August 2015

GeneRead™ DNAseq Library Quant Array Handbook

For reliable quantification of Ion Torrent™ or Illumina® libraries and target enrichment QC for GeneRead DNAseq Targeted Panels using real-time PCR

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Contents

Kit Contents

GeneRead DNAseq Library Quant Array							
Catalog no.	**180601 (Varies depending on format)**						
Format	**A**	**C**	**D**	**E**	**F**	**G**	**R**
Plate containing dried assays	96-well (2)	96-well (2)	96-well (2)	384-well (1)	96-well (2)	384-well (1)	100-well (2)
Optical Thin-Wall 8-Cap Strips (12 per plate)	12 x 2	–	12 x 2	–	–	–	–
Optical adhesive film	–	1 x 2	–	1	1 x 2	1	–
Rotor-Disc Heat-Sealing Film	–	–	–	–	–	–	1 x 2
Handbook				1			

Array formats for use with real-time PCR cyclers

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

Storage

The GeneRead DNAseq Library Quant Array is shipped at ambient temperature, on ice, or on dry ice depending on the destination and accompanying products. Upon receipt, store at –20°C. If stored under these conditions, GeneRead DNAseq Library Quant Array is stable for 6 months after receipt.

Quality Control

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

Intended Use

The GeneRead DNAseq Library Quant Array 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 the GeneRead DNAseq Library Quant Array, 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

GeneRead DNAseq Targeted Panels use multiplex PCR-based target enrichment technology to amplify and enrich genes of interest or targeted regions in the human genome for detecting genetic variation using next-generation sequencing (NGS). After multiplex PCR is performed to generate amplicons for target enrichment, the amplicons are pooled and purified and are then subjected to NGS library construction.

One of the most important factors in a NGS 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 DNAseq Library Quant Array uses real-time PCR to quantify NGS libraries. The GeneRead DNAseq Library Quant Array 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 DNAseq Library Quant Array 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.

The GeneRead DNAseq Library Quant Array also includes sets of QC primers to monitor the results of the target enrichment procedure. A target enrichment QC score can be calculated for each library construct. This score serves as a checkpoint before sequencing setup. Appropriate usage of the score enables early identification of poor samples or library constructs, helping to prevent setup of costly sequencing runs on samples that would not generate meaningful results.

Principle and Workflow

The GeneRead DNAseq Library Quant Array for the Ion Torrent platform contains five pre-dispensed, sequential 10-fold dilutions of Ion Torrent DNA Standard mixed with a PCR primer assay in triplicates, and PCR primer assays in the remaining wells of a 96-well, 384-well or 100-well PCR plate. The

predispensed, serially diluted DNA standards and PCR primer assay are highly convenient for quantification of library input.

The Ion Torrent DNA standard harbors a 130 bp target flanked with the "A" and "trP1" adaptor sequences for Ion Torrent sequencing libraries. The Ion Torrent DNA Standard generates a 183 bp amplicon using the provided PCR assay. This 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 DNAseq Library Quant Array for Illumina contains five pre-dispensed, sequential 10-fold dilutions of Illumina DNA Standard mixed with a PCR primer assay in triplicates, and PCR primer assays in the remaining wells of a 96-well, 384-well or 100-well PCR plate. The predispensed, serially diluted DNA standards and PCR primer assay are highly convenient for quantification of library input.

The Illumina DNA Standard harbors a target flanked by "P5" and "P7" primer sequences for Illumina sequencing libraries. The Illumina DNA Standard generates a 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'

Each GeneRead DNAseq Targeted Panel contains a set of spike-in controls, and the GeneRead DNAseq Library Quant Array provides a set of wells designated to measure those controls. Based on the representation of the controls in the overall library, a target enrichment QC score can be calculated. Briefly, NGS library abundance is determined by the C_T of primers targeting NGS library adaptor sequences. The experimental representation of the control target in the library is calculated based on those C_Ts. The theoretical representation of the controls in the library is determined based on the total number of controls and total number of different amplicons in each panel. The target enrichment QC score is then generated by calculating the deviation between the experimental and the theoretical numbers. The data analysis then provides a final call for the

target enrichment procedure as "Pass" or "Fail" based on the QC score (Table 1).

Table 1. Explanation of QC scores

QC Score	QC results	Recommendations
1–8	Pass	Proceed with sequencing. Sequencing results will mostly meet standard specifications for specificity and coverage.
>8	Fail	Do not sequence the library. Sequencing results will not meet standard specifications. Check sample quality or workflow.

The GeneRead DNAseq Library Quant Array workflow (Figure 1) is very simple. The procedure begins with two 10-fold dilutions of a sample library (the sample library must be diluted to a concentration within the range of the serially diluted standards). Next, appropriate mixtures of PCR are prepared. These mixtures are aliquotted into the wells of a real-time PCR plate. PCR is performed and C_T values are exported to an Excel® data analysis file to calculate the Template Dilution Factor (for the Ion Torrent platform) or the concentration of the library (for the Illumina platform). In addition, the data analysis file will provide a final call for the target enrichment procedure as "Pass" or "Fail."

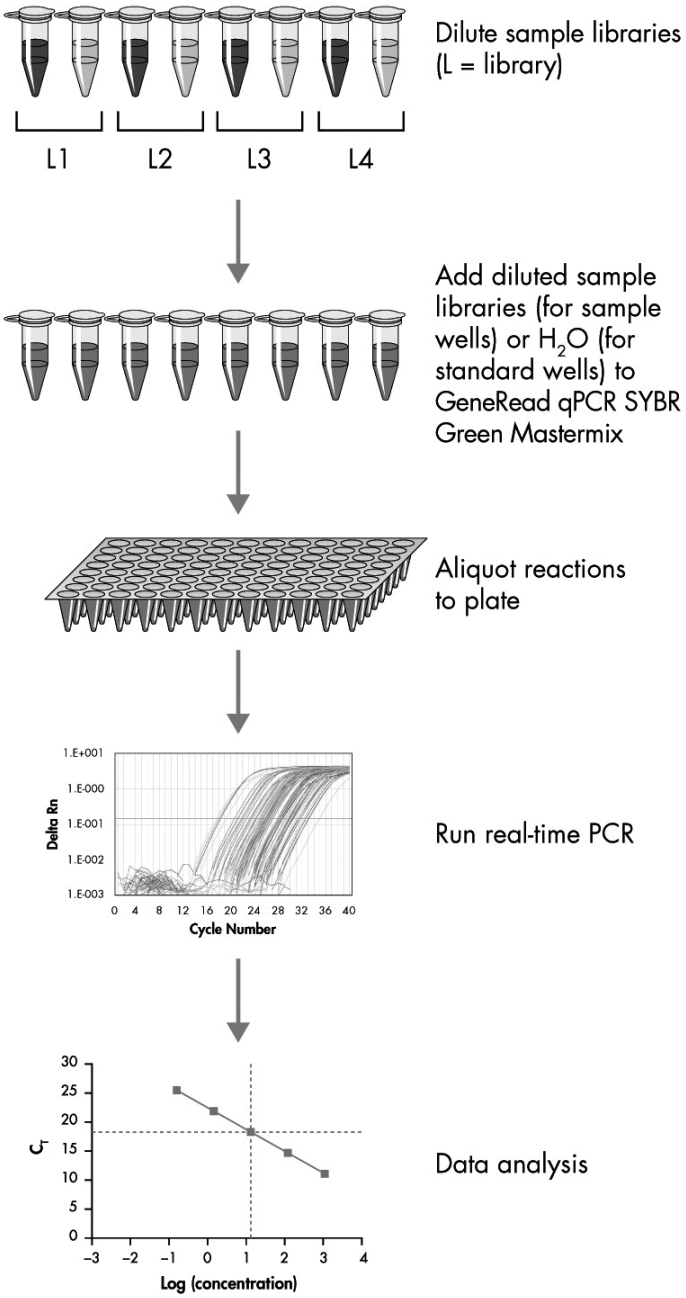

Figure 1. GeneRead DNAseq Library Quant Array workflow.

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.

- Appropriate GeneRead qPCR SYBR Green Mastermix (be sure to select the correct format for the PCR instrument):

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 II 480 (96- and 384-well)
GeneRead qPCR SYBR Green ROX FAST Mastermix	180850	Rotor-Gene Q and Rotor-Gene 6000

- 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
- Dilution buffer (10 mM Tris-Cl, pH7.8 or QIAGEN EB buffer)
- Rotor-Disc® heat sealer (if using RGQ).

Important Notes

DNA contamination

For reliable results, it is very important to prevent contamination of 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 the 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 GeneRead DNAseq Library Quant Arrays have been tested on the following cyclers:

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

- Refer to tables 4, 5, 6 and 9 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

Protocol 1: Real-Time PCR for GeneRead DNAseq Library Quant Array 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 11) and the plate format are suitable for your real-time cycler (see page 4). An incorrect format will not fit the real-time cycler properly and may damage the real-time cycler.
- If you are dealing with a new library prep without prior measurement 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 sample library dilution

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

3. 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).

4. Make PCR mix for standards or sample libraries (according to Table 3a or 3b).

Table 3a. PCR mix for standard or sample libraries* for 96-well plate (formats A, C, D, F)

Component	For standards and NTC	For sample libraries	
	Rows A and B	Dilution 1 (9 wells)	Dilution 2 (3 wells)
RNase-/DNase-free water	325 µl	96 µl	32 µl
GeneRead qPCR SYBR Green Mastermix	325 µl	126 µl	42 µl
Template (sample library)	–	30 µl	10 µl
Final volume	**650 µl**	**252 µl**	**84 µl**

Table 3b. PCR mix for standards or sample libraries* for 384-well plate (formats E, G)

Component	For standard and NTC	For sample libraries	
	Rows A and B (wells 1–12)	Dilution 1 (8 wells)	Dilution 2 (4 wells)
RNase-/DNase-free water	150 µl	38 µl	19 µl
GeneRead qPCR SYBR Green Mastermix	150 µl	50 µl	25 µl
Template (sample library)	–	12 µl	6 µl
Final volume	**300 µl**	**100 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.

5. Add 25 µl PCR mix to each well for the 96-well plate (see Figure 2 for layout) or 10 µl PCR mix to each well for the 384-well plate (see Figure 3 for layout).

   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	Standard 2	Standard 2	Standard 2	Standard 3	Standard 3	Standard 3	Standard 4	Standard 4	Standard 4
B	Standard 5	Standard 5	Standard 5	NTC	NTC	NTC	NTC for QC	NTC for QC	NTC for QC	NTC for QC	NTC for QC	NTC for QC
C	Library 1 dilution 1	Library 1 dilution 1	Library 1 dilution 1	Library 1 dilution 1	Library 1 dilution 1	Library 1 dilution 1	Library 1 dilution 1	Library 1 dilution 1	Library 1 dilution 1	Library 1 dilution 2	Library 1 dilution 2	Library 1 dilution 2
D	Library 2 dilution 1	Library 2 dilution 1	Library 2 dilution 1	Library 2 dilution 1	Library 2 dilution 1	Library 2 dilution 1	Library 2 dilution 1	Library 2 dilution 1	Library 2 dilution 1	Library 2 dilution 2	Library 2 dilution 2	Library 2 dilution 2
E	Library 3 dilution 1	Library 3 dilution 1	Library 3 dilution 1	Library 3 dilution 1	Library 3 dilution 1	Library 3 dilution 1	Library 3 dilution 1	Library 3 dilution 1	Library 3 dilution 1	Library 3 dilution 2	Library 3 dilution 2	Library 3 dilution 2
F	Library 4 dilution 1	Library 4 dilution 1	Library 4 dilution 1	Library 4 dilution 1	Library 4 dilution 1	Library 4 dilution 1	Library 4 dilution 1	Library 4 dilution 1	Library 4 dilution 1	Library 4 dilution 2	Library 4 dilution 2	Library 4 dilution 2
G	Library 5 dilution 1	Library 5 dilution 1	Library 5 dilution 1	Library 5 dilution 1	Library 5 dilution 1	Library 5 dilution 1	Library 5 dilution 1	Library 5 dilution 1	Library 5 dilution 1	Library 5 dilution 2	Library 5 dilution 2	Library 5 dilution 2
H	Library 6 dilution 1	Library 6 dilution 1	Library 6 dilution 1	Library 6 dilution 1	Library 6 dilution 1	Library 6 dilution 1	Library 6 dilution 1	Library 6 dilution 1	Library 6 dilution 1	Library 6 dilution 2	Library 6 dilution 2	Library 6 dilution 2

Figure 2. GeneRead DNAseq Library Quant Array 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 B	standard1	standard2	standard3	standard4	standard5	NTC	Library 1 diultion 1	Library 1 diultion 1	Library 1 diultion 2	Library 2 diultion 1	Library 2 diultion 1	Library 2 diultion 2
C D	Library 3 diultion 1	Library 3 diultion 1	Library 3 diultion 2	Library 4 diultion 1	Library 4 diultion 1	Library 4 diultion 2	Library 5 diultion 1	Library 5 diultion 1	Library 5 diultion 2	Library 6 diultion 1	Library 6 diultion 1	Library 6 diultion 2
E F	Library 7 diultion 1	Library 7 diultion 1	Library 7 diultion 2	Library 8 diultion 1	Library 8 diultion 1	Library 8 diultion 2	Library 9 diultion 1	Library 9 diultion 1	Library 9 diultion 2	Library 10 diultion 1	Library 10 diultion 1	Library 10 diultion 2
G H	Library 11 diultion 1	Library 11 diultion 1	Library 11 diultion 2	Library 12 diultion 1	Library 12 diultion 1	Library 12 diultion 2	Library 13 diultion 1	Library 13 diultion 1	Library 13 diultion 2	Library 14 diultion 1	Library 14 diultion 1	Library 14 diultion 2
I J	Library 15 diultion 1	Library 15 diultion 1	Library 15 diultion 2	Library 16 diultion 1	Library 16 diultion 1	Library 16 diultion 2	Library 17 diultion 1	Library 17 diultion 1	Library 17 diultion 2	Library 18 diultion 1	Library 18 diultion 1	Library 18 diultion 2
K L	Library 19 diultion 1	Library 19 diultion 1	Library 19 diultion 2	Library 20 diultion 1	Library 20 diultion 1	Library 20 diultion 2	Library 21 diultion 1	Library 21 diultion 1	Library 21 diultion 2	Library 22 diultion 1	Library 22 diultion 1	Library 22 diultion 2
M N	Library 23 diultion 1	Library 23 diultion 1	Library 23 diultion 2	Library 24 diultion 1	Library 24 diultion 1	Library 24 diultion 2	Library 25 diultion 1	Library 25 diultion 1	Library 25 diultion 2	Library 26 diultion 1	Library 26 diultion 1	Library 26 diultion 2
O P	Library 27 diultion 1	Library 27 diultion 1	Library 27 diultion 2	Library 28 diultion 1	Library 28 diultion 1	Library 28 diultion 2	Library 29 diultion 1	Library 29 diultion 1	Library 29 diultion 2	Library 30 diultion 1	Library 30 diultion 1	Library 30 diultion 2

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

6. 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.

7. 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.

8. 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.

9. 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* cycler

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	HotStarTaq DNA 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.

10. 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 DNAseq Library Quant Array 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 you are dealing with a new library prep without prior measurement 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 sample library dilution

1. Prepare a starting 1:20 dilution of the sample library in dilution buffer (2 µl sample library + 38 µl dilution buffer).
2. Prepare two working dilutions of sample library, Dilution 1 and Dilution 2, as indicated in Table 7.

Table 7. 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

3. 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).
4. Make PCR mix for standards and sample libraries according to Table 8.

Table 8. PCR mix for standards or sample libraries*, Rotor-Disc 100

Component	For standard and NTC	For sample library	
	A1–B12 and R	**Dilution 1 (9 wells)**	**Dilution 2 (3 wells)**
RNase-/DNase-free water	336 µl	81 µl	27 µl
GeneRead qPCR SYBR Green ROX FAST Mastermix	336 µl	108 µl	36 µl
Template	–	27 µl	9 µl
Final volume	**672 µl**	**216 µl**	**72 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.

5. Slide the plate into the Rotor-Disc 100 Loading Block using the tab at position A1 and the tube guide holes.
6. Add 20 µl PCR mix to each well (see Figure 4 for layout).

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

   The layout of figure 4 must be followed as shown for appropriate data analysis.

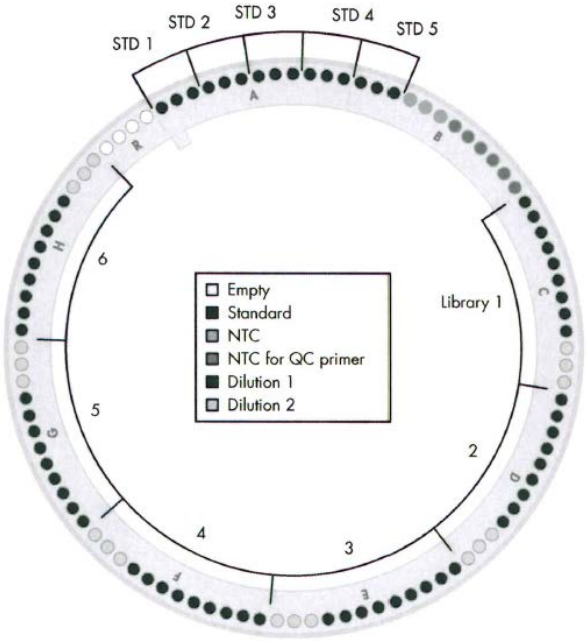

Figure 4. GeneRead DNAseq Library Quant Array layout (Format R).

7. 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*.
8. Program the real-time cycler according to Table 9.

   Note: For additional help with instrument setup, see our instrument-specific setup instructions and protocol files at (under 'Supplementary Protocols'): **https://www.qiagen.com/us/shop/assay-technologies/real-time-pcr-and-rt-pcr-reagents/rt2-profiler-pcr-arrays/#resources**

Table 9. Cycling conditions for Rotor-Gene cyclers

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.

9. Insert the plate into the Rotor-Disc 100 Rotor and secure with the Rotor-Disc 100 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 file

First, download the GeneRead DNAseq Library Quant Array 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 DNAseq Library Quant Array Excel-based data analysis file. Enter the required run parameters.

1. Dilution fold
    a. If you are using the default dilutions (2000 and 20000), no changes are necessary
    b. If you are not using the default dilutions (2000 and 20000), enter your library dilutions
2. Total primer pair
    a. Enter the total number of primer pairs (amplicons) of the panel you are using
        i. If using a cataloged panel, refer to table 1 in the data analysis file

ii. If using a Mix-n-Match or Custom panel, the total number of amplicons is designated by the last digits of the panel number. For example, a panel with a catalog number of CNGHS-99999X-1000 has 1000 primer pairs (amplicons).

3. 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 the GeneRead DNAseq panels with a different library prep system, enter the appropriate size of library fragments.

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

Third, check the final call for the target enrichment procedure. If "pass" is shown, proceed to sequencing. Otherwise, if "fail" is shown, perform target enrichment PCR again.

Finally, use the "template dilution factor" to dilute the original library to perform emPCR (for Ion Torrent platform) or dilute the original library to designed range for cluster generation (for Illumina platform). Refer to Appendix F of the *GeneRead DNAseq Targeted Panels V2 Handbook 06/15.*

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 12 for details).
b) Primer dimers or false amplification is generated.	Primer dimers or false amplification may occur in NTCs. As long as the C_T is high (e.g. >29), this 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) PCR program is wrong.	Pay close attention to different PCR programs for different machines.
C_T of sample library is out of the range of standard curve (C_T between 6–25, depending on real-time cycler used)	

	Comments and suggestions
a) Library concentration is too high	Dilute further to ensure that at least one of the diluted concentrations is within the range of the 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 the 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 DNAseq Library Quant Array	Two arrays in Formats A,C,D, F, R or 1 array in Format E,G	180601
Related products		
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	180612
GeneRead qPCR SYBR Green Mastermix (2)*	For 2 x 96 assays in 96-well plates; suitable for use with real-time cyclers that do not require a reference dye, including: Bio-Rad models CFX96, CFX384, Bio-Rad/MJ Research Chromo4, Bio-Rad/MJ Research Opticon 2; Roche LightCycler 480 II (96-well and 384-well); all other cyclers	180820
GeneRead qPCR SYBR Green ROX Mastermix (2)*	For 2 x 96 assays in 96-well plates; suitable for use with the following real-time cyclers: Applied Biosystems models 5700, 7000, 7300, 7500 [Standard and Fast], 7700, 7900HT 96-well block [Standard and Fast] and 384-well block, StepOnePlus; Eppendorf Mastercycler ep realplex models 2, 2S, 4, 4S; Stratagene models Mx3000P, Mx3005P, Mx4000	180840
GeneRead qPCR SYBR Green Fluor Mastermix (2)*	For 2 x 96 assays in 96-well plates; suitable for use with the following real-time cyclers: Bio-Rad models iCycler, iQ5, MyiQ, MyiQ2	180830

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
GeneRead qPCR SYBR Green ROX FAST Mastermix (2)*	For 2 x 96 assays, suitable for use with Rotor-Gene Q and Rotor-Gene 6000	180850

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Notes

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