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SHORT COMMUNICATION

A rapid and inexpensive genotyping method using dried blood spots for mutational analysis in a mutant mouse model: an update

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Abstract

Background Dried blood spot (DBS) testing is a well-known method of bio-sampling by which blood samples are blotted and dried on filter paper. The dried samples can then be analyzed by several techniques such as DNA amplification and HPLC. We have developed a non-invasive sampling followed by an alternative protocol for genomic DNA extraction from a drop of blood adsorbed on paper support. This protocol consists of two separate steps: (1) organic DNA extraction from the DBS, followed by (2) DNA amplification by polymerase chain reaction (PCR). The PCR-restriction fragment length polymorphism (PCR-RFLP) is an advantageous and simple approach to detect single nucleotide polymorphisms (SNPs).

Results We have evaluated the efficiency of our method for the extraction of genomic DNA from DBS by testing its performance in genotyping mouse models of obesity and herein discuss the specificity and feasibility of this novel procedure.

Conclusions Our protocol is easy to perform, fast and inexpensive and allows the isolation of pure DNA from a tiny amount of sample.

Keywords Dried blood spots · Genotyping · Genomic DNA extraction · SNP-RFLP

Abbreviations

DBS	dried blood spots
PCR	polymerase chain reaction
SNP-RFLP	Single Nucleotide Polymorphisms-Restriction Fragment Length Polymorphism

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Background

DNA extraction is a routine procedure of molecular biology used in research laboratories and forensic analyses. The process is composed of a combination of physical and chemical steps [1]. Solution-based methods using organic solvents consists of the organic extraction and subsequent salting-out. The chemical steps include lysis, phenol-chloroform extraction, ethanol precipitation and washing. Organic extraction is mostly used in laboratories because it is affordable and allows the production of large quantities of pure DNA. The salting out method exploits the dependence of protein solubility on their chemical-physical characteristics, temperature, pH and saline concentration of the solution. These two methods involve the use of toxic chemicals and increase the risk of contamination because of the DNA transfer between multiple tubes. Other procedures of DNA isolation, such as commercially available kits, Chelex 100

extraction and Solid Phase Extraction (SPE) are based on chromatographic principles. These methods differ in quality and quantity of extracted DNA but are all very expensive and poorly reproducible.

The choice of the most suited DNA extraction method depends on several factors, including cost, time, easiness of execution, quality and quantity of DNA isolated, safety and risk of contamination. In addition, the type of sample from which the DNA is extracted must also be considered [2–5]. DNA can be extracted from diverse sources of samples through different methods depending on the selected tissue. In animals, peripheral blood leukocytes are the usual source of genomic DNA. The most common blood collection procedures in mice (e.g. withdrawal from the orbital sinus) are usually painful and stressful for the animal; the performing personnel must be appropriately trained and compliant with guidelines for anesthesia and/or analgesic use and maximal sample volumes and phlebotomy frequency [6].

In detail, blood collection volume should not exceed 10% of total blood volume. Common DNA extraction methods require large amounts of blood (about 500 µL), but mice have on average about 55 mL of blood *per* kg of bodyweight, so usually a mouse only has few milliliters of blood in total. For this reason, it is difficult to get satisfactory results with commercial kits.

Different mouse models are commonly used for the study of diabetes, including the non-obese diabetic (NOD) mice, a useful animal model for the study of the immunopathogenetic events leading to autoimmune type 1 diabetes development, and the obese mice (Lep^{ob}, referred to as *ob* or *ob*/*ob*) and their genotyping is of paramount importance. In the SNP-RFLP enzymatic method a region containing a specific SNP allele is targeted and amplified by PCR and subsequently digested using an endonuclease enzyme [7]; the length variation of the digested PCR fragments is then distinguished by gel electrophoresis [8]. It is a relatively simple, rapid, inexpensive, and convenient method frequently used for to the detection of those small genetic differences between individuals which can lead to physiological or pathological changes [9, 10].

In NOD/LtJ mice, a spontaneously G→T transversion mutation (designated as $Lepr^{db-5\,J}$) occurs at position 640 of the leptin receptor gene (at the protein level, the mutation causes a valine-to-glycine substitution) and mutant mice become obese and hyper-insulinemic [11, 12].

We have here developed an up-dated method for the extraction of genomic DNA from mouse whole blood, obtained by puncturing the tail tip, and letting blood drops to be adsorbed and dry on Whatman 3 MM paper. This method may substantially facilitate SNP-based genotyping by presenting many advantages such as cost reduction, the employment of a less invasive practice for blood sample collection in small laboratory animals and the necessity of reduced blood volumes (about 50 µL); furthermore, it also allows easy blood transportation among laboratories. We have tested this method for NOD $Lepr^{db5j}$ mice genotyping.

Methods

Reagents

- Whatman 3 MM paper support.
- HCl 0.4 N.
- NaOH 10 N.
- SDS 10%
- $CHCl_3$.
- LiCl 4 M.
- Ethanol absolute.
- 10X PCR buffer (usually supplied by the manufacturer with the Taq polymerase).
- Taq polymerase (Taq DNA Polymerase, dNTPack, Sigma-Aldrich).
- dNTP mixture (dATP, dCTP, dGTP, dTTP; usually supplied by the manufacturer with the Taq polymerase; Sigma-Aldrich).
- PCR primers (forward and reverse): 5 µM in Tris/EDTA (TE) buffer; primers are usually obtained from Integrated DNA Technologies, Inc.
- Nuclease-Free Water (not DEPC-Treated) (ThermoFisher Scientific).
- Template DNA (see Experimental design for further information).
- Restriction enzyme *Hae* III (10U/µL) (New England Biolabs).
- Restriction digestion buffer CutSmart 10X (usually supplied by the manufacturer with the restriction enzyme).
- Agarose (UltraPure™ Agarose, ThermoFisher Scientific) CRITICAL Usually discriminates DNA fragments of less than 1,000 bp. Within a given range of differently sized DNA fragments, the smaller DNA fragments of less than 1,000 bp are best separated in TBE buffer.
- Tris base (Sigma).
- Boric acid (Sigma).
- 0.5 M EDTA pH 8.0 (Sigma).
- EtBr (0.5 mg mL^{-1}; Sigma; stock solution is prepared as 10 mg mL^{-1} in water and stored in a light-resistant bottle at room temperature).
- Bromophenol blue (Sigma).
- Xylene cyanol FF (Sigma).
- Glycerol (Sigma).
- An optimal DNA size marker, 1Kb Plus DNA Ladder (Invitrogen).

Equipment

- Scissors.
- Tweezers.
- PCR thermal cycler.
- Pipettors and tips DNase and RNase free.
- Horizontal minigel electrophoresis apparatus (Sub-Cell GT Cell, BioRad).
- Thermostatic bath.

Solutions

- TE buffer (pH 8.0) 10 mM Tris-HCl, 1 mM EDTA.
- NaOH 0.1 N / SDS 1% solution.
- 10X stock solution electrophoresis buffer (TBE), 1 L: 108 g Tris base, 55 g boric acid, 40 mL 0.5 M EDTA, pH 8.0.
- Gel-loading dye: 0.25% bromophenol blue, 0.25% xylene cyanol, 50% glycerol, 1 mM EDTA (pH 8.0).

Samples

Individual blood samples were taken from NOD/ShiLtJ-Lepr$^{db-5\ J}$/LtJ mice through a puncture of the tail tip. For each animal a blood drop was collected on pieces of Whatman 3 MM paper, placed in sterile boxes and left to dry for at least 24 h at room temperature. The area of each drop was about 80 mm^2. In addition, a clean piece of Whatman 3 MM paper support (without blood) was used as negative control to evaluate the contamination degree of the procedure, while genomic DNA obtained through classical organic extraction from mice tails served as positive control (25 ng of DNA for each PCR reaction).

DNA extraction

A blood drop adsorbed on a Whatman 3MM paper support was used as starting sample (Fig. 1 A).

- Scissors and tweezer were sterilized through a treatment in HCl 0.4 N for 5 min, then washed in water;
- One fourth of the drop was cut with scissors. Then, excess of paper, without mouse blood, was carefully removed and the sample placed in a 1.5 mL conical tube containing 40 µL of TE buffer.
- Firstly, samples were incubated at 65 °C for 15 min, then another incubation at 95 °C for 2 min was required to allow the detachment of blood from the paper.

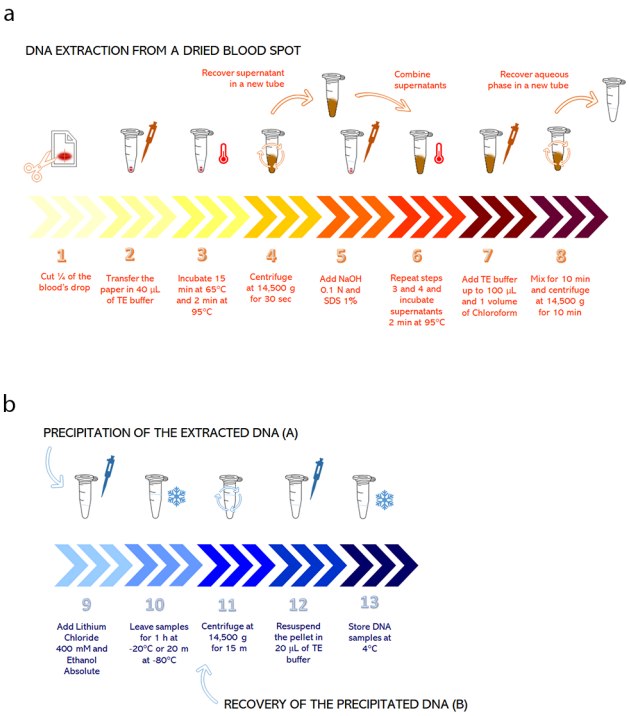

Fig. 1 DNA extraction from dried blood spots, precipitation and recovery (a) Schematic representation of experimental workflow for extraction of DNA from dried blood spots. Steps from 1 to 8 describe the procedure from the cut of the filter paper to the recovering of the aqueous phase containing DNA after chloroform extraction. (b) Schematic representation of experimental workflow to recover DNA extracted in previous steps. Steps from 9 to 13 describe the protocol to precipitate DNA with LiCl and ethanol and to recover it after centrifugation and TE resuspension

- Samples were centrifuged at 14,500 g for 30 s and the supernatant transferred to new tubes.
- Forty µL of NaOH 0.1 N and SDS 1% solution were added on the pieces of paper in the first tube, in order to lyse cells, degrade RNA and denature proteins.
- The previous steps were repeated (incubations at 65 °C for 15 min and at 95 °C for 2 min and centrifugation at 14,500 g for 30 s) and then the new supernatant was added to the former.
- Samples were incubated at 95 °C for 2 min once again.
- TE buffer was added to reach the volume of 100 µL and then 1 volume of chloroform (100 µL) was added to the samples to remove lipids and hydrophobic proteins from DNA.
- Samples were mixed by inversion for 10 min and then centrifuged at 14,500 g for 10 min.

DNA precipitation, recovery and quantification

- Aqueous phase was transferred to new tubes and DNA was precipitated with LiCl 400 mM and 2.5 volumes of absolute Ethanol (final concentration 70%) (Fig. 1B).
- DNA precipitation was carried out at -20 °C for 1 h or at -80 °C for 20 min.

Samples can be stored at -20 °C.

- Samples were centrifuged at 14,500 g for 15 min, the supernatant was removed by a vacuum pump and DNA pellet was washed twice with ethanol 70% (centrifugation at 14,500 g for 15 min is repeated).
- The DNA pellet was dissolved in 20 µL of TE buffer.
- The concentration/quantity and purity of the extracted DNA were assessed by spectrophotometric measurements, using a Beckman Coulter DU730 UV-Vis Spectrophotometer. On average, the yield of DNA extracted from each sample was approximately 3 micrograms, with an absorbance ratio (260 nm /280nm) of 1.45.
- DNA samples were stored at 4 °C until further use.

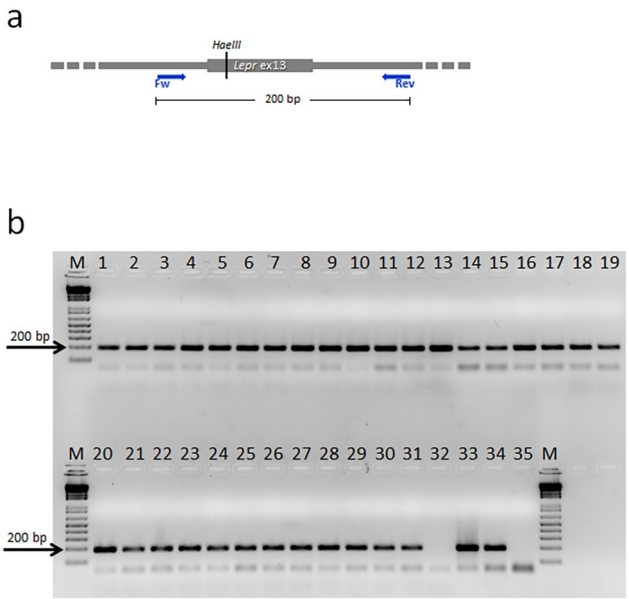

Fig. 2 PCR analysis for *Lepr*$^{db-5J}$ mice. (a) The restriction enzyme sequence recognized by *HaeIII* (b) A schematic representation of the PCR-amplified region spanning exon 13 of mouse leptin receptor gene is shown, the cleavage site for the restriction enzyme *HaeIII* is indicated. Primer positions are represented as blue arrows. (c) The gel picture displays the PCR-amplified DNA of the region of the extracellular domain of leptin receptor that includes the point mutation (G→T transversion); the presence of a band of 200 bp indicates the successful amplification. In upper gel, lane M shows the molecular weight marker 1 Kb plus, lanes indicated as 1 to 35 contain PCR-products obtained by the amplification of DNA extracted from blood's drops; lane 32 and 35 correspond to negative controls (amplified from paper without blood in lane 32, PCR sample without template DNA in lane 35), lanes 33 and 34 represent positive controls (amplified-DNA extracted from mouse tails)

DNA amplification by PCR

PCR-RFLP analysis always needs specific amplification primers pairs and appropriate restriction enzymes to identify the SNP in the PCR product. In this mouse model, *Lepr*$^{db-5j}$, we have designed a primer pair that amplifies a part of the extracellular domain of mouse leptin receptor, including *HaeIII* restriction enzyme site, to identify the presence of a specific SNP (G→T transversion mutation). In particular, the amplified region comprised exon 13. Primer sequences were: Forward 5' -CGAGAACATGTGTCTGACTCAG – 3', Reverse 5' – GGTAATGACAGGGCTATGAACA – 3'.

The PCR reaction was set up in a final volume of 30 µL for each DNA sample and, to amplify more than one sample, a master reaction mixture was prepared in a 1.5 mL tube, then the mixture was aliquoted in 0.2 mL micro-amplification tubes, and each template DNA was added individually to PCR tubes. The total volume of the master mixture was calculated considering an extra 10% to account for loss of liquid during pipetting steps. The final concentration of the PCR mixture for the amplification was 1× PCR reaction buffer, 200 nM dNTP, 250 nM of each primer (F/R), and 0.02 units/µL of Taq polymerase. Four microliters of DNA template were added to a reaction volume of 26 µL.

PCR-amplification of template DNA is performed using an automated thermal cycler (SimpliAmp, Applied Biosystems) with cycle conditions as follows: 95 °C 5 min; 35×(95 °C 45 s, 60 °C 60 s, 72 °C 60 s); 72 °C 10 min. The program was designed according to the manufacturer's instructions of Taq polymerase and primers used in PCR.

Restriction enzyme digestion of PCR products

To detect the point mutation, the PCR-amplified product was digested adding a restriction enzyme to a reaction tube containing an aliquot of the PCR product. The restriction enzyme we used is *Hae* III; this enzyme recognizes a specific palindromic sequence of 4 nucleotides and performs a symmetrical cut on DNA, producing flat ends and allowing SNP-genotyping. The palindromic sequence recognized is shown in Fig. 2 (panel a). The digestion was carried out in a 25 µL reaction volume containing 1x enzyme buffer, 0.2 units/µL restriction enzyme and twenty microliters of PCR product. Reaction mixture was incubated on thermostatic bath at optimal temperature (in this case 37 °C) for appropriate time (1 h and 30 min).

DNA sequencing

The authenticity of isolated DNA was checked by sequence analysis of PCR amplified products. Each sample was sequenced with both forward and reverse primers. The editing and alignment of DNA sequences were performed using using MegAlign software (a module of the Lasergene Software Suite for sequence analysis by DNASTAR).

Results

PCR-RFLP analysis of mouse DNA from *Lepr*$^{db-5j}$ mice.

We analysed 31 samples of *Lepr*$^{db-5j}$ mice by PCR and subsequent enzymatic digestion. The PCR-amplified region covers exon 13 of mouse leptin receptor gene (Fig. 2b), in the extracellular domain of leptin receptor and it includes the point mutation (G→T transversion). After the PCR reaction, samples were subjected to electrophoresis analysis (Fig. 2c). The results were visualized using UV-transilluminator (GelDoc system, Biorad) comparing the PCR products with DNA size marker. In addition to the samples, positive and negative controls were loaded. Positive controls (samples named 33 and 34) are represented by the amplification of DNA extracted from mice tails through classical organic extraction protocol (25 ng of DNA used for each PCR), while as negative controls we amplified the sample eluted from paper without blood (lane indicated as 32) and a sample without template DNA in lane indicated as 35. The presence of an intense band of 200 bp for all the samples and the two positive controls indicates the successful amplification. The same genotyping procedure was performed multiple times, as shown in Fig. 1 S, demonstrating the reproducibility of our method.

Furthermore, to analyze the correctness of the procedure and verify the repeatability and reproducibility data for the study we applied the MSA (Measurement System Analysis) evaluation method. According to the results of the measurement system analysis (MSA), the ratio between the Total Gage R&R and the tolerance is less to 10% (%Tolerance = 8.2%) indicating that the measurement system is acceptable.

In alleles carrying the G→T mutation at position 640 of the leptin receptor gene, a *HaeIII* restriction enzyme site is lost, and this leads to a replacement of glycine by valine in the protein sequence (Fig. 3a). To determine the genotype for each mouse specimen, the remaining volume of PCR samples (20 µL) was digested with *HaeIII* enzyme (5 enzymatic units for each sample) for 90 min. After the digestion was completed, samples were loaded onto 2% (wt/vol) agarose gels (stained with EtBr 0.4 µg/mL). The results were visualized comparing the digestion products with DNA size marker (Fig. 3b and c). In wild type mice, the *HaeIII* site is preserved in both alleles, so the amplified band of 200 bp is cut in two bands of 150 bp and 50 bp. In heterozygous mice, one allele is cut by the restriction enzyme while the other allele contains the point mutation causing the loss of the restriction site (arising in three bands of 200 bp, 150 bp and 50 bp respectively). In homozygous mutants, the restriction enzyme recognition site is lost and the digestion cannot occur; in these samples only the band of 200 bp (undigested PCR product) is detectable. Genotyping data are summarized in Tables 1 and 2.

To further demonstrate the validity of our method, we performed Sanger DNA sequencing on the PCR products verifying the accuracy of the genotypes. By this approach,

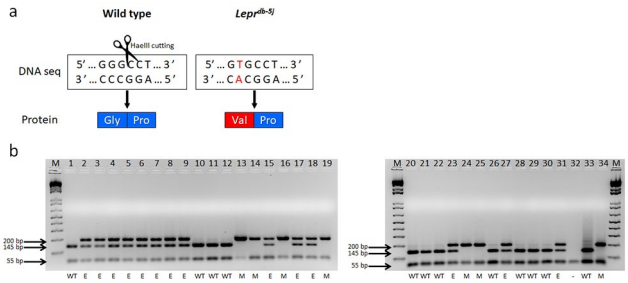

Fig. 3 NOD-*Lepr*$^{db-5J}$ mice genotyping by PCR-RFLP. (a) Single nucleotide change (a G to T transversion in position 640) causes a glycine to valine substitution (G640V). This substitution is discriminated using a mismatch PCR-RFLP method because this SNP deletes the restriction site of *HaeIII* enzyme. (b,c) Representative results from gel electrophoresis analysis of 31 DNA samples after digestion of the PCR products by *HaeIII* enzyme. Lanes indicated with M display molecular weight marker 1 Kb plus; lanes indicated as WT are wild-type mice in which the enzymatic cut produces two bands of 145 bp and 55 bp respectively. In NOD-*Lepr*$^{db-5J}$ heterozygous mice (lanes indicated as E) *HaeIII* digestion produces three bands of 200 bp (allele with SNP), 145 bp and 55 bp (wild type allele) respectively; in NOD-*Lepr*$^{db-5J}$ mutant mice the lack of the restriction enzyme site means that the only visible band is the 200 bp one (lanes indicated as M). Lane 32 in (c) represents a negative control and lanes 33–34 are positive controls: genomic DNA (from mouse tails) extracted from a wild type mouse (lane 33) or a mutant mouse (lane 34), amplified and subjected to *HaeIII* digestion

Table 1 Genotyping results, band lengths of RFLP and number of cases

Genotype	band lenghts	n. of cases
Wild Type	150 bp, 50 bp	12
Heterozygous	200 bp, 150 bp, 50 bp	14
Homozygous Mutant	200 bp	7

Table 2 Genotyping results after digestion analysis

Sample no.	Genotype
1, 10, 11, 12, 20, 21, 22, 26, 28, 29, 30, 33	Wild type
2, 3, 4, 5, 6, 7, 8, 9, 15, 17, 18, 23, 27, 31	Heterozygous
13, 14, 16, 19, 24, 25, 34	Homozygous mutant
32	Negative control

we found a complete agreement of our digest method with the DNA sequencing results when representative mice samples were tested. As showed in figure S2, we obtained distinct peaks of high quality, allowing us to distinguish between wild type, heterozygous and mutant mice.

Discussion

This study aimed at developing a sensitive, rapid and cost-effective method for the extraction of DNA from mouse whole blood on filter paper. Compared with conventional plasma sampling, DBS requires much smaller blood volumes (50 vs. 500 µL). The method described here aims to improve the TE buffer DNA extraction method, since in addition to drop blood incubation with TE buffer, there is a second incubation with a NaOH/SDS solution which allows to extract greater quantities of DNA from DBS. Sodium Hydroxide is a DNA chemical denaturant and allows to remove DNA molecules stuck on the paper. In addition, extraction of samples with chloroform and their precipitation with ethanol allow to obtain DNA that can be used for genotyping. Previous studies developed similar extraction methods for detection and genotyping of parasites [13–15]. For Panda BB et al. the mean DNA concentration was 56.8 using the Chelex-100 method and 27.7 ng/µl from the TE method (14), while for Sharma S. et al. DNA concentration varies from 80 to 189 ng/µL depending on the eluting solution and the storage temperature and time (15), but the advantage of our method is the ability to obtain significant quantities of DNA (mean DNA yields were 2–3 µg, with a mean concentration of 150 ng/µL), a result usually obtained only with commercial kits [16–19]. Moreover, it was possible to obtain a sufficient quantity of DNA for the amplification and genotyping even from a quarter of the blood drop, corresponding to about 12–13 µL. The only limitation of the procedure described here is that extracted DNA is not pure enough to allow safe long storage.

Advantages and disadvantages of the protocol are summarized in Table 3, including a comparison with a commercial kit.

Table 3 Comparison between commercial kits and our method with reference to different parameters

	"PureLink Genomic DNA Mini Kit" from Invitrogen	This method
DNA yield	++	+++
Purity	+	+
Extraction Time	+++ (≈ 90 min)	++ (≈ 120 min)
Number of steps involved	18 steps	17 steps
Cost	+	+++
Ease of doing	++	+++
Animal welfare	+	+++
Reproducibility	+++	+++
DNA stability	+	+

Conclusions

In summary, the protocol described here is rapid and simple and it confers a reduced risk for cross-contamination due to minimum manipulation of samples during extraction; moreover, it requires relatively limited equipment or funds resulting very inexpensive. All these aspects contribute to make it very advantageous in genotyping studies in which the model organisms (such as mice) have limited amount of blood, moreover it involves the use of a less invasive practice resulting less stressful and painful for the animals.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07649-x.

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Authors' contributions A Porcellini designed the experiments. AR, GZ, RG, CF and CZ performed the experiments. GZ, A Pezone and CZ prepared figures. AR, GZ, SM, PdC and GM were involved in the writing of the manuscript. All authors were involved in the review and editing of the manuscript.

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Data Availability All relevant data generated during this study are included in this published article.

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Ethics approval All of the experiments were performed according to the international guidelines for animal research and approved by the Animal Care Committee of "Federico II" University of Naples, Italy and Ministry of Health, Italy.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Chacon-Cortes D, Haupt LM, Lea RA, Griffiths LR (2012) Comparison of genomic DNA extraction techniques from whole blood samples: a time, cost and quality evaluation study. Mol Biol Rep 39(5):5961–5966
2. Mayjonade B, Gouzy J, Donnadieu C, Pouilly N, Marande W, Callot C, Langlade N, Muños S (2016) Extraction of high-molecular-weight genomic DNA for long-read sequencing of single molecules. Biotechniques 61(4):203–205
3. Wang TY, Wang L, Zhang JH, Dong WH (2011) A simplified universal genomic DNA extraction protocol suitable for PCR. Genet Mol Res 10(1):519–525
4. Psifidi A, Dovas CI, Banos G (2010) A comparison of six methods for genomic DNA extraction suitable for PCR-based genotyping applications using ovine milk samples. Mol Cell Probes 24(2):93–98
5. Bezerra CS, Portilho MM, Frota CC, Villar LM (2021 Mar) Comparison of four extraction methods for the detection of hepatitis B virus DNA in dried blood spot samples. Microbiologyopen 10(2):e1161
6. Parasuraman S, Raveendran R, Kesavan R (2010) Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 1(2):87–93
7. Ota M, Fukushima H, Kulski JK, Inoko H (2007) Single nucleotide polymorphism detection by polymerase chain reaction-restriction fragment length polymorphism. Nat Protoc 2(11):2857–2864
8. Saifullah TT (2018) Genotyping of single nucleotide polymorphisms using the SNPRFLP method. Biosci Trends 12(3):240–246
9. Sobin K, Ashish M (2007) SNP Genotyping: Technologies and Biomedical Applications. Annu Rev Biomed Eng 9:289–320
10. Kwok PY, Chen X (2003) Detection of single nucleotide polymorphisms. Curr Issues Mol Biol 5(2):43–60
11. Lee CH, Reifsnyder PC, Naggert JK, Wasserfall C, Atkinson MA, Chen J, Leiter EH (2005) Novel leptin receptor mutation in NOD/LtJ mice suppresses type 1 diabetes progression: I. Pathophysiological analysis. Diabetes 54(9):2525–2532
12. Matarese G, Sanna V, Lechler RI, Sarvetnick N, Fontana S, Zappacosta S, La Cava A (2002) Leptin accelerates autoimmune diabetes in female NOD mice. Diabetes 51(5):1356–1361
13. Bereczky S, Mårtensson A, Gil JP, Färnert A (2005) Short report: Rapid DNA extraction from archive blood spots on filter paper for genotyping of Plasmodium falciparum. Am J Trop Med Hyg 72(3):249–251
14. Panda BB, Pradhan N, Hazra RK (2018) Comparative analysis of three methods from dried blood spots for expeditious DNA extraction from mosquitoes; suitable for PCR based techniques. Mol Biol Rep 46(1):151–160
15. Sharma S, Mann R, Kumar S, Mishra N, Srivastava B, Valecha N, Anvikar AR (2019 Jan-Mar) A Simple and Cost-Effective Freeze-Thaw Based Method for Plasmodium DNA Extraction from Dried Blood Spot. Iran J Parasitol 14(1):29–40
16. Cler L, Bu D, Lewis C, Euhus D (2006) A comparison of five methods for extracting DNA from paucicellular clinical samples. Mol Cell Probes 20(3–4):191–196
17. Singh UA, Kumari M, Iyengar S (2018) Method for improving the quality of genomic DNA obtained from minute quantities of tissue and blood samples using Chelex 100 resin. Biol Proced Online 20:12
18. Hendrix MM, Cuthbert CD, Cordovado SK (2020) Assessing the Performance of Dried-Blood-Spot DNA Extraction Methods in Next Generation Sequencing. Int J Neonatal Screen 6(2):36
19. Kumar A, Mhatre S, Godbole S, Jha P, Dikshit R (2019) Optimization of extraction of genomic DNA from archived dried blood spot (DBS): potential application in epidemiological research & bio banking. Gates Open Res 2:57