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

DAY 1:

1) Create solutions of LB + appropriate antibiotic (amp$_{50}$, amp$_{50}$+kan$_{35}$, tet$_{10}$)
    e.g. LB+amp50 solution
        (4columnsX4rows)+1 = 17mL LB
                                17 μL amp$_{50}$
2) Put 1mL of appropriate solution in each well being used

Example: Shows 4 gates being tested by themselves and then with their 4 inputs, hence four columns of LB+amp$_{50}$ for the gates only, and four columns of LB+amp$_{50}$+kan$_{35}$ for gates+input and then in column 10 usually XL1-B is used for a blank so LB+tet$_{10}$.

LB+amp50	LB+amp50	LB+amp50	LB+amp50	LB+amp50+kan35	LB+amp50+kan35	LB+amp50+kan35	LB+amp50+kan35		LB+tet10		
LB+amp50	LB+amp50	LB+amp50	LB+amp50	LB+amp50+kan35	LB+amp50+kan35	LB+amp50+kan35	LB+amp50+kan35		LB+tet10		
LB+amp50	LB+amp50	LB+amp50	LB+amp50	LB+amp50+kan35	LB+amp50+kan35	LB+amp50+kan35	LB+amp50+kan35		LB+tet10		
LB+amp50	LB+amp50	LB+amp50	LB+amp50	LB+amp50+kan35	LB+amp50+kan35	LB+amp50+kan35	LB+amp50+kan35		LB+tet10		

3) Pick 4 colonies from plates containing appropriate plasmids and place 1 colony under each appropriate column in the four different rows.
4) Leave O/N to grow at 37°C and 320rpm.

DAY 2:

1) Create solutions of LB+appropriate antibiotics+IPTG+glucose/arabinose(2%)
    e.g. LB+amp$_{50}$+IPTG+glucose
        (4columnsX4rows)+1 = 17mL LB
                                17μL amp$_{50}$
                                17μL IPTG
                                1700μL glucose(20%)

2) Put 1mL of solutions into appropriate columns/rows of 96-well plate

LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose		LB+tet10 +IPTG+glucose		
LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose		LB+tet10 +IPTG+glucose		
LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose		LB+tet10 +IPTG+glucose		
LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+ IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose	LB+amp50+kan35 +IPTG+glucose		LB+tet10 +IPTG+glucose		
LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose		LB+tet10+ IPTG+arabinose		
LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose		LB+tet10+ IPTG+arabinose		
LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose		LB+tet10+ IPTG+arabinose		
LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+ IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose	LB+amp50+kan35 +IPTG+arabinose		LB+tet10+ IPTG+arabinose		

3) Inoculate 1mL with 100µL of each O/N (multi-channel pipettor)
    e.g. row 1+5 same O/Ns
        row 2+6 same O/Ns
        row 3+7 same O/Ns
        row 4+8 same O/Ns
4) Incubate at 37°C and 320rpm until A600~0.3
5) Make ONPG 4mg/mL in 0.1M phosphate buffer (20µL/well)

Measuring Cell Concentration A_{600}:

1) Transfer 150µL of cells from each well into clear, flat bottom plate. (multi-channel pipettor)
2) Put LB in column 11 as blank
3) Read A_{600}

Lyse Cells:

1) Aliquot 1mL Z buffer + 20µL (0.1%) SDS + 40µL chloroform into a different, empty 96-well plate
    *chloroform not in tray
2) Using multi-channel pipettor transfer 175µL of culture from other 96-well plate into this plate and pipette up and down 15 times to lyse cells
    *Pipette from middle and dispense into bottom of the well where chloroform is
3)Leave for a while (5-10 minutes so chloroform settles to bottom)

LacZ Assay:

1) Transfer 200µL (middle to top) from lysed cells 96-well plate into clear 96-well plate.
2) As a blank, transfer 200µL of Z buffer into last column of clear plate
3) Add 20µL ONPG to all wells recording t_0
4) As colour develops add 50µL 1MNa_2CO_3, record t_f
5) Once all reactions are stopped use plate reader to record A_{550}(cell debris) and A_{420}(yellow product)

Calculations:

Miller Units = $1000\times [(A_{420}-(1.75\times A_{550}))]/(T\times V\times 4\times A_{600})$

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
V=volume of culture used in the assay in mLs
Units give the change in A_{420}/min.mLL of cells/A_{600}