Dorman:Chloroquine gel analysis

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This is a method used to monitor the superhelicity of a reporter plasmid such as pUC18 or pBR322. Larger plasmids can be used but the resolution of topoisomers in the gel is better with smaller plasmids. Also, pUC18 is very high copy number which makes it easy to extract from low culture volumes. A standard mini-prep from 1 or 2 mls of overnight culture usually provides plenty of plasmid for chloroquine gel analysis.

Procedure: Gel and buffer are made of 2X TBE.

1. Make up 2 litres of 2X TBE buffer (we buy ours as a 10X stock) and about 1 ml of chloroquine (25 mg/ml, made up in water). This is a 10,000X stock.
2. Add 3 g agarose to 300 mls buffer to make a 1% gel. Melt the agarose solution and allow cool slightly before adding 30 µl chloroquine (gives a final concentration of 2.5 µg/ml).
3. Tape the ends of the big gel tank and pour the chloroquine. Put in the comb, cover the gel and allow it tosolidify. When solid, add 170 µl chloroquine to the 1.7 litres of buffer. Pour this into the gel tank so it covers the gel.
4. Remove the comb and load samples (the wells take 20 µL). If using the 6X dye provided by promega with DNA ladders, you need to use it as a 1X dye (2X TBE has a different buoyancy to normal electrophoresis conditions). Alternatively, make up a dye by heating 100% glycerol (heat to allow easy pipetting) and any dye (to allow sample visualization in the lane). Keep the dye in a heating block at 70 degrees C beside the gel tank when loading. The easiest way to load is first to spot 20 µL of each sample onto parafilm. With a second p20 pipette, add 3 µL heated loading dye to the first sample, swap to the pipette set at 20 µL, mix the sample and load into the first lane. Continue doing this with the rest of the samples.
5. Run the gel at 100V (3V/cm) overnight (~16 hours).
6. Remove gel from the tank and wash repeatedly in tap water for at least 2 hours. This is essential to remove the choroquine from the gel before staining with ethidium bromide (~500 mL, 1 µg/mL). Keep the stain covered in tin foil during storage and use as it is light sensitive as repeated exposure to light will reduce its shelf life.
7. Stain the gel for at least half an hour, pour the ethidium bromide back into the bottle carefully and wash the gel once (for a few seconds) to remove excess stain before visualization under UV.

At chloroquine concentrations of 2.5 µg/mL, more negatively supercoiled molecules migrate further through the gel and more relaxed molecules migrate slower. At a higher concentration (25 µg/mL) this will be reversed, with more relaxed molecules migrating further.

NB: Samples on different chloroquine gels can only be compared if a reference sample is used that is common to all the gels. This is easily done and definitely worth while. (It doesn't matter what the reference conditions are as long as they're consistent.)