

# High Quality and High Throughput Solutions for Gene Expression Analysis

## Extending PCR Detection

Applied Biosystems has been at the forefront of real-time PCR innovation, with the first instrument, the ABI PRISM® 7700 Sequence Detection System, and the first chemistry, TAMRA™-labeled TaqMan® probes for 5' nuclease assays, introduced in 1996. Since then we have continuously introduced improved models of instruments and chemistries to meet the increasing demand for quality, reproducibility, and throughput.

Today our real-time PCR solution for gene expression is an optimized workflow that extends from sample preparation to data analysis. And it has been expanded to include new areas of interest for the study of functional biology. The workflow covers sample collection and nucleic acid extraction, reverse transcription (RT), amplification, and analysis in a series of optimized and coordinated steps for gene expression and gene regulation applications (Figure 1).

This article describes how Applied Biosystems products can enable two real-time PCR workflows commonly used in scientific research (Figure 2):

- The “few samples, many genes” workflow—characterized by the need to analyze multiple genes or “gene signatures” in a relatively limited number of samples.
- The “many samples, few genes” workflow—in which the number of gene targets is limited but the number of samples is high.

In the “few samples, many genes” workflow, typical of cancer research, it is important to accurately extract and isolate RNA from each sample. A single RT reaction is performed on each RNA sample, followed by real-time PCR

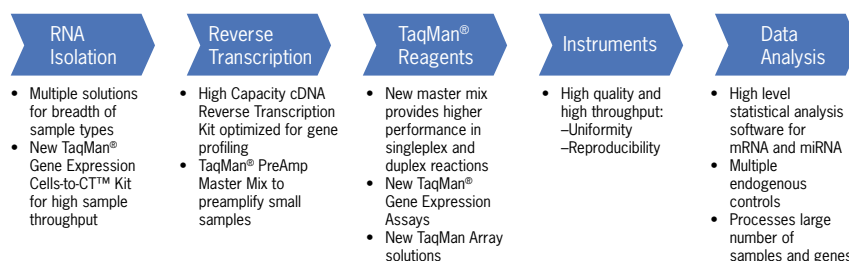


Figure 1. A Complete Gene Expression Analysis Workflow.

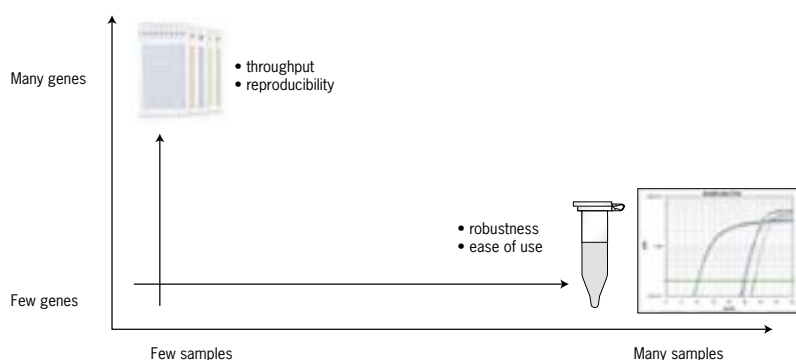


Figure 2. Design Workflow to Meet Scope of Project.

analysis of multiple genes. The single RT reaction helps provide homogenous conditions to minimize bias. The TaqMan assay design process optimizes every assay to the same PCR conditions, again minimizing bias. It is therefore important to have an efficient RT reaction capable of uniformly reverse transcribing both low and high expressors.

The subsequent real-time PCR step is best performed in individual reactions for each gene and endogenous control (EC), using three replicates. This allows for the use of multiple ECs and the ability to decide, post-PCR, the optimal combination of genes and ECs for downstream analysis of results.

In the “many samples, few genes” workflow, typical of metabolic disease research, the number of samples can be quite high, with each sample typically

requiring analysis of <10 genes. In this case, it is more desirable to have a rapid and automatable solution for RNA extraction from the samples, followed by direct RT and real-time PCR. Moreover, since often just one or two genes will be analyzed, the researcher may want to run duplex reactions, i.e., simultaneous real-time PCRs for each gene and EC in the same tube, thus decreasing costs and increasing throughput.

## Applied Biosystems Solutions for “Few Samples, Many Genes” Workflows (See Figure 3)

### RNA Isolation

Applied Biosystems offers a broad set of solutions for RNA extraction from cells, tissues, and paraffin embedded-material that are all compatible with the Applied Biosystems High Capacity cDNA Reverse Transcription Kit, a technology ideal for

## Pushing the Sensitivity of Gene Expression Analysis

An area of increasing scientific focus is small sample (including single cell) analysis, in which the sample is precious or limited, yet there is a desire to analyze a large number of genes. This includes applications that use laser capture microdissection (LCM), needle biopsy, and formaldehyde or paraformaldehyde-fixed, paraffin-embedded (FFPE) tissues. The Applied Biosystems PreAmp™, PCR-based enrichment technology makes it possible to analyze hundreds of genes by real-time PCR from a theoretical single cell (see Innovations 4, MicroRNA and mRNA Expression—One Stem Cell at a Time, at [info.appliedbiosystems.com/innovations](http://info.appliedbiosystems.com/innovations)). The PreAmp Master Mix Kit provides a 200–400 fold increase in sensitivity with negligible enrichment efficiency bias. It is compatible with any combination of up to 100 TaqMan® Gene Expression Assays and with the new TaqMan MicroRNA Assays megaplexed for single cell miRNA profiling. For more information see [www.allgenes.com](http://www.allgenes.com).

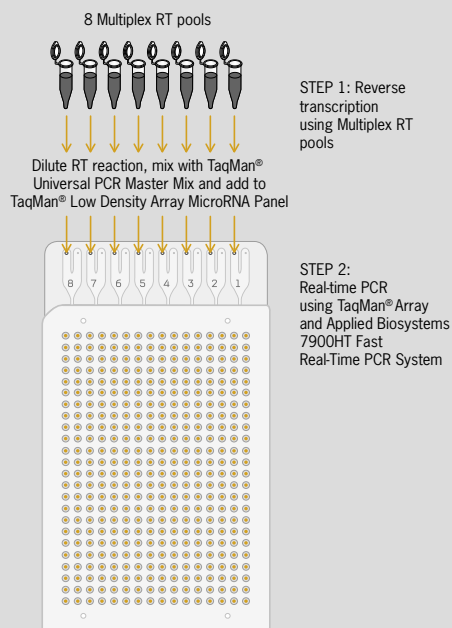


Figure 1. Fast, Sensitive miRNA Profiling by Real-Time RT-PCR.

## miRNAs and Gene Regulation Analysis

miRNAs play an important role in the biology of cells. Accurate miRNA measurement is possible using TaqMan® technology and the specially designed TaqMan MicroRNA Assays. The assays are available for every miRNA published in the Sanger database and can be used in single tubes or, for screening purposes, on the new miRNA TaqMan Low Density Arrays. These arrays can run 365 unique miRNA assays in parallel, with minimal sample input, in <3 hours.

### Recent Publications Using TaqMan miRNA Technology Include:

#### Tumor Suppressor miRNAs

Kumar SM, Lu J, Mercer KL, Golub TR, Jacks T. (2007) Impaired microRNA processing enhances cellular transformation and tumorigenesis *Nat Genetics* **39**:673–677.

He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA, Hannon GJ (2007) A microRNA component of the p53 tumour suppressor network. *Nature* **47(7148)**:1130–1134. (This work is summarized on page 15 of this issue).

Mayr C, Hemann MT, Bartel DP. (2007) Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. *Science* **315**:1576–1579.

#### Regulation/control of Immune Systems

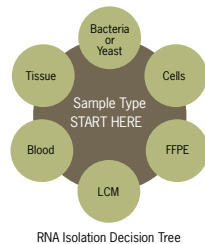
Li Q, Chau J, Ebert P, Sylvester G, Min H, Liu G, Braich R, Manoharan M, Soutschek J, Skare P (2007) miR-181a Is an intrinsic modulator of T cell sensitivity and selection. *Cell* **129(1)**:147–161.

Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, Vetrie D, Okkenhaug K, Enright AJ, Dougan G, Turner M, Bradley B (2007) Requirement of bic/microRNA-155 for Normal Immune Function. *Science* **316(5824)**:608–611.

Thai T-H, Calado DP, Casola S, Ansel KM, Xiao C, Xue Y, Murphy A, Friendewey D, Valenzuela D, Kutok JL, Schmidt-Suppran M, Rajewsky N, Yancopoulos G, Rao A, Rajewsky K (2007) Regulation of the Germinal Center Response by MicroRNA-155. *Science* **316(5824)**:604–608.

Wang Y, Medvid R, Melton C, Jaenisch R, and Blillock R (2007) DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nat Genetics* **39(3)**:380–385.

preparing small and large amounts of cDNA for subsequent real-time PCR analysis. Visit the RNA Isolation Decision Tree at [www.ambion.com/techlib/trees/RNA/](http://www.ambion.com/techlib/trees/RNA/) to choose the RNA isolation method that best suits your system.



### Reverse Transcription

In this workflow, multiple genes are analyzed from the same sample. It is important to maintain an unbiased ratio between target genes and ECs, i.e., the presence of highly expressed transcripts must not inhibit the transcription efficiency of low expressors during the RT step. A system that ensures high capacity, allowing higher amounts of RNA to be loaded so that multiple PCR reactions can be run, should therefore be used. The Applied Biosystems High Capacity cDNA Reverse Transcription Kit offers superior capacity, efficiency, and linearity for accurate quantitation of multiple RNA targets.



Figure 3. "Few Samples, Many Genes" Workflow.

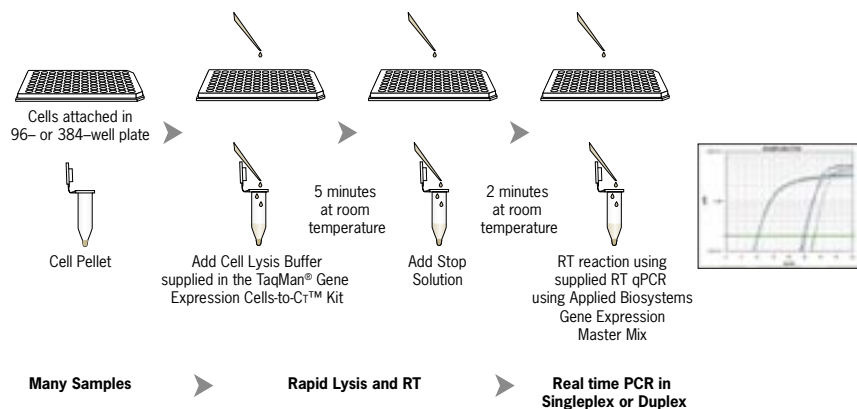


Figure 4. "Many Samples, Few Genes" Workflow.

### Real-Time PCR

Applied Biosystems offers more than 750,000 TaqMan Gene Expression Assays in eight species—the most comprehensive set of pre-designed real-time PCR assays available. All TaqMan Gene Expression Assays have been designed using Applied Biosystems' validated bioinformatics pipeline, and can be run with the same PCR protocol, eliminating the need for primer design or PCR optimization. TaqMan Gene Expression Assays were used as the gold standard in the MicroArray Quality Control (MAQC) Project, which compared data from seven microarray platforms [1]. These assays have the highest specificity, highest sensitivity, and the largest dynamic range of any gene expression technology.

TaqMan Gene Expression Assays are available in multiple formats, allowing the researcher to select the one that best suits their experimental workflow, e.g. tubes for many samples, and TaqMan Arrays for many genes. TaqMan Arrays offer the benefits of high throughput, low sample consumption, high reproducibility,

and easy set up. They are also offered in fixed menu configurations, a ready solution for the analysis of common gene signatures or pathways.

For more information on TaqMan Gene Expression Assays, TaqMan Arrays, and TaqMan Gene Signature Panels, visit [www.allgenes.com](http://www.allgenes.com).

### Applied Biosystems Solutions for the "Many Samples, Few Genes" Workflows (See Figure 4)

Applied Biosystems provides the simplest, most sensitive, and most integrated solution for workflows with high sample numbers. The TaqMan Gene Expression Cells-to-Ct™ Kit offers a 7 minute, isothermal procedure for the preparation of cell lysates and subsequently cDNA directly from cultured cells—no RNA isolation necessary. The cDNA generated is compatible with the new Applied Biosystems Gene Expression Master Mix, which enables high efficiency both

in singleplex and duplex reactions. With an input range of 10–10<sup>5</sup> cells, the TaqMan Gene Expression Cells-to-Ct Kit is also ideal for increasing sensitivity when only a few cells are available. Decreased sample handling minimizes RNA loss.

**Reference**  
1. MAQC project. (2006) *Nature Biotechnology* 24:1105–1150.