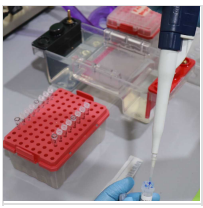

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Visualization of DNA of low concentration and molecular weight (DNA gel stain)

In 1 collection

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ABSTRACT

After PCR amplification of DNA, an 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 upon the 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 steps in using the agarose gel electrophoresis to show that the BenBio DNA gel stain can allow the visualization of DNA of low molecular weight and concentration, and therefore good alternative to EtBr based DNA gel stains.

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

- 10000x TO-DMSO gel stain stock
- DNA amplicon (*in this case 50 or 100bp DNA ladder*)
- 10x TBE buffer
- Horizontal agarose gel electrophoresis system
- Visualization systems
- Beaker
- Micropipette and tips
- Agarose gel electrophoresis system
- Gel visualization system

BEFORE STARTING

Ensure all materials, equipment and chemicals to be used for this experiment are all in place before starting.

Visualization of DNA of low concentration and molecular weight

1

The confirmation of visualization tests are applicable for DNA gel stains, Electrophoresis buffers and DNA loading dye. The visualization tests could be modified slightly to suit a particular product.

The protocol described below describes the QC steps in confirming a products ability to allow visualization of low molecular weight DNA and low DNA concentration.

2 To confirm visualization of DNA of low molecular weight DNA gel stain

1. Follow the steps in preparing a 2% agarose gel as described in this protocol,
2. We use the TO-DMSO DNA gel stain as "test DNA gel stain" and an EtBr based DNA gel stain as "positive control or standard DNA gel stain" (*meaning 2 gels will be prepared*).
3. Load the gels (*in this case a loading dye would not be used since the amplicon is a 50 or 100bp DNA ladder*) therefore it can be loaded without loading dye needing 3-5ul of the ladder. Load directly into the gel wells in replicates.
4. Run the 2 gels simultaneously under the same conditions to finish and visualize the resulting band separation to confirm separation of the ladder into visible distinct bands (from the lowest to highest base pair bands) using a UV transilluminator or blue gel system.

To confirm visualization of a low concentration DNA

Make 1:5 serial dilutions of the 100bp DNA ladder or any DNA amplicon and use the different dilutions as amplicons to load a gel well.
Allow the gel to run through and visualize to determine the lowest concentration detectable by the gel stain.