July 2020

Quick-Start Protocol

QIAseq® FastSelect™ –5S/16S/23S Kit with the NEBNext® Ultra™ II Directional Library Prep Kit

The QIAseq FastSelect –5S/16S/23S Kit (cat. nos. 335925, 335927, 335929) can be used for bacterial 5S/16S/23S rRNA removal with the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs cat. no. E7760S, E7760L). Upon receipt, the FastSelect 5S/16S/23S tube should be immediately stored at –30 to –15°C in a constant-temperature freezer. All remaining components should immediately be stored in a refrigerator at 2–8°C.

Further information

- *QIAseq FastSelect –5S/16S/23S Handbook*: **www.qiagen.com/HB-2695**
- 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.
- For more information, please refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina* instruction manual (Version 2.2_05/19).

Procedure

1. Thaw template RNA on ice. Mix gently, centrifuge briefly to collect residual liquid from the sides of the tubes, and then return to ice.

2. Prepare reagents required for RNA fragmentation and QIAseq FastSelect rRNA removal:
   2a. Bring FastSelect 5S/16S/23S, FastSelect FH Buffer, and Nuclease-free Water to room temperature.
   2b. **Important**: Only for FastSelect 5S/16S/23S, incubate the tube at 37°C for 5 min, and then vortex to dissolve the precipitate.
   2c. Mix all other reagents vigorously by vortexing, and then briefly centrifuge.
3. On ice, prepare the fragmentation/depletion reaction according to Table 1. Briefly centrifuge, mix by pipetting up and down 10 times, and centrifuge briefly again.
4. Incubate as described in Table 2.

   Important: Perform steps 2–9 regardless of the time and temperature in step 1.
5. Add 19.5 µl QIAseq Beads (prewarmed to room temperature and mixed by vortexing) to the 15 µl reaction. Mix thoroughly by vortexing, and incubate for 5 min at room temperature.
6. Centrifuge in a table-top centrifuge until the beads are completely pelleted (~2 min).
7. Place the tubes/plate on a magnetic rack for 2 min. Once the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant. **Important**: Do not discard the beads, because they contain the RNA of interest.
8. Add 15 µl of Nuclease-free Water and 19.5 µl of QIAseq Bead Binding Buffer (prewarmed to room temperature and mixed by vortexing). Mix vigorously by vortexing, and incubate for 5 min at room temperature.
9. Centrifuge in a table-top centrifuge until the beads are completely pelleted (~2 min).
10. Place the tubes/plate on a magnetic rack for 2 min. Once the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant. **Important**: Do not discard the beads, because they contain the RNA of interest.
11. With the beads still on the magnetic stand, add 200 µl of 80% ethanol. Wait 30 s. Carefully remove and discard the wash.
12. Repeat the ethanol wash.
    Important: Completely remove all traces of ethanol after this second wash. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol that will settle.
13. With the beads still on the magnetic stand, air-dry at room temperature for 6–10 min. (until all liquid has evaporated but without overdrying the beads).
    Note: Visually inspect the pellet to confirm that it is completely dry.

Table 1. Setup of fragmentation/depletion reactions

Component	Volume/reaction
Total RNA (20 ng – 1 µg)	Variable
FastSelect FH Buffer	1.5 µl
FastSelect 5S/16S/23S*	1 µl
Nuclease-free Water	Bring total reaction volume to 15 µl
Total volume	**15 µl**

* If needed, add 1 µl QIAseq FastSelect –rRNA HMR, 1 µl QIAseq FastSelect –Globin, 1 µl QIAseq FastSelect –rRNA Plant, and/or 1 µl QIAseq FastSelect –Yeast. To keep the total volume at 15 µl, correspondingly reduce the Nuclease-free Water by the volume of additional FastSelect products that have been added.

Table 2. Combined QIAseq fragmentation and FastSelect hybridization protocol

Note	Step	Mean insert size ~175–225 bp
RNA fragmentation	1*	8 min at 89°C*†
Steps 2–9 are performed regardless of input RNA quality. They need to be performed whether the RNA is high quality, moderate quality, FFPE, or degraded.	2	2 min at 75°C
	3	2 min at 70°C
	4	2 min at 65°C
	5	2 min at 60°C
	6	2 min at 55°C
	7	2 min at 37°C
	8	2 min at 25°C
	9	Hold at 4°C

* For high-quality RNA (RIN ≥8), 8 min at 89°C is the basic recommendation. Alternatively, 4 min at 89°C provides an average insert size of ~325–375 bp, and 12 min at 89°C provides an average insert size of ~125–175 bp.

† If working with RNA with RIN values <8, please refer to the *QIAseq FastSelect –5S/16S/23S Handbook*, Table 1, for fragmentation recommendations.

14. Remove the beads from the magnetic stand, and elute the RNA from the beads by adding 7 µl Nuclease-free Water. Mix well by pipetting or vortexing, and then allow the tubes to sit at room temperature for 2 min to appropriately hydrate the beads.
15. Return the tubes/plate to the magnetic rack until the solution has cleared.
16. Transfer 5 µl of the supernatant to clean tubes/plate. Alternatively, the samples can be stored at –90 to –65°C in a constant-temperature freezer.
17. Set up the first strand synthesis associated with the NEBNext Ultra II Directional RNA Library Prep Kit as described in Table 3. Briefly centrifuge, mix by pipetting up and down 10 times, and centrifuge briefly again.

Table 3. NEBNext Ultra II Directional RNA Library Prep Kit first-stranded synthesis setup

Component	Volume/reaction
RNA from bead cleanup reaction	5 µl
(lilac) NEBNext First Strand Synthesis Reaction Buffer*	4 µl
(lilac) Random Primers*	1 µl
(brown) NEBNext Strand Specificity Reagent*	8 µl
(lilac) NEBNext First Strand Synthesis Enzyme Mix*	2 µl
Total volume	**20 µl**

* All designated components are from the NEBNext Ultra II Directional RNA Library Prep Kit.

18. Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* and immediately proceed to and perform step 4.2.3 under "First Strand cDNA Synthesis Reaction".
19. Follow the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* to perform all remaining library construction steps.
    Important: If starting with 20 ng or less of total RNA, 2 additional cycles of library amplification must be performed.

Document Revision History

Date	Changes
07/2020	Clarified storage condition. Included 37°C incubation for FastSelect 5S/16S/23S as a default step in the procedure. Included options to include FastSelect –rRNA Plant and/or FastSelect –rRNA Yeast in the procedure. Replaced all precise storage temperatures with temperature ranges.

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