Agarose gel electrophoresis, 1.2% with Ethidum bromide

Harold Bien

Abstract

Separates molecules based on size. Great for checking DNA after a Restriction Digest. This protocol is for a 1.2% agarose gel, sufficient for resolving DNA 400bp to 7kb (conservatively). The gel electrophoresis system is a 7.5cm x 10cm gel bed area with approximately 15cm electrode distance. The minimum volume required for a 7.5cm x 10cm x 0.5cm gel is 37.5mL. The maximum gel height for 50mL is 0.67cm.

Citation: Harold Bien Agarose gel electrophoresis, 1.2% with Ethidum bromide. **protocols.io** dx.doi.org/10.17504/protocols.io.ds76hm
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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 using sterile filtered water. For the dual-sided 1.0mm gel comb MHC-10-0816 the maximum volume is 35/13µL. For the dual-sided 1.5mm gel comb MHC-15-1014, the maximum sample volume is 38/25µL.

Materials

- 2-Log DNA Ladder - 100-200 gel lanes N3200S by New England Biolabs
- Gel Loading Dye, Purple (6X), no SDS - 4.0 ml B7025S by New England Biolabs

GeneMate LE Quick Dissolve Agarose, 500g E-3119-500 by Bioexpress

Ethidium bromide, 10mg/mL, 10mL X328-10ML by Amresco

TAE (TRIS-ACETATE-EDTA) Buffer, 50x K915 by Amresco

Horizontal mini-gel kit MHU-202 by C.b.s Scientific

Protocol

Prep Work

Step 1.
Weigh out 0.6 g (1.2% w/v of 50mL) agarose and add it to the Erlenmeyer Flask.

AMOUNT
1 g Additional info:

REAGENTS
GeneMate LE Quick Dissolve Agarose, 500g E-3119-500 by Bioexpress

Prep Work

Step 2.
Add 50mL of 1x TAE buffer

AMOUNT
50 ml Additional info:

REAGENTS
TAE (Tris-Acetate-EDTA) buffer, 1x by Contributed by users

Prep Work

Step 3.
Place Erlenmeyer Flask in microwave. Set to wait 30 seconds, then full power (P10, 1250W) for 20 seconds followed by low power (P1, 125W) for 30 seconds or until solution is clear and agarose is completely dissolved.

DURATION
00:00:50

NOTES
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Press 'Timer', enter 30 seconds. Then press 'Power', enter 20 seconds. Then press 'Power' 10 times to select P1, then enter 20 seconds. Press 'START'.

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
00:03:00

Prep Work

Step 5.
Add 1µL concentrated ethidium bromide (10mg/mL) into the flask and swirl to mix, taking care not to introduce bubbles.

AMOUNT
1 µl Additional info:

REAGENTS
Ethidium bromide, 10mg/mL, 10mL X328-10ML by Amresco

NOTES
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Final concentration of ethidium bromide will be 10µg/50mL or 0.2µg/mL

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.

NOTES
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For **MHC-10-0816 1mm thick dual-sided 8/16 well gel combs**, the maximum recommended sample volume is ***35µL for 8 wells*** and ***13µL for 16 wells***.
For **MHC-10-1014 1mm thick dual-sided 10/14 well gel combs**, the maximum recommended sample volume is ***26µL for 10 wells*** and ***17µL for 14 wells***.
For **MHC-15-0816 1.5mm thick dual-sided 8/16 well gel combs**, the maximum recommended

sample volume is ***52µL for 8 lanes*** and ***19µL for 16 lanes***.
For **MHC-15-1014 1.5mm thick dual-sided 10/14 well gel combs**, the maximum recommended sample volume is ***38µL for 10 lanes*** and ***25µL for 14 lanes***.

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.

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.

Loading the Gel

Step 11.
Prepare DNA ladder and samples by adding 6x blue dye

REAGENTS
Gel Loading Dye Blue (6X) - 4.0 ml B7021S by New England Biolabs

NOTES
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Recommend diluting DNA ladder 1:10 in sterile filtered water and loading 200ng of DNA ladder for the 1.5mm thick 14-well lane (3.5mm wide).

Loading the Gel

Step 12.
Pipette your samples into each well.

NOTES
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For the 1.5mm gel comb MHC-15-1014, recommended DNA mass is 200-500ng for 14-well.

Running the Gel

Step 13.
Place lid on apparatus and plug cables into high voltage power supply. Run at 100V (6.6V/cm) for 45-60 minutes or until the loading dye has sufficiently migrated down the gel.

DURATION
00:45:00

NOTES
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Ensure that the negative terminal (typically black) is plugged into the negative/black terminal on the power supply and the loading well are closest to the black/negative side.

Visualizing DNA bands

Step 14.
Gel can be imaged on UV transilluminator through the UV-transparent gel tray or removed and wrapped in plastic wrap for storage at 4°C for later use.

Warnings

Ethidium Bromide potentially acts as a mutagen or carcinogen.