Incubation of Synechocystis sp. PCC 6803 colonies for plate reader measurement

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Abstract

In this protocol, the incubation of *Synechocystis* sp. PCC 6803 colonies on agarplates and further incubation in flasks for further plate reader measurements for fluorescence detection are described. The protocol was handed over by Anna Behle MSc.

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Before start

Prepare BG-11 media-based agar plates.You can use the recipe for standard BG-11 media, which includes the recipe for BG-11 media based agar plates.

Order/borrow **agar plates, fresh cultures or cryo cultures** with your **desired colonies**. If your agar plates are contaminated, make sure that you only pick **uncontaminated** colonies.

Protocol

Inoculation of BG-11 media based agar plates

Step 1.

Make sure to work under sterile conditions!

Use fresh BG-11 media based agar plates for your inoculation. If your cultures contain a plasmid with a certain antibiotic resistance, prepare your agar with appropriate antibiotica. Pick your desired colony from an incubated agar plate and inoculate your agar plate with this colony. The wider you inoculate, the more cell-material you will earn.

If you inoculate from fresh cultures or thawed cryo cultures, pipette **100µl** of your cultures on the plate. Spread your culture **steady** with a pipette or spreader on your agar.

Incubation of agar plates

Step 2.

Incubate your colonies for **seven days** under appropriate wavelenth and temperatures.

Your colonies should have a **fresh, rich green colour**. If your colonies are just light green, use more cell-material for your further inoculation in flasks.

Inoculation in liquid medium (BG-11)

Step 3.

Use inoculation loops. Take an **appropriate amount** of cell-material and inoculate a certain volume of 1x BG-11 media. Use the **same amount** of cell-material in each sample. Incubate your cultures under appropriate wavelengths, rpm and temperatures for **seven days**.

Check your cultures

Step 4.

Check your flasks after **24 hours**. Each sample should have the same, fresh green colour. If certain cultures show a lighter colour, you can re-inoculate them with appropriate colonies.

Write your plate reader protocol

Step 5.

Write an appropriate plate reader measurement protocol or ask your colleagues for protocols.

Dilution

Step 6.

Check your cultures. They should show a uniform, fresh to deep green colour. They shouldn't be light green or even dark green.

Measure the absorption under the OD750 via photometry. Your cultures should have an absorption between 0.4 and 0.6. I strongly recommend 0.4.

If your cultures have an absorption higher than 0.6 and lower than 1.0, dilute them with 1x BG-11. **If you use an inducible promoter, make sure that your BG-11 media doesn't contain that certain chemicals i.e. nickel, cobalt or copper.**

For example: My plasmid contains a cobalt-inducible promoter. Therefore I induce my cultures with 20µM cobalt(II)-chloride.

Measure the absorption under the OD750 afterwards. If each sample shows the same absorption, you can go to step 6.

Induction

Step 7.

Induce your cultures with your appropriate chemicals, if you use an inducible promoter. Afterwards **mix your cultures very well**. Do not vortex.

Incubate your colonies for your desired period under appropriate wavelengths, rpm and temperatures. You can measure now for the first time after 0h after induction.

Measurement

Step 8.

Take your samples out of the incubator.

Pipette **triplicates** of each sample **quickly** on a 96 well plate / appropriate well plate.

Remember to pipette **negative controls** i.e. empty vector controls / **media only** and **positive controls**.

Measure your samples.

If you want to extract RNA afterwards, you can use the Isolation of total RNA from *Synechocystis* protocol by Anna Behle MSc.

Make sure follow the first three steps of Anna's protocol quickly after the plate reader measurement to catch the cells in the current stage.

Warnings

Make sure to **work under sterile conditions**! It's also recommended to work in cold seasons or in a climated environment to **avoid fungal contaminations**.

Wear gloves when preparing stocks!

Heavy metals are toxic for the environment and need to be discarded accordingly.

Make sure to **check your reagents and chemicals for safety warnings and correct waste disposal!**