Protocol for Shimadzu HPLC RF–20A/20Axs

Seven modules of the instrument:
LC-20AB (pump)
SIL-20AC (autosampler)
FCV-20AH$_6$ (valve unit)
CBM-20A (communication bus module)
SPD-20AV (UV/Vis detector)
RF-20A xs (fluorescence detector)

Start the instrument and software:
1. Turn on the power for each of the 6 modules (bottom left hand corner; top left hand corner for the FCV-20AH$_6$).
2. Open the Lab Solutions software located on the desktop. Sign in as the administrator with no password (just hit ok).
3. The software will then connect with the instrument. A 'beep' will sound. Confirm the software is connected by looking at the top left hand corner of the software window once it opens. It should say LC==Ready==. (If not connected, it will read LC`NotConnected`. Restart the Dell computer and reopen the software if this happens).
4. The 'remote' LEDs on each module should be lit up green. The pump will also have the G.E. led button lit, and the CBM will have 'connect' lit up.

Before the run, create or edit a method:
1. Go to 'File' and choose to open a method file.
2. Open the 'Superdex200_EGFP' method from the Lab Methods folder located on the desktop.
3. The method editor will display automatically upon opening the method. (If you need to call up the method editor once this window has been closed, there is a button at the top of the bottom screen on the left hand side that says 'Edit Instrument Parameters' which will load the same window.)

Data acquisition tab
- This is where you set the length of the run in 'mins'. This should be adjusted according to the column volume for whichever column you're using. (usually want to run ~1.2 CVs).
- You also have the option to change the acquisition time for the different detectors here. Probably best to acquire signal on all detectors for your entire run. Hit 'apply to all acquisition time', and make sure the box for each detector you plan on using is checked.

LC Time prop.
- We don't need to mess around with anything in here (it's for more complex HPLC runs).
- *Make sure that the command for stopping the pump is there!*
   [Time:50.00/Module:Controller/Command:Stop]

Pump
- This is where you set the flow rate for the run. This is also dependent on whichever column you are using. We always use Line A for our running buffer, so ensure that B concentration remains at 0%.

- The pressure limit set for the pump is the standard, do not change this (4500 psi).

Detector A
- This is the UV/Vis detector.
- You have the option to change sensitivity, etc..., but these are the standard settings, and should be fine for anything we'll need it for. We don't recommend changing anything in this tab.

Detector B
- This is the tab that controls the fluorescence detector settings.
- Ensure that the box is checked next to 'Lamp on'. The standard response is set for 1 sec.
- The cell temperature can be changed, I keep it off since we run the column at room temperature anyways.
- The sensitivity of the output can be tuned by adjusting the gain and sensitivity drop down options. Gain x16 = more intense signal, Gain x1 = less intense. (see pg. 4-36 in manual for specifics)
- The instrument can monitor two fluorescence signals at once. The excitation and emission wavelengths are set in the box. (**EGFP** - excitation: 488 nm, emission: 512 n; **mCherry** – excitation: 587 nm, emission: 610nM; **GFP** – excitation: 398nm, emission: 508nm)

Controller
- This is the tab where you designate what position your column is in.
- Not exactly straightforward. Power on should be checked in all cases. In order to switch positions, a combination of certain events will be checked:

Column position	Power on	Event 1	Event 2	Event 3
1				
2				
3				
4				
5				
6				

(ie: if your column is hooked up to position 1, you want to ensure that only 'Power on' checked. The correct position will be lit up with the LED light on the valve unit, make sure your column is in the correct position).
- Ignore the acquisition cycle time optimization option.

Autosampler
- This is where you can change options for your injection.
- There is the option to rinse the needle either before, after, or both before and after aspiration.
- This is where the rack temperature can be set. Ensure that 'cooler temperature' is checked and it's set for 4 degrees.

AutoPurge
- This is where we might be able to change to an option that purges the pump for us so we don't have to do it manually, but it hasn't been looked into or tried out yet so leave this tab alone for now.

Equilibration of the column:
1. Purge the pump
   - o Ensure that Line A is placed into your running buffer.
   - o Open the drain on the LC-20AB by turning the knob counter-clockwise.
   - o Hit the 'Purge' button on both the LC-20AB and the SIL-20AC.
   - o When the purging is done, close the drain knob.
2. Run a prewash method. Ensure that
   - i) the column position is correct
   - ii) the lamps are on during the wash to warm up before your run

Starting a single run:
1. Pull up the appropriate method and modify according to your needs. If you want to save your changes, hit 'download and close' in the method editor, then go to 'File' and choose 'Save method as...'. **DO NOT OVERWRITE THE LAB METHOD.** This is a common mistake, so I suggest copying the lab method to your own folder, and opening it from your own directory before you start making changes.
2. Ensure the parameters shown in the bottom window are what you want for your run. Make sure the correct column position is lit up on the valve unit.
3. Place your sample securely in slot #1.
4. On the left hand side of the screen, hit start single run. Choose a name and folder for your data file. Ensure you adjust the vial# and the amount of sample you want injected (located at the bottom of the window) before hitting 'ok'.

Starting a sequence:
1. Go to Realtime Batch on the left. Set up the rows according the amount of samples you have.
2. Make sure to set the vial # for different samples, and set vial # to -1 if no sample injection is required.
3. "Tray name" set to 1.
4. Choose "Method file" to load the method you want to use.
5. Set "Data file" to save your data to desired destination.
6. Make sure to set an injection volume (I usually do 50-100ul).
7. Make sure to have a shut down method after the run just to make sure it won't go forever.
8. After everything has been set up, go to "File→Save Batch File As" to save the sequence run.
9. Press "Start Real time batch" to start the sequence run.

After your run:
1. Place Line A into water.
2. Purge the pump and autosampler (as described above).
3. Open the Endwash method. Ensure that in the column position is correct and that the lamps are not on.
4. Open the Shutdown sequence and ensure the Endwash method has been replaced with one containing the correct column information.
5. Run the sequence.
6. Repeat the steps above with Line A now in 20% EtOH.
7. Exit the software by choosing "File→Exit".
8. Turn off the power on each module.