Quick-Start Protocol October 2017

QIAseq™ Immune Repertoire RNA Library Kit

Part 2: Adapter ligation, cleanup of adapter-ligated DNA, target enrichment

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.

Adapter ligation

1. On ice, prepare the adapter ligation reactions as described in Table 1.
   Important: Only one single-indexed adapter should be used per ligation reaction. Each sample will have a different IL-N7## adapter. For 12-index adapter, open one tube at a time. For 96-index adapters supplied in a plate (layout described in the *QIAseq Immune Repertoire RNA Library Kit Handbook*), use a multichannel pipettor.
   Important: Pipet slowly to mix. The reaction mix is very viscous. Do not vortex.

Table 1. Preparation of adapter ligation reactions

	1 reaction (µl)
End-repair and A-addition reaction (from step 16 of Part 1)	50
IL-N7## adapter*	2.5
5x Ligation Buffer	20
DNA Ligase	10
Ligation solution†	15
Nuclease-free water	2.5
Total	**100**

* This component applies to QIAseq IL-N7## adapters with up to a 12 sample index for QIAseq 12-index I or QIAseq 96-index I A, B, C or D set.

† Ligation solution is very viscous. It should be added into each reaction individually and not pre-mixed with other components for a master mix. Do not coat the outside of the pipet tip with ligation solution or excess volume may be added.

2. With the thermal cycler lid open, incubate for 15 min at 20°C.

   Important: Do not use a heated lid.

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

Cleanup of adapter-ligated DNA

4. Transfer the 100 µl adapter ligation product into a 1.5 ml LoBind® tube or a 300 µl 96-well PCR plate.
5. 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/plate on a magnetic rack. After the solution has cleared (10 min for 1.5 ml LoBind tube or ~15 min for 300 µl plate), carefully remove and discard supernatant.

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

8. With the beads still on the magnetic stand, completely remove residual supernatant.

9. 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.
10. Repeat the ethanol wash.

    Important: Completely remove all traces of the ethanol wash after this second wash.
11. 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.
12. Remove the tube from the magnetic stand, and elute the DNA from the beads by adding 52 µl nuclease-free water. Mix well by pipetting.
13. Return the tubes/plate to the magnetic rack until the solution has cleared.
14. Transfer 50 µl of the supernatant to clean tubes/plate.
15. Add 35 µl QIAseq Beads to the 50 µl supernatant. Pipet up and down 10 times to mix.
16. Incubate for 5 min at room temperature.
17. Place the tubes/plate on a magnetic rack for 5 min. Once 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.
18. 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.
19. Repeat the ethanol wash.

    Important: Completely remove all traces of the ethanol wash after this second wash.
20. 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 target enrichment PCR step will affect PCR efficiency.
21. Remove the beads from the magnetic stand, and elute cDNA from beads by adding 12.4 µl nuclease-free water. Mix well by pipetting.
22. Return the tube/plate to the magnetic rack until the solution has cleared.
23. Transfer 10.4 µl supernatant to a clean tube or plate.

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

Target enrichment

25. Prepare the target enrichment reactions as described in Table 2.

Table 2. Preparation of target enrichment reactions

	1 reaction (µl)
Adapter-ligated DNA (from "Cleanup of adapter-ligated DNA")	10.4
QIAseq RNA Buffer, 5x	4
QIAseq TCR panel	4
IL-Forward primer	0.8
HotStarTaq® DNA Polymerase	0.8
Total	**20**

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

Table 3. Cycler setting for SPE target enrichment

Step	Cycles	Incubation temperature	Incubation time
1	1	95°C	15 min
2	8	95°C 68°C	15 s 10 min
3	1 1	72°C 4°C	5 min Hold

27. Place the target enrichment reaction in the thermal cycler and start the run.

28. Proceed with "Cleanup of target enrichment" in Quick-Start Protocol Part 3. Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer for up to 3 days.

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