QIAseq FX DNA Library Kits

Store the QIAseq FX DNA Library Kit (cat. nos. 180475 and 180473) at –30 to –15°C upon receipt. This protocol is for enzymatic DNA fragmentation and library construction for Illumina® NGS platforms using up to 24 or 96 uniquely barcoded adapters.

Further information

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

Notes before starting

- Quantitate DNA using a fluorometric method. Begin with 1 ng – 1 µg purified DNA in 5–35 µl 10 mM Tris•HCl (pH 8.0), free of cations or chelators (such as EDTA).
- Refer to the kit handbook for equipment and reagents required.

FX single tube fragmentation, end repair and A-addition (60 min)

1. Thaw all reagents, including the adapter, on ice and program a thermocycler with a heated lid according to Table 1.

Table 1. Reaction mix for end-repair FX reaction cycling conditions

Step	Temperature	Incubation time
1	4°C	1 min
2	32°C	3–20 min
3	65°C	30 min
4	4°C	Hold

Note: For >10ng input DNA, 15 min in step 2 produces a fragment distribution around 250 bp. For <10ng input or other fragment sizes, please see the *Handbook*.

2. Start the program. When the thermocycler block reaches 4°C, pause the program.
3. Prepare the FX reaction mix in a PCR plate or tube on ice according to Table 2. For FX reaction setup using <10ng input DNA, include the FX Enhancer as described in the kit handbook. Mix by gently pipetting (do not vortex to mix).

Table 2. FX reaction mix setup (per sample) for >10 ng input DNA

Component	Volume/reaction (µl)
FX Buffer, 10x	5
Purified DNA	Variable
Nuclease-free water	Variable
Total reaction volume	**40**

4. Add 10 µl FX Enzyme Mix to each reaction on ice and mix well by pipetting 6-8 times.
5. Briefly spin down the PCR plate/tubes and immediately transfer to the pre-chilled thermocycler (4°C). Resume the cycling program and when complete, place samples on ice. Immediately proceed with adapter ligation.

Adapter ligation (45 min)

1. Vortex and spin down the thawed adapter plate. Remove the protective adapter plate lid, pierce the foil seal and transfer 5 µl of one DNA adapter well to each sample. Track the barcodes used.
2. Replace the adapter plate lid and freeze unused adapters.
3. Prepare the ligation master mix (per DNA sample) in a separate PCR plate or tube on ice according to Table 3. Mix well by pipetting.
4. Add 45 µl of ligation master mix to each sample and mix well. Incubate ligation reaction at 20°C for 15 min. Do not use thermocycler with a heated lid.
5. Proceed immediately to adapter ligation cleanup.

Table 3. Ligation master mix (per sample)

Component	Volume/reaction (µl)
DNA Ligase Buffer, 5x	20
DNA Ligase	10
Nuclease-free water	15
Total reaction volume	**45**

6. Add 80 µl of resuspended Agencourt® AMPure® XP beads to each sample and mix well by pipetting.
7. Incubate for 5 min at room temperature. Pellet the beads on a magnetic stand for 2 min, then carefully discard the supernatant.
8. Wash the beads by adding 200 µl 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
9. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry. Over-drying may result in lower DNA recovery. Remove from magnetic stand.
10. Elute by resuspending in 52.5 µl Buffer EB. Pellet beads on the magnetic stand. Carefully transfer 50 µl supernatant into a new plate or tube.
11. Add 50 µl of resuspended Agencourt AMPure XP beads to each sample.
12. Repeat steps 7-9. Elute DNA by adding 26 µl Buffer EB. Pellet the beads and carefully collect 23.5 µl of purified DNA for storage or for library amplification.

Library amplification (45 min, recommended for DNA inputs of <100 ng)

1. Program a thermocycler with a heated lid according to Table 4.
2. Prepare a reaction mix on ice according to Table 5 in a PCR tube or 96-well PCR plate.
3. Transfer the PCR tube or plate to the thermocycler and start the program.
4. Once PCR is complete, remove the PCR plate from the thermocycler and add 50 µl of AMPure XP beads to each reaction (50 µl) and pipet up and down thoroughly to mix.

Table 4. Library amplification cycling conditions

Incubation time	Temperature	Number of cycles
2 min	98°C	1
20 s	98°C	6 (100 ng input DNA) 10 (10 ng input DNA) 12 (1 ng input DNA)
30 s	60°C	
30 s	72°C	
1 min	72°C	1
∞	4°C	Hold

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**

5. Follow steps 7-9 from adapter ligation protocol above.
6. Elute by resuspending in 25 µl Buffer EB. Pellet the beads on the magnetic stand. Carefully transfer 23 µl of supernatant into a new tube.
7. Assess the library quality using the QIAxcel, a capillary electrophoresis device or a comparable method. The median library size will be the fragment size plus 120 bp for the adapters. The library can be quantified with qPCR using the QIAseq Library Quant Assay Kit (not provided) or a comparable method.
8. 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.

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