December 2014

QIAGEN GeneRead™ Library Prep (L) Handbook

For preparation of DNA libraries for next-generation sequencing (NGS) applications that use Ion Torrent™ instruments from Life Technologies®

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QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

GeneRead™ DNA Library L Core Kit	**(12)**
Catalog no.	**180462**
Number of reactions	**12**
End Repair Buffer, 10x	50 µl
Ligation Buffer, 2x	600 µl
End Repair Enzyme Mix	24 µl
Ligation and Nick Repair Mix	48 µl
dNTP Mix (10 mM)	55 µl
RNase-free Water	1.9 ml
Quick Start Protocol	1

GeneRead DNA L Amp Kit	**(100)**
Catalog no.	**180485**
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

GeneRead DNA Adapter L Set 1-plex	**(12)**
Catalog no.	**180922**
Number of reactions	**12**
Adapter, 50 µM	24 µl
Quick Start Protocol	1

GeneRead DNA Adapter L Set 12-plex*	**(72)**
Catalog no.	**180994**
Number of reactions	**72**
Adapter BcGen, 25 µM	144 µl
Adapter Bc1, 25 µM	12 µl
Adapter Bc2, 25 µM	12 µl
Adapter Bc3, 25 µM	12 µl
Adapter Bc4, 25 µM	12 µl
Adapter Bc5, 25 µM	12 µl
Adapter Bc6, 25 µM	12 µl
Adapter Bc7, 25 µM	12 µl
Adapter Bc8, 25 µM	12 µl
Adapter Bc9, 25 µM	12 µl
Adapter Bc10, 25 µM	12 µl
Adapter Bc11, 25 µM	12 µl
Adapter Bc12, 25 µM	12 µl
Quick Start Protocol	1

* For adapter sequences, refer to Appendix A, page 18.

Shipping and Storage

The GeneRead DNA Library L Core Kit and GeneRead DNA L Amp Kit are shipped on dry ice and should be stored immediately upon receipt at –15 to –30°C in a constant-temperature freezer. The GeneRead Adapter L Set 1-plex and the GeneRead Adapter L Set 12-plex are shipped at ambient temperature and should be stored immediately upon receipt at –15 to –30°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.

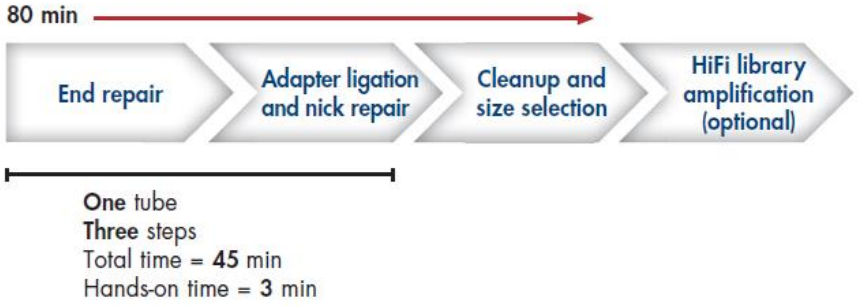
Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of GeneRead DNA Library L Core Kit, GeneRead DNA L Amp Kit, GeneRead Adapter L Set 1-plex, and GeneRead Adapter L Set 12-plex are tested against predetermined specifications to ensure consistent product quality.

Introduction

Next-generation sequencing (NGS) is a driving force for numerous applications, including cancer research, stem cell research, metagenomics, population genetics, and medical research. While NGS technology is continuously improving, library preparation is 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 each step, saving time and preventing handling errors as well as loss of valuable samples. The efficient procedure includes an optional, high-fidelity amplification step to ensure high yields of DNA library that are reproducibly generated with minimal sequence bias and low error rates.



Principle and procedure

QIAGEN GeneRead Library Prep (L) Kits use a fast, one-tube procedure with fewer cleanup steps than library preparation workflows from other suppliers and an optional, high-fidelity library amplification step.

Samples consisting of longer DNA fragments are first sheared into a random library of fragments that are a median fragment size of 400 bp (when using the Ion Torrent PGM instrument), or 200 bp (when using the Ion Proton™ instrument), in length. Following fragmentation, the ends of the DNA fragments are repaired and adaptors, which are necessary for amplification and sequencing, are ligated to both ends of the DNA fragments. Barcode adapters, which contain a unique identifying sequence, are also available with the GeneRead Library Prep (L) Kit and enable multiplex sequencing reactions to be performed. The fragments are then size selected and purified. To ensure maximum yields from minimum amounts of starting material, an optional, high-fidelity amplification step can also be performed that provides highly accurate amplification of library DNA with low error rates and minimum bias.

Description of protocols

This handbook contains 2 protocols for generation of DNA libraries that are for use on instruments from Life Technologies. The first protocol (page 10) describes end repair, 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 14) describes an optional, high-fidelity amplification step that can be used to ensure high amounts of DNA library from minimum amounts of starting material.

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., sonication) to shear DNA
- PCR tubes or plates
- Pipette tips and pipettes
- Microcentrifuge
- Thermocycler
- MinElute® PCR Purification Kit (cat. no. 28004)
- Agilent® Bioanalyzer® or a comparable method to assess the quality of DNA library
- Optional: GeneRead Library Quantification Kit (cat. no. 180612)
- Optional: GelPilot® 50 bp Ladder (cat. no. 239025) or GelPilot 100 bp Plus Ladder (cat. no. 239045)

Important Notes

DNA preparation and quality control

High-quality DNA is essential for obtaining good 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 block completely, the enzyme activities necessary for optimal library preparation.

Recommended genomic DNA preparation method

The QIAamp® DNA Mini Kit (cat. no. 51304) and QIAamp DNA FFPE Tissue Kit (cat. no. 56404) are highly recommended for the preparation of genomic DNA samples from fresh tissues and FFPE tissue samples. Ensure that samples have been treated for the removal of RNA, as RNA contamination will cause inaccuracies in DNA concentration measurements. Do not omit the recommended RNase treatment step to remove RNA.

Recommendations for DNA fragmentation

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 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 Agilent Bioanalyzer.

Recommended library quantification method

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

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

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

Important points before starting

- The median fragment size of DNA should be compatible with the read-length of the sequencing platform you are using (e.g., when using the Life Technologies Ion Torrent PGM instrument, use a median fragment length of 400 bp. When using the Ion Proton instrument, use a median fragment length of 200 bp). Specific median fragment length sizes of DNA can be prepared using a Covaris instrument, according to the manufacturer's instructions.
- GeneRead Adapter L Set 1-plex, as well as the GeneRead Adapter L Set 12-plex, are dissolved in duplex buffer (30 mM Hepes, pH 7.5, 100 mM Potassium Acetate). The adapters are pre-annealed and are provided ready-to-use.
- GeneRead Adapter L Set 1-plex contains both adapter duplices mixed together in one tube at a concentration of 50 µM for each adapter duplex. GeneRead Adapter L Set 12-plex contains the universal adapter BcGen pre-annealed and ready to use, as well as the barcode adapters 1–12 in 13 separate tubes at a concentration of 25 µM.
- The library adapters are fully compatible with Life Technologies instruments, such as the Ion Torrent PGM or the Ion Proton, and do not require nick translation during the enrichment step.

Things to do before starting

- Shear 100 ng – 1 µg DNA using either an enzymatic method or a physical method (e.g., sonication).

Procedure

End repair of DNA

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

   Note: The reaction mix should be prepared on ice.

Table 1. Reaction mix for end-repair

Component	Volume/reaction (µl)
DNA (100 ng – 1 µg sheared DNA)	Variable
RNase-free water	Variable
End-Repair Buffer, 10x*	2.5
End-Repair Enzyme Mix	2
Total reaction volume	**25**

* Contains dNTPs.

2. **Mix thoroughly.**
3. **Program a thermocycler to incubate for 20 min at 25°C, followed by 10 min at 70°C.**

Adapter ligation

4. **Prepare a reaction mix for adapter ligation according to Table 2, adding the components to the PCR tube containing the end-repaired DNA (step 3).**

- **Note**: When using barcode adapters, open one adapter tube at a time and change gloves between pipetting the different barcode adapters to avoid cross-contamination.
- **IMPORTANT**: Only one of the 12 adapters (Adapter Bc1–Bc12) should be used per ligation reaction, in combination with the universal adapter BcGen.

Table 2. Reaction setup for adapter ligation

Component	Singleplex adapter mix Volume/reaction	Multiplex adapter mix Volume/reaction (µl)
End-repaired DNA (from step 3)	25	25
Ligation Buffer, 2x	40	40
Adapter mix (singleplex)	Variable (0.5 µM)*	–
Universal Adapter BcGen	–	Variable (0.5 µM)*
Barcode Adapter 1–12	–	Variable (0.5 µM)*
Ligation and Nick Repair Mix	4	4
dNTP Mix (10 mM)	1	1
RNase-free water	Variable	Variable
Total volume	**80**	**80**

* Use 0.5 µM final concentration of GeneRead Adapter I Set 1-plex or GeneRead Adapter L Set 12-plex. Alternatively, add the correct amount of adapter according to supplier's directions.

5. **Mix thoroughly**
6. **Program a thermocycler to incubate for 10 min at 25°C, followed by 5 min at 72°C.**

   IMPORTANT: Do not use a thermocycler with a heated lid.
7. **Purify adapter-ligated library fragments. For libraries with a median fragment size below 200 bp, use the MinElute PCR Purification Kit (not supplied; cat. no. 28004). For libraries with a median size of >200 bp, QIAGEN's GeneRead Size Selection Kit (not supplied; cat. no. 180514) can be used.**

Fine size selection

8. **For DNA that was sheared to a median size of 150 bp, select adapter-ligated DNA in the 210–250 bp range for 100 bp read lengths or select adapter-ligated DNA in the range of 280–320 bp for 200 bp read lengths. Yields may vary depending on the size selection method used. Size selection can be performed using a**

standard 2% agarose gel or alternative, gel-based separation methods.

Note: Ensure that the library is sufficiently well separated to allow selection of an exact fragment size. Use a DNA ladder with size markers between 50 bp and 500 bp (e.g., GelPilot 50 bp Ladder; cat. no. 239025) or between100 bp and 1500 bp for larger fragment sizes (e.g., GelPilot 100 bp Plus Ladder, cat. no. 239045).

IMPORTANT: When handling multiple libraries in parallel, avoid cross contamination during gel excision by using a new scalpel for each sample.

9. Isolate the DNA from the gel using the MinElute Gel Extraction Kit (cat. no. 28604).

Note: Dissolve the gel at room temperature, as this will result in higher library yields.

Note: Following isolation, purified DNA can be stored at –15 to –30°C.

10a. If sequencing the library directly (i.e., without further amplification), proceed directly to step 10.

10b. If amplifying the library prior to sequencing, proceed to step 1 of the protocol "Optional Amplification of Library DNA", page 14).

- **a. Clean up the amplified DNA using the MinElute PCR Purification Kit (not provided; cat. no. 28004).**
- **b. Assess the quality of the library using an Agilent Bioanalyzer or a comparable method. Check for the correct size distribution (see Figure 1, page 15) of library fragments and for the absence of free library adapters.**

Note: The median fragment size can be used for subsequent qPCR-based quantification methods (step 12).

11. Quantify the library using the GeneRead Library Quantification Kit (not provided; cat. no. 180612), or a comparable method.

Note: Store the DNA at –15 to –30°C until ready to sequence.

Protocol: Optional Amplification of Library DNA

This protocol is for optional, high-fidelity amplification of the DNA library.

Important points before starting

- The Primer Mix for library enrichment (see Table 3) is provided as a ready-to-use premix with a final concentration of 10 µM.

Things to do before starting

- Prepare library DNA using the protocol "End Repair, Adapter Ligation, and Cleanup and Size Selection of DNA", page 10.

Procedure

1. Prepare a reaction mix according to Table 3.

Table 3. 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 10b, page 14)	Variable
RNase-free water	Variable
Total reaction volume	**50**

2. Program a thermocycler according to Table 4.

Table 4. Cycling conditions

Time	Temperature	Number of cycles
2 min	98°C	1
20 s	98°C	
30 s	60°C	8–10*
30 s	72°C	
1 min	72°C	1

* We recommend using 8–10 amplification cycles. Additional cycles may be required to ensure robust performance. However, too many cycles should be avoided to prevent over-amplification.

3. **Clean up the amplified DNA using the MinElute PCR Purification Kit (not provided; cat. no. 28004).**
4. **Assess the quality of the library using an Agilent Bioanalyzer or a comparable method. Check for the correct size distribution (Figure 1) of library fragments and for the absence of free library adapters.**

   Note: The median fragment size can be used for subsequent qPCR-based quantification methods (step 5).
5. **Quantify the library using the GeneRead Library Quantification Kit (cat. no. 180612; not provided), or a comparable method.**

   Note: Store the library DNA at –15 to –30°C until ready to sequence.

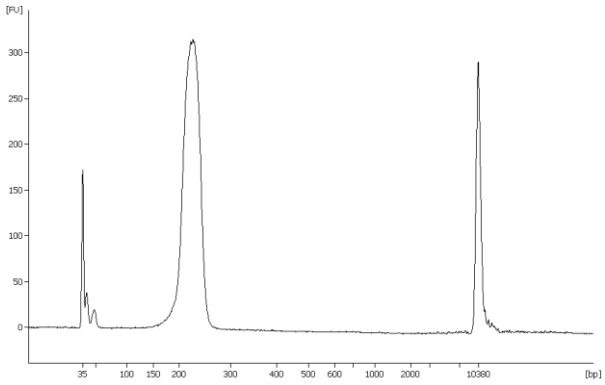


Figure 1. Agilent trace data showing the correct size distribution of library fragments and the absence of adapters or adapter-dimers.

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, 800–1000 ng of genomic DNA generates enough Ion-Torrent-compatible library to use the library 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 fragment size selection.
c) Insufficient amount of starting DNA	RNA from the sample material is 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 Agilent Bioanalyzer traces	
a) Library fragments are of unexpected size after gel size selection	Make sure to excise the recommended size ranges for subsequent emulsion PCR and sequencing. Fragments that are too large may negatively influence the efficiency of the downstream procedures.

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: Barcode Sequences for the GeneRead Adapter L Set 12-Plex

The barcode sequences used in the GeneRead Adapter L set 12-plex are listed in Table 5. Barcodes 1–12 correspond to the respective Ion Torrent adapter barcodes.

Table 5. Barcode adapter name

Barcode adapter name	Barcode sequence
Adapter Bc1 Ion Torrent	CTAAGGTAAC
Adapter Bc2 Ion Torrent	TAAGGAGAAC
Adapter Bc3 Ion Torrent	AAGAGGATTC
Adapter Bc4 Ion Torrent	TACCAAGATC
Adapter Bc5 Ion Torrent	CAGAAGGAAC
Adapter Bc6 Ion Torrent	CTGCAAGTTC
Adapter Bc7 Ion Torrent	TTCGTGATTC
Adapter Bc8 Ion Torrent	TTCCGATAAC
Adapter Bc9 Ion Torrent	TGAGCGGAAC
Adapter Bc10 Ion Torrent	CTGACCGAAC
Adapter Bc11 Ion Torrent	TCCTCGAATC
Adapter Bc12 Ion Torrent	TAGGTGGTTC

Ordering Information

Product	Contents	Cat. no.
GeneRead DNA Library L Core Kit (12)	For 12 reactions: Buffers and reagents for end-repair, ligation, and nick repair, for use with Ion Torrent Instruments from Life Technologies	180462
GeneRead DNA L Amp Kit (100)	For 100 reactions: Buffers and reagents for library amplification, for use Ion Torrent Instruments from Life Technologies	180485
GeneRead Adapter I Set 1-plex (12)	For 12 reactions: Adapters for ligation to DNA library, for use Ion Torrent Instruments from Life Technologies	180922
GeneRead Adapter L Set 12-plex (72)	For 72 reactions: 12 barcoded adapters for ligation to DNA library, for use with Illumina instruments	180994
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 FFPE Tissue Kit	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404

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.

Notes

Limited License Agreement for QIAGEN GeneRead Library Prep (I) Kits

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

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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

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