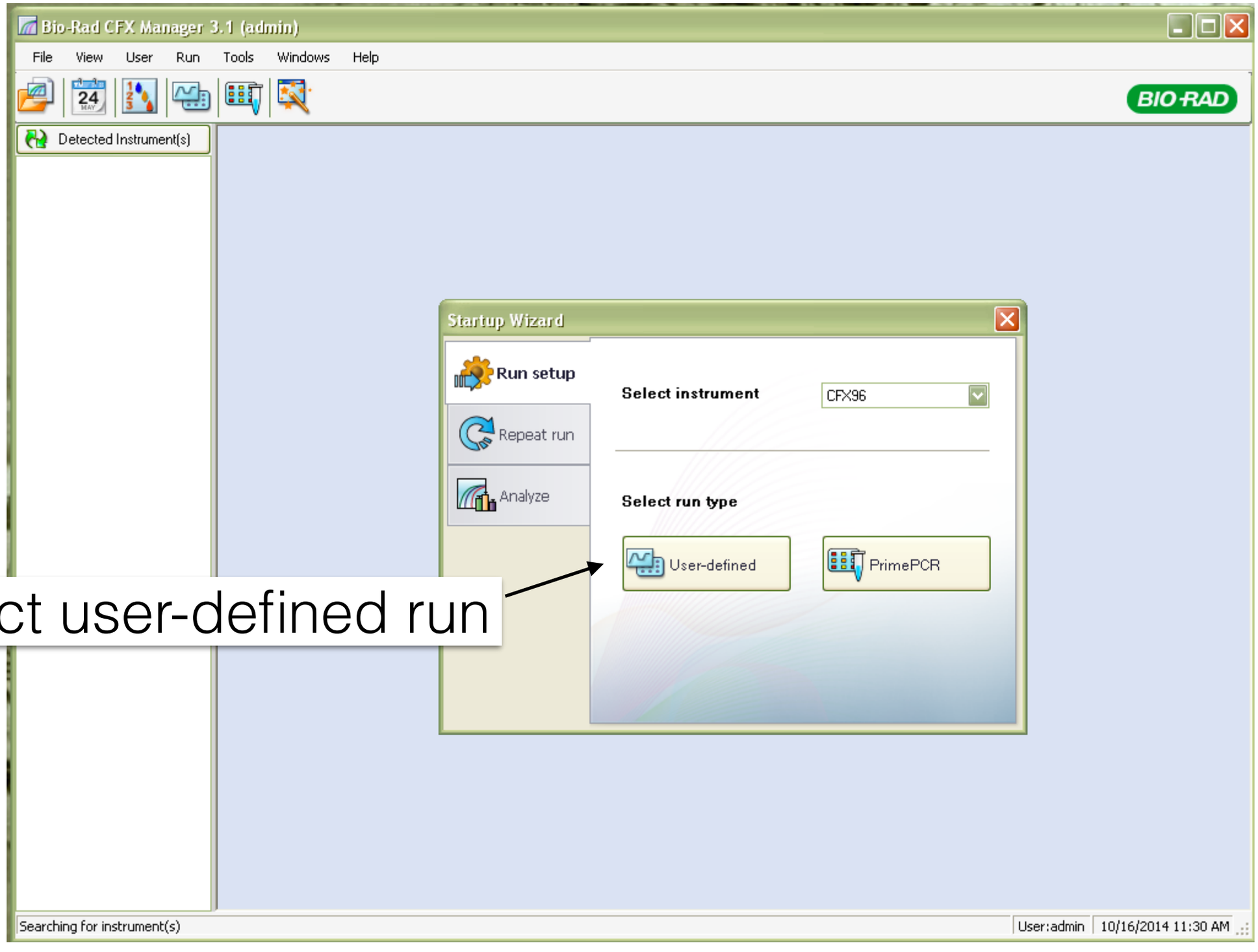
CFX Manager

Run set up

Protocol must be defined BEFORE run
please check your kit manual for guidelines

Plate Layout can be edited after the run

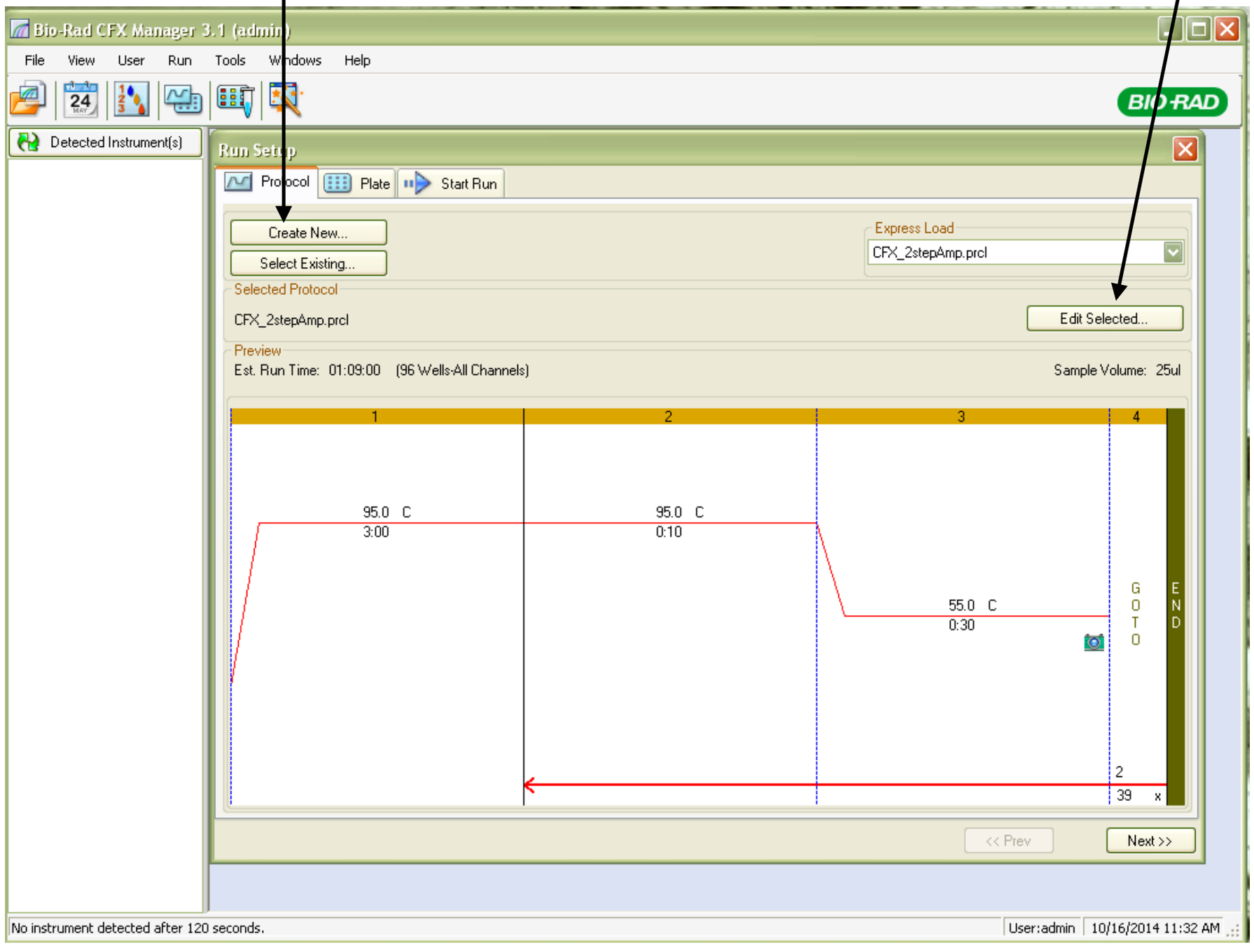
Run set up


Bio-Rad CFX Manager 3.1 (admin)
File
View
User
Run
Tools
Windows
Help
BIO-RAD
Detected Instrument(s)
Startup Wizard
Run setup
Repeat run
Analyze
Select instrument
CFX96
Select run type
User-defined
PrimePCR
1. select user-defined run
Searching for instrument(s)
User:admin
10/16/2014 11:30 AM

Run set up: protocol

2. make your own protocol or select existing

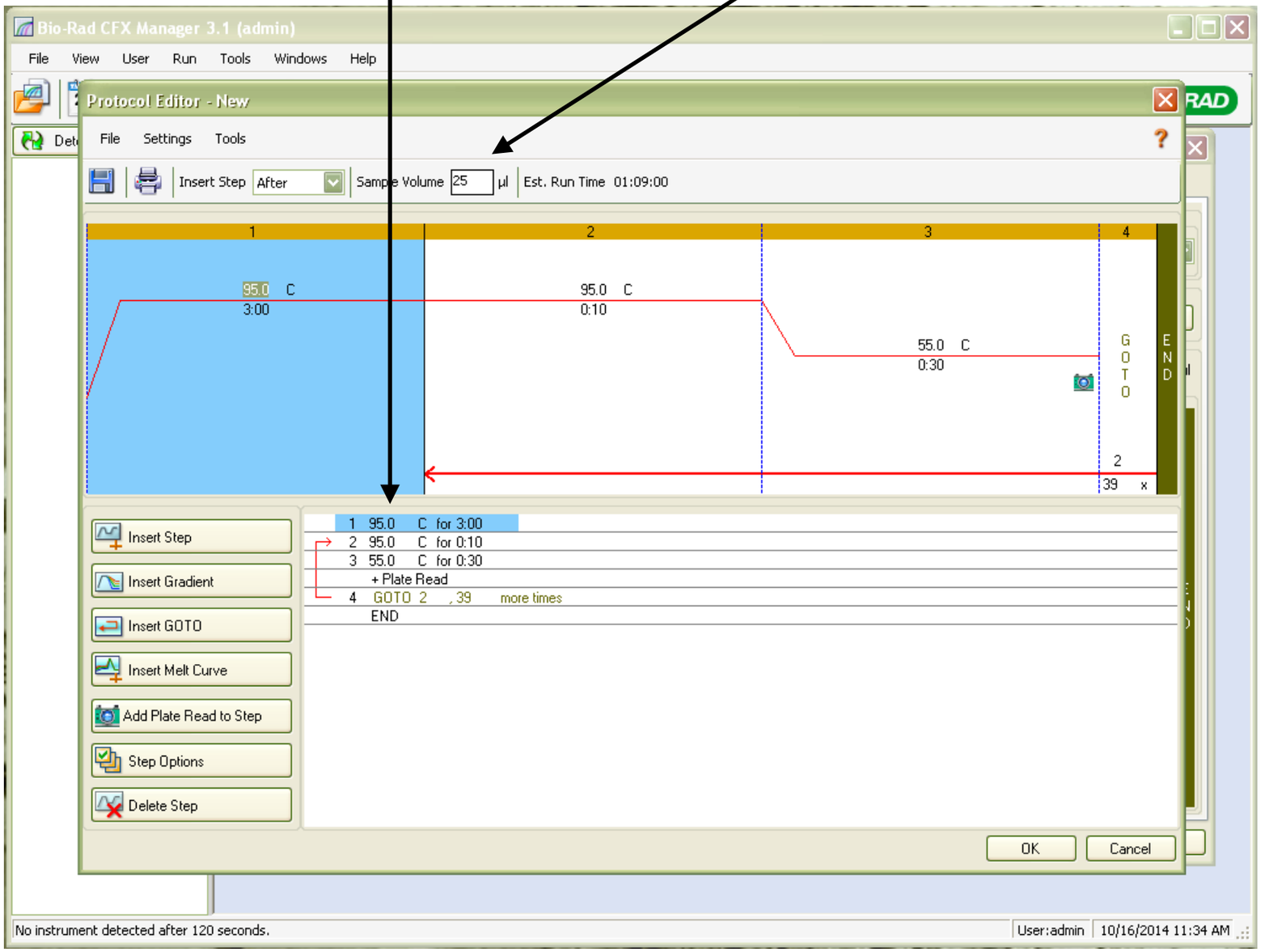
3. you can edit existing protocol



Run set up: protocol

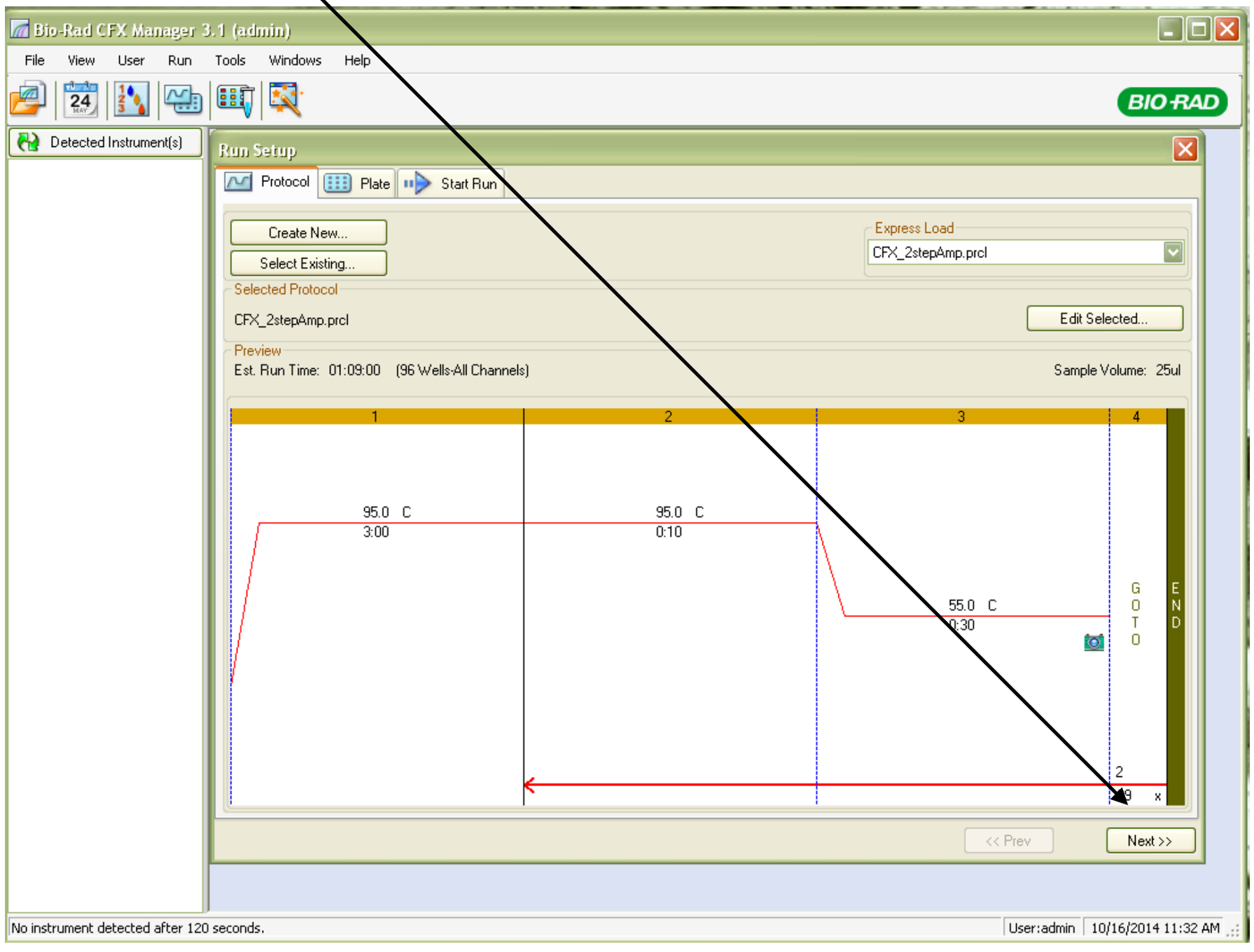
4. type in your protocol

5. type in the volume of your reactions!!



Run set up: protocol

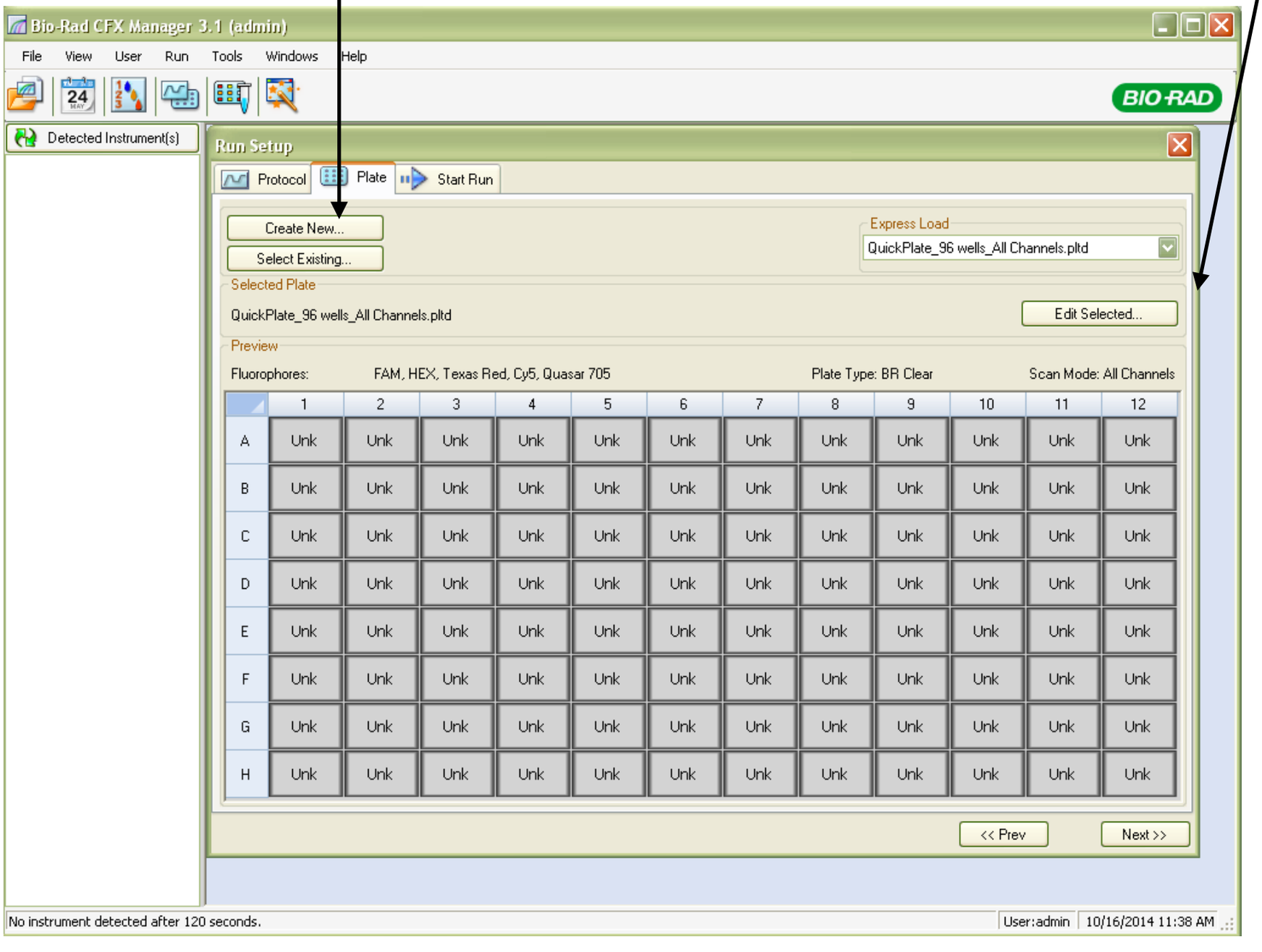
6. save your protocol and click on NEXT



Run set up: plate

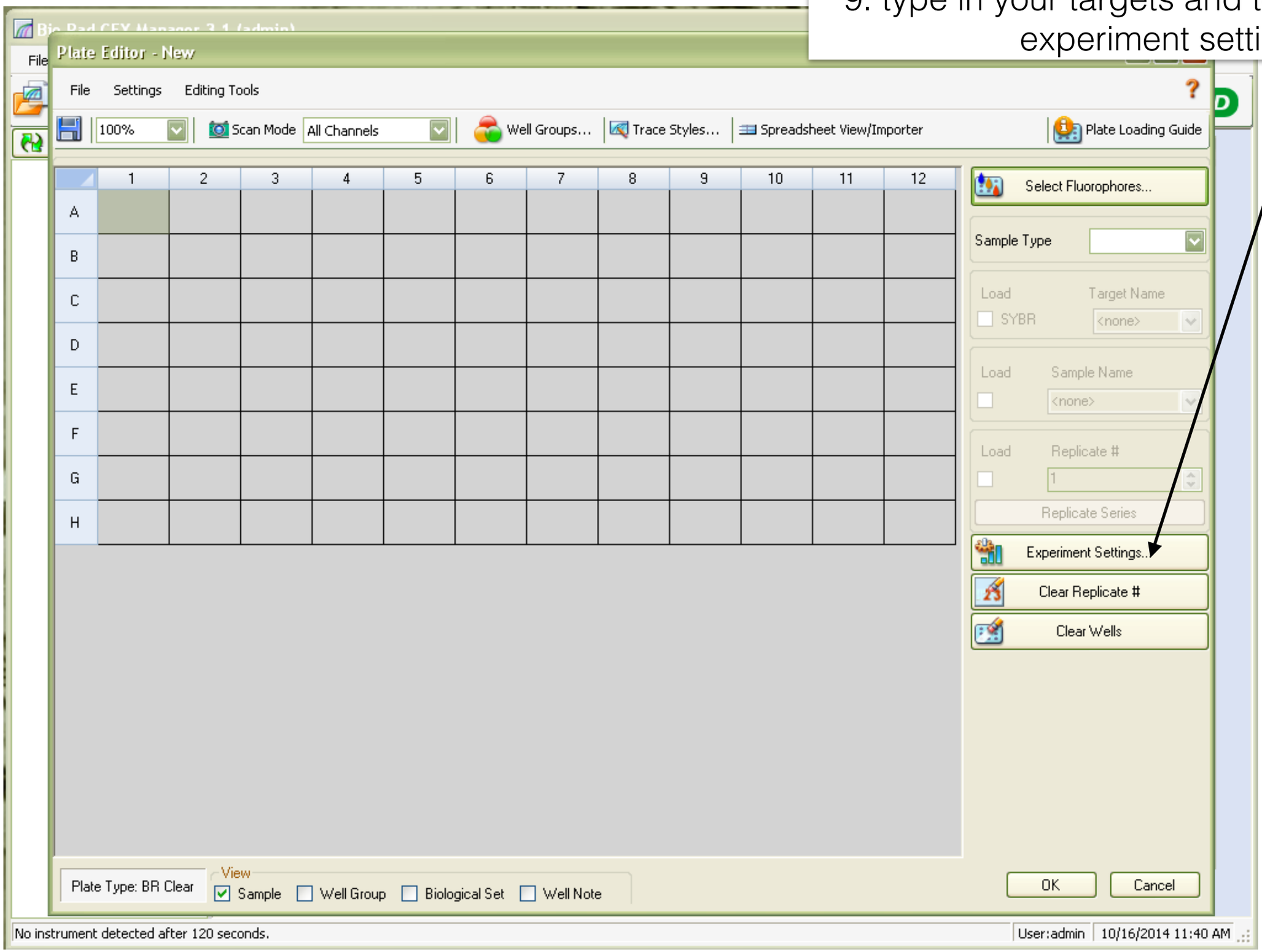
7. make your own plate layout or select existing

8. you can edit existing layout



Run set up: plate

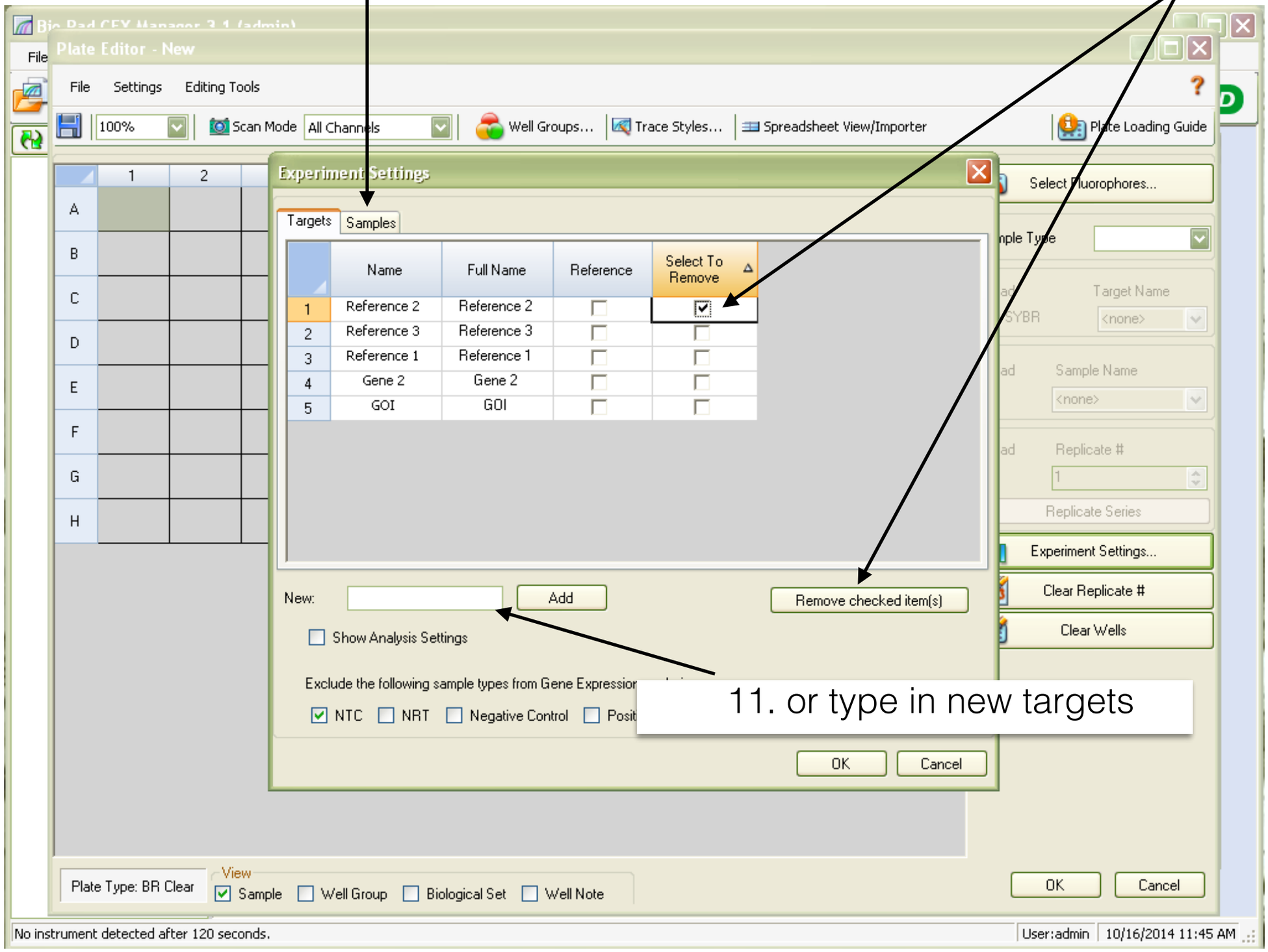
9. type in your targets and templates in the experiment settings



Run set up: plate

12. and do the same for your templates

10. you can remove target names from the list

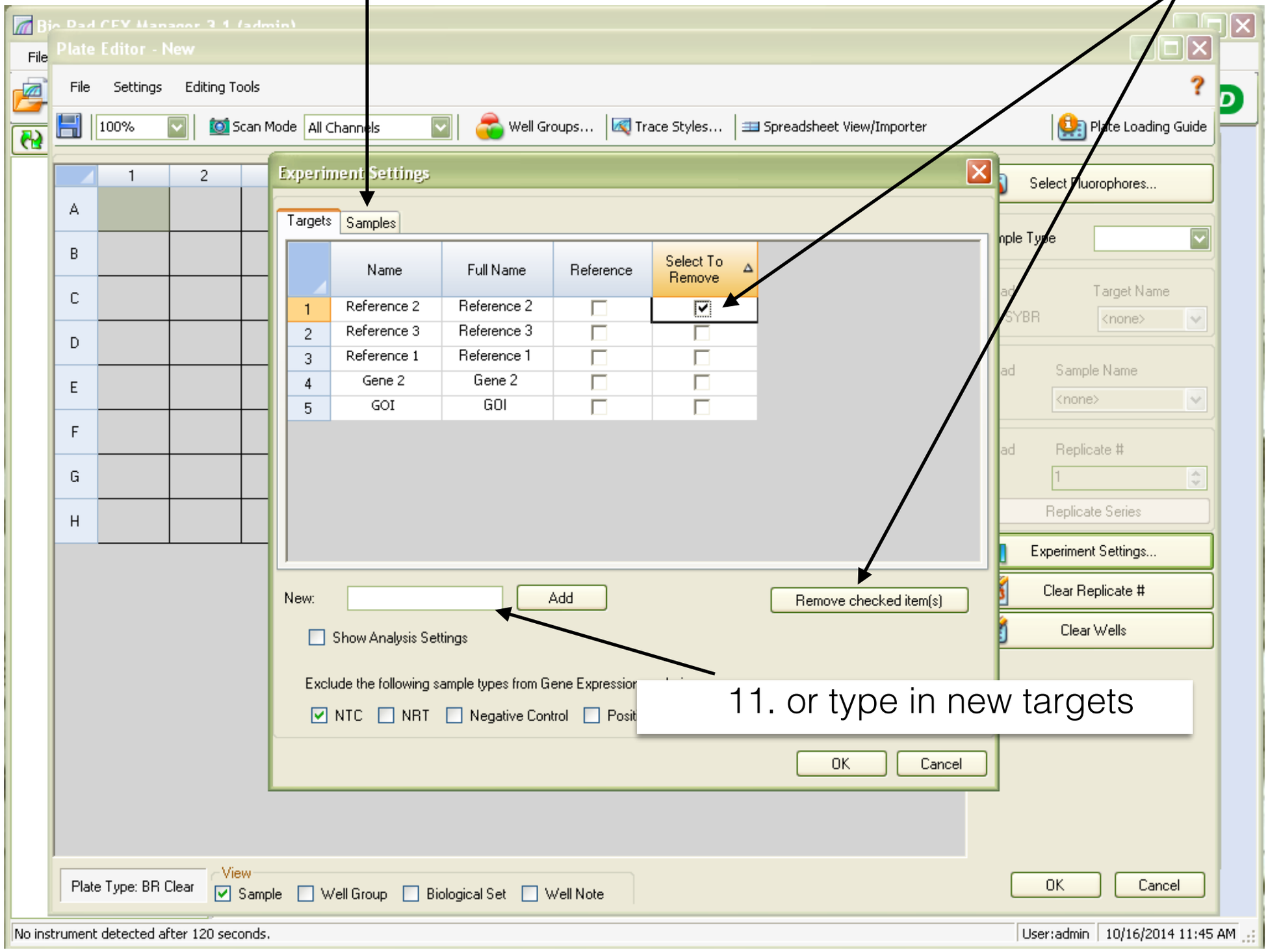


11. or type in new targets

Run set up: plate

12. and do the same for your templates

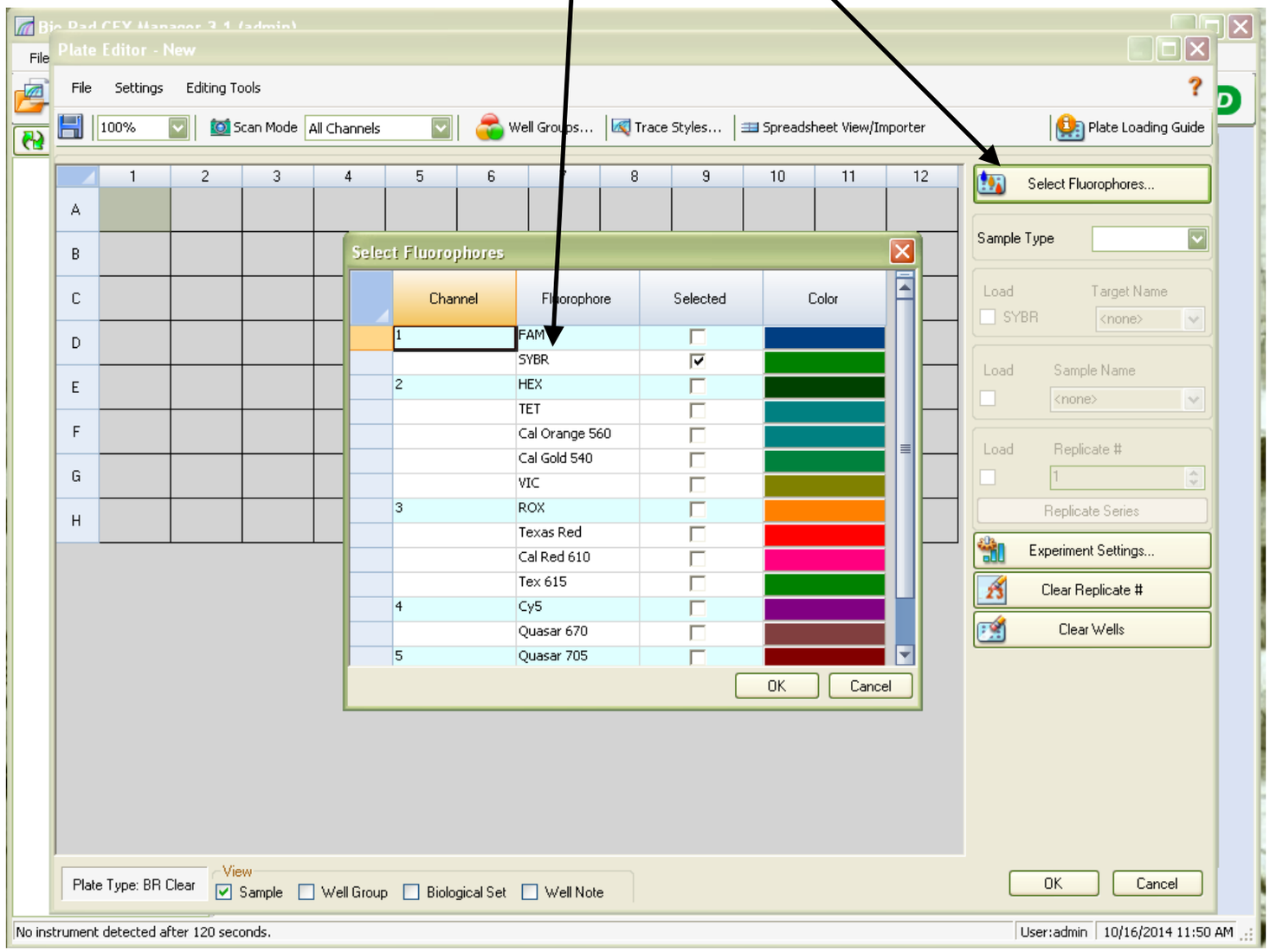
10. you can remove target names from the list



11. or type in new targets

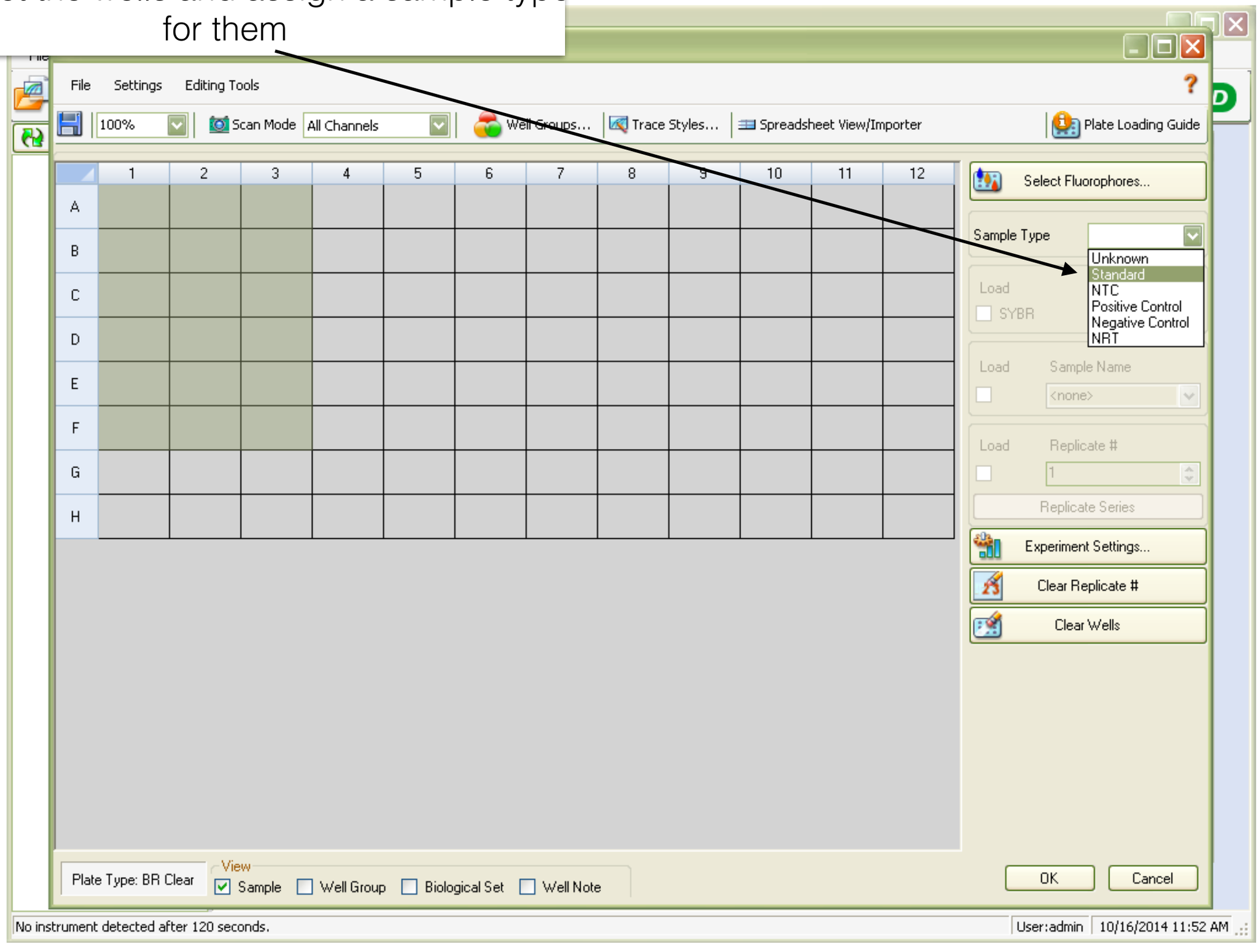
Run set up: plate

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



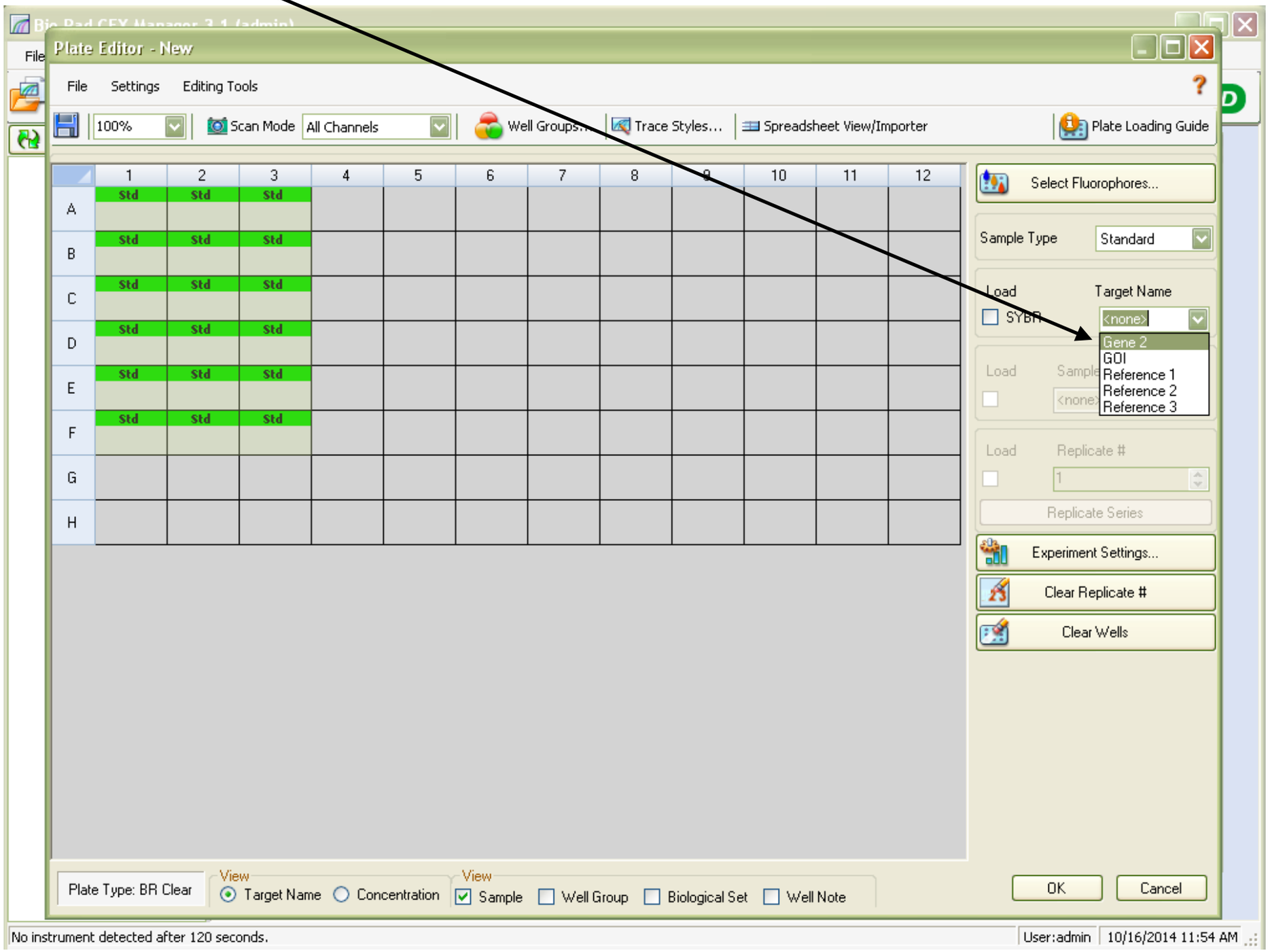
Run set up: plate

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



Run set up: plate

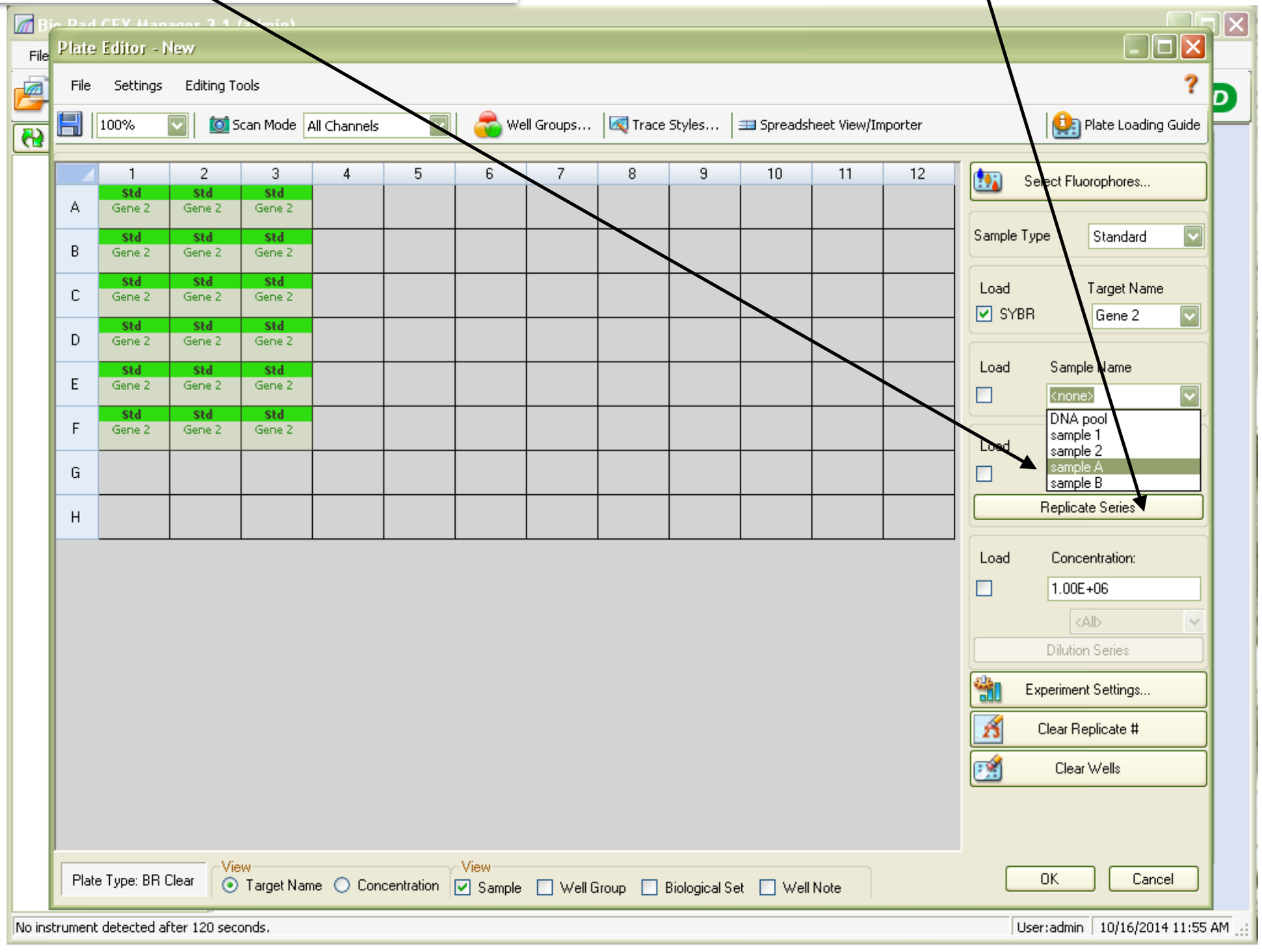
15. select the wells and assign the target gene for them (you can type the name here directly)



Run set up: plate

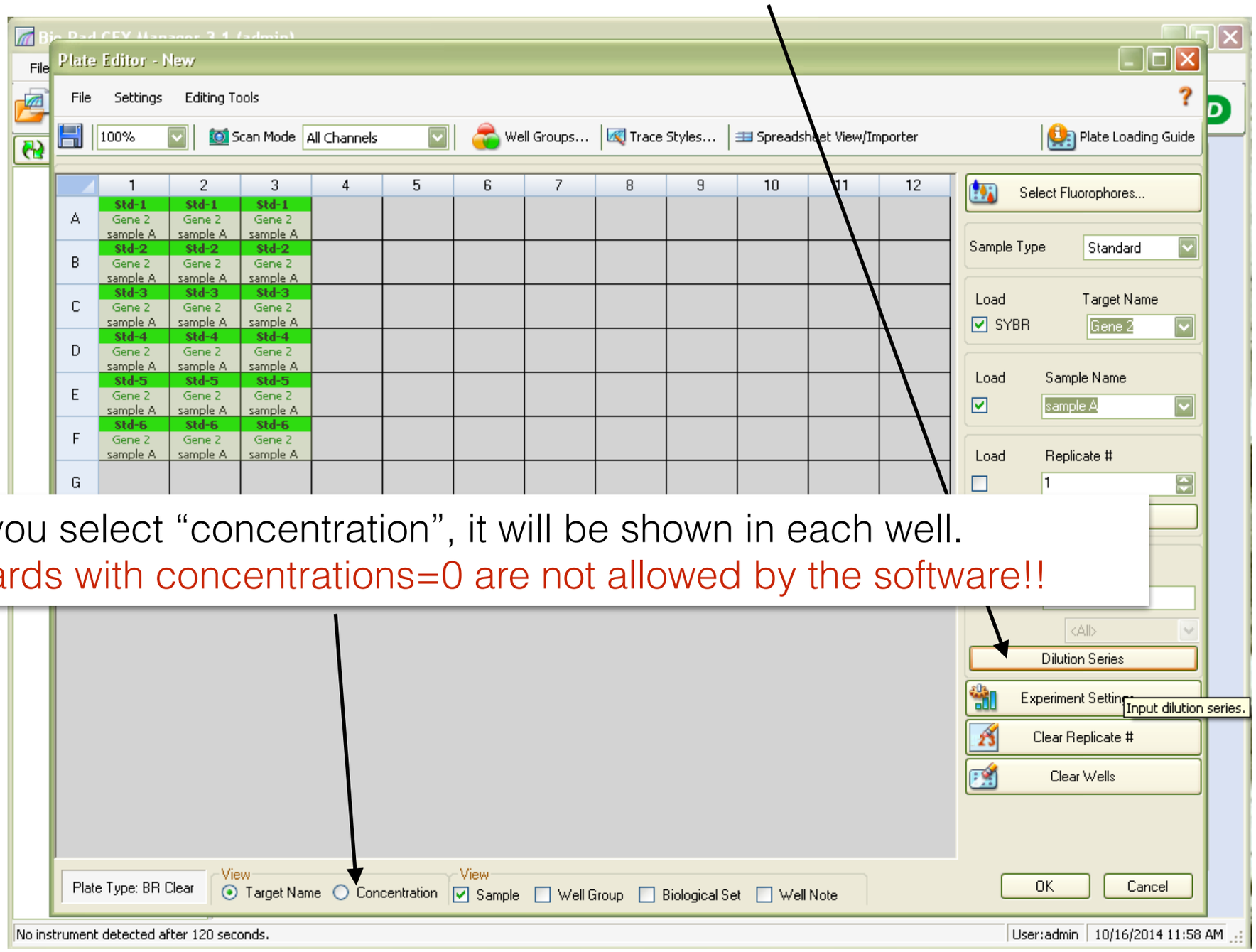
16. select the wells and assign the template for them (you can type it in here directly)

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



Run set up: plate

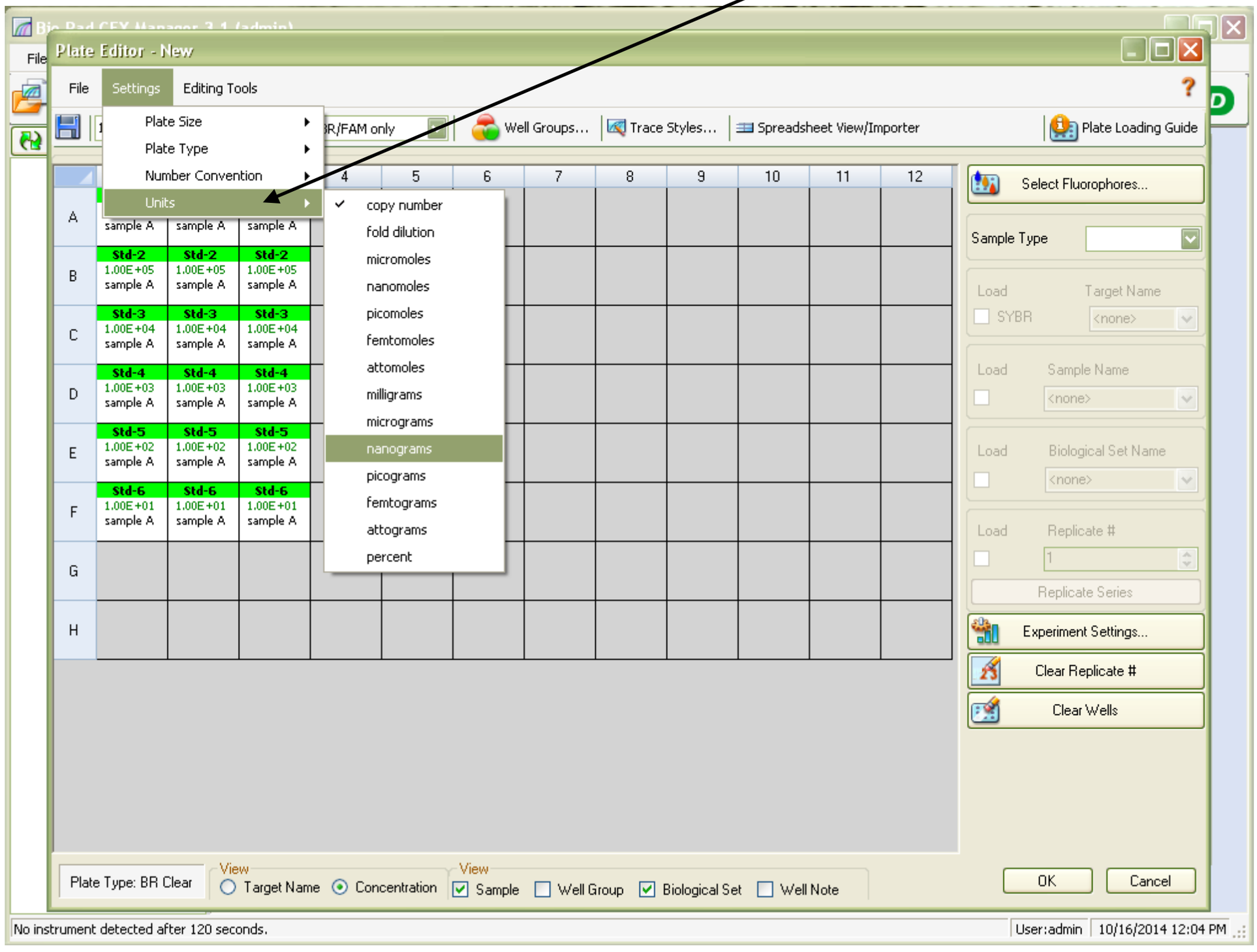
18. in dilution series you can type in initial concentration (for primer efficiency use a random number) and the dilutions factor. and click on Apply



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

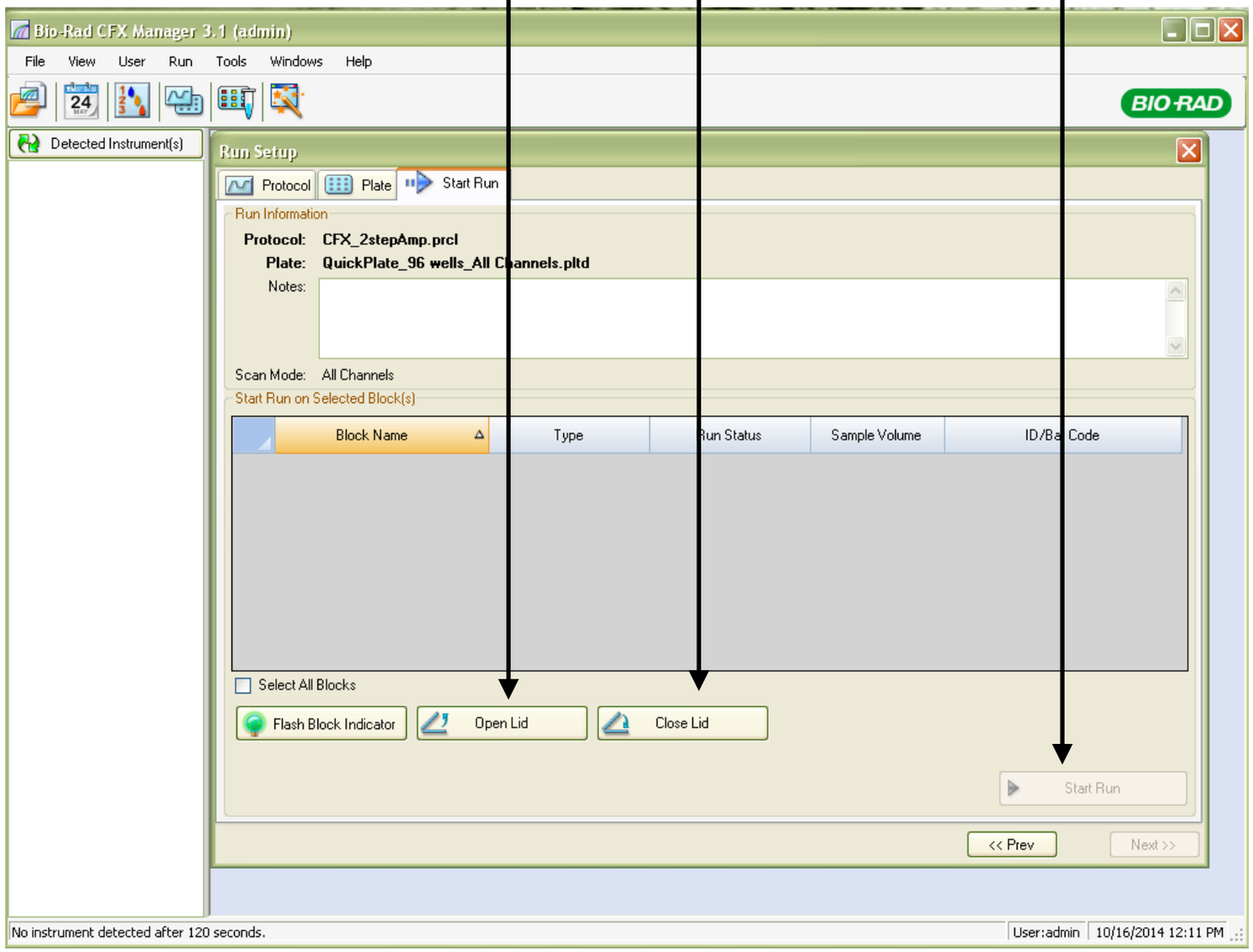
Run set up: plate

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



Run

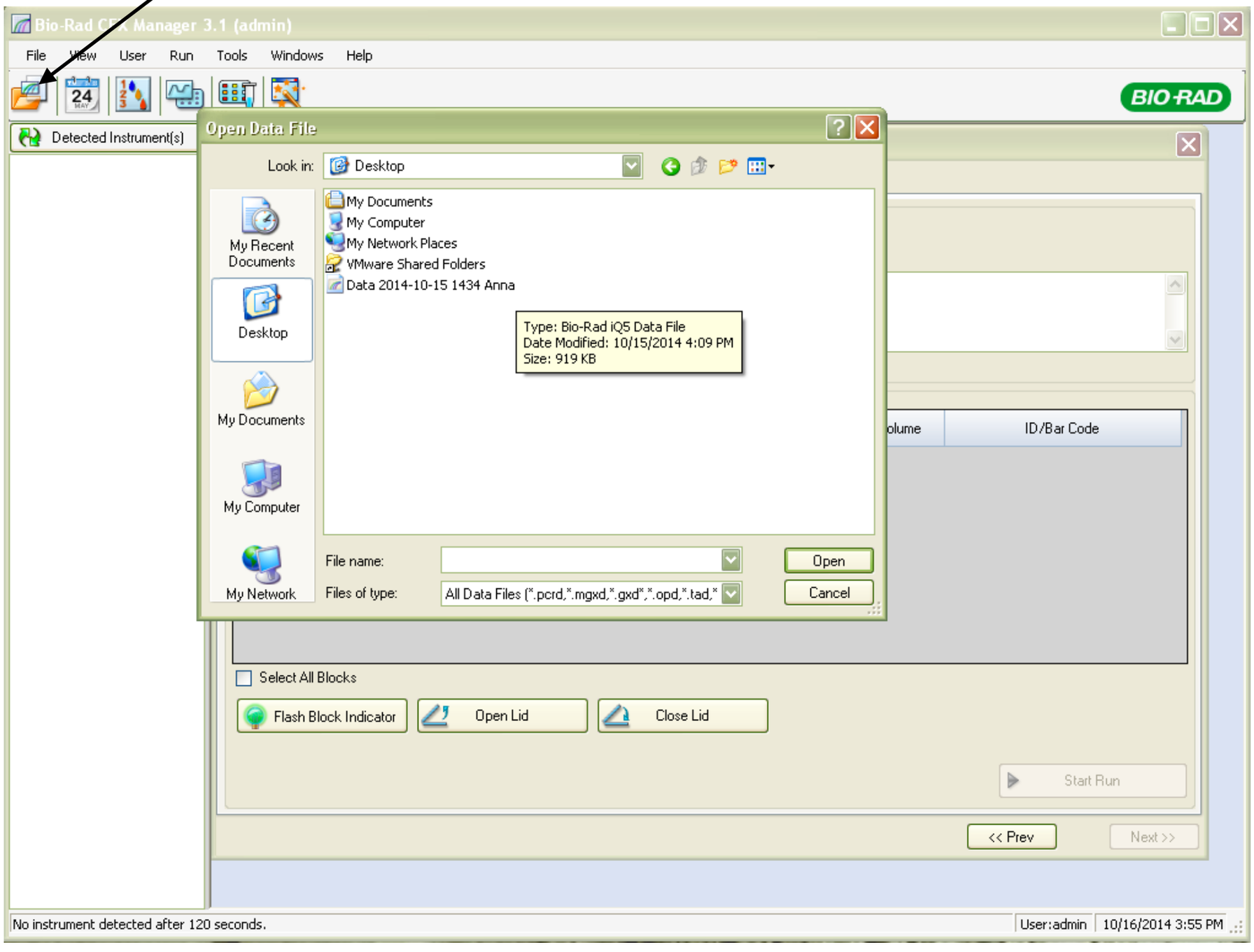
21. open the lid, place your plate on the thermoblock , close the lid and start run



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

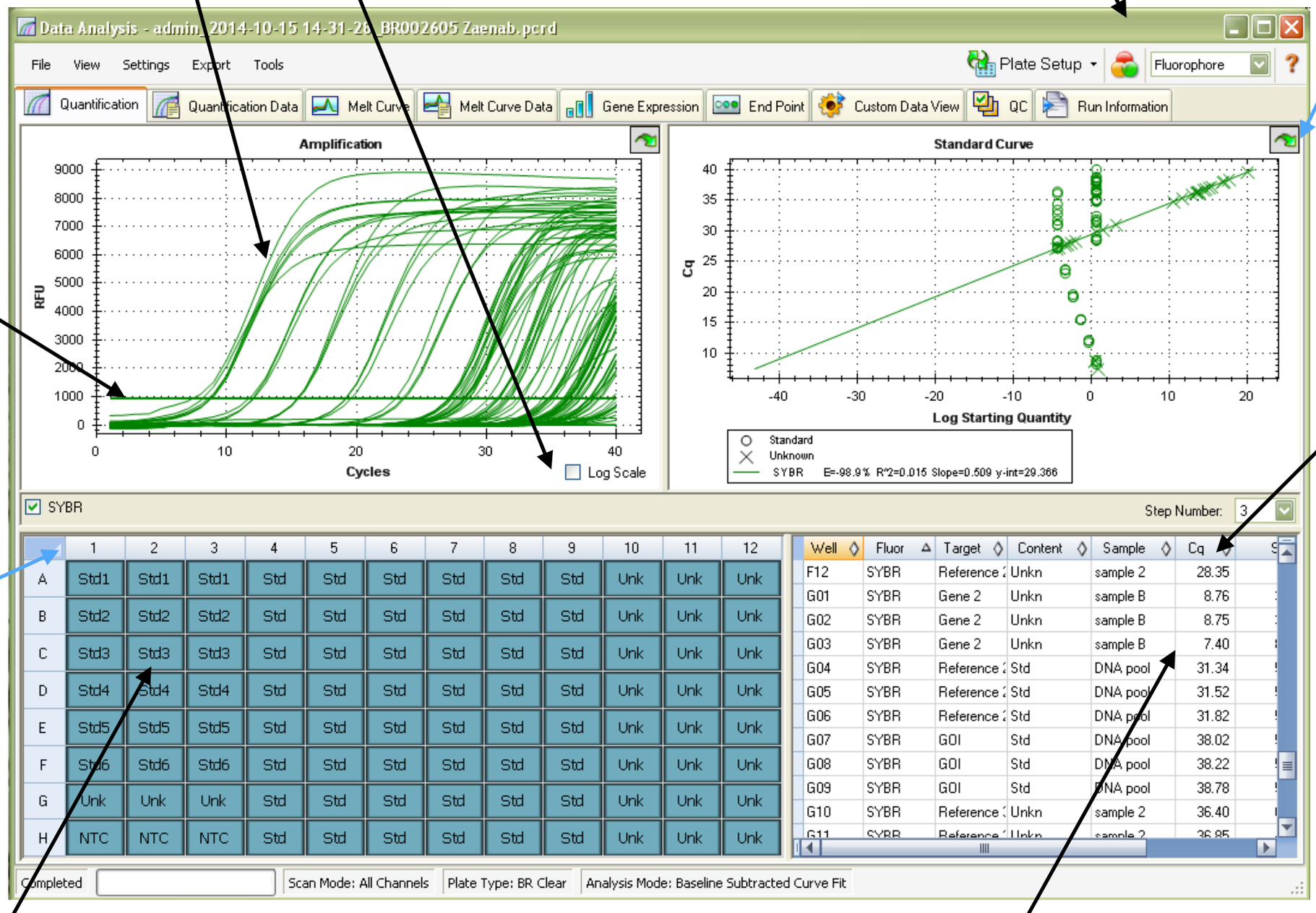
Do not painc! now the standard curve is calculated for both your experiments mixed together

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

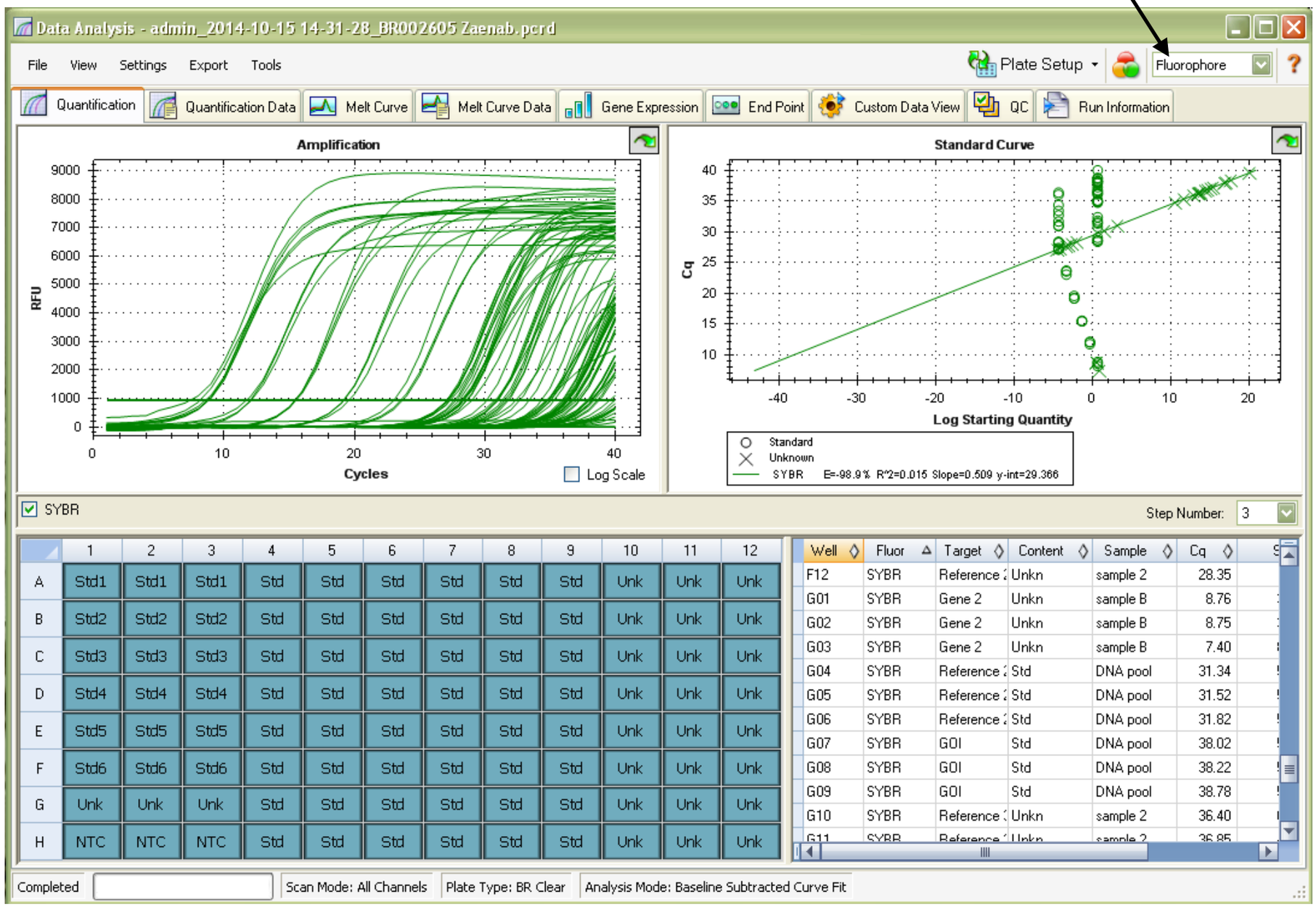
you can select/deselect wells you want to see

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

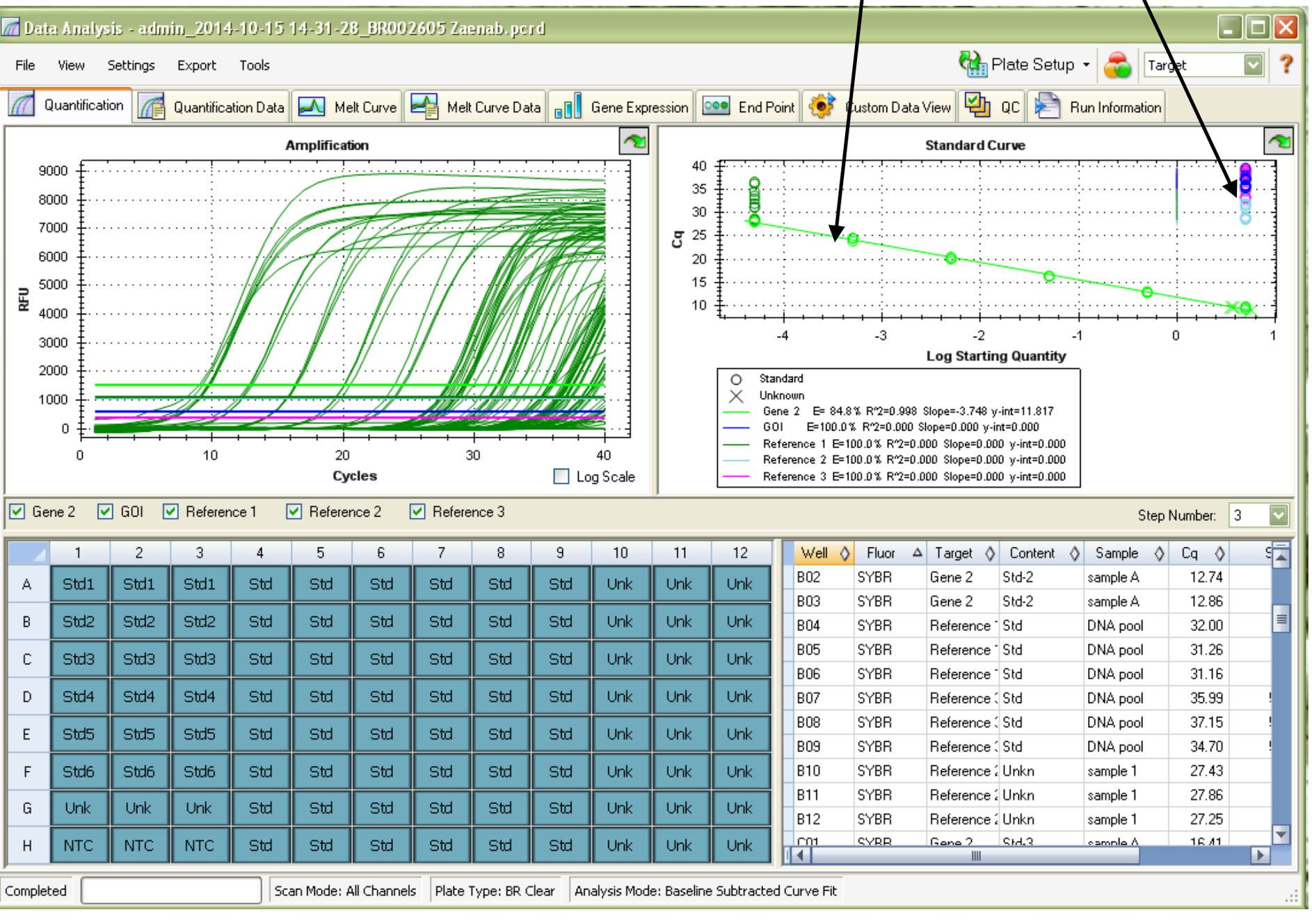


	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1	Std1	Std1	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
B	Std2	Std2	Std2	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
C	Std3	Std3	Std3	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
D	Std4	Std4	Std4	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
E	Std5	Std5	Std5	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
F	Std6	Std6	Std6	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
G	Unk	Unk	Unk	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
H	NTC	NTC	NTC	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk

Well	Fluor	Target	Content	Sample	Cq
F12	SYBR	Reference 2	Unkn	sample 2	28.35
G01	SYBR	Gene 2	Unkn	sample B	8.76
G02	SYBR	Gene 2	Unkn	sample B	8.75
G03	SYBR	Gene 2	Unkn	sample B	7.40
G04	SYBR	Reference 2	Std	DNA pool	31.34
G05	SYBR	Reference 2	Std	DNA pool	31.52
G06	SYBR	Reference 2	Std	DNA pool	31.82
G07	SYBR	GOI	Std	DNA pool	38.02
G08	SYBR	GOI	Std	DNA pool	38.22
G09	SYBR	GOI	Std	DNA pool	38.78
G10	SYBR	Reference 1	Unkn	sample 2	36.40
G11	SYBR	Reference 1	Unkn	sample 2	36.85

Data analysis

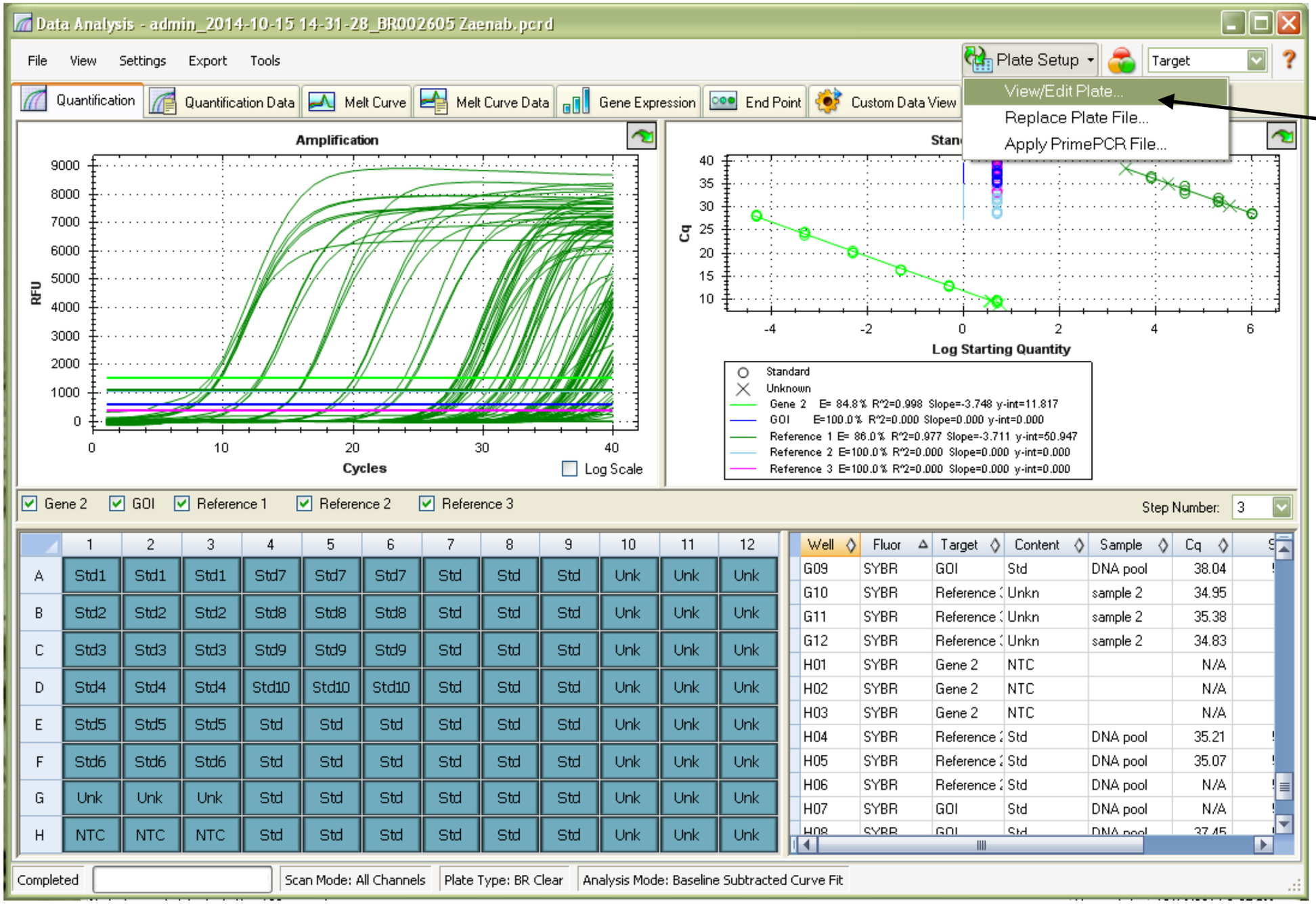
the curve for Gene 2 looks fine, but other targets curve are screwed up



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

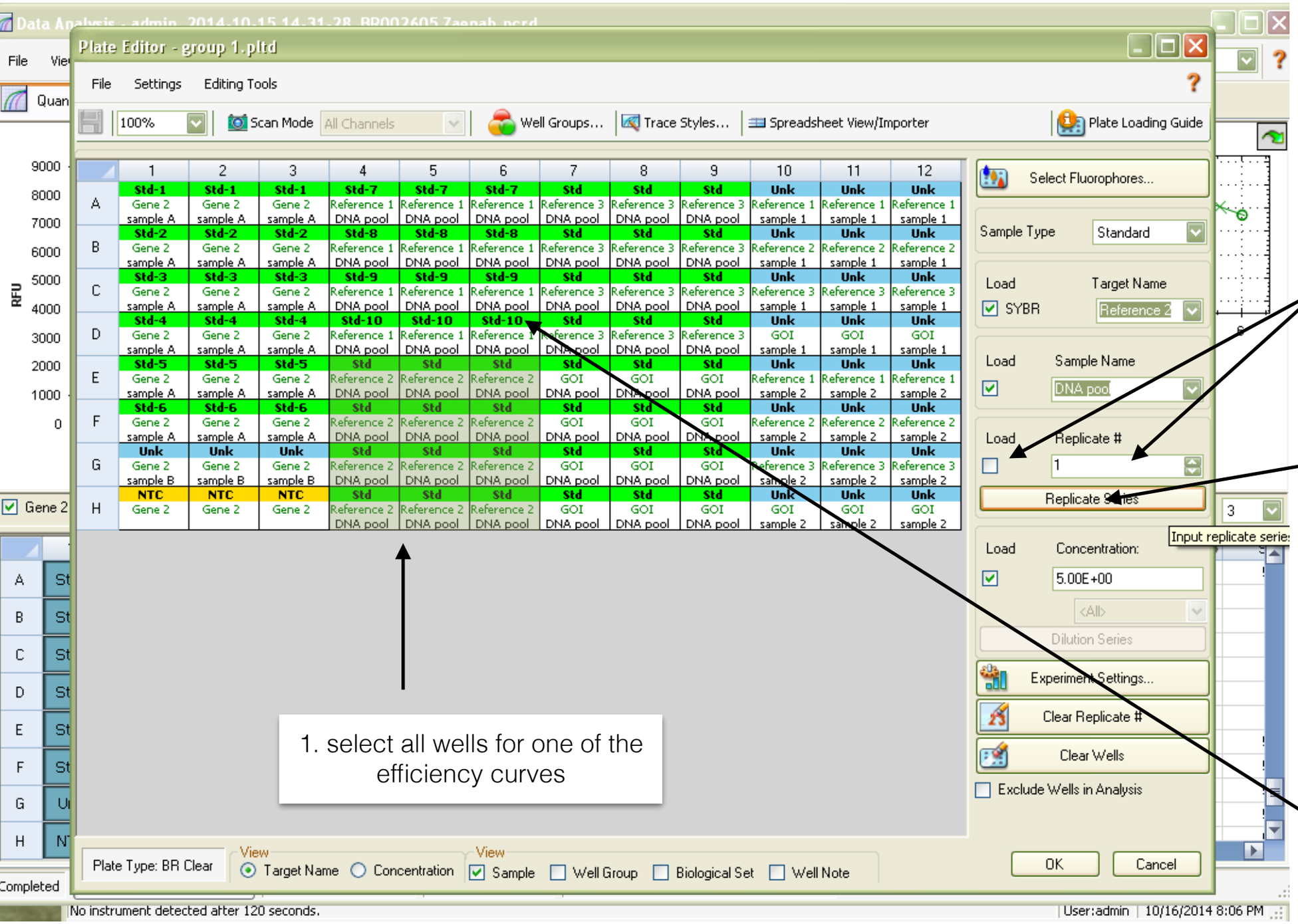


	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1	Std1	Std1	Std7	Std7	Std7	Std	Std	Std	Unk	Unk	Unk
B	Std2	Std2	Std2	Std8	Std8	Std8	Std	Std	Std	Unk	Unk	Unk
C	Std3	Std3	Std3	Std9	Std9	Std9	Std	Std	Std	Unk	Unk	Unk
D	Std4	Std4	Std4	Std10	Std10	Std10	Std	Std	Std	Unk	Unk	Unk
E	Std5	Std5	Std5	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
F	Std6	Std6	Std6	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
G	Unk	Unk	Unk	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk
H	NTC	NTC	NTC	Std	Std	Std	Std	Std	Std	Unk	Unk	Unk

Well	Fluor	Target	Content	Sample	Cq
G09	SYBR	GOI	Std	DNA pool	38.04
G10	SYBR	Reference 1	Unkn	sample 2	34.95
G11	SYBR	Reference 1	Unkn	sample 2	35.38
G12	SYBR	Reference 1	Unkn	sample 2	34.83
H01	SYBR	Gene 2	NTC		N/A
H02	SYBR	Gene 2	NTC		N/A
H03	SYBR	Gene 2	NTC		N/A
H04	SYBR	Reference 2	Std	DNA pool	35.21
H05	SYBR	Reference 2	Std	DNA pool	35.07
H06	SYBR	Reference 2	Std	DNA pool	N/A
H07	SYBR	GOI	Std	DNA pool	N/A
H08	SYBR	GOI	Std	DNA pool	37.45

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

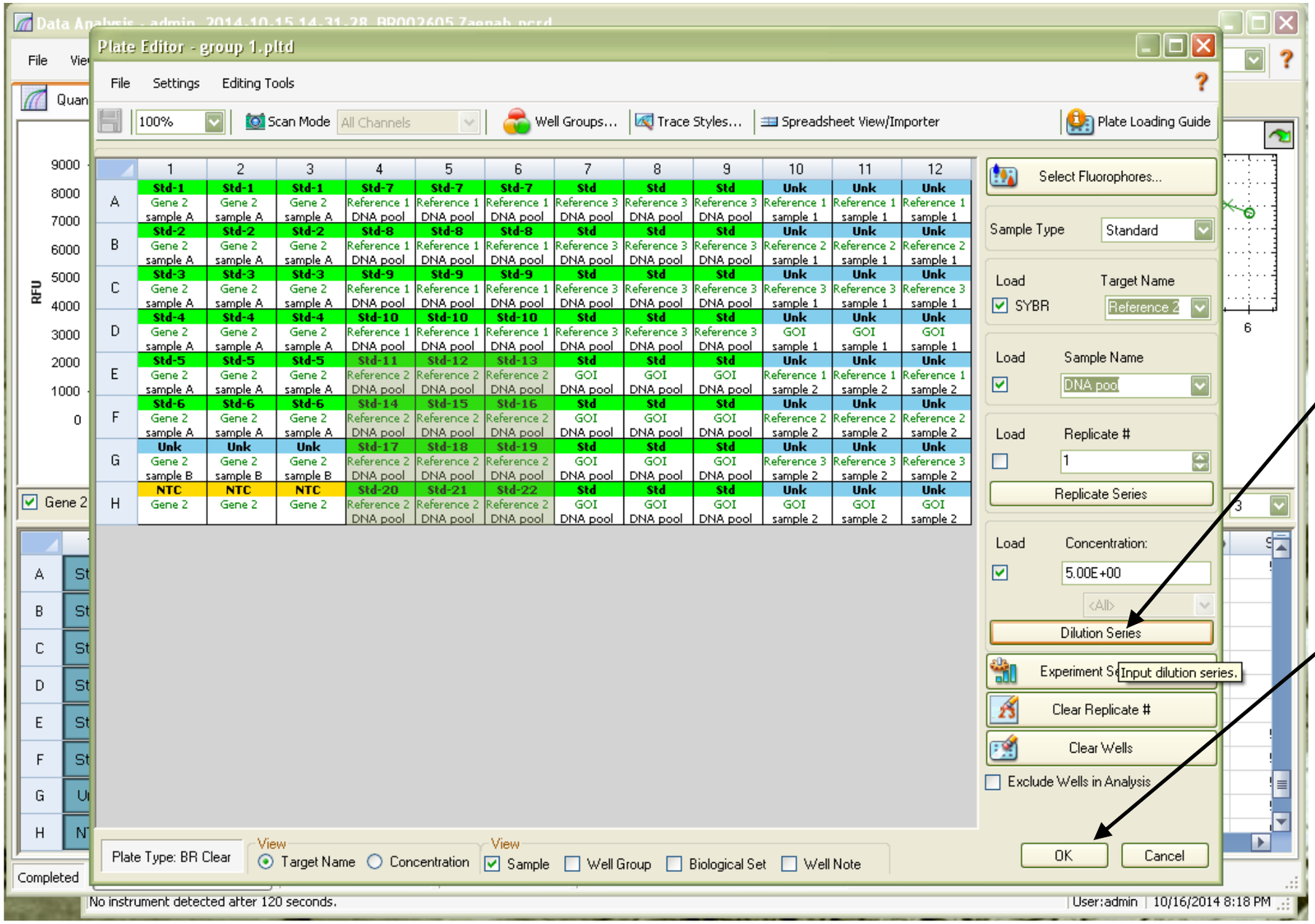
Data analysis



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



open the dilution series

->type in a starting concentration

->type in dilution factor

->click on Apply

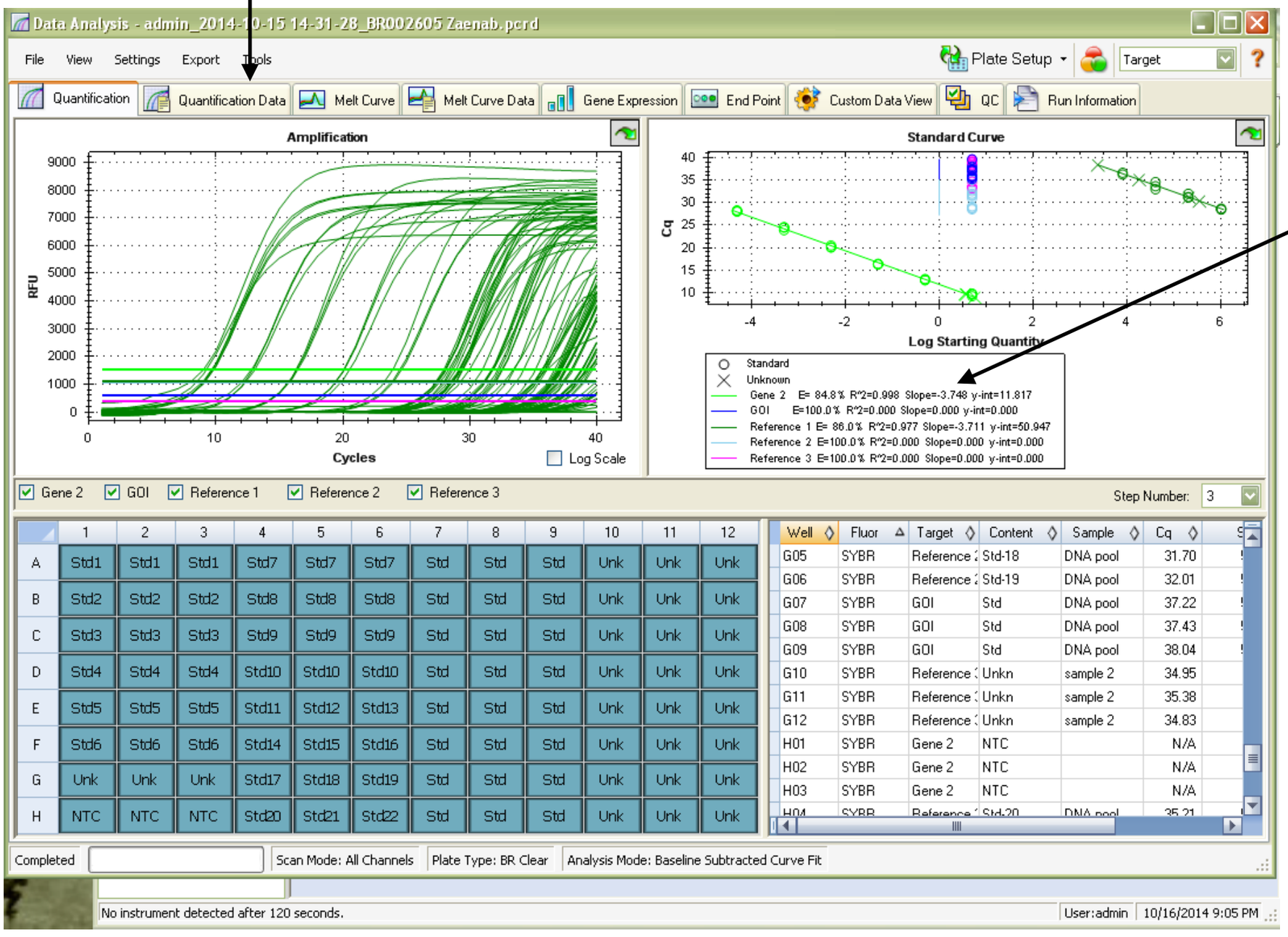
-> ok

->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 be clicking on the small arrows in the header row. For example you can sort all data according to the target name



	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1	Std1	Std1	Std7	Std7	Std7	Std	Std	Std	Unk	Unk	Unk
B	Std2	Std2	Std2	Std8	Std8	Std8	Std	Std	Std	Unk	Unk	Unk
C	Std3	Std3	Std3	Std9	Std9	Std9	Std	Std	Std	Unk	Unk	Unk
D	Std4	Std4	Std4	Std10	Std10	Std10	Std	Std	Std	Unk	Unk	Unk
E	Std5	Std5	Std5	Std11	Std12	Std13	Std	Std	Std	Unk	Unk	Unk
F	Std6	Std6	Std6	Std14	Std15	Std16	Std	Std	Std	Unk	Unk	Unk
G	Unk	Unk	Unk	Std17	Std18	Std19	Std	Std	Std	Unk	Unk	Unk
H	NTC	NTC	NTC	Std20	Std21	Std22	Std	Std	Std	Unk	Unk	Unk

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

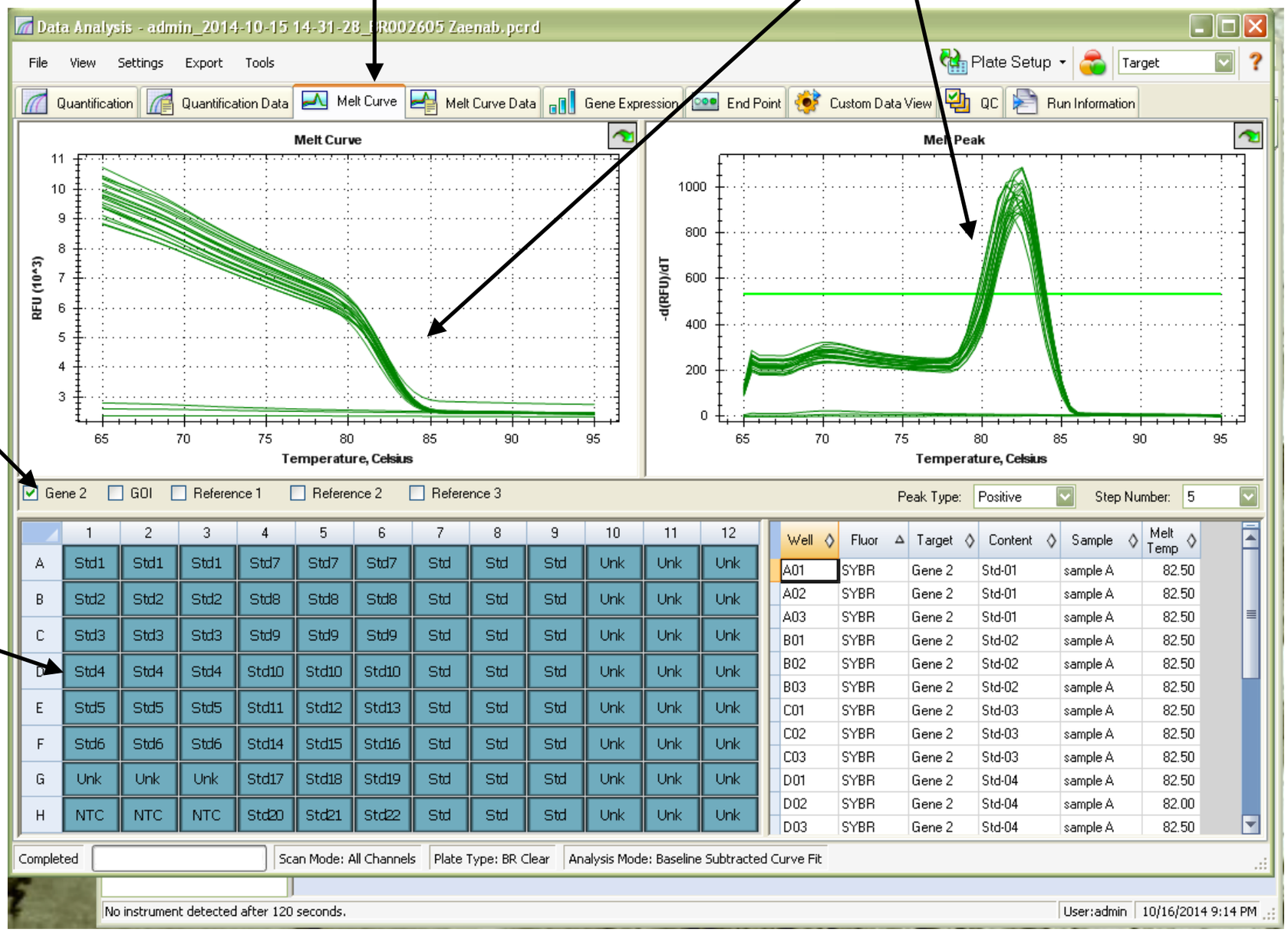
Data analysis

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

Melt curve

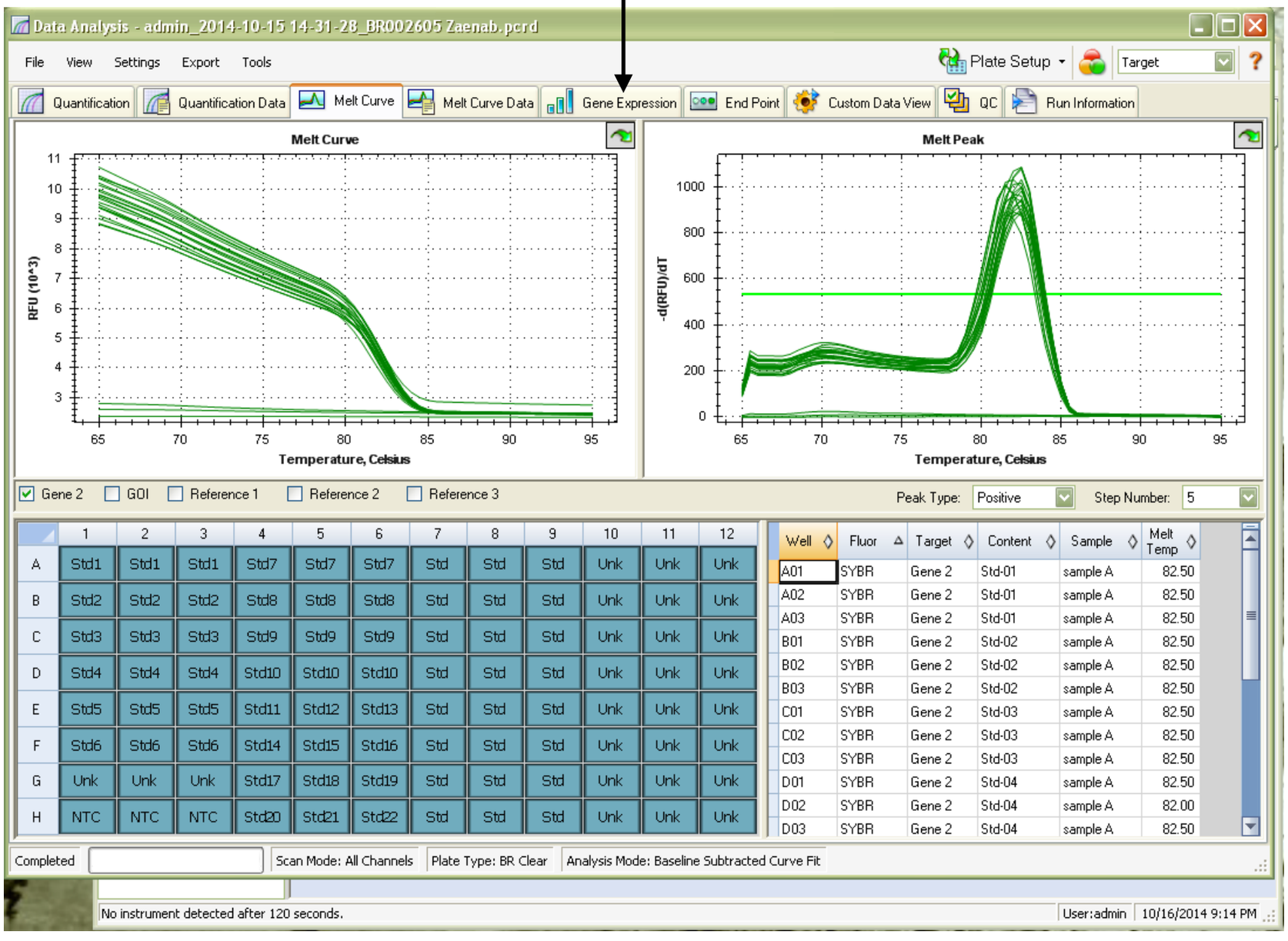
you can select curve for which target you want to see

and also curves for which wells should be shown

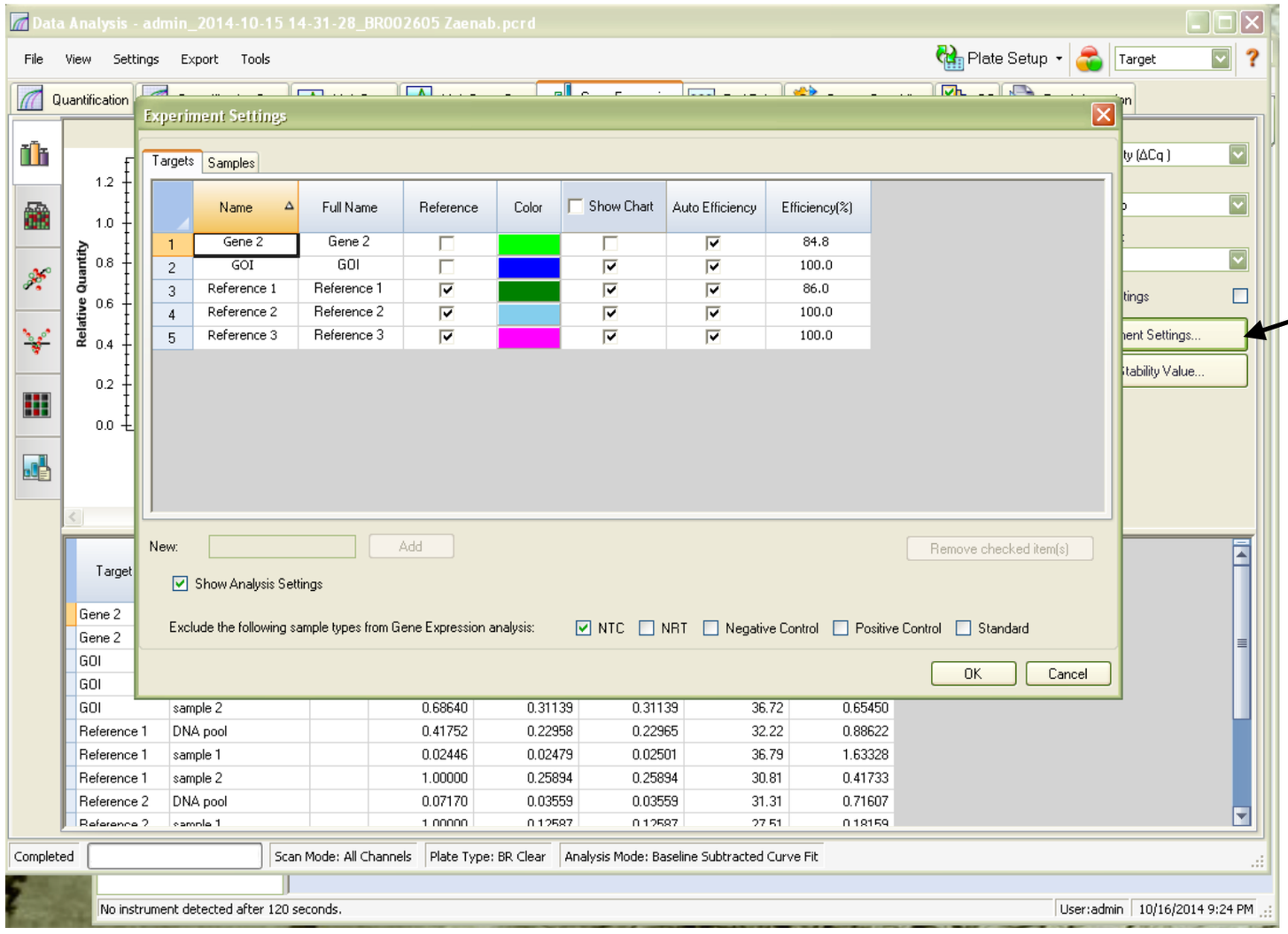


Data analysis

Here you run Relative qPCR analysis



Data analysis

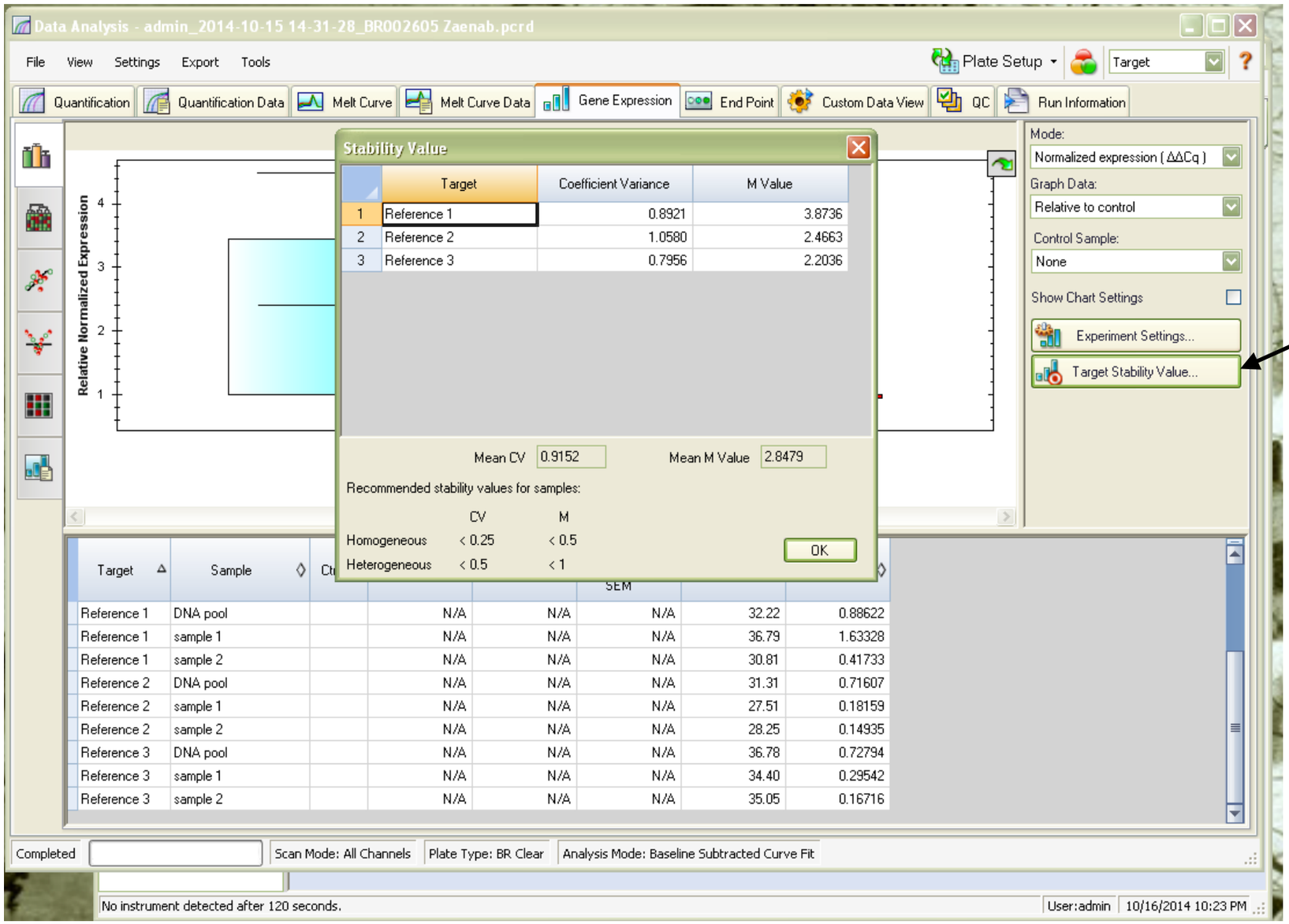


Click on the Experiment setting

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