

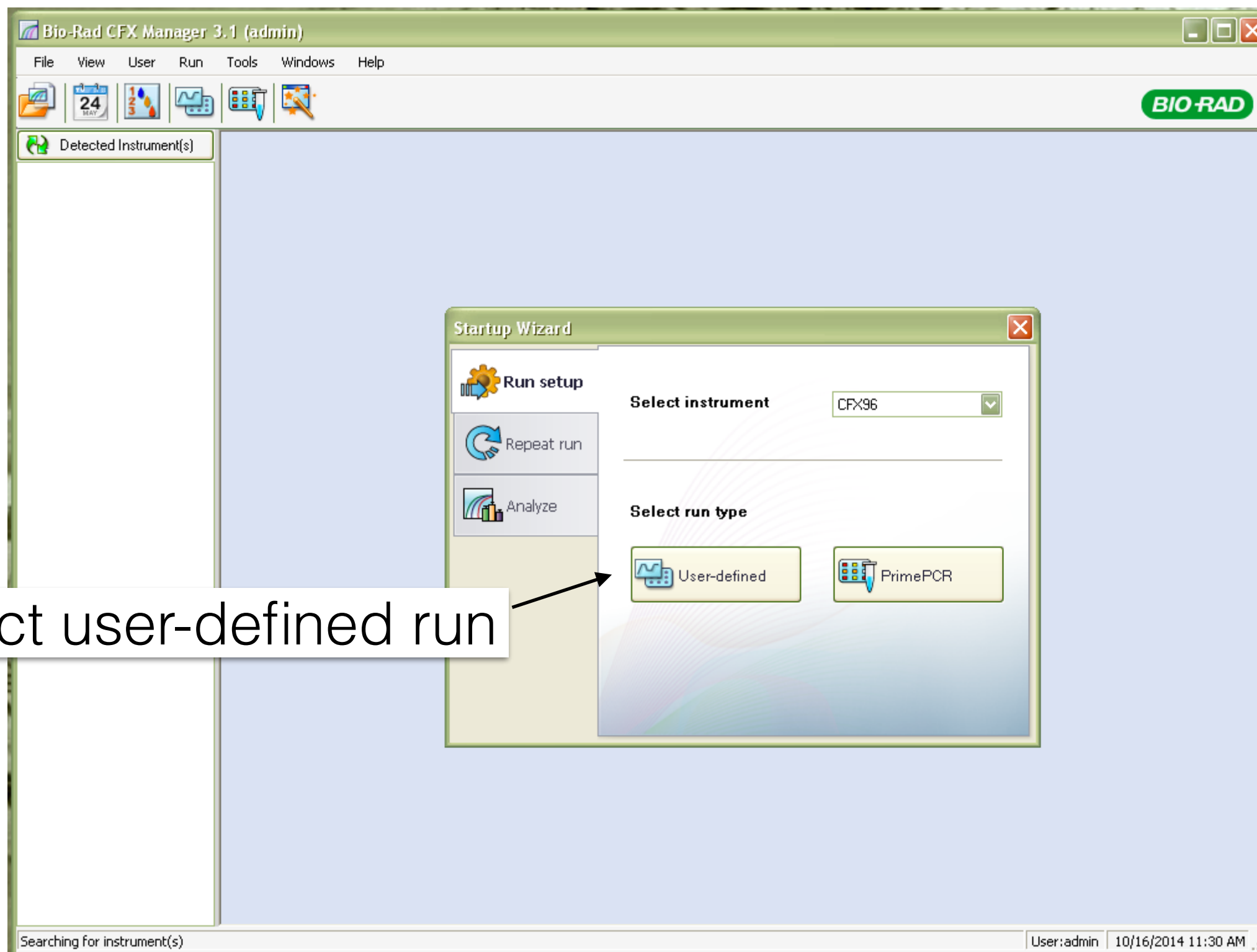
# CFX Manager

# Run set up

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

Plate Layout can be edited after the run

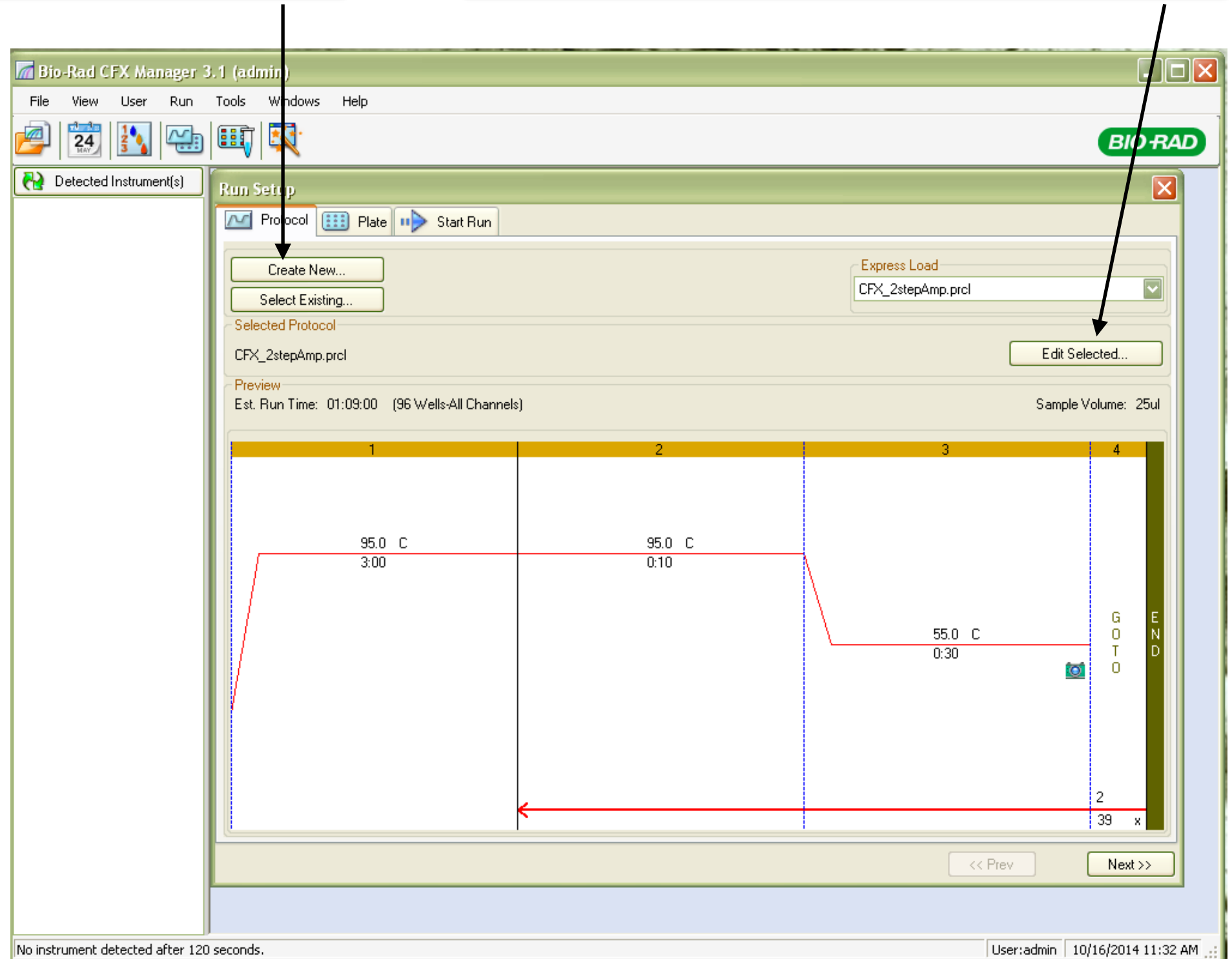
# Run set up



# Run set up: protocol

2. make your own protocol or select existing

3. you can edit existing protocol



# Run set up: protocol

4. type in your protocol

5. type in the volume of your reactions!!

The screenshot displays the Bio-Rad CFX Manager 3.1 (admin) interface, specifically the Protocol Editor - New window. The window features a menu bar (File, View, User, Run, Tools, Windows, Help) and a toolbar with icons for saving, printing, and inserting steps. The main area shows a thermal protocol graph with four steps: 1 (95.0 C for 3:00), 2 (95.0 C for 0:10), 3 (55.0 C for 0:30), and 4 (GOTO 2, 39 more times). A red line indicates the temperature profile. The bottom panel lists the steps in a table format.

Step	Temperature (C)	Time	Notes
1	95.0	3:00	
2	95.0	0:10	
3	55.0	0:30	
4	GOTO 2	39 more times	

Annotations: A black arrow points from the text '4. type in your protocol' to the step list area. Another black arrow points from the text '5. type in the volume of your reactions!!' to the 'Sample Volume' field, which is set to 25 µl.

At the bottom of the window, a status bar indicates 'No instrument detected after 120 seconds.' and the user information 'User: admin 10/16/2014 11:34 AM'.

# Run set up: protocol

6. save your protocol and click on NEXT

Bio-Rad CFX Manager 3.1 (admin)

File View User Run Tools Windows Help

Detected Instrument(s)

Run Setup

Protocol Plate Start Run

Create New... Select Existing...

Express Load

CFX\_2stepAmp.prc

Edit Selected...

Selected Protocol

CFX\_2stepAmp.prc

Preview

Est. Run Time: 01:09:00 (96 Wells-All Channels) Sample Volume: 25ul

1 2 3 4

95.0 C 3:00 95.0 C 0:10 55.0 C 0:30 GOTO

2

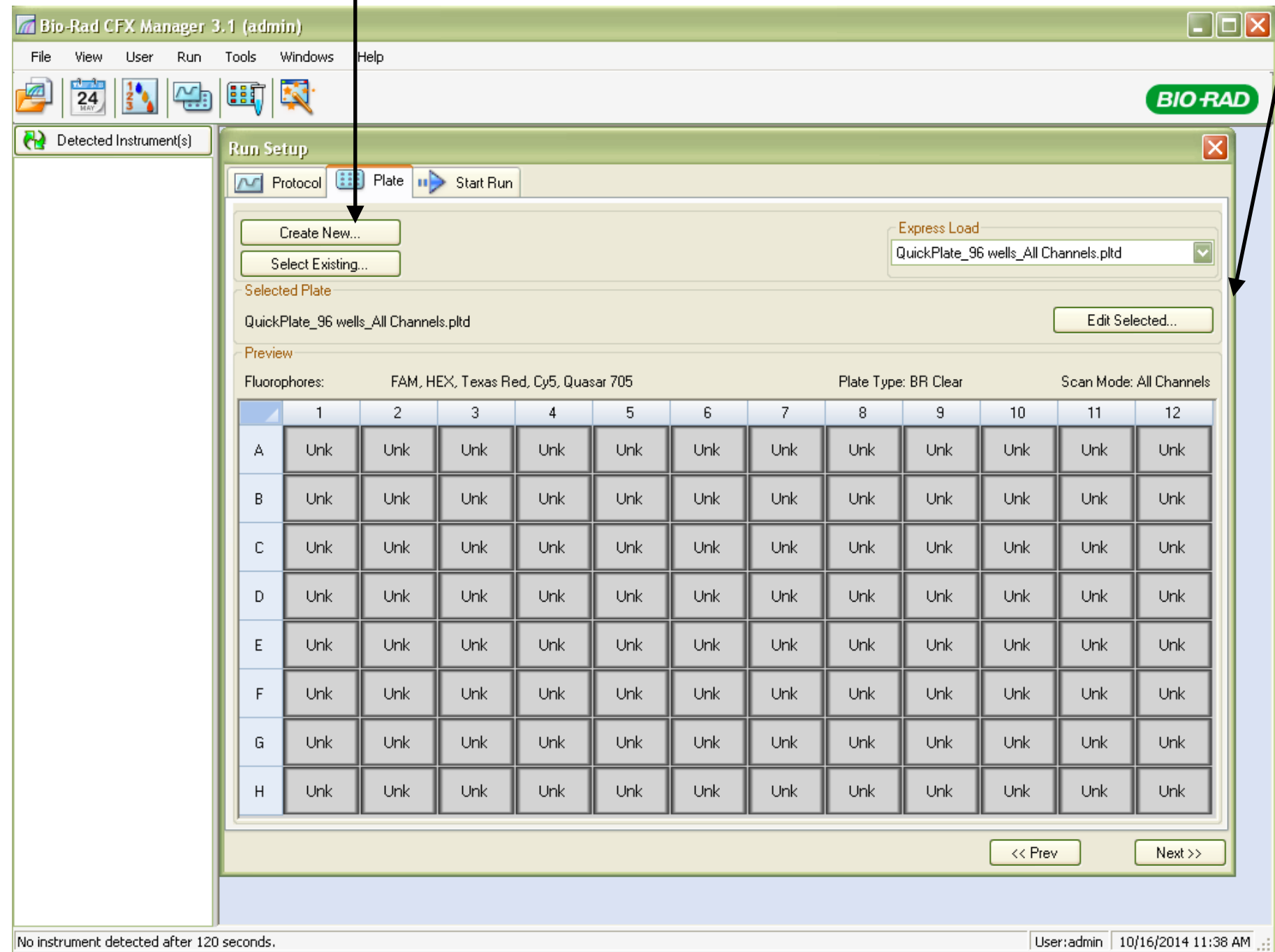
<< Prev Next >>

No instrument detected after 120 seconds. User:admin 10/16/2014 11:32 AM

## Run set up: plate

7. make your own plate layout or select existing

8. you can edit existing layout



# Run set up: plate

9. type in your targets and templates in the experiment settings

Plate Editor - New

File Settings Editing Tools

100% Scan Mode All Channels Well Groups... Trace Styles... Spreadsheet View/Importer Plate Loading Guide

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

Select Fluorophores...

Sample Type

Load ☐ SYBR Target Name

Load ☐ Sample Name

Load ☐ Replicate #

Replicate Series

Experiment Settings...

Clear Replicate #

Clear Wells

Plate Type: BR Clear View ☒ Sample ☐ Well Group ☐ Biological Set ☐ Well Note

OK Cancel

No instrument detected after 120 seconds. User:admin 10/16/2014 11:40 AM



# Run set up: plate

12. and do the same for your templates

10. you can remove target names from the list

The screenshot shows the 'Plate Editor - New' window in the Bio-Rad CFX Manager software. The 'Experiment Settings' dialog box is open, with the 'Targets' tab selected. The 'Targets' table lists five items: Reference 2, Reference 3, Reference 1, Gene 2, and GOI. The 'Select To Remove' column for 'Reference 2' is checked. An arrow points from the '12. and do the same for your templates' text to the 'Targets' tab. Another arrow points from the '10. you can remove target names from the list' text to the 'Select To Remove' checkbox. A third arrow points from the '11. or type in new targets' text to the 'New:' text box. The 'Remove checked item(s)' button is also visible.

	Name	Full Name	Reference	Select To Remove
1	Reference 2	Reference 2	<input type="checkbox"/>	<input checked="" type="checkbox"/>
2	Reference 3	Reference 3	<input type="checkbox"/>	<input type="checkbox"/>
3	Reference 1	Reference 1	<input type="checkbox"/>	<input type="checkbox"/>
4	Gene 2	Gene 2	<input type="checkbox"/>	<input type="checkbox"/>
5	GOI	GOI	<input type="checkbox"/>	<input type="checkbox"/>

New:

☐ Show Analysis Settings

Exclude the following sample types from Gene Expression:

☒ NTC ☐ NRT ☐ Negative Control ☐ Positive Control

Plate Type: BR Clear ☒ Sample ☐ Well Group ☐ Biological Set ☐ Well Note

No instrument detected after 120 seconds. User:admin 10/16/2014 11:45 AM

# Run set up: plate

12. and do the same for your templates

10. you can remove target names from the list

The screenshot shows the 'Plate Editor - New' window in the Bio-Rad CFX Manager software. The 'Experiment Settings' dialog box is open, with the 'Targets' tab selected. The 'Targets' table lists five items: Reference 2, Reference 3, Reference 1, Gene 2, and GOI. The 'Select To Remove' column for 'Reference 2' is checked. An arrow points from the '12. and do the same for your templates' text to the 'Targets' tab. Another arrow points from the '10. you can remove target names from the list' text to the 'Select To Remove' checkbox. A third arrow points from the '11. or type in new targets' text to the 'New:' text box. The 'Remove checked item(s)' button is also visible.

	Name	Full Name	Reference	Select To Remove
1	Reference 2	Reference 2	<input type="checkbox"/>	<input checked="" type="checkbox"/>
2	Reference 3	Reference 3	<input type="checkbox"/>	<input type="checkbox"/>
3	Reference 1	Reference 1	<input type="checkbox"/>	<input type="checkbox"/>
4	Gene 2	Gene 2	<input type="checkbox"/>	<input type="checkbox"/>
5	GOI	GOI	<input type="checkbox"/>	<input type="checkbox"/>

New:

☐ Show Analysis Settings

Exclude the following sample types from Gene Expression:

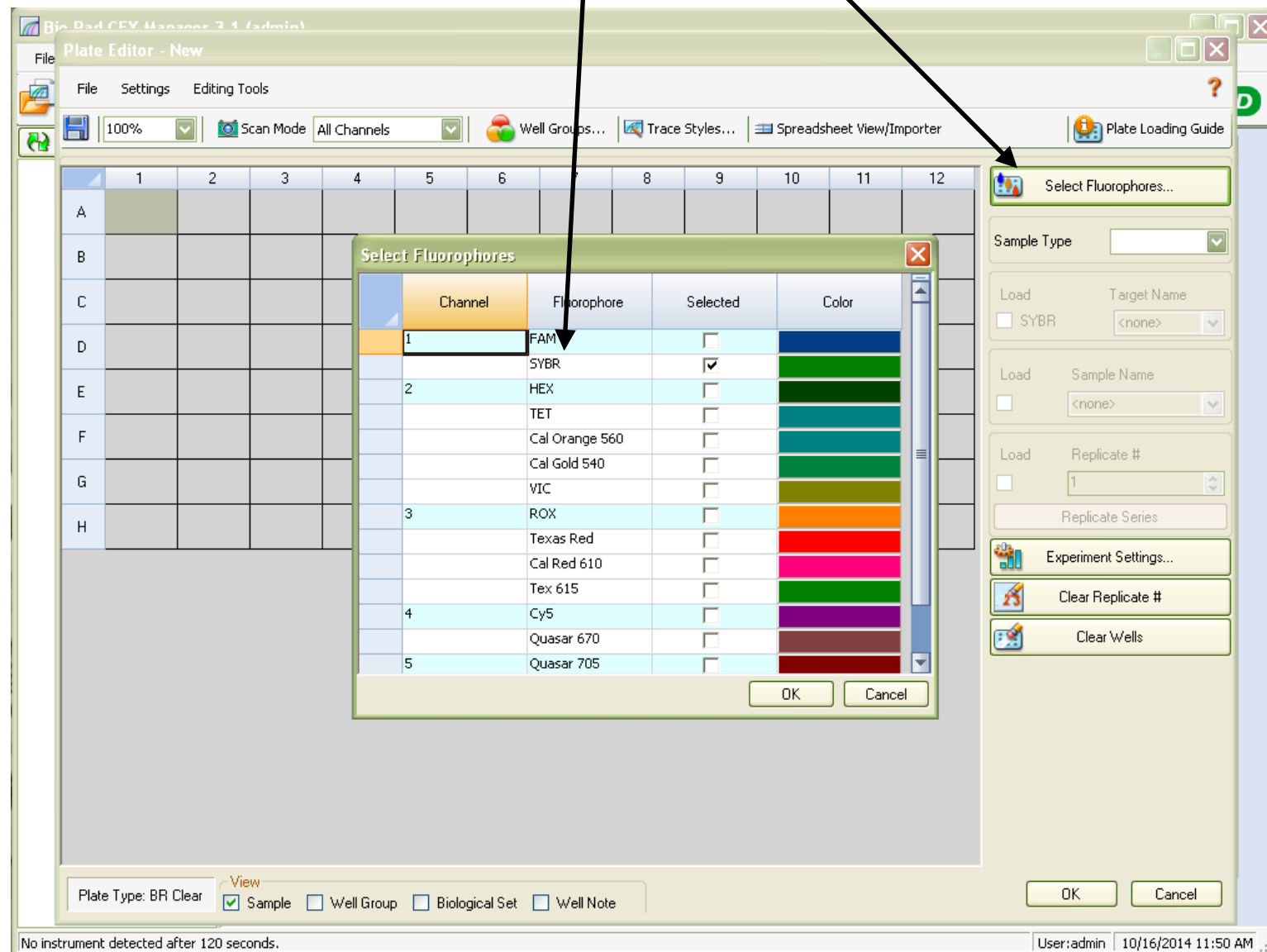
☒ NTC ☐ NRT ☐ Negative Control ☐ Positive Control

Plate Type: BR Clear ☒ Sample ☐ Well Group ☐ Biological Set ☐ Well Note

No instrument detected after 120 seconds. User:admin 10/16/2014 11:45 AM

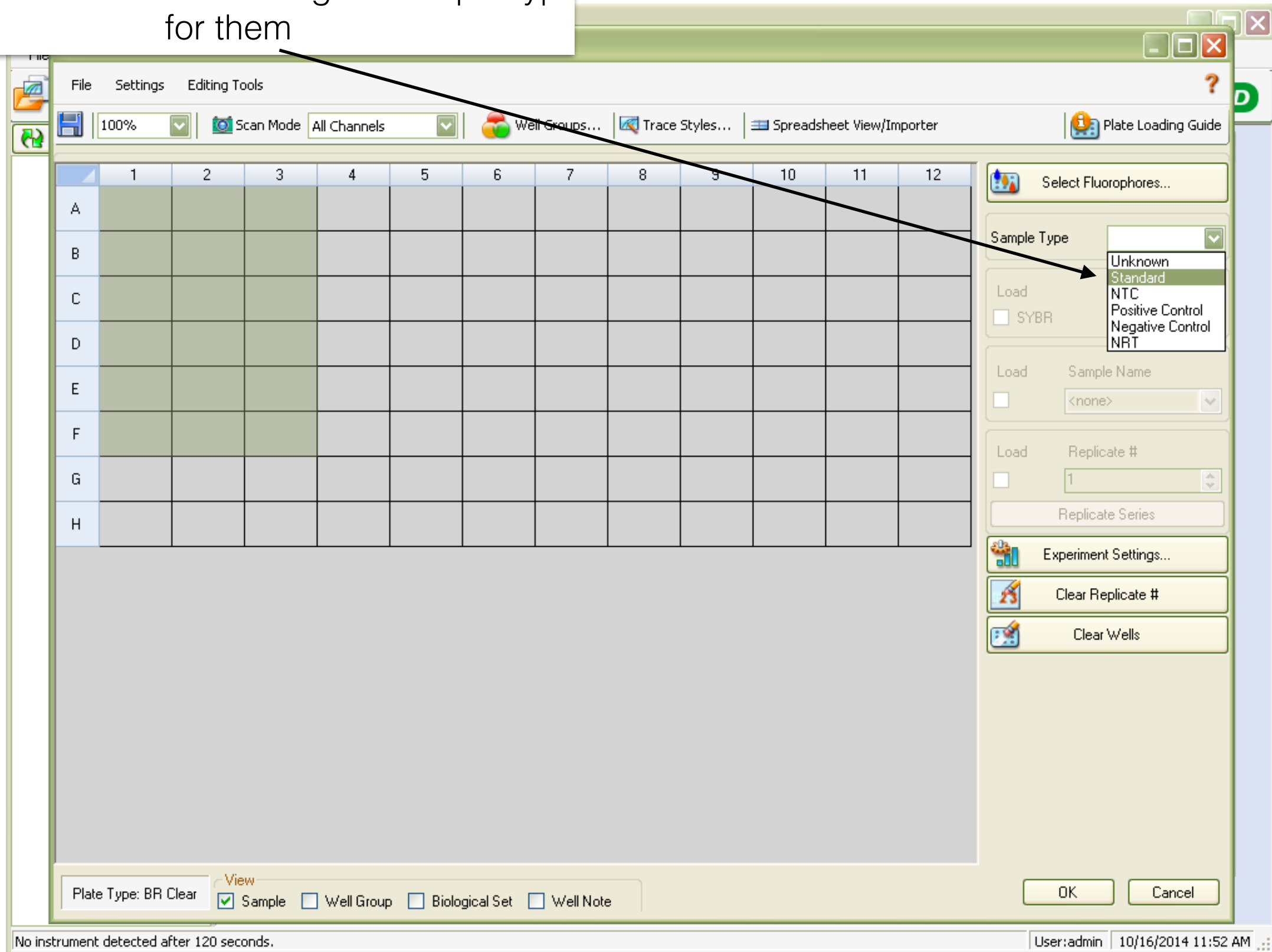
## Run set up: plate

13. make sure you will detect the correct fluorophore (in our case it is SYBR green)



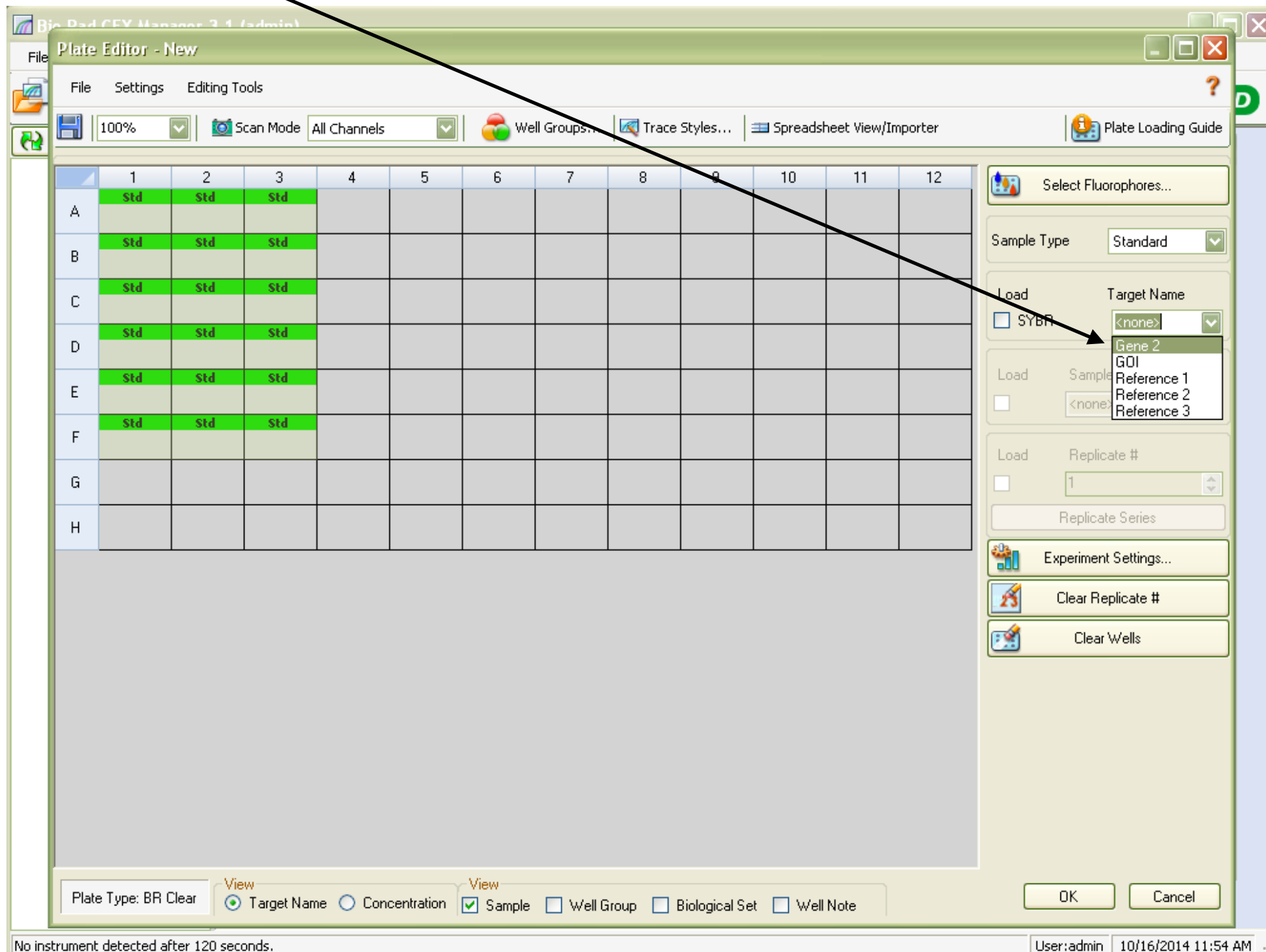
# Run set up: plate

14. select the wells and assign a sample type for them



# Run set up: plate

15. select the wells and assign the target gene for them (you can type the name here directly)



# Run set up: plate

16. select the wells and assign the template for them (you can type it in here directly)

17. in this case we have 3 technical replicates, which you can assign here

**Plate Editor - New**

File Settings Editing Tools

100% Scan Mode All Channels Well Groups... Trace Styles... Spreadsheet View/Importer Plate Loading Guide

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

Select Fluorophores...

Sample Type: Standard

Load: ☒ SYBR Target Name: Gene 2

Load: ☐ Sample Name: <none>

DNA pool  
sample 1  
sample 2  
sample A  
sample B

Replicate Series

Load: ☐ Concentration: 1.00E+06

Dilution Series

Experiment Settings...

Clear Replicate #

Clear Wells

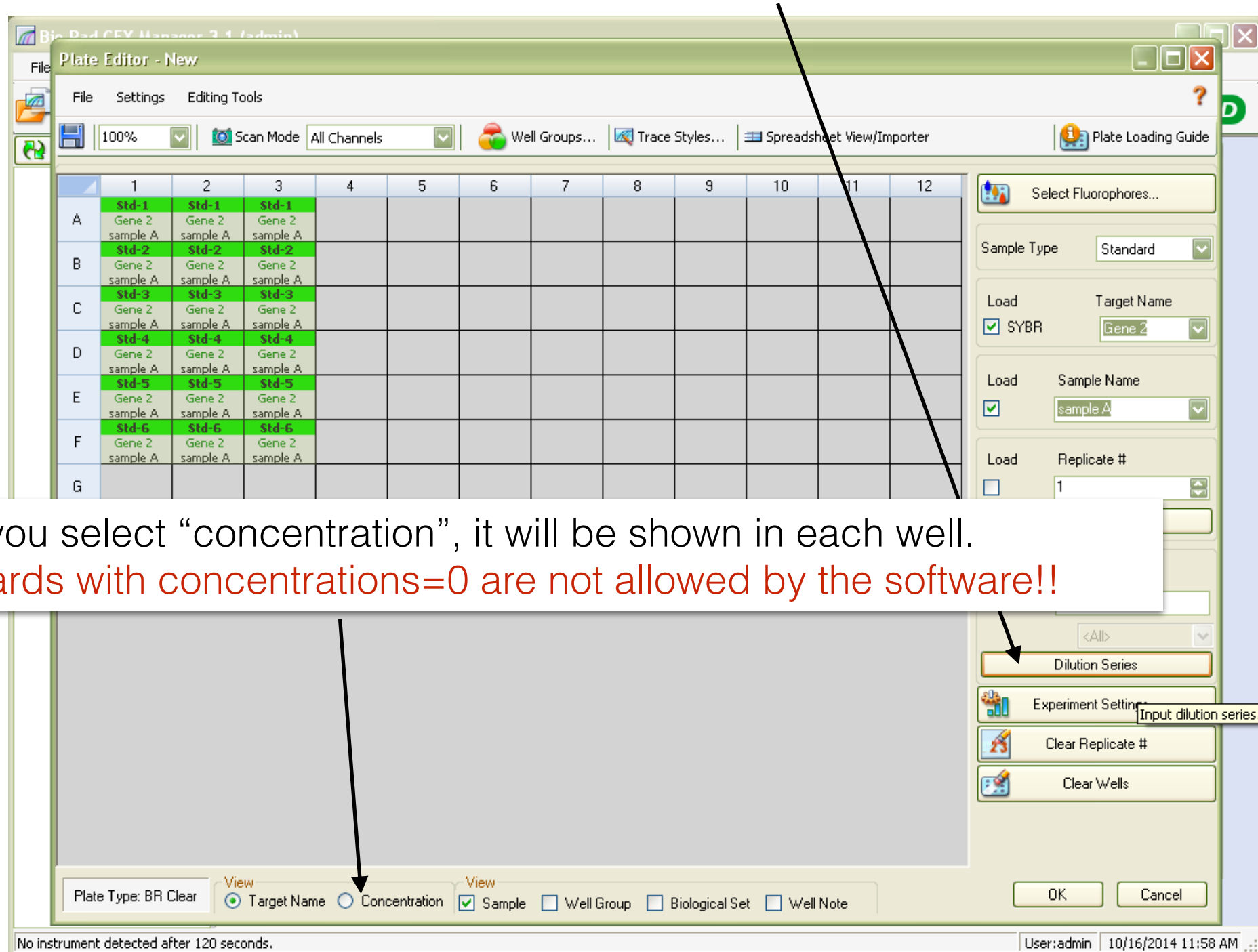
Plate Type: BR Clear View Target Name Concentration View Sample Well Group Biological Set Well Note

OK Cancel

No instrument detected after 120 seconds. User:admin 10/16/2014 11:55 AM

# Run set up: plate

18. in dilution series you can type in initial concentration (for primer efficiency use a random number) and the dilutions factor. and click on Apply



19. If you select "concentration", it will be shown in each well.  
sic! standards with concentrations=0 are not allowed by the software!!

# Run set up: plate

20. in the settings you can define what units you are going to use for your standards

Plate Editor - New

File Settings Editing Tools

Plate Size Plate Type Number Convention Units

copy number  
fold dilution  
micromoles  
nanomoles  
picomoles  
femtomoles  
attomoles  
milligrams  
micrograms  
nanograms  
picograms  
femtograms  
attograms  
percent

Sample Type  
Load Target Name  
SYBR <none>  
Load Sample Name  
<none>  
Load Biological Set Name  
<none>  
Load Replicate #  
1  
Replicate Series  
Experiment Settings...  
Clear Replicate #  
Clear Wells

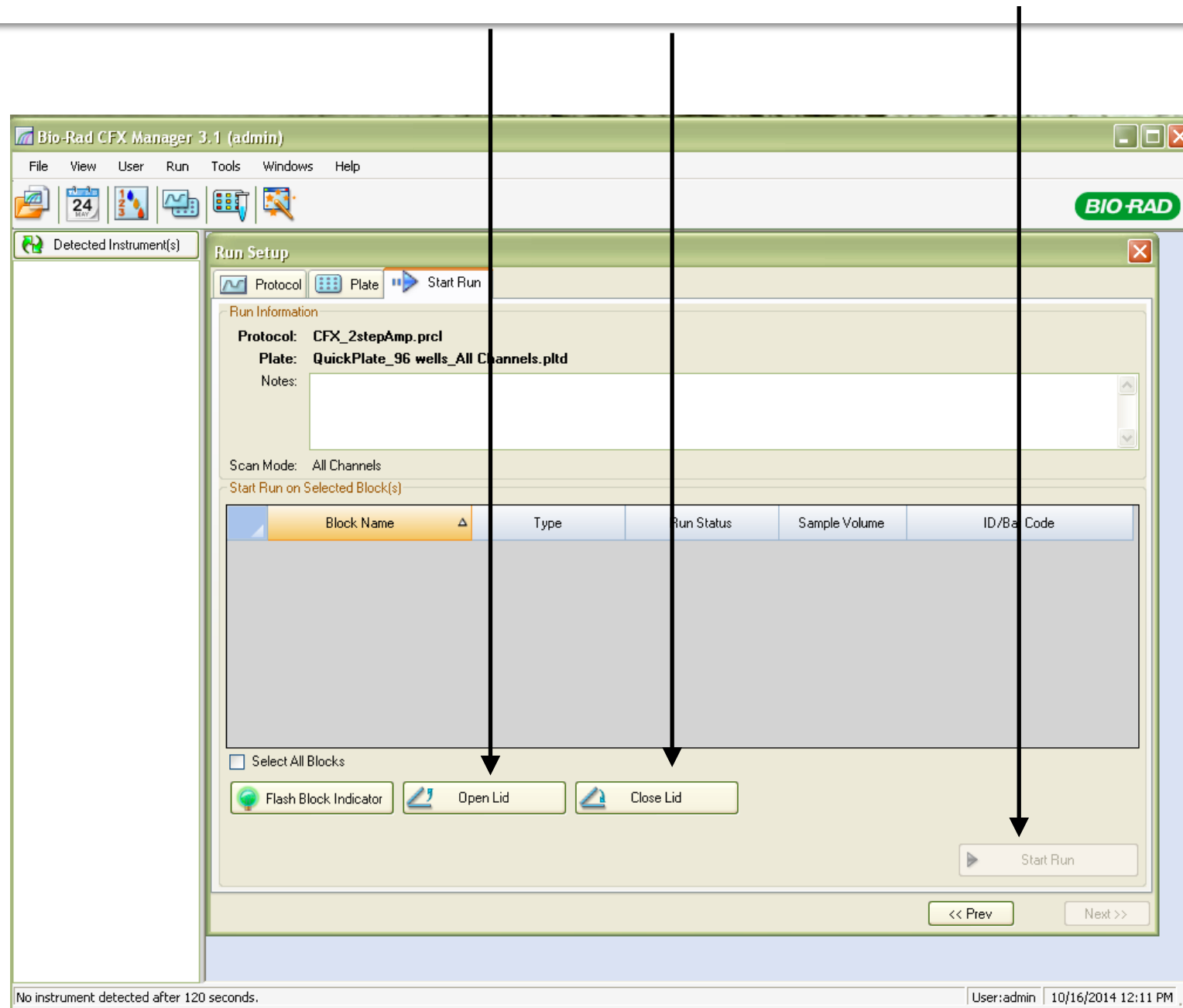
Plate Type: BR Clear  
View  
Target Name Concentration  
Sample Well Group Biological Set Well Note

No instrument detected after 120 seconds. User:admin 10/16/2014 12:04 PM



# Run

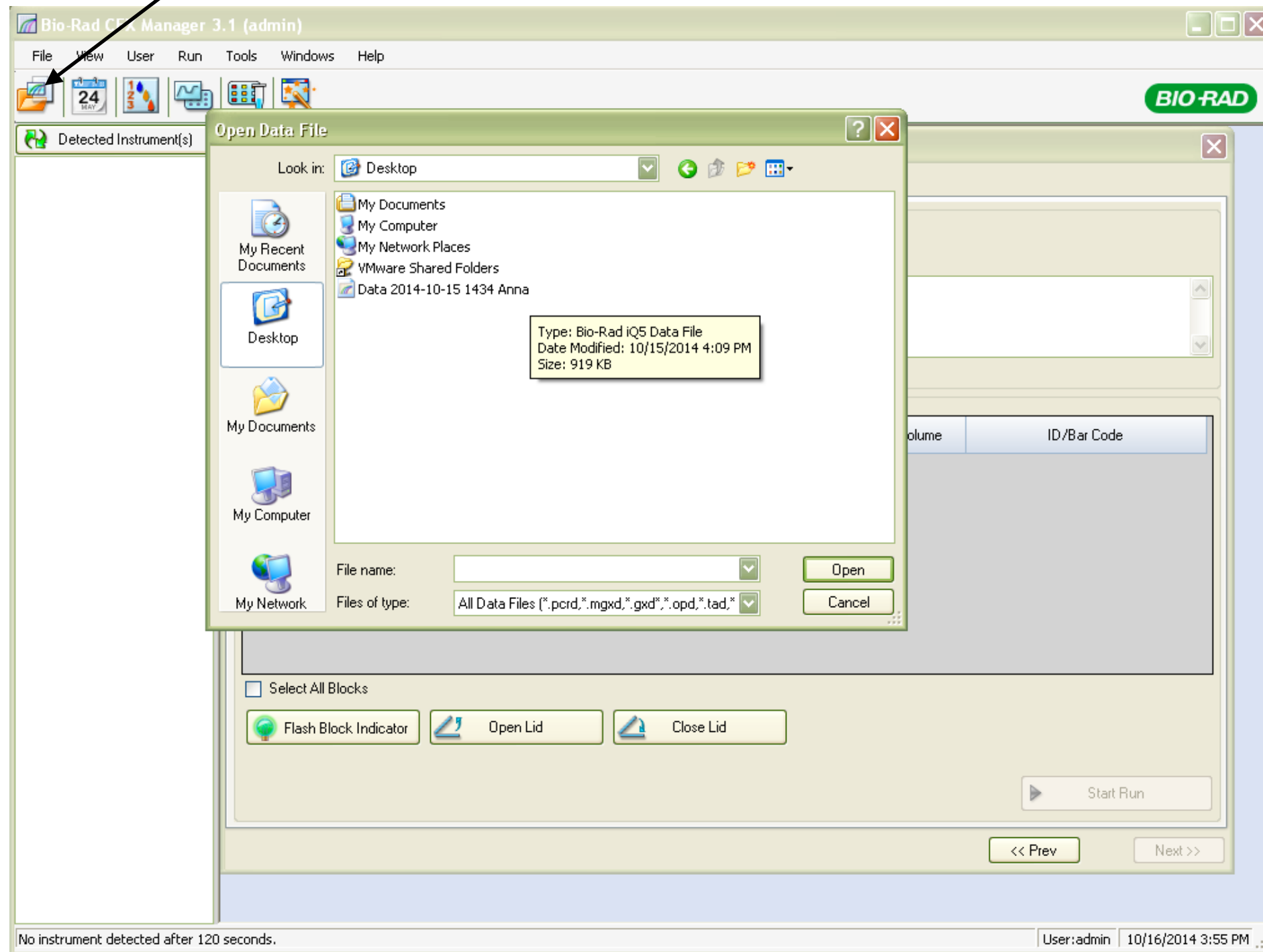
21. open the lid, place your plate on the thermoblock , close the lid and start run



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

# Data analysis

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



# Data analysis

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

Do not painc! now the standard curve is calculated for both your experiments mixed together

please remind yourself, why using log scale here is handy

all graphs can be exported to Microsoft Office

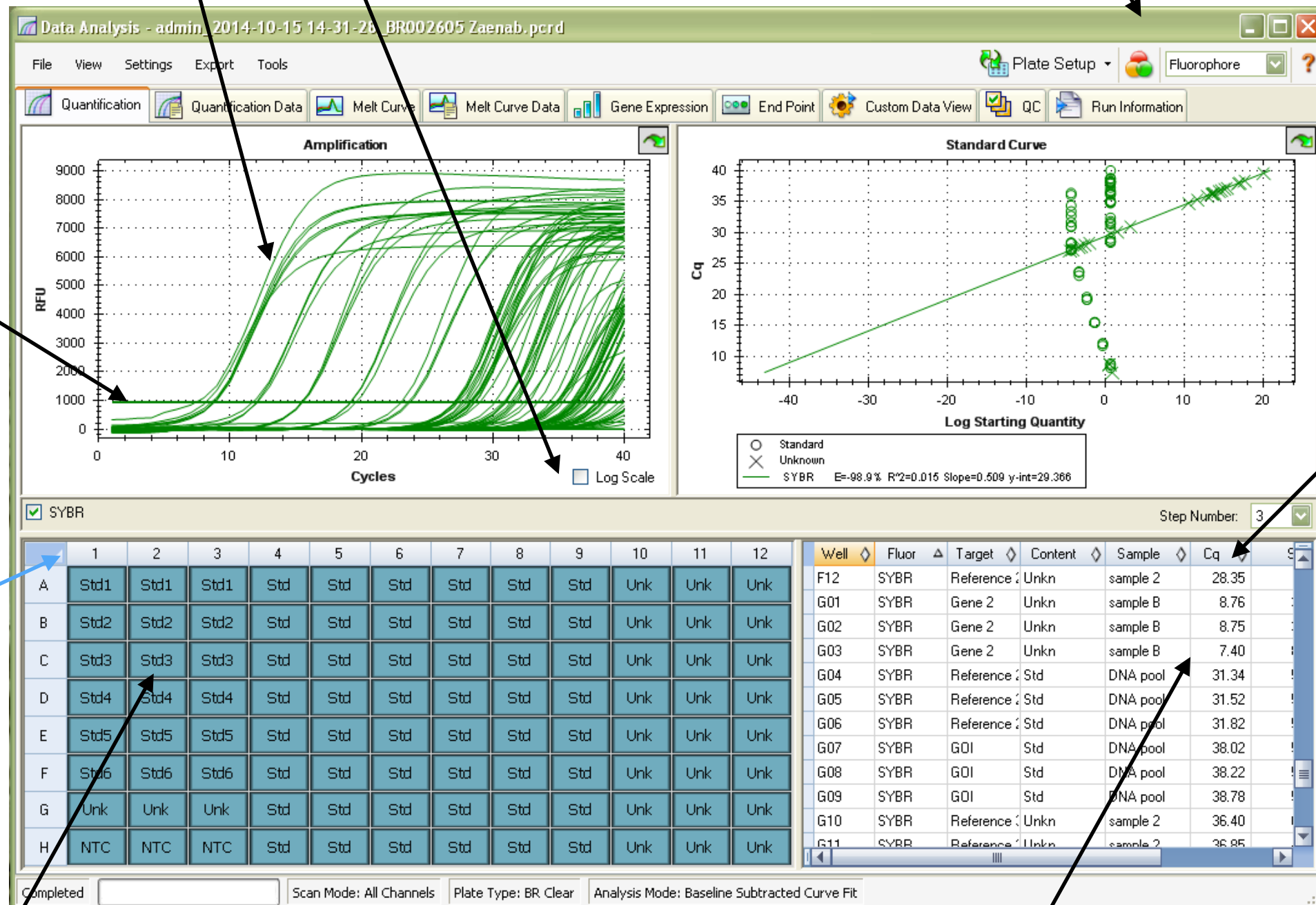
please remind yourself, what is this line and why is it here

please remind yourself, what is a Ct(Cq) value

all tables can be exported to Excel

you can select/deselect wells you want to see

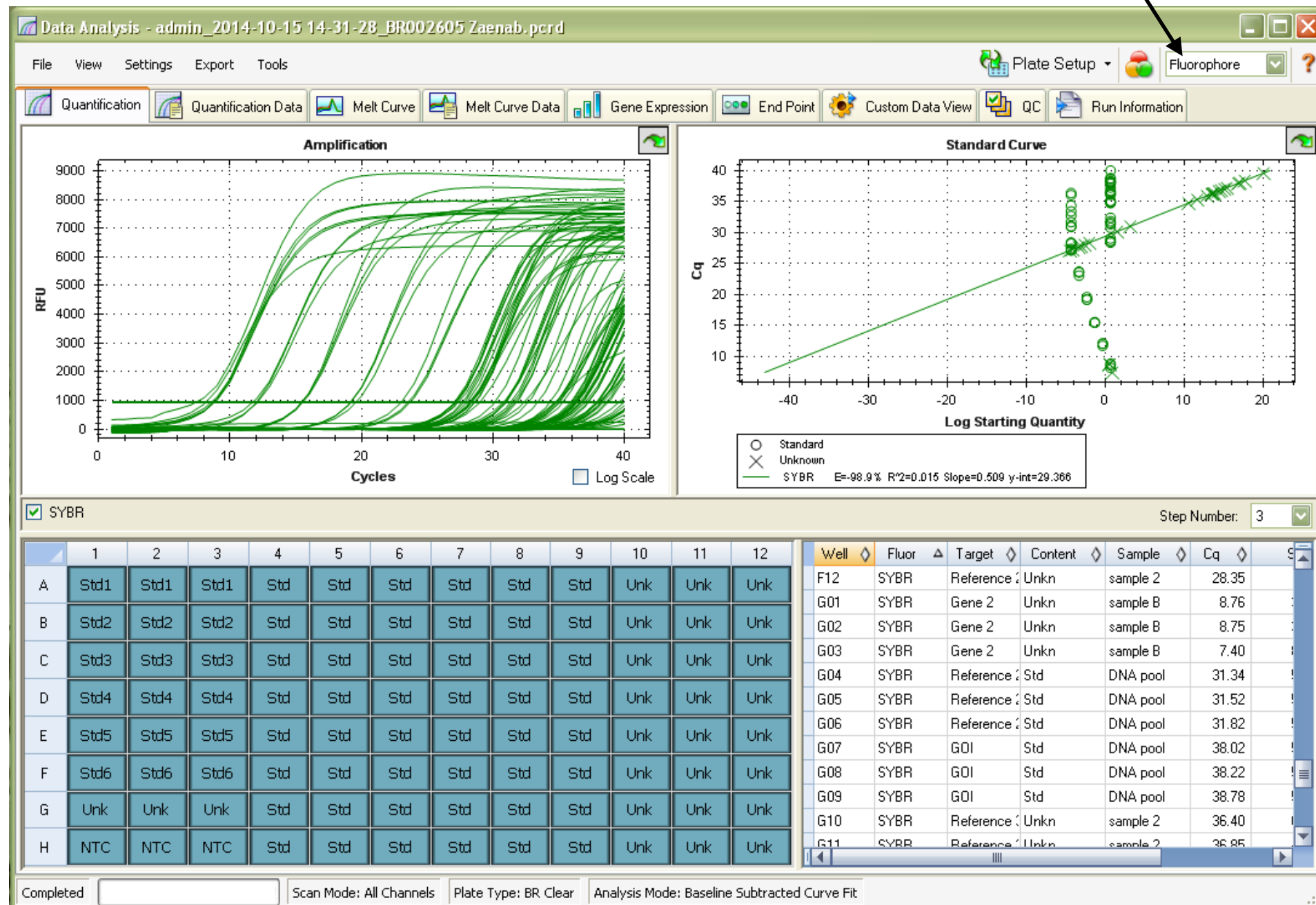
and see the Ct (Cq) values for each reaction



# Data analysis

Switch here to the Target to calculate a standard curve for each target individually

please remind yourself, what is a target

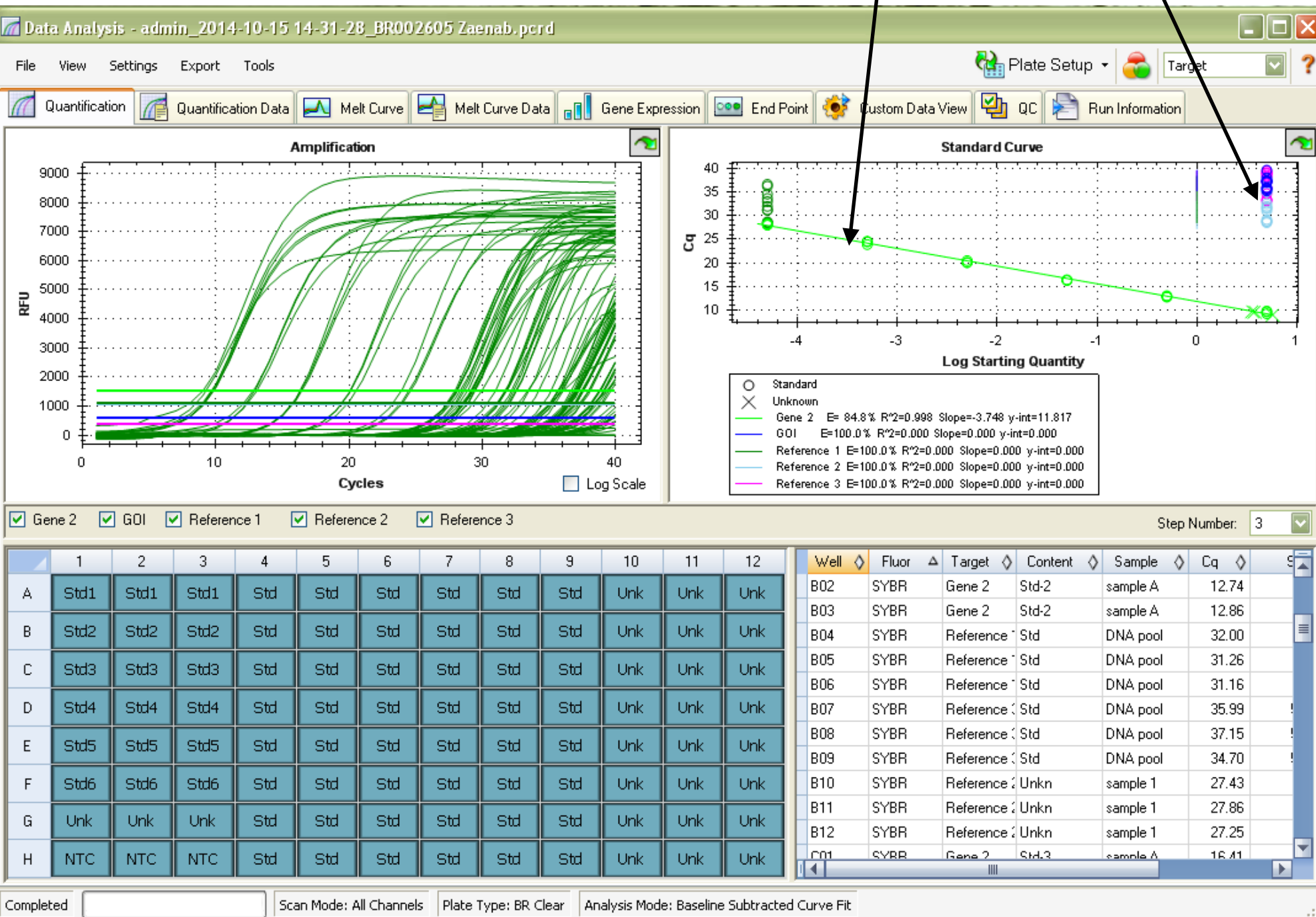


# Data analysis

the curve for Gene 2 looks fine, but other targets curve are screwed up

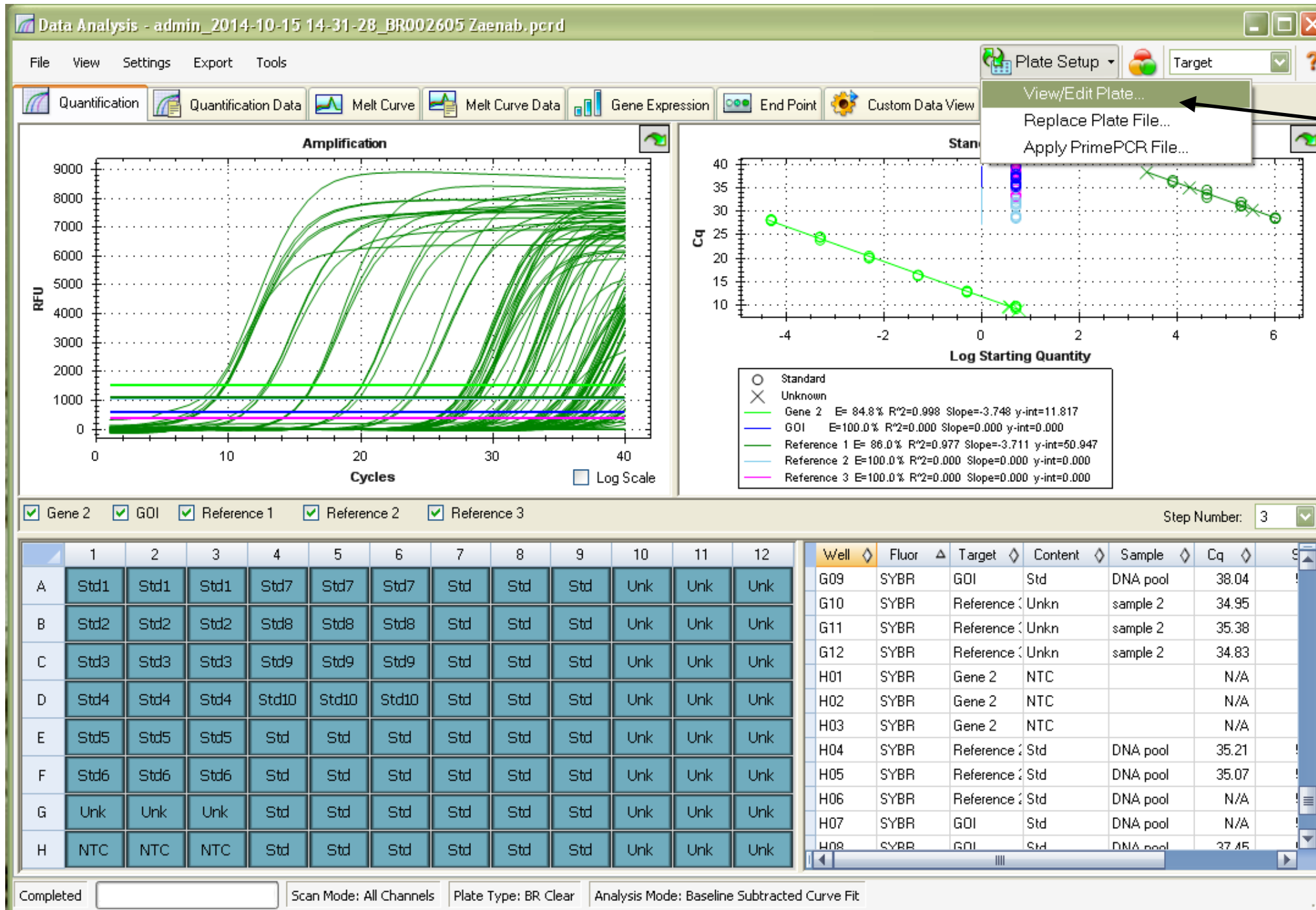
Do not panic  
If you remember we  
didn't insert proper  
dilution factor for your  
primer efficiency  
curves

You need to go to the Plate Setup  
and change it





# Data analysis



Please go to the Plate Setup  
->View /edit plate

# Data analysis

Plate Editor - group 1.pltd

File Settings Editing Tools

100% Scan Mode All Channels Well Groups... Trace Styles... Spreadsheet View/Importer Plate Loading Guide

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std-1	Std-1	Std-1	Std-7	Std-7	Std-7	Std	Std	Std	Unk	Unk	Unk
B	Gene 2 sample A	Gene 2 sample A	Gene 2 sample A	Reference 1 DNA pool	Reference 1 DNA pool	Reference 1 DNA pool	Reference 3 DNA pool	Reference 3 DNA pool	Reference 3 DNA pool	Reference 1 sample 1	Reference 1 sample 1	Reference 1 sample 1
C	Std-2	Std-2	Std-2	Std-8	Std-8	Std-8	Std	Std	Std	Unk	Unk	Unk
D	Gene 2 sample A	Gene 2 sample A	Gene 2 sample A	Reference 1 DNA pool	Reference 1 DNA pool	Reference 1 DNA pool	Reference 3 DNA pool	Reference 3 DNA pool	Reference 3 DNA pool	Reference 2 sample 1	Reference 2 sample 1	Reference 2 sample 1
E	Std-3	Std-3	Std-3	Std-9	Std-9	Std-9	Std	Std	Std	Unk	Unk	Unk
F	Gene 2 sample A	Gene 2 sample A	Gene 2 sample A	Reference 1 DNA pool	Reference 1 DNA pool	Reference 1 DNA pool	Reference 3 DNA pool	Reference 3 DNA pool	Reference 3 DNA pool	Reference 3 sample 1	Reference 3 sample 1	Reference 3 sample 1
G	Std-4	Std-4	Std-4	Std-10	Std-10	Std-10	Std	Std	Std	Unk	Unk	Unk
H	Gene 2 sample A	Gene 2 sample A	Gene 2 sample A	Reference 2 DNA pool	Reference 2 DNA pool	Reference 2 DNA pool	GOI DNA pool	GOI DNA pool	GOI DNA pool	Reference 1 sample 2	Reference 1 sample 2	Reference 1 sample 2
	Std-5	Std-5	Std-5	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
	Gene 2 sample A	Gene 2 sample A	Gene 2 sample A	Reference 2 DNA pool	Reference 2 DNA pool	Reference 2 DNA pool	GOI DNA pool	GOI DNA pool	GOI DNA pool	Reference 2 sample 2	Reference 2 sample 2	Reference 2 sample 2
	Std-6	Std-6	Std-6	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
	Gene 2 sample A	Gene 2 sample A	Gene 2 sample A	Reference 2 DNA pool	Reference 2 DNA pool	Reference 2 DNA pool	GOI DNA pool	GOI DNA pool	GOI DNA pool	Reference 2 sample 2	Reference 2 sample 2	Reference 2 sample 2
	Unk	Unk	Unk	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
	Gene 2 sample B	Gene 2 sample B	Gene 2 sample B	Reference 2 DNA pool	Reference 2 DNA pool	Reference 2 DNA pool	GOI DNA pool	GOI DNA pool	GOI DNA pool	Reference 3 sample 2	Reference 3 sample 2	Reference 3 sample 2
	NTC	NTC	NTC	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
	Gene 2	Gene 2	Gene 2	Reference 2 DNA pool	Reference 2 DNA pool	Reference 2 DNA pool	GOI DNA pool	GOI DNA pool	GOI DNA pool	GOI sample 2	GOI sample 2	GOI sample 2

1. select all wells for one of the efficiency curves

2. click on Replicate Series to tell the software, that you have technical triplicates loaded horizontally:

Replicate size =3  
Horizontal

Please note, that the software cannot have standards with overlapping numeration  
your starting replicate # should be greater than the number of Standards already assigned  
in this case you salary have 10 standards, so you should sign the following starting from "starting replicate 11"

Plate Type: BR Clear

View Target Name Concentration Sample Well Group Biological Set Well Note

OK Cancel

if you add a #3 here and click on Load, all selected wells will be considered as a replicate #3 (you don't want it)

2. click on Replicate Series to tell the software, that you have technical triplicates loaded horizontally:  
Replicate size =3  
Horizontal  
Please note, that the software cannot have standards with overlapping numeration  
your starting replicate # should be greater than the number of Standards already assigned  
in this case you salary have 10 standards, so you should sign the following starting from "starting replicate 11"

# Data analysis

Plate Editor - group 1.pltd

File Settings Editing Tools

100% Scan Mode All Channels Well Groups... Trace Styles... Spreadsheet View/Importer Plate Loading Guide

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std-1 Gene 2 sample A	Std-1 Gene 2 sample A	Std-1 Gene 2 sample A	Std-7 Reference 1 DNA pool	Std-7 Reference 1 DNA pool	Std-7 Reference 1 DNA pool	Std Reference 3 DNA pool	Std Reference 3 DNA pool	Std Reference 3 DNA pool	Unk Reference 1 sample 1	Unk Reference 1 sample 1	Unk Reference 1 sample 1
B	Std-2 Gene 2 sample A	Std-2 Gene 2 sample A	Std-2 Gene 2 sample A	Std-8 Reference 1 DNA pool	Std-8 Reference 1 DNA pool	Std-8 Reference 1 DNA pool	Std Reference 3 DNA pool	Std Reference 3 DNA pool	Std Reference 3 DNA pool	Unk Reference 2 sample 1	Unk Reference 2 sample 1	Unk Reference 2 sample 1
C	Std-3 Gene 2 sample A	Std-3 Gene 2 sample A	Std-3 Gene 2 sample A	Std-9 Reference 1 DNA pool	Std-9 Reference 1 DNA pool	Std-9 Reference 1 DNA pool	Std Reference 3 DNA pool	Std Reference 3 DNA pool	Std Reference 3 DNA pool	Unk Reference 3 sample 1	Unk Reference 3 sample 1	Unk Reference 3 sample 1
D	Std-4 Gene 2 sample A	Std-4 Gene 2 sample A	Std-4 Gene 2 sample A	Std-10 Reference 1 DNA pool	Std-10 Reference 1 DNA pool	Std-10 Reference 1 DNA pool	Std Reference 3 DNA pool	Std Reference 3 DNA pool	Std Reference 3 DNA pool	Unk GOI sample 1	Unk GOI sample 1	Unk GOI sample 1
E	Std-5 Gene 2 sample A	Std-5 Gene 2 sample A	Std-5 Gene 2 sample A	Std-11 Reference 2 DNA pool	Std-12 Reference 2 DNA pool	Std-13 Reference 2 DNA pool	Std Reference 2 DNA pool	Std Reference 2 DNA pool	Std Reference 2 DNA pool	Unk Reference 1 sample 2	Unk Reference 1 sample 2	Unk Reference 1 sample 2
F	Std-6 Gene 2 sample A	Std-6 Gene 2 sample A	Std-6 Gene 2 sample A	Std-14 Reference 2 DNA pool	Std-15 Reference 2 DNA pool	Std-16 Reference 2 DNA pool	Std Reference 2 DNA pool	Std Reference 2 DNA pool	Std Reference 2 DNA pool	Unk Reference 2 sample 2	Unk Reference 2 sample 2	Unk Reference 2 sample 2
G	Unk Gene 2 sample B	Unk Gene 2 sample B	Unk Gene 2 sample B	Std-17 Reference 2 DNA pool	Std-18 Reference 2 DNA pool	Std-19 Reference 2 DNA pool	Std Reference 2 DNA pool	Std Reference 2 DNA pool	Std Reference 2 DNA pool	Unk Reference 3 sample 2	Unk Reference 3 sample 2	Unk Reference 3 sample 2
H	NTC Gene 2	NTC Gene 2	NTC Gene 2	Std-20 Reference 2 DNA pool	Std-21 Reference 2 DNA pool	Std-22 Reference 2 DNA pool	Std Reference 2 DNA pool	Std Reference 2 DNA pool	Std Reference 2 DNA pool	Unk GOI sample 2	Unk GOI sample 2	Unk GOI sample 2

Plate Type: BR Clear View Target Name Concentration Sample Well Group Biological Set Well Note

No instrument detected after 120 seconds. User: admin 10/16/2014 8:18 PM

Select Fluorophores...

Sample Type Standard

Load SYBR Target Name Reference 2

Load Sample Name DNA pool

Load Replicate # 1

Replicate Series

Load Concentration: 5.00E+00

Dilution Series

Experiment Set Input dilution series.

Clear Replicate #

Clear Wells

Exclude Wells in Analysis

OK Cancel

open the dilution series

->type in a starting concentration

->type in dilution factor

->click on Apply

-> ok

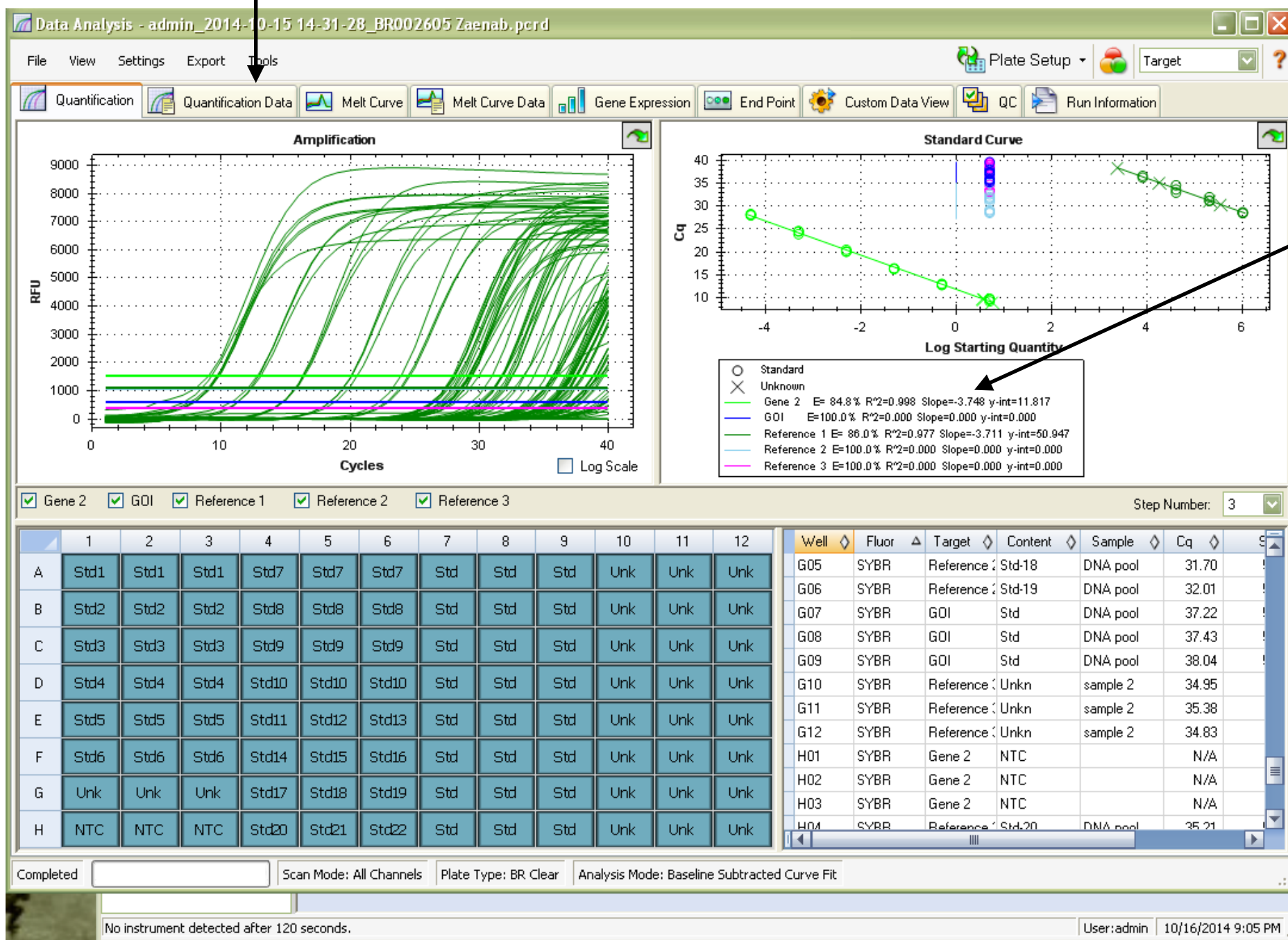
->and apply changes made in the plate layout

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



# Data analysis

go to the quantification data to see more information, you can sort the whole table by clicking on the small arrows in the header row. For example you can sort all data according to the target name



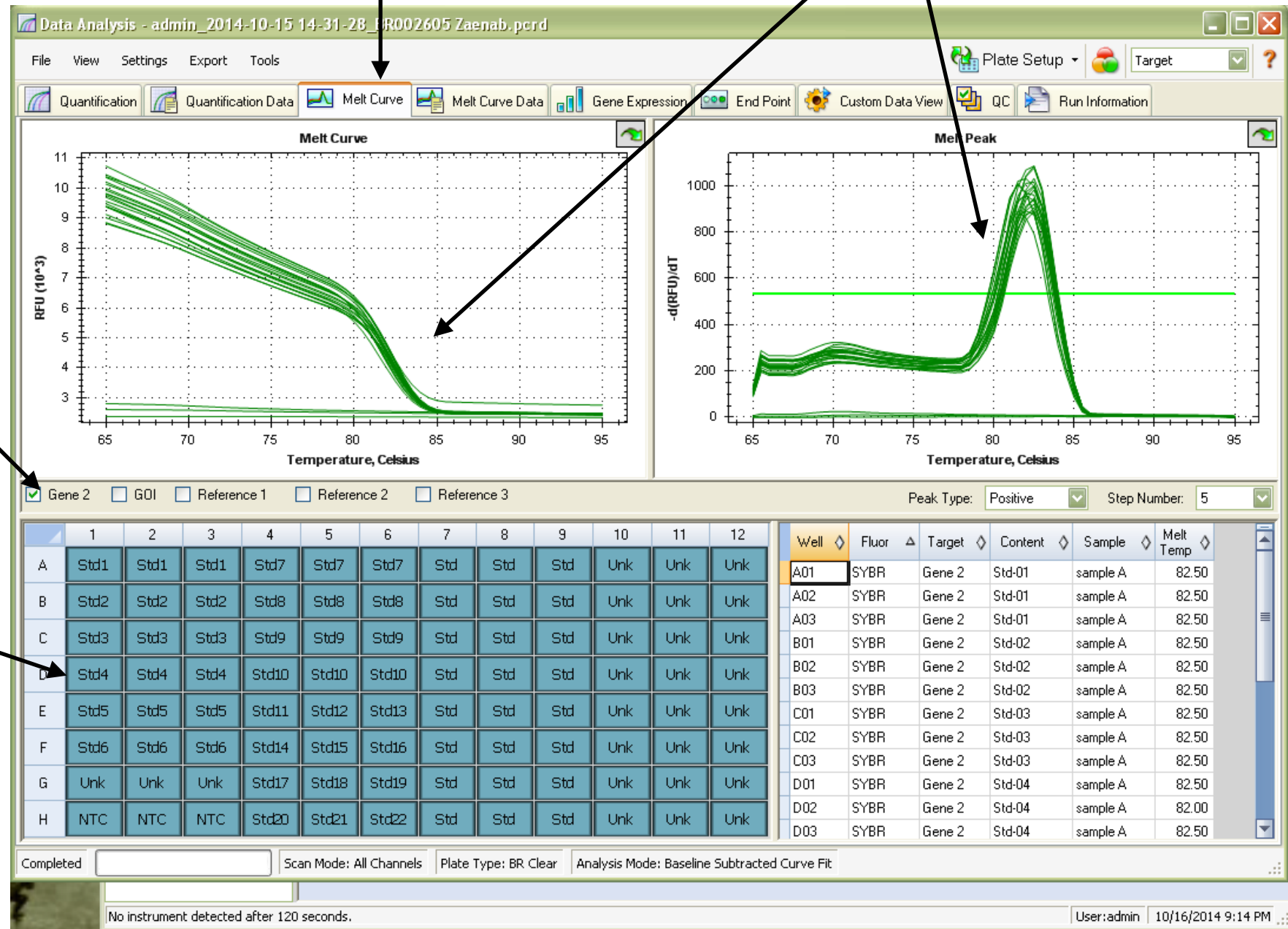
on the graph you will see the standard curve, Efficiency,  $R^2$ , slope and y intercept values

**please remind yourself, what are the desired and what are the allowed values for Efficiency and  $R^2$**

# Data analysis

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

Melt curve

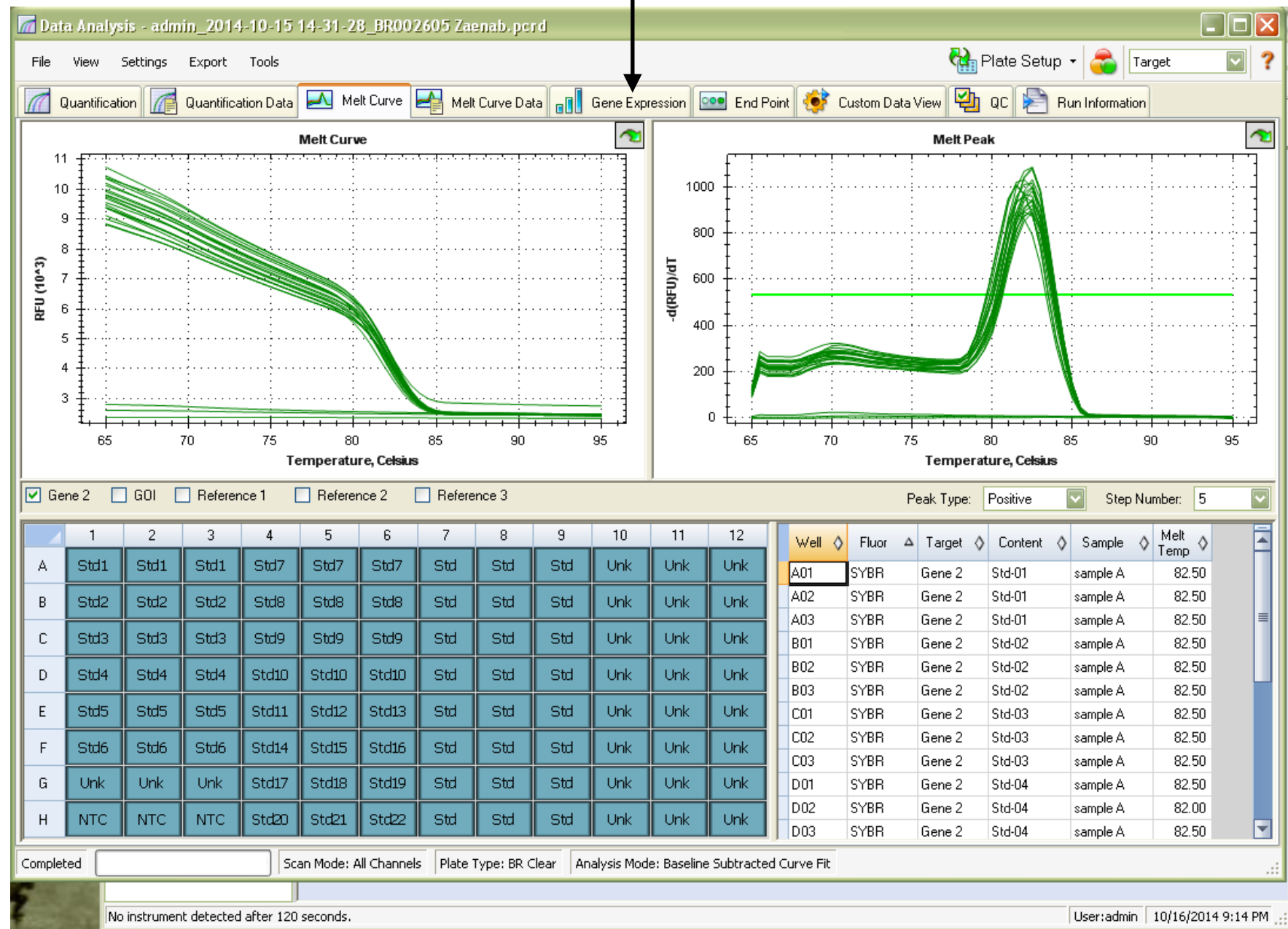


you can select curve for  
which target you want to see

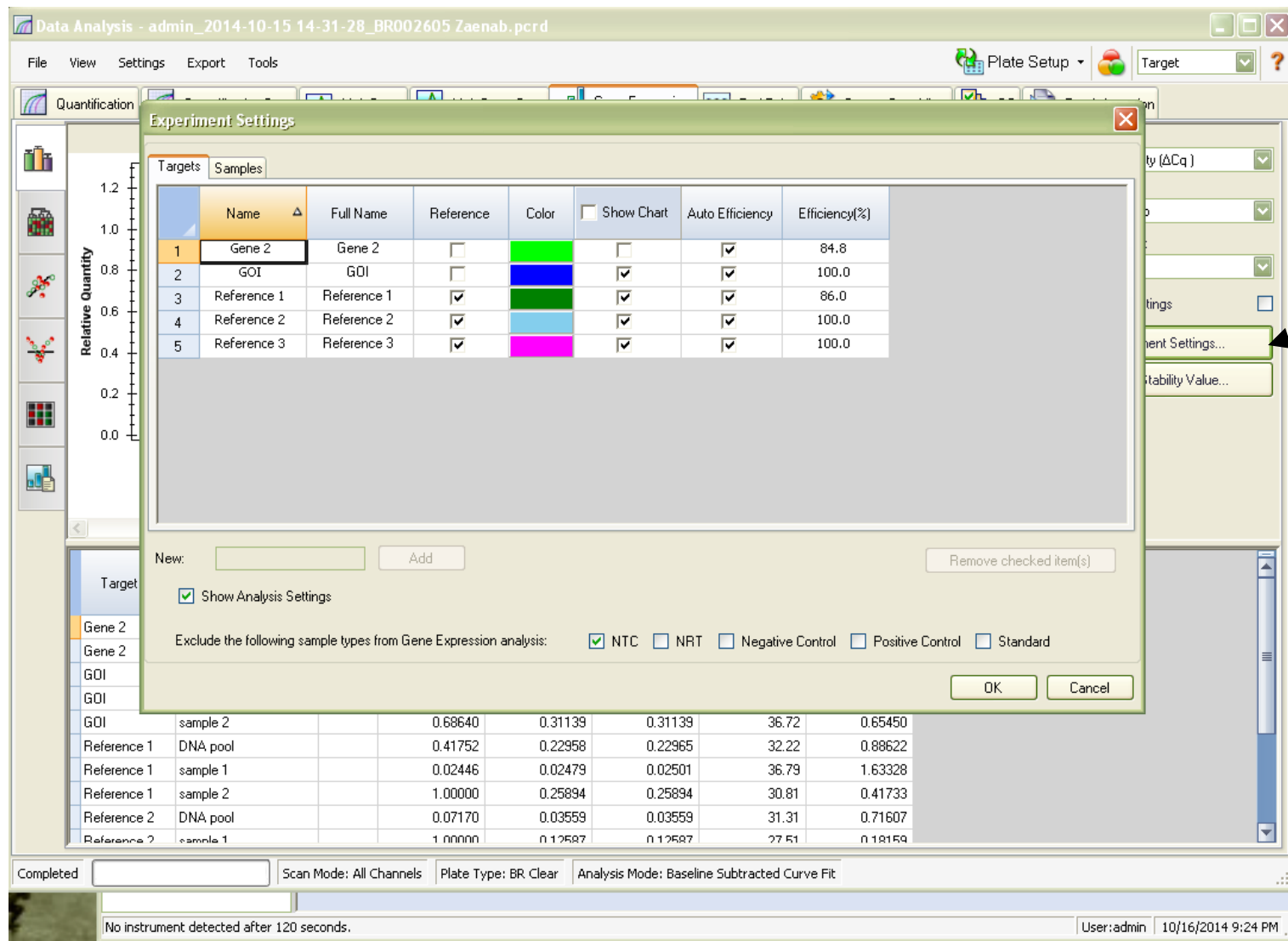
and also curves for which  
wells should be shown

# Data analysis

Here you run Relative qPCR analysis



# Data analysis



Click on the Experiment setting

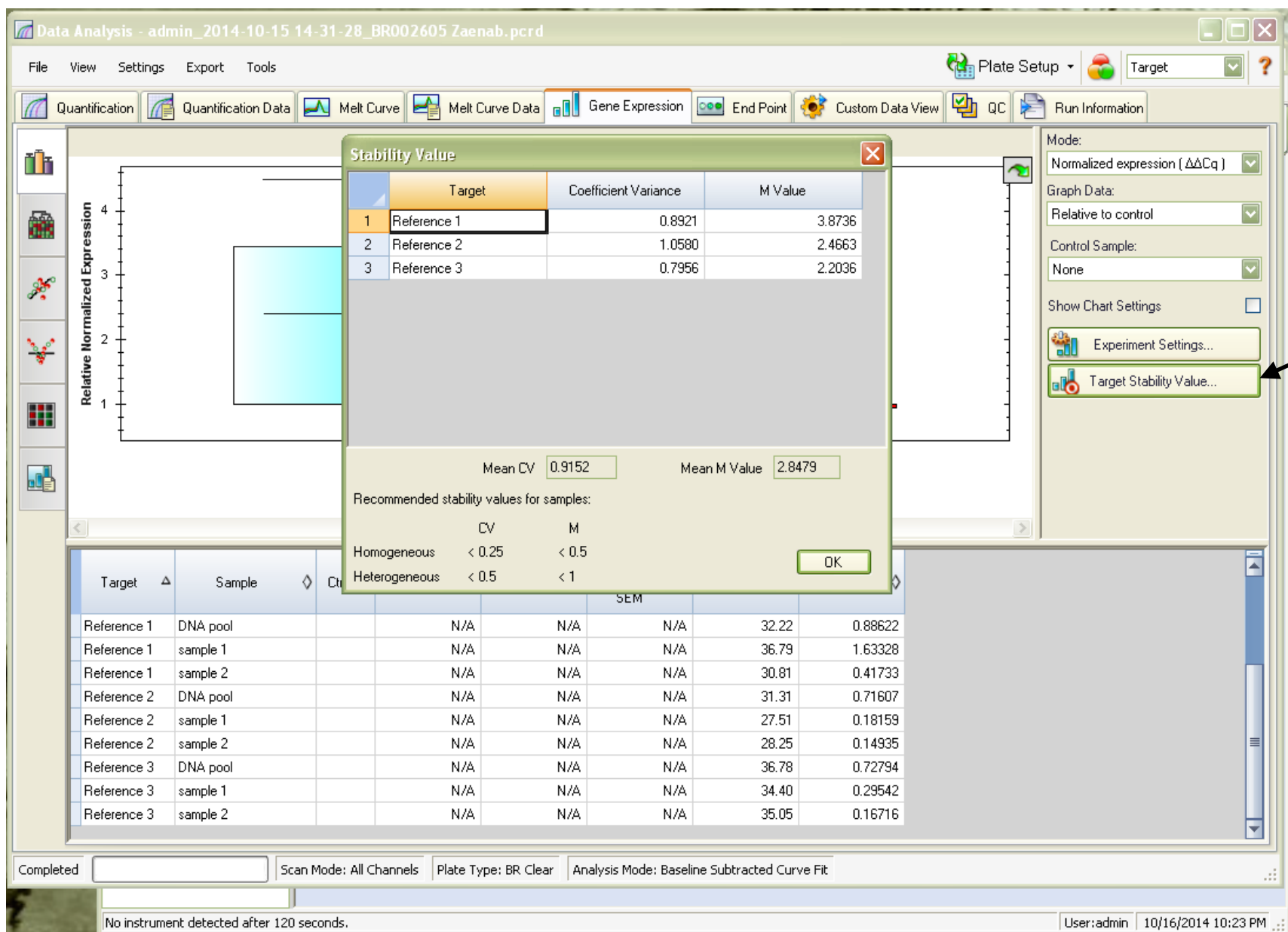
in the Target tab:

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 Sample tab:

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)

# Data analysis



1. Click on the Target stability value

please remind yourself, what is it for and what are the desirable values

2. adjust your selections in the Experiment Settings based on the M values you get