Plaque and Spot Assay

To check the concentration of infectious phages.

Materials

- LB plates
- Soft LB-agar (0.6 %)
- Bacterial culture at OD ≈ 1
- Phage dilution series in LB

Spot Assay Protocol

1. Prewarm the LB plates at 37°C.
2. Add 100µL of bacterial culture to 3mL of pre-melted soft agar and mix by vortexing.
3. Pour the soft agar mix on the top of a plain LB plate and let it solidify.
4. Spot small volume (1-3µL) of the diluted phage solution on the top of soft agar and incubate at 37°C overnight.
5. Determine an appropriate concentration of phage for plague assay, usually the highest dilution with which plaques appear.

Plaque Assay Protocol

1. Melt soft LB-agar. Wait for it to cool so you can touch it without burning your hands.
2. Prewarm the LB plates at 37°C.
3. Put 100µL bacterial culture, 1µL of phage dilution in LB and 3mL soft agar in a tube and vortex.
4. Spread the mixture quickly on the pre-warmed LB-plate and wait for it to solidify.
5. Incubate plates at 37°C for 4-6 h.