AGAROSE GEL MIGRATION PROTOCOL

Purpose

This protocol explains how to prepare an agarose gel that will be used in an electrophoresis to separate DNA fragments in regard of their length. It allows to identify, quantify and even purify given linear DNA fragments.

KEYWORDS: DNA, electrophoresis, agarose.

Total time: 90 minutes

Waiting time: 60 minutes

Resume

Gel preparation → Gel migration → Gel coloration → Gel visualization

Materials

Pipettes

Microwave

- DNA
- Agarose DNA grade
- H_2O
- Loading dye
- DNA Ladder
- Gel Red
- TAE 1X Buffer: Tris 40mM, acetic acid 20mM, EDTA 1mM

Protocol

Gel preparation

1. Determine the theoric length of the DNA predicted fragments, as it will determine the agarose concentration in the gel.

2. Put 25 mL of 1X TAE for a small gel (6 wells) in an Erlenmeyer; 50 mL for a medium gel (13 wells) or 100 mL for a giant gel (2 x 13 wells).

3. Put the wanted agarose quantity according to the following table in the Erlenmeyer and put it in the microwave at maximum power for 30s. Take it out with a glove and mix it to homogenize the solution.

Tips and tricks

If you see unmelted particles, put it back for 10s. Repeat until the solution is perfectly translucid.

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Protocol

DNA Size (bp)	Agarose (%)	Agarose quantity (g)		
		6 wells gel	13 wells gel	26 wells gel
1 000 – 30 000	0,5	0,125	0,25	0,5
800 – 12 000	0,7	0,175	0,35	0,7
500 – 10 000	1	0,25	0,5	1
400 – 7 000	1,2	0,3	0,6	1,2
200 – 3 000	1,5	0,375	0,75	1,5
50 – 2 000	2	0,5	1	2

4 Pour the solution into the mold, then add the brush to create the wells and let it solidify for approximately 15-20 min, depending on the room temperature.

Tips and tricks
If you want to extract DNA use the lowest agarose % possible (<1%)

Gel migration

5 Put the gel into the electrophoresis cuve and make sure it is entirely covered in TAE 1X.

6 Add 5 µL of DNA Ladder to the first well, useful to determine the DNA sample size of the bands.

7 Mix the DNA sample with the loading dye in the appropriate proportion (the loading dye should be at a final concentration of 1x in the mixture).

Tips and tricks
If you want to add a bigger volume, 2 wells can be merged by putting tape around the brush during gel preparation.

8 Add a maximum of 25 µL of sample in a single well, being careful not to contaminate the other wells with the sample.

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Protocol

9 Set the migration settings to 100V for 30 min. The migration should be stopped when the colored marker have passed approximately 75% of the gel.
Beware, the colored marker does NOT correspond to the migration front as in SDS-PAGE electrophoresis.

10 Set the migration settings to 100V for 30 min. The migration should be stopped when the colored marker have passed approximately 75% of the gel.

Gel analysis

11 Put the gel in 3X GelRed Dye to stain it for 20 min with mild agitation on.

12 Take it out and wash it with distilled water.

13 Take a picture of the stained gel using a ChemiDoc or a classic UV plate. The gel can be used for a gel purification, or thrown out in the carcinogenic bin.

Note
WARNING: GelRed is toxic, protect yourself with gloves!

Troubleshooting

- *If the bands are not visible enough, try to put more sample, leave it longer in the GelRed or turn the negative colors on the ChemiDoc logicial: ImageLab.*
- *If the following gel extraction doesn't give correct results, it may be linked to an overexposure to UV light that have degraded the DNA. In this case, retry with a shorter exposure to UV light.*