**Genetic markers-based DNA detective activity to solve phyto-forensics case**

**Plant genetics tools used to resolve farmers’ proprietary disputes**

(Grades 9th-12th)

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| **Introduction**: Pull key information from wiki <https://en.wikipedia.org/wiki/DNA_profiling> **Video:** <https://www.youtube.com/watch?v=tpPkmDeS3Dg> **Hands On**: (A) <https://www.fybikon.no/file/andre/06061_dna-fingerprintingbyggesett_molymod.pdf> (B) Solving “The Phyto-Forensics Case” using DNA Fingerprints <https://www.tnstate.edu/tsuaged/PhytoForensics%20Class%20Activity.pdf> |

 What is DNA Marker’s Detection by its Duplication?

### Purpose

Students will become familiar with the process of PCR (Polymerase Chain reaction) and simulate the steps involved in making multiple copies of the targeted DNA fragment. Activities include conducting PCR with a DNA sample either from Food or Biofuel Plant as well as the unknown seed source.

### Essential Files (maps, charts, pictures, or documents)

* [DNA Fingerprinting](https://www.genome.gov/genetics-glossary/DNA-Fingerprinting#:~:text=DNA%20fingerprinting%20is%20a%20laboratory,evidence%20came%20from%20that%20suspect)

* [Explore PCR and Electrophoresis](https://www.brightstorm.com/science/biology/molecular-biology/pcr-dna-fingerprinting)
* [Three PCR Steps](https://www.tnstate.edu/tsuaged/Three%20PCR%20Cycles%206%20and%2010%20bp.pdf)

### Vocabulary

**DNA** **Template:** DNA strand portion used during replication to attach complementary bases as per its sequence for creating a growing nucleic acid.

**Primer:** A short nucleic acid sequence that complimentarily hydrogen bonds with beginning end of DNA template and provides a starting point for DNA synthesis.

**Taq Polymerase:** A large hot-spring bacterium (**T**hermus **aq**uaticus) enzyme that facilitates DNA replication for a new strand synthesis as per sequence of template.

**Extending Strand:** A DNA polymer synthesized by joining the 5'-carbon of a new nucleotide (A, C, G & T) to the 3'-carbon of last nucleotide of existing strand.

**5' to 3' direction:** The template DNA sequence is used to attach complementary primer and other bases, for synthesizing new strand in 5' to 3' direction.

**Polymerase Chain Reaction** (**PCR):** A cycle is composed of three temperature regimes for melting template (950C), annealing primers (540C) and extending new strand (720C).

**PCR Cycle:** DNA melts (two strands separate @950C), then primers anneal to template (~540C) and finally polymerase extends primer (720C) by adding nucleotides (5' to 3').

**Amplification Product:** Two new DNA strands synthesized from original separated (melted) strands cause doubling the number of DNA molecules after each PCR cycle.

### Background/Agricultural Connections

This lesson is the middle one in a series of related topics to provide students basics of phytoforensics and molecular science concepts. Polymerase Chain Reaction (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 can distinguish one individual’s DNA from another’s. To isolate and amplify a specific gene or segment of DNA, the PRIMERS (single stranded DNA molecules that are needed to initiate PCR process and are designed to isolate the specific gene for amplification) must be added to the sample. PRIMERS are sequences of DNA bases that are complementary to the sequences of DNA bases on either side of the gene segment targeted, also called genetic marker. 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 of the PRIMERS. The DENATURATION or MELTING step causes the two strands of DNA to separate at high temperature (usually 95° C). Then in HYBRIDIZATION or ANNEALING, the primers attach (Hydrogen bonds) to the complementary bases of the strands (template) created during denaturation at a lower temperature (usually around 54° C). Finally, through DNA Synthesis (EXTENSION or POLYMERIZATION), the DNA polymerase (Taq enzyme) facilitates (catalyzes) the attachment of the new nucleotides (around 72° C) to the primer and complementary nature of the DNA bases allows to construct a new double stranded DNA molecule from single strand template.

### Interest Approach – Engagement

1. Ask students if they know what in vivo DNA replication is and introduce them to the process of PCR for DNA fingerprinting technology in identifying BioFuel (BF), Traditional Food (TF) sorghum and suspicious sample (Unknown Seeds-'XS'). The semi conservative DNA replication in organisms ensures that genetic information is transferred from one generation to other. The same process is repeated in the laboratory to make several copies of the DNA fragments known as genetic markers, which can be used as biological evidence in any proprietary related legal issues.
2. Watch the [DNA-Fingerprinting](https://youtu.be/AkBUriMK9u8) and PCR video clip, [Markers’ Amplification](https://www.youtube.com/watch?v=Je3xO8e-MvQ).
3. Inform your students they will:
	* Explore sequences of two genes; the amelogenin and D16S539 (short tandem repeat), allowing determination of unknown DNA sample origin
	* Learn Biofuel (BF) sorghum has a 6 base pair deletion in amelogenin gene
	* Research that the Traditional Food (TF) sorghum has D16S539 gene with only one short tandem repeat sequence (GATA)
	* Use online resources to identify amelogenin and D16S539 gene sequences giving different size DNA products after PCR amplifications
	* Each group conducts three cycles of PCR amplifications with the MDNA-STR-408 pieces to generate specific DNA-product to solve forensics case

### Procedures

**Materials**

**For the class:**

* Discovering DNA Ltd. MDNA-STR-408 (molymod®/miniDNA®, Spicing Enterprises Limited, UK) kit (two boxes and instruction booklet)
* Internet access for research (this part may be done at home for homework)
* Instructions on the “Solving the Phyto-Forensics Case” activity

**For BioFuel (BF) sorghum group** **(DNA-2):**

* Cytosine (C- Yellow) pieces (C embossed on edge of the base)- 7
* Guanine (G- Green) pieces (G embossed on edge of the base)- 7
* Adenine (A- Blue) pieces (A embossed the edge of the base)- 10
* Thymine (T- Orange) pieces (T embossed the edge of the base)- 12
* Deoxyribose Sugar (White) puzzle pieces- 8
* Deoxyribose Sugar (Black) puzzle pieces- 32
* Phosphate (Purple) puzzle pieces- 36

**For Traditional Food (TF) sorghum group (DNA-1):**

* Cytosine (C- Yellow) pieces (C embossed on edge of the base)- 6
* Guanine (G- Green) pieces (G embossed the edge of the base)- 6
* Adenine (A- Blue) pieces (A embossed the edge of the base)- 15
* Thymine (T- Orange) pieces (T embossed the edge of the base)- 16
* Deoxyribose Sugar (White) puzzle pieces- 8
* Deoxyribose Sugar (Black) puzzle pieces- 40
* Phosphate (Purple) puzzle pieces- 44

**For suspicious sample (Unknown Seeds-'XS') group (DNA-2):**

* Cytosine (C- Yellow) pieces (C embossed on edge of the base)- 7
* Guanine (G- Green) pieces (G embossed the edge of the base)- 7
* Adenine (A- Blue) pieces (A embossed the edge of the base)- 10
* Thymine (T- Orange) pieces (T embossed the edge of the base)- 12
* Deoxyribose Sugar (White) puzzle pieces- 8
* Deoxyribose Sugar (Black) puzzle pieces- 32
* Phosphate (Purple) puzzle pieces- 36

**For each student:**

* Pencil for Lab drawings
* Lab worksheet/ Drawing paper

**Preparation**

* Before the lesson, confirm all the contents of Discovering DNA Ltd. MDNA-STR-408 (molymod®/miniDNA®, Spicing Enterprises Limited, UK) kit have been restored in two boxes after previous activity.
* Per respective assigned forensics’ sample, distribute on benches for each the three students’ groups, A (Adenine, Blue), T (Thymine, Orange), C (Cytosine, Yellow) and G (Guanine, Green) puzzle pieces along with that for Deoxyribose Sugar (Black and White) as well as for Phosphate (Purple).
* Check for each the three students’ groups per their assigned forensics’ sample, that they know one of the Two DNA sequences to amplify;
	1. 5' - A AAG GATA GTA - 3’ 2) 5' - CA GAT GTT TC - 3’

3' - T TTC CTAT CAT - 5’ 3’ - GT CTA CAA AG - 5’

* For yielding cycles of denaturation, hybridization and DNA synthesis; only the specific genetic marker we want will be replicated using either of the DNA primer pairs below;
	1. 5' - AAG - 3’ 2) 5' - GAT - 3’

 3' - CAT - 5’ 3’ - CAA - 5’

* After completing three PCR cycles, resulting amplification products would appear like as one of the two DNA sequences below;
	1. 5' - AAG GATA GTA - 3’ 2) 5' - GAT GTT - 3’

3' - TTC CTAT CAT - 5’ 3’ - CTA CAA - 5’

**Hands-On Activity:**

1. Tell students that in this lab they will be duplicating DNA markers from three samples for resolving plant identification case in farmers’ proprietary dispute.
2. Ask students if they know that in all living cells, the DNA double helix is duplicated by a mechanism called semiconservative replication. Thus, replication occurs separately on each of the parent strand in antiparallel directions while each copy is conserving the information from one half of the original DNA molecule.
3. Inform students that per semiconservative DNA replication in lab (in vitro), each parent strand of the DNA double helix acts as a template for the synthesis of a new, complementary or daughter strand.
4. Tell students that they will be assembling DNA molecules per three cycles of denaturation, hybridization and extension of nucleic acid that will be used in DNA fingerprinting. Demonstrate to the students how to conduct cycles of semiconservative DNA replication through pieces of Discovering DNA Ltd. MDNA-STR-408 model sequentially from each of the three steps;
	* In the first step of DENATURATION the two strands of DNA double helix originally assembled are separated (hydrogen bonds broken) from each other to act as single-stranded templates for next step.
	* In the HYBRIDIZATION or ANNEALING step, the single-stranded primers are assembled and then attached (hydrogen bonding established) to the complementary bases (A-T and C-G) of the two single-stranded templates created during denaturation.
	* For the final step of DNA Synthesis (extension or polymerization), complementary nature of the DNA bases (A-T and C-G) is used to complete a new double stranded DNA structure. Thus, the complementary nucleotide bases per two opposite single stranded DNA templates are added beyond the primers.
5. Instruct students that they will conduct three replications while disassembling other single-stranded DNAs except the selected templates that will be used in the next cycle. They can draw the information on semiconservative DNA replication beyond three cycles for discussion in class as groups sharing their concepts.

### Concept Elaboration and Evaluation:

After conducting these activities, review and summarize the following key concepts:

* Compare the components of cellular machinery with laboratory reagents needed for semiconservative DNA replication and PCR cycles.
* After recording the number double stranded DNA fragments from each of the three PCR cycles before any disassembly, deduce the pattern.
* Since PCR usually runs for 40 cycles, use the mathematical pattern per three cycles to calculate the number of single stranded DNA molecules produced at the end of 40th cycle.

### Variations:

* Instead of using DNA templates and primers provided, use random sequences for PCR cycles.
* Discovering DNA Ltd MDNA-STR-408 (molymod ®/miniDNA®, Spicing Enterprises Limited, UK) booklet has other DNA-markers listed, which can be used for DNA-replication activity.

### Sources/Credits

This lesson was developed per Formats of Utah Agriculture and California Foundation for Agriculture in the Classroom

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