

TaqMan[®] Gene Expression Assays

Protocol

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Preface


This preface contains:


Safety v
How to Obtain Support ix


Safety Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined below:


IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

 **CAUTION** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

 **WARNING** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

 **DANGER** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

 **WARNING CHEMICAL HAZARD.** Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “About MSDSs” on page vi.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to <https://docs.appliedbiosystems.com/msdssearch.html>

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2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
 3. Find the document of interest, right-click the document title, then select any of the following:
 - **Open** – To view the document
 - **Print Target** – To print the document
 - **Save Target As** – To download a PDF version of the document to a destination that you choose
 4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
 5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Waste Hazard



WARNING CHEMICAL WASTE HAZARD. Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.

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- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
 - Handle chemical wastes in a fume hood.
 - After emptying the waste container, seal it with the cap provided.
 - Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument 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, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; <http://bmbi.od.nih.gov>)

-
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).

Additional information about biohazard guidelines is available at: <http://www.cdc.gov>

How to Obtain Support

For the latest services and support information for all locations, go to <http://www.appliedbiosystems.com>, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.



Product Overview

Product Description TaqMan[®] Gene Expression Assays are a comprehensive collection of predesigned primer and probe sets, which help researchers quickly and easily perform quantitative gene expression studies on human, mouse, and rat genes.

Product Properties TaqMan Gene Expression Assays are built on Applied Biosystems 5' nuclease chemistry. Each assay consists of two unlabeled PCR primers and a FAM[™] dye-labeled TaqMan MGB probe. All components are quality control-tested and formulated as a single 20X mix.

TaqMan Gene Expression Assays are designed to

- Run under universal conditions for two-step RT-PCR.
- Work with TaqMan Universal PCR Master Mix (with or without AmpErase[®] UNG)
- Amplify target cDNA without amplifying genomic DNA (*m* suffix in assay ID), when possible. This is achieved by designing probes that cross exon-exon junctions.

The latest information on specific product uses is available on the Applied Biosystems Web site:

<http://www.allgenes.com>

Available TaqMan Gene Expression Assays Products TaqMan Gene Expression Assays (PN 4331182) are available for human, mouse, and rat genes. The prefix of the assay name indicates the species for which the assay was designed: *Hs* for *Homo sapiens* (human), *Mm* for *Mus musculus* (mouse), and *Rn* for *Rattus norvegicus* (rat).

Visit our Web site to view the available products:

<http://www.allgenes.com>

For more information about ordering TaqMan Gene Expression Assays, see “Ordering Assays” on page 8.

Related Products **TaqMan Low Density Array**

TaqMan Low Density Arrays are 384-well microfluidic cards, delivered with customer-selected TaqMan Gene Expression Assays dried into each of the 2 μ L-reaction wells.

The TaqMan Array enables researchers to manually perform small-volume quantitative real-time PCR without the need for robotic pipetting stations. Additionally, the design of the low-density array eliminates other problems associated with small volume reactions, such as evaporation and pipetting errors.

Custom TaqMan Gene Expression Assays

Each Custom TaqMan Gene Expression Assay is a probe and primer set that Applied Biosystems designs for a specific user-submitted sequence, supplied in single-tube format. You can submit sequences for any species, gene, or splice variant.

TaqMan Endogenous Controls

TaqMan Endogenous Controls are predesigned probe and primer sets for human, mouse, and rat genes. TaqMan Endogenous Controls, supplied in single-tube format, are available in both large- and small-scale synthesis, with either VIC[®] or FAM dye-labeled TaqMan probes.

About This Protocol

This protocol provides the following:

- Background information about gene expression assays
- A list of materials and equipment that can be used with TaqMan Gene Expression Assays
- Guidelines for synthesizing cDNA from total RNA using the High Capacity cDNA Archive Kit (PN 4322171)
- Instructions for amplifying cDNA using TaqMan Gene Expression Assays on an Applied Biosystems Sequence Detection System (SDS)
- General guidelines for data analysis

Chemistry Overview

Reaction Components

Reaction components include:

- TaqMan Gene Expression Assay:
 - Two unlabeled primers for amplifying the sequence of interest (final concentration of 900 nM each)
 - One TaqMan MGB probe (6-FAM dye-labeled) for detecting the sequence of interest (final concentration of 250 nM)
- A PCR Master Mix and DNA polymerase, which you supply
- A cDNA for other target, which you supply

Two-Step RT-PCR

In the reverse transcription (RT) step, cDNA is reverse transcribed from total RNA samples using random primers from the High Capacity cDNA Archive Kit. In the PCR step, PCR products are synthesized from cDNA samples using the PCR Master Mix (with or without AmpErase UNG).

Figure 1 illustrates the assay steps.

Note: Figure 1 does not show hybridization of the TaqMan MGB probe (6-FAM dye-labeled).

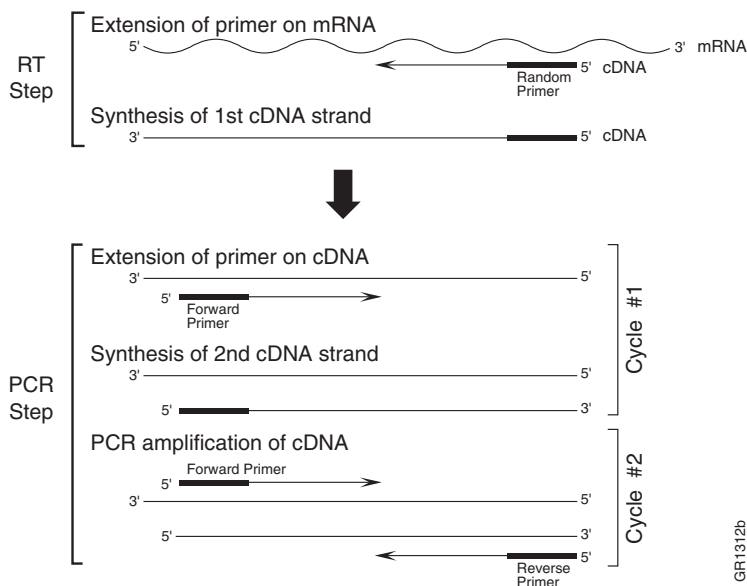


Figure 1 Two-step RT-PCR process

GR1312b

About the Probes

The TaqMan MGB probes contain:

- A reporter dye (6-FAM) linked to the 5' end of the probe.
- A minor groove binder (MGB).

MGBs increase the melting temperature (T_m) without increasing probe length (Afonina *et al.*, 1997; Kutuyavin *et al.*, 1997); they also allow the design of shorter probes.

- A nonfluorescent quencher (NFQ) at the 3' end of the probe.

5' Nuclease Assay Process

The 5' nuclease assay process (Figures 2 through 6) takes place during PCR amplification. This process occurs in every cycle and does not interfere with the exponential accumulation of product.

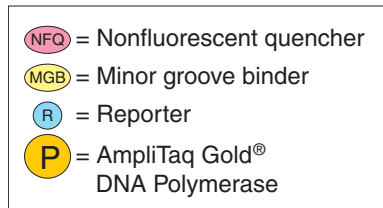


Figure 2 Legend for 5' nuclease assay process figures

During PCR, the TaqMan MGB probe (6-FAM dye-labeled) anneals specifically to a complementary sequence between the forward and reverse primer sites (Figure 2).

When the probe is intact (Figures 2 and 3), the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence primarily by Förster-type, or fluorescent resonance energy transfer (FRET) (Förster, 1948; Lakowicz, 1983).

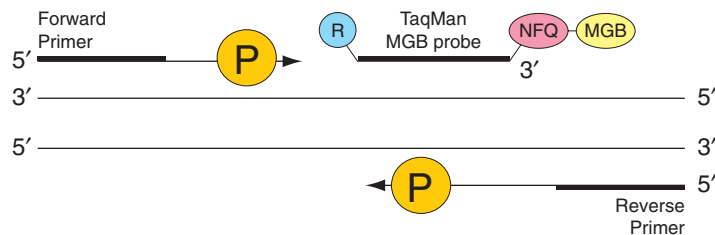


Figure 3 Polymerization

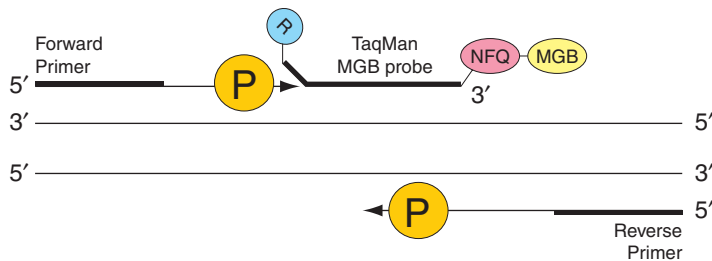


Figure 4 Strand displacement

The DNA polymerase cleaves only probes that are hybridized to the target (Figure 4). Cleavage separates the reporter dye from the quencher dye, resulting in increased fluorescence by the reporter. The increase in fluorescence signal occurs only if the target sequence is complementary to the probe and is amplified during PCR. Because of these requirements, any nonspecific amplification is not detected.

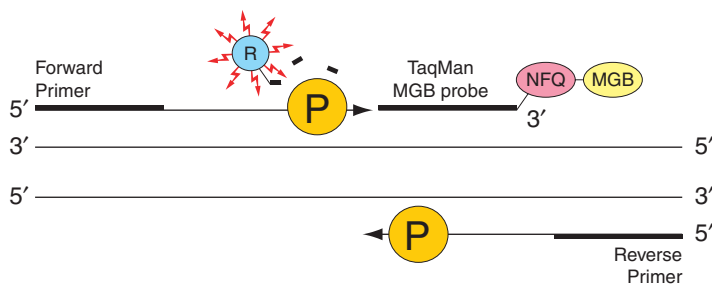


Figure 5 Cleavage

Polymerization of the strand continues, but the 3' end of the probe is blocked to prevent extension of the probe during PCR (Figure 5).

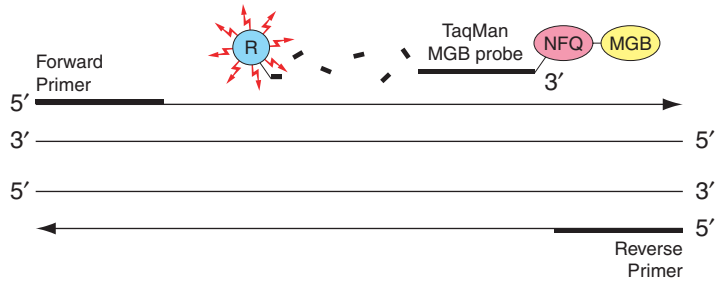


Figure 6 Completion of polymerization

Definitions of Terms

This protocol assumes these definitions:

- **Endogenous control**—A gene present at a consistent expression level in all experimental samples. By using an endogenous control as an active reference, you can normalize quantification of a cDNA target for differences in the amount of cDNA added to each reaction.

An endogenous control assay is performed in a separate well from the target assay in a singleplex reaction.

- **Target**—The genetic sequence of interest

Materials and Equipment

Available Products The TaqMan Gene Expression Assays are available as shown in the table below.

Product	Part Number	Contents	Volume/# of Rxns
TaqMan Gene Expression Assays	4331182	20X formulation of sequence-specific primers (unlabeled) and TaqMan MGB probe (6-FAM dye-labeled)	250 μ L
TaqMan Endogenous Controls	Multiple. Visit http://www.allgenes.com for more information.	20X formulation of sequence-specific probe and primer sets for human, mouse, and rat genes commonly used as endogenous controls. Available in both large- and small-scale synthesis, with either VIC or FAM dye	200 or 1000

Assay Naming TaqMan Gene Expression Assays are named using the following 13-digit format:

Abnnnnnnnn_XY

Where

- *Ab* corresponds to the species: *Hs* for *Homo sapien*(human), *Mm* for *Mus musculus* (mouse), *Rn* for *Rattus norvegicus* (rat)
- *nnnnnnnn* corresponds to the eight digits identifying the assay
- *X* corresponds to the exons
 - *m* for assays over an exon-exon boundary
 - *s* for assays within an exon or to a single-exon gene
 - *g* for assays that are over an exon-exon boundary but that may still detect genomic DNA
 - *mH*, *sH*, or *gH* for assays that detect transcripts that belong to gene families with high sequence homology.

These assays yield a 10 to 15Ct difference between the target gene and the gene with the closest sequence homology. They detect the target transcript with 1,000 to 30,000-fold greater sensitivity than the closest homologous transcript, when both transcripts are present in the same copy number in a sample.

- *Y* corresponds to the chemistry version

Ordering Assays Visit our Web site to order the available assays:

<http://www.allgenes.com>

You can search our online catalogs using gene symbols, public accession numbers, or protein classifications.

Storage and Stability

Follow the guidelines below for storing TaqMan Gene Expression Assays:

- Store the assay at –15 to –25 °C.
- Minimize freeze-thaw cycles.
- Keep all TaqMan Gene Expression Assays protected from direct exposure to light. Excessive exposure to light may affect the fluorescent probes.

Equipment and Materials Not Included

The following tables include equipment and materials for using TaqMan Gene Expression Assays. Unless otherwise noted, many of the items listed are available from major laboratory suppliers (MLS).

Instruments from Applied Biosystems

Instruments	Source
ABI PRISM® 7900HT Sequence Detection System	Contact your local Applied Biosystems sales office.
ABI PRISM 7700 Sequence Detection System	
ABI PRISM 6700 Automated Nucleic Acid Workstation	
Applied Biosystems 7300 Real-Time PCR System	
Applied Biosystems 7500 Real-Time PCR System	
ABI PRISM 6100 Nucleic Acid PrepStation	
GeneAmp® PCR System 9700 thermal cycler	
GeneAmp PCR System 9600 thermal cycler	

User-supplied materials

Materials	Source
ABI PRISM 96-Well Optical Reaction Plate With Barcode (code 128)	Applied Biosystems (PN 4306737)
ABI PRISM 96-Well Optical Reaction Plate With Barcode (code 128)	Applied Biosystems (PN 4326659)

User-supplied materials (continued)

Materials	Source
ABI PRISM 384-Well Clear Optical Reaction Plate With Barcode (code 128)	Applied Biosystems (PN 4309849)
ABI PRISM Optical Adhesive Covers	Applied Biosystems (PN 4311971)
ABI PRISM Optical Caps, 8 caps/strip	Applied Biosystems (PN 4323032)
ABI PRISM Cap Installing Tool	Applied Biosystems (PN 4330015)
High-Capacity cDNA Archive Kit	Applied Biosystems (PN 4322171)
MicroAmp® Multi Removal Tool	Applied Biosystems (PN 4313950)
Reagent Tubes With Caps, 10-mL	Applied Biosystems (PN 4305932)
RNase inhibitor	Applied Biosystems (PN 8080119)
TaqMan Universal PCR Master Mix	Applied Biosystems (PN 4304437)
TaqMan Universal PCR Master Mix, No AmpErase UNG	Applied Biosystems (PN 4324018)
Accessories for tubes of assay mixes <ul style="list-style-type: none"> • Decapper for single caps (PN 54000) • Decapper for eight caps (PN 54001) • TPE cap cluster for simultaneously capping of 96 individual polypropylene tubes, 50 capmats/bag (PN 53001) 	Micronic BV ^a PO Box 604 8200 AP Lelystad Netherlands Telephone: +31(0)320.277077 Fax: +31(1)320.277088
Centrifuge with plate adapter	MLS
Disposable gloves	MLS
Microcentrifuge	MLS
Pipet tips, aerosol resistant	MLS
Pipettors: <ul style="list-style-type: none"> • Positive-displacement • Air-displacement • Multichannel 	MLS
Polypropylene tubes	MLS
RNase-free, sterile-filtered water	MLS

User-supplied materials (*continued*)

Materials	Source
Vortexer	MLS

a. Other vendors supply similar products

Applied Biosystems Documents

Documents	Part Number
<i>High-Capacity cDNA Archive Kit Protocol</i>	4322169
<i>User Bulletin #2: Relative Quantitation of Gene Expression</i>	4303859
<i>ABI PRISM® 7900HT Sequence Detection System and SDS Enterprise Database User Guide</i>	4317596

Preventing Contamination

Overview PCR assays require special laboratory practices to avoid false positive amplifications (Kwok and Higuchi, 1989). The high throughput and repetition of these assays can lead to amplification of a single DNA molecule (Saiki *et al.*, 1985; Mullis and Faloona, 1987).

AmpErase UNG AmpErase uracil-N-glycosylase (UNG) is a pure, nuclease-free, 26-kDa recombinant enzyme encoded by the *Escherichia coli* uracil-N-glycosylase gene. This gene has been inserted into an *E. coli* host to direct expression of the native form of the enzyme (Kwok and Higuchi, 1989).

UNG acts on single- and double-stranded dU-containing DNA by hydrolyzing uracil-glycosidic bonds at dU-containing DNA sites. The enzyme causes the release of uracil, thereby creating an alkali-sensitive apyrimidic site in the DNA. Apyrimidinic sites block replication by DNA polymerases. The enzyme has no activity on RNA or dT-containing DNA (Longo *et al.*, 1990).

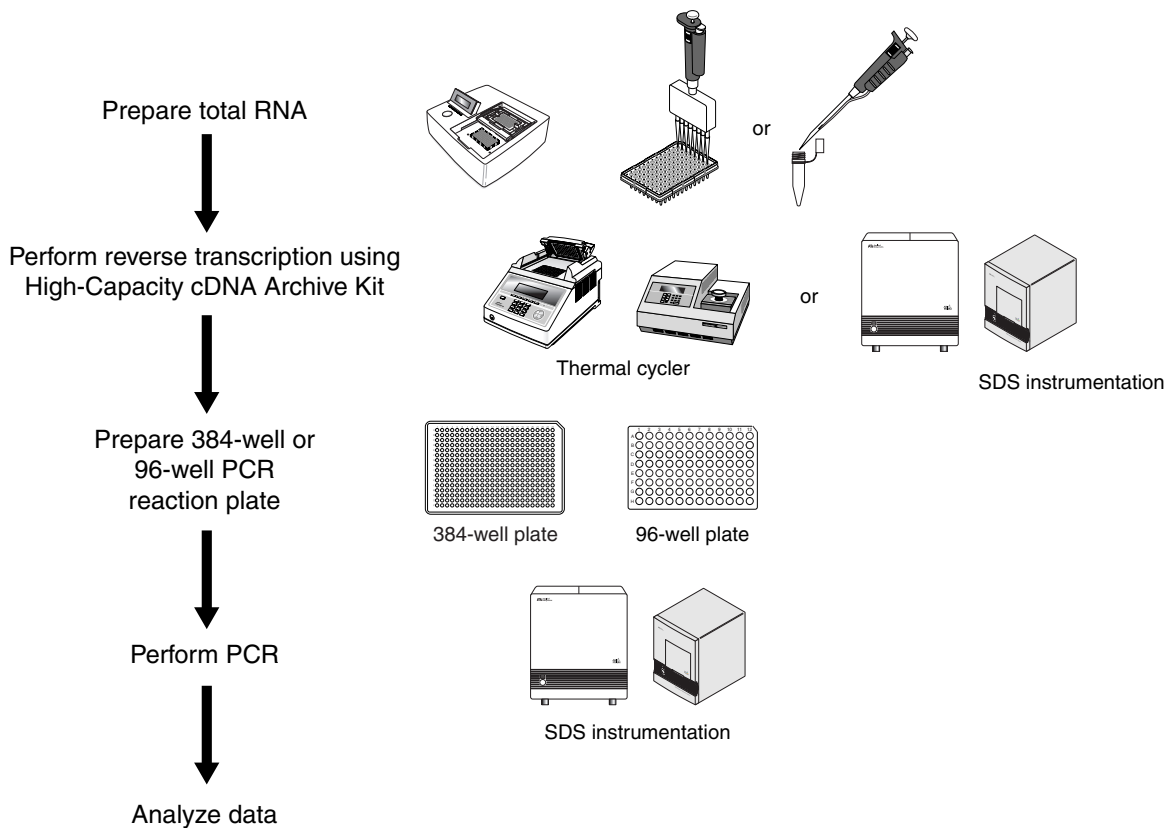
General PCR Practices

Follow these recommended procedures:

- Maintain separate areas, dedicated equipment, and supplies for:
 - Sample preparation
 - PCR setup
 - PCR amplification
 - Analysis of PCR products
- Do not bring amplified PCR products into the PCR setup area.
- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves when preparing samples for PCR amplification.
- Change gloves whenever you suspect that they are contaminated.
- Open and close all sample tubes and reaction plates carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use positive-displacement pipets or aerosol-resistant pipet tips.
- Clean lab benches and equipment periodically with freshly diluted 10% bleach solution.

Procedural Overview

The following diagram provides a simplified overview of the procedure for using TaqMan Gene Expression Assays.



Performing Reverse Transcription

Overview Synthesis of cDNA from total RNA samples is the first step in using TaqMan Gene Expression Assays. Applied Biosystems recommends using the High-Capacity cDNA Archive Kit to obtain cDNA from total RNA samples.

IMPORTANT! Applied Biosystems has designed and developed TaqMan Gene Expression Assays for use with samples reverse transcribed from total RNA using the High Capacity cDNA Archive Kit. Other protocols have not been tested for use with TaqMan Gene Expression Assays.

General Process Use the High-Capacity cDNA Archive Kit to synthesize single-stranded cDNA from total RNA samples. The process involves the following procedures:

1. Preparing the RT master mix
2. Preparing the cDNA archive reaction plate
3. Performing reverse transcription

Note: Refer to the *High-Capacity cDNA Archive Kit Protocol* (PN 4322169) for additional guidelines and instructions. The protocol is not shipped with the High Capacity cDNA Archive Kit. You must download the protocol from the Applied Biosystems Documents on Demand Web site at

<http://docs.appliedbiosystems.com/search.taf>

RNA Template Guidelines For optimal performance of the High-Capacity cDNA Archive Kit and of the TaqMan Gene Expression Assays, Applied Biosystems recommends using RNA with the following characteristics:

- Greater than 60 μL of sample
- Between 0.002 and 0.2 $\mu\text{g}/\mu\text{L}$ in concentration of RNA
- Less than 0.005% of genomic DNA by weight
- Free of inhibitors of reverse transcription and PCR
- Dissolved in PCR-compatible buffer

-
- Free of RNase activity

Note: If you suspect that the RNA contains RNase activity, add RNase inhibitor to the reverse transcription reaction at a final concentration of 1.0 U/ μ L. It is not necessary to add RNase inhibitor to the reverse transcription reaction if the RNA was purified using the ABI PRISM 6700 Automated Nucleic Acid Workstation or the ABI PRISM 6100 Nucleic Acid PrepStation and Applied Biosystems nucleic acid purification reagents.

- Nondenatured

IMPORTANT! It is not necessary to denature the RNA. Denaturation of the RNA may reduce the yield of cDNA for some gene targets.

Reagent and Sample Preparation Guidelines

Follow the guidelines below to ensure optimal performance of the High-Capacity cDNA Archive Kit and of the TaqMan[®] Gene Expression Assays.

- Use nuclease-free pipet tips and reagents to minimize degradation of the RNA.
- Observe standard laboratory practices when handling RNA.

Manual Method Overview

Use the manual method for converting total RNA into cDNA, as specified in the *High Capacity cDNA Archive Kit Protocol* (PN 4322169). Briefly:

1. Prepare 2X RT master mix.
2. Prepare the cDNA archive reaction plate manually.
3. Place the plate in the thermal cycler or SDS instrument for reverse transcription.

Refer to the *High Capacity cDNA Archive Kit Protocol* for detailed procedures.

Performing PCR Amplification

Overview Target amplification, using cDNA as the template, is the second step in using TaqMan Gene Expression Assays. In this step, the DNA polymerase (from the TaqMan Universal PCR Master Mix) amplifies target cDNA synthesized from the RNA sample, using sequence-specific primers and TaqMan MGB probe (6-FAM dye-labeled) from the TaqMan Gene Expression Assay Mix.

IMPORTANT! You must perform the PCR step on a real-time sequence detection system instrument. Traditional thermal cyclers cannot be used because they cannot detect and record the fluorescent signals generated by the cleavage of TaqMan probes.

PCR Process Performing the PCR step for singleplex assays in 384-well or 96-well formats requires the following procedures:

1. Configuring the sequence detector plate document
2. Preparing the reaction plate
3. Running the plate

Configuring the Plate Document Refer to the appropriate instrument user guide for instructions on how to configure the plate document.

cDNA Template Guidelines For optimal performance of TaqMan Gene Expression Assays, use 1 to 100 ng of RNA converted to cDNA per 50- μ L reaction, when using the TaqMan Universal PCR Master Mix.

Reagent Preparation Guidelines Following these guidelines ensures optimal PCR performance:

- Keep all TaqMan Gene Expression Assays protected from light, in the freezer, until you are ready to use them. Excessive exposure to light may affect the fluorescent probes.
- Prior to use:
 - Mix the PCR master mix thoroughly by swirling the bottle.
 - Resuspend the primer and probe mixture by vortexing and then centrifuge the tube briefly.

- Thaw any frozen cDNA samples by placing them on ice. When thawed, resuspend the samples by vortexing and then centrifuge the tubes briefly.
- Prepare the PCR reaction mix before transferring to the reaction plate for thermal cycling and fluorescence analysis.

PCR Reaction Mix Components

The recommended reaction sizes vary depending on the PCR master mix used. Prepare the plate so that each PCR reaction contains the components listed in the following table.

Component	Volume (μL) / Reaction	
	20- μL Reactions (384-Well Setup)	50- μL Reactions (96-Well Setup)
20X TaqMan Gene Expression Assay	1.0	2.5
cDNA template ^a	9.0	22.5
2X TaqMan Universal Master Mix (with or without AmpErase UNG)	10.0	25.0
Total Volume	20.0	50.0

a. Use 1 to 100 ng of RNA converted to cDNA plus RNase-free water if using the TaqMan Universal Master Mix.

⚠ CAUTION CHEMICAL HAZARD. TaqMan Universal PCR Master Mix may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparing the PCR Reaction Plate

Applied Biosystems recommends performing four replicates of each reaction.

To prepare the reaction plate:

<p>1. Prepare the PCR reaction mix for each sample (in quadruplicate) separately:</p>	TaqMan Universal Master Mix		
	Component	Vol (μL) for Four 20-μL Reactions	Vol (μL) for Four 50-μL Reactions
	20× TaqMan Gene Expression Assay	5.0	12.5
	cDNA template ^a	45.0	112.5
	2× TaqMan Universal Master Mix (with or without AmpErase UNG)	50.0	125.0
	Total Volume	100.0	250.0
<p>a. Use 1 to 100 ng of RNA converted to cDNA plus RNase-free water if using the TaqMan Universal Master Mix.</p> <p>Note: An additional reaction is included in the calculations to provide excess volume for the loss that occurs during reagent transfers.</p> <p>⚠ CAUTION CHEMICAL HAZARD. TaqMan Universal PCR Master Mix may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</p>			
2.	Cap the tubes and mix the solutions by gentle inversion.		
3.	Centrifuge the tubes briefly to spin down the contents and eliminate any air bubbles from the solutions.		

To prepare the reaction plate: *(continued)*

4.	Transfer the appropriate volume of each reaction mixture to wells of an optical reaction plate, as specified in the following table. <table border="1" data-bbox="482 348 1193 583"><thead><tr><th data-bbox="482 348 751 414">If using...</th><th data-bbox="751 348 1002 414">With...</th><th data-bbox="1002 348 1193 414">Transfer...</th></tr></thead><tbody><tr><td data-bbox="482 414 751 499">TaqMan Universal Master Mix</td><td data-bbox="751 414 1002 499">384-well optical reaction plates</td><td data-bbox="1002 414 1193 499">20 μL</td></tr><tr><td data-bbox="482 499 751 583"></td><td data-bbox="751 499 1002 583">96-well optical reaction plates</td><td data-bbox="1002 499 1193 583">50 μL</td></tr></tbody></table>	If using...	With...	Transfer...	TaqMan Universal Master Mix	384-well optical reaction plates	20 μ L		96-well optical reaction plates	50 μ L
If using...	With...	Transfer...								
TaqMan Universal Master Mix	384-well optical reaction plates	20 μ L								
	96-well optical reaction plates	50 μ L								
5.	Cover the plate with an optical adhesive cover or with optical caps.									
6.	Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles from the solutions.									

Running the Plate See the appropriate instrument user guide for help with programming the thermal cycling conditions or with running the plate.

To run the plate:

1.	Place the reaction plate in the sequence detector.																												
2.	<p>Set the thermal cycling conditions.</p> <p>When using the TaqMan Universal PCR Master Mix, use the default PCR thermal cycling conditions specified in the following table:</p> <table border="1"> <thead> <tr> <th rowspan="2">Step</th> <th>AmpErase UNG Activation^a</th> <th>AmpliTaq Gold Enzyme Activation</th> <th colspan="2">PCR</th> </tr> </thead> <tbody> <tr> <td>HOLD</td> <td>HOLD</td> <td colspan="2">CYCLE (40 cycles)</td> </tr> <tr> <td></td> <td></td> <td></td> <td>Denature</td> <td>Anneal/Extend</td> </tr> <tr> <td>Time</td> <td>2 min</td> <td>10 min</td> <td>15 sec</td> <td>1 min</td> </tr> <tr> <td>Temp</td> <td>50 °C</td> <td>95 °C</td> <td>95 °C</td> <td>60 °C</td> </tr> </tbody> </table> <p>a. The 2-min, 50 °C step is required for optimal AmpErase UNG activity when using TaqMan Universal PCR Master Mix (PN 4304437). This step is not needed when using the TaqMan Universal PCR Master Mix, No AmpErase UNG (PN 4324018).</p>					Step	AmpErase UNG Activation ^a	AmpliTaq Gold Enzyme Activation	PCR		HOLD	HOLD	CYCLE (40 cycles)					Denature	Anneal/Extend	Time	2 min	10 min	15 sec	1 min	Temp	50 °C	95 °C	95 °C	60 °C
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Time	2 min	10 min	15 sec	1 min																									
Temp	50 °C	95 °C	95 °C	60 °C																									
3.	<p>Set the reaction volume according to the following table.</p> <table border="1"> <thead> <tr> <th>Master Mix</th> <th>Plate Format</th> <th>Reaction Volume (µL)</th> </tr> </thead> <tbody> <tr> <td rowspan="2">TaqMan Universal PCR Master Mix</td> <td>384-well</td> <td>20</td> </tr> <tr> <td>96-well</td> <td>50</td> </tr> </tbody> </table>					Master Mix	Plate Format	Reaction Volume (µL)	TaqMan Universal PCR Master Mix	384-well	20	96-well	50																
Master Mix	Plate Format	Reaction Volume (µL)																											
TaqMan Universal PCR Master Mix	384-well	20																											
	96-well	50																											
4.	Start the run.																												

Analyzing Results

Overview Data analysis varies depending on the instrument. Refer to the appropriate instrument user guide for instructions on how to analyze your data.

General Process The general process for analyzing the data from gene expression assays involves the following procedures:

1. Viewing the amplification plots for the entire plate
2. Setting the baseline and threshold values
3. Using the relative standard curve method or the comparative C_T method to determine relative quantification

Resources for Data Analysis Refer to the following documents for more information about analyzing your data:

- The appropriate instrument user guide
- *User Bulletin #2: Relative Quantitation of Gene Expression* (PN 4303859)
- Livak, K.J., and Schmittgen, T.D. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. *Methods* 25:402–408.

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