Quick-Start Protocol October 2017

QIAseq™ Immune Repertoire RNA Library Kit

Part 3: Cleanup of target enrichment, universal PCR, cleanup of universal PCR

Further information

- *QIAseq Immune Repertoire RNA Library Kit Handbook*: **www.qiagen.com/HB-2479**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Prepare the reagents as described in the handbook.
- **Important**: Ensure reactions are thoroughly mixed (7 to 8 times unless otherwise stated), prepared and incubated at recommended temperatures.

Cleanup of target enrichment

1. Transfer the completed target enrichment reaction (from Step 28 of Part 2) into a 1.5 ml LoBind® tube or a 96-well PCR plate.
2. Add 80 µl nuclease-free water to bring each sample to 100 µl.
3. Add 70 µl QIAseq Beads. Mix well by pipetting up and down 10 times.
4. Incubate for 5 min at room temperature.
5. Place the tubes/plate 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.

   Important: Do not discard the beads as they contain the DNA of interest.

6. With the beads still on the magnetic stand, add 200 µl 80% ethanol. Rotate the tube (2 to 3 times) or move the plate side-to-side between the two column positions of the magnet to wash the beads. Carefully remove and discard the wash.
7. Repeat the ethanol wash.

   Important: Completely remove all traces of the ethanol wash after this second wash.
8. With the beads still on the magnetic stand, air dry at room temperature for 10 min.

   Note: Visually inspect that the pellet is completely dry. Ethanol carryover to the next universal PCR step will affect PCR efficiency.
9. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 15.4 µl nuclease-free water. Mix well by pipetting.
10. Return the tube/plate to the magnetic rack until the solution has cleared.
11. Transfer 13.4 µl supernatant to a clean tube or plate.
12. Proceed with "Universal PCR". Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer for up to 3 days.

Universal PCR

13. Prepare the Universal PCR as described in Table 1 or Table 2.

    Important: The IL-N7 Adapter Plate A, B, C or D set used in the adapter ligation reaction must be paired with the matchingIL-S5 Index Primer Plate A, B, C or D set used in the Universal PCR reaction.

    Important: If using QIAseq 96-index I set A, B, C or D, mix components directly in IL-S5 Index Primer Plate A, B, C or D set which contains dried IL-S5 index and Universal PCR primers. See Figure 4 in the handbook for layout of the index primers.

Table 1. Preparation of universal PCR reactions if using QIAseq 12-index I

	1 reaction (µl)
Purified sample (already in tube)	13.4
QIAseq RNA Buffer, 5x	4
IL-Universal Primer	0.8
IL-S5## Index Primer	0.8
HotStarTaq® DNA Polymerase	1
Total	**20**

Table 2. Preparation of Universal PCR reactions if using QIAseq 96-index I Set A, B, C or D*

	1 reaction (µl)
Purified sample	13.4
QIAseq RNA Buffer, 5x	4
HotStarTaq DNA Polymerase	1
Nuclease-free water	1.6
Total	**20**

* Applies to QIAseq IL-S5 Index Primer Plate in A, B, C or D set. The final library dual sample index is determined by the combination of the IL-N7 Adapter Plate and the QIAseq IL-S5 Index Primer Plate. Total sample index level can be up to 384-plex if using QIAseq 96-index A, B, C and D sets together.

14. Program a thermal cycler using the cycling conditions in Table 3.

Table 3. Cycling conditions for universal PCR

Step	Cycles	Incubation temperature	Incubation time
1	1	95°C	15 min
2	27 or 30	95°C 60°C	15 s 2 min
3	1 1	72°C 4°C	5 min Hold

Note: Cycle numbers can be adjusted based on sample type and user experience. Library yield is related to input amount and sample type. It is recommended to use 27 cycles for fresh, high-quality RNA samples with ≥ 100 ng input or 30 cycles for input amounts less than 100 ng.

15. Proceed with "Cleanup of universal PCR". Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer for up to 3 days.

Cleanup of universal PCR

16. Transfer the completed universal PCR reaction into a 1.5 ml LoBind tube or a 96-well PCR plate.
17. Add 80 µl nuclease-free water to bring each sample to 100 µl.
18. Add 70 µl QIAseq Beads. Mix well by pipetting up and down 10 times.
19. Incubate for 5 min at room temperature.
20. Place the tubes/plate 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.
21. With the beads still on the magnetic stand, add 200 µl 80% ethanol. Rotate the tube (2 to 3 times) or move the plate side-to-side between the two column positions of the magnet to wash the beads. Carefully remove and discard the wash.
22. Repeat the ethanol wash. Completely remove all traces of the ethanol wash.
23. With the beads still on the magnetic stand, air dry at room temperature for 10 min.

    Note: Visually inspect that the pellet is completely dry.
24. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 30 µl nuclease-free water. Mix well by pipetting.
25. Return the tubes/plate to the magnetic rack until the solution has cleared.
26. Transfer 28 µl supernatant to a clean tube or plate.
27. The library can be stored at –30 to –15°C for up to 3 months prior to quantification using the QIAseq Library Quant System (QIAGEN cat. no. 333304). Once quantification is performed, proceed with "Protocol: Sequencing Setup on Illumina® MiSeq® and NextSeq®". Refer to the *QIAseq Immune Repertoire RNA Library Kit Handbook* for details.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. Trademarks: QIAGEN®, QIAseq™, Sample to Insight® HotStarTaq® (QIAGEN Group); LoBind® (Eppendorf AG); Illumina®, MiSeq®, NextSeq® (Illumina, Inc.). 1108395 10/2017 HB-2472-001 © 2017 QIAGEN, all rights reserved.