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QIAGEN[®] QIAseq FX DNA Library Kit (96) Handbook

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

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Kit Contents

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
Primer Mix Illumina Library Amp, 10 μ M	Clear	1 tube
Quick-Start Protocol	N/A	1

Storage

Store the QIAseq FX DNA Library Kit (96) (cat. no. 180475) at -30 to -15°C upon receipt.

Intended Use

The QIAseq FX DNA Library Kit (96) is 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 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 Kit (96) 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 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 in the same tube following enzymatic shearing, making the DNA fragments ready for ligation. Following this step, platform-specific adapters are ligated to both ends of the DNA fragments. These adapters contain sequences essential for binding up to 96 dual-barcoded libraries to a flow cell for sequencing, binding sequencing primer and allowing for PCR amplification of adapter-ligated fragments.

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 (96). 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 (96). Each adapter well contains a single-use adapter consisting of a unique combination of two eight-nucleotide identification barcodes. By combining one of eight D5 barcodes and one of twelve D7 barcodes in each ready-to-use adapter, this kit supports up to 96-plexing 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 the GeneRead™ Size Selection Kit (cat. no. 180514), which is based on a simple, easy and precise, silica column-based method, **or** by using Agencourt® AMPure® XP Beads (Beckman Coulter, cat. no. A63880).

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 size selection **OR** the GeneRead Size Selection Kit (QIAGEN, cat. no. 180514) for column-based size selection
- 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/Life Technologies, DynaMag™-2 Magnet, cat.no. 12321D)
- Agilent® 2100 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 such as RNase Away® (Molecular BioProducts, Inc. San Diego, CA).
- For consistent library construction and amplification, ensure the thermal cycler used in this protocol is in good working order and has been calibrated to 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, if not completely block, 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. 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

We recommend the following QIAGEN kits:

- 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 the preparation of NGS-ready genomic DNA from FFPE tissue samples
- 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

For accurate DNA quantification, we recommend the QIAxpert® (cat. no. 9002340).

It is critical to determine the input DNA concentration accurately, 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.

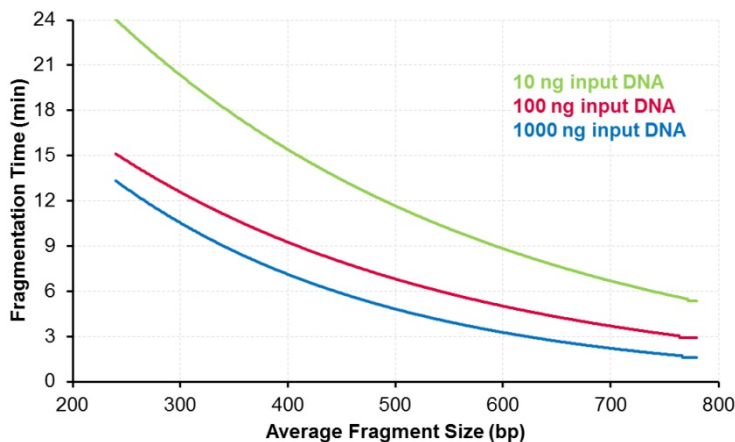


Figure 1. Fragmentation profile of different amounts of input DNA.

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 exact reaction time may need to be optimized for different amounts of input DNA.

[†] For input DNA <10 ng, 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 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 thermal cyclers. 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 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	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	(32.5–x)
Total	40

4. 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.
5. Briefly spin down the PCR plate/tubes and immediately transfer to the pre-chilled thermocycler (4°C). Resume the cycling program.
6. When thermocycler program is complete and sample block has returned to 4°C, remove samples and place 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 and transfer 5 μ l from one DNA adapter well to each 50 μ l sample from the previous protocol. Track the barcodes 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.

3. Prepare the ligation master mix (per DNA sample) in a separate tube on ice and mix well by pipetting.

Table 5. Ligation master mix setup (per sample)

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

4. Add 45 μ l of the ligation master mix to each sample 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 μ l) Agencourt AMPure XP beads.
 6. Add 80 μ l of resuspended Agencourt AMPure XP beads slurry 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 μ l 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.
 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 μ l of Buffer EB or 10 mM Tris-HCl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 50 μ l of supernatant into a new tube.
 11. Perform a second purification using 1x (50 μ l) Agencourt AMPure XP beads. Elute DNA by adding 26 μ l Buffer EB. Pellet beads and carefully collect 23.5 μ l 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. This protocol is for high-fidelity amplification of the DNA library using the reagents provided in the QIAseq FX DNA Library Kit (96).

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	6–12*
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. In general, 6 amplification cycles are sufficient for 100 ng input DNA, 10 cycles are sufficient for 10 ng input DNA and 12 cycles are sufficient for 1 ng input DNA.

2. Prepare a reaction mix on ice according to Table 7.

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

- Transfer the PCR plate to the thermocycler and start the program.
- 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 the beads and PCR mix.
- Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.
- 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 from the magnetic stand.
- 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.
- Elute by resuspending in 20 µl of Buffer EB. Pellet the beads on the magnetic stand. Carefully transfer 17 µl of supernatant into a new tube.
- Assess the quality of the library using a capillary electrophoresis device such as the Agilent BioAnalyzer. Check for the expected size distribution (see Figure 2) of library fragments and for the absence of adapters or adapter-dimers.
Note: The library should show a distribution centred around the size of the fragmented DNA plus 120 bp (see Figure 2). This reflects the addition of sequencing adapter length.
Note: The median fragment size can be used in subsequent qPCR-based quantification methods to calculate library concentration (step 10).
- Quantify the library using the GeneRead Library Quant Kit (cat. no. 180612 [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

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|---|--|
| 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 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 μ l, adjust the volume to 50 μ l with nuclease-free water.
- A2. Add 90 μ l of resuspended Agencourt AMPure XP beads slurry to the reaction for a ratio of 1.8x and mix well by pipetting. If DNA is in a volume greater than 50 μ l, 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 μ l 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.
- 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 μ l 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 μ l of supernatant into a new tube.
- A7. Determine the concentration of the purified DNA using Qubit, PicoGreen or other fluorometric method.

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

Follow in 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 according as described in the next protocol.

Appendix C: Adapter Barcodes for the QIAseq FX Library Kit 96-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 single use adapter plate is displayed in Figure 3.

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	D50X barcode name	i7 bases for entry on sample sheet
D501	TATAGCCT	D701	ATTACTCG
D502	ATAGAGGC	D702	TCCGGAGA
D503	CCTATCCT	D703	CGCTCATT
D504	GGCTCTGA	D704	GAGATTCC
D505	AGGCGAAG	D705	ATTCAGAA
D506	TAATCTTA	D706	GAATTCGT
D507	CAGGACGT	D707	CTGAAGCT
D508	GTACTGAC	D708	TAATGCGC
		D709	CGGCTATG
		D710	TCCGCGAA
		D711	TCTCGCGC
		D712	AGCGATAG

	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.

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

This protocol is optimized for the size selection of sheared DNA in common elution buffers (Buffers EB, TE and AE), and DNA libraries prepared with, for example, the QIAseq FX DNA Library Kit. See the *GeneRead Size Selection Handbook* for additional protocols using other kits.

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.
- Symbols: ▲ size selection of sheared DNA; ■ size selection of DNA libraries prepared with the QIAseq FX DNA Library Kit.

Procedure

- D1. Add 4 volumes of Buffer SB1 to 1 volume of ▲ the sheared DNA sample or ■ adapter-ligated DNA library sample prepared using the QIAseq FX DNA Library Kit, and mix. For example, add 360 μ l Buffer SB1 to a 90 μ l 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 μ l 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 ▲ 17 μ l Buffer EB or ■ 90 μ l 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.
- D8. ▲ Sheared DNA is now size selected and ready for use.
- For the DNA library sample prepared with the QIAseq FX DNA Library Kit, proceed with steps D9–D15.
- IMPORTANT:** Keep the spin column and the flow-through.
- D9. Place the MinElute spin column from step D8 into a new 2 ml collection tube (provided). Add 4 volumes of Buffer SB1 (~360 μ l) to 1 volume of the flow-through, and mix.
- D10. Re-apply the mixture to the MinElute spin column and centrifuge for 1 min. Discard the flow-through.
- D11. To wash, add 700 μ l 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.
- D12. Repeat step D11.
- D13. Centrifuge the MinElute spin column for an additional 1 min at maximum speed.

D14. Place the MinElute spin column in a clean 1.5 ml microcentrifuge tube (provided).

D15. For elution, add 17 μ l 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 centre 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 GeneRead DNaseq Library Quant Array. With this array, 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
Related products		
QIAGEN GeneRead Kits – for next-generation sequencing applications		
GeneRead Size Selection Kit (50)	For 50 reactions: Spin columns and buffers	180514
GeneRead Library Quant 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	180612
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 Kit (48)	For 48 DNA preps: MagAttract Suspension G, Buffer ATL, Buffer AL, Buffer MB, Buffer MW1, Buffer PE, Proteinase K, RNase A, Buffer AE, Nuclease-Free Water	67563
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)*	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

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