August 2018

Quick-Start Protocol

QIAseq® miRNA Library Kit

Part 3a: Library amplification using HT plate indices

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
- Do not vortex reactions or reagents unless instructed
- When performing bead cleanups in plates, centrifuge briefly at 2000 rpm for 2 min

1. On ice, prepare the library amplification reaction using Table 1 for Illumina HT plate indices (cat. no. 331565) or Thermo Fisher Scientific HT plate indices (cat no. 331585).

   Note: Layouts of HT index plates are described in their respective *QIAseq miRNA Library Kit* handbooks (i.e., *Illumina NGS Systems* or *Thermo Fisher Scientific NGS Systems*).

   Note: Indexing primers and a universal primer are pre-dried as single-use plates. During reaction setup, components are added directly to the plate. There is no need to reconstitute and transfer indices to a separate plate.

Table 1. Setup of library amplification reactions using Illumina (331565) or Thermo Fisher Scientific (331585) plate indices

Component	Volume/rxn
Product from cDNA cleanup protocol	15 µl
QIAseq miRNA NGS Library Buffer	8 µl
HotStarTaq® DNA Polymerase	1.5 µl
Nuclease-free water	15.5 µl
Total volume	**40 µl**

2. Program the thermal cycler according to Table 2. The correct number of cycles depends on the original RNA input and is shown in Table 3.

Table 2. Library amplification protocol

Step	Time	Temperature
Hold	15 min	95°C
3-step cycling		
Denaturation	15 s	95°C
Annealing	30 s	60°C
Extension	15 s	72°C
Cycle number (see Table 3)		
Hold	2 min	72°C
Hold	∞*	4°C

* At least 5 min.

Table 3. Cycles of library amplification

Original RNA input (total RNA)	Cycle number
500 ng	13
100 ng	16
10 ng	19
1 ng	22
Serum/plasma	22

3. Place the library amplification reaction in the thermal cycler and start the run.
   Important: Upon completion of the protocol, hold at 4°C for at least 5 min.
4. Briefly centrifuge the 40 µl library amplification reactions.
5. Add 37.5 µl of QMN Beads to the plates containing the cDNA reactions. Vortex for 3 sec, and then centrifuge briefly.
   Note: When working with plates, centrifuge at 2000 rpm.
   Note: If plates become warped, simply transfer mixtures to new plates.
6. Incubate for 5 min at room temperature (15–25°C).
7. Place the plates on a magnet stand for ~4 min or until the beads have fully migrated.
   Transfer 72.5 µl of the supernatant to new plates. Discard the plates containing the beads.
   Important: Do not discard the supernatant at this step.
8. To the 72.5 µl supernatant, add 65 µl of QMN Beads. Vortex for 3 sec, and then briefly centrifuge.
9. Incubate for 5 min at room temperature.
10. Place the plates on a magnet stand until the beads have fully migrated.
    Note: Ensure beads have fully migrated before proceeding.
11. Discard the supernatant, keep the beads, and keep the plates on the magnet stand.
12. Add 200 µl of 80% ethanol into the plates, and then immediately remove and discard the ethanol wash.
13. Repeat the 80% ethanol wash: Add 200 µl of 80% ethanol into the plates again, and then immediately remove and discard this second ethanol wash.
    Note: It is important to completely remove all traces of the ethanol after the second wash. To do this, briefly centrifuge the plates and then return them to the magnetic stand. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.
14. With the bead pellets still on the magnetic stand, air dry them at room temperature for 10 min.
    Note: Visually inspect to ensure that each pellet is completely dry.

15. With the bead pellets still on the magnetic stand, elute the DNA by adding 17 µl of nuclease-free water into the plates. Subsequently cover and remove the plates from the magnetic stand.
16. Carefully pipet the mixture up and down until all the beads are thoroughly resuspended. Briefly centrifuge, and then incubate at room temperature for 2 min.
17. Place the plates on the magnetic stand for ~2 min or until the beads have fully migrated.

    Note: Ensure that the beads have fully migrated before proceeding.
18. Transfer 15 µl of eluted DNA to new plates. This is the miRNA sequencing library.
19. Store the completed miRNA sequencing library at –20°C in a constant-temperature freezer, or proceed to the *miRNA Library Pre-Sequencing QC* protocol in the *QIAseq miRNA Library Kit Handbook* for either the Illumina NGS systems or the Thermo Fisher NGS systems.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.