Protocols #XXX : Study of M15 [pRep4;pQE30::KR] cell response to light illumination
iGEM Grenoble-EMSE-LSU

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Materials and reagents:

- M9 minimum medium (with iron sulfate and elementary traces)
- M15[pRep4;pQE30::KR] cells
- Sterile LB medium
- Sterile LB-Agar
- Ampicillin (100µg/mL), Kanamycin (50µg/mL)
- IPTG (0.05M)
- 1.5 mL Eppendorf tubes
- 50 mL Falcon tubes
- 90 mm Petri dishes
- Incubator
- LED Lamp (specifications)
- Spectrophotometer (for fluorescence and OD610 measurements)
- 3 Autoclaved 100 mL Erlenmeyers
- Aluminum fold
- Spreader
- Black 96-well plates
- Disposable absorbance cuvettes
- Pipettes, with sterile pipet tips

Protocol

Day 1 (in the evening):

1) Pre culture M15[pRep4;pQE30::KR] cells ON (37°C, 200 rpm) in a 50 mL Falcon tube, filled with 10mL M9 medium, supplemented with 200µg/µL Ampicillin and 50µg/µL Kanamycin.
2) Wrap the 3 Erlenmeyers in aluminum fold
3) Prepare 100-150 LB-Agar plates, supplemented with 200µg/µL Ampicillin and 50µg/µL Kanamycin

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Day 2:

1) Supplement 100 mL M9 minimum medium with iron sulfate, elementary traces, 200µg/µL Ampicillin, 50µg/µL Kanamycin and 0.05mM IPTG.
2) Pre warm the mix at 37°C
3) Measure the OD610 of your M15[pRep4;pQE30::KR] pre culture, and re suspend it at 0.015 in each of the Erlenmeyer, filled with pre warmed and complete M9 medium.
4) Immediately measure OD610 and fluorescence (540/630 nm). Spread 50µL of diluted cell samples on agar plates, using 10^4, 10^5 and 10^6 dilutions (made in sterile LB medium). Incubate the resulting plates ON (37°C)

Note: Let agar plates open under a sterile environment for 30 min before performing the plating, to dry them up.

5) Repeat step 4) every 30min-1h for 3 hours.
6) At t = 180 min, unwrap one of the Erlenmeyer, and start illuminating it in the incubator with the LED lamp.
7) Repeat step 4) every 30-1h for 8 hours.

Day 3:

8) Count the number of colonies on each of the agar plates (discard those displaying more than 300 colonies or less than 30 colonies)
9) Calculate the number of living cells per µL using the following formula

   #cells/µL = (#colonies*dilution)/50