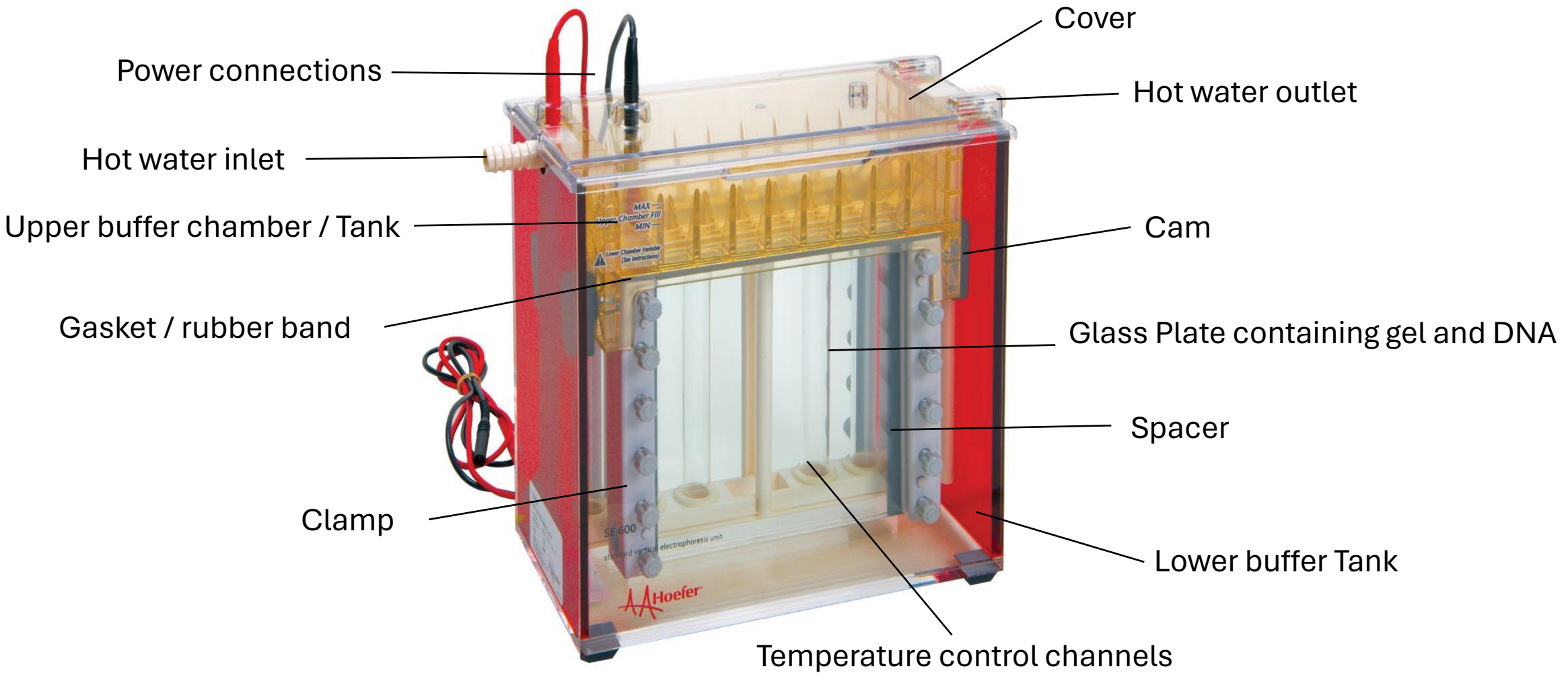
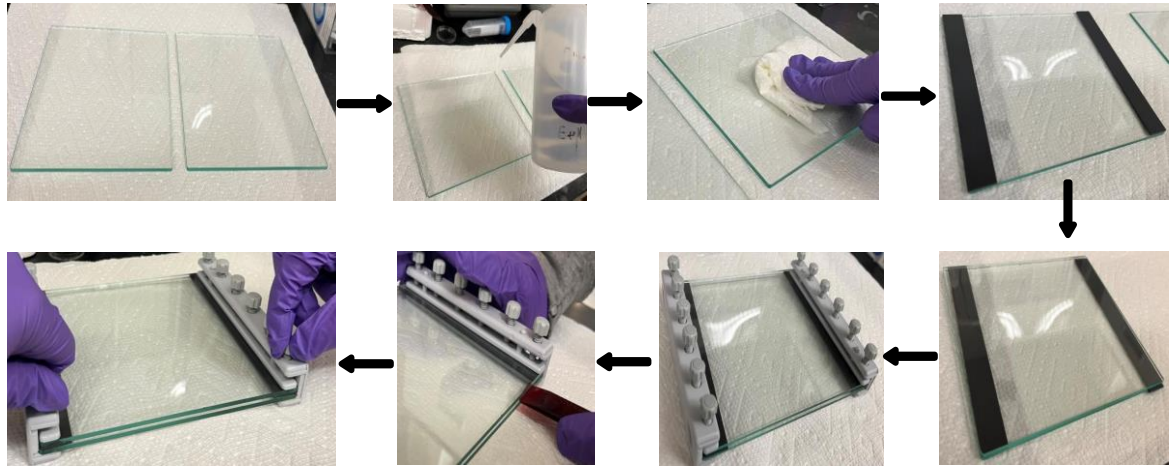
Denaturing Polyacrylamide Gel Electrophoresis (DPAGE)



SE600 Standard Dual Cooled Vertical Protein Electrophoresis Unit

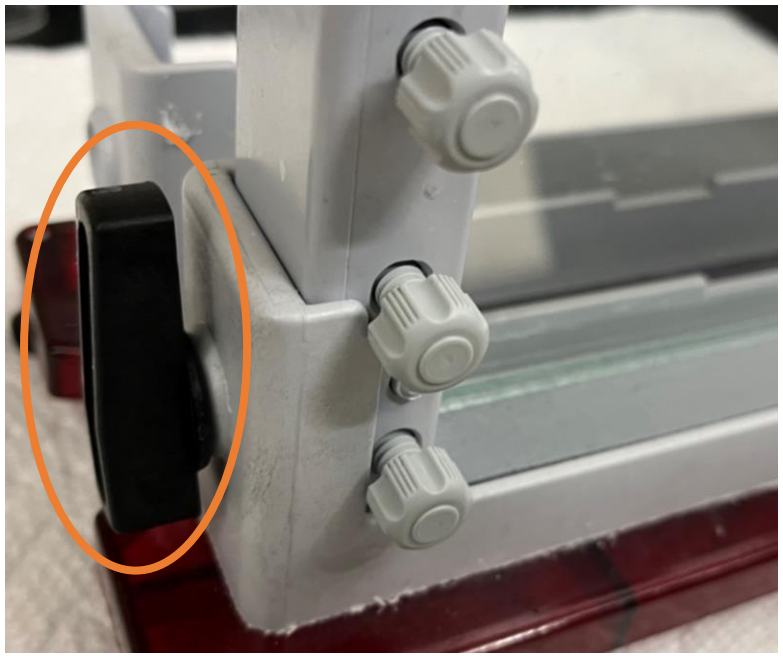
STEP 1: Check the tank condition and Glass plates assembling

- Check the buffer tank, if many particle present then discard buffer in the sink, rinse with water, wipe with paper towels, rinse 3 times with DI water, fill with ~ 5 liters of 0.5X or 1X TBE fresh buffer. And upper chamber rinse with DI water and load 500 ml of 0.5X or 1X TBE fresh buffer.
- Preheat water bath to 50 °C, ~1 hour ahead of planned run time is recemented but not necessary. Note: Max. temp. 50 °C. Preheat reduce the electrolysis time after adding the sample solutions in to the wells.
- Take two glass plates and clean them using paper towel and ethanol.
- Do the same cleaning process with water at least twice to make the surfaces hydrophobic
- Use the two spaces at the end and overlay the other glass slide to a make a sandwich. Clamp the two glass slides using the clampers and tighten them.



STEP 2: Glass slides / Gel setting holder

- Use the holder base to place the glass sandwich on the slot with rubber pad and force it down using the cams.
- You need to see the rubber get slightly into the space between glass at the bottom to ensure that the gel doesn’t flow out when introduced in it.
- If the spacer is not properly aligned or cleaned it might cause the leak at the bottom corner of the glass sandwich holder. Make sure everything is cleaned properly.

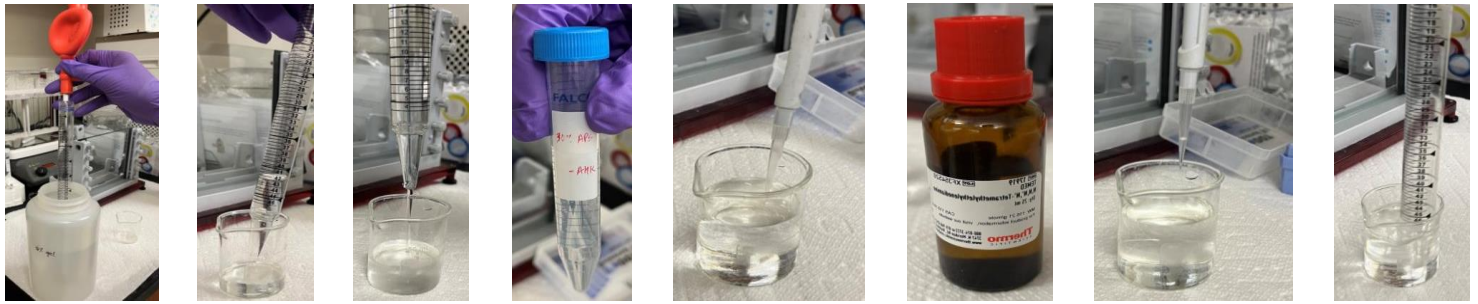


STEP 3: Gel Preparation

- Decide the percentage of gel you want to run based on the length of the DNA strands, the DNA purification guide table provided in next slide for reference. For e.g., a 46 nt strand required 10 % gel. Use the stock 20% and 0% solutions to make the desired concentration of gel. *E.g.*, 20 mL of 20% and 20 mL of 0% to make a 40 mL of 10% gel (if the solution terns pale/Light yellow color then need to prepare fresh gel solution as the protocol as mentioned in the table below.
- Note: 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.
- Add 300 µL APS solution (Not more than 6 months), 30 µL TMEDA and give it a quick mix.

Gel in good condition (transparent)

Decomposed Gel (light yellow color)



Gel stock solution	20% of 500 mL gel	0% of 500 mL gel
40% of acrylamide/bis solution 19:1	250 mL	0 mL
Urea	250 mg	250 mg
10X TBE	50 mL	50 mL
DI water	To make 500 mL	To make 500 mL

Migration of marker dyes in polyacrylamide denaturing gels (in TBE)

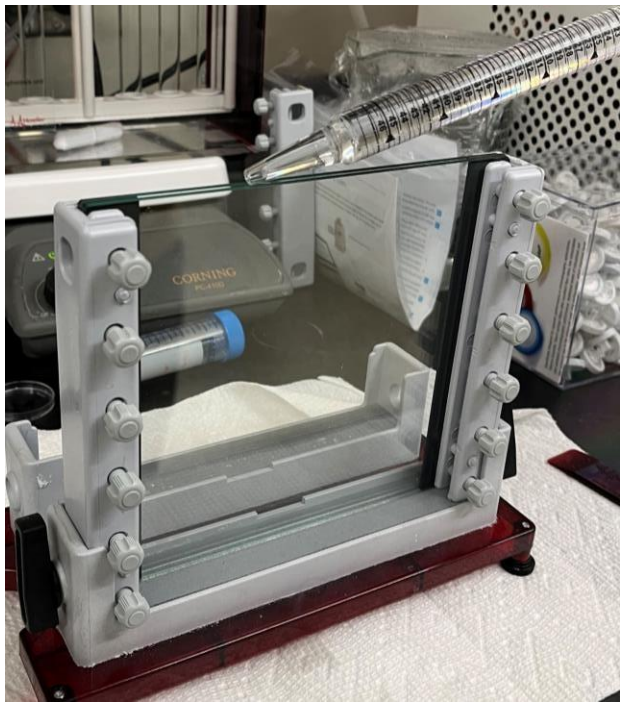
Gel %	Bromophenol Blue (BP)	Xylene Cyanole (XC)
4.0		160
5.0	35	130
6.0	26	106
8.0	19	75
10.0	12	55
12.0		50
15.0		36-42
17.0		
20.0	8	28

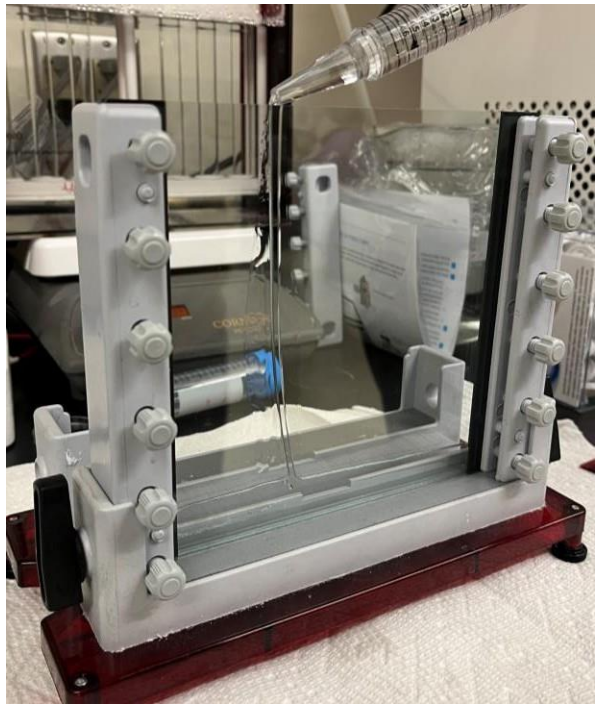
DNA Purification Guide

DNA Length (nt)	Gel %	Bromophenol Blue (BP)	Xylene Cyanole (XC)
10	20	3 cm to bottom	
15	20	2 cm to bottom	
20	20		
25	20		4 cm to bottom
30	20		2 cm to bottom
35	20		
40	20		
45	20		
50	20		

STEP 4: Gel Preparation

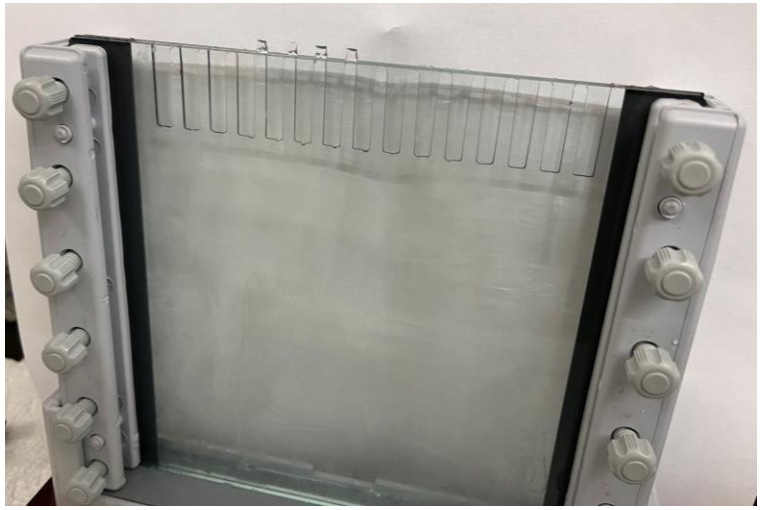
- Make sure to immediately transfer the gel into the glass assembly using 50 mL pipette inclined at an angle.
- Make sure you shouldn’t get any bubbles in the gel. If at all, bubbles appeared then shake the total gel assembly without disturbing the alignments.
- Insert the comb immediately to make the wells to load the sample. Clean the gel on glass plates and bottom of glass holder assembly spilled while pouring the gel. If not, these gel dissociate in the buffer tank and you need to change with fresh buffer every time.





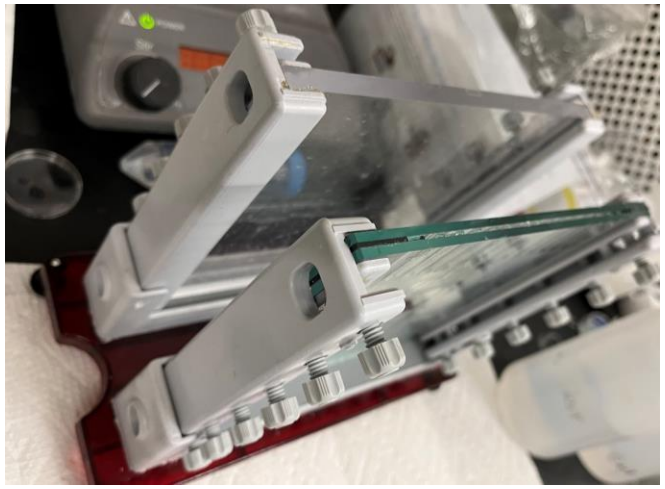
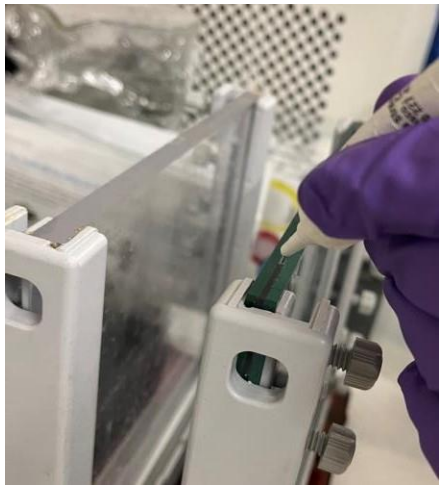
STEP 5: Gel polymerization and wells cleaning

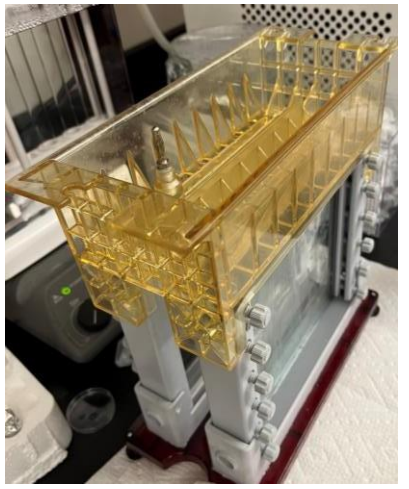
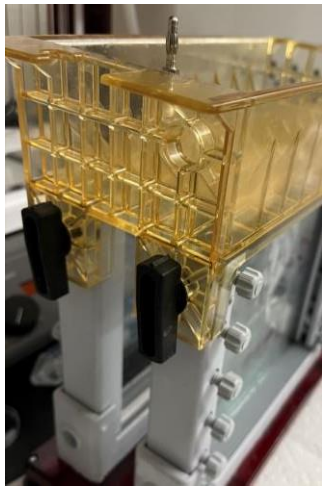
- In about 10-15 min the gel gets polymerized, and one can notice it becomes solid at the well boundaries.
- Remove the comb and immediately flow water (0.5X or 1X TBE recommended instead of DI water) by using transfer pipette into wells so that they do not collapse. Using force action, drain all the buffer and pour fresh buffer again. Repeat the step for 2-3 times.
- You could physically notice if any unsolidified solution and buffer in the well. The gel sandwich assembly is ready and can be used to assemble for buffer tank.



STEP 6: Buffer tank assembly

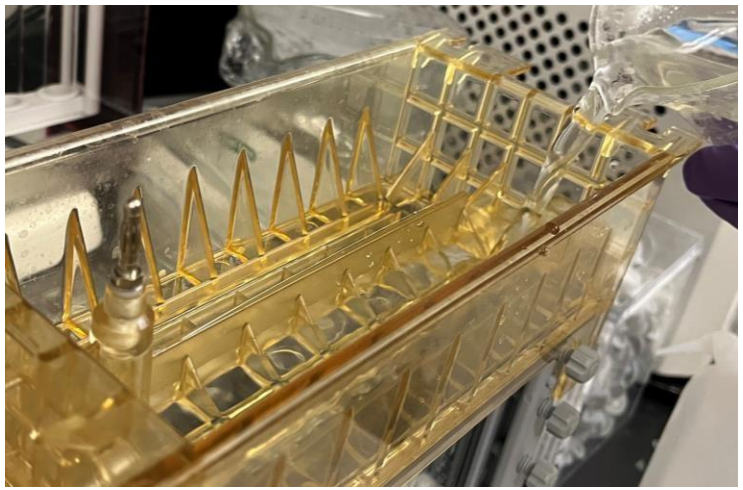
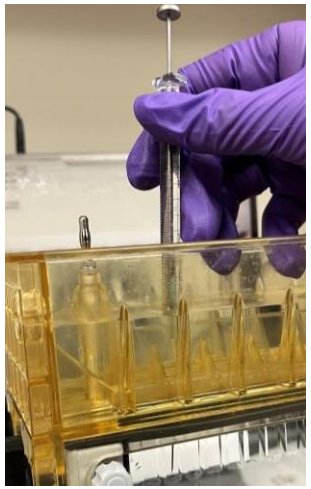
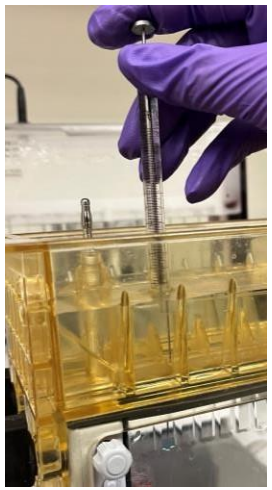
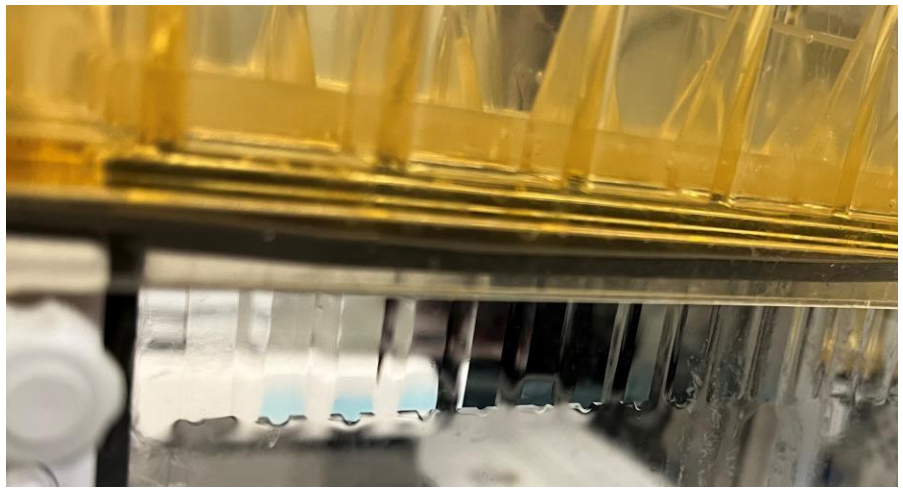
- Use the gel sandwich assembly and a dummy plastic assembly to connect to the buffer tank.
- Make sure to apply vacuum grease at the ends to avoid any leakage of buffer.
- Place the gaskets onto the slots of buffer tank and make sure they are well aligned.
- Assemble the buffer tank onto the glass sandwich.
- Use the cams to tighten the contact between them and make sure the cams point downward direction.

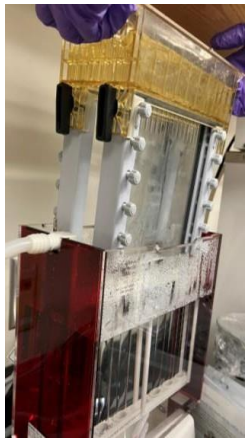
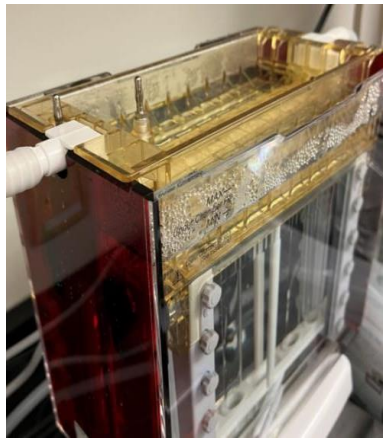
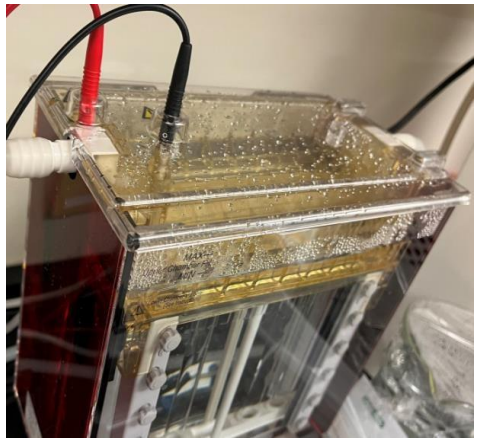
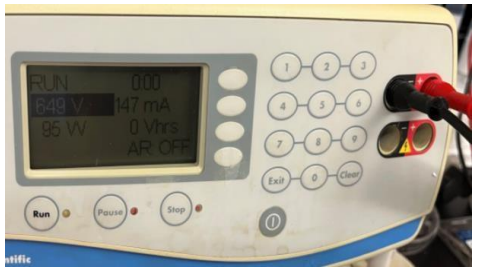
STEP 7: Buffer and sample loading

- Pour 0.5X or 1X TBE buffer into buffer tank till the electrode submerges.
- It is always good to do a 20-30 minute pre-run of gel prior to running with samples. This will ensure that if there is any running issue, you will aware prior to sample being in the wells and if there is any fast-moving contaminant, that it will move through the gel and await from your samples.
- Run the gel Initially 50 °C
- Use syringe and insert the prepared sample (10 µL to 50 µL max.) into each of wells excluding first and last for dye markers to go during electrophoresis.
- You should not use dye in all wells, only first and last wells are filled with dye formamide, and rest of the wells are filled with DNA solution and formamide. The below images are shown for easier understanding.

STEP 8: Power supply

- Make sure the buffer level meets the minimum requirement.
- 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. If you forget to load the buffer on the top chamber or cap with power connections, you will see the error message in the power source. 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 voltmeter. If the power source gives an error or the measured current readout is more than 50 mA, the gel setting probably has leakage. Run the gel without samples for 20-30 minutes, then the current readout comes down to ~35 mA, pause the power supply and add the samples (DNA) then on the power.
- Monitor the rate of movement of the dye markers and estimate the time and position of desired strand relative to the marker. Do not let the gel run overtime and loose the sample. Typical time for 46 nt strand is ~30 min in 10% gel.

STEP 9: Disassembly of gel setup

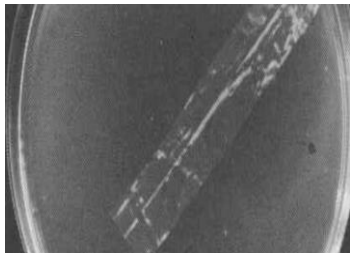
- Stop the hot water circulation and stirring bar. Remove the Buffer tank and glass plates assembly.
- Pour back the buffer in the stock solution. Disassemble the buffer tank, glass sandwich assembly. Remove the spaces from the glass sandwich and carefully remove the upper glass plate.
- Now the gel is ready to be transferred.

STEP 10: Gel stain

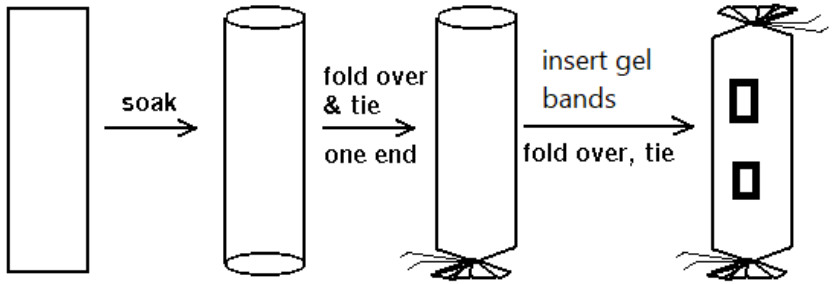
- Carefully remove the gel from glass plates and soak it in concentrated Et-Br bath for 25 - 30 min. Et-Br stock concentration (10mg/ml), take 50 µl for each 100 mL of DI water. The stain in the covered chamber and shake lightly every 5 to 10 min. so that impurities in the stain tray do not settle onto your gel.
- Ethidium bromide is a carcinogen and should be handled with care. If you get any on you, wash immediately.
- Do not remove the gel immediately because all strands may not be visualized, and this leads to separation of wrong bands.
- Under the UV see for the desired bands. (Check the sample gel positions to get an idea in the last slide). Minimize the UV exposer time to avoid the DNA damage.
- Cut the gel into 2 rectangular strips and use for extraction in further steps.

STEP 11: Dialysis

- To make the electrodialysis setup, pour 0.5X or 1X TBE buffer into the chamber to the required level.
- Cut 5 cm of 3.5 kDa dialysis tube and soak in DI water for 5-10 minutes so that it becomes softer and will be easy to handle it. Fold over and tie in one end.
- Introduce 1mL of 0.5X or 1X TBE buffer into bag initially. Try to insert the 2 gel cut pieces into bag. Use extra TBE buffer whenever needed to make a good volume of dialysis bag.
- Tie the other end once the gel is transferred in and make sure there are no air bubbles. You may also use clips. I prepared to use both, First tie then clips, so that no chances of leakage.
- Run the dialysis at 100 V for 1 hr. for all the DNA to be transferred from gel into the TBE solution in the bag. More than 1 hr. dialysis heat up the buffer which will damage the DNA. You could also cross-check the DNA in the gel bands (if at all) by exposing to UV light.



Dialysis tube soak in DI water in Petri dish



Tie both ends of dialysis bag

Clips

Dialysis set-up

STEP 12: DNA Purification

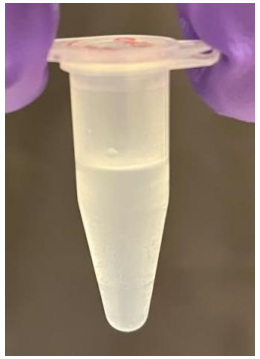
- Transfer the solution from dialysis bag into 15 mL tube.
- Load syringe with 0.45 µm porous filter. Transfer this solution into syringe and pass the solution through the filter. Add an additional 1mL of TBE buffer in the syringe and let it pass and wash any DNA that sticks in wall or filter.
- (It will work without filtering since the gel bands are not crushed)
- Using the Butanol concentration table fill up the tube with butanol to desired level. Shake it vigorously to see a mixture and centrifuge for 5 min at max speed.
- Remove the supernatant butanol and redisperse with fresh butanol and repeat the above step.
- Do not perform this purification more than 2-3 times. You might loose whole sample sometimes.

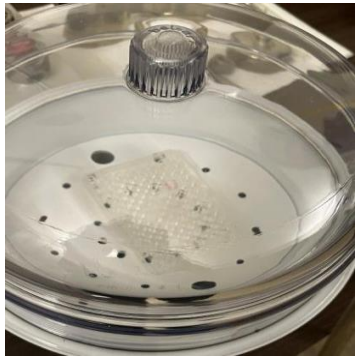
DNA Concentration via Butanol Purification Guide

Buffer containing DNA	Butanol addition to make final volume
2.4 mL	10 mL
2 mL	8 mL
1.5 mL	6 mL
1.2 mL	5 mL
800 µL	3 mL

STEP 13: DNA purification

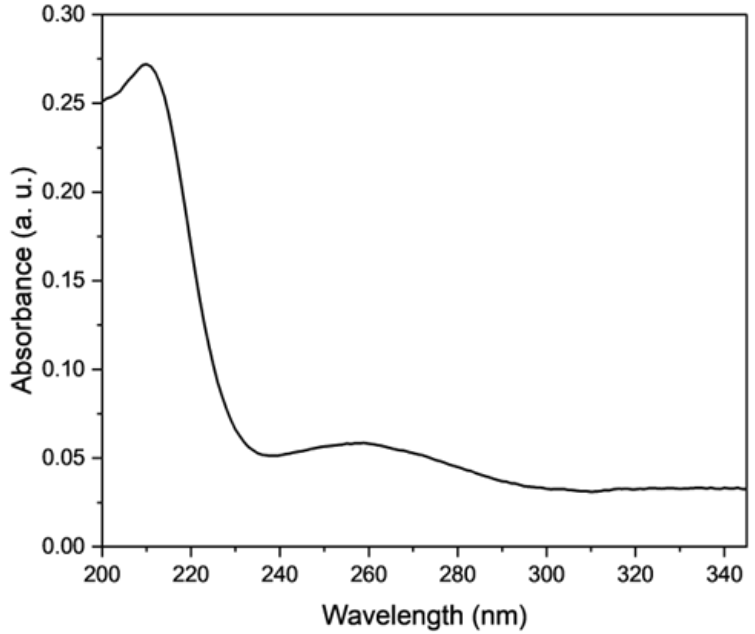
- Transfer the final solution into an Eppendorf tube.
- Add 83 µL of 3M Sodium Acetate, 20 µL of 500 mM Magnesium Acetate and Ethanol to make a volume of 1 mL solution.
- Give a quick vortex and store at -20 ^{O}C overnight.
- Perform centrifugation at max. rpm for 25-30 min to see a DNA concentrate pellet.
- Wash away ethanol and introduce fresh ethanol and perform centrifugation again.
- Remove the ethanol carefully without disturbing the pellet and vacuum dry for 25-30 min





STEP 14:

- Dissolve the dried pellet in 50 µL of DI water and measure the concentration using absorbance measurements.
- Store it at -20 ^{o}C for future use.



STEP 15:

- Et-Br Dye stained gel for visualizing the DNA bands under UV light