iGEM 2017 Collaboration

User guide

Utrecht University

Wageningen University

iGEM Utrecht

iGEM Wageningen 2017 mantis

modular antigen-based test for infectious diseases

iGEM WUR – UU BioBrick exchange | CpxR BiFC measurement

Materials:

- LB agar plate + chloramphenicol
- Saltless LB liquid medium + chloramphenicol
- Greiner tubes (15 mL)
- Erlenmeyers (250 mL)
- Polystyrene Round-Bottom tubes (14 mL)
- L-arabinose (10% w/v)
- KCl (5 M)
- 96-wells flat bottom black microplate (Corning)
- Synergy MX platereader

Protocol:

1. Transform *E. coli* K12 with pSB1C3-araCpBAD-CpxReYFPn-CpxReYFPc
2. Plate on LB-Chloramphenicol, grow overnight at 37 °C.
3. Inoculate a 3 mL saltless LB starter culture with a single colony, grow in 15 mL Greiner tube overnight at 37 °C, 250 rpm.
4. Inoculate a 25 mL saltless LB culture with 100 uL of starter culture. Grow in 250 mL Erlenmeyer at 37 °C, 180 rpm until OD_{600} = 0.6.
5. Transfer cells to fresh saltless LB until OD_{600} = 0.2 in a total volume of 3 mL. Grow in 14 mL Polystyrenne Round-Bottom Tubes. Induce with 0.2 % L-arabinose (unless stated otherwise). Grow for 3-4 hours.
6. Transfer 100 uL samples to 96-wells flat bottom black microplate (Corning) (in triplo) and measure fluorescence using SynergyMx platereader using the following settings.
    a. Set temperature at 30 °C to improve fluorophore recomplementation.
    Continuous shaking (medium)
    Measure initial fluorescence and cell density, re-read every 10 minutes.
        Measure OD_{600}
        Measure fluorescence (ex: 513/9.0 nm, em: 532/9.0 nm)
    b. After 20 minutes, activate Cpx pathway by adding KCl (use range of 25 mM-100 mM final concentration).
        Measure OD_{600} and fluorescence as before, every 15 minutes, for 6 hours.

iGEM WUR – UU BioBrick exchange | Leucine Zipper BiFC measurement

Materials:

- LB agar plate + chloramphenicol
- LB liquid medium + chloramphenicol
- Greiner tubes (15 mL)
- Centrifuge adapted to Greiner tubes.
- L-arabinose (10% w/v)
- MiliQ Water
- 96-wells flat bottom black microplate (Corning)
- Synergy MX platereader

Protocol:

1. Transform *E. coli* BL21 with:
    a. Full sfGFP (Control)
    b. Full Venus (Control)
    c. Split sfGFP
    d. Split Venus
2. Plate on LB-Chloramphenicol, grow overnight at 37 °C.
3. Inoculate a 5 mL LB-Chloramphenicol starter culture with a single colony, grow in 15 mL Greiner tube overnight at 37 °C, 250 rpm.
4. Inoculate a 5 mL LB-Chloramphenicol-0.2% Arabinose culture with the required volume from the overnight culture to reach an OD_{600}=0.1.
5. Incubate for 3 hours at 37 °C at 250 rpm for 3 hours.
6. Store in the fridge overnight.
7. Centrifuge the cultures at 4700 rpm for 12 minutes.
8. Remove the supernatant.
9. Lyse the cells with a procedure that maintain proteins in their native structure (e.g. B-Per).
10. Collect the lysate.
11. Make two decimal dilutions for each culture. For each sample you will have 1:1, 1:10 and 1:100.
12. Transfer 100 uL samples to 96-wells flat bottom black microplate (Corning) (in triplo).
    a. Use miliQ water as blank control
    b. BL21 without any plasmid may be used as a negative control.
13. Measure fluorescence using SynergyMx platereader using the following settings.
    a. Set temperature to 30°C
    b. Medium shaking
    c. Measure fluorescence:
        i. sfGFP: (ex: 485/9.0 nm, em: 510/9.0 nm)
        ii. Venus: (ex: 510/9.0 nm, em: 530/9.0 nm)

The Eppendorf tubes contain approximately 250ng of DNA, dried according to the iGEM Protocol. It is recommended to spin the tubes at max RPM in a table top centrifuge for 2 min, then resuspend the pellet in 10ul TE or H_2O. Use 2ul for electroporation (~50ng)