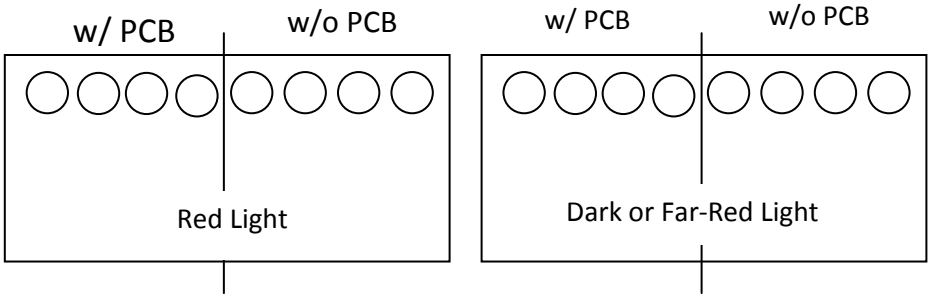
Functional Assay

Developed from the Quail Laboratory – University of California, Berkeley

ONPG assay from Yeast Protocols Handbook from Clontech Laboratories

1. Inoculate 5 mL culture of L6457 yeast culture with BNT and GP3 in SD (leu- trp-). Leave overnight until saturated (dense culture, white, about OD_{600} ≈2)
2. In 48-well plate, grow pre-cultures in 4 wells (4 wells for culture and 4 wells for experiment). In each well have the following set-up [Want PCB conc. to be 2 uL/mL]:
    a. 374 uL SD (leu- trp-) ; 1 uL PCB ; 125 uL starter culture
    b. 375 uL SD (leu- trp-) ; 125 uL starter culture
3. Let this grow for 16-24 hours
4. 1:4 dilution into YPD culture, 2 different wells, as shown below:



5. Grow up for 5 hours
6. While growing for 5 hours put ONPG into Z-Buffer (4 mg/mL), vortex for 1 min, but want to let dissolve for a while
7. Take the OD_{600} of each. Should be between 0.5-0.8. (Remember to blank with YPD w/ PCB for cultures that have PCB in it.
    a. *PCB absorbs at 600 nm*
8. Pool the 4 of each type into a 1.5 mL eppendorf tube. Note the total amount of pooled cells.
9. Centrifuge for 30 seconds at 14,000 rpm (10,000 x g). Discard of supernatant
10. Resuspend with 1.5 mL of Z-Buffer.
11. Centrifuge for 30 seconds at 14,000 rpm (10,000 x g). Discard supernatant.
12. Resuspend each pellet in 300 uL of Z-Buffer. (Note that this is a 5-fold concentration, 1.5 mL to .3 mL).
13. Transfer 0.1 mL of the cell suspension to a fresh eppendorf tube.
14. Place cells in liquid nitrogen until the cells are frozen (0.5-1 min).
15. Place frozen tubes in a 37°C water bath for 0.5 – 1 min to thaw.
16. Repeat the freeze/thaw cycle (Steps 14 and 15) two more times to ensure that the cells have been lysed.
17. Set up a blank tube with 100 uL of Z buffer.
18. Add 0.7 mL of Z Buffer + β-mercaptoethanol to the reaction and blank tubes.
19. Start timer. Immediately add 160 uL of ONPG in Z-Buffer to the reaction and blank tubes.
20. Place tubes in a 30°C incubator

21. After the yellow color develops, add 0.4 mL of 1 M Na_2CO_3 to the reaction and blank tubes. Record elapsed time in minutes.
    a. *The time needed will vary, no longer than 24 hours for a very weak reaction.*
    b. *The yellow color is not stable and will become more intense with time.*
22. Centrifuge tubes for 10 minutes at 14,000 rpm to pellet cell debris
23. Using a spectrophotometer, measure the OD_{420} of the samples.
24. Calculate the β-galactosidase units.
    a. *1 unit of β-galactosidase is defined as the amount which hydrolyzes 1 µmol of ONPG to o-nitrophenol and D-galactose per min per cell (Miller 1972; Miller, 1992):*

$$\beta - galactosidase\ units\ = 1000\ \times {}^{OD_{420}}/_{(t\ \times V\ \times OD_{600})}$$

Where:

$$t = \ elapsed\ time\ (in\ \mathrm{min}) of\ incubation$$
$$V = 0.1\ mL\ X\ Concentration\ Factor\ *$$
$$OD_{600} = A_{600}\ of\ 1\ mL\ of\ culture$$

* The concentration factor from step 12 usually calls for a conc. factor of 5, but in case this is not the case, please take this into account. Also if need to dilute cell culture to remain within the linear range, it should be taken into account here as well.