Last Update: April 10, 2023

Denaturing Polyacrylamide Gel Electrophoresis DPAGE

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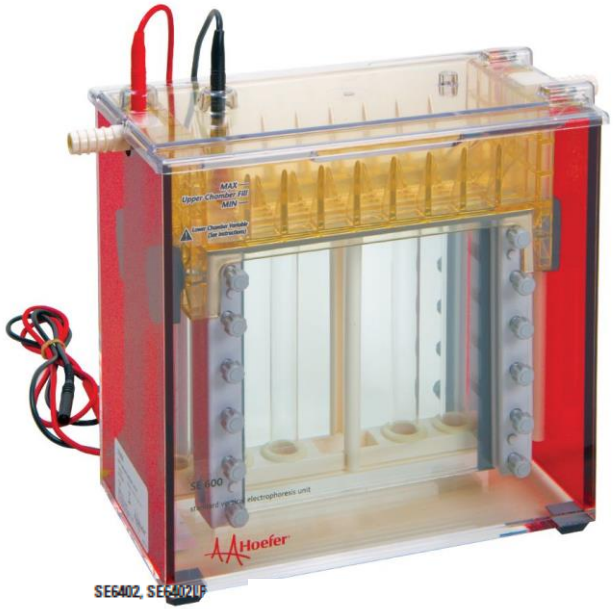
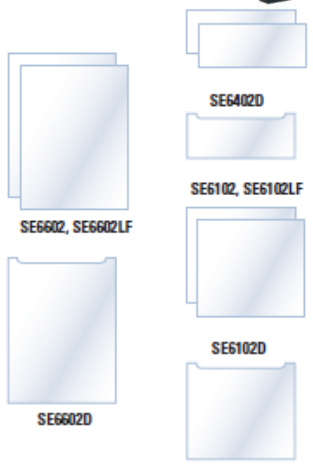
Apparatus Required

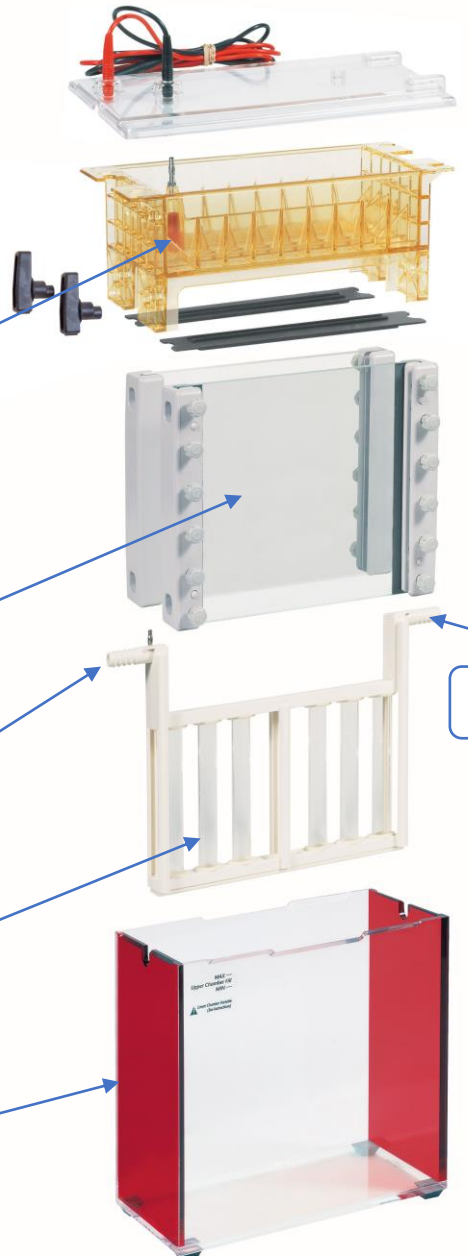
SE600 Standard Dual Cooled Vertical Protein Electrophoresis Unit





Glass Plates

Combs



Buffer Tank

Glass plates containing GEL and DNA samples

Hot Water IN

Temperature Control Channels

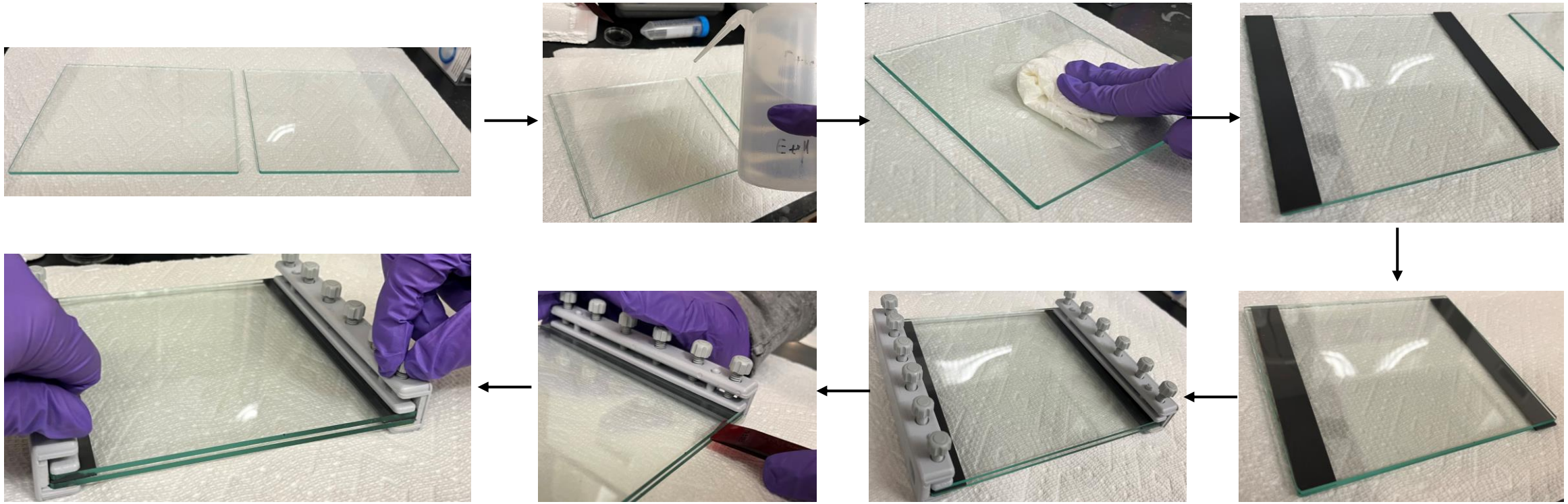
Buffer Tank

Hot Water OUT

Glass Sandwich Preparation

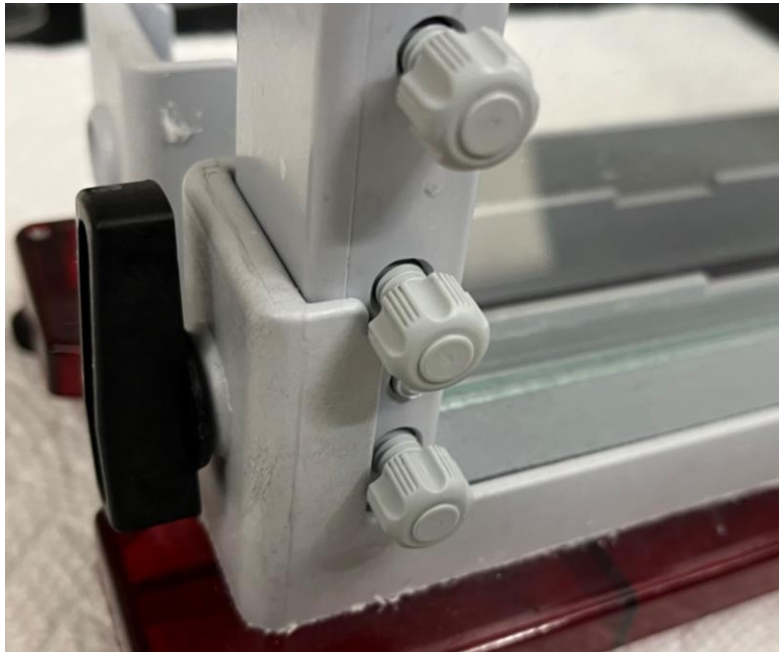
STEP 1:

- 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 hydrophilic.
- 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:

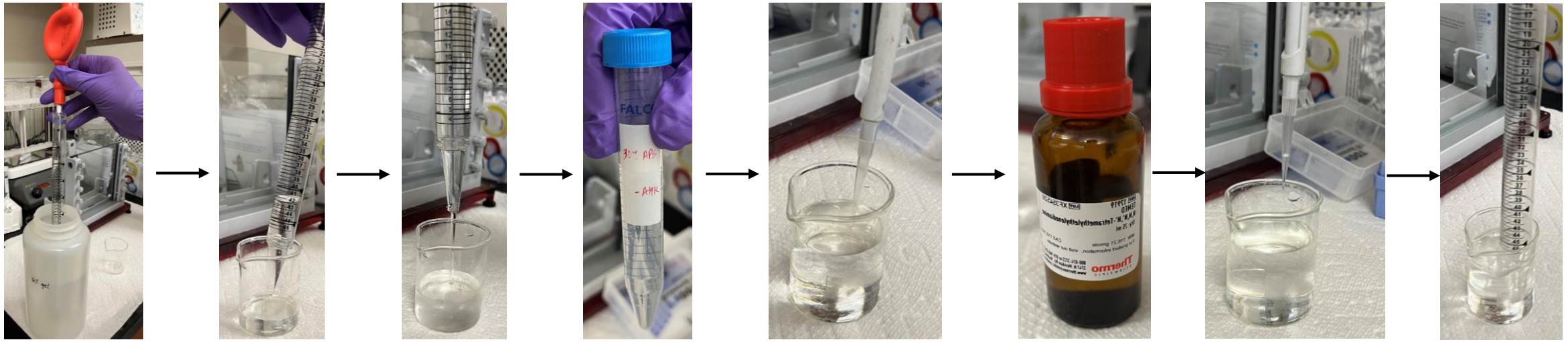
- Use the holder base to place the glass sandwich on the slot with rubber pad and force it down using the cams.
- You need to see the rubber get slightly into the space between glass at the bottom to ensure the gel doesn’t flow out when introduced.



Gel Preparation

STEP 3:

- Decide the percentage of gel you want to run the purification from the table provided in next slide. For example, a 46 nt strand may require 10 % gel.
- Use the stock 20% and 0% solutions to make the desired concentration in a 40 mL volume. Example 20 mL of 20% and 20 mL of 0% to make a 40 mL 10% gel.
- Add 300 µL APS solution, 30 µL TMEDA and give it a mix.



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

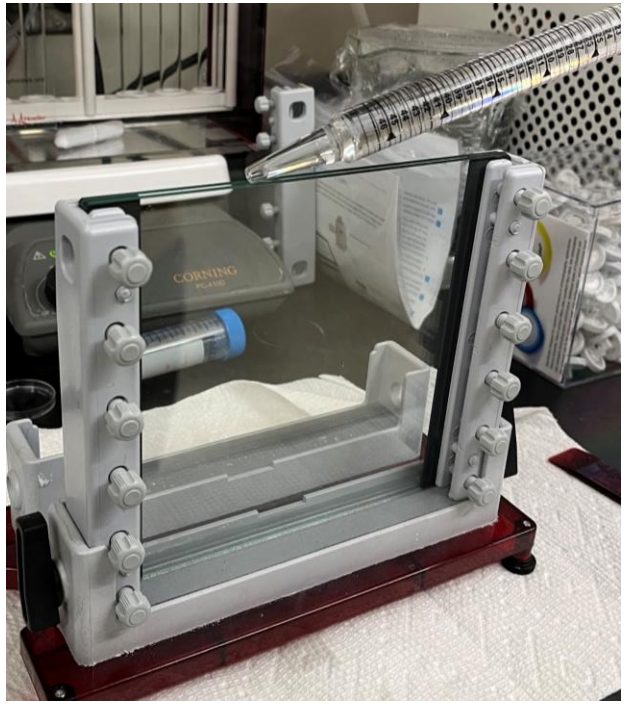
Gel %	*Bromophenol Blue (BP)*	*Xylene Cyanole (XC)*
4.0		160
5.0	35	130
6.0	26	106
8.0	19	75
10.0	12	55
12.0		50
15.0		36-42
17.0		
20.0	8	28

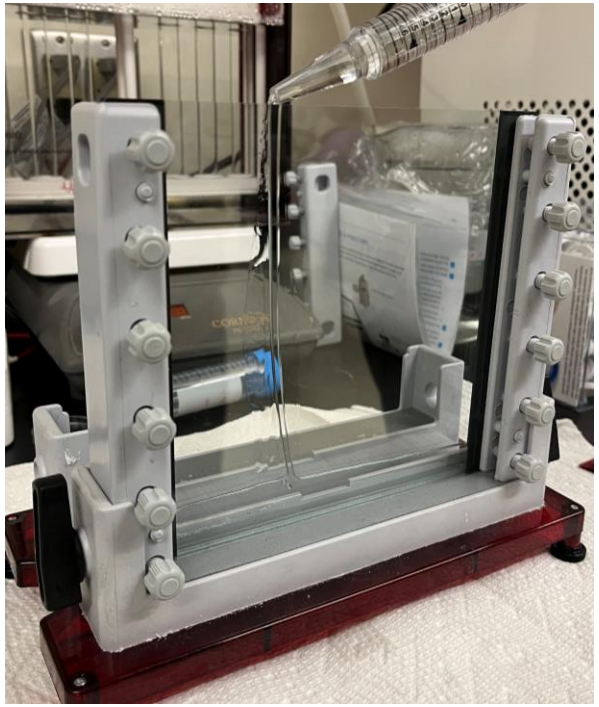
DNA Purification Guide

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:

- Make sure to immediately transfer the gel into the glass assembly using 50 mL pipette inclined at an angle.
- Insert the comb immediately to make the wells to load the sample.





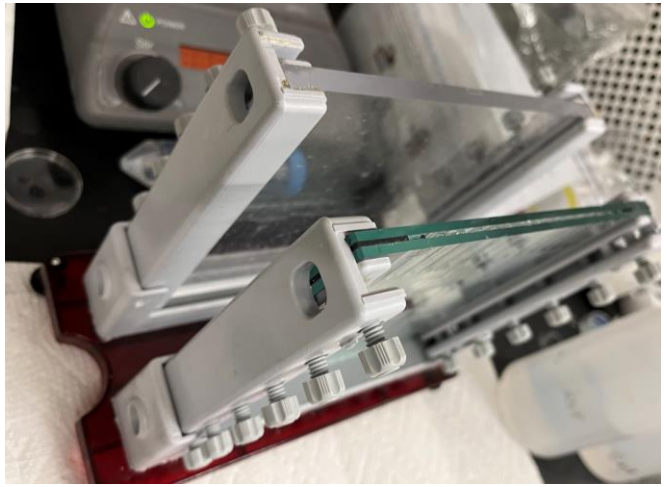
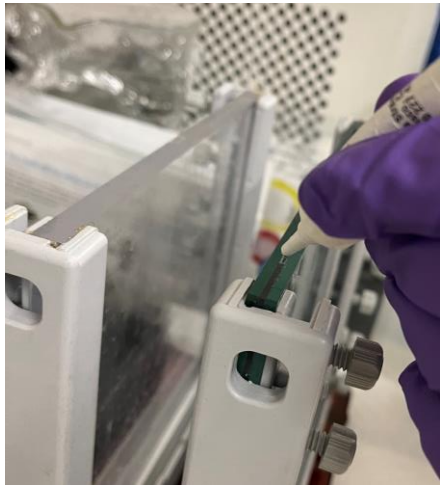
STEP 5:

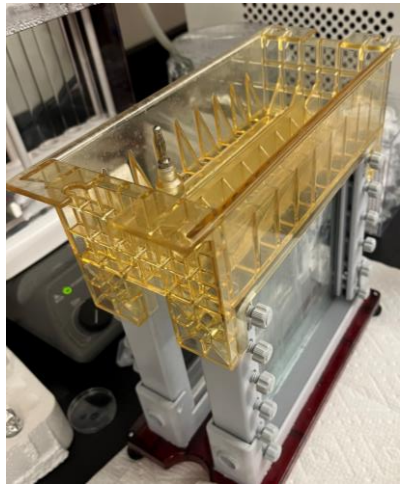
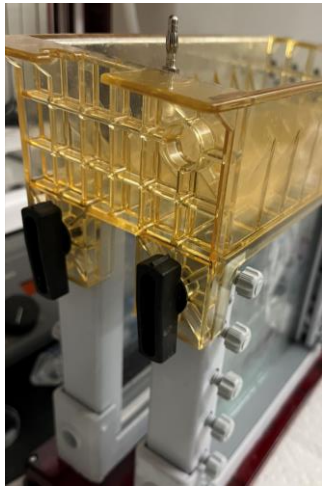
- In about 10-15 min the gel gets polymerized, and one can notice it becomes solid at the well boundaries.
- Remove the comb and immediately flow water into wells so that they do not collapse. Using force action, drain all the water and pour fresh water again. Repeat the step for 2-3 times.
- The gel sandwich assembly is ready and can be used to assemble for buffer tank.

Solution Placement

STEP 6:

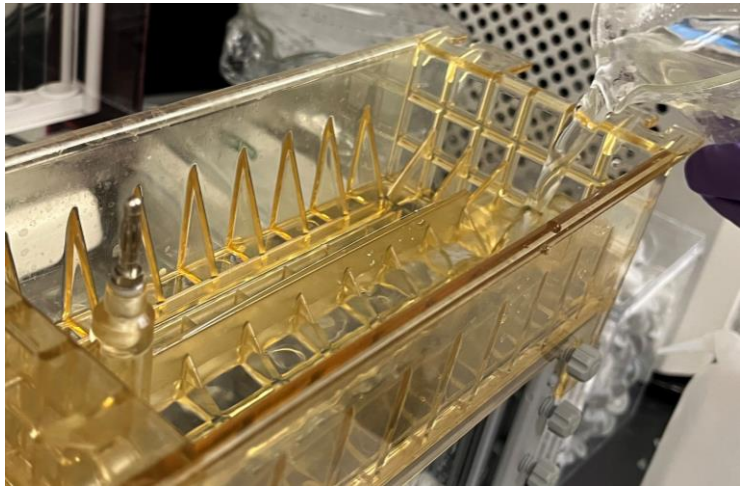
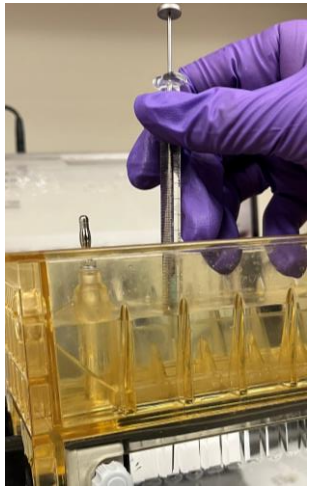
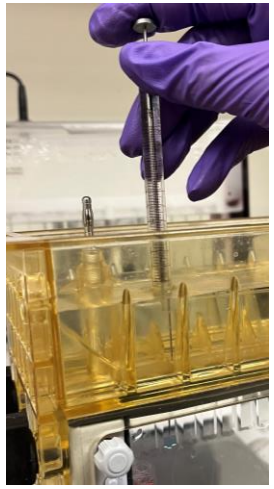
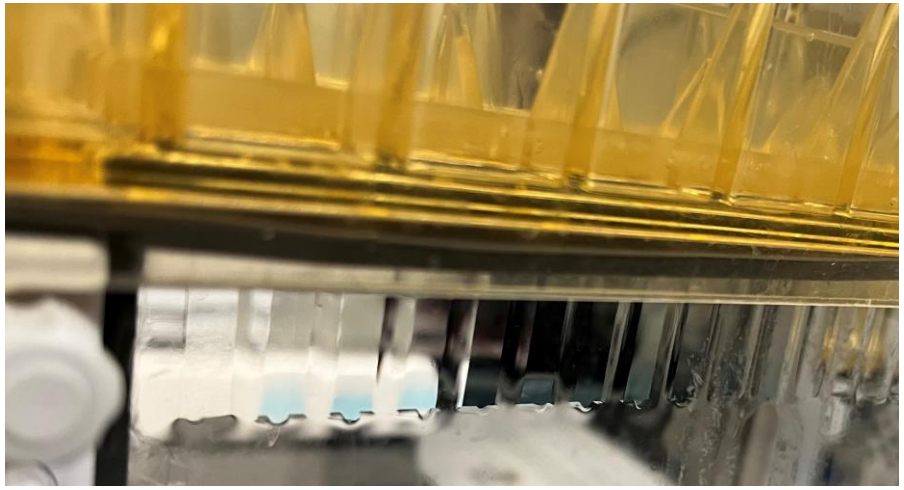
- Use the gel sandwich assembly and a dummy plastic assembly to connect to the buffer tank.
- Make sure to apply vacuum grease at the ends to avoid any leakage of buffer.
- Place the gaskets onto the slots of buffer tank and make sure they are well aligned.
- Assemble the buffer tank onto the glass sandwich.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

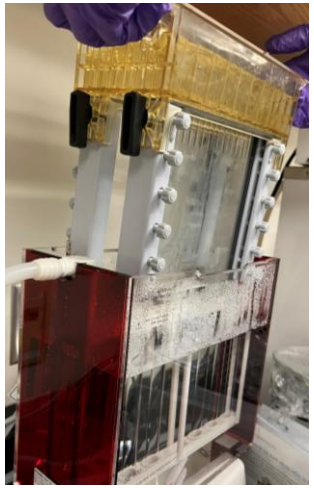
STEP 7:

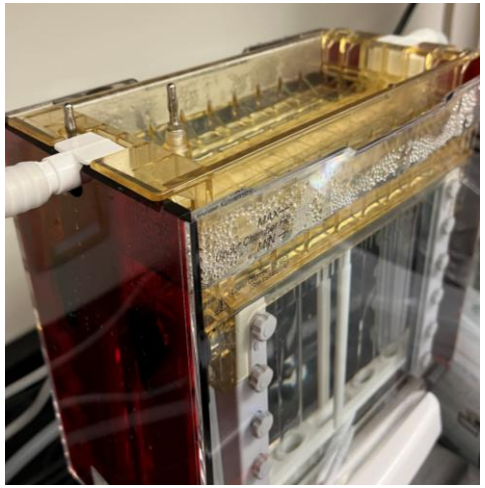
- Pour 1X TBE buffer into buffer tank till the electrode submerges.
- Use syringe and insert 20 µL of prepared sample into each of wells excluding first and last for dye markers to go.
- Leave the first and last well and use the rest of them for electrophoresis
- You should not use dye in all wells, 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.

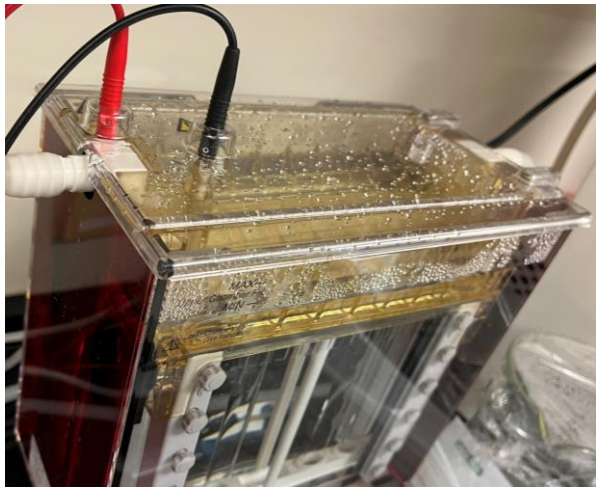





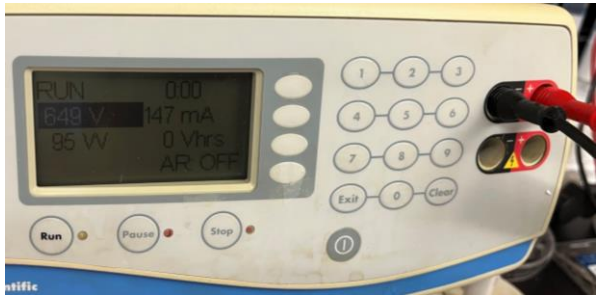
STEP 8:

- Arrange the whole assembly into the electrophoresis chamber and close the lid.
- Make sure the buffer level meets the minimum requirement by adding extra volume.
- Run the gel at 650 V at 45^0 C.
- 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 45 min in 10% gel.









Running & Visualizing the Bands in Gel

STEP 9:

- 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:

- Carefully remove the gel from glass plates and soak it in concentrated EtBr bath for 15-20 min. 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 : Slide x)
- Cut the gel into 2 rectangular strips and use for extraction in further steps.

Sample Recovery

STEP 11:

- To make the electrodialysis setup, pour 1X TBE buffer into the chamber to the required level.
- Cut 5 cm of 3.5 kDa dialysis tube and clip it at one end. Using tweezers try to open the dialysis bag and let it sit inside the electrodialysis setup so that the bag becomes softer and will be easy to handle ahead.
- Introduce 1mL of 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.
- Clip the other end once the gel is transferred in and make sure there are no air bubbles.
- Run the dialysis at 100 V 4.5 mA for 1.5 hr for all the DNA to be transferred from gel into the TBE solution in the bag.

STEP 12:

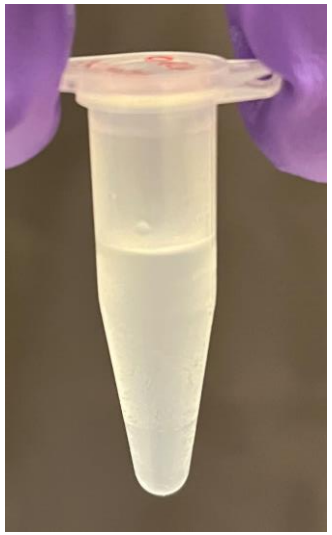
- Transfer the solution from dialysis bag into 15 mL tube.
- Load syringe with 0.45 μm porous filter. Transfer this solution into syringe and pass the solution through the filter.
- Add an additional 1mL of TBE buffer in the syringe and let it pass and wash any DNA that sticks in wall or filter.
- Using 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.

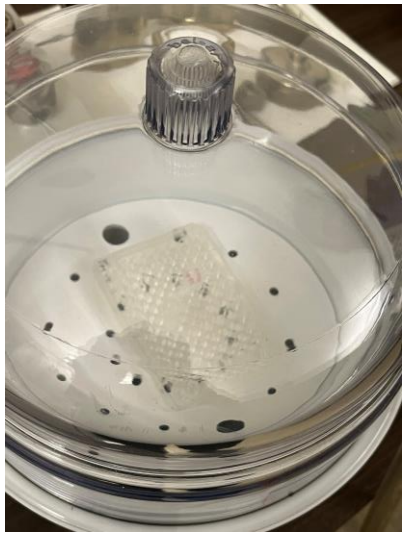
DNA Concentration via Butanol Purification Guide

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:

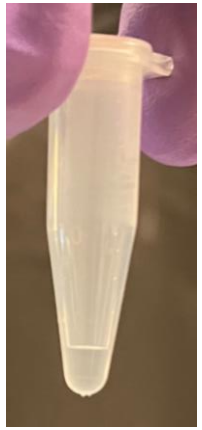
- Transfer the final solution into an Eppendorf tube.
- Add 83 µL of 3M Sodium Acetate, 20 µL of 500 mM Magnesium Acetate and Ethanol to make a volume of 1 mL.
- Give a quick vortex and store at -20^0 C overnight.
- Perform centrifugation at max. rpm for 25-30 min to see a DNA concentrate pellet.
- Wash 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 -20^0 C for future use.



Notes

Visualising gel in all-stains dye