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Supplementary Protocol

QIAseq® FastSelect™ –rRNA HMR and/or –Globin with the Truseq® Stranded Total RNA Library Prep

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 Total RNA Library Prep (Illumina, cat. nos. 20020596, 20020597, 20020598, 20020599) to remove human, mouse, or rat rRNA and/or globin.

IMPORTANT: Please consult "Safety Information" and "Important Notes" in the *QIAseq FastSelect –rRNA HMR and –Globin Handbook*, **www.qiagen.com/HB-2670**, before beginning this procedure.

Important points before starting

- TruSeq Stranded Total RNA Library Prep is required for use with this protocol.
  Note: Ribo-Zero® Gold and Ribo-Zero Human/Mouse/Rat or Globin are not required for this protocol.
- TruSeq Stranded mRNA may be substituted for TruSeq Stranded Total RNA Library Prep. Please consult the *QIAseq FastSelect –rRNA HMR and –Globin Handbook* for details.
- **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.
- Refer to the *TruSeq Stranded Total RNA Reference Guide* (1000000040499) available at **support.illumina.com**

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 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 QIAseq FastSelect –rRNA HMR
   Option 2 (remove Globin): Add 1 µl QIAseq FastSelect –Globin
   Option 3 (remove rRNA and Globin): Add 1 µl QIAseq FastSelect –rRNA HMR and 1 µl QIAseq FastSelect –Globin

3. From the TruSeq Stranded Total RNA Library Prep, add 2.5 µl ELB (when using option 1 or option 2 above) or add 1.5 µl ELB (when using option 3 above) to bring the volume of the reaction to 8.5 µl.
4. From the TruSeq Stranded Total RNA Library Prep, add 8.5 µl EPH to bring the volume of the reaction to 17 µl.
5. Mix thoroughly by pipetting up and down several times, and then briefly centrifuge to collect residual liquid from the sides of the tubes.
6. Incubate in a thermal cycler with a heated lid as described in Table 1.
   Important: Table 2 can be consulted to adjust RNA insert size. Irrespective of time at 94°C, steps 2–9 listed in Table 1 must be performed.

Table 1. TruSeq Stranded fragmentation and 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

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

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.

7. Using 17 µl of the fragmented/hybridized RNA, refer to the *TruSeq Stranded Total RNA Reference Guide* and immediately proceed to "Synthesize First Strand cDNA".
8. Follow the *TruSeq Stranded Total RNA 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 reference guide.

IMPORTANT 	When removing globin, 2 additional cycles of library amplification may need to be performed.

Document revision history

Date	Changes
12/2019	Initial release

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.