

Primer design using the qScript® miRNA Quantification System

A key component for quantification of microRNA (miRNA) using real-time quantitative PCR (RT-qPCR) is proper primer design. In order to assist you with using the qScript miRNA quantification system we have prepared this technical guide, which provides details on how to design assay primers specific to your miRNA of interest.

The basic procedure for the qScript microRNA quantification system involves 2-step RT-qPCR, and is comprised of three components:

RT

- cDNA synthesis using qScript microRNA cDNA Synthesis Kit (Quantabio #95107)
 - o includes addition of a poly-A tail



qPCR

- miRNA-specific primers (from this document)
- PerfeCta® SYBR® Green SuperMix (Quantabio #95053, 95054, 95055, 95056)

As noted above, two of the components are retail products available through Quantabio's distribution partners.

Visit www.quantabio.com for ordering information, and downloadable product manuals. The third component, miRNA-specific primers, are the subject of this document. The oligonucleotides resulting from this procedure may be ordered from the vendor of your choice.

Basic Steps for Primer Design

1. Convert miRNA sequence to a DNA sequence
2. Append the reverse complement of the oligo-dT adapter primer (sequence provided below) to its 3' end.
3. Using primer design software or web-based tool, design the miRNA-specific FORWARD PRIMER that is compatible and T_m -balanced with the REVERSE PRIMER (universal primer, sequence provided below).

Example – human miR-193P

1.

miRNA sequence:

5'-UGUGCAAUCUAUGCAAACUGA-3'



Convert to DNA sequence

5'-TGTGCAAATCTATGCAAACCTGA-3'



2.

Oligo dT Adapter Primer:

5'-GCATAGACCTGAATGGCGGTAAGGGTGTGGTAGGCGAGACATTTTTTTTTTTTTTTTTTTT-3'



Make reverse complement

5'-AAAAAAAAAAAAAAAAAAAAATGTCTCGCTACCACACCTTACCGCCATTAGGTCTATGC-3'

Template created by appending the RC of adapter primer to miRNA (DNA seq):

5'-TGTGCAAATCTATGCAAACCTGAAAAAAAAAAAAAAAAAAAAAATGTCTCGCTACCACACCTTACCGCCATTAGGTCTATGC-3'

3.

Design FORWARD PRIMER

TGTGCAAATCTATGCAAACCTGA →

5'-TGTGCAAATCTATGCAAACCTGAAAAAAAAAAAAAAAAAAAAAATGTCTCGCTACCACACCTTACCGCCATTAGGTCTATGC-3'

← ATGGCGGTAAGTCCAGATACG

Select last 21 bases as REVERSE PRIMER (Universal Primer)

When designing the forward primer, specific to your miRNA, restrict the search to 1-25 bases. In the example above the software (Oligo7) determined the optimal primer sequence (green) to be in effect the miRNA sequence. In other cases, depending on T_m balancing and compatibility with the universal primer, it may be longer or shorter.

Once you have designed the your miR-specific forward primer, you can order it through the oligonucleotide vendor of your choice. The reverse (universal) primer is included with the qScript microRNA cDNA synthesis kit. The experimental protocol for using the primers is available at www.quantabio.com/resources, along with protocols for the qScript microRNA cDNA synthesis kit and PerfeCta SYBR Green SuperMix.

Key Sequences

Oligo-dT adapter primer

5'-GCATAGACCTGAATGGCGGTAAGGGTGTGGTAGGCGAGACATTTTTTTTTTTTTTTTTTTT-3'

Primer design: Used in step 2 for creating the sequence template. Included as part of the qScript microRNA cDNA synthesis kit.

Universal primer

5'-GCATAGACCTGAATGGCGGTA-3'

Primer design: Used in step 3 as the reverse primer. Included with the qScript microRNA cDNA synthesis kit.

Developed Assays

A list of primers for more than 1500 miRNAs have already been designed and are available for download at www.quantabio.com/products/microrna-profiling. If you are a customer who previously purchased our PerfeCta microRNA Assays, you will find the sequence for the assays of interest in this document. Use the ctrl-F function within excel to search by ID, accession number, or miRNA sequence.

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