

the  
**Chestnut Tree Lab**

---

**An Environmental Application of Molecular DNA Technologies**

**Maryland Loaner Lab Teacher Packet**



*Written by the MaBio Foundation and Towson University.*

# Table of Contents

## TEACHER MATERIALS

---

Loaner Lab Overview	2
Loaner Lab Kit Inventory	3
Maryland Science Core Learning Goals	4
Background information	5
Introduction	7
Pre-laboratory Activity	10
Laboratory Explanation	13
Micropipette Challenge Explanation	15
Teacher Laboratory Preparation	17
Data/Observation Sheet Answer Key	21
Extension Activities	22

## STUDENT ACTIVITY HANDOUTS

---

Gel Report	S-1	<i>Used in the Pre-Laboratory Activity</i>
cDNA Strips	S-2	<i>Used in the Pre-Laboratory Activity</i>
Additional DNA Fragment Exercise	S-3	<i>Used in the Pre-Laboratory Activity</i>
Micropipette Challenge	S-4	<i>Used in the Pre-Laboratory Activity</i>
Practice Gel Loading Exercise	S-5	<i>Used in the Pre-Laboratory Activity</i>
Laboratory Protocol	S-6	<i>Used in the Laboratory Activity</i>
Data/Observation Sheets	S-8	<i>Used in the Laboratory Activity</i>



### **Maryland Chapter**

*The Maryland Chapter of The American Chestnut Foundation (MD-TACF) has awarded a grant to the University of Maryland Biotechnology Institute's Education & Outreach Program for the purchase of additional Chestnut Tree Laboratory kits.*

# Chestnut Tree Lab

---

## Loaner Lab Overview

### ***The Chestnut Tree Lab* has three parts:**

- The background information includes an introductory power point presentation that outlines a portion of the research being done at University of Maryland Biotechnology Institute (UMBI) under the direction of Dr. Donald Nuss. The purpose of this power point presentation is to give students a history of the American chestnut tree, show its economic importance, and explain how an invasive species of fungus caused the chestnut tree blight that is responsible for the loss of the native chestnut in the United States. The presentation then goes on to explain how research into viruses that reduce the ability of the fungus to cause disease, is leading to possible treatments. In this lab students will use some of the same molecular DNA techniques used by plant pathologists in the field who are trying to restore the American chestnut tree.
- The pre-lab activity will allow students to: 1) Identify a need for DNA restriction analysis; 2) Model the concept of DNA restriction analysis; 3) Apply DNA restriction analysis in the actual lab to determine the source of possible viral infection within the fungus found in a chestnut tree canker.
- The Chestnut Tree Laboratory is an activity to facilitate learning about genetic engineering and the connection of laboratory science and the study of environmental biotechnology. Students will use cDNA produced from reverse transcriptase-polymerase chain reactions (RT-PCR) from chestnut blight fungi to identify if cankers have been infected by a debilitating virus. The virus is debilitating to the fungus not the tree, and actually can help stop the growth of the chestnut tree cankers. Through the use of DNA restriction enzymes and gel electrophoresis students will determine if the fungus from their canker samples is infected with the virus and, if so, if the virus is a wild-type (naturally occurring) or a genetically tagged virus (altered in the laboratory). Both viruses can convert the fungus to a less pathogenic form – called hypovirulent. As a treatment for the trees, an application of a vegetative compatible virus-containing fungus to the margin of a canker results in fusion of the resident virulent fungal strain with the hypovirulent treatment strain, cytoplasmic transmission of the hypovirus, conversion of the resident strain to hypovirulent and containment of the canker, i.e., prevents additional expansion. It is important to determine if the canker has the hypovirus to predict if the canker will continue to grow and if it needs to be further treated. The differentiation of the origin of the hypovirus (if it is wild-type or genetically tagged ) is useful for the researchers to track the transmission of the virus and in the understanding of how to better treat other chestnut trees with blight.

**Teachers and students who will be performing *The Chestnut Tree* laboratory activity using the Maryland Loaner Lab must first complete the Pre-Laboratory classroom activity. The conceptual aspects of the curriculum will be reinforced with the laboratory activity.**

## Loaner Lab Kit Inventory

### Supplied by Maryland Loaner Lab Program:

Description	Quantity	Comments	Must Be Returned
Gel Electrophoresis box	1	With lid	Yes
Power supply	1	With black cord	Yes
Gel trays	6	Use with combs provided	Yes
Gel combs	6	To make 5 gels + 1 extra	Yes
Black rubber gel tray ends	12	Used to cast gels	Yes
Bottle and 1 bag of 1.2g agarose	1	To make 6 gels	Rinse and Return
2-liter Container	1	To mix 10X TAE & H <sub>2</sub> O	Rinse and Return
10X TAE Buffer	150 ml	Follow Teacher Prep for dilution	Rinse and Return
20 ul Micropipettes	10	1 per group	Yes
Micropipette tips	5 boxes	1 box per two groups	Yes
Staining Trays	2	For five gels	Rinse and Return
Carolina Blu™ Stain Kit	1	Dropper bottle and Stain bottle	Yes
Non-latex gloves	1 pair	Use with gel stain	No
Uninfected fungal cDNA	10 (“UF”)	1 per group <b>Refrigerate until use</b>	No
WT viral infected fungal cDNA	10 (“WT”)	1 per group <b>Refrigerate until use</b>	No
Genetic-tagged viral infected fungal cDNA	10 (“GT”)	1 per group <b>Refrigerate until use</b>	No
Unknown sample: new canker cDNA sample	10 (“NC”)	1 per group <b>Refrigerate until use</b>	No
Sample Loading Dye	10 (“LD”)	1 per group <b>Refrigerate until use</b>	Yes, <b>Keep cold until returned</b>
DNA marker	1 (“DM”)	Teacher uses <b>Refrigerate until use</b>	No
Foam microtube racks	10	1 per group	Yes
Practice Gels	10	1 per group <b>Refrigerate until use</b>	Empty and Return
Practice Loading Dye	10 tubes	1 per group <b>Refrigerate until use</b>	Yes, <b>Keep cold until returned</b>
Microcentrifuge	1	Used to spin samples down in tubes	Yes
Spatula	1	For removing gels from staining tray	Rinse and Return

### Supplied by the Teacher:

Description	Quantity	Comments
Safety goggles	Enough for class	Each student working with the kit’s contents should wear safety goggles
Test Tubes	30	Each group needs 1 empty and 2 with food colored water
Test Tube Rack	10	1 per group
Food Coloring	1 each color	Teachers need to make yellow and blue water for <i>Micropipette Challenge</i>
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 <sub>2</sub> O)	2000 ml	Used to dilute 10X TAE buffer and destain gels
Graduated cylinders	2	One needs to hold 300 ml, One needs to hold 15 ml

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

# Chestnut Tree Lab

## Maryland Science Core Learning Goals

These laboratory activities address several of the Maryland Science Core Learning Goals.

### Goal 1.0 Skills and Processes

1.3.1 <sup>NTB</sup>	<i>The student will develop and demonstrate skills in using lab and field equipment to perform investigative techniques.*/**</i>
1.1.1	The student will recognize that real problems have more than one solution and decisions to accept one solution over another are made on the basis of many issues
1.2.1	The student will identify meaningful, answerable scientific questions.
1.2.6	The student will identify appropriate methods for conduction and investigation (independent and dependent variables, proper controls, repeat trials, appropriate sample size, etc).
1.3.2	The student will recognize safe laboratory procedures. **
1.3.3 <sup>NTB</sup>	The student will demonstrate safe handling of the chemicals and materials of science. **
1.3.4 <sup>NTB</sup>	The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction. **
1.4.2	The student will analyze data to make predictions, decisions, or draw conclusions.
1.5.1	The student will demonstrate the ability to summarize data.
1.5.2	The student will explain scientific concepts and processes through drawing, writing, and/or oral communication.
1.5.4	The student will use tables, graphs, and displays to support arguments and claims in both written and oral communication.
1.5.5	The student will create and/or interpret graphics. (scale drawings, photographs, digital images, field of view, etc.)
1.7.1	The student will apply the skills, processes and concepts of biology, chemistry, physics, or earth science to societal issues.
1.7.5	Students will investigate career possibilities in the various areas of science. ***

### Goal 3.0 Concepts of Biology

3.3.4	<i>The student will interpret how the effects of DNA alteration can be beneficial or harmful to the individual, society, and/or the environment. (lab activity focus) *</i>
3.5.3	<i>The student will investigate how natural and man-made changes in environmental conditions will affect individual organisms and the dynamics of populations. (pre-lab activity focus) *</i>

3.1.1	The students will be able to describe the unique characteristics of chemical substances and macromolecules utilized by living systems.
3.4.2	The student will estimate degrees of relatedness among organisms or species.
3.5.1	The student will analyze the relationships between biotic diversity and abiotic factors in environments and the resulting influence on ecosystems.
3.5.2	The student will analyze the interrelationships and interdependencies among different organisms and explain how these relationships contribute to the stability of the ecosystem.

\* Italicized CLG's are the primary focus of the laboratory activity. The other indicators are addressed, but can also be used as a guide to determine the focus of the lesson plans for the pre-lab and post-lab extension activities.

\*\* All labs include all assessment limits/indicators for Goal 1.Expectation 3: The student will carry out scientific investigations effectively and employ the instruments, systems of measurement, and materials of science appropriately.

# The Chestnut Tree Lab

---

## Background Information

### Terminology:

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

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

**Gel Electrophoresis:** A method of separating molecules based on their size and electric charge. Molecules are forced to run through a gel by placing them in an electric field. The speed at which they move depends on their size and charge.

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

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

**Genetically Tagged:** To introduce a genetic change that a researcher can use to track a specific portion of the nucleic acid. This does not alter any of the natural functions of the genetic information.

**Genotype:** Genetic make-up of an organism.

**Hypovirulence:** Where a disease-causing organism is less able to cause the disease, or where the disease which results is milder. Decreased virulence in a pathogen.

**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.

**PCR:** Polymerase Chain Reaction – 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’s 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:** 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.

**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 which may be used to prevent the genetic exchange between dissimilar strains.

**Wild-Type (WT):** Naturally occurring organism.

## Concepts and Web Resources:

**Biology of Chestnut Tree Blight:** *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.

**Biocontrol issues:** Discuss how the introduction of a virus to the fungi, the fungi that infects the chestnut trees, can act as a method of biocontrol for the blight disease that kills the trees.

<http://www.acf.org/>  
<http://ipm.ppws.vt.edu/griffin/blight.html>  
<http://lamar.colostate.edu/~samcox/chestnut.htm>  
<http://www.apsnet.org/online/feature/chestnut/top.html>  
[http://www.forestpathology.org/dis\\_chestnut.html](http://www.forestpathology.org/dis_chestnut.html)  
<http://www.msu.edu/~borelama/presentation.html>  
[http://botit.botany.wisc.edu/toms\\_fungi/may98.html](http://botit.botany.wisc.edu/toms_fungi/may98.html)  
[http://www.mdinvasivesp.org/species/other/Chestnut\\_Blight.html](http://www.mdinvasivesp.org/species/other/Chestnut_Blight.html)

**Fungi Characteristics:** Basic structure and life cycle. Discussion 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.doctorfungus.org/thefungi.index.htm>  
<http://www.herbarium.usu.edu/fungi/funfacts/factindx.htm>  
[http://bugs.bio.usyd.edu.au/Mycology/Reprodn\\_Dispersal/compatibility.shtml](http://bugs.bio.usyd.edu.au/Mycology/Reprodn_Dispersal/compatibility.shtml)

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

<http://www.microbe.org/microbes/virus1.asp>  
<http://www.ucmp.berkeley.edu/allife/virus.html>  
<http://science.howstuffworks.com/virus-human1.htm>  
<http://library.thinkquest.org/23054/basics/index.html>

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

<http://people.ku.edu/~jbrown/pcr.html>  
<http://www.accessexcellence.org/RC/VL/GG/polymerase.html>  
<http://allserv.rug.ac.be/~avierstr/principles/pcr.html>  
<http://www.faseb.org/opar/bloodsupply/pcr.html>  
[http://www.bio.davidson.edu/courses/Immunology/Flash/RT\\_PCR.html](http://www.bio.davidson.edu/courses/Immunology/Flash/RT_PCR.html)  
<http://www.mywiseowl.com/articles/RT-PCR>

**Gel Electrophoresis:** Basics concepts and applications.

[http://en.wikipedia.org/wiki/Agarose\\_gel\\_electrophoresis](http://en.wikipedia.org/wiki/Agarose_gel_electrophoresis)  
<http://gslc.genetics.utah.edu/units/biotech/gel/>  
<http://www.bergen.org/AAST/Projects/Gel/index.html>  
<http://www.life.uiuc.edu/molbio/geldigest/electro.html>  
<http://arbl.cvmb.colostate.edu/hbooks/genetics/biotech/gels/agardna.html>

# The Chestnut Tree Lab

---

## Introduction

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 explain each aspect for a complete understanding of why DNA technology is being employed in this laboratory investigation. See the Pre-Laboratory Engagement section 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. Student 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 (RT) and then performing PCR (polymerase chain reaction) to obtain a significant amount of cDNA (together a two-step process referred to as 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 and specific DNA primers to define the ends of the region of which to amplify. It 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.

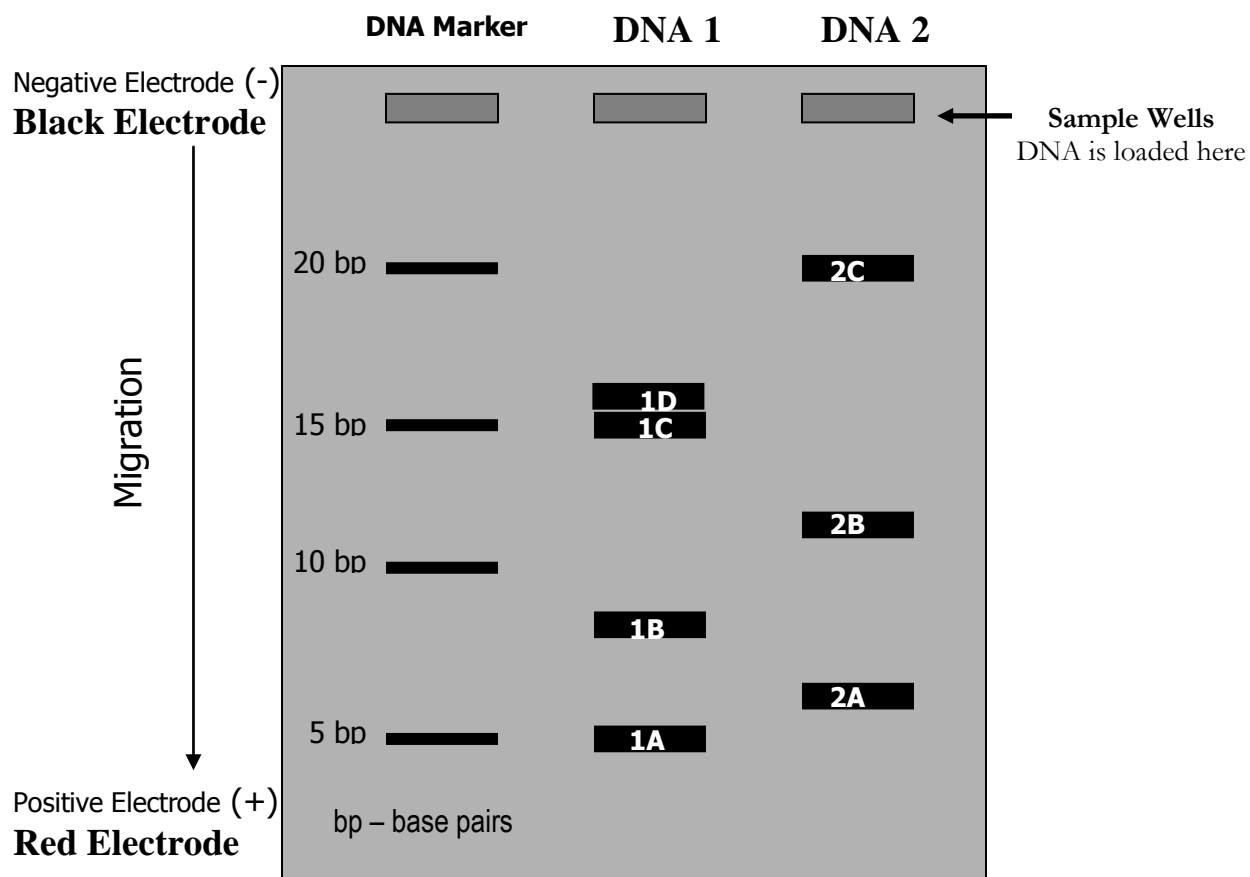
Students should have a basic understanding of fungus and how two fungi samples must have vegetative compatibility for them to share cytoplasmic elements such as viruses. This lab is modeled after what researchers have tried to do by using molecular DNA technology and by applying it to an environmental problem in hopes of solving it. Researches have tried to treat the fungus found in the cankers on the chestnut trees with a vegetative compatible fungus that naturally contains a virus (called hypovirulent) 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. The biologists have resulted in genetically modifying a hypovirulent virus that they can track and use this to transfect (a way to initiate viral infection) the fungus from the new canker. In order to follow this genetically modified virus in nature the viral RNA has been genetically tagged to contain a restriction site, which is not found in the wild-type viral strain. The students should also know that they could 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 analysis is a technique with wide ranging applications in all kinds of research, medicine, forensics, paternity/pedigree testing, etc. DNA restriction 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
- 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 size fragments of DNA can be separated using gel electrophoresis

Gel electrophoresis is a technique for separating 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) towards the positive electrode as shown in Figure 1. The positive electrode is colored red and electrophoresis of DNA is always “Running towards 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 when run on an agarose gel appear as a ladder with many rungs. 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: Gel Electrophoresis of Fragments from Restriction Digest with *Not I***



This lesson is organized into two parts: a Pre-Laboratory activity and a laboratory investigation. During the Pre-Laboratory activity students work in groups to simulate a DNA restriction analysis using paper DNA sequences and scissors 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 genetic tagged virus. 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 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.

The laboratory investigation requires the use of micropipettes for accurately measuring small volumes. ***The Micropipette Challenge*** is an additional Pre-Laboratory activity included with this packet that will allow students practice in using this piece of equipment. If your students are unfamiliar with the use of micropipettes provide additional time to do this activity before students perform the laboratory investigation. All students must be reminded about the proper usage of micropipettes to prevent damage to the equipment and also to provide students with accurate results during the lab.

Another pre-laboratory activity allows the students to practice loading agarose gels as it can be a difficult skill to initially acquire. The ***Practice Gel Loading Exercise*** instructs students to use proper technique when loading gels and gives them the opportunity to practice before loading the samples involved in the laboratory investigation.

# The Chestnut Tree Lab

---

## Pre-Laboratory Activity

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

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

- Identify a need for RT-PCR, DNA restriction digestion, and Gel Electrophoresis
- Model the concepts of DNA restriction 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 per group)
- 5 copies of the **Gel Report** with instructions for each envelope set (one per group)
- 5 copies of the cDNA strips sheet (includes “**PCR Product-Canker #1**”, “**PCR Product-Canker #2 A and B**”, “**PCR Product- Canker #3 A and B**”) (one per group)
- 5 rolls of tape (one per group)
- 5 **poster-sized Gel Chart** as shown on **Gel Report**. (one per group)

### Teacher Preparation (for 5 student groups)

- Prepare a **poster-sized Gel Chart** as shown on the bottom of the **Gel Report** (one per group)
- Photocopy of **Gel Report** with instructions for each envelope set (one per group)
- Label 5 sets of 3 envelopes labeled: “**Canker #1**”, “**Canker #2**”, and “**Canker #3**” (one set of three 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 (one set per group)

### Pre-laboratory Engagement (30 minutes)

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. Tell the students that they are part of this team and they are investigating whether the fungus from three cankers found on one tree are infected with this hypovirulent virus. One of the biologists believed that they have previously treated one of these cankers with the genetic tagged virus and that one of the other cankers may have had the naturally occurring wild-type virus and 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 genetic 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 pairs (bp) viral cDNA PCR product with a restriction enzyme called Not I. The genetically tagged viral cDNA can be cut by the Not I restriction enzyme because it contains the Not I enzyme DNA sequence (added 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 pairs (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 by gel electrophoresis followed by a DNA staining technique (because DNA is invisible in an agarose gel unless stained).

### **Pre-laboratory Exploration (15 minutes)**

Ask the students to follow the instructions on the **Gel Report** to try to determine whether viral infection of the chestnut blight fungus is present, as well as the source of infection (wild-type or tagged virus). The instructions on the **Gel Report** will guide them through the process of DNA restriction analysis. As 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** 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 (15 minutes)**

After each group has finished putting 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 3000bp and are uninfected fungal cDNA. Canker #2 B has 1200bp 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 502bp and 706bp, which means it is the fungus infected with the genetic tagged virus. The Pre-Laboratory activity is directly modeled after the actual laboratory activity and gel results can be seen on page 20 under Teacher lab preparation 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”. 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’s the purpose of using the DNA restriction enzymes?**  
The restriction enzymes cut at very specific DNA recognition sequences and allow us to determine if a virus present in our canker sample was the laboratory modified or tagged virus.
- **What is the purpose of the DNA marker?**  
DNA markers are made up of standard DNA fragments of known sizes and they assist 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 3000bp band is seen then it can be determined that no viral cDNA was present and, therefore, the fungus in the tree canker wasn't infected either naturally from a WT virus or from the treatment with the genetically modified virus that came from the lab.

Emphasize that the distinguishing characteristic of DNA is its size (number of basepairs) and the specific sequence of nucleotide bases. Note that the technique modeled does not sequence the DNA. The technique, DNA restriction 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.

### **Assessment**

Give an index card to each student. Have them describe in their own words what they learned from the activity and discuss briefly other applications for which DNA restriction analysis and gel electrophoresis could be used.

# The Chestnut Tree Lab

---

## Laboratory Explanation

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 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 which will be analyzed
- Perform a restriction digest on cDNA
- Perform agarose gel electrophoresis
- Apply DNA restriction analysis to determine if viral infection (and its source) occurred in fungal samples

### Developing the concept for the Laboratory Activity

This laboratory 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, the concept of RT-PCR, background information regarding fungus and viruses, the concept of DNA restriction digestion using restriction enzymes, the 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.

### Additional pre-laboratory activities (to be performed in advance to the Laboratory Investigation)

Before students perform the laboratory activity discuss with students how to use micropipettes properly (see Micropipette Challenge Explanation). Students will practice using the micropipette by performing *The Micropipette Challenge* prior to the lab. Because loading the wells of agarose gels can be difficult at first, students will be given an opportunity to practice loading dye samples into a practice agarose gel by performing the *Practice Gel Loading Exercise*.

### The Laboratory Investigation

The students will perform a mock restriction digestion on real (pre-digested) DNA samples. Even though the restriction digestion is a mock simulation it will reinforce for students the actual steps necessary to digest or cut DNA using restriction enzymes. The protocol involves adding “Restriction Buffer” and “Restriction Enzyme” to each of their four cDNA sample tubes (“UF”= uninfected fungal DNA, “WT”= fungus infected with wild-type virus, “GT”= fungus infected with genetic-tagged virus, and “NC”= unknown sample: fungus from new canker). The first 3 samples are controls that will be used for comparison of the fourth unknown sample. All samples will undergo the same restriction digestion. The addition of the restriction buffer to the enzymatic reaction is to provide the necessary salts to maintain proper pH for the enzyme to function optimally. The “reaction” in the protocol will take place during the 15 minutes incubation time. Restriction enzyme digestions generally take place in a 37°C water bath for a minimum of an hour for the reaction to occur. This is to ensure complete digestion of the DNA.

Loading dye is added to the samples to help visualize the samples while loading into the wells of the gels and the loading dye also increases the sample density and keeps the samples in the wells while adding the running buffer. When the gels start to run, the loading dye starts to separate into three separate dyes that each have their own sizes based on their molecular weight (blue the largest, purple in the middle, and the yellow is the smallest). The yellow

dye will come very close to the end of the gel after running 30 minutes. It is important for the students to understand that this is not the stained cDNA, it is the loading dye.

Groups will be assigned four wells in a gel to load 20ul 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 20ul of DNA marker to one well in every gel, they will be run for approximately 30 minutes at 200 volts in **cold** 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 stained with the Carolina Blu™, which is mixed into both the agarose gel and the electrophoresis running buffer. A final Carolina Blu™ staining procedure will be performed to darken the bands on the gel and to allow the loading dye to fade in the gel.

# The Chestnut Tree Lab

---

## Micropipette Challenge Explanation

**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. They are expensive and must be used with care. Their accuracy is dependent 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.

### Solution Preparation for Challenge

Make up the colored water solutions (yellow and blue) by adding food coloring to water (for 10 ml of water, add 1 drop of food coloring). Allow 1 ml of each colored solution for each student group.

### Student Stations for Challenge

1 empty test tube            test tube rack            20 ul micropipette            micropipette tips  
2 tubes of 1 ml each (pre-made yellow and blue solutions)

### Basic Directions for Micropipette Use

#### Golden Rules of Pipetting

- 1. Don't rotate the volume adjuster beyond the upper or lower range of the pipette, this can damage it.*
- 2. Don't use a pipette without a tip on it. If this happens, liquid gets into the opening of the pipette and can damage the mechanism inside.*
- 3. Don't lay down a pipette that has a tip filled with liquid. If this happens, liquid can get inside the pipette and can damage it.*
- 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  $\mu$ l) 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 depress 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 depress the plunger control 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 requiring use of the pipettes.

### **Using the Micropipette:**

1. Select the pipette that includes the volume range you will need.
2. Adjust the pipette to the desired volume by turning the dial. Do not turn beyond the volume range for the pipette.
3. Press a new tip onto the pipette firmly (gently tap the pipette into a tip while in the box). Get a tip without touching it with your hands; this is to prevent contamination of the samples.
4. To draw liquid into the micropipette tip:
  - a) Depress the plunger to the first stop to measure the desired volume and hold in that position.
  - b) Holding the pipette vertically, immerse the tip 1-3 mm into the liquid to be transferred.
  - c) Draw the fluid into the tip by slowly releasing the plunger. Wait 1-2 seconds to be sure that the full volume of sample is drawn into the tip. 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 (either your tip wasn't immersed far enough down into the liquid or you perhaps raised your arm while releasing the plunger).
5. To dispense the liquid:
  - a) Place the tip into the container where the liquid is to be released, near the bottom.
  - b) Slowly depress the plunger to the second stop to blow out all of the liquid in the tip. Be careful to not to suck liquid back into the tip by releasing the plunger while the tip is in the liquid you just dispensed.
  - c) Eject the tip when done into a waste container by pressing the separate ejector plunger found on the top or side of the micropipette (depends on the brand of micropipette).

# The Chestnut Tree Lab

## Teacher Laboratory Preparation

Maryland Loaner Lab will supply reagents, equipment, and instruction for the laboratory activity for 10 groups. **Teachers must supply distilled water used for dilutions.**

**Teachers must supply the students with the following handouts:**

Micropipette Challenge, Practice Gel Loading Exercise, Laboratory Protocol, and Data/Observation Sheet

### Prepare Student Stations (for 10 groups):

- One pair of safety goggles per student
- One foam microtube rack
- One tube each: “UF”, “WT”, “GT”, “NC”, “RE”, and “LD”
- One 20 ul micropipette
- One box of micropipette tips (1 box/2 student groups)
- One practice gel
- One practice gel loading dye tube

Activity:	Time needed:
Preparing gels & student stations	30 minutes
Pre-Lab activity	60 minutes
Micropipette Challenge and Practice Gel Loading Exercise	30 minutes
Laboratory activity	90 minutes
Post-Lab activity	10 minutes

**(Use the microcentrifuge to spin down all cDNA and dye samples. Centrifuge for only a few seconds)**

### Shared equipment for multiple groups:

- One agarose gel for every two groups (each group has 4 wells plus the addition of the DNA marker well)
- One gel electrophoresis chamber (gel box) for all 6 gels (5 for the activity with 1 extra.)
- One tube of DNA marker “DM” (teacher will load 20ul 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, and not water, must be used to make and run the gels. The buffer supplies the necessary ions to conduct the electricity. The buffer received in the kit is 10X Tris-Acetate-EDTA (TAE) with 150 ml in three conical tubes, and needs to be diluted with distilled water (dH<sub>2</sub>O) to make a 1X concentrated solution.

1. Add the entire 150 ml of 10X TAE buffer to 1350 ml of distilled water in the 2-liter container provided, 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. **Keep this buffer cold in a refrigerator.**

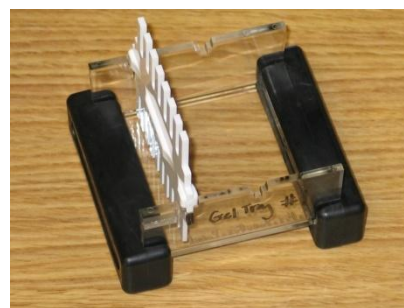
#### Step 2 - Prepare a set of six 1.0 % agarose gels (5 for the activity with 1 extra gel.)

Agarose gels and running buffer should be made the night before use or the day of use, to prevent fading of the Carolina Blu™ stain. This prep will make 5 small gels. Each gel will have 10 wells and will accommodate two groups of students with 4 samples each and a well for the DNA marker. Before making the agarose solution have casting trays prepared and ready to be used (see Step 3).

1. Pour the 1.20g of agarose into the bottle marked “Agarose” and dilute it with the 120 ml of the 1X TAE buffer from Step 1 (use a graduated cylinder to fill the bottle to 120 ml). Add the buffer to the bottle and mix well with the agarose.
2. 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 over heat** as the liquid will boil out of the bottle and spill. It is best to microwave in small time intervals and to mix, then continue heating. The agarose must be completely in solution and well mixed. No particulate matter should be visible.
3. Cool the agarose solution to about 60°C by placing the melted agarose in a 60°C water bath or by allowing it to stand 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, in which it starts 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).
4. Add 5 drops of Carolina Blu™ stain (from the dropper bottle) to the bottle of 120 ml of the cooled agarose and swirl to mix. This stain is not toxic; however, it will stain your hands so wear gloves. Immediately pour into gel trays with rubber dams (see next step).

### 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 then using 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, with the small teeth down, over the black mark (**Use the smallest 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 cooled agarose solution into the 6 casting trays using a graduate 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. Refrigeration is best but not required.
8. Gels stored longer than two to three days tend to fade and the DNA may not be visible during electrophoresis. These gels are still usable, but will require longer staining after electrophoresis.

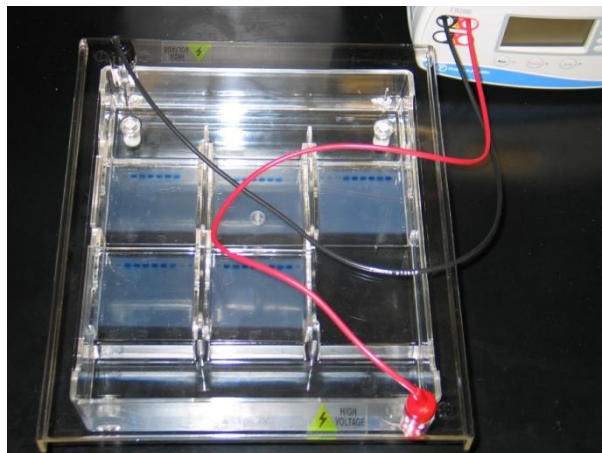


### Step 4 – Prepare Electrophoresis Running Buffer

1. To the 1000 ml of 1X TAE buffer from Step 1 add 49 drops of Carolina Blue and mix well. This now is the electrophoresis running buffer that will be used to run the gels. The gel boxes require approximately 1000 ml of running buffer. **It is very important to keep the running buffer cold in the refrigerator until used. Cold TAE running buffer is needed due to the heat that is generated while running the gels at 200 volts.**

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

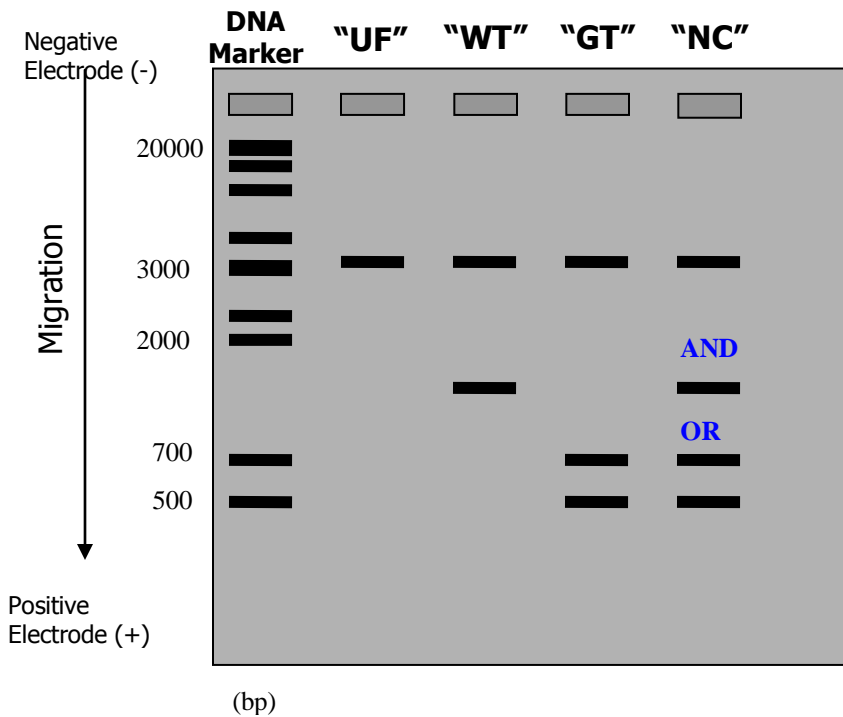
1. The electrophoresis gel box will hold all six gel trays. The gel trays are labeled “1-6”. Assign up to two student groups on one gel tray and assign four wells per group. Each gel has 10 wells, so assign wells #2-5 and wells #7-10 to the two student groups as well #1 will hold the DNA marker that the teacher will add after students have loaded their samples. There will be an empty well between student groups.
2. Next, the gels will be loaded dry at the student’s tables. Students will load 20  $\mu$ l of the DNA samples to their assigned wells then the teacher will add 20ul of the DNA marker “DM” to the first well of each gel. Finally, 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” into place in the gel box).
3. 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 picture).
4. Next, slowly pour 1000 ml of the 1X TAE COLD **running buffer** into the bottom chamber of the gel box (nearest the red electrode). **Do not pour the buffer directly onto the gel** 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.
5. Once gels have been placed in the gel box and the running buffer added, be careful not to disturb the electrophoresis apparatus.
6. Place the cover on the gel box matching black and red electrodes.
7. 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.
8. Follow the printed directions found on the top of the power supply to start the run. The voltage selector on the power supply should be set to 200V and the timer should be set for 30 minutes.
9. 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”)
10. When the gels are done turn off the power supply and disconnect the lid of the gel box from the power supply.
11. Remove the gel trays from the box. See if the DNA bands are visible and considered dark enough to obtain results. Usually final staining is necessary. The DNA bands are best visualized when viewed against a white background or even better on a light box.
12. If DNA bands on the gel are not visible (or dark enough) then place the gels into the two staining trays and cover them with the Carolina Blu™ Final Stain (large bottle split between two staining trays) for 20 minutes. After staining and with gloves on, remove the gels and place them on a plastic surface or plastic wrap. **The used final stain should be poured back into its bottle and returned with the equipment (please mark on the bottle if you used it)**. Place the gels back into the staining tray and rinse them off with **distilled water** and pour it out. Destain them by adding **distilled water** to the staining tray and allow them to sit for 15 minutes. Change the distilled water and repeat. Destain can be poured down a sink drain.
13. When done with the running buffer it may be poured down a sink drain. Used gels can be disposed of in the trash.
14. After use, the gel box and trays should be rinsed with tap water and allowed to air dry.



### Step 6 – Interpretation of gels

After running and staining, the pattern of cDNA bands resulting from restriction analysis should be analyzed. The loading dye that separated into three colors while the gels ran should have faded during the final stain procedure. 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 genetic-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). 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 recognizes and the DNA 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** (as reflected in figure 2) and the teacher must verify individual group results.

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 *Data/Observation Sheet*. To facilitate analyzing the sizes of the bands produced choose a gel and put it on an overhead projector and point out the sizes of the DNA marker bands as labeled in figure 2.



**FIGURE 2: Restriction Analysis of fungal cDNA samples**

# Chestnut Tree Lab

---

## Data/Observation Sheet Answer Key

### Questions:

1. Does the fungus from the new canker “NC” sample have a virus? If the fungus does have a virus, is the virus wild-type or genetically tagged? Results will vary for each student group. You must determine your own results from your gel and **verify with your teacher**.

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

A. The “NC” sample is compared against the DNA marker.

B. It is also compared against the control samples (“UF”, “WT”, and “GT”).

3. What is the difference between a wild-type and a genetically-tagged virus?

The wild-type virus (WT) is the naturally occurring form of the virus and does not contain the genetically modified DNA tag. It should have 2 bands (at 3000 bp and 1296 bp). The genetically tagged virus (GT) has been modified to contain a genetic tag that makes it easier for researchers to identify. It should have 3 bands (at 3000 bp, 750 bp and 546 bp).

4. What 3 DNA technologies are used in this laboratory (hint: one was done for you)? Briefly in one sentence describe what they are used for.

A. **RT-PCR:** The enzyme reverse transcriptase is used to produce a complementary DNA (cDNA) product from viral RNA. The cDNA is then copied or multiplied to produce a useable amount.

B. **Restriction Enzyme Digestion:** A restriction enzyme is used to cut the DNA at specific locations.

C. **Gel Electrophoresis:** An electrical charge is used to push DNA fragments through a gel. Different size fragments will move through the gel at different rates. Larger fragments will not move as far as smaller fragments.

# The Chestnut Tree Lab

---

## Extension Activities

The following extension activities may be used to reinforce the concepts introduced during the Pre-Laboratory activity and the Laboratory activity.

- I Ask the students to write a letter to a friend and describe what they did in the laboratory, which is modeled after actual research, attempting to help the devastated American chestnut tree.
  
- II Biocontrol is an approach of trying to use 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 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. Good links are:  
<http://www.nysaes.cornell.edu/ent/biocontrol/>  
<http://en.wikipedia.org/wiki/Biocontrol>  
<http://www.anbp.org/a-bioinfo.htm>
  
- III Have students investigate and report on the work being done by biologists in back-cross breeding programs with the blight resistant Asian chestnut trees and the blight susceptible American chestnut trees. Early breeding efforts simply tried to make hybrids of the Asian trees with the 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 the American chestnut trees, which increased the percentage of American tree genes into the hybrids with the resistance genes. The goal is to produce a mostly American chestnut tree, exhibiting its unique physical characteristics, that has the resistance genes to chestnut tree blight. Good links are:  
[http://www.acf.org/r\\_r.htm](http://www.acf.org/r_r.htm)  
<http://www.apsnet.org/online/feature/chestnut/top.html>  
[http://www.patacf.org/bc\\_pgm.htm](http://www.patacf.org/bc_pgm.htm)
  
- IV There are many examples of invasive species (plants, weeds, animals, aquatic species, insects, and microbes) which 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 attempts to fix an environmental problem and they would not be considered “invasive”. Have students research and give a report on an invasive species. Good links are:  
[http://www.mdinvasivesp.org/what\\_is\\_an\\_invasive\\_species.html](http://www.mdinvasivesp.org/what_is_an_invasive_species.html)  
<http://www.ioe.ucla.edu/reportcard/report03/InvasiveSpecies.htm>  
<http://nature.org/initiatives/invasivespecies/about/>

# GEL REPORT

**Instructions:** 1) 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 Not I restriction enzyme as molecular scissors) to cut your DNA strip **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' ATAC**GCGGCCGC**CATCC becomes ATAC**GC** and **GGCCGC**CATCC 3' (7 and 10 base pairs, respectively)  
 3' TATGG**GCGGCCGC**TAGG TATGG**GC**CCGG and **CGTAGG** 5' (11 and 6 base pairs, respectively)

2) 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.

3) 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
---------------------------------------	-------------------------	-------------------------	-------------------------



**PCR PRODUCT CANKER#1 (ENVELOPE 1)**

<b>1490 Base Pair DNA Sequence</b>	<b>ATCCGATTCCATTTAACGGT TAGGCTAAGGTAAATTGCCA</b>	<b>1490 Base Pair DNA Sequence</b>
--	--	--

Canker #1= \_\_\_\_\_bp total

1 (IN ENVELOPE 1)

**PCR PRODUCT CANKER#2 A (ENVELOPE 2)**

<b>1490 Base Pair Sequence</b>	<b>ATCCGATTCCATTTAACGGT TAGGCTAAGGTAAATTGCCA</b>	<b>1490 Base Pair Sequence</b>
------------------------------------	--	------------------------------------

Canker #2 A= \_\_\_\_\_ bp total

2A ( IN ENVELOPE 2)

**PCR PRODUCT CANKER#2 B (ENVELOPE 2)**

<b>690 Base Pair DNA Sequence</b>	<b>ATACCGGGGCTGCATCCATA TATGGCCCCGACGTAGGTAT</b>	<b>490 Base Pair DNA Sequence</b>
---------------------------------------	--	---------------------------------------

Canker #2 B= \_\_\_\_\_ bp total

2B (IN ENVELOPE 2)

**PCR PRODUCT CANKER#3 A (ENVELOPE 3)**

<b>1490 Base Pair Sequence</b>	<b>ATCCGATTCCATTTAACGGT TAGGCTAAGGTAAATTGCCA</b>	<b>1490 Base Pair Sequence</b>
------------------------------------	--	------------------------------------

Canker #3 A= \_\_\_\_\_ bp total

3A (IN ENVELOPE 3)

**PCR PRODUCT CANKER#3 B (ENVELOPE 3)**

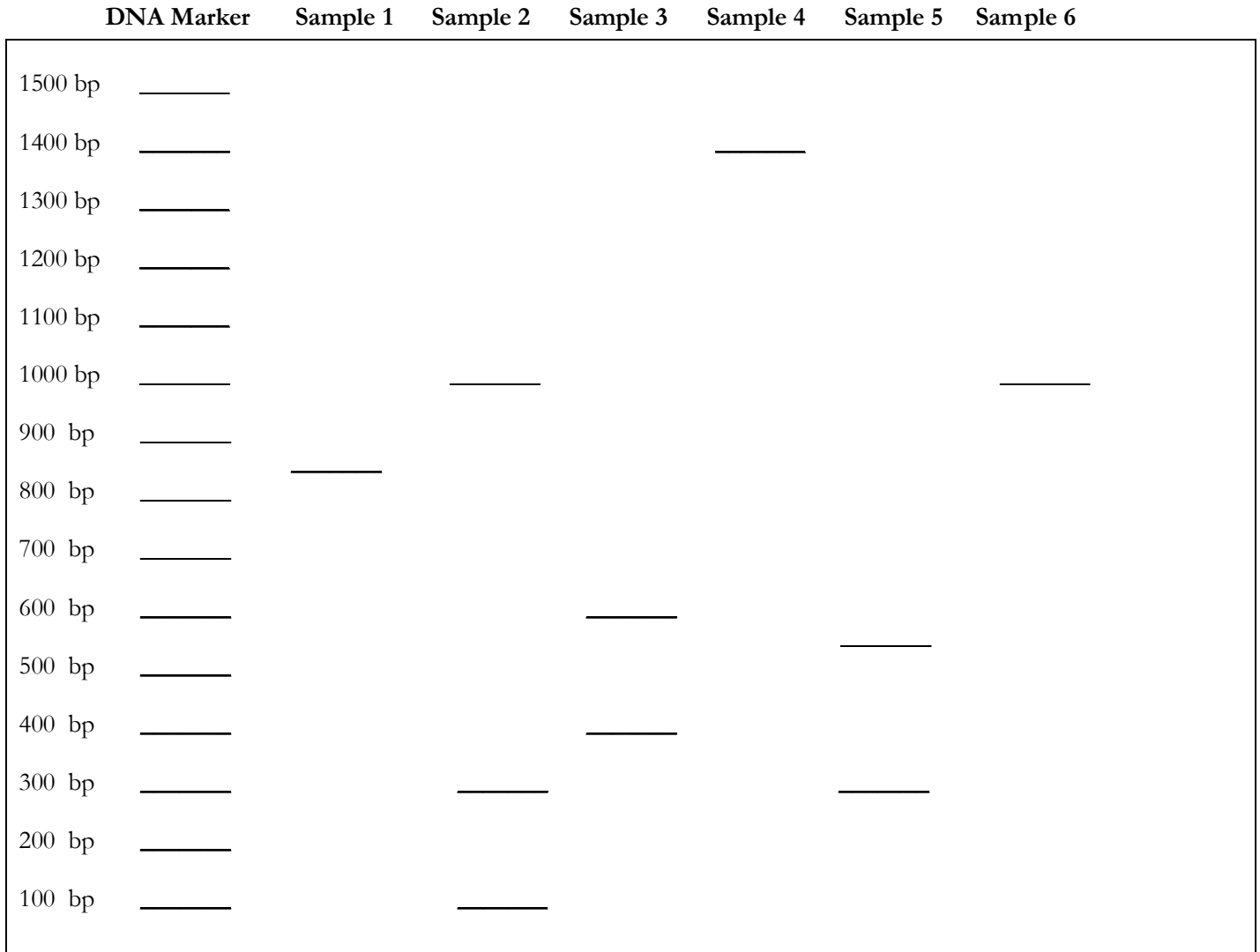
<b>492 Base Pair DNA Sequence</b>	<b>GATATACCGCGGCCGCATCC ATATATGGCGCCGCGTAGG</b>	<b>696 Base Pair DNA Sequence</b>
---------------------------------------	---	---------------------------------------

Canker #3 B= \_\_\_\_\_ bp total

3B (IN ENVELOPE 3)

## 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 matches 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 Challenge

Laboratory science often involves working with very small volumes of liquid; frequently millionths of liters are used. One millionth of a liter is equal to one microliter, abbreviated 1  $\mu\text{l}$ . (1 liter = 1,000 ml = 1,000,000  $\mu\text{l}$ )

You can imagine that it would be very difficult to measure such small volumes without a very accurate and precise instrument. The instrument most often used to measure microliters is called a micropipette. Micropipettes differ in the volume of liquid they can accurately measure.

To help you become accustomed to using micropipettes you will be given two tubes, one has blue food coloring; the other has yellow food coloring. Practice using the micropipettes by adding the amounts listed to an empty tube. Notice how very small the volumes really are. Discuss with your class what is considered good micropipette technique and how to use micropipettes properly.

Remember to change pipette tips between different color solutions, so not to contaminate them. (option: you could pipette all the yellow first, and then change the tip and pipette the blue last) Watch each other pipette and check each other's technique. Ask your teacher for help if you have questions about using the micropipettes.

## Example:

Amount to add to tube	Color	Record the setting as it appears in the window
15 $\mu\text{l}$	blue	15.0

Add the following amounts to an empty tube – all of the amounts will be added to the same tube:

Amount to add to tube	Color	Record the setting as it appears in the window
12 $\mu\text{l}$	yellow	
5 $\mu\text{l}$	blue	
8 $\mu\text{l}$	yellow	

How many microliters ( $\mu\text{l}$ ) should you have in the test tube when you are done? \_\_\_\_\_

How many milliliters (ml) should you have in the test tube when you are done? \_\_\_\_\_

# The Chestnut Tree Lab

---

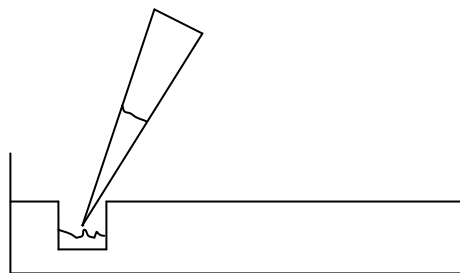
## Practice Gel Loading Exercise

“Loading gels”, or 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. Take your time, figure out how you feel most comfortable doing this (example: some people like to rest their elbow on the counter while loading), and practice filling a few different wells of the practice gels. Remember, this is for practice so don't get frustrated if you spill out of a well or if you accidentally tear the edge of the agarose gel - try it again.

**An important thing to note about 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 else the liquid will seep into the bottom of the dish and not stay in the well.

### Loading the practice gels

1. Become familiar with the feel of the two stops on the micropipette by pressing on the plunger a few times. You should notice a difference in resistance and the amount of pressure required between the two stops. The first stop is used to suck the liquid into the tip and the second stop is to let the liquid completely out of the tip.
2. Adjust micropipette to 15  $\mu$ l.
3. Make sure you gently tap a tip onto the end of the micropipette.
4. Remove the lid of the practice agarose gel and make sure you can clearly see the wells.
5. To suck up the practice loading dye into the tip, press down to the first stop outside of the tube then place your tip into the liquid dye and slowly lift up your thumb. (Be careful not to raise your hand while lifting your thumb or you'll get air bubbles and the volume will be incorrect)
6. Select a well to pipette the dye into.
7. Lower the tip filled with the dye into a well to be filled. 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.



8. To release the dye from the tip, press down to first stop and then keep pressing down until you reach the bottom of the second stop. While the second stop is still pressed in lift up the micropipette so the tip is no longer in the well (or you may accidentally suck the liquid back into the tip).
9. Look to see if all of the dye went into the well.
10. Repeat this 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. You do not need to change tips since you will be using the same liquid between group members.

## Laboratory Protocol

### Scenario

As an environmental researcher, you have been asked to save a large surviving chestnut tree 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 the tree, which you have sampled for vegetative compatibility with treatment fungi that contains the wild-type hypovirulent virus that is available in the lab for treating cankers. It was found to be incompatible to the available fungi that contain the hypovirus. In the laboratory, you had to genetically engineer a traceable (tagged) hypovirulent virus and use it to infect a fungus that was compatible with the canker fungus. This was used to treat the canker that you found on the tree.

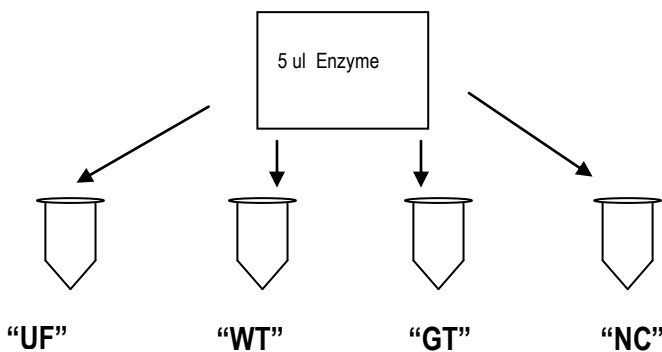
After six months, you return to the tree to observe the status of canker. The canker had stopped expanding and was showing new bark tissue (callus) at the edges. This suggests that the treatment was working. However, you also notice that a new canker has appeared. Since it takes some time before a new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

**Note:** the 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 these tubes in your rack: “UF”=uninfected fungus, “WT”=fungus infected with wild-type virus, “GT”=fungus infected with genetically-tagged virus, “NC”=fungus from new canker, “RE”=restriction enzyme and “LD”=loading dye.

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

2. **To set up a Restriction Enzyme Digest:** Add 5 ul of restriction enzyme “RE” to each of the four samples “UF”, “WT”, “GT”, and “NC”, by adding it to the bottom of the tube with the cDNA. Remember to change your tips.



### Assign Gels and Sample Wells:

Gel Tray # \_\_\_\_\_

“UF” well # \_\_\_\_\_

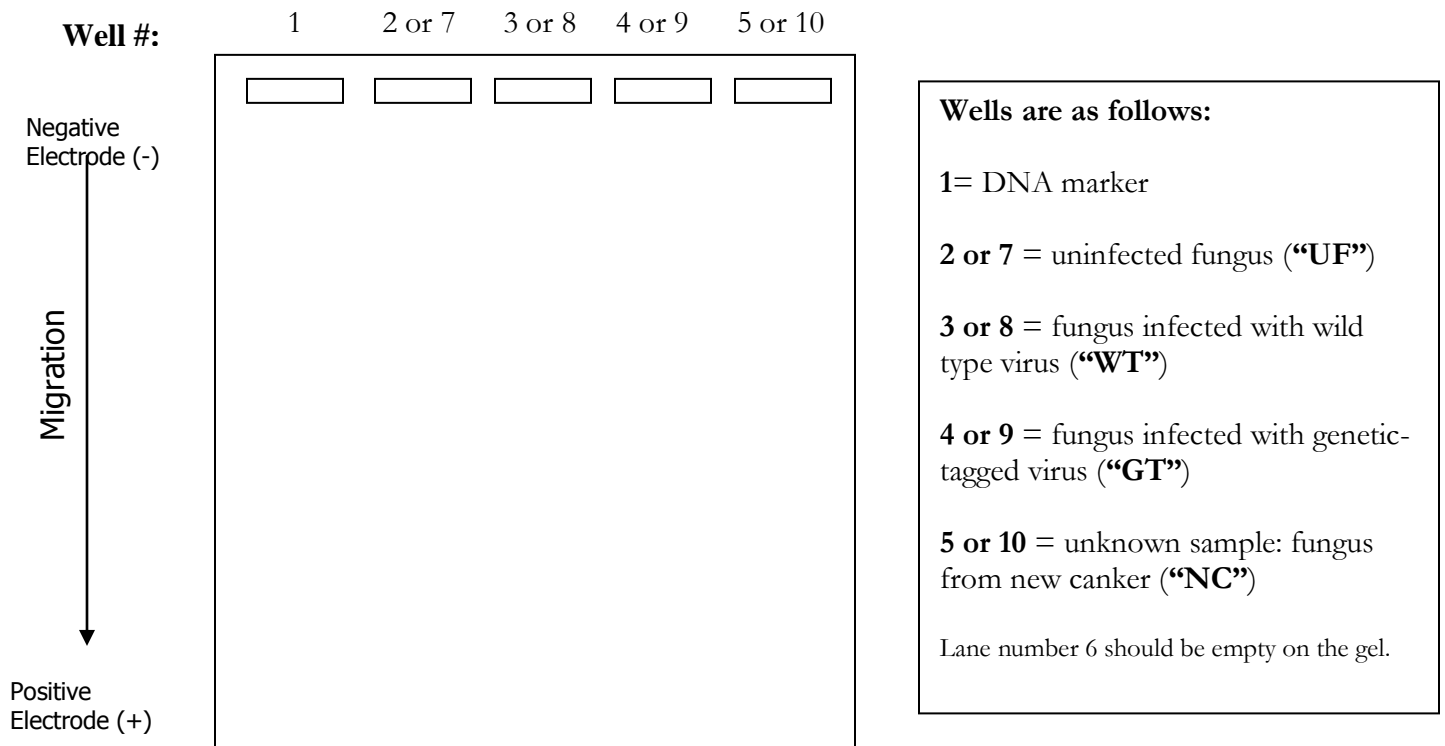
“WT” well # \_\_\_\_\_

“GT” well # \_\_\_\_\_

“NC” well # \_\_\_\_\_

3. Incubate all the samples at room temperature for **5** minutes for the enzymatic reaction to occur.
4. Add 5 ul of loading dye **“LD”** to each of the four samples “UF”, “WT”, “GT”, and “NC” by adding it to the bottom of the tube with the DNA. Remember to change your tips.
5. Load 20 µl of each of the four samples “UF”, “WT”, “GT”, and “NC” (**in that exact order**) into the wells of gels you have been assigned by your teacher. Remember to change your tips.
6. After all student samples are added your teacher will come around and pick up your gel and add 20ul 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 with the lid. Your teacher will connect the gel box to the power supply and check it for the correct settings to be sure the gel runs properly. The gel will run for about 30 minutes at 200 volts.
8. After the gel is done running your teacher will turn off the power supply and remove the lid of the gel box. If the gels need to be stained to make the DNA bands darker your teacher will do this for your group.
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 bands contained in the DNA marker. Record your results on the diagram below. Complete the questions on the Data/Observation Sheet.

**Results:** Draw the DNA bands you see for your samples and the DNA marker on your gel.  
Your samples are either in wells # 2-5 or #7-10.



# Chestnut Tree Lab

---

## Data/Observation Sheet

### Questions:

1. Does the fungus from the new canker “NC” sample have a virus? If the fungus does have a virus, is the virus wild-type or genetically tagged? Results will vary for each student group. You must determine your own results from your gel and **verify with your teacher**.
2. From the gel, what two things (DNA marker, control samples, and/or unknown “NC” new canker sample) get compared when determining whether the new canker sample “NC” has a virus and what type of virus may be present?
  - A.
  - B.
3. What is the difference between a wild-type and a genetically-tagged virus?
4. What 3 DNA technologies are used in this laboratory (hint: one was done for you)? Briefly in one sentence describe what they are used for.
  - A.
  - B.
  - C.