miRCURY LNA™ Universal RT microRNA PCR
Supplement to Instruction manual version 4.0, May 2011

Quick-guide for individual microRNA LNA™ primer sets

Before starting the experiment

We recommend mixing your reagents with excess volume for pipetting. Typically, 10-25% excess is needed, depending on pipetteing system. Prepare the **microRNA LNA™ PCR primer set:** Add 110 µl nuclease free water to each tube, vortex and spin down. Mix Fwd and Rev Primer 1:1 and spin down. Prepare the **Reference gene primer mix:** Add 220 µl nuclease free water, vortex and spin down. **Keep all reagents on ice while setting up.**

Phase I: prepare RNA sample (see Tips section, page 51-52 for tips).

Phase II (Steps 1-5): cDNA synthesis (see page 22 for details).

- Adjust each template RNA to 5 ng/µl
- Prepare and mix:

Reagent	Volume (µl)
5x Reaction buffer	4
Nuclease-free water	9
Enzyme mix	2
Synthetic spike in, optional replace with H_2O if omitted	1
Template total RNA (adjusted)	4
Total volume	20

Incubate for **60 min at 42°C** followed by heat-inactivation of the reverse transcriptase for 5 min at 95°C. Immediately cool to 4°C. If not used immediately, store at 4°C or freeze.

Phase III (Steps 6-10): real-time PCR amplification (see page 24 for details).

- When ready to set up the qPCR, dilute your cDNA 80x add 1580 µl H_2O pr 20 µl RT. Add passive reference dye if recommended by instrument manufacturer.
- To each well add:

Reagent	Volume (µl), 96/384 well plate, tubes or strips
SYBR® Green master mix	5
PCR primer mix	1
Diluted cDNA template	4
Total volume	10

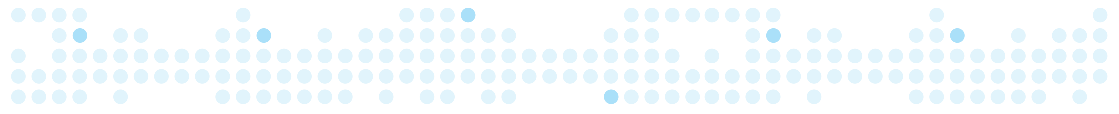
Insert plate, tubes or strips in cycler, and run according to the following settings:

Polymerase Activation/Denaturation	95°C, 10 min
40 amplification cycles*	95°C, 10 s 60°C, 1 min, ramp-rate 1.6°C/s (100% standard on ABI instruments) Optical read
Melting curve analysis	

*45 amplification cycles are required for LC480 instruments to allow collection of data for Cp values up to 40.

Phase IV (Step 11): Data analysis

ABI instruments: use manual baseline and threshold settings (see Tip 10, page 55).



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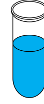
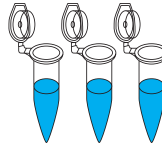
Workflow for individual primer sets



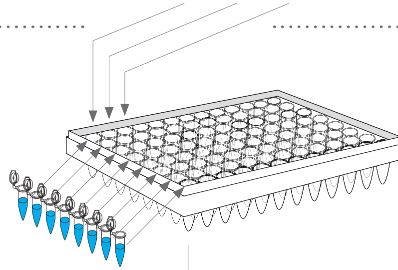
Phase I: Prepare RNA sample

See protocol page 51 for recommendations



Phase II: cDNA synthesis

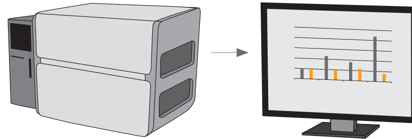
See protocol page 22.
- Triplicate RT per sample is recommended
- a no enzyme RT negative control per study is recommended



Phase III: real-time PCR amplification

See protocol page 24.
- Resuspend and mix PCR primers
- Add primers, cDNA:SYBR® Green mix to PCR plates

ROX: The SYBR® Green master mix, Universal RT does not include the ROX passive reference dye. Add as needed.



Phase IV: Data analysis

Export data for further analysis:
- See data analysis guide online
- Data pre-processing, normalization and statistical analysis

ABI 7900 and 7900 HT: 96- and 384-well sds template files with cycling conditions and analysis settings are available at www.exiqon.com/sds. Plate layout and detector must be added manually.

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exiqon.com/mirna-pcr

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