Quick-Start Protocol July 2015

GeneRead™ DNA Library I Kit (96)

Store the GeneRead DNA Library I Kit (96) immediately upon receipt at –20°C. If stored under these conditions, kits are stable until the date indicated on the QC label inside the kit lid.

Further information

- *QIAGEN GeneRead Library Prep (I) Handbook*: **www.qiagen.com/handbooks**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000, or **www.qiagen.com/contact**

Notes before starting

- This protocol is for constructing barcoded sequencing libraries for Illumina® NGS platforms with up to 96 individual barcodes.
- For information on additional kits that are needed, see the kit handbook.
- Vortex and spin down the library GeneRead Adapter I 96-plex Plate prior to use.

	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 1. GeneRead Adapter I 96-plex Plate layout, combination of dual barcodes.

For adapter sequences to be entered on the sample sheet, please refer to Appendix C of the *QIAGEN GeneRead Library Prep (I) Handbook*.

End repair

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

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. Program a thermocycler to incubate for 30 min at 25°C, followed by 20 min at 75°C to inactivate the enzymes.

A-addition

3. Prepare a reaction mix for A-addition according to Table 2, by adding the components to the PCR tube containing the end-repaired DNA from step 2 and mix thoroughly.

Table 2. Reaction mix for A-addition

Component	Volume/reaction (µl)
End-repaired DNA (from step 2)	25
A-Addition Buffer, 10x	3
Klenow Fragment (3′→5′ exo–)	3
Total reaction volume	**31**

4. Program a thermocycler to incubate for 30 min at 37°C, followed by 10 min at 75°C to inactivate the enzyme.

Adapter ligation

5. 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 4) and mix thoroughly. **Note**: Make sure to use each well of the adapter plate only once to avoid cross-contamination. Pierce the protective foil of the individual wells using the pipet tip that is also used for transferring the adapter solution. Adapter

volumes to be transferred are optimized for multi-channel pipetting. **IMPORTANT**: Only a single adapter should be used per ligation reaction.

Table 3. Reaction setup for adapter ligation

Component	Volume/reaction (µl)
DNA from step 4 (has undergone end-repair and A-addition)	31
Ligation Buffer, 2x	45
GeneRead Adapter	5*
T4 DNA Ligase	4
RNase-free water	5
Total reaction volume	**90**

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

6. Program a thermocycler (without a heated lid) to incubate for 10 min at 25°C.

Reaction cleanup and removal of adapter dimers

7a. If sequencing the library directly (i.e., without further amplification), clean up DNA from step 6 using the GeneRead Size Selection Kit (not provided; cat. no. 180514), then proceed to step 8. **Note**: Following purification, the DNA can be stored at –20°C.

7b. If amplifying the library prior to sequencing, clean up the DNA from step 6 using the GeneRead Size Selection Kit (not provided; cat. no. 180514), and proceed to step 10.

8. Assess the quality of the library using a capillary electrophoresis device or comparable method. Check for the correct size distribution (see Figure 2) 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 96-plex, add 120 bp). This median fragment size is needed for subsequent qPCR-based library quantification (step 9).

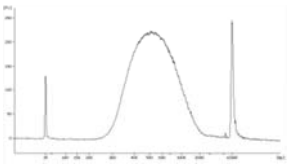

Figure 2. Agilent® trace data showing the correct size distribution of library fragments and the absence of adapters or adapter dimers.

9. Quantify the library using the GeneRead Library Quantification Kit (cat. no. 180612 [not provided]), or a comparable method.
10. 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 7b)	Variable
RNase-free water	Variable
Total reaction volume	**50**

11. 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.

11. Clean up the amplified DNA using the GeneRead Size Selection Kit (not provided; cat. no. 180514).
12. Assess the quality and quantity of the library as outlined in steps 8 and 9. Store the adapter-ligated library at –20°C until ready to use for sequencing.