# Overlap growth curve (24 hours)

## ESSENTIALS (not including the test-run):

9 | 100ml LB broth flasks: 6 used 3 back up

12 | 10ml LB broth universal bottles: 2 first run and 3-4 for second batch

6 |LB agar plates per sampling hour per replicate per batch

~18 | microcentrifuge tubes per sampling time per batch (650 ml ¼ strength ringer solution)

Filled with 900ul ringer solution/550-650 tubes

4 | 1000ul tip boxes (blue)

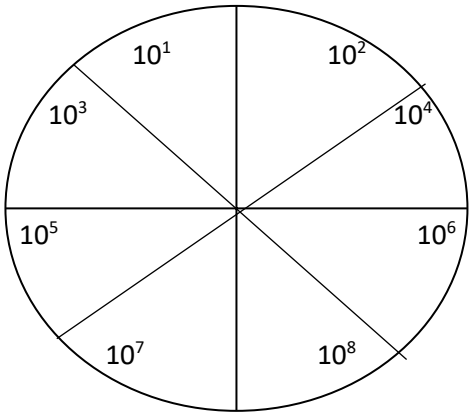
8 | 100ul tip boxes (yellow)

1 box of cuvettes

*Spectrophotometer measurements @550nm

*Use drop plate technique (Miles & Misra) for CFU counts

- Select target bacterial strain and grow it on recommended medium (broth and agar) and growth parameters (oxygen, temp etc.)
- Subculture the original plate onto (1st subculture) LB medium (broth + agar), and store original plate in cold room
- Compare the growth on the recommended medium and the LB medium,
    - Note:
        - Check any observation in turbidity in broth
        - Colony morphology, colour and size
- From the LB agar take a loop and dispense the bacteria into 10ml LB broth and incubate it for 12 hours
- Measure the OD then add 1ml and 0.1ml from the overnight growth into 100ml LB flasks.
- Incubate the flasks for a test run (200 rpm, 25C) and take samples every 2 hours, starting from second hour until the 8th hour. Also, one sample at the 24th hour.
- Take the OD and CFU for each sample point, write any observation and note the observable dilutions for the bacteria (see figure)



- Plan the growth curve for the target bacterial strain based on the growth observations
  {Note take pictures of the colonies and the growth on the culture in the flasks}

**GROWTH CURVE PROTOCOL**

- From the 1st subculture LB plate make a streak and an LB agar (don't do more than 2 subcultures – to reduce the probability of creating mutants which can be different from the original).
- After 24 hours check the LB agar for pure colonies (sometimes it takes longer)
- Take two 10ml LB universal bottles, use a 100ul loop and scrape off 1 whole colony and place it in LB broth (10 ml), and half a colony and place it in the other LB broth.
- Incubate the universal bottles aerobically at 25C until mid or late exponential phase, this is based on the test run results (if slow grower it might take longer time to reach that phase, whereas fast growers will reach the exponential phase faster)
- After incubation is done measure the OD of both universal bottles, choose the one that is nearest to the log phase and plate the overnight culture into LB agar. [NOTE THE CULTURE USED WITH OD AND CFU/ML, THIS IS ESSENTIAL INFO FOR THE SECOND BATCH RUN]
- After measuring the OD take 100ul of one of the overnight cultures and place them in 100ml LB broth to start the first batch of the bacterial strain.
  {At this point prepare the second batch by repeating the previous steps}
- Start the run starting from the 0 hour up to 12-14 hours.
- Place the flasks in the shaker at 25C, 200 rpm, and measure the optical density by taking 1ml into a clean cuvette and measure it into a BLANKED spectrophotometer at 550nm.
  (Report the blank OD just to keep track of any changes)
- For the CFU/ml, prepare a ten-fold serial dilution using buffered solution. Take 10ul of the selected dilution and make three drops onto LB agar plates.
- Carefully transfer the plates into a 25C incubator overnight (or when the colonies are CLEARLY visible).
- After the 1st batch is done commence the second batch