BANANA GUARD

Protocols

Protocol Congo Red Assay

received by iGEM 2014 TU Delft – Leiden University

DAY 1

1. Inoculate cells from plate/stock in 10ml LB with antibiotic stated in [.] and grow O/N cultures of

- ΔCsgB + GG50 [chloroamphenicol]
- ΔCsgB + GG51 [chloroamphenicol]
- ΔCsgB + GG52 [chloroamphenicol]
- ΔCsgB [none]

DAY 2

2. EARLY IN THE MORNING! (9am); transfer 1 ml of the O/N cultures in 30ml LB including relevant antibiotic; twice for each of the O/N cultures (=8 cultures total, 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b).
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:
   - ΔCsgB + GG50 [Chloramphenicol] + (0.8% rhamnose w/w)
   - ΔCsgB + GG51 [Chloramphenicol] + (0.8% rhamnose w/w)
   - ΔCsgB + GG52 [Chloramphenicol] + (0.8% rhamnose w/w)
   - ΔCsgB 'empty' [No AB] + (0.8% Rhamnose w/w)
   - ΔCsgB + GG50 [Chloramphenicol] + (No inducer)
   - ΔCsgB + GG51 [Chloramphenicol] + (No inducer)
   - ΔCsgB + GG52 [Chloramphenicol] + (No inducer)
   - ΔCsgB 'empty' [No AB] + (No inducer)
4. Wait 3h after induction
5. Take 1 ml samples of all 8 flasks and measure OD600 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 14k 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 E to H every 45min for all 8 samples.
10. After overnight incubation, repeat step E to H for all 8 samples.