May 2016

QIAGEN® QIAseq FX DNA Library Kit Handbook

For combined DNA fragmentation and preparation of DNA libraries for next generation sequencing (NGS) applications that use Illumina® instruments

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAseq FX DNA Library Kit (24)		
Catalog no.	**180473**	
Number of reactions	**24**	
Component	**Tube cap color**	**Quantity**
FX Enzyme Mix (24 reactions)	Violet	1 tube
FX Buffer, 10x (24 reactions)	Blue	1 tube
FX Enhancer (24 reactions)	Black	1 tube
DNA Ligase (24 reactions)	Red	1 tube
DNA Ligase Buffer, 5x (24 reactions)	Yellow	1 tube
Adapter Plate 24-plex Illumina	N/A	1 plate
RNase-Free Water (1.9 ml/2)	Clear	2 tubes
HiFi PCR Master Mix, 2x, (0,30/2), KG	Green	2 tubes
Primer Mix Illumina Libr. Amp 12rxn(20µl)	Clear	2 tubes
Quick-Start Protocol	N/A	1

QIAseq FX DNA Library Kit (96)		
Catalog no.	**180475**	
Number of reactions	**96**	
Component	**Tube cap color**	**Quantity**
FX Enzyme Mix (96 reactions)	Violet	1 tube
FX Buffer, 10x (96 reactions)	Blue	1 tube
FX Enhancer (96 reactions)	Black	1 tube
DNA Ligase (96 reactions)	Red	1 tube
DNA Ligase Buffer, 5x (96 reactions)	Yellow	2 tubes
Adapter Plate 96-plex Illumina	N/A	1 plate
RNase-Free Water (1.9 ml/2)	Clear	3 tubes
HiFi PCR Master Mix, 2x (1.25/2 ml),KG	Green	2 tubes

QIAseq FX DNA Library Kit (96)		
Catalog no.	**180475**	
Number of reactions	**96**	
Primer Mix Illumina Library Amp, 10 µM	Clear	1 tube
Quick-Start Protocol	N/A	1

Storage

Store the QIAseq FX DNA Library Kit (cat. nos. 180473 and 180475) at –30 to –15°C upon receipt.

Intended Use

QIAseq FX DNA Library Kits are intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in a convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAseq FX DNA Library Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

Next-generation sequencing (NGS) is a driving force for numerous new and exciting applications, including cancer research, stem cell research, metagenomics, population genetics and medical research. While NGS technology is continuously improving, library preparation remains one of the biggest bottlenecks in the NGS workflow and includes several time-consuming steps that can result in considerable sample loss and the potential to introduce handling errors. QIAGEN's QIAseq FX technology incorporates enzymatic DNA fragmentation into a streamlined, optimized protocol that does not require sample cleanup between fragmentation and adapter ligation, saving time and preventing errors. Optimized enzyme and buffer compositions ensure high sequencing library yield. Streamlined library construction protocols also enable straightforward automation of library prep on different liquid-handling platforms.

Principle and procedure

The QIAGEN QIAseq FX DNA Library Kit provides a fast, fully enzymatic procedure from DNA fragmentation to NGS library with no cleanup steps until after adapters have been ligated to the sample DNA.

Samples consisting of longer DNA fragments, such as genomic DNA or amplicons from long-range PCR, are first enzymatically sheared into smaller fragments. The median fragment size is dependent on the applications and sequencing read length, and can be adjusted by varying the QIAseq FX DNA fragmentation reaction conditions. The fragmented DNA is directly end-repaired and an 'A' is added to the 3' ends during the FX reaction, making the DNA fragments ready for adapter ligation. Following this step, platform-specific adapters are ligated to both ends of the DNA fragments. These adapters contain sequences essential for binding dual-barcoded libraries to a flow cell for sequencing, allowing for PCR amplification of adapter-ligated fragments, and binding standard Illumina sequencing primers.

To ensure maximum yields from limited amounts of starting material, a high-fidelity amplification step can be performed using the reagents included in the QIAseq FX DNA Library Kit. The proprietary HiFi PCR Master Mix can evenly amplify DNA regions with vastly different GC contents, minimizing sequencing bias caused by PCR.

Dual-barcoded, plate-format adapters are included with the QIAseq FX DNA Library Kit. Each well contains a single-use adapter consisting of a unique combination of two eight-nucleotide identification barcodes. By combining one D5 barcode and one D7 barcode in each ready-to-use adapter, QIAseq FX kits support up to 24-plex or 96-plex pooling prior to sequencing (see Appendix C for barcode IDs).

Following library construction, the reaction cleanup and removal of adapter-dimers can be achieved by using Agencourt® AMPure® XP Beads (Beckman Coulter, cat. no. A63880), which enables easy automation on various high throughput automation platforms.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Agencourt® AMPure® XP Beads (cat. no. A63880, A63881) for bead-based library purification
- 100% ethanol (ACS grade)
- Nuclease-free water
- Buffer EB (QIAGEN, cat. no. 19086)
- PCR tubes or plates
- Pipet tips and pipets
- DNA LoBind tubes (from Axygene or Eppendorf)
- Vortexer
- Microcentrifuge
- Thermocycler
- Magnetic racks for magnetic beads separation (e.g., Thermo Fisher Scientific DynaMag™ Magnet)
- Capillary electrophoresis device, e.g. QIAGEN QIAxcel, Agilent® Bioanalyzer or similar to evaluate the DNA fragmentation profile (optional)

Important Notes

General precautions

- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microcentrifuge tubes and pipet tips that are certified sterile, DNase- and RNase-free.
- Before starting, wipe down work area and pipets with an RNase and DNA cleaning product
- For consistent library construction and amplification, ensure the thermocycler used in this protocol is in good working order and has been calibrated within the manufacturer's specifications.
- Read the entire protocol before beginning. Take note of stopping points where samples can be frozen at –20°C and plan your workflow accordingly.
- Enzyme-based DNA fragmentation is sensitive to many factors, such as reaction temperature, time and setup conditions, as well as the quality of the input DNA.

DNA preparation and quality control

High-quality DNA is essential for obtaining reliable sequencing results. The most important prerequisite for any DNA sequence analysis experiment is consistent, high-quality DNA from every experimental sample. Therefore, sample handling and DNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts or other contaminants will degrade the DNA or decrease the efficiency of the enzymatic activities necessary for optimal library preparation.

It is important to remove all cations and chelators from DNA preparations, therefore make sure DNA is eluted in QIAGEN's Buffer EB or H_2O, not 1x TE buffer containing 1mM EDTA. If the DNA was eluted or dissolved in 1x TE, or if you are not certain about the EDTA

concentration in the input DNA, we strongly recommend purifying the input DNA using Agencourt® AMPure® XP beads, following the instructions in Appendix A. Alternatively, we recommend setting up the FX reaction using the FX Enhancer as described in Appendix B.

Recommended genomic DNA preparation method

To prepare purified DNA, we recommend using an appropriate QIAGEN DNA purification kit that supports DNA elution in Buffer EB or 10mM Tris pH 8.0. For the best FX fragmentation performance, do not elute samples in a buffer containing >0.1mM EDTA.

- QIAamp® DNA Mini Kit (cat. no. 51304) for the preparation of genomic DNA samples from fresh tissues and cells
- GeneRead DNA FFPE Kit (cat. no. 180134) for efficient recovery of high-quality gDNA from FFPE tissue
- MagAttract® HMW DNA Kit (cat. no. 67563) for isolation of high-molecular-weight genomic DNA
- QIAamp DNA Microbiome Kit (cat. no. 51704) for isolation of bacterial microbiome DNA from mixed samples

It is critical to accurately determine the input DNA concentration, especially when the input amount is below 100 ng. We recommend using Qubit®, PicoGreen® or another fluorometric method to accurately quantify DNA with a concentration below 1.5 ng/µl.

Protocol: Fragmentation, End-Repair and A-addition

This protocol describes the FX reaction for single-tube fragmentation, end-repair and A-addition.

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, 10mM Tris, QIAGEN's Buffer EB or low TE (0.1X TE, 0.1mM EDTA). If input DNA is in 1x TE, please set up the FX reaction according to the protocol in Appendix B.

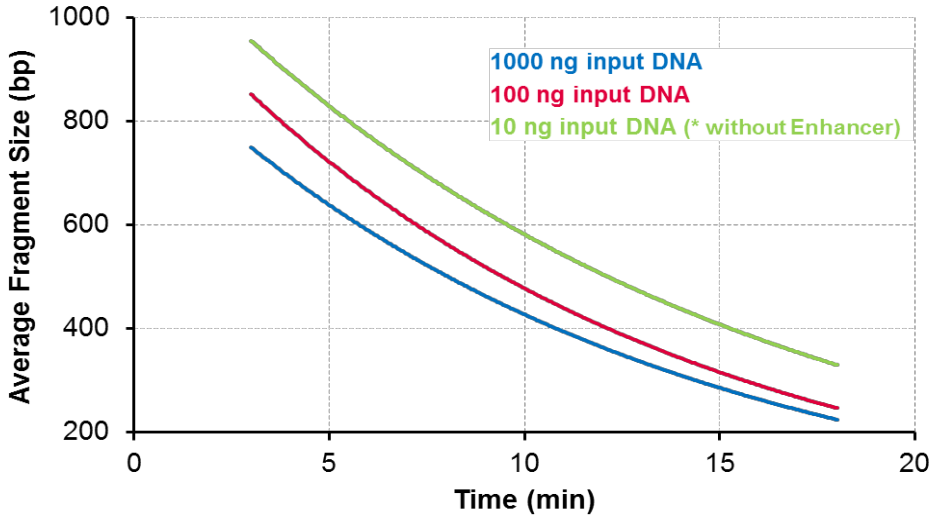

Figure 1. Fragmentation profile of different amounts of input DNA.

Table 1. Guideline for choosing the initial fragmentation time

Fragment peak size	250 bp	350 bp	450 bp	550 bp
	Fragmentation time (min) at 32°C			
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 five-fold range of input DNA amounts. The exact reaction time may need to be optimized for DNA samples of variable quality.

† 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 Figure 1 and 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.

Procedure

1. Program a thermocycler according to Table 2 using the pre-determined 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 (1–1000 ng) free of EDTA, Buffer EB, or in 0.1x TE.

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

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 1–10ng 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 1–10 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 6–8 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 pre-chilled 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 ligation as described in the next protocol.

Protocol: Adapter Ligation

This protocol describes adapter ligation.

Things to do before starting

- Equilibrate Agencourt® AMPure® XP beads to room temperature for 20–30 min before use.
- Vortex and spin down the thawed adapter plate before use.

Procedure

1. 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.
2. Replace the adapter plate lid and freeze unused adapters. The adapter plate is stable for a minimum of 10 freeze-thaw cycles.

   IMPORTANT: Only one single adapter should be used per ligation reaction. If adapters from another supplier are used, follow the manufacturer's instructions. Do not re-use adapter wells once the foil seal has been pierced.
3. Prepare the ligation master mix (per DNA sample) in a separate PCR plate or tube on ice and mix well by pipetting.

Table 5. Ligation master mix setup (per sample)

Component	Volume (µl)
Ligation Buffer, 5x	20
DNA ligase	10
Nuclease-free water	15
Total	**45**

4. 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.

5. Proceed immediately to adapter ligation cleanup using 0.8x (80 µl) Agencourt® AMPure® XP beads.
6. Add 80 µl of resuspended Agencourt® AMPure® XP beads to each ligated sample and mix well by pipetting.
7. 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.
8. 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.
9. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery. Remove from the magnetic stand.
10. Elute by resuspending in 52.5 µl of Buffer EB or 10 mM Tris-HCl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 50 µl of supernatant into a new plate or tube.
11. Perform a second purification using 1x (50 µl) Agencourt® AMPure® XP beads following steps 7–9 for DNA binding and washing. Elute DNA by adding 26 µl Buffer EB or 10 mM Tris-HCl, 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 –20°C.

Protocol: 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.

Procedure

1. Program a thermocycler with a heated lid according to Table 6.

Table 6. 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)
30 s	72°C	
1 min	72°C	1
∞	4°C	Hold

* **Note**: 6–12 amplification cycles are recommended based on the input DNA amount and quality.

2. Prepare a reaction mix on ice according to Table 7. Mix the components in a PCR tube or 96-well PCR plate.

Table 7. 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 50 µl of resuspended Agencourt® AMPure® XP 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 two ethanol washes. Remove as much excess ethanol as possible.
7. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery. Remove from the magnetic stand.
8. Elute by resuspending in 25 µl of Buffer EB or 10 mM Tris-HCl, 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 2) of library fragments and for the absence of an adapters or adapter-dimers peak around 120bp.

   Note: The library should show a distribution centered around the size of the fragmented DNA plus 120 bp (see Figure 2). 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 –20°C in a DNA LoBind tube until ready to use for sequencing or other applications.

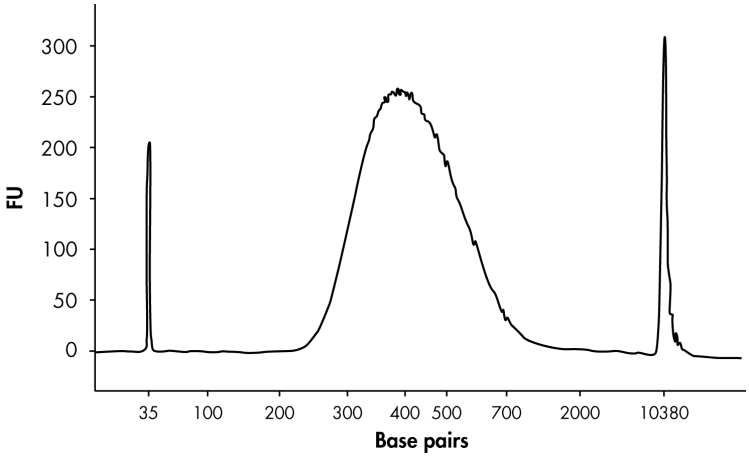

Figure 2. Capillary electrophoresis device trace data.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
Low library yields		
a)	Suboptimal reaction conditions due to low DNA quality	Make sure to use high-quality DNA to ensure optimal activity of the library enzymes.
b)	Insufficient amount of starting DNA for direct sequencing without library amplification	Typically, 100 ng of sheared genomic DNA generates enough Illumina-compatible library to use directly for sequencing without amplification. If the final library yield is not sufficient for the expected number of sequencing runs, a library amplification step can be performed following the adapter ligation step.
c)	Inaccurate quantification of starting DNA due to RNA contamination.	RNA from the sample material can be co-purified with genomic DNA. This contaminating RNA will affect the accuracy of DNA quantification. To remove RNA during the sample preparation protocol, it is recommended to perform RNase A treatment of the DNA.
Unexpected signal peaks in capillary electrophoresis device traces		
a)	Presence of shorter peaks between 60 and 120 bp	These peaks represent library adapters and adapter-dimers that occur when there is no, or insufficient, adapter depletion after library preparation. As adapter-dimers can form clusters on the flow cell and will be sequenced, this will reduce the capacity of the flow cell for the library fragments, even though a low ratio of adapter-dimers versus library will not be a problem. Agencourt® AMPure® XP Beads or GeneRead Size Selection Kit (cat. no. 180514) efficiently remove adapter-dimers, as well as free adapter molecules.
b)	Presence of larger library fragments after library enrichment	If the fragment population shifts higher than expected after adapter ligation and PCR enrichment (e.g., more than the expected 120 bp shift), this can be a PCR artifact due to over-amplification of the DNA library. Make sure to use as few amplification cycles as possible to avoid this effect.

		Comments and suggestions
c)	Incorrect library fragment size after adapter ligation	During library preparation, adapters of approximately 60 bp are ligated to both ends of the DNA library fragments. This should be reflected on a capillary electrophoresis device by a shift in size of all library fragments of 120 bp. If using library adapters from other suppliers, please refer to the size information given in the respective documentation. The absence of a clear size shift may be indicate no, or only low, adapter ligation efficiency. Make sure to use the parameters and incubation times described in the handbook for end-repair, A-addition and ligation, as well as the correct amount of starting DNA.
d)	Incorrect DNA fragment size prior to adapter ligation	The wrong DNA fragment size prior to adapter ligation can be due to the wrong conditions used for enzymatic DNA fragmentation. The reaction time should be optimized for different amount of input DNA. For input DNA >10 ng, we recommend 12 min as a starting point as it produces fragmentation size centers around 300 to 500 bp. Depending on the size requirement and type of input DNA, either increase or decrease reaction time by 2–4 min incrementally until expected size range is achieved.

Appendix A: Removal of Divalent Cations and EDTA from Input Nucleic Acid

Refer to manufacturer's protocol for details on methods of purification

A1. If DNA is in a volume of less than 50 µl, adjust the volume to 50 µl with nuclease-free water.

A2. Add 90 µl of resuspended Agencourt® AMPure® XP beads to the reaction for a ratio of 1.8x and mix well by pipetting. If DNA is in a volume greater than 50 µl, scale the volume of Agencourt® AMPure® XP beads appropriately such that the ratio of beads to DNA is 1.8x.

A3. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand for 2–4 min and carefully discard the supernatant without disturbing the beads.

A4. Wash the beads with 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once for a total of two ethanol washes. Remove as much excess ethanol as possible.

A5. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry. Remove from the magnetic stand.

A6. Elute by resuspending in 45 µl of QIAGEN's Buffer EB or 10 mM Tris-HCl, pH 8.0. Pellet the beads on the magnetic stand for 2 min. Carefully transfer 42.5 µl of supernatant into a new tube.

A7. Determine the concentration of the purified DNA using Qubit, PicoGreen or another fluorometric method.

Appendix B: Fragmentation, End-repair and A-addition of DNA in 1X TE

Follow the instructions below for input DNA in 1X TE buffer.

B1. Enter the following program into a thermocycler (Table 8 below). Make sure to use the instrument's heated lid, and if possible, set the temperature of the heated lid to ~70°C.

Table 8. Input DNA (1–1000 ng) in 1X TE

Step	Incubation temperature	Incubation time
1	4°C	1 min
2	32°C	15–25 min*
3	65°C	30 min
4	4°C	Hold

* To determine the reaction time for step 2, please refer to Figure 1 and Table 1 on pages 11–12.

B2. Prepare the FX reaction mix in a PCR plate on ice according to Table 9 for >10 ng input DNA or Table 10 for 1–10 ng input DNA. Mix well by gently pipetting (do not vortex). The reaction can be scaled as needed for the desired number of samples.

Table 9. Input DNA (10–1000 ng) in 1X TE

Component	Volume (µl)
FX Buffer, 10x	5
DNA in 1X TE	x
FX Enhancer	2.5
Nuclease-free water	(32.5–x)
Total	**40**

Table 10. Input DNA 1–10 ng in 1X TE

Component	Volume (µl))
FX Buffer, 10x	5
DNA in 1x TE	x
FX Enhancer	5
Nuclease-free water	(30–x)
Total	**40**

B3. Add 10 µl FX Enzyme Mix to reach reaction and mix well by pipetting up-and-down 6–8 times. It is critical to keep the PCR tube on ice for the entire time during reaction setup.

B4. Briefly spin down the PCR plate/tubes and immediately transfer to the pre-chilled thermocycler (4°C). Resume the cycling program.

B5. When thermocycler program is complete and sample block has returned to 4°C, remove samples and place on ice.

B6. Immediately proceed to adapter ligation as described in the adapter ligation protocol.

Appendix C: Adapter Barcodes for the QIAseq FX Library Kit 96-plex and 24-plex Adapter Plate

The barcode sequences used in the QIAseq FX DNA Library Kit 96-plex adapter plate are listed in Table 11. Indices 501–508 and 701–712 correspond to the respective Illumina adapter barcodes. The layout of the 96-plex and 24-plex single use adapter plate is displayed in Figure 3 and Figure 4.

Table 11. Adapter barcodes used in the QIAseq FX DNA Library Kit 96-plex Adapter Plate

Codes for entry on sample sheet				
D50X barcode name	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/ 2500)**	**i5 bases for entry on sample sheet (MiniSeq, NextSeq, HiSeq 3000/ 4000)***	**D50X barcode name**	**i7 bases for entry on sample sheet**
D501	TATAGCCT	AGGCTATA	D701	ATTACTCG
D502	ATAGAGGC	GCCTCTAT	D702	TCCGGAGA
D503	CCTATCCT	AGGATAGG	D703	CGCTCATT
D504	GGCTCTGA	TCAGAGCC	D704	GAGATTCC
D505	AGGCGAAG	CTTCGCCT	D705	ATTCAGAA
D506	TAATCTTA	TAAGATTA	D706	GAATTCGT
D507	CAGGACGT	ACGTCCTG	D707	CTGAAGCT
D508	GTACTGAC	GTCAGTAC	D708	TAATGCGC
			D709	CGGCTATG
			D710	TCCGCGAA
			D711	TCTCGCGC
			D712	AGCGATAG

*Note: Sequencing on the MiniSeq, NextSeq, and HiSeq 3000/4000 systems follow a different dual-indexing workflow than other Illumina systems, which requires the reverse complement of the i5 index adapter sequence.

	1	2	3	4	5	6	7	8	9	10	11	12
A	501/701	501/702	501/703	501/704	501/705	501/706	501/707	501/708	501/709	501/710	501/711	501/712
B	502/701	502/702	502/703	502/704	502/705	502/706	502/707	502/708	502/709	502/710	502/711	502/712
C	503/701	503/702	503/703	503/704	503/705	503/706	503/707	503/708	503/709	503/710	503/711	503/712
D	504/701	504/702	504/703	504/704	504/705	504/706	504/707	504/708	504/709	504/710	504/711	504/712
E	505/701	505/702	505/703	505/704	505/705	505/706	505/707	505/708	505/709	505/710	505/711	505/712
F	506/701	506/702	506/703	506/704	506/705	506/706	506/707	506/708	506/709	506/710	506/711	506/712
G	507/701	507/702	507/703	507/704	507/705	507/706	507/707	507/708	507/709	507/710	507/711	507/712
H	508/701	508/702	508/703	508/704	508/705	508/706	508/707	508/708	508/709	508/710	508/711	508/712

Figure 3. QIAseq FX 96-plex Adapter Plate layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	501/701	501/702	501/703	empty	empty	empty	empty	empty	empty	empty	empty	empty
B	502/701	502/702	502/703	empty	empty	empty	empty	empty	empty	empty	empty	empty
C	503/701	503/702	503/703	empty	empty	empty	empty	empty	empty	empty	empty	empty
D	504/701	504/702	504/703	empty	empty	empty	empty	empty	empty	empty	empty	empty
E	505/701	505/702	505/703	empty	empty	empty	empty	empty	empty	empty	empty	empty
F	506/701	506/702	506/703	empty	empty	empty	empty	empty	empty	empty	empty	empty
G	507/701	507/702	507/703	empty	empty	empty	empty	empty	empty	empty	empty	empty
H	508/701	508/702	508/703	empty	empty	empty	empty	empty	empty	empty	empty	empty

Figure 4. QIAseq FX 24-plex Adapter Plate layout.

Appendix D: Column-based Reaction Cleanup with the GeneRead Size Selection Kit.

This protocol is optimized for the removal of primers and adapter dimers from DNA libraries prepared from at least 100ng of DNA using the QIAseq FX DNA Library Kits. This protocol may not remove all adapter dimer from libraries prepared from less than 100ng of DNA.

Notes before starting

- All centrifugation steps should be performed at full speed (maximum 20,000 x *g*) in a conventional, table-top centrifuge.
- Wash steps should be performed using 80% ethanol prepared from 96–100% ethanol.

Procedure

D1. Add 4 volumes of Buffer SB1 to 1 volume of adapter- ligated DNA library prepared using the QIAseq FX DNA Library Kit, and mix. For example, add 360 µl Buffer SB1 to a 90 µl sample.

D2. To bind DNA, apply the sample to the MinElute® spin column and centrifuge for 1 min. Discard the flow-through. Place the MinElute spin column back into the same tube.

D3. To wash, add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min. Discard the flow-through. Place the MinElute spin column back into the same tube.

D4. Repeat step D3.

D5. Centrifuge the MinElute spin column for an additional 1 min at maximum speed.

D6. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).

D7. Add 90 µl Buffer TE to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min.

IMPORTANT: Ensure that the buffer is dispensed directly onto the center of the membrane.

IMPORTANT: Keep the spin column and the flow-through.

D8. Place the MinElute spin column from step D7 into a new 2 ml collection tube (provided). Add 4 volumes of Buffer SB1 (~360 µl) to 1 volume of the flow-through, and mix.

D9. Re-apply the mixture to the MinElute spin column and centrifuge for 1 min. Discard the flow-through.

D10. To wash, add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min. Discard the flow-through. Place the MinElute spin column back into the same tube.

D11. Repeat step D10.

D12. Centrifuge the MinElute spin column for an additional 1 min at maximum speed.

D13. Place the MinElute spin column in a clean 1.5 ml microcentrifuge tube (provided).

D14. For elution, add 17 µl Buffer EB to the center of the membrane, let the MinElute spin column stand for 1 min, and then centrifuge for 1 min.

IMPORTANT: Ensure that the buffer is dispensed directly onto the center of the membrane for complete elution of the bound DNA.

Appendix E: Library Quantification and Quality Control

Quality control for the library construction process can be performed using QIAGEN's QIAseq Library Quant Assay Kit (cat. no. 333314). With this assay, the correct dilution of the library can also be determined for sequencing. Please refer to the corresponding handbook for library quantification and quality control.

Ordering Information

Product	Contents	Cat. no.
QIAseq FX DNA Library Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180475
QIAseq FX DNA Library Kit (24)	For 24 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation and library amplification; for use with Illumina instruments; includes a plate containing 24 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180473
Related products		
QIAseq Library Quantification Kits for use with Illumina instruments		
QIAseq Library Quant Assay Kit	Laboratory-verified forward and reverse primers for 500 x 25 µl reactions (500 µl); DNA Standard (100 µl); Dilution Buffer (30 ml); (1.35 ml x 5) GeneRead qPCR SYBR® Green Mastermix	333314
QIAamp Kits – for genomic DNA purification		
QIAamp DNA Mini Kit	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA Microbiome Kit	For 50 DNA preps: 50 QIAamp UCP Mini Columns, 50 Pathogen Lysis Tubes L, buffers, reagents, Collection Tubes (2 ml)	51704
MagAttract Kits – for high-molecular-weight genomic DNA purification		
MagAttract HMW DNA	For 48 DNA preps: MagAttract Suspension G,	67563

Kit (48)	Buffer ATL, Buffer AL, Buffer MB, Buffer MW1, Buffer PE, Proteinase K, RNase A, Buffer AE, Nuclease-Free Water	
MagAttract Magnetic Rack	Magnetic rack for convenient processing of up to 12 samples	19606

Product	Contents	Cat. no.
REPLI-g® Kits – for MDA-based whole genome amplification		
REPLI-g Mini Kit (100)1	DNA Polymerase, Buffers, and Reagents for 100 x 50 µl whole genome amplification reactions (typical yield 10 µg per reaction)	150025
REPLI-g Single Cell Kit (96)*	REPLI-g sc Polymerase, Buffers, and Reagents for 96 whole genome amplification reactions (yields up to 40 µg/reaction)	150345
REPLI-g Mitochondrial DNA Kit (25)	DNA Polymerase, Buffers, and Reagents for 25 x 50 µl mitochondrial DNA specific whole genome amplification reactions	151023

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

1 Other kit sizes available; **see www.qiagen.com**.

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