

Denaturing Polyacrylamide Gel Electrophoresis (dPAGE).

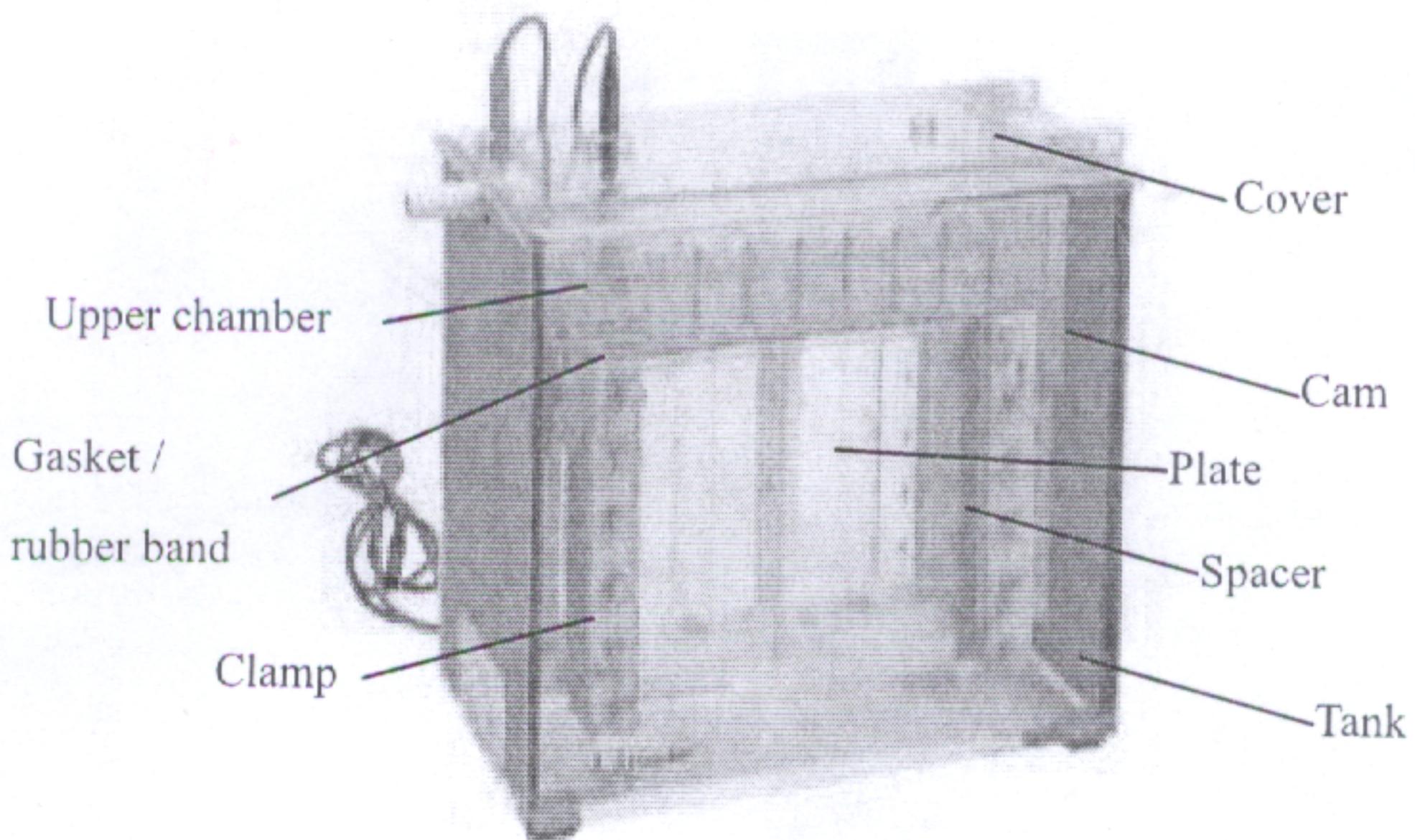


Figure 1. Gel setting schematic.

1. Check:
 - 1.1. Preheat water bath to 50 °C 1 – 2 hours ahead of planned run time. Note that the bath warms up a lot faster than it cools, so count 2 times longer to decrease temperature compared to increasing temperature. The max heat setting allowed is 50 °C.
 - 1.2. All gel tanks should be reserved appropriately ahead of time and with heating / cooling times accounted for in the MaoGroup358 calendar “Page + dPage”. You must list dPAGE, buffer tank type, temperature, name, and total time needed.
2. Prepare your samples.
 - 2.1. Determine your DNA amount. Use a rule of thumb: $N = L / 10$, where N is the maximum amount of purified DNA per gel, L is the sequence’s length in nt.
 - For example, for Y-1H purification with length of 78 nt, you can purify up to 7.8 OD per gel.
 - 2.2. Mix 50 µL of DNA solution and 150 µL denaturing loading buffer. 20 µL for each well, 10 wells.
 - If you add too much water relative to formamide, the solution will diffuse into the upper chamber so make sure you have at least 1:3 ratio, but higher amounts of formamide is fine.
 - Denaturing loading buffer consists mostly of formamide.
 - 2.3. Make the marker (DNA ladder) by mixing 2 µL of ssDNA marker with 18 µL of denaturing loading buffer (per each well).
 - Typically, 1 well with DNA ladder is enough per gel.
3. Assemble the gel setting (Figure 1).
 - 3.1. To assemble one gel, the following equipment will be required *per gel*:
 - 3.1.1. 2 plates (clean by first removing remaining grease with paper towel, then with detergent, ddH₂O, ethanol, and ddH₂O again, wiping vertically).
 - 3.1.2. 3 spacers (same length and thickness) (clean with ddH₂O, ethanol, and ddH₂O again).
 - 3.1.3. 2 clamps (clean with ddH₂O, ethanol, and ddH₂O again).
 - 3.1.4. 1 comb (clean with ddH₂O, ethanol, and ddH₂O again).
 - 3.1.5. 4 cams.
 - 3.1.6. 2 gaskets with wider slits from the thin rubber strip side (clean without much force with a dry paper towel).
 - 3.1.7. 1 gel setting holder.
 - 3.1.8. 1 upper chamber (rinse with ddH₂O).
 - 3.1.9. 500 mL of TBE.
 - 3.1.10. 10% APS (Ammonium persulfate), self-made refrigerated.
 - 3.1.11. TEMED. (Shown Right, Figure 2)
 - 3.1.12. 50 µL glass syringe.

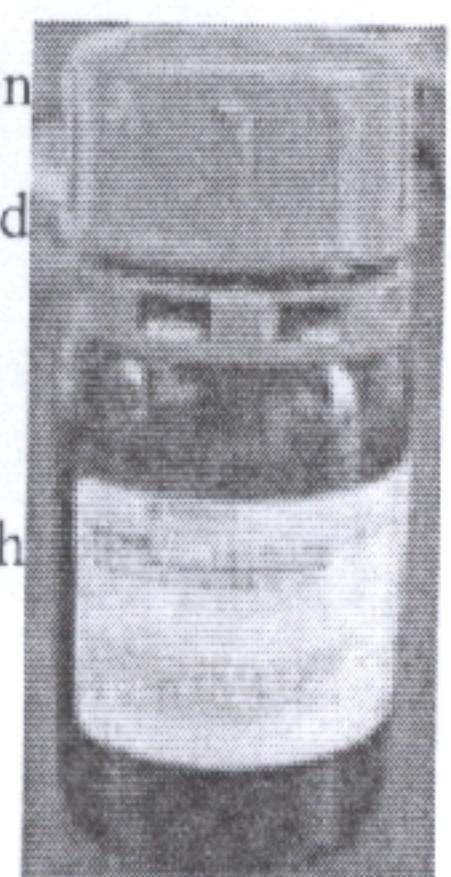


Figure 2: TEMED

- 3.2. Place the spacers on the thinner edge of the 1st plate, push to the edges (Figure 2 left), and place the 2nd plate on top in the same orientation as the first (Figure 2 right).

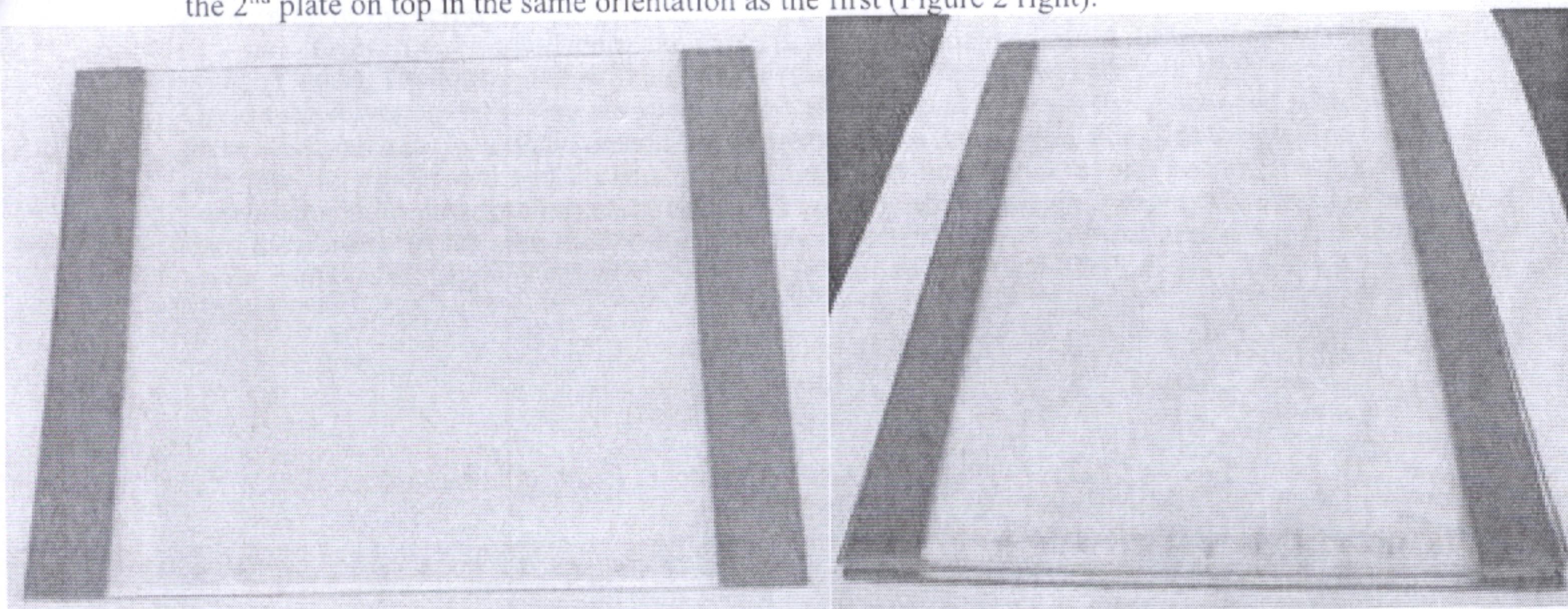


Figure 3. First Plate + Spacers (left). Both Plates + Spacers (right)

- 3.3. Add the clamps on the sides with the spacers, slightly tighten the first and the last screws. Using the third, extra, spacer push the 2 spacers between the plates towards the edges against the clamps (Figure 4 left).

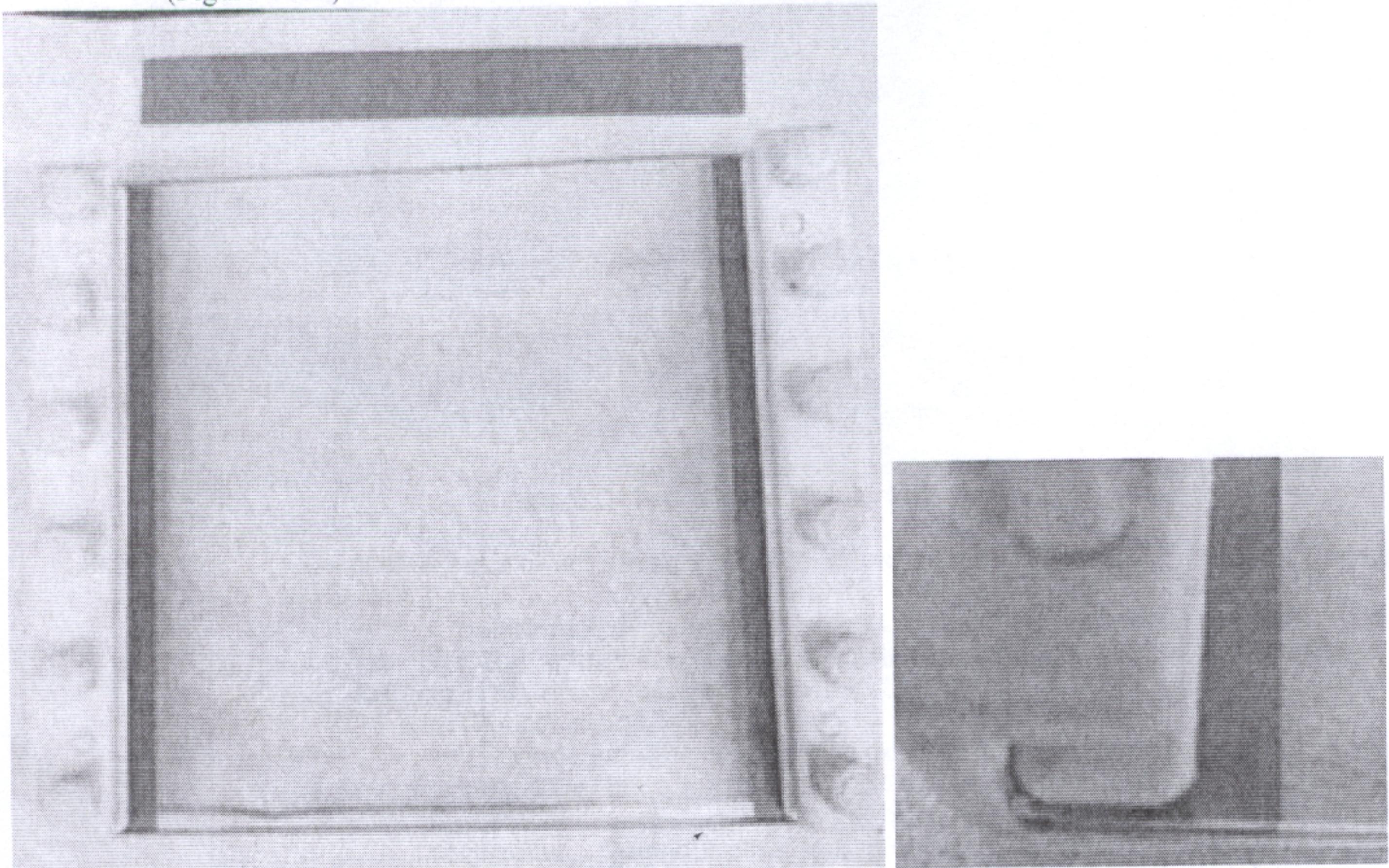


Figure 4. Assembly (left). Close up of corner spacer lineup with bottom edge (right).

- 3.4. Adjust the plates, spacers, and clamps so that the clamps and plate are aligned at the top and bottom and so that the spacers are sealed against the clamps and down to the bottom of the plates (Figure 4 right).

- If the spacers are too high, the gel solution will be able to leak under the spacers, so it is most important that the gel plate, spacers, and clamps are all even at the base of the set up.

- 3.5. Tighten all the screws once the individual components are aligned and make sure the tightness is even on all of the screws.
- Uneven screws will cause the gel to be asymmetrical and run at different speeds on either side. The looser side will be thicker (faster) and the tighter side will be thinner (slower).
- 3.6. Grease the lower corners. You are specifically trying to seal between the spacer and plate in case there is a small gap. You do not need a lot of grease, just a thumb-pad-full. Slot the greased bottom side into the holder and add 2 cams into the holes, on the outside of the holder, starting in the “down” position, then rotating up to tighten. You may need to apply a slight downward pressure onto the plate to fit the cam through all the way. Rotate the cams to tighten, making sure they’re on the same level / angle. Typically, $2/3 - 3/4$ rotation to up is enough to seal (Figure 5).

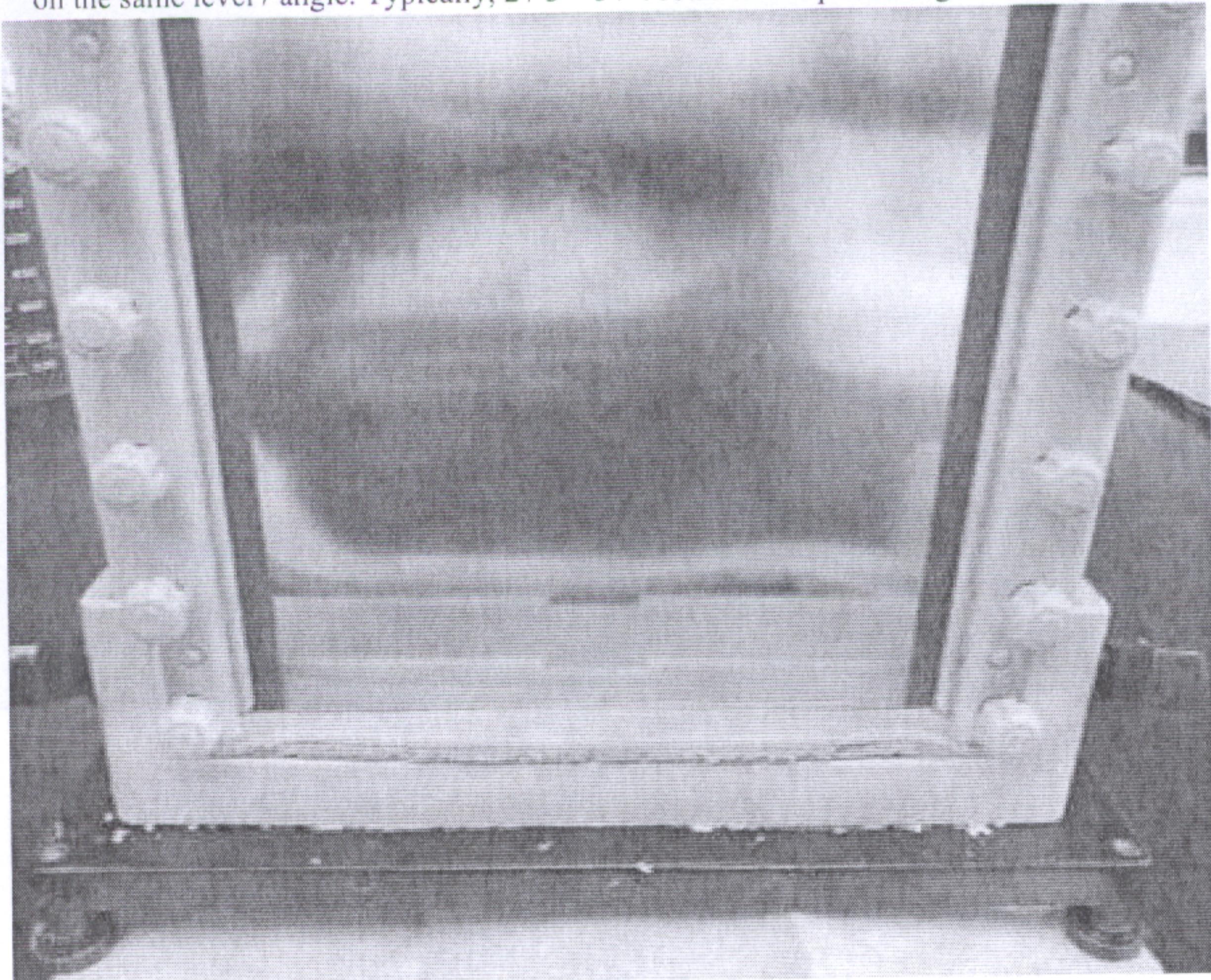


Figure 5. Gel secured in Holder with Cams.

- 3.7. Prepare the gel:
- 3.7.1. Gels need 20 mL of solution. Use 10/25 mL serological pipettes to measure volumes larger than 1 mL.
- dPAGE solutions are created from the 20 % and 0 % denaturing solutions. These are TBE and 500 g / L urea, which creates an optimal chemical environment for keeping DNA denatured. Mix 15 mL of 20 % and 5 mL 0 % solutions to achieve 15 % gel concentration.
 - Acrylamide solution is highly toxic: it causes skin and eye irritation, it is acutely toxic upon inhalation, toxic to reproduction and peripheral nervous systems, and causes cancer. To minimize the exposure of accidentally spilled acrylamide, assemble your gel and keep all beakers and bottles with acrylamide on a designated absorbing pad.
- 3.7.2. The following steps until the comb is set (3.7.5) will need to be done in a quick and efficient manner. Add 150 μ L of 10 % APS and mix.
- 3.7.3. Add 15 μ L of TEMED, mix.
- Once TEMED and 10 % APS were added, you will need to pour the gel solution into the setting quickly, otherwise it would solidify too rapidly. Do not add TEMED and 10 % APS until you are ready to pipette solution into gel (prepare 25 mL serological pipette and comb).

- 3.7.4. Add the polymerizing gel solution into the setting with your serological pipette. To prevent bubbles in the gel, pour the solution on the corner of the cast, next to the spacer, and if a bubble forms, tilt the whole apparatus to move the solution away and expose the bubble to air. You can also tap the plate near the bubble to try to pop it.
- 3.7.5. Insert the comb in the solution to form the wells. The recommended depth is 1.5 cm lower once the comb teeth are completely entered into the gel. Make sure the comb is centered and level, so your lanes are even and in the centermost part of the gel (Figure 6).

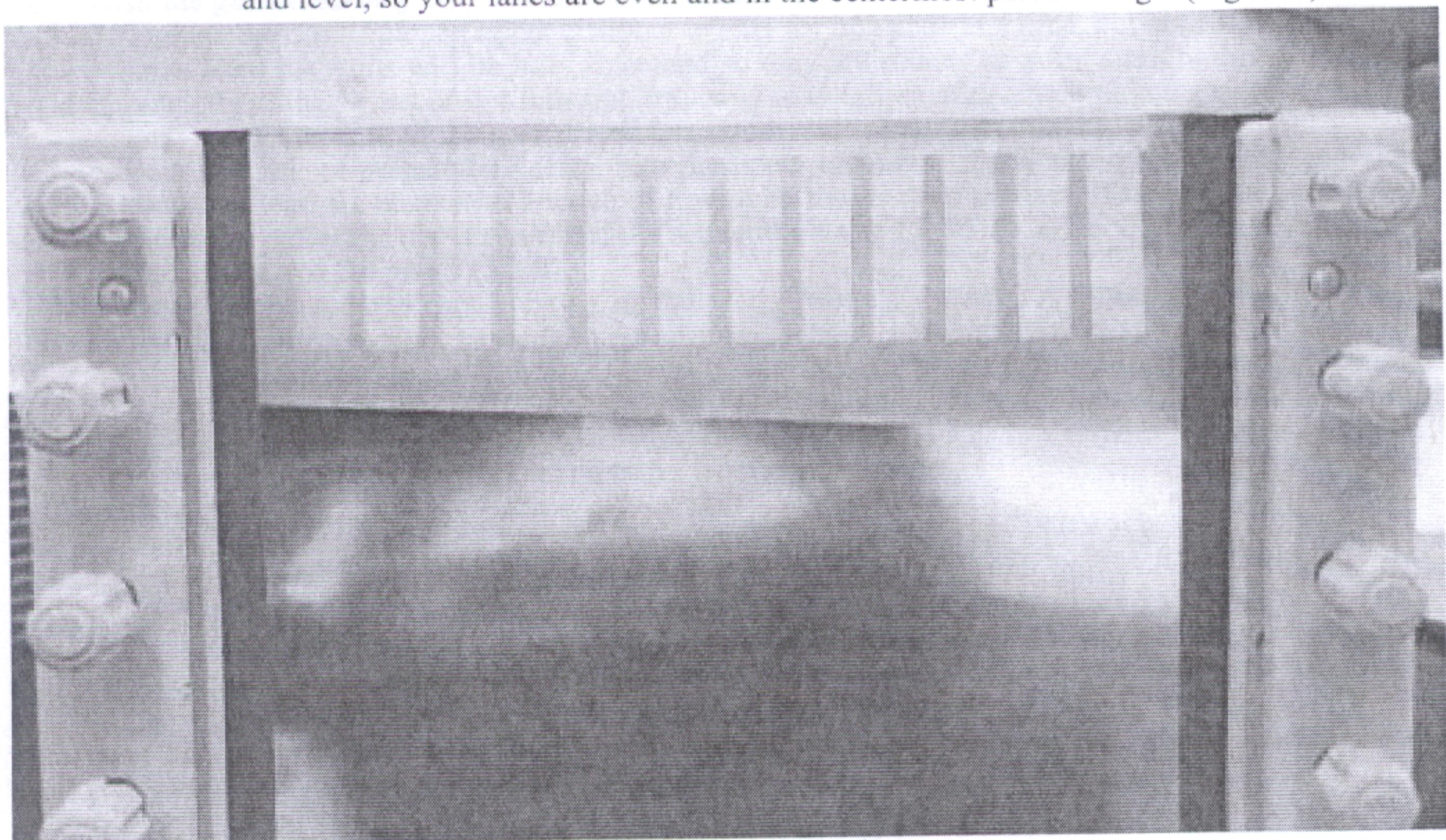


Figure 6. Example of comb placement.

- 3.7.6. Leave the gel to set until the wells' walls and bases are visible around the comb. Typically, around 15 minutes is enough but may vary so check often.
 - Over polymerizing dries out the gel and will cause the lanes to crack (get destroyed) when removing the comb. Under polymerizing will cause the lanes to flow as a liquid and either be uneven or completely gone.
- 3.7.7. Once polymerized, release the cams, and take the plate to the sink with a squeeze water bottle.
- 3.7.8. Remove the comb out by pulling directly up out of the position and, as quick as you can, immediately flush the lanes with ddH₂O to agitate the remaining solution in the lanes. Then remove the water by flicking the gel away from you into the sink. Repeat 3 times (the speed is most critical for the first time) and put the plate back into the holder once the lanes have been flushed well.
 - Be careful with the flicked water, since the first wash contains significant unpolymerized acrylamide.
- 3.7.9. Check the height of the top side glass plate relative to the clamp. If the plate is lower than the clamp, it will not be able to form a seal with the gasket in the next step. Raise the plates to approximately 1-2 mm above the clamps by carefully loosening one side of the clamp, shifting it down, and retightening before adjusting the other one in the same way. Be wary of releasing too much pressure between the plates as air bubbles may enter into the set up. Make sure both sides are even and reset the tightness of the screws on the clamps.
 - Do not over tighten if you need to shift the clamp. You risk warping the gel. Keep the same or slightly weaker pressure on the knobs.
- 3.7.10. Label your plates and wells, especially if you have multiple ones. Remember you should not add your samples in the outermost wells.
- 3.8. Grease the top corners similarly to the bottom ones. Here you may need a little more grease but again we are hoping to seal the connection between the glass plate and the clamp, as well as to the



gasket we are about to add. Leaks are most likely to occur as a result of poorly doing any of the following steps.

- 3.8.1. If you are only running one gel, put a blank plate (bulk foggy plastic piece with clamps on the side) into the empty slot of the holder and grease it lightly as well.
 - 3.9. Rinse the upper chamber with ddH₂O. Flip the upper chamber and place the two gaskets so the slits line up with the upper chamber. Flip the upper chamber back over and onto the two plates and fit the gaskets so they are even and sit correctly.
 - 3.10. Tighten the cams onto the upper chamber evenly for one plate at a time, but both sides at the same time. Here the cams will be fully tightened so they are rotated to point straight down. If there is pinching of the gasket or if it falls out, try again to retighten after adjusting the gasket. It may take multiple attempts to fit the gasket well but a poorly fitted gasket is the biggest problem.
 - 3.11. Pour 500 mL of prepared 1X TBE into the upper chamber. If any small leaks (slow individual drops) appear, fix them with a touch of grease on the point of contact. If that doesn't work, pour the buffer out of the upper chamber, and repeat the steps 3.8 – 3.10. Also, check the level between the clamps and the plates (3.7.9).
 - 3.12. Once stable, remove any bubbles in the wells using a syringe. Additionally, flush the wells manually with a syringe twice to make sure everything is homogenous.
 - It is always good to do a 20-30 minute pre-run of gels prior to running with samples. This will ensure that if there is any running issue, you will be aware of it prior to sample being loaded in the wells. Additionally, if there is any fast-moving contaminant, it will move through the gel and away from your samples.
4. Run the gel.
- 4.1. Denature the samples prior to loading by placing them at 95 °C for 5 minutes.
 - 4.2. Prior to loading, flush your syringe 3 times with the upper chamber buffer. For each new sample (different mixture), flush 3 times with upper chamber buffer to not cross-contaminate.
 - If all lanes contain the same sample, you can load without cleaning between two lanes. Though, you may want to wipe the syringe with a paper towel so the buffer from the upper chamber doesn't mix with and dilute your sample. The buffer tends to pool on the syringe tip.
 - 4.3. Slowly load the samples in the wells so that there is a clear rectangular setting of your loading solution at the bottom of the lane (Figure 7).

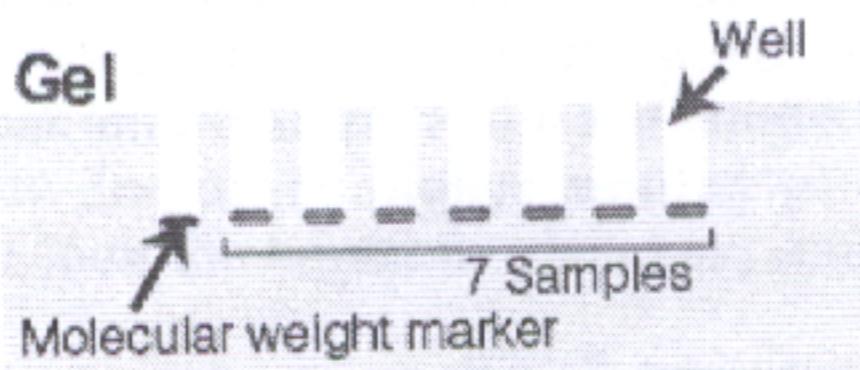


Figure 7: Example of Loading Visual

- Loading too quickly will make the 'rectangle' of solution larger (more mixed with buffer) while better loading will result in a compact solution. Aim to have all lanes compact and even. This will make gels more reliable to compare between lanes.
 - Be sure to run your gel shortly after loading your samples. Rule of thumb is the warmer the gel, the quicker you need to get your gel running. Do not leave samples in wells without current driving them for longer than 20 minutes at room temperature.
- 4.4. Once loaded, put the assembled apparatus in the buffer tank and put on the lid, making sure the two contacts are correctly attached from the cover seal with the upper chamber correctly. The black (negative) wire should be attached to the upper chamber, and the red wire (positive) – to the buffer tank. Note that the contact point of the upper chamber and the tank will be on the same side. You may have to flip the apparatus around 180° if you see your electrical contact points are on opposite sides. If the gel is 'too wide', it may be necessary to push the cams in to clear the walls of the buffer chamber.
 - 4.4.1. Check that the buffer level of the upper chamber is both above the metal wire of the upper chamber and consistent (not leaking). You may have to check a few times especially if you jostled the gel significantly while placing in the tank.
 - 4.4.2. Check that the buffer level of the tank is above that of the gel, especially above the samples of the gel.
 - If it is too low, temperature fluctuations will be significant in different parts of the gel causing it to run very asymmetrically.
 - 4.4.3. Place the cover connections into the appropriate color input (red to red, black to black) and turn on the power source to V = 500 V. Wait until it reaches the desired value before

leaving. Maximum voltage setting allowed is 500 V. If you use higher voltage settings than 500 V it will wear the power source significantly faster and cause damage to the power source.

- 4.4.4. If the power source gives an error or the measured current readout is more than 50 mA per plate, the gel setting probably has leak.
 - 1.1.1.2. Turn off the power source, take out the gel assembly.
 - 1.1.1.3. Watch the contact points between the upper chamber and gel plate for dripping or leaking.
 - 1.1.1.4. Apply grease where the upper chamber is leaking.
 - 1.1.1.5. If that doesn't help, put the gel setting in an empty, clean tank or the sink. Remove all the cams and let the buffer flow out of the upper chamber.
 - The lanes themselves should remain unperturbed so you can try to reset the rubber bands and upper chamber. Do not tilt the plates during this time.
 - 1.1.1.6. You should be able to reassemble the upper chamber with fresh buffer carefully with new buffer once you have reassembled everything.
- 4.5. Wait for the desired amount of time for the gel to run. Good practice is to check every 15 to 30 minutes and compare the markers relative travel rate against our known associated lengths by gel percent. You can assess the gel's progress by comparing the dyes' mobilities to your bands of interest (see Table 1).
 - The recommended gel stopping time is when the lowest band of interest is no lower than 2.5 cm / 1 inch from the bottom of the gel. Bands lower than this distance tend to compress and vary largely in travel speed, thus are no longer accurately represented in the gel.
 - Remember, if you do need to correct anything in your gel, you must first turn off the power before attempting to touch any part of the system.
- 4.6. When done, turn off the voltage, take out the gel assembly. Pour the buffer from the upper chamber in the nearest sink, doing your best to not allow any residual dripping on the floor or surface.
 - 4.6.1. Clean any buffer dripping from the floor / surfaces if they do appear.
- 4.7. Disassemble the upper chamber by opening cams.
- 4.8. Take the plate, once detached from upper chamber, and run tap water onto the glass for a couple of minutes until the temperature of the plates are very similar to room temperature.
 - Failure to do so often makes the gels stick to the plates when trying to remove the plate from the gel if coming from cold temperature. From hot temperature, failing to do so will make your bands will start to migrate due to the higher motility of the material at hot temperatures and cooling it with water will lock the bands in place and make the gel itself less elastic (easier to handle).
- 4.9. Once the temperature is equal to room temperature, loosen the clamps and remove them.
- 4.10. To separate the plates, use a spacer and wedge them between the two plates in free space above the wells. Do not make contact with the gel itself at this time as you may cut or tear it. Pull the spacers out by pressing them away from the gel, use your fingers once the spacers are far enough. Then lift the top plate directly up, careful not to apply pressure on any size of the gel as it is likely to squish and deform it if you do.
 - Alternatively, you can first remove the top plate by tilting a wedged spacer / tweezers and carefully lift the top plate away, followed by removing the spacers from one of the corners by pulling them directly up off the plate.

1. Once the gel is open to air and sitting on only one plate, stain your gel.

- 1.1. If ethidium bromide tank is dry:
 - 1.1.1. Clean it by adding a little bit of bleach, wait for the ethidium bromide to fully dissolve (add more bleach if needed), and pour down the sink with a lot of water.
 - 1.1.2. Rinse the tank with ddH₂O 3 times.
 - 1.1.3. For every 100 mL of ddH₂O, add 50 µL of 10 mg / mL ethidium bromide solution. (Usually use 500 mL of ddH₂O, and add 200 µL of ethidium bromide)
 - Ethidium bromide is a carcinogen and should be handled with care. If you get any on you, wash immediately with soap and water.
- 1.2. Carry your gel over to the ethidium bromide stain tray.
 - 1.2.1. Pick up the gel from the two outer bottom corners (corners opposite from the lanes), peel it off the plate, and place directly into the tank.

- You should not touch the gel anywhere you intend to include in your image as your gel stain will be affected by your gloves and will be marked. Stick to corners or the extreme left and right margins.
- 1.3. Stain in the covered chamber for 15 mins. Shake lightly every 5 minutes so that impurities in the stain tray do not settle onto your gel. Note that the stain tank needs to be periodically cleaned.
 2. Now, cut out your gel bands from the gel.
 - 2.1. Clean the UV illuminator with ethanol and water, wipe it. Place a large piece of plastic wrap film on top, stretched taught. Take the gel out of the staining tank, and place it on the transparent film flat, remove any bubbles.
 - 2.2. Get UV protection (the UV shield, UV glasses / face shield, lab coat for arms), and prepare a razor.
 - UV radiation is dangerous. Cover skin with sleeves and high-cut clothing. Additionally, use the UV shield and glasses to protect any remaining exposed skin.
 - 2.3. Turn off the lights and turn on the UV illuminator.
 - If your bands do not clearly show up as orange bands, you may need to stain for longer time. If the problem persists, adjust the UV light with the toggle.
 - ssDNA markers in the edge wells may help to identify the correct band.
 - 2.4. Cut out the band of interest as tightly as possible to maintain the purity of the sample. Also, cut the band quickly, since UV light degrades DNA over time. As soon as you have cut out the band, turn off UV to minimize DNA damage.
 - Be careful to limit ethidium bromide contamination. DO NOT touch the light switch with your contaminated glove. Throw away everything that has touched the ethidium bromide in the solid waste in the same fume hood.
 - 2.5. Turn the lights back on. Isolate and remove the bands of interest, discard the rest of the gel so you don't confuse it in regular light.
 - This can be done by wrapping the transparent film around everything and placing it in the ethidium bromide waste in one go.
 - 2.6. Clean the plates, clamps, rubber bands, spacers and return them to their regular stations.
 7. Now, you need to elute your DNA from the gel.
 - 1.1. Prepare per each gel:
 - 1.1.1. Cut-out gel band.
 - 1.1.2. A dialysis bag ~10 cm / 4 inches (half the length of the gel horizontally per gel).
 - 1.1.3. 2 dialysis clamps (with magnetic weights attached).
 - Using clamps without the weights still work but will float due to buoyancy and need to be weighed down with another item.]
 - 1.1.4. 2 empty 15 mL tubes.
 - 1.1.5. ~2 mL of 0.1x TBE.
 - 1.1.6. A separate tube full of 0.1x TBE to the top for soaking
 - 1.1.7. A 6 mL luer-lock tip monoinject syringe.
 - 1.1.8. A 0.2 µm cellulose syringe filter.
 - 1.1.9. 0.1x TBE electrophoresis chamber (each chamber needs approximately 500mL to fill completely).
 - 1.2. Prepare the dialysis bag:
 - 1.2.1. Soak one end of the dialysis bag in the 0.1x TBE tube for 30 seconds to [1 minute until soft].
 - 1.2.2. Attach one dialysis clamp to the soaked end to seal and submerge the bag into the tube with the clamp resting on the open tube neck to soak until you are ready to use it (at least for 5 minutes).
 - 1.3. Stack one of the strips onto the other to create a pseudo rectangular prism "brick". This rectangle of stacked bands will be what you place into the dialysis bag.
 - Note that you should not let the cut bands sit idly for more than 5 minutes; so, prepare to perform the next steps efficiently to not waste time before removing the gel from the EtBr stain bath.
 - 1.4. Retrieve the bag from soaking and lightly open the non-clamped end (previously submerged the furthest in. It should open with minimal force and should be soaked for a longer time if it does not.
 - 1.5. Pipette 2 mL of 0.1x TBE from your other tube (not the "soaking tube") to create volume in the bag into which you can submerge the rectangle bands.
 - 1.6. Carefully place the stacked, cut-out gel bands into the bag. Here you will need to be careful not to break the bands with too much pressure.

- Typically, “pumping” up and down the solution with one hand while you lead the bands with your other hand can help to draw the bands further in until they completely enter solution.
 - This step is most challenging until the bands no longer peek above the dialysis bag. This will require the most patience and practice as you must juggle multiple components at once.
- 1.7. Once the bands have been coaxed to reach the bottom of the bag and to sit on the bottom clamp, carefully (with barely any pressure) squeeze the bag into a thinner position with lower volume. Here your goal is to remove stray air bubbles and to remove excess liquid if there is enough.
- The goal is to have only between 1-1.5 mL of 0.1x TBE once sealed.
 - Removal of too much will be clear by the fact that air bubbles will refuse to exit. You can always add more 0.1x TBE if you accidentally removed too much.
 - Too much liquid will cause problems during butanol extraction due to higher volume of starting solution. It will also dilute your ethidium bromide and make it harder to track DNA within the dialysis bag.
- 1.8. While maintaining the ‘squeezed’ position of the bag, close the bag with the second clamp allowing for excess buffer to escape, creating a sealed dialysis bag with no air bubbles.
- Undo the clip and try again to seal the bag if you see bubbles.
 - If it is too difficult to remove the air bubbles on second attempt, you may need to add back some buffer again.
- 1.9. Once a good seal has been made, double check that your “brick” formation of the two pieces is still maintained. You can carefully shift the two gel pieces to again arrange them onto each other if they have moved significantly. Position the gel bands towards the negative (black contact) (left) picture side of the dialysis bag if you were to lay it down with the clips oriented North / South. This will allow for the current to pull the gel-locked DNA into the right-side free solution, and out of the bands in a consistent manner.
- If the gel bands are spread out or not stacked properly, one band may successfully elute its DNA while the other keeps it locked, so it is important to have them positioned in a stacked and with equal thickness and position.
- 1.10. Place the dialysis bag with the gel band in the prepared 0.1x TBE dialysis chamber. The volume of the chamber should be high enough to completely submerge the dialysis bag.
- The gel stack side of the dialysis bag should be closer to the cathode (typically oriented to the left when looking at the dialysis chamber) as the current will pull the DNA material into the solution on the right (the anode).
- 1.11. Turn on the voltage, V = 100 V.
- Voltage lower than 75 V will waste a lot of your time and higher than 100 V may damage your DNA due to heating.
- 1.12. Wait for 60 minutes to allow as much DNA to exit the gel as possible.
- 1.13. Turn off the voltage, remove cover and check if DNA left the gel by illuminating it with a ‘hand-held’ UV lamp. Use eye protection before turning on the UV lamp and make sure there is no one else who can get accidental exposure.
- If you see DNA heavily coated the side of the dialysis bag (the one opposite to the gel band), reverse the anode and cathode connections and run the voltage for 2 minutes.
 - Again, lower DNA concentrations will be difficult to see here so you may want to continue, nonetheless. The major concern is if you see significant amounts of the DNA in the gel still.
- 1.14. When done, carefully lift dialysis bag vertically again and open from the “top” clamp.
- 1.15. Pipette out all the liquid into 15 mL centrifuge tube.
- 1.16. Rinse the dialysis bag with 300 μ L of ddH₂O three times (total volume of 900 μ L): once inside each side of the dialysis bag and once over the gel. Pipette the resulting accumulated solution from the bottom of the bag into your centrifuge tube. Discard the dialysis bag into the ethidium bromide waste container.
- If you suspect your DNA is sticking to the dialysis bag, you can use the UV lamp to look for traces of concentrated ethidium bromide on the bag. Though this will not help you for the current round, you can adjust your next run by running reverse longer. Similarly, if you see evidence of Ethidium bromide in your bands, you can choose to run longer than you did for this attempt.
- 1.17. Filter the DNA solution:
- 1.17.1. Centrifuge the DNA solution in the 15 mL tube. Use a counterbalance filled with equal amounts of water if needed to balance the centrifuge.
 - 1.17.2. Attach the syringe filter to the syringe. Take out the plunger and pipette (or pour) the solution into the syringe. Resecure the plunger back and push the solution through the filter into a new 15 mL tube.

- 1.17.3. Rinse the old tube with 0.5 to 0.8 mL of ddH₂O, centrifuge, and repeat step 6.18.2.
 - 1.17.4. Discard the tubes (except for the one with solution) and the syringe. This sample is now ready for butanol extraction.
2. Perform butanol extraction:
- 2.1. Prepare:
 - 2.1.1. Eluted samples.
 - 2.1.2. Butanol.
 - 2.1.3. Butanol waste container.
 - Make sure to use an appropriate and marked butanol waste container (glass).
 - 2.2. Repeat the following sequence until the sample's volume is ≤ 0.5 mL:
 - 2.2.1. Depending on the sample's starting volume, add butanol to the following mark. See table 2 below as a reference.
 - 2.2.2. Shake it well, holding the cap, then centrifuge for 20-30 seconds at the maximum setting. The resulting solution is a butanol / water fraction with butanol on top.
 - 2.2.3. Without disturbing the bottom layer, carefully remove from the centrifuge and pipette off as much of the top layer as possible into the butanol waste container. Keep the bottom layer with as little butanol as possible.
 - 1.1.1.1. If you do disturb the solution, re-centrifuge it to reform the fractions if needed.
 - 1.1.1.2. If the bottom layer is absent (or if your solution is homogenously cloudy), it means that too much butanol was added. Add 50-200 μ L of ddH₂O and centrifuge again to form the fraction.
 - 2.3. When the lower fraction's final volume is ≤ 0.5 mL and butanol is removed, centrifuge the tube and pipette the bottom layer into a 1.5 mL tube. Leave as much of the butanol as possible as it will lower your yield.
3. Perform ethanol precipitation.
- 3.1. Ethanol precipitation equipment (centrifuge and vacuum centrifuge) should be reserved appropriately ahead of time and with times accounted for in the MaoGroup358 calendar "EtOH Precipitation Equip". You must list your name and total time needed to prevent possible time conflicts.
 - 3.2. Prepare for each sample:
 - 3.2.1. The sample from butanol extraction.
 - 3.2.2. 83.3 μ L of 3 M NaOAc.
 - 3.2.3. 20 μ L of 0.5 M Mg(OAc)₂.
 - 3.2.4. 1 mL of 100 % ethanol.
 - 3.2.5. 3 mL of 80 % ethanol.
 - 3.2.6. 1-3 lb of dry ice (Fisher Store).
 - 3.3. In the 1.5 mL tube mix ≤ 0.5 mL of sample, 83.3 μ L of 3 M NaOAc, and 20 μ L of 0.5 M Mg(OAc)₂. Add ddH₂O to 0.5 mL volume. Add 1 mL of 100 % ethanol. Lower amounts may accidentally cause salt imbalances based on the calculated volumes.
 - 3.4. Put the sample and 3 mL of 80 % ethanol (in a 15 mL tube) in crushed dry ice for minimum 1 hour. Dry ice can be crushed using the mallet in the cold room and should be stored in the cold room.
 - 3.5. Centrifuge the sample at $\omega = 21130$ rcf (the maximum speed setting) for 30 minutes.
 - As soon as the centrifuge is close slowing down you need to do all the following steps quickly as the longer you wait the worse your yield gets.
 - 3.6. Remove your ethanol tube from dry ice and open it.
 - 3.7. Carefully take the sample from the centrifuge, invert the tube above the sink and inspect the bottom of the tube while the liquid is against the cap. Make sure the DNA pellet is visible and is still stuck to the bottom of the tube. It may be clear or white or tan/brown. If the pellet is visible and secured proceed to the next step.
 - 3.8. If there is no pellet, you may proceed if you are working with small quantities of DNA. Alternatively, you may decide to re-pellet it by going back to step 8.3.
 - If you see no pellet, you may also wish to pour the solution into a clean tube. Maybe you realize your forgot something, hence no pellet.
 - 3.9. Open the tube while inverted so the ethanol goes into the sink and leave the pellet with as little warmed ethanol as possible.
 - 3.10. Add 1 mL of the cold 80 % ethanol solution to the tube, adding the solution on the same side as the pellet. Restart the centrifuge the sample at $\omega = 21130$ rcf for 10 minutes, pellet facing inwards the centrifuge. Don't forget to return the 80 % ethanol back into dry ice during the centrifugation.

- We are washing down any remaining DNA into the pellet however, we must make sure the pellet and tube stays as cold as possible during these steps as higher temperature leads to more solubility and less pelleting.
- 3.11. Once the centrifuge is again ending, repeat the steps 8.5 – 8.8, this time washing down the other half of the tube (the side away from the pellet formation).
- 3.12. Once done with the second wash and 10-minute centrifugation, pour the liquid in the sink for a final time, leaving as little liquid as possible, without losing the pellet. Quickly make your way to the vacufuge (vacuum centrifugation).
- 3.13. Dry the samples in the vacufuge for 20 minutes at 45 °C.
- 3.14. Rehydrate the pelleted sample by adding ddH₂O. The water volume is determined by the amount of DNA purified in OD. Typical volumes are 50-200 μL. 50 μL should always be used if the pellet was not visible and can be adjusted based on UV-vis. quantification if necessary.
- 3.15. Invert, flick with your finger, vortex, and centrifuge the tube multiple times and leave for 10 minutes to make sure your DNA has fully dissolved from the pellet before storing or quantifying.
4. Congratulations! Your sample is ready for UV characterization.

| Gel % | Bromophenol blue (BP) | Xylene cyanole (XC) |
|-------|--------------------------|------------------------|
| 2.5 | >100 | |
| 3 | 80 | |
| 4 | 55 | |
| 5 | 35 | 130 |
| 6 | 26 | 106 |
| 8 | 19 | 75 |
| 10 | 12 | 55 |
| 12 | | 50 |
| 15 | | 36-42 |
| 17 | | |
| 20 | 8 | 28 |

Table 1. Mobilities of marker dyes in TBE dPAGE.

| V _{sample} , mL | V _{butanol} , mL |
|--------------------------|---------------------------|
| 0.8 | 3 |
| 1.2 | 5 |
| 1.5 | 6 |
| 2 | 8 |
| 2.4 | 10 |
| >3 | 15 |

Table 2. Butanol extraction reference table.

Note that V_{butanol} refers to the volume of the total solution after butanol addition. So, for example, for 2mL of sample, add until the 8mL mark on the 15 mL tube with butanol.