2021-02-10 Yeast Sensitivity

* Test only one concentration (1mM) for each insecticide at the top end of the concentration range. 5mM started to have solubility issues for Thiacloprid.
* Add 1x105 cells per well. Count them in a haemocytometer instead of relying on an inaccurate spectrophotometer (in Peter’s lab). Should remove much of the day variance.
* Use two strains and 11 treatments across 10 days.
* Model will be
  + auc ~ treatment \* strain + (1 | day)
  + both factors
  + if interaction term not significant remove to become auc ~ treatment + strain + (1 | day)
  + day random effect
  + treatment and strain fixed effects
* Correct for lower concentration with lower solvent concentration (0.2%).
* Plate design
  + Each day one plate with 6 rows, 3 for each strain.
  + Within each row each well is a treatment.
  + Therefore, each treatment is repeated 3 times a day (technical replicates) on 10 days (number of biological replicates).
  + Use mean of 3 daily technical replicates to produce biological replicate? Or use middle technical replicate?
  + Each day the 3 row strain block is alternated.
* Treatments now include a +ve control and a -ve control with and without solvent.
* Include blanks around perimeter.

Stock Solutions

For all wells 100µl of stock is added to 100µl of yeast culture to result in 1mM insecticide + 0.2% DMSO/0.2% Methanol + 1x105 cells/well

For insecticides:

* Make 1ml of 2mM insecticide stock solution
  + 992µl media + 4µl 0.5M insecticide stock + 4µl other solvent
* 100µl 2mM stock + 100µl 1x106 cells/ml yeast culture

For -ve control

* Make 1ml of 0.4% DMSO + 0.4% Methanol
  + 992µl media + 4µl DMSO + 4µl MeOH
* 100µl solvent stock + 100µl 1x106 cells/ml yeast culture

For +ve control

* 991µl media + 4µl DMSO + 4µl MeOH + 1µl NAT 200mg/ml
* 100µl 0.2mg/ml NAT + 100µl 1x106 cells/ml yeast culture
* Final conc 0.1mg/ml NAT

For -ve -ve control

* 100µl media + 100µl 1x106 cells/ml yeast culture

1x106 Yeast Stock

* Overnight culture x1, x5, x25, x50, x125 yeast stock for both strains.
* Measure on inaccurate spectrophotometer to identify dilution that is ~0.6OD.
* Use the dilution closest to this value and dilute to theoretical 1x106 cell/ml.
* Count on haemocytometer.
* Use this to calculate actual cell/ml of dilution, then make 10ml of 1x106 cell/ml solution.
* Add 100µl of the respective strains to the appropriate wells last using a multichannel.

Contamination

* After identifying correct overnight dilution check it for contamination.
* After 16h assay randomly select 3 wells and check for contamination (microscope).