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# Local Material-Based DNA Experiment (LMBE):

## Viable Alternative Approach for Biochemistry Laboratory

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***Abstract*—The COVID-19 pandemic has led to the implementation of e-learning to ensure the teaching process does not halt utterly due to the crisis. However, e-learning courses alone are less adequate to provide hands-on skills regarding laboratory experiments. The course designed emphasizes the development of student practical chemistry skills and creativity in conducting isolation and detection of DNA at home. The context of "varieties of tomatoes" provides students with an opportunity to study how genetics affects phenotype and how to see fruits DNA through an experiment. In this experiment, students were exposed to scientific methods and started their activity by defining the problem. The lab format also provides students to analyze the basic principle functions of each reagent used for DNA extraction and was challenged to explore alternative compounds to substitute standard chemicals. Afterwards, the students designed local material-based experiments (LMBE), as the way to develop creative and practical chemistry skills including critical analysis on the alternative experimental design, primary results interpretation and analysis, and scientific writing. In the second part, the students were introduced to learn the principles of electrophoresis as one of the molecular genetics techniques and built their homemade electrophoresis to visualize the extracted DNA from previous activity. To this end, the LMBE is serving an alternative approach to the aim of developing students' practical and creativity skills in biochemistry laboratory during stay at home.**

***Keywords—biochemistry laboratory, creative thinking, DNA, electrophoresis, hands-on learning, local material-based experiment***

## I. INTRODUCTION

The term DNA recently becomes more familiar since nuclei acid-based technology is being used to diagnostic the Covid-19. This technology utilizes the specific genetic material such as DNA/RNA with homologous strands. Structurally, DNA has an extremely long twist strand in which two strands are bind about one to another. Understanding DNA is important to understand life at the molecular level. However, some students found lack ability to easily comprehend the key concept of DNA due to its abstract concepts. Conducting experiential learning about DNA will help students to solve their learning problems and be independent learners. Yet, another problem is to conduct this experiments required invariable expensive materials. Therefore, this experiment mostly skips [1] for chemistry students.

Experiential activities play significance role in facilitating students to gain practical and high order thinking skills through some experience of science is all about [2]. An effective biochemistry laboratory could facilitate the strengthening of concept learning [3]. Ideally, the laboratory exercises should be designed to promote students to be able to formulate question related to the observed-phenomenon, capable to design and carryout an experimental strategy, to collect and critically analyse the results, to reach conclusion, and to decide whether further experiments should be conducted to answer the questions or new additional questions that may have come from the investigations [4].

The Covid-19 pandemic has caused enormous impact on the implementation of routine classroom teaching. The online learning has rapidly developed to make sure that learning process does not completely stop due to the crisis [5]. Many options have been carried out to encounter the situations, ranging from cancelling the laboratory activities, holding virtual experiments, guiding students to design experimental procedures, and encourage students to critically analyse available data [6]. However, thus processes less adequate to facilitate students to earn significant laboratory experience as

 

one of the learning outcome for Biochemistry laboratory (KI409) in Chemistry Department of Universitas Pendidikan Indonesia. The real experiment at the campus was also closed to prevent the spread of Covid-19. Experiment is an important and proper instrument for developing students' creativity [7]. Toward this purpose, we designed at home experiments using local material not only to facilitate hands-on activities, but also to develop students' creative thinking in searching alternative materials for their experiments. The experiments covered the following concepts: Basic principal for extracting and isolating DNA and DNA separation using gel electrophoresis.

DNA has been known as blueprints of all living things. The order of long-chain nucleotide makes organisms similar to other of their species, but different as an individual. DNA is located exclusively in the nucleus in eukaryotic cells, while in prokaryotic is organized in rings or circular plasmid. To study DNA, we have to extract DNA from the cells and then isolates the DNA from other molecules. There are three basic steps to extract DNA: 1) lysis, 2) precipitation, and 3) purification. All the steps were carried out by using simple and low-cost material from daily life.

Once the DNA successful to be extracted, the next step is separation and characterization. Electrophoresis is one of the methods for separating DNA by charge, mass, and shape by employing an electrical voltage to a gel in a buffered solution. The problems have arisen is high running costs and exposure of hazard material such as ethidium bromide when visualization of DNA. Here, we boosted students to construct their own electrophoresis using homemade apparatus and readily available chemicals.

In this work, the local material-based experiments (LMBE) was designed to ensure the students to have real lab experience as the way to develop their creativity and practical skills. Students were encouraged to search alternative materials to replace the standard chemicals used for conducting DNA experiments.

## II. Materials and Methods

### *A. At-home Laboratory Contents*

The "at-home" laboratory contents include total DNA extraction and purification from fruits and vegetables, comparing the amount of DNA qualitatively from both DNA sources, and DNA characterization using gel electrophoresis.

### *B. LMBE DNA Extraction and Purification at Home*

*1) Preparing lysis buffer:* Prepare a beaker glass containing about 90 ml of distilled water. Add 1.5 g table salt (either iodized or non-iodized will work) then stir until completely dissolved. Add washing up liquid until the volume reached about 100 ml. Stir gently the mixture and avoiding making too many bubbles during the stirring.

*2) Preparing DNA sample from fruits or vegetables. Basically:* DNA can be extracted from any living thing sources. Here, we selected fruits and vegetables due to it highly yield and easily to prepare. Break the cells mechanically by using blender/mortar and pestle/ cutting the tissue into small pieces. Put the sample into plastics bag and sealed it for further use.

*3) Preparing chilled alcohol:* Put the whole container of 95-99% ethanol or isopropanol in the freezer for about 24 hours.

*4) Extracting DNA:* Add about 50 ml buffer lysis into the fruit/vegetable mush and then sealed again the plastic. Firmly squeeze the mixture using your hands and fingers to pound, crush, and homogenize the mixture in plastics until there are no large pieces left and a "pulp" is formed. To completely extract the DNA, incubate the mixture in a container filled with warm water at a temperature of ± 600C for 15 minutes. Make sure it's mixed submerged in water. After 15 minutes, pour and filter the solution using a soft cotton cloth to obtain fruit/vegetable filtrate and place another clean beaker glass and let it cool.

*5) DNA precipitation:* Pour 20 ml of chilled alcohol into a beaker with the DNA filtrate in it. Wait for the DNA to start precipitating out in the alcohol (the process begins almost immediately and the DNA will continue to condense for the next few minutes). Fish the mash of white stringy stuff at the top of the beaker (the DNA!) using chopstick and place it onto a clean chamber for further analysis.

### *C. LMBE Electrophoresis*

*1) Gel comb, gel box, electrode, and Power supply:* Prepare a gel comb using wood coffee sticks to obtain a comb shape to make gel well for inserting the samples. Electrophoresis chamber in the standard kit was replaced by household plastic container that fitted to the prepared gel. The stainless steel electrodes were positioned along the bottom of each end of the gel box. Place carefully the anode at least 2 cm away from the end of the gel as oxidation products. Five-six batteries were used for yielding a voltage with a low and safe current.

*2) Gel and running buffer:* The gel was used agar to replace the agarose and baking soda (sodium bicarbonate) for preparing running buffer. Prepare 0.2% running buffer by dissolving 1 g of sodium bicarbonate into 500 ml of water. The 1% gel was prepared by dissolving 1 g agar into 100 ml water, and continued by heating the mixture until all agar completely dissolved and you resulted in a colorless solution. Left the solution in room temperature. Once it gets warm, pour the solution into the plastic box. Set the comb on the plastic box about 2 cm from the edge of the plastic box and let the gel become solidified and compacted.

*3) Setting electrode and well:* When the gel has solidified completely, prepare the electrodes by bending stainless wire to

stick firmly along bottom of each side of the plastic box. Cut the gel on the opposite sides of the plastic box with a width about 1 cm. Set electrodes to stick firmly along bottom of each side of the plastic box. The two electrodes act as positive and negative electrodes. Remove gently the comb from the gel, and make sure the gel is not damaged. Now the gel is ready to use for DNA separation.

*4) DNA separation:* Fill the plastic box with running buffer to completely soaking the gel. The empty part of the well will be visible when the buffer solution is added. Prepare a DNA sample by mixing 2 ml of the DNA sample with 1-2 drops of food coloring, 1 ml glycerin, and 1 ml of water on plastic painting ink. Loading about 20 μl of the samples using a plastic pipette with a needle. The tip of the pipetting needle contains about 10 microliters. Connected the electrode with the 45 V of voltage from a series of batteries. Run the electrophoresis for about 15-20 minutes.

## III. Results and Discussion

### *A. LMBE : The development of students' creativity*

The focused of this activity was to explore students' creativity in conducting DNA experiment using household materials. The student worksheet was used to guide students for critically evaluate the use of each component in DNA extraction and purification and gel electrophoresis, and to assess available substitutes for compatibility and practicality. The essential considerations were to search local materials which are safely and inexpensive for conducting DNA experiment at home since the materials needed to perform this experiments are invariably high cost [8][9]. Setting students to explore their ability to construct experiment using local materials may boost their awareness with the local resources in their environments and stimulate their creativity [1].

The LMBE worksheet stimulate students' inquiry by providing phenomenon about the varieties of tomatoes to show that the order of nucleotides in their DNA makes thus species fruits are different. The ability of students to set experiment for scientifically explain the phenomenon may lead the multiple solutions or products [10]. The worksheet also requested students to formulate the questions related to the phenomenon, designed the experiment, searching local materials for substitute the laboratory components, and conducting experiments to answer the questions. This strategy was designed to experience students to solve problems using scientific method and to systematically generate new ideas through "at-home" laboratory experience. This steps may lead students to gain creative thinking skills [1]. Laboratory-based learning experience students to have basic skills and performance ranging from laboratory techniques, research designing, conducting experiments, and academic writing.

From students' report, it was found that LMBE facilitates the development of students' creativity. Students' ability to express the reason why they select sample A/B/C/D/etc. as DNA sources and how they would carry out the experiment using local materials meet fluency indicator of creativity. Deciding the household materials for replacing standardized chemicals or even modified the standardized chemicals concentration reflect flexibility indicator of creativity. Originality indicator of creativity was evidently shown by the ability of students to discovered the alternatives chemicals from household materials. The ability of students to elaborate on the discussion in the laboratory report, instead of constructing experiments were clearly demonstrated the elaboration element of creativity.

### *B. DNA Isolation*

The LMBE DNA extraction and purification experiment was designed to ensure that laboratory activities do not totally halt due to the Covid-19 pandemic. In addition, this strategy intent to develop students' creative thinking skills. The term of DNA is fairly familiar. The blue print of all living system. In order to study more about DNA, we have to get the DNA out of the cells and isolate it from other unwanted molecules.

Here, the students selected varieties of fruits or vegetables such as oranges, bananas, strawberries, kiwis, eggplants, spinach, and tomatoes as sources to obtain DNA extract. Observation on different DNA extract from various sources, leads to an elaboration between unity and diversity in living systems. Experience to "seeing" DNA from fruits or vegetables using household materials should make the abstract concepts of DNA become more real for students fostering better comprehension of DNA concepts. An example of DNA extract appearance obtained by student is shown in Fig. I. The figure shown that a mass of white stringy stuffs at the top of beaker are onion DNA.

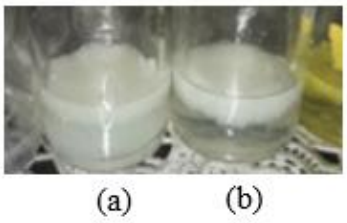


Fig. 1. Extract DNA from (a) Bombay onion and (b) Garlic onion.

The questions for comparing the basic protocol for extracting DNA from fruits and vegetables promoted students to evaluate whether different tissues may require modification in the basic protocol in order to completely disrupt the cell walls, inactivation of nuclease that probably available and can degrade DNA, etc. Table I is an example of student's report on DNA extract from fruits and vegetable.

TABLE I. DNA YIELD AND ITS CHARACTERISTIC

| Source of DNA | Characteristic and yield of DNA | | |
|---|---|---|---|
| | ***Profile*** | ***Amount (point) per 20g raw mass*** | ***time to precipitate (second)*** |
| Tomato | a mass of white stringy stuff at the top | ++ | 240 |
| Banana | a mass of white stringy stuff at the top of | +++ | 34 |
| Red onion | a mass of white stringy stuff at the top of | ++ | 370 |

The purpose of this study was to develop a local material-based procedure for extracting DNA from fruits or vegetables. From the results obtained (Table I), it was showed that all the sources could be successfully lysed using the washing up liquid as a detergent component for lysis buffer. The key consideration in selecting the materials is the function of each chemical in each process. Washing up liquid reacted with cell membranes resulted in pores on the surface of the membrane lead to the release of intracellular such as DNA, RNA, protein, lipid, carbohydrate, etc. [11]. Table salt was added during the lysis process to allow the DNA molecules to get together instead of deterring each other. The cations of the salt neutralize the negative electrical charge of the DNA. For complete recovery of the DNA, the procedure was also designed to apply additional incubation at about 60ºC for 15 minutes. This step was intended to inactivate nucleases that may degrade the DNA [12]. Further identification for suitable nucleases treatment by adding meat tenderizer or contact lens cleaning solution may worthy to get an effective and efficient protocol for DNA extraction. The final step is DNA purification using 95-99% of chilled alcohol even 75% alcohol also works well. The local materials used for alternative of DNA extraction experiment are shown in Table 2.

TABLE II. MATERIALS LIST FOR LMBE DNA ISOLATION

| Step of DNA isolation | Laboratory chemicals | Local-materials |
|---|---|---|
| Lysis cells | **Buffer lysis:** 1.0% lauryl sulphate 1x buffer Tris-EDTA (pH 7.6) Proteinase K | **Buffer lysis:** Washing up liquid 1.5% NaCl (table salt) |
| Precipitation | 90-95% of chilled ethanol or isopropyl alcohol | 70-95% of either chilled alcohol or room temperature |

Table II shows that the invariably and expensive chemicals for conducting DNA experiments can be replaced by household materials. Constructing LMBE experiments may foster students' creative thinking skills to solve the problem because this activity closely mimics research where the reagents or laboratory equipment may not be available [9].

### *C. Electrophoresis*

In order to design homemade electrophoresis, students have to concern on the following components: (1) buffer (s), (2) gel, (3) an electric current, and (4) molecules (here is DNA) visualization. To meet the four key elements of creativity, each of the creativity indicators were developed through guided-inquiry worksheet that supplemented by Science Writing Heuristic (SWH) syntax.

The electrophoresis experiment was designed to better visualize the chemical properties of DNA as polymers with different lengths (size). DNA has negative electrical charge due to the phosphate group on the DNA backbone [9]. Thus charge make DNA could separate using electrophoresis and thus would migrate toward the anode [13]. Electrophoresis is quite common techniques to separate molecules. However, for DNA separation, the application of this technique requires expensive materials. The DNA chelating agent, ethidium bromide EtBr, is too hazardous for this setting. Here, we promote students to construct their own electrophoresis apparatus using low-cost, safe, and locally obtained materials. The important steps in setting electrophoresis is constructing gel box and preparing gel. Here, the students learned the basic principal of the equipment operation and gained a comprehensive understanding related the concepts and processes. An example of students' exploration for constructing local –based material for electrophoresis components is shown in Table 3.

TABLE III. MATERIALS LIST FOR LMBE ELECTROPHORESIS

| Component of Electrphoresis | Local-materials | Laboratory standard | Function |
|---|---|---|---|
| Gel | Agar-agar (sold at food store) | Agarose | Matrix for DNA separation |
| Gel tray | Plastic box | High quality acrylic | Casting gel |
| Chamber | | High quality acrylic | Chamber to filled in the electrophoresis buffer |
| Electrode | Stainless steel/bare copper wire/paper clips | Platinum wire | Anode and catode |
| Power supply | Battery | Direct current (DC) | Source of electrical current |
| Gel comb | Wood coffee sticks | High quality acrylic | To form the well in which the DNA will be loaded |
| Gel buffer | Baking soda (sodium bicarbonate) | TAE (tris-acetate EDTA) or TBE (tris-borate EDTA) | Maintain the pH solution |
| Running buffer | Baking soda (sodium bicarbonate) | TAE (tris-acetate EDTA) or TBE (tris-borate EDTA) | Maintain the pH solution |
| Loading buffer | Food coloring glycerol | Bromphenol blue/methyl red/other dyes Glycerol | Visulization for DNA migration during electrophoresis |

The local materials used in the homemade electrophoresis (Table 3) suggesting that students are able to discovered the alternatives components for establishing the electrophoresis

equipment and evidently met the originality indicator of creativity. Fig. 2 show an example of LMBE electrophoresis constructed by student.

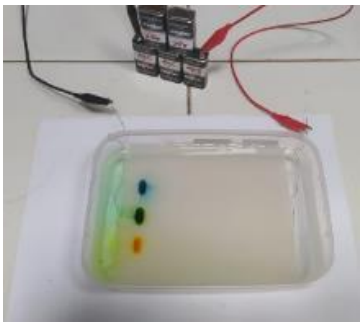

Fig. 2. The construction of LMBE electrophoresis.

It was observed from students' report that optimization of the buffer concentration and electrical current used for better separation of DNA. Gel electrophoresis system apply the same buffer for both casting and running gel. Buffer system is critical to prevent the changes of pH that may degraded DNA [13]. Another thing to be highlighted is the color migration could not be used to determine the size of the DNA fragment and the use of food coloring in this experiment is different from staining DNA for visualization purpose. Further exploration should be carried out construct standard curve of distance migrated against the molecular weight of the unknown DNA using dye-based gel electrophoresis. Boosting student to generate alternative DNA ladder by degrading the DNA using acids or base and utilizing this fragment to be made as standard.

## IV. Conclusion

This study presents a complete set of effective substitute materials for simulating DNA extraction and DNA separation using gel electrophoresis at home to keep ensure the practical skills obtained from hands-on activities do not utterly halt due to the Covid-19 crisis. The current results also show that the local material-based experiment (LMBE) worksheet may foster students' creativity and hope better comprehension about DNA and its nature. Students' answer in the LMBE worksheet met the four key components of creativity: fluency, flexibility, originality, and elaboration. Altogether, LMBE could assist students to develop their creativity and serve an alternative approach for conducting biochemistry experiment at home.

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