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TECHNICAL NOTE

Extraction of PCR-amplifiable DNA from a variety of biological samples with uniform success rate

Sandeep Kumar Gupta · Ajit Kumar · Syed Ainul Hussain

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Abstract This study describes the comparison of three DNA extraction protocols for successful extraction of PCR amplifiable quality DNA from bones, antlers and feces samples of Sambar deer (*Rusa unicolor*). Three different DNA extraction protocols were compared in this study including Phenol–Chloroform (PC), column based Qiagen kit, and Guanidine hydrochloride (Gu-HCl) based in-house method. The effectiveness of the protocols was compared for higher success rate of PCR amplification from the extracted DNA. This study highlights that silica based indigenous DNA extraction protocol using Gu-HCl chaotropic salts yields better quality DNA with higher PCR amplification success rate.

Keywords DNA extraction protocol · PCR · Non-invasive wildlife samples · PCR inhibitors

Introduction

The extraction of DNA from variety of biological samples is the first and important step in the field of molecular genetics. In the field of molecular genetics, the quantity and quality of extracted DNA and the success rate of PCR amplification with extracted DNA has great importance (Gupta et al. 2011). DNA extraction from intricate biological samples including bone, antlers and fecal matters with higher PCR success rate is a challenging task. The DNA extracted from these samples contains inhibitors which affect the PCR success (Rohland and Hofreiter 2007a, b). The conundrum in selecting the better DNA extraction protocol with higher PCR success rate is one of the frequently arising situations in conservation genetics. The chaotropic salts [Guanidine thiocyanate (Gu-SCN)] and silica binding based DNA extraction technique has been used for the extraction of DNA from hard tissue including bone and teeth (Höss and Pääbo 1993; Rohland and Hofreiter 2007a), and feces (Wehausen et al. 2004). The eluted DNA contains minimum inhibitory effect but these protocols are based on Gu-SCN, which is an expensive and hazardous reagent. This study highlights the use of more efficient, less toxic and low-cost Guanidine hydrochloride (Gu-HCl).

We are conducting research to address the phylogeny of Sambar deer (*Rusa unicolor*) populations across India. As a part of this work we have to standardize the protocol for the extraction of good quality DNA from various biological samples of Sambar deer. The antler, tissue and bone samples of Sambar deer collected by forest department from samples confiscated for forensics investigation were used for optimizing the DNA extraction. The antlers and bone were stored at room temperature and tissues were stored at −20 °C. The feces of Sambar deer were collected in 70 % ethanol and stored at room temperature. For uniformity and comparability of the results from the tested protocols, same samples in equal quantity were used for the extraction of DNA in equal final volume (80 µl). For the experiment, 1.5 g of bone and antler were pulverized and incubated with 0.5 M EDTA for 48 h for decalcification. 0.5 g of the surface layer of feces was taken for each extraction. The three DNA extraction methods used in this study are Phenol–Chloroform (PC) (Sambrook et al. 1989), QIAamp DNA Stool Mini/blood and tissue kit (Qiagen, Germany), and Gu-HCl based silica binding protocol. For Gu-HCl based DNA extraction, 0.5 g of above sample was mixed

S. K. Gupta (✉) · A. Kumar · S. A. Hussain
Wildlife Institute of India, Post Box # 18, Chandrabani, Dehra Dun 248 001, Uttarakhand, India
e-mail: skg@wii.gov.in; skg.bio@gmail.com

with 500 µl of lysis buffer (6 M Gu-HCl) and 20 µl of Proteinase-K (from 20 mg/ml stock) in a 2 ml centrifuge tube and incubated at 56 °C in an hybridization oven with continuous rotation for 24–48 h (till complete lysis). The tubes were centrifuged at 13,000 rpm for 2 min and clear solutions were transferred to a fresh tube. 30 µl of silica suspension (SiO_2 powder in equal volume of distilled water) was added and incubated with constant rotation at room temp for 15 min. The tubes were centrifuged at 13,000 rpm for 2 min and the supernatant were discarded. For washing the DNA–silica pellet, 500 µl of wash buffer (20 mM Tris–HCl, pH 7.8, 1 mM EDTA, 50 mM NaCl, 50 % ethanol) was added and centrifuged at 13,000 rpm for 2 min and the supernatant were discarded. The washing was repeated twice. The silica pellets were dried in heating block at 60 °C for 10 min. 80 µl of TE buffer (10 mM Triss pH 7.8 and 1 mM EDTA) was added in each tube and mixed gently. The tubes were then centrifuged at 13,000 rpm for 5 min to collect the aqueous DNA solution.

The DNA extracted from above three methods was used for amplification of 472 bp long mtDNA cyt *b* gene fragment with universal primers (Verma and Singh 2003). This primer has been used in investigation of various crime cases related to species identification (Gupta et al. 2005, 2012). PCR reactions were carried out in 20 µl reaction volume by using Hot Start Multiplex PCR kit (Qiagen, Germany) with 4 pmol of each primer and 1 µl template DNA. The PCR conditions were: 95 °C for 15 min, followed by 35 cycles each 95 °C for 45 s, 55 °C for 40 s, and 72 °C for 1.5 min. The final extension was at 72 °C for 10 min. The PCR products were electrophoresed on 2 % agarose gel, stained with ethidium bromide (0.5 mg/ml) and visualized under U.V. transilluminator (Fig. 1).

All positive PCR products were treated with *Exonuclease-I* (*Exo-I*) and *Srimp Alkaline Phosphatase* (*SAP*) to clean the unused primers and dNTP's. Cleaned PCR products were used for sequencing with BigDye sequencing kit and 3130 ABI Genetic Analyzer (Applied Biosystem). The sequence resolved was aligned and cleaned by using Sequencher 4.7 (Gene Code Corporation, Ann Arbor, USA). The DNA sequences were used for Blast search (at http://blast.ncbi.nlm.nih.gov/) to confirm the origin of the sample and to check the purity of the DNA extraction and amplification.

Results and conclusion

The three different DNA extraction protocols yielded different PCR outcomes. PCR amplification in bone samples was negligible in PC method, low in Qiagen kit and high with Gu-HCl method (Fig. 1). Poor amplification was observed for DNA extracted from bone, antler, and feces using PC method (Fig. 1). In antlers, PCR amplifications were detected in all the three methods. For feces samples, Qiagen Stool kit and Gu-HCl method show comparable PCR amplification. The PC method was found inconsistent with the fecal samples (Fig. 1). Although 7–8 samples of each group including bone, antler, feces and tissue were used for assessment of the result, the results from only two samples of each group are shown in Fig. 1.

This study evidently indicates that two different Qiagen kits (blood/tissue and stool) were required for obtaining the higher PCR success rate from different sample types. However, Gu-HCl based DNA extraction protocol illustrated uniformly higher PCR success from all types of biological samples. The amplified PCR product yielded good DNA sequence, which showed authentic matching of the source of its origin and confirmed that the same can be applied on a variety of biological samples with uniform success rate. The uniform success rate in PCR and sequencing evidenced that Gu-HCl method is fast, low-cost, and less hazardous. This protocol was also successfully tested on antler and bone samples of Swamp deer (*Rucervus duvaucelii*) and critically endangered Sangai deer (*Rucervus eldii eldii*).

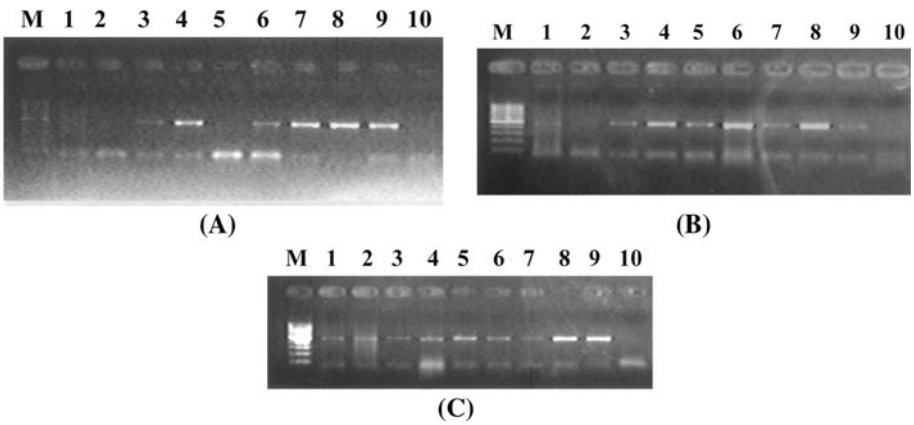

Fig. 1 PCR amplification with the DNA extracted from various sample using PC (**a**), Qiagen kit (**b**), and Gu-HCl method (**c**). *Lane M* 100 bp ladder; *Lane 1–2* Bone; *Lane 3–v4* Antler; *Lane 5–6* Feces; *Lane 7–8* Tissue; *Lane 9* and *10* are positive control and extraction negative control, respectively

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