IQ5

Run set up

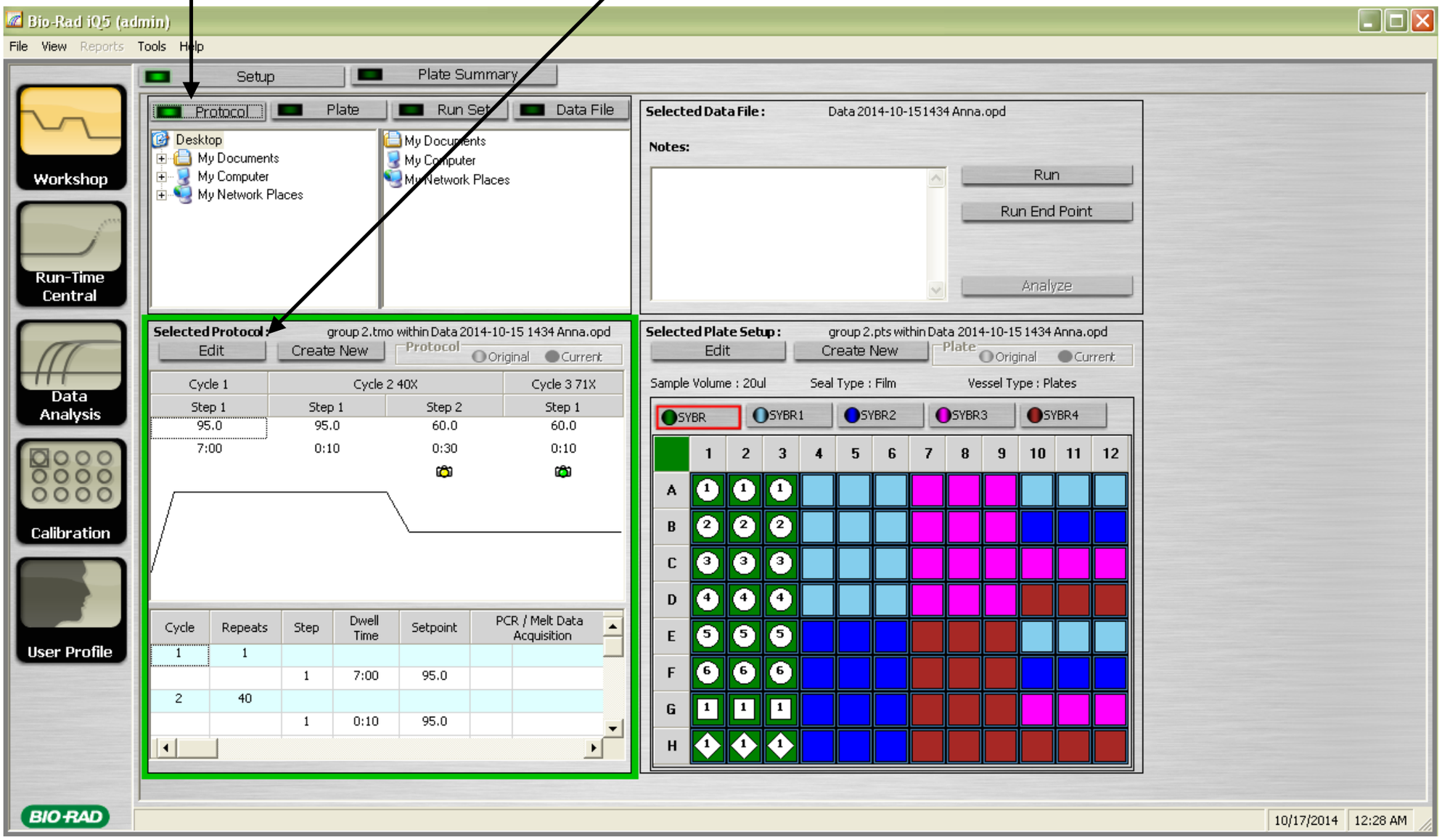
Protocol must be defined BEFORE run
please check your kit manual for guidelines

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

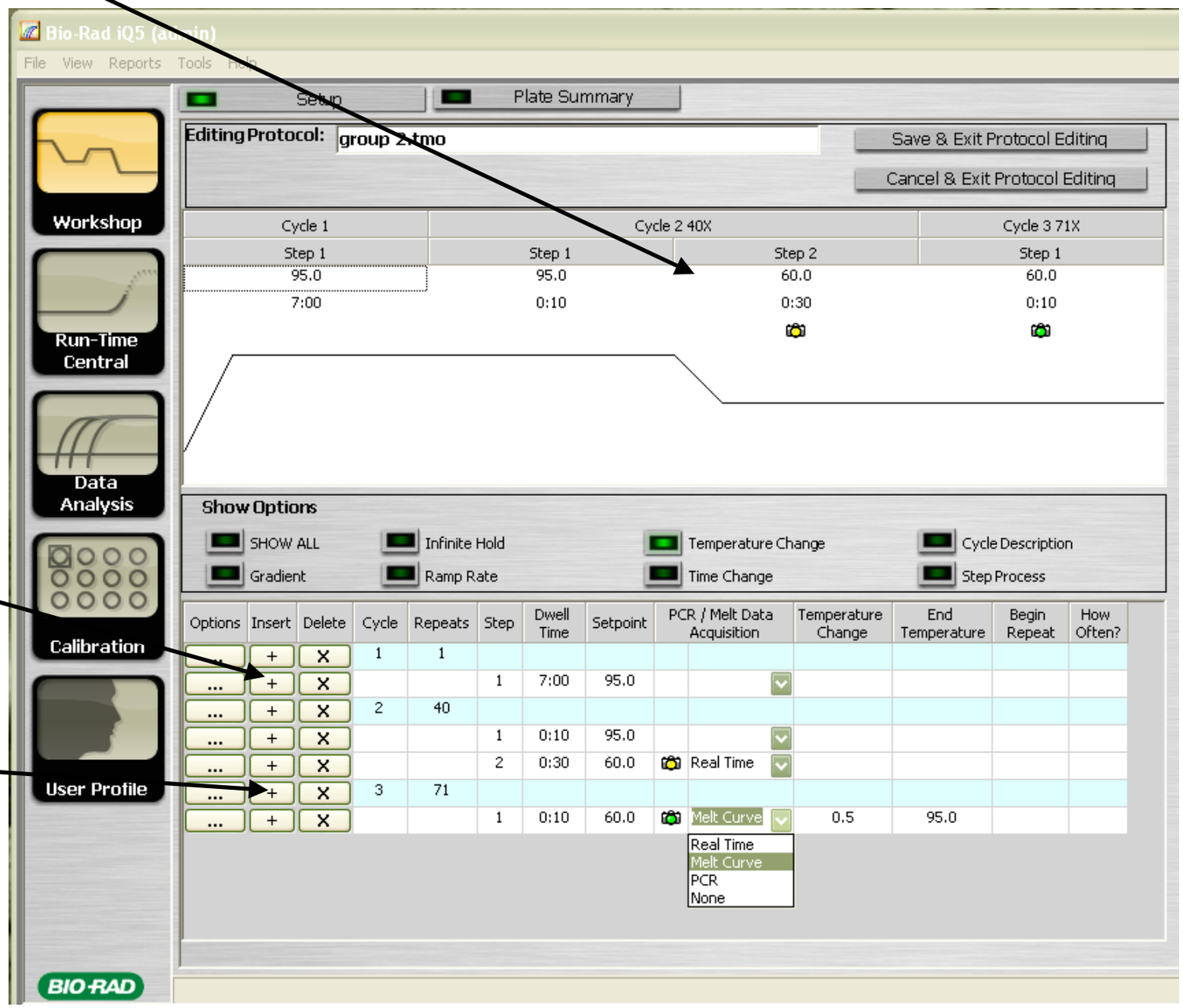


Run set up: protocol

3. type in your protocol

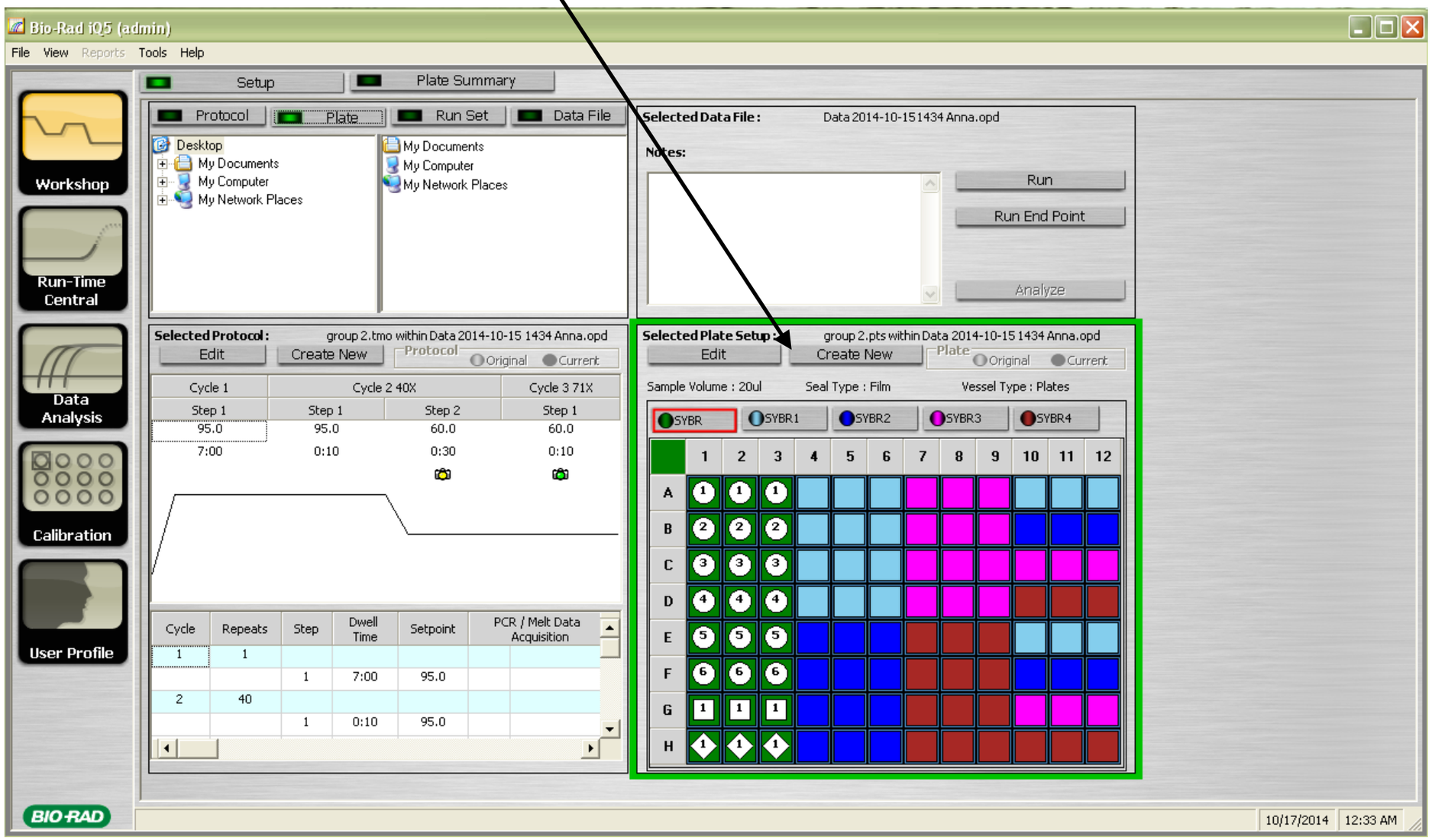
to add a white row (step) click on + on a white row

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



Run set up: plate

4. click on edit plate or create new



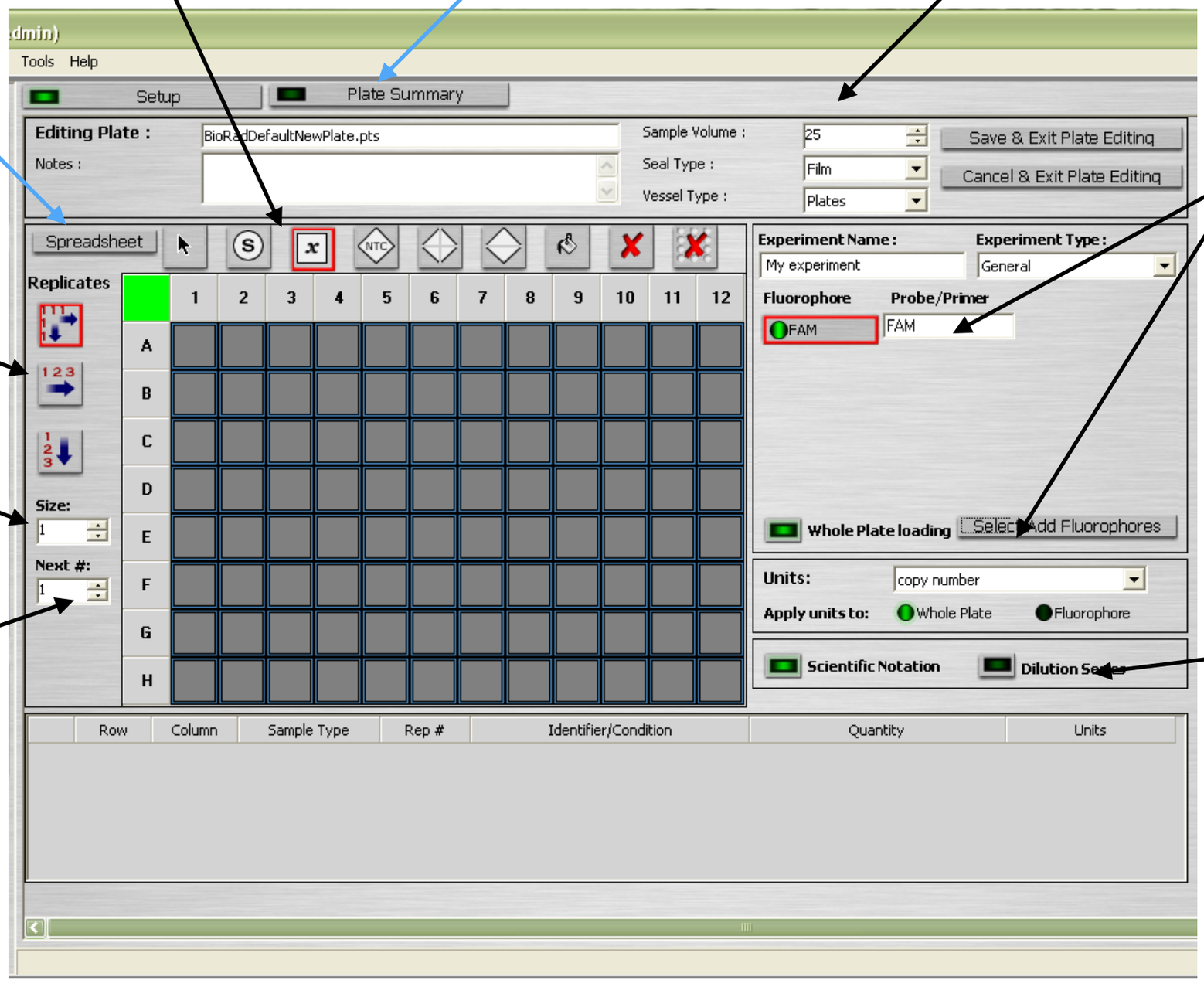
Run set up: plate

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



5. define whether your replicates are horizontal

6. define how many replicates you have

7. define how do you want to number your replicates

4. if you can assign each target as individual fluorophore

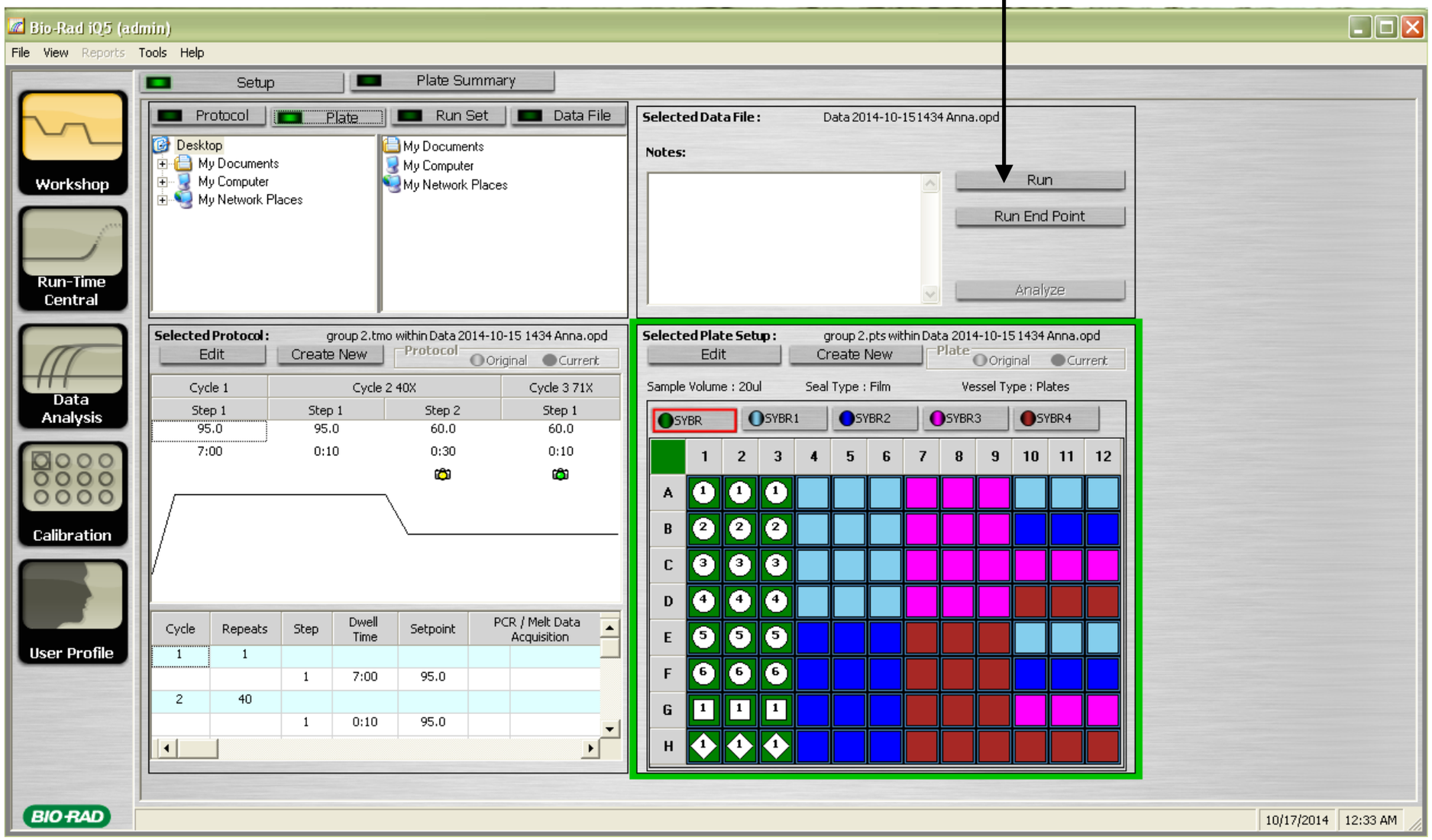
8. add info about your standards dilutions and units you are using

Sic! if you do not add concentration for dilutions the software will not save the plate

Run

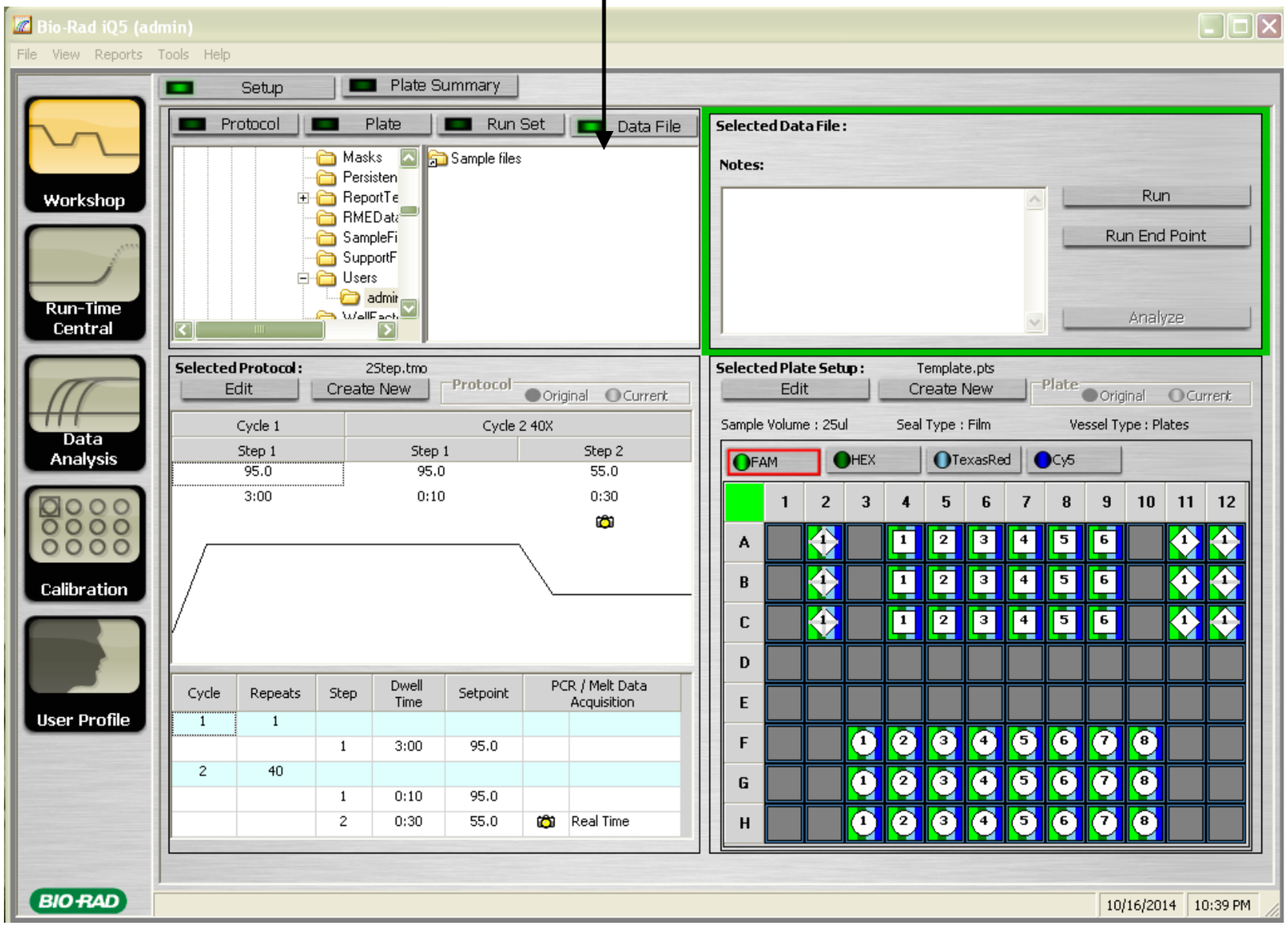
please make sure, that you spun down the plate before running the reaction

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



Data analysis

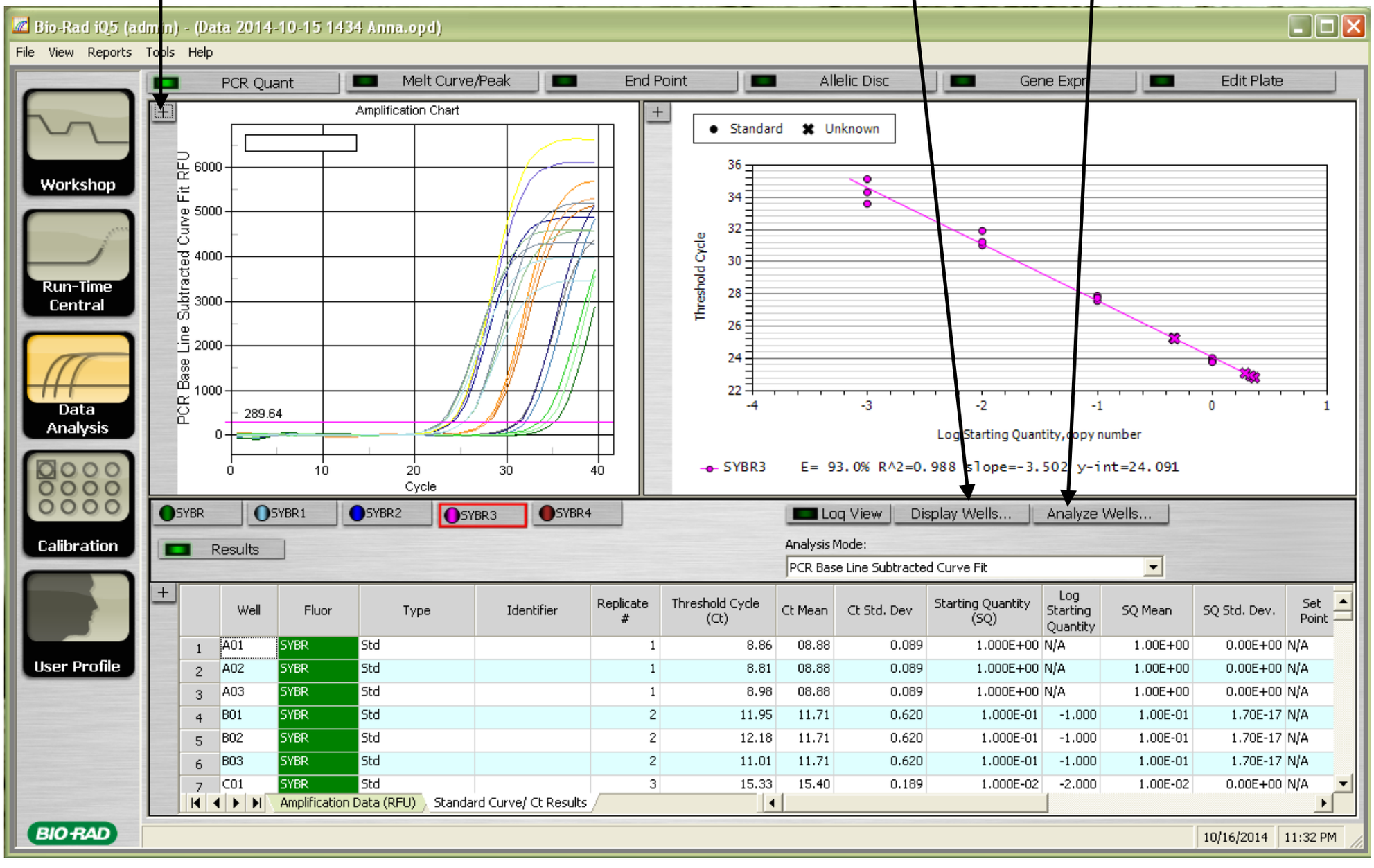
1. open your file (you can also drag and drop your data file it into the data file window)



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



	Well	Fluor	Type	Identifier	Replicate #	Threshold Cycle (Ct)	Ct Mean	Ct Std. Dev	Starting Quantity (SQ)	Log Starting Quantity	SQ Mean	SQ Std. Dev.	Set Point
1	A01	SYBR	Std		1	8.86	08.88	0.089	1.000E+00	N/A	1.00E+00	0.00E+00	N/A
2	A02	SYBR	Std		1	8.81	08.88	0.089	1.000E+00	N/A	1.00E+00	0.00E+00	N/A
3	A03	SYBR	Std		1	8.98	08.88	0.089	1.000E+00	N/A	1.00E+00	0.00E+00	N/A
4	B01	SYBR	Std		2	11.95	11.71	0.620	1.000E-01	-1.000	1.00E-01	1.70E-17	N/A
5	B02	SYBR	Std		2	12.18	11.71	0.620	1.000E-01	-1.000	1.00E-01	1.70E-17	N/A
6	B03	SYBR	Std		2	11.01	11.71	0.620	1.000E-01	-1.000	1.00E-01	1.70E-17	N/A
7	C01	SYBR	Std		3	15.33	15.40	0.189	1.000E-02	-2.000	1.00E-02	0.00E+00	N/A

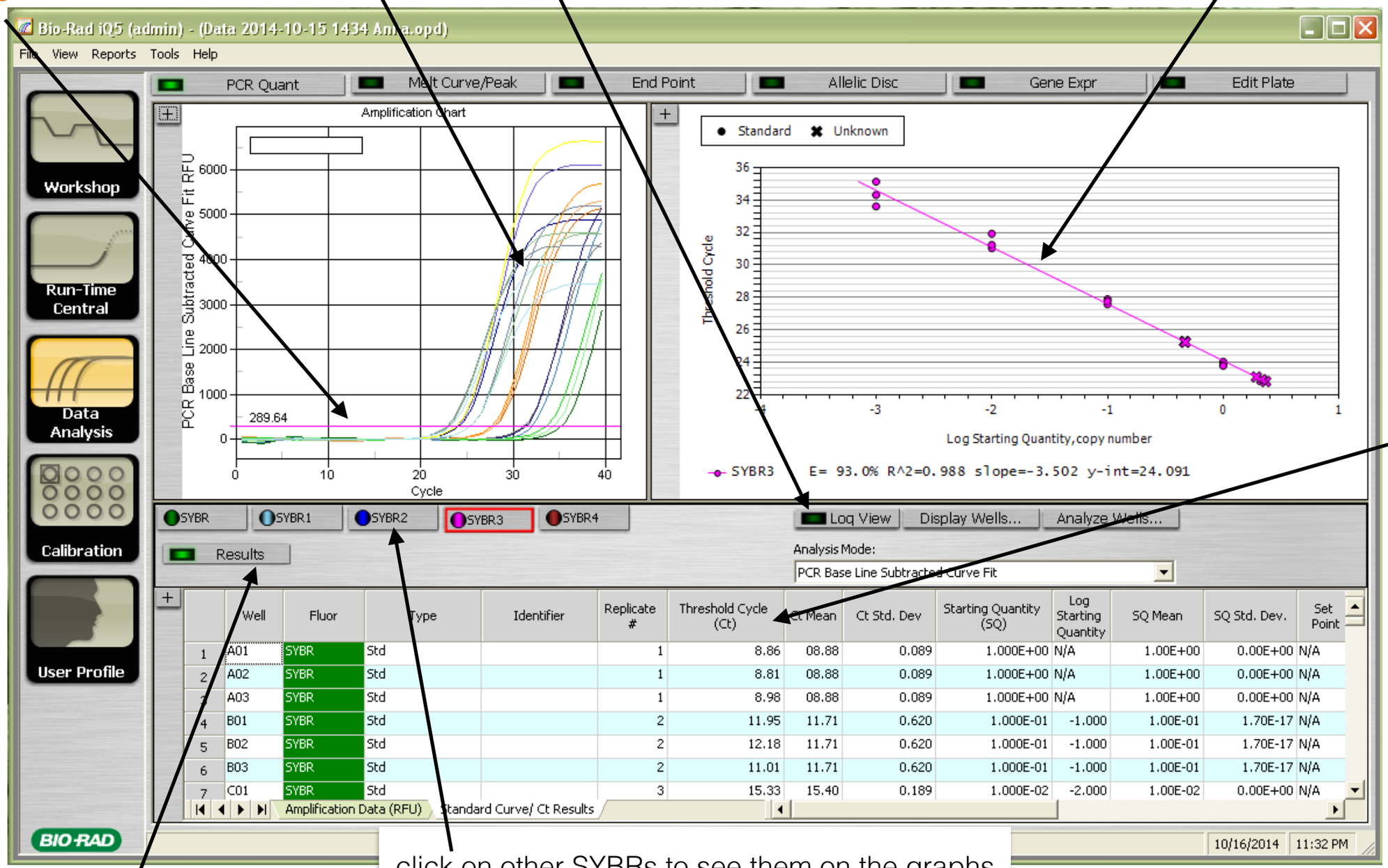
Data analysis

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

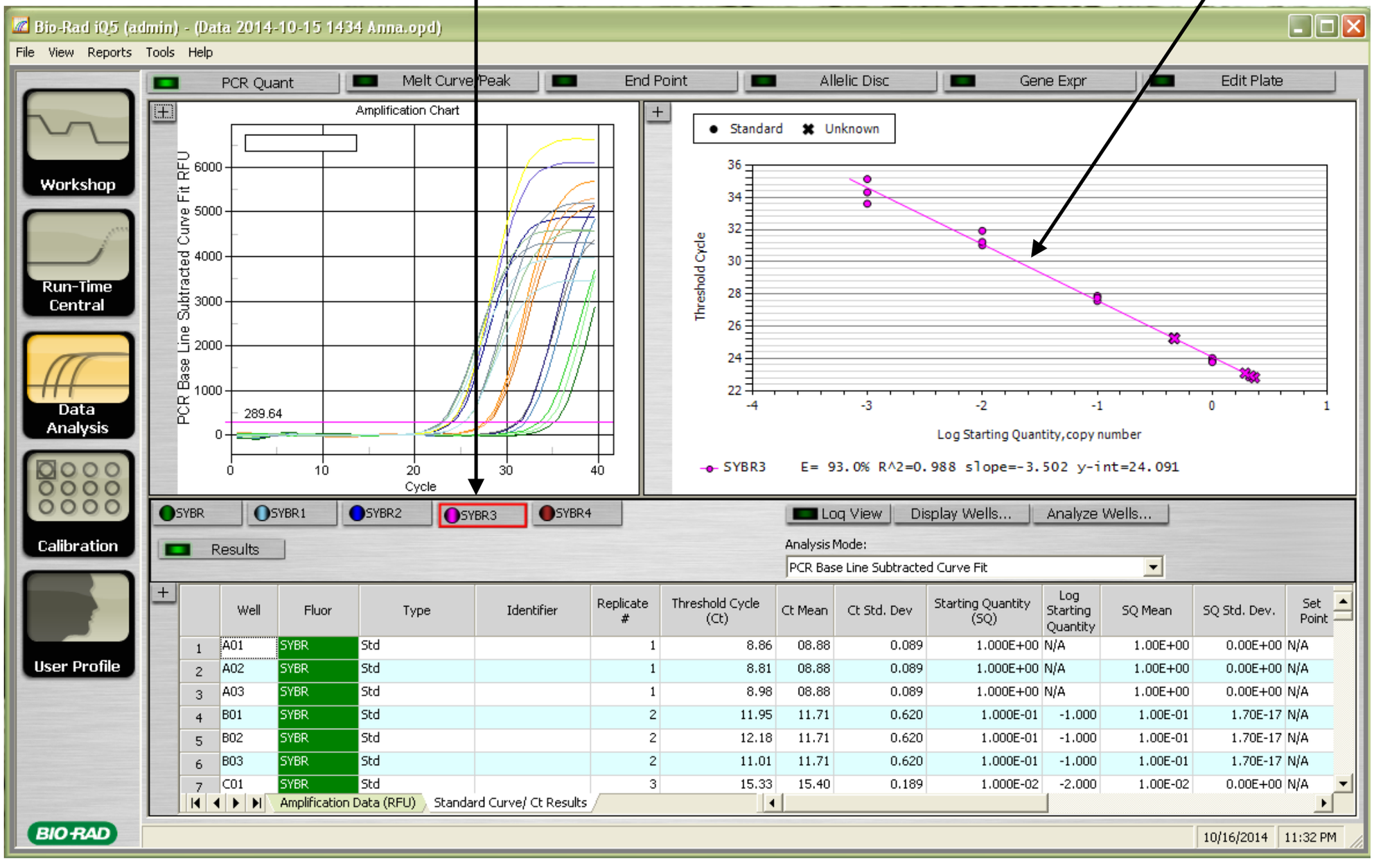
click on other SYBRs to see them on the graphs

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



	Well	Fluor	Type	Identifier	Replicate #	Threshold Cycle (Ct)	Ct Mean	Ct Std. Dev	Starting Quantity (SQ)	Log Starting Quantity	SQ Mean	SQ Std. Dev.	Set Point
1	A01	SYBR	Std		1	8.86	08.88	0.089	1.000E+00	N/A	1.00E+00	0.00E+00	N/A
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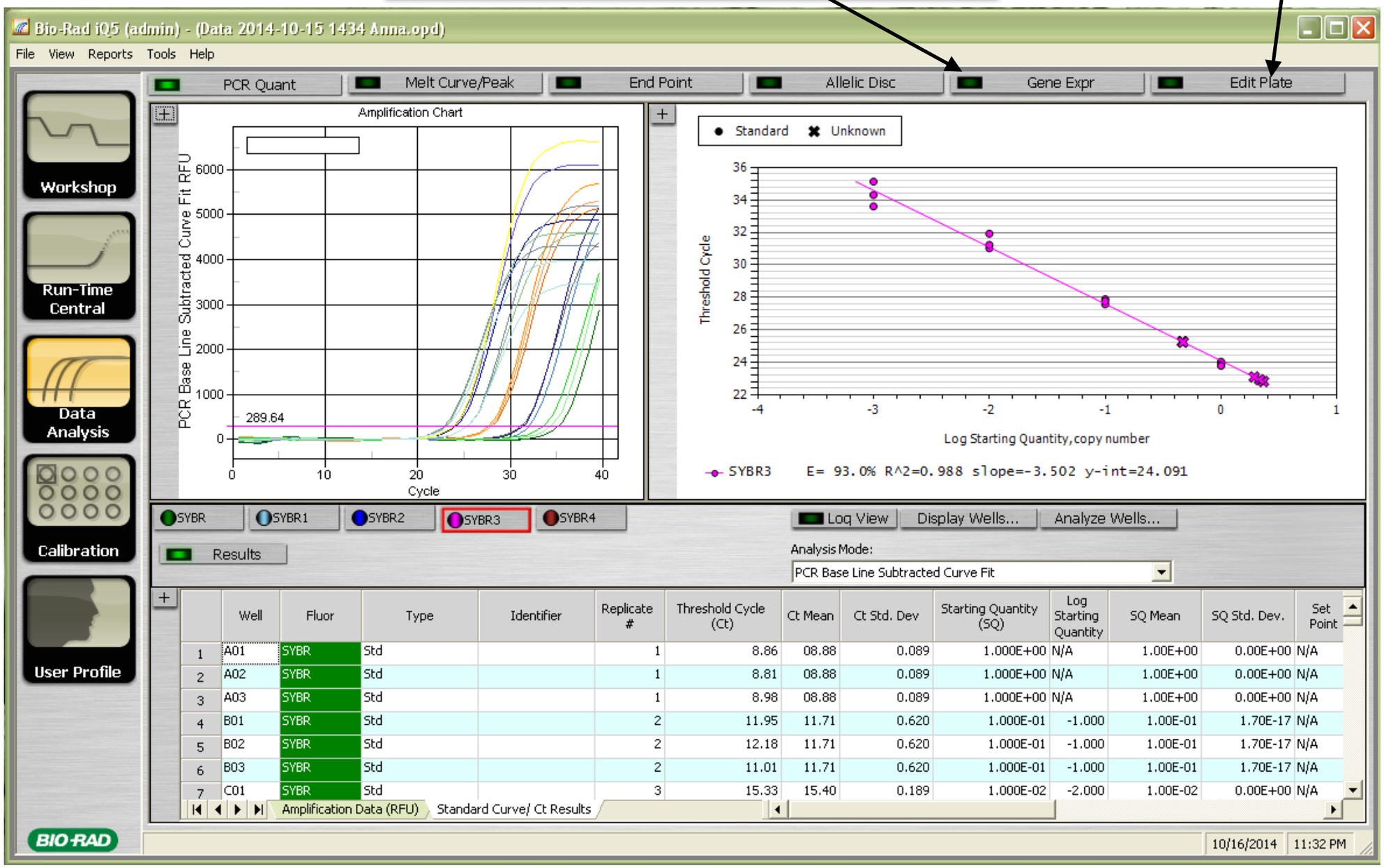
5	B02	SYBR	Std		2	12.18	11.71	0.620	1.000E-01	-1.000	1.00E-01	1.70E-17	N/A
6	B03	SYBR	Std		2	11.01	11.71	0.620	1.000E-01	-1.000	1.00E-01	1.70E-17	N/A
7	C01	SYBR	Std		3	15.33	15.40	0.189	1.000E-02	-2.000	1.00E-02	0.00E+00	N/A

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



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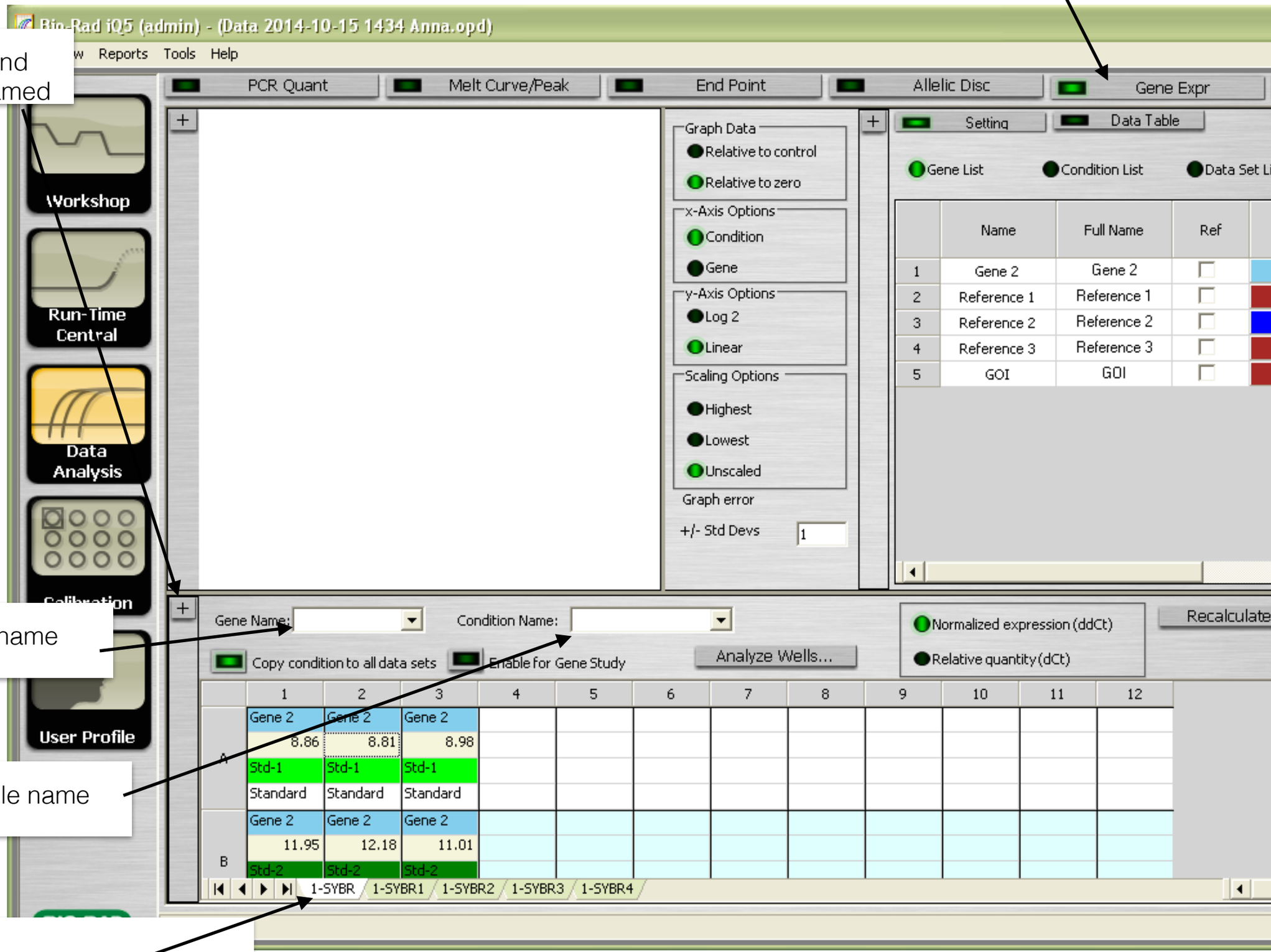
2	A02	SYBR	Std		1	8.81	08.88	0.089	1.000E+00	N/A	1.00E+00	0.00E+00	N/A
3	A03	SYBR	Std		1	8.98	08.88	0.089	1.000E+00	N/A	1.00E+00	0.00E+00	N/A
4	B01	SYBR	Std		2	11.95	11.71	0.620	1.000E-01	-1.000	1.00E-01	1.70E-17	N/A
5	B02	SYBR	Std		2	12.18	11.71	0.620	1.000E-01	-1.000	1.00E-01	1.70E-17	N/A
6	B03	SYBR	Std		2	11.01	11.71	0.620	1.000E-01	-1.000	1.00E-01	1.70E-17	N/A
7	C01	SYBR	Std		3	15.33	15.40	0.189	1.000E-02	-2.000	1.00E-02	0.00E+00	N/A

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 florophores

Data analysis

you can select curve for which target you want to see

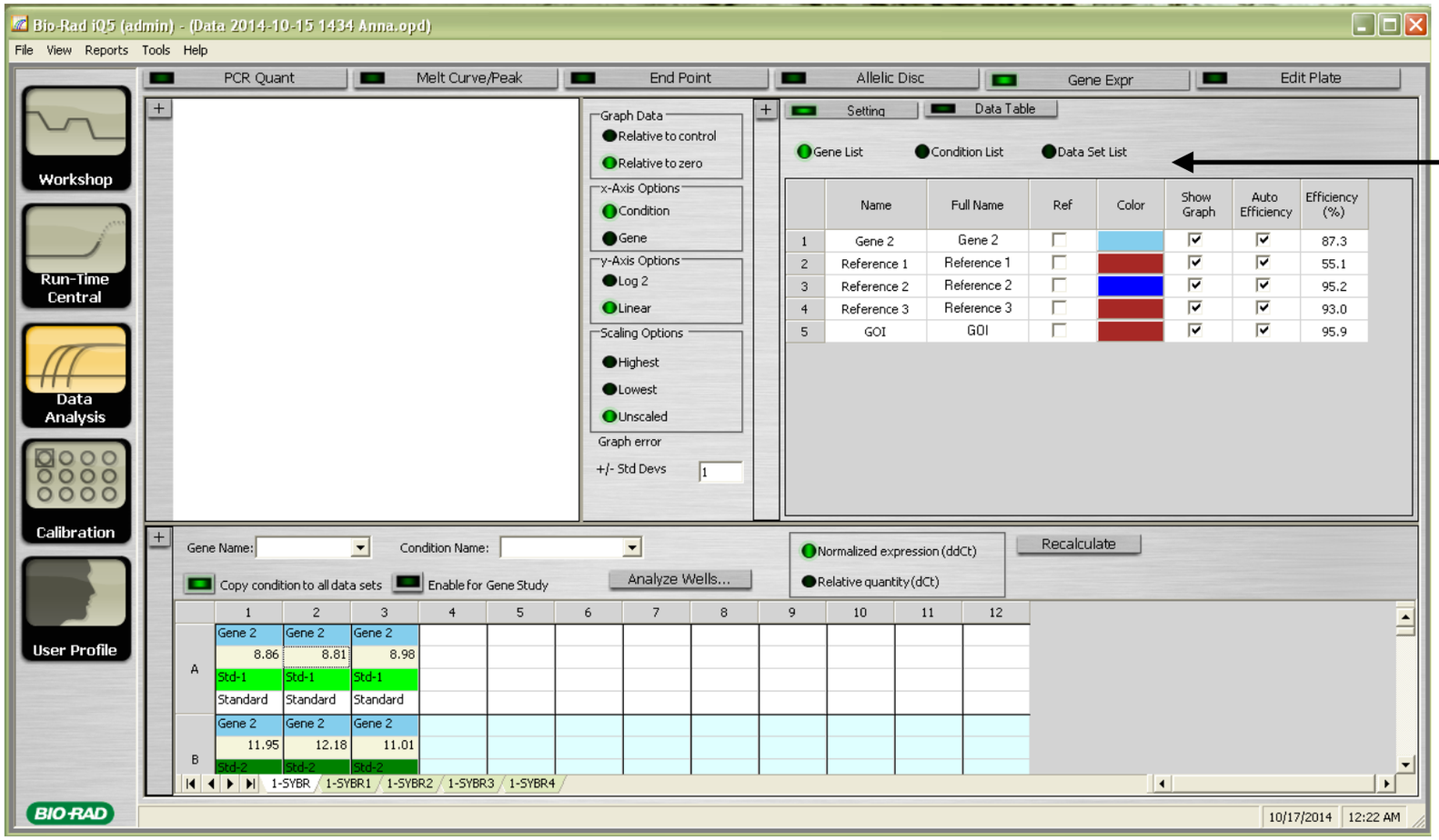
Melt curve

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