Size Determination of DNA Restriction Fragments

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of principles involved in estimating the size of unknown DNA fragments by agarose gel electrophoresis.

This document includes instructions for both EDVOTEK Experiment # 104 and # 104-Q. Please follow instructions for the appropriate experiment. Experiment #104 is designed for DNA staining with InstaStain® Methylene Blue. Experiment # 104-Q is designed for DNA staining with InstaStain® Ethidium Bromide.
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This document includes instructions for both EDVOTEK Experiment # 104 and # 104-Q. Please follow instructions for the appropriate experiment. Experiment # 104 is designed for DNA staining with InstaStain® Methylene Blue. Experiment # 104-Q is designed for DNA staining with InstaStain® Ethidium Bromide.

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DNA samples are stable at room temperature. However, if the experiment will not be conducted within one month of receipt, it is recommended that the DNA samples be stored in the refrigerator.

Over time, some evaporation of samples may occur. Before distributing reagents to students, check sample volumes as described in the Instructor’s Pre-Lab Preparation section.

Store entire experiment at room temperature. DNA samples do not require heating prior to gel loading.

**Components & Requirements**

**Experiment Components**

**READY-TO-LOAD™ DNA SAMPLES FOR ELECTROPHORESIS**

- A Standard DNA Fragments
- B Unknown DNA 1
- C Unknown DNA 2

**REAGENTS & SUPPLIES**

- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- Practice Gel Loading Solution
- 1 ml pipet
- 100 ml graduated cylinder (packaging for samples)
- Microtipped Transfer Pipets
- DNA Stain for Standard Series 100 experiments
  - InstaStain® Methylene Blue
  - Methylene Blue Plus™
- DNA Stain for Series 100-Q experiments
  - InstaStain® Ethidium Bromide

**Requirements**

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Distilled or deionized water
- For gel staining with InstaStain® Methylene Blue
  - Small plastic trays or large weigh boats for destaining
  - White light DNA visualization system
- For gel staining with InstaStain® Ethidium Bromide
  - UV Transilluminator
  - Photodocumentation system (optional)
This document includes instructions for both EDVOTEK Experiment # 104 and # 104-Q. Please follow instructions for the appropriate experiment.

Experiment #104

Designed for DNA staining and visualization with InstaStain® Methylene Blue. This experiment requires a 0.8% gel with the following volume:

30 ml (7 x 7 cm) or 60 ml (7 x 14 cm)

Refer to Table A.1 or A.2 in Appendix A for agarose gel preparation specifications.

Experiment # 104-Q

Designed for DNA staining and visualization with InstaStain® Ethidium Bromide. This experiment requires a 0.8% gel with the following volume:

25 ml (7 x 7 cm) or 50 ml (7 x 14 cm)

Refer to Table A.3 or A.4 in Appendix B for agarose gel preparation specifications.
Size Determination of DNA Restriction Fragments

Size determination of DNA fragments is essential to DNA mapping and analyzing restriction enzyme cleavage patterns. Restriction enzymes are endonucleases that cleave both strands of DNA at very specific sequences within DNA. The location of their cleavage sites are important for DNA fingerprinting, determination of genetic diseases and in formulating strategies for DNA analysis.

Agarose gel electrophoresis is a convenient analytical method for determining the size of DNA molecules in the range of 500 to 30,000 base pairs. Samples of DNA are loaded into wells made in an agarose gel, which is placed in an electrophoresis chamber containing a buffer solution and electrodes. Direct current (D.C.) is applied from a power source. Since DNA is negatively charged at neutral pH, it will migrate through the gel towards the positive electrode. The agarose gel consists of microscopic pores that act as a molecular sieve that separates DNA molecules according to their size and shape. The migration rate of DNA molecules of the same shape is inversely proportional to their size. This results in the smaller DNA molecule to migrate faster through the gel. The charge to mass ratio is the same for different sized DNA molecules.

Nucleotides in DNA are linked together by negatively charged phosphodiester bonds. For every base pair (average molecular weight of approximately 660) there are two charged phosphate linkages. Therefore, the negative charge in DNA is accompanied by approximately the same mass. The absolute amount of charge in DNA is not a critical factor in the separation process. Separation occurs because smaller molecules pass through the gel pores more easily than larger ones (i.e., the gel is sensitive to the physical size of the molecule). DNA fragment migration rate is inversely proportional to the log\(_{10}\) of its size in base pairs.

In this experiment, DNA fragments of unknown size and Standard DNA fragments are submitted to electrophoresis. The unknown DNA fragments will migrate through the gel according to their respective sizes and relative to the Standard DNA bands are visualized. The migration distances of the known and unknown fragments are measured and plotted on semi-log graph paper according to their size on the y-axis versus the migration distance on the x-axis. The size of the fragments on the y-axis are expressed as the log of the number of base pairs. This allows the data to be plotted as a straight line. The DNA fragments of known size (Standard DNA fragments) are used to make a standard curve. The migration distance of the unknown DNA fragments are extrapolated and estimated from the standard curve.

Quick Reference:

Standard DNA fragments, which were generated by restriction enzymes are provided in this experiment. A standard curve will be made on semi-log graph paper. The following are the Standard DNA fragment sizes - length is expressed in base pairs.

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<thead>
<tr>
<th>Size</th>
<th>23130</th>
<th>9416</th>
<th>6557</th>
</tr>
</thead>
<tbody>
<tr>
<td>4361</td>
<td>3000</td>
<td>2322</td>
<td></td>
</tr>
<tr>
<td>2027</td>
<td>725</td>
<td>570</td>
<td></td>
</tr>
</tbody>
</table>

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Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of principles involved in estimating the size of unknown DNA fragments by agarose gel electrophoresis.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:
- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:
- Record (draw) your observations, or photograph the results.

Following the Experiment:
- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.
Experiment Overview and General Instructions

EXPERIMENT OVERVIEW: FLOW CHART

1. Prepare agarose gel in casting tray.
2. Remove end blocks, comb and submerge gel under buffer in electrophoresis chamber.
3. Load each sample in consecutive wells.
4. Attach safety cover, connect leads to power source and conduct electrophoresis.
5. After electrophoresis, transfer gel for staining.
6. Analysis on white light source after destaining.
7. Analysis on UV Transilluminator no destaining.
8. Gel pattern will vary depending on experiment.

100 Series (Standard) InstaStain® Methylene Blue
100-Q Series InstaStain® Ethidium Bromide
Experiment Overview and General Instructions

ABOUT THE ELECTROPHORESIS SAMPLES

Samples in EDVOTEK Series 100, 100-Q and Sci-On® Series electrophoresis experiments are packaged in individual 1.5 ml or 0.5 ml microtest tubes.

Individual 1.5 ml or 0.5 ml microtest tubes

- Your instructor may have aliquoted samples into a set of tubes for each lab group. Alternatively, you may be required to withdraw the appropriate amount of sample from the experiment stock tubes.

- Check the sample volume. Sometimes a small amount of sample will cling to the walls of the tubes. Make sure the entire volume of sample is at the bottom of the tubes before starting to load the gel.

- Briefly centrifuge the sample tubes, or tap each tube on the tabletop to get all the sample to the bottom of the tube.
Size Determination of DNA Restriction Fragments

Agarose Gel Electrophoresis

AGAROSE GEL REQUIREMENTS FOR THIS EXPERIMENT

- Recommended gel size: 7 x 7 cm or 7 x 14 cm
- Number of sample wells required: 3
- Placement of well-former template: first set of notches
- Agarose gel concentration: 0.8%

PREPARING THE GEL BED

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.

   A. Using Rubber dams:
      - Place a rubber dam on each end of the bed. Make sure the dam fits firmly in contact with the sides and bottom of the bed.

   B. Taping with labeling or masking tape:
      - With 3/4 inch wide tape, extend the tape over the sides and bottom edge of the bed.
      - Fold the extended edges of the tape back onto the sides and bottom. Press contact points firmly to form a good seal.

2. Place a well-former template (comb) in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.

CASTING AGAROSE GELS

- If you will be staining the gel after electrophoresis with InstaStain® Methylene Blue, you should be using Table A.1 or A.2 found in Appendix A.
- If you will be staining the gel after electrophoresis with InstaStain® Ethidium Bromide, you should be using Table A.3 or A.4 found in Appendix B.

3. Use a 250 ml flask or beaker to prepare the gel solution.

4. Use the appropriate Reference Table for agarose gel preparation provided by your instructor. Add the specified amount of agarose powder and buffer and swirl the mixture to disperse clumps of agarose powder.

5. With a marking pen, indicate the level of the solution volume on the outside of the flask.
**Agarose Gel Electrophoresis**

At high altitudes, it is recommended to use a microwave oven to reach boiling temperatures.

6. Heat the mixture to dissolve the agarose powder.

   **A. Microwave method:**
   - Cover the flask with plastic wrap to minimize evaporation.
   - Heat the mixture on High for 1 minute.
   - Swirl the mixture and heat on High in bursts of 25 seconds until all the agarose is completely dissolved.

   **B. Hot plate method:**
   - Cover the flask with aluminum foil to minimize evaporation.
   - Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

   Check the solution carefully and continue heating until the final solution appears clear (like water). If "crystal" particles are visible, the agarose is not completely dissolved.

7. Cool the agarose solution to 60°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume marked in step 5.

---

**Important Note**

8. Seal the interface of the gel bed and tape to prevent the agarose solution from leaking.

   - Use a transfer pipet to deposit a small amount of cooled agarose to both inside ends of the bed.
   - Wait approximately 1 minute for the agarose to solidify.

9. Place the bed on a level surface and pour the cooled (60°C) agarose solution into the bed.

10. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

---

**PREPARING THE GEL FOR ELECTROPHORESIS**

11. After the gel is completely solidified, carefully and slowly remove the rubber dams or tape from the gel bed.

   Be especially careful not to damage or tear the gel wells when removing the rubber dams. A thin plastic knife, spatula or pipet tip can be inserted between the gel and the dams to break the surface tension.
Agarose Gel Electrophoresis

12. Remove the comb by slowly pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.

13. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.

14. Fill the electrophoresis apparatus chamber with the appropriate amount of diluted (1x) electrophoresis buffer (refer to Table B (found in Appendix A or B) on the instruction sheet provided by your instructor).

15. Make sure that the gel is completely submerged under buffer before proceeding to loading the samples and conducting electrophoresis.

LOADING THE SAMPLES

Samples should be loaded into the wells of the gel in consecutive order:

• For gels to be stained with InstaStain® Methylene blue, the amount of sample that should be loaded is 35-38 µl.

• For gels to be stained with InstaStain® Ethidium Bromide, the amount of sample that should be loaded is 18-20 µl.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Tube</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>Standard DNA Fragments</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Unknown 1</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Unknown 2</td>
</tr>
</tbody>
</table>
Agarose Gel Electrophoresis

RUNNING THE GEL

1. After the DNA samples are loaded, carefully snap the cover down onto the electrode terminals. Make sure that the negative and positive color-coded indicators on the cover and apparatus chamber are properly oriented.

2. Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).

3. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor.

4. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.

5. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.

6. Remove the gel from the bed for staining.

STAINING AND VISUALIZATION OF DNA

After electrophoresis, agarose gels require staining to visualize the separated DNA samples. Various options are available for DNA staining. Your instructor will provide instructions for the DNA staining method you will be using.

InstaStain is a registered trademark of EDVOTEK, Inc. Patents Pending.
Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. How should the x and y axes of the semi-log graph paper used in this experiment, be labeled?

2. Determine unknown 1 and unknown 2 DNA fragment sizes in base pairs according to your standard curve, then determine your percentage error.

3. Use the example of the standard curve to determine:
   A. the base pair size if the migration distance is 2.8 centimeters.
   B. the migration distance if the fragment contains 5,500 base pairs.
Material Safety Data Sheet

IDENTITY (As Used on Label and/or)

Section I - Material and Company Identification

EDVOTEK

Section II - Hazardous Ingredients/Identify Information

Hazardous Components

- 3.7 Bis (Dimethylamino) Phenothiazin 5 IUM   Chloride

Chemical Identity;  Common Name(s)

CAS # 61-73-

Vapor Density (AIR = 1)

No data

Boiling Point

No data

Melting Point

No data

Flammable Limits

No data

Other Limits

No data

Recommended Extinguishing Media

Dry chemical, carbon dioxide, water spray or foam

Unusual Fire and Explosion Hazard

Emits toxic fumes under fire conditions

Hazardous Decomposition or Byproducts

Toxic fumes of Carbon monoxide, Carbon dioxide, Sulfur oxides, and bromides

Exposure Limits

OSHA PEL

No data

ACGIH TL

No data

Recommended Personal Protective Equipment

Respiratory Protection (Specify Type)

MIOSH/OSHA approved, SCBA

Other Protective Clothing or Equipment

None

Environmental considerations

Blue liquid, no odor

Details of first aid measures

Inhalation:  Cyanosis

Health Hazards (Acute and Chronic)

None reported

Medical Conditions Generally Aggravated by Exposure

None

Emergency and First Aid Procedures

Rinse contacted area

Precautions to be Taken in Handling and Storage

Avoid eye and skin contact

Other Precautions

None

Control Measures

Ventilation

Local Exhaust:  Yes

Mechanical (General):  Yes

Protective Glasses

Yes

Chemical splash proof goggles

None

Other Protective Clothing or Equipment

None required

Working Procedures

Avoid eye and skin contact

Section III - Physical/Chemical Characteristics

Appearance and Odor

Blue liquid, no odor

Flash Point (Method Used)

No data

Extinguishing Media

Dry chemical, carbon dioxide, water spray or foam

Special Fire Fighting Guidance

Use agents suitable for type of surrounding fires. Keep spilled, acid breathing hazardous sulfur oxides and bromides. Wear SCBA.

Evaporation Rate

No data

Solubility in Water

Soluble

Appearance and Odor

Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

Technical Grade Material Used

No data

Flammable Limits

No data

Other Limits

No data

Solubility in Water

Soluble

Appearance and Odor

Blue liquid, no odor

Page 1 of 8

Material Safety Data Sheets

Material used to comply with OSHA’s Hazard Communication Standard. 29 CFR 1910.1200. Standards must be consulted for specific requirements.

Section V - Reactivity Data

Incompatibilities

Strong oxidizing agents

Hazardous Decomposition or Byproducts

Toxic fumes of Carbon monoxide, Carbon dioxide, Sulfur oxides, and bromides

Stable

Health Hazards (Acute and Chronic)

None

Medical Conditions Generally Aggravated by Exposure

None

Emergency and First Aid Procedures

Rinse contacted area

Precautions to be Taken in Handling and Storage

Avoid eye and skin contact

Other Precautions

None

Control Measures

Ventilation

Local Exhaust:  Yes
Mechanical (General):  Yes

Protective Gloves

Rubber

Working Procedures

Avoid eye and skin contact

Section VI - Health Hazard Data

Reactivity

Hazardous Polymers

No data available

Health Hazards (Acute and Chronic)

None

Medical Conditions Generally Aggravated by Exposure

None

Emergency and first aid procedures

Rinse contacted area

Precautions to be Taken in Handling and Storage

Avoid eye and skin contact

Other Precautions

None

Control Measures

Ventilation

Local Exhaust:  Yes
Mechanical (General):  Yes

Other Protective Clothing or Equipment

None required

Working Procedures

Avoid eye and skin contact

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in Case Material Is Released or Spilled

Ventilate area and wash spill site

Waste Disposal Method

Observe all federal, state, and local regulations.

Material Safety Data Sheets

Material used to comply with OSHA’s Hazard Communication Standard. 29 CFR 1910.1200. Standards must be consulted for specific requirements.

EDVOTEK  Hazardous Ingredients/Identify Information

Hazardous Components

- 3.7 Bis (Dimethylamino) Phenothiazin 5 IUM   Chloride

Chemical Identity;  Common Name(s)

CAS # 61-73-

Vapor Density (AIR = 1)

No data

Boiling Point

No data

Melting Point

No data

Flammable Limits

No data

Other Limits

No data

Recommended Extinguishing Media

Dry chemical, carbon dioxide, water spray or foam

Unusual Fire and Explosion Hazard

Emits toxic fumes under fire conditions

Hazardous Decomposition or Byproducts

Toxic fumes of Carbon monoxide, Carbon dioxide, Sulfur oxides, and bromides

Exposure Limits

OSHA PEL

No data

ACGIH TL

No data

Recommended Personal Protective Equipment

Respiratory Protection (Specify Type)

MIOSH/OSHA approved, SCBA

Other Protective Clothing or Equipment

None

Environmental considerations

Blue liquid, no odor

Details of first aid measures

Inhalation:  Cyanosis

Health Hazards (Acute and Chronic)

None

Medical Conditions Generally Aggravated by Exposure

None

Emergency and First Aid Procedures

Rinse contacted area

Precautions to be Taken in Handling and Storage

Avoid eye and skin contact

Other Precautions

None

Control Measures

Ventilation

Local Exhaust:  Yes
Mechanical (General):  Yes

Other Protective Clothing or Equipment

None required

Working Procedures

Avoid eye and skin contact

Section VIII - Control Measures

Regulatory Requirement by Type

OSHA approved, SCBA