

Custom TaqMan® Small RNA Assays

Design and Ordering Guide

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About This Guide

Purpose

The *Custom TaqMan[®] Small RNA Assays Design and Ordering Guide* provides information for ordering Custom TaqMan Small RNA Assays from the Applied Biosystems website.

Prerequisites

This guide uses conventions and terminology that assume a working knowledge of the Microsoft[®] Windows[®] operating system, the internet, and internet-based browsers.

Custom TaqMan[®] Small RNA Assays

Product information

Purpose of the product

With Custom TaqMan[®] Small RNA Assays, you can obtain custom-designed small RNA assays based on the chemistries for TaqMan[®] MicroRNA Assays. You can use Custom TaqMan Small RNA Assays to validate and quantify small RNA sequences, including:

- Mature microRNA (miRNA)
- Small interfering RNA (siRNA)
- Processed short hairpin RNA (shRNA)
- Piwi-interacting RNA (piRNA)
- Any other small RNA sequence that is 17 to 200 bases long

After you select small RNA sequences to study, you can submit your sequences to the Custom TaqMan[®] Small RNA Assay Design Tool on the Applied Biosystems website. If a predesigned assay is not yet available, Applied Biosystems will create an assay using our proprietary Custom TaqMan Small RNA Assay Design pipeline. After the design is complete, you will receive an email with a link to a Custom TaqMan Small RNA Assay Design page that lists the assay designed for each sequence you submitted. From this page, you can select assays to order, then submit your order. Applied Biosystems manufactures, packages, and ships the Custom TaqMan Small RNA Assays to you.

Assay contents

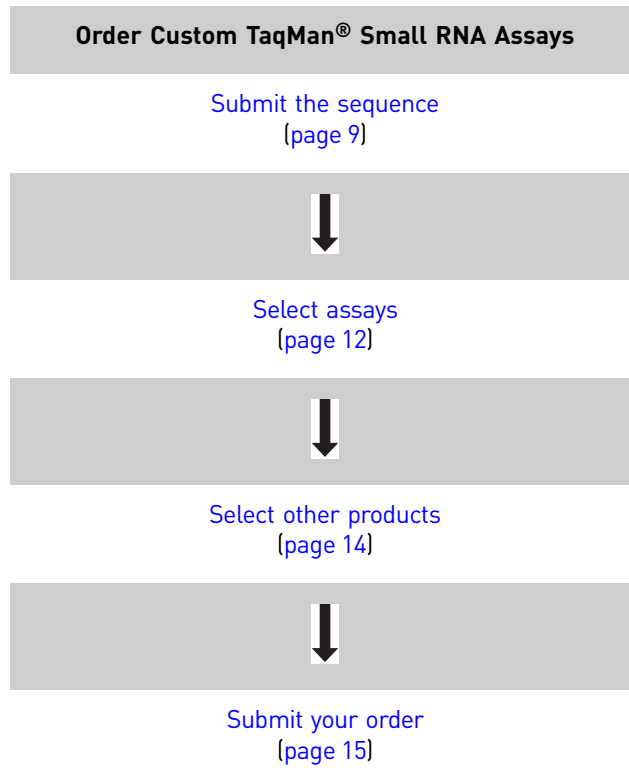
Custom TaqMan Small RNA Assays use a stem-looped primer for reverse transcription (RT) and a sequence-specific TaqMan[®] assay to accurately detect small RNAs.

Each Custom TaqMan[®] Small RNA Assay contains two tubes:

- One tube containing a stem-loop RT primer specific to the small RNA of interest
- One tube containing a mixture of:
 - Small RNA-specific forward PCR primer
 - Specific reverse PCR primer
 - TaqMan[®] MGB probe specific to the small RNA of interest



Workflow





Submit the sequence

Sequence format guidelines

You can enter your sequences directly on the Custom TaqMan® Small RNA Assay Design page or you can upload a text file (*.txt) that contains the sequences in FASTA format. With either method, follow these guidelines:

- Sequences must contain only A, C, G, T, and U bases.
- Sequences must be between 17 and 200 bases.



Note: All sequences are designed to the 3' end of the sequence, regardless of the length of the sequence submitted. If your sequence is up to 100 bases, consider ordering a Custom TaqMan® Gene Expression Assay instead of a Custom TaqMan® Small RNA Assay.

- For double-stranded sequences that contain chemically modified bases, submit the sequence of the unmodified strand.



Note: For siRNAs, consider that chemical modifications of bases may affect the performance of the assay.

- Limit the name to 20 characters and do not include spaces or special characters (such as ; : " < > + = \ | ? ,) . Assay names can include underscores (_), periods (.), dashes (-), and asterisks (*).

Uploading sequences

You can upload multiple sequences into the Custom TaqMan® Small RNA Assay Design page from a text file (*.txt) that contains the sequences in FASTA format:

- The first line is called the header line, which begins with a greater-than symbol (>), followed by a unique identifier for the sequence. For example, the miRBase ID can be used as the unique identifier.
- The second line contains the small RNA sequence.

For example:

	Greater-than symbol	Unique identifier
Header line –	>	gga-miR-1749*
(used as the assay name)	UUGGCUCUGUCCCCAUUUCC	
		Small RNA sequence



Note: When uploading sequences from a text file, the name is uploaded from the header line of each assay entry.

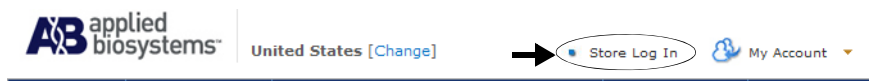
The figure below shows an example of a text file containing small RNA sequences that can be uploaded by the tool.

```
>kshv-miR-k12-9
CUGGGUAUACGCAGCUGCGUAA
>gga-miR-1749*
UUGGCUCUGUCCCCAUUUCC
>sirna
TTATTTGGTGCTGTAAACCTG
```

Enter sequences

In the Submit Sequences screen of the Custom TaqMan Small RNA Assay Design page, enter or upload the names and sequences for the small RNA you want to order.

1. Go to: www.appliedbiosystems.com
2. If you are not logged into the Applied Biosystems Website Store, click **Store Log In** at the top of the Applied Biosystems web page, then log into the website as instructed.



3. Access the Custom TaqMan Small RNA Assay Design page by going to: www.appliedbiosystems.com/smallrna
4. In the Submit Sequences screen, enter or upload the name and sequence for each small RNA.

To *manually* enter the name and sequence for each small RNA:

- a. In the Name field, enter a unique name to identify the small RNA.
The name can be up to 20 characters and cannot include spaces or special characters (such as ; : " < > + = \ | ? ,).
- b. In the Sequence field, enter the sequence of the small RNA in the 5' to 3' orientation. Acceptable sequences are 17- to 200-bases long and consist only of: A, C, G, T, and U.

For example:

Open/Import File		
Name	Sequence	Remove
kshv-miR-k12-9	CUGGGUAUACGCAGCUGCGUAA	Remove
gga-miR-1749*	UUGGCUCUGUCCCUAUUUCC	Remove
sirna	TTATTTGGTGCTGTAACCTG	Remove

To *automatically* upload sequences from a FASTA-formatted text file (*.txt):

- a. Click **Open/Import File**.



- b. In the FASTA File Import dialog box, click **Browse**.
- c. Select the text file (*.txt) that contains your sequences in FASTA format, then click **Open**.
- d. Click **Import File**.

The website automatically populates the Name and Sequence fields with the data in the imported file.

>kshv-miR-k12-9 CUGGGUAUACGCAGCUGCGUAA	→	Name	Sequence
>gga-miR-1749* UUGGCUCUGUCCCUAUUUCC		kshv-miR-k12-9	CUGGGUAUACGCAGCUGCGUAA
>sirna TTATTTGGTGCTGTAACCTG		gga-miR-1749*	UUGGCUCUGUCCCUAUUUCC
		sirna	TTATTTGGTGCTGTAACCTG
		+ Enter More Sequences	



5. Click **Submit Sequences(s)**. If you have not yet logged into the Applied Biosystems Store, you will be instructed to do so.
After submitting your sequence(s), you can close the web browser without losing your submission. If you choose to leave your browser open, click **Refresh Batch List** to refresh the Design Job Details page. Applied Biosystems will email you a link to the design report when the website confirms your submission.

Confirm the status of your submission

After you submit your sequences:

1. The website sends you an email message confirming the status of your submission. The confirmation email contains a link to the Select Assays screen of the Custom TaqMan Small RNA Assay Design page. To view the status of your submission, click the email link to open the Custom TaqMan Small RNA Assays Design page.

```
Dear <Your Name>,  
  
Your Custom TaqMan(R) Small RNA Assay Design has been submitted.  
  
Design Batch ID: w0909103133000  
Submission Date: 2009-10-28  
  
For quick access to the Design report in your Applied Biosystems workspace follow  
this link https://XXXXXX.appliedbiosystems.com:443/ab/en/US/direct/  
ab?cmd=ABCSDTDesignViewer&WorkspaceItemId=XXXX&Operation=viewCustomDesign  
  
Thank you,  
  
The Custom TaqMan(R) Technical Support Team  
(abtechnicalsupport@appliedbiosystems.com)
```

2. Applied Biosystems screens the TaqMan® Small RNA Assays for predesigned assays that match your submitted sequence(s). If predesigned assays exist for the submitted sequence(s), then the Custom TaqMan Small RNA Assay Design page displays the matching assays.
3. If no predesigned assays match your sequence(s), the sequence(s) is submitted to the design pipeline, which can take up to several hours to complete.
4. After the assay design is complete, you will receive an email message containing a link to the Select Assays tab of the Custom TaqMan Small RNA Assays Design page.

Select assays

The Select Assays screen displays assay information for each of the small RNA sequences that you submitted for Custom TaqMan® Small RNA Assay Design. The screen divides the information into two or more tables that list the submitted sequences and the results of the assay design process. For a complete description of the Design Job Details and Design Results tables, see [Appendix A, “About the Design Tables” on page 17](#).

Assay design failure

If an assay design for a small RNA sequence fails, the Custom Assays table on the Select Assays tab shows “FAIL” in the Status column for the sequence. Error messages for failures include:

- **Error Message 1** – We have been unable to design a high-performing assay for this sequence in the past. Please contact technical support if you would like to consider purchasing an assay with relaxed performance standards.
- **Error Message 2** – The submitted sequence failed design. The design may have failed due to high G/C content, high A/T content, or the potential of the sequence to create self-dimers.
- **Error Message 3** – One or more of the assay oligonucleotides cannot be manufactured in a way that meets Applied Biosystems high-performance standards. Contact technical support to see if it is possible to redesign the assay.

Select assays to order

1. After the assay design is complete, follow the email notification link to access the Custom TaqMan Small RNA Assay Design Tool page.

```
Dear <Your Name>,  
  
Your Custom TaqMan(R) Small RNA Assay Design report is ready for review.  
  
Design Batch ID: w0909103132000  
Submission Date: 2009-10-28  
Design Date: 2009-09-28T20:46:42.000979  
  
For quick access to the Design report in your Applied Biosystems workspace follow  
this link https://XXXXXX.appliedbiosystems.com:443/ab/en/US/direct/  
ab?cmd=ABCSDesignViewer&WorkspaceItemId=XXXXX&Operation=viewCustomDesign  
  
Thank you,  
  
The Custom TaqMan(R) Technical Support Team  
(abtechnicalsupport@appliedbiosystems.com)
```

2. Select the batch of interest in the Design Job Details table to display the results of the assay design.
3. Review the list of custom and predesigned assays in the Design Results tables.



4. For each assay that you want to order:
 - a. Select the assay size from the drop-down list in the Size column.
 - Extra Small (XS) – Sufficient reagents to perform 75 PCR reactions.
 - Small (S) – Sufficient reagents to perform 150 PCR reactions.
 - Medium (M) – Sufficient reagents to perform 750 PCR reactions.
 - Large (L) – Sufficient reagents to perform 2900 PCR reactions.
 - b. Enter the quantity to order in the **Quantity** column.
 - c. Click **Add** next to an assay to add it to the Shopping List.

Shopping List (4 items)

Order Now
Clear List

Custom Small RNA Assays

[1] CSR4YY5 - madeup - medium ✕

Pre-designed Assays

TaqMan® MicroRNA Assays

[1] 197243_mat - small ✕

[1] 00006850 - medium ✕

TaqMan® siRNA Assays

[1] s1629_asy - large ✕

Custom Assays					
Name	Assay ID	Status	Size	Quantity	Add All
madeup View Target Sequence	CSR4YY5	DESIGN SUCCESS	4398987: SM (150 RT, 150 PCR rxns)	1	Add

Pre-designed Assays						
The sequences you entered matched the following pre-designed assays. A custom assay is not necessary.						
Name	Assay ID	Type	Availability	Size	Quantity	Add All
kshv-miR-k12-9 View Target Sequence	197243_mat	miRNA Assay	Inventoried	4427975: S (50 RT, 150 PCR rxns)	1	Add
gga-miR-1749* View Target Sequence	00006850	miRNA Assay	Made To Order	4440886: S (50 RT, 150 PCR rxns)	1	Add
siRNA View Target Sequence	s1629_asy	siRNA Assay	Made To Order	4440878: S (50 RT, 150 PCR rxns)	1	Add

Select other products

The Select Other Products tab displays assays that can be used to detect candidate endogenous controls for normalizing the small RNA sequences that you submitted for Custom TaqMan® Small RNA Assay Design. A valid normalization or endogenous control is needed to correct for differences in RNA sampling and sample variation. The ideal control is expressed consistently under experimental conditions and is sufficiently abundant across all tissues and cell types studied.



Note: We recommend that you experimentally validate all candidate genes to be used as endogenous controls.

Add a control assay to the order

1. After you add Custom TaqMan® Small RNA Assays to your order, choose the **Select Other Products** tab to add control assays to your order.
2. Review the list of assays on the Select Other Products tab.
 - a. Click a species name to expand the list.
 - b. Click the assay name of any control assay to see more information on the assay of interest.

Endogenous Control Assays

Endogenous control assays simplify data normalization and are designed against carefully selected, small, non-coding RNAs that are unrelated to miRNAs. These controls are expressed at consistent levels across a wide variety of cell types, tissues, and experimental conditions.

- Human
- Mouse
- Rat
- A. thaliana

Control Assays for Species: A. thaliana			
snoR101	50 RT/150 TaqMan® rxns	<input type="text" value="1"/>	<input type="button" value="Add"/>
snoR41Y	50 RT/150 TaqMan® rxns	<input type="text" value="1"/>	<input type="button" value="Add"/>
snoR66	50 RT/150 TaqMan® rxns	<input type="text" value="1"/>	<input type="button" value="Add"/>
snoR85	50 RT/150 TaqMan® rxns	<input type="text" value="1"/>	<input type="button" value="Add"/>


- C. elegans
- D. melanogaster

3. For each assay that you want to order:
 - a. Enter the number of assays to order in the **Quantity** column.
 - b. Click **Add** next to an assay to add it to the Shopping List.



Submit your order

Review and submit order

1. In the Shopping List, click **Order Now**.
2. On the Review & Order tab, review your order. To add, change, or delete assays from your order:
 - **Add assays** – Return to the **Select Assays** tab, select the size, enter the Quantity, then click **Add**. In the Shopping List, click **Order Now** to return to the Review & Order tab.
 - **Change an assay scale** – Select the correct scale from the drop-down list for the appropriate assay.
 - **Change an assay quantity** – Enter the new quantity in the Quantity field for the appropriate assay.
 - **Delete assays** – Click the  next to the unwanted assay.
3. When you are ready to order, click **Add To Basket**.
4. Complete the order as instructed by the website.
5. Click **Submit Order**. Applied Biosystems will send you an email to confirm your order.


Download supporting documentation

You can download the *TaqMan® Small RNA Assay Protocol*, the *TaqMan® Small RNA Assay Quick Reference Guide*, and other supporting documentation from the Applied Biosystems website. The documents provide instructions for using TaqMan® Small RNA Assays and detailed information on related products. See [“Documentation and Support” on page 29](#) for instructions on finding and downloading the documentation.

Reorder assays

With the Assay ID, you can reorder Custom TaqMan® Small RNA Assays using the Quick Order feature of the Applied Biosystems website.

To reorder an assay:

1. Go to: www.appliedbiosystems.com
2. If you are not logged into the Applied Biosystems Store, click **Store Log In** at the top of the Applied Biosystems web page, then log in as instructed.
3. After you log in, click  **Quick Order** at the top of the Applied Biosystems web page.

4. In the Quick Order screen, enter the part number and Assay ID that you want to order, then follow the instructions to complete the order.

Quick Order

Quickly and easily add products to your shopping basket by manually entering **and/or** by copying and pasting the desired part numbers (or part number + ID).

Have you placed this order before? You can reorder it by using your [Order History](#).

Manual Entry			Copy/Paste Entry	
Individually enter the desired Part Number (or Part Number + ID). Learn more			Copy the desired Part Numbers (or Part Numbers + IDs) from your files and then paste the data into the box provided below. Learn more	
Part Number	ID*	Qty	Part Numbers/IDs*	
<input type="text" value="4398989"/>	<input type="text" value="CSR4YY5"/>	<input type="text" value="1"/>	<p>Each Part Number (or Part Number + IDs) must be separated by a return or a comma. Part Numbers must be separated from their IDs by a single space.</p> <p>EXAMPLE:</p> <p>4304437 AM16708 16214 Hs00198914_m1</p> <p>OR</p> <p>4304437, AM16708 16214, Hs00198914_m1</p>	
<input type="text"/>	<input type="text"/>	<input type="text" value="1"/>		
<input type="text"/>	<input type="text"/>	<input type="text" value="1"/>		
<input type="text"/>	<input type="text"/>	<input type="text" value="1"/>		
<input type="text"/>	<input type="text"/>	<input type="text" value="1"/>		
<input type="text"/>	<input type="text"/>	<input type="text" value="1"/>		
<input type="text"/>	<input type="text"/>	<input type="text" value="1"/>		
<input type="text"/>	<input type="text"/>	<input type="text" value="1"/>		
<input type="text"/>	<input type="text"/>	<input type="text" value="1"/>		
<input type="text"/>	<input type="text"/>	<input type="text" value="1"/>		
[+] Add more rows		Clear	Clear	
			My Basket03/18/2010 21:10:33 <input type="button" value="Add to Basket"/>	

Appendix A About the Design Tables

Design Job Details table

The Design Job Details table displays all assay design jobs that you have submitted to Applied Biosystems. Each row summarizes the design results for the sequences that you submitted to Applied Biosystems at the same time.

Batch ID	Submitted	Status	Details 
w0912103623000	2009-12-16 18:28:58	PENDING	2 Pending, 0 Failed, 1 Pre-designed
w0909103133000	2009-10-28 23:08:04	COMPLETED	1 Passed, 0 Failed, 0 Pre-designed
w0909103132000	2009-09-28 23:06:49	COMPLETED	3 Passed, 0 Failed, 0 Pre-designed

Batch ID – Unique identifier assigned by Applied Biosystems to each design job.

Submitted – The date and time that the design job was submitted.

Status – The status of the design job, where:

- COMPLETED indicates the design job is complete.
The Custom TaqMan® Small RNA Assay Design Tool has either:
 - Found pre-designed assays that match the associated sequence(s).
 - Designed an assay for the associated sequence(s).
 - Failed to design an assay for the associated sequence(s).
- PENDING indicates the assay design is in progress. The design process can take up to several hours.



Note: If the tool has found pre-designed assays for the associated sequences, select the row to display the assays in the Pre-Designed Assay table (see [page 18](#)).

Details – Displays the status of sequences for the assay design, where:

- Pending indicates the number of sequences that are still in the design process.
- Passed indicates the number of sequences that have completed the design process and now have a custom assay.
- Failed indicates the number of sequences that have failed the design process.
- Pre-designed indicates the number of sequences that have matched one or more pre-designed TaqMan® Small RNA Assays.

Design Results table

The Design Results table lists the TaqMan® assays that have been designed or found for your submitted sequences. Select a row in the Design Job Details table to display the corresponding assays in the Design Results table. For each assay, the design tool displays the following information.

Design Job Details

[Click here to show/hide Batch Query XML details](#)

Batch ID	Submitted	Status	Details
w1007118344000	2010-07-07 17:31:20	PENDING	1 Pending, 0 Failed, 0 Pre-designed
w1007118343000	2010-07-07 17:23:20	COMPLETED	1 Passed, 0 Failed, 4 Pre-designed
w1007118341000	2010-07-07 15:02:56	COMPLETED	0 Passed, 0 Failed, 4 Pre-designed

[Go To Workspace](#)

Design Results for Batch ID: w1007118343000

[Click here to show/hide Batch Query XML details](#)

Save Results to File

Custom Assays					
Name	Assay ID	Status	Size	Quantity	Add All
madeup View Target Sequence	CSR4YY5	DESIGN SUCCESS	4398987; SM (150 RT, 150 PCR rxns)	1	Add

Pre-designed Assays						
The sequences you entered matched the following pre-designed assays. A custom assay is not necessary.						
Name	Assay ID	Type	Availability	Size	Quantity	Add All
kshv-miR-k12-9 View Target Sequence	197243_mat	miRNA Assay	Inventoried	4427975; S (50 RT, 150 PCR rxns)	1	Add
gga-miR-1749* View Target Sequence	00006850	miRNA Assay	Made To Order	4440886; S (50 RT, 150 PCR rxns)	1	Add
siRNA View Target Sequence	s1629_asy	siRNA Assay	Made To Order	4440878; S (50 RT, 150 PCR rxns)	1	Add

Name – Identifier for the sequence that you submitted to Applied Biosystems.



Note: Hover the mouse pointer over **View Target Sequence** to view the small RNA sequence you submitted to Applied Biosystems.

Assay ID – A unique identifier assigned to the assay by Applied Biosystems. You can use the Assay ID to reorder the TaqMan® assay.

Type – (Pre-designed assays only) The classification of the small RNA target to which the assay is pre-designed (for example, miRNA or siRNA).

Availability – (Pre-designed assays only) The availability of the assay, where:

- Inventoried indicates the assay is currently stocked and shipped at the time of order.
- Made To Order indicates the assay is manufactured at the time of order.

Status – (Custom assays only) The status of the custom assay design, where:

- DESIGN SUCCESS indicates the assay design for the associated sequence is complete and the algorithm was able to design an optimal assay for the sequence.
- DESIGN PENDING indicates the assay design for the associated sequence is in progress. The design process may take several hours.
- DESIGN FAILED indicates the assay design is complete, but the algorithm was unable to design an optimal assay for the sequence. For more information, see [“Assay design failure” on page 12](#).

Size – The size of the assay to order. Available sizes, including:

- Extra Small (XS) – Sufficient reagents to perform 75 PCR reactions.
- Small (S) – Sufficient reagents to perform 150 PCR reactions.
- Medium (M) – Sufficient reagents to perform 750 PCR reactions.
- Large (L) – Sufficient reagents to perform 2900 PCR reactions.

Quantity – The number of assays to order.



Appendix A About the Design Tables
Design Results table

Appendix B Ordering Information

Materials and equipment

	Product	Quantity/Package
RNA Isolation Kits	mirVana™ miRNA Isolation Kit	<ul style="list-style-type: none"> Kit, 40 purifications (PN AM1560) Kit without Phenol, 40 purifications (PN AM1561)
	TRI Reagent®	<ul style="list-style-type: none"> 100 mL (PN AM9738)
	TaqMan® MicroRNA Cells-to-CT™ Kit	<ul style="list-style-type: none"> Kit, 100 rxns (PN 4391848) Kit, 400 rxns (PN 4391996) Cells-to-CT™ Stop Solution, 1 mL (PN 4402960) Cells-to-CT Bulk Lysis Reagents, 2500 rxns (PN 4391851)
RT Kits	TaqMan® MicroRNA Reverse Transcription Kit‡	<ul style="list-style-type: none"> Kit, 200 rxns (PN 4366596) Kit, 1000 rxns (PN 4366597)
PCR Master Mixes	TaqMan® Universal PCR Master Mix II, No UNG§	<ul style="list-style-type: none"> Kit, Mini-Pack, 1 × 1-mL tube (PN 4440043) Kit, 1-Pack, 1 × 5-mL bottle (PN 4440040) Kit, 2-Pack, 2 × 5-mL bottles (PN 4440047) Kit, 5-Pack, 5 × 5-mL bottles (PN 4440048) Kit, 10-Pack, 10 × 5-mL bottles (PN 4440049)
	TaqMan® Universal PCR Master Mix II, with UNG§	<ul style="list-style-type: none"> Kit, Mini-Pack, 1 × 1-mL tube (PN 4440042) Kit, 1-Pack, 1 × 5-mL bottle (PN 4440038) Kit, 2-Pack, 2 × 5-mL bottles (PN 4440044) Kit, 5-Pack, 5 × 5-mL bottles (PN 4440045) Kit, 10-Pack, 10 × 5-mL bottles (PN 4440046)
	TaqMan® 2X Universal PCR Master Mix, No AmpErase® UNG§	<ul style="list-style-type: none"> Kit, 1-Pack, 1 × 5-mL bottle (PN 4324018) Kit, 2-Pack, 2 × 5-mL bottles (PN 4364341) Kit, 5-Pack, 5 × 5-mL bottles (PN 4364343) Kit, 10-Pack, 10 × 5-mL bottles (PN 4324020) Kit, Bulk, 1 × 50-mL bottle (PN 4326614)
	TaqMan® 2X Universal PCR Master Mix, with AmpErase® UNG§	<ul style="list-style-type: none"> Kit, 1-Pack, 1 × 5-mL bottle (PN 4304437) Kit, 2-Pack, 2 × 5-mL bottles (PN 4364338) Kit, 5-Pack, 5 × 5-mL bottles (PN 4364340) Kit, 10-Pack, 10 × 5-mL bottles (PN 4305719) Kit, Bulk, 1 × 50-mL bottle (PN 4326708)

‡ TaqMan® Small RNA Assays are specifically optimized to work with the MuLV Reverse Transcriptase contained in the TaqMan® Micro RNA Reverse Transcription Kit. Applied Biosystems cannot guarantee assay performance if you use other reverse transcriptase enzymes.

§ We strongly recommend that you use these Applied Biosystems reagents with all TaqMan® Small RNA Assays.

B

Appendix B Ordering Information *Materials and equipment*

Appendix C Chemistry Overview

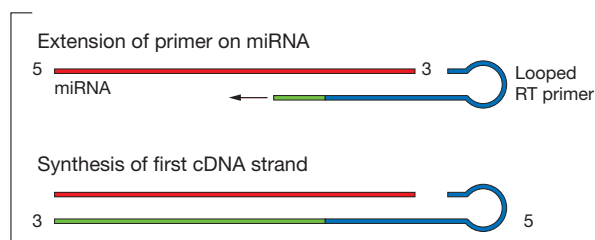
Two-step RT-PCR

Quantification using the TaqMan[®] Small RNA Assays is done using two-step RT-PCR:

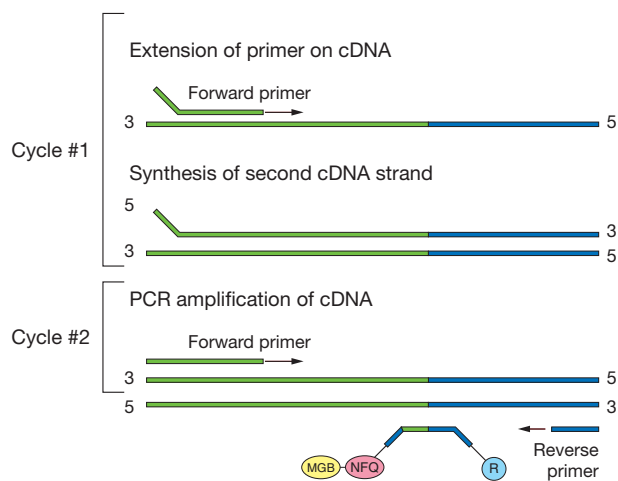
1. In the reverse transcription (RT) step, cDNA is reverse transcribed from total RNA samples using a small RNA-specific, stem-loop RT primer from the TaqMan[®] Small RNA Assays and reagents from the TaqMan[®] MicroRNA Reverse Transcription Kit.
2. In the PCR step, PCR products are amplified from cDNA samples using the TaqMan[®] Small RNA Assay together with the TaqMan[®] Universal PCR Master Mix II.

Figure 1 Two-step RT-PCR

Step 1: Reverse Transcription



Step 2: Real-Time PCR



About the probes

The TaqMan[®] MGB probes contain:

- A reporter dye (FAM[™] dye) linked to the 5' end of the probe.
- A minor groove binder (MGB) at the 3' end of the probe.

This modification increases the melting temperature (T_m) without increasing probe length (Afonina et al., 1997; Kutuyavin et al., 1997), which allows the design of shorter probes.





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

Because the quencher does not fluoresce, Applied Biosystems sequence detection systems can measure reporter dye contributions more accurately.

5' nuclease assay process

The 5' nuclease assay process (Figure 2 through Figure 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

-  = Nonfluorescent quencher
-  = Minor groove binder
-  = Reporter
-  = Hot-start DNA polymerase

During PCR, the TaqMan[®] MGB probe anneals specifically to a complementary sequence between the forward and reverse primer sites (Figure 3).

When the probe is intact (Figure 3 and Figure 4), the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence, primarily by Förster-type energy transfer (Förster, 1948; Lakowicz, 1983).

Figure 3 Polymerization

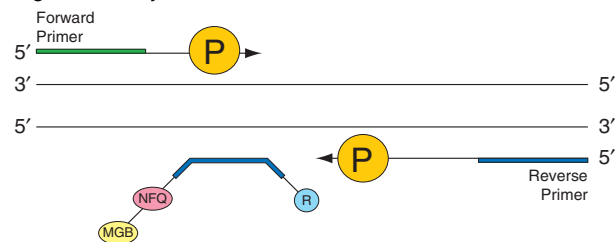
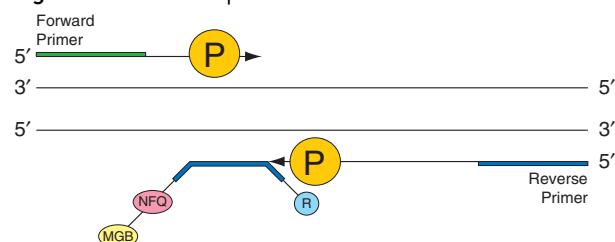
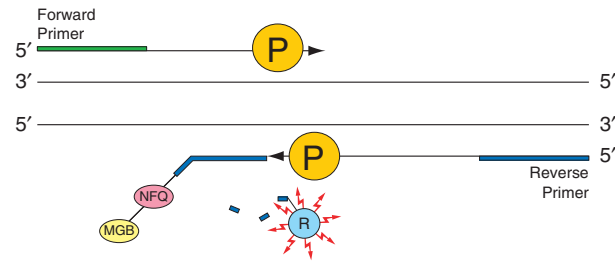


Figure 4 Strand displacement



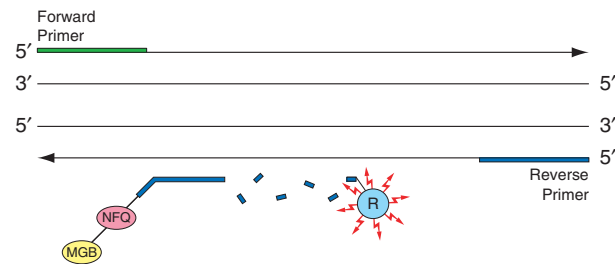
The DNA polymerase cleaves only probes that are hybridized to the target (Figure 5). Cleavage separates the reporter dye from the quencher dye; the separation of the reporter dye from the quencher dye results in increased fluorescence by the reporter. The increase in fluorescence signal during occurs only if the target sequence is complementary to the probe and is amplified during PCR. Because of these requirements, nonspecific amplification is not detected.

Figure 5 Cleavage



Polymerization of the strand continues, but because the 3' end of the probe is blocked, there is no extension of the probe during PCR (Figure 6).

Figure 6 Completion of polymerization





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Lakowicz, J.R. 1983. Energy Transfer. In: *Principles of Fluorescence Spectroscopy*, New York: Plenum Press 303–339.

Documentation and Support

Related documentation

The following related documents are available from the Applied Biosystems website:

Document	Part number
<i>TaqMan[®] Small RNA Assays Protocol</i>	4364031
<i>TaqMan[®] Small RNA Assays Quick Reference Card</i>	4412551



Note: For additional documentation, see “[Obtaining support](#)” below.

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Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone 760.603.7200
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