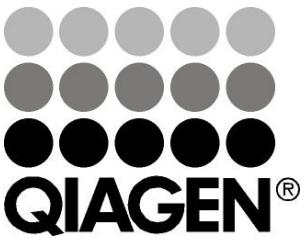
September 2015

QIAGEN® GeneRead™ Library Prep (I) Handbook

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



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

If pooling up to 96 different libraries in the same sequencing run, the following all-in-one library prep kit is required:

GeneRead DNA Library I Kit (96)*	**(96)**
Catalog no.	**180435**
Number of reactions	**96**
T4 DNA Ligase	2 x 204 µl
Klenow Fragment	1 x 192 µl
A-Addition Buffer, 10x	2 x 200 µl
End Repair Buffer, 10x	2 x 200 µl
Ligation Buffer, 2x	3 x 1.7 ml
End Repair Enzyme Mix	2 x 104 µl
RNase-Free Water	1.9 ml
Primer Mix, 10 µM	150 µl
HiFi PCR Master Mix, 2x	2 x 1.25 ml
GeneRead Adapter I 96-plex Plate (96)	1 x 96-well plate
Quick-Start Protocol	1

* For adapter sequences, refer to Appendix C, page 32.

The following modular library prep kits can be used with QIAGEN adapter sets (to be ordered separately) or custom adapters to construct NGS libraries for use with Illumina sequencing platforms:

GeneRead DNA Library I Core Kit	**(12)**	**(48)**
Catalog no.	**180432**	**180434**
Number of reactions	**12**	**48**
T4 DNA Ligase	48 µl	204 µl
Klenow Fragment	36 µl	192 µl
A-Addition Buffer, 10x	50 µl	200 µl
End Repair Buffer, 10x	50 µl	200 µl
Ligation Buffer, 2x	600 µl	2 x 1.7 ml
End Repair Enzyme Mix	24 µl	104 µl
RNase-Free Water	1.9 ml	1.9 ml
Quick-Start Protocol	1	1

GeneRead DNA I Amp Kit	**(100)**
Catalog no.	**180455**
Number of reactions	**100**
Primer Mix, 10 µM	150 µl
HiFi PCR Master Mix, 2x	2 x 1.25 ml
RNase-Free Water	1.9 ml
Quick-Start Protocol	1

The following non-barcoded adapter sets are required for use with modular library prep kits for applications that do not require pooling libraries together in the same sequencing run:

GeneRead Adapter I Set 1-plex	**(12)**
Catalog no.	**180912**
Number of reactions	**12**
Adapter Solution	30 µl
Quick-Start Protocol	1

If pooling up to 12 different libraries in the same sequencing run, one of the following are required. To pool up to 24 different libraries in the same sequencing run, combine both Set A and Set B.

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 adapter sequences, refer to Appendix A, page 30.

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 adapter sequences, refer to Appendix A, page 30.

Shipping and Storage

The GeneRead DNA Library I Core Kit and GeneRead DNA I Amp Kit, GeneRead Adapter I Set 1-plex, GeneRead Adapter I Set A 12-plex and GeneRead Adapter I Set B 12-plex are shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. Store the GeneRead DNA Library I Kit (96) immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. If stored under these conditions, the kits are stable until the date indicated on the QC label inside the kit lid.

Intended Use

GeneRead DNA Library Prep 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 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 GeneRead DNA Library I Core Kit, GeneRead DNA I Amp Kit, GeneRead Adapter I Set 1-plex, GeneRead Adapter I Set A 12-plex, GeneRead Adapter I Set B 12-plex and GeneRead DNA Library I Kit (96) are 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 GeneRead Library Prep Kits use a streamlined, optimized one-tube protocol that does not require sample cleanup between steps, saving time and preventing handling errors. Optimized enzyme and buffer compositions ensure high yield of sequencing library. The GeneRead DNA I Amp Kit is used for an optional, high-fidelity library amplification step. The proprietary HiFi PCR Master Mix can evenly amplify DNA regions with vastly different GC contents, minimizing sequencing bias caused by PCR.

Streamlined GeneRead library construction protocols also enable straightforward automation of library prep on different liquid-handling platforms.

Principle and procedure

QIAGEN GeneRead Library (I) Kits provide a fast, one-tube procedure without intermediate cleanup steps.

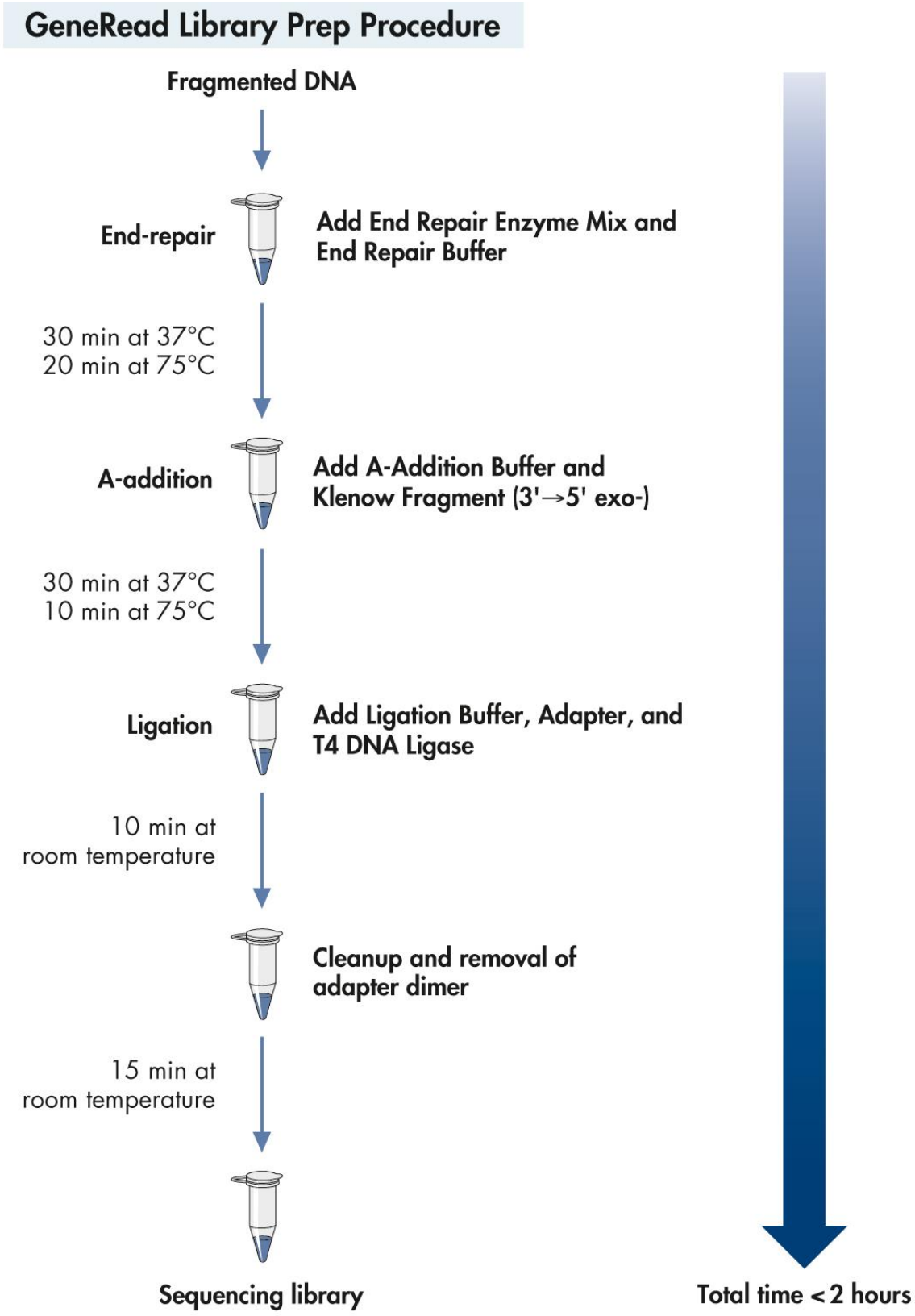
Samples consisting of longer DNA fragments, such as genomic DNA or amplicons from long-range PCR, are first sheared into random fragments. The median fragment size is dependent on the applications and sequencing read length, typically between 150–600 bp. A tight distribution of fragment sizes is recommended for optimal library sequencing performance. Afterwards, platform-specific adapters are ligated to both ends of the DNA fragments. These adapters contain sequences essential for binding library 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, an optional, high-fidelity amplification step can also be performed using the GeneRead Library I Amp Kit (cat. no. 180455) for highly accurate amplification of library DNA with low error rates and minimum bias. High-fidelity amplification reagents are included in the GeneRead DNA Library I Kit (96).

Barcoded adapter sets, which contain proprietary modifications, are also available as ready-to-use solutions at concentrations optimized for a wide range of input DNA amounts — from 50 ng to 1 µg (GeneRead Adapter Sets, cat. nos. 180985, 180986, 180912). Each of these adapters has a unique

identification barcode comprising six nucleotides, allowing samples to be pooled and sequenced in the same run. 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.

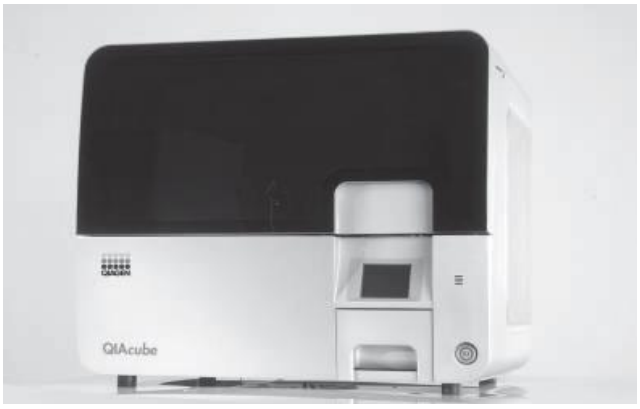
Dual-barcoded, plate-format adapters are included with the GeneRead DNA Library I Kit (96). Each adapter well contains a single-use adapter consisting of a unique combination of two eight-nucleotide identification barcodes. By combining one of eight D5 barcodes and one of twelve D7 barcodes in each ready-to-use adapter, this kit supports up to 96-plexing prior to sequencing (see Appendix C for barcode IDs and sequence information).


GeneRead Library Prep Procedure
Fragmented DNA
End-repair
Add End Repair Enzyme Mix and End Repair Buffer
30 min at 37°C
20 min at 75°C
A-addition
Add A-Addition Buffer and Klenow Fragment (3'→5' exo-)
30 min at 37°C
10 min at 75°C
Ligation
Add Ligation Buffer, Adapter, and T4 DNA Ligase
10 min at room temperature
Cleanup and removal of adapter dimer
15 min at room temperature
Sequencing library
Total time < 2 hours

Automated library construction

Due to the highly streamlined, one-tube protocol and unique column-based size selection to remove adapters and adapter-dimers, GeneRead Library preparation protocols can be automated on the QIAcube®. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated sample prep into your laboratory workflow for up to 12 samples.

Additionally, the QIAcube is preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, plus DNA and RNA cleanup, and serves as an optimal platform to automate pre-analytic NGS sample and library preparations. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at www.qiagen.com/MyQIAcube.



The QIAcube.

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 18) describes end repair, A-addition, adapter ligation and cleanup and size selection of DNA, to generate libraries that are ready to quantify and use in next-generation sequencing. The second protocol (page 24) describes an optional, high-fidelity amplification step that can be used to ensure high amounts of DNA library from minimum amounts of starting material. If performing library construction for Illumina platforms using GeneRead Library Prep Kits, following target enrichment with GeneRead DNAseq Targeted Panels V2, please refer to the *GeneRead DNAseq Targeted Panels V2 Handbook* for additional information.

Compatible sequencing platforms

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

Starting materials

- Genomic DNA, sheared
- REPLI-g® amplified DNA, sheared
- Double-stranded cDNA
- PCR amplicons

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 or physical method (e.g., Covaris® instrument) to shear DNA
- PCR tubes or plates
- Pipet tips and pipets
- Microcentrifuge
- Thermocycler
- GeneRead Size Selection Kit (cat. no. 180514)
- Capillary electrophoresis device or comparable method to assess the quality of DNA library
- GeneRead Library Quant Kit (cat. no. 180612)
- DNA LoBind tubes (from Axygene or Eppendorf)

Important Notes

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.

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
- QIAamp Circulating Nucleic Acids Kit (50) (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 reactions

To ensure complete fragmentation of the DNA that is needed for library preparation, only use 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.

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

Use the GeneRead Size Selection Kit (cat. no. 180514) to clean up the DNA following fragmentation if the desired median fragment size is above 200 bp. Use the QIAquick® PCR Purification Kit to clean up the DNA following fragmentation if the desired median fragment size is below 200 bp.

Handling information for the GeneRead DNA Library I Kit (96) Adapter Plate

The wells of the GeneRead DNA Library I Kit (96) Adapter Plate are sealed with a thin pierceable aluminum foil. This sealing of the single-use library adapter plate minimizes the risk of cross-contamination of different adapters as each well is only used once and opened immediately before use by piercing the foil at that individual position. Each well contains a ready-to-use adapter duplex and the amount of reagent is sufficient for one ligation reaction.

It is recommended to vortex and spin down the library adapter plate prior to use. If only parts of the plate have been used in parallel, the plate can be stored at –20°C until further use. QIAGEN adapters are specially designed to be robust for up to 10 freeze-thaw cycles after the kit has been opened. Prevent accidental damage to the foil seal by replacing the protective plate lid immediately after use.

Recommended library quantification method

QIAGEN's GeneRead Library Quant Kit (cat. no. 180612), which contains laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate qPCR quantification of the prepared library.

Protocol: End Repair, A-Addition, Adapter Ligation, Cleanup and Size Selection of DNA

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

Important points before starting

- This protocol is for constructing sequencing libraries for Illumina NGS platforms. The following QIAGEN products are required for this protocol: GeneRead DNA Library I Kit (96) (cat no 180435) or GeneRead DNA Library I Core Kit (cat. no. 180432) and GeneRead Adapters (cat. nos. 180985 or 180986).
- For reaction cleanup and removal of adapter-dimers following library construction, the GeneRead Size Selection Kit (cat. no. 180514) is required and should be ordered separately.
- Use 50 ng–1 µg of sheared sample gDNA, double-stranded cDNA or 10–200 ng amplicon DNA (e.g., from GeneRead DNAseq Targeted Panels) as input materials for this library prep protocol.
- Median fragment sizes depend on the applications and read length. Please refer to the recommendations from the sequencer manufacturer for suitable fragment size.
- GeneRead Adapters are dissolved in duplex buffer and are ready to use.
- GeneRead Adapters are fully compatible with all Illumina instruments, including MiSeq, NextSeq and HiSeq instruments. The PCR enrichment step is not required to complete the adapter sequences.
- Do not use a thermal cycler with a heated lid during the adapter ligation step.

Things to do before starting

- Fragment sample DNA using either an enzymatic 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).
- The fragmented DNA sample should be in EB/Tris buffer or H_2O before starting.
- Thaw reagents on ice. Once reagents are thawed, mix them thoroughly by quick vortexing to avoid any localized concentrations.

- Program cycles. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved on a thermal cycler. See Table 1 for thermal cycling for each enzymatic step in the library prep procedure.

Table 1. Thermal cycling parameters

Step	Time	Temperature	Additional comments
End repair	30 min	25°C	Polishing the ends of DNA fragments
	20 min	75°C	Inactivation of end repair enzymes
	∞	4°C	Hold
A-addition	30 min	25°C	Adding A to the 3′ of the DNA fragments
	10 min	75°C	Inactivation of A-addition enzymes
	∞	4°C	Hold
Ligation	10 min	25°C	Ligation of the adapters to the DNA fragments
	∞	4°C	Hold

Procedure

End repair of DNA fragments

1. **Prepare a reaction mix for end-repair according to Table 2, dispensing the reagents into a PCR tube or the well of a PCR plate.**

   Note: The reaction mix should be prepared on ice.

Table 2. Reaction mix for end-repair

Component	Volume/reaction (µl)
DNA	Variable
RNase-free water	Variable
End-Repair Buffer, 10x*	2.5
End-Repair Enzyme Mix	2
Total reaction volume	**25**

* Genomic DNA and double-stranded cDNA: 50 ng–1 µg; GeneRead DNAseq Targeted Panel amplicon DNA: 10–200 ng.

2. **Mix thoroughly.**
3. **Program a thermocycler to incubate for 30 min at 25°C, followed by 20 min at 75°C to inactivate the enzyme.**

A-addition

4. **Prepare a reaction mix for A-addition according to Table 3, adding the components to the PCR tube containing the end-repaired DNA from step 3.**

Table 3. Reaction mix for A-addition

Component	Volume/reaction (µl)
End-repaired DNA (from step 3)	25
A-Addition Buffer, 10x	3
Klenow Fragment (3'→5' exo-)	3
Total reaction volume	**31**

5. **Mix thoroughly**
6. **Program a thermocycler to incubate for 30 min at 37°C, followed by 10 min at 75°C to inactivate the enzyme.**

Adapter ligation

7. **Prepare a reaction mix for adapter ligation according to Table 4, adding the components to the PCR tube containing DNA that has undergone end repair and A-addition (step 6).**

   Note: When using GeneRead barcoded adapters in tubes, open one adapter tube at a time and change gloves between pipetting the different barcode adapters to avoid cross-contamination. When using GeneRead barcoded adapters in plate format, only pierce the wells containing the adapters that are to be used. Use the same pipet tip to pierce the foil seal that will be used to transfer the adapter to the reaction tube.

   IMPORTANT: Only one single adapter should be used per ligation reaction. If adapters from another supplier are used, follow the manufacturer's instructions.

Table 4. Reaction setup for adapter ligation

Component	Volume/reaction (µl)	Volume/reaction (µl)
	Adapter tube	GeneRead DNA Library I Kit (96) Adapter Plate
Reaction mix from step 6	31	31
Ligation Buffer, 2x	45	45
GeneRead Adapter	2.5*	5
T4 DNA Ligase	4	4
RNase-free water	Variable	5
Total reaction volume	**90**	**90**

* Alternatively, add the correct amount of adapter according to supplier's directions.

8. **Mix thoroughly.**
9. **Program a thermocycler to incubate for 10 min at 25°C.**

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

Reaction cleanup and removal of adapter-dimers

10. **GeneRead Size Selection Kit (not provided; cat. no. 180514), is required to clean up the ligation reaction and remove adapter-dimers.**

11. **Add 360 µl Buffer SB1 to 90 µl DNA library sample ligation reaction from step 9 and mix thoroughly.**
12. **To bind DNA, apply the mixture to the MinElute® spin column and centrifuge for 1 min.**

    For maximum recovery, transfer all traces of the sample to the column.
13. **Discard the flow-through and place the MinElute spin column back into the same tube.**
14. **To wash, add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.**
15. **Discard the flow-through and place the MinElute spin column back into the same tube.**
16. **Add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.**
17. **Discard flow-through and place the MinElute spin column back into the same tube.**
18. **Centrifuge the MinElute spin column for an additional 1 min.**

    IMPORTANT: Residual ethanol will not be completely removed unless the flow-through is discarded before this additional centrifugation step.
19. **Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).**
20. **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. Keep the spin column and the flow-through.
21. **Place the MinElute spin column into a new 2 ml collection tube (provided). Add 360 µl Buffer SB1 to 90 µl of the flow-through, and mix.**
22. **Re-apply the sample to the same MinElute spin column and centrifuge for 1 min.**

    For maximum recovery, transfer all traces of the sample to the column.
23. **Discard the flow-through and place the MinElute spin column back into the same tube.**
24. **To wash, add 700 µl 80% ethanol to the MinElute spin column and centrifuge for 1 min.**
25. **Discard the flow-through and place the MinElute spin column back into the same tube.**
26. **Add 700 µl 80% ethanol to the MinElute spin column and centrifuge for 1 min.**

27. **Discard the flow-through and place the MinElute spin column back into the same tube.**
28. **Centrifuge the MinElute spin column for an additional 1 min.**

    IMPORTANT: Residual ethanol will not be completely removed unless the flow-through is discarded before this additional centrifugation step.
29. **Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).**
30. **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 bound DNA.
31. **Assess the quality of the library using a capillary electrophoresis device or comparable method. Check for the correct size distribution (see Figure 1, page 25) of library fragments and for 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 the GeneRead Adapter I Set 1-plex or the GeneRead Adapter I Set 12-plex, add 120 bp).

    Note: The median fragment size can be used for subsequent qPCR-based quantification methods (step 32).
32. **Quantify the library using the GeneRead Library Quant Kit (cat. no. 180612 [not provided]), or a comparable qPCR-based method.**

    Note: If the library will not be amplified, store the DNA at –20°C until ready to use for sequencing. If amplifying the library, proceed to the protocol "Optional Amplification of Library DNA", page 24).
33. **The purified library can be safely stored at –20°C until further applications or amplifications. DNA LoBind tubes are recommended for long-term storage.**

Protocol: Optional Amplification of Library DNA

PCR-based library amplification is normally required if the input DNA amount is below 500 ng. This protocol is for optional, high-fidelity amplification of the DNA library using the GeneRead DNA I Amp Kit (cat. no. 180455) or the amplification reagents included in the GeneRead DNA Library I Kit (96) (cat. no. 180435).

Important points before starting

- For reaction cleanup and removal of adapter-dimers following library construction, the GeneRead Size Selection Kit (cat. no. 180514) is required and should be ordered separately.

Things to do before starting

- Prepare library DNA using the protocol "End Repair, A-Addition, Adapter Ligation and Cleanup and Size Selection of DNA", page 18.
- Thaw all reagents on ice. Once reagents are thawed, mix them thoroughly by quick vortexing to avoid any localized concentrations.
- For PCR reaction cleanup and removal of primer-dimers following library construction, the GeneRead Size Selection Kit (cat. no. 180514) is required and should be ordered separately.

Procedure

1. Prepare a reaction mix according to Table 5.

Table 5. Reaction mix for library enrichment

Component	Volume/reaction (µl)
HiFi PCR Master Mix, 2x	25
Primer Mix (10 µM each)	1.5
Library DNA (from step 33, page 22)	15
RNase-free water	8.5
Total reaction volume	**50**

2. Program a thermocycler according to Table 6.

Table 6. Cycling conditions

Time	Temperature	Number of cycles
2 min	98°C	1
20 s	98°C	
30 s	60°C	5–10*
30 s	72°C	
1 min	72°C	1
∞	4°C	Hold

* We recommend 5–10 amplification cycles depending on the DNA input amount and quality. Generally, 8 amplification cycles are sufficient for >50 ng input DNA.

3. **Once PCR is finished, clean up the PCR reaction and remove any residual adapter-dimers with the GeneRead Size Selection Kit (cat. no. 180514) following the instructions below.**
4. **Add 200 µl Buffer SB1 to 50 µl of the DNA library sample prepared at step 2, and mix.**
5. **To bind DNA, apply the mixture to the MinElute spin column and centrifuge for 1 min.**

   For maximum recovery, transfer all traces of the sample to the column.
6. **Discard the flow-through and place the MinElute spin column back into the same tube.**
7. **To wash, add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.**
8. **Discard the flow-through and place the MinElute spin column back into the same tube.**
9. **Add 700 µl of 80% ethanol to the MinElute spin column and centrifuge for 1 min.**
10. **Discard flow-through and place the MinElute spin column back into the same tube.**
11. **Centrifuge the MinElute spin column for an additional 1 min.**

    IMPORTANT: Residual ethanol will not be completely removed unless the flow-through is discarded before this additional centrifugation step.
12. **Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube (provided).**

13. **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 bound DNA.

14. **Assess the quality of the library using a capillary electrophoresis device or comparable method. Check for the correct size distribution (see Figure 1) of library fragments and for 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 the GeneRead Adapter I Sets or the GeneRead Adapter I Set 12-plex, add 120 bp).

    Note: The median fragment size can be used in subsequent qPCR-based quantification methods to calculate library concentration (step 15).

15. **Quantify the library using the GeneRead Library Quant Kit (cat. no. 180612 [not provided]), or a comparable method.**

    Note: The library DNA can be stored at –20°C until ready to use for sequencing.

16. **The purified library can be safely stored at –20°C in a DNA LoBind tube until further applications.**

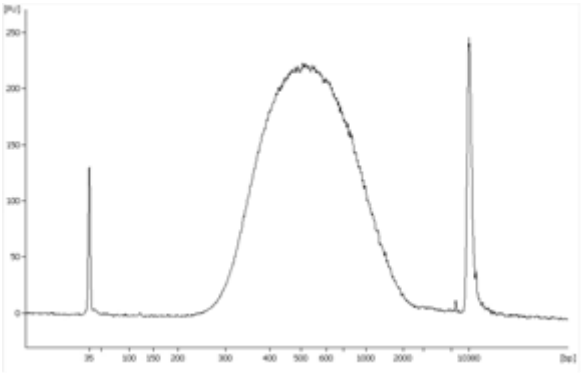


Figure 1. Capillary electrophoresis device trace data showing the correct size distribution of 400 bp fragments ligated to GeneRead 120 bp adapters. Note that the library is free of a ~120 bp peak indicating residual adapter-dimers.

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 protocols in this handbook or sample and assay technologies (for contact information, see back cover or 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, 500 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 cleanup with the GeneRead Size Selection Kit (cat. no. 180514).
c) Insufficient amount of starting DNA for direct sequencing without library amplification	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.

	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 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. The GeneRead Size Selection Kit (cat. no. 180514) efficiently removes 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 (8–10) to avoid this effect.
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.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

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

The barcode sequences used in the GeneRead Adapter I Set 12-plex are listed in Table 7. Barcodes 1–12 correspond to the respective Illumina adapter barcodes.

Table 7. Adapter barcodes

Adapter name	Barcode
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 Barcodes for the GeneRead Adapter I Set B 12-Plex

The barcode sequences used in the GeneRead Adapter I Set 12-plex B are listed in Table 8. Barcodes 13–27 correspond to the respective Illumina adapter barcodes.

Table 8. Adapter barcodes

Adapter name	Barcode
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 GeneRead DNA Library I Kit (96) Adapter Plate

The barcode sequences used in the GeneRead DNA Library I Kit (96) Adapter Plate are listed in Table 9. Barcodes 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 2.

Table 9. Adapter barcodes used in the GeneRead DNA Library I Kit (96) Adapter Plate

D501–D508 adapters			
AATGATACGGCGACCACCGAGATCTACAC[**D50X**]ACACTCTTTCCCTACACGACGCTCTTCCGATCT			
D701–D712 adapters			
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC[**D70X**]ATCTCGTATGCCGTCTTCTGCTTG			
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 2. GeneRead DNA Library I Kit (96) Adapter Plate layout.

Appendix D: Library Quantification and Quality Control

Quality control for the targeted enrichment and 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.
GeneRead DNA Library I Kit (96)	For 96 reactions: Buffers and reagents for 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)	180435
GeneRead DNA Library I Core Kit (12)	For 12 reactions: Buffers and reagents for end-repair, A-addition and ligation, for use with Illumina instruments	180432
GeneRead DNA Library I Core Kit (48)	For 48 reactions: Buffers and reagents for end-repair, A-addition and ligation, for use with Illumina instruments	180434
GeneRead DNA I Amp Kit (100)	For 100 reactions: Buffers and reagents for library amplification, for use with Illumina instruments	180455
GeneRead Adapter I Set 1-plex (12)	For 12 reactions: Adapters for ligation to DNA library, for use with Illumina instruments	180912
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

Product	Contents	Cat. no.
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
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
QIAamp Circulating Nucleic Acid Kit (50)	For 50 preps: QIAamp Mini Columns, Tube Extenders (20 ml), QIAGEN Proteinase K, Carrier RNA, Buffers, VacConnectors, and Collection Tubes (1.5 ml and 2 ml)	55114

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.

Limited License Agreement for GeneRead Library Prep (I) Kits

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.qiagen.com.

www.qiagen.com

Australia ■ techservice-au@qiagen.com

Austria ■ techservice-at@qiagen.com

Belgium ■ techservice-bnl@qiagen.com

Brazil ■ suportetecnico.brasil@qiagen.com

Canada ■ techservice-ca@qiagen.com

China ■ techservice-cn@qiagen.com

Denmark ■ techservice-nordic@qiagen.com

Finland ■ techservice-nordic@qiagen.com

France ■ techservice-fr@qiagen.com

Germany ■ techservice-de@qiagen.com

Hong Kong ■ techservice-hk@qiagen.com

India ■ techservice-india@qiagen.com

Ireland ■ techservice-uk@qiagen.com

Italy ■ techservice-it@qiagen.com

Japan ■ techservice-jp@qiagen.com

Korea (South) ■ techservice-kr@qiagen.com

Luxembourg ■ techservice-bnl@qiagen.com

Mexico ■ techservice-mx@qiagen.com

The Netherlands ■ techservice-bnl@qiagen.com

Norway ■ techservice-nordic@qiagen.com

Singapore ■ techservice-sg@qiagen.com

Sweden ■ techservice-nordic@qiagen.com

Switzerland ■ techservice-ch@qiagen.com

UK ■ techservice-uk@qiagen.com

USA ■ techservice-us@qiagen.com

1096182 09/2015 HB-1501-006

Sample & Assay Technologies