Colony PCR (DreamTaq) with Synechocystis sp. PCC 6803 and gel electrophoresis

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Abstract

In this protocol, the extraction of template DNA out of *Synechocystis* sp. PCC 6803 samples, the PCR of the template and the application on a agarosegel are described. The protocol was handed over by Maximilian Dietsch MSc.

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Before start

Culture your desired *Synechocystis* colonies for **at least 7 days** on BG11 agar plates.

You can use the recipe for standard BG-11 media, which includes the recipe for BG-11 media based agar plates, of Anna Behle MSc.

Always **work under sterile conditions** while inoculating your agar plates!

Protocol

Inoculation

Step 1.

Inoculate **50µl sterile water** in a tube with your **desired colony**.

It would be good if you use cell-material from a agar plate with an applied clone from an agar plate, so you have enough cell-material.

The inoculated water should have a **fresh, light green colour**.

Work under **sterile conditions** if you need your colonies on your agar plates for a further use.

AMOUNT
50 µl Additional info: nuclease-free water

Heating

Step 2.

Heat your samples up to **95 °C** for **10 minutes**.

Make sure that you **don't burn yourself**! The samples are **hot**!

TEMPERATURE
95 °C Additional info:

Centrifugation

Step 3.

Centrifuge your samples at **13,000 rpm** for 1 minute.

Pipette your samples

Step 4.

Pipette **1-2µl** of the supernatant into a fresh PCR tube.

You can **freeze the rest of your samples** (including the pallet) at **-20° C** for a further use. Afterwards just **thaw** your sample by **room temperature** and **centifuge** it at **13,000 rpm** for **1 min**. Now you can pipette **1-2µl** of the supernatant again for a later colony PCR.

Pipette your master mix

Step 5.

Pipette your master mix. In this case, we use the Dream Taq DNA Polymerase Kit from Thermo Fisher Science.

Use the following contents for one sample:

10x DreamTaq buffer: 2µl

dNTP Mix: 0.4µl

Forward primer: 2µl

Reverse primer: 2µl

DreamTaq DNA Polymerase: 0.2µl

Water (nuclease-free): 12.4µl (for 1µl template) or 12.3µl (for 2µl template)

Total: 18-19µl

Pipette the Dream Taq DNA Polymerase in the end.

You can also use the Thermo Fisher DreamTaq Master Mix, which contains the buffer, dNTP Mix and the Dream Taq Polymerase. In this case, you just have to pipette The Master Mix,

the forward primer, the reverse primer. the template DNA and nuclease-free water. Use the related protocol from your DreamTaq PCR package leaflet.

ANNOTATIONS

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I use a primer concentration of 1µM in this step. Therefore I pipette 2µl of 10µM primer (forward and reverse).

Pipette your master mix to your sample

Step 6.

Pipette **18-19µl** of your **master mix** to your template DNA, which contains 1-2µl (from the previous steps).

Run your PCR

Step 7.

Run a PCR with your samples.

Check the Tm of your primers to use the right annealing temperature. The DreamTaq Polymerase uses the annealing temperature which is 5 °C lower than your Tm.

Therefore I use a temperature that is 5 °C lower than my lowest primer Tm. For example: My lowest Tm is 63 °C. Therefore I use 58 °C as my annealing temperature.

You can also check your optimal annealing temperature with various mathematical equations or the Thermo Fisher Tm calculator.

https://www.thermofisher.com/de/de/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/tm-calculator.html

In my case I use the following PCR protocol:

1. Initial denaturation: 98° C - 2 min.

2. Denaturation: 98° C - 30 sec.

3. Annealing: Tm - 5° C - 30 sec -> I use 58° C here.

4. Extension: 72° C - 1 min.

5. Final extension: 72° C - 10 min.

5. 4° C hold

Rerun the second to forth step for 35 times.

Note from Thermo Fisher:

The recommended extension step is 1 min. for PCR products up to 2kb. For longer products, the extension time should be prolonged by 1 min/kb.

Prepare your agarose gel

Step 8.

Prepare an appropriate gel for gel electrophoresis.

I prepare an 1%, small agarose gel. Therefore put **0.5g** of **agarose** in **50ml 1x TAE** (TRIS-Acetat-EDTA) buffer.

Afterwards heat it up in a microwave with 360 watt until the agarose is completely dissolved and the **solution** is **clear**.

Cool it down at room temperature until it's hotter than lukewarm but still touchable and not burning your skin!

Shake it some times while it's cooling down and check the solution for lump formation. It should be clear!

Add your **dye** to your agarosegel. I use **midoriGreen Advance** with an 1:20,0000 dilution factor.

Therefore I pipette 2.5µl in my agarose gel solution.

Afterwards pour your prepared agarose gel in a gel carrier.

Don't forget to put your appropriate gel combs in your agarose gel before it hardens.

When your gel is hardened, take out your gel combs and put appropriate buffer in the gel chamber until the agarose gel is completely soaked.

Load your samples

Step 9.

Load your samples with an appropriate **loading dye**. I use the **6x loading dye** of Thermo Fisher.

Therefore I pipette **4µl** to each sample.

Load your gel

Step 10.

Pipette your samples carefully up and down to mix your samples with the loading dye.

Load your hardened agarosegel with your samples. Adjust the loading volume to the size of your gel comb.

Don't forget to use an appropriate **ladder** for your samples.

I use the Gene Ruler **1kb Plus DNA ladder** (ready to use) to detect a fragment of a size of 708 bp.

Therefore I pipette **5µl** of my ladder in one slot of the agarose gel.

Run your gel

Step 11.

Run your gel with your desired voltage. I use **120 V**.

Adjust your running time to your fragment size.

I run my gel until it reaches **2/3** of the gel.

Check your results

Step 12.

Check your results with your imaging system.

I use the ChemyDoc MP System of Bio-Rad to check my gel for my fragments. I use midoriGreen Advance to dye my fragments in the agarose gel.

Therefore I use the Nucleic Acid Gels -> GelGreen channel in my Image Lab 5.0 software.

Good luck!

Warnings

Work under sterile conditions in the first step if you need your colonies for a further use.

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

disposal!