Halo Assay

* Everything next to flame.
* Heat up YPD agar to >50oC.
* Pour agar plates. 25ml of YPD + agar.
* Allow to dry.
* Add 250µl (3ml for all plates) of cellular stock containing 5x106 cells. Remember that 0.6OD ~ 2x107. Spread evenly with glass spreader that has been sterilised (dunk in ethanol then into flame).
* 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 (ethanol/flame), place sterile filter disks onto the dish and add 15µl 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. Have a control plate.
* 9 plates, 27 disks.
* 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

Wednesday – Start overnight culture, pour agar plates.

Thursday – Make insecticide stock solutions and begin halo assay

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

Protocol partially based on:

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