

Sample and assay loading protocol for the QuantStudio™ 12K Flex Real-Time PCR System

Using the QuantStudio™ 12K Flex Real-Time PCR System with the OpenArray® Block and digital PCR plates, you have great flexibility for running assays of your choice with a variety of sample types. QuantStudio™ OpenArray® Digital PCR Plates contain 48 empty subarrays where a unique combination of sample and assay can be combined and filled using the AccuFill™ System. With designated template files for QuantStudio™ 12K Flex Software, you can run three samples per subarray. With this technique, you can achieve 16 replicates per sample/assay combination for a genotyping or gene expression experiment, providing the precision you need.



Loading and running digital PCR plates

- Using a 384-well OpenArray® digital PCR plate, mix:
 - 1.5 µL of sample (50 ng/µL)
 - 1.5 µL of the appropriate master mix for the application you are running (i.e., use OpenArray® Genotyping Master Mix for genotyping, TaqMan® OpenArray® Master Mix for gene expression, or Digital PCR Master Mix for digital PCR applications).
- Pipet samples in an OpenArray® digital PCR plate. (Figure 1 shows how to pipet three samples per subarray in a 384-well OpenArray® plate)
- Use the AccuFill™ System and software to load sample and assays onto an OpenArray® digital PCR plate.
- Seal the OpenArray® plate according to the directions in the User Manual (Cat. No. 4470935).
- Run the loaded OpenArray® plate using the ThreeSample.edt template file downloaded from lifetechnologies.com.

Downloading and using an EDT or EDS file

- Download the three sample load template (ThreeSample.edt) from lifetechnologies.com/openarrayplatefiles, according to your application of choice (gene expression or genotyping).
- Save the template file to your desktop.
- Open QuantStudio™ 12K Flex Software.
- Load the ThreeSample.edt file.
- In the samples section of the .edt file, rename samples and assays according to your experiment (or use the OpenArray® Sample Tracker Software to easily import your sample names from a .csv file as described in the section “Loading three samples using OpenArray® Sample Tracker Software”).
- Save the .edt file in .eds format according to the barcode of the OpenArray® digital PCR plate:
File → Save As → EDS file type
- From the **Home Screen** in QuantStudio™ Software, select **Run OpenArray**.
- After the instrument has located the barcode, browse for the .eds file you just created.

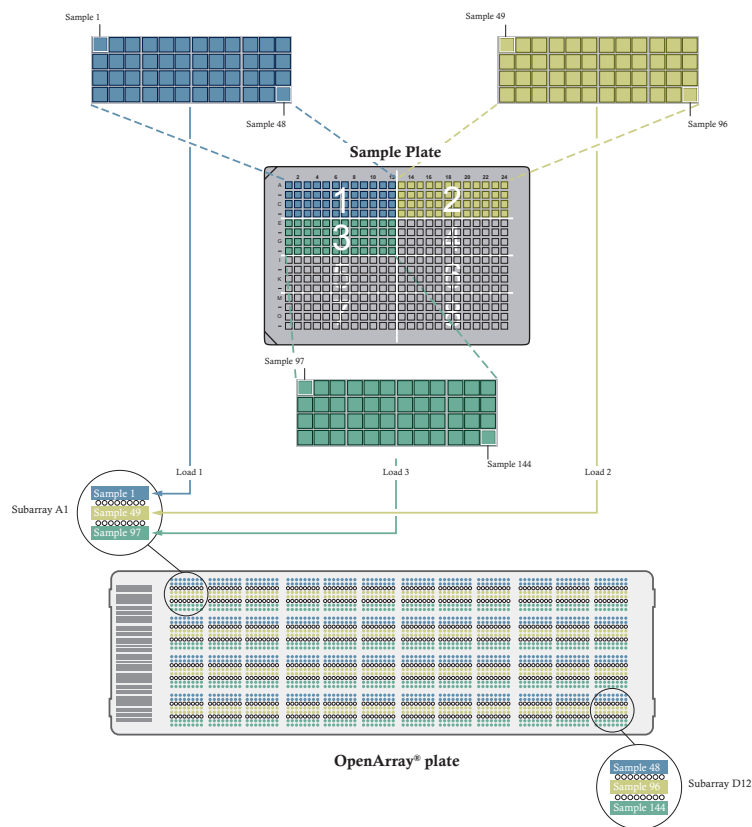


Figure 1. How to physically load samples and assays onto a 384-well OpenArray® digital PCR plate. To load three samples per subarray, pipet 1.5 µL of each sample into the array in sections 1, 2, and 3 of a 384-well OpenArray® plate.

Loading three samples using OpenArray® Sample Tracker Software

Using OpenArray® Sample Tracker Software, load three samples per subarray:

1. Select Genotyping 16 format.
2. Upload your 144 sample names (yellow) corresponding to the three 96-well plates (Figure 2).

Ordering information

Product	Cat. No.
QuantStudio™ Digital PCR Kit, 4 Pack (includes 1.5 mL Digital PCR Master Mix)	4470185
TaqMan® OpenArray® Real-Time Master Mix (1.5 mL)	4462159
TaqMan® OpenArray® Genotyping Master Mix (5 mL)	4404846

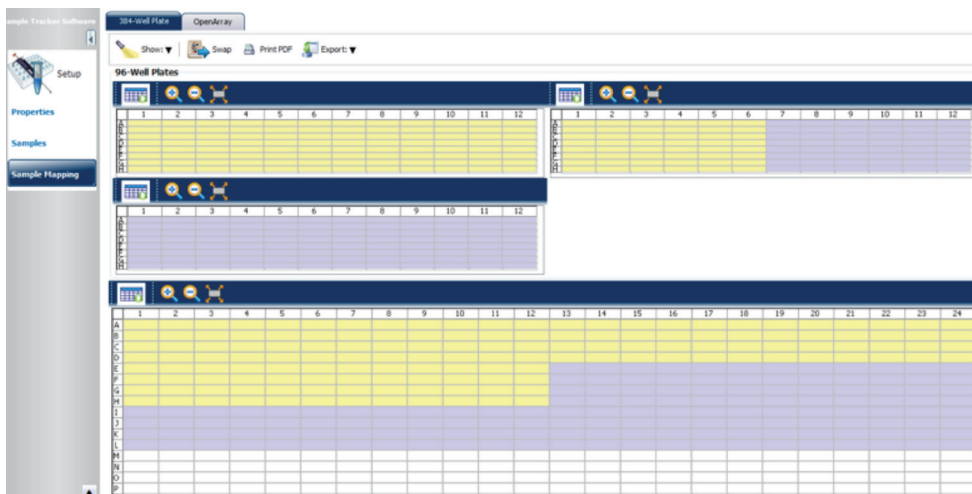


Figure 2. Use of OpenArray® Sample Tracker Software to import 144 sample names (in the yellow quadrants) from the .csv file to create three samples per subarray on a single OpenArray® digital PCR plate.