DNA Fingerprinting

ESTABLISH LINKS ● COLLECT EVIDENCE ● MAKE AN ARGUMENT

Biotechnology Outreach

featuring the
blueGel™ electrophoresis system
by miniPCR bio™

_____ _____

student name date

BE A SCIENTIST

- ✓ Listen to and read instructions closely
- ✓ Follow all directions, including safety precautions
- ✓ Wear appropriate safety equipment, including medical gloves
- ✓ Collaborate with your team/group to ensure all work is done efficiently and correctly
- ✓ Take time to think about…
  - …WHAT you're doing.
  - …HOW you're supposed to do it.
  - …WHY you're doing it.

A special "thank you" to the team at miniPCR bio™ for their contributions to this project including blueGel™ electrophoresis system instructions and diagrams.

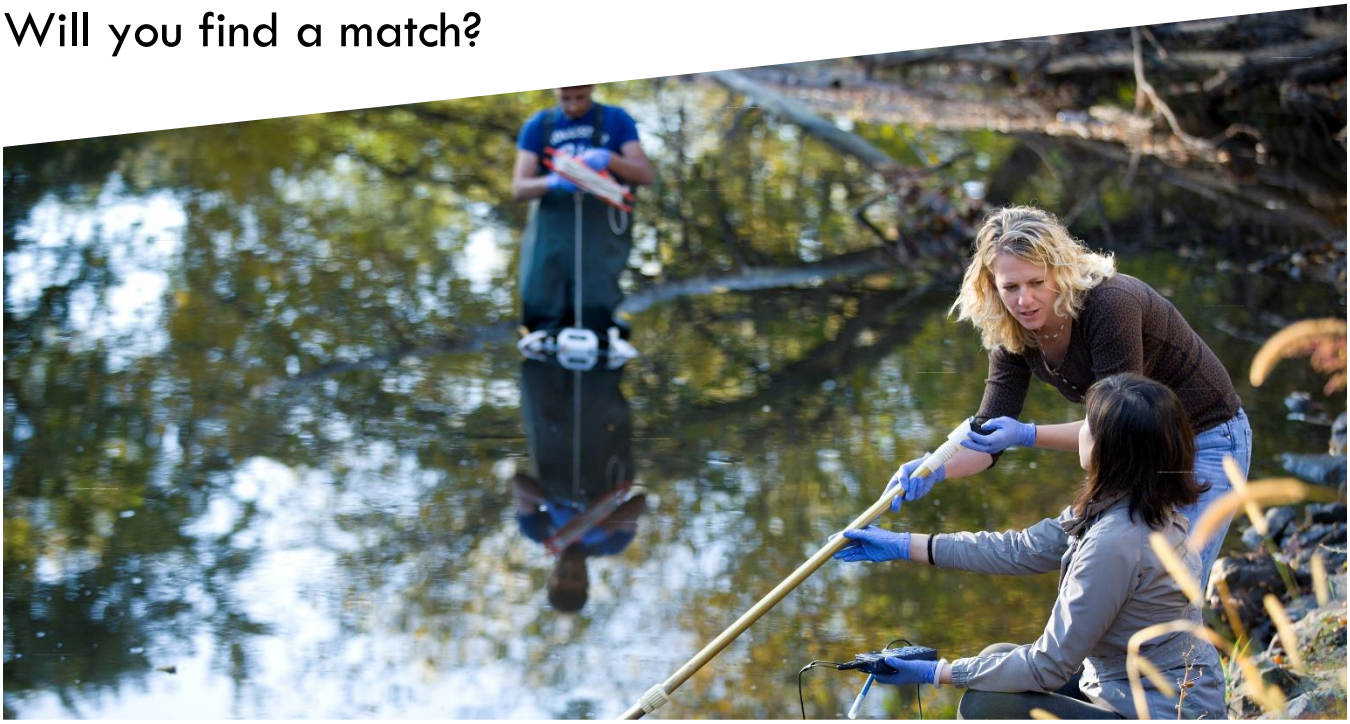
A MATCH GAME

Bacteria are all around you! Whether they're on your skin, on your cell phone, or your cute puppy's tongue when she licks you…they are everywhere! Some bacteria cause disease, but many are helpful. They can live in your body and help you digest food and eliminate other disease-causing organisms. And, some bacteria play important roles in the ecosystems around us.

As a water quality researcher, you see a lot of different kinds of bacteria in your lab. Samples of water from streams, ponds, and even soil from farmland contain many different types of bacteria. One particular type of bacteria interests you more than the others. You know that these bacteria efficiently remove nitrates from groundwater and farmland run-off, but you're unsure what kind of bacteria it is. So today, you plan to investigate and find out!

You have collected several different known kinds of bacteria, along with your unknown bacteria sample from a super-efficient woodchip bioreactor. You extracted the DNA from the bacterial cells and collected it in small microcentrifuge tubes. Now, you need to use high-tech biotechnology processes and equipment to determine if your unknown sample matches any of the known bacteria.

Will you find a match?



STUDENT INSTRUCTIONS

Step 1. Put on medical gloves and wear them throughout the experiment. The gloves will protect the DNA samples from contaminates that may be on your hands.

Step 2. Your group has a sample of the unknown bacterial DNA in a 1.5 ml microcentrifuge tube labeled U and samples from each of the known bacteria in tubes labeled with A - D. Keep the tubes upright throughout all the steps of the experiment to keep the DNA off the sides of the tube. Into each of the five tubes, pipette 3 ul of the restriction endonuclease Bgl I from the tube labeled N. Use a fresh pipette tip when adding Bgl 1 to each tube. To rinse the pipette tip and mix the DNA and Bgl 1, fill and unload the pipette with the sample three times. Label the five tubes with the letter assigned to your group.

Step 3. Place the tubes in a rack provided by the instructor and incubate them at 37°C for 15 - 30 minutes.

SCIENCE BREAK

Bgl 1 is isolated from the bacteria Bacillus globigii. The restriction endonuclease protects the bacteria from foreign DNA, such as from a virus, by cutting it up and rendering it ineffective. The endonuclease cuts (↑↓) the DNA at each site where the following sequences occur:

5'-GCCN NNN↓NGGC-3'
3'-CGGN↑NNN NCCG-5'

N can be any nucleotide, but the location and order of G (guanine) and C (cytosine) is very specific to this restriction endonuclease.

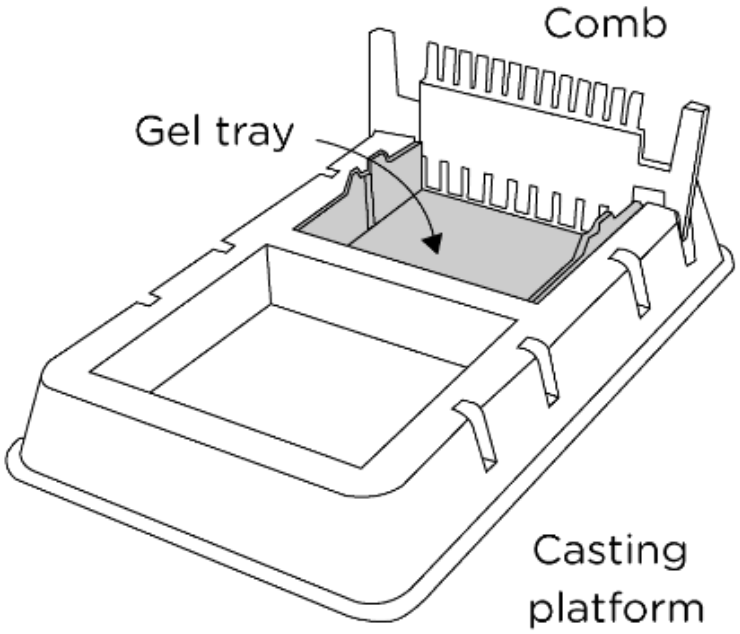
Step 4. Place the gel tray inside the casting platform and add combs. Place on a level surface to ensure uniform gel thickness. Determine the percentage gel to make.

Gel %	**Per 1 SeeGreen™ Tab**	**Yield (no. of gels)**
1.0%	40 mL distilled water	2 blueGel gels
1.5%	27 mL distilled water	2 blueGel gels
2.0%	20 mL distilled water	1 blueGel gel

Step 5. Soak one SeeGreen™ all-in-one agarose tab in distilled water according to the table above. Use a container at least three times larger than the desired gel volume.

Step 6. Swirl about 3 minutes until the Tab is fully dissolved.

Step 7. Heat the solution until it is clear and all particles are dissolved (typically 30-40 seconds per 20 mL gel in a high-power microwave). Allow to cool to 60-70°C. DO NOT add any DNA stain.



Consider these questions as you investigate the identity of your unknown bacteria sample:

1) What claim can you make about the identity of the unknown bacteria sample after running the gel?

2) What specific evidence from the investigation supports your claim?

3) Are there any possible variables or effects that may have impacted the evidence you collected?

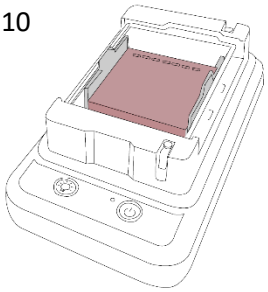
4) What limitations exist in this kind of investigation? Where might mistakes have been made?

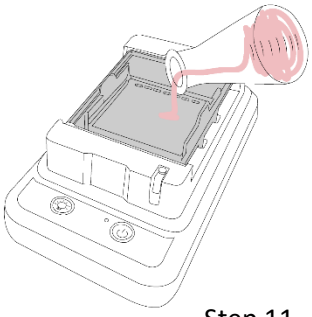
INVESTIGATION NOTES

Step 8. Pour the solution into the gel tray to a depth of approximately 8 mm. 2-3mm of the comb should be below the level of the solution. Allow the gel to completely set before moving on.

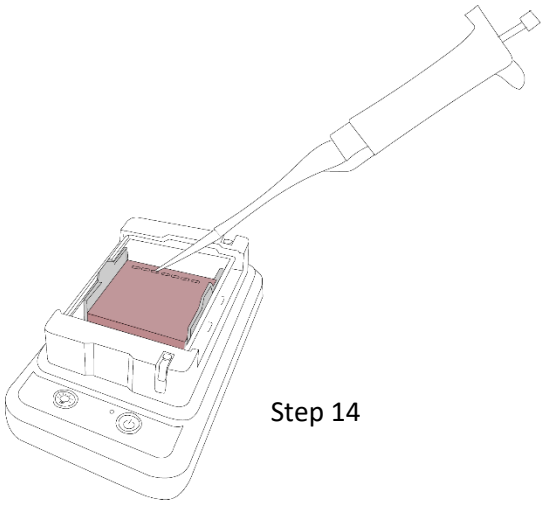
Step 9. Remove the gel tray from the casting platform. If a small amount of gel has formed underneath the gel tray, wipe it off and discard it. Note: you may store gels in a cool, dark place for up to 5 days. Keep the gel moist (in a resealable zip bag with paper towel saturated with water).

Step 10



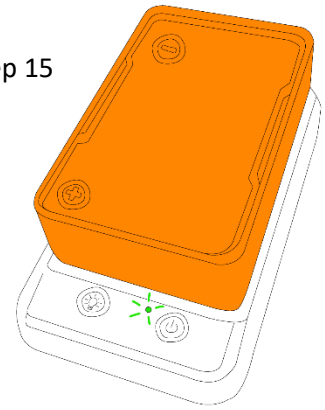


Step 11



Step 14

Step 15



Step 10. Place the gel tray containing a gel in the buffer chamber and place the buffer chamber inside the blueGel™ base. The wells should be closest to the (-) end.

Step 11. Add 30 ml of 1X TBE buffer in the buffer chamber. The buffer should just cover the agarose gel. CAUTION: Do not overfill the gel chamber as it may overflow when the cover is placed over the gel.

Step 12. Remove air bubbles (if any) trapped between the gel and the gel tray, or between the gel tray and the buffer chamber.

Step 13. If you have not already, remove the DNA tubes from the incubator and keep them upright. Into each of the five tubes, pipette 4 ul of loading/migration dye. Use a fresh pipette tip when adding dye to each tube. To rinse the pipette tip and mix the DNA and the dye, fill and unload the pipettor with the sample three times. The blue dye is used to monitor the migration of the DNA during electrophoresis.

Step 14. Load the DNA samples in the wells using a micropipette. 9-well combs hold up to 20 µl and 13-well combs hold up to 10 µl. Be careful not to puncture the gel with the micropipette tip.

Step 15. Place the orange cover on the blueGel™ base. The cover contains the electrodes and will only fit in one direction, with the (+) electrode positioned to attract the negatively charged DNA.

Step 16. Press the power button  to start the run. The green LED indicator located next to the power button should light up. Small bubbles will form near the electrodes.

SAFETY NOTE

For safety, the blueGel™ system's power will not turn on if:

a. The cover is not correctly placed on the base, and electrodes are not making contact
b. There is no buffer in the buffer chamber
c. Using the incorrect buffer (too diluted or too concentrated)

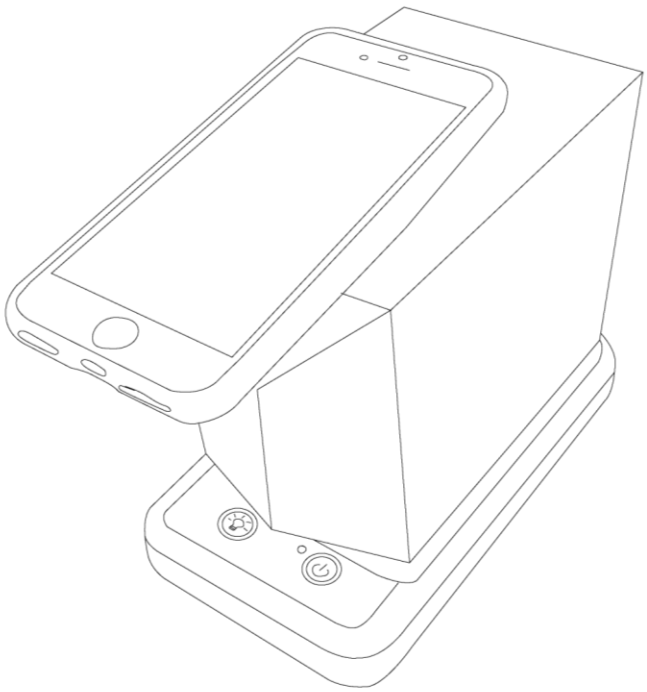
Step 17. At any time during the run press the lightbulb button to visualize the DNA. The orange cover filters the excess blue light allowing easier visualization of the fluorescence emitted by DNA.

TECH TIP

To document the run, turn on the blue light and take a picture with a smartphone, tablet or other camera device.

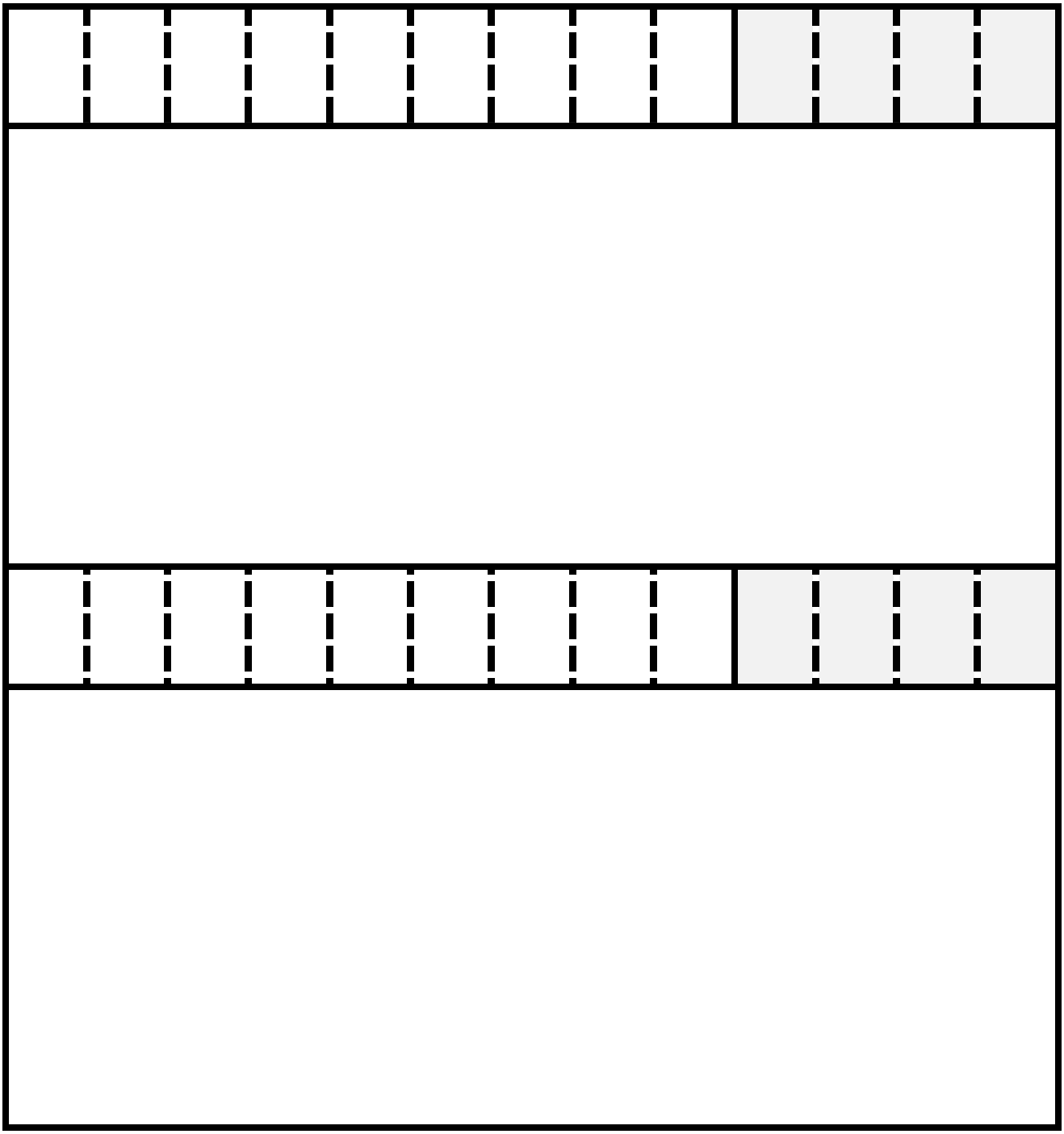
Tip: If DNA is not easily visible, dim or turn off ambient light. To document gels in bright ambient light, use the supplied Fold-a View™ photo documentation hood. Pop up the Fold-a-View™ following the instructions on its side and place it on the blueGel™ orange cover, sliding it down until it fits snugly around the cover's edges. Place your camera on top, and align the camera lens with the circular opening on the Fold-a-View™.

If needed, softly wipe condensation off the inside of the orange cover with the supplied lens cleaning cloth to improve visibility



LABEL & SKETCH YOUR GEL

Use the diagram below to label the contents of each well on your gel. Space for 2 rows have been provided – large combs create 9 wells, small combs create 13 wells each. You should also sketch the results (bands) of your run.



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Eric Hall, Program Coordinator
ethall@iastate.edu
(515) 294-5949