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DYNAMICS IN DNA EXTRACTION

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ABSTRACT

Since the first DNA extraction by Friedrich Miescher in 1869, scientists have made extraordinary strides in developing extraction methods that are more reliable, easier and faster to perform, less expensive, and yield higher yields. A fast, simple, and inexpensive DNA extraction method with greater yield and purity of DNA would aid in rapid diagnosis, large population screening, and other routine PCR-based applications. The present study evaluated and standardized a rapid and zero-cost DNA extraction from targeted organism for routine molecular studies. Four DNA extraction methods i.e. 1.4 M sodium chloride (NaCl), 20 mM ethylene diaminetetraacetic acid (EDTA, pH 8.0), and elution from blotted nitrocellulose membrane (NCM) were compared with standard CTAB extraction buffer, EtBr-CsCl Gradient Centrifugation Method and salting-out method is a non-toxic DNA extraction method. There has been an increasing demand for reliable and efficient DNA isolation methods that can yield sufficient amounts of high quality DNA with minimal contamination. The current review addresses the development of different DNA extraction techniques from solvent-based methods to physical extraction methods, each with their distinct advantages and limitations.

KEYWORD- DNA, DNA extraction method, Friedrich Miescher, Rapid and zero cost extraction method, Standard method, Salting-out method.

1. Introduction

A crucial advance made in the 1940s was the realization that deoxyribonucleic acid (DNA) is the likely carrier of this genetic information. But the mechanism by which genetic information is copied for transmission from one cell generation to the next, and how proteins are specified by the instructions in DNA, remained the same until 1953, when the structure of DNA was determined by James Watson and Francis Crick , completely mysterious . The structure immediately revealed how DNA could be copied or replicated and provided the first clues as to how a DNA molecule could encode the instructions for making proteins. Today, the fact that DNA is the genetic material is so fundamental to our understanding of life that it is difficult to appreciate the enormous intellectual gap this discovery filled(1).

Now, the extraction of nucleic acids is the starting point of any molecular biology study and is therefore considered a crucial process. The Swiss doctor Friedrich Miescher carried out the first crude DNA extraction in 1869. While examining proteins from leukocytes, he accidentally purified DNA from the cell nucleus and found that the property of this substance is fundamentally different from that of proteins, hence the term nuclein (2).

Double-stranded DNA is present in almost all organisms, single-stranded DNA is found in very few pathogenic viruses, such as members of the families Parvoviridae, Circoviridae, Anelloviridae, and Geminiviridae (5). Miescher owed much of his success in isolating and characterizing DNA to the choice of cells for his experiments. Both leukocytes and spermatozoa are not embedded in tissue or extracellular matrix and can therefore be easily purified. Furthermore, in both, but especially in spermatozoa, the nuclei are large compared to the cytoplasm, facilitating enrichment of nucleus components in purification protocols (2).

When choosing an appropriate DNA extraction method, it is crucial to ensure the quality and quantity of the isolated DNA to perform the intended downstream applications. Other factors that should be considered to optimize the DNA extraction method include time, cost, potential toxicities, yield, laboratory equipment and expertise, and the amount of sample required for the protocol (6). There are numerous other potential uses for isolated nucleic acid, but one innovation that has revolutionized research in genetics is the advent of precision gene editing technology, which has made significant contributions to the fields of medicine, agriculture, and synthetic biology has (7).

Current review discusses the method yields a more quantity of DNA that is comparable to standard protocols and suitable.

2. Materials and Methods

- **EtBr-CsCl Gradient Centrifugation Method.**

This method was developed back in 1957 by Matthew Meselson, Franklin W. Stahl, and Jerome Vinograd (9). DNA is first mixed With cesium chloride (CsCl), the solution is then ultra-centrifuged at high speed (10,000 to 12,000 rpm) for more than 10 hours. With centrifugation, DNA separates from the rest of the substances Based on its density. Depending on DNA types varying by density, One or more DNA bands appear upon reaching the isopycnic Point. Ethidium bromide (EtBr) acts as an intercalating agent and is incorporated comparatively more into non-supercoiled than supercoiled DNA molecules, hence allowing supercoiled DNA to Accumulate at lower densities. Location of the DNA can be easily Visualized under ultraviolet light. EtBr and CsCl are removed prior to precipitation of DNA with ethanol. This method can be used to extract DNA from bacteria, but a Large amount of the material source is required. Moreover, this method is complicated, time consuming and costly due to the long Duration of high-speed ultra-centrifugation required (8).

- **Salting-out method.**

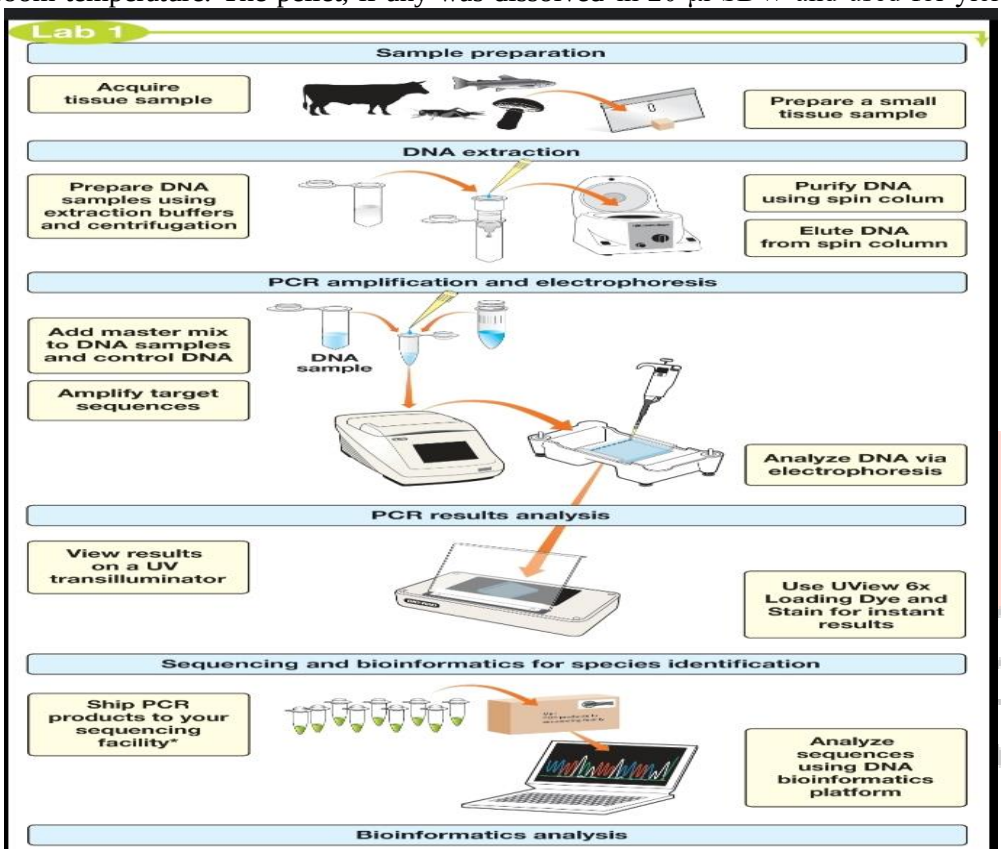
The salting-out method is a non-toxic DNA extraction method described by Miller, Dykes, and Polesky in 1988. The DNA-containing Sample is added to 3mL of lysis buffer (0.4 M NaCl, 10 mM Tris–HCl pH 8.0 and 2 mM EDTA, pH 8.0), SDS and proteinase K. The mixture is incubated at 55–65°C overnight. Next, about 6M of saturated NaCl is added and the mixture is shaken for 15 seconds then centrifuged at 2500 rpm for 15 minutes. The high salt concentration decreases protein solubility, resulting in its precipitation. The DNA-containing supernatant is pipetted into a fresh tube and can be precipitated using ethanol (10). The salting-out method has been reported to yield high quality DNA comparable to that obtained using the phenol-chloroform method, and is superior than the latter in that it is more time Efficient and cost effective and most importantly the reagents used are nontoxic (11). It is also used to extract DNA from blood, Suspension culture, or tissue homogenate (10).

- **Rapid DNA extraction.**

For simple and rapid DNA extraction, individual insects were collected in 1.5 ml microcentri-fuge tubes and crushed separately in 20 µl of SDW, 1X PBS (containing 0.137 M NaCl, 0.0027M KCl, 0.01 M Na2HPO4, and 0.0018 M KH2PO4), 1.4 M NaCl, and 20 mM EDTA using sterile micro-pestles. All the lysates were incubated at 98˚C for 2 min in a water bath and assessed for purity, quantity, and PCR applications. DNA extraction was performed in 10 replicates.
In the case of NCM, individual adults were blotted on the membrane using sterile micropestles. The blotted membrane was air-dried and stored at room temperature. A single dot was cut and taken in a microcentrifuge tube. DNA was eluted in 20 µl SDW by incubating at 98˚C for 2 min and assessed for purity, quantity, and PCR applications (3).

- **DNA extraction using standard methods.**

CTAB extraction buffer, DNA was extracted from individual specimens in CTAB extraction buffer. Briefly, 10 ml CTAB extraction buffer contained 3.5 ml of 10% CTAB, 2.8 ml of 5 M NaCl, 1 ml of 1 M Tris-HCl, 400 µl of 0.5 M EDTA (pH 8.0), 20 µl β-mercaptoethanol, and 2.28 ml SDW. Single adult females of respective species were taken in l.5 ml micro-centrifuge tubes separately and crushed in 100 µl of extraction buffer using micro-pestles. The lysate was incubated at 65˚C for 1 hr with intermittent vortexing at every 10 min interval. An equal volume of chloroform: isoamyl alcohol (24: 1) was added to the lysate, mixed, and centrifuged at 14,000 xg for 15 min. The upper aqueous layer was transferred to a new micro-centrifuge tube A 0.8 volume of chilled isopropanol was added and kept at -20˚C for 30 min. The DNA was pelleted by centrifuging at 14,000 xg for 10 min. The supernatant was decanted gently without disturbing the pellet. The DNA pellet was washed with 70% ethanol. The residual ethanol was removed by allowing the samples to dry at room temperature. The pellet, if any was dissolved in 20 µl SDW and used for yield, purity assessment, and PCR amplification (3).



Source:- https://www.bio-rad.com/en-in/education/overlay/dna-barcoding-kit-activity-flowchart?ID=DNA-Barcod_1627941095

Conclusion

Many techniques for purifying biomolecules have been developed. The automated nucleic acid extraction system was developed due to the influence of the rapid growth of today's automation technology. Recent techniques that are more reliable and efficient have facilitated advances in knowledge of the human genome and played an important role in the emergence of various fields of science such as gene editing and personalized medicine. However, at present it appears that there is no single method applicable to all contexts of DNA extraction due to its limitations in generating yields of optimal purity and convenience of use. Therefore, solutions to overcome the limitations of these techniques are needed to achieve better results and simplify DNA manipulation. Improving the design of existing methods and developing new techniques will be the driving force for the direct advancement of future DNA extraction technology.

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