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7.13 Experimental Microbial Genetics

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Agarose Gel Electrophoresis and DNA Band Excision

How it works:

Agarose gel electrophoresis is a simple and highly effective method for separating, identifying, and purifying 0.5 to 25 kb DNA fragments. Voltage applied at the ends of an agarose gel generates an electric field with a strength defined by the length of the gel and the potential difference at the ends (V/cm). DNA molecules exposed to this electric field migrate toward the anode (positive end) due to the negatively charged phosphates along the DNA backbone. The migration velocity is limited by the frictional force imposed by the gel matrix. While charge and/or size can affect the rate at which macromolecules will pass through the gel, the charge to mass ratio is the same for DNA molecules of different lengths. It is the size of the DNA, therefore, that determines the rate at which it passes through the gel, thereby allowing an effective separation of DNA fragment-length mixtures by electrophoresis. To visualize the DNA, the gel is treated with ethidium bromide. This dye intercalates between the stacked bases of nucleic acids and fluoresces red-orange (560 nm) when illuminated with UV light (260 to 360 nm).

Reagents and materials:

50 X TAE buffer (agarose gel running buffer)

Ethidium bromide 10 mg/ml (20,000x stock) - *wear gloves when handling EtBr.*

Since it is a DNA-intercalating molecule, EtBr is a mutagen! Dispose of all tips etc in labeled receptacle in fume hood.

6X Gel loading buffer

Agarose gel:

1. To an Erlenmeyer flask, add agarose to an appropriate weight to volume (see below). You will want the final gel to be about 8mm thick so calculate how much agar solution you will need.

Agarose (%)	Size of fragment separated
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0.5	15 kb to 30 kb
0.7	1 kb-12 kb
1.0	500 bp to 8 kb
1.2	400 bp to 5 kb
1.5	200 bp to 3 kb

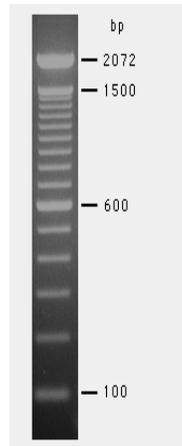
2. Add appropriate amount of 1X TAE to the flask. ****Do not make your agarose in water!**** Bring slurry to a boil in a microwave (heat about 1.5 minute on high power), swirl, and repeat as necessary until no "streaks" or chunks remain.

*** Be careful with molten agar and agarose. It can boil over suddenly and cause nasty burns.**

3. Once agarose is completely dissolved, add ethidium bromide (10 mg/ml; a 10,000 X stock) to a final concentration of 0.1µg/ml (WEAR GLOVES!). Swirl to mix.
4. Pour molten agar into a gel box.
5. The agar is completely set when it becomes opaque (< 20 min).
6. If you prepare your gel ahead of time, wrap it in saran wrap and store it in the cold room.
7. Add the loading dye to your DNA samples to a final concentration of 1X.
8. Load the gel. Remember to also load a molecular weight marker!!! See end of this protocol for 1 kb and 100 bp molecular weight marker patterns.
9. Run gel with an overlay of 1X TAE (about 5-8 mm deep above the gel) at 80-100V.
10. The bromphenol blue in the loading dye will migrate with an apparent molecular weight of 500 bp and the xylene cyanol of 2 kb.
11. When the gel has run far enough, so that your bands have clearly separated and you can tell what size they are, take a picture of the gel.

Cutting out DNA band:

Take the gel to the dark room and use a hand-held UV-light transilluminator to visualize the DNA bands. In order to minimize damage to the DNA, use a long-wave UV light for as short a time as possible. ***Make sure to use protective glasses during this procedure since the UV light will burn your retina.*** Excise the desired band using a razor blade. Place the band in an eppendorf tube. Take a picture of the gel without the band in it to be sure the band is successfully recovered.



100 bp DNA Ladder
0.5 µg/lane
2% agarose gel stained with ethidium bromide.

Note:

During 2% agarose gel electrophoresis with tris-acetate (pH 7.6) as the running buffer, bromophenol blue migrates near the 100-bp fragment. The 100-bp band migrates behind the bromophenol blue marker on 6% polyacrylamide gels with tris-borate (pH 8.0) as the running buffer.

Part of the 600-bp band may migrate anomalously slowly in polyacrylamide gels (1,2,3). This band may appear as an extra band near or on top of the 700-bp band.

References:

1. Hsieh, C., et al. (1991) *Mol. Gen. Genet.* 225, 25.
2. Stellwagen, N.C. (1983) *Biochemistry* 22, 6186.
3. Jordan, H. and Hartley, J. (1997) *Focus*® 19, 9.

