Gel Electrophoresis

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Abstract

Separates molecules based on size. Great for checking DNA after a Restriction Digest.

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Before start

Have a DNA Sample ready, typically either from PCR or a recently performed Restriction Digest. Dilute down the 50X TAE Buffer to 1X.

Materials

- Ethidium View by P212121
- 1 kb DNA Ladder - 1,000 gel lanes N3232L by New England Biolabs
- Gel Loading Dye, Purple (6X), no SDS - 4.0 ml B7025S by New England Biolabs
- TAE Buffer (Tris-acetate-EDTA) B49 by Contributed by users
- Agarose A5304 by Contributed by users

Protocol

Prep Work

Step 1.

Pour 50 mL of 1X TAE Buffer into an Erlenmeyer Flask.

AMOUNT

50 ml Additional info:

REAGENTS

- TAE Buffer (Tris-acetate-EDTA) B49 by Contributed by users

Prep Work

Step 2.

Weigh out 0.5 g Agarose and add it to the Erlenmeyer Flask.

AMOUNT

1 g Additional info:

REAGENTS

- Agarose A5304 by Contributed by users

Prep Work

Step 3.

Place Erlenmeyer Flask in a microwave on high power for two minutes or until solution is clear and agarose is completely dissolved.

DURATION

00:02:00

NOTES

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Every time it starts to boil open the microwave and swirl the flask around then place the flask back in the microwave and continue heating.

Prep Work

Step 4.

Remove Erlenmeyer Flask from microwave and let it sit on the lab bench to cool just until you can comfortably pick it up.

DURATION

00:05:00

Prep Work

Step 5.

Add 5 uL Ethidium into the flask and swirl to mix, taking care not to introduce bubbles.

AMOUNT

5 µl Additional info:

REAGENTS

Ethidium View by P212121

NOTES

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Ethidium intercalates with DNA and floureses orange under UV light.

ANNOTATIONS

Olga Biskou 10 Jul 2015

Nowdays there are other substances that do the same work without being toxic.

Prep Work

Step 6.

Place gel tray on clamp and clamp securely. Add well plates where you want wells and use a level to ensure it is balanced.

Prep Work

Step 7.

Pour contents of the Erlemeyer Flask into the gel tray and let it sit for 30 minutes, or until a blue tint appears.

DURATION

00:30:00

Loading the Gel

Step 8.

Remove the well plates carefully as to not tear the gel and remove the tray from the clamp, but ensure the gel remains in the tray.

Loading the Gel

Step 9.

Place gel tray into gel electrophoresis apparatus with the wells closer to the negative/black end.

NOTES

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As DNA is negatively charged it will be attracted to the positive end and repelled from the negative end.

Loading the Gel

Step 10.

Pour additional TAE Buffer to fill each side of the apparatus and to create a thin layer of buffer covering the top of the gel.

AMOUNT

10 µl Additional info:

REAGENTS

1 kb DNA Ladder - 1,000 gel lanes N3232L by New England Biolabs

Loading the Gel

Step 11.

Pipette 10 uL of the 1kb DNA Ladder with Loading Dye into a well. Typically this is placed into one of the wells near an edge.

AMOUNT

10 µl Additional info:

REAGENTS

1 kb DNA Ladder - 1,000 gel lanes N3232L by New England Biolabs

NOTES

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Be careful when loading not to puncture the sides or bottom of the wells as the sample may then leak out.

Loading the Gel

Step 12.

Pipette your DNA with Loading Dye mixture into another well. Repeat for each sample.

AMOUNT

5 µl Additional info:

REAGENTS

Gel Loading Dye, Purple (6X), no SDS - 4.0 ml B7025S by New England Biolabs

NOTES

Alan Cone 08 Jul 2015

Make sure you pipette to mix your sample before loading it into the well. Loading Dye contains glycerol which will sink to the bottom of your sample and not appropriately stain your DNA or ensure it stays in the well.

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For a 25 uL PCR reaction you can add 5 uL of the Loading Dye to yield a final volume of 30 uL with 1/6 of the mixture being Loading Dye.

Running the Gel

Step 13.

Place lid on apparatus and plug cables into amplifier. Set amplifier to stay at a constant voltage of 100 V.

Running the Gel

Step 14.

Let run for 30 minutes or until the loading dye has sufficiently moved.

DURATION

00:30:00

Running the Gel

Step 15.

Remove gel from gel tray after draining excess TAE Buffer and place on plastic wrap.

Reading the Gel

Step 16.

Place gel with plastic wrap on UV lamp to view bands, or store in the plastic wrap at +4 C for later use.

Warnings

Ethidium Bromide potentially acts as a mutagen or carcinogen.