Quick-Start Protocol November 2015

QIAseq 1-Step Amplicon Library Kit

Upon receipt, store the QIAseq 1-Step Amplicon Library Kit (cat. nos. 180412 and 180415) at –30 to –15°C. This protocol is for the preparation of sequencing libraries for Illumina® NGS platforms from amplicons generated using GeneRead™ DNAseq Targeted Panels V2 or other comparable PCR panels.

Further information

- *QIAseq 1-Step Amplicon Library Kit Handbook*: **www.qiagen.com/HB-2028**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Quantitate DNA using a fluorometric method or with an appropriate microfluidics platform such as QIAxpert® (cat. no. 9002340). Begin with 10–100 ng multiplex PCR product in 5–30 µl nuclease-free water or Buffer EB (QIAGEN).
- Requires Agencourt® AMPure® XP beads (Beckman Coulter, cat. no. A63880 or A63881). Refer to the kit handbook for other equipment and reagents required.

End-repair and adapter ligation in one step

1. Prepare a reaction mix for adapter ligation according to Table 1, adding the components to the PCR tube or plate containing purified gene panel PCR products.

   Note: When using barcoded adapters, open one adapter tube at a time and change gloves between pipetting the different barcode adapters to avoid cross-contamination.
2. Mix components by pipetting up and down several times and incubate in a thermocycler at 25°C for 30 min. **IMPORTANT**: Do not use a thermocycler with a heated lid.

Table 1. Reaction mix for one-step adapter ligation

Component	Volume/reaction (µl)
Multiplex PCR product	Variable, 10–100 ng
1-Step Amplicon Library Buffer, 4x	12.5 µl
QIAseq Adapter (96-plex)*	4 µl
1-Step Amplicon Enzyme Mix	2 µl
DNase-free water	Variable
Total reaction volume	**50 µl**

* If using GeneRead I Adaptor Set A/B (cat. no. 180985/180986), add 2 µl to each reaction. For adapters from another supplier, add adapter to 1 µM final concentration, or according to the supplier's directions.

3. After the reaction is complete, place the reactions on ice and proceed with purification using Agencourt AMPure XP beads.

Reaction cleanup and removal of adapter dimers

4. Transfer the 50 µl ligation reaction from step 3 to a labeled 1.5 ml LoBind tube or 96-well plate. Add 50 µl nuclease-free water and 40 µl (0.4x volume) Agencourt AMPure XP beads to each ligation reaction. **Note**: The addition of water ensures the correct polyethylene glycol (PEG) concentration for bead:DNA binding.
5. Mix well by pipetting up and down several times.
6. Incubate the mixture at room temperature for 5 min to bind DNA to the beads.
7. Place the tube on a magnetic rack and wait until the liquid is clear (about 5 min).
8. Carefully transfer 133 µl supernatant to a new LoBind tube or 96-well plate without disturbing the beads. This will leave behind about 7 µl supernatant. Discard the beads, which contain unwanted large DNA fragments. **Note**: Do not discard the supernatant.
9. Add 40 µl Agencourt AMPure XP beads to the supernatant, mix well and incubate for 5 min at room temperature.
10. Pulse-spin the tube. Pellet the beads on a magnetic stand. Carefully remove and discard supernatant. Do not disturb the beads and bound DNA. **Note**: Do not discard the beads.
11. Keep the tube on the magnetic rack and add 200 µl fresh 80% ethanol to the tube. Rotate the tube to wash the beads, then carefully remove and discard the supernatant.

12. Repeat the previous step once, for a total of 2 ethanol washes.
13. Remove residual ethanol without disturbing the beads. On the magnetic stand, dry beads for 5–10 min while the tube is open. Remove from the magnetic stand.
14. Elute by resuspending in 26 µl nuclease-free water. Mix well by pipetting and incubate at room temperature for 2 min to elute the DNA from the beads.
15. Place the tube back on the magnetic rack to pellet the beads, and collect 23.5 µl of the supernatant for library amplification.

Library amplification

16. Prepare a reaction mix according to Table 2.

Table 2. Reaction mix for library amplification

Component	Volume/reaction (µl)
HiFi PCR Master Mix, 2x	25
Primer Mix	1.5
Library DNA (from previous step)	23.5
Total reaction volume	**50**

17. Program a thermocycler according to Table 3.

Table 3. Cycling conditions

Incubation time	Temperature	Number of cycles
2 min	98°C	1
20 s	98°C	10*
30 s	60°C	
30 s	72°C	
1 min	72°C	1
∞	4°C	Hold

* 10 cycles are sufficient for 10 ng input multiplex PCR products. PCR cycle number can be reduced accordingly if higher amount of input DNA is used.

18. Transfer the PCR plate to the thermocycler and start the program.

19. When the program is complete, add 50 µl of resuspended Agencourt AMPure XP beads to each 50 µl PCR sample. Mix well by pipetting up and down several times.
20. Incubate for 5 min at room temperature.
21. Pellet the beads on a magnetic stand, then carefully remove and discard supernatant.
    Note: Do not discard or disturb the beads.
22. Add 200 µl fresh 80% ethanol to the tube or well while it is on the magnetic rack. Rotate the tube to wash the beads, then carefully remove and discard the supernatant.
23. Repeat the previous step once, for a total of 2 ethanol washes.
24. Remove residual ethanol without disturbing the beads. On the magnetic stand, dry beads for 5–10 min while the tube is open. Remove from the magnetic stand.
25. Elute by resuspending in 30 µl nuclease-free water. Pellet the beads on a magnetic stand. Carefully transfer 28 µl of supernatant into a new LoBind tube or 96-well plate.
26. The library can be stored in a –20°C freezer prior to analysis on the capillary electrophoresis device and quantification using the GeneRead DNAseq Library Quant Array or a comparable method.

    Note: The median size of the library will be the median size of the PCR products plus 120 bp, which is the size of adapters.

Scan QR code for handbook.

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