Quick-Start Protocol March 2019

QIAseq® UPX 3' Transcriptome Library Kit

Part 2: Template amplification and fragmentation, end-repair, and A-addition

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.

Template amplification

1. Prepare the library amplification reaction on ice as described in Table 1.

Table 1. Preparation of template amplification reactions

Component	Volume per reaction
Product from reverse transcription cleanup	10 µl
2x QIAGEN HiFi PCR MM	25 µl
UPX AMP Primer	4 µl
Nuclease-Free Water	11 µl
Total volume	**50 µl**

2. Incubate the reaction according to the amplification protocol described in Table 2.

Table 2. Template amplification protocol

Step	Time	Temperature
Hold	2 min	98°C
3-step cycling:		
Denaturation	20 s	98°C
Annealing	45 s	65°C
Extension	3 min 30 s	72°C
Cycle number	4 cycles	
3-step cycling:		
Denaturation	20 s	98°C
Annealing	20 s	67°C
Extension	3 min 30 s	72°C
Cycle number	Varies*	
Hold	∞	4°C

* Number of cycles depends on the results from Protocol: Quantitative Template Amplification. Refer to the *QIAseq UPX 3′ Transcriptome Library Handbook* for details.

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 minutes at room temperature (15–25°C).
6. Place the tube on a magnetic rack for 5 minutes. 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 3 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 minutes.

   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. Determine the concentration of the sample using a Qubit® fluorometer.
14. Proceed with fragmentation, end-repair, and A-addition. Alternatively, store the samples at –30 to –15°C in a constant-temperature freezer.

Fragmentation, end-repair, and A-addition

1. Prepare the reaction on ice according to Table 3.

Table 3. Preparation of fragmentation, end-repair, and A-addition reaction

Component	Volume per reaction
Product from template amplification cleanup (50 ng)	Variable
Fragmentation Buffer, 10x	2.5 µl
FERA Solution	0.75 µl
Nuclease-Free Water	Variable
Fragmentation Enzyme Mix	5 µl
Total volume	**25 µl**

2. Program the thermal cycler according to Table 4. Use the instrument's heated lid.

Table 4. Cycling conditions for fragmentation, end-repair, and A-addition

Step	Incubation temperature	Incubation time
1	4°C	1 min
2	32°C	5 min
3	65°C	30 min
4	4°C	Hold

3. Prior to placing the tubes/plate into the thermal cycler, start the program. When the thermal cycler reaches 4°C, pause the program.

   Important: The thermal cycler must be pre-chilled and paused at 4°C.

4. Transfer the tubes/plate prepared in step 1 to the pre-chilled thermal cycler, and resume the cycling program.
5. Upon completion of the program, allow the thermal cycler to return to 4°C.
6. Place the samples on ice and immediately proceed with "Part 3: Adapter ligation and universal PCR".

Scan QR code for handbook.

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