August 2018

Quick-Start Protocol

QIAseq® miRNA Library Kit

Part 2: QIAseq miRNA NGS (QMN) Bead preparation, cDNA cleanup

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
- Bead cleanups can be performed in tubes or plates. When working with plates, perform brief centrifugations at 2000 rpm for 2 min
- Do not vortex reactions or reagents unless instructed

QMN Bead preparation

1. Thoroughly vortex the QIAseq Beads and the QIAseq miRNA NGS Bead Binding Buffer.
2. Carefully add 400 µl of QIAseq Beads (bead-storage buffer is viscous) to a 2 ml microfuge tube. This amount of beads is sufficient to perform cDNA cleanup and library cleanup protocols for one sample. Briefly centrifuge and immediately separate beads on a magnet stand.

3. When beads have fully migrated, carefully remove and discard the supernatant.
4. Remove the tube from the magnet stand, and carefully pipet (buffer is viscous) 150 µl of the QIAseq miRNA NGS Bead Binding Buffer onto the beads. Thoroughly vortex to completely resuspend the beads. Briefly centrifuge and immediately separate the beads on a magnet stand.
5. When beads have fully migrated, carefully remove and discard the supernatant.

   Note: Remove as much supernatant as possible without disturbing the beads.
6. Remove the tube from the magnet stand and carefully pipet 400 µl of the QIAseq miRNA NGS Bead Binding Buffer onto the beads. Thoroughly vortex to completely resuspend.
7. Preparation of the QMN Beads is now complete. Proceed to cDNA cleanup.

cDNA cleanup

1. Ensure that QMN Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads.
2. Centrifuge the tubes/plates containing the cDNA reactions.
3. Add 143 µl of QMN Beads to tubes/plates containing the cDNA reactions. Vortex for 3 s and centrifuge briefly again.
   Note: When working with plates, centrifuge at 2000 rpm for 2 min.
   Note: If plates are warped, simply transfer mixtures to new plates.
4. Incubate for 5 min at room temperature (15–25°C).
5. Place tubes/plates on a magnet stand for ~4 min or until beads have fully migrated.

   Note: Ensure beads have fully migrated before proceeding.
6. Discard the supernatant, keep the beads and do not remove the tube from the magnet stand.
7. With the beads still on the magnet stand, add 200 µl of 80% ethanol. Immediately remove and discard the ethanol wash.

8. Repeat the 80% ethanol wash. Immediately remove and discard the ethanol wash.

   Important: To remove all ethanol, briefly centrifuge (centrifuge plates at 2000 rpm), and then return the tubes/plates 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.

9. With the beads still on the magnetic stand, air dry at room temperature for 10 min.

   Note: Visually inspect to confirm that the pellet is completely dry.

10. With the bead pellet still on the magnetic stand, elute the DNA by adding 17 µl of nuclease-free water to the tubes/plates. Close the tubes or cover the plates, and then remove them from the magnetic stand.
11. Carefully pipet up and down until all the beads are thoroughly resuspended. Briefly centrifuge, and then incubate at room temperature for 2 min.
12. Return the tubes/plates to the stand for ~2 min or until beads have fully migrated.

    Note: Ensure that the beads have fully migrated before proceeding.

13. Transfer 15 µl of eluted DNA to a clean tube.
14. Store the completed cDNA cleanup product at –20°C in a constant-temperature freezer, or proceed to library amplification:
    - If using single-use plate indices, refer to the *QIAseq miRNA Library Kit Quick-Start Protocol*, Part 3a: Library amplification using HT plate indices.
    - If using tube indices, refer to the *QIAseq miRNA Library Kit Quick-Start Protocol*, Part 3b: Library amplification using tube indices.

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; removed Library Amplification section and transferred it to its own document; inserted new bullet into "Notes before starting"; made multiple adjustments in protocol steps.

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.