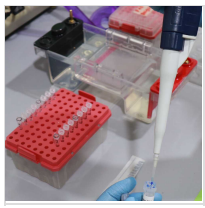

Version 2

Jul 28, 2022

Visualization of a low concentration and molecular weight DNA (DNA gel stain) V.2

In 1 collection

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Low-cost, high-quality ...

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 low molecular weight and low concentration DNA with agarose gel electrophoresis. The BenBio DNA gel stain is therefore, a good alternative to EtBr-based DNA gel stains.

DOI

dx.doi.org/10.17504/protocols.io.5jyl89x46v2w/v2

PROTOCOL CITATION

Nadine Mowoh, Stephane Fadanka, Shalo Minette 2022. Visualization of a low concentration and molecular weight DNA (DNA gel stain). **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl89x46v2w/v2
Version created by Nadine Mowoh

COLLECTIONS

Beneficial Bio: Quality control tests

KEYWORDS

Quality control test of DNA gel stain

CREATED

Jul 27, 2022

LAST MODIFIED

Jul 28, 2022

PROTOCOL INTEGER ID

67728

PARENT PROTOCOLS

Part of collection

Beneficial Bio: Quality control tests

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.

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MATERIALS TEXT

Reagents

- 10000x TO-DMSO gel stain stock (test sample)
- DNA amplicon (*in this case 50 or 100bp DNA ladder*)
- 10x TBE buffer
- EtBr based gel stain (positive control or standard)

Materials and equipment

- Glass beaker
- Micropipette and tips
- Micropipette and tips
- Microwave
- Gel casting tray
- Well comb
- UV transilluminator
- Voltage source (Electrophoresis unit)

SAFETY WARNINGS

- If using UV transilluminator to visualize, care must be taken in order not to expose yourself to UV rays.
- A safer alternative to use is the blue light transilluminator.
- Care should also be taken when handling EtBr as it is a know mutagen.

BEFORE STARTING

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

Visualization of a low concentration and molecular weight DNA

1

The test for visualization of a low concentration and molecular weight DNA 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 product's ability to allow visualization of low molecular weight DNA and low concentration DNA.

2 **To confirm visualization of a low molecular weight DNA**

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- Follow the steps in preparing a 2% agarose gel as described in this protocol,

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 and run simultaneously, where possible.*
If this is not possible, run one gel to finish and run the next.

- Load the gel (made from the test gel stain) by pipetting 3 to 5 uL of sample into the wells (*in this case a loading dye would not be used since the amplicon/ sample is a DNA ladder that is made with the tracking dye in it.*
- Load the second gel *(made from the control gel stain)* by pipetting 3 to 5 uL of sample into the wells (*in this case a loading dye would not be used since the amplicon/ sample is a DNA ladder that is made with the tracking dye in it).*
- Connect the electrophoresis units to a power pack and run the 2 gels simultaneously under the same conditi*ons (when using TBE running buffer, we run at 80 to 100 Volts for 15 to 20 minutes)* to finish.
- Transfer the gel unto a UV transilluminator and visualize the resulting band separation to confirm separation of the ladder into visible distinct bands (from the lowest to highest base pair bands).

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.

  - 2 gels would be prepared, one with the test gel stain and another with the control gel stain.
  - These gels could be run simultaneously or one after the other.

- Repeat the steps described above in loading a gel using the different dilutions of the DNA.
- Connect the electrophoretic unit to the power source and allow the gel to run through (when using TBE buffer, we run at 80 to 100 Volts for 15 to 20 minutes).
- Transfer the gel unto a UV transilluminator and visualize to identify visible, sharp bands which determine the highest dilution of the DNA that is detectable by the gel stain.

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