

TaqMan® MicroRNA Assays FAQs

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TaqMan® MicroRNA Assay Naming

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What is miRBase?

miRBase (www.miRBase.org) is the primary online repository for all miRNA sequences and annotation: Each entry represents a predicted hairpin portion of a miRNA transcript, with information on the location and sequence of the mature miRNA(s) derived from that hairpin. miRBase was established in 2002 and is maintained by the Sanger Institute. It provides miRNA researchers with universal and unique gene names, and an archive of all miRNA sequences.

miRBase ID

The **miRBase ID** is the official miRBase name given to a mature miRNA sequence. There may be multiple miRBase IDs for a given mature miRNA sequence because many miRNAs are conserved across species (for example, several hundred human miRNAs have the identical sequence in common with the mouse species). The miRBase ID consist of a 3 letter species identifier, including the first letter of the genus and the first two letters of the species, followed by the expression “miR”, and then a numeric suffix, e.g.: hsa-miR-212. Since two distinct mature miRNAs can be derived from the different arms of the same stem-loop precursor (see figure below), a -5p or -3p suffix specifies the particular arm of the stem from which they come, e.g.: hsa-miR-501-5p is derived from the 5’ end of the stem, while hsa-miR-501-3p is derived from the 3’ end (see figure below). The miRNA gene and hairpin precursor locus name is given lower case: hsa-mir-212. If a mature miRNA is predicted to be expressed from more than one hairpin precursor, then a numeric suffix is added to the precursor name. For example, hsa-miR-101 is expressed from two precursor loci, hsa-mir-101-1 and hsa-mir-101-2. Closely related miRNAs are given lettered suffixes (hsa-miR-19a-3p and hsa-miR-19b-3p) and are expressed from similarly named precursors (hsa-mir-19a-3p and hsa-mir-19b-3p). miRBase continues to re-annotate the miRNA sequences as more information becomes available. Updated versions of miRBase are released on a regular basis. At the time of print (June 2013), it was up to v20.



Figure depicting mature miRNAs derived from the same arm of a stem-loop pri-miRNA. The pink line on the left is annotated with -5p and the purple on the right with -3p

What is a “star” sequence?

Earlier naming convention used the **miR/miR*** (a.k.a. “non-star/star”) nomenclature to identify the mature miRNA that was predominantly expressed from a precursor stem loop. This nomenclature has been phased out and replaced with -5p/-3p in miRBase, as explained earlier. Life Technologies continues to use the “star” in the Assay Names for those assays that were released when the “star” convention was in use by miRBase.

What is the miRBase Accession Number?

Accession numbers are unique identifiers assigned to both hairpin (e.g.: MI0000015) and mature (e.g.: MIMAT0000029) sequences. While miRBase names may change, the accession numbers are stable and remain associated with a specific sequence.

TaqMan® MicroRNA Assay naming principles and special cases

TaqMan® MicroRNA Assays are given a unique and invariable **Assay ID**. The Assay ID, as well as the assay design, i.e. the sequences of the oligos composing the assay (RT, forward and reverse primers, and TaqMan® probe), do not change. The Assay Name is the miRBase ID for the targeted miRNA sequence when the assay was first released. miRBase IDs are known to be unstable as there have been multiple changes over time: Annotation changes include revised miRNA sequences for the same miRBase ID, and updated miRBase IDs. Thus, a sequence may have a different miRNA ID from the one it originally had, or a given miRNA ID may have a modified sequence. The assay, however, is still completely functional for the sequence for which it was originally designed. It is always recommended to use the miRNA sequence or miRBase Accession Number to confirm that a given assay will target a specific miRNA.

Why do some assays no longer map to an annotated miRNA sequence?

miRBase continues to re-annotate the miRNA sequences as more information becomes available. A sequence that once mapped to a given species (e.g.: human) may no longer exist, so it is removed from miRBase. Accordingly, the original assay for that sequence no longer maps to an annotated miRNA, and as a result the annotation fields on the search results page on the Life Technologies portal are empty. An example of this is Assay “hsa-miR-505” (Assay ID 001049).

☐ 001049
 [View Assay on Map](#)

Assay Name	miRBase ID	Species	Mature miRNA Sequence	Inventoried Cat. # 4427975
hsa-miR-505			GUCAACACUUGCUGGUUCCUC	S (50 RT/150 PCR rxns) ▼

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Another example is when a miRNA sequence no longer maps to a certain species (e.g. human) but still maps to other species, such as rat and mouse. In this case, the Assay Name will not change. Assay “hsa-miR-674” (Assay ID 002021) is an example of this type of annotation change.

Examples of Assay Searches including Special Cases of Annotation:

- Search for **Assay ID 002021** with **Species** selected for **mouse or rat**: The assay annotation is for rodent species (mmu -, rno -).

Search Term: 002021

☐ 002021
 [View Assay on Map](#)

Assay Name	miRBase ID	Species	Mature miRNA Sequence	Inventoried Cat. # 4427975
hsa-miR-674	mmu-miR-674-5p rno-miR-674-5p	Mouse Rat	GCACUGAGAUGGGAGUGGUGUA	S (50 RT/150 PCR rxns) ▼

[Details ▼](#)
[Mimics/Inhibitors ▼](#)
[Pri-miRNA Assays ▼](#)
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- Search for **Assay ID 002021** with **Species** selected for **human**: There is no annotation because the sequence does not map to human.

☐ 002021
 [View Assay on Map](#)

Assay Name	miRBase ID	Species	Mature miRNA Sequence	Inventoried Cat. # 4427975
hsa-miR-674		Mus musculus Rattus norv...	GCACUGAGAUGGGAGUGGUGUA	S (50 RT/150 PCR rxns) ▼

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How are the assays named when the same sequence is common across multiple species?

When a sequence that maps to multiple species is introduced into miRBase, the assay name is given according to the following hierarchy: hsa, mmu, rno, dre, cel, ath, and then alphabetically for the remaining species. Thus if an assay maps to human, mouse and c. elegans simultaneously, it will be named hsa-miR-*nnn*.

Why is an assay for human named with the name of another species?

An assay for a human miRNA may have a lower species name because the target sequence was first introduced and published in miRBase for that species and only later found in human. For example, mmu-miR-124a was first introduced as a mouse miRNA. The human miRNA was introduced later.

☐ 001182 View Assay on Map

Assay Name	miRBase ID	Species	Mature miRNA Sequence	Inventoried Cat. # 4427975
mmu-miR-124a	hsa-miR-124-3p	Human	UAAGGCACGCGUGAAUGCC	S (50 RT/150 PCR rxns) ▼

Details ▼

Mimics/Inhibitors ▼

Pri-miRNA Assays ▼

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What is an "alias"?

An **alias** is the miRBase ID for a given miRNA from an earlier version. Alias information is found in the **Details** section of the search results. The miRBase release version shown in parentheses represents when the alias miRBase ID was last listed.

☐ 000385 View Assay on Map

Assay Name	miRBase ID	Species	Mature miRNA Sequence	Inventoried Cat. # 4427975
hsa-miR-1	asu-miR-1-3p cbr-miR-1 cel-miR-1-3p View more...	Ascaris suum Caenorhabdi... Caenorhabdi... View more...	UGGAAUGUAAAGAAGUAUGUA	S (50 RT/150 PCR rxns) ▼

Details ▲

Mimics/Inhibitors ▼

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Species:

miRBase Accession Numbers:

Mapped to miRBase Version:

miRBase Alias:


Ascaris suum, Caenorhabditis briggsae, Caenorhabditis elegans, Chicken, Dog, Gorilla gorilla, Monodelphis domestica, Oryzias latipes, Pan paniscus, Pig, Xenopus tropicalis
MIMAT0021424, MIMAT0000465, MIMAT0000003, MIMAT0001127, MIMAT0006656, MIMAT0024078, MIMAT0004085, MIMAT0022558, MIMAT0002621, MIMAT0010187, MIMAT0003551
v19
hsa-miR-1 (v9.2)
mmu-miR-1 (v9.2)
asu-miR-1 (v18)
cel-miR-1 (v17)
gga-miR-1 (v7.1)
ssc-miR-1a (v14)

[View Full Details](#)

How come the annotation of some assays on the portal show only blanks?

An assay would lose its annotation when it no longer maps to an annotated miRNA (i.e. the miRNA was removed from miRBase); as a result, the annotation fields on the website are empty. For example, the sequence UGUAAACAUCUUGACUGGA was last mapped to hsa-miR-30e-5p in miRBase v9.2. It has since been removed from the database and replaced with a sequence that is 2 bases longer at the 3' end: UGUAAACAUCUUGACUGGAAG.

☐ 000421

 [View Assay on Map](#)

Assay Name	miRBase ID	Species	Mature miRNA Sequence	Inventoried Cat. # 4427975
hsa-miR-30e-5p			UGUAAACAUCUUGACUGGA	S (50 RT/150 PCR rxns) ▼

[Details](#) ▼

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TaqMan® MicroRNA Assay search

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How do I search for a TaqMan® MicroRNA Assay on the Life Technologies website?

Go to the URL: www.lifetechnologies.com/taqmanmirna

Assay Search Tool:

What type of experiment are you conducting?

Gene Expression

SNP Genotyping

Copy Number

siRNA

MicroRNA

Mutation Detection

What type of miRNA are you interested in?

Mature miRNA

Pri-miRNA

Mimics/Inhibitors

Controls

What species do you want to target? (Select one or more)

Human

Mouse

Rat

More (191)

All

Enter target information

e.g., Assay ID, miRBase ID, miRBase Accession #

OR

Enter Single Sequence

Enter / Upload Multiple Targets

Select a single species to search by location

Enter / Upload Multiple Sequences

na-mrna-assay.html

Search

1. Select **MicroRNA** under “What type of experiment are you conducting”?
2. Select **Mature miRNA** under “What type of miRNA are you interested in”?
3. Select the name of the **Species** that the assay needs to target.
4. Specify the assay or the mature miRNA targeted by entering one of the following pieces of **information**:
Assay ID, miRBase ID, Accession #, or miRNA target sequence.

Below is a screen shot from the results of a search using generic name, **miR-21**, species: **human**. Two assay results were obtained with unique Assay ID numbers, as indicated by the example in the red rectangle below. If

the search returns a large number of results, these can be narrowed with the filters in the left hand of the screen, e.g.: by selecting for Inventoried Assays only, or by selecting specific species.

The screenshot shows the TaqMan MicroRNA Assays search results page. At the top, there is a search bar with the text '000397'. Below the search bar, there are several filters: 'Species' (Human) and 'miRNA Type' (TaqMan® MicroRNA Assays). The results are displayed in a table with columns: Assay Name, miRBase ID, Species, Mature miRNA Sequence, and Inventory/Catalog info. Two results are shown: hsa-miR-21 (Assay ID 000397) and hsa-miR-21* (Assay ID 002438). Each result has a 'Details' dropdown menu and an 'Add To Cart' button.

- Assay ID:** A unique 6-digit identifier for each assay design (RT, qPCR primers and probe sequence). The Assay ID and the assay target sequences are invariable. Recent Assay IDs present the suffix “_mat”. For example: 000430 (hsa-miR-92) or 463010_mat (hsa-miR-4443).
- Assay Name:** this name is given to the assay when the assay is **first** designed and is based upon the miRBase name current at that time. **Note:** the names within miRBase may change, thus a given Assay Name may not match the present miRBase Name.
- miRBase ID:** this is the name of the miRNA from the current miRBase version that corresponds to the miRNA sequence. All species that have a miRNA that maps to this sequence are listed.
- Sequence information:** The sequence of RNA targeted by the Assay.
- Cat. #** (Catalog or Product Part Number), **Assay Availability**, and **Assay Size**. **Cat. #** corresponds to the Product Part Number. There is a different Catalog Number for each different available Assay Size (i.e.: Extra Small, Small, Medium, or Large). Assays are available as either **Inventoried** (INV), meaning that they are in stock and ready to ship, or as **Made to Order** (MTO), meaning that they will be manufactured upon order. INV and MTO assays carry different Catalog Numbers.
- Details and Full Details:** Under the **Details** drop down button are listed all species, miRBase Accession numbers and Aliases associated with that miRNA. An **Alias** is the miRBase ID for a given miRNA from an earlier version. The **Full Details** drop down button shows the precursor stem-loop sequence and related information.
- When available, drop down menus for related TaqMan® **Pri-miRNA Assays**, miRNA **Mimics/Inhibitors** products, and/or literature **Citations** are displayed.

What is the best way to search for a TaqMan® MicroRNA Assay?

Because of the potential instability of miRBase, names can sometimes be misleading; therefore searching by miRNA sequence is the most accurate method to search for an assay.

I have found more than one assay for my query. How do I know which one is the right assay?

Check the target sequence listed in the search details to ensure it corresponds to your needs. If you are not sure about the sequence of your miRNA of interest, first verify it by looking up the associated miRNA on miRBase.org and reviewing the reference information to make sure you select the correct miRNA.

I searched with the Assay Name and the search returned two different assays: Which one should I order?

Searching by sequence of a given miRNA is the most accurate way to ensure finding the right assay for that particular miRNA. In some cases, when searching by Assay Name, multiple assays are found bearing that Assay name. Two different assays may have the same Assay Name when miRBase modifies the sequence of an existing miRNA but retains the same miRNA ID as the old one for the new sequence. As an example, Assay 000385 was designed to target hsa-miR-1, the human miR-1 introduced with miRBase v2.0, and assigned “hsa-miR-1” as the Assay Name to reflect the annotation in miRBase. When the sequence for the human miR-1 was changed in a later release of miRBase (v10.1), Assay 002222 was designed to target the new sequence. As a result, both assays are named “hsa-miR-1” in alignment with miRBase. Due to the change in sequence for human, Assay 000385 no longer maps to the human miRNA. However, this assay still targets the miR-1 in all those species whose sequence has not changed since miRBase 2.0, as shown in the picture below.

[Change Your Search](#)
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Your Results

Species

☐ Ascaris suum
 ☐ Caenorhabditis briggsae
 ☐ Caenorhabditis elegans
 ☒ Chicken
 [+ Show 7 More Species](#)

miRNA Type

TaqMan® MicroRNA Assays

[Reset Filters](#)

000385

[View Assay on Map](#)

Assay Name	miRBase ID	Species	Mature miRNA Sequence	Inventoried Cat # 4427975
hsa-miR-1	cbr-miR-1 ola-miR-1-3p cfa-miR-1 View more...	Caenorhabdi... Monodelphis... Pan paniscus View more...	UGGAAUGUAAAGAAGUAUGUA	S (50 RT/150 PCR rxns) ▼

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Controls

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What endogenous control to use for non-human and non-mouse miRNA assays?

It is important to select the appropriate endogenous control to normalize your data. Life Technologies has identified a number of small non-coding RNAs (snoRNAs, snRNAs) that show stable, moderate expression across a large number of tissues for human and mouse that are therefore good candidates for an endogenous control. Candidate controls are also available for rat, *Drosophila*, *Arabidopsis* and *C. elegans*. An updated list of available controls can be found on our portal under the URL www.lifetechnologies.com/taqmanmirna by selecting **MicroRNA** and then **Controls**, followed by the species of interest as search options.

In general, any miRNA can be used as an endogenous control to normalize results across different samples as long as it meets the criteria of a good endogenous control: providing stable expression levels with minimal variation across the different sample and conditions being used in your study. Since a control for one experimental study/treatment may not be appropriate for another one, a control should be validated before use. One approach is to select the control(s) based on your data set. An Application Note that describes how to perform validation using simple statistical methods, i.e. by measuring the Standard Deviation of the average C_t, is available: [Endogenous Controls for Real-Time Quantitation of miRNA Using TaqMan® MicroRNA Assays \(Application Note: TaqMan® MicroRNA Assays, 2007\)](#). Alternatively, you can use geNorm (Vandensompele et al. 2002 Genome Biology, <http://genomebiology.com/2002/3/7/research/0034>) to determine which of the candidate controls is best to use for your study.

What endogenous control to use for human miRNA from blood (serum, plasma)?

Small RNAs such as snRNAs or snoRNAs are usually not present in serum or other body fluids. Spike in controls can be used to monitor sample preparation. Any miRNA that is present in your serum samples can be used as a control as long as it is stably expressed across all the sample types in your study. You can refer to the literature for candidate miRNAs to test or you can select of a control from your data set to use in your analysis.

What endogenous control to use for pri-miRNA?

TaqMan® endogenous control assays are compatible for pri-miRNA (visit the [TaqMan® Endogenous Control Assays](#) web portal for more information).

What is the expected C_t value of U6 in my sample?

As with any other targets, the particular C_t value will depend on multiple factors, such as the tissue type and its condition, the input amount of RNA, the sample preparation method, etc. In the tissue samples we have looked at the U6 is moderately expressed.

When are spike-ins recommended? How?

Spike-ins or exogenous controls are recommended when it is necessary to monitor for extraction efficiency or sample input amount with difficult samples (for example with serum/plasma, other biofluids). A spike in control should be a target sequence that is not present in your sample. For example, ath-miR-159a is not present in humans so is a good spike in control for human. Exogenous controls are synthetic RNA oligonucleotides that match the target sequence. The RNA oligo does not require a 5' phosphate and HPLC purification is not necessary. We recommend using 5-10 pM for each spike in control. We have TaqMan(R) MicroRNA Assays available for a number of miRNAs that can be used as spike in controls with human samples (ath-miR-159a; cel-miR-39-3p cel-miR-2-3p).

Data Normalization

What is the best way to normalize results?

Selecting a good endogenous control is imperative for good data normalization; see [Endogenous Controls for Real-Time Quantitation of miRNA Using TaqMan® MicroRNA Assays \(Application Note: TaqMan® MicroRNA Assays, 2007\)](#). For a large number of targets (e.g., beyond 380), you can use the Global Mean Normalization method (Mestdagh et al. 2009 Genome Biology, <http://genomebiology.com/2009/10/6/R64>). Expression Suite, a free gene expression data analysis tool, provides global normalization when using large number of samples. It also provides the selection of single or multiple endogenous controls.

Individual TaqMan® MicroRNA Assays - Pooled RT and PreAmp primer reactions

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Is it possible to combine several miRNA RT primers together for reverse transcription, and if so, how?

Yes. If you are using individual TaqMan® MicroRNA Assays, you can prepare your own RT and PreAmp pools. Refer to [Protocol for Creating Custom RT and Preamplification Pools using TaqMan® MicroRNA Assays \(User Bulletin PN 4465407\)](#) for detailed information. Although this protocol has been tested by our development group, we recommend that you validate the performance of the particular pool that you are interested in working with. At a minimum, we recommend running a No Template Control (NTC). The NTC is crucial to identify any primer interactions that may increase the background.

Can I run preamp with individual TaqMan® MicroRNA Assays?

Yes. Refer to [Protocol for Creating Custom RT and Preamplification Pools using TaqMan® MicroRNA Assays \(User Bulletin PN 4465407\)](#) for detailed information. When pooling fewer than 12 assays together, you can reduce the final volume but keep the final concentration of each assay in the pool at 0.2X (i.e. by diluting each 20X TaqMan(R) MicroRNA Assay 1:100X)

Pre-amplification was developed for use with small sample size as a means to “stretch” your sample. At the same time, the variability of the C_t value is reduced for low copy number transcripts.

Reagents Compatibility

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Can one use the reagents from High Capacity RT kit to make cDNA for TaqMan(R)MicroRNA Assay?

Yes. You can use the reagents from the High Capacity cDNA Kit PN 4368814 (without RNase Inhibitor) or 4374966 (with RNase Inhibitor).

Do I have to use the Universal Master Mix (which one?) with my TaqMan® MicroRNA Assays?

Yes. This is the recommended master mix for TaqMan® MicroRNA Assays. You can use Universal Master Mix or Universal Master Mix II (with or without UNG).

Packaging Scales

What are the different packaging options for TaqMan® MicroRNA Assays?

- Extra Small (XS)
- Small (S)
- Medium (M)
- Large (L)

What number of RT reactions and TaqMan® reactions are in the different packaging options?

Assay Size	no. of RT reactions	no. of PCR reactions
Extra Small (XS)	25	75
Small (S)	50	150
Medium (M)	750	750
Large (L)	2900	2900

Custom TaqMan® Small RNA Assays

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What does it mean when I receive an Error message for a new Custom TaqMan® Small RNA Assay design?

The error message means a high performing assay cannot be designed to that sequence.
Error messages may include the following:

- Error Message 1** – We have been unable to design a high-performing assay for this sequence in the past.
- 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 submitted sequence to create self-dimers.
- Error Message 3** – One or more of the assay oligonucleotides cannot be manufactured in a way that meets Life Technologies high-performance standards.

Errors may also be generated under these particular cases:

- The submitted sequences is less than 15 nucleotides in length
- There is presence of palindromes at 3' end of the submitted sequence
- The assay sequences may generate excessive self-dimerization
- The submitted sequence has a high G/C content
- The submitted sequence has a high A/T content

If an error message occurs, is there anything else that can be done?

Any alternative designs may not meet our strict specifications for specificity or performance in general.

What is the ordering process for a custom Small RNA Assay?

Custom TaqMan® Small RNA assays can be designed using the Custom Small RNA Design Tool by entering the RNA sequence of interest: <https://www5.invitrogen.com/custom-genomic-products/tools/small-rna/>

Reproducibility and Lot-to-Lot Variation

How are my results going to be affected by using assays of different manufacturing lots?

TaqMan® MicroRNA Assays provide excellent lot-to-lot reproducibility, with a maximum discrepancy of $\pm 0.5 C_t$'s between different lots. We advise running a control sample with both lots in parallel to ensure continuity in your experiments.

What I can do to minimize variability when using assays?

Use multiple replicates and consider, when possible, using an overall study control that is used in every assay to monitor potential day-to-day/run-to-run/across study variability.

Assay Performance

What about isomiRs? Will Life Technologies assays give me good quantification if they only detect one isomer and not all of them? 14

Do you have data to back up your claim that your miRNA assays can accurately distinguish miRNA targets that differ by a single base? Have you tested each miRNA Assay that's designed to one of two or more closely-related target sequences? 15

What about isomiRs? Will Life Technologies assays give me good quantification if they only detect one isomer and not all of them?

Deep sequencing analysis of mature miRNAs revealed that many miRNAs have either an addition or deletion of 1-3 bases at the 3' and less frequently at the 5' terminal end. These are often referred to as isomiRs. The sequence deposited in miRBase is the canonical sequence derived by aligning sequences from current deep

sequencing data. Thus far, there has been no biological relevance attached to these different forms since they exclusively occur outside the seed sequence. For that reason, the changes detected in the expression level of one isomer are proportional to changes within the entire pool. As a result, there may be a shift in raw C_t value using assays targeting two separate isomiRs. However, the relative expression ($\Delta\Delta C_t$) has been demonstrated to be roughly the same. It should be noted that, although TaqMan® MicroRNA Assays are designed to be sequence specific, they will detect a small spectrum of isomiRs. Depending on the number and composition at the 3' end, an assay may detect the +1 and +2 isomiRs but not the -1 or -2 forms.

Do you have data to back up your claim that your TaqMan® MicroRNA Assays can accurately distinguish miRNA targets that differ by a single base? Have you tested each TaqMan® MicroRNA Assay that is designed to one of two or more closely-related target sequences?

It is well understood within the miRNA community that designing assays for miRNAs is challenging due to their short length (<22 bases) and closely related sequences. Although we have not tested cross reactivity of every closely related species, we have demonstrated that we can achieve <5% cross reactivity between a single nucleotide mismatch. Specificity of an assay depends on the number of mismatches to its closest homologue, the location of the mismatch, and the surrounding bases, making cross reactivity difficult to predict. As a general rule, the most difficult miRNA targets to discriminate are those with minimal mismatches localized to the 5' region of the sequence, and it is close to impossible to design an assay that discriminates between a single mismatch at the 5' most base. In addition, the assays in our catalog have been designed to provide a balance between specificity and sensitivity: an assay may be very specific but lack the needed sensitivity, or vice versa. To achieve this balance, and to ensure the highest sensitivity and to reduce false positives, TaqMan® MicroRNA Assays must have an NTC background $C_t > 38.0$ and display good linearity across at least 3 \log_{10} (ideal $R^2 > 0.98$).

Data Analysis

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Can C_t 's greater than a cut-off be considered valid results? 16

What data analysis tools do you recommend?

If you are using an Applied Biosystems instrument, we recommend using [Expression Suite](#) to analyze data from the TaqMan® MicroRNA Array Cards. After importing the .sds or .eds files, you can perform all the QC and data analysis within the tool.

What is a good C_t cut-off for the TaqMan® MicroRNA Array Cards? In other words, beyond what C_t do I not want to trust the data?

The typical C_t cut-off on TaqMan® Array Cards is 32, which is equivalent to C_t 35 on a plate (10µl reaction). Previous studies show that if you use pre-amplification, a C_t cut-off of 29 or 30 can be used to reduce numbers of false positives (see Technical Note [Optimized protocols for human or rodent microRNA profiling with precious samples](#)). To ensure that you have selected a correct cut-off, you should run replicates of the same sample and use C_t cut-off before you see an increase in the Standard Deviation.

Can C_t 's greater than a cut-off be considered valid results?

Yes they can. However, it is important to recognize the true linearity and detection limits of your assay: C_t values above the cut-off can indicate non-specific amplification, unless your NTC is a true- no-target control, and you have run a statistically significant number of replicates. Any results with C_t above the recommended cut-off need to be validated with individual assays on plates.

TaqMan® Pri-miRNA Assays

Do the TaqMan® MicroRNA Assays detect only the mature form of a miRNA? Vice versa, do the TaqMan® Pri-miRNA Assays detect only the pri-form?

Yes. The specific stem-loop chemistry of the TaqMan® MicroRNA Assay and its design prevent amplification of the pri-form. Vice versa, the chemistry of the TaqMan® Pri-miRNA Assays is not amenable to amplify the mature form. In particular cases, assays designed to detect mature miRNAs derived from the 3' arm of the stem loop may also detect pre-miRNA.

Can a TaqMan® Pri-miRNA Assay detect more than a single stem-loop?

TaqMan® Pri-miRNA Assays are designed to detect the transcript that contains the targeted stem loop. In some instances, e.g. when stem-loop sequences are clustered together and are transcribed on the same transcript, a TaqMan® Pri-miRNA Assay targeting one of the stem-loops will also detect the other stem loops in the cluster.

Performance Specifications for TaqMan® MicroRNA Assays

Note: See [TaqMan® Assays QPCR Guarantee white paper](#) for details

Performance Feature	Specification
Dynamic Range	$\geq 7 \log_{10}$ with ≥ 0.98 linearity (R^2 value)
Specificity	Majority of assays have < 5% cross reactivity with closely related sequences. NTC background: $C_t > 38.0$
Lot-to-Lot Reproducibility	Difference between C_t 's $\leq 0.5 C_t$ when different lots of an assay are run with the same sample and master mix from the same lot on the sample plate
Amplification Efficiency	$100\% \pm 15\%$ across $5 \log_{10}$
TaqMan® Assays QPCR Guarantee	Assay replacement if performance specs are not met to customer satisfaction if protocol is adhered to and sample is of good quality

Index of Resources

Introductory and Overview Materials:

Brochures

[Illuminate the Mystery of Biological Dark Matter: miRNA Expression Analysis Research Tools](#) (PRODUCT BROCHURE: Applied Biosystems/Ambion, 2006)

[MicroRNA: having a big impact on biology: A collection of innovative tools for microRNA research](#) (PRODUCT BROCHURE: Life Technologies 2012)

Bulletins

[TaqMan\(R\)MicroRNA Assays and Arrays](#) – (PRODUCT BULLETIN, TaqMan® MicroRNA Assays, Life Technologies 2011)

[TaqMan® Pri-miRNA Assays: Detect the Origins of MicroRNA Expression](#) (PRODUCT BULLETIN TaqMan® Pri-miRNA Assays, Life Technologies 2010)

Product Overviews

Highly Accurate and Cost-effective global MicroRNA Profiling Using qPCR Nanofluidic Technology - Iain Russell, Ph.D., David N. Keys, Ph.D., Dana Donnenwirth, and Yu Liang, Ph.D, **International Drug Discovery –Technote**, 2011 p26

[MicroRNA Quantitation by RT-PCR](#) (POSTER: Applied Biosystems, 2005: Caifu Chen, Dana Ridzon, Adam Broomer, Hui Zhou, Ruoying Tan, Danny Lee, Julie Nguyen, Kelly McDonald, Nan Lan Xu, Kai Lao, Karl Guegler)

[TaqMan® MicroRNA Assays Product Overview \(Applied Biosystems, 2010\)](#)

[TaqMan® MicroRNA Assays: The Gold Standard](#) (PRODUCT DATA COMPARISON, Applied Biosystems, 2010)

Product Information and Product Guides:

Application Notes

[Design Pipeline for TaqMan® Small RNA Assays](#) (TECHNOTES NEWSLETTER, Small RNA Analysis, Volume 16 no 3)

[Endogenous Controls for Real-Time Quantitation of miRNA Using TaqMan® MicroRNA Assays](#) (APPLICATION NOTE: TaqMan® MicroRNA Assays, 2007)

Manuals & Protocols

[Custom TaqMan® Small RNA Assays Design and Ordering Guide](#) (PRODUCT MANUAL PN4412550 RevE, 2011)

[Megaplex™ Pools For microRNA Expression Analysis](#) (PROTOCOL PN4399721 Rev. C, 2010)

[TaqMan® Small RNA Assays : TaqMan® MicroRNA Assays, TaqMan® siRNA Assays, Custom TaqMan® Small RNA Assays](#) (PROTOCOL PN4364031 RevE, 2011)

[TaqMan® Pri-miRNA Assays](#) (PROTOCOL PN4427719 Rev. D, 2010)

Quick Reference Cards

[Megaplex™ Pools for microRNA Expression Analysis](#) (QUICK REFERENCE CARD PN4399813 Rev. B, 2008)

[TaqMan® Small RNA Assays, TaqMan® MicroRNA Assays, TaqMan® siRNA Assays, and Custom TaqMan® Small RNA Assays](#) (QUICK REFERENCE CARD PN4412551 Rev. C, 2011)

[TaqMan® Pri-miRNA Assays](#) (QUICK REFERENCE CARD PN 4427720 Rev. B, 2009)

Specific Applications and Protocols:

Application Notes & Collaborative Studies

[Expression analysis of both mRNA and miRNA on the same TaqMan® Array Card: Development of a pancreatic tumor tissue classification methodology](#) (APPLICATION NOTE: TaqMan® Array Cards for combined mRNA and miRNA analysis, 2013)

[High correlation of miRNA quantitation data from matched FFPE and snap-frozen tissues using TaqMan® MicroRNA Assays](#) (APPLICATION NOTE: TaqMan® MicroRNA Assays, 2012)

MicroRNA Isolation from Human Plasma/Serum (APPLICATION NOTE: Applied Biosystems/Ambion)

[Optimized protocol with low sample input for profiling human microRNA using the OpenArray® platform](#) (APPLICATION NOTE OpenArray® Real-Time PCR System, 2011)

[Optimized protocols for human or rodent microRNA profiling with precious samples](#) (TechNote qPCR microRNA Profiling, Life Technologies 2012)

[Applied Biosystems® TaqMan® MicroRNA Assays help researchers validate miRNA markers for forensic body fluid identification](#) (YOUR INNOVATIVE RESEARCH: Applied Biosystems® TaqMan® MicroRNA Assays, 2011)

Protocols & Bulletins

[Gene Expression Assay Performance Guaranteed With the TaqMan® Assay QPCR Guarantee Program](#) (WHITE PAPER: TaqMan® Assay QPCR Guarantee Program, Applied Biosystems, 2010)

[MicroRNA detection in single-cell samples using Megaplex™ RT and PreAmp Primers](#) (minimized volumes) (PROTOCOL Single-Cell microRNA expression analysis, 2011)

[Protocol for Creating Custom RT and Preamplification Pools using TaqMan® MicroRNA Assays](#) (USER BULLETIN PN 4465407, 2013)

[Protocol for Running Custom RT and Preamplification Pools on Custom TaqMan® Array MicroRNA Cards](#) (QUICK REFERENCE PN 4478705 RevA, 2013)

[Quantitate and Profile miRNA without RNA Isolation](#): TaqMan® microRNA Cells-to-Ct™ Kit (PRODUCT DATA COMPARISON, Life Technologies)

Posters

[Characterization of microRNA expression profiles in normal human tissues identifies immune response as a potential microRNA target in normal and neoplastic brain](#) (POSTER: Applied Biosystems, 2006. Yu Liang, Dana Ridzon, Caifu Chen)

[MicroRNA Expression Signature in Human Glioblastoma Multiforme Brain Tumor](#) (POSTER: 1. Applied Biosystems 2. Dartmouth Medical School and the Norris Cotton Cancer Center, 2005. Dana Ridzon¹, Ruoying Tan¹, Julie Nguyen¹, Adam Broome¹, Caifu Chen¹, Victor Ambros² and Mark Israel²)

[Quantitation of Plant miRNAs by RT-PCR](#) (POSTER: 1. Applied Biosystems & 2. Delaware Biotechnology Institute, 2004. Karl J Guegler¹, Julie Nguyen¹, Cheng Lu², Blake Meyers², Pamela Green², Kelly K McDonald¹, Caifu X Chen¹)

[Single cell microRNA and mRNA profiling reveals unique gene expression signatures and heterogeneities in mouse ES cells](#) (POSTER: 1. Applied Biosystems 2. Department of Molecular, Cellular, & Developmental Biology, University of Colorado, 2006. Caifu Chen¹, Ruoying Tan¹, Dana Ridzon¹, Leila Bahreinifar¹, Linda Wong¹, Karl Guegler¹, William M. Strauss²)

[TaqMan®-based miRNA profiles classify mouse ES and differentiated cells](#) (POSTER, 1. Applied Biosystems & Department of Molecular, Cellular 2. Developmental Biology, University of Colorado, 2005. Caifu Chen¹, Dana A. Ridzon¹, Yu Liang¹, Linda Wong¹, Karl J. Guegler¹, William M. Strauss²)