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# An Efficient and Optimized Protocol for DNA Extraction from Animal Tissues

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**ABSTRACT**

Extraction of deoxyribonucleic acid (DNA) molecule is a critical and basic step affecting molecular-based techniques. Therefore, researchers have been used different modified and optimized protocols for efficient genomic DNA extraction from biological samples. In this research, we reported a rapid and optimized protocol with cost-efficient and non-poisonous reagents for extraction of DNA molecule from animal tissue sample. This efficient protocol was optimized based on combination of salting-out and boiling methods. Extracted DNA with current protocol was qualified using gel electrophoresis, spectrophotometric and PCR analysis. Our results showed that by this protocol high quantity and high quality of DNA was extracted and isolated DNA was successfully can be used for molecular and PCR-based techniques. In conclusion, we reported a rapid, cost-efficient and non-toxic procedure for high yield DNA extraction from animal tissues.



## 1. Introduction

DNA extraction from biological samples is a basic and essential method in the molecular and clinical laboratories (Moradi et al., 2014). Also, molecular experiments need to template DNA with high-quality and high-quantity that influence the subsequent downstream applications in molecular genetics purposes such as PCR based methods (Wang et al., 2011). However, in 1869, DNA extraction method was reported by Swiss physician, Friedrich Miescher (Dahm, 2008), but today researchers need to DNA extraction procedure for certain biological organism that is relatively rapid, inexpensive with high yielded and high quality of extracted DNA.

Currently, there are many known conventional protocols and commercial kits for DNA extraction from biological samples; such as phenol- chloroform, salt extraction method and theirs modifications that generally divided into solution-based or column-based however, these methods are laborious, time consuming (at least 2 days) and poisonous (Ghaheri et al., 2016; Wang et al., 2011; Tan and Yiap, 2009; Moradi et al., 2014). Also, commercial DNA extraction kits have been produced that are fast and safe, but usually are expensive and low yielded. In general, choice of good DNA extraction method depends on required quantity and purity of extracted DNA, time and expanse factors.

Tissues are a general source of DNA when performing genetic analysis. In the current study the optimized protocol for the DNA extraction from animal tissue sample is described, which is high yielded, inexpensive, cost efficient, simple and also quick protocol without using of hazardous chemical substances.

## 2. Materials and Methods

The all chemical reagents such as NaCl, Tris, $Na_2EDTA$, Ammonium acetate, SDS and chloroform were purchased from Merck company (Germany).

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*2.1. Modified Protocol for DNA Extraction*

1- About 50-100 mg of fresh tissue (human breast) was placed into 2 ml Eppendorf tube that labeled with an identification number.

2- Liquid nitrogen was added and grinded the tissue for 1 min with a pestle.

3- One ml of tissue digestion buffer (10 mM $Na_2EDTA$, 20 mM Tris-HCL and 100 mM NaCl (pH 7.4) was added and mixed with vortex for breakup and rinse.

4- 100 µl of SDS (10%) and 20 µl of proteinase K were added and suspended with vortexing or shaking.

5- Microtube was incubated in 55° C for 60 min.

6- It was centrifuged in 5000 rpm for 10 min and then supernatant was removed.

7- Microtube was put into ice tray for 5 min and 350 µl of chloroform and 350 µl of saturated NaCl (6 M) were added.

8- Microtube gently was shacked for 30 sec and centrifuged in 5000 rpm for 10 min at 4° C.

9- Upper phase (about one ml) was transferred to new 2 ml tube and one ml of cold absolute ethanol and 150 µl of ammonium acetate (5 M) were added.

10- Gently shacked the tube; in this step, DNA was appeared as a white skein.

11- Again tube was centrifuged for 10 min at 13000 rpm at 4° C.

12- The supernatant was discarded and pellet was washed with 1 ml of chilled 70% ethanol and centrifuged again at 10000 rpm for 10 min.

13- The supernatant was discarded and pellet was left at room temperature to be dried.

14- 50-100 µl of $ddH_2O$ was added to dissolve the pellet and DNA solution stored at -20° C until use.

*2.2. Assessment of Extracted DNA*

The yield and purity of extracted DNA was estimated and confirmed using agarose gel electrophoresis (0.1%). The concentration and purity of extracted DNA were estimated using NanoDrop (Thermo) with measuring A260 and A260/A280 ratio, respectively.

*2.3. PCR and Analysis of Human MMP-2 Gene*

Quality of the isolated DNA was evaluated by polymerase chain reaction (PCR) method. PCR is a powerful method for molecular analysis technology, theoretically enabling the detection of a single copy sequence (Klein et al., 1997). About 100-500 ng of extracted DNA was used as a template for PCR.

The expected amplicon size of the PCR product was 300 bp that amplified with the following primer pair: 5´ ATAGGGTAAACCTCCCCACATT 3´ and 5´ GGTAAAATGAGGCTGAGACCTG 3´ (Yari et al., 2014). PCR was performed using thermocycler with 20 pmol of each forward and reverse primer, 100-500 ng of extracted DNA, 200 µM of dNTPs, 1.3 mM of $MgCl_2$, 2 U of Taq polymerase and 2.5 µl of 10X PCR buffer with 25 µl final volume. The PCR thermal cycling parameters were: 1 cycle at 94° C for 5 min that followed by 35 cycles at 94° C for 60s, 62° C for 45s and 72° C for 60s, then the final cycle was continued for 10 min at 72° C as the last extension (Yari et al., 2014).

## 3. Results and Discussion

In our study, quality of extracted DNA was analyzed on 1% agarose gel electrophoresis and intense band observed. Our results showed that the yield and the quality of the isolated DNA by this optimized protocol are adequate (above 2-3 µg per 100 mg tissue) to perform hundreds of PCR reaction and can be used in other PCR-based techniques. The pattern of gel electrophoresis of extracted DNA that band of extracted genomic DNA was sharp and there is no sign of degradation and smear DNA (Fig. 1).

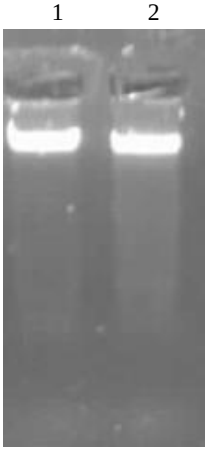


**Fig. 1.** Agarose gel electrophoresis (1%) for extracted DNA. Lanes 1 and 2 show extracted DNA by optimized protocol.

The purity of the extracted DNA was assessed with NanoDrop. Based on results obtained from NanoDrop assay, extracted DNA with current protocol was

approximately free from protein and polysaccharide impurity (A260/ A280 ratio of 1.80 to 2).

Then, successful DNA isolation was confirmed with PCR amplification of human MMP2 gene. Fig. 2 shows the agarose gel electrophoresis pattern (2%) for fragment (300 bp) of MMP2 gene that amplified with PCR. In PCR amplification, 100-500 ng of isolated DNA was used as a template DNA (Fig. 2).

Molecular genetics methods require extraction of genomic DNA with suitable yield and efficient purity (Ghaheri et al., 2016; Aljanabi and Martinez, 1997). DNA isolation has been reported as primary and critical method in wide range of applications in molecular genetics technology such as PCR and PCR based methods. Therefore, an optimized protocol requires for DNA isolation from biological samples for PCR based downstream applications (Devi et al., 2013). In our study we developed a modified and optimized protocols for DNA extraction from animal tissue samples. In recent decade, researchers reported different optimized protocols for DNA extraction for different biological samples (Sepp et al., 1994; Moradi et al., 2014). However, in our protocol no toxic chemical substances such as phenol and β-mercaptoethanol were used.

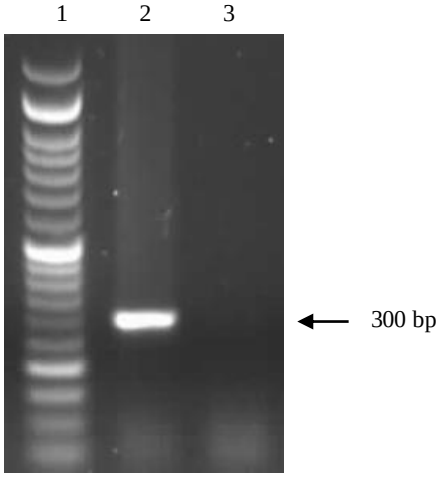


**Fig. 2.** Agarose gel (2%) electrophoresis of PCR product (300 bp) for human MMP2 gene. Lane 1 shows 50 bp molecular DNA marker, lane 2 shows 300 bp PCR product band, lane 3 shows negative control.

Previously, like with current protocol. Moradi et al. 2014 reported a novel, efficient, fast and inexpensive DNA extraction protocol from whole blood sample (Moradi et al., 2014). In accordance with our study, Konat et al. 1990 have reported for rapid isolation of genomic DNA from liver tissues (Konat et al., 1990) and Wang et al. 2011 reported a simplified universal genomic DNA extraction protocol suitable for PCR from various types of organism (Wang et al., 2011). Also, Amani et al. 2011 reported a simple and rapid protocol for DNA extraction from leaf (Amani et al., 2011).

In conclusion, in the work presented here we reported a rapid, cost efficient and optimized protocol for high yield extraction of genomic DNA from animal tissues. The isolated DNA is suitable for PCR amplification and other subsequent methods.

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Conflict of interest: The authors declare that they have no conflict of interest.

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