

User Bulletin #5

ABI PRISM[®] 7700 Sequence Detection System

August 10, 1998 (updated 01/2001)

SUBJECT: Multiplex PCR with TaqMan[®] VIC Probes

Overview Applied Biosystems now offers probes constructed with the new TaqMan[®] VIC reporter dye. The characteristics of the VIC dye make it an excellent candidate to replace existing TaqMan[®] JOE and HEX reporter dyes. The increased signal strength and improved spectral resolution also make VIC-labeled probes the ideal second probe for a multiplex PCR system.

This user bulletin describes the characteristics of VIC probes in relation to the existing JOE probes. It also contains guidelines for defining limiting primer concentrations in a one- or two-step multiplex reverse transcription-polymerase chain reaction (RT-PCR) system using VIC probes.

The following topics are covered in this user bulletin:

Topic	See Page
Characteristics of TaqMan VIC Probes	2
Multiplex RT-PCR	5
Technical Support	13

IMPORTANT To use VIC probes on the ABI PRISM[®] 7700 Sequence Detection System (SDS), you must first calibrate the instrument with the Sequence Detection Systems Spectral Calibration Kit (P/N 4305822). This kit contains the new SYBR[®] Green and VIC fluorescent dye standards used to update the spectra components file in the SDS software. See *User Bulletin #4: Generating New Spectra Components* (P/N 4306234).

Note All documents referred to in this user bulletin are available though the internet at the Applied Biosystems technical support documentation library or through Fax-on-Demand (see "To Obtain Documents on Demand" on page 16 for information).

The technical support documentation library is located at:

◆ www.appliedbiosystems.com/techsupport

Characteristics of TaqMan VIC Probes

Introduction Applied Biosystems designed the VIC dye as a replacement for the TaqMan® JOE and HEX dyes currently in use with ABI PRISM 7700 instruments. The VIC dye features improvements in fluorescent signal strength, spectral resolution, and production cost. This section describes these characteristics of the VIC reporter dye in comparison to the existing reporters.

Increased Signal Strength The fluorescent signal from VIC dye is nearly four times stronger than JOE. Figure 1 illustrates the difference between the emissions of the two dyes. In combination with the improved spectral resolution, the increased signal strength of the VIC dye is extremely important in multiplex systems.

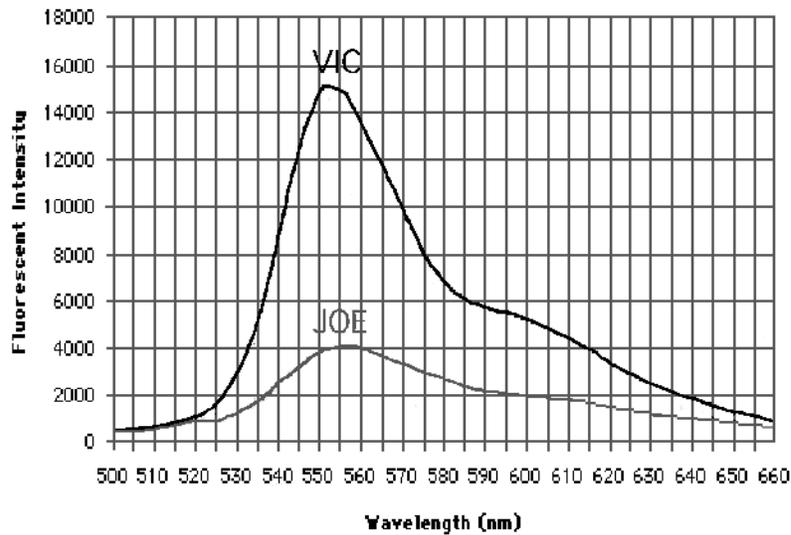


Figure 1. Emission spectra of VIC and JOE dye standards (200nM)

Improved Spectral Resolution

Spectral resolution refers to the degree of separation between the spectra of dyes in a set. Figure 2 illustrates the spectral resolution of four dyes used in TaqMan assays.

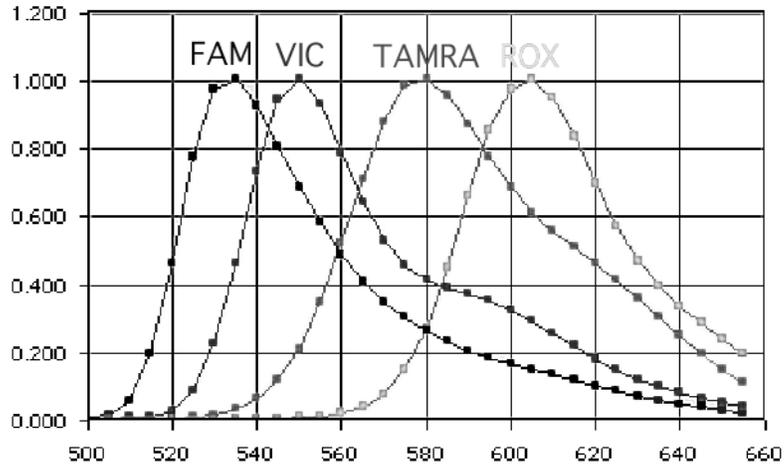


Figure 2. Dual probe (VIC and FAM) system pure dye view

Because of experimental variation in measuring multiple dye spectra, multicomponenting introduces some error into the determination of each dye's contribution. The degree of error depends on how well the various dyes are spectrally resolved. The less the spectral overlap between the dyes, the less the error. Therefore, reporter dyes with the largest difference in emission maxima provide the most accurate quantitation with two probes in one tube (i.e. 6-FAM and VIC).

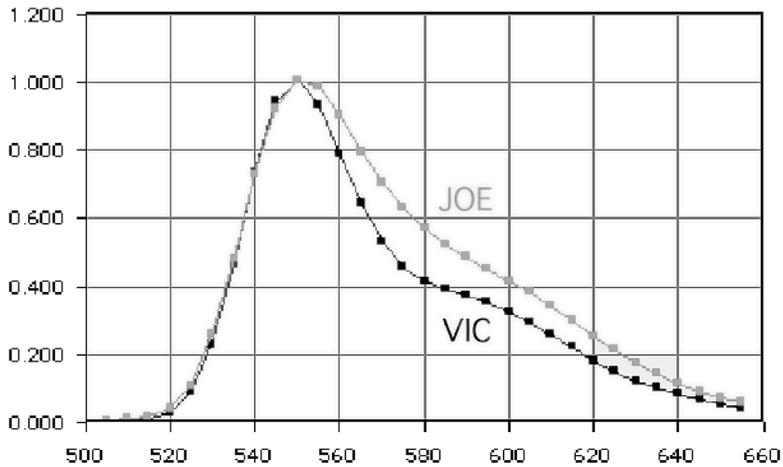


Figure 3. VIC and JOE pure dye view

Figure 3 illustrates the improved spectral resolution of the VIC dye. The VIC spectrum consists of a narrow peak with a half band width 15 percent less than that of JOE. Consequently, the VIC spectrum overlaps less with surrounding dyes and introduces less error into the system.

**Probe Availability
and Production Cost**

Unlike the JOE and HEX reporters, probes labeled with the VIC dye are available at three synthesis scales. Probes labeled with HEX and JOE are only available in the 50,000–100,000 pmol scale because they are difficult to synthesize and must undergo additional purification. VIC dye synthesis chemistry however, is similar to that of TaqMan® FAM and TET dyes. Consequently, Applied Biosystems can offer VIC probes in the three quantities listed below at prices lower than current HEX and JOE labeled probes.

Part #	Description	Quantity (pmol.)
450025	TaqMan Probe, HPLC purified 5'-Fluorescent label: FAM, VIC or TET 3'-Fluorescent label: TAMRA + HPLC Purification	5,000–6,000
450024	TaqMan Probe, HPLC purified 5'-Fluorescent label: FAM, VIC or TET 3'-Fluorescent label: TAMRA + HPLC Purification	15,000–25,000
450003	TaqMan Probe, HPLC purified 5'-Fluorescent label: FAM, VIC or TET 3'-Fluorescent label: TAMRA + HPLC Purification	50,000–100,000

Multiplex RT-PCR

Introduction The increased signal strength and improved spectral resolution of VIC probes make them ideal second probes for a multiplex system. This section contains brief instructions for defining limiting primer concentrations using one- and two-step RT-PCR.

Multiplex PCR is the use of more than one primer pair in the same tube. You can use this method for relative quantitation where one primer pair amplifies the target and another primer pair amplifies the endogenous reference. You can perform a multiplex reaction using either the Standard Curve Method or the Comparative C_T Method. See “Multiplex PCR (Same Tube)” on page 16 of the *ABI PRISM 7700 Sequence Detection System User Bulletin #2* (P/N 4303859) for a detailed description of multiplex PCR.

Multiplex Strategy The strategy for performing two independent reactions in the same tube is to adjust primer concentrations such that accurate threshold cycles (C_T) are obtained for both targets. In order to do this, it is necessary to limit the primer concentrations for the majority species. By doing so, the amplification of the majority species can be stopped before it can limit the common reactants available for amplification of the minority species.

Limiting Primer Protocol To define limiting primer concentrations, perform the following experiments:

Step	Action	See Page
1	Evaluate the Relative Abundance of Target and Reference Species	6
2	Determine the Optimal Primer Concentrations	6
3	Determine the Linear Dynamic Range	7
4	Define Limiting Primer Concentrations	8
5	Verify the Limiting Primer Concentrations	11

Limitations of the Limiting Primer Protocol The following limiting primer protocol does not guarantee successful determination of limiting primer concentrations for all assays. If you are unable to define limiting primer concentrations after completing the limiting primer protocol, use one of the following alternative methods for quantitation:

- ◆ Run the reactions in separate tubes. By separating the reactions you permit each reaction to proceed unaffected by the other, thereby eliminating the competition for common reagents.
 - ◆ Redesign and retest the primers to find limiting concentrations. If either primer used in the reaction has an actual melting temperature (T_m) lower than expected, it may preclude determination of limiting primer concentrations. In this case, Applied Biosystems generally recommends that you lengthen the primer by one or two nucleotides to increase its T_m . By increasing the T_m of the primer, you are able to achieve robust amplification at lower primer concentrations.
-

Evaluate the Relative Abundance of Target and Reference Species

Understanding Relative Abundance By understanding the relative abundance of target and reference species, it is possible to control the competition between the reactions. Once the relative abundance of the competing species are known, preventing domination by the abundant species becomes a simple matter of limiting the primers. Thus, it is important that you understand the relative abundance of your target and reference species before conducting the limiting primer protocol.

If...	Then...
the concentration of one species is always greater than the other Note For example, the concentration of rRNA is always greater than mRNA for total RNA systems.	limit the concentrations of the abundant species primers.
the abundance of one RNA species is unknown	limit the primer concentrations for both amplicons

Determine the Optimal Primer Concentrations

Optimal Primer Concentrations Applied Biosystems suggests that you establish the optimal probe and primer concentrations for your reaction before attempting to determine limiting primer concentrations. The optimal primer concentrations are those that yield the maximum ΔR_n and the lowest C_T values for your assay. These values are used to verify the results from the “Define Limiting Primer Concentrations” experiment on page 8.

Determining the Optimal Primer Concentrations To determine the optimal primer concentrations for your RT-PCR system, follow the “Optimize Primer Concentrations” procedure on page 18 of the *TaqMan® Universal PCR Master Mix Protocol* Revision A (P/N 4304449) with the following changes.

Step 4 of the Optimal Primer procedure now reads:

Step	Action
4	At the end of runs, tabulate the results for ΔR_n and C_T . Choose the minimum forward- and reverse- primer concentrations that yield the lowest C_T and the maximum ΔR_n .

(Changes to the procedure are shown in bold text.)

Determining the Optimum Probe Concentration Optimal probe concentration should be determined for each quantitative assay with the procedure outlined on pages 20–21 of the *TaqMan® Universal PCR Master Mix Protocol* (P/N 4304449).

Determine the Linear Dynamic Range

Linear Dynamic Range and RT-PCR

The linear dynamic range refers to the range of initial template concentrations over which accurate C_T values are obtained. By defining the linear dynamic range for your system, you can identify the possible starting nucleic acid concentrations for your sample.

Defining the Linear Dynamic Range

To determine the linear dynamic range of your multiplex system, perform the template titration experiment on pages 16–29 of the *TaqMan® Gold RT-PCR Kit Protocol* Revision A (P/N 402876). This experiment will define the upper and lower boundaries for the dynamic range of your system.

Selecting an Initial Template Concentration

The minimum template concentration for your system can be defined by constructing a standard curve from the results of the defining the linear dynamic range experiment (see page 7 of the *ABI PRISM 7700 Sequence Detection System User Bulletin #2* for instructions on how to construct the plot).

Although any initial template concentration within the dynamic range can be used to determine limiting primer concentrations, lower initial template concentrations will yield greater sensitivity for your assay. Therefore, select an initial template concentration within the dynamic range of your assay that represents the lowest concentration of input nucleic acid that you can expect for any sample.

Example 1: Linear Dynamic Range Analysis

Figure 4 illustrates an example of the linear dynamic range for a one-step RT-PCR run using a $1-10^5$ pg range of initial template concentrations.

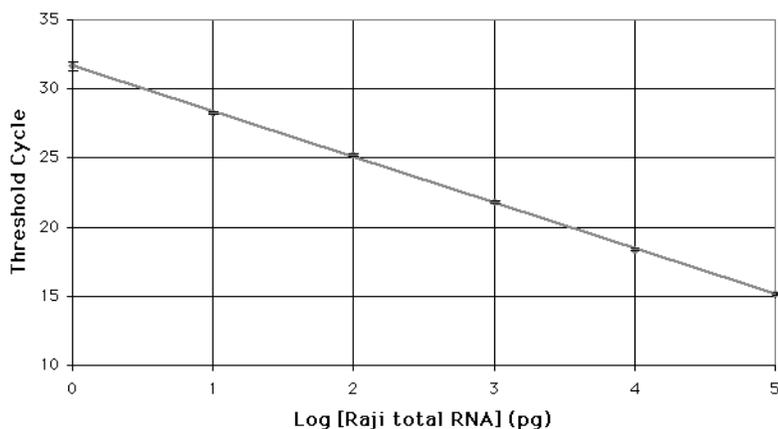


Figure 4. Standard Curve (Human GAPDH mRNA System). Each data point represents the average of triplicate reactions.

All initial template concentrations plotted on the graph appear to be in the linear dynamic range for the system. Therefore, any total RNA concentration within the $1-10^5$ pg range can be used. However, the greatest sensitivity will be achieved if the limiting primer experiment is run at 1 pg initial total RNA.

Define Limiting Primer Concentrations

Description Once you establish the initial template concentration for your system, defining limiting primer concentrations becomes a matter of varying the primer concentrations and observing the trends in ΔR_n and C_T . Because initial template concentration is held constant for all wells in the matrix, the forward and reverse primers will be directly responsible for any variation in the data.

Limiting Primer Matrix To define limiting primer concentrations, run a matrix of forward and reverse primer concentrations using the value of the minimum initial template found in the previous section. Figure 5 illustrates a recommended matrix of forward and reverse primers varying in concentration from 20–100 nM.

For 100 nM Rev 100 nM	For 100 nM Rev 80 nM	For 100 nM Rev 60 nM	For 100 nM Rev 40 nM	For 100 nM Rev 20 nM
For 80 nM Rev 100 nM	For 80 nM Rev 80 nM	For 80 nM Rev 60 nM	For 80 nM Rev 40 nM	For 80 nM Rev 20 nM
For 60 nM Rev 100 nM	For 60 nM Rev 80 nM	For 60 nM Rev 60 nM	For 60 nM Rev 40 nM	For 60 nM Rev 20 nM
For 40 nM Rev 100 nM	For 40 nM Rev 80 nM	For 40 nM Rev 60 nM	For 40 nM Rev 40 nM	For 40 nM Rev 20 nM
For 20 nM Rev 100 nM	For 20 nM Rev 80 nM	For 20 nM Rev 60 nM	For 20 nM Rev 40 nM	For 20 nM Rev 20 nM

Figure 5. Limiting primer matrix: A matrix of varying concentrations of forward and reverse primers (20–100 nM).

Perform triplicate reactions for each primer combination within the matrix. As in the previous step, follow the procedure outlined in pages 16–29 of the *TaqMan® Gold RT-PCR Kit Protocol*.

Interpreting Results Ideally, limiting primer concentrations will have negligible effects on the C_T while decreasing product yield. Therefore, desirable primer concentrations are those that show a reduction in ΔR_n but no effect on C_T .

Graphically, the primer concentrations of interest will be those combinations that:

- ◆ fall within the plateau region of the forward and reverse primer concentration vs. C_T graph
- ◆ achieve a ΔR_n lower than the maximum value for the assay

IMPORTANT If the graph of forward and reverse primer concentration vs. C_T does not plateau, then limiting primer concentrations are unobtainable. See “Limitations of the Limiting Primer Protocol” on page 5 for alternative methods for quantitation.

**Example 2:
One-Step Multiplex
GAPDH RT-PCR
Amplification**

Figures 6 and 7 illustrate the ΔR_n and C_T trends found by varying concentrations of forward and reverse primers (20–100 nM). Figure 6 demonstrates that the C_T is affected by variations in the concentrations of both primers. By decreasing the forward primer concentration below 40 nM or the reverse primer concentration below 60 nM, the C_T increased by at least one cycle.

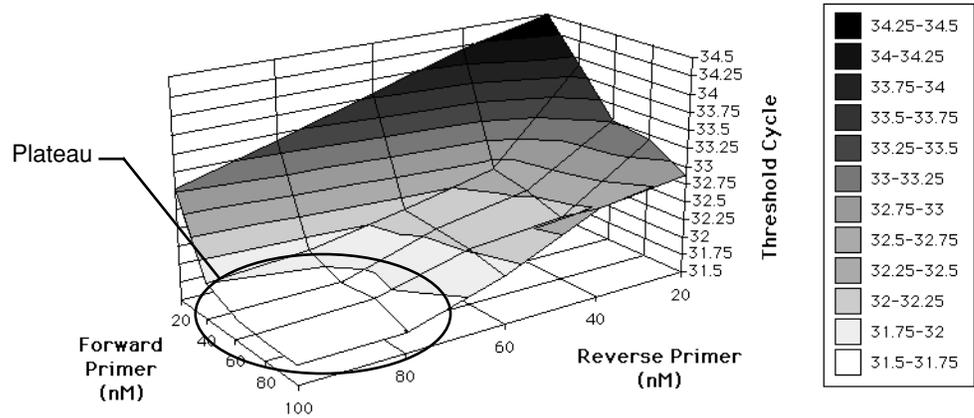


Figure 6. Comparison of primer concentrations and C_T from one-step Human GAPDH mRNA RT-PCR amplification using combinations of forward and reverse primers (20–100 nM).

Figure 7 demonstrates that lower ΔR_n s are achieved by decreasing both primer concentrations. Therefore, final product yields can be regulated by limiting the primer concentrations.

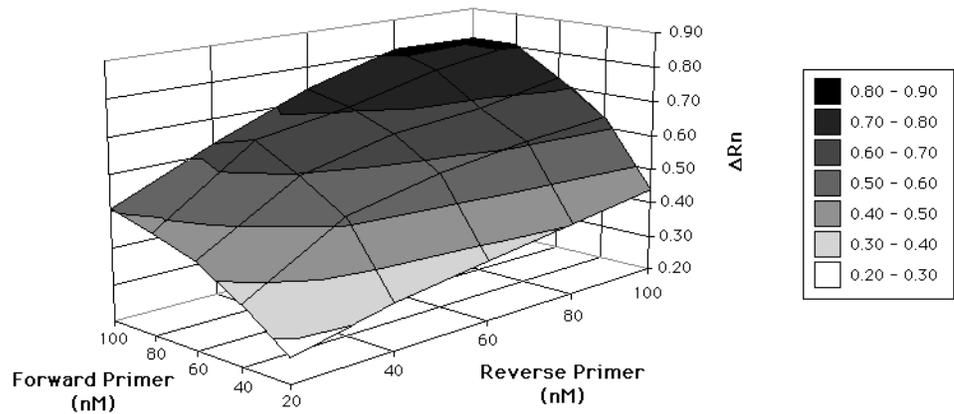


Figure 7. Comparison of primer concentrations and ΔR_n from one-step Human GAPDH mRNA RT-PCR amplification using combinations of forward and reverse primers (20–100 nM).

Note For the example above, 50 nM forward primer and 80 nM reverse primer were selected as limiting primer concentrations in order to provide an experimental margin for error.

**Example 3:
Two-Step Multiplex
GAPDH RT-PCR
Amplification**

Figures 8 and 9 illustrate the ΔR_n and C_T trends found in two-step RT-PCR by varying the concentrations of forward and reverse primers (20–100 nM). Unlike the one-step RT-PCR in Example 2, the concentrations of forward and reverse primers can be reduced to 50 nM for the corresponding two-step system. Typically, the reverse primer concentration is higher for one-step RT-PCR than for the corresponding two-step system. This is because the reverse primer is responsible for both reverse transcription and PCR priming in one-step RT-PCR.

Figure 8 shows that although the C_T was unchanged by the decrease in reverse primer concentration, the change in forward primer concentration had a significant effect. For example, the C_T rose nearly two cycles when the forward primer concentration fell below 40 nM.

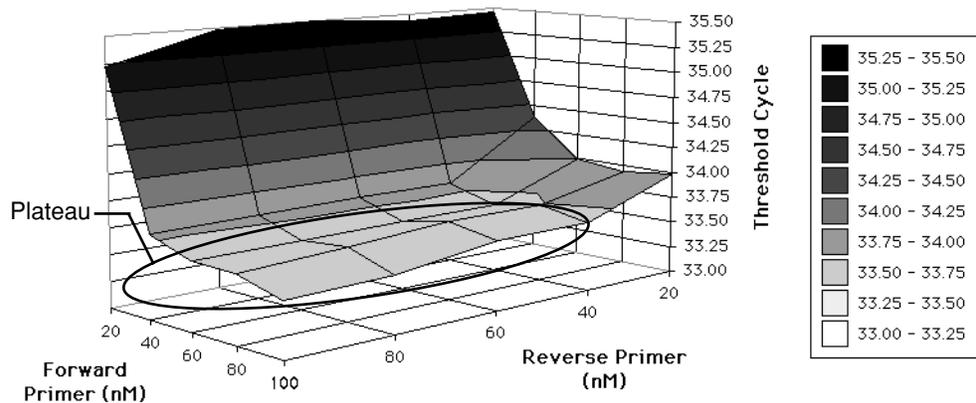


Figure 8. Comparison of primer concentrations and C_T from two-step Human GAPDH mRNA RT-PCR amplification using combinations of forward and reverse primers (20–100 nM).

Figure 9 shows the corresponding relationship between primer concentrations and ΔR_n . It demonstrates that lower product yields can be achieved by decreasing forward and reverse primer concentrations.

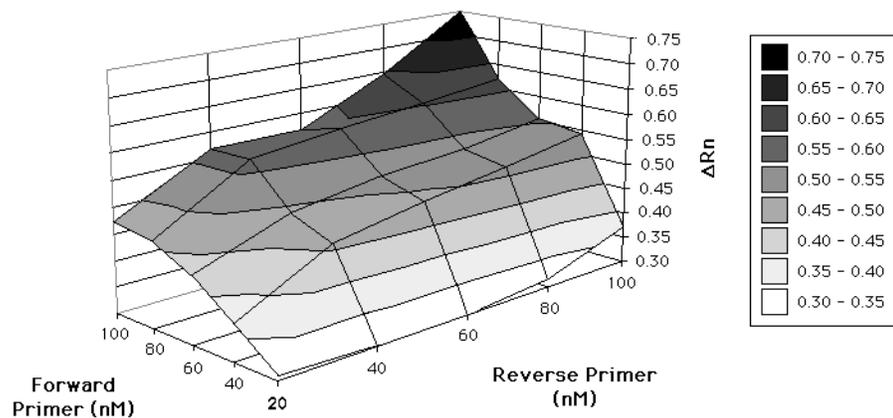


Figure 9. Comparison of primer concentrations and ΔR_n from two-step Human GAPDH mRNA RT-PCR amplification using combinations of forward and reverse primers (20–100 nM).

Note For the example above, 50 nM forward primer and 50 nM reverse primer were selected as limiting primer concentrations in order to provide an experimental margin for error.

Verify the Limiting Primer Concentrations

Confirming Results After defining limiting primer concentrations in the previous step, they should be verified experimentally. This is accomplished by running primer limited multiplex RT-PCR in parallel with non-primer limited RT-PCR in separate tubes. Because limiting primer concentrations should not affect the C_T , the values obtained from both the multiplex and separate tube experiments should match. As in the previous step, follow the procedure outlined in pages 16–29 of the TaqMan® *Gold RT-PCR Kit Protocol*.

Example 4: Primer Verification Figure 10 illustrates the comparison of the standard curves for primer limited (multiplex) and non-primer limited (separate tubes) RT-PCR. The results verify that the C_T values determined for this target were not affected by limiting the primer concentrations.

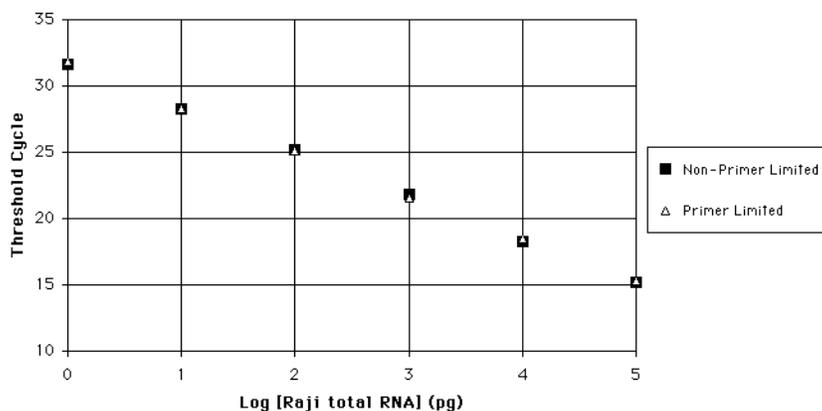


Figure 10. Threshold cycle vs. initial template concentration (Human GAPDH mRNA System). Each data point represents the average of triplicate reactions.

**RT-PCR With
Known Limiting
Primers**

Once you define the limiting primers in your system, perform the RT-PCR assay as described on pages 16–29 of the *TaqMan® Gold RT-PCR Kit Protocol* with the modifications to the reaction mix shown in Tables 1 and 2 (changes shown in bold print).

Table 1. One-step Multiplex RT-PCR Reaction Mix Components

Component	Volume/Tube (μL)	Final Concentration
RNase-free water	see below ^a	—
10X TaqMan Buffer A	5.0	1X
25mM MgCl ₂	11	5.5 mM
10mM deoxy ATP	1.5	300 μM
10mM deoxy CTP	1.5	300 μM
10mM deoxy GTP	1.5	300 μM
20mM deoxy UTP	1.5	600 μM
Target Primers and Probe	x	(see below^b)
Endogenous Reference Primers and Probe	y	(see below^b)
AmpliQ Gold DNA Polymerase (5.0 U/μL)	0.5	0.05 U/μL
MultiScribe Reverse Transcriptase (MuLV) (50 U/ μL)	0.25	0.25 U/ μL
RNase Inhibitor	1.0	0.4 U/ μL

a. Volume of RNase-free water (μL) = 26.25–(RNA Sample Volume+x+y)

b. Depending on the relative abundance of the target and reference (see table on page 6), use either the optimal or limiting primer concentrations as determined on page 6 or page 8.

Table 2. Two-step Multiplex PCR Mix Reaction Components

Component	Volume/Tube (μL)	Final Concentration
RNase-free water	see below ^a	—
TaqMan Universal PCR Master Mix (2X)	25.0	1X
Target Primers and Probe	v	(see below^b)
Endogenous Reference Primers and Probe	w	(see below^b)

a. Volume of RNase-free water (μL) = 25.0–(cDNA Sample Volume+v+w)

b. Depending on the relative abundance of the target and reference (see table on page 6), use either the optimal or limiting primer concentrations as determined on page 6 or page 8.

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section "To Obtain Documents on Demand" following the telephone information below).

To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pclab@appliedbiosystems.com
Protein Sequencing, Peptide and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor [®] , FMAT [™] , Voyager [™] , and Mariner [™] Mass Spectrometers	tsupport@appliedbiosystems.com
Applied Biosystems/MDS Sciex	api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

Hours for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

Product	Hours
Chemiluminescence	8:30 a.m. to 5:30 p.m. Eastern Time
Framingham support	8:00 a.m. to 6:00 p.m. Eastern Time
All Other Products	5:30 a.m. to 5:00 p.m. Pacific Time

To Contact Technical Support by Telephone or Fax

In North America

To contact Applied Biosystems Technical Support, use the telephone or fax numbers given below. (To open a service call for other support needs, or in case of an emergency, dial **1-800-831-6844** and press 1.)

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM [®] 3700 DNA Analyzer	1-800-831-6844 , then press 8	1-650-638-5981
DNA Synthesis	1-800-831-6844 , then press 21	1-650-638-5981
Fluorescent DNA Sequencing	1-800-831-6844 , then press 22	1-650-638-5981
Fluorescent Fragment Analysis (includes GeneScan [®] applications)	1-800-831-6844 , then press 23	1-650-638-5981
Integrated Thermal Cyclers (ABI PRISM [®] 877 and Catalyst 800 instruments)	1-800-831-6844 , then press 24	1-650-638-5981

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM [®] 3100 Genetic Analyzer	1-800-831-6844 , then press 26	1-650-638-5981
BioInformatics (includes BioLIMS [™] , BioMerge [™] , and SQL GT [™] applications)	1-800-831-6844 , then press 25	1-505-982-7690
Peptide Synthesis (433 and 43X Systems)	1-800-831-6844 , then press 31	1-650-638-5981
Protein Sequencing (Procise [®] Protein Sequencing Systems)	1-800-831-6844 , then press 32	1-650-638-5981
PCR and Sequence Detection	1-800-762-4001 , then press 1 for PCR, 2 for the 7700 or 5700, 6 for the 6700 or dial 1-800-831-6844 , then press 5	1-240-453-4613
Voyager [™] MALDI-TOF Biospectrometry and Mariner [™] ESI-TOF Mass Spectrometry Workstations	1-800-899-5858 , then press 13	1-508-383-7855
Biochromatography (BioCAD [®] Workstations and Poros [®] Perfusion Chromatography Products)	1-800-899-5858 , then press 14	1-508-383-7855
Expedite [™] Nucleic acid Synthesis Systems	1-800-899-5858 , then press 15	1-508-383-7855
Peptide Synthesis (Pioneer [™] and 9050 Plus Peptide Synthesizers)	1-800-899-5858 , then press 15	1-508-383-7855
PNA Custom and Synthesis	1-800-899-5858 , then press 15	1-508-383-7855
FMAT [™] 8100 HTS System and Cytofluor [®] 4000 Fluorescence Plate Reader	1-800-899-5858 , then press 16	1-508-383-7855
Chemiluminescence (Tropix)	1-800-542-2369 (U.S. only), or 1-781-271-0045	1-781-275-8581
Applied Biosystems/MDS Sciex	1-800-952-4716	1-650-638-6223

Outside North America

Region	Telephone Dial...	Fax Dial...
Africa and the Middle East		
Africa (English Speaking) and West Asia (Fairlands, South Africa)	27 11 478 0411	27 11 478 0349
South Africa (Johannesburg)	27 11 478 0411	27 11 478 0349
Middle Eastern Countries and North Africa (Monza, Italia)	39 (0)39 8389 481	39 (0)39 8389 493
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	61 3 9730 8600	61 3 9730 8799
China (Beijing)	86 10 64106608	86 10 64106617

Region	Telephone Dial...	Fax Dial...
Hong Kong	852 2756 6928	852 2756 6968
Korea (Seoul)	82 2 593 6470/6471	82 2 593 6472
Malaysia (Petaling Jaya)	60 3 758 8268	60 3 754 9043
Singapore	65 896 2168	65 896 2147
Taiwan (Taipei Hsien)	886 2 22358 2838	886 2 2358 2839
Thailand (Bangkok)	66 2 719 6405	66 2 319 9788
Europe		
Austria (Wien)	43 (0)1 867 35 75 0	43 (0)1 867 35 75 11
Belgium	32 (0)2 712 5555	32 (0)2 712 5516
Czech Republic and Slovakia (Praha)	420 2 61 222 164	420 2 61 222 168
Denmark (Naerum)	45 45 58 60 00	45 45 58 60 01
Finland (Espoo)	358 (0)9 251 24 250	358 (0)9 251 24 243
France (Paris)	33 (0)1 69 59 85 85	33 (0)1 69 59 85 00
Germany (Weiterstadt)	49 (0) 6150 101 0	49 (0) 6150 101 101
Hungary (Budapest)	36 (0)1 270 8398	36 (0)1 270 8288
Italy (Milano)	39 (0)39 83891	39 (0)39 838 9492
Norway (Oslo)	47 23 12 06 05	47 23 12 05 75
Poland, Lithuania, Latvia, and Estonia (Warszawa)	48 (22) 866 40 10	48 (22) 866 40 20
Portugal (Lisboa)	351 (0)22 605 33 14	351 (0)22 605 33 15
Russia (Moskva)	7 095 935 8888	7 095 564 8787
South East Europe (Zagreb, Croatia)	385 1 34 91 927	385 1 34 91 840
Spain (Tres Cantos)	34 (0)91 806 1210	34 (0)91 806 1206
Sweden (Stockholm)	46 (0)8 619 4400	46 (0)8 619 4401
Switzerland (Rotkreuz)	41 (0)41 799 7777	41 (0)41 790 0676
The Netherlands (Nieuwerkerk a/d IJssel)	31 (0)180 331400	31 (0)180 331409
United Kingdom (Warrington, Cheshire)	44 (0)1925 825650	44 (0)1925 282502
All other countries not listed (Warrington, UK)	44 (0)1925 282481	44 (0)1925 282509
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	81 3 5566 6230	81 3 5566 6507
Latin America		
Del.A. Obregon, Mexico	305-670-4350	305-670-4349

To Reach Technical Support Through the Internet

We strongly encourage you to visit our Web site for answers to frequently asked questions and for more information about our products. You can also order technical documents or an index of available documents and have them faxed or e-mailed to you through our site. The Applied Biosystems Web site address is

<http://www.appliedbiosystems.com/techsupp>

To submit technical questions from North America or Europe:

Step	Action
1	Access the Applied Biosystems Technical Support Web site.
2	Under the Troubleshooting heading, click Support Request Forms , then select the relevant support region for the product area of interest.
3	Enter the requested information and your question in the displayed form, then click Ask Us RIGHT NOW (blue button with yellow text).
4	Enter the required information in the next form (if you have not already done so), then click Ask Us RIGHT NOW . You will receive an e-mail reply to your question from one of our technical experts within 24 to 48 hours.

To Obtain Documents on Demand

Free, 24-hour access to Applied Biosystems technical documents, including MSDSs, is available by fax or e-mail or by download from our Web site.

To order documents...	Then...
by index number	<ol style="list-style-type: none"> Access the Applied Biosystems Technical Support Web site at http://www.appliedbiosystems.com/techsupp Click the Index link for the document type you want, then find the document you want and record the index number. Use the index number when requesting documents following the procedures below.
by phone for fax delivery	<ol style="list-style-type: none"> From the U.S. or Canada, call 1-800-487-6809, or from outside the U.S. and Canada, call 1-858-712-0317. Follow the voice instructions to order the documents you want. <p>Note There is a limit of five documents per request.</p>
through the Internet for fax or e-mail delivery	<ol style="list-style-type: none"> Access the Applied Biosystems Technical Support Web site at http://www.appliedbiosystems.com/techsupp Under Resource Libraries, click the type of document you want. Enter or select the requested information in the displayed form, then click Search. In the displayed search results, select a check box for the method of delivery for each document that matches your criteria, then click Deliver Selected Documents Now (or click the PDF icon for the document to download it immediately). Fill in the information form (if you have not previously done so), then click Deliver Selected Documents Now to submit your order. <p>Note There is a limit of five documents per request for fax delivery but no limit on the number of documents you can order for e-mail delivery.</p>

© Copyright 2001, Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures..

Applied Biosystems and ABI PRISM are registered trademarks of Applied Biosystems Corporation or its subsidiaries in the U.S. and certain other countries.

ABI, MicroBlotter and VIC are trademarks of Applied Biosystems Corporation or its subsidiaries in the U.S. and certain other countries.

TaqMan is a registered trademark of Roche Molecular Systems, Inc.

All other trademarks are the sole property of their respective owners.

P/N 4306236B; Stock Number 777805-002