July 2016

QIAseq™ Ultralow Input Library Kit Handbook

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

Sample to Insight

QIAGEN

Contents

Kit Contents 4

Shipping and Storage 7

Intended Use 7

Safety Information 7

Quality Control 7

Introduction 8

- Principle and procedure 8
- Automated library construction 11
- Description of protocols 11
- Compatible sequencing platforms 11
- Starting materials 11

Equipment and Reagents to Be Supplied by User 12

Important Notes 13

- DNA preparation and quality control 13

Protocol: End-Polishing and Ultralow Input Ligation 16

Protocol: Amplification of Library DNA 21

Troubleshooting Guide 25

Appendix A: Adapter Indices for the GeneRead Adapter I Set A 12-Plex 28

Appendix B: Adapter Indices for the GeneRead Adapter I Set B 12-Plex 29

Appendix C: Adapter Barcodes for the 96-plex Adapter Plate 30

Appendix D: Adapter Plate Preparation for Ultralow Input Ligation 32

Appendix E: GeneRead Adapter Preparation for Ultralow Input Ligation 35

Ordering Information 37

Kit Contents

QIAseq Ultralow Input Library Kit **Catalog no.** **Number of reactions**		**(12)** **180492*** **12**	**(96)** **180495** **96**
Component	**Tube cap color**	**Quantity in kit**	**Quantity in kit**
End-Polishing Enzyme Mix	Violet	1 tube	1 tube
End-Polishing Buffer, 10X	Blue	1 tube	1 tube
Ultralow Input Ligase	Orange	1 tube	1 tube
Ultralow Input Ligation Buffer, 4X	Yellow	1 tube	3 tubes
HiFi PCR Master Mix, 2x	Green	1 tube	2 tubes
Primer Mix Illumina Libr. Amp	Clear	1 tube	1 tube
RNase-Free Water (1.9 mL)	Clear	1 tube	4 tubes
Adapter Plate 96-plex Illumina®	N/A	N/A	1 plate
Quick-Start Protocol	N/A	1	1

* Adapters are not included in the QIAseq Ultralow Input Library Kit (12) (cat. 180492). The following adapters can be ordered separately to construct NGS libraries in combination with the QIAseq Ultralow Input Library Kit for use with Illumina sequencing platforms.

GeneRead Adapter I Set A 12-plex*	**(144)**
Catalog no.	**180985**
Number of reactions	**144**
Adapter Bc1	33 µl
Adapter Bc2	33 µl
Adapter Bc3	33 µl
Adapter Bc4	33 µl
Adapter Bc5	33 µl
Adapter Bc6	33 µl
Adapter Bc7	33 µl
Adapter Bc8	33 µl
Adapter Bc9	33 µl
Adapter Bc10	33 µl
Adapter Bc11	33 µl
Adapter Bc12	33 µl
Quick-Start Protocol	1

* For barcode sequences, refer to Appendix A.

GeneRead Adapter I Set B 12-plex*	**(144)**
Catalog no.	**180986**
Number of reactions	**144**
Adapter Bc13	33 µl
Adapter Bc14	33 µl
Adapter Bc15	33 µl
Adapter Bc16	33 µl
Adapter Bc17	33 µl
Adapter Bc18	33 µl
Adapter Bc19	33 µl
Adapter Bc20	33 µl
Adapter Bc21	33 µl
Adapter Bc22	33 µl
Adapter Bc23	33 µl
Adapter Bc25	33 µl
Adapter 27	33 µl
Quick-Start Protocol	1

* For barcode sequences, refer to Appendix A.

Shipping and Storage

The QIAseq Ultralow Input Library Kit is shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer.

Intended Use

QIAseq Ultralow Input Library Kits are intended for molecular biology applications. These products are 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 QIAseq Ultralow Input Library Kit 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 a process bottleneck for many labs and a limiting factor in the types of samples that can successfully generate NGS data.

QIAGEN QIAseq Ultralow Input Library Kits have been designed to be the definitive solution for generating high quality libraries from even very challenging NGS samples. Intended for NGS researchers who seek a single library prep kit compatible with a wide range of Ultralow, low and standard input fragmented DNA (including circulating cell free DNA, fragmented DNA, ancient DNA and ChIP DNA), QIAseq Ultralow Input Library Kits enable new insights by maximizing performance particularly from limited and damaged DNA sample types. The streamlined, 2.5-hour protocol for generating libraries from fragmented DNA using QIAseq Ultralow Input Library Kits also enables straightforward automation on different liquid-handling platforms.

Principle and procedure

Starting from enzymatically, chemically, mechanically or naturally fragmented double-stranded DNA, QIAseq Ultralow Input Library Kits use an optimized end-polishing reaction and a new Ultralow Input Ligation formulation along with QIAGEN's proprietary HiFi PCR Master Mix. This combination maximizes the conversion rate of sample DNA into NGS library while efficiently and evenly amplifying even high and low GC content regions of the genome. This protocol enables the highest possible yield sequencing library free of adapter dimer contamination starting from a little as 10–100 pg* of DNA input. Due to the kit's flexible protocol, the same kit can also be used for higher DNA input amounts of up to 100 ng, including PCR-free library preparation from as little as 10 ng DNA input.

Important note

Note that the genomic complexity necessary for a given experiment will vary depending on the genome size of the organism and the fraction of that genome included in the target region of interest. For example, 100 pg of DNA represents approximately 30 unique copies of the human haploid genome, but over 5000 copies of a small genome such as *E. coli*. A conversion rate of 0.3 from sample to library can be applied to these calculations to roughly estimate the maximum genomic complexity of the expected NGS data. For most whole genome sequencing experiments from human or other organisms with haploid genome sizes over ~1 gigabase, QIAGEN recommends starting the QIAseq Ultralow Input protocol from at least 100 pg of input DNA.

Dual-barcoded, plate-format adapters are included with the 96-reaction size QIAseq Ultralow Input Library Kit. Each well in the 96-plex adapter plate 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, the 96-reaction QIAseq Ultralow Input Kits supports up to 96-plex pooling of libraries prior to sequencing (see Appendix C for adapter barcode sequencing information).

While adapters are not included in the 12-reaction size kit, two sets of 12-plex adapters with a single six-nucleotide barcode each can be ordered separately (QIAGEN cat. nos. 180985, 180986).

QIAGEN's QIAseq Ultralow Input Library Kit, with its innovative buffer and enzyme formulations, provides an optimized solution to efficiently construct Illumina libraries from as little as 10 pg input DNA. Following adapter ligation and library amplification steps, reaction cleanup and removal of residual adapter dimers can be achieved by using Agencourt AMPure XP beads, which enable easy automation on various high throughput automation platforms.

QIAseq Ultralow Input Library Preparation Workflow

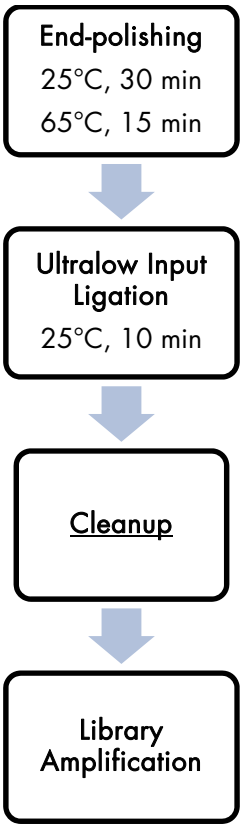

Figure 1. QIAseq Ultralow Input Library Kit Workflow. Starting from enzymatically, chemically, mechanically or naturally fragmented double-stranded DNA, QIAseq Ultralow Input Library Kits use an optimized end-polishing reaction and a new Ultralow Input Ligation formulation along with QIAGEN's proprietary HiFi PCR Master Mix.

Automated library construction

Due to the streamlined protocol and magnetic bead-based purification, QIAseq Ultralow Input library preparation protocols can be easily automated on most commonly used liquid handling platforms.

Description of protocols

This handbook contains two protocols for generation of DNA libraries that are for use on NGS platforms from Illumina. The first protocol (page 16) describes end-polishing, adapter ligation and cleanup of DNA – to generate libraries that are ready to quantify and use in next-generation sequencing. The second protocol (page 21) describes an optional, high-fidelity amplification step that can be used to ensure sufficient library for sequencing from as little as 10 pg of starting material.

Compatible sequencing platforms

- Illumina MiniSeq®
- Illumina MiSeq®
- Illumina NextSeq®
- Illumina HiSeq®

Starting materials

- Fragmented genomic DNA
- Fragmented FFPE DNA
- Circulating cell free DNA (cfDNA)
- Ancient DNA (naturally fragmented)
- DNA from ChIP (Chromatin Immunoprecipitation)
- Fragmented double-stranded cDNA
- Fragmented REPLI-g amplified DNA

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.

- Enzymatic, mechanical (e.g., Covaris® instrument) or other method to fragment high molecular weight gDNA samples
- Agencourt™ AMPure™ XP Beads (Beckman Coulter Inc., 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
- Pipette tips and pipettes
- Vortexer
- Microcentrifuge
- Thermocycler
- Magnetic stand (e.g., DynaMag™-2 Magnet or DynaMag™-96 Side Skirted Magnet, Thermo Fisher, cat. no. 12027)
- QIAGEN QIAxcel, Agilent Bioanalyzer or similar method to assess the quality of DNA library
- qPCR instrument and QIAseq Library Quant Assay Kit (QIAGEN, cat. no. 333314) or a similar method for qPCR-based library quantitation

Important Notes

DNA preparation and quality control

High-quality DNA is always the best choice for obtaining reliable sequencing results. Sample handling and DNA isolation procedures are also critical to the success of the experiment and maximizing the performance of real-world samples. 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.

Recommended 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
- QIAamp DNA Micro Kit (cat. no. 56304) for purification of genomic and mitochondrial DNA from small samples
- QIAamp Circulating Nucleic Acid Kit (cat. no. 55114) for isolation of free-circulating DNA and RNA from human plasma or serum
- 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

Recommendations for DNA fragmentation

DNA can be fragmented using one the following methods:

- Acoustic shearing (e.g., Covaris Adaptive Focused Acoustics™ (AFA) technology)
- Nebulization
- Sonication
- Enzymatic methods

To ensure complete fragmentation of the DNA that is needed for library preparation, follow the recommended parameters given in the manufacturer's instructions. Using too much DNA in a Covaris instrument may, for example, lead to incomplete shearing of the DNA. Check the fragmented DNA for the correct size distribution using an agarose gel or capillary electrophoresis device.

We recommend the QIAxpert System for convenient quantitation of higher-concentration dsDNA samples. For DNA samples at low concentrations or containing both dsDNA and ssDNA – such as FFPE DNA or cfDNA – we recommend Qubit®, PicoGreen® or another fluorometric method to accurately quantitate input DNA.

Recommended library quantification method

We recommend final library quantification by qPCR using primers complementary to the platform-specific adapters. This allows measurement of only complete library molecules, which are the only molecules able to perform bridge PCR on Illumina instruments. QIAGEN's QIAseq Library Quant Array Kit (cat. no. 333304) is highly recommended for accurate qPCR quantification of the prepared library. The QIAseq Library Quant Array Kit is compatible with all major NGS platforms and qPCR instruments, and includes pre-dispensed, sequentially diluted DNA standard to eliminate manual titration steps.

For the most accurate qPCR analysis, the library fragment size distribution should be as narrow as possible, with a known mean value in base pairs. Wide fragment distributions are more difficult to accurately quantitate by qPCR, and the smallest library fragments will be over-represented in NGS data due to their higher efficiency of amplification during bridge PCR.

Important points before starting

- This protocol is for constructing sequencing libraries for Illumina NGS platforms.
- QIAseq and GeneRead adapters are dissolved in duplex buffer and are ready to use.
- Adapters are fully compatible with all Illumina instruments, including MiniSeq, MiSeq, NextSeq and HiSeq instruments. A PCR step is not required to complete the adapter sequences – they are full length and ready for sequencing following the ligation step.
- The protocol can be used with 10 pg–100 ng double-stranded DNA with suitable fragment sizes for Illumina sequencing. The ideal fragment size will depend on the application and read length. Please refer to the recommendations from the sequencer manufacturer.
- The majority of circulating cell free DNA (cfDNA) fragments are approximately 170 bp and do not require further fragmentation prior to library preparation.
- Ancient DNA is normally fragmented and does not require further fragmentation prior to library preparation.
- Do not use a heated lid during the adapter ligation step.

Protocol: End-Polishing and Ultralow Input Ligation

This protocol describes end repair, A-addition, adapter ligation and library cleanup. It generates libraries that are ready to quantify and use in next-generation sequencing on instruments from Illumina.

Things to do before starting

- Fragment high molecular weight DNA before starting this protocol, using either an enzymatic method or a physical method (e.g., DNA with specific median fragment length sizes can be prepared using a Covaris instrument, according to the manufacturer's instructions).
- Sample DNA should be dissolved EB/Tris buffer or H_2O before starting.
- Thaw frozen reagents on ice. Once thawed, buffers should be mixed thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use. Do not vortex enzymes.
- Prepare fresh 80% ethanol

Procedure

1. Program a thermocycler according to Table 1.

Table 1. Thermal cycling parameters

Step	Time	Temperature	Additional comments
End- polishing	30 min	25°C	End repair
	15 min	65°C	Inactivation of end-repair enzymes; A-addition
	∞	4°C	Hold
Ligation	10 min	25°C	Ligation of the adapters to the prepared DNA fragments
	∞	4°C	Hold

2. Prepare the End-Polishing Reaction Mix according to Table 2, adding the components to the PCR tube or plate containing DNA fragments.

   Note: The reaction mix should be prepared on ice.

3. Mix components by pipetting up and down several times and transfer to the thermocycler for end polishing at 25°C followed by incubation at 65°C to inactivate the End-Polishing Enzyme Mix.

Table 2. End-Polishing Reaction Mix setup (per sample) for 10 pg – 100 ng input DNA

Component	Volume/reaction (µl)
100 pg – 100 ng Sample DNA (fragmented)	Variable
End-Polishing Buffer, 10X	5
End-Polishing Enzyme Mix	2
Nuclease-free water	Variable
Total reaction volume	**50**

4. During the end-polishing step, prepare and dilute adapters according to the instructions in Appendix D/E and Table 3:

Table 3. Adapter dilution factors

Sample DNA Amount	QIAseq or GeneRead Adapters (Plate or Set)
10–99 pg	1:1000 dilution
100–999 pg	1:100 dilution
1–9 ng	1:10 dilution
10– 100 ng	No dilution
	See Apppendix D or Appendix E

5. After the end-polishing steps are complete, remove the reaction tubes from the thermocycler and place them on ice.
6. Proceed immediately to Ultralow Input Adapter Ligation.
7. Add components for the adapter ligation reaction to end-polished samples according to Table 4:

Table 4. Adapter ligation setup (per sample) for end-polished DNA

Component	Volume/reaction (µl)
End-Polished DNA	50
Ultralow Input Ligation Buffer, 4X	25
Ultralow Input Ligase	5
QIAseq or GeneRead Adapter, prepared and diluted adapter; See Table 3 and Appendix D	2
DNase-Free Water	18
Total reaction volume	**100**

8. Transfer reactions to the thermocycler to incubate at 25°C for 10 min. Do not use a heated lid.

   Important: Do not attempt to use diluted adapters more than once due to the risk of barcode cross-contamination and lower than expected adapter concentration after storage of very dilute oligos.

9. Once ligation is complete, place the reactions on ice and proceed with purification using Agencout AMPure XP beads.
10. Add 80 µl resuspended Agencourt AMPure XP beads to each sample and mix well by pipetting.
11. Incubate the mixture at 5 min at room temperature.
12. Pellet the beads on a magnetic stand (e.g., DynaMag) for 2 min, then carefully discard the supernatant.
13. Wash the beads by adding 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 as much excess ethanol as possible.
15. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Over-drying of the beads may result in lower DNA recovery. Remove from the magnetic stand.
16. Elute by resuspending in 52.5 µl QIAGEN Buffer EB or 10mM Tris-HCl, pH 8.0. Pellet beads on the magnetic stand. Carefully transfer 50 µl of supernatant to a new plate or tube.
17. Perform a second purification using 1X AMPure beads: Add 50 µl resuspended Agencourt AMPure XP beads to each sample and mix well by pipetting.
18. Repeat steps 11–15. Elute by resuspending in 26 µl QIAGEN Buffer EB or 10 mM Tris-HCl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 23.5 µl of supernatant into a new PCR plate or tube for QC and storage or amplification.
19. Assess the quality of the library using a capillary electrophoresis device or comparable method. Check for the correct size distribution of library fragments and for the absence of adapters or adapter dimers.
20. If the library will not be amplified, store the DNA at –20°C in a DNA Lo-bind tube until ready for sequencing. If amplifying the library, proceed to the protocol "Amplification of Library DNA").

Note: Libraries generated from less than 10 ng input DNA may not be visible by capillary electrophoresis prior to library amplification.

Protocol: Amplification of Library DNA

PCR-based library amplification is normally required if the input DNA amount is below 100 ng for genomic DNA or below 10 ng for cfDNA. This protocol is for optional, high-fidelity amplification of the DNA library using the QIAseq HiFi PCR Master Mix included in the kit.

Things to do before starting

- Prepare library DNA using the protocol "End-Polishing and Ultralow Input Ligation."
- Thaw frozen reagents on ice. Once thawed, buffers should be mixed thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use. Do not vortex enzymes.

Procedure

Table 5. Cycling conditions

Time	Temperature	Number of cycles
2 min	98°C	1
20 s	98°C	
30 s	60°C	Variable
30 s	72°C	
1 min	72°C	1
∞	4°C	Hold

Note: Use the minimum number of cycles necessary to generate sufficient library yield. We suggest a starting point of 16 cycles for libraries created from 10 pg DNA, 14 cycles for 100 pg, 10 cycles for 1 ng input and 8 cycles for 10 ng.

21. Program a thermocycler according to Table 5.
22. Prepare the library amplification reaction mix on ice according to Table 6.

Table 6. Reaction mix for library amplification

Component	Volume/reaction (µl)
HiFi PCR Master Mix, 2x	25
Primer Mix (10 µM each)	1.5
Library DNA (from Step 19)	23.5
Total reaction volume	**50**

23. Transfer the PCR plate to the thermocycler and start the program.
24. When the program is complete, add 50 µl of resuspended Agencourt AMPure XP beads to each 50 µl PCR sample. Mix well by pipetting up and down several times.
25. Incubate the mixture at 5 min at room temperature.
26. Pellet the beads on a magnetic stand (e.g., DynaMag) for 2 min, then carefully discard the supernatant.
27. Wash the beads by adding 200 µl fresh 80% ethanol to each pellet. Pellet the beads on the magnetic stand and carefully discard the supernatant.
28. Repeat step 27 for a total of 2 ethanol washes. Remove as much excess ethanol as possible.
29. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Overdrying of the beads may result in lower DNA recovery. Remove from the magnetic stand.
30. Elute by resuspending in 25 µl nuclease-free water or QIAGEN Buffer EB. Pellet the beads on a magnetic stand. Carefully transfer 23 µl of supernatant into a new LoBind tube.
31. Store libraries at –20°C in a DNA Lo-bind tube until ready for QC, library quantification, and sequencing.

Library QC and quantification

1. Assess the quality of the library using a capillary electrophoresis device or comparable method. Check for the correct size distribution of library fragments and for the absence of adapters or adapter dimers.

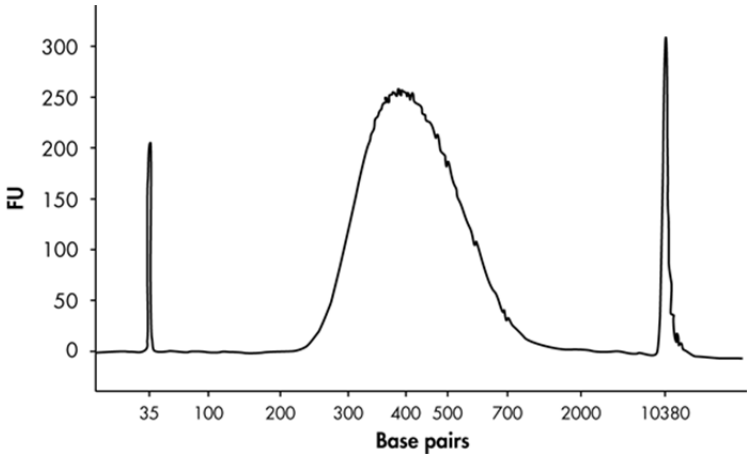

Figure 2. Capillary electrophoresis device trace data showing the correct size distribution of library fragments and the absence of adapters or adapter dimers.

Note: The median size of the DNA fragment should be shifted by the size of the adapters that were ligated to the library fragments (e.g., for QIAGEN adapters, add 120 bp). For PCR-free libraries, be aware that 1) the adaptors contain non-complementary regions prior to PCR amplification, which makes the DNA library migrate slower on the capillary electrophoresis than completely complementary dsDNA, and 2) in addition to insert molecules with adaptors on both ends, the ligation product may also contain unligated DNA fragments as well as DNA fragments ligated with only one adaptor.

Note: The median fragment size can be used for subsequent qPCR-based quantification methods.

2. Quantify the library using the QIAseq Library Quant Array Kit or a comparable qPCR-based method.
3. The purified library can be safely stored at –20°C in DNA LoBind tubes until downstream hybrid capture or sequencing.

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 the highest quality sample DNA available to ensure optimal activity of the library enzymes.
b)	Insufficient amount of starting DNA for direct sequencing without library amplification	Typically, 10 ng of cfDNA or 100 ng of fragmented genomic DNA generates enough Illumina-compatible library to use directly for sequencing without amplification. Make sure to use an accurate DNA quantification method (eg. QIAxpert or a fluorometric method) to ensure sufficient DNA input. If the final library yield is not sufficient for the expected number of sequencing runs or applications (e.g., hybrid capture), a library amplification step can be performed.
c)	Insufficient amount of starting DNA due to inaccurate DNA quantification.	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. For low concentration DNA samples and FFPE samples, use fluorescence-based methods such as PicoGreen measurement to accurately and sensitively quantify double-stranded DNA.
d)	Overdrying of the Ampure beads during cleanup steps.	Overdrying of the Ampure beads can make it difficult to elute the DNA off the beads. Do not dry beads for more than 10 min at room temperature.
e)	Wrong reaction volumes or conditions used for end polishing, ligation or PCR amplification.	Make sure to use the exact conditions in the protocol. Make sure not to use heated-lid for ligation.

		Comments and suggestions
f)	Lower than expected library yield with extensive adaptor dimer.	Excessive adaptor dimers can compete with the amplification of the real libraries during library amplification PCR step and lead to low yield of specific libraries. Make sure the right dilution of the adaptor is used. Make sure the correct AMPure bead purification protocol is used.

		Comments and suggestions
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 Illumina flow cell and will be sequenced, this will reduce the capacity of the flow cell for the library fragments. A low ratio of adapter dimers versus library will not be a problem. Please make sure the correct dilution of the adaptors and the correct volume of the AMPure beads is used for the cleanup steps.
b)	Presence of larger library fragments after library enrichment	If the fragment population shifts higher than expected after adapter ligation and library amplification (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.
c)	Presence of larger library fragments after library enrichment	If the fragment population shifts higher than expected, this can also be due to the carry-over of the AMPure beads. Make sure not to aspirate beads while taking supernatant during the cleanup steps.
d)	Presence of larger library fragments prior to library enrichment.	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.

Appendix A: Adapter Indices for the GeneRead Adapter I Set A 12-Plex

The index sequences used in the GeneRead Adapter I set 12-plex are listed in Table 4. Indexes 1–12 correspond to the respective Illumina adapter indexes.

Table 7. Adapter indices

Adapter name	Indices
Adapter Bc1 Illumina	ATCACG
Adapter Bc2 Illumina	CGATGT
Adapter Bc3 Illumina	TTAGGC
Adapter Bc4 Illumina	TGACCA
Adapter Bc5 Illumina	ACAGTG
Adapter Bc6 Illumina	GCCAAT
Adapter Bc7 Illumina	CAGATC
Adapter Bc8 Illumina	ACTTGA
Adapter Bc9 Illumina	GATCAG
Adapter Bc10 Illumina	TAGCTT
Adapter Bc11 Illumina	GGCTAC
Adapter Bc12 Illumina	CTTGTA

Appendix B: Adapter Indices for the GeneRead Adapter I Set B 12-Plex

The index sequences used in the GeneRead Adapter I set 12-plex B are listed in Table 5. Indices 13–27 correspond to the respective Illumina adapter indices.

Table 8. Adapter indices

Adapter name	Indices
Adapter Bc13 Illumina	AGTCAA
Adapter Bc14 Illumina	AGTTCC
Adapter Bc15 Illumina	ATGTCA
Adapter Bc16 Illumina	CCGTCC
Adapter Bc18 Illumina	GTCCGC
Adapter Bc19 Illumina	GTGAAA
Adapter Bc20 Illumina	GTGGCC
Adapter Bc21 Illumina	GTTTCG
Adapter Bc22 Illumina	CGTACG
Adapter Bc23 Illumina	GAGTGG
Adapter Bc25 Illumina	ACTGAT
Adapter Bc27 Illumina	ATTCCT

Appendix C: Adapter Barcodes for the 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 9. Adapter barcodes used in the QIAseq Ultralow Input Library Kit (96) 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. 96-plex Adapter Plate layout.

Appendix D: Adapter Plate Preparation for Ultralow Input Ligation

Prepare Adapters for Ultralow Input Ligation by thawing and diluting in nuclease-free water prior to Adapter Ligation setup. QIAGEN recommends the following dilution factors to achieve an optimum molar ratio between fragmented sample DNA and adapter oligos:

Table 10. Adapter dilution factors

Sample DNA Amount	QIAseq Adapter Plate Dilution
10–99 pg	1:1000 dilution
100–999 pg	1:100 dilution
1–9 ng	1:10 dilution
10–100 ng	No dilution

Figure 4: QIAseq Adapter Plate 96-plex Illumina (included in cat. no. 180495).

1. Thaw the Adapter Plate on ice. Vortex and spin down thawed adapters before use.
2. Remove the clear protective adapter plate lid and carefully pierce only the foil seal for each adapter well to be used. Use a fresh pipette tip to pierce each well to avoid cross-contamination.
3. Dilute the adapters according to Table 10 to ensure a correct molar ratio with fragmented sample DNA. If necessary, use fresh PCR tubes or 96-well PCR plate for dilutions.
    a. To achieve a 1:10 Adapter Plate dilution:
        i. Add 90 µl nuclease-free water to each adapter well to be used. With a multi-channel pipettor set to 30 µl, pipet up and down 5–6 times to mix. 1:10 diluted adapter is ready for use.
    b. To achieve a 1:100 Adapter Plate dilution:
        i. Add 90 µl nuclease-free water to each adapter well to be used. With a multi-channel pipettor set to 30 µl, pipet up and down 5–6 times to mix.
        ii. Using a multi-channel pipettor, transfer 10 µl 1:10 diluted adapter to a fresh 96-well plate. Carefully track the location of each adapter barcode in the new plate.
        iii. Add 90 µl nuclease-free water to each well in the 96-well PCR plate. With a multi-channel pipettor set to 30 µl, pipet up and down 5–6 times to mix. 1:100 diluted adapter is ready for use.
    c. To achieve a 1:1000 Adapter Plate dilution:
        i. Add 90 µl nuclease-free water to each adapter well to be used. With a multi-channel pipettor set to 30 µl, pipet up and down 5–6 times to mix.
        ii. In a fresh 96-well PCR plate, add 198 µl nuclease-free water.
        iii. Using a multi-channel pipettor, remove 2 µl 1:10 diluted adapter and add to the PCR tubes or wells in the 96-well PCR plate that already

contain 198 µl nuclease-free water. Carefully track the location of each adapter barcode in the new plate.

iv. With a multi-channel pipettor set to 30 µl, pipet up and down 5–6 times to mix. 1:1000 diluted adapter is now ready for use.

4. After adapter ligation, replace the adapter plate lid and freeze unused adapters at –15°C to –35°C. If desired, residual diluted adapter can be removed and discarded before plate storage.
5. Do not attempt to reuse diluted adapter due to the risk of barcode cross-contamination and lower than expected adapter concentration after storage of very dilute oligos.

Appendix E: GeneRead Adapter Preparation for Ultralow Input Ligation

This appendix describes how to prepare the following QIAGEN adapter products for Ultralow Input Ligation:

- GeneRead Adapter I Set A 12-plex (144) cat. no 180985
- GeneRead Adapter I Set B 12-plex (144) cat. no 180986

Adapter preparation includes thawing and diluting in nuclease-free water prior to adapter ligation setup. QIAGEN recommends the following dilution factors to achieve an optimum molar ratio between fragmented sample DNA and adapter oligos:

Table 11. Adapter dilution factors

Sample DNA Amount	GeneRead Adapter I Dilution
10–99 pg	1:1000
100–999 pg	1:100
1–9 ng	1:10
10–100 ng	No dilution

Adapter Preparation using the GeneRead Adapter I Set A (QIAGEN cat. no. 180985) or GeneRead Adapter I Set B (QIAGEN cat. no. 180496)

Note: When using GeneRead Adapters, open one tube at a time and change gloves between pipetting the different barcode adapters to avoid cross-contamination.

1. Thaw the GeneRead Adapter tubes ice. Vortex and spin down thawed adapters before use.
2. Transfer 2 µl of each adapter to be used to a new PCR tube or wells in a 96-well PCR plate. Carefully track the location of each adapter barcode in the new plate.

3. Re-cap each GeneRead Adapter tube immediately after use. Freeze unused adapters at –15°C to –35°C.
4. Dilute the adapters in the PCR tubes or plate according to Table 11 to ensure a correct molar ratio with fragmented sample DNA.

   To achieve a 1:10 GeneRead Adapter dilution:

   i. Add 18 µl nuclease-free water to each adapter tube or well to be used. Pipet up and down 5–6 times to mix. 1:10 diluted adapter is ready for use.

   To achieve a 1:100 GeneRead Adapter dilution:

   i. Add 18 µl nuclease-free water to each adapter tube or well to be used. Pipet up and down 5–6 times to mix.

   ii. Using a multi-channel pipettor, remove and discard 10 µl 1:10 diluted adapter.

   iii. Add 90 µl nuclease-free water to each adapter tube or well to be used. Pipet up and down 5–6 times to mix. 1:100 diluted adapter is ready for use.

   To achieve a 1:1000 GeneRead Adapter dilution:

   i. Add 18 µl nuclease-free water to each adapter well to be used. Pipet up and down 5–6 times to mix.

   ii. Using a multi-channel pipettor, transfer 2 µl 1:10 diluted adapter to a fresh tube or well. Carefully track the location of each adapter barcode in the new plate.

   iii. Add 198 µl nuclease-free water to the 2 µl 1:10 adapter. Pipet up and down 5–6 times to mix. 1:1000 diluted adapter is ready for use.
5. Return to Protocol: Adapter Ligation
6. Discard residual diluted adapter.
7. Do not attempt to reuse diluted adapter due to the risk of barcode cross-contamination and lower than expected adapter concentration after storage of very dilute oligos.

Ordering Information

Product	Contents	Cat. no.
QIAseq Ultralow Input Library Kit (96)	For 96 reactions: Buffers and reagents for Ultralow Input End Polishing, Ultralow Input Ligation and HiFi library amplification. Includes 96-plex Adapter Plate with individually pierceable foil sealed wells. For use with Illumina instruments.	180495
QIAseq Ultralow Input Library Kit (12)	For 12 reactions: Buffers and reagents for Ultralow Input End Polishing, Ultralow Input Ligation and HiFi library amplification. 12-plex adapters sold separately. For use with Illumina instruments.	180492
Related products		
GeneRead Adapter I Set A 12-plex (144)	For 144 reactions: 12 barcoded adapters for ligation to DNA library, for use with Illumina instruments	180985
GeneRead Adapter I Set B 12-plex (144)	For 144 reactions: 12 barcoded adapters for ligation to DNA library, for use with Illumina instruments	180986
GeneRead Size Selection Kit (50)	For 50 reactions: Spin columns and buffers	180514
QIAseq Library Quant Array 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	333304

Product	Contents	Cat. no.
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
GeneRead DNA FFPE Kit	For 50 preps: QIAamp MinElute Columns, Collection Tubes, Deparaffinization Solution, Uracil-N-Glycosylase, RNase-Free Water, RNase A, and Buffers	180134
QIAamp DNA FFPE Tissue Kit	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404
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

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.

Trademarks: QIAGEN®, Sample to Insight®, QIAamp® (QIAGEN Group), QIAseq™, GeneRead™, QuantiNova™, Illumina®, PicoGreen®, Qubit®, SYBR® (Life Technologies Corporation), MiniSeq® (Illumina, Inc.), MiSeq® (Illumina, Inc.), NextSeq® (Illumina, Inc.), HiSeq® (Illumina, Inc.), DynaMag™, Covaris®, Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

1103469 HB-2155-001 07/2016