Halo Assay

* Everything next to flame.
* Heat up YPD agar to >50oC.
* Pour agar plates. 25ml of YPD + agar.
* Allow to dry.
* Add 4ml of cellular stock containing 5x106 cells. Remember that 0.6OD ~ 2x107. Swirl gently to ensure an even distribution of cells.
* Dry next to flame.
* Make up 5mM stocks of each insecticide (2µl 0.5M stock + 196µl media + 2µl other solvent).
* Make up control (196µl media + 2µl DMSO + 2µl MeOH).
* Using an empty petri dish and sterile tweezers, place sterile filter disks onto the dish and add 10µl of the 5mM insecticide stock or control to the centre of the disk.
* Place saturated filter disks onto the inoculated agar plates, 3 to each plate in a triangle. Add a control disk to each plate in the centre of the triangle.
* Label all plates clearly.
* Invert plates to avoid condensation from disrupting lawn, place inside bag and incubate for 16h at 30oC.
* Scan plates in batches of three and remember which plates correspond to which insecticide when scanning.

Day Plan

Monday – Start overnight culture, pour agar plates.

Tuesday – Make insecticide stock solutions and begin halo assay

Wednesday – Scan plates. Create key to associate plate with insecticide.

Protocol partially based on:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5448412/>