applied biosystems

Introduction to Gene Expression
GETTING STARTED GUIDE

Planning your experiment

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Revision B

Thermo Fisher
SCIENTIFIC

Manufacturer:
Life Technologies Corporation |
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Pleasanton, CA 94566

Products:
TaqMan® Gene Expression Assays
TaqMan® Gene Expression Assays, Primer-Limited (PL)
TaqMan® Non-coding RNA Assay
Custom Plus TaqMan® RNA Assay
Custom PlusTaqMan® RNA Assay, Primer-Limited (PL)
Custom TaqMan® Gene Expression Assays
Custom TaqMan® Gene Expression Assay, Primer-Limited (PL)

Manufacturer:
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Products:
TaqMan® Gene Expression Assays
TaqMan® Gene Expression Assays, Primer-Limited (PL)
Custom Plus TaqMan® RNA Assay
Custom TaqMan® Gene Expression Assays

Manufacturer:
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Kingdom

Products:
Custom TaqMan® Gene Expression Assays

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Revision	Date	Description
B	24 January 2018	• Added information for VIC™-labeled assays. • Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A	May 2010	New document.

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Introduction to Gene Expression Getting Started Guide

1 Background information

What is gene expression?

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as rRNA genes or tRNA genes, the product is a structural or housekeeping RNA. In addition, small non-coding RNAs (miRNAs, piRNA, siRNA) and various classes of long non-coding RNAs are involved in multiple regulatory functions.

When studying gene expression with real-time polymerase chain reaction (PCR), scientists usually investigate changes, increases or decreases, in the expression of a particular gene or set of genes by measuring the abundance of the gene-specific transcript. The investigation monitors the response of a gene to treatment with a compound or drug of interest, under a defined set of conditions. Gene expression studies can also involve looking at profiles or patterns of expression of several genes. Whether quantitating changes in expression levels or looking at overall patterns of expression, real-time PCR is used by most scientists performing gene expression.

Real-time PCR concepts

Real-time PCR, also known as quantitative reverse transcription PCR (RT-qPCR) or quantitative PCR (qPCR), is one of the most powerful and sensitive gene analysis techniques available. Real-time PCR is used for a broad range of applications including quantitative gene expression analysis, genotyping, copy number, drug target validation, biomarker discovery, pathogen detection, and measuring RNA interference. Real-time PCR measures PCR amplification as it occurs, so that it is possible to determine the starting concentration of nucleic acid. In traditional PCR, which is based on endpoint detection, results are collected after the reaction is complete, making it impossible to determine the starting concentration of nucleic acid.

Every real-time PCR contains a fluorescent reporter molecule to monitor the accumulation of PCR product, for example, a TaqMan® probe or SYBR™ Green dye. As the quantity of target amplicon increases, so does the amount of fluorescence emitted from the fluorophore.

Advantages of real-time PCR include:

- Generation of accurate quantitative data
- Increased dynamic range of detection
- Elimination of post-PCR processing
- Detection down to one copy
- Increased precision to detect smaller-fold changes
- Increased throughput

PCR phases

There are three phases in a basic PCR run:

- **Exponential phase**—Cycle range characterized by a high and constant amplification efficiency. When plotted on a log scale of DNA versus cycle number, the curve generated by the exponential phase should approximate a straight line with a slope. Exact doubling of product occurs at every cycle (assuming 100% reaction efficiency). Exponential amplification occurs because all the reagents are fresh and available, and the kinetics of the reaction push the reaction to favor doubling of amplicons.

- **Linear (high variability) phase**—Cycle range characterized by a leveling effect, where the slope of the amplification curve decreases steadily. This phase is termed linear, because amplification approximates an arithmetic progression, rather than a geometric increase. As the reaction progresses, reagents are consumed as a result of amplification and the reactions start to slow down. The PCR product is no longer doubled at each cycle.

- **Plateau (endpoint) phase**—The reaction has stopped, no more products are made, and the normalized reporter (R_n) signal remains relatively constant. If left longer, the PCR products start to degrade. Each tube or reaction plateaus at a different point, due to the different reaction kinetics for each sample. These differences can be seen in the plateau phase. The plateau phase is where traditional PCR takes its measurement, often with gel detection.
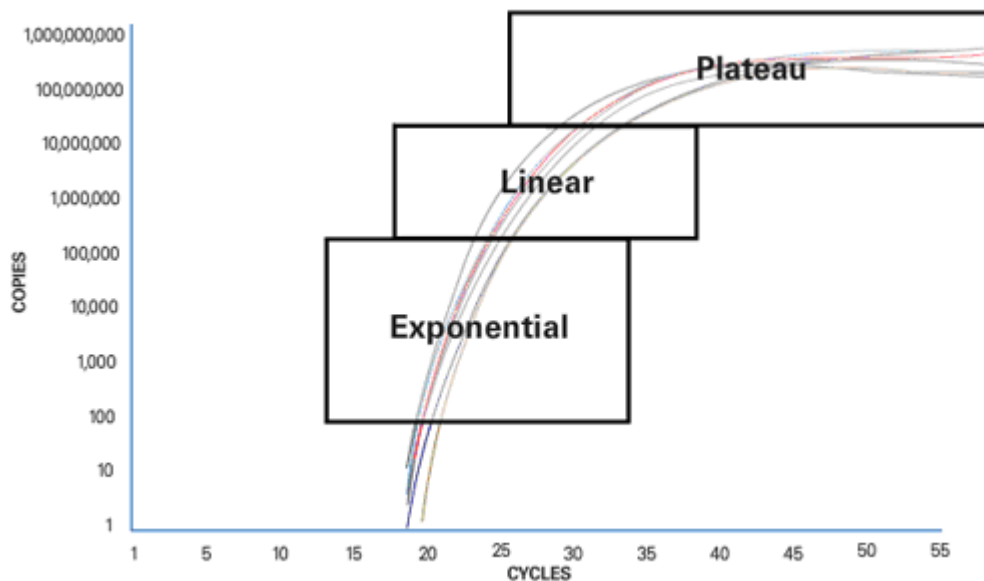
Exponential phase measurement in real-time PCR

Real-time PCR focuses on the exponential phase, which provides the most precise and accurate data for quantitation. During the exponential phase, the real-time PCR instrument calculates two values:

- **Threshold**—The level of detection at which a reaction reaches a fluorescent intensity above background.

- C_t—The PCR cycle at which the sample reaches the threshold. The C_t value is used in absolute and relative quantitation.
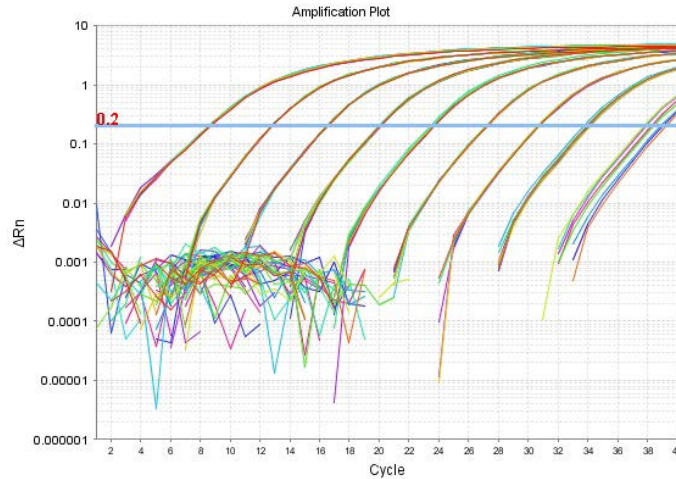
Threshold cycles (C_t)

Data analysis associated with quantitative real-time PCR (qPCR) depends upon the concept of threshold cycle (C_t): the cycle at which the level of fluorescence from accumulating amplicons crosses a defined threshold. The most common method of quantitation, based on this measurement, can be referred to as the Ct method, or "baseline threshold" method.

Test samples containing a greater initial template number cross the detection threshold at a lower cycle than samples containing lower initial template. The C_t method uses a threshold setting to define the level of detectable fluorescence. The threshold cycle (C_t) for a given amplification curve occurs at the point that the fluorescent signal grows beyond the value of the threshold setting. The C_t is dependent on two factors:

- Starting template copy number

- Efficiency of DNA amplification on the PCR system

To determine the C_t for an amplification plot, the software uses data collected from a predefined range of PCR cycles called the "baseline" (the default baseline occurs between cycles 3 and 15). First, the software calculates a mathematical trend using the baseline cycles' R_n values to generate a baseline subtracted amplification plot (showing ΔR_n on the Y-axis). Then, an algorithm searches for the point on the amplification plot at which the ΔR_n value crosses the default threshold setting of 0.2. The fractional cycle at which this occurs is defined as the C_t .

Note: It may be necessary to adjust baseline and threshold settings to obtain accurate and precise data.

Relative threshold cycles (C_{rt})

An alternative method called the C_{rt}, or "relative threshold" method can also be used to analyze real-time data. The relative threshold method calculates C_{rt} values for each individual amplification curve, and no information is needed from the other curves. The amplification curve is first set to a relative scale by setting the minimum relative fluorescence value to 0 and the maximum value to 1. A curve that models the reaction efficiency is calculated on a 0-1 scale such that the early cycles are around 1 and the later cycles are close to 0. Using an empirically predetermined reference fluorescence value and a proprietary algorithm, a common point on the reaction efficiency curve is identified and used to map back to the original amplification curve. This fractional cycle value is ultimately reported as the C_{rt}.
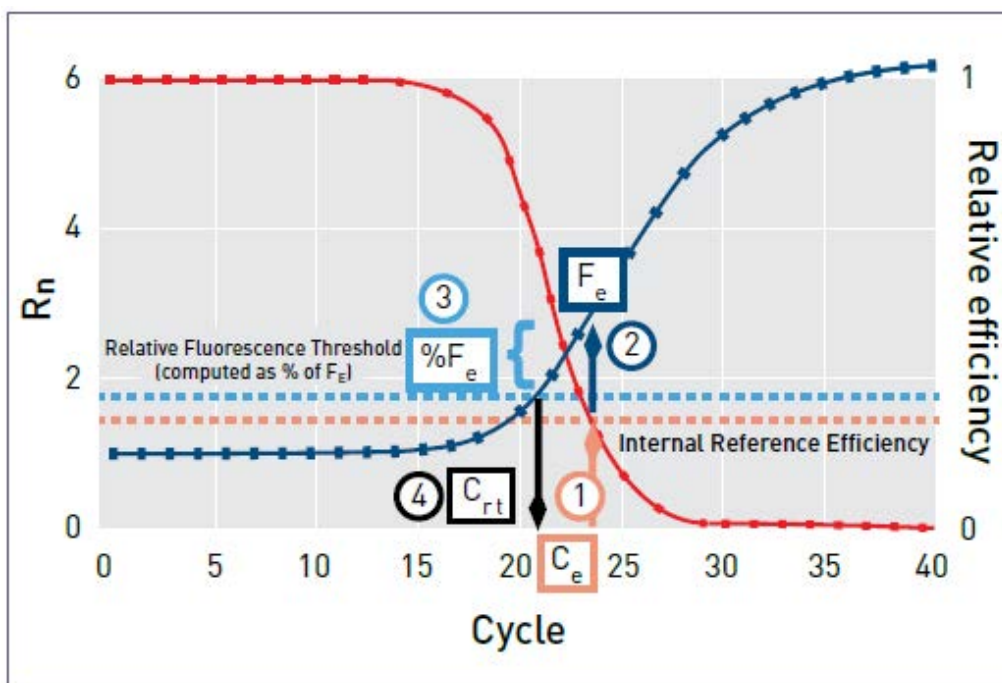
Figure 1 The C_{rt} method

C_{rt} is calculated with the following steps:

① A predetermined internal reference efficiency level (pink dotted line) is used to identify the fractional cycle (C_e) where the reaction efficiency curve (model) reaches a specific value.

② The fluorescence level (F_e) corresponding to the fractional cycle C_e on the amplification curve is determined.

③ The relative fluorescence threshold (light blue dotted line) is a curve-specific threshold that is computed as a specific percentage of F_e (%F_e).

④ The C_{rt} is computed as the fractional cycle where the amplification curve crosses the Relative Fluorescence Threshold.

Workflow

Select the chemistry	TaqMan / SYBR
▼	
Select the reverse transcription method	One-step vs. Two-step
▼	
Select or design the assays	Preformulated vs. Custom-designed
▼	
Select the quantitation method	Relative or Absolute
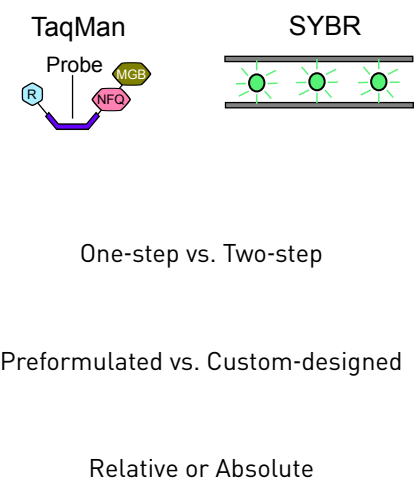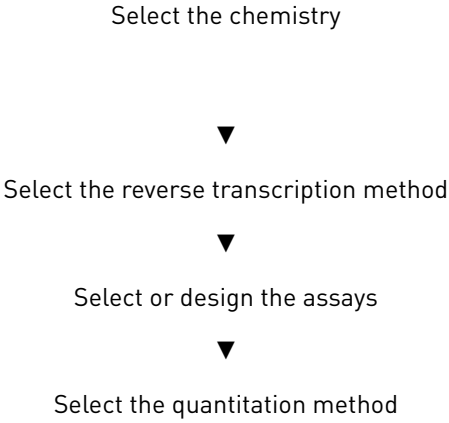
2 Plan the experiment

Select the chemistry

Two types of gene expression chemistries have been developed for real-time PCR studies.

- TaqMan® chemistry (also known as "fluorogenic 5′ nuclease chemistry")
- SYBR™ Green dye chemistry

TaqMan® chemistry

The introduction of fluorogenic-labeled probes that use the 5′ nuclease activity of Taq DNA polymerase has improved real-time PCR systems. The availability of these fluorogenic probes enabled the development of a real-time method for detecting specific amplification products.

TaqMan® probes

TaqMan® probes are dual-labeled hydrolysis probes that increase the specificity of real-time PCR assays. TaqMan® probes contain:

- A reporter dye (for example, FAM™ dye) linked to the 5′ end of the probe
- A nonfluorescent quencher (NFQ) at the 3′ end of the probe
- MGB moiety attached to the NFQ

TaqMan® MGB probes also contain a minor groove binder (MGB) at the 3′ end of the probe. MGBs increase the melting temperature without increasing probe length, allowing for the design of shorter probes.

Introduction to Gene Expression Getting Started Guide

How TaqMan® chemistry works

1. An oligonucleotide probe is constructed with a fluorescent reporter dye that is bound to the 5′ end and a quencher on the 3′ end.

   While the probe is intact, the proximity of the quencher greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer through space.

2. If the target sequence is present, the probe anneals between primer sites and is cleaved by the 5′ nuclease activity of the Taq DNA polymerase during primer extension. This cleavage of the probe:

   - Separates the reporter dye from the quencher, increasing the reporter dye signal.

   - Removes the probe from the target strand, allowing primer extension to continue to the end of the template strand. Therefore, inclusion of the probe does not inhibit the overall PCR process.

3. Additional reporter dye molecules are cleaved from their respective probes with each cycle, resulting in an increase in fluorescence intensity proportional to the amount of amplicon produced. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed.

SYBR™ Green dye chemistry

An important difference between the TaqMan® probes and SYBR™ Green dye chemistries is that the SYBR™ Green dye chemistry binds all double-stranded DNA, including nonspecific reaction products. A well-optimized reaction is therefore essential for accurate results.

How SYBR™ Green dye chemistry works

The SYBR™ Green dye chemistry uses the SYBR™ Green dye to detect PCR products by binding to the double-stranded DNA formed during PCR. Here is how this chemistry works:

1. SYBR™ Green dye fluoresces when bound to double-stranded DNA.

2. During the PCR, Taq DNA Polymerase amplifies the target sequence, which creates the PCR product, or amplicon.

3. As the PCR progresses, more amplicons are created. SYBR™ Green binds to all double-stranded DNA, resulting in an increase in fluorescent intensity proportional to the amount of PCR product produced.

Comparison of TaqMan® and SYBR™ Green dye chemistries

TaqMan® probe-based assay chemistry

Step 1. Polymerization: A fluorescent reporter (R) dye and a quencher (NFQ) are attached to the 5′ and 3′ ends of a TaqMan® probe, respectively.
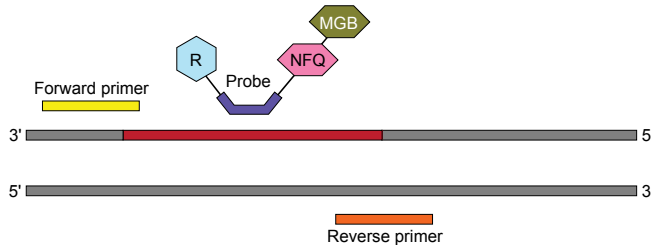
Step 2. Strand displacement: When the probe is intact, the reporter dye emission is quenched.

Step 3. Cleavage: During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe.
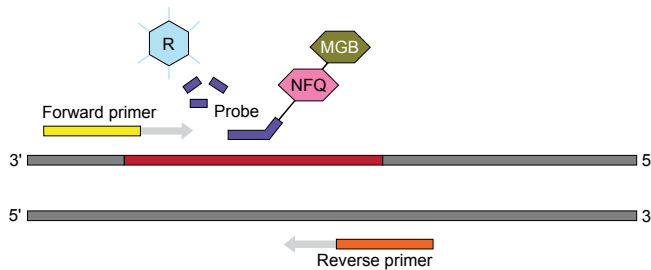
Step 4. Polymerization completed: After separation from the quencher, the reporter dye fluoresces.
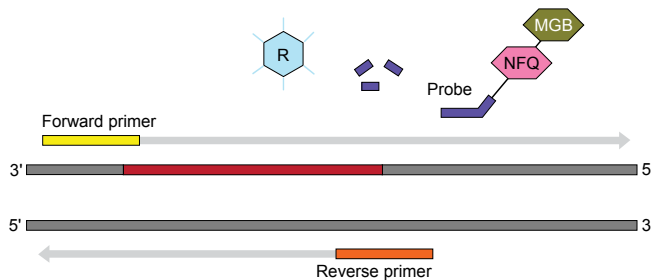
SYBR™ Green dye assay chemistry

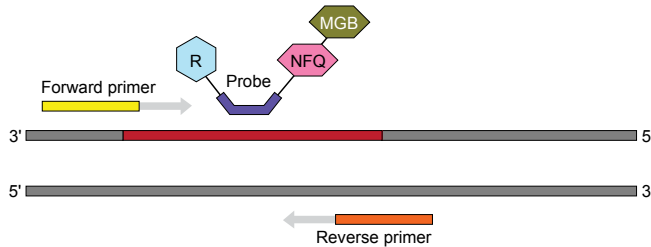Step 1. Reaction setup: The SYBR™ Green dye fluoresces when bound to double-stranded DNA.
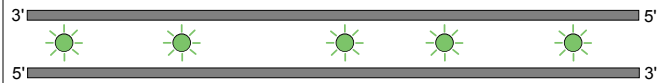
Step 2. Denaturation: When the DNA is denatured, the SYBR™ Green dye is released and the fluorescence is drastically reduced.
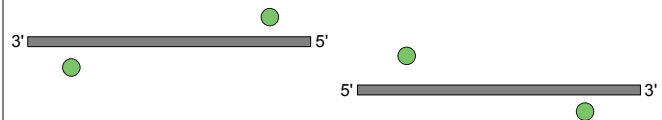
Step 3. Polymerization: During extension, primers anneal and PCR product is generated.
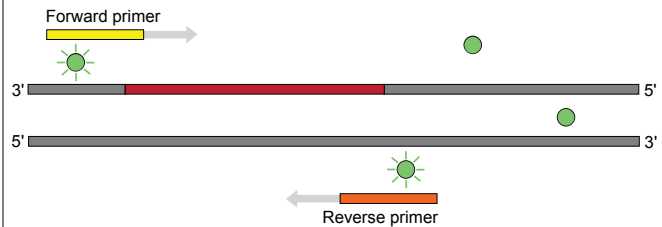
Step 4. Polymerization completed: When polymerization is complete, SYBR™ Green dye binds to double-stranded product, resulting in a net increase in fluorescence detected by the instrument.

Advantages and limitations of TaqMan® and SYBR™ Green chemistries

Consider the following aspects of each chemistry type when selecting between TaqMan® probe-based and SYBR™ Green chemistry for your quantitation assays.

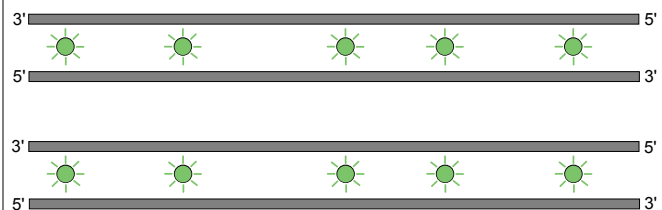| Detection	Description	Advantages	Limitations
TaqMan®	Uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR cycles. Detects specific amplification products only.	• Specific hybridization between probe and target is required to generate fluorescent signal, significantly reducing background and false positives. • Two or more specific targets can be detected in the same reaction when the probes are labeled with different dyes. Multiplex PCR can reduce cost and improve precision. • Post-PCR processing is eliminated, saving time.	A different probe has to be synthesized for each unique target sequence.
SYBR™ Green	Uses SYBR™ Green dye, a double-stranded DNA binding dye, to detect PCR product as it accumulates during PCR cycles. Detects all double-stranded DNA, including both specific and nonspecific reaction products.	• Enables you to monitor the amplification of any double-stranded DNA sequence. • Does not require probes, so your assay setup and running costs are reduced. • Multiple dye molecules can bind to a single amplified target sequence, increasing sensitivity for detecting amplification products.	• Because SYBR™ Green dye binds to any double-stranded DNA—including nonspecific double-stranded DNA sequences—it may generate false-positive signals. • Primer optimization is sometimes necessary to improve the performance of SYBR™ Green assays. • Multiplex PCR cannot be done when using SYBR™ Green. • A dissociation or "melt" of the PCR products is highly recommended for SYBR™ Green assays, which lengthens the protocol, and requires visual analysis of the peaks.

Select the reverse transcription method

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is used to quantify RNA. RT-qPCR can be performed as a one-step or two-step procedure.

The most common method for looking at gene expression is two-step RT-qPCR.

One-step RT-qPCR   With one-step RT-qPCR, the reverse transcription and PCR amplification steps are performed in a single buffer system.
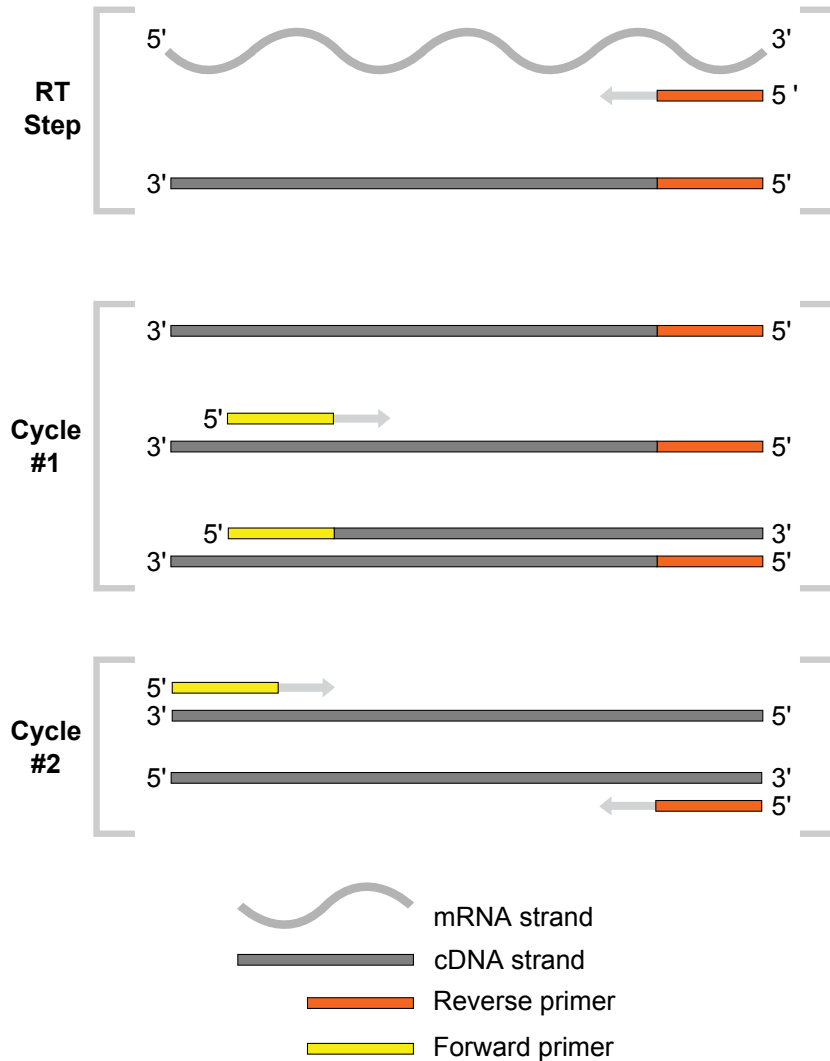
The reaction proceeds without the addition of reagents between the RT and PCR steps. One-step RT-qPCR offers the convenience of a single-tube preparation for RT and PCR amplification. This method is target- or gene-specific. Only the specific target is transcribed because one of the PCR primers is used to prime the reverse transcription. This approach is useful when studying a single gene in many samples.

Two-step RT-qPCR   With two-step RT-qPCR, the reverse transcription and PCR amplification steps are performed in two separate reactions.

mRNA strand

cDNA strand

RT primer

Reverse primer

Forward primer
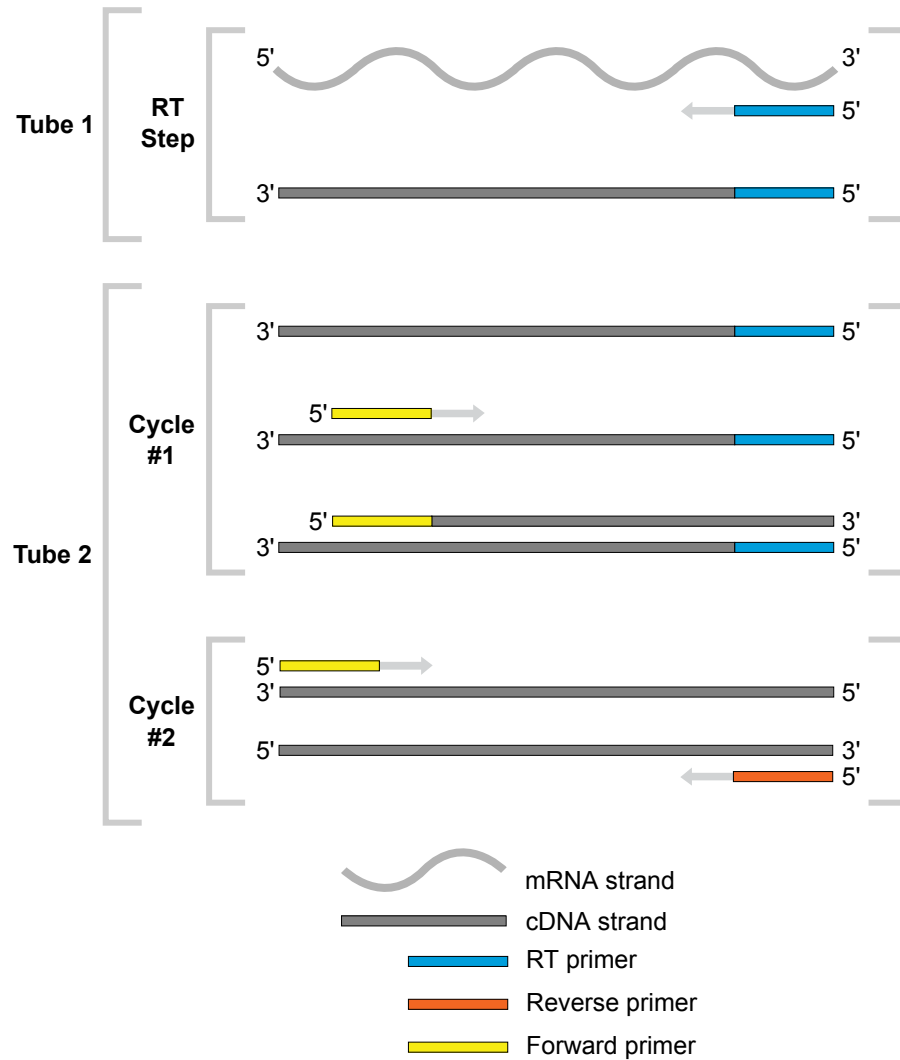
Two-step RT-qPCR is useful when detecting multiple transcripts from a single sample, or when storing a portion of the cDNA for later use. In a two-step approach, the reverse transcription is primed with either oligo $d(T)_{16}$ or random primers. Oligo $d(T)_{16}$ binds to the poly-A tail of mRNA, and random primers bind across the length of the RNA being transcribed.

Guidelines for selecting reagents   For guidelines on selecting the reverse transcription and amplification reagents, see the *TaqMan® Gene Expression Assays User Guide—single-tube assays* (Pub. No. 4333458) or go to **thermofisher.com/reverse-transcription**.

Preamplification   If you have limited samples or low expressing samples, use TaqMan® PreAmp Master Mix, which preamplifies small amounts of cDNA without introducing amplification bias to the sample. Preamplification enables you to stretch your limited sample into many more real-time PCR reactions. For more information, see the *TaqMan® PreAmp Master Mix User Guide* (Pub. No. 4384557).

Select or design the assays

When you are deciding whether to select a predesigned assay or to design a custom assay, think about your goals for the assay and consider the following factors.

Considerations for optimal assay performance

Consideration	Action
Target of interest	Identify the genes or pathway of interest.
Specificity	Select or design the assay to: • Detect all known transcripts of your gene of interest (gene-specific detection). • Detect a unique splice variant (transcript-specific detection). • Discriminate between closely related members of a gene family (homologs and potentially orthologs). Ensure specificity by checking against known sequences databases such as NCBI and Ensembl.
Efficiency	Ensure that your assay has an amplification efficiency close to 100%. Less efficient assays can result in reduced sensitivity and linear dynamic range, limiting your ability to detect low abundance transcripts.
Reproducibility	Repeating your experiment should produce the same results. Factors that could affect reproducibility are oligo manufacturing and assay formulation, and primer-dimer formation.

Preformulated assays and PCR arrays

Whether you are studying single genes or whole pathways, there are many choices of preformulated assays and PCR arrays.

- Preformulated assays in tubes are appropriate when you are a studying small number of genes or when you need maximum flexibility.
- PCR arrays are 96- or 384-well plates or microfluidic cards that are loaded with assays corresponding to pathways or other common gene sets. This format is appropriate when you are studying many genes or when you are trying to narrow down the number of genes you want to focus on in your experiment.

If your gene targets are not available as commercial primer or probe sets, you could use either of the following tools to design customized assays:

- A commercial assay design tool or service, such as the Custom TaqMan® Assay Design Tool (**thermofisher.com/cadt**)
- A public primer or probe design tool

Endogenous controls

In any gene expression study, selecting a valid normalization or endogenous control to correct for differences in RNA sampling is critical to avoid misinterpretation of results. TaqMan® Endogenous Controls consist of the most commonly used housekeeping genes in human, mouse, and rat, and these controls are provided as a preformulated set of predesigned probe and amplification primers.

For more information on selecting or ordering endogenous control assays, go to **thermofisher.com/taqmancontrols**.

Singleplex PCR vs. duplex PCR

Duplex PCR is the simultaneous amplification of two target sequences in a single reaction.

Duplex real-time PCR is possible using TaqMan® probe–based assays, in which each assay has a specific probe that is labeled with a unique fluorescent dye, resulting in different observed colors for each assay. Real-time PCR instruments can discriminate between the different dyes. The signal from each dye is used independently to quantitate the amount of each target. Running both assays in a single tube reduces both the running costs and the dependence on accurate pipetting when splitting a sample into two separate tubes.

Typically one probe is used to detect the target gene and another probe is used to detect an endogenous control (reference gene). For example, a FAM™-labeled assay could be used for the gene of interest and a VIC™-labeled assay could be used for the endogenous control.

Duplex PCR is not possible when using SYBR™ Green chemistry.

Consider the following advantages and limitations when selecting between duplex and singleplex PCR:

PCR	Description	Advantage	Limitation
Singleplex	A reaction in which a single target is amplified in the reaction tube or well.	• No optimization is required for TaqMan® assays. • Flexibility to use TaqMan® or SYBR™ Green reagents.	Requires separate reactions for the target and the endogenous control assay.
Duplex	A reaction in which two targets are amplified in the same reaction tube or well.	Reduces the: • Running costs. • Dependence on accurate pipetting.	Requires validation and optimization.

Select the quantitation method

Methods for relative quantitation of gene expression enable you to quantify differences in the expression level of a specific target (gene) between different samples. The data output is expressed as a fold-change or a fold-difference of expression levels. For example, you can look at the change in expression of a particular gene over a given time period in treated versus untreated samples.

Select the quantitation method:

- Comparative C_t ($\Delta\Delta\ C_t$) method (relative quantitation)
- Relative standard curve method (relative quantitation)
- Standard curve method (absolute quantitation)

Comparative C_t method

Relative quantitation is a technique that is used to analyze changes in gene expression in a given sample relative to a reference sample (such as an untreated control sample).

Uses

Comparative C_t experiments are commonly used to:

- Compare expression levels of a gene in different tissues.
- Compare expression levels of a gene in treated versus untreated samples.
- Compare expression levels of genes in samples that are treated with a compound under different experimental conditions, over a time-course and study-defined length of time.

Experimental validation

To use the comparative C_t method, the efficiencies of the target and endogenous control amplifications should be approximately equal. If you are using a pre-designed Thermo Fisher ScientificTaqMan® Gene Expression Assay, there is no need to run a validation experiment. A validation experiment is recommended if you are multiplexing, if you are using a Custom TaqMan® Gene Expression Assay, or if you purchase assays from another source.

Relative standard curve method

Similar to the comparative C_t method, the relative standard curve method can be used to determine fold changes in gene expression. Generally, use the relative standard curve method when you use two assays for quantitation (an assay for the target gene and an assay for endogenous control) that did not have equivalent amplification efficiency. A dilution series is created from a common sample and run with both the target and the endogenous control gene. For all experimental samples, a quantity is determined from this dilution series, and a fold change in expression can be calculated from this data.

For more information, go to **thermofisher.com/qpcreducation**.

Standard curve method

Use the standard curve method to determine the absolute target quantity in samples. With the standard curve method, the real-time PCR system software measures amplification of the target in samples and in a standard dilution series of known copy number. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates the absolute quantity of target in the samples. The standard curve method is the least common method for quantitation of gene expression.

Advantages and limitations of the quantitation methods

Consider the following advantages and limitations when selecting the quantitation method:

Experiment type	Advantage	Limitation
Comparative C_t ($\Delta\Delta C_t$)	• Relative levels of target in samples can be determined without the use of a standard curve or dilution series. • Requires reduced reagent usage. • More space is available in the reaction plate. • Because a standard curve is not needed, throughput can increase. • Dilution errors made in creating the standard curve samples are eliminated. • The target and endogenous control can be amplified in the same tube, increasing throughput and reducing pipetting errors.	• Suboptimal (low PCR efficiency) assays can produce inaccurate results. • The PCR efficiencies for the target assay and the endogenous control assay must be approximately equal. Therefore, validation experiments are recommended when using multiplex assays, Custom TaqMan® Gene Expression Assays, and assays purchased from another source.
Relative standard curve	Requires the least amount of validation because the PCR efficiencies of the target and endogenous control do not need to be equivalent.	A dilution series must be run for each target; a series requires more reagents and more space in the reaction plate.
Absolute quantitation (standard curve)	Absolute, instead of relative, quantities of transcripts are calculated.	• The required standard curve for each target requires more reagents and more space in the reaction plate. • Often a standard with known copy numbers is not available.

Resources for data analysis

For detailed information about data analysis, see the appropriate documentation for your instrument. Use the absolute or relative quantification ($\Delta\Delta C_t$) methods to analyze results.

The general guidelines for analysis include:

- View the amplification plot; then, if needed:
  - Adjust the baseline and threshold values.
  - Remove outliers from the analysis.
- In the well table or results table, view the C_t values for each well and for each replicate group.

Perform additional analysis using any of the following software:

Software	Resource
Relative Quantification app	thermofisher.com/us/en/home/cloud.html
Standard Curve app	
ExpressionSuite™ Software[1]	thermofisher.com/us/en/home/technical-resources/software-downloads/expressionsuite-software.html

[1] Can automatically define the baseline. Files from a QuantStudio™ 3 or 5 System are not compatible.

For more information about real-time PCR, see page 31 or go to **thermofisher.com/qpcreducation**.

For more information about real-time PCR, go to: **thermofisher.com/qpcreducation** or to "Related documentation" on page 31.

A
Ordering Information

TaqMan® Gene Expression Assay formulations

To find and order predesigned, preformulated primer and probe sets in a variety of species, go to **thermofisher.com/taqmangeneexpression**, then use the Assay Search Tool.

Table 1 Standard formulations

Product	Dye	Size	Number of 20-µL reactions	Cat. No.	Concentration
TaqMan® Gene Expression Assays	FAM™	Extra Small	75	4453320[1] or 4448892	20X
		Small	250	4331182[1]	
		Small	360	4351372	
		Medium	750	4351370	
		Large	2900	4351368	60X
	VIC™	Small	360	4448489	20X
		Medium	750	4448490	
		Large	2900	4448491	60X
TaqMan® Gene Expression Assays, Primer-Limited (PL)	VIC™	Small	360	4448484	20X
		Medium	750	4448485	
		Large	2900	4448486	60X
TaqMan® Non-coding RNA Assay	FAM™	Small	360	4426961	20X
		Medium	750	4426962	
		Large	2900	4426963	60X

[1] This product is inventoried.

Custom TaqMan® Gene Expression Assay formulations

Use the Custom TaqMan® Assay Design Tool (**www.thermofisher.com/cadt**) to enter and submit sequences for new assay design. The tool also supports submission files created using FASTA file format. For details, see the *Custom TaqMan® Assays Design and Ordering Guide* (Pub. No. 4367671).

For a comparison of Custom and Custom Plus Assay products go to **thermofisher.com/customtaqmangex**.

Table 2   Custom formulations

Product	Dye	Size	Number of 20 µL reactions	Cat. No.	Concentration
Custom Plus TaqMan® RNA Assay	FAM™	Small	360	4441114	20X
		Medium	750	4441117	
		Large	2900	4441118	60X
	VIC™	Small	360	4448514	20X
		Medium	750	4448515	
		Large	2,900	4448516	60X
Custom PlusTaqMan® RNA Assay, Primer-Limited (PL)	VIC™	Small	360	4448511	20X
		Medium	750	4448512	
		Large	2900	4448513	60X
Custom TaqMan® Gene Expression Assays	FAM™	Small	360	4331348	20X
		Medium	750	4332078	
		Large	2900	4332079	60X
	VIC™	Small	360	4448508	20X
		Medium	750	4448509	
		Large	2900	4448510	60X
Custom TaqMan® Gene Expression Assay, Primer-Limited (PL)	VIC™	Small	360	4448487	20X
		Medium	750	4448488	
		Large	2900	4448492	60X

Assay primer and probe concentrations

Assay type	Concentration					
	Forward primer		Reverse primer		Probe	
	1X	20X	1X	20X	1X	20X
TaqMan® Gene Expression Assays (FAM™ or VIC™)	900 nM	18 µM	900 nM	18 µM	250 nM	5 µM
TaqMan® Gene Expression Assays (VIC™, Primer Limited)[1]	150 nM	3 µM	150 nM	3 µM		

[1] Recommended for multiplexing. For more information, see ¨Guidelines for duplex reactions using TaqMan® Gene Expression Assays¨ on page 26.

TaqMan® Array Plates

TaqMan® Array Plates are MicroAmp™ Optical 96-Well Reaction Plates, Standard or Fast, that contain dried-down TaqMan® Gene Expression Assays. The gene expression assays are a collection of predesigned, gene-specific primer and probe sets for performing quantitative gene expression studies on cDNA. The assays are available for multiple species. For more information, see the *TaqMan® Gene Expression Assays User Guide—TaqMan® Array Plates* (Pub. No. 4391016).

Table 3 Available plate formats and configurations

Description	Customizable	Standard (0.2-mL) plates	Fast (0.1-mL) plates
Fixed-content plate	—	✓	✓
Preconfigured (fixed-content) plates with the most sought-after predefined gene panels, categorized by specific disease, pathway, or biological process.			
Flexible-content plate	✓	✓	✓
Modifiable plates configured with a suggested selection of assays from predefined gene panels, categorized by specific disease, pathway, or biological process. Allows the substitution of preselected assays with other predesigned assays that target more relevant genes or specific markers of interest for experiments.			
Custom-configured plate	✓	✓	✓
Fully customizable plates. Allows the configuration of the plate with any predesigned assays.			

TaqMan® Array Card overview

TaqMan® Array Cards are 384-well microfluidic cards prepared with dried-down TaqMan® Assays. With array cards, gene expression is measured using the comparative C_t ($\Delta\Delta C_t$) method of relative quantitation. For more information about relative quantitation, see the **Resources** section at **thermofisher.com/taqman**.

Advantages of using TaqMan® Array Cards include:

- Small-volume design minimizes sample and reagent consumption.
- Streamlined reaction setup saves time and reduces labor-intensive steps.
- Access to high-throughput, 384-well format without liquid-handling robotics.
- Two-fold discrimination detection at the 99.7% confidence level.
- Standardization across multiple samples in multiple laboratories.

You can run 1 to 8 samples per card, against 12 to 384 TaqMan® Assay targets (including controls).
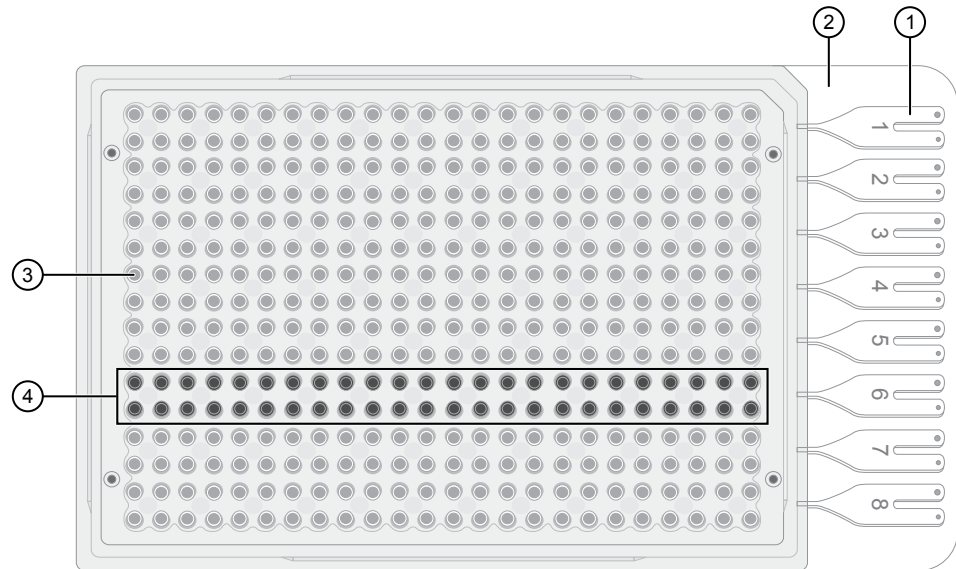
① **Fill reservoir**—Each reservoir is loaded with a sample-specific PCR reaction mix; the associated reaction wells fill with that sample (8 total reservoirs)

② **Fill reservoir strip**—Support strip for fill reservoirs; removed before running the card

③ **Reaction well**—Each well contains dried-down assay (384 total reaction wells)

④ **Reaction well row**—A set of reaction wells that fill with the same sample-specific PCR reaction mix (8 total rows, each row associated with a single fill reservoir)

B   Design TaqMan® Assays, Primers, and Probes

Design Custom TaqMan® Gene Expression Assays

Use the Custom TaqMan® Assay Design Tool (**www.thermofisher.com/cadt**) to enter and submit sequences for new assay design. The tool also supports submission files created using FASTA file format. For details, see the *Custom TaqMan® Assays Design and Ordering Guide* (Pub. No. 4367671).

See Table 2 for available formulations.

Guidelines for duplex reactions using TaqMan® Gene Expression Assays

Duplex real-time PCR is the simultaneous amplification and measurement of two target sequences in one reaction. TaqMan® Gene Expression Assays can be used in duplex real-time PCR when using a FAM™ dye-labeled assay in combination with a primer-limited, VIC™ dye-labeled assay. When setting up a duplex reaction:

- Validate that your duplex assay combinations provide similar results to your singleplex reactions.
- Consider the relative expression levels of each target.
- Perform serial dilutions of your sample in both singleplex and duplex reactions, and compare the results for relative expression.
- Select the higher-expressing target as the primer-limited, VIC™ dye-labeled assay.
- Use TaqMan® Fast Advanced Master Mix, which has been optimized for duplexing reactions.

For more details on how to validate your duplex assay reactions and interpret the results, see *TaqMan® Multiplex PCR Optimization User Guide* (Pub. No. MAN0010189) or go to **thermofisher.com/us/en/home/life-science/pcr/real-time-pcr.html**.

Design TaqMan® custom primers

Primers can be designed using Primer Express software as described in the *Primer Express Software Version 3.0 Getting Started Guide* (Pub. No. 4362460). Follow these guidelines when designing primers:

- Choose the primers after selecting the probe.
- Design the primers as close as possible to the probe without overlapping the probe.
- Keep the G-C content in the 20% to 80% range.
- Avoid runs of an identical nucleotide, especially for guanine, where runs of four or more Gs should be avoided.
- Set the melting temperature of each primer between 58°C and 60°C.

- Avoid having more than two G and/or C bases in the five nucleotides at the 3′ end of each primer.
- Set amplicon length betweem 50 and 150 bases for optimum PCR efficiency.

Design TaqMan® probes

TaqMan® fluorogenic probes enable the detection of a specific PCR product as it accumulates during PCR cycles. The Thermo Fisher Scientific patented fluorogenic probe design, which incorporates the reporter dye on the 5′ end and the quencher dye on the 3′ end, has greatly simplified the design and synthesis of effective 5′ fluorogenic nuclease assay probes (Livak, Flood, et al., 1995).

TaqMan® MGB probes

TaqMan® minor groove binder (MGB) probes contain:

- A reporter dye (for example, FAM™ dye) linked to the 5′ end of the probe.
- A nonfluorescent quencher (NFQ) at the 3′ end of the probe.
  Because the quencher does not fluoresce, Thermo Fisher Scientific real-time PCR systems can measure reporter dye contributions more accurately.
- MGB moiety attached to the NFQ.
  MGBs increase the melting temperature without increasing probe length (Afonina et al., 1997, Kutyavin et al., 1997). They also allow for the design of shorter probes.

Guidelines for designing custom TaqMan® probes

Probes can be designed using Primer Express software, as described in the *Primer Express Software Version 3.0 Getting Started Guide* (PN *4362460*). Follow these guidelines when designing probes:

- Design the probe length to be between 13 to 25 bases (13 to 30 bases if using conventional TaqMan® probes).
- Design the melting temperature (T_m) to be 68°C–70°C.
- For a primer, set the guanine-cytosine (GC) content between 30% and 80%.
- The 5′ end cannot be a guanosine residue. A guanosine residue adjacent to the reporter dye somewhat quenches the reporter fluorescence, even after cleavage.
- Avoid including long sequences of identical nucleotides.

C

Safety

⚠️ **WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

⚠ **WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:

  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:

  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Pub. No.
TaqMan® Gene Expression Assays Quick Reference—single-tube assays	4401212
TaqMan® Gene Expression Assays User Guide—single-tube assays	4333458
Understanding Your Shipment	MAN0017153
Custom TaqMan® Assays Design and Ordering Guide	4367671
TaqMan® Multiplex PCR Optimization User Guide	MAN0010189
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
QuantStudio™ Design and Analysis desktop Software User Guide	MAN0010408
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide	4489821
QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Getting Started Guide	4489822
QuantStudio™ 12K Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide	4470689
QuantStudio™ 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470050
StepOne™ or StepOnePlus™ Real-Time PCR System	
StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking and Maintenance User Guide	4376782
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide	4376785
ViiA™ 7 Real-Time PCR System	
Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security	4442661
Applied Biosystems™ ViiA™ 7 Real-Time PCR System Getting Started Guide	4441434

Document	Pub. No.
7500/7500 Fast Real-Time PCR System	
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4347828
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Standard Curve and Comparative C_t Experiments	4387783
Data analysis	
Real-Time PCR Systems Chemistry Guide: Applied Biosystems™ 7900HT Fast Real-Time PCR System and 7300/7500 Real-Time PCR Systems	4348358
Applied Biosystems™ 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide	4364016
Applied Biosystems™ 7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide	4364014
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve	4347825
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Quantitation using Comparative C_t	4347824
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide	4376785
Applied Biosystems™ Relative Quantitation Analysis Module Help	MAN0010505
Applied Biosystems™ Relative Quantitation Analysis Module User Guide	MAN0014820
Applied Biosystems™ Standard Curve Analysis Module User Guide	MAN0014819

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.