

Chestnut Tree Lab

An Environmental Application of Molecular DNA Technologies

Maryland Loaner Lab Teacher Packet



Written by the MdBio Foundation and Towson University.

Chestnut Tree Lab

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STUDENT ACTIVITY HANDOUTS AND LABORATORY PROTOCOL

Jigsaw	S1	<i>Used in the Pre-Laboratory Activity</i>
Jigsaw KLEW Chart	S4	<i>Used in the Pre-Laboratory Activity</i>
Investigation Design	S5	<i>Used in the Pre-Laboratory Activity</i>
Paper DNA Activity	S8	<i>Used in the Pre-Laboratory Activity</i>
Gel Report	S9	<i>Used in the Pre-Laboratory Activity</i>
Additional DNA Fragment Exercise	S10	<i>Used in the Pre-Laboratory Activity</i>
Micropipette Use	S11	<i>Used in the Pre-Laboratory Activity</i>
Practice Gel Loading Exercise	S12	<i>Used in the Pre-Laboratory Activity</i>
Laboratory Protocol	S13	<i>Used in the Laboratory Activity</i>
Post-Lab Questions	S15	<i>Used in the Laboratory Activity</i>
Vocabulary & Web Resources	S17	

CORRELATION TO STANDARDS

Next Generation Science Standards	A1
Advanced Placement Biology Correlation	A5



The Maryland Chapter of The American Chestnut Foundation (MD-TACF) provided funds for Chestnut Tree Laboratory kits.

Chestnut Tree Lab

Introduction for Teachers

The Chestnut Tree lab explores the use of DNA biotechnology in ecology and conservation. Prior to 1904, the American chestnut (*Castanea dentata*) served a large role in forest ecosystems and local economies through much of its range. A fungus accidentally introduced to New York in 1904 infected the chestnuts, and within 40 years, the fungus drastically reduced the chestnut population across their range. The fungus causes cankers that kill the trees. This disease process caused by the fungus is called “chestnut blight”. Conservation efforts include hybridizing the American chestnut with a blight resistant Asian chestnut variety, cultivating the American variety in western areas where the fungus has not spread, and targeting the fungus with a biocontrol application.

A biocontrol uses a living organism or natural methods to limit pathogens or fight a “disease process”. In the case of blight, a naturally occurring virus readily infects the fungus that causes blight. Researchers can transfer viral DNA or RNA to an uninfected canker in a process called transfection. It is helpful to track viral spread and to compare natural spread with researcher-applied transfers. To do so, researchers employed a technique called restriction enzyme analysis of genetically-tagged viruses. In restriction enzyme analysis, a genetic tag is inserted into the viral genome, and the tag matches the cut sequence for a specific restriction enzyme. When researchers sample cankers, they can expose the genetic material to the restriction enzyme, then run the cut DNA through gel electrophoresis. By comparing the resulting bands to the wild-type (naturally occurring) viral DNA and the genetically-tagged viral DNA, researchers can determine if the canker had a viral infection as well as the type of virus that infected the canker (wild-type vs. genetically-tagged).

This lab provides an opportunity for students to discover the historic importance of the American chestnut and the impacts of blight on chestnut trees; explore the concepts of biocontrol, restriction enzyme analysis, and genetic tagging; and run a wet lab that employs several DNA biotechnology techniques to differentiate between blight with genetically-tagged viruses, wild-type viruses, and uninfected cankers.

Overview

This lab is designed in the following three parts:

- **Pre-Lab Jigsaw.** This activity engages student interest and promotes active learning as students explore and then share their new knowledge of the history of the American chestnut, blight, viruses, and biotechnology techniques used in this activity. This jigsaw activity divides students into groups to become “experts” on a topic, then returns the students to their “home” groups, which consist of “experts” from each topic area. The experts share the discovered information with their “home” groups. Alternatively, “expert” groups can simply present their findings to the class (45-60 min).
- **Paper Pre-Lab.** The second part of the pre-lab allows students time to work through concepts with a paper version of the wet lab and practice skills necessary to complete the wet lab (30-45 min).
- **Wet Lab Activity.** Students conduct the wet lab investigation, which employs restriction enzyme analysis to identify which canker is infected with a wild-type virus, which canker is infected with a genetically-tagged virus, and which canker does not have a viral infection (90 min; including ~30 minutes to run the gel).

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Introduction for Teachers

Natural Stopping Points

If you will be completing this lab over several class periods, there are several places that act as natural stopping points.

- You can stop at any point during the jigsaw activity, paper DNA gel electrophoresis pre-lab activity, the micropipette instructions and the practice gel activity.
- Ideally, you would have enough time left for students to view the gels immediately after running the gel electrophoresis experiment. It takes about 30-45 minutes to run the gels in the gel electrophoresis boxes, not including the time it takes for the students to actually load the gels. You can find more information on gel running time in Step 10 of the Laboratory Preparation instructions (Page 25). However, if you run out of time, you can complete the gel electrophoresis and then save the gels overnight in with ~2 tbsps of buffer in a sealed Tupperware or Ziplock to keep them moist. Then students can visualize them the next day. We do suggest if you do this, to go ahead and visualize and take pictures of each before storing them just in case the stain fades.

Inquiry-based instruction promotes exploration and discovery before explanation and a student-centered approach to teaching. This lab encourages inquiry-based instruction by allowing students to explore concepts, discover history, and connect techniques to ecological conservation applications. It is student-centered by allowing students to invest personally in the learning process by connecting to prior knowledge and experience and by using active learning of doing and exploring to teach the concepts. The instructor can continue inquiry-based, student-centered instruction through the paper lab and wet lab experiences by asking questions and promoting student thinking throughout the lab.

Opportunity exists to foster cross-curricular connections in courses in history, social studies, biotechnology, ecology, and writing. Cross-curricular studies can enhance the learning experience by assisting students to build connections with the content and deepen understanding of the concepts. The more connections made for and by a learner, the better the student retains the information learned!

Thank you for selecting the Chestnut Tree Lab for your classroom!

Loaner Lab Learning Goals:

This lesson aims to have students explore the use of DNA technologies and biocontrols in the real-world ecological problem of the chestnut blight. To achieve this overarching goal, students will:

1. Understand and describe the processes of DNA extraction, RT-PCR, DNA restriction enzyme digestion, and agarose gel electrophoresis (Jigsaw – Explore).
2. Understand and describe biocontrols, with a focus on viral biocontrols (Think-Pair-Share, Discussion – Explain).
3. Explain the use of and importance of genetic tags in tracking biocontrol dispersal (Jigsaw – Explore).
4. Build connections between the role of a specific organism and its ecological, economic, and aesthetic impacts (Discussion – Engage; Jigsaw – Engage).
5. Plan and carry out investigations on the presence of and, if present, origination of a viral biocontrol agent (Paper DNA and Wet Labs – Explore).

Chestnut Tree Lab

Introduction for Teachers

6. Analyze and interpret data collected during an investigation on viral presence and origination (Wet Lab Data – Explain).
7. Extend ecological applications of biocontrols to novel situations (Post-Lab – Extension).

Lab Flow and Time Needed

- Initial Engagement: 5-10 minutes
- Jigsaw Activity: 60-75 minutes
- Expert Group Summary: 10-5 minutes
- Investigation Design: 20 minutes
- Pre-Lab for Paper DNA Extraction: 30 minutes
- Paper DNA Lab: 30 minutes
- Wet Lab: 60-75 minutes
- Discuss Applications: 10 minutes
- Post-Lab: 10+ minutes
- Other Extension Activities: As time allows

Teachers and students who will be performing the *Chestnut Tree* laboratory activity from the Maryland Loaner Lab must first complete the pre-laboratory classroom activity. Conceptual aspects of the curriculum will be reinforced with the laboratory activity.

Chestnut Tree Lab

Materials and Supplies

SAFETY: The classroom teacher must instruct students with basic laboratory safety rules and provide gloves and goggles for student use with the laboratory activity.

Supplied by the Teacher:

Description	Quantity	Comments
Student Worksheets	1/student	Copy from laminated template in binder (or can be found online)
Safety Goggles	Enough for entire class	Each student working with the kit's contents should wear safety goggles
Gloves	≥1 pair/student	For student use with main laboratory activity
Scissors	5 pairs	1 per group
Rolls of Tape	5	1 per group
Poster Board	5 medium sheets	Use with pre-laboratory activity (can also use large sheets of paper)
Distilled Water (dH ₂ O)	1,350 ml/class set	Used to dilute 10X TAE buffer. Will need an additional 1,350 ml if requesting more than one class set.
Lab Microwave or Hot Plate	1	Used to melt the agarose solution
Waste Containers	1/group	Used for discarded pipette tips, tubes, etc.

Supplied by Maryland Loaner Lab:

Description	Quantity	Comments	Return Directions
Teacher Binder and CD	1	Background info, instructions, and student worksheet templates	Return
Gel Electrophoresis Box	1	Contains 1 box with a lid, (6) combs, (6) trays, (12) stoppers and (2) slats	Rinse, dry, and return. Refer to binder for packing instructions.
Blue Light Transilluminator	5	For visualizing DNA bands	Wipe clean and return
Power Supply with Cord	1	Supply cord is separated to prevent breakage	Return
Orange Capped Agarose Bottle	1	To make 6 gels, contains tube with premeasured agarose powder	Rinse, dry, and return
Agarose Powder (1.8 g)	1 tube	Shipped inside orange-capped bottle. Used to make agarose gels.	Discard tube, return labeled bag
SYBR Safe DNA Stain	1 tube	12 ul of SYBR Safe DNA stain	Discard empty tube
m2 Liter Container	1	To mix 10X TAE & H ₂ O for dilution	Rinse, dry, and return

Description	Quantity	Comments	Return Directions
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10X TAE Buffer	150 ml (1, 150ml bottle)	Follow directions in binder for dilution. If you requested multiple class sets: <ul style="list-style-type: none"> Running buffer should be re-used for each class An extra 150 ml 10X TAE buffer is supplied in case of spills & for making more gels 	Rinse and return
20 µl Micropipettes	10	1 per group	Return
Micropipette Tip Boxes	5	1 box per two groups	Return only unused tips
Foam Microtube Racks	10	1 per group	Return
DuraGel practice Gels	11	1 per group	Rinse, dry and return
Class set of "Chestnut Tree Reagents"	1/class set	<ul style="list-style-type: none"> (10) RE (restriction enzyme) Student Tubes (10) Prac Dye (Practice Dye) Student Tubes 	Return all tubes. Do not empty or rinse.
Graduated Cylinder (100 ml)	1	Used to pour gels	Rinse, dry, & return
Disinfectant Wipes	1	Used to wipe pipettes after student use	Return
Class Set of DNA "Chestnut Tree DNA and LD" Samples	1/class set	<ul style="list-style-type: none"> (10) UF Student DNA Samples (10) WT Student DNA Samples (10) GT Student DNA Samples (10) NC Student DNA Samples (10) LD Student Loading Dye Tubes 1 Extra LD for Teacher Use (1) DM (DNA marker, teacher uses to run one lane (15 µl) on each gel) Refrigerate until use.	Discard tubes, return labeled bags
Microcentrifuge	1	Used to spin samples down in tubes prior to setting out at student stations	Return
Spatula	1	For moving gels to and from transilluminator	Rinse, dry, and return
Insulated Bag & Freezer Pack(s)	varies	Holds DNA and all reagents & dyes	Return

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Background Information for Teachers

The Chestnut Tree Lab covers many concepts and applications of DNA technology, including RT-PCR, DNA restriction enzyme digestion, and agarose gel electrophoresis. The details about the fungus that causes chestnut tree blight and the treatment of the cankers with a different fungus that contains a virus found to be debilitating to the original canker-causing fungus are very complicated. Time will need to be taken in the pre-laboratory to allow students to explore and develop a complete understanding of why DNA technology is being employed in this laboratory investigation. See the Jigsaw Section (Page 14) for a full explanation. The pre-laboratory activity models what will be performed in the actual laboratory activity.

Students should be familiar with DNA and RNA. Students should understand that while the DNA code normally results in an RNA product and then a protein, it is possible to obtain a cDNA (complementary DNA) product from RNA by using an enzyme known as Reverse Transcriptase and then performing PCR (polymerase chain reaction) to obtain a significant amount of cDNA (together a two-step process referred to as Reverse Transcription PCR or RT-PCR). PCR is a test tube reaction in which a specific region of DNA is amplified many times by repeated synthesis of DNA using a heat-stable enzyme called DNA polymerase taq and specific DNA primers to define the ends of the region to amplify. PCR is used to make a larger quantity of a small original amount of DNA and would be analogous to photocopying a worksheet to have many identical copies. The hypovirulent strain that targets the chestnut blight fungus is an RNA virus. The heating-cooling cycles required for PCR degrade RNA. Therefore, the cDNA produced during reverse transcriptase is amplified using PCR.

Students should have a basic understanding of fungus and how two fungi samples must have vegetative compatibility for them to share cytoplasmic elements such as viruses. This lab is modeled after what researchers have tried to do by using molecular DNA technology and by applying it to an environmental problem in hopes of solving it. Researchers have tried to treat the fungus found in the cankers on chestnut trees with a vegetative compatible fungus that naturally contains a virus (called a hypovirulent strain/virus) that is debilitating to the original fungus that caused the canker. The hope is to use the treatment fungus that has the hypovirulent virus to stop the growth and spread of the cankers on the chestnut trees. Often, the fungi found in new cankers are not of the same vegetative compatibility with the treatment fungi kept in the lab. As a result, biologists have genetically modified a hypovirulent virus that they can track and use to transfect (a way to initiate viral infection) the fungus of the new canker. In order to follow this genetically modified virus in nature, the viral RNA has been genetically modified to contain a restriction site that is not found in the wild-type viral strain, which results in a genetically 'tagged' virus. The students should also know that they can determine whether the virus is natural (wild-type) or genetically-tagged (a virus that has a restriction enzyme sequence added) by using restriction enzyme analysis.

DNA restriction enzyme analysis is a technique with wide ranging applications in all types of research, medicine, forensics, paternity/pedigree testing, etc. DNA restriction enzyme analysis is based on the following assumptions:

- Various DNA molecules can be identified by a difference in the sequence of bases.
- DNA restriction enzymes, which are produced naturally by bacteria, cut DNA molecules at specific sites denoted by specific base sequences.

Background Information for Teachers

- When a restriction enzyme is used to cut different DNA molecules (in a restriction digestion), the size of the fragments generated will be unique to each molecule because of its difference in sequence.
- After being cut by restriction enzymes, DNA fragments remain mixed in solution and indistinguishable from one another. One way to distinguish between the different fragments created is to compare them by size. Different sized fragments of DNA can be separated using gel electrophoresis.

Gel electrophoresis is a technique used to separate molecules based on the differential movement of charged particles through a matrix when subjected to an electric field. In basic terms, DNA is negatively charged, and in the presence of an electric current DNA will travel according to size—the number of base pairs (smallest pieces first) toward the positive electrode as shown in Figure 1. The positive electrode is colored red, and electrophoresis of DNA is always “Running toward Red”. The sizes of the DNA fragments can be determined by comparing them to a DNA Marker (often called a “DNA ladder”), which has standard DNA fragments of known size and appear as a ladder with many rungs when run on an agarose gel. By comparing the resulting pattern of the DNA fragments on the gel (looking at both the number of bands and the corresponding sizes) the different DNA molecules may be differentiated.

Figure 1 below shows examples of the samples that students will be running in this investigation. Lane one contains the DNA Marker (DM), which is DNA of known sizes used to determine the size of the samples being run. Lane 2 is a sample that contains DNA from a fungal sample that is not infected with the virus (UF). It has a single band at 3,000 bp. Lane 3 is a sample that was taken from a fungus that contained the wild-type virus (WT). The fungus DNA band is 3,000 bp, and the wild-type virus band is ~1,200 bp. Lane 4 is an example of a sample taken from a fungal canker (fungal DNA band is at 3,000 bp) that contains a genetically-tagged virus (GT) and was cut with restriction enzymes, resulting in two bands: sizes 706 bp and 502 bp. Lane 5 is an example of a new canker (NC) that is being tested. In this case, the new canker has a DNA banding pattern that is consistent with a fungal canker that contains a wild-type virus.

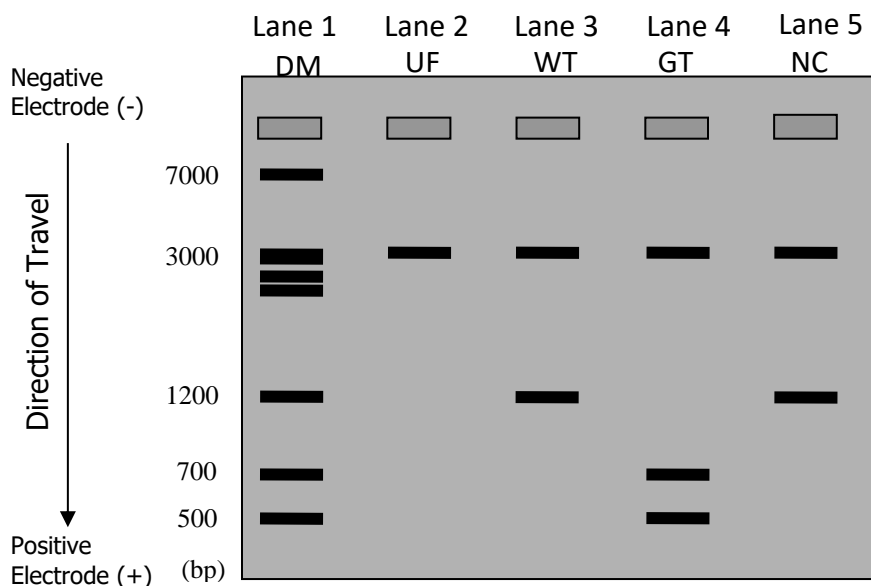


Figure 1. Examples of different types of DNA that students will run on gels in this investigation.

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Background Information for Teachers

This lesson is organized into two parts: a pre-laboratory and a laboratory investigation. The pre-laboratory investigation contains four activities:

- The Jigsaw allows students to actively explore and build a conceptual basis for this activity. The Jigsaw and the pre-Jigsaw activities encourage student engagement and develop a personal interest as an investment in the activity and the ecological effects of ecological change.
- Simulating a DNA restriction enzyme analysis using paper DNA sequences and scissors helps to determine if treated blight cankers have been infected with a hypovirulent virus and if so, whether it's a wild-type virus or a genetically-tagged virus.
- *Micropipette Use* is an activity designed to familiarize students with micropipettes, as accurate pipetting is critical in the laboratory activity.
- The *Practice Gel Loading Exercise* allows students to gain practice using proper techniques when loading gels and gives them the opportunity to hone their skills before loading the samples involved in the laboratory investigation.

Following the pre-laboratory activity, students work in the laboratory where they apply the concepts acquired in the pre-lab to actually test cDNA samples. The restriction digestion in the laboratory investigation is a mock exercise (the restriction enzyme, 'RE', provided is actually water) due to the limitations of a loaner lab program (issues of keeping restriction enzymes frozen as needed, classrooms not being equipped with the necessary water baths, etc.). The actual cDNA samples used in the lab are pre-digested with restriction enzymes.

A PowerPoint presentation is available at <https://www.towson.edu/fcsm/centers/stem/loanerlab/highschool.html> to guide students through some of the background information and laboratory activities.

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Chestnut Tree Lab Facilitation Guide

Engage (5-10 minutes; S1)

1. Get students thinking about their own connections to woodlands, what makes a forest location unique, and how species composition contributes to the uniqueness of that woodland or forest. Eventually, students should come to recognize that part of what makes their forest connection identifiable is species composition.
 - a. The first slide of the PowerPoint presentation presents pictures of different woodland environments. Ask students to describe species and feature differences that make each pictured location unique. Have students use words or sketches to capture their favorite or iconic woodland image. Discuss what makes each area different and unique.
 - b. Foster a cross-curricular connection by sharing literature or art from another subject or class that captures a woodland environment. Have students identify key characteristics of the environment. Compare and contrast with the woodlands nearest them.
2. Ask students to imagine and then describe how their forests would feel, aesthetics, products or what would change if one of the key species disappeared.

Explore (60-75 minutes; S1-4)

3. Tell students we're going to explore an ecological problem that created a huge forest species composition shift right here in Maryland and the mid-Atlantic states. Then, assign students into jigsaw groups. These "expert" groups of 4-5 students will research a particular aspect of the niche (both ecological and human-oriented) of the chestnut tree, chestnut blight, and DNA technologies used in the lab. They will then report their findings back to the class. Alternatively, each student can research one of the subtopics to form an "expert" group where students share information gathered on the same topic. "Expert" groups then present the information to their classmates.
4. Summarize the Jigsaw activity on the KLEW chart.

Explain (10-15 minutes; S1-4)

5. After the "expert" groups present their findings, the instructor should confirm understanding. A PowerPoint presentation is available for use. This PowerPoint presentation can be used throughout the activity to guide you. There is a slide set up for each of the "expert" groups. These slides can be used by the instructor as a backdrop for discussion or by the groups to assist their discussion. The goal of this section is to clarify the exploration activity and be sure students understand the concepts so they can further explore and then apply these concepts in the next activity.

Explore (20 minutes; S5-6)

6. Ask students to form new groups with a representative from each "expert" group. These integrated groups represent real science teams where researchers from diverse backgrounds work together to study issues. In the integrated groups, have students

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develop ideas on how biocontrols and genetically-tagged viruses could be used to fight chestnut blight issues. Key questions include:

- a. Would the virus pose a risk to the chestnut tree?
 - b. Why track the spread of the genetically-tagged virus?
 - c. How can we test a canker to see if it has a virus and if the virus is a wild-type virus or a genetically-tagged virus?
7. Ask students to develop a lab investigation plan using a genetically-tagged virus, a wild-type virus, DNA gel electrophoresis, RT-PCR, and DNA restriction enzyme digestion to counter the chestnut blight. The goal here is for students to develop the framework of this investigation. As such, they should recognize that they would sample the unknown canker and compare the bands produced against a positive (GT virus) and a negative (wild-type) virus. DNA extraction should happen first so the DNA is available. RT-PCR happens next, using the primer associated with the genetic tag, so the amplification (copies) of the DNA section are of those that identify the virus as GT or WT. Students **SHOULD NOT** try to note amounts pipetted or other specifics as to the protocols of the lab, as this is meant to be a more general overview of the process.

Explain (30 minutes for steps 8-10, 60-75 minutes for step 11; student pages are noted below)

8. Explain the lab procedures we will use to complete this lab. There is a scenario on page S5 to explain students' role as researchers.
9. Tell students that in order to prepare to run these samples they need to complete a practice run. The paper lab activity provides a "dry run" of the wet lab. Complete the paper lab activity described in Paper DNA Activity (S8-10).
10. Complete the Practice Loading Gel Exercise (S12) to develop the skills necessary for this lab.
11. NOW you're ready to run the wet lab. Follow the directions on pages S13-14.

Extend (Variable time, 10 minutes minimum to complete step 12)

12. After completing the lab, have students regroup and discuss their results. Options include:
 - a. Discuss which cankers were infected with a WT virus and which were infected with a GT virus and what evidence supported these conclusions.
 - b. Speculate how the virus spreads from tree to tree.
13. The following extension activities may be used to reinforce concepts introduced during the pre-laboratory activity and the laboratory activity.
 - a. Ask the students to write a letter to a friend and describe what they did in the laboratory, which is modeled after actual research, as they attempt to help the devastated American chestnut tree.
 - b. A biocontrol is an approach using living organisms or natural methods to fight a "disease process". It is often used in terms of agriculture and pest management. This laboratory looks at the use of hypovirulent viruses to weaken the fungus that causes chestnut tree blight. Another possible biocontrol method is to use soil organisms that naturally produce "antibiotic" products that can also suppress the

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fungus in the cankers. This was discovered because the roots and root collars from the trees are protected from the fungus when they are covered by soil. What biologists have done as a method of treatment is to mud-pack the cankers found on the trees. Ask students to research additional examples of biocontrol treatments used to solve an environmental problem.

- c. Have students investigate and report on the work being done by biologists in back-cross breeding programs with blight resistant Asian chestnut trees and blight susceptible American chestnut trees. Early breeding efforts simply tried to make hybrids of Asian trees with American trees. Later, it became clear that multiple genes were responsible for the resistance found in Asian trees. A back-cross breeding system was developed where partially resistant hybrids were repeatedly crossed with American chestnut trees, which increased the percentage of American tree genes into hybrids with resistance genes. The goal is to produce a mostly American chestnut tree, exhibiting its unique physical characteristics, that has resistance genes to chestnut tree blight.
- d. There are many examples of invasive species (plants, weeds, animals, aquatic species, insects, and microbes) that are found in the United States. Usually, “invasive species” are considered negative because their introduction causes or is likely to cause economic or environmental harm or harm to human health. Occasionally, “non-native” plants or organisms are introduced into a region in an attempt to fix an environmental problem and they would not be considered “invasive”. Have students research and give a report on an invasive species.

Evaluate (10 min for post-lab; S15-16)

14. Students can complete the attached post-lab materials or prepare a formal lab report.

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Pre-Lab Teacher Instructions: Jigsaw

The purpose of this pre-lab activity is to help students recognize that different tree species have unique characteristics. This initial activity engages students by connecting prior knowledge, developing a sense of personal connection to a specific woodland area, and providing an opportunity for students to become subject “experts” and share with the class.

Materials Needed:

- PowerPoint slides
- Projector for slides
- Student Jigsaw Worksheets
- Student access to internet resources or other resources
- Student access to materials for presentation (computers or flipchart or similar)

Teacher Preparation:

- Run and provide copies of the Jigsaw activity (one per student)
- Reserve computers and/or library for student research OR have materials available for research

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Pre-Lab Teacher Instructions: Paper DNA Gel Electrophoresis

NOTE: Groups using the loaner lab must first complete this pre-laboratory classroom activity. The conceptual aspects of the curriculum will be reinforced with the laboratory activity.

The purpose of this pre-laboratory activity is to explore how using the cDNA product from Reverse Transcription Polymerase Chain Reaction (RT-PCR) on the nucleic acid found in the chestnut blight fungus allows different blight cankers to be identified as having been infected by a debilitating virus (debilitating for the fungus, not the tree). This activity provides students with the opportunity to investigate the application of RT-PCR, DNA restriction enzymes, and gel electrophoresis to generate data when determining whether a viral infection is present as well as if the source of infection is caused either through natural means with a wild-type virus or through a genetically-tagged virus modified in the laboratory. The objectives of this pre-laboratory activity are:

- Identify a need for RT-PCR, DNA restriction digestion, and gel electrophoresis
- Model the concepts of DNA restriction enzyme analysis, which includes gel electrophoresis

Pre-Laboratory Materials (for 5 student groups)

- 5 pairs of scissors (one per group)
- 5 sets of 3 envelopes labeled: “**Canker #1**”, “**Canker #2**”, and “**Canker #3**” (one set of three envelopes per group)
- 5 copies of the **Gel Report** with instructions for each envelope set (one copy per group)
- 5 copies of the cDNA strips sheet that include “**PCR Product-Canker #1**”, “**PCR Product-Canker #2 A and B**”, “**PCR Product-Canker #3 A and B**” (template found on the next page)
- 5 rolls of tape (one roll per group)
- 5 **poster-sized Gel Charts** as shown on the **Gel Report** (one chart per group)

Teacher Preparation (for 5 student groups)

- Prepare a **poster-sized Gel Chart** as shown on the bottom of the **Gel Report** (one chart per group)
- Photocopy of the **Gel Report** with instructions for each envelope set (one per group)
- Cut up the cDNA strips labeled: “**PCR Product-Canker #1**”, “**PCR Product-Canker #2 A and B**”, “**PCR Product-Canker #3 A and B**” and place into the properly labeled envelopes (“**Canker #1**”, “**Canker #2**”, and “**Canker #3**”). You will need one set of three envelopes for each group.

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Pre-Lab Teacher Instructions: Paper DNA Gel Electrophoresis

PCR PRODUCT CANKER #1 (ENVELOPE 1)

1490 Base Pair DNA Sequence	ATCCGATTCCATTTAACGGT TAGGCTAAGGTAAATTGCCA	1490 Base Pair DNA Sequence
--------------------------------	--	--------------------------------

Canker #1= _____ bp total

1 (IN ENVELOPE 1)

PCR PRODUCT CANKER #2 A (ENVELOPE 2)

1490 Base Pair DNA Sequence	ATCCGATTCCATTTAACGGT TAGGCTAAGGTAAATTGCCA	1490 Base Pair DNA Sequence
--------------------------------	--	--------------------------------

Canker #2 A= _____ bp total

2A (IN ENVELOPE 2)

PCR PRODUCT CANKER #2 B (ENVELOPE 2)

690 Base Pair DNA Sequence	ATACCGGGGCTGCATCCATA TATGGCCCCGACGTAGGTAT	490 Base Pair DNA Sequence
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Canker #2 B= _____ bp total

2B (IN ENVELOPE 2)

PCR PRODUCT CANKER #3 A (ENVELOPE 3)

1490 Base Pair DNA Sequence	ATCCGATTCCATTTAACGGT TAGGCTAAGGTAAATTGCCA	1490 Base Pair DNA Sequence
--------------------------------	--	--------------------------------

Canker #3 A= _____ bp total

3A (IN ENVELOPE 3)

PCR PRODUCT CANKER #3 B (ENVELOPE 3)

492 Base Pair DNA Sequence	GATATACCGCGGCCGCATCC ATATATGGCGCCGGCGTAGG	696 Base Pair DNA Sequence
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Canker #3 B= _____ bp total

3B (IN ENVELOPE 3)

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Pre-Lab Teacher Instructions: Paper DNA Gel Electrophoresis

The following scenario is presented to the students for their investigation.

A team of biologists is trying to treat the fungus found in blight cankers on chestnut trees with a vegetative compatible fungus containing a virus that is debilitating to the original fungus that caused the canker (called a hypovirulent virus). The hope is to use the treatment fungus that contains the hypovirulent virus to stop the growth and spread of the cankers on the chestnut trees. Tell the students that they are part of this team and they are investigating whether the fungi from three cankers found on one tree are infected with this hypovirulent virus.

The biologists believed that they have previously treated one of these cankers with the genetically-tagged virus, that one of the other cankers may have had the naturally occurring wild-type virus, and that there is a new canker. The goal for the students is to determine from the three canker samples if they are infected at all with a virus and if so, if it is with a wild-type or genetically-tagged virus. Any uninfected fungal samples would later be treated by this team. Students must understand what RT-PCR is and how the cDNA in this lab was obtained.

To determine whether or not the virus is genetically modified or tagged involves successfully cutting the 1200 base pair (bp) viral cDNA PCR product with a restriction enzyme called NotI (pronounced 'not-one'). The genetically-tagged viral cDNA can be cut by the NotI restriction enzyme because it contains the NotI enzyme DNA sequence (added by researchers in the lab). It is important to confer to the students that while the search for the viral cDNA is important, they should realize that the fungus that causes the cankers also has DNA that will be copied in the PCR reactions as a 3000 base pair (bp) cDNA product. After the restriction digestion of the different DNA samples, agarose gel electrophoresis is used to analyze the results. The size of the different fragments generated by restriction enzyme digestion should total the size of the original uncut cDNA and will be visualized (using a Blue Light transilluminator) after gel electrophoresis.

Students should understand that the different types of DNA they are examining in this investigation are of different lengths (different numbers of base pairs or 'bp'). By running their samples on an agarose gel using gel electrophoresis, they will be able to visualize the size of the DNA fragments and draw conclusions about the type of DNA in their samples. To be able to do this, they will need to know:

- Fungal DNA is 3000 bp
- Wild-type (WT) viral cDNA is 1200 bp
- Genetically-tagged (GT) viral cDNA, after being cut by restriction enzymes, shows up as two bands, 706 bp and 502 bp

Pre-Laboratory Exploration

Ask the students to follow the instructions on the **Gel Report** (S9) to determine whether viral infection of the chestnut blight fungus is present as well as the source of infection (a wild-type or genetically-tagged virus). The instructions on the **Gel Report** will guide them through the process of DNA restriction enzyme analysis. As the facilitator, be prepared to assist the students and address any misconceptions. This activity is set up for 5 student groups. There are 5 cDNA strips that need to be analyzed by each group. As students complete the activity as instructed they will tape the resulting cDNA fragments on the **poster-sized Gel Chart**

Chestnut Tree Lab

Pre-Lab Teacher Instructions: Paper DNA Gel Electrophoresis

in the appropriate place using the DNA marker (made up of standard DNA fragments of known sizes) to help them place the cDNA fragments correctly.

Pre-Laboratory Explanation

After each group has finished placing the cDNA fragments on the chart, have each student complete the **Gel Report** by drawing in the correct cDNA positions for each canker sample. The PCR product seen in cDNA strips from cankers #1, #2 A, and #3 A all have 3000 bp and are uninfected fungal cDNA. Canker #2 B has 1200 bp and is the fungus infected with the wild-type virus. The only cDNA strip to be cut by the restriction enzyme is Canker #3 B that results in two fragments of 502 bp and 706 bp, which means it is the fungus infected with the genetically-tagged virus. The pre-laboratory activity is directly modeled after the actual laboratory activity. An example of the actual gel results can be seen on page 26 in the Teacher Laboratory Preparation section, where Canker #1 will look like the sample "UF", Canker #2 will look like the sample "WT", and Canker #3 will look like the sample "GT". The new canker (NC) samples will be a mix of UF, GT, and WT, so each groups' results may vary. Lead a class discussion regarding their conclusions and the process they employed to determine whether viral infection was present and, if so, whether it was from a naturally occurring or genetically modified virus. Possible discussion questions could be:

- **Why is performing RT-PCR on the nucleic acid from the cankers necessary?**
To obtain large quantities of cDNA for additional testing purposes.
- **What is the purpose of using the DNA restriction enzymes?**
The restriction enzymes cut at very specific DNA recognition sequences and allow us to determine if the virus present in our canker sample was the laboratory modified tagged virus.
- **What is the purpose of the DNA marker?**
A DNA marker is made up of standard DNA fragments of known sizes, and it assists in estimating the sizes of unknown DNA fragments through comparison.
- **Why would no viral cDNA be obtained from a canker sample?**
If only the fungal cDNA 3000 bp band is seen, then it can be determined that no viral cDNA was present and, therefore, the fungus in the tree canker was not infected either naturally from a wild-type (WT) virus or from the treatment with the genetically-modified virus (GT) that came from the lab.

Emphasize that distinguishing characteristics of DNA are its size (number of base pairs) and the specific sequence of nucleotide bases. Note that the technique modeled here does not sequence the DNA. The technique, DNA restriction enzyme analysis, provides indirect evidence that a particular sequence of DNA exists based on the recognition of the restriction enzymes and the ability to differentiate between different DNA samples based on the different size fragments produced.

Evaluation

Give an index card to each student. Have them describe in their own words what they learned from the activity.

Micropipette Instructions

NOTE: This activity may be performed the day of the laboratory activity or any time in advance.

Micropipettes

Micropipettes are precision instruments designed to measure and transfer small volumes of liquid. They are expensive and must be used with care. Their accuracy depends upon their proper use. Different brands of micropipettes vary in the volume range they will measure, the type of tips they fit, and the type of device used to set the volume. Be sure that everyone understands how to operate the micropipettes correctly.

Basic Directions for Micropipette Use

Golden Rules of Pipetting

- 1. Be aware of the upper and lower range of the pipette. Going above or below the range will damage the micropipette.*
- 2. Always use the micropipette with a micropipette tip. Without a tip on the end, liquid can get into the opening of the pipette and damage the mechanism inside.*
- 3. Always hold the micropipette straight up to prevent liquid from getting into the micropipette.*
- 4. Use new pipette tips between different samples to prevent contamination.*

Setting the Volume

All micropipettes have a volume control dial. Determine whether the volume window on your pipette shows tenths of microliters (0.1 μl) or whole microliters in the smallest place, so that you can read the scale correctly (it varies with different brand micropipettes).

Drawing Up and Expelling Liquid

Micropipettes have 2 stops as you press down on the plunger to expel liquid. The first stop corresponds to the volume set in the window. The second stop gives a little puff of air to blow out any remaining liquid upon delivery. To draw liquid into the pipette tip, press down on the plunger only to the first stop. If you go to the second stop you will draw too much liquid into the tip. The most common pipetting error is to go past the first stop to the second stop for drawing liquid into the tip (which gives an inaccurate volume). When you are letting the liquid out of the tip, then you go to the second stop. It is worthwhile to check each student for correct technique before beginning laboratory procedures that require the use of the pipettes.

Chestnut Tree Lab

Pre-Lab Teacher Instructions: Micropipette Use & Practice Gel Loading

Preparation of Student Stations for Micropipette Use and Practice Gel Loading Exercise:

- 1 tube of “Prac Dye” (practice loading dye)
- 1 copy of student worksheet pages S11-12
- 1 20 µl micropipette
- micropipette tips (1 box for every 2 student stations)
- 1 practice loading gel (square reusable gel)

Micropipette Use Activity:

The purpose of this activity is to give students an opportunity to practice using the micropipettes to draw up and expel liquid. Please go over the instructions regarding proper pipetting use with your students prior to having them complete this activity.

Practice Gel Loading Exercise:

The purpose of this activity is to allow students to practice loading samples into the tiny wells of an agarose gel. We have supplied you with 10 practice gels (agarose gels cast into small, round petri dishes) so that each group of students can practice pipetting before handling their DNA samples. Students will use the tube of dye labeled “Prac Dye” (practice dye) for this activity. See the student sheet (S6) for detailed instructions on how to load a well.

“Loading gels” (filling the wells of a gel) can be a challenging task, especially if one has never done it before. This is an opportunity for students to practice before they are asked to load the actual samples involved in the laboratory activity. Tell students to take their time, figure out how they feel most comfortable doing this (i.e., some people like the rest their elbow on the counter while loading), and practice filling a few different wells of the practice gels. Remind students that this is for practice and not to get frustrated if liquid spills out of a well or if they accidentally tear the edge of the agarose gel. They can just try again.

An important thing to note about the gels: the wells appear as holes, but they really aren't. They are more like indentations that do not go through completely to the bottom of the petri dish. This is why it is so important not to poke the micropipette tip through the bottom of the well, or the liquid will seep into the bottom of the dish and not stay in the well.

Chestnut Tree Lab

Laboratory Activity Overview for Teachers

The purpose of the laboratory activity is to apply the concepts outlined in the pre-laboratory activity to an actual application in which students use DNA restriction enzyme analysis to determine if their treated canker samples have successfully been infected by a hypovirulent virus, and if so, to determine the origins of the virus. The objectives of the laboratory component are as follows:

- Discuss the need for RT-PCR to produce the cDNA that will be analyzed
- Perform a restriction enzyme digest on cDNA
- Perform agarose gel electrophoresis
- Analyze results to determine if a viral infection occurred in new canker fungal samples, and, if so, whether the infection is caused by a wild-type virus or a genetically-tagged virus

Developing the Concept for the Laboratory Activity

This lab involves many detailed concepts that will need to be addressed with the students prior to the laboratory activity. Topics and concepts include: basics about DNA and RNA; RT-PCR; background information regarding fungi and viruses; DNA restriction enzyme digestion; fundamentals of agarose gel electrophoresis and its application; and finally, an understanding of chestnut tree blight and the use of hypovirulent viruses as one method of biocontrol for the possible treatment of the cankers produced by the blight.

The Laboratory Investigation

Students will perform a mock restriction digestion on real (pre-digested) DNA samples. The restriction enzyme (RE) used in this mock digestion is actually just water. Even though the restriction digestion is a mock simulation it will reinforce the actual steps necessary to digest or cut DNA using restriction enzymes. The protocol involves adding “Restriction Enzyme” to each of the four cDNA sample tubes (“UF”= uninfected fungal DNA, “WT”= fungus infected with a wild-type virus, “GT”= fungus infected with a genetically-tagged virus, and “NC”= unknown sample: fungus from a new canker). The first three samples are the controls that will be used for comparison of the fourth unknown sample. All samples will undergo the same restriction digestion. The “reaction” in the protocol will take place during the five-minute incubation time.

Loading dye is added to the samples to help visualize the samples while loading into the wells of the gels and to increase the sample density to keep the samples in the wells while adding the running buffer. A DNA stain (SYBR Safe) was added to the gels when they were poured and this will allow students to visualize the DNA fragments using a transilluminator once electrophoresis has been performed. Groups will be assigned four wells in a gel to load 15 µl of their four cDNA samples all in the same order: “UF”, “WT”, “GT”, and “NC”. Once all gels have been loaded with the student samples and the teacher has added 15 µl of the DNA marker to one well in every gel, the gels will run for approximately 30 minutes at 75 -100 volts in 1X TAE running buffer. The agarose gels act as a sieve to separate the different sized cDNA fragments. The cDNA samples in the gels are invisible but they will become visible when placed on the Blue Light transilluminator.

Chestnut Tree Lab

Laboratory Preparation Instructions for Teachers

Prepare Student Stations (for 10 groups):

- One pair of safety goggles and gloves/student (provided by the teacher)
- One foam microcentrifuge tube rack
- One tube* of each of the following: “UF”, “WT”, “GT”, “NC”, “RE”, and “LD”
- One 20 µl micropipette
- One box of micropipette tips (1 box/2 student groups)
- One practice gel
- One practice loading dye tube
- One disposable cup (waste container for tips) (provided by the teacher)
- 1 copy/student of student worksheets pages S13-16

Activity:	Time Needed:
Preparing Gels and Student Stations	60-90 minutes
Pre-Lab Activity	60 minutes
Micropipette Use and Practice Gel Loading Exercise	30-45 minutes
Laboratory Activity	90 minutes
Post-Lab Activity	10 minutes

*Use the microcentrifuge to spin down all cDNA and dye samples prior to student use. Centrifuge for only 1-2 seconds.

Shared Equipment for Multiple Groups:

- One agarose gel for every two groups (each group will use 4 wells)
- One gel electrophoresis chamber (gel box) for all gels
- One tube of DNA marker “DM” (teacher will load 15 µl into one well on each gel after students have loaded samples)

Electrophoresis: Agarose Gel Preparation and Directions for Running Gels

Step 1 – Prepare 1X TAE Buffer (for making agarose gels and for use as a running buffer)
Buffer (not water) must be used to make and run the gels. The buffer supplies the necessary ions to conduct electricity. The buffer received in the kit is 10X Tris-Acetate-EDTA (TAE) in a 150 ml bottle (150 ml total), and needs to be diluted with distilled water (dH₂O) to make a 1X concentrated solution.

Add the entire 150 ml of 10X TAE buffer (the entire bottle) to 1350 ml of distilled water in the 2-liter container provided and mix well. From this now diluted 1X TAE buffer, 120 ml will be used to make the agarose gels and 1000 ml will be used as the electrophoresis running buffer.

Step 2 – Prepare a set of six 1.0 % agarose gels (5 gels for the activity with 1 extra gel).
Agarose gels and running buffer may be 1-2 days before use. This prep will make 6 small gels. Each gel will have 10 wells and will accommodate 2 groups of students with 4 samples each. Before making the agarose solution, have casting trays prepared and ready to be used (see Step 3).

Chestnut Tree Lab

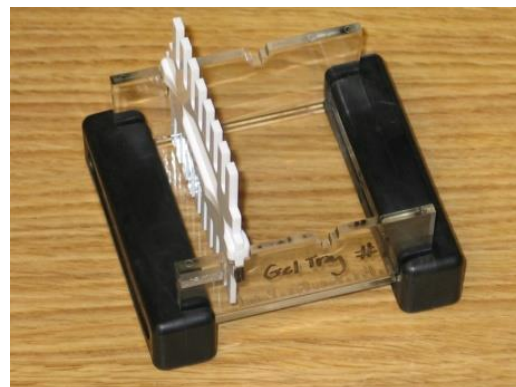
Laboratory Preparation Instructions for Teachers

Pour the entire contents of the microcentrifuge tube containing 1.20 g of agarose powder found in the powder bag into the orange-capped glass bottle. Then, add 120 ml of the diluted 1X TAE buffer from Step 1 (use the graduated cylinder). Add the buffer to the glass bottle, and mix well with the agarose powder by swirling the bottle.

1. Dissolve the agarose in a microwave or on a hot plate. **The orange bottle cap must be removed before heating.** The power of the microwave may vary, but to prepare 120 ml of agarose it generally takes 1.5-2 minutes on high power. For best results place the bottle in the microwave for one minute, stir and heat for 30 more seconds, stir and heat another 30 seconds only if needed. **Do not overheat** as the liquid will boil out of the bottle and spill. It is best to microwave in small time intervals and mix, then continue heating. The agarose must be completely dissolved in solution and well mixed. No particulate matter should be visible.
2. Cool the agarose solution to about 60°C by placing the melted agarose in a 60°C water bath or by allowing it to sit at room temperature for several minutes. **Swirl occasionally** while it is cooling to avoid rapid cooling of the agarose in the bottom of the bottle so that the agarose does not start to solidify (or reheating will be necessary). The bottle of melted agarose solution is ready to be used when it is warm to the hand but not too hot to handle (if it's too hot it can warp the comb and gel tray).
3. Once the gel has cooled to ~60°C (still liquid, but cool enough to pour) add 12 ul of SYBR Safe DNA stain to melted agarose. Swirl to mix.

Step 3 – Casting Agarose Gels

1. Place the rubber dams onto the ends of each gel tray (it is easiest to lay the rubber dam on a table and, holding the gel tray, carefully press it into one corner and then use your weight to “roll” the gel tray into the second corner and repeat with the other rubber dam). **Use caution to prevent breaking the gel tray.**
2. Place the gel trays with rubber dams onto a flat surface.
3. Position the comb teeth down over the black mark. **Use the small teeth only, as it will create 10 wells of the needed size.**
4. Swirl the mixture and slowly (to avoid air bubbles) pour 20 ml of cooled agarose solution into each of the 6 casting trays using a graduated cylinder (use a pipette tip to pop any air bubbles).
5. After the gel has hardened (about 30 minutes), gently remove the comb. It is important that the gels have completely solidified before the comb is removed.
6. Being very careful so that the gel does not slide off the gel tray, remove the two rubber dams from each end of the gel tray.
7. The gels may be stored by placing them in a zip-lock bag or other plastic container with ~5 ml of 1X TAE buffer for moisture. If storing gels for more than 1-2 hours,



Chestnut Tree Lab

Laboratory Preparation Instructions for Teachers

keep in dark place, as DNA stain is light sensitive. Refrigeration is best, but not required.

Step 4 – Prepare Electrophoresis Running Buffer

Measure out 1000 ml of 1X TAE buffer from Step 1. This now is the electrophoresis running buffer that will be used to run the gels. The gel box requires approximately 1000 ml of running buffer. The buffer may be stored at room temperature or in a refrigerator.

Step 5 – Electrophoresis of the Samples (following student Laboratory Protocol)

1. The electrophoresis gel box will hold all five gel trays (see Figure 3). The gel trays are labeled “1-6” (there is one extra tray that won’t be used). Assign up to two student groups on one gel tray and assign four wells per group. Each gel has 10 wells, so assign wells #2-5 and wells #7-10 to the two student groups and well #1 will hold the DNA marker that the teacher will add after the students have loaded their samples. There will be an empty well between student groups.
2. After the students have prepared their samples by adding the restriction enzyme, they will need to add 4 μ l of loading dye (step 4 of student protocol). **Accurate pipetting is critical at this step.** If students are not pipetting correctly, they will not have enough loading dye for all of their samples. One of the most common mistakes is students will push the pipette plunger to the second stop when drawing up liquid. This results in them drawing up more than they intended, which will leave them short of loading dye for the rest of their samples. Note that we have included a single “Extra LD for Teacher Use” tube that the teacher can use if students pipette incorrectly and run out of loading dye at their individual stations. Before allowing students to access extra loading dye, the teacher must check student pipetting technique. If the samples get stuck on the sides of the tubes, you may need to centrifuge the samples to bring all the liquid to the bottom of the tube. Once loading dye has been added to the gels, the samples will be loaded dry into the gel at the student’s tables. Students will load 15 μ l of the DNA samples to their assigned wells.
3. The teacher must add a single lane of 15 μ l of DNA Marker to each gel.

Chestnut Tree Lab

Laboratory Preparation Instructions for Teachers

4. Prepare the gel box by inserting the two plastic gel slats (Figure 3). Next, the teacher will very carefully pick up the gel trays and add them to the gel box. Notice there is a notch at the top of the gel tray that fits or “locks” the gel tray into place with the gel slats..
5. Be sure to **place the gel trays in the gel box so the ends containing the wells are closest to the black electrode** or the samples will run backwards. This gel box holds two rows of gel trays, so both rows must be oriented the same way in the gel box (see Figure 3).
6. Next, slowly pour 1000 ml of the 1X TAE running buffer into the bottom chamber of the gel box (nearest the red electrode). **Do not pour the buffer directly onto the gels** or the samples may come out of the wells. The gels in the trays need to be completely submerged to run, but the top of the trays (sides) will be exposed out of the buffer while running.
7. Once gels have been placed in the gel box and the running buffer has been added, be careful not to disturb the electrophoresis apparatus.
8. Place the cover on the gel box matching the black and red electrodes.
9. Connect the gel box with lid to the power supply, again matching black and red electrodes to the colors marked on the ports of the power supply.
10. Follow the printed directions found on the top of the power supply to start the run. For optimal results, the voltage selector on the power supply should be set to 75 or 100 volts and the timer should be set for ~30 minutes. If time is a critical issue, you can check the results sooner, but it may be hard to see the two smaller bands in the GT samples.
11. To confirm proper operation of the power supply, look for bubbles rising from the electrodes and that the samples are moving in the proper direction (“running towards red”).
12. When the gels are done, turn off the power supply and disconnect the lid of the gel box from the power supply.
13. Remove the gel trays from the box. Have each group use the transilluminator to visualize the gels. This is a Blue Light transilluminator, not a UV light so students do **not** need to wear special UV safety goggles.
14. Re-use the running buffer if you are performing this lab with multiple classes. When completely done with the running buffer, it may be poured down a sink drain. Used gels can be disposed of in the trash.
15. After use, the gel box and trays should be rinsed with tap water and allowed to air dry.

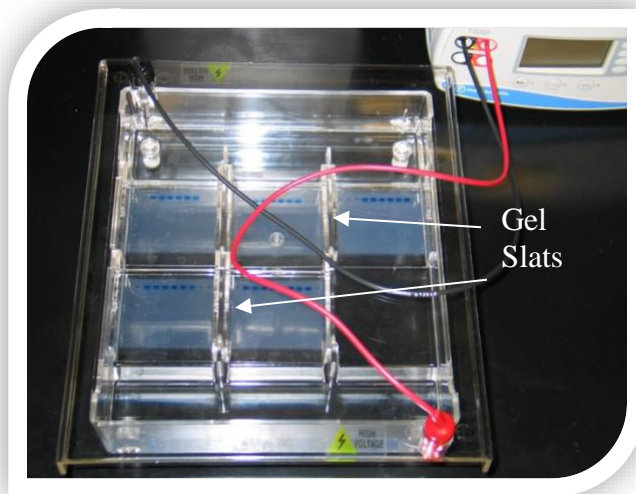


Figure 3. Gel box connected to power supply.

Step 6 – Interpretation of gels

Chestnut Tree Lab

Laboratory Preparation Instructions for Teachers

After running the gels, the pattern of cDNA bands resulting from restriction enzyme analysis should be analyzed. The “UF”, “WT”, and “GT” cDNA samples are controls being run to make sure that the RT-PCR and restriction enzyme digestion is done properly and also to use for comparison of the unknown sample results. The students will be comparing their unknown fungus sample from the new canker to these three control samples. Based on both the number of bands and their sizes (bp=base pairs), they will be able to determine if the fungus cDNA from the new canker has been infected with a hypovirulent virus and if so, whether it is a wild-type or genetically-tagged virus. The DNA marker that consists of standard DNA fragments of known sizes (bp) will be used to determine the cDNA fragment sizes of all four student samples (larger DNA fragments are closest to the wells and the smallest are closest to the bottom of the gel, as they run faster). See the photo in Figure 4 on page 26 for information on the sizes of the bands in the DNA Marker. Remind the students that if only one band of cDNA is seen in a sample, then the restriction enzyme never saw the DNA sequence it can recognize and it was never cut. Therefore, there is only one piece of cDNA. The unknown “NC” new canker samples will be a mixture of “UF”, “WT”, and “GT”, so **results will vary for each student group**.

The students should write their analysis in a lab notebook with evidence to support their results. Students should complete the gel diagram with their results and should also answer the questions on the *Post-Lab Questions* sheet. To facilitate analyzing the sizes of the bands produced, choose a gel, place it on an Elmo (if available), and point out the sizes of the DNA marker bands as labeled in Figure 4.

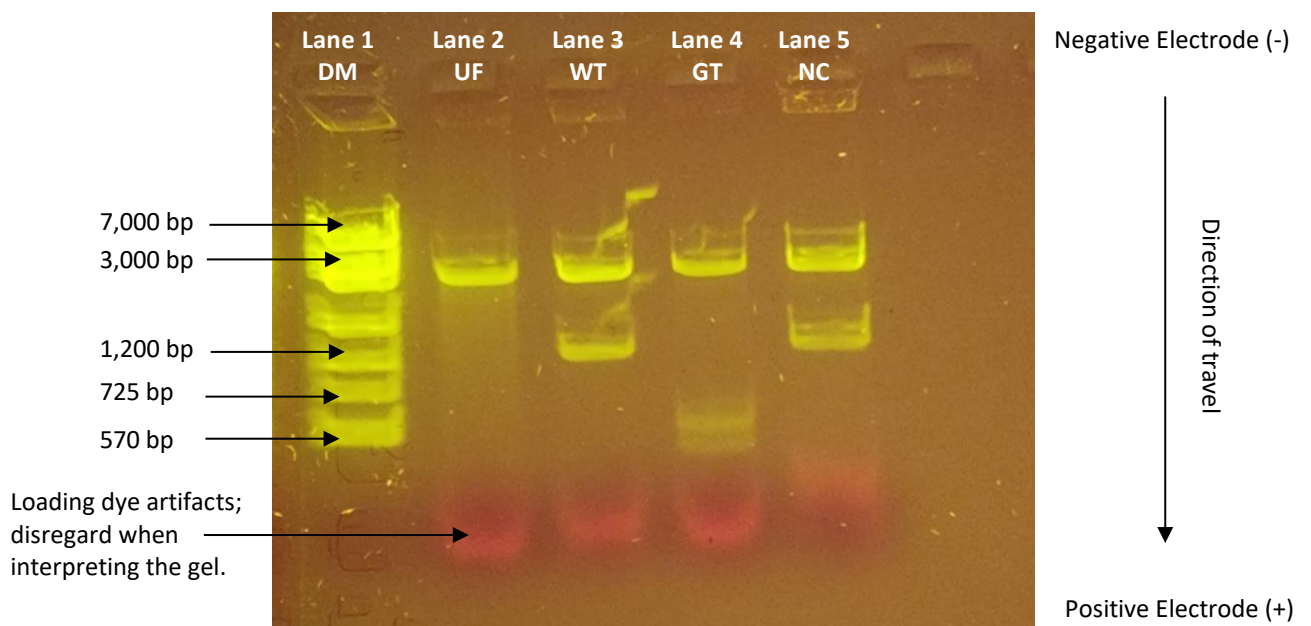


Figure 4. Restriction enzyme analysis of fungal cDNA samples. Lane 1 is uninfected fungal DNA (UF) (3000 bp). Lane 2 is fungal DNA (3000 bp) infected with wild-type (WT) viral cDNA (1200 bp). Lane 3 is fungal DNA (3000 bp) infected with genetically-tagged (GT) viral cDNA (725 bp and 500 bp). Lane 4 is from a new canker (NC). In this example, the new canker contains DNA from the fungus (3000 bp) as well as from genetically-tagged viral DNA (725 bp and 500 bp). Lane 5 is the DNA marker loaded by the teacher and is used as a standard, allowing us to estimate the size in base pairs of the bands from the other samples on the gel.

Answers to Investigation Design

1. Why might a researcher want to make additional copies of cDNA? Are there other applications of PCR?

Answers will vary. This is an example: Additional copies of cDNA are helpful because they can be used to make more of the targeted RNA, can be manipulated (i.e., adding a genetic tag), and then used to make the targeted RNA. Applications of PCR include gaining more DNA for genetic testing, sharing evidence in crime investigations, getting enough DNA to run gels, etc.

2. Does the virus harm the tree? Why or why not?

No, the virus can only infect the fungus. Viruses infect a host by working with specific receptors on host cells. The tree cells do not have the same receptors as the fungus, so the virus cannot infect the tree.

3. Based on your notes and knowledge of viruses, would you expect that the virus could spread naturally to other trees or would it have to be individually and artificially transferred? Explain your thinking.

Answers will vary. The WT virus can transfer without assistance because it has shown up in new places. If the GT virus could spread artificially, why would researchers track GT dispersal?

4. Would it be beneficial for researchers to track the spread of wild-type and genetically-tagged viruses? Explain.

Answers will vary. It is helpful for researchers to track viral spread to learn about how viruses spread and to predict dispersal rates to better determine when and where researchers need to artificially infect cankers.

5. Will the restriction enzyme cut the fungal DNA into smaller pieces? Why or why not?

Not1 will not cut fungal DNA into smaller fragments because the fungal DNA does not have the genetic tag that the restriction enzymes requires.

6. After PCR for each canker sample, the PCR products are exposed to the Not1 restriction enzyme. The genetic tag (cDNA component) will be cut into two segments. How many bands of DNA will be present if there is no virus present? How many bands of DNA will appear if a WT virus is present? If a GT virus is present?

If there is no virus present, then that means there is only one band containing fungal DNA. The WT virus lacks the genetic tag, so it will have 1 band for the fungus and 1 band for the virus (2 bands total). The GT virus has the fungal band and TWO viral bands because Not1 was able to cut the viral genome into two sections (3 bands total).

7. Briefly outline an investigation plan on how you can use the technologies you studied to identify viruses in the cankers. You don't need to worry about specifics like how to run a gel electrophoresis or how much to pipette. Instead focus on the investigation framework starting with the question you want to answer about each canker.

Question: Is there a virus in the canker? If there is a virus in the canker, is it a GT or a WT virus?

Investigation: Compare the DNA from the unknown canker to a WT and a GT virus. How?

1. Collect DNA and RNA from canker.
2. Extract DNA and RNA from the canker.
3. Run RT-PCR.
4. Expose amplified product to Not1.
5. Run gel electrophoresis with cDNA, WT and GT viral samples.

Positive control: GT virus

Negative control: WT virus

Chestnut Tree Lab

Answers to Post-Lab Questions

1. Why were some chestnut tree cankers infected with a virus?

The cankers are caused by a fungus that was accidentally introduced in 1904. This fungus causes cankers to grow and eventually kills the tree. New trees develop cankers after exposure to the fungal pathogen.

2. Why are some viral DNA strands genetically tagged? What is the difference between a genetically-tagged virus and a wild-type virus?

Researchers insert a small DNA sequence into an existing genome in order to identify the virus or other organism or to differentiate the wild-type virus from the virus that the lab created. In the case of chestnut blight, the genetic tag allows researchers to see if the canker is infected by a wild-type fungus or if it was a researcher-planted virus that infected the canker. Being able to identify if a canker is infected with a wild-type or genetically-tagged virus allows researchers to track the spread of the virus.

3. Why must the DNA samples have loading dye added to them?

Loading dye helps weigh the samples so they stay in the wells.

4. Why did the procedure require a DNA marker (DM) well?

The DM well contains a DNA marker. This marker has known lengths (bp) of DNA and is used to compare the sample to estimate the base pairs (bp) in each strand.

5. From the gel, what two things (DNA marker, control samples, and/or unknown "NC" new canker sample) are compared when determining whether the new canker sample "NC" has a virus and what type of virus may be present?

The NC is compared the control samples WT and GT to see if it contains a virus (as determined by bands at ~1200 bp or ~702 and 506 bp. NC should also contain a band at ~3000 bp that indicates that fungal DNA has been amplified. If no fungal DNA is amplified, this indicates something went wrong with your samples or the procedure, as we expect all samples to contain fungal DNA (they may or may not contain viral DNA). If there is band at ~1200 bp, that means the virus is a wild-type virus. If there are two bands at ~702 and ~506, that means the virus was genetically tagged and cut by the restriction enzyme.

6. Remember, DNA is negatively charged, so it travels toward the positive anode on the gel electrophoresis box. What would happen if you forgot to stop the gel and allowed it to run overnight?

The DNA bands would run off the gel, and the gel would look "empty" the next day.

7. If the virus that infects a fungus is naturally occurring, why would scientists want to genetically tag and track it?

Answers will vary. Scientists may need to spread the virus more rapidly than it spreads naturally and want to know whether a particular tree has a natural or researcher-caused infection. Scientists can use data collected to learn about the spread of viral infections in trees and fungi. Without genetic tagging, researchers cannot identify whether the virus is wild-type or lab-based.

8. Summarize the results of your samples and sketch them in the box provided.

Results will vary with each sample.

Chestnut Tree Lab

Answers to Post-Lab Questions

9. Interpret your results, using evidence from your investigation.

Two bands mean the virus was genetically tagged whereas one band means that a virus is present, but it is a WT virus. No bands mean that the canker does not have a viral infection at all. (Bands described do not include the 3000 bp band indicating the presence of fungal DNA.)

10. Biocontrol is the term used to describe living organisms or natural methods to limit growth or fight a “disease process”. In what other applications could biocontrols be used? How else could viruses be used to fight diseases or treat illnesses?

Answers will vary.

Introduction

Think of your favorite wooded location. Alternatively, think of a stereotypical forest in Pennsylvania, the Rocky Mountains, or another iconic wooded location. Describe the forest or sketch it below, noting the types of trees that make that area unique for you.

Now, imagine that a disease or infection significantly impacted one of the key types of trees found there. This is what happened to our area of the woods in the early 1900s. The woods in our area used to be identified as primarily Chestnut-Oak forests. However, a fungal infection was accidentally introduced due to an import of Japanese chestnut trees. The fungus causes a disease in American chestnut trees called “chestnut blight”. Many trees died of the infection, and the current classification of our forests is now Mixed Oak forests.

Scientists began working on the problem of chestnut blight not long after the disease was first presented. Please answer the following questions to prepare for the lab by understanding the disease, the importance of chestnut trees, and the biotechnology currently in use to help combat the disease.

Jigsaw: You will become a class expert on one of the topics below! Research your assigned topic and be prepared to present to your class.

1. **Chestnut Tree:** What contributions did the chestnut tree make to the economy? To local ecosystems? Was it important enough to have any social/song/media references? Explain.

Jigsaw KLEW Chart

KLEW Chart: After completing the Jigsaw, please complete the KLEW chart below.

Know: What do you know about this topic?	Learn: What did you learn?	Evidence: What is your evidence?	Wondering: What are you still wondering?

You will be developing the investigation design for the scenario below after working through the following questions:

Scenario: There is a team of biologists trying to treat the fungus found in blight cankers on chestnut trees with a vegetative compatible fungus that contains a virus, which is debilitating to the original fungus that caused the canker. The hope is to use the treatment fungus that contains the hypovirulent virus to stop the growth and spread of the cankers on the chestnut trees. YOU are part of this team and are investigating whether the fungi from three cankers found on one tree are infected with this hypovirulent virus.

The biologists believed that they have previously treated one of these cankers with a genetically-tagged virus, that one of the other cankers may have had the naturally occurring wild-type virus, and there is a new canker. Your goal is to determine from the three canker samples if they are infected at all with a virus and if so, if it is with a wild-type or genetically-tagged virus. Any uninfected fungal samples would later be treated by the team. You must understand what RT-PCR is, how the cDNA in this lab was obtained, and how restriction enzyme analysis will allow researchers to distinguish between the genetically-tagged (GT) and wild-type (WT) viruses.

RT-PCR. The viral genome is RNA not DNA. RNA is easily degraded and so it cannot withstand the heat used in PCR. To copy the viral genome, researchers must first make DNA from viral RNA. This process is called reverse transcription and produces cDNA.

Once the cDNA has been produced, a technique called PCR can amplify the cDNA to make additional copies. This two-step process of using reverse transcriptase followed by PCR is called reverse transcription PCR or RT-PCR.

1. Why might a researcher want to make additional copies of cDNA? Are there other applications of PCR?

Genetically-Tagged Virus. When researchers infected the original canker on the tree, they used a virus that they genetically modified in the lab to contain a short sequence of DNA that could be cut by a restriction enzyme. Both the genetically-tagged (GT) and naturally occurring (wild-type or WT) viruses infect *Cryphonectria parastica*, the fungus that causes chestnut blight.

2. Does the virus harm the tree? Why or why not?

Investigation Design

Design Your Investigation. Remember to include your research question, the controls, and an outline of broad steps.

7. Briefly outline an investigation plan on how you can use the technologies you studied to identify viruses in the cankers. You don't need to worry about specifics like how to run a gel electrophoresis or how much to pipette. Instead focus on the investigation framework starting with the question you want to answer about each canker.

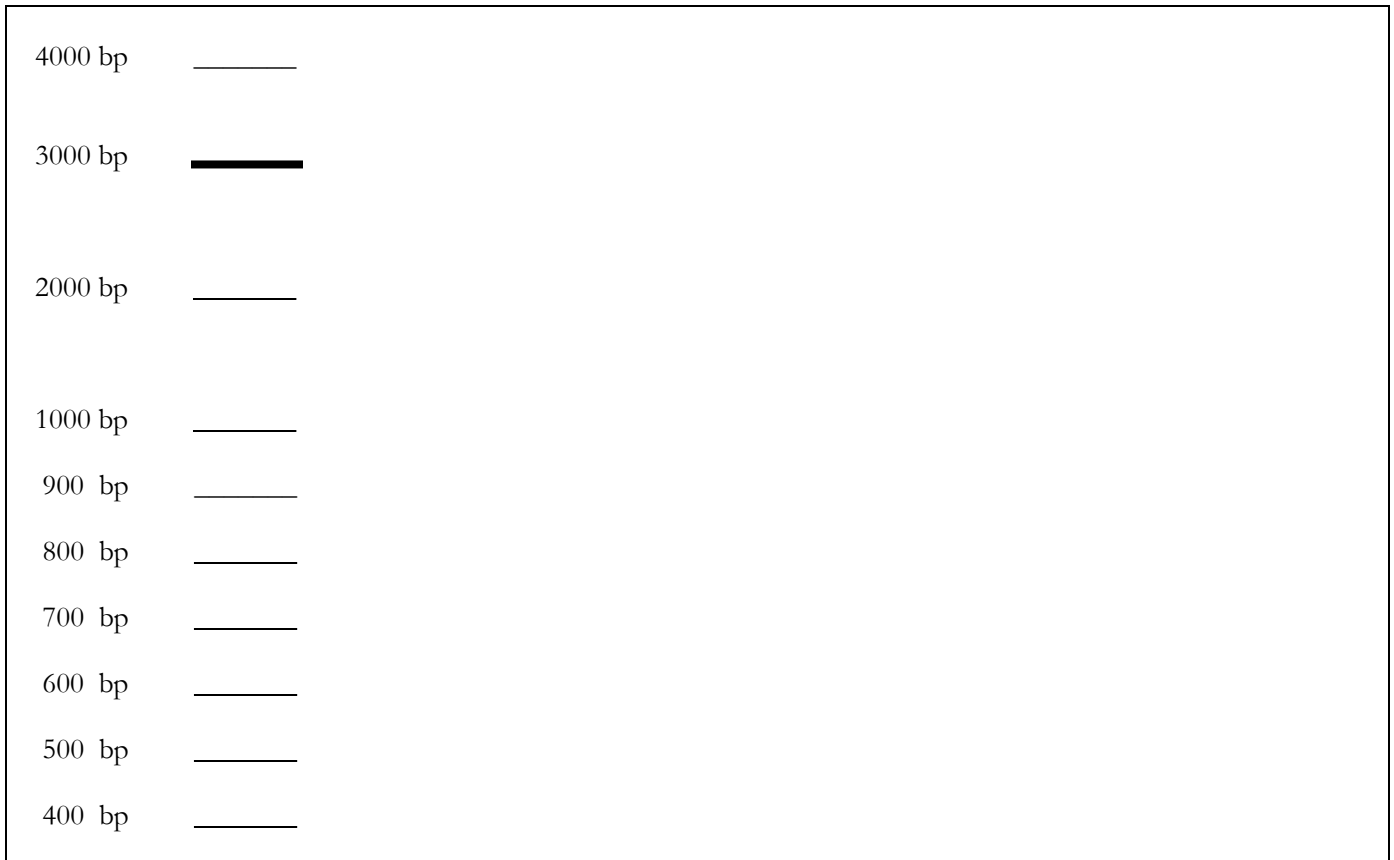
Gel Report

- Examine the DNA base sequences within the 5 cDNA strips from each of the three cankers. Add up the end DNA sequences and the highlighted sequence in the middle for a total base pair count; record the number on the strip. Use a pair of scissors (representing the NotI restriction enzyme as molecular scissors) to cut your DNA strips **only** where you see this base sequence: **GCGGCCGC**. Cut right after the first **GC** in the **GCGGCCGC** sequence (5' to 3') as shown in this 17 base pair (bp) example:

5' ATACCG**GCGGCCGC**CATCC becomes ATACCGC and **GGCCGC**CATCC 3' (7 and 10 base pairs, respectively)
 3' TATGG**GCGGCCGC**TAGG becomes TATGG**CGCCGG** and **CGTAGG** 5' (11 and 6 base pairs, respectively)

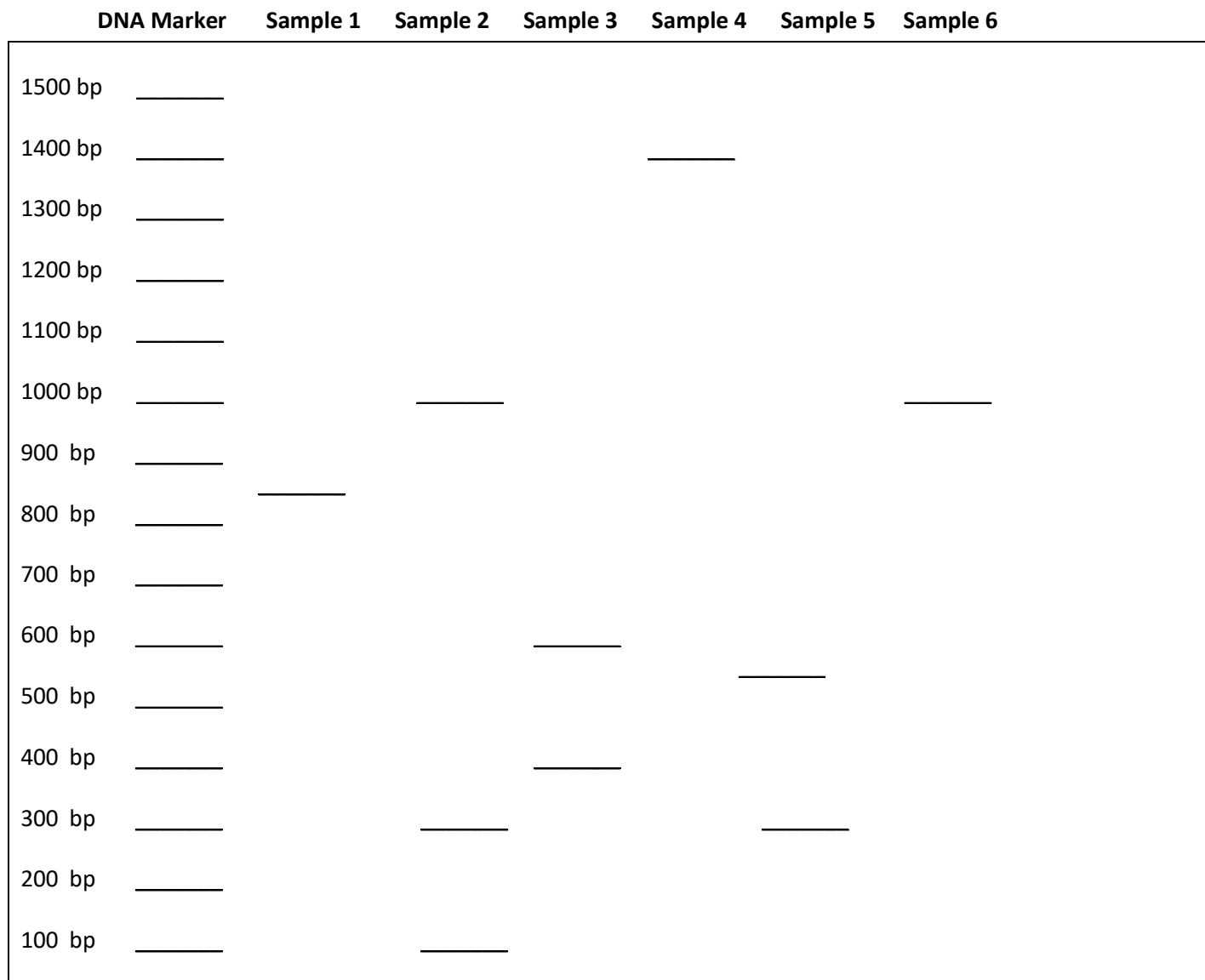
- Some canker cDNA strips may not have the correct DNA sequence to be cut by the restriction enzyme. For each canker cDNA strip that is cut by the restriction enzyme count the number of base pairs on the strips after they were cut and write the new number on the back of each fragment. Fold all cDNA strips into small rectangles so that they will fit in their respective column and represent bands on an agarose gel.
- Using the DNA marker (with standard DNA size fragments) tape your cDNA fragments on the poster sized Gel Chart according to the number of base pairs in each. Be sure to put your canker cDNA fragments in the appropriately marked columns (Hint: PCR product Canker #2 A and B both belong on the gel under Canker #2).

DNA Size Marker (bp=base pairs)	PCR Product Canker #1	PCR Product Canker #2	PCR Product Canker #3
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Additional DNA Fragment Exercise

Instructions: You are given six DNA samples in unlabeled tubes. Three of the six samples are uncut DNA fragments and three have been digested with restriction enzymes. By performing gel electrophoresis on the samples along with a DNA size marker, can you determine which samples are the uncut and cut DNA samples based on the results below? You will need to estimate the size in base pairs (bp) of all fragments in each sample.



Match#1: Sample# _____ (uncut) and Sample# _____ (cut)

Match#2: Sample# _____ (uncut) and Sample# _____ (cut)

Match#3: Sample# _____ (uncut) and Sample# _____ (cut)

Micropipette Use

Micropipette Skills

Laboratory science often involves working with very small volumes, requiring careful measurement of less than one milliliter. The micropipette is a precision instrument used to accurately measure and transfer volumes up to 1 ml.

$$1 \text{ liter} = 1,000 \text{ ml} = 1,000,000 \mu\text{l}$$

How to use a Micropipette

1. Adjust the pipette to 20 μl by turning the dial. Do not turn beyond the volume range for the pipette. Push down on the plunger, locating the first and second stops.
2. Adjust the pipette volume to 4 μl , and again find the first and second stops. What is different this time compared to the first time?
3. Press a new micropipette tip onto the pipette firmly (gently tap the pipette into a micropipette tip while in the box). To prevent contamination, do not touch the micropipette tips with your hands.
4. To practice micropipetting, keep the volume set to 4 μl . To draw liquid into the micropipette tip:
 - a. Press the plunger down with your thumb to the **first stop** to measure the desired volume and hold in that position.
 - b. Holding the pipette vertically, immerse the micropipette tip into the prac dye tube.
 - c. Draw the prac dye into the tip by slowly releasing the plunger. If you see air bubbles, there is a problem with your volume and you will need to repeat this step to get the correct volume.
 - d. To verify the correct volume was extracted, compare the volume in your micropipette tip with the group next to you.
5. To dispense the liquid:
 - a. Place the micropipette tip into the prac dye tube.
 - b. Slowly press the plunger down to the **second stop** to blow out all of the liquid. Keep the plunger pressed down after releasing the liquid until the micropipette tip is out of the tube. This ensures that you do not suck liquid back into the micropipette tip.
 - c. Eject the micropipette tip when done into a waste container by pressing the separate ejector plunger on the side of the micropipette.
6. Repeat steps 4 and 5, but pipette incorrectly by going to the second stop to extract the liquid. Was a different volume drawn up into the micropipette tip compared with the amount drawn initially?
7. Change micropipette tips between each use to prevent contamination.



Practice Gel Loading Exercise

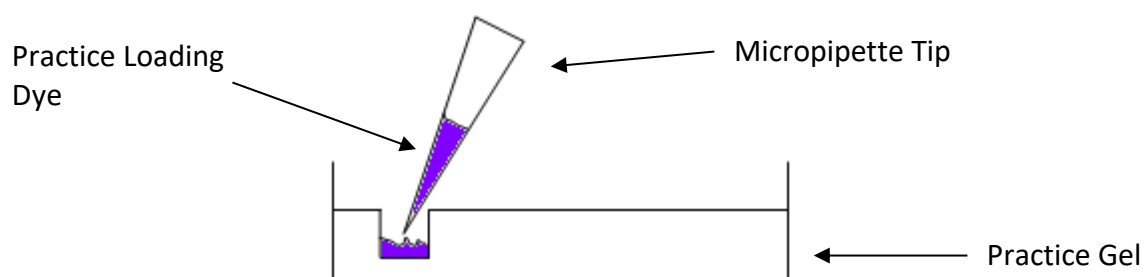
Loading gels skills:

“Loading gels” (filling the wells of a gel) can be a challenging task, especially if one has never done it before. This is an opportunity to practice before you are asked to load the actual samples involved in the laboratory activity. Many people’s hands shake a bit, so people often prefer to stabilize the micropipette while loading the gel. For example, try resting your elbow on the counter while loading, and steady the micropipette by bracing the pipette body against a finger of the other hand, or try placing both elbows on the workbench and work with both hands to raise and lower the micropipette like a drill. Take your time, try different stabilizing positions, and practice filling a few different wells of the practice gels. This is for practice so don’t get frustrated if you spill out of a well or accidentally tear the edge of the agarose gel. Just try again!

An important thing to note about gels: The wells appear as holes but they are not holes. They are more like indentations that do not go through completely to the bottom of the Petri dish. This is why it is so important not to poke the micropipette tip through the bottom of the well or the liquid will seep into the bottom of the dish and not stay in the well.

Loading the practice gels:

1. Adjust your micropipette to 15 μ l and gently tap a micropipette tip onto the end of the micropipette.
2. Remove the lid of the practice agarose gel and make sure you can clearly see the wells.
3. Draw 15 μ l of practice dye (“Prac Dye”) into the micropipette tip.
4. Select a well to pipette the dye into.
5. Lower the micropipette tip filled with the dye into a well to be filled, and gently expel the liquid into the well. Be careful not to poke through the bottom of the well or rip between the wells, or the liquid will not stay in the individual well you chose.



6. Look to see if all of the dye went into the well.
7. Repeat Steps 5-6 at least two or three times until you feel comfortable loading samples into a well. Each person in the group needs to practice loading wells in the practice gel.

Chestnut Tree Lab

Laboratory Protocol

Scenario

As an environmental researcher, you have been asked to save three large surviving chestnut trees that could be used in the blight resistance backcross breeding program with Asian chestnut trees that are resistant to the blight fungus. You have identified a canker on each of the trees that were sampled for vegetative compatibility with a treatment fungus that contains the wild-type hypovirulent virus available in the lab for treating cankers. Each canker was found to be incompatible with the available fungus that contains the hypovirus. As a result, you had to genetically engineer a traceable (tagged) hypovirulent virus in the laboratory to infect a fungus that was compatible with the canker fungus. This genetically-tagged virus was used to treat the cankers that you found on each tree.

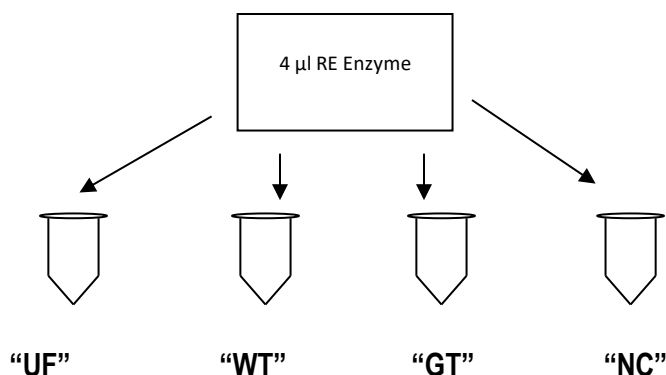
After six months, you returned to the trees to observe the status of the cankers. The cankers stopped expanding and were showing new bark tissue (callus) at the edges. This suggests that the treatment was working. However, you also notice that a new canker appeared on each tree. Because it takes some time before a new canker shows signs of a hypovirulent viral infection (callus growth), the new blight cankers must be tested. If they are infected with a virus, it will be important to determine if the canker contains the wild-type virus or the genetically-tagged virus that was introduced by your lab.

Note: RT-PCR has already been done. You must do the restriction enzyme digestion and gel electrophoresis on all four of your cDNA samples following the directions below:

1. Be sure you have the following tubes: “UF”=uninfected fungus, “WT”=fungus infected with a wild-type virus, “GT”=fungus infected with a genetically-tagged virus, “NC”=fungus from a new canker, “RE”=restriction enzyme, and “LD”=loading dye.

When working with micropipettes, be sure to change your micropipette tip between samples to prevent contamination of your samples and reagents. When adding a small amount of a reagent to your sample, be sure to add the reagent to the bottom of your sample tube and pipette up and down once or twice to mix.

2. **To set up a Restriction Enzyme Digest:** Add 4 μ l of restriction enzyme “RE” to each of the four samples (“UF”, “WT”, “GT”, and “NC”) by adding RE to the bottom of each tube. Remember to change your micropipette tips for each sample tube.



Assign Gels and Sample Wells:

Gel Tray # _____

“UF” well # _____

“WT” well # _____

“GT” well # _____

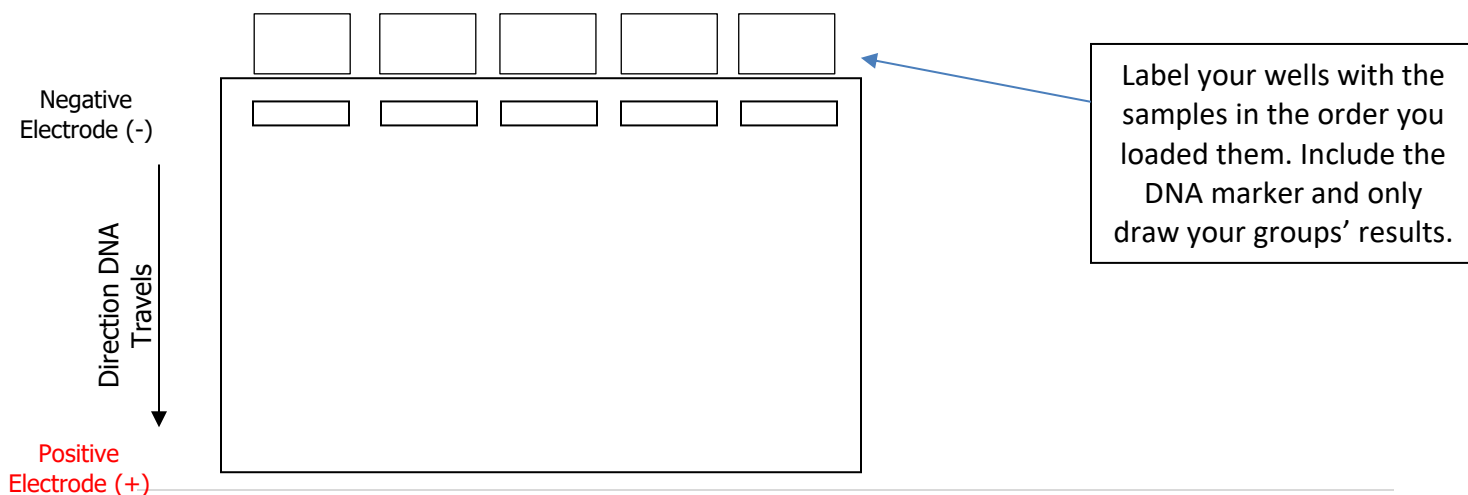
“NC” well # _____

Chestnut Tree Lab

Laboratory Protocol

3. Incubate all sample tubes at room temperature for **5** minutes to allow the enzymatic reaction to occur.
4. Add 4 μl of loading dye "**LD**" to each of the four sample tubes ("UF", "WT", "GT", and "NC") by adding LD to the bottom of each tube. Remember to change your micropipette tips for each sample. Make sure to use proper pipetting technique. Push down to FIRST stop when preparing to draw up liquid, not the second stop, or you will run out of loading dye. Gently pipette up and down to mix each sample. All of the liquid should be at the bottom of the tube. Let your teacher know if you are having trouble because some of the sample is stuck to the sides of the tube. You may need to use the microcentrifuge.
5. Your teacher will assign you wells to load your samples into. Write the wells you have been assigned under #2 on page S13. Load 15 μl of each of the four samples "UF", "WT", "GT", and "NC" (**in that exact order**) into the wells of the gel you have been assigned. Remember to change your micropipette tip for each sample.
6. After all student samples are added, your teacher will come around and pick up your gel, add 15 μl of DNA marker to well #1, and add your gel tray to the gel box.
7. After all gel trays are added to the gel box, your teacher will add electrophoresis buffer to the gel box and cover the box with the lid. Your teacher will connect the gel box to the power supply and check the power supply for the correct settings to be sure the gel runs properly.
8. After the gel is done running, your teacher will turn off the power supply and remove the lid of the gel box. Your teacher will tell you when it is your group's turn to use the Blue Light transilluminator to visualize your DNA bands.
9. Analyze the DNA bands for each sample. Remember to inspect both the **number of bands and the different sizes**. Your teacher will tell you the sizes of the bands contained in the DNA marker. Record your results on the diagram below. Complete the post-lab questions.

Results: Draw the DNA bands you see for your samples and the DNA marker on your gel.



Chestnut Tree Lab

Post-Lab Questions

Directions: Answer the following questions after you have completed the lab.

1. Why were some chestnut tree cankers infected with a virus?
2. Why are some viral DNA strands genetically tagged? What is the difference between a genetically-tagged virus and a wild-type virus?
3. Why must the DNA samples have loading dye added to them?
4. Why did the procedure require a DNA marker (DM) well?
5. From the gel, what two things (DNA marker, control samples, and/or unknown "NC" new canker sample) are compared when determining whether the new canker sample "NC" has a virus and what type of virus may be present?
6. Remember, DNA is negatively charged, so it travels toward the positive anode on the gel electrophoresis box. What would happen if you forgot to stop the gel and allowed it to run overnight?

Chestnut Tree Lab

Post-Lab Questions

7. If a virus that infects a fungus is naturally occurring, why would scientists want to genetically tag and track it?

8. Summarize the results of your samples and sketch them in the box provided.



9. Interpret your results, using evidence from your investigation.

10. **Biocontrol** is the term used to describe using living organisms or natural methods to limit growth or fight a “disease process”.

In what other applications could biocontrols be used?

- 1.
- 2.

How else could viruses be used to fight diseases or treat illness?

- 1.
- 2.

Biocontrol: An approach using living organisms or natural methods to fight a “disease process”.

cDNA: Complementary DNA strand made from RNA using an enzyme called reverse transcriptase.

DNA Gel Electrophoresis: A method of separating DNA molecules based on their size. DNA molecules are forced to run through a gel by placing them in an electric field. The distance they move depends on their size.

Genetic Engineering: Human manipulation of the genotype of an organism or virus that may or may not affect the phenotype. Genetic engineering may include simple methods, such as selective breeding of organisms, or complicated ones like gene cloning.

Genetically Modified Organism (GMO): Use of genetic engineering to produce a desired genotype and/or phenotype. A genetically modified organism is denoted by the acronym GMO.

Genetically Tag (GT): Introducing a genetic change that a researcher can use to track a specific portion of the nucleic acid. A genetic tag does not alter any of the natural functions of the genetic information.

Genotype: Genetic make-up of an organism.

Hypovirulence: A biological control process where a disease-causing organism (i.e., a virus) is debilitated due to an infection caused by a fungal virus (aka hypovirus).

Invasive species: An alien species (plant or animal), also called non-native, non-indigenous, or exotic, is one that is introduced, accidentally or purposefully, into an ecosystem in which it did not evolve and is likely to cause economic or environmental harm or harm to human health.

Polymerase Chain Reaction (PCR): A test tube reaction in which a specific region of DNA is amplified many times by repeated synthesis of DNA using DNA polymerase and specific primers to define the ends of the amplified region. It is a technique used to make more of a very small quantity of DNA in a sample.

Phenotype: Visible or measurable expression of the genes of an organism.

Restriction Enzyme (RE): An enzyme that cuts DNA at specific nucleotide sequences. The function of these enzymes inside cells is to protect the cells from foreign DNA. Researchers have utilized them in genetic engineering techniques.

Chestnut Tree Lab

Vocabulary and Web Resources

Reverse Transcription (RT): Production of cDNA from RNA by reversing the natural transcription process and using an enzyme called reverse transcriptase.

Transfection: Initiating viral infection by introducing viral nucleic acid (purified RNA or DNA) into cells of an organism by chemical or mechanical means. After this, viral replication occurs and viral progeny are generated.

Vegetative Compatibility (VC): A self/non-self-recognition system in fungi that may be used to prevent the genetic exchange between dissimilar strains.

Wild-Type (WT): A naturally occurring organism.

Web Resources

Chestnut Tree Laboratory PowerPoint: A PowerPoint presentation that can be used in your classroom to introduce students to the ideas covered in the MDLL Chestnut Tree laboratory activity can be found here:

<http://www.towson.edu/fcsm/centers/stem/loanerlab/highschool.html>

Biology of Chestnut Tree Blight: Information about the *Cryphonectria parasitica* fungus, which is an invasive species to North America, tree symptoms of blight, and how the fungal infection is transmitted to other trees.

- <http://www.acf.org/>
- <http://www.apsnet.org/publications/apsnetfeatures/Pages/ChestnutBlightDisease.aspx>
- http://www.forestpathology.org/dis_chestnut.html
- <http://www.accf-online.org/Blight/Control/integrat.html>
- http://botit.botany.wisc.edu/toms_fungi/may98.html
- http://www.mdinvasivesp.org/species/other/Chestnut_Blight.html

Fungi Characteristics: Basic structure and life cycle of fungi as well as discussions about vegetative compatibility/incompatibility between different fungal strains.

- <http://www.ucmp.berkeley.edu/fungi/fungi.html>
- <http://ag.arizona.edu/classes/plp427L/Lecture.html>
- <http://www.herbarium.usu.edu/fungi/funfacts/factindx.htm>

Virus Characteristics: Viral composition (viruses can be double or single stranded RNA or DNA) and life cycle.

- <http://www.ucmp.berkeley.edu/alllife/virus.html>
- <http://science.howstuffworks.com/virus-human1.htm>

Chestnut Tree Lab

Vocabulary and Web Resources

PCR and RT-PCR: Basics of Polymerase Chain Reaction and Reverse Transcription-Polymerase Chain Reaction (a two-step process of reverse transcription of RNA first to produce cDNA, then PCR of the cDNA).

- <http://www.accessexcellence.org/RC/VL/GG/polymerase.html>
- http://www.bio.davidson.edu/courses/Immunology/Flash/RT_PCR.html

Gel Electrophoresis: Basic concepts and applications.

- http://en.wikipedia.org/wiki/Agarose_gel_electrophoresis
- <http://www.life.uiuc.edu/molbio/geldigest/electro.html>

Chestnut Tree Lab

Next Generation Science Standards

Performance Expectations: Students’ ability to complete the following performance expectations will be supported by participation in this activity.

HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

HS-LS2-6: Evaluate the claims, evidence, and reasoning that the complex interactions in ecosystems maintain relatively consistent numbers and types of organisms in stable conditions, but changing conditions may result in a new ecosystem.

HS-LS2-7: Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.

HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.

HS-LS4-6: Create or revise a simulation to test a solution or to mitigate adverse impacts of human activity on biodiversity.

HS-ETS1-3: Evaluate a solution to a complex real-world problem based on prioritized criteria and trade-offs that account for a range of constraints, including cost, safety, reliability, and aesthetics as well as possible social, cultural, and environmental impacts.

Dimension	NGSS Code or citation	Corresponding student task in activity
Disciplinary Core Idea	LS1.A Structure and Function <ul style="list-style-type: none"> All cells contain genetic information in the form of DNA molecules. 	In the jigsaw, students will explore how DNA can be used to identify species.
	LS2.C Ecosystem Dynamics, Functioning, and Resilience <ul style="list-style-type: none"> Disruptions in the physical and biological components of an ecosystem—which can lead to shifts in the types and numbers of the ecosystem’s organisms, to the maintenance or the extinction of species, to the migration of species into or out of the region, or to the formation of new species (speciation)—occur 	In the jigsaw, students will explore how the American chestnut tree was an integral part of the ecosystem in the Eastern US. They will consider how the accidental introduction by human of an exotic fungus severely impacted the population of Chestnut trees and disrupted the ecosystem. They will also explore how the presence of naturally occurring virus can render the fungus

Chestnut Tree Lab

Next Generation Science Standards

	<p>for a variety of natural reasons. But many changes are induced by human activity, such as resource extraction, adverse land use patterns, pollution, introduction of nonnative species, and global climate change. Extinction of species or evolution of new species may occur in response to significant ecosystem disruptions.</p>	<p>hypovirulent and might offer a solution for preserving remaining American chestnuts.</p>
	<p>LS3.A Inheritance of Traits</p> <ul style="list-style-type: none">• Each gene on the chromosome is a particular segment of that DNA.	<p>In the jigsaw, students will explore a variety of techniques used by biotechnologists to study DNA, including PCR, cDNA, RT-PCR and restriction enzymes.</p>
	<p>LS4.D Biodiversity and Humans</p> <ul style="list-style-type: none">• Human activity is also having adverse impacts on biodiversity.	<p>In the jigsaw, students will explore how an introduced fungus resulted in the “Chestnut Blight” that has decimated the population of American chestnuts.</p>
	<p>HS-ETS1.A</p> <ul style="list-style-type: none">• Humanity faces major global challenges today, such as the need for supplies of clean water and food or for energy sources that minimize pollution, which can be addressed through engineering.	<p>Students will explore how scientists are using biomarkers and biocontrol techniques to understand and fight the spread of chestnut blight.</p>
Practice	<p>Planning and conducting an investigation</p> <ul style="list-style-type: none">• Ask questions that can be investigated within the scope, of the school laboratory, research facilities, or field, (e.g., outdoor environment), with available resources and, when appropriate, frame a hypothesis based on a model or theory.	<p>Students are asked to outline an investigative plan that takes into account various biotechnology techniques (such as DNA extraction, PCR, RT-PCR) to determine if three canker samples are infected with a virus, and whether the virus is wild-type or genetically-tagged.</p>
	<p>Analyzing and Interpreting Data</p>	<p>Students will analyze the DNA data from the gels they ran using electrophoresis to</p>

Chestnut Tree Lab

Next Generation Science Standards

	<ul style="list-style-type: none"> Analyze data using tools, technologies, and/or models (e.g., computational, mathematical) in order to make valid and reliable scientific claims. 	determine if newly detected cankers contain viral DNA, and if so, if the viral DNA is wild-type or genetically-tagged.
	<p>Construct an explanation</p> <ul style="list-style-type: none"> Apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena and solve design problems, taking into account possible unanticipated effects 	Students will construct an explanation for why scientists would want to genetically tag and track viral DNA found in cankers on American chestnut trees.
Crosscutting Concept	<p>Patterns</p> <ul style="list-style-type: none"> Empirical Evidence is needed to identify patterns. 	Students will examine DNA banding patterns created by using gel electrophoresis for evidence of whether or not a canker found on an American chestnut tree is infected by a hypovirulent virus and if so, whether the virus is wild-type or genetically-tagged.
	<p>Cause and Effect</p> <ul style="list-style-type: none"> Cause and effect relationships can be suggested and predicted for complex natural systems by examining what is known about smaller-scale mechanisms within the system. 	Students will explore the causes and effects that led to the demise of the American chestnut tree.
	<p>Stability and Change</p> <ul style="list-style-type: none"> Much of science deals with constructing explanations of how things change and how they remain stable. 	Students will explore how stable ecosystems can change when non-native organisms are introduced.
<p><u>Nature of Science</u></p> <p>Scientific Knowledge is Based on Empirical Evidence</p> <ul style="list-style-type: none"> Science knowledge is based upon logical and conceptual connections between evidence and explanations. Science arguments are strengthened by multiple lines of evidence supporting a single explanation. <p>Scientific Investigations Use a Variety of Methods</p> <ul style="list-style-type: none"> New technologies advance scientific knowledge. 		

Chestnut Tree Lab

Next Generation Science Standards

Connections to [Common Core State Standards](#)

English Language Arts/Literacy

RST.9-10.2

RST.9-10.3

RST.9-10.4

RST.9-10.7

RST.9-10.10

RST.11-12.2

RST.11-12.3

RST.11-12.4

RST.11-12.7

RST.11-12.8

RST.11-12.10

Advanced Placement Biology Correlation

Standard	Associated Chestnut Tree Activity
APB-1 (whole standard): Scientific Inquiry The learner will develop abilities necessary to do and understand scientific inquiry.	This lab assists in meeting this standard. Specifically, the following activities relate to the standard: Jigsaw, Investigation Design, Wet Lab, Post Lab, and Extension.
APB-5.04: Biological Evolution Analyze the mechanics of evolution, their role, results and implications.	The teacher can guide the Jigsaw and Post Jigsaw Discussion to highlight this standard.
APB-6.04: The Unity & Diversity of Life Analyze evolutionary relationships.	The Jigsaw and Post Jigsaw Discussion particularly highlight this standard.
APB-6.06: The Unity & Diversity of Life Examine past and present research on the unity and diversity of life.	The Jigsaw and Extension activities meet this standard.
APB-7: Ecological Principles Examine the actions and interactions of communities and ecosystems.	The Jigsaw, Post Jigsaw Discussion, and some Extension activities meet this standard.
APB-8: Connecting Themes The major themes of this course are expected to permeate the entire course: science as a process, evolution, interdependence of nature.	This lab assists in meeting this standard throughout the lab activities, specifically in the Jigsaw, Discussion, Wet Lab, Post Lab, and Extension activities.