PyroMark® Q24 Advanced

For advanced methylation and mutation quantification in long sequence runs

PyroMark Q24 Advanced has improved Pyrosequencing® technology and provides even better real-time, sequence-based detection and quantification than before. PyroMark Q24 Advanced features advanced technology, software and chemistry and is highly suited for analyzing any kind of sequence variation, particularly DNA methylation at CpG or CpN sites, complex mutations or for *de novo* sequencing applications such as microbial typing.

PyroMark Q24 Advanced provides:

- Advanced technology, software and chemistry for long sequence runs
- Quantitative methylation analysis at consecutive CpG and CpN sites
- Improved quantification of sequence variations at any sequence position
- Easy and improved base calling functionality

Improved methylation analysis at any position

PyroMark Q24 Advanced enables improved methylation quantification in long sequence runs at any sequence position. Previously, analysis of methylation sites further away from the sequencing primer could be uncertain, but now with longer read lengths and higher accuracy, methylation quantification is highly reliable throughout the entire sequencing run (see Figure 1).

Bisulfite conversion in DNA methylation analysis leads to frequent poly T stretches in the nucleotide sequence, and analysis of CpG sites directly after such T homopolymers has previously been challenging due to uncertain quantification of the light signal at these sites. The increased accuracy of PyroMark Q24 Advanced enables reliable quantification of CpG methylation behind and even within a stretch of 8 T nucleotides (see Figure 2).

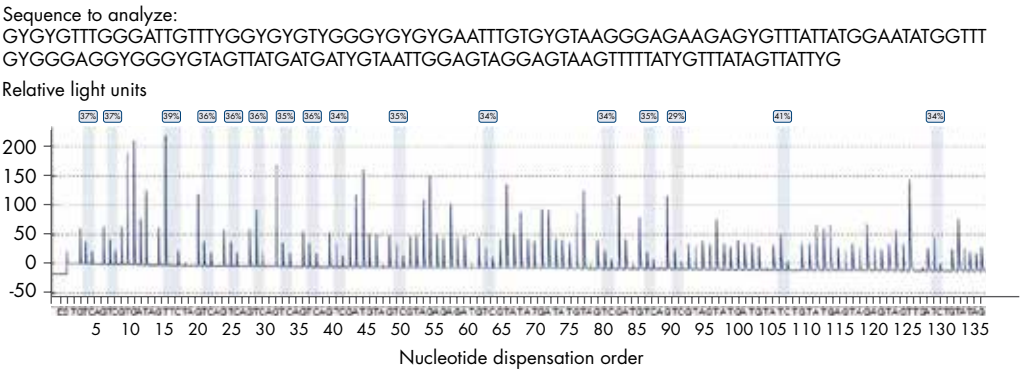

Figure 1. Analysis of 16 CpG sites in a long sequence run. PyroMark Q24 Advanced increases both read length and reliability of methylation analysis at positions later in the sequence. This example demonstrates 135 nucleotide dispensations and the accurate analysis of 16 different CpG positions in a single PyroMark Q24 Advanced CpG reaction.

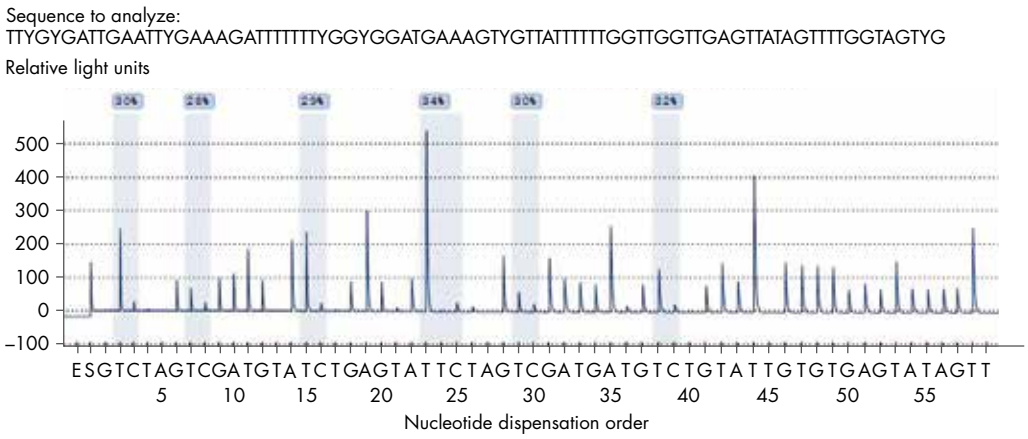

Figure 2. Improved methylation quantification in homopolymers. This example shows the analysis of a CpG site within a stretch of 8 T nucleotides using PyroMark Q24 Advanced.

Longer sequencing runs and higher accuracy

PyroMark Q24 Advanced features improved chemistry and instrument operation algorithms that significantly increase assay read length and accuracy in base calling, enabling easy analysis of long *de novo* sequencing runs. Assay read length was previously limited by background peaks and reduced light signals in the sequencing reaction. The updated chemistry and algorithms of PyroMark Q24 Advanced reduce this background, thereby increasing read length and reliability. Depending on the sequence to be analyzed, highly accurate read lengths of 140 or more bases can be obtained in just a single PyroMark Q24 Advanced reaction (see Figure 3).

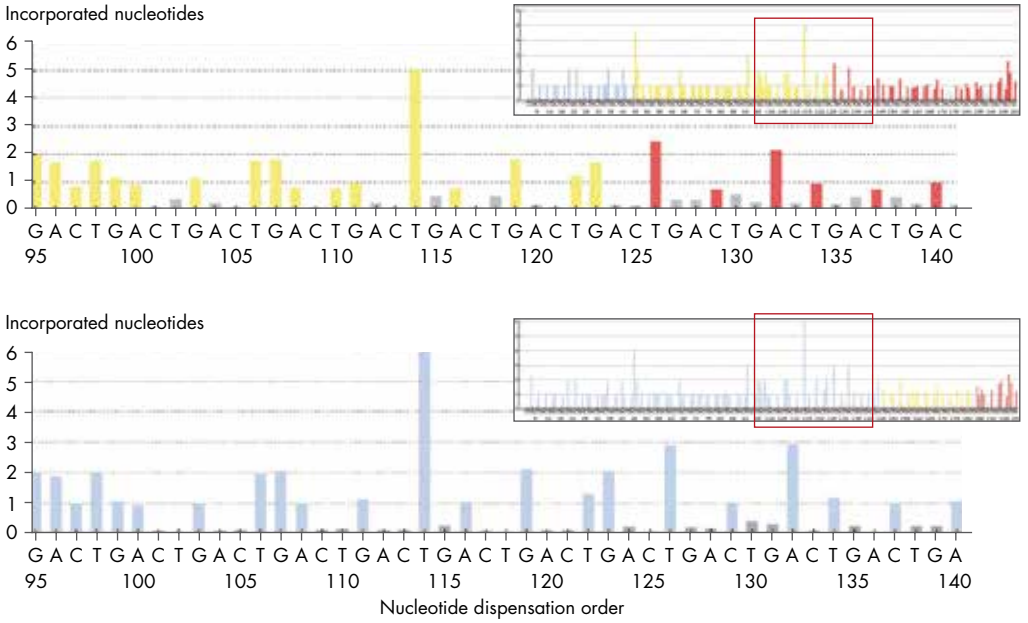

Figure 3. Long *de novo* sequencing runs. PyroMark Q24 (upper panel) and PyroMark Q24 Advanced (lower panel) were used for *de novo* sequencing of 140 bases using the same assay. The detailed sequence between dispensations 95 and 140 is enlarged. Blue bars indicate reliably detected bases; yellow bars indicate bases that might have been determined correctly but should be checked by the user; red bars indicate unreliable readings; gray bars indicate background noise. The improved algorithm of PyroMark Q24 Advanced leads to longer reads, clearer data and minimized background.

Analysis of mutations over long sequences

PyroMark Q24 Advanced also provides reliable quantification of multiple polymorphisms in a single assay. Since single nucleotide polymorphisms (SNPs) and other mutations are often not located close to one another, traditional Pyrosequencing chemistry usually requires separate assays for each mutation site to be analyzed. The new chemistry of PyroMark Q24 Advanced allows much longer runs, enabling reliable analysis of more than one mutation or SNP in the same run (see Figure 4).

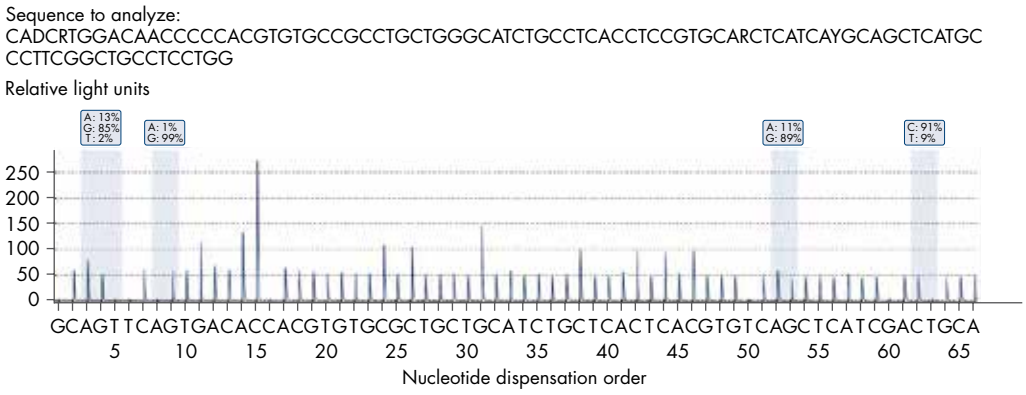

Figure 4. Quantitative mutation analysis in long sequence runs.
This example shows the analysis of a defined DNA sample containing a 90:10 mixture of wild-type and mutated EGFR. Even after 60 nucleotide dispensations, the mutation quantification is accurate.

Quantification of CpN methylation

The new CpN mode of PyroMark Q24 Advanced now enables methylation analysis of cytosine residues that are not part of CpG sites. Analysis of CpN and CpG sites can be performed together in a single Pyrosequencing reaction. Each of these positions can be selected individually in the software during the run setup. The example in Figure 5 shows the analysis of a sequence with a CpN site (CpA in this case) in the first position, followed by 2 classical CpG sites.

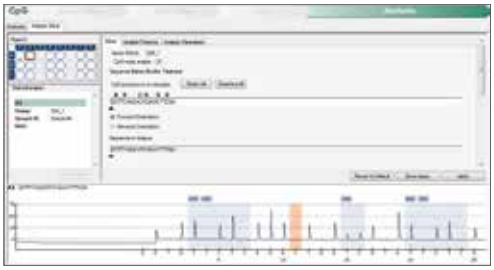

Figure 5. New mode for analyzing CpN methylation.

PyroMark Q24 Advanced Software

The PyroMark Q24 Advanced analysis software is user-friendly and intuitive, and now provides convenient and improved tools for run analysis. If a problem occurs during the run, or if the system detects an inconsistency with an assay, the software provides specific warning information for each individual well (see Figure 6). A "Warning Info" button now gives access to additional information about the warning along with recommendations for troubleshooting and preventing its occurrence in subsequent assays.

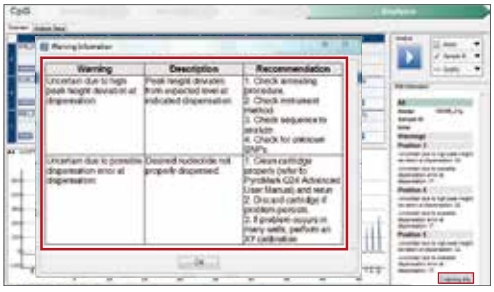

Figure 6. New warning information window with troubleshooting recommendations.

Ordering Information

Product	Contents	Cat. no.
PyroMark Q24 Advanced	Instrument, software, and installation for advanced Pyrosequencing analysis of 24 samples in parallel	9002270
PyroMark Q24 Vacuum Workstation	Vacuum Workstation for preparing 24 samples in parallel from PCR product to single-stranded template	Varies
PyroMark Q24 Advanced Software	Analysis software for upgrading PyroMark Q24 systems	9022779
PyroMark Q24 Advanced Reagents (4 x 24)*	Enzyme mix, substrate mix, nucleotides, buffers	970902
PyroMark Q24 Advanced CpG Reagents (4 x 24)*	Enzyme mix, substrate mix, nucleotides, and buffers for assays requiring longer sequence read lengths	970922
PyroMark Assay Design Software 2.0	Software for convenient design of PCR and sequencing primers, optimized for Pyrosequencing analysis	9019077
PyroMark Q24 Plate (100)	24-well plate for samples, for use with PyroMark Q24 and PyroMark Q24 Advanced; 100 plates in each package	979201
PyroMark Q24 Cartridge (3)	Reusable dispensing tool for nucleotide and reagent delivery, for use with PyroMark Q24 and PyroMark Q24 Advanced	979202
PyroMark PCR Kit (200)	PCR Master Mix (includes HotStarTaq DNA Polymerase and optimized PyroMark Reaction Buffer) for 200 highly specific PyroMark PCR reactions	978703
PyroMark OneStep RT-PCR Kit (50)†	OneStep RT-PCR Mix, RT-PCR Buffer, 10x CoralLoad Concentrate, 5x Q-Solution, dNTP Mix, and RNase-Free Water for highly specific PyroMark RT-PCR reactions	978801
PyroMark Control Oligo	For installation check of system	979203
PyroMark Q24 Validation Oligo	For performance confirmation of system	979204

* PyroMark Q24 Advanced Reagents should only be used with PyroMark Q24 Advanced Software, which is included with the PyroMark Q24 Advanced system, or available separately to upgrade existing PyroMark Q24 systems.

† Larger kit sizes available.

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** or can be requested from QIAGEN Technical Services or your local distributor.

Advance your Pyrosequencing analyses at **www.qiagen.com/PyroMarkQ24Advanced**!