Plaque Drop Assay

**Introduction**
With this assay you can determine the phage titer concentration

**Materials**

- sterile MYPGP Agar plate
- sterile dd. H₂O - 30 mL
- BHI Media - 20 mL to dissolve the phages
  - BHI Powder 37 g/L
  - Yeast Extract 3 g/L
- BHI Top agar - 20 mL
  - BHI Powder 37 g/L
  - Yeast Extract 3 g/L
  - 0.7 % Agar

**Procedure**

- **Day 1**
  1. Take one row of the bacteria lawn from a fresh (max. 2 days old) *P.larvae* streak plate and dissolve them in 200 µL sterile dd.H₂O
  2. Dissolve the bacteria (resuspend, vortex)
  3. Plate out the 200 µL bacteria suspension onto a MYPGP agar plate
  4. Incubate overnight at 37 °C

- **Day 2**
  1. Prepare and autoclave BHI Top Agar and cool it at 55 °C
  2. Turn the photometer on
  3. warm the MYPGP Plates at 37 °C
4. Resuspend the *P. larvae* ONC Plate with 2 mL ddH₂O (sterile) and pipette it in a 1.5 mL reaction tube

5. Centrifuge 5,000 x g, 5 min in the eppifuge

6. Discard the supernatant, and dissolve the Pellet in ca. 250 µL ddH₂O (sterile)

7. Create 1:50 and 1:500 dilutions from the Probe and measure OD₆₀₀

8. Set the OD₆₀₀ of the bacterial probe to be OD₆₀₀ = 15

9. Pipette 50 µL OD₆₀₀ = 15 bacterial suspension into a glass eprouvette, add 4 mL BHI-Top agar (~55 °C), mix, pour it quickly on a MYPGP agar plate
   • the plate should look smooth (no air bubbles)

10. Cool the MYPGP Plate at RT for 10 min

11. Prepare the phage dilutions (dilute in BHI media)
   • Dilutions from 10⁻¹ to 10⁻⁹

12. Pipette a 5 µL drop from the phage dilution on the cooled MYPGP-Top agar plate

13. Let it sit at RT for 20-30 min

14. Incubate overnight at 37 °C, do not invert the plate

• Day 3

15. Count the plaques

16. Determine the Phage titer
   • PFU/mL = (Plaque Count * 200) / Dilution