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Assessing Tissue Lysis with Sodium Dodecyl Sulphate for DNA Extraction from Frozen Animal Tissue

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Abstract

Background: Many molecular biology experiments start with DNA extraction. The quality and the quantity of the extracted DNA are very important for downstream analyses. Therefore, the DNA extraction process is very important and efforts should be aimed at constant improvement of the available protocols.

Objective: The first objective of this study is to compare various lysing agents that use Sodium Dodecyl Sulphate (SDS) in DNA extraction process. The second objective is to report detailed protocol for the extraction of DNA from frozen animal sample.

Methods: The efficiencies of four different lysing reagents involving SDS and a commercial lysing agent were compared. The reagents tested were ethylenediaminetetraacetic acid (EDTA), Sodium Chloride-Tris-EDTA (STE), Sodium chloride (NaCl) and tris acid (Tris). The four lysing agents investigated were SDS+EDTA+NaCl (SEN), SDS +EDTA+Tris (SET), SDS+STE+NaCl (SSN) and SDS+STE+Tris (STT). In addition, Qiagen (Q) lysing buffer was also included. The experiments were conducted in duplicates. The five lysing reagents were used to extract DNA from homogenized muscles and bones from the sternum of juvenile Japanese giant flying squirrel that had been frozen for four years.

Results: With the protocol presented here, gel electrophoresis, nanospec, bioanalyzer and PCR showed that all the five lysing reagents were able to extract DNA from the homogenized muscles and sternum bones of frozen rodent species. Large DNA molecules could be retrieved from all the lysing reagents. However, A_{260}/A_{280} shows that SSN and STT had the purest extracted DNA molecules.

Conclusion: We present a protocol that successfully extracted DNA with good integrity from frozen animal sample. The comparison of five lysing reagents showed that although all the reagents successfully extracted DNA, the purest extracted DNA came from SDS+STE+NaCl (SSN) and SDS+STE+Tris (STT). Interestingly, DNA from all the reagents was successfully amplified with PCR suggesting that the impurities may not be of significant impacts to the downstream analyses.

Keywords: DNA extraction; Sodium Dodecyl Sulphate (SDS); Frozen tissue; DNA quality; Extraction protocol

Introduction

Molecular experiments involving DNA typically starts with DNA extraction [1]. In the process of DNA extraction, getting the adequate amount of pure DNA is the goal. This is because the quantity, quality and integrity of the extracted DNA are important for subsequent analyses [2,3]. Therefore, DNA quantity and quality are usually evaluated before downstream analyses [4,5]. DNA quality is usually measured in terms of purity and intactness [2]. This is because DNA might be contaminated in the process of sample collection, sample preparation and DNA extraction process. Therefore, tissue lipid, carbohydrate or proteins, salts, RNA, organic salts, inorganic materials and even residual reagents from the extraction process might constitute contamination to DNA [3]. DNA quality is usually assessed by the ratio of absorbance at certain wavelengths, typically 230, 260 and 280 nm [6,7]. Low value of A_{260}/A_{280} may indicate protein contamination while low values of A_{260}/A_{230} may indicate salt, carbohydrate or reagent contamination [7,8]. The length of DNA is another measure of DNA quality. Appropriate DNA extraction method should minimize DNA fragmentation and promote DNA intactness [9]. DNA fragmentation can be evaluated using gel electrophoresis or Qubit bioanalyzer [10].

DNA can be extracted from any species. In animals, DNA can be extracted from any cell or tissue type. For example, DNA has been extracted from feather [11,12], hair [3,13], stool [14,15], saliva [16], tissues [4,17], blood [7,18] and others [19–22], including ancient samples [23–26]. Perhaps one of the most commonly used tissues is blood [27]. However, less invasive methods involving sources such as saliva and feces are now gaining popularity in DNA extraction from human populations [28,29] and other animal species [30-32]. One major source of concern in such methods, however, is the presence of microbial DNA, which is sometimes the target [14]. Although DNA extraction from blood is more common and less problematic as the

cells are free, there may be instances in which DNA extraction from tissues might be inevitable. For example, the extraction of DNA from dead animals might be exclusively dependent on tissues as blood might not be available. Also, DNA extraction using blood is impossible in animals with no blood. Therefore, extraction from other tissues might sometimes be inevitable.

Many commercial DNA extraction kits are now available for the extraction of DNA from different tissues [2,10,14,33]. One major concern of the kit is the cost [34]. DNA extraction kits are usually designed for extraction from many samples, and become cost-effective only when the number of sample is large. Even with larger sample size, DNA yield from commercial kits is limited. Furthermore, the identities of the reagents in kits are not usually disclosed, making it difficult to troubleshoot. Therefore, traditional extraction techniques are still sometimes used. DNA extraction from tissues starts with tissue lysis. In this study, the use of SDS as lysing buffer in the extraction of DNA from a frozen rodent sample was investigated.

Material and Methods

Design

Five different lysing reagent combinations of SDS and other reagents were investigated. The reagents included ethylenediaminetetraacetic acid (EDTA), Sodium Chloride-Tris-EDTA (STE), sodium chloride (NaCl) and tris acid (Tris). First combination was 20% SDS, 125mM EDTA and 5M NaCl in ratio 1:1:1 hereafter abbreviated as SEN. Second combination involved 20% SDS, 125mM EDTA and pH 7.5 Tris acid in ratio 1:1:1 hereafter abbreviated as SET. The third combination was 20% SDS, STE and 5M NaCl in ratio 1:1:1 hereafter abbreviated as STN. The fourth combination was 20% SDS, STE and 5M NaCl in ratio 1:1:1 hereafter abbreviated as STT. As a control, Qiagen ATL (Q) was used. Each lysing combination was prepared in duplicates. These lysing buffers were used to lyse the sternum tissue of a juvenile Japanese giant flying squirrel which was preserved in freezer for about four years.

Reagents

- Lysing buffer
- Protinase K@
- RNase A@
- Ultrapure Phenol:Chloroform:Isomyl Alcohol (25:24:1) [Caution: This reagent is very hazardous. It should be handled carefully inside biological chamber].
- Sodium acetate
- Chilled 95% ethanol [Note: Isoproanol could be used in place of 95% ethanol but elusion seems to be slightly affected].
- 70% Ethanol

Equipment

- Chopping tools such as ultra-clean scissors, clean dish etc.
- Weighing scale
- Vortex machine
- Incubator
- Centrifuge
- Biological chamber
- 1.5 ml microtubes
- Micropipettes
- Thermo Scientific nanodrop spectrophotometer
- Bioanalyzer
- PCR machine
- Electrophoresis chamber
- UV transilluminator

Protocol

- Defreeze and chop the tissue to be lysed into small sizes using sterilized scissors. [Note: Adequate chopping makes lysing faster.]
- Measure 70 mg of the sample into 1.5 ml microtube.
- Add 300 µl of the lysing buffer and 40 µl proteinase K.
- Vortex rigorously for 15 seconds.
- Incubate at 56°C until complete dissolution.
- Add 8 µl of 100 mg/ml RNase A.
- Vortex briefly and incubate for 30 minutes at 37°C. [Note: Steps 6-7 can be skipped for downstream analyses that are not affected by the presence of RNA].
- Add 450 µl of Ultrapure Phenol:Chloroform:Isomyl Alcohol (25:24:1).
- Vortex briefly and centrifuge for 10 minutes at 15,000G.
- Carefully transfer the clear supernatant into a new tube without disturbing the interphase layer. [Caution: Disturbing the interphase would reduce DNA purity.]
- Add 1/10 of the supernatant volume of sodium acetate and gently tap the tube to mix well. [Caution: Vortexing should be avoided to prevent DNA fragmentation.]
- Add twice the total volume of 95% chilled ethanol and tap the tube to mix well.
- Incubate on ice or freezer for 15 minutes.
- Centrifuge at 15,000G for 10 minutes and discard the ethanol supernatant. [Note: DNA pellet might be visible as whitish wool-like material at the base of the tube.]
- Add 300 µl 75% ethanol.
- Centrifuge at 12,000G for 2 minutes and discard the ethanol supernatant. [Caution: DNA pellet might not stick to the tube. Be careful not to discard the DNA pellet with ethanol.]
- Air dry the pellet for about 5 minutes. [Caution: Overdrying tends to affect the DNA solubility during elusion. It is okay to have some few drops of ethanol. Elusion buffer will dilute it during elusion.]
- Elute with 300 µl ultrapure water.

Gel electrophoresis

Gel electrophoreses were run for the extracted intact DNA and PCR products. For the intact DNA, the concentration of the gel was 0.8% whereas for the PCR product the gel concentration was 1.5%. For the two electrophoreses, the processes were run for 30 minutes. Thereafter, the products were stained with two drops of Ethidium bromide in 50 ml ultra-pure water for 30 minutes followed by 15 minutes of de-staining in ultra-pure water. The gel was then visualized under UV transilluminator.

PCR amplification

PCR amplification was run for all the designs and replicates with two blanks. The total volume of the PCR mix was 25 µl. The reaction mix composition per sample was 19.75 µl DDW, 2.5 µl buffer, 0.5 µl 10 mM dNTPs, 0.5 µl forward primer, 0.5 ml reverse primer and 0.25 µl Ex-Taq polymerase. The reaction mix was prepared for all the samples and blanks on ice in the clean bench. Thereafter, 24 µl of the PCR mix was transferred into the PCR tubes on ice. For each design and replicate, 1 µl of the template was introduced and tapped by finger. For

blanks, 1 µl of DDW was introduced into each PCR tube. The tubes were then spinned and transferred into 95°C pre-heated PCR machine.

Results and Discussion

DNA extracted from all the designs

The protocol presented here does not guarantee a fix amount of DNA yield. A lot of factors can affect the final DNA yield. However, 200 µl of supernatant in step 10 of the protocol was taken from all the designs and replicates without disturbing the layer. The disturbance of the layers should be avoided to avoid contaminants which may affect the purity of the extracted DNA. One of the ways to check the success of DNA extraction is through gel electrophoresis. Gel electrophoresis result shows the availability of the DNA, and reveals some information about the size of the DNA molecules extracted. The gel electrophoresis result (Figure 1) shows that there were DNA molecules extracted from all the designs. Because a preserved sample was used, lambda Hind III and 1k Plus ladders were used. These ladders can measure up to 23 kb and 12 kb, respectively. The gel result shows that DNA molecules of longer than 23 kb fragments could be extracted. However, gel electrophoresis does not reveal much about DNA quantification and purity. Therefore, DNA quantification and quality assessment was done with nanodrop spectrophotometer at 2 mm wavelength. Typical of successful DNA extraction, there was a bump at the wavelength of 260 nm for the DNA extracted from all the designs (Figure 2). The concentrations of DNA measured using nanodrop Spectrophotometer are presented in Table 1. All the designs and duplicates had the concentration of >390 ng/ul. We then proceeded to examine the purity of the extracted DNA.

Variables	Nanospec concentration (ng/µl)	Bioanalyzer concentration (ng/µl)	OD 260/280	OD 260/230
Q1	701.56	250	1.76	0.82
Q2	498.58	244	1.78	0.61
SEN1	455.15	186.8	1.87	1.03
SEN2	449.29	154.4	1.86	0.79
SET1	550.32	324.9	1.85	1.32
SET2	506.17	268	1.82	1.1
SSN1	393.75	173.4	1.94	1.87
SSN2	555.55	276	1.92	2
SST1	468.12	222	1.89	1.83
SST2	391.89	200	1.81	1.83

Table 1: The quantity and the quality of extracted DNA.

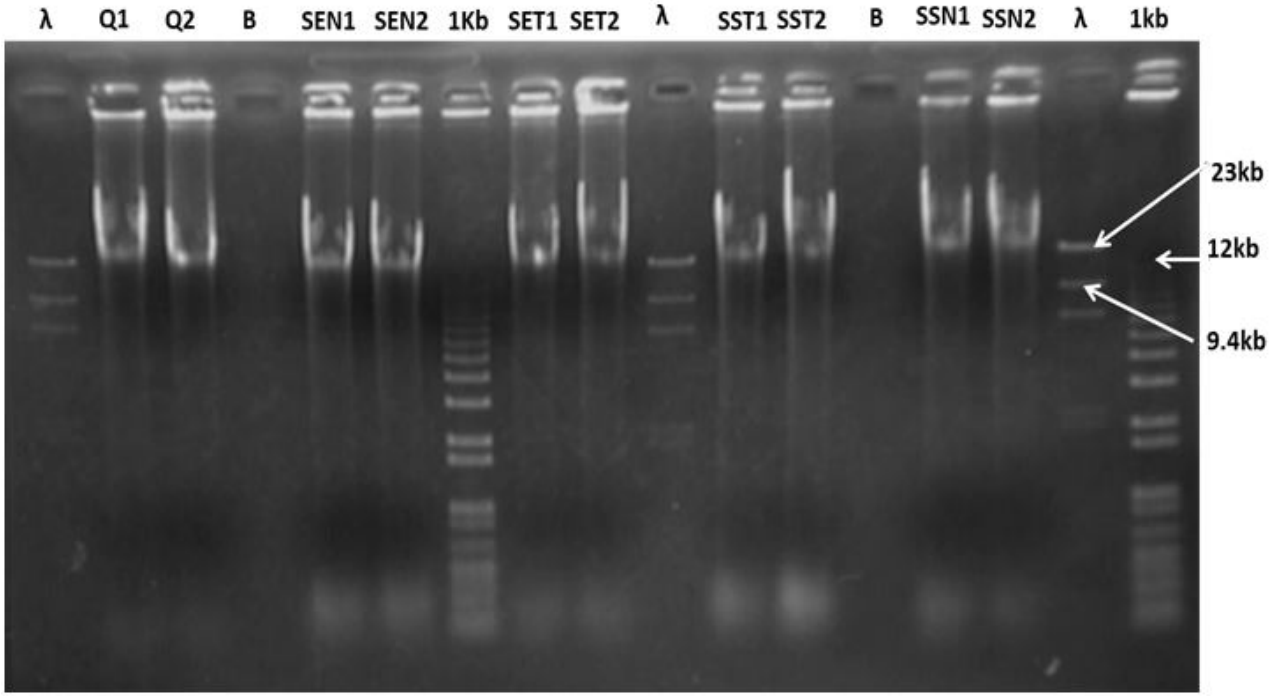

Figure 1: The extraction of large fragments of DNA molecules from frozen animal tissues. DNA molecules larger than 23kbp could be extracted from all the reagents investigated. B wells are represent the blanks. Lambda Hind III (λ) and 1k Plus (1kb) ladders were used.

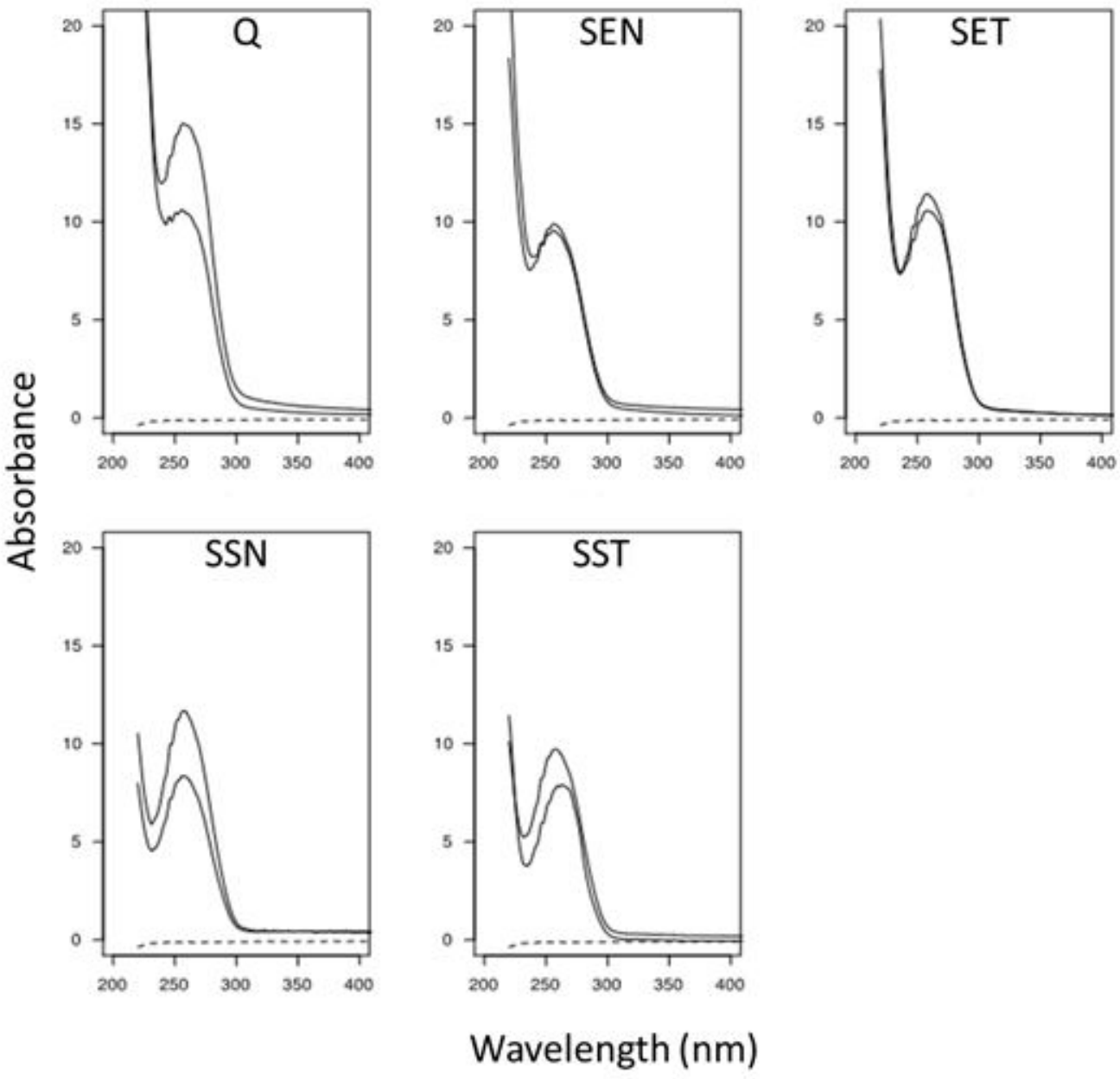

Figure 2: DNA absorbance to investigate DNA quality. DNA extracted from the five lysing reagents were checked with nanospec. The results show that SSN and SST have purer DNA.

The purities of the DNA were different

One of the ways to examine the purity of extracted nucleic acid is by measuring the absorbance at 260 nm wavelengths, relative to 230 nm and 280 nm. Whereas low A_{260}/A_{230} ratio suggests the presence of EDTA, carbohydrates and phenol which have absorbance near 230 nm, low A_{260}/A_{280} suggests the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. All designs and replicates have the A_{260}/A_{280} ratio of around 1.8 or higher suggesting that the extracted DNA was free from protein or phenol. However, the A_{260}/A_{230} ratio for Q, SEN and SET are low, suggesting the presence of contaminants with 230 nm absorbance. Since SEN and SET both contains EDTA which absorbs around 230 nm, this may be the reason for the low A_{260}/A_{230} for these designs. The low ratio in these designs, suggestive of the presence of contaminant suggests that the concentration estimates from nanospec might not be so accurate. We therefore measured the concentration and estimated the length distributions using bioanalyzer. The estimate of the double stranded DNA concentrations using bioanalyzer was generally lower than the concentrations from nanospec (Table 1). For example, while the nanospec concentration for Q1 was 701 ug/ml, the concentration with bioanalyzer was 250 ug/ml. The length distributions shown in Figure 3, show that longer fragments were indeed extracted.

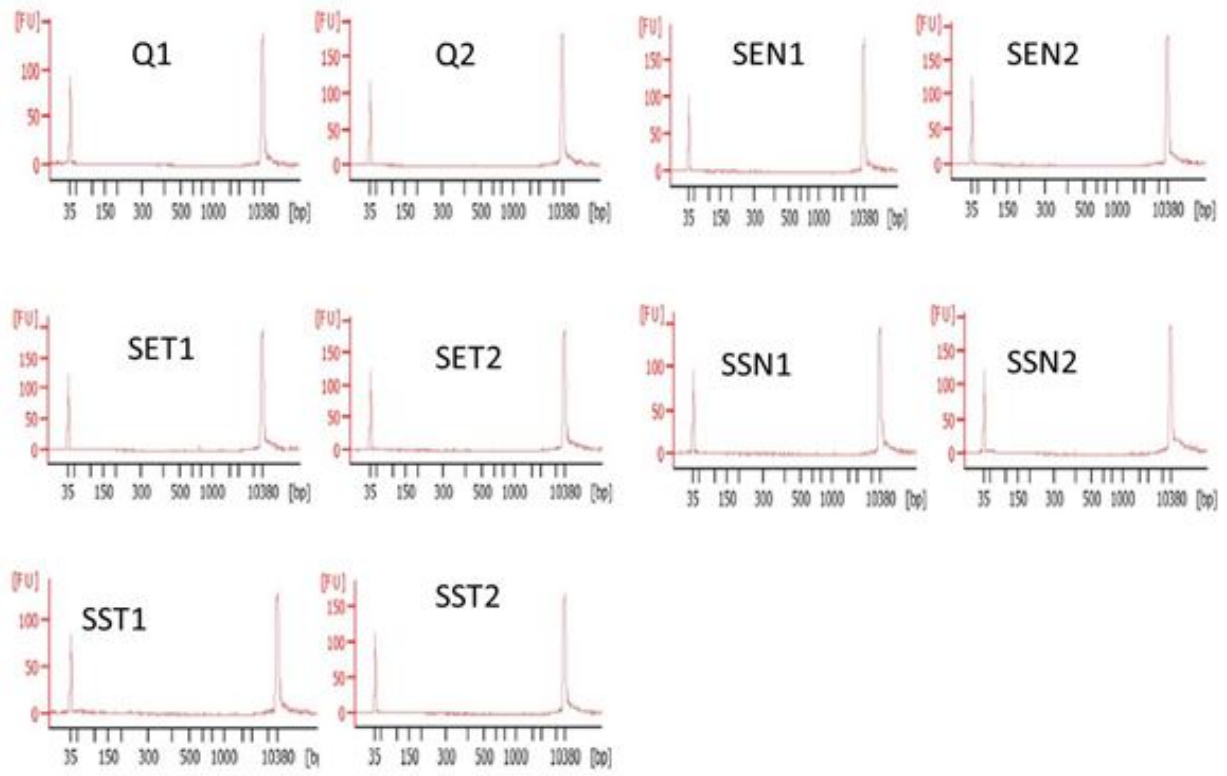

Figure 3: Estimating fragment sizes under Qubit bio-analyzer. The extracted DNA was diluted to 1:500. The bioanalyzer result shows the presence of double-stranded large-size DNA molecules in all the reagents tested.

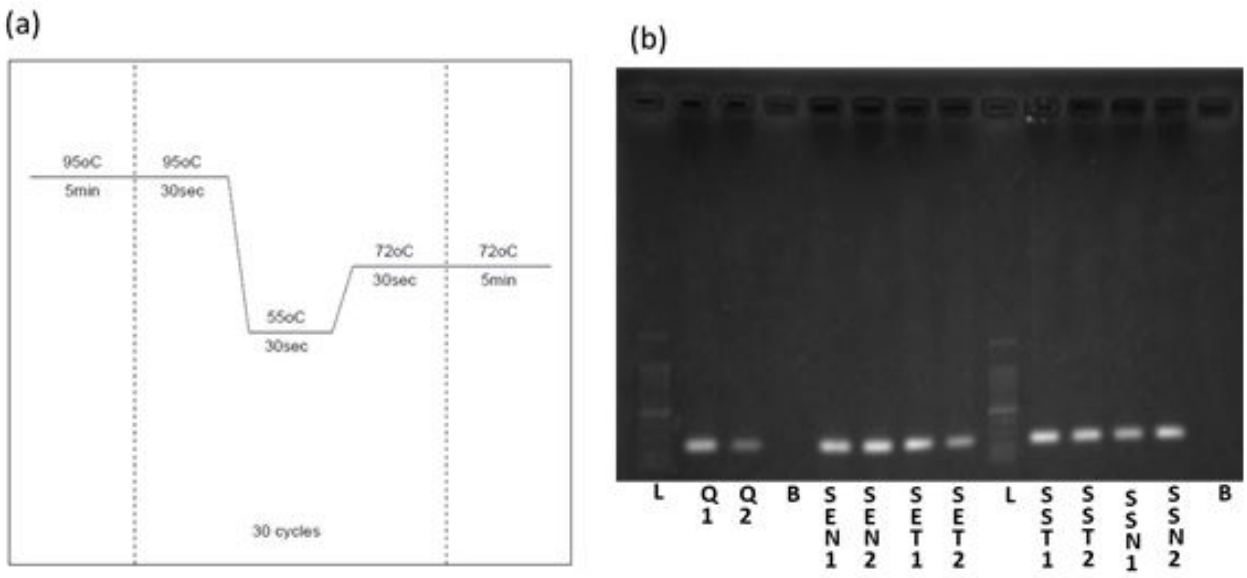

Figure 4: Successful PCR amplification of extracted DNA. (a) The PCR condition for the amplification is shown. Thirty cycles of PCR was run. (b) Gel electrophoresis results shows that the amplification from all the reagents were successful. However, there was no amplification in blank, eliminating the possibility of contaminants. The ladders, represented by L, estimate the expected DNA size to be around 240bp.

Successful PCR amplification from all the designs

The impurities suggested in Figure 2 and Table 1 can be reduced or removed by purifying the extracted DNA. However, the process of purification usually leads to the loss of DNA. This is of a concern especially in the case of precious DNA samples such as ancient samples. Moreover, proper A_{260}/A_{230} and A_{260}/A_{280} ratios do not always imply that that the downstream analyses would be successful. We therefore proceeded to carry out the polymerase chain reaction (PCR) amplification for all the designs and replicates. The choice of PCR as a candidate downstream analysis is because it is mostly used in many biological experiments, including many next generation sequencing library preparation techniques. The PCR was run with a universal primer reported by Kitano et al. [35] to amplify 244 bp region of 16S rRNAs mitochondrial region. PCR conditions are presented in Figure 4a. The gel electrophoresis result for the PCR products shows that amplification was successful in all the designs and replicates (Figure 4b). Also, the estimated fragment size was around the expected 244 bp. The primer set used for amplification was reported to be successful for many vertebrate species. Therefore, it was important to exclude the possibility of the amplification of contaminant DNA, especially from human sources. If there was contaminant, we should see the amplification in blank. However, Figure 4b shows that there was no amplification in blank, indicating that the amplifications were indeed from Japanese giant flying squirrel DNA.

Conclusion

Many molecular biology experiments begin with the extraction of nucleic acids in good quality and quantity. Cell lysis is an important step in DNA extraction. Although numerous commercial extraction kits are available, there are sometimes needs to extract DNA in the absence of kits. Therefore, we presented the comparison of different combination of lysing reagents in the context of SDS-based DNA extraction protocol using the juvenile of Japanese giant flying squirrel that had been frozen for four years. As a reference, we also included a commercial kit. The protocol could be used to extract DNA from the tissues of various animal species.

We have shown that with the protocol, reasonable amount of large sized DNA could be extracted from all lysing reagents investigated. However, the measures of DNA purity through absorbance show that reagents including Tris acid were the purest. Therefore, the lysing reagents that gave the purest DNA are SDS+STE+NaCl (SSN) and SDS +STE+Tris (STT). The exact compositions of these two lysing reagents are presented in the Design section. The results for the duplicates of all the lysing reagents are similar (Table 1), indicating that the observations were not a product of stochastic effects. It is interesting that PCR amplification was successful for all the lysing agents. This suggests that the presence of impurities in the other lysing reagents did not affect PCR. By extension, the impurities might not also affect other downstream analyses. In the cases where extremely pure DNA is required, DNA extracted with any of the lysing reagents could be further purified. Although the process of further purification would lead to DNA loss, purer DNA could be extracted.

The protocol presented here has been used in extracting DNA from the masseter muscle of a capybara individual which died of undisclosed reason. Also, this protocol was used to extract DNA from another Japanese giant flying squirrel which was preserved for 25 years. Although the DNA molecules from the 25-year old preserved sample were degraded, they could still be amplified and sequenced using next generation sequencing platforms. DNA extracted with the protocol has been successfully sequenced by new generation sequencing techniques such as Miseq and Hiseq using paired-ends and mate pairs library preparation procedures. In conclusion, we have compared several lysing reagents using SDS-based DNA extraction protocol, presented its usefulness in extracting DNA from relatively old but well prepared animal sample.

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