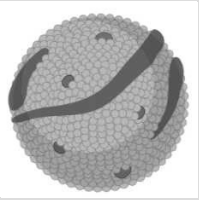

Jan 31, 2022

Quantification of Isolated Circulating MicroRNAs using Qiagen LNA Panels

Dakota Gustafson[1]

[1]University of Toronto

Dakota Gustafson

A protocol for quantification of circulating microRNA using the Qiagen LNA Low Density Array MicroRNAs isolated from platelet-poor plasma. During the purification step samples are spiked with cel-miR-39 as a mean of technical normalization. Post-collected normalization is performed using the 'Global Mean Normalization' methodology.

Dakota Gustafson 2022. Quantification of Isolated Circulating MicroRNAs using Qiagen LNA Panels. **protocols.io** https://protocols.io/view/quantification-of-isolated-circulating-micrornas-u-b4huqt6w

MicroRNA, Circulating, PCR, Qiagen, LNA, Quantification, Normalization

protocol ,

Jan 31, 2022

Jan 31, 2022

57620

This protocol is for use with the miRCURY SYBR® Green PCR Kit (cat. nos. 339345, 339346, 339347) on any real-time PCR cycler. This protocol is used for conducting real-time PCR using the following PCR panels in 96- or 384-well format. This protocol is optimized for detection of miRNA targets with any real-time cycler and conditions for fluorescence normalization. The amount of required ROX dye varies, depending on the instrument used.

5x miRCURY RT SYBR® Green Reaction Buffer **Qiagen Catalog #339340** Step 2

10x miRCURY RT Enzyme Mix **Qiagen Catalog #339340** Step 2

UniSp6 RNA Spike-in Template dried down **Qiagen Catalog #339340** Step 2

Nuclease-Free Water **Contributed by users** In 2 steps

2x miRCURY SYBR® Green PCR Master Mix **Qiagen Catalog #339346** Step 7

SYBR Green PCR Buffer **Qiagen Catalog #339346** Step 7

ROX™ Reference Dye **Qiagen Catalog #339346** Step 7

miRCURY LNA miRNA Panels **Qiagen** Step 7

Thaw reagents on ice prior to beginning experiment.

Reverse Transcription 1h 5m

1 Dilute each template RNA sample to 5 ng/µl using nuclease-free water.

2 Prepare the reverse transcription master mix using:
5x miRCURY RT SYBR® Green Reaction Buffer **Qiagen Catalog #339340**
10x miRCURY RT Enzyme Mix **Qiagen Catalog #339340**
UniSp6 RNA Spike-in Template dried down **Qiagen Catalog #339340**
Nuclease-Free Water **Contributed by users** .

3

Component	miRNA PCR Assay	miRNome PCR Panels : Human, Mouse & Rat (Panel I)	miRNome PCR Panels : Human, Mouse & Rat (Panel I+II)	Focus PCR Panel: Cancer (1 x 96 assays)	Custom PCR Panel: ≤192 miRNAs analyzed per sample	Custom PCR Panel: 193–384 miRNAs analyzed per sample
5x miRCURY SYBR® Green RT Reaction Buffer	2 µl	4 µl	8 µl	2 µl	2 µl	4 µl
RNase-free water	4.5 µl	9 µl	18 µl	4.5 µl	4.5 µl	9 µl
10x miRCURY RT Enzyme Mix	1 µl	2 µl	4 µl	1 µl	1 µl	2 µl
UniSp6 RNA spike-in (optional)	0.5 µl	1 µl	2 µl	0.5 µl	0.5 µl	1 µl
Template RNA (5 ng/µl)	2 µl	4 µl	8 µl	2 µl	2 µl	4 µl
Total reaction volume	**10 µl***	**20 µl***	**40 µl***	**10 µl***	**10 µl***	**20 µl***

* All volumes refer to corresponding PCR reaction volumes of 10 µl. For the Rotor-Disc 100, a reaction volume of 20 µl is recommended, double the amount of all reagents in the Reverse Transcription setup.

4 Incubate for **01:00:00** at **42 °C** , then **00:05:00** at **95 °C** , and immediately cool to **4 °C** .

1h 5m

5 6. Place the reverse-transcription reactions on ice and proceed directly with real-time PCR. Follow the recommendations for proper cDNA dilution provided in the protocol for the PCR Assay or Panel to be used.
Note: If you do not plan to use the cDNA immediately, store it undiluted at 2–8ºC for up to 4 days or at –30 to –15ºC for up to 5 weeks. We recommend storing synthesized cDNA in low-nucleic acid binding tubes or plates.

Quantitative, Real-Time PCR Using miRCURY LNA miRNA Custom PCR Panels

6 Dilute the cDNA 1:80 according to the table immediately before use. We do not recommend storing this 1:80 dilution of cDNA.

	Custom PCR panel configuration	Suggested cDNA dilution for each sample, 1:80 (cDNA + nuclease-free water, µl)	Volume of 1:80 diluted cDNA needed for each sample on a custom PCR panel plate
96	12 x 8 (12 samples)	2 + 158	32 µl
96	8 x 12 (8 samples)	2 + 158	48 µl
96	6 x 16 (6 samples)	2 + 158	64 µl
96	4 x 24 (4 samples)	2 + 158	96 µl
96	3 x 32 (3 samples)	2 + 158	128 µl
96	2 x 48 (2 samples)	3 + 237	192 µl
96	1 x 96 (1 sample)	5 + 395	384 µl
384	48 x 8 (48 samples)	2 + 158	32 µl
384	32 x 12 (32 samples)	2 + 158	48 µl

7 Prepare the master reaction mix according to the table using:

☒ Nuclease-Free Water **Contributed by users** Step 2

☒ 2x miRCURY SYBR® Green PCR Master Mix **Qiagen Catalog #339346**

☒ SYBR Green PCR Buffer **Qiagen Catalog #339346**

☒ ROX™ Reference Dye **Qiagen Catalog #339346**

☒ miRCURY LNA miRNA Panels **Qiagen**

Component	Volume per reaction	Volume Rotor-Disc 100
2x miRCURY SYBR® Green Master Mix	5 µl	10 µl
ROX Reference Dye (ABI instruments only)	0.5 µl/0.05 µl*	
cDNA template (diluted 1:80)	4 µl (diluted 1:80)	8 µl (diluted 1:80)
RNase-free water	1 µl*	2 µl
Total reaction volume	**10 µl†**	20 µl

8 Vortex the reaction mix thoroughly and dispense 10 µl per well into the PCR panel plate(s).

Note: The experiment can be paused at this point. Store the reactions protected from light at 2–8°C for up to 24 h.

Seal the plate. Carefully vortex it to dissolve the primers (optional). Briefly centrifuge the

9 plate(s) at room temperature. Wait 5 min while the primers dissolve in the reaction mix.

10 Program the real-time cycler according to the table.
Note: Data acquisition should be performed during the annealing/extension step.

Step	Time	Temperature	Ramp rate
PCR initial heat activation	2 min	95°C	Maximal/fast mode
2-step cycling			
Denaturation	10 s	95°C	Maximal/fast mode
Combined annealing/extension	60 s	56°C	Maximal/fast mode
Number of cycles	40*		
Melting curve analysis		60–95°C	

11 Perform the initial data analysis using the software supplied with your real-time PCR

12 Save data and export.

Data Analysis

13 Obtain raw Cq values (Cp or CT, depending on PCR instrument) and verify the run quality (i.e., checking for values >35; depending on the threshold being used).

14 Perform data management according to Qiagen's website to facilitate import:

https://geneglobe.qiagen.com/us/analyze

15 Import data and conduct analysis appropriate to the method used (i.e., normalization to reference gene or global mean normalization), and explore downstream analysis.