

SOP#: 005	Date Issued: 8/03/2021	Date Revised: 7/08/2021
TITLE:	CFX Connect Real Time PCR	
SCOPE:	Research Personnel	
RESPONSIBILITY:	BORC staff	
PURPOSE:	To outline the proper procedures for use and maintenance of the CFX Connect Real Time PCR.	

1 PURPOSE

This SOP explains how to use CFX Connect Real Time PCR to measure RNA expression level in cells and tissues.

2 RESPONSIBILITY

It is the responsibility of the BORC staff to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

3 BEFORE CONDUCTING YOUR EXPERIMENT

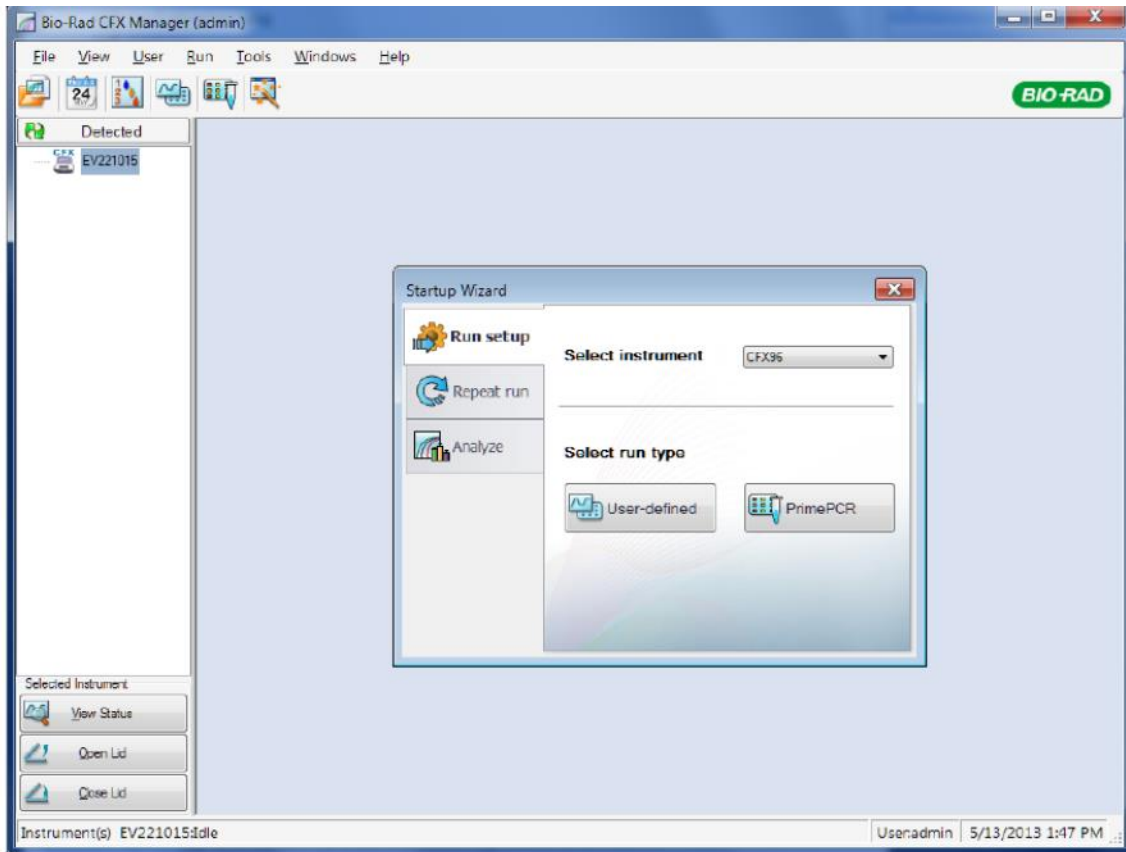
Reserve CFX Connect on iLAB before you preparing for your experiment.

4 PROCEDURES

4.1 Turn on CFX Connect.

Press the power switch on the back panel of the CFX Connect chassis to turn on the system.

Start Bio-Rad CFX manager.



4.2 Load reaction samples in the block.

4.2.1 Click the Open Lid button located on software's Start Run, or press the lid button on the front of the system to start opening the motorized lid.

4.2.2 Place the 0.2 ml microplate or tube strips with sealed lids in the block. Check that the tubes are completely sealed to prevent leakage. Close the lid. For optimal results load sample volumes of 20–25 μ l.

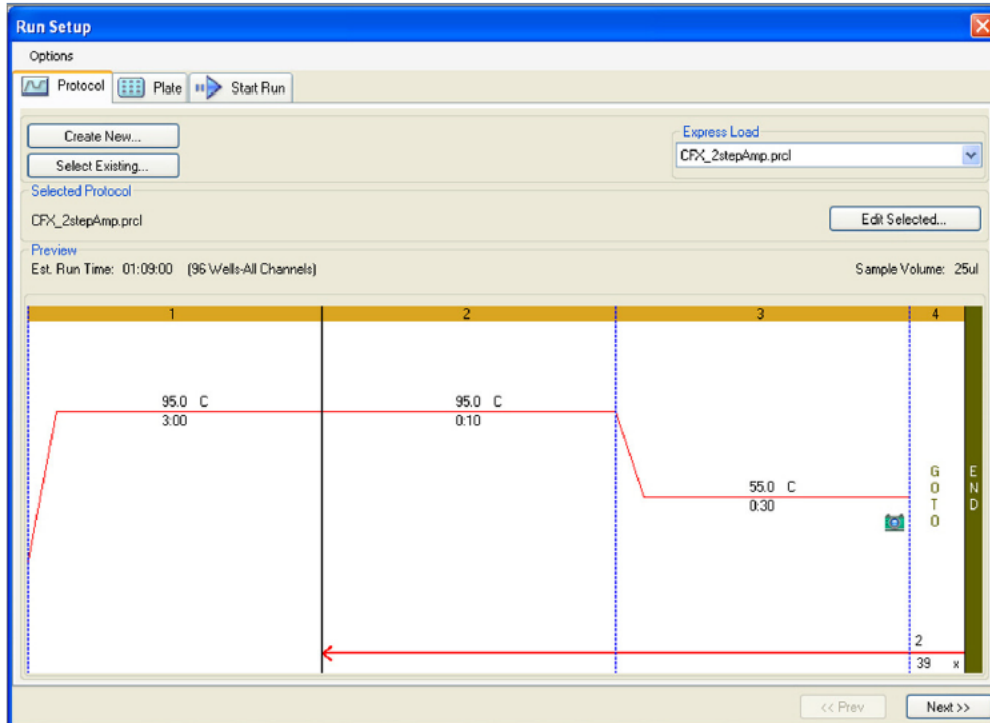
4.3 Run setup

The Run Setup window provides quick access to the files and settings needed to set up and start a run. To open the Run Setup window, perform one of these options:

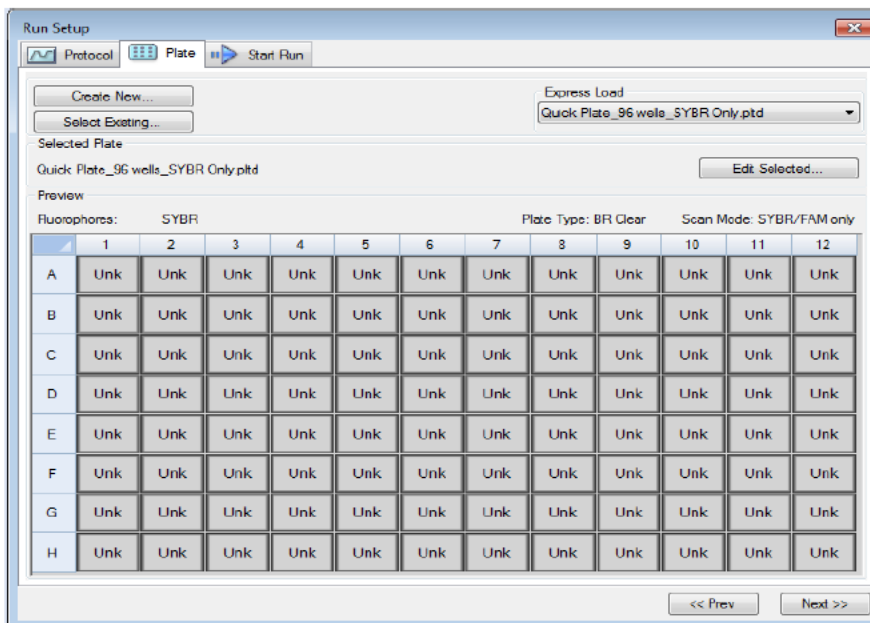
- Click the User-defined or the PrimePCR button in the Run Setup tab of the Startup Wizard.
- Click the User-defined Run Setup or PrimePCR Run Setup button in the main software toolbar.
- Select File > New > User-defined Run or PrimePCR Run in the main software menu bar

The Run Setup window includes three tabs:

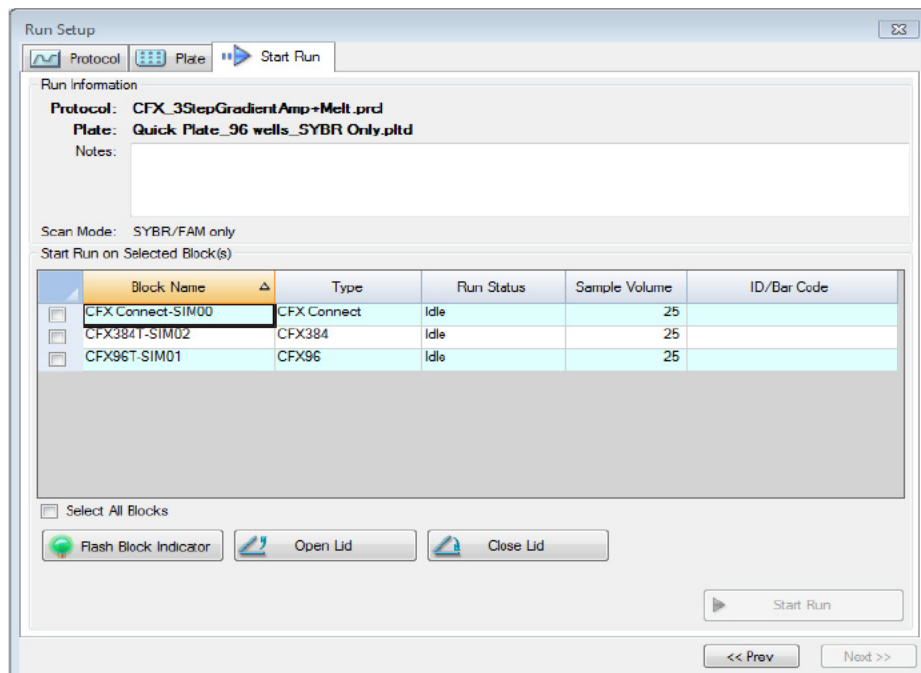
- **Protocol.** Click the **Protocol** tab to select an existing protocol to run or edit, or to create a new protocol in the Protocol Editor window.



- **Plate.** Click the **Plate** tab to select an existing plate to run or edit, or to create a new plate in the Plate Editor window. In a real-time PCR run, the plate file contains a description of the contents of each well, the scan mode, and the plate type. CFX Manager software uses these descriptions for data collection and analysis.



- **Start Run.** Click the **Start Run** tab to check the run settings, select one or more instrument blocks, and begin the run.



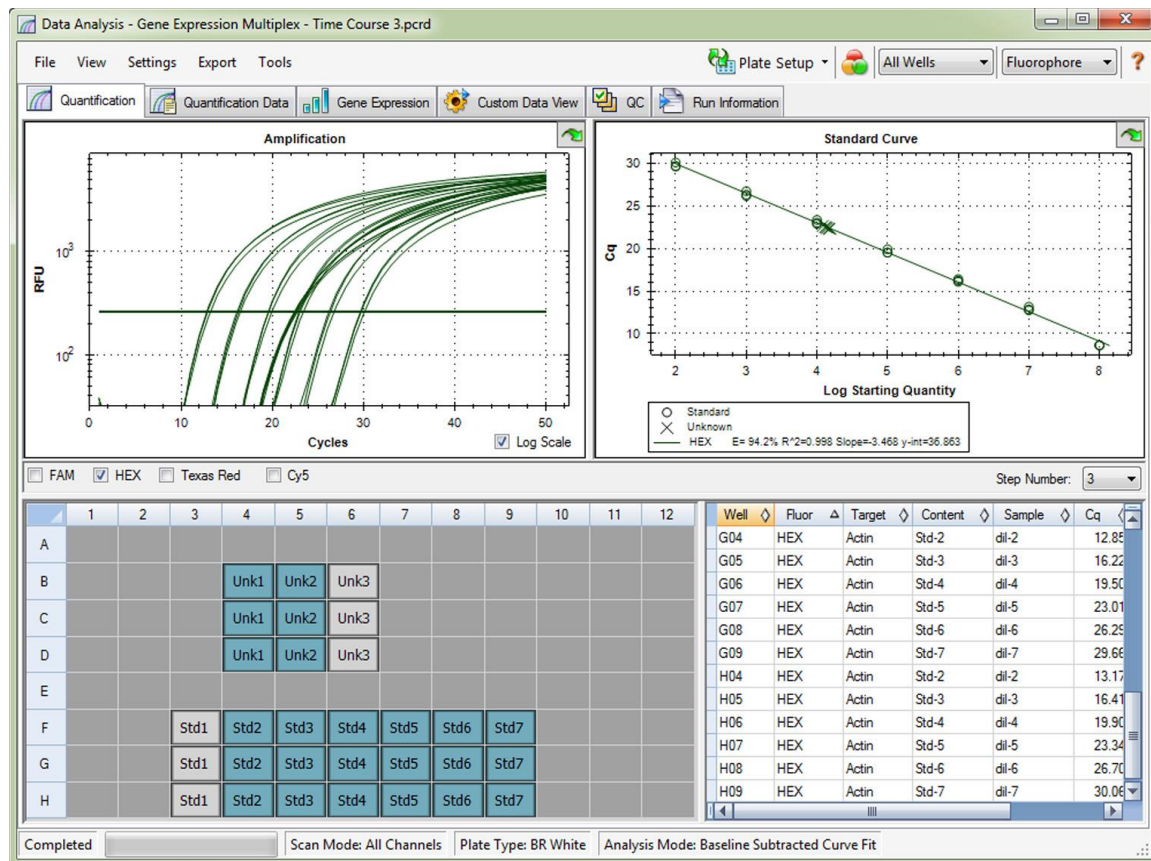
4.4 Data Analysis

4.4.1 Data Analysis Windows

CFX Manager™ software processes real-time PCR data automatically at the end of each run and opens the Data Analysis window to display these data. Choose one of these methods to open existing data files in the Data Analysis window:

- Drag a data file (.pcrd extension) over the main software window and release it
- Select Analyze tab in the Startup Wizard window and either select from Recent Files or click the Browse button to find.
- Select File > Open > Data File in the main software window to select a file in the Windows browser
- Click the Data Analysis button in the main software window toolbar to select a file in the Windows browser
- Select File > Recent Data Files to select from a list of the ten most recently opened data files

During data analysis, changing the way the data are displayed by changing the contents of the wells in the Plate Editor never changes the fluorescence data that were collected from each well during the run. Once the module collects fluorescence data, you cannot delete those data but you can choose to remove data from view and analysis.



4.4.2 Data Analysis Settings

The Amplification chart data in the Quantification tab show the relative fluorescence (RFU) for each well at every cycle. Each trace in the chart represents data from a single fluorophore in one well. These data are used to determine Cq values for each well on a per fluorophore basis.

Select modes to determine Cq values:

The software uses one of two modes to determine Cq values:

- Regression. This mode applies a multivariable, nonlinear regression model to individual well traces and then uses this model to compute an optimal Cq value
- Single Threshold. This mode uses a single threshold value to calculate the Cq value based on the threshold crossing point of individual fluorescence traces

Select Settings > Cq Determination Mode to choose the Cq determination mode.

Adjusting the Threshold

- Select one fluorophore in the fluorophore selector in the Quantification tab by clicking the boxes next to the fluorophore name located under the Amplification chart.
- Select **Settings > Baseline Threshold...** in the menu bar to open the Baseline Threshold window.
- Adjust the crossing threshold (Figure 53) for the fluorophore by clicking **User Defined** and entering a threshold number.
- Click **OK** to confirm the change and close the window

Baseline Settings

Select Settings > Baseline Setting to choose one of these three options:

- No Baseline Subtraction. The software displays the data as relative fluorescence traces. Some analysis is not possible in this analysis mode and therefore the software does not display the Gene Expression, End Point, and Allelic Discrimination tabs.
- Baseline Subtracted. The software displays the data as baseline subtracted traces for each fluorophore in a well. The software must baseline subtract the data to determine quantification cycles, construct standard curves, and determine the concentration of unknown samples. To generate a baseline subtracted trace, the software fits the best straight line through the recorded fluorescence of each well during the baseline cycles and then subtracts the best fit data from the background subtracted data at each cycle
- Baseline Subtracted Curve Fit. The software displays the data as baseline subtracted traces and the software smoothes the baseline subtracted curve using a centered mean filter. This process is performed so that each Cq is left invariant

4.4.3 Melt Curve Tab

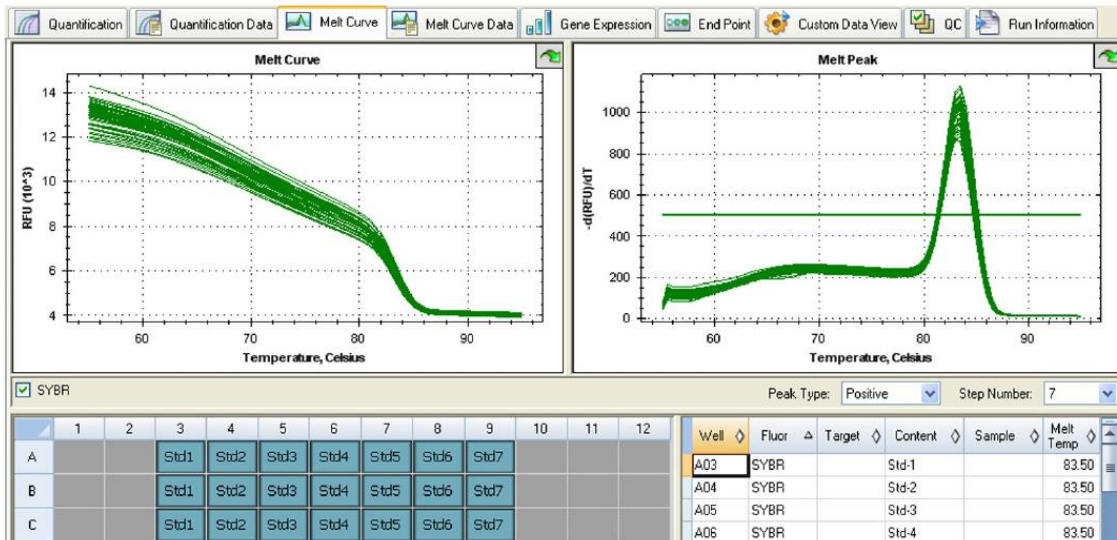
For DNA-binding dyes and noncleavable hybridization probes, the fluorescence is brightest when the two strands of DNA anneal. Therefore, as the temperature rises towards the melting temperature (T_m), fluorescence decreases at a constant rate (constant slope). At the T_m , there is a dramatic reduction in the fluorescence with a

noticeable change in slope. The rate of this change is determined by plotting the negative first regression of fluorescence versus temperature ($-d(\text{RFU})/dT$). The greatest rate of change in fluorescence results in visible peaks and represents the T_m of the double-stranded DNA complexes.

The software plots the RFU data collected during a melt curve as a function of temperature. To analyze melt peak data, the software assigns a beginning and ending temperature to each peak by moving the threshold bar. The floor of the peak area is specified by the position of the melt threshold bar. A valid peak must have a minimum height relative to the distance between the threshold bar and the height of the highest peak.

Open the Melt Curve tab (Figure 68) to determine the T_m of amplified PCR products. This tab shows the melt curve data in these four views:

- Melt Curve. View the real-time data for each fluorophore as RFUs per temperature for each well
- Melt Peak. View the negative regression of the RFU data per temperature for each well
- Well selector. Select wells to show or hide the data
- Peak spreadsheet. View a spreadsheet of the data collected in the selected well



4.4.4 Create a Data Analysis Report

To create a report in the Data Analysis window, follow these steps:

- Make final adjustments to the well contents, selected wells, charts, and spreadsheets in the Data Analysis window before creating the report.
- Select Tools > Reports in the Data Analysis menu bar to open the Report window.
- Change the options you want to include in the report. The report opens with default options selected. Click the checkboxes in the report options list to change whole categories or individual options within a category.

- The ordering of categories and items within a report can be changed by clicking and dragging these to the desired relative position. Items can be reordered only within the categories to which they belong.
- Click the Update Report button to update the Report Preview with any changes.
- Print or save the report. Click the Print Report button in the toolbar to print the current report. Select File > Save to save the report in PDF (Adobe Acrobat Reader file), MHT (Microsoft document), or MHTML (Microsoft document) file format and select a location to store the file. Select File > Save As to save the report with a new name or in a new location.

4.5 Shut Down the System

After a run, click the open lid button on the front of the system to access the samples loaded in the thermal cycler block. Remove the samples from the block and click the close lid button to close the lid. Press the power switch on the back panel to power down the system.

5 MAINTENANCE

- Always clean the outside of any containers before placing them in the block
- Never run a reaction with a seal that is open, loose, punctured, or otherwise damaged because you could contaminate the block, inner lid, and optical system
- Never run a PCR or real-time PCR reaction with volatile reagents that could explode and contaminate the block, inner lid, and optical system
- Clean the block and inner lid periodically to prevent the buildup of dirt, biohazardous material, or fluorescent solutions.
- Never clean or otherwise touch the optical system behind the heater plate holes in the inner lid.
- Clean the outer lid and CFX Connect base on a regular schedule.

6 REFERENCES

Refer to the manufacturer's manual for additional information.

END OF DOCUMENT