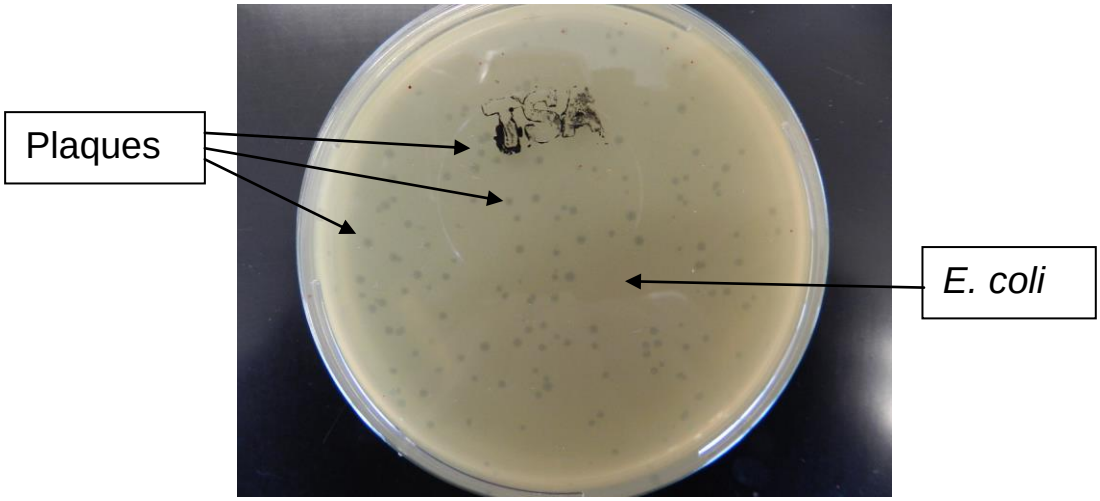
Bacteriophage Plaque Assay Substitute

Live Organisms: *E. coli B, Bacteriophage T_2 or T_4*

Phage and *E. coli B* are mixed together using soft agar. The soft agar mixture is poured and swirled onto the surface of TSA plates. The soft agar solidifies. After incubation, we see that the *E. coli* have covered the entire surface of the TSA plate (lawn of bacteria) and the viruses have reproduced killing *E. coli* and leaving holes in the growth called plaques.

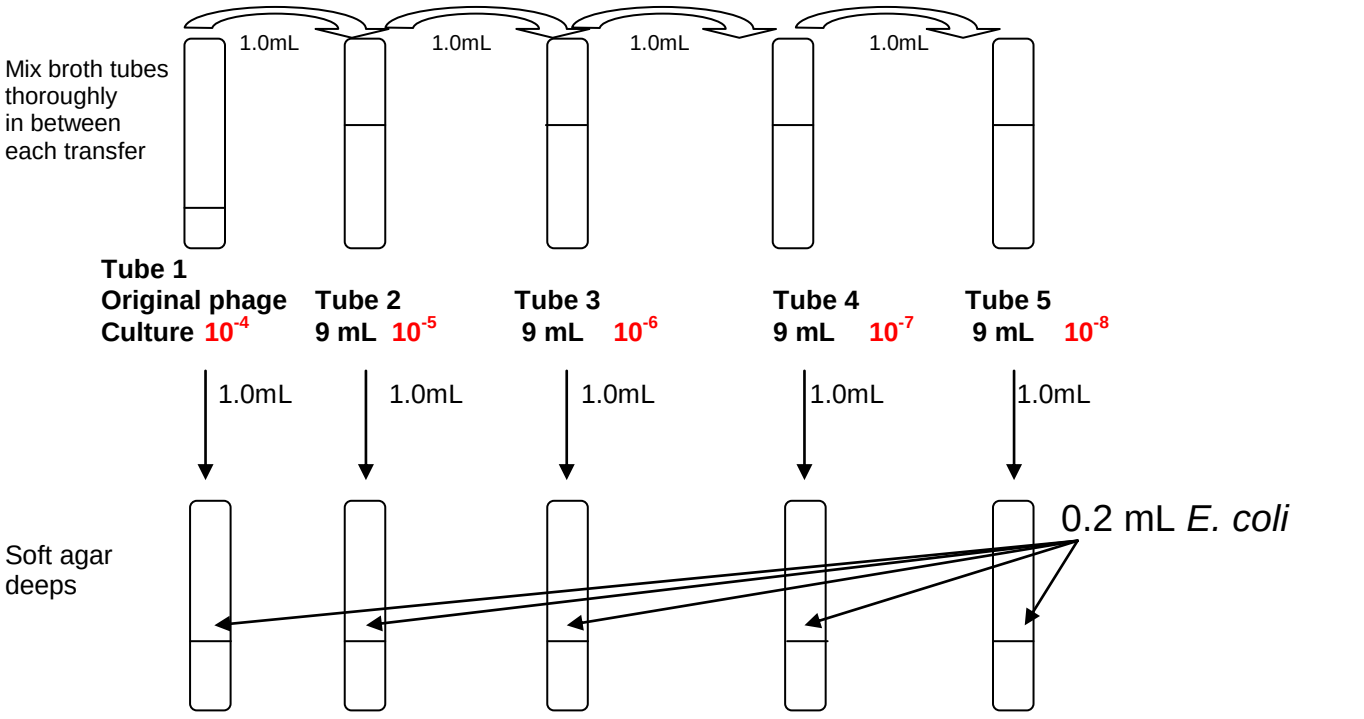
The plaques are counted (30-300). The numbers of plaques are multiplied by the dilution factor (DF) of the plate to find pfu/mL. PFU = plaque forming units. One virus particle (virion) produces each plaque. pfu/mL is the same as viruses or phages/mL. This represents the concentration of phages/mL in the original broth culture. The calculations are the same as cfu/mL for determining the concentration of bacteria in a broth culture.

The plaques stay small and will not spread and kill all of the *E. coli* on the TSA plate. The reason why is because the phages want to infect log phase bacteria (high metabolism and reproductive rate). Within hours, the bacteria enter into stationary phase to be followed by death. It is not advantageous for the phages to infect stationary phase bacteria so the phages sit and wait for better hosts to come along.



Take Home Lesson: Define: plaque, PFU, TNTC, and TFTC. As with the previous serial dilution, the number of plaques must fall between 30 and 300. Calculate the number of pfu's per mL of stock phage culture by multiplying the number of pfu's on a plate times the dilution factor of that plate. What type of phage are we using? T-even bacteriophage What life cycle does it use? Lytic; otherwise plaques would not be present. Explain the steps in this life cycle.

1. **Put all dilution tubes including the original phage tube into water bath.**
2. **Do all dilutions first and then transfer 1.0mL of each dilution to the corresponding soft agar deep.**
3. **Then, add 0.2mL *E. coli* to each soft agar deep.**
4. **Remove soft agar deep from water bath, dry tube with paper towel, then pour each soft agar deep (phage dilution + *E. coli*) on top of an agar plate and <u>immediately</u> gently swirl a few times to spread mixture onto agar surface.**
5. **Wait 5 minutes before turning agar plates upside down.**



Note: the "effective" dilution on the surface of the plate is the same as it is in the tubes because we multiply the volume placed on the plate (1 mL) times the dilution in the tube. 1 x anything = the same thing!

Plate 1 10^{-4}	Plate 2 10^{-5}	Plate 3 10^{-6}	Plate 4 10^{-7}	Plate 5 10^{-8}
DF= 10^{4}	10^{5}	10^{6}	10^{7}	10^{8}

Example: There are 147 plaques on plate 4. Plates 1, 2, 3 are TNTC, plate 5 is TFTC.

Therefore: 147 x 10^{7} = **1.47 x 10^{9} pfu/mL**