

A Short note on DNA Isolation in Plants and Single Cellular Organisms

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Abstract

A fundamental step in molecular biology is DNA isolation, which is used to extract DNA from plants and other biological samples. Due to the presence of various polysaccharides, proteins, and secondary metabolites that can impede DNA extraction, it can be challenging to isolate DNA from plants. However, a number of techniques for efficiently isolating DNA from plant tissues have been developed as a result of advancements in molecular biology research. In this article, we'll go over a few different approaches to separating DNA from plants. Selecting a suitable plant tissue sample for DNA extraction is the first step in DNA isolation. The tissue ought to be brand-new, undamaged, and abundant in DNA. Leaves, stems, roots, and flowers are the plant tissues that are utilized the most frequently for DNA extraction.

Keywords: DNA extraction; Tissue; CTAB buffer; Centrifugation

Introduction

The CTAB (Cetyltrimethylammonium bromide) method is the most established one for removing DNA from plants. A CTAB-based buffer is used in this method to help dissolve the plant cell walls and let the DNA out. The process involves grinding the plant tissue, adding CTAB buffer, centrifuging, and DNA precipitation, among other steps. Due to its effectiveness and capacity to isolate DNA of high quality, the CTAB method has been widely used for DNA isolation in plants [1]. The DNeasy Plant Mini Kit is another widely used method for isolating DNA from plants. The spin column-based method used in this kit makes it possible to effectively isolate DNA from plant tissues. The plant tissue is ground up, a lysis buffer is added, and the lysate is put into a spin column. After that, the column is centrifuged, and an elution buffer is used to elute the DNA. Using the DNeasy Plant Mini Kit, you can quickly and effectively isolate DNA from a variety of plant tissues. Another method for separating DNA from plants is the modified CTAB method. PVP (Polyvinylpyrrolidone) and NaCl (Sodium Chloride) are two additional reagents that are utilized in this modified version of the CTAB method. The PVP assists with eliminating the auxiliary metabolites and colors, while NaCl assists with hastening the DNA. Because it makes it possible to efficiently isolate DNA of high quality in plants, the modified CTAB method is widely used for DNA isolation. Another method for separating DNA from plants is the Chelex method. This technique includes the utilization of Chelex pitch, which assists with chelating the metal particles present in the plant tissue, consequently delivering the DNA [2, 3]. The DNA is released by heating the mixture, adding Chelex resin, and grinding the plant tissue. The Chelex method is a quick and simple way to isolate DNA from a variety of plant tissues.

Method

For DNA isolation from plants: Sample Preparation: Collect plant tissue, such as leaves or young shoots, and finely grind or chop it to increase the surface area and facilitate the release of DNA.

Cell lysis: Add a lysis buffer containing a combination of detergents, enzymes (e.g., cellulose, pectinase), and salts to break down the cell walls and release the cellular contents. The choice of lysis buffer depends on the plant species and tissue type [4].

Protein removal: Add protein precipitation reagents (e.g., phenol-chloroform) to the lysate to remove proteins, polysaccharides, and other contaminants. Centrifuge the mixture to separate the aqueous

DNA-containing phase from the organic phase.

DNA precipitation: Transfer the aqueous phase to a clean tube and add cold ethanol or isopropanol along with a salt (e.g., sodium acetate). The DNA will precipitate out of the solution due to the alcohol and salt combination. Centrifuge the mixture to collect the DNA pellet.

DNA wash: Wash the DNA pellet with a solution containing ethanol to remove any residual salts or contaminants. Centrifuge the mixture again and carefully remove the supernatant without disturbing the DNA pellet.

DNA resuspension: Dissolve the DNA pellet in a suitable buffer, such as Tris-EDTA (TE) buffer, to obtain a concentrated DNA solution. The DNA can be stored or used directly for downstream applications. For DNA isolation from single-cellular organisms (e.g., bacteria, yeast):

Sample preparation: Collect the single-celled organisms by centrifugation or filtration and pellet them.

Cell lysis: Add a lysis buffer containing detergents (e.g., SDS, Triton X-100) and enzymes (e.g., lysozyme, proteinase K) to disrupt the cell membrane and release the DNA. The lysis buffer composition may vary depending on the organism [5, 6].

Protein removal: Similar to plant DNA isolation, use protein precipitation reagents (e.g., phenol-chloroform) to remove proteins and contaminants. Centrifuge the mixture to separate the DNA-containing phase.

DNA precipitation: Add cold ethanol or isopropanol along with a salt (e.g., sodium acetate) to the DNA-containing phase. Centrifuge the mixture to collect the DNA pellet.

DNA wash: Wash the DNA pellet with ethanol to remove any

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Table 1: The key steps involved in DNA isolation from plants and single-cellular organisms.

Step	Description
Step 1: Sample Preparation	Collect plant tissue or culture cells and homogenize them to break open the cells and release the DNA.
Step 2: Cell Lysis	Use a lysis buffer containing detergents and enzymes to disrupt the cell membrane and nuclear envelope, releasing the DNA into the solution.
Step 3: Protein Removal	Add protein precipitation reagents, such as phenol-chloroform or proteinase K, to remove proteins and other cellular contaminants from the DNA solution.
Step 4: DNA Precipitation	Add cold ethanol or isopropanol to the DNA solution to cause the DNA to precipitate out of the solution. Centrifuge the mixture to collect the DNA pellet.
Step 5: DNA Wash	Wash the DNA pellet with a solution containing ethanol to remove any residual contaminants or salts.
Step 6: DNA Resuspension	Dissolve the DNA pellet in a suitable buffer, such as Tris-EDTA (TE) buffer, to obtain a concentrated DNA solution.
Step 7: DNA Quantification	Measure the concentration and purity of the isolated DNA using spectrophotometry or fluorometry.
Step 8: DNA Storage	Store the isolated DNA at -20°C or -80°C to maintain its stability and integrity for future analysis or experiments.

residual salts or contaminants. Centrifuge the mixture, remove the supernatant, and let the pellet air-dry briefly [7].

DNA re suspension: Dissolve the DNA pellet in a suitable buffer, such as TE buffer, to obtain a concentrated DNA solution. The DNA can be stored or used for downstream applications.(Table 1).

Discussion

Researchers in genetics, microbiology, and molecular biology all rely on DNA isolation as a crucial technique. DNA is extracted from cells or tissues and then purified for use in other applications. DNA isolation is a well-established method for working with organisms with multiple cells [8, 9], but it presents unique difficulties when working with organisms with just one cell. Organisms with a single cell are referred to as single-cell organisms or unicellular organisms. Bacteria, archaea, and some types of algae are examples of organisms with one cell. Because they provide insight into fundamental biological processes like gene expression, cell division, and evolution, these organisms are an important focus of scientific research. Due to the small cell size and low DNA content, DNA isolation from single-cell organisms is difficult. Additionally, it is challenging to gain access to the DNA in single-cell organisms due to their rigid cell walls or membranes. However, there are a number of approaches that have been developed for isolating DNA from single-cell organisms, each of which has its own set of benefits and drawbacks [10, 11]. Lysis, in which the DNA is extracted from a single cell organism by breaking it open, is a common method for isolating DNA from single-cell organisms. Chemical disruption, such as detergents or enzymes, or mechanical disruption, like sonication or grinding, can both lead to lysis. The DNA can be purified in a variety of ways after the cell is lysed [12], such as precipitation or column-based purification. Whole-genome amplification (WGA) is another method for isolating DNA from single-cell organisms [13, 14]. WGA is a method that lets researchers amplify a single cell's entire genome. Multiple displacement amplification (MDA) is the process of synthesizing multiple DNA copies with a DNA polymerase. WGA can be used to generate enough DNA for sequencing or PCR to use in the future [15]. Methods based on microfluidics have recently emerged as a promising strategy for separating DNA from single-cell organisms. High-throughput analysis of single-cell genomics is made possible by these strategies, which make use of microscale devices to manipulate and isolate individual cells. Droplet-based microfluidics [16], for instance, can be used to enclose individual cells in droplets so that they can be lysed and their DNA amplified with WGA.

Conclusion

In conclusion, DNA isolation from single-cell organisms is necessary for studying fundamental biological processes, despite the unique difficulties it presents. For isolating DNA from single-cell organisms,

lysis, whole-genome amplification, and microfluidics-based methods have been developed. Researchers must carefully consider which method is best suited for their specific research question because each of these methods has its own advantages and disadvantages. The study of single-cell genomics stands to make significant contributions to our comprehension of biology and evolution if these techniques are further developed and refined. Various applications of molecular biology, such as PCR, gene cloning, and sequencing, all require the isolation of DNA from plants. Several techniques for efficiently isolating DNA from plant tissues have been developed as a result of advancements in molecular biology techniques. The type of plant tissue, the quantity and quality of the required DNA, and the applications that follow are all important considerations when choosing a method for DNA isolation. The CTAB method, the DNeasy Plant Mini Kit, the modified CTAB method, and the Chelex method are the most frequently used methods for isolating DNA from plants.

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Conflict of Interest

None

References

1. Kieper WC, Burghardt JT, Surh CD (2004) A role for TCR affinity in regulating naive T cell homeostasis. J Immunol 172: 40–44.
2. Kassiotis G, Zamoyska R, Stockinger B (2003) Involvement of avidity for major histocompatibility complex in homeostasis of naive and memory T cells. J Exp Med 197: 1007–1016.
3. Moulias R, Proust J, Wang A, Congy F, Marescot MR, et al. (1984) Age-related increase in autoantibodies. Lancet 1: 1128–1129.
4. Goronzy JJ, Weyand CM (2005) T cell development and receptor diversity during aging. Curr Opin Immunol 17: 468–475.
5. Weyand CM, Goronzy JJ (2003) Medium- and large-vessel vasculitis. N Engl J Med 349: 160–169.
6. Goronzy JJ, Weyand CM (2005) Rheumatoid arthritis. Immunol Rev 204: 55–73.
7. Koetz K, Bryl E, Spickschen K, O'Fallon WM, Goronzy JJ, et al. (2000) T cell homeostasis in patients with rheumatoid arthritis. Proc Natl Acad Sci USA 97: 9203–9208.
8. Surh CD, Sprent J (2008) Homeostasis of naive and memory T cells. Immunity 29: 848–862.
9. Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, et al. (2003) Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 289: 179–186.
10. Rivetti D, Jefferson T, Thomas R, Rudin M, Rivetti A, et al. (2006) Vaccines for preventing influenza in the elderly. Cochrane Database Syst Rev 3: CD004876.
11. Green NM, Marshak-Rothstein A (2011) Toll-like receptor driven B cell activation in the induction of systemic autoimmunity. Semin Immunol 23: 106–112.

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12. Hakim FT, Memon SA, Cepeda R, Jones EC, Chow CK, et al. (2005) Age-dependent incidence, time course, and consequences of thymic renewal in adults. *J Clin Invest* 115: 930–939.
 13. Naylor K, Li G, Vallejo AN, Lee WW, Koetz K, et al. (2005) The influence of age on T cell generation and TCR diversity. *J Immunol* 174: 7446–7452.
 14. Shlomchik MJ (2009) Activating systemic autoimmunity: B's, T's, and tolls. *Curr Opin Immunol* 21: 626–633.
 15. Doran MF, Pond GR, Crowson CS, O'Fallon WM, Gabriel SE (2002) Trends in incidence and mortality in rheumatoid arthritis in Rochester, Minnesota, over a forty-year period. *Arthritis Rheum* 46: 625–631.
 16. Goronzy JJ, Weyand CM (2001) T cell homeostasis and auto-reactivity in rheumatoid arthritis. *Curr Dir Autoimmun* 3: 112–132.