Quick-Start Protocol April 2016

QIAseq Ultralow Input Library Kits

Store the QIAseq Ultralow Input Library Kit (cat. no. 180495 or 180492) at –30 to –15°C upon receipt. This protocol is for preparation of DNA libraries with minimal input DNA for next-generation sequencing with Illumina® instruments.

Further information

- *QIAseq Ultralow Input Library Handbook:* **www.qiagen.com/handbooks**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Quantitate purified double-stranded DNA fragments using a fluorometric method. Begin with 10 pg – 100 ng DNA in Buffer EB, Nuclease-free H_2O or 10 mM Tris•HCl (pH 8.0).
- Refer to the kit handbook for equipment and reagents required.

End-polishing

Table 1. End-polishing and adapter ligation thermal cycling conditions

Program	Temperature	Time	Additional Comments
End-Polishing	25°C	30 min	Polishing the ends of DNA fragments
	65°C	15 min	Inactivation of end-polishing enzymes; A tailing
	4°C	∞	Hold
Ligation	25°C	10 min	Ligation of the adapters to the DNA fragments
	4°C	∞	Hold

1. Set up the End-polishing Reaction Mix on ice according to Table 2.

Table 2. End-polishing Reaction Setup

Component	Volume/reaction (µl)
Input DNA (fragmented)	variable
End-polishing Buffer 10X	5 µl
End-polishing Enzyme Mix	2 µl
Nuclease-free water	variable
Total reaction volume	**50 µl**

2. Mix by gently pipetting. Do not vortex.
3. Load into the thermal cycler and start the End Polishing program. Proceed to the next step.

Adapter ligation

4. During the end-polishing reaction, vortex and spin down the thawed adapter plate. Remove the protective adapter plate lid, pierce the foil seal for each well to be used and prepare diluted adapter with TE buffer as directed in the kit handbook.
5. When the thermal cycler reaches the 4°C hold, stop the program.
6. Remove reactions from the thermal cycler and add ligation components on ice according to Table 3. Return any unused 96-plex adapter to –20°C.

Table 3. Ligation Reaction Setup

Component	Volume/reaction (µl)
End-polished DNA	50 µl
Ultralow Input Ligation Buffer, 4X	25 µl
Ultralow Input Ligase	5 µl
QIAseq Adapter (96-plex plate) or	2 µl (pre-diluted or undiluted)*
Nuclease-free water	18 µl
Total reaction volume	**100 µl**

*Adaptor dilution depends on input DNA amount: 10–100 ng input: undiluted adaptor; 1–9 ng: 1:10 diluted; 100–999 pg: 1:100 diluted; 10-99 pg: 1:1000 diluted. With user-provided adaptors: see handbook for instructions.

7. Mix adapter ligation reactions by pipetting 5–6 times.
8. Incubate at 25°C for 10min.

   IMPORTANT: Do not use a thermocycler with a heated lid.
9. When the program is complete, proceed immediately to adapter ligation cleanup.
10. Add 80 µl resuspended Agencourt® AMPure® XP beads to each sample and mix.
11. Incubate for 5 min at room temperature.
12. Pellet the beads on a magnetic stand and carefully discard the supernatant.
13. Add 200 µl fresh 80% ethanol to each pellet. Pellet the beads on the magnetic stand and carefully discard the supernatant.
14. Repeat step 13 for a total of 2 ethanol washes. Remove excess ethanol.
15. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Over-drying may result in lower DNA recovery. Remove from the magnetic stand.
16. Elute by resuspending in 52.5 µl Buffer EB. Pellet beads on the magnetic stand. Carefully transfer 50 µl supernatant to a new PCR plate.
17. Add 50 µl of resuspended Agencourt AMPure XP beads to each sample.
18. Follow steps 11-15. Elute by resuspending in 26 µl Buffer EB. Pellet the beads on the magnetic stand. Carefully transfer 23.5 µl of supernatant into a new PCR plate.

Library amplification

19. Program a thermocycler with a heated lid according to Table 4.

Table 4. Library amplification cycling conditions

Incubation time	Temperature	Number of cycles
2 min	98°C	1
20 s	98°C	variable depending on DNA input*
30 s	60°C	
30 s	72°C	
1 min	72°C	1
∞	4°C	Hold

*Number of PCR cycles depends on input DNA amount and quality. Start with 8 cycles for 10 ng input, 10 cycles for 1ng input, 14 cycles for 100 pg input and 16 cycles for 10 pg input.

20. Prepare reactions on ice according to Table 5. Pipet 6–8 times to mix.

Table 5. Amplification reaction setup

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

21. Transfer the PCR plate to the thermocycler and start the program.
22. When the program is complete, remove the plate and add 50 µl resuspended Agencourt AMPure XP beads to each amplified library. Follow steps 11–15 above.
23. Elute by resuspending in 25 µl nuclease-free water or Buffer EB. Pellet the beads on the magnetic stand. Carefully transfer supernatants to new tubes for storage.
24. Assess the library quality using a capillary electrophoresis method. The median library size will be the fragment size plus 120 bp for the adapters. Libraries can be quantified with qPCR using a QIAseq Library Quant Array (sold separately).
25. Purified libraries can be stored at –20°C until ready for sequencing or hybrid capture.

Scan QR code for handbook.

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