3]. The guidelines recommend that the choice of method should minimize adverse effects to the animals. When there is a need to both identify individuals and collect a DNA sample for genotyping, the chosen method should meet both goals [2,3].

Here we describe an animal-friendly approach to both identify and genotype mice in a single workflow by combining toe tattooing with direct PCR from mice buccal swab and hair samples. Among permanent identification methods, toe tattooing is a good compromise between minimum animal pain and reliability of identification. The use of anesthesia enables the combination of permanent tattooing using a tattoo machine with the least invasive sampling methods (buccal swab or hair samples).

The Thermo Scientific™ Phire™ Tissue Direct PCR Master Mix allows DNA amplification directly from the sample, omitting the DNA purification step and thus significantly reducing genotyping workflow time. Additionally, the Phire Tissue Direct PCR Master Mix supports consistent PCR from buccal swab and hair samples, which enables gentler approaches to sampling from the animal compared to tail clips or ear punches. The data show that both types of samples are reliable alternative sources of DNA template for genotyping of transgenic mouse strains. Our approach therefore fulfills the requirement of improving animal welfare without compromising the quality of genotyping results.

Materials and methods

Materials

- AIMS™ ATS-3 General Rodent Tattoo System (Fisher Scientific, Cat. No. 14-370-133)
- Phire Tissue Direct PCR Master Mix (Thermo Fisher Scientific, Cat. No. F170S)
- Applied Biosystems™ StepOnePlus™ Real-Time PCR System, 96-well block (Thermo Fisher Scientific, Cat. No. 4376600)
- Applied Biosystems™ MicroAmp™ Fast Reaction Tube with Cap, 0.1 mL (Thermo Fisher Scientific, Cat. No. 4358297)
- Puritan™ Sterile Mini-Tip Rayon Swabs (Puritan Medical Products, Cat. No. 25-800 R 50)
- Mouse tail tissue
- Mouse buccal cells
- Mouse hair samples (with follicles)
- Purified mouse tail DNA

Toe tattooing

The needle of the tattoo machine was disinfected prior to each use, and short-term isoflurane anesthesia was used during the procedure. After the area was gently cleansed with alcohol, the surface of the toe was tattooed with five back-and-forth movements. The needle tip of the tattoo machine was held perpendicular to the skin in order to place pigments in both the epidermis and dermis (Figure 1), thereby ensure a permanent marking. The toe was disinfected immediately afterwards. Figure 1 shows the quality of the tattoo one day later.

Sample preparation

Tail tips: A 1 mm sample of tail tip was taken under isoflurane anesthesia using sterile scissors. The sample was placed in 20 µL of Dilution Buffer containing 0.5 µL of DNA Release Additive from the Phire Tissue Direct PCR Master Mix kit.

Buccal cells: A sterile cotton swab with a miniature tip was gently rubbed and rotated 5–10 times along the inside of the mouse cheek. The buccal swab was then rotated 5–10 times in a 1.5 mL tube containing 50 µL Dilution Buffer, 1.5 µL DNA Release Additive, and 250 µL TE buffer, pH 8. The swab was gently pressed against the side of the tube before removing it from the tube.

Hairs: Sterile tweezers were used to collect 5–10 mouse hairs. The hairs were directly placed into 20 µL of Dilution Buffer containing 0.5 µL of DNA Release Additive.

After collection, sample tubes were briefly mixed and spun down. Samples were incubated for 2–5 min at room temperature, then 2 min at 98°C in a preheated block.

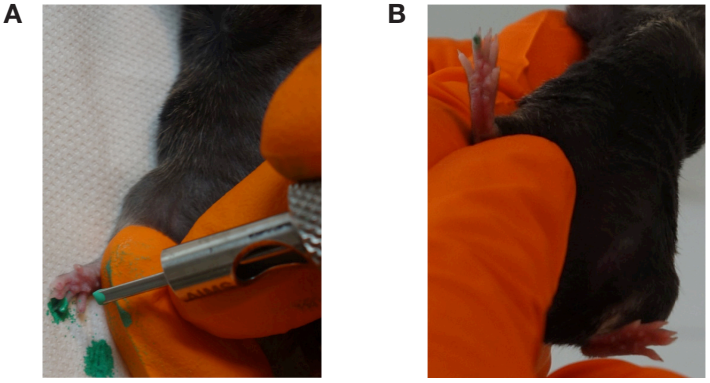

Figure 1. Tattooing of mouse toe. (A) Procedure using a dermograph. **(B)** Tattoo one day later.

PCR

For each sample type, 1 µL of Dilution Buffer supernatant was used as template in a 20 µL PCR reaction as described in Table 1 for conventional PCR and Table 2 for direct PCR. Cycling conditions were as described in Table 3, and PCR products were directly loaded onto a 1% agarose gel for electrophoretic separation.

Results and discussion

Phire Tissue Direct PCR Master Mix allows quick and animal-friendly mouse genotyping using saliva or hair samples, with the same efficiency and yield as conventional PCR using purified DNA from mouse tail (Figure 2). With respect to identification methods, there is currently no means to ensure a permanent marking without invasiveness and pain at application [2]. Electrical toe tattooing was chosen because the size of the needle was adapted to the animal and allowed proper ink injection in the dermis, which limits fading. This ensured a permanent identification that can last for months compared to microtattoo systems. However, as this procedure requires restraining the animal for a few minutes and may be painful, we refined it by using isoflurane anesthesia. The main drawback of this identification method is that it does not provide biopsies for genotyping [2], which was circumvented here by sampling hair or buccal cells directly after the tattooing procedure while the mouse was still anesthetized. We therefore limited the overall duration of the procedure to the minimum.

Table 1. Conventional PCR reaction conditions.

Component	20 µL reaction	Final conc.
H_2O	Add to 20 µL	–
10X PCR buffer	2 µL	1X
dNTP mix, 10 mM	0.4 µL	200 µM of each dNTP
Forward primer	X µL	1 µM
Reverse primer A	X µL	0.5 µM
Reverse primer B	X µL	0.5 µM
DNA polymerase	0.2 µL	–
Sample	1 µL*	–

* Concentrations of purified DNA: wild type, 39 ng/µL; heterozygous, 30.4 ng/µL; knockout, 26.8 ng/µL.

Table 2. Direct PCR reaction conditions.

Component	20 µL reaction	Final conc.
H_2O	Add to 20 µL	–
2X Phire Tissue Direct PCR Master Mix	10 µL	1X
Forward primer	X µL	1 µM
Reverse primer A	X µL	0.5 µM
Reverse primer B	X µL	0.5 µM
Sample	1 µL	–

Table 3. PCR cycling conditions.

	Conventional PCR			Direct PCR		
Cycle step	Temp.	Time	Cycles	Temp.	Time	Cycles
Initial denaturation	94°C	3 min	1	98°C	15 min	1
Denaturation	94°C	30 sec		98°C	15 sec	
Annealing	55°C	30 sec	30	69.7°C	15 sec	40
Extension	72°C	45 sec		72°C	1 min	
Final extension	72°C	5 min	1	72°C	1 min	1
Hold	4°C	∞	–	4°C	∞	–

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Among sampling techniques for genotyping, buccal swabs are considered noninvasive whereas hair collection is minimally invasive [3]. Hair is fast to collect but there is a risk of cross-contamination between samples, primarily because hair sticks electrostatically to plastics. However, disposable tweezers can be used for each sample to prevent contamination, or researchers may limit this sampling method to purposes of resampling as previously suggested [3]. Buccal swabs serve as yet another alternative method having a lower risk of contamination, and this method is particularly useful for hairless models such as nude strains. Figure 2 shows that robust amplification was achieved with all samples. When using purified DNA from mouse tail, the DNA amount for PCR can be controlled, but both buccal swabs and hair samples also provide sufficient amounts of DNA for PCR. For all samples, PCR products corresponding to the wild type and knockout alleles were clearly distinguishable, enabling reliable identification of the 3 different genotypes of our transgenic mouse strain.

Tail biopsies still remain the most-used source of DNA for genotyping, but tail biopsies should be discouraged as the first option for genotyping when identification is also required, to minimize adverse effects to the animals [3]. The present work demonstrates that this process can be replaced by less invasive sampling methods using either buccal swabs or hair samples.

Conclusion

The approach described here combines a reliable identification method with a noninvasive sampling method for genotyping, using the Phire Tissue Direct PCR Master Mix in the same workflow. In accordance with FELASA recommendations, this method helps improve animal welfare by reducing animal handling to a minimum for the management of transgenic strains.

References

1. European Union (2010) Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. Retrieved from **http://eur-lex.europa.eu/**.
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3. Bonaparte D, Cinelli P, Douni E et al. (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(3):134–45.

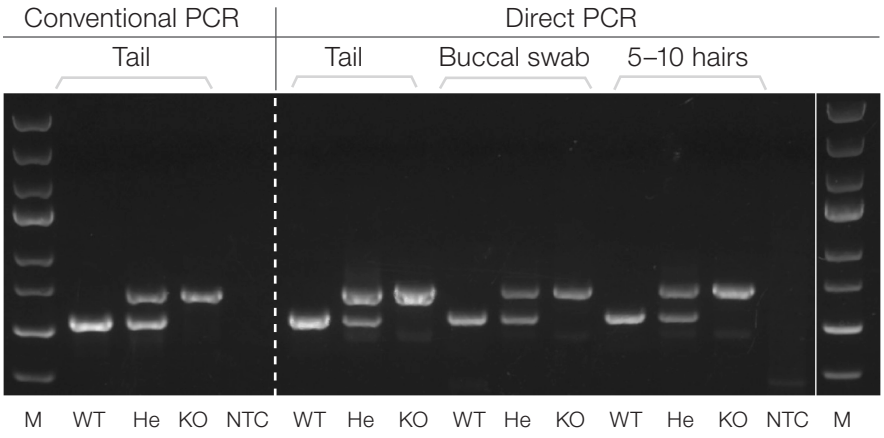

Figure 2. Genotyping of transgenic mice. Genotypes were determined using a conventional *Taq* PCR kit with DNA from mouse tail, or Phire Tissue Direct PCR Master Mix with DNA from mouse tail, buccal swab, or hair. Fragment sizes: 600 bp and 750 bp. WT: wild type; He: heterozygous; KO: knockout; NTC: no-template control; M: Thermo Scientific™ O'GeneRuler™ Express DNA Ladder.

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