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Quick-Start Protocol

QIAseq® FastSelect™ –rRNA HMR and/or –Globin with TruSeq® Stranded Library Preparation

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 TruSeq Stranded mRNA Library Prep (Illumina, cat. nos. 20020594 and 20020595) 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 TruSeq Stranded mRNA Library Prep is required for use with this protocol.

  Note: With this protocol, do not perform mRNA purification. Instead, follow the steps outlined below before proceeding to "Synthesize First Strand cDNA" in the *TruSeq Stranded mRNA Reference Guide*. By doing this, a stranded, total RNA library preparation will be performed.
- Refer to the *TruSeq Stranded mRNA Reference Guide* (1000000040498).

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. To 100 ng – 1 µg of total RNA, which is required to be in a maximum volume of 5 µl, 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
3. From the TruSeq Stranded mRNA Library Prep, add 14.5 µl FPF (when using option 1 or 2 above), or add 13.5 µl FPF (when using option 3 above), to bring the volume of the reaction to 20.5 µl.
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 1 (next page).

   Important: Table 2 (next page) can be consulted to adjust RNA insert size. Irrespective of time at 94°C, steps 2–9 listed in Table 1 must be performed.
6. Use 17 µl of the fragmented/hybridized RNA, refer to the *TruSeq Stranded mRNA Reference Guide* and immediately proceed to "Synthesize First Strand cDNA."
   Note: From the *TruSeq Stranded mRNA Reference Guide*, the procedural step "Place the RBP plate on the magnetic stand and wait until the liquid is clear (~5 minutes)" is not applicable.
7. Follow the *TruSeq Stranded mRNA Reference Guide* to perform all remaining library construction steps.

   Important: It is highly recommended to dilute the Illumina adapters 2-fold compared to what is suggested in the default Illumina protocol.

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

Table 1. Combined TruSeq Stranded fragmentation and FastSelect hybridization protocol

Step	Time and temperature
1*	8 min at 94°C*
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

* The initial step at 94°C can be modified to permit longer RNA insert sizes. Refer to Table 2 for recommendations.
Note: The remaining steps 2–9 are performed regardless of the time at 94°C.

Table 2. Fragmentation time at 94°C for alternative RNA insert sizes

Time at 94°C*	Range of insert length (bp)	Median insert length (bp)	Average final library size (Bioanalyzer bp)
0 min	130–350	200	467
1 min	130–310	190	439
2 min	130–290	185	410
3 min	125–250	165	366
4 min	120–225	160	326
8 min	120–210	155	309
12 min	115–180	140	272

* The remaining steps 2–9 from Table 1 must be performed regardless of the time at 94°C.

Revision History

Date	Changes
10/2019	Initial release

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