Protocol plate reader

Introduction

This is the protocol Sophie and Lieke discussed with Sonja and used for the plate reader experiments

Materials

- GFP E.coli strain
- RFP E.coli strain
- mcherry - pTet4 E. coli strain
- mcherry - pTet5 E. coli strain
- LB with ampiciline
- LB with Kan
- anhydrotetracyclin (aTET) (2.5 mg/ml)
- tetracyclin (5 mg/ml)
- plate reader
- testtubes
- pipettes

Procedure

Dilutions:

1. Tetracycline: 5 mg/ml --> 100 ng/ml = 50000x dilution

   First diluted 100x = 10µl in 1 ml autoclaved deionized water
   This got diluted 5x again = 200µl (of the 100x dilution) in 1 ml autoclaved deionized water (end up with a 500x dilution)
   Last step: dilute 100x again, but now in the test tube = 32µl (of the 500x dilution) in 3ml LB (with amp) + 200µl of the overnight (ON) culture

2. aTET: 2.5 mg/ml --> 125 ng/ml = 20000x dilution

   First diluted 100x = 10µl in 1 ml autoclaved deionized water
   This got diluted 2x again = 500µl (of the 100x dilution) in 1 ml autoclaved deionized water (end up with a 200x dilution)
   Last step: dilute 100x again, but now in the test tube = 32µl (of the 200x dilution) in 3ml LB (with amp) + 200µl of the overnight (ON) culture

3. aTET: 2.5 mg/ml --> 250 ng/ml = 10000x dilution

   First diluted 100x = 10µl in 1 ml autoclaved deionized water
   Last step: dilute 100x again, but now in the test tube = 32µl (of the 100x dilution) in 3ml LB (with amp) + 200µl of the overnight (ON) culture

4. aTET: 2.5 mg/ml --> 500 ng/ml = 5000x dilution

   First diluted 50x = 20µl in 1 ml autoclaved deionized water
   Last step: dilute 100x again, but now in the test tube = 32µl (of the 50x dilution) in 3ml LB (with amp) + 200µl of the overnight (ON) culture

Protocol:

5. Create a overnight culture of each strain (minimum 3 ml)

6. Day 2: add to testtube:

   - 3 ml LB (with the right antibiotic)
   - 200µl of ON culture
   - 32µl of the correct inducer (make a extra testtube without inducer).

7. Add 200µl of each testtube to the microplate reader in triplo, and measure the t0 at the correct wavelength.

8. After t0 measurement put the testtubes in the shaker at 37 degrees celcius and ... rpm. After 4 hours measure the t4, after 8 hours measure the t8 and after 12h measure the t12.

Evaluating results:

9. Take the average background noise of all the empty wells and substract this number from the values in the filled wells.

10. Divide the Fluorescence by the OD and calcualte the average of every triplo.

11. Plot the values in a graph and compare the no inducer to the values of the inducer.

    Now you can tell someone what concentration of inducer and what incubation time they should use.