Structural DNA Nanotechnology Laboratory
General Protocol

WEBSITE: http://seemanlab4.chem.nyu.edu/NedlabTrainingProtocols.htm

2. Preparing a "Gel Sandwich"

1. Each gel box can run two gel sandwiches; each sandwich has two plates. Clean 2 glass plates with soap and warm water for each gel that you want to run. **The sink has EB, be careful.**
2. Cover the desk with paper towel. Place the plates on and clean the side facing up (inside) with acetone.
3. Get 2 spacers and 1 comb for each 2 plates as well as 1 additional spacer; clean all spacers and combs with acetone. **Make sure they are the appropriate spacers for your experiment** (thin vs. thick)
4. Place 2 spacers on either side of one of the plates and flip the other plate on top of it so that the side cleaned with acetone is on the **inside**.
5. Get 2 clamps and attach them to the plates.
6. Using a clean spacer, make sure that the 2 spacers between the plates are straight and all the way to the sides. Leave little space the top to be sealed with grease.
7. Make sure that the glass plates are parallel and that they stick out a bit at the bottom and top.
8. Use your hands to add grease to the four corners of the glass sandwich; make sure that any gaps not covered by the spacers are filled out by the grease.
9. Get a stand and place your gel sandwich on it.
10. Get 2 ears and insert them facing down into both sides of the stand.
11. Twist the ears into the upward position, and push inwards if necessary. **It should feel tight**.

3. Purification (via Denaturing Gel Electrophoresis) *Purpose: to isolate strands of a desired length.

Before you do anything, make sure that the water circulator is on.

- If off, check the water level in the circulator. If the water level is under half, refill with dH_2O.
- Check that the back switch is turned on, turn on the circulator from the front panel, then check that the temperature is set to 55.0°C.

Make 1 gel sandwich for each gel you want to run.

1. Get a 40 mL beaker and rinse it with distilled water.
2. Get a bottle of **20% Acrylamide Denaturing Solution** and pour approximately 20 mL per gel into the beaker (40 mL for two gels).
3. For one gel, use 100 λ of 10% ammonium persulfate (APS) and one tenth that of Temed, 10 λ.
   a. If you are only making two gels, use 150 λ of 10% APS and 15 λ of Temed.
   b. **DO NOT** ADD APS OR TEMED UNTIL YOU HAVE A 10 mL LOADING PIPETTE AND BULB READY.
   c. APS can be found in the glass tray at the back of the gel preparation bench. Check the date for fresh APS (3 days old at most). 10% APS can be made with 1-1.5 g of APS and 10-15 mL ddH_2O in a 15 mL Falcon tube.
4. Attach a bulb to the top of a 10 mL loading pipette and squeeze the bulb.
5. When you are ready to load your gel, **add the APS and then Temed at the same time quickly**. Stir the solution with your loading pipette and press the up button on the squeezed bulb to suck up solution into the pipette.
6. Place the tip of the pipette between the gel plates and press the down button gently to release the solution.
7. Tap the stand against the bench to dislodge any air bubbles in the gel before it polymerizes.

8. Insert the comb between the glass plates.
9. Allow 15-20 minutes for your gel to polymerize. You can pour the rest gel on stand to check polymerizing.
10. While you are waiting, prepare **1x TBE buffer** for the upper chamber.
    a. For each box (2 gels), you need 500 mL of buffer.
    b. For 1 box: Measure 50 mL 10x TBE using a graduated cylinder and pour it into a 500 mL volumetric flask. Fill the flask up to the line with single distilled water.
    c. For 2 boxes: Measure 100 mL 10x TBE using a graduated cylinder and pour it into a 1 L volumetric flask. Fill the flask up to the line with single distilled water.
11. After your gel has polymerized, remove the combs and shake the plates over a trash can to remove any liquid from the wells. **DO NOT** leave the wells dry after removing the combs; the wells must be kept wet.
12. Fill the wells with ddH_2O or 1x TBE buffer and again shake the plates over a trash can until all liquid is expelled from the wells.
13. Re-fill the wells with 1x TBE buffer using a Pasteur pipette, 1 mL pipette, or loading pipette.
14. Heat up the DNA samples you wish to run for 3 minutes prior to loading at 90° C.
15. Load the gel using a 10 λ pipette; load 10 λ of your sample into each well.
16. Get an upper chamber and fit it on top of your gel plate(s). **If you are only running one gel, be sure to get an upper chamber with a backer.**
17. Insert the ears in the upwards position and twist them so that into the downwards position. Push in if necessary and make sure that they feel tight.
18. Get a glass tray and stand your gel(s) with the upper chamber up inside of it. Pour buffer into the upper chamber, enough so that it completely covers the bottom, and make sure that there are no leaks.
19. Open up a gel-running box and place your gel(s) inside. Pour the remaining buffer in so that the wire in the upper chamber is completely submerged.
20. Close the box and attach it to a voltage generator. Switch the machine on set it to 600 V. Press the "On" button.
21. Allow about 45-60 minutes for your gel to run, depending on the gel concentration. Check on it regularly to make sure that it is running properly.
    a. Generally, it will be ready to be taken out once the top dye has run about halfway down the gel. To be safe, allow the bottom dye to reach near the bottom.
22. After the gel(s) is done running, take it out and dump the buffer from the upper chamber into a sink.
23. Allow cold water to run over the gel for about 5 minutes. Or place the plates into the cold room.
24. During this time, clean out a glass tray and fill it with single distilled water. **Wear another pair of gloves for EB staining.** Add 1000 λ of ethidium bromide (EB) and stir with the pipette tip. Cover with a tray and label it with your name and "EB".
25. Prepare Eppendorf tubes and label them appropriately. Place them on a stand and put it in the cold room.
26. Remove your gel from between the plates using a spacer, and place your gel in the EB for about 5-10 minutes.
27. Rinse off the cutting board from the cold room. First, run tap water over it then wipe it down with ddH_2O.
28. Place your EB-stained gel on the cutting board and bring it into the cold room. **DO NOT TOUCH ANYTHING WHILE YOU HAVE EB-STAINED GLOVES.**
29. Switch the sign in front of the cold room to "STOP" and make sure that no one is inside. Put on a UV protection visor.
30. Turn on the UV switch and then turn off the light in the cold room.
31. Using a razor, cut the band as close as possible. Discard excess gel in the waste bin by the door.
32. Cut the band in half and then cut each half into thin strips. **Do not cut too thin to split with the solution.** Using the razor, slide each half into one appropriately labeled tube.
33. Make sure to change your gloves before you touch anything and to rinse the cutting board between gels.
34. Clean the plate with dH_2O when you are finished cutting and return it to the cold room.
35. Add 600 λ of **elution buffer** to each tube and place the tubes on the cold room shaker overnight.
    a. Turn off the shaker first, and then put your tubes on it. Make sure to turn the shaker back on when you are finished.

4. Butanol Extraction *Purpose: to remove EB, tracking dye, and other impurities from DNA solution.

1. Take your tubes off the shaker. Make sure to turn the shaker back on when you are finished.
2. Get the same number of Eppendorf tubes that you have now and label them identically.
3. Pipette the buffer (now containing your DNA) from the tubes with the gel into the empty tubes of the same label. **Make sure not to get any gel pieces into the new tubes.**
    a. Make sure to change the pipette tips between separate strands.
4. Add 100 λ of elution buffer to the tubes with the gel pieces. Vortex and centrifuge the tube, and then pipette the buffer into the new tube. You can dispose of the gel tubes in the appropriate waste bins.
5. Move your experiment into the fume hood; ALL PROCEDURES INVOLVING BUTANOL MUST BE PERFORMED IN THE FUME HOOD.
6. Get a small beaker to use as a waste beaker, a 1000 λ pipette, and the necessary pipette tips.
7. Start by adding about 800 λ of butanol to the tubes with buffer. Vortex and centrifuge the tubes.
    a. Be careful not to confuse your tubes, **use stickers**; butanol will markings on the top of your tubes.
8. Notice that there is now a distinct layering within the tube. Remove the supernatant (top layer) and pipette it into the waste beaker.
9. **Repeat this process until you have about 100 λ of buffer remaining**. This is indicated by the bottom line on the Eppendorf tube.
    a. **DO NOT** continue adding 800 λ of butanol; add less as the bottom layer in the tube gets lower.
10. Once you reach the bottom, use a 100 λ or 10 λ pipette to remove any supernatant from the sides so that there is no longer a top layer.
    a. If you accidentally go below the 100 λ line, add elution buffer so that the level reaches the line.

5. Ethanol Precipitation

1. Immediately after butanol extraction, add 1000 λ of 100% ethanol to each of the tubes.
2. Get a Styrofoam bucket from the cold room and fill it with dry ice (on the 4th floor in the Brown building).
3. Embed your tubes in the dry ice and close the lid. Place it on the floor in the cold room for 45 minutes.
4. After 45 minutes has elapsed, remove the tubes from the dry ice and place them into one of the centrifuges in the cold room.
    a. Make sure that the centrifuge is **BALANCED** and is set to spin at 16.1 rct/sec.
5. Allow the centrifuge to run for 30 minutes.
    a. Do not set the timer for 30 minutes; rather, set it at infinite or a number significantly greater than 30 and come back after 30 minutes has elapsed. This is so that the DNA does not go back into solution if you are not exactly on time.
6. After 30 minutes has elapsed, get the following materials **BEFORE** you go into the cold room:
    a. 1000 λ pipette (set at 1000 λ)
    b. Stand
    c. 70% ethanol (found on the top shelf of the refrigerator on the left)
    d. Waste beaker
7. Stop the centrifuge and **QUICKLY** open the tubes and fully decant the supernatant into the waste beaker.
8. Pipette 1000 λ of 70% ethanol into each of the tubes.
9. Close them and place them back into the centrifuge. Allow the centrifuge to run for another 10 minutes.
10. After 10 minutes has elapsed, stop the centrifuge and **QUICKLY** open the tubes and **partially decant** the supernatant so that a little liquid remains in the tube.
11. Place your tubes in the vacuum concentrator (CentriVap) to dry. This usually takes 30-45 minutes, depending on how much ethanol is left in the tubes.
12. Take your tubes out of the CentriVap after they have dried and add 60 λ of ddH_2O to each tube.
13. Combine the samples of tubes that contain the same DNA using a pipette.
14. Measure the OD if you need to.

6. Using the Centrivap

1. Open the lid of the Centrivap and place your tubes inside; Make sure that the centrifuge is **BALANCED**.
2. Close the lid carefully then press the run/stop button on the right side of the front panel to start the Centrivap. Wait until you can hear a single beep.
3. **Close the valve on the vacuum pump THEN turn on the vacuum.**
4. After 30-45 minutes, use the light gun on the right-hand side of the Centrivap to check if your tubes have completely dried.
5. When completely dry, turn off the pump **THEN** open the valve. If you do not turn it off first, the valve will not open.
6. Stop the Centrivap and wait for it to stop spinning for the lid to open. Remove your tubes.
    a. If there are tubes that do not belong to you in the Centrivap, turn the Centrivap back on.

7. Using the RotoVap

1. Make sure the knob leading to the RotoVap is open and that the Rotovap is at 55° C.
2. Get a spherical round bottom flask and rinse it with ddH_2O.
3. Turn on the water (cools down machine).
4. Turn on the pump (cabinet under RotoVap on the left side).
5. Start spinning; turn the knob to 10 (or lower if appropriate).
6. Close the exhaust knob.
7. Slowly close/adjust the knob on the RotoVap so that the sample inside bubbles slightly.
8. Wait for your sample to dry completely, then detach the round bottom flask.

8. Purity Check

1. Prepare a 20% acrylamide denaturing gel.
2. Pipette 60-80 pmol of each strand whose purity you wish to verify into new appropriately labeled eppendorf tubes.
    a. 1 µM = 1 pmol/λ
3. Pipette denaturing dye into your tubes so that the total volume is 10 λ.
4. Prepare a 10 base pair ladder marker.
    a. 2 λ 10 bp ladder (found in public enzyme box)
    b. 8 λ denaturing dye
5. Heat tubes for 3 minutes at 90° C and load the gel. Make sure that you know which wells contains which strands, especially if they are all of similar length.
6. After running the gel, clean a glass tray with soap and water. Rinse it with ddH_2O and fill it halfway with Stains-All solution.
    a. Make sure to cover the tray with aluminum foil; Stains-All is light-sensitive and will lose its staining properties when exposed to light.
7. Open your gel plates and place the gel along with the bottom glass plate into the Stains-All.
8. Wait for about 1 hour. If you want the dye to wash off, leave your gel in the Stains-All for longer or overnight.
9. Get another tray, rinse it, and fill it with dH_2O or tap water.
10. Move the gel + plate from the Stains-All to the water in order to de-stain the gel (the Stains-All will wash off the gel but the DNA will remain stained).
11. Put the tray on the light table and turn the light on. Allow 15-20 minutes for the gel to fully de-stain.
12. Carefully move the gel onto a transparency film.
13. Dry the gel **GENTLY** using kimtech wipes.
    a. Place the wipes on top of the gel, pat down, and slowly peel them off.
14. Place another transparency film on top and place it inside the scanner with the wells towards you.
15. Take off your glove(s) before using the computer.

16. Open the “Epson Scan” program and double-click on the “Preview” button.
17. After the preview scan is finished, crop a close area around your gel and double-click the “Scan” button.
18. Title the file with an appropriate name and save the picture into your folder.
19. When you view your gel on the computer, you may want to increase the contrast.
    a. To do this, click on Tools>>Image Correction
    b. Increase the contrast and lower the brightness
20. If it is necessary, print a picture of your gel.

9. Fast Annealing (Times are approximate)

- 90° C – 5 minutes
- 65° C – 15 minutes
- 45° C – 20 minutes
- 37° C – 20 minutes
- Room Temperature – 30 minutes
- Cold Room – 30 minutes

10. Slow Annealing

1. Get a 2L beaker and rinse it out with ddH_2O, then fill it with ddH_2O.
2. Place it in the microwave and heat it up for 20 minutes.
3. Take it out using with mitts and place it in a Styrofoam box.
4. Place your samples on a floater and place the floater in the beaker.
    a. Your tubes should be capped and covered with parafilm to prevent evaporation.
5. Allow the water to slowly return to room temperature over 2 days. Be sure to label the box with your name and the dates it will be in use.

11. Measuring Optical Density (OD)

1. Be sure to vortex and centrifuge your samples prior to measuring the OD.
2. Turn on the spectrophotometer (the switch is on the left side in the back). The machine should be set at 260 nm.
3. Take out the cuvette from the drawer labeled “Cuvettes.” DO NOT touch the transparent sides of the cuvette.
4. Rinse the cuvette out with ddH_2O and pour it out.
5. Pipette 990 λ of ddH_2O into the cuvette.
6. Clean the transparent sides by wiping them with a kimtech wipe.
7. Place the cuvette inside the spectrophotometer, close the lid, and press the “Measure Blank” button to calibrate the machine.
8. Take the cuvette out of the spectrophotometer. Using a 10 λ pipette, take 10 λ of your sample and submerge the tip into the water of the cuvette and pipette out your sample.
9. Cut a piece of parafilm, place it on top of the cuvette, and shake it to mix the sample. Make sure there are no air bubbles in the cuvette.
10. Place the cuvette back into the spectrophotometer and close the lid.
11. Record the OD measurement for the sample.
12. Pour out the liquid in the cuvette into a waste bin and rinse out the cuvette with ddH_2O.
13. In order to convert OD to µM, we assume 1 OD = 35 mg of DNA:

$$\left[\frac{(x)\left(3.5 * 10^{-6}\frac{g}{OD}\right)}{\left(330\frac{g}{mol}\right)(n)(10 * 10^{-6}L)}\right] \qquad x = OD \qquad n = \#\ of\ bases$$

Your answer will be in mol/L. In order to convert to μM, multiply by 106.

13. Turning the AFM on

1. The computer should be on whenever the controller is on! Turn the computer on before the nanoscope controller.
2. On the scan control panel, avoid OFFSET voltages outside of +/- 150V for periods of more than about an hour. When possible, reset them to zero.
3. Avoid using maximum scan size for long periods (for instance, size greater than 40 – 120 um) if not necessary.
4. Avoid leaking when organic solvents were used (isopropanol) during imaging.
5. The **right** way to start and shut down the scope should be as following:
   ON: Main power --- computer --- controller
   OFF: Controller --- computer --- main power

14. AFM Imaging

1. Tip: For the tapping mode imaging in buffer, use the short cantilever (either skinny or fat) on the AFM chip.
2. Sample preparations: The protocol for preparing sample is to put 5 ul DNA lattice in TAE/Mg or HEPES/Mg on the mica, let it sit for 0.5 – 1 min, then add 25 ul of buffer. An additional 30 ul buffer will be applied to the tip with fluid cell "right side up" so that the drop is hanging.
3. Laser align Make sure to align the photodiodes both side to side (horizontal difference signal) by aligning the laser in "AFM & LFM" mode and aligning the laser in top to bottom (vertical different signal) in "TM AFM" mode. Both vertical and horizontal difference should be around 0. When laser is properly aligned, go to auto tune icon and ask for an amplitude of 0.5 volts. The resonance for the small skinny tip should fall between 9 and 9.5 kHz.

4. Imaging: Settings for tapping in buffer:
   Scan size: 5um – 10 um
   Scan angle: 0
   Scan rate: 3 – 6 Hz

5. Feedback control:
   Integral gain: 0.4 – 0.7
   Proportional gain: 0.6 – 0.8
   Channel 1: 10 nm of height contrast (for DNA)
   Channel 2: 1 volt of amplitude contrast