DNA Fingerprinting

ESTABLISH LINKS ● COLLECT EVIDENCE ● MAKE AN ARGUMENT

Biotechnology Outreach

TEACHER BOOKLET

featuring the
blueGel™ electrophoresis system
by miniPCR bio™

_____ _____

student name date

Updated 1/2024

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.

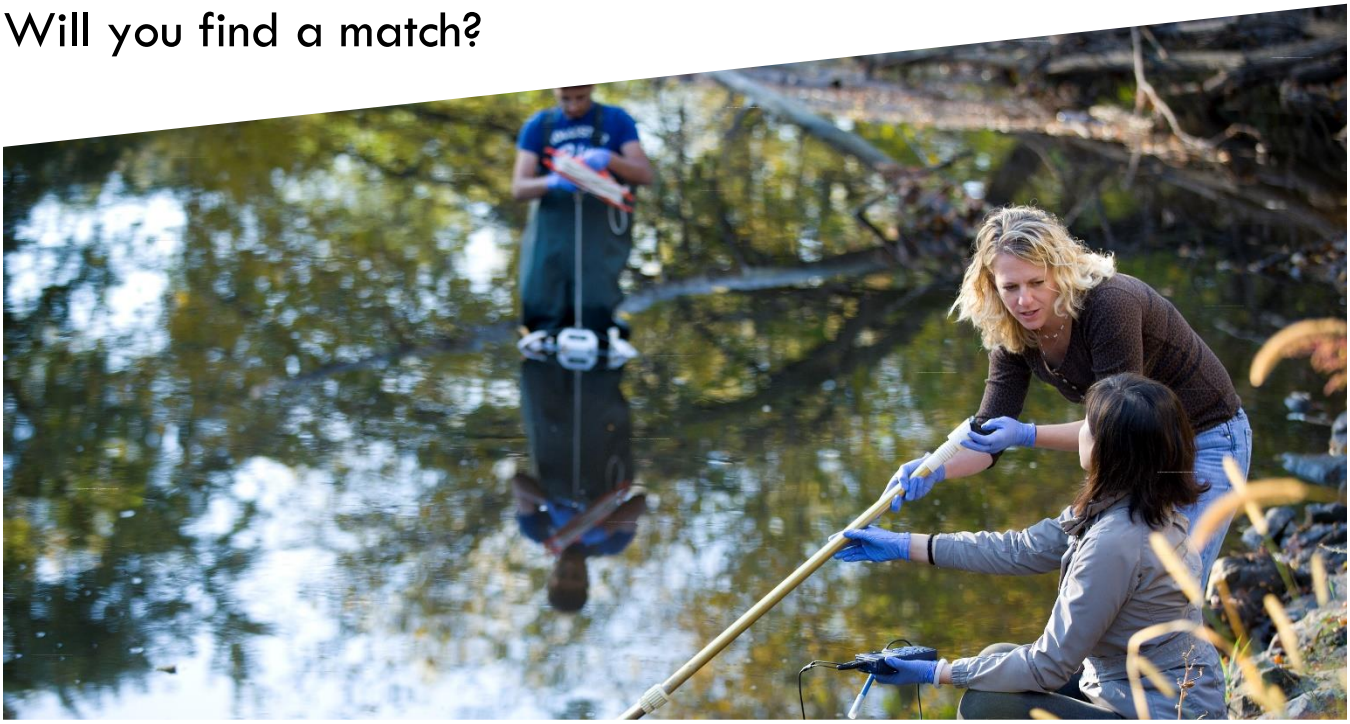
A MATCH GAME

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?



TEACHER PREP INSTRUCTIONS

BACKGROUND

DNA fingerprinting is a technique used in biotechnology laboratories to establish links between various samples of DNA. These samples would likely be taken from several different sources and compared to see if – or how well – they match. Scientists are able to determine relationships among the sources of DNA depending on the results of the "fingerprint" using gel electrophoresis.

DESCRIPTION

In this activity, students will prepare several DNA samples and analyze them using gel electrophoresis.

CONNECTIONS WITH STANDARDS

Teachers should consider these possible links with the Next Generation Science Standards (NGSS). Depending on how, when, and why this investigation is done there will likely be other meaningful connections with standards, too.

Science and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
Planning and Carrying Out Investigations • Plan an investigation or test a design individually and collaboratively to produce data to serve as the basis for evidence as part of building and revising models, supporting explanations for phenomena, or testing solutions to problems. (INV-H1, pages 8-9) Engaging in Argument from Evidence • Construct, use, and/or present an oral and written argument or counter-arguments based on data and evidence. (ARG-H4, pages 16-17)	LS3.A – Inheritance of Traits • Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. (page 66) PS1.A – Structure of Matter • The sub-atomic structural model and interactions between electric charges at the atomic scale can be used to explain the structure and interactions of matter. (page 38) PS2.B – Types of Interaction • Attraction and repulsion between electric charges at the atomic scale explain the structure, properties, and transformations of matter, as well as the contact forces between material objects. (page 44)	Energy and Matter: Flows, Cycles, and Conservation • Changes of energy and matter in a system can be described in terms of energy and matter flows into, out of, and within that system. (EM-H3, pages 30-31) Scale, Proportion, and Quantity • The significance of a phenomenon is dependent on the scale, proportion, and quantity at which it occurs. (SPQ-H1, pages 26-27)
In support of planning by teachers and PLCs, we have provided reference pages from The NSTA Atlas of the Three Dimensions by Ted Willard, available at NSTA.org		

GRADE LEVELS
High school

TIME REQUIRED
3 – 45-minute periods (see page 8 for recommended schedule)

ADDITIONAL SUPPLIES AVAILABLE*
Teachers may request a miniPCR blueGel electrophoresis kit from the BOEC. The kit contains (2) electrophoresis systems and (6) 20µL micropipettors, if needed. Visit https://boec.biotech.iastate.edu/lending/ for additional information.

PREPARATION OF THE STUDENT MATERIALS
The supplies can best be provided to the class in groups of five students. The DNA samples should be kept in the refrigerator until the class is set up. It is too expensive for the Office of Biotechnology to provide DNA for every student in a class. *The BOEC will provide enough DNA, restriction endonuclease, and reaction buffer for a minimum of two groups of five students or it will provide enough for one group of five students in every class section, whichever is greater.* For the remaining groups of students, use distilled water to replace the DNA, restriction endonuclease, and reaction buffer. Every group of students should be provided with the blue migration dye.

- 1 microcentrifuge tube (1.5 ml) containing 17ul of a 0.025 ug/ul concentration of pBR322 DNA and labeled "U" (unknown). The label should be written on the cap and body of the tube with a felt tip pen.
- 4 microcentrifuge tubes (1.5 ml), each containing 17ul of one of four different DNA samples. The tube labeled "A" should contain 0.15 ug/ul concentration of λ DNA, the tube labeled "B" should contain 0.075 ug/ul concentration of Ad-2 DNA , the tube labeled "C" should contain 0.025 ug/ul concentration of pBR322 DNA, and the tube labeled "D" should contain 0.025 ug/ul concentration of pUC19 DNA.
- 1 microcentrifuge tube (1.5 ml) containing 18ul of a mixture of 3ul Bgl 1 and 15ul of reaction buffer. The tube should be labeled "N".
- 1 microcentrifuge tube (1.5 ml) containing 40ul of blue migration/loading dye
- 1 2-20ul pipettor
- 10 sterile pipette tips of 200ul in an appropriate container
- 1 container to hold the used pipette tips
- 5 copies of the laboratory instructions. Each group should have a letter assigned to it in the upper right hand corner of the instruction sheet.

The teacher should have available for the entire class:

- blueGel electrophoresis system(s)
- An incubator at 37°C and rack to hold the microcentrifuge tubes of the students
- 1 Sharpie marking pen
- Enough small, medium, large, and extra large medical gloves for the students.

Supplies provided* by the Office of Biotechnology, Iowa State University
Teachers should request the following supplies at least two weeks in advance. Supplies should be ordered from: https://boec.biotech.iastate.edu/lending/

- Boxes of pipette tips (already autoclaved)
- 34µl of 0.025 µg/µl pBR322 per class section (minimum of 68µl per school)
- 17µl of 0.075 µg/µl Ad-2 DNA per class section (minimum of 34µl per school)
- 17µl of 0.15 µg/µl λ DNA per class section (minimum of 34µl per school)
- 17µl of 0.025 µg/µl pUC19 DNA per class section (minimum of 34µl per school)
- 3µl BgI 1 per class section (minimum of 6µl per school)
- 15µl Reaction buffer per class section (minimum of 30µl per school)
- SeeGreen gel tablet(s) (contains agarose, stain, buffers)
- TBE buffer (either powdered or liquid)
- Migration/loading dye for each group of 5 students
- Microcentrifuge tubes for each group of 5 students in all sections (autoclaved)
- A packing list with note to send the unused pipette tips/boxes, 10X TBE bottles, electrophoresis equipment, and micropipettors back to the Office of Biotechnology when finished, including a paid shipping return label (usually FedEx).

PLASMID DNA PREPARATION
It is best if medical or dishwashing gloves are worn when preparing supplies for the laboratory to prevent the teacher's fingers from contaminating the DNA samples. The DNA will not harm the teacher, but the teacher can harm the DNA.

The DNA samples are prepared from plasmid DNA provided at the appropriate concentration by the Office of Biotechnology. The DNA should be kept refrigerated, except when it is being used to prepare and conduct the laboratory.

The 17ul of the plasmid DNA should be put into sterile 1.5ml microcentrifuge tubes for the students. The tubes can be prepared up to 24 hours in advance and kept in a refrigerator until the class period. The number of tubes of each type for each group of five students is described above in the PREPARATION OF THE STUDENT MATERIALS.

RESTRICTION ENDONUCLEASE PREPARATION

The restriction endonuclease used for the laboratory is Bgl 1, commonly referred to as Bagel 1. The endonuclease and the reaction buffer can be prepared for the students up to 24 hours in advance and kept in a refrigerator until the class period. The mixture should be prepared in separate tubes for each group of students, i.e. a large amount should not be prepared and subdivided into different tubes. To obtain 18ul of the mixture for a group of five students, 3ul of Bgl 1 is added to a sterile 1.5 microcentrifuge tube, then 15ul of the reaction buffer is added to the tube. To rinse the pipette tip and mix the Bgl 1 and reaction buffer, fill and unload the pipette with the sample three times. The tube should be clearly labeled as "N".

MIGRATION DYE PREPARATION

A blue migration dye is used to monitor the movement of the DNA during electrophoresis. The migration dye is provided by the Office of Biotechnology ready for use. Each group of students should receive the dye, *even if they are given placebos of water* (instead of DNA, restriction endonuclease, and reaction buffer).

STUDENT INSTRUCTIONS

Step 1. Put on medical gloves and wear them throughout the experiment. The gloves will protect the DNA samples from contaminants 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 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 floating rack provided by the instructor and incubate them in a 37°C water bath 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.

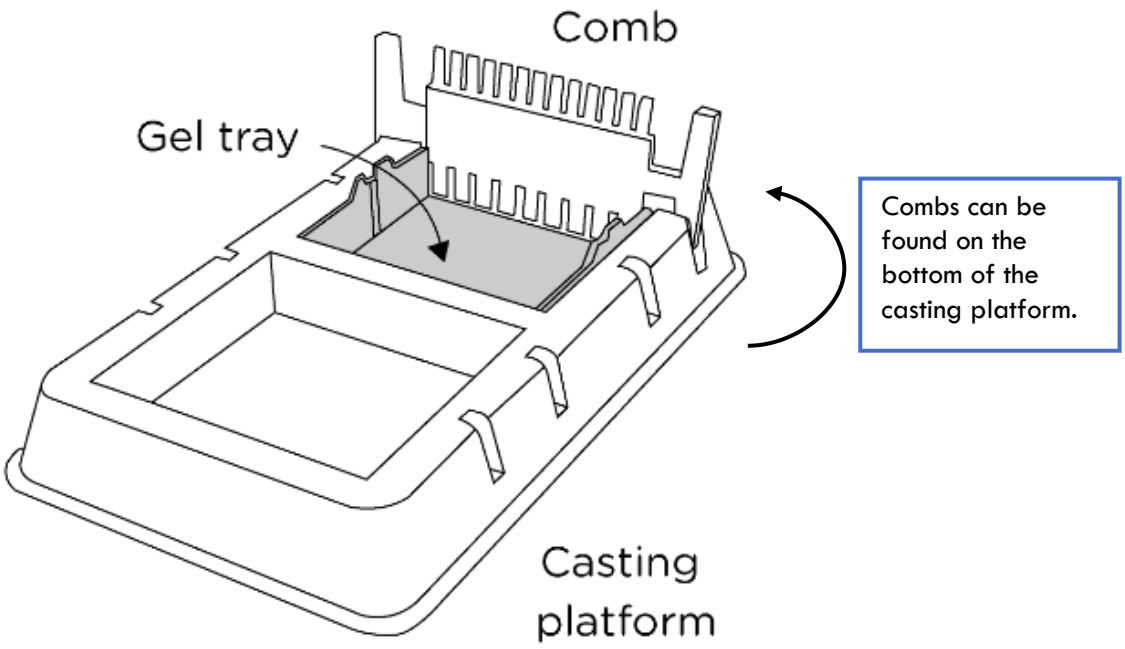
Step 4. Place the gel tray inside the casting platform and add combs from the bottom side of the tray (we will use the wider, 9-well comb). Place on a level surface to ensure uniform gel thickness. Determine the percentage gel to make; we recommend a 2.0% gel for this lab.

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 for about 3 minutes until the Tab is fully dissolved.

Step 7. Wearing heat-resistant gloves, heat the solution until it is clear and all particles are dissolved. To do this, microwave on high for 15 seconds, remove and swirl the flask, and then heat for another 15 seconds. Allow cooling to 60-70°C (or when the bottom of the flask is comfortable to touch). 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?

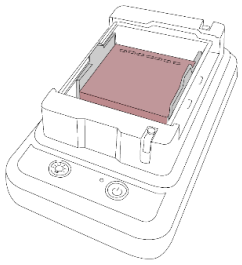
4) What limitations exist in this kind of investigation? Where might mistakes have been made?

INVESTIGATION NOTES

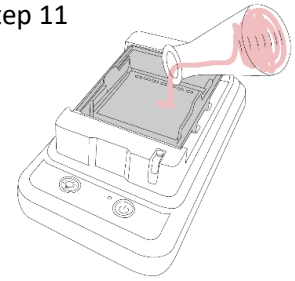
Step 8. Pour the solution into the gel tray to a depth of approximately 8mm; if using a 2.0% gel, this will be the whole solution. 2-3mm of the comb should be below the level of the solution. Allow the gel to set completely before moving on (approximately 20 minutes).

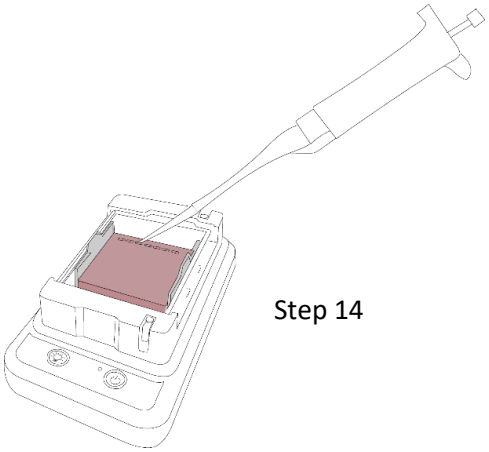
Step 9. Slowly remove the combs from the gel. Remove the gel tray from the casting platform. If a small amount of gel has formed underneath the gel tray, wipe it off and discard it. Note: you may store gels in a cool, dark place for up to 5 days. Keep the gel moist in a resealable zip bag with a paper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

Step 10



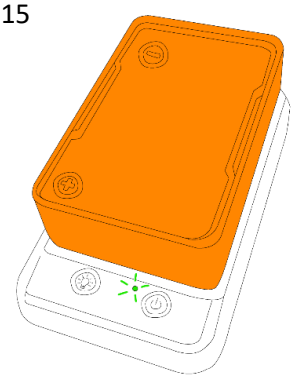
Step 11





Step 14

Step 15



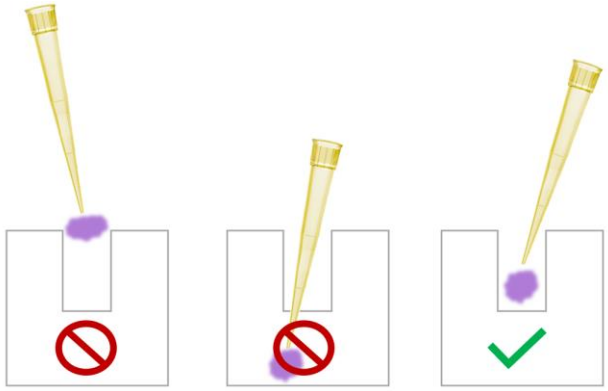
Step 10. Place the gel tray containing a gel in the buffer chamber and place the buffer chamber inside the blueGel™ base. The wells should be closest to the (-) end.

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. Record the contents of each well in the diagram on page 11.



Step 14

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. Run for approximately 35-50 minutes.

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)

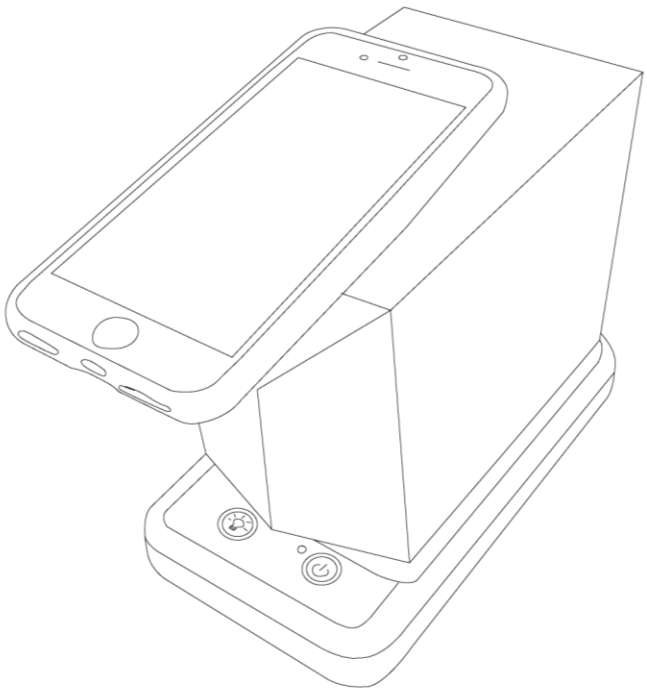
Step 17. . At any time during the run, press the lightbulb button to visualize the DNA. The orange cover filters the excess blue light allowing easier visualization of the fluorescence emitted by DNA. To protect the bulb, only turn the light on while you are viewing the gel.

TECH TIP

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

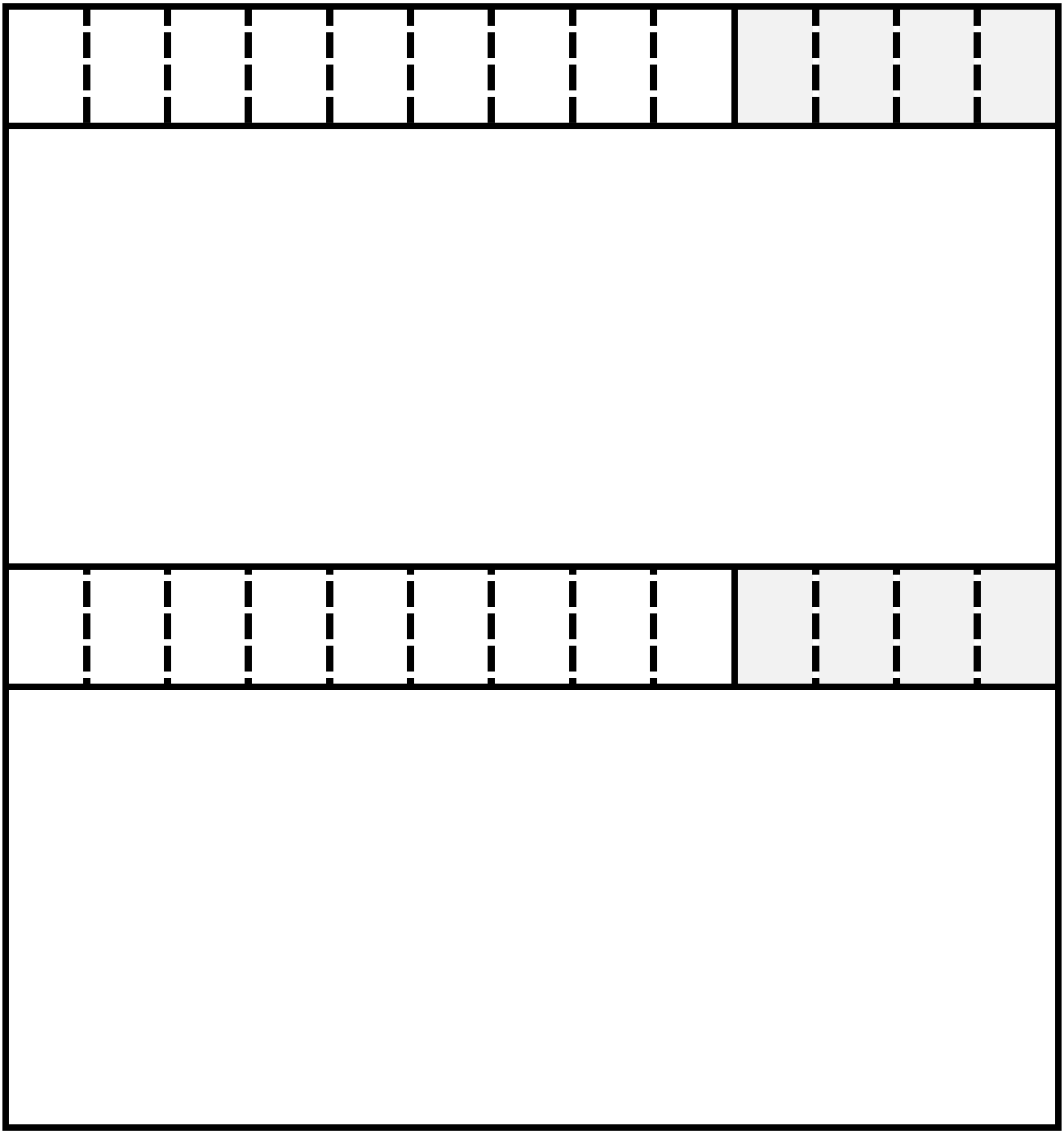
Tip: If DNA is not easily visible, dim or turn off ambient light. To document gels in bright ambient light, use the supplied Fold-a View™ photo documentation hood. Pop up the Fold-a-View™ following the instructions on its side and place it on the blueGel™ orange cover, sliding it down until it fits snugly 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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