

Extraction and Purification of DNA from *Escherichia coli* by Kit Promega and Electrophoresis

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Abstract:

Orphan Extraction DNA from *Escherichia coli* by kit promega (# A1120) and accentuated this method positive result to procuration across being quantum from DNA, After that to knowledge any DNA we use Electrophoresis By Agarose gel and accentuated result being genomic DNA and compare with DNA Marker (10 kb) and find that genomic DNA heavydid n't migrant distance about well.

Keywords: E.coli , Genomic DNA , Kit Prpmega (#A1120) , Agarose gel.

1- Introduction

Agarose gel electrophoresis is a quick and easy molecular technique used to analyze and separate nucleic acids based on their size (i.e.how many base pairs a molecule is composed of). Electrophoresis takes advantage of the fact that DNA's phosphate backbone is negatively charged. Thus when DNA is placed in an electric field, it will migrate toward the positive electrode [1], the differential ability of DNA to move through a gel based on its size doesn't really depend on the electric field or the charged properties of DNA, but more importantly on the composition of the gel, Agarose is a complex polymer that forms a matrix through which DNA travels when subjected to an electric field. I like to think of agarose as a big3-D net, where a higher concentration of agarose means that the net is more tightly woven. If you were a giant molecule of DNA, like the human X chromosome which is 1.5×10^8 base pairs (bp), you would have a very difficult time getting through this net and wouldn't be able to travel very far down the gel [2].

In fact, if you were that big, you probably wouldn't even be able to get through the net at all, you'd get stuck in the well. On the other hand, if you were one of the little 750 base pair PCR products that we look at on gels all the time in the Treseder Lab, you would beat the X chromosome every time because it would be relatively easy to maneuver your spry little self through the net. So the moral of the story is: Smaller DNA runs FARTHER down the gel than bigger DNA [3].

In general, the rate of travel of linear DNA in an electric field varies inversely with its molecular weight (I think it is easier to think of it as "size" in base pairs, which is related to molecular weight) and depends on 4 things, the strength of the E field, or voltage applied – DNA will run "faster" in a stronger field. You may think that larger DNA runs faster than smaller DNA as more voltage is applied because larger DNA is more negatively charged simply because it is larger. But this is not necessarily the case [4], as explained above, DNA of different sizes move at different rates because of their relative ease at which they can get through the agarose matrix, not because a big DNA is more charged than as mall DNA. The only time the charge of large DNA molecules makes a difference is at very high voltages. Therefore, gels are usually run at voltages less than 125V [5].

Concentration of agarose – DNA runs "slower" in a more tightly woven net (i.e more concentrated agarose), Buffer used – the same DNA will run slightly different in different types and concentrations of buffer, Conformation of DNA – Circular and supercoiled DNA run faster than linear DNA. Thus if you have a 1000 bp linear fragment and a 1000 bp bacterial plasmid which is circular, the plasmid will migrate farther down the gel than the linear molecule even though they are the same molecular weight. In the Treseder Lab, we usually only deal with this issue when analyzing clones which contain plasmids, in the Treseder lab, there are essentially two different purposes for "doing a gel" [6,7].

2- Research material

Micro centrifuge, Eppendorf tubes 1,5-2 ml, Electro micro pipett, Biohazard Bags, Nutrient Broth, cell lysis solution ,RNase, Ethanol , isopropanol , DNA Rehydration, Gel box/tank, gel tray, gel comb(s), power supply, UV viewing table, camera (or more sophisticated gel viewing and image producing equipment), pipettes, pipette tips, Kim Wipes, gloves, goggles, 250 ml Erlenmeyer flask, graduated cylinder- Ethidium Bromide (10mg/ml), Agarose, DNA ladder/marker,10kbp, Promega (# A1120), (TAE) 50x in 1 liter, 242 gr Tris Base + 57,1 ml Acetic acid + 100 ml pH =8 (EDTA), 2 ml promo phenol BLUE.

3- Research Methodologies

Proliferate *Escherichia coli* in Nutrient broth for 24 hours 37 C° then dealings this liquid, in refill , after that we taken 1 ml from colony ,centrifuge 13000 g for 2 min,add 600 µl cell lysis solution ,incubate 80 c° for 5 min,

Add 3 μ l RNase, add 200 μ l protein precipitation solution, incubate ice for 5 min, transmit to pipe contain 600 μ l isopropanol, centrifuge 13000 g for 2 min liquidation from fluid, add 600 μ l Ithanol 70%, add 50 μ l DNA Rehydration, after that we use Electrophoresis technique.

To use Electrophoresis:

First, get an idea of what you want to view in the gel and how many lanes you will need (i.e. how many samples you have). Don't forget to leave at least one well for DNA ladder/marker. If you plan to purify the DNA bands out of this gel via gel extraction, see below. We have 2 gel apparatuses: large and small. I only use the large one when I have more than 22-24 lanes to load, when I need to skip lanes, or when I want to load more than 15-20 μ l of sample. The small gel is easier and faster to deal with, so use that one if you can, the gels have several combs - choose the ones appropriate for how many lanes you want your gel to have. You can do a double-decker gel if you need that many lanes or you can do just one row of lanes.

A single row is sometimes helpful if you need to run the gel for a long time in order to get good double row gel and a single row gel except that in a double-row gel you just have to be sure that your top samples don't run down into the bottom section. The other important thing to note about the combs is that the teeth on the different sides of the comb have different widths. With the small gel, use the fat side of the comb if you want to load up to 15-20 μ l of sample. The skinnier side creates wells that can only reliably hold up to 10 or 12 μ l. But if you want to have deeper wells to accommodate more sample volume, you can also increase the gel height by using more buffer to create the gel. Or just use the fat side of the comb, or use the large gel which creates larger wells

4- Result and discussion:

Figure out the best concentration of agarose to use based on what size DNA you want to visualize, the DNA will show up pretty much on any gel, but the resolution will be better if you follow the table below. On that note, however, don't worry about measuring exactly when making the gel, because a small difference in concentration doesn't matter that much. Only worry about exact measurements when you need to be super consistent between gels (e.g. RFLP). Also, agarose is a little expensive, so don't make a 2 or 3 % gel unless you really need to separate fragments that only differ in size by a few base pairs. Gels less than 1% are flimsy, however, so handle them carefully. The more concentrated a gel is, the stiffer and easier to handle it is. Good rules of thumb are: to look at PCR products make a 1.5 % gel and to look at genomic DNA make a 1% gel.

Table 1. Separation of DNA in agarose.

Agarose in gel (percent)	Efficient range of separation of linear DNA molecules (kilo-bases)
0,3	60- 5.0
0,6	20- 1.0
0,7	10- 0.8
0,9	7 - 0.5
1,2	6 - 0.4
1,5	4 - 0.2
2.0	3 - 0.1

Then Make the melted agarose solution. The small gel is made with 35-40 mL of buffer and the large gel is made with 80-100 mL of buffer. The percent agarose is calculated in g/mL as in the following: to make a 1% gel in the small gel tray, use 0.35 grams of agarose in 35 mL of buffer $0.35\text{g} / 35\text{ml} = .01$ or 1% Weigh out the appropriate amount of agarose using the designated agarose weigh boat that is on the first shelf above the electrophoresis bench. Put the agarose in a 250 ml erlenmeyer flask. In a graduated cylinder, measure out the appropriate volume of 0.5 X TBE Buffer for the appropriate size gel tray and pour it into the flask. Swirl a little bit. Stuff 2 crumpled kimwipes into the opening of the flask Microwave for 30 sec then swirl, and then microwave another 30 sec, or until it boils but before it tries to bubble out of the flask. Use a paper towel or flask holder to grab the flask (watch out, it's hot!)

and swirl it around well. Make sure there are no little undissolved agarose floaties if there are, microwave again. Remove the kimwipes and let cool on bench for a few minutes.

While the melted agarose is cooling, tape up the open ends of the gel tray with lab labeling tape. Leave the rubber gaskets on the ends of the tray, just make sure to press down on the tape so there is no way for the agarose to ooze out when you pour it into the tray. Also, choose the combs you want to use and put them into the slots on the tray.

If there is still visible steam coming out of the flask, let it cool a few more minutes on the bench, run it under cool tap water for a bit, or hold it in a cool water bath for a bit. If it gets too cool it will harden up, which is not a problem - just microwave it again. The melted gel must be a little cooled off before you add Ethidium Bromide (EtBr is toxic, so avoid creating toxic vapor by adding EtBr to hot liquid) and before you pour it into the tray (it can crack the tray if it's too hot). You can be pretty certain the gel is cool enough if you can hold the

flask at the bottom comfortably

Add Ethidium Bromide. EtBr is necessary to stain the DNA so we can see it under UV light. Because it works by intercalating the base pairs in the major groove of double-stranded DNA, Ethidium Bromide is also a mutagen. Therefore, PLEASE USE CAUTION WHEN USING ETHIDIUM BROMIDE. Use gloves, and proper disposal procedures. Ethidium Bromide can also be used as a stain after the gel has already been run. We do this occasionally in the Treseder lab, but not that often because it tacks on another hour to the procedure. Gels tend to look nicer and clearer if stained afterwards, but just including it inside the gel is easier and gets the job done. If you need to stain the gel afterwards, refer to the gel staining protocol.

The final concentration of EtBr in the gel should be about 0.3 - 0.5 $\mu\text{g}/\text{mL}$. The stock solution is 10 mg/mL . So for the small gel consisting of 35 mL, I add 1.1 – 1.25 μL of EtBr. Pipette up the amount of EtBr you need, with any pipettor directly out of the stock solution. Pipette it out into the E-flask, and pipette up and down and submerge the pipette tip to get all the EtBr off the tip. If you successfully got off all the EtBr go ahead and put the tip in the regular tip waste container. If not, put the tip in the EtBr waste. Swirl the melted gel slurry very well.

Cast the gel. Pour the slurry slowly into the taped gel tray. Add combs if you haven't already. Tip the tray slightly to get the slurry into every crevice and pop any bubbles with the corner of a kimwipe. Be sure to check for bubbles around the teeth of the comb(s). Let the tray stand on a level surface for about 20 minutes or until the gel has that plastic-y sheen to it. (Before the gel is hard, it is perfectly transparent, but becomes less clear as it hardens.)

While you are waiting for the gel to set, get out all the things you need to thaw, and let them thaw on the bench. (If you are going to store the gel overnight, don't do this step until you are actually going to run the gel.) Things that will need to thaw include: 6 X loading dye/buffer DNA ladder/molecular marker DNA samples you want to run.

Prepare the gel box. Carefully lift up the combs from the hardened gel. If you don't have time to run the gel today, you can wrap it up with plastic wrap and store it overnight in the refrigerator. Otherwise, go ahead and remove the tape from the sides and place the entire tray into the gel box/tank.

Place the gel tray containing the hardened gel into the box/tank so that the top of the gel (top = the end with the wells nearest to the edge) is at the negative (black) electrode-end of the tank. Pour enough 0.5 X TBE (use the same batch of buffer as used to create the gel) into the tank such that the gel is completely submerged. It only needs to be covered in about a mm of buffer. Try to place the tank in a location that will be convenient for loading and running it is not a good idea to move the entire tank once you've already loaded the wells.



Figure 1. Kit promega (#A1120)



Figure 2. bacteria in nutrient broth.

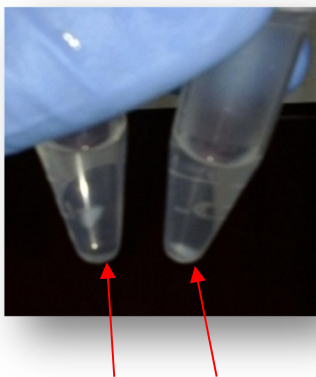


Figure 3. DNA from E.coli

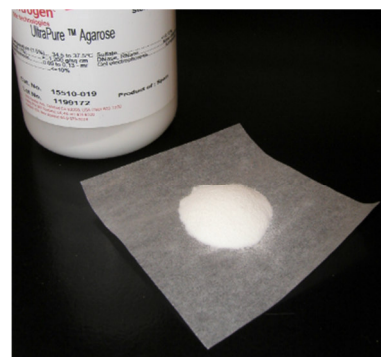


Figure 4. Making Agarose gel

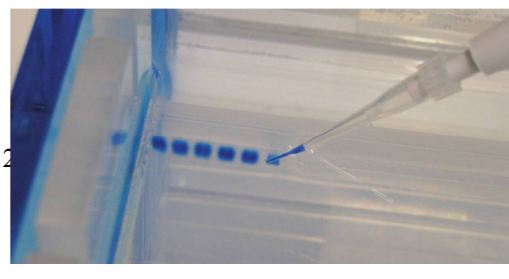


Figure 5. Agarose gel in buffer solution.

Figure 6. DNA+Bromophenol.

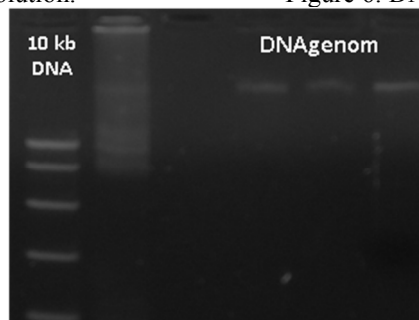


Figure 7. 10 kb + Genomic DNA.

5- Conclusion

point at results being genomic DNA and comparative with DNA Marker 10 Kb but genomic DNA was heavy didn't travel for far distance about wall .

Please use the appropriate set of pipettors based on what type of DNA you are dealing with. If you are looking at PCR products, use the “PCR products only” pipettors and barrier tips. Never use the “PCR products only” pipettors when working with non-amplified DNA.

- i. Mix the DNA with the loading dye and load the wells. This can be done either on a piece of parafilm (good for running small volumes of sample quickly) or in separate eppendorf tubes. I usually use parafilm because it is quicker. Decide what volume of DNA you need to load – this is usually dependent on the concentration of the DNA, but one often does not know what concentration the sample is in before it is run on a gel. (It is probably a good idea to measure your DNA concentration at least roughly in the spec beforehand.) Usually a couple of uLs is enough to see on a gel. for PCR products and total genomic DNA, 3-4 uL is sufficient. If you are doing a gel extraction, you will probably want to use 15 or 20 uL. Pipette out the appropriate volume of 6 X loading buffer in little beads on the parafilm. Because it is 6X concentration, use about 1 ul of dye for every 5 ul of DNA that you will be loading. But beware: the dye is mostly glycerol and does not like to pipette very accurately. Also, it is better to use too much dye than too little. If you don't have enough dye, the DNA won't sink into the well. So, considering all these things, I like to set the pipettor for about 1 ul when pipetting the dye, even if I am only going to load 3 or 4 ul of DNA.

Work quickly or only pipette a few dye aliquots at a time because the dye likes to evaporate off the parafilm. Next pipette out the volume of DNA you want to use (if you are using PCR products, please use the PCR pipettors) and pipette it up and down with a bead of dye on the parafilm to mix. If you don't want to waste any DNA, then change your pipettor setting up one ul and then suck up all the DNA/dye mixture into the tip and carefully dip the pipette into one of the wells in the gel and pipette it out slowly. You should see the colored solution literally look as though it is sinking. If you change the pipette setting in order to get up all the DNA, just be sure to stay consistent between all the samples you will be loading. Otherwise, you can leave the pipette's setting as is, and just leave a little unused DNA/dye on the parafilm. Continue this procedure with all your samples. Try to keep your loading technique consistent between samples and always have some mechanism to remember what sample each well contains. And always change your pipette tip between samples. If you have trouble getting the DNA solution into the wells, not to worry – it does take a bit of practice. Just take note if some stray DNA gets into a neighboring well – you can even load every other well if this is a concern. Try standing/sitting in different positions, or sliding the gel tray slightly within the tank – sometimes there is a glare that can make loading difficult. Also, make sure not to stick the tip down into the well too far or you may puncture the bottom of the well. And don't forget that you can use your non-pipetting hand to rest or stabilize the barrel of the pipette while pipetting. Another trick that sometimes makes loading easier, is to just pipette up and down the buffer inside each well before loading to “rinse” out the well

Don't forget to load a DNA ladder into at least one of your wells. DNA ladders, also called molecular weight markers, are used as a standard so that you know how far DNA of a known size migrates in your gel. We have 3 different ladders:

1. 100 bp ladder – good for fragments that are 100 bp – 1 or 2 kilobases (kb)
 2. 1 kb ladder – good for fragments larger than 500 bp
 3. Quant-it Ladder – quantitative ladder for determining approximate DNA concentration. This is the one with the purple top, and already contains dye. So do NOT add dye to this one. For Ladders 1 and 2 (both have a pale green top), use 5 ul of ladder + 1 ul dye. For the Quant-it ladder, do not add anything, just pipette out 10 ul of ladder and load directly.
- ii. After loading the wells, I usually add Ethidium Bromide to the positive end (red electrode, or end opposite the wells in the gel) of the gel tank. Because EtBr runs toward the negative electrode, the EtBr inside the gel will move towards the top of the gel, leaving the bottom half of the gel a different color. To correct for this, add about 1.25uL of EtBr to the buffer in the positive end of the gel tank. Swish it around with the pipette tip to mix. Remember to dispose of EtBr waste appropriately
 - iii. Place the lid on the gel tank and be sure the electrode connections are secure and tight.
 - iv. Turn on the power supply by flipping the switch. Set the voltage appropriately. Quick diagnostic gels are usually run at 90-105 V. But for clearer bands and a prettier picture, you can use as low as 60 V. The lower the voltage, the longer you will have to run the gel. Also, if you need to resolve very small DNA fragments or fragments that are close in length, the voltage should be lower.
 - v. Set your timer. For quick diagnostic gels in the small gel box, I usually run then at 95 V for 25-35 minutes. Run longer to resolve bands better. Do not run too long or at too high of voltage that you DNA runs off the gel!
 - vi. When timer is done, turn off the power supply. Take off the gel box lid and remove the gel tray. Careful here: remember to wear gloves, and do not let the gel slip out of the tray! Transfer the gel in it's tray to the UV-viewing device. Slide the gel out of the tray and directly onto the UV table Cover with the plexi-glass cover attached to the UV-table. Turn on the UV. Remember to wear goggles! Note: there are 2 UV settings. One makes the bands look brighter use this one to take picture. The lower intensity setting is useful for gel extractions, such that the DNA is not exposed to high UV while you are cutting out the gel.
 - vii. Take a polaroid picture of your gel. Wearing goggles, place the tube-like end of the camera so it is standing upright on the UV table, directly over the gel, with the handle end of the camera facing up. Being as still as possible, pull the trigger on the handle to take the picture. Pull the strip of paper out of the camera to release the exposed Polaroid. Do NOT peel off the paper yet. Let the picture set for 5 minutes before opening. When peeling the paper off the photo, try not to touch the liquidy stuff on the sides – the exposure chemicals are skin irritants.
 - viii. If you aren't doing a gel extraction, dispose of the gel in the EtBr hazardous waste. You can leave the used buffer in the gel tank for several more runs, just be sure to add to the buffer to completely submerge the gel. For best gel results, use completely fresh buffer. To clean: wash gel tray, lid, and tank with mild soap and warm water (not hot!) and rinse with DI.
 - ix. To do a gel extraction: Label appropriate number of 1.5 ml tubes for each band you will be extracting. Put UV setting at the lower intensity. Using a clean razor blade or scalpel, carefully cut out the piece of gel containing the band(s) of interest. Place in tubes. Perform Qiagen gel extraction kit. Dispose of razor or scalpel in hazardous sharps waste.

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