Gel electrophoresis

by Howard Judelson

What is Gel Electrophoresis?

The movement of small particles through a gel by the application of electricity. The particles can be DNA, RNA, or proteins. The matrix nature of the gel (a semi-solid colloidal system consisting of a solid dispersed in a liquid) acts as a sieve to slow the progress of the molecules. Smaller molecules move more rapidly and thus further. Gel electrophoresis separates molecules on the basis of size, but is also influenced by molecule shape and charge density.

Caution!

EtBr is typically used to visual DNA in gels. It is a known mutagen. It will adhere to your DNA just as it will adhere to the DNA on your gel. Always wear gloves when handling your gel.

Also, be careful not to come in contact with the electrophoresis buffer during runs, to avoid electrocution.

Running an agarose Gel

Gels are normally run at from 0.6% to 1.2% agarose. 0.8% is a good all-around choice, good for resolving and visualizing fragments from 350 to 10 kb. If you are expecting fragments from 150 to 400 bp, and it is important to be able to accurately visualize and size them, run a 1.2% gel. Make the gel as follows:

1. Pouring the gel

a) Calculate the required amount of buffer (usually 1X TBE) and agarose for your experiment and the gel tray being used.
b) To a clean flask, add the buffer and then the agarose. Try to pour the agarose straight into the bottom of the flask; particles that stick to the sides of the glass may result in lumps, which should be avoided. Swirl the flask to mix up the agarose; this also helps avoid lumps.
c) I typically make a 40-80 ml gel in a 250 ml flask, a 300 ml gel in a 1 liter flask, etc.
d) Weigh the flask.
e) Microwave until agarose is completely dissolved. The time varies depending on the volume. Typically, 3 min at full power is good for 300 mls, and 2 min at 50% power is good for about 50 ml. The idea is to avoid boil-over.
f) After the initial microwaving, carefully examine the flask to ensure that all of the particles are dissolved. For large volumes (300 ml), usually it helps to swirl the flask after 3 min and then microwave for another 1 min.
g) After microwaving, weigh the flask and add water to the original volume. This helps result in a perfect gel with nice, straight bands.
h) Cool the flask in the 50-60C water bath.
i) optional step: add 10 mg/ml ethidium bromide to the gel before pouring (0.1 microliter per ml of gel, i.e. 2 µl per 40 ml gel).

j) After a minimum of 10 minutes, pour the gel in a prepared apparatus. (each apparatus is prepared differently; the idea is to generate a leak-proof vessel with a comb 1 to 2 mm above the base. ALSO, ensure that the gel tray is level).
k) Wait for the gel to solidify, usually about 15 minutes (you can speed this up by pouring the gel in the cold. For low melting temperature agarose gels, do all of the pouring in the cold).
l) When you are finished pouring your gel, rinse out your Erlenmeyer really well with water before you put it in the wash tub, because whoever is doing the dishes will not want to get EtBr all over him/herself. Also, hardened agarose is hard to clean off of glassware.

2. Preparation of samples and loading of gel

a) The maximal sample volume for each comb varies from about 8 to 35 microliter—plan in advance what you will be using.
b) Carefully remove the comb from the gel. If you go too quickly, you will pull out the bottom of each well, and your sample will leak out! If in doubt, you can load a little sample dye into questionable wells, to see if they are OK. Leaks can also be sealed with a little melted agarose (For very low percent gels, try refrigerating the gel first to prevent damage when removing the comb).
c) Place the gel in the buffer tank and cover with a minimal amount of buffer, 1-2 mm. (too much buffer needlessly increases the amperage and consequently heating of the gel, reducing resolution).
d) Pipette 1/10 volume of gel buffer into each sample and mix.
e) Carefully load each sample into the wells.

3. Running the gel

Electrophorese at 5-7.5 v/cm (don't go too high, to avoid overheating).
The length of time needed depends on what you are actually trying to see, but normally I run gels until the bromophenol blue tracking dye goes 70% of the length of the gel. You can estimate how long the gel will run, but measuring movement of the dye after 15 minutes and then extrapolating (note that this will just be an estimate, as phenomena such as gel heating during the run will affect mobility).

Ethidium bromide staining

19)place gel in Ethidium bromide bath (10 µl ethidium bromide (10mg/ml) in 500ml H2O)
(Let the gel gently slip from gel tray)
20)stain for 1 h on the shaker
21)visualise results under UV + take photograph
Note: Always use Nitril gloves while working with ethidium bromide

Disposal of Ethidium-bromide waste

22) All items that were in contact with EthBr must be disposed of in the designated waste
container (marked with "Ethidium bromide waste") within the Gel-Doc-Area, including gels, tissue paper to clean UV table, and Nitril-gloves
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PAGE: Poly-Acrylamid-Gel-Electrophoresis

Pouring of PAGE-gels

1) set-up of gel-plates in gel-caster as illustrated in manufacturer's instructions
2) premix 10 ml Acrylamid-Gel-solution, using 5ml-Pipet (sufficient for 2 mini-gels):

Acrylamide concentration	10.5%	5.1%
	5.4 ml	7.2 ml ds H_2O
	3.5 ml	1.7 ml 30% Acrylamide solution
	1 ml	1 ml 10X TBE-buffer
	20 µl	20 µl TEMED
	70 µl	70 µl 10% APS

(Add APS directly before pouring the gel!)
3) pour gel using 5ml-pippette up to the top of the short plate (Avoid bubbles!)
4) insert comb
5) leave for 1h to polymerise

Preparation of samples

6) mix 2 µl of bromo-phenol-blue (BPB) loading buffer and 1-5 µl of sample DNA
7) similarly prepare 5 µl pGEM marker DNA (see below)

Gel-Electrophoresis

8) set-up gel in gel-tank as illustrated in manufacturer's instructions
9) add 130 ml 1X TBE to inner chamber & 200 ml 1X TBE to outer chamber of gel-tank
10) slowly remove comb
11) thoroughly rinse sample wells using a syringe
12) load samples & close lid of gel-tank
13) run the gel for 1h (45 min) at 80V
14) switch off power & remove gel-cassettes as illustrated in manufacturer's instructions

Staining of gel

17) place gel cassette in water bath & carefully remove short plate
18) Stain PAGE-gel on large plate in SYBR-green (1:10,000 SYBR Green in 100 ml H_2O) for
1 h (30 min if solution is fresh)
19) transfer PAGE-gel from large plate onto the UV-table using fish slice
20) check results + take photograph

Note: Always wear Nitril gloves while working with SYBR-Green

Disposal of SYBR-Green waste

21) All items that were in contact with SYBR Green must be disposed of in the designated
waste container (marked with "SYBR Green waste") within the Gel-Doc-Area, including gels, tissue paper to clean UV table, and Nitril-gloves

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Electrophoresis-Gel-Photography

Before starting

(prior to contact with ethidium bromide or SYBR Green)
1) wear Nitril gloves & eye protection
2) switch on camera, monitor, and thermo printer
3) press release button of camera remote control to initialise camera settings

Note: camera release button may be contaminated! Therefore, wear Nitril gloves!

4) choose the appropriate filter (red filter for EthBr-gels, green filter for SYBR-Green gels)
5) if necessary, adjust aperture to a value between 5.0 and 6.0 using wheel in front of camera
6) switch on table lamp (“clean” button)
7) switch off main light
8) close the curtains

Visualisation of results

9) **ALWAYS** wear Nitril gloves while working with ethidium bromide or SYBR-Green
ALWAYS wear eye protection while UV light is on
10) place gel on UV table
11) lower camera to 50 cm-mark
12) zoom in or out using camera remote control
13) further adjust height of camera until picture is focused
14) turn on UV light
15) switch off table lamp (“dirty” button)
16) press release button of printer remote control to obtain print-out of results
17) if necessary, take photograph using release button of the camera remote control
Note: if photographs were taken, write your name, date and the picture number on FlashCard in the photo-book; otherwise, your photo may get deleted
18) switch on table lamp (“dirty” button)
19) switch off UV light

Before leaving

20) dispose of gels in designated waste containers
21) clean UV table using tissue paper
22) dispose of tissue paper in designated waste containers
23) take off Nitril gloves and dispose of them in designated waste containers
24) Only then:
switch on main light
switch off table lamp (“clean” button)
open curtains
take off eye protection

Note: Avoid unnecessary contamination of lab with ethidium bromide and SYBRGreen

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Marker DNA for Agarose Gel Electrophoresis

λ-DNA cut with *Hin*dIII [λ-*Hin*dIII]

size of bands [kb] weight of bands in [ng] (per 100ng loaded)
23.130 50.0
9.400 20.0
6.560 13.5
4.360 10.0
2.300 5.0
2.030 5.0
0.564 1.2

0.125 0.3

λ-DNA cut with *Hin*dIII, *Eco*RI, *Bam*HI pGEM-marker
[λ-BEH] [PGM]
size of bands [kb] size of bands [kb]
16.270 2.645
8.950 1.605
5.540 1.198
3.680 0.676
3.410 0.517
2.790 0.460
2.580 0.396
2.470 0.350
2.450 0.222
1.980 0.179
1.570 0.126
1.320 0.075
1.130 0.065
0.930 0.051
0.840 0.036
0.780
0.560
0.490
0.140
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