Using the Hitachi HPLCs (Pauling & Maniatis)

Start up protocol

1. Turn on the power to all four units and wait for the "D-Line not ready" message to appear in the LCD displays
2. Start up the D-7000 HSM software (not the administrator software) on the computer
3. Initialize the software and hardware connection by going to the " **i** " icon, and selecting initialize. This process takes about 2 minutes to complete the connection. Hit "OK" once complete.
4. **Purge** (optional) each of the 4 inlet lines before pumping any buffers through the column: on the pump unit, select "manual set" and pump each buffer one at a time at 100% for 20-30 seconds or until all of the bubbles are out of the line. Make sure the purge valve is open and turn the pump on by pressing the "pump on/off" key
5. **Clean the column before use**:
   a) Turn the pump off, close the purge valve, and set the injection port valve in the "inject" position
   b) Pump 100% D (95% Isopropanol/H_2O) for 20 min at a flow rate of 7-8 ml/min
   c) Pump 95% B (80% Acetonitrile/0.1% TFA/H_2O) and 5% A (0.1% TFA/H_2O) for 10 minutes at 9.6 ml/min (note that the flow rates in steps b and c are halved for 10 mm diameter columns)

Performing a run

1. After cleaning the column select the "acquire data" icon ("eye ball"), and open the sample table and method appropriate for your protein. (when opening the "acquire data" window, be sure the pump parameters are correct in the method setup)
2. Equilibrate the column by pumping at the starting conditions for 10 minutes
3. **During this time, clean the needle and the sample loop**:
   a) Assemble the needle on a new syringe and clean the needle several times by drawing up about 5 ml of water into the syringe and dispensing the water into the waste container
   b) Turn the injection port valve up to the "load" position
   c) Using the injection port cleaner syringe, wash the loop by injecting 3x the loop volume
   d) Turn the valve back down to the "inject" position
   e) Continue pumping with the starting conditions and wait ~10 minutes for a return to baseline
4. After the column has been equilibrated, inject your protein onto the column:
   a) Turn the injection valve up to the "load" position to access the sample loop
   b) Load your sample into the loop using the needle and a new syringe. Load no more than 75% of the loop volume (e.g. 15 ml for the 20 ml loop and that too very slooooowly).
   c) Return the injection valve down to the "inject" position
   d) Wait for approximately 10 minutes to observe your injection peak and a return to baseline
   e) It's a good idea to collect your injection peak if it has strong absorbance at 280 nm (Maniatis)
5. Make sure the pump is out of "Manual" mode and click on "start run" to begin. Check to make sure the %B is increasing, because occasionally, the run is not triggered
6. Collect the peak(s) during the run in 15 ml tubes (no more than 8 ml per tube)
7. Using black ink, label each sample with the protein name, run number, date and the time range along with the corresponding %B value
8. Freeze sample tubes at –80 ^{o}C; for faster freezing put tubes in Nalgene beakers in the freezer
9. After your protein has been collected, manually set the pump to 95% B for 10 minutes
10. Cancel the run and the pump will reset to your starting conditions

Shut down protocol

1. Clean the column with 95% Buffer B for 10 minutes after the last run for the day.
2. Manually set the pump to 100% C (50% Acetonitrile/50% H_2O) and pump for 10 minutes
3. Turn the pump off using the "pump on/off" button; close software and power all 4 units off