SHORT COMMUNICATION
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TROUBLE SHOOTING THE EXTRACTION OF DNA FROM AVIAN BLOOD SAMPLES WITH A SOLID PHASE EXTRACTION KIT

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ABSTRACT

Solid phase DNA isolation kit was used to isolate genomic DNA (gDNA) from duck blood samples. The manufacturer's protocol was modified by reducing the quantity of blood from 200 µL to 10 µL. Normal saline was used to extend the volume to 200 µL. DNA was isolated with the modified protocol and was used for amplifying a segment of exon 2 of duck growth hormone gene.

Keywords: Trouble shooting, DNA isolation, Silica membrane-based kit

INTRODUCTION

Isolation of genomic DNA (gDNA) from blood is a standardized procedure. The classical phenol-chloroform method (Sambrook *et al*., 1989) is a reliable and reproducible technique. It is often considered as the 'gold standard' of DNA isolation protocols, even though it requires toxic reagents and is time consuming (Dairawan and Shetty, 2020). Different methods used to isolate DNA from biological samples were reviewed by Ali *et al*. (2017).

Whole blood is a commonly used material in several laboratories. Solid phase extraction methods using silica membranes became very popular, since many of the commercially available kits use this process. Such kits employ positively charged silica matrices to achieve separation of DNA. Chaotropic reagents in lysis solution of kits release DNA from cells in clinical specimen. The lysate is placed over a silica matrix and centrifuged at high speed. Debris and protein are filtered out. DNA could be released from silica by using a hypo osmotic solution like nuclease free water or alkaline TRIS EDTA buffer (Padhye *et al*., 1997).

Some solid phase DNA extraction kits do not provide extraction procedure for species with nucleated RBC's like

birds. The short communication discusses trouble shooting of DNA isolation process from duck blood using mammalian DNA isolation kits and the utility of normal saline (NS) in place of phosphate buffered saline (PBS) as diluent for the process.

MATERIALS AND METHODS

Blood (1 ml) was collected from the wing web vein of Kuttanad ducks maintained at Avian Research Station, Thiruvazhamkunnu under Kerala Veterinary and Animal Sciences University in sterile vials containing one ml of one milligram percent EDTA solution.

Isolation of genomic DNA from blood was attempted using the procedure mentioned in the kit (SRL-Biolit Whole blood DNA extraction minikit). The kit contained lysis solution, wash buffer and elution solution. Other items in the kit were tubes fixed with silica membrane and receiving tubes for elutes. Whole blood (200 µL) was added to lysis solution in a micro-centrifuge tube, vortexed briefly and incubated at 65^0 C for 10 minutes in a water bath. Binding buffer was added into the tube and vortexed for 30 seconds. The lysate was loaded atop the silica membrane and was attached to its receptacle. The assembly was centrifuged at 12,500 rpm for two minutes. The column was washed twice with 750 µL of wash buffer by centrifugation at 12,500 rpm for two minutes. Elution buffer (60 µL) was layered over the membrane and incubated at room temperature for 5 minutes. A new receptacle was fixed and gDNA was eluted by centrifugation at 12,500 rpm for two minutes. The modified procedure was exactly same except that 10 µL of blood was mixed with 190 µL of either normal saline (pH:6.36) or phosphate buffered saline (pH:7.4) being used as starting material. Normal saline (NS) was prepared by dissolving 0.9 g of sodium chloride in 100 ml of double distilled water and was used as such. pH of the solutions was measured using Eutech pH meter (Thermoscientific, USA).

Isolated DNA (4 µL) was mixed with 6x gel loading dye (2 µL) and was loaded into 0.8 per cent agarose gel, pre-stained with ethidium bromide. Electrophoresis was performed in a mini submarine gel apparatus (Bio-Rad, USA) attached with a power pack (Bio-Rad, USA) at 60 V for 45 minutes. The images of gels were captured using Geldoc Go imaging system (Bio-Rad, USA). Absolute quantification of nucleic acids from the gel image was done using the software, Image Lab Version 6.1 (Bio-Rad, USA). Statistical analysis of concentrations was performed using Mann-Whitney U test. Suitability of DNA isolated using NS as diluent for downstream applications was tested by using it as template in polymerase chain

reaction (PCR). Amplification of a segment from exon 2 of duck growth hormone gene was attempted using the primers and procedure described by Wu *et al*. (2012). Gradient PCR was performed in a thermal cycler (Bio-Rad T-100, USA). Master mix (Taq mix, SRL-Biolit) was used to set up reactions as per Wu *et al*. (2012). PCR products were subjected to electrophoresis alongside 100 bp ladder (ProxiB, SRL-Biolot) in 1.4 per cent agarose gel.

RESULTS AND DISCUSSION

On addition of lysis buffer to 200 µL of blood as mentioned in the kit protocol, the cells immediately coagulated into a thick, viscous gel, which rendered downstream processing of the sample impossible. Considering the normal white blood count of mammals in the range of 10,000 cells/ microlitre, the kit was designed to extract gDNA from about two million cells. Avian red blood cells (RBC) also contains nucleus with DNA, in contrast to their mammalian counterparts. The reported value for RBC count in ducks was between 2.56 x 10^6 to 2.94 x 10^6 cells per microlitre (Roger and Carlton, 1966). The viscosity of DNA solutions remain high at room temperature in pH neutral conditions and are also influenced by temperature and salt concentration among other conditions (Bravo-Anaya *et al*., 2016). Therefore upon lysis, DNA would have been released from about 512 million cells by using 200 µL duck blood, which was 256 times the capacity of the kit. It is thus reasonable to assume that the increased viscosity observed was due to release of DNA from cells.

Just 10 µL of whole blood from duck would provide about 25.6 million cells and the procedure was modified accordingly as part of the trouble shooting plan. Literature from another kit suggested PBS as a volume extender for species with nucleated RBC's (GeneJET genomic DNA purification mini kit, Thermo Scientific). The utility of NS in place of PBS and its effect on PCR was studied. Either NS or PBS (190 µL) was used to expand the volume to 200 µL. The results from the isolation process are presented in Table 1.

Higher mean yields were obtained with PBS, even though the differences were not statistically significant. The buffering action could be assumed to be beneficial

Table 1: Concentration of isolated genomic DNA

Diluent	pH	Concentration (ng/µl) mean±SE	P
Normal Saline (NS)	6.36	216.80±88.10 (n=6)	0.485
Phosphate buffered saline (PBS)	7.4	258.49±61.53 (n=6)	

Figure 1: Lanes 1 & 2- DNA isolated with NS
Lanes 3 to 5- DNA isolated with PBS

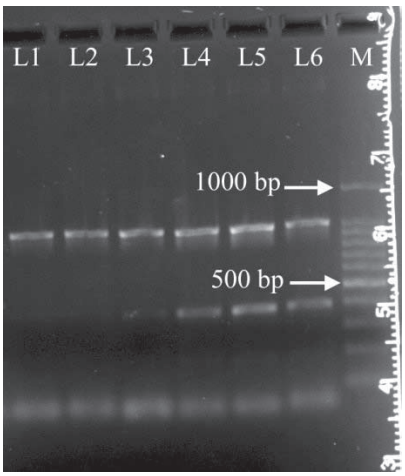

Figure 2 : Results from gradient PCR
Lanes 1-3 : Specific products &primer dimers
Lanes 4-6 : Non specific product near 400 bp
Lane M : 100 bp marker

for higher recovery of nucleic acids from the columns. However this aspect could be ascertained only through studies with larger sample size. It was therefore concluded that both NS and PBS provided comparable DNA concentrations and that NS could be used if the latter is not readily available.

The gel picture of extracted DNA and PCR products are presented in Figure 1 and Figure 2 respectively. The temperature range used for gradient PCR was from 56 ^{o}C to 62 ^{o}C. Specific PCR products (769 bp) were observed in the gel, even though non specific bands also appeared when the annealing temperature was below 58.3 ^{o}C. Results indicated that DNA isolated using NS as diluent could be used for PCR.

CONCLUSION

A mammalian DNA isolation kit was used to isolate gDNA from ducks by using 10 µL of blood diluted with 190 µL of normal saline. The isolated DNA was successfully used for amplification of a part of exon 2 of duck growth hormone gene. It was concluded that normal saline could be used in place of PBS for isolation of DNA from silica membrane based kits, which can be used for PCR.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with respect to the findings of the study.

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