**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>  |

 How DNA Fingerprints Identify Similar Seeds?

### Purpose

Students will understand that after PCR steps to isolate and amplify a specific gene, the resulting products (amplified double stranded DNA fragments) can be compared for their sizes. Activities include simulating gel electrophoresis to compare the PCR products for identifying DNAs obtained from suspect or crime location.

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

* [DNA Analysis Techniques](https://www.agclassroom.org/matrix/lesson/661/)
* [How to Extract DNA](https://learn.genetics.utah.edu/content/labs/extraction/howto/)
* [PCR and Gel Electrophoresis](https://www.brightstorm.com/science/biology/molecular-biology/pcr-dna-fingerprinting)

### Vocabulary

**Genetic Marker:** Identifiable DNA sequences found at specific locations of the genetic material and transmitted from one generation to the next by the law of inheritance.

**DNA Lengths:** The DNA molecule is a double helical structure, the size of which is measured by counting total number of base pairs per its length.

**Size Fractionation:** The separation of DNA molecules based on their sizes or lengths measured in number of nucleotides or base-pairs.

**Gel Electrophoresis:** A size fractionation technique where DNA molecules are moved through a gel under the influence of an electric field separating them by lengths.

**Electrophoresis Band:** A well-defined “line” of DNA fragments of the same size that have all traveled as a group to the same position on gel. Larger or longer DNAs travel short distance while smaller length DNAs move furthest.

**DNA Fingerprinting:** A laboratory-based genetic profiling to establish a link between biological evidence and a suspect in a criminal investigation.

**Genetic Profile:** A unique pattern of DNA bands generated by gel electrophoresis for identifying a biological sample.

### Background/Agricultural Connections

This lesson is the final one in a series of related lessons to introduce students to markers’ based genetic profiles that are identifiable as DNA bands after gel electrophoresis. Students will compare DNA fingerprints or migration patterns on gel (bands on poster board) from the three PCR samples of previous activity. The 6 base pair PCR product obtained per deletion in amelogenin gene of Bio-Fuel sorghum is smaller in size and expected to run faster, compared to the 12bp DNA from Traditional-Food sorghum D16S539 gene with only one short tandem repeat sequence (GATA). Thus, it would need to be resolved whether the seeds found at the crime location are identifiable as Traditional-Food or Bio-Fuel sorghum.

### Interest Approach – Engagement

1. Ask students if they know what a forensics geneticist does and introduce them to this career field which deals with application of genetic knowledge for identifying samples and helping to solve crimes. The forensics geneticists are expert in the analyses of different types of biological materials and create genetic profiles. They work in areas of genetics, biological evidence, and DNA testing. They help farmers know genetic makeup of their plants and obtain DNA evidence for any propriety related legal issues.
2. Watch the [DNA Analysis Techniques](https://www.agclassroom.org/matrix/lesson/661/) and [PCR and Gel Electrophoresis](https://www.brightstorm.com/science/biology/molecular-biology/pcr-dna-fingerprinting) video clip.
3. Inform your students they will:
	* Explore the DNA Size Fractionation and Gel Electrophoresis concepts
	* Learn how DNA fragments of the same size travel as a group to the same position on electrophoresis gel and make a well define band
	* Use online resources to identify kinds of DNA molecular weight standards or size ladders are available (e.g., 1kb ladder has DNA bands 1000bp apart on an electrophoresis gel)
	* Research the size of PCR product generated in the previous activity by counting its base pairs and draw its electrophoresis position on the poster board while considering it as an electrophoresis gel

### Procedures

**Materials**

**For the class:**

* poster board and markers
* Ruler to show 1, 5, 10, 15 and 20 bp DNA bands
* Internet access for research (this part may be done at home for homework)

**For BioFuel (BF) sorghum group:**

Six (6) base pair (bp) DNA product from previous activity after completion of three polymerase chain reaction (PCR) cycles using Discovering DNA Ltd. MDNA-STR-408 (molymod®/miniDNA®, Spicing Enterprises Limited, UK) kit

**For Traditional Food (TF) sorghum group:**

12 (twelve) base pair (bp) DNA product from previous activity after completion of three polymerase chain reaction (PCR) cycles using Discovering DNA Ltd. MDNA-STR-408 (molymod®/miniDNA®, Spicing Enterprises Limited, UK) kit

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

Six (6) base pair (bp) DNA product from previous activity after completion of three polymerase chain reaction (PCR) cycles using Discovering DNA Ltd. MDNA-STR-408 (molymod®/miniDNA®, Spicing Enterprises Limited, UK) kit

**For each student:**

* Pencil for Lab drawings
* Lab worksheet/ Drawing paper

**Preparation**

* Before the lesson, confirm that the 6bp and 12 bp specific genetic markers that were replicated after completing three PCR cycles from previous activity are available. resulting
* On benches for each the three students’ groups, confirm that the DNA sequences of the amplification products are as one of the two below;
	1. 5' - AAG GATA GTA - 3’ 2) 5' - GAT GTT - 3’

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

* Check if materials to make paper replicas for the lengths of 6bp and 12 bp DNA products of PCR process are available on benches for each the three students’ groups.

**Hands-On Activity:**

1. Tell students that in this lab they will act as DNA detectives, solving a plant identification case and resolving farmers’ proprietary dispute.
2. Ask students if they know chromatography and size fractionation where components of a mixture are separated based on their size-based speeds of passage through a material and accordingly displayed as different bands.
3. Ask students why electrophoresis which is based on the motion of dispersed and charged particles under electrical charge, through a gel where its pores work like a sieve, is used to separate DNA molecules based on their size (measure in number of base pairs).
4. Tell students that they will be separating PCR products (amplified double stranded DNA fragments) per for their sizes to identify DNAs obtained from two plant seeds sources as well as crime location through simulating gel electrophoresis conditions.
	* Count the DNA size (6bp or 12bp) per sequences of the amplification products are as one of the two available below;
	1. 5' - AAG GATA GTA - 3’ 2) 5' - GAT GTT - 3’

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

* + Use paper replicas for the lengths of 6bp and 12 bp DNA products obtained from two plant seeds sources as well as crime location.
	+ Arrange or draw DNA bands from Bio-Fuel (BF) sorghum, Traditional-Food (TF) sorghum and crime scene (unknown seeds-'XS') per respective columns on the poster-board (with 1, 5, 10, 15 & 20 bp bands in the column marked as DNA-standards) according to their size.
1. Instruct students to draw the poster-board information on their notes, while resolving plant identification case where two farmers claimed the ownership of seeds found at the crime scene.

### Concept Elaboration and Evaluation:

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

* DNA strands of different lengths can be used to represent and identify plant samples from varied sources and origins.
* Obtaining purified DNA samples and amplifying specific DNA markers through PCR is essential for resolving a forensics case to identify biological samples.
* Gel electrophoresis cause DNAs of different lengths to move through a gel, where its pores work like a sieve, while separating them by allowing smaller molecules to move faster than the larger ones.

### Variations:

* Instead of using three samples provided, vary the DNA fingerprints with multiple gel-bands to solve a forensics case or proprietary dispute.
* In addition to phytoforensics scenario for resolving proprietary dispute between two farmers, explore other avenues where these techniques can help detect transport of illegal plants.

### Sources/Credits

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

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