Bulletin of Pharmaceutical Research 2011;1(3):4-9
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH ARTICLE

ISOLATION, QUANTIFICATION AND PURITY ESTIMATION OF DNA FROM VARIOUS SOURCES

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Received: October 18, 2011 / Revised: November 01, 2011 / Accepted: November 02, 2011

DNA was isolated from various sources such as bacteria, fungi, blood, fish tissue and onion. The entire DNA that has been extracted was found to differ in their molecular weight and was seen as separate bands when viewed under UV light. The concentration of the DNA was estimated by using the UV spectrophotometer. The concentration of DNA was found to be more from onion and the ratio of their absorbance at 260 and 280 nm, was 1.9 which showed slight contamination. The concentration of DNA was found to be the least from fungi and ratio was found to be 1.58 indicating the presence of contamination. The DNA isolated from the bacteria and blood, was found to be 100% pure and free from contamination.

Key words: DNA isolation, Blood, Fish tissue, Onion, Bacteria, Yeast.

INTRODUCTION

Deoxyribonucleic acid is a genetic material which can able to store the information which has to be transferred from one generation to another. Methods used to isolate the DNA depend on the source, age and size of the sample. Principle behind the separation of DNA which is present in the cells is to make the DNA free from the other cellular components (Saenger, 1984). Isolation of DNA is needed for the genetic analysis, which is used for scientific, medical or forensic purpose. Scientists use DNA in a number of applications, such as introduction of DNA into the cells and animals or plants, or for diagnostic purposes. In medicine, diagnostic purpose is the most common. Forensic science needs to recover DNA for identification of individuals; for example rapists, petty thieves, accidents, or war victims, paternity determination, plant and animal identification (Bruce *et al* 2002).

Presence of proteins, lipids, polysaccharides and some other organic or inorganic compounds in the DNA preparations can interfere with DNA analysis methods, especially with the polymerase chain reaction (PCR). They can also reduce the quality of DNA leading to its shortest storage life (Bauer and Patzelt, 2003). Sources of DNA isolation are very diverse. Basically it can be isolated from any living or dead organism. Common sources for DNA isolation includes the whole blood, hair sperm, bones, nails, tissues, blood stains, saliva, buccal (cheek) swabs, epithelial cells, urine, paper cards used for sample collection, bacteria, animal tissue or plants (Mandelkern *et al* 1981). It is quite clear that the extraction methods from various sources have to be adopted in such a way that they can be efficiently purified. Another important factor is the sample size. If the sample is small, as for example, sperm or single hair, the method has to be different to the methods used in isolating DNA from a couple of milligram of tissue or milliliters of the blood. Another important factor is whether the sample is fresh or has been stored. Stored samples can come from archived tissue samples, frozen blood or tissue, exhumed bone or tissues and ancient human, animals or plant samples (Gregory, 2006).

Isolation of DNA usually begins with lysis or breakdown of tissue or cells. This process is

essential for the destruction of protein structure and allows for the release of nucleic acid from the nucleus. Lysis is carried out in salt solution containing detergents to denature the proteins or proteases, the enzymes for digesting proteins such as proteinase K or in some cases both are used. Usage of these enzymes results in breakdown of the cells and dissolving of membrane (Watson and Crick, 1953). Lysis of soft tissues or cells is easy. DNA also has to be isolated from hard tissue such as bone, wood and various plant materials. Most plant samples are required to be treated with liquid nitrogen and subsequently pulverizing the tissue to fine powder. On the other hand, bones are highly mineralized and the ions have to be removed from the samples before extraction so that they do not later interfere with dealing with PCR. Once the samples are partly processed, they are homogenized in lysis buffer using a mechanical homogenizer (Psifidi *et al* 2010).

DNA isolation is a simple process and can be performed in a kitchen using the household appliances and chemicals. Vegetables or meat can be homogenized with salt and water. Afterwards, by application of a detergent, cellular proteins and lipids are separated away from DNA. Enzymes found in the meat tenderizer or pine apple juice allows precipitation of proteins and frees DNA into the solutions. By adding alcohol to the mix, nucleic acid is brought to the top of the container and can be spooled onto a stick as a visible white string (Ghosh and Bansal, 2003).

A number of commercial DNA purification kits use the very same principle as that of household method but with different reagents. In a commercial kit, the common lysis solution contain sodium chloride, tromethamine (also known as Tris) which is a buffer to retain constant pH, ethylenediaminetetracetic acid (EDTA) which binds metal ions; and sodium dodecyl sulphate (SDS), which is a detergent. A common enzyme used in DNA extraction is proteinase K (Wiesinger-Mayr *et al* 2011).

Although the plants are not a common source of DNA for forensic investigation, analysis of their DNA is very common in science. From the plants it is very difficult to isolate the DNA than any other samples because of the two reasons. First, plant cells have cell wall, which has to be at least partly destroyed before the cytoplasm with the DNA can be accessed. Second, plants often have high levels of sugars like starch and fructose in their tissues or other organic compounds such as polyphenols (Kumar and Chattopadhyay, 2007). Grinding the samples in liquid nitrogen helps to destroy the cell wall, but the organic compounds including sugars still remain. As a result, methods were developed that use chloroform-octanol mix, hexadecyltrimethylammonium bromide (CTAB) with high salt to remove the poly saccharides and polyvinylpyrrolidone (PVP) to remove polyphenols (Shyur *et al* 2005). All these methods are successfully used in various laboratories and with various samples. The methods have to be properly selected to optimize the yield and quality of the DNA extracted. This paper deals with the isolation of DNA from various sources such as blood, bacteria, yeast, onion, fish and reports the molecular weight of the DNA that has been extracted from the various sources.

MATERIALS AND METHODS

Tris, EDTA, methyl blue, ethanol, lifton buffer, potassium acetate, sodium acetate, spectrophotometer, tissue homogenizer, onions and fish tissue were purchased from the local market. The microorganism such as bacteria and yeast were purchased from NCIM, Pune.

Extraction of DNA from the fish tissue

Small piece of fish tissue was placed in 200 μl of Lifton buffer and 20 μl of 20 mg/ml proteinase K solution was added. With the help of the pipette the tissues were crushed and incubated at 55-65°C for a minimum of one hour with gentle rocking. The yield of the extraction increases with the amount of incubation time. Next step can be carried out only after the tissue is completely dissolved. To this, 300 μl of phenol and 25 μl of 5 M potassium acetate pH 4.8 was added and shaken vigorously and then kept for incubation for 5-10 min with gentle rocking. Then, the solution was centrifuged at 14,000 rpm, the aqueous phase remained at the top and interface which appeared whitish in color and contained the denatured proteins and carbohydrates. The aqueous layer was removed and transferred to the new tube. To this tube 300 μl of chloroform was added, mixed gently and incubated for 15 min. After incubation, 1 ml of 100% ethanol was added to the mixture and then once again centrifuged. The aqueous phase was removed and placed at -20°C for a period of 30 min. Sometimes, storage at -20°C for overnight may be necessary for the extraction. After removing from -20°C, it has to be centrifuged for 5 min at 14,000 rpm.

Supernatant solution was removed in such a way that the pellets should not be discarded. The pellets were resuspended in 200 µl of RNAse free sterile water and then 500 µl of 100% ethyl alcohol and 25 µl of 7.5 M ammonium acetate was added and placed at -20°C for 20 min. Once again, centrifugation was performed for 5 min at 14,000 rpm. The supernatant was removed and tube was dried completely by applying speed vacuum without using heat for 1-2 min and the pellets were resuspended in 50 µl of sterile water (Schander, 2003).

Extraction of DNA from the bacteria

Hundred ml of the broth inoculated with the microorganism for 20 min was centrifuged at 14,000 rpm so that the microorganisms settle down in form of pellets. The pellets were transferred to an eppendroff tube along with a small quantity of broth and again centrifuged. The pellets were resuspended in 500 µl of ice cold solution containing lysozyme at the concentration of 2 mg/ml and then incubated on ice for 10 min. After the incubation, 50 µl of 10% SDS buffer was added and incubated at 37°C for 5-10 min until clear and viscous solution was obtained. Phenol (550 µl) saturated with equal volume of 0.3 M sodium acetate was added and mixed gently by inversion and centrifuged at 4°C for 15 min, top layer was removed and once again treated with 550 µl of phenol, centrifuged and top layer was transferred to a new eppendroff tube. To this, 1/10 volume of 3 M sodium acetate solution was added and spinned for 3 minutes and the supernatant was transferred to another eppendroff tube. In this eppendroff tube, 100% ethanol was added and mixed gently. The sample was kept at -80°C for 5 min and then centrifuged for 15 min at 4°C. The supernatant was discarded and the pellets were vacuum dried and resuspended in 50 µl of water (Munroe, 2004).

Extraction of genomic DNA from the fungus

Two hundred mg of mycelium was harvested from the liquid media and broken up by using the spatula. Four ml of CTAB extraction buffer was added and the mycelium was wetted by the buffer and kept on a water bath at 65°C for 30 min. After cooling, equal volume of chloroform-isoamyl alcohol (24:1) was added, mixed and centrifuged at 10,000 rpm for 10 min. Supernatant was removed and treated with equal volume of isopropanol. After centrifugation for 2-5 min at 10,000 rpm, the DNA was spool out with glass rod and washed with 70% ethanol. Tubes were drained on a paper towel and dried. To this, 200 µl of TE containing 20 µ of RNAse was added and placed in a water bath at 65°C and then kept at 4°C for overnight. DNA was precipitated which was resuspended in 50 µl of water (Hwang-Lee *et al* 1983).

Extraction of DNA from the onion

Onion was treated with salt, distilled water and detergents and the filtrate obtained was transferred to 50 ml test tube. To this, 1 ml of 10% SDS and 4 ml of meat tenderizer solution was added and placed on ice for 5 min. Add 15-20 ml of ice cold ethanol to the test tube slowly. The tube was left aside for 3-5 min without disturbing to precipitate out the DNA (when the ethanol is added to the mixture, all the components of the mixture except DNA stay in the solution while the DNA precipitates out of solution and becomes visible as white strings in the ethanol layer). DNA was spooled out by using the glass rod and stored after resuspending in 50 µl of water (Gadegaard *et al* 2008).

Extraction of genomic DNA from the blood

Blood contain number of enzyme inhibitors that can interfere with the isolation of DNA. 0.8 ml of 1X SSC (which contain 3 M NaCl, 0.3 M sodium citrate, dissolved in 80 ml of distilled water and pH adjusted to 7.0 with conc. HCl) buffer was added to 1 ml of the blood and centrifuged for 1 min at 12,000 rpm in micro centrifuge. The supernatant solution was discarded. Again 1 ml of 1X SSC buffer was added and centrifuged for 1 min at 12,000 rpm and the supernatant solution was discarded so that the pellets remained at the bottom. 370 µl of 0.2 M sodium acetate was added to the pellet and mixed by inverting the tube. Then 25 µl of 10% SDS and 5 µl of proteinase K in the concentration of (20 mg/ml) was added and mixed by inverting the tubes and then incubated for 1 h at 55°C. After incubation 100 µl of phenol-chloroform-isoamyl alcohol was added and mixed for 30 sec and centrifuged for 2 min at 12,000 rpm; aqueous layer was transferred to the new tube and to that 1 ml of 100% cold ethanol was added and then kept at -20°C for 15 min. Then it was centrifuged for 2 min at 12,000 rpm and supernatant was removed. 180 µl of 1X TE buffer was added and incubated at 55°C for 10 min. After incubation, 370 µl of 2 M sodium acetate solution was added and mixed. To this mixture, 500 µl of cold 100%

ethanol was added and centrifuged for 1 min at 12,000 rpm. Supernatant was discarded and the pellets were washed with 1 ml of 70% ethanol and then centrifuged for 1 min at 12,000 rpm. The supernatant was then discarded and pellets were vacuum dried for 10 min and resupended in 200 μl of 1X TE buffer (Psifidi, 2010).

Agarose gel electrophoresis

Agarose gel electrophoresis was used for the separation of the DNA fragments by using 0.7% of agarose. The sample DNA which was isolated from various sources are mixed with ethidium bromide, a marker dye and then loaded well in agarose gel which was then kept in position in the electrophoresis chamber filled with buffer and current was applied (typically 100 V for 30 min). The marker dye had a low molecular weight and migrated faster than the DNA. When the marker dye approached the end of the gel, the current was stopped and viewed under ultra violet light (Devenish *et al* 1982).

Estimation of the purity of the DNA

DNA concentration can be accurately measured by the UV absorbance spectrophotometer. The amount of UV radiations absorbed by solution of DNA is directly proportional to the amount of DNA in the sample. Usually, the absorbance is measured at 260 nm at which wavelength of absorbance A 260 of 1.0 corresponds to 50 μg. UV absorbance is also used to check the purity of DNA with the pure sample of DNA where the ratio of the absorbance at 260 nm and 280 nm (A260/A280) was 1.8. The ratio less than 1.8 indicated that the preparation was contaminated either with proteins or with phenol. A standard of known DNA concentration was prepared and the readings were measured by UV spectrophotometer at 260 nm. As the DNA concentration increased the OD value also increased. OD is directly proportional to DNA concentration (Psifidi, 2010).

RESULTS AND DISCUSSION

The entire DNA that has been extracted found to differ in their molecular weight. DNA was seen as separate bands when it was viewed under UV light (**Figure 1**). The concentration of the DNA was estimated by UV spectrophotometer using the formula 50 × OD × 20 μl/4000 and the values were recorded in **Table 1**.

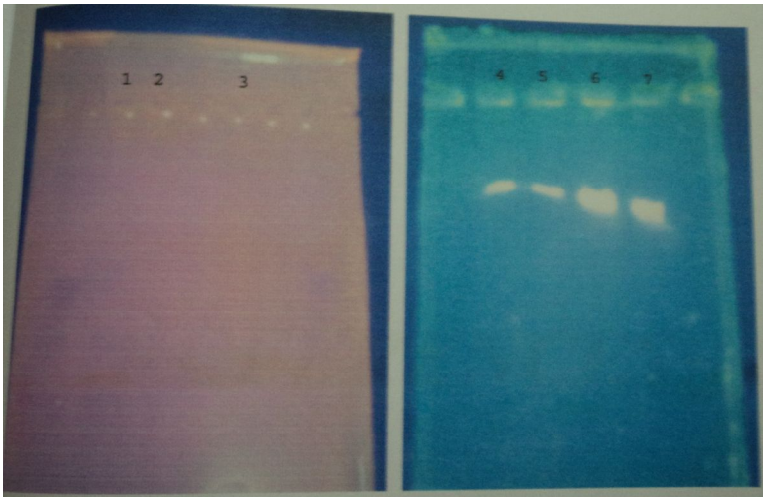

Fig. 1. Agarose gel electrophoresis of DNA isolated from various sources (Lane 1: DNA from fungi, Lane 2: DNA from human blood, Lane 3: DNA from onion, Lane 4 and 5: DNA from fish tissue, Lane 6 and 7: DNA from bacteria)

Table 1. Characterization of extracted DNA from various sources

S. No.	Samples	OD value	Concentration of DNA (μg/ml)	A260/A280
1.	Bacteria	0.562	2,8	1.81
2.	Fungi	0.057	2.7	1.58
3.	Blood	0.066	3.6	1.88
4.	Fish tissue	0.632	3.3	1.59
5.	Onion	0.083	5.3	1.92

The concentration of DNA was found to be more from onion and the ratio of their absorbance at 260 and 280 nm is 1.9 which showed slight contamination, the concentration of DNA was found to be least from fungi and ratio was found to be 1.58 showed the presence of contamination. The DNA which was isolated from the bacteria and blood was found to be 100% pure which was free from contamination. In molecular biology, isolation of purified DNA is required for numerous manipulations. For large scale DNA isolation, several procedures relatively rapid mini preparations have been developed. The time required for extraction and purification depends upon the purity of DNA and its suitability for various procedures such as restricted enzyme cleavage, ligation and cloning. Banding of DNA in CSCl or similar density slat gradients is required when the highest purity of DNA is prepared. For the isolation of Plasmid DNA a rapid two layer CSCl gradient techniques was developed. For the isolation of DNA from eukaryotic cells, no equally rapid procedures have been developed. 100,000 rpm table top centrifuge reduced the time required for preparing CSCl-purified DNA within 5 h (Pramanick *et al* 1976). For the detection and identification of fish species in the processed food products, DNA is reported to be the most appropriate molecule. In all the tissue types, DNA has a greater stability at high temperature and differentiation of closely related species is offered by the genetic code (de Kloet, 1984).

CONCLUSION

Various pathological conditions and changes that occur during the diseased states get reflected in the sequence of the DNA which produces the unwanted reactions in the body. So, it is becoming necessary to isolate the DNA in the pure form to study the various pathological conditions that occur due to the effect of external compounds inside the body as well as to develop the new strains which contain positive characteristic in themselves.

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