Antibiotic Screening Protocol

Plate bacteria in a gradient of antibiotic concentration. Controls: no bacteria and no antibiotic

Question: At what concentration of antibiotics do bacteria become sensitive?

Anticipated results:

* Bacteria do not grow differently across the gradient: they are not sensitive to the antibiotic at these concentrations
* Bacteria grow less as antibiotic concentration increases: they are sensitive to the antibiotic
* Bacteria grow more as antibiotic concentration increases: they somehow derive nutrients or otherwise grow because of the antibiotic (unlikely)

Day 1:

* For two species, make agar plates and streak from glycerol stocks

Day 3:

* Culture species in media

Day 4:

* From liquid culture, transfer 1.5 mL of each species to microcentrifuge tubes and centrifuge at 2000 RCF for approx. 30 sec
* Remove supernatant and dilute the remaining bacteria in PBS to 0.1 OD600; 1.5 mL is required
* Fill a 10 mL centrifuge tube with media and antibiotics to achieve the maximum antibiotic concentration; 6 mL per plate is required
* Fill another microcentrifuge with 500 µL PBS
* In a 96 well helper plate:
  + For each of the two species, a column (8 wells) should contain 200 µL of the species; the last row should be filled with 200 µL PBS for control
* For each half of a 96 well experimental plate use a multichannel pipette to add:
  + 180 µL media to the last 5 rows
  + 180 µL antibiotic media to the first row
  + 180 µL antibiotic media to the second row; pipette mix to dilute, and use the dilution to dilute the next row, etc. Leave the last row plain for control
  + 20 µL of the species from the helper plate
* Apply EasyBreathe to top of plate; poke holes in each well for oxygen diffusion
* Read OD600 with plate reader (30C, 48 hr, read every 5 min)

Day 6:

* Pull data and dispose of plate