Quick-Start Protocol April 2016

GeneRead™ DNA Library Prep I Kit

Store the GeneRead DNA Library I Core Kit, GeneRead DNA I Amp Kit and GeneRead Adapters immediately upon receipt at –30 to –15°C in a constant-temperature freezer. If stored under these conditions, kits are stable until the date indicated on the QC label inside the kit lid.

Further information

- *GeneRead DNA Library Prep I Handbook*: **www.qiagen.com/HB-1501**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes 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 Core Kit (cat. no. 180432 or180434), GeneRead DNA I Amp Kit (cat. no. 180455), GeneRead Adapters (cat. nos. 180985, 180986, 180984, 180912).
- For reaction cleanup and removal of adaptor dimers following library construction, the GeneRead Size Selection Kit (cat. no. 180514) is required and should be ordered separately.
- Shear 10 ng – 1 µg DNA using either an enzymatic method or a physical method (e.g., sonication).
- Median fragment sizes depend on the applications and read length.
- GeneRead Adapters are dissolved in duplex buffer and ready to use.
- GeneRead Adapters are fully compatible with Illumina instruments, such as MiSeq®, NextSeq® or HiSeq® instruments. The enrichment step is not required to complete the adapter sequences.
- The Primer Mix for library enrichment (see Table 4) is provided as a ready-to-use premix with a concentration of 10 µM for each primer.

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*	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; gene panel amplicons: 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 2, adding the components to the PCR tube containing the end-repaired DNA from step 3.

Table 2. 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 3, adding the components to the PCR tube containing DNA that has undergone end-repair and A-addition (step 6).

   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 single adapter should be used per ligation reaction. If adaptors from another supplier are used, follow the manufacturer's instructions.

Table 3. Reaction setup for adaptor ligation

Component	Volume/reaction (µl)
DNA from step 6 (has undergone end-repair and A-addition)	31
Ligation Buffer, 2x	45
GeneRead Adapter	2.5*
T4 DNA Ligase	4
RNase-free water	Variable
Total reaction volume	**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 adaptor dimers

10. If sequencing the library directly (i.e., without further amplification), follow step 10a. If amplifying the library prior to sequencing, follow step 10b.

    10a. Clean up DNA from step 9 using the GeneRead Size Selection Kit (not provided; cat. no. 180514), then proceed to step 11.

    Note: Following purification, the DNA can be stored at –20°C.

    10b. If amplifying the library prior to sequencing, clean up the DNA from step 9 using the GeneRead Size Selection Kit (not provided; cat. no. 180514), and proceed to step 13.

11. 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 Set 12-plex, add 120 bp).

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

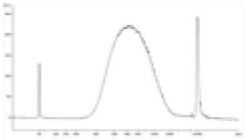

Figure 1. Agilent® trace data showing the correct size distribution of library fragments and the absence of adapters or adapter dimers.

12. Quantify the library using the GeneRead Library Quantification Kit (cat. no. 180612 [not provided]), or a comparable method.
13. To amplify the library, prepare a reaction mix according to Table 4.

Table 4. 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)	Variable
RNase-free water	Variable
Total reaction volume	**50**

14. Program a thermocycler according to Table 5.

Table 5. 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

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

15. Clean up the amplified DNA using the GeneRead Size Selection Kit (not provided; cat. no. 180514).
16. Assess the quality and quantity of the library as outlined in steps 11 and 12. Store the adapter-ligated library at –20°C until ready to use for sequencing.

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