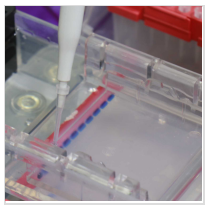

Jul 09, 2022

Functionality test (DNA loading dye)

In 1 collection

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This protocol is published without a DOI.

Nadine Mowoh

ABSTRACT

Beneficial Bio 6X DNA Loading Dye is a premixed loading buffer used to prepare samples for loading onto agarose and polyacrylamide gels. It contains bromophenol blue and Xylene cyanol dyes for visual tracking of DNA during electrophoresis. It is made with high concentrations of trehalose to ensures that DNA forms a layer at the bottom of the well and facilitates sample loading. The EDTA in it chelates metal ions and stops metal-dependent enzymatic reactions. The solution contains SDS which results in sharper bands by removing restriction enzymes from DNA.

This protocol describes the steps in testing the functionality of our Beneficial Bio DNA loading dye (to show it can help DNA samples to sink to the bottom of a gel well and allow tracking of the DNA during migration on an agarose gel without interfering with the visualization of the resulting bands.

PROTOCOL CITATION

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COLLECTIONS

Beneficial Bio: Quality control tests

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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)
"Standard" DNA loading dye (6x NEB)
"Test" DNA loading dye (BenBio 6x DNA loading)
DNA ladder(Bioline 1kb)
Standard DNA gel stain (SYBR Safe or other Ethidium bromide, EtBr stain)

Equipment

Micropipette
Microwave
Gel casting tray
Well comb
UV transilluminator
Voltage source

SAFETY WARNINGS

- When using UV light, protect your skin by wearing safety goggles or a face shield, gloves and a lab coat.
- EtBr is a known mutagen. Wear a lab coat, eye protection and gloves when working with this chemical.

BEFORE STARTING

Make sure to have a 10mg/mL stock of Ethidium bromide DNA gel stain, 6x loading dye to act as standard, 10x TBE buffer and all materials to be used in preparing the agarose

gel.

Functionality test of BenBio 6x DNA loading dye

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

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.

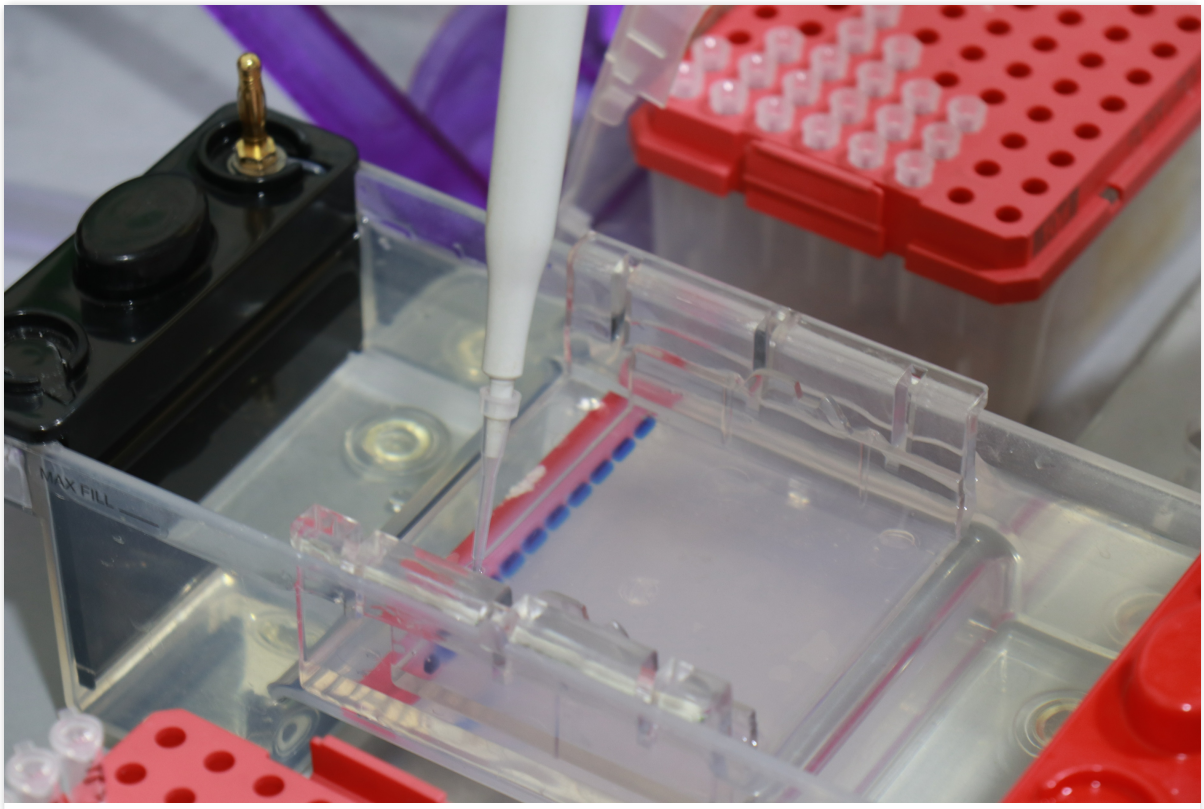
1. Use a weighing balance to weigh **0.375 g** of agarose powder and pour into a 150 ml or any appropriate size glass beaker.
2. Use a measuring cylinder to measure **25 mL** of 1x TBE buffer and add to the agarose powder in the beaker (The volume of gel you will need to make will depend on the size 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 **00:01:00** 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 EtBr gel stain to a final concentration of 0.5 µg/mL (usually about **2.5 µL** to **3 µL** of lab stock solution per **25 mL** gel). EtBr 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).

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 "test" 6x DNA loading dye and 5parts of the DNA amplicon, mix and load into the next 4 wells.
6. Pipette 1 part of the "standard" 6x DNA loading dye and 5parts of the DNA amplicon, mix and load into the last 4 wells.
7. 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. Run to finish (we adopt 80 volts for 20 minutes run).

The Xylene cyanol and bromophenol blue dyes in the BenBio loading dye 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.

Compare the migration pattern of the DNA and visibility of the DNA bands of the "standard" to that of the "test" DNA loading dyes to show that the BenBio 6x Loading dye is able to allow tracking and migration of DNA without hindering visualization of the DNA bands on the gel.