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A brief analysis brief analysis on available methods for genomic DNA exaction from various sources:

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ABSTRACT: Deoxyribonucleic acid is a molecule composed of two polynucleotide chains that coil around each other to form a double helix carrying genetic instructions for the development, functioning, growth and reproduction of all known organisms and many viruses. The ability to extract DNA is of primary importance to studying various aspects associated with detection and diagnosis of genetic causes of disease, development of diagnostics methods and formulation of drugs. In the era of genomics, DNA extraction from different sources is requiring to further utilize this DNA molecule. Extracted DNA may be used in forensic science, sequencing genomes, detecting bacteria and viruses in the environment and for determining paternity. Due to all this application, it is need of an hour to have rapid and single step DNA extraction method from various sources including Prokaryotes and Eukaryotes. DNA extraction procedure proposed byEdward et al., 1991 was modified to yield DNA extraction from Prokaryotes and Eukaryotes. Edward et al. uses combination of four chemical namely Tris HCl, NaCl, EDTA and SDS as extraction buffer followed by Precipitation of DNA by Isopropanol and DNA precipitate were dissolved in T.E. buffer. The modification involves alteration and elimination of some steps in Extraction process. The Extraction of DNA from Prokaryotes (E.coli) and Eukaryotes (Yeast, Plant) was carried out using the modified technique. The purity of extracted DNA was measured by taking ratio of Absorbance at 260/280 nm. The validation of extracted DNA was performed by agarose gel electrophoresis. Through modification of the Edwards method of extraction, a protocol that greatly improves the efficiency of Prokaryotic and Eukaryotic DNA extraction and reduces the potential or sample contamination while simultaneously yields high quality DNA. This extraction method yields high quality of Prokaryotic and Eukaryotic DNA with improved efficiency while greatly minimizing the potential for cross contamination. This low-cost and environment-friendly method is widely applicable for plant molecular biology research.

Keywords: DNA extraction, Rapid Method, Eukaryotes, Prokaryotes.

I. INTRODUCTION

The ability to extract and detect specific DNA sequences is now a powerful tool used in a wide variety of applications such as disease detection, qualitative trait loci (QTL) selection and mutant screening. As in diagnosis of disease, nucleic acid-based analysis has many advantages over traditionally used methods such as enzyme or antibody-based assays offering increased sensitivity, faster sample-to-answer results, and flexibility as it can be rapidly modified to meet new challenges as they arise (1).

Previous methods for the extraction of DNA from prokaryotes and eukaryotes take several hours to complete. These methods include the use of bead-vortexing, lysozyme, CTAB and mechanical lysis using high speed cell disruption. Although those methods can be used to extract DNA, they still have the drawbacks including laborious manipulations, such as four to six changes of microcentrifuge tubes, incubation, precipitation, elution or washing and drying steps or even special equipment. Thus, the release of DNA is often poor due to multiple manipulations (2). The hypothesis was drafted to develop relatively simple method for the extraction of DNA from Prokaryotes and Eukaryotes.

II. BRIEF DESCRIPTION OF AVAILABLE METHODS

All the existing methods involve 1. Breakdown of cell wall 2. Separation of thesoluble DNA from cell debris and other insoluble material 3. Precipitation of DNA that hasbeen extracted from cell and 4. Purification of DNA that is precipitated. The cell wall isfrequentlybroken down using physical,chemical,or enzymatic digestion.Physically thecell is broken down with a bead-beating technique or crushed with a mortar and pestle,chemical lysisis can be doneby usingionicdetergents, and forenzymatic digestion,

lysozyme can be used. Separation of DNA from cell debris can be done utilizing phenol and chloro form. DNA is precipitated with the help of alcohol (3).

2.1 CHEMICAL EXTRACTION METHODS

These methods rely on biochemical properties of cellular components to elicit desired molecular separation and might exhibit preference or exclusively in extracting DNA or RNA, depending on intrinsic characteristics (4).

2.2 Phenol: Chloroform Extraction

Organic extraction procedure for the recovery of DNA commonly employs a buffer containing one or several detergents, which include sodium dodecyl sulfate (SDS), dithiothreitol (DTT), ethylenediaminetetra acetic acid (EDTA) and NaCl. During the process SDS serves two functions. First it helps in the disruption of polar Interactions in the cell membrane for facilitating its breakdown and secondly it causes the lipids and proteins to precipitate out of the solution (5,6).

Additionally, DTT is combined into the reaction mix, when the Desired outcome is differential separation of male and female DNA as observed in Sexual assault or rape cases. By virtue of DDT, protein disulfide bridges that are Unique to sperm cell nuclear membranes are broken down (7).

Addition of EDTA during the extraction process has shown to inhibit DNA degradation within the Sample. EDTA acts as a chelator and binds divalent cations comprising Mg2+ and Ca2+, both of which play critical roles in cell membrane stability. In addition to this, Mg2+ is a cofactor for the cellular nucleases. While this cation is bound by EDTA, Nuclease can no longer degrade the DNA, hence preservation of DNA‟s integrity is maintained. Moreover, NaCl causes proteins to coagulate together once they interact with water molecules and in this fashion, proteins are precipitated. Following these processes, an enzyme namely proteinase K, is added to the solution. Proteinase K is a serine protease, an enzyme that catalyzes the hydrolytic cleavage of peptide bonds between the amino acids known as the building blocks of proteins. It has been observed that Proteinase K exhibits broad cleavage specificity for cellular proteins, wherein it cleaves peptide bonds of aliphatic and aromatic amino acids (8).

This enzyme in addition, works over a wide range of pH values besides functions in the presence of detergents like SDS. These factors make it an extremely useful enzyme for general digestion of proteins during nucleic acid purification. After addition of the proteinase K, many extraction protocols call for an extended incubation of the sample at 56°C. At this temperature many proteins are partially or fully denatured, facilitating their digestion by proteinase K, which achieves close to its maximum activity between 20°C and 60°C. In addition, at such high temperatures, enzymes (including DNAase) are denatured and inactivated (9).

Once all of the above-mentioned reagents are utilized in the preparation of cell lysate, an equal volume of phenol/chloroform is next added to this cell lysate, also called as the aqueous phase (10).

When together, these organic solvents (phenol and chloroform) work effectively to denature proteins and aid their precipitation from the lysate. It is worth to mention here that phenol itself is a good solvent for protein denaturation but is known to dissolve small amounts of DNA. Fortunately, in a phenol/chloroform mixture, DNA does not dissolve (11).

In the process, phenol gets stabilized by chloroform and simultaneously increases the density of the organic solvent, as well as denaturation of proteins and facilitates removing lipids from the lysate. Additionally, isoamyl alcohol aided to reduce the foaming of proteins that occurs during the extraction process and as such phenol/chloroform/isoamyl mixture is used (12).

The organic phase having a higher specific gravity, settles to the bottom of the tube, while as the lighter aqueous phase remains on the top. This principle is enunciated for the separation as the unwanted proteins and cellular debris collect in the organic phase or at the interface and the double-stranded, negatively charged DNA remains in the aqueous phase (top of the tube). This allows an easy transfer of the DNA. Note worthily, it has been observed that 7 to 8.5 are the ideal pH values that in addition ensures that nucleic acids of the DNA molecule are negatively charged, hydrophilic and optimally soluble in the aqueous phase (13).

The aqueous phase containing DNA is transferred to a new tube and an equal volume of chloroform (or chloroform- isoamyl alcohol) may be added to the solution. Consequently, the organic phase settles to the bottom of the tube while the DNA containing aqueous phase remains again on top and is accordingly transferred. This step is utilized to remove the traces of phenol that might have transferred with the aqueous phase in previous

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steps. In order to precipitate the DNA out of the solution, a salt (commonly sodium acetate) and an alcohol (commonly isopropanol or ethanol) is added to the aqueous phase. The addition of salt facilitates, neutralization of charged DNA which occurs when positively charged sodium ions approach the negatively charged phosphate group on the DNA backbone. This very association makes DNA less hydrophilic and hence, less soluble in water (14).

In addition, alcohol reduces the solubility of DNA by facilitating the attraction of positive sodium ions (salt) and negative phosphate ions of the DNA backbone (15).

Following this, sample is subjected to centrifugation. Supernatant is consequently removed and the DNA pellet is rinsed with alcohol (usually 70 -80% ethanol). Ethanol washes the sample besides dissolving the salt without resolublizing nucleic acids, hence the probabilities of co-precipitation are eliminated. The tube containing DNA pellet is air dried for the removal of excess ethanol. Following complete evaporation of alcohol, the pellet is dissolved in water or a low ionic strength neutral buffer (TE buffer) and storage of DNA is likewise enhanced (16).

2.3 SALTING OUT DNA EXTRACTION METHOD

Salting Out represents a very inexpensive protocol that has been found Suitable for DNA purification after proteinase K and SDS digestion. Since the Solubility of proteins relies on several parameters which include: pH, temperature and Ionic strength of the solution, this forms the basic principle in salting out DNA Extraction and accordingly precipitation of protein occurs at a high salt concentration (17).

It is worth to mention here that low salt concentration causes an Increase in protein solubility (salting in) whereas high salt concentration leads to a Rapid decrease in protein solubility thereby facilitating precipitation of proteins (salting out). Hence salting out remains the simplest and most affordable DNA extraction method, wherein the principle of insolubility of long strands of DNA in a specific salt concentration is evoked (Rivero et al., 2006). Following the complete digestion of sample by Proteinase K, saturated NaCl solution is added to the tubes for facilitating protein precipitation. While the precipitated protein mass (pellet) remains at the bottom of the tube, supernatant containing the DNA is transferred to another fresh tube. The DNA is recovered from this tube by the precipitation of DNA which is brought about by the addition of ethanol or isopropanol (18).

2.4 CESIUM CHLORIDE DENSITY GRADIENT METHOD

Matthew Meselson, Franklin Stahl, and Jerome Vinograd developed this method at the California Institute of technology. Density gradient centrifugation is based on the principle that the substances are separated based on their size, shape, and density (19).

Meselson and Stahl invented a specific type of centrifuge called isopycnic centrifuge that extracted DNA based on density alone using a solution of cesium chloride. By providing high centrifugal force, the solution of cesium chloride molecules can be dissociated (20).

The heavyweight Cs^+ atoms will be enforced away from the center in the direction of the outer end of the tube, thus forming a shallow density gradient. DNA molecules will be placed during this gradient will travel to the resolution where they find an equivalent density (21).

2.5 GUANIDINIUM THIOCYANATE-PHENOL-CHLOROFORM METHOD

GuSCN-Phenol-Chloroform- is a liquid-liquid extraction technique initially developed by Piotr Chomczynski and Nicoletta Sacchi. It is largely used for RNA as well as DNA isolation (22).

This method has a higher purity and advantage of high recovery that is desired for downstream applications. It depends on phase separation by centrifugation of a combination of the aqueous sample and a solution comprising phenol and chloroform, giving rise to the upper aqueous phase and lower organic phase (23).

Guanidinium thiocyanate, a chaotropic agent, is added to the organic phase to assist in the denaturation of proteins. The nucleic acid parted into the aqueous phase and proteins in the organic phase. Nucleic acid gets purification is regulated by the pH of the mixture. Under acidic conditions (pH 4-6), DNA gets into the organic phase while under neutral pH both DNA and RNA partition into the aqueous phase. Nucleic acid was recovered from the aqueous phase through precipitation with 2% isopropanol (24).

This method gives decent outcomes but requires a lot of time and a large number of samples. This method was later was modified by different researchers for quick extraction of RNA

from small amounts of samples. One of the kit-based procedures that involved prompt isolation of RNA by adjusting this method involves 6 steps i.e., 1. Homogenization 2. Extraction 3. Precipitation 4. Washing and 5. Solubilization. The whole method took about 4 hrs. to complete. The entire range of RNA molecules was obtained with (4S and 5S) RNAs (25).

2.6 CETYL TRIMETHYL AMMONIUM BROMIDE METHOD

DNA extraction from plant tissue is tough owing to occurrence of firm cell wall adjacent to plant cells. Essentially any mechanical means of breaking down the cell membrane and membranes to permit access to nuclear material, without its degradation is required. CTAB is a detergent used to break open plant cells and then solubilize their insides (26).

Cell disruption is improved by denaturing and precipitating the cell wall lip polysaccharides and proteins with the help of CTAB. In the occurrence ofNa+ concentrations above 0.5M, the DNA will remain soluble. EDTA stops nuclease activity by binding divalent cations. The CTAB technique can be enhanced to obtainbetter and quicker outcomes. In one of the experiments, it was modified by increasing the temperature to reduce incubation time and the quality of DNA yield was still excellent (27).

2.7 ALKALINE LYSIS METHOD

For extracting plasmid DNA, or RNA from the bacterial cells alkaline lysis is the preferred method. It is possibly one of the most suitable methods as it is a fast, consistent, and relatively clean way to acquire DNA from cells (28).

The alkaline lysis method relies on the property of plasmid DNA to rapidly bind following denaturation. With the help of strong alkali and detergent, bacterial cells are lysed and cell content is released into the medium. High alkali concentration denatures both genomic and plasmid DNA, after the addition of neutralization buffer genomic DNA is precipitated while plasmid DNA remains in the solution. Soluble plasmid DNA can be precipitated by alcohol (29).

Enough quantity of DNA could be isolated with this procedure. Later this process was modified to make it more versatile, cost-effective, and rapid. In the enhanced technique, the alkaline lysis method was combined with Guanidinium HCl. The alkaline condition causes denaturation of chromosomal DNA and Guanidinium HCl precipitates of protein- SDS complexes and in this way, major parts constituting contaminants are reduced to yield high-quality desired molecules (30).

III. SOLID PHASE EXTRACTION METHOD

The solid-phase extraction of the nucleic acid protocol is comparable to the traditional phenol extraction for DNA purification, apart from the phenol is substituted by insoluble particulate materials that are chemically similar to phenol and hence perform similarly. Proteins have a comparatively high affinity for these particulate molecules, whereas nucleic acids have a very low affinity (31).

It is feasible to isolate enormous amounts of proteins (tens of milligrams) from tiny amounts of nucleic acid (sub microgram) and quantitatively recover the nucleic acid in a biologically active state by using these materials. In comparison to alternative methods for purifying nucleic acids now in use, protocols based on these materials offer speed, quantitative DNA recovery, safety, and suitability (32).

3.1 SILICA BASED EXTRACTION METHOD

The use of harmful chemicals is not essential in this method. The unique feature of the silica matrix is the foundation for all products for quick and effective DNA purification. Nucleic acids are attracted to silica beads under high chaotropic salt concentration (33).

At optimal pH and salt concentration silica is shielded with positive ions allowing negatively charged nucleic acid to bind with the matrix and the remaining cell components and chemicals are washed out of the matrix. Later nucleic acid is extracted by altering the concentration of the solution. Sodium ions play a vital role in this procedure by binding to a negatively charged phosphate group of nucleic acid and neutralizing it, a process is known as salting out. High purity of DNA is obtained using this method (34).

The major disadvantage of this procedure is that the silica matrix can be used only once because for a second-time silica binding capacity gets reduced. To overcome this problem this technique was modified such that the silica matrix can regain its original binding capacity multiple times. In this method, two new buffers were added to the silica matrix and DNA purification was done in only 6 minutes. A single DNA binding column

can be used 20 times (35).

3.2 MAGNETIC BEAD BASED METHOD

Magnetic separation is an evolving process that utilizes the property of magnetism i.e., magnetism for the active isolation of micrometer-sized and ferromagnetic particles from chemical or biological suspensions. The clue of utilizing magnetic separation procedures to decontaminate biologically active compounds (nucleic acids, proteins, etc.), cells, and cell organelles directed in the direction of a regrowing curiosity over the last decade. Newly evolved magnetic particles have enhanced properties for the partly complex isolation procedures in these areas. For the isolation process, magnetic carriers which are immobilized and possess affinity ligands or biopolymers with affinity to the target nucleic acid are utilized (36).

Many magnetic carriers are available commercially and can also be made in the lab. Magnetic particles made from various synthetic polymers, biopolymers, porous glass, or magnetic particles made from inorganic magnetic materials like surface-modified iron oxide are examples of such materials. Superparamagnetic particles, which do not interact with one another without a magnetic field, are particularly well suited. When exposed to a high magnetic field, these particles get magnetized but lose their magnetism when the field is removed. The particles that are present in suspended form and steady extraction of nucleic acids are ensured when magnetic aggregation and clumping of the particles are avoided during the procedure (37,38).

In these systems, the magnet is used to attract and hold the paramagnetic beads to the side of the tube to allow supernatant removal during the wash and elution steps. This technique can be useful to extract DNA from eukaryotic samples. Kits are available in the market that combines surface chemistry and lysis solution to allow the binding of soluble genomic DNA to the surface of superparamagnetic, monodisperse particles. The method is simple and easy to perform, giving a suitable amount of DNA for downstream processing (39).

3.3 ANION EXCHANGE METHOD

Ion exchange chromatography has emerged as a reliable alternative to classic CsCl-ethidium bromide gradients for isolating nucleic acids of high purity. This process is similar to the silica-based method except the silica is negatively charged and here the matrix is positively charged (40).

Anion exchange is a method that parts substances by their charges by making use of an ion-exchange resin comprising positively charged groups, such as diethyl-aminoethyl groups (DEAE) (41).

In the solution, the resin is covered with positively charged and negatively charged ions (cations). Anion exchange resins will bind to molecules that contain a negative charge, relocating the counter-ion. Anion exchange chromatography is commonly used to decontaminate proteins, amino acids, sugars/carbohydrates, and other acidic substances with a negative charge at higher pH levels. The tightness of the binding between the substance and the resin is based on the strength of the negative charge of the substance (42).

For DNA purification, binding, washing, and elution, conditions are optimized to achieve efficient isolation of DNA. This method is well suited to eukaryotic systems (43).

3.4 CELLULOSE MATRIX-BASED METHOD

The cellulose matrix is used as an adsorbent for the purification of molecules. cellulose being a polymer of glucose, it is highly hydroxylated, which leads to a polar attraction that is strong enough to bind nucleic acids under certain chemical circumstances. In a study performed a simple nucleic acid purification method was developed that does not require modern laboratory equipment. In this method, the property of DNA to bind with cationic chemicals that could help in capturing DNA or RNA was used. DNA was purified by making use of Chitosan and polyethyleneimine (PEI) because these compounds show strong attraction with nucleic acid and the purified DNA was tested for its ability to get consistent results in PCR. In this research, was designed a simple nucleic acid extraction procedure was tested to get whether it was likely to remove PCR-inhibiting chemical/biological contaminants present in a plant crude extract while retaining DNA for amplification. It has been stated that the use of cellulose-based paper can be appropriate as it can bind with DNA and RNA and purifies nucleic from a wide variety of plant and animal tissues (44).

3.5 OTHER METHODS

In the field of molecular biology, the extraction of DNA from various sources is often the first step and the application follows DNA extraction. In a study, performed, DNA extraction

was carried out from samples of varied origins and the quality and amount of obtained DNA were assessed using agarose gel electrophoresis and 260/280 ratio respectively (45).

The same method was able to extract genomic DNA from both gram-positive and gram-negative. However, since various samples were used various types of inhibitors were co-extracted with DNA that could interfere with the downstream processing. Those contaminants were removed by different chemical treatments and later PCR of extracted DNA gave satisfactory results (46).

In a defined study for optimization of various DNA extraction procedures from soil samples. In this particular study, utmost common elements of DNA extraction and decontamination procedures were compared and the data was used to frame an all- inclusive purification method for attaining whole community DNA from soil and sediment samples encompassing diverse amounts of organic matter. about this procedure nine different nucleic acid purification methods were optimized intended for DNA extraction nevertheless an in-detail assessment of discrete procedures hasn't been accomplished. A different set of chemical treatments were used at each stage to evaluate(i) efficiency of cell lysis (ii) total yield of DNA, and (iii) molecular sizes of DNA fragments. The result found was an optimized isolation of DNA and purification protocol for soil and sediment having varied organic matter content (47).

A quick process intended at the direct extraction of DNA from soil and sediments was developed by Yu-li Tsai and Betty H. Olson, 1991 in 1991 (48). The application DNA extraction methods to environmental samples can obviate the need for cell cultivation, since cell cultivation has the disadvantage of obtaining only a very small proportion of total microbial community.

In a study performed a similar kind of study on comparison of DNA extraction methods for food analysis. He carried out two DNA extraction and purification for food and compared vis-à-vis extraction efficiency, DNA purity and DNA appropriateness for amplification. Silica matrix was used in both the methods as affinity matrix for binding of nucleic acids. The difference was that one of it used a mobile solid phase, while other is a column-based system. Concentration of DNA was determined by taking the absorbance reading at 260nm. DNA purity was determined by calculating the ratio of absorbance values obtained at 260-280nm. The comparison of these two extraction methods, highlighted a different efficiency in extraction and removing the inhibitors interfering in the PCR test. This technique is less time consuming and technically demanding. This study emphasized the necessity of a appropriate extraction method to get highly purified nucleic acids with no inhibitor, which if not removed may be inhibitory to downstream PCR application (49).

Wen-ping Cheng and Tsong in 1993 assessed two main protocols for the preparation of bacterial genomic DNA and presented a simple and rapid method for extraction of bacterial genomic DNA. In this study four different gram-negative organisms were used. The organisms used in this method produce copious amounts of polysaccharide, which can affect DNA purity. Method described here is relatively short and simple and also avoids use of enzymes for cell lysis. It needs a single chloroform extraction, and the process takes approximately 1 hour to complete (50).

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