Viral Infection Lab Activity

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WARD'S is working with educators to design our literature to meet the many challenges of today's teachers. Included in every activity are correlations to National Science Education Content Standards and easy to follow instructions, as well as diversified assessments that cater to students of all levels and learning styles.

MATERIALS INCLUDED IN THE KIT

This lab activity is designed for fifteen groups of students

Materials Checklist

□	40	Transfer pipettes
□	30	Disposable inoculating loops
□	30	1.5 ml microfuge tubes
□	30	Bacterial cell spreaders
□	1	Tube of sterile water
□	4	Bottles of luria agar
□	1	Redemption coupon for:
		1 *E. coli* BL21-GFP Culture
		1 tube of T7 phage (10^{-6} dilution)
		4 vials of ampicillin
		4 tubes of luria broth

MATERIALS NEEDED BUT NOT PROVIDED

- Incubator (Optional)

Consult your WARD'S catalog for additional products you may need

SPECIAL HANDLING INSTRUCTIONS

- Redeem the enclosed coupon for the perishable components: *E.coli*, T7 phage, luria agar, and ampicillin.
- Store the vials of ampicillin frozen until needed.
- Store the *E. coli* culture refrigerated: use or subculture on LB/AMP plate within two weeks.
- All other components may be stored at room temperature.

NATIONAL SCIENCE EDUCATION CONTENT STANDARDS

Standard K-12: Unifying Concepts and Processes	Evidence, models, and explanation
	Form and function
Standard A: Science as Inquiry	Abilities necessary to do scientific inquiry
	Understandings about scientific inquiry
Standard C: Life Science	The cell
	Matter, energy, and organization in living systems
Standard E: Science & Technology	Understandings about science and technology
Standard F: Science in Personal & Social Perspectives	Personal and community health
Standard G: History & Nature of Science	Science as a human endeavor
	Nature of scientific knowledge
	History perspectives

OBJECTIVES

- Students will be able to define the term virus.
- Students will be able to explain why viruses are not considered living organisms.
- Students will be able to explain the relationship between a virus and its host cell.
- Students will learn how to culture *E. coli*.
- Students will infect bacteria with the virus bacteriophage T7.
- Students will be able to compare and contrast T7 with other viruses such as Human Immunodeficiency Virus (HIV) and the coronavirus that causes Severe Acute Respiratory Syndrome (SARS-CoV)

TIME REQUIREMENTS

Day 1: 45 minutes
Day 2: 30 minutes

BACKGROUND

Based on the traditional qualities used to define life, such as the independent ability to carry out metabolism and reproduction, among other qualities, viruses would not be considered alive. Unlike living organisms, viruses require the molecular machinery of a living host cell in order to replicate. Due to this deficiency, scientists often describe viruses as "a type of cellular parasite." However, like living organisms, all viruses posses a genetic code.

The general parasitic reproductive strategy of all viruses is to insert their genetic code into a host cell, use the machinery of the cell to express their genetic code, assemble new virus particles, and exit the cell. However, viruses demonstrate amazing diversity in how they accomplish this strategy. Some viruses have a genetic code of DNA, while others, such as the Human Immunodeficiency Virus (HIV) posses a genetic code of RNA. Viruses can vary in the shape and composition of their protective coat that stores the genetic code. How they exit a cell also varies. Viruses may bud from a host cell, taking a piece of the cell membrane with it to form an envelope. Other viruses may break open, or "lyse," the cell releasing the virus particles into the environment at once. Some viruses may even remain dormant, simply using the host cell to replicate their genetic code, only to activate when specific environmental conditions occur.

DID YOU KNOW?

T7 can complete its life cycle in just 25 minutes at 30ºC and can produce about 100 new virus particles from a single infected *E. coli* cell.

In this lab you will use the bacteriophage T7 as a model for learning about viruses. The word bacteriophage, coined by Felix d'Herelle in 1917, comes from the word "bacteria" (the host cell) and the Greek word "Phagein," meaning "to eat." While the viruses do not eat the host cell, the exact nature of the relationship between bacteriophage, or simply phage, and their host was not clear until the invention of the electron microscope and the development of the field of molecular genetics in the 1940s and 50s. In fact, it would be the famous phage experiment by Alfred Hershey and Martha Chase in 1952 that would prove, beyond a reasonable doubt, that the genetic code was made of DNA. Modern biology, especially molecular genetics, owes much to the basic research of bacteriophages.

The host for T7 phage is the prokaryotic bacteria *E. coli*. T7 is the type of phage that lyses its host when the new virus particles leave the cell. Scientists, therefore refer to T7 as a "lytic" phage. When *E.coli* are cultured on an agar plate in the presence of T7, large clear holes, or "plaques" form where the bacteria are growing. Each plaque represents a place where a single virus infected a single cell and replicated more virus particles. These viruses infect neighboring cells, produce more virus particles, and so on, until all of the bacteria in this section of the agar plate are killed producing a clear spot.

The bacteria and T7 phage stock supplied with this lab, come from the Brookhaven National Laboratory (BNL) research lab of biophysicist F. William Studier. T7 has been a continuing interest of Studier's since the 1960s. Processes studied in his lab have included the entry of T7 DNA into its host cell, *E. coli*; overcoming host restriction; expression of T7 genes and shut-off of host functions; replication, processing and packaging of T7 DNA; and structure and assembly of phage particles. A highlight of BNL's contribution to phage research includes the determination of the complete nucleotide sequence of T7 DNA, 39,937 base pairs. Through the T7 genome project, BNL scientists William Studier and John Dunn revealed the DNA coding sequences for more than fifty T7 proteins and the arrangement of signals that direct T7 gene expression.

Today, Studier continues to do basic research on T7 phage at BNL. In 2004, he was awarded an R&D 100 Award by R&D Magazine for his development of a gene expression system that originated from his basic research of T7. Studier's T7 expression system, is now used worldwide by academia and industry to produce proteins of interest within bacterial cells.

References:

Studier FW. 1972 Bacteriophage T7. *Science* 176. p. 367-376.

Studier FW. 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *J Mol Biol* 79. p. 237-248.

Dunn JJ. and FW Studier. 1983. Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. *J Mol Biol* 166. p. 477-535.

Studier FW. and BA Moffatt. 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J Mol Biol* 189. p. 113-130.

Studier FW. 2005. Protein production by auto-induction in high-density shaking cultures. *Protein Expr Purif* 41. p. 207-234.

Demerec M. and U. Fano. 1945. Bacteriophage-resistant mutants in *Escherichia coli*. *Genetics* 30. p. 119-136.

Delbrück M. 1946. Bacterial viruses or bacteriophages. *Biol. Rev.* 21. p. 30-40.

Alberts B., Bray, D., Lewis J.,Ra M., Roberts K., Watson J. D. 1994. *Molecular Biology of The Cell* 3rd Edition. Garland Publishing.

William Studier

DID YOU KNOW?

William Studier developed a system that uses T7's genetic "switch" for expressing genes, called the T7 promoter, to help express genes from many different organisms- including humans. This system played a key role in the development of the biotechnology industry. William Studier is still researching T7 phage at Brookhaven National Laboratory.

PRE-LAB PREP

This experiment utilizes a strain of *E. coli* (*E. coli* BL21) that has been genetically engineered to express Green Fluorescent Protein (GFP). GFP, from the pacific jellyfish, has been added to the help students visualize the bacteria when grown on an agar plate. Under UV light, the bacteria will fluoresce a bright green color. The bacteria will also look green under ambient light conditions. It is important to grow the bacteria on LB+AMP plates, as the ampicillin selects for the maintenance of the plasmid that contains GFP.

Two days before the lab:

You may wish to have students prepare the ampicillin solution and pour their own plates the day before the experiment, or you may prepare them yourself in advance.

Sterile technique should be used throughout the pre-lab preparation and procedure in this lab activity. If students are not familiar with sterile technique, a brief review before conducting the procedure would be beneficial.

Preparation of Ampicillin Stock Solution

Add 2 ml sterile distilled water to each of the vials containing the ampicillin. Cap and shake gently to mix. Label and store frozen until needed. Remove from the freezer 30 minutes prior to pouring your antibiotic plates.

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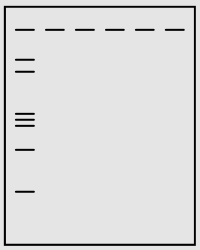


DID YOU KNOW?

Slab Gel Electrophoresis, a method for separating proteins and nucleic acids, was developed at BNL in 1970 by William Studier, Jake Maizel, and William Siegelman. This technique, developed for Studier's research on T7 phage, is considered one of the most important technical advancements in molecular biology.

Melting Prepared Media

Always use aseptic technique. Melt prepared agar using one of the following methods:

Always wear heat-protective gloves when handling heated bottles.

A. Hot Water Bath Method

1. Loosen the cap on the bottle.
2. Place the bottle in a large beaker or saucepan. Add water until the level rises above the level of medium in the bottle.
3. Place a thermometer in the water and heat the water on a hot-plate until it boils.
4. Boil gently for several minutes. Swirl the bottle occasionally to ensure the medium is completely liquefied.
5. Turn off the heat and remove the bottle from the water bath.

B. Microwave Method

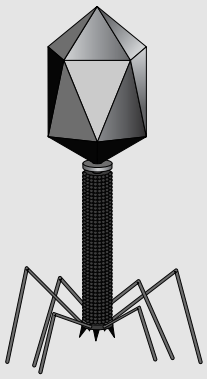
1. Loosen the cap on the bottle.
2. Microwave on high for two minutes.
3. Remove the bottle from microwave. Squeeze it to help break apart the agar and swirl gently.
4. Continue microwaving the agar in 30-second intervals until it is completely liquefied. Swirl the bottle gently after each 30 second interval.

Pouring Media Plates

Always wear heat-protective gloves when handling heated bottles.

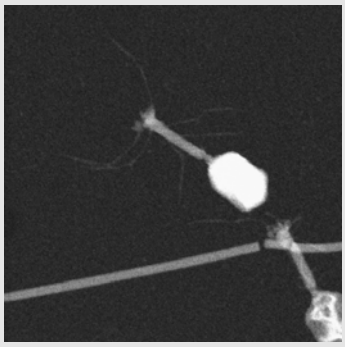
Each bottle of Luria agar contains enough medium for ten plates.

1. Once the Luria agar bottles have cooled to 50-55ºC, pour one vial of ampicillin solution (2 mL) into each bottle of Luria agar. Swirl each bottle gently to mix the ampicillin solution into the agar.



DID YOU KNOW?

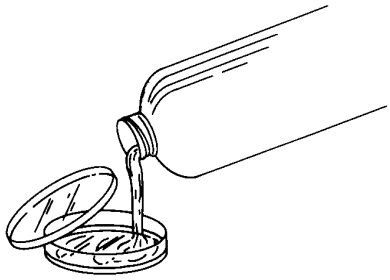
The genome, or genetic code, of T7 contains 56 genes. The human genome is estimated to contain between 20,000-25,000 genes.



DID YOU KNOW?

92% of T7's genetic code actually codes for proteins while only 1.5% of the human genetic code is estimated to code for proteins.

2. Lift the lid of a Petri dish just enough to admit the neck of the bottle. Avoid touching the neck of the bottle to any surface.



3. Pour approximately 15 to 20 ml of the liquefied agar into the Petri dish; this should be slightly more than enough to cover the bottom of the plate. Replace the lid. Swirl gently to ensure the agar completely covers the bottom of the plate.

4. Repeat until you have poured all forty plates.

5. Label all of these plates "Luria agar w/ampicillin" and allow the plates to remain undisturbed until the agar solidifies. This should take approximately 20-30 minutes.

6. Once the agar has solidified, store the plates in the refrigerator upside down until needed. If you are not using the plates that day, place them in a plastic bag or wrap them with plastic wrap prior to placing them in the refrigerator.

Subculture *E. coli*

The day before the experiment, obtain eight agar plates. Using a sterile loop, streak some bacteria on eight plates. Incubate these plates at 37° in an incubator overnight or at room temperature for two days. These plates will be used as starter plates.

One day before the lab:

Aliquot the reagents to be used in the experiment for each pair of students. Use sterile technique to the best of your ability. For each pair of students, aliquot into separate 1.5 ml tubes the following:

- 150 µl of T7 phage
- 1200 µl of luria broth

These aliquots should be stored in a refrigerator until ready for use the next day.

MATERIALS

MATERIALS NEEDED PER GROUP

4 transfer pipettes
2 disposable inoculating loops
2 1.5 ml microfuge tubes
2 bacterial cell spreaders for plating cells
2 LB+AMP plates
1 tube of luria broth (1.2 ml)
1 tube of dilute T7 phage (150 ul)

SHARED MATERIALS

"Starter plates" of *E. coli*

DID YOU KNOW?

T7 can produce about 100 new virus particles from a single infected *E. coli* cell.

DID YOU KNOW?

Two historic places of phage research, Brookhaven National Laboratory and Cold Spring Harbor Laboratory, are both located on Long Island, NY

PROCEDURE

1. Using a transfer pipette, transfer 500 µl of luria broth to two separate 1.5 ml microcentrifuge tubes. Label one tube "+virus" and the other tube "– control."

2. Using a plastic inoculating loop, transfer 1-2 "loop-fulls" of GFP expressing *E. coli* from your starter plate (green in color) to each tube.

3. Use the transfer pipette to resuspend the cells in the luria broth. Both tubes must be cloudy with *E. coli* cells to get good results.

4. Use the transfer pipette to transfer 0.1 ml of virus stock to the "+ virus" tube only. Close the top of the microfuge tube and "hand incubate" both tubes for 5 minutes (hold in hand).

5. Transfer 0.1 ml of the "+ virus" tube to a LB + AMP plate. Spread cells evenly with a bacteria spreader (hockey stick). Label this plate "+ virus."

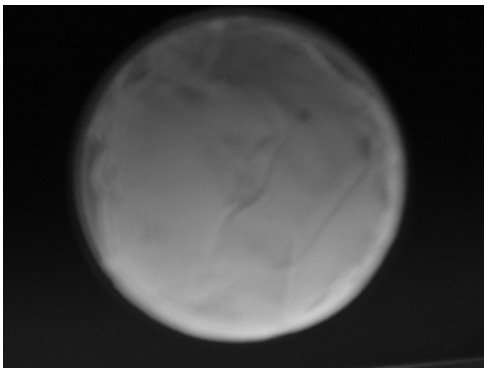
6. Transfer 0.1 ml of the "- control" tube to a LB + AMP plate. Spread cells evenly with the a bacteria spreader (hockey stick). Label this plate "- control."

Make sure you do not cross-contaminate the two different tubes/plates/spreaders.

7. Place the plates upside down in a 37°C incubator to grow overnight. Results will be visible by the next day.

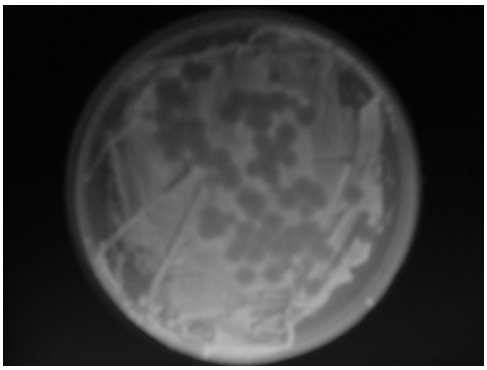
Grow plates at room temperature for visible results in 2-3 days.

ANALYSIS



- control

The "- control" plate will just have a green lawn of *E. coli* covering the plate.



+ virus

The "+ virus" plate will contain a green *E. coli* lawn with large plaques (clear spots).

Calculate the number of plaque-forming viruses (pfu) per µl for your viral stock. This is referred to as the phage titer.

1. Count the number of viral plaques on your "+ virus" phage plate.

2. Determine how many µl of viral stock were actually plated.

   Hint: you added 100 µl of virus stock into 500 µl of LB broth. You then plated 100 µl of this dilution on your final plate.

3. Determine the number of plaque forming viruses per µl for your viral stock.

   phage titer: µl of stock solution X plating dilution factor = # of plaques

   For example:
   If you counted 40 plaques per plate.

   $20\mu l \times 10^{-6} = 40$ plaques

   or

   phage titer $= \frac{40 \text{ plaques}}{20\mu l \times 10^{-6}}$ OR $\frac{2 \text{ plaques}}{1\mu l \times 10^{-6}}$ OR 2 plaques x $1\mu l \times 10^{6}$ OR $2pfu/\mu l \times 10^{6}$

ASSESSMENT

1. What does the T7 phage insert into the *E. coli* cell during infection?

   Its genetic code- DNA

2. Are bacteriophages, such as T7, harmful to human cells?

   No. T7 is cell-specific. While T7 can infect *E. coli*, it can not infect human cells.

3. After T7 successfully infects a cell, describe how the virus "hijacks" the cell to make new virus particles.

   Using the cellular machinery of *E. coli*, the viral genetic code (DNA) is transcribed into RNA . The viral RNAs are then translated, via the *E. coli* ribosomes, into viral proteins. The viral proteins are assembled and packaged with copies of the T7 genome. The cell fills with new virions and lyses.

4. Why must you use sterile technique during this lab?

   LB plates are an ideal growing medium for many environmental microorganisms, including microorganisms that may be harmful to your *E. coli.*

5. Why did you setup a second tube containing diluent buffer and *E. coli*, but without adding virus? What is this tube called?

   This tube is called a (negative) control. When performing the experiment, the negative control was needed to demonstrate that T7 phage were responsible for plaque formation.

6. If you were to add T7 phage to a turbid, (cloudy) stationary culture of *E. coli* and incubate for 10 hours, would you expect the culture to maintain its turbidity, become clearer, or become more cloudy? Explain.

   You would expect a decrease in turbidity since the bacterial cells are lysed by the phage.

7. What does each clear plaque on your "+ virus" plate actually represent?

   It represents a location on the plate where a single *E. coli* cell was successfully infected by T7, which then made new virus particles, which then infected more cells, spreading out and repeating this process, until you are ultimately left with a circular location on the plate that contains no visible *E. coli* growth.

8. Why do you grow the *E. coli* LB+AMP plates upside down?

   If you do not place the plates upside down, moisture from the LB agar will evaporate and condense on the lid. The condensation looks like water droplets. Due to gravity, the droplets can fall back down on the plate and inhibit the growth of the *E. coli* cells. Growing the plates upside down prevents this.

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