β-Galactosidase Assay for *B. subtilis* (Miller, 1972)

Example of culture preparation

- Inoculate LB medium 1:100 with a fresh overnight culture carrying a promoter-*lacZ*-fusion and incubate on a shaker at 37°C
- At OD_{600} 0.4-0.5 split the culture to 2 ml samples, induce one sample with e. g. an antibiotic, leave one sample as an uninduced control
- After 30 min, harvest cells by centrifugation and store the pellet at -20°C or continue directly with the assay

β-Galactosidase Assay

- Resuspend the cell pellet in 1 ml working buffer
- In a cuvette dilute the samples with working buffer until OD_{600} is between 0.2 and 0.8 in a final volume of 800 µl (usually 500 µl working buffer and 300 µl of cells)
- Measure OD_{600}, use 800 µl working buffer as blank
- Add 10 Lysozyme, vortex and incubate at 37°C for 15-45 min, check if the sample is clear
- Add 150 µl ONPG, mix well and record time (=t_0)
- Incubate at room temperature until the sample turns yellow
- Stop the reaction by adding 400 µl Na_2CO_3, mix well and record time (=t_s)
- If the samples do not turn yellow, stop the reaction after 60 min
- Measure OD_{420} and OD_{550} of each sample, use a cuvette with everything but the cells as blank
- Calculate promoter activity according to the formula:

$$Miller\ Units = \frac{1000 * \left(A420 - \left(1{,}75 * OD550\right)\right)}{\left(t * v * OD600\right)}$$

A420	absorption at 420 nm	t	time of reaction (T_s - T_0)
A550	absorption at 550 nm	v	volume of sample (usually 0.8 ml)
A600	absorption at 600 nm		

Solutions

Lysozyme	15 mg/ml in Z-buffer
Na_2CO_3	1 M
ONPG (2-nitrophenyl-β-D-galactopyranoside)	4 mg/ml in Z-buffer

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

Working buffer (prepare fresh)	Z-buffer 20 mM β-Mercaptoethanol (139,5 µl to 100 ml Z-buffer)

Protocol generously provided by the lab
Prof. Thorsten Mascher
Großhadernerstr. 2-4
82152 Planegg-Martinsried
www.syntheticmicrobe.bio.lmu.de