Comprehensive miRNA Research Technologies

QIAGEN®

Sample & Assay Technologies

QIAGEN solutions for advancing microRNA research

In the last few years, the identification of microRNA (miRNA) and the recognition of its important role in regulation of gene expression have led to increasing interest in the identification and characterization of miRNAs (1–3). A growing body of evidence suggests that miRNAs play a role in many diverse biological processes such as development, differentiation, and apoptosis. Misregulation of miRNA expression is reported to be associated with several cancers (1) and other diseases.

The miRNA system is an endogenous mechanism of regulation of gene expression. miRNA precursors are transcribed from genomic DNA in the nucleus. The mature miRNAs contribute to the regulation of endogenous genes, primarily by translational repression (see flowchart, opposite). In addition, miRNAs can mediate mRNA destruction by rapid deadenylation and/or decapping. While siRNAs and miRNAs act on endogenous mRNAs through integration into a multiprotein silencing complex (RISC), siRNAs require a perfect match with the targeted mRNA, leading to cleavage and subsequent destruction, whereas miRNAs are often only partially complementary to target mRNAs. Naturally occurring miRNA-binding sites are typically found in the 3' untranslated regions (UTRs) of target mRNAs. Their partial complementarity has made positive identification of true binding sites difficult and imprecise.

To support this exciting new field of research, QIAGEN has introduced innovative miRNeasy and miScript technologies for miRNA purification and detection. miRNeasy Kits (page 4) enable purification of total RNA, including miRNAs and other small RNAs, from all types of animal tissues and cells. Small RNAs, including miRNA can be enriched in a separate fraction if desired. Using the miScript System (page 6), real-time PCR analysis can be used to detect hundreds of miRNAs, as well as mRNAs, from a single cDNA synthesis reaction.

As well as miRNAs, knowledge about other classes of small, noncoding RNAs is currently emerging (4). miRNeasy Kits and the miScript System have been tested and verified for purification and detection of other noncoding RNAs (e.g., snoRNAs, piRNAs). For more details and application data, visit www.qiagen.com/miRNA .

References

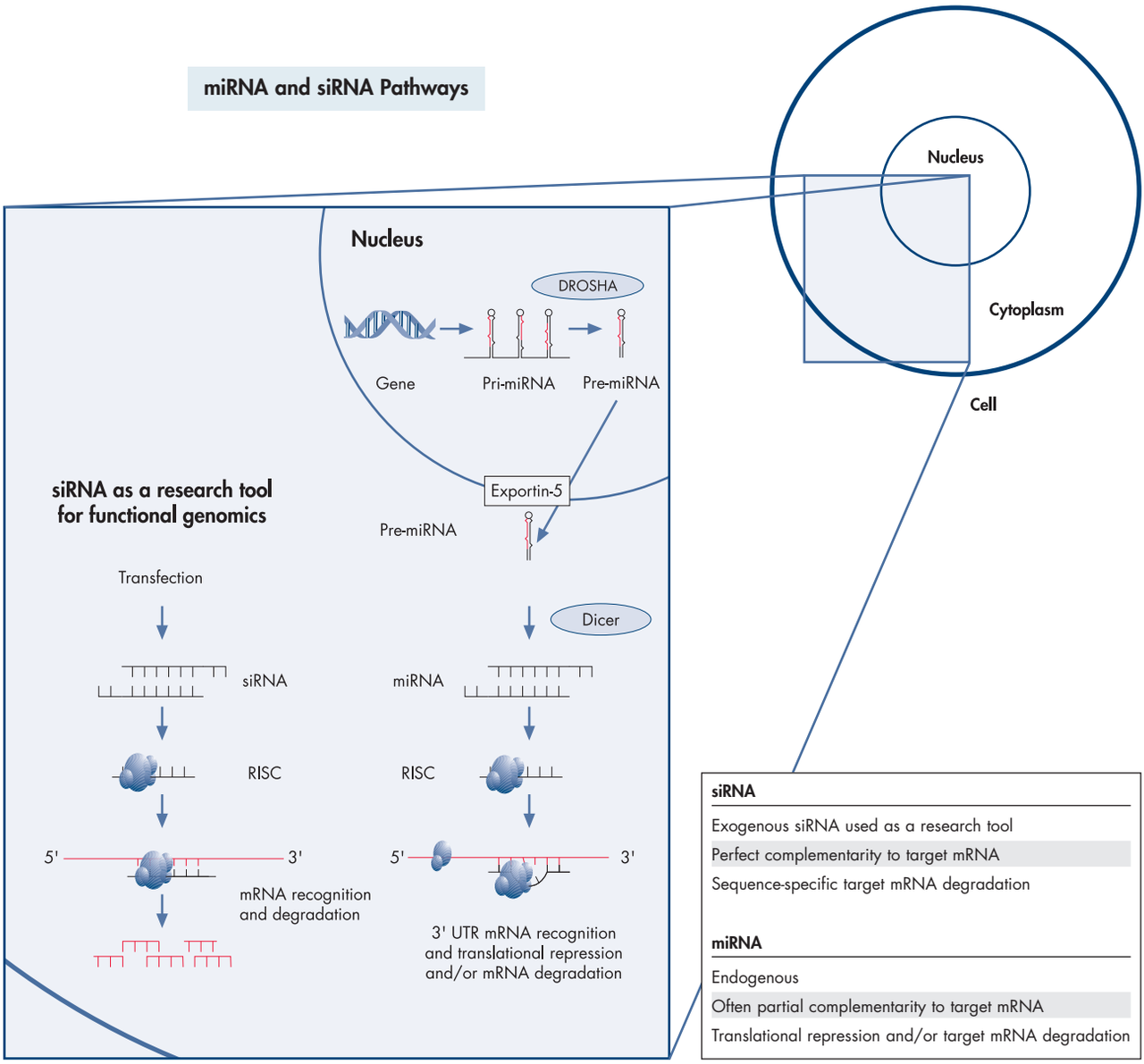
1. Cummins, J.M. and Velculescu, V.E. (2006) Implications of micro-RNA profiling for cancer diagnosis. Oncogene **25,** 6220.
2. microRNAs supplement (2006) Nature Genetics **38,** 6s.
3. Kloosterman, W.P. and Plasterk, R.H. (2006) The diverse functions of microRNAs in animal development and disease. Dev. Cell **11,** 441.
4. Mattick, J.S. and Makunin, I.V. (2005) Small regulatory RNAs in mammals. Hum. Mol. Genet. **14,** R121.

miRNeasy Mini Kit	miScript Reverse Transcription Kit	HiPerFect Transfection Reagent*
miRNeasy 96 Kit	miScript SYBR® Green PCR Kit	Coming soon:
	miScript Primer Assays	miRNA Inhibitors†
		miRNA Synthesis†

* Visit www.qiagen.com/goto/HiPerFect for more information.

† Please inquire for more information about these products.

miRNA and siRNA Pathways



miRNAs are first transcribed in the nucleus as long, primary miRNAs (pri-miRNA). While still in the nucleus, they are processed into precursor miRNAs (pre-miRNA) by nuclear Drosha, a dsRNA-specific ribonuclease. Pre-miRNAs are subsequently transported from the nucleus into the cytosol by Exportin-5. Dicer (an RNase III-like enzyme) processes the pre-miRNAs into ~22 nt mature miRNAs that are subsequently incorporated into the RNA-induced silencing complex (RISC). When the RISC identifies the complementary or partially complementary mRNA target, it inhibits gene expression by translational repression or by mRNA degradation.

miRNA resources

Visit www.qiagen.com/miRNA for useful miRNA resources including:

- Protocols
- Application notes
- References
- Background information
- Links to databases

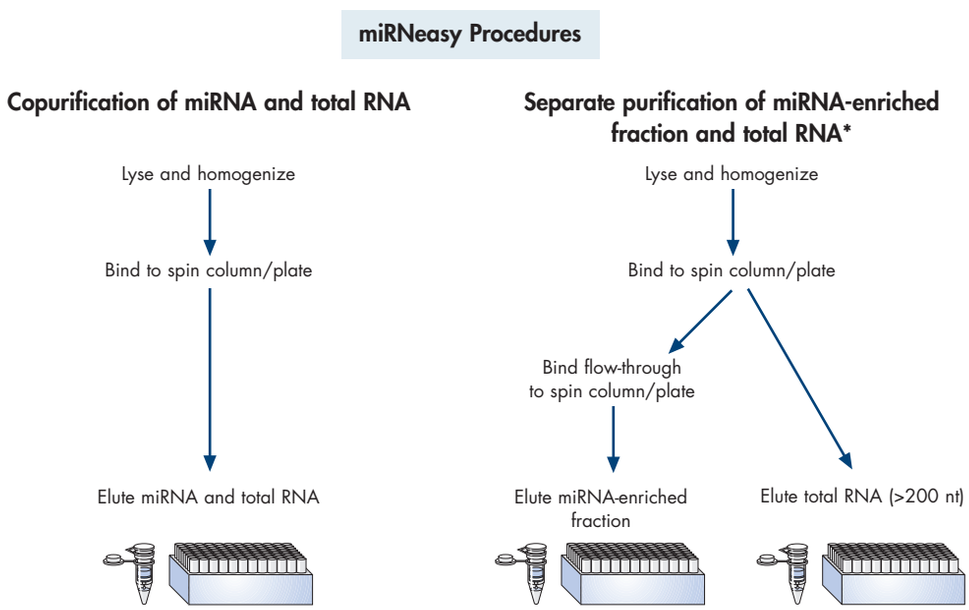
miRNeasy Mini Kit and miRNeasy 96 Kit

For effective purification of total RNA including miRNA or enrichment of miRNA in a separate fraction

- Effective purification from all types of animal tissues and cells
- Efficient enrichment of miRNA and other small RNAs in a separate fraction
- High-purity RNA suitable for all downstream applications
- Flexibility of low-throughput or 96-well formats

Purification of total RNA or enrichment of miRNA

miRNeasy Kits enable purification of total RNA which includes RNA from approximately 18 nucleotides (nt) upwards. Alternatively, an miRNA-enriched fraction (<200 nt) and a total RNA fraction (>200 nt) can be purified separately (see flowchart). The miRNeasy Mini Kit provides low-throughput RNA purification using spin columns. The miRNeasy 96 Kit is used for high-throughput purification in a 96-well format. Purified total RNA is suitable for use in downstream applications such as northern blot analysis and quantitative, real-time RT-PCR. Enrichment of small RNAs may provide better results for some applications such as microarray analysis.



* Separate purification using the miRNeasy Mini Kit requires additionally the RNeasy® MinElute® Cleanup Kit. For separate purification using the miRNeasy 96 Kit, an additional RNeasy 96 plate is required. For economical purchase of an additional RNeasy 96 plate, we recommend ordering an RNeasy 96 Kit (see Ordering Information, page 10).

Effective purification from animal tissues and cells

miRNeasy Kits efficiently purify RNA from tissues and cells, even when low amounts of starting material are used. In the results shown in Figure 1, C_T values increased linearly when RNA was purified from decreasing amounts of cells or tissue, indicating effective miRNA purification from a wide range of amounts of starting material.

RNA can be purified from a variety of tissues and cells, including difficult-to-lyse tissues (Figure 2). Purification of total RNA including miRNA allows direct comparison of miRNA expression levels with those of housekeeping reference genes or any other mRNA of interest (Figure 2).

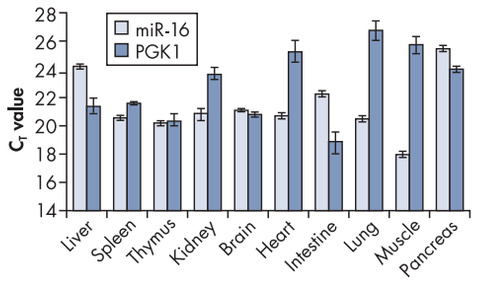


Figure 2. Efficient copurification from a wide range of tissues. Total RNA including miRNA was purified from 25 mg of a range of RNA*later*® stabilized rat tissues using the miRNeasy 96 Kit. Purified RNA was used as a template in quantitative, real-time RT-PCR assays for the miRNA miR-16 and for the larger mRNA of the PGK1 gene. Results showed successful detection of both PGK1 mRNA (large RNA) and miR-16 (small RNA) from the same eluates.

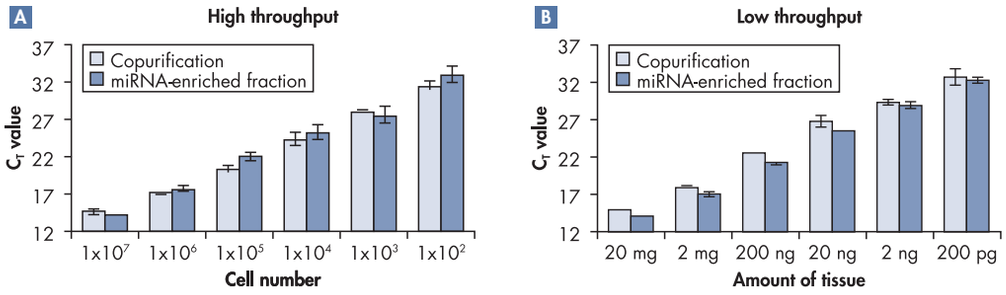


Figure 1. Effective purification from a range of starting amounts. Total RNA was purified from A 10^2–10^7 Jurkat cells using the miRNeasy 96 Kit or B a dilution series of rat lung tissue homogenate from 20 mg to 200 pg tissue equivalent, using the miRNeasy Mini Kit. miRNA-enriched fractions (<200 nt) were also isolated from the same samples. Purified RNA was used as a template in quantitative, real-time RT-PCR assays for the miRNA miR-16.

High-purity RNA

RNA prepared using miRNeasy Kits is highly pure and ready for use in sensitive downstream applications. miRNeasy procedures eliminate the possibility of contamination with salts or phenol which could interfere with later analyses (Figure 3). In addition to higher purity, miRNeasy Kits offer superior yields to alternative methods of miRNA purification such as using TRIzol Reagent (Figure 4).

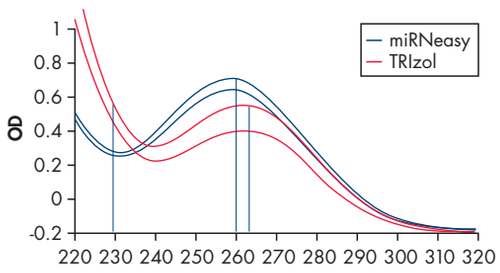


Figure 3. Highly pure RNA without phenol carryover. Total RNA including miRNA was purified from 1x10⁶ Jurkat cells using the miRNeasy Mini Kit or precipitation from TRIzol Reagent. The absorbance spectra showed the OD maximum for RNA purified using the miRNeasy Kit was at 260 nm. In contrast, the OD maximum was greater than 260 nm when TRIzol was used for purification, indicating phenol carryover. In addition, the OD_{230} measurement was higher in the TRIzol-prepared RNA, indicating salt carryover from the TRIzol Reagent.

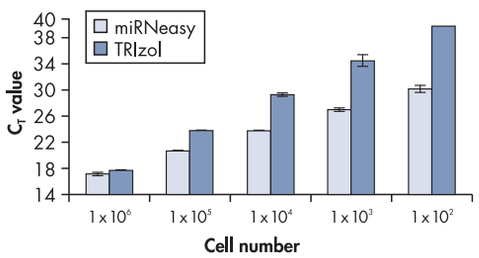


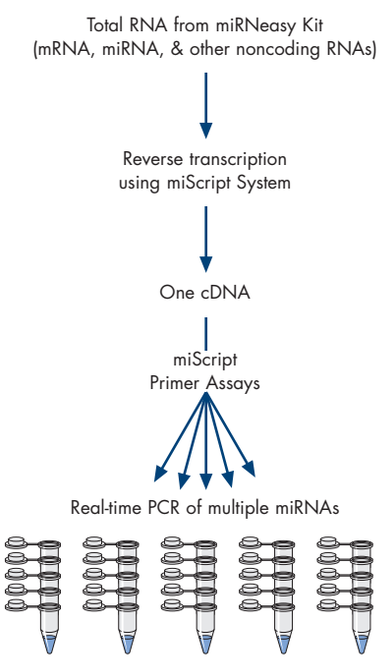
Figure 4. miRNeasy Kit outperforms TRIzol. Total RNA including miRNA was purified from a range of amounts of Jurkat cells using the miRNeasy Mini Kit or precipitation from TRIzol Reagent. Purified RNA was used as a template in quantitative, real-time RT-PCR assays for the miRNA miR-16. Results showed that C_T values were lower after purification using the miRNeasy Kit, indicating that higher amounts of miRNA were purified than when using TRIzol. miRNA was effectively purified from as little as 1x10² cells using the miRNeasy Kit. In contrast, no miRNA was detected after 40 PCR cycles from 1x10² cells when TRIzol was used for purification.

The miScript System

For detection of hundreds of miRNAs from a single cDNA synthesis reaction using SYBR Green based, real-time PCR

- Fast, simple quantification of multiple miRNAs from a single cDNA synthesis reaction reducing variability and saving precious sample
- Quantification of miRNA and mRNA from the same cDNA synthesis reaction allowing simultaneous detection of reference genes or other mRNAs of interest
- Hundreds of validated and predesigned miRNA-specific assays available for human, mouse, and rat via the GeneGlobe™ Web portal
- Sensitive and specific detection and quantification of miRNA

Quantify Hundreds of miRNAs from a Single cDNA Synthesis Reaction



Principle of the miScript System

The miScript System is a three-component system which covers all the steps of conversion of miRNA and mRNA into cDNA and detection of miRNAs in SYBR Green based, real-time PCR. The miScript Reverse Transcription Kit, miScript SYBR Green PCR Kit, and miScript Primer Assay allow sensitive and specific detection and quantification of miRNA. The modular system enables detection of individual miRNAs of interest using miScript Primer Assays or screening of multiple human, mouse, or rat miRNAs using miScript Primer Assay Sets. Alternatively, researcher-designed assays can be used for newly discovered miRNAs. For this reason, the three components can be purchased separately.

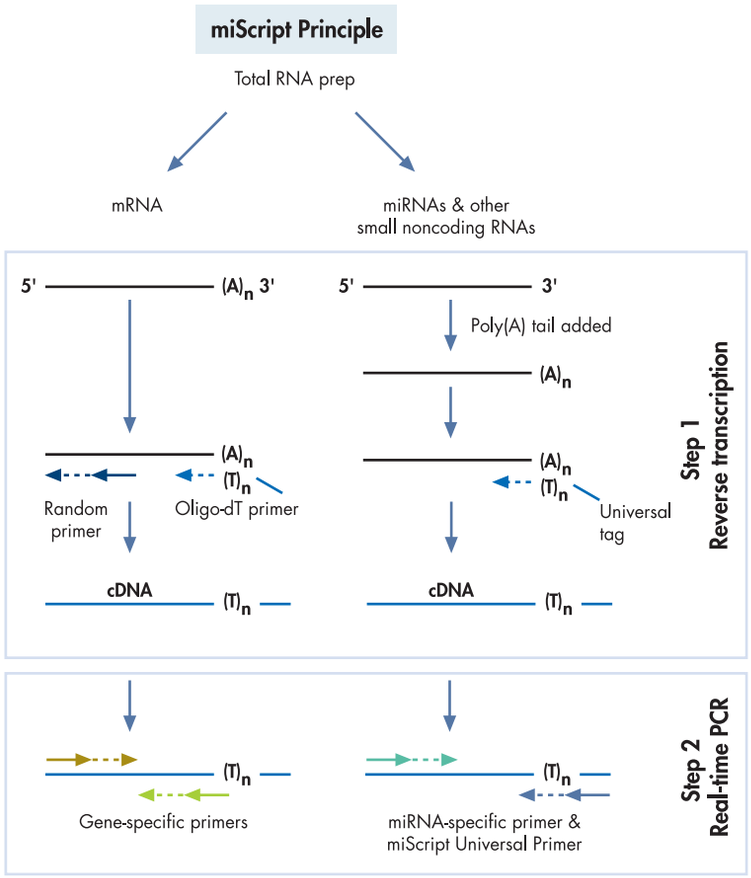
The miScript System comprises the following components:

- **miScript Reverse Transcription Kit**
  This kit enables simple, single-step cDNA synthesis.
- **miScript SYBR Green PCR Kit**
  This kit includes the miScript Universal Primer which allows detection of miRNAs in combination with an miRNA-specific primer.
- **miScript Primer Assay**
  The assay comprises an miRNA-specific forward primer which is used in combination with the miScript SYBR Green PCR Kit.

miScript Reverse Transcription Kit

The miScript Reverse Transcription Kit includes miScript Reverse Transcriptase Mix and miScript RT Buffer. miScript Reverse Transcriptase Mix is an optimized blend of enzymes comprising a poly(A) polymerase and a reverse transcriptase. miScript RT Buffer has been developed specifically for use with miScript Reverse Transcriptase Mix to enable maximum activity of both enzymes. It includes Mg^{2+}, dNTPs, oligo-dT primers, and random primers.

Unlike mRNAs, miRNAs are not polyadenylated in nature. During the reverse-transcription step, miRNAs are polyadenylated by poly(A) polymerase. In parallel, reverse transcriptase converts both miRNA and mRNA to cDNA (see flowchart, Step 1). The parallel reactions make miRNA conversion into cDNA a very fast and simple one-tube procedure. The oligo-dT primers carry a universal tag sequence on the 5' end which allows amplification in the real-time PCR step (see flowchart, Step 2).



miScript SYBR Green PCR Kit

The miScript SYBR Green PCR Kit is based on the proven technology of QuantiTect® SYBR Green Kits. The miScript SYBR Green PCR Kit includes QuantiTect SYBR Green PCR Master Mix and the miScript Universal Primer which is specific for the universal tag sequence on the 5' end of the cDNA.

The cDNA serves as the template for real-time PCR analysis using the miScript Primer Assay in combination with the miScript SYBR Green PCR Kit. miRNAs are amplified using the miScript Universal Primer together with the miRNA-specific primer (the miScript Primer Assay).

A single cDNA synthesis reaction is sufficient to interrogate hundreds of miRNAs by real-time PCR using different miScript Primer Assays, allowing comprehensive expression profiling of known miRNAs. The cDNA can also be used for detection of mRNA using gene-specific primer pairs (instead of the miScript Primer Assay and Universal Primer), allowing simultaneous detection of reference genes, such as GAPDH, or any other mRNAs of interest, such as the mRNA targeted by a particular miRNA (Figure 5). QIAGEN offers QuantiTect Primer Assays for real-time PCR detection of mRNA.

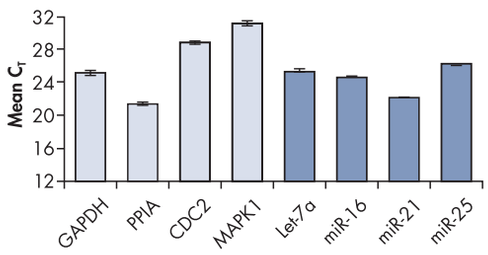


Figure 5. A single cDNA synthesis reaction enables detection of multiple miRNAs and mRNAs. Total RNA was prepared from HeLa S3 cells using the miRNeasy Mini Kit. The miScript System was used for real-time PCR analysis of 4 miRNAs (Let-7a, miR-16, miR-21, and miR-25). QuantiTect Primer Assays were used for real-time PCR analysis of 4 mRNAs (GAPDH, PPIA, CDC2, and MAPK1).

miScript Primer Assay

miScript Primer Assays are miRNA-specific primers used in combination with the miScript SYBR Green PCR Kit for real-time PCR of miRNA using SYBR Green detection. The miScript SYBR Green PCR Kit includes the miScript Universal Primer which is necessary for successful amplification.

miScript Primer Assays are available in single tubes and can be ordered online from the GeneGlobe Web portal (www.qiagen.com/GeneGlobe). miScript Primer Assays for the detection of human miRNAs have been experimentally validated. miScript Primer Assays for mouse and rat are predesigned and a proportion has been validated. Human, Mouse, or Rat miScript Primer Assay Sets provide assays for >95% of the miRNAs listed in miRBase version 9.0 (http://microrna.sanger.ac.uk/sequences/).

Linearity of cDNA synthesis enables sensitive detection

Using the miScript System, the cDNA synthesis reaction is highly linear which ensures sensitive, accurate quantification in subsequent real-time PCR. In the results shown in Figure 6, C_T values from real-time PCRs were highly linear following cDNA synthesis reactions from a range of amounts of starting RNA.

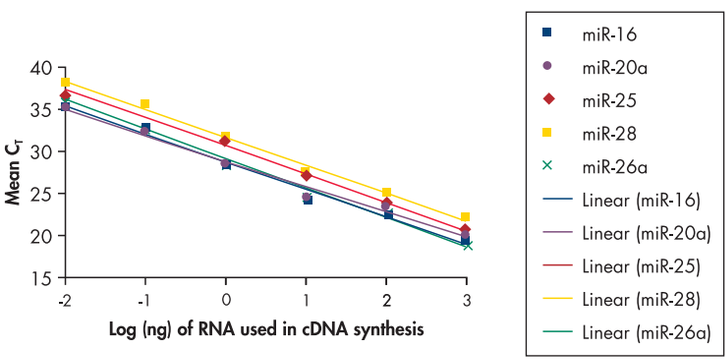


Figure 6. Highly linear cDNA synthesis reactions. RNA was purified from HeLa S3 cells using the miRNeasy Mini Kit. A range of amounts of RNA from 10 pg to 1 µg were used in cDNA synthesis reactions using the miScript Reverse Transcription Kit. cDNA was used as a template in quantitative, real-time PCR assays for 5 miRNAs (miR-16, miR-20a, miR-25, miR-28, and miR-26a).

Highly specific miRNA detection

The existence of multiple miRNA isoforms presents a significant challenge in miRNA quantification. miScript Primer Assays are highly specific and can distinguish between isoforms as shown for the Let-7 family. Human Let-7 isoforms with mismatches and/or differing lengths were used in these experiments (Table 1). In most cases, cross reactivity was very low and insignificant. Where cross reactivity was observed, it was at low levels (e.g., ~6% for Let-7a miScript Primer Assay with Let-7f cDNA). These results indicate that miScript Primer Assays are isoform specific.

Table 1. Isoforms of human Let-7 family

	miRNA sequence
Let-7a	UGAGGUAGUAGGUUGUAUAGUU
Let-7b	UGAGGUAGUAGGUUGUGUGGUU
Let-7c	UGAGGUAGUAGGUUGUAUGGUU
Let-7d	AGAGGUAGUAGGUUGCAUAGU•
Let-7e	UGAGGUAGGAGGUUGUAUAGU•
Let-7f	UGAGGUAGUAGAUUGUAUAGUU
Let-7g	UGAGGUAGUAGUUUGUACAGU•
Let-7i	UGAGGUAGUAGUUUGUGCUGU•

These sequences show the Let-7 isoforms. Base changes are red and underlined. Changes in length are indicated by a red dot.

Table 2. Specificity of miScript Primer Assays

	Relative detection (as % of perfect match)							
cDNA used	miScript Primer Assay used							
in PCR	Let-7a	Let-7b	Let-7c	Let-7d	Let-7e	Let-7f	Let-7g	Let-7i
Let-7a	**100.00**	0.00	0.29	0.33	**2.44**	0.01	0.00	0.00
Let-7b	0.00	**100.00**	1.68	0.00	0.00	0.01	0.00	0.00
Let-7c	0.27	0.14	**100.00**	0.00	0.00	0.00	0.00	0.00
Let-7d	**4.11**	0.00	0.03	**100.00**	0.01	0.00	0.00	0.00
Let-7e	1.23	0.00	0.01	0.01	**100.00**	0.00	0.00	0.00
Let-7f	**5.77**	0.00	0.00	0.00	0.00	**100.00**	0.00	0.00
Let-7g	0.01	0.00	0.00	0.00	0.00	0.00	**100.00**	0.00
Let-7i	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**100.00**

Synthetic miRNAs of each Let-7 isoform were used in cDNA synthesis reactions performed with the miScript Reverse Transcription Kit. An aliquot of the resultant cDNA was used as a template in real-time PCR reactions with a miScript Primer Assay for each isoform and the miScript SYBR Green PCR Kit. The % relative detection was calculated using the differences between the C_T values achieved from the mismatching miScript Primer Assays and those from the perfectly matching miScript Primer Assays (% relative detection = $2^{-\Delta C_T}$ x 100).

Ordering Information

Product	Contents	Cat. No.
miRNeasy Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217004
miRNeasy 96 Kit (4)	4 RNeasy 96 Plates, Collection Microtubes (racked), Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217061
miScript Reverse Transcription Kit (10)	For 10 reactions: miScript Reverse Transcriptase Mix, miScript RT Buffer, RNase-Free Water	218060
miScript Reverse Transcription Kit (50)	For 50 reactions: miScript Reverse Transcriptase Mix, miScript RT Buffer, RNase-Free Water	218061
miScript Primer Assay (100)	10x miScript Primer Assay (contains one miRNA-specific primer)	Varies*
Human miScript Primer Assay Set V1.0	452 miScript Primer Assays targeting human miRNAs provided in 96-well plates	218411
Mouse miScript Primer Assay Set V1.0	356 miScript Primer Assays targeting mouse miRNAs provided in 96-well plates	218412
Rat miScript Primer Assay Set V1.0	226 miScript Primer Assays targeting rat miRNAs provided in 96-well plates	218413
miScript SYBR Green PCR Kit (200)	For 200 reactions: QuantiTect SYBR Green PCR Master Mix, miScript Universal Primer	218073
miScript SYBR Green PCR Kit (1000)	For 1000 reactions: QuantiTect SYBR Green PCR Master Mix, miScript Universal Primer	218075
Additional kits for purification of separate fractions		
RNeasy MinElute Cleanup Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free Reagents and Buffers	74204
RNeasy 96 Kit (4)†	4 RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, RNase-Free Reagents and Buffers	74181
For miRNA purification from formalin-fixed, paraffin-embedded tissue		
RNeasy FFPE Kit (50)‡	50 RNeasy MinElute Spin Columns, 50 gDNA Eliminator Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74404
Related products		
QuantiTect Primer Assay (200)	10x QuantiTect Primer Assay (contains a mix of forward and reverse primers for a specific target)	Varies*
QuantiTect SYBR Green PCR Kit (200)†	For 200 x 50 µl reactions: 3 x 1.7ml 2x QuantiTect SYBR Green PCR Master Mix, 2 x 2 ml RNase-Free Water	204143

* Visit www.qiagen.com/GeneGlobe to search for and order these Primer Assays.

† Larger kit size available; please inquire.

‡ Requires a supplementary protocol. Visit www.qiagen.com/miRNA to download the protocol.

miRNeasy Kits, the RNeasy MinElute Cleanup Kit, the RNeasy 96 Kit, the RNeasy FFPE Kit, the miScript System, HiPerFect Transfection Reagent, QuantiTect SYBR Green Kits, and QuantiTect Primer Assays are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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