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Molecular Testing for Crop Viruses

By Tracey Mason, ABE Ireland

ABE Master Teacher Fellowship Program

AMGEN Biotech Experience

Scientific Discovery for the Classroom

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We are grateful to the ABE Master Teacher Fellows for sharing their work with the ABE community. If you have questions about any of the project components, please reach out to us at ABEInfo@edc.org, and we will be happy to connect you with the author and provide any assistance needed.

AMGEN Biotech Experience

Scientific Discovery for the Classroom

Ireland

Molecular Testing for Crop Viruses

TIME FRAME: 5–6 class sessions

SUGGESTED AGE RANGE: 14–18 yrs

SUGGESTED COURSE OR CONTENT AREA:

Connection Descriptions:

- Molecular modeling
- Evolution and population genetics
- Career pathways for bioscience careers
- Connections/tie-ins between ABE and physics, chemistry, and Earth science, marine biology, and neuroscience
- Integrating inquiry
- Data analysis/Data literacy
- Project or problem-based learning
- Professional skills in STEM/profiles in STEM
- Eye on the news- current real-world applications for ABE concepts, content, and technologies

AUTHOR: Tracey Mason, ABE Ireland

Background:

Importance of Studying Viruses in Crops

Plant viruses are a major threat to agriculture and global food security. These viruses can reduce crop quality and yields, resulting in significant economic losses for farmers and food producers. Unlike fungal or bacterial diseases, plant viral infections often have no cure once established, making early detection and prevention critically important.

Aphids and other insect vectors can rapidly spread plant viruses across large fields, especially in intensive farming systems. Some viruses remain hidden within plant tissues without obvious symptoms, making them difficult to detect without molecular tools like PCR. Understanding plant-virus-vector relationships enables scientists to design resistant plant varieties, develop diagnostic kits, and implement more effective pest management strategies.

By studying how viruses spread and how they interact with their hosts and vectors, researchers help safeguard food production, support sustainable agriculture, and mitigate the impact of plant diseases on ecosystems and economies.

Lesson 1: Introduction to DNA, Viruses & Molecular Diagnostics

Overview

This foundational lesson introduces students to the structure and function of DNA and RNA, the nature of viruses (especially plant viruses), and how molecular diagnostics such as PCR are used to detect viral infections in organisms like aphids. The lesson includes videos and interactive content from LabXchange.

Learning Goals

By the end of the lesson, students will be able to:

- Describe the basic structure and function of DNA and RNA
- Explain how viruses infect host organisms
- Understand how PCR is used in molecular diagnostics to detect viral infection in vectors like aphids

Key Words

DNA, RNA, virus, genome, host, vector, molecular diagnostics, aphid, pathogen, nucleic acid

Assessment Outcome

Students will complete a short digital quiz explaining:

- What a virus is.
- How PCR can detect viruses.
- Why aphids are important in plant virus transmission.

Teacher Preparation

- Create a class group on LabXchange.org.
- Assign the following resources:
  - LabXchange Video: *"DNA and Genes"* – The Secondary Structure of DNA - LabXchange
  - Optional reading: The First Survey Using High-Throughput Sequencing of Cereal and Barley Yellow Dwarf Viruses in Irish Spring and Winter Barley Crops – *ScienceOpen*
- Prepare a short formative quiz and vocabulary handout.
- Projector or screen to show video segments in class.

Lab Safety

- No hands-on work this session, but review general lab behavior expectations:
  - No food or drinks
  - Respect for shared equipment
  - Intro to PPE (lab coats, gloves, goggles)

Lesson 1 Quiz: DNA, Viruses, and Molecular Diagnostics

Instructions: Answer the following questions in full sentences.

1. What is DNA and what is its role in living organisms?

2. How are viruses different from bacteria?

3. Why are aphids important in the spread of plant viruses?

4. What is the purpose of PCR in virus detection?

5. Imagine a farmer suspects a viral infection in crops. How could scientists use aphids to help diagnose the problem?

Bonus (Vocabulary Match): Match the terms with the correct definitions:

A. Vector
B. Pathogen
C. Genome
D. Primer
E. Host

1. __ A sequence that initiates DNA replication during PCR.
2. __ An organism that carries and transmits a virus.
3. __ The complete genetic material of an organism.
4. __ An organism that is infected by a virus.
5. __ A microorganism that causes disease.

Lesson 2: Understanding PCR — Theoretical and Virtual Application

Lesson Overview

Students will explore how PCR (Polymerase Chain Reaction) works through detailed explanation and a virtual LabXchange simulation. They'll learn the role of primers, polymerase, nucleotides, and thermal cycling in amplifying DNA.

Learning Goals

Students will:

- Identify and describe the main components of a PCR reaction.
- Understand the steps of the PCR cycle: denaturation, annealing, and extension.
- Complete a virtual PCR reaction and troubleshoot basic problems.

Key Words

PCR, DNA polymerase, primers, nucleotides (dNTPs), thermal cycler, amplification, template DNA, denaturation, annealing, extension

Assessment Outcome

Students will complete a Placemat Activity in groups of 4 students.

- Label the components of a PCR setup.
- Describe each step of the PCR cycle.
- Summarize what happens to DNA after 30 cycles of amplification.

Teacher Preparation

- Assign or prepare to guide the LabXchange simulation:
    - LabXchange Simulation: *"PCR: Polymerase Chain Reaction"*
    - Questions: How does PCR amplify DNA? How Does PCR Amplify DNA? - LabXchange
    - Prepared A3 sheets for placemat activity in small groups

Lab Safety

- Discuss lab safety around handling PCR reagents:
    - Importance of ice and cold blocks for enzyme stability.
    - Avoiding contamination with gloves and filter tips.
    - Pipetting safety and avoiding cross-contamination.

Lesson 2 Worksheet: Understanding PCR

Part A: PCR Cycle Steps
Fill in the blanks with the correct step: (Denaturation, Annealing, Extension)

1. _____: The double-stranded DNA is heated to 94°C to separate strands.
2. _____: The temperature is lowered to allow primers to bind.
3. _____: DNA polymerase extends the new DNA strand by adding nucleotides.

Part B: Short Answer

1. Why is Taq polymerase used in PCR instead of regular DNA polymerase?
2. What happens to the amount of DNA after 30 cycles of PCR?
3. What would happen if primers were not added to the PCR mix?

Lesson 3: Running a PCR (Wet Lab)

Molecular Detection of Virus in Aphids

1. Overview

Aphids are small, sap-sucking insects that pose a significant threat to barley crops by transmitting plant viruses. They act as vectors, carrying viruses such as Barley Yellow Dwarf Virus (BYDV) and Cereal Yellow Dwarf Virus (CYDV), which can severely impact crop yield and quality. Grain and bird cherry oat aphids are major vectors of Barley Yellow Dwarf Virus. BYDV can cause yield losses up to 80% in Ireland (Teagasc).

This activity describes a protocol that can be carried out in a classroom environment for educational purposes. It enables students to get hands-on practice of running a PCR to identify if an insect has a virus. Control material can be provided along with unknown samples for testing. A primer set targeting an aphid gene is also provided to help the students reduce false negatives (for example due to extraction failures, or PCR inhibitors being present in the sample). This can be carried out as a simplex PCR reaction (testing one primer at a time), and once evaluated, students can investigate how they might reduce workload and move to a multiplex PCR reaction (where the control gene and virus are amplified simultaneously).

The students will be provided with eight samples that need to be tested to determine if they are positive or negative for the sample. They will also be provided with positive and negative controls to run with each batch of samples. The students should first test the samples with primers targeting the virus and compile the results. They then should test the samples with the primers targeting an aphid gene to make sure all samples are OK and amplify a product as expected.

The details on the sequence of the virus targeted can be found in OQ686648 and further information on the source of this data can be found Molecular testing for crop viruses (School Genetics Club). The students should try and locate the sequence of the primers used in the assay or use the virus genome to design new primers for a PCR assay.

2. Learning Goals

Learn Molecular Lab Techniques

- Practice pipetting, sample preparation, nucleic acid extraction.
- Perform PCR, including setting up reactions and using thermocyclers.

Handle Biological Samples Safely and Accurately

- Learn aseptic techniques and biosecurity measures for working with potentially infectious materials.

3. Assessed Outcome

Students will successfully extract nucleic acids from aphid samples and amplify a target viral sequence using PCR

4. Key Vocabulary

BYDV, aphids, PCR, master mix

5. Materials

	Component	Quantity per Reaction
1	**Classic Taq++**	12.5 µL
2	**Molecular-grade H_20**	9.5 µL
3	**Forward primer**	1 µL
4	**Reverse primer**	1 µL
5	*Template*	1 µL
6	Total	25 µL

Micropipettes, tips, empty beaker, gloves, lab coats, Thermocycler, PCR tubes, mini-centrifuge, ice, Chromebook (LabXchange)

6. Teacher Preparation

Set up 8 stations around the classroom, each with micropipettes, tips, beaker, centrifuge, tube rack with primers, positive and negative controls.

7. Lab Safety Considerations

- Wear appropriate PPE: lab coat, gloves, and safety goggles.
- Tie back long hair and avoid loose clothing.
- Do not eat, drink, or chew gum in the lab.
- Be familiar with the location and proper use of safety equipment (e.g., eye wash station, fire extinguisher, first aid kit).
- Dispose of all waste in the correct biohazard or chemical waste containers.
- Treat all aphid and plant material as potentially biohazardous.
- Use aseptic technique when handling samples to avoid contamination.
- Dispose of aphid material and used pipette tips in designated **biohazard waste**.
- Disinfect work surfaces **before and after** the experiment using ethanol or appropriate disinfectant.

8. Sequence of Activities

Activity Description	*Time*	*Materials*
1. Review Micropipetting Technique from ABE Lab 1. Instructions for Students - Lab 1 Micropipetting - LabXchange	10 min	Micropipettes
2. Discuss the task to find out whether the eight unknown samples: a) Are aphids b) Are testing positive for viruses	25 min	Make up master mix and load into PCR tubes. Centrifuge
3. Carry out a PCR to amplify genetic code specific to aphids and viruses.	20 min	Set and leave thermocycler to run for estimated time. Centrifuge
4. Place PCR tubes in freezer until next lesson.	5 min	Freezer

Method:

1. Identify the templates you want to use as input for your PCR amplification - these will consist of:

   - Samples you want to test for yellow dwarf virus (these will be labelled US01, US02, etc.)
   - Positive controls (representing virus infected aphids to help the researcher know that their protocol worked, these will be labelled PC-MAV)
   - A negative control (representing virus free aphids to help the researcher ensure that PCR amplification will not show a product for the virus when an aphid is not infected, this will be labelled NC-SA)
   - A no template control (this has no template DNA available for amplification by PCR and help the researcher ensure that their reagents are free from contamination, it is labelled as NTC)

2. Identify the primers you want to use in the test and thaw on ice. The following table shows the primer details that can be used to identify if an aphid has the virus BYDV-MAV. There are also primers that target a gene in the aphid (GAPDH gene) and this can be used as a control to make sure your extraction worked (so help to reduce false negatives). The choice of primers to use will depend on your target assay and each primer is provided as a working solution.

   Primers: Each PCR will require a forward and reverse primer: if testing for the virus then you can use MAV-For and MAV-Rev, if amplifying the aphid gene GAPDH then you can use GAPDH-SA-For and GAPDH-SA-Rev

3. Identify the remaining ingredients required to run the PCR and place on ice: (i) the enzyme master mix to carry out the amplification and (ii) molecular biology grade water. The enzyme master mix is called Classic Taq++ (Tonobo) and this contains Taq polymerase (to amplify the DNA), dNTPs (nucleotide building blocks used during amplification), and buffers. This is supplied in a 2X concentration (so for example, to get 1X in a final reaction of amount 25 µL you would need to add 12.5 µL of Classic Taq++).

4. Identify the total number of reactions that will be carried out (this is the number of test sample plus the controls) and select tips and tubes that will be used to prepare and hold reactions during the PCR.

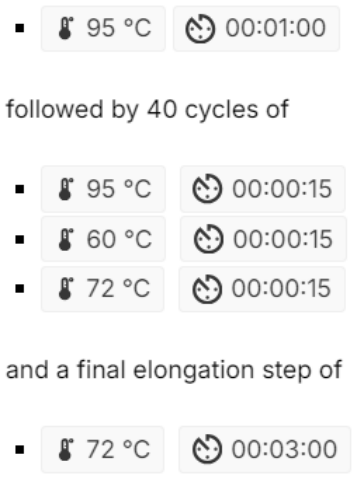
PCR Protocol:

1. Create a master mix with all ingredients necessary for all reactions together. Quantities per reaction below:

	Component	Quantity per Reaction
1	**Classic Taq++**	12.5 µL
2	**Molecular-grade H_20**	9.5 µL
3	**Forward primer**	1 µL
4	**Reverse primer**	1 µL
5	*Template**	1 µL
6	Total	25 µL

*The Template is the only component that changes across reactions; this will either be test sample, control, or water.

2. Add 24 mL of master mix into each reaction tube.
3. Add your respective template (aphid/virus sample or controls). Place the reactions in a thermocycler and run the PCR cycle at right.
4. Once the PCR is finished, place the reactions in the freezer.
5. To test whether the amplification has worked, we will run a gel electrophoresis in the following week.



Lesson 4: Gel Electrophoresis (Wet Lab)

Learning Goals:

Understand the purpose of gel electrophoresis:

- Explain how gel electrophoresis separates DNA fragments based on size.

Set up and run a gel electrophoresis experiment:

- Demonstrate correct use of equipment (gel rig, power supply, micropipettes, loading buffer, DNA ladder).

Interpret DNA banding patterns:

- Analyse results to determine whether a virus was detected in aphid samples.

Compare unknown DNA samples to a DNA ladder:

- Estimate the size of DNA fragments by comparing them to known standards.

Practice safe lab techniques:

- Follow lab safety procedures for handling DNA stains and electrical equipment.

Apply observation and analysis skills:

- Use careful observation of gel results to draw conclusions about virus presence.

Assessed Outcome:

Students will accurately interpret the results of a gel electrophoresis experiment by identifying DNA bands in aphid samples, comparing them to a DNA ladder, and correctly determining which samples tested positive for a plant virus.

Teacher Preparation:

Pre-Lab Setup

- **Prepare agarose gel:**
  - Make gels in advance (e.g., 1% agarose in buffer), pour into casting trays with combs, and allow to solidify.
  - You may also allow students to pour the gel as part of the lab if time allows.
- **Prepare buffer solution:**
  - Fill gel rigs with buffer before students arrive or have students do this if part of the skills focus.
- **Load some pre-run gels for demo (optional):**
  - Having a backup gel already run helps in case student gels fail.
- **Label all sample tubes clearly:**
  - Including ladder, controls, and test samples.

Materials:

- Agarose powder
- TAE or TBE buffer
- Gel casting trays and combs
- Gel electrophoresis chambers (rigs)
- Power supply units
- DNA samples (student samples + positive control)
- DNA loading dye
- DNA ladder (size marker)
- DNA stain (e.g. SYBR Safe or GelRed)
- Micropipettes and tips
- Gloves, lab coats, and safety goggles
- UV or blue light transilluminator (if needed to visualize DNA)

Method:

1. Prepare a 1.2 percent TBE agarose gel according to standard protocol.
2. Use 5 µL of PCR product together with 5 µL of water and 2 µL of 6X loading buffer.
3. Load the samples into the gel alongside a 100 bp DNA ladder for time appropriate for voltage and gel size.
4. Prepare a 3X GelGreen staining solution and place the gel into solution (for example in an old lunch/take-away box) with gentle agitation for approximately 00:30:00 min.
5. Image the gels with a light transilluminator.

Results:

	Sample										
Group 1 (aphid)											
Group 2 (virus)											

Discussion:

Based on your findings, try to explain to the winter barley farmer whether the crop is at risk of yield losses (or not).

Lesson 5: Research Task – Can Viruses Affect Behaviour in Aphids?

Overview:

Aphids that are infected with Barley Yellow Dwarf Virus (BYDV) exhibit several behavioral changes compared to uninfected aphids. These changes are largely due to the virus's impact on the aphid's physiology and the plant they are feeding on.

Learning Goals:

- Conduct Research
- Design an experiment
- Engage in class discussion on findings

Materials: Digital tablet, White cardboard, black marker, BYDV virus infected aphid, virus free aphid, magnifying glass, stopwatch

Teacher Preparation: Ask students to explore the link between viruses and changes in behaviour in animals/insects and get them to share their research with the class using at least one example.

Using the link below, ask students to review and carry out the task between aphids with the virus and without. State the importance of observation as foundation in the scientific method and ask students to form their own hypothesis and design an experiment to investigate if the BYDV affects behaviour in aphids.

- Download *Do Viruses Affect Behaviour?*

Acknowledgments:

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