

Genaxxon BioScience

Pyrosequencing - Applications

fon: +49 (0)7357 - 91 63 77
 fax: +49 (0)7357 - 91 63 78
 eMail: info@genaxxon.com
 internet: www.genaxxon.com

Pyrosequencing in Microbiology As Reliable as Sequencing, as Easy as PCR

Identify species and detect resistance from the DNA sequence

New technologies are enabling DNA sequence information to become the new gold standard for microbial species identification and resistance detection. By sequencing well-characterized hypervariable regions of genes such as 16S rRNA or rnpB, sequence data provides, either on its own or with supporting biochemical data, unambiguous and discriminatory information for microbial identification.

Pyrosequencing is highly suited to providing reliable sequence data in clinical situations. In roughly the time it takes to run a PCR reaction, Pyrosequencing reads a discriminatory stretch of DNA of up to 96 samples in parallel. Depending on the assay design, the sequence can be used to discriminate microbial species, types and strains, or detect genetic mutations that confer resistance to antibiotics and anti-viral drugs.

The obtained sequences are rapidly matched against a local database using software algorithms optimized for Pyrosequencing data. The raw data together with the matched hits are presented in identification reports.



Benefits

Pyrosequencing delivers the microbial sequence in 1 hour.

This means

- True DNA Sequence data in a clinically-relevant timeframe, not just a yes/no answer
- Ideal for rapid identification, epidemiological studies and resistance detection
- Species identification and resistance characterisation with 1 system
- Fulfils 2 fundamental applications with 1 simple method
- Mutation-Tolerant Assays
- Unlike hybridisation, sequencing yields sequence data despite unpredicted mutations
- Quantification of multi-copy genes
- Antibiotic resistance characterization, measuring viral and fungal loads
- Built-in Quality Control
- Seeing the mutation in the context of the DNA sequence ensures a correct analysis

Pyrosequencing for bacterial identification and typing

Pyrosequencing gives a unique opportunity to determine any bacterial species. Whenever reliable identification is needed.

Pyrosequencing is a proven technology for sequence identification of species as well as in typing applications. A variety of assays, broad-range as well as targeting specific species, are available using discriminatory genetic regions for *Salmonella*, *Staphylococcus*, *Neisseria*, *Mycobacteria*, *Nocardia*, *Helicobacter*, *Lactobacillus*, or to identify bacteria in e.g. neonatal sepsis, rapidly and accurately, alive or dead. Pyrosequencing uses a single PCR reaction to discriminate among a variety of species.

Pyrosequencing for fungal identification

Pyrosequencing identifies clinically important fungal species, dramatically shortening the time from sample collection to identification. Pyrosequencing is more efficient in identification than hybridization techniques, since a number of species can be discriminated from a single PCR product. One sequencing primer identifies a large number of species, instead of using one dual-labelled probe for each species. Pyrosequencing therefore works elegantly in combination with routine PCR screening.

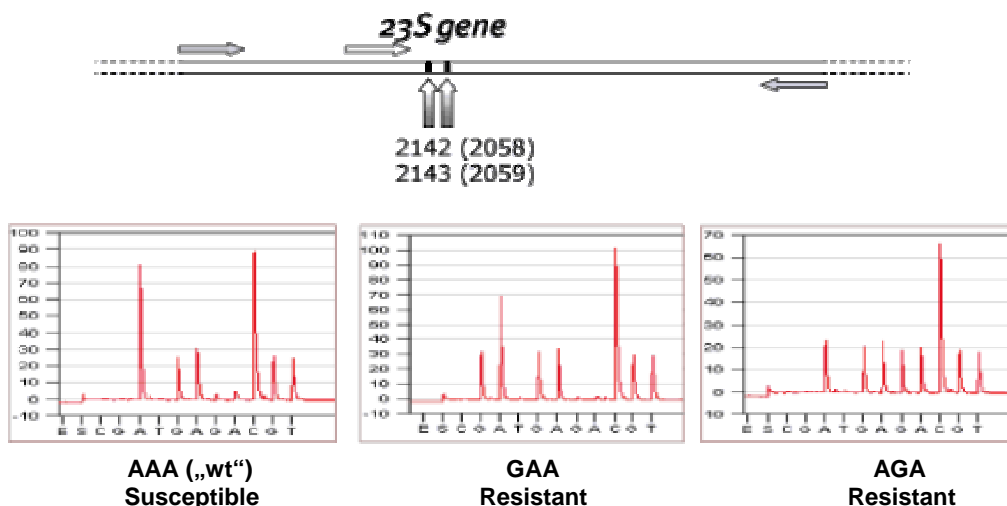
Fungal identification by Pyrosequencing offers major benefits of speed, ease and accuracy.

Pyrosequencing for resistance detection

Pyrosequencing gives all the advantages of high quality sequencing, yet in real-time. It gives both ease of use and quality control that is unavailable by any other genotyping method. Pyrosequencing delivers meaningful data despite the high rate of mutation by microorganisms, making it a very powerful platform for the development of methods to detect antibiotic resistance.

Detection of a Range of Possible Resistance Mutations with a Single Assay

Many common resistance mutations are single nucleotide polymorphisms (SNPs) or clustered mutations in mutation "hot spots", which makes sequencing an appropriate choice to characterize them. Sequencing has the advantage over hybridization-based approaches in that a single assay can detect a range of possible mutations in a region, as well as new and unexpected mutations that hybridization-based methods would miss (e.g. Q-PCR).



154 isolates were genotyped - 154 isolates were correctly genotyped (100%)

Compared to traditional biochemical and sequencing methods, Pyrosequencing offers significant advantages for detecting resistance: it is accurate with built-in QC, takes less than one hour to perform an analysis, and offers high throughput (96 samples in parallel) at significantly lower cost and manpower.

An Efficient Alternative to Culture-based Resistance Screening

One major advantage of applying molecular biological techniques for resistance screening is the avoidance of culturing, which is time-consuming for slow-growing organisms like *Mycobacteria*. Several studies have demonstrated that Pyrosequencing is useful for rapid screening of resistance in *Mycobacterium tuberculosis*. Arnold *et al.* (2005) performed both species identification and a rapid screen for drug resistance to rifampicin and isoniazid. Since identification and resistance screening could be performed directly from sputum, they suggest that this could function as a low-cost replacement of the existing time-consuming tests based on culture.

High throughput Genetic Surveillance of Antiviral Drug Resistance in Influenzavirus

A global surveillance study performed with Pyrosequencing (Bright *et al.*, 2005) revealed an alarming increase in Asia of resistance in human influenza A(H3N2) over the last decade to the antiviral drug class the adamantanes. In the largest study to date on the emergence of drug resistance in influenza virus, The Centers for Disease Control and Prevention (CDC) in Atlanta, GA were able to reliably and with high throughput sequence the specific region of DNA that contained the mutations conferring drug resistance. Using Pyrosequencing, two laboratory workers screened for adamantane resistance in more than 7,000 isolates of normal circulating human H3N2 influenza A viruses in less than 3 months. In comparison, the previous surveillance study using traditional methods took 4 workers over 4 years to complete.

In January 2006, Pyrosequencing was again used to detect a significant increase in adamantane resistance in circulating influenza A(H3N2) viruses in the USA. The CDC found that 109 of the isolates (91%) were resistant to amantadine and rimantadine, leading to a recommendation by the CDC that these drugs should not be used during the 2005-06 influenza season (Bright *et al.*, 2006).

Quantify DNA methylation in a flash, like never before

Varioscan CpG is an accurate and fast analysis method for quantifying CpG methylation in epigenetic studies. Since its inception in 2002, it has established itself among leading researchers as the gold standard of DNA methylation analysis. Varioscan CpG has revealed correlation between DNA methylation of genes to tumor type and gene expression, measured the response to treatment with demethylating agents, as well as changes in methylation state in relation to tumor genesis and tumor progression, genetic imprinting and exposure to environmental toxins.

The most appreciated property of Varioscan CpG is its highly reproducible quantification of methylation frequencies in individual consecutive CpG sites, enabling reproducible measurement of even small changes in methylation levels. The reproducibility of Varioscan CpG is a result of a quantitative measurement principle, inherent quality controls (QC), and few processing steps. QC is inherent because CpG sites are presented in the context of the DNA sequence, and controls for completion of the bisulfite conversion step can be integrated into the analysis process.

Based on Pyrosequencing® technology, Varioscan CpG analyzes and presents the individual methylation levels of multiple consecutive CpG sites in the context of the DNA sequence. Single CpG sites can be analyzed as easily as multiple sites, and methods are established for estimating global methylation.

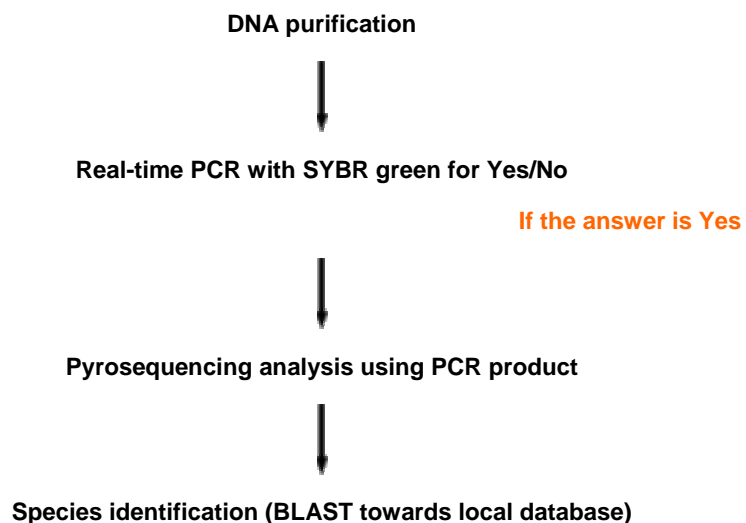
Pyrosequencing in Clinical Genetics Applications

Pyrosequencing produces valuable data in a range of studies of clinical genetic syndromes. Here, we illustrate the performance of Pyrosequencing in quantitative measurement of methylation in Prader-Willi & Angelmann syndromes, the identification of multiple polymorphisms in the APOE gene, simple genotyping of adult hypolactasia, and a single test to characterise the complex cancer mutations involved in Multiple Endocrine Neoplasia (MEN2).

All pictures and information are taken from the Biotage pyrosequencing internet page: www.pyrosequencing.com

How can sequential PCR tests for Microbial Determination be improved?

- QPCR is sensitive and highly quantitative, however one probe is required for each species - time-consuming and costly
- With Pyrosequencing, only 1 sequencing primer is needed for species identification
- Combining QPCR for screening with Pyrosequencing gives high-speed, high resolution species identification at lower cost



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