Santander lab

How to make a template? – QuantStudio 3

Contribuited by

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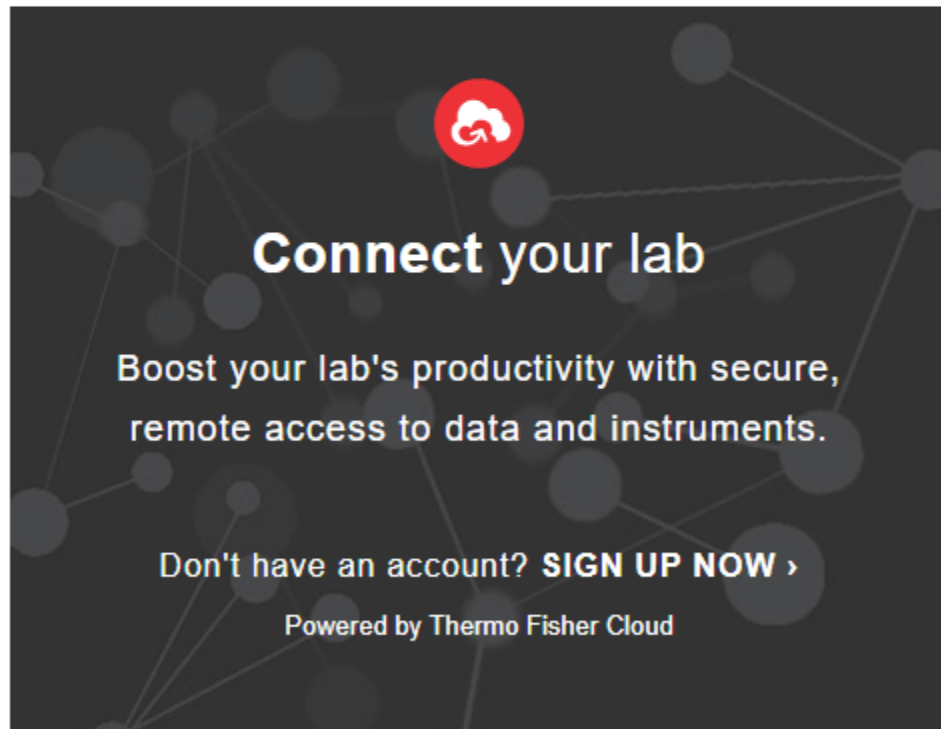
- Go to https://www.thermofisher.com/ca/en/home.html and press in the tab "Connect your lab".

• Press in the tab "Sign in".

- Introduce your Username and Password to start the connection. If you do not have and account, you must create one before follow the next steps.

- This is the main tab. At the beginning no files going to be show. To work in template creation and/or work on plate already measured, the main tool to be use will be "DESIGN AND ANALYSIS APPLICATION". Press on this application.

- If this is your first time, you should start to create a file from the beginning, so press in the "new" tab. If you already have an existing file and the next measure will be similar in layout, you can use an existing file as a reference to don't complete again certain parameters. In this example we go to create a new file.

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1st Step to create the template - properties

Name, Bar code, User name, instrument type

- <u>Name</u>: should be something easy to remember to you about what was that plate measured. Usually the date goes first and them the experiment description. i.e. "20180920 Cod gene expression plate 1", in this case you must know which genes you put on plate 1 .

- <u>Barcode and user name</u> are optional and is not necessary to put nothing here.

- <u>Instrument type</u>: the equipment used in the Dr. Javier Santander Lab is the QuantStudio 3, so you must put that equipment as the option here.

Block type and experiment type

- <u>Block type:</u> The QuantStudio 3 can be purchase in two different block type formats, 0.1 or 0.2 mL 96-well Block. In the case of the equipment purchase by Dr. Santander, the block type of that equipment is 0.1.mL Block, so be sure that you choose that option. Wrong measures will be conducted if you choose the other one.

- <u>Experiment type</u>: for efficiency of primers you have to select the option "STANDARD CURVE". For primers test and also for relative expression, the cycle used will be always "Comparative Ct (ΔΔCt)".

Chemistry and Run mode

- <u>Chemistry</u>: This mean the reagent that you are using for gene measure. Actually we are using only SYBR green so you must select this option. If you start to use TaqMan in some point, you must change to that option.

- <u>Run mode</u>: One great thing of the QuantStudio 3 is read plates in fast mode (45 min approx.), but be careful because this is combined only with the PowerUp SYBR green master mix, so if you use an older/different version of SYBR you will have to check which mode will be necessary to use.

- What you choose here is totally up to you, but usually the most common things are send notifications when: the run is completed and also is there is any instrument error. Add your email to be notified about any of these situations.

2nd Step to create the template - method

- The method is the most important step to create the template. Any difference with the protocol of the SYBR will finish in wrong measures.

- Please follow the instruction shown in the protocol and make double check to each step.

2nd Step to create the template - method

- In the case of the PowerUp SYBR green master mix to be used in fast mode, the protocol describe the next method (check the protocol online or inside the SYBR green boxes).
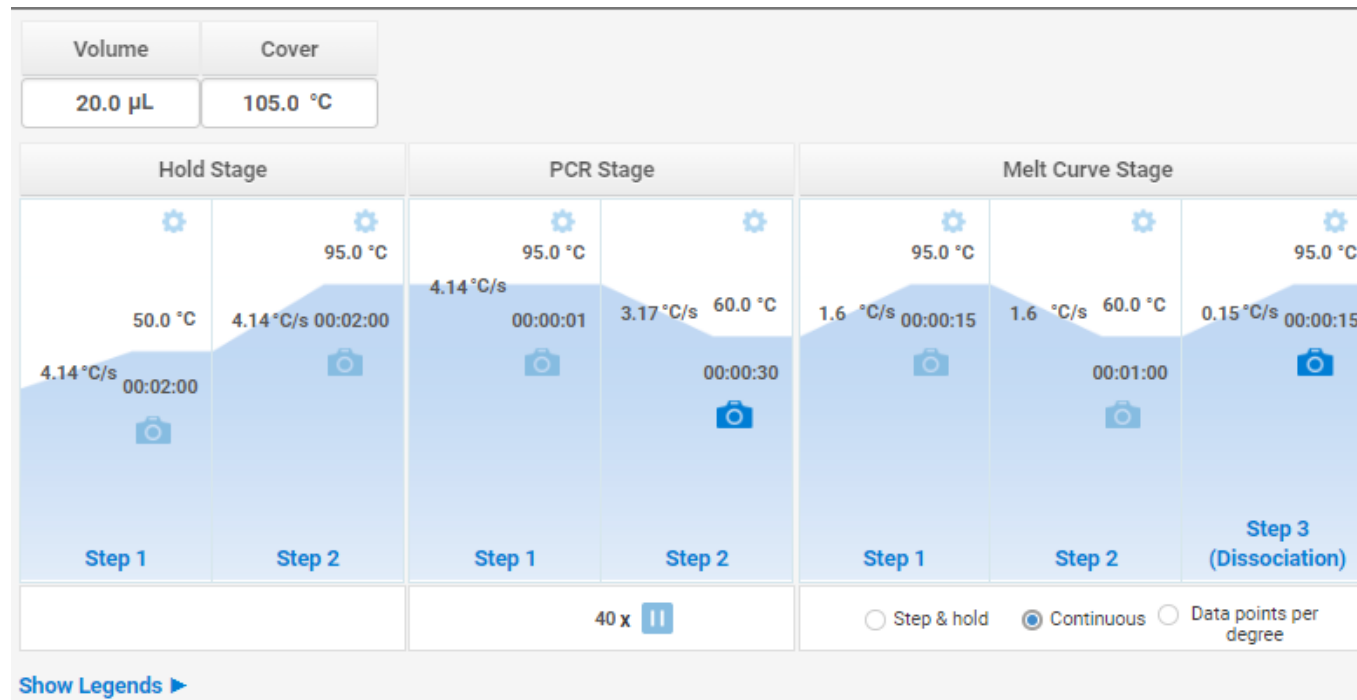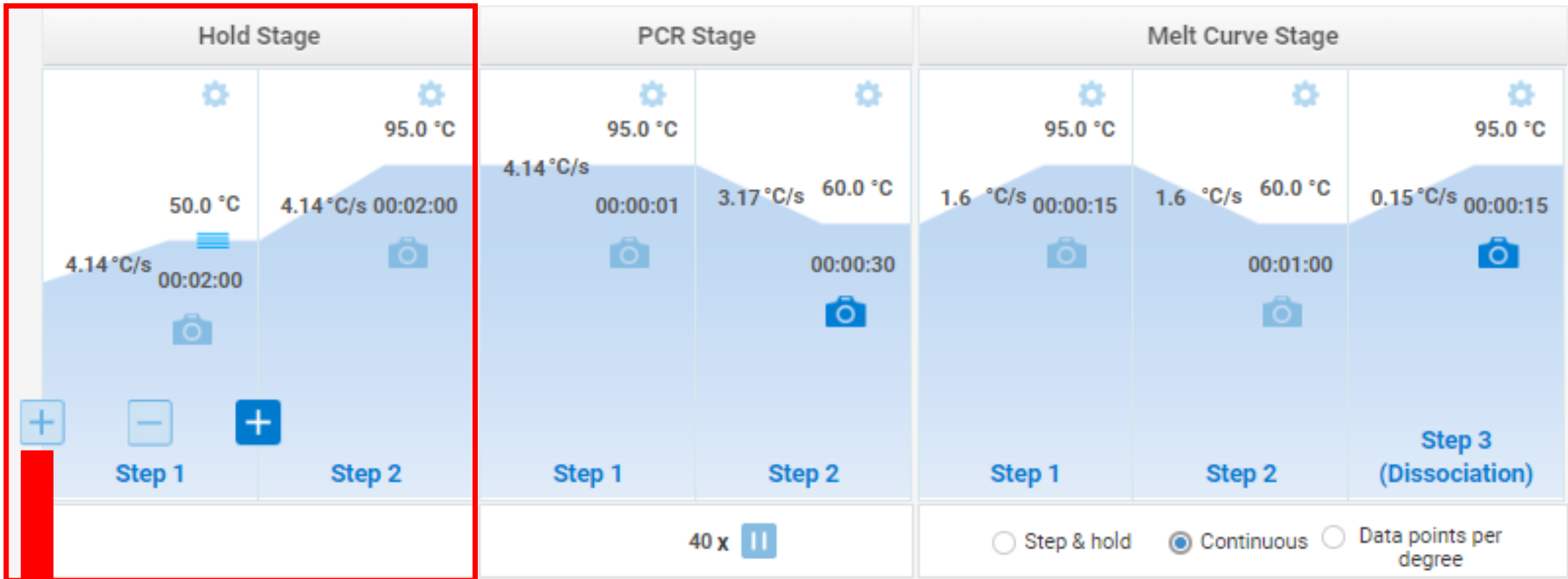
- To add or remove a hold stage you must press in the + or – sign respectively.
- There are two hold stages steps using the PowerUp SYBR, the first one 50°C for 2 minutes, and the second one 95°C for 2 minutes. The ramp rate (4.14°C/s) is calculated automatically for the equipment, so don't change this parameter.
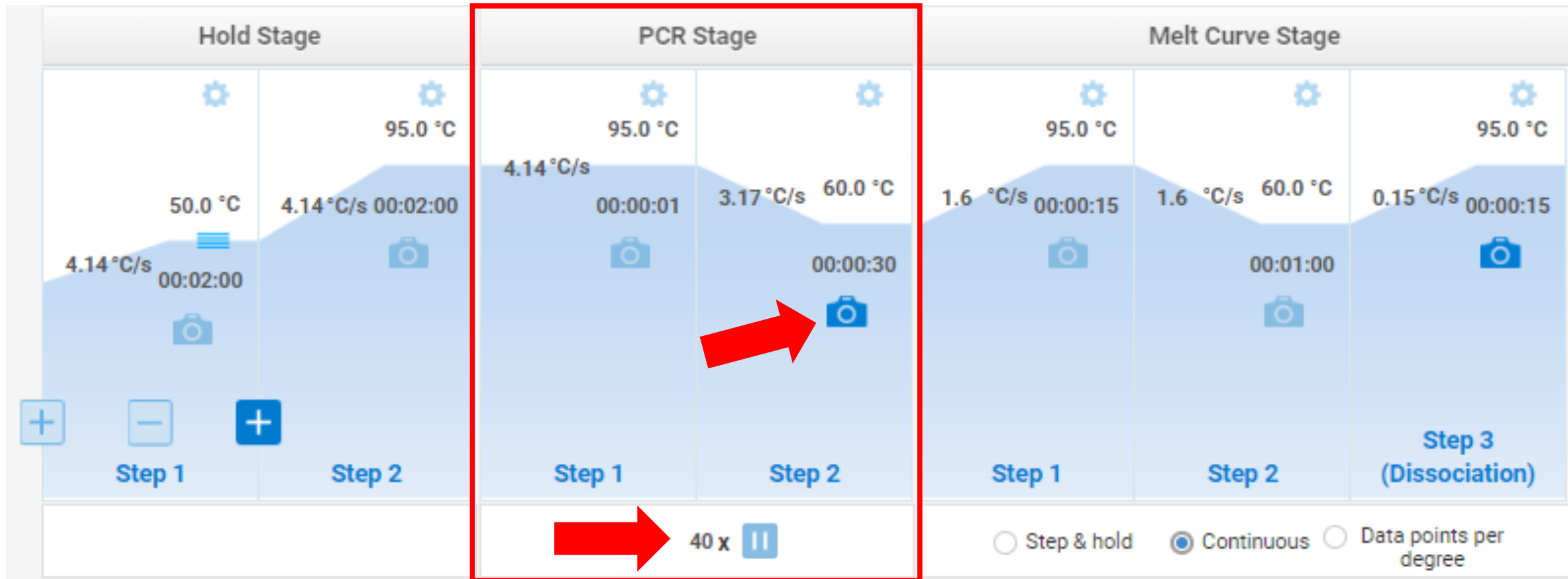
- There are two PCR stage steps using the PowerUp SYBR, the first one 95°C for 1 second, and the second one 60°C for 30 seconds. The ramp rate is calculated automatically for the equipment, so don't change this parameter.

- The camera must be selected in the second step and 40 cycles must be added (40x).
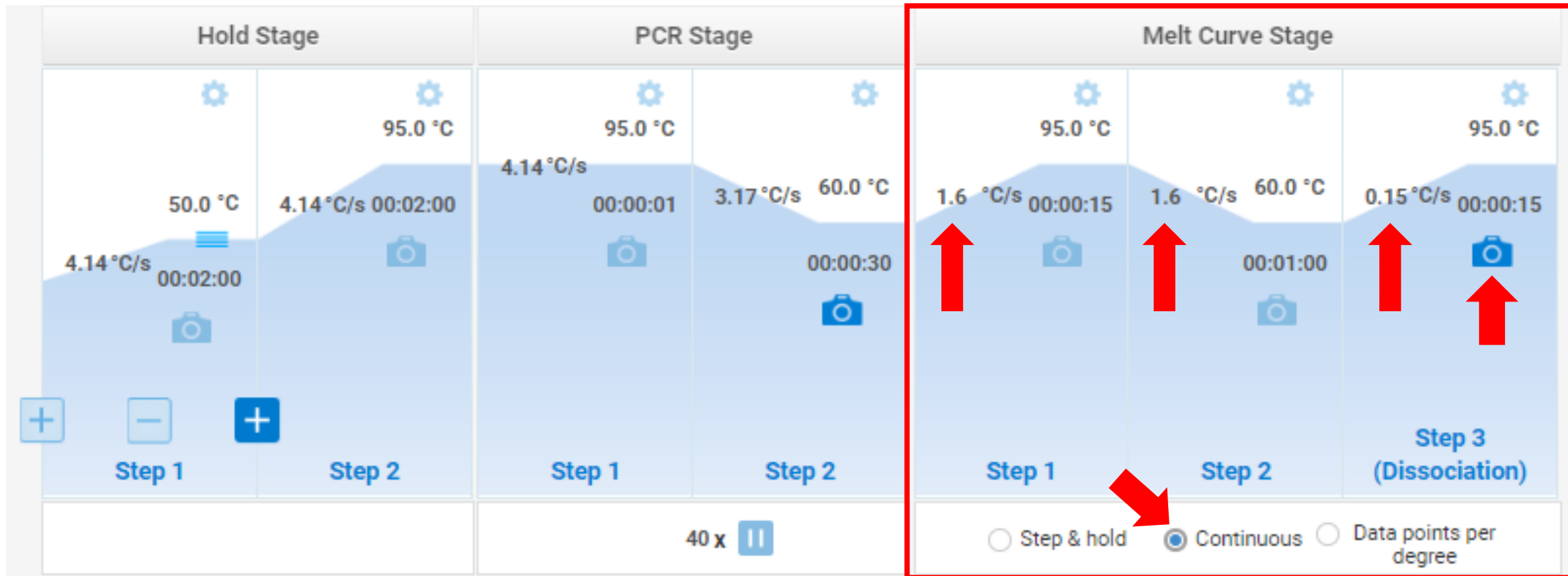
- There are 3 melt curve stage steps using the PowerUp SYBR, 1st 95°C for 15 seconds, 2nd 60°C for 1 minute and 3rd 95°C for 15 seconds. The ramp rate must be setup manually, 1st 1.6°C/s; 2nd 1.6°C/s and 3rd 0.15°C/s.

- The camera must be selected in the third step and the continuous cycle must be selected.

3rd Step to create the template - plate
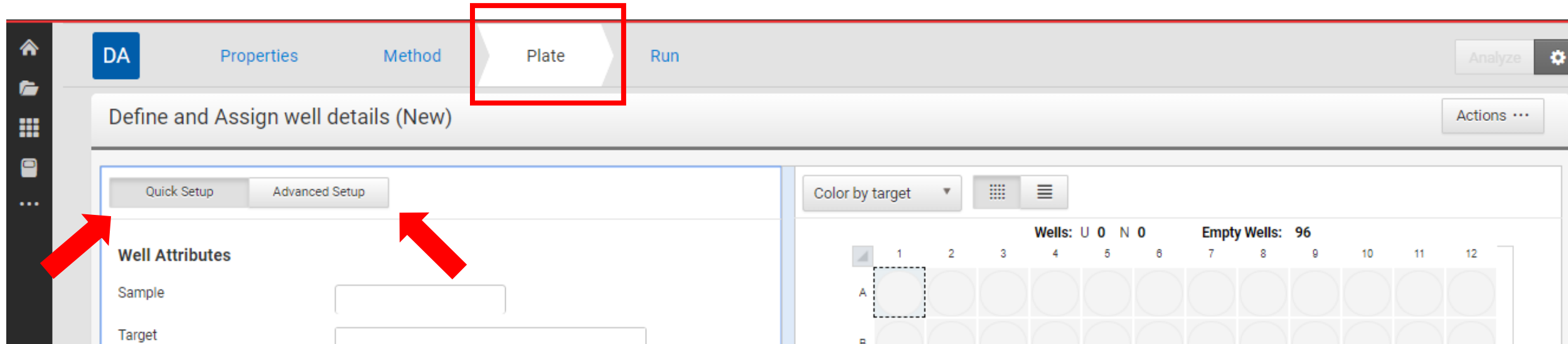
- There are two ways to create a layout of the plate, the quick setup and the advanced setup. You must select the advanced setup always since this mode give you more flexibility to create your layout.

- In the left side you going to put all the information about the experiment and the way how you going to load your samples.

- In the right side, you can see all the time how the final layout of your plate will be.
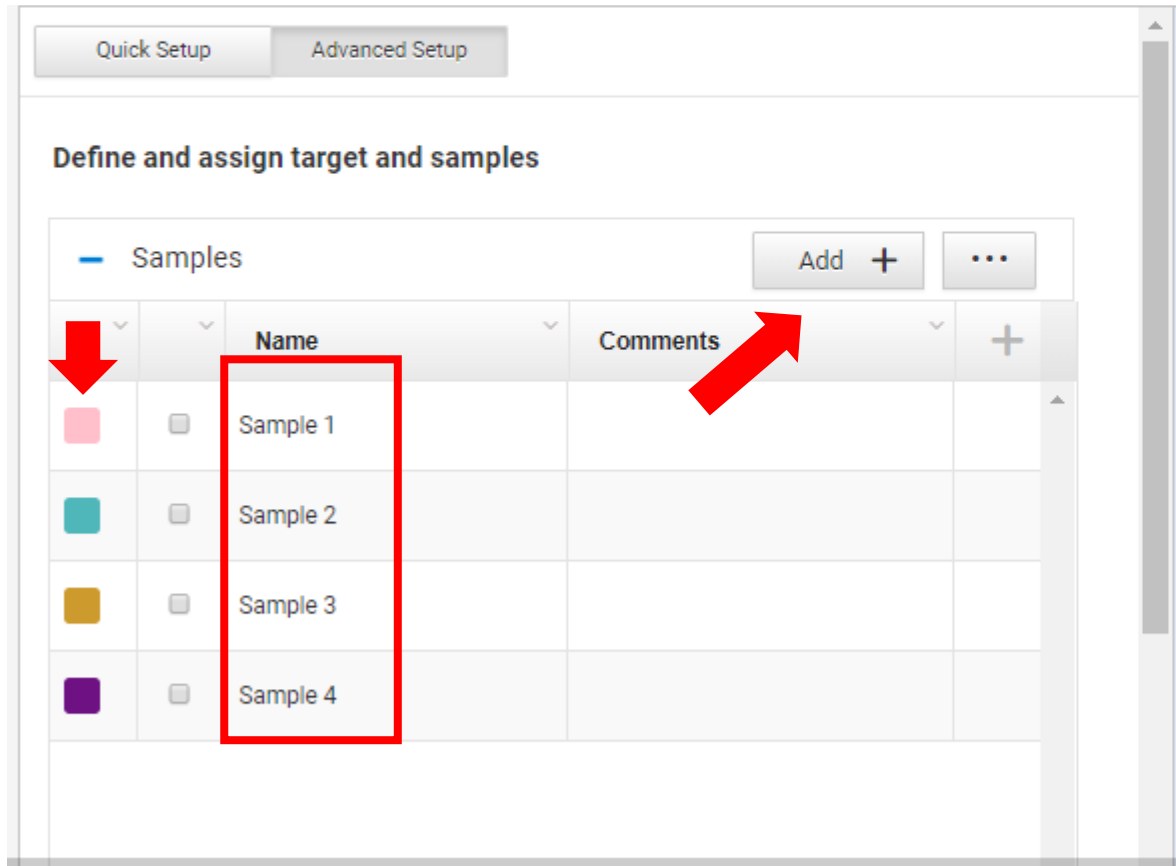
- FOCUS FIRST ON THE LEFT SIDE!

- Samples are referred also to treatments. i.e. if you have a control and 3 different times of infection, you must add until 4 samples like in the picture. Just press "add"

- If you want it, you can change the colors of the samples, and you must every time to change the names, i. e. sample 1 will be control; sample 2 will be 1h post infection, etc, etc.

- Targets are referred also to genes. i.e. if you have to measure 3 different genes, add 3 different targets. Just press "add"

- If you want it, you can change the colors of the targets, and you must every time to change the names, i. e. sample 1 will be B-actin; sample 2 will be IL-10, etc, etc.

- FOCUS FIRST ON THE LEFT SIDE!
- If you scroll down you will see now the target.

Let's put these in context

- Example of experiment:

You have 3 fish per tank, and 2 treatments (1 tank per treatment). One tank is the control group, and one tank is 1h post infection. You have to measure 2 genes (IL-8 and IL-10) for your thesis and an extra gene that will be the housekeeping (EF-1a).

Samples first

- You have to put "color by sample" to see the 96-wells plate showing the layout for samples

- You have 3 fish, so each sample will be 1 fish. Choose different colors for each fish.
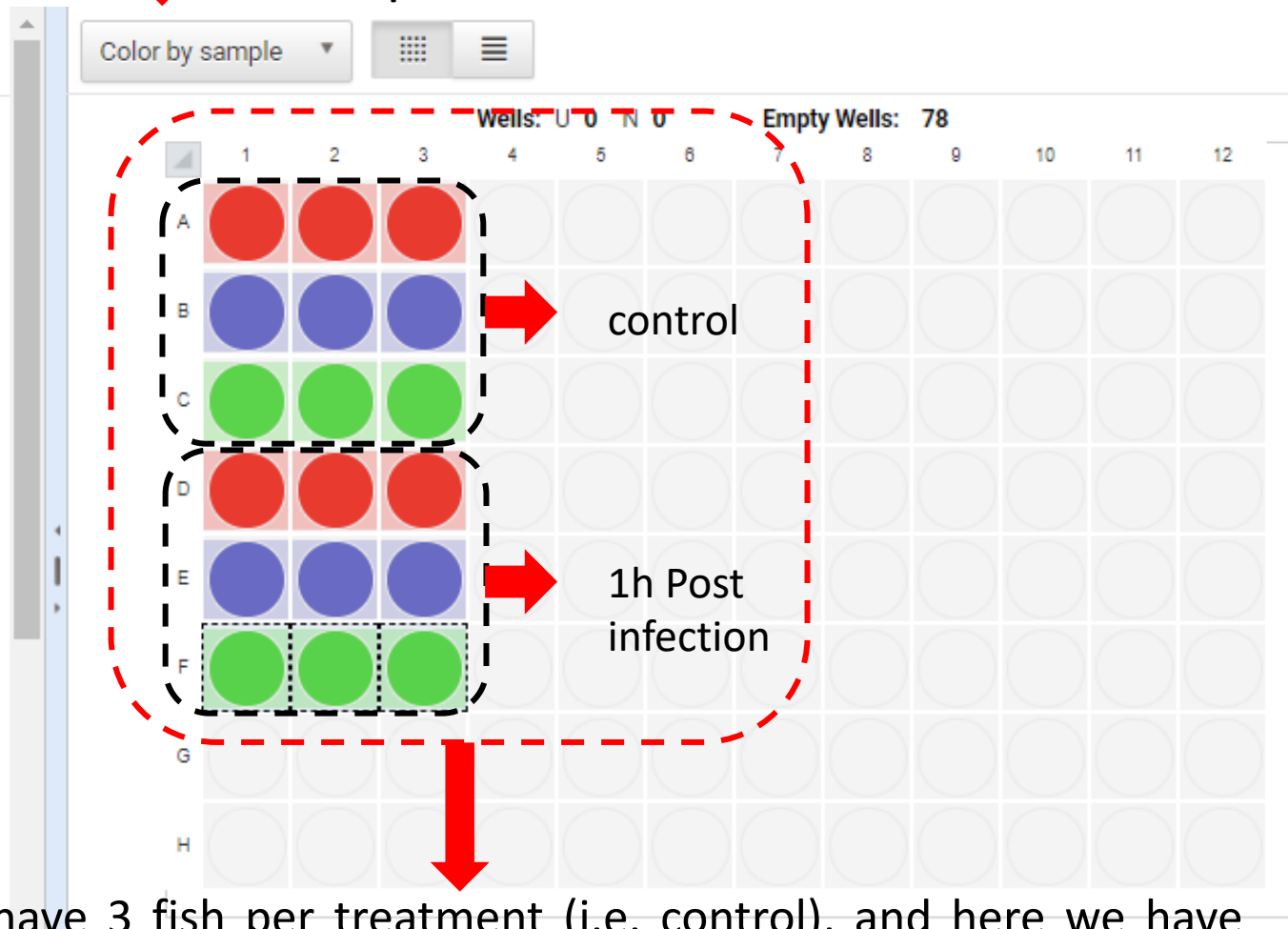
- We have 3 fish per treatment (i.e. control), and here we have already the two treatments.

- Both black squares make the measure of one gene, so the final layout to measure the 2 genes + the housekeeping will be…..

….this.

- Remember that this is only about samples, but we don't include the targets yet.

Now about targets (genes)

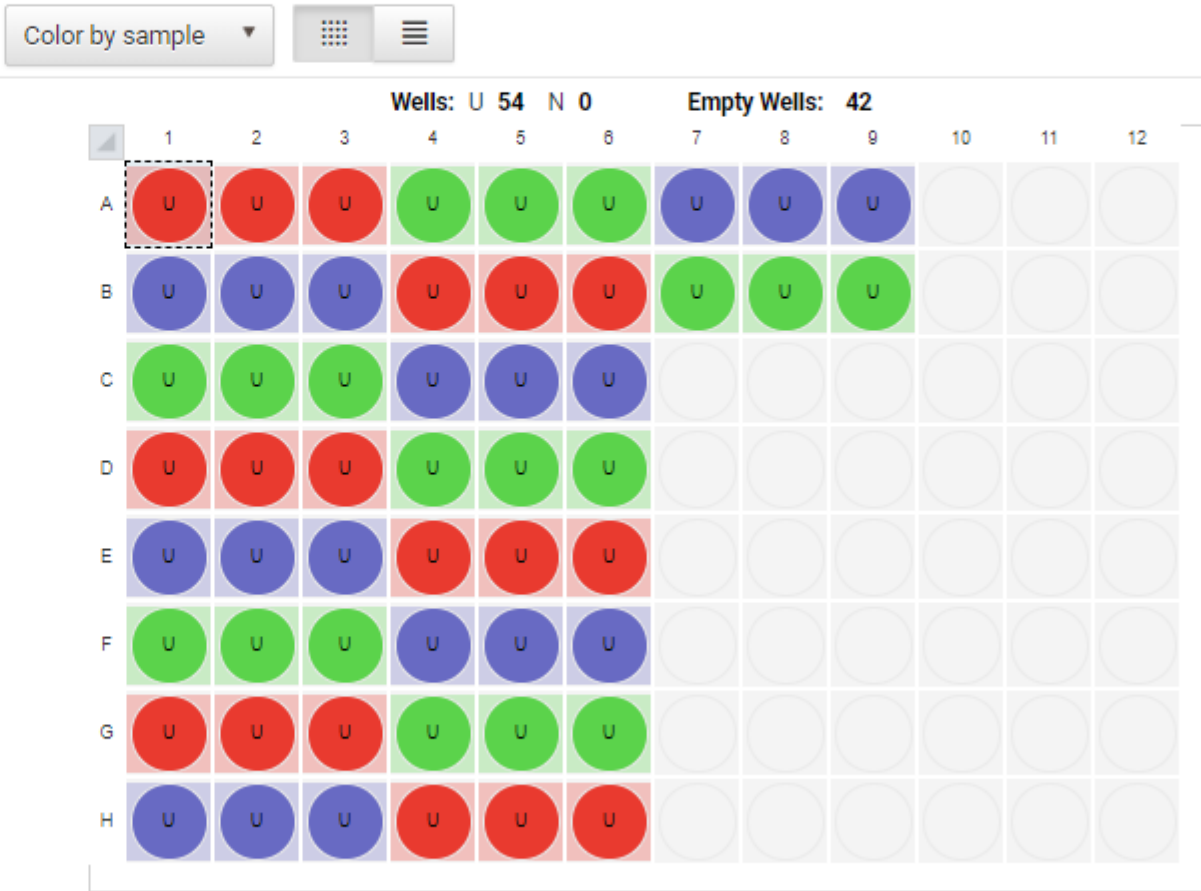- You have to put "color by target" to see the 96-wells plate showing the layout for genes.

- You have 3 genes (targets). Put the name of each one and select different colors.
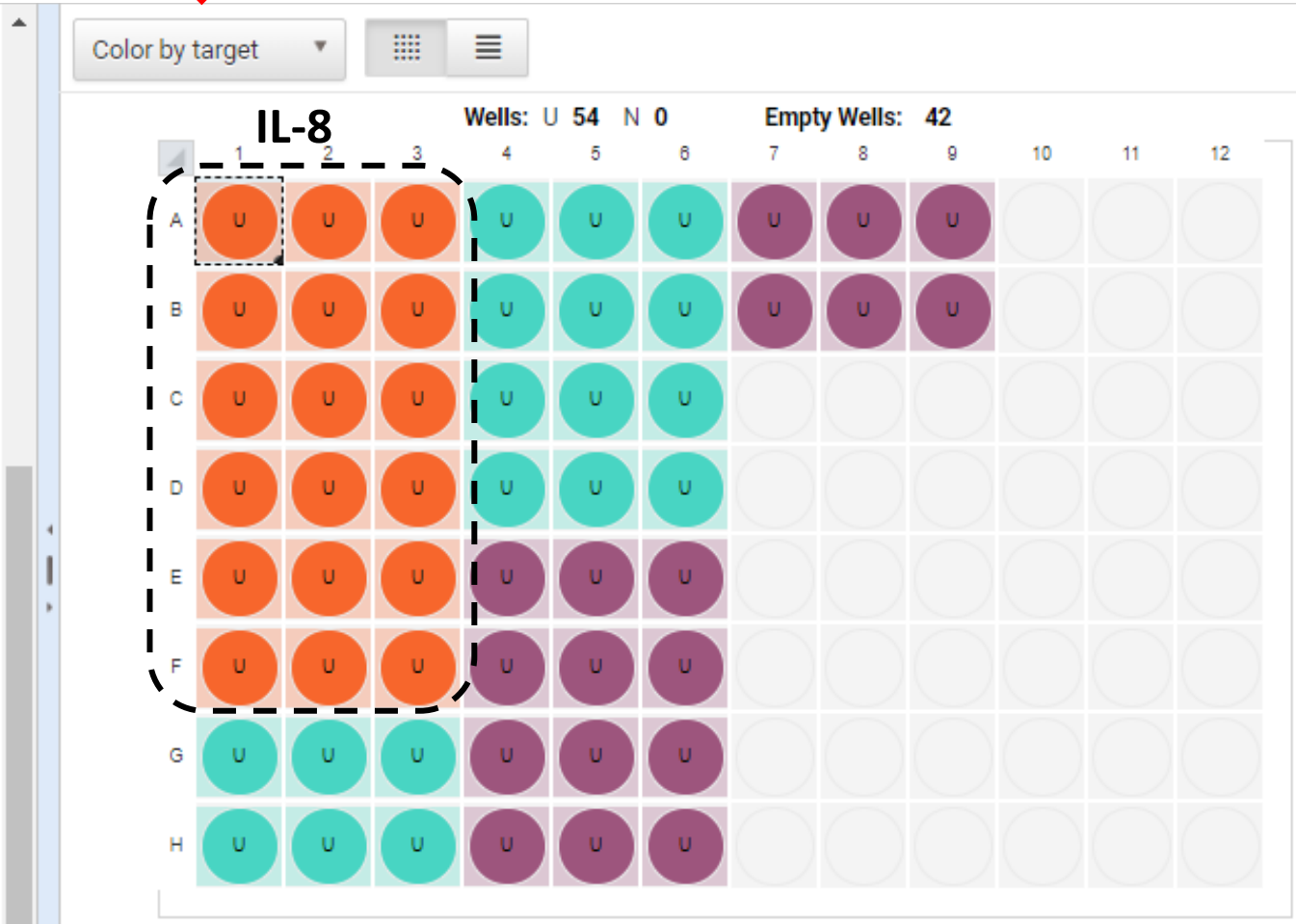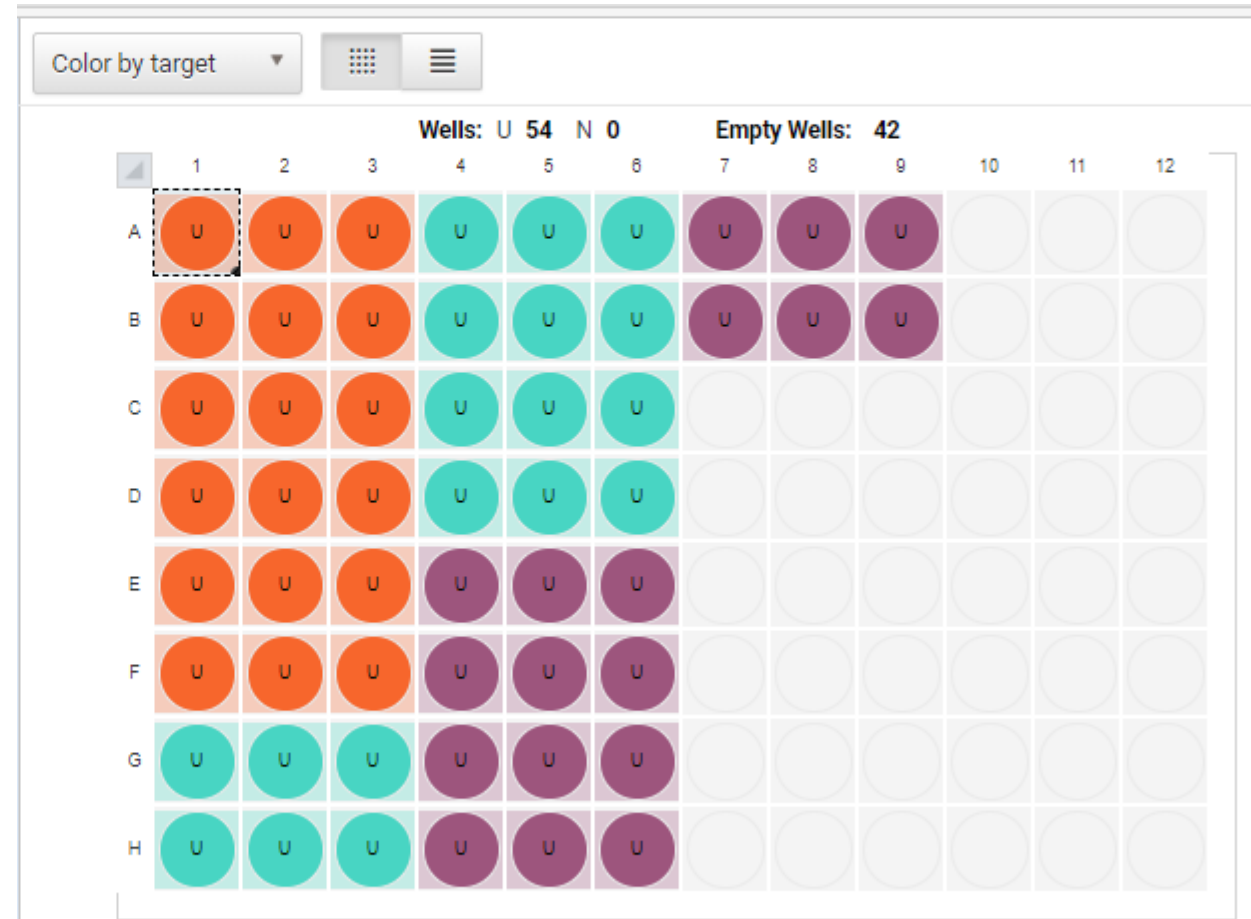
- We have 2 genes of interest and the housekeeping. Usually you put at the end the housekeeping, but the way how you design the layout is up to you.

Final layout for samples and target

By sample

By target

Add controls to test the mix

- Add at the end of the 96-well, one well per gene to test if the master mix is contaminated. These wells must contain only the same amount of master mix used per well + the same amount used of cDNA but instead of cDNA, must be filled with water.

- Put the mouse over 1 well, select the gene and then change the task from unknown to NTC (not treatment control).

Last step - Run

- Press "next".

- The system will give the option to save the file.

- Choose the same file name used at the beginning.

Last step - Run

Last step - Run

- After you press "save", the file will be upload to your account and will be available in your user account of the equipment to be measured.

- The file created is an .EDT file in case to need be used again as a template for further analysis.

- Finally you can see the option to "monitor my run". If you click there meanwhile you are running your plate, you can see the time left, if the run goes ok and inclusive how goes your measurements on each well.