PyroMark Q24

For quantitative methylation and mutation analysis using Pyrosequencing® technology in a 24-well format

The PyroMark Q24 uses proven Pyrosequencing technology for real-time, sequence-based detection and quantification in genetic analysis and epigenetic methylation studies. The unique PyroMark system combines reliable quantification and sequencing results in minutes. The platform consists of PyroMark Q24 which is the sequencing instrument, the PyroMark Q24 Vacuum Workstation for preparation of single-stranded DNA, reagents and controls, as well as PyroMark Q24 Software for analysis.

Benefits of the PyroMark Q24 include:

- Highly accurate results in epigenetics and cancer research
- Reliable quantification of allele representation and methylation status
- Sequence information enables discovery of rare mutations and CpG sites
- Methylation analysis can be combined with SNP typing in one assay
- 1–24 samples can be analyzed in as little as 15 minutes

Figure 1. PyroMark Q24.

Rapid results compared to classical sequencing methods

Mutation and methylation analysis by traditional Sanger sequencing only offers qualitative or semiquantitative information. Quantitative Sanger sequencing requires cloning of PCR products prior to sequencing and is labor-intensive and time-consuming. In contrast, Pyrosequencing offers easy and flexible sequencing combined with highly accurate quantification, in as little as 15 minutes (see Figure 2).

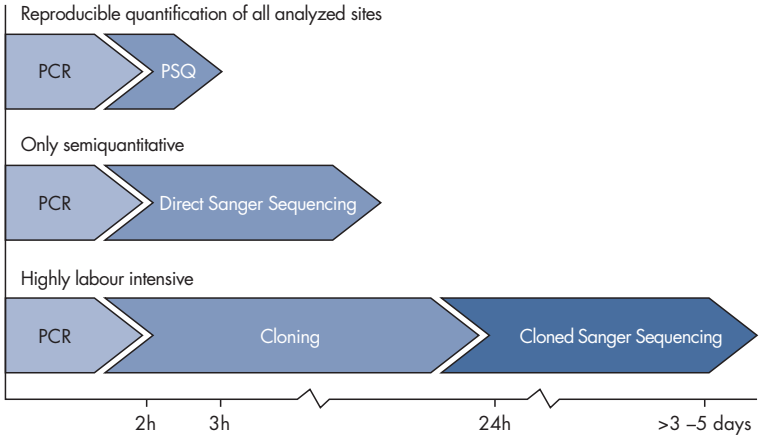


Figure 2. Rapid results compared to classical sequencing methods. PSQ®: Pyrosequencing

QIAGEN®

A fully integrated system

PyroMark Q24 is a fully integrated system that provides real-time sequence information and is highly suitable for epigenetics research and genetic analysis. It accurately quantifies individual CpG sites in methylation experiments. It is also a powerful tool for genetic analysis and allows quantification of common and rare mutations as well as allele representation in mixed cell populations.

The system includes PyroMark Q24, PyroMark Q24 Vacuum Workstation, PyroMark Q24 Software, PyroMark Gold Q24 Reagents, PyroMark Control Oligo, and PyroMark Q24 Validation Oligo (see Table 1). Sample preparation solutions are also supplied to enable preparation of single-stranded DNA using the PyroMark Q24 Vacuum Workstation.

Primers can be designed using Pyrosequencing Assay Design Software. One of the primers must be labeled with biotin so that the PCR product that is generated can be immobilized to beads during sample preparation.

Table 1. PyroMark Q24 system

System component	Description
PyroMark Q24	Sequencing instrument for quantitative mutational and methylation analysis
PyroMark Q24 Vacuum Workstation	Workstation for sample preparation of up to 24 samples in parallel
PyroMark Q24 Software	Analysis software; provided in 2 analysis modes (for CpG analysis and allele quantification)
PyroMark Gold Q24 Reagents	Enzymes, substrates, and nucleotides
PyroMark Control Oligo	Control for verification of proper installation and operation of the system
PyroMark Q24 Validation Oligo	Control for performance confirmation of the system

Streamlined workflow — from sample to result

The versatile PyroMark Q24 seamlessly integrates into epigenetics and genetic analysis workflows, and complements QIAGEN's advanced technologies for sample preparation, bisulfite conversion, and PCR amplification. The highly reliable instrument enables sequence-based detection and quantification of CpG sites as well mutations. The streamlined workflow means that results can be achieved faster.

PyroMark Q24 Vacuum Workstation — sample preparation for the PyroMark Q24

From PCR product to single-stranded template ready for sequencing — up to 24 samples can be prepared in parallel using the PyroMark Q24 Vacuum Workstation, in less than 15 minutes. The workstation ensures easy handling and the actual hands-on time is less than 5 minutes.

Biotinylated PCR products are bound to Streptavidin-coated Sepharose beads. The beads are then captured with the Vacuum Prep Tool and thoroughly washed, generating single-stranded DNA suitable for sequencing.

The robust procedure helps to avoid DNA cross-contamination between sample batches and enables highly sensitive results.

Figure 3. PyroMark Q24 Vacuum Workstation.

PyroMark Q24 Software

Easy-to-use PyroMark Q24 software, installed on a PC, enables analysis of your results. The software contains two analysis modes, CpG and AQ (Allele Quantification). Both of these modes can be used to analyze samples on the same plate, enabling different types of samples to be run at the same time. The AQ mode can be used for analysis of single and multivariable positions as well as di-, tri- , and tetra- allelic mutations. The CpG mode enables analysis of multiple consecutive CpG sites and provides a built-in control of the bisulfite treatment.

This is based on the fact that all unmethylated C's not followed by a G are completely converted to a T after bisulfite treatment and PCR. In addition, the software provides a reference sequence pattern, delivering information about the location of expected peaks and expected peak heights. Post-run, the software performs a quality assessment of individual sites as well as the sequence context based on this information.

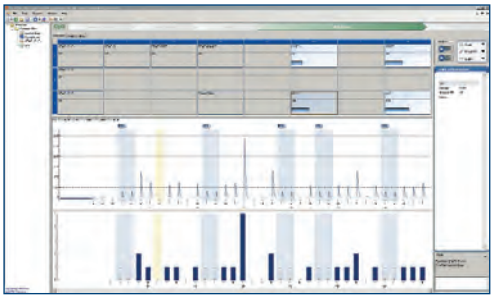


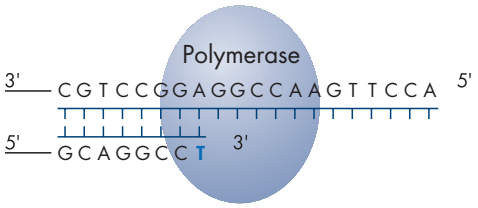
Figure 4. Fully comprehensive and flexible, PyroMark Q24 Software.

Increased flexibility

In addition to a 24-well format, Pyrosequencing is also available in 96-well format for higher throughput needs, while providing the same accuracy, flexibility, and convenience. For more information, contact your local sales representative.

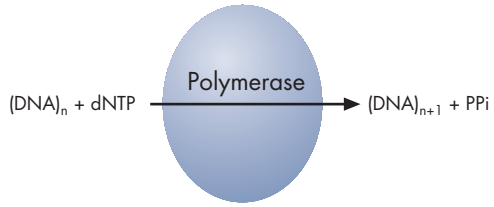
Principle of Pyrosequencing

Pyrosequencing technology, which is based on the principle of sequencing by synthesis, provides quantitative data in sequence context within minutes.



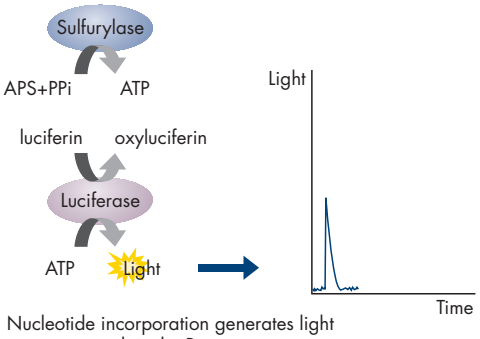
Step 1

A sequencing primer is hybridized to a single-stranded PCR amplicon that serves as a template, and incubated with the enzymes, DNA polymerase, ATP sulfurylase, luciferase, and apyrase as well as the substrates, adenosine 5' phosphosulfate (APS), and luciferin.



Step 2

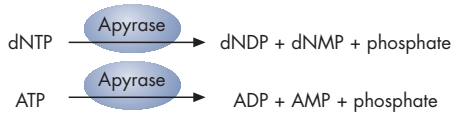
The first deoxribonucleotide triphosphate (dNTP) is added to the reaction. DNA polymerase catalyzes the incorporation of the deoxyribo-nucleotide triphosphate into the DNA strand, if it is complementary to the base in the template strand. Each incorporation event is accompanied by release of pyrophosphate (PPi) in a quantity equimolar to the amount of incorporated nucleotide.



Nucleotide incorporation generates light seen as a peak in the Pyrogram trace

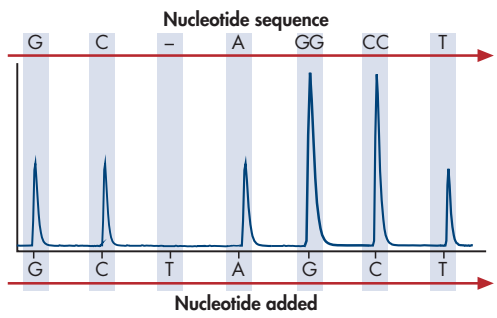
Step 3

ATP sulfurylase converts PPi to ATP in the presence of adenosine 5' phosphosulfate (APS). This ATP drives the luciferase-mediated conversion of luciferin to oxyluciferin that generates visible light in amounts that are proportional to the amount of ATP. The light produced in the luciferase-catalyzed reaction is detected by a charge coupled device (CCD) chip and seen as a peak in the raw data output (Pyrogram®). The height of each peak (light signal) is proportional to the number of nucleotides incorporated.



Step 4

Apyrase, a nucleotide-degrading enzyme, continuously degrades unincorporated nucleotides and ATP. When degradation is complete, another nucleotide is added.



Step 5

Addition of dNTPs is performed sequentially. It should be noted that deoxyadenosine alfa-thio triphosphate (dATP·S) is used as a substitute for the natural deoxyadenosine triphosphate (dATP) since it is efficiently used by the DNA polymerase, but not recognized by the luciferase. As the process continues, the complementary DNA strand is built up and the nucleotide sequence is determined from the signal peaks in the Pyrogram trace.

A detection tool highly suited for epigenetics research

Pyrosequencing complements QIAGEN's epigenetics portfolio and enables accurate and sensitive quantification of the methylation status by providing highly reliable sequence data (see Figure 5). It even allows the identification of novel mutations as well as detection of aberrant DNA methylation patterns present at low levels. PyroMark Q24 includes a complete software package for CpG methylation analysis as well as a built-in control for bisulfite treatment.

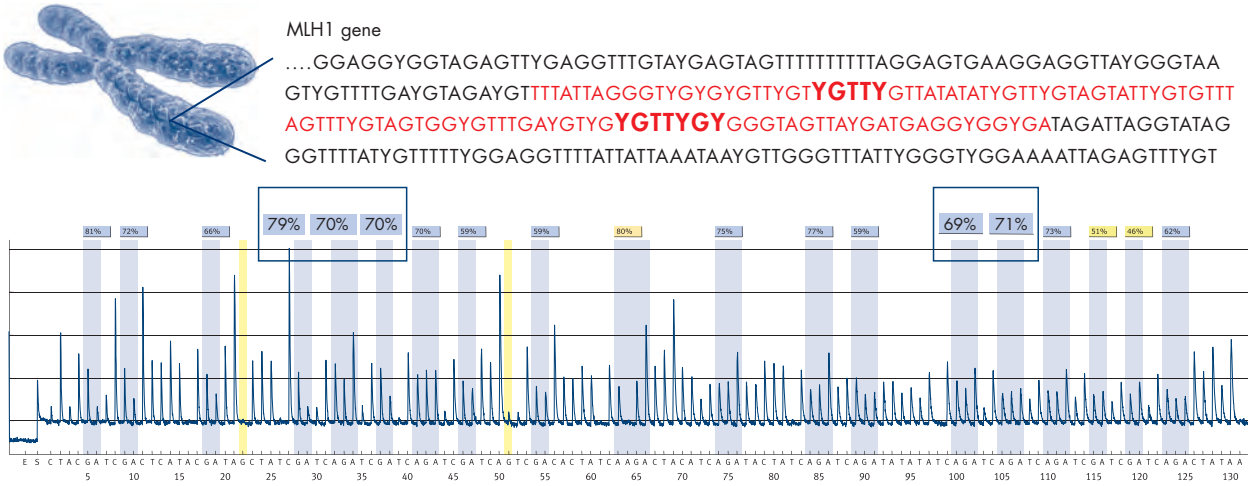


Figure 5. Pyrogram trace illustrating CpG methylation analysis of the MLH1 gene. Highlighted areas in the Pyrogram trace indicate variable CpG positions (light gray) and built-in bisulfite treatment controls (yellow). The methylation level of each CpG site is indicated in blue boxes on top of the Pyrogram trace. *Data kindly provided by Dr. Triantafillos Liloglou, Roy Castle Lung Cancer Foundation, Molecular Oncology, Liverpool.*

Quantification of individual CpG sites

Analysis of single CpG sites is of crucial importance when studying differential gene expression in various tumors (see Figure 6). Traditional methods often fail to provide this degree of sensitivity since the data obtained is of lower resolution. Pyrosequencing technology overcomes this challenge and enables analysis of single variations in the methylation pattern of multiple sites with high accuracy.

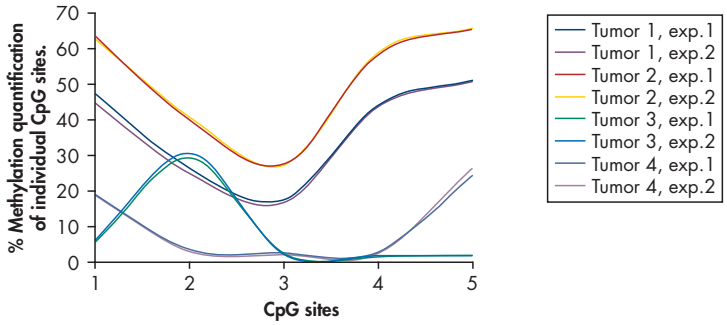


Figure 6. Pyrosequencing analysis of CpG methylation pattern in the RASSF1A gene. The figure illustrates the variation in methylation level between 5 different CpG sites in 4 individual tumor samples. Each sample was run in duplicate and the concordance between the two samples clearly illustrates the reproducibility of Pyrosequencing technology.

A highly suitable platform for genetic analysis

Genetic analysis comprises multiple applications to analyze differences in genomic DNA, including mutation detection and SNP typing. PyroMark Q24 facilitates accurate and highly sensitive mutational analysis of any gene of interest and enables quantification of allele representation in mixed cell populations. Pyrosequencing technology offers optimized and validated RUO tests for mutation analysis of genes such as KRAS.

KRAS assay

Mutations in the KRAS gene result in a KRAS protein that is permanently active. Such oncogenic activation has been suggested to be involved in many aspects of the development and progression of cancer, including abnormal cell growth, proliferation, and differentiation, as well as increased invasion and metastasis. Consequently, mutations in the KRAS gene are found in many types of human cancers with the most common mutations found in codons 12, 13, and 61.

The PyroMark Q24 KRAS v2.0 test enables reliable detection and quantification of mutations in these codons in a single run. In addition, the flexible assay set up provided by PyroMark Q24 allows the detection of additional, rare mutations (see Figures 7 and 8).

Analysis method

Fragments spanning either codons 12 and 13 or codon 61 are amplified by PCR. Pyrosequencing analysis is performed to detect and quantify the common mutations. The assay design even enables detection of additional, rare mutations in codons 12, 13, and 61. The surrounding sequence context serves as a built-in quality control of the results.

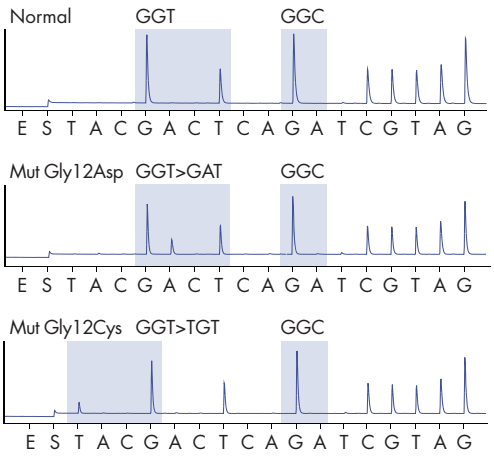


Figure 7. Results from mutation analysis of codons 12 and 13 using PyroMark KRAS v2.0 test and PyroMark Q24. The upper Pyrogram trace shows a sample with a normal genotype. The middle Pyrogram trace shows mutation analysis in a sample with a G to A mutation in position 2 of codon 12, and the lower Pyrogram trace shows a sample with a G to T mutation in position 1 of codon 12 identified post-run by the altered sequence. Light blue areas indicate the variable position.

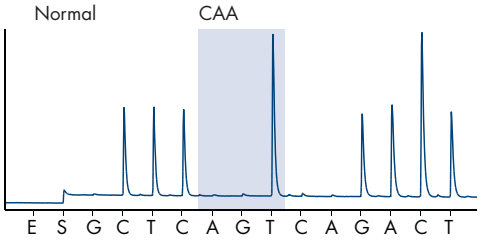


Figure 8. Results from mutation analysis of codon 61 using PyroMark Q24 KRAS v2.0 test and PyroMark Q24. Analysis of codon 61 is set up as a reverse assay, which means that CAA is read as TTG. The Pyrogram trace shows a sample with a normal genotype. Light blue areas indicate the variable position.

Unrivaled instrument and application support

QIAGEN offers comprehensive Service Support Agreements, including Warranty Extensions, Full Cover Support Agreements, and instrument/application training, including on-site installation. Service Support Agreements maximize productivity and ensure high performance from your instrument. In addition, service histories are fully documented and all parts are certified and guaranteed.

Table 2. PyroMark Q24 specifications

PyroMark Q24 Instrument	
Input voltage and current	100–240 V AC, 47–63 Hz, 1.1–0.45 A (grounded) From the external power supplies to the instrument: 12 VDC and 24 VDC nominal
Power consumption	Maximum 160 Watt
Laboratory environment	Ambient temperature: 15–32°C (59–90°F) Ambient humidity: 20–90% relative humidity
Dimensions with the lid closed (H x W x D)	420 x 390 x 525 mm (16.5 x 15.4 x 20.7 in.)
Weight	27.5 kg (60.6 lb.)
Connections	One USB port (2.0)
Capacity	1–24 samples/run
Batch format	24-well microtiter plate, PyroMark Q24 Plate
Process temperature	28°C (82.4°F) +/-1%
Process time	Depending on the number of dispensations (20 dispensations take 24 minutes)
PyroMark Q24 Vacuum Workstation	
Laboratory environment	Ambient temperature: 15–32°C (59–90°F) Ambient humidity: 20–90% relative humidity
Dimensions (H x W x D)	Vacuum Prep Worktable 68 x 95 x 353 mm (2.7 x 3.7 x 13.9 in.)
Weight	Vacuum Prep Worktable 2.1 kg (4.6 lb.), Vacuum Prep Tool 300 g (0.66 lb.)
Process time	Less than 13 minutes for up to 24 samples in parallel
PyroMark Q24 Software	
The office computer used to set up runs and analyze data should be a personal computer with the following minimum specifications:	
Operating system	Microsoft® Windows® XP or Windows Vista, English versions
Processor	Intel Pentium® 4, 3 GHz
RAM	1 GB
Free hard disk space	100 MB
Graphics card	Supporting the resolution of the monitor
Monitor	1280 x 1024 pixels
Pointer device	Mouse or similar
Interfaces	USB port and CD-ROM

Ordering Information

Product	Contents	Cat. no.
PyroMark Q24*	Instrument, for laboratory use only	9001514
PyroMark Q24 Vacuum Workstation	Vacuum Workstation for preparing 24 samples in parallel from PCR product to single-stranded template	Varies†
PyroMark Q24 Software	Analysis software, for laboratory use only	9019062
Related products		
PyroMark Gold Q24 Reagents (5 x 24)	Enzyme mix, substrate mix, nucleotides	970802
PyroMark Control Oligo	For installation check of system and troubleshooting	979203
PyroMark Q24 Validation Oligo	For performance confirmation of system	979204
PyroMark Assay Design SW	Assay design software for genetic analysis	Inquire
EpiTect® Bisulfite Kit (48)	48 EpiTect Bisulfite Spin Columns, Reaction mix, DNA Protect Buffer Carrier RNA, Buffers	59104
EpiTect Control DNA Set (100)	Human control DNA set (containing bisulfite converted methylated and unmethylated DNA and unconverted DNA for 100 control reactions)	59695
PyroMark Q24 KRAS v2.0 (4 x 24)	Mutation detection assay	970452
PyroMark Q24 BRAF (4 x 24)	Mutation detection assay	970412
PyroMark Q24 CpG p16 (4 x 24)	Methylation detection assay	970012
PyroMark Q24 CpG MLH1 (4 x 24)	Methylation detection assay	970022
PyroMark Q24 CpG MGMT (4 x 24)	Methylation detection assay	970032
PyroMark Q24 CpG LINE-1 (4 x 24)	Methylation detection assay	970042

* Available for IVD applications. Not available in all countries; please inquire.
† 9001518 (220V); 9001516 (110V); 9001519 (100V).

PyroMark Q24 is intended to be used only in combination with QIAGEN kits indicated for use with the PyroMark Q24 for the applications described in the respective kit handbooks. PyroMark Gold Q24 Reagents (5 x 24), PyroMark Control Oligo, PyroMark Q24 Validation Oligo, EpiTect Bisulfite Kit, and EpiTect Control DNA Set are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products. PyroMark Q24 KRAS v2.0, PyroMark Q24 BRAF, PyroMark Q24 CpG p16, PyroMark Q24 CpG MLH1, PyroMark Q24 CpG MGMT, PyroMark Q24 CpG LINE-1, and PyroMark Q24 CpG MLH1 are intended for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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