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

Example of culture preparation

- Inoculate 10 ml LB medium 1:100 or to an OD_{600} 0.1 with a fresh overnight culture carrying a promoter-*lacZ*-fusion and incubate on a shaker at 37°C
- At OD_{600} 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_{600} 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 (=t_0)
- Incubate at room temperature until the sample turns yellow
- Stop the reaction by adding 500 µl Na_2CO_3, mix well and record time (=t_s)
- If the samples do not turn yellow, stop the reaction after 60 min
- Centrifuge (7 min, 13000 rpm, RT)
- Measure OD_{420} of the supernatant, use a cuvette with everything but the cells as blank

- Calculate promoter activity according to the formula:

$$Miller\,Units = \frac{OD_{420} * V * 1000}{t * OD_{600}}$$

t time of reaction in min (T_s - T_0)
V dilution factor

Solutions

Na_2CO_3	1 M
ONPG (2-nitrophenyl-β-D-galactopyranoside)	4 mg/ml in Z-buffer
SDS	0.1% (w/v)
Chloroform	

Z-buffer (pH 7.0)	$Na_2HPO_4 * 2\ H_2O$	60 mM	10.68 g
	$NaH_2PO_4 * H_2O$	40 mM	5.52 g
	KCl	10 mM	0.75 g
	$MgSO_4$	1 mM	0.24 g
	H_2O		ad 1000 ml

In the original protocol, the Z-buffer contains 100 µg/ml Chloramphenicol. I did the assay without Chloramphenicol and it worked.

Protocol generously provided by the lab
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