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DNA Gel Electrophoresis Summative Assessment

By Euginia (Gina) Revelas Nicoletti, ABE Canada

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We are grateful to the ABE Master Teacher Fellows for sharing their work with the ABE community. If you have questions about any of the project components, please reach out to us at ABEInfo@edc.org, and we will be happy to connect you with the author and provide any assistance needed.

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DNA Gel Electrophoresis Summative Assessment

Teacher Guide

By Euginia (Gina) Revelas Nicoletti | Modified from the ABE/UTM lab series

LABORATORY: DNA GEL ELECTROPHORESIS SUMMATIVE ASSESSMENT

In this assessment, students demonstrate a range of lab skills including micro pipetting and loading DNA samples in gel wells, running a gel electrophoresis and knowledge on how DNA fragments are separated, identifying unknown DNA fragments by comparing to the DNA ladder. Students also demonstrate their analytical skills through the measurements of the distance the fragments travelled, calculating Rf values and plotting a standard curve to determine the molecular size of the unknown fragments.

LEARNING GOALS

By the end of this lab, students will be able to do the following:

- Predict the relative speed of DNA fragments through a gel during gel electrophoresis
- Separate DNA fragments using gel electrophoresis
- Calculate Rf values of DNA fragments
- Plot a standard curve to calculate the molecular size of the unknown DNA fragment

ASSESSED OUTCOMES

By the end of this lab, students will be assessed on their ability to:

- accurately pipette and load DNA samples on a gel
- run a gel electrophoresis to effectively separate the DNA fragments in the DNA ladder and unknown DNA sample
- measure the distance travelled for each DNA fragment
- calculate the Rf values for each DNA fragment
- plot a standard curve and best fit line to determine the estimated molecular size (bp) of the unknown fragments
- accurately list and explain sources of error

TEACHER PREPARATION

Before you begin this summative lab, you will need to:

- Obtain the equipment and materials from the host site including prepared DNA ladder (M) and unknown DNA samples for each student. The unknown will be determined by host site. Each student will need one sample for each: DNA ladder (M) and unknown DNA.
- Make Agarose Gels as per ABE Laboratory 4. This includes preparing electrophoresis gel trays and preparing the agarose solution containing GelGreen®. Plan for one gel per 2 or 3 students.
- Provide the students a copy of the student guide (electronically or print)

BEFORE THE LAB

Students to review the following:

1. Micro pipetting techniques. Refer to ABE lab 1.
2. Running a gel electrophoresis. Refer to ABE lab 4.
3. How to measure migration of DNA fragments and complete Rf calculations.
4. Plotting a standard curve with the best fit line.

MATERIALS

Reagents

- A plastic microfuge tube rack with the following
  - Microfuge tube of DNA Marker solution (M)
  - Microfuge tube of unknown DNA fragment (U)
    - The unknown used in the example was a digested K+ and the theoretical molecular sizes of the respective fragments are 4705 bp and 807 bp
- 50-mL flask containing 1x sodium borate buffer (1x SB)

Equipment and Supplies

- P-20 micropipette (measures 2.0–20 µL)
- Tip box of disposable pipette tips
- Electrophoresis box loaded with 0.8% agarose gel containing GelGreen® (will be shared among students)
- Microcentrifuge (will be shared among students)
- Waste container for used tips and microfuge tubes (will be shared among students)
- DNA Ladder Diagram

SAFETY:

- All appropriate safety precautions and attire required for a science laboratory should be used, including safety goggles and gloves. Please refer to your teachers' instructions.
- Wash your hands well with soap after completing the lab.

METHOD

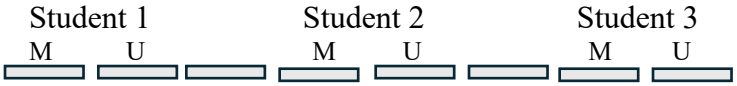
1. Check your rack to make sure that you have all the reagents listed.
2. Spin the microfuge tubes (M and U) in the microcentrifuge for several seconds to pool the reagents at the bottom of each tube.

   LAB TECHNIQUE: Distribute the tubes evenly in the microcentrifuge so that their weight is balanced, making sure they are directly opposite one another.

3. Distribute gels (one gel per 3 students). Make sure that the wells in your gel are located at the top near the negative (black) electrode when placed in the gel electrophoresis unit.
4. Fill the box with 1x SB to a level that just covers the entire surface of the gel. If you see any "dimples" over the wells, add more buffer.

LAB TECHNIQUE: If there are "dimples" add very small amounts of buffer to the electrophoresis box. While the gel needs to be completely under the buffer, you don't want too much buffer in the box, as this will cause the electrical current to run through the buffer and not the gel.

5. Make a drawing in your notebook that shows the location of the wells in the electrophoresis box. There will be 3 students using the same gel.

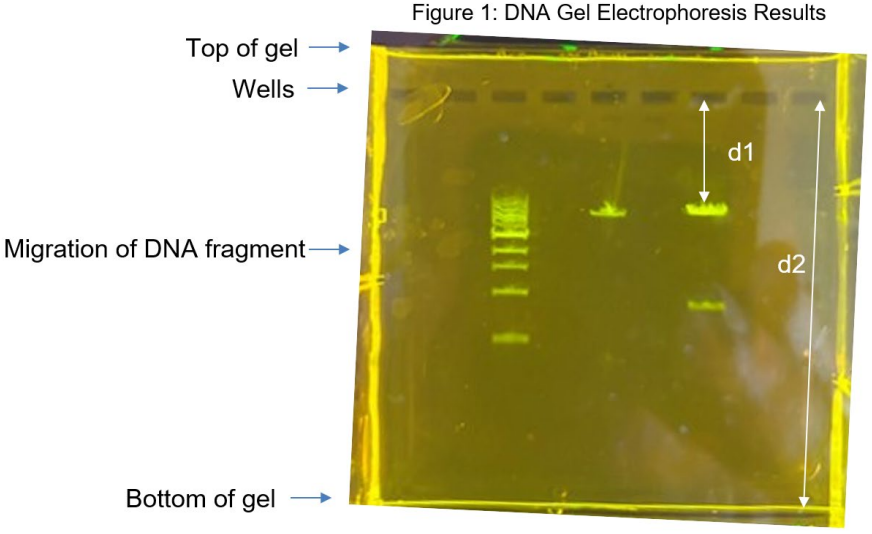


6. Using a fresh pipette tip for each sample, dispense 10.0 uL of the DNA ladder (M) and unknown DNA fragment (U). For each sample, do the following:
    a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
    b. Lower the pipette tip until it is under the buffer but just above the well.

LAB TECHNIQUE: Do not puncture the gel, or it will become unusable. Gently press the plunger to slowly dispense the sample. To avoid getting air into the buffer, do not go past the first stop. The sample will sink into the well.

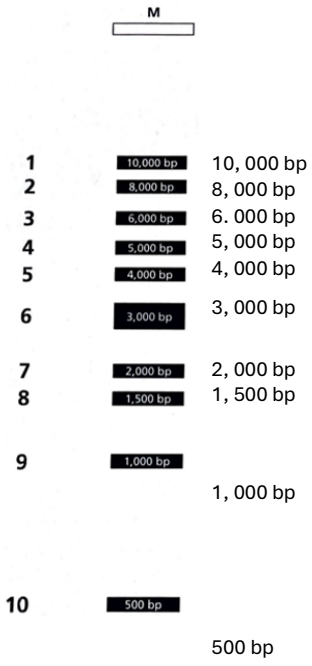
7. When all the samples have been loaded, close the cover tightly over the electrophoresis box.
8. Turn on the power supply and set the voltage to 130–135 V.
9. After two or three minutes, check to see if the loading dye is moving toward the positive electrode.
10. The loading dye will need to run just near the end of the gel, about 40–50 minutes.
11. After the gel has finished running, view the gel under the transilluminator to show the DNA fragments and unknown fragment.
12. Take a photo of the gel. Your teacher will explain how to dispose of the gel. *The following steps may be completed the next day. Please check with your teacher.*
13. Print the photo of the gel results. Colour print shows the fragments more than black and white print.
14. Record in **TABLE FORMAT** all your results and calculations.
15. On the printed photo of the gel results, measure the distance travelled for each DNA fragment in the DNA ladder (M) and in the unknown sample.
16. Measure the distance from the middle of the well to the bottom of the gel. Record in table.
17. Calculate the Rf value for each DNA fragment in the DNA ladder (M) and in the unknown sample. See Figure 1. See NOTES Rf information.

Figure 1: DNA Gel Electrophoresis Results



18. Plot a standard curve using the DNA Ladder (M) Rf values (y-axis) versus the logarithm of the corresponding molecular size (x-axis). See Figure 2 to obtain the molecular size for each fragment in the DNA Ladder (M).

Figure 2: DNA Ladder (M) molecular size (bp) for each fragment



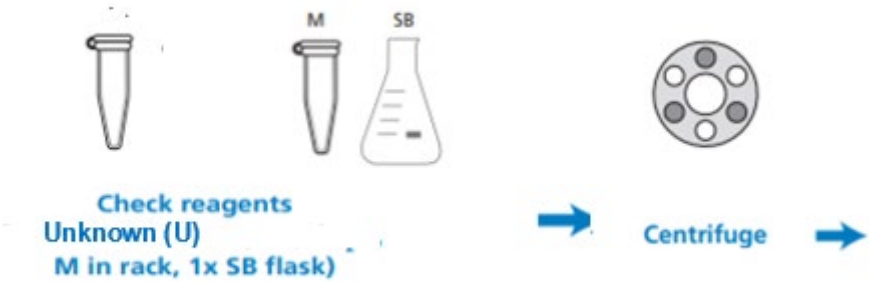
19. Draw the best fit line.
20. Using slope = rise/run or a graphing program (i.e. desmos), determine the best fit line equation, y = mx + b, where m is the slope of the line and b is the y-intercept. See video Calculating a Line of Best Fit with Desmos to learn how to use Desmos calculator to find the line of best fit equation.

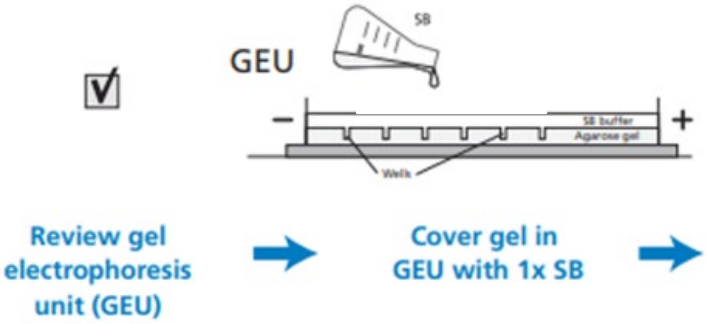
21. On the graph, plot the Rf values of the unknown DNA fragments and intersect the best fit line.
22. Extrapolate to determine the logarithm molecular size (bp) on the x-axis.
23. Using anti-log, calculate the molecular size (bp) of each unknown DNA fragment.
24. For each unknown DNA fragment, enter the value of the Rf value as y in the best fit line equation and solve for x (logarithm molecular size (bp). Then solve for the estimated molecular size (bp).
25. Calculate the % difference of the bp value determined from the graph extrapolated versus the bp value determined from the line of best fit equation.
26. Obtain from your teacher the theoretical value for the molecular size (bp) for each unknown fragment and calculate the % error. Use bp calculated from best fit formula. **Percent Error = | Experimental Value – Actual Value | / Actual Value x 100%**
27. Explain a minimum of two sources of error.

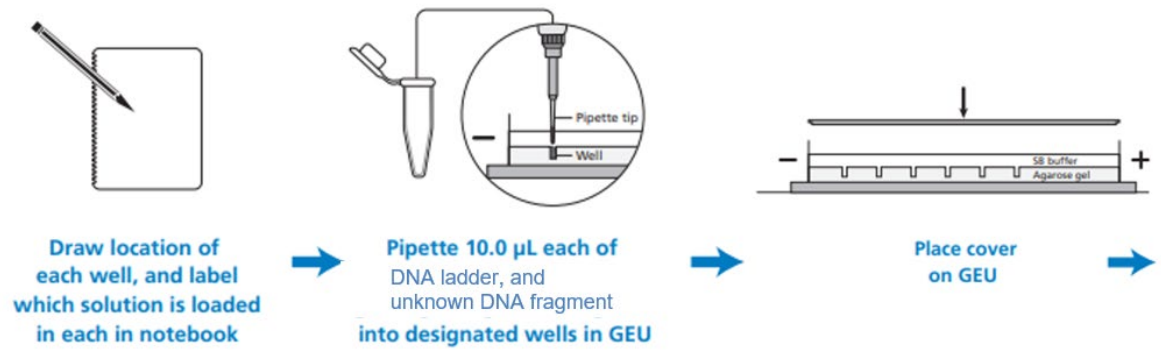
NOTES:

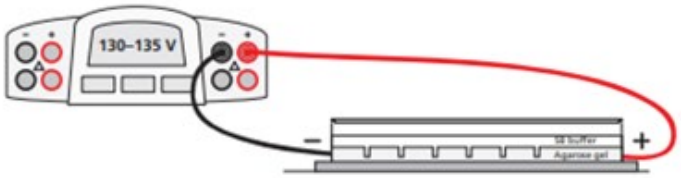
- Rf values are used to confirm identity of unknown DNA samples.
- Rf = distance the DNA fragment has travelled from the origin (gel well) / distance from the origin to the reference point (*dye front) = $\frac{d_1}{d_2}$
- Refer to the following video on how to calculate Rf values. https://youtu.be/z8Hz2WNnGY4
- For d_1, the migration of a DNA fragment/band may be measured starting from the middle of the well to the middle of each DNA fragment.
- *For d_2, instead of using the dye front (not visible under transilluminator), use the bottom of gel to obtain the d_2 measurement.

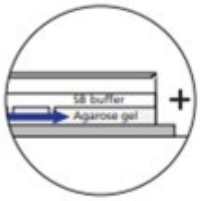
Flow Chart









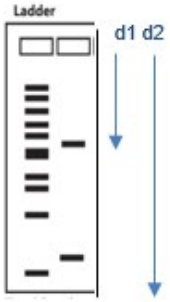


Connect leads to power supply → Turn on power supply and set voltage to 130–135 V → Check to see if the purple loading dye is moving toward the positive (red) electrode →

Run the gel until the DNA fragments are separated (viewed under transilluminator) → Take a photo & print → Measure the distance the DNA fragments travelled through the gel. (d1)

Measure the distance from the well to the bottom of the gel. (d2) → Calculate the Rf values of the unknown DNA fragments & ID.

Rf = d1 / d2



Plot a standard curve by plotting the Rf value (y-axis) of each standard DNA fragment (from Marker) versus the logarithm of its molecular size (x-axis) → On this graph plot the Rf value of the unknown DNA fragment on y-axis and extrapolate from the standard curve to determine molecular size of the unknown (anti-log of value from graph) → Continue as per procedure step 20

THEORETICAL DNA UNKNOWN SAMPLES USED

The unknown DNA samples used in the pilot lab included digested K^+ and A^+. These samples were prepared by the host site. The A^+ samples did not produce reliable results (i.e. not visible on the gel). Consequently, A^+ results were omitted and not recommended to be used in future summative assessment labs.

See Figure 3 for the sequences of digested K^+ and A^+ fragments and corresponding molecular size (bp). For the calculations only the digested K^+ fragments were incorporated.

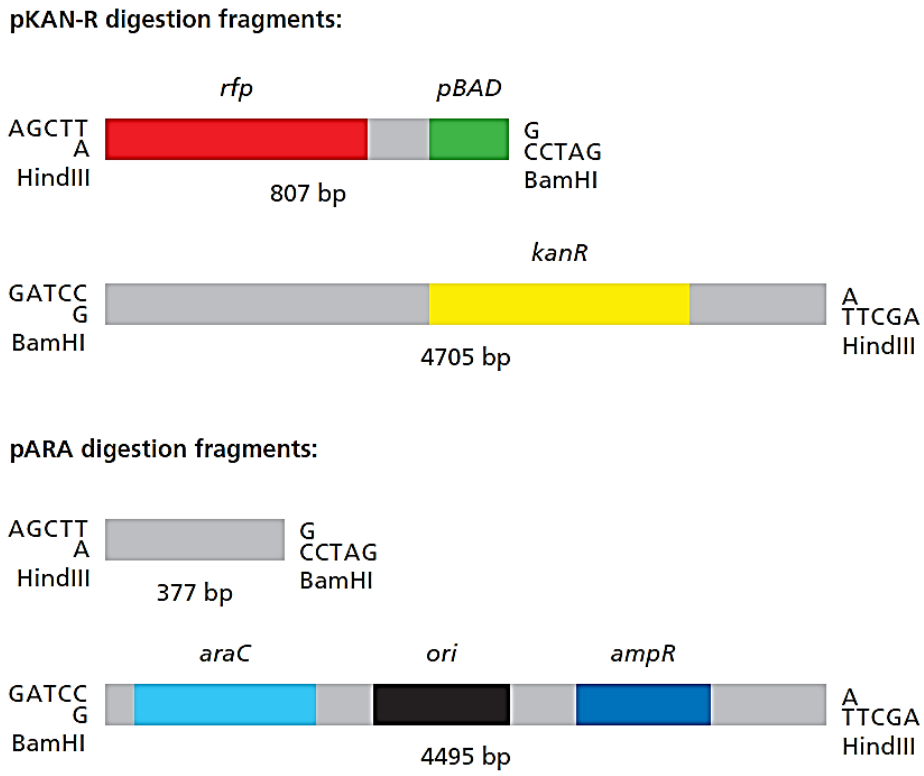
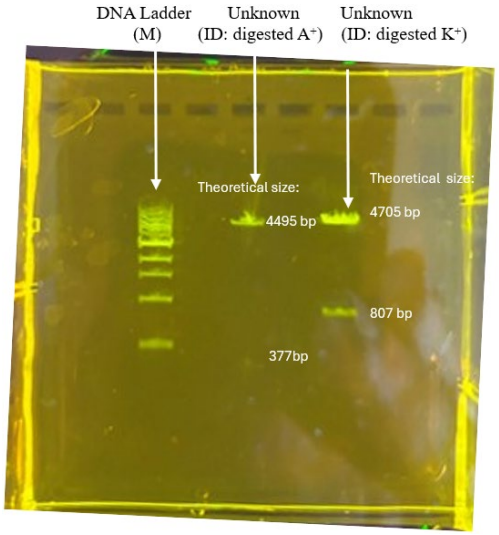


Figure 3: Sequences of digested K+ and A+ fragments and corresponding molecular size (bp) for each fragment

EXAMPLE GEL RUN:

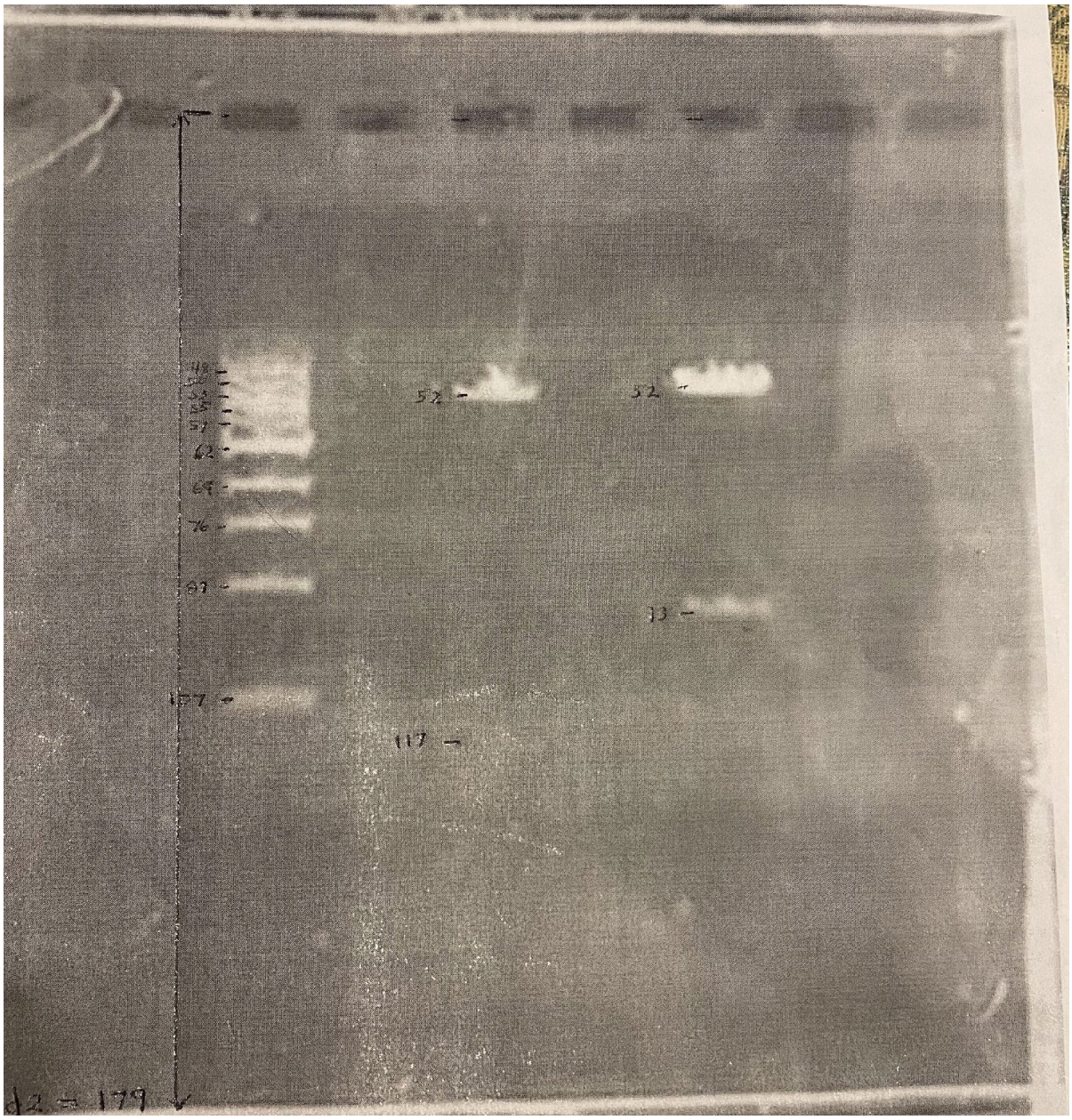
See Figure 4: Example gel run with DNA Ladder (M) and 2 unknown samples. The unknown samples contain the digested fragments of A^+ and K^+. The small fragment of A+ is not clearly visible. Consequently, using a digested A^+ as an unknown is not recommended for future labs.

Figure 4: Example of a gel run (M and 2 Unknown samples)



EXAMPLE PHOTO PRINTED COPY OF GEL RUN:

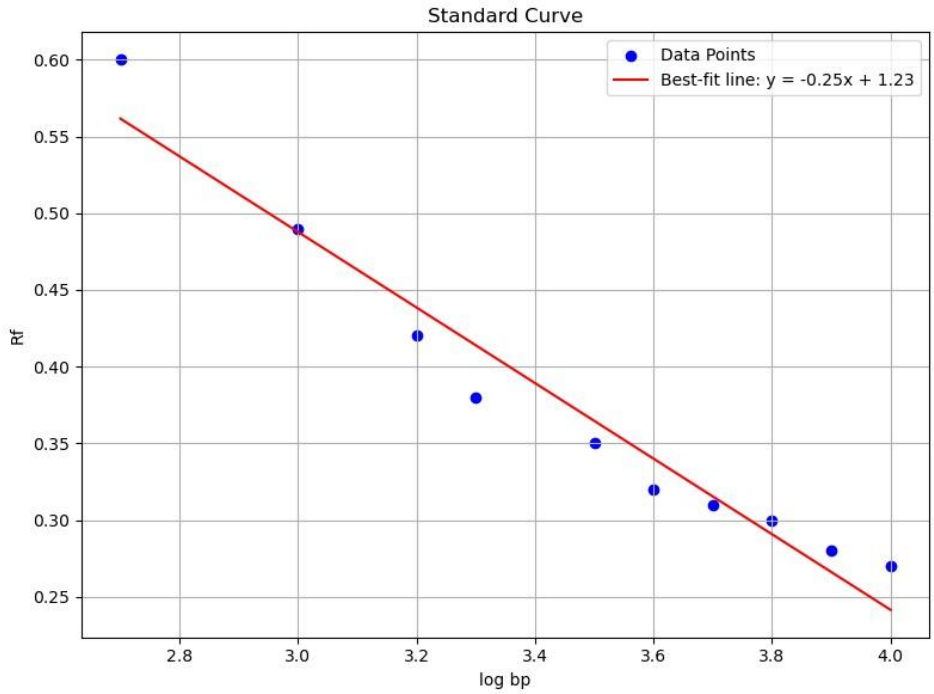
Figure 5: Printed copy of gel run with d_1 and d_2 measurements



Note: Black/white photocopying is not recommended. Colour print-out gives better visibility for the fragments.

EXAMPLE GRAPH:

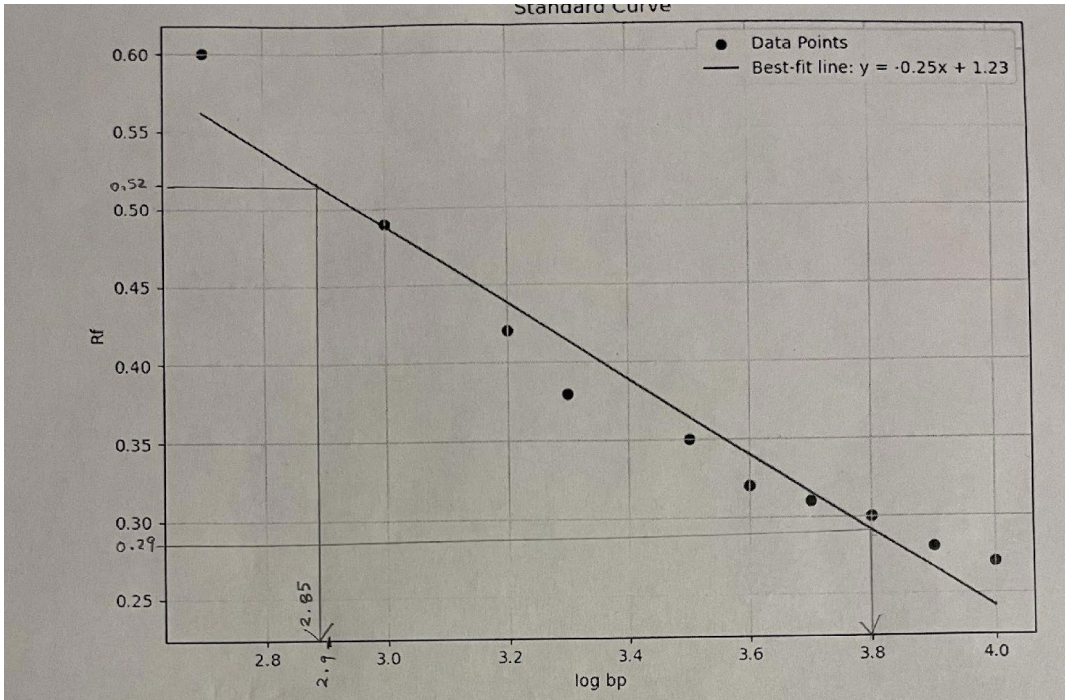
Figure 6: Standard Curve and Best-Fit Line graph



log bp	Rf
x-axis	y-axis
4.0	0.27
3.9	0.28
3.8	0.30
3.7	0.31
3.6	0.32
3.5	0.35
3.3	0.38
3.2	0.42
3.0	0.49
2.7	0.60

EXAMPLE GRAPH - EXTRAPOLATION

Figure 7: Graph showing extrapolation of Unknown (K+ fragments only)



log bp x-axis	Rf y-axis
4.0	0.27
3.9	0.28
3.8	0.30
3.7	0.31
3.6	0.32
3.5	0.35
3.3	0.38
3.2	0.42
3.0	0.49
2.7	0.60

EXAMPLE DATA TABLE

Figure 8: Completed data table of measured results and calculated results

Marker	Length of Fragment (bp)	Log bp	Distance migrated (mm) = d1	Rf	Unknown Sample (A+) (Challenge – omitted)	Distance migrated (mm) = d1	Rf	From graph x-value Log bp	x-value calculated from best fit line equation	Calculated bp (anti-log)
Fragment 1	10, 000	4.0	48	0.27	Unk Fragment 1	52	0.29	3.8	3.8	6310
Fragment 2	8, 000	3.9	50	0.28	Unk Fragment 2	~117 difficult to see	0.65	-	2.32	209
Fragment 3 (estimated)	6, 000	3.8	53	0.30	Unknown Sample (K+)	Distance migrated (mm)	Rf	From graph x-value Log bp	x-value calculated from best fit line equation	Calculated bp (anti-log)
Fragment 4	5, 000	3.7	55	0.31						
Fragment 5	4, 000	3.6	57	0.32	Unk Fragment 1	52	0.29	3.8	3.8	6310
Fragment 6	3, 000	3.5	62	0.35	Unk Fragment 2	93	0.52	2.85	2.84	708
Fragment 7	2, 000	3.3	69	0.38						
Fragment 8	1, 500	3.2	76	0.42						
Fragment 9	1, 000	3.0	87	0.49						
Fragment 10	500	2.7	107	0.60						
Distance from well to bottom of gel (mm) = d2 = 179										

EXAMPLE: BEST FIT LINE EQUATION CALCULATIONS

Best-Fit Line equation calculations for UNKNOWN (ID: K+ fragments)

$y = -0.25x + 1.23$

1. For Rf = 0.29 = y

$0.29 = -0.25x + 1.23$

$0.29 - 1.23 = -0.25x$

$X = \frac{0.29 - 1.23}{-0.25} = 3.8$ (Note: 3.8 is also the extrapolated value obtained from graph)

$\%\ \text{difference} = \frac{\text{measured experiment value-calculated value}}{\text{calculated value}} \times 100\% = 0\%$

2. For Rf = 0.52 = y

$0.52 = -0.25x + 1.23$

$0.52 - 1.23 = -0.25x$

$X = \frac{0.52 - 1.23}{-0.25} = 2.84$ (Note: 2.85 is the extrapolated value obtained from graph)

$\%\ \text{difference} = \frac{\text{measured experiment value-calculated value}}{\text{calculated value}} \times 100\%$

$= \frac{2.85-2.84}{2.84} \times 100\% = 0.35\%$

EXAMPLE CALCULATIONS: % ERROR

There is a discrepancy when comparing the actual value obtained from experiment to the expected theoretical value for the unknown fragments that were identified as digested fragments for K^+.

$\%\ \text{error} = \left| \frac{\text{theoretical value} - \text{calculated experimental value}}{\text{Theoretical value}} \right| \times 100\%$

For Unknown Fragment 1: ID K^+ large fragment

$\%\ \text{error} = \left| \frac{4705 - 6310}{4705} \right| \times 100\% = 234\%$

For Unknown Fragment 2: ID K+ small fragment

$\%\ \text{error} = \left| \frac{807 - 708}{807} \right| \times 100\% = 12\%$

POSSIBLE SOURCES OF ERROR:

Depending on the results obtained by the students when running the gel electrophoresis summative lab, there are a range of errors that could have occurred. When students list and explain the sources of errors, they must be specific and explain the errors that may have impacted their results. The students are not to be randomly listing errors that did not pertain to their own experiment. Some errors are explained below.

1. If fragments were calculated to be larger than the expected theoretical results, then incomplete digestion when using restriction enzymes to prepare the unknown DNA fragments before the lab may have resulted in larger than expected fragments. Some reasons for this include an insufficient amount of restriction enzyme added for digestion, a short incubation time, experimental temperatures during digestion were not optimal etc.

2. If students' results do not show separation of DNA Ladder fragments, then the gel electrophoresis run time was too short. Then the standard curve would not be accurate to calculate the unknown DNA fragments that were to be analyzed.

3. If the fragments were <u>not</u> visible under the transilluminator then the GelGreen® may have not been added to the gel when being prepared or the gel was exposed to direct light degrading the GelGreen®.

4. If the DNA ladder was separated successfully but an unknown was not visible, then the student must examine for evidence of puncturing of gel wells which would have dispersed the sample; causing an unsuccessful run.

5. If the student's results are not strong enough to see the bands clearly, there is a chance that the student did not load enough sample in the gel wells. This could be either a result of having some sample spill over out of the gel wells or the wrong amount of sample was loaded (i.e., 10 µL required to load).

6. If air bubbles were introduced by dispensing to full stop instead of 1st stop then this would result in the inaccurate pipetting of samples into the wells reducing the intensity of the fragments when running through the gels.

DISCUSSION/CONCLUSION:

The purpose of this lab experiment was to provide teachers with a summative assessment that was practical. The students were assessed on their individual ability to run a gel electrophoresis to separate and analyze unknown DNA fragments and determine their respective molecular sizes by comparing their migration distance to the fragments of the DNA ladder with known base pairs. The students are evaluated on their micropipetting techniques, loading samples in the gel wells and successfully observing the dye front to run their gels long enough to get optimal separation of DNA fragments. Introducing the graphic analysis through the creation of a standard curve and best-fit line has students experience how unknown DNA fragments can be identified using the Rf calculated values measured from the migration of each fragment and dye front. To minimize error, the migration of the dye front was not used as d_2. Instead, the distance from the

middle of the gel well to the bottom of the gel was used to ensure consistency especially since the loading dye is not visible under the transilluminator where students view their DNA fragments and take a photo for the calculation section of this lab. The teacher must provide to the students the molecular sizes of the fragments contained in the DNA ladder (see procedure) so that students can complete all respective calculations and determine the number of base pairs of their samples. The teacher must also provide students with the theoretical molecular size of each fragment of the unknown DNA sample so they can properly assess their results and incorporate all sources of errors during the experiment. It is important for students to compare their extrapolated results with calculations using the best-fit equation to see how close their results were to each other. If the DNA fragments were calculated to be larger than the expected theoretical molecular size (bp) this is not seen as a failure but an opportunity for students to think about the digestion step and any sources of errors that could have occurred when the unknown DNA samples were being prepared. In conclusion, this summative DNA Gel Electrophoresis lab enables students to (1) predict the relative speed of DNA fragments through a gel during gel electrophoresis, (2) separate DNA fragments using gel electrophoresis, (3) Calculate Rf values of DNA fragments, (4) Plot a standard curve to calculate the molecular size of the unknown DNA fragment and (5) analyze results to determine sources of error.

EXAMPLE RUBRICS

Rubric: DNA Gel Electrophoresis

Criteria	Excellent (4 pts)	Good (3 pts)	Satisfactory (2 pts)	Needs Improvement (1 pt)
Sample Preparation	Samples are prepared accurately with proper labeling and documentation.	Samples are mostly prepared correctly with minor errors.	Some errors in sample preparation or labeling are evident.	Samples are poorly prepared or mislabeled; major errors present.
Loading Samples and Marker	Samples and marker are loaded accurately without contamination or spillage or puncturing the gel.	Minor loading errors or slight contamination or slight puncturing of gel.	Some samples are misloaded or contaminated or gel punctured.	Significant loading errors or contamination or gel fully punctured.

Criteria	Excellent (4 pts)	Good (3 pts)	Satisfactory (2 pts)	Needs Improvement (1 pt)
Running the Gel	Gel is run at correct time; buffer is used appropriately.	Minor deviations in timing.	Incorrect timing affects results.	Gel is run improperly or not at all.
Use of Standard Marker	Marker is used correctly and clearly labeled for size comparison.	Marker is used but not clearly labeled or slightly misloaded.	Marker is present but difficult to interpret.	Marker is missing or unusable.
Interpretation of Results	Results are interpreted accurately with clear comparison to marker.	Interpretation is mostly accurate with minor errors.	Some misinterpretation of band sizes or results.	Results are misinterpreted or not analyzed.

Rubric: Standard Curve and Data Extrapolation

Criteria	Excellent (4 pts)	Good (3 pts)	Satisfactory (2 pts)	Needs Improvement (1 pt)
Sample Preparation	Samples are prepared accurately with proper labeling and documentation.	Samples are mostly prepared correctly with minor errors.	Some errors in sample preparation or labeling are evident.	Samples are poorly prepared or mislabeled; major errors present.
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Criteria	Excellent (4 pts)	Good (3 pts)	Satisfactory (2 pts)	Needs Improvement (1 pt)
Running the Gel	Gel is run at correct time; buffer is used appropriately.	Minor deviations in timing.	Incorrect timing affects results.	Gel is run improperly or not at all.
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Interpretation of Results	Results are interpreted accurately with clear comparison to marker.	Interpretation is mostly accurate with minor errors.	Some misinterpretation of band sizes or results.	Results are misinterpreted or not analyzed.
Sources of Error	Sources of errors accurately identified and explained.	Minor issue in sources of error and explanations.	Some errors in the sources of errors identified.	Sources of errors missing or errors in explanation or identifying.

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BEFORE THE LAB

Review the following:
1. Micropipetting techniques. Refer to ABE Lab 1.
2. Running a gel electrophoresis. Refer to ABE Lab 4.

MATERIALS

Reagents
- A plastic microfuge tube rack with the following
  - Microfuge tube of DNA Marker solution (M)
  - Microfuge tube of unknown DNA fragment (U)
- 50-mL flask containing 1x sodium borate buffer (1x SB)

Equipment and Supplies
- P-20 micropipette (measures 2.0–20 µL)
- Tip box of disposable pipette tips
- Electrophoresis box loaded with 0.8% agarose gel containing GelGreen® (will be shared among students)
- Microcentrifuge (will be shared among students)
- Waste container for used tips and microfuge tubes (will be shared among students)
- DNA Ladder Diagram

SAFETY:
- All appropriate safety precautions and attire required for a science laboratory should be used, including safety goggles and gloves. Please refer to your teachers' instructions.
- Wash your hands well with soap after completing the lab.

METHOD

1. Check your rack to make sure that you have all the reagents listed.
2. Spin the microfuge tubes (M and U) in the microcentrifuge for several seconds to pool the reagents at the bottom of each tube.

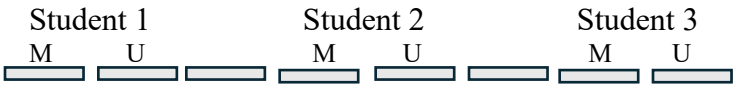
   LAB TECHNIQUE: Distribute the tubes evenly in the microcentrifuge so that their weight is balanced, making sure they are directly opposite one another.

3. Obtain gel from the teacher. Make sure that the wells in your gel are located at the top near the negative (black) electrode when placed in the gel electrophoresis unit.

4. Fill the box with1xSB to a level that just covers the entire surface of the gel. If you see any "dimples" over the wells, add more buffer.

   LAB TECHNIQUE: If there are "dimples" add very small amounts of buffer to the electrophoresis box. While the gel needs to be completely under the buffer, you don't want too much buffer in the box, as this will cause the electrical current to run through the buffer and not the gel.

5. Make a drawing in your notebook that shows the location of the wells in the electrophoresis box. There will be 3 students using the same gel.

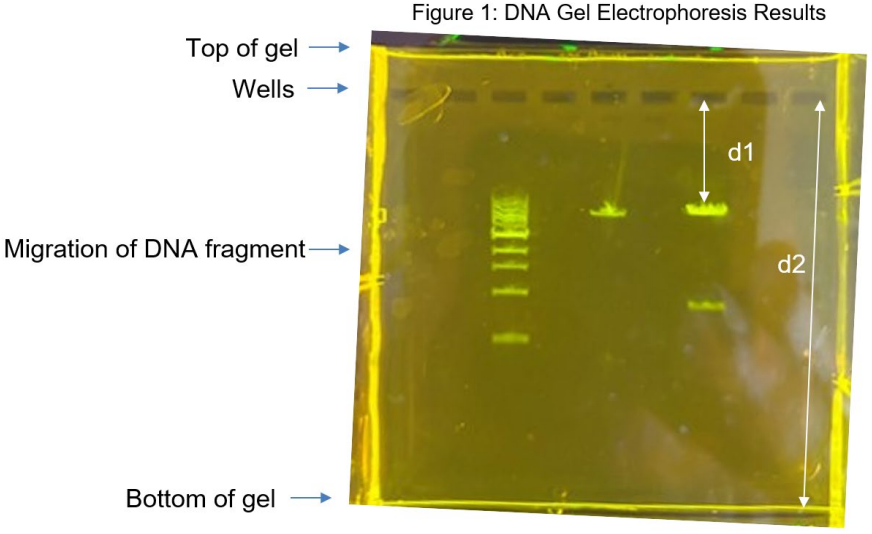


6. Using a fresh pipette tip for each sample, dispense 10.0 µL of the DNA ladder (M) and unknown DNA fragment (U). For each sample, do the following:
   a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
   b. Lower the pipette tip until it is under the buffer but just above the well.

   LAB TECHNIQUE: Do not puncture the gel, or it will become unusable. Gently press the plunger to slowly dispense the sample. To avoid getting air into the buffer, do not go past the first stop. The sample will sink into the well.

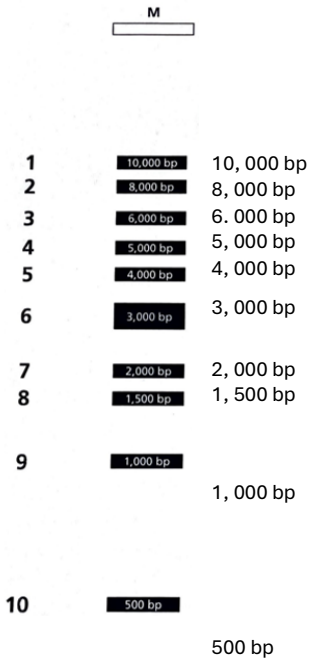
7. When all the samples have been loaded, close the cover tightly over the electrophoresis box.
8. Turn on the power supply and set the voltage to 130–135 V.
9. After two or three minutes, check to see if the loading dye is moving toward the positive electrode.
10. The loading dye will need to run just near the end of the gel, about 40–50 minutes.
11. After the gel has finished running, view the gel under the transilluminator to show the DNA fragments and unknown fragment.
12. Take a photo of the gel. Your teacher will explain how to dispose of the gel. *The following steps may be completed the next day. Please check with your teacher.*
13. Print the photo of the gel results. Colour print shows the fragments more than black and white print.
14. Record in **TABLE FORMAT** all your results and calculations.
15. On the printed photo of the gel results, measure the distance travelled for each DNA fragment in the DNA ladder (M) and in the unknown sample.
16. Measure the distance from the middle of the well to the bottom of the gel. Record in table.
17. Calculate the Rf value for each DNA fragment in the DNA ladder (M) and in the unknown sample. See Figure 1. See NOTES Rf information.

Figure 1: DNA Gel Electrophoresis Results



18. Plot a standard curve using the DNA Ladder (M) Rf values (y-axis) versus the logarithm of the corresponding molecular size (x-axis). See Figure 2 to obtain the molecular size for each fragment in the DNA Ladder (M).

Figure 2: DNA Ladder (M) molecular size (bp) for each fragment



19. Draw the best fit line.
20. Using slope = rise/run or a graphing program (i.e. desmos), determine the best fit line equation, y = mx + b, where m is the slope of the line and b is the y-intercept. See video Calculating a Line of Best Fit with Desmos to learn how to use Desmos calculator to find the line of best fit equation.

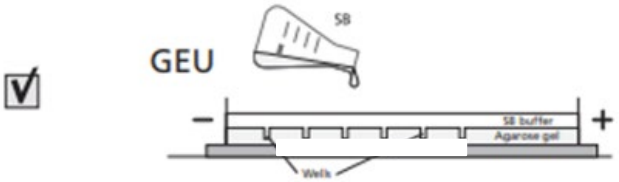
21. On the graph, plot the Rf values of the unknown DNA fragments and intersect the best fit line.
22. Extrapolate to determine the logarithm molecular size (bp) on the x-axis.
23. Using anti-log, calculate the molecular size (bp) of each unknown DNA fragment.
24. For each unknown DNA fragment, enter the value of the Rf value as y in the best fit line equation and solve for x (logarithm molecular size (bp). Then solve for the estimated molecular size (bp).
25. Calculate the % difference of the bp value determined from the graph extrapolated versus the bp value determined from the line of best fit equation.
26. Obtain from your teacher the theoretical value for the molecular size (bp) for each unknown fragment and calculate the % error. Use bp calculated from the best fit formula. **Percent Error = (| Experimental Value – Actual Value | / Actual Value) x 100%**
27. Explain a minimum of two sources of error.

NOTES:

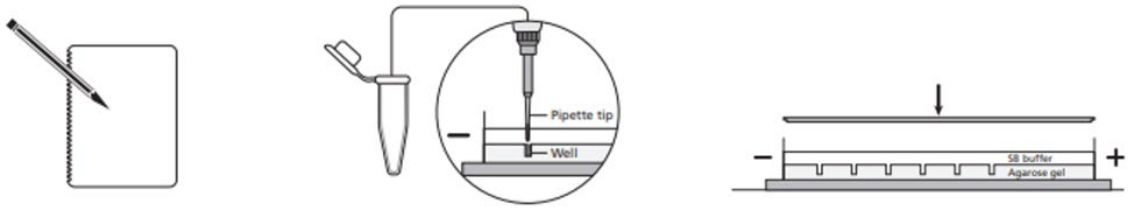
- Rf values are used to confirm the length of unknown DNA samples.
- Rf = distance the DNA fragment has travelled from the origin (gel well) / distance from the origin to the reference point (*dye front) = $\frac{d_1}{d_2}$
- Refer to the following video on how to calculate Rf values. https://youtu.be/z8Hz2WNnGY4
- For d_1, the migration of a DNA fragment/band may be measured starting from the middle of the well to the middle of each DNA fragment.
- *For d_2, instead of using the dye front (not visible under transilluminator), use the bottom of the gel to obtain the d_2 measurement.

Flow Chart

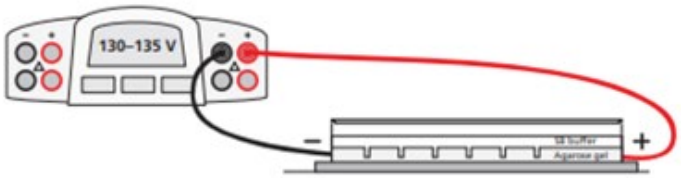
Check reagents
Unknown (U)
M in rack, 1x SB flask) → **Centrifuge** →

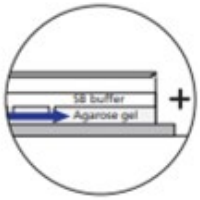


Review gel electrophoresis unit (GEU) → **Cover gel in GEU with 1x SB** →



Draw location of each well, and label which solution is loaded in each in notebook → **Pipette 10.0 µL each of** DNA ladder, and unknown DNA fragment **into designated wells in GEU** → **Place cover on GEU** →



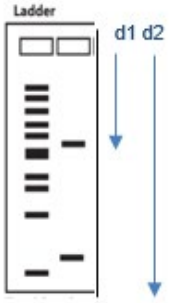


Connect leads to power supply → Turn on power supply and set voltage to 130–135 V → Check to see if the purple loading dye is moving toward the positive (red) electrode →

Run the gel until the DNA fragments are separated (viewed under transilluminator) → Take a photo & print → Measure the distance the DNA fragments travelled through the gel. (d1)

Measure the distance from the well to the bottom of the gel. (d2) → Calculate the Rf values of the unknown DNA fragments & ID.

Rf = d1 / d2 →



Plot a standard curve by plotting the Rf value (y-axis) of each standard DNA fragment (from Marker) versus the logarithm of its molecular size (x-axis) → On this graph plot the Rf value of the unknown DNA fragment on y-axis and extrapolate from the standard curve to determine molecular size of the unknown (anti-log of value from graph) → Continue as per procedure step 20

