Informational

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Extracting plant DNA can be a little easier than RNA because DNA is double-stranded, making it more stable and less prone to degradation. However, don't be overconfident. Several aspects should still be considered before extracting DNA to avoid common mistakes and wasting excessive time.

There are several common obstacles that arise when extracting plant DNA. The basis behind these obstacles are usually a result of polysaccharides, polyphenols and DNases.

As you may know, one DNA extraction protocol can work for a specific plant species but may not work for others. Researchers still need to search deeply to understand the plant genotype, check the proper reagents and equipment, and have a very clear application where the extracted DNA will be used.

In this article, we'll guide you through common DNA extraction obstacles, applications for plant DNA, extraction tips, and so much more.

Common issues found in plant DNA extraction

Generating sufficient yield and quality of DNA during plant DNA extraction is more difficult than in animals because of the plant's rigid cell wall.
Furthermore, plants also contain varying levels of carbohydrates or polyphenols which combine with nucleic acids during DNA isolation and further affect the quality of the extracted DNA.

These plant components have a similar nucleic acid structure that allows secondary metabolites and polysaccharides to interfere with total DNA isolation.

Due to their chemical properties, polysaccharides co-precipitate with genomic DNA, giving viscous solutions.

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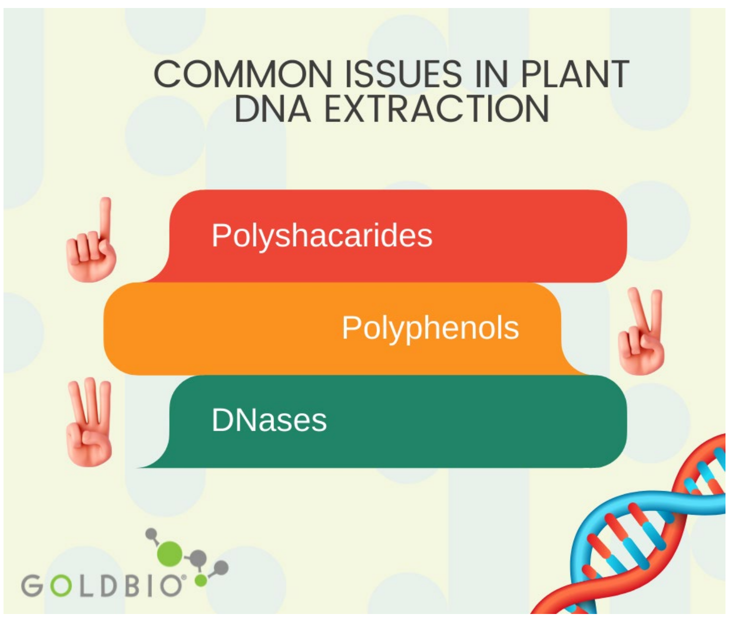
Phenolics are chemicals which, once released from plant tissue, irreversibly bind to the phosphate backbone of DNA and generate the typical browning observed in plant tissues. **Both contaminants hamper the use of DNA for molecular biology purposes**, such as restriction digest, PCR, or sequencing (like Next Generation Sequencing), **by inhibiting the action of polymerases or endonucleases.**

Some plant taxa are also more likely to contain a high level of specific metabolites, making DNA extraction harder. For instance, cereals are rich in carbohydrates, whereas stressed plants are rich in polyphenols.

A way to overcome these issues is to look for protocols that specialize in eliminating these contaminants. Some references for these protocols are listed below.
If you are under a tight budget and your plant is known to have a lot of contaminants, you might want to avoid column-based kits at first and instead optimize our GoldBio DNA extraction protocol for your specific plant.



Sources of plant DNA

Choosing the right plant tissue for DNA extraction is often one of the most critical decisions to obtain large amounts of DNA for a wide variety of downstream applications.

In general, younger tissue is preferred since it usually contains smaller amounts of secondary metabolites. However, DNA has also been obtained from milligram amounts of herbarium and mummified tissues.

As DNA is the same in all somatic cells (all cells composing the plant except sexual cells), most researchers use young leaves as starting material. However, when leaves are not available, or in the case of gymnosperms, researchers have used other types of tissues. These tissue types include seeds, embryogenic axes, buds, and stems.

Furthermore, cell suspensions can serve as a DNA source as well. A good review that shows DNA extraction from different plant tissues and a large variety of plant species can be found in Tamari and Hinkley, 2016 (see reference section).

The following figure shows resources of plant DNA for different species.

Applications of plant DNA

Historically, plant researchers have focused on understanding the function of one or a few genes at a time. However, with the advent of sequencing technologies, DNA is being used in broader studies to refine and redefine our understanding of plant evolution and adaptation while also providing information for conservation, crop breeding, and food security.

When considering your applications, choosing the best extraction method is critical to your success. Some of the applications given for the DNA include:

- Conventional PCR
- PCR-based applications like
    - Inter simple sequence repeats (ISSR)
    - Random amplified polymorphic DNA (RAPD)
    - Real-time PCR
    - Sequence related amplified polymorphisms
    - Simple sequence repeats (SSR)
    - Single nucleotide polymorphisms (SNP)
- Amplified fragment length polymorphisms (AFLP)
- Cleaved amplified polymorphic sequences (CAPS)
- Restriction fragment length polymorphisms (RFLP)

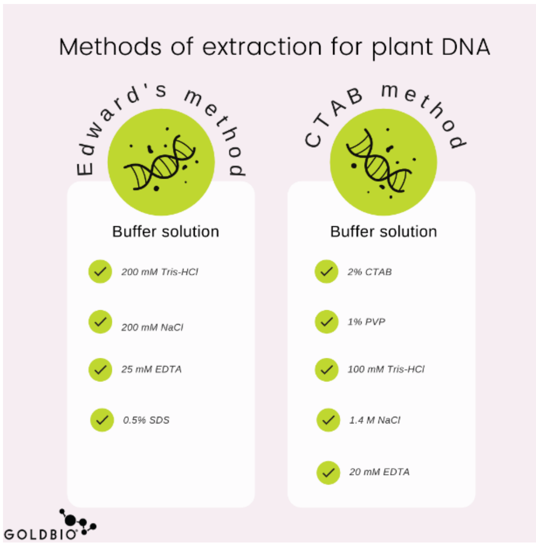
Some other modern applications for the extracted DNA include:

- Construction of a bacterial artificial chromosome library
- Digestion with a restriction endonuclease
- Southern blotting
- Next-Generation Sequencing
- PacBio Sequencing

It's undeniable that genetically modified organisms (GMO) have been a great promoter in a large number of applications developed to detect these organisms, especially in food products. Thus, new and even more exciting scientific frontiers will be coming for plant DNA research.

Plant DNA extraction methods

Thousands of protocols have been published for plant DNA extraction. However, most of the reported protocols are based on three methods:

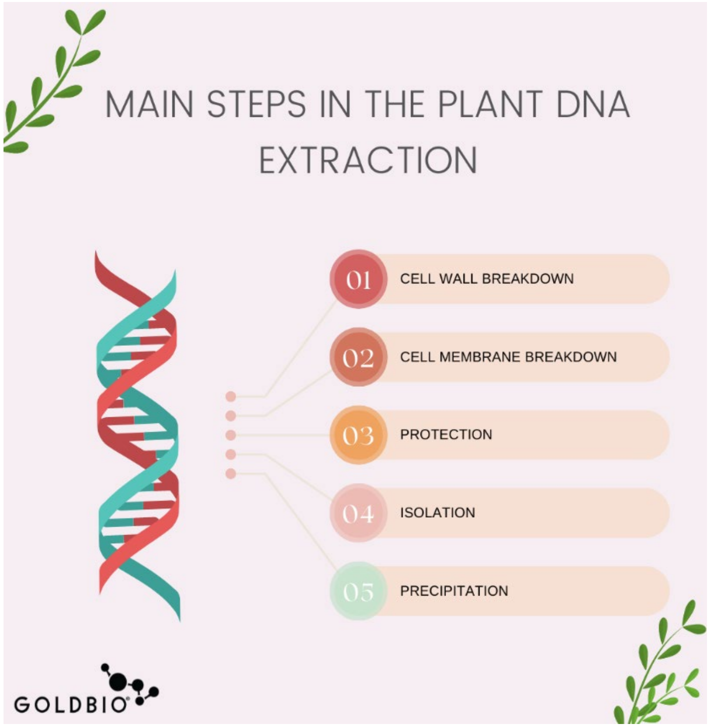


1. **Edward's-based method:** This method has been used for many tissues, including tissues from dead or dying plants. This protocol is recommended when using young tissues or reproductive organs of angiosperms. The advantage of this protocol is that it does not involve the use of organic compounds that may affect human health. The extraction buffer is composed of 200mM **Tris-HCl**, 200mM NaCl, 25mM **EDTA**, and 0.5% SDS (sodium dodecyl sulfate). The SDS is a detergent that forms complexes with proteins, thus removing protein contaminants from the extraction.

2. **CTAB-based method:** This method has been used in many protocols with some variations, and it is widely used for DNA obtained from diverse plant species and taxa. However, it includes the use of organic compounds and is potentially more toxic than the Edwards method. This method is advisable for plants containing high polysaccharides and polyphenol content. The CTAB (cetyltrimethylammonium bromide) extraction buffer is composed of 2% cetyltrimethylammonium bromide, 1% polyvinylpyrrolidone (PVP), 100mM Tris-HCl, 1.4M NaCl, and 20mM EDTA. This method starts by removing the polysaccharides, then proteins, and then finally secondary metabolites like flavonoids.

Main Steps in plant DNA extraction

Extraction procedures for plant DNA, in general, must accomplish the following five steps:



1. **Cell wall breakdown:** Grind the tissue in dry ice or liquid nitrogen with a mortar and pestle to break down the cell walls and release the cellular contents.

2. **Cell membrane breakdown:** After the cell wall is broken, the cell membranes must be disrupted too, so that the DNA is released into the extraction buffer. Researchers accomplish this by using a detergent, usually CTAB or SDS buffers.

3. **DNA protection:** Once DNA is released into the extraction buffer, endogenous nucleases can degrade the DNA. There are some reagents used to prevent DNA degradation, such as EDTA (ethylenediaminetetraacetic acid), which is a chelating agent that binds magnesium ions that are necessary cofactors for most nucleases.

4. **DNA isolation:** The DNA in the extraction buffer is isolated from other components (like proteins, polysaccharides, and phenolics). The buffer/tissue mixture is emulsified with either chloroform or phenol to denature and separate the contaminants from DNA to achieve isolation.

5. **DNA precipitation:** After removing contaminants, the DNA is precipitated with ethanol (generally 70%) and dissolved later in the water. Finally, DNA is stored at -20°C.

Key reagents for plant DNA extraction

- CTAB: Hexadecyltrimethyl-ammonium bromide or CTAB is a cationic detergent that constitutes a long hydrophobic hydrocarbon chain (water-repelling) and a hydrophilic head (soluble in water). As cell membranes are composed of diverse phospholipids and proteins, a detergent can be an option to dissolve them. This is where CTAB plays a vital role in breaking the cell membranes.

- **NaCl**: Salt is used to remove proteins that are bound to DNA. It also helps keep the proteins dissolved in the aqueous layer, avoiding DNA co-precipitate with proteins.

- **Tris**: The (hydroxymethyl) aminomethane is a buffer favoring the stability of DNA once it is released. The cell pH gets altered when cytoplasmic material is released, consequently, the pH change can hamper the DNA.

- **EDTA**: EDTA chelates divalent cations, such as Mg2+ and Ca2+, which are cofactors of DNases, and make DNases non-functional.

- β-Mercaptoethanol (BME): BME is a potent reducing agent to clean tannins and polyphenols. Furthermore, it favors the dissolution of globular proteins in water.

- **PVP**: PVP is added to remove phenolic compounds from plant DNA extracts.

- Phenol: Phenol is an organic solvent, immiscible with water. It is used with chloroform and isoamyl alcohol to purify the DNA and remove proteins and polysaccharide contaminants.

Measuring the quantity and quality of the extracted plant DNA

To measure the DNA quantity and quality parameters, researchers often use the Nanodrop, a device that can quickly determine the DNA absorbance with less sample loss than traditional spectrometers. Generally, DNA is estimated to be pure if the ratios A260/A280 and A230/A280 are around 1.9.

Higher values can be indicative of the presence of contaminants.

Furthermore, an agarose gel is also performed for visualization. Poor quality DNA can be observed on the gel as smeared bands.

Tips for extracting plant DNA

Tip 1: Have a written protocol before you start

It is essential to have a written protocol before starting the extraction, and check the steps as you go. It will allow you to verify errors if results are not achieved. It also provides an opportunity for improvement as changes or deviations can easily be noted on the protocol and referenced with respect to better or poorer yields.

Tip 2: Prepare all materials in advance
All DNA protocols require sterilized and cleaned material, free from DNases and RNases. It generally takes time to be prepared. So, in advance, sterilize mortar, pestle, and be ready with aseptic tips, falcon tubes, microcentrifuge tubes, among others.

Tip 3: Set a time for DNA extraction
If you are optimizing a protocol, block enough time for the DNA extraction. Some protocols may require rounds of cleaning or overnight incubations. Starting early is advisable because you may find some issues that could be hard to solve after work-hours (e.g., after 5 pm).

Tip 4. Review after failing
Don’t get desperate after a failed DNA extraction. It is normal to feel frustration after many failed attempts. Follow each step in your DNA extraction protocol carefully, and if you followed our first tip, you can easily go back and find the probable cause of the error.

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