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RESEARCH NOTE

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A universal, rapid, and inexpensive method for genomic DNA isolation from the whole blood of mammals and birds

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Abstract

There is no ‘one’ procedure for extracting DNA from the whole blood of both mammals and birds, since each species has a unique property that require different methods to release its own DNA. Therefore, to obtain genomic DNA, a universal, rapid, and noncostly method was developed. A very simple biological basis is followed in this procedure, in which, when the blood is placed in water, it rapidly enters the RBCs by osmosis and causes cells to burst by hemolysis. The validity of extracting genomic DNA was confirmed by several molecular biological experiments. It was found that this method provides an efficient and versatile alternative for extracting bulk amounts of highly-qualified DNA from the blood of a wide range of species. This is the first manuscript that describes use of distilled water as the only eliminator of RBCs among all other known DNA extraction techniques.

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Introduction

There are many available nonorganic low cost protocols that avoid the use of hazardous organic solvents to extract nucleic acids from blood samples (Chacon-Cortes and Griffiths 2014). But, some of these methods require large amounts of blood samples and they are not convenient for low volume DNA extraction procedures (Lahiri and Nurnberger 1991). While other methods are relatively expensive (Miller *et al.* 1988; Grimberg *et al.* 1989), and require specific reagents and their preparation is a relatively time-consuming (Bailes *et al.* 2007). To overcome these problems, some researchers have used laundry detergents to extract genomic DNA (Nasiri *et al.* 2005), but it may be difficult to standardize. However, the validity of all these previously mentioned methods for DNA extraction from several species of both mammals and birds was not reported. Thus, it is so mandatory to find a multispecies and simple method for isolation of DNA from a variety of sources that should reduce costs, efforts, time and reagents. Besides, it may be important to minimize specialized equipment to perform such tasks. Accordingly, it is basically aimed to develop an easy extraction genomic DNA procedure that suits several desired molecular biological experiments, such as high-throughput polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), single stranded conformation polymorphism (SSCP), or sequencing in the variable species in a situation where the overall costs must be minimized. Moreover, it is satisfactory for many researchers to prepare reagents for ‘all in one’ / ready to use tool to extract genomic DNA (gDNA) from a very wide spectrum of blood samples. To meet these criteria, a universal and versatile DNA extraction procedure should be developed with minimal chemicals and equipment. On the other hand, the interesting natural relation between RBCs and water was exploited in this aspect, since RBCs are rapidly disrupted once they are placed in distilled water (DW) (Lemery 1998). Therefore, the objective of this work was to employ the naturally encrypted power of water in the rapid disruption of RBCs to develop a wide-range, easy to use, and reliable method for the extraction of high quality and large quantity of DNA from blood of both mammals and birds with a little effort, expense, and steps.

Materials and methods

Blood sampling

Blood samples were isolated from randomly chosen mammals and hens from Babylon governorate, Iraq. In 135 human volunteers, 2.5 mL of blood were collected. The same amount of blood was collected from the jugular veins of 60 cows,

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178 sheep, and six goats. While, only 50 μL of the blood was isolated from 81 local hens by puncturing the main vein in the inner wing region. In all cases, once blood isolation is performed, the whole blood was placed in anticoagulation tubes.

DNA isolation

Only 2.5 mL of blood of humans, cows, sheep, and goats, or 50 μL of blood of hens were placed into a 15 mL centrifuge tube. Then, 10 mL of DW was added and mixed well by inverting several times and centrifuged for 4 min at 3461 g in a clinical centrifuge (EBA 20, Hettich, Germany). The supernatant was discarded, and this step was repeated. The supernatant was poured off and the pellet was resuspended in 1 mL of TNES lysis buffer (10 mM Tris-Cl pH 7.7, 0.4 M NaCl, 2 mM EDTA, 0.5% SDS), and then, when the suspension was not visually homogenized, the whole suspension was mixed thoroughly by pipetting back and forth for several times. Once the suspension is homogenized it was incubated for 5 min at 55°C. Only 0.30 mL of 6 M NaCl was added and mixed well. The suspension was centrifuged at 17135 g for 5 min in a microcentrifuge (Prism R, Labnet, USA). The supernatant containing DNA was saved and the precipitated protein pellet at the bottom of the tube was discarded. To the supernatant, 2 V of 100% ethanol at room temperature were added and the tube was inverted several times until the DNA precipitated. The precipitated DNA strands were removed by a wide orifice pipette and placed in a microcentrifuge tube containing 1 mL of ice-cold 70% ethanol. Microcentrifugation was performed for 5 min at 17135 g at room temperature. After discarding ethanol, DNA was resuspended in 0.5 mL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at 65°C for 15 min. To ensure further dilution, this suspension was pipetted back and forth for sometimes.

Evaluation of DNA extraction

After DNA extraction, the concentration and purity of DNA were measured by a nanodrop (BioDrop μLITE, Biodrop, UK). Then, the DNA degradation probability was checked by a standard 0.8% (w/v) agarose gel electrophoresis that was prestained with a higher concentration of ethidium bromide (0.7 μg/mL) in TAE (40 mM Tris acetate; 2 mM EDTA, pH 8.3) buffer (figure 1a).

Digestion with restriction enzymes

The gDNA digestion with restriction enzymes was performed to confirm the absence of any inhibitor(s) for restriction endonucleases that might be available in the extracted DNA. Each genomic DNA that prepared from each different organism was digested with several restriction enzymes (*Hinf*I, *Hpa*II, *Rsa*I, *Hae*II, and *Hha*I) according to the manufacturer's instruction (New England BioLabs, Hitchin, UK). After digestion, DNA samples were resolved in 0.8% agarose gel electrophoresis and stained with ethidium bromide (figure 1b).

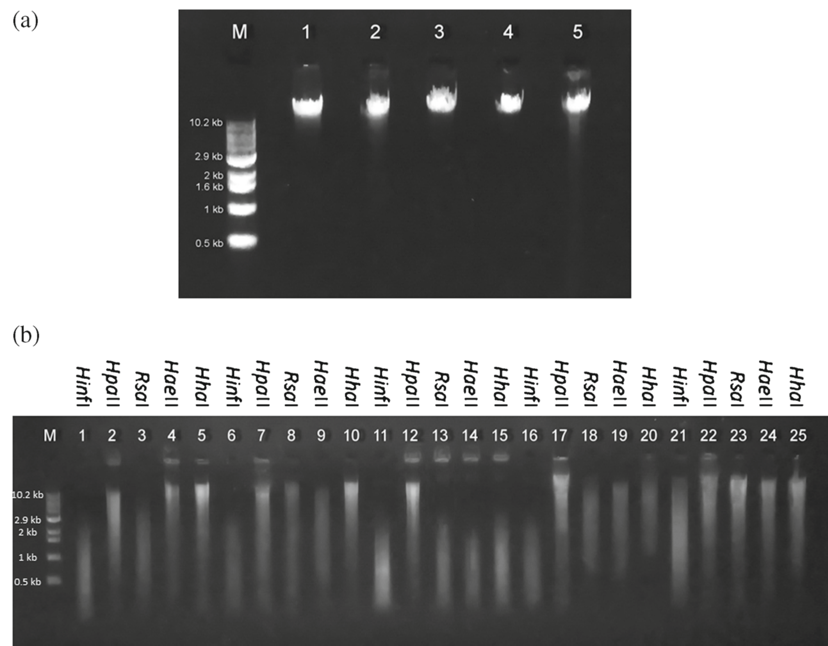

Figure 1. Electrophoresis evaluation of five different samples sources of gDNA that extracted by the universal method. (a) Agarose gel electrophoresis of 0.5 μg of uncleaved blood gDNA. Lane M, 1-kb ladder; lanes 1–5, example of gDNA isolated genomic DNA from human, cows, sheep, goats, and hens, respectively. (b) Agarose gel electrophoresis of restriction enzymes cleaved blood gDNA. Lane M, 1-kb ladder. Lanes 1–5, digested one human DNA sample. Lanes 6–10, digested one cow's DNA sample. Lanes 11–15, digested one sheep DNA sample. Lanes 16–20, digested one goat DNA sample. Lanes 21–25, digested one hen's DNA sample. Five different restriction enzymes (*Hinf*I, *Hpa*II, *Rsa*I, *Hae*II, and *Hha*I) were used to digest each five lanes sequentially.

Table 1. List of the designed primers that are tested in the universal DNA extraction method.

GenBank accession no.	Sequence (5′–3′)	Annealing temp. (°C)	Product length (bp)	Amplified segment	Organism
NG_016441.1	F: GTAGCTCCTTGCTTGCATCC R: GCATGGTGAAGAACATGGTG	58.1	255	MC4R	*Homo sapiens*
D13897.1	F: ACGCACTGGCTGGGTATAAG R: CTTGTGAAGCAGACGAGCAG	54.9	230	YY	
AJ512638.1	F: GGATGCGGGTGGTAACGGAGCAC R: CGGGATGGCCACGGTTCTACCTC	61.0	293	LEP, exon 2	*Bos taurus*
AJ580801.1	F: GCCCTCTTCTTTTGGAGCCT R: GCTGTCTCCTGCTCTCATCC-	62.0	222	LEPR, exon 20	
AY455984.1	F: CCCAGCTCAGCTCAATTCCA R: CATGTGACTTCGGCCTGTCT	64.1	196	Ghrelin, exon 3	*Ovis aries* and *Capra aegagrus*
	F: GCCAAACTGGATGGCAACAG R: AACAGACAGGTGGTTGGTCC	62.0	262	Ghrelin, exon 4	
AY303688.1	F: CACAGCACTAGATGGCCTGA R: AAGCCTACACGTCAGCCTGT	59.5	174	Ghrelin, exon 5	*Gallus gallus*

PCR

The gDNA amplification was performed to make sure the absence of any inhibitor(s) for *Taq* DNA polymerase. In each organism, several primers that cover several genetic loci were designed using the NCBI primer BLAST program (https://www.ncbi.nlm.nih.gov/tools/primer-blast), such as 255 bp of mutated melanocortin receptor 4 (*MC4A*) and 230 bp of YY peptide (for human), 293 bp of exon 2 / leptin (*LEP*), and 222 bp of exon 20 / leptin receptor (*LEPR*) (for cattle), 196 bp of exon 3 / ghrelin and 262 bp of exon 4 / ghrelin (for both sheep and goats), and 174 bp of exon 5 / ghrelin (for hens) as described in table 1. PCR reaction was performed using AccuPower PCR premix (Bioneer, Daejeon, South Korea). Each 20 μL of PCR premix contained 1 U of *Top* DNA polymerase, 250 μM of dNTPs, 10 mm of Tris-HCl (pH 9.0), 30 mm of KCl, and 1.5 mm of $MgCl_2$. The reaction mixture was completed with 10 pmol of each primer and 50 ng of genomic DNA. The optimum annealing temperatures were determined empirically using gradient PCR (Mastercycler-nexus, Eppendorf, Hamburg, Germany). The amplification began by initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing (empirical) for 30 s, and elongation at 72°C for 30 s, and was concluded with a final extension at 72°C for 5 min. After performing PCR thermocycling, PCR products were tested by 1.5% agarose gel electrophoresis. In each case, the optimum amplification of PCR products was chosen (figure 2).

SSCP

The post-PCR SSCP experiments were performed on 60 samples of cows' gDNA to provide further molecular indication for the suitability of the extracted gDNA to the post-PCR analysis. SSCP was performed according to the standard protocol of Orita *et al.* (1989). Briefly, 5 μL of each amplification product was mixed with 15 μL of SSCP denaturing loading buffer (95% formamide, 20 mM EDTA pH 8, 0.05% xylene cyanol and 0.05% bromophenol blue). The samples were heat-denatured at 95°C for 10 min and chilled on ice for at least 5 min. Then, the PCR amplicons were separated in a mini vertical gel electrophoresis format, gel size (W×L) cm: 10×10, and gel thickness: 1 mm (OmniPAGE, Cleaver Scientific, Warwickshire, UK). Denatured PCR products were loaded in 8% (37.5 acrylamide / 1 bis) polyacrylamide gels, containing 7% glycerol, and 1×TBE buffer. The gels were run under 200 V / 100 mA at room temperature until the tracking dye reached the end of the gel (100 min). Gels were stained by PAGE GelRed dye (Cat # 41014, Biotium, Hayward, USA).

Sequencing

Each different SSCP samples' set for the amplified *LEP* gene 293-bp PCR fragment was sequenced from both ends (Macrogen, Seoul, Korea). The cattle reference sequences (AJ512638.1), as long as with its intron 1 / exon 2 / intron 2 positions were retrieved from the NCBI website (http://www.ncbi.nlm.nih.gov). The sequencing results of the PCR products of different SSCP patterns were edited, aligned and analysed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment editor software ver. 7.1. (DNASTAR, Madison, USA).

Results and discussion

This method has been applied successfully in variable numbers of peripheral blood samples that were manually collected from 135 human volunteers, 60 cows, 178 sheep, six goats and 81 hens. Despite the significant biological differences that were usually observed among variable blood samples, the average quantity of the isolated genomic DNA in this method as its measured by a nanodrop is estimated about 60, 160, 140, 120, 230 μg/mL for human, cow, sheep, goat,

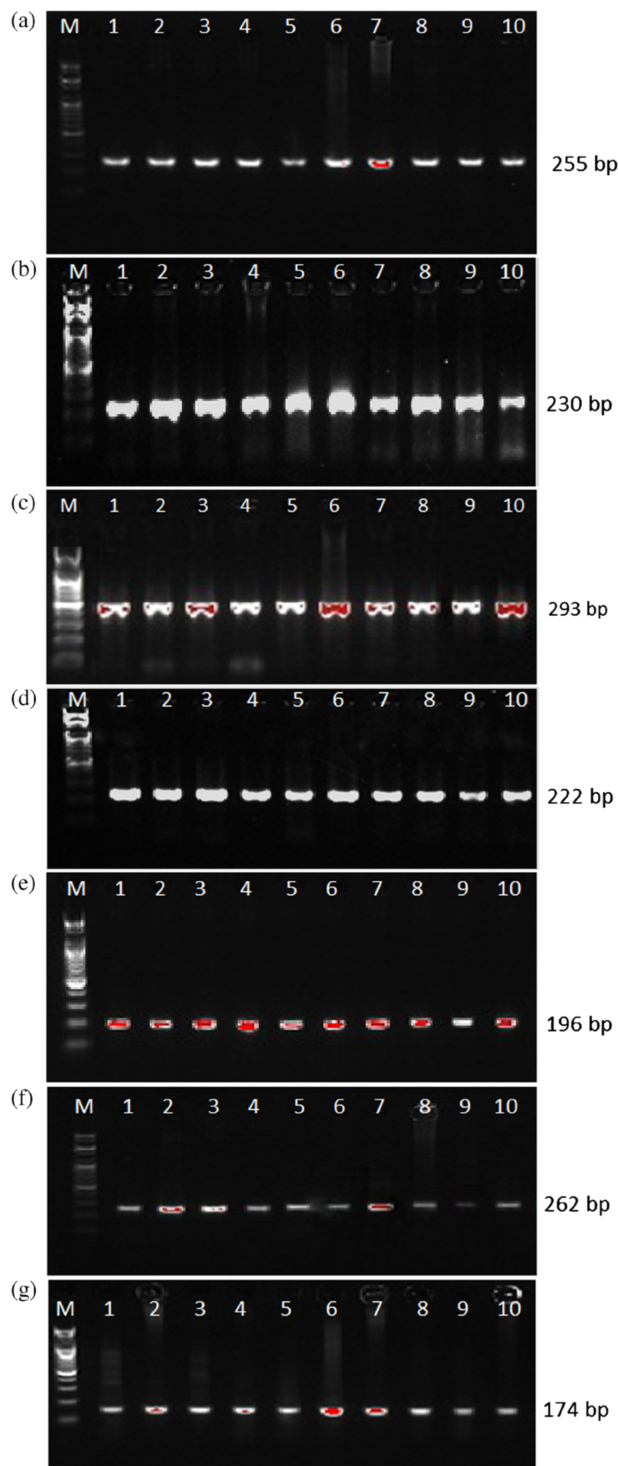

Figure 2. PCR for 5 μL of some selected amplicons of the genomic DNA samples were prepared from several mammals and hens using the universal DNA extraction method. (a) MC4R (255 bp) amplicons amplified from humans' gDNA. (b) YY segment (230 bp) amplicons amplified from humans' gDNA. (c) *LEP*, exon 2 (293 bp) amplicons amplified from cows' gDNA. (d) *LEPR*, exon 20 (222 bp) amplicons amplified from cows' gDNA. (e) ghrelin, exon 3 (196 bp) amplicons amplified from sheep's gDNA. (f) ghrelin, exon 4 (262 bp) amplicons amplified from goats' gDNA. (g) ghrelin, exon 5 (174 bp) amplicons amplified from hens' gDNA. M, ladder marker. Lanes 1–10, amplicons.

and hen, respectively, and the quality of this DNA as measured by a nanodrop ranged between 1.7 and 1.8. However, very few samples qualities ranged between 1.9 and 1.6. Despite a variety of the species from which gDNA was isolated by this method, no noticeable differences were observed regarding the quality of isolated gDNA. The integrity of the obtained genomic DNA and the absence of RNA and protein contamination were further examined by agarose gel electrophoresis (figure 1a). The same results were obtained even after prolonged incubation with ethidium bromide. But, as long as the isolation of high quantities of pure, intact and noncontaminated gDNA is a prerequisite for successful and reliable subsequent genotyping analysis (Psifidi *et al.* 2015), several molecular biological experiments were performed to confirm the validity of this universal method. The results obtained in PCR, restriction enzymes digestion, SSCP, and sequencing experiments demonstrated the absence of any significant inhibitor(s) for the enzymes used in molecular biology reactions and indicated that the high quantity isolated DNA was of high quality as well. This method has proven efficient in the downstream application of molecular biology experiments, such as restriction enzyme digestion (figure 1b). Add to that, through the successful amplification of several sets of primers (figure 2), it was demonstrated that this method is highly suited PCR experiments. Besides, the feasibility of these amplicons for postPCR analysis, such as SSCP (figure 3a) and sequencing (figure 3b) was confirmed. The sequencing pattern showed clear chromatograms and several SNPs that contributed in the genotyping of resolved SSCP haplotypes were identified using the multiple sequence alignment method. Several SNPs were discovered between the *LEP* reference sequence and the corresponding SSCP three genotypes that positioned only in the introns (both introns 1 and 2) regions, while the exonic region did not exert any SNP. However, sequencing results have given further confirmation about the competency of this method to be applied for post-PCR diagnostics techniques.

The application of the universal method for DNA extraction does not restrict to routine mediocre budget labs, but it is proved to be efficient enough to be applied for several highly demanded molecular biology facilities. It was found that the versatility of this universal method has extended from fresh samples into frozen counterparts.

On the other hand, the universal method has proven its feasibility to be utilized instead of many commercial DNA extraction kits. Although the commercial kits are available at relatively low costs (QIAamp DNA Blood Mini Kit, Qiagen Inc. Germany, Geneaid™ DNA Isolation Kit, Geneaid Biotech., New Taipei City, 22180 Taiwan, Wizard® Genomic DNA Purification Kit, Promega, Madison, USA), most of the DNA extraction kits require repeated centrifugation steps, followed by removal of supernatants and additional mechanical treatment (Tan and Yiap 2009). Further, they can isolate DNA that suffices only few PCR reactions (Bailes *et al.* 2007). Thus, it was shown that the gDNA that isolated by this universal method has an adequate quantity that is large

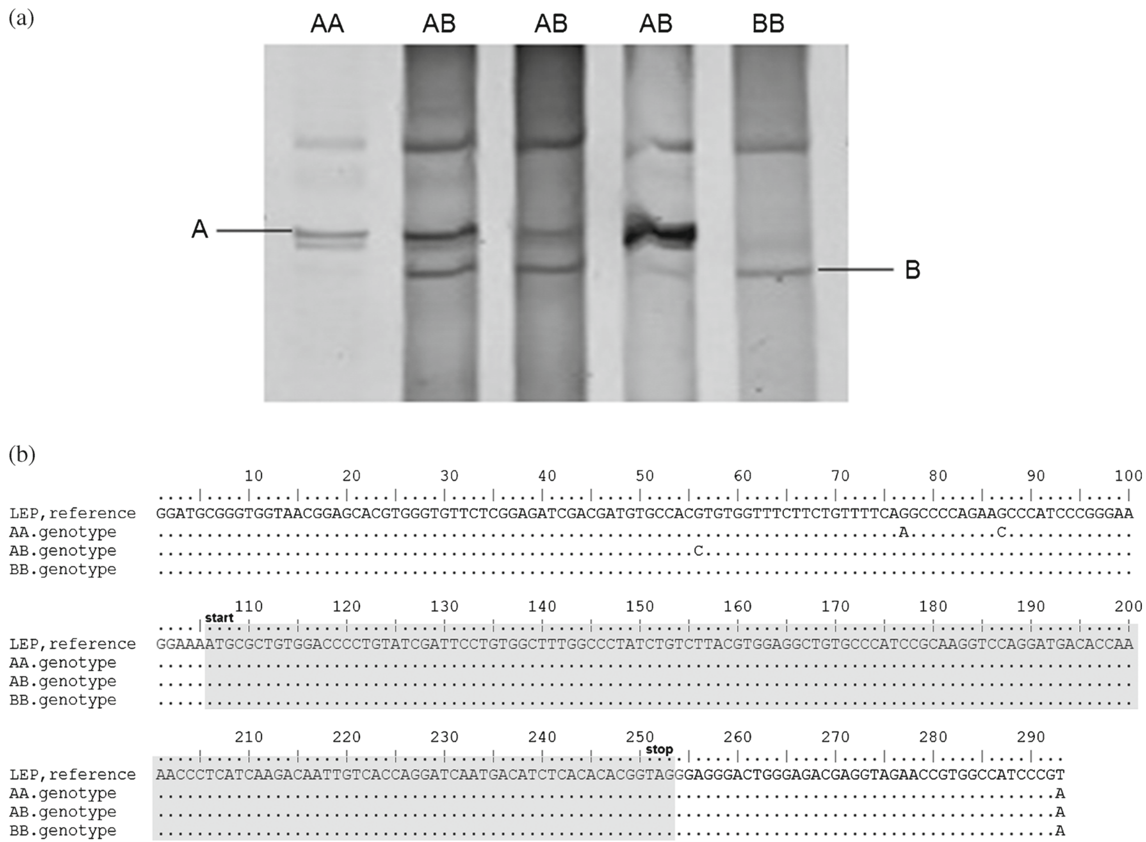

Figure 3. SSCP-sequencing of one example species of gDNA samples that isolated by the universal method. (a) SSCP reaction: three genotypes and two alleles that were observed in *Bos taurus* leptin (LEP), exon 2 gene fragment (293 bp) after its being extracted using the universal DNA extraction method. The concentration of SSCP gel is 8%, and the voltage applied is 16.6 V/cm. SSCP gel stained by PAGE GelRed dye. (b) Sequences alignment results for *Bos taurus LEP* gene three SSCP genotypes with their reference sequence (GenBank accession no. AJ512638.1) using DNA Star EditSeq software. Only intronic SNPs are observed. The word 'START' refers to the start codon of exon 2, while the word 'STOP' refers to the termination codon of the same exon.

enough to perform about 10-folds, a greater number of PCR reactions since it has a greater extracted volume of high quantity DNA. However, the superiority of the universal method is not restricted over the commercial kit, rather its reproducibility was proven in comparison with the main known manual procedures for DNA extraction that require greater labour input through having more steps and chemicals, particularly in removing RBCs (Kumar *et al.* 2006). Actually, this universal method can be standardized and easily compared with the other nonorganic methods that also reduce the number of steps required for DNA isolation. It was noticed that this method requires a half volume of the starting materials of the mammalian whole blood compared with other inorganic methods (Nasiri *et al.* 2005). Moreover, the later method includes the use of laundry powder which varies according to various brands with different chemicals and enzymatic compositions and this may show different results (Kumar *et al*. 2006). The developed universal method of this study has many other advantages, such as the reduced time involved in the nonorganic extraction as less than half an hour is required to isolate DNA until it has been dissolved in TE buffer. While the other common inorganic methods require much more time and steps (Lahiri and Nurnberger 1991; Lahiri *et al.* 1992). Moreover, this method does not require an extended incubation with the relatively high-cost proteinase K as some nonorganic extraction procedures require (Grimberg *et al.* 1989). This method is also characterized by its considerable low costs because it uses fewer chemicals than many published methods with which it was compared (Bartlett and White 2003), although the actual cost–benefit of this would be difficult to calculate. However, very few chemicals were used in this method, since in addition to ethanol, only four chemicals were used in this method (tris-HCl, EDTA, NaCl and SDS), which are available in every routine laboratory around the world at a low cost. While, in the case of DNA extraction from hens, this method provides a nonenzymatic tool that does not rely on the relatively costly enzymes (i.e. proteinase K) in lysing cells as compared with some commercial kits (QuickPick^{TM} gDNA kit, BioNobile Co. 21600 Pargas, Finland). Further, this method does not rely on filter columns in its extraction procedure. This, in turn, simplifies the extraction of hen's DNA since there is a difficulty of passing the hen's blood lysate through the spin column beds, as it is

noticed in some commercially available kits (GF-1 Nucleic Acid Extraction Kits, Vivantis Tech. 47600 Subang-Jaya, Malaysia). This difficulty is due to the high viscosity of hen's blood that attributed to the presence of nucleated RBCs (Gaehtgens *et al.* 1981). Actually, this universal method has a wide versatility in variable genomic DNA based experiments around the world. Pairwise, in addition to the application of this method to human samples, it can be applied to other mammals, such as cows, sheep, goats, and hens with very high efficiency. As long as this method has shown desired results in all the examined species, it is highly advisable to extend this universal DNA extraction technique to screen further species that have not yet been tested to such simple method to provide a wide utilization of a broader spectrum of this highly dependable procedure.

Since DW is the main RBCs remover in this method with a significant reduction in handover time, it might be concluded that this method is one of the simplest nonorganic tools for DNA extraction from the blood. It was proven that this method is one of the most broadened and guaranteed protocols of the isolation of mammalian and hen's blood in routine laboratories. Accordingly, I suggest using this universal, rapid, simple and low-cost method in the DNA isolation experiments of mammalian and bird's blood.

References

Bailes S. M., Devers J. J., Kirby J. D. and Rhoads D. D. 2007 An inexpensive, simple protocol for DNA isolation from blood for high-throughput genotyping by polymerase chain reaction or restriction endonuclease digestion. *Poult. Sci.* **86**, 102–106.

Bartlett J. M. and White A. 2003 Extraction of DNA from whole blood. In: *Methods in molecular biology* (ed.: J. M. S. Bartlett and D. Stirling) vol. 226, 2nd edition. Humana Press, Totowa, USA.

Chacon-Cortes D. and Griffiths L. R. 2014 Methods for extracting genomic DNA from whole blood samples: current perspectives. *J. Biorepos. Sci. Appl. Med.* **2**, 1–9.

Gaehtgens P., Schmidt F. and Will G. 1981 Comparative rheology of nucleated and non-nucleated red blood cells. *Pflügers Arch.* **3**, 278–282.

Grimberg J., Nawoschik S., Belluscio L., McKee R., Turck A. and Eisenberg A. 1989 A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucleic Acids Res.* **17**, 8390.

Kumar O. S., Sharma M. K. and Singh D. 2006 DNA isolation from goat blood using different brands of household detergents and its downstream application. *Indian J. Exp. Biol.* **44**, 852–854.

Lahiri D. K. and Nurnberger J. I. 1991 A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* **19**, 5444.

Lahiri D. K., Bye S., Nurnberger J. I., Hodes M. E. and Crisp M. 1992 A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested. *J. Biochem. Biophys. Methods* **25**, 193–205.

Lemery L. 1998 Oh, no! it's hemolyzed!' what, why, who, how? *Adv. Med. Lab. Prof.* **15**, 24–25.

Miller S. A., Dykes D. D. and Polesky H. F. 1988 A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **18**, 1215.

Nasiri H., Forouzandeh M., Rasaee M. J. and Rahbarizadeh F. 2005 Modified salting-out method: high-yield, high-quality genomic DNA extraction from whole blood using laundry detergent. *J. Clin. Lab. Anal.* **19**, 229–232.

Orita M., Iwahana H., Kanazawa H., Hayashi K. and Sekiya T. 1989 Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc. Natl. Acad. Sci. USA* **86**, 2766–2770.

Psifidi A., Chrysostomos I., Dovas I., Bramis G., Lazou T., Russel C. L. and Arsenos G. 2015 Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using blood samples. *PLoS One* **10**, e0115960, doi: 10.1371/journal.pone.0115960.

Tan S. C. and Yiap B.C. 2009 DNA, RNA, and protein extraction: the past and the present. *J. Biomed. Biotech.* **2009** e574398.

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