

# Agarose Gel Electrophoresis

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## I. Theory

In theory, electrophoresis should be a wondrously simple technique that allows us to determine the charges and molecular weights of all sorts of macromolecules. The basic tenet is a simple one: more negatively charged molecules will migrate in an electric field, over time, toward the positively charged cathode. In practice, however, it is not that simple. A matrix (such as agarose or polyacrylamide) must be used to conduct heat evenly and provide an extra sieving effect. Frictional forces that act on the molecules are difficult to estimate because few molecules can be approximated as a simple sphere (most are ellipsoid). Coulombic forces are even more difficult to calculate because counterions in solution will interfere with the field, and also partially shield the charges of the macromolecules being separated. These complications and others mean that electrophoresis is a very poor quantitative tool. Electrophoresis is still somewhat useful as a qualitative tool for estimation of molecular weights, but its real power is in separation of complex mixtures of macromolecules into their components.

In particular, agarose gel electrophoresis is generally used to separate DNA (single-stranded, double-stranded, and supercoiled) and RNA. Since DNA is negatively charged, it migrates in an electric field toward the positively charged cathode. The agarose matrix retards DNA migration roughly proportionally to DNA length when the DNA being separated is small. Longer oligonucleotides have a harder time traveling through the matrix, while shorter oligonucleotides (and small molecules such as ATP) breeze right through it.

### A. Gel concentration

The concentration of agarose in the gel can be fine-tuned to achieve optimal separation for a specific range of sizes. The general equation for the relationship between electrophoretic mobility ( $\mu$ ) and the gel concentration ( $C$ ) is:

$$\log \mu = \log \mu_0 - K_r C$$

where  $\mu_0$  is the “free” (matrix-free) electrophoretic mobility, and  $K_r$  is the retardation coefficient (a scaling factor) which is related to the properties of the gel and the size and shape of the migrating molecules (see Maniatis, p. 6.5). A plot of  $\log \mu$  versus  $C$  is called a Ferguson plot and can be used to optimize gel concentration for difficult separations. A steep slope on such a plot (large  $K_r$ ) usually indicates a larger molecule, so that sieving effects become more pronounced at higher gel concentrations. A higher intercept (large  $\mu_0$ ) usually indicates a more charged molecule.

For the typical DNA separation experiment, however, this simple chart is sufficient for selecting a gel concentration:

Agarose Gel Concentration (%w/v)	DNA Size Range for Optimal Separation
0.3	5,000 – 60,000
0.6	1,000 – 20,000
0.7	800 – 10,000
0.9	500 – 7,000
1.2	400 – 6,000

1.5	200 – 3,000
2.0	100 – 2,000

Usually 1 to 2% gels are used for detecting plasmids (several kb long) or their fragments (ie. from digestions). For resolving much shorter DNAs, use polyacrylamide gel electrophoresis (PAGE, see separate section). Gels with a lower percentage of agarose tend to be flimsy, so if you do use them run them at low temperature (4°C).

## B. Agaroses

There are a few different types of agarose available. For analytical purposes, such as running digested plasmids to see whether a ligation was successful, you can usually use agarose from USB. However, if you want to recover your DNA and/or perform some in-gel reactions, you should use the low melting agaroses (the NuSieve GTG, etc). These specific agarose protocols are usually provided with the reagent and are available online.

### 1. Agarose Gel Electrophoresis Protocol

#### a. Equipment

To run a gel you will need the following:

1. Two 1L orange cap bottles.
2. 250 mL flask
3. Volumetric cylinders
4. Spatula
5. Gel casting tray
6. Gel combs
7. Tape
8. Electrophoresis tank
9. Power supply and cables

The first six items are used to pour the gel, and the last three are required for running the gel.

#### b. Buffers

There are several buffers that can be used. TAE is typically used, but TBE and others can be used also (again, see Maniatis). Making a stock of 50x TAE for yourself saves time and prevents variations in salt concentration from gel to gel. Also, make or get 0.5 M EDTA ahead of time and adjust pH to 8.0 (it can be somewhat time consuming).

<u>50x TAE</u> 242 g Tris-base 57.1 mL Acetic Acid, glacial 100 mL 0.5 M EDTA Filter	<u>6x Loading Dye</u> 0.25% Bromophenol blue – BB— (or tiny amount on the spatula tip) 0.25% Xylene cyanol FF –XC— (or same as BB) 15% Ficoll 120mM EDTA (240 µL of 0.5 M EDTA in 1 mL total 6x loading dye) <i>Note:</i> Very little loading dye is used; 1 mL of 6x dye should last a long time!!
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*c. Pouring the gel*

1. Tape both sides of the casting tray so that it will hold the liquid gel. It is good to do this step first when you're pouring the gel the first time, as it may require some practice. Also, select a comb. It should have enough teeth to make wells for all your samples, but keep in mind that the more wells, the less sample each will hold.
2. Make 1 L of 1x TAE (20 mL into 1 L).
3. Pour 100 mL of 1x TAE into a clean 250 mL flask (or any other container that is >>100mL and fits well into the microwave) and save the other 900 mL for running buffer. Add 1 g of Agarose to make 1% gel (w/v), 2g for a 2% gel, etc.
4. Microwave until solution is clear, and just starting to bubble. **\*\*IT WILL BE HOT\*\***. Use insulated mitts, a paper towel folded several times over, or the like to transport the flask. Be careful as it may bubble or spill over. If you prefer, you can eschew the microwave method and instead stir the agarose on a hot plate until it is liquid.
5. Check for undissolved agarose. It will appear as floating "lenses".
6. If the solution is clear and fully liquefied, bring it to your bench and let it cool to about 60°C – it should feel quite warm to your hand, but not hot. If you are impatient, you can cool it under running water or in the deli case, but if you cool it too much it will solidify, and you will need to re-heat it. It is better if the gel is warm during pouring, but if it is too hot, it may melt the glue holding the casting tray together. With practice you will be able to recognize the proper pouring temperature.
7. Add Ethidium Bromide (EtBr) to a final concentration of 0.05 µg/mL. So for a 100 mL gel add 5 µL of 10 mg/mL stock (you can also stain later). Note that EtBr is a carcinogen (it intercalates DNA) and so must be handled with care and gloves at all times!
8. Pour the warm liquid agarose. Place the comb into the casting tray by placing the sides into the notches.
9. Wait until the gel polymerizes. It usually takes about an hour. The gel should look opaque and uniform. Use this time to do other things, finish preparing samples, or get the power supply if you haven't done so already.
10. Carefully remove the comb to expose the sample wells.

*d. Running the gel*

1. Place the gel into the tank.
2. Add EtBr to the ~900 mL 1x TAE to make it 0.05 µg/mL (45 µL of a 10 mg/mL stock solution). Pour the buffer into the tank high enough to cover the gel. Be careful since the buffer now has EtBr in it.
3. Add 1 µL loading dye per 5 µL sample (because the dye is 6x).
4. Add samples:
  - a) Loading 100-500 ng of DNA per lane is usually sufficient.
  - b) Total sample volume should be from 10-35 µL (depends on the gel thickness of the gel and well size used).
  - c) One of the samples should be a marker that contains DNA fragments of known lengths that are in the range of your samples.
5. Connect the tank to the power supply:
  - a) Set the voltage at ~150 V. The passage of current will produce bubbles at the electrodes. Also, flipping the display switch to mA should show you a value (usually 2 or 3 digits). If you have no current, check the connections. The samples will migrate towards the "+" electrode.

- b) Watch the gel carefully in first couple of minutes to ensure that the dyes are migrating in the correct direction. If they are not, turn off the power, switch the electrodes and turn the power back on. The gel should still come out reasonably well.
6. Run for about an hour or until the faster dye (BB) migrates most of the way through the gel. You can monitor the progress of the DNA directly (if the EtBr was added) by shining UV light on the gel as it is running. Just be careful with the UV lamp.

*e. Staining a gel*

If you did not add the ethidium bromide earlier, you will need to do so before you can visualize it. The advantage of staining it after running is reduced probability of DNA damage and perturbed migration. The disadvantage, however is that you can't visualize the DNA directly during the run. If you didn't add EtBr, put the unstained gel in a container and pour some TAE buffer (you can reuse the one from the tank you just used to run the gel) just enough to cover the gel, and add ~50 $\mu$ L EtBr (from 10 mg/mL stock solution). Incubate for about an hour with mild shaking.

*f. Imaging the gel*

To visualize the DNA bands, you must look at them under UV light. Use the UV transilluminator next to the STORM in the Schepartz hot room. **\*\*WEAR GLOVES\*\***. You can carry your gel in the casting tray or transfer it onto saran wrap first. Be very careful with gels, as they can be very fragile.

1. Open the plastic cover.
2. Transfer gel from the casting tray by sliding onto the transilluminator (if you're using saran wrap, you can leave it on the saran wrap).
3. Close the plastic cover. This should protect you from UV, but you can use a face mask, a plastic shield, or other protection as well.
4. Turn on UV to 100%. You should see bands at this point.
5. **TURN OFF UV**. You want to minimize the UV exposure time as it may damage DNA. Turn off UV **before** you open the plastic cover.

If you want to take a picture of your gel and save the image:

1. Make sure you have a directory on the E: drive on Pompeii (that's the computer next to the STORM). Your TA will create one designated for Chemical Biology students.
2. Place the digital camera (with its black "hood") onto the gel so that the four corners of the hood align with the marks on the transilluminator.
3. Open Adobe Photoshop on Pompeii.
4. Go to File  $\rightarrow$  Import and click on TWAIN\_32. You'll get a window called "Kodak DC120 Digital Access (Twain Acquire)." Click "Camera Functions" and set the following:
  - ⌚ Single Spot (Auto Focus)
  - ⌚ Best (Quality)
  - ⌚ Flash OFF

- ⌚ Shutter speed to 1/2 second (in the Manual Exposure box); this is a good starting point as it works for most gels; you can optimize it for your gel, but keep in mind that you can bring out a lot of details in image processing later.
  - ⌚ Click “Update Camera”. **\*\*VERY IMPORTANT\*\*** Settings will not take effect unless you update camera. Update after you have made all desired changes.
  - ⌚ Close the Camera Functions window.
5. Turn on UV. Click on “Take a picture!” and wait; you should see a small picture of your gel. If it is all black, you forgot to turn on the UV. If it is faint, you can change the shutter speed, or you can try to see whether you’ll be able to recover it in Photoshop (usually you can). If that doesn’t work, double check all previous steps and try again.
  6. Now click on “Transfer picture” and wait for the picture to transfer to Photoshop. When it does, close the “Kodak DC120” window.
  7. Go to Image → Mode → and click on Grayscale; click OK (discard color information). You don't have to make it black-and-white, but it will make the file smaller, and only need to determine the positions of the bands.
  8. Go to Image → Adjust → and click on Auto Levels. This will work great most of the time. If you still don’t like the way your gel looks, you can change contrast and brightness manually (under the Image → Adjust menu).
  9. Also, **invert the image** so that the bands are black and the gel is white/gray. This will save the ink when you print.
  10. Save the image in your directory on the E: drive.
  11. Print the image to include in your lab notebook. Remember to always label each gel image with the contents of each lane so you know what you are looking at.