Biotech in the Classroom: Laboratory Manual

Tomato Spotted Wilt Virus

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Cover Art: Brandon Laufenberg @iStockphoto.com

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http://biotechintheclassroom.webs.com
How to Use this Book

SECTION ONE
Section One acquaints the user to the curriculum. This section contains an Introduction to provide background and rational for the curriculum’s development and use, as well as the individuals and groups that provided support.

SECTION TWO
The second section is the heart of the Biotech in the Classroom Laboratory Manual. It contains complete lesson plans, student worksheets, and teacher answer keys for the four laboratory experiments. Each lesson plan is arranged in the same format and includes the following components:

Key Question(s): Describes the primary focus of the experiment in question form. The key question(s) can be used to engage the learners.

Overall Time Estimate: Provides time estimates for advance preparation and actual lab activity.

Vocabulary: Lists terms that are unique to the laboratory experiment. These terms are defined and bolded in the background information section of each lab.

Lesson Summary: Provides a brief explanation of the laboratory experiment, including techniques used.

Student Learning Objectives: Describes desired student learning outcomes.

Next Generation Florida Science Standards: Identifies the Florida Science Standards addressed in experiment.

Materials: Provides a list of materials and equipment and quantities of each needed for each student workstation and common area.

Background Information: Summarizes research and background information related to the experiment.

Advanced Preparation: Describes what the teacher needs to do in advance to prepare for the experiment.

Procedure: Outlines the steps of implementing the laboratory with time estimates for each step and suggested laboratory wrap-up and assessment.

1. Pre lab: Includes Background Information, Setting the Stage vignette, focus questions, and suggested demonstrations
   • Background Information: Provides the students general background knowledge concerning the biotechnology tools used in the laboratory.
   • Setting the Stage: Orients the students to the problem they need to solve through experimentation. Similar to a case study approach, the students become vested in helping our local peanut farmer, Mr. John Bailey.
• **Focus Questions:** These questions help focus student learning and complement information provided through teacher instruction. Expected answers are provided in the *Teacher’s Edition.*

2. **Lab:** Formatted for student use to implement experiment
   - **Workstation Checklist:** Lists all materials needed during the laboratory experiment. This allows students to familiarize themselves with all reagents and equipment prior to use.
   - **Laboratory Method:** Includes step-by-step instructions to perform the experiment.
   - **Data Collection & Analysis:** Lab 1 and Lab 4 contain a data collection and analysis section as they will have experimental results to record. Labs 2 and 3 build on each other, with the final results evident in Lab 4. Expected answers are provided in the *Teacher’s Edition.*

3. **Post lab:** Includes review questions, suggested assessment, and whole-class sharing and data table use
   - **Review Questions:** These review questions ask students to reflect upon and explain elements of the procedure. Expected answers are provided in the *Teacher’s Edition.*

**Assessment:** A suggested assessment is included for each experiment. The suggested assessments offer a variety of tradition and non-traditional tools to evaluate student learning. Additionally, the teacher may wish to utilize additional components of the laboratory experiment, **Focus Questions, Data Collection & Analysis,** and/or **Review Questions** for assessment.

**SECTION THREE**
The third section of the book contains support materials designed to make implementation easier.

**Companion Website:** Describes how to access the companion website and the resources available.

**Equipment and Supplies:** Provides a detail list of all materials needed to complete the full laboratory sequence, including ordering information and prices for required laboratory reagents.

**Next Generation Science Standards Correlated to Experiments:** Identifies the Grade 9-12 science standards addressed in the curriculum.

**Resources:** Includes links and descriptions of images, presentations, and supplemental information.

**References:** Lists citations for technical and research literature used to develop the experiments. These references can also provide additional background information for the teacher.

**Assessment:** A content assessment is provided as a pre/post testing instrument to measure student learning gains. The instrument can also be used as a summative assessment.

**Evaluation:** Includes forms to solicit student, teacher, and content expert feedback about the content validity and usability of the curriculum.

http://biotechinthe classroom.webs.com
Introduction

The Biotech in the Classroom Laboratory Manual is designed using the plant pathogen tomato spotted wilt virus as a model system of an emerging pathogen that can be used in classrooms. It consists of four experiments that build students’ knowledge of technologies used to diagnose and combat plant pathogens. As sequenced, lessons have increasing levels of complexity and equipment requirements.

Biotech in the Classroom Laboratory Manual began as a collection of company protocols photocopied for teacher use during a two-week professional development institute at the University of Florida (UF). The teachers performed the experiments successfully and enjoyed the experience, but only one teacher incorporated a component of the laboratory sequence into her classroom curriculum the following year.

My frustration and entry into graduate school spurred the development of a user-friendly laboratory manual that teachers could use as a student lab manual during the Institute at UF and then easily incorporate into their own classroom curriculum. The concept of the Biotech in the Classroom Laboratory Manual was started for the 2009 summer institute with one experiment developed into a full lesson. The remaining three experiments functioned well as a student manual, but teacher support was still lacking.

The 2009 cohort of teachers used the manual in draft form and provided valuable comments on the design and usability. Perhaps more importantly, they provided positive reinforcement that the development of the lab manual and associated lessons was useful. Nine teachers included elements of the biotechnology laboratory experience into their curriculum the following school year.

Eight of the teachers have used one or more of the experiments as written in the Biotech in the Classroom Laboratory Manual, however, even with all of reagents, equipment, and on-site assistance provided to the teachers by the grant, only a four teachers have implemented the genetic engineering section (DNA extraction, PCR, gel electrophoresis) in their classroom. One additional teacher brought her students to UF for the genetic engineering sections.

Biotech in the Classroom Laboratory Manual and Biotech in the Classroom Curriculum Guide were developed to try to reach as many teachers as possible and provide lessons and activities that can be incorporated into any classroom. Biotech in the Classroom Laboratory Manual provides teachers who have access to the necessary equipment and supplies a complete guide to successfully implementing a hands-on wet lab biotechnology experience. As written, experiment two requires UF collaboration due to the use of genetically engineered peanut seed.

The laboratory experiments were designed with high school (grades 9-12) Biology and Biotechnology classes in mind. There are certainly applications to other subjects such as Agriscience and Environmental Science and teachers may wish to adjust the focus of the experiments to better suit the context of his/her class. Some sample science concepts addressed in the Biotech in the Classroom Laboratory Manual include, but are not limited to, viruses, plant pathogens, disease vectors, biotechnology, immunoassays, polymerase chain reaction, DNA, and gel electrophoresis.

http://biotechintheclassroom.webs.com
Acknowledgements

A precollege award from Howard Hughes Medical Institute (HHMI) to the University of Florida Center for Precollegiate Education and Training was the impetus for this particular manual. Without funding for the Interdisciplinary Center for Ongoing Research / Education: Emerging Pathogens (ICORE) partnership program for Florida science teachers, I might never have discovered Dr. Maria Gallo who introduced me to the world of tomato spotted wilt virus. The protocols contained within originated in her laboratory, modified from manufacturers’ protocols. I merely attempted to make them classroom friendly and encourage teachers to include biotechnology in their classrooms.

Dr. Linda Cronin-Jones has served as my tireless advisor through my journey, helping me mold this guide into a teacher-friendly product while staying true to the authentic research techniques it was built upon. Her level of experience, expertise, enthusiasm, and commitment is unmatched.

Dr. Mary Jo Koroly has cheered the development of this manual into a valuable curriculum guide, transporting a portion of the two-week ICORE professional development institute back into the classroom. As PI on the HHMI ICORE grant and my center Director, she has given me the freedom to create.

The ICORE teachers of 2008 and 2009 offered initial feedback on the feasibility of the laboratory sequence and individuals will be included as the manual moves into the evaluation and field testing phase.

Many thanks to the Mrs. Mary Russ and her AP Biology students at Williston High School who allowed me to observe their use of Experiment 1 with peanut plants they grew on the school campus. Thanks also to the Mount Dora High School students from Mrs. Melissa Guinta AP Biology class for allowing me to lead them through Experiments 2-4 at the University of Florida and providing me valuable feedback.

Special thanks to the UF researchers and staff who donated their time to assist with the ICORE program.

And of course, my family.
Experiment 1: Mr. Bailey’s Peanut Plants – ImmunoStrip Assays

Lesson Plan

Key Question: To what extent can TSWV be identified by direct observations? Is there a quick diagnostic tool that can be used to confirm the field observations?

Overall Time Estimate:

- Advanced preparation: 15 minutes
- Student procedure: 45 minutes

Vocabulary:

Immunoassay - biochemical test that measures the concentration of a substance in a biological substance, using the reaction of an antibody to its antigen.

Tomato spotted wilt virus (TSWV) - a negative RNA virus vectored by thrips affecting ~800 species of agricultural and ornamental plants. It causes spotting, stunted growth, loss of production, and eventual plant death.

Lesson Summary:

Students are given peanut plants suspected of having TSWV, and they are to observe stems, leaves, and roots to determine if they are infected. The students will perform an immunoassay to test the plant material for the presence of TSWV.

Student Learning Objectives:

The student will be able to:

1. Describe the physical characteristics of a plant suspected of viral infection
2. Use technology to test for the presence of TSWV.
3. Explain antigen/antibody interactions and how their properties are used in diagnostics

Next Generation Florida Science Standards:

Benchmark (SC.912.N.1.6): Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied

Benchmark (SC.912.L.14.52): Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics

Benchmark (SC.912.L.16.7): Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology

Benchmark (SC.912.L.17.6): Compare and contrast the relationships among organisms, including predation, parasitism, competition, commensalism, and mutualism.
Materials:

<table>
<thead>
<tr>
<th>Source</th>
<th>Student workstation</th>
<th>Number required per group</th>
<th>Number required for class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut plants showing signs of TSWV infection – local grower, UF collaborator, class-grown plants</td>
<td>Farmer John’s plant samples</td>
<td>Variable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Test strips</td>
<td>2-4</td>
<td></td>
</tr>
<tr>
<td>Classroom laboratory</td>
<td>Sharpie</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Classroom laboratory</td>
<td>Scissors</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Classroom laboratory</td>
<td>Colored pencils/crayons</td>
<td>Assortment</td>
<td></td>
</tr>
</tbody>
</table>

Background Information:

**Tomato spotted wilt virus (TSWV)** in peanut displays a wide array of symptoms that range from minor spotting on leaves to severe plant stunting and death as the root crown can become severely infected. Standard detection of spotted wilt in the field is through a visual disease intensity rating that corresponds to both incidence and severity. However, peanuts can be asymptomatic, yet still contain TSWV. **Imunoassays** of peanut leaf and root tissues from plants not displaying visible symptoms have detected the presence of TSWV.

A common type of immunoassay is an enzyme-linked immunosorbant assay (ELISA) which has an enzyme that causes a color change when a specific antigen and antibody bind. The ELISA test system for tomato spotted wilt virus utilized in this activity is an immunostrip: the components (antibodies and color-linked enzyme) are in a strip that if positive (antigens in leaf tissue) causes a colored line to appear, not unlike a pregnancy test kit. Other widely used applications of immunoassays include diagnostics of other infectious diseases such as HIV and the rapid strep test.

Advanced Preparation:

Order Agdia ImmunoStrip™ Tests (see resources below)
Obtain peanut plants suspected of infection (see resources below)

Procedure:

I. **Pre-lab**
   1. (3 minutes) Have students form groups around the student workstations (see student workstations below).
   2. (5 minutes) Ask students to read the Background Information, Setting the Stage, and Laboratory Methods to themselves. (Alternative: Assign for homework the previous class; read aloud to the students; have students read aloud; develop a presentation to introduce immunostrip assays)
3. (3 minutes) Allow time for students to answer the *Focus Questions*.
4. (3 minutes) Review answers to *Focus Questions* as a class.
5. (3 minutes) Review Laboratory Method, indicating reagents and materials needed.

II. Laboratory
6. (15 minutes) Allow the students to work through *Experiment 1: Mr. Bailey’s Peanut Plants* in groups and complete the *Data Collection and Analysis* section.

III. Post-lab
7. (15 minutes) As groups finish and clean up, ask each group to report their findings on the board or overhead transparency, making a chart with the class results. (Chart template available at [www.biotechintheclassroom.com](http://www.biotechintheclassroom.com))
8. (15 minutes) Have each group discuss their conclusions. Summarize the class data and clarify any misconceptions. Help students establish that visual observation correlates with TSWV infection but is not necessarily causative.
9. Assign the assessment (summary report to Farmer John) for homework.

**Assessment:**
Students write a report for summarizing the steps taken to diagnose the pathology in the samples he provided. A suggested rubric is provided to guide students and assessment.
Figure 1. TSWV in tomato plant.

Figure 2. Foliar symptoms of TWSV infection in peanut plants

Figure 3. Healthy peanut plant (left) and TSWV infected peanut plant (right)
Control line: will usually appear in 3 to 5 minutes. The control line assures that the test is working properly. If the control line does not appear, the test is invalid.

Positive result: the test line will also appear. The test line will be red to purple in color just as the control line. The color intensity of the test line will vary.

Negative result: the test line will not appear.

Figure 4: Actual ImmunoStrip results compared to manufacturer image
<table>
<thead>
<tr>
<th>Group Number/Name</th>
<th>Sample Number and type</th>
<th>Visual appearance (Infected or healthy?)</th>
<th>ImmunoStrip result (Positive or negative?)</th>
</tr>
</thead>
<tbody>
<tr>
<td># 0 Virus Hunters</td>
<td>1 – root</td>
<td>Healthy</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>2 – peanut leaf</td>
<td>Infected</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>3 – peanut leaf</td>
<td>Healthy</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>4 – peanut stem</td>
<td>Healthy</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Student Pages

Experiment 1: Mr. Bailey’s Peanut Plants – ImmunoStrip Assays

Background Information

Tomato spotted wilt virus (TSWV) in peanut displays a wide array of symptoms that range from minor spotting on leaves to severe plant stunting and death as the root crown can become severely infected. Standard detection of spotted wilt in the field is through a visual disease intensity rating that corresponds to both incidence and severity. However, peanuts can be asymptomatic, yet still contain TSWV. Immunoassays of peanut leaf and root tissues from plants not displaying visible symptoms have detected the presence of TSWV.

A common type of immunoassay is an enzyme-linked immunosorbant assay (ELISA) which has an enzyme that causes a color change when a specific antigen and antibody bind. The ELISA test system for tomato spotted wilt virus utilized in this activity is an immunostrip: the components (antibodies and color-linked enzyme) are in a strip that if positive (antigens in leaf tissue) causes a colored line to appear, not unlike a pregnancy test kit. Other widely used applications of immunoassays include diagnostics of other infectious diseases such as HIV and the rapid strep test.

Setting the Stage

A local peanut farmer, Mr. John Bailey, comes to see you at the Plant Pathogen Diagnostic Laboratory (PPDL). He has observed that some of his peanut plants are not thriving and brings samples of them to the PPDL. Based on years of field experience, Mr. Bailey has a good idea what the culprit is, but it is suggested that laboratory confirmation be made so the best management practices can be utilized. He has asked you to determine what is afflicting his peanut plants.

You decide the best test to use is an ImmunoStrip Assay. This simple test will detect if tomato spotted wilt virus antigens are present in the plant tissue. If antigens are present, they will bind to the specific antibodies on the test strip and result in a line appearing in the test window.

Focus Questions

1. List and illustrate all observable traits or characteristics for each sample. Consider the whole plant as well as individual portions of the plant.

2. Using your observations and knowledge, write an inference indicating the cause of Farmer John’s problem.
ImmunoStrip Assay Laboratory

Workstation Checklist

<table>
<thead>
<tr>
<th>Student workstation</th>
<th>Number required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmer John’s plant samples</td>
<td>Variable</td>
</tr>
<tr>
<td>Sample extract bags</td>
<td>2-4</td>
</tr>
<tr>
<td>Test strips</td>
<td>2-4</td>
</tr>
<tr>
<td>Sharpie</td>
<td>1</td>
</tr>
<tr>
<td>Scissors</td>
<td>1</td>
</tr>
<tr>
<td>Colored pencils/crayons</td>
<td>Assortment</td>
</tr>
</tbody>
</table>

Laboratory Method for Agdia ImmunoStrip™ Test

1. Each sample bag contains 3 ml of sample extract buffer. For optimal performance, the sample to be tested should be about 0.15 g (leaf area about 3 to 5 cm² or 1 inch²).
2. Cut off the top of the sample extract bag, being careful not to spill the buffer.
3. Place sample between the mesh linings of the bag.
4. Rub the bag with a pen or blunt object to completely crush sample. Use only one sample per bag and be sure to label each bag.
5. Remove one strip from the packaging. When handling the strip, always grasp the top of the strip marked with the test name. Do not remove protective covering.
6. Keep the strip in a vertical position; insert the end of the strip marked “sample” into the extract.
7. Do not allow much more than 0.5 cm or 1/4 inch of the end of the strip to be submerged in the extract. Be sure the strip remains in the extract during the test.

RESULTS:

Control line: will usually appear in 3 to 5 minutes. The control line assures that the test is working properly. If the control line does not appear, the test is invalid.

Positive result: the test line will also appear. The test line will be red to purple in color just as the control line. The color intensity of the test line will vary.

Negative result: the test line will not appear.

If you wish to keep the strips as permanent records, cut off the sample pads (green ends marked “sample”) and discard. Then blot the ImmunoStrips between paper towels to remove excess liquid.
Data Collection and Analysis

1. a. What do you observe?
   b. Draw or affix your strips and describe them below.

2. What are the results of the tests?

Review Questions

1. Did your observations and predictions match your immunostrip results? How were they different?

2. Do you think observation alone is an accurate predictor of TSWV infections? Why or why not?

Assessment
Write a report to send to Mr. Bailey detailing your findings. Be sure to include the methods used to reach your conclusion. Discuss the use of the ImmunoStrip, how it works, and the results obtained from Mr. Bailey’s samples.
Answer Key

Focus Questions
1. List and illustrate all observable traits or characteristics for each sample. Consider the whole plant as well as individual portions of the plant.

   Answers and illustrations will vary. Likely answers: yellow spots, stunted growth, yellow rings, looks sick or unhealthy

   Encourage students to describe as many observations as they can, using words and illustrations.

   Peanut leaf with concentric ring spots caused by Tomato spotted wilt virus (TSWV). (Courtesy A. Culbreath, J. Todd, and H. Pilcher)

2. Using your observations and knowledge, write an inference indicating the cause of Farmer John’s problem.

   Tomato Spotted Wilt Virus
   *students will not know this answer unless they have been previously introduced to TSWV

   Students may also suggest nutrient deficiency or other insect disease depending on the prior knowledge of the student.

Data Collection and Analysis
1. a. What do you observe?
   b. Draw or affix your strips and describe them below.

   Answers will vary. Hopefully students will have at least one positive result and be able to describe how they know it is positive.

2. What are the results of the tests?
   Answers will vary. Students usually shout out their results as they perform the lab. Try to help students identify at least one positive result.

Review Questions
1. Did your observations and predictions match your immunostrip results? How were they different?
   Students tend to be very critical and suspect of all leaves. Most likely, their predictions will not match the immunostrip results unless they have very obviously infected plants.

2. Do you think observation alone is an accurate predictor of TSWV infections? Why or why not?
   Only in cases of severe infection. A slight infection might show only a few symptoms that can be overlooked or misdiagnosed.
**TSWV ImmunoStrip Report Rubric**

**Student Name: __________________________**

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question/Purpose</td>
<td>The purpose of the experiment is clearly identified and stated.</td>
<td>The purpose of the experiment is identified, but is stated in a somewhat unclear manner.</td>
<td>The purpose of the experiment is partially identified, and is stated in a somewhat unclear manner.</td>
<td>The purpose of the experiment is erroneous or irrelevant.</td>
</tr>
<tr>
<td>Drawings/Diagrams</td>
<td>Clear, accurate diagrams are included and make the experiment easier to understand. Diagrams are labeled neatly and accurately.</td>
<td>Diagrams are included and are labeled neatly and accurately.</td>
<td>Diagrams are included and are labeled.</td>
<td>Needed diagrams are missing OR are missing important labels.</td>
</tr>
<tr>
<td>Procedures</td>
<td>Procedures are listed in clear steps. Each step is numbered and is a complete sentence.</td>
<td>Procedures are listed in a logical order, but steps are not numbered and/or are not in complete sentences.</td>
<td>Procedures are listed but are not in a logical order or are difficult to follow.</td>
<td>Procedures do not accurately list the steps of the experiment.</td>
</tr>
<tr>
<td>Materials</td>
<td>All materials and setup used in the experiment are clearly and accurately described.</td>
<td>Almost all materials and the setup used in the experiment are clearly and accurately described.</td>
<td>Most of the materials and the setup used in the experiment are accurately described.</td>
<td>Many materials are described inaccurately OR are not described at all.</td>
</tr>
<tr>
<td>Conclusion</td>
<td>Conclusion includes whether the findings supported the hypothesis, possible sources of error, and what was learned from the experiment.</td>
<td>Conclusion includes whether the findings supported the hypothesis and what was learned from the experiment.</td>
<td>Conclusion includes what was learned from the experiment.</td>
<td>No conclusion was included in the report OR shows little effort and reflection.</td>
</tr>
</tbody>
</table>

Maximum points: 20
Experiment 2: Getting the DNA Out – DNA Extraction

Lesson Plan

Key Question(s): Where is DNA located in a cell? How can DNA be extracted for use in other procedures? What experiments can be performed with DNA?

Overall Time Estimate:

- Advanced preparation: 30 minutes
- Student procedure: 45 minutes

Vocabulary:

DNA extraction: process of removing DNA from a cell

Lesson Summary: Using peanut seeds that are genetically modified, students will extract DNA using a commercial DNA extraction kit. For comparison, wild-type peanut seeds will also be used. The DNA extracted will provide the template DNA for Experiment 3.

Student Learning Objectives:
The student will be able to:

1. Explain parts of cell relevant to DNA extraction (cell wall, cellular and nuclear membrane, nucleus)
2. Illustrate key cell structures (cell wall, cellular and nuclear membrane, nucleus)
3. Describe the DNA extraction process
4. Articulate use of DNA in biotechnology

Next Generation Florida Science Standards:

Benchmark (SC.912.N.1.6): Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied

Benchmark (SC.912.L.14.2): Relate structure to function for the components of plant and animal cells. Explain the role of cell membranes as a highly selective barrier.

Benchmark (SC.912.L.14.3): Compare and contrast the general structures of plant and animal cells.

Benchmark (SC.912.L.16.7): Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology

Benchmark (SC.912.L.18.3): Describe the structures of fatty acids, triglycerides, phospholipids, and steroids. Explain the functions of lipids in living organisms. Identify some reactions that fatty acids undergo. Relate the structure and function of cell membranes.
## Materials:

<table>
<thead>
<tr>
<th>Source</th>
<th>Student workstation</th>
<th>Number required per group</th>
<th>Number required for class</th>
</tr>
</thead>
</table>
| Sigma Aldrich  
http://www.sigmaaldrich.com  
REDExtract-N-Amp Seed™ PCR Kit  
10 extractions/ 10 PCR reactions :  
XNASS-1KT, $26.50  
100 extractions/ 100 PCR reactions :  
XNAS-1KT, $213.00 | Seed extraction buffer, 100µl  
1 tube  
Seed preparation buffer, 15µl  
1 tube  
Neutralization buffer, 120µl  
1 tube |  
| Fisher Scientific  
http://www.fishersci.com/  
Cat. No. K749520-0090, $132.23  
PK of 100 individually wrapped  
microtubes and micropestles | 1.5ml microcentrifuge tubes  
2 |  
| Fisher Scientific  
http://www.fishersci.com/  
Cat. No. 14-230-225, $125.00  
Case of 1000 | Sterile blue micropestle  
2 |  
| Classroom laboratory | Sterile distilled H₂O, 200µl  
1 tube |  
| Bio-Rad  
http://www.bio-rad.com  
2–20 µl Digital Micropipet  
166-0506EDU, $221.00  
OR  
Classroom 20–200 µl Digital Micropipet  
166-0551EDU, $111.00 | P20 pipette  
1 |  
| Bio-Rad  
http://www.bio-rad.com  
20–200 µl Digital Micropipet  
166-0507EDU, $221.00  
OR  
Classroom 20–200 µl Digital Micropipet  
166-0552EDU, $111.00 | P200 pipette  
1 |  
| Fisher Scientific  
http://www.fishersci.com/  
Cat. No. 50863814  
Pack of 960 for $120.99  
(10 boxes of tips with 96 tips per box) | P20 pipette tips  
1 box |  
| Fisher Scientific  
http://www.fishersci.com/  
Cat. No. 50863815  
Pack of 960 for $120.89  
(10 boxes of tips with 96 tips per box) | P200 pipette tips  
1 box |
### Source

<table>
<thead>
<tr>
<th>Source</th>
<th>Instructor’s (common) workstation</th>
<th>Number required for class</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF Collaborator</td>
<td>Genetically engineered peanut seeds</td>
<td>1</td>
</tr>
<tr>
<td>Local grocery store, unsalted peanuts</td>
<td>Wild-type peanut seeds</td>
<td>1</td>
</tr>
<tr>
<td>MJ Mini 48-Well Personal Thermal Cycler PTC-1148EDU, $3,196.00</td>
<td>70% ethanol</td>
<td>1 squeeze bottle</td>
</tr>
<tr>
<td>Classroom laboratory</td>
<td>Analytical scale</td>
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<td>Classroom laboratory</td>
<td>Scalpel</td>
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<tr>
<td>Classroom laboratory</td>
<td>Kimwipes</td>
<td>1 box</td>
</tr>
<tr>
<td>100–1000 μl Digital Micropipet Cat. No. 166-0508EDU, $221.00</td>
<td>P1000 pipette tips</td>
<td>1 box</td>
</tr>
<tr>
<td>Fisher Scientific <a href="http://www.fishersci.com/">http://www.fishersci.com/</a> Cat. No. 50863812 Pack of 560 for $66.98</td>
<td>1.5ml microcentrifuge tubes</td>
<td>Variable; need at least four per group for reagent aliquots</td>
</tr>
</tbody>
</table>

### Background Information:

**DNA extraction** is a basic procedure in scientific laboratories and is important to the study of heredity as well as the treatment of many diseases and determining evolutionary relatedness. Extracted DNA can also be used to create DNA fingerprints to help diagnose genetic diseases, solve criminal cases, identify victims of disaster and war, and establish paternity or maternity. Scientists can genetically engineer changes in DNA to create more productive or disease-resistant genetically modified plants and animals. DNA extraction is also necessary in order to sequence the DNA of different organisms (Human Genome Project, *E. coli* Genome Project) and compare different species.

In this experiment, DNA is extracted from plant cells (peanut seeds). Although all cells are surrounded by a cellular membrane that protects and contains its contents, plant cells have an additional barrier surrounding the cell membrane. The cell wall gives plants their rigidity and strength. Animal cells do not have cell walls. In a eukaryotic cell, DNA is located within the cell’s nucleus which is surrounded by the nuclear membrane.

There are three layers that must be broken to release the DNA into solution: nuclear membrane, cellular membrane, and cell wall. The cell wall is composed of cellulose, making it very tough. The physical or mechanical force of the micropestle will break open the cell wall. The cellular and nuclear membranes are composed of a phospholipid bi-layer. Chemicals and heat are used to disrupt their
structure. Heating the cells to 55°C softens the membranes, allowing them to become more fluid and pores to open. This breaks or lyses the cell.

For the purposes of this experimental sequence, the amount of DNA needed is very small. The reaction is heated to 95°C to destroy DNA chewing enzymes (DNase) and neutralization buffer added to prevent other proteins and ions from inhibiting the subsequent amplification procedure.

**Advanced Preparation:**

1. Aliquot reagents into individual sterile 1.5ml microcentrifuge tubes.
   - # groups x 100µl Seed extraction buffer
   - # groups x 15µl Seed preparation buffer
   - # groups x 120µl Neutralization buffer
   - # groups x 200µl Sterile water
   *Sterile water is used in Experiments 3 and 4 as well. You may wish to aliquot 1000µl and have students keep their water for use in the following experiments.

2. Set up student workstations, or have general area for student groups to collect supplies.

3. Set up common workstation. If equipment and supplies are available, multiple stations will expedite procedure.
   *Alternative method: Weigh peanut samples for each student group in advance, place in labeled 1.5 microfuge tubes. Have students begin procedure at step number 4.

4. Program thermal cycler with the following conditions; name program “Extraction.”
   
<table>
<thead>
<tr>
<th>Step 1:</th>
<th>55°C</th>
<th>10 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 2:</td>
<td>95°C</td>
<td>3 mins</td>
</tr>
<tr>
<td>Step 3:</td>
<td>4°C</td>
<td>forever</td>
</tr>
</tbody>
</table>

**Procedure:**

1. (3 minutes) Have students form groups around the student workstations (see student workstations below).
2. (5 minutes) Ask students to read the vignette to themselves, or read aloud to the class.
3. (5 minutes) As a class, work through the pre-lab focus questions.
   *Alternatively: The pre-lab focus questions can be assigned for homework the previous class.
4. (25 minutes) Allow the students to work through Experiment 2: Getting the DNA Out of Mr. Bailey’s Seeds in groups.
5. (5 minutes) When groups have finished and cleaned up, provide wrap-up for Experiment 2 by discussing the review questions.
6. Assign the assessment (DNA extraction crossword) for homework, or if time allows, students may work on it during class.

**Assessment:** A seven-item crossword is provided for student assessment of the DNA extraction laboratory.
Figure 6: Basic plant cell model. Key structures for DNA extraction are indicated.
Student Pages

Experiment 2: Getting the DNA Out – DNA Extraction

Background Information

DNA extraction is a basic procedure in scientific laboratories and is important to the study of heredity as well as the treatment of many diseases and determining evolutionary relatedness. Extracted DNA can also be used to create DNA fingerprints to help diagnose genetic diseases, solve criminal cases, identify victims of disaster and war, and establish paternity or maternity. Scientists can genetically engineer changes in DNA to create more productive or disease-resistant genetically modified plants and animals. DNA extraction is also necessary in order to sequence the DNA of different organisms (Human Genome Project, E. coli Genome Project) and compare different species.

In this experiment, DNA is extracted from plant cells (peanut seeds). Although all cells are surrounded by a cellular membrane that protects and contains its contents, plant cells have an additional barrier surrounding the cell membrane. The cell wall gives plants their rigidity and strength. Animal cells do not have cell walls. In a eukaryotic cell, DNA is located within the cell’s nucleus which is surrounded by the nuclear membrane.

There are three layers that must be broken to release the DNA into solution: nuclear membrane, cellular membrane, and cell wall. The cell wall is composed of cellulose, making it very tough. The physical or mechanical force of the micropestle will break open the cell wall. The cellular and nuclear membranes are composed of a phospholipid bi-layer. Chemicals and heat are used to disrupt their structure. Heating the cells to 55°C softens the membranes, allowing them to become more fluid and pores to open. This breaks or lyses the cell.

For the purposes of this experimental sequence, the amount of DNA needed is very small. The reaction is heated to 95°C to destroy DNA chewing enzymes (DNase) and neutralization buffer added to prevent other proteins and ions from inhibiting the subsequent amplification procedure.
**Setting the Stage**
Mr. Bailey is a very successful peanut farmer, who is farming the same land his grandfather planted many years ago. He has many friends and fellow growers across the country that call on him for advice and assistance. One such collaboration is with a university researcher.

Mr. Bailey assists the researcher by performing field trials of new seed and donating some of his land and labor. He received peanut seeds from a researcher to plant a test field. Both genetically engineered and wild-type seed were sent. Unfortunately, the labels that indicated which bag contained which seeds came off during the shipping process.

The researcher is in another state, and it will take considerable time to have her lab analyze them. Since you did such a good job detecting TSWV in his previous peanut plants, Mr. Bailey is again asking for your help. You are familiar with the research project, and your task is to set up an experiment to extract DNA and amplify a portion of the peanut genome to determine which seeds are genetically engineered.

**Focus Questions**
1. Why does the research design include genetically engineered and wild-type peanut seeds?

2. Where in a peanut seed is the DNA located? Draw a picture of a plant cell, labeling key structures related to DNA instruction.

3. How would you extract the DNA from the plant cell?
DNA Extraction Laboratory

Workstation Checklist

Materials and supplies that should be present at your workstation prior to beginning this lab are listed below:

<table>
<thead>
<tr>
<th>Student workstation</th>
<th>Number required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed extraction buffer</td>
<td>1 tube</td>
</tr>
<tr>
<td>Seed preparation buffer</td>
<td>1 tube</td>
</tr>
<tr>
<td>Neutralization buffer</td>
<td>1 tube</td>
</tr>
<tr>
<td>1.5ml eppendorf tubes</td>
<td>2</td>
</tr>
<tr>
<td>Sterile blue micropestle</td>
<td>2</td>
</tr>
<tr>
<td>0.2ml PCR tubes</td>
<td>2</td>
</tr>
<tr>
<td>Sterile distilled H₂O</td>
<td>1 tube</td>
</tr>
<tr>
<td>P20 pipette</td>
<td>1</td>
</tr>
<tr>
<td>P200 pipette</td>
<td>1</td>
</tr>
<tr>
<td>P20 pipette tips</td>
<td>1 box</td>
</tr>
<tr>
<td>P200 pipette tips</td>
<td>1 box</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Instructor’s (common) workstation</th>
<th>Number required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal cycler</td>
<td>1</td>
</tr>
<tr>
<td>70% ethanol</td>
<td>1 squeeze bottle</td>
</tr>
<tr>
<td>Analytical scale</td>
<td>1</td>
</tr>
<tr>
<td>Scalpel</td>
<td>1</td>
</tr>
<tr>
<td>Kimwipes</td>
<td>1 box</td>
</tr>
</tbody>
</table>

Laboratory Method for DNA Extraction

Since even the slightest trace of unwanted DNA will give a false positive result in the subsequent PCR, always wear gloves, clean scalpel between samples, and use new pipette tips for different samples or if tip accidentally touches anything.

1. Obtain peanut seeds and identify one as Seed A and the other as Seed B.

   **Note:** The DNA extraction steps below should be performed for each sample.

2. Remove 0.02g peanut seed using a clean scalpel. Clean blade between samples using 70% ethanol.
3. Place sample in a 1.5ml microcentrifuge tube. Be sure to label the tube.
4. Add 80μl sterile distilled H₂O to microcentrifuge tube.
5. Using a sterile blue micropestle, grind seed to a white, milky solution.
6. Label a sterile 0.2ml PCR tube with the seed sample id and group number.
7. Place 45µl Seed Extraction Solution + 5µl Seed Preparation Solution into the labeled PCR tube.
8. Add 5µl white peanut seed extract to the labeled PCR tube and mix by pipetting up and down.
9. Place sample in thermal cycler. Once all samples are collected, the instructor will start the thermal cycler “Extraction” program. (55°C for 10 minutes; 95°C for 3 minutes)
10. Remove samples from thermal cycler and add 50µl Neutralization Buffer.
11. Mix by pipetting up and down.
12. Samples can be stored at 4°C or used immediately.
**Review Questions**

1. What was the purpose of using the micropestle?

2. What does heating at 55°C accomplish? 95°C?

**Assessment**

Complete TSWV crossword activity about the extraction lab
**TSWV Crossword Puzzle**

**Across**

4. technique used to soften cellular membranes

5. surround plant cells and give rigid structure

6. genetic material found in cells

7. organelle which houses the genetic material of a cell

**Down**

1. removal of genetic material

2. technique used to mechanically break open a cell

3. nuclear and cellular
Answer Key

Focus Questions

1. Why does the research design include genetically engineered and wild-type peanut seeds?

   *Wild-type seeds function as the control group to compare what the normal infectivity rate for the field would be. The genetically engineered seeds are the test group.*

2. Where in a peanut seed is the DNA located? Draw a picture of a plant cell, labeling key structures related to DNA instruction.

   *Illustrations will vary. Instructor may wish to indicate key structures important in DNA extraction such as cell wall, cellular membrane, nuclear membrane, nucleus, and DNA.*

3. How might you extract the DNA from the plant cell?

   *Answers will vary based on the prior knowledge and experience of the students. If they have performed a DNA extraction from fruit, wheat germ, or cheek cells, they should be able to explain the steps well.*

   b) *Break open the cell wall using mechanical force.*

   c) *Break open the cell membrane and nuclear membrane using detergent which disrupts the phospholipid bi-layer.*

   d) *DNA is now free in solution. Filter plant debris (cell wall, seeds, etc) to separate the DNA.*

   e) *Precipitate the DNA with ethyl alcohol.*

   f) *Collect the DNA.*

Review Questions

1. What was the purpose of using the micropestle?

   *Grind the peanut seed, mechanically breaking open the cell wall.*

2. What does heating at 55°C accomplish? 95°C?

   *55°C softens the membranes and breaks-up connective materials in the cells. 95°C destroys DNase -- an enzyme that chews up DNA.*
**TSWV Crossword Puzzle**

**Across**

4. technique used to soften cellular membranes (*heating*)

5. surround plant cells and give rigid structure (*wall*)

6. genetic material found in cells (*DNA*)

7. organelle which houses the genetic material of a cell (*nucleus*)

**Down**

1. removal of genetic material (*extraction*)

2. technique used to mechanically break open a cell (*grinding*)

3. nuclear and cellular (*membrane*)
Experiment 3: Making Lots of Copies – DNA Amplification

Lesson Plan

Key Question(s): What is polymerase chain reaction, and what are the necessary components of a PCR reaction? How can PCR be used to answer biological questions?

Overall Time Estimate:

- Advanced preparation: 30 minutes
- Student procedure: 45 minutes

Vocabulary:

PCR: polymerase chain reaction, technique used to produced millions of copies of a target sequence

Thermal cycler: a programmable laboratory instrument used to perform PCR. The thermal block of the machine is programmed to cycle rapidly through different temperatures and hold for specific lengths of time

Lesson Summary: Students use the DNA extracted from transgenic peanut seed and wild-type peanut seed from Experiment 2 as the template for polymerase chain reaction.

Student Learning Objectives:

The student will be able to:

1. Define polymerase chain reaction
2. Illustrate the amplification process
3. Explain the specificity of PCR
4. Describe usefulness of PCR

Next Generation Florida Science Standards:

Benchmark (SC.912.L.16.3): Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information.

Benchmark (SC.912.L.16.9): Explain how and why the genetic code is universal and is common to almost all organisms.

Benchmark (SC.912.L.16.12): Describe the basic DNA technology (restriction digestion, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules.

Benchmark (SC.912.L.18.1): Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.

Benchmark (SC.912.L.18.11): Explain the role of enzymes as catalysts that lower the activation energy of biochemical reactions. Identify factors, such as pH and temperature, and their effect on enzyme activity.
### Materials:

<table>
<thead>
<tr>
<th>Source</th>
<th>Student workstation</th>
<th>Number required per student group</th>
<th>Number required for class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed A, Seed B from Experiment 2 Positive control from UF collaborator</td>
<td>DNA samples, 5µl each (Seed A, Seed B, Positive Control)</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
| Sigma Aldrich REDExtract-N-Amp Seed™ PCR Kit [Access to Sigma Aldrich website](http://www.sigmaaldrich.com) 
10 extractions/ 10 PCR reactions: Cat. No. XNASS-1KT, $26.50
OR 100 extractions/ 100 PCR rxns: Cat. No. XNAS-1KT, $213.00 | PCR reaction mix, 60µl (Contains reaction buffer, Mg2+, dNTPs, Taq polymerase) | 1 tube |  |
| Integrated DNA Technologies (IDT) [Access to IDT website](http://www.idtdna.com )
257: GCCAAGACAAACCTGATCTC | Primer 1, 5µl | 1 tube |  |
| Integrated DNA Technologies (IDT) [Access to IDT website](http://www.idtdna.com )
259: TGACCTTCAGAAGGCTTGATAGC | Primer 2, 5µl | 1 tube |  |
Cat. No. 02-681-5, $17.67
Pack of 250 | 1.5ml microcentrifuge tubes | 1 |  |
Cat. No. 14-230-225, $125.00
Case of 1000 | 0.2ml PCR tubes | 5 |  |
| Classroom laboratory OR Invitrogen [Access to Invitrogen website](http://www.invitrogen.com )
UltraPure™ DNase/RNase-Free Distilled Water
Cat. No. 10977-023, $134.00
10 x 500 ml
OR Cat. No. 10977-015, $24.72
1 x 500ml | Sterile distilled H2O, 50µl | 1 tube |  |
| Bio-Rad [Access to Bio-Rad website](http://www.bio-rad.com )
Classroom 20–200 µl Digital Micropipet
166-0551EDU, $111.00 | P20 pipette | 1 |  |
| Bio-Rad [Access to Bio-Rad website](http://www.bio-rad.com )
Classroom 20–200 µl Digital Micropipet
166-0552EDU, $111.00 | P200 pipette | 1 |  |
Cat. No. 50863814
Pack of 960 for $120.99 (10 boxes of tips with 96 tips per box) | P20 pipette tips | 1 box |  |
Background Information:
To study genes scientists need a large quantity of DNA. **PCR** is a biotechnology technique that can produce many copies of a targeted DNA sequence. The process will replicate a small quantity of DNA in just a few hours. All you need is template DNA, a few reagents and three different temperatures. A **thermal cycler** is essentially a computerized hot block which is able to alternate between the three crucial temperatures rapidly.

Polymerase Chain Reaction, or PCR, was developed by Dr. Kerry Mullis in 1983. He later received the Nobel Prize in Chemistry in 1993 for his invention of the polymerase chain reaction, arguably one of the most important scientific advances of the 20th century. PCR is a technique which produces millions of copies of a specific portion of a DNA strand starting from just a single DNA template strand. PCR amplifies the DNA exponentially in just a few hours.

PCR became possible with the discovery of an enzyme found in bacteria which live in the hydrothermal vents at the bottom of the oceans and hot springs. The temperature of the water in these hydrothermal vents is near boiling. Therefore, their DNA polymerase (the enzyme which copies their DNA) is stable and functional at very high temperatures. This enzyme is called Taq polymerase (named after the organism *Thermus aquaticus*). The DNA polymerases found in other organisms, such as humans, which do not live in boiling water, are destroyed at temperatures not far above the organism's normal range. DNA polymerases are the enzymes which build a new strand of DNA along the old strand in DNA replication.
The reagents for PCR: small amount of template DNA + master mix

Master mix:

- buffer (contains water and MgCl₂)
- an excess of primer molecules (forward and reverse primers)
- an excess of each of the four nucleotides, dNTPs (dATP, dTTP, dGTP, dCTP)
- Taq polymerase

There are three steps in PCR, which are repeated over and over again (cycled):

(1) Denature, 94°C: In this step double-stranded DNA molecules are heated to 94°C which separates or denatures them so that they become single-stranded. These single strands of DNA then become the templates for the new DNA strands to be made upon.

(2) Annealing, 60°C: In order for the Taq polymerase to start building a new strand of DNA, it must have something to hook onto. A primer is used to start the process. A primer is a short (20-25 base-pairs long) piece of DNA which will anneal, or stick, to the DNA template strand where it finds a sequence complementary to its own sequence. Scientists use specific primers which flank the portion of the DNA they are interested in amplifying (forward and reverse primers). In order for the primer to anneal to the template strand, the temperature must be lowered to a temperature which will allow it to bind. 50 - 65°C for 30-60 seconds is the range for annealing primers. (The time and temperature is dependent on the nucleotide composition of the primer sequence. The optimal conditions for our experiment are 60°C for 30 seconds.)

(3) Extension, 72°C: During this step of the cycle, the Taq polymerase will extend the primer by bringing in complementary nucleotides as it moves along the template strand. The Taq polymerase works best at 72°C; the temperature is raised so the enzyme can work efficiently. All four nucleotides are free in solution and are added as Taq builds a new strand complementary to the template strand.

More resistant strains of peanuts have been produced through traditional breeding programs. However, even these are still susceptible to TSWV. Research is ongoing to create a viable and high-yielding genetically engineered peanut plant that is resistant to TSWV. Inserting part of the viral construct into the plant genome conveys increased resistance to tomato spotted wilt virus. One method that can be used to analyze seeds and determine if the viral construct has been incorporated into the peanut genome is PCR. Using the DNA extracted in the previous experiment, students now perform PCR to determine if the gene of interest is present and if so, in which peanut seed.
Advanced Preparation:

1. Aliquot reagents into individual sterile 1.5ml microcentrifuge tubes.
   — # groups X 5µl positive control DNA (label “+”)
   — # groups X 60µl PCR reaction mix (label “PCR mix”)
   — # groups X 5µl Primer 1 (label “P1”)
   — # groups X 5µl Primer 2 (label “P2”)
   — # groups X 50µl Sterile water (label “H2O”)
   *Keep cold until ready to use. If preparing more than one hour in advance, keep in freezer.

2. Set up student workstations, or have general area for student groups to collect supplies.

3. Program the thermal cycler with the following conditions; name the program “TSWV”:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94°C</td>
<td>5 mins</td>
</tr>
<tr>
<td>2</td>
<td>94°C</td>
<td>1 min</td>
</tr>
<tr>
<td>3</td>
<td>60°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>4</td>
<td>72°C</td>
<td>2 mins</td>
</tr>
<tr>
<td>5</td>
<td>Go to step 2; repeat 34 times</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>72°C</td>
<td>3 mins</td>
</tr>
<tr>
<td>7</td>
<td>4°C</td>
<td>forever</td>
</tr>
</tbody>
</table>

Procedure:

1. (3 minutes) Have students form groups around the student workstations. One person from each group should obtain DNA extracts from Experiment 2 (see student workstations below).

2. (5 minutes) Ask students to read the vignette to themselves, or read aloud to the class.

3. (5 minutes) As a class, work through the pre-lab focus questions.
   *Alternatively: The pre-lab focus questions can be assigned for homework the previous class.

4. (15 minutes) Allow the students to work through Experiment 3: Making Lots of Copies in groups.

5. (5 minutes) When groups have finished and cleaned up, provide wrap-up for Experiment 3 by discussing the review questions.

6. Assign the assessment (PCR report) for homework, or if time allows, students may begin work on it during class.

Assessment: Suggested assessment given instructs students to write a report for Mr. Bailey detailing the process of PCR. A rubric is included below.
Figure 7: Polymerase chain reaction produces new copies of DNA exponentially
Student Pages

Experiment 3: Making Lots of Copies – DNA Amplification

Background Information

To study genes, scientists need large quantities of DNA. Polymerase Chain Reaction (PCR) is a technique that produces many copies of a targeted DNA sequence starting with a small quantity of DNA in just a few hours. All you need is template DNA, a few reagents and three different temperatures. A thermal cycler is used and is essentially a computerized hot block that alternates between the three crucial temperatures rapidly.

The reagents for PCR: small amount of template DNA + master mix

Master mix:

- buffer (contains water and MgCl₂)
- an excess of primer molecules (forward and reverse primers)
- an excess of each of the four nucleotides, dNTPs (dATP, dTTP, dGTP, dCTP)
- Taq polymerase

There are three steps in PCR, which are repeated over and over again (cycled):

1. Denature, 94°C: In this step double-stranded DNA molecules are heated to 94°C which separates or denatures them so that they become single-stranded. These single strands of DNA then become the templates for the new DNA strands to be made upon.

2. Annealing, 60°C: In order for the Taq polymerase to start building a new strand of DNA, it must have something to hook onto. A primer is used to start the process. A primer is a short piece of DNA which anneals, or sticks, to the DNA template strand where it finds a complementary sequence. Scientists use specific primers which flank the portion of the DNA they are interested in amplifying (forward and reverse primers – much like book-ends). In order for the primer to anneal to the template strand, the temperature must be lowered to 60°C.

3. Extension, 72°C: During this step of the cycle, the Taq polymerase will extend the primer by bringing in complementary nucleotides as it moves along the template strand. The Taq polymerase works best at 72°C. All four nucleotides (A, T, C, and G) are free in solution and are added as Taq builds a new strand complementary to the template strand.

More resistant strains of peanuts have been produced through traditional breeding programs. However, even these are still susceptible to TSWV. Research is ongoing to create a viable and high-yielding genetically engineered peanut plant that is resistant to TSWV. Inserting part of the viral construct into the plant genome conveys increased resistance to tomato spotted wilt virus. One method used to analyze seeds and determine if the viral construct has been incorporated into the peanut genome is PCR. Using the DNA extracted in the previous experiment, you will now perform PCR to determine if the gene of interest is present and if so, in which peanut seed.
Setting the Stage
DNA has been extracted from each of Mr. Bailey’s peanut seeds, successfully, you hope. You must now perform PCR to amplify a portion of the extracted DNA to determine which seed is transgenic. The researcher hopes to confer resistance in peanut plants to the tomato spotted wilt virus (TSWV). The transgenic seed has a segment DNA which is complementary to a portion of the virus genome that codes for a nucleocapsid protein. If the transgenic plant is exposed to virus, the nucleic acid of TSWV is bound to the decoy strand of DNA and cannot replicate.

Focus Questions
1. What is PCR?
2. What are the key steps in the PCR process?
DNA Amplification Laboratory

Workstation Checklist

Materials and supplies that should be present at your workstation prior to beginning this lab are listed below:

<table>
<thead>
<tr>
<th>Student workstation</th>
<th>Number required</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA samples</td>
<td>3</td>
</tr>
<tr>
<td>(Seed A, Seed B, Positive Control)</td>
<td></td>
</tr>
<tr>
<td>PCR reaction mix (labeled PCR mix)</td>
<td>1 tube</td>
</tr>
<tr>
<td>(Contains reaction buffer, Mg2+, dNTPs, Taq polymerase)</td>
<td></td>
</tr>
<tr>
<td>Primer 1 (labeled P1)</td>
<td>1 tube</td>
</tr>
<tr>
<td>Primer 2 (labeled P2)</td>
<td>1 tube</td>
</tr>
<tr>
<td>1.5ml eppendorf tubes</td>
<td>1</td>
</tr>
<tr>
<td>0.2ml PCR tubes (or one tube strip)</td>
<td>5</td>
</tr>
<tr>
<td>Sterile distilled H2O</td>
<td>1 tube</td>
</tr>
<tr>
<td>P20 pipette</td>
<td>1</td>
</tr>
<tr>
<td>P200 pipette</td>
<td>1</td>
</tr>
<tr>
<td>P20 pipette tips</td>
<td>1 box</td>
</tr>
<tr>
<td>P200 pipette tips</td>
<td>1 box</td>
</tr>
<tr>
<td>Vortex</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Instructor’s (common) workstation</th>
<th>Number required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal cycler</td>
<td>1</td>
</tr>
<tr>
<td>Mini centrifuge (0.2ml tubes)</td>
<td>1</td>
</tr>
</tbody>
</table>

Laboratory Method for DNA Amplification

Note: Since PCR amplifies a very small amount of DNA to a quantity that can easily be detected, even trace amounts of unwanted DNA can produce false positive results. Take care not to cross-contaminate samples and reagents by changing pipette tips between reagents and DNA extracts. Barrier or filter tips are used to prevent DNA contamination of pipettors. Always wear gloves.

1. Obtain DNA samples to be amplified from 4°C.
2. Label 0.2ml PCR tubes with DNA sample identifier and your group number.
   • Seed A DNA (A)
   • Seed B DNA (B)
   • Positive control (+)
   • Negative control (-)
3. Vortex and centrifuge all master mix reagents (PCR reaction mix, Primer 1, Primer 2, H2O).
4. Prepare one master mix containing all of the PCR reagents (PCR reaction mix, Primer 1, Primer 2, H₂O). Calculate the amount of master mix reagents needed using the volumes for the basic reaction for one sample below.

\[
\# \text{PCR samples} + \text{one (to allow for pipetting error)} = 5 \text{ total sample prep}
\]

<table>
<thead>
<tr>
<th>PCR reaction</th>
<th>1</th>
<th>5 PCR reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR reaction mix</td>
<td>10μl</td>
<td>μl</td>
</tr>
<tr>
<td>Primer 1</td>
<td>0.5μl</td>
<td>μl</td>
</tr>
<tr>
<td>Primer 2</td>
<td>0.5μl</td>
<td>μl</td>
</tr>
<tr>
<td>Sterile distilled H₂O</td>
<td>5μl</td>
<td>μl</td>
</tr>
<tr>
<td>Total master mix volume per sample</td>
<td>16μl</td>
<td>μl</td>
</tr>
</tbody>
</table>

5. Add master mix reagents to a 1.5ml microcentrifuge tube.
6. Mix by vortexing briefly.
7. Aliquot 16μl master mix into each labeled PCR tube.
8. Add 4μl DNA to correspondingly labeled PCR tube.
9. Add 4μl Sterile distilled H₂O to negative control PCR tube.
10. Mix samples by pipetting up and down or flicking the tube.
11. Centrifuge samples in the mini centrifuge and place in thermal cycler.
12. Once all samples are collected, the instructor will start the thermal cycler “TSWV” program.

**PCR cycling conditions (The complete program takes ~ 2h 40 mins.)**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Duration</th>
<th>Action</th>
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<tbody>
<tr>
<td>1</td>
<td>94°C</td>
<td>5 mins</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>94°C</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60°C</td>
<td>30 sec</td>
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</tr>
<tr>
<td>4</td>
<td>72°C</td>
<td>2 mins</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>Go to step 2; repeat 34 times</td>
</tr>
<tr>
<td>6</td>
<td>72°C</td>
<td>3 mins</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4°C</td>
<td>forever</td>
<td></td>
</tr>
</tbody>
</table>

http://biotechinthe classroom.webs.com
Review Questions
1. The Taq polymerase used in Experiment 3 has an antibody attached that requires a “hot-start” to activate the polymerase. What does this mean? Why is it usually important to keep PCR reagents cold?

2. What is the purpose of the positive and negative controls?

Assessment
Mr. John Bailey is very interested in the technique you are using to test the peanuts. Illustrate and describe the method used to amplify the gene of interest. Provide Mr. Bailey a summary of PCR and include the key steps of the technique.
Answer Key

Focus Questions

1. What is PCR?

   *Polymerase chain reaction: a method for amplifying a portion of a genome.* The great power of PCR is the small amount of template needed which yields millions of copies of the targeted section of the genome.

2. What are the key steps in the PCR process?

   - **Denature:** the reaction is heated to ~94°C, causing the double stranded DNA template to separate into single strands
   - **Anneal:** temperature is brought down to ~60°C and the forward and reverse primers bind to the single stranded DNA
   - **Extend:** temperature is raised to 72°C and Taq polymerase extends the single strands of DNA to form the complement strand

   These steps cycle 30-35 times, producing millions of copies of DNA, much like a photocopier copying a page from a book.

Review Questions

1. The Taq polymerase used in Experiment 3 has an antibody attached that requires a “hot-start” to activate the polymerase. What does this mean? Why is it usually important to keep PCR reagents cold?

   The initial 5 minutes at 94°C disassociates the antibody from the Taq polymerase. Once the Ab has been removed, the Taq can function enzymatically and extend the new growing DNA strands. Having the antibody bound prevents premature degradation of the Taq enzyme and limits non-specific binding. Keeping the reagents cold prevents them from ‘working’ before all components of the PCR reaction are mixed.

2. What is the purpose of the positive and negative controls?

   The positive control provides DNA template for the reaction that is known to contain the gene of interest. This will show that all reagents are working properly.
   No template DNA was added to the negative control reaction. If amplification occurs in this reaction it must be due to contamination, nullifying the results of the co-reactions.
### PCR Report Rubric

**Student Name:** __________________________________________

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<tr>
<th>CATEGORY</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
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<tr>
<td><strong>Question:</strong> What is PCR?</td>
<td>The question to be answered during the report is clearly identified and stated.</td>
<td>The question to be answered during the report is identified, but is stated in a somewhat unclear manner.</td>
<td>The question to be answered during the report is partially identified, and is stated in a somewhat unclear manner.</td>
<td>The question to be answered during the lab is erroneous or irrelevant.</td>
</tr>
<tr>
<td>Spelling, Punctuation and Grammar</td>
<td>One or fewer errors in spelling, punctuation and grammar in the report.</td>
<td>Two or three errors in spelling, punctuation and grammar in the report.</td>
<td>Four errors in spelling, punctuation and grammar in the report.</td>
<td>More than 4 errors in spelling, punctuation and grammar in the report.</td>
</tr>
<tr>
<td>Drawings/Diagrams</td>
<td>Clear, accurate diagrams are included and make the experiment easier to understand. Diagrams are labeled neatly and accurately.</td>
<td>Diagrams are included and are labeled neatly and accurately.</td>
<td>Diagrams are included and are labeled.</td>
<td>Needed diagrams are missing OR are missing important labels.</td>
</tr>
<tr>
<td>Summary</td>
<td>Summary describes the information learned and some applications to real life situations.</td>
<td>Summary describes the information learned and a possible application to a real life situation.</td>
<td>Summary describes the information learned.</td>
<td>No summary is written.</td>
</tr>
<tr>
<td>Components of the report</td>
<td>All required elements are present and additional elements that add to the report (e.g., thoughtful comments, graphics) have been added.</td>
<td>All required elements are present.</td>
<td>One required element is missing, but additional elements that add to the report (e.g., thoughtful comments, graphics) have been added.</td>
<td>Several required elements are missing.</td>
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</tbody>
</table>

Maximum points: 20
Experiment 4: Visualizing the DNA – Gel Electrophoresis

Lesson Plan

Key Question(s): How can PCR results be visualized?

Overall Time Estimate:

- Advanced preparation: 15 minutes
- Student procedure: 50 minutes

Vocabulary:

Gel electrophoresis: literally, to carry with electricity. Technique used to separate molecules through a gel matrix.

Lesson Summary: Polymerase chain reaction amplifies a targeted region of a genome, creating millions of copies of the target. To determine if amplification was successful, gel electrophoresis is performed. The fragment length and band intensity reveals an approximate qualitative estimate of PCR. Students will use their amplified samples from Experiment 3.

Student Learning Objectives:
The student will be able to:

1. Explain the process of gel electrophoresis
2. Explain why DNA is a negatively charged molecule and its importance in gel electrophoresis
3. Describe what the banding pattern represents
4. Interpret a gel

Next Generation Florida Science Standards:

Benchmark (SC.912.L.16.12): Describe the basic DNA technology (restriction digestion, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules

Benchmark (SC.912.L.18.1): Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.

Materials:

<table>
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<tr>
<th>Source</th>
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<th>Number required for class</th>
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<td>PCR samples (Seed A, Seed B, Positive Control, Negative Control)</td>
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<td>Invitrogen <a href="http://www.invitrogen.com/">http://www.invitrogen.com/</a> E-Gel® 50 bp DNA Ladder Cat. No. 10488-099, $117.00</td>
<td>Molecular weight marker, 25µl aliquot</td>
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<td>Classroom laboratory</td>
<td>Sterile distilled H₂O, 500µl aliquot</td>
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<td>Bio-Rad</td>
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<tr>
<td>E-Gel® 1.2% with SYBR Safe™ Starter Kit Cat. No. G6206-01, $109.00 (includes PowerBase, adaptor plug, and six 1.2% E-Gels)</td>
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<td>Invitrogen</td>
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<td>E-Gel® 1.2% with SYBR Safe™18-Pak Cat. No. G5218-01, $170.00 (Gels only)</td>
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<td>Mini centrifuge (0.2ml tubes)</td>
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<td>Fisher Scientific Cat. No. FD11430, $856.06</td>
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<td>Invitrogen E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit Cat. No. G6465, $824.00</td>
<td>E-Gel Safe Imager Transilluminator and iBase</td>
<td>1-4</td>
</tr>
<tr>
<td>Classroom or personal Digital camera (optional)</td>
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</tbody>
</table>

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**Background Information:** DNA molecules can be visualized as dark bands in an agarose matrix using gel electrophoresis. Gel electrophoresis is a molecular technique that allows DNA to be separated based on size. All DNA is negatively charged, and when an electrical current is applied, DNA migrates through the gel to the positive electrode. The DNA has to move through the pores in the agarose matrix. Large pieces of DNA move slowly; small fragments of DNA move quickly.

In research laboratories, DNA is commonly stained with ethidium bromide (EtBr). Ethidium is a positively charged molecule that binds to DNA in an intercalating manner; EtBr sits between the steps of the DNA ladder. Ethidium bromide is a known mutagen and potential carcinogen and therefore is not recommended for use in secondary school classrooms. The stain used in Experiment 4 functions in the same manner, but is believed to be safer. Sybr Safe is a proprietary dye and is contained within a closed gel system. Like EtBr, Sybr Safe is visualized with UV light.

Traditionally, the agarose gel is cast and placed in a horizontal electrophoresis chamber filled with buffer. A power supply is connected via the electrodes on the electrophoresis unit and an electrical current moves through the buffer and gel. These relatively slow, bulky systems are gradually being replaced with small, fast, bufferless systems such as the E-Gel used in this experiment.

**Advanced Preparation:**

1. Aliquot reagents into individual sterile 1.5ml microcentrifuge tubes.
   - # groups X 25µl molecular weight marker (label “marker”)
   - # groups X 500µl Sterile water (label “H2O”)
   *Keep marker at 4°C until ready to use.

2. Set up student workstations, or have general area for student groups to collect supplies.

**Procedure:**

1. (3 minutes) Have students form groups around the student workstations. One person from each group should obtain PCR products from Experiment 3 (see student workstations below).
2. (2 minutes) Ask students to read the vignette to themselves, or read aloud to the class.
3. (15 minutes) Allow the students to work through *Experiment 4: Visualizing the DNA* in groups.
4. (15 minutes) While gels are running, work through the pre-lab focus questions as a class.
   *Alternatively: The pre-lab focus questions can be assigned for homework the previous class.*
5. (10 minutes) Gels can be stopped after ~15-20 minutes running. Allow students to visualize gels on illuminators. Take photos if camera is available.
6. (5 minutes) When groups have finished and cleaned up, assign review questions for homework.

**Assessment:** Assessment can be used as an in-class quiz or assigned for homework.
Figure 8: Movement of DNA fragments through the pores in a gel
Student Pages

Experiment 4: Visualizing the DNA – Gel Electrophoresis

Background Information
DNA molecules can be visualized as dark bands in an agarose matrix using gel electrophoresis. Gel electrophoresis is a molecular technique that allows DNA to be separated based on size. All DNA is negatively charged, and when an electrical current is applied, DNA migrates through the gel to the positive electrode. The DNA has to move through the pores in the agarose matrix. Large pieces of DNA move slowly; small fragments of DNA move quickly.

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Traditionally, the agarose gel is cast and placed in a horizontal electrophoresis chamber filled with buffer. A power supply is connected via the electrodes on the electrophoresis unit and an electrical current moves through the buffer and gel. These relatively slow, bulky systems are gradually being replaced with small, fast, bufferless systems such as the E-Gel used in this experiment.
Experiment 4: Visualizing the DNA – Gel Electrophoresis

Setting the Stage
Polymerase chain reaction amplifies a targeted region of a genome, creating millions of copies of the target. To determine if amplification was successful, gel electrophoresis is performed. The fragment length and band intensity reveals an approximate qualitative estimate of PCR. Use your amplified samples from Experiment 3 and determine which of Mr. Bailey’s seeds is genetically engineered.

Focus Questions
1. a. What is gel electrophoresis?
   b. Describe how gel electrophoresis works.
DNA Gel Electrophoresis Laboratory

Workstation Checklist

Materials and supplies that should be present at your workstation prior to beginning this lab are listed below:

<table>
<thead>
<tr>
<th>Student workstation</th>
<th>Number required</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR samples (Seed A, Seed B, Positive Control, Negative Control)</td>
<td>4</td>
</tr>
<tr>
<td>Molecular weight marker</td>
<td>1 tube</td>
</tr>
<tr>
<td>Sterile distilled H₂O</td>
<td>1 tube</td>
</tr>
<tr>
<td>P20 pipette</td>
<td>1</td>
</tr>
<tr>
<td>P20 pipette tips</td>
<td>1 box</td>
</tr>
<tr>
<td>E-Gel® PowerBase™ and adaptor plug</td>
<td>1</td>
</tr>
<tr>
<td>E-Gel® 0.8% or 1.2% with SYBR Safe™</td>
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</tr>
<tr>
<td>Vortex</td>
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<td>UV Transilluminator</td>
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</tr>
<tr>
<td><strong>OR</strong></td>
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</tr>
<tr>
<td>E-Gel Safe Imager Transilluminator</td>
<td>4</td>
</tr>
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</table>

**Laboratory Method for DNA Gel Electrophoresis using the E-Gel System**

Prepare samples for loading

1. Obtain PCR samples from thermal cycler or 4°C.
2. Centrifuge samples in the mini centrifuge.

Prepare gel

1. Plug PowerBase™ into an electrical outlet.
2. Remove gel cassette from package and insert the gel (with comb in place) into the base right edge first. The Invitrogen logo should be located at the bottom of the base. Press firmly at the top and bottom to seat the gel cassette in the PowerBase™. A steady, red light will illuminate if the gel cassette is correctly inserted.
3. Pre-run the gel (with comb in place) by pressing and holding either the 15 minute or 30 minute button until the red light turns to a flashing green light indicating the start of the 2 minute pre-run. Release the button. At the end of the pre-run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping.
Load prepared samples

1. Remove and discard comb from the E-Gel® cassette.
2. Add 10μl sterile distilled H₂O to wells 1-5.
3. Add 20μl sterile distilled H₂O to wells 6-12.
4. Add 10μl PCR samples to wells 1-4 (Seed A, Seed B, Positive Control, Negative Control).
5. Add 10μl molecular weight marker to well 5.

<table>
<thead>
<tr>
<th>Well #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>10μl Seed A</td>
<td>10μl Seed B</td>
<td>10μl positive control</td>
<td>10μl negative control</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Marker</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>10μl</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<td>--</td>
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</tr>
<tr>
<td>H₂O</td>
<td>10μl</td>
<td>10μl</td>
<td>10μl</td>
<td>10μl</td>
<td>10μl</td>
<td>20μl</td>
<td>20μl</td>
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<td>20μl</td>
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</tr>
</tbody>
</table>

Run gel

1. Press and release the 30 minute button on the E-Gel® PowerBase™ to begin electrophoresis.
2. At the end of the run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping.
3. Remove the gel cassette and analyze your results by viewing on one of the transilluminators. The instructor will take a picture of your gel as well.

Data Collection and Analysis

1. Draw or affix a picture of your gel. Label each of the lanes.

2. Discuss your results and explain any results not expected.
Review Questions

1. What is the chemical used to visualize the DNA bands in the agarose gel? What type of light must be used?

2. What is the function of the molecular weight marker?

3. How does the band intensity relate to the amount of DNA present?

Assessment: Gel Electrophoresis Quiz
Gel Electrophoresis Quiz

1. What is the function of each of the following in gel electrophoresis of DNA?
   a. Agarose gel:
   b. Electric current:
   c. "Wells" in the gel:

2. Toward which pole (positive or negative) does DNA migrate when electric current is run through the gel? _____________ Why do the DNA molecules move toward this pole?

3. Describe how different sized DNA fragments are separated by the agarose gel matrix.

4. Examine the diagram of an agarose gel and answer the following questions.
   a. What do the bands in the drawing of the agarose gel represent?
   b. Which band(s) traveled slowest?
   c. Which band(s) traveled fastest?
   d. On the drawing, label the positive and negative ends of the gel.
Focus Questions

1. a. What is gel electrophoresis?
   b. Describe how gel electrophoresis works.

Gel electrophoresis is a method used to separate nucleic acids and/or proteins. Nucleic acids are generally separated using an agarose gel. Protein separation makes use of acrylamide gels.

The agarose gel is a colloidal mixture, with small agarose beads essentially suspended as a matrix. DNA samples are added at the top of the gel in wells. The wells are small divots in the gel that allow the DNA to sit and then move through the gel once an electrical current is applied. DNA is negatively charged, so it will move toward the positive electrode. As DNA fragments move through the matrix, smaller fragments move through faster. This is what allows DNA to separate based on size.
Data Collection and Analysis

1. Draw or affix a picture of your gel. Label each of the lanes.

   Lane 1, 7, 8: Molecular weight marker
   Lane 2: Seed A
   Lane 3: Seed B
   Lane 4, 5: Positive control
   Lane 6: Negative control

2. Discuss your results and explain any results not expected.

   Students should note the presence of a band in lane 2 = Seed A. Therefore, Seed A contains the transgenic insert. Seed B did not amplify = wild-type.

   Answers will vary. Possible errors: incorrect DNA used during PCR, not changing tips during PCR set-up causing cross-contamination, general procedural errors.

Review Questions

1. What is the chemical is used to visualize the DNA bands in the agarose gel? What type of light must be used?

   SYBR Safe is used to stain the DNA in this activity. This is a safe alternative to ethidium bromide, with integrates with the DNA and fluoresces. UV or blue light needs to be used to visualize SYBR Safe.

2. What is the function of the molecular weight marker?

   The molecular weight marker acts as an internal ruler for the gel with bands of known size. Since all gels will run differently, the standard allows us to determine the approximate size of our unknown DNA samples.

3. How does the band intensity relate to the amount of DNA present?

   The SYBR Safe binds to the DNA. The more DNA present, the more SYBR Safe is bound, causing the band to fluoresce more brightly.
**Gel Electrophoresis Quiz**

1. What is the function of each of the following in gel electrophoresis of DNA?
   - d. **Agarose gel**: *jell-o like substance that allows DNA to separate based on size.* Colloidal mixture with small pores which DNA moves through.
   - e. **Electric current**: *Drives the negatively charged DNA through the gel matrix*
   - f. "**Wells**" in the gel: *Divots that sit in the gel and act as a holding area for the DNA samples before the current is applied. The DNA can then move through the gel.*

2. Toward which pole (positive or negative) does DNA migrate when electric current is run through the gel? __________ Why do the DNA molecules move toward this pole?
   - DNA moves toward the positive pole due to its negative charge. The phosphate groups of the DNA backbone confer an overall negative charge to the DNA molecule.

3. Describe how different sized DNA fragments are separated by the agarose gel matrix.
   - All DNA is negatively charged and all will move toward the positive electrode. The smaller pieces of DNA can snake through the pores in the gel, moving faster through the gel. The smaller pieces will be at the bottom of the gel.

4. Examine the diagram of an agarose gel and answer the following questions.
   - e. What do the bands in the drawing of the agarose gel represent?
     - *Many fragments of same-size DNA co-migrating*
   - f. Which band(s) traveled slowest?
     - 1
   - g. Which band(s) traveled fastest?
     - 6
   - h. On the drawing, label the positive and negative ends of the gel. *Negative electrode at the top by the wells/sample ID; positive electrode at the bottom.*
Companion Website

All components of the Biotech in the Classroom Curriculum are available at the companion website www.biotechintheclassroom.webs.com.

Images, presentations, useful websites, and resources are also available for use.
## Equipment and Supplies

*In order of appearance*

Lesson plans provide lab set-up information.

<table>
<thead>
<tr>
<th>Source</th>
<th>Student workstation</th>
<th>Number required per group</th>
<th>Experiment</th>
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<td>Farmer John’s plant samples</td>
<td>Variable 2-4</td>
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<td>Sample extract bags</td>
<td>2-4</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>Test strips</td>
<td>2-4</td>
</tr>
<tr>
<td>Classroom laboratory</td>
<td>Sharpie</td>
<td>1</td>
<td>1, 2, 3, 4</td>
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<tr>
<td>Classroom laboratory</td>
<td>Scissors</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Classroom laboratory</td>
<td>Colored pencils/crayons</td>
<td>Assortment</td>
<td>1</td>
</tr>
<tr>
<td>Sigma Aldrich <a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a></td>
<td>Seed extraction buffer, 100µl</td>
<td>1 tube</td>
<td>2, 3</td>
</tr>
<tr>
<td>REDExtract-N-Amp Seed™ PCR Kit &lt;br&gt;10 extractions/ 10 PCR reactions : XNAS-1KT, $26.50 &lt;br&gt;100 extractions/ 100 PCR reactions : XNAS-1KT, $213.00</td>
<td>Seed preparation buffer, 15µl</td>
<td>1 tube</td>
<td>2, 3</td>
</tr>
<tr>
<td></td>
<td>Neutralization buffer, 120µl</td>
<td>1 tube</td>
<td>2, 3</td>
</tr>
<tr>
<td>Cat. No. K749520-0090, $132.23 &lt;br&gt;PK of 100 individually wrapped microtubes and micropestles</td>
<td>Sterile blue micropestle</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Fisher Scientific <a href="http://www.fishersci.com/">http://www.fishersci.com/</a></td>
<td>0.2ml PCR tubes</td>
<td>2</td>
<td>2, 3</td>
</tr>
<tr>
<td>Cat. No. 14-230-225, $125.00 &lt;br&gt;Case of 1000</td>
<td>Sterile distilled H₂O, 200µl</td>
<td>1 tube</td>
<td>2, 3, 4</td>
</tr>
<tr>
<td>Classroom laboratory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Rad <a href="http://www.bio-rad.com">http://www.bio-rad.com</a></td>
<td>P20 pipette</td>
<td>1</td>
<td>2, 3, 4</td>
</tr>
<tr>
<td>Classroom 20–200 µl Digital Micropipet 166-0551EDU, $111.00</td>
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<td></td>
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<td>Classroom 20–200 µl Digital Micropipet 166-0552EDU, $111.00</td>
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<td>Cat. No. 50863814 &lt;br&gt;Pack of 960 for $120.99 &lt;br&gt;(10 boxes of tips with 96 tips per box)</td>
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<tr>
<td>Item</td>
<td>Category</td>
<td>Description</td>
<td>Quantity</td>
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<td>UF Collaborator or UF CPET</td>
<td>Genetically engineered peanut seeds</td>
<td>1 box</td>
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<td>Local grocery store, unsalted peanuts</td>
<td>Wild-type peanut seeds</td>
<td>1 box</td>
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<td>Bio-Rad</td>
<td>Thermal cycler</td>
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<td>MJ Mini 48-Well Personal Thermal Cycler</td>
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<td>Classroom laboratory</td>
<td>70% ethanol</td>
<td>1 squeeze bottle</td>
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<td>Classroom laboratory</td>
<td>Analytical scale</td>
<td>1 box</td>
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<td>Classroom laboratory</td>
<td>Scalpel</td>
<td>1 box</td>
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<tr>
<td>Classroom laboratory</td>
<td>Kimwipes</td>
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<tr>
<td>Bio-Rad</td>
<td>P1000 pipette</td>
<td>1 box</td>
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<tr>
<td>100–1000 μl Digital Micropipet</td>
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<td>Cat. No. 166-0508EDU, $221.00</td>
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<tr>
<td>Fisher Scientific</td>
<td>P1000 pipette tips</td>
<td>1 box</td>
<td></td>
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<tr>
<td>Cat. No. 50863812</td>
<td></td>
<td>Pack of 560 for $66.98</td>
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<tr>
<td>Fisher Scientific</td>
<td>1.5ml microcentrifuge tubes</td>
<td>Variable; need at least four per group for reagent aliquots</td>
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<tr>
<td>Cat. No. 02-681-5, $17.67</td>
<td></td>
<td>Pack of 250</td>
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<tr>
<td>Seed A, Seed B from Experiment 2</td>
<td>DNA samples, 5μl each</td>
<td>(Seed A, Seed B, Positive Control)</td>
<td>3</td>
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<td>Positive control from UF collaborator</td>
<td></td>
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<tr>
<td>Sigma Aldrich</td>
<td>PCR reaction mix, 60μl</td>
<td>(Contains reaction buffer, Mg2+, dNTPs, Taq polymerase)</td>
<td>1 tube</td>
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<tr>
<td><a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a></td>
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<td>REDExtract-N-Amp Seed™ PCR Kit</td>
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<td></td>
<td>100 extractions/ 100 PCR reactions:</td>
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<tr>
<td>Invitrogen</td>
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<td><a href="http://www.invitrogen.com">http://www.invitrogen.com</a></td>
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<td>UltraPure™ DNase/RNase-Free Distilled Water</td>
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<tr>
<td>Cat. No. 10977-015, $24.72</td>
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<td>Integrated DNA Technologies (IDT)</td>
<td>Primer 1, 5μl</td>
<td>1 tube</td>
<td>3</td>
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<tr>
<td>257: GCCAAGACAACACTGATCATCTC</td>
<td></td>
<td></td>
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<tr>
<td>OR</td>
<td></td>
<td>Primer 2, 5μl</td>
<td>3</td>
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<td>Invitrogen</td>
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<td><a href="http://www.invitrogen.com">http://www.invitrogen.com</a></td>
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<tr>
<td>UltraPure™ DNase/RNase-Free Distilled Water</td>
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<td></td>
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<tr>
<td>Cat. No. 10977-015, $24.72</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Integrated DNA Technologies (IDT)</td>
<td>Primer 2, 5μl</td>
<td>1 tube</td>
<td>3</td>
</tr>
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<td>259: TGACCTTCAGAAGGCTTGATAGC</td>
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<tr>
<td>Classroom laboratory</td>
<td>Sterile distilled H2O, 50μl</td>
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<td>OR</td>
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<tr>
<td>Invitrogen</td>
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<td><a href="http://www.invitrogen.com">http://www.invitrogen.com</a></td>
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<tr>
<td>UltraPure™ DNase/RNase-Free Distilled Water</td>
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<td>Cat. No. 10977-015, $24.72</td>
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<tr>
<td>Item Description</td>
<td>Supplier</td>
<td>Cat. No.</td>
<td>Price</td>
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<tr>
<td>------------------</td>
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<td>1 x 500ml</td>
<td>Bio-Rad</td>
<td><a href="http://www.bio-rad.com">http://www.bio-rad.com</a></td>
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<td>BR-2000 Vortexer</td>
<td>166-0610EDU, $275.00</td>
<td>Vortex (optional)</td>
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<td><a href="http://www.invitrogen.com/">http://www.invitrogen.com/</a></td>
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<td>E-Gel® 50 bp DNA Ladder</td>
<td>Cat. No. 10488-099, $117.00</td>
<td>Molecular weight marker, 25μl aliquot</td>
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<td>Invitrogen</td>
<td><a href="http://www.invitrogen.com/">http://www.invitrogen.com/</a></td>
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<td>E-Gel® 1.2% with SYBR Safe™ Starter Kit</td>
<td>Cat. No. G6206-01, $109.00</td>
<td>E-Gel® PowerBase™ and adaptor plug</td>
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<td>Invitrogen</td>
<td><a href="http://www.invitrogen.com/">http://www.invitrogen.com/</a></td>
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<td>E-Gel® 1.2% with SYBR Safe™-18-Pak</td>
<td>Cat. No. G5218-01, $170.00</td>
<td>E-Gel® 1.2% with SYBR Safe™</td>
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<td></td>
<td>Fotodyne* FOTO/Phoresis* UV Transilluminator – minigel model</td>
<td>Cat. No. FD11430, $856.06</td>
<td>UV Transilluminator</td>
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<tr>
<td><strong>OR</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Invitrogen</td>
<td><a href="http://www.invitrogen.com/">http://www.invitrogen.com/</a></td>
<td></td>
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<tr>
<td></td>
<td>E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit</td>
<td>Cat. No. G6465, $824.00</td>
<td>E-Gel Safe Imager Transilluminator and iBase</td>
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<td>Classroom or personal</td>
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<td>Digital camera (optional)</td>
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</table>
## Next Generation Science Standards Correlated to Experiments

<table>
<thead>
<tr>
<th>Benchmark</th>
<th>Exp 1</th>
<th>Exp 2</th>
<th>Exp 3</th>
<th>Exp 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SC.912.N.1.6</strong>: Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>SC.912.L.14.2</strong>: Relate structure to function for the components of plant and animal cells. Explain the role of cell membranes as a highly selective barrier.</td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
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<tr>
<td><strong>SC.912.L.14.3</strong>: Compare and contrast the general structures of plant and animal cells.</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td><strong>SC.912.L.14.52</strong>: Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SC.912.L.16.3</strong>: Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information.</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td><strong>SC.912.L.16.7</strong>: Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
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<tr>
<td><strong>SC.912.L.16.9</strong>: Explain how and why the genetic code is universal and is common to almost all organisms.</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td><strong>SC.912.L.16.12</strong>: Describe the basic DNA technology (restriction digestion, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules.</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>SC.912.L.17.6</strong>: Compare and contrast the relationships among organisms, including predation, parasitism, competition, commensalism, and mutualism.</td>
<td></td>
<td></td>
<td>✓</td>
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</tr>
<tr>
<td><strong>SC.912.L.18.1</strong>: Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td><strong>SC.912.L.18.3</strong>: Describe the structures of fatty acids, triglycerides, phospholipids, and steroids. Explain the functions of lipids in living organisms. Identify some reactions that fatty acids undergo. Relate the structure and function of cell membranes.</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>SC.912.L.18.11</strong>: Explain the role of enzymes as catalysts that lower the activation energy of biochemical reactions. Identify factors, such as pH and temperature, and their effect on enzyme activity.</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
Resources

General TSWV:
- Background information: http://www.caes.uga.edu/topics/diseases/tswv/peanut/intro.html
- Tomato Spotted Wilt Virus PowerPoint: Presented by Dr. Maria Gallo
- TSWV images: http://www.caes.uga.edu/topics/diseases/tswv/peanut/images.html
- Figure 1. TSWV in tomato http://www.ces.ncsu.edu/fletcher/programs/plantpath/2006-11-tomato-disease/tdw-agenda.html
- Figure 2. Typical foliar symptoms of tomato spotted wilt virus (TSWV) on peanut http://www.caes.uga.edu/topics/diseases/tswv/peanut/foliar.html
- Figure 3. Leaf with symptoms of tomato spotted wilt virus (right) and healthy leaf (left). http://www.caes.uga.edu/topics/diseases/tswv/peanut/tswvimage.html

Experiment 1, Immunostrips:
- Peanut plants provided by Dr. Maria Gallo and Dr. Barry Tillman, University of Florida. Teachers may also wish to grow their own as part of a class project, have students collect samples, or contact a local grower for samples.
- Agdia ImmunoStrip results image: http://www.agdia.com/cgi_bin/catalog.cgi/39300

Experiment 2, DNA Extraction:
- REDExtract-N-Amp Seed™ PCR Kit from Sigma Aldrich: http://www.sigmaaldrich.com
- Transgenic peanut seed provided by Dr. Peggy Ozias-Akins via Dr. Maria Gallo, University of Florida.
- Wild-type peanut seed: unsalted peanuts from grocery store
- Figure 5. Membrane image: http://floriascience.spaces.live.com/
- Figure 6. Plant cell image: http://syd15.wordpress.com/category/uncategorized/

Experiment 3, PCR:
- Figure 7. PCR image: http://www.bio.miami.edu/~cmallery/150/gene/Taq.htm
- PCR Virtual Lab: http://learn.genetics.utah.edu/content/labs/pcr/

Experiment 4, Gel Electrophoresis:
- Figure 8. Agarose gel: www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mcb&part=A1637&rendertype=figure&id=A1649
References

General TSWV:
- UF IFAS Extension Fact Sheets: http://edis.ifas.ufl.edu/

Experiment 1, Immunostrips:
- Technical sheet for Agdia ImmunoStrip™ Tests http://www.agdia.com/cgi_bin/catalog.cgi/39300

Experiment 2, DNA Extraction:

Experiment 3, PCR:
- Primer sequences and PCR cycling conditions: Dr. Peggy Ozias-Akins, University of Georgia

Experiment 4, Gel Electrophoresis:
Assessment
CONTENT ASSESSMENT

Student name: ___________________________ Date: __________________

Circle One: Pre-test Post-test

Part I. True-False
Directions: Write True or False in the blank next to each statement.

_____ 1. Tomato spotted wilt virus is a pathogen.
_____ 2. An immunology based assay is used to detect tomato spotted wilt virus.
_____ 3. DNA cannot be extracted from seeds due to the tough cell wall.
_____ 4. Peanut plants can be genetically engineered for resistance to tomato spotted wilt virus.
_____ 5. Polymerase chain reaction cannot be used to test genetically engineered peanut seeds.
_____ 6. Agarose separates DNA molecules based on size.

Part II. Multiple Choice
Directions: Write the letter of the correct answer in the blank next to each item.

_________ 1. Tomato spotted wilt virus is:
    A. Vectored by mosquitoes
    B. Vectored by spiders
    C. Vectored by ticks
    D. Vectored by thrips

_________ 2. An immunoassay detects an interaction between:
    A. Acid and base
    B. Antigen and antibody
    C. DNA and RNA
    D. Negative and positive

_________ 3. A condition not able to be detected by an immunoassay is:
    A. Cancer
    B. HIV infection
    C. Pregnancy
    D. Strep throat

_________ 4. When extracting DNA from plant cells, heat is used to:
    A. Hybridize the DNA
    B. Prevent the DNA from mixing in solution
    C. Soften membranes allowing DNA to be free in solution
    D. Trap the energy in the cell wall

Content Assessment: Pre and Post Test
5. To perform polymerase chain reaction, your template is:
   A. Antigen
   B. ATP
   C. DNA
   D. Protein

6. The electrical current in gel electrophoresis causes DNA to move towards the:
   A. Iso-electric point
   B. Positive
   C. Negative
   D. Neutral

Part III. Short answer
Directions: Write your answers in the spaces below each item.
1. How is an immunoassay used to detect the presence of a condition?

2. Draw a plant cell. Identify the key structures involved in DNA extraction.

3. What is PCR? Identify the acronym and describe the three-step process with words and illustration.
CONTENT ASSESSMENT: ANSWER KEY

Student name: __________________________ Date: __________________

Circle One: Pre-test  Post-test

Part I. True-False
Directions: Write True or False in the blank next to each statement.

True___ 1. Tomato spotted wilt virus is a pathogen.

True___ 2. An immunology based assay is used to detect tomato spotted wilt virus.

False___ 3. DNA cannot be extracted from seeds due to the tough cell wall.

True___ 4. Peanut plants can be genetically engineered for resistance to tomato spotted wilt virus.

False___ 5. Polymerase chain reaction cannot be used to test genetically engineered peanut seeds.

True___ 6. Agarose separates DNA molecules based on size.

Part II. Multiple Choice
Directions: Write the letter of the correct answer in the blank next to each item.

_____ D _____ 1. Tomato spotted wilt virus is:
   A. Vectored by mosquitoes
   B. Vectored by spiders
   C. Vectored by ticks
   D. Vectored by thrips

_____ B _____ 2. An immunoassay detects an interaction between:
   A. Acid and base
   B. Antigen and antibody
   C. DNA and RNA
   D. Negative and positive

_____ A _____ 3. A condition not able to be detected by an immunoassay is:
   A. Cancer
   B. HIV infection
   C. Pregnancy
   D. Strep throat

_____ C _____ 4. When extracting DNA from plant cells, heat is used to:
   A. Hybridize the DNA
   B. Prevent the DNA from mixing in solution
   C. Soften membranes allowing DNA to be free in solution
   D. Trap the energy in the cell wall
5. To perform polymerase chain reaction, your template is:
   A. Antigen
   B. ATP
   C. DNA
   D. Protein

6. The electrical current in gel electrophoresis causes DNA to move towards the:
   A. Iso-electric point
   B. Positive
   C. Negative
   D. Neutral

Part III. Short answer
Directions: Write your answers in the spaces below each item.

1. How is an immunoassay used to detect the presence of a condition?
   The test strip contains antigens specific to the disease or condition of interest. The host’s immune system creates antibodies to try to fight the foreign body. A fluid or tissue sample is taken from the host and exposed to the antigens in the immunoassay test strip. If there are antibodies present in the test subject, they will bind to the antigens in the test strip and cause a chemical reaction, creating a visible detection line.

2. Draw a plant cell. Identify the key structures involved in DNA extraction.
   Key structures include: cell wall, cell membrane, nuclear membrane. The students may also list that the nucleus contains the DNA.

3. What is PCR? Identify the acronym and describe the three-step process with words and illustration.
   Polymerase Chain Reaction
   1. 94°C denature – double-stranded DNA template melts apart to produce single-stranded DNA
   2. 60°C anneal – primers bind to their complementary sequence, creating a short double-stranded DNA piece that shows DNA polymerase where to begin
   3. 72°C extend – Taq polymerase moves down the DNA template strand, allowing the complementary bases to bind, creating a new double-stranded DNA molecule
Evaluations
### Student Feedback Form: Biotech in the Classroom Laboratory Manual

**Student name:** ___________________________  **Date:** ____________  **Student grade level:** ____________  **Circle one:** Male  Female

**School name:** ___________________________  **Teacher’s name:** ___________________________  **Subject:** ___________________________

#### Part I: Evaluation of individual experiments

**Section A:** For each question below, please indicate your response for each **specific experiment** by marking High, Moderate, Low, or Not Applicable (NA).

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1: ImmunoStrip Assays</th>
<th>Experiment 2: DNA Extraction</th>
<th>Experiment 3: DNA Amplification</th>
<th>Experiment 4: Gel Electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>NA</td>
</tr>
<tr>
<td>1. Is the amount of background information sufficient?</td>
<td></td>
<td></td>
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<td>2. Do you feel you were provided adequate advance instruction?</td>
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<td>3. Do you feel you were provided enough opportunities to practice using equipment?</td>
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<tr>
<td>4. Were you provided enough time to perform the experiment?</td>
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<tr>
<td>5. Do the focus questions prepare you for the experiment?</td>
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<tr>
<td>6. Is the experimental procedure clearly written?</td>
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<tr>
<td>7. Does the data collection/analysis section assist documentation of your observations?</td>
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<td>8. Do the review questions help clarify thinking?</td>
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<td>9. Are the assessment instructions clearly stated?</td>
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<tr>
<td>10. Are the illustrations/charts/tables helpful?</td>
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</tbody>
</table>
**Section B:** Please provide additional comments pertaining to each specific experiment.

<table>
<thead>
<tr>
<th>Experiment 1: ImmunoStrip Assays</th>
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<th>Experiment 3: DNA Amplification</th>
<th>Experiment 4: Gel Electrophoresis</th>
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</thead>
<tbody>
<tr>
<td>1. Are there any topics/sections that should be added or deleted? If so, please explain.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Additional comments</td>
<td></td>
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</tbody>
</table>

**Part II.** Please evaluate the Biotechnology in the Classroom Curriculum overall. For each item below, indicate your personal response checking one of the five boxes below.

<table>
<thead>
<tr>
<th></th>
<th>1 Strongly agree</th>
<th>2 Agree</th>
<th>3 Unsure</th>
<th>4 Disagree</th>
<th>5 Strongly disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Do you think emerging pathogens are an important topic for scientific research?</td>
<td></td>
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<tr>
<td>2. Do you think emerging pathogens are an interesting topic?</td>
<td></td>
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<tr>
<td>3. Do you think emerging pathogens are relevant to your own life?</td>
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<td>4. Did you enjoy the experiments?</td>
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<td>5. Did performing the experiments increase your knowledge of emerging pathogens?</td>
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<tr>
<td>6. Do you feel the experiments reflect actual research practice?</td>
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</tbody>
</table>

Student Feedback Form: Biotech in the Classroom Laboratory Manual
Part III. Please rank order your topic preferences for other materials in the Biotechnology in the Classroom series. (1 = most preferred; 6 = least preferred)

_____ Animal pathogens

_____ Endocrine disruptors (introduced chemicals in the environment that interfere with development)

_____ Global health issues such as avian flu, malaria, or tuberculosis

_____ History of evolutionary thought: how our modern theory of evolution was shaped by the contributions of several key individuals

_____ Human pathogens

_____ Translational research: taking a research discovery through clinical trials to a final therapeutic treatment

_____ Other suggested topics? ________________________________

_____ No thanks. Labs aren’t my thing.

Part IV. Do you have any questions or is there anything you don’t understand related to the experiments you performed? ________________________________

______________________________

______________________________

Thank you!
TEACHER FEEDBACK FORM: BIOTECH IN THE CLASSROOM LABORATORY MANUAL

Thank you for reviewing the Biotech in the Classroom Laboratory Manual. Please review the entire manual and then complete the questions below. You are welcome to insert comments directly in the manual as well as in the section provided below. Comments and suggestions are greatly appreciated!

Teacher name: _____________________________
Subjects taught: ___________________________ Grade levels taught: ______________________
School: _____________________________ Email: _____________________________

Part I: Evaluation of the entire curriculum

Section A: For each item below, please indicate your response to each question as it relates to the curriculum overall by marking Strongly Agree (SA), Agree (A), Undecided (U), Disagree (D), or Strongly Disagree (SD).

<table>
<thead>
<tr>
<th></th>
<th>SA</th>
<th>A</th>
<th>U</th>
<th>D</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Are the experimental procedures appropriate for your students?</td>
<td></td>
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<tr>
<td>2. Are the topics addressed important for your course objectives?</td>
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<tr>
<td>3. Are the topics addressed relevant to your students’ lives?</td>
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<tr>
<td>4. Are the topics addressed interesting to your students?</td>
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<tr>
<td>5. Is the depth of coverage of topics appropriate?</td>
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<td>6. Is the overall quality of the laboratory manual satisfactory?</td>
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<td>7. Is the content in the manual properly sequenced?</td>
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<tr>
<td>8. Is the content in the manual adaptable for a range of student ability levels?</td>
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</tbody>
</table>

Section B: Please indicate your views regarding the usefulness of each of the following curriculum support materials by marking Extremely Useful, Moderately Useful, or Not Useful.

<table>
<thead>
<tr>
<th></th>
<th>Extremely Useful</th>
<th>Moderately Useful</th>
<th>Not Useful</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is the “How to Use this Manual” section useful?</td>
<td></td>
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<tr>
<td>2. Is the “Introduction” section useful?</td>
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<td>3. Is the “Glossary” section useful?</td>
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<tr>
<td>4. Is the “Equipment and Supplies List” section useful?</td>
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<td>5. Is the “Master Standards Correlated to Experiments” section useful?</td>
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<tr>
<td>6. Is the “Resources” section useful?</td>
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<tr>
<td>7. Is the “Reference” section useful?</td>
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<tr>
<td>8. Is the “Biotechnology in the Classroom” website useful?</td>
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</tbody>
</table>
Section C: Please provide additional comments pertaining to the laboratory manual overall.

1. Are there any topics/sections that should be added to/deleted from the laboratory manual? If so, please explain. 

2. Additional comments 

Section D: Please rank order your topic preferences for other materials in the Biotechnology in the Classroom series. (1 = most preferred; 6 = least preferred)

_____ Animal pathogens
_____ Endocrine disruptors (introduced chemicals in the environment that interfere with development)
_____ Global health issues such as avian flu, malaria, or tuberculosis
_____ History of evolutionary thought: how modern theory of evolution was shaped by several key individuals
_____ Human pathogens
_____ Translational research: taking a research discovery through clinical trials to a final therapeutic treatment
_____ Other suggested topics?

Part II: Evaluation of individual experiments (following page)
### Part II: Evaluation of individual experiments

**Section A:** For each question below, please indicate your response for each specific experiment by marking High, Moderate, Low, or Not Applicable (NA).

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1: ImmunoStrip Assays</th>
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<th>Experiment 3: DNA Amplification</th>
<th>Experiment 4: Gel Electrophoresis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
<td>NA</td>
</tr>
<tr>
<td>1. Is the amount of teacher background information sufficient?</td>
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<tr>
<td>2. Do the time estimates seem reasonable?</td>
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<tr>
<td>3. Is the amount of advance preparation reasonable?</td>
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<tr>
<td>4. Do the focus questions prepare students for the experiment?</td>
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<tr>
<td>5. Is the student procedure clearly stated?</td>
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<tr>
<td>6. Do the review questions help students clarify their thinking?</td>
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<tr>
<td>7. Does the data collection/analysis section help students organize their thoughts?</td>
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<tr>
<td>8. Is the suggested assessment of sufficient quality?</td>
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<tr>
<td>9. Are the assessment instructions clearly stated?</td>
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<td>10. Is the assessment rubric provided useful?</td>
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<tr>
<td>11. Are the illustrations/charts/tables helpful?</td>
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</tbody>
</table>
Section B: Please provide additional comments pertaining to each specific experiment.

| 1. | Are there any topics/sections that should be added or deleted? If so, please explain. |
| 2. | Additional comments |

| **Experiment 1:** ImmunoStrip Assays | **Experiment 2:** DNA Extraction | **Experiment 3:** DNA Amplification | **Experiment 4:** Gel Electrophoresis |
CONTENT AREA EXPERT EVALUATION: BIOTECH IN THE CLASSROOM LABORATORY MANUAL

Please review the entire manual and then complete the questions below. Comments may be inserted directly in the manual as well as in the section provided below. Comments and suggestions are greatly appreciated!

Reviewer name: ___________________________ Date reviewed: ___________________________

Email: ___________________________ Employer: ___________________________

Department/Division: ___________________________ Job title: ___________________________

**Part I:** For each item below, please indicate your response to each question as it relates to the curriculum overall by circling Yes (Y), No (N), or Undecided (U).

<table>
<thead>
<tr>
<th>Question</th>
<th>Y</th>
<th>N</th>
<th>U</th>
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<tbody>
<tr>
<td>1. Is the science content in the lab manual accurate?</td>
<td></td>
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<tr>
<td>2. Is the science content in the lab manual current?</td>
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<tr>
<td>3. Is the science content in the lab manual important for science literacy?</td>
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<tr>
<td>4. Is the content in the manual related to major biological concepts? (e.g., molecular genetics)</td>
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<tr>
<td>5. Is the content coverage in the manual thorough and complete?</td>
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<tr>
<td>6. Are potential misconceptions adequately addressed?</td>
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<tr>
<td>7. Is the content in the lesson properly sequenced for a novice?</td>
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<tr>
<td>8. Do the experiments model authentic research?</td>
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<tr>
<td>9. Are there additional concepts that should be included? (If yes, please elaborate below.)</td>
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</table>

**Part II:** Please include below any comments or suggestions about the curriculum.

1. General comments about the overall curriculum ____________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

2. Comments regarding individual experiments

<table>
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Content Expert Evaluation Form: Biotech in the Classroom Laboratory Manual