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QIAseq™ DNA QuantiMIZE Assay Handbook

For advanced qualification and quantification of amplifiable genomic DNA for PCR-based targeted enrichment prior to next-generation sequencing （assay format）

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Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Kit Contents

QIAseq DNA QuantiMIZE Assay Kit	(Variable)
Catalog no.	333414
Assay 100 for 400 x 25 µl reactions (400 µl)	1
Assay 200 for 400 x 25 µl reactions (400 µl)	1
QuantiMIZE Control gDNA (50 µl)	1
RNase- and DNase-free water (1 ml)	4
GeneRead qPCR SYBR® Green Mastermix (1.35 ml)	4
Handbook	1

Storage

The QIAseq DNA QuantiMIZE Assay Kit is shipped on blue ice. Upon receipt, store at −30°C to −15°C. If stored under these conditions, QIAseq DNA QuantiMIZE Assay Kit is stable for 6 months after receipt.

Intended Use

Purchaser agrees that use of this product and data therefrom is limited solely to the purchaser and for only the purchaser's own internal molecular biology research applications ("Permitted Use"), and shall not be re-sold or used for any other purposes (all of which are expressly prohibited), including without limitation diagnostic purposes, uses that could require regulatory approval for diagnostics from an agency of any government or regulatory entity anywhere in the world, diagnosis, prevention, or treatment of disease, and the right to perform commercial services of any kind, including without limitation, reporting the results of purchaser's activities, including without limitation, for a fee or other commercial consideration. Except for the Permitted Use, no rights, titles or interests in or to any tangible or intangible property rights are conveyed or shall be deemed conveyed by implication, estoppel or otherwise. The performance characteristics of the product other than for the Permitted Use are unknown.

The QIAseq DNA QuantiMIZE Assay Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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 DNA QuantiMIZE Assay Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAseq DNA QuantiMIZE System utilizes a qPCR-based approach to determine the quantity and quality of sample DNA that is amenable to PCR-based targeted enrichment prior to next-generation sequencing (NGS). This allows optimization of cycling conditions and input DNA amounts. The system provides the most cost-effective approach to qualify and quantify DNA isolated from biological sources, especially those preserved as formalin-fixed, paraffin-embedded (FFPE) samples.

FFPE tissue archives are an invaluable source for the molecular characterization of disease using NGS. Unfortunately, genomic DNA present in FFPE samples is damaged and fragmented to varying extents depending on fixation and storage conditions. Because of this damage, only a fraction of the DNA may be usable as template for PCR. Commonly used DNA quantification methods, including mass measurements by spectrometry or fluorometry, do not differentiate between amplifiable and non-amplifiable DNA. Therefore, they cannot reliably measure the amplifiable amounts of DNA that are able to participate in the multiplex PCR-based targeted enrichment step in the NGS workflow. To overcome this challenge, the QIAseq DNA QuantiMIZE System utilizes a simple qPCR protocol to determine the amplifiable fraction of DNA present in a sample, and provide guidance to NGS users for the appropriate amount of input DNA and number of targeted enrichment cycles needed for successful NGS runs.

The QIAseq DNA QuantiMIZE System can be integrated into the complete NGS workflow with QIAGEN's GeneRead DNAseq Targeted Panels (Figure1). This method can be applied to a variety of human gDNA samples (including single cells, sorted cells, blood, free circulating or sonicated gDNA, etc.) and multiplex PCR-based targeted enrichment strategies for NGS.

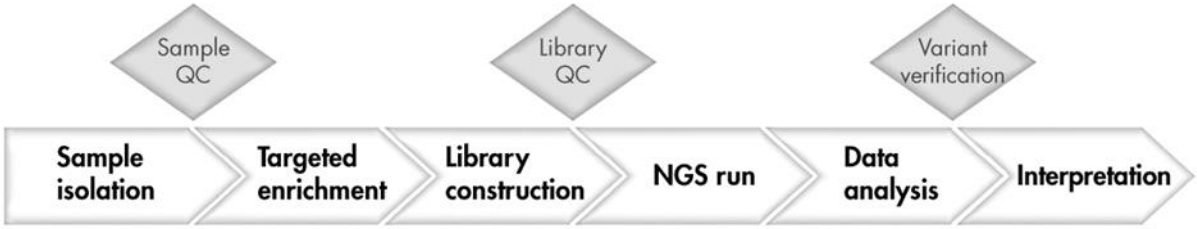


Figure 1. The QuantiMIZE System fits easily into the NGS workflow with GeneRead DNAseq Targeted Panels. The procedure involves DNA extraction, followed by QuantiMIZE analysis (Sample QC), targeted enrichment with GeneRead DNAseq Targeted Panels, NGS

library construction, sequencing and data analysis using the QIAGEN NGS Data Analysis Web Portal. Follow-up experiments or variant confirmation against specific variants can be performed with qBiomarker Somatic Mutation qPCR Assays and Arrays.

Principle and procedure

Unlike other approaches that target a single genomic locus, the QIAseq DNA QuantiMIZE System uses two qPCR assays (Assay 100 or Assay 200, generating amplicon sizes around 100 bp and 200 bp, respectively) to query more than 40 discrete genomic loci that are randomly distributed in the genome for the quantification and qualification of DNA samples. This proprietary design ensures minimal variation caused by local genome events, like copy number changes or SNPs.

For high-quality gDNA, C_T values obtained using Assay 100 will be similar to those obtained from Assay 200. For low-quality gDNA, C_T values obtained from Assay 200 will be higher than that from Assay 100, meaning that larger amplifiable fragments are less common than smaller ones in this sample. When plotting the C_T of Assay 200 (C_T 200) and Assay 100 (C_T 100) against amplicon sizes, the slope provides size distribution information for targets in each genomic DNA sample, thus serving as a quality control indicator for gDNA.

Meanwhile, use of the included high-quality reference gDNA allows quantification of amplifiable molecules in gDNA samples, without the need for serial dilutions. Therefore, QuantiMIZE assays are highly suited for determining the correct input amount of DNA, in particular from FFPE samples, for the multiplex PCR-based targeted enrichment step in the NGS workflow. For samples with insufficient numbers of amplifiable molecules, additional PCR cycles are added to compensate for the low amount of suitable starting material. In this way, most precious FFPE samples can be rescued for NGS analysis. However, while PCR can compensate for low DNA mass amounts, it cannot compensate for lower variant call sensitivity for severely damaged samples, like FFPE samples. Therefore, the ability to call low frequency mutations might not be rescued by the additional PCR cycles.

The workflow for the QIAseq DNA QuantiMIZE Assay Kit is simple and convenient (Figure 2). After extracting gDNA from biological samples, as long as the gDNA concentration is between 10 pg/µl to 2.5 µg/µl, simply mix sample or control gDNA with qPCR mastermix and QuantiMIZE assays, and load on plates. Real-time PCR is performed according to manufacturer's

recommendations, and C_T values are exported to an Excel® data analysis file to calculate 1) the volume and number of cycles needed for targeted enrichment PCR for each sample, and 2) the absolute quantities of amplifiable DNA. In addition, the data analysis file will provide a QC score (derived from the slope of C_T 200 and C_T 100) and a final quality call for "high" or "low" sample classification. Please refer to Table 1 for explanation of QC scores and quality calls (QC scores do not apply to UNG-treated gDNA samples).

Table 1. Explanation of QC scores

QC Score	QC call	Recommendations
<=0.04	High	Proceed – the gDNA is of good quality
>0.04	Low	Highly fragmented/damaged DNA; proceed with caution if working with FFPE samples. The gDNA may only be suitable for detection of high-frequency variants. Most low-frequency C>T or G>A variants are not reliable. Repeat gDNA extraction with GeneRead DNA FFPE Kit (including UNG treatment step) to reduce artificial "U" in the FFPE sample.

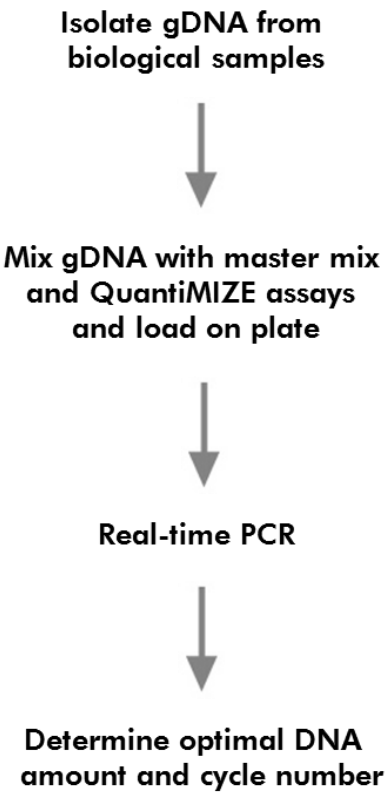


Figure 2. QIAseq DNA QuantiMIZE Assay Kit workflow.

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.

- 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 (8-tube or 12-tube strip)
- Molecular biology grade RNase- and DNAase-free water
- Rotor-Disc Heat Sealer (if using the RGQ)

Important Notes

Recommended genomic DNA preparation method

QIAamp DNA FFPE Tissue Kit (cat. no. 56404) or GeneRead DNA FFPE Kit (including UNG treatment, cat. no. 180134) are highly recommended for the preparation of genomic DNA samples from FFPE tissue. If genomic DNA needs to be harvested from biological samples for which kits are not available, please contact Technical Support representatives for suggestions. For best results, all DNA samples should be resuspended in DNase-free water, EB or ATE buffer. Do not use DEPC-treated water.

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 mastermix tubes, warm the reagents at 42°C for 1 min and vortex briefly to dissolve. Repeat if necessary.
- The appropriate GeneRead qPCR SYBR Green Mastermix is already included in the kit. Please ensure that the correct mastermix, according the table below, has been included in your order before starting your runs.

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

■ Select the correct plate format for your cycler:

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

■ The QIAseq DNA QuantiMIZE Array 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 5, 6 and 7 in this handbook for 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: Real-time PCR for QIAseq DNA QuantiMIZE Assay Kit

Important points before starting

- Ensure that the GeneRead qPCR SYBR Green Mastermix and the plate format are suitable for your real-time cycler (see pages 11 and 12). An incorrect plate format will not fit the real-time cycler properly and may damage the real-time cycler.
- When NTC is desired, simply replace one sample with double-distilled water.

Procedure

Note: FFPE gDNA samples can be used without any prior measurement as long as the yield of gDNA is between 10 pg/µl to 2.5µg/µl. DNA extracted using methods and samples that yield high amounts of DNA might need to be diluted first. If you are using a new method to extract DNA, serial dilutions of the DNA sample are recommended to ensure at least one concentration is within the above range and to test if any PCR inhibitors are present in the DNA sample.

Note: Always include QuantiMIZE Control gDNA in every run, regardless of the number of gDNA samples processed. Calculations cannot be performed in the absence of the Control gDNA.

Note: Each gDNA sample is interrogated by 6 reactions: 3 (triplicate) reactions using Assay 100 and 3 using Assay 200.

1. Prepare 2 PCR components mixes: mix 1 for primer Assay 100 and mix 2 for primer Assay 200, as described in Table 2 or 3. Volume calculations are automatically performed in the data analysis file (Quick start guide sheet) for your convenience.

Table 2. PCR components mix for 96-well or 384-well plate format

	For 96-well plates (A, C, D, F)	For 384-well plates (E, G)
Component	***n* sample (µl)**	
RNase/DNase-free water	21.45 µl∗(*n* + 1)	8.58 µl∗(*n* + 1)
GeneRead qPCR SYBR Green Mastermix	41.25 µl∗(*n* + 1)	16.5 µl∗(*n* + 1)
Primer assay	3.3 µl ∗(*n* + 1)	1.32 µl ∗(*n* + 1)
Final volume	**66 µl *(*n* + 1)**	**26.4 µl *(*n* + 1)**

∗ 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.

Table 3. PCR components mix for Rotor-Disc 100 or 72-well Rotor

Component	***n* sample (µl)**
RNase/DNase-free water	17.16 µl ∗(*n* + 1)
GeneRead qPCR SYBR Green ROX FAST Mastermix	33 µl ∗(*n* + 1)
Primer assay	2.64 µl ∗(*n* + 1)
Final volume	**52.8 µl *(*n* + 1)**

∗ 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.

2. Dispense PCR components mix 1 (Assay 100) or mix 2 (Assay 200) into each well: 20 µl for 96-well plate (Figure 3), 8 µl for 384-well plate (Figure 4) or 16 µl for Rotor-Disc 100 (Figure 5) and 72-well Rotor (Figure 6). The layouts of Figures 3 through 6 must be followed as shown for appropriate data analysis.
3. Dilute each sample or Control gDNA with RNase-/DNase-free water according to Table 4 and then dispense diluted sample or control into each well: 5 µl for 96-well plate (Figure 3), 2 µl for 384-well plate (Figure 4) or 4 µl for Rotor-Disc 100 (Figure 5) or 72-well Rotor (Figure 6).

Table 4. Prepare dilution of each sample or control

Component	96-well plate	384-well plate	Rotor-Disc 100-/72-well Rotor
Genomic DNA (control or sample)*	4 µl	2 µl	4 µl
RNase-/DNase-free water	28 µl	14 µl	28 µl
Final volume	**32 µl**	**16 µl**	**32 µl**

* Sample and control volume can be further decreased (down to 1 µl) if desired, as long as both are treated in the same manner.

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	S1			S9			S1			S9		
B	S2			S10			S2			S10		
C	S3			S11			S3			S11		
D	S4			S12			S4			S12		
E	S5			S13			S5			S13		
F	S6			S14			S6			S14		
G	S7			S15			S7			S15		
H	S8			Control			S8			Control		

Figure 3. Layout for 96-well plate. Sample 1 (S1) occupies wells A1–3 for Assay 100 (white cells) and A7–9 for Assay 200 (shaded cells), while Sample 2 (S2)

occupies wells B1–3 and B7–9, and so on. The Control gDNA occupies wells H4–6 for Assay 100 and H10–12 for Assay 200.

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	S1			S17			S33			S49			S1			S17			S33			S49		
B	S2			S18			S34			S50			S2			S18			S34			S50		
C	S3			S19			S35			S51			S3			S19			S35			S51		
D	S4			S20			S36			S52			S4			S20			S36			S52		
E	S5			S21			S37			S53			S5			S21			S37			S53		
F	S6			S22			S38			S54			S6			S22			S38			S54		
G	S7			S23			S39			S55			S7			S23			S39			S55		
H	S8			S24			S40			S56			S8			S24			S40			S56		
I	S9			S25			S41			S57			S9			S25			S41			S57		
J	S10			S26			S42			S58			S10			S26			S42			S58		
K	S11			S27			S43			S59			S11			S27			S43			S59		
L	S12			S28			S44			S60			S12			S28			S44			S60		
M	S13			S29			S45			S61			S13			S29			S45			S61		
N	S14			S30			S46			S62			S14			S30			S46			S62		
O	S15			S31			S47			S63			S15			S31			S47			S63		
P	S16			S32			S48			Control			S16			S32			S48			Control		

Figure 4. Layout for 384-well plate. Sample 1 (S1) occupies wells A1–3 for Assay 100 (white cells) and A13–15 for Assay 200 (shaded cells), and so on. The Control gDNA occupies wells P10–12 for Assay 100 and P22–24 for Assay 200.

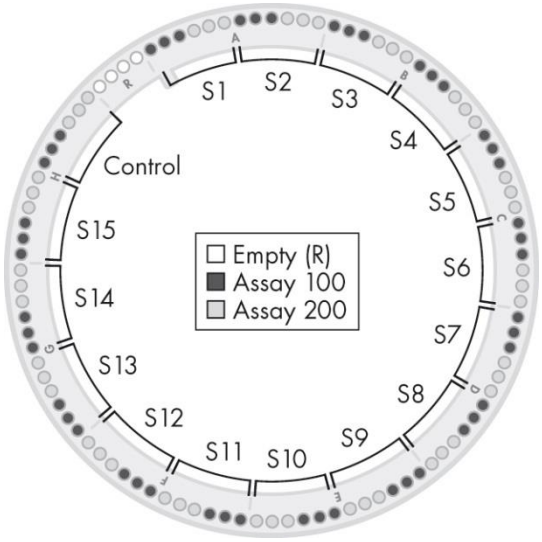


Figure 5. Layout for Rotor-Disc 100. Sample 1 (S1) occupies wells 1–6, Sample 2 (S2) occupies wells 7–12 and so on. The Control gDNA occupies wells 91–96 and wells 97–100 are empty.

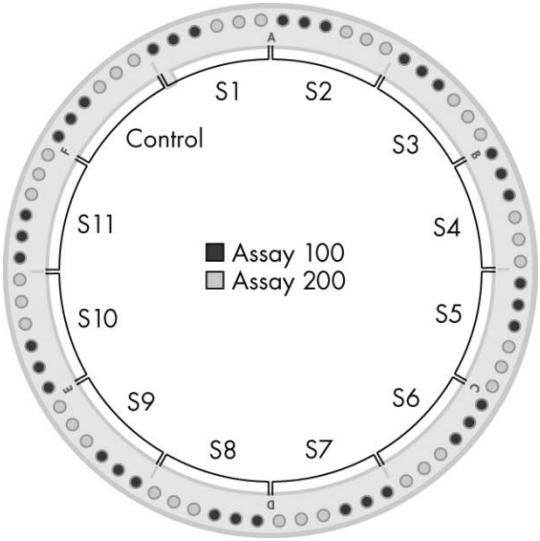


Figure 6. Layout for 72-well Rotor. Sample 1 (S1) occupies wells 1–6, Sample 2 (S2) occupies wells 7–12 and so on. The Control gDNA occupies wells 67–72.

4. Carefully and tightly seal the plate with Optical Thin-Wall 8-Cap Strips (Formats A and D), Optical Adhesive Film (Formats C, E, F and G) or Rotor-Disc Heat-Sealing Film using the Rotor-Disc Heat Sealer (Rotor Disc-100 or 72-well Rotor; see the Rotor-Gene Q User Manual for detailed instructions).

5. Centrifuge for 1 min at 1000 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 will interfere with results.

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

   Note: The plates containing PCR components mix may be stored at −15°C to −30°C wrapped in aluminum foil for up to 1 week if desired.

7. Program the real-time cycler according to Table 5 for 96-well or 384-well plates, Table 6 for the Rotor-Disc 100 or Table 7 for the 72-well Rotor.
8. Place the plate in the real-time cycler and start the run.

Table 5. Cycling conditions for 96-well or 384-well plates

Cycles	Duration	Temperature	Comments
1	10 min	95°C	HotStarTaq® DNA Polymerase is activated by this heating step
40	15 s	95°C	
	2 min	60°C	Perform fluorescence data collection

(If using 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).

Table 6. Cycling conditions for Rotor Disc-100

Cycles	Duration	Temperature	Comments
1	10 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step
40	15 s	95°C	
	5 s	68°C	
	5 s	65°C	
	60 s	60°C	Perform fluorescence data collection

Table 7. Cycling conditions for 72-well Rotor

Cycles	Duration	Temperature	Comments
1	10 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step
40	15 s	95°C	
	60 s	60°C	Perform fluorescence data collection

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

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 setting the baseline and threshold values as follows:

Baseline: Use automatic baseline.

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

Exporting C_T values

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

Excel-based data analysis template

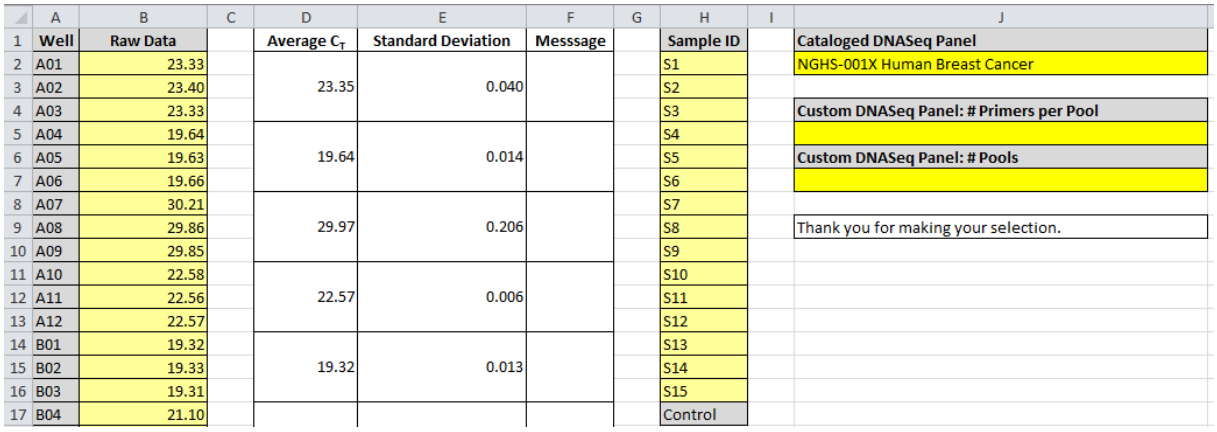
First, download the QIAseq DNA QuantiMIZE Assay Kit Excel-based data analysis file that corresponds to the plate format used, which is available at: https://www.qiagen.com/us/shop/sequencing/next-gen-sequencing/generead-dna-quantimize-kits?autoSuggest=true#resources

The "Assay Quick Start Guide" worksheet provides a simplified experimental setup protocol for any number of sample(s).

Second, referring to Figure 7, paste the raw C_T values (for the whole plate, even if only part of the plate was used) into Column B of the "Raw Data & Analysis Setup" sheet of the QIAseq DNA QuantiMIZE Assay Kit Excel-based data analysis file. The following inputs are also required:

1. Sample ID (Column H): Assign a unique name for each sample. Do not use redundant names.
2. If using a cataloged GeneRead panel downstream of QuantiMIZE, select the panel you are using from the "Cataloged DNASeq Panel" dropdown menu (Column J, row 2).

3. If using a Custom or Mix-n-Match GeneRead panel, specify the following inputs using the appropriate dropdown menus:
    a. Number of primers per pool (Column J, row 5).
    b. Number of pools (Column J, row 7).



	A	B	C	D	E	F	G	H	I	J
1	Well	Raw Data		Average C_T	Standard Deviation	Messsage		Sample ID		Cataloged DNASeq Panel
2	A01	23.33		23.35	0.040			S1		NGHS-001X Human Breast Cancer
3	A02	23.40						S2		
4	A03	23.33						S3		Custom DNASeq Panel: # Primers per Pool
5	A04	19.64		19.64	0.014			S4		
6	A05	19.63						S5		Custom DNASeq Panel: # Pools
7	A06	19.66						S6		
8	A07	30.21		29.97	0.206			S7		
9	A08	29.86						S8		Thank you for making your selection.
10	A09	29.85						S9		
11	A10	22.58		22.57	0.006			S10		
12	A11	22.56						S11		
13	A12	22.57						S12		
14	B01	19.32		19.32	0.013			S13		
15	B02	19.33						S14		
16	B03	19.31						S15		
17	B04	21.10						Control		

Figure 7. Required inputs for the QIAseq DNA QuantiMIZE Excel-based data analysis sheets.

Third, refer to the "Results" sheet (Figure 8) to determine next steps. The results sheet calculates the following outputs:

1. Quality control results:
    a. Assay QC: If the Assay QC for all Samples and the Control gDNA reports "Pass", then the assays performed optimally. If the Control gDNA Assay QC reports "Fail", double check the assay setup and real-time PCR program. If any sample's Assay QC reports "Fail", the sample may contain PCR inhibitor contaminants. We recommend making serial dilutions (1:10 and 1:100) of the sample and re-running QuantiMIZE.
    b. QC Score and Call: QC Score provides an indication of DNA sample damage or fragmentation. Samples with a QC Score <= 0.04 are labeled with "High", meaning high quality and low degree of damage or fragmentation. Samples with a QC Score > 0.04 are labeled with "Low", meaning low quality and a high degree damage or fragmentation. Samples with a "Low" QC Call may only be suitable for detection of high-frequency variants.
        i. This output can be applied to any multiplex PCR-based targeted enrichment strategy for NGS.
2. Amplifiable: This is the concentration of amplifiable DNA in the sample.

    a. This output can be applied to any multiplex PCR-based targeted enrichment strategy for NGS.
3. Added cycles: This is the number of cycles we recommend adding to the amplification program for each sample. Use this number in the GeneRead DNAseq Gene Panel PCR setup. No number is displayed in "Added cycles" column for samples that are too dilute. We do not recommend proceeding with such samples.
    a. This output applies to enrichment using the GeneRead DNAseq panels only.
4. # Cycles: The total number of PCR cycles we recommend for the targeted enrichment for the specific sample for the specific GeneRead panel.
    a. This output applies to enrichment using the GeneRead DNAseq panels only.
5. Dilution setup
    a. Dilute each sample according to the recipe in Columns H and I unless not required as indicated by the "No dilution required" text in Column H. "No dilution required" means that samples can be used as is, without any further dilutions.
        i. This output applies to enrichment using the GeneRead DNAseq panels only.
6. Total (Diluted) Sample Volume Needed (Column J): this shows the total volume needed depending on the number of PCR pools.
    a. This output applies to enrichment using the GeneRead DNAseq panels only.
7. Comments: If you are still concerned about additional amplification cycles, this column will indicated which Samples may be used, after following the dilution recipe, without additional cycles beyond the standard GeneRead DNAseq panel protocol.
    a. This output applies to enrichment using the GeneRead DNAseq panels only.

All calculations and formulas can be found in the "Calculations" sheet.

Finally, follow the protocols in the *GeneRead DNAseq Targeted Panels V2 Handbook* for PCR setup for targeted enrichment.

	A	B	C	D	E	F	G	H	I	J	K
1	Sample ID	Quality Control Results					# Cycles	Dilution Setup		Total (Diluted) Sample Volume Needed (µL)	Comment
2		Assay QC	QC Score	QC Call	Amplifiable (ng/µL)	Added Cycles		Sample Volume (µl)	ddH_2O (µl)		
3	S1	Pass	0.060	Low	0.023	6	24	No dilution required		19.2	
4	S2	Pass	0.023	High	1.356	2	20	No dilution required		19.2	
5	S3	Pass	0.018	High	3.566	2	20	13.5	5.7	19.2	
6	S4	Pass	0.055	Low	0.005						We do not recommend proceeding with this sample
7	S5	Pass	0.043	Low	0.015						We do not recommend proceeding with this sample
8	S6	Pass	0.017	High	4.018	2	20	11.9	7.3	19.2	
9	S7	Pass	0.012	High	5.876	2	20	8.2	11.0	19.2	If prefered, only 18 cycles may be used
10	S8	Pass	0.007	High	18.730	2	20	2.6	16.6	19.2	If prefered, only 18 cycles may be used
11	S9	Pass	0.023	High	1.083	2	20	No dilution required		19.2	
12	S10	Pass	0.024	High	0.407	2	20	No dilution required		19.2	
13	S11	Pass	0.034	High	0.245	6	24	No dilution required		19.2	
14	S12	Pass	0.020	High	5.310	2	20	9.0	10.2	19.2	If prefered, only 18 cycles may be used
15	S13	Pass	0.019	High	2.168	2	20	No dilution required		19.2	
16	S14	Pass	0.019	High	2.223	2	20	No dilution required		19.2	
17	S15	Pass	-0.001	High	19.919	2	20	2.4	16.8	19.2	If prefered, only 18 cycles may be used
18	Control	Pass									

Figure 8. Example of a "Results" sheet. This screenshot shows a sample "Results" worksheet. For a 4-pool targeted enrichment panel, a total of four 20 µl reactions are set up (16 µl PCR mix plus 4 µl DNA). Therefore, 16 µl (4 reactions x 4 µl DNA) of each diluted sample is needed. The grey columns list the number of cycles, "Dilution Setup" and "Total (Diluted) Sample Volume Needed (µl)" for each sample with excess volume for multiple tubes. Refer to the data analysis files for detailed explanations. In this example, 15 samples (S1 to S15) and the Control gDNA (Control) were run. All 16 samples passed the Assay QC test (Column B), indicating that assays on all samples performed optimally. Three samples (S1, S4, and S5) showed "Low" QC calls (Column D), indicating that these DNA samples exhibit low quality and a high degree of damage or fragmentation, and might be suitable only for detecting of high-frequency variants. Of these three samples, sample S1 can be rescued by adding 6 additional PCR cycles, despite having a low QC call. However, the other two samples (S4 and S5) are so heavily damaged and of low quantity that even the addition of 6 PCR cycles won't rescue them (QC calls and Amplifiable amounts are two independent measurements). This is indicated by the blank cells in the "Added cycles" column (Column F). All rescuable samples are placed into 2 bins to simplify the setup process: a bin of 2 added cycles and a bin of 6 added cycles. The added cycles are added to the standard number of PCR cycles for the specific panel being used. This is shown in Column G. For example, sample S1 requires 6 additional cycles, for a total of 24 PCR cycles, while sample S2 requires 2 additional cycles, for a total of 20 PCR cycles. High-quality intact DNA samples might not require additional PCR cycles, and, therefore, could be run using the standard number of PCR cycles. The comments section will indicate which of your DNA samples can be run using the standard number of PCR cycles.

Troubleshooting Guide

For technical support, please call us at 1-888-503-3187 or 1-301-682-9200. 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**).

References

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Ordering Information

Product	Contents	Cat. no.
QIAseq DNA QuantiMIZE Assay Kit	Assay100 for 400 x 25 µl reactions (400 µl), Assay200 for 400 x 25 µl reactions (400 µl), QuantiMIZE Control gDNA (50 µl), RNase- and DNase-free water (1 ml x 4), GeneRead qPCR SYBR Green Mastermix (1.35 ml x 4) (varies depending on plate format)	333414
Related products		
QIAseq DNA QuantiMIZE Array Kit	2 arrays (Formats A, C, D, F), or 2 discs (Format R), or 1 array (Format E or G), in addition to QuantiMIZE Control gDNA (50 µl) and GeneRead qPCR SYBR Green Mastermix (1.35 ml x 2) (varies depending on plate format)	333404
QIAamp DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Collection Tubes (2 ml), buffers	56404
GeneRead DNA FFPE Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, UNG, Proteinase K, Collection Tubes (2 ml), buffers	180134

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