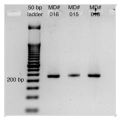

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2% Agarose Gel Electrophoresis

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1 Works for me dx.doi.org/10.17504/protocols.io.bdmki44w

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ABSTRACT

This protocol describes the procedure for electropohresing DNA through an agarose gel to examine fragment size.

GUIDELINES

Use this protocol at your own risk.

MATERIALS

NAME	CATALOG #	VENDOR
Gel Loading Dye Blue (6X) - 4.0 ml	B7021S	New England Biolabs
MilliQ water		
TAE, 40X (Tris-acetate-EDTA), 1000ml	V4281	Promega
SYBR SAFE DNA stain	S33102	Life Technologies
50 bp DNA Ladder	10416014	Thermo Fisher
Agarose I (Molecular Biology Grade)	17850	Thermo Fisher

MATERIALS TEXT

Mandatory PPE

Eye protection
Nitrile gloves
Lab coat
Enclosed footwear

Equipment

Electronic balance
UV transilluminator (BioRad)
Electrophoresis tank
20-well gel combs
Power pack
Gel casting tray
Microwave
Conical flask
Graduated measuring cylinder
Weigh boats
Spirit level
Oven mitts
Spatula
Vortex

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Minispin
96-well plate rack
Parafilm
P10, P100 pipettes and filter tips
Biohazard bin bags (for incineration)
Sharps bin

SAFETY WARNINGS

Key hazards

Harmful in conact with skin (buffers)
Electrical hazards (electrophoresis tank, microwave, UV transilluminator)
UV hazards (UV transilluminator)
Burning hazard (boiling agarose)
Ergonomic hazard (pipetting)
Sharps (pipette tips)

This requires UV safety training, and working with electricity.

Ensure chemical spill kit and first aid kit is within reach and that eye wash stations and safety showers are easily accessible.

Do not seal the flask when microwaving agarose! Steam needs to escape so pressure is not built up from boiling agarose.

Take care with SYBRSafe (or any gel stain alternative, such as Ethidium Bromide). Check local guidelines for disposal of these chemicals.

Do not pour liquid agarose down the sink.

Spill response: apply full PPE, mop up with absorbant material (e.g., paper towel), place in a sealed container and discard in a biohazard bin for incineration.

BEFORE STARTING

Apply PPE.
Prepare instruments and regents.
Read all relevent MSDSs for reagents and follow the safety guidelines stipulated therein.
Read and undestand relevent risk assessments.
Requires appropriate training and instruction provided by a competent person.
Read and undsertand equipment operating manuals provided by the supplier.

Cast a 2%, medium sized agarose gel 30m

1

Make up 2 L of 1X TAE buffer:

50 ml of 40X TAE buffer
1950 ml of MilliQ water

Mix by inversion.

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2 Using an electronic balance, weigh out 2.2 g of agarose powder on to a weigh boat using a spatula.

Note that gel concentration can be adjusted. The more concentrated the gel, the greater the resolution of small fragment sizes.

3 Transfer the powder to a 200 ml conical flask.

4 

Using the graduated measurng cylinder, measure out 110 ml of 1xTAE buffer. Add to the conical flask containing the agarose powder. Swirl the flask gently to mix.

5 Microwave the flask uncovered for 1 minute.

A lid can loosly be placed over the flask but DO NOT tighten--allow steam to escape. Do not microwave for more than 1 minute at a time.

Do not fill flasks or beakers/Schott bottles more than half full with liquid!

6 

Remove the flask from the microwave using oven mitts and swirl gently.

The liquid is boiling! Use oven mitts to handle flask. Do not put your face over the opening to the flask as liquid can splash out!

7 

Microwave the flask for a further minute but remove from the microwave if the agarose appears to boil excessively. Swirl to mix and examine near a light source to ensure the agarose has melted. Allow to cool for 5 minutes.

8 Place the gel casting tray into a rubber vice that will seal the ends tightly, or tape the ends with masking tape.

9 Place the assembly on a flat bench and use the spirit level to check it is level--adjust if needed.

10 Place a 20-well comb into the casting tray.

11 When the flask is cool to the touch, add 5 ul of SYBR Safe and swirl gently to mix. Avoid generating bubbles.

Wait until the liquid is warm (not boiling) to cast the gel, or the tray may crack!

12 Pour the liquid gel slowly into the casting tray. Pop any bubbles that have formed using a clean pipette tip.

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13 Let the gel set for 20-30 minutes at room temperature.

14 Allow residual gel to set in the flask, then scrape into the bin. Fill the flask half full with water and microwave until the water boils. Pour the water down the sink and clean the flask using a bottle brush.

Load and run the gel 1h 25m

15 When the gel is set, remove the combs gently.

16 Place the casting try and gel in the electrophoresis tank.

17 Fill the electrophoresis tank with 1 X TAE buffer to the fill line indicated on the tank.

18 

Pipette 3 ul of 50 bp DNA ladder into the first well of the gel.

The recommended volume will depend on the concentration of the ladder. Check the manufacterer's recommendations. If the ladder is not pre-mixed with loading dye, be sure to add 1-2 ul of loading dye before loading into the gel.

19 Place some Parafilm across a 96-well PCR plate rack and press down firmly to create small wells.

20 

For each sample, pipette 1-2 ul of loading dye onto the Parafilm, taking care not to pierce the Parafilm.

21 

Mix 10 ul of PCR product with the loading dye by pipetting gently up and down.

22 

Transfer the 12 ul of PCR product/loading dye to the wells of the gel, taking care not to pierce the bottom of the well with the pipette tip.

The volume each well can take will depend on the size of the comb used. Be sure not to overload the wells or product will float out the top of the well.

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23 ⚠

Place the lid on the gel tank and plug the electrodes into the appropriate power slots. Ensure the positive electrode is at the base of the gel.

Take care when working with electricity and water!

Check electrical cords of all equipment and ensure none are damaged and that cords are not a tripping hazard. Do not use if the electrical cord is damaged in any way. Tag the instrument with warnings, make the area safe, and notify your line manager and anyone else in the immediate area that may be affected.

Use electrical equipment indoors only in an area free of explosive material, corrosive gas, powerful vibrations, direct exposure to sunlight, and temperature fluctuations. Use in a space where cables will not come into contact with liquids, be manually damaged, or interfere with other workplace operations.

Do not use electrical equipment with any other power adapter or cord than the one supplied.

24 Switch the power pack on a set the voltage to 80 V and the time to 1 hr and 10 min.

Note that the voltage and time can be adjusted to suit what you are running on the gel. For amplicons (one small product), I will run the gel at 96 V for 30-40 min. For shotgun libraries, I will run the gel as above. The lower the voltage and longer it is run, the greater the separation of fragments will be.

25 Press 'Run' or 'Start' on the power pack and check to see that bubbles are rising from electrodes.

26 When the run is over, switch of the power pack, remove the lid, and remove the gel from the tank, taking care not to let it slide off the tray.

Do not remove the lid to the electrophoresis tank until the power pack is switched off.

Photograph the gel 10m

27

Place the gel on the UV transilluminator and photograph using the attached camera. Follow the manufactuerer's instructions to use the equipment.

Take care working with UV. You should have UV safety training. Do not open the transilluminator while the UV is on! Use signage to warn others when the UV is on.

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28 Discard the gel into a designated biohazard bin, and clean the UV dock with 70% ethanol.

Finishing work 10m

29 Dispose of used tips into a designated sharps container.

30 Dispose of gel waste into a biohazard bag.

31 Used combs, beakers, flasks, and tray should be washed with warm water and placed on a rack to dry.

32 Gloves and chemical waste should be sealed in a biohazard bag for incineration.

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