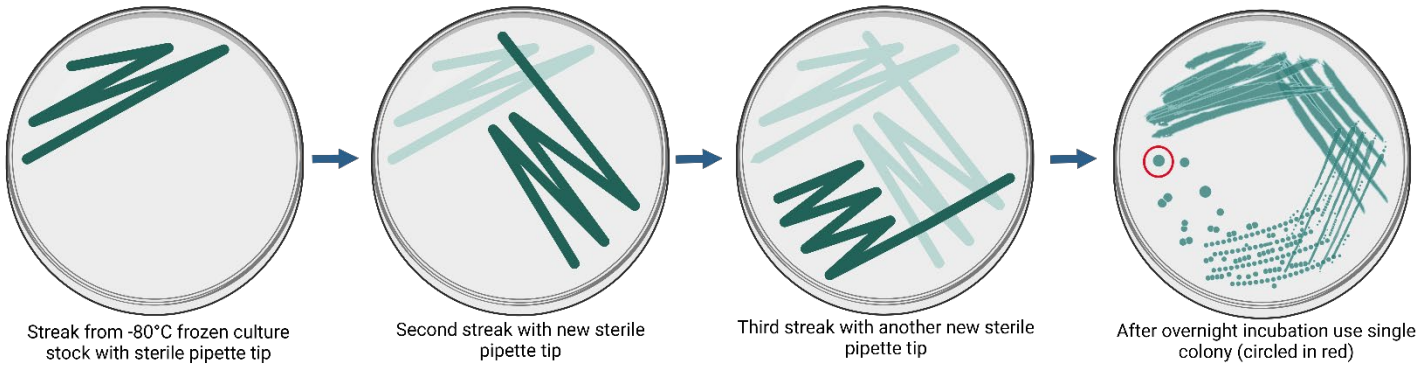
Antimicrobial Column Flow-through Protocol for Flow Clear

Materials:

- Sterile Double LB
- Sterile MilliQ/Evian water
- Sterile LB
- EPOCH Plate Reader
- 96-well plate with lid (round bottom)
- Anti-Fog Solution (Recipe Below)
- LB agar plates
- Culture tubes
- 15mL falcon tubes

Methods:

Day1 → 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.



Day2 → 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 overnight.

Day3 → Confirm that the negative control LB is still clear and sterile.

Wash the bacteria by centrifuging the overnight culture in 15 mL falcon tube at 8,000 xg for 8 min to pellet the bacteria. Discard the supernatant and resuspend in sterile MilliQ/Evian water.

Measure OD_{600} of washed overnight culture in 1:10 dilution (900µL water + 100µL ON culture), blank with 1mL of water. Remember to multiply measured value by 10 to get actual OD value.

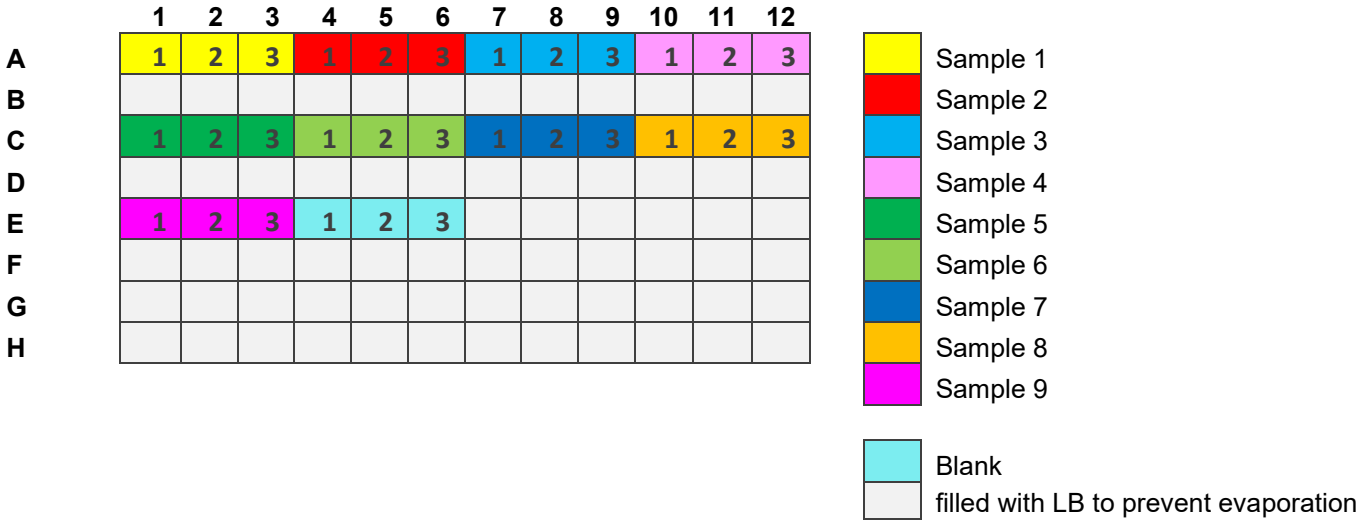
Note: 1.0 OD_{600} = 2.66 x 10^9 cells/mL of E. coli

Calculate your bacterial concentration using the above relationship. Dilute to a concentration of 1.0 x 10^9 cells/mL with a final volume of 5-10mL using $C_1V_1=C_2V_2$ formula.

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™.

Apply culture to the column and collect flow-through for testing.

In a 96-well plate, apply 100µL of double LB in each well that is to be used for analysis. To the double LB filled wells add 100µL of sample from column flow-through, 3 replicates for each flow-through sample being tested. Don't forget the blank control in triplicate (100µL double LB and 100µL water). For easier analysis use the set-up below in a 96-well plate.



Extend the template based on the number of samples you have.

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.

Day4 → Export data into excel, save and analyze.