β-Galactosidase Assay for *E. coli* (Miller, 1972)

**Example of culture preparation**

- Inoculate 10 ml LB medium 1:100 or to an OD<sub>600</sub> 0.1 with a fresh overnight culture carrying a promoter-<i>lacZ</i>-fusion and incubate on a shaker at 37°C
- At OD<sub>600</sub> 0.4-0.8 split the culture to 3 ml samples, induce one sample with e. g. an antibiotic, leave one sample as an uninduced control (or whatever conditions you need)
- After induction (for example, 30 min), *measure OD<sub>600</sub>* and harvest 2 ml of cell culture by centrifugation
- Store cell pellets at -20°C or continue directly with the assay

**β-Galactosidase Assay**

- Resuspend the cell pellet in 2 ml Z-buffer
- In a 2 ml eppendorf cup, prepare three different dilutions with Z-buffer (for example, 0, 1:5 and 1:10, final volume 1 ml), use 1 ml Z-buffer as reference
- Add 25 µl SDS and 50 µl chloroform (from here on, work under fume hood) and mix by vortexing
- Incubate 5 min at room temperature
- Add 200 µl ONPG, mix well and record time (=<i>t</i><sub>0</sub>)
- Incubate at room temperature until the sample turns yellow
- Stop the reaction by adding 500 µl Na<sub>2</sub>CO<sub>3</sub>, mix well and record time (=<i>t</i><sub>s</sub>)
- If the samples do not turn yellow, stop the reaction after 60 min
- Centrifuge (7 min, 13000 rpm, RT)
- Measure OD<sub>420</sub> of the supernatant, use a cuvette with everything but the cells as blank
- Calculate promoter activity according to the formula:

\[
\text{Miller Units} = \frac{OD_{420} \times V \times 1000}{OD_{600} \times (T_s - T_0)}
\]

- <i>t</i> time of reaction in min (T<sub>s</sub> - T<sub>0</sub>)
- V dilution factor
**Solutions**

- \( \text{Na}_2\text{CO}_3 \) 1 M
- \( \text{ONPG} \) (2-nitrophenyl-\( \beta \)-D-galactopyranoside) 4 mg/ml in Z-buffer
- \( \text{SDS} \) 0.1% (w/v)
- Chloroform

**Z-buffer (pH 7.0)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Na}_2\text{HPO}_4 \cdot 2 \text{H}_2\text{O} )</td>
<td>60 mM</td>
<td>10.68 g</td>
</tr>
<tr>
<td>( \text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O} )</td>
<td>40 mM</td>
<td>5.52 g</td>
</tr>
<tr>
<td>KCl</td>
<td>10 mM</td>
<td>0.75 g</td>
</tr>
<tr>
<td>MgSO(_4)</td>
<td>1 mM</td>
<td>0.24 g</td>
</tr>
<tr>
<td>H(_2)O</td>
<td></td>
<td>ad 1000 ml</td>
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</tbody>
</table>

In the original protocol, the Z-buffer contains 100 \( \mu \)g/ml Chloramphenicol. I did the assay without Chloramphenicol and it worked.

Protocol generously provided by the lab
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