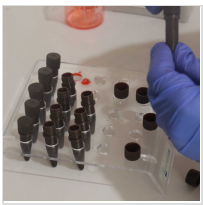

Jul 09, 2022

Functionality test (DNA gel stain)

In 1 collection

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Nadine Mowoh

ABSTRACT

After PCR amplification of DNA, agarose gel electrophoresis is run to separate the DNA based on their size.
The agarose gel consists of microscopic pores that act as a molecular sieve which separates molecules based on their charge, size and shape. Agarose gel electrophoresis can also be used to separate other charged biomolecules such as RNA and proteins. Agarose is isolated from the seaweed genera Gelidium and Gracilaria and consists of repeated agarobiose (L- and D-galactose) subunits. The concentration of agarose in a gel depends on the sizes of the DNA fragments to be separated, with most gels ranging between 0.5%-2%.

This protocol describes the use of BenBio DNA gel stain for the visualization of DNA with agarose gel electrophoresis (to show its functionality), and that it can be a good (cheaper and safer) alternative to EtBr-based DNA gel stains.

PROTOCOL CITATION

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COLLECTIONS

Beneficial Bio: Quality control tests

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Functionality test for DNA gel stain, using Thiazole Orange DNA gel stain, TO-DMSO based DNA gel stain

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GUIDELINES

This procedure can be performed by laboratory staff that have been trained and have theoretical and practical skills in good laboratory practices. It can also be performed by molecular biology students or students of related fields under the supervision of a laboratory staff.

MATERIALS TEXT

Reagents

Agarose (electrophoresis grade)
DNA template PCR amplicon (lambda 0.5 and 1kb or other)
1x TBE buffer (Recipe here)
Thiazole Orange gel stain (13 mg/ml stock concentration)
DNA loading dye(BenBio 6x DNA loading dye)
DNA ladder(Bioline 1kb)
DNA gel stain (SYBR Safe or other Ethidium bromide based gel stain as standard)

Equipment

Micropipette
Microwave
Gel casting tray
Well comb
UV transilluminator
Voltage source

BEFORE STARTING

Make sure to have a 13 mg/mL stock of DNA gel stain, 10x TBE buffer and all materials to be used in preparing the agarose gel.

Functionality test of Thiazole Orange-DMSO based DNA gel stain

1 Preparing DNA amplicons

To have the DNA amplicons to use for this experiment, follow the steps described in this protocol to amplify a specific region of a DNA template.

2 Preparing 1.5 % agarose gel (using the test DNA gel stain)

Prepare a 1.5 % agarose gel for electrophoresis as follows:

Agarose gels are commonly used in concentrations of 0.5% to 2% depending on the size of bands needed to be separated. Simply adjust the mass of agarose in a given volume to make gels of other agarose concentrations (e.g. 0.5g of agarose in 25 mL of TBE will make a 2% gel).

Make sure the buffer used in preparing the gel is the same buffer to be used as the gel running buffer, do not mix buffers and do not use water, as it might affect migration of the DNA samples.

1. Use a weighing balance to weigh **0.375 g** of agarose powder and pour into a 150 ml or any appropriate size glass beaker.
2. Use a measuring cylinder to measure **25 mL** of 1x TBE buffer and add to the agarose powder in the beaker (The volume of gel you will need to make will depend on the size of the casting tray.)
3. Swirl the beaker for about 5 seconds to dissolve the powder
4. Put the beaker into the Microwave and heat at medium high for 1 minute until it boils
5. Remove the molten agar from the micro wave and allow to cool for 30 seconds (about when you can comfortably keep your hand on the beaker).
6. Add Thiazole orange gel stain to a final concentration of 1.3 µg/mL (usually about **2.5 µL** of lab stock solution per **25 mL** gel). Thiazole orange binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light or Blue light.
7. Swirl the beaker gently to mix and distribute the DNA gel stain (swirl gently to avoid bubbles).

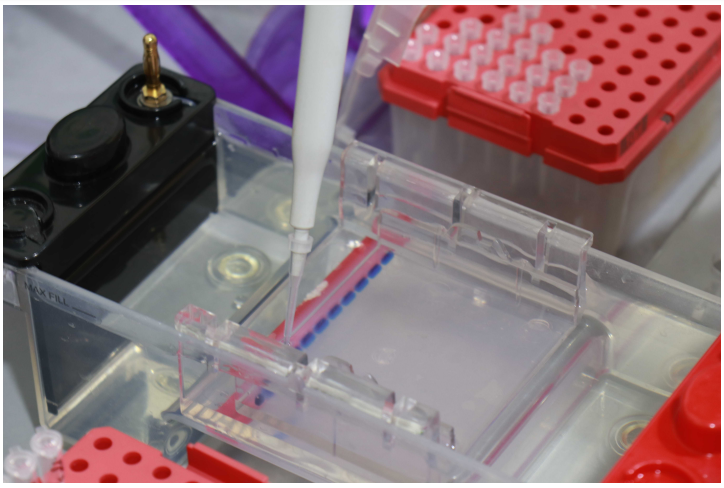
Prepare 1.5 % agarose gel using SYBR Safe as standard by adding 3 µL of the gel stain from the lab stock to 25 mL molten agar to make a final concentration of 0.5 µg/mL following the steps described above.

3 Casting and loading the Electrophoresis gel

1. Pour the molten agar into the gel casting tray with the well combs in place. (Pour slowly to

avoid bubbles which will disrupt the gel), allow to solidify for about 15 to 20 minutes.
2. After the gel is solidified, remove the comb and place the casting tray into the gel box or electrophoresis unit.
3. Fill the electrophoresis unit with 1x TBE running buffer to cover the gel (the amount of TBE to use will depend on the size of the electrophoresis unit).
4. Pipette 3 µLof DNA ladder (a collection of DNA fragments of known lengths that helps you determine the approximate length of a DNA fragment by running it on an agarose gel alongside the DNA amplicon), into the first well.
5. Pipette 1 part of the DNA loading dye and 5parts of the DNA amplicon, mix and load into the subsequent wells.
6. Connect the electrophoresis unit to a power or voltage source by connecting the electrodes from the power source to the electrophoresis unit (*Black is negative, red is positive). The DNA is negatively charged and will run towards the positive electrode.* ***Always Run to Red.*** *R*un to finish (we adopt 80 volts for 20 minutes run).
7. Run the second gel (from the standard DNA gel stain at the same conditions.

DNA loading dye serves two purposes: 1) it provides a visible dye that helps with gel loading and allows you to gauge how far the DNA has migrated; 2) it contains a high percentage of trehalose that increases the density of your DNA sample causing it to settle to the bottom of the gel well, instead of diffusing in the buffer.



4 Visualizing and Analyzing the gel

1. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box unto a gel visualization system.
2. Using any device that has UV light (UV transilluminator), visualize your DNA fragments. The fragments of DNA are usually referred to as ‘bands’ due to their appearance on the gel.
3. Using the DNA ladder in the first lane as a guide (the manufacturer's instruction will tell you the size of each band), you can infer the size of the DNA in your sample lanes.

Visualize the gel from the standard gel stain and compare with that of the test DNA gels stain to confirm the functionality of the Thiazole orange (determined by

showing that the Thiazole orange dye is able to bind to DNA and allows visualization using UV or blue light.