**LI-6800 Dynamic CO2 Response Curve Protocol**

**LI-6800 Initial setup**

1. Connect CO2 cylinder, water input attachment, tubing between console and measurement head
2. Turn on machine
   1. Recommended to allow machine/IRGAs to warm up for 40min before beginning warmup tests. This can be skipped in the field to expedite fieldwork, but is not recommended.
3. Make sure all chamber conditions are turned off except fan and flow function
4. Conduct warmup tests (with cuvette clamped shut)
   1. Startup window -> Warmup tests -> Start (should take 20 mins)
5. Turn on Dynamic equations
   1. Check "Add Dynamic Equations" after navigating to Start Up -> Chamber Setup
   2. Check "Use Dynamic" after navigating to Constants -> Gas Exchange
   3. This **must be checked** before opening a log file

**Starting curves (open log file, know about user defined vars, etc.)**

1. Open log file by going to "Log Setup" (“Log Setup -> Open a Log File”)
2. Turn off point matching requirements by going to "Log Setup -> Matching Options", and select "Never match" for H2O and CO2
3. Be aware of the user defined columns written into all machines, which can be accessed from by navigating to "Constants -> User".
   1. There should be two columns added to each file- a machine name that auto-populates and an empty id column. The empty id column is for adding unique identifiers for each curve, and should be updated with each curve/measurement

**Range matching (required for dynamic curves)**

1. Range matching is required for running dynamic response curves, but is recommended to wait until the chamber warms up (see vignette: <https://www.licor.com/env/support/LI-6800/topics/matching-the-analyzers.html#Rangematching>)
   1. Can be circumvented by performing an auto match between response curves
2. Navigate to Flow window in "Environment window". Note the ratio of Flow\_r to Flow\_s
3. Navigate to range matching window in Constants -> Range Match
4. Start with the CO2 range match window from the dropdown window, press start and enter the Flow\_s:Flow\_r ratio
   1. Range matching will take 5 minutes to complete. CO2 range match should exceed 2000 μmol mol-1 without problem. If not, troubleshooting may be needed
   2. Upon completion of the range match program, a prompt will ask whether you want to keep the range match. Any range match with lower standard deviation than the previous range match (noted on right side of window) is acceptable.
   3. May need to repeat range match if standard deviation is higher than previous range match
5. Repeat process with H2O by navigating to the H2O range match window through the same dropdown menu. Enter Flow\_s:Flow\_r and follow same advice as CO2 range matches
   1. H2O range match should exceed 17 μmol mol-1 without problem. If not, troubleshooting or additional warmup time may be needed

**Dynamic tuning**

1. Tuning should always occur after range matching is complete. This feature tunes time lag and dynamic coefficients used for determining flux differences between Adyn and Asty
2. Navigate to Dynamic Tuning test window (Constants -> Dynamic -> Utilities/Tests on lower right hand side of screen)
3. Tune CO2 coefficients with the "CO2 Test Current" option in the dropdown menu. Press start. A window will pop up to prompt you to start the dynamic tuning BP
   1. Once complete, you will be prompted to accept or reject tuning results. As with range matching, we are wanting the standard deviation to be lower than the previous dynamic tuning
4. Tune H2O coefficients with the "H2O Test Current" option
   1. **Note that you will need to turn off temperature control for this before starting the tuning process**
5. After tuning and range matching is finished, the machine is prepped and ready to start dynamic CO2 response curves

**Set Conditions**

1. Set chamber conditions in "Environment" window.
   1. We usually use the following settings:
      1. **Flow**: Flow = 500 μmol s-1; ΔPcham = 0.1 kPa
      2. **H2O**: 1.5 kPa (VPDleaf) or 50% relative humidity (1.5 VPDleaf is recommended by LICOR for the LI-6800)
      3. **CO2**: CO2\_r = 420 μmol mol-1
      4. **Fan**: Fan speed = 10000 rpm
      5. **Temperature**: Tleaf = 25.0°C
      6. **Light**: Setpoint = 1600-2000 μmol m-2s-1 (optional: do a light response curve before starting to ensure light saturation)
2. Let chamber stabilize
   1. Note: you may need to set Tleaf and let it stabilize before setting VPDleaf – doing both at the same time may result in high exchanger RH and possible condensation buildup inside console
   2. If RH persists above 90% for more than a few seconds, set RH\_air to 50% and allow the temperature to stabilize and the console to dehumidify before re-attempting to set VPDleaf

**Dynamic CO2 response curves**

1. Clamp a leaf, wait until the leaf acclimates. Usually, a good measure of this is less than a 0.5 μmol m-2s-1 change (A) across 4 minutes, but this is species-dependent
2. Once ready to start a dynamic curve (i.e. a leaf has stabilized), navigate to the BP program selection
   1. Programs -> BP Builder -> Dynamic subfolder
3. Select "DAT\_CO2\_Split.py" and press "Start BP". This will bring up a screen that prompts user to select dynamic curve conditions. The lab has found the following combination to be successful:
   1. Low CO2 = 20 μmol mol-1 (do not put low CO2 to 0 μmol mol-1 as this will cause issues with mixing fan/boundary layer)
   2. High CO2 = 1620 μmol mol-1
   3. Ramp rate: 200 μmol mol-1 min-1
   4. Recovery rate: 90 seconds
   5. Logging interval: 5 seconds (not advised do more frequent logging interval than this on old computer chips)

**Final tasks (closing log files, etc.)**

1. Once finished with curves, close log file by navigating to "Log Setup -> Open a Log File -> Close Log (button on right side of screen)"
2. Turn off all environment settings
3. Turn off machine by navigating to "Start Up -> Power Off"
4. **Remove water column and CO2 cartridge**; leave chamber head gasket slightly ajar (or "park" mode)

**LI-6800 Startup Timeline**

[Basically the Quick Start Guide but letting you know how long each step will likely take]

**T = 0min** => Turn On Machine

* Set Fan = 10000 rpm, Flow = 500 μmol s-1; ΔPcham = 0.1 kPa

**T = 40min** => Warmup Tests

* Turn on Dynamic Equations (Start Up -> Chamber Setup -> Gas Exchange)

**T = 60min** => Open a Log File

* Log Setup -> Open a Log File 🡪 Select your folder or make a new one (“New Folder”), then select “New File”
* Set matching options to Never (Log Setup -> Options)

**T = 80min** => Range Matching

* Turn on CO2 and H2O controls if they have been turned off
* Note **Flow\_s/Flow\_r** in Environment -> Flow using calculator and save value
* Constants -> Range Match
  + Run both CO2 and H2O range matches, ensuring that sd < “Old” value
  + Ensure CO2 range match exceeds 2000 μmol mol-1
  + Ensure H2O range match maximum value exceeds 17-20 μmol mol-1, depending on the set Tleaf you intend to use (higher value for lower temperature) to prevent suspension of H2O range match

**T = 90min** => Dynamic Tuning

* Contants -> Dynamic -> Utilities/Tests (on bottom right)
* Run CO2\_Test\_Current and ensure sd < “Old” value
* Run H2O\_Test\_Current and ensure sd < “Old" value

**T = 95min** => Set Conditions

* CO2\_r = 420 ppm
* Tleaf = [your value]
  + Default is 25°C, but can vary depending on your experiment
* Light (Environment -> Light -> Flourometer, on right side) = [your value]
  + Default is 2000 μmol m-2s-1, but can vary based on light saturation point
* **NOTE: Let temperature stabilize or near stabilization before setting VPDleaf**
* VPDleaf = 1.5 kPa (OR RH\_air = 50%)

**T = 110min** => First Clamp; Measurements Begin

* Select green, unbroken leaf to clamp & note the name of the individual as “id” under Constants -> User
* Ensure the leaf clamped has contact with the diode in the cuvette (only applicable for leaves that do not fill the cuvette)
* Allow chamber & plant to stabilize – gsw varies < 0.3 over 4min, A varies < 0.5 over 4min – then navigate Programs -> BP Builder -> Dynamic subfolder -> "DAT\_CO2\_Split.py"