Gel Electrophoresis

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Abstract

Separates molecules based on size.

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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 100 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 1 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, occasionally stirring.

DURATION

00:02:00

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.

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 6.

Pour contents of the Erlemeyer Flask into the gel tray and let it sit for 10 minutes, or until solid.

DURATION

00:30:00

Loading the Gel

Step 7.

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 8.

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

Loading the Gel

Step 9.

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 10.

Pipette 5 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

Loading the Gel

Step 11.

Pipette your DNA, approximately 20 uL, 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

Running the Gel

Step 12.

Place lid on apparatus and plug cables into amplifier. Set amplifier to stay at a constant voltage of 70 V.

Running the Gel

Step 13.

Let run for 45 minutes or until the loading dye has sufficiently moved.

DURATION

00:45:00

Running the Gel

Step 14.

Remove gel from gel tray after draining excess TAE Buffer.

Running the Gel

Step 15.

Place gel into a bin with 1% Ethidium Bromide, ensuring the solution does not come into contact with skin. Clean all surfaces that may have come in contact with ethidium bromide, place gloves in a bin labelled "Ethidium Bromide", and wash hands repeatedly afterwards. Let gel sit for 15 minutes.

DURATION

00:15:00

Running the Gel

Step 16.

Carefully removing the gel, to avoid splashes, place on top of a UV light source with a camera. In as short a time as is possible, turn on the UV light to capture a photograph of the gel.

Running the Gel

Step 17.

Remove band if purifying DNA. Otherwise, carefully dispose of the gel into a wastebin labelled "Ethidium Bromide."

Warnings

Ethidium Bromide potentially acts as a mutagen or carcinogen.