Streamline high-throughput gene expression studies with SuperScript® III Platinum® One-Step Quantitative RT-PCR and OpenArray® technology on the QuantStudio™ 12K Flex system

One-step reverse transcription (RT) quantitative polymerase chain reaction (qPCR) is a widely accepted technique that combines RT and real-time qPCR in the same reaction well. The advantage of this approach is a reduction in sample manipulations required, which minimizes human error and contamination. This approach requires only half the number of reactions compared to a conventional two-step qRT-PCR reaction, resulting in an easier and faster workflow. Here, we validated the SuperScript® III Platinum® One-Step Quantitative RT-PCR Kit performance using high-throughput OpenArray® technology on the QuantStudio™ 12K Flex Real-Time PCR System. High-throughput gene expression profiling of RNA from various tissues using SuperScript® one-step reagents on an OpenArray® plate exhibits a wide dynamic range and generates highly reproducible results. The SuperScript® III Platinum® One-Step Quantitative RT-PCR System is as efficient as two-step qRT-PCR and equally compatible on the QuantStudio™ 12K Flex Real-Time PCR System with OpenArray® technology.

Optimal specificity, consistency, and efficiency

The SuperScript® III Platinum® One-Step Quantitative RT-PCR System is a one-step reaction strategy that combines the high-temperature reverse transcription (RT) capability of SuperScript® III Reverse Transcriptase with the hot-start PCR capability of Platinum® *Taq* DNA Polymerase for optimal specificity, consistency, and efficiency [1]. The two major components of the system are a SuperScript® III Reverse Transcriptase/Platinum® *Taq* DNA Polymerase mix and a 2X reaction mix. SuperScript® III Reverse Transcriptase is an engineered form of M-MLV reverse transcriptase with reduced RNase H activity that provides increased thermal stability [2, 3]. The ability of the enzyme to synthesize cDNA at 45–60°C provides a high yield of cDNA with increased sensitivity and more full-length product than provided by other reverse transcriptases [1]. The SuperScript® III Platinum® One-Step Quantitative RT-PCR System has been specifically formulated to be compatible with fluorogenic hybridization probe-based detection methods such as TaqMan® probes and molecular beacons [1].

OpenArray® technology

The QuantStudio™ 12K Flex Real-Time PCR System with OpenArray® technology is a high-throughput nanoliter-fluidic qPCR system that utilizes a stainless-steel microscopic-sized plate. Each plate has 3,072 through-holes and is laid out in 48 subarrays, with each subarray containing 64 through-holes in an 8 x 8 array grid. Each through-hole holds 33 nL of reaction mix through surface tension as a result of hydrophilic coating of the through-holes and hydrophobic coating on the outside (Figure 1).

life technologies™

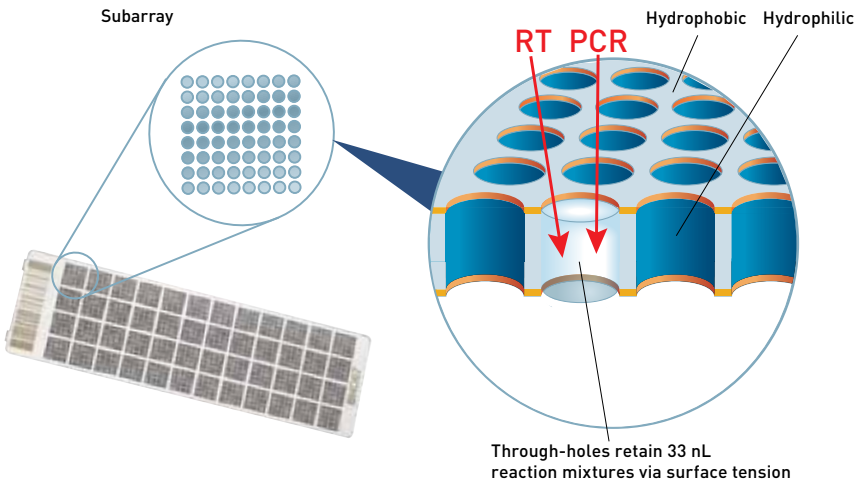


Figure 1. SuperScript® III Platinum® one-step quantitative RT-PCR reaction in a single OpenArray® through-hole. This system combines the RT and the PCR amplification step in a single through-hole of an OpenArray® plate containing a total reaction volume of 33 nL.

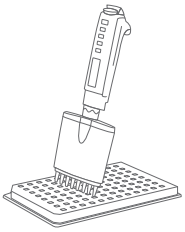
QuantStudio™ 12K Flex OpenArray® plates are delivered preloaded with TaqMan® Gene Expression Assays of your choice in each through-hole. The assays are available in different assay layout formats. The OpenArray® plates are also available without any assays spotted in through-holes, thus providing the flexibility to run any assay/sample combination with a large number of replicates or to validate assay performance and compatibility with the OpenArray® platform. The QuantStudio™ 12K Flex Real-Time PCR System with OpenArray® block provides a simple workflow and the highest sample throughput for mid-density qPCR analysis, as over 43,000 gene expression reactions and more than 110,000 genotyping reactions can be run in a single day (Figure 2).

Methods and protocols

The use of SuperScript® III Platinum® one-step qRT-PCR reagents on an OpenArray® plate simplifies qRT-PCR, as the RT and PCR occur in a single through-hole of an OpenArray® plate (Figure 1). The reaction volume of one-step qRT-PCR for one sample or subarray is 5 µL, in which 1.2 µL of RNA sample is mixed with 3.8 µL of SuperScript® III Platinum® one-step qRT-PCR reaction mix (Table 1A). Addition of 0.25 µL of 20X TaqMan® Assay to 3.55 µL of SuperScript® III Platinum® one-step reaction mix is required when using the OpenArray® plate without an assay preloaded in any of the through-holes (Table 1B).

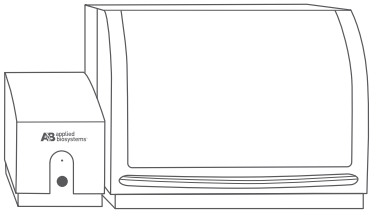
The reaction mix containing SuperScript® III Platinum® one-step reagents and samples is loaded first on 384-well sample plates (Figure 2A). The samples on the 384-well plate are then loaded onto the OpenArray® plate with a robotic QuantStudio™ 12K Flex AccuFill™ System (Figure 2B). Finally, the OpenArray® plate is processed with a one-step RT-qPCR protocol containing all information about the RT and cycling conditions on the OpenArray® block of the QuantStudio™ 12K Flex Real-Time PCR System (Figure 2C and 2D). Data are collected using the QuantStudio™ 12K Flex system analysis software and analyzed by ExpressionSuite™ Software, provided with the system.

A



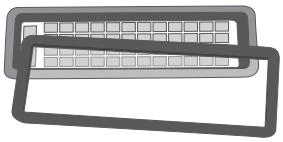
For gene expression and genotyping applications, mix cDNA or DNA sample with master mix in 384-well sample plates. For digital PCR applications, mix assays, samples, and master mix in 384-well sample plates.

B



Load sample mixes onto an QuantStudio™ 12K Flex OpenArray® plate with the QuantStudio™ 12K Flex AccuFill™ System.

C



The QuantStudio™ 12K Flex OpenArray® plate, encased in an alloy bottom for easy handling; simply apply the adhesive lid and fill the case with immersion fluid.

D

Easily run up to 4 QuantStudio™ 12K Flex OpenArray® plates for any application on the QuantStudio™ 12K Flex instrument in stand-alone mode.

Figure 2. The OpenArray® System workflow. This workflow involves **(A)** mixing sample with PCR master mix in a 384-well plate, **(B)** loading the samples with the AccuFill™ System onto a TaqMan® OpenArray® plate, **(C)** encasing the plate with immersion oil, then **(D)** running up to 4 plates on the QuantStudio™ 12K Flex instrument.

Table 1A. The SuperScript® III Platinum® one-step qRT-PCR reaction mix.

Component	Final concentration	Volume for one subarray (µL)	Volume for one OpenArray® plate (µL)
2X SuperScript® buffer	1X	2.5	130
25X SuperScript® enzyme	1	0.2	10.4
Ectoine* (0.71 g/mL)		0.5	26
Water		0.6	31.2
Reaction mix volume		3.8	197.6
RNA**		1.2	62.4
Total volume		5.0	260

Table 1B. The SuperScript® III Platinum® one-step qRT-PCR reaction mix with TaqMan® Assay.

Component	Final concentration	Volume for one subarray (µL)	Volume for one OpenArray® plate (µL)
2X SuperScript® buffer	1X	2.5	130
25X SuperScript® enzyme	1	0.2	10.4
20X TaqMan® assay	1X	0.25	13
Ectoine* (0.71 g/mL)		0.5	26
Water		0.35	18.2
Reaction mix volume		3.8	197.6
RNA**		1.2	62.4
Total volume		5.0	260

*Weigh out 0.71 g ectoine (CAS Number 96702-03-3, Sigma-Aldrich (81619)), then add 1 mL of purified water and mix until the solution becomes clear.
**The optimal RNA concentration should be determined for each sample.

Comparison of performance of Superscript® III Platinum® One-Step RT-PCR Kit vs. High-Capacity cDNA kit on OpenArray® platform

The SuperScript® III Platinum® One-Step qRT-PCR Kit and the traditional two-step High Capacity cDNA Reverse Transcription Kit were compared on the QuantStudio™ 12K Flex Real-Time PCR System with OpenArray® technology. Four different total RNAs, kidney, liver, lung, and spleen, at concentrations of 150 ng/µL, were directly tested on a TaqMan® OpenArray® HS Endogenous Control Panel for the QuantStudio™ 12K Flex system, which contains 56 housekeeping genes, using the SuperScript® III Platinum® one-step protocol. For the two-step approach, RNA samples at the same concentration were reverse-transcribed using a High Capacity cDNA Reverse Transcription Kit and the cDNA samples were tested on the same TaqMan® panel using TaqMan® Real-Time OpenArray® Master Mix. Each RNA and cDNA sample was loaded in 12 subarrays, generating 12 replicates for each sample/assay combination. As shown in Figure 3A, the performance of the SuperScript® III Platinum® one-step kit is comparable to the conventional two-step qRT-PCR kit.

Another experiment confirming the performance of the SuperScript® III Platinum® one-step kit was conducted on the QuantStudio 12K Flex system using a TaqMan® OpenArray® Human Cancer Panel containing a wide range of genes with different expression levels (N = 649). Human

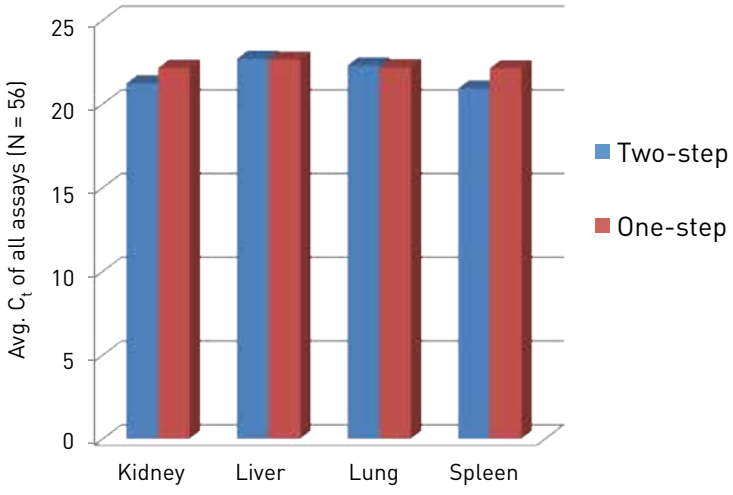


Figure 3A. Four different human tissue RNA samples (150 ng/µL) were processed in a single TaqMan® OpenArray® HS Endogenous Control Panel plate (N = 12). There are 2,688 total data points per OpenArray® plate. Each bar represents the average of 672 data points per sample (56 assays x 12 replicates). The results generated using reverse-transcribed cDNA (two-step) and RNA (one-step) samples were comparable and exhibited similar average C_t values for the same RNA input.

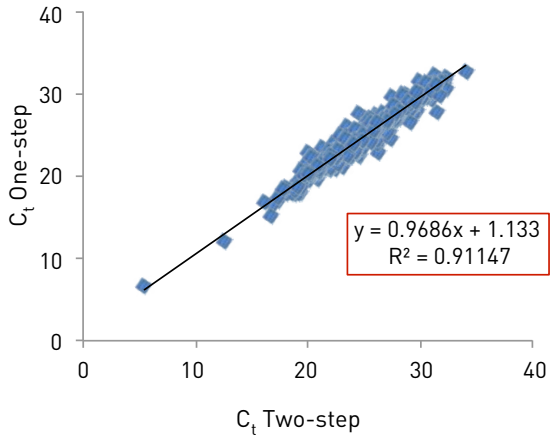


Figure 3B. Gene expression profiling of 649 target genes or samples was performed on a TaqMan® OpenArray® Human Cancer Panel for the QuantStudio™ 12K Flex system with the SuperScript® III Platinum® One-Step Quantitative RT-PCR Kit, the High Capacity cDNA Reverse Transcription Kit, and TaqMan® Real-Time OpenArray® Master Mix (two-step) using liver total RNA (~100 ng/µL). Samples were run in duplicate (N = 2). Comparison of average C_t values for 649 targets between SuperScript® one-step and the High Capacity cDNA Reverse Transcription Kit (two-step) generates $R^2 = 0.91$.

liver total RNA (100 ng/µL) was tested in duplicate using analogous one-step and two-step protocols on a QuantStudio™ OpenArray® block. Figure 3B shows a scatter plot comparing the SuperScript® III Platinum® one-step kit with the two-step approach using the High Capacity cDNA Reverse Transcription Kit on the TaqMan® OpenArray® Human Cancer Panel. The correlation coefficient, R^2, was 0.91 when average C_t values of 649 targets were compared between the SuperScript® III Platinum® one-step reaction and the two-step reaction. Thus, the performance of the SuperScript® III Platinum® One-Step Quantitative RT-PCR Kit proves to be as efficient and sensitive as the traditional two-step approach, with the advantage of reduced reaction time and decreased potential of errors due to handling.

Assessment of dynamic range and reproducibility

A standard curve analysis was conducted using the SuperScript® III Platinum® one-step qRT-PCR protocol on a TaqMan® OpenArray® HS Endogenous Control Panel for the QuantStudio™ 12K system (56 assays). Human liver total RNA was diluted 4-fold in the range from 500 ng/µL to 0.5 ng/µL. Each RNA dilution was loaded in 6 subarrays, thereby generating 6 replicates for each assay/sample combination. Samples were run on a QuantStudio™ OpenArray® block using a gene expression cycling protocol. The linearity of the results generated by standard curve analysis using the SuperScript® III Platinum® one-step kit for four assays, RPLPO, B2M, PPIA, and MT-ATP6, is shown in Figure 4. For most of the assays, the slopes of the reactions varied from 3.1 to 3.45, the efficiency of the reactions ranged from 94 to 110%, and the assays had an $R^2 = 0.99$. The SuperScript® III Platinum® one-step qRT-PCR reaction mix and the QuantStudio™ 12K Flex system produce a wide dynamic range of detection as low as 3.96 pg of total RNA input per reaction, which

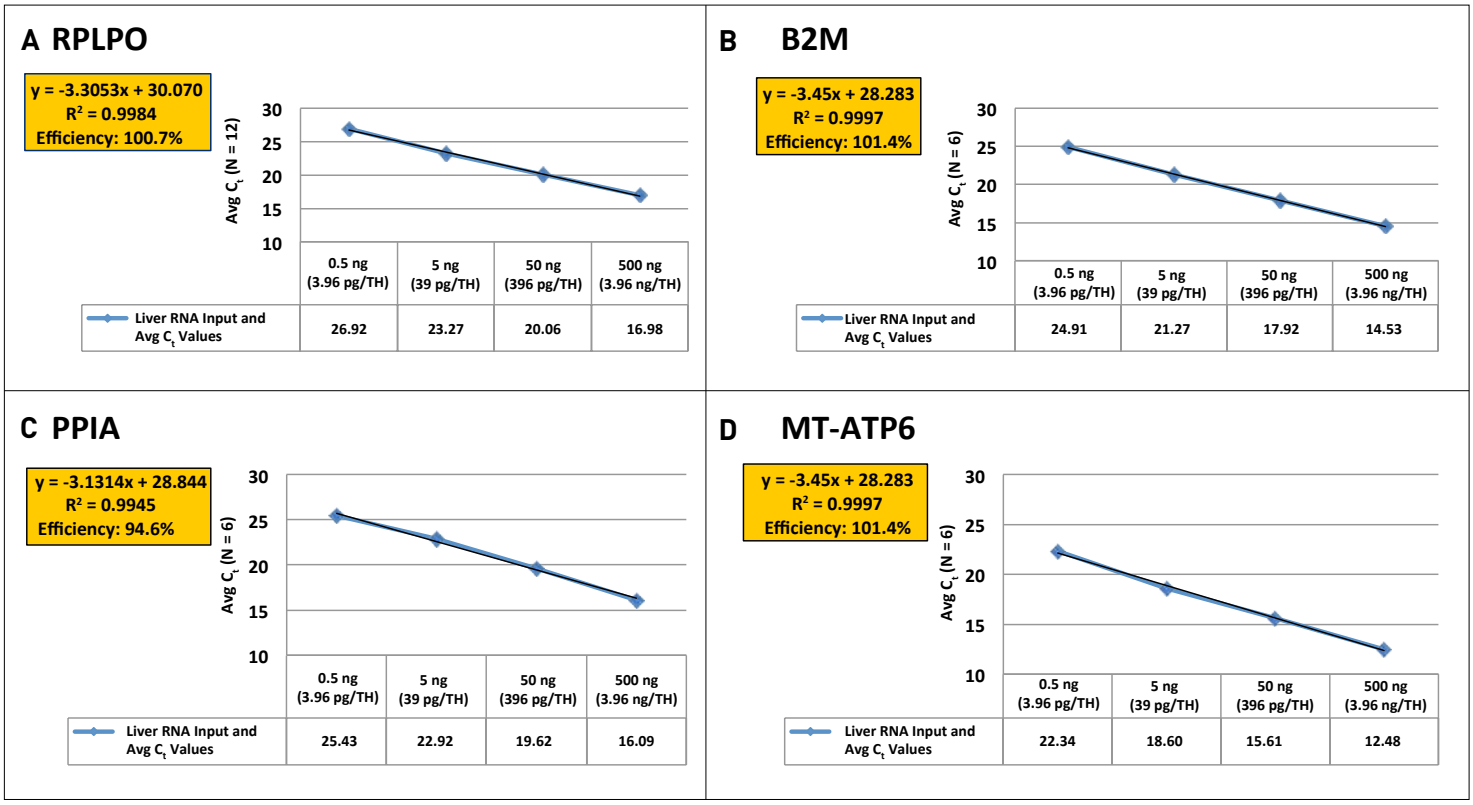


Figure 4. Standard curve analysis. A set of 4-fold serial dilutions of human total RNA ranging from 500 ng/µL to 0.5 ng/µL were used to profile 56 housekeeping genes on a TaqMan® OpenArray® HS Endogenous Control Panel. A 1.2 µL aliquot of each diluted template is added to 3.8 µL of SuperScript® III Platinum® One-Step qRT-PCR mix for each subarray/sample combination. The efficiency, slope, and R^2 for 4 different assays, RPLPO **(A)**, B2M **(B)**, PPIA **(C)**, and MT-ATP6 **(D)**, are shown, with R^2 >0.99 for all of the assays.

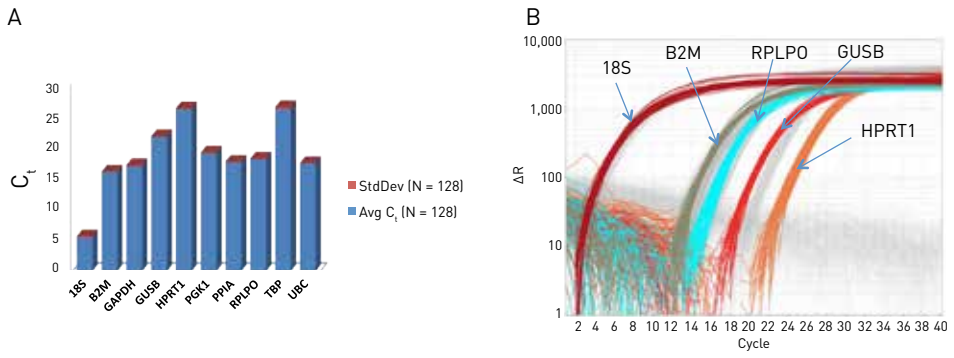


Figure 5. SuperScript® III Platinum® one-step qRT-PCR reaction mix generates reproducible results using OpenArray® plates. A set of 10 different TaqMan® housekeeping assays was analyzed on an OpenArray® plate using 100 ng/µL of liver total RNA. **(A)** Each bar represents the average C_t (N = 128) of sample/assay combinations with respective standard deviation on top. **(B)** An example of QuantStudio™ 12K Flex system amplification plots of 5 TaqMan® housekeeping assays shows the performance of 128 replicates/assays.

supports accurate quantification of high- and low-copy number mRNA [1].

SuperScript® III Platinum® one-step qRT-PCR reaction mix can also be used on OpenArray® plates containing no preloaded assays. A set of 10 TaqMan® housekeeping assays was tested with human liver total RNA (100 ng/µL) using the SuperScript® III Platinum® one-step protocol with the assay added to the master mix (Table 1B) on blank OpenArray® PCR plates. Each sample/assay combination was added to two subarrays, hence generating 128 replicates per tested sample. Figure 5A shows a bar chart for the 10 genes, with their average C_t values and a standard deviation ranging from 0.08 to 0.26 for the 128 data points for each assay. Figure 5B shows an example of amplification curves for five different assays (N = 128) with different levels of gene expression, ranging from C_t values of ~6 (18S) to ~26 (HPRT1).

Conclusions

Use of the SuperScript® III Platinum® One-Step Quantitative RT-PCR Kit on OpenArray® plates simplifies qRT-PCR and accelerates high-throughput gene expression with the additional benefit of using a low amount of sample. The combination of SuperScript® III one-step RT-qPCR and OpenArray® technology delivers precise and accurate analysis of gene expression in a convenient one-step format with reduced hands-on time. The ability to efficiently and sensitively amplify a broad range of genes with a wide dynamic range, using reagents that are compatible with OpenArray® technology, makes the SuperScript® III Platinum® One-Step Quantitative RT-PCR System a well-accepted application for high-throughput one-step qRT-PCR on the QuantStudio™ 12K Flex Real-Time PCR System.

References

1. SuperScript III Platinum® One-Step Quantitative RT-PCR System, Manual and Protocol (lifetechnologies.com)
2. Kotewicz ML, D'Alessio JM, Driftmier KM, et al. (1985) Cloning and overexpression of Moloney murine leukemia virus reverse transcriptase in *Escherichia coli*. *Gene* 35(3):249–258.
3. Gerard GF, D'Alessio JM, Kotewicz ML, et al. (1986) Influence on stability in *Escherichia coli* of the carboxy-terminal structure of cloned Moloney murine leukemia virus reverse transcriptase. *DNA* 5(4):271–279.

Ordering information

Product name	Cat. No.
SuperScript III® Platinum® One-Step Quantitative RT-PCR System	11732-020
High Capacity cDNA Reverse Transcription Kit	4368814
TaqMan® OpenArray® Real-Time PCR Master Mix	4462164
Human Liver Total RNA	AM7960
FirstChoice® Human Total RNA Survey Panel	AM6000
TaqMan® OpenArray® HS Endogenous Control Panel, QuantStudio™ 12K Flex	4471226
TaqMan® OpenArray® Human Cancer Panel, QuantStudio™ 12K Flex	4475391
QuantStudio™ Digital PCR Kit	4470184
QuantStudio™ 12K Flex Real-Time PCR System with OpenArray® Block (with AccuFill™ System)	4471090
QuantStudio™ 12K Flex OpenArray® AccuFill™ System	4471021
QuantStudio™ 12K Flex OpenArray® Accessories Kit	4469576

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