Quick-Start Protocol May 2018

QIAseq™ UPX 3' Targeted RNA Panel

Part 2: Single primer extension

Upon receipt, store the QIAseq UPX 3' Targeted RNA Panel at –30 to –15°C. The QIAseq Beads and Cell Index (ID) RT Plate should be stored at 4°C.

Further information

- *QIAseq UPX 3' Targeted RNA Library Handbook*: **www.qiagen.com/HB-2480**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Ensure reactions are thoroughly mixed (12 times). Do not vortex.

Single primer extension

1. On ice, prepare the SPE reactions as described in Table 1.

Table 1. Preparation of SPE reactions

Component	Volume/reaction
Product from cDNA cleanup (from QSP Part 1)	10 µl
SPE Buffer	8 µl
HotStarTaq® DNA Polymerase	1.6 µl
UPX 3' Targeted Panel Pool	8 µl
UPX AMP Primer	1.6 µl
Nuclease-free Water	10.8 µl
Total volume	**40 µl**

2. Incubate as described in Table 2.

Table 2. SPE cycling protocol

Step	Time	Temperature
Hold	15 min	95°C
2-step cycling		
Denaturation	15 s	95°C
Annealing/Extension	10 min	68°C
10 cycles		
Hold	5 min	72°C
Hold	∞	4°C

3. Add 120 µl nuclease-free water to bring each sample to 160 µl.
4. Add 208 µl QIAseq Beads. Mix well by pipetting up and down 12 times.
5. Incubate for 5 min at room temperature.
6. Place the tubes on a magnetic rack for 5 min. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.
7. With the beads still on the magnetic stand, add 200 µl of 80% ethanol. Rotate the tube (3 times) to wash the beads. Carefully remove and discard the wash.
8. Repeat the ethanol wash. Completely remove all traces of the ethanol wash.
9. With the tube (caps opened) still on the magnetic stand, air dry for 10 min.

   Note: Visually inspect that the pellet is completely dry.
10. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 12 µl nuclease-free water. Mix well by pipetting.
11. Return the tubes to the magnetic rack until the solution has cleared.
12. Transfer 10 µl of the supernatant to clean tubes.

13. Proceed with "Universal PCR". Alternatively, samples can be stored at –30 to –15°C.

Note: Consult "*Optional Protocol: Quantitative Universal PCR*", in the handbook, to determine the cycles required for universal amplification.

Universal PCR

14. On ice, prepare the library amplification reactions as described in Table 3. When using the QIAseq UPX 3' Targeted 96 Index A, B, C or D, add reagents directly to the plate.

Table 3. Preparation of universal PCR reactions

Component	Volume/reaction Index Tubes	Volume/reaction Index Plate
Product from SPE cleanup	9 µl	9 µl
UPX 3' uPCR Buffer	5 µl	5 µl
HotStarTaq DNA Polymerase	1 µl	1 µl
QIAseq UPX 3' Targeted Index*	5 µl	–
Nuclease-free water	5 µl	10 µl
Total volume	**25 µl**	**25 µl**

* QIAseq UPX 3' Targeted RNA 12 Index (Tubes) or QIAseq UPX 3' Targeted 96 Index A, B, C or D (Plate).

15. Incubate the reaction in a real-time PCR instrument as described in Table 4.

Table 4. Universal PCR cycling

Step	Time	Temperature
Hold	15 min	95°C
3-step cycling		
Denaturation	15 s	95°C
Annealing/Extension	2 min	65°C
Cycle number	**Based on "Optional Protocol: Quantitative Universal PCR"***	
Hold	∞	4°C

*See the *QIAseq UPX 3' Targeted RNA Library Handbook*.

16. Add 75 µl nuclease-free water to bring each sample to 100 µl.
17. Add 130 µl QIAseq Beads. Mix well by pipetting up and down 12 times.
18. Incubate for 5 min at room temperature.
19. Place the tubes on a magnetic rack for 5 min. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.
20. With the beads still on the magnetic stand, add 200 µl of 80% ethanol. Rotate the tube (3 times) to wash the beads. Carefully remove and discard the wash.
21. Repeat the ethanol wash. Completely remove all traces of the ethanol wash.
22. With the tube (caps opened) still on the magnetic stand, air dry for 10 min.

    Note: Visually inspect that the pellet is completely dry.
23. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 22 µl nuclease-free water. Mix well by pipetting.
24. Return the tubes to the magnetic rack until the solution has cleared.
25. Transfer 20 µl of the supernatant to clean tubes. Alternatively, the samples can be stored at –30 to –15°C.
26. Proceed with "Protocol: Library QC and Concentration Determination", in the *QIAseq UPX 3' Targeted RNA Library Handbook*.

Scan QR code for handbook.

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