Bauer Core Standard Protocol						
Title: CFX Touch Real-Time PCR Det	Detection Systems Protocol					
Pages: 4	Revision: 1.0	Date: 2/13/17				
Author(s): Nicole Che El-Ali	Reviewers: Claire I	Hartmann, Patrick Dennett				
Contact: ncelali@fas.harvard.edu	Comment:					

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 <u>\\rcstore.02.rc.fas.harvard.edu\data\Softwares</u>
- 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



of 5

Choose Protocol

4.6. Select or create a protocol

Figure 1. Startup Wizard Menu

- 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

3. CFX_2stepAmp.prcl
Edit Selected
4.

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 figure4) and then system will auto calculateincrements based off this desired range andstarting temperature.
- 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.

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

Run Setup			23
M Protocol	💷 Plate 🕪 Start Run		
1. Create M	ew	Express Load	
Select Ex	sting	GuickPlate_96 wells	_All Channels.pltd V
Selected Plate			Ethol
QuickPlate_9	wells_All Channels.pitd	4.	Edit Selected
Preview Fluorophores:	FAM, HEX, Texas Red, Cy5, Quasar 705	Plate Type: BR Clear	Scan Mode: All Channels
Figure 6.	Plate Setup Menu		

Protocol Editor - New

3.00

Delete Step

File Settings Tools

lnsert Step After

Figure 3. Protocol Editor Menu

	1	95.0	С	for 3:0	0			Gradient
	2	Gradi	ient 5	5.0	/ 65	5.0	Ste	o 2
\rightarrow	3	95.0	C	for 0:1	0		Δ	55.0
	4	55.0	C	for 0:3	0		2	
		+ Plat	e Rei	ad			в	64.5
	5	GOT	D 3	, 39		more	С	63.3
		END					D	61.4
							Е	59.0
							F	57.0
							G	55.7
							н	55.0
							Rar	nge
								10.0
					OF	<		Cancel

Figure 4. Gradient step in protocol edit menu

Sample Volume 25 μl

GOTO

OK Cancel

95.0 C

Step 4				Gradient
	Plate P	lead	A	
Temperature	95.0	°C	в	
Gradient		°C	С	
Increment		°C/cyde	D	
Ramp Rate		°C/sec	E	
Time	0:10	sec/cycle	F	
Extend		sec/cycle	G	
	Веер	1	Н	

Figure 5. Step Options menu



Using Plate Editor

- 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
- 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.
- 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.
- 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.
- 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



 Select Ruorophores
 X

 Ownel
 Ruorophore
 Selected
 Color

 1
 74M
 Г
 F

 2
 HK
 Г
 F

 2
 HK
 Г
 F

 2
 HK
 Г
 F

 3
 ROM
 Г
 F

 4
 Cy5
 Г
 F

 4
 Cy5
 Г
 F

 5
 Quaser 705
 Г
 F





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



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



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

Figure 12. Start run tab

5. Software: Analysis of run

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" \rightarrow 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)

14. 1	tes Sett	a beat	Tools					61 Plate	Setup + 📇 AES	iele -	Target	
1	atom 1	and -	ten Data La	Net Cane	A Matter	e Dea	al free box	-	on Data View Links on	A Beiten	1	
un,tu	at the second	- Sap)	uter 1				-	and the second				
Wel	0 R.0	a Tarpel	d Crowt	0 Sarate	0 0	a. 0	OsNen Ø	Callet Dev ()	Desirg Question of	Log Setting 0	SO Mean	1
N01	sman	Actin	Uneral	04		22.41	22.43	0.072	8 2862-64	430	3.032-04	
R2	SYMP	Acte	Union-32	114		22.59	22.45	0.118	8.2895-04	4.822	3,288,-04	
A01	SYBR	Ade	Union-20	24		22.55	22.49	0.059	8.5485-01	4.932	8.910-04	
AG4	5784	Takan .	Unio-56	.04		16.78	15 68	0.087	11905-05	5.945	\$ 528-45	
ACS .	SYDE	Salain	Univ-35	110		10.72	91.63	0.079	92790-05	5.907	1.0205	
N/K	(FYBE)	Tubulin .	Union-DG	24		16.82	15.58	0 132	24545-08	\$.907	\$55-05	
ACT .	STUP	1,16+10	UNIT-27	34		11.85	11.68	0.151	76716-06	6.005	8,596-06	
ACE.	15Y8R	3. Mate	Unio-08	114		13.94	13.87	0.083	13645-05	6.270	1.995-06	
A(5	SYDE	8.18ets	UH-00	214		15.98	11.95	0.601	47975-05	5.671	4.510-05	
100	STOP	/ide	Union DR	0+		22.55	22.48	0.012	85170-04	4,902	5.000-04	
912	SYBR	Acte	Union-02	14		22.44	22.46	0118	82270-04	4.965	1.08-04	
803	SYDE	Atte	Ution-30	24		22.48	22.49	0.059	1,9905-01	4.954	6.915-04	
NG4	51/56	Table	Uner-58			15.41	10.42	9.687	11145-05	5.993	5.528+00	
005	SYDE	Table	Unio-75	118		1558	15.03	0.076	10145-00	6.000	0.002-05	
000	SYUE	Tubuin	Union OG	24		15.57	95.68	0.132	10255-06	6.011	158-25	
124	SYBE	1,16412	Unor-37	0.4		11.55	11.62	0.161	82616-05	6.971	8.506-06	
808	5786	1.1840	Uner-58	114		13.84	13.87	0.080	1 9995-08	6.900	1996-04	
809	5784	2.18eta	04-08	218		15.94	15.95	0.631	4 9385-05	5.883	4.915-05	
C21	5100	Acto	Unier-01	01		22.47	22,43	0.072	30410-04	4.955	2.002-04	ã
012	SYIP	Acto.	Union-32	THE .		22.26	22.46	0.118	85756-04	4305	3.08-04	
000	SYBR	Adm	Uner-00	24		22.44	22.49	0.059	8 1890-01	6.902	1,910-04	
024	SYM	7.0.00	Uikr-58	04		15.61	95.63	3 687	10431-05	5.905	5.528-405	
025	SYM	-date	UN-75	116		15.55	10 29	0.070	10127-06	6.005	1 077-05	1
CDG	SYDE	Tubun	Univer-DG	24		15.64	15.68	0.102	3 7995-05	5.901	\$53,45	8
CET	SY84	2,164ta	Uner-07	24		1185	11.68	0.181	8 7326-08	6.911	5.5%-05	đ
000	\$786	K, 18eta	Uner-38	1147		13.80	13.87	0.085	25715-08	6.376	1.905-05	đ
						-						-

A. Quantification Data

FM. 16	in Set	ingi lipor	Teen				the rises a	etup + 🍣 AttWels	· · Targe	n 93
and Ques	Acaim.	Cartho Cartho	nier Cata 🛃 I	Net Carve 🛃 H	at Gave Data	A Gen Luna	en 😻 Casio	Data Hev 🐏 GC 🐔	flue Homation	
like Peaks		- 3m	Neter 7 Peak	Type Postaw						
Well	- Fiyar	4 Taget	O Curtere D	Sample 0	Nat 0	Peak Height 👌	Soph 0	End O		
401	SYER.	Am	Union-Ot	04	\$1.00	1497.18	78.00	88.90		
#82	SYER.	Am	Univ-C2	114	84.95	1425.57	78.50	94.00		
483	SYDR	Adin	Unic-CD	21	54.00	1432 53	78.50	51.05		
484	SYER	Tubulin	Urier-04	24	84.50	1750.14	78.50	20.16		
405	SYER	Tubuin	Unice-05	lie .	01.50	(797 53	80.50	91.02		
404	SYER	Tab.in	Unior-Of-	24	84.50	1726-08	88.00	90.00		
A07	SYDR	6.10ex	Unic-07	24	03.50	1043.50	79.50	88.55		
108	SYER	£18mm	Unio-00	lie .	\$3.50	1263.98	7940	13.50		
429	SYER	L'Bea	Unior-09	24	\$3.52	1430.69	73.50	88.50		
881	SYER	Am	Unio-01	5+	84.05	1458 75	78.50	92.55		
E02	SYER	Adm	Uniciti	519	54.00	1513.77	78.00	10.01		
603	SYER	Azin	Unio (0)	2+	94.92	1480.25	79.00	18.55		
804	SYER	Fubulin	Unior-04	04	58.50	1815 18	79.50	13.55		
805	SYEE	Tain	Union-05	lie .	84.50	1875.70	80.00	54 OZ		
806	SYER	Take	Unio-00	24	54.50	1879.25	88.00	93.02		
807	SYER	L'úra	Unio-07	0+	02.50	1045 18	78.50	30.08		
800	SYER	1,18em	Unior OE	118	83.50	1443.15	79.50	91.00		
809	SYER	Libes.	Univ.25	24	43.50	1496.55	78.50	88.50		
C91	SYER	Am	Une-01	0+	84.05	1521 98	78.50	9150		
092	\$100	Adin	Unie (2)	11er	94,00	1613.79	78.00	20.00		
C83	SYER	Adin	Unio-33	24	\$4.00	1521.56	78.00	10.05		
C94	SYER	Tutuin	Unker-04	0e	\$4.50	1851.00	88.00	92.00		
C\$5.	SITR	Taulm	Unice-00	De .	84.50	1854.01	88.00	90.50		
C86	STER.	Tabulin	U-Mr-00	24	04.50	1913.68	79.50	90.50		
087	\$158	L'Ben	Unier-07	0+	\$3.50	1477.36	78.50	91.00		
008	SYER	L'Bes	Uner-OI	19	\$3.52	1901.40	78.50	91.50		
C09	TYER	8.18eia	Uki-OS	2+	83.50	1527.95	79.00	90.00		
emplated			Scan Mi	Ide SYSRUBAM and	Plate Topo B	it unto Analy	us Mode Baude	a Subtracted Curve Fit		

C. Melt Curve Data



E. Custom Data View



B. Melt Curve



D. Gene Expression



F. OC 😋 Rate Selap - 🍮 Al Web - Tayat Luon Nob 🚮 Quantification () and () an Godon Data har 🗟 ac 🏟 Anithme 950 C 950 C 95.0 C 550 C 56.0 C 230 94.0 C 0.05 **I**DI 6A.0 C 1-00 1 80 C for 300 1 80 C for 300 1 93 C for 30 1 93 ar Mode 1988/SIM only Pate Type IP White Stealy is Mode Baselin

G. Run Information