Efficient gene expression profiling for biomarker identification using multiplex quantitative PCR

In this application note, we show:

- How efficient gene expression analysis can be achieved using samples with limited quantities, such as needle biopsies and FFPE tissue sections
- Similar performance of singleplex and multiplex gene expression assays
- A complete workflow for multiplex gene expression analysis, from sample preparation through data analysis
- Integrated solutions from Thermo Fisher Scientific that can facilitate molecular analysis of tumor tissues
- Options for automating your qPCR and digital PCR (dPCR) gene expression analysis workflows

Introduction

Quantitative PCR (qPCR) has long been recognized as a powerful tool for analyzing nucleic acid levels. When coupled with reverse transcription of RNA to cDNA prior to qPCR (RT-qPCR), the relative quantities of mRNA transcripts in samples can be sensitively and specifically determined [1,2]. With RT-PCR, analysis of a few gene targets, each in a separate reaction (singleplex qPCR), is relatively simple and cost-effective. However, as our understanding of biological pathways and how they interact advances, it is becoming more desirable to analyze more than one or two targets simultaneously. Working against this need, sophisticated sample collection techniques like fine-needle biopsy provide limited sample quantities for downstream genetic analysis. As translational research laboratories must analyze a large number of samples to detect statistically and biologically significant trends and rare events, there is a need for DNA and RNA analysis solutions that can yield large amounts of scientific data from limited amounts of sample in a simple workflow.

One way to maximize the amount of scientific information obtained from a sample is to analyze multiple gene targets in one multiplex qPCR reaction. In a multiplex qPCR reaction, different fluorescent dyes known as reporters are used to label different target amplicons. A single qPCR reaction containing differentially labeled assays can provide information about all targets in a sample, as long as the instrument can accurately measure each fluorophore. A multiplex reaction that queries six different targets can drastically reduce the complexity of pipetting and minimize the amount of sample needed for the experiment. This can be a tremendous benefit because it enables rapid acquisition of data from precious samples.

applied biosystems

We have developed several integrated solutions to facilitate isolation and analysis of nucleic acids. Thermo Scientific™ KingFisher™ automated nucleic acid extraction systems are designed for magnetic bead–based purification of DNA and RNA from various sample types [3]. The trusted and intuitive **Applied Biosystems™ QuantStudio™ family** of qPCR instruments [4] enables detection and quantitation of up to six targets in a single reaction when used with established and highly specific Applied Biosystems™ TaqMan™ Assays [5]. The **Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System** allows highly sensitive and precise quantitation of specific sequences in samples, and is ideally suited for situations in which sample quantities are limited or transcript copy numbers are very low [6]. We have also developed software solutions for efficient downstream analysis, allowing researchers to gain significant insights from their results [7].

Here we demonstrate an integrated workflow for multiplex qPCR gene expression analysis with formalin-fixed paraffin-embedded (FFPE) sections of various tumors (Figure 1). Using the Applied Biosystems™ MagMAX™ FFPE DNA/RNA Ultra Kit and the **Thermo Scientific™ KingFisher™ Flex Purification System**, we purified DNA and RNA from 24 different tumors. We designed three different multiplex qPCR panels to test commonly used normalizer genes, genes linked to cancer and immuno-oncology, and mismatch repair enzyme genes. We also analyzed genomic DNA from the samples to assess microsatellite instability. We were able to obtain precise transcript levels for mismatch repair genes in colon tumor samples that showed microsatellite instability. Together, these techniques can enable researchers to quickly and easily derive valuable gene expression profiles from precious research samples.

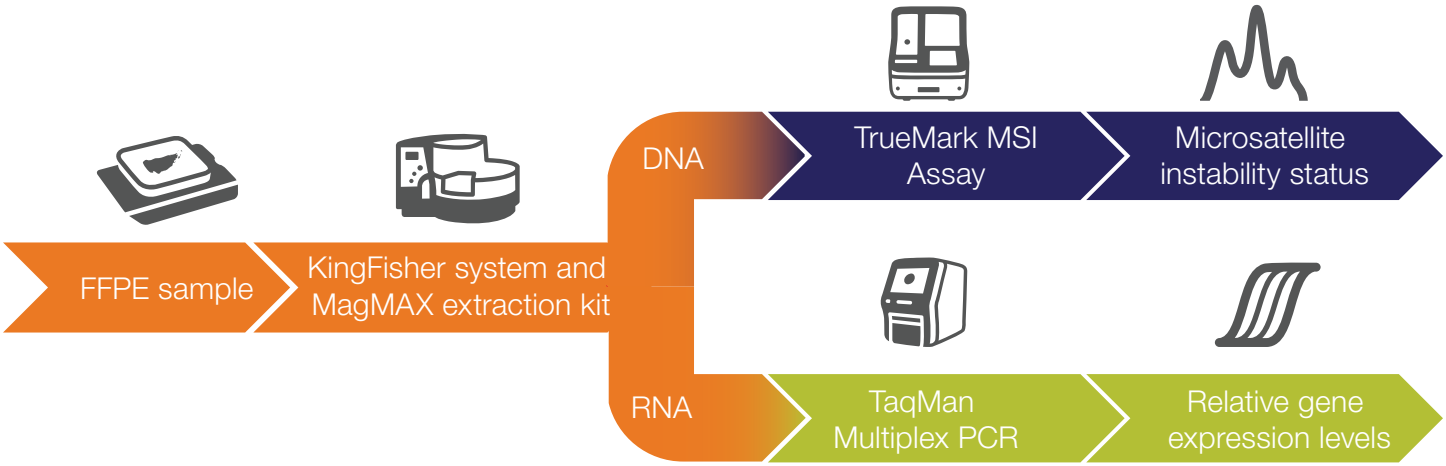

Figure 1. Workflow for multianalyte analysis of gene expression in FFPE tumor samples in this study. Starting from FFPE samples, DNA and RNA were extracted from the same slice using the MagMAX FFPE DNA/RNA Ultra Kit. The DNA was used for MSI analysis, and the RNA was used for multiplex RT-qPCR gene expression analysis.

Results

Design of multiplex panels

A multiplex qPCR panel can be designed easily using predesigned TaqMan Assays. When multiplexing **TaqMan Assays**, any potential interactions between the various **PCR primers and probes** need to be considered. To assist with this through our newly established **assay design hub**, we have a dedicated team of bioinformaticians that will check for interactions between assays and suggest redesigns or alternative assays that reduce undesirable interactions [8]. Once the panel design has been optimized, it can be forwarded to the assay synthesis team for formulation of the multiplex assay. When completing the assay design hub request form, simply enter the TaqMan Assay ID and desired reporter dye. Alternatively, **custom primers and probes** can be requested if the sequences are known.

For the experiments described here, three different sets of **predesigned TaqMan Assays** were submitted for panel design (Table 1). Note that assays that include cyanine 5 (Cy®5) or cyanine 5.5 (Cy®5.5) dye require a different quencher, and a sequence may need to be redesigned by the bioinformatics team relative to the inventoried assay, in some cases. Each of the multiplex panels was ordered as a premixed 20X assay, so a single pipetting step was sufficient to add all primers and probes to each qPCR reaction. However, each of the assays can be ordered individually and combined as needed. This was done for the reference gene panel, in parallel to pooling at the time of manufacture, to compare singleplex and multiplex efficiencies (Figure 2). The identities of the assays and makeup of each panel used in this study are shown in Table 1.

Table 1. Composition of the multiplex assay panels used in this study. The TaqMan IDs correspond to the inventoried assays in our portfolio.

Multiplex assay	Gene	TaqMan Assay ID	Probe label	Quencher	Notes
Reference gene	*ACTB*	Hs99999903_m1	VIC	NFQ-MGB	
	B2M	Hs99999907_m1_qsy	Cy5	QSY	
	GAPDH	Hs99999905_m1	JUN	NFQ-MGB	
	GUSB	Hs99999908_m1	ABY	NFQ-MGB	
	PPIA	Hs99999904_m1	FAM	NFQ-MGB	
	TUBB	Hs00917771_g1_qsy	Cy5.5	QSY2	
Cancer	*VEGF-A*	Hs00900055_m1	VIC	NFQ-MGB	Angiogenesis
	PD-L1	Hs00204257_m1	ABY	NFQ-MGB	Checkpoint inhibitor
	PPIA	Hs99999904_m1	FAM	NFQ-MGB	Reference
	HER2	Hs01001580_m1	JUN	NFQ-MGB	Growth factor
	MHL1	Hs00979919_m1_qsy	Cy5	QSY2	Mismatch repair
	CTLA4	Hs00175480_m1_qsy	Cy5.5	QSY2	Checkpoint inhibitor
Mismatch repair	*MSH2*	Hs00953527_m1	VIC	NFQ-MGB	
	MLH1	Hs00979919_m1	ABY	NFQ-MGB	
	PPIA	Hs99999904_m1	FAM	NFQ-MGB	
	MSH6	Hs00943000_m1	JUN	NFQ-MGB	
	PMS2	Hs00241052_m1_qsy	Cy5	QSY	

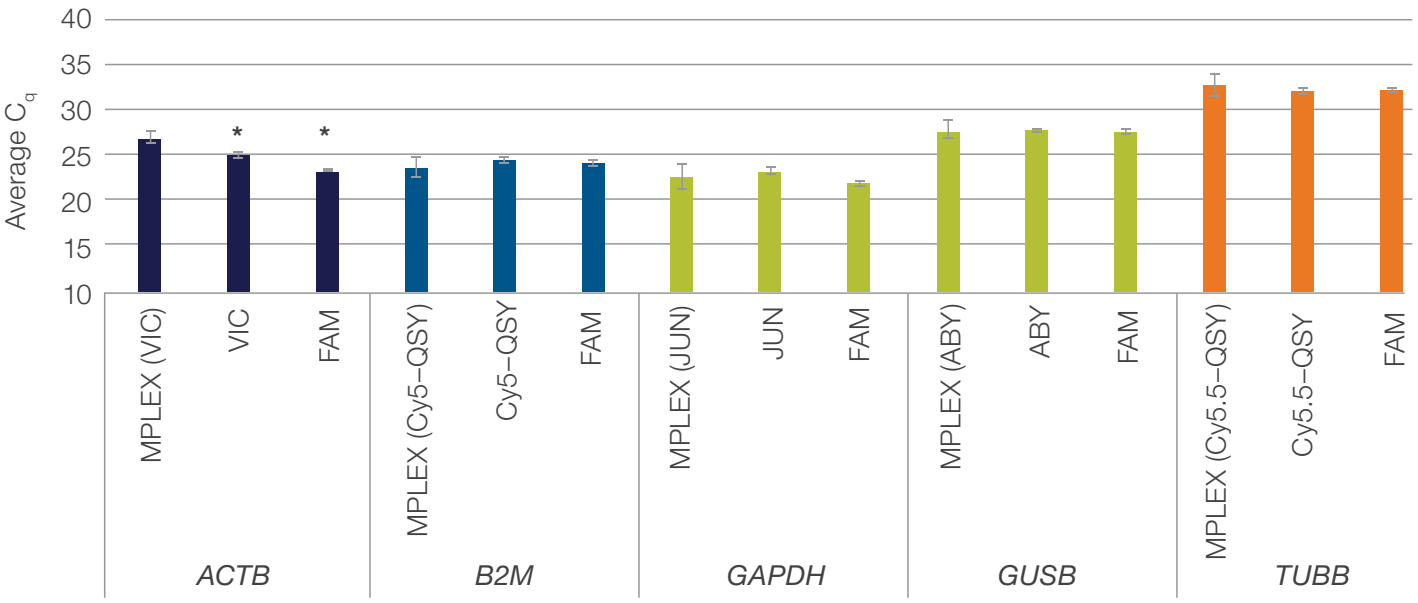

Figure 2A. Fluorophores conjugated to probes have little effect on assay performance. For each assay, C_q values were obtained in a multiplex reaction (MPLEX, first bar in each set), a singleplex reaction with the same reporter/quencher combination (second bar in each set), and a singleplex reaction using off-the-shelf assays with FAM dye (third bar in each set). The *PPIA* assay was labeled with FAM dye in the multiplex reaction, so it is not present in this comparison. Each assay was analyzed in triplicate. The *ACTB* assays showed statistically significant differences in C_q values when compared (*$p < 0.01$), but no other differences were statistically significant.

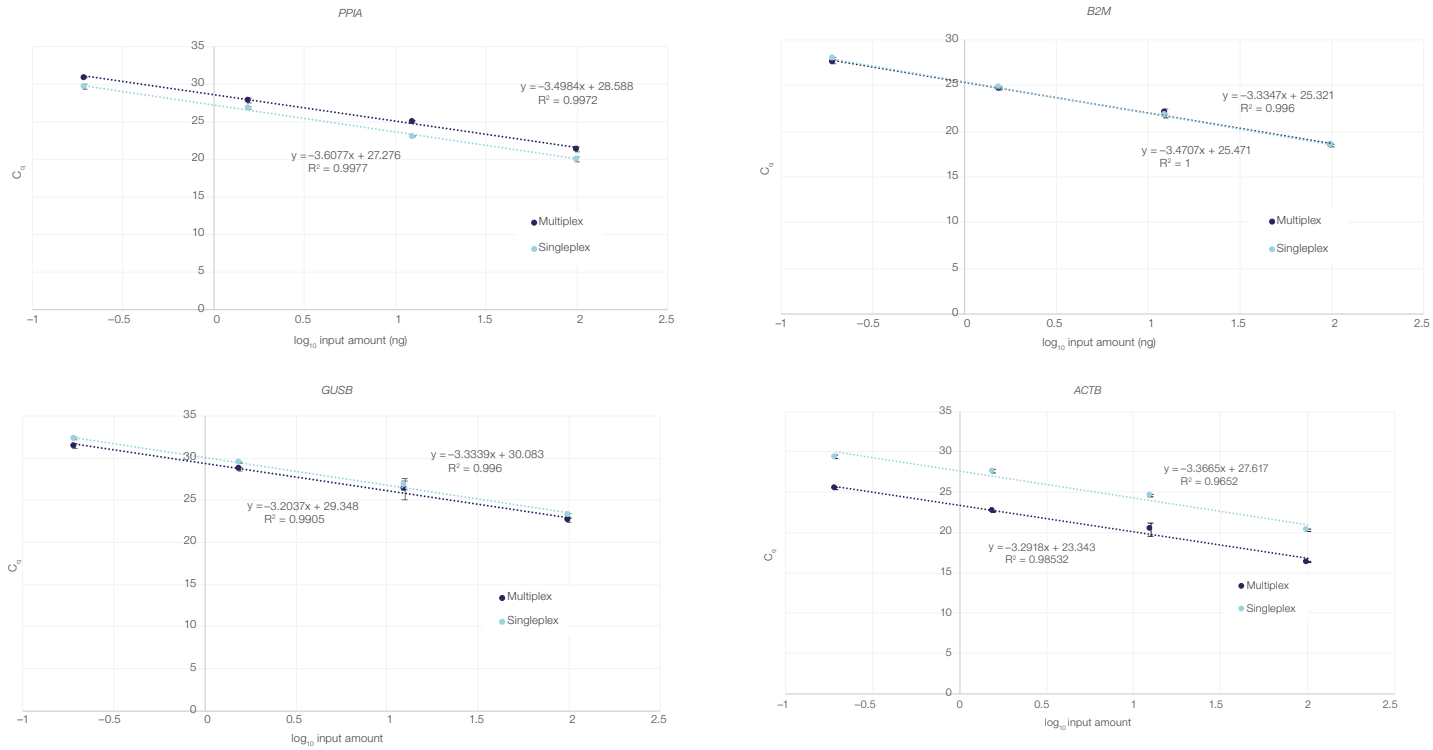

Figure 2B. Efficiency of singleplex vs. multiplex reactions. Each primer/probe combination was analyzed in triplicate with differing amounts of input RNA. Regression analysis was performed to determine PCR efficiency, indicated by the slope of the line (dark blue: multiplex reaction; light blue: singleplex reaction). Ideal efficiency corresponds to a slope of $-1/\log_{10}(2)$, or about –3.3. In each case, assay efficiency was near –3.3, and R^2 (linear correlation) was greater than 0.95. The y-intercepts for the *ACTB* assays differed significantly, suggesting differences in assay performance.

RT-qPCR conditions

We recently introduced **Applied Biosystems™ TaqPath™ DuraPlex™ 1-Step RT-qPCR Master Mix**, a benchtop-stable, single-tube master mix that includes a reverse transcriptase and *Taq* DNA polymerase. It has been optimized for rapid, sensitive, and reproducible detection of RNA, even in the presence of inhibitors. This new master mix allows multiplexing of up to six targets in one reaction, and the concentrated 4X formulation helps ensure that the mix constitutes only 25% of the overall reaction volume. TaqPath DuraPlex 1-Step RT-qPCR Master Mix can be ordered with or without Applied Biosystems™ ROX™ passive reference dye, freeing the ROX channel on the qPCR instrument for detection of an additional gene in multiplex.

For all RT-qPCR reactions described below, 1–3 µL of RNA was combined with 5 µL of 4X master mix without ROX dye, 1 µL of the 20X multiplex TaqMan Assay, and water to a final volume of 20 µL. The concentrations of each primer and probe in the 20X multiplex assay were 900 nM and 250 nM, respectively. These were combined at the time of manufacture in a single tube, so the assay could be added in a single pipetting step. Reactions were run on the QuantStudio 7 Pro Real-Time PCR System in 96-well plates with the following thermal cycling profile: 50°C for 15 min; 95°C for 5 min; then 45 cycles of 95°C for 5 sec followed by 60°C for 1 min. Each sample was analyzed in triplicate reactions.

The data were analyzed using Applied Biosystems™ Design and Analysis v2 (DA2) software and exported to Microsoft™ Excel™ software for regression and statistical analysis.

We first examined the performance of the assays in singleplex and multiplex reactions. For this, we compared two different singleplex conditions: one in which the assay was labeled with FAM dye, and the other in which the assay was labeled with the same reporter dye and quencher as in the multiplex panel. For targets, we used assays in the reference gene multiplex set (Table 1) and 10 ng of Invitrogen™ Universal Human Reference RNA as the RNA source. Four of the assays produced nearly identical C_q values, whether they were labeled with FAM dye or the alternative fluorophore and whether they were singleplex or multiplex (Figure 2A). The *ACTB* assay was the exception. The C_q values were statistically significantly different when the three different assay combinations were compared. We also compared the efficiency and linear range of the assays in singleplex and multiplex reactions. For this analysis, we titrated the RNA and obtained C_q values in multiplex and singleplex reactions while keeping the fluorophore on the probe constant (Table 1). Five of the six assays performed equivalently over the concentrations tested, with linear correlations (R^2 values)

close to 0.99 and slopes close to the ideal. Perfect reaction efficiency would have a slope of $-1/\log_{10}(2)$, or –3.32 (Figure 2B). Again, the *ACTB* assay was an exception. Although the enzyme efficiency was acceptable, the y-intercepts were different, indicating there were quantitative differences.

The results of the *ACTB* assay suggest the need to prequalify an assay before use for some experiments. For example, given that the enzyme efficiency is close to 100%, *y*-intercept differences may have a negligible impact if this panel is used to compare relative expression across different samples. However, it could introduce problems if comparing expression levels with singleplex reactions or using the same *ACTB* primer/probe combination in different multiplex panels. Thus, some assays might need further optimization during design. For this reason, the *ACTB* assay results were not used in subsequent experiments.

Tumor sample extraction

We purchased 24 commercially available FFPE tumor samples representing five different tissues. Each sample was a 5 µm section mounted on a glass slide. The tissues were deparaffinized by incubating the slides in xylene for 5 min, followed by two 95% ethanol incubations of 5 min each and 5–10 min of air-drying according to established methods used with the **Invitrogen™ RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE**. Tissue curls were scraped off the slides with a razor blade into microcentrifuge tubes. For each sample, the tissue curls were incubated with 110 µL protease solution from the **MagMAX FFPE DNA/RNA Ultra Kit** at 60°C for 4 hr with periodic vortexing, followed by 60 min at 90°C. The samples were loaded onto the **KingFisher Flex Purification System** along with the **reagents** in the **MagMAX FFPE DNA/RNA Ultra Kit** and run with the appropriate script for DNA purification [9]. When DNA purification was complete, the reserved sample fractions were reloaded onto the KingFisher Flex system and run using the reagents provided in the kit and the script supplied for RNA purification [10]. The DNA and RNA were quantified using Invitrogen™ Qubit™ kits (Table 2). Given the varying sizes and quality of the sections used, differences in the amounts of nucleic acid obtained were to be expected. However, yields were sufficient for subsequent testing.

Table 2. Tissue sources and nucleic acid recovery from FFPE tissue slices using the KingFisher Flex Purification System and the MagMAX FFPE DNA/RNA Ultra Kit.

Sample name	Tumor source	DNA concentration (ng/µL)	RNA concentration (ng/µL)
Colon 25	Colon	0.705	31.9
Colon 26	Colon	0.839	30.7
Colon 27	Colon	0.347	37.2
Colon 28	Colon	0.245	13.6
Colon 29	Colon	0.334	35.0
Gastric 30	Stomach	0.356	10.1
Gastric 31	Stomach	1.50	24.3
Gastric 32	Stomach	0.483	7.66
Gastric 33	Stomach	0.593	16.5
Endometrial 34	Endometrium	0.749	18.1
Endometrial 35	Endometrium	3.51	86.0
Endometrial 36	Endometrium	1.55	33.8
Endometrial 37	Endometrium	1.95	53.0
Endometrial 38	Endometrium	1.30	52.0
Brain 39	Brain	0.599	17.6
Brian 40	Brain	0.263	6.03
Lung 41	Lung	0.500	17.1
Brain 42	Brain	0.863	29.0
Brain 43	Brain	0.489	27.4
Brain 44	Brain	0.540	33.5
Lung 45	Lung	0.308	25.0
Lung 46	Lung	3.23	35.1
Lung 47	Lung	1.92	24.1
Lung 48	Lung	0.902	40.6

Normalizer gene panel results

We first examined the transcript abundance in each RNA purification with the normalizer gene panel (Figure 3). These genes are commonly used, either alone or in combination, to normalize for differential input of RNA or cDNA in gene expression assays. One microliter of each RNA sample was analyzed per reaction, and each sample was analyzed in triplicate using the thermal profile described previously. The C_q values were calculated by DA2 software using the automatic baseline and threshold settings. DA2 software can calculate the relative differences in target abundance using the well-established $\Delta\Delta C_t$ method. For these experiments, *PPIA* was used as the normalizer gene, and calculated fold changes were expressed relative to a representative sample in the data set normalized to a fold change or relative quantity (RQ) of 1. For example, Brain 40 was chosen as the representative brain sample, and Colon 26 was chosen as the representative colon sample. These experiments demonstrated that Brain 42 and Brain 43 had approximately twice as much amplifiable *GAPDH* transcript as Brain 39, Brain 40, and Brain 44. Similarly, Endometrial 36 had smaller amounts of three gene transcripts after *PPIA* normalization relative to the representative Endometrial 34 sample. Note that the sources of RNA in these samples were sections of advanced tumors that might have had varying amounts of normal stromal tissue and heterogeneity in the tumor cell genotypes. A uniform tissue-specific expression pattern might not be expected. Some of the samples failed to produce results, likely because of the extremely low RNA yields, and were omitted. Nevertheless, these results demonstrate that multiple transcript targets can be analyzed simultaneously by multiplexing TaqMan Assays.

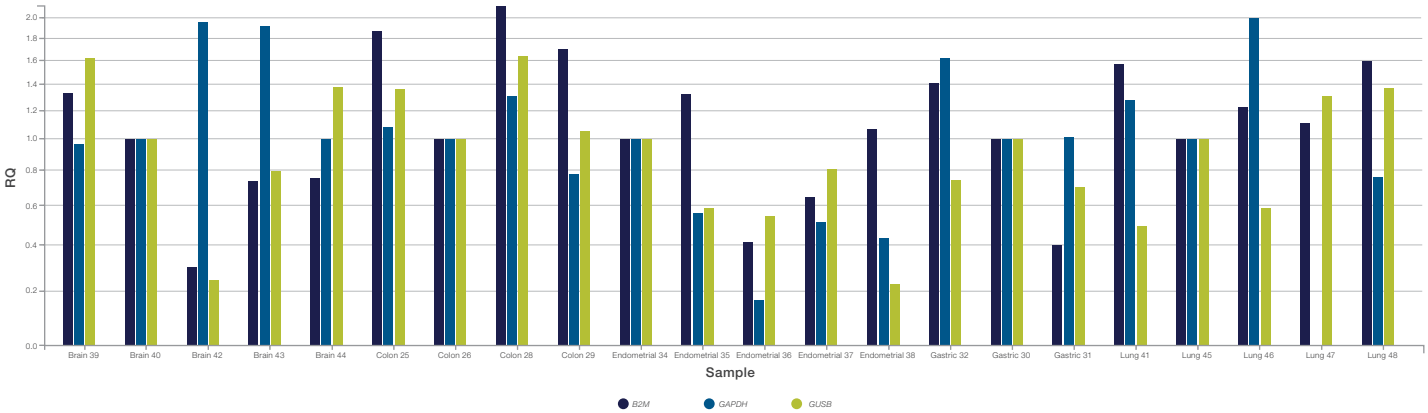

Figure 3. Reference gene expression levels determined using a multiplex assay panel and RNA extracted from FFPE samples. Relative quantities (RQs) were determined by normalizing to *PPIA*, followed by normalization to a representative sample in each tissue group. For the representative samples, RQ = 1. The other values are expression levels relative to the representative sample.

Cancer-related gene panel results

In translational research, there is interest in determining the expression levels of certain cancer-associated genes. Therefore, we put together a demonstration set of genes that might be of interest to oncology researchers. This multiplex panel included immuno-oncology markers, a growth factor marker, and a marker for angiogenesis. RNA from the samples was analyzed as previously described. As expected, there was variability in the expression of these markers (Figure 4). Notably, not all of the samples expressed *CTLA4*, suggesting treatment with *CTLA4* inhibitors would have had no effect on those tumors. Some of the tumors had high levels of *PD-L1*, suggesting they would have been more sensitive to *PD1* inhibitors. Interestingly, samples Colon 25 and Colon 29 had high levels of *VEGF* (see MSI results in next section). Thus, multiplex gene expression analysis can yield information about several specific targets in a single reaction, possibly leading to faster identification of pathways that could be evaluated for interventions.

Mismatch repair deficiencies are commonly seen in cancers of the colon [13] and other tissues [14,15]. Some of these were represented in the samples analyzed in this study. To determine whether expression of mismatch repair enzyme genes might be affected in these samples, we built a multiplex panel of probes that interrogated common mismatch repair enzymes. We used this panel to examine expression as described previously, except that we increased the amount of RNA analyzed to 5 µL per reaction to reduce the C_q values. Expression was normalized to *PPIA*, then to each of the representative samples. Most of the transcripts were detected in the different samples (Figure 5). Interestingly, *MLH1* was not detectable in six of the samples. Brain and lung tissues are not known for mismatch repair–deficient tumorigenesis, so *MLH1* deficiency might be compensated for by other mechanisms. However, two colon tumor samples (Colon 25 and Colon 29) were also lacking *MLH1*. Lack of expression of mismatch repair enzymes is commonly seen in colon cancers, suggesting this pathway may have been disrupted in these samples.

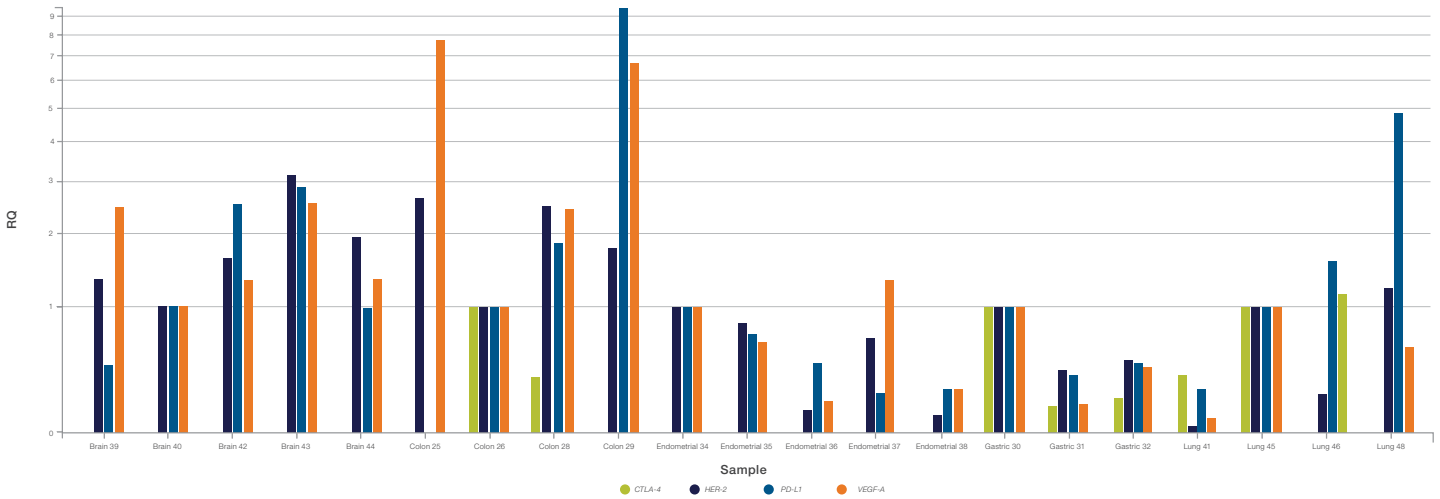

Figure 4. Cancer-related gene expression levels determined using a multiplex assay panel and RNA extracted from FFPE samples. RQs were determined by normalizing to *PPIA*, followed by normalization to a representative sample in each tissue group. For the representative samples, RQ = 1. The other values are expression levels relative to the representative samples.

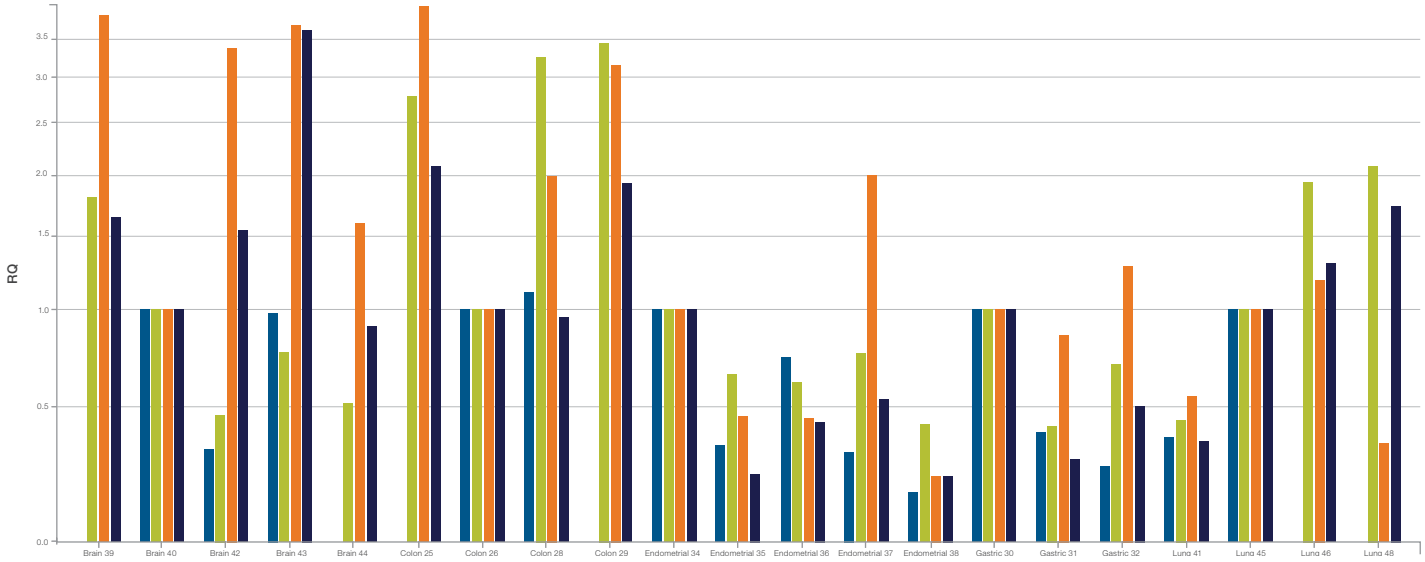

Figure 5. Mismatch repair enzyme gene expression levels in RNA extracted from FFPE samples, determined using a multiplex assay panel. RQs were determined by normalizing to *PPIA*, followed by normalization to a representative sample in each tissue group. For the representative samples, RQ = 1. The other values are expression levels relative to the representative samples.

Characterizing the mismatch repair pathway by microsatellite instability (MSI) analysis

To further characterize the mismatch repair pathway in these samples, we analyzed microsatellite status using the Applied Biosystems™ TrueMark™ MSI Assay. This kit analyzes 13 loci, including the Bethesda panel of markers [16,17], for microsatellite instability [18]. The assay also includes two highly variable short tandem repeats (STRs) that can be used to track sample identity. Building on an extensive database of normal samples, Applied Biosystems™ TrueMark™ MSI Analysis Software does not require side-by-side analysis of normal, non-tumor tissue to make MSI calls. This simplified workflow enables scientists to conserve material and quickly determine the MSI status of samples.

One microliter of gDNA from individual FFPE extractions and Applied Biosystems™ Control DNA (from CEPH Individual 1347-02) were analyzed using the protocol and reagents provided in the TrueMark MSI kit. The PCR products were run on the Applied Biosystems™ SeqStudio™ Flex Genetic Analyzer with a 50 cm capillary and Applied Biosystems™ POP-7™ polymer. Results were analyzed using TrueMark MSI Analysis Software, and MSI status was called by the software. No calls (NCs) were reviewed and called manually if peaks were present but below the threshold of detection.

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The results of the microsatellite analysis are shown in Table 3. The normal CEPH gDNA showed no MSI, as expected. Five of the samples (Colon 25, Colon 28, Colon 29, Gastric 31, and Gastric 32) showed high levels of instability. Manual examination of the fragment peaks confirmed the instability calls (Figure 6). Of the remaining 19 tumor samples, 4 were no calls, 12 were stable, and 5 had low levels of instability.

Table 3. Microsatellite instability status of tissues used in this study. MSI-H: high instability at all loci examined; MSI-L: some instability seen at some loci; MSS: stable at all loci; NC: no call made due to low signal. Also shown is the *MLH1* expression status (presence/absence) of each sample: present (+), not detected (–), or no *PPIA* detected (0).

Sample name	Tumor source	MSI status	MLH1
Colon 25	Colon	MSI-H	–
Colon 26	Colon	MSS	+
Colon 27	Colon	MSS	0
Colon 28	Colon	MSI-H	+
Colon 29	Colon	MSI-H	–
Gastric 30	Stomach	MSS	+
Gastric 31	Stomach	MSI-H	+
Gastric 32	Stomach	MSI-H	+
Gastric 33	Stomach	MSI-L	0
Endometrial 34	Endometrium	MSI-L	+
Endometrial 35	Endometrium	MSS	+
Endometrial 36	Endometrium	MSS	+
Endometrial3 7	Endometrium	MSS	+
Endometrial 38	Endometrium	MSS	+
Brain 39	Brain	MSS	–
Brian 40	Brain	NC	+
Lung 41	Lung	MSS	+
Brain 42	Brain	NC	+
Brain 43	Brain	MSI-L	+
Brain 44	Brain	MSS	–
Lung 45	Lung	MSS	+
Lung 46	Lung	MSS	+
Lung 47	Lung	NC	+
Lung 48	Lung	NC	–
CEPH	Normal white blood cells	MSS	+

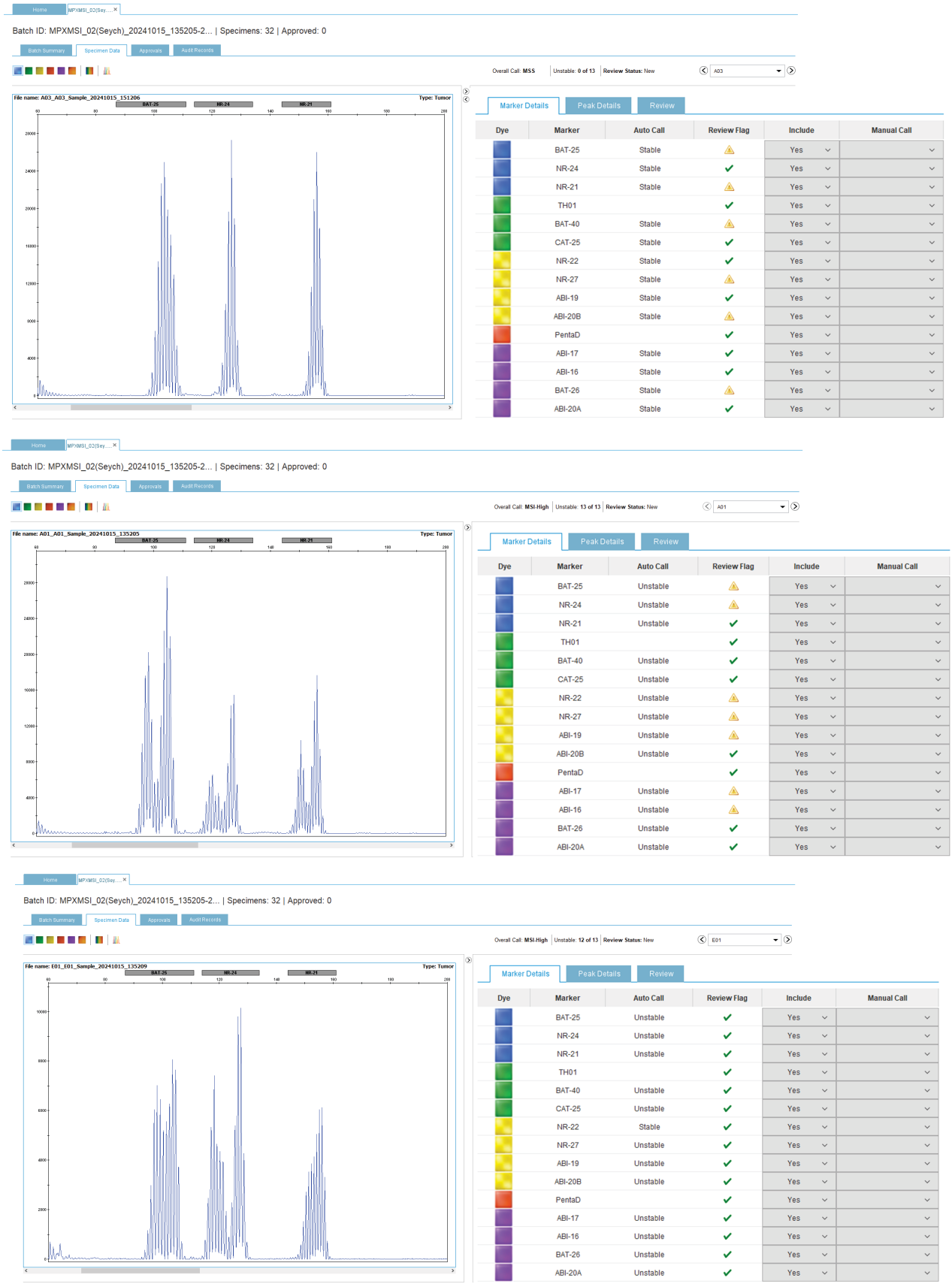

Figure 6. MSI results for normal CEPH gDNA (top) and Colon 25 and Colon 29 tumor samples (middle and bottom). Note the smaller fragments detected in the colon samples at these loci, indicating instability. Only the peaks in the blue channel querying three loci are shown. Results for all other channels and loci are shown in the tables to the right of the electropherograms.

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Summary

Here we have shown how multiplex qPCR can be used to address questions about gene expression in translational research. We have described how a multiplex qPCR panel can be designed and how it can be used on the latest **QuantStudio qPCR instruments**. Although most TaqMan Assays perform equivalently in singleplex and multiplex reactions, occasional optimization might be required. We have also described how DA2 software facilitates analysis of gene expression assays. Finally, we have shown how results from such experiments can be further analyzed using other solutions from Thermo Fisher Scientific. This workflow, from sample preparation to follow-up analysis, can simplify the steps needed in a translational research program (Figure 7).

Our results provide some interesting insights that illustrate how integrated workflows could be used in translational research. For example, even though some brain and lung samples lacked *MLH1*, they did not show MSI. This suggests that other mechanisms might compensate to keep microsatellite sequences stable [18]. On the other hand, two gastric and one colon sample demonstrated MSI even though they had *MLH1* transcripts. This suggests that there might be loss-of-function mutations in the coding sequences of *MLH1* and/or other MMR enzymes that may not alter transcript abundance. Finally, two of the colon samples lacked *MLH1* transcripts and had high levels of MSI. It is tempting to suggest that a lack of *MLH1* expression was responsible for the high MSI. Interestingly, the same samples had very high levels of *VEGF* transcription. That there is a possible link between these observations shows how integrated workflows can quickly lead to novel observations and testable hypotheses.

Several factors should be considered when designing a high-order multiplex TaqMan qPCR assay. First, the primers and probes in the assay should be specific for the target sequences. Second, they should not cross-react with each other under standard qPCR conditions. Third, primer and probe sequences should be modified if necessary to optimally perform with the fluorophores and quenchers to be used in the assay. Finally, the efficiency of the polymerase should not be significantly affected by the fluorophores, quenchers, or multiplex level in the reaction.

We have established solutions to assist with each of these steps. Our proprietary assay design algorithms, optimized for TaqMan chemistry, allow input of target sequences into a web-based, self-service design tool to obtain assay sequences. Our multiplex assay design portal leverages our bioinformatics proficiency to assess the compatibility of multiple assays or primer/probe pairs in a multiplex reaction. If needed, this service can also suggest sequence changes when converting minor groove binder (MGB) quenchers to Applied Biosystems™ TaqMan™ QSY™ quenchers for higher-degree multiplexing.

Our **Multiplex Performance Assessment (MPA) service** can assist with validating untested multiplex panels and address issues that may arise. This service includes control materials and master mix to run experiments on-site, and consultation with a trained application scientist who will evaluate the performance of multiplex execution of the experiment. It also includes analysis of the data by a technical project manager, and a readout that indicates how the assays performed and whether moving to analytical validation is recommended. Our **Analytical Validation Consulting Services** follow an industry-accepted process and adhere to stringent guidelines, including CAP, CLIA, CLSI, NYSDoH, and ISO 15189.

Automation and multiplexing options for biomarker identification

Biomarker identification often requires screening of numerous samples with qPCR for relative quantitation of nucleic acids, or digital PCR for absolute quantitation when appropriate. In addition to high-order multiplexing tools, we are excited to offer automation-friendly qPCR and dPCR tools that assist in expediting the high-throughput sample screening process for biomarker identification.

The automation of qPCR and dPCR workflows can help transform the field of bioanalysis and markedly enhance the speed and efficiency of biotherapeutics development and manufacturing. By streamlining sample processing and downstream analysis, these technologies help minimize human error, boost throughput, enhance data accuracy, and improve reproducibility. Integrating automated sample preparation with high-throughput qPCR and dPCR equips researchers and manufacturers with tools to expedite the development and production of biotherapeutic products.

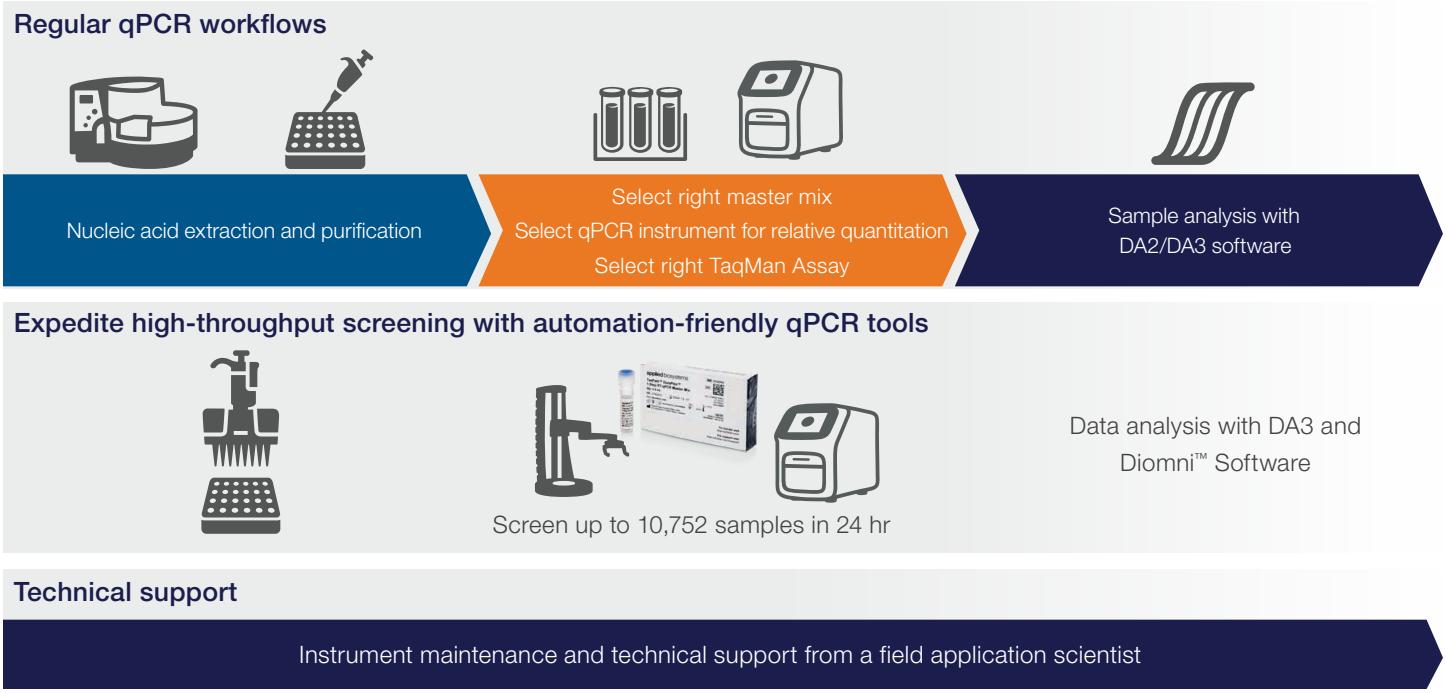


Figure 7. qPCR workflows for nucleic acid quantitation.

qPCR tools

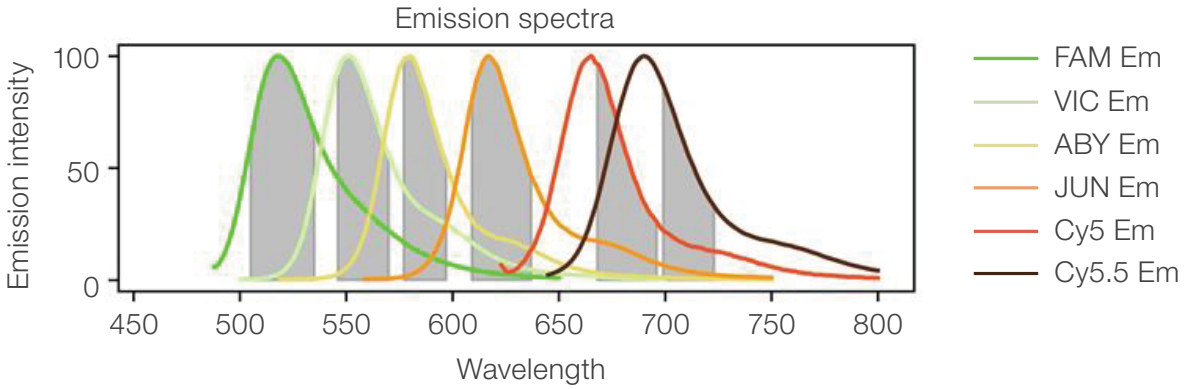
QuantStudio instruments are compatible with the **Thermo Scientific™ Orbitor™ RS2 Microplate Mover**, a benchtop device designed for use with the QuantStudio 12K Flex, QuantStudio 7 Pro, and QuantStudio 7 Flex Real-Time PCR Systems (Figure 8). This integration enables real-time PCR in automated environments, significantly increasing sample throughput. Screen up to 10,000 samples in 24 hr with QuantStudio qPCR instruments and the Orbitor RS2 Microplate Mover.

The Orbitor RS2 Microplate Mover is a robust, efficient, and reliable benchtop mover known for its outstanding speed and accuracy. It is compatible with most SBS footprint plates and is powered by Momentum™ workflow scheduling software.

Figure 8. QuantStudio 7 Pro Real-Time PCR System with the Orbitor RS2 Microplate Mover.

TaqMan Assays

We offer custom probes for multiplexing with up to six targets, enabling easy conversion from Black Hole Quencher™ (BHQ) and Iowa Black™ probe sequences (Figure 9). Applied Biosystems™ TaqMan™ QSY™ probes are designed for multiplexing with up to four targets using Applied Biosystems™ ABY™, JUN™, FAM™, and VIC™ dyes. Applied Biosystems™ TaqMan™ QSY™2 probes can be used for five- and six-target multiplexing with cyanine 5 and cyanine 5.5 dyes for relative quantitation (qPCR).



	Absorbance	Emission	MGB probe	QSY probe	QSY2 probe
FAM dye	496 nm	520 nm	✓	✓	
VIC dye	532 nm	552 nm	✓	✓	
ABY dye	568 nm	583 nm	✓	✓	
JUN dye	606 nm	618 nm	✓	✓	
Cyanine 5 dye	649 nm	667 nm			✓
Cyanine 5.5 dye	678 nm	695 nm			✓

Figure 9. Dye compatibility for multiplexing with TaqMan Assays.

TaqPath DuraPlex 1-Step RT-qPCR Master Mix is specifically formulated for enhanced benchtop stability, making it an automation-friendly solution for high-throughput gene expression analysis (Figure 10).

Figure 10. Automation-friendly TaqPath DuraPlex 1-Step RT-qPCR Master Mix.

TaqPath DuraPlex 1-Step RT-qPCR Master Mix is a benchtop-stable, single-tube master mix optimized for rapid, sensitive, and reproducible detection of viral and bacterial pathogens even in the presence of PCR inhibitors. This automation-friendly master mix is ideally suited for testing and developing high-throughput molecular diagnostics, as well as for gene expression analysis for high-throughput biologics screening. The 4X concentrated formulation can multiplex six targets in a single well and has intuitive user handling.

Benefits of TaqPath DuraPlex 1-Step RT-qPCR Master Mix

- Enables workflow automation with benchtop stability of fully assembled reactions for a full workday
- Optimizes your assay for high-order multiplexing of up to six targets in a single reaction
- Improves handling, in-use stability, and freeze-thaw conditions
- Increases confidence in detecting both RNA and DNA targets in samples containing challenging PCR inhibitors
- Ensures reproducible results with consistent lot-to-lot performance

For Laboratory Use.

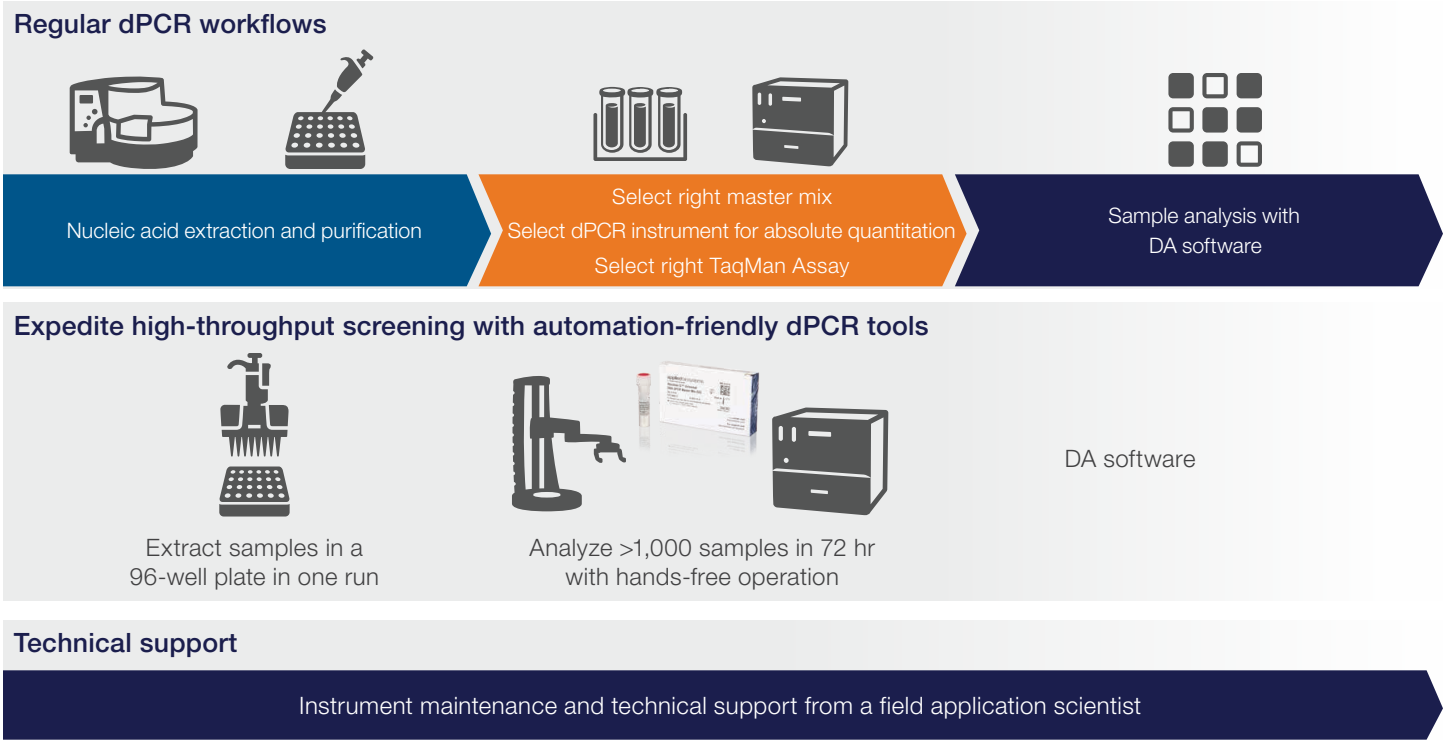


Figure 11. Digital PCR (dPCR) workflows for absolute nucleic acid quantitation.

The **Applied Biosystems™ QuantStudio™ Absolute Q™ AutoRun™ Digital PCR Suite** is an automated dPCR solution designed to scale nucleic acid quantitation with efficiency and ease (Figure 11). The QuantStudio Absolute Q AutoRun dPCR Suite streamlines high-throughput multiplex dPCR with an easy-to-use walkaway workflow, allowing you to reclaim valuable time and resources while maximizing throughput (Figure 12).

Applied Biosystems™ Absolute Q™ Universal DNA Digital PCR Master Mix is compatible with a broad range of applications and over 20 million predesigned and performance-backed TaqMan Assays, reducing the need to design custom assays (Figure 13).

Figure 12. QuantStudio Absolute Q AutoRun Digital PCR Suite for automation.

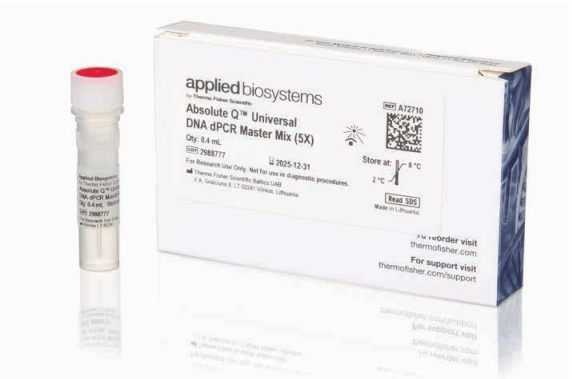


Figure 13. Absolute Q Universal DNA Digital PCR Master Mix for absolute quantitation.

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