Quick-Start Protocol

July 2023

QIAseq FX DNA Library Preparation – Use of QIAseq beads for library size selection and cleanups

This protocol describes the QIAseq FX library preparation workflow employing QIAseq beads for all size selection and cleanup steps. If using Ampure XP beads for purification, please follow the protocol described in the kit handbook: **QIAGEN QIAseq FX DNA Library Handbook**.
Store the QIAseq FX DNA Library Kit at –30°C to –15°C upon receipt. This protocol is for enzymatic DNA fragmentation and library construction for Illumina® NGS platforms using uniquely barcoded adapters.

Fragmentation, End-Repair, Addition and Adapter Ligation

Important points before starting

- Before setting up the reaction, it is critical to accurately determine the amount of the input DNA.
- Ensure input DNA is in water, 10 mM Tris, QIAGEN's Buffer EB or low TE (0.1x TE, 0.1 mM EDTA). If input DNA is in 1x TE, please set up the FX reaction according to the protocol in Appendix B of the QIAseq FX handbook.

Table 1. Guideline for choosing initial fragmentation time

Fragment peak size	250 bp	350 bp	450 bp	550 bp
Fragmentation time (min) at 32°C				
50 pg –1 ng input DNA*	14	4	1	–
10 ng input DNA†	24	16	14	10
100 ng input DNA	16	10	8	6
1000 ng input DNA	14	8	6	4

Note: The same FX fragmentation time will produce a consistent fragment size within an approximately 5-fold range of input DNA amounts. The exact reaction time may need to be optimized for DNA samples of variable quality.

* For input DNA amounts between 20 and 50 pg, incubate the FX reaction including the FX Enhancer for 25 min to produce a fragment distribution centered around 250 bp.

† For input DNA <10 ng, FX Enhancer is required for optimal performance (Table 4). To produce a fragment distribution centered around 300 bp from 1 ng input, incubate the FX reaction including FX Enhancer for 10 min.

Things to do before starting

- Refer to Table 1 to determine the time required to fragment input DNA to the desired size. If input DNA is less than 10 ng, add FX Enhancer according to the protocol and use half the reaction time listed for 10 ng input DNA. For example, to produce a fragment distribution centered around 300 bp from 1 ng input, incubate the FX reaction including FX Enhancer for 10 min.
- Make fresh 80% ethanol.
- Thaw reagents on ice. Once reagents are thawed, mix buffers thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use.
- Program thermocyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advance.
- Equilibrate QIAseq beads to room temperature (15–25°C) for 20 – 30 min before use.
- Vortex and spin down the thawed adapter plate before use.

Procedure

1. Program a thermocycler according to Table 2 using the predetermined FX fragmentation time for step 2. Be sure to use the instrument's heated lid, and if possible, set the temperature of the heated lid to 70°C.

Table 2. Input DNA (20 pg –1000 ng) free of EDTA, Buffer EB, or in 0.1x TE

Step	Incubation temperature	Incubation time
1	4°C	1 min
2	32°C	1–30 min*
3	65°C	30 min
4	4°C	Hold

* To determine the reaction time for step 2, refer to Table 1.

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 3 for >10 ng input DNA or Table 4 for <10 ng input DNA. Mix well by gently pipetting (do not vortex to mix).

Table 3. FX reaction mix setup (per sample) for >10 ng input DNA

Component	Volume (µL)
FX Buffer, 10x	5
Purified DNA	Variable
Nuclease-free water	Variable
Total without FX Enzyme Mix	40

Table 4. FX reaction mix setup (per sample) for <10 ng input DNA

Component	Volume (µL)
FX Buffer, 10x	5
Purified DNA	Variable
FX Enhancer	2.5
Nuclease-free water	Variable
Total without FX Enzyme Mix	40

4. Add 10 µL FX Enzyme Mix to each reaction and mix well by pipetting up and down 20 times. It is critical to keep the reactions on ice for the entire time during reaction setup.
5. Briefly spin down the PCR plate/tubes and immediately transfer to the prechilled thermocycler (4°C). Resume the cycling program.
6. When the thermocycler program is complete and the sample block has returned to 4°C, remove samples and place them on ice.
7. Immediately proceed with adapter litigation.
8. Remove the protective adapter plate lid, pierce the foil seal for each adapter well to be used, and transfer 5 µL from one DNA adapter well to each 50 µL sample from the previous protocol. Track the barcodes from each adapter well used for each sample.

Note: If your DNA input is <10 ng, dilute the adapters according to Table 5.

Table 5. Adapter dilution factors

Sample DNA amount	Adapter dilution
20–99 pg	1:1000
100–999 pg	1:100
1–9 ng	1:10

9. Replace the adapter plate lid and freeze unused adapters. The adapter plate is stable for a minimum of 10 freeze-thaw cycles.

Important: Only 1 single adapter should be used per ligation reaction. If adapters from another supplier are used, follow the manufacturer's instructions. Do not reuse adapter wells once the foil seal has been pierced.

10. Prepare the ligation Master Mix (per DNA sample, Table 6) in a separate PCR plate or tube on ice, and mix well by pipetting.

Table 6. Ligation master mix setup (per sample)

Component	Volume (µL)
Ligation buffer, 5x	20
DNA ligase	10
Nuclease-free water	15
Total	45

11. Add 45 µL of the ligation Master Mix to each sample, for a total of 100 µL, and mix well by pipetting. Incubate the ligation reaction at 20°C for 15 min.

Important: Do not use a thermocycler with a heated lid.

12. Proceed immediately to adapter ligation cleanup using 0.8x (80 µL) QIAseq beads.
13. Add 80 µL of resuspended QIAseq beads to each ligated sample and mix well by pipetting.
14. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) for 2 min, then carefully discard the supernatant.
15. Wash the beads by adding 200 µL of 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.
16. Incubate the QIAseq beads on the magnetic stand for 10 min. Visually inspect that the pellet is completely dry. Over drying the QIAseq beads will not affect the DNA elution. Remove from the magnetic stand.
17. Elute by resuspending in 52.5 µL of Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 50 µL of supernatant into a new plate or tube.
18. Perform a second purification using 1.1x (55 µL) QIAseq beads following steps 14–16 for DNA binding and washing. Elute DNA by adding 26 µL Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads and carefully collect 23.5 µL of purified DNA sample in a DNA LoBind tube for library amplification. If not proceeding immediately, the sample can be stored at –30°C to –15°C.

Amplification of Library DNA

PCR-based library amplification is normally required if the input DNA amount is below 100 ng or if large amounts of libraries are required for downstream hybrid capture. This protocol is for high-fidelity amplification of the DNA library using the amplification reagents provided in the QIAseq FX DNA Library Kit.

Things to do before starting

- Thaw QIAseq HiFi PCR Master Mix and Primer Mix on ice. Once reagents are thawed, mix them thoroughly by quick vortexing to avoid any localized concentrations.
- Always start with the cycling conditions specified in this protocol. The cycling has been optimized for use with QIAseq HiFi PCR Master Mix for even and high-fidelity amplification of sequencing libraries.
- Equilibrate QIAseq beads to room temperature (15–25°C) for 20–30 min before use.

Procedure

1. Program a thermocycler with a heated lid according to Table 7.

Table 7. Library amplification cycling conditions

Time	Temperature	Number of cycles
2 min	98°C	1
20 s	98°C	
30 s	60°C	6 (100 ng input DNA) 10 (10 ng input DNA) 12 (1 ng input DNA) 14 (100 pg input DNA) 16 (20 pg input DNA)
30 s	72°C	
1 min	72°C	1
∞	4°C	Hold

Note: 6–16 amplification cycles are recommended based on the input DNA amount and quality.

2. Prepare a reaction mix on ice according to Table 8. Mix the components in a PCR tube or 96-well PCR plate.

Table 8. Reaction mix for library enrichment

Component	Volume (µL)
HiFi PCR Master Mix, 2x	25
Primer Mix (10 µM each)	1.5
Library DNA	23.5
Total reaction volume	50

3. Transfer the PCR tube or plate to the thermocycler and start the program.
4. Once PCR is complete, add 60 µL of resuspended QIAseq Beads to each reaction (50 µL) and pipet up and down thoroughly to mix.
5. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.
6. Wash the beads by adding 200 µL of 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.
7. Incubate on the magnetic stand for 10 min. Visually inspect that the pellet is completely dry. Over drying the QIAseq beads will not affect the DNA elution.
8. Elute by resuspending in 25 µL of Buffer EB or 10 mM Tris·Cl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 23 µL of the supernatant into a new tube.

9. Assess the quality of the library using a capillary electrophoresis device such as QIAGEN QIAxcel or Agilent BioAnalyzer. Check for the expected size distribution (see Figure 1) of library fragments and for the absence of an adapters or adapter-dimers peak around 120 bp.

   Note: The library should show a distribution centered around the size of the fragmented DNA plus 120 bp (see Figure 1). The increase in library length reflects the addition of sequencing adapters to the DNA fragments.

   Note: The median fragment size can be used in subsequent qPCR-based quantification methods to calculate library concentration (step 10).

10. Quantify the library using a qPCR-based method such as the QIAseq Library Quant Assay Kit (cat. no. 333314; not provided), or a comparable method.

11. The purified library can be safely stored at –30°C to –15°C in a DNA LoBind tube until ready to use for sequencing or other applications.

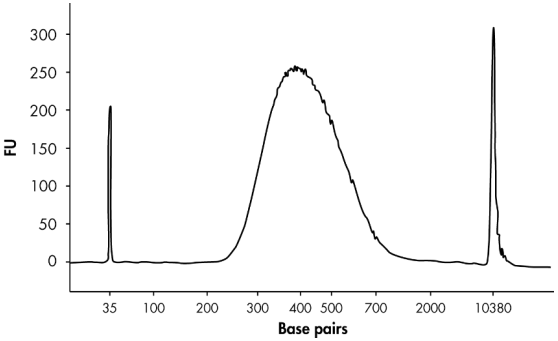

Figure 1. Capillary electrophoresis device trace data.

Document Revision History

Date	Changes
06/2016	Initial release
06/2023	Added alternative protocol using QIAseq beads for cleanup instead of Ampure XP beads.