Name: _____( ) **Class:**_____ **Date:**_____

Topic: Gel Electrophoresis

Activity 1: Procedures — Setting Up Gel Electrophoresis

What You Will Do

In this activity, you will follow five steps to set up and run a gel electrophoresis experiment. Complete each step in order and read the feedback carefully before moving on.

Guided Questions

Before you begin:

1. What type of molecule is being separated in gel electrophoresis?

   _____

2. DNA carries a _____ charge. This means it will migrate toward the _____ electrode when a voltage is applied.

Step 1 — Prepare the Agarose Gel

3. What is the purpose of the comb in gel casting?

   _____

   _____

   _____

4. Suggest why a higher percentage agarose gel (e.g. 2%) would be used instead of a lower percentage (e.g. 0.5%).

   _____

   _____

   _____

   _____

Step 2 — Load DNA Samples

5. Why is loading dye mixed with the DNA sample before it is pipetted into the wells?

_____

_____

6. Why is a DNA ladder loaded into one of the wells alongside the samples?

_____

_____

Step 3 — Fill Tank with Buffer

7. State the role of TAE or TBE buffer in gel electrophoresis.

_____

_____

_____

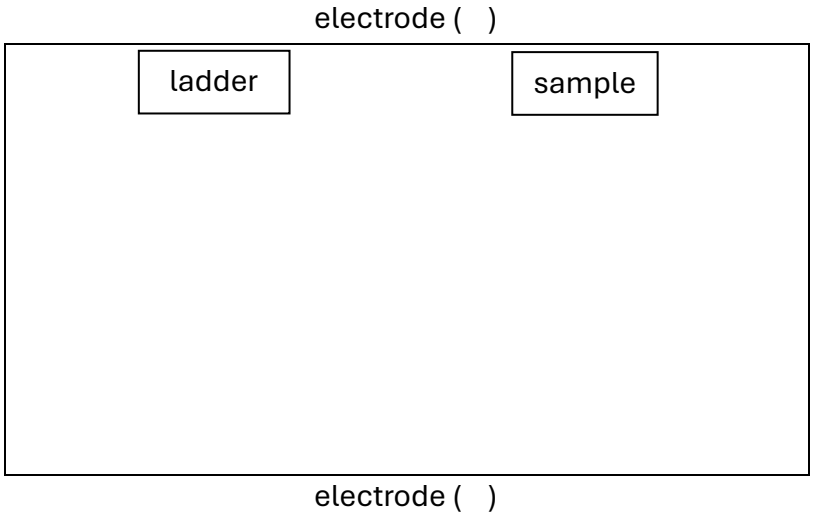
8. What would happen if the gel was not fully submerged in buffer before running?

_____

_____

Step 4 — Connect Electrodes

9. Label the diagram below to show which end is the negative electrode (−) and which is the positive electrode (+). Draw an arrow to show the direction of DNA migration.



10. A student accidentally reverses the electrodes. What would happen to the DNA in the wells?

_____

_____

_____

Step 5 — Run the Gel

11. Observe the animation. Which fragments move the furthest from the wells — larger or smaller ones? Explain why.

_____

_____

_____

12. After running the gel for DNA Fingerprinting (Example 1), four bands are visible. List them from the band closest to the wells to the band furthest from the wells.

_____ bp → _____ bp → _____ bp → _____ bp

Closest to wells Furthest from wells

Reflection

13. Write the correct order of steps for gel electrophoresis by numbering the following from 1 to 5:

Step	**Order**
Connect electrodes and run the gel	
Pour buffer into the tank	
Cast the agarose gel and form wells	
Load DNA samples into the wells	
Observe and analyse the band pattern	

Activity 2: Investigations — Identifying Unknown Samples

What You Will Do

In this activity, you will compare an unknown DNA sample's gel pattern against three reference samples to identify a match. Study the band positions carefully before selecting your answer.

Guided Questions

Before you begin:

1. List **three** features of a gel band pattern that you should compare when identifying an unknown sample.

   (i) _____

   (ii) _____

   (iii) _____

Example 1 — Crime Scene DNA Profiling

2. How many bands does the crime scene sample show? _____
3. Using the DNA ladder, estimate the sizes of the bands in the crime scene sample.

   Band 1: _____ bp Band 2: _____ bp Band 3: _____ bp
4. Which suspect's profile matches the crime scene sample? _____
5. Explain how you reached this conclusion.

   _____

   _____

   _____
6. Why is it important that the same restriction enzyme is used to digest all DNA samples before running the gel?

   _____

   _____

   _____

Example 2 — Paternity Testing

7. The child's DNA profile is shown as the unknown sample. Which person is identified as the biological parent? _____

8. In paternity testing, a child inherits DNA from both parents. Would you expect the child's profile to be identical to the parent's profile? Explain.

_____

_____

_____

Example 3 — GMO Food Testing

9. What is the expected band size for the GMO gene? _____ bp

10. The food sample shows two bands. What does the second band (not the GMO band) represent?

_____

_____

_____

11. If the food sample showed **no** band at 400 bp, what conclusion would you draw?

_____

_____

_____

Reflection

12. Suggest **one** limitation of using gel electrophoresis alone to confirm the identity of an unknown DNA sample.

_____

_____

_____

_____

Activity 3: Fragment Sizing — Using the DNA Ladder

What You Will Do

In this activity, you will estimate the sizes of unknown DNA fragments by comparing their positions on the gel to the DNA ladder. Use the ladder band positions to interpolate the sizes of the unknown bands.

Guided Questions

Before you begin:

1. What is a DNA ladder?

   _____

   _____

   _____

2. The ladder used in this activity contains bands at: 3000, 1500, 1000, 500, 200, and 100 bp. Which band will appear closest to the wells? Explain.

   _____

   _____

   _____

Example 1 — Single Unknown Band

3. Look at Band X on the gel. Which two ladder bands does it fall between?

   Between _____ bp and _____ bp

4. Is Band X closer to the upper ladder band or the lower ladder band? _____
5. Based on its position, estimate the size of Band X: _____ bp
6. Enter your answer into the simulation and check it. Were you within the accepted range? _____

Example 2 — Two Unknown Bands

7. For each band, identify the two ladder bands it falls between and estimate its size.

Band	Between (bp)	Estimated Size (bp)	Accepted?
A			
B			

8. Band A is closer to the wells than Band B. What does this tell you about their relative sizes?

_____

_____

_____

Example 3 — Three-Fragment Digest

9. Complete the table below for all three fragments.

Fragment	Between (bp)	Estimated Size (bp)	Accepted?
1			
2			
3			

10. Calculate the total size of the three fragments combined: _____ bp
    This total represents the approximate size of the original _____ before it was cut.

Going Further

11. Explain why migration distance is **not** directly proportional to fragment size (i.e. why the spacing between ladder bands is uneven).

    _____

    _____

    _____

12. A more accurate method of estimating fragment size is to plot **log(fragment size)** against **migration distance** and draw a best-fit line. Explain why this produces a straight line.

    _____

    _____

    _____

13. Two fragments of 480 bp and 520 bp are run on the same gel. Suggest why they may be difficult to distinguish as separate bands.

    _____

    _____

    _____