
	<p align="center">SKILLS CENTER STANDARD OPERATING PROCEDURE</p>	<p align="center">A BIOFIZZ  PRODUCTON</p>
<p>DNA Agarose Gels & Electrophoresis Module Hours: 3</p>	<p align="center">Effective Date: 9/13/2021 PRQs: Lab Safety Pipetting Buffers and Stock Solutions</p>	<p align="center">Revision # 1.1 M. Guzie Checked: M. Stowell</p>

BACKGROUND

Gel electrophoresis is a critical technique in molecular biology used to separate molecule fragments by size. This is done by using a polarized system with a positive electrode at one end, which takes advantage of the net negative charge of DNA and proteins. The negatively charged macromolecules are attracted to the positive end of the system but move very slowly through a gel. This allows the molecules to be separated by size, as smaller fragments can move through the small holes in the gel with more ease and thus are closer to the positive end, and larger fragments struggle and do not travel as far toward the positive end. Approximations of DNA fragment sizes can then be made against a control, a “DNA Ladder”, which is a well loaded with DNA in fragments of known sizes that can then be compared to the other DNA wells.

Different methods of electrophoresis used to separate macromolecules by size have become critical and one of the most commonplace experimental techniques in molecular biology. The variations and improvements of this techniques would not be possible without the original work of Arne Tiselius, a biochemist from Sweden who published his work on electrophoresis techniques in the 1930s and then was awarded the nobel prize for his work in 1948 (Faculty of Biosciences and Medical Engineering).

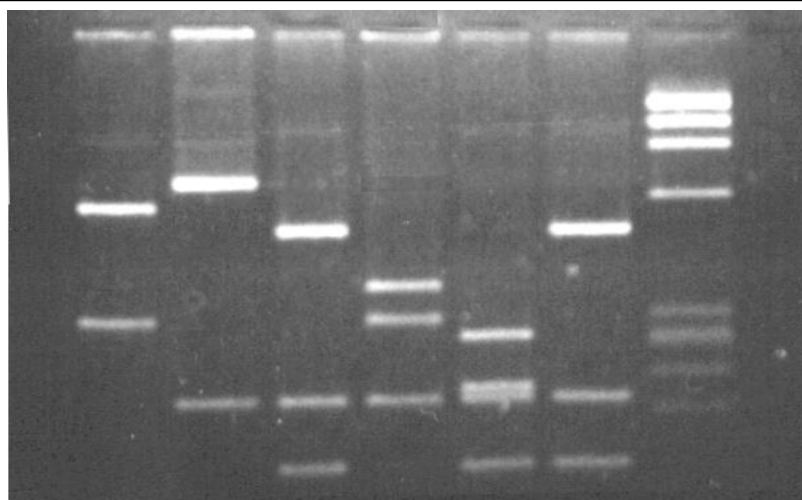




Figure 1: An example of DNA bands on a gel electrophoresis (Carr, 2012).

This module will describe how to make and pour a gel and run a gel electrophoresis using a given predigested sample and determine its length against a DNA ladder.

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1. PURPOSE

The purpose of this procedure is to learn the fundamentals of gel electrophoresis through creating, pouring, loading, and running a gel; and then interpreting results of DNA fragment sizes against a control DNA ladder.

2. SCOPE



This procedure applies to qualified skills center users.

3. RESPONSIBILITY

- 3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
- 3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
- 3.3. It is the responsibility of the user to become trained on the casting and loading a agarose gel, setting up the reaction chamber, loading the wells with DNA, and making comparative assessments of DNA band length against a control DNA ladder.

4. DEFINITIONS

- 4.1. Agarose: a polysaccharide which is used for the separation of macromolecules in the form of a gel in electrophoresis.
- 4.2. Gel Electrophoresis: A common laboratory technique in molecular biology which utilizes an agarose gel to separate macromolecules (nucleic acids and proteins) by size using an electrode to pull molecules through the gel.
- 4.3. DNA Ladder: A control when running a gel electrophoresis to compare other samples against; includes DNA digested into known sizes which creates standardized bands to compare experimental samples against.
- 4.4. Kilobases (kb): A unit typically used to measure DNA, in thousands of base pairs. 1 kb=1000 base pairs.
- 4.5. Restriction Enzyme: "A protein isolated from bacteria that cleaves DNA sequences at sequence-specific sites, producing DNA fragments with a known sequence at each end. The use of restriction enzymes is critical to certain

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laboratory methods, including recombinant DNA technology and genetic engineering” (<https://www.genome.gov/genetics-glossary/Restriction-Enzyme>).

4.6. Sonication: The process of using high frequency sound waves to break molecular bonds and disrupt the structure of small molecules. Two main types: Direct and Indirect sonication, where the source of the sound waves comes in direct contact with your sample, or travels through a secondary medium to access your sample, respectively.

5. MATERIALS/EQUIPMENT

- 5.1. Casting tray
- 5.2. Well combs
- 5.3. Voltage source
- 5.4. Gel box
- 5.5. UV light source
- 5.6. Microwave/Heat source
- 5.7. DNA sample (sheered Calf Thymus or Salmon/Herring sperm DNA)
- 5.8. Restriction Enzyme Digest Reagents: 10X NEBuffer, Restriction enzyme of choice, & nuclease free water
- 5.9. 6X DNA Purple Loading Dye + GelRed DNA stain

6. RECIPES

6.1. Buffer

For 1L of 10X TAE Buffer (<https://www.sigmaaldrich.com/US/en/technical-documents/protocol/protein-biology/gel-electrophoresis/tae-and-tbe-running-buffers-recipe>): 48.5 g tris, 11.4 ml glacial acetic acid, 20 ml 0.5M EDTA (pH 8.0) up to 1000 mL with mqH2O

6.2. Agarose Gel

Gel concentrations are chosen to suit the size range of the expected DNA product. For the standard sample of sheered thymus DNA, use 0.8%.

Agarose (%), Size (kb), B1 (small gel box), B2 (large gel box)



0.5 %, 2-50 kb: 0.375 g /75 mL, 0.75 g /150 mL

0.8 %, 0.8-12 kb: 0.6 g /75 mL, 1.2 g /150 mL

1.2 %, 0.3-7 kb: 0.9 g /75 mL, 1.8 g /150 mL

1.5 %, 0.2-3 kb: 1.125 g 75 mL, 2.25 g /150 mL

2.0 %, 1-2 kb: 1.5 g /75 mL, 3.0 g /150 mL

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7. PROCEDURE

7.1. Pre-procedure planning

7.1.1. In the below procedure, you will be comparing two different methods for cutting and analyzing samples of genomic DNA. Read through the procedures below and generate a

7.2. Agarose Gel Preparation

7.2.1. To cast gels, place the UVT gel tray into the buffer chamber in the casting position, making sure the gel tray rests level and centered on the platform. Be sure the gasketed ends of the gel tray press against the walls of the buffer chamber.

7.2.2. Reference chart below to determine the volume of agarose gel you wish to cast. Top Row: Specifications of *gel electrophoresis combs* (the comb looking part placed in the gel as it casts to generate wells for loading). Comb thickness in millimeters is shown first, B1A/B2 represents the size of the gel box, and the 10/12/20 that follows indicates the number of wells you wish to cast per comb. Below each header is the information on the volume of each well, given the volume of gel you cast paired with the top-row specifications. (**Figure 2.**)



7.2.3.

Determine if you will use the B1 or B2 gel box. Prepare the appropriate volume and %agarose (see recipe 6.2) of buffer fitting with the gel box size (see above table). Add agarose to a microwavable flask. Add the desired volume of 1X TAE buffer. Microwave for 1min (or until agarose is dissolved). Remove ***HOT*** glass with heat resistant glove. (NOTE) In the case where your DNA stain and loading buffer are separate (common), you will add the appropriate amount of DNA stain directly to the cooling agarose prior to casting your gel. Not needed with the reagents we use here.

7.2.4. Pour the warm agarose into the UVT gel tray that has been placed in the casting position in the buffer chamber. Immediately after pouring, insert the desired comb or combs into the comb slots to form the sample wells.

7.2.5. Standard agarose should solidify completely in about 30 minutes.

7.2.6. Once the gel is completely solidified, lift the tray out of the chamber, turn it 90° in the running position and replace it in the chamber with the first comb

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closest to the cathode (negative = black) side of the chamber. This running position exposes the open ends of the agarose to the buffer.

7.2.7. Pour enough compatible running buffer into the unit to fill the buffer chamber and completely cover and submerge the gel. Correct buffer level is clearly marked on the units side wall as "FILL LINE".

7.2.8. Carefully remove the comb (or combs) by tapping lightly to loosen, and slowly lifting straight up out of the gel tray to avoid damage to the wells.

7.3. DNA Sample Preparation

7.3.1. Restriction Digest

7.3.1.1. Obtain an aliquot of an available restriction digest enzyme from your lab proctor. Look up the enzyme you are given and document how the enzyme functions.

7.3.1.2. Search for your enzyme using the NEBcloner website (<https://nebcloner.neb.com/#!/redigest>) to determine the reagents and protocol needed to run the restriction digest.

7.3.1.3. Collect needed reagents, your DNA sample, and run the restriction enzyme digest per the protocol from NEBcloner

7.3.2. Indirect Sonication

7.3.2.1. Many protocols exist for sonicating genomic DNA, which differ based on what the sheared DNA will be used for. In the case here, we only need to shear our DNA sample enough to be noticeably different from unsheared and enzyme digest treated DNA.

7.3.2.2. Aliquot 50-100 μ l of your sample DNA in thin-walled 0.5 ml pcr tubes (the thinner walls allow sound waves to pass through easier). Place samples in foam floating tube holder, the tube holder in the Elmasonic water bath, and cover with the lid to the water bath.



7.3.2.3. Sonicate at the preset frequency of the Elmasonic Sonicator (37 kHz) for 20-30 minutes.

7.3.3. Preparation of samples for gel electrophoresis

7.3.3.1. Aliquot 10 μ l of each DNA sample into new tubes.

7.3.3.2. Add Pre-prepared 6X Concentration Loading Dye + DNA Stain(NEB 6X Purple Loading Dye #B7024S + 1:10 Gel Red) to a final concentration of 1X.

7.4. Running Gel Electrophoresis

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- 7.4.1. Add 6X Purple Dye/Stain to a sample of your DNA ladder as described above.
- 7.4.2. Carefully pipette 10µl of the DNA ladder and each prepared DNA sample into each well of the casted agarose gel. Label order of all samples in planning stage of experiment.
- 7.4.3. Plug gel box into power source in the right orientation (consider the charge of DNA and which direction it needs to run on the gel).
- 7.4.4. Run gel at 150-200 V until dye front indicates desired separation (typically 30 min – 1 hour)

7.5. Imaging Gel

- 7.5.1. After electrophoresis is complete, your gel can be imaged using a UV light source.
- 7.5.2. Carefully remove gel from the casting tray and place on the UV light box. Taking the proper safety precautions (UV protective glasses for open UV box; Correctly closed and sealed chamber for closed UV light box), turn on UV source and capture image of your results with your phone or the camera of a closed UV light box.
- 7.5.3. Clean up and return equipment to its original condition.

8. TROUBLE SHOOTING



- 8.1. If there is no current observed ensure that sufficient running buffer is in the gel tank and that it contacts the agarose gel.

9. REFERENCES

Faculty of Biosciences and Medical Engineering. (n.d.) *How Was Gel Electrophoresis Developed?* Faculty of Biosciences and Medical Engineering. Retrieved from <https://gel-electrophoresis.wixsite.com/gel-electrophoresis/history>

Carr, Steven M. (2012). *DNA electrophoresis*. Newfoundland and Labrador's University. Retrieved from https://www.mun.ca/biology/scarr/Gel_Electrophoresis.html

10. Module Methods Task

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- 10.1. Describe how DNA processing via sonication differs from restriction enzyme digestion. Provide a practical application for each.
- 10.2. Describe the foundational principles of DNA electrophoresis (the conceptual biology and chemistry allowing this procedure to work). In your description, please describe the value/use of the agarose gel, the components of the DNA loading dye and stain (both 6X purple dye and Gel Red), as well as how a voltage difference across the agarose gel allows for electrophoresis.
- 10.3. Follow the procedure to make a 0.8% agarose gel
- 10.4. Load a DNA ladder, an unprocessed (negative) control, 4, 1:2 dilutions of sheered DNA, and 4, 1:2 dilutions of restriction enzyme digested DNA (calf thymus, salmon sperm, etc)
- 10.5. Run the gel according to the SOP.
- 10.6. Take a photo of the results of the gel using the gel scanner. If you need, turn off lights to visualize the gel.
- 10.7. Submit your photo with an analysis of your results to a proctor. Using the DNA ladder as comparison, identify the sizes of the major DNA fragments in kb.