Quick-Start Protocol May 2018

QIAseq™ UPX 3' Transcriptome Library Kit

Part 3: Adapter ligation and universal PCR

Upon receipt, store the QIAseq UPX 3' Transcriptome Library Kit at –30 to –15°C. QIAseq Beads and the Cell Index (ID) RT Plate should be stored at 2–8°C.

Further information

- *QIAseq UPX 3' Transcriptome Library Handbook*: **www.qiagen.com/HB-2485**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- **Important**: Rebuffered QIAseq Beads (RQ Beads) must be used for the cleanup reactions. Consult the kit handbook for rebuffering details.
- Ensure that the reactions are thoroughly mixed (12 times). Do not vortex.

Adapter ligation

1. Prepare the adapter ligation reaction on ice as described in Table 1.

Table 1. Preparation of adapter ligation reactions

Component	Volume per reaction
Fragmentation, end-repair, and A-addition reaction already in tube)	25 µl
Ligation Buffer, 5x	10 µl
DNA Ligase	5 µl
UL Adapter	1.4 µl
Ligation Solution	7.2 µl
Nuclease-Free Water	1.4 µl
Total volume	**50 µl**

2. Incubate the reaction for 15 min at 20°C.

   Important: Do not use a heated lid.
3. Add 50 µl nuclease-free water to bring each sample to a final volume of 100 µl.
4. Add 60 µl RQ Beads. Mix well by pipetting up and down 12 times.
5. Incubate for 10 min at room temperature (15–25°C).
6. Place the tube on a magnetic rack for 5 min. After the solution has cleared, leave the tube on the magnetic stand, and carefully remove and discard the supernatant.
7. Add 200 µl of 80% ethanol. Rotate the tube three times. Carefully remove the wash.
8. Repeat the ethanol wash in step 7. Completely remove all traces of the ethanol wash.

   Important: Briefly centrifuge and return the tubes to the magnetic stand. Remove the ethanol by pipetting.
9. Air dry the opened tube on the magnetic stand at room temperature (15–25°C) for 10 min.

   Note: Visually inspect that the pellet is completely dry.
10. Remove the tube from the magnetic stand, and elute the DNA from the beads by adding 13 µl Nuclease-Free Water. Mix well by pipetting.
11. Return the tube to the magnetic rack until the solution has cleared.
12. Transfer 11 µl of the supernatant to a clean microcentrifuge tube.
13. Proceed with universal PCR. Alternatively, store the samples at –30 to –15°C in a constant-temperature freezer.

Universal PCR

1. Prepare the universal PCR reactions on ice as described in Table 2.

Table 2. Preparation of universal PCR reactions

Component	Volume/reaction Index tubes	Volume/reaction Index plate
Product from adapter ligation cleanup	10 µl	10 µl
UPCR Buffer, 5x	5 µl	5 µl
HotStarTaq DNA Polymerase	1 µl	1 µl
QIAseq UPX 3' Trans Index*	2.5 µl	0 µl
Nuclease-free water	6.5 µl	9 µl
Total volume	**25 µl**	**25 µl**

* QIAseq UPX 3' Trans 12 Index (tubes) or QIAseq UPX 3' Trans 48 Index (plate). For the QIAseq UPX 3' Trans 48 Index, a universal primer and an indexing primer are pre-dried in single-use plates. During reaction setup, components are added directly to the plate. There is no need to reconstitute and transfer indices to a separate plate.

2. Incubate the reaction as described in Table 3.

Table 3. Universal PCR protocol

Step	Time	Temperature
Hold	15 min	95°C
3-step cycling:		
Denaturation	15 s	95°C
Annealing/Extension*	2 min	65°C
Cycle number	Varies*	
Hold	∞	4°C

* Number of cycles depends on the results from "Protocol: Quantitative Universal PCR". Refer to the *QIAseq UPX 3' Transcriptome Library Handbook* for details.

3. Add 75 µl Nuclease-Free Water to bring each sample to a final volume of 100 µl.
4. Add 60 µl RQ Beads. Mix well by pipetting up and down 12 times.
5. Incubate for 10 min at room temperature (15–25°C).
6. Place the tube on a magnetic rack for 5 min. After the solution has cleared, leave the tube on the magnetic stand, and carefully remove and discard the supernatant.

7. Add 200 µl 80% ethanol. Rotate the tube three times. Carefully remove the wash.
8. Repeat the ethanol wash in step 7. Completely remove all traces of the ethanol wash.
9. Air dry the opened tube on the magnetic stand at room temperature (15–25°C) for 10 min.

   Note: Visually inspect that the pellet is completely dry.
10. Remove the tube from the magnetic stand, and elute the DNA from the beads by adding 13 µl Nuclease-Free Water. Mix well by pipetting.
11. Return the tube to the magnetic rack until the solution has cleared.
12. Transfer 11 µl of the supernatant to a clean microcentrifuge tube.
13. Proceed with "Protocol: Library Quantification and QC" in the *QIAseq UPX 3' Transcriptome Library Kit Handbook*. Alternatively, store the samples at –30 to –15°C in a constant-temperature freezer.

Scan QR code for handbook.

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