Solving "The Phyto-Forensics Case" using DNA Fingerprints

(Adapted from year 2000 document of woodrow.org while using Discovering DNA Ltd MDNA-STR-408 (molymod ®/miniDNA®, Spicing Enterprises Limited, UK) puzzle pieces.

The Objectives:

1. To become familiar with the process of PCR (Polymerase Chain reaction).
2. To conduct PCR with one DNA sample (from Food/Biofuel Plant or the unknown seed source) 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 neighboring 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 neighbors. He used male-sterile lines so that his sorghum wouldn't breed with other varieties. 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 neighbor selling harvest at great prices to a company he didn't recognize. TF decides to steal seeds from his neighbor 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 forensics geneticist) gets a statement from BF about the theft and specially notes BF's concerns that Bio-Fuel (BF) sorghum shouldn't get crossbreed with Traditional-Food (TF) 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.

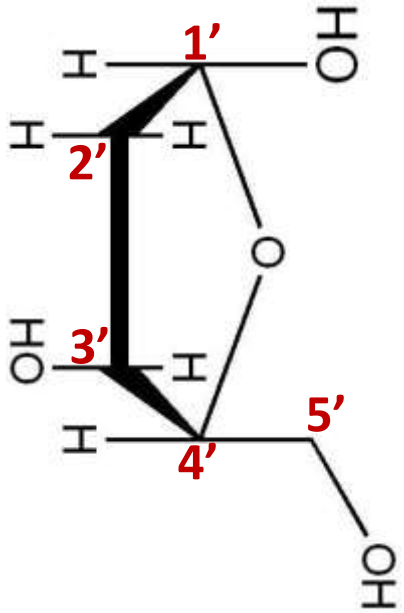
After the search warrant Mario's big break arrived: He did find a burnt-out bag and a container with seeds in TF's old barn; however, TF claimed that those were from his own crop of last year. Mario is an expert in phyto-forensic science and criminology to recognize this opportunity to use the latest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

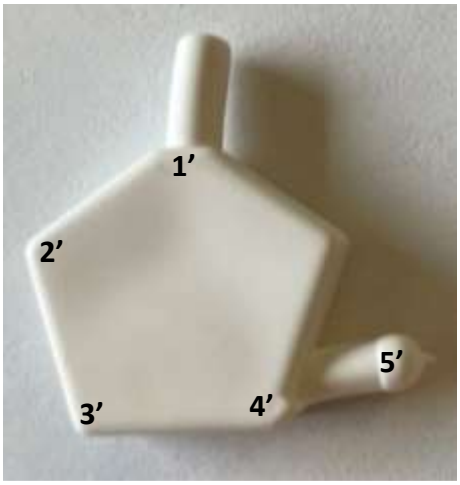
You are Mario's assistant, and your mission is to use this technology to uncover whether the seeds in the container have origin being BF (Biofuel Sorghum) or TF (Food Plant). This information is vital and is needed immediately for Judge O'Sullivan to issue the verdict. Since you are new to the crime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

PCR Simulation

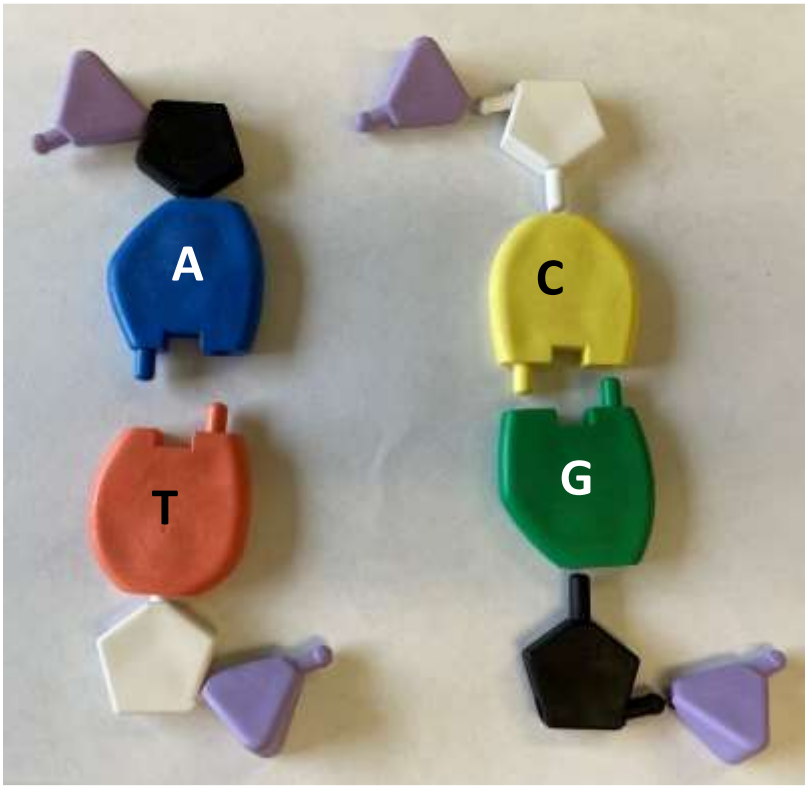
Background

Discovering DNA Ltd. MDNA-STR-408 (molymod®/miniDNA®, Spicing Enterprises Limited, UK) puzzle pieces explains the structure of hereditary molecule well. Basic unit (monomer) of a DNA strand is nucleotide comprise of a sugar (black or white), a phosphate (purple) and a base (yellow, green, blue or orange). Please note the different sugar colors are included to make it easier to differentiate the primer and template DNA-strands and they do not represent different chemicals. When assembling a nucleotide through puzzle pieces, attach the purple phosphate to the black/white sugar by pushing the bent knob from the sugar into the hole in the purple phosphate. Make sure you add the sugar to the 5' prime end as shown, so the knob still sticks out of the purple phosphate not out of the sugar. Push the colored base (green, orange, blue or yellow) onto the straight knob on the sugar so that the final two protruding knobs (from phosphate and base) on an assembled nucleotide, are on same sides.



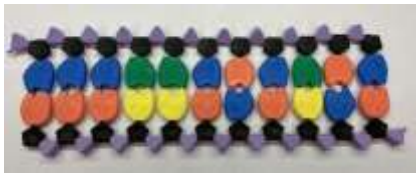


Deoxy Ribose (Sugar)



Base Pair (Complimentary Nucleotides)

The joining (ligation from 5' to 3') of nucleotides through covalent bonds yield single stranded DNA molecule. The hydrogen bonds between the complementary nucleotides (A-T and C-G) joins two single stranded DNA molecules yielding a DNA double helix structure. The unit of this DNA double helix structure is called base pair (bp) which has two complementary nucleotides (A-T and C-G) joined through hydrogen bonds between them. When using the sugar (black), phosphate (purple) and base (yellow, green, blue or orange) pieces of Discovering DNA Ltd. MDNA-STR-408 to make a double stranded DNA helix structures, following two kinds would result for this exercise:



5' - A AAG GATA GTA - 3'
3' - T TTC CTAT CAT - 5'

5' - CA GAT GTT TC - 3'
3'- GT CTA CAA AG - 5'

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 target is the section of DNA that will be isolated segment by designing PRIMERS complementary to the flanking sequence of the gene we want.

Primers are single stranded DNA molecules that are needed to initiate PCR process. For this exercise students need to use the sugar (two white for flanking nucleotides and one black for the middle nucleotide), phosphate (purple) and base (yellow, green, blue or orange) pieces of Discovering DNA Ltd. MDNA-STR-408 to make 3-nucleotide long single stranded DNA molecules. This activity will be using three (3) R-primers with *AAC* sequence (flanking *A* and *C* have white sugar and middle *A* has black sugar) and three (3) R-primers with *TAC* sequence (flanking *T* and *C* have white sugar and middle *A* has black sugar), as well as three (3) F-primers with *GAT* sequence (flanking *G* and *T* have white sugar and middle *A* has black sugar) and three (3) F-primers with *AAG* sequence (flanking *A* and *G* have white sugar and middle *A* has black sugar).

Students need to assemble all 12 single stranded primers using the DNA puzzle pieces provided. Following four kinds (x3) of primers would result for this exercise:

[F-Primer] 5' -AAG -3'

[R-Primer] 5' - TAC - 3'

[R-Primer] 5' -AAC -3'

[F-Primer] 5' - GAT - 3'

Procedure

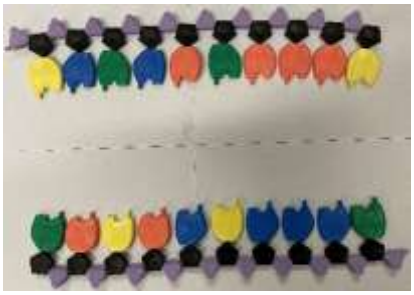
The Discovering DNA Ltd. MDNA-STR-408 Kit has following contents:

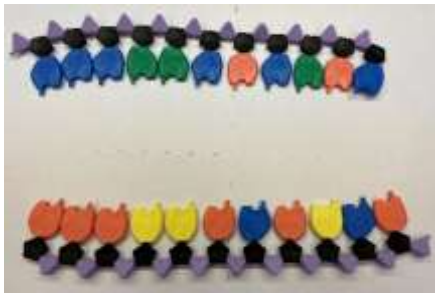
		C, G, A, and T are embossed on the edge of the base	
Cytosine (Yellow)	22		
Guanine (Green)	22	White Sugars	24
Adenine (Blue)	50	Black Sugars	112
Thymine (Orange)	40	Phosphate (Purple)	136

Students should look for two genes, i.e. and the amelogenin and the D16S539 that allows the determination of DNA sample origin. The Biofuel (BF) Sorghum chromosome has a 6 base pair deletion in amelogenin while the Traditional Food (TF) Sorghum chromosome has D16S539 gene with only one short tandem repeat sequence (GATA), so both give different size DNA bands on an electrophoresis gel after PCR amplification. Each student group will complete two cycles of PCR with their assigned DNA sample. Each PCR cycle consists of three steps.

PCR 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 the single-strands of your double-stranded DNA model, creating two templates of single stranded DNA as shown below for both kinds of scenarios:





PCR Step 2: This step is called HYBRIDIZATION or ANNEALING. 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 these templates 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 template strand.

- Anneal single stranded primers to the complementary bases of template - one primer per strand.
- 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).
- Following is how the two kinds of DNA-models would appear after annealing:
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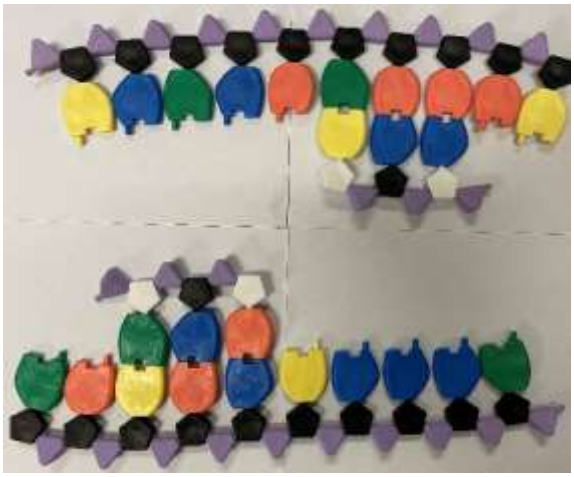
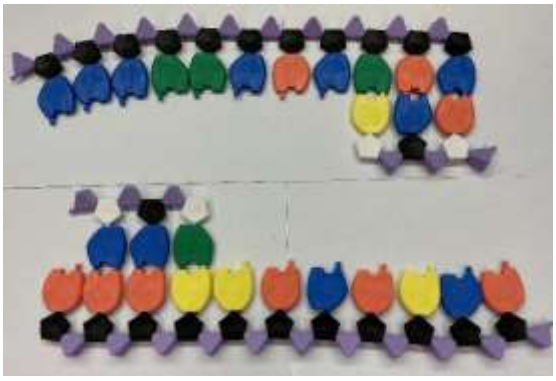


- 

PCR Step 3: This final step is called DNA Synthesis (extension or polymerization). The complementary nature of the DNA bases allows us to construct a new double stranded DNA molecule from a single strand. When the primers are added to the DNA samples for PCR, simultaneously additional bases (A's, T's, C's, G's) and an enzyme called DNA polymerase are also added. When the temperature is raised to around 72° C, the DNA polymerase (Taq polymerase) facilitates (catalyzes) the attachment of the 5'-ends 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.

- Start by adding complementary nucleotide at the primer's 3' end.
- Next complementary nucleotide should be added so that your DNA synthesis direction is from 5' to 3'.
- By using parts of DNA-puzzle pieces, make complete model of double stranded PCR product as per the length of the template.

Notice how not all of the original DNA strand has been copied. Only the portion extending from the primer has been copied. Since the DNA polymerase will only start adding new bases to the end of the primer, we will not copy all of a sample's DNA. The primers have been designed to isolate the genetic marker we want to copy. It is in this way that we will control which part of a sample's DNA (genetic marker) is copied.

- As the thermal cycler goes through further cycles of denaturation, hybridization and DNA synthesis; only the specific genetic marker we want will be replicated. After completing three PCR cycles, resulting DNA would appear like as one of the models shown below:

Eventually, after approximately 30-40 cycles, the majority of DNA stands present will represent the specific genetic marker which we were looking for.

- Count the TOTAL number of DOUBLE-stranded DNA molecules from first 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 two cycles of PCR.
- Continue to record your data in table 1, also fill anticipated numbers for cycle 3 and 4.

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

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 base pairs). 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 as electrophoresis Gel material
- Markers
- Tape
- DNA Products (assembled puzzle pieces) from the PCR Activity

Procedure

- Group students together who have amplified DNA strands from unknown seeds-XS, 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 with DNA that 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 gel looks like clear Jell-O). This is done using an instrument called a micropipette, which transfers very small quantities of liquid in units known as microliters (µL). The DNA isolated from unknown seeds-XS, BF plants and TF plants will have separate wells.

- Each group should place their DNA strands from unknown seeds-XS, 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 XS (unknown 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 DNA products 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 Amplified DNA Markers

1. Samples from Traditional Food (TF), Biofuel Sorghum (BF) or the seed-X gave three different DNA molecules (double stranded) and after PCR, portion of DNA molecule (called marker) is amplified.

2. You can identify three DNAs on your page (next) based on sequence of nucleotides (A, C, G and/or T). The DNA-marker for each sequence is the amplifiable region that is between the nucleotides complimentary to primers for double stranded molecules

3. Based on your counting of the As, Cs, Gs & Ts in-between the bold nucleotides, identify the size (6 or 10 bp) of DNA marker from seed samples (TF, BF or XS).

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?

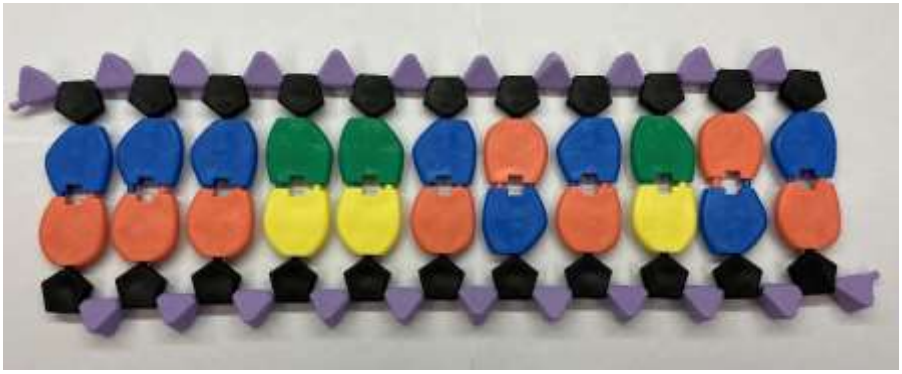
2. Based on the evidence from the agarose gel, whose seeds are in the container?

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;

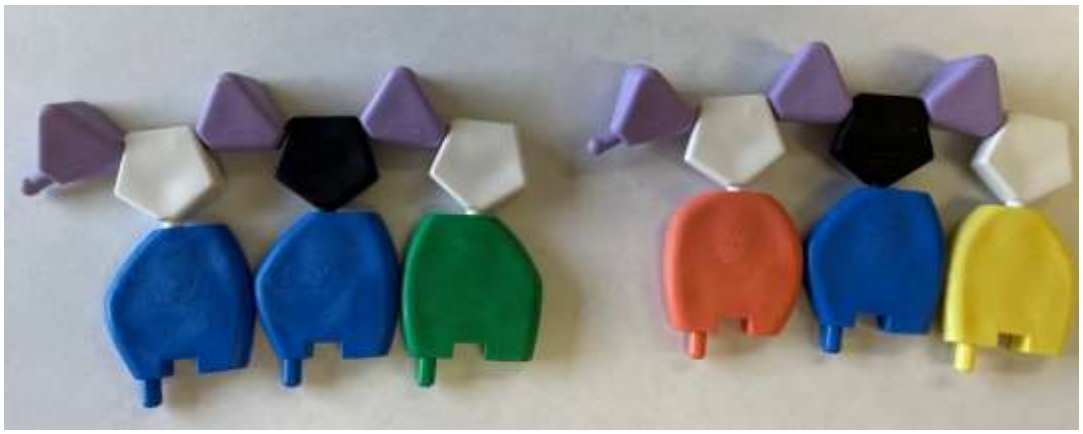
DNA from Bio-Fuel (BF) Plants

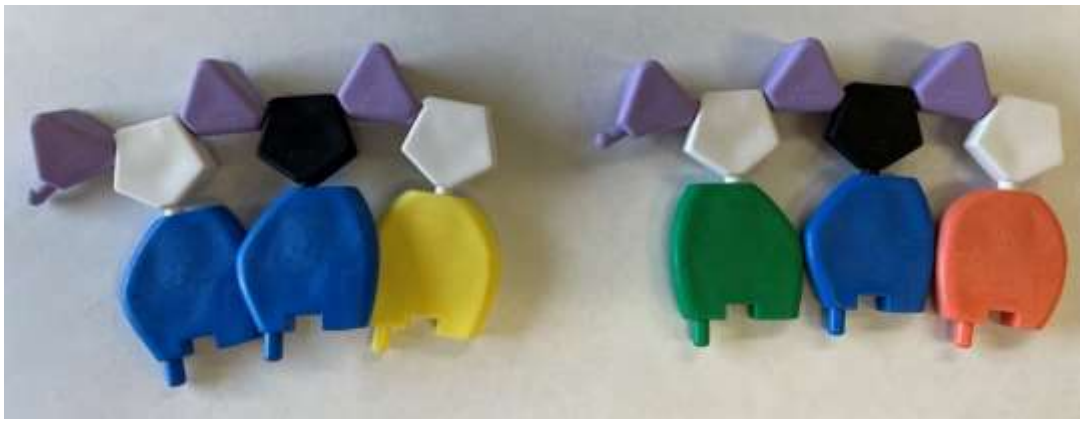
DNA from Traditional-Food (TF) Plants



DNA from Seeds of Unknown Source (XS)

Use one of the following Primer Pairs for the PCR cycles to amplify the 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