

Supplementary Protocol

QIAseq® FastSelect™ with SMARTer® Total RNA-Seq Kit v3 – Pico Input Mammalian

Most QIAseq FastSelect Kits may be used with SMARTer Total RNA-Seq Kit v3 – Pico Input Mammalian (Takara, cat. nos. 634485, 634486, 634487, 634488).

For QIAseq FastSelect -5s/16s/23s Kits, please contact QIAGEN technical support for a modified protocol.

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

Further information

- QIAseq FastSelect Handbook: www.qiagen.com/HB-2929
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Important points before starting

- The SMARTer Total RNA-Seq Kit v3 - Pico Input Mammalian is required for use with this protocol. Due to the template switching-based approach of SMARTer, it is important to follow the modified protocol. Otherwise, the library prep will not work.
- Refer to “V. Protocols” section of the SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian User Manual (www.takarabio.com/assets/114949).

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. Make 0.1x FastSelect by combining 1 μ L of FastSelect with 9 μ L Nuclease-Free Water (scale up as needed). Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
3. In place of step 1 in the Protocol: First-Strand cDNA Synthesis section of the *SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian User Manual*, mix the following components on ice:

Table 1. Sample preparation guidelines including FastSelect

Components	1 rxn (μ L)
RNA	1–7
SMART Pico Oligos Mix v3	1
Nuclease-Free Water	0–6
FastSelect rRNA Removal reagent (diluted to 0.1x)	1
5X First-Strand Buffer	4
Total Volume	13

4. In place of step 2 in the Protocol: First-Strand cDNA Synthesis section of the *SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian User Manual*, incubate the samples as mentioned in Table 2, next page.

Table 2. Combined QIAseq Stranded fragmentation and FastSelect hybridization protocol

Step	Time and temperature
1	Variable 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

* See Table 1 in the *SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian User Manual*.

5. Continue with Step 3 in the *SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian User Manual* for all the remaining steps.

Document Revision History

Date	Changes
10/2024	Initial release.



Scan QR code for the *QIAseq FastSelect Handbook*.

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