December 2018

Quick-Start Protocol

QIAseq® miRNA Library Kit

Part 1: 3' Ligation, 5' ligation, reverse transcription

Further information

- When using Illumina® NGS systems, refer to the *QIAseq miRNA Library Kit Handbook: Illumina NGS Systems*: **www.qiagen.com/HB-2157**
- When using Thermo Fisher Scientific® NGS systems, refer to the *QIAseq miRNA Library Kit Handbook: Thermo Fisher Scientific NGS Systems*: **www.qiagen.com/HB-2573**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Prepare the reagents according to the *QIAseq miRNA Library Kit* handbooks
- Ensure reaction components are added in the order listed
- **Important:** Ensure reactions are thoroughly mixed (pipet up and down 15–20 times), prepared at recommended temperatures and incubated at recommended temperatures

3' ligation

1. If working with low RNA inputs (≤10 ng) or serum/plasma samples, dilute the QIAseq miRNA NGS 3' Adapter using nuclease-free water according to Table 1.

Table 1. Dilution of the QIAseq miRNA NGS 3' Adapter

Template RNA input (total RNA)	Adapter dilution
10 ng	1:5
1 ng	1:10
Serum/plasma	1:5

2. On ice, prepare the 3' ligation reaction according to Table 2.

Table 2. Setup of 3' ligation reactions

Component	Volume/rxn
Nuclease-free water	Variable
QIAseq miRNA NGS 3' Adapter*	1 µl
QIAseq miRNA NGS RI	1 µl
QIAseq miRNA NGS 3' Ligase	1 µl
QIAseq miRNA NGS 3' Buffer	2 µl
2x miRNA Ligation Activator	10 µl
Template RNA (added in step 3)	Variable
Total volume	**20 µl**

* For low input and serum/plasma RNA, the QIAseq miRNA NGS 3' Adapter must be diluted according to Table 1.

3. Add template RNA to each tube containing the 3' ligation Master Mix.
4. Incubate for 1 h at 28°C, and then for 20 min at 65°C, and then hold at 4°C.
5. **Important:** Hold at 4°C for at least 5 min.
6. Proceed immediately to 5' ligation.

5' ligation

1. If working with low RNA inputs (≤10 ng) or serum/plasma samples, dilute the QIAseq miRNA NGS 3' Adapter using nuclease-free water according to Table 3.

Table 3. Dilution of the QIAseq miRNA NGS 5' Adapter

Template RNA input (total RNA)	Adapter dilution
10 ng	1:2.5
1 ng	1:5
Serum/plasma	1:2.5

2. On ice, prepare the 5' ligation reaction according to Table 4.

Table 4. Setup of 5' ligation reactions

Component	Volume/rxn
3' ligation reaction (already in tube)	20 µl
Nuclease-free water	15 µl
QIAseq miRNA NGS 5' Buffer	2 µl
QIAseq miRNA NGS RI	1 µl
QIAseq miRNA NGS 5' Ligase	1 µl
QIAseq miRNA NGS 5' Adapter*	1 µl
Total volume	**40 µl**

* For low-input and serum/plasma RNA, the QIAseq miRNA NGS 5' Adapter must be diluted according to Table 3.

3. Incubate for 30 min at 28°C, and then 20 min at 65°C, and then hold at 4°C.
4. Proceed immediately to reverse transcription.

Reverse transcription

1. Add 2 µl QIAseq miRNA NGS RT Initiator to each tube.
2. Incubate the tubes as described in Table 5.

Table 5. Incubation of tubes with QIAseq miRNA NGS RT Initiator

Temperature	Duration
75°C	2 min
70°C	2 min
65°C	2 min
60°C	2 min
55°C	2 min
37°C	5 min
25°C	5 min
4°C	∞

3. If working with low RNA inputs (≤10 ng) or serum/plasma samples, dilute the QIAseq miRNA NGS RT Primer using nuclease-free water according to Table 6.

Table 6. Dilution of the QIAseq miRNA NGS RT Primer

Template RNA input (total RNA)	RT primer dilution
10 ng	1:5
1 ng	1:10
Serum/plasma	1:5

4. On ice, prepare the reverse-transcription reaction according to Table 7.

Table 7. Setup of reverse-transcription reactions

Component	Volume/rxn
5' ligation reaction (already in tube)	42 µl
QIAseq miRNA NGS RT Primer*	2 µl
Nuclease-free water	2 µl
QIAseq miRNA NGS RT Buffer	12 µl
QIAseq miRNA NGS RI	1 µl
QIAseq miRNA NGS RT Enzyme	1 µl
Total volume	**60 µl**

* For low input and serum/plasma RNA, the QIAseq miRNA NGS RT Primer must be diluted according to Table 6.

5. Incubate for 1 h at 50°C, 15 min at 70°C, and hold at 4°C.
6. **Important:** Hold at 4°C for at least 5 min.
7. Proceed to *QIAseq miRNA Library Kit, Part 2: QMN Bead Preparation* Quick-Start Protocol.

Revision History

Revision no.	Description of change
R3 08/2018	Added separate handbook references for Illumina and Thermo Fisher Scientific NGS systems users; updated Technical Assistance contact details; revised Table 5 title
R4 12/2018	Changed name of QIAseq miRNA NGS Ligation Activator to 2x miRNA Ligation Activator

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