Quick-Start Protocol September 2017

QIAseq™ Stranded Total RNA Library Kit

Part 2: Strand-specific ligation

Immediately upon receipt, store the QIAseq Stranded Total RNA Kit (cat. nos. 180753 and 180755) at –30°C to –15°C. QIAseq Beads (cat. nos. 1107149, 1107460) should be stored at 4°C (**do not freeze**). If stored under these conditions, kits are stable until the date indicated on the QC label.

Further information

- *QIAseq Stranded Total RNA Library Handbook*: **www.qiagen.com/HB-2465**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- To maximize output yields and minimize adapter dimer formation, use 2 ml tubes.
- **Important**: Ensure reactions are thoroughly mixed by pipetting up and down, prepared and incubated at recommended temperatures.
- Ligation Initiator is a very viscous solution. Pipet slowly and carefully to ensure the addition of the correct volume into the ligation reaction.

Strand-specific ligation

1. Thaw and keep Ligation Initiator at room temperature until the ligation mix is prepared.
2. Vortex briefly and centrifuge the adapter plate. Remove the protective lid on the adapter plate, pierce the foil seal and dilute the QIAseq Adapters as described in Table 1 (Dilution of QIAseq adapters for total RNA input following mRNA enrichment)

or Table 2 (Dilution of QIAseq adapters for mRNA/depleted rRNA input).

3. For each sample, add 2 µl of diluted adapter. Ensure that only one adapter is used per sample, and be sure to track the barcodes. **Important**: To avoid cross-contamination, do not reuse diluted adapters.

Table 1. Dilution of QIAseq Adapters for total RNA input following mRNA enrichment

Input RNA (total RNA) amount	Volume of QIAseq Adapter	Volume of RNase-free water	Adapter dilution
100 ng	10 µl	990 µl*	1:100
500 ng	10 µl	240 µl*	1:25
1000 ng	10 µl	115 µl	1:12.5
5000 ng	10 µl	40 µl	1:5

Table 2. Dilution of QIAseq Adapters for mRNA/depleted rRNA input

Input RNA (total RNA) amount	Volume of QIAseq Adapter	Volume of RNase-free water	Adapter dilution
1 ng	10 µl	1240 µl*	1:100
10 ng	10 µl	240 µl*	1:25
50 ng	10 µl	40 µl	1:5
100 ng	10 µl	15 µl	1:2.5

* Detailed dilution steps are described in the *QIAseq Stranded Total RNA Library Handbook*.

4. Prepare ligations as described in Table 3 and incubate at 25°C for 10 min.
   Important: Do not use a heated lid.

Table 3. Strand-specific ligation reaction setup

Component	Volume/reaction
Pre-mixed sample	50 µl
Diluted adapter	2 µl
Ultralow Input Ligation Buffer, 4X	25 µl
Ultralow Input Ligase	5 µl
Ligation Initiator	6.5 µl
RNase-free water	11.5 µl
Total reaction volume	**100 µl**

5. After the ligation reaction, add 80 µl QIAseq Beads. Mix well by pipetting up and down 10 times.
6. Incubate for 5 min at room temperature.
7. Place the tubes on a magnetic rack. After the solution has cleared (~10 min or longer), carefully remove and discard the supernatant.

   Important: Do not discard the beads as they contain the DNA of interest.
8. With the tubes still on the magnetic stand, add 200 µl of 80% ethanol. Rotate the tubes (2 to 3 times) to wash the beads. Carefully remove and discard the wash.
9. Repeat the ethanol wash.

   Important: Completely remove all traces of the ethanol wash 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.
10. With the tubes (caps opened) still on the magnetic stand, air dry at room temperature for 5 to 10 min.

    Note: Visually inspect that the pellet is completely dry, but avoid over-drying.
11. Remove the tubes from the magnetic stand, and elute the DNA from the beads by adding 92 µl nuclease-free water. Mix well by pipetting.
12. Return the tubes to the magnetic rack until the solution has cleared.
13. Transfer 90 µl supernatant to clean tubes.
14. Add 108 µl QIAseq Beads.
15. Incubate for 5 min at room temperature.
16. Place the tubes on a magnetic rack. After the solution has cleared (~10 min or longer), carefully remove and discard the supernatant.

    Important: Do not discard the beads as they contain the DNA of interest.
17. With the tubes still on the magnetic stand, add 200 µl of 80% ethanol. Rotate the tubes (2 to 3 times) to wash the beads. Carefully remove and discard the wash.
18. Repeat the ethanol wash.

Important: Completely remove all traces of the ethanol wash 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.

19. With the beads (caps opened) still on the magnetic stand, air dry at room temperature for 5 to 10 min.

    Note: Visually inspect that the pellet is completely dry, but avoid over-drying.

20. Remove the tubes from the magnetic stand, and elute the DNA from the beads by adding 25 µl nuclease-free water. Mix well by pipetting.
21. Return the tubes to the magnetic rack until the solution has cleared.
22. Transfer 23.5 µl to clean tubes/plate.
23. Proceed to CleanStart library amplification in part 3 of the protocol. Alternatively, the samples can be stored at –20°C in a constant-temperature freezer overnight.

Scan QR code for handbook.

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