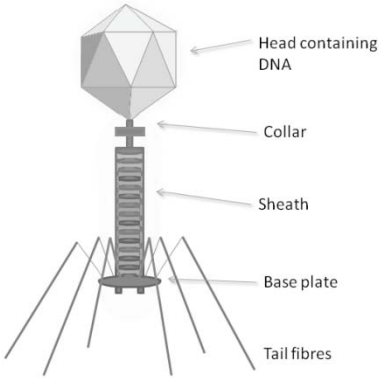
BACTERIOPHAGE ENUMERATION

Viruses were first discovered in the late 19th century by Dimitri Ivanovski and Martinus Beijerinck with the discovery of the tobacco mosaic virus. Since this initial discovery, seven classifications of viruses have been defined based on the makeup of their genomes, as follows:

[1] double stranded (ds) DNA viruses
[2] single stranded (ss) DNA viruses
[3] dsRNA viruses
[4] + sense ssRNA viruses
[5] – sense ssRNA viruses
[6] ssRNA-RT retroviruses (uses reverse transcriptase to create a DNA intermediate)
[7] dsDNA-RT viruses

Some common recognizable viruses include the tobacco mosaic virus (Group 4), HIV (Group 6), and lambda phage (Group 1). Even though some diseases are caused by a specific virus, they are more often caused by any of several members from a viral family. This is the case for the common cold (>200 viruses including rhinoviruses & coronaviruses; Group 4) and the flu (Orthomyxoviridae; Group 5).

All viruses consist of a protein coat (called a **capsid**) encapsulating the viral genetic material. Some are also surrounded by a lipid envelope derived from the host cell membrane. Viruses that infect bacteria are called **bacteriophages**. Some bacteriophages, such as the P1 phage used in this lab, have a complex structure consisting of an icosahedral head, collar, contractile tail, base plate, and tail fibers (seen at right). The **P1 phage** is a Group 1 (dsDNA) virus of the Myoviridae family.



The viral life cycle can involve the lytic **and/or** lysogenic cycles. In the **lytic cycle**, the virus uses the host's cellular machinery to copy the viral genome and other viral components. Upon completion, the genome is packaged into a viral particle (**virion**). When enough copies of the virus are made, host cell lysis is triggered and the virions are released to infect new hosts. Some bacterial viruses also have a **lysogenic cycle** where the viral genome is inserted into the host genome by homologous recombination, producing a prophage. As the host cell divides, the viral genome is also copied. An external cue, often a stressor such as UV radiation, subsequently causes the prophage to be excised from the host genome and the virus enters the lytic cycle.

For this lab, we will be estimating the number of phage particles used to infect the *E. coli* recipient cells. Estimates of phage density are calculated using a protocol known as a **phage titer**. This method is very similar to the viable cell counts we performed during the bacterial enumeration lab. Dilutions of phage lysate are plated onto a soft agar lawn of *E. coli* cells. A viral particle will infect a single host where it lands. It will then reproduce, exit the host during the lytic cycle, and diffuse to infect nearby host cells. The result is a clearing of *E. coli* cells known as a **plaque**. Like colonies, plaques should only be counted between 30 and 300 on a plate to give statistically viable results. From these results, the density of phage particles in the phage lysate can be estimated by calculating **plaque forming units (PFUs)** per ml. These calculations are identical to those we have already learned to find colony forming units per ml (CFU/ml).

FIRST PERIOD

Phage Enumeration

Material:

1. Recipient *E. coli* broth culture
2. P1 bacteriophage transducing lysate (10 μl)
3. Six preheated LB plates
4. Six 3 ml tubes of H top agar
5. Sterile saline and 13 microfuge tubes for dilutions
6. Preheated (37˚C & 60˚C) water baths

Protocol: (working in pairs)

1. Prepare serial dilutions (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}) of the P1 bacteriophage lysate in the supplied microfuge tubes. Note: 10 μl P1 plus 990 μl saline = 10^{-2} dilution.

2. Add 100 μl of each dilution to six separate microfuge tubes with 100 μl recipient *E. coli* broth. Note: this will make your plate 10^{-3} for the total dilution.

3. Incubate mixtures for 15 minutes at 37˚C. (This time allows the virus to adsorb to the *E. coli* cells.)

4. Add each phage-host mixture to a 3 ml H top agar tube. Mix by rolling the tube between your fingers. Do not allow the agar to solidify before pouring!

5. Pour the H top agar/phage/host mixture onto the prepared preheated LB agar plates.

6. Allow the agar to solidify completely (~5 minutes). Incubate plate agar-side up at 37˚C for 24 - 48 hours.

SECOND PERIOD

Phage Enumeration

Protocol: (working in pairs)

1. Examine the phage titer plates for the appearance of plaques (clearing zones in the *E. coli* lawn). Record plaque counts and a description of the plate for each dilution below.

Dilution	Plaque forming units (PFUs)	Description of Plate
10^{-3}		
10^{-4}		
10^{-5}		
10^{-6}		
10^{-7}		
10^{-8}		

2. Calculate the number of plaque forming units per ml of the original phage lysate (PFUs/ml).

$$\text{Phage Titer} = \frac{\text{PFU}}{\text{Original sample volume (ml)}}$$

3. Why might this be important information?