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RAPID GENOTYPING OF GENETICALLY MODIFIED LABORATORY ANIMALS FROM WHOLE BLOOD SAMPLES WITHOUT DNA PREPARATION

SHORT COMMUNICATION

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A new, rapid method is described which permits the genotyping of genetically modified animals from a microlitre volume of whole blood samples via one step polymerase chain reaction amplification. The major advantage of the presented method is the exclusion of a DNA preparation step, which significantly reduces the time expenditure and work load of the genetic testing. Pilot studies indicate, that this method is efficient and applicable also on tissue biopsies and larger amount of blood providing a rapid and reliable new technique over conventional genotyping approaches.

Keywords: PCR – genotyping – direct – blood – mice – knock-out animals

Genetically modified animals are widely used in biomedical research which has necessitated the routine genotyping of large number of offspring's to select the individuals suitable for further experiments or to verify the genetic stability of a breeding colony. Tissue samples obtained from the tail tip or earlobe [1], and anticoagulated whole blood samples are most frequently used for further PCR-based genetic testing [4]. The conventional methods use different protocols to isolate and purify genomic DNA from these samples in an amount and quality sufficient for further nucleotide analysis. To reduce the duration and improve the reliability of genotyping new PCR techniques omitting the DNA preparation step have been developed. This progress could be made by the use of special buffers [5] or modified polymerases [10]. Recently, PCR-mastermixes containing these or similar components have

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appeared in the market to enable direct DNA amplification of highly inhibitory samples [6]. Here we describe a rapid, simple and accurate procedure for genotyping from a single drop of animal blood without DNA preparation steps.

Twelve heterozygous N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD$^{+/-}$) genetic knock-out C57BL/6 mice [7] were used in these experiments. Under brief ether anaesthesia, the skin was disinfected with ethanol and the tail vein of the animals was punctured with a 22G hypodermic needle. In one group of the animals 5–8 µl of venous blood was collected with a sterile micropipette tip and transferred immediately into 500 µl Eppendorf tubes pre-filled with 3 µl sterile heparin solution (Heparin Na 5000, Ratiopharm). In the second group of animals after the phlebotomy the blood droplet was absorbed with a sterile dental absorbent paper point (size 80; Meta Biomed) and transferred into sterile 1.5 ml Eppendorf tubes. The samples were dried over silica gel at 4 °C for 4 hours. The samples were stored at –70 °C until further analysis.

The used primers [7] and the calculated melting temperatures (Tm) are listed in Table 1. MJ Mini Instrument (Bio-Rad, Hercules, CA) was used for investigation. Two mastermixes were suitable for DNA amplification from highly inhibitory samples (SsoFast Supermix, Bio-Rad, and Phusion Blood Direct PCR Kit, Finnzymes). Both mastermixes were used according to the manufacturer's instruction. Primers were added to reach final concentration of 0.5 µM and 1 µl diluted (10×) blood samples used as template. Alternatively, a 0.5–1 mm long piece from the tip of the dental absorbent paper points were cut and placed directly into the reaction mix to provide genomic DNA samples from the blood. The PCR parameters are described previ-

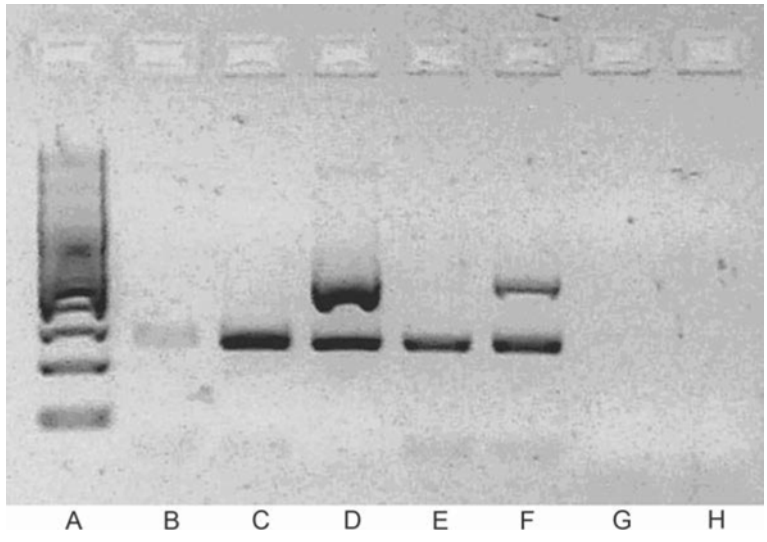

Fig. 1. Comparison of efficieny of PCR mastermixes. Lines: A – Size marker (100 bp DNA ladder, Fermentas); B – Water control; C – wild-type mouse, SsoFast Supermix; D – NAPE-PLD$^{+/-}$, SsoFast Supermix; E – wild-type animal, Phusion Blood Direct PCR Kit; F – NAPE-PLD$^{+/-}$, Phusion Blood Direct PCR Kit; G–H – Control, commercial MMX

Table 1
Primers and calculated melting temperatures

NAPE-PLD mice primers	Sequences	Tm (°C)
NAPE-PLD +/+ forw	GAG CTG GAC TGG TGG GAG GAG	61.6
NAPE-PLD +/+ rev	GCT CCG ATG GGA ATG GCC GC	64.0
NAPE-PLD –/– forw	CTG CAC ACT TGT TCC CCG AGC	61.1
NAPE-PLD –/– rev	GCT GCT ATT GGC CGC TGC	60.3
HD-N171-82Q mice primers		
MORP-SENSE	CCT CTT TGT GAC TAT GTG GAC TGA TGT	58.2
HD-591-5	AGA ACT TTC AGC TAC CAA GAA AGA CCG TGT	61.2/58.9
MORP-ANTISENSE	GTG GAT ACC CCC TCC CCC AGC CTA GAC	66.8/60.2

ously [7]. The amplicons were separated by electrophoresis on 1.5% agarose gels containing GelRed Nucleic Acid Stain (Biotium) and visualized by UV illumination (Fig. 1). Reaction products of 250 bp and 450 bp were obtained corresponding to the wild type and the transgenic allele, respectively.

Not all used blood sampling techniques are fully in accordance with animal welfare principles discussed in guidelines and workshops dedicated to the use of laboratory animals [9]. We have selected this complicated-looking way because of other, parallel chemical and physiological investigations. There are another, minimally traumatic techniques which are suitable for intravenous blood sampling [3].

We have obtained similar results with both mastermixes and both with whole blood samples and with the blood specimen dried on the absorbent paper. Advantage of the SsoFast Supermix is the easy-to-use formulation because this 2× concentrated mastermix contains all of the necessary components. Under the conditions used in the present experiments detectable amplification was not observed with the other commercially available PCR kits tested.

We have got better results with diluted blood samples although there were amplicons with concentrated blood samples, too. The maximum dilution was 100× at which the reaction can be reliably operated. The inhibition was same as described previously [1] after the different DNA preparation methods from mouse tail samples. Therefore, recommended to insert as small as possible pieces into the reaction mix when using dental absorbent paper points. For the same reason the PCR reactions from whole blood are more sensitive for the primer Tm differentiations as the conventional PCR with prepared DNA samples. It is very frequent that the primers propound to distinguish the wild type and the mutant animals are hardly differs in Tm. If the calculated Tm with nearest neighbour method vary more than 3 °C it is advisable to design new primers. One example has been presented in the second part of Table 1. Here, we shortened the primers described previously [8] which are distin-

guishing the huntingtin-producing transgenic (HD-N171-82Q) and wild type mice. The redesigned primers are underlined.

Although, similar paper-disc method have been used previously for blood collection [2] the PCR procedure omitting sample DNA preparation makes this genotyping method efficient and time sparing. Whereas conventional procedures for genotyping take about a day to complete, the new technique described in this paper can be accomplished within two hours. The investigations were performed with codified samples in two independent laboratories to maintain blinding during laboratory processing. Conventionally prepared DNA was used as control and same results were obtained in all cases. Pilot studies indicate, that this method can be also applied for genetic analysis of tissue biopsies or blood samples of larger volumes providing a rapid and reliable solution over current genotyping methods.

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