Sample protocol for obtaining assimilation-temperature (AT) response curves using the Fast Assimilation-Temperature Response (FAsTeR) method with the LI-6800.

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1. Open a log file.
    a. Under Log Files > Logging Options: Select "Also log to Excel file" and "Use additional averaging time" set to 1 sec. We use minimal signal averaging time because we are measuring in non-equilibrium conditions, but using 1 sec averaging time substantially reduces noise compared with no signal averaging.
    b. Under Log Files > Matching Options: Select "Never match" for both CO2 and H2O.
    c. Under Log Files > Fluorometer Options: Select "0: Nothing" from the drop-down menu.
    d. Open a new file to store data in. Under Log Files > Open a log file: Select a folder/make a folder, and press "New File" to make a new logfile. Use desired naming convention for the logfile.
2. Set Environment controls
    a. Flow: Set Flow to 600 µmol s^{-1} and ΔP to 0.1 kPa.
    b. H2O: Set H2O on; set RH to 35%.
    c. CO2: Set CO2 injector to on; set reference CO2R to 420ppm.
    d. Under Environment > Fan: Set mixing fan to on; set fan speed to 10,000 rpm.
    e. Under Environment > Temperature: Set Temperature on; Set Txchg = 0 °C
    f. Under Environment > Light > Fluorometer: Set control mode to "Setpoint"; Set Setpoint to saturating PAR.
3. If Tair <= ambient, insert a leaf and close the chamber, ensuring that the leaf is in contact with the leaf thermocouple.
4. Under Environment > Flow, check that Leak % < 2-3%. (Do not use chamber leak test.) If Leak % is too high, fix leaks by reclamping or sealing around stems/midribs/veins with putty. You may also be able to reduce leak % by manually pressing on the chamber, but this may damage fragile leaves. Once Leak % < 2-3%, proceed.
5. Under Measurements: Monitor A, gsw, Txchg, and Tleaf for a **minimum of 20 minutes**, and then ensure stability using graph zoom functions (flat lines for 5 minutes). Some species may take more than 45 minutes to stabilize.
6. Ensure LI-COR is able to hit all setpoints: Go to the Environment tab, and check each environmental variable that you have set (e.g. RH, CO2R, Txchg) and ensure that the actual value is the same as the setpoint/requested value (or very close).
7. Check ΔCO2 < -8 µmol s-1. If not, reduce flow rate until ΔCO2 < -8 µmol s-1 is achieved, to a minimum of ~250 µmol s-1 (flow in both sample and reference IRGAs must exceed 200 µmol s-1). If ΔCO2 << -8 µmol s-1, increase flow rate to bring ΔCO2 close to (but below) -8 µmol s-1. High flow rates are desirable in this method because they reduce the mixing and transit time for gas in the chamber, which reduces the temporal "mismatch" between the CO2 measurements and the Tleaf measurement. A flow in excess of 1000 µmol s-1 is likely unnecessary.
8. When Txchg is stable at its minimum temperature, set Txchg to its current value.
9. Configure temperature ramp.

a. Under Environment > auto controls, select “Txchg”. Set F(t) to linear. Set F(0) to the current Txchg value and set F(end) to the desired maximum Txchg temp (usually 40 degrees above minimum).
b. Set Period based on desired ramping speed.
    a. If flow >= 600, use 2 degree/min, which is a period of 20 minutes for a 40 degree span. This is the fastest ramping speed that is reliably achievable by the machine.
    b. If using a lower flow rate, use 1.5 degree/min, which is a period of 30 min for a 40 degree span. This helps reduce “mismatch” between measured temperatures and gas exchange rates.
c. **Press the right-facing arrow on the bottom left.** This ensures that you start the ramp from the bottom.

10. Configure autologging program
    a. Under the Auto Programs tab, select AutoLog from the drop-down box.
    b. Select desired logging interval. Logging every 2 seconds is suggested – you can log every 1 s, but this causes the machine to bog down.
    c. Select total time to equal or exceed the Period selected in Step 9. – If using a fast ramping speed, add an extra 5 minutes (total 25 min), and if using a slower ramping speed, add an extra 3 min (total 33 min) to the ramping period to ensure that a complete curve is logged, since often Tleaf lags behind Txchg by a few minutes.
11. Monitor Txchg, A, and gsw using charts (flat line for 5 minutes) and stability metrics (line e, “Stable” = “3/3’). When stability is achieved, proceed.
12. **Match**. Wait for stability.
13. Begin data collection. Go to Environment > Auto Controls and press **start**. Immediately go to Auto Programs and press **start**. The machine will begin ramping the temperature and logging automatically.
14. Wait while data collection is carried out.
15. **Match again at the end and log once** to see difference in match offsets.
17. Close Log file.