Quick-Start Protocol March 2016

GeneRead™ DNA Library Prep L Kit

The GeneRead DNA Library L Core Kit (cat. no. 180462), GeneRead DNA Library L Amp Kit (cat. no. 180485) and the GeneRead Adapter L Set 1-plex (cat. no. 180922) or 12-plex (cat. no. 180994) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. The DNA library prepared using this protocol is for use in next-generation sequencing using instruments from Life Technologies.

Further information

- *GeneRead DNA Library Prep L Handbook*: **www.qiagen.com/HB-1502**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Shear 100 ng – 1 µg DNA using an enzymatic physical method.
- The median fragment size of DNA should be compatible with the read-length of the sequencing platform you are using (e.g., with the Life Technologies® Ion Torrent® PGM instrument, use a median fragment length of 400 bp. With 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.
- GeneRead Adapter L Set 1-plex is 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, as well as the barcode adapters 1–12 in 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.
- The Primer Mix for library enrichment (see Table 3) is provided as a ready-to-use premix with a final concentration of 10 µM.

- **IMPORTANT**: 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.

End-repair

1. Prepare a reaction mix for end-repair according to Table 1, dispensing the reagents into a PCR tube or 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 from step 3.
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, the GeneRead Size Selection Kit (not supplied; cat. no. 180514) can be used.

Table 2. Reaction setup for adaptor ligation

Component	Singleplex adapter mix Volume/reaction (µl)	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 reaction volume	**80**	**80**

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

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 –20°C.

10. If sequencing the library directly (i.e., without further amplification), proceed directly to step 14. If amplifying the library prior to sequencing, proceed to step 11.

11. To amplify the library, 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 8)	Variable
RNase-free water	Variable
Total reaction volume	**50**

12. 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.

13. Clean up the amplified DNA using the MinElute PCR Purification Kit (not provided; cat. no. 28004).
14. Assess the quality of the library using an Agilent® Bioanalyzer or comparable method. Check for the correct size distribution 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 15).
15. Quantify the library using the GeneRead Library Quantification Kit (not provided; cat. no. 180612), or a comparable method.

    Note: Store the DNA at –20°C until ready to sequence.

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