

# Isolation of plant dna from onions essay sample

[Science](#), [Biology](#)



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## **Title: Isolation of plant DNA from onion**

### **Introduction**

DNA is the hereditary material of all living organisms and therefore the isolation of DNA is essential to geneticists and molecular biologists and scientists interested in studying hereditary diseases. Almost all cells contain DNA, but not all have equal amounts and therefore it is important to select the source of DNA carefully. DNA is an actual thing, found in our bodies, in very large quantities. In fact, it is found in every living thing except bacteria (Walter, 2017). It is DNA that determines who we are, but DNA is made up of the same basic materials as are found in the rest of bodies. It has the biochemical property of being polar, which allows us to separate it from a solution using the electrochemical properties of other things found in our bodies, such as proteins (Switzer, 2009).

DNA extraction is a fairly simple procedure that requires a few steps: The lysis buffer (detergent) breaks open the cells by destroying the fatty membranes that enclose the cells as well as the nuclei membranes within the cells. DNA is released into the solution (Tijssen, 2003). The process of extracting DNA from a cell is the first step for many laboratory procedures in biotechnology. The scientist must be able to separate DNA from the unwanted substances of the cell gently enough so that the DNA is not broken up. Isolation of DNA from cells is the first step in many investigations in molecular biology. First, the tissue is broken up mechanically. Cell fragments are separated by filtration; the DNA and soluble proteins remain. An enzyme removes the protein, then the DNA is precipitated using ice-cold ethanol (Rice, 2010).

TBE or Tris/Borate/EDTA is a buffer solution containing a mixture of Tris base, boric acid, and EDTA. In molecular biology, TBE and TAE buffers are often used in procedures involving nucleic acids, the most common being electrophoresis. It is used in agarose electrophoresis typically for the separation of nucleic acids such as DNA and RNA (Philip, 2003).

### **Abstract**

DNA is the hereditary material of all living organisms and therefore the isolation of DNA is essential to geneticists and molecular biologists and scientists interested in studying hereditary diseases. In this experiment, the aim is to isolate DNA from plants, in this case, an onion, and to discover the fundamental properties of DNA and the scientific methods required to isolate it. Isolation of DNA will be isolated from a fine onion. The onion will be mixed with water and salt. After filtering the homogenate meat tenderizer will be added; whose function is to act as a catalyst to break down, it breaks an open the cell nucleus to release the DNA. The isopropanol will be added because it causes the DNA to precipitate. When DNA comes out of solution it tends to clump together, which makes it visible. To evaluate the integrity of the DNA 0.8% of agarose gel will be prepared by adding 0.4g of powdered agarose to 50 ml of TBE buffer then heating the slurry in a microwave on full power. After cooling the solution the comb will be placed thus creating wells in which the DNA will be loaded. After loading the DNA samples and adding enough buffer to cover the tank current will be applied until the bromophenol blue migrates to the anode. The gel will be photographed with the use of UV illuminator.

Aim

The aim of this practice is to isolate DNA from plants, in this case, an onion, and to discover the fundamental properties of DNA and the scientific methods required to isolate it.

### Objectives

- To experience the firsthand isolation of DNA from plant tissue (onion) without destroying its structure and sequences
- To become familiar with the physical properties of DNA by isolating it from living tissue
- Be able to evaluate the integrity of the extracted DNA from onion by photographing it under UV light.

## **Materials and methods**

### **1. Isolation of DNA**

Water was measure out to 80 ml then 10 ml of dishwashing liquid and 10 g of non-iodized salt were also measured then placed into a 250 ml beaker. Into the beaker, 50 g of finely grated onion were added. The beaker was incubated in a 60oC water bath for exactly 15 min. The homogenate was filtered through four layers of cheesecloth and the filtered solution, which contains the DNA, was saved. 1 g of meat tenderizer was added to the strained homogenate then let stand for 5 minutes at room temperature. The homogenate was cooled to 10oC on ice. Very slowly 50 ml of ice-cold isopropanol was added by pouring it down the side of the tilted beaker. It is essential that the isopropanol and homogenate form separate layers with the homogenate on the bottom.

The white stringy DNA that appears at the interface was spooled out by gently swirling a glass rod around at the isopropanol/homogenate interface. The rod was always turned in the same direction. The DNA looked like a blob of mucus on the glass rod. The DNA was suspended in 500  $\mu$ l TE.

## **2. Preparation of a 0. 8% agarose gel**

The edges of a clean, dry, plastic tray were sealed with tape so as to form a mold. The mold was set on a horizontal section of the bench. 0. 4 grams of powdered agarose was added to 50 ml of TBE buffer in an Erlenmeyer flask. The neck of the flask loosely plugged with cotton wool. The slurry was heat in a microwave oven on full power for 20 – 60 seconds. Note: heat the slurry for the minimum time required to allow all the grains of agarose to dissolve. Wearing an oven glove, the flask was carefully swirled from time to time to make sure that any grains sticking to the walls enter the solution. The solution was cooled to approximately 60oC (just cool enough to hold). Ethidium bromide was added to a final concentration of 0. 5  $\mu$ g/ml and then mixed thoroughly. The gel comb was positioned near one end of the mold and then approximately 40 ml of the warm agarose solution was poured into the mold. The gel was between 3 mm and 5 mm thick. It was checked to see there were no air bubbles under or between the teeth of the comb. The gel was then to set for approximately 45 minutes.

## **3. Evaluation of DNA integrity by agarose gel electrophoresis**

After the gel was completely set, the comb and tape were carefully removed and the gel mounted in the electrophoresis tank. Enough electrophoresis buffer was added to cover the gel to a depth of about 1 mm. In a sterile microcentrifuge tube (1. 5 ml), a sample of isolated DNA was mixed with gel

loading buffer (10 µl of 6 x DNA loading buffer per 50 µl DNA solution). Slowly 30 µl was loaded of the mixture into the slots of the submerged gel using a micropipette. A sample of DNA marker was loaded in lane 1. This provided a ladder of DNA bands of defined sizes for use as molecular size markers for determining the size of the DNA. The lid of the gel tank closed and the leads attached so that the DNA will migrate towards the anode (red label). A voltage of 1 – 5 V/cm applied (measured as the distance between the electrodes; normally 50 – 100 V). The gel was electrophoresed until the bromophenol blue had migrated three-quarters the length of the gel, approximately for 1 hour. The electric current was turned off and the leads and lid from the gel tank removed. The gel was examined using an ultraviolet (UV) illuminator then photographed the using the departmental gel documentation system.

## **Results**

Figure 1: Diagrammatical representation of Genomic DNA sample isolated from an onion after the gel containing the DNA sample was exposed to ultraviolet light

## **Discussion**

An onion is used because it has a low starch content, which allows the DNA to be seen clearly. The salt shields the negative phosphate ends of DNA, which allows the ends to come closer to the DNA can precipitate out of a cold alcohol solution.

Gel electrophoresis is one of the most common tools of molecular biology. Depending on the size of DNA being analyzed, the correct concentration of agarose in the gel is essential to permit an effective resolution of the DNA

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under consideration. One of the most powerful tools used in molecular biology is agarose gel electrophoresis. Gel electrophoresis separates DNA molecules based on size. However, the concentration of agarose used in the gel is an important factor that must be taken into consideration when dealing with DNA of specific sizes. The concentration of the gel affects the ability of fragments to be separated and resolved on the gel (Lee et al., 2012).

The detergent causes the cell membrane to break down by dissolving the lipids and proteins of the cell and disrupting the bonds that hold the cell membrane together. The detergent then forms complexes with these lipids and proteins, causing them to precipitate out of solution (Switzer, 2009). The detergent helps to degrade both the cell membranes and those surrounding the nuclei. In place of dishwashing liquid, another commercial reagent that could be used is shampoo.

According to (Brody & Kern, 2004), the source of Enzyme Meat Tenderizer Results Meat tenderizer acts as a catalyst to break down, it breaks an open the cell nucleus to release the DNA. During DNA isolation protocols, another commercial reagent that is normally used is baking soda. Ethanol or isopropyl alcohol causes the DNA to precipitate. When DNA comes out of solution it tends to clump together, which makes it visible.

Buffers in gel electrophoresis are used to provide ions that carry a current and to maintain the pH at a relatively constant value. There are a number of buffers used for electrophoresis. The most common being, for nucleic acids Tris/Acetate/EDTA (TAE), Tris/Borate/EDTA (TBE). TBE is a buffer solution

containing a mixture of Tris base, boric acid, and EDTA. Loading buffer also increases the density of the sample. Recall that denser objects sink, so adding loading buffer to the DNA samples will enable the DNA molecules to sink into the wells in the gel in preparation for gel electrophoresis (Aaij & Borst, 2002).

DNA ladder is made up of different fragments with known sizes. DNA ladder is added in a different lane from the DNA sample by running both DNA ladder and DNA sample in the agarose gel electrophoresis; this allows comparing and estimates the size of the DNA sample (Mustafa et al., 2007).

Conventional approaches to DNA isolation and purification are based on multi-step procedures involving phenol/chloroform, anion exchange, or silica gel exchange systems. DNA can be isolated from almost any cellular or tissue source. Purification of DNA requires removing it from the cell while protecting against degradation. Isolation procedures must also be gentle enough to protect the long DNA strands from mechanical stress (Walter, 2017).

## **Conclusion**

DNA is an actual thing, found in our bodies, in very large quantities. In fact, it is found in every living thing except bacteria. It is DNA that determines who we are, but DNA is made up of the same basic materials as are found in the rest of bodies. It has the biochemical property of being polar, which allows us to separate it from a solution using the electrochemical properties of other things found in our bodies, such as proteins. DNA is important in several other branches of science, such as genetic engineering.



**References**

- Aaij C. and Borst P. (2002). " The gel electrophoresis of DNA". Biochim Biophys Acta. 269 (2): 192–200. PMID 5063906. doi: 10. 1016/0005-2787(72)90426-1.
- Brody J. R. and Kern S. E. (2004). " History and principles of conductive media for standard DNA electrophoresis" (PDF). Anal. Biochem. 333 (1): 1–13. PMID 15351274. doi: 10. 1016/j. ab. 2004. 05. 054.
- Lee P. Y, Costumbrado J., Hsu CY, Kim Y. H. (2012). " Agarose gel electrophoresis for the separation of DNA fragments". J Vis Exp. 62 (3923). PMC 4846332 Freely accessible. PMID 22546956. doi: 10. 3791/3923.
- Mustafa U., Morgan M. E, Minden J. S (2007) Electrophoresis. Gel methods for detecting changes in protein extracts. 18
- Philip S. (2003). " Agarose gels: Properties and use for electrophoresis". Electrophoresis. 4 (6): 375–382. doi: 10. 1002/elps. 1150040602
- Rice, M. (2010), DNA Extraction Principles: How do you get DNA out of a whole organism? from [http://www. slideshare. net/meganrice/dna-extraction-principles](http://www.slideshare.net/meganrice/dna-extraction-principles)
- Switzer, R. (2009). Experimental Biochemistry. New York, U. S. A.: WHFreeman and Company.
- Tijssen, P. (2003). Laboratory Techniques in Biochemistry and Molecular Biology Amsterdam, Netherlands: Elsevier Science Publishers B. V
- Walter R. Fehr (2017). Extracting onion DNA. Office of Biotechnology, Iowa State University. Retrieved from [https://www. sciencebuddies. org/science-fair-projects/project-ideas/BioChem\\_p001/biotechnology-techniques/extracting-onion-dna](https://www.sciencebuddies.org/science-fair-projects/project-ideas/BioChem_p001/biotechnology-techniques/extracting-onion-dna)