Gel Electrophoresis - TBE 0.5X

Forked from Gel Electrophoresis

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ABSTRACT

Separates molecules based on size.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

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

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Agarose	A5304	
1 Kb Plus DNA Ladder	10787018	Invitrogen - Thermo Fisher
DNA Gel Loading Dye (6X)	R0611	Thermo Fisher Scientific

SAFETY WARNINGS

Ethidium Bromide potentially acts as a mutagen or carcinogen.

BEFORE STARTING

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

Prep Work

1 Pour 150 mL of 0.5X TBE Buffer into a 250 ml Duran Bottle.

150 ml 0.5X TBE buffer

2 Weigh out 1.5 g Agarose and add it to the Duran bottle.

1.5 g agarose

Agarose
Catalog #: A5304

3 Place Duran bottle in a microwave on medium power for two minutes or until solution is clear and agarose is completely dissolved, occasionally stirring.
00:02:00 agarose melting

4 Remove the Duran bottle from the microwave with a glove/pot holder and let it cool under tap water until you can comfortably pick it up without protection.
00:02:00 gel cooling down

5 Place gel tray on clamp.
Add well combs and use a level to ensure it is balanced.
Pour 40 ml (small gel) or 70 ml (big gel) of melted agarose into the gel tray and let it sit for 15 minutes, or until solid.
00:15:00 gel solidification

Loading the Gel

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

7 Place the gel tray into the gel electrophoresis apparatus with the wells closer to the negative/black end.
Pour additional 0.5X TBE Buffer to fill each side of the apparatus and to create a thin layer of buffer covering the top of the gel.
Pipette 5 µL of the 1kb DNA Ladder with Loading Dye into a well.
Prepare your DNA samples by adding an adequate amount of Gel Loading Dye (6X) and load them into the wells.

1 Kb Plus DNA Ladder
by Invitrogen - Thermo Fisher
Catalog #: 10787018

DNA Gel Loading Dye (6X)
by Thermo Fisher Scientific
Catalog #: R0611

Running the Gel

8 Place lid on apparatus and plug cables into the power supply. Set the power supply to stay at a constant voltage of 90 V (small gel) - 120 V (big gel).
Let the gel run until the bromophenol blue reaches the end of the gel

Staining

9 Remove the gel from the gel tray after draining excess of TBE Buffer.
Place gel into a tray with 1% Ethidium Bromide ensuring the solution does not come into contact with your skin, then place the tray over a shaker with gentle agitation.
Let the gel stain for 15 minutes.
00:15:00

Destaining

10 Carefully remove the gel from the Ethidium Bromide solution and place it ina tray with distilled water, then place the tray over a shaker with

gentle agitation.
Let the gel destain for 10 min

UV exposure

11 Place the gel on top of a UV light source with a camera.
Turn on the UV light and capture a picture of the gel.