iGEM TU/e 2017
Biomedical Engineering

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Streaking Glycerol Stock

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1 Streaking Glycerol Stock

Estimated bench time: 60 minutes
Estimated total time: 40 hours
Purpose: Reviving your glycerol stock in order to make your stored bacteria ready to be used again; you streak to make sure that your new culture contains bacteria with the same DNA.

It is essential to work sterile, thus disinfect your hands and work near a Bunsen Burner.

It is not necessary to streak; you can create a small culture from your glycerol stock directly, but it is scientifically more correct to do it this way.

1.1 Materials

- Antibiotic stock
- Bunsen Burner
- LB-agar plates with bacteria
- LB Medium
- Pipetboy and pipettes
- Pipettes and tips
- Shake incubator and regular incubator
- Sterile culture tubes

1.2 Setup & Protocol

- Take a LB-agar plate with the right antibiotic(s) and label it.
- From now on: work near a Bunsen burner flame.
- Take your glycerol stock from the -80 °C freezer and transfer it to ice.
- Use a (sterile) pipet tip, tooth pick or sterile loop and jab the point in the glycerol stock.
- Streak gently with the point across the agar plate according to path 1 as seen below.

- Use a new sterile tip, tooth pick or loop to create streak 2; make sure you cross streak 1.
- Make streak 3 using another new sterile tip, tooth pick or loop.
- Grow the bacteria overnight at 37 °C.

- The next day, take your plate out the incubator and look if you can find a single colony for you to pick (not touching other colonies).
- Work near a Bunsen burner flame again.
- Fill a sterile culture tube with LB-agar medium and the right antibiotic(s).
- Use a sterile pipet tip tooth pick or sterile loop, pick your chosen colony with it and deposit it in your medium.
- Grow the bacteria overnight at 37 °C and 250 rpm.