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QIAseq™ Library Quant Assay Kit

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

Sample to Insight

QIAGEN

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QIAGEN Sample to Insight

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Intended Use

QIAseq Library Quant Assay Kit is 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 these 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.

24-hour emergency information

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

CHEMTREC

USA and Canada: 1-800-424-9300

Outside USA and Canada: +1-703-527-3887 (collect calls accepted)

Kit Contents

QIAseq Library Quant Assay Kit	Catalog no. 333314
Laboratory-verified forward and reverse primers for 500 × 25 µl reactions (500 µl)	1
DNA standard (100 µl)	1
Dilution buffer (30 ml)	1
SYBR® Green Mastermix (1.35 ml)	5
Handbook	1

Storage

The QIAseq Library Quant Assay Kit is shipped on blue ice. Upon receipt, store at –20°C. If stored under these conditions, the QIAseq Library Quant Assay Kit is stable for up to six months from the date of receipt.

Quality Control

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

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 as well as the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAseq Library Quant Assay 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**).

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 QIAseq Library Quant Assay Kit uses real-time PCR to quantify NGS libraries. The QIAseq Library Quant Assay Kit specifically quantifies DNA molecules with adaptors at both ends, and these are the only amplifiable molecules during emulsion PCR (Ion Torrent platform) or bridge PCR (Illumina platform). The QIAseq Library Quant Assay Kit 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 QIAseq Library Quant Assay Kit is optimized with 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 QIAseq Library Quant Assay 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 sequencing 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 QIAseq Library Quant Assay 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 QIAseq Library Quant Assay 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 must 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 SYB 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 Microsoft® Excel®-based data analysis file to calculate the Template Dilution Factor (Ion Torrent) or the concentration of the library (Illumina).

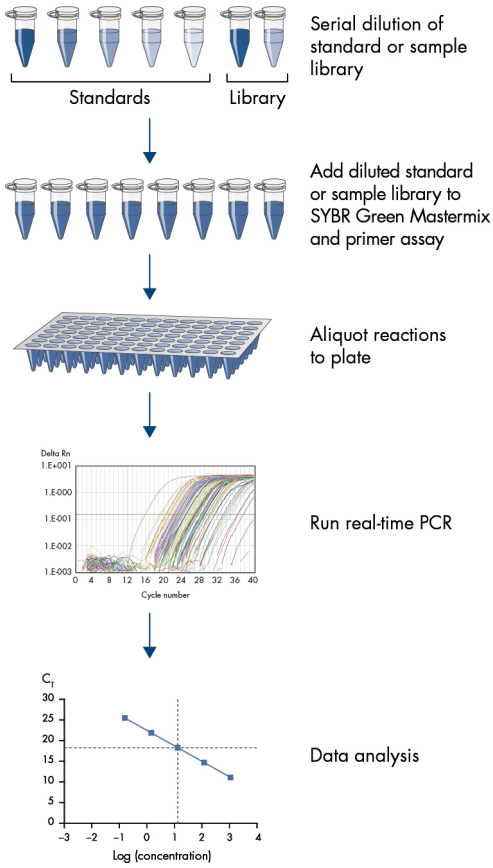

Figure 1. QIAseq Library Quant Assay Kit workflow.

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.

In addition to the QIAseq Library Quant Assay Kit, the following supplies are required:

- 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 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	Instrument
SYBR Green ROX™ Mastermix	All Applied Biosystems® and Stratagene® instruments, and Eppendorf® Mastercycler® ep *realplex* instruments with a ROX filter set
SYBR Green Fluor Mastermix	Bio-Rad® iCycler®, MyiQ™, MyiQ2™ and iQ™ 5
SYBR Green Mastermix	Bio-Rad models CFX96™, CFX384™, Bio-Rad/MJ Research Opticon 2, and Bio-Rad/MJ Research Chromo4™, Roche® LightCycler® 480 (96- and 384-well)
SYBR Green ROX FAST Mastermix	Rotor-Gene® Q and Rotor-Gene 6000

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

Important points before starting

- Ensure that the SYBR Green Mastermix (see page 12) and plate format are suitable for your real-time cycler. An incorrect format will not fit the real-time cycler properly and may damage the instrument.
- If dealing with 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
Dilution 1 (1:2000)	2 µl 1:20	198 µl
Dilution 2 (1:20000)	5 µl 1:2000	45 µl

Note: Always use freshly made dilutions.

Setting up the PCR

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

   Note: Because SYBR Green Mastermix contains HotStarTaq® DNA Polymerase, which is heat-activated, reactions can be prepared at room temperature (15–25°C).

6. Make PCR reaction 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	Volumes for 96-well plate (A, C, D, F)	Volumes for 384-well plate (E, G)
RNase- / Dnase-free water	30.6 µl	17 µl
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 PCR mix per well to three wells for the 96-well plate (see Figure 2 for layout) or 10 µl PCR mix per well to four wells for the 384-well plate (see Figure 3 for layout) for each sample dilution.

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1	Standard 1	Standard 1	Sample2 dilution 1	Sample2 dilution 1	Sample2 dilution 1	Sample6 dilution 1	Sample6 dilution 1	Sample6 dilution 1	Sample10 dilution 1	Sample10 dilution 1	Sample10 dilution 1
B	Standard 2	Standard 2	Standard 2	Sample2 dilution 2	Sample2 dilution 2	Sample2 dilution 2	Sample6 dilution 2	Sample6 dilution 2	Sample6 dilution 2	Sample10 dilution 2	Sample10 dilution 2	Sample10 dilution 2
C	Standard 3	Standard 3	Standard 3	Sample3 dilution 1	Sample3 dilution 1	Sample3 dilution 1	Sample7 dilution 1	Sample7 dilution 1	Sample7 dilution 1	Sample11 dilution 1	Sample11 dilution 1	Sample11 dilution 1
D	Standard 4	Standard 4	Standard 4	Sample3 dilution 2	Sample3 dilution 2	Sample3 dilution 2	Sample7 dilution 2	Sample7 dilution 2	Sample7 dilution 2	Sample11 dilution 2	Sample11 dilution 2	Sample11 dilution 2
E	Standard 5	Standard 5	Standard 5	Sample4 dilution 1	Sample4 dilution 1	Sample4 dilution 1	Sample8 dilution 1	Sample8 dilution 1	Sample8 dilution 1	Sample12 dilution 1	Sample12 dilution 1	Sample12 dilution 1
F	NTC	NTC	NTC	Sample4 dilution 2	Sample4 dilution 2	Sample4 dilution 2	Sample8 dilution 2	Sample8 dilution 2	Sample8 dilution 2	Sample12 dilution 2	Sample12 dilution 2	Sample12 dilution 2
G	Sample1 dilution 1	Sample1 dilution 1	Sample1 dilution 1	Sample5 dilution 1	Sample5 dilution 1	Sample5 dilution 1	Sample9 dilution 1	Sample9 dilution 1	Sample9 dilution 1	Sample13 dilution 1	Sample13 dilution 1	Sample13 dilution 1
H	Sample1 dilution 2	Sample1 dilution 2	Sample1 dilution 2	Sample5 dilution 2	Sample5 dilution 2	Sample5 dilution 2	Sample9 dilution 2	Sample9 dilution 2	Sample9 dilution 2	Sample13 dilution 2	Sample13 dilution 2	Sample13 dilution 2

Figure 2. QIAseq Library Quant Assay 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 B	standard1	Sample 2 dilution 1	Sample 6 dilution 1	Sample 10 dilution 1	Sample 14 dilution 1	Sample 18 dilution 1	Sample 22 dilution 1	Sample 26 dilution 1	Sample 30 dilution 1	Sample 34 dilution 1	Sample 38 dilution 1	Sample 42 dilution 1
C D	standard2	Sample 2 dilution 2	Sample 6 dilution 2	Sample10 dilution 2	Sample 14 dilution 2	Sample 18 dilution 2	Sample 22 dilution 2	Sample 26 dilution 2	Sample 30 dilution 2	Sample 34 dilution 2	Sample 38 dilution 2	Sample 42 dilution 2
E F	standard3	Sample 3 dilution 1	Sample 7 dilution 1	Sample11 dilution 1	Sample 15 dilution 1	Sample 19 dilution 1	Sample 23 dilution 1	Sample 27 dilution 1	Sample 31 dilution 1	Sample 35 dilution 1	Sample 39 dilution 1	Sample 43 dilution 1
G H	standard4	Sample 3 dilution 2	Sample 7 dilution 2	Sample 11 dilution 2	Sample 15 dilution 2	Sample 19 dilution 2	Sample 23 dilution 2	Sample 27 dilution 2	Sample 31 dilution 2	Sample 35 dilution 2	Sample 39 dilution 2	Sample 43 dilution 2
I J	standard5	Sample 4 dilution 1	Sample 8 dilution 1	Sample 12 dilution 1	Sample 16 dilution 1	Sample 20 dilution 1	Sample 24 dilution 1	Sample 28 dilution 1	Sample 32 dilution 1	Sample 36 dilution 1	Sample 40 dilution 1	Sample 44 dilution 1
K L	NTC	Sample4 dilution 2	Sample 8 dilution 2	Sample12 dilution 2	Sample 16 dilution 2	Sample 20 dilution 2	Sample 24 dilution 2	Sample 28 dilution 2	Sample 32 dilution 2	Sample 36 dilution 2	Sample 40 dilution 2	Sample 44 dilution 2
M N	Sample 1 dilution 1	Sample 5 dilution 1	Sample 9 dilution 1	Sample13 dilution 1	Sample 17 dilution 1	Sample 21 dilution 1	Sample 25 dilution 1	Sample 29 dilution 1	Sample 33 dilution 1	Sample 37 dilution 1	Sample 41 dilution 1	Sample 45 dilution 1
O P	Sample 1 dilution 2	Sample 5 dilution 2	Sample 9 dilution 2	Sample 13 dilution 2	Sample 17 dilution 2	Sample 21 dilution 2	Sample 25 dilution 2	Sample 29 dilution 2	Sample 33 dilution 2	Sample 37 dilution 2	Sample 41 dilution 2	Sample 45 dilution 2

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

8. Carefully, tightly seal the plate with Optical Thin-Well 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 instrument has been calibrated to use clear, flat optical caps with plates prior to initiating the run.

9. Centrifuge for 1 min at 1000 × g at room temperature (15–25°C) to remove any 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.

11. Program the real-time cycler according to Table 4, 5 or 6 depending on the instrument used. If prompted by your cycler software, select "Absolute Quantification" 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 the Roche LightCycler 480II*

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, Takara 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	
	60 s	72°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.

* 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 for a plate sealed with optical adhesive film (formats C, E, F and G). Start the run.

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

Important points before starting

- Ensure that SYBR Green ROX FAST Mastermix is used with a Rotor-Gene cycler.
- If dealing with 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 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 1:20	198 µl
Dilution 2 (1:20000)	5 µl 1:2000	45 µl

Note: Always use freshly made dilutions.

Setting up the PCR

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

   Note: Because SYBR Green Mastermix contains HotStarTaq® DNA Polymerase, which is heat-activated, 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
SYBR Green ROX FAST Mastermix	40 µl
Primer mix (10 µM)	3.2 µl
Template (standard 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 PCR components mix per well to three wells (see Figure 4 or Figure 5 for layout) for each sample dilution or standard.

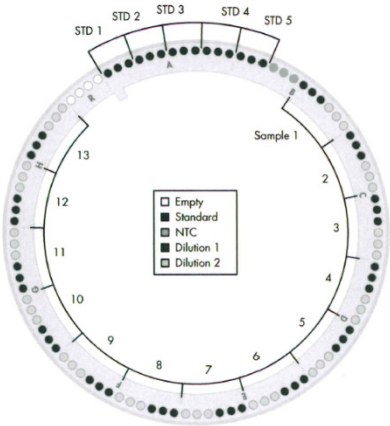

Figure 4. QIAseq Library Quant Assay Kit layout (Format R; Rotor-Disc 100).

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

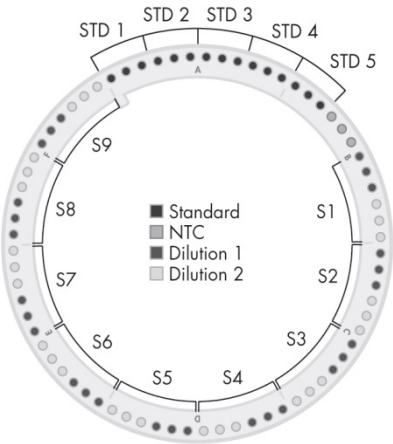

Figure 5. QIAseq Library Quant Assay Kit layout (Format R; 72-well Rotor).

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 1. 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 to the manufacturer's instructions for the real-time PCR instrument.

Excel-based data analysis template

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

Second, paste the raw C_T values (for the entire plate, even if only part is used) into the QIAseq Library Quant Assay Kit Excel-based data analysis file. Enter the required run parameters.

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 (Ion Torrent) or dilute the original library to designated range for cluster generation (Illumina). 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 HotStarTaq in the SYBR Green Mastermix.
Signal is detected for no-template control (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 reagents 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 programs for different machines
C_T of sample library is out of the range of standard curve (C_T 6–25 depending on instrument)		
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

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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.
QIAseq Library Quant Assay Kit	Laboratory-verified forward and reverse primers for 500 × 25 µl reactions (500 µl); standard (100 µl); dilution buffer (30 ml); SYBR Green Mastermix (1.35 ml × 5) (type varies based on plate format)	333314
Related products		
QIAseq Library Quant Array Kit	Two arrays in Formats A, C, D, F or R or one array in Format E or G; SYBR Green Mastermix (1.35 ml × 2) (type varies based on plate format)	333304

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