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Chloroquine agarose gel

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ABSTRACT

DNA is not only separated by size, but also by conformation. This can be used as advantage when it comes to separate and visualize topoisomers of the same plasmid: the more supercoiled a plasmid, the more compact it is. A standard agarose gel shows supercoiled plasmid as one blurred band; adding an intercalating agent such as ethidium bromide or chloroquine can resolve this. Chloroquine is able to unwind the DNA locally, releasing torsional stress and neutralizing negative supercoils, or even introducing positive supercoils. This circumstance alters the electrophoretic mobility. Low negative supercoiled plasmids become positive supercoiled and move faster through the agarose grid. Highly negative supercoiled plasmids become less negative supercoiled and move slower. This way we are able to see individual topoisomer bands on the gel.

This protocol is calibrated for pUC19 extracted from *E. coli* and the native plasmids of *Synechocystis sp.* PCC 6803 pCC5.2, pCA2.4 and pCB2.4.

Preparing a fresh 1.2% agarose gel

1 A 1.2% agarose gel consists of:

- **2.7 g** agarose
- **225 ml** 0.5x TBE

Mix both together in an **open 500 ml bottle** and boil it up in a microwave. The agarose should be dissolved and the hot solution completely clear.

Mix the solution with putting it on a magnetic stirrer with a stir bar. The solution will cool down faster and is mixed with less incurrence of air bubbles. Wait until the solution is warm to the touch and take care that the solution keeps liquid and clear.

Adding Chloroquine

2 When the solution is warm to the touch, add the needed amount of chloroquine* and let it stir a bit.

*Stock solution: 10 mg/ml chloroquine

- 1 µg/ml in 225 ml solution --> 22.5 µl
- 8 µg/ml in 225 ml solution --> 180 µl
- 10 µg/ml in 225 ml solution --> 225 µl
- 15 µg/ml in 225 ml solution --> 337.5 µl

Based on experience, 8 µg/ml always brought good separation of topoisomers.

Pour the mixture into a gel chamber with fitting gel carrier. We are using the Peqlab chamber 40-1515.
Occurring air bubbles can be eliminated or pushed to the gel edges with a pipette tip.
Put a proper comb in your liquid gel before it hardens.

Preparing Running buffer

3 Prepare 1 L of 0.5 x TBE buffer with the same chloroquine concentration as in the gel.

*Stock solution: 10 mg/ml chloroquine

- 1 µg/ml in 1 L TBE --> 100 µl
- 8 µg/ml in 1 L TBE --> 800 µl
- 10 µg/ml in 1 L TBE --> 1000 µl
- 15 µg/ml in 1 L TBE --> 1500 µl

When the gel hardens put out the comb and pour the prepared 0.5x TBE in the gel chamber until the gel, especially the gel slots, are completely covered.

Preparing samples

4 Per Lane, 250 ng of pUC19 or 1 µg of *Synechocystis sp.* plasmid DNA is recommended.

To ballast our samples we use 6x Purple Loading Dye (Thermo Fischer).
The amount of Loading Dye in your sample should be **1/6** of your sample volume.

Application on gel

5 Pick a proper ladder. We are using GeneRuler^{TM} 1 kb DNA Ladder from Thermo Scientific, which shows the best resolution if it is mixed 1:1:3 with Loading Dye and Millipore filtered water (one ladder lane: 1 µl ladder + 1 µl Loading Dye + 3 µl MilliQ).

Load your gel with your samples. Here it is the best to use only the first push of your pipette to avoid pipetting your samples out of the gel slots.

Gel run

6 Close the gel chamber cover it from the light (chloroquine is light-sensitive) and make sure that the voltage source is plugged.
Running your gel should take place at 1.8 V/cm. With the chamber we are using, the run takes place at 40 V for 24 h.
It is always better to run the gel for longer and less voltage to seperate the topoisomers better.

Washing and dying the chloroquine agarose gel

7 After stopping the run, the gel is washed two times for 30 minutes in 250 ml 0.5x TBE buffer to remove the chloroquine. After that, the gel is stained with 25 µl Sybr Gold in 225 ml 0.5x TBE buffer for at least 1 h.

Saving you Results

8 Use a matching imaging system for your application.
We are using ChemiDoc^{TM} MP Imaging System from BioRad to analyze our agarose gel.
Make sure to pick -->N*ucleic Acid Gel* -->'*Sybr Gold*" for our usage in the Image Lab software. Click "*Position Gel*" and place your gel according to what is best for your application. Then click "*Run Protocol*" and wait for your image.