October 2019

Quick-Start Protocol

QIAseq® FastSelect™ –rRNA HMR and/or –Globin with the NEBNext® Ultra II Directional Library Prep Kit

The QIAseq FastSelect Kits for –rRNA HMR (cat. nos. 334386, 334387, 334388), –Globin (cat. nos. 334376, 334377, 334378), and –rRNA/Globin (cat. nos. 335376, 335377, 335378) may be used with the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina® (NEB cat. nos. E7760S and E7760L) to remove human, mouse, or rat rRNA and/or globin.

All components of QIAseq FastSelect should be stored at –30 to –15°C in a constant-temperature freezer.

Further information

- *QIAseq FastSelect –rRNA HMR and –Globin Handbook*: **www.qiagen.com/HB-2670**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- The NEBNext Ultra II Directional RNA Library Prep Kit for Illumina is required for use with this protocol.
- Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* (Version 2.2).

Procedure

1. Thaw the tube(s) from the QIAseq FastSelect kit. Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
2. Referring to Section 4 from the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual*, perform the following in place of steps 4.1.1 through 4.1.4:

   2a. Assemble the fragmentation and priming reaction described in Table 1 on ice in a nuclease-free tube.

Table 1. NEBNext Ultra II Stranded fragmentation and priming mix

Component	Volume/reaction
Total RNA (5 ng–1 µg)	4 µl
(lilac) NEBNext First Strand Synthesis Reaction Buffer*	4 µl
(lilac) Random primers*	1 µl
Total volume	**9 µl**

* From NEBNext Ultra II Directional Library Prep Kit.

   2b. To the assembled fragmentation and priming mix, add QIAseq FastSelect as follows:
   - **Option 1 (remove rRNA)**: Add 1 µl of QIAseq FastSelect –rRNA HMR
   - **Option 2 (remove globin)**: Add 1 µl of QIAseq FastSelect –Globin
   - **Option 3 (remove rRNA and globin)**: Add 1 µl of QIAseq FastSelect –rRNA HMR and 1 µl of QIAseq FastSelect –Globin

   2c. Mix thoroughly by pipetting up and down several times and then briefly centrifuge to collect residual liquid from the sides of the tubes

   2d. Incubate in a thermal cycler with a heated lid as described in Table 2, according to your input RNA quality.

   Important: Regardless of time and temperature chosen in step 1, steps 2–9 must be performed.

Table 2. Combined NEBNext Ultra II fragmentation and FastSelect hybridization protocol

Step	Intact RNA (RIN >7)	Partially degraded RNA (RIN 2–6)
1	15 min at 94°C	7–8 min at 94°C
2	2 min at 75°C	2 min at 75°C
3	2 min at 70°C	2 min at 70°C
4	2 min at 65°C	2 min at 65°C
5	2 min at 60°C	2 min at 60°C
6	2 min at 55°C	2 min at 55°C
7	2 min at 37°C	2 min at 37°C
8	2 min at 25°C	2 min at 25°C
9	Hold at 4°C	Hold at 4°C

3. Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* and immediately proceed to "First Strand cDNA Synthesis Reaction".
   Note: "First Strand cDNA Synthesis Reaction" is chapter 4.2 in Version 2.2 of the instruction manual.
4. Follow the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* to perform all remaining library construction steps.

   Important: When removing globin, 2 additional cycles of library amplification need to be performed.

Revision History

Date	Changes
10/2019	Initial release

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For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.