**Notes on Using the FPLC**

**Updated November 2011**

**Instrument info**

* We have a Bio-Rad BioLogic DuoFlow, serial number 484BR1743
* Two F10 pump heads
* QuadTec UV-Vis detector
* Conductivity monitor
* AVR7-3 sample inject valve
* BioFrac fraction collector
* Two SV5-4 buffer select valves (each with four positions)

**Priming the system with water**

1. Turn on the computer, main instrument, fraction collector, and QuadTec (order doesn’t matter).
2. Open software: BioLogic DuoFlow.
3. In the “manual” screen of the software you can select the positions for buffer select valves A and B.
	1. Valve A is hooked up at instrument port 1, valve B is at port 2.
	2. Position 1 on both valves should always be water and position 2 should always be 20% ethanol.
4. Check that position 1 is chosen for both valves and that the corresponding inlet lines are immersed in water. Water should be freshly filtered and degassed. Use syringe marked for priming pumps to draw water through the priming ports on the fronts of the pumps until no air bubbles come out (press the syringe onto the port, unscrew counter-clockwise and slowly pull out the plunger. Rescrew port closed before removing the syringe). Draw several syringe-fuls of water through on both pumps.
5. If a bubble gets trapped in the pump head: disconnect the pump head outlet tube and use a syringe full of water to inject into the priming port and dislodge any trapped air bubbles.
6. Use software to switch AVR7-3 inject valve to **Purge** position. Press purge buttons A and B next to power switch and run for 1 min (check that liquid is going to waste). Change inject valve back to Load. Flow rate will now be at 10 ml/min so remember to change it when actually running liquid through the column.

**Hooking up a column and equilibrating it**

1. Purging the system of air bubbles before running through the column is extremely important because bubbles with damage the column.
2. Unscrew lines from other column and replace with black plugs. Unscrew plugs from column to be used.
3. Set flow rate to 1 ml/min and start flow. Water will begin dripping out of the unattached line.
4. Hold the dripping end (tubing coming from inject valve) over the top opening of the column and let it fill with water. Once liquid has reached the top, screw in incoming tubing. Once drips start coming out of bottom of column, screw in outgoing tubing (going to QuadTec).
5. Run water through the column at the appropriate flow rate and volume to remove 20% ethanol and prepare for buffer equilibration. Can do this with manual screen or create and run a method (see below).
6. When system is not running, hook up experimental buffers. Immerse inlet tubes for positions 3 or 4 of both valves into appropriate buffers. Prime the system as before (select appropriate valve position on manual screen, pull liquid through priming ports with syringe). Switch inject valve to Purge and purge both pumps for 1 min. Typically the B pump buffer has salt in it, so let pump A purge alone after switching off B to remove salt from the lines. Switch inject valve back to Load.
7. Run buffer through the column at appropriate flow rate and long enough to equilibrate it. Can do this with manual screen or make it part of the experimental method (but don’t start it running until have loaded sample in sample loop).

**AVR7-3 sample inject valve**

* “Load” position: injected sample goes into sample loop and excess goes to waste. Workstation pump goes directly to column and detector.
* “Inject” position: sample loop is put in line with column so workstation pump flows through loop and onto column.
* “Purge” position: workstation pump goes directly to waste. Nothing is hooked up to the column.
* Valve should be on “Load” most of the time, including when sample is loaded into the sample loop. Should be on “Purge” when priming the system. Should only be on “Inject” during a run when sample is being injected onto the column. Syringe with injection needle should be left in the injection port during injection or air bubbles will get sucked into line and injected onto the column!

**Loading a sample into the sample loop**

1. Select desired size sample loop and screw into ports on inject valve.
2. Only special blunt needle should ever be inserted into injection port on inject valve!
3. Screw injection needle onto a syringe and rinse sample loop with water or buffer. Valve should be on Load!
4. When loading actual sample into sample loop, avoid injecting air bubbles into the loop using the following techniques:
	1. Make sure valve is in Load position.
	2. Have a higher volume of sample in the syringe than would fit in the sample loop (for example, have 5.5 ml in the syringe for a 5 ml sample loop).
	3. After filling syringe with sample, tap air bubbles to the top and push them out of the end.
	4. While injecting sample into sample loop, stop injecting while there is still liquid in the syringe (since you should have extra!). Also watch for sample to come out of exit tubing to know you’ve reached the end of the sample loop.
	5. Leave the syringe sticking out of the injection port until the injection step of the run is over so no air gets injected onto the column.

**Running a method**

1. Once the system has been primed with the appropriate buffers, sample has been loaded in the sample loop, and collection tubes placed in the fraction collector, a method can be started running.
2. Use the Browser to navigate the saved methods and choose the desired one, or create a new one (see below).
3. When a method is edited, it must be given a new name. Each run must be given a new name and can have comments entered.
4. Double clicking on the fraction collection scheme line will open a window that tells you the fractions to be collected and the number of tubes required.
5. Always check that the inject valve is on Load before beginning the method, because it won’t set itself back automatically.
6. When ready, press the Run button at the top, give the run a name, then press the green “start” button and watch it go!
7. Monitor the pressure to make sure it does not exceed the limit of the column. An upper limit can be entered in the run screen so that the instrument shuts down if this pressure is exceeded.
8. You can look at other methods or finished runs while a method is running. These will open in a separate tab.
9. After a run is complete, you can export the chromatogram and method by clicking “Report”. Choose to include in the report “Run data” and “Method” and click OK. In the screens that appear, enter in a name and choose where to save them (the chromatogram will come up first). It will write these to PDFs which you can copy to your own computer and print.
10. If you want the actual data points, go to File🡪Export Data and choose which data to export and points/sec (default is A LOT of points so should reduce this).

**Creating a method**

1. In the Browser screen, highlight the project folder under the appropriate user’s name.
2. Click New and select Method. Enter a name for the method.
3. First it will take you to the Setup screen where you select the devices to be used in the method.
	1. Highlight UV Detector and Delete it. Click on Detectors on left and choose QuadTec. Enter in wavelengths to be monitored.
	2. Conductivity monitor and inject valve are already chosen.
	3. Click on Fraction collector and choose BioFrac and 12-13 mm tubes.
	4. Click on SV5-4 valve and enter in buffers for valve A. After this valve is added, click SV5-4 valve button again and enter in buffers for valve B.
4. Click Protocol button at top. Begin entering steps for the method.
	1. Can enter steps for isocratic flow, sample injection, and linear gradient.
	2. For each step added, the buffers to be used in valves A and B should be selected, then the percentage of each, the volume, and the flow rate entered.
	3. Can also put in Pause steps, zero the baseline at the beginning of the run, or turn off the detector lamp at the end of the run.
	4. To set up fraction collection, click Fraction Collection button and choose Collection Windows. Click Collection Windows tab and enter in starting and ending values and fraction sizes. The Fraction Collection Scheme window will tell you the total number of tubes required.
	5. Double click on any line to edit it.
5. When finished creating the method, click Run button at top and run the method!

**Cleaning the FPLC after runs are finished**

1. Column should be rinsed with water and stored in 20% ethanol (can set up a method to do this overnight and turn of QuadTec at end).
	1. Prime and purge the system with water (position 1 on both valves) before beginning cleaning method to save time.
2. For short-term storage, aqueous buffers may be left in buffer select valve lines but for long-term storage all lines should be rinsed and stored in 20% ethanol. Urea solutions should NEVER be left in lines and should be rinsed out with copious amounts of water.
3. Sample loop should be rinsed several times with water.
4. VERY IMPORTANT: should rinse behind pump heads with water at the end of every day of use, especially if salt buffers were run through them.
	1. Using special syringe, inject water through holes on top of pump heads.
	2. Water will come out of washout outlet in between pump heads. Place paper towels and a beaker under it to catch the liquid.
	3. Rinse each pump head at least **10 times** with syringe full of water (20 times if used urea buffers).
5. After all rinsing and cleaning methods are done, turn everything off.