



NSF Engineering Research Center

From Invisible Viruses to Powerful Biotechnology Tools Lesson Plan: Isolating, Growing, and Studying Bacteriophages Using the Basic bacteriophage Culture Set A

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Overview

Bacteriophages (phages) are viruses that infect and destroy specific bacteria. Scientists study phages to understand virus–host interactions, develop alternatives to antibiotics through phage therapy, and explore biotechnology applications. In this lesson, students use the [Basic Bacteriophage Culture Set A](#) to explore the process scientists use to isolate, purify, and study lytic bacteriophages.

Students learn how microbiologists prepare bacterial growth media, create conditions for phage infection, identify phage activity through plaque formation, isolate individual phages through plaque picking, increase phage numbers through propagation, collect phage lysates, and estimate phage concentration using spot titer analysis.

The lesson introduces students to real-world microbiology workflows used in research laboratories, biotechnology companies, and pharmaceutical development. Students connect laboratory techniques to careers such as microbiologist, bioprocess technician, quality control scientist, and biotechnology researcher.

Key Search Words

Bacteriophage, phage isolation, lytic phage, phage propagation, plaque assay, plaque picking, bacterial culture, LB broth, LB agar, top agar, bacterial lawn, lysate collection, spot titer, microbiology, biotechnology, aseptic technique, viral replication, phage therapy, laboratory skills, STEM careers, 5E instructional model.

Learning Objectives

Students will be able to:

1. Explain how bacteriophages infect bacteria and reproduce through the lytic cycle.
 2. Describe the purpose of preparing bacterial growth media for phage isolation.
 3. Explain how environmental samples can contain naturally occurring bacteriophages.
 4. Demonstrate understanding of the steps involved in phage isolation, propagation, and purification.
 5. Interpret plaque formation as evidence of phage activity.
 6. Explain the importance of plaque picking in obtaining isolated phage populations.
 7. Describe how phage lysates are collected and used for further analysis.
 8. Explain the purpose of spot titer assays in determining phage concentration.
 9. Identify biotechnology applications of bacteriophages, including phage therapy and antimicrobial research.
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Curriculum Alignment (North Carolina Science Standards 2025)

NC Biology Standards

LS.Bio.8.2 Biotechnology Applications

Biotechnology has applications in medicine, agriculture, and environmental science. Biotechnology applications create access to diagnoses, treatments, and products that improve quality of life.

Students:

- Investigate how bacteriophages are isolated and studied for medical applications.
- Explain how biotechnology uses microorganisms and biological systems to develop solutions.
- Explore phage therapy as an alternative approach for controlling bacterial infections.

LS.Bio.2.1 Structure and Function of Cells

Students understand that cells contain structures and processes necessary for growth and reproduction.

Students:

- Explain bacterial growth requirements.
- Connect bacterial reproduction to phage infection and cell lysis.

LS.Bio.9.1 Analyze and interpret data to summarize how various factors such as geographic isolation, pesticide resistance, antibiotic resistance can influence natural selection.

LS.Bio.9 Understand natural selection as a mechanism for biological evolution.

LS.Bio.10 Analyze evolutionary relationships among organisms.

Students:

- Analyze plaque assay data to explain how antibiotic resistance has driven interest in bacteriophage therapy as an alternative treatment.
 - Explain how genetic variation and environmental changes drive natural selection, leading to adaptations, shifts in population dynamics, and the emergence or extinction of species, and use models to classify and illustrate evolutionary relationships among organisms.
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Classroom Time Required

Laboratory Investigation (5–7 class periods)

Day 1:

Preparing Media and Introduction to Phage
(95 minutes)

Day 2:

Phage Enrichment and Preparation for Isolation
(95 minutes)

Day 3:

Plaque Assay and Detection of Phage Activity
(95 minutes)

Day 4:

Picking Individual Plaques and Phage Purification
(95 minutes)

Day 5:

Phage Propagation and Lysate Preparation
(95 minutes)

Day 6:

Lysate Collection and Spot Titer Analysis
(95 minutes)

Materials and Technology**Basic Bacteriophage Culture Set A Materials****Media Preparation**

- LB broth powder
- Agar
- Calcium chloride (CaCl₂)
- Sterile water
- Weigh boats
- Balance
- Bottles/flasks
- Magnetic stirrer
- Stir bars
- Labels

Phage Culture Materials

- Environmental phage samples
- Bacterial host culture
- LB agar plates
- LB top agar
- Petri dishes
- Pipettes
- Pipette tips
- Microcentrifuge tubes
- Syringes
- 0.22 µm filters

Equipment

- Incubator
- Centrifuge
- Vortex mixer
- Micropipettes
- Tube racks

Technology

Teacher:

- Computer
- Projector
- Biotechnology videos
- Phage lifecycle diagrams

Students:

- Chromebooks/tablets
 - Digital lab notebook
 - Google Classroom/Canvas
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Safety

Students must:

- Wear gloves, goggles, and lab coats during laboratory activities.
 - Wash hands before and after laboratory work.
 - Never consume laboratory materials.
 - Practice aseptic technique to prevent contamination.
 - Handle bacterial cultures and biological materials according to classroom safety rules.
 - Use micropipettes correctly to avoid contamination.
 - Keep centrifuge lids closed during operation.
 - Balance centrifuge tubes before spinning.
 - Dispose of biological waste according to teacher instructions.
 - Disinfect laboratory surfaces after activities.
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Teacher Preparation for Activity

- Prepare biotechnology introduction slides.
 - Review bacteriophage structure and lytic cycle.
 - Prepare LB broth and LB agar materials.
 - Demonstrate proper measurement using a balance.
 - Prepare bacterial host cultures according to classroom procedures.
 - Label laboratory stations.
 - Prepare student worksheets and observation tables.
 - Demonstrate:
 - Aseptic technique
 - Micropipette use
 - Plate handling
 - Centrifuge safety
 - Review safety expectations.
 - Prepare examples of real-world phage applications.
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Student Preparation for Activity

Students should:

- Review cell structure and viruses.
 - Understand bacterial reproduction.
 - Learn laboratory safety expectations.
 - Review aseptic technique.
 - Understand measurements including grams, milliliters, and microliters.
 - Learn vocabulary:
 - Bacteriophage
 - Host bacteria
 - Plaque
 - Lysate
 - Propagation
 - Titer
 - Bacterial lawn
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Procedure

Day 1: Preparing Growth Media

Engage: “Invisible Viruses Around Us”

Teacher asks:

“How can a virus that is invisible to the human eye be discovered and studied?”

Students discuss.

Teacher introduces bacteriophages and explains:

- Phages infect bacteria.
 - Lytic phages destroy bacterial cells.
 - Scientists grow bacteria on nutrient media to study phages.
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Activity 1: Preparing LB Media

Students prepare media used for bacterial growth.

LB Agar Preparation

Students:

1. Measure LB agar components.
2. Mix LB broth and agar.
3. Heat mixture until dissolved.
4. Sterilize according to laboratory procedure.
5. Add calcium chloride after sterilization.
6. Pour into Petri dishes.
7. Allow plates to solidify.

Discussion:

Why do bacteria require nutrient media?

Why is agar used?

Day 2: Preparing Phages for Isolation

Phage Enrichment

Students learn:

Environmental samples contain mixtures of microorganisms and phages.

Phage enrichment increases the number of phages by allowing them to infect suitable bacterial hosts.

Students:

- Combine environmental sample with bacterial host culture.
 - Allow phage infection and replication.
 - Prepare enriched phage sample.
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Day 3: Plaque Assay and Phage Detection

Students create bacterial lawns.

Students:

1. Mix bacterial culture with soft agar.
2. Pour onto LB agar plates.
3. Add enriched phage sample.
4. Incubate plates.

Observation:

Students look for clear zones called plaques.

Discussion:

A plaque indicates:

- Phages infected bacteria.
 - Bacterial cells were destroyed.
 - New phages were released.
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Day 4: Picking a Phage

Plaque Isolation

Teacher explains:

A plate may contain multiple phage types. Scientists isolate individual phages by selecting a single plaque.

Students:

- Observe plaque morphology.
- Compare:
 - Size
 - Shape
 - Clarity
- Select individual plaques.
- Transfer plaque material into phage buffer.

Discussion:

Why is isolating one plaque important?

Day 5: Phage Propagation

Increasing Phage Numbers

Students learn:

Propagation allows scientists to produce enough phage material for further study.

Students:

- Combine isolated phage with bacterial host.
 - Allow phage infection.
 - Prepare additional plates.
 - Observe increased plaque formation.
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Day 6: Flooding and Lysate Collection

Collecting Phage Lysate

Students:

1. Add phage buffer to plates containing phage plaques.
2. Allow phages to diffuse into buffer.
3. Collect liquid containing phages.
4. Filter lysate.

Teacher explains:

The collected liquid contains millions of phage particles and is called a lysate.

Day 7: Spot Titer Analysis

Students determine phage concentration.

Students:

- Prepare serial dilutions.
- Apply phage samples to bacterial lawns.
- Observe plaque formation.

Students calculate:

Approximate number of phages present in sample.

Differentiation

English Language Learners (ELL)

Strategies:

- Provide vocabulary cards with images.
- Use diagrams of phage infection.
- Provide sentence starters:

“Phages infect bacteria by _____. ”

“A plaque shows _____. ”

- Allow students to explain concepts verbally.
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Students with Learning Disabilities

Modifications:

- Provide step-by-step procedure checklist.
- Assign laboratory roles:
 - Recorder
 - Equipment manager
 - Observer
- Provide enlarged diagrams.
- Allow additional time.
- Use teacher demonstrations before independent work.

Alternative assessment:

Students label diagrams instead of writing explanations.

Advanced/Gifted Students

Extensions:

- Research phage therapy.
- Compare bacteriophages with antibiotics.

- Analyze plaque morphology.
 - Design a phage-based treatment proposal.
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Assessment / Check for Understanding

Formative Assessment

Teacher observes:

- Proper laboratory technique.
 - Accurate measurements.
 - Safety practices.
 - Student explanations.
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Student Laboratory Notebook

Students record:

- Media preparation observations.
 - Plate observations.
 - Plaque descriptions.
 - Lysate collection results.
 - Spot titer data.
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Exit Ticket Questions

1. Why are bacteria needed for phage isolation?
 2. What does a plaque represent?
 3. Why is plaque picking necessary?
 4. Why do scientists collect lysates?
 5. How can phages benefit human health?
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Final Assessment

Students create a flowchart:

Environmental Sample → Enrichment → Plaque Assay → Plaque Picking → Propagation → Lysate Collection → Spot Titer

Rubric:

Criteria	Points
Correct sequence of phage isolation steps	5
Explanation of purpose of each step	5
Laboratory vocabulary used correctly	5
Biotechnology connection explained	5

Required Resources

1. SEA-PHAGES Discovery Guide
<https://seaphagesphagediscoveryguide.helpdocsonline.com/>

Used for:

- Phage isolation concepts
 - Plaque assays
 - Phage enrichment procedures
2. HHMI BioInteractive
<https://www.biointeractive.org/>

Used for:

- Virus biology resources
 - Biotechnology lessons
3. Basic Bacteriophage Culture Set A Laboratory Manual

Used for:

- Media preparation
 - Phage culture procedures
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Supplemental Resources

- Phage therapy research articles
 - Biotechnology career resources
 - Pharmaceutical microbiology videos
 - Bacteriophage lifecycle animations
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Author Comments

This lesson was designed to introduce high school students to authentic biotechnology laboratory practices. The investigation connects microbiology concepts with careers in biotechnology, pharmaceutical manufacturing, and biomedical research.

The activity can be shortened by focusing only on plaque assays and phage visualization or expanded into a multi-week biotechnology research project.

Sources (APA Format)

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Appendix: Student Worksheet

Basic Bacteriophage Culture Investigation

Name: _____ Date: _____

Part 1: Phage Knowledge

1. What is a bacteriophage?
 2. What organism does a bacteriophage infect?
 3. What evidence shows that phages are present on a bacterial plate?
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Part 2: Laboratory Steps

Complete the flow chart:

Environmental Sample

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↓

Plaque Assay

↓

↓

Propagation

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↓

Spot Titer

Part 3: Observation

Describe your plaque:

Size:

Shape:

Color:

Clarity:

Part 4: Reflection

Why are bacteriophages important in biotechnology?

What biotechnology career uses skills from this laboratory?