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Dose response assay for inducible promoters in *Synechocystis* sp. PCC 6803

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ABSTRACT

Inducible promoters are an important tool for synthetic biology. They enable temporal control of gene expression, as well as controlled expression of toxic genes.

This protocol describes the methodology for fluorescence-based reporter assays in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 to quantify the dose-dependent response of an inducible promoter to its inducer.

GUIDELINES

For comparability, all cultures that are to be compared to each other should be similar in terms of optical density. To achieve this, it is important to adjust preculture optical densities until they are in the same growth phase.

MATERIALS

NAME	CATALOG #	VENDOR
ethanol		
Nuclease-free water (e.g. MilliQ or HPLC grade water)		
Anhydrotetracycline hydrochloride	37919-100MG-R	Sigma Aldrich
L-rhamnose	W373011-100G-K	Sigma Aldrich
Vanillic acid	H36001-25G	Sigma Aldrich
6-well plate	92406	Techno Plastic Products (tpp)
Vision Plate 96 well	4ti-0221	4ti

MATERIALS TEXT

Other materials required:

- Erlenmeyer flasks, wide neck
- BG11 media
- antibiotic stocks
 - Spectinomycin 20 mg/mL
 - Kanamycin 25 mg/mL
 - Chloramphenicol 10 mg/mL

BEFORE STARTING

Prepare all stock solutions, including one stock of the solvent the inducer is dissolved in (e.g. H₂O or ethanol).

Important: If a large range of inducer concentrations is used, prepare multiple stock dilutions so that a similar amount of inducer is pipetted for each concentration. For example, if you require a final concentration of 1 μ M and 1 nM, prepare 1:1000 stock solutions for both concentrations, i.e. 1 mM and 1 μ M, respectively. Make sure to prepare all dilutions from the same stock solution to minimise technical errors.

Inoculation of preculture

- 1 Inoculate an appropriate amount of BG11 with your cyanobacterial strain of choice. Include appropriate controls, i.e. an empty vector control. The inoculation volume depends on the number of concentrations to be tested. For example: 6 concentrations x 3 replicates x 5 mL = 90 mL of preculture. Since the culture will be diluted ~1:3 before starting, 35 mL culture volume should be enough.



Include appropriate antibiotics, especially when using plasmid-based systems, since the plasmid will be lost in the absence of selection pressure.

Grow culture for 3-5 days, until an OD₇₅₀ of ~1 is reached.

Dilution of preculture



- 2 Dilute precultures to an OD₇₅₀ of 0.2. Include the appropriate antibiotics.




This step is important for comparability. In order to compare cultures, precultures should be relatively fresh, i. e., no older than 1 week and no further than early stationary phase. Furthermore, their OD₇₅₀ should be roughly the same.

- 3 Grow preculture for 2-3 additional days, until OD₇₅₀ has reached ~ 0.6.

Dilution of main culture

- 4 In a large sterile vessel, dilute the preculture to OD₇₅₀ = 0.2, making sure to prepare a sufficient amount of culture. Include appropriate antibiotics.
Aliquot this main culture into clear 6-well culture plates, with 5 mL per well and at least three biological replicates per inducer concentration.

Induction

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At the end, the total volume added should be equal for each replicate. This can be achieved either by preparing one stock solution for each concentration, or adding the difference in volume of the solvent used.

The following three tables show the pipetting schemes for the three inducible promoters P_{rha} , P_{L03} and P_{vanCC} over a range of inducer concentrations.

L-rhamnose, final concentration [mM]	0	0.01	0.05	0.1	0.5	1	2	5	10	20
L-rhamnose stock solution	-	10	10	10	100	100	100	1000	1000	1000
μ L stock solution	0	5	25	50	25	50	100	25	50	100
μ L MilliQ water, sterile	100	95	75	50	75	50	0	75	50	0

Table 1: Pipetting scheme for dose response assay with L-rhamnose as inducer.

Inducer amounts are calculated for 5 mL culture volume in a 6-well plate format. Total inducer volume per well is 100 μ L.

aTc, final concentration [nM]	0	10	50	100	500	1000
aTc stock solution [μ M]	-	10	50	100	500	1000
μ L stock	0	5	5	5	5	5
μ L EtOH, 100 %	5	0	0	0	0	0

Table 2: Pipetting scheme for dose response assay with aTc as inducer.

Inducer amounts are calculated for 5 mL culture volume in a 6-well plate format. Total inducer volume per well is 5 μ L.

vanillate, final concentration [μ M]	0	100	200	500	1000	2000
vanillate stock solution [mM]	-	100	100	250	250	250
μ L stock	0	5	10	10	20	40
μ L EtOH, 100 %	40	35	30	30	20	0

Fluorescence measurement

- To monitor fluorescence development, fluorescence measurements should be performed 6h, 24h and 48h post-induction. For each sample, pipette 150 μ L into a black-walled, clear-bottom 96-well plate in technical triplicates.

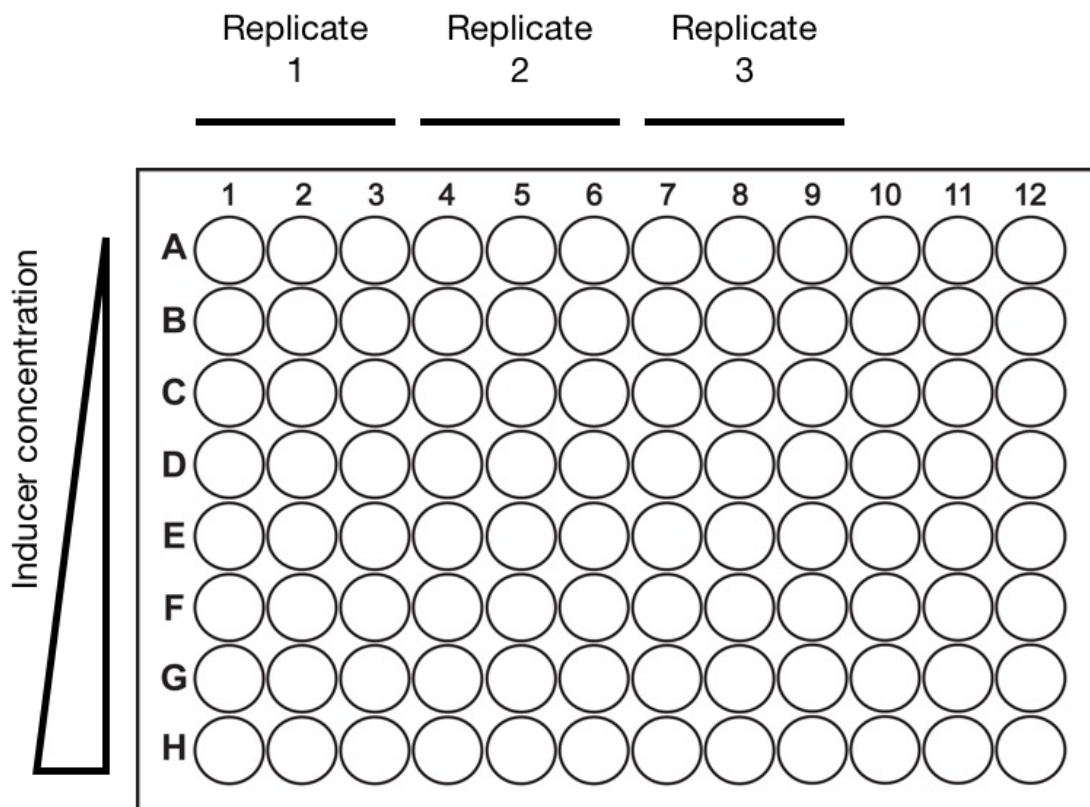


Figure 1: Pipetting scheme for plate reader measurement.

Include a well with an appropriate blank (BG11)

Fluorescence measurement settings in the BMG Clariostar:

Top optic

Excitation λ : 511/12 nm

Excitation λ : 552/20 nm

of flashes: 20

To quantify cell density, measure the absorption at 750 nm.

Shake before plate reading at 500 rpm for 30 seconds (double orbital)



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