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

Topic: Gel Electrophoresis (Answers)

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?

   DNA (deoxyribonucleic acid) / DNA fragments

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

Step 1 — Prepare the Agarose Gel

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

   The comb creates wells in the gel for loading DNA samples.

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

   A higher percentage agarose gel has a denser matrix with smaller pores. This gives better resolution (separation) of smaller DNA fragments, as they are slowed down more and the differences in their migration distances become more distinguishable.

Step 2 — Load DNA Samples

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

   Loading dye makes the sample denser than the buffer so it sinks into the well instead of diffusing away. It also contains a coloured dye that migrates with the DNA, allowing the progress of the run to be monitored visually.

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

   The DNA ladder contains fragments of known sizes. It is run alongside samples so that the sizes of unknown fragments can be estimated by comparing their band positions to the ladder.

Step 3 — Fill Tank with Buffer

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

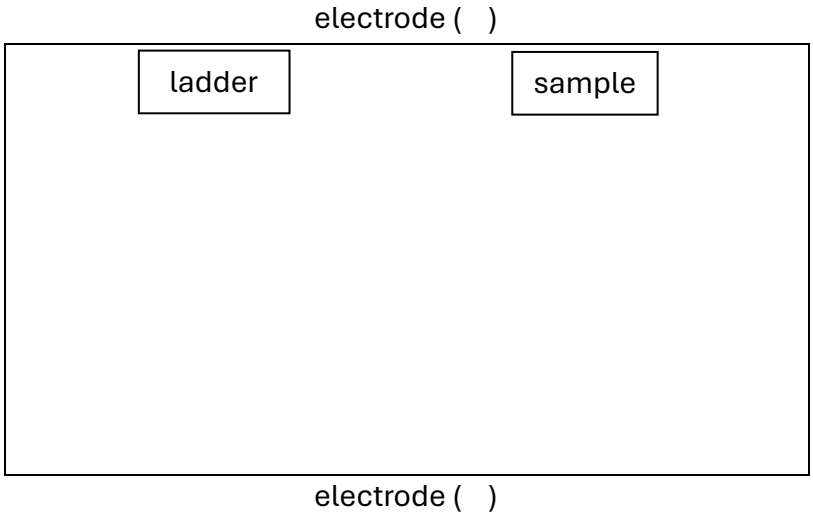
   Buffer (TAE or TBE) conducts electricity, allowing current to flow through the gel. It also maintains a stable pH, which keeps DNA negatively charged so it migrates consistently.

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

   If the gel is not fully submerged, current cannot flow evenly through the gel. This would result in uneven or no migration of DNA fragments.

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.



   The negative electrode (−) should be at the same end as the wells. The positive electrode (+) should be at the opposite end. The arrow showing DNA migration should point from the wells toward the positive electrode.

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

    If the electrodes are reversed, DNA (which is negatively charged) would migrate toward the negative electrode — away from the wells in the wrong direction — and could be pushed out of the wells back into the buffer.

Step 5 — Run the Gel

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

    Smaller fragments move the furthest. Smaller fragments experience less resistance as they pass through the pores of the agarose gel matrix, so they migrate faster and travel a greater distance in the same amount of time.

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.

    2000 bp → 800 bp → 300 bp → 100 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	**4**
Pour buffer into the tank	**3**
Cast the agarose gel and form wells	**1**
Load DNA samples into the wells	**2**
Observe and analyse the band pattern	**5**

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:

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

    1. (i) Number of bands
    2. (ii) Position / height of each band (i.e. migration distance / estimated size in bp)
    3. (iii) Colour/intensity of bands *(Accept any two of the above for full marks; all three listed here for completeness)*

Example 1 — Crime Scene DNA Profiling

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

    Band 1: ~**300 bp** Band 2: ~**800 bp** Band 3: ~**2000 bp**

17. Which suspect's profile matches the crime scene sample? **Suspect A**
18. Explain how you reached this conclusion.

    Suspect A's profile shows three bands at the same positions as the crime scene sample — approximately 300 bp, 800 bp, and 2000 bp. The number of bands, their sizes, and their positions all match, indicating the same restriction fragment pattern.

19. Why is it important that the same restriction enzyme is used to digest all DNA samples before running the gel?
20. Each restriction enzyme cuts DNA at a specific recognition sequence. Using the same enzyme on all samples ensures that the same cut sites are used, producing fragments that can be directly compared. Using different enzymes would produce different fragment sizes, making comparison meaningless.

Example 2 — Paternity Testing

21. The child's DNA profile is shown as the unknown sample. Which person is identified as the biological parent? **Person B**

22. 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.

    No, the child's profile would not be identical to either parent's profile. A child inherits half of their DNA from each parent, so the child's profile would share some bands with the biological parent but not all. The profile is unique to the individual.

Example 3 — GMO Food Testing

23. What is the expected band size for the GMO gene? **400 bp**

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

    The second band (at ~1200 bp) represents an **internal control / housekeeping gene** — a gene that is present in all cells regardless of GMO status. It confirms that the DNA extraction was successful and the PCR reaction worked correctly.

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

    If no band appeared at 400 bp, the conclusion would be that the GMO gene is **absent** from the food sample — i.e. the food does not contain the GMO. *(Accept: the food is not genetically modified for this specific gene.)*

Reflection

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

    One limitation: Two different DNA samples could produce bands at similar positions by coincidence, especially if only a small number of bands are compared. Gel electrophoresis alone cannot confirm identity with 100% certainty — additional testing (e.g. DNA sequencing) would be needed for conclusive identification. *(Accept also: gel resolution may not be sufficient to*

distinguish fragments of very similar sizes; band intensity differences are not considered.)

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:

27. What is a DNA ladder?

    A DNA ladder is a mixture of DNA fragments of known sizes (in base pairs), run alongside unknown samples on a gel. It serves as a size reference to estimate the sizes of unknown fragments.

28. 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.

    The **3000 bp** band will appear closest to the wells. Larger fragments migrate more slowly through the gel matrix due to greater resistance, so they travel a shorter distance from the wells in the same time.

Example 1 — Single Unknown Band

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

    Between **500** bp and **1000** bp

30. Is Band X closer to the upper ladder band or the lower ladder band?

    500 (i.e. it has migrated further, indicating it is closer in size to 500 bp than to 1000 bp)

31. Based on its position, estimate the size of Band X:

    ~**700 bp** *(Accept any value between 595–805 bp, i.e. ±15% of 700 bp)*

32. Enter your answer into the simulation and check it. Were you within the accepted range?

(Student to check against simulation — accept "yes" if estimate is within ±15%.)

Example 2 — Two Unknown Bands

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

Band	Between (bp)	Estimated Size (bp)	Accepted?
A	200 – 500	~350 bp	*(±15%: 298–403 bp)*
B	1000 – 1500	~1200 bp	*(±15%: 1020–1380 bp)*

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

    Band A is closer to the wells than Band B, which means Band A has migrated less. This indicates that Band A is a **larger** fragment than Band B. *(Note: closer to wells = larger size = slower migration.)*

Example 3 — Three-Fragment Digest

35. Complete the table below for all three fragments.

Fragment	Between (bp)	Estimated Size (bp)	Accepted?
1	100 – 200	~150 bp	*(±15%: 128–173 bp)*
2	500 – 1000	~600 bp	*(±15%: 510–690 bp)*
3	1500 – 3000	~2500 bp	*(±15%: 2125–2875 bp)*

36. Calculate the total size of the three fragments combined: **~150 + 600 + 2500 = 3250 bp** . This total represents the approximate size of the original **DNA molecule / plasmid / gene** before it was cut.

Going Further

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

38. Migration distance is not directly proportional to fragment size because the gel matrix acts as a **molecular sieve**. The relationship between fragment size and

migration distance follows a **log-linear** pattern — larger fragments experience disproportionately greater resistance. The difference in migration between small fragments (e.g. 100 bp vs 200 bp) is much greater than between large fragments of the same size difference (e.g. 2000 bp vs 2100 bp).

39. 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.

    Because migration distance is proportional to **log(fragment size)**, plotting log(size) against distance produces a straight line (linear relationship). This means a best-fit straight line can be drawn through the ladder points and used to read off the log(size) of any unknown band, which can then be converted back to a size in bp. This is more accurate than visually interpolating between curved points.

40. 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.

    Fragments of 480 bp and 520 bp are very similar in size (only ~8% difference). They would migrate almost the same distance through the gel, so their bands would overlap or appear as a single thick band. The resolution of the gel is insufficient to separate fragments so close in size unless an unusually high-percentage gel or a very long run time is used.