Gene expression runs on Rob™.

Gene expression using qPCR or PCR is commonly used in research on both 96 and 384 platforms. Rob™ is very suitable for improving results by eliminating pipetting mistakes and at the same time improving accuracy. The software allows for very easy set-up of various kinds of experiments, as will be shown in this application guide.

In addition will the OneDip™ function allow for savings on both reagents and pipette tips compared to manual pipetting and other robotic systems. The low cost of Rob™ compared to competition opens for most research labs to automate their pipetting. Cost of Rob™ is in parity to a good 96-well qPCR instrument as a comparison.

The key is the precision of Rob™ allowing for accurate and fast multi-/ serial-dispensing of reagent/ qPCR mix volumes from 3 µl and sample volumes from 2 µl. This allows for use of single tubes for the mix while multi-channel pipettes/ heads requires more wells and increase waste for each mix. Only one tip is used for each mix or sample creating substantial tip savings. In addition will through-put be better than most (all?) multi-channel robotic systems on the market. It is of course an option to use single dispense in precision critical steps but normally will OneDip™ provide sufficient precision.

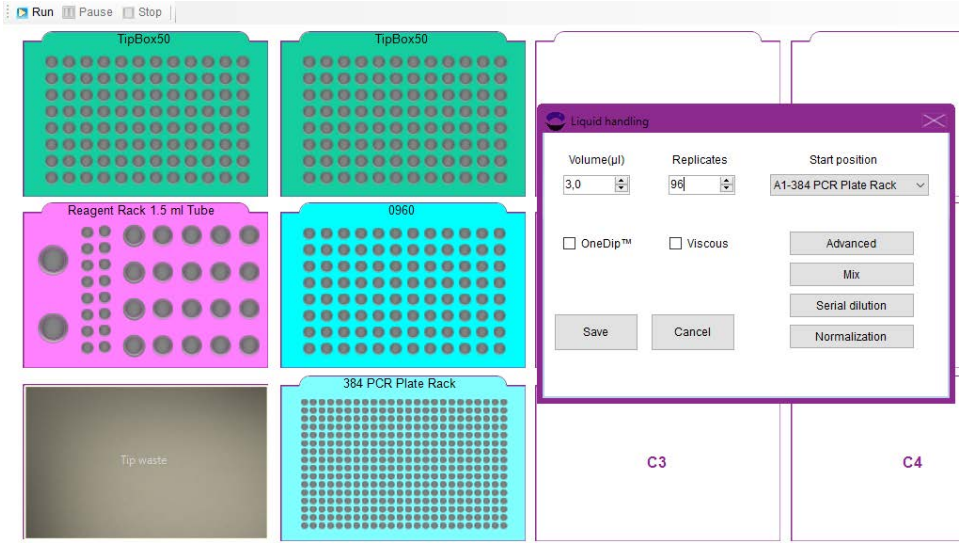
The examples on set-up below is followed by instruction on how to add sample name and target genes for data interpretation as well as easy preparation of qPCR mixes by Rob™.

Examples on common set-up methods.

In this document we present easy set-up solutions for various gene expression methods. Some divide a 384 plate in four 96 parts, column or row wise and express against four genes. Others like to see all genes expressed to the sample in wells next to each other for easier interpretation of data. NGS re-sequencing using arrays of forward and reverse primers is a growing method, also described here.

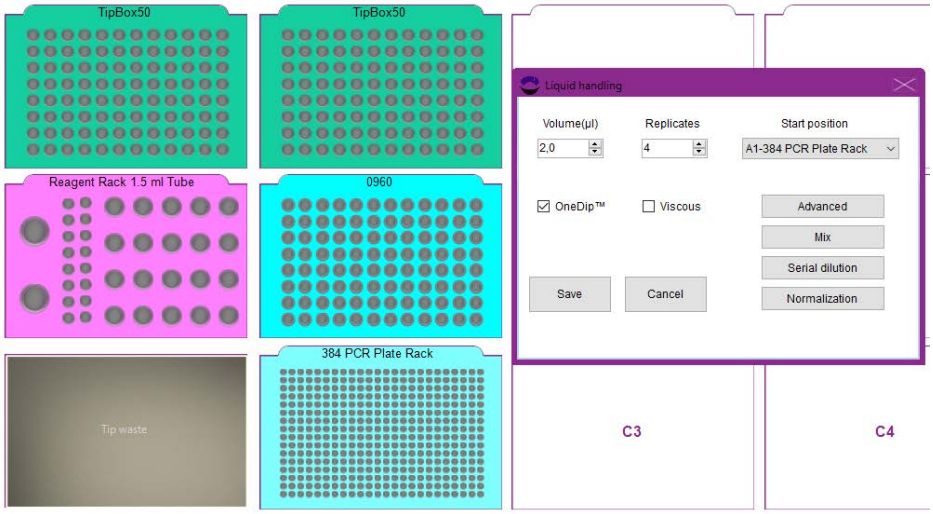
Column dispensing set-up, example below on 384 plate.

Make a layout like below. Use four tubes for the qPCR mix, one 96 plate for samples and two tip boxes. Samples can also be in three rack of 32 tubes or other formats.
1. Mark the four with the mix, drag them to the 384 plate to open the liquid handling window.
2. Set volume to ≥3 µl, replicates to 96 and select OneDip™ in the liquid handling window, see screen capture below.

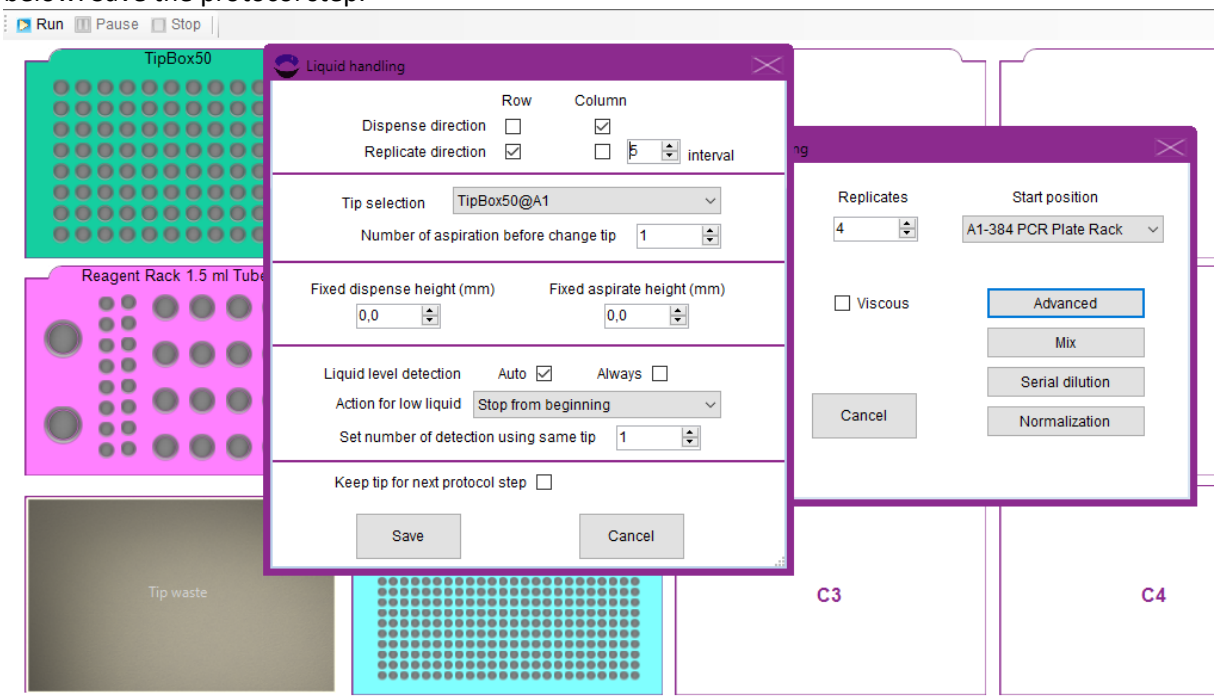


Mix one is now dispensed between well A1 and P6, mix two between A7 and P12 and so on.

3. Mark the sample plate and drag that to the 384 plate to open the liquid handling window.
4. Set volume to ≥ 2 µl, replicates to 4 and select OneDip™, see screen capture below.



5. Open the “Advanced” menu. Set Replicate direction to row and interval to 5, see screen capture below. Save the protocol step.



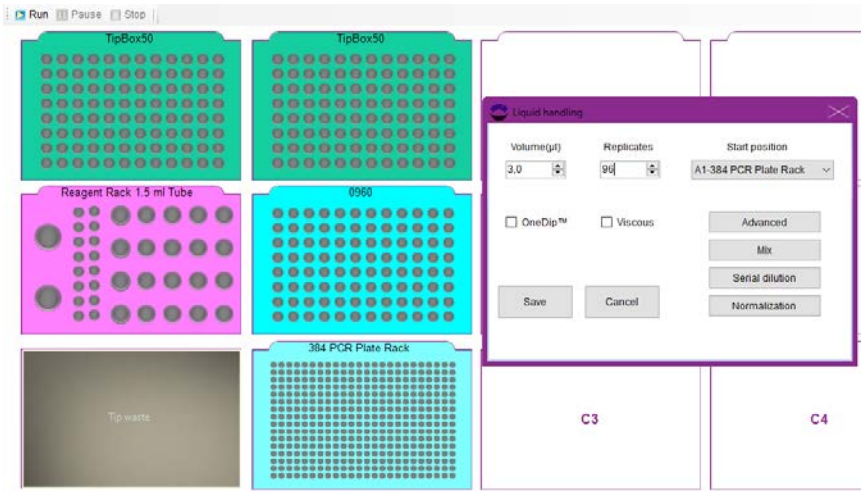
Sample in well A1 is now dispensed in wells A1, A7, A13 and A19. Sample in well B1 is now dispensed in wells B1, B7, B13 and B19.

If samples are in three 32 tube racks you have to mark and drag each sample rack individually. Start position for rack two (A3) and three (A5) must be selected manually.

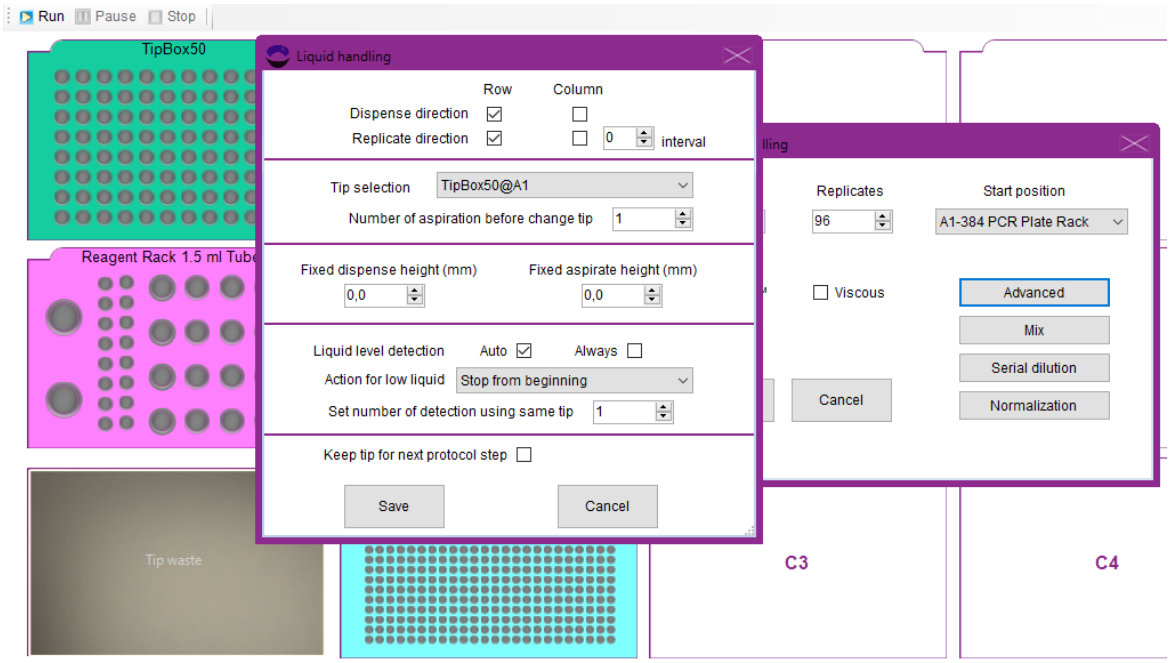
NTC can either be water in a well in the sample plate or in another tube/well. The later will require that only 95 samples are market in step 3 and that the start well P6 has to be selected manually followed by selecting row Replicate direction and interval 5.

Row set-up, example below on 384 plate.

Make a layout like below. Use four tubes for the qPCR mix, one 96 plate for samples and two tip boxes.
1. Mark the four tubes with the mix, drag them to the 384 plate to open the liquid handling window.
2. Set volume to ≥3 µl, replicates to 96 and select OneDip™ in the liquid handling window, see below.

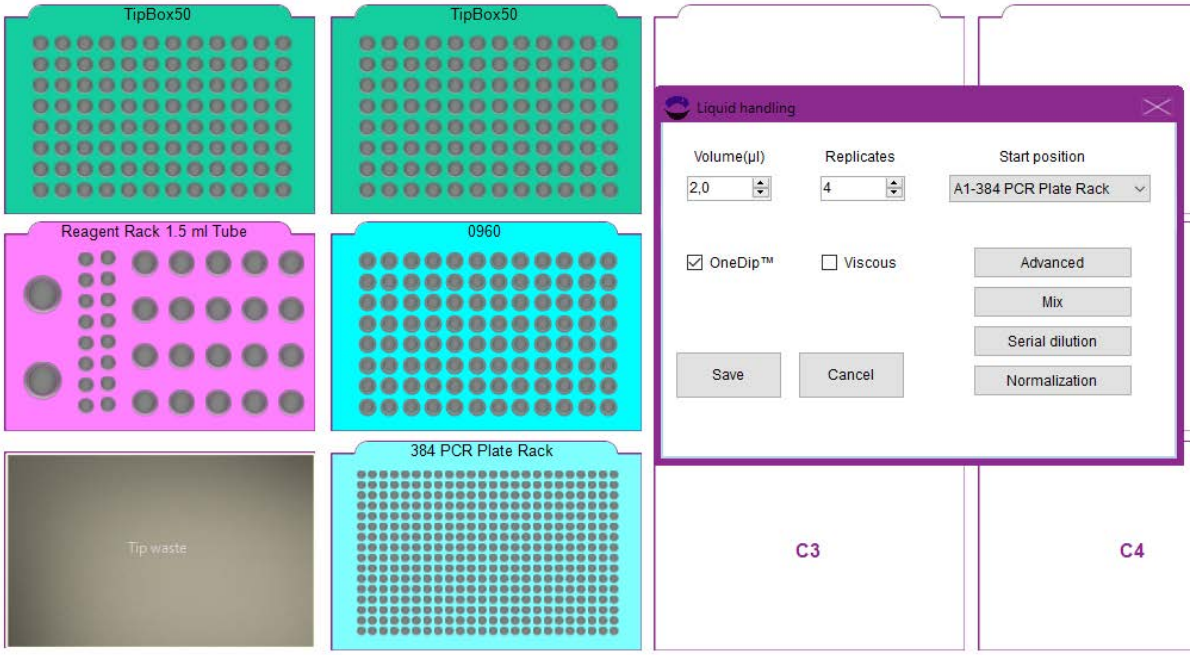


3. Open the Advance menu. Set Dispense and Replicate direction to row, see screen capture below. Save the protocol step.

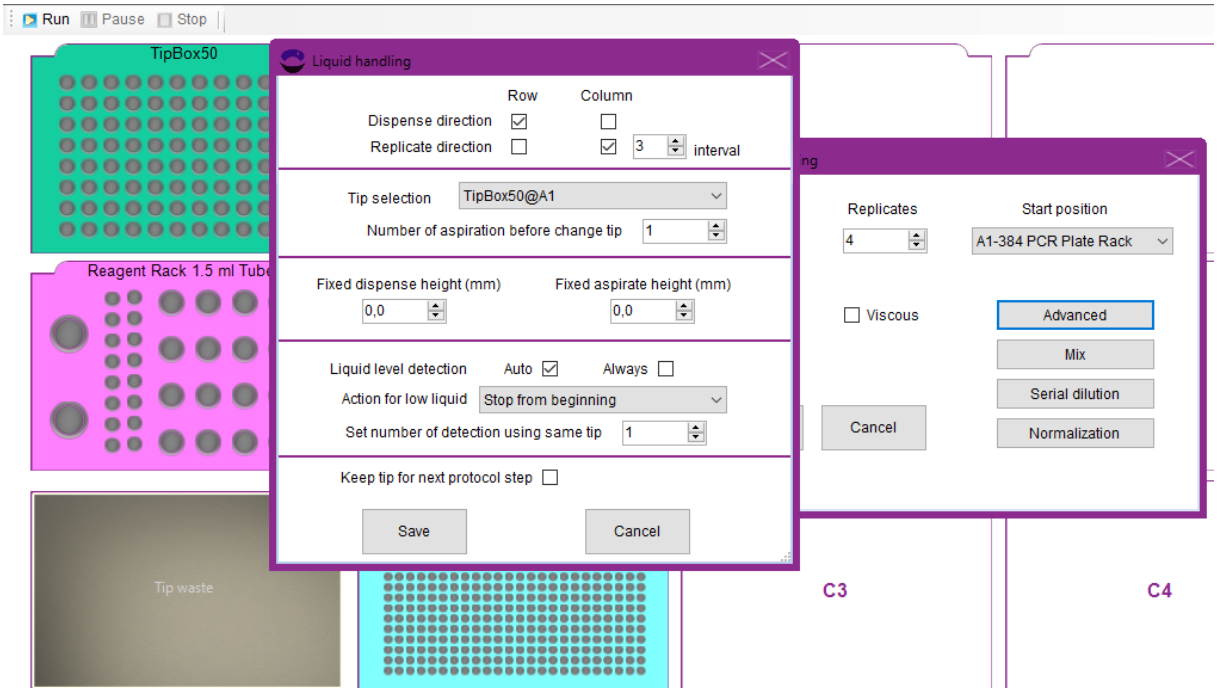


Mix one is now dispensed in wells A1 to D24, mix two in wells E1 to H24 and so on.

4. Mark sample plate and drag that to the 384 plate to open the liquid handling window.
5. Set volume to ≥ 2 µl, replicates to 4 and select OneDip™, see screen capture below.



7. Open Advanced menu. Select row for Dispense direction, keep column as Replicate direction and set interval to 3, see screen capture below.



Sample in well A1 is now dispensed in wells A1, E1, I1 and M1. Sample in well B1 is now dispensed in wells A2, E2, I2 and M2.

NTC, no template control, can either be water in a well in the sample plate or in another tube/well. The later will require that only 95 samples are market in step 3 and that the start well (D24) has to be selected manually.

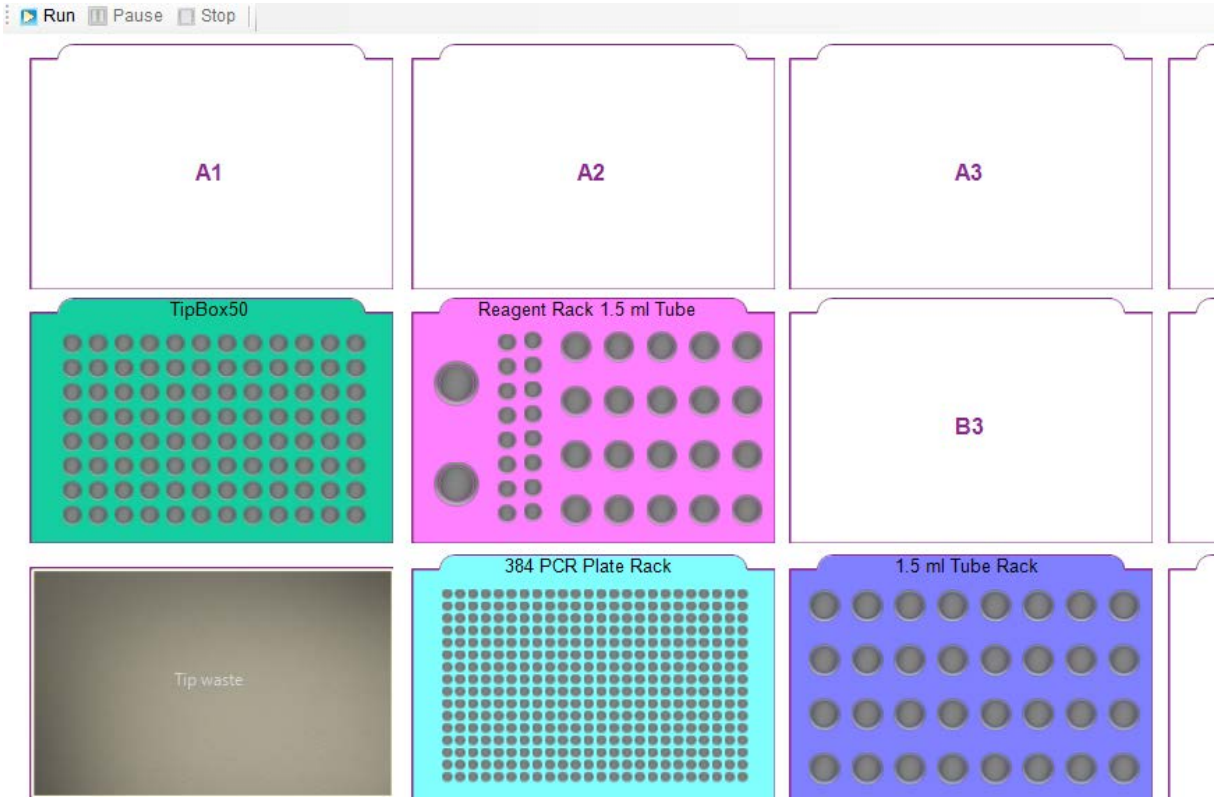
Expressed genes next to each other for same sample.

This method will have the genes that the sample is expressed for next to each other. The method is also suitable for making arrays of forward and reverse primers and express them against a sample.

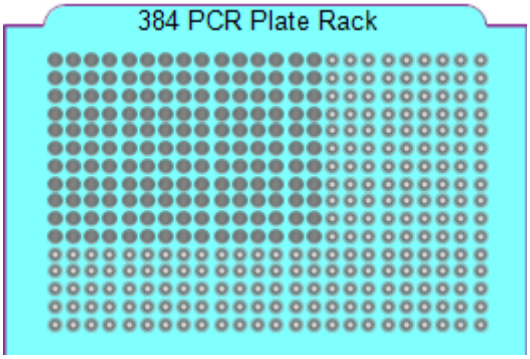
In this example we run 7 samples and a NTC in row direction to match the qPCR instrument set-up. Samples and NTC are dispensed in duplicates. Samples are expressed against 13 genes.

To allow for easy protocol set-up we need to calculate how many wells that is required in row and column direction.
Each mix will be dispensed in (No of samples x No of replicates) + No of NTC's wells = (7 x 2) + 2 = 16 columns will be required.
Each gene will be dispense 11 rows so 11 rows is required.
In this example are 11 tubes for gene mixes and 7 + 1 tubes for samples and NTC required.

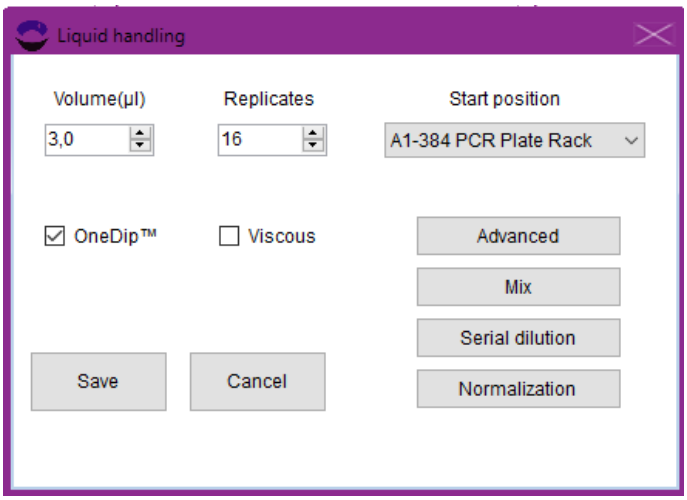
1. Make a layout like the screen capture below.



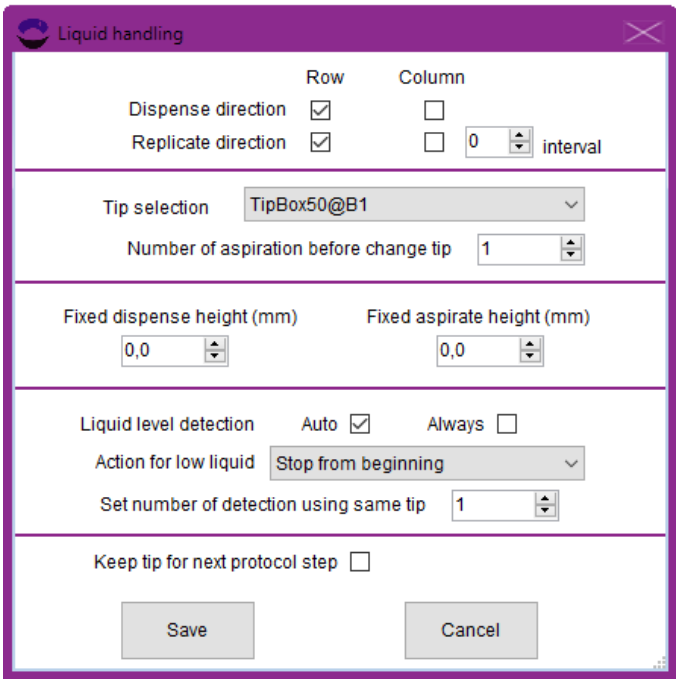
2. Create a partial 384 plate by marking wells A1 to K16 and then use a right click and select Dispense pattern. Screen capture below shows how the plate looks like.



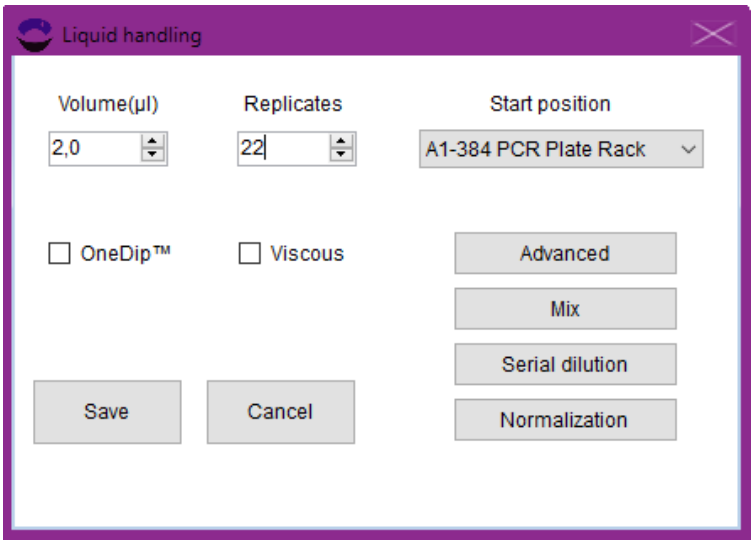
2. Mark the 13 tubes containing the gene mixes and drag them to the 384 plate to open the Liquid handling window. Set volume to ≥3 µl, replicates to 16 ((No samples x No of replicates) + No of NTC's) and select OneDip, see screen capture below.



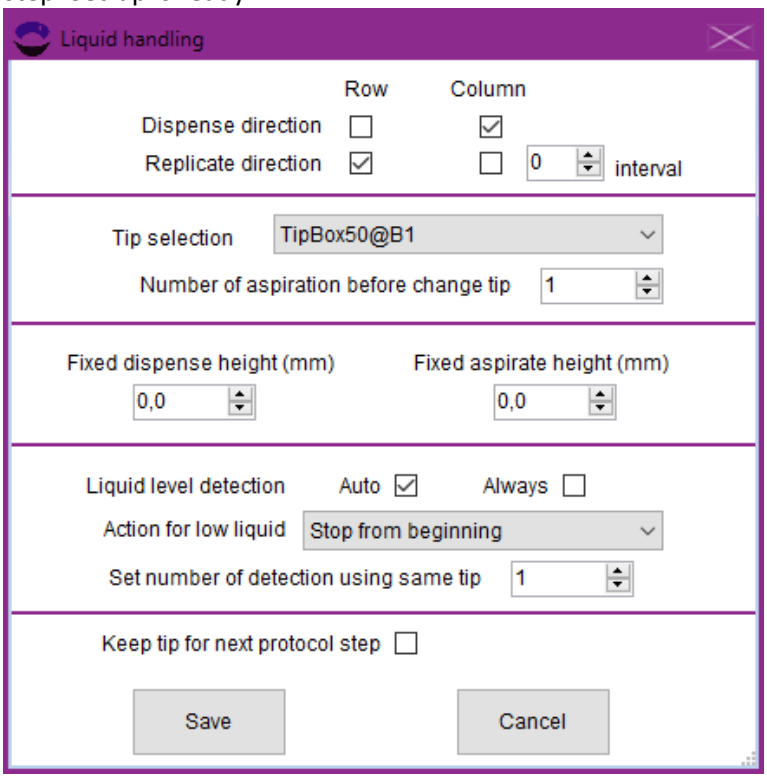
3. Open Advanced menu. Set both Dispense and Replicate direction to row, see screen capture below. Save the protocol step.



4. Mark sample plus NTC water tubes and drag them to the 384 plate to open the liquid handling window. Set volume to ≥2 µl, replicates to 2 and select OneDip™, see screen capture below.



3. Open Advanced menu. Set Replicate direction to row, see screen capture below. Save the protocol step. Set-up is ready.



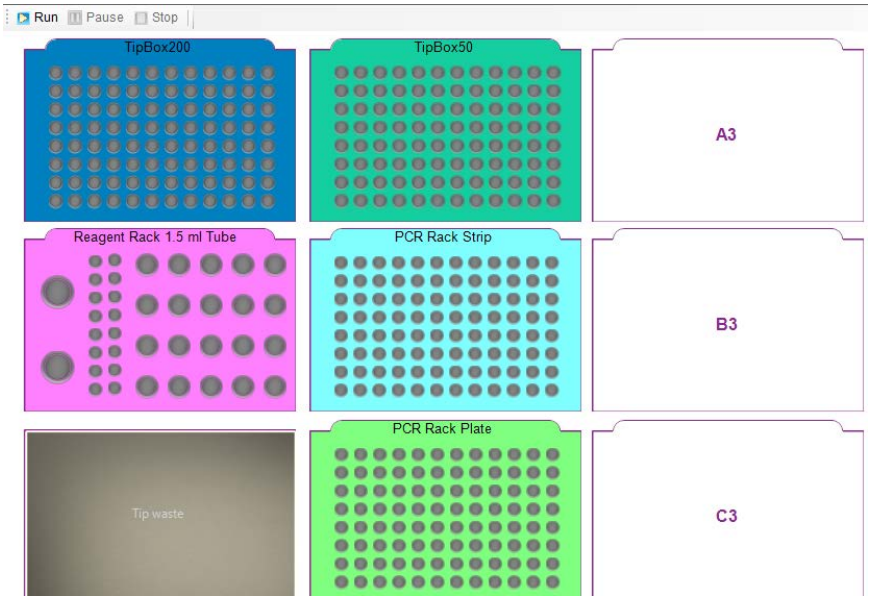
Additional information.

Number of tips used: 8 + 11 = 19
Estimated run time: < 20 min.
Precision: Ct ≤ ± 0.5

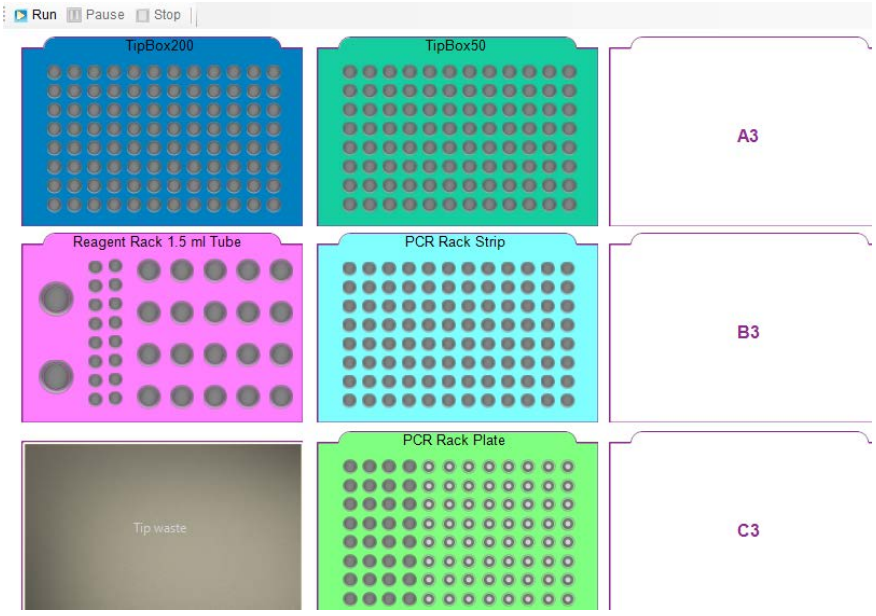
Making primer arrays for NGS re-sequencing or gene expression on a 96 plate.

In this example we are running 8 forward primers and pool them against 4 reverse primers so all combinations are created so forward primer one will be mixed with reverse primer 1, 2, 3 and 4. NTC is not used because of practical reason. Reagent volume is 16, primer volume is 2 and sample volume is 5 µl in this example. Primers in this example is place in 200 µl PCR tube strips in order to minimize spill. The PCR/qPCR mix is placed in a 1.5 ml tube on the reagent rack. The sample can also be dispensed by Rob™ but as that in some cases is a PCR product it may not be allowed in due to contaminations risks. Sample can be place in a 1.5 ml tube on the reagent rack.

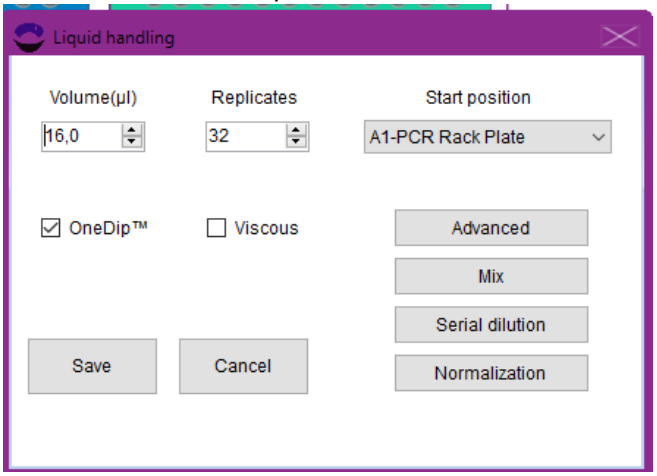
1. Make a layout like below screenshot.



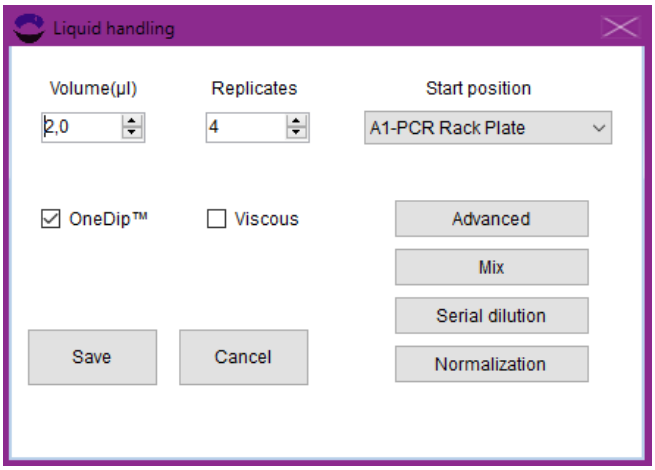
2. Make a plate that will fit 8 X 4 primer pairs. See screen capture below.



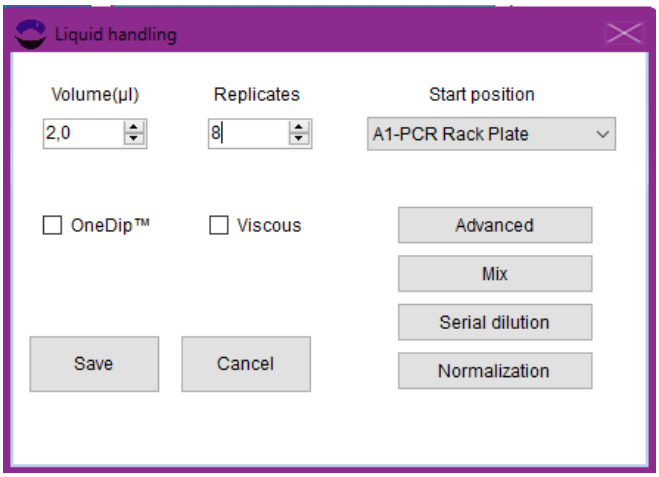
3. Mark the reagent tube and drag that to the PCR plate. To open the liquid handling window. Set volume to 16 ul and replicates to 32, see screenshot below.



4. Mark the fw primers and drag them to the PCR plate to open the liquid handling window. Set volume to 2, replicates to 4 and select OneDip™ in order to save time and tips, see screenshot below. Always start to dispense the primer type (forward/ reverse) that have more variants, in this case fw primer. This will save tips and time.



5. Mark the fw primers and drag them to the PCR plate to open the liquid handling window. Set volume to 2 and replicates to 8, see screenshot below. Do <u>not</u> use OneDip™ because of possible contamination issues.



Using sample names and target genes.

A double click on the plate/rack containing target gene mixes or samples allows for entering corresponding names. It is also possible to enter both sample names and target gene in sample column and export that to the qPCR instrument. Next version of software will make it possible to have two columns, one for sample name and one for target gene/test type. We will also add more dedicated export files that fits directly to the qPCR instruments.