[Review Jacob and Monod’s work.] This is a preliminary experiment to learn how to assay levels of \( \beta \) galactosidase in \( E. coli \) B. From our data, we should be able to answer the question “What are the optimum conditions to assay \( \beta \) galactosidase levels in repressed and derepressed \( E. coli \)?”

### EQUIPMENT FOR CLASS:
- Clinical centrifuge, balance
- Two vortices set up on side benches

### EQUIPMENT FOR TEAMS OF TWO:
- 200, 1000 & 5000 ul micropipets & tips
- Spectrophotometer, warmed up
- Two cuvettes, “B” and “S” in rack
- Two 16x150 mm test tubes, with rack
- Eleven 13x100 mm test tubes, with rack
- 37°C hot block, warmed up

### SUPPLIES PER TEAM OF TWO:
- 5 mL glucose-grown cells, \( A_{660} \) at least 0.5
  (Csh \( A^1 + 0.1\% \) glu)
- (In shaken 125 mL flasks, grow 60 mL)
- 5 mL lactose-grown cells (Csh \( A^1 + 1.0\% \) lac)
  (In shaken 125 mL flasks, grow 60 mL)
- 2% \( K_2CO_3 \) (at least 15 mL/team) in 2 mL repipet
- 20 mM ortho nitrophenyl \( \beta \)-D-galactoside
  (1 mL per team, in 13x100 mm)
- 0.1 M PO\(_4\) buffer, pH 6.5
- Toluene
- Parafilm and scissors
- Sterile dH\(_2\)O in repipetter

1. **Prepare a table in your lab book** with the additions you will make to each reaction tube:

<table>
<thead>
<tr>
<th>Tube</th>
<th>dH(_2)O buffer</th>
<th>growth condition</th>
<th>A(_{660}) of cells</th>
<th>20 mM ONPG</th>
<th>Start</th>
<th>Finish</th>
<th>( \Delta A_{415})</th>
<th>Sp.Act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0.4</td>
<td>glucose</td>
<td>3.00</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>3.3</td>
<td>lactose</td>
<td>0.10</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>2.9</td>
<td>lactose</td>
<td>0.50</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. **Label eleven 13x100 tubes:**
   - a) 2 culture dilution tubes for the carbon source, glucose or lactose: Glu and Lac
   - b) 3 enzyme Rxn tubes: R1, R2 & R3
   - c) 6 \( K_2CO_3 \) tubes: 1S, 1F, 2S, 2F, 3S, 3F (A Start and Finish for each reaction tube)

3. Spin down 5 mL cells in 16x150 mm tubes in the clinical centrifuge, speed 4 for 10 minutes. Decant and discard supernatant. Resuspend in 10 mL sterile dH\(_2\)O. Read \( A_{660} \): Perform dilution to make 4 mL of cell suspension (Glu and Lac) with an \( A_{660} = 0.2 \). Read and record \( A_{660} \).

4. **Toluenize the cells:** Add 3 drops of toluene to the diluted cells, cover with parafilm, hold thumb securely over parafilm, shake well and place on ice.

5. **Add 2 mL 2% \( K_2CO_3 \) to each of the 6 \( K_2CO_3 \) tubes.** [Use a repeater pipet.]

6. Set up enzyme reaction tubes: Add three ingredients to the enzyme Rxn tubes as in the table in this order: 1) dH\(_2\)O (use 5 mL pipet), 2) buffer, 3) toluenized cells. Do not add ONPG yet.

7. **Prewarm** the three prepared enzyme Rxn tubes in 37°C hot block for two minutes.

8. **Start the reaction** by adding 0.2 mL ONPG to each tube, vortex, return to 37°C hot block.

9. **Withdraw the starting blanks** (a single 1 mL micropipet may be used for this stage if done in sequence): take 1.00 mL from Rxn tube 1, add to tube 1S (containing 2 mL \( K_2CO_3 \)), start stopwatch. Return tube Rxn1 to 37°C hot block. At 30 second subsequent intervals, take 1 mL from Rxn tube 2 add to tube 2S, then 1 mL from 3 to 3S, each with \( K_2CO_3 \). Flick tubes to mix.

10. **Incubate Rxn tubes 1, 2 and 3 for 15 minutes.**

11. **Withdraw the finish samples:** using a fresh tip and in sequence, take 1.00 mL from Rxn tube 1, add to tube 1F. At 30 second subsequent intervals, withdraw 1 mL aliquots from Rxn tube 2, add to 2F, repeat with Rxn tube 3 to tube 3F. Flick tubes to mix.

12. **Read and record the \( A_{415} \) of the six tubes** (1S, 1F, 2S, 2F, 3S, 3F). (REMEMBER: \( A_{415} \)!)

13. Determine the change in \( A_{415} \) (\( \Delta A_{415} \)) for each of the three enzyme reaction tubes.

14. **Calculate specific activities** for each sample:

\[
(1/mL \text{ aliquot of cells tested}) \times (\Delta A_{415} \text{ from assay})/(A_{660} \text{ of the diluted culture})
\]

### 'REAGENTS FOR LACTASE ENZYME ASSAY:
- **0.1 M PO\(_4\) buffer, pH 6.5:**
  - 0.5 g KH\(_2PO_4\) + 0.9 g Na\(_2HPO_4\), dissolve in 100 mL dH\(_2\)O
- **20 mM o-nitrophenyl-\( \beta \)-D galactoside (ONPG):**
  - 181 mg ONPG, dissolve 30 mL dH\(_2\)O, warm slightly & swirl to dissolve.
- **2% \( K_2CO_3 \):**
  - dissolve 5 g \( K_2CO_3 \) in 250 mL dH\(_2\)O, stir to dissolve.
  (Or dilute 4% 1:1.)
- **10x Cold Spring Harbor A Medium**
  - \( (NH_4)_2SO_4 \) 10 g
  - \( K_2HPO_4 \) 105 g
  - \( KH_2PO_4 \) 45 g
  - MgSO\(_4\)\(_2\) \( H_2O \) 1g
dissolve in dH\(_2\)O, q.s. to 1 L, autoclave
- **dilute 1:10 into sterile dH\(_2\)O**
- add sterile carbon source after dilution