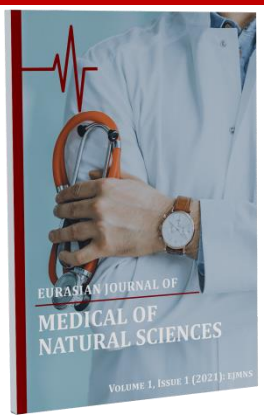
EURASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
Innovative Academy Research Support Center
UIF = 8.3 | SJIF = 5.995 www.in-academy.uz



METHODS FOR ISOLATING NUCLEIC ACIDS

D.D. Dehqonboeva
Sh.Yu. Menglieva
Tashkent Pharmaceutical Institute, Tashkent, Uzbekistan
https://www.doi.org/10.5281/zenodo.10548904

ARTICLE INFO

Received: 14th January 2024
Accepted: 21th January 2024
Online: 22th January 2024

KEY WORDS

Nucleic acids, DNA, RNA, sorbent isolation method, precipitation.

ABSTRACT

Nucleic acids (DNA and RNA) are the most important biomolecules that play a key role in the transmission of hereditary information, protein biosynthesis and other biological processes. To carry out various studies such as sequencing, cloning and transfection, it is necessary to obtain pure nucleic acids. This article examined the literature data on the main methods for isolating nucleic acids, analyzed the advantages and disadvantages of methods for isolating nucleic acids, and also considered the prospects for the development of new methods for isolating nucleic acids.

Introduction

Nucleic acid isolation is a process by which nucleic acids (DNA, RNA) are separated from other components of biological material. Isolation of nucleic acids is an important step in many molecular biological methods, such as PCR, sequencing, hybridization, etc. Traditional methods of isolating nucleic acids, such as mechanical destruction of cells, chemical or enzymatic digestion of cellular components, have a number of disadvantages, such as low efficiency, duration and complexity [1].

Purpose of the study. The purpose of the study is to study a variety of methods for isolating nucleic acids. Review of modern methods for isolating nucleic acids.

Materials and methods. The research material is an analysis of existing protocols, literature data on methods for isolating NA.

Results. The main methods for isolating nucleic acids can be divided into two groups:

Nonsorption methods are based on differences in the physicochemical properties of nucleic acids and other components of biological material.

Sorption methods are based on the ability of nucleic acids to bind to certain molecules or particles.

Non-sorption methods include mechanical, chemical, biochemical methods. Mechanical methods are based on cell destruction and mechanical separation of nucleic acids from other components. Mechanical methods include: homogenization, centrifugation, filtration. Mechanical methods for isolating nucleic acids are based on the use of physical forces to destroy cells and release nucleic acids from the cell matrix [2,3].

EURASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
Innovative Academy Research Support Center
UIF = 8.3 | SJIF = 5.995 www.in-academy.uz

The main mechanical methods for isolating nucleic acids include:

- The homogenization method involves grinding the cells using a homogenizer. In this case, the cells are destroyed, and the nucleic acids are released in the form of a solution.

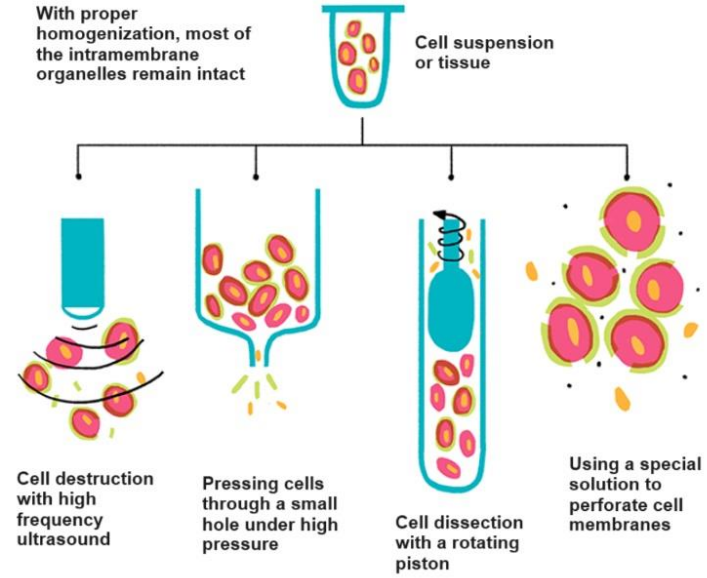


Fig.1. Homogenization. General scheme of destruction of biological material to obtain individual subcellular fractions [12].

- The centrifugation method involves centrifuging cells or cell lysate. In this case, cells or cell fragments settle to the bottom of the tube, and nucleic acids remain in the supernatant. Can be repeated to increase purity.

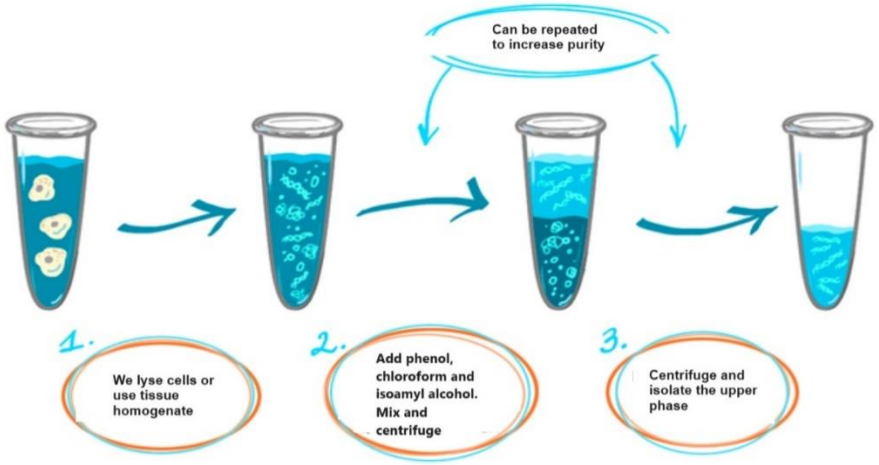


Fig.2. Stages of NC isolation using the phenol-chloroform method [13]

- The filtration method involves passing the cell lysate through a filter with a pore size smaller than the size of the cells. In this case, the cells are retained on the filter, and nucleic acids pass through the filter [4,5].

Mechanical methods for isolating nucleic acids are simple to perform and do not require the use of chemical reagents. However, they may be less effective than chemical methods and produce more impurities.

The most common mechanical methods for nucleic acid isolation are used to isolate nucleic acids from bacteria, yeast and other microorganisms. Chemical methods are usually used to isolate nucleic acids from plants and animals [6].

Chemical methods rely on the use of chemical reactions to separate nucleic acids from other components. Chemical methods include:

• Disintegration of nucleic acids using alkali or acid.
• Isolation of nucleic acids using organic solvents.

Biochemical methods rely on the use of enzymes to separate nucleic acids from other components. Biochemical methods include:

• Degradation of proteins using proteases.
• Degradation of lipids by lipases.

Sorption methods

- Electrophoresis is based on the movement of charged particles in an electric field. Nucleic acids generally have a negative charge, so they move towards the positive electrode.
- Adsorption is based on the ability of nucleic acids to bind to certain molecules or particles. Adsorption methods include:

• Sorption of nucleic acids on columns with silica gel, cellulose acetate or other sorbents.
• Sorption of nucleic acids on magnetic particles.

The choice of nucleic acid extraction method depends on the following factors:

Type of biological material. Different methods are used to isolate nucleic acids from different biological materials. For example, mechanical methods are usually used to isolate nucleic acids from blood or tissues, and sorption methods are used to isolate nucleic acids from bacteria or viruses.

Number of nucleic acids. To isolate small quantities of nucleic acids, fast and simple methods such as mechanical or chemical methods are usually used. To isolate large quantities of nucleic acids, more complex methods such as electrophoresis or adsorption are usually used [7,8].

Purpose of nucleic acid isolation. If it is necessary to obtain nucleic acids in their pure form, then more complex methods are used, such as electrophoresis or adsorption. If it is necessary to obtain nucleic acids in crude form, then simpler methods can be used, such as mechanical or chemical methods.

Modern methods for isolating nucleic acids

In recent years, new methods for isolating nucleic acids have emerged, which are based on the use of new technologies. For example, magnetic separation methods are used to isolate nucleic acids from complex biological materials. These methods allow the rapid and efficient isolation of nucleic acids from any biological material, including cells, tissues, liquids and solid samples.

Polymerase chain reaction (PCR) methods are also used to isolate nucleic acids. PCR methods allow the isolation of nucleic acids from a very small amount of biological material [9].

Sorption of nucleic acids on magnetic particles. Sorption of nucleic acids on magnetic particles is a method for isolating nucleic acids based on the ability of nucleic acids to bind to certain molecules deposited on the surface of magnetic particles. After this, magnetic particles with nucleic acids are easily separated from the solution using a magnetic field.

EURASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
Innovative Academy Research Support Center
UIF = 8.3 | SJIF = 5.995 www.in-academy.uz

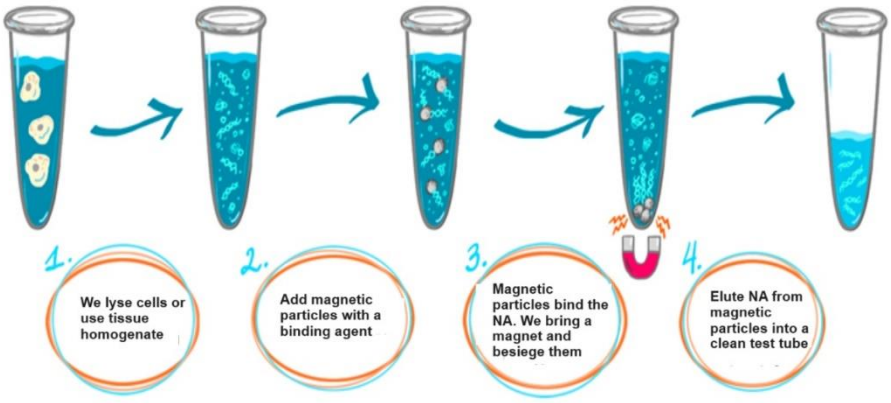


Fig.3. Stages of NA isolation on magnetic particles [12,13]

The mechanism of sorption of nucleic acids on magnetic particles. Nucleic acids have a cationic charge, which may be due to the presence of charged groups in the nucleotides. Molecules deposited on the surface of magnetic particles can also have a cationic charge. As a result, electrostatic attractive forces arise between the nucleic acid molecules and the molecules deposited on the surface of the magnetic particles.

Types of molecules deposited on the surface of magnetic particles

Various substances with a cationic charge can be used as molecules deposited on the surface of magnetic particles. The most common substances are the following:

- Amino acids such as lysozyme, glutamine, asparagine.
- Proteins such as albumin, globulin.
- Polysaccharides such as chitosan, dextran.
- Lipids such as phospholipids.

Selecting the type of molecules deposited on the surface of magnetic particles

The choice of the type of molecules applied to the surface of magnetic particles depends on the following factors:

Type of nucleic acids. Different types of molecules are used to isolate different types of nucleic acids. For example, molecules with a positive charge are used to isolate DNA, and molecules with a negative charge are used to isolate RNA.

Amount of nucleic acids. Molecules with high affinity for nucleic acids are used to isolate small amounts of nucleic acids, and molecules with lower affinity for nucleic acids are used to isolate large amounts of nucleic acids [9, 10, 11].

Purpose of nucleic acid isolation. If it is necessary to obtain nucleic acids in a pure form, then molecules with high specificity for nucleic acids are used, and if it is necessary to obtain nucleic acids in an unpurified form, then molecules with lower specificity for nucleic acids can be used.

Advantages of the method of sorption of nucleic acids on magnetic particles

The method of sorption of nucleic acids on magnetic particles has a number of advantages over other methods of isolating nucleic acids:

High efficiency. The method allows you to obtain nucleic acids with high concentration.

Rapidity. The method allows you to obtain nucleic acids in a short time.

Simplicity. The method does not require the use of complex equipment.

Disadvantages of the method of sorption of nucleic acids on magnetic particles

The method of sorption of nucleic acids on magnetic particles also has a number of disadvantages:

Dependence on the type of molecules deposited on the surface of magnetic particles. The choice of the type of molecules applied to the surface of the magnetic particles depends on the type of nucleic acids that need to be isolated.

The difficulty of purifying nucleic acids from molecules deposited on the surface of magnetic particles. After isolating nucleic acids using magnetic particles, it is necessary to purify them from molecules deposited on the surface of the magnetic particles. This can be difficult if the molecules deposited on the surface of the magnetic particles have high specificity for nucleic acids.

Application of the method of sorption of nucleic acids on magnetic particles

The method of sorption of nucleic acids on magnetic particles is widely used for the isolation of nucleic acids from various biological materials, including cells, tissues, liquids and solid samples. This method is used in various fields such as biotechnology, medicine, pharmaceuticals and agriculture [6, 12].

In biotechnology, the method of sorption of nucleic acids on magnetic particles is used to isolate nucleic acids from various sources, such as cells, tissues, bacteria, viruses, etc. These nucleic acids can be used for various purposes such as:

Carrying out genetic research. Nucleic acids can be used to study the structure and function of genes, as well as to develop new treatments for diseases.

Production of biotechnological products. Nucleic acids can be used to produce various biotechnology products such as vaccines, drugs and nutritional supplements.

In medicine, the method of sorption of nucleic acids on magnetic particles is used to isolate nucleic acids from various biological fluids, such as blood, urine, saliva, etc. These nucleic acids can be used to diagnose diseases, as well as to develop new treatments for diseases.

In pharmaceuticals, the method of sorption of nucleic acids on magnetic particles is used to isolate nucleic acids from various biological sources, such as cells, tissues, bacteria and viruses. These nucleic acids can be used to develop new drugs.

In agriculture, the method of sorption of nucleic acids on magnetic particles is used to isolate nucleic acids from various biological materials such as eggs, milk and meat. These nucleic acids can be used to develop new methods for diagnosing animal diseases, as well as for the production of vaccines and other biotechnological products [12, 13].

Conclusion. Isolation of nucleic acids from biological materials is an important step in various fields of science and technology. For this, various methods are used, which can be divided into two main groups: non-sorption and sorption.

The method of sorption of nucleic acids on magnetic particles is one of the most widely used methods for isolating nucleic acids. It has a number of advantages over other methods, such as high efficiency, speed and simplicity. However, this method also has a number of disadvantages, such as dependence on the type of molecules deposited on the surface of magnetic particles and the difficulty of purifying nucleic acids from these molecules.

Currently, new methods for isolating nucleic acids are being developed, which should be more efficient, faster and simpler, and also less dependent on the type of biological material.

References:

1. Ali, N., Rampazzo, R. D. C. P., Costa, A. D. T., & Krieger, M. A. (2017). Current nucleic acid extraction methods and their implications to point-of-care diagnostics. BioMed research international, 2017.
2. Hagarová, I. (2020). Magnetic solid phase extraction as a promising technique for fast separation of metallic nanoparticles and their ionic species: a review of recent advances. Journal of Analytical Methods in Chemistry, 2020.
3. Ma, W., Li, X., Bai, Y., & Liu, H. (2018). Applications of metal-organic frameworks as advanced sorbents in biomacromolecules sample preparation. TrAC Trends in Analytical Chemistry, 109, 154-162.
4. Rahman, M. M., & Elaissari, A. (2012). Nucleic acid sample preparation for in vitro molecular diagnosis: from conventional techniques to biotechnology. Drug discovRery today, 17(21-22), 1199-1207.
5. Антонова, О. С., Корнева, Н. А., Белов, Ю. В., & Курочкин, В. Е. (2010). Эффективные методы выделения нуклеиновых кислот для проведения анализов в молекулярной биологии (обзор). Научное приборостроение, 20(1), 3-9.
6. Аукенов, Н. Е., Масабаева, М. Р., & Хасанова, У. У. (2014). Выделение и очистка нуклеиновых кислот. Состояние проблемы на современном этапе. Наука и здравоохранение, (1), 51-53.
7.
8. Егунова, О. Р., Константинова, Т. А., & Штыков, С. Н. (2014). Магнитные наночастицы магнетита в разделении и концентрировании. Изв. Сарат. ун-та. Нов. сер. Сер. Химия. Биология. Экология, 14(4), 27-34.
9. Yu, M. S. (2023). HYDROLYZATION FROM EGGSHELL MEMBRANE UNDER THE INFLUENCE OF PAPAIN ENZYME AND DETERMINATION OF TOTAL PROTEIN AMOUNT. Asian journal of pharmaceutical and biological research, 12(2).
10. Менглиева, Ш. Ю., & Зоирова, Х. Т. ЯИЧНАЯ СКОРЛУПА КАК АЛЬТЕРНАТИВНЫЙ И ДЕШЁВЫЙ ОБЪЕКТ ДЛЯ ПОЛУЧЕНИЯ БИОЛОГИЧЕСКИ АКТИВНЫХ ДОБАВОК.
11. Tursunova Solikha Zafar qizi. (2023). TO STUDY THE MEDICINAL PROPERTIES OF THE GINGER PLANT. Modern Scientific Research International Scientific Journal, 1(2), 134–144. https://doi.org/10.5281/zenodo.7932573
12. Mamatkulov Z.U., K. G. G. (2023). DETERMINATION OF THE CHEMICAL COMPOSITION OF THE PLANT CALENDULA OFFICINALIS. INTERNATIONAL BULLETIN OF MEDICAL SCIENCES AND CLINICAL RESEARCH, 3(9), 134–138. https://doi.org/10.5281/zenodo.8405927
13. https://biomolecula.ru/articles/metody-v-kartinkakh-ochistka-molekul-i-razdelenie-smesei
14. https://www.helicon.ru/support/methodological/life-sciences/vydelenie-i-ochistka-nukleinovykh-kislot-kak-pravilno-vybrat-metod/