Bauer Core Standard Protocol		
Title: CFX Touch Real-Time PCR Detection Systems Protocol		
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1. Purpose: Navigate and set up qPCR experiments using the Bio-Rad CFX Manager Software for CFX Touch real-time PCR instruments (available in 96 & 384 well formats)

2. Materials

All materials (qPCR plate, seal, set-up master mixes, etc…) provided by user.

Consumables: low profile plates (skirted, semi-skirted, or non-skirted) with no raised lip around edge

Master Mix: no reference dye needed (if mix contains ROX it is okay, just ignore signal)

3. Instrumentation (available in core facility)

CFX96 Touch: ramps at 5°C/sec, 5 colors

CFX384 Touch: ramps at 2.5°C/sec, 4 colors

4. Software: Run, Protocol, Plate Setup

4.1. Bio-Rad CFX Manager

4.1.1. Open license, can be found at \\rcstore.02.rc.fas.harvard.edu\data\Softwares

4.2. Open Bio-Rad CFX Manager Software from Desktop

4.3. Login under user name (user name created during training)

Setup Run

4.4. From the Startup Wizard under the "Run setup" tab select "User-defined" for run type

4.5. Run set-up window will open

Figure 1. Startup Wizard Menu

Choose Protocol

4.6. Select or create a protocol

4.6.1. To create a NEW protocol: Select "Create New" (1.): This will open the Protocol Editor to write your protocol

4.6.2. To choose an EXISTING protocol: Choose "Select Existing" (2.) if you have a saved protocol: This will open the file browser to choose file to load

4.6.3. Use the "Express Load" (3.) drop down menu if you have saved a protocol to this menu

4.6.4. To EDIT an existing protocol: Choose "Edit Selected" (4.) and the editing window will open

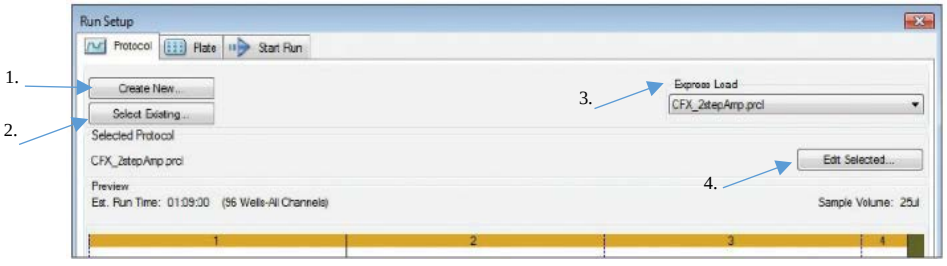


Figure 2. Run Setup Menu

Using Protocol Editor

4.7. Protocol editor is used when creating or editing a protocol

4.7.1. Change sample volume: enables cycling to quickly change temperature.

4.7.2. To edit a step: make sure step is highlighted blue then edit parameters for that step.

4.7.1.1. To edit the parameters be sure to click and select (highlight blue) the temperature or time you want to edit.

4.7.3. Insert step: to insert a step after highlighted step

4.7.4. Delete step: to delete highlighted step

4.7.5. Insert Gradient: Set rows at defined temperature increments

4.7.5.1. User can set a range (10 degrees in figure 4) and then system will auto calculate increments based off this desired range and starting temperature.

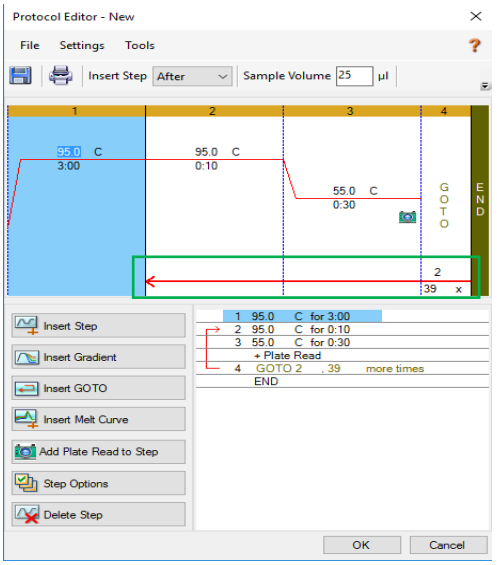


Figure 3. Protocol Editor Menu

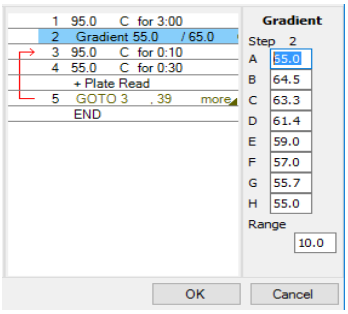


Figure 4. Gradient step in protocol edit menu

4.7.6. Insert GOTO: inserts a step to repeat cycles up to desired step.

4.7.6.1. In figure 3 GOTO is to Step 2 with 39 repeated cycles, 40 cycles total.

4.7.7. Insert Melt Curve: inserts dissociation/melt curve after highlighted step.

4.7.7.1. Default melt curve is normal 2 step, however, reading time and temperatures can be modified.

4.7.8. Add Plate Read to Step: designates when fluorescence data will be acquired during protocol.

4.7.8.1. Will change to “remove plate read” if highlighted step is a plate read step. Select to remove step.

4.7.9. Step Options: Menu with options to change opens. (Figure 5)

Note: Do not select “Beep” option as this will cause machine to beep at end of this step.

4.7.10. Estimated run time: will update as you edit the protocol.

4.7.11. Select “Next” at bottom right corner to move to plate setup.

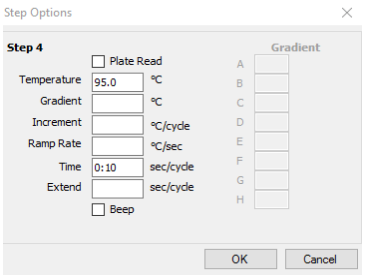


Figure 5. Step Options menu

Choose Plate

4.8. The “Plate” tab displays the plate layout for the run.

4.9. Select or create a plate

4.9.1. To create a NEW plate: Select “Create New”: This will open the Plate Editor to create your plate layout.

4.9.2. To choose an EXISTING plate: Choose “Select Existing” if you have a saved plate: This will open the file browser to choose file to load

4.9.3. Use the “Express Load” drop down menu if you have saved a plate to this menu

4.9.4. To EDIT an existing plate: Choose “Edit Selected” and the editing window will open

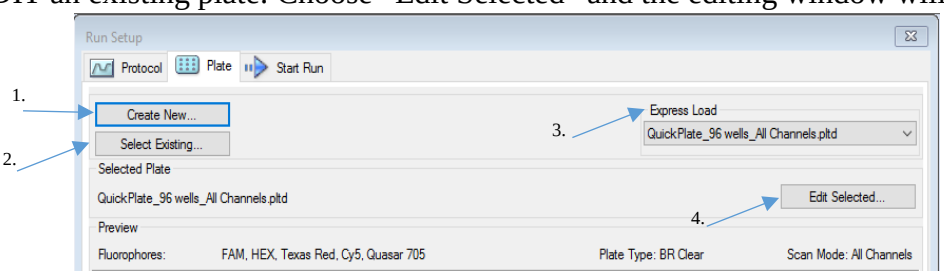


Figure 6. Plate Setup Menu

Using Plate Editor

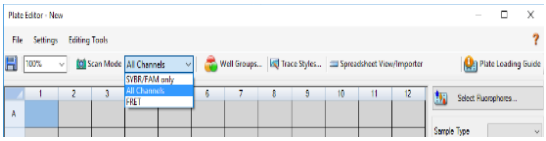


Figure 7. Plate Editor Menu

4.10. Set Scan Mode: Select from dropdown menu (Fig.7)

4.11. Select Fluorophores: indicate which to use in the run (Fig.8)

4.11.1. Default is SYBR

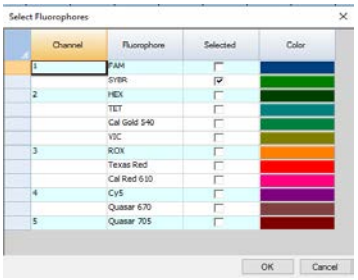


Figure 8. Select Fluorophore Menu

4.12. Select wells to load in plate diagram. (See Fig. 9 for 4.12-4.17)

4.13. Select sample type from dropdown menu.

4.14. Check off fluorophores to load in selected wells.

4.15. Indicate “Target Name” by typing and press enter or select existing.

4.16. Type sample name of selected wells and press enter (required for gene expression analysis).

4.17. For gene expression analysis, select “Experiment Settings…” to assign reference targets and controls.

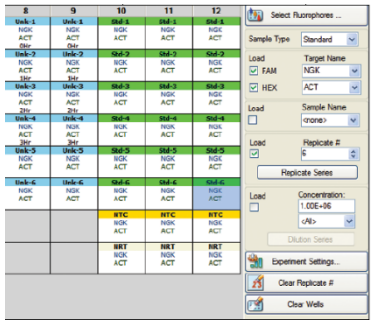


Figure 9. Plate editor figure from Bio-Rad Software Plate Quick Guide

4.18. Set Replicates: can be done individually or in subsets (duplicates, triplicates, etc…)

4.18.1. Individually: Select wells (using Ctrl key) and input replicate number manually in replicate # box press enter to load.

4.18.2. Subsets: Select wells (using Ctrl key or Shift key) and select “Replicate Series”

4.18.2.1. Input replicate group size, starting replicate number, and orientation of loaded samples (horizontal/vertical) Fig. 10. Select Apply to set.

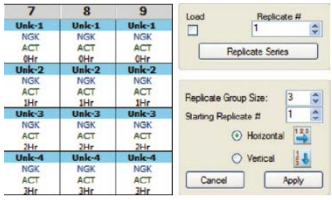


Figure 10. Replicate series figure from Bio-Rad Software Plate Quick Guide

4.19. Create a Standard Curve (Fig. 11)

4.19.1. Select standard wells (usually with replicates) and select dilution series.

4.19.2. In dilution series window, enter:

4.19.2.1. Starting concentration

4.19.2.2. Number of replicates (# of standards used)

4.19.2.3. Dilution factor

4.19.2.4. Whether the dilutions are increasing or decreasing

4.19.3. Select Apply to set.

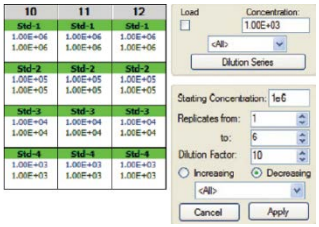


Figure 11. Standards figure from Bio-Rad Software Plate Quick Guide

4.20. Create Well Groups: groups to be analyzed independently

4.20.1. Select Well Groups at top of plate editor, window will open

4.20.2. Select Add to create new group and select wells to add to group.

4.20.3. Select OK to return to plate editor.

4.21. Select OK once plate is completed and save plate

4.22. Select Start Run

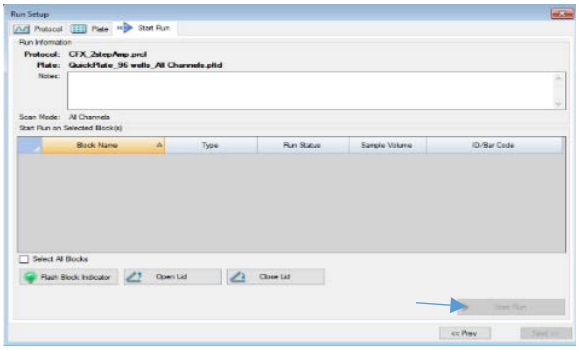


Figure 12. Start run tab

5. Software: Analysis of run

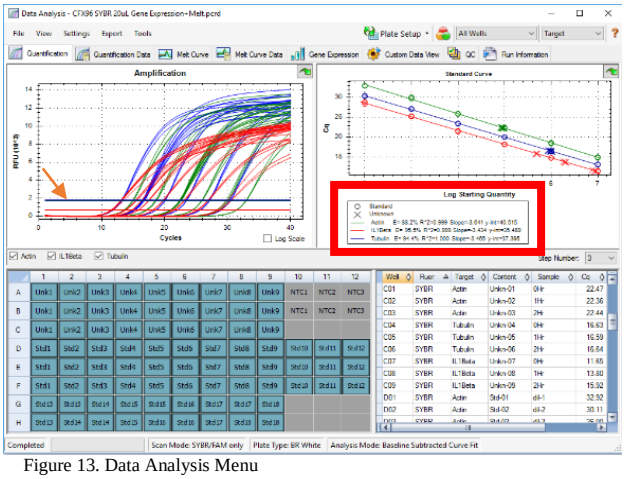


Figure 13. Data Analysis Menu

5.1. Data Analysis window will open after run is complete.

5.2. Check Efficiency (E) & R^2 value to make sure they are within expected specs (Red box, Fig. 13)

5.3. Cq Determination Mode

- 5.3.1. Under "Settings" → Cq Determination Mode→Single Threshold or Regression
  - 5.3.1.1. To set single threshold drag threshold line (orange arrow Fig. 13)

5.4. Baseline Settings

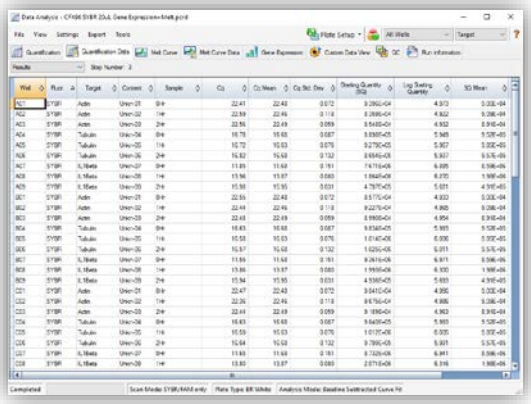
- 5.4.1. Under "Settings" → Baseline Settings→Choose between no baseline, baseline subtracted, and baseline subtracted curve fit.

5.5. Tab Descriptions:

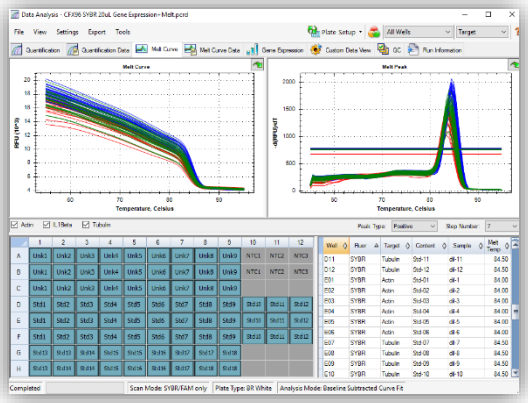
- 5.5.1. Quantification: compiled overview of run, includes plate layout, standard curve, amplification plots, and a small window of data. (Fig. 13) To exclude any samples from analysis, select well(s) → right click → choose exclude wells from analysis.
- 5.5.2. Quantification Data: data from run, can export data into various desired formats (Fig. 14a)
- 5.5.3. Melt Curve: compiled overview of dissociation data (Fig. 14b)
- 5.5.4. Melt Curve Data: data from run, can export data into various desired formats (Fig. 14c)
- 5.5.5. Gene Expression: Basic analysis tools and results such as bar chart (Fig. 14d)
- 5.5.6. Custom Data View: Choose all the data you would like to see on one page (Fig. 14e)
- 5.5.7. QC: displays any flagged QC samples (Fig. 14f)
- 5.5.8. Run Information: details of run protocol information (Fig. 14g)

5.6. Export Data

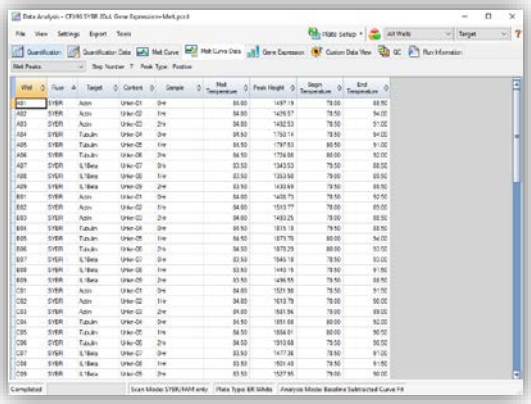
- 5.6.1. From top menu tab select Export dropdown menu → Select custom export and choose from list all the items you want to export into a file type of your choice or choose to export all data sheets (select type)
- 5.6.2. Alternatively, from Quantification Data menu, right click and select export to Excel (file will open once selected)



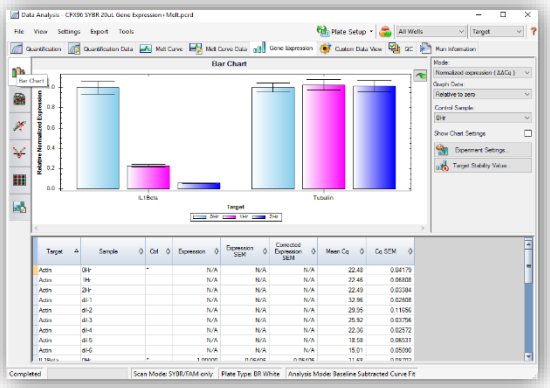
A. Quantification Data



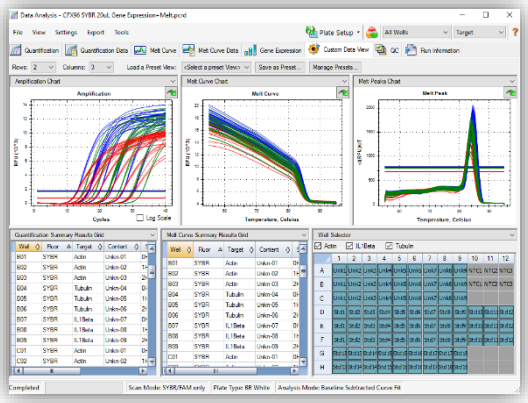
B. Melt Curve



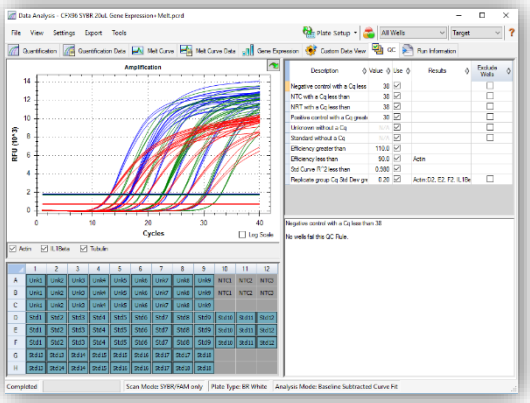
C. Melt Curve Data



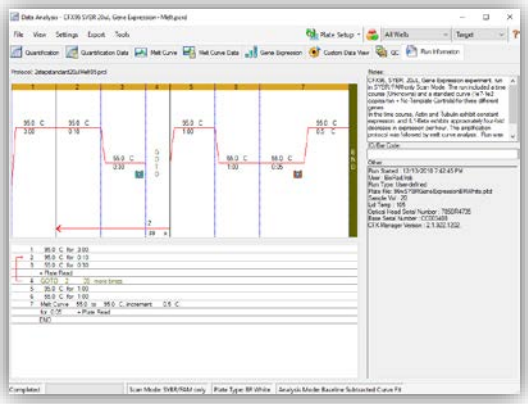
D. Gene Expression



E. Custom Data View



F. QC



G. Run Information

Figure 14. Tab Description Images