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GeneRead™ Library Quant Kit Handbook

For reliable quantification of Ion Torrent™ or Illumina® libraries using real-time PCR (assay format)

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Kit Contents

GeneRead Library Quant Kit (varies)	Catalog no. 180612
Laboratory-verified forward and reverse primers for 500 x 25 µl reactions (500 µl)	1
DNA standard (100 µl)	1
Dilution buffer (30 ml)	1
GeneRead qPCR SYBR® Green Mastermix (1.35 ml)	5
Handbook	1

Storage

The GeneRead Library Quant Kit is shipped on blue ice. Upon receipt, store at –20°C. If stored under these conditions, GeneRead Library Quant Kit is stable for 6 months after receipt.

Quality Control

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

Intended Use

The GeneRead Library Quant Kit is intended for molecular biology applications. This product is 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.

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding GeneRead Library Quant Kit, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support** or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

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.

24-hour emergency information

Chemical emergency or accident assistance is available 24 hours a day from:

CHEMTREC

USA & Canada ■ Tel: 1-800-424-9300

Outside USA & Canada ■ Tel: +1-703-527-3887 (collect calls accepted)

Introduction

One of the most important factors in a next-generation sequencing experiment is accurate quantification of the prepared library. The accurate quantification of amplifiable library molecules is essential for ensuring optimal quality reads and efficient data generation. Underestimation of amplifiable library molecules leads to mixed signals and non-resolvable data; conversely, overestimation results in poor yield of template-carrying beads (Ion Torrent platform) or clusters (Illumina platform) and reduced usage of sequencing capacity.

The GeneRead Library Quant Kit uses real-time PCR to quantify NGS library. The GeneRead Library Quant Kit specifically quantifies DNA molecules with adaptors at both ends, which are the only amplifiable molecules during emulsion PCR (Ion Torrent platform) or bridge PCR (Illumina platform), and therefore provides highly accurate quantification of amplifiable library molecules. The high sensitivity of real-time PCR allows quantification of libraries with very low concentrations, even below the detection threshold of conventional spectrophotometric methods.

The GeneRead Library Quant Kit is optimized with GeneRead qPCR SYBR Green Mastermixes to provide superior sensitivity and wide linear dynamic ranges. It can be easily automated for high-throughput applications.

Principle and Workflow

The GeneRead Library Quant Kit for Ion Torrent provides a DNA standard and PCR primer assay for reliable and convenient quantification of library input for the Ion Torrent semiconductor sequencing platforms. The Ion Torrent DNA Standard harbors a 130 bp target flanked with the “A” and “trP1” adaptor sequences used in Ion Torrent sequencing libraries. The Ion Torrent DNA Standard generates 183 bp amplicon using the provided PCR assay. The PCR assay is experimentally verified to specifically amplify Ion Torrent library molecules flanked with the “A” and “trP1” adaptor sequences. The primer sequences used in the PCR assay are as follows:

Ion Torrent forward: 5'-CCA TCT CAT CCC TGC GTG TC-3’

Ion Torrent reverse: 5'-CCT CTC TAT GGG CAG TCG GTG AT-3'

The GeneRead Library Quant Kit for Illumina provides a DNA standard and PCR primer assay for reliable and convenient quantification of library input for the Illumina NGS sequencing platforms. The Illumina DNA Standard harbors a

target flanked by “P5” and “P7” primer sequences for Illumina sequencing libraries. The Illumina DNA Standard generates 426 bp amplicon using the provided PCR assay. This PCR assay is experimentally verified to specifically amplify Illumina library molecules with the “P5” and “P7” sequences. The primer sequences used in the PCR assay are as follows:

Illumina forward: 5'-AAT GAT ACG GCG ACC ACC GA-3'

Illumina reverse: 5'-CAA GCA GAA GAC GGC ATA CGA-3'

The GeneRead Library Quant Kit workflow (Figure 1) is simple. The procedure begins with preparing five sequential 10-fold dilutions of DNA Standard and two 10-fold dilutions of sample library (the sample library has to be diluted to a concentration within the range of the serially diluted standards). Next, diluted DNA standards and sample libraries are mixed with the provided PCR assay and the appropriate GeneRead qPCR SYBR Green Mastermix. This mixture is aliquoted into the wells of a real-time PCR plate. PCR is performed and C_T values are exported to a provided Excel-based data analysis file to calculate the Template Dilution Factor (for Ion Torrent platform) or the concentration of the library (for Illumina platform).

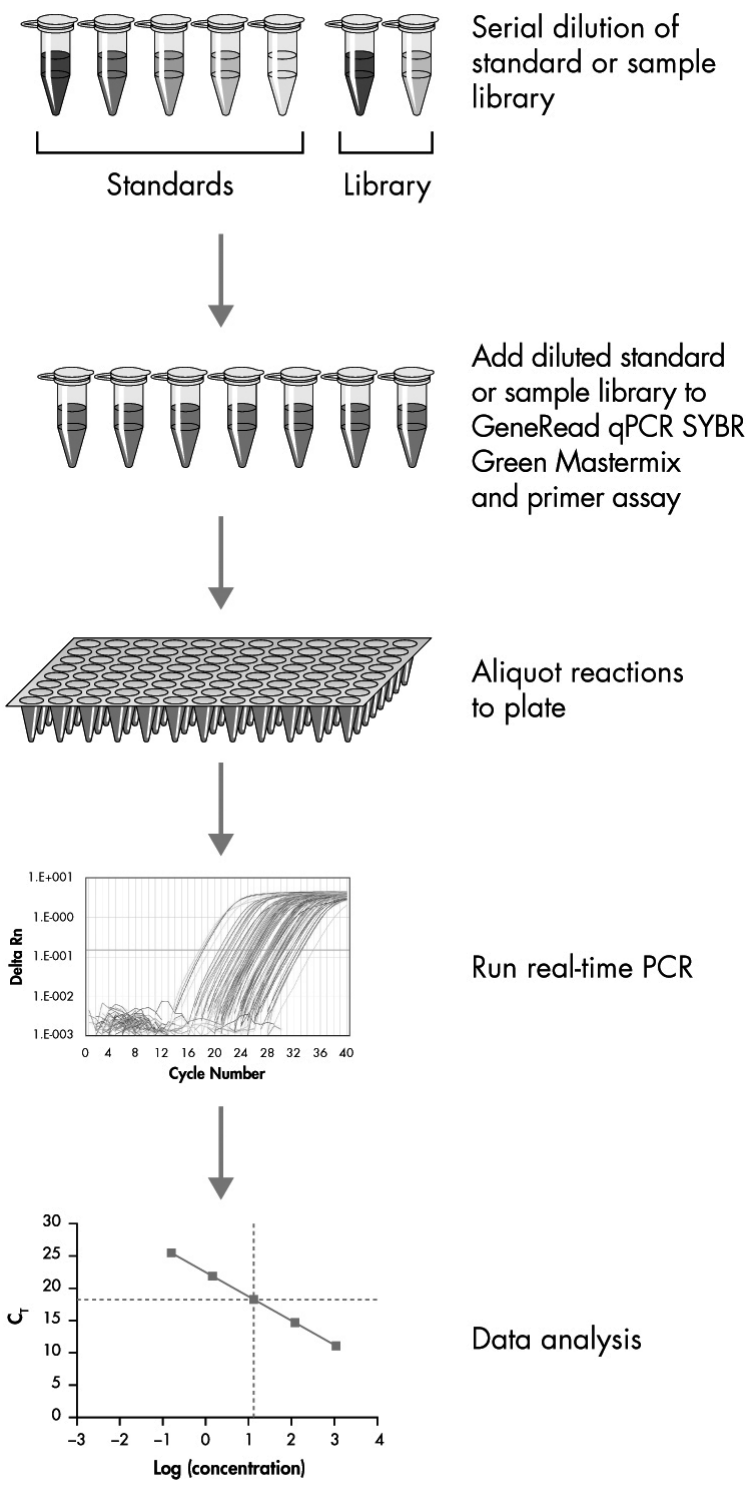

Figure 1. GeneRead Library Quant Kit workflow.

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.

- Real-time PCR instrument
- Calibrated single- and multi-channel pipets
- RNase-/DNase-free pipet tips and tubes
- RNase-/DNase-free 200 µl regular PCR tubes, or 8-tube or 12-tube strips
- Molecular biology grade RNase- and DNase-free water
- Rotor-Disc® heat sealer (if using RGQ)

Important Notes

DNA contamination

For reliable results, it is very important to prevent contamination with foreign DNA. Even very small amounts of foreign DNA can artificially inflate SYBR Green signals, yielding false positive results. The most common source of contamination in PCR reagents comes from the products of previous PCR experiments in your working area. To minimize contamination, follow the recommendations below:

- Wear gloves throughout the entire procedure
- Use only fresh PCR-grade reagents and labware
- Physically separate the workspace for PCR setup and post-PCR work
- Before setting up an experiment, decontaminate the PCR workspace and labware (pipet barrels, tube racks, etc.) with 10% bleach and UV light. Preferentially set up reactions in a PCR workstation.
- Close all tubes containing PCR products as soon as possible after use
- Treat any labware (tips or tubes) containing PCR products or other DNA with 10% bleach before discarding

General considerations

- For accuracy and precision, ensure that micropipettors are calibrated before beginning the protocol. Be sure not to introduce bubbles into the wells when pipetting.
- Do not use DEPC-treated water. Use high-quality, nuclease-free water.
- If precipitates are present in the master mix tubes, warm the reagents at 42°C for 1 min and vortex briefly to dissolve. Repeat if necessary.
- The appropriate GeneRead qPCR SYBR Green Mastermix is already included in the kit. Please ensure that the correct master mix, according to the list that follows, has been included in your order before starting your runs.

Master mix	Cat. no.	Instrument
GeneRead qPCR SYBR Green ROX™ Mastermix	180840	All Applied Biosystems® and Stratagene® instruments, and Eppendorf® Mastercycler® ep *realplex* instruments with a ROX filter set
GeneRead qPCR SYBR Green Fluor Mastermix	180830	Bio-Rad® iCycler®, MyiQ™, MyiQ2™ and iQ™ 5
GeneRead qPCR SYBR Green Mastermix	180820	Bio-Rad models CFX96™, CFX384™, Bio-Rad/MJ Research Opticon 2, and Bio-Rad/MJ Research Chromo4™, Roche® LightCycler® 480 (96- and 384-well)
GeneRead qPCR SYBR Green ROX FAST Mastermix	180850	Rotor-Gene® Q and Rotor-Gene 6000

- The GeneRead Library Quant Kits have been tested on the following cyclers:

Cycler	Plate
Vii7A/Stratagene	A
ABI7500	C
ABI7900	E
Roche-LightCycler II	F
RGQ	Rotor-Disc 100
RGQ	72-well Rotor

- Refer to tables 4, 5, 6 and 10 in this handbook for cycler-specific cycling programs.
- Instrument setup protocols can be found at:

  https://www.qiagen.com/us/shop/assay-technologies/real-time-pcr-and-rt-pcr-reagents/rt2-profiler-pcr-arrays/#resources
- Cyclers for use for different plate formats:

Format	Suitable real-time cyclers	Plate
A	Applied Biosystems 5700, 7000, 7300, 7500 Standard, 7700, 7900HT Standard; Bio-Rad® iCycler, iQ™ 5, MyiQ, MyiQ2, Bio-Rad/MJ Research Chromo4™; Eppendorf Mastercycler® ep realplex 2, 2s, 4, 4s; Stratagene® Mx3005P®, Mx3000P®	96-well
C	Applied Biosystems 7500 FAST, 7900HT FAST, StepOnePlus™	96-well
D	Bio-Rad CFX96™, Bio-Rad/MJ Research Opticon 2®; Stratagene Mx4000®	96-well
E	Applied Biosystems 7900HT (384-well block); Bio-Rad CFX384™	384-well
F	Roche LightCycler 480 II (96-well block)	96-well
G	Roche LightCycler 480 II (384-well block)	384-well
R	QIAGEN Rotor-Gene Q and Rotor-Gene 6000	100-well/ 72-well

Protocol 1: Real-Time PCR for GeneRead Library Quant Kit for Ion Torrent or Illumina (Formats A, C, D, E, F, G)

Important points before starting

- Ensure that the GeneRead qPCR SYBR Green Mastermix (see page 12) and the plate format are suitable for your real-time cycler. An incorrect format will not fit the real-time cycler properly and may damage the real-time cycler.
- If dealing with a new library prep without prior determination of the final concentration, we recommend checking the concentration using Agilent® BioAnalyzer or QIAxcel® Advanced and diluting the library to about 1 nM before proceeding with the protocol given below.

Procedure

Preparing serial dilutions of Ion Torrent or Illumina DNA Standard

1. Thaw Ion Torrent or Illumina DNA Standard on ice.
2. Prepare five sequential 10-fold dilutions from Ion Torrent or Illumina DNA Standard in dilution buffer using PCR tubes or strips according to Table 1.

Table 1. Standard dilution

Standard	Ion Torrent or Illumina DNA Standard	Dilution buffer
Std1	5 µl undiluted	45 µl
Std2	5 µl Std1	45 µl
Std3	5 µl Std2	45 µl
Std4	5 µl Std3	45 µl
Std5	5 µl Std4	45 µl

Preparing sample library dilution

3. Prepare a starting 1:20 dilution of the sample library in dilution buffer (2 µl sample library+ 38 µl dilution buffer).

4. Prepare two working dilutions of sample library, Dilution 1 and Dilution 2, as indicated in Table 2.

Table 2. Sample library dilution

Dilution	Library	Dilution buffer
Dilution1 (1:2000)	2 µl of 1:20	198 µl
Dilution2 (1:20000)	5 µl of 1:2000	45 µl

Note: Always use freshly made dilutions.

Setting up the PCR

5. Briefly centrifuge the GeneRead qPCR SYBR Green Mastermix (10–15 s) to bring the contents to the bottom of the tube.

   Note: As the GeneRead qPCR SYBR Green Mastermix contains HotStarTaq® DNA Polymerase, which is active only after heat activation, reactions can be prepared at room temperature (15–25°C).

6. Make PCR mix for each sample (for triplicates on a 96-well plate or quadruplicates on a 384-well plate) using PCR tubes or strips according to Table 3.

Table 3. PCR mix for each sample (standard or sample library)*

Component	For 96-well plate (A,C,D,F)	For 384-well plate (E,G)
RNase-/DNase-free water	30.6 µl	17 µl
GeneRead qPCR SYBR Green Mastermix	45 µl	25 µl
Primer mix (10 µM)	3.6 µl	2 µl
Template (standard or sample library)	10.8 µl	6 µl
Final volume	**90 µl**	**50 µl**

* For multiple wells, an excess volume is provided to allow for pipetting errors. Perform pipetting steps as precisely as possible to ensure that each well receives the required volume. For NTC, add dilution buffer as template.

7. Add 25 µl per well PCR mix to three wells for the 96-well plate (see Figure 2 for layout) or 10 µl/per well PCR mix to four wells for the 384-well plate (see Figure 3 for layout) for each sample dilution.

   The layouts of figures 2 and 3 must be followed as shown for appropriate data analysis.

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1	Standard 1	Standard 1	Library 2 dilution 1	Library 2 dilution 1	Library 2 dilution 1	Library 6 dilution 1	Library 6 dilution 1	Library 6 dilution 1	Library 10 dilution 1	Library 10 dilution 1	Library 10 dilution 1
B	Standard 2	Standard 2	Standard 2	Library 2 dilution 2	Library 2 dilution 2	Library 2 dilution 2	Library 6 dilution 2	Library 6 dilution 2	Library 6 dilution 2	Library10 dilution 2	Library10 dilution 2	Library10 dilution 2
C	Standard 3	Standard 3	Standard 3	Library 3 dilution 1	Library 3 dilution 1	Library 3 dilution 1	Library 7 dilution 1	Library 7 dilution 1	Library 7 dilution 1	Library11 dilution 1	Library11 dilution 1	Library11 dilution 1
D	Standard 4	Standard 4	Standard 4	Library 3 dilution 2	Library 3 dilution 2	Library 3 dilution 2	Library7 dilution 2	Library7 dilution 2	Library7 dilution 2	Library 11 dilution 2	Library 11 dilution 2	Library 11 dilution 2
E	Standard 5	Standard 5	Standard 5	Library 4 dilution 1	Library 4 dilution 1	Library 4 dilution 1	Library 8 dilution 1	Library 8 dilution 1	Library 8 dilution 1	Library 12 dilution 1	Library 12 dilution 1	Library 12 dilution 1
F	NTC	NTC	NTC	Library 4 dilution 2	Library 4 dilution 2	Library 4 dilution 2	Library 8 dilution 2	Library 8 dilution 2	Library 8 dilution 2	Library12 dilution 2	Library12 dilution 2	Library12 dilution 2
G	Library 1 dilution 1	Library 1 dilution 1	Library 1 dilution 1	Library 5 dilution 1	Library 5 dilution 1	Library 5 dilution 1	Library 9 dilution 1	Library 9 dilution 1	Library 9 dilution 1	Library13 dilution 1	Library13 dilution 1	Library13 dilution 1
H	Library 1 dilution 2	Library 1 dilution 2	Library 1 dilution 2	Library 5 dilution 2	Library 5 dilution 2	Library 5 dilution 2	Library9 dilution 2	Library9 dilution 2	Library9 dilution 2	Library 13 dilution 2	Library 13 dilution 2	Library 13 dilution 2

Figure 2. GeneRead Library Quant Kit Layout (96-well plate).

Well	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	standard1		Library 2 dilution 1		Library 6 dilution 1		Library 10 dilution 1		Library 14 dilution 1		Library 18 dilution 1		Library 22 dilution 1		Library 26 dilution 1		Library 30 dilution 1		Library 34 dilution 1		Library 38 dilution 1		Library 42 dilution 1	
B																								
C	standard2		Library 2 dilution 2		Library 6 dilution 2		Library10 dilution 2		Library 14 dilution 2		Library 18 dilution 2		Library 22 dilution 2		Library 26 dilution 2		Library 30 dilution 2		Library 34 dilution 2		Library 38 dilution 2		Library 42 dilution 2	
D																								
E	standard3		Library 3 dilution 1		Library 7 dilution 1		Library11 dilution 1		Library 15 dilution 1		Library 19 dilution 1		Library 23 dilution 1		Library 27 dilution 1		Library 31 dilution 1		Library 35 dilution 1		Library 39 dilution 1		Library 43 dilution 1	
F																								
G	standard4		Library 3 dilution 2		Library 7 dilution 2		Library 11 dilution 2		Library 15 dilution 2		Library 19 dilution 2		Library 23 dilution 2		Library 27 dilution 2		Library 31 dilution 2		Library 35 dilution 2		Library 39 dilution 2		Library 43 dilution 2	
H																								
I	standard5		Library 4 dilution 1		Library 8 dilution 1		Library 12 dilution 1		Library 16 dilution 1		Library 20 dilution 1		Library 24 dilution 1		Library 28 dilution 1		Library 32 dilution 1		Library 36 dilution 1		Library 40 dilution 1		Library 44 dilution 1	
J																								
K	NTC		Library4 dilution 2		Library 8 dilution 2		Library12 dilution 2		Library 16 dilution 2		Library 20 dilution 2		Library 24 dilution 2		Library 28 dilution 2		Library 32 dilution 2		Library 36 dilution 2		Library 40 dilution 2		Library 44 dilution 2	
L																								
M	Library 1 dilution 1		Library 5 dilution 1		Library 9 dilution 1		Library13 dilution 1		Library 17 dilution 1		Library 21 dilution 1		Library 25 dilution 1		Library 29 dilution 1		Library 33 dilution 1		Library 37 dilution 1		Library 41 dilution 1		Library 45 dilution 1	
N																								
O	Library 1 dilution 2		Library 5 dilution 2		Library 9 dilution 2		Library 13 dilution 2		Library 17 dilution 2		Library 21 dilution 2		Library 25 dilution 2		Library 29 dilution 2		Library 33 dilution 2		Library 37 dilution 2		Library 41 dilution 2		Library 45 dilution 2	
P																								

Figure 3. GeneRead Library Quant Kit layout (384-well plate).

8. Carefully, tightly seal the plate with Optical Thin-Wall 8-Cap Strips (Formats A and D) or Optical Adhesive Film (Formats C, E, F and G).

   Note: Users of Bio-Rad and Eppendorf real-time cyclers must ensure that the real-time cycler has been calibrated to use clear, flat optical caps with plates prior to initiating the run.

9. Centrifuge for 1 min at 1000 x *g* at room temperature (15–25°C) to remove bubbles. Visually inspect the plate from underneath to ensure no bubbles are present in the wells.

   Note: The presence of bubbles in the wells interferes with results.

10. Place the plate on ice while setting up the PCR cycling program.

    Note: The plates containing PCR components mix may be stored at –20°C wrapped in aluminum foil for up to one week if desired.

11. Program the real-time cycler according to Table 4, 5 or 6, depending on the real-time cycler used. If prompted by your cycler software, select "Absolute Quantitation" to begin.

Table 4. Cycling conditions* for Applied Biosystems, Bio-Rad,† Stratagene and Eppendorf‡ cyclers

Cycles	Duration	Temperature	Comments
1	10 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step.
30	15 s	95°C	
	30 s	60°C	
	2 min	72°C	Perform fluorescence data collection.

* Recommended for the following cyclers: Applied Biosystems models 5700, 7000, 7300, 7500, 7700, 7900HT, StepOnePlus, ViiA 7; Bio-Rad models iCycler, iQ5, MyiQ, MyiQ2, CFX96, CFX384; Stratagene models Mx3000P, Mx3005P, Mx4000P; Eppendorf Mastercycler ep realplex models 2, 2S, 4, 4S.

† For Bio-Rad models CFX96 and CFX384: adjust the ramp rate to 1°C/s.

‡ For Eppendorf Mastercyler ep realplex models 2, 2S, 4 and 4S: for the Silver Thermoblock, adjust the ramp rate to 26%; for the Aluminum Thermoblock, adjust the ramp rate to 35%. Refer to the Instrument Setup Guide at **https://www.qiagen.com/us/shop/assay-technologies/real-time-pcr-and-rt-pcr-reagents/rt2-profiler-pcr-arrays/#resources** for detailed setup instructions.

Table 5. Cycling conditions for Roche LightCycler 480 II*

Cycles	Duration	Temperature	Comments
1	10 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step.
35	15 s	95°C	
	30 s	60°C	
	2 min	72°C	Perform fluorescence data collection.

* Recommended for the Roche LightCycler 480 II. If using a Roche LightCycler 480 II, adjust the ramp rate to 1.5°C/s for 96-well plate and 2°C/s for 384-well plate. Refer to the "Instrument Setup Guide" at **https://www.qiagen.com/us/shop/assay-technologies/real-time-pcr-and-rt-pcr-reagents/rt2-profiler-pcr-arrays/#resources** for more information on other required changes to settings for melt curve acquisition.

Table 6. Cycling conditions for Bio-Rad and Takara cyclers and all other cyclers†

Cycles	Duration	Temperature	Comments
1	10 min	95°C	HotStart DNA *Taq* Polymerase is activated by this heating step.
30	15 s	95°C	
	30–40 s	55°C	Perform fluorescence data collection. Different cyclers need different lengths of time to detect the fluorescent signal. Choose the appropriate time for the annealing step (55°C) for your cycler.
	60 s	72°C	

† Recommended for the following cyclers: Bio-Rad/MJ Research models Chromo4, DNA Engine Opticon, DNA Engine Opticon 2; Takara TP-800; all other cyclers.

12. Place the plate in the real-time cycler. If recommended by the cycler user manual, use a compression pad with plate sealed with optical adhesive film (formats C, E, F and G). Start the run.

Protocol 2: Real-Time PCR for GeneRead Library Quant Kit for Ion Torrent or Illumina (Format R)

Important points before starting

- Ensure that GeneRead qPCR SYBR Green ROX FAST Mastermix (180850) is used with a Rotor-Gene cycler.
- If dealing with new library prep without prior experience of final concentration, we recommend you to check concentration using Agilent BioAnalyzer or QIAxcel Advanced and dilute the library to about 1nM and proceed with protocol given below.

Procedure

Preparing serial dilutions of Ion Torrent or Illumina DNA Standard

1. Thaw Ion Torrent or Illumina DNA Standard on ice.
2. Prepare five sequential 10-fold dilutions from Ion Torrent or Illumina DNA Standard in dilution buffer using PCR tubes or strips according to Table 7.

Table 7. Standard dilution

Standard	Ion Torrent or Illumina DNA Standard	Dilution buffer
Std1	5 µl undiluted	45 µl
Std2	5 µl Std1	45 µl
Std3	5 µl Std2	45 µl
Std4	5 µl Std3	45 µl
Std5	5 µl Std4	45 µl

Preparing sample library dilution

3. Prepare a starting 1:20 dilution of the sample library in dilution buffer (2 µl sample library + 38 µl dilution buffer).
4. Prepare two working dilutions of sample library, Dilution 1 and Dilution 2, as indicated in Table 8.

Table 8. Sample library dilution

Dilution	Library	Dilution buffer
Dilution 1 (1:2000)	2 µl of 1:20	198 µl
Dilution 2 (1:20000)	5 µl of 1:2000	45 µl

Note: Always use freshly made dilutions.

Setting up the PCR

5. Briefly centrifuge the GeneRead qPCR SYBR Green ROX FAST Mastermix (10–15 s) to bring the contents to the bottom of the tube.

   Note: As the GeneRead qPCR SYBR Green ROX FAST Mastermix contains HotStarTaq DNA Polymerase, which is active only after heat activation, reactions can be prepared at room temperature (15–25°C).

6. Make PCR reaction mix for each sample (for triplicates) using PCR tubes or strips according to Table 9.

Table 9. PCR mix for each sample dilution (Dilution 1 and 2) and standard*

Component	Rotor-Disc 100 or 72-well Rotor
RNase-/DNase-free water	26.8 µl
GeneRead qPCR SYBR Green ROX FAST Mastermix	40 µl
Primer mix (10 µM)	3.2 µl
Template (standards or sample library)	10 µl
Final volume	**80 µl**

* For multiple wells, an excess volume is provided to allow for pipetting errors. Perform pipetting steps as precisely as possible to ensure that each well receives the required volume. For NTC, add dilution buffer as template.

7. Slide the plate into the Rotor-Disc 100 or 72-well Rotor Loading Block using the tab at position A1 and the tube guide holes.
8. Add 20 µl per well PCR components mix to three wells (see Figure 4 for layout) for each sample dilution or standard.

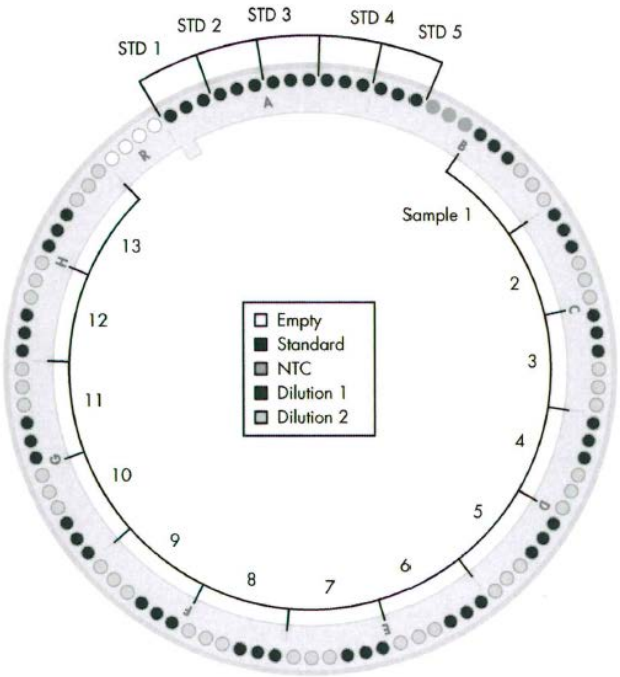

Figure 4. GeneRead Library Quant Kit layout (Format R; Rotor-Disc 100).

Note: Although wells 97–100 do not contain assays, it is essential to add PCR components mix for optimized balancing.

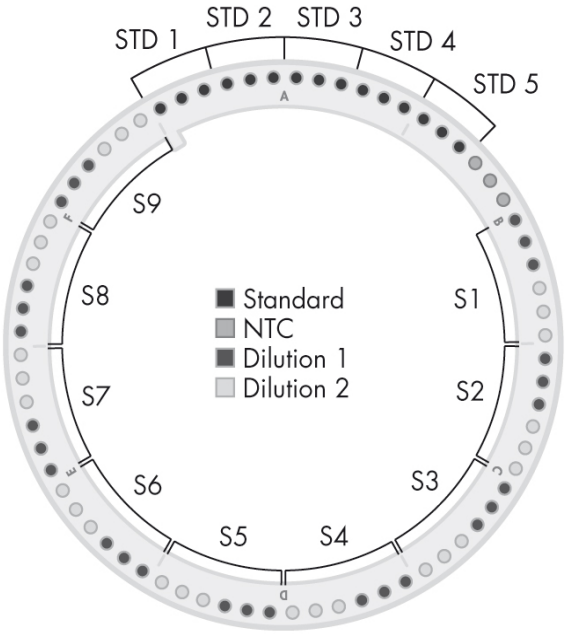

Figure 5. GeneRead Library Quant Kit layout (Format R; 72-well Rotor).

The layouts of figures 4 and 5 must be followed as shown for appropriate data analysis.

9. Carefully seal the plate with Rotor-Disc Heat-Sealing Film using the Rotor-Disc Heat Sealer. For detailed instructions, see the *Rotor-Gene Q User Manual*.
10. Program the real-time cycler according to Table 10.

    Note: For additional help with instrument setup, see our instrument-specific setup instructions and protocol files at:

    https://www.qiagen.com/us/shop/assay-technologies/real-time-pcr-and-rt-pcr-reagents/rt2-profiler-pcr-arrays/#resources

Table 10. Cycling conditions for Rotor-Gene cyclers (both Rotor-Disc 100 and 72-well Rotor)

Cycles	Duration	Temperature	Comments
1	10 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step.
30	15 s	95°C	
	5 s	68°C	
	5 s	65°C	
	60 s	60°C	Perform fluorescence data collection.

11. Insert the plate into the Rotor-Gene cycler and secure with the Rotor-Disc 100 or 72-well Rotor Locking Ring. Start the run. For detailed instructions, see the *Rotor-Gene Q User Manual*.

Data Analysis

Obtaining raw threshold cycle (C_T) values

After the cycling program has completed, obtain the C_T values according to the instructions provided by the manufacturer of the real-time PCR instrument. We recommend manually setting the baseline and threshold values as follows.

Baseline: Use automatic baseline.

Threshold value: Using the Log View of the amplification plots, place the threshold above the background signal but within the lower third of the linear portion of the amplification curves.

Exporting C_T values

Export and/or copy/paste the C_T values from the instrument software to a blank Microsoft® Excel® spreadsheet according the manufacturer's instructions for the real-time PCR instrument.

Excel-based data analysis template

First, download the GeneRead Library Quant Kit Excel-based data analysis file that corresponds to the plate format used, which is available at:
https://www.qiagen.com/us/shop/sample-technologies/dna-sample-technologies/genomic-dna/generead-library-quant-system/#resources

Second, paste the raw C_Ts (for the whole plate, even if you only use part of the plate) into the GeneRead Library Quant Kit Excel-based data analysis file. Enter the required run parameters.

1. Dilution fold
    a. If you are using the default dilutions (2000 and 20,000), no changes are necessary
    b. If you are not using the default dilutions (2000 and 20,000), enter your library dilutions
2. Library size
    a. If you used the GeneRead DNAseq panels with the GeneRead Library Prep system, do not change the library size in the data analysis file for either Illumina or Ion Torrent.
    b. If you used a different target enrichment or library construction method, enter the appropriate size of library fragments.

Analyze the automatically generated results by following the directions in the "Instructions" worksheet of the Excel file.

Finally, dilute the original library to perform emPCR (for Ion Torrent platforms) or dilute the original library to designed range for cluster generation (for Illumina platforms). If you used the GeneRead DNAseq panels with the GeneRead Library Prep system, refer to Appendix F of the all-bead *GeneRead DNAseq Targeted Panels V2 Handbook*).

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
No PCR product is generated	
a) Assay reagents or master mix are incorrectly stored	Storing assay reagents or master mix at inappropriate temperature for extended periods may cause degradation or reduce their activity and PCR amplification efficiency.
b) Incorrect real-time PCR cycling program is used	Be sure to use the correct cycling program, including 10 minutes at 95°C to fully activate the hot start enzyme in the GeneRead qPCR SYBR Green Mastermix.
Signal is detected for no-template controls (NTC)	
a) Reaction is contaminated by nucleic acids	Follow recommendations for PCR handling (see page 11 for details).
b) Primer dimers or false amplification are generated	Primer dimers or false amplification may occur in NTCs. As long as the C_T is high (>29), it is far enough above the C_T range for the actual template that it will have no effect on quantification.
PCR efficiency is out of the range of 90%–110%	
a) PCR conditions are not optimal	Ensure that all the regents are stored properly and reactions are set correctly
b) Dilution is not performed correctly	Pay attention to pipetting steps and use dilution buffer included in the kit for dilution.
c) PCR program is wrong.	Pay close attention to the different PCR program for different machines.

Comments and suggestions	
C_T of sample library is out of the range of standard curve (C_T between 6–25 depending on the real-time cycler)	
a) Library concentration is too high	Dilute more to ensure that at least one of the diluted concentrations is within the range of serial dilution standards.
b) Library concentration is too low	Dilute less to ensure that at least one of the diluted concentrations is within the range of serial dilution standards.

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.

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Cited references

1. Buehler, B. (2010) Rapid quantification of DNA libraries for next-generation sequencing. Methods 50, S15.

Ordering Information

Product	Contents	Cat. no.
GeneRead Library Quant Kit	Laboratory-verified forward and reverse primers for 500 x 25 µl reactions (500 µl) Standard (100 µl); Dilution buffer (30ml) GeneRead qPCR SYBR Green Mastermix (1.35 ml x 5) (varies depend on plate format)	180612
Related products		
GeneRead Library Quant Array	Two arrays in Formats A, C, D, F, R or 1 array in Format E or G	180611

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