Toxicity assay for inducer compounds 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.
However, prior to working with an inducible promoter system, it is important to assess whether the inducer compount might positively or negatively affect growth or viability of the cell.
This protocol describes the methodology for toxicity assays in the unicellular cyanobacterium *Synechocystis sp*. PCC 6803 to quantify its growth in the presence of different concentrations of inducer.

GUIDELINES

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

MATERIALS

NAME	CATALOG #	VENDOR
Cobalt(II) chloride hexahydrate	60820	Sigma Aldrich
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

MATERIALS TEXT

Other materials required:
- Erlenmeyer flasks, wide neck
  - 100 mL: vwr, 214-1171
  - 250 mL: vwr, 214-1172
- BG11 media
- antibiotic stocks
  - Spectinomycin 20 mg/mL
  - Kanamycin 25 mg/mL
  - Chloramphenicol 10 mg/mL

SAFETY WARNINGS

Handle inducer stocks with care, using appropriate safety equipment. Some compounds (such as $CoCl_2$) may be toxic to humans or the environment and may need to be disposed of properly and without endangering the environment.

BEFORE STARTING

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 pre-culture

1 Inoculate an appropriate amount of BG11 with your cyanobacterial strain of choice. The inoculation volume depends on the number of concentrations to be tested. For example: 3 concentrations x 3 replicates x 30 mL = 270 mL of preculture. Since the culture will be diluted ~1:3 before starting, 100 mL culture volume in a 250 mL Erlenmeyer flask should be enough.

If using a strain carrying a plasmid, make sure to include appropriate antibiotics, 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 pre-culture

2 Dilute pre-cultures to an OD_{750} of 0.2. Include the appropriate antibiotics.

This step is critical for comparability. In order to compare cultures, pre-cultures 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 pre-culture 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} = 0.1**, making sure to prepare a suffient amount of culture. Include appropriate antibiotics if necessary.

In order to compare cultures, it is important that all cultures are in the same growth phase and that you start the experiment at the same OD_{750}!

Aliquot this main culture into smaller, 100 mL Erlenmeyer flasks, with 30 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 four tables show the pipetting schemes for the four inducer compounds L-rhamnose, aTc, vanillic acid and $CoCl_2$, over a range of concentrations.

L-rhamnose, final concentration [mM]	0	1	10
L-rhamnose stock solution [mM]	-	1000	1000
µL stock solution	0	30	300
µL MilliQ water, sterile	300	270	0

Table 1: Pipetting scheme for toxicity assay with L-rhamnose as inducer.
Inducer amounts are calculated for 30 mL culture volume in a flask format. Total inducer volume per well is 300 µL.

aTc, final concentration [nM]	0	100	1000
aTc stock solution [µM]	-	100	1000
µL stock	0	30	30
µL EtOH, 100 %	30	0	0

Table 2: Pipetting scheme for toxicity assay with aTc as inducer.
Inducer amounts are calculated for 30 mL culture volume in a flask format. Total inducer volume per well is 30 µL.

vanillate, final concentration [µM]	0	1000	2000
vanillate stock solution [mM]	-	250	250
µL stock	0	120	240
µL EtOH, 100 %	240	120	0

Table 3: Pipetting scheme for toxicity assay with vanillate as inducer.
Inducer amounts are calculated for 30 mL culture volume in a flask format. Total inducer volume per well is 240 µL.

Cobalt (III) chloride, final concentration [µM]	0	5	10
Cobalt (III) chloridestock solution [mM]	-	5	10
µL stock	0	30	30
µL MilliQ water, sterile	30	0	0

Table 4: Pipetting scheme for toxicity assay with $CoCl_2$ as inducer.
Inducer amounts are calculated for 30 mL culture volume in a flask format. Total inducer volume per well is 30 µL.

Growth monitoring

6 Measure OD_{750} of each culture every day. If the OD is above 1.0, an additional dilution step is necessary for precise measurement.
Monitor growth over at least 7 days, or until the culture becomes stationary.

If growing your cultures in an incubator with CO_2-enriched air, it could be beneficial to measure the OD twice a day, since the growth rate is much higher under these conditions.

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