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Strategies for optimal DNA extraction from Prokaryotic Cells: A Comprehensive review

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Abstract : DNA extraction from prokaryotic cells is a fundamental step in molecular biology and biotechnology research. It is challenging to achieve high-quality DNA yields with minimal contamination. This review emphasis on a comprehensive design approach for optimizing the DNA extraction procedure from prokaryotic cells. The methodology involves systematically assessing and fine-tuning various extraction parameters, including the choice of cell lysis method, selection of DNA purification techniques, optimization of reaction conditions (pH, temperature, incubation time), and integration of suitable controls to minimize experimental variability. The design approach emphasizes the importance of cell wall composition, DNA-binding properties, and enzymatic activity specific to prokaryotic cells. Additionally, advanced techniques such as bead beating, enzymatic digestion, and magnetic bead-based purification enhances DNA extraction efficiency and purity. The framework provides researchers with a systematic methodology to optimize DNA extraction protocols tailored to the unique characteristics of prokaryotic cells, enabling reliable and reproducible DNA isolation for downstream applications in genomics, metagenomics, and microbial ecology.

Keywords: Extracted DNA, Optimization, Purification, High-quality DNA and Prokaryotes.

Introduction

Prokaryotes are organisms whose cells lack a nucleus and different organelles. The majority of prokaryotes have a tiny quantity of genetic material in the form of a circular DNA single molecule, or chromosome. The DNA in prokaryotes in contained in a leading location of the mobile known as the nucleoid, which is not surrounded by way of a nuclear membrane. (Kobilinsky, L. F., et al., 2005).The size of bacterial genomes stages from sort of 0.4 $*10^9$ to 8.6 $*10^9$ daltons (Da), with a number of the smallest being obligate parasites (*Mycoplasma*) and the most crucial belonging to bacteria capable of complicated differentiation, such *Myxococcus*. The maximum quantity of information that the genome can encode relies upon on the quantity of DNA there is in it. The majority of bacteria have haploid genomes, which might be made from a single round, double-stranded chromosome. However, Gram-positive *Borrelia*. Had been suggested to have linear chromosomes, at the same time as the Gram-negative bacterium *Agrobacterium tumefaciens* has ever a linear and a round chromosome. (Chaudhuri, S. R., et al., 2006)

I. The molecular approach in bacteriology involves the extraction and purification of bacterial DNA, which is essential for maintaining biological function. Different isolation methods have been developed, including the traditional phenol: chloroform extraction method . (Gross-Bellard M et al., 1973, Sambrook J, et al., 1989). Emphasize the importance of DNA in this process, as it stores biological information through its linear sequence of polynucleotides. DNA is translated into mRNA and transcribed into amino acids, determining the protein's three-dimensional structure and its biological function. Over time, different isolation methods have evolved, each with its advantages and disadvantages (Alberts et al.,2002).

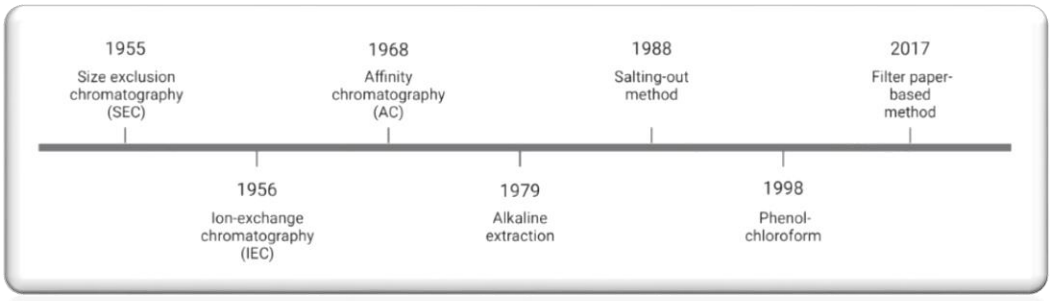

Fig:1 Timeline of the development of different DNA extraction techniques and their inventors.

2. Extraction of DNA

DNA extraction is a crucial step in processing biological samples for PCR-based forensic analysis1869 (Gupta, N. 2019). It involves separating DNA from cellular membranes, proteins, and other biological components using bodily and chemical methods. Conventional methods like phenol and chloroform are time-consuming and carcinogenic (Silva, A. Et al., 2021). Mobile extraction and direct lysis are the two primary techniques for isolating microbial DNA from soil (Saano, A., et al., 1995). Both techniques use strategies like repeated freezing and thawing cycles, sonication, boiling, liquid nitrogen, bead beating, osmotic stress, SDS, and lysozyme (Sørensen, S. J., et al., 2002). Separating soil microorganisms is essential before mobile lysis and DNA recovery. Bacterial genomes range from 0.4 *109 to 8.6 *109 daltons (Da), with some being obligate parasites and others being complex bacteria like Myxococcus (de Lipthay, et al., 2004).

The process of separating DNA via physical or chemical means from proteins, cell membranes, and other biological components is known as DNA extraction. Solid phase extraction, inorganic extraction, and organic extraction are common techniques. The four stages of DNA purification include:

1. Cell lysis of cell membrane
2. Cellular proteins degradation and precipitate
3. Dissociation of cell proteins and other cellular factors from nucleic acid
4. Nucleic acid precipitation (El-Ashram, et al., 2016).

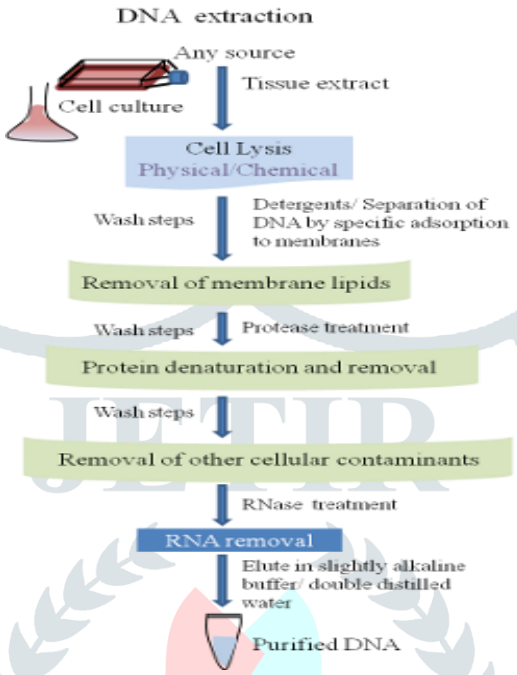

Fig:2 Basic step in DNA Extraction (Dhaliwal, A, 2013).

Cell lysis

Three types of mobile lysis, bodily, chemical, and enzymatic, along with membrane disruption, are currently used in soil treatment. Physical treatments that alter soil shape are most accessible to the entire bacterial community, including microorganisms deeply embedded in soil micro aggregates. The most famous bodily disruption techniques include bead-mill homogenization (Niemi, R. M, et al., 2001, Miller, D. N., 2001, Miller, D. N., et al., 1999 and Steffan, R. J., et al.,1988) and freezing-thawing or freezing-boiling (Degrange, V. And Bardin, R., 1995, Moré, M. I. Et al., 1994, Tsai, Y. L., et al., 1991). A "bead beating" approach has been tested, revealing that DNA yields increase with longer beating intervals, excessive beating speeds, and smaller extraction buffer volumes, but it causes DNA shearing (Bürgmann, H., et al., 2001).

Sodium dodecyl sulphate (SDS) is commonly used to dissolve hydrophobic cell membranes. Detergents, including chelating agents like EDTA ,Chelate 100 (Herron, P. R., and Wellington, E. M., 1990, Jacobsen, C. S. And Rasmussen, O. F., 1992), Tris buffers, sodium phosphate buffers and heat treatment, are often used together. Studies have shown that increasing EDTA concentration strengthens extraction and lysis buffers, resulting in higher yields but less natural isolated nucleic acids. This suggests that the choice of buffer represents a trade-off between the desired DNA purity and the predicted DNA amount (Krsek, M. And Wellington, E. M. H., 1999).

Methods of DNA Extraction

Techniques for extracting DNA include non-natural methods like salting out and proteinase K remedy in addition to organic techniques like the phenol-chloroform method and adsorption strategies like silicone-gel membrane (Gupta, N. 2019).

1. **CHROMATOGRAPHY BASED DNA EXTRACTION:**

DNA extraction techniques are primarily based on chromatography (M. Carpi, F., et al., 2011)., including size exclusion chromatography (SEC), ion exchange chromatography (IEC), and affinity chromatography (AC). SEC separates molecules based on length and type, while gel filtration chromatography uses an aqueous solution to transport DNA-containing fabric down the column. Ion-trade chromatography (IEC) is a great chromatography-based approach for removing DNA. To equilibrate the column, a solution containing DNA anion change resin is used to bind DNA with diethylamino ethyl cellulose (DEAE) corporation (Peterson, E. A. And Schorr, J., 1956, Cuatrecasas, P., et al., 1968). Natural factors such as proteins, lipids, carbohydrates, metabolites, and RNA are eluted simultaneously while DNA is stored in the column. The pH can be decreased or DNA can be recovered using immoderate salt buffers (Budelier, K. And Schorr, J., 1998). This method is easier than other excellent DNA extraction techniques like CsCl-gradient centrifugation (M Carpi, F., et al., 2011). This water-based DNA purification approach is less difficult, purifier, and more experienced, and does not use ethanol salts, natural solvents that affect the quality of extracted DNA, or the time-consuming precipitation step.

2. SALTING OUT METHOD:

The salting out technique is a method used for DNA extraction where high salt concentration is added to the DNA sample, causing it to precipitate out. This process increases protein solubility when the salt concentration is low, and decreases when it is excessive. After digestion with proteinase okay, the sample is salted out by adding saturated NACL and shaking for 15 seconds, followed by centrifugation at 3000 x g for 15 minutes. The DNA-containing supernatant is then transferred to a separate tube and induced with ethanol or isopropanol to clean the DNA, leaving the protein pellet at the bottom (Mwer, S., et al., 1988).

3. SDS PROTEINASE K:

Proteinase K is a broad-spectrum serine protease used to degrade proteins and remove contaminants from nucleic acid preparations. It is active in the presence of protein denaturing agents like SDS, UREA, and EDTA and strong across a pH range of 4-12. Ebeling., et al discovered Proteinase K in the fungus Engyodontium album in 1974 (Thomas, S. M., et al., 1989).

4. CTAB EXTRACTION METHOD:

The cetyltrimethylammonium bromide (CTAB) method is used to extract and purify DNA from vegetation and food products. It was developed by Murray and Thompson in 1980 and is particularly useful for removing polysaccharides and polyphenolic substances that could compromise DNA purity. CTAB buffer is used to lyse mobile membranes. CTAB is a first-class technique for challenging materials, aiding polysaccharide separation and nucleic acid recovery. It prevents DNA breakdown by chelating magnesium ions needed by DNA-breaking enzymes (K.L. Tiwari, et al., 2012). Beta-mercaptoethanol, when combined with CTAB, facilitates protein breakdown after polysaccharide breakdown of the mobile membrane. CTAB partially denatures different proteins while inhibiting a few. It is related to excessive salts acting as reducing agents and selective nucleic acid precipitants (Lekgari, G. L. P., 2010).

5. MAGNETIC BEADS

In 1998, Trevor Hawkins filed a patent application for the separation and purification of DNA using magnetic particles. Magnetic separation is an evolving process that uses magnetium's properties to isolate micrometer-sized and ferromagnetic DNA from chemical or biological suspensions suspension (Berensmeier 2006; Olsvik et al., 1994). DNA is attached to magnetic nanoparticles by coating them with a DNA-binding antibody or polymer (Elkins, K. M., 2013). The core of magnetic beads is typically magnetite or maghemite, with silica and functional groups like sulphate and hydroxyl groups . (Dairawan, M. and Shetty, P. J., 2020). A magnetic field is created at the bottom of the tube using an external magnet to separate the DNA-bound magnetic beads from the cell lysate. The supernatant can be removed by rinsing. The ethanol precipitation method is used to elute the magnetic pellet, which is then subjected to an incubation stage at 65°C to separate the magnetic particles from the DNA (Peterson, E. and Sober, H. A., 1956). The process can be completed in 15 minutes, making it quicker than other conventional methods. The yield of DNA produced by this method is comparable to other conventional procedures (El-Aal, A., et al., 2010). It is ideal for automation and requires less equipment. However, it is less economical than centrifugation-based methods (M Carpi, F., et al., 2011, Fomovskaia, G., et al., 2004).

6. EtBr-CsCl Gradient Centrifugation Method

A method for separating DNA from bacteria was created in 1957 by Jerome Vinograd, Franklin W. Stahl, and Matthew Meselson. This entails mixing DNA with cesium chloride (CsCl) and rapidly ultracentrifuging the mixture for more than ten hours (10,000 to 12,000 rpm). Depending on density, one or more DNA bands may appear as DNA separates from other components. Supercoiled DNA can assemble at lower densities thanks to the intercalating agent EtBr. DNA is visible in the UV light, and EtBr and CsCl are removed prior to ethanol precipitation (Meselson et al.,1957). The lengthy period of high-speed ultra-centrifugation makes this procedure difficult, expensive, and time-consuming (Leland et al., 2012).

7. **Chelex-100 Extraction**

A chelex-100-based DNA extraction method was patented in 2011 by Xiong Hui, Xie Liqun, and Chen Jiayi. This technique chelates metal ions that function as DNase cofactors by using chelex resin, a styrenedivinylbenzene copolymer. A 5% chelex solution is added, then proteinase K is added to break down the DNases. The tissue sample is brought to a boil, the proteins and DNA are denatured, and the membranes are lysed. Chelex stops any leftover DNases from destroying DNA, stabilising the preparation. From the supernatant, the single-stranded DNA is separated and concentrated (Ashry, et al., 2018). By using a single test tube and fewer manipulation steps, this approach lowers the risk of contamination and improper pipetting. However, it may be unstable for RFLP analysis and is ineffective in eliminating PCR inhibitors (Shi and Panthee., 2017).

8. **Filter paper-based DNA extraction method**

In 2017, Rui Shi and Dilip Panthee presented a DNA extraction method based on filter paper. The method uses a 96-well plate with a bottom hole drilled to accommodate an 8 mm-diameter Whatman TM filter paper disc. Centrifugation is then employed to filter the samples, which is far less expensive than utilising glass fibre filters found in commercial kits. DNA extracted from plant sources may be separated using this procedure, which is very helpful (Rui and Dilip., 2017).

9. **Phenol- Chloroform DNA Extraction method:**

The phenol-chloroform DNA extraction method, introduced in 1998 by Barker et al., (Elkins K., 2013) is an organic extraction process that involves the addition of detergents such as sodium dodecyl sulfate (SDS), dithiothreitol (DDT), ethylenediamine tetra acetic acid (EDTA), minute acetic acid, and NACL to a buffer. SDS plays two roles in this process: breaking down polar interactions in the cell membrane and precipitating proteins and lipids out of the solution (Gill et al., 1985). For differential separation of male and female DNA, more DTD is added to the process to break down the virtual disulphide bridge of DDT protein, which is specific to the nuclear membrane of sperm cells (Buttler., 2005). EDTA acts as a chelator and binds divalent cation, which play a critical role in cell membrane stability. It also causes protein cogulation when they interact with water molecules, resulting in protein precipitation. Proteinase K is added to the solution, which catalyzes the hydrolytic cleavage of peptide bonds between amino acids, making it an effective enzyme for general digestion of protein during nucleic acid purification (Nelson and Cox., 2005). After adding the reagents, an equal volume of phenol/chloroform is added to the cell lysate (Tan and Yiap., 2009)., also known as the aqueous phase. These organic solvents effectively denature proteins and facilitate their precipitation from the lysate. Although phenol is an effective solvent for denaturing proteins, it is also known to dissolve trace amounts of DNA (Walsh et al., 1992 ; Butler, 2005). However, DNA does not disintegrate when combined with phenol and chloroform (Funabashi et al., 2012). Phenol stabilizes the organic solvent, increases the density of the organic solvent, and facilitates lipid removal from the lysate. Isoamyl alcohol is used to reduce foaming of protein during the extraction process used (Hoff-Olsen et al., 1999). The organic phase with higher specific gravity settles to the bottom of the tube while the lighter aqueous phase remains on the top (Greene, 1996). This principle allows for easy buffering of the DNA, as unwanted proteins and cellular debris collect in the organic phase or at the interface, while double-stranded, charged DNA remains in the aqueous phase.To precipitate the DNA out of the solution, salt (usually sodium acetate) and alcohol (usually isopropanol or ethanol) are added to the aqueous phase. The addition of salt facilitates neutralization of charge DNA, making it less hydrophyllic and less soluble in water (Raspaud, 1998). Alcohol reduces DNA solubility by attracting positive sodium ions (salt) and negative phosphate ions of the DNA backbone (Hengen, 1996). The sample is subjected to centrifugation, and the DNA pellet is rinsed with alcohol (usually 70-80% Ethanol). Ethanol washers the sample, removes excess ethanol, and after complete evaporation, the pellet is dissolved in water or a low-strength neutral buffer (TE buffer) to enhance DNA storage (Sambrook and Russel., 2001).

10. **Silica- based extraction method:**

This method of DNA purification uses a unique silica matrix, which attracts nucleic acids under high chaotropic salt concentration (Esser et al., 2006). The matrix is shielded with positive ions, allowing the negatively charged nucleic acid to bind with the matrix and washes out the remaining cell components and chemicals. The nucleic acid is extracted by altering the solution concentration. Sodium ions play a crucial role in this process by binding to the negatively charged phosphate group of the nucleic acid and neutralizing it, known as salting out (Tagliavia et al., 2009). This method achieves high DNA purity. However, the silica matrix can only be used once, and a modified technique has been developed to regain its original binding capacity multiple times. This method requires adding two new buffers to the silica matrix, allowing DNA purification in just six minutes. A single DNA binding column can be used 20 times (Shao et al., 2008).

Drawbacks in current methods

The process of extracting DNA is intricate and requires a number of processes and techniques from different sources, which increases expenses and time (Rapp, 2010). The usage of toxic substances makes it risky. DNA extraction takes a long period, which makes it challenging for forensic investigations and medical diagnostics (Sa et al., 2011). DNA that has been further extracted is frequently tainted with phenol and protein, producing dirty DNA. Absorbance at 260/280 is a useful tool for evaluating DNA purity; high-preity DNA usually has a ratio of 1.8 (Weir, 1999).

Future challenges:

Due to its importance in polymerized chain reaction and rapd analysis, the DNA section's subsequent application necessitates DNA of suitable quality. The process of authentication is hampered by the poor quality of DNA taken from diverse sources. The cost and toxicity of the chemicals used are among the factors considered while choosing an adequate DNA extraction technique (Chemat et al ., 2019). For instance, chemicals like phenol and chloroform are utilized in our modified methods of extracting DNA, which greatly increases the risk of contaminating DNA and health. The quality of extracted DNA is more important than quantity for a successful PCR amplification process. During the PCR process, several of the chemicals employed in the DNA extraction process may impede DNA polymerase. Additionally contaminating and impeding downstream application are phenol and chloroform(Rapp, 2010).

Downstream application

1. **In disease diagnosis:**

Since the discovery of DNA in 1953—a vital component of modern technology—molecular diagnostics has been a quick diagnostic method. Currently, it makes it possible to identify a variety of mutations and hundreds of diagnostic responses at once (Lucena- Aguilar et al., 2016). In addition to people, viruses, and bacteria are now additional diagnostic targets due to the quick growth of molecular diagnostic testing. Patient treatment has been immensely enhanced by the expanding family tree of inherited illnesses (Huijsmans et al., 2010). Stressing that genetic testing will eventually

encompass a wide spectrum of studies with different consequences for Native Americans and their families is vital. Still grounded in positive promises, this expectancy is more rational in its meaning(Azmat et al., 2012).

2. Crime investigation:

In forensic investigations concerning criminalities and medico-legal identification, forensic identification is an essential approach for verifying the truth (Bukya et al., 2021). The specialist's ability to match trace evidence from the crime scene with reference evidence is critical to the identification procedure (Stepaniuk et al., 2019). Because molecular genetic testing may accurately and successfully solve criminal identification cases, its application in investigative procedures is growing. Because DNA analysis techniques identify the recovered object based on its distinguishing qualities, they are essential for solving major crimes such as rape and murder. An individual's distinctiveness stems from their specific genetic code, which is a reflection of their DNA profile. The common identification study model developed in research theory is used to carry out the identification procedure in molecular genetic analysis (Lewontin R.C., Hartl D.L., 1991). Experts analyze the study results to detect different and coincidening traits and determine their implications for solving the research problem (Tobe and Linacre, 2010).

Conclusion

Since Friedrich Miescher carried out the first DNA extraction in 1869, scientists have made incredible strides in creating extraction techniques that are more dependable, simpler, quicker, more economical, and generate better yields. Improved and more dependable methods have contributed to our understanding of the human genome and helped give rise to new scientific disciplines like gene editing and personalized medicine. The one-step DNA isolation method presents a streamlined and efficient approach to extracting DNA from samples. By combining cell lysis, protein denaturation, and DNA purification into a single step, this method significantly simplifies the process of DNA extraction. The elimination of multiple reagents and steps not only saves time but also reduces the likelihood of errors and contamination. Future DNA extraction technology development will be mostly driven by creating new procedures and refining the design of current ones.

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