

Santander lab

How to make a template? – QuantStudio 3

Contributed by

Manuel Soto-Davila

MSc. student Aquaculture

2019



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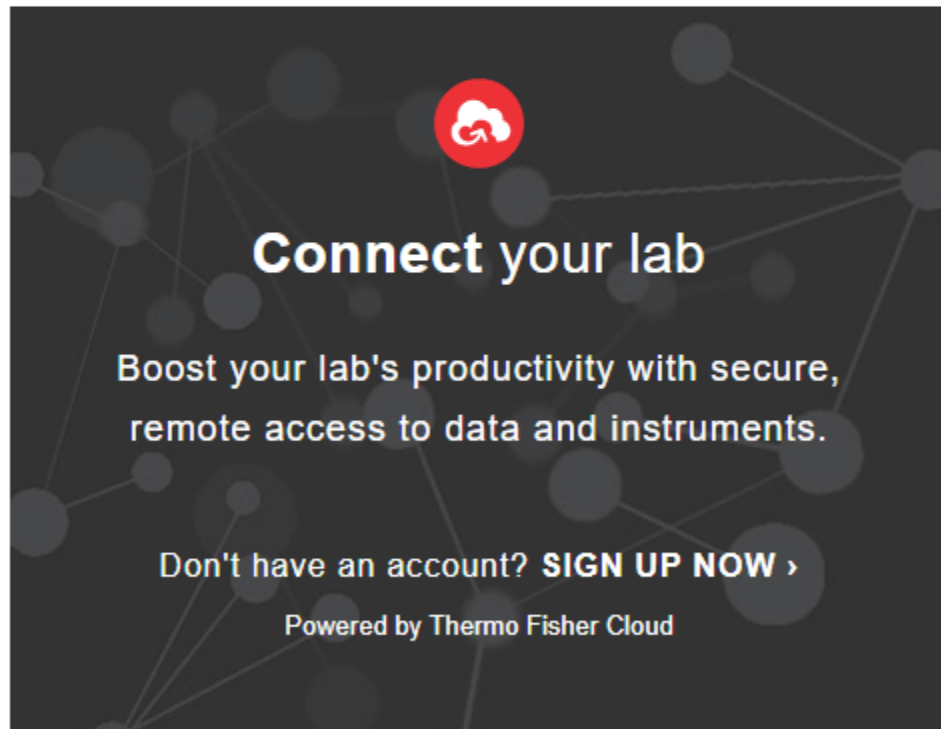
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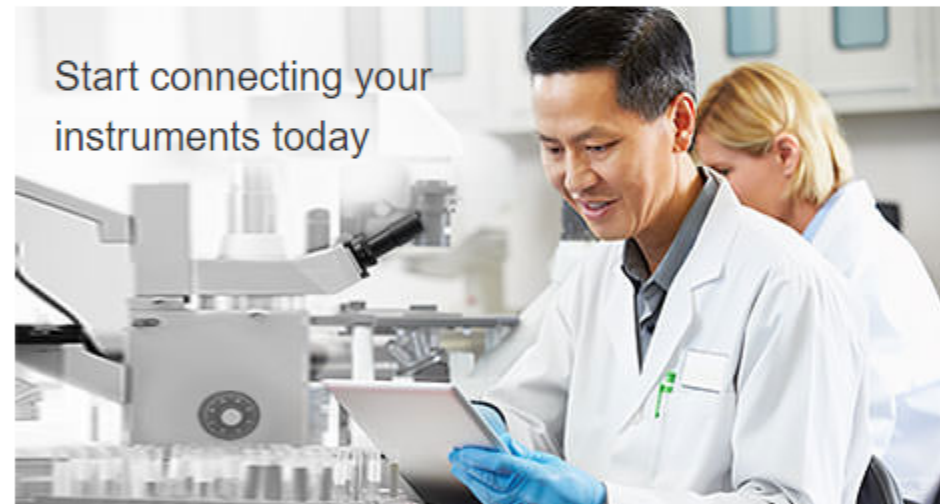


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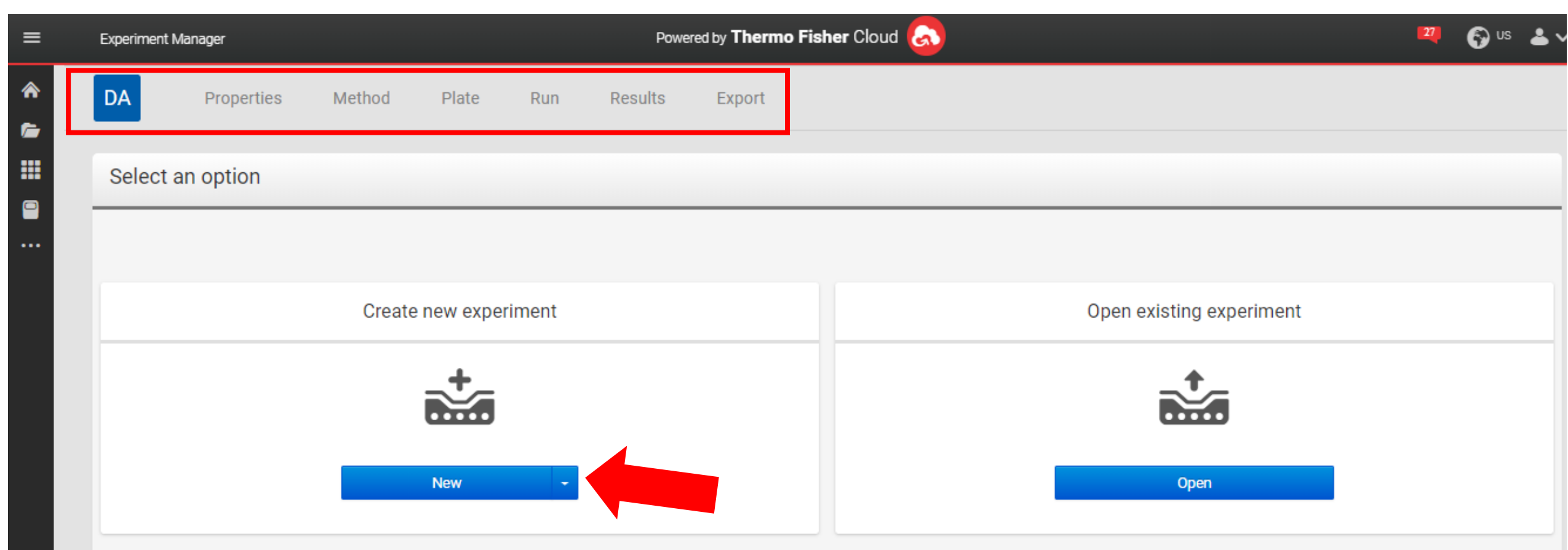
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- Introduce your Username and Password to start the connection. If you do not have an account, you must create one before following the next steps.

- 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.

DA

Properties

Method

Plate

Run

Results

Export

Select an option

Create new experiment



New

New

New from an existing file

New from a template

Open existing experiment



Open

- 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.

1st Step to create the template - properties

Experiment Manager Powered by Thermo Fisher Cloud 27 US

DA **Properties** Method Plate Run Analyze ⚙️

Experiment Properties (New) Actions ...

| | | |
|-----------------|--|---|
| Name | <input type="text" value="2018-09-20 011759"/> | <input type="text" value="Comments - optional"/> |
| Barcode | <input type="text" value="Barcode - optional"/> | |
| User name | <input type="text" value="User name - optional"/> | |
| Instrument type | <input type="text" value="QuantStudio® 3 System"/> | <input type="checkbox"/> Notifications <input type="text" value="Email addresses (separated by commas)"/> |
| Block type | <input type="text" value="96-Well 0.2-mL Block"/> | Progress <input type="checkbox"/> Run started <input type="checkbox"/> PCR cycling started <input type="checkbox"/> Run paused <input type="checkbox"/> Run stopped <input type="checkbox"/> Run about to end <input type="checkbox"/> Run completed |
| Experiment type | <input type="text" value="Comparative Ct (ΔΔCt)"/> | Conditions <input checked="" type="checkbox"/> Instrument error |
| Chemistry | <input type="text" value="TaqMan® Reagents"/> | |
| Run Mode | <input type="text" value="Standard"/> | |

[Manage Chemistry Details](#)

Next

Name, Bar code, User name, instrument type

The screenshot shows the 'Experiment Manager' interface. The 'Properties' tab is selected and highlighted with a red box. Below the tab, the 'Experiment Properties (New)' form is visible. The form contains the following fields:

| Field | Value |
|-----------------|-----------------------|
| Name | 2018-09-20 011759 |
| Barcode | Barcode - optional |
| User name | User name - optional |
| Instrument type | QuantStudio® 3 System |

- Name: should be something easy to remember to you about what was that plate measured. Usually the date goes first and then the experiment description. i.e. “20180920 Cod gene expression plate 1”, in this case you must know which genes you put on plate 1 .
- Barcode and user name are optional and is not necessary to put nothing here.
- Instrument type: 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

The image shows a software interface with the following settings:

- Block type:** 96-Well 0.1-mL Block
- Experiment type:** Comparative Ct ($\Delta\Delta C_t$)
- Chemistry:** (Not explicitly set, but the dropdown menu is open)
- Run Mode:** (Not explicitly set)

The dropdown menu for 'Experiment type' contains the following options:

- Custom Experiment
- Standard Curve
- Relative Standard Curve
- Comparative Ct ($\Delta\Delta C_t$)
- Melt Curve
- Genotyping
- Presence/Absence

- Block type: The QuantStudio 3 can be purchased 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.
- Experiment type: 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 ($\Delta\Delta C_t$)”.

Chemistry and Run mode

Chemistry SYBR® Green Reagents

Run Mode Fast

[Manage Chemistry Details](#)



- Chemistry: This means the reagent that you are using for gene measurement. Actually, we are using only SYBR green, so you must select this option. If you start to use TaqMan at some point, you must change to that option.
- Run mode: One great thing about the QuantStudio 3 is that it reads 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.

Comments - optional

Notifications

Email addresses (separated by commas)

Progress

Run started

PCR cycling started

Run paused

Run stopped

Run about to end

Run completed

Conditions

Instrument error

Next

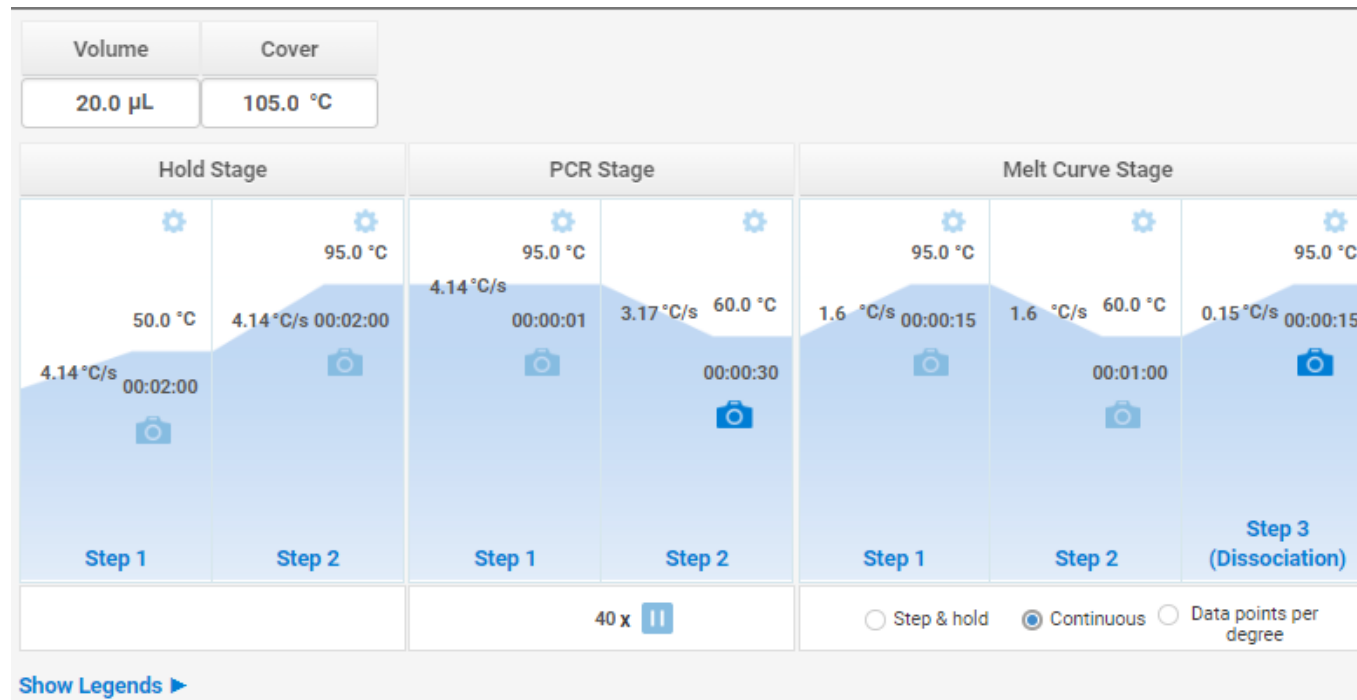
- What you choose here is totally up to you, but usually the most common things are send notifications when: the run is completed and also is there is any instrument error. Add your email to be notified about any of these situations.

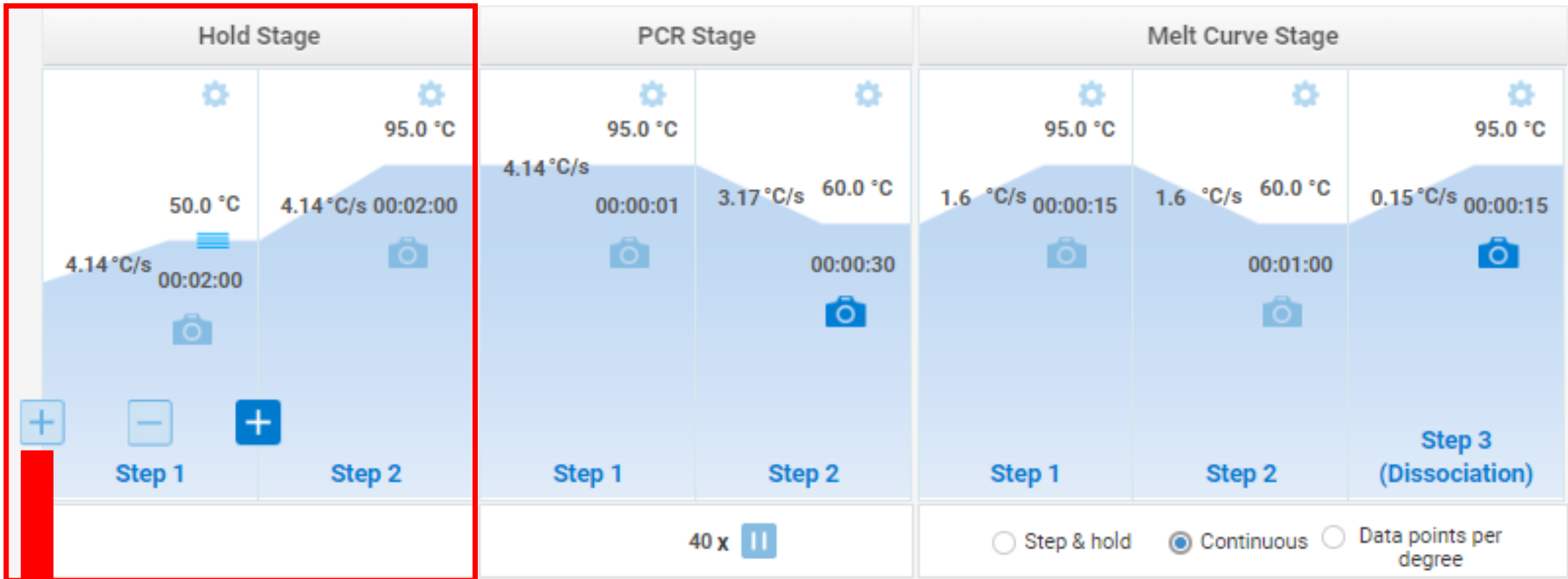
2nd Step to create the template - method

- 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.

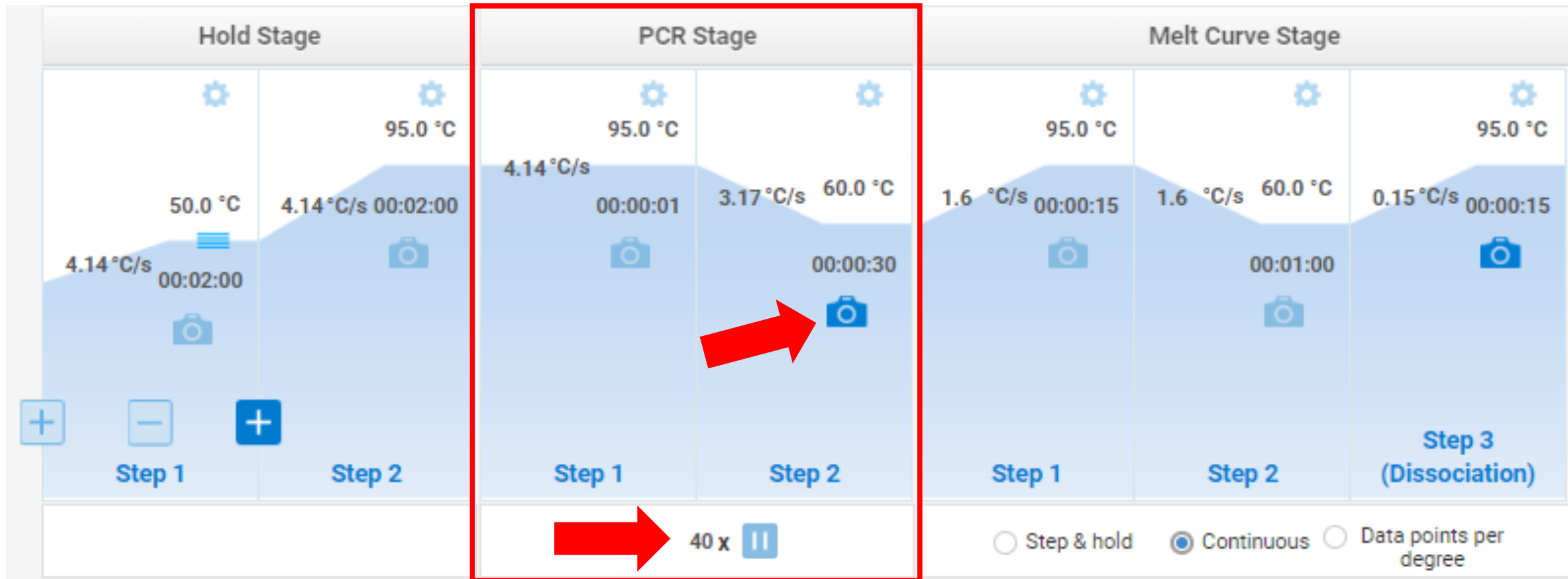
2nd Step to create the template - method

- 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).

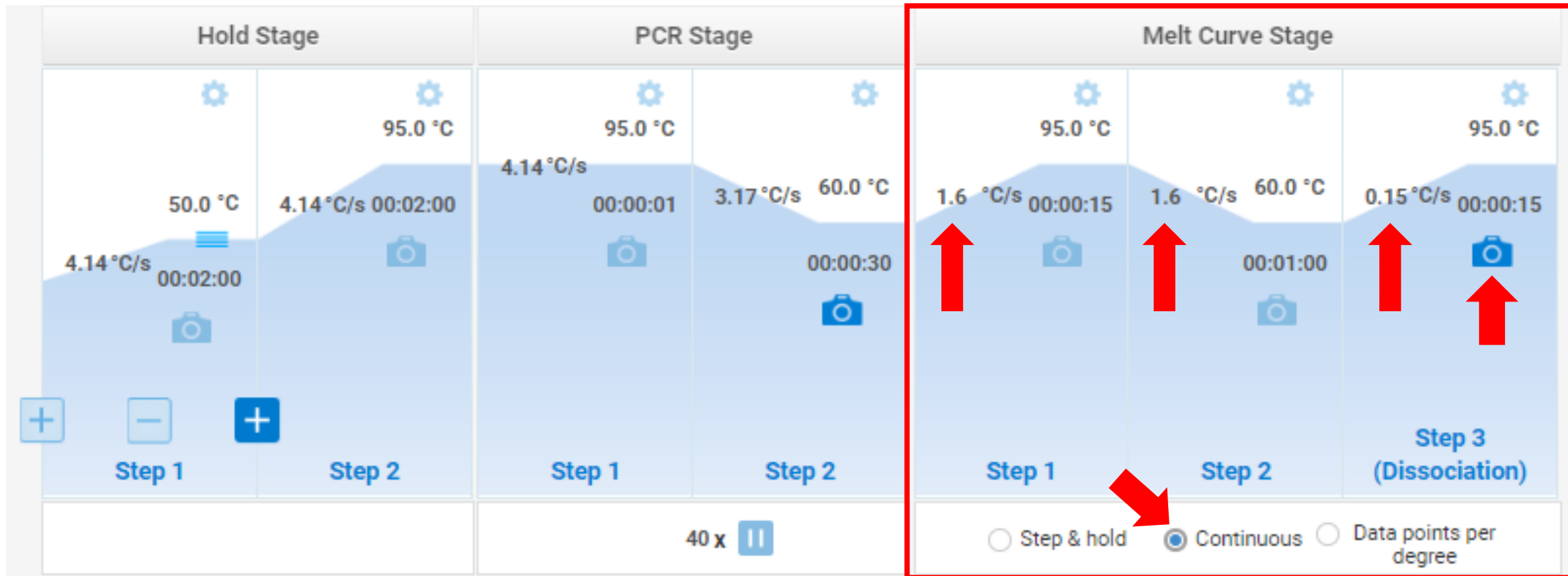




- To add or remove a hold stage you must press in the + or – sign respectively.
- There are two hold stages steps using the PowerUp SYBR, the first one 50°C for 2 minutes, and the second one 95°C for 2 minutes. The ramp rate (4.14°C/s) is calculated automatically for the equipment, so don't change this parameter.

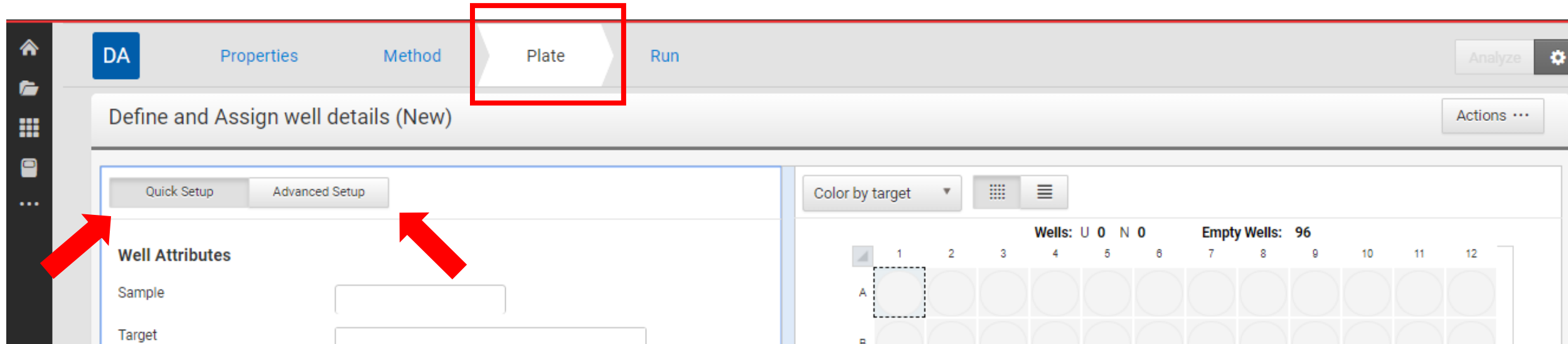


- There are two PCR stage steps using the PowerUp SYBR, the first one 95°C for 1 second, and the second one 60°C for 30 seconds. The ramp rate is calculated automatically for the equipment, so don't change this parameter.
- The camera must be selected in the second step and 40 cycles must be added (40x).



- 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.

DA Properties Method Plate Run

Define and Assign well details (New)

Quick Setup **Advanced Setup**

Define and assign target and samples

Samples

| Name | Comments |
|----------|----------|
| Sample 1 | |

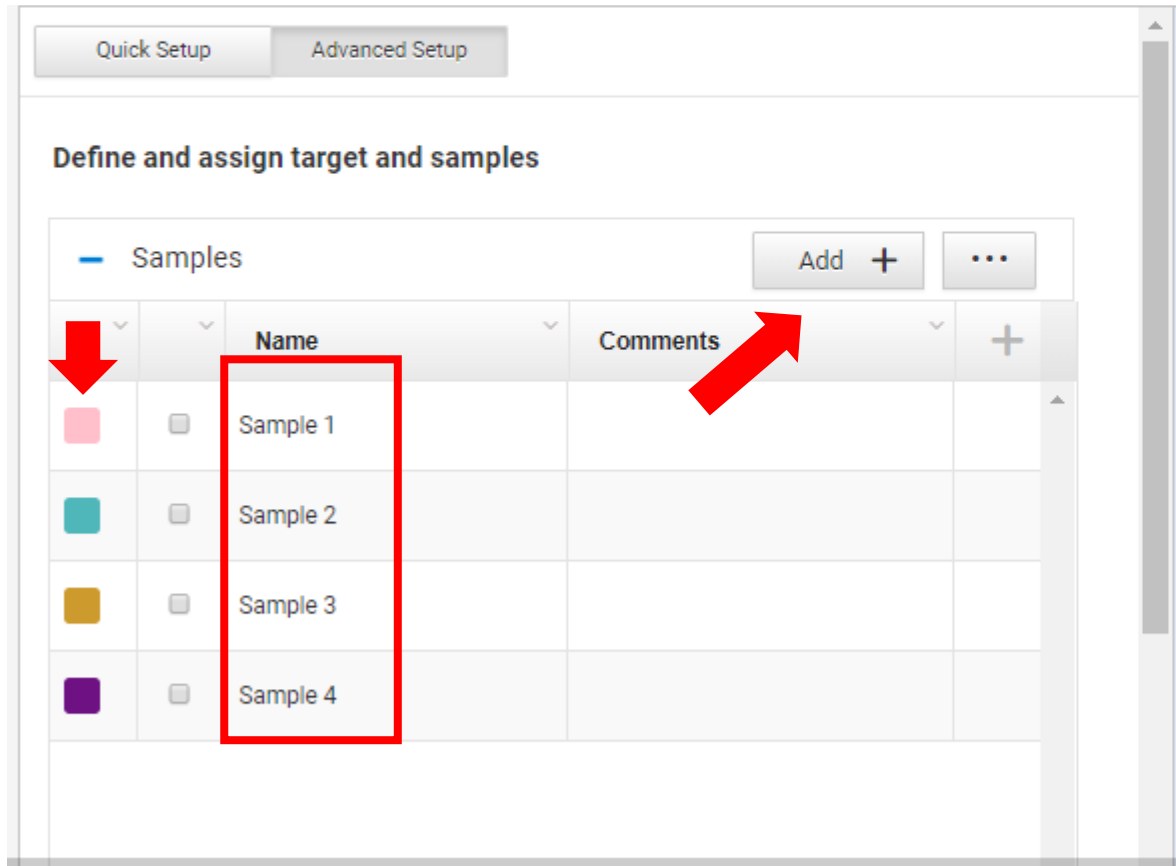
Color by target

Wells: U 0 N 0 Empty Wells: 96

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




- 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 | | Name | Task | Reporter | Quencher | Commen |
|--|--------------------------|----------|---------|----------|----------|--------|
|  | <input type="checkbox"/> | Target 1 | Unknown | SYBR | NFQ-MGB | |
|  | <input type="checkbox"/> | Target 2 | Unknown | SYBR | NFQ-MGB | |
|  | <input type="checkbox"/> | Target 3 | Unknown | SYBR | NFQ-MGB | |

Previous

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

- 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.

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

Quick Setup Advanced Setup

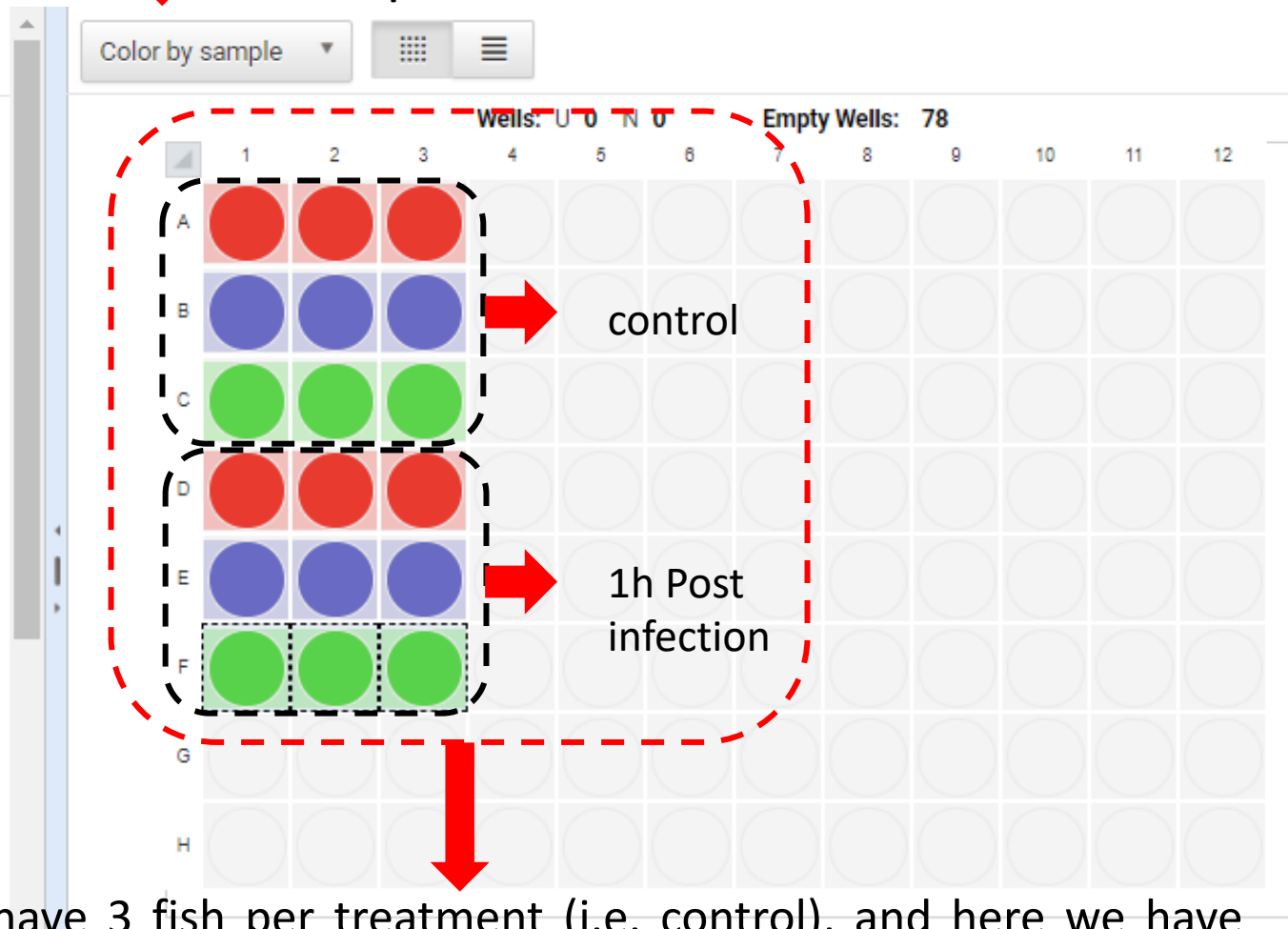
Define and assign target and samples

Samples Add + ...

| | Name | Comments |
|-------------------------------------|--------|----------|
| <input type="checkbox"/> | Fish 1 | |
| <input type="checkbox"/> | Fish 2 | |
| <input checked="" type="checkbox"/> | Fish 3 | |

- You have 3 fish, so each sample will be 1 fish. Choose different colors for each fish.

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



- 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.

The screenshot displays a software interface for defining and assigning target and samples. On the left, a table lists three samples: Fish 1 (red), Fish 2 (blue), and Fish 3 (green). On the right, a well plate layout is shown with 8 rows (A-H) and 12 columns (1-12). The layout is color-coded by sample: Fish 1 (red) occupies columns 1-3 of rows A, D, and H; Fish 2 (blue) occupies columns 1-3 of rows B, E, and F; Fish 3 (green) occupies columns 4-6 of rows A, C, and G. A dashed box highlights a 3x3 area in the top-left corner (rows A-C, columns 1-3). The interface also shows a 'Color by sample' dropdown and a summary of 'Wells: U 0 N 0' and 'Empty Wells: 42'.

| Sample | Color | Assigned Wells (Row, Column) |
|--------|-------|---|
| Fish 1 | Red | (A,1), (A,2), (A,3), (D,1), (D,2), (D,3), (H,1), (H,2), (H,3) |
| Fish 2 | Blue | (B,1), (B,2), (B,3), (E,1), (E,2), (E,3), (F,1), (F,2), (F,3) |
| Fish 3 | Green | (A,4), (A,5), (A,6), (C,4), (C,5), (C,6), (D,4), (D,5), (D,6), (E,4), (E,5), (E,6), (G,4), (G,5), (G,6) |




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

Now about targets (genes)

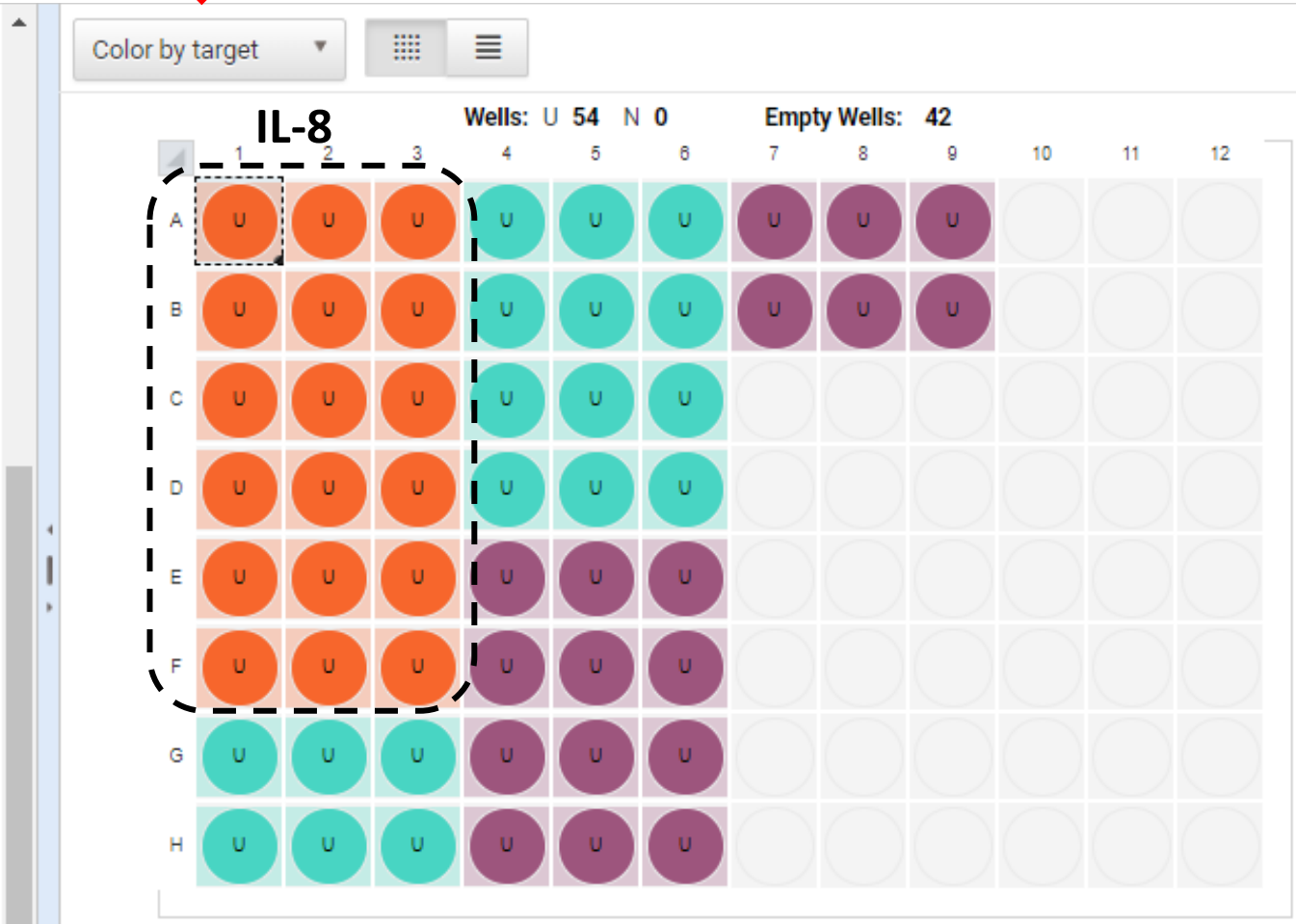
- You have to put "color by target" to see the 96-wells plate showing the layout for genes.



Targets

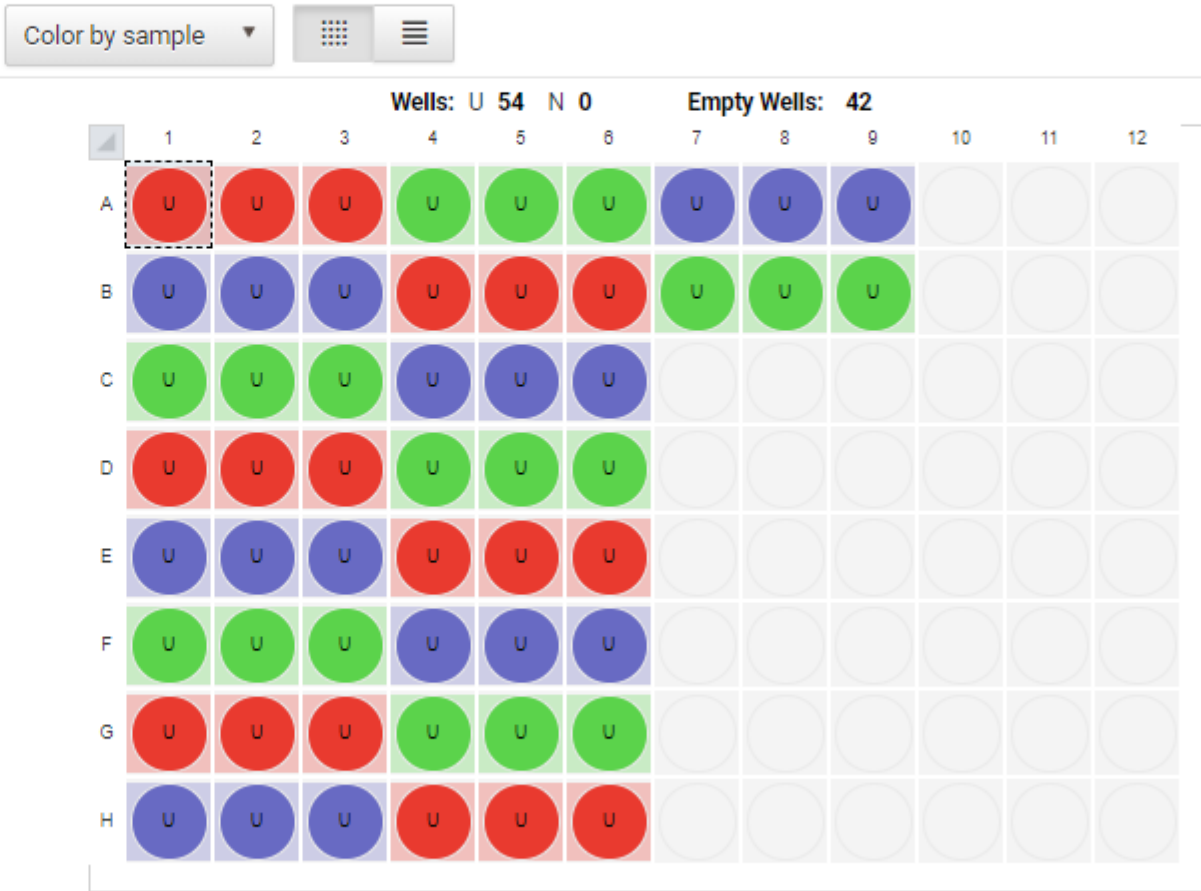
| | | Name | Task | Reporter | Quencher | Comment |
|---|-------------------------------------|-------|---------|----------|----------|---------|
|  | <input checked="" type="checkbox"/> | IL-8 | Unknown | SYBR | NFQ-MGB | |
|  | <input type="checkbox"/> | IL-10 | Unknown | SYBR | NFQ-MGB | |
|  | <input type="checkbox"/> | EF-1a | Unknown | SYBR | NFQ-MGB | |

- You have 3 genes (targets). Put the name of each one and select different colors.

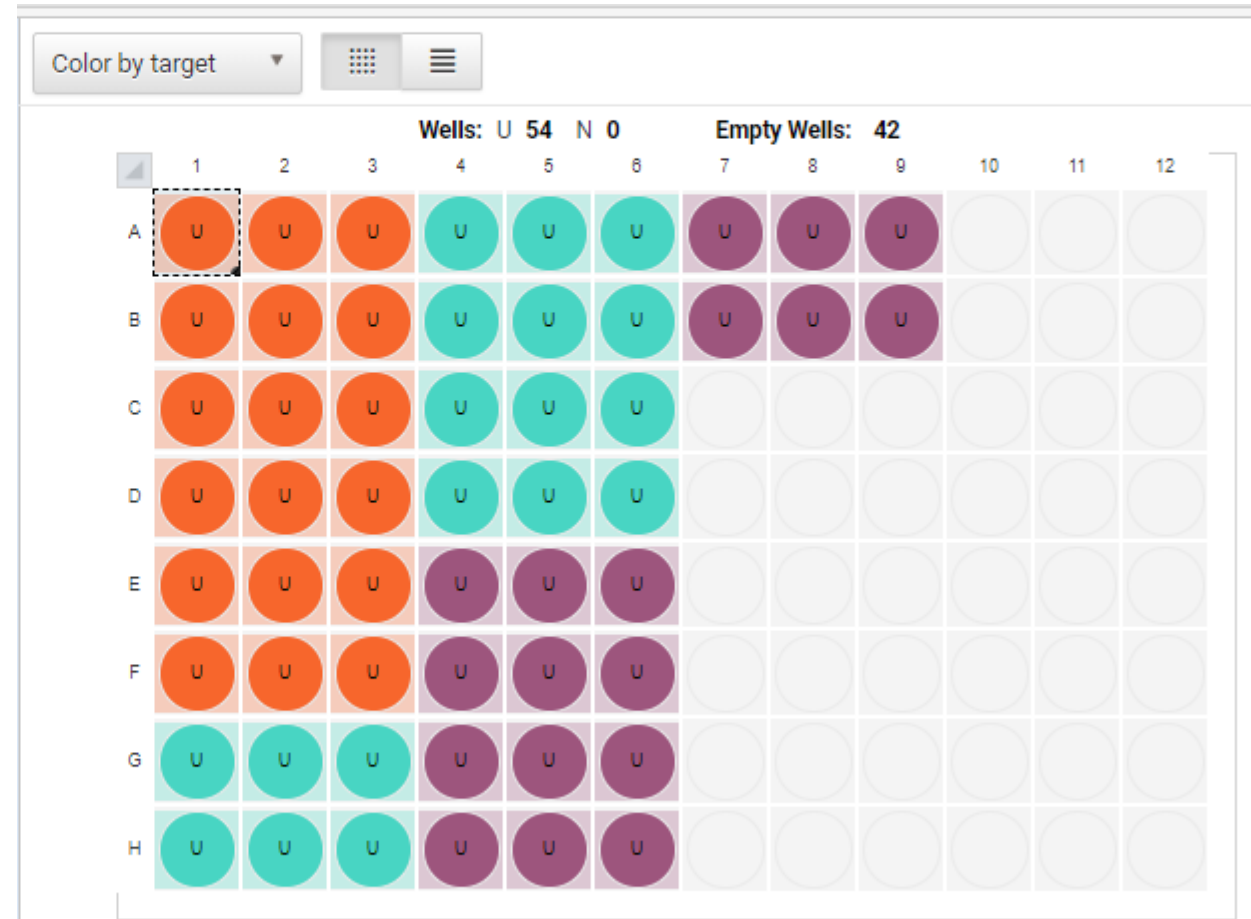


- We have 2 genes of interest and the housekeeping. Usually you put at the end the housekeeping, but the way how you design the layout is up to you.

Final layout for samples and target






By sample



By target

Add controls to test the mix

Targets

| | | Name | Task | Reporter | Quencher | Comment |
|---|-------------------------------------|-------|---------|----------|----------|---------|
|  | <input type="checkbox"/> | IL-8 | Unknown | SYBR | NFQ-MGB | |
|  | <input type="checkbox"/> | IL-10 | Unknown | SYBR | NFQ-MGB | |
|  | <input checked="" type="checkbox"/> | EF-1a | NTC | SYBR | NFQ-MGB | |

Dropdown menu for EF-1a Task:

- Unknown
- NTC



- 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

The screenshot displays the 'Experiment Manager' interface, powered by Thermo Fisher Cloud. The main navigation bar includes 'DA', 'Properties', 'Method', 'Plate', and 'Run'. The 'Run' step is currently active, showing a 'My Plate (New)' section with a checklist for 'Step 1' and 'Step 2'. A 'Save as' dialog box is open in the foreground, containing a text input field with the text '20180920 Cod gene expression plate 1', a 'Save as a Locked Template' checkbox, and a 'Save' button. The background interface includes a 'Monitor my run' button and a 'Close' button at the bottom right.

Experiment Manager

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27 US

DA Properties Method Plate Run Analyze

My Plate (New)

Step 1

✓

Your experiment is now

1. Go to a Cloud-linked QuantStudio
2. Load the experiment file from your T
3. Insert your plate, the

Step 2

Monitor my run

Save as

20180920 Cod gene expression plate 1

Save as a Locked Template

Save

Create new experiment

Close

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.