

IQ5

# Run set up

Protocol must be defined BEFORE run  
please check [your kit manual](#) for guidelines

Plate Layout can be edited after the run

# Run set up: protocol

1. open the software and select Protocol

2. you can edit existing protocol or create a new one

The screenshot shows the Bio-Rad iQ5 software interface. The 'Protocol' tab is selected in the top navigation bar. The 'Selected Protocol' section is highlighted with a green border. The 'Selected Plate Setup' section is also visible, showing a 96-well plate layout with various colored wells (green, blue, red, yellow) indicating different sample types or conditions. The 'Selected Data File' is 'Data 2014-10-15 1434 Anna.opd'. The 'Notes' section is empty. The 'Run' button is visible. The 'Selected Protocol' section shows a table with columns for Cycle, Repeats, Step, Dwell Time, Setpoint, and PCR / Melt Data Acquisition. The table has two rows: Cycle 1 with 1 repeat, Step 1, Dwell Time 7:00, Setpoint 95.0; and Cycle 2 with 40 repeats, Step 1, Dwell Time 0:10, Setpoint 95.0.

Cycle	Repeats	Step	Dwell Time	Setpoint	PCR / Melt Data Acquisition
1	1	1	7:00	95.0	
2	40	1	0:10	95.0	

# Run set up: protocol

3. type in your protocol

to add a white row  
(step) click on + on a  
white row

to add a blue row  
(cycle) click on = on a  
blue row

Bio-Rad iQ5 (admin)

File View Reports Tools Help

Setup Plate Summary

Editing Protocol: group 2.tmo

Save & Exit Protocol Editing

Cancel & Exit Protocol Editing

Cycle 1		Cycle 2 40X		Cycle 3 71X
Step 1	Step 1	Step 2	Step 1	
95.0	95.0	60.0	60.0	
7:00	0:10	0:30	0:10	

to add a white row (step) click on + on a white row

to add a blue row (cycle) click on = on a blue row

Workshop

Run-Time Central

Data Analysis

Calibration

User Profile

BIO-RAD

Show Options

SHOW ALL Infinite Hold Temperature Change Cycle Description

Gradient Ramp Rate Time Change Step Process

Options	Insert	Delete	Cycle	Repeats	Step	Dwell Time	Setpoint	PCR / Melt Data Acquisition	Temperature Change	End Temperature	Begin Repeat	How Often?
...	+	X	1	1								
...	+	X			1	7:00	95.0					
...	+	X	2	40								
...	+	X			1	0:10	95.0					
...	+	X			2	0:30	60.0	Real Time				
...	+	X	3	71								
...	+	X			1	0:10	60.0	Melt Curve	0.5	95.0		

Real Time

Melt Curve

PCR

None

# Run set up: plate

4. click on edit plate or create new

The screenshot displays the Bio-Rad iQ5 software interface. The 'Plate Summary' tab is active, showing the 'Selected Plate Setup' section. This section includes buttons for 'Edit' and 'Create New', and radio buttons for 'Original' and 'Current'. Below these, the 'Sample Volume' is set to 20ul, 'Seal Type' is Film, and 'Vessel Type' is Plates. A color-coded plate layout grid is shown, with columns 1-12 and rows A-H. The grid is color-coded by well: green for SYBR, blue for SYBR1, magenta for SYBR2, red for SYBR3, and dark red for SYBR4. The 'Edit' button in the 'Selected Plate Setup' section is highlighted with a green border, and an arrow points to it from the text '4. click on edit plate or create new'.

**Selected Protocol:** group 2.tmo within Data 2014-10-15 1434 Anna.opd

Cycle	Repeats	Step	Dwell Time	Setpoint	PCR / Melt Data Acquisition
1	1	1	7:00	95.0	
2	40	1	0:10	95.0	

# Run set up: plate

you can type in the names of your samples here. **Sic!** if you want to rename them after run you will need to do it in Gene expression tab

7. select wells with standards or other type of samples and annotate them using this tools from this panel

you can see here an analogue of the excel file I asked you to make

9. type in your reactions volume

4. if you can assign each target as individual fluorophore

5. define whether your replicates are horizontal

6. define how many replicates you have

7. define how do you want to number your replicates

8. add info about your standards dilutions and units you are using  
**Sic! if you do not add concentration for dilutions the software will not save the plate**

The screenshot shows the 'Plate Summary' tab of a software interface. At the top, there are tabs for 'Setup' and 'Plate Summary'. Below these, there's a section for 'Editing Plate' with a text field for the plate name ('BioRadDefaultNewPlate.pts') and a 'Notes' field. To the right of this are fields for 'Sample Volume' (25), 'Seal Type' (Film), and 'Vessel Type' (Plates), along with 'Save & Exit Plate Editing' and 'Cancel & Exit Plate Editing' buttons. Below the editing section is a 'Spreadsheet' view showing a grid of wells (A-H, 1-12). A red box highlights the 'x' icon in the toolbar above the grid. To the left of the grid are controls for 'Replicates' (horizontal/vertical arrows), 'Size' (1, 2, 3), and 'Next #' (1). To the right of the grid are fields for 'Experiment Name' (My experiment), 'Experiment Type' (General), 'Fluorophore' (FAM), and 'Probe/Primer' (FAM). Below these are checkboxes for 'Whole Plate loading', 'Scientific Notation', and 'Dilution Series'. At the bottom, there's a table with columns: Row, Column, Sample Type, Rep #, Identifier/Condition, Quantity, and Units.

Row	Column	Sample Type	Rep #	Identifier/Condition	Quantity	Units
A	1					
A	2					
A	3					
A	4					
A	5					
A	6					
A	7					
A	8					
A	9					
A	10					
A	11					
A	12					
B	1					
B	2					
B	3					
B	4					
B	5					
B	6					
B	7					
B	8					
B	9					
B	10					
B	11					
B	12					
C	1					
C	2					
C	3					
C	4					
C	5					
C	6					
C	7					
C	8					
C	9					
C	10					
C	11					
C	12					
D	1					
D	2					
D	3					
D	4					
D	5					
D	6					
D	7					
D	8					
D	9					
D	10					
D	11					
D	12					
E	1					
E	2					
E	3					
E	4					
E	5					
E	6					
E	7					
E	8					
E	9					
E	10					
E	11					
E	12					
F	1					
F	2					
F	3					
F	4					
F	5					
F	6					
F	7					
F	8					
F	9					
F	10					
F	11					
F	12					
G	1					
G	2					
G	3					
G	4					
G	5					
G	6					
G	7					
G	8					
G	9					
G	10					
G	11					
G	12					
H	1					
H	2					
H	3					
H	4					
H	5					
H	6					
H	7					
H	8					
H	9					
H	10					
H	11					
H	12					

# Run

please make sure, that you spun down the plate before running the reaction

10. click on Run, select well factor you want and click on Begin Run

The screenshot shows the Bio-Rad iQ5 software interface. The 'Run' button is highlighted with a black arrow. The 'Selected Plate Setup' section shows a 96-well plate layout with SYBR dyes. The 'Selected Protocol' section shows the following steps:

Cycle	Repeats	Step	Dwell Time	Setpoint	PCR / Melt Data Acquisition
1	1	1	7:00	95.0	
2	40	1	0:10	95.0	

The 'Selected Plate Setup' section shows a 96-well plate layout with SYBR dyes. The 'Run' button is highlighted with a black arrow.



# Data analysis

1. open your file ( you can also drag and drop your data file it into the data file window)

The screenshot shows the Bio-Rad iQ5 software interface. The 'Data File' window is highlighted with a green border, and an arrow points to it from the instruction text. The 'Selected Plate Setup' window is also visible, showing a 96-well plate layout with FAM, HEX, TexasRed, and Cy5 channels. The 'Selected Protocol' window shows a 2-step protocol: Cycle 1 (Step 1, 95.0, 3:00) and Cycle 2 (40X, Step 1, 95.0, 0:10; Step 2, 55.0, 0:30). The 'Selected Plate Setup' window shows a 96-well plate layout with FAM, HEX, TexasRed, and Cy5 channels. The 'Selected Plate Setup' window also shows a 96-well plate layout with FAM, HEX, TexasRed, and Cy5 channels.

**Bio-Rad iQ5 (admin)**

File View Reports Tools Help

Setup Plate Summary

Protocol Plate Run Set Data File

Workshop

Run-Time Central

Data Analysis

Calibration

User Profile

**Selected Protocol:** 2Step.tmo

Edit Create New Protocol Original Current

Cycle 1		Cycle 2 40X	
Step 1	Step 1	Step 2	
95.0	95.0	55.0	
3:00	0:10	0:30	

**Selected Plate Setup:** Template.pts

Edit Create New Plate Original Current

Sample Volume : 25ul Seal Type : Film Vessel Type : Plates

FAM HEX TexasRed Cy5

	1	2	3	4	5	6	7	8	9	10	11	12
A		1		1	2	3	4	5	6		1	1
B		1		1	2	3	4	5	6		1	1
C		1		1	2	3	4	5	6		1	1
D												
E												
F			1	2	3	4	5	6	7	8		
G			1	2	3	4	5	6	7	8		
H			1	2	3	4	5	6	7	8		

**PCR / Melt Data Acquisition**

Cycle	Repeats	Step	Dwell Time	Setpoint	PCR / Melt Data Acquisition
1	1				
		1	3:00	95.0	
2	40				
		1	0:10	95.0	
		2	0:30	55.0	Real Time

BIO-RAD

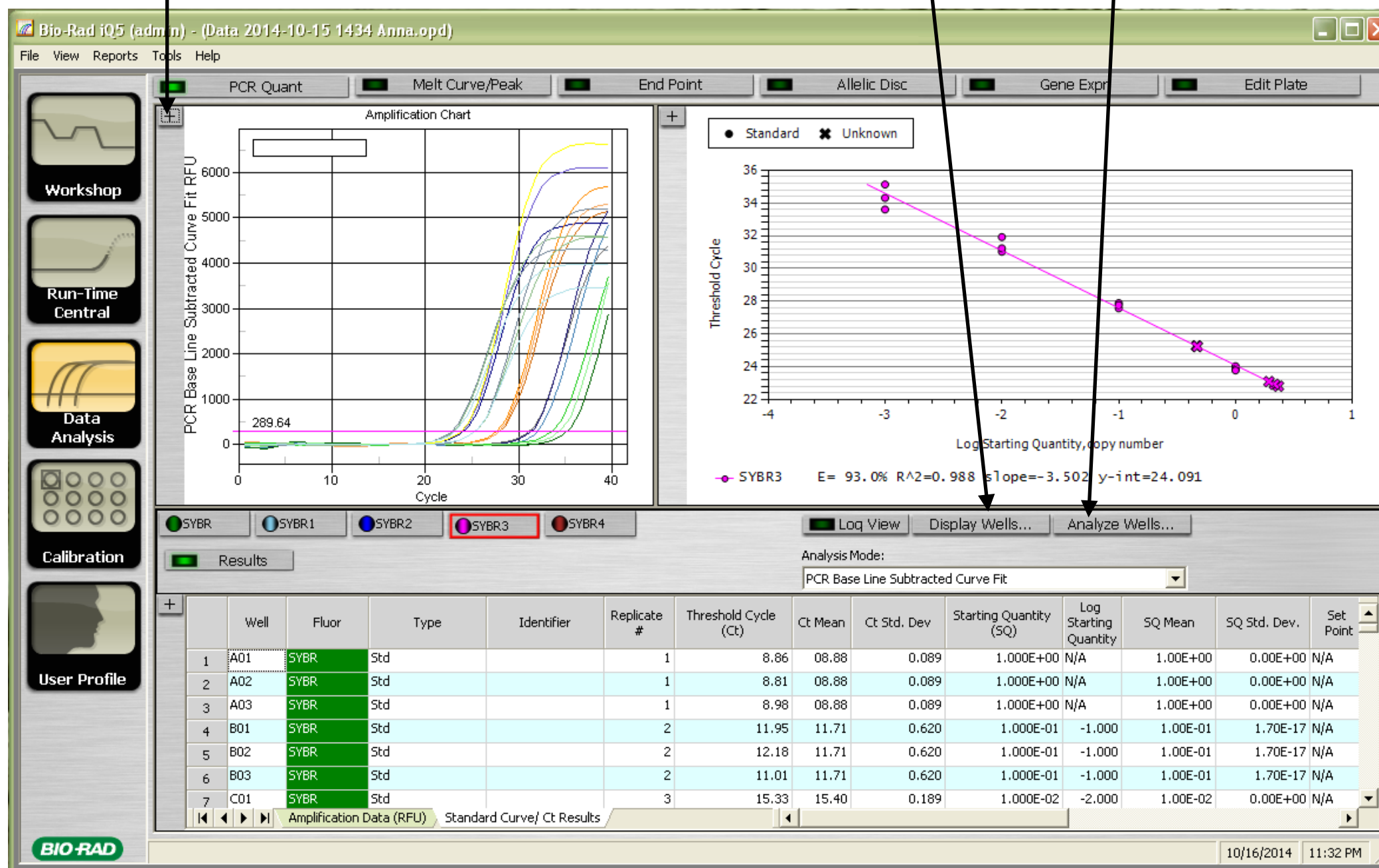
10/16/2014 10:39 PM



# Data analysis

everywhere in the software +/- is to zoom in/out into the a window

you can selected which wells you wanted to not be shown to you or also excluded from the analysis



# Data analysis

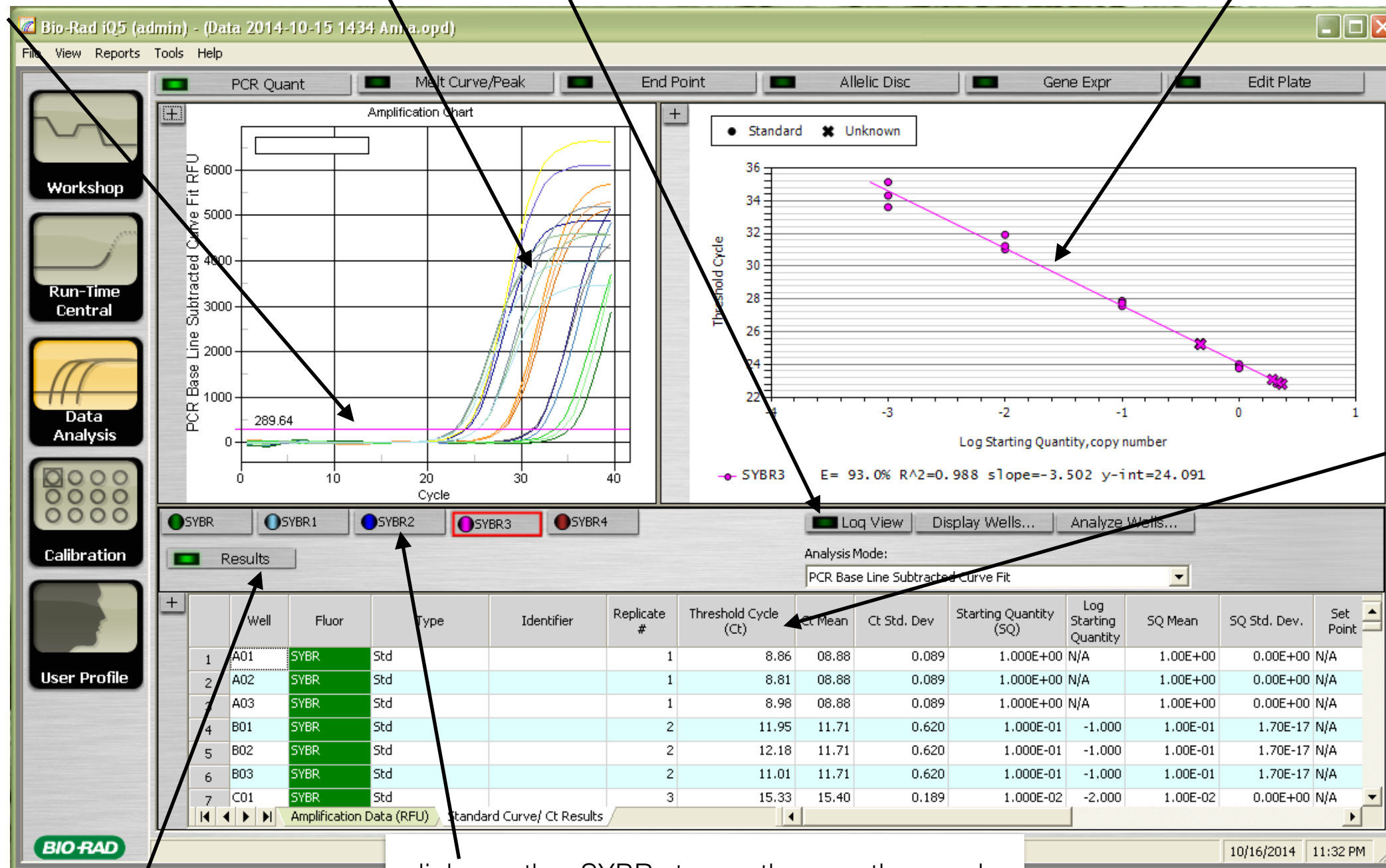
this are fluorescence intensities detected in each well of the plate for each cycle

if standards were annotated in the plate layout  
standard curve will be calculated  
automatically

please remind  
yourself, what is  
this line and why is  
it here

please remind yourself, why using log scale here is handy

please remind  
yourself, what is  
a threshold  
cycle



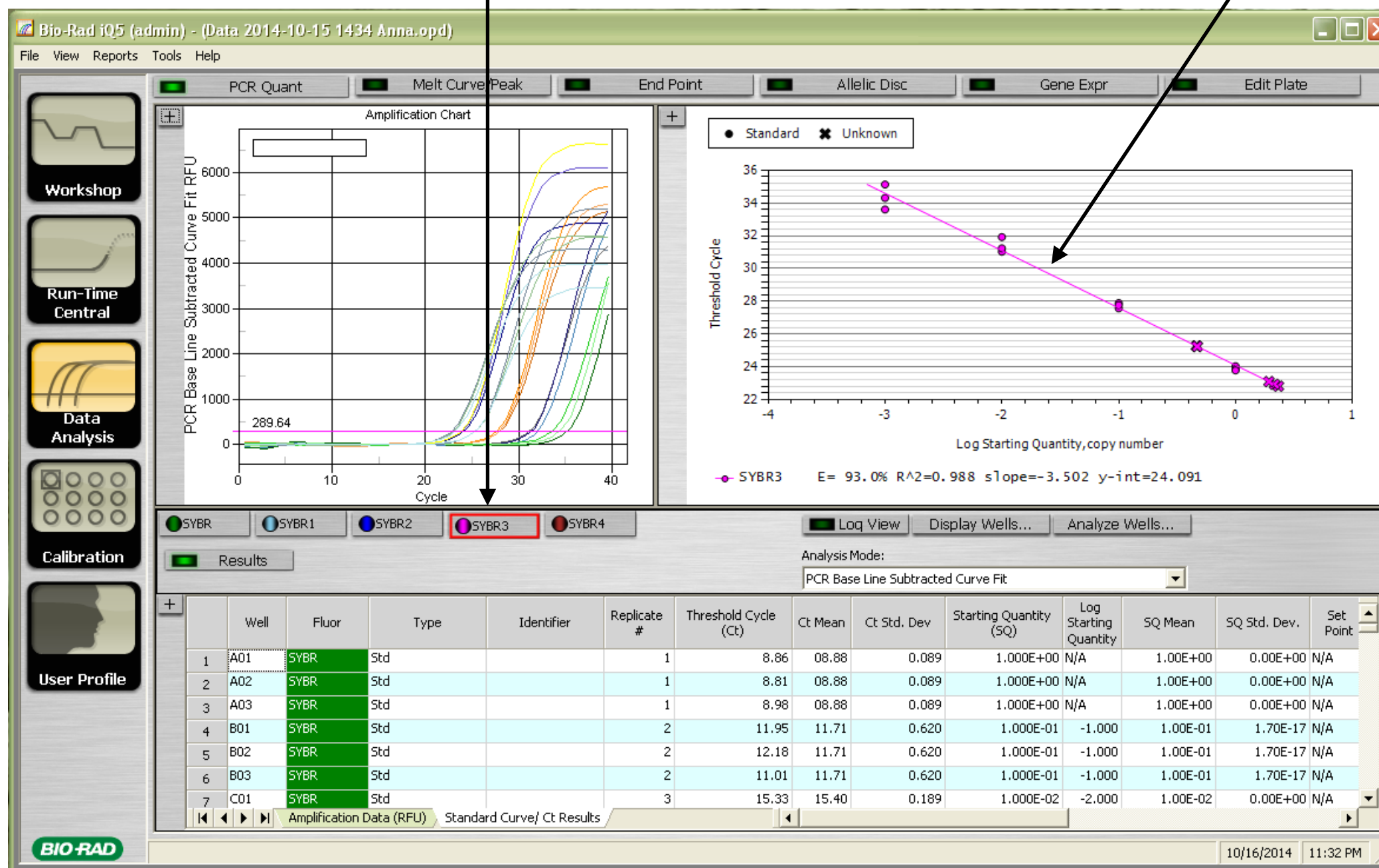
click on other SYBRs to see them on the graphs

click here to see your results

# Data analysis

please note, if you wouldn't annotate your targets as individual fluorophores all your standards would be in the same curve

if standards were annotated in the plate layout standard curve will be calculated automatically

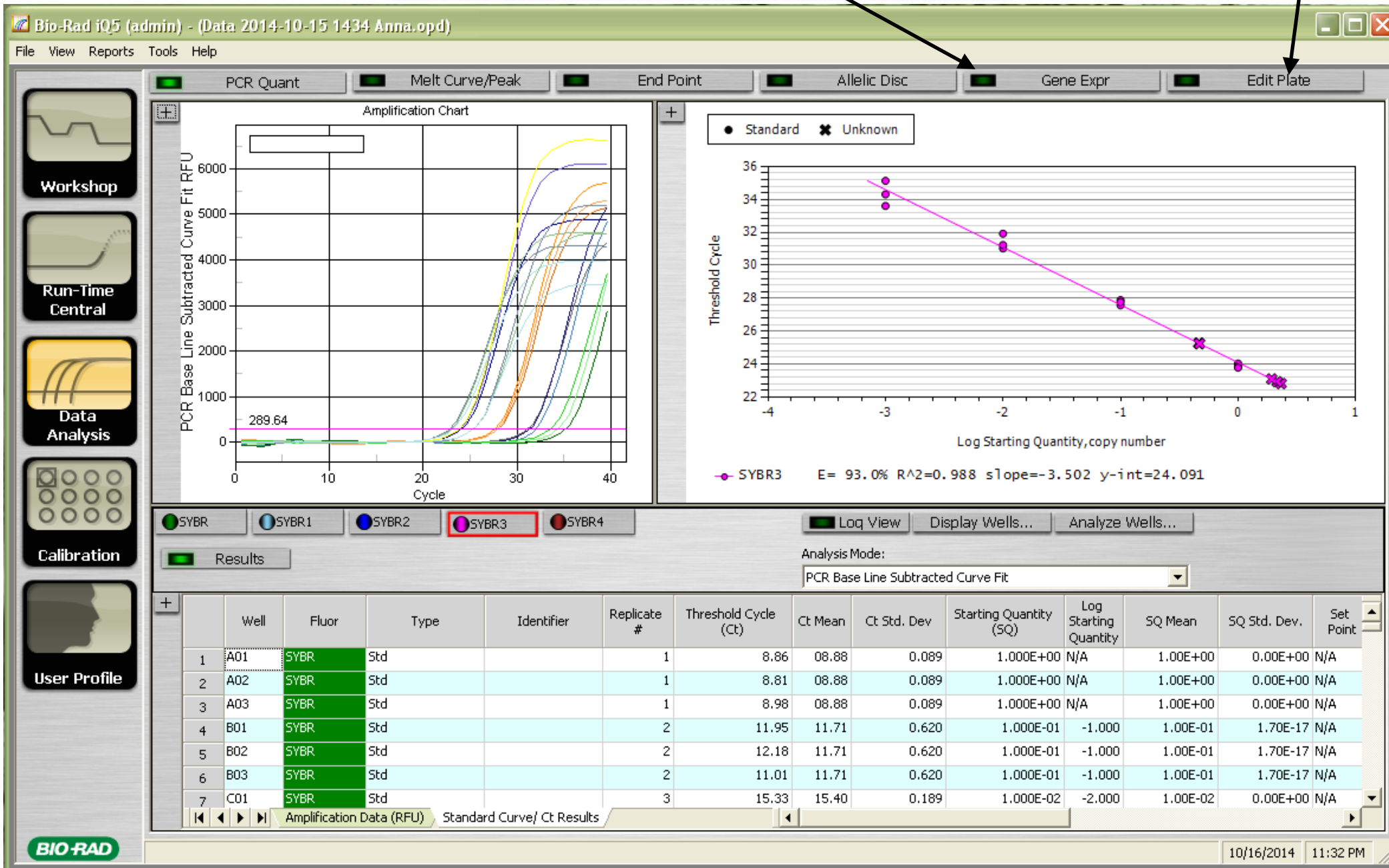


# Data analysis

if you want forename your samples you need to go here

here you can change dilution factors for your efficiency curve

please remind yourself, why you do not need to know the concentration of the sample you used for the primer efficiency curve





# To rename your samples

in the gene expression tab

1. enlarge the window and select the wells to be renamed

2. type in the target name

3. type in the sample name

you can display only individual fluorophores

Bio-Rad iQ5 (admin) - (Data 2014-10-15 1434 Anna.opd)

File View Reports Tools Help

PCR Quant Melt Curve/Peak End Point Allelic Disc Gene Expr

Graph Data  
☒ Relative to control  
☒ Relative to zero

x-Axis Options  
☒ Condition  
☒ Gene

y-Axis Options  
☒ Log 2  
☒ Linear

Scaling Options  
☒ Highest  
☒ Lowest  
☒ Unscaled

Graph error  
+/- Std Devs 1

Setting Data Table

☒ Gene List ☒ Condition List ☒ Data Set L

	Name	Full Name	Ref
1	Gene 2	Gene 2	<input type="checkbox"/>
2	Reference 1	Reference 1	<input type="checkbox"/>
3	Reference 2	Reference 2	<input type="checkbox"/>
4	Reference 3	Reference 3	<input type="checkbox"/>
5	GOI	GOI	<input type="checkbox"/>

Gene Name: Condition Name: Analyze Wells...

☒ Copy condition to all data sets ☒ Enable for Gene Study

☒ Normalized expression (ddCt) ☒ Relative quantity (dCt) Recalculate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Gene 2 8.86	Gene 2 8.81	Gene 2 8.98									
	Std-1	Std-1	Std-1									
	Standard	Standard	Standard									
B	Gene 2 11.95	Gene 2 12.18	Gene 2 11.01									
	Std-2	Std-2	Std-2									

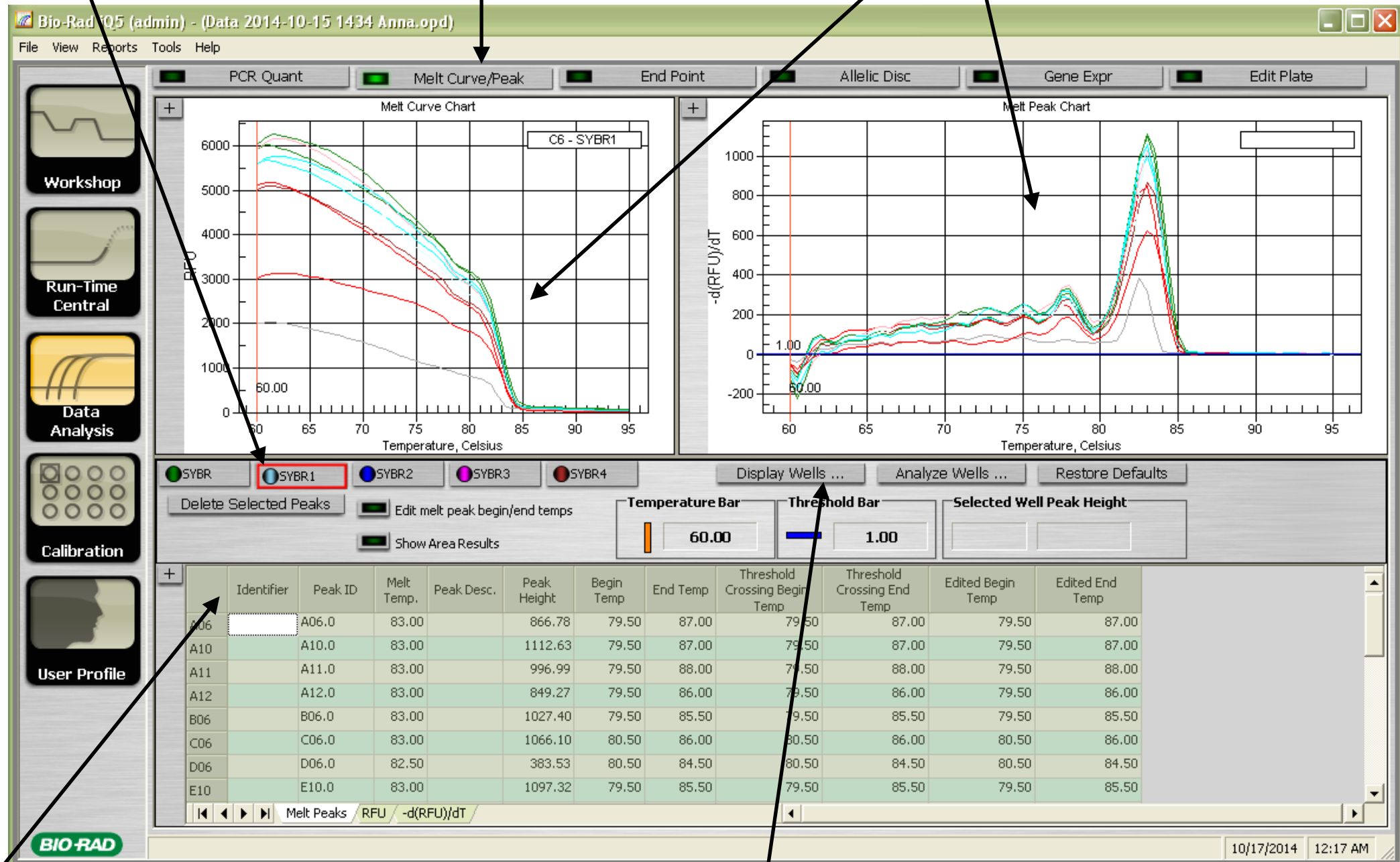
1-SYBR 1-SYBR1 1-SYBR2 1-SYBR3 1-SYBR4

# Data analysis

you can select curve for which target you want to see

Melt curve

please remind yourself,  
what are these two  
representations of the  
melt curve

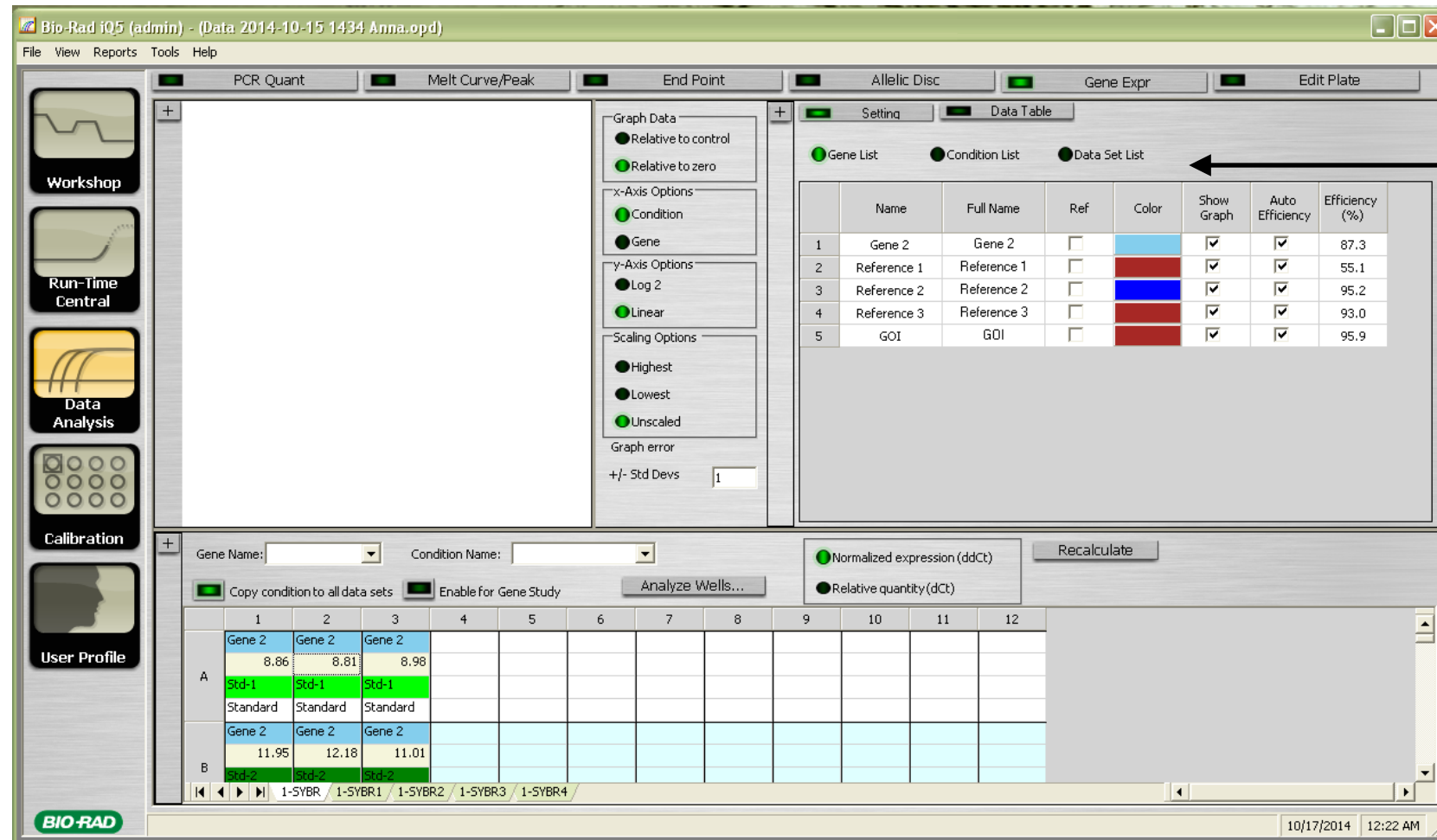


all tables can be  
exported to Excel

and also curves for which wells should be shown



# Data analysis



In Gene Expression you can run delta Ct and delta delta Ct tests

in the Gene list:

1. Select the genes you want to use as a reference
2. Select the genes you want to have on the chart
3. Make sure all the efficiencies are correctly annotated

in the Condition list:

1. Select samples you want to analyse
2. Check in a sample which you want to use as a control (if you have any controls)

# M-value

This software doesn't calculate stability values, please check Daniel's lecture and the link in the "useful stuff" on the course site

Adjust your selection of references according to M-values results