

Introduction to TaqMan[®] and SYBR[®] Green Chemistries for Real-Time PCR

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Further information regarding 5' nuclease licensing program and commercial service licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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Part Number 4407003 Rev. B
06/2010

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Safety information


For general safety information, see this Preface. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix of the protocol provided with the kit for the complete alert on the chemical or instrument.


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.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see the “Safety” Appendix of the protocol provided with the kit.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

How to use this guide

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:
Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis.
For example:
Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▶) separates successive commands you select from a drop-down or shortcut menu. For example:
Select **File ▶ Open ▶ Spot Set**.
Right-click the sample row, then select **View Filter ▶ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

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

How to obtain support

For the latest services and support information for all locations, go to www.appliedbiosystems.com.

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- 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

Introduction to TaqMan[®] and SYBR[®] Green Chemistries for Real-Time PCR

Overview

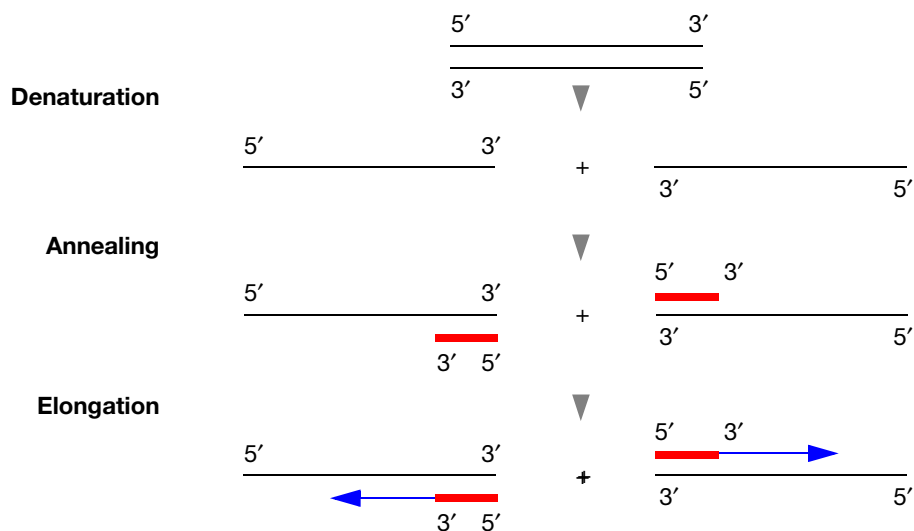
Real-time PCR is detected and measured using TaqMan[®] and SYBR[®] Green chemistries.

Polymerase chain reaction (PCR)

PCR is a method to amplify, or increase, the amount of a specific DNA sequence. Typically, the target DNA sequence is amplified using a solution containing DNA polymerase and nucleotides, and primers that are complementary to the target DNA sequence. When this solution is heated, the double-stranded DNA (dsDNA) denatures, separating into two separate strands. As the solution cools, the primers anneal to the target sequences in the separated DNA strands. The DNA polymerase then forms a new strand by extending the primers with nucleotides, creating a complementary copy of the target DNA sequence. When repeated, this cycle of denaturing, annealing, and extending increases exponentially the number of target DNA sequences. Ideally, no amplification occurs if the target DNA sequence is not present.

PCR reaction components

- DNA polymerase
- Primer mix containing primers, and dNTPs
- Sample DNA isolated from media, cell culture, or other sources



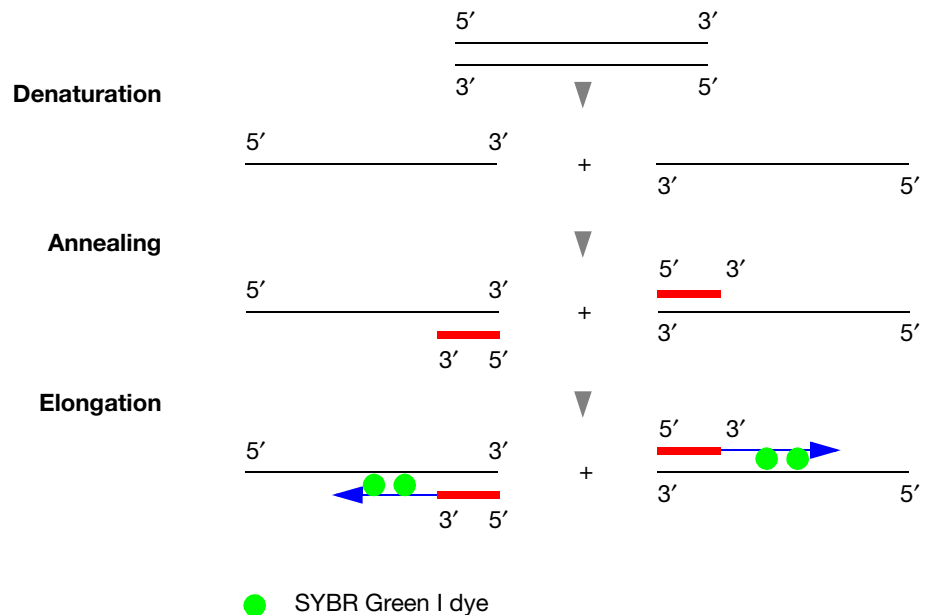
Power SYBR® Green dye fluorescence detection

SYBR® Green chemistry is a method for performing real-time PCR analysis. SYBR Green dye binds the minor groove of double-stranded DNA. When SYBR Green dye binds to double-stranded DNA, the intensity of the fluorescence increases. As more double-stranded amplicons are produced, SYBR Green dye fluorescence increases. SYBR Green dye binds to any double-stranded DNA molecule.

The Power SYBR® Green PCR Master Mix can detect as few as 1 to 10 copies of a target gene over a wide range of DNA template concentrations. The master mix formulation contains a blend of dTTP and dUTP, which ensures optimal PCR results and compatibility with AmpErase® UNG treatment. In addition, the master mix includes AmpliTaq Gold® DNA Polymerase, LD (Low DNA), a highly purified version of AmpliTaq Gold® DNA Polymerase that minimizes nonspecific, false-positive DNA products. The enzyme is provided in an inactive state to automate the Hot Start PCR technique and allow flexibility in the reaction setup, including pre-mixing of PCR reagents at room temperature.

PCR reaction components

- Power SYBR® Green PCR Master Mix containing AmpliTaq Gold® DNA Polymerase, LD (Low DNA); SYBR Green I dye; and a blend of dTTP and dUTP
- Primer mix containing primers and dNTPs
- Sample DNA that is isolated from media, cell culture, or other sources

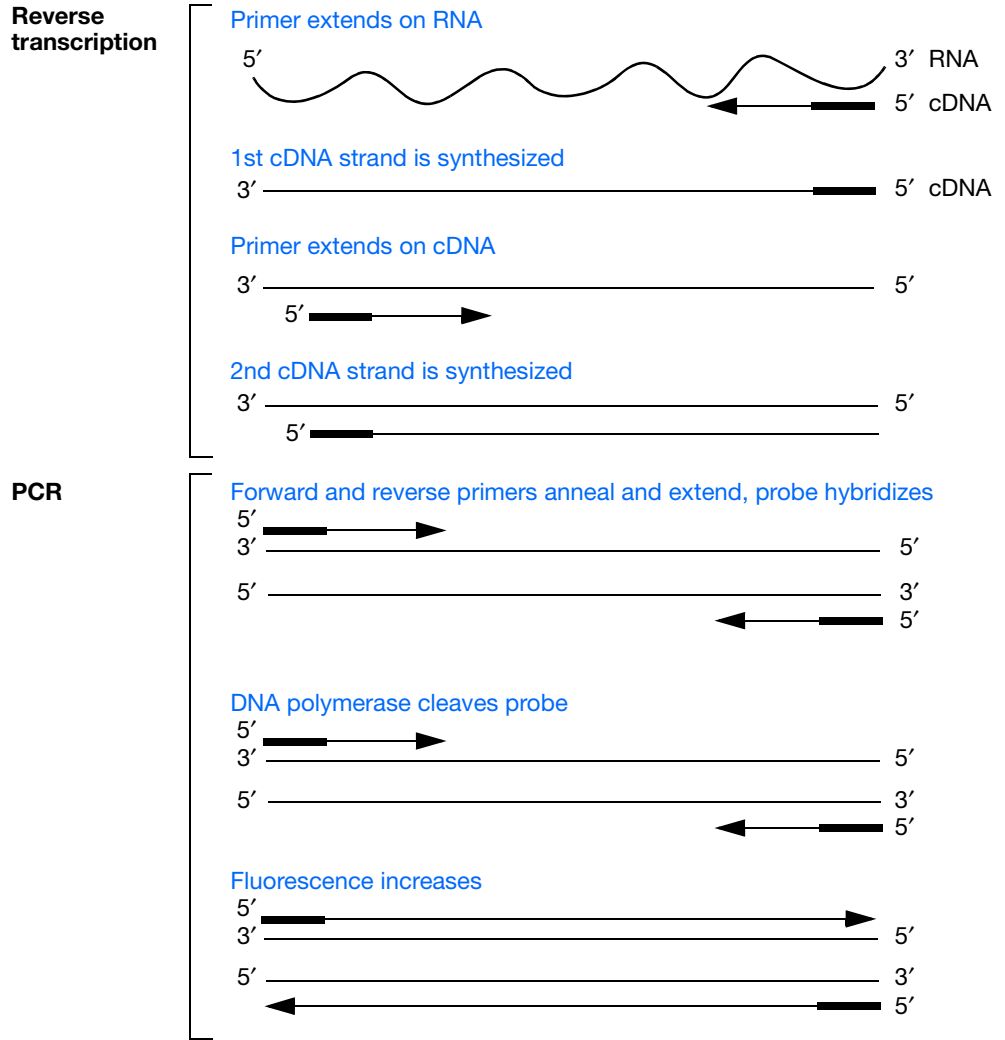


Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR is a method to amplify, or increase, the amount of a specific RNA sequence. Typically, the target RNA sequence is reverse transcribed into its complementary DNA (cDNA). The cDNA is subsequently amplified using a solution containing DNA polymerase and nucleotides, and primers that are complementary to the target DNA sequence. When this solution is heated, the dsDNA denatures, separating into two separate strands. As the solution cools, the primers anneal to the target sequences in the separated DNA strands. The DNA polymerase then forms a new strand by extending the primers with nucleotides, creating a complementary copy of the target DNA sequence. When repeated, this cycle of denaturing, annealing, and extending increases exponentially the number of target DNA sequences. Ideally, no amplification occurs if the target DNA sequence is not present.

RT-PCR reaction components

- DNA polymerase and reverse-transcriptase
- Primer mix containing primers and dNTPs
- Sample RNA



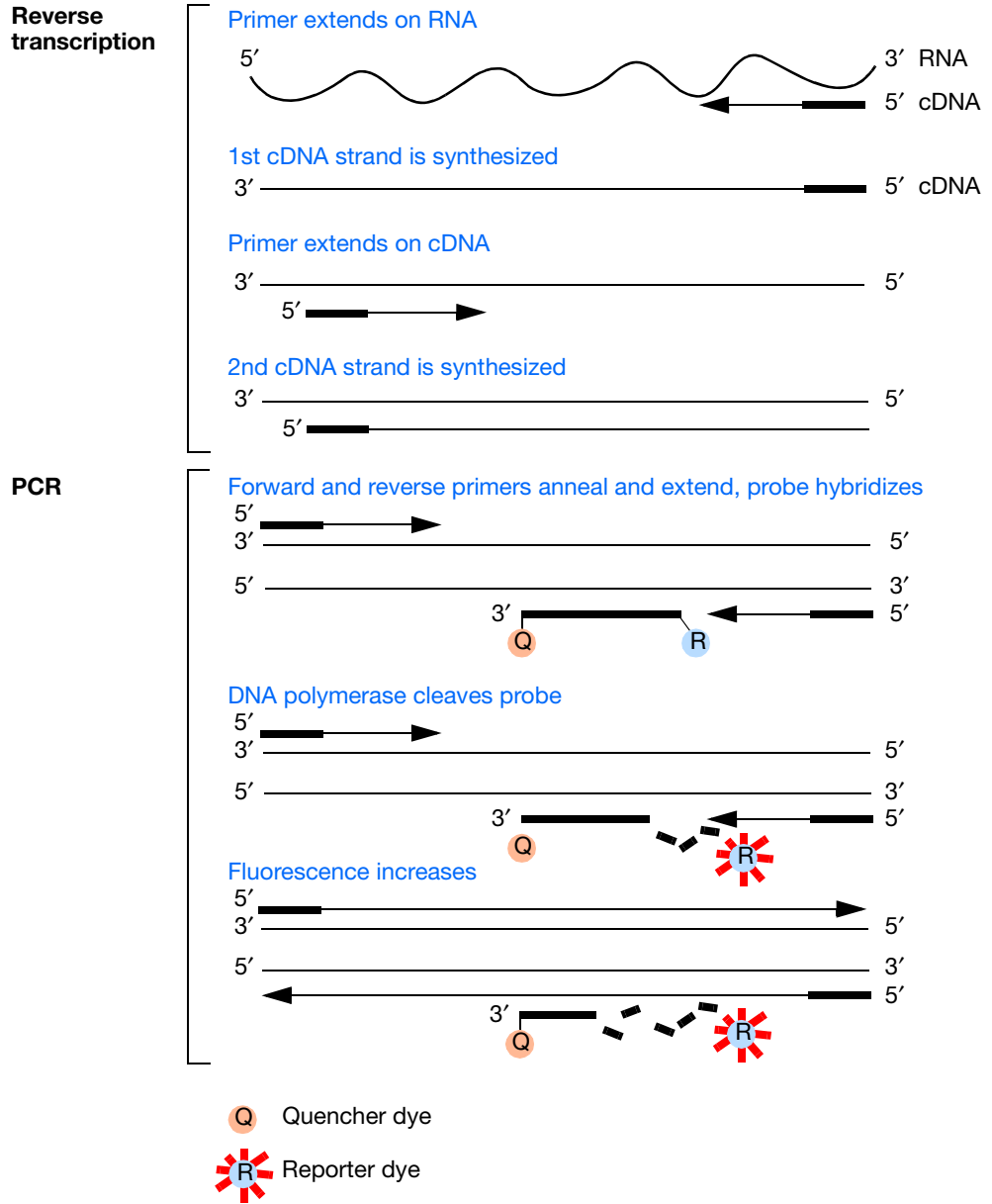
TaqMan® fluorescence detection

TaqMan® reactions can use either a one- or two-step reverse transcription-polymerase chain reaction. The RNA is reverse-transcribed to cDNA, which then undergoes standard PCR. If the cDNA target of interest is in the amplification product, the TaqMan probe hybridizes to the sequence. The 5' to 3' nucleolytic activity of the DNA polymerase cleaves the hybridized fluorogenic probe between the reporter dye and the quencher dye. The fragments of reporter dye are displaced from the target, resulting in an increase in fluorescence. This step, which occurs in every cycle, does not interfere with the exponential accumulation of product. The polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. The increase in fluorescence occurs only if the target sequence is complementary to the probe and is amplified during PCR. Nonspecific amplification is not detected in the absence of a probe-binding site. The 5' nuclease assay is specific to a pre-determined target.

The TaqMan® probe contains a fluorescent reporter dye at the 5' end of the probe and a quencher dye at the 3' end of the probe. When the probe is intact, the proximity of the reporter dye to the quencher dye suppresses the reporter fluorescence. Probe cleavage during the PCR reaction spatially separates the reporter dye from the quencher dye, thereby allowing detection of the reporter dye fluorescence.

RT-PCR reaction components

- DNA polymerase
- Primer mix containing primers, dNTPs, and TaqMan probes
- Sample RNA



Good PCR Practices

Prevent contamination and nonspecific amplification

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.

PCR good laboratory practices

When preparing samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
 - Sample preparation
 - PCR setup
 - PCR amplification
 - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution.

IMPORTANT! To avoid false positives due to cross-contamination:

- Prepare and close all negative control and unknown sample tubes before pipetting the positive control.
 - Do not open tubes after amplification.
 - Use different sets of pipettors when pipetting negative-control, unknown, and positive-control samples.
-

Plate layout suggestions

- For each plate row, dispense in sequence from left to right the: negative controls, unknown samples, inhibition controls, and positive controls (at the end of the row or column).
- Place positive controls in one of the outer columns.
- If possible, separate all samples from each other by at least one well; if space is limiting, place at least one well between unknown samples and controls.
- Be aware that caps come in strips of 8 or 12.

Related documentation

For additional documentation, see “How to obtain support” on page vi.

Real-time PCR system	Document	PN
All real-time PCR systems	<i>MicroSEQ[®] Mycoplasma Real-Time PCR Detection Kit Protocol</i>	4393111
	<i>MicroSEQ[®] Mycoplasma Real-Time PCR Detection Kit Quick Reference Card</i>	4393471
7900 Fast system	<i>Applied Biosystems 7900 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide</i>	4364014
7300, 7500, and 7500 Fast systems	<i>Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide</i>	4347825

Portable document format (PDF) versions of this guide and the documents listed above are available at www.appliedbiosystems.com

Note: To open the documentation available from the Applied Biosystems web site, use the Adobe[®] Acrobat[®] Reader[®] software available at www.adobe.com

Part Number 4407003 Rev. B 06/2010



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