Quick-Start Protocol September 2018

QIAseq® FastSelect™ RNA Removal Kit

NEBNext Ultra II Directional RNA Library Prep Kit: rRNA and/or Globin removal

Further information

- *QIAseq FastSelect RNA Removal Kit Handbook*: **www.qiagen.com/HB-2580**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000 or **www.qiagen.com/contact**

Notes before starting

- The NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs, cat. no. E7760S, E7760L) is required for use with this protocol.

Procedure

1. Thaw the tube(s) from the QIAseq FastSelect RNA Removal Kit. Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
2. From the NEBNext Ultra II Directional RNA Library Prep Kit, assemble the fragmentation and priming reaction described in Table 1 on ice in a nuclease-free tube.

Table 1. NEBNext Ultra II Directional RNA fragmentation and priming mix

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

* From NEBNext Ultra II Directional RNA Library Prep Kit.

3. To the assembled fragmentation and priming mix, add QIAseq FastSelect as follows:
   Option 1 (remove rRNA): Add 1 µl of rRNA Removal.
   Option 2 (remove Globin): Add 1 µl of Globin Removal.
   Option 3 (remove rRNA and Globin): Add 1 µl of rRNA Removal and 1 µl Globin Removal.
4. Mix thoroughly by pipetting up and down several times and then briefly centrifuge to collect residual liquid from the sides of the tubes.
5. 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. NEBNext Ultra II Directional RNA fragmentation and 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	5 min at 37°C	5 min at 37°C
8	5 min at 25°C	5 min at 25°C
9	Hold at 4°C	Hold at 4°C

6. 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 Instruction Manual 1.0.
7. Follow the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* to perform all remaining library construction steps.
   Note: When removing Globin, 2 additional cycles of library amplification may need to be performed.

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