

# A New Standard in Epigenetic and Genetic Analysis!

Pyrosequencing® — the synergy  
of sequencing and quantification



Sample & Assay Technologies

# Quantitative data for new horizons in genetic research

The development of sequencing technology has greatly expanded our understanding of how biological information is coded in the sequence of nucleotides of DNA. This knowledge has also led to new questions: What is the significance of subtle sequence changes, especially in non-coding regions? When do interactions of regulatory elements impact gene expression? How does nonsequence-based information — epigenetic information — contribute to developmental programming and cell differentiation? Finally, how can answers to these questions benefit applied sciences? These questions are the new horizons in genetic research. Researchers are uncovering new layers of biological information stored and inherited in genetic and epigenetic modifications of DNA. Characterization of this information requires next-generation research tools that enable application flexibility and acquisition of quantitative data.

## Pyrosequencing with QIAGEN® PyroMark® products meets these requirements

Integrating detection and quantification of genetic variation into one powerful system, Pyrosequencing with the PyroMark platform outperforms other sequence-based solutions in the analysis of targeted short DNA sequences. For a range of applications, this means reduced cost and time investment (Figure 1).

### Advantageous features of Pyrosequencing include:

- [Quantification of alleles](#), even when found at low frequencies
- Detection of [unknown sequence](#) variants
- [Assay versatility](#) on the same instrument and even in the same run
- [Built-in assay controls](#) guaranteeing data accuracy and reliability
- Fast delivery of direct and unambiguous sequence data
- [Intuitive software](#) that enables powerful analyses and user-designed assays

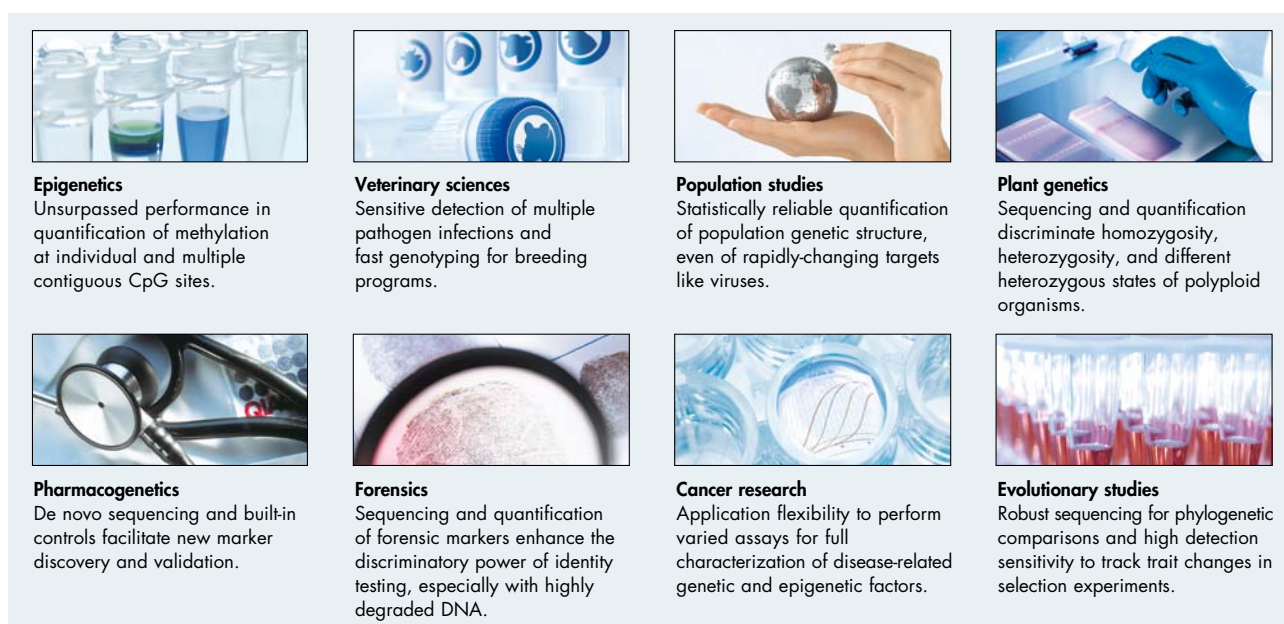


Figure 1. The advantages of Pyrosequencing in different areas of research.

## Elegant chemistry and sensitive technology enable quantification of sequence variation

### Step 1

A DNA segment is amplified and the strand to serve as the Pyrosequencing template is biotinylated. After denaturation, the biotinylated single-stranded PCR amplicon is isolated and allowed to hybridize with a sequencing primer.

### Step 2

The hybridized primer and single-stranded template are incubated with the enzymes DNA polymerase, ATP sulfurylase, luciferase, and apyrase, as well as the substrates adenosine 5' phosphosulfate (APS) and luciferin.

### Step 3

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

### Step 4

ATP sulfurylase converts PPi to ATP in the presence of 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 CCD sensors 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 5

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

### Step 6

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

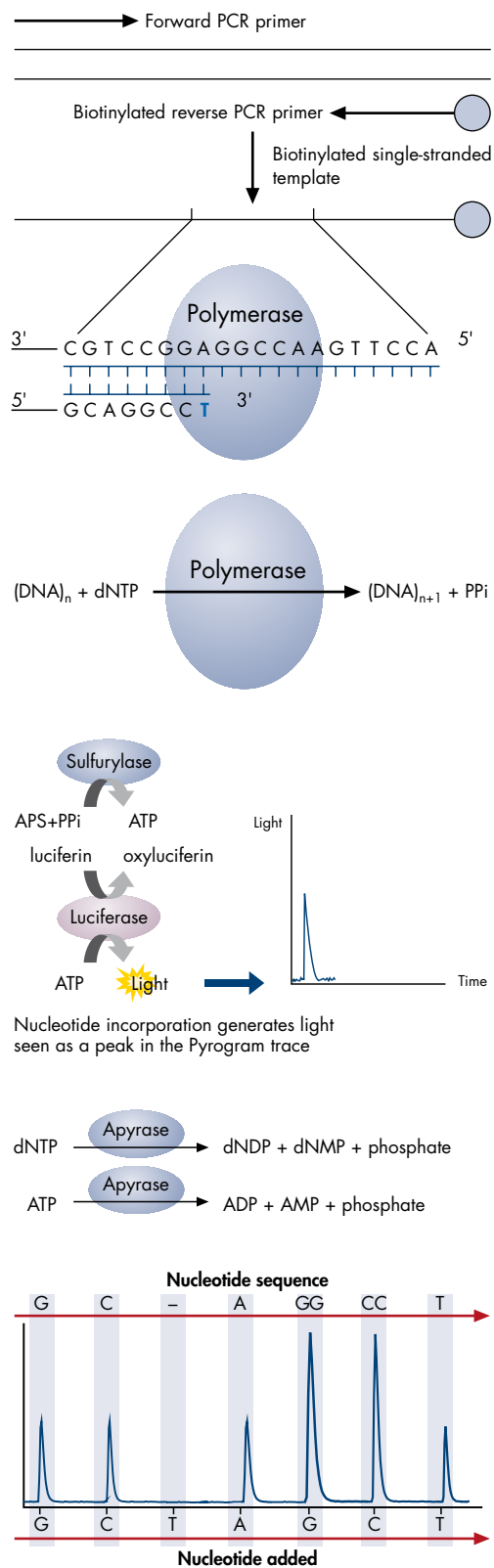


Figure 2. The Pyrosequencing reaction cascade.

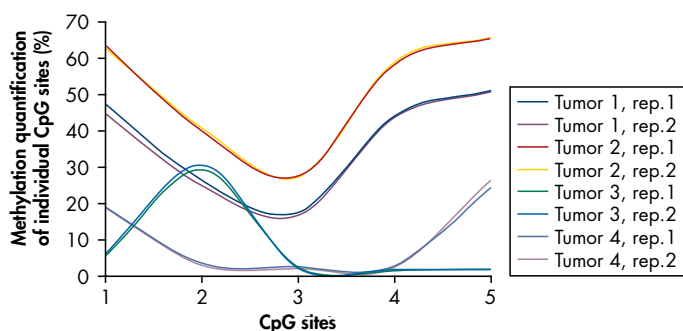


## DNA methylation analysis

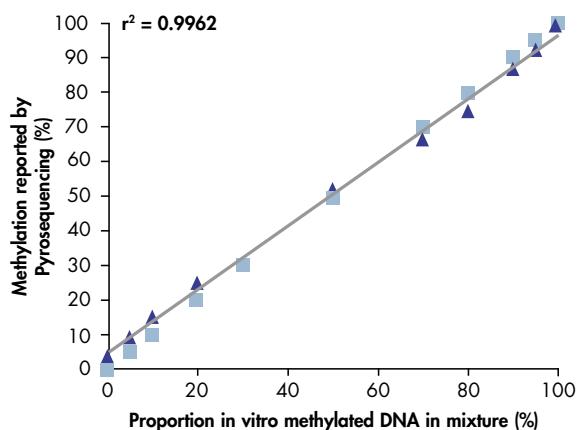
Standard methylation analysis methods provide only qualitative or semi-quantitative data, which can lead to inaccurate conclusions regarding the effects of [epigenetic DNA methylation](#) on cell cycle and metabolism. Without quantification of methylation levels, it is not possible to distinguish physiologically relevant methylation from background methylation.

Pyrosequencing solves this limitation by generating highly reproducible quantification of methylation frequencies at individual consecutive CpG sites (Figures 3 and 4). As such, Pyrosequencing can detect and quantify even small changes in methylation levels. Other valuable features include the inherent quality control afforded by the sequence context of results and the ability to compare results to expected methylation levels. Built-in controls for the bisulfite treatment eliminate manual estimation of non-converted DNA levels and prevent false-positive methylation detection, thereby ensuring the reliability of results.

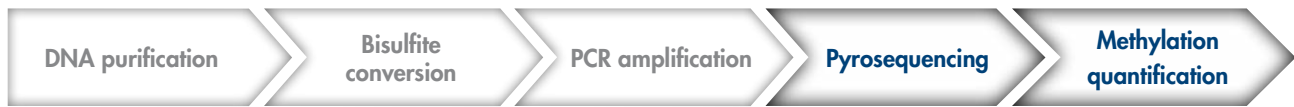
These features have established Pyrosequencing as the gold standard for DNA methylation analysis. This technology has been used to correlate DNA methylation to tumor type and gene expression, to measure cellular response to treatment with demethylating agents, and to assess changes in methylation state in relation to tumorigenesis, genetic imprinting, and exposure to environmental toxins (1).



**Figure 3. Pyrosequencing analysis of CpG methylation pattern in the RASSF1A gene.** Variation in methylation levels at five different CpG sites in four tumor samples. Sample replicates were run on different days and still show high repeatability.



**Figure 4. Linearity of methylation quantification by Pyrosequencing.** PCR products from varying mixtures of unmethylated genomic DNA and methylated DNA (EpiTect Control DNAs) were analyzed by Pyrosequencing. A tight correlation between the known percentage of methylated DNA in the mixtures (■) and the methylation percentage reported by Pyrosequencing (▲) was observed ( $r^2 = 0.9962$ ). The graph represents the quantification of methylation at a single CpG site in the p16 gene.



## Valuable quality control of bisulfite conversion

To characterize the methylation status of a DNA sequence via Pyrosequencing, the DNA is first incubated with sodium bisulfite. As a result, unmethylated cytosine residues are converted into uracil while methylated cytosines remain unchanged, giving rise to two different sequences that can be distinguished. An internal control for bisulfite treatment is incorporated into analysis. Cytosines that are not followed by guanine in template sequences are not methylated, and should therefore be converted to thymine by bisulfite treatment and PCR (Table 1). Full bisulfite conversion is confirmed if all templates show thymine and no cytosine in these positions. With unique DNA Protect technology, QIAGEN [EpiTect® Bisulfite Kits](#) facilitate complete conversion and minimal degradation of the treated DNA.

**Table 1. Sequences resulting from bisulfite conversion and PCR**

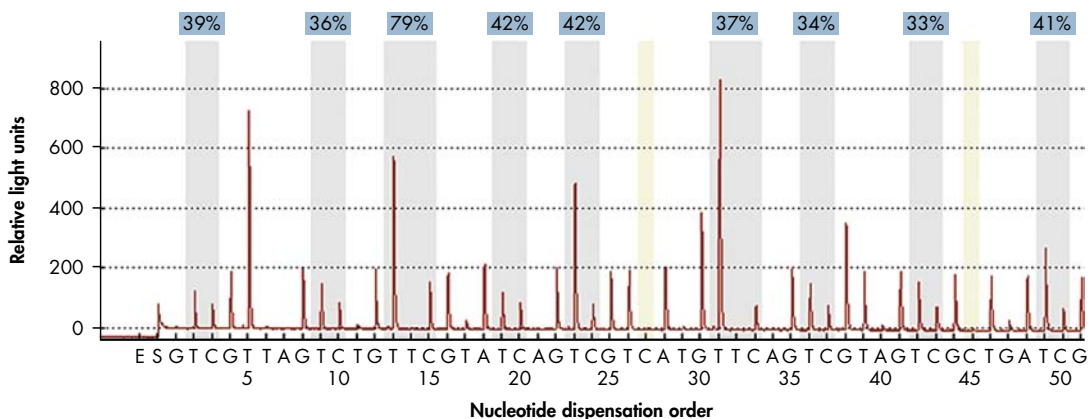
	Original sequence	After bisulfite treatment	After PCR amplification
Unmethylated DNA	A-C-G-T-C-G-T-C-A	A-U-G-T-U-G-T-U-A	A-T-G-T-T-G-T-T-A
Methylated DNA	A-C-G-T-C-G-T-C-A	A-C-G-T-C-G-T-U-A	A-C-G-T-C-G-T-T-A

## Flexible analysis of methylation patterns

The analysis of methylation status exploits the quantitative nature of Pyrosequencing data. Unlike Sanger sequencing, the peak heights in the resulting Pyrogram report the ratio of cytosine to thymine at each analyzed CpG site, which reflects the proportion of methylated DNA. Assay design is flexible — it can be performed in forward or reverse orientations, on either the top or the bottom strands. In addition, contiguous CpG sites are analyzed independently and within the same run, which enables assessment of sequence-wide methylation patterns while retaining details of position-specific methylation (Figure 5).

### Sequence to analyze:

C/TGTTTGC/TGTTTC/TGAC/TGTTT/TGAGGTTTC/TGC/TGGTGC/TGATC/TGTT



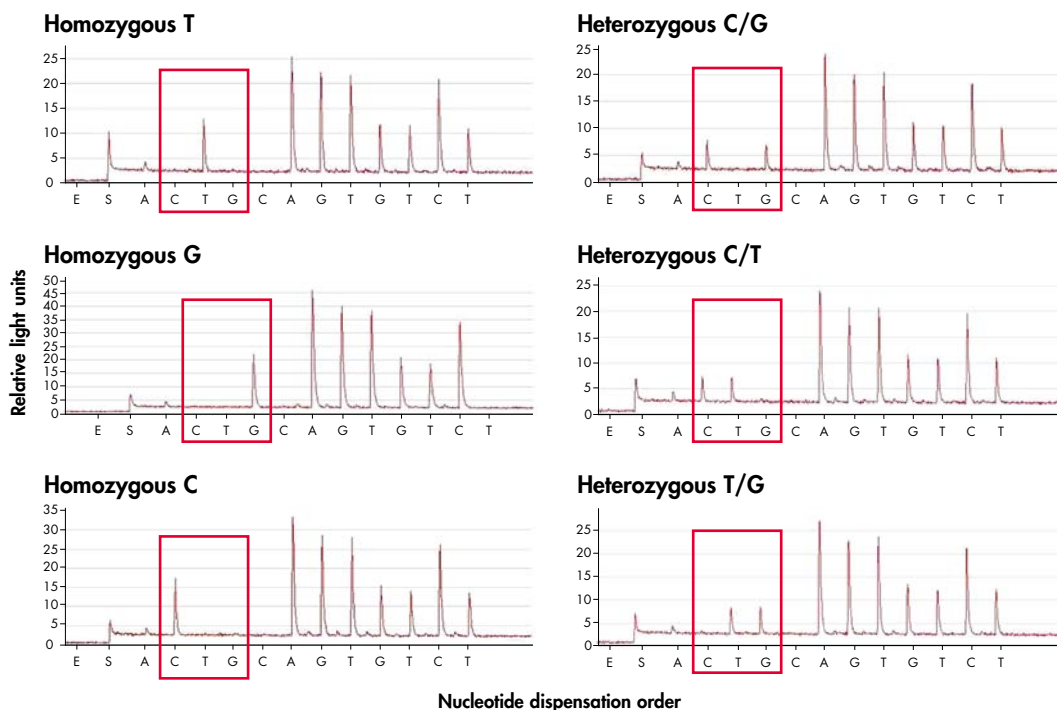
**Figure 5. Analysis of multiple contiguous CpG sites.** Methylation at nine independent CpG sites (highlighted in gray) is quantified in a single Pyrosequencing run. Position-specific information in the context of an analyzed sequence presents broad sequence methylation patterns. Note the built-in quality control sites (highlighted in yellow) consisting of cytosines converted to thymines, demonstrating full bisulfite conversion of the treated DNA.



## Insertion-deletion and SNP analysis

[Genetic testing](#) is an important component of many applications. For example, developing effective therapeutic agents requires information about how gene polymorphisms impact metabolism; understanding genetic contributions to a disease involves characterizing linked mutations; finally, analysis of forensic DNA evidence relies on accurate detection of sequence variation.

Many mutation types are utilized for these applications and new markers are continuously being validated in areas such as epidemiology, pharmacogenetics, and animal husbandry. Correspondingly, a variety of analysis methods are used to detect this genetic variability. This can complicate comparison of results obtained by different methods and different researchers. Ideally, a single technology could be adapted for all applications to standardize results. Pyrosequencing offers precisely that versatility. Because of the flexibility of primer placement in Pyrosequencing reactions, virtually all genetic markers, those currently used and those to be identified, can be assayed. Alleles of variable loci are accurately quantified, and heterozygosity is easily resolved (Figure 6 and 7). In addition, because Pyrosequencing delivers sequence information, various types of genetic variation can be evaluated — insertion-deletions, single nucleotide polymorphisms, single tandem repeats, and variable gene copy number — and it is possible to assay several contiguous sequence variants in a single run.

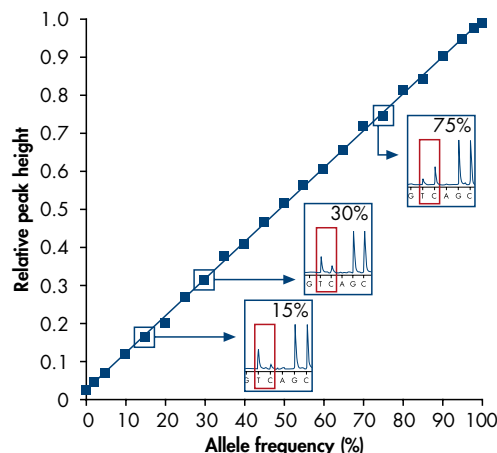


**Figure 6. Analysis of a tri-allelic SNP.** Detection of tri- and tetra-allelic SNPs can be difficult with commonly used methods. This series of Pyrograms illustrates the ease of Pyrosequencing based detection of a tri-allelic SNP (red outline). **C**, **T** and **G** are serially dispensed in the Pyrosequencing reaction and only the incorporated nucleotides will elicit a signal peak. The result is a different peak pattern for homozygous samples of each allele (three Pyrograms on the left) or compound peak patterns for heterozygous samples (three Pyrograms on the right).



## Versatility at your fingertips

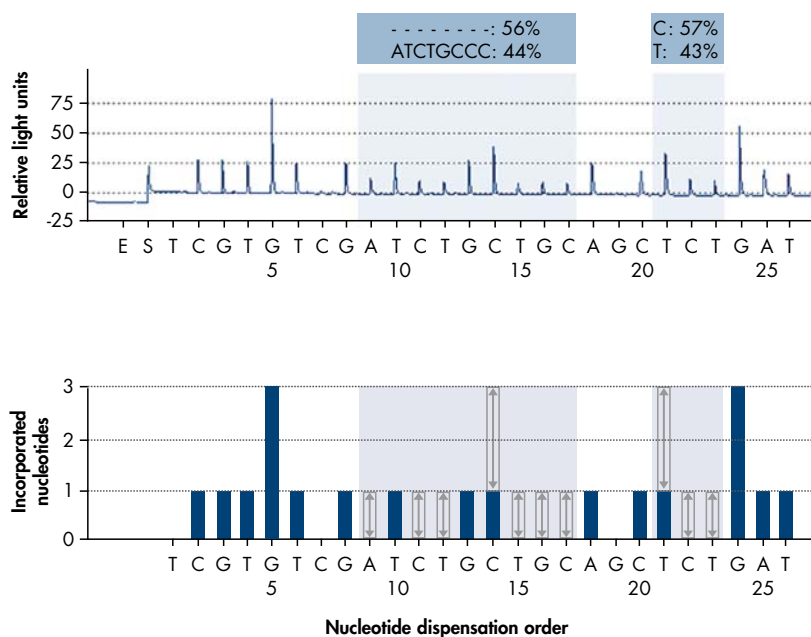
Regardless of the marker or mutation being analyzed, preparation of templates for Pyrosequencing and the subsequent analysis of the resulting sequence information are quick and easy, saving time and valuable resources. However, the strength of Pyrosequencing for genetic testing lies in the elegance of its output. Because results are simply the true sequence of the DNA in a sample, the user can examine multiple mutation sites within a specified region, and even multiple variation types, all in the same run (Figure 8). Furthermore, the straightforward results are easily interpreted. Pyrosequencing enables de novo sequencing which, coupled with the built-in control afforded by the sequence surrounding the variable site, is a guaranteed way of validating newly identified markers. The high throughput facilitates rapid compilation of the population data needed to establish reference databases for these markers. This feature makes Pyrosequencing a powerful and versatile tool for the development of pharmacogenetic and forensic markers (2).



**Figure 7. Quantitative mutation analysis.** Pyrogram peak heights are proportional to the frequency of an allele in the sample. Therefore, it provides accurate measures of the proportion of, for example, a mutation in a blood sample.

## Sequence to analyze:

CGTGGGTG[ATCTGCC]TGCCTTGGGATA



**Figure 8. Two mutation types quantified in a single Pyrosequencing reaction.** Pyrogram of a DNA sequence featuring an insertion-deletion mutation (ATCTGCC) and a somatic mutation involving a single base pair substitution (C vs. T). The variable regions are highlighted in blue and the allele frequencies are given above the indicated sites. The histogram (lower graph) indicates the number of nucleotides incorporated at each nucleotide dispensation. The dark blue bars represent the nucleotide positions conserved between alleles and arrowed empty bars portray the quantified variation.



## Microbial identification and drug resistance typing

Sequence information provides reliable data for microbial genotyping applications. However, standard methods used to assess discriminatory regions of microbial genes can be time-consuming, may require species-specific probes or gel electrophoresis, or are susceptible to the presence of unknown mutations (e.g., hybridization). Pyrosequencing is a rapid and accurate alternative method. Because this technology [sequences by synthesizing](#) new copies of the DNA template, the results provide unambiguous information since users can check the sequence surrounding the variable site to ensure that the correct DNA region has been analyzed.

### Identify multiple species in one run and with one primer set

Unlike hybridization techniques, Pyrosequencing allows the identification of a large number of species using a single conserved sequencing primer (Figure 9). Consequently, DNA extracted from multiple microbe species can be sequenced in the same Pyrosequencing run. PyroMark Identifire SW 1.0 facilitates the compilation of a local sequence database against which imported Pyrosequencing output are rapidly aligned. The raw data, matched hits, and percent concordance of each hit are presented in detailed identification reports.

These reliable data are provided within short timeframes. In less than one hour, Pyrosequencing reads a discriminatory stretch of DNA of up to 96 samples in parallel (Figure 10). Depending on assay design, the sequence can be used to discriminate microbial species, types and strains, or detect genetic mutations that confer resistance to antibiotics and antiviral drugs.



**Figure 9. Pyrosequencing based bacterial identification.** PCR primers were designed for conserved regions of 16S rRNA and the sequencing primer was placed immediately upstream of the hypervariable region V1 (blue). One set of PCR and sequencing primers is sufficient to discriminate the listed species (as well as others not included in the alignment).



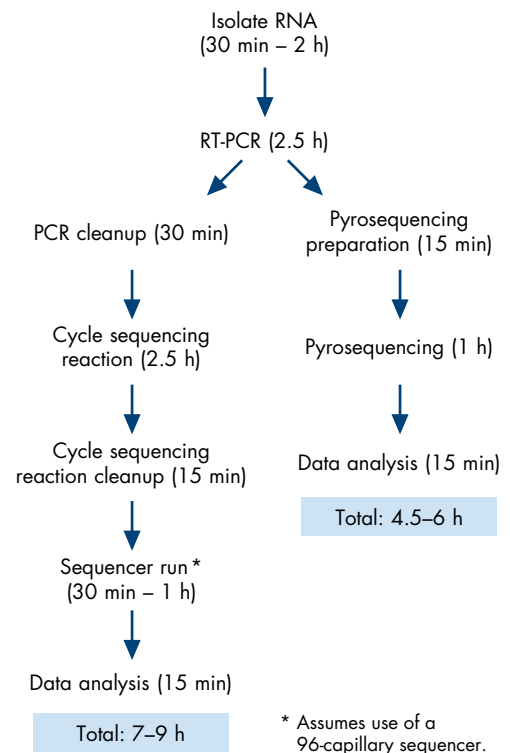


## Tracking drug resistance development

Multiple samples can be concurrently assayed for common drug resistance mutations (Figure 11). Since Pyrosequencing reports the actual sequence of the locus, a single assay can detect a range of possible mutations including new and unexpected mutations. Instrumentation and optimization of assays make Pyrosequencing technology routinely capable of detecting sequence variants present at frequencies as low as 5%, and potentially lower for some targets. Furthermore, heterogenic variation among several gene copies, which accounts for different resistance patterns, is reliably quantified. Visualizing the mutation in the context of the DNA sequence permits the user to verify the analysis. Coupled with the short time to obtain results and the ease of use, this analysis flexibility and detection sensitivity make Pyrosequencing an informative complement or even alternative to culture-based and hybridization-based resistance screening.

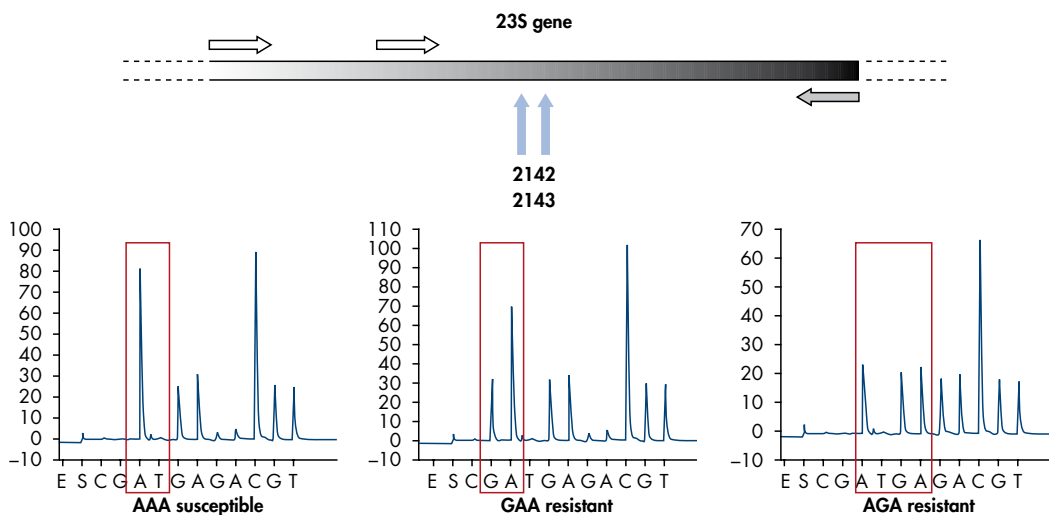
## A growing library of species-specific assays

While broad-range identification strategies using signature sequences in well-characterized hypervariable regions of microbial genes have been described, there are a growing number of assays that specifically target commonly studied pathogenic species and strains of bacteria, viruses, and fungi.



**Figure 10. Time saving afforded by Pyrosequencing.**

From RNA isolation to data analysis, with Pyrosequencing detection of resistance-conferring mutations in a RNA virus can easily be completed within a single workday. Times given reflect sequencing of a 44 base-long region in 96 viral samples.



**Figure 11. Analysis of mutations in the 235S genes that confer antibacterial resistance in *Helicobacter pylori*.**



Figure 12. Sequence analysis in PyroMark Q24 Software.



Figure 13. PCR and sequencing primer design in PyroMark Assay Design Software.

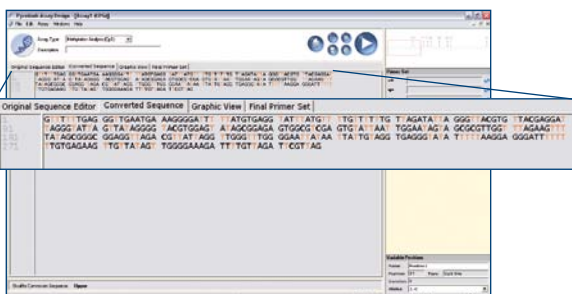


Figure 14. Predicted bisulfite converted sequence in PyroMark Assay Design Software.

## Intuitive software designed to meet your needs

The application and supplementary software that accompany PyroMark systems are user-friendly interfaces granting access to assay design, run setup, and various analyses of the obtained results. The software is driven by drop-down menus that ensure the correct selection of parameters and analysis modes for any assay, enabling the user to perform Pyrosequencing runs almost immediately (Figure 12). Finally, the software includes flexible report formats and a variety of different data export functions.

## Integrated assay design saves time and money

Time and money spent on trial-and-error design and optimization of assays is minimized with flexible [PyroMark Assay Design Software 2.0](#). Featuring algorithms for full quality control and smooth import of assays into any PyroMark instrument, this software was constructed based on years of expert knowledge regarding Pyrosequencing and PCR optimization. With a few clicks, the software designs PCR and sequencing primers for genotyping, allele quantification, sequence analysis, and methylation analysis (Figure 13). For the latter application, the software predicts the bisulfite converted sequence and highlights CpG sites and non-CpG cytosines that can serve as controls (Figure 14).

## Software for high-quality and high-throughput methylation analysis

When analyzing multiple samples, each with several methylation sites, it is important to have the aid of software that enables examination of sequence-wide patterns as well as position-specific details. The software should also provide meaningful statistics. CpG analysis with PyroMark Q24 Software or [PyroMark CpG SW 1.0](#) for the [PyroMark Q96 ID](#) and [PyroMark Q96 MD](#) provide that functionality. Standard calculations include methylation frequency, quality assessment, mean methylation values per well and replicates, statistical methylation patterns of multiple consecutive sites, and deviation from expected methylation patterns. Results are displayed in easily understood graphical and numerical presentations.

## PyroMark Q24 — small footprint, great versatility

Pyrosequencing of up to 24 samples for a multitude of applications is the strength of the [PyroMark Q24](#). PyroMark Q24 Software offers three assay types to accommodate your research needs — allele quantification (AQ), methylation analysis (CpG), and sequence analysis (SQA). Thus, in minutes you can characterize DNA methylation patterns, quantify allele frequencies, detect rare mutations, perform de novo sequencing, test for markers linked to specific phenotypes, and assay for disease-related mutations. This compact sequencing platform occupies little workbench space and yet streamlines all workflow steps, from PCR products to sequence variant detection and quantification, so that several runs can be completed in a workday. Furthermore, assays designed on the PyroMark Q24 are compatible with the large-format PyroMark instruments, giving you the option to validate new assays on this smaller platform and then upgrade to high throughput.

A screen on the instrument displays real-time sequencing information for a selected well as samples are processed, so you can track the progress of a run. All collected data are stored simultaneously on the instrument hard drive and on a USB stick. This allows you to transfer run files to any computer with an installed copy of PyroMark Q24 Software for analysis.

In addition to the sequencing instrument and software, the PyroMark Q24 System features a [vacuum workstation](#) to rapidly prepare the single-stranded DNA templates for the Pyrosequencing reactions. The system also includes [PyroMark Gold Q24](#) Reagents that optimize Pyrogram peak clarity, and a variety of test kits of QIAGEN Quality® (Figure 15).

If the trade-off between workbench space, time limitation, and application diversity is an issue in your lab, the PyroMark Q24 offers an optimal solution to meet the needs of varied research and testing programs.



Figure 15. PyroMark Q24 Instrument and PyroMark Q24 Vacuum Workstation — a complete Pyrosequencing system.



## PyroMark Q96 ID — versatility and high throughput

Applications that require large sample sizes are best performed on the [PyroMark Q96 ID](#). Examples include microbial identification and drug-resistance typing, population-wide referencing of DNA methylation levels, phylogenetic or evolutionary studies based on gene polymorphisms, Y chromosome haplotype analysis, and assessment of mitochondrial heteroplasmy in determining myopathy symptom manifestation (3).

This multiapplication platform offers the same versatility as the PyroMark Q24. With its 96-well format, automatic base-calling function, and dedicated software solutions for methylation analysis and assay design, the PyroMark Q96 ID can handle any research question requiring true sequence information and quantification of genetic variation. In addition, the PyroMark Q96 ID interfaces directly with PyroMark IdentiFire SW 1.0, using algorithms optimized for Pyrosequencing data to match sequencing results to a local database.

Accessories that facilitate efficient workflow are available in this high-throughput format as well. For example, the [PyroMark Q96 Vacuum Workstation](#) eliminates tedious pipetting by enabling parallel generation of 96 samples of purified single-stranded DNA ready for Pyrosequencing (Figure 16).

The PyroMark Q96 ID System is the solution for a research or testing program that places high value on the statistical reliability afforded by large sample sizes or high replicate number, and demands an investigative tool that adapts to current and future research questions.



Figure 16. PyroMark Q96 ID and PyroMark Q96 Vacuum Workstation — parallel preparation and analysis of 96 samples.

## PyroMark Q96 MD — highest throughput, added sensitivity

Maximizing throughput can become prohibitively expensive when considering the larger volume of reagents needed to process hundreds to thousands of samples. Equipped with a highly sensitive light-detecting camera, the [PyroMark Q96 MD](#) accurately sequences small amounts of template DNA with reduced quantities of reagents. The effect is to make Pyrosequencing a cost-effective alternative to any other high-throughput sequencing or detection platform.

This instrument is also available in a fully automated format — the PyroMark Q96 MD Automated. Up to ten plates held in an integrated plate stacker are fed by a precise robotic arm into the instrument process chamber. The plates are identified by bar code, permitting automated tracking of samples and results. Simply load the desired number of 96-well plates into the automated module and walk away (Figure 17).

The PyroMark Q96 MD offers similar [system components](#) featured by the PyroMark Q96 ID, with software that handles allele frequency quantification, genotyping, and methylation analysis. The ability to detect and quantify virtually any sequence variation coupled with increased sensitivity and an automation option, make the PyroMark Q96 MD highly suited for the larger-scale sequencing projects typical of polyploid plant genetics and pharmacogenetic marker development, as well as sensitive sequencing projects such as methylation analysis of low-yield samples.

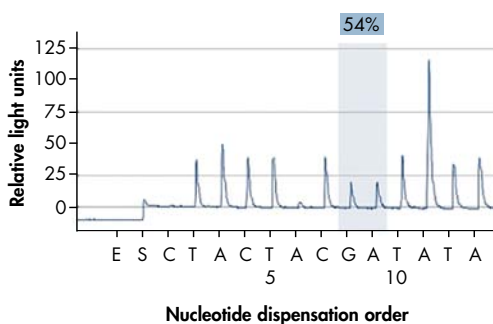


Figure 17. PyroMark Q96 MD Automated and PyroMark Q96 Vacuum Workstation — automate the analysis of up to 960 samples.



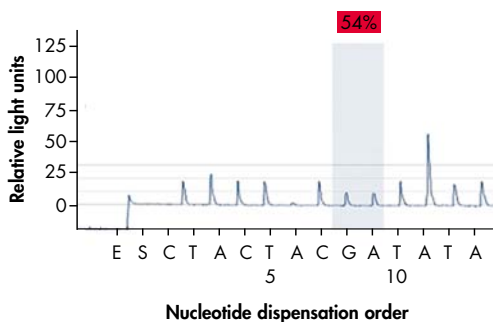
## Sequence to analyze:

TACTCR TAAATA



## Sequence to analyze:

TACTCR TAAATA



**Figure 18. Improved Pyrogram peaks enabled by the PyroMark PCR Kit.** Pyrosequencing results from DNA amplified with standard hot-start PCR (bottom) and PyroMark PCR Kit (top). Not only are peaks enhanced, but the resulting allele quantification is more reliable, as indicated by the “passed” quality assessment (blue box) of the frequency percentage calculated in the upper Pyrogram and the “failed” assessment (red box) in the lower Pyrogram.

## An optimized product for each workflow step

The components of the PyroMark product line are designed to make your research workflow straightforward and efficient. Each step is supported by software, kits, reagents, and sample preparation instrumentation optimized for Pyrosequencing.

To start, design an assay using the dedicated [PyroMark Assay Design Software](#), which generates PCR and sequencing primers suited for the selected DNA region. Then, amplify the DNA sequence to be analyzed. QIAGEN has developed the [PyroMark PCR Kit](#), which includes unique PCR reagents and yields biotinylated high-quality DNA that guarantees the clearest and strongest Pyrogram peaks (Figure 18).

Isolate the template DNA for all your samples in parallel on the PyroMark vacuum workstation. Sepharose® beads held by vacuum on probes bind and immobilize the biotinylated PCR amplicons. Through a series of steps whereby you dip the vacuum probes into carefully formulated solutions, the amplicons are denatured and washed, leaving only the desired single-stranded DNA attached to the beads. You then release the template DNA into specially designed 24- or 96-well plates holding sequencing primers and you are ready to go!

After you complete the Pyrosequencing run, end your workflow with the versatile analyses incorporated into the PyroMark application and [supplementary software](#).

Whichever format PyroMark system you choose to launch your investigative work into new horizons of genetic and epigenetic research, you know you are purchasing a robust and powerful research tool that meets stringent QIAGEN quality standards.

## References

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- Divne, A.-M., Edlund, H., and Allen, M. (in press) Forensic analysis of autosomal STR markers using Pyrosequencing. *Forensic Sci. Int. Genet.*
- Ballana, E. et al. (2009) Detection of unrecognized low-level mtDNA heteroplasmy may explain the variable phenotypic expressivity of apparently homoplasmic mtDNA mutations. *Hum. Mutat.* **29**, 257.

## Ordering Information

Product	Contents	Cat. no.
PyroMark Q24	Instrument, software, and installation for Pyrosequencing	9001514
PyroMark Q96 ID	Instrument, software, and installation for Pyrosequencing	9001525
PyroMark Q96 MD	Instrument, software, and installation for Pyrosequencing	9001526
PyroMark Q96 MD Automated	Instrument, software, and installation for automated Pyrosequencing	9001527
PyroMark Q24 Vacuum Workstation	Workstation for preparing 24 samples in parallel from PCR product to single-stranded template	Varies*
PyroMark Q96 Vacuum Workstation	Workstation for preparing 96 samples in parallel from PCR product to single-stranded template	Varies†
PyroMark Gold Q24 Reagents (5 x 24)	Enzyme Mixture, Substrate Mixture, and nucleotides for Pyrosequencing reactions on the PyroMark Q24	970802
PyroMark Gold Q96 Reagents (5 x 96)	Enzyme Mixture, Substrate Mixture, and nucleotides for Pyrosequencing reactions on the PyroMark Q96 ID (5 x 96) or PyroMark Q96 MD (15 x 96)	972804‡
PyroMark PCR Kit (200)	For 200 reactions: 2x PyroMark PCR Master Mix (includes HotStarTaq® DNA Polymerase and optimized PyroMark Reaction Buffer containing 3 mM MgCl <sub>2</sub> and dNTPs), 10x CoralLoad Concentrate, 5x Q-Solution, 25 mM MgCl <sub>2</sub> , and RNase-Free Water	978703‡
PyroMark Assay Design SW 2.0	Software for convenient design of PCR and sequencing primers, optimized for Pyrosequencing analysis	9019077§
PyroMark CpG SW 1.0	CpG methylation analysis software for PyroMark Q96 ID and PyroMark Q96 MD	9019067§
PyroMark Identifire SW 1.0	Software for comparing and matching Pyrosequencing data to a local database	9019087
EpiTect Bisulfite Kit (48)	48 EpiTect Bisulfite Spin Columns, Reaction Mix, DNA Protect Buffer, Carrier RNA, Buffers	59104‡

\* 9001518 (220V); 9001516 (110V); 9001519 (100V). † 9001529 (220V); 9001528 (110V); 9001740 (100V).

‡ Larger kit sizes available; please inquire. § Multiple software licenses available; please inquire.

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

**Discover new possibilities with sequence-based detection and quantification at [www.qiagen.com/goto/Pyrosequencing](http://www.qiagen.com/goto/Pyrosequencing).**

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