PYROSEQUENCING

May 2008

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Highlights
Pyrosequencing Newsletter

In this issue of Highlights

Biotage

In this issue of Highlights

Pyrosequencing technology makes an entry into diagnostics and personalized medicine

I may be the editor of Highlights but I work mainly in the R&D department at Biotage. It is certainly challenging and fun to work on projects, especially the one leading to our new product PyroMark™Q24. But at regular intervals I get the welcome chance to put down the pipette and pick up the pen (or in this day and age, the keyboard), and gather together interviews from Pyroneers, take stock of the literature, and bring in interesting snippets of information. This is a privilege: the chance of getting a broader view, instead of being confined to microliters and picomoles. I hope that you get the chance to pause and take stock now and then.

In this issue we have put together a diverse selection of articles, as usual. It reflects the broad range of questions that you, the Pyroneers, use Pyrosequencing® technology to help answer. We have interviews with Pyroneers working on pharmacogenetics, DNA methylation, and mutation detection in mixed cell populations. There are also articles that help to confirm the place Pyrosequencing technology has in the microbiology laboratory. A summary of the recent literature celebrates the fact that you have together published over 1000 articles where Pyrosequencing technology played a significant role in DNA analysis! And of course we have a report from the AACR meeting in San Diego, where PyroMark Q24 was officially launched.

We have reports from three Pyroneers who have been test pilots for PyroMark Q24. All three are very positive and excited with the new product. We would like to thank them for their efforts and valuable input into the project. The work of one group is particularly relevant to the current news in personalized medicine: they are using PyroMark Q24 to detect *KRAS* mutations in mixed samples from, for example colon cancer tumors. On the 16th of May, ImClone sent out a press-release detailing the results of new studies on the implications of *KRAS* mutations for those colorectal-cancer patients who are treated with their big-selling drug Erbitux. Simply put, if the patient has a *KRAS* mutation then the expensive drug treatment will not work, only result in side-effects. The company now states that the drug should only be given to patients with normal *KRAS*. A similar press-release was sent out by Amgen in December last year, concerning a competing drug, Vectibix. This is a good example of the enormous value genotyping can have in the choice of therapy for individual patients, or 'theranostics' as it is called.

Our new product, PyroMark Q24, has been launched into the IVD marketplace in Europe*. Let's hope that it helps make a difference in diagnostics, personalized medicine and theranostics.

With best wishes from everyone at Biotage.

Nigel Tooke

Editor

United States: *PyroMark™Q24 System is designed for Laboratory Use Only, which means it may be used for either research purposes or by high complexity CLIA certified laboratories.*

Europe: *PyroMark™Q24 System is available for research and, in certain European countries*, for in vitro diagnostic applications. PyroMark™Q24 System meets the requirements of Annex III of the European Directive for In Vitro Diagnostic Medical Devices 98/79/EC.*

** For more information, see www.biotagebio.com.*

AACR

This year the American Association for Cancer Research Annual meeting was held in San Diego in sunny California, with over 17 000 participants. Biotage was well represented in a frequently visited booth where we presented the new member of the Pyrosequencing® family of instruments: PyroMark™Q24. PyroMark Q24 gained a lot of interest and the launch of PyroMark Q24 was also celebrated with a launch party in connection to the meeting.

Scientifically Biotage was represented on 29 posters where Pyrosequencing technology had been used for various applications, showing the flexibility of the technology. Some examples of the posters where Pyrosequencing was the method of choice are described below. All abstracts can be downloaded from the AACR official webpage.

http://www.aacr.org/home/scientists/meetings--workshops/annual-meeting-2008.aspx

Genotyping of mutation hotspots in OGG1 and *MYH* in Barrett's carcinomas was described by Diana Walluscheck, and co-workers: **Oxidative stress-induced DNA-damage in the Barrett´s carcinogenesis**

Determination of HPV infection status in head and neck squamous cell carcinomas: *Quynh-Thu Le et al* **Potential mechanisms for improved outcomes in Human Papilloma Virus (HPV)- related head and neck squamous cell carcinomas (HNSCC)**

The most common application overall for Pyrosequencing technology was the study of DNA methylation (21 of the 29 posters). These studies included gene-specific assays as well as assays for global methylation using the repetitive elements LINE and/or SINE.

Ryan J. Castoro and co-workers at Anderson Cancer Center, Houston, Texas presented a poster concerning methylation of microRNA and its influence on gene expression: **MicroRNA 124 and its role in response to epigenetic therapy in acute myelogenous leukemia and myelodysplastic syndrome.** The conclusion was that methylation of miR-124 and subsequent demethylation may play a critical role in response to epigenetic therapy in acute myelogenous leukemia and myelodysplastic syndrome by decreasing CDK6 levels.

Another study using Pyrosequencing technology for gene-specific DNA methylation was presented in a poster by T. Liloglou and co-workers: **Aberrant methylation of both Tp73 promoters in non-small cell lung cancer.** In this study they showed different methylation patterns for the two promoters for Tp73, which gave rise to two isoforms of the protein. The results suggest that methylation status of the two promoters might be of importance during treatment of NSCLC patients with demethylating agents.

Global methylation was studied using both LINE-1 and Alu in multiple myeloma by Bollati and co-workers: **Repetitive DNA hypomethylation in multiple myeloma.** The poster presented results that showed that repetitive DNA hypomethylation is a feature of Multiple Myeloma, particularly in subtypes with the poorest prognosis.

Highlights on PyroMark™Q24

It is hardly surprising that a major part of this issue of Highlights concerns our new system, PyroMark™Q24:

The Small Smart PyroMark™Q24 system
Pyrosequencing® technology in a 24-well format

- Analyze methylation in the presence of SNPs
- Run mutation analysis and CpG assays on the same plate
- Run a typical SNP plate in only 15 minutes
- Prepare 24 single-stranded DNA templates in less than 15 minutes

PyroMark™Q24 provides high resolution quantitative data for individual sites with built-in quality control

Pyrosequencing® technology is ideal for samples from paraffin embedded tissue and as it is a non-destructive technology it enables further template analysis

PyroMark™Q24 includes a complete software package for the study of CpG methylation, allele quantification and mutation analysis.

These studies are used for a wide range of clinical research, including the identification and tracking of changes involved in various forms of cancer. Studying these changes using Pyrosequencing technology, which is based on the principle of sequencing by synthesis, is an ideal choice for genetic analysis in clinical research. The output information is based on real sequence data providing integrated quality control and confidence in the quality of the results.

The 24-well format and the ability to run both mutation analysis and CpG assays on the same plate makes PyroMark Q24 ideal for fewer samples, while providing the accuracy, flexibility and convenience of Pyrosequencing technology.

The results are shown in real time on the instrument display and as it takes only 15 minutes to run a typical SNP plate, many plates can be run each day.

The system is extremely easy to use. A control oligonucleotide delivered with the instrument ensures proper installation and a validation oligonucleotide is available to check the performance of the system.

PyroMark Q24 takes very little bench space, measuring only H420xW390xD525mm. It can be run at ambient temperatures between 15°C and 32°C.

PyroMark Q24 is a small and robust tool for efficient handling of mixed samples and low sample numbers

Quantitative methylation and mutation analysis of several contiguous sites can be run in parallel

A continually updated database of almost 1000 assay designs is available online

The full list can be seen at www.biotagebio.com

PyroMark™Q24 Software

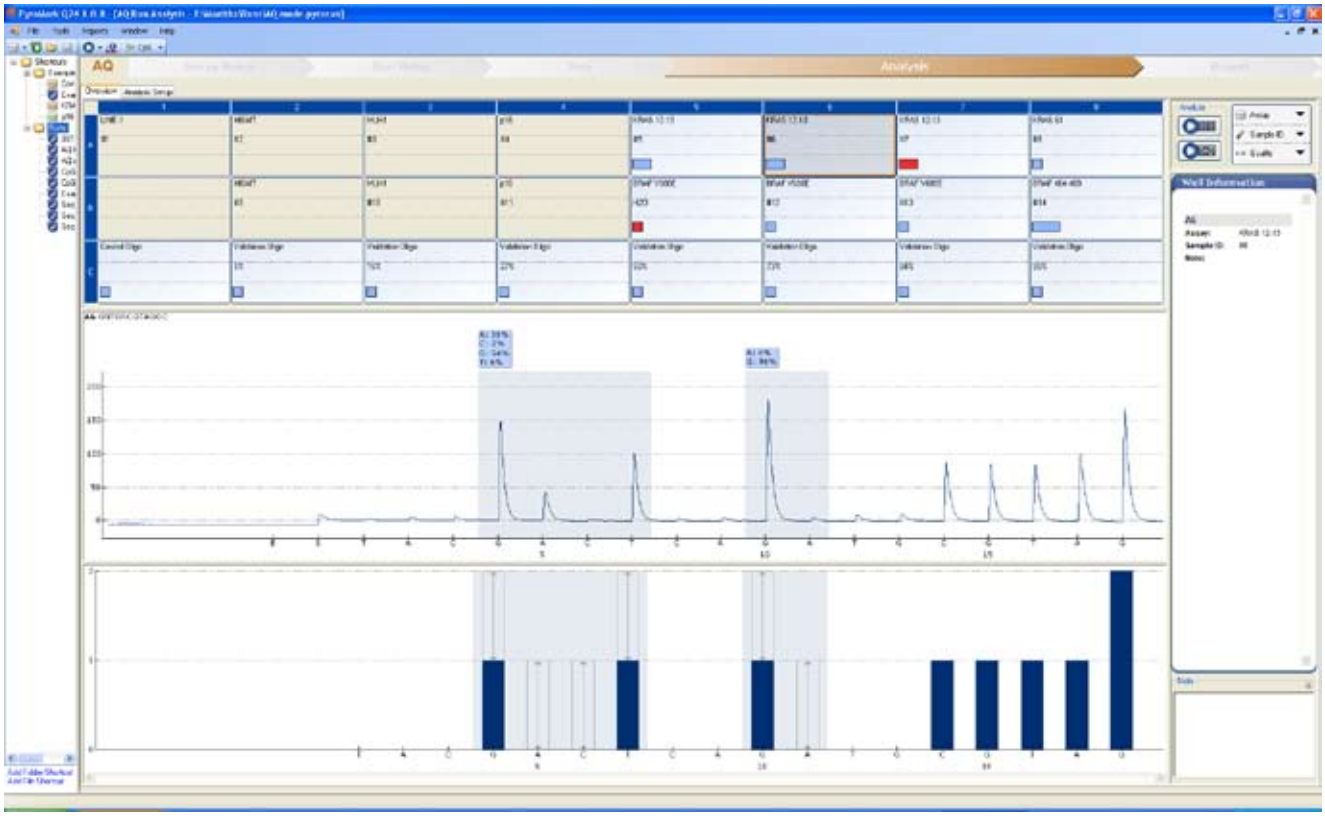
PyroMark™Q24 software can be installed on any computer that has the required specifications; it does not need to be connected to the PyroMark Q24 instrument. Information is transferred between the instrument and computer via a USB memory stick.

The system is delivered with two software 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 software offers detailed report information that can be exported to html, Excel, text and pdf formats.

AQ mode

Frequency calculations of variable positions in sequence context including:

- Single variable position AG/TC
- Multiple variable positions AG/TCAG/TCAG/T/AC
- Di- Tri- or Tetra-allelic mutations GA/C/G/TA
- High resolution of individual sites
- Quality assessment of individual sites and sequence context



Results from PyroMark Q24 software illustrating an analysis of mutations in codons 12 and 13 in KRAS. The software automatically analyzes mutation levels and gives a quality score for all results. It also provides an informative plate overview, raw data (Pyrogram®), theoretical histogram, and information that can be exported to html, Excel, text and pdf formats.

CpG mode

Quantification of multiple consecutive CpG sites in sequence context:

- High resolution of individual sites
- Built-in control for bisulfite treatment
- Analyze methylation in the presence of SNPs
- Presents an overview of the deviation from specified methylation ranges
- Quality assessment of individual sites and sequence context
- Mean methylation for the whole assay can be shown for a quick overview of results

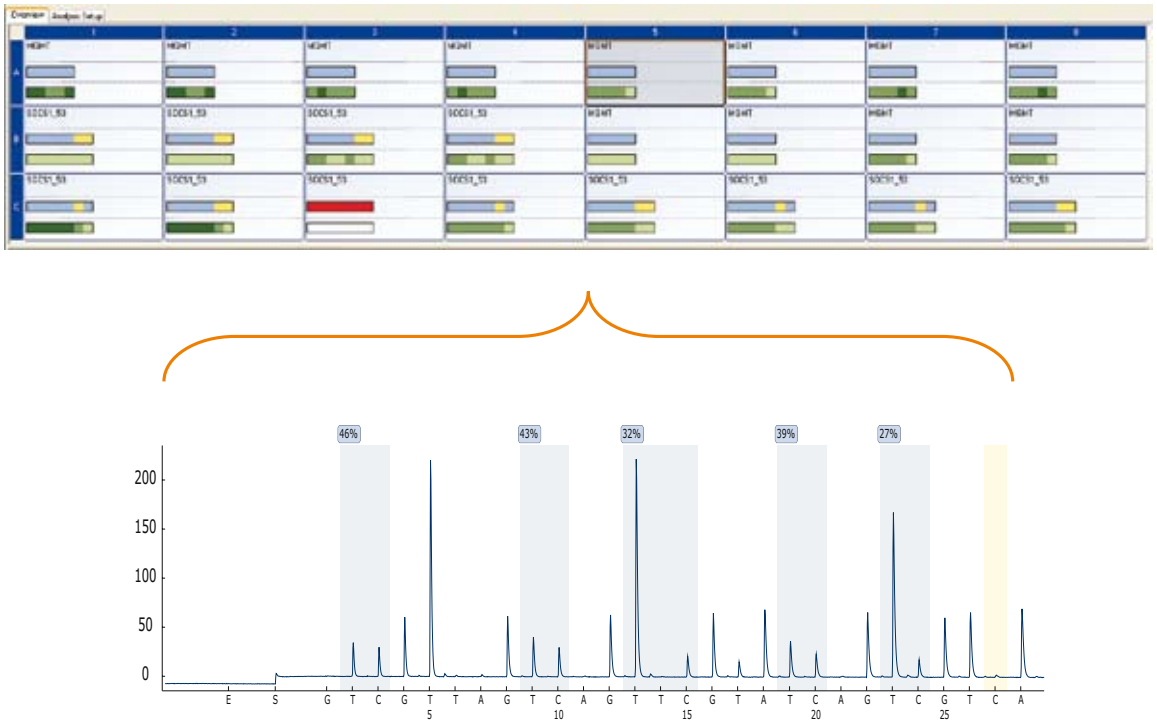
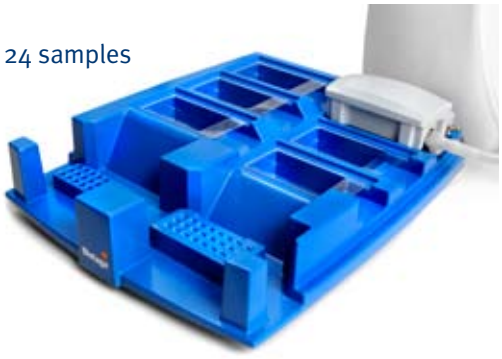


Plate overview showing individual methylation frequencies and quality assessment for multiple, adjacent CpG sites. The above Pyrogram shows the raw data from well A5, (MGMT analysis). Blue areas indicate variable CpG positions; yellow area indicates the built-in bisulfite treatment control.

PyroMark™Q24 Vacuum Prep Workstation - Sample Preparation for PyroMark Q24

In less than 15 minutes, PyroMark Q24 VPW (Vacuum Prep Workstation) prepares 24 samples in parallel from PCR product to single stranded template ready for sequencing.

- Reliable and simple preparation of DNA for Pyrosequencing
- Process up to 24 samples in parallel in less than 15 minutes
- Only 2 pipetting steps
- Vacuum Prep Tool designed for repeated use with re-usable filter probes



PyroMark™Q24 Validation Oligo
PyroMark™ Control Oligo

Two easy-to-use products are made available for functional testing of PyroMark™Q24 System. Both products consist of biotinylated oligonucleotides for an all-in-one verification of the entire system, including sample preparation. The need for a sequencing primer is eliminated by the formation of an internal stem-loop structure.

What do the products offer?

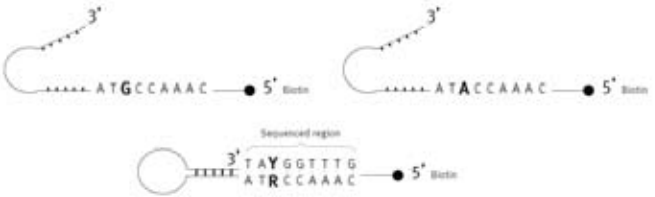
PyroMark™ Control Oligo provides you a simple and easy confirmation of successful installation of your PyroMark Q24 system. The product is also a useful tool for troubleshooting of all PyroMark systems.

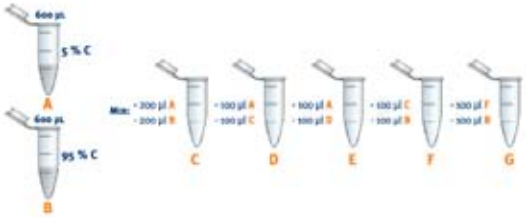
PyroMark™Q24 Validation Oligo consists of two oligonucleotides that differ in sequence in a single position. When mixed at defined ratios, they present performance characteristics in terms of linearity, bias and repeatability of your PyroMark Q24 system to assure you of the accuracy of Pyrosequencing technology in your own lab.

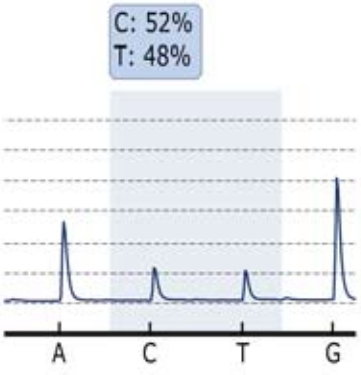
PyroMark™ Control Oligo

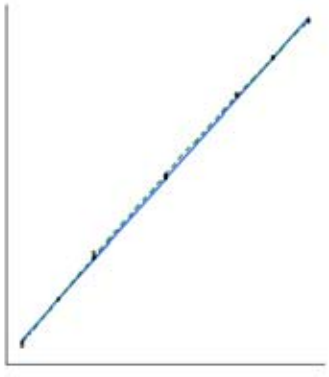
- for a simple installation check of your system, and even for troubleshooting

PyroMark™Q24 Validation Oligo – for a thorough performance confirmation of your system









PyroMark™Q24 Research Use Only tests

A range of optimized, validated Research Use Only tests for quantitative mutation and CpG methylation analysis now available for PyroMark™Q24

Each test offers

- Full confidence in assay results by scoring polymorphic sites within the context of the surrounding DNA sequence
- Built-in quality control for completion of bisulfite treatment in methylation assays
- Quality controlled and tested reagents, ensuring the successful implementation of the tests, and reducing the time to get the assay up and running

The capabilities of the new PyroMark™Q24 Software give additional value to the PyroMark Research Use Only (RUO) tests. The software now has functionality for re-analyzing samples after run completion, using an altered dispensation order. This allows for automatic analysis of common mutations in close proximity to rare mutations. As always, Pyrosequencing analysis of mutations is quantitative.

Cancer mutations -
KRAS* and *BRAF

Simple and reliable determination of contiguous, multi-variable mutations

Methylation -
LINE-1, *p16*, *MGMT* and *MLH1*

Individual methylation results for all CpG sites provides high resolution results

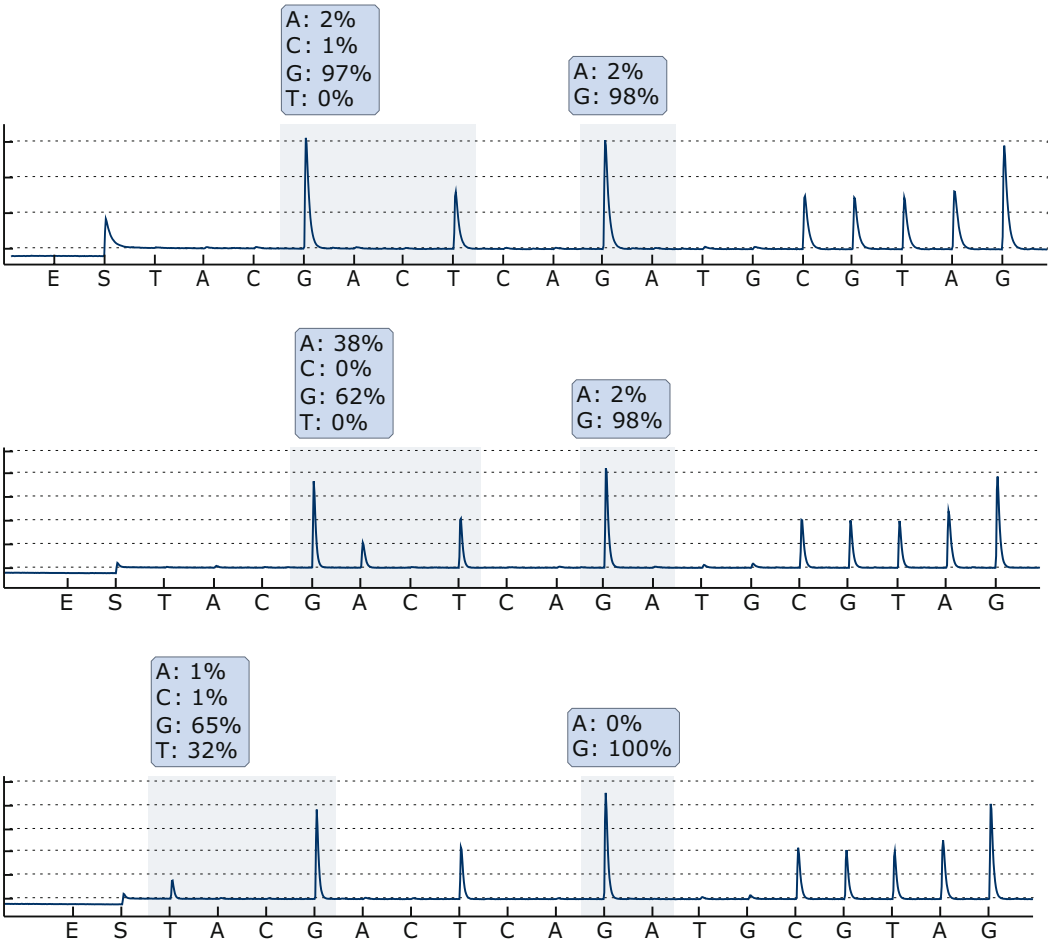
PyroMark™Q24 RUO tests

Designed for PyroMark™Q24 System

Mutation assays

- Simple and reliable determination of contiguous, multi-variable mutations
- Mutations shown in sequence context
- High resolution of individual sites
- Flexibility of PyroMark Q24 Software enables detection and analysis of less common surrounding mutations, as well as more frequently occuring mutations

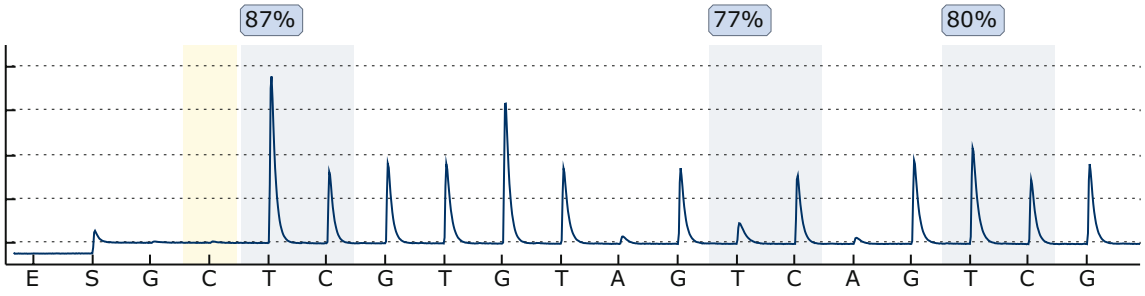
KRAS (codon 12+13 and codon 61)



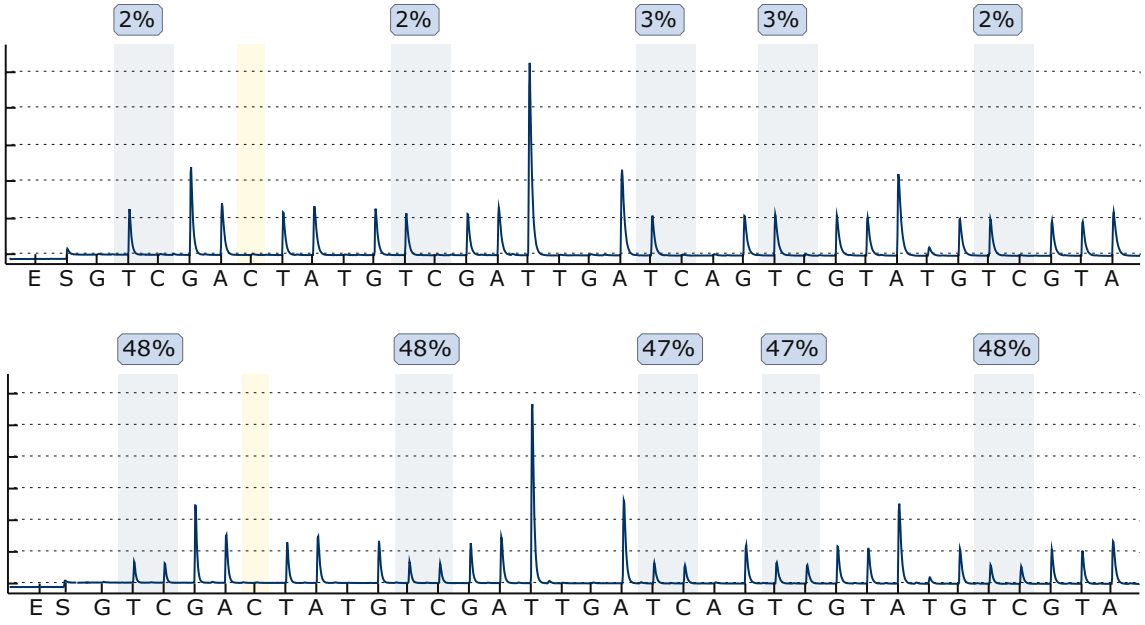
Results from analysis of KRAS. Upper Pyrogram® trace shows a wild-type sample, middle Pyrogram trace shows a sample with a G to A mutation in position 2 of codon 13, and lower Pyrogram trace shows a sample with a G to T mutation in position 1 of codon 12. Light-blue areas indicate the variable positions. The flexible PyroMark Q24 Software can type all these mutations using a single assay.

Methylation assays

- Individual methylation results for all CpG sites provides high resolution results
- Mean methylation for all CpG sites shown for quick overview of results
- Quality score of individual sites is provided by sequence context
- Built-in control for bisulfite treatment improves accuracy and reproducibility of the results
- Possibility to present deviation from predefined methylation ranges
- Assay for Line-1, *p16, MGMT* and *MLH1* now available



Pyrogram trace from LINE-1 analysis. The LINE-1 assay amplifies a region in the LINE-1 retrotransposable elements and serves as a marker for global methylation. Light-blue boxes indicate CpG positions, yellow box indicates position of bisulfite conversion control.



Results from MLH1 methylation analysis. Upper Pyrogram trace shows a non-methylated sample, lower Pyrogram trace shows an almost 50% methylated sample. Light-blue boxes indicate CpG positions, yellow boxes indicate position of bisulfite conversion control.

Click here for online -catalogue

> We have had very good experiences with PyroMark systems in an earlier clinical research project. We were therefore interested in increasing the level of collaboration towards clinical diagnostics

The Molecular Pathology unit in Uppsala evaluates PyroMark Q24 prior to launch

The Molecular Pathology unit at the Institute for Pathology, Uppsala University Hospital has been closely involved in the evaluation of PyroMark Q24 prior to its launch in April. Lead by Dr Patrick Micke, they have been using Pyrosequencing technology for nearly a year now and are very satisfied with the results.

They originally used Sanger sequencing for their *KRAS* mutation analyses but found that the mutation detection limit of 25% was too restrictive for the diagnostic samples they had to analyze: samples that consisted of a mixture of cancer cells and normal cells, often with cancer cells as the minor component. The challenge was to find a more sensitive method, and one that could work on formalin-fixed paraffin-embedded tissue. Pyrosequencing technology is promising, even though the need for high analytical sensitivity is still an issue. Method improvements will hopefully solve this problem.

From left to right: Monica Lindell, Patrick Micke, Ingrid Thörn, Magnus Sundström

One example is the need by molecular pathologists for sensitive detection of *KRAS* mutations in tumor biopsies. Magnus Sundström, PhD, and Monica Lindell, PhD, at the Molecular Pathology unit in Uppsala evaluated PyroMark Q24 initially with the help of Biotage's product 'PyroMark KRAS' for clinical diagnostics on patient material. The mutation status of the *KRAS* gene has been shown to be very important in the treatment of a number of cancer forms. For example, new drugs aimed at the growth hormone receptor *EGFR* have been cleared for use in the treatment of colon cancer. The efficacy of these drugs is strongly related to the mutation status of the *KRAS* gene. Many studies have shown that patients who have a mutation in *KRAS* do not respond to treatment with *EGFR*-inhibitors. In contrast, patients who do not carry such mutations are more likely to respond to such treatment. PyroMark KRAS can be used to analyze contiguous multi-variable mutations in a single assay. It covers codons 12 and 13 in one assay, and codon 61 in another.

Dr Micke emphasized the clinical value of being able to implement robust and sensitive molecular analyses in cancer diagnostics. 'We have had very good experiences with PyroMark systems in an earlier clinical research project. We were therefore interested in increasing the level of collaboration towards clinical diagnostics. This has given us the possibility of providing the results of mutation analyses to active clinicians. During the coming months we will be carefully evaluating the system and will collaborate with other pathology centers to compare the system's performance with alternative analysis methods'.

The Department of Molecular Pathology provides molecular testing services for a large range of conditions. Analyses range from FISH analysis, to assays of mutations, loss-of-heterozygocity, microsatellite instability and minimal residual disease. Pyrosequencing technology has been part of their instrument base for some time. In addition, they have RT-PCR, an ABI 3130 Genetic Analyser, FISH imaging microscope, and a laser capture microdissection system (PALM). Biotage is confident that PyroMark Q24 will find a prominent position in their lab. The initial results are very promising.

More information on the work of the Molecular Pathology unit can be found here:

www.akademiska.se/upload/klinisk%20patologi/Molecular.pdf

> PyroMark Q24 provides unique and automated sequence-based assays for methylation profiling and detection of mutations in cancer cells in the clinical setting

Dr Bin Yang, a Pyroneer who helped us to test PyroMark Q24, stresses the advantages of a flexible system

Dr Bin Yang is a highly merited pathologist at the Cleveland Clinic, with expertise in gynecologic pathology and cytopathology. In recent years he has deepened his knowledge of molecular pathology, including epigenetics, and we welcomed his help in assessing the performance of PyroMark Q24 prior to launch. He now leads the Cancer Epigenetics Core Laboratory at Cleveland Clinic.

The laboratory focuses on translating and validating advanced molecular technologies into useful clinical tests, with a particular interest in molecular cytology. They use methylation-specific PCR (MSP), a real-time PCR based quantitative methylation assay, and a few other technologies.

Why did you choose Pyrosequencing technology and how does it compare with other technologies?

Pyrosequencing technology provides the possibility of running unique quantitative sequence-based methylation assays. We use it to run methylation analysis, SNP genotyping, mutation detection and short sequencing.

Do you see a future for Pyrosequencing technology in clinical diagnostics?

Simultaneously analyzing both methylation level and SNP genotyping is the most unique technical advantage of Pyrosequencing technology has over other technologies. This gives Pyrosequencing technology a great potential in clinical testing.

We have combined methylation and mutation analysis on PyroMark Q24 to analyze the most common genetic and epigenetic alterations in thyroid papillary carcinoma, using fine-needle aspirates. Using a multiplex PCR approach, we have tested the plausibility of analyzing four CpG islands simultaneously, including those of *P16*, *E-cadherin*, *RASSF1a* and *MGMT*, along with mutation assays for *BRAF* and *RAS* genes. Our preliminary studies demonstrate that simultaneous analysis of both genetic and epigenetic changes significantly increased the sensitivity of detecting thyroid papillary carcinoma.

We think that PyroMark Q24 provides unique and automated sequence-based assays for methylation profiling and detection of mutations in cancer cells in the clinical setting.

What would you like to do with Pyrosequencing technology in the future?

Developing early detection molecular panels targeting to specific clinical entities.

How can we as a company help you in the future?

Assay design and troubleshooting are the most challenging aspects of Pyrosequencing analysis due to the unique design of the technology. Biotage should try to make all the software even easier to master if they are to be applied in a clinical testing. Prompt and thorough technical support for users will be essential.

Is there any particular improvement to our product line that you would like to see?

Firstly, add more automated software for simultaneous duplex and triplex assays for SNP genotyping. Secondly, the 24-well plate seems a little limited for testing samples. A plate with 36 to 48 wells would be more suitable to a clinical setting.

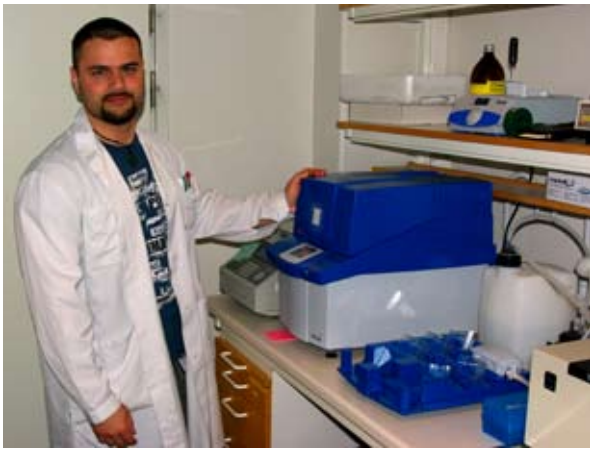
More information on Dr Yang's work can be found at the following links:

http://cms.clevelandclinic.org/giving/body.cfm?id=248

http://www.clevelandclinic.org/pathology/uploads/PathologyResearch-Spring2006.pdf

> PyroMark Q24 has already catalyzed several promising collaborations with other groups

Nimrod Kiss, an experienced user of Pyrosequencing technology reports on beta-site testing of PyroMark Q24



PyroMark Q24 pilot number 1 has been in use in the Endocrine Tumors Group at the world-renowned Karolinska Institute since January of this year. During this time several assays have been designed and used to determine tumor suppressor gene methylation. PyroMark Q24 has also been used in collaboration with another group to assess promoter methylation levels for genes other than tumor suppressors.

Please describe your group and the key activities in your lab

I work at the Karolinska Institute, Center for Molecular Medicine and Surgery (CMM), in the Endocrine Tumors group under Professor Catharina Larsson. The group studies different aspects of endocrine tumor genetics. I investigate tumors of the adrenal gland, with focus on mapping epigenetic changes that predispose cancer formation. Other members of the group work with thyroid and parathyroid tumors, cervical cancer, melanoma and lymphoma.

What technologies do you use in your group other than Pyrosequencing technology?

In the past I have worked with different PCR-based methods for detecting methylation, including COBRA (combined bisulfite restriction analysis) and MSP (methylation specific PCR). Other techniques frequently employed in our lab include Western Blot, immunohistochemistry, 2D-gels, aCGH (array comparative genome hybridization), FISH (fluorescent in-situ hybridization), RNA/miRNA microarrays, to name just a few. My colleagues have different backgrounds, so our group benefits from having members with broad areas of expertise.

Why did you choose Pyrosequencing technology and how does it compare with other technologies?

It was a lucky shot that we came into contact with Pyrosequencing technology and Biotage. My colleague and co-supervisor attended a big cancer meeting where he met representatives from Biotage who introduced him to Pyrosequencing technology. At that time we were working together on a project where we used MSP in order to detect methylation changes in the promoters of the *p16INK4A* and *p14ARF* tumor suppressor genes, so we were very excited about trying the new technique on our samples. Since then we have come to appreciate the precision and reliability of Pyrosequencing technology, and gradually we have all but eliminated the more arbitrary techniques we employed in the past. The big advantage of Pyrosequencing analysis over, say, MSP or COBRA is that it is quantitative, not just qualitative. It is also both sensitive and specific, not to mention that it is one of the new “hot” techniques.

Which assays do you run using Pyrosequencing technology?

We use both our own in-house designs and assays designed by Biotage.

Are there any assays that became possible or much easier due to Pyrosequencing technology?

Pyrosequencing technology allows the researcher to study a much larger number of CpG sites than is possible using MSP. Also, the technique makes methylation studies of individual CpG sites possible. The quantitative nature of Pyrosequencing analysis also allows the researcher to study the effects of "methylation dose". However, one should be careful to draw too daring assumptions from such studies, as tissue heterogeneity may be an obscuring factor.

It would, of course, be very interesting to hear how PyroMark Q24 has performed for you in these tests, and how it compares with the performance of our other systems.

PyroMark Q24 has performed well in our lab. Overall, the PyroMark Q24 instrument and accompanying software are easy to use by anyone with moderate laboratory and computer skills. Runs are easily set up, and analysis of the results is straightforward. As our experience increases, so does the quality of the assays. After a period of getting used to the new machine and optimization of the new assays we have reached a point where we feel that PyroMark Q24 is working flawlessly. During the time we have had PyroMark Q24 a lot of people have been asking about it. It is a new and exciting addition to the array of techniques available to us researchers. PyroMark Q24 has already catalyzed several promising collaborations with other groups.

Do you see a future for Pyrosequencing technology in clinical diagnostics? If so, how could the system be used, and what would be needed to succeed in this area?

Definitely. One of the projects that I have been involved in showed clear correlations between distinct methylation patterns and certain tumor features. In order for this to work as a diagnostic tool one would need to develop robust assays for the genes in question, which should be supplied by Biotage as ready-made master mixes (just add DNA), so that they can be used by lab technicians without the need for all the hassle associated with setting up PCRs.

Is your Pyrosequencing System used by other labs in your facility? What do they do?

Yes, but I'm not very familiar with their research. There is also another Pyrosequencing instrument at CMM, which is used for SNP analysis.

Is there any particular improvement to our product line that you would like to see, if so, what e.g. software, instrument changes, reagents, kits?

I would like to have animated tutorials for the software.

For more information on the work of the Endocrine Tumors group at the Center for Molecular Medicine and Surgery visit the following website: **http://ki.se/ki/jsp/polopoly.jsp?d=5239&a=131061=en**

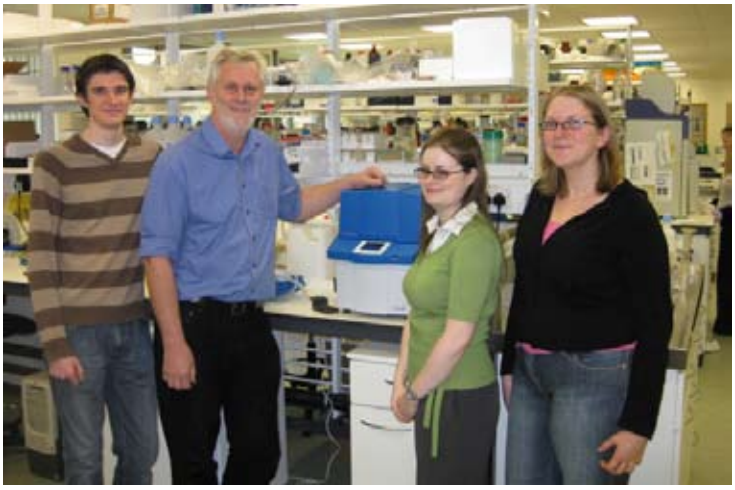
> PyroMark Q24 allows research-based mutation assays to be migrated into clinical diagnostics since the instrument and reagents are IVD quality

An interview with Professor Ken Mills, our first customer for PyroMark Q24

The 17th of April was a memorable day for Pyrosequencing technology. This was the day the first PyroMark Q24 instrument was installed in a customer's laboratory: that of Professor Ken Mills at The Center for Cancer Research and Cellular Biology (CCRCB) at Queen's University, Belfast, Northern Ireland.

CCRCB has scientific teams from a broad range of disciplines including Biomedical Science, Clinical Medicine, Chemistry, Immunology, Virology, Mathematics, Engineering and Radiobiology. Clinical and laboratory experts collaborate to increase the quality and scope of basic research and clinical investigations on cancer and other diseases.

The principal investigators in the Cancer Cell & Molecular Biology division conduct a variety of activities designed to promote basic and clinical research in cancer and other serious diseases. Strong emphasis is placed on the elucidation of the mechanisms of disease and translational research.



Professor Ken Mills is particularly interested in translational research in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). These studies are aimed at improving diagnosis, prognosis and prediction of therapeutic responses.

What technologies do you use in your group other than Pyrosequencing technology?

The myeloid leukemia group in the haematology section of the CCRCB uses a wide range of techniques including gene expression profiling, quantitative RT-PCR and PCR, CpG arrays, tissue culture and in vitro drug response.

Why did you choose Pyrosequencing technology and how does it compare with other technologies?

Pyrosequencing technology offers several advantages over conventional technologies for both mutation detection and methylation status analysis. The quantitative aspects of the technology are very important particularly in determining methylation changes.

Which assays do you run using Pyrosequencing technology?

We run assays for several mutations that are common in myeloid leukemia including JAK2 mutations which are relatively simple as they involve a point mutation. We have also used the technology to detect several different mutations that occur within a 9 base-pair region of the tyrosine kinase domain of the *FLT3* gene and also to detect the 4 or 8 base-pair insertion mutations in the *NPM1* gene. In addition, we are using Pyrosequencing technology to determine the methylation status at different CpG sites across a number of genes in leukemia, head and neck tumors, and in prostate cancer.

Are there any assays that became possible or much easier due to Pyrosequencing technology?

The advantage of Pyrosequencing technology is that it enables rapid determination of the type of mutation with an indication of the level of mutation which may be as low as 5% or as high as 100%. The detection of *FLT3* or *NPM1* mutations, of which there are between 5-20 variations, has been streamlined by the technology.

What would you like to do with Pyrosequencing technology in the future?

The technology has already been stretched in our lab by trying to detect insertions and deletions – this means adapting the SNP or point mutation assays. It would be interesting to try to combine mutation analysis with methylation analysis in some genes to see their direct relationships. In addition, the detection of low levels of mutations would be valuable – maybe by the use of allele specific primers.

What is the value of PyroMark Q24 to your lab?

The mutation assays have been developed by using research samples from clinical trials, but these assays now need to be moved into a clinical diagnostic or monitoring situation. PyroMark Q24 allows these to be migrated into that environment since the instrument and reagents are IVD quality. On a research front, the ability to mix SNP and methylation assays on the same smaller plate reduces reagent consumption and time spent as compared to the larger Pyrosequencing machines.

What would be needed for Pyrosequencing technology to succeed in clinical diagnostics?

Pyrosequencing systems can certainly have a place in the diagnostic laboratory. The chances of success have been increased by PyroMark Q24, but the availability of relevant and validated assays for the increasing range of mutations and assays will need to be increased so that the clinical scientists do not need to design, optimize and validate the kits themselves. Also mutations are not just single point mutations and the analysis software needs to be able to analyze these too.

Are your Pyrosequencing Systems used by other labs in your facility?

The Pyrosequencing systems are used by research groups in Belfast on colon cancer, head and neck tumors and also by colleagues from Dublin. They will also be used for collaborative studies involving Nottingham and Dublin.

How can we as a company help you in the future?

By continuing to be responsive and available for technology support and also to discuss with customers the availability and development of assays. The database of published primers for mutations and methylation assays needs to be more interactive and up-to-date as it is usually the first place I look for assays before designing them in-house.

Is there any particular improvement to our product line that you would like to see?

The software for PyroMark Q24 needs be updated to include insertions and deletions. And as I said before, the number of relevant and validated assays for the increasing range of mutations and assays will need to be increased.

For more information on Professor Mills and his group's work please visit the website for the Centre for Cancer Research and Cell Biology: **www.qub.ac.uk/ccrcb**

Rapid and accurate typing of the protozoan parasite *Giardia lamblia* by Pyrosequencing® analysis

Pyrosequencing® analysis using PyroMark™Q24 is a rapid and accurate way to obtain both qualitative (sequence) and quantitative (ratio of mixed sequences) information regarding microbiological infections.

- *Giardia lamblia* is one of the most common causes of waterborne outbreaks of diarrhea
- Rapid and accurate detection and genotyping is very important for the prevention and control of outbreaks
- Single clinical or environmental samples often contain several types of *Giardia lamblia,* highlighting the need for high precision typing

Figure 1. The parasite *Giardia lamblia*

> The specificity and sensitivity of Pyrosequencing technology using PyroMark Q24 makes it a suitable tool to detect and genotype protozoa from clinical and environmental samples.

Dr. Staffan Svärd
Professor of Eukaryotic Microbiology
Department of Cell and Molecular Biology Uppsala University, Sweden

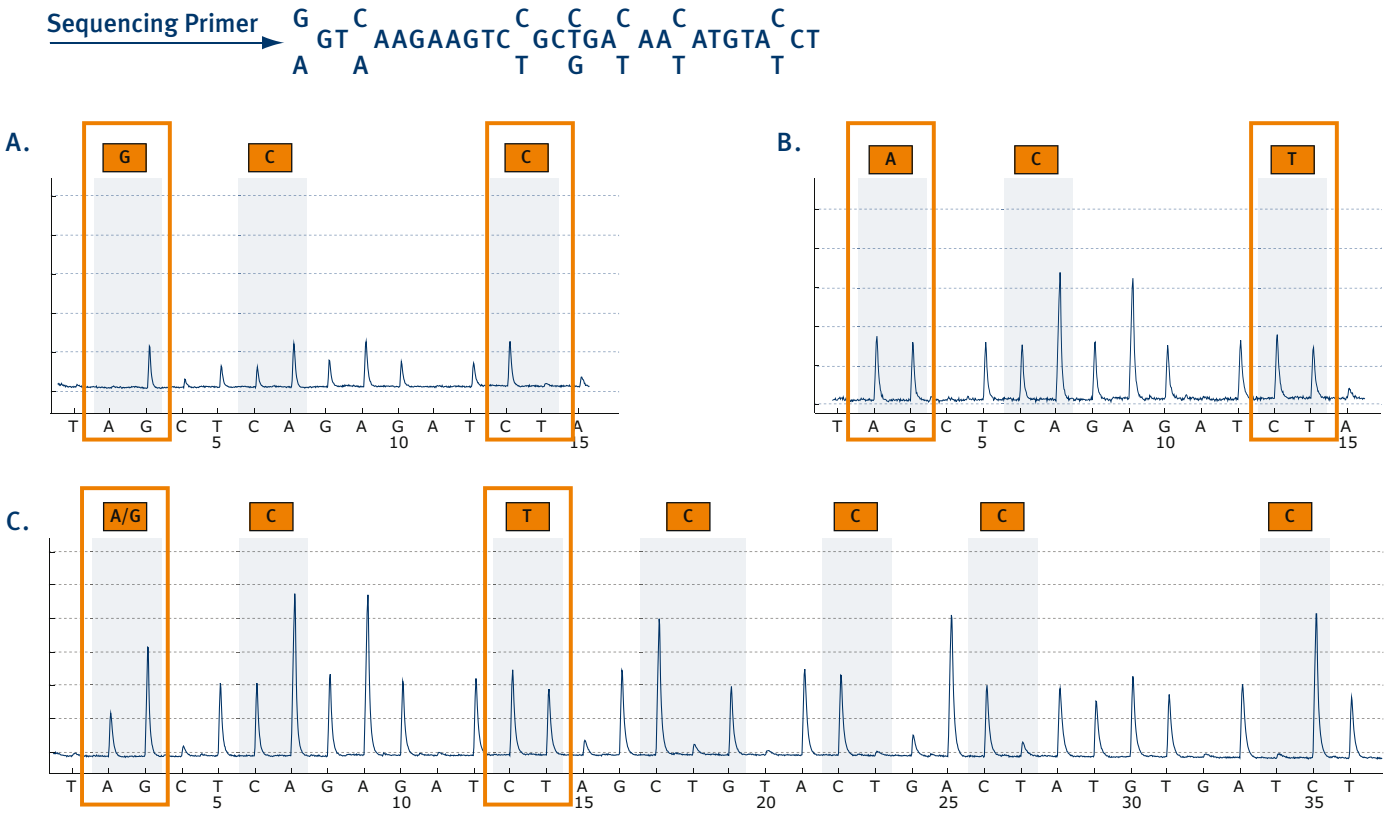


Figure 2: The sequence analysis of Giardia lamblia
The resulting Pyrogram® traces of the investigated sequence segment are shown above. There are seven sites in the segment that vary in the different types of the parasite. Only the first three polymorphisms are shown in a) and b).

Assay Results

DNA was extracted from human fecal samples using the QIAGEN DNA Tissue Kit. The amplification primers covered a region of the *Giardia*-specific virulence factor beta-giardin (Fig. 2) and the resulting amplicon sequence was analyzed for several known polymorphisms using PyroMark Q24.

Three different samples are shown in Figure 2. In one sample the *Gardia lamblia* sequence contained a guanosine (G) at the first position and a cytosine (C) at the third polymorphic position, strongly indicating a Type A infection (Fig 2a). Another sample showed a variation consistent with a Type B *Giardia lamblia* infection with an adenosine (A) at the first position and a thymine (T) in the third polymorphic site (Fig 2 b).

Interestingly, the analysis result shown in Fig 2c indicated a mixed infection of two different strains. This was observed as a 50% mixture of adenosine (A) or guanine (G) in position one of the analyzed sequence. Furthermore, the sample showed a thymine (T) in the third polymorphic site indicating that the patient is co-infected with two different B-type strains of *Giardia lamblia*.

An Interview with Professor Alain Morel, Angers University and INSERM

> Pyrosequencing technology is the corner stone of our clinical pharmacogenetic effort

From left to right: Pr Erick Gamelin, Dr Michele Boisdron-Celle and Pr Alain Morel

Please describe your group and the key activities in your lab

Our group is led by Professor Erick Gamelin and is part of Angers University and INSERM, located in the Anticancer Center Paul Papin in Angers, France. We have been involved for several years in complementary approaches aimed at optimizing treatment of colorectal cancers. We characterize mainly the germinal genetic and epigenetic factors leading to a wide variability of drug outcomes that result in either toxicity or, on the contrary, tumor progression. We have been working on the variability of cytotoxic drug metabolism and tumor resistance between individuals.

The main drugs used in the treatment of colorectal cancers are fluoropyrimidines, oxaliplatin and irinotecan and more recently targeted therapies such as cetuximab, which is targeted at the EGF receptor. We have characterized the individual pharmacogenetic factors of drug-induced toxicity and tumor resistance, through the study of individual polymorphisms of metabolic enzymes. These include dihydropyrimidine dehydrogenase (DPD) methyl tetrahydrofolate reductase, glutathione S transferase, uridine di-phosphoglycerate (UGT1A1), or targets such as thymidylate synthetase, topoisomerase 1, and EGFR.

We now provide clinicians the opportunity to use individualized and optimal doses of cytotoxic drugs in combination with new targeted therapies. Our goal is the clinical application of our findings i.e. to treat patients for colorectal cancers at an individual level. This will involve optimizing drug efficacy and tolerance, with the goal of avoiding severe toxic side-effects. Pyrosequencing technology is the corner stone of our clinical pharmacogenetic effort.

What technologies do you use in your lab other than Pyrosequencing technology?

We use the Maxwell automation system from Promega for nucleic acid extraction and an Eppendorf thermocycler for DNA amplification. Amplification products are checked with the help of an eGene capillary electrophoresis apparatus. In addition to SNP analysis on Pyrosequencing technology, we use TaqMan technology and an automated DNA sequencer, both from Applied Biosystems.

Why did you choose Pyrosequencing technology and how does it compare with other technologies?

We chose Pyrosequencing technology because, in addition classical SNP identification, we needed to characterize some repetitive sequences, deletions and insertions. Pyrosequencing technology fulfills our needs for clinical use as it is fast and robust, and offers high throughput. More importantly, the results are as reliable as Sanger sequencing technology, the gold standard in the characterization of DNA polymorphisms. In addition, Pyrosequencing analysis also costs less and is faster.

Which assays do you run using Pyrosequencing technology?

In our laboratory we have set up numerous assays: we can characterize most of the *DPD* polymorphisms as well as *UGT1A1* and *1A7* and *MTHFR,TS, KRAS, EGFR1,* DNA repair genes, some methylation modifications and so on.

Are there any assays that became possible or much easier due to Pyrosequencing technology?

Most of the assays are easier and this is not limited to SNP characterization. Moreover, there is no need to quantify precisely the amount of input DNA in the assays. For certain tough characterizations, we use LNA oligonucleotides to overcome difficulties in detecting variants.

What would you like to do with Pyrosequencing technology in the future?

We want to use it in medium throughput sequencing projects following dHPLC analysis.

Do you see a future for Pyrosequencing technology in clinical diagnostics?

We have been using Pyrosequencing technology in our laboratory as a standard for diagnostic analysis for six years and we have been using it to control the results obtained by TaqMan technology.

Is your Pyrosequencing System used by other labs in your facility? What do they do?

Yes, our Pyrosequencing system is available to other laboratories, either for clinical applications or for any other projects, such as genotyping in the study of plant diversity.

How can we, as a company, help you in the future?

We would appreciate some development and/or support in quantitative sequence determination as, in some cases, it is difficult to clearly read quantitative results.

Is there any particular improvement to our product line that you would like to see?

Improvement of multiplex assays in a single run could be very helpful, as well as a fully automated sample preparation solution, even if the prep tool is very easy to use.

More information on the work of Professor Morel can be found here:

Web site address www.centrepaulpapin.fr/

or see publications on next page →

Publications:

Gamelin L, Capitain O, Morel A, Dumont A, Traore S, Anne le B, Gilles S, Boisdron-Celle M, Gamelin E.
Predictive factors of oxaliplatin neurotoxicity: the involvement of the oxalate outcome pathway.
Clin Cancer Res. 2007 Nov 1;13(21):6359-68.

Capitain O, Boisdron-Celle M, Poirier AL, Abadie-Lacourtoisie S, Morel A, Gamelin E.
The influence of fluorouracil outcome parameters on tolerance and efficacy in patients with advanced colorectal cancer.Pharmacogenomics J. 2007 Aug 14

Morel A, Boisdron-Celle M, Fey L, Soulie P, Craipeau MC, Traore S, Gamelin E.
Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance.
Mol Cancer Ther. 2006 Nov;5(11):2895-904.

Boisdron-Celle M, Remaud G, Traore S, Poirier AL, Gamelin L, Morel A, Gamelin E.
5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency.
Cancer Lett. 2007 May 8;249(2):271-82.

Morel A, Boisdron-Celle M, Fey L, Lainé-Cessac P, Gamelin E.
Identification of a novel mutation in the dihydropyrimidine dehydrogenase gene in a patient with a lethal outcome following 5-fluorouracil administration and the determination of its frequency in a population of 500 patients with colorectal carcinoma.
Clin Biochem. 2007 Jan;40(1-2):11-7.

Rouits E, Boisdron-Celle M, Dumont A, Guérin O, Morel A, Gamelin E.
Relevance of different UGT1A1 polymorphisms in irinotecan-induced toxicity: a molecular and clinical study of 75 patients.
Clin Cancer Res. 2004 Aug 1;10(15):5151-9.

Previous interviews with Pyroneers

December 2007

An interview with Jörg Tost, a Pyroneer in DNA methylation analysis

June 2007

Dr. Mark Bouzyk, USA, translating assays into clinical tests.

Professor Maria Zambon, UK, investigating respiratory viral infections.

Dr. Suman Lee, Korea, on stem-cell epigenetics.

Dr. Richie Soong, Singapore, speeding up translational research.

Dr. Cath Arnold, UK, with Julie Ormond, educating future Pyroneers.

December 2006

Dr. Véronique Quillien, France, working on an aggressive type of brain tumor

Dr. Richard Shaw, UK, analyzing epigenetic markers in head and neck cancer

Dr. Somvong Tragoonrung, Thailand, who develops genotyping assays for agricultural applications

Dr. Michael Burczynski and Jennifer Isler, USA, develop genotyping biomarkers to improve the success rate of drug candidates

May 2006

Dr. Ulrich Lehmann, Germany, Quantitative Mapping of Methylation in Whole CpG Islands

Dr. Lakis Liloglou, UK, The Hunt for Methylation Markers for Early Prediction of Lung Cancer

December 2005

MDL's American Success Story: a Short History, Clear Vision and a Total Lack of Culture

Dr. Martin E. Adelson and Dr. Eli Mordechai, USA

May 2005

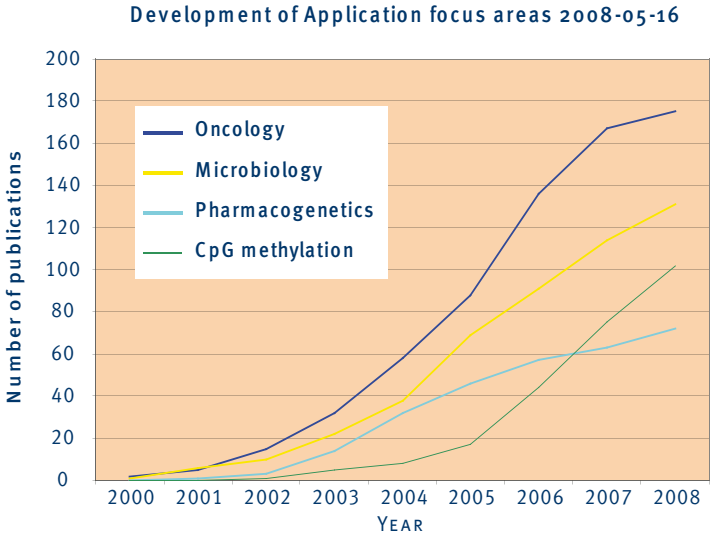
Dr. Jean Jordan, USA, tackling neonatal sepsis

Dr. Lena Klingspor, Sweden, identifying fungal infections

Download Highlights December 2007
Download Highlights June 2007
Download Highlights December 2006
Download Highlights May 2006
Download Highlights December 2005
Download Highlights May 2005

Pyrosequencing in the Press: now over 1000 publications

The figure shows development of literature based on Pyrosequencing technology for four key areas. All shown continuous growth. And we have something extra to celebrate: the users of Pyrosequencing technology have now managed to break the 1000 publications 'barrier' with a total of 1031 publications!!!



Here are brief reviews of a few publications in our focus areas:

CpG Methylation analysis

Geli, J., Kiss, N., Karimi, M., Lee, J.-J., Backdahl, M., Ekstrom, T. J. and Larsson, C. "Global and Regional CpG Methylation in Pheochromocytomas and Abdominal Paragangliomas: Association to Malignant Behavior." Clin. Cancer Res. 2008. 14, 2551-2559.

Pyrosequencing technology was used to study methylation of seven gene promoters and also global DNA methylation using both LINE-1 and LUMA (LUminometric Methylation Assay). A CpG Island Methylator Phenotype (CIMP) was identified in abdominal parangliomas and this phenotype was strongly associated with malignant behavior and young age at presentation.

Lee, E.-S., Issa, J.-P., Roberts, D. B., Williams, M. D., Weber, R. S., Kies, M. S. and El-Naggar, A. K. "Quantitative Promoter Hypermethylation Analysis of Cancer-Related Genes in Salivary Gland Carcinomas: Comparison with Methylation-Specific PCR Technique and Clinical Significance." Clin. Cancer Res. 2008. 14, 2664-2672.

DNA methylation in *RARß2, RASSF1A, MGMT* and *E-cadherin* was studied in salivary carcinoma cell lines as well as in human salivary gland carcinoma specimens using both the MSP method and Pyrosequencing technology. The conclusion was that Pyrosequencing technology offered a more sensitive method than the MSP method and that the methylation of RARß2 and RASSF1A was significantly associated with aggressive tumor phenotypes and patient survival.

Shaw, R. J., Hall, G. L., Lowe, D., Liloglou, T., Field, J. K., Sloan, P. and Risk, J. M. "The Role of Pyrosequencing in Head and Neck Cancer Epigenetics: Correlation of Quantitative Methylation Data With Gene Expression." Arch Otolaryngol Head Neck Surg 2008. 134, 251-256.

Promoter methylation was correlated with mRNA expression in samples from head and neck squamous cell carcinoma. Three genes, *MGMT, cyclin A1* and *RARB*, showed the expected negative correlation between levels of methylation and mRNA expression. *E-cadherin* showed no correlation between methylation levels and mRNA expression and surprisingly *p16* showed a positive correlation between methylation level and mRNA expression.

Shen, L., Toyota, M., Kondo, Y., Lin, E., Zhang, L., Guo, Y., Hernandez, N. S., Chen, X., Ahmed, S., Konishi, K., Hamilton, S. R. and Issa, J.-P. J. "Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer." PNAS 2007. 104, 18654-18659.

Genetic and epigenetic analyses, using among other methods Pyrosequencing technology for both mutation and methylation analyses, revealed that colon cancer comprises three molecularly distinct subclasses of disease. Genetic alterations (mutations of *BRAF, KRAS* and *p5s* and microsatellite instability (MSI)) and epigenetic alterations (DNA methylation of 27CpG island promoter regions) were analyzed in colorectal cancer patients. Three distinct groups of colon cancers were identified: CpG island methylator phenotype (CIMP) 1, CIMP2 and CIMP negative. The three groups had very distinct genetic profiles. CIMP1 was characterized by *MSI* and *KRAS* mutations, CIMP2 was associated with *KRAS* mutations, and the CIMP negative group showed a high rate of *p53* mutations.

Microbiology

Cristea-Fernstrom , M., Olofsson, M., Chryssanthou, E., Jonasson, J. and Petrini, B. "Pyrosequencing of a short hypervariable 16S rDNA fragment for the identification of nontuberculous mycobacteria -a comparison with conventional 16S rDNA sequencing and phenotyping." Apmis 2007. 115, 1252-9.

We at Biotage often get questions about the read-length of Pyrosequencing technology in comparison with conventional Sanger sequencing, especially in the context of species identification. This publication was therefore very welcome. The authors present data on sequencing a region of 16S rDNA that can be used to identify nontuberculous mycobacteria (NTM). The conclusion was that even if Pyrosequencing analysis generated no more than 60 bases of high-quality sequence, in contrast with the conventional "full gene" sequencing method, 86% of the 312 isolates were identified with identical results by both methods. In other words, in 86% of cases the 60 bases from Pyrosequencing analysis were representative of the full NTM 16S rRNA gene. Pyrosequencing analysis was sufficient to identify over 76% of the NTM isolates when complemented with two phenotypic tests. This compared to 87 % for conventional sequencing. Pyrosequencing data gave no erroneous identifications. Moreover, Pyrosequencing technology was considered to be more rapid and more easily performed than conventional sequencing.

Kramski, M., Meisel, H., Klempa, B., Kruger, D. H., Pauli, G. and Nitsche, A. "Detection and Typing of Human Pathogenic Hantaviruses by Real-Time Reverse Transcription-PCR and Pyrosequencing." Clin. Chem. 2007. 53, 1899-1905.

There is no doubting the power of real-time PCR as a method for detecting lower levels of DNA. And there is no doubting the power of Pyrosequencing technology in providing rapid sequence data for more stringent identification. This group combines the two methods to detect and identify Hantavirus. They used real-time RT-PCR assays to detect down to 10 copies of virus genome. All PCR products could then be used for Pyrosequencing analysis to generate at least 30 bases for further, detailed identification.

Heller, L. C., Jones, M. and Widen, R. H. "Comparison of DNA pyrosequencing with alternative methods for identification of mycobacteria." J. Clin. Microbiol. 2008. [Epub ahead of print]

Another paper that concerns mycobacteria identification. The authors have used Pyrosequencing technology to sequence the hypervariable A region of the 16s rDNA gene of mycobacteria and compared the results with those from other identification methods. Greater than 90% of isolates were correctly identified to complex or species by Pyrosequencing analysis with a single sequencing primer. Less than 5% of isolates were truly discordant between Pyrosequencing analysis and alternative methods.

A full list of all references to Pyrosequencing technology in the scientific literature can be found at:
WWW.BIOTAGEBIO.COM/PUBLIST

Work on rapid identification of pathogenic yeasts using Pyrosequencing assay wins award at mycology conference

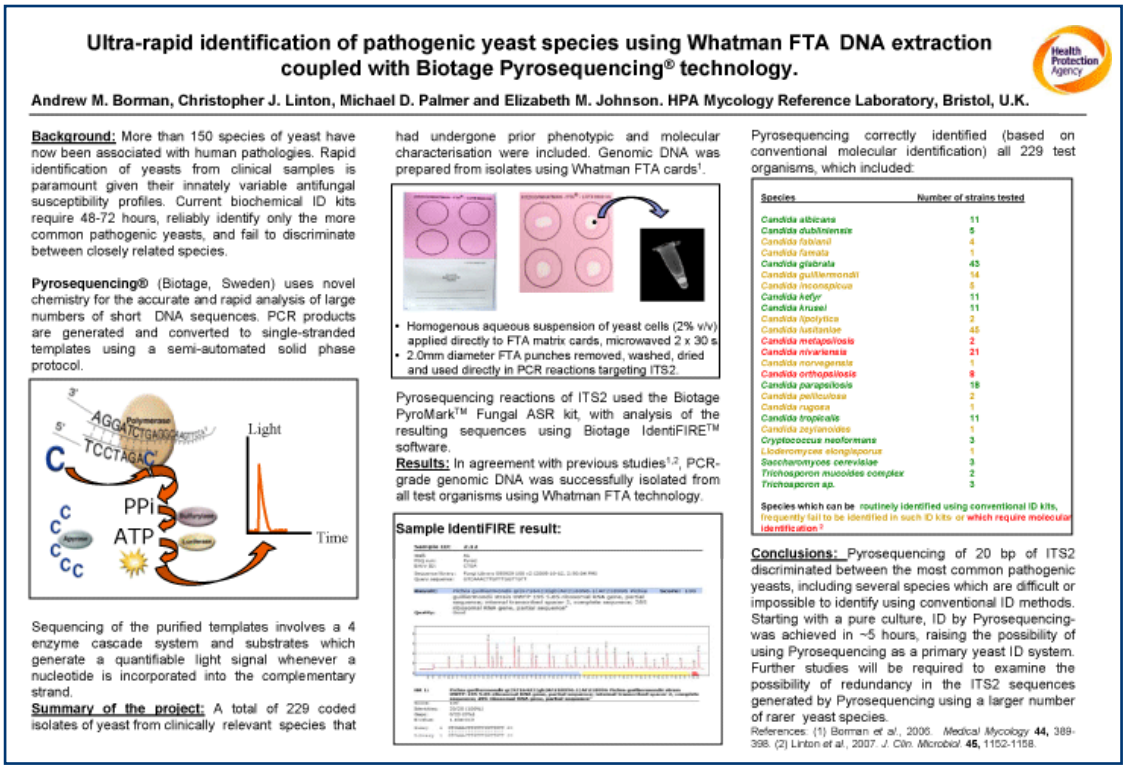
A group of Pyroneers at the Health Protection Agency Mycology Reference Laboratory in Bristol, England presented an award-winning abstract and poster at the meeting 'Focus on Fungal Infection 18' held in San Antonio, Texas, USA, March 6-8. The poster generated interest from delegates from both Europe and the US.

The work concerned identification of pathogenic yeast species using a novel combination of DNA extraction method and Pyrosequencing analysis. The assay was performed with PyroMark Fungi ASR, which the group had evaluated prior to launch.

The Thomas J. Walsh Mycology Award was established to encourage and recognize scientific research excellence. The award is presented to the best overall abstract presented at the meeting.

So congratulations go to Andrew Borman, Christopher Linton, Michael Palmer and Elizabeth Johnson!

The poster can be viewed on www.biotagebio.com/highlights >>



Ultra-rapid identification of pathogenic yeast species using Whatman FTA DNA extraction coupled with Biotage Pyrosequencing® technology.

Andrew M. Borman, Christopher J. Linton, Michael D. Palmer and Elizabeth M. Johnson. HPA Mycology Reference Laboratory, Bristol, U.K.

Background: More than 150 species of yeast have now been associated with human pathologies. Rapid identification of yeasts from clinical samples is paramount given their innately variable antifungal susceptibility profiles. Current biochemical ID kits require 48-72 hours, reliably identify only the more common pathogenic yeasts, and fail to discriminate between closely related species.

Pyrosequencing® (Biotage, Sweden) uses novel chemistry for the accurate and rapid analysis of large numbers of short DNA sequences. PCR products are generated and converted to single-stranded templates using a semi-automated solid phase protocol.

Sequencing of the purified templates involves a 4 enzyme cascade system and substrates which generate a quantifiable light signal whenever a nucleotide is incorporated into the complementary strand.

Summary of the project: A total of 229 coded isolates of yeast from clinically relevant species that had undergone prior phenotypic and molecular characterisation were included. Genomic DNA was prepared from isolates using Whatman FTA cards[1].

- Homogenous aqueous suspension of yeast cells (2% v/v) applied directly to FTA matrix cards, microwaved 2 x 30 s.
- 2.0mm diameter FTA punches removed, washed, dried and used directly in PCR reactions targeting ITS2.

Pyrosequencing reactions of ITS2 used the Biotage PyroMark™ Fungal ASR kit, with analysis of the resulting sequences using Biotage IdentiFIRE™ software.

Results: In agreement with previous studies[1,2], PCR-grade genomic DNA was successfully isolated from all test organisms using Whatman FTA technology.

Sample IdentiFIRE result:

Pyrosequencing correctly identified (based on conventional molecular identification) all 229 test organisms, which included:

Species	Number of strains tested
Candida albicans	11
Candida dubliniensis	5
Candida fabianii	4
Candida famata	1
Candida glabrata	43
Candida guilliermondii	14
Candida inconspicua	5
Candida kefyr	11
Candida krusei	11
Candida lipolytica	2
Candida lusitaniae	45
Candida metapsilosis	2
Candida nivariensis	21
Candida norvegensis	1
Candida orthopsilosis	9
Candida parapsilosis	18
Candida pelliculosa	2
Candida rugosa	1
Candida tropicalis	11
Candida zeylanoides	1
Cryptococcus neoformans	3
Lodderomyces elongisporus	1
Saccharomyces cerevisiae	3
Trichosporon mucoides complex	2
Trichosporon sp.	3

Species which can be routinely identified using conventional ID kits, frequently fail to be identified in such ID kits or which require molecular identification[3]

Conclusions: Pyrosequencing of 20 bp of ITS2 discriminated between the most common pathogenic yeasts, including several species which are difficult or impossible to identify using conventional ID methods. Starting with a pure culture, ID by Pyrosequencing was achieved in ~5 hours, raising the possibility of using Pyrosequencing as a primary yeast ID system. Further studies will be required to examine the possibility of redundancy in the ITS2 sequences generated by Pyrosequencing using a larger number of rarer yeast species.

References: (1) Borman *et al.*, 2006. *Medical Mycology* **44,** 389-398. (2) Linton et al., 2007. *J. Clin. Microbiol.* **45,** 1152-1158.

The value of Pyrosequencing technology in monitoring resistance development in Influenza A has been proven yet again – this time in Europe

An international group has reported the emergence of resistance to oseltamivir (Tamiflu) in Influenza A H1N1 viruses in Europe. The ongoing work includes the use of Pyrosequencing technology to detect resistance mutations.

Over 400 Influenza A (H1N1) isolates from the period November 2007-January 2008 were analyzed for sensitivity of neuraminidase to oseltamivir; the presence of mutations in the neuraminidase gene (NA) was confirmed using Pyrosequencing technology. At the date of publication resistant isolates had been detected in nine countries, and particularly high proportions of isolates from Norway, France, Germany and the UK carried the mutation (H274Y) known to confer high resistance to the drug. The researchers also concluded that the mutated virus can be readily transmitted between individuals.

One of the authors, Angie Lackenby states **'Our Pyrosequencing equipment has been in use constantly and will be for the foreseeable future. At the peak of the influenza season, we are getting 150 to 300 samples a week to test, which would be totally impossible without Pyrosequencing technology'**.

This work is now in press [1]. In the meantime more information can be found in the Eurosurveillance report detailed below [2].

Pyrosequencing technology has been used in the past to detect the massive development of resistance to adamantanes in H3N2 and H1N1 isolates from Asia and the US (see references 3-5, and also earlier numbers of Highlights).

[1] Lackenby A, Democratis J, Siqueira MM and Zambon MC. Rapid Quantitation of Neuraminidase Inhibitor Drug Resistance in Influenza Virus Quasispecies. Antiviral Therapy (in press).

[2] Lackenby A, Hungnes O, Dudman SG, Meijer A, Paget WJ, Hay AJ, Zambon MC. Emergence of resistance to oseltamivir among influenza A(H1N1) viruses in Europe. Euro Surveill. 2008;13(5):pii=8026. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8026

[3] Bright RA, Medina MJ, Xu X, Perez-Oronoz G, Wallis TR, Davis XM, Povinelli L, Cox NJ, Klimov AI. Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. Lancet. 2005 Oct 1;366(9492):1175-81. Epub 2005 Sep 22

[4] Bright RA, Shay DK, Shu B, Cox NJ, Klimov AI. Adamantane resistance among influenza A viruses isolated early during the 2005-2006 influenza season in the United States. JAMA. 2006 Feb 22;295(8):891-4. Epub 2006 Feb 2.

[5] Deyde V, Xu X, Bright R, ShawM, Smith C, Zhang Y, Shu Y, Gubareva L, Cox N, and Klimov A. Surveillance of Resistance to Adamantanes among Influenza A(H3N2) and A(H1N1) Viruses Isolated Worldwide. J Infect Dis 2007.196, 249-57.

Pyrosequencing® technology offers an established genetic analysis method based on the principle of sequencing by synthesis. It is the only genetic analysis technology capable of delivering explicit sequence information within minutes. Pyrosequencing analysis is an ideal choice for genetic analysis in clinical research. The output data from Pyrosequencing analysis is the gold standard of genetic information: real sequence data. This is the best possible assurance of a correct genetic test.

Pyrosequencing technology is brought to you by Biotage, a global company focused on life sciences.
More information at **www.biotagebio.com**

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United States: *PyroMark™Q24 System is designed for Laboratory Use Only, which means it may be used for either research purposes or by high complexity CLIA certified laboratories.*

Europe: *PyroMark™Q24 System is available for research and, in certain European countries*, for in vitro diagnostic applications. PyroMark™Q24 System meets the requirements of Annex III of the European Directive for In Vitro Diagnostic Medical Devices 98/79/EC.*

** For more information, see www.biotagebio.com.*

Pyrosequencing sequencing by synthesis systems enable DNA to be analyzed either bound to solid support or in solution. A review of DNA purification methods can be found in Fakhrai-Rad, et al. Hum Mutation (2002) and other papers listed on the Biotage web site.

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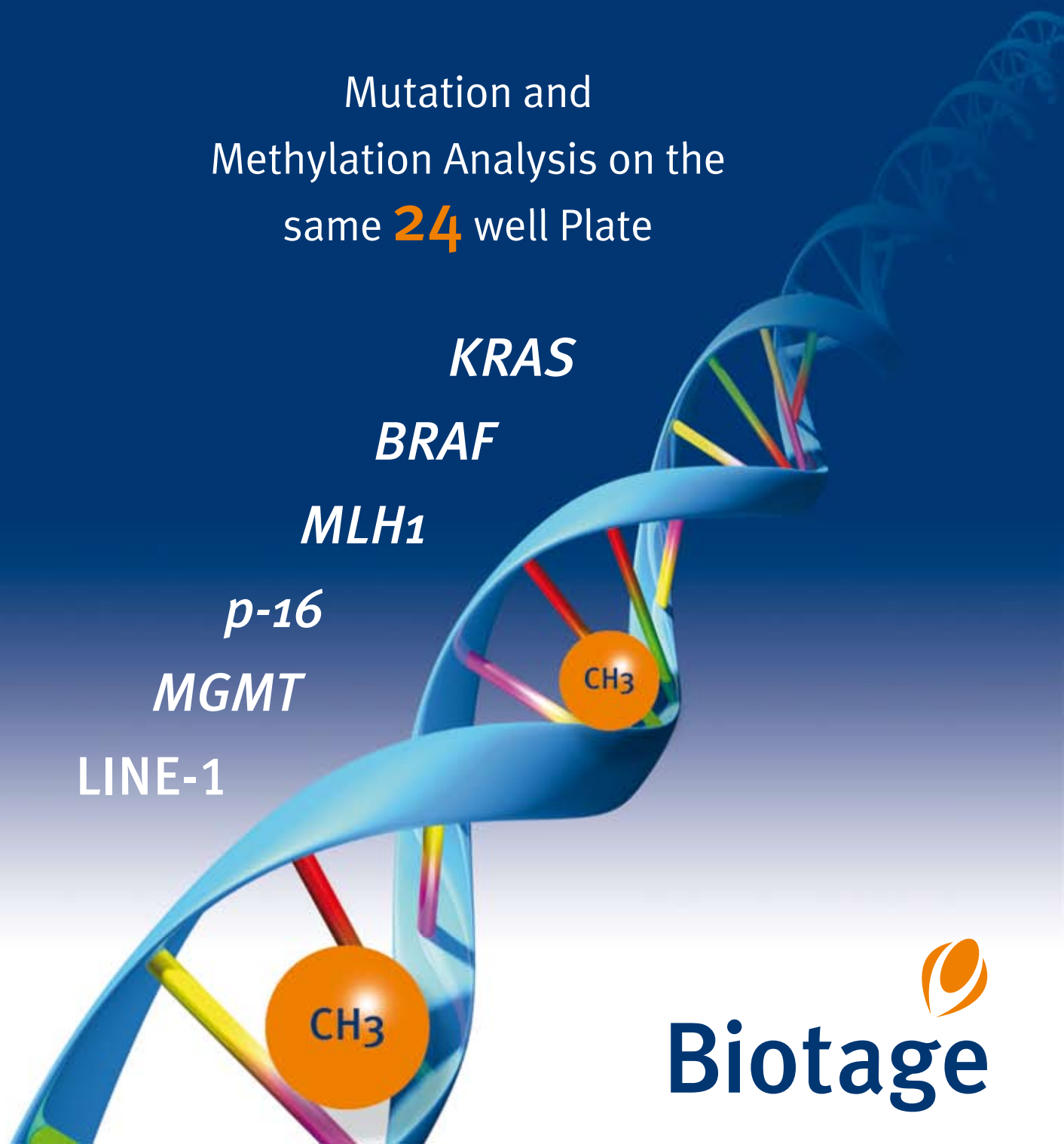
LINE-1



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