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Extraction of genomic DNA from formalin fixed tissues of different wild avian species

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ABSTRACT

Museum specimens are generally preserved for public viewing or as academic models, but they can be a good source of genomic DNA, especially in the case of wild animals. In the current study, we evaluated the usefulness of one such protocol described by France & Kocher (1996) with little modifications, in isolation of DNA from museum specimens of different avian species. Formalin fixed tissues were used as a source of DNA. Approximately 100mg of heart muscle or leg muscle were weighed and used for DNA extraction. The Universal primers of 12S rRNA gene of genomic DNA were used for PCR amplification of the isolated DNA. All the samples subjected for DNA isoltion yielded varying amount of DNA by using the current protocol. There was red/ brown discolouration of some DNA pellets as well as smearing in 0.8% gel but that didnot affected the PCR amplification. We were able to amplify the 12s rRNA region in all species, the size of PCR product ranged from 457 to 488.

Key words Formaline fixed tissue, DNA, Wild avians

INTRODUCTION

Obtaining genomic DNA for molecular genetic studies in wild animals is a difficult task. Wild animal populations are generally scattered among large areas where sampling is a major problem, isolation of DNA from blood of wild animals is seldom possible, it can be done only in zoos or small captive areas with necessary facilities, moreover such populations are not large enough to make broader inferences. Therefore adoption of non invasive techniques for DNA typing of animals is necessary. Terrestrial wild animals such as wild carnivores, rhodents etc. are difficult to catch & obtaining DNA from such animals requires extensive planning. Similarly, DNA isolation from cryptic and rare species that are found in deep seas, marine carnivores, wild birds etc. is often problematic due to lack of facilities, time and more so over they are difficult to reach. In such cases museum specimens can act as a good source of DNA for molecular biology studies. Museum specimens are generally preserved for public viewing or as academic models, but they can form an excellent source of genomic DNA, especially in the case of wild animals. Formalin or formaldehyde was first reported in 1859 by Russian chemist Aleksandr Butlerov and was conclusively identified in 1869 by August Wilhelm von Hofmann. Since then it has been widely used in preservation of biological specimens throughout the world. Initial attempts to use formaldehyde-fixed material for molecular studies

were made in the medical field to study genetic diseases [3]. The yields of DNA in the early attempts were generally low, and the conclusion was that the results were largely dependent on fixation time and type of storage. In the current study, we evaluated the usefulness of one such protocol [2] in isolation of DNA from museum specimens of different wild avian species.

MATERIALS AND METHODS

Source of DNA

Formalin fixed tissues were used as a source of DNA. Approximately 100 mg of heart muscle or leg muscle were cut and weighed and used for DNA extraction. All the tissues were fixed in 10 % of buffered formalin. The samples were obtained from college museum at Department of Veterinary Anatomy & Histology, College of Veterinary Science & A.H., Jabalpur, M.P and Center for Wildlife Forensic & Health, MPPCVV, Jabalpur, M.P.

DNA extraction

DNA extraction was carried out by protocol described earlier [2] with some modifications. The method in brief goes as, approx. 100 mg of intact muscle (from heart or forelimb) was first submerged in TE9 buffer (500 mM Tris pH 9.0; 20 mM EDTA; 10 mM NaCl) at 37^{o}C for 24 hours, with single buffer change. Tissues were then minced thoroughly and added to 1 ml of TE9 buffer plus 50 µl of 20% SDS, 0.5% Triton X 100, 15 µl Dithiothreitol (8mg/ml) and 25 µl of 20 mg/ml proteinase K (Sigma Aldrich) and incubated at 55 °C in a water bath. Proteinase K solution and 20 % SDS were prepared fresh prior to the DNA isolation. After 24 hours, another aliquot of 25 µl of 20% SDS and 25 µl of 20 mg/ml proteinase K were added, and incubation was continued for another 48 hours at 55 °C in a water bath shaker with gentle shaking. The tubes were then centrifuged at 10000 rpm for 15 min. at room temperature, the supernatant was taken in a separate 2ml microcentrifuge tube and DNA was extracted twice with phenol:chloroform (1:1, v:v), and once with chloroform washing, DNA was then precipitated by adding double amount of ice cold ethanol and 3M sodium acetate (1/20 of total volume) to the supernatant, followed by an 18 hr precipitation at –20 °C. After precipitation, the solution was centrifuged at 10000 rpm for 15 min. at 4^{o}C to pellet down the DNA. The DNA pellet were given 2-3 washings of freshly prepared 70 % ethanol, air dried and re-suspended in 100 µl of 1X TE buffer (10 mM Tris, pH 8.0; 1 mM EDTA).

PCR reaction

The quality and quantity check of DNA was done on nanodrop spectrophotometer (ND-1000) and 0.8 % agarose gel electrophoresis. The samples were diluted to working concentration of 30 ng/µl and set up in a PCR reaction. Universal primers of 12S rRNA gene were used for PCR amplification, with the following sequence, *forward* 5'-CAA ACT GGG ATT AGA TAC CCC ACT AT -3' and *reverse* 5'- GAG GGT GAC GGG CGG TGT GT -3'. A typical PCR cocktail consisted of DNA sample 3µl (30ng/µl), PCR master mix 12.5 µl, nuclease free water 7.5 µl and forward primer and riverse primer (10 pmole/µl) 1 µl each. Total 25 µl of reaction mixture was briefly centrifuged at 1000 rpm to mix the contents and then subjected to PCR. The PCR cycle consisted of following steps, initial denaturation at 95^{o}C for 5 min., denaturation at 94 ^{o}C for 30 seconds, annealing at 56^{o}C for 30 seconds, extension at 72^{o}C for 1 min. (40 cycles) , and final extension at 72^{o}C for 5 min. The products obtained were then subjected to 1.5 % agarose gel electrophoresis to check for amplification, band sizing was done with the help of 50 bp DNA ladder and Gel analyser 2010 software.

RESULTS AND DISCUSSION

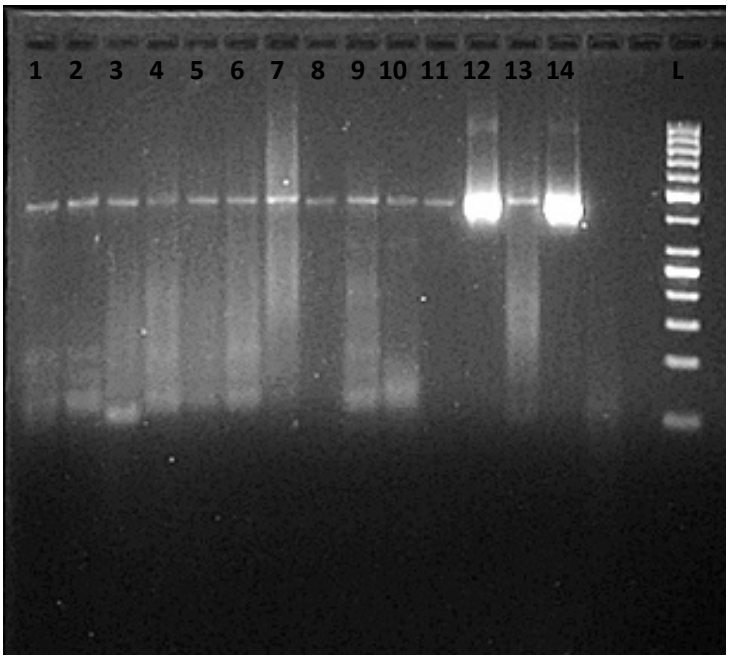
All the samples subjected for DNA isolation yielded varying amount of DNA by using the current protocol. The 260/230 OD was in the range of 1.28 to 1.8, with concentration varying from 92.7 ng/µl to 329.6 ng/µl (Table 1). Although the fixation conditions clearly influence the quality of formaldehyde-fixed DNA, optimization of extraction procedures can be critical to obtaining usable amounts of DNA. Extraction methods can differ in performance with different species [6]. The present protocol of DNA extraction has yielded sufficient quality and quantity of DNA from formaline fixed tissues, that can be easily used in PCR amplification. These DNA samples can be used for further molecular genetic studies and forensic analysis. Previously described such methods used commercial kits [1], other similar methods [5], [4] were able to amplify fragments of 570 bp to 450 bp mostly in amphibian tissues and amphipods [2]. The basic protocol that we tested in this study [1] was successfully able to isolate DNA from formalin fixed avian tissues with the aforementioned modifications. During the DNA extraction process some of the DNA pellets retained red or brown colouration, addition of 0.5 % Triton X 100 reduced the reddish brown discolouration to some extent. 0.8 % Agarose gel electrophoresis to assess the genomic DNA size

doesnot showed a clear disticnt band in most of the cases and smearing in the gel was a common problem encountered, specially in older tissues, it might have occurred due to the precipitation of proteins but that didnot affected the PCR amplification. We were able to amplify the 12s rRNA region in all species (Figure 1), the size of PCR product ranged from 457 to 488 (Table 1).

Table 1. Details of DNA yield, quality and PCR product size of different samples

S.No.	Species	Duration of preservation (in years)	Tissue	O.D. at 260/230	O.D. at 260/280	Concentration (ng/µl)	PCR product size (bp)
1.	Dove	6	Forelimb muscle	1.25	1.5	173.1	457
2.	Cattle Egret	3	Forelimb muscle	1.5	1.5	283.7	479
3.	Spotted Owlet	6	Forelimb muscle	1.5	1.7	121.6	479
4.	Pariah kite	1	Heart muscle	1.3	1.5	207.8	488
5.	Intermediate Egret	3	Forelimb muscle	1.28	1.52	258.6	488
6.	Barned owl	1	Forelimb muscle	1.4	1.63	329.6	488
7.	Eagle	25	Forelimb muscle	1.4	1.5	92.3	488
8.	Spotted owl	6	Forelimb muscle	1.8	1.7	144.2	488
9.	Blue rock pigeon	6	Forelimb muscle	1.7	1.4	113.2	488
10.	Swift	3	Pectoral muscle	1.3	1.5	110.3	488
11.	Pheasant	2	Forelimb muscle	1.7	1.6	157.2	479

Fig 1. Gel photograph of 1.5 % agarose gel electrophoresis of different samples



In figure – 1 to 11 are samples, (*1- Dove; 2-Cattle egret; 3-Spotted Owlet; 4-Pariah kite; 5- Intermediate Egret; 6- Barned owl; 7- Eagle; 8- Spotted owl; 9- Blue rock pigeon; 10- Swift; 11- Pheasant*). 12 - 14 are positive control; L is a 50 bp DNA marker.

CONCLUSION

Formaline fixed tissues found in biological museums can form a valualble source of DNA for genetic and forensic analysis. There are plenty of protocols available for such extractions, choice of protocol depends upon the availability of chemicals and rapidity of the procedure. The main problem in such extractions are short size of DNA, sometimes fragmented, poor quality or quantity, but these are mainly due to the conditions at the time of preservation of tissue. There are, very often, low intensity bands after PCR as well as false negative results, for this

standardization of PCR protocol and setting up of duplicate tubes in PCR is done. Such extractions however can be very effectively applied for study of rare and cryptic species, for studying their genome profile or in evolutionary studies or for forensic applications.

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