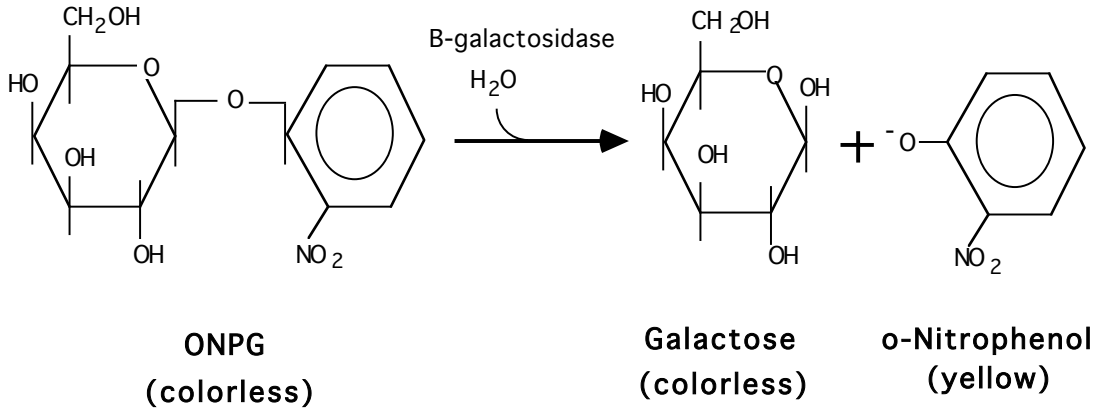
ß-galactosidase assay

The purpose of this experiment is to measure the relative transcription of a Xgal$^+$ MudJ insertion mutant and regulatory mutants. Since the MudJ operon fusions express the *lacZ* gene product (ß-galactosidase;) from the promoter of the mutated gene, transcription of the mutant gene can be quantitated by determining the ß-galactosidase activity expressed in the MudJ insertion mutants (Beckwith, 1981). By determining the ß-galactosidase activity expressed in regulatory mutants or under different growth conditions, the transcriptional regulation of the mutant gene can be studied. ß-galactosidase assay. ß-galactosidase can be assayed by measuring hydrolysis of the chromogenic substrate, o-nitrophenyl-ß-D-galactoside (ONPG) as shown below (Miller, 1972).



The amount of o-nitrophenol formed can be measured by determining the absorbance at 420 nm. If excess ONPG is added, the amount of o-nitrophenol produced is proportional to the amount of ß-galactosidase and the time of the reaction. The reaction is stopped by adding Na_2CO_3 which shifts the reaction mixture to pH 11. At this pH most of the o-nitrophenol is converted to the yellow colored anionic form and ß-galactosidase is inactivated. The reaction can be run using whole cells that have been permeabilized to allow ONPG to enter the cytoplasm. However, since whole cells are present, the absorbance at 420 nm is the sum of the absorbance due to o-nitrophenol and light scattering due to the cells. The contribution of light scattering can be determined by measuring the absorbance at 550 nm where o-nitrophenol doesn't absorb. The light scattering at 420 nm is 1.75x the light scattering at 550 nm, so the absorbance of o-nitrophenol is determined by subtracting 1.75 x OD_{550} nm. The corrected absorbance is then used to calculate the activity of ß-galactosidase.

REFERENCE

Miller, J. 1972. Experiments in Molecular Genetics, p. 352-355. Cold Spring Harbor Laboratory, NY.

ß-galactosidase assay:

1. Subculture each strain in 2 ml of the appropriate medium (plus any required supplements). Grow overnight at 37°C.
2. Add 1.5 ml of the culture to a sterile microfuge tube. Spin for 20 sec in the microfuge. Pour off the supernatant and resuspend the cell pellet in 1 ml 0.85% NaCl. Vortex until completely resuspended.
3. Subculture each cell suspension into 5 ml medium in a Klett flask. (Try to start each culture at about 10 KU.) Grow at 37°C to exponential phase (100-120 KU).
4. Prepare triplicate dilutions of 0.5 ml cells with 0.5 ml complete Z-buffer in test tubes. Also prepare two controls with only 1.0 ml Z-buffer. (Save the cell suspension on ice!)
5. To permeabilize cells add 1 drop of 0.1% SDS and 2 drops of chloroform using a Pasteur pipet. Vortex.
6. Place the tubes in a 30°C water bath and allow to equilibrate for about 2 min.
7. Add 0.2 ml ONPG to each tube and vortex to initiate the reaction. Return to the 30°C shaker. Note the time.
8. When a yellow color develops, stop the reaction by adding 0.5 ml of 1 M Na_2CO_3. Note the time that each reaction is stopped.
9. Return to the shaker for about 5 min.
10. Determine the absorbance within 1 hr. Measure OD_{420} and OD_{550} for each tube.
11. Measure OD_{650} of the cell suspension. If the OD_{650} is greater than 1.2, dilute the cells (0.5 ml cells + 0.5 ml 0.85% NaCl) and reread the absorbance. If the cells are diluted, remember to correct the OD by the dilution factor before calculating ß-galactosidase activity.
13. Using the equation shown below, calculate the ß-galactosidase activity of each sample. Determine the mean and standard deviation of the triplicates.

$$\text{Activity} = \frac{OD_{420} - (1.75\ OD_{550})}{OD_{650} \times \text{time} \times \text{vol}} \times \frac{1\ \text{nmol}}{0.0045\ \text{ml cm}} \times 1.7\ \text{ml}$$

Where:

time =	time of reaction in min
vol =	ml cells added to the assay tubes
0.0045 OD_{420}/nmol =	e_{420} o-nitrophenol
1.7 ml =	total vol
cuvette =	1 cm path length
Activity =	nmol / min / OD_{650} ml

REAGENTS

Z-buffer stock solution

4.27 g Na_2HPO_4

2.75 g NaH_2PO_4 H_2O

0.375 g KCl

0.125 g $MgSO_4$ $7H_2O$

Adjust to pH 7.0.

Bring to 500 ml with dH_2O. Do not autoclave. Store at 4°C.

For complete Z-buffer -- Prior to daily use mix:

50 ml Z-buffer

0.14 ml ß-mercaptoethanol

ONPG (4 mg/ml) (Sufficient for 100 assays -- make fresh daily)

80 mg o-nitrophenyl-ß-D-galactoside (o-nitrophenyl-ß-D-galactopyranoside)

20 ml dH_2O

1 M Na_2CO_3 (Sufficient for 100 assays -- store in refrigerator)

5.3 g Na_2CO_3

50 ml dH_2O