Scientific article

Rapid and accurate cancer somatic mutation profiling with the qBiomarker Somatic Mutation PCR Arrays

Samuel Long, Min You, Liang Wang & Yexun Wang
QIAGEN, 6951 Executive Way, Frederick, Maryland 21703, USA

Abstract: QIAGEN has developed real-time PCR-based qBiomarker Somatic Mutation PCR Arrays for pathway- and disease-focused mutation profiling. By combining allele-specific amplification and 5' hydrolysis probe detection, the PCR assays on these arrays detect as little as 0.01% somatic mutation in a background of wild-type genomic DNA. These assays have consistent and reliable mutation detection performance in different sample types (including fresh, frozen, or formalin-fixed samples), and with varying sample quality. In application examples, the PCR-based mutation detection results are consistent with Pyrosequencing results for the same samples. The qBiomarker Somatic Mutation PCR Arrays, combining laboratory-verified assays, comprehensive content, and integrated data analysis software, are highly suited for identifying somatic mutations in the context of biological pathways and diseases.

Introduction

Advances in sequencing technology have led to a significant increase in the number of known somatic mutations involved in cancer. Translating such information into therapeutic benefits requires the development of research tools that enable simultaneous interrogation of multiple mutations. Mutation detection has been applied to 3 separate applications. The first application is personalized medicine, where clinicians treat patients based on their genotype or somatic mutations. This application is first applied during drug development studies for absorption, distribution, metabolism, and excretion (ADME) and toxicology studies. The second application is the identification of genotypes that correlate with clinical outcomes and prognosis. This application requires the flexibility to conduct current as well as retrospective studies. The last application allows basic researchers to link mutations with specific molecular mechanisms. For example, chronic lymphocytic leukemia is a heterogeneous disease with at least 2 subtypes, but lacked known genomic drivers. A recent report has demonstrated that mutations in specific genes define specific subgroups of patients. The study also suggested that mutations in 2 of these genes are activating events, and therefore are putative therapeutic targets (1). Each of these applications requires sensitive, verified assays with focused, disease-relevant content.

Contents

In order to be applied to these different applications, a research tool should contain carefully selected mutation content, must unambiguously distinguish between mutant alleles and wild-type alleles (i.e., high assay specificity), and must detect the presence of a low percentage of mutant cells among non-malignant cells in tumor tissue (i.e., high assay sensitivity). This tool must account

for the confounding impact of gene copy number changes on data interpretation. To meet these research needs, QIAGEN developed pathway- and disease-focused qBiomarker Somatic Mutation PCR Arrays that simultaneously and accurately detect 80 to 370 somatic mutations in the same sample. The pathways covered include major receptor tyrosine kinase pathways and non-receptor kinase pathways, as well as additional oncogene and tumor suppressor pathways. The targeted diseases include all major cancer types. In addition, a collection of more than 900 pre-verified somatic mutation assays enables researchers to study single mutations or to customize the mutation panels or collections according to their research needs. The required sample input is 2 to 5 nanograms of genomic DNA (including DNA from FFPE samples) for each mutation detection reaction. The simple workflow involves mixing the DNA sample of interest with ready-to-use qBiomarker Probe PCR Mastermix, aliquoting the mixture into the array plate wells, performing real-time PCR, and making mutation/genotype calls using Web-based data analysis software or Excel® templates that compare the allele-specific C_T values between the test sample and a wild-type control sample. The simplicity of the qBiomarker Somatic Mutation PCR Arrays format and operating procedure allows routine somatic mutation profiling in any research laboratory with access to a real-time PCR instrument (Figure 1).

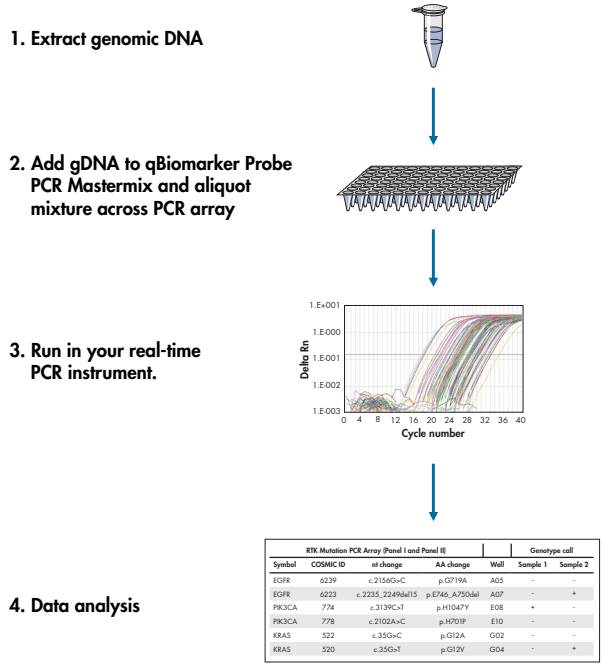


Figure 1. qBiomarker Somatic Mutation PCR Array workflow. The procedure involves DNA extraction, qPCR detection on qBiomarker Somatic Mutation PCR Arrays, and data analysis (using the qBiomarker Somatic Mutation Data Analysis Template or Web portal).

Technological principles

The qBiomarker Somatic Mutation PCR Arrays contain panels of bench-verified hydrolysis probe-based real-time PCR assays. By combining allele-specific amplification and hydrolysis probe detection, QIAGEN has developed real-time PCR assays that detect as low as 0.01% mutant DNA in a wild-type background. Allele-specific amplification is achieved by Amplification Refractory Mutation System (ARMS®) technology (2), which is based on Taq polymerase discriminating between a match and a mismatch at the 3' end of the PCR primer (Figure 2). These assays are optimized to work under standard cycling conditions, enabling a large number of assays to be analyzed simultaneously.

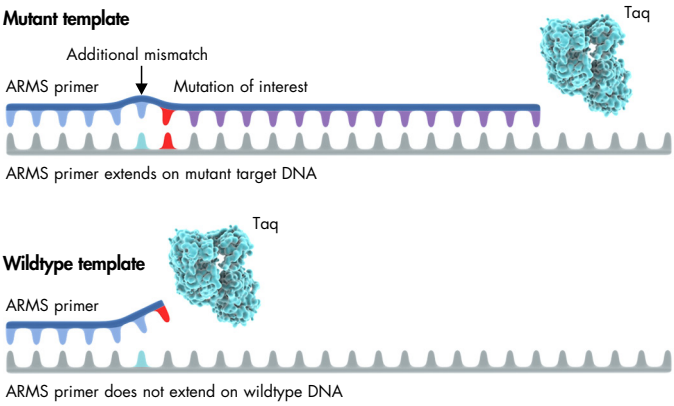
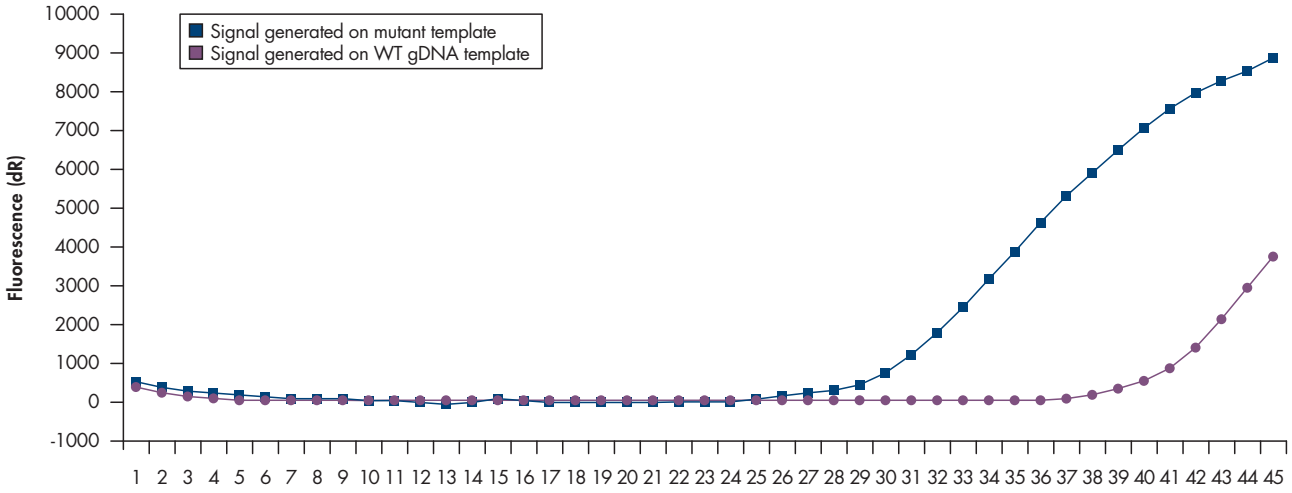


Figure 2. Allele discrimination with ARMS primers. ARMS technology differentiates between mutant and wild-type alleles using template matches and mismatches at a primer's 3' end. An ARMS primer that has a match to the mutant template at the 3' end, which is mismatched with the wild-type, will extend on the mutant instead of the wild-type template. Additional mismatch(es) can be included in the ARMS primer to increase allele discrimination. Allele-specific amplification results, revealing the presence of mutant alleles.

Assay statistics and performance

To ensure assay sensitivity (i.e., the detection of low-percentage mutations) and assay specificity (i.e. the detection of only the mutant allele), we determine for each mutation assay the C_T value difference between equal copy numbers of mutant synthetic and wild-type templates (~3000 genome copies, equivalent to 12,000 single-stranded copies). Our assay verification quality control criteria mandate that the mutation assay must have a minimum difference, or detection window, of 8 cycles (Figure 3A). Assays on average demonstrate a detection window of 13.6 cycles, allowing for very sensitive detection of low-percentage mutations (Figure 3B). The calculated average assay sensitivity is 0.008%, and the calculated median assay sensitivity is 0.024% (Figure 3B and 3C). We also verify that all assays have close to 100% amplification efficiency (Figure 3D).

A

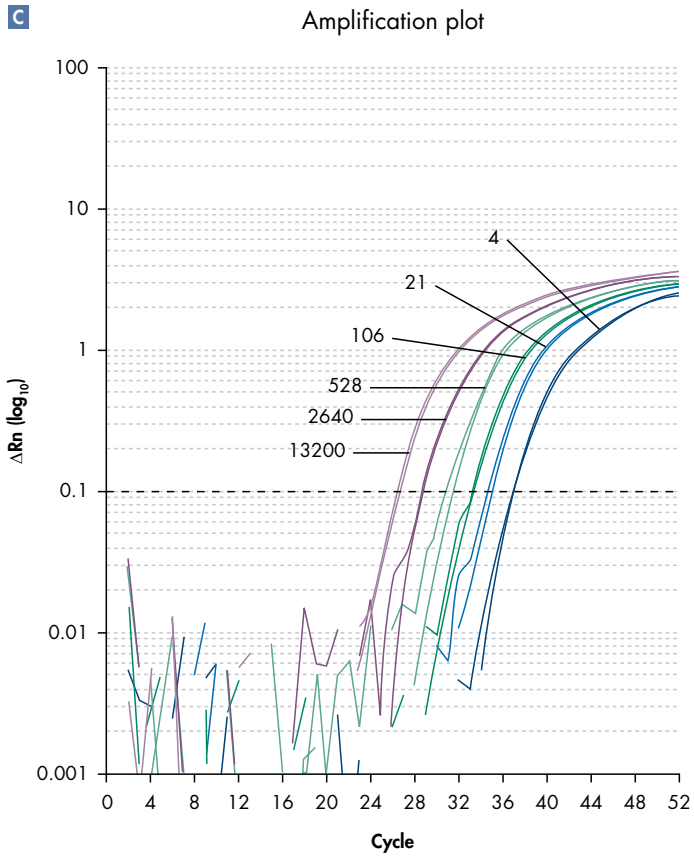


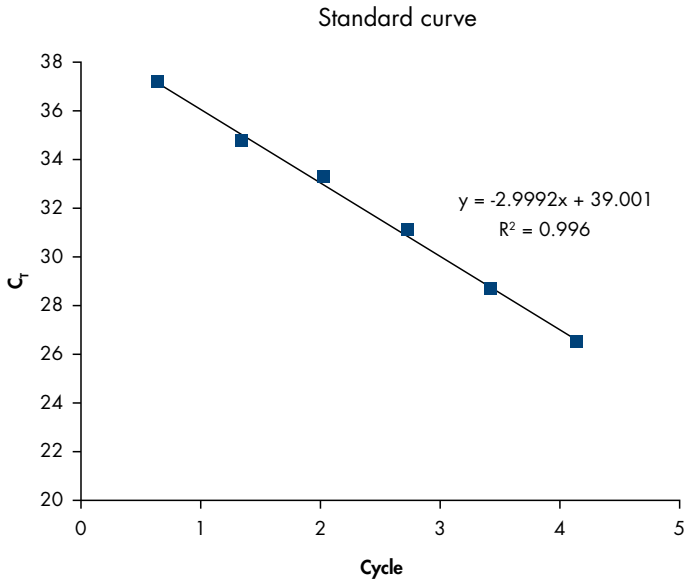
B

Window average	13.6
Window median	12.1
Calculated average sensitivity	0.008%
Calculated median sensitivity	0.024%

Figure 3. Assay statistics. A Assay differentiation window (ADW). The ADW was defined as the C_T difference between the signals generated on a wild-type genomic DNA background and on 100% mutant template by a mutation assay. Assays were verified to have a minimal ADW of 8 but are usually higher. B Average and median assay differentiation window, and average, median assay sensitivities based on 406 qBiomarker Somatic Mutation PCR Assays. C Assay sensitivity test for p53 R280K qBiomarker Somatic Mutation PCR Assay. A series of 10 ng genomic DNA samples, which contain 4, 21, 106, 528, 2640 and 13200 copies of mutant DNA template respectively, from MDA-MB-231 cell line (mutant harboring the p53 R280K mutation) mixed with genomic DNA from the Coriell GM00131 cell line (wild-type), were tested on qBiomarker Somatic Mutation PCR Assay for p53 R280K. Assays were performed in duplicate. Amplification plots for all assays (top) and linear plot using the average of duplicate assay C_Ts (bottom) are shown. Mutation detection limit for this assay is determined to be 0.03%. D Summary of average ADW and assay efficiencies on 4 of the qBiomarker Somatic Mutation PCR Arrays.

C





D

	Average ADW	Average efficiency
EGFR pathway	11.15	104.7%
ERBB2 pathway	12.33	107.1%
PDGFR pathway	11.41	102.3%
FLT3 pathway	11.73	102.5%

Content selection and array format

The development of the qBiomarker Somatic Mutation PCR Arrays and Assays achieves two important goals. Firstly, researchers can screen 80 to 370 cancer mutations per sample in a single PCR run using the whole-plate array format, or alternatively, screen a smaller number of mutations for a greater number of samples using the custom array feature. Each of these options is available on a 96-well or 384-well plate. Secondly, this system provides comprehensive coverage of the most frequently detected, functionally significant cancer mutations in public databases, with ready-to-use single assays for cancer research and drug development. To these ends, we based our assay development content selection on the published functional significance of cancer somatic mutations and the mutations' relative reporting frequency in the literature. For pathway-focused arrays, we included assays for detecting the most frequent and functionally verified mutations for multiple genes within a specific pathway implicated in a variety of cancers. Additional assays are also available for each gene to allow array customization. For disease-focused arrays, we drew from between 2000 to 40,000 published tumor samples per disease type and selected the most frequently reported somatic mutations for each.

Each array contains reference assays for each gene represented by the array. These assays target non-variable regions of the genes and measure input DNA quality and quantity. 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 (SPC) 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 its primer set (Figure 4).

Layout of qBiomarker Somatic Mutation PCR Array

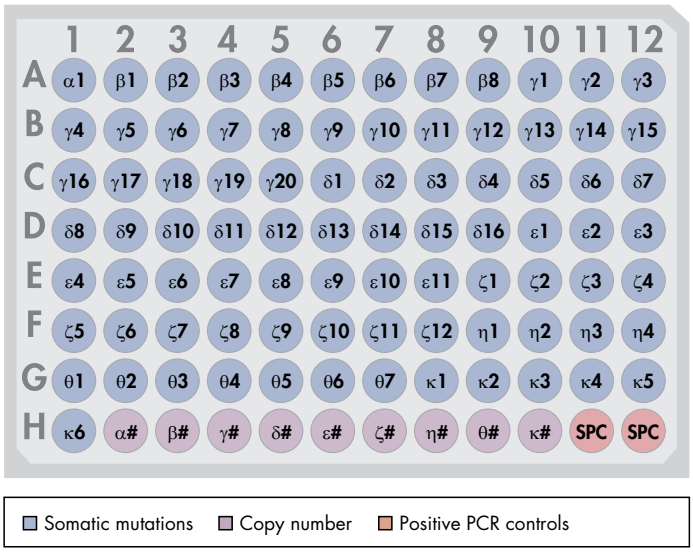


Figure 4. qBiomarker Somatic Mutation PCR Array layout (96 well format). Wells A1 through H1 contain assays for somatic mutations in the same biological pathway or cancer type. Wells H2 through H10 contain gene copy number reference assays to normalize mutation assay data. Depending on the specific array content, slight variations in plate layout can occur. Wells H11 and H12 contain replicate Positive PCR Controls (SPC) to test for the presence of inhibitors in the sample or efficiency of the polymerase chain reaction itself using a pre-dispensed artificial DNA sequence and the primer set that detects it.

The 96-well format usually allows 1 (96 assays per sample) to 12 (8 assays per sample) samples to be analyzed on each plate, while a 384-well format plate allows 1 (384 assays per sample) to 48 (8 assays per sample) samples to be analyzed on each plate.

Data analysis

The qBiomarker Somatic Mutation PCR Arrays utilizes allele-specific primer design to maximize the detection of mutant DNA with minimal or no detection of the wild-type DNA template. The choice between one of two data analysis methods depends on the experimental setup and sample type.

1. $\Delta\Delta C_T$ method (Figure 5A)

Recommended for experiments using:

- **A small number (4 or less) of fresh, frozen samples with known wild-type samples**
- **A large number of samples with similar DNA quality with known wild-type samples**

The C_T value from a mutation detection assay (C_T^{MUT}) for a sample is inversely and exponentially proportional to the abundance of DNA containing this mutation in the sample. A separate simultaneous copy number reference assay using the same amount of DNA as in the mutation detection assay accounts for potential gene copy number variation in different samples. This reference assay targets a non-variable region of the same gene which carries the mutation, and a sample's C_T value on this assay (C_T^{REF}) is inversely and exponentially proportional to the total copies of DNA for that gene in this sample.

The relative abundance of mutant DNA templates in a given test sample has a reverse correlation with $\Delta C_T^{TEST} = C_T^{MUT} - C_T^{REF}$.

To reliably determine the mutation status for a specific allele in a test sample, a wild-type allele control sample(s) must also be tested with the same mutation detection and copy number reference assays. The resulting ΔC_T^{CTRL} (= $C_T^{MUT} - C_T^{REF}$) establishes a window into which mutation-positive samples fall. When multiple wild-type allele control samples are present, an average of the ΔC_T^{CTRL} will be calculated and used in downstream comparison.

When ΔC_T^{TEST} is significantly smaller than ΔC_T^{CTRL} ($\Delta C_T^{TEST} < \Delta C_T^{CTRL}$) by statistical analysis or a preset threshold (for example 4 cycles), a positive mutation call ("+") can be made. A borderline ("+/–") mutation call will be made if the difference is between –3 and –4. Otherwise, the sample is considered wild-type ("–") for the assayed allele.

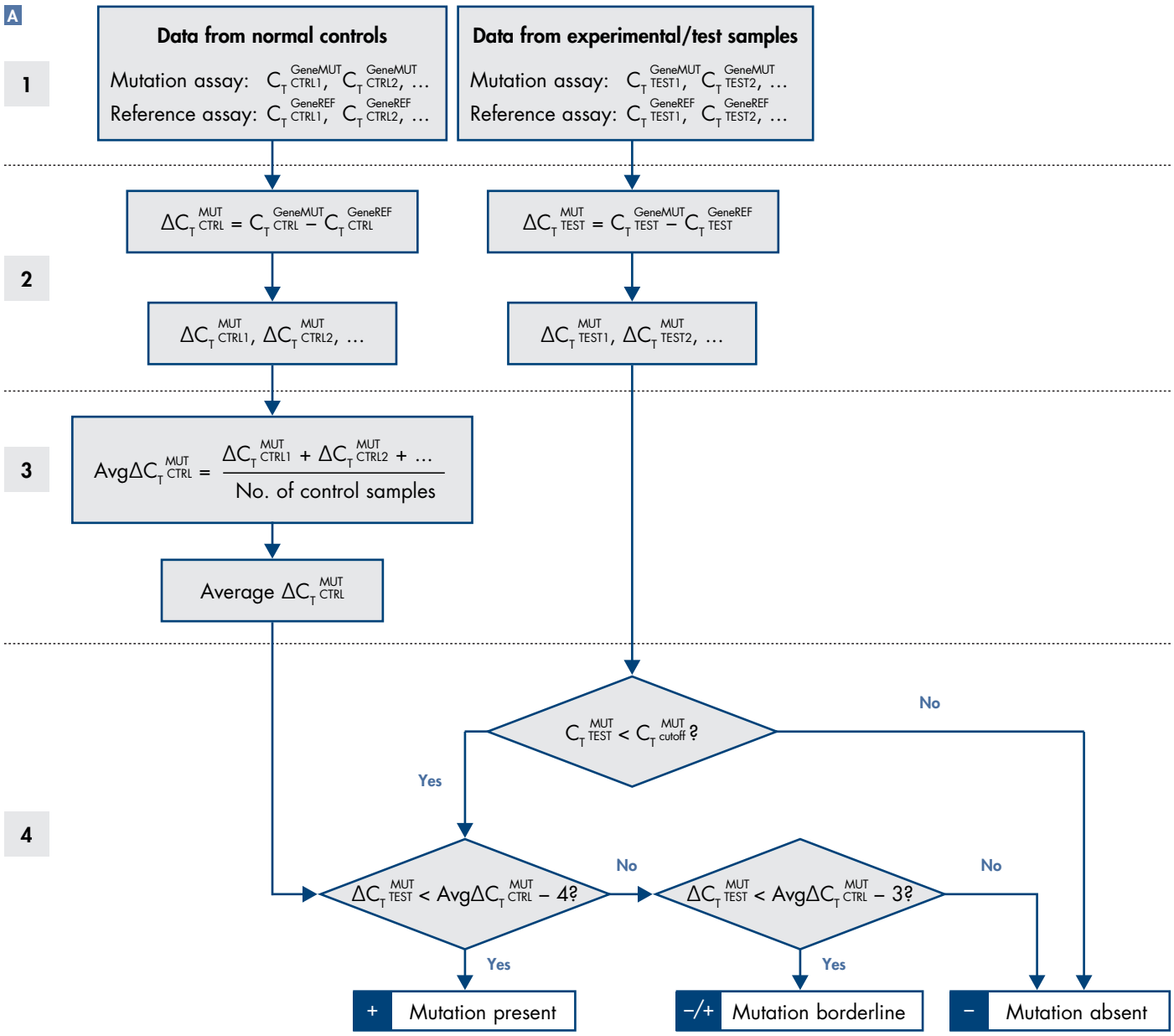


Figure 5A. Data analysis workflow for the $\Delta\Delta C_T$ method.

Note: A wild-type reference control is required for this method.

1. Raw C_T data for each mutation assay are grouped by two conditions.

2. ΔC_T for each mutation assay in each sample is calculated as the difference of the mutation assay (C_T GeneMUT) and corresponding gene copy reference assay (C_T GeneREF) (e.g. BRAF V600E assay C_T and BRAF copy assay C_T).

3. The average ΔC_T for each mutation assay in all normal control samples is calculated.

4. The raw C_T for a given mutation assay in a test sample is compared with a predefined C_T cutoff. If the outcome is "No", the mutation is considered "Absent". If "Yes", the ΔC_T for that mutation in that test sample is compared with the corresponding average ΔC_T of the normal control samples. Based on the difference, the mutation can be considered as "Present", "Borderline", or "Absent".

2. Average C_T method (Figure 5B)

Recommended for experiments using:

- **FPPE samples, a large number of samples with varying DNA quality, or samples without wild-type controls**

The average C_T method assumes that for a given locus, mutations only occur in a small percentage of tested samples. Thus, the average C_T value for that locus across all the samples analyzed may represent the mutation assay background in a wild-type sample. The C_T value from a mutation detection assay in a test sample will be compared with this average C_T value across all samples tested. If a particular mutation assay in a test sample yields a much lower C_T (according to a pre-set threshold of 4 cycles) than the average C_T value for that locus, then the sample carries a mutation at that locus. A borderline ("+/–") mutation call will be made if the difference is between 3 and 4.

For both data analysis methods, a C_T value greater than 37 for a mutation assay in a sample generally indicates that the mutant allele is not detected in that sample. A small number of assays will have a raw C_T cutoff of 35 or 36. Each assay's C_T cutoff value was individually defined in a variety of DNA samples that are wild-type for the locus of interest and represent the lowest C_T value that can be obtained for a sample that is wild-type at this locus. The C_T cutoff values are embedded in the qBiomarker Somatic Mutation PCR Arrays data analysis software.

The control assays contained on each array plate test each sample's quality to ensure reliable mutation calls. The gene copy reference assays mentioned above sensitively indicate gene dosage differences. Homozygous deletions register as a lack of detection by both the mutation detection and copy number reference assays, and no call can be made. A good measure of input DNA amount and quality is the average C_T of all gene copy reference assays on the array (excluding any aberrantly high C_T values indicative of homozygous deletions). The duplicate SPC assays indicate PCR reaction success. The complimentary data analysis software provides a report on sample quality and SPC results to allow researchers to qualify or disqualify a particular sample from further analysis.

Data analysis can either be performed on the qBiomarker Somatic Mutation PCR Arrays Data Analysis Web Portal or by downloading the Excel data analysis templates at www.sabiosciences.com/somaticmutationdataanalysis.php

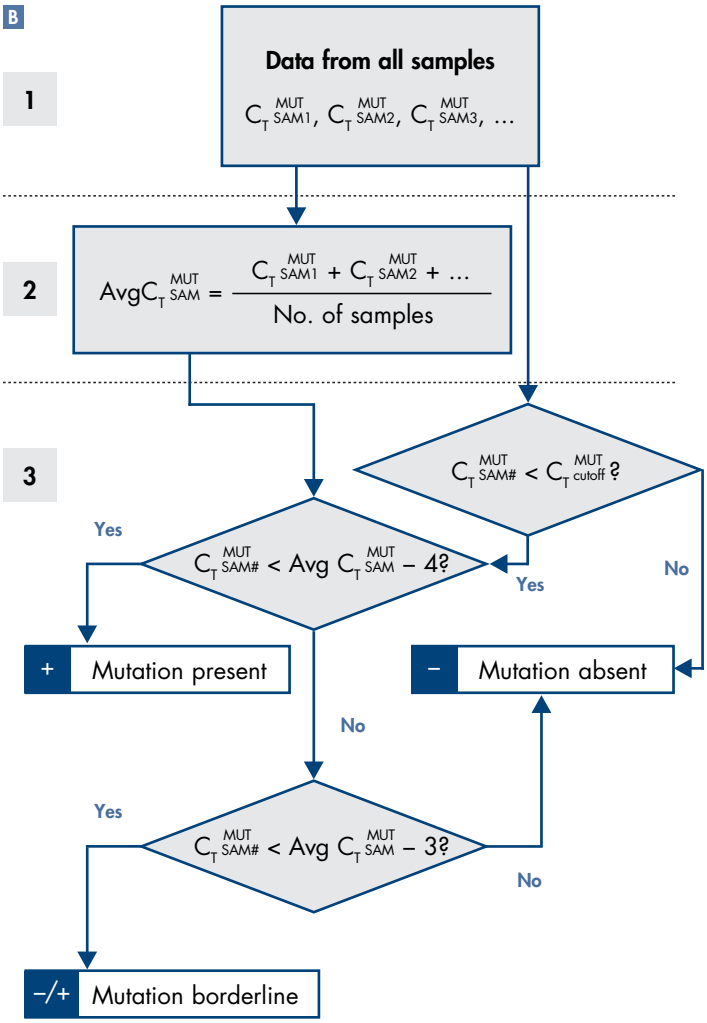


Figure 5B: Data analysis workflow for the average C_T method.

1. Raw C_T data for each mutation assay from all samples regardless of conditions is entered into the data analysis software.

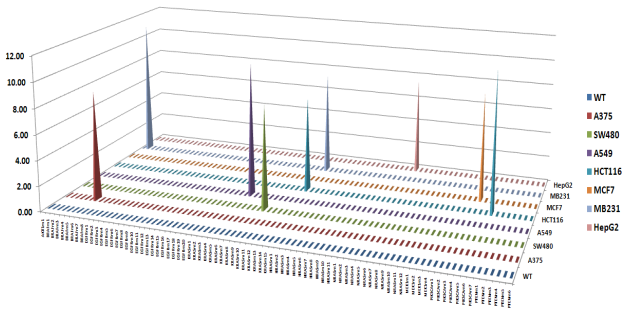
2. The average C_T for each mutation assay in all samples is calculated. Later, this value is used to estimate the assay background in the wild-type sample. The underlying assumption is that the frequency of a mutation is always low (e.g. <20%) in a large enough sample pool. Thus the majority of test samples are wild-type samples for that mutation locus.

3. The raw C_T for a given mutation assay in a test sample is compared with a predefined C_T cutoff. If the outcome is "No", the mutation is considered "Absent". If "Yes", the C_T for that mutation in that test sample is compared with the corresponding average C_T from all samples. Based on the difference, the mutation can be considered as "Present", "Borderline", or "Absent".

Array performance

The qBiomarker Somatic Mutation PCR Arrays and Assays yield accurate and verifiable results in various sample types, including fresh frozen cell lines and tissue samples, cell line admixtures, FFPE cell line samples and FFPE tissue samples from various sources. The examples presented below provide representative mutation profiling results for cancer cell lines, cell line admixtures and FFPE tissue samples.

A



B

Gene	Nucleotide change
AKT1	c.49G>A
BRAF	c.1391G>T, c.1397G>T, c.1406G>C, c.1789C>G, c.1798G>A, c.1799T>A, c.1799T>C, c.1799T>G
EGFR	c.2155G>A, c.2155G>T, c.2156G>C, c.2235_2249del15, c.2236_2250del15, c.2236_2253del18, c.2237_2251del15, c.2238_2248>GC, c.2238_2255del18, c.2239_2247del9, c.2239_2256del18, c.2240_2254del15, c.2303G>T, c.2307_2308insGCCAGCGTG, c.2310_2311insGGT, c.2319_2320insCAC, c.2369C>T, c.2572C>A, c.2573T>G, c.2582T>A
KRAS	c.182A>G, c.182A>T, c.183A>T, c.34G>A, c.34G>C, c.34G>T, c.35G>A, c.35G>C, c.35G>T, c.37G>A, c.37G>C, c.37G>T, c.38G>A, c.38G>C, c.38G>T, c.64C>A
HRAS	c.181C>A, c.182A>G, c.182A>T, c.183G>T, c.34G>A, c.34G>C, c.34G>T, c.35G>A, c.35G>T, c.37G>C, c.37G>T
NRAS	c.181C>A, c.182A>C, c.182A>G, c.182A>T, c.34G>A, c.35G>A, c.35G>C, c.37G>C, c.38G>A, c.38G>C, c.38G>T, c.52G>A
MEK1	c.167A>C, c.171G>T, c.199G>A, c.371C>T
PIK3CA	c.1616C>G, c.1624G>A, c.1633G>A, c.1634A>G, c.1635G>T, c.3140A>G, c.3140A>T
PTEN	c.389G>A, c.388C>G, c.388C>T, c.517C>T, c.518G>A, c.697C>T

Figure 6. qBiomarker Somatic Mutation PCR Array performance in cancer cell lines. A 200 ng genomic DNA from WT or well-characterized cancer cell lines was profiled on the Human EGFR Pathway qBiomarker Somatic Mutation PCR Arrays. All previously identified mutations in the EGFR pathway in these cell lines were called correctly. B List of assays included in the Human EGFR Pathway qBiomarker Somatic Mutation PCR Array.

Cancer cell lines and cell line admixtures

To validate mutation call accuracy, we analyzed a control B cell line (WT) and 7 cancer cell lines (A375, SW480, A549, HC_T116, MCF7, MDA-MB231 and HepG2) with 9 known mutations in the EGFR pathway on the Human EGFR Pathway qBiomarker Somatic Mutation PCR Arrays. Cell line DNA (200 ng each) was profiled against a total of 85 mutations in the EGFR pathway. All 9 mutations were readily detected (Figure 6).

To test sensitivity, a 100% wild-type sample and 3 DNA admixtures mimicking 3 different mutant samples containing 10%, 5%, and 2% of each of the above 7 cell lines in a wild-type DNA background were profiled on the Human EGFR Pathway qBiomarker Somatic Mutation PCR Arrays. The array detected all mutations in all mutant sample scenarios except the PIK3CA E545K mutation at the 2% level (Figure 7).

Symbol	COSMIC ID	Nt change	AA change	Well	10%	5%	2%
BRAF	450	c.1391G>T	p.G464V	A02	+	+	+
BRAF	476	c.1799T>A	p.V600E	A07	+	+	+
KRAS	517	c.34G>A	p.G12S	C09	+	+	+
KRAS	520	c.35G>T	p.G12V	D02	+	+	+
KRAS	532	c.38G>A	p.G13D*	D06	+	+	+
NRAS	583	c.182A>T	p.Q61L	E12	+	+	+
PIK3CA	763	c.1633G>A	p.E545K	G03	+	+	–
PIK3CA	775	c.3140A>G	p.H1047R	G06	+	+	+

Figure 7. qBiomarker Somatic Mutation PCR Arrays performance in cell line admixtures. Three DNA admixtures were profiled on the Human EGFR Pathway qBiomarker Somatic Mutation PCR Arrays. They mimic 3 different heterogeneous samples, which contain 10%, 5%, and 2% of each mutation in the wild-type DNA background, respectively (*except for KRAS G13D, which is at 20%, 10% and 4% because it is carried by 2 cell lines in the admixture). The array was able to detect all mutations in all mutant sample scenarios except the PIK3CA E545K mutation at 2%.

To test the performance of the qBiomarker Somatic Mutation PCR Arrays on FFPE samples, we used the Human EGFR Pathway qBiomarker Somatic Mutation PCR Arrays to profile somatic mutations in 4 FFPE cell line samples, a wild-type FFPE placenta sample (control), and 9 lung adenocarcinoma FFPE tissue samples (Asterand®). The array detected BRAF and KRAS mutations in the FFPE MDA-MB231 cell line (Figure 8), which were independently confirmed by QIAGEN KRAS PCR Kit (formerly DxS KRAS Mutation Kit) and Pyrosequencing (data not shown). The array also detected one KRAS somatic mutation and one EGFR somatic mutation in 2 of the FFPE lung adenocarcinoma samples. The EGFR mutation rate is in agreement with the reported EGFR mutation rate (~10%) in lung cancer adenocarcinoma samples of such origin (3, 4). These results show that qBiomarker Somatic Mutation PCR Arrays detects both known and unknown mutations as well as co-occurring mutations.

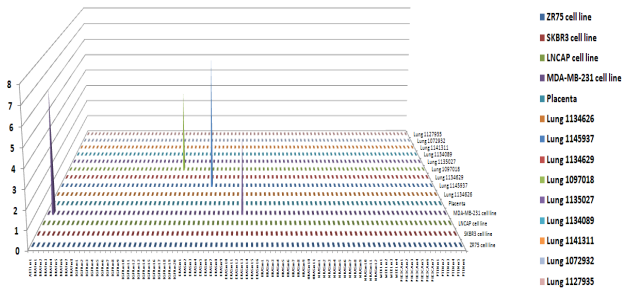
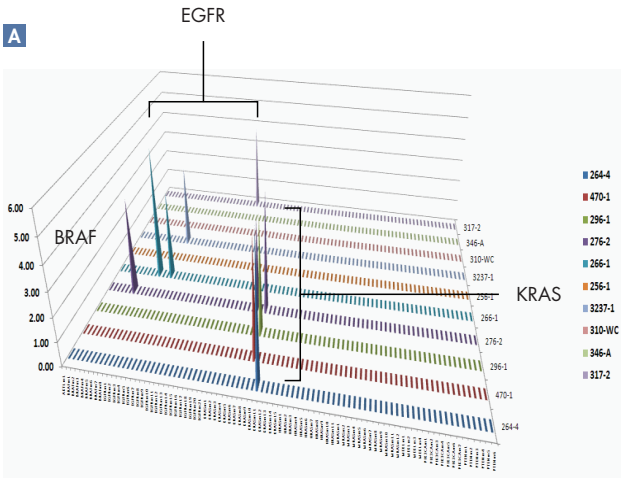


Figure 8. qBiomarker Somatic Mutation PCR Arrays performance in FFPE samples. The Human EGFR Pathway qBiomarker Somatic Mutation PCR Array was used to profile somatic mutations in 4 FFPE cell line samples, a wild-type FFPE placenta sample (control), and 9 lung adenocarcinoma FFPE tissue samples (Asterand). The array verified known mutations and also identified mutations in previously uncharacterized samples (see text for detailed results).

To systematically test if mutations detected by qBiomarker Somatic Mutation PCR Arrays can be verified by another platform, we compared the qBiomarker Somatic Mutation PCR Array results and Pyrosequencing results in 10 previously untested FFPE lung adenocarcinoma samples (Cybrdi). The Human EGFR Pathway qBiomarker Somatic Mutation PCR Array detected a total of 9 mutations in these samples including 1 BRAF mutation in 1 sample, 4 EGFR mutations in 3 samples and 4 KRAS mutations in 4 samples. EGFR mutations were detected in 3 out of 10 samples (Figure 9A), in agreement with the literature-reported mutation rate (~30%) for samples of similar origin (4). The 10 samples were then subjected to Pyrosequencing analysis with QIAGEN's KRAS Pyro Kit (which identifies mutations in codons 12 and 13) and EGFR Pyro Kit (which identifies mutations in exon 19). Seven of 8 of the EGFR and KRAS mutations detected by qBiomarker Somatic Mutation PCR Arrays were confirmed by Pyrosequencing analysis (Figure 9B). Interestingly, one mutation, KRAS c.37G>C, p.G13R in sample 264-4 was detected by the Human EGFR Pathway qBiomarker Somatic Mutation PCR Array but was not detected by Pyrosequencing. The apparent discrepancy is likely due to the lower sensitivity of Pyrosequencing (5% detection limit) relative to the corresponding real-time PCR assay for this mutation, which indicated an occurrence of less than 5% of cells within this sample.

Table 1. List of qBiomarker Somatic Mutation PCR Arrays

Disease/Pathway	Species	Array ID
Brain Cancer	Human	SMH-025
Breast Cancer	Human	SMH-020
Colon Cancer	Human	SMH-021
Hematopoietic Neoplasms	Human	SMH-022
Lung Cancer	Human	SMH-023
Lymphoid Neoplasms	Human	SMH-026
Melanoma	Human	SMH-029
Skin Cancer	Human	SMH-024
Soft Tissue Tumors	Human	SMH-027
Thyroid Cancer	Human	SMH-028
Bladder Cancer	Human	SMH-030
Endometrial Cancer	Human	SMH-032
Liver Cancer	Human	SMH-034
Ovarian Cancer	Human	SMH-039
Gastric Cancer	Human	SMH-041
Pancreatic Cancer	Human	SMH-035
APC/CTNNB1	Human	SMH-010
c-MET	Human	SMH-007
ErbB2	Human	SMH-002
EGFR	Human	SMH-001
FGFR	Human	SMH-005
FLT3	Human	SMH-003
KIT	Human	SMH-006
p53/Rb	Human	SMH-011
PDGFR	Human	SMH-004
PI3K-PTEN	Human	SMH-012
Ras-Raf	Human	SMH-013
RTK Panel I	Human	SMH-008
RTK Panel II	Human	SMH-009



Additional advantages

In addition to qPCR-based superior detection sensitivity and straightforward data analysis procedure, the qBiomarker Somatic Mutation PCR Arrays and Assays have several major advantages over other currently available mutation detection platforms/methods. Firstly, the workflow is very simple, involving only one setup step. No multi-step handling is involved, and hands-on time is less than any other method available. Secondly, reactions involved are all closed-tube reactions avoiding sample contamination. Thirdly, the DNA sample input is low: as little as 5-10 ng of genomic DNA from fresh or frozen samples when using the Repli-g® UltraFast Kit for whole genome amplification. For FFPE samples, just 200 ng of genomic DNA is needed. Finally, the hardware involved in analysis using the qBiomarker Somatic Mutation PCR Arrays and Assays is highly accessible, enabling such analysis for any laboratory with access to a real-time PCR instrument.

Summary

The QIAGEN qBiomarker Somatic Mutation PCR Arrays were designed to provide highly sensitive detection of cancer somatic mutations. The arrays correctly identify known mutations in previously characterized samples and identify mutations in previously uncharacterized samples from various sources that subsequent pyrosequencing analyses confirm. The cataloged arrays cover a large number of oncogenes, tumor suppressors, cancer pathways and disease types (Table 1), while customized array design can draw from the availability of more than 1000 mutation-specific assays. Analyzing 80 to 370 cancer somatic mutations simultaneously by real-time PCR provides a fast and accurate tool to profile the existence of functionally significant mutations which could be relevant to cancer progression and/or disease outcome.

Figure 9. qBiomarker Somatic Mutation PCR Arrays results verified by Pyrosequencing. A The Human EGFR Pathway qBiomarker Somatic Mutation PCR Array detected BRAF, EGFR, and KRAS mutations in FFPE lung adenocarcinoma samples (Cybrdi). Sample quality varies by 8 C_T as measured by gene copy assays on the array. B KRAS G12 somatic mutations and EGFR deletion mutations in these samples were confirmed by Pyrosequencing. Representative pyrograms are shown.

References

1. Puente, X.S. et al. (2011) Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature **475**, 101.

2. Newton, C.R. et al. (1989) Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Res. **17**, 2503.

3. Fukuoka, M. et al. (2003) Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial). J. Clin. Oncol. **21**, 2237.

4. Paez, J.G. et al. (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science **304**, 1497.

Ordering Information

Product	Contents	Cat. no.
qBiomarker Somatic Mutation PCR Arrays	PCR plate and mastermix	337021
qBiomarker Somatic Mutation PCR Assays	PCR assay and mastermix for 100 assays	337011
Related products		
QIAamp® DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404
REPLI-g UltraFast Mini Kit (25)	DNA Polymerase, Buffers, and Reagents for 25 x 20 µl ultrafast whole genome amplification reactions	150033

The qBiomarker Somatic Mutation PCR Arrays and Assays are for research use only, not for use in in vitro diagnostics.

Learn more, visit www.sabiosciences.com/somaticmutation.php today!

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