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GeneRead™ DNA Library Q Handbook

Σ 48

For preparation of DNA libraries for next-generation sequencing (NGS) applications that use the QIAGEN GeneReader® instrument

For Research Use Only. Not for use in diagnostic procedures.

REF 185444

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Sample to Insight

QIAGEN

Contents

Kit Contents

The following QIAGEN® kits are required to construct NGS libraries for use with the QIAGEN GeneReader instrument.

GeneRead DNA Library Q Kit (48)	
Catalog no.	**185444**
Number of reactions	**48***
GeneRead DNA Library Q Kit†	Cat. no. 1094933
GeneRead Size Selection Q Kit†	Cat. no. 1094984

* Total reaction number is calculated for 8 automated runs of 6 samples processed on QIAcube® Connect. If runs with <6 samples are performed, the total number of processed samples could be less than stated.

† Kit is a component of the GeneRead DNA Library Q Kit (48).

GeneRead DNA Library Q Kit*	
Catalog no.	**1094933**
End Repair Buffer, 10x	200 µl
Ligation Buffer, 2x	2 x 1.25 ml
End Repair Enzyme Mix	150 µl
Ligation + Nick Repair Enzyme Mix	250 µl
RNase-Free Water	3 x 1.9 ml
dNTP Mix (10 mM)	2 x 55 µl
Universal Adapter Q, 2.5 µM	252 µl
Adapter Q BC1, 2.5 µM†	22 µl
Adapter Q BC2, 2.5 µM†	22 µl
Adapter Q BC3, 2.5 µM†	22 µl
Adapter Q BC4, 2.5 µM†	22 µl
Adapter Q BC5, 2.5 µM†	22 µl
Adapter Q BC6, 2.5 µM†	22 µl
Adapter Q BC7, 2.5 µM†	22 µl
Adapter Q BC8, 2.5 µM†	22 µl
Adapter Q BC9, 2.5 µM†	22 µl
Adapter Q BC10, 2.5 µM†	22 µl
Adapter Q BC11, 2.5 µM†	22 µl
Adapter Q BC12, 2.5 µM†	22 µl
Primer Mix, Library Amplification	70 µl
Multiplex PCR Mastermix	2 x 850 µl

* Not for individual sale; to order reagents, see cat. no. 185444.

† For adapter indices, refer to "Appendix A: Adapter Indices for the GeneRead DNA Library Q Kit", page 43.

GeneRead Size Selection Q Kit*	
Catalog no.	**1094984**
Buffer EBA, Elution Buffer	70 ml
Diluent (for 80% ethanol formulation)	3 x 25 ml
Buffer SB2, Size Selection Buffer	2 x 60 ml
MinElute® Spin Columns	2 x 50 tubes
Collection Tubes, 1.5 ml†	4 x 50 tubes

* Not for individual sale; to order reagents, see cat. no. 185444.

† Collection Tubes, 1.5 ml and 1.5 ml Elution Tubes (provided with QIAcube Rotor Adapters) can be used interchangeably in the library preparation workflow.

For manual library preparation (see "Manual protocol", page 24, and "Manual protocol", page 38), the following must be ordered separately:

Collection Tubes (2 ml)*	
Catalog no.	**19201**
Collection Tubes, 2ml (50)	20 x 50 tubes

* Collection Tubes, 2.0 ml, are ordered to support manual library preparation. If library preparation is automated on QIAcube Connect, these tubes are not required.

Storage

The GeneRead DNA Library Q Kit (cat. no. 1094933) is shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. If stored under these conditions, the kit is stable until the date indicated on the kit label.

The GeneRead Size Selection Q Kit (cat. no. 1094984) is shipped at ambient temperature. Upon receipt, open the kit and store the MinElute spin columns at 2–8°C in a constant-temperature refrigerator. The remaining kit components can be stored at room temperature (15–25°C). If stored under these conditions, the kit is stable until the date indicated on the kit label. Check buffers for precipitates before use and re-dissolve at 37°C if necessary. After prolonged storage in light, Buffer SB2 may turn yellow. This does not affect buffer or kit performance. However, light exposure should be avoided, and it is recommended to store Buffer SB2 in the dark.

Collection Tubes, 2 ml, (cat. no. 19201) are shipped at ambient temperature and can be stored at room temperature (15–25°C; required for manual library preparation only; ordered separately).

Intended Use

The GeneRead DNA Library Q Kit and the GeneRead Size Selection Q Kit are intended for Research Use Only. Not for use in diagnostic procedures.

QIAcube Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

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.

CAUTION: DO NOT add bleach or acidic solutions directly to the library preparation waste.

Buffer SB2 contains guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. In case liquid containing this buffer is spilt, clean with suitable laboratory detergent and water.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the GeneRead DNA Library Q Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

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 potential introduction of handling errors. The GeneRead DNA Library Q Kit uses a streamlined, optimized one-tube protocol, saving time and preventing handling errors. Optimized enzyme and buffer compositions ensure high yields of sequencing library and are ready for use on the GeneReader™ instrument.

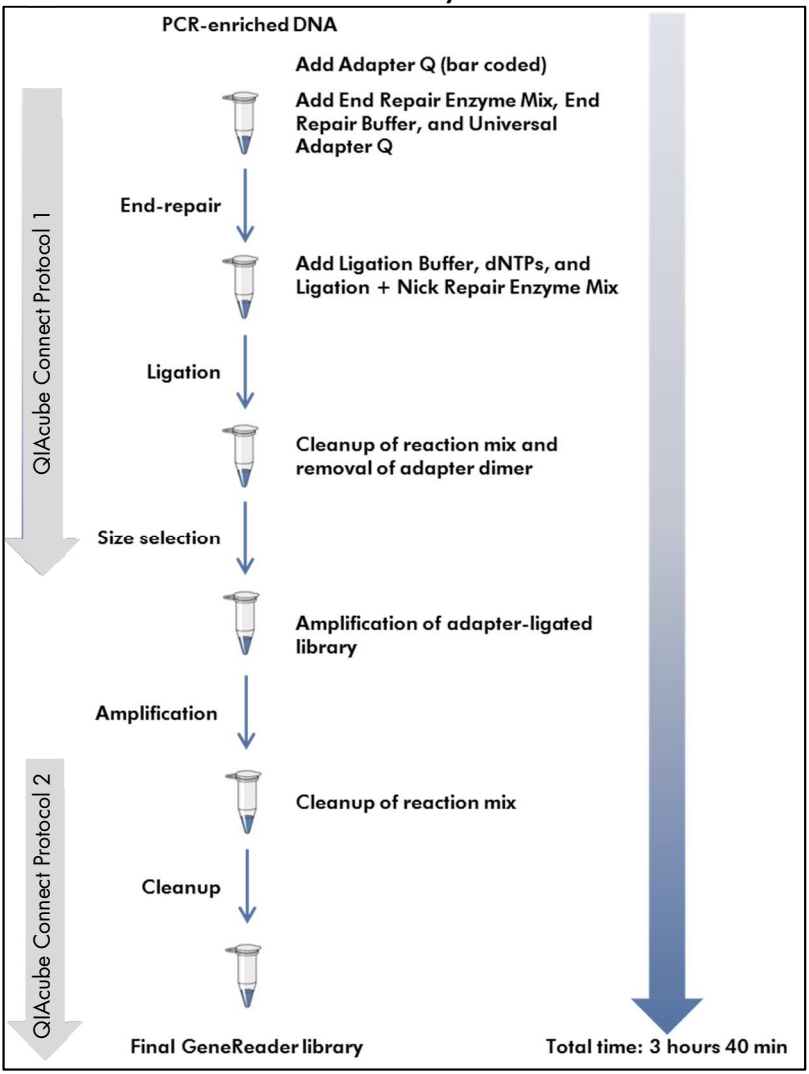
GeneRead library construction protocols are designed to enable straightforward automation on QIAcube Connect.

Principle and procedure

The GeneRead DNA Library Q Kit provides a fast, automated (or manual, single-tube) procedure for library construction from PCR-enriched DNA samples.

Following determination of DNA concentration, the ends of the DNA fragments are repaired and adapters, which are necessary for sequencing template preparation and sequencing steps, are ligated to both ends of the DNA fragments. The bar code adapters supplied with the kit (Adapter Q BC1–BC12) contain a unique identifying 6 bp bar code sequence and are used in combination with the Universal Adapter Q to allow analysis of multiple DNA libraries in one GeneReader sequencing run. Unincorporated adapters or adapter dimers are depleted from the samples by simple, easy and precise silica-based size separation using the MinElute spin column. To ensure maximum yields from minimum amounts of starting material, an additional amplification step is performed.

GeneRead DNA Library Q Procedure



Automated library construction

Due to the highly streamlined, one-tube protocol and unique column-based size selection to remove adapters and adapter dimers, GeneRead DNA Library preparation protocols can be automated on QIAcube Connect. The innovative QIAcube Connect 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, QIAcube Connect is preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA and viral nucleic acids, as well as 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/qiacubeprotocols**.

QIAcube Connect.

Description of protocols

This handbook contains three protocols for generation of DNA libraries that are for use on the QIAGEN GeneReader platform. The first protocol ("Automated protocol on QIAcube Connect" automated version, page 14, or manual version, page 24) describes end repair, adapter ligation and size selection of DNA. The second protocol ("Protocol: Amplification of Library DNA", page 30) describes an amplification step that is used to ensure high amounts of DNA library from minimum amounts of starting material. The third protocol ("Protocol: Cleanup of Final Library DNA" automated version, page 33, or manual version, page 38) describes the cleanup procedure after library amplification.

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.

- PCR tubes (200 µl) or plates
- Pipet tips and pipets
- Microcentrifuge (maximum 20,000 x *g*)*
- Vortexer*
- Thermal cycler*
- Ethanol (96–100%, non-denatured); do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone
- Analytical device to assess the quality of DNA library (e.g., QIAxcel® Advanced instrument [see, visit **www.qiagen.com**] and QIAxcel DNA High Resolution Kit [cat. no. 929002])*

The following products are only required for automated QIAcube Connect procedures:

- QIAcube Connect instrument* (for information, visit **www.qiagen.com**)
- QIAcube Filter Tips, 1000 µl (cat. no. 990352)
- QIAcube Filter Tips, 200 µl (cat. no. 990332)
- QIAcube Rotor Adapters (containing 1.5 ml Elution Tubes) (cat. no. 990394)
- QIAcube Reagent Bottles (cat. no. 990393)
- Sample Tubes RB (2 ml) (cat. no. 990381)

* Make sure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Important Notes

Compatible sequencing platform

- QIAGEN GeneReader instrument (cat. no. 9002312)

Starting materials

- PCR-enriched DNA. For further information, see GeneRead QIAact Panels, Powered by QCI, (cat. no. 181910) at **www.qiagen.com**.

Preparation of final 80% ethanol

Add 4 volumes (100 ml) ethanol (96–100%, non-denatured) to the bottle containing 25 ml Diluent (for 80% ethanol formulation), and mix well by inverting the bottle 5 times. Tick the check box on the bottle label to indicate that ethanol has been added. Store the 80% ethanol at room temperature (15–25°C).

Note: Using lower concentrations of ethanol during library preparation might lead to loss of library DNA. Make sure to add the proper 100 ml volume of 96—100% ethanol (non-denatured) to the Diluent bottle. Always close the bottles immediately after use to avoid evaporation.

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

Automated protocol on QIAcube Connect

This automated protocol describes end repair, adapter ligation and size selection of DNA, and generates adapter-ligated libraries that are ready to use for library amplification (see "Protocol: Amplification of Library DNA" on page 30).

Important points before starting

- This protocol is for constructing sequencing libraries for the QIAGEN GeneReader instrument. The following QIAGEN products are required for this protocol: GeneRead DNA Library Q Kit and GeneRead Size Selection Q Kit, both included in the GeneRead DNA Library Q Kit (48) (cat. no. 185444).
- PCR-enriched DNA, 4–40 ng (from GeneRead QIAact Panels, Powered by QCI) in a total volume of 8 µl, should be used with the library prep protocol.
- GeneRead Adapters are ready to use. The GeneRead DNA Library Q Kit contains the Universal Adapter Q and bar-coded Adapter Q (BC1–BC12) in separate tubes at a concentration of 2.5 µM.
- Prepare 80% ethanol in the Diluent buffer bottle (see "Preparation of final 80% ethanol", page 14); do not forget to tick the check box on the lid after preparation.
- **IMPORTANT**: When handling bar code adapters, open one adapter tube at a time and pay close attention when handling different bar code adapters; do not touch the rim of the tube or the inner part of the lid with gloves to avoid cross-contamination; we recommend changing gloves between pipetting different bar code adapters.
- **IMPORTANT**: Only 1 of the 12 bar code adapters (Adapter Q BC1–BC12) should be used for each sample.

- **IMPORTANT**: Running less than 6 samples in a QIAcube Connect run might reduce the total number of sample preparations per kit.

Things to do before starting

- Thaw buffers, dNTPs, adapters, primer mix, PCR master mix, RNase-free water and samples at room temperature (15–25°C). Once reagents and samples are thawed, mix them thoroughly by vortexing quickly to avoid localized concentrations, briefly centrifuge, and immediately place them on ice.
- Enzyme mixes should be kept on ice during preparation of samples and reaction mixes.
- All reagents and samples should be stored at –30°C to –15°C immediately after use.

Procedure

Preparation of Rotor Adapters

1. For each sample, prepare one Rotor Adapter according to the following steps.
2. Remove the 2 ml collection tube from a MinElute spin column. Cut off the lid of the column as close to the rim as possible. Place the prepared MinElute spin column in position 1 of the adapter (see Figure 1). Position L1 is not used.

   Note: Not cutting off the lid might result in the run being aborted and instrument damage.
3. Leave position 2 of the adapter empty.
4. Place one 1.5 ml elution tube (supplied with the Rotor Adapter) into position 3 and put the lid of the tube in slot L3.

   Note: Label elution tubes before loading onto QIAcube Connect to avoid mixing up the samples.

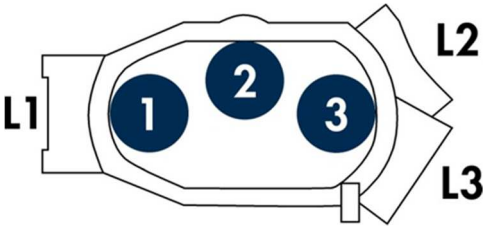


Figure 1. Rotor Adapters with tube positions (1–3) and lid positions (L1–L3) indicated. Positions L1, 2 and L2 are not used in this protocol.

Preparation of Reagent Bottle Rack

5. Prepare the bottles according to the volumes in Table 1. Positions 5 and 6 remain empty in the bottle rack and do not require reagent bottles (Figure 2).

   Note: Each reagent bottle can hold a total reagent volume of 30 ml.

   Note: Calculate the volume of buffer for the corresponding number of samples and add the reserve volume for each bottle to ensure the correct final volume for each buffer.

   For example, the following volume for Buffer EBA should be loaded onto the instrument for 3 samples: 3 × 200 µl + 2500 µl = 3100 µl

Table 1. Reagent bottle preparation

Position	Reagent	Volume per sample*	Reserve volume per bottle
1	Buffer SB2	800 µl	2500 µl
2	80% ethanol	1600 µl	2500 µl
3	80% ethanol	1600 µl	2500 µl
4	Buffer EBA	200 µl	2500 µl
5	—	—	—
6	—	—	—

* Not the actual volume pipetted by the instrument

Figure 2. Reagent Bottle Rack. Positions 5 and 6 are not used in this protocol.

Preparation of master mix MM-A

6. Prepare a reaction mix for end-repair according to Table 2 using the 1.5 ml microcentrifuge tubes supplied with the GeneRead DNA Library Q Kit (48). Select the correct number of samples to run on QIAcube Connect to ensure the correct volume is prepared.

Table 2. Master mix MM-A

No. of samples	RNase-free water, µl (clear lid)	End Repair Buffer, µl (yellow lid)	End Repair Enzyme Mix, µl (black lid)	Universal Adapter Q, µl (green lid)
2	23	9.0	7.2	11.5
3	29	11.7	9.3	14.9
4	36	14.4	11.5	18.4
5	43	17.1	13.7	21.9
6	50	19.8	15.8	25.3
7	57	22.5	18.0	28.8
8	63	25.2	20.1	32.2
9	70	27.9	22.3	35.7
10	77	30.6	24.5	39.1
12	91	36.0	28.8	46.0

7. Mix thoroughly, spin down, and place on ice until ready for use on QIAcube Connect.

   Note: Store reagents at –30°C to –15°C immediately after use.

Preparation of master mix MM-C

8. Prepare a reaction mix for adapter ligation according to Table 3, using the 1.5 ml microcentrifuge tubes supplied with the GeneRead DNA Library Q Kit (48). Select the correct number of samples to run on QIAcube Connect to ensure the correct volume is prepared.

Table 3. Master mix MM-C

No. of samples	RNase-free water, µl (clear lid)	Ligation Buffer, µl (red lid)	Ligation + Nick Repair Enzyme Mix, µl (brown lid)	dNTP Mix (10 mM), µl (purple lid)
2	25	101	10.1	2.5
3	36	144	14.4	3.6
4	47	187	18.7	4.7
5	58	231	23.1	5.8
6	68	274	27.4	6.8
7	79	317	31.7	7.9
8	90	360	36.0	9.0
9	101	403	40.3	10.1
10	112	447	44.7	11.2
12	133	533	53.3	13.3

9. Mix thoroughly, spin down, and place on ice while preparing QIAcube Connect.

   Note: Store reagents at –30°C to –15°C immediately after use.

Preparation of samples

10. Prepare 8 µl sample. Each sample should contain a total of 4–40 ng PCR-enriched DNA. We recommend starting with 8 ng PCR-enriched DNA in 8 µl. Pipet the sample to the bottom of a Sample Tube RB (2 ml) (cat. no. 990381). According to quantification results, dilutions of the sample may be required. RNase-free water should be used for dilution steps.

    Note: Do not dilute the complete sample volume; only use dilution volumes sufficient for 1–2 library preparation reactions.

    Note: Less than 4 ng PCR-enriched DNA can be used for library preparation, but might lead to low library outputs. Using more than 40 ng might lead to formation of unwanted fragments.

    IMPORTANT: PCR-enriched DNA samples usually contain amplified DNA fragments of high concentrations and should be handled with care.

11. Add 3.2 µl of the corresponding Adapter Q (bar-coded) to the sample.

    Note: Only use one Adapter Q (bar-coded) per sample. Pay close attention when handling different bar code adapters; do not touch the rim of the tube or the inner part of the lid with gloves. We recommend changing gloves between handling of the different bar code adapters.

    Note: Universal Adapter Q is added to the end-repair master mix (see step 6, page 18).

12. After preparation of samples, pulse-spin each sample tube to ensure that DNA and bar code adapters are properly combined, and place the tubes on ice until placing them on QIAcube Connect (see step 13 below).

Preparation of QIAcube Connect

13. QIAcube Connect should be handled according to the user manual available online at **www.qiagen.com/QIAcube**.

14. Place the prepared master mixes into the microcentrifuge tube slots A (MM-A) and C (MM-C). Put the lids of the tubes into the corresponding slots (Figure 3).

    Note: Microcentrifuge tube slot B remains empty.

Figure 3. Microcentrifuge tube slots. Microcentrifuge tube slot B is not used in this protocol.

15. Place the reagent bottle rack onto the instrument, and remove the screw caps.

    Note: Do not discard the screw caps of the buffer bottles. Immediately close the buffer bottles using the screw caps after the protocol is completed to avoid evaporation of buffers.

16. Distribute the Rotor Adapters into the centrifuge buckets according to the number of samples and balance scheme of QIAcube Connect (see *QIAcube Connect User Manual*).

17. Put one tip rack with 1000 µl tips (do not use wide-bore tips) and one rack with 200 µl tips into the tip rack positions on the work deck. See Table 4 for the number of tips required according to the number of samples.

    Note: The position of the tips is recognized by the instrument. Make sure to click the tip racks properly into the recess.

Table 4. Number of tips used

No. of samples	1000 µl tips	200 µl tips
2	11	5
3	13	7
4	15	9
5	17	11
6	19	13
7	21	15
8	23	17
9	25	19
10	27	21
12	31	25

18. Use the shaker adapter marked "2" for 2 ml microcentrifuge tubes.
19. Place each sample tube into a shaker position according to the number of samples and balance scheme of QIAcube Connect (see *QIAcube Connect User Manual*).

    Note: The lid of each tube is held in a slot at the edge of the shaker rack to ensure that tubes cannot be displaced during sample processing and to enable loading check of the instrument.
20. To start the protocol, select the **DNA** application.
    - Select the kit name: **GeneRead DNA Library Q Kit**
    - Select the material: **DNA**
    - Select the protocol name: **Standard**
22. Follow the instructions displayed on the touchscreen and check all steps. Close the door of QIAcube Connect prior to pressing the **Start** button.

    Note: For 12 samples, QIAcube Connect run time is approximately 1 hour and 50 minutes.

    Note: Elution of the adapter-ligated library samples is performed using 20 µl Buffer EBA.

23. After the protocol is completed, remove the 1.5 ml microcentrifuge tubes containing residual master mixes MM-A and MM-C and discard.
24. Remove the sample tubes from the shaker position and discard.
25. Close the buffer bottles with the corresponding screw caps.
26. Remove the Rotor Adapters following the correct orientation of the samples, and remove the 1.5 ml microcentrifuge tube containing the ligated library sample. Proceed with the "Protocol: Amplification of Library DNA", page 30.

    Optional stopping point: Adapter-ligated samples can be stored for up to 2 weeks at –30°C to –15°C until they are used in the amplification step.

    Note: Always check the position of the MinElute spin columns after QIAcube Connect run. The column should be placed on top of the 1.5 ml microcentrifuge tube to ensure DNA elution from the column into the tube.

Manual protocol

This manual protocol describes end repair, adapter ligation and size selection of DNA, and the generation of adapter-ligated libraries that are ready to use for library amplification (see "Protocol: Amplification of Library DNA" on page 30).

Important points before starting

- This protocol is for constructing sequencing libraries for the QIAGEN GeneReader instrument. The following QIAGEN products are required for this protocol: GeneRead DNA Library Q Kit and GeneRead Size Selection Q Kit, both are included in the GeneRead DNA Library Q Kit (48) (cat. no. 185444) and Collection Tubes (2ml).
- PCR-enriched DNA, 4–40 ng (from GeneRead QIAact Panels, Powered by QCI) in a total volume of 14.1 µl, should be used with the library prep protocol.
- GeneRead Adapters are ready to use. The GeneRead DNA Library Q Kit contains the Universal Adapter Q and bar-coded Adapter Q (BC1–BC12) in separate tubes at a concentration of 2.5 µM.
- Prepare 80% ethanol in the Diluent buffer bottle (see "Preparation of final 80% ethanol", page 14); do not forget to tick the check box on the lid after preparation.
- **IMPORTANT**: When handling bar code adapters, open one adapter tube at a time and pay close attention when handling different bar code adapters; do not touch the rim of the tube or the inner part of the lid with gloves to avoid cross-contamination; we recommend changing gloves between pipetting different bar code adapters.
- **IMPORTANT**: Only 1 of the 12 bar code adapters (Adapter Q BC1–BC12) should be used for each sample.

Things to do before starting

- Thaw buffers, dNTPs, adapters, primer mix, PCR master mix, RNase-free water and samples at room temperature (15–25°C). Once reagents and samples are thawed, mix them thoroughly by vortexing quickly to avoid localized concentrations, and immediately place them on ice.
- Enzyme mixes should be kept on ice during preparation of samples and reaction mixes.
- All reagents and samples should be stored at –30°C to –15°C immediately after use.

Procedure

End repair and adapter ligation

1. Prepare 14.1 µl sample containing 4–40 ng PCR-enriched DNA using a 0.2 ml PCR tube. According to quantification results, dilutions of the sample may be required. RNase-free water should be used for dilution steps.

   Note: Do not dilute the complete sample volume; only use dilution volumes sufficient for 1–2 reactions.

   Note: Less than 4 ng PCR-enriched DNA can be used for library preparation but might lead to low library outputs. Using more than 40 ng might lead to formation of unwanted fragments.

   IMPORTANT: PCR-enriched DNA samples usually contain amplified DNA fragments of high concentrations and should be handled with care.

2. Prepare a reaction mix according to Table 5. The total volume of PCR-enriched DNA, End Repair Buffer 10x, End Repair Enzyme Mix and water should be 18.6 µl. Add 3.2 µl of the corresponding Adapter Q (bar-coded) to each sample and 3.2 µl Universal Adapter Q to bring the total volume to 25 µl.

   Note: Only use one Adapter Q (bar-coded) per sample. Pay close attention when handling different bar code adapters; do not touch the rim of the tube or the inner part of the lid with gloves. We recommend changing gloves between handling of the different bar code adapters.

Table 5. Reaction mix for end repair

Reagent	Volume per sample
PCR-enriched DNA (4–40 ng) and water	14.1 µl
End Repair Buffer, 10x (yellow lid)	2.5 µl
End Repair Enzyme Mix (black lid)	2.0 µl
Add Adapter Q (bar-coded) (green lid)	3.2 µl
Add Universal Adapter Q (green lid)	3.2 µl
Total volume	**25 µl**

3. After preparation of the end repair reaction mixture, mix thoroughly by vortexing for 5 seconds, and pulse-spin each sample tube to remove any liquid from inside the lid.
4. Program a thermal cycler according to Table 6.

   IMPORTANT: Do not use a heated lid.

Table 6. Incubation temperature and time for end repair

Temperature	Time
25°C	20 minutes

5. After end repair, the adapter ligation step follows. Prepare a reaction mix according to Table 7, adding the components directly to the sample in the PCR tube.

Table 7. Reaction mix for adapter ligation

Reagent	Volume per sample
Reaction mixture after end repair	25 µl
Ligation Buffer, 2x (red lid)	40 µl
Ligation + Nick Repair Enzyme Mix (brown lid)	4 µl
dNTP mix (10 mM) (purple lid)	1 µl
RNase-Free Water (clear lid)	10 µl
Total volume	80 µl

6. After preparation of the adapter ligation reaction mixture, mix thoroughly by vortexing for 5 seconds, and pulse-spin each sample tube to remove any liquid from inside the lid.
7. Program a thermal cycler according to Table 8.

   IMPORTANT: Do not use a heated lid. Make sure that the lid does not emit indirect heat to the sample tube. The reaction can be performed with the lid open.

Table 8. Incubation temperature and time for adapter ligation

Temperature	Time
25°C	10 minutes
72°C	5 minutes

8. After adapter ligation, directly proceed to the "Size selection with Buffer SB2" procedure (below) using the prepared DNA samples.

   IMPORTANT: Continue immediately with the size-selection procedure.

Size selection with Buffer SB2

After adapter ligation, impurities, such as enzymes from previous reactions, should be removed, as well as adapter monomers and dimers. This can be accomplished by removing DNA fragments of defined sizes.

Note: All of the following centrifugation steps should be performed at full speed (maximum 20,000 x *g*) in a microcentrifuge at room temperature (15–25°C).

9. Transfer the adapter-ligated DNA from step 8 to a 1.5 ml tube. This DNA will be used for size selection.
10. Add 4 volumes of Buffer SB2 to 1 volume of adapter-ligated DNA, and mix by vortexing for 5 seconds.

    Note: Add 320 µl Buffer SB2 to 80 µl sample.
11. To bind DNA, apply the mixture to the MinElute spin column and centrifuge for 1 minute.

    Note: Transfer all traces of sample to the column for maximum recovery.
12. Discard the tube and place the MinElute spin column into a new 2 ml collection tube.
13. To wash, add 700 µl 80% ethanol (using the Diluent bottle prepared for formulation of 80% ethanol) to the MinElute spin column, and centrifuge for 1 minute.
14. Discard the collection tube, and place the MinElute spin column into a new 2 ml collection tube.
15. Add 700 µl 80% ethanol to the MinElute spin column, and centrifuge for 1 minute.
16. Discard the collection tube, and place the MinElute spin column into a new 2 ml collection tube.
17. Centrifuge the MinElute spin column for 1 minute to remove residual ethanol.
18. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube.
19. Add 90 µl Buffer EBA to the center of the membrane, let the column stand for 1 minute, and then centrifuge for 1 minute.

20. Save the collection tube and flow-through for step 21. Place the MinElute spin column into a new 2 ml collection tube.
21. Add 4 volumes of Buffer SB2 to 1 volume of the flow-through from step 20 (sample), and mix by vortexing for 5 seconds.

    Note: Add 360 µl Buffer SB2 to 90 µl sample.
22. Apply the sample–Buffer SB2 mixture from step 21 to the same MinElute spin column from step 20, and centrifuge for 1 minute.

    Note: Transfer all traces of sample to the column for maximum recovery.
23. Discard the collection tube, and place the MinElute spin column into a new 2 ml collection tube.
24. To wash, add 700 µl 80% ethanol to the MinElute spin column, and centrifuge for 1 minute.
25. Discard the collection tube, and place the MinElute spin column into a new 2 ml collection tube.
26. Add 700 µl 80% ethanol to the MinElute spin column, and centrifuge for 1 minute.
27. Discard the collection tube, and place the MinElute spin column into a new 2 ml collection tube.
28. Centrifuge the MinElute spin column for 1 minute to remove residual ethanol.
29. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube.
30. To elute adapter-ligated DNA, add 20 µl Buffer EBA to the center of the membrane, let the MinElute spin column stand for 1 minute, and then centrifuge for 1 minute.
31. Proceed with the protocol "Protocol: Amplification of Library DNA", page 30.

    Optional stopping point: Adapter-ligated DNA samples can be stored for up to 2 weeks at –30°C to –15°C until they are used in the amplification procedure.

Protocol: Amplification of Library DNA

PCR-based library amplification is required after adapter ligation to DNA samples. Ten cycles of PCR are mandatory using the GeneRead DNA Library Q Kit.

Important points before starting

- This protocol for library amplification ensures high amounts of DNA library from a minimum amount of starting material for the QIAGEN GeneReader instrument. The following QIAGEN products are required for this protocol: GeneRead DNA Library Q Kit, which is included in the GeneRead DNA Library Q Kit (48) (cat. no. 185444).
- The Primer Mix, Library Amplification and Multiplex PCR Mastermix are ready-to-use reagents and do not require pre-dilution.

Things to do before starting

- Prepare adapter-ligated library DNA according to “Important Notes” using either the automated version, page 14, or the manual version, page 24.
- Thaw all reagents at room temperature (15–25°C). Once reagents are thawed, mix them thoroughly by quick vortexing to avoid localized concentrations, and immediately place them on ice.

Procedure

1. Prepare a reaction mix according to Table 9.

Table 9. PCR mix for library amplification

Reagent	Volume per reaction (µl)
Multiplex PCR Mastermix	25
Primer Mix, Library Amplification	1
RNase-Free Water	7
Library DNA	17
Total reaction volume	**50**

Note: After transferring 17 µl library DNA, 2–3 µl sample will remain; this remnant can be discarded.

2. Program a thermal cycler according to Table 10 (total run time is approximately 45 minutes).

Table 10. Cycling conditions

Time	Temperature	Number of cycles
15 minutes	95°C	1
30 seconds	94°C	
30 seconds	52°C	10
30 seconds	72°C	
5 minutes	72°C	1
Hold	4°C	

Note: Make sure to close the PCR tubes or plate properly to avoid evaporation during cycling.

3. Remove samples from the thermal cycler, and proceed with "Protocol: Cleanup of Final Library DNA" using either the automated version, page 33, or the manual version, page 38.

   Optional stopping point: Amplified library samples can be stored overnight at 2–8°C until they are used in the cleanup procedure.

Protocol: Cleanup of Final Library DNA

Automated protocol on QIAcube Connect

This automated protocol describes cleanup of amplified library DNA and results in ready-to-use GeneReader sequencing libraries.

Important points before starting

- This protocol is for cleaning up amplified sequencing libraries for the QIAGEN GeneReader instrument. The following QIAGEN product is required for this protocol: GeneRead Size Selection Q Kit, which is included in the GeneRead DNA Library Q Kit (48) (cat. no. 185444).

Things to do before starting

- Amplify library DNA according to "Protocol: Amplification of Library DNA", page 30.

Procedure

1. For each sample, prepare one Rotor Adapter according to the following steps.
2. Remove the 2 ml collection tube from the MinElute spin column. Place the prepared MinElute spin column in position 1 of the adapter. Position L1 is not used.
3. Position 2 of the adapter remains empty.
4. Place one 1.5 ml elution tube (supplied with the Rotor Adapter) into position 3, and put the lid of the tube in slot L3.

   Note: Label elution tubes before loading onto QIAcube Connect to avoid mixing up the samples.
5. Distribute the Rotor Adapters into the centrifuge buckets according to the number of samples and balance scheme of QIAcube Connect (see *QIAcube Connect User Manual*).

6. Prepare reagent bottles according to the volumes in Table 11. Positions 2, 5 and 6 remain empty in the bottle rack and do not require reagent bottles.

   Note: Calculate the volume of buffer for the corresponding number of samples, and add the reserve volume per bottle for the final volume of each buffer.

   For example, the following volume of Buffer SB2 is loaded onto the instrument for 4 samples: 4 × 400 µl + 2500 µl = 4100 µl.

Table 11. Reagent bottle preparation

Position	Reagent	Volume per sample*	Reserve volume per bottle
1	Buffer SB2	400 µl	2500 µl
2	—	—	—
3	80% ethanol	1600 µl	2500 µl
4	Buffer EBA	100 µl	2500 µl
5	—	—	—
6	—	—	—

* Not the actual volume pipetted by the instrument

7. Place the reagent bottle rack onto the instrument, and remove the screw caps.

   Note: Do not discard the screw caps of the buffer bottles. Immediately close the buffer bottles using the screw caps after the protocol is completed to avoid evaporation of buffers.

8. Put one tip rack with 1000 µl tips (do not use wide-bore tips) and one rack with 200 µl tips into the tip rack positions on the work deck. See Table 12 for the number of tips required according to the number of samples.

   Note: The position of the tips is recognized by the instrument. Make sure to click the tip racks properly into the recess.

Table 12. Number of tips used

No. of samples	1000 µl tips	200 µl tips
2	5	1
3	6	1
4	7	1
5	8	1
6	9	1
7	10	1
8	11	1
9	12	1
10	13	1
12	15	1

9. After the PCR is completed, spin down the sample tubes or plate, and transfer the complete volume of amplified library sample (approximately 50 µl) to a Sample Tube RB (2 ml) (cat. no. 990381).

   Note: If available sample volume is <50 µl due to pipetting losses, adjustment of sample volume is not required.

10. Use the "2" position of the shaker adapter of QIAcube Connect for 2 ml microcentrifuge tubes.

11. Place each sample tube into the corresponding position on the shaker according to the number of samples and balance scheme of QIAcube Connect (see *QIAcube Connect User Manual*).

    Note: The lid of each tube is held in a slot at the edge of the shaker rack to ensure that tubes cannot be displaced during sample processing and to enable loading check of the instrument.

12. To start the protocol, select the **Cleanup** application.
    - Select the kit name: **GeneRead Size Selection Q Kit**
    - Select the material: **Enriched library**
    - Select the protocol name: **Standard**
13. Follow the instructions displayed on the touchscreen and check all steps. Close the door of QIAcube Connect prior to pressing the **Start** button.

    Note: For 12 samples, the run time on QIAcube Connect is approximately 27 minutes.

    Note: Elution of the final library samples is performed using 20 µl Buffer EBA.
14. After the protocol is completed, remove the sample tubes from the shaker position and discard.
15. Close the buffer bottles with the corresponding screw caps.
16. Remove the Rotor Adapters following the correct orientation of the samples, and take out the 1.5 ml microcentrifuge tube that contains the final library sample.

    Note: Final library samples can be stored for up to 12 months at –30°C to –15°C until further processing.

    Note: Always check the position of the MinElute spin columns after QIAcube Connect run. The column should be placed on top of the 1.5 ml microcentrifuge tube to ensure DNA elution from the column into the tube.
17. Assess the quality of the library using an analytical device (e.g., QIAxcel Advanced). Check the final library by assessing the concentration, the correct size distribution (see Figure 4, page 37) of library fragments, and the absence of adapters or adapter dimers (<170 bp).

    Note: The median DNA fragment size should be increased by the size of the adapters that were ligated to the library fragments (for the GeneRead DNA Library Q Kit, the sized is increased by 92 bp).

    Note: Depending on the GenePanel primer mix used, the size distribution of fragments might differ.

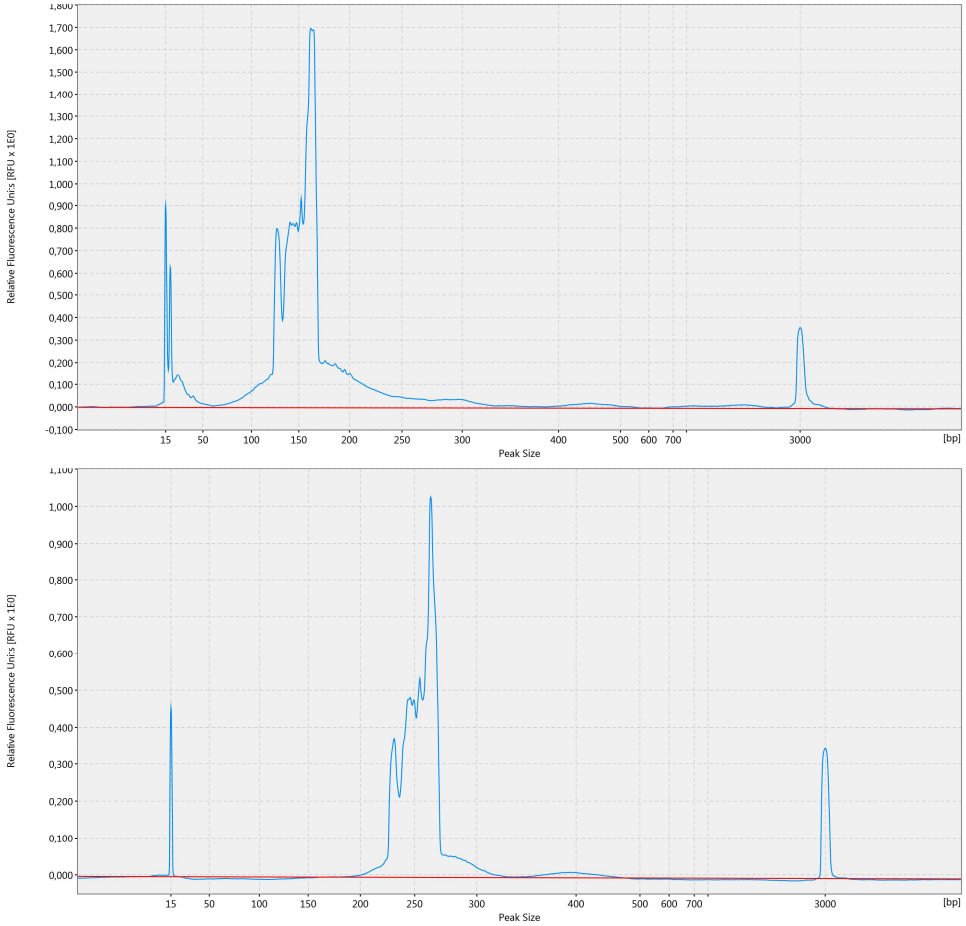


Figure 4. Trace data from a capillary electrophoresis device QIAxcel Advanced, showing the PCR-enriched samples (above), as well as the correct size distribution of final library fragments after library preparation and the absence of adapters or adapter-dimers.

Note: For further information regarding library control methods and data analysis, see "Appendix B: Library Control Methods", page 44.

Manual protocol

This manual protocol describes cleanup of amplified library DNA and results in ready-to-use GeneReader sequencing libraries.

Important points before starting

- This protocol is for cleaning up amplified sequencing libraries for the QIAGEN GeneReader instrument. The following QIAGEN product is required for this protocol: GeneRead Size Selection Q Kit, which is included in the GeneRead DNA Library Q Kit (48) (cat. no. 185444) and Collection Tubes (2ml).

Things to do before starting

- Amplify library DNA according to "Protocol: Amplification of Library DNA", page 30.

Note: All of the following centrifugation steps should be performed at full speed (maximum 20,000 x *g*) in a microcentrifuge at room temperature (15–25°C).

Procedure

1. Add 4 volumes of Buffer SB2 to 1 volume of amplified library, and mix sample by vortexing for 5 seconds.

   Note: Add 200 µl Buffer SB2 to 50 µl amplified library.
2. To bind DNA, apply the sample to the MinElute spin column, and centrifuge for 1 minute.
3. Discard the collection tube, and place the MinElute spin column into a new 2 ml collection tube.
4. To wash, add 700 µl 80% ethanol to the MinElute spin column, and centrifuge for 1 minute.
5. Discard the collection tube, and place the MinElute spin column into a new 2 ml collection tube.
6. Add 700 µl 80% ethanol to the MinElute spin column, and centrifuge for 1 minute.

7. Discard the collection tube, and place the MinElute spin column into a new 2 ml collection tube.
8. Centrifuge the MinElute spin column for 1 minute to remove residual ethanol.
9. Place the MinElute spin column into a clean 1.5 ml microcentrifuge tube.
10. To elute amplified library DNA, add 20 µl Buffer EBA to the center of the membrane, let the MinElute spin column stand for 1 minute, and then centrifuge for 1 minute.

    Note: Final library samples can be stored for up to 12 months at –30°C to –15°C until further processing.
11. Assess the quality of the library using an analytical device (e.g., QIAxcel Advanced). Check the final library by assessing the concentration, the correct size distribution (see Figure 4, page 37) of library fragments, and the absence of adapters or adapter dimers (<170 bp).

    Note: The median DNA fragment size should be increased by the size of the adapters that were ligated to the library fragments (for the GeneRead DNA Library Q Kit, the size is increased by 92 bp).

    Note: Depending on the GenePanel primer mix used, the size distribution of fragments might differ.

    Note: For further information regarding library control methods and data analysis, see "Appendix B: Library Control Methods", page 44.

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/or protocols in this handbook or sample and assay technologies (for contact information, 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)	No library DNA present after automated cleanup protocol	Make sure the MinElute spin column has been transferred to the 1.5 ml microcentrifuge tube of the rotor adapter at the end of QIAcube Connect run. If the spin column is not placed on top of the 1.5 ml tube, no DNA is eluted from the column, and only elution buffer has been transferred into the tube. Check if the lid of the spin column was cut off properly (as close to the rim as possible), and repeat the library preparation using the same sample. Check if the MinElute spin column has been stored as instructed (upon arrival store MinElute spin columns at 2–8°C). If columns have not been stored as instructed, contact QIAGEN Technical Services.
c)	No library DNA present after automated cleanup protocol	Inhibitors, such as ethanol, which might be present in the PCR-enriched sample (e.g., from targeted enrichment) used for library preparation, can influence the library reactions. Check if inhibitors are present in the sample and clean-up the PCR-enriched sample if appropriate (e.g., by using the MinElute PCR Purification Kit, cat. no. 28004). Afterwards, repeat the library construction.
d)	No library DNA present after manual cleanup protocol	Always check if the MinElute spin column has been stored as instructed (upon arrival store MinElute spin columns at 2–8°C). If columns have not been stored as instructed, contact QIAGEN Technical Services.
e)	No library DNA present after manual cleanup protocol	Make sure the final concentration of the 80% ethanol made from the supplied Diluent is correct. The use of lower concentrations of ethanol might lead to loss of library DNA. Make sure to use 96–100% non-denatured ethanol to prepare the final 80% ethanol.

		Comments and suggestions
f)	No library DNA present after manual library construction	Make sure that the lid of the thermal cycler was powered off during end repair and adapter ligation reactions.
Unexpected signal peaks in capillary electrophoresis device traces		
a)	Presence of peaks smaller than 170 bp	These peaks represent artifacts (e.g., library adapters and adapter dimers) that are present when depletion after library preparation is not sufficient. As these artifacts can be amplified during sequencing-template preparation and will be sequenced, the capacity of the flow cell for the library fragments will be reduced. A low ratio of artifact to library fragments will not interfere with sequencing. If a large number of artifacts is present in the sample, check the pH of Buffer SB2. If the pH value is below 7.9, contact QIAGEN Technical Services.
b)	Presence of peaks larger than 300 bp	These peaks represent artifacts (e.g., adapter or library multimers) that occur when wrong concentrations of adapters are used during library preparation or when the input DNA amount exceeds 40 ng. As these artifacts can amplify during sequencing-template preparation and will be sequenced, the capacity of the flow cell for the library fragments will be reduced. A low ratio of artifacts to library fragments will not interfere with sequencing.
c)	Incorrect library fragment size after adapter ligation	During library preparation, adapters of approximately 92 bp in size are ligated to both ends of the DNA library fragments. This should be apparent from capillary electrophoresis as an increase in size of all library fragments by 92 bp. The absence of a clear size shift might indicate low adapter ligation efficiency. Make sure to use the protocols, reagents and volumes described in these instructions, as well as the correct amount of starting DNA. Make sure to add the bar code adapter directly to the sample prior to starting QIAcube Connect.

Symbols

Symbol	Symbol definition
	Contains reagents sufficient for <N> tests
	Catalog number
	Manufacturer

Appendix A: Adapter Indices for the GeneRead DNA Library Q Kit

The index sequences used in the GeneRead DNA Library Q Kit are listed in Table 13. Indices 1–12 correspond to the corresponding GeneReader adapter indices.

Table 13. Adapter indices

Adapter name	Indices
Adapter Q BC1	ATCACG
Adapter Q BC2	CGATGT
Adapter Q BC3	TTAGGC
Adapter Q BC4	TGACCA
Adapter Q BC5	ACAGTG
Adapter Q BC6	GCCAAT
Adapter Q BC7	CAGATC
Adapter Q BC8	ACTTGA
Adapter Q BC9	GATCAG
Adapter Q BC10	TAGCTT
Adapter Q BC11	GGCTAC
Adapter Q BC12	CTTGTA

Appendix B: Library Control Methods

Recommended method for library control

- To assess the quality and quantity of the final library, we recommend the following analytical device: QIAGEN's QIAxcel Advanced instrument in combination with the QIAxcel DNA High Resolution Kit (cat. no. 929002). Refer to the corresponding instrument user manual and kit handbook for setting up the DNA analyses.
- For sample preparation and data analysis, we recommend the QIAxcel Advanced instrument setups.
- For final library analysis, we recommend diluting the sample 1:2. Therefore, add 5 µl Buffer EBA to 5 µl sample, for a final volume of 10 µl.

  Note: Make sure to calculate the dilution factor for final library quantification results.

  Note: The QIAxcel Advanced instrument requires a minimum volume of 10 µl for analysis.

  Note: The QX DNA Size Marker should be diluted with Buffer EBA for analysis.

  Note: According to QIAxcel analysis results, higher or lower dilutions of the library sample might be required.
- Use the QIAxcel ScreenGel Software version 1.5 or higher.
- QIAxcel DNA High Resolution Kit (cat. no.929002) should be used for final library analyses.
- QX DNA Size Marker 50–800 bp (50 µl) v2.0 (cat. no. 929561) and QX Alignment Marker 15 bp/3 kb (1.5 ml) (cat. no. 929522) should be used for final library analyses.
- For further guidance for final library analysis, refer to the QIAxcel Advanced System guide "NGS Sample Quality Control using the QIAxcel Advanced System" available from **www.qiagen.com**.

Alternative methods for library control

- Agilent® Bioanalyzer instrument in combination with the Agilent High-Sensitivity DNA Kit (Agilent Technologies cat. no. 5067-4626).
- Qubit® Fluorometer in combination with the Qubit dsDNA HS Assay Kit (Life Technologies cat. no. Q32851).

Agilent Bioanalyzer instrument

- The Agilent Bioanalyzer instrument uses 1 µl sample. For final library analysis, pre-dilute 1 µl by bring the volume up to 10 µl with RNase-free water.

  Note: Diluting 1 µl sample with 10 µl RNase-free Water results in a final dilution of 1:10. Make sure to recalculate the dilution factor for final library quantification results.
- Agilent High-Sensitivity DNA Kit (cat. no. 5067-4626) should be used for final library analyses.

Qubit Fluorometer instrument

- The Qubit Fluorometer instrument uses sample volumes ranging from 1–10 µl in a total volume of 200 µl for analysis. For final library analysis, sample volumes of 1–3 µl are sufficient.

  Note: Pre-dilutions of samples are not necessary.
- The Qubit dsDNA High-Sensitivity Assay Kit (cat. no. Q32851) should be used for final library analyses.

Ordering Information

Product	Contents	Cat. no.
GeneRead DNA Library Q Kit (48)	For 48 reactions: Buffers and reagents for end-repair, adapter ligation, library amplification, size selection and cleanup; for use with the QIAGEN GeneReader instrument	185444
Collection Tubes (2ml)	Disposable 2ml Collection Tubes; for use with the manual library preparation protocol	19201
Filter-Tips, 1000 µl (1024)	Disposable Filter-Tips, racked; (8 x 128); for use with QIAcube instruments	990352
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128).; for use with QIAcube instruments	990332
Rotor Adapters (10 x 24)	Rotor Adapters for use with the centrifuge buckets in QIAcube instruments	990394
Reagent Bottles, 30 ml (6)	Reagent Bottles for use with buffers of preparation kits for QIAcube instruments	990393
Sample Tubes RB (2 ml)	Sample Tubes round-bottom for use with sample material on QIAcube instruments	990381
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

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.

Document Revision History

Date	Changes
April 2018	Updated sample dilution for final library analysis. Added revision history
February 2020	Updated text, ordering information and intended use for QIAcube Connect.

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Notes

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