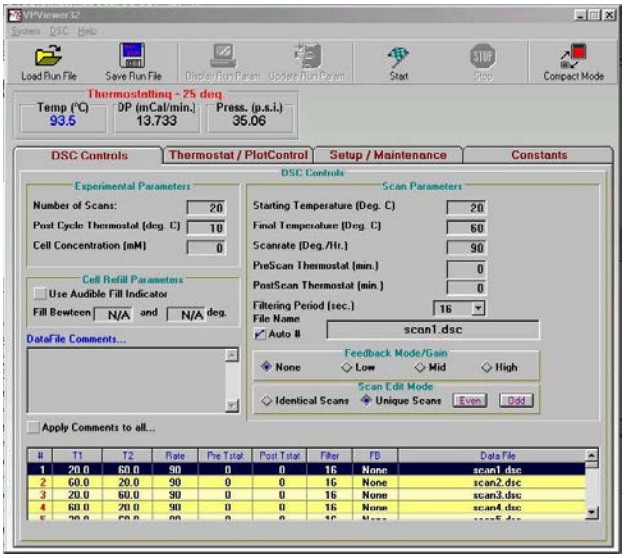
# Simplified MicroCal VP-DSC protocol

**STOP** This protocol is for qualified users operating VP-DSC in IBC 402 only. Dr. Jao accepts no responsibility for actions taken as a result of using this protocol. Reading the manufacturer's User's Manual is highly recommended.

## Sample preparation:

1. For each cell, prepare >750 μL of solution (buffer or sample), degassed at starting temperature for at least 5 min. (1mL is a lot easier for loading).
2. Protein concentrations are usually 0.1 mg/mL to 1mg/mL.
3. Prepare 30-50 mL of buffer for baseline scans and cleaning purpose.
4. Please consult manager if you intend to use non-aqueous solutions.

## Start up:




5. Boot up the control PC.
6. Log in to Windows.
7. Power up the instrument. Do not be alerted if the light on the instrument front panel is off at this point.
8. After several seconds, initialize "VPViewer2000 DSC".
9. The software contains four tabs: **DSC Controls, Thermostat/Calib., Setup/Maintenance** and **Constants.** The description of the tabs can be found in VP-DSC MicroCalorimeter User's Manual Section 3.
10. Basic parameters for a DSC scan may be set up by loading a previously saved setup Run files (.scn) or data files (.dsc).
11. Set jacket temperature according to the starting temperature using thermostat control in the **Thermostat/Calib.** tab.

## Sample loading and start:

12. Drain water from both reference and sample cells using Hamilton syringe. Rinse both cells twice with your buffer.
13. Load buffer (degassed) to both reference and sample cells. Before loading, ***insert the filling tube into the cell reservoir***. Pull 750 μL of buffer into the 2mL Hamilton syringe, slowly Insert the syringe to the bottom of the cell, raise the tip about 1mm and fill the cell until buffer spills out the top of cell stem. Gently oscillate 3 times and stir to remove bubbles. Oscillate again if necessary.

Pictures adopted from Micro VPDSC manual M110SP.pdf



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Slowly raise the filling syringe and remove excess buffer from cell top reservoir. Drain excess buffer using the "filling adjust syringe", which would draw the top 7/8" of solution out of cell stem. Finally, detach the filling tube.

14. Cap the cell port to apply positive pressure (~23psi) by turning the lower part of the cap first then the plastic upper part.
15. Set up experiment parameters and start a set of 20 scans. Please note that a temperature scan of **5-110** ℃ is usually sufficient for most protein samples. Never freeze your sample in filled cells. A pre-scan thermostat period of 15 minutes is recommended.
16. After about 3 scans of buffer/buffer scans (or an overnight experiment of several buffer/buffer scans), check for baseline repeatability. If the baseline is stable, you may refill the sample cell at the appropriate time and temperature (about +5 to +15 of the starting temperature). Refilling reference cell is not necessary, as it might introduce loading errors.
17. After loading, recap the cell port. The cycle should go on continuously.
18. Delete unwanted scans. It requires longer and harsher method to clear out precipitating samples caused by back and forth "cooking".
19. You should rinse Hamilton syringe with buffer and water now.

**Cleaning Procedure:**

The following is adopted from **Section 4.5** of VP-DSC User's Manual.

20. After cells cool down, withdraw your sample from sample cell using Hamilton syringe and check for precipitates. Make sure the filling tube is installed into the cell reservoir before starting manual wash procedure. Buffer rinse is usually adequate between scans if no precipitation occurs.
21. If precipitation occurs or when you are finished, detergent soak of 10% Decon-90 is carried out at 60 ℃ for one hour. ***Make sure the filling tube is installed*** into the cell reservoir before loading detergent solution. The cells are not capped but covered with a damp paper towel. Once cell temperature cools down to room temperature, flush both cells with 250 – 500 mL dd$H_2O$ using cell clean apparatus (VP-DSC User's manual Section 1.5).
22. Fill both cells with degassed dd$H_2O$ and perform water scans to check water baseline. Acidic solution might be needed if the baselines are not stable. Please contact lab manager for assistance.
23. When the cells are clean, depressurize cells and leave the instrument on.
24. For analysis, please refer to the **VP-DSC Data Analysis in Origin® Tutorial Guide.**