Standard Operating Procedure

Thermal shifts (DSF) using the Bio-Rad CFX Connect

Preparing the plate:

Prepare your plate using the supplied plates and plate seals in the cupboard above the instrument. Recommended total well volume = 25 μL (5 μL min – 50 μL max; better results are achieved with ≥ 20 μL).

Suggested protein concentration is ~ 5 μM (> 1 μM for a 20 kDa protein).

Suggested Sypro Orange dilution 1:~2000, e.g. prepare a fresh 1:400 stock of dye (usually supplied as a 5000X stock in DMSO) in buffer, and add 5 μL to each well.

Pipette with care to avoid bubbles, or use a plate-spinner prior to the run.

Press the seal down thoroughly to avoid evaporation losses.

Always load duplicate/triplicate wells.

Turning on the instrument:

Turn on the instrument (at the rear, bottom RH corner) and boot up the PC.

Log in using your research group username and password (e.g. username "<RGH> Group", password: one4<RGH> (where <RGH> are the initials of the research group head, capitalised). Ask Simon Quick (sq204@cam.ac.uk, 33685) or Katherine Stott (ks123@cam.ac.uk, 33669) if your group does not have an account.

The instrument uses LEDs and therefore is ready to use after two minutes of being switched on. No calibration is necessary.

Experiment set-up:

Start the software using shortcut icon on the Desktop: "Bio-Rad CFX Manager".

Select run type "User-defined" in the Start-Up Wizard.

Use the buttons in the bottom left of the panel, "Open Lid" and "Close Lid" to load your plate, ensuring it is properly seated.

First tab – "Protocol"

You have the option to "Create New" or "Select Existing" protocol. If this is your first time using the instrument, then "Select Existing" and choose the protocol called "Starting_Protocol" from the C:\Users\Public\Public Documents\Bio-Rad\CFX\Users\admin\ directory.

Once the protocol is loaded, click the “Edit Selected” button on the right, and use “File” -> “Save As” to make a copy of the file in your own directory (put this in C:\Users\Public\Public Documents\Bio-Rad\CFX\Users\<RGH>\<your name>).

Input the “Sample Volume” in μL. You can also change the starting or final temperatures, temperature increments and settling times (recommended minimum = 30s) by clicking and editing directly in the graphical window or in the table below, hitting return after each edit.

Save any changes.

Click “OK” to close the protocol-editing window.

Click “Next” (bottom right) or select the second tab “Plate”, directly.

Second tab – “Plate”

Again you have the option to “Create New” or “Select Existing” plate. If this is your first time using the instrument, then “Create New”.

Under “Settings”, set the “Plate Type” to “BR Clear”.

In the main panel, set the “Scan Mode” to “FRET”.

In the main panel, you can use the mouse to select sample wells by clicking individual wells or dragging using the LH button to select multiple wells.

Select wells containing the same protein target and set the “Sample Type” to “Unknown”.

Use the “Target Name” to name the wells according to your protein target and check the “FRET” box under “Load”. The wells should now be named in green. Name any different protein targets using the same process.

Select wells containing the same ligand/condition, set the “Sample Names”, and check “Load” to apply them to the selected wells.

The plate can also be edited in “Spreadsheet view”.

Use “File” -> “Save As” to save your plate file in your own directory.

Click “OK” to close the plate-editing window.

NB: The plate can be edited at any time during or after the run if you are short of time and wish to start your experiment straight away. All 96 wells are measured and the data is stored in every run, regardless of the initial user plate settings.

Click “Next” (bottom right) or select the third tab “Start Run”, directly.

Third tab – “Start Run”

Click the button “Start Run” in the bottom RH corner to start the run.

Viewing and exporting the data:

Once the run has started, three tabs are available for following the progress of the run: “Run Status” (progress in time and temperature), “Real-time Status” (data) and “Time Status” (a countdown to the experiment finish).

The data can be viewed as it is generated in “Real-time Status”. Selecting single or multiple wells causes them to appear in the table to the bottom right as well as the melt curve window. Hovering over a well will highlight the relevant data in the melt curve window. Deselecting them causes them to disappear from the window and table (although the data from all 96 wells is *always* stored, regardless of the plate settings or well selections).

NB: Your plate can be edited at any time during or after the run, using the “Plate Setup” button. “View/Edit Plate…” to make your changes, and them “Replace Plate File…” to apply them.

When the run has finished, a new “Data Analysis” window opens. The derivative of the melting curve is now shown in the first “Melt Curve” tab. Set the “Peak Type” to “Negative” for melting transitions resulting in an increase in fluorescence (the norm). All significant minima are then reported in the data table (if a well produces more than one minimum, multiple rows appear in the table). The “Melt Curve Data” tab contains the full data table and the “Custom Data View” allows the user to freely define and arrange a selection of data windows of different types.

Check that replicates show reproducible melting temperatures as a guide to the quality of the data.

The “Export” menu allows exporting in several formats (.xls, .csv …)

Export your processed data as an appropriately-named file in your user group directory.

Data transfer is *via* **clean** USB stick (ports behind the flap at the front of the PC) or *via* the network (e.g. WinSCP).

When you have finished your experiments:

Remove and dispose of your plate.

Exit the software and shut down the PC. Switch off the instrument (switch at rear).

Clean up the bench area.

Please inform Simon Quick (sq204@cam.ac.uk) when stocks of the plates or plate seals are < 10.

Katherine Stott, 13th October 2014