β-Galactosidase Activity Assay (96-well plate format)

(Adapted from Kevin L. Griffith and Richard E. Wolf, Jr. (2002) Measuring β-galactosidase activity in bacteria: cell growth, permeabilization and enzyme assay in 96-well arrays; Bioch. & Biophys. Res. Comm., 290; 397-402)

Example (below): Shows two mono-input gates (Gate 1 & Gate 2) and one dual-input gate (Gate 3) being assayed on a single 96-well plate. Single column tests one Gate + input combination. First four rows contain no arabinose in media but last four have arabinose.

BBa_I732901 or 2021	Gate1 + 2011	Gate1 + input	Gate2 + 2011	Gate2 + input	Gate3 + 2011	Gate3 + input A	Gate3 + input B	Gate 3 + input A&B		blank

- BBa_I732901 is P_{Lac}::,lacZ BioBrick which is a positive control.
- BBa_K102021 is a TRUE gate which should be always ON (abbr. 2021), also a positive control.
- In order to properly test the response of a gate, its activation by the proper input must be compared to its activation by the null input (TA0In or 2011). This will adjust for differences in the response of individual transformants. Activation resulting from an input is tested by comparing the LacZ activity when the input is activated by arabinose (bottom 4 rows) to when there is no input (no arabinose in the top 4 rows). The ratio of [activation by correct input] / [activation by TA0In] gives the Activation Ratio due to the correct input. Activations are measured for a population of transformants by inoculating with multiple colonies as specified below. Assays in all four cells of one condition (ara- or ara+) are based on a single culture.

DAY 1:
1) Co-transform 0.75 μl gate test plasmids and input test plasmids into XL1-B cells and plate on appropriate media.

DAY 2:
1) Aliquot 1.5 ml LB + appropriate antibiotic (amp_{50}, amp_{50}+kan_{35}) into top row of deep, 96-well polypropylene plates. Only one sample of each transformant is required, thus only one row is used.

2) In order to correctly control for random variations of expression in different transformants, inoculate O/Ns from plated transformants by scraping across multiple colonies (~10 or more). Incubate O/N at 37ºC with 320 rpm shaking.

DAY 3:

1) Aliquot solutions of LB+appropriate antibiotics+IPTG [+ /arabinose(0.2%)] into two rows of the same deep, 96-well plate used for O/N. First row contains no arabinose, second row has arabinose.

2) Using multi-channel pipettor, inoculate each well with 50μl from corresponding well in top row containing O/N culture.

3) Incubate at 37ºC with 320 rpm shaking until A_{600} 0.4-0.5 (approximately 3-4 hours). Plate should look something like this:

O/N culture	O/N culture	O/N culture	O/N culture	O/N culture	O/N culture	O/N culture	O/N culture	O/N culture		O/N culture
Ara-	Ara-	Ara-	Ara-	Ara-	Ara-	Ara-	Ara-	Ara-		Ara-
Ara+	Ara+	Ara+	Ara+	Ara+	Ara+	Ara+	Ara+	Ara+		Ara+

4) Make ONPG 2mg/mL in 100 mM phosphate buffer (40μL/well). Vortex well for 10 seconds, then wrap in aluminum foil and rock for >30 minutes to dissolve.

Measure Cell Concentration A_{600}:
1) Transfer 150μL of cells from each well into a clear, flat bottom plate. (multi-channel pipettor)
2) Read A_{600} using plate reader.

Lyse Cells:
1) Aliquot 1 mL room temperature Z buffer + 20μL (0.1%) SDS + 50μL chloroform into a separate, deep, 96-well plate (polypropylene).
2) Using multi-channel pipettor transfer 2 X 150μL of culture into this plate and pipette up and down 15-20 times to lyse cells
        *Pipette from middle and dispense into bottom of the well where chloroform is
3) Allow to sit 5-10 minutes for chloroform to settle to bottom

LacZ Assay:
1) Using multi-channel pipettor, transfer 200μL (middle to top, leaving chloroform behind) from lysed cells into clear 96-well plate.
2) Using repeat-pipettor, add 40μL ONPG to all wells recording t_0
4) As colour develops add 50μL 1MNa$_2$CO$_3$ (columnwise with 8-channel multi-channel pipettor or rapidly with repeat-pipettor), record t_f for the column.
5) Once all reactions are stopped use plate reader to record A_{550}(cell debris) and A_{420}(yellow product)

Calculations:

Miller Units $= 1000 \times [(A_{420} - (1.75 \times A_{550}))]/(T \times V \times A_{600})$

A_{420} and A_{550} are read from the reaction mixture
A_{600} reflects cell density in the washed cell suspension
T=time of the reaction in minutes before adding $1M Na_2CO_3$
V=volume of culture used in the assay in ml (=0.35, above)
Units give the change in A_{420} normalized to total cells used and time.

Solutions for β-galactosidase assays:

Z buffer, per 50 mL:

- O.80g $Na_2HPO_4.7H_2O$ (0.06M)
- 0.28g $NaH_2PO_4.H_2O$ (0.04M)
- 0.5 mL 1M KCl (0.01M)
- 0.05 mL 1M $MgSO_4$ (0.001M)
- 0.135 mL β -mercaptoethanol (BME) (0.05M)
- bring to approximately 40 mL with H_2O, dissolve all the salts
- adjust the pH to 7.0
- use a graduated cylinder to bring the buffer to 50 mL
- store at 4 C.

Note: BME is added to the reaction buffer to stabilize the β-galactosidase enzyme. The important part of BME is a reactive thiol (SH group). Thiols react with oxygen in the air and oxidize (inactivate) over time. Therefore, try not to make much more Z buffer than you will use in a few days. Store the unused portion at 4 C.

0.1M Phosphate buffer, per 100 mL:

- 1.61g $Na_2HPO_4.7H_2O$ (0.06M)
- 0.55g $NaH_2PO_4.H_2O$ (0.04M)
- adjust the pH to 7.0
- phosphate buffer is stable at room temperature and does not need to be made fresh each time.