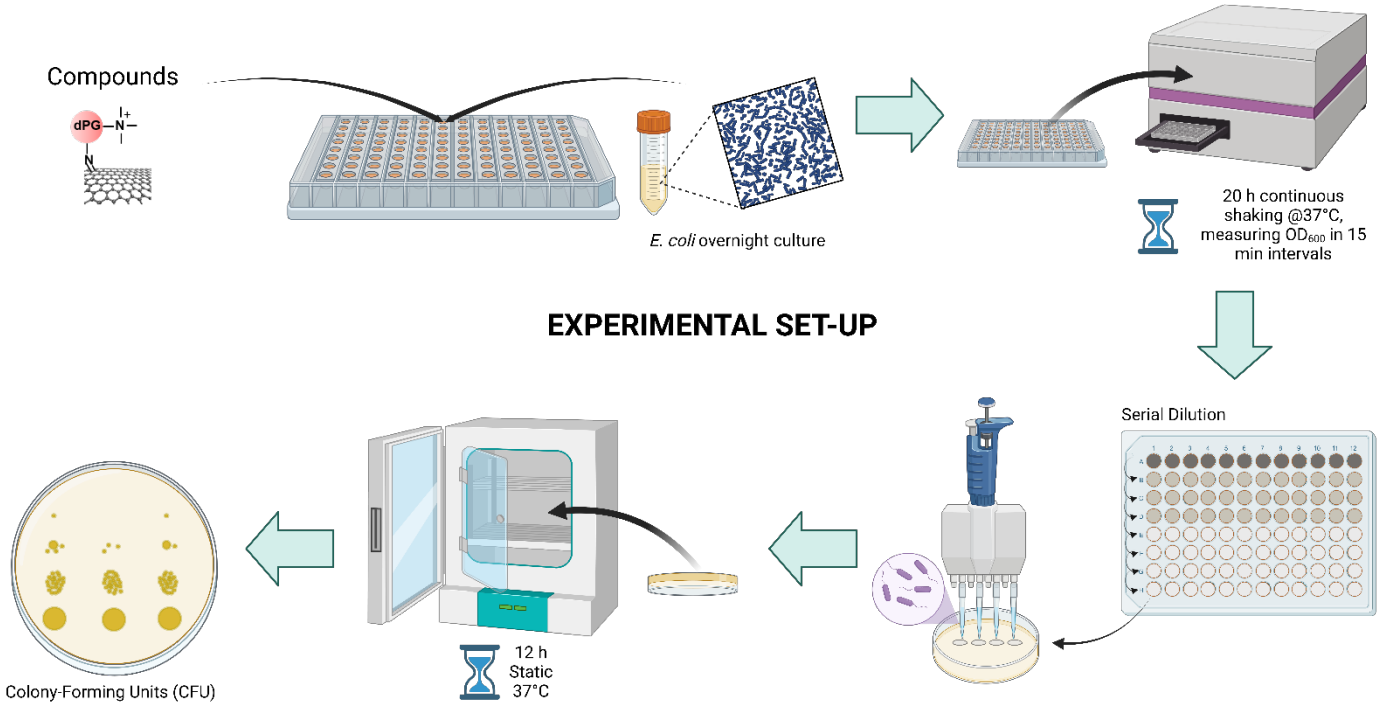
Assay for determining antimicrobial effects of polymer compounds on bacteria via growth curve & CFU

Graphical Schematic of Procedure:



Materials:

- Sterile LB
- DPBS -$CaCl_2$, -$MgCl_2$ (Gibco Cat. No. 14190-094)
- 14 ml bacterial culture tubes with lids
- Falcon tubes 15 ml & 50 ml
- Pipette tips
- multichannel pipette
- EPOCH Plate Reader
- 96-well plate with lid (round bottom)
- Anti-Fog Solution (Recipe Below)
- LB agar plates
- Compounds for testing at desired concentration in LB
- Bacterial strain *E.coli*-AR3110 (or other) (-80°C stock)
- Eppendorf Bio Photometer
- Plastic cuvettes 1 ml
- Reservoir

Work under sterile conditions

Methods:

Day 1 → Streak out (single-colony method) bacterial strain (*E. coli*) on LB Agar plate as shown below (can use tip instead of toothpick) and allow to incubate overnight (ON) at 37°C.

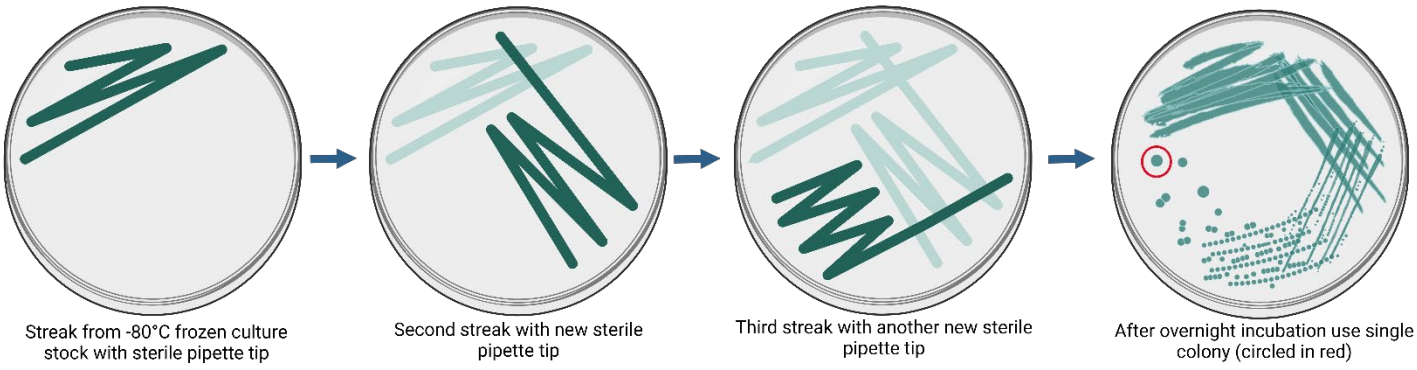
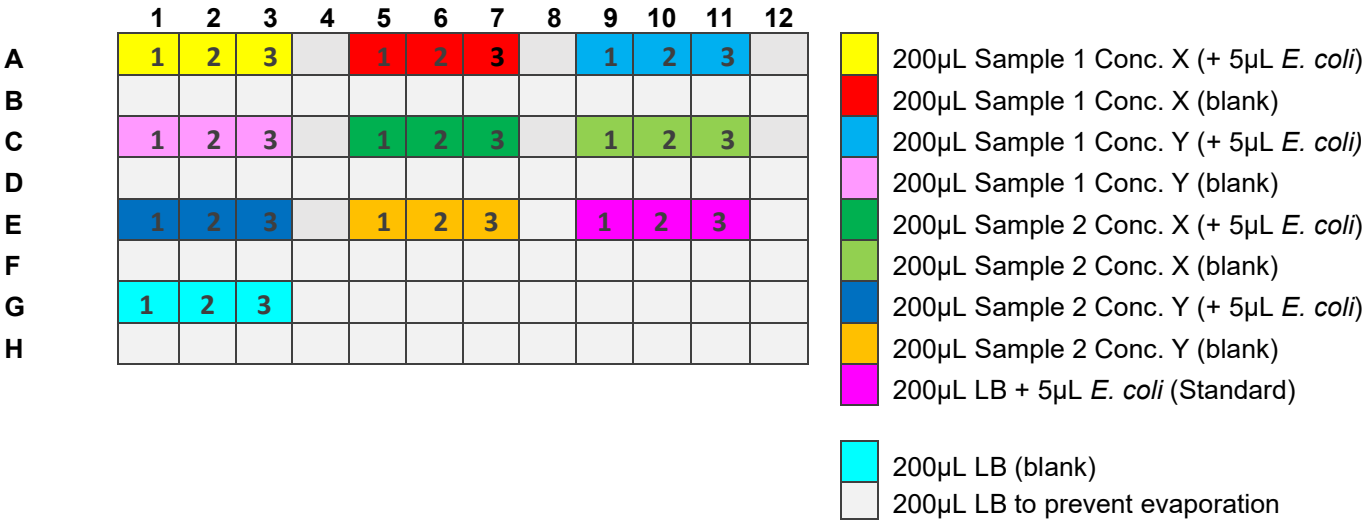


Figure 1: Single colony streak out method

Day 2 → Prepare bacterial culture by inoculating 5mL LB with single colony from plate. Prepare negative control of only LB (3mL). Grow shaking 180-250 rpm at 37°C and allow to grow 6-16 hrs.

Confirm that the negative control LB is still clear and sterile. In the clean bench, apply antifog solution (0.05% TritonX-100, 20% Ethanol in water) to the inside of 96 well lid, fully cover with solution and pour back the excess solution. Allow the lid to fully dry (or alternatively dry with kimwipes™.

Add 200µL of compound solution to the 96-well plate, at least 3 replicates for each compound. Add 5µL of bacterial ON culture to each compound to be tested. Always make bacterial standard of only 200 µL LB medium and 5µL of bacteria. Make sure to create a blank by adding 200µL of compound without E. coli. For easier analysis use the set-up below in the 96-well plate.



Extend the template based on the number of samples you have. (Having a well of space ensures that no cross contamination can occur between samples and blanks (though this should not happen, if you have the space this layout is recommended)

Cover plate and place in EPOCH 2 plate reader with continuous shaking (double orbital) at 37°C measuring at OD_{600} in 15 min intervals for 20 hours.

Day 3 → Export data into excel, save and analyze for growth curve analysis portion.

Fill a 96-well plate with 180µL PBS (-,-) buffer using multichannel pipette via a reservoir. Add 20µL of incubated compound-bacterial solution that grew from previous day, to row A, each well has a different replicate/compound, and mix thoroughly. Change tips between compounds.

Serially dilute 20µL of mixture from row A to H, changing tips between rows and mixing thoroughly by pipetting up and down at least 10 times per well. May use multichannel pipette. Can throw away last 20µL from row H, to ensure same volume in each of the wells. (See diagram below).

Meanwhile dry LB-agar plates thoroughly on clean bench.

Mark LB-agar plates with a line using marker to be able to orient the lid.

Plate 20µL drops onto LB-agar starting from H to E, can use the same tip for each compound when increasing the dilution. Place lid on top of plate and trace where the droplets were placed onto the lid and label each drop. Carefully allow the drops to fully dry in clean bench, the colonies will become invisible once fully dry. See potential layout of 20µL drops below (Figure 3). Once dry cover and place upside down to grow ON in the incubator at 37°C.

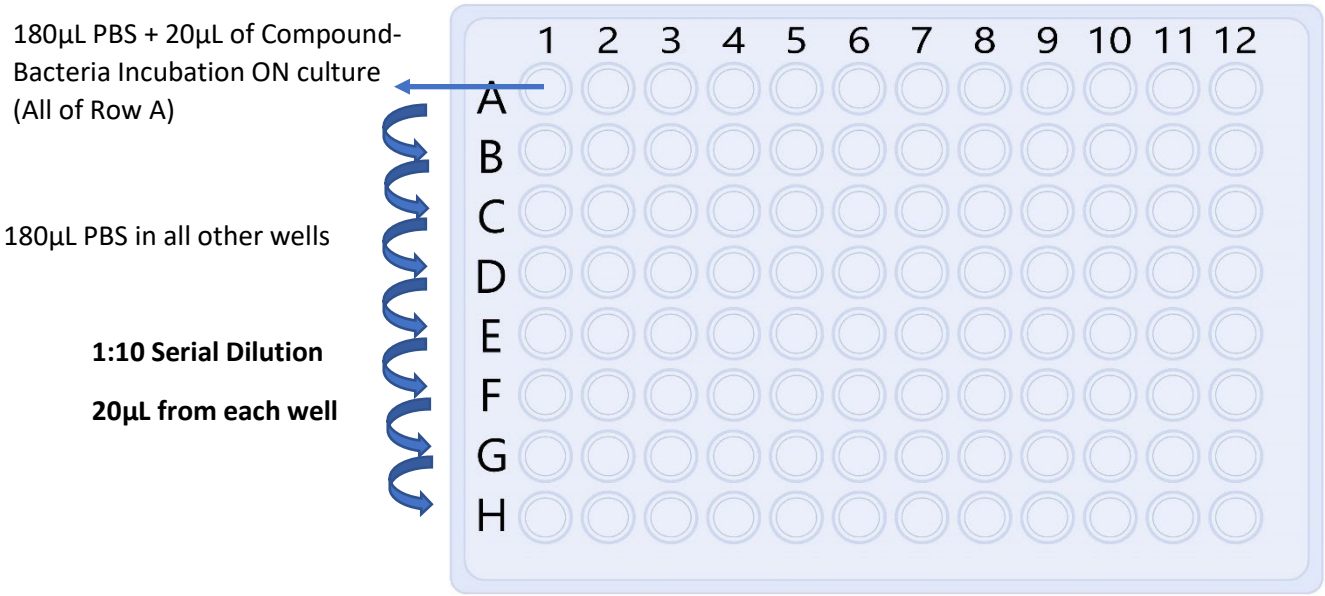


Figure 2: 96-Well Plate Set-Up of Serial Dilution

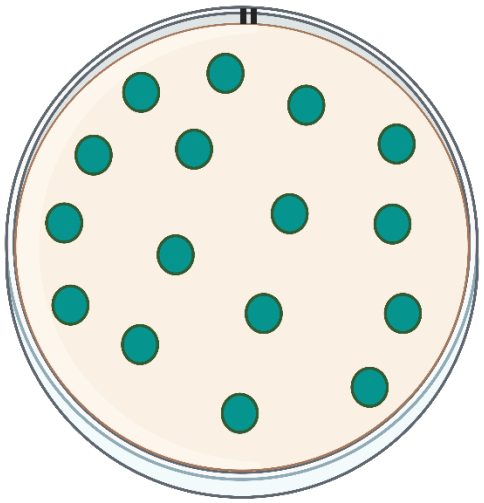


Figure 3: Drop Colony Method for CFU. Each colony is 20µL. A standard LB plate can have between 16-25 20µL colonies.

Day 4 → Using colony counter and marker, count the colonies in each drop and write the numbers down and the corresponding dilution. Record the numbers in lab notebook. Analyze values in excel applying the following formula for the relationship between number of colonies, volume plated (20µL) and dilution factor:

$$\frac{CFU}{mL} = \frac{(number\ of\ colonies\ x\ dilution)}{volume\ plated}$$

Then graph the resulting CFU/mL as bar graphs using standard deviation values as error bars values.

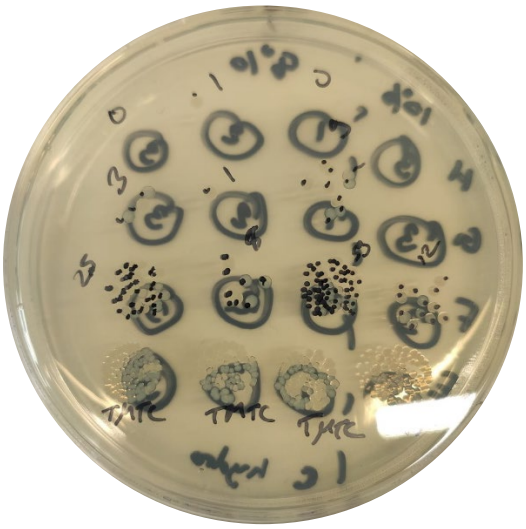


Figure 4: LB Agar plate after colonies have formed and counted with marker