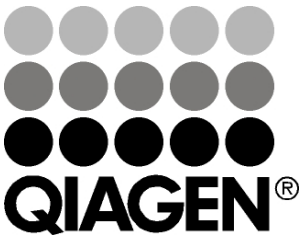
August 2012

qBiomarker Somatic Mutation PCR Handbook

qBiomarker Somatic Mutation PCR Array

qBiomarker Somatic Mutation PCR Assay

For real-time PCR-based, pathway- or disease-focused somatic mutation profiling



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

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Kit Contents

qBiomarker Somatic Mutation PCR Arrays							
Catalog no.	**337021**						
Format	**A**	**C**	**D**	**E**	**F**	**G**	**R**
96-well plate containing dried assays	1	1	1	–	1	–	–
384-well plate containing dried assays	–	–	–	1	–	1	–
Rotor-Disc® 100 containing dried assays	–	–	–	–	–	–	1
Optical Thin-Wall 8-Cap Strips (12 per plate)	12	–	12	–	–	–	–
Optical Adhesive Film (1 per plate	–	1	–	1	1	1	
Rotor-Disc Heat Sealing Film (1 per Rotor-Disc)	–	–	–	–	–	–	1
384EZLoad Covers* (1 set of 4 per plate)	–	–	–	1	–	1	–
qBiomarker Probe Mastermix (tube)†	1	1	1	2	1	2	1
Handbook	1	1	1	1	1	1	1

* Must be purchased separately for custom arrays; for single use only

† qBiomarker Probe Mastermix is available with ROX™ or fluorescein passive reference dye

qBiomarker Somatic Mutation PCR Assay	**(100)**
Catalog no.	**337011**
Number of samples	**100**
qBiomarker Somatic Mutation PCR Assay	1
qBiomarker Somatic Mutation PCR Reference Assay	1
qBiomarker Probe Mastermix	2
Handbook	1

Cyclers for use with array formats

Format	Suitable real-time cyclers	Plate
A	Applied Biosystems® (Standard 96-well block) 5700, 7000, 7300, 7500, 7900HT, ViiA™ 7; Bio-Rad® Chromo4™, iCycler®, iQ™5, MyiQ™, MyiQ2; Eppendorf® Mastercycler® ep *realplex* 2, 2S, 4, 4S; Agilent® Mx3005P®, Mx3000P®	96-well
C	Applied Biosystems (Fast 96-well block) 7500 Fast, 7900HT Fast, StepOnePlus™, ViiA 7 Fast	96-well
D	Bio-Rad CFX96™, Opticon®, Opticon 2; Agilent Mx4000®	96-well
E	Applied Biosystems (384-well block) 7900HT (384-well block), ViiA 7; Bio-Rad CFX384™	384-well
F	Roche® LightCycler® 480 (96-well block)	96-well
G	Roche LightCycler 480 (384-well block)	384-well
R	QIAGEN Rotor-Gene® cyclers	Rotor-Disc 100

Note: qBiomarker Somatic Mutation PCR Arrays cannot be used with the Cepheid SmartCycler® or the Roche LightCycler 2.0.

Cyclers compatible with ROX or fluorescein reference dye

Master mix	Reference dye	Suitable real-time cyclers
qBiomarker Probe Mastermix	ROX	All Applied Biosystems, Agilent (formerly Stratagene) and QIAGEN instruments; BioRad Opticon, Opticon 2, and Chromo 4; Roche LightCycler 480; Eppendorf Mastercycler ep *realplex* 2, 2S, 4, 4S
qBiomarker Probe Mastermix	Fluorescein	BioRad iCycler, MyiQ, and iQ5

Shipping and Storage

qBiomarker Somatic Mutation PCR Arrays are shipped at ambient temperature or on ice, depending on the destination and accompanying products. qBiomarker Somatic Mutation PCR Assays are shipped on ice. Upon receipt, store at –20°C.

qBiomarker Probe Mastermixes are shipped on ice. Upon receipt, store at 4°C.

If stored under these conditions, qBiomarker Somatic Mutation PCR Arrays and qBiomarker Somatic Mutation PCR Assays are stable for 6 months after receipt.

Intended Use

qBiomarker Somatic Mutation PCR Arrays and qBiomarker Somatic Mutation PCR Assays are intended for molecular biology applications. These products are 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.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

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 qBiomarker Somatic Mutation PCR Arrays, qBiomarker Somatic Mutation PCR Assays, 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

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's Quality Management System, each lot of qBiomarker Somatic Mutation PCR Array and qBiomarker Somatic Mutation PCR Assay is tested against predetermined specifications to ensure consistent product quality.

Introduction

qBiomarker Somatic Mutation PCR Arrays are translational research tools that allow rapid and accurate profiling of the somatic mutation status for a pathway- or disease-focused set of genes. Each array is comprised of a panel of qBiomarker Somatic Mutation PCR Assays that are designed to detect as low as 1% somatic mutations in the background of wild-type genomic DNA. qBiomarker Somatic Mutation PCR Arrays enable the somatic mutations reported in important genes related to a biological pathway or disease to be analyzed in a single experiment. Mutations are selected from comprehensive somatic mutation databases (e.g., COSMIC) and peer-reviewed scientific literature based on their clinical or functional relevance and frequency of occurrence.

Acquisition of somatic mutations in human genomic DNA is an important event during tumorigenesis and cancer progression. Somatic mutations can occur as single mutations within a gene, multiple mutations within a gene, or mutations present across related genes in a variety of cancers. Cells may respond differently to treatment regimens based on their somatic mutation profile. For example, the EGFR Pathway qBiomarker Somatic Mutation PCR Array, with its comprehensive content coverage, is designed for studying mutations in the context of the EGFR pathway. Use of the array enables potential discovery and verification of drug target biomarkers for targeted therapy research involving the EGFR signaling pathway and downstream effectors.

Studying the most common and clinically relevant mutations within the context of biological pathways has the potential to rapidly advance discovery and verification of important clinical biomarkers. Analyzing the status of individual or multiple somatic mutations can provide valuable information for identifying key signaling transduction disruptions.

Principle

By combining allele-specific amplification and hydrolysis probe detection, qBiomarker Somatic Mutation PCR Assays can detect as low as 1% somatic mutations in a wild-type genomic DNA background. Allele-specific amplification is achieved by Amplification Refractory Mutation System (ARMS®) technology, which is based on the discrimination by *Taq* polymerase between a match and a mismatch at the 3' end of a PCR primer (Figure 1).

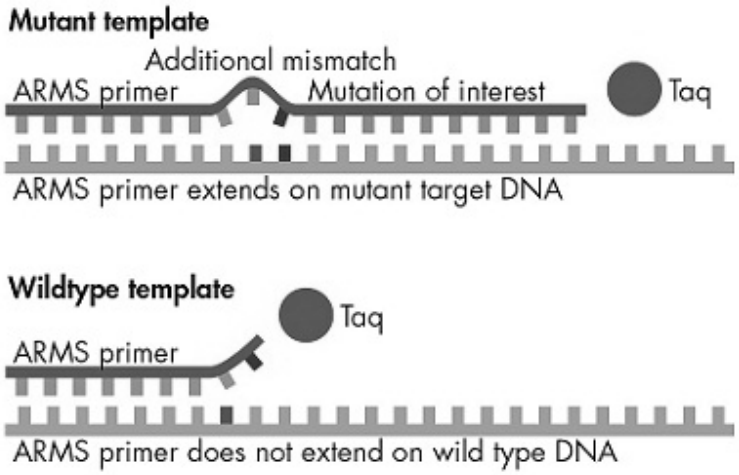

Figure 1. Principle of mutant allele discrimination with ARMS. The ARMS PCR primer is designed to specifically amplify mutant target DNA. An additional mismatch between the ARMS primer and template DNA that is close to the mutation of interest does not inhibit ARMS primer extension of the mutant target DNA. However, PCR amplification of the wild-type sequence is prevented.

qBiomarker Somatic Mutation PCR Arrays

Each qBiomarker Somatic Mutation PCR Array contains a panel of hydrolysis probe (FAM™ labeled) qBiomarker Somatic Mutation PCR Assays for a stringently selected set of pathway- or disease-focused somatic mutations, gene copy number controls, and PCR quality controls. All assays on the arrays have been wet-bench validated for hydrolysis probe-based real-time PCR detection. The assays have been optimized to work under standard cycling conditions, enabling a large number to be analyzed simultaneously. They can be used on almost any real-time cycler.

Pathway-focused arrays contain assays for detecting the most frequent and functionally verified mutations for multiple genes within a specific pathway implicated in a variety of cancers. These pathways include major receptor tyrosine kinase pathways and non-receptor kinase pathways, as well as additional oncogene and tumor suppressor pathways.

Disease-focused arrays include the most common/best characterized somatic mutations for a specific disease type. The targeted diseases include all major types of cancer.

In addition, a collection of more than 800 prevalidated somatic mutation assays enables researchers to study single mutations or to customize the mutation panels or collections according to their research needs.

qBiomarker Probe Mastermix

HotStart DNA Polymerase

HotStart DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperatures. This prevents the formation of misprimed products and primer–dimers during reaction setup and the first denaturation step. Competition for reactants by PCR artifacts is therefore avoided, enabling high PCR specificity and accurate quantification. The enzyme is activated by a 10 minute, 95°C incubation step, which is easily incorporated into existing thermal cycling programs.

Passive reference dye

For certain real-time cyclers, the presence of a passive reference dye in real-time PCR compensates for non-PCR–related variations in fluorescence detection. Two versions of qBiomarker Probe Mastermix are available, which contain either ROX passive reference dye or fluorescein passive reference dye. For a list of cyclers that can be used with each master mix, refer to the table on page 6. The presence of ROX dye does not interfere with the function of real-time PCR cyclers that do not require passive reference dye (e.g., Rotor-Gene cyclers).

Procedure

The qBiomarker Somatic Mutation PCR Array procedure involves DNA purification, an optional amplification step for DNA isolated from fresh samples, real-time PCR detection using qBiomarker Somatic Mutation PCR Arrays or Assays, and data analysis. An optional DNA sample quality control step can also be performed immediately before the detection array or assay setup to determine the quality of the DNA samples (see Appendix C, page 47).

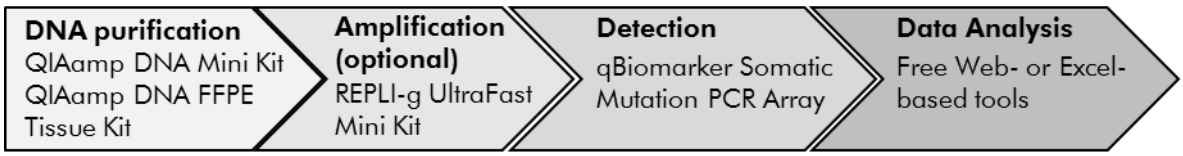

Figure 2. Overview of the qBiomarker Somatic Mutation PCR procedure. The simple workflow involves mixing the genomic DNA sample of interest with ready-to-use qBiomarker Probe Mastermix, aliquoting the reaction mixture into the array plate wells, performing real-time PCR and making mutation/genotype calls using Web-based data analysis software or Excel® based templates.

qBiomarker Somatic Mutation PCR Arrays and Assays yield accurate and verifiable results using a variety of sample types. These include fresh-frozen cell lines and tissue samples, cell line admixtures, formalin-fixed, paraffin-embedded (FFPE) cell line samples, and FFPE tissue samples. The procedure can be performed with 5 to 10 ng genomic DNA isolated from fresh (unfrozen)

or frozen human tissues, or as little as 200 ng genomic DNA from FFPE sections. Optionally, genomic DNA from fresh tissues can be uniformly amplified (e.g., using the QIAGEN REPLI-g® UltraFast Mini Kit). Genomic DNA is added to the ready-to-use qBiomarker Probe Mastermix and aliquoted into each well of the plate, which contains pre-dispensed gene-specific primer and hydrolysis probe sets. The mutation status of a particular sample is determined using real-time PCR to compare the allele-specific C_T values between the test sample with a wild-type control sample (see Appendix B, page 45).

qBiomarker Somatic Mutation PCR Array plate layout

qBiomarker Somatic Mutation PCR Arrays are available in 96-well plate, 384-well plate, and Rotor-Disc 100 formats (Figures 3-5).

96-well format

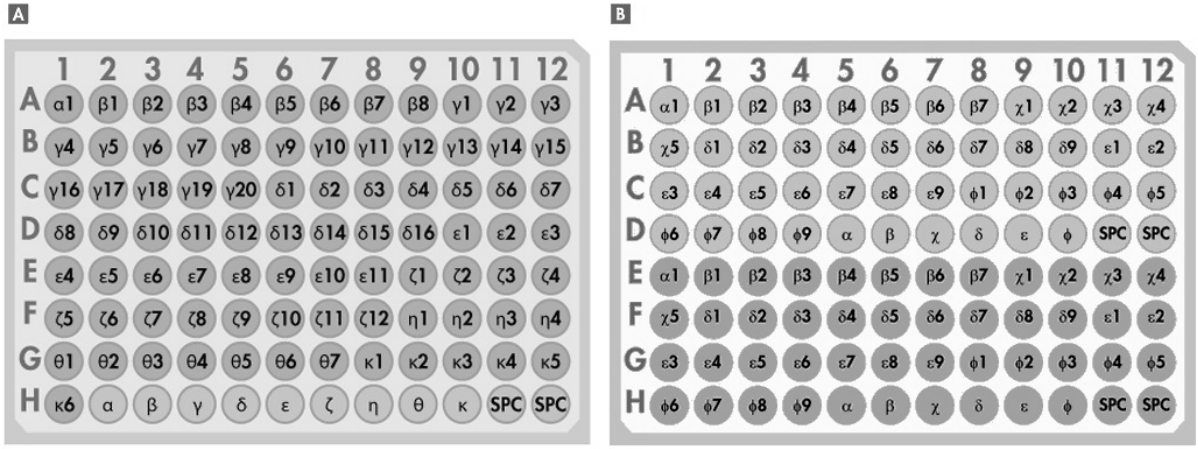

Figure 3. qBiomarker Somatic Mutation PCR Array layout for plate formats A, C, D, F. **A** Typically, wells A1– H1 of the standard qBiomarker Somatic Mutation PCR Array 96-well format contain assays for somatic mutations in the same biological pathway or disease. Wells H2 – H12 contain PCR controls. Depending on the specific array content, slight variations in plate layout may occur. **B** An alternative 2 x 48 option enables 2 samples to be profiled on each plate (sample 1: wells A1 to D12; sample 2: wells E1 to H12).

384-well format

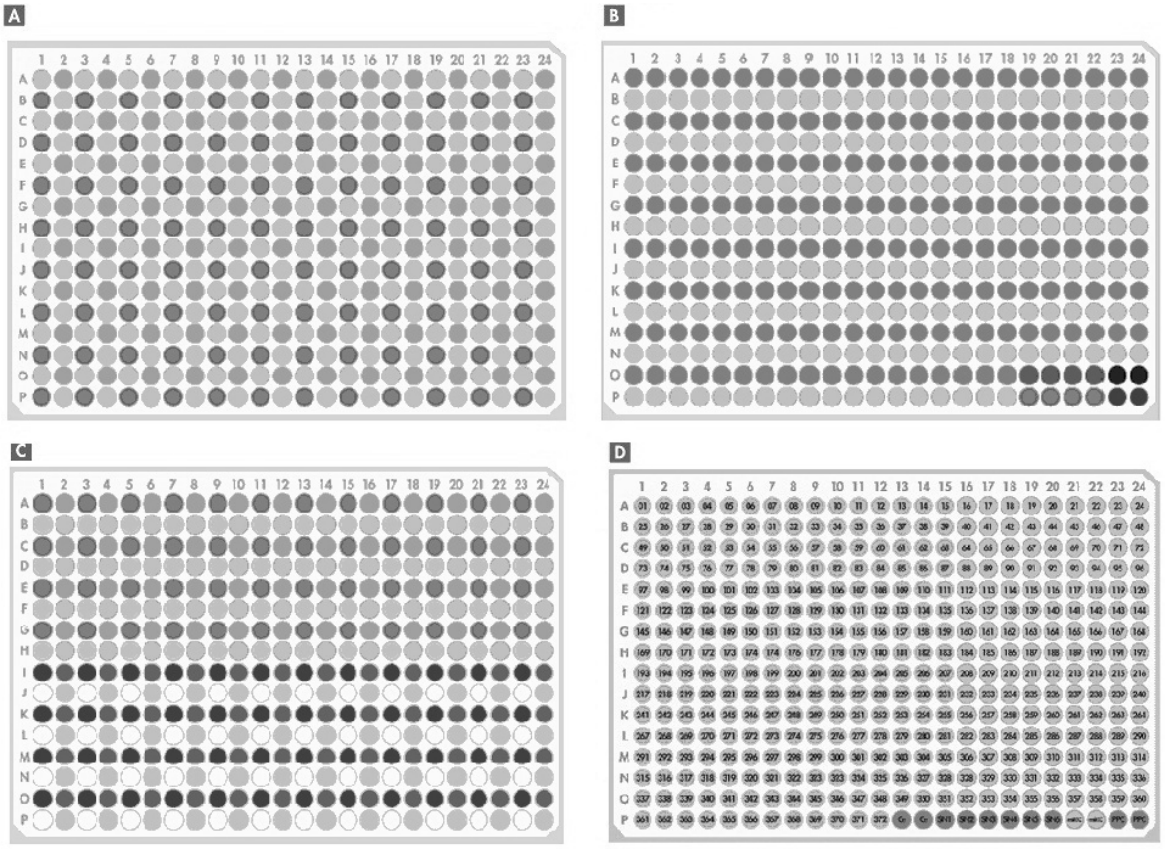

Figure 4. qBiomarker Somatic Mutation PCR Array layout for plate formats E and G. **A** The standard 384-well qBiomarker Somatic Mutation PCR Array format includes 4 replicates of the equivalent 96-well plate format, enabling 4 samples to be tested. Alternative 384-well array options include **B** a 2 x 192 option that allows 2 samples to be profiled on each plate, **C** 8 x 48 option that allows 8 samples to be profiled on each plate, and **D** a 384HT option that allows 1 sample to be profiled on each plate.

Rotor-Disc 100 format

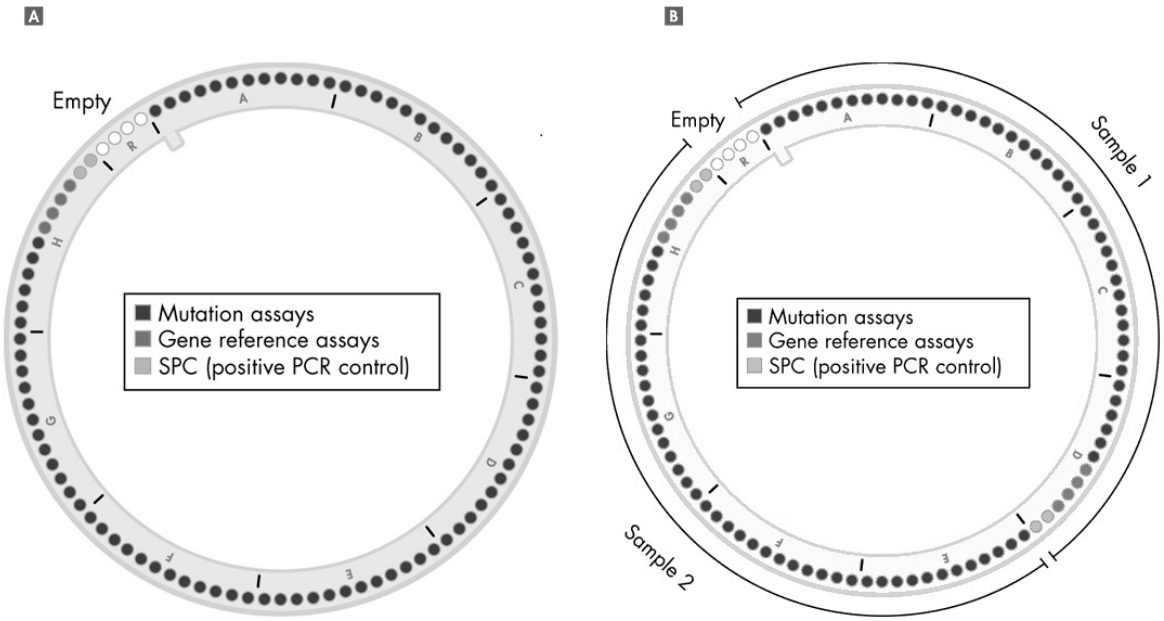

Figure 5. qBiomarker Somatic Mutation PCR Array layout for Rotor-Disc format R. Two array options are available. **A** The 96 option array contains 96 assays (from well position 1 to 96) and can be used for profiling one sample, and **B** the 2 x 48 option array, which contains a duplicate set of 48 assays (set 1is at well positions 1 to 48; set 2 is at well positions 49 to 96) and can be used for profiling 2 samples.

Controls

Each array contains gene copy reference assays for each gene represented by the array. These assays target non-variable regions of the genes and measure input DNA quality and amount. In addition, these assays sensitively measure gene dosage to normalize mutation assay data against the gene copy number. Each array also contains positive PCR controls (SMPC) to test for the presence of inhibitors in the sample or the efficiency of the polymerase chain reaction itself using a pre-dispensed artificial DNA sequence and the primer set that detects it.

Negative and positive control DNA can also be used to ensure that the experimental conditions and PCR setup are correct (see Appendix D, page 53 for more information).

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 material safety data sheets (MSDSs), available from the product supplier.

- Genomic DNA isolation kit (we recommend the QIAamp® DNA Mini Kit (cat. no. 51304) or the QIAamp DNA FFPE Tissue Kit (cat. no. 56404)
- Real-time PCR cycler; the table on page 6 indicates the appropriate real-time cycler for each array format

  Note: qBiomarker Somatic Mutation PCR Arrays are not recommended for the Cepheid SmartCycler or the Roche LightCycler 2.0 due to the heat block format of these instruments.
- Multichannel pipettor
- Nuclease-free pipet tips and tubes
- High-quality nuclease-free water

  Note: Do not use DEPC water
- Optional: REPLI-g UltraFast Mini Kit (cat. no. 150033)

Important Notes

General precautions

For accurate and reproducible PCR array results, it is essential to avoid contamination of the assay with foreign DNA, especially PCR products from previously run plates. The most common sources of DNA contamination are the products of previous experiments.

To maintain a working environment free of DNA contamination, we recommend the following precautions:

- Wear gloves throughout the procedure. Use only fresh PCR-grade reagents (water) and labware (tips and tubes).
- Use sterile pipet tips with filters.
- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents.
- Physically separate the workspaces used for PCR setup and post-PCR processing operations. Decontaminate your PCR workspace and labware (pipets, tube racks, etc.) with UV light before each new use to render any contaminated DNA ineffective in PCR through the formation of thymidine dimers or with 10% bleach to chemically inactivate and degrade any DNA.
- Do not open any previously run and stored PCR array plate. Removing the thin-wall 8-cap strips or the adhesive film from PCR arrays releases PCR product DNA into the air where it can contaminate the results of future experiments. In the event that PCR products need to be analyzed by an independent method, ensure that any labware and bench surfaces are decontaminated.
- Do not remove the PCR array plate from its protective sealed bag until immediately before use.

DNA purification

High-quality DNA is a required starting material for qBiomarker Somatic Mutation PCR Arrays and Assays. QIAGEN provides a range of solutions for genomic DNA purification from various types of samples (Table 1).

Table 1. DNA purification kits recommended for use with qBiomarker Somatic Mutation PCR Arrays and Assays

Sample material	DNA purification kit*	Catalog number
Fresh or frozen tissue or cultured cells	QIAamp DNA Mini Kit	51304
Formalin-fixed, paraffin-embedded (FFPE tissues)	QIAamp DNA FFPE Tissue Kit	56404

* Do not omit the recommended RNase treatment step to remove RNA. RNA contamination will cause inaccuracies in DNA concentration measurements.

PCR setup

For additional assistance with instrument setup, see our instrument-specific setup instructions and protocol files at: www.SABiosciences.com/pcrarrayprotocolfiles.php.

Data analysis

Free data analysis software for qBiomarker Somatic Mutation PCR Arrays is available at www.sabiosciences.com/somaticmutationdataanalysis.php. At this Web page, both the qBiomarker Somatic Mutation PCR Array Web-based software and the qBiomarker Somatic Mutation PCR Array Excel template can be accessed. Both tools will automatically perform genotype/mutation calls using the data analysis method of the user's choice (for more details, see Appendix B, page 45).

Protocol: Real-Time PCR Using the qBiomarker Somatic Mutation PCR Array A, C, D, and F Formats

This protocol is for use with qBiomarker Somatic Mutation PCR Arrays formats A, C, D, and F, using the 96 option array (1 sample per plate) or the 2 x 48 option array (2 samples per plate).

Important points before starting

- Before beginning the procedure, read "Important Notes", page 16.
- It is essential to start with high-quality DNA. For recommended genomic DNA preparation methods, refer to Table 1, page 17.
- For best results, all DNA samples should be resuspended in DNase-free water or, alternatively, in DNase-free 10 mM Tris buffer, pH 8.0. Do not use DEPC-treated water.
- Ensure that you are using the correct master mix for your real-time instrument before beginning this procedure. For a list of cyclers that can be used with each master mix, refer to the table on page 6.
- PCR array plates should only be used in the compatible real-time PCR cycler listed in the table on page 6. The PCR array plates will not fit properly into incompatible real-time PCR cyclers and may cause damage to the cyclers.
- Pipetting accuracy and precision affects the consistency of results. Be sure that all pipets and instruments have been checked and calibrated according to the manufacturer's recommendations.
- For best results, use an 8–channel pipettor to load the PCR array. Alternatively, use 8 tips of a 12–channel pipettor.
- Change pipette tips following each addition of master mix to the PCR array to avoid cross-contamination between the wells or PCR.

Things to do before starting

- Determine DNA concentration and purity by preparing dilutions and measuring absorbance in 10 mM Tris, pH 8.0 buffer. For best results, the concentration measured at A_{260} should be greater than10 μg/ml DNA, the A_{260}/A_{280} ratio should be greater than 1.8, and the A_{260}/A_{230} ratio should be greater than 1.7.
- Determine DNA integrity. To achieve the best results when using a sample containing as little as 10 ng genomic DNA (which requires whole genome amplification), genomic DNA should be greater than 2 kb in length, with some fragments greater than 10 kb. This can be verified by running an

aliquot of each DNA sample on a 1% agarose gel. For DNA extracted from FFPE sections, we recommend omitting the amplification process.

- DNA quality and consistency can also be checked on the qBiomarker Somatic Mutation PCR Array Human DNA QC Plate (cat. no. 337021), which measures 7 reference genes in real-time PCR. For more information, refer to Appendix C, page 47).
- If performing whole genome amplification on your sample, refer to the protocol in Appendix A, page 43.
- Thaw genomic DNA sample and qBiomarker Probe Mastermix at room temperature (15–25°C) prior to starting the procedure. Mix well after thawing.

Procedure

1. **Prepare a reaction mix according to Table 2. For non-amplified genomic DNA, use the following amounts:**

   Fresh tissue samples: add 500 ng DNA for each 96 option array or 250 ng DNA per sample tested using the 2 x 48 option array.

   FFPE samples: add 500 ng to 3 µg of DNA for each 96 option array or 250 ng to 1.5 µg DNA per sample tested using the 2 x 48 option array.

Table 2. Reaction mix

Array format	**96 option**	**2 x 48 option**	**96 option**	**2 x 48 option**
No. samples	**1**	**2**	**1**	**2**
Component	**Amplified DNA**		**Non-amplified DNA**	
qBiomarker Probe Mastermix	1275 µl	680 µl	1275 µl	680 µl
Genomic DNA	15 µl	8 µl	500 ng to 3 µg	250 ng to 1.5 µg
Nuclease-free water	1260 µl	672 µl	Variable	Variable
Total volume per sample*	2550 µl	1360 µl	2550 µl	1360 µl

* Provides an excess volume of 150 µl (96 option array) or 160 µl (2 x 48 option array). Care should be taken when adding the reaction mix to the qBiomarker Somatic Mutation PCR Array to ensure each well receives the required 25 µl volume.

2. **Remove the qBiomarker Somatic Mutation PCR Array from its sealed bag.**

3. **Dispense reaction mix into an RT² PCR Array Loading Reservoir (ordered separately; cat. no. 338162).**

   Use of the RT² PCR Array Loading Reservoir is recommended to assist in loading.

4. **Add reaction mix to the qBiomarker Somatic Mutation PCR Array as follows:**

   For 96 option array (1 sample): add 25 µl to each well.

   For 2 x 48 option array (2 samples): add 25 µl reaction mix for sample 1 into each well of rows A, B, C, and D and 25 µl reaction mix for sample 2 into each well of rows E, F, G, and H.

5. **Tightly seal the qBiomarker Somatic Mutation PCR Array with the optical thin-wall 8-cap strips (A and D formats) or the optical adhesive film (C and F formats).**

   Note: Ensure that no bubbles remain in any of the wells of the array. To remove bubbles, tap the plate gently on the bench top and centrifuge the plate at 1000 rpm for 1 minute.

6. **Program the PCR cycler as described in Table 3.**

   The PCR array plate should be placed on ice until the PCR cycler is set up.

   Arrays that are not processed immediately may be stored wrapped in aluminum foil at –20°C for up to one week.

Table 3. Cycling conditions

Step	Time	Temperature	Number of cycles
Initial PCR activation step	10 min	95°C	1
2-step cycling:			
Denaturation	15 sec	95°C	40
Annealing and extension	60 sec*	60°C	

* Detect and record FAM fluorescence from every well during the annealing/extension step of each cycle.

7. **Place one plate in the real-time thermal cycler. Use a compression pad with the optical film-sealed plate formats (C and F formats) if recommended in the cycler's user manual. Start the run.**

8. **Calculate the threshold cycle (C_T) for each well using the cycler's software (see Table 5 for examples of cycler settings).**

   For best results, we recommend manually setting the baseline and threshold values (see Table 4 for examples of settings for selected real-time cyclers).

   To define the baseline value, use the linear view of the amplification plots and set the cycler to use the readings from cycle 5 up to 2 cycles before the earliest visible amplification, usually around cycle number 15, but not more than cycle number 20.

   To define the threshold value, use the log view of the amplification plots and place the threshold value above the background signal but within the lower half to one-third of the linear phase of the amplification plot.

   Note: Ensure the baseline and threshold settings are the same across all PCR array runs in the same analysis. If the DNA sample quality has been adequately controlled and the cycling program has been executed correctly, then the C_T value for the control sample SMPC should be 22±2 across all arrays or samples. If not, consult the "Troubleshooting Guide", page 41.

Table 4. Example values for threshold and baseline settings

Instrument	Baseline setting	Threshold setting
Applied Biosystems 7900 HT	8–20 cycles	0.1
Applied Biosystems 7500	8–20 cycles	0.1
Agilent Mx3000P and Mx3005P	Varies	0.1

9. **Export the resulting threshold cycle values for all wells to a blank Excel spreadsheet for data analysis (refer to Appendix B, page 45).**

Protocol: Real-Time PCR Using the qBiomarker Somatic Mutation PCR Array E and G Formats

This protocol is for use with qBiomarker Somatic Mutation PCR Array formats E and G, using the 4 x 96 option array (4 samples per plate), the 2 x 192 option array (2 samples per plate), or the 8 x 48 option (8 samples per plate). For formats E and G 384HT option arrays, refer to the protocol on page 29.

Important points before starting

- Before beginning the procedure, read "Important Notes", page 16.
- It is essential to start with high-quality DNA. For recommended genomic DNA preparation methods, refer to Table 1, page 17.
- For best results, all DNA samples should be resuspended in DNase-free water or, alternatively, in DNase-free 10 mM Tris buffer, pH 8.0. Do not use DEPC-treated water.
- Pipetting accuracy and precision affects the consistency of results. Be sure that all pipets and instruments have been checked and calibrated according to the manufacturer's recommendations.
- Ensure that you are using the correct master mix for your real-time instrument before beginning this procedure. For a list of cyclers that can be used with each master mix, refer to the table on page 6.
- PCR array plates should only be used in the compatible real-time PCR cycler listed in the table on page 6. The PCR array plates will not fit properly into incompatible real-time PCR cyclers and may cause damage to the cycler.
- For best results, use a 12–channel pipettor to load the PCR array.
- Change pipette tips following each addition of master mix to the PCR array to avoid cross-contamination between the wells or PCR.

Things to do before starting

- Determine DNA concentration and purity by preparing dilutions and measuring absorbance in 10 mM Tris, pH 8.0 buffer. For best results, the concentration measured at A_{260} should be greater than10 μg/ml DNA, the A_{260}/A_{280} ratio should be greater than 1.8, and the A_{260}/A_{230} ratio should be greater than 1.7.
- Determine DNA integrity. To achieve the best results when using a sample containing as little as 10 ng genomic DNA (which requires whole genome amplification), genomic DNA should be greater than 2 kb in length, with some fragments greater than 10 kb. This can be verified by running an

aliquot of each DNA sample on a 1% agarose gel. For DNA extracted from FFPE sections, we recommend omitting the amplification process.

- DNA quality and consistency can also be checked on the qBiomarker Somatic Mutation PCR Array Human DNA QC Plate (cat. no. 337021), which measures 7 reference genes in real-time PCR. For more information, refer to Appendix C, page 47).
- If performing whole genome amplification on your sample, refer to the protocol in Appendix A, page 43.
- Thaw genomic DNA sample and the qBiomarker Probe Mastermix at room temperature (15–25°C) prior to starting the procedure. Mix well after thawing.

Procedure

1. **Prepare a reaction mix according to Table 5. For non-amplified genomic DNA, use the following amounts:**

   Fresh tissue samples: add 200 ng DNA per sample using the 4 x 96 option array, 400 ng DNA per sample using the 2 x 192 option array, or 100 ng DNA per sample using the 8 x 48 option array.

   FFPE samples: add 200 ng to 1.2 µg DNA per sample using the 4 x 96 option array, 400 ng to 2.4 µg DNA per sample using the 2 x 192 option array, or 100 ng to 600 ng DNA per sample using the 8 x 48 option array.

Table 5. Reaction mix

Array format	4 x 96 option	2 x 192 option	8 x 48 option	4 x 96 option	2 x 192 option	8 x 48 option
No. samples	**4**	**2**	**8**	**4**	**2**	**8**
Component	**Amplified DNA**			**Non-amplified DNA**		
qBiomarker Probe Mastermix	550 µl	1100 µl	275 µl	550 µl	1100 µl	275 µl
Genomic DNA	7 µl	14 µl	3.5 µl	200 ng to 1.2 µg	400 ng to 2.4 µg	100 ng to 600 ng
Nuclease-free water	543 µl	1086 µl	271.5 µl	Variable	Variable	Variable
Total volume per sample*	1100 µl	2200 µl	550 µl	1100 µl	2200 µl	550 µl

* Provides an excess volume of 140 µl (4 x 96 option array), 280 µl (2 x 192 option array), or 70 µl (8 x 48 option array). Care should be taken when adding the reaction mix to the qBiomarker Somatic Mutation PCR Array to ensure each well receives the required 10 µl volume.

2. **Carefully remove the qBiomarker Somatic Mutation PCR Array from its sealed bag.**
3. **Dispense reaction mix into an RT² PCR Array Loading Reservoir (ordered separately; cat. no. 338162).**

   Use of the RT² PCR Array Loading Reservoir is recommended to assist in loading.
4. **Add reaction mix to the qBiomarker Somatic Mutation PCR Array as follows using 384EZLoad Covers (see Figure 6):**

   Note: The spacing between the tips of standard multi-channel pipettors will enable you to skip rows or columns when adding each sample.

   Place cover 1 (white) on the plate.

   For 4 x 96 option array (4 samples): add 10 µl sample 1 reaction mix to the open wells (odd numbered wells of rows A, C, E, G, I, K, M, and O). Remove and discard the cover.

   For 2 x 192 option array (2 samples): add 10 µl sample 1 reaction mix to the open wells (odd numbered wells of rows A, C, E, G, I, K, M, and O). Remove and discard the cover.

For 8 x 48 option array (8 samples): add 10 µl sample 1 reaction mix to the open wells of rows A, C, E, G; add 10 µl sample 2 reaction mix to the open wells of rows I, K, M, and O. Remove and discard the cover.

Place cover 2 (yellow) on the plate.

For 4 x 96 option array (4 samples): add 10 µl sample 2 reaction mix to the open wells (even numbered wells of rows A, C, E, G, I, K, M, and O). Remove and discard the cover.

For 2 x 192 option array (2 samples): add 10 µl sample 2 reaction mix to the open wells (even numbered wells of rows A, C, E, G, I, K, M, and O). Remove and discard the cover.

For 8 x 48 option array (8 samples): add 10 µl sample 3 reaction mix to the open wells of rows A, C, E, and G; add 10 µl sample 4 reaction mix to the open wells of rows I, K, M, and O. Remove and discard the cover.

Place cover 3 (black) on the plate.

For 4 x 96 option array (4 samples): add 10 µl sample 3 reaction mix to the open wells (odd numbered wells of rows B, D, F, H, J, L, N, and P). Remove and discard the cover.

For 2 x 192 option array (2 samples): add 10 µl sample 1 reaction mix to the open wells (odd numbered wells of rows B, D, F, H, J, L, N, and P). Remove and discard the cover.

For 8 x 48 option array (8 samples): add 10 µl sample 5 reaction mix to the open wells of rows B, D, F, and H; add 10 µl sample 6 reaction mix to the open wells of rows J, L, N, and P. Remove and discard the cover.

Place cover 4 (red) on the plate.

For 4 x 96 option array (4 samples): add 10 µl sample 4 reaction mix to the open wells (even numbered wells of rows B, D, F, H, J, L, N, and P). Remove and discard the cover.

For 2 x 192 option array (2 samples): add 10 µl sample 2 reaction mix to the open wells (even numbered wells of rows B, D, F, H, J, L, N, and P). Remove and discard the cover.

For 8 x 48 option array (8 samples): add 10 µl sample 7 reaction mix to the open wells of rows B, D, F, and H; add 10 µl sample 8 reaction mix to the open wells of rows J, L, N, and P. Remove and discard the cover.

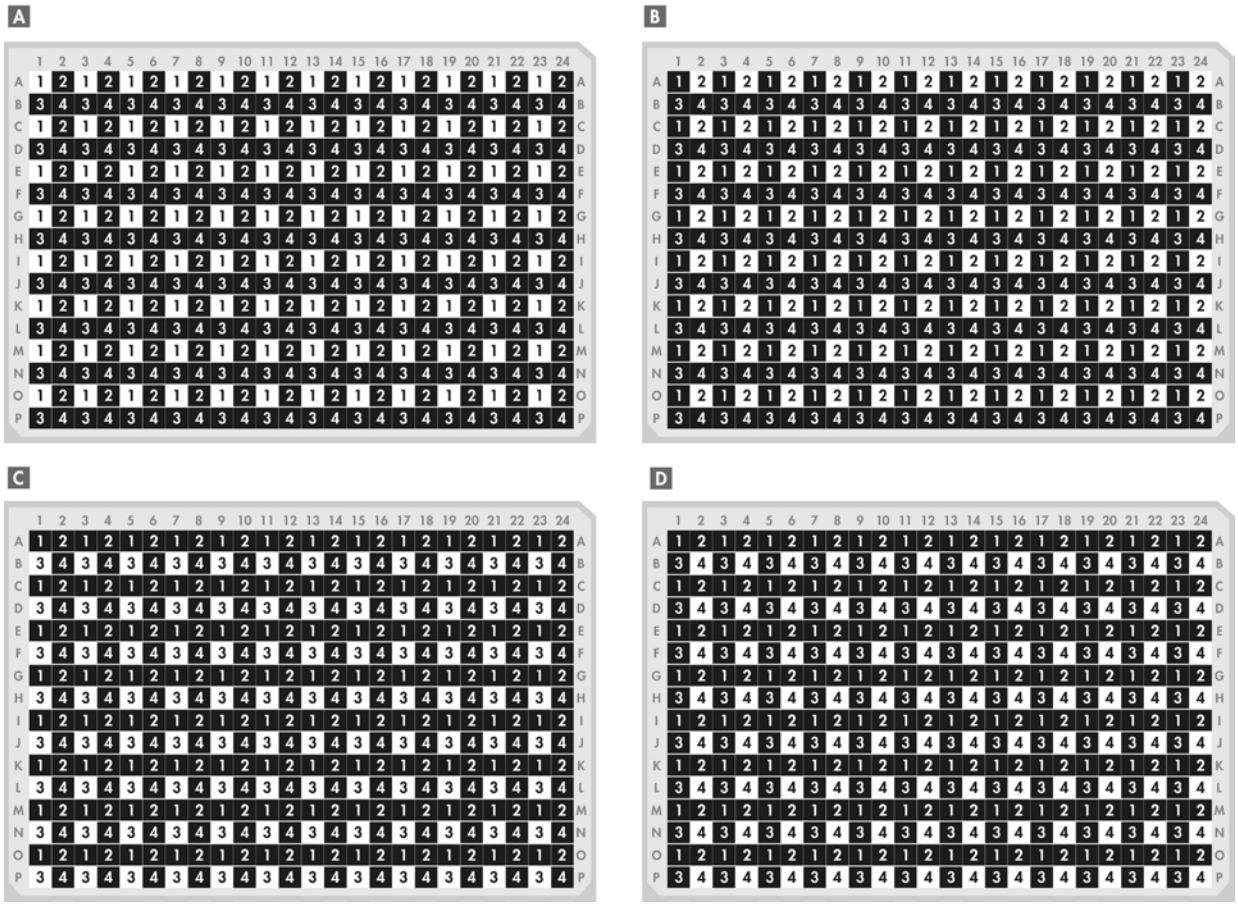

Figure 6. Loading qBiomarker Somatic Mutation PCR Array formats E or G, 4 x 96 option array, 2 x 192 option array, or 8 x 48 option array. For the 4 x 96 option array: Add 10 µl reaction mix from each numbered sample into the staggered wells with the same number as indicated in the figure. For the 2 x 192 option array: use covers 1 and 3 to add 10 µl reaction mix from sample 1 and use covers 2 and 4 to add 10 µl reaction mix from sample 2. For the 8 x 48 option array: use cover 1 to add 10 µl reaction mix from samples 1 and 2; use cover 2 to add 10 µl reaction mix from samples 3 and 4; use cover 3 to add 10 µl reaction mix from samples 5 and 6; use cover 4 to add 10 µl reaction mix from samples 7 and 8. **A** Cover 1; **B** cover 2; **C** cover 3; **D** cover 4

5. **Tightly seal the qBiomarker Somatic Mutation PCR Array with the optical adhesive film.**

   Note: Ensure that no bubbles remain in any of the wells of the array. To remove bubbles, tap the plate gently on the bench top and centrifuge the plate at 2000 rpm for 2 minutes.

6. **Program the PCR cycler as described in Table 6.**

   The PCR array plate should be placed on ice until the PCR cycler is set up.

   Arrays that are not processed immediately may be stored wrapped in aluminum foil at –20°C for up to one week.

Table 6. Cycling conditions

Step	Time	Temperature	Number of cycles
Initial PCR activation step	10 min	95°C	1
2-step cycling:			
Denaturation	15 sec	95°C	40
Annealing and extension	60 sec*	60°C	

* Detect and record FAM fluorescence from every well during the annealing/extension step of each cycle.

7. **Place one plate in the real-time thermal cycler. Use a compression pad with the optical film-sealed plate if recommended in the cycler's user manual. Start the run.**
8. **Calculate the threshold cycle (C_T) for each well using the cycler software (See Table 7 for examples of settings for select real-time cyclers).**

   For best results, recommend manually setting the baseline and threshold values (see Table 7 for examples of settings for select real-time cyclers).

   To define the baseline value, use the linear view of the amplification plots and set the cycler to use the readings from cycle 5 up to 2 cycles before the earliest visible amplification, usually around cycle number 15, but not more than cycle number 20.

   To define the threshold value, use the log view of the amplification plots and place the threshold value above the background signal but within the lower half to one-third of the linear phase of the amplification plot.

   Note: Ensure the baseline and threshold settings are the same across all PCR array runs in the same analysis. If the DNA sample quality has been adequately controlled and the cycling program has been executed correctly, then the C_T value for the control sample SMPC should be 22±2 across all arrays or samples. If not, consult the "Troubleshooting Guide", page 41.

Table 7. Examples values for threshold and baseline settings

Instrument	Baseline setting	Threshold setting
Applied Biosystems 7900 HT	8–20 cycles	0.1
Applied Biosystems 7500	8–20 cycles	0.1
Agilent Mx3000P and Mx3005P	Varies	0.1

9. Export the resulting threshold cycle values for all wells to a blank Excel spreadsheet for data analysis (refer to Appendix B, page 45).

Protocol: Real-Time PCR Using the qBiomarker Somatic Mutation PCR Array E and G Formats 384HT Option

This protocol is for use with the qBiomarker Somatic Mutation PCR Array formats E and G, using the 384HT option array (1 sample per plate).

Important points before starting

- Before beginning the procedure, read "Important Notes", page 16.
- It is essential to start with high-quality DNA. For recommended genomic DNA preparation methods, refer to Table 1, page 17.
- For best results, all DNA samples should be resuspended in DNase-free water or, alternatively, in DNase-free 10 mM Tris buffer, pH 8.0. Do not use DEPC-treated water.
- Pipetting accuracy and precision affects the consistency of results. Be sure that all pipets and instruments have been checked and calibrated according to the manufacturer's recommendations.
- Ensure that you are using the correct master mix for your real-time cycler before beginning this procedure. For a list of cyclers that can be used with each master mix, refer to the table on page 6.
- PCR array plates should only be used in the compatible real-time PCR cycler listed in the table on page 6. The PCR array plates will not fit properly into incompatible real-time PCR cyclers and may cause damage to the cycler.
- For best results, use a 12–channel pipettor to load the PCR array.
- Change pipette tips following each addition of master mix to the PCR array to avoid cross-contamination between the wells or PCR.

Things to do before starting

- Determine DNA concentration and purity by preparing dilutions and measuring absorbance in 10 mM Tris, pH 8.0 buffer. For best results, the concentration measured at A_{260} should be greater than10 μg/ml DNA, the A_{260}/A_{280} ratio should be greater than 1.8, and the A_{260}/A_{230} ratio should be greater than 1.7.
- Determine DNA integrity. To achieve the best results when using a sample containing as little as 10 ng genomic DNA (which requires whole genome amplification), genomic DNA should be greater than 2 kb in length, with some fragments greater than 10 kb. This can be verified by running an

aliquot of each DNA sample on a 1% agarose gel. For DNA extracted from FFPE sections, we recommend omitting the amplification process.

- DNA quality and consistency can also be checked on the qBiomarker Somatic Mutation PCR Array Human DNA QC Plate (cat. no. 337021 SMH-999AFA), which measures 7 reference genes in real-time PCR. For more information, refer to Appendix C, page 47).
- If performing whole genome amplification on your sample, refer to the protocol in Appendix A, page 43.
- Thaw genomic DNA sample and the qBiomarker Somatic Probe Mastermix at room temperature (15–25°C) prior to starting the procedure. Mix well after thawing.

Procedure

1. **Prepare a reaction mix according to Table 8. For non-amplified genomic DNA, use the following amounts:**

   Fresh tissue samples: add 800 ng DNA per sample.

   FFPE samples: add 800 ng to 4.8 µg DNA per sample.

Table 8. Reaction mix

Component	Amplified DNA	Non-amplified DNA
qBiomarker Probe Mastermix	2080 µl	2080 µl
Genomic DNA	20 µl	400 ng to 4.8 µg
Nuclease-free water	2060 µl	Variable
Total volume per sample*	4160 µl	4160 µl

* Provides an excess volume of 320 µl. Care should be taken when adding the reaction mix to the qBiomarker Somatic Mutation PCR Array to ensure each well receives the required 10 µl volume.

2. **Carefully remove the qBiomarker Somatic Mutation PCR Array from its sealed bag.**
3. **Dispense reaction mix into an RT² PCR Array Loading Reservoir (ordered separately; cat. no. 338162).**

   Use of the RT² PCR Array Loading Reservoir is recommended to assist in loading.

4. **Load 10 µl reaction mix into each well of the qBiomarker Somatic Mutation PCR Array.**
5. **Tightly seal the qBiomarker Somatic Mutation PCR Array with the optical adhesive film.**

   Note: Ensure that no bubbles remain in any of the wells of the array. To remove bubbles, tap the plate gently on the bench top and centrifuge the plate at 2000 rpm for 2 minutes.
6. **Program the PCR cycler as described in Table 9.**

   The PCR array plate should be placed on ice until the PCR cycler is set up.

   Arrays that will are not processed immediately may be stored wrapped in aluminum foil at –20°C for up to one week.

Table 9. Cycling conditions

Step	Time	Temperature	Number of cycles
Initial PCR activation step	10 min	95°C	1
2-step cycling:			
Denaturation	15 sec	95°C	40
Annealing and extension	60 sec*	60°C	

* Detect and record FAM fluorescence from every well during the annealing/extension step of each cycle.

7. **Place one plate in the real-time thermal cycler. Use a compression pad with the optical film-sealed plate if recommended in the cycler's user manual. Start the run.**
8. **Calculate the threshold cycle (C_T) for each well using the cycler software (see Table 10 for examples of settings for select real-time cyclers).**

   For best results, recommend manually setting the baseline and threshold values (see Table 10 for examples of settings for select real-time cyclers).

   To define the baseline value, use the linear view of the amplification plots and set the cycler to use the readings from cycle 5 up to 2 cycles before the earliest visible amplification, usually around cycle number 15, but not more than cycle number 20.

   To define the threshold value, use the log view of the amplification plots and place the threshold value above the background signal but within the lower half to one-third of the linear phase of the amplification plot.

Note: Ensure the baseline and threshold settings are the same across all PCR array runs in the same analysis. If the DNA sample quality has been adequately controlled and the cycling program has been executed correctly, then the C_T value for the control sample SMPC should be 22±2 across all arrays or samples. If not, consult the "Troubleshooting Guide", page 41.

Table 10. Examples values for threshold and baseline settings

Instrument	Baseline setting	Threshold setting
Applied Biosystems 7900 HT	8–20 cycles	0.1
Applied Biosystems 7500	8–20 cycles	0.1
Agilent Mx3000P and Mx3005P	Varies	0.1

9. Export the resulting threshold cycle values for all wells to a blank Excel spreadsheet for data analysis (refer to Appendix B, page 45).

Protocol: Real-Time PCR Using the qBiomarker Somatic Mutation PCR Array R Format

This protocol is for use with qBiomarker Somatic Mutation PCR Array format R, using the 96 option array (1 sample per Rotor-Disc 100) or the 2 x 48 option array (2 samples per Rotor-Disc 100).

Important points before starting

- Before beginning the procedure, read "Important Notes", pages 16.
- Two array options are available for the format R arrays: the 96 option array, which contain 96 assays (from well position 1 to 96) and can be used for profiling one sample, and the 2 x 48 option array, which contains a duplicate set of 48 assays (set 1is at well positions 1 to 48; set 2 is at well positions 49 to 96) and can be used for profiling 2 samples. Be sure to check the array format to identify the appropriate reaction mix preparation and sample loading procedure.
- It is essential to start with high-quality DNA. For recommended genomic DNA preparation methods, refer to Table 1, page 17.
- For best results, all DNA samples should be resuspended in DNase-free water or, alternatively, in DNase-free 10 mM Tris buffer, pH 8.0. Do not use DEPC-treated water.
- Ensure that you are using the correct master mix for your real-time cycler before beginning this procedure. For a list of cyclers that can be used with each master mix, refer to the table on page 6.
- Pipetting accuracy and precision affects the consistency of results. Be sure that all pipets and instruments have been checked and calibrated according to the manufacturer's recommendations.

Things to do before starting

- Determine DNA concentration and purity by preparing dilutions and measuring absorbance in 10 mM Tris, pH 8.0 buffer. For best results, the concentration measured at A_{260} should be greater than10 μg/ml DNA, the A_{260}/A_{280} ratio should be greater than 1.8, and the A_{260}/A_{230} ratio should be greater than 1.7.
- Determine DNA integrity. To achieve the best results when using a sample containing as little as 10 ng genomic DNA (which requires whole genome amplification), genomic DNA should be greater than 2 kb in length, with some fragments greater than 10 kb. This can be verified by running an aliquot of each DNA sample on a 1% agarose gel. For DNA extracted from FFPE sections, we recommend omitting the amplification process.

- DNA quality and consistency can also be checked on the qBiomarker Somatic Mutation PCR Array Human DNA QC Plate (cat. no. 337021 SMH-999AFA), which measures 7 reference genes in real-time PCR. For more information, refer to Appendix C, page 47.
- If performing whole genome amplification on your sample, refer to the protocol in Appendix A, page 43.
- Thaw genomic DNA sample and the qBiomarker Probe Mastermix at room temperature (15–25°C) prior to starting the procedure. Mix well after thawing.

Procedure

1. **Prepare a reaction mix according to Table 11. For non-amplified genomic DNA, use the following amounts:**

   Fresh tissue samples: add 400 ng DNA per sample (96 option array), or 200 ng DNA per sample (2 x 48 option array).

   FFPE samples: add 400 ng to 2.4 µg DNA per sample (96 option array) or 200 ng to 1.2 µg DNA per sample (2 x 48 option array).

Table 11. Reaction mix

Array format	**96 option**	**2 x 48 option**	**96 option**	**2 x 48 option**
No. samples	**1**	**2**	**1**	**2**
Component	**Amplified DNA**		**Non-amplified DNA**	
qBiomarker Probe Mastermix	1100 µl	550 µl	1100 µl	550 µl
Genomic DNA	10 µl	5 µl	400 ng to 2.4 µg	200 ng to 2.4 µg
Nuclease-free water	1090 µl	545 µl	Variable	Variable
Total volume per sample*	2200 µl	1100 µl	2200 µl	1100 µl

* Provides an excess volume of 200 µl (96 option array) or 320 µl (2 x 48 option array). Care should be taken when adding the reaction mix to the qBiomarker Somatic Mutation PCR Array to ensure each well receives the required 20 µl volume.

2. **Carefully remove the qBiomarker Somatic Mutation PCR Array from its sealed bag. Slide the array into the Rotor-Disc 100 Loading Block using the tab at position 1 and the tube guide holes.**

3. **Dispense reaction mix into an RT² PCR Array Loading Reservoir (ordered separately; cat. no. 338162).**

   Use of the RT² PCR Array Loading Reservoir is recommended to assist in loading.

4. **Add reaction mix to the qBiomarker Somatic Mutation PCR Array as follows.**

   For the 96 option array (1 sample): add 20 µl reaction mix to each well starting from position 1.

   For the 2 x 48 option array (2 samples): add 20 µl sample 1 reaction mix into wells 1 to 48, 99, and 100 of the PCR array. Add 20 µl sample 2 reaction mix into wells 49 to 98 of the PCR array.

   Reaction mix can be dispensed manually or using the QIAgility (www.qiagen.com/goto/QIAgility).

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

5. **Tightly seal the qBiomarker Somatic Mutation PCR Array with Rotor-Disc Heat-Sealing Film using the Rotor-Disc Heat Sealer.**

6. **Program the Rotor-Gene Q cycler as described in Table 12.**

   The PCR array should be placed on ice until the PCR cycler is set up

   Arrays that are not processed immediately may be stored wrapped in aluminum foil at –20°C for up to one week.

Table 12. Cycling conditions

Step	Time	Temperature	Number of cycles
Initial PCR activation step	10 min	95°C	1
2-step cycling:			
Denaturation	15 sec	95°C	40
Annealing and extension	60 sec*	60°C	

* Detect and record FAM fluorescence from every well during the annealing/extension step of each cycle.

7. **Insert the Rotor-Disc 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*.

8. **Calculate the threshold cycle (C_T) for each well using the Rotor-Gene software.**

   To define the baseline value, select "Ignore First". Fluorescent signal from the initial cycles may not be representative of the remainder of the run. Therefore, better results may be achieved if the initial cycles are ignored. Up to 5 cycles can be ignored.

   Note: Ensure the settings are the same across all PCR array runs in the same analysis.

   Manually define the threshold value by using the log view of the amplification plots. Select a threshold value above the background signal. The threshold value should be in the lower half of the linear phase of the amplification plot. A threshold setting of 0.03 is recommended as a reference.

   Note: Ensure the threshold values are the same across all PCR array runs in the same analysis. The absolute position of the threshold is less critical than its consistent position across the arrays. If the DNA sample is of sufficient quality, the cycling program has been carried out correctly, and the threshold values have been defined correctly, the C_T value for the SMPC control should be 21 ± 2 for all arrays or samples.

9. **Export the resulting threshold cycle values for all wells to a blank Excel spreadsheet for data analysis (refer to Appendix B, page 45).**

Protocol 5: Real-Time PCR Using the qBiomarker Somatic Mutation PCR Assay

This protocol is for use with qBiomarker Somatic Mutation PCR Assays.

Important points before starting

- Before beginning the procedure, read "Important Notes", pages 16.
- It is essential to start with high-quality DNA. For recommended genomic DNA preparation methods, refer to Table 1, page 17.
- For best results, all DNA samples should be resuspended in DNase-free water or, alternatively, in DNase-free 10 mM Tris buffer, pH 8.0. Do not use DEPC-treated water.
- Pipetting accuracy and precision affects the consistency of results. Be sure that all pipets and instruments have been checked and calibrated according to the manufacturer's recommendations.
- Ensure that you are using the correct master mix for your real-time cycler before beginning this procedure. For a list of cyclers that can be used with each master mix, refer to the table on page 6.

Things to do before starting

- Determine DNA concentration and purity by preparing dilutions and measuring absorbance in 10 mM Tris, pH 8.0 buffer. For best results, the concentration measured at A_{260} should be greater than10 µg/ml DNA, the A_{260}/A_{280} ratio should be greater than 1.8, and the A_{260}/A_{230} ratio should be greater than 1.7.
- Determine DNA integrity. To achieve the best results when using a sample containing as little as 10 ng genomic DNA (which requires whole genome amplification), genomic DNA should be greater than 2 kb in length, with some fragments greater than 10 kb. This can be verified by running an aliquot of each DNA sample on a 1% agarose gel. For DNA extracted from FFPE sections, we recommend omitting the amplification process.
- DNA quality and consistency can also be checked on the qBiomarker Somatic Mutation PCR Array Human DNA QC Plate (cat. no. 337021 SMH-999AFA), which measures 7 reference genes in real-time PCR. For more information, refer to Appendix C, page 47.
- Thaw genomic DNA sample and the qBiomarker Probe Mastermix at room temperature (15–25°C) prior to starting the procedure. Mix well after thawing.

Procedure

1. **Set up 2 reaction mixes according to Table 13 for the specific somatic mutation assay and the corresponding reference gene copy assay.**

   Note: Do not forget to include a wild-type control sample.

   To detect a single somatic mutation using an individual PCR assay, there is no need to use amplified genomic DNA. For best results when using genomic DNA from FFPE samples, use 4–30 ng (for reactions performed using Rotor-Gene cyclers) or 5–30 ng (for reactions performed using all other cyclers) per PCR assay.

Table 13. Reaction mix

Component	Rotor-Gene cyclers	All other cyclers
qBiomarker Probe Mastermix	10 µl	12.5 µl
qBiomarker Somatic Mutation PCR Assay	1 µl	1 µl
DNA sample	4 ng	5 ng
Water	Variable	Variable
Total volume per sample*	20 µl	25 µl

* If setting up more than one reaction, prepare a reaction mix 10% greater than that required for the total number of reactions to be performed.

2. **Dispense reaction mixes into the PCR wells.**
3. **Tightly seal the PCR plate with optical thin-wall 8-cap strips or optical adhesive film. If using a Rotor-Disc, tightly seal the Rotor-Disc or Strip Tubes using the Rotor-Disc Heat Sealing Film or caps.**

   Note: Ensure that no bubbles remain in any of the PCR wells. To remove bubbles, tap the PCR plate or tube gently on the bench top and centrifuge at 1000 rpm for 1 minute.

4. **Program the PCR cycler as described in Table 14.**

   Reactions should be placed on ice until the PCR cycler is set up.

   Assays that are not processed immediately may be stored wrapped in aluminum foil at –20°C for up to one week.

Table 14. Cycling conditions

Step	Time	Temperature	Number of cycles
Initial PCR activation step	10 min	95°C	1
2-step cycling:			
Denaturation	15 sec	95°C	40
Annealing and extension	60 sec*	60°C	

* Detect and record FAM fluorescence from every well during the annealing/extension step of each cycle.

5. **Place PCR tubes/plate/Rotor-Disc into the real-time thermal cycler. Use a compression pad with the optical film-sealed plate formats if recommended in the cycler's user manual. Start the run.**
6. **Calculate the threshold cycle (C_T) for each well using the cycler software.**

   Note: Ensure the settings are the same across all PCR assay runs in the same analysis.

   For assays performed using a Rotor-Gene cycler:

   To define the baseline value, select "Ignore First". Fluorescent signal from the initial cycles may not be representative of the remainder of the run. Therefore, better results may be achieved if the initial cycles are ignored. Up to 5 cycles can be ignored.

   Manually define the threshold value by using the log view of the amplification plots. Select a threshold value above the background signal. The threshold value should be in the lower half of the linear phase of the amplification plot. A threshold setting of 0.03 is recommended as a reference.

   For assays performed using all other cyclers:

   For best results, recommend manually setting the baseline and threshold values (see Table 15 for examples of settings for select real-time cyclers).

   To define the baseline value, use the linear view of the amplification plots and set the cycler to use the readings from cycle 5 through 2 cycles before the earliest visible amplification, usually around cycle number 15, but not more than cycle number 20.

To define the threshold value, use the log view of the amplification plots and place the threshold value above the background signal but within the lower half to one-third of the linear phase of the amplification plot.

Table 15. Examples values for threshold and baseline settings

Instrument	Baseline setting	Threshold setting
Applied Biosystems 7900 HT	8–20 cycles	0.1
Applied Biosystems 7500	8–20 cycles	0.1
Agilent Mx3000P and Mx3005P	Varies	0.1

7. Export the resulting threshold cycle values for all wells to a blank Excel spreadsheet (refer to Appendix B, page 45).

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 product, or product detected late in real-time PCR for positive controls	
a) PCR annealing time too short	Use the annealing time specified in the protocol.
b) PCR extension time too short	Use the extension time specified in the protocol.
c) Pipetting error or missing reagent when setting up PCR	Check the concentrations and storage conditions of reagents, including primers and cDNA.
d) HotStart DNA Polymerase not activated with a hot start	Ensure that the cycling program includes the 10 minute hot start activation step for HotStart DNA polymerase.
e) No detection activated	Check that fluorescence detection was activated in the cycling program.
f) Wrong detection step	Ensure that fluorescence detection takes place during the extension step of the PCR cycling program.
g) Wrong dye layer/filter chosen	Ensure that the appropriate layer/filter is activated.
h) Insufficient starting template	Increase the amount of template genomic DNA.

The average C_T^{SMPC} value varies by more than 2 across the qBiomarker Somatic Mutation PCR Arrays being compared and/or is greater than 24

a) Cycler sensitivity levels vary	If the average C_T^{SMPC} value of 22±2 is difficult to obtain for your cycler, the observed average C_T^{SMPC} value should be acceptable as long as it does not vary by more than 2 cycles between the qBiomarker Somatic Mutation PCR Arrays being compared.

Poor sample quality resulting in high C_T values

a) Mutations are not identified when using the average C_T method because the mutation locus C_T value is too high	Use the average C_T value for gene copy number assays on the array to gauge the sample quality (or run the sample on a DNA QC plate before testing the samples on the array). For FFPE samples, we recommend for the average C_T to be below 32 to allow sensitive detection of mutations. Samples that meet this criterion perform robustly on the arrays.

Varying fluorescence intensity

a) Real-time cycler contaminated	Decontaminate the real-time cycler according to the supplier's instructions.
b) Real-time cycler no longer calibrated	Recalibrate the real-time cycler according to the supplier's instructions.

Appendix A: Whole Genome Amplification of Genomic DNA

Whole genome amplification (WGA) can dramatically reduce the required amount of starting material. This protocol is for amplifying DNA from fresh or frozen tissue samples for subsequent use with qBiomarker Somatic Mutation PCR Arrays.

Note: The following procedure is a quick setup guide for whole genome amplification using the REPLI-g UltraFast Mini Kit. For detailed instructions, refer to the *REPLI-G UltraFastMini Handbook*.

Important points before starting

- WGA is intended for fresh or frozen cell and tissue samples that contain 5–10 ng genomic DNA. If 200–500 ng genomic DNA is extracted from fresh tissue and is of high quality, it is not necessary to perform WGA (see "Important Notes", page 16).
- WGA is not recommended for DNA extracted from FFPE sections.

Procedure

A1. Prepare sufficient Buffer D1 for the total number of WGA reactions, including a wild-type control sample, according to Table 16.

Table 16. Preparation of Buffer D1

Component	Volume
Reconstituted Buffer DLB	5 µl
Nuclease-free water	35 µl
Total volume*	40 µl

* The total volume is sufficient for 40 samples.

A2. Prepare sufficient Buffer N1 for the total number of WGA reactions, including a wild-type control sample, according to Table 17.

Table 17. Preparation of Buffer N1

Component	Volume
Reconstituted Buffer DLB	8 µl
Nuclease-free water	72 µl
Total volume*	80 µl

* The total volume is sufficient for 40 samples.

A3. Dilute genomic DNA to 10 ng/µl in nuclease-free water.

A4. Add 1 µl genomic DNA into a microcentrifuge tube.

A5. Add 1 µl Buffer D1 to the genomic DNA and mix by gentle pipetting.

A6. Incubate the samples at room temperate (15–25°C) for 3 min.

A7. Add 2 µl Buffer N1 to the samples and mix by gentle pipetting.

A8. Prepare a reaction mix on ice according to Table 18.

Mix and centrifuge briefly.

Table 18. Preparation of reaction mix

Component	Volume
REPLI-g UltraFast Reaction Buffer	15 µl
REPLI-g UltraFast DNA Polymerase	1 µl
Total volume	16 µl

A9. Add 16 µl master mix to 4 µl sample.

A10. Incubate the samples at 30°C for 1.5 hours.

A11. Incubate the samples at 65°C for 3 min to inactivate the DNA polymerase.

A12. Store the amplified DNA at –20°C until use.

There is no need to repurify the DNA.

Appendix B: Data Analysis

Free data analysis software for qBiomarker Somatic Mutation PCR Arrays is available at www.SABiosciences.com/somaticmutationdataanalysis.php.

Procedure

Excel-based PCR array Data Analysis template

B1. Download the Excel-based PCR array Data Analysis Template from www.sabiosciences.com/somaticmutationdataanalysis.php.

B2. Save the Excel file to your local computer. Open the file in Excel.

Note: For analyzing data generated on Rotor-Gene cyclers, use the Rotor-Gene–specific Data Analysis Template.

B3. Follow the instructions for using the template provided in the "instructions" Excel worksheet.

B4. If using a 384-well (E or G format), download the 384-well format E data analysis patch to convert a 384-well dataset into the correct 4 x 96-well dataset for each of the 4 samples.

Web-based PCR array Data Analysis tool

B5. Access the Web-based PCR array Data Analysis Tool from www.sabiosciences.com/somaticmutationdataanalysis.php.

Principles for qBiomarker Somatic Mutation PCR Array Data Analysis

The qBiomarker Somatic Mutation PCR Assay utilizes allele specific primer design. Each mutation assay maximizes the detection of mutant DNA with minimal or no detection of the wild-type DNA template. The C_T value from the mutation assay (C_T^{MUT}) is inversely correlated to the abundance of mutant DNA in the sample.

$\Delta\Delta C_T$ method (recommended for experiments using fresh or frozen samples or a smaller [≤4] number of samples)

To account for the different starting amounts of DNA copies used in the experiment, a separate reference assay is setup using the same amount of DNA that is used in the mutation assay. This reference assay is designed on a non-variable region of the same gene that carries the mutation. The C_T value (C_T^{REF}) of the reference assay essentially correlates to the total number of DNA copies used in the mutation-specific assay.

Note: Higher than normal C_T^{REF} values mean that the starting DNA amount and/or quality is significantly lower than optimal. This will reduce the ability to detect 1% mutant DNA in the sample. For 5 ng genomic DNA isolated from fresh tissue, the C_T^{REF} value typically ranges from 25 to 29 (depending on target genes). However, if only one C_T^{REF} shows an aberrantly high value (i.e., >35), while C_T^{REF} values for other genes are in the normal range, this may indicate a homologous deletion in that gene. None of the loci for a deleted gene will be assigned a genotype in downstream analysis.

The relative abundance of mutant DNA templates in a given test sample can be represented by: $\Delta C_{T\,TEST} = C_T^{MUT} - C_T^{REF}$.

In order to reliably determine the mutation status for a specific allele in the test sample, a control sample that has the wild-type sequence for the corresponding allele also needs to be tested with the same mutation assay and reference assay. The resulting $\Delta C_{T\,CTRL}$ ($= C_T^{MUT} - C_T^{REF}$) establishes the wild-type background relative to the total DNA input for the mutation-specific assay.

When $\Delta C_{T\,TEST}$ is significantly smaller than $\Delta C_{T\,CTRL}$ ($\Delta C_{T\,TEST} < \Delta C_{T\,CTRL}$) by statistical analysis or a preset threshold, a positive mutation call can be made. Otherwise, the sample is considered to be wild-type for the assayed allele.

Average C_T method (recommended for experiments using FFPE samples, a large number of samples, or samples without wild-type controls)

The average C_T method assumes that for a given locus, mutation only occurs in a small percentage of tested samples. Thus the average C_T for that locus across all the samples analyzed can be used to represent the mutation assay background in the wild-type sample. The C_T from a mutation assay in a test sample will be compared with this average C_T. If a particular mutation assay in a test sample yields a much lower C_T (according to a present threshold) than the average C_T for the same locus, then this suggests that the sample carries a mutation at that locus.

Limited by the accuracy of the real-time PCR chemistry, any sample with a C_T value greater than 35 for the mutation assay indicates that the mutation is not detected for the corresponding allele in that sample. A small number of assays will have a raw C_T cutoff of 36 or 37. These C_T cutoff values will be embedded in the qBiomarker Somatic Mutation PCR Array data analysis template.

Note: Some assays' raw C_T cutoff values are different for the Rotor-Gene cycler (format R). The Rotor-Gene–specific set of raw C_T cutoff values are imbedded in Rotor-Gene–specific Excel- and Web-based data analysis tools.

Appendix C: Quality Control Using the qBiomarker Somatic Mutation PCR Array Human DNA QC Plate

Sample DNA quality can affect the performance of the qBiomarker Somatic Mutation PCR Array. For DNA purified from FFPE sections, different degrees of cross linkage and fragmentation may cause the mutation detection window to decrease, consequently the mutation analysis for certain low-quality samples may be compromised, especially for mutant alleles that are present at a lower percentage in the sample. Thus when unsure of sample quality, it is recommended to perform quality control using a qBiomarker Somatic Mutation PCR Array Human DNA QC Plate (cat. no. 337021).

The DNA QC Plate is designed to measure the C_T of 7 reference genes. When the DNA is highly cross-linked or fragmented, C_Ts from these 7 genes will be much higher than those from the same amount of high quality DNA. Each 96-well format DNA QC Plate can be used for quality control of 12 DNA samples. DNA QC Plates are available in formats A, C, D, and F (96-well plates; see Figure 7), formats E, G (384-well plates), or format R (Rotor-Disc 100, see Figure 8).

	1	2	3	4	5	6	7	8	9	10	11	12
A	BRAF	BRAF	BRAF	BRAF	BRAF	BRAF	BRAF	BRAF	BRAF	BRAF	BRAF	BRAF
B	KRAS	KRAS	KRAS	KRAS	KRAS	KRAS	KRAS	KRAS	KRAS	KRAS	KRAS	KRAS
C	HRAS	HRAS	HRAS	HRAS	HRAS	HRAS	HRAS	HRAS	HRAS	HRAS	HRAS	HRAS
D	NRAS	NRAS	NRAS	NRAS	NRAS	NRAS	NRAS	NRAS	NRAS	NRAS	NRAS	NRAS
E	MEK1	MEK1	MEK1	MEK1	MEK1	MEK1	MEK1	MEK1	MEK1	MEK1	MEK1	MEK1
F	PIK3CA	PIK3CA	PIK3CA	PIK3CA	PIK3CA	PIK3CA	PIK3CA	PIK3CA	PIK3CA	PIK3CA	PIK3CA	PIK3CA
G	PTEN	PTEN	PTEN	PTEN	PTEN	PTEN	PTEN	PTEN	PTEN	PTEN	PTEN	PTEN
H	SMPC	SMPC	SMPC	SMPC	SMPC	SMPC	SMPC	SMPC	SMPC	SMPC	SMPC	SMPC

Figure 7. qBiomarker Somatic Mutation PCR Array Human DNA QC Plate layout (formats A, C, D, and F). Each plate enables quality control of 12 samples (one sample per column). Formats E and G consist of 4 replicates of the 96-well format plates, enabling quality control of 48 samples (not shown).

Well	1	2	3	4	5	6	7	8
Sample 1	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	9	10	11	12	13	14	15	16
Sample 2	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	17	18	19	20	21	22	23	24
Sample 3	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	25	26	27	28	29	30	31	32
Sample 4	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	33	34	35	36	37	38	39	40
Sample 5	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	41	42	43	44	45	46	47	48
Sample 6	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	49	50	51	52	53	54	55	56
Sample 7	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	57	58	59	60	61	62	63	64
Sample 8	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	65	66	67	68	69	70	71	72
Sample 9	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	73	74	75	76	77	78	79	80
Sample 10	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	81	82	83	84	85	86	87	88
Sample 11	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC
Well	89	90	91	92	93	94	95	96
Sample 12	BRAF	KRAS	HRAS	NRAS	MEK1	PIK3CA	PTEN	SMPC

Figure 8. qBiomarker Somatic Mutation PCR Array Human DNA QC Plate layout (format R). Each plate enables quality control of 12 DNA samples.

Important points before starting

- Text marked with a ■ denotes instructions for 96-well and 384-well plates (Formats A, C, D, E, F, G) and text marked with a ▲ denotes instructions for use with Rotor-Disc 100 (format R).

Procedure

C1. Prepare enough reaction mix for 8.4 reactions per sample (Table 19).

Table 19. Reaction mix

Component	98-well plate	384-well plate	Rotor-Disc 100
DNA sample	40 ng	16 ng	32 ng
qBiomarker Probe Mastermix	105 µl	42 µl	84 µl
Water	Variable	Variable	Variable
Total volume in 8.4 reactions	210 µl	84 µl	168 µl

C2. Add reaction mix to each well of the DNA quality control plate as follows:

For 98-well plate: add 25 µl

For 384-well plate: add 10 µl

For Rotor-Disc 100: add 20 µl

C3. Tightly seal the array with the ■ optical adhesive films or optical thin-wall 8-cap strips or with ▲Rotor-Disc Heat-Sealing Film using the Rotor-Disc Heat Sealer.

Note: Ensure that no bubbles remain in any of the wells of the array. To remove bubbles, tap the plate/disc gently on the bench top and centrifuge at 2000 rpm for 2 minutes.

C4. Program the PCR cycler as described in Table 20.

The PCR array plate should be placed on ice until the PCR cycler is set up.

Arrays that are not processed immediately may be stored wrapped in aluminum foil at –20°C for up to one week.

Table 20. Cycling conditions

Step	Time	Temperature	Number of cycles
Initial PCR activation step	10 min	95°C	1
2-step cycling:			
Denaturation	15 sec	95°C	40
Annealing and extension	60 sec*	60°C	

* Detect and record FAM fluorescence from every well during the annealing/extension step of each cycle.

C5. ■ Place one plate in the real-time cycler. Use a compression pad with the optical film-sealed plate formats if recommended in the cycler's user manual. ▲Insert the Rotor-Disc into the Rotor-Disc 100 Rotor and secure with the Rotor-Disc 100 Locking Ring. Start the run.

C6. Calculate the threshold cycle (C_T) for each well using the cycler software.

Note: Ensure the settings are the same across all PCR assay runs in the same analysis.

For formats A, C, D, E, F, G:

We highly recommend manually setting the baseline and threshold values. See Table 21 for examples of settings for select real-time cyclers.

To define the baseline value, use the linear view of the amplification plots and set the instrument to use the readings from cycle 5 through 2 cycles before the earliest visible amplification, usually around cycle number 15, but not more than cycle number 20.

To define the threshold value, use the log view of the amplification plots and place the threshold value above the background signal but within the lower half to one-third of the linear phase of the amplification plot.

For format R:

To define the baseline value, select "Ignore First". Fluorescent signal from the initial cycles may not be representative of the remainder of the run. Therefore, better results may be achieved if the initial cycles are ignored. Up to 5 cycles can be ignored.

Manually define the threshold value by using the log view of the amplification plots. Select a threshold value above the background

signal. The threshold value should be in the lower half of the linear phase of the amplification plot. A threshold setting of 0.03 is recommended as a reference.

Table 21. Examples values for threshold and baseline settings

Instrument	Baseline setting	Threshold setting
Applied Biosystems 7900 HT	8–20 cycles	0.1
Applied Biosystems 7500	8–20 cycles	0.1
Agilent Mx3000P and Mx3005P	Varies	0.1

C7. Export the resulting threshold cycle values for all wells to a blank Excel spreadsheet.

Data analysis of the DNA QC Plate

To determine the quality of DNA samples based on the C_T results, first ensure that the C_T of SMPC assay for each sample is consistent at ~22 (formats A, C, D, E, F, G) or ~21 (format R). If not, adjust the baseline and threshold setting to achieve that value. Then calculate the average for the lowest 6 C_Ts among the gene copy number assays for each sample. (The highest C_T is removed from the average calculation as some samples may contain homozygous deletion for one of the 7 genes included on the DNA QC Plate. The deleted gene will give a high C_T value.) The typical average C_T for high-quality DNA from fresh tissue samples should be below 29 (based on the baseline and threshold setup outlined in Appendix B, page 45). Samples of lower quality (i.e. average C_T value higher than 29) may not yield optimal results.

For DNA extracted from FFPE samples, an average C_T value of lower than 32 for the lowest 6 C_Ts (using 5 ng genomic DNA in 25 µl reaction volume, 2 ng genomic DNA input in 10 µl reaction volume, or 4 ng genomic DNA in a 20 µl reaction volume) indicates sufficient quality for mutation profiling analysis. Samples of lower quality (i.e., average C_T value higher than 32) may not yield optimal results or require more input materials (to make the average C_T value lower than 32).

Appendix D: qBiomarker Somatic Mutation Control DNA

Positive and negative control DNAs can be used to ensure that the experimental conditions and PCR setup are correct.

Positive control DNA

qBiomarker Mutation Positive Control DNA is a mixture of DNA containing 33 mutations in 14 genes. These genes are listed in Table 22.

Table 22. Mutations present in positive control DNA

COSMIC ID	Gene Symbol	Mutation CDS	Mutation AA	Catalog#
483	HRAS	c.35G>T	p.G12V	SMPH006497A
496	HRAS	c.181C>A	p.Q61K	SMPH006505A
553	KRAS	c.182A>T	p.Q61L	SMPH007544A
563	NRAS	c.34G>A	p.G12S	SMPH010075A
574	NRAS	c.38G>T	p.G13V	SMPH010082A
580	NRAS	c.181C>A	p.Q61K	SMPH010073A
760	PIK3CA	c.1624G>A	p.E542K	SMPH010629A
775	PIK3CA	c.3140A>G	p.H1047R	SMPH010630A
783	FLT3	c.2503G>T	p.D835Y	SMPH005661A
4898	PTEN	c.950_953delTACT	p.V317fs*3	SMPH011511A
4986	PTEN	c.741_742insA	p.P248fs*5	SMPH011468A
5039	PTEN	c.518G>A	p.R173H	SMPH011472A
5662	CTNNB1	c.110C>T	p.S37F	SMPH003946A
5667	CTNNB1	c.134C>T	p.S45F	SMPH003953A
5670	CTNNB1	c.101G>T	p.G34V	SMPH003948A

Table continued on next page.

Table 22 (continued). Mutations present in positive control DNA

COSMIC ID	Gene Symbol	Mutation CDS	Mutation AA	Catalog#
5677	CTNNB1	c.98C>G	p.S33C	SMPH003963A
5708	CTNNB1	c.119C>T	p.T40I	SMPH003987A
5661	CTNNB1	c.94G>T	p.D32Y	SMPH003956A
13127	APC	c.4348C>T	p.R1450*	SMPH000539A
5888	PTEN	c.723_724insTT	p.E242fs*15	SMPH011734A
6137	BRAF	c.1799T>G	p.V600G	SMPH001912A
6147	PIK3CA	c.1636C>G	p.Q546E	SMPH010708A
1311	KIT	c.2446G>C	p.D816H	SMPH007137A
10648	TP53	c.524G>A	p.R175H	SMPH014921A
10654	TP53	c.637C>T	p.R213*	SMPH014928A
10656	TP53	c.742C>T	p.R248W	SMPH014929A
517	KRAS	c.34G>A	p.G12S	SMPH007533A
10670	TP53	c.469G>T	p.V157F	SMPH014984A
10704	TP53	c.844C>T	p.R282W	SMPH014941A
10758	TP53	c.659A>G	p.Y220C	SMPH014964A
10808	TP53	c.488A>G	p.Y163C	SMPH014931A
12476	CDKN2A	c.341C>T	p.P114L	SMPH002680A
28749	IDH1	c.394C>G	p.R132G	SMPH006592A

Each aliquot contains 1000 ng DNA in a 50 µl volume. The recommended amount to use in an assay setup is as follows:

- 5 ng positive control DNA per 25 µl reaction
- 4 ng positive control DNA per 20 µl reaction
- 2 ng positive control DNA per 10 µl reaction

Under proper assay setup and PCR conditions, the corresponding wells on the PCR arrays will yield a C_T value between 25 and 31 for the positive control DNA.

Negative control DNA

qBiomarker Mutation Negative Control DNA is derived from an EBV-transformed lymphoblast cell line. This DNA serves as a negative control for all qBiomarker Somatic Mutation PCR Arrays and Assays. Each aliquot contains 500 ng DNA in a 25 µl volume. The recommended amount to use in an assay setup is as follows:

- 5 ng negative control DNA per 25 µl reaction
- 4 ng negative control DNA per 20 µl reaction
- 2 ng negative control DNA per 10 µl reaction

Under proper assay setup and PCR conditions, each negative control DNA assay will yield a C_T value that is above the raw C_T cutoff value (usually ~35) for that assay.

References

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Ordering Information

Product	Contents	Cat. no.
qBiomarker Somatic Mutation PCR Array	PCR plate and master mix	337021
qBiomarker Somatic Mutation PCR Assay	PCR assay and master mix	337011
qBiomarker Mutation Assay Control DNA	Positive and negative control DNA set	337016
Related products		
REPLI-g UltraFast Mini Kit	For 25 preps: DNA Polymerase, Buffers, and Reagents for 25 x 20 µl ultrafast whole genome amplification reactions	150033
QIAamp DNA Mini Kit	For 50 preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA FFPE Tissue Kit	For 50 preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404

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