Effect of Temperature and Storage Time on DNA Quality and Quantity from Normal and Diseased Tissues

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Abstract: DNA extraction and purification is an initial step for authentic results in advance molecular biology, therefore DNA degradation is unavoidable. The aim of present study is to investigate the DNA quality and quantity in terms of shorter time preservation with normal and diseased tissue, therefore tissues of normal (*n* = 18) and diseased (*n* = 18) liver, lung and heart was collected from goat after slaughtered. For DNA extraction Gene JET Genomic DNA Purification Kit protocol was followed, then stored at -20 ^{o}C and -04 ^{o}C temperatures for 24hrs and 48hrs period of time. The concentration and purity of the extracted DNA were measured with Spectrophotometer and purity confirmed at an absorbance ratio of 260 or 280. It was observed that at a -20 ^{o}C temperature for 24hours the concentration of DNA yield was numerically higher than at -04 ^{o}C temperature for tissue stored at 48hrs, whereas absorbance was higher, however in normal tissues in contrast with diseased the concentration and absorbance of DNA was somehow same at -20 and -04 ^{o}C but different in storage time. On the basis of these findings, it was concluded that time elapsed between sampling with the storage condition and with normal or diseased samples for DNA extraction will largely depend on the experiment. If tissue preservative conditions and sampling are appropriate, storage time will not be a factor at least for short storage periods.

Keywords: DNA, degradation, concentration, purity, temperature, spectrophotometer.

INTRODUCTION

Extraction and purification of DNA is one of the basic steps in molecular biology and recombinant DNA techniques. The preparation of high quality DNA from various sources such as, tissue samples (fresh and frozen), whole blood etc is the most important first step [1]. With affection to DNA features, it is essential to maintain appropriate near-physiological conditions to prevent degradation of DNA molecules. Similarly to proteins, DNA molecules denature at higher temperatures, extreme pH values, and in the presence of organic solvents and some tensides [2].

Therefore reliable approaches to determine the accurate concentration of DNA are essential for several biological applications, ranging from traditional molecular biological manipulations, such as restriction digest analysis, Southern blotting, and polymerase chain reaction (PCR), to diagnostic techniques, including quantification of genetically modified organism content of samples, detection of DNA contamination in drug preparations produced from recombinant organisms, and medical diagnosis of virus and cancer [3, 4]. Previous studies have evaluated the efficiency of number of DNA extraction methods based on, for example, DNA yield, extent of DNA shearing, and use as template for subsequent PCR [5-8]. Commonly three methods used to investigate the DNA concentration: ultraviolet (UV) absorbance, fluorescence, and diphenylamine reaction. Currently, the most common procedure to define DNA concentration is the measurement of absorbance of UV light at 260 nm [19]. The DNA extraction by using these methods are based on the same principles are used: (i) disruption of the cell membrane or cell wall; (ii) inhibition of nucleases, released after the cell lysis; (iii) removal of cell debris; and (iv) final purification of the DNA isolate, removal of low molecular contaminations, desalting, and precipitation [9]

The aspects that influence DNA quality and quantity are based on DNA degradation that starts occurring at the moment an organism dies, when cell membranes break down. This allows entrance of bacteria and other threats to the cell and release of DNAase, enzymes that sequentially cut single nucleotides from DNA until the molecule is too little that cannot longer be called DNA. The speed of the DNA degradation process depends on many factors, and can be slowed down by keeping the samples cold and/or dry or by using preservative agents that prevent DNAase activity. Refrigeration, by storage at – 20 ºC, -80 ºC or in liquid nitrogen (-196 ºC), and desiccation, by drying at 70 ºC or placement in silica desiccant, impede degradation by microbes and enzymes, which require temperatures above 0 ºC and the presence of water to be active [10]. Commonly used preservative agents not only dehydrate the sample by permeating the tissue and

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Table 1: DNA Concentration and Absorbance Mean Values of Normal and Diseased Tissues with Different Time of Storage and Temperature

No. of Samples	TISSUE		Storage Temperature	Storage Time	DNA Concentration ng/µl	DNA Absorbance Ratio 260/280nm
03	Normal	Liver	-20 °C	24hr	6.6	1.85
03	Normal	Lung	-20 °C	24hr	13.6	1.75
03	Normal	Heart	-20 °C	24hr	90.6	1.84
03	Suspected	Liver	-20 °C	24hr	6.0	1.08
03	Suspected	Lung	-20 °C	24hr	6.1	1.17
03	Suspected	Heart	-20 °C	24hr	102	1.8
03	Normal	Liver	-04 °C	48hr	5.0	1.88
03	Normal	Lung	-04 °C	48hr	8.2	1.79
03	Normal	Heart	-04 °C	48hr	88.7	1.85
03	Suspected	Liver	-04 °C	48hr	2.4	1.09
03	Suspected	Lung	-04 °C	48hr	5.9	1.18
03	Suspected	Heart	-04 °C	48hr	79.4	1.8

displacing water, but have also other properties that prevent DNA degradation; for example, ethanol precipitates enzymes and kills bacteria and fungi, DESS (dimethyl sulfoxide combined with NaCl and hylene diamine tetra acetic acid) chelates metals on which enzyme action depends [20], Number of studies have been performed comparing different methods of sample storage to prevent DNA degradation [10-13].

The goal of this study was to compare the DNA quality and quantity extracted from normal, and diseased tissue with different storage temperature for two time intervals which help in future to adopt the best suited condition for better yield with best quality to get the more valuable detailed results in relations for PCR techniques.

MATERIALS AND METHODS

Samples of normal and diseased tissue of liver, lungs, and heart from slaughtered goats were collected and wrapped in aluminum foil and kept on ice and transferred to the laboratory. For DNA extraction about 30mg tissues transferred into Eppendorf tubes and further procedure were followed according to the Gene JET Genomic DNA Purification Kit # K0721 (Fementas, EU), supplied by Thermo scientific. Briefly, the samples were homogenized and transferred into individual 1.5ml Eppendorf tube and 180µl digestion buffer was added in each of the tube and were re-suspended. After re-suspension 20µl Proteinase K was added and mixed thoroughly by vortexing till a uniform solution obtained. The tissues were lysed by incubating at 56ºC for 2hrs. After that 20µl of RNase A solution was added and were vortexed, the samples were incubated for 10 minutes at room temperature. 200µl of lysis buffer was added and mixed thoroughly by vortexing for 15 seconds till a homogenous mixture was obtained. 400µl of 50% ethanol was added and mixed by pipetting. The prepared lysate was transferred to Gene JET Genomic DNA purification column inserted in a collection tube; the column was centrifuged at 600xg for 60 seconds. After centrifugation flow through was discarded along with the collection tube, the Gene JET Genomic DNA purification column was placed into a new collection tube. 500µl of wash buffer I was added and centrifuged at 800xg for 60 seconds, the flow through was discarded and the column was placed back into the collection tube. 500µl of was buffer II was added in each tube and centrifuged at 12000xg for 03 minutes, the collection tube and flow through were discarded and the column was placed in a new sterilized 1.5ml Eppendorf collection tube.

After placing the column in a new collection tube, 200µl of Elution Buffer was added in each in center of the Gene JET Genomic DNA purification column membrane to elute genomic DNA, they were incubated for 02 minutes at room temperature and centrifuged for 01 minutes at 8000xg. The purification column was discarded and the purified DNA was immediately stored at -20 °C and -04 °C for the time period of 24hrs and 48hrs further experiments.

The concentration and purity of the extracted DNA were measured with the help of Nano drop 1000

Spectrophotometer (Thermo Scientific) whereas purity of DNA was confirmed by the absorbance ratio of 260/280.

RESULTS & DISCUSSION

The concentration and absorbance (260/280) of DNA measured after 24hrs and 48hrs of normal and diseased tissue at -20 ^{o}C and -04 ^{o}C stored. It was observed that at a -20 ^{o}C temperature for 24 hours the concentration of DNA yield was numerically higher than at -04 ^{o}C temperature for tissue stored at 48hrs, whereas absorbance (260/280) was higher, however in normal tissues in contrast with diseased the concentration and absorbance (260/280) of DNA was somehow same at -20 ^{o}C and -04 ^{o}C but different in storage time presented in Table **1**.

DNA degradation is unavoidable in DNA extraction and storage for molecular studies [14], furthermore samples stored at -20 ºC without any preservative show higher yield of DNA quantity than samples stored with a liquid preservative [15]. This might be due to the fact that the lysis buffer can easier penetrate the sample when no fixative agent has previously done so and therefore more efficiently release DNA.

During DNA extraction, DNA extraction kit left some residues of chemical reagents, RNA, and protein in the DNA elution [16], which would interfere with the measurement of DNA concentration to different degrees. It was speculated that the dramatic differences in the estimated concentration of the same sample were derived not only from the methods themselves, but also from the interference from impurities in the DNA samples which was shown in the current study.

The time interval since death of the organism to a collection of samples up to the of measurement of DNA yield, quality or quantity, in that period the fact about the samples were defrost was known, but in other cases, even if samples arrive frozen to the laboratory, froze-thaw processes the researcher is unaware of can occur during transportation, making the causes of DNA degradation difficult to determine. Additionally, samples stored at -20 ºC without preservative need to be more carefully handled in the laboratory, where they cannot stand on the bench outside the fridge even for a few minutes like samples that contain preserving agents [15]. Although a number of studies have been performed comparing different methods of sample storage to prevent DNA degradation [10-13].

According to the relationship that the absorbance value of 1 at 260 nm is equivalent to 50 µg/ml pure dsDNA, 40 µg/ml single-stranded DNA, and 33 µg/ml oligonucleotides [17], the absorbance at 260 nm would increase with the degradation of DNA into oligonucleotides. Moreover, the added enzyme also absorbs UV light and so interferes with the measurements [17]. Therefore, DNA degradation triggered the standards of measurements by the UV absorbance technique to increase rather than decrease resulted in DNA of less purity. However, for high valuable results in advance studies needed than every condition should be in consideration for better understanding the results, because the [18] DNA with good integrity can be obtained from samples stored for months or even years.

CONCLUSIONS

Time elapsed between sampling with the storage condition and with normal or diseased samples for DNA extraction will principally depend on experiment. If tissue preservative conditions and sampling are appropriate, storage time will not be a factor at least for short storage periods.

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