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?
Learning target	Students use PCR and gel electrophoresis to diagnose a pathogen that affects crops.
Vocabulary	Amplify, thermocycler, primer, DNA polymerase, dNTPs, target DNA, melting, anneal, extension, pathogen, disease, elution

HS.PS.1 Chemical Reactions

Performance expectation HS.PS1-4, HS-PS1-6	**Classroom connection:** The changes to the physical bonds in DNA during polymerase chain reaction (PCR) can be explained in detail using additional activities; the model of the changes made in PCR will become clear through the use of this model.
Performance expectation HS-LS2-1	**Classroom connection:** Students look for evidence in extracted materials that identify specific pathogens that may affect yield (carrying capacity) of a crop.

Science and engineering practices

Developing and Using Models	**Classroom connection:** Students will interact with a paper model to explain the changes to DNA during PCR.
Constructing Explanations and Designing Solutions	**Classroom connection:** Students will be able to explain the steps in PCR and gel electrophoresis.
Using Mathematical and/or Computational Thinking	**Classroom connection:** Students will interact with a paper model to explain the changes to DNA during PCR.

Disciplinary core ideas

PS1.B Chemical Reactions	**Classroom connection:** Students will use the model to show how PCR exponentially creates more molecules of the desired DNA molecule and how molecules of DNA are moved through gel using electrophoresis.
LS2.A Interdependent Relationships in Ecosystems	**Classroom connection:** Students look at the average yield of crop(s) to determine the effect of a pathogen's presence on yield

Cross-cutting concepts

Stability and Change	**Classroom connection:** The resulting copies of the desired DNA is a result of added nucleotides (dNTPs), but the rearrangement of the molecules is determined by the primers and the predictable action of DNA as a molecule.
Scale Proportion and Quantity	**Classroom connection:** The effect on a field "ecosystem" from the presence of a pathogen can be calculated to represent an entire farm.

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, 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, 1) the target DNA is made into single strands during the **melting** step, 2) primers bind to these single strands during the **annealing** step, and 3) each primer-strand complex is made into a double strand by DNA polymerase during the **extension** step. These three steps are cycled many times to produce amplified target DNA in the form of a liquid **elution**.

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.

Prior knowledge

Students should be able to micropipette, prepare agar, and load wells for gel electrophoresis. Students should research common pathogens found in crops in their area. This presentation focuses on two corn diseases, Bacterial Leaf streak and gray leaf spot, but there are many other pathogens in soils that affect different crops. Students will need to investigate the differences between these two pathogens—how they reproduce, their genetic make-up, their habitat for living, etc. Here is a general resource that describes potential disease-causing organisms: **intechopen.com/books/plant-growth/plant-pathogens**.

Free information on these and other pathogens and pests can be found in literature produced by colleges and universities. For example, Iowa State University Extension and Outreach has a Corn Field Guide that contains information on the corn disease in the Midwest and recommendations for treatment. This free resource can be found at: **lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1023&context=extension_pubs**. The best and most reliable sources will be produced by universities with .edu domains.

Materials

- Materials for soil sample collection:
  - Small shovel or spade
  - Bucket (1 or 5 gallon)
  - 1-cup measuring cup
  - Zipper bag
  - Marker pen
- Materials for DNA extraction:
  - Centrifuge (2mL tube capacity)
  - DNeasy PowerSoil Kit (see detailed protocol)
  - Gloves
  - Heat block (or water bath)
  - Marker pen
  - Pipettes (p1000, p100, p10)
  - Pipette tips (1000µl, 100µl, 10µl)
  - Refrigerator
  - Scale
  - Tube racks
  - Vortex
- Materials for PCR amplification:
  - 2mL microcentrifuge tubes
  - GoTaq Master Mix (Promega Cat No: M7122)
  - Molecular grade water
  - pipette tips
  - Positive Amplification Controls
  - 10X Primer Mixes
  - Thermocycler
  - Tube rack that holds PCR tubes (0.5 or 0.2 mL) and 1.5mL tubes (PCR reagents)
- Materials for electrophoresis:
  - Electrophoresis setup:
    - Gel electrophoresis chamber
    - Gel casting tray
    - Gel comb
    - Power supply
  - Erlenmeyer flask or beaker (250mL capacity)
  - Graduated cylinder
  - Pipettes
  - Scale
  - Spatula
  - UV-Transilluminator
  - Weigh boat
- Materials for agar preparation:
  - Agarose
  - Hot plate or microwave
  - Scale
  - 250 mL flask
  - TAE or TBE buffer
  - SYBR safe DNA gel stain
- DNA ladder
- Positive samples of pathogens
- Micropipettes
- Pipette tips

Teacher preparation

If you choose to do the protocol with all steps—soil collection, DNA extraction using the DNeasy PowerMax Soil Kit, and amplifying and running your own samples on gel electrophoresis, this activity may take up to five days or longer depending on how much time is given to students for research.

Qiagen sells a DNeasy PowerMax Soil Kit. It is available at **qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dneasy-powermax-soil-kit/**

It is possible to request a soil DNA extraction from the National Agricultural Genotyping Center (NAGC) and to request 10X primer mix and positive DNA samples for the pathogens mentioned in the presentation. Contact Zack Bateson (**zack.bateson@genotypingcenter.com**) for more information.

Another option would be to show the presentation (see slide notes below), obtain samples from NAGC, have students complete the Understanding PCR card sort while running the PCR, then set up and run the samples for gel electrophoresis to determine a diagnosis of pathogen presence. It is possible for a classroom to go through the 2–3 day process and find no positive results, which may be frustrating. However, this is a real example of science in action in agriculture. Have students brainstorm what may have happened—there may be none of the pathogens tested for, but there is evidence for something causing issues. Ask for ideas about next steps such as testing for different pathogens, looking for outside events that may have caused the damage, etc.

Procedure

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 hosts, but may be found in the same field or location if both corn and soybeans have been grown in the area.

1. Use the small shovel or spade to dig up a 2 cup sample of soil at least 4–6 inches below the surface.
2. Add it to the bucket.
3. Choose another spot in the field; repeat steps 1 and 2.
4. Choose a third spot in the field; repeat steps 1 and 2.
5. Mix the soil in the bucket together well.
6. Bag and label the soil sample with the field name/location.

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.

PCR amplification

The 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. Follow the steps below to determine whether a particular pathogen is present in the extract soil sample.

1. Select primers based on the pathogen.
2. Completely thaw the reaction components.
3. Determine the number of reactions for Master Mix calculation. This should include positive and negative control reactions, and add 10% for pipetting error (i.e., if performing 10 PCRs, add another reaction for a total of 11 on the master mix tables below). Use Table 1 as an outline to prepare the Reaction Master Mix for PCR amplification.

Example master mix table

Component	Volume/ rxn	No. rxns	Reaction master mix volume
Molecular grade water	5µl	11	55µl
2X GoTaq Master Mix	12.5µl	11	137.5µl
10X Primer Mix	2.5µl	11	27.5µl
Reaction Master Mix per sample	20µl	11	220µl*
Template DNA	5µl**	11	60.5µl
Total Volume per sample	*25µl*	*11*	*275µl*

* A good check involves adding up the entire column of reagents (55 + 137.5 + 27.5) to make sure it matches 20 x 11 for the Reaction Master Mix.
If it doesn't the calculations are off and need to be checked again.

** Note: The 5µl of Template DNA for each sample is added at step 5, after the 20µl Reaction Master Mix (step 4) is aliquoted into individual PCR tubes.

Component	Volume/ rxn	No. rxns	Reaction master mix volume
Molecular grade water	5µl		
2X GoTaq Master Mix	12.5µl		
10X Primer Mix	2.5µl		
Reaction Master Mix per sample	20µl		
Template DNA	5µl	xx	xxxxx
Total volume per sample	*25µl*		

4. Add 20µl of Reaction Master Mix to each PCR tube.
5. Add 5µl of soil DNA extracts (template DNA) to each PCR tube.
6. For the positive control, add 5µl of Positive Amplification Control (PAC) to the appropriate tube.
7. For the negative amplification control (or No Template Control - NTC), add 5µl of molecular grade water instead of soil DNA extracts to the appropriate tube.
8. Place tubes in thermal cycler with the following conditions:

Temp*	Time	Cycles	Reason
95° C	3 min	1	Activate taq, initial melt
95° C	15 sec	40	Amplification steps
60° C	1 min		
16° C	Hold		Temporary storage on cycler

* The temperatures given above are specific to these particular pathogens. These may change if you test for different pathogens in your soil samples.

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. Follow the steps below to cast gel and run PCR samples.

1. Make a 2% agarose gel using a standard protocol. See Appendix A for an example.
2. Add 8µl of SYBR Safe Stain (10,000X) directly to the molten agarose.
3. After gels have set:
4. Add 10µl of DNA ladder to lane #1 of each gel.
5. Add 10µl of PS or GLS electrophoresis control to lane #2 of each gel.
6. Add 2µl of 6x DNA loading buffer to PCR samples.
7. Load 15µl of each PCR sample (containing 6x loading dye) to agarose gel.
8. Run gel at 150V for 35–40 minutes.
9. View gel under UV or Blue LED Viewer.

Appendix A: How to prepare agarose gel

Choose a flask that is 2–4 times the volume of the solution.

- Mass the correct amount of agarose (0.8% gel = 0.8g of agarose in 100 ml 1X buffer).
- Sprinkle in the agarose powder while solution is rapidly stirred.
- Cover with plastic wrap and puncture hole for ventilation.
- Heat flask on high in a microwave until bubbles appear.*
- Remove beaker and GENTLY swirl the beaker to resuspend any settled powder and gel pieces.
- Reheat the flask until the solution comes to a boil. It may be safe to use short time periods depending on the microwave. Hold the boil for about one minute.
- Gently swirl the flask (USING SAFETY OVEN MITTS/GLOVES) to mix the agarose solution.
- Cool solution to 60° C (about 5 min) before adding stain and casting.

*Alternatively, use a hot plate with a magnetic stir bar turned on high to bring the solution to a boil. (**home.sandiego.edu/~josephprovost/AGAROSE%20GELS_Short%20Protocol.pdf**)

Differentiation

- **Local community:** Students may visit a local biotech lab, department of agriculture lab, or look for a virtual tour of a biotech lab where pcr is being practiced.
- **Students with special needs (language/reading/auditory/visual):** Results of the gels can be projected or magnified for students to compare.
- **Extra support:** Complete Understanding PCR as part of this lab. Also, students may watch: Gel Electrophoresis at `youtu.be/ZDZUAleWX78`.
- **Extensions:** Students may do their own DNA extraction from soils in their areas.

Assessments

Rubric for assessment

Skill	Developing	Satisfactory	Exemplary
Refine the design of a chemical system by specifying a change in conditions that would produce increased amounts of products at equilibrium.	Student can successfully illustrate the steps in PCR.	Student can successfully illustrate the steps in PCR and identify and use gel electrophoresis as a tool to determine the presence of a pathogen.	Student can successfully illustrate the steps in PCR and identify and explain how gel electrophoresis is a tool to determine the presence or absence of a pathogen.

Rubric for self-assessment

Skill	Yes	No	Unsure
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.			