**Bacterial Activation**

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**DAY-1:**
1. Make LB-agar 10 cm plates.
2. Warm-up up LB-agar and take 15 ml and add respective antibiotics (if needed) and mix it well and put in the 10 cm plate.
3. Let this cool till it is solidified.
4. Using a sterile loop touch the bacteria from the glycerol stock.
5. Gently spread the bacteria over a section of the plate, as shown in the diagram above, to create horizontal streaks all over the plate.

![Diagram showing bacterial streaking](image)

6. Incubate plate upside down overnight at 37 °C.

**DAY-2:**
7. In the morning of day 2, single colonies should be visible. A single colony should look like a white dot growing on the solid medium.
8. Place the plates with parafilm wrapped around them and place them at 4°C to stop their further growth.
9. Take 3-ml LB media (or another amount as required) in a snap cap tube and add the respective antibiotic (if needed).
10. With a sterile loop take one single colony of bacteria from the streaked plate and place it into the snap cap tubes containing LB media and antibiotics.
11. Incubate it overnight at 37 °C (without shaking).
12. **Pre-equilibration of DMEM.** Take plain DMEM (5 ml – for infection of cells cultured 96 well plate, 24 well plate, 12 well plate, 6 well plate; 10 ml - 10 cms plate; 20 ml- 15 cms plate) and place in a 50 ml sterile tube with a blue cap. **Important:** do not screw the cap tightly, but keep the cap loose to allow medium equilibration with CO2.
13. Keep these tubes in the CO2 incubator at 37 °C overnight.

**DAY-3:**
14. Dilute the overnight bacterial culture (step 12) at 1:50 with the preequilibrated DMEM.
15. Incubate for 3-3.5 hrs. in the CO2 incubator at 37 °C for bacterial activation.