News

Barcoding update

Constance – GATC Biotech has developed a platform-independent barcoding system that allows an additional level of parallel processing with a virtually unlimited increase in the number of samples processed. GATC's system is suitable for use with the Roche GS FLX and Illumina Genome Analyzer. The barcoding method is especially well suited to DNA smaller than whole genomes (e.g. cDNA libraries, *de-novo* sequencing of BACs, fosmids, viruses) where sequencing would otherwise be impractical and expensive. The technique is highly efficient, the company says, and results in 99.9% of sequences successfully tagged, which can cut sequencing costs dramatically. The tags are nucleotides which are attached to the samples during the production of the shotgun libraries and read during the sequencing process. Following sequencing, samples are sorted according to their tags. The new system will also be applied to the recently installed ABI SOLiD System. The tags are very robust to sequencing errors caused by homopolymers.

Sequencing market

Dublin – The global DNA sequencing market is projected to grow at 4.63% compounded annually from 2005 to 2012, according to an analysis released by Research and Markets at the beginning of March. The US and Europe are forerunners in DNA Sequencing-based applications on automated and non-automated (Sanger's, Maxam-Gilbert and Pyrosequencing) DNA sequencing methods, and with regard to the 430 companies engaged in DNA sequencing services and instrumentation, the report says.

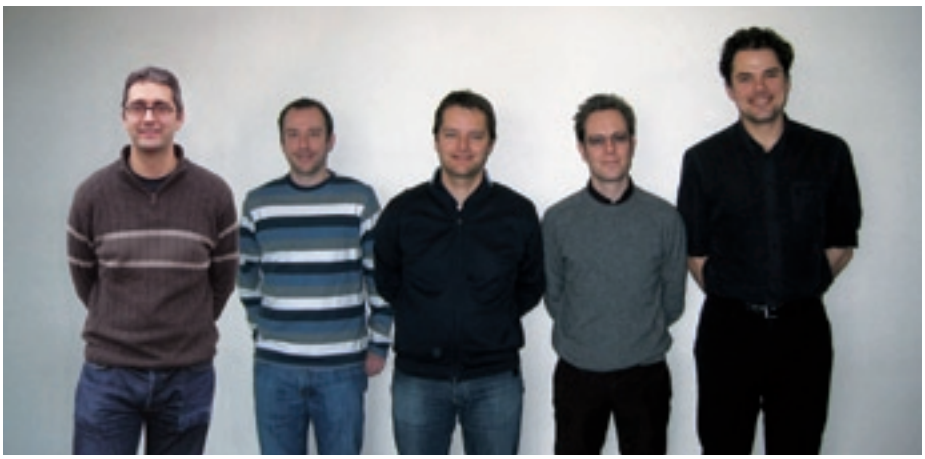
PCR

A new standard for qPCR data: RDML

Dr. Andreas Untergasser, Wageningen University; Steve Lefever, Dr. Filip Pattyn, Dr. Jan Hellemans und Prof. Dr. Jo Vandesompele, University Ghent

The RDML-consortium was founded to develop a universal data format for real-time PCR data, termed RDML (Real-time PCR Data Markup Language). The impulse for this initiative came from difficulties experienced when trying to share qPCR data between different laboratories and users, or when exchanging data between different software packages or analysis tools. The RDML-consortium designed RDML as a universal standard containing sufficient information to understand the experimental setup, re-analyse the data and interpret the results. Because of the XML nature of RDML, data are stored in a self documenting, open format which guarantees future accessibility and allows easy implementation in existing software. We are confident that RDML will promote the development of innovative third party software and new analysis methods.

Quantitative PCR is a widely used method for measuring levels of gene expression and determining gene copy numbers, and it is a crucial tool in medical diagnostics for quantifying viral or bacterial loads. Those interested in purchasing a qPCR instrument can currently choose between several manufacturers. A variety of different models are available – either with or without a gradient, with glass capillaries or with plastic wells. Some manufacturers offer up to several thousand wells. The diversity of PCR machines is also reflected in the software delivered along with the instruments. Each vendor has its own programmes and makes use of a propri-



The RDML key developer group (left to right): Dr. Andreas Untergasser, Steve Lefever, Prof. Dr. Jo Vandesompele, Dr. Jan Hellemans and Dr. Filip Pattyn.

etary format to store data. These data formats are largely incompatible with one another – limiting the scientists to a single software for analysing results. At the moment, scientists are also unable to exchange data if they do not use the same qPCR instruments. Finally, it makes little sense to publish unmodified raw data as supplementary data in papers if very few scientists can read it.

The RDML Consortium

RDML was designed to break these dependencies and to establish a truly open format. The initiative was founded by Jan Hellemans and Jo Vandesompele (both from Ghent University in Belgium, and CEOs at Biogazelle, a real-time PCR data analysis company, *www.biogazelle.com*). It was initially presented at the 2nd International qPCR Symposium (Hellemans et al., September 5-9, 2005, Freising, Germany) and further discussed at the 3rd International qPCR Symposium (Weihenstephan, Germany 2007). Also from Ghent University, Filip Patyn who is the developer and administrator of the Real-time PCR Primer and Probe Database (*http://medgen.ugent.be/rtprimerdb/*). Andreas Untergasser from Wageningen University in the Netherlands, who developed primer3plus and is involved in the further development of primer3, joined the project in 2007. The international RDML-consortium was founded in 2007 to develop and maintain the RDML data format. Interested individuals or organisations can join the consortium free of charge; it is organised into a key developer group, a member community and supporters (*www.rdml.org*). The role of the key developer group is to discuss comments and requests with the members, and collate and distill these into new RDML recommendations. The process of creating these recommendations passes through four subsequent levels: Working Draft, Candidate Recommendation, Proposed Recommendation, and finally RDML Recommendation.

Only the RDML Recommendation is binding. Until this version is released, all features within a proposed standard can change at every level. The RDML-consortium leaves it up to manufacturers to follow the RDML Recommendations. The RDML format allows easy exchange of raw annotated data between different laboratories. It is a flat text file in Extensible Markup Language (XML), termed RDML with a *.rdml or *.rdm file extension. The format is not dependent on any specific computer hardware, operating system or software package, and can be extended in the future to include additional information if required. Details of the standard will be published soon[1], with the final RDML Recommendation available in spring or summer 2008.

RDML will also make it possible to include qPCR data in scientific papers, allowing both reviewers and readers to reanalyze the data, similar to the MIAME guidelines proposed for microarray experiments[2].

The RDML-consortium is also interested in promoting software development. Steve Lefever (Ghent University) is currently working on a conversion tool that will allow users to write RDML files. A validator tool for checking whether the RDML files are in accordance with the standard is available as well. More tools will be developed and made available under open source license, including a library for reading/writing this file format and a programme for simple data analysis.

The RDML standard will extend the possibilities of qPCR and result in new analysis methods that are not publicly available at this time. If you would like to find out more or join the RDML-consortium, please visit *www.rdml.org*

References

[1] in preparation

[2] Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Ansorge W, Ball CA, Causton HC, Gaasterland T, Glenisson P, Holstege FC, Kim IF, Markowitz V, Matese JC, Parkinson H, Robinson A, Sarkans U, Schulze-Kremer S, Stewart J, Taylor R, Vilo J, Vingron M., Nat Genet. 2001 Dec;29(4):365-71.

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