DNA Fingerprinting

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 also request an electrophoresis kit from the BOEC. The kit contains (2) electrophoresis gel boxes with combs, (1) power supply, (6) 20µL micropipettors, and (4) plastic trays for staining. Use the form linked on page 3 to request kits.

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TEACHER PREPARATION AND INSTRUCTION GUIDE FOR USE WITH CAROLINA BLU STAIN

The preparation and conduct of the DNA fingerprinting laboratory is divided into the following sections: Preparation of the student materials, plasmid DNA preparation, restriction endonuclease preparation, migration dye preparation, and the preparation, loading and running of an agarose gel for use with Carolina Blu Stain.

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 DNAfor every student in a class. It 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 17 ul of a 0.025 ug/ul concentration of pBR322 DNA andlabeled "C". 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 17 ul of one of four different DNA samples. The tube labeled "1" should contain 0.15 ug/ul concentration of λ DNA, the tube labeled "2" should contain 0.075 ug/ul concentration of Ad-2 DNA, the tube labeled "3" should contain 0.025 ug/ul concentration of pBR322 DNA, and the tube labeled "4" should contain 0.025 ug/ulconcentration of pUC19 DNA.
- 1 microcentrifuge tube (1.5 ml) containing 18 ul of a mixture of 3 ul Bgl 1 and 15 ul of reaction buffer. The tube should be labeled "N".
- 1 microcentrifuge tube (1.5 ml) containing 40 ul of blue migration dye and labeled "D".
- 1-20 ul pipettor
- 10 sterile pipette tips of 200 ul 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:

- Electrophoresis gel
- Electrophoresis power supply
- 1-20 ul pipettor and 1 box of sterile 200 ul pipette tips for each gel box
- An incubator at 37°C and rack to hold the microcentrifuge tubes of the students
- 1 Sharpie marking pen
- 1 sheet of paper for each gel box that has numbered lines or boxes corresponding to the lanes on the gel
- (Optional) 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 by filling out this online form: https://forms.office.com/r/CBDnAyQg1J

- 6 boxes of pipette tips (already autoclaved)
- 34 µl of 0.025 µg/µl pBR322 per class section (minimum of 68 µl per school)
- 17 µl of 0.075 µg/µl Ad-2 DNA per class section (minimum of 34 µl per school)
- 17 µl of 0.15 µg/µl λ DNA per class section (minimum of 34 µl per school)
- 17 µl of 0.025 µg/µl pUC19 DNA per class section (minimum of 34 µl per school)
- 3 µl Bgl 1 per class section (minimum of 6 µl per school)
- 15 µl Reaction buffer per class section (minimum of 30 µl per school)
- 144 µl Carolina Blu Gel Stain per gel (minimum of 2 gels)
- 732 µl Carolina Blu Buffer Stain per gel (minimum of 2 gels)
- 1 bottle Carolina Blu DNA Stain
- 0.7 g Agarose per gel (minimum of 2 gels)
- 70 ml 10X TBE per gel (minimum of 2 gels)
- 40 µl blue migration dye for each group of 5 students in all sections.
- 8 Microcentrifuge tubes for each group of 5 students in all sections (autoclaved)
- Fingerprinting instructions
- A note to send the unused pipette tips/boxes, 10X TBE bottles, Carolina Blu DNA Stain, and Styrofoam container back to the Office of Biotechnology when finished, including a paid shipping return label (usually FedEx).

**free supplies are available only to those teachers who have successfully completed the corresponding BOEC workshop*

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 canharm the DNA.

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

The 17 ul of the plasmid DNA should be put into sterile 1.5 ml 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 oftubes 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 18 ul of the mixture for a groupof five students, 3 ul of Bgl 1 is added to a sterile 1.5 microcentrifuge tube, then 15 ul 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 migrationdye 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.

PREPARATION, LOADING, AND RUNNING OF AN AGAROSE GEL FOR USE WITH CAROLINA BLUSTAIN

The gel can be prepared up to 2 days in advance of the period in which the DNA is loaded into it. If prepared in advance, 1) place the electrophoresis box in the refrigerator with the cover in place or 2) the casting tray and gel can be removed from the electrophoresis box, sealed in a plastic bag, and stored in a refrigerator.

The following description applies to the 12 cm wide x 14 cm long electrophoresis unit provided by the Office of Biotechnology, Iowa State University. The general procedures would be the same for any gel apparatus, except for the volumes of gel and buffer that are used.

1) Put on a pair of medical or dishwashing gloves and wear them throughout the procedure, including during the laboratory and the clean-up. None of the chemicals used are toxic, but the gloves provideprotection for persons who may have sensitive skin.

2) The electrophoresis box will come completely assembled. To prepare the box for casting a gel, followthe directions below.
    a. To remove the lid from the box, face the box with the electrode plugs pointing to the back, and place fingers on each end of the unit while pressing thumbs against the front edge of the lid. Push thumbsagainst the lid toward the rear to disconnect the power supply leads from the plugs. Lift lid to remove from system.

b. Remove the gel tray by grasping each side and lifting at an angle to ease the tray out of the system (See figure 1). Rinse the combs, gel tray and gel box in distilled water to remove any residue. It is not necessary to dry the pieces.

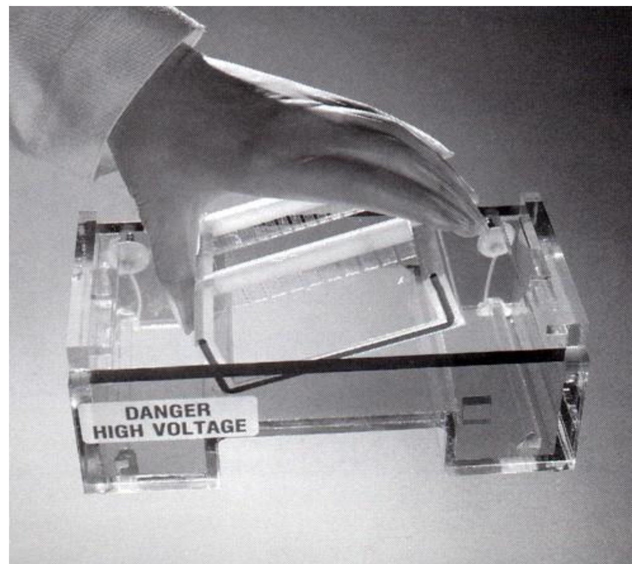
c. Replace the gel tray in the system by carefully lowering the tray at an angle so the gaskets fit againstthe front and rear of the gel box wall (See figure 1). This should provide an efficient seal that prevents leakage of the warm agarose when poured into the tray. If the gasket has slipped out of the groove, push it back in place before lowering the tray into the box.

3) Prepare 700 ml of 1X TBE electrophoresis buffer by diluting 70 ml of 10X TBE stock solution with 630 ml of distilled water.

4) Weigh 0.7 g of agarose and pour into a 250 ml flask or beaker containing 45 ml of 1X TBE electrophoresis buffer prepared in step 3. Swirl the agarose suspension to disperse the powder.

5) Put on a heat-resistant glove. Microwave the suspension until it boils (about 30 seconds to 1 minute), swirl the flask, and alternate boiling and swirling at 15 second intervals until the solution has boiled a total of 1 minute or until there are no visible solids.

Figure 1



6) Cool the agarose solution by adding 45 ml of the 1X TBE buffer from step 3, bringing the volume inthe container to 90 ml. Swirl the solution gently to avoid trapping air bubbles.

7) Add 144 µl of Carolina Blu Gel Stain to the agarose solution. The Gel Stain will be provided from the Office of Biotechnology in a microcentrifuge tube. To get all the stain out of the tube, rinse the tube with 1X TBE buffer or distilled water and pour it in the agarose solution. Swirl the agarose solution gently until it has a uniform light blue color.

8) Slowly pour the agarose solution into the gel tray taking care not to allow formation of any bubbles within the gel. If bubbles form, tap them with a finger until they disappear.

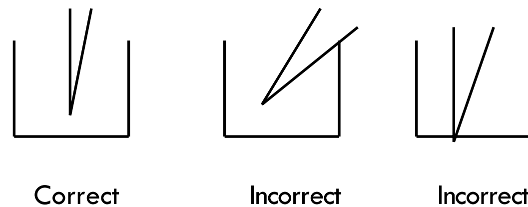
9) Rinse the flask or beaker immediately with plenty of tap water to prevent the agarose from hardening init or the sink.

10) Immediately place a comb(s) of choice into the tray slot, attempting to avoid air bubble formation in the wells. Allow the gel to sit at room temperature for about 30 minutes until solid (gel will appear slightly milky).

11) After the gel is set, remove the comb(s). To remove a comb, grasp both ends of the comb and gently lift straight up with a slight back and forth rocking motion. To orient the gel tray in the running position, grasp the sides of the tray and gently lift at an angle (See figure 1). Rotate the tray 90 degrees to position the open ends toward the platinum electrodes. DNA is negatively charged and will migrate toward the positive (red) pole during electrophoresis. The wells of the gel should be nearest the negative electrode (black) end of the electrophoresis box. Carefully lower the tray into position, and secure the tray between the gel tray tabs. To verify if the gel tray is properly oriented,place the lid loosely on the box. The wells should be nearest the negative electrode (black).

12) Load the DNA samples by placing the pipette tip into the top of the well and slowly releasing the solution into the well (See figure 2). The pipettor can be kept steady by holding the barrel like a pool stick and leaning on the gel box. Put the tip into the well as vertically as possible. Do not gotoo deep into the well to avoid puncturing the gel.

Figure 2



Correct Incorrect Incorrect

13) After the students have loaded their samples into the wells, add 732 µl of Carolina Blu Buffer Stain to the 610 ml of 1X TBE buffer that remained after step 6. The Buffer Stain will be provided from the Office of Biotechnology in a microcentrifuge tube. To get all the stain out of the tube, rinse the tubewith distilled water and pour it into the buffer. Swirl the buffer until it is a uniform light blue color.

14) Slowly fill the gel box with all the 1X TBE electrophoresis buffer to cover the gel to about a 2mm depth. **Do not pour the buffer directly on the gel.**

15) Make sure the switch on the power supply is in the "Off" position before connecting the electrophoresischamber. When ready for electrophoresis, place the lid tightly on the chamber and plug the electrical leads into the recessed output jacks of the power supply. Plug the red (+) lead into the red jack, and the black (-) lead into the black jack.

16) For operation of the power supply, follow the instructions provided with it.

17) Select the desired voltage on the power supply. A voltage of 150 will permit the electrophoresis run tobe completed in about an hour. Lower voltages also can be used. The lower the voltage, the slower the DNA will migrate. For example, at a voltage of 10 the electrophoresis run will be completed in about 24 hours. The band of migration dye marks the leading edge of the DNA. The electrophoresisis complete when the leading edge of the dye has migrated 5 to 6 cm from the wells.

18) Proceed with electrophoresis: Check to be sure the blue migration dye is moving toward the positiveelectrode (red). If it is migrating toward the negative electrode (black), turn off the power supply, remove the lid, lift out the gel tray, turn it 180˚, and repeat steps 16 and 17. **CAUTION: Never remove the electrophoresis chamber lid while the power supply is on.**

19) When electrophoresis is completed, turn off the power supply.

20) To remove the lid from the box, face the box with the electrode plugs pointing to the back, and place fingers on each end of the unit while pressing thumbs against the front edge of the lid. Push thumbsagainst the lid toward the rear to disconnect the power supply leads from the plugs. Lift the lid to remove it from the box.

21) After the electrophoresis is complete, some DNA bands will be visible. To darken the bands and makemore of them visible, remove the gel tray from the electrophoresis unit and place the tray into a plastic container. Slide the gel off of the tray by pushing on one end of it. Add the Carolina Blu DNA Stain, making sure the gel is completely immersed. **Do pour the stain directly on the gel.**

22) Stain the gel for 15 minutes. Agitate gently, if possible.

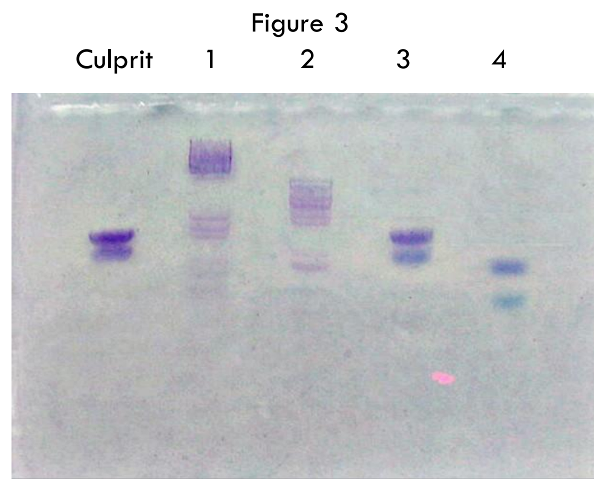
23) Pour the stain back into the bottle. The stain can be reused 6-8 times.

24) Cover the gel with distilled water to destain. (*Tap water contains chloride ions that can partially remove the stain from the DNA bands and give inferior results.)* Agitate gently, if possible. During the 30 to 40 minutes of destaining, change the water every 10 minutes, if possible. During the destaining process, the bands of DNA will become clearer as the stain is removed from the remainder of the gel. It is possible to destain the gel for up to 24 hours. If the DNA bands becometoo light, the gel can be stained and destained again by repeating steps 21 through 24.

25) The gel can be displayed to the class by sliding it on to a piece of plexiglass and placing it on a whitesheet of paper or on a white-light viewing box. Figure 3 illustrates the expected results. Dog 3 is the culprit.

Figure 3

Culprit 1 2 3 4



26) The gel can be saved for at least a month in a refrigerator by sliding it into a clear plastic bag andsealing the bag. The gel can be viewed on a white sheet of paper or a white-light viewing box without removing it from the bag.

27) None of the chemicals used in the experiment are toxic. Solutions can be poured down a conventionaldrain. The gel can be disposed of with other trash.

FITTING THE DNA FINGERPRINTING EXPERIMENT INTO 45-MINUTE PERIODS

PERIOD 1: Teach how to use the pipettor and make the agarose gel.

- *After the gel is made, the teacher has two options:*
  - *Place the electrophoresis box in the refrigerator with the cover in place until period 2.*
  - *The casting tray and gel can be removed from the electrophoresis box, sealed in a plastic bag, and stored in a refrigerator until period 2. The gel can be kept in the refrigerator for up to 2 days before it is used.*

PERIOD 2: Remove the container with the gel from the refrigerator. Conduct steps 1 (optional) through 6 of the student instructions. In step 3, the incubation at 37˚C can be done for up to 45 minutes, if desired by the teacher. If the incubated DNA is not going to be loaded by the students into the gel immediately, it can be stored in a refrigerator until the migration dye is added and the gel is loaded.

- *After the DNA is loaded into the gel by the students, the teacher has three options:*
  - *Carry out the electrophoresis.*
  - *If students in another section are to load DNA into the same gel on the same day, place thecover on the electrophoresis box and store it in a refrigerator until the next class period. After the gel is loaded by the last section, add the electrophoresis buffer and carry out the electrophoresis.*
  - *If another gel is to be loaded on the same day by another class section, place the cover on theelectrophoresis box with the loaded gel and store it in a refrigerator until both gels can be run. After both gels are ready, add the electrophoresis buffer and carry out the electrophoresis.*

- *After the electrophoresis is completed, there are two options for staining:*
  - *Stain the gel immediately and place it in the refrigerator, as described in step 26 of the instructions.*
  - *Pour enough of the electrophoresis buffer out of the electrophoresis box so that the gel is notimmersed in it. Place the cover on the box and store it in the refrigerator for up to a day until it is convenient to stain it.*

PERIOD 3: Stain the gel if it has not already been done, view the gel and discuss the results.

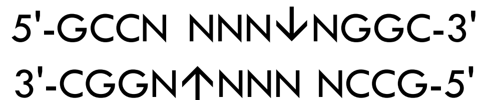
DNA FINGERPRINTING STUDENT INSTRUCTIONS **Group letter: _____**

A farmer owned four dogs. One of the dogs chewed on her new pair of boots, which made the farmer unhappy. She wanted to pen up the culprit, but did not know which dog had done it. Fortunately, the culprit had left some strands of hair on the boot. The farmer put the hair in a plastic bag and labeled it "Hair of the culprit". She took hair from each of the four dogs and labeled the samples "Dog 1", "Dog 2", "Dog 3", and "Dog 4". She took the samples to Iowa State University and asked a scientist to determine which dog had chewed up her boots. The scientist extracted DNA from the sample of hair labeled "Hair of the culprit" and labeled it "C". The scientist extracted DNA from samples of hair from each dog and labeled them with the dog number. The teacher is providing you and your colleagues with the samples and wants you to determine which dog was the culprit. Eachgroup of up to five students will have samples to analyze together.

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

Step 2. Your group has a sample of the culprit DNA in a 1.5 ml microcentrifuge tube labeled C and samples from each of the four dogs in tubes labeled with the dog number. 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 toeach 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 and written in the upper right-hand corner of this instruction sheet.

Step 3. Place the tubes in a rack provided by the instructor and incubate them at 37˚C for 10 minutes. *Bgl 1 is isolated from the bacteria Bacillus globigi. 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.*



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

Step 4. Remove the tubes from the incubator and keep them upright. Into each of the five tubes, pipette 4 µl ofblue dye from the tube labeled D. 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 5. Go to the electrophoresis box and record the identity of your samples before loading them on the gel. The gel has lanes on which individual samples will be run, therefore, there are numbered lines on the sheet of paper. Record on the sheet the identity of the sample that corresponds to the lane into which the sample will be loaded.

Step 6. Using a pipettor set for 20 µl, transfer your sample into the well of the appropriate lane in the gel. Place the top of the pipette tip into the top of the well and dispense the 20 µl of solution into it slowly. Do not let the pipette tip touch the bottom of the well because it will puncture the gel. Discard the pipette tip in the designated container after a sample has been put in the well and use a new one for the next sample.

The gel will be run by the instructor.

Step 7. After the gel is run, the bands on it will be viewed. From the DNA patterns on the gel, determine which of the four dogs chewed up the boots.