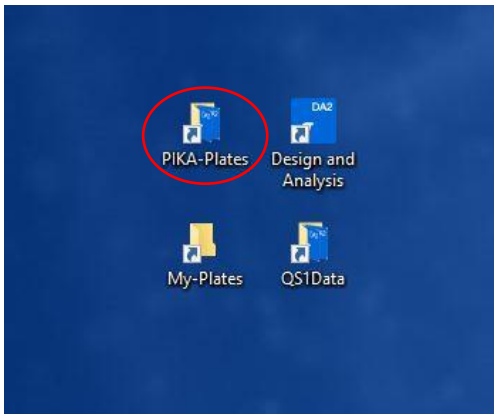
TECHNICAL PAPER #05

RUN MULTIPLE 4EVERYONE DETECTION KITS ON QUANTSTUDIO PLUS-MINUS SOFTWARE

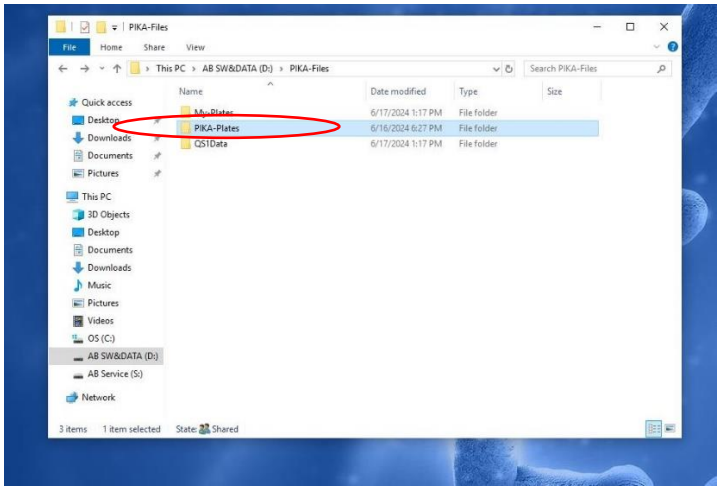
Section 1

Set up an Experiment

- Switch on your laptop
- Log in as INSTR-USER, password INSTR-USER
- On the desktop, three folders are displayed together with the Design and Analysis program
- If your desktop is not assigned in that way, have a look on the second picture how to prepare shortcuts to these folders



- Double click on the folder "PIKA-Plates"



- If the shortcuts to these three folders are not available on the desktop, you can find the folders here: "This PC →AB SW&DATA (D:)→PIKA-Files"
- Create a desktop shortcut for the folder
    - Right-click on the folder and select "Send to" -> "Desktop (create shortcut)"

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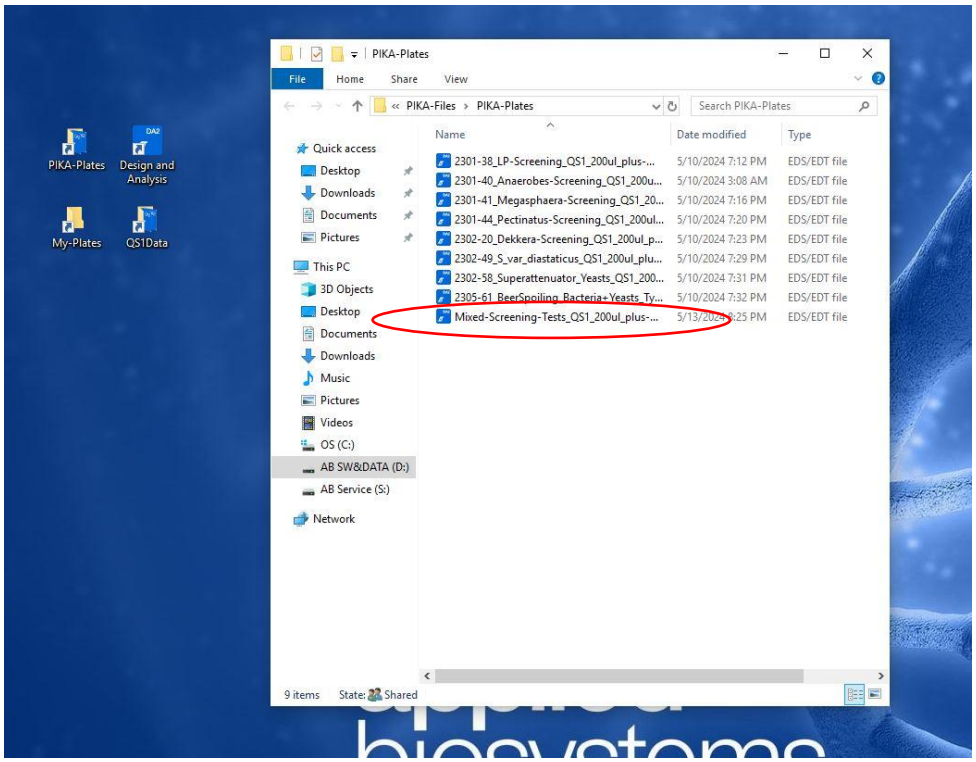
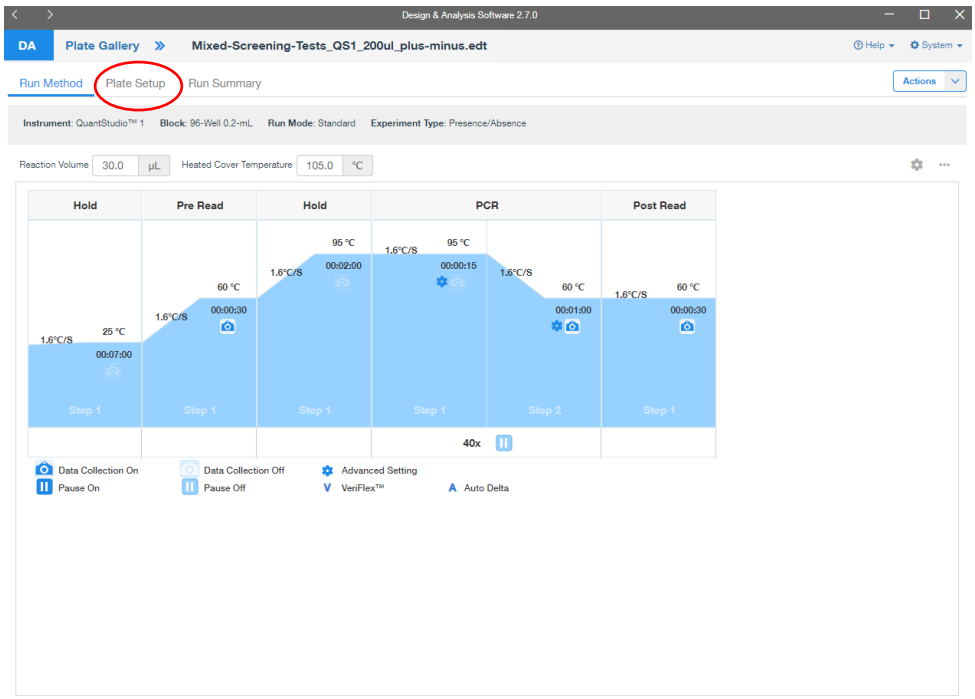


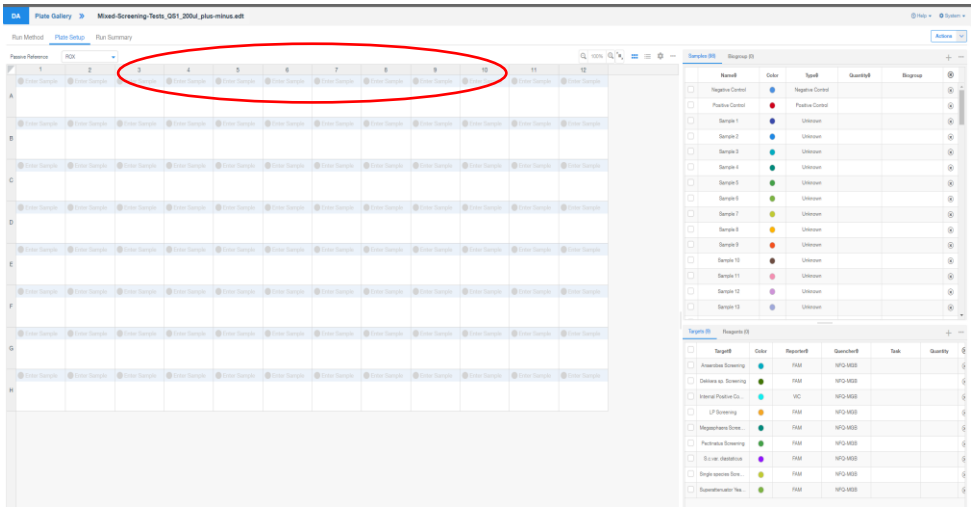
- In the folder PIKA-Plates, all 4everyone Detection Kits are displayed with their product numbers
- Double click on the kit you want to run, here "Mixed-Screening-Tests_QS1_200ul_plus-minus"
- The DA2 software opens automatically in a new window

Section 2

Program Your Test

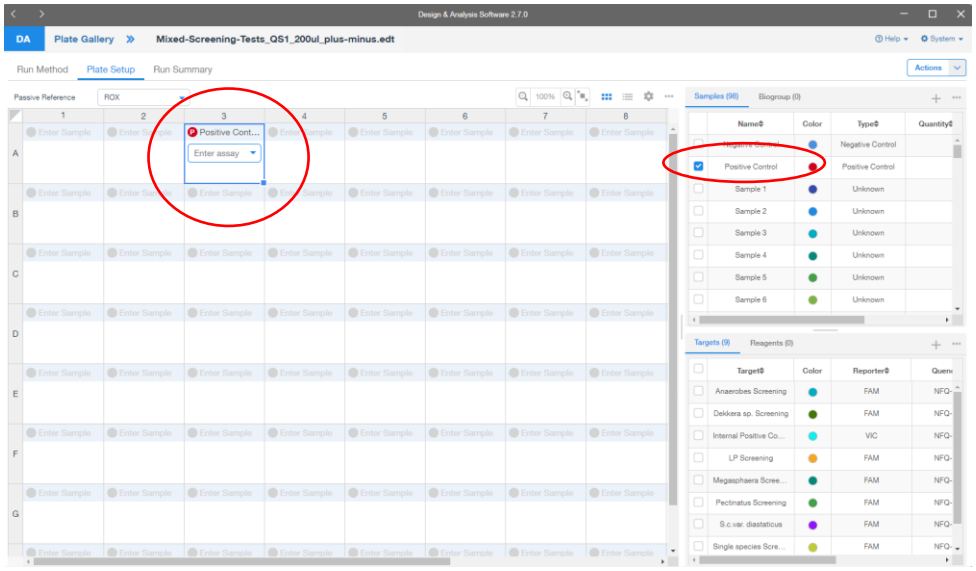


- Switch to Plate Setup by a click on the table

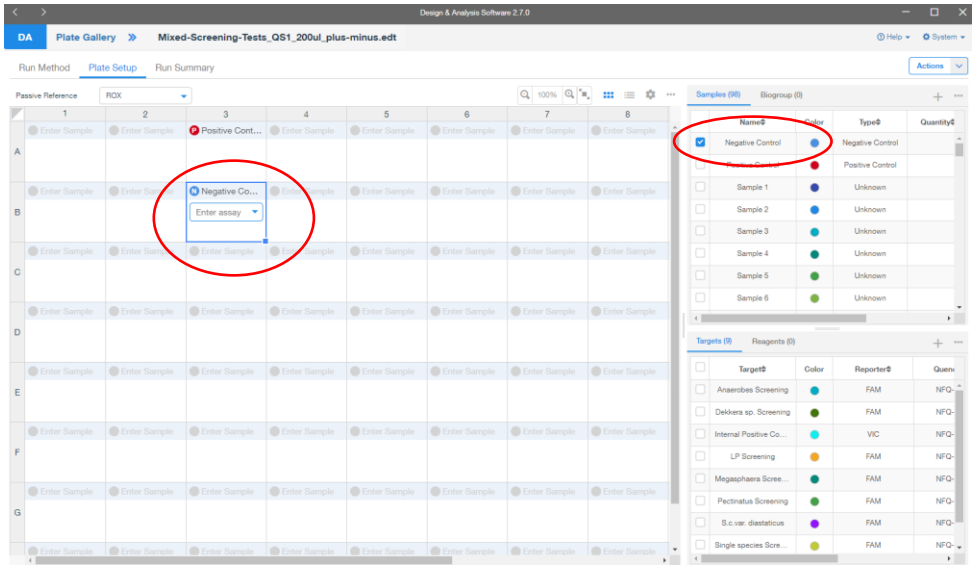


- If you don't use a full 96 well plate
  - select the positions in the center of the thermoblock to position the samples
  - you should always insert one full empty tube strip (closed with a matching cap strip) into each of the rows 1 (positions A-H) and 12 (positions A-H) of the thermoblock. Doing so will allow better pressure distribution of the heated cover during the measuring process.
- This table basically works like an Excel table

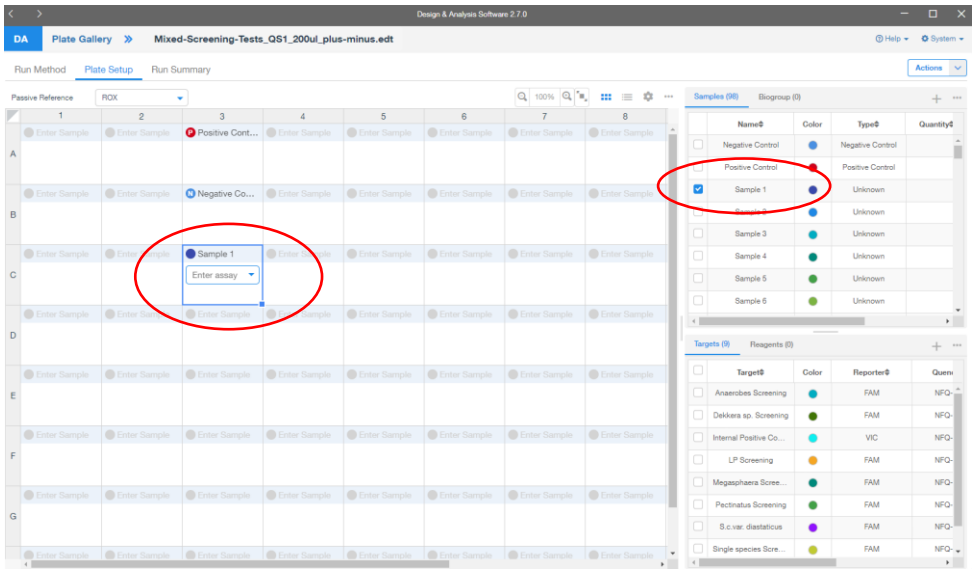
- Although you are generally free to create the plate setup, we recommend to position the positive control first, then the negative control and then the samples



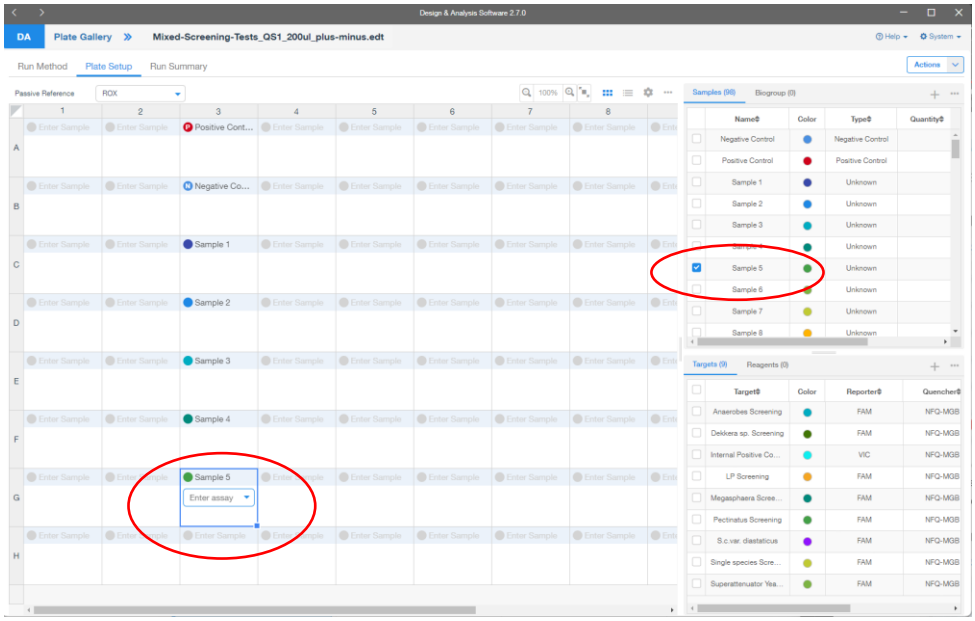
- Select one field in row A (here for example column 3) with a single click and assign the positive control by a single click followed by ticking "Positive Control"



- Use the second position of a column for the negative control, so you have it as a barrier between your samples and the positive control DNA to avoid cross contamination from the DNA to a sample
- Select the second position with a single click and tick "Negative Control"



- Select the next field below (here for example C3) and start adding your samples
- For the first, activate the corresponding position with a single click and tick "Sample 1"



- Repeat activation as described for the following sample positions according to the number of samples to be tested (here for example 5 samples) , assign one sample number after the other

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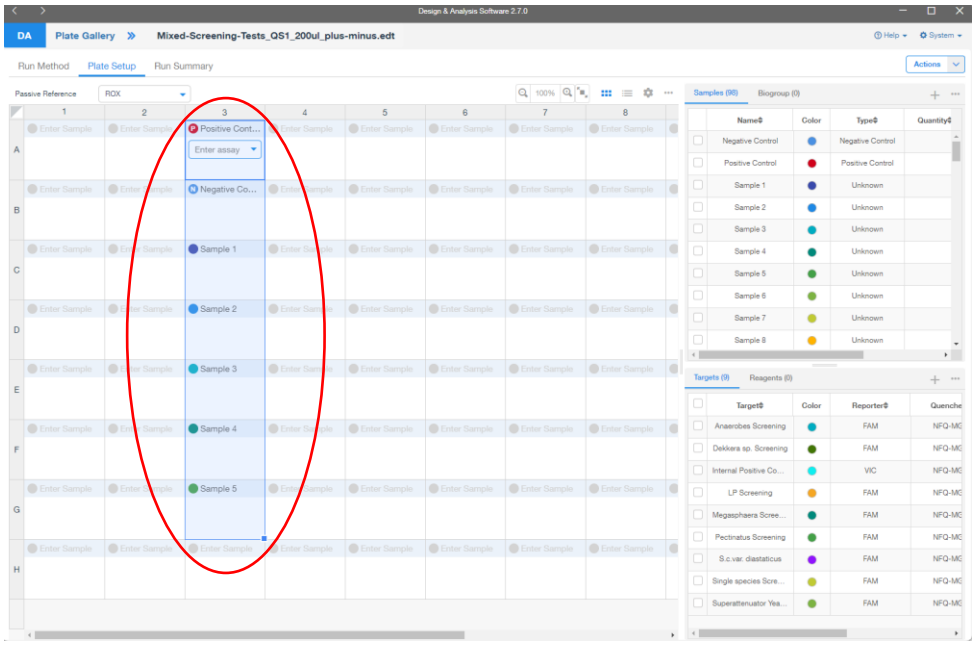
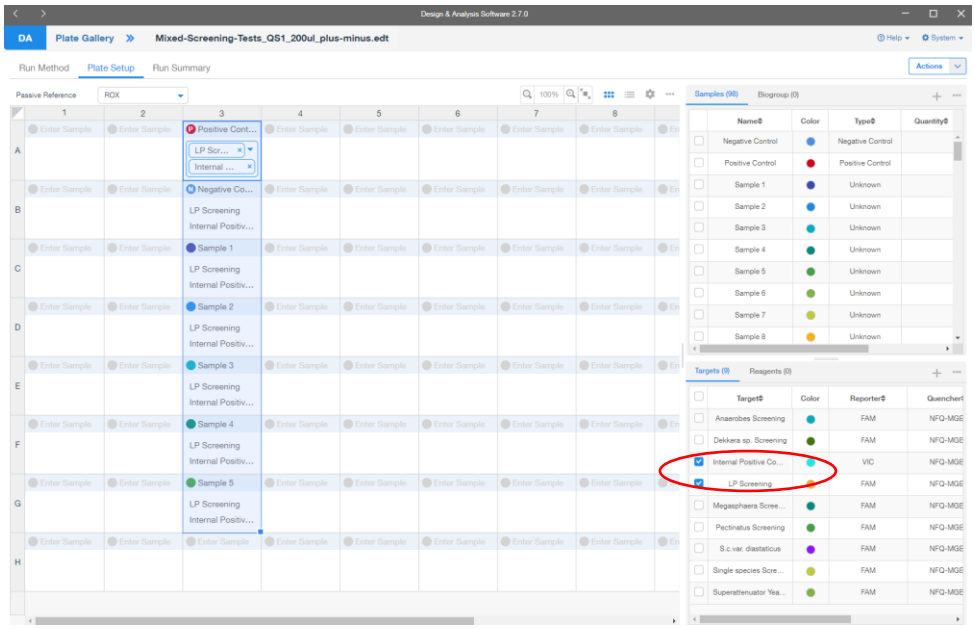
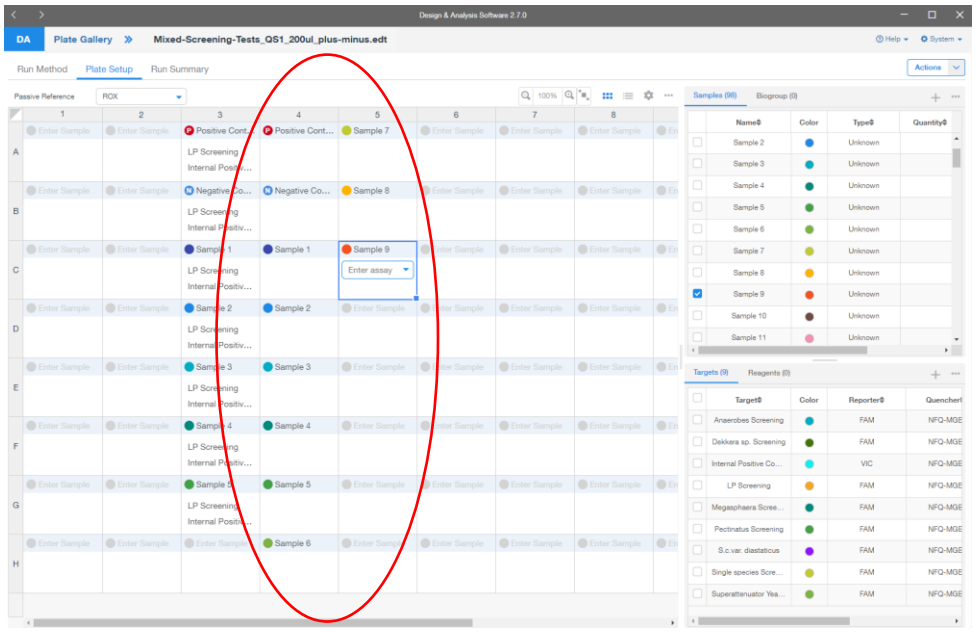


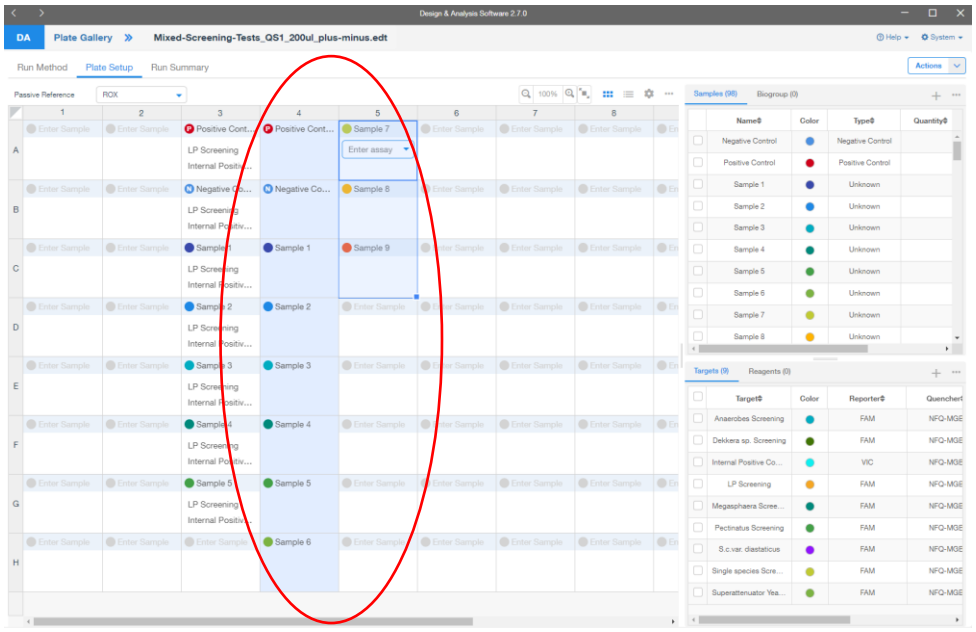
- Align samples and tests
    - Select all samples and both controls which you want to run in the first test
    - To activate multiple positions, press the "shift" key and hold it while clicking on the two controls and samples to select them all



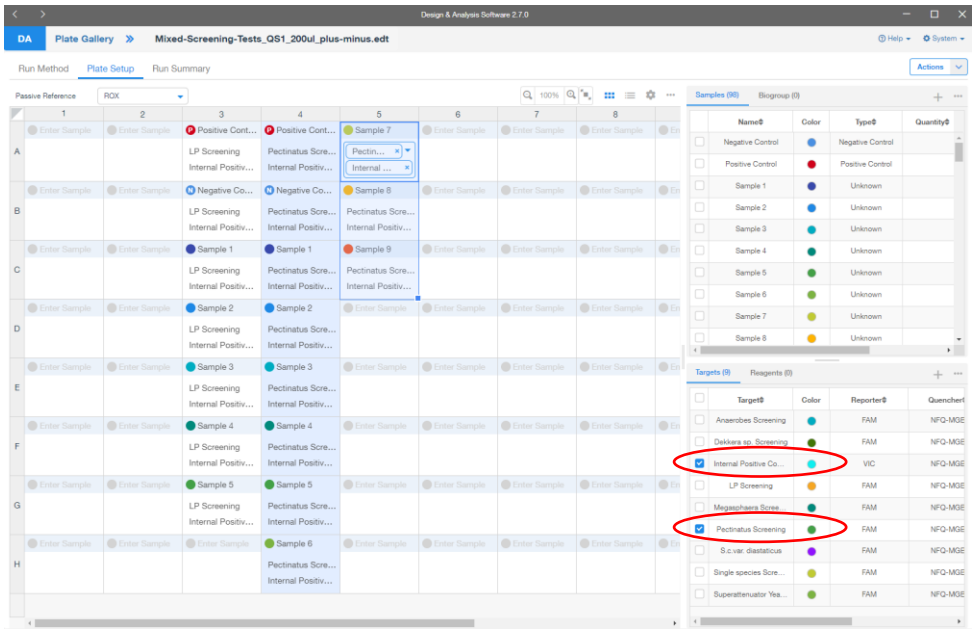
- To assign the test to the samples
    - tick the the kit name which you want to run (here for example “LP Screening”)
    - additionally tick “Internal Positive Control”
- Now both channels (VIC and FAM) are activated for the LP Screening measurement in all PCR tubes positions which are in use for the first test



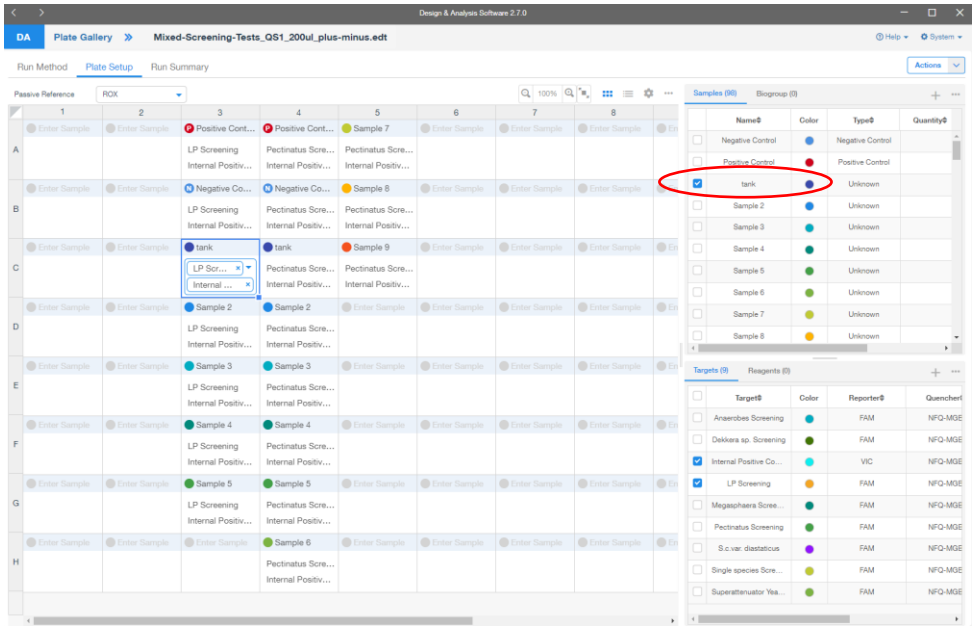
- To add a second or more PCR tests, continue with the following columns (here for example columns 4 and 5)
- Proceed with adding the sample names and assingment of the tests to the sample positions in the same way as shown for column 3 (here for example 9 samples), but assign those to the name of the second test kit



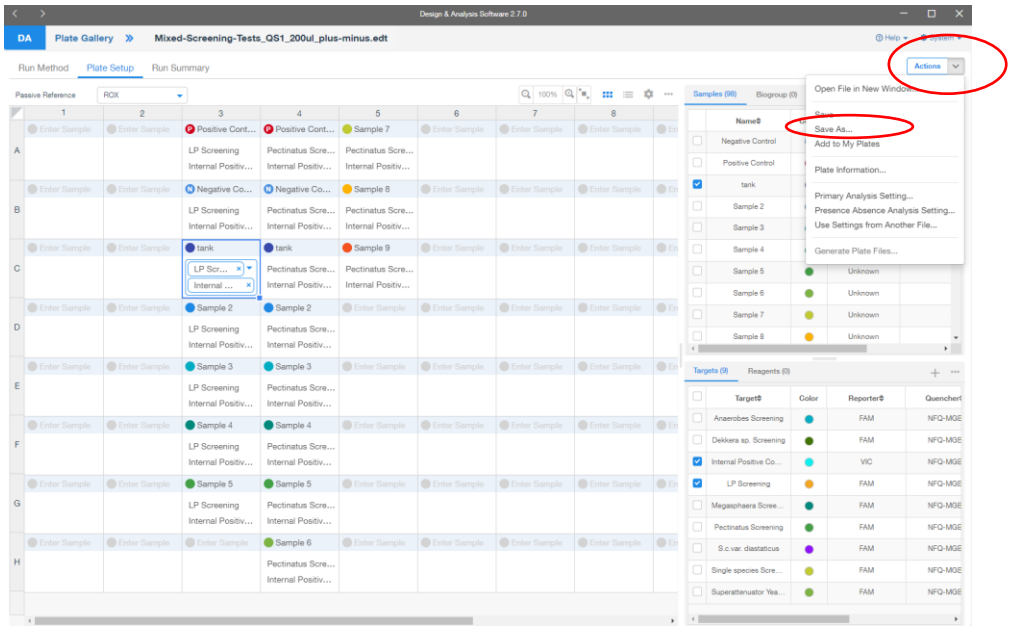
- Add a second test
  - Select all new added samples and both controls
  - To select multiple positions together, press the "shift" key and hold it while clicking on the two controls and samples to select them



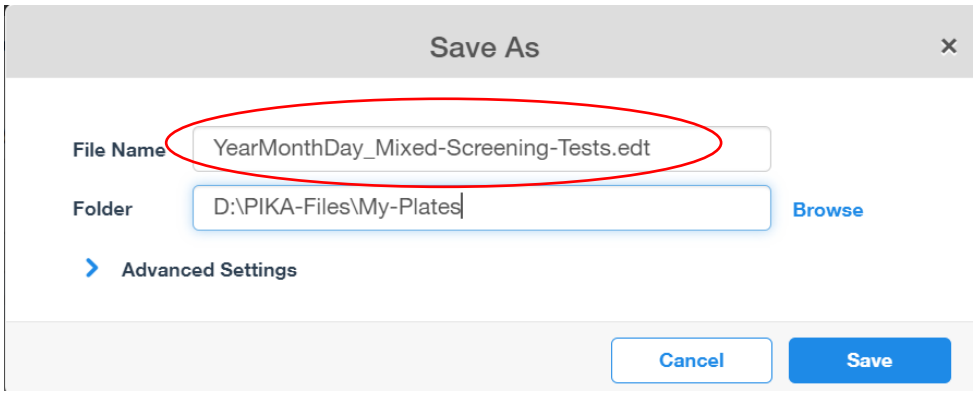
- To assign the second test to the samples
  - tick the the kit name you which you want to run (here for example "Pectinatus Screening")
  - tick "Internal Positive Control"
- Now both channels (VIC and FAM) are activated for the Pectinatus measurement in all PCR tubes positions which are in use



- The samples' names can be changed according to your real sample names
  - Click on "Sample 1" in the right table and enter the name of the sample (here for example "tank")
  - Repeat re-naming for all samples
  - New sample names will appear automatically in the positions as were selected before for the different sample numbers



- After all samples and controls are activated and show the correct corresponding targets, save your plate file
    - click on "Actions"
    - Then click on "Save As…", and type a unique name for the run into the field "File Name"



- We recommend to save the file with a name starting with the year, month, day, followed by the name of the test, as they will always be sorted accordingly
- Make sure that the folder path which is displayed is correct as shown above, if not, then browse to the folder "My-Plates"
- Click on "Save"

Please make sure now that your laptop is connected to your QuantStudio with the blue cable. The laptop must remain powered on when you start the run on QuantStudio, so the saved data can be automatically transferred to the thermocycler.

Section 3

Run Your Samples on QuantStudio

- Switch on the instrument by pressing the power button in the back of QuantStudio
- Log in as INSTR-USER, password is 2024
- Click "Load Experiment"
- Click "Network Drive"
    - 3 folders (My-Plates/, PIKA-Plates/, QS1Data/) are displayed
- Select the folder "My-Plates/"
    - Now you see all runs which you had saved so far
- Click on the file with the run you want to start, for example here "YearMonthDay_Mixed-Screening-Tests.edt"
- Now there are three tabs shown on the screen, click on "Plate"
    - The positioning of the preselected samples and controls is shown on display now
- Open the drawer of the instrument, be careful that you generally shouldn't leave the drawer open longer than necessary to avoid contamination of the thermocycler block by dust
- Place the PCR strips into the positions as shown on the display
    - Check that the PCR strips are inserted correctly and are tightly closed
    - Positive control should be in the first position (here for example A4)
    - If not using a full plate, you should always insert one full empty tube strip (closed with a matching cap strip) into each of the edge rows 1 (positions A-H) and 12 (positions A-H) of the thermoblock. This allows better pressure distribution of the heated lid during the measuring process
- **IMPORTANT NOTICE:**
    - NEVER use the cover foil for PCR which is mounted on the plates with oligomix when these are delivered in the kit. This foil is NOT temperature proof and will melt when used in the thermocycler!
    - **Melting of the foil will cause irreversible damage to the thermocycler optics and ruin the Quant Studio instrument!!!**
- Do not make any changes of names or positions on the Quant Studio screen, always use the software and data on the laptop to make changes
- Close the drawer
- Click on "Start Run"
    - Now the run starts automatically
    - After a short time, the display shows the time remaining until the run will finish
- Data collection and read out of results
    - The data from the QuantStudio will be automatically transmitted to the laptop as soon as the run has finished if a connection to the laptop is available
- After the run has finished, click on button "Done" and switch off the Quant Studio instrument.

Optional: It is not mandatory to have the laptop connected to the QuantStudio after the run has started as all data are always saved on the QuantStudio instrument.

If QuantStudio was not connected with the laptop when the run ends, you'll receive an alert message "Failed to transfer the file to Network drive" on the display of your QuantStudio and have to transfer the data file manually as follows:

- Click on "Transfer File" in the center of the circle
- A new window "Select Destination" opens
- Scroll down to Network and click on icon "Network"
- Click on "Destination" in the field next to "Network" (If you see a "/" then the connection between QuantStudio and the laptop is established, Status: Connected)
- In the new screen "Network" you see three folders (My-Plates/, PIKA-Plates/, QS1Data/), click on "QS1Data"
- The "/QS1Data/" folder opens now
- Click on"OK"
- This will bring you back again to the "Select Destination" window showing the path "/QS1Data/"
- Click on "Transfer" to complete the transfer of the data file to the laptop
- The transfer is completed once you see the message "Transfer Complete" on the screen
- Click on "Done"
- Now you can switch off the QuantStudio instrument
- Read out the results on the laptop