

Microarray- and deep sequencing-based profiling approaches: the technological evolution continues...

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: Thursday, July 09, 2009

This one-day meeting aims at providing the audience with a comprehensive overview and in-depth comparison of currently available research tools, including array-, bead- or massive parallel sequencing-based platforms as well as experimental considerations in relation to expression-, genomic-, and epigenetic-profiling. Illustrated by real-life examples, various internationally acknowledged speakers will provide the attendee with critical experimental design parameters. Pitfalls associated with specific technologies as well as their solution will be discussed extensively.

This meeting has CPD approval

- 9:00 – 9:45 **Registration**
- 9:45 – 10:00 **Introduction by the Chair**
Professor Eric F.P.M. Schoenmakers, Radboud University Nijmegen Medical Centre (RUNMC)
& Nijmegen Centre for Molecular Life Sciences (NCMLS), Nijmegen, The Netherlands.
- 10:00 – 10:30 **Identification of novel biomarkers by high-resolution copy number profiling and homozygosity mapping in hematologic malignancies**
Dr Roland P. Kuiper, Microarray Facility Nijmegen, *Oncology Research, Radboud University Nijmegen Medical Centre (RUNMC)* & Nijmegen Centre for Molecular Life Sciences (NCMLS), Nijmegen, The Netherlands
Recent progress in genomics technology has made detailed characterization of the cancer genome feasible. One example involves the development of high-resolution SNP-based genotyping arrays for detecting regions of genomic amplifications, deletions, and copy-neutral homozygosity. Application of these arrays has revealed major new insights into the field of cancer genomics, particularly in hematologic malignancies, which has led to the discovery of several new biomarkers. In this presentation, examples will be presented for childhood acute lymphoblastic leukemia and myelodysplastic syndrome.
- 10:30 – 11:00 ***Deciphering the role of miRNAs in hypoxia by Digital Gene Expression profiling***
Dr. Ioannis Ragoussis, Wellcome Trust Centre for Human Genetics, University of Oxford, UK
Hypoxia in tumours may confer resistance to conventional therapies and is associated with a poorer prognosis. MicroRNA expression alterations have been described in cancer and certain microRNAs have shown regulation by hypoxia.
We performed a time course exposition to hypoxia (1% oxygen for 16h, 32h and 48h) using MCF7 cancer cell cultures. We also investigated the effect of VHL suppression in RCC4 renal cancer cells by comparing to RCC4 cells transfected with VHL. The microRNA fraction was isolated from total RNA and sequenced using the GA II analyser. Gene expression profiles were determined using Illumina WG-6 v3 arrays and ImicroRNA arrays v.1. This led to the identification of 376 different microRNAs and microRNA variants in MCF7 samples and 283 in RCC4 and RCC4+VHL cells. Relative microRNA expression analysis showed a set of 36 microRNAs dysregulated in all 3 hypoxia times compared to normoxia. A second set of 62 microRNAs appeared to be dysregulated from 32h of hypoxia onwards, suggesting a more severe reaction to hypoxia. Concerning RCC4 cells, 102 microRNAs showed differential levels of expression compared to RCC4+VHL cell. New microRNAs were identified using a novel machine learning algorithm and are being validated. MicroRNA target sequences were identified among genes differentially expressed in hypoxia and correlated with microRNA expression. All this information will enhance our understanding of hypoxia mediated regulation of gene expression.
- 11:00 - 11:15 **Speakers photo**
- 11:15 – 11:45 **Mid-morning break**

- 11:45 – 12:15 ***Methylome analysis using array and sequencing based approaches***
Professor Stephan Beck, Cancer Institute, University College London, UK
DNA methylation plays an essential role in biology with wide-ranging implications for human health and disease. To understand the rules governing DNA methylation and the consequences if DNA methylation is perturbed requires genome-wide analysis of its temporal and spatial plasticity. Almost 60 years after the discovery of 5-methyl cytosine and about 25 years since the discovery that altered DNA methylation plays a role in disease aetiology, particularly in cancer, technologies have finally become available for whole-genome DNA methylation profiling (methylome analysis) with ever increasing resolution. I will present data from our efforts using array- and sequencing-based platforms for high-throughput DNA methylation analysis, discuss some of the lessons learnt and give an outlook on how the data may be used in an integrated approach – termed ‘reverse phenotyping’ – to analyse and better understand the (epi)genomics of phenotypic plasticity in health and disease.
- 12:15 – 12:45 ***A holistic view on differential gene expression through deep sequencing***
Professor Henk Stunnenberg, Radboud University, The Netherlands
The regulation of gene expression is paramount in growth, development, differentiation, signaling, adaptation to the environment and many other processes. Gene expression is regulated at many levels, but primarily by binding of specific transcription factors to regulatory regions, resulting in the recruitment of activating or repressive factors and subsequent changes in mRNA levels and gene activity. Identification of the target gene and binding site networks of transcription factors is vital to understand its role. The presence or absence of a protein (or histone modification) at a specific genomic location is typically determined using chromatin immunoprecipitation (ChIP). The application of massively parallel sequencing to ChIP (ChIP-Seq) has opened up new avenues at the genome-wide scale to elucidate entire regulatory networks and pathways. So far mostly static views of transcription factor binding has been described, usually restricted to one cell line. The increasing sequence capacity enables for the first time the genome wide identification of transcription factor binding sites, histone marks, DNA methylation as well as RNA polymerase II occupancy and quantitative transcriptome sequencing (RNA-seq) at different time points, conditions and cell lines.
- 12:45 – 14:00 **Lunch**
- 14:00 – 14:30 **Troubleshooting Panel Discussion**
Delegates are welcome to submit questions to the expert panel either before this session (before the event or at the event) or during the session
- 14:30 – 15:00 ***A comparison of expression profiling by deep sequencing and microarrays***
Professor Johan den Dunnen, Center for Human and Clinical Genetics, Leiden University Medical Center (LUMC), Leiden, The Netherlands
We have done the first large-scale comparison between deep sequencing and microarray-based expression profiling. With the Illumina digital gene expression assay, we obtained ~2.4 million sequence tags per sample, their abundance spanning four orders of magnitude. Results were highly reproducible, even across laboratories. The correlation with five different microarray platforms was modest and most significant for Affymetrix. The changes in expression observed by deep sequencing were larger than observed by microarrays or quantitative PCR. While undetectable by microarrays, antisense transcription was found for 51% of all genes and alternative polyadenylation for 47%. Deep sequencing provides a major advance in robustness, comparability and richness of expression profiling data and is expected to boost collaborative, comparative, and integrative genomics studies.
- 15:00 – 15:30 ***Use of new sequencing technologies for the annotation of cancer genomes***
Dr. Peter J. Campbell, Sanger Institute, Cambridge, UK
We are now entering an era in which it will be feasible to catalogue every genetic event in a cancer. Next generation sequencing platforms already offer the capacity to generate gigabases (Gb) of sequence each week at a cost of less than 1 cent per kilobase (kb). Techniques have been developed which allow the detection of genomic rearrangements, copy number changes, point mutations and small insertions and deletions as well as epigenetic alterations on a single instrument. This will be a significant advance on existing approaches to cancer genomics. The analysis will be genuinely genome-wide, cataloguing genetic changes not only in coding sequence but also the other 98% of the human genome including, for example, promoters, enhancers and non-coding RNAs. At the Cancer Genome Project, we have developed protocols for mapping acquired rearrangements to the base-pair level, providing insights into the diversity of aberrant processes sculpting the genome which underlie the evolution and development of cancer.
- 16:00 – 16:30 **Chairman's summing up.**

This meeting was organised by Euroscicon (www.euroscicon.com), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry. The event was hosted by 'BioPark (www.biopark.co.uk), a research and development centre in Welwyn Garden City providing specialist facilities and support for bioscience and health technology businesses to grow, and to develop new products and technologies

About the chair

Professor Eric F.P.M. Schoenmakers obtained his PhD in Medical Sciences (Molecular Oncology) from the University of Leuven in Belgium (1997), where he studied the molecular basis of benign mesenchymal solid tumor development, and identified HMGA2 as the most frequently targeted oncogene in humans (Schoenmakers et al., Nature Genetics 1995). In 2000 he was appointed assistant professor and scientific board member at the department of Human Genetics at the University of Nijmegen, The Netherlands, where he is currently studying the genetic basis of cancer and (other) genetic diseases using high-throughput molecular cytogenetic approaches including array-based comparative genomic hybridisation (CGH). His main research-focus is on brain and urogenital cancers, including kidney cancer and gynecological neoplasms. In 2004 he was appointed Strategic Advisor to the Scientific Director. He is currently the chairman of the Dutch Cancer Society for Tumor Cell Biology, member of several international review boards, and has (co-) authored over 100 international peer-reviewed scientific publications. In addition, Eric is a full-blown "Tango Argentino" addict, and never travels without his "zapatos para bailar".

About the Speakers

Dr. Roland Kuiper studied Biology in Nijmegen where he graduated in 1994. He performed a PhD at the department of Animal Physiology in Nijmegen (prof. dr G. Martens) on selective intracellular prohormone transport in endocrine cells. In 2001, he joined the department of Human Genetics for a post-doc, where he focused on the characterization of renal cell carcinomas carrying t(6;11)(p21;q11) chromosomal translocations. Since 2005, he heads the tumor cell genetics group within the microarray facility at the department of Human Genetics, Nijmegen. His research focuses on genomic aberrations in hereditary kidney and colon cancers, head and neck cancers, childhood cancers and hematologic malignancies

Dr. Jiannis (Ioannis) Ragoussis is Head of Genomics at the Wellcome Trust Centre for Human Genetics and Oxford University Reader (Associated Professor) in Genomics. He studied Biochemistry at the University of Tuebingen, Germany where he also received his PhD in Biochemistry in 1988. He received a two year EMBO fellowship to perform post-doctoral studies at the ICRF Laboratories in London, UK, where he became an ICRF fellow. In 1992 he became Lecturer in Medical Genetics at Guy's hospital, where he set up the Genomic Research laboratory and became Reader in Genomic Research at King's College London. He joined Oxford University's Wellcome Trust Centre for Human Genetics in October 2001 and set up the microarray and genotyping facilities. Since then he has been working on Functional genomic methodology, including approaches for the quantitative analysis of protein-DNA interactions and the transcriptional response to hypoxia. He is also working on numerous genetics projects (autism, asthma, chlamydia infection) and is involved in the development of methods utilising genomic SNP arrays for copy number analysis. Recently he has set up the Centre's Next Generation Sequencing facility and is collaborating with Bioinformatics, Statistics and the Biomedical Research Centre on what is now becoming one of the UK's largest high-throughput sequencing facilities. He has been senior instructor in the Wellcome Trust Advanced Courses since 1992, teaching genomic and functional genomic technologies at the Sanger Institute to doctoral, post-doctoral and senior researchers. Dr. Ragoussis is the recipient of a South East of England Development Agency funded business training fellowship in order to promote interactions between the University and industry

Professor Stephan Beck is Professor of Medical Genomics at the University College London Cancer Institute. His laboratory has broad interests in the genomics and epigenomics of phenotypic plasticity in health and disease. He received his PhD in 1985 from the University of Konstanz where he studied DNA structure. After appointments at the MRC Laboratory of Molecular Biology in Cambridge, Millipore Corporation in Boston and the Imperial Cancer Research Fund in London, he joined the Sanger Institute in 1996. During his tenure as Head of Human Sequencing (1998-2006), he contributed to the sequencing and analysis of the human, mouse and zebrafish genomes.

The research of **Professor Stunnenberg** focuses on deciphering the genetic and epigenetic mechanisms of gene regulation during development, differentiation and in cancer performing His group is performing ChIP-seq of histone modifications in normal and cancer cells and wild type and KO lines. His group was amongst the first in Europe to establish ChIP in combination with next generation sequencing platform, and he acquired and is routinely running an Illumina GAI platform with paired-end module since 2007. The group participates in EU consortia focusing on ChIP-seq profiling of oncogenic translocation fusion proteins in AML and histone marks. His lab has established genome wide DNA methylation profiling at high resolution (MeDIP-seq and MethylCap-seq and is performing genome wide DNA methylation profiling in cancer. His lab is also involved in consortia aiming at establishing and implementing novel, highly sensitive protocols and novel cross-linking methods for ChIP and ChIP-seq on very small numbers of cells.

Professor Johan den Dunnen leads the research group Genome Technology and Genetic Disease, specialising in the development and implementation of new technologies in research and diagnosis. He heads the Leiden Genome Technology Center (LGTC), the genomics & transcriptomics facility of the LUMC, performing DNA sequencing (incl. next-generation), array-technology, SNP-typing and mutation detection.

His research focuses on genetic disease in general, muscular dystrophy and mental retardation in particular. His group is involved in the development and improvement of tools for clinical diagnostics, functional analysis of the genes involved using animal models and gene expression profiles and the development of strategies for DMD gene therapy ("exon skipping"). His group developed the open source LSDB-in-a-Box software (LOVD) and he initiated the Leiden Muscular Dystrophy pages (<http://www.DMD.nl>), incl. ~ 40 gene sequence variant databases (LSDBs).

Dr Peter Campbell's primary research interest is in cancer genomics. In the last two years, based at the Cancer Genome Project at the Sanger Institute, he has concentrated on the application of new sequencing technologies to the annotation of cancer genomes

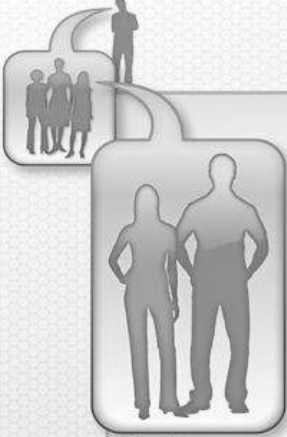
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