Pyrosequencing® and its applications

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Pyrosequencing principle

Pyrosequencing technology is based on the sequencing by synthesis principle. After successful incorporation of a nucleotide by a polymerase using a single-stranded PCR (or RT-PCR) fragment as template, the released PPi is converted to light by an enzyme cascade: 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, which is detected by CCD sensors and seen as a peak in the raw data output (Pyrogram®). Pyrosequencing continuously degrades unincorporated nucleotides and ATP. The height of each peak (light signal) is proportional to the number of nucleotides incorporated. Sequential addition of nucleotides allows quantitative decoding of the sequence to analyze.

Methylation analysis

Pyrosequencing generates highly reproducible quantification of methylation frequencies at individual contiguous Cpg sites. Pyrosequencing can detect and quantify even small changes in methylation levels. Other features include the inherent quality control afforded by the sequence context of results. Built-in methylation sensitivity and specificity allows detection and quantification of virtually any sequence variation. The PyroMark® product line includes analysis of KRAS, EGFR, BRAF, NRAS, APOE, HFE, and MTHFR. In addition, easy assay design with PyroMark software enables detection and quantification of virtually any sequence variation.

Mutation analysis

Pyrosequencing ensures precision and accuracy when performing highly sensitive mutational analysis or when quantifying small changes in nucleic acid levels of the DNA template. Results provide unambiguous information. Users can check the sequence surrounding the variable site ensuring that the correct DNA region was analyzed. Unlike hybridization techniques, Pyrosequencing allows the identification of a large number of species using a single conserved primer. Consequently, DNA extracted from multiple microbe species can be sequenced in the same Pyrosequencing run. PyroMark identify Software complex local sequence database against which imported Pyrosequencing outputs are rapidly aligned. The raw data, matched hits, and percentage concordance of each kit are presented in detailed reports.

Genetic testing

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 diseases involving characterizing linked SNPs, finally, analysis of forensic DNA evidence relies on accurate detection of sequence variation.

Microbial identification

Sequence information provides reliable data for microbial genotyping applications. Since Pyrosequencing sequences by synthesizing new copies of the DNA template, results provide unambiguous information. Users can check the sequence surrounding the variable site ensuring that the correct DNA region was analyzed. Unlike hybridization techniques, Pyrosequencing allows the identification of a large number of species using a single conserved primer. Consequently, DNA extracted from multiple microbe species can be sequenced in the same Pyrosequencing run. PyroMark identify Software complex local sequence database against which imported Pyrosequencing outputs are rapidly aligned. The raw data, matched hits, and percentage concordance of each kit are presented in detailed reports.

The Pyrosequencing applications presented here are for research purposes. Not for use in diagnostic procedures.