ISSN: 2582-4686 SJIF 2021-3.261,SJIF 2022-2.889,
2024-6.875 ResearchBib IF: 8.848 / 2024

THE MULTIDISCIPLINARY JOURNAL OF SCIENCE AND TECHNOLOGY

VOLUME-4, ISSUE-5

MODERN METHODS OF EXTRACTING NUCLEIC ACIDS FROM BIOLOGICAL OBJECTS

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Abstract. The study of nucleic acids began many years ago, and each scientist used unique methods in the study. To study nucleic acids, it is necessary to isolate them first. For this reason, several DNA or RNA isolation methods have been created, each of which has its own advantages. This article teaches the most convenient, easiest, most effective methods of extracting nucleic acids.

Key words: Nucleic acids, extracting, isolation, biopolymer, white blood cell, cell lysis, elution, cleaning, SDS, centrifugation, chromatography, salting method

Introduction. Nucleic acids are high-molecular biopolymers with a molecular weight from 250 to 1.2 x 10^5 kDa. Nucleic acids are the only macromolecule that stores information about the structure of a living organism and transmits it to the next generation. A person reading about nucleic acids will certainly try to see them and study them more deeply. For this reason, the isolation of nucleic acids from living cells is currently the first task facing molecular biologists. The extraction of nucleic acids from living objects in its pure form presents some problem. Because nucleic acids bind to protein in eukaryotes, forming a complex complex compound. Even when isolating nucleoproteins, it will be necessary to separate the protein from the DNA, which is the product of nucleotide polymerization.

The isolation of nucleic acids at different times had its own methods, and with them its own difficulties. For example, if laboratory personnel consisting of representatives of different fields are going to work with nucleic acids, then geneticists from a Medical center require DNA extraction from blood to study a particular syndrome. In another corner of the world, a lab technician wearing headphones under loud music is trying as carefully as possible to extract DNA from canned meat. Representatives of criminology tried to isolate DNA through a thin sample taken from the suspect's fingernail.

Analysis and results. The most important event in the history of the study of DNA was the discovery of its structure by Watson and Crick in 1953. However, in fact, the history of the relationship between scientists and nucleic acids began much earlier — in 1869. A young Swiss doctor, Fredrich Mischer, in the process of studying proteins, discovered substances that are not similar in composition to proteins, precipitating when acid is added and dissolving again when alkali is added. These substances isolated from the nucleus of leukocytes, Mischer called nucleins (inside the nucleus).

Initially, Mischer needed to extract leukocytes from the blood serum. He solved this problem with the help of dilute sodium sulfate. The solution was left for 1-2 hours, precipitated and observed under a microscope. Mischer washed the cell with hydrochloric acid diluted 6-10 times in cold conditions for several weeks to separate the cell nucleus from the cytoplasm. After

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making sure that the cytoplasm was completely washed out with the help of an iodine-stained solution, Mischer applied an aqueous solution of ether to the nuclear mixture to dissolve the oils and membranes of the nucleus. He noticed that a certain part of the core accumulates, and the rest goes into solution, and then, under the action of alkali on the mixture, they began to stand translucent. Finally, when acetic or hydrochloric acid is added, sedimentary nucleic acids, similar to cotton fibers, are released. Later, Richard Altman, a student of Mischer, gave these substances the name nucleic acids.

In 1957, Meselson and Stahl developed a different protocol for DNA extraction, in which density gradient centrifugation was used to isolate DNA from E. coli bacteria. In 1988, Miller and colleagues used proteinase K for DNA extraction, an enzyme that cleaves peptide bonds in proteins and promotes their separation from nucleic acids. And even later, Sambrook and Russell proposed one of the most popular methods of DNA extraction using phenol-chloroform and isoamyl alcohol.

The isolation of nucleic acids is divided into three main stages

- **Cell lysis –** physical or chemical. So, if we isolate nucleic acids from the leaves of a plant, then first we need to wipe them (physically disrupt the integrity of the cells), and then use a special buffer. Heating may also be needed (to accelerate lysis) or centrifugation of samples (for separation into fractions). Special buffers are used as a chemical agent for lysis. They may contain buffer salts (e.g. Tris-HCl) and ionic salts (e.g. NaCl) to regulate the pH and osmolarity of the lysate. Sometimes detergents (such as Triton X-100 or SDS) are added to destroy the cell membrane.
- **Cleaning.** After the destruction of the cell and nuclear membranes, it becomes necessary to remove impurities — other substances that make up cells. The addition of concentrated saline solution leads to precipitation, which contains proteins, lipids and sugars. Remember that if they stay together with DNA, they will do a lot of harm during PCR and/or sequencing. Nucleic acids remain in solution, and centrifugation contributes to their better separation from the sediment. The removal of proteins is also helped by the addition of protease, which cleaves them and allows them to be separated from nucleic acids. So you can get rid, for example, of histone proteins, on which DNA is "wound" in chromosomes.
- **Elution.** Now the nucleic acids must be removed from the solution. Here, knowledge of the chemical properties of the acids we are interested in is useful — they are soluble in water, but not in alcohol. It turns out that once we add ethanol or isopropyl alcohol, a precipitate will appear — this is our DNA (or RNA). The precipitate can be separated from the solution by centrifugation. After we remove the alcohol, and that's it**.**

When isolating nucleic acids, it is also important to separate DNA from RNA. To do this, the resulting sample of nucleic acids can be treated with ribonucleases leading to RNA degradation — then whole DNA will remain in the solution; or add deoxyribonucleases — so we get RNA.

DNA yield can be estimated using various methods: optical density determination, electrophoresis in agarose gel or using fluorescent DNA-binding dyes. All three methods are convenient, but have different requirements in terms of the necessary equipment, differ in ease of use and calculations.

The optical density is measured at 260 nm (A260). At this wavelength, DNA absorbs light most strongly, and the resulting number allows us to estimate the concentration of the solution. A strong absorption of about 230 nm may indicate that organic compounds or chaotropic salts are present in the purified DNA. The ratio of values obtained at 260 nm and 230 nm shows the

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contamination of DNA with salts. The concentration and yield can be determined after the completion of gel electrophoresis by comparing the intensity of the glow of the strip with the DNA of the sample with the intensity of the standard sample (marker).

There are three basic principles underlying the different allocation methods, but this is not all that unites them. Many approaches are also similar in the composition of the "actors". Here are the most common:

• tris buffer - controls pH, interacts with lipopolysaccharides and increases permeability, and also lyzes the cell membrane;

• EDTA — works as a chelating agent, blocking the need for a DNase enzyme cofactor, thereby preventing DNA degradation;

• SDS — solubilizes proteins of nuclear and cell membranes;

• NaCl — neutralizes the negative charge of DNA, stabilizes the molecule;

• $MgCl_2$ is an agent that protects and stabilizes DNA by blocking the negative charge of lipoproteins;

• phenol — precipitates protein impurities.

Several modern methods of nucleic acid isolation have been created. One of them is the chromatography method. Probably no one expected that this method, originally used to separate plant pigments, would later become the basis of chemical, biological experiments. One is new methods based on the addition of additional fundamentally different stages appear in the second half of the XX century. And Grant Henry Lathe and Colin Ruthven discovered the "arms race" among molecular biologists and geneticists in 1955. They start using exclusive, or gel-filtering chromatography (SEC, size-exclusion chromatography). First, the column was filled with starch gel, later dextran, and then the turn came to the now familiar agarose and polyacrylamide (Tab. 1).

The next method was ion exchange chromatography (IEC). In 1956, E.A. Peterson and G.A. Sober published an article in which they talked about the application of the method for working with proteins. Diethylaminoethyl cellulose was used as a matrix inside the column, which binds to DNA molecules and holds them for a long time, while proteins, lipids, metabolites and RNA are washed out by a salt buffer (Tab. 1).

The centrifugation method is also used in the separation of nucleic acids. The method created by Svedberg is now widely used to separate the desired part or molecule of a cell. The capacity of modern centrifuge machines also exceeds 200,000 g. Under the influence of such a force, any molecule can be precipitated. Nucleic acids are no exception. During the centrifuged isolation of nucleic acids from all living organisms, it is necessary to prepare their extract (homogenate). The homogenate is placed in a centrifuge in a filtered state. During centrifugation, successive cell components are deposited. Nucleoproteins remain in the supernatant. The addition of CsCl leads to the fact that DNA molecules are deposited only to the position in the test tube at which the gradient density will be equal to its own density, and remain there, forming a strip. After the formation of the DNA strip, it is removed from the centrifuge tube. The CsCl itself can be removed from the sample by DNA deposition with ethanol. The main advantage of this method is the possibility of separating plasmid DNA from genomic DNA due to their differences in nucleic acid compactification. When visualized with the addition of ethidium bromide, plasmid DNA is easily distinguished from genomic DNA.

Although the centrifugation method has many positive aspects, DNA extraction will require 16 hours of centrifugation with 60,000 rotation per minute. It's been a while long. (Tab. 1)

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VOLUME-4, ISSUE-5

Another simple way to extract DNA is by alkali ectraction. In 1979, in the journal Nuclear Acids Research, J. Doli and H.C. Birnboim published an article describing the alkaline extraction of nucleic acids.

But this method has now been proven to be convenient in the separation of plasmid DNA. NaOH solution and SDS (sodium salt of laurylseric acid) are used to lysis the cell. In doing so, NaOH serves to degrade cell membranes as a whole, while SDS serves to defrost fats in the cell and cell membranes (Tab. 1).

SDS Detergent is found in soap, shampoo and toothpaste. For this reason, this method can also be used at home.

Table 1. Advantages and disadvantages of nucleic acid extraction methods

Extraction method	Objects	Advantages	Disadvantages
Chromatography SEC	Any	Easy to use, relatively fast	Relatively low yield and quality of NA. Cannot effectively separate DNA from RNA
Chromatography IEC	Any	Easy to use, relatively fast. Separates DNA from RNA well	Relatively low yield and quality of NA
Centrifugation + EtBr-CsCl	Any	Can effectively isolate plasmids as well as bacterial genomic DNA	Toxic reagents, expensive and time-consuming. The purity and yield of NA are relatively small
Alkaline	Preferably bacteria	Better for isolating plasmid DNA	Contamination of the output solution of chromosomal DNA
Spin columns	Any	Fast, simple and safe. Filters can be changed and genomic DNA can be separated from plasmid or RNA.	Expensive, relatively low yield
Salting out	Blood, cell culture, homogenate	Low-cost, safe reagents	May take a long time

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Extraction method	Objects	Advantages	Disadvantages
CTAB	Plants	An effective method for working with plant tissues rich in polyphenols, etc.	Time-consuming method, possible combination with other approaches using toxic reagents
Enzymes	Any	Simple, safe, gives good quality and NA output	More expensive for consumables, sometimes requires a long incubation
Phenol-chloroform	Any	Good yield and NA quality	Toxic reagents, duration
Magnetic particles	Any	Simple, fast, accurate, safe	Setting up a magnetic device is required
Glass Beads	Any	Can be combined with non-toxic buffers, good yield	Contamination of the final eluate with beads
Chelex-100	Any	Fast, economical, safe	Does not remove impurities enough for further use using many other methods
Intelligent extraction	Any	No need for a lot of equipment, non-toxic, good yield	
Enzymatic temperature-dependent	Any	Fast, inexpensive	For a small sample quantity
Filter paper	Any	Economical, simple, safe, good output	Does not work with small volumes

Using the alkaline extraction method, it is easier to extract nucleoproteins even from plants such as bananas, tomatoes, onions; even from animal tissues such as liver, spleen, etc. For example, to extract DNA from a banana, 10-20 g of banana is crushed in a porcelain cup and 100 ml of an aqueous solution of liquid soap and NaCl are added to it. The mixture is left for a few minutes. In

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this case, nucleic acids are released into the solution due to the dissolution of cell membranes and the disintegration of the nuclear envelope. The solution is filtered. To separate the nucleic acids from the filtrate, they are slowly poured into ethanol or isopropyl alcohol cooled to 0 ^{O}C. A little later, a layer of nucleic acids forms on the alcohol. (Pic.1)

To isolate nucleoproteins, you can also use large cells (bananas, tomatoes) or large nuclei (yeast).

Picture 1. DNA of bananas

The subsequent addition of potassium acetate leads to the renaturation of DNA molecules and the accidental formation of long networks, as well as their joint precipitation together with SDS and proteins. In 1988, S.A. Miller, D.D. Dykes and H.F. Poleski described a non—toxic approach to DNA isolation - the salting method. It does not involve either chloroform or ethidium bromide, which are poisonous to humans and require additional precautions when working. The sample is incubated in a salt buffer that promotes the precipitation of protein molecules from the solution. Subsequent centrifugation makes it possible to separate the protein precipitate from the DNA in solution. The method is used when working with blood, cell culture, tissue homogenate. It is simple, cheap and safe. However, since the effective removal of protein molecules requires a long incubation — say, a whole night - it is not suitable for fast work.

A completely different approach was the use of silica filters in spin columns. Spin columns are modified test tubes in which the volume of the test tube is separated by a silicate filter. It has already been shown that DNA binds to silicate in the presence of holotropic salts; this principle was used in the process of creating spin columns. The new approach involved simple and affordable materials and allowed to isolate nucleic acids of sufficiently high quality. For the first time, the use of silica (silicate) when working with DNA was described in 1979 by B. Fogelstein and D. Gillespie. They showed that in a buffer solution containing NaCl, nucleic acids can bind to the silica surface. Accordingly, DNA molecules "anchored" in this way can be washed from impurities and further "detached" from the carrier using an eluting buffer or distilled water.

The essence of the method is the binding of nucleic acid molecules with immobilized silicon in a column. The sample is first subjected to lysis (and everything is standard here), and then the lysate interacts with silicon in a spin column. To pass the lysate through a silicon filter, the tube is centrifuged. Then the washing steps follow, then the nucleic acids are eluted. A significant advantage of the method now is the ability to use different filters that are able to separate genomic and plasmid DNA, as well as RNA from each other.

Isolation of DNA from plant material using CTAB buffer. CTAB buffer is added to the sample after lysis, which forms complexes with nucleic acids after incubation. After centrifugation, the bound nucleic acids are precipitated, and the supernatant with the remaining cellular components can be removed. This is followed by the stages of washing with chloroform and ethanol. The isolated nucleic acids form a visible precipitate at the bottom of the tube, the supernatant is removed.

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Isolation of nucleic acids using magnetic particles. Magnetic particles are added to the cellular lysate, on the surface of which nucleic acids are adsorbed. The creation of a magnetic field by an external source leads to the concentration of magnetic particles at the bottom of the tube, after which the remaining lysate is removed. After washing and incubation, the nucleic acids are separated from the magnetic particles and can be transferred to another tube.

For the first time, anion exchange resins were used to isolate DNA from forensic samples - semen and dried blood stains. The presented approach turned out to be simple and fast, did not require repeated transfer of samples from test tube to test tube and, accordingly, reduced the likelihood of loss of valuable biomaterial.

Chelex 100 is a styrene copolymer with divinylbenzene, containing iminodiacetate as chelate groups for binding polyvalent metal ions. The principle of operation is the ability of anion exchange resin to bind charged cellular components (as well as enzymes capable of destroying nucleic acid or interfering with further PCR), leaving nucleic acids in solution. That's it! Thus, a typical protocol for obtaining DNA or RNA includes: adding a resin suspension to the sample, boiling to release nucleic acids, separating the nucleic acid solution from the resin

Conclusion. Extracting DNA from living organisms is a complex process, all based on three basic rules: cell membrane degradation, protein degradation, and nucleic acid separation from the rest of the components. The difference between them is only in the number of nucleotides when extracting nucleic acids from living organisms cleanly. The DNA of bacteria is extremely high in the G-C pair, and a person has a high number of A-T pairs. Many methods have been used to date to isolate nucleic acids, each of which is important. The longer it takes to extract DNA, the cleaner it will separate. The gradual emergence and development of PCR, sequencing and other methods of working with nucleic acids led to the natural evolution of isolation approaches. One way or another, they were aimed at reducing the cost and labor of isolation, using non-toxic reagents and increasing the quality and quantity of nucleic acid yield.

In parallel with the approaches to the isolation of nucleic acids, other areas of the arsenal of molecular biology, such as sequencing, PCR and electrophoresis, have also developed. It became increasingly clear that pure source material was needed for high-quality results, and the evolution of methods for isolating nucleic acids continued. The first methods have not gone into the shadows, they have been modified, improved, and only for some specific tasks are inferior to more modern ones in the purity of the final product and performance.

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