Making OP50 solution from Frozen Stock

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Behavioural Genomics

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

1 Day: 1

- Get LB Agar from the media kitchen and autoclave it for 2hrs
- Post autoclave, pour the agar on large petri dishes (60mm) and leave to dry overnight to be transferred to the cold room the next day

2 Day: 2

- Get the frozen tube out of the freezer (Freezer 12, SD Box)
- Take a sterile pipette tip and get some frozen sample
- Streak the pipette on the LB agar plate
- Take another new pipette and streak across the old streak and then at a separate spot streak again
- Repeat this step for one more time
- Keep the plate at 37°C incubator overnight for the bacteria to grow
- Transfer the plate to the fridge the next day (This plate can be used for about 1 month to inoculate bacterial culture)

3 Day:3

- Purchase LB Broth from the Media kitchen (Don't use less than 200ml)
- Get flat bottomed conical flasks from the glassware kitchen (For 200ml LB Broth take 400/500ml flask to allow enough headspace for the bacteria)
- From the culture plate, select and circle a single colony
- Using aseptic technique, carefully scoop the single colony and mix it with the LB Broth that is already poured into the conical flask
- Label the flask and put it on the 37°C shaker for overnight incubation

4 Day:4

- Following overnight incubation, take the flask out of the incubator and measure the OD using a spectrophotometer
- Record the average of the OD600, use LB Broth as Blank
- Aliquot the solution into labelled 15ml falcon tubes and keep them in the 4°C Fridge