Solving "The Phyto-Forensics Case" using paper PCR

(Adapted from year 2000 document of woodrow.org.)

The Objectives:

1. To become familiar with the process of PCR
2. To conduct PCR with one DNA sample (from Food/Biofuel Plant or the seed 'X') and simulate the steps involved in making multiple copies of the DNA fragment
3. To use PCR to isolate a specific gene and amplify it
4. To compare the PCR products (amplified DNA fragments) by simulating gel electrophoresis
5. To identify what DNA was obtained from suspect location

"The Crime"

Bonan Fuji (BF) and Terri Fernandes (TF) are farmers who grow their crops in almost neighbouring fields. BF, who is college educated and believes in environment protection uses innovative plant breeding methods to address fuel problems through agriculture. He planted a non-sweet sorghum that contains high levels of syrup that is not fit for human consumption but is a best source for producing Bio-Fuels. BF had followed all regulations for planting his crop, to legally separate it from his neighbours. He used male-sterile lines so that his sorghum wouldn't breed with other varities. BF keeps his fertile seeds in a secure bag inside the barn close to his house.

TF on the other hand grows only Traditional Food sorghum. His crop yields have not been promising in recent years and he saw his neighbour selling harvest at great prices to a company he didn't recognize. TF decides to steal seeds from his neigbour not knowing that such sorghum can be toxic to consumers. He breaks the lock and takes away the bag of Bio-Fuel sorghum seeds. On returning home that evening BF discovers the break ins and immediately suspects TF for the theft due to history of their encounters.

At the investigation agency, Mario (the geneticist) gets a statement from BF about the theft and specially notes BF's concerns that Bio-Fuel sorghum shouldn't get cross-breed with Traditional-Food sorghum that TF intends to do. With consumer safety on stake Mario knew there was only a small chance of recovering concrete evidence, but it would take a miracle at this point to avoid the disaster.

After the search warrant Mario's big break arrived: He did find a burnt out bag and a container with seeds in TF's old barn; however, TF claimed that those were from his own crop of last year. Mario is expert in phyto-forensic science and criminology, and recognized this opportunity to use the latest DNA fingerprinting technology: PCR and gel electrophoresis to identfy the suspicious seeds (seeds-'X') in the container .

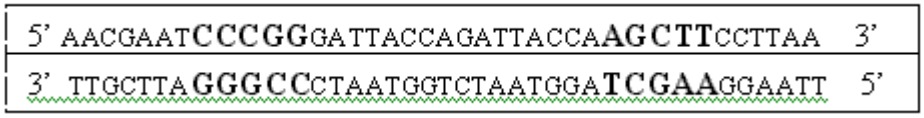
You are Mario's assistant, and your mission is to use this technology to uncover whether the seeds in the container belonged to BF or TF. This information is vital, and is needed immediately for Judge O'Sullivan to issue the verdict. Since you are new to the crime lab, Mario is going to explain how PCR works.

PCR Paper Simulation

Background

Polymerase Chain Reaction (PCR) and gel electrophoresis are very important tools used by biologists to study organisms at the genetic level. PCR is the process by which a very small quantity of DNA is amplified (multiplied) into literally millions of copies. During PCR, only specific sections of the DNA are amplified. With millions of copies of a specific gene, scientists are able to distinguish one individual's DNA from another's. To isolate and amplify a specific gene or segment of DNA, PRIMERS must be added to the sample. The PRIMERS are designed to isolate the specific gene we wish to amplify. PRIMERS are sequences of DNA bases that are complementary to the sequences of DNA bases on either side of the gene (or segment) you want to isolate. The gene we want to isolate in the example below is the section of DNA between the bold pieces. We will isolate the segment by designing PRIMERS that are complementary to the sections of DNA just beyond the gene we want (in bold).

Original Strand of DNA



Gene of Interest

Single Stranded Primers

Materials (Per group of students)

- A handout that contains an original DNA strand, specific primers, and extension DNA strips.
- Scissors
- Tape

Procedure

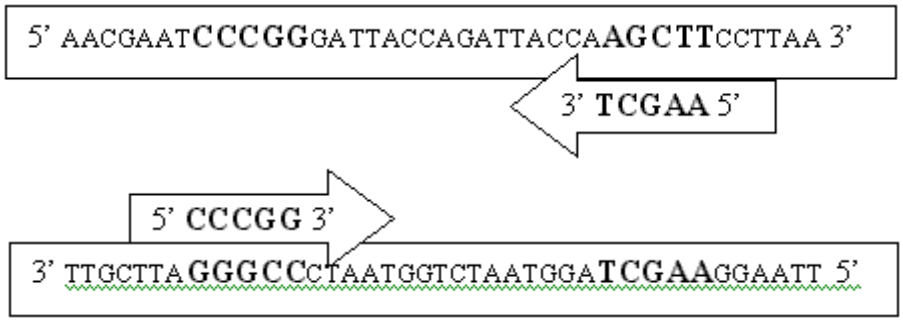
Each student group will complete four cycles of PCR with their DNA sample. Each cycle consists of three steps.

Step1: This step is called DENATURATION. *In this step, the thermal cycler raises the DNA to a high temperature (usually 95° C), causing the two strands of DNA to separate.*

- Simulate denaturation by separating (cutting) your double-stranded DNA, creating two strands of single stranded DNA.

Step 2: This step is called HYBRIDIZATION. In this step, the primers attach to the complementary bases of the strands created during denaturation. This happens at a lower temperature (usually around 54° C). The primers bind to the DNA strands before the strands can bind back to each other. Notice that the primers attach to the complementary base sequences even though they may not be at the end of the strand.

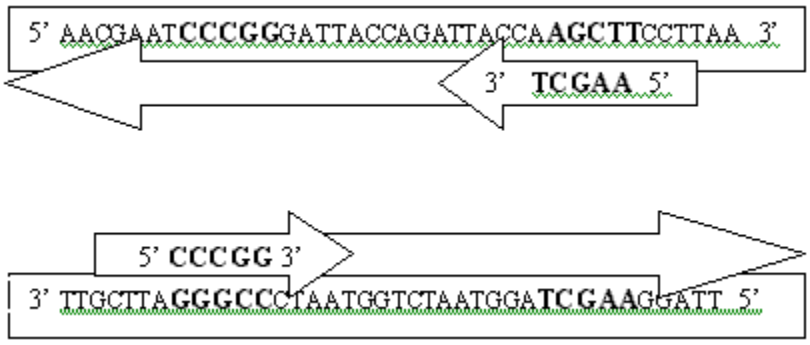
- Cut out your primers.
- Match the complementary bases of a 5' to 3' primer to a 3' to 5' DNA strand.
- Repeat this step for ALL DNA strands from denaturation (step 1).



Step 3: This step is called DNA Synthesis. The complementary nature of the DNA bases allows us to construct a new double stranded DNA molecule from a single strand. When the primers were added to the DNA sample, we also added additional bases (A's, T's, C's, G's) and an enzyme called DNA polymerase. When the temperature is raised to around 72° C, the DNA polymerase (Taq polymerase) facilitates (catalyzes) the attachment of the new bases to the primer. Because of the molecular structure of the DNA bases, new DNA bases can only be added to the 3' end of the primer. Therefore, the DNA polymerase extends the primer creating the second strand of DNA.

- Cut out a blank DNA strip and attach it to the primer's 3' end.
- Tape the three pieces together.
- Write in the complementary base pairs. Make sure to "add" DNA bases in the direction of the arrow (starting at the primer's 3' end)

- Complete the steps for all hybridized strands (strands from step 2).



What you have now is the product of one cycle.

Notice how not all of the original DNA strand has been copied. Only the portion extending from the primer has been copied. It is in this way that we will control which part of an individual's DNA is copied. The primers have been designed to isolate the gene we want to copy. Since the DNA polymerase will only start adding new bases to the end of the primer, we will not copy all of an individual's DNA. As the thermal cycler goes through more cycles of denaturation, hybridization and DNA synthesis, only the specific gene we want will be copied. Eventually, after approximately 30-40 cycles, the majority of DNA stands present will be the specific gene for which we were looking. (Note: The number of cycles needed may vary)

Table 1	**# of Double Stranded DNA Fragments**
Beginning	
After Cycle 1	
After Cycle 2	
After Cycle 3	
After Cycle 4	

- Count the TOTAL number of DOUBLE-stranded DNA molecules from this cycle. Fill in data table 1 for cycle 1.
- Now take your products from the end of step 3 and go back to step 1. Go through the steps again (with ALL of your DNA molecules) until you have completed 4 cycles of PCR.
- Continue to record your data in table 1.

You are now ready to simulate gel electrophoresis using your products from PCR.

Gel Electrophoresis Paper Simulation

Background

Gel electrophoresis is a technique that is used to separate DNA fragments based on their size (number of bases). In this exercise, you will separate, by size, the DNA strands made during the PCR simulation. Because DNA is a negatively charged molecule, it will always move toward the positive end of the gel electrophoresis box (red electrode). We remember this by the phrase "**R**un toward the **R**ed." However, because the DNA strands are of different lengths (sizes), they will move at different speeds. The agarose gel acts as a type of sieve through which the smaller pieces of DNA can move more easily. Therefore, the shorter strands will move faster and the longer strands will move slower.

Materials

- Poster Board and Gel template
- Markers
- Tape
- DNA strands from the PCR Activity

Procedure

- Group together students who have amplified DNA strands from seeds-X, BF plants and TF plants.
- Each group should use the poster board and markers to create an agarose gel just like on the template.

Step 1: Loading the gel wells. DNA is a colorless material; therefore, we must stain it. In order to run it through the gel, the DNA strands must be loaded into a cavity, or well, in the agarose gel (the agarose jell looks like clear Jell-O). This is done using an instrument called a micropipetter, which transfers very small quantities of liquid in units known as microliters (mL). The DNA isolated from seeds-X, BF plants and TF plants will have separate wells.

- Each group should place their DNA strands from seeds-X, BF plants and TF plants into the corresponding agarose well.

Step 2: Running the gel. When an electrical charge is created across the gel, the DNA strands move toward the positive end. The strands will run in a straight line from their well toward the positive charge. They move at different speeds depending upon their size. Strands of the same size will travel the same distance and create a visible stripe (band) in their lane. Therefore, the group of DNA strands for X-seeds, will create a pattern of bands unique to Bio-Fuel plants and distinct from Traditional-Food plants.

- Arrange your DNA strands on the gel according to their size. Use the standards on the left side of the gel as a guide for the distance each size will travel after 30 minutes.
- Strands of the same size should be stacked on top of each other.
- Tape the strands at the appropriate place on the gel.

Step 3: Analyzing the gel. Compare the patterns created by the different DNA groups and note your findings.

- Analyze your gel and answer the gel electrophoresis analysis questions.

PCR Analysis Questions

1. Using Table 1, what is the pattern you observe in the number of DNA molecules (double stranded) after each cycle of PCR? Express this with a mathematical formula.

2. PCR usually runs for 40 cycles. Based on your formula in #1, how many single stranded DNA molecules would you have at the end of 40 cycles if you started with just one copy of the double stranded gene?

3. Based on your understanding of the simulation, why do scientists use PCR (the "real power" of PCR)? List three circumstances where this would be extremely helpful.

Gel Electrophoresis Analysis Questions

1. Which length of DNA would produce the darkest band on the gel (after staining)? Consult with your classmates and find out what the darkest band would be for the other samples of DNA. Why is this band the darkest?

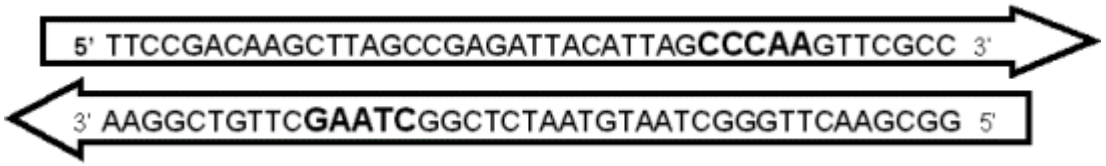
2. Based on the evidence from the agarose gel, whose seeds are in the conatiner?

3. Would you recommend to the judge to issue arrest orders and penalty for TF? Why or why not?

Choose any ONE of the following three DNAs for your group;

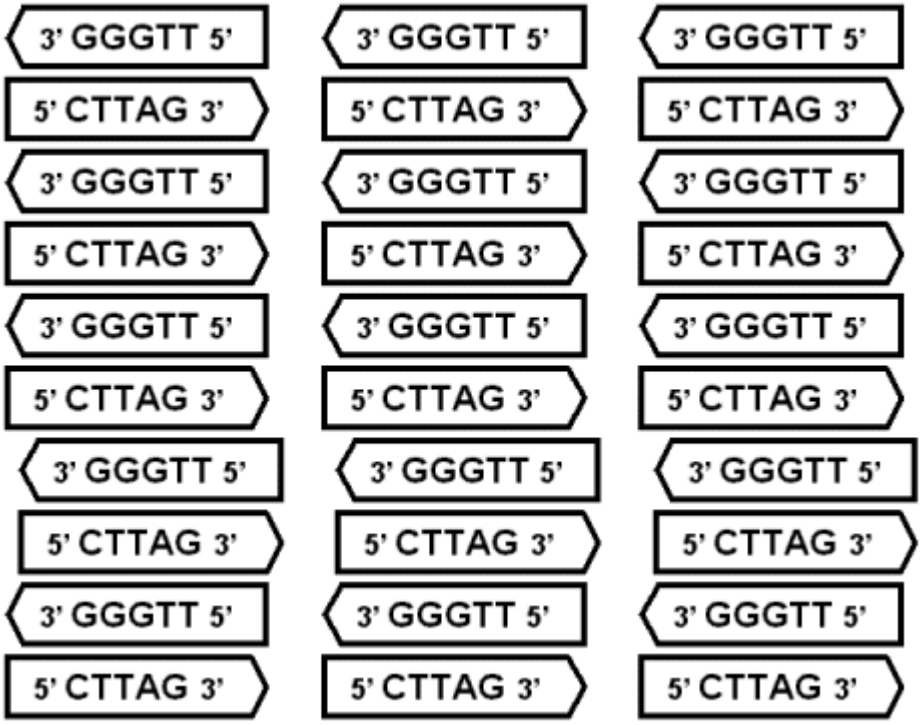
DNA from Bio-Fuel Plants

DNA from Traditional-Food Plants

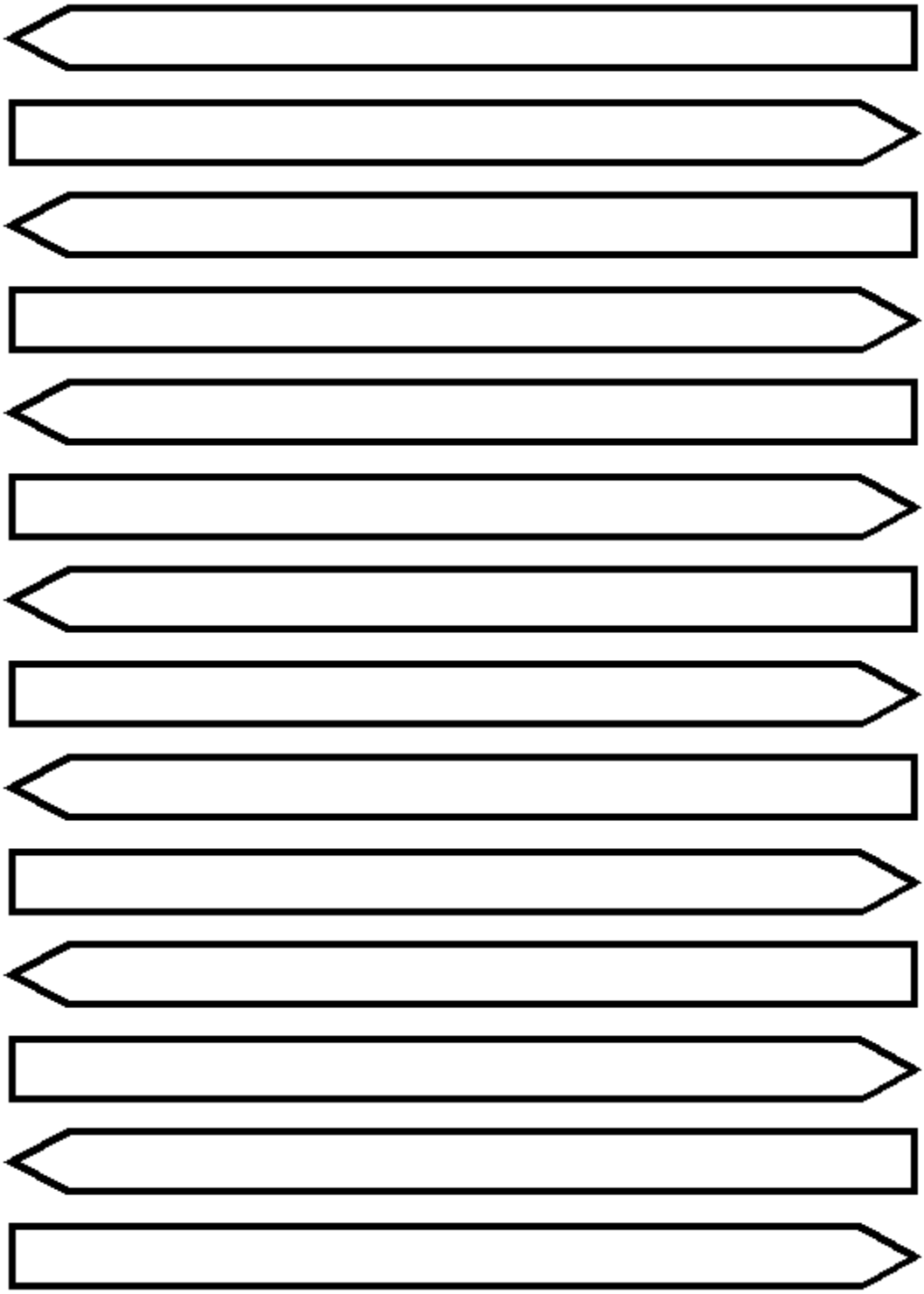


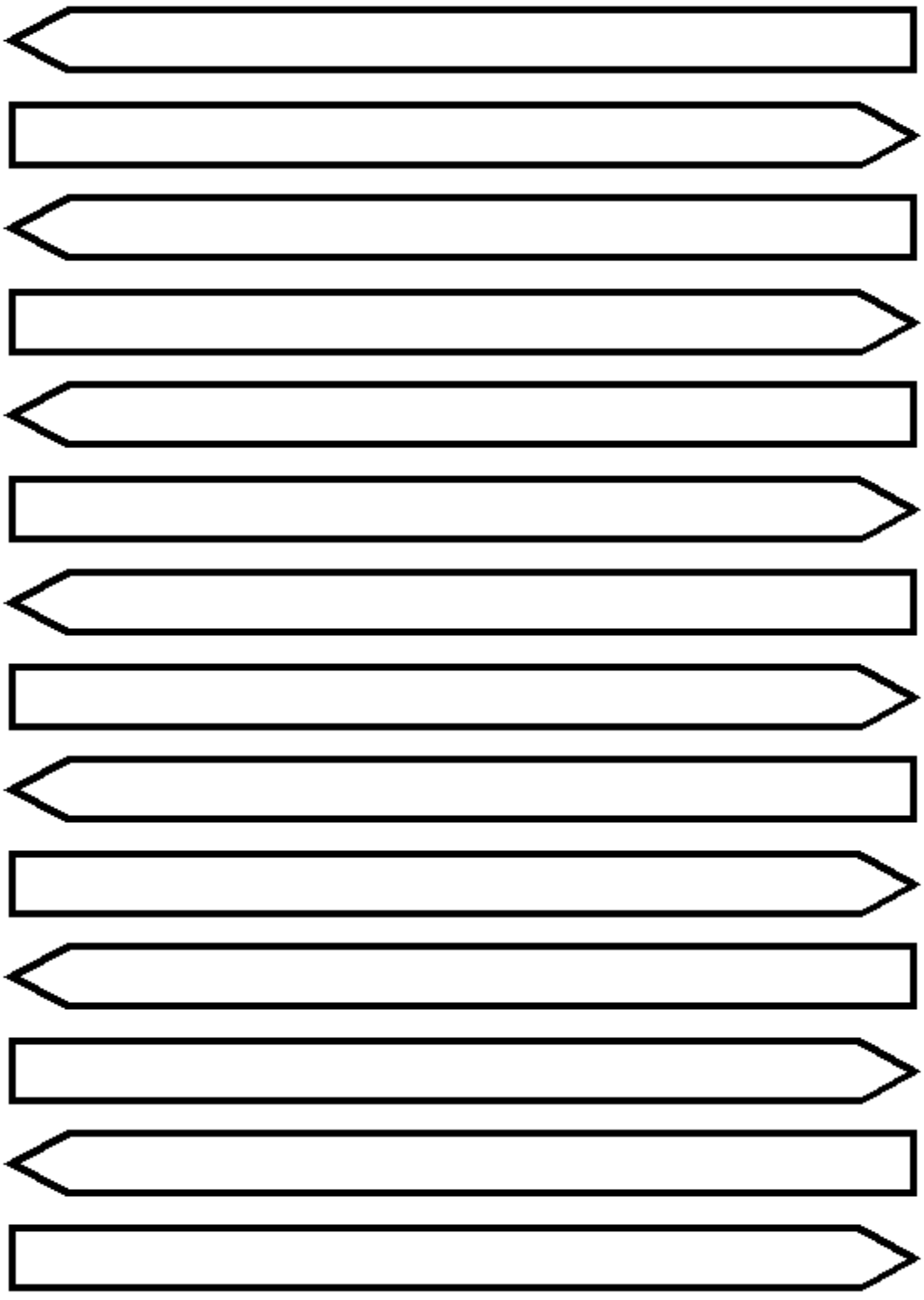
DNA from Seeds of Unknown (X) Source

Use following Primers for the four PCR cycles to amplify the DNA your group has chosen;



During four PCR amplification cycles use following blank DNA strips to amplify DNA your group has chosen;





Electrophoresis Gel

- Directions: Enlarge this diagram to poster size so that DNA sequences can be sorted by size.
- Note: bp stands for number of base pairs