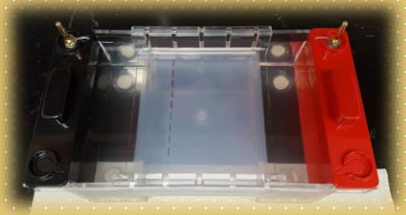
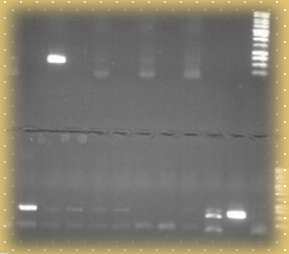
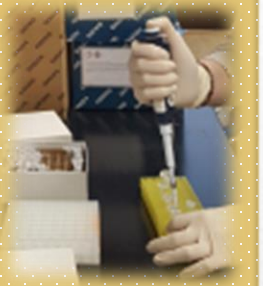
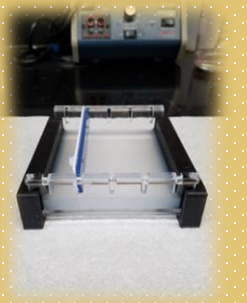
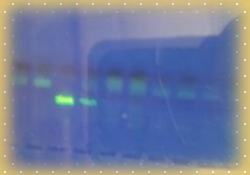


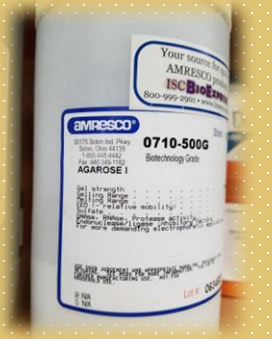


Gel Electrophoresis (optional step)

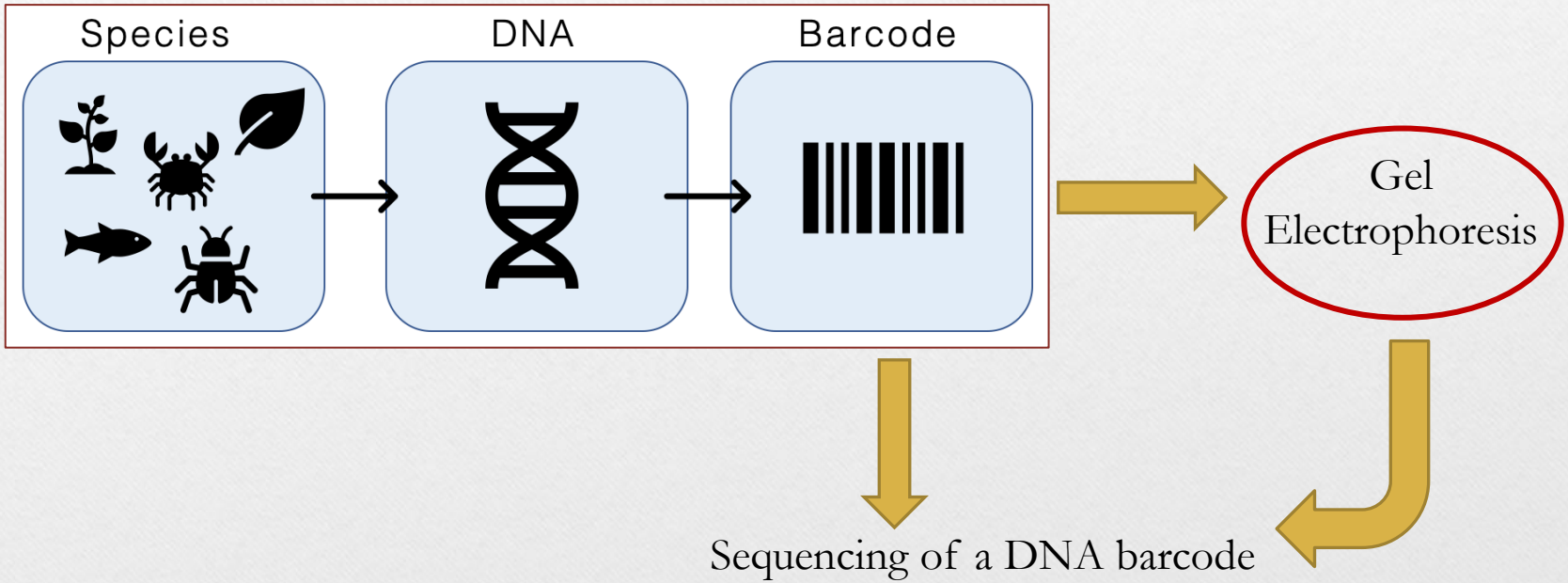








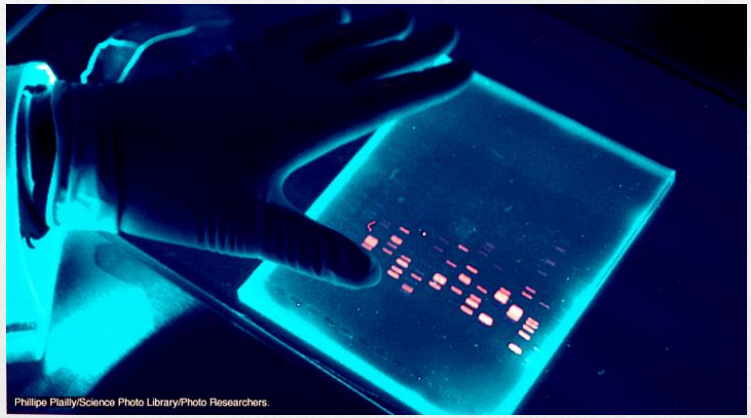
Gel Electrophoresis: when do we do it?



Images: https://en.wikipedia.org/wiki/DNA_barcoding

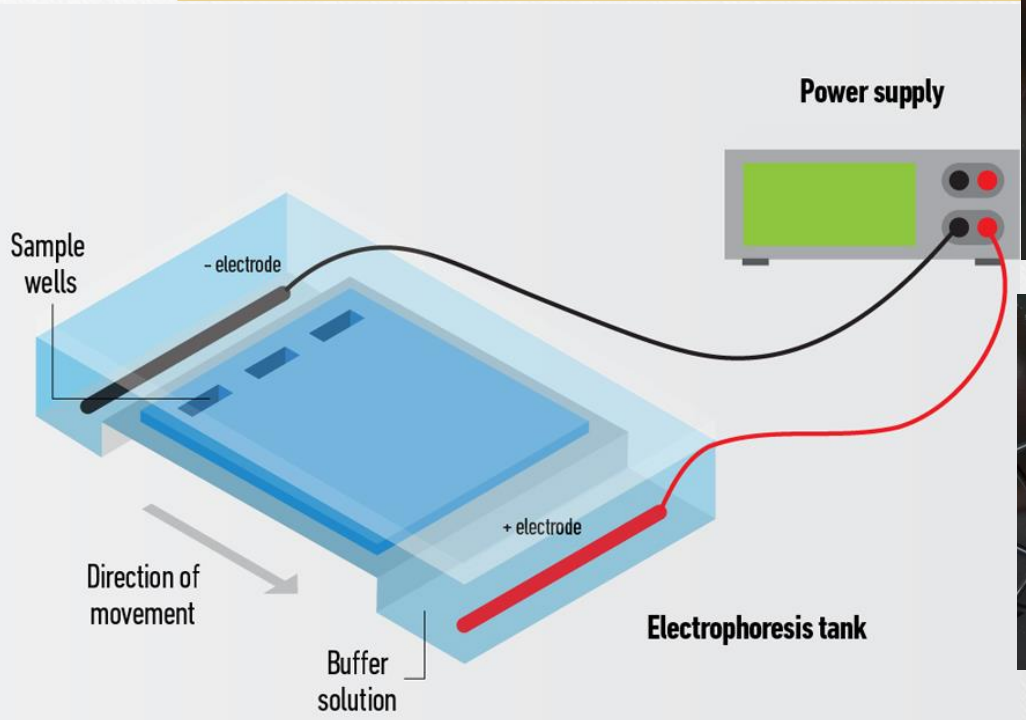
Gel Electrophoresis: why do we do it?

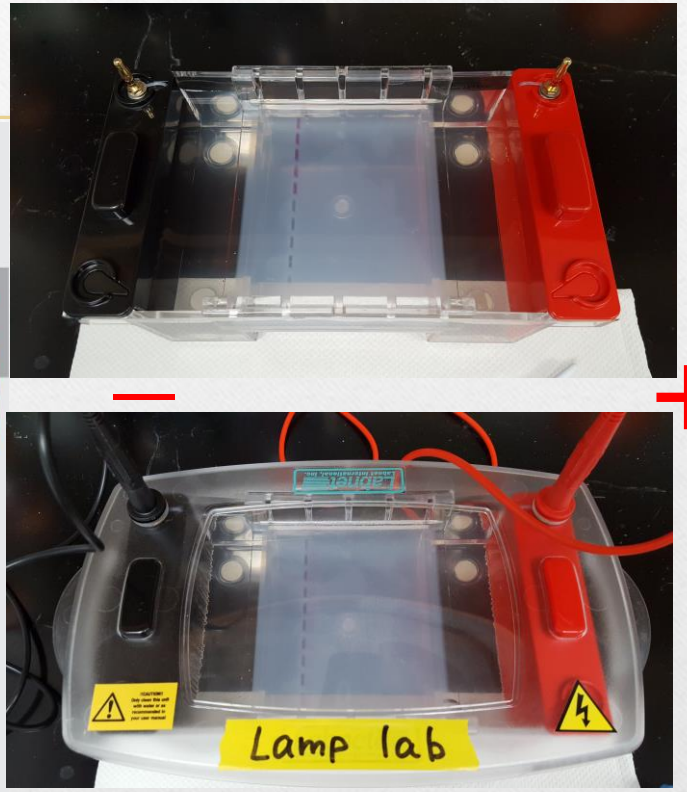
Gel electrophoresis allow us to visualize DNA migration



- To confirm presence of the DNA template in the PCR product
- To validate the length of the DNA barcode we amplified during the PCR

DNA migration



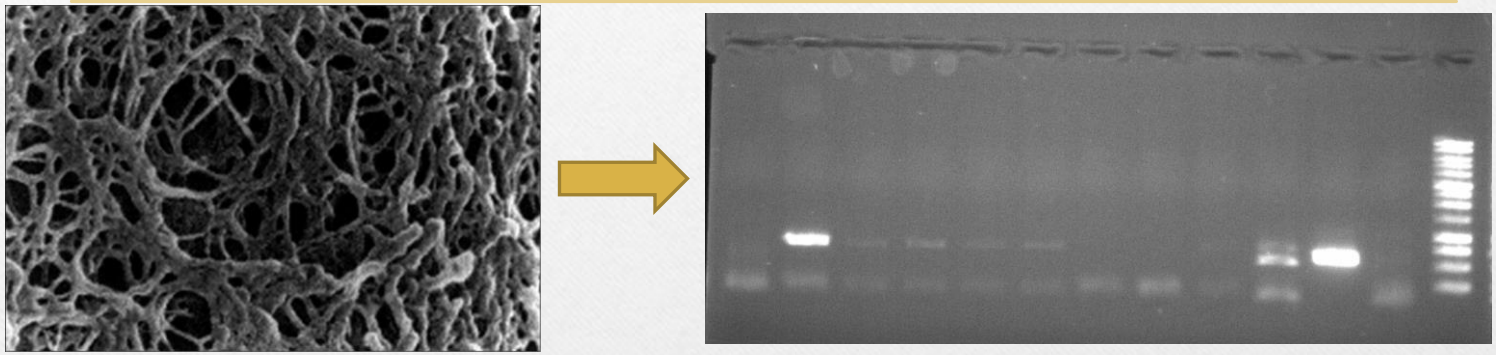


Run to the red!

DNA is strongly negative => it will migrate through the gel towards the positive electrode (from cathode to anode)

https://www.technologynetworks.com/

DNA migration in Agarose Gel

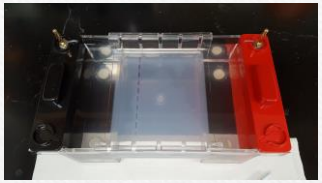


- The pores in the gel separate the DNA molecules according to their size and shape
- The smaller the DNA molecule, the faster it migrates through the gel
- Supercoiled DNA migrates faster than its linear form
- Linear DNA migrates faster than its nicked circular form

https://users.cs.duke.edu

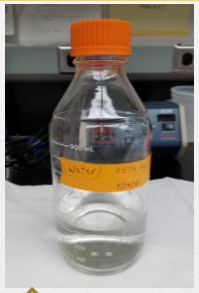
Gel Electrophoresis: Equipment and Materials

Gel box



Power source

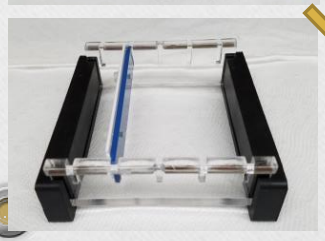
Distilled water

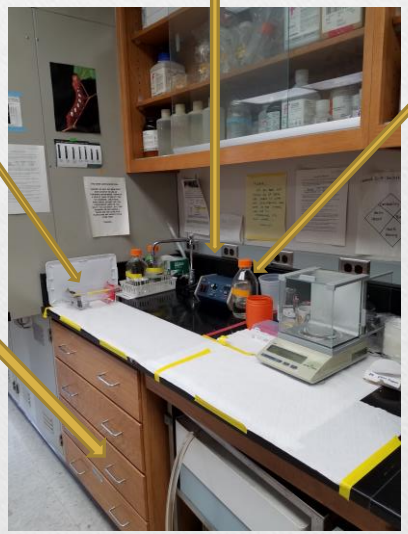


50x TAE

Agarose

Gel tray, gel comb



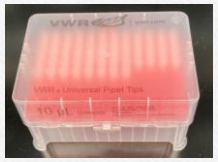


Gel Electrophoresis: Equipment and Materials

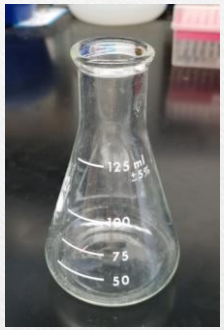
Pipette

p20

Pipette tips



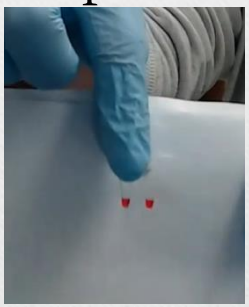
Flask



Graduated cylinder

Parafilm

PCR tube strips with PCR products



Loading dye

SYBR green

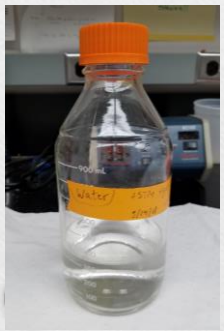
Ladder

Gel Electrophoresis: Protocol

Steps 1, 2

10 ml

490 ml



Gel Electrophoresis

1. Use the designated bench (on the right).
2. Prepare a new 1xTAE buffer: **490ml dH_2O + 10 ml 50xTAE**
3. Make 1% agarose solution in 150-200 ml flask; mix these reagents I the following order:

75 ml 1xTAE
7.5 µl SYBR green
0.75 g agarose

4. Heat to boiling in microwave (~1.5 min) swirling each 20 sec.
5. Place flask on the bench to cool down to ~56^{0}C.

6. Prepare a small gel box (in the drawer, labeled "Lamp lab").
7. Prepare a gel tray. Attach the rubber walls, make sure they are tight. Place a comb in the desired place.
8. Pour gel solution into the gel tray. Let set about 30-60 min.

9. When ready, carefully take the comb out (one single movement), remove the rubber walls, and place the gel tray in the gel box to run the gel ("RUN TO THE RED").
10. Fill the gel box with the prepared 1x TAE solution until the gel is completely submerged (covered with the buffer for ~3-4mm).

11. Take the PCR samples, SYBR green, the ladder and the loading buffer out from the freezer. Let it thaw.
12. (If needed) Load **5 µl** of the ladder into the 1st well.
13. On parafilm, mix 1 **µl SYBR green + 9 µl 6x loading buffer**
14. On parafilm, mix 1 **µl SYBR/buffer mixture (from step 13) + 5 µl PCR product**
15. Load **5 µl** of each PCR sample/SYBR mixture (from step 14) into the rest of the wells.
16. Cover the gel box with the lid.

17. Plug into the power pack and run at constant voltage approx. 130V until bands have migrated to approximately 1 inch from the end (~15-20 min).
18. When it is done, turn off the power pack and remove the gel.
19. Place gel onto UV illuminator to see the results.

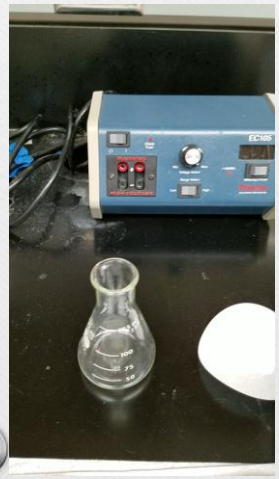
Gel Electrophoresis: Protocol

0.75g

75ml

7.5 µl

Steps 3-5



Gel Electrophoresis

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2. Prepare a new 1xTAE buffer: **490ml dH_2O + 10 ml 50xTAE**
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   7.5 µl SYBR green
   0.75 g agarose

4. Heat to boiling in microwave (~1.5 min) swirling each 20 sec.
5. Place flask on the bench to cool down to ~56^{0}C.

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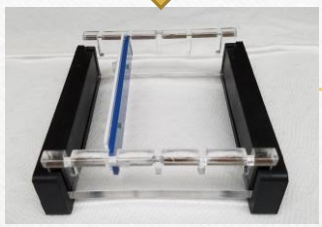
8. Pour gel solution into the gel tray. Let set about 30-60 min.

9. When ready, carefully take the comb out (one single movement), remove the rubber walls, and place the gel tray in the gel box to run the gel ("RUN TO THE RED").
10. Fill the gel box with the prepared 1x TAE solution until the gel is completely submerged (covered with the buffer for ~3-4mm).

11. Take the PCR samples, SYBR green, the ladder and the loading buffer out from the freezer. Let it thaw.
12. (If needed) Load **5 µl** of the ladder into the 1st well.
13. On parafilm, mix 1 **µl SYBR green + 9 µl 6x loading buffer**
14. On parafilm, mix 1 **µl SYBR/buffer mixture (from step 13) + 5 µl PCR product**
15. Load **5 µl** of each PCR sample/SYBR mixture (from step 14) into the rest of the wells.
16. Cover the gel box with the lid.

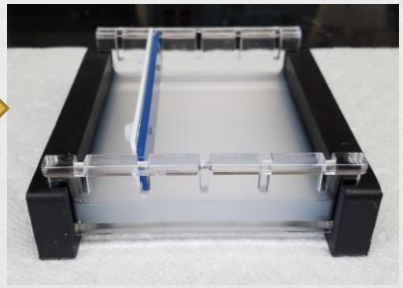
17. Plug into the power pack and run at constant voltage approx. 130V until bands have migrated to approximately 1 inch from the end (~15-20 min).
18. When it is done, turn off the power pack and remove the gel.
19. Place gel onto UV illuminator to see the results.

Gel Electrophoresis: Protocol



Steps 6-10

~30-60 min later



Gel Electrophoresis

1. Use the designated bench (on the right).
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10. Fill the gel box with the prepared 1x TAE solution until the gel is completely submerged (covered with the buffer for ~3-4mm).

11. Take the PCR samples, SYBR green, the ladder and the loading buffer out from the freezer. Let it thaw.
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13. On parafilm, mix 1 **µl SYBR green + 9 µl 6x loading buffer**
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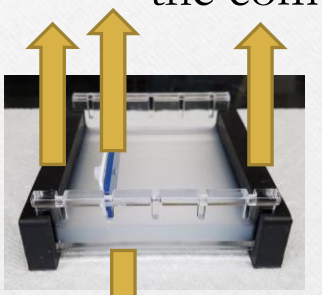
15. Load **5 µl** of each PCR sample/SYBR mixture (from step 14) into the rest of the wells.
16. Cover the gel box with the lid.

17. Plug into the power pack and run at constant voltage approx. 130V until bands have migrated to approximately 1 inch from the end (~15-20 min).
18. When it is done, turn off the power pack and remove the gel.
19. Place gel onto UV illuminator to see the results.

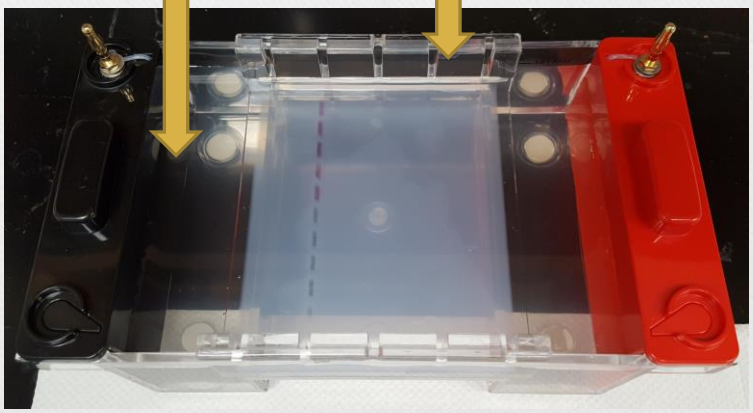
Gel Electrophoresis: Protocol

~400 ml

Remove the walls and the comb



Steps 9-10



Gel Electrophoresis

1. Use the designated bench (on the right).
2. Prepare a new 1xTAE buffer: **490ml dH_2O + 10 ml 50xTAE**
3. Make 1% agarose solution in 150-200 ml flask; mix these reagents I the following order:

   75 ml 1xTAE
   7.5 µl SYBR green
   0.75 g agarose

4. Heat to boiling in microwave (~1.5 min) swirling each 20 sec.
5. Place flask on the bench to cool down to ~56^{0}C.

6. Prepare a small gel box (in the drawer, labeled "Lamp lab").
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8. Pour gel solution into the gel tray. Let set about 30-60 min.

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10. Fill the gel box with the prepared 1x TAE solution until the gel is completely submerged (covered with the buffer for ~3-4mm).

11. Take the PCR samples, SYBR green, the ladder and the loading buffer out from the freezer. Let it thaw.
12. (If needed) Load **5 µl** of the ladder into the 1st well.
13. On parafilm, mix 1 **µl SYBR green + 9 µl 6x loading buffer**
14. On parafilm, mix 1 **µl SYBR/buffer mixture (from step 13) + 5 µl PCR product**
15. Load **5 µl** of each PCR sample/SYBR mixture (from step 14) into the rest of the wells.
16. Cover the gel box with the lid.

17. Plug into the power pack and run at constant voltage approx. 130V until bands have migrated to approximately 1 inch from the end (~15-20 min).
18. When it is done, turn off the power pack and remove the gel.
19. Place gel onto UV illuminator to see the results.

Gel Electrophoresis: Protocol

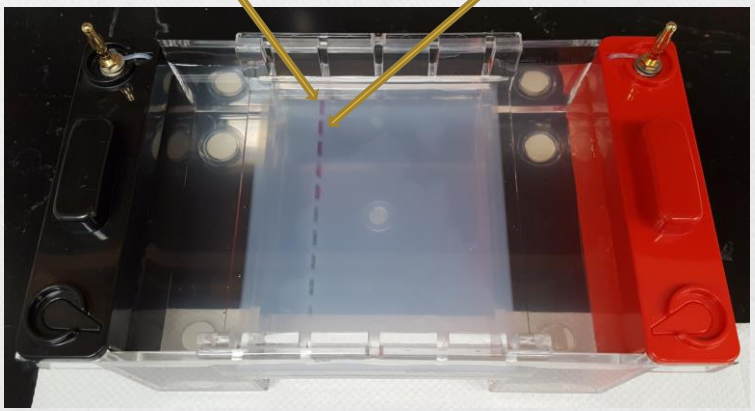
9 µl

1 µl

1 µl

5 µl

5 µl



Steps 11-16

Gel Electrophoresis

1. Use the designated bench (on the right).
2. Prepare a new 1xTAE buffer: **490ml dH_2O + 10 ml 50xTAE**
3. Make 1% agarose solution in 150-200 ml flask; mix these reagents I the following order:

   75 ml 1xTAE
   7.5 µl SYBR green
   0.75 g agarose

4. Heat to boiling in microwave (~1.5 min) swirling each 20 sec.
5. Place flask on the bench to cool down to ~56^{0}C.

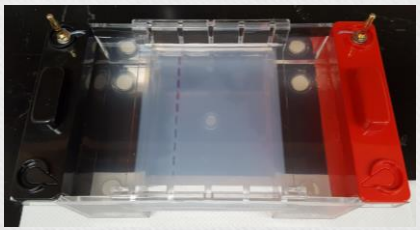
6. Prepare a small gel box (in the drawer, labeled "Lamp lab").
7. Prepare a gel tray. Attach the rubber walls, make sure they are tight. Place a comb in the desired place.
8. Pour gel solution into the gel tray. Let set about 30-60 min.

9. When ready, carefully take the comb out (one single movement), remove the rubber walls, and place the gel tray in the gel box to run the gel ("RUN TO THE RED").
10. Fill the gel box with the prepared 1x TAE solution until the gel is completely submerged (covered with the buffer for ~3-4mm).

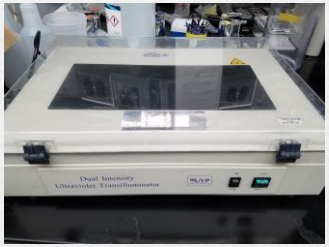
11. Take the PCR samples, SYBR green, the ladder and the loading buffer out from the freezer. Let it thaw.
12. (If needed) Load **5 µl** of the ladder into the 1st well.
13. On parafilm, mix 1 **µl SYBR green + 9 µl 6x loading buffer**
14. On parafilm, mix 1 **µl SYBR/buffer mixture (from step 13) + 5 µl PCR product**
15. Load **5 µl** of each PCR sample/SYBR mixture (from step 14) into the rest of the wells.
16. Cover the gel box with the lid.

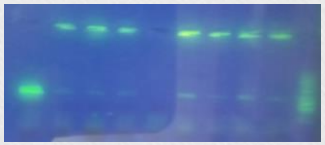
17. Plug into the power pack and run at constant voltage approx. 130V until bands have migrated to approximately 1 inch from the end (~15-20 min).
18. When it is done, turn off the power pack and remove the gel.
19. Place gel onto UV illuminator to see the results.

Gel Electrophoresis: Protocol



~15-20 min later





Steps 17-19

Gel Electrophoresis

1. Use the designated bench (on the right).
2. Prepare a new 1xTAE buffer: **490ml dH_2O + 10 ml 50xTAE**
3. Make 1% agarose solution in 150-200 ml flask; mix these reagents I the following order:

75 ml 1xTAE
7.5 µl SYBR green
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4. Heat to boiling in microwave (~1.5 min) swirling each 20 sec.
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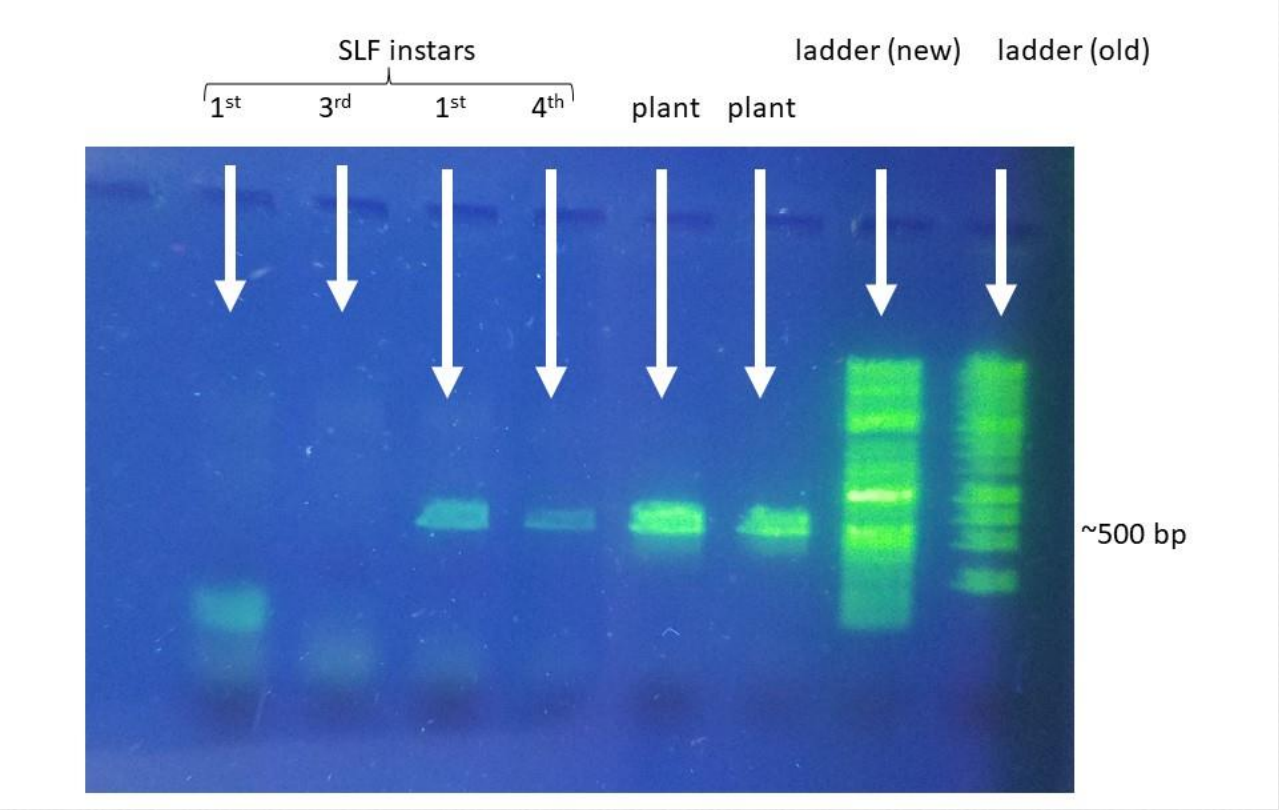
15. Load **5 µl** of each PCR sample/SYBR mixture (from step 14) into the rest of the wells.
16. Cover the gel box with the lid.

17. Plug into the power pack and run at constant voltage approx. 130V until bands have migrated to approximately 1 inch from the end (~15-20 min).
18. When it is done, turn off the power pack and remove the gel.
19. Place gel onto UV illuminator to see the results.

Gel Image Interpretation



Gel electrophoresis is done!

After you obtained the gel image, you can either store the PCR products at -20^{0}C until you need to send them for sequencing, or you can proceed immediately to sample preparation for sequencing (~10 min; please see the protocol).

Helpful resource

- https://users.cs.duke.edu/~reif/courses/molcomplectures/MolecularImaging/GelElectrophoresis/Agarose%20Gel%20Electrophoresis%20Handout(JosephProvost,USD).pdf

Image and video credits

- Videos: gel preparation, recording, and editing were done by Alina Avanesyan
- Photos: preparing and editing by Anya Wilkinson and Alina Avanesyan
- Gel electrophoresis protocol: prepared by Alina Avanesyan
- Videos were recorded and photos were taken in Dr. David Hawthorne's lab: 4172 Plant Science Building, Department of Entomology, University of Maryland, College Park, MD

Acknowledgements

- We thank Dr. David Hawthorne (Department of Entomology, University of Maryland) for providing lab equipment and lab space for our DNA barcoding work; for providing lab space to take the photos and record the videos needed for developing this course; and for continuous support and encouragement!