

Phage spot assay

Daniel Padfield

Outline

Phage spot assays allow for the abundance of phage (as plaque forming units, PFUs) to be estimated

Beforehand

- Prepare some overnight stocks of bacteria

Method

- Set a water bath to ~50 °C. Place enough soft agar (~5 mL per plate) into falcon tubes and place to keep warm in the water bath
- Place plates (with hard agar in) in the hood and make sure they are dry before pouring on soft agar. **This is especially important if the plates have been stored in the cold room.**
- Place 50 µl of bacteria into a 12 mL centrifuge tube. Use 1 centrifuge tube per plate.
- Place 5 mL of soft agar into each tube
- Vortex
- Pour soft agar onto hard agar plates and let dry
- In the meantime create a serial dilution of your phage using M9.
- Pipette 10 µl of your phage dilution onto the plate. Can pipette multiple dilutions onto a single plate just make sure there is enough space between spots.
- Let dry
- Place either upside down or not in the incubator
- **Make sure to know which dilutions were placed where so back calculations of phage abundance can be made retrospectively**