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EXPLORING DNA ISOLATION TECHNIQUE: A GUIDE FOR EDUCATORS AND STUDENTS

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Abstract: *This study is intended to guide educators or students who will conduct DNA isolation experiments to prove and view the presence of DNA contained in fruit using a simple method. DNA isolation is a method to obtain or research deoxyribonucleic acid from an organism. The basic principle in DNA isolation is to break or extract a tissue so that a cell extract contains DNA, RNA, and other essential substances will be formed. DNA isolation experiment is intended to determine a simple method of isolating DNA in fruit and to determine the effectiveness of detergents used for DNA isolation. In this experiment, to observe DNA, DNA isolation was carried out on the crushed fruits to form juice from the fruit. Then, make a solution from a mixture of detergent and water. Later, mix the fruit juice with a detergent solution and add salt to it. Once the solution is homogeneous, filter it and add cold ethanol. Then, the DNA strands will appear because of the reaction. The results obtained are influenced by the type of detergent used, the type of fruit used, the alcohol concentration, and the results of the filtering performed.*

Abstrak : Penelitian ini merupakan panduan bagi para pendidik atau siswa yang akan melakukan eksperimen isolasi DNA dengan tujuan untuk membuktikan dan melihat keberadaan DNA yang terkandung dalam buah dengan metode yang cukup sederhana. Isolasi DNA adalah metode yang digunakan untuk mendapatkan atau melakukan penelitian tentang asam deoksiribonukleat dari suatu organisme. Prinsip dasar dalam isolasi DNA adalah mengekstrak jaringan sehingga akan terbentuk ekstrak sel yang mengandung DNA. Percobaan isolasi DNA dimaksudkan untuk menentukan metode sederhana untuk mengisolasi DNA dalam buah dan untuk menentukan efektivitas deterjen yang digunakan untuk isolasi DNA. Dalam percobaan ini dilakukan isolasi DNA yang diperoleh dari hasil jus buah. Kemudian, membuat larutan dari campuran deterjen dan air, lalu campurkan jus buah dengan larutan sabun dan tambahkan garam ke dalamnya. Setelah larutan homogen, saring larutan dan tambahkan etanol dingin ke dalamnya. Kemudian untaian DNA akan muncul sebagai hasil dari reaksi. Hasil yang diperoleh dipengaruhi oleh jenis deterjen, jenis buah, dan konsentrasi alkohol, serta hasil penyaringan yang dilakukan.

INTRODUCTION

Teaching science through inquiry enables students to formulate a question and explore potential explanations to answer it. This type of science learning fills a student's head with vocabulary words such as "DNA being the genetic material" and "mitochondria being the powerhouses of the cell." This kind of science teaching has many problems. Students will fail to see how this type of knowledge will be helpful in the future, and it will not allow them to practice critical thinking, problem-solving, and communication. Therefore, in science education, students must be able to conduct investigations so they can easily understand and retain the concepts they have learned (Olson & Loucks-Horsley, 2000). This study is a guide for educators or students who will conduct DNA isolation experiments. DNA isolation experiments can be conducted for grade 12 high schools or university students to prove and view the presence of DNA contained in fruit by a reasonably simple method.

DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms (Masruroh & Darmanto, 2024). DNA is one of the most important macromolecules in the cell. DNA carries genetic information to guide cells to perform RNA and protein synthesis (Liang et al., 2024). DNA is found in the cell's nucleus as nuclear DNA. An organism's complete set of nuclear DNA is called its genome. Besides the DNA in the nucleus, humans and other complex organisms also have a small amount of DNA in cell structures known as mitochondria. This is because most genetic information has been transferred to the nucleus or lost during mitochondrial evolution. However, mitochondria maintain their genomes (Filograna et al., 2021).

Extraction of DNA, RNA, and proteins is the basic method used in molecular biology. These biomolecules can be isolated from any biological material for subsequent downstream processing, analytical purposes, or preparation. In the past, extracting and refining nucleic acids was complex, time-consuming, labor-intensive, and limited overall throughput. DNA extraction is the first stage of molecular research, significantly affecting the quality of DNA isolation. DNA extraction removes DNA from the source, cell nucleus, or cell organelle (chloroplast DNA, mitochondrial DNA). DNA extraction can be obtained from tissue samples that are still fresh, frozen, dried, or stored in alcohol or buffers (Nouws et al., 2020). Currently, many specialized methods can be used to extract pure biomolecules, such as solution-based and column-based protocols (Masoomi-Aladizgeh et al., 2023).

DNA isolation is a method for obtaining deoxyribonucleic acid from a living thing. DNA isolation is a fundamental technique required in molecular biology laboratories (Bruegmann et al., 2022). DNA isolation consists of three main procedures: cell lysis, removing the contaminant, and purification of DNA. DNA isolation method was chosen based on some considerations, such as the amount and molecular weight, purity, time, species, and cost (Arfa et al., 2018). DNA isolation can be accomplished through stages such as preparation of cell extracts, purification of DNA from cell extracts, and DNA precipitation. Although DNA isolation can be done in various ways, each type or part of the plant can produce different results because there are high concentrations of polyphenols and polysaccharides that inhibit DNA purification (Abdel-Latif & Osman, 2017). If DNA isolation is done with fruit samples, then the water level of each fruit is different, which can also give different results. Fruit with high water content will produce different isolates when compared to fruit with low water content. The higher the water level of dissolved cells in the extract will be less, so the DNA precipitation will also be less.

Manual methods have certainly evolved rapidly over time with various commercial offerings that include complete kits containing most of the components needed to isolate nucleic acids. However, most require repeated centrifugation steps, followed by removal of the supernatant depending on the specimen type and additional mechanical treatment. Automated systems designed for medium-to-large laboratories have been in increasing demand over the last few years. It is an alternative to the labor-intensive manual method. Technology must allow high sample throughput, yield, purity, reproducibility, and scalability of biomolecules. The tests' speed, accuracy, and reliability must be maximized while minimizing the risk of cross-contamination (Bosetto et al., 2017).

The principal isolation method is to use a high concentration of SDS for cell lysis, followed by adding chloroform-isoamyl alcohol to remove non-DNA biomolecules such as proteins and lipids and then precipitating DNA with isopropanol. Adding detergent in DNA isolation can cause cell membrane damage through the structure formed through the hydrophobic side of the detergent with proteins and fats on the membrane of complex detergent-lipid protein compounds. These compounds can be formed because proteins and lipids have hydrophilic and hydrophobic ends, as well as detergents, so they can form a chemical bond (Shariatpanahi et al., 2018).

In DNA isolation, the material we use is usually plant tissue or animal tissue, so the first step is to break the tissue into independent cells. The process is carried out

mechanically or physically by mashing or grinding the ingredients we will use with a mortar or blender. The second is to break the DNA wrapping layer's cell wall and cell membrane. The main structure forming the membrane and cell walls is fat; we use detergents and table salt. These two materials are used to perforate and damage cells so that the contents of the cell nucleus or DNA can come out. The next stage is the separation of DNA from other materials. Separation is carried out using cold ethanol with a 90-95% concentration. Ethanol or Alcohol does not dissolve DNA, and the specific gravity of alcohol, which is lighter than water, makes DNA rise and hover on the surface (El-Ashram et al., 2016).

The detergent method is one of the methods developed for DNA isolation. The detergent method uses detergents in cell wall separation and protein removal in the sample (Acton, 2013). DNA is then physically isolated from the buffy coat and used as the starting material for DNA isolation by cell lysis in a detergent buffer. In a second general protocol, a nonionic detergent is added to whole juice to lyse the cell membrane, followed by pelleting of intact nuclei. In either method, the nuclei are lysed in a buffer containing NaCl and EDTA, and the nuclear protein fraction is digested with proteinase K buffer containing SDS. Proteins are then removed by either salting out or phenol-chloroform extraction. The resulting supernatants are ethanol precipitated to obtain the final DNA (Kieleczawa, 2016).

In the lysis and extraction step, the cell wall and/or membrane disrupted in the previous step are now being dissolved, and the cell releases its content, thus facilitating DNA extraction from the nucleus. For this purpose, lysis buffers are used. Although their composition might vary depending on the extraction method, they must contain the following components: detergents, which are compounds able to remove or solubilize membrane lipids (Radovic et al., 2019).

In the lysis process using detergents, sodium dodecyl sulfate (SDS) is often used as a step for coating cell membranes. Apart from having a role in lysing the cell membrane, this detergent can also play a role in reducing the activity of the enzyme nucleases, which are DNA-degrading enzymes. Apart from being used by SDS, other detergents, such as cetyltrimethylammonium bromide (CTAB), are also often used to lyse cell membranes in plant DNA isolation (Allen et al., 2006). The success parameters in using CTAB depend on several things. First, the NaCl concentration must be above 1.0 M to prevent the formation of CTAB-DNA complexes. Because the amount of water in cell pellets is difficult to predict, the use of CTAB as a solution breaker must be with

NaCl with a minimum concentration of 1.4 M. Second, extracts and cell solutions containing CTAB must be stored at room temperature because the CTAB-DNA complex is insoluble at temperatures below 15 ° C. Third, the use of CTAB with good purity will determine the purity of the DNA obtained and with very little polysaccharide contamination. After adding CTAB, the samples were incubated at room temperature. This incubation aims to prevent the deposition of CTAB because CTAB will settle at a temperature of 15°C. Because of its effectiveness in removing polysaccharides, CTAB is widely used for DNA purification in cells that contain many polysaccharides, such as those found in plant cells and gram-negative bacteria such as Pseudomonas, Agrobacterium and Rhizobium (Xia et al., 2019).

The presence of the chelating agent EDTA protects the DNA from damage by DNases present in DNA preparations intended for long-term storage. EDTA is a component of TE buffer (10 mM Tris, one mM EDTA) and other resuspension buffers. EDTA will also inhibit enzyme activity when the DNA is used in various procedures, such as restriction enzyme digestion or PCR. One must be careful not to dilute the DNA too far so that large volumes (e.g., more than 10% of a reaction volume) of the DNA-EDTA solution are required for subsequent analysis methods. When DNA yield is low, as with some clinical samples, it is better to dissolve it in water. More of this can be used in analysis procedures without adding excess amounts of EDTA. Because most of or the entire sample will be used for analysis, protection in storage is not a concern (Buckingham, 2019).

METHOD

It is necessary to carry out a systematic procedure to get maximum and accurate results from DNA isolation experiments. The first procedure is to make a solution with a mixture of detergent or soap with 60 ml of distilled water, then stir it for 15 minutes slowly until it becomes a homogeneous and non-foaming solution. Then, make juice by crushing 100 grams of the determined fruit pulp with 100 ml of distilled water in a blender for 50 seconds. After that, mix each 4 ml of soap solution and 4 ml of fruit juice and add one spatula of table salt. Stir slowly for 10 minutes until a homogeneous and even mixture is produced. After mixing everything well, filter it using filter paper twice to produce a good extract. Then, take 6 ml of the filtered solution and put it in a test tube, then add 5 ml of cold ethanol with different concentrations. After everything is mixed,

observe the process of DNA emergence, including the time required and how much DNA is formed.

RESULTS AND DISCUSSION

The materials used in this experiment have their respective functions. The fruit is used as a DNA isolation material by taking its extract. Detergent or soap functions as a material that will lyse the cell walls of the fruit because in the detergent or soap, there is an SDS compound that can lyse the cell walls faster, plus there is an EDTA compound to remove Mg2+ ions, which maintain the cell wall structure in the fruit. Ethanol functions to bind DNA so that it can be seen and easily observed. Salt functions as a concentrator because it contains Na+ ions, which can form bonds at the negative pole in the DNA phosphate bond so that DNA can gather and be easily observed. Aquadest are used to make solutions, whether fruit juices or soap solutions. Filter paper is used to filter soap solutions that have been mixed with fruit juice and salt, the function of which is to filter so that other organelles do not participate in the DNA isolation process so that they can produce accurate data.

Apart from materials, tools that have their function were also used in this experiment. The knife serves to cut the fruit into smaller pieces, making it easy to put in the blender. The glass is used to hold the solution and mix the solution. Stirrer to mix the solution so that it becomes a homogeneous solution. Blender to crush the fruit so that it can be extracted. Spatula to pick up ingredients. Test tube for reacting to the solution. After running the entire experimental procedure, the form of data obtained by the student is as follows.

Table 1 Experiment Result

No	Material			Estimated time of appearance of DNA	Amount of DNA
	Fruit	Soap	Alcohol concentration		
1	Papaya	Bukrim	70%	3 minutes	++
		Bukrim	95%	2 minutes	++
		Bukrim	70%	3 minutes	+++
2	Papaya	Bukrim	70%	1 minutes	+++
		Bukrim	70%	6 minutes	+++
		Wing Ekonomi	70%	3 minutes	+++
		Wing Ekonomi	70%	2 minutes	+++

3	Pear	Rinso	70%	2 minutes	++
	Pear	Soklin	70%	3 minutes	++
	Pear	Daia	70%	2 minutes	+++
	Pear	Rinso	70%	5 minutes	+++
	Pear	Soklin	70%	4 minutes	+++
4	Pear	Rinso	70%	4 minutes	++
	Pear	Rinso	70%	18 minutes	+++
	Pear	Soklin	70%	10 minutes	+
	Pear	Rinso	98%	2 minutes	+++
5	Tomato	Mama lime	95%	5 minutes	++
			70%	7 minutes	++
			70%	6 minutes	++
			95%	5 minutes	++
			95%	30 minutes	++
6	Tomato	Mama lime	70%	7 minutes	++
	Tomato	Mama lime	95%	5 minutes	++
	Tomato	Mama Lime	70%	5 minutes	++
	Tomato	Mama lime	70%	3 minutes	++

Description:
+ = very less
++ = less
+++ = much
++++ = very much

In DNA isolation, the material used is plant tissue in the fruit that has been determined. The first step we must take is to break the tissue into independent cells. The process is carried out mechanically or physically by mashing or grinding the ingredients we will use with a mortar or blender. The second is a chemical process that breaks the DNA covering layer's cell walls and membranes. The main structure forming the membrane and cell wall is fat; for that, we use detergents with SDS compounds that can lyse the cell walls more quickly; plus, there are EDTA compounds to remove Mg2+ ions that maintain the cell wall structure in the fruit. In addition to detergents, table salt also contains Na+ ions, which can form bonds at the negative pole in the DNA phosphate bond so that DNA can gather and be easily observed. These two materials are used to perforate and damage cells so that the contents of the cell nucleus or DNA can come out. The next stage is the separation of DNA from other materials. Separation was carried out using 96% cold ethanol concentration. Ethanol does not dissolve DNA, and the specific

gravity of alcohol, which is lighter than water, makes the DNA rise and hover on the surface.

From the table of observations, there are differences in results in the aspect of the amount of DNA seen and the estimated time of appearance of DNA on the surface. Several factors influence the difference in results. The first factor is the type of fruit used. Fruit with high water content will produce different isolates when compared to fruit with low water content. The higher the water content, the fewer cells will be dissolved in the extract, so less DNA is precipitated. The second factor is in the filtering process; if the filtering is done incorrectly, many other organelles will participate in the solution to be isolated. The third factor is using a type of soap or detergent, where the higher the SDS content, the faster the lysis process on the fruit cell walls, and if the higher the EDTA content, the fruit cell walls will also be destroyed more quickly. The fourth factor is the concentration of ethanol used, where ethanol with a concentration of 96% will bind DNA faster than ethanol with a concentration of 70%.

By paying attention to the SDS and EDTA content in soap and detergent, it is known that the SDS and EDTA content in detergents is higher than that of soap. Therefore, detergents will be more effective in DNA isolation experiments than soap. The main structure forming the membrane and cell wall is fat; for that, we use detergents in which there are SDS compounds that can lyse the cell walls more quickly, plus there are EDTA compounds to remove Mg2+ ions that maintain the cell wall structure in the fruit. By using detergents in DNA isolation experiments, the process of lysis of fruit cell walls will be faster, and the destruction of cell walls will also be faster than using soap.

CONCLUSION

In conclusion, by developing questions and investigating possible answers, teaching science through inquiry helps students participate actively in teaching-learning processes. Science education should, however, inspire students to do practical research if we are to have a more thorough knowledge. This method develops critical thinking, problem-solving, and communication abilities, helping pupils better remember the ideas they acquire. Students can immediately witness scientific ideas and better understand complex subjects like genetics and molecular biology by applying these abilities in hands-on activities, including DNA isolation. Therefore, using experimental techniques

like DNA isolation is a necessary approach to link theoretical knowledge with practical applications, improving student involvement and comprehension and bridging this gap. Ultimately, this approach fosters a deeper connection between students and scientific practices, preparing them for future challenges in science.

The study on DNA isolation from fruit emphasizes applying the correct tools and materials for correct outcomes. Elements including fruit type, detergent composition, ethanol concentration, and filtering method have influenced the yield and quality of the obtained DNA. Higher SDS and EDTA content detergents are more effective in lysing cell walls and promoting DNA isolation than soap. These results show how little changes in experimental techniques could affect the result, so both teachers and students will be offered important new perspectives. These kinds of tests support scientific ideas and equip students for more complex molecular biology and genetics courses. Moreover, it highlight the importance of precision in experimental procedures, which is crucial for obtaining reliable and reproducible results in scientific research.

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