Growth Kinetics

Procedure:

Day 1

1. Prepare 3 ONCs for each bacterium to be tested (3 Biological Replicates).

Day 2

2. Adjust the OD_{600} of all ONCs to 0.05 after subtracting the OD_{600} of just LB.
3. The 3 Biological Replicates (BR) are to be tested with and without 1 mM IPTG.
    a) No IPTG: add 200 µL of diluted culture to a 96-well plate.
    b) With IPTG: add 198 µL of diluted culture and 2 µL of 1 M IPTG.
4. Plate 2 Technical Replicates for each condition.
5. Run the experiment on a plate reader for 24 h following these instructions:
    a) Temperature: Setpoint 37 °C
    b) Read: (A) 600 – makes a reading at time zero
    c) Start Kinetic [Run 24:00:00, Interval 0:20:00]
        i) Shake: Orbital (Continuously)
        ii) Read: (A) 600
    d) End Kinetic

Day 3

6. After the run is finished, plate the remaining culture volume from the 96-well plate to perform colony counts – to confirm if they are consistent with the final OD values.

Day 4

7. Count CFUs.
8. Draw Growth Kinetics from raw data.