Reviving Glycerol Stock
## Table of contents

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<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reviving Glycerol Stock</td>
<td>3</td>
</tr>
<tr>
<td>1.1</td>
<td>Materials</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>Setup &amp; Protocol</td>
<td>3</td>
</tr>
</tbody>
</table>
1 Reviving 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.

![streaking diagram]

- 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.