Using PCR to detect threats to food supplies

Focus questions	What are some threats to food supplies? How might we diagnose threats from pathogens in soil?
Vocabulary	Amplify, thermocycler, primer, DNA polymerase, dNTPs, target DNA, melting, anneal, extension, pathogen, disease, elution

Background

Global threats to food supplies are not just from climate (i.e. droughts, flooding, and damage from storms). Many threats may be within the soil. Most fungi and bacteria that live in soil are helpful. However, the harmful types, called **pathogens**, can cause **disease** that decrease yield or kill crops. These pathogens are specific to certain types of crops, so farmers can reduce the risk of damage if they are regularly rotating crops. However, pathogens in the soil can survive through multiple seasons. If a specific pathogen is detected with diagnoses, farmers can take that information to help control or manage the disease and minimize future crop damage.

This activity uses samples of soil taken from two different "farms" (or fields) to determine if a pathogen is present and what type of pathogen it is, in order to direct treatment. The DNA extraction from soil is a complicated and intense process. Once the DNA is extracted as a **liquid elution**, specific primers are used to **amplify** (make many copies) of the DNA from the potential pathogen(s) through the process of **polymerase chain reaction** (PCR). Labs test for specific DNA sequences from pathogens by choosing the appropriate **primers** before beginning PCR.

PCR brings together the necessary ingredients for DNA replication and amplification, which includes the **target DNA** from the soil extraction along with pathogen-specific primers, **dNTPs** (nucleotides), and **DNA polymerase**. Once these PCR ingredients are combined into a single tube, the PCR samples are placed into a **thermocycler**, a machine that provides the necessary temperature conditions for DNA amplification. In thermocycling, the target DNA is made into single strands during the **melting** step, primers bind to these single strands during the **annealing** step, and each primer-strand complex is made into a double strand by DNA polymerase during the **extension** step. These three steps are cycled up to 40 times to produce amplified target DNA, which is also called the PCR product.

Once the target DNA is amplified, the PCR samples can be checked or visualized using gel electrophoresis and compared to positive controls to determine if the pathogen was present in the original soil sample.

Materials

Soil sample collection:

- Small shovel or spade
- Bucket (1 or 5 gallon)
- 1-cup measuring cup
- Zipper bag
- Marker pen

Procedure

1. After watching the presentation, investigate the two pathogen possibilities in this case study and the diseases they cause, bacterial leaf streak and gray leaf spot. Identify the type of disease-causing organism, what crop it affects, its habitat and life span/cycle. Make some notes about what you find and note your references.

2. Investigate other common pathogens and corresponding diseases that affect crops in your area.

3. Soil collection: The best collection method is to make a composite or mixture of multiple soil samples from different places in a field. The two potential pathogens for testing have different host crops, but may be found in the same field or location if both corn and soybeans have been grown in the area.
    a. Use the small shovel or spade to dig up a 2 cup sample of soil at least 4-6 inches below the surface.
    b. Add it to the bucket.
    c. Choose another spot in the field; repeat steps a and b.
    d. Choose a third spot in the field; repeat steps a and b.
    e. Mix the soil in the bucket together well.
    f. Bag and label the soil sample with the field name/location.

4. DNA extraction: Soil often contains high levels of organic compounds that can severely reduce the ability of PCR to amplify pathogen DNA. Thus, specialized DNA extraction kits are used for testing soil. These DNA extraction kits contain multiple washing steps that help remove PCR-inhibiting compounds that are found in the soil. Follow the step-by-step instructions in the DNeasy PowerSoil DNA Isolation Kit.

5. PCR amplification: The liquid elution from the DNA extraction kit contains DNA from all of the organisms within the starting soil sample. PCR allows scientists to target the pathogen of interest by specifically amplifying pathogen DNA to concentrations that can be detected through gel electrophoresis.
    a. Once extracted products are loaded into the thermocycler, complete *Understanding PCR*.

6. Electrophoresis of PCR products: If the pathogen was present in the soil subsample used in the DNA extraction, PCR should have amplified a portion of the pathogen DNA to detectable levels for visualization on an agarose gel.

Rubric for self-assessment

Skill	**Yes**	**No**	**Unsure**
I discovered the characteristics of two pathogens that live in soil and impact field crops.			
I successfully collected soil samples.			
I was able to extract DNA from soil (if applicable).			
I can explain how PCR uses the physical properties of DNA to amplify the number of copies of DNA in a sample.			
I can explain how DNA is separated as it flows through a gel as a result of an electrical current.			
I can give a pathogen diagnosis (present or absent) on each test sample by interpreting the banding pattern on a gel following electrophoresis.			