Protocol Congo Red

This protocol is used for the Conductive curli module

Materials

- LB media
- 0.8% rhamnose w/w
- 5x Congo Red solution
- Chloramphenicol (34 ug/ml final concentration)

Method

1. Inoculate cells from plate/stock in 10 ml LB with antibiotic stated in (…) and grow O/N cultures of the strain with the right plasmids.
    a. ΔCsgB + Biobrick BBa_K1316013 (CamR)
    b. ΔCsgB + Biobrick BBa_K1316014 (CamR)
    c. ΔCsgB + Biobrick BBa_K1316015 (CamR)
    d. ΔCsgB (none)
2. Start early in the morning by transferring 1 ml of the O/N cultures in 30 ml LB including relevant antibiotic; twice for each of the O/N cultures
3. Once cultures reach OD600 between 0.25 and 0.4 induce 4 cultures (1a-4a) with 0.8% rhamnose w/w end concentration. This yields 8 flasks with the following contents:
    a. ΔCsgB + Biobrick BBa_K1316013 (CamR) + (0.8% rhamnose w/w)
    b. ΔCsgB + Biobrick BBa_K1316014 (CamR) + (0.8% rhamnose w/w)
    c. ΔCsgB + Biobrick BBa_K1316015 (CamR) + (0.8% rhamnose w/w)
    d. ΔCsgB [none] + (0.8% Rhamnose w/w)
    e. ΔCsgB + Biobrick BBa_K1316013 (CamR) + (No inducer)
    f. ΔCsgB + Biobrick BBa_K1316014 (CamR) + (No inducer)
    g. ΔCsgB + Biobrick BBa_K1316015 (CamR) + (No inducer)
    h. ΔCsgB [none] + (No inducer)
4. Wait 3 hours after induction
5. Take 1 ml samples of all 8 flasks and measure OD_{600} with LB + [AB] as blank /reference.
6. Add 5x Congo Red solution to a final concentration of 20 µg/ml, mix and incubate for 5 min at Room Temperature (easiest is to do step E and F in the cuvette).
7. Transfer sample from step F to Eppendorf and centrifuge at 14000 rpm for 5 min.
8. Measure absorption at 480nm (A480) of the supernatant with LB + (…) + 20 µg/ml Congo Red as a reference. (The A480 will be negative since the Congo Red will get stuck in the produced Curli)
9. Repeat from step 4 every 45 min for all 8 samples.

References

Chen et al., Synthesis and patterning of tunable multiscale materials with engineered cells. *Nature Materials* 13, 515–523 (2014)