aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 6 (9): 958-965, 2011
ISSN 1683-9919 / DOI: 10.3923/ajava.2011.958.965

Rapid Salt-Extraction of Genomic DNA from Formalin-Fixed Toad and Frog Tissues for PCR-Based Analyses

[1,2]Wu-Yi Liu and [3]Ke-Jun Zhang

[1]Department of Biology Science, Fuyang Normal College, Fuyang City 236041, China
[2]Department of Scientific Research, Fuyang Normal College, Fuyang City 236041, China
[3]Qingdao Institute of Biomass Energy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao City 266101, China

Corresponding Author: Wu-Yi Liu, Department of Biological Sciences, Fuyang Normal College, No. 100 West Qing He Road, Fuyang City 236041, People's Republic of China Tel: +86-558-2596561, +86-15505588508 Fax: +86-558-2593670

ABSTRACT

The technical procedures for extraction of DNA from formahn-fixed tissues include many steps such as chemical treatment, enzymatic digestion, phenol-chloroform purification and alcohol precipitation. Formalin-fixed specimens used in molecular cell and DNA studies have shown shortcomings with respect to the efficacy of DNA isolation and subsequent PCR (Polymerase Chain Reaction) amplification. This study was designed to simplify and maximize recovery of PCR-amplifiable DNA from formalin-fixed toad and frog specimens and also to minimize co-extraction of substances that inhibit PCR amplification. This is achieved by a combination of DNA extraction from formalin-fixed muscle tissues using a salt-out buffer consisting of EDTA and proteinase K and NaCl. All steps are performed at room temperature (20-25°C), thereby reducing further degradation of the already damaged fragile specimen DNA and providing an optimal trade-off between DNA release and degradation. The salt-extraction method of genomic DNA presented here allows DNA isolation from formalin-fixed tissues with a minimum of working steps and equipment and rapidly yields much DNA.

Key words: Toad and frog, salt-extraction, genomic DNA, formalin, PCR amplification

INTRODUCTION

Molecular phylogenetics, behavioral ecology and population biology has increased dramatically during the last decades. For the application of these techniques, it is essential to obtain tissue samples allowing proper extraction of nucleic acids. Genomic DNA or RNA usually obtained from fresh or frozen tissues. Although the extraction of high-quality nucleic acid may be problematic from formalin-fixed tissues because of cross-linking between DNA and proteins or impurities, there are many studies on PCR-based analysis using formalin-fixed tissues have been published by Jackson *et al.* (1998), Harty *et al.* (2000), Lewis *et al.* (2001), Specht *et al.* (2001), Drabkova *et al.* (2002), Shi *et al.* (2002, 2004), Cao *et al.* (2003), Bibikova *et al.* (2004), Bahador *et al.* (2004), Rivero *et al.* (2006), Negishi *et al.* (2009) and April *et al.* (2009).

The Polymerase Chain Reaction (PCR) is an *in vitro* amplification technique that depends on adequate storages of samples and good protocols for DNA extraction. Methods for DNA extraction from fresh tissue and cytological preparation have been described and adapted for use in some

archival specimens (Shibata *et al.*, 1988; Coates *et al.*, 1991; Akao *et al.*, 1991; Foss *et al.*, 1994; Frank *et al.*, 1996; Mao *et al.*, 1996; Adams *et al.*, 1996; Diaz-Cano and Brady, 1997). The most common archival specimens are formalin-fixed and/or Paraffin-Embedded Tissues (PETs). DNA can be extracted from PETs but archival tissues may be unsuitable for many molecular techniques which require high molecular weight genomic DNA, as slow degradation of DNA occurs with time. However, short segments of genomic DNA are useful as a substrate for PCR amplification (Foss *et al.*, 1994; Mies, 1994; Diaz-Cano and Brady, 1997; Akalu and Reichardt, 1999; Sato *et al.*, 2001; Drabkova *et al.*, 2002; Cao *et al.*, 2003) and many researchers have also shown that PCR can be performed successfully on nucleic acids (DNA or RNA) that are partially degraded over time.

Formalin is the most acceptable fluid for soft tissue preservation and is by far one of the most widely fixatives used in specimen collections, particularly for toads and frogs. Formalin has been used as a fixative in archival specimens for more than a hundred years. During the research practice of last century, a large number of formalin-fixed tissue banks have been established. These tissue banks form invaluable resources of samples for various translational studies of molecular genetics and evolution and other interesting topics. The accessibility of macromolecules in fixed tissue specimens is a critical issue, as exemplified by the growth of PCR-based analyses. Although several DNA extraction methods for formalin-fixed and/or paraffin-embedded tissues were previously proposed by Rogers *et al.* (1990), Stein and Raoult (1992), Forsthoefel *et al.* (1992), Freeman *et al.* (1997), Merkelbach *et al.* (1997), Lum and Marchand (1998), Mulot *et al.* (2005), Huang *et al.* (2005), Bremmer *et al.* (2005), Cao *et al.* (2003) and Rivero *et al.* (2006), few studies have been conducted to compare these existing methods in order to identify a better method for DNA isolation. The present study was designed to rapidly isolate genomic DNA with salt-extraction method from formalin-fixed tissues of toads and frogs. We also evaluated the quality of genomic DNA extracted from toads and frogs fixed for five to ten years with PCR amplification.

MATERIALS AND METHODS

Tissues and DNA extraction: The study was conducted from October, 2009 to December, 2010. Formalin-fixed toad and frog muscle tissues were obtained from the Experimental Center of Fuyang Normal College from 2001 to 2010. All samples were routinely fixed in 10% neutral buffered formalin (average period of fixation was 24 h at room temperature, 20-25°C). All fixed tissues were processed routinely as required by the Experimental Center. All protocols were approved by the Institutional Review Board and the Institutional Animal Care and Use Committee of Fuyang Normal College.

The muscles of one back leg and/or part body of a toad or frog were used for DNA extraction. DNA was extracted using a modified salt-extraction method (Aljanabi and Martinez, 1997; Sambrook, 2001; Rivero *et al.*, 2006). The formalin-fixed muscle tissue was homogenized in 450 mL of sterile salt homogenizing buffer (0.4 M NaCl 10 mM Tris-HCl pH 8.0 and 2 mM EDTA pH 8.0) for 10-15 s. Then, 40 mL of 20% SDS (2% final concentration) and 8 mL of 10 mg mL^{-1} protenase K (200 mg mL^{-1} final concentration) were added and mixed well. The samples were incubated at 55-56°C overnight, after which 300 mL of 6 M NaCl solution (NaCl saturated H_2O) was added to each sample. Completed genomic DNA extraction was performed by the salt-extraction method, according to Aljanabi and Martinez (1997). Genomic DNA purity was assessed with a spectrophotometer and calculated by the ratio of DNA optical density (A260) and protein optical density (A280). Genomic DNA yield was calculated from DNA optical density (OD 260) for clean DNA samples. The purity of genomic DNA, determined from the A260/A280 ratio was averaged >1.71 for all samples. There was no RNA contamination in all samples during preparation.

Table 1: Primers of 12 S rRNA used in this study

Primer name	Primer sequence
12S1091	5'-AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT-3'
12S1092	5'-TGACTGCAGAGGGTGACGGGCGGTGTGT-3'

Following extraction, 4 to 5 mL samples were run on agarose electrophoresis gels (1%) containing ethidium bromide, with a 15000 bp ladder (marker D15000+2000) and were photographed under UV light to estimate the size range of genomic DNA fragments. Later, 1-2 μL DNA was used for PCR amplifications. The amount of tissue required for this method is minimal and the average number of PCR amplifications that can be performed using DNA extracted from 50 mg tissue was >1000.

PCR analysis: Each DNA extract was used as a template for PCR amplification, using a primer pair of 12 S rRNA genes as listed in Table 1. PCR tests were carried out based on groups of DNA samples extracted from eight formalin-fixed muscle tissues and a total of 16 PCR test results were evaluated by gel electrophoresis. PCR was performed by standard protocols. Briefly, the DNA sample diluted in 1-2 μL of distilled water containing 100 ng as template was added to the PCR reaction Mixture. PCR amplifications were carried out in a total volume of 25 μL. The PCR reaction mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.25 mM of dNTP each, 0.01 mg Bovine Serum Albumin (BSA), 50 ng of each primer, 0.05 units of Taq polymerase and about 50 ng of genomic DNA.

The PCR amplification program was designed with an initial denaturing step at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 52°C for 40 sec and 72°C for 30 sec, with a final hold at 4°C for 10 min to complete the program. The PCR amplification products were then discerned by electrophoresis on 2% agarose gels for about 30 min at 120 V and stained with ethidium bromide for visualization under UV light.

RESULTS AND DISCUSSION

The total DNA extracted from formalin-fixed toad and frog tissues were 14 out of 16 specimens examined, as shown in Fig. 1a and b. The toad DNA was successfully extracted from 8/8 samples (Fig. 1b) with about two folds of the amount of frog muscle tissues used in Fig. 1a. The failures were the two older samples, collected 10 years ago.

All the samples of genomic DNA extracted from formalin-fixed toad and frog tissues had been PCR amplified (Fig. 2). The PCR products amplified of toad and frog 12 S rRNA genes were a fragment of 350 bp. Toad 12S rRNA gene were sequenced successfully for 15 out of 16 PCR products (Fig. 2b). The PCR products were shown as specific. Frog samples 12S rRNA gene were sequenced successfully for all PCR products but some unspecific DNA bands appeared (Fig. 2a). The results revealed that the genomic DNA extracts from formalin-fixed tissues of toad and frog were about 10000 bp (Fig. 1) and none of PCR negative controls or extraction blanks exhibited signs of contamination with RNA or fungi DNA (Fig. 2).

As demonstrated in Fig. 1 and 2, the samples exhibited no significant external change/damage post extraction and the PCR products were satisfying (Fig. 2). All genomic DNA extracts produced a clear, sharp and reproducible PCR amplification product pattern. We had the same results after we repeated the PCR experiment over a period. As regards to these results, the modified salt-extraction method was validated for DNA extraction from formalin-fixed tissues. These results

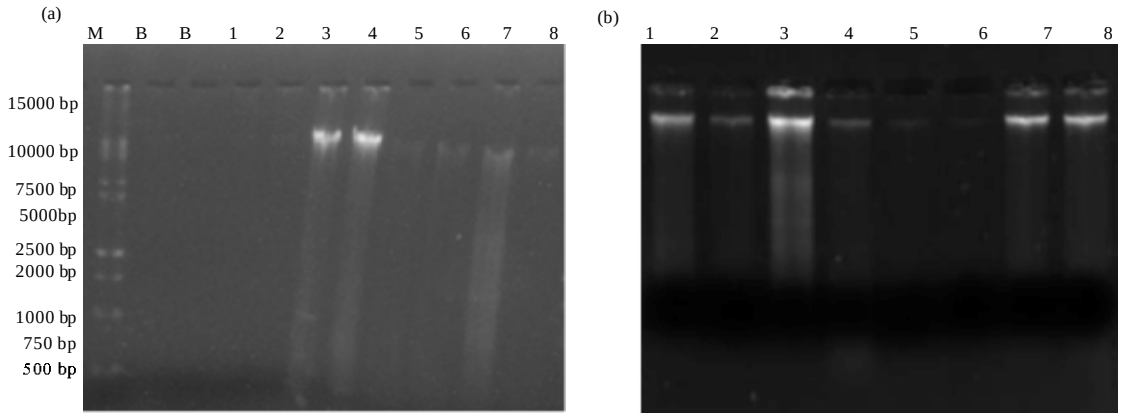

Fig. 1 (a-b): (a) Frog and (b) Toad DNA extracts from formalin-fixed muscle tissues, discerned by electrophoresis on 1% agarose gels with a 15000-bp ladder (D15000+2000, noted as M). Marker B shows blank lanes working as negative controls

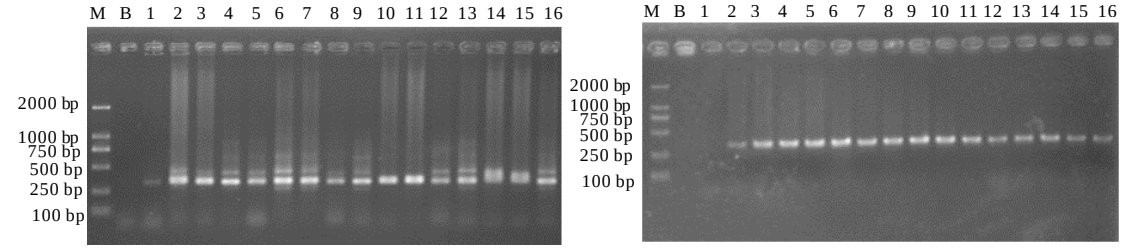

Fig. 2 (a-b): PCR products of 12S rRNA gene sequences from formalin-fixed tissues of (a) Frog and (b) Toad, amplified with the primers 12S1091/12S1092, were discerned by electrophoresis on 2% agarose gels with a 2000-bp ladder (DL2000, noted as M). Marker B shows blank lanes working as negative control

were in agreement with Drabkova *et al.* (2002) and Cao *et al.* (2003). Although the methods used in their reports were not salt-based types. The present study also examined the efficacy of salt-extraction method derived from Aljanabi and Martinez (1997) and Rivero *et al.* (2006). In the present study, sufficient DNA of samples was retrieved to enable us to provide enough DNA for PCR-based analyses.

Pervious methods for sample preparation of DNA from formalin-fixed and/or paraffin-embedded tissues are time consuming. These methods involved many steps and require several centrifugations and washes and multiple tube transfers which increase opportunities for the introduction of contaminant (Shibata *et al.*, 1988; Rogers *et al.*, 1990; Stein and Raoult, 1992; Forsthoefel *et al.*, 1992; Freeman *et al.*, 1997; Huang *et al.*, 2005).

Most of the recent studies used the DNA extraction with modified phenol–chloroform protocol, boiling method and commercial DNA Extraction Kit (Shi *et al.*, 2002, 2004; Drabkova *et al.*, 2002; Cao *et al.*, 2003). However, there are few reports of DNA extraction methods similar to our protocol. Rivero *et al.* (2006) studied a simple method of DNA extraction from formalin-fixed and paraffin-embedded tissues using a salt solution to precipitate protein and isopropanol to precipitate DNA. They focused on samples from Paraffin-Embedded Tissues (PETs). Their samples were tissues from

small biopsies of three oral Inflammatory Fibrous Hyperplasia (IFH) and three oral Squamous Cell Carcinomas (SCC), first fixed in 10% buffered formalin and then embedded in paraffin. They compared the salting-out DNA extraction method with a phenol–chloroform extraction method and a commercial DNA isolation kit. According to their results, the extraction method using proper concentrations of ammonium acetate proved to be simple and suitable for obtaining high quality DNA.

Usually, blood and leaf samples have been the specimens of choice for genomic DNA in molecular genetics and/or molecular biology studies (Bahador *et al*., 2004; Eshraghi *et al*., 2006; Bailes *et al*., 2007; Khairalla *et al*., 2007; Dehestani and Kazemi Tabar, 2007; Sahasrabudhe and Deodhar, 2010; Shankar *et al*., 2011; Chaudhary *et al*., 2011). Various methods are currently available to extract DNA from blood lymphocytes and other animal tissues with phenol-chloroform (Bailes *et al*., 2007; Khairalla *et al*., 2007; Chamani-Tabriz *et al*., 2007). However, collecting these samples is invasive and expensive and none of DNA extraction methods are ideal or universal. In comparison with phenol-chloroform based methods, salt-extraction or salt-out method is relatively simple, feasible, rapid and more acceptable by museum and field research participants (Drabkova *et al*., 2002; Cao *et al*., 2003; Aljanabi and Martinez, 1997; Rivero *et al*., 2006). The use of molecular techniques on archival materials has been limited due to the difficulty in obtaining consistent results. It is accepted that genomic DNA extracted from formalin-fixed and PETs archived specimens is not well preserved or is degraded but some molecular techniques require high molecular weight DNA (Shi *et al*., 2002, 2004; Drabkova *et al*., 2002; Cao *et al*., 2003; Bahador *et al*., 2004; Rivero *et al*., 2006; April *et al*., 2009). The reasons why formalin-fixed and PETs undergo degradation include insufficient neutralization of the formalin, causing acid depurination of DNA and preventing amplification (Shibata *et al*., 1988; Drabkova *et al*., 2002; Cao *et al*., 2003; Bahador *et al*., 2004). Present results showed that despite degradation, it is possible to use the genomic DNA from formalin-fixed for the past five to ten years in PCR amplification of short specific gene sequences. In the present study, amplification of a 350 bp fragment of 12 S rRNA genes was successful in all the 16 samples extracted by the salt-extraction method. Another important problem is the toxicity of phenol. Procedures using salt have been used to extract DNA from blood and other samples and were proved to be less laborious and non-toxic than the phenol-chloroform techniques. This makes the method an attractive optional method of genomic DNA isolation.

CONCLUSION

Present results proved that the modified simple salt-extraction method was considered proper and satisfying as one of the rapid methods for DNA extraction from formalin-fixed archival specimens or tissues.

ACKNOWLEDGMENT

We are grateful to the anonymous reviewers for their constructive comments and suggestions. This work was supported by Chinese grants from Anhui Educational Research Funds to LWY (2005QL11, 2006jql222, 2006KJ224B).

REFERENCES

Adams, V., M.A. Hany, M. Schmid, S. Hassam, J. Briner and F.K. Niggli, 1996. Detection of t (11;22) (q24;ql2) translocation breakpoint in paraffin-embedded tissue of the ewing's sarcoma family by nested reverse transcription-polymerase chain reaction. Diagn. Mol. Pathol., 5: 107-113.

Akalu, A. and J.K. Reichardt, 1999. A reliable PCR amplification method for microdissected tumor cells obtained from paraffin-embedded tissue. Genet. Anal. Biomol. Eng., 15: 229-233.

Akao, I., Y. Sato, K. Mukai, H. Uhara and S. Furuya *et al.*, 1991. Detection of epstein-barr virus DNA in formalin-fixed paraffin-embedded tissue of nasopharyngeal carcinoma using polymerase chain reaction and in situ hybridization. Laryngoscope, 101: 279-283.

Aljanabi, S.M. and L. Martinez, 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acid Res., 25: 4692-4693.

April, C., B. Klotzle, T. Royce, E. Wickham-Garcia and T. Boyaniwsky *et al.*, 2009. Whole-genome gene expression profiling of formalin-fixed, paraffin-embedded tissue samples. PloS one, 4: e8162-e8162.

Bahador, A., H. Etemadi, B. Kazemi and R. Ghorbanzadeh, 2004. Comparison of five DNA extraction methods for detection of Mycobacterium tuberculosis by PCR. J. Med. Sci., 4: 252-256.

Bailes, S.M., J.J. Devers, J.D. Kirby and D.D. Rhoads, 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.

Bibikova, M., D. Talantov, E. Chudin, J.M. Yeakley and J. Chen *et al.*, 2004. Quantitative gene expression profiling in formalin-fixed, paraffin-embedded tissues using universal bead arrays. Am. J. Pathol., 165: 1799-1807.

Bremmer, J.F., B.J.M. Braakhuis, H.J. Ruijter-Schippers, A. Brink and H.M.B. Duarte *et al.*, 2005. A noninvasive genetic screening test to detect oral preneoplastic lesions. Lab. Invest., 85: 1481-1488.

Cao, W., M. Hashibe, J.Y. Rao, H. Morgenstern and Z.F. Zhang, 2003. Comparison of methods for DNA extraction from paraffin-embedded tissues and buccal cells. Cancer Detection Prevention, 27: 397-404.

Chamani-Tabriz, L., M.J. Tehrani, M.M.A. Akhondi, A. Mosavi-Jarrahi and H. Zeraati *et al.*, 2007. Chlamydia trachomatous prevalence in Iranian women attending obstetrics and gynaecology clinics. Pak. J. Biol. Sci., 10: 4490-4494.

Chaudhary, P.P., S.K, Sirohi and S. Kumar, 2011. Improved extraction of quality DNA from methanogenic archaea present in rumen liquor for PCR application. Asian J. Anim. Sci., 5: 166-174.

Coates, P.J., A.J. d'Ardenne, G. Khan, H.O. Kangro and G. Slavin, 1991. Simplified procedures for applying the polymerase chain reaction to routinely fixed paraffin wax sections. J. Clin. Pathol., 44: 115-118.

Dehestani, A. and S.K. Kazemi Tabar, 2007. A rapid efficient method for DNA isolation from plants with high levels of secondary metabolites. Asian J. Plant Sci., 6: 977-981.

Diaz-Cano, S.J. and S.P. Brady, 1997. DNA extraction from formalin-fixed, paraffin-embedded tissues: Protein digestion as a limiting step for retrieval of high-quality DNA. Diagn. Mol. Pathol., 6: 342-346.

Drabkova, L., J. Kirschner and C. Vicek, 2002. Comparison of seven DNA extraction and amplification protocols in historical herbarium specimens of Juncaceae. Plant Mol. Biol. Rep., 20: 161-175.

Eshraghi, P., R. Zarghami and H. Ofoghi, 2006. RAPD analysis of micropropagated plantlets in date palm. Pak. J. Biol. Sci., 9: 111-114.

Forsthoefel, K.F., A.C. Papp, P.J. Snyder and T.W. Prior, 1992. Optimization of DNA extraction from formalin-fixed tissue and its clinical application in Duchenne muscular dystrophy. Am. J. Clin. Pathol., 98: 98-104.

Foss, R.D., N. Guha-Thakurta, R.M. Conran and P. Gutman, 1994. Effects of fixative and fixation time on the extraction and polymerase chain reaction amplification of RNA from paraffin-embedded tissue: Comparison of two housekeeping gene mRNA controls. Diagn. Mol. Pathol., 3: 148-155.

Frank, T.S., S.M. Svoboda-Newman and E.D. Hsi, 1996. Comparison of methods for extracting DNA from formalin-fixed paraffin sections for nonisotopic PCR. Diagn. Mol. Pathol., 5: 220-224.

Freeman, B., J. Powell, D. Ball, L. Hill, I. Craig and R. Plomin, 1997. DNA by mail: An inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. Behav. Genet., 27: 251-257.

Harty, L.C., M. Garcia-Closas, N. Rothman, Y.A. Reid, M.A. Tucker and P. Hartge, 2000. Collection of buccal cell DNA using treated cards. Cancer Epidemiol. Biomarkers Prev., 9: 501-506.

Huang, Q., P. G. Sacks, J. Mo, S.A. McCormick and C.E. Iacob *et al.*, 2005. A simple method for fixation and microdissection of frozen fresh tissue sections for molecular cytogenetic analysis of cancers. Biotech. Histochem., 80: 147-156.

Jackson, P.J., M.E. Hugh-Jones, D.M. Adair, G. Green and K.K. Hill *et al.*, 1998. PCR analysis of tissue samples from the 1979 Sverdlovsk anthrax victims: The presence of multiple Bacillus anthracis strains in different victims. Proc. Natl. Acad. Sci., 95: 1224-1229.

Khairalla, K.M.S., I.E. Aradaib, A.O. Bakhiet, T. Hassan and B.E. Hago, 2007. A simple and rapid assay for specific identification of bovine derived products in biocomplex materials. Pak. J. Biol. Sci., 10: 1170-1174.

Lewis, F., N.J. Maughan, V. Smith, K. Hillan and P. Quirke, 2001. Unlocking the archive-gene expression in paraffin-embedded tissue. J. Pathol., 195: 66-71.

Lum, A. and L.L. Marchand, 1998. A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. Cancer Epidemiol. Biomarkers Prev., 7: 719-724.

Mao, E.J., D. Oda, W.G. Haigh and A.M. Beckmann, 1996. Loss of the adenomatous polyposis coli gene and human papillomavirus infection in oral carcinogenesis. Eur. J. Cancer B Oral Oncol., 32: 260-263.

Merkelbach, S., J. Gehlen, S. Handt and L. Fuzesi, 1997. Novel enzyme immunoassay and optimized DNA extraction for the detection of polymerase-chain-reaction-amplified viral DNA from paraffin-embedded tissue. Am. J. Pathol., 150: 1537-1546.

Mies, C., 1994. Molecular biological analysis of paraffin-embedded tissues. Hum. Pathol., 25: 555-560.

Mulot, C., I. Stucker, J. Clavel, P. Beaune and M.A. Loriot, 2005. Collection of human genomic DNA from buccal cells for genetics studies: Comparison between cytobrush, mouthwash and treated card. J. Biomed. Biotechnol., 2005: 291-296.

Negishi, A., M. Masuda, M. Ono, K. Honda and M. Shitashige *et al.*, 2009. Quantitative proteomics using formalin-fixed paraffin-embedded tissues of oral squamous cell carcinoma. Cancer Sci., 100: 1605-1611.

Rivero, E.R.C., A.C. Neves, M.G. Silva-Valenzuela, S.O.M. Sousa and F.D. Nunes, 2006. Simple salting-out method for DNA extraction from formalin-fixed, paraffin embedded tissues. Pathol. Res. Practice, 202: 523-529.

Rogers, B.B., L.C. Alpert, E.A. Hine and G.J. Buffone, 1990. Analysis of DNA in fresh and fixed tissue by the polymerase chain reaction. Am. J. Pathol., 136: 541-548.

Sahasrabudhe, A. and M. Deodhar, 2010. Standardization of DNA extraction and optimization of RAPD-PCR conditions in *Cinia indica*. Int. J. Bot., 6: 293-298.

Sambrook, J., 2001. Molecular Cloning: A Laboratory Manual. (2nd Ed.)., Cold Spring Harbor. Laboratory Press, New York.

Sato, Y., R. Sugie, B. Tsuchiya, T. Kameya, M. Natori and K. Mukai, 2001. Comparison of the DNA extraction methods for polymerase chain reaction amplification from formalin-fixed and paraffin-embedded tissues. Diagn. Mol. Pathol., 10: 265-271.

Shankar, K., L. Chavan, S. Shinde and B. Patil, 2011. An improved DNA extraction protocol from four, *in vitro* banana cultivars. Asian J. Biotechnol., 3: 84-90.

Shi, S.R., R.J. Cote, L. Wu, C. Liu and R. Datar *et al*., 2002. DNA extraction from archival formalin-fixed, paraffin-embedded tissue sections based on the antigen retrieval principle: Heating under the influence of pH. J. Histochem. Cytochem., 50: 1005-1011.

Shi, S.R., R. Datar, C. Liu, L. Wu, Z. Zhang, R.J. Cote and C.R. Taylor, 2004. DNA extraction from archival formalin-fixed, paraffin-embedded tissues: Heat-induced retrieval in alkaline solution. Histochem. Cell Biol., 122: 211-218.

Shibata, D., W.J. Martin and N. Arnheim, 1988. Analysis of DNA sequences in forty-year-old paraffin-embedded thin-tissue sections: A bridge between molecular biology and classical histology. Cancer Res., 48: 4564-4566.

Specht, K., T. Richter, U. Muller, A. Walch, M. Werner and H. Hifler, 2001. Quantitative gene expression analysis in microdissected archival formalin-fixed and paraffin-embedded tumor tissue. Am. J. Pathol., 158: 419-429.

Stein, A. and D. Raoult, 1992. A simple method for amplification of DNA from paraffin-embedded tissues. Nucl. Acids Res., 20: 5237-5238.