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

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We use this protocol and it's working

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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

MATERIALS

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

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

Before start

Prepare all stock solutions, including one stock of the solvent the inducer is dissolved in (e.g. H_2O 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 µM and 1 nM, prepare 1:1000 stock solutions for both concentrations, i.e. 1 mM and 1µM, 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.

> Note
>
> 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 OD_{750} of ~1 is reached.

Dilution of preculture

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

> Note
>
> 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 OD_{750} should be roughly the same.

3 Grow preculture for 2-3 additional days, until OD_{750} has reached ~ 0.6.

Dilution of main culture

4 In a large sterile vessel, dilute the preculture to OD_{750} = 0.2, making sure to prepare a suffient 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

5

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
	µL stock solution	0	5	25	50	25	50	100	25	50	100
	µL 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 µL.

	aTc, final concentration [nM]	0	10	50	100	500	1000
	aTc stock solution [µM]	-	10	50	100	500	1000
	µL stock	0	5	5	5	5	5
	µL 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 µL.

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

Fluorescence measurement

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

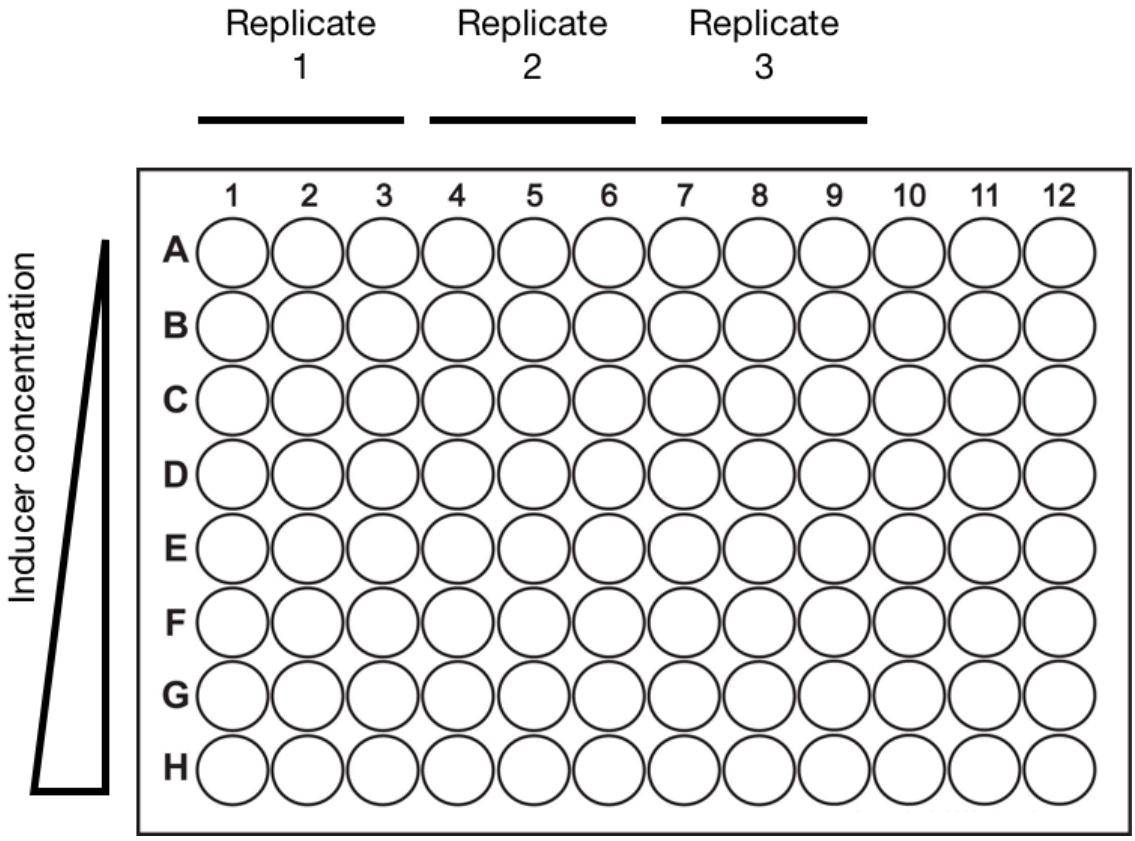

Figure 1: Pipetting scheme for plate reader measurement.

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)