May 2017

QIAseq™ Library Quant Array Handbook

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

Sample to Insight

QIAGEN

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Kit Contents

QIAseq Library Quant Array Kit				Catalog no. 333304 (contents vary 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 × 2	–	12 × 2	–	–	–	–
Optical adhesive film	–	1 × 2	–	1	1 × 2	1	–
Rotor-Disc Heat-Sealing Film	–	–	–	–	–	–	1 × 2
SYBR® Green Mastermix	1.35 ml × 2, type varies depending on plate format						

Storage

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

Intended Use

QIAseq Library Quant Array Kits are 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.

Quality Control

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

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 Array Kit uses real-time PCR to quantify NGS libraries. The QIAseq Library Quant Array 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 Array 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 Array 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 procedure

The QIAseq Library Quant Array Kit for Ion Torrent contains five predispensed, sequential 10-fold dilutions of Ion Torrent DNA Standard mixed with a PCR primer assay in triplicates, as well as 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 provide a highly convenient method for quantification of library input.

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. 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 QIAseq Library Quant Array for Illumina contains five predispensed, sequential 10-fold dilutions of Illumina DNA Standard mixed with a PCR primer assay in triplicates, as well as 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 provide a highly convenient method for quantification of library input.

The Illumina DNA Standard harbors a target flanked by the "P5" and "P7" primer sequences used in 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 Array Kit workflow (Figure 1) is simple. The procedure begins with two 10-fold dilutes of the sample library (the sample library must be diluted to a concentration within the range of the serially diluted standards). Next, appropriate mixtures of PCR reactions are prepared and aliquotted into the wells of a real-time PCR plate. PCR is performed and C_T values are exported to the provided Microsoft® Excel®-based data analysis file to calculate the Template Dilution Factor (Ion Torrent) or library concentration (Illumina).

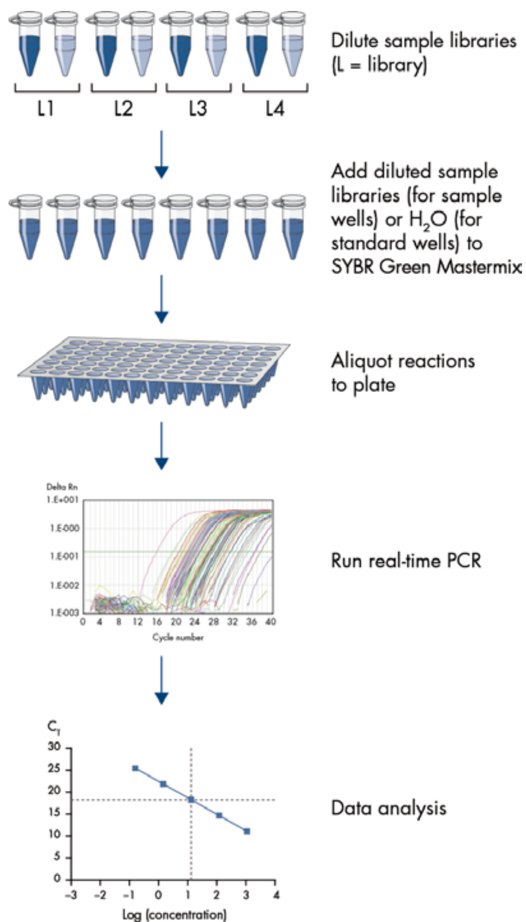

Figure 1. QIAseq Library Quant Array Kit workflow.

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 Array Kit, the following supplies are required.

- Real-time PCR instrument
- Calibrated single- and multichannel pipets
- RNase-/DNase-free pipet tips and tubes
- RNase-/DNase-free 200 µl regular PCR tubes or 8- or 12-tube strips
- Molecular-biology-grade RNase- and DNase-free water
- Dilution buffer (10 mM Tris·Cl, pH 7.8 or QIAGEN Buffer EB)
- Rotor-Disc® heat sealer (if using the Rotor-Gene® Q)

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 minute 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 (Table 1) is included before starting PCR runs.

Table 1. Mastermixes required for each instrument

Master mix	Instrument
SYBR Green ROX™ Mastermix	All Applied Biosystems® and Agilent® (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™, DNA Engine Opticon® 2 and Chromo4™; Roche® LightCycler® 480 (96- and 384-well)
SYBR Green ROX FAST Mastermix	Rotor-Gene Q and Rotor-Gene 6000

- QIAseq Library Quant Array Kits have been tested on the cyclers shown in Table 2.

Table 2. Cyclers tested with QIAseq Library Quant Array Kits

Cycler	Plate
Vii7A™/Agilent	A
Applied Biosystems 7500	C
Applied Biosystems 7900	E
Roche LightCycler II	F
Rotor-Gene Q	Rotor-Disc 100

- See Table 6, Table 7, Table 8 or Table 11 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 with different plate formats can be found in Table 3.

Table 3. Array formats for use with different real-time cyclers

Format	Suitable real-time cyclers	Plate
A	Applied Biosystems 5700, 7000, 7300, 7500 Standard, 7700, 7900HT Standard Bio-Rad iCycler, iQ5, MyiQ, MyiQ2, Chromo4 Eppendorf Mastercycler ep realplex 2, 2s, 4, 4s Agilent Mx3005P®, Mx3000P®	96-well
C	Applied Biosystems 7500 FAST, 7900HT FAST, StepOnePlus™	96-well
D	Bio-Rad CFX96, DNA Engine Opticon 2; Agilent 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

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

Important points before starting

- Ensure that the SYBR Green Mastermix and plate format are suitable for your real-time cycler (see pages 10 and 11). 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 below.

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 4.

Table 4. Sample library dilution

Dilution	Library	Dilution buffer
Dilution 1 (1:2000)	2 µl 1:20	198 µl
Dilution 2 (1:20,000)	5 µl 1:2000	45 µl

Note: Always use freshly made dilutions.

Setting up the PCR

3. 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).

4. Make PCR reaction mix for each sample dilution (triplicates on plate formats A, C, D and F; quadruplicates on plate formats E and G) or standards/NTC wells according to Table 5.

Table 5. 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)	
	For standard and NTC (18 wells)	For sample library Dilution 1 and 2 (3 wells for each dilution)	For standard and NTC	For sample library Dilution 1 and 2 (4 wells for each dilution)
RNase-/DNase-free water	240 µl	32 µl	150 µl	19 µl
SYBR Green Mastermix	240 µl	42 µl	150 µl	25 µl
Template (sample library)	–	10 µl	–	6 µl
Final volume	**480 µl**	**84 µl**	**300 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 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. Add 25 µl (96-well plate, Figure 2) or 10 µl (384-well plate, Figure 3) PCR reaction mix (without template) into each well for standards and NTC.

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 Array 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 Array Kit layout (384-well plate).

6. 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.

7. 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.

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 1 week.

9. Program the real-time cycler according to Table 6, Table 7 or Table 8 depending on the instrument used. If prompted by your cycler software, select "Absolute Quantification" to begin.

Table 6. Cycling conditions* for Applied Biosystems, Bio-Rad†, Agilent and Eppendorf‡ cyclers

Cycles	Duration	Temperature	Comments
1	10 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step.
30	15 s 30 s 2 min	95°C 60°C 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; Agilent 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 7. 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 30 s 2 min	95°C 60°C 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 8. 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 30–40 s 60 s	95°C 55°C 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 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 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 Array Kit for Ion Torrent or Illumina (Format R)

Important points before starting

- 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.

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 9.

Table 9. Sample library dilution

Dilution	Library	Dilution buffer
Dilution 1 (1:2000)	2 µl 1:20	198 µl
Dilution 2 (1:20,000)	5 µl 1:2000	45 µl

Note: Always use freshly made dilutions.

Setting up the PCR

3. 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).

4. Make PCR reaction mix for each sample (for triplicates) using PCR tubes or strips according to Table 10.

Table 10. PCR mix for standard or sample libraries*

Component	Rotor-Disc 100	
	For standard and NTC **Wells 1–18 (A1–A12, B1–B6) and R (97–100)**	**For sample library** **For Dilutions 1 and 2 (3 wells for each dilution)**
RNase-/DNase-free water	240 µl	27 µl
SYBR Green ROX FAST Mastermix	240 µl	36 µl
Template (sample library)	–	9 µl
Final volume	**480 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 components mix per well to three wells for each sample dilution or standard (see Figure 4 for layout). Add 20 µl PCR mix (without template) into each of wells 1–18 (A1–A12, B1–B6) and 97–100 (R).

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

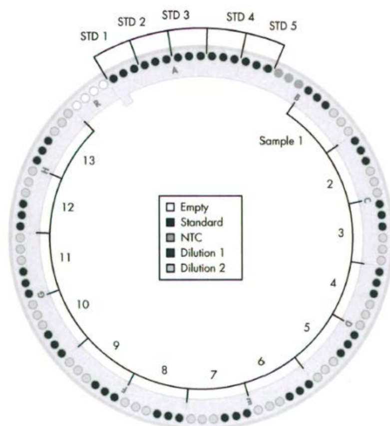

Figure 4. QIAseq Library Quant Array Kit layout (Format R; Rotor-Disc 100).

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 11.

   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 11. 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 5 s 5 s 60 s	95°C 68°C 65°C 60°C	Perform fluorescence data collection.

9. Insert the plate into the Rotor-Gene cycler and secure with the Rotor-Disc 100. 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 and 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 Array Kit Excel-based data analysis file that corresponds to the plate format used, available at **https://www.qiagen.com/us/shop/sample-technologies/dna/genomic-dna/qiaseq-library-quant-system/#resources**.

Then, paste the raw C_T values (for the entire plate, even if only part is used) into the QIAseq Library Quant Array 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 *GeneRead DNAseq Targeted Panels V2 Handbook*. If you used QIAseq Targeted DNA panels, refer to the *QIAseq Targeted DNA Panel Handbook* for sequencing instrument setup protocols.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
No PCR product is generated		
a)	Array reagents or master mix are incorrectly stored	Storing array 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 "Important Notes", page 9 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)	Suboptimal PCR conditions	Ensure that all the reagents are stored properly and reactions are set correctly.
b)	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.

Ordering Information

Product	Contents	Cat. no.
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 × 5) (type varies based on plate format)	333304
Related products		
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

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Notes

Notes

Limited License Agreement for QIAseq Library Quant Array Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at **www.qiagen.com**. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
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