Meeting report

Chips to hits

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A report on the IBC USA International Microtechnology Conference 'Chips to hits', San Diego, USA, 27 October to 1 November 2001.

The 'Chips to hits' meeting gathered approximately 1,200 scientists and covered recent advances in the fields of gene expression, protein arrays and molecular diagnostics. There were also three pre-conference sessions on surface chemistry, bioinformatics and nano-biotechnology.

Surface chemistry

In order to develop protein arrays, specialized coatings are needed for slides; there is currently rapid growth in the coating chemistry field. Several investigators, including Malcolm Pluskal (Proteome Systems, Acton, USA) and Karin Hughes (ProlinX, Bothell, USA) talked about their ongoing coating chemistry projects. Pluskal gave an overview of the basic properties of surfaces and how they have been modified to immobilize cDNAs, oligonucleotides, proteins and antibodies. He described the coatings used on metal surfaces, especially on noble metals such as gold, including long-chain alkyl thioester monomers and self-assembling monolayers. Glass surfaces can be treated with thin layers of silane reagents (aminopropyl-, mercaptopropyl-, methylpropyl-, and epoxy-silane), avidin and streptavidin, poly-L-lysine, glutaraldehyde, or polyacrylamide. These coating chemistries have been applied in the development of DNA chips, protein arrays and surface plasmon resonance sensors. Pluskal also discussed piezoelectric liquid dispensing technology, which has applications in peptide-mass fingerprinting, mapping sites of *N*-glycosylation, antigen discovery and immunodiagnostics. 'Chemical printing' by *in situ* tryptic digestion of membrane-bound proteins directly on the blot was highlighted as a very useful approach for quantitating proteins, because it needs only small amounts of reagents that can be delivered precisely, archiving of the results is possible and, most importantly, it can be automated for high-throughput quantitation purposes.

Hughes presented information on her company's Versalinx™ protein array technology platform, which uses glass slides coated with phenyl-(di)-boronic acid to bind reversibly to salicyl hydroxamic acid. This non-biological surface chemistry has several advantages, such as excellent surface stability, good retention of proteins, the capacity to bind all different proteins, and a uniform signal strength and spot morphology. This type of self-assembled, monolayer surface chemistry is highly compatible with types of mass spectrometry such as matrix absorption laser time-of-flight (MALDI) and surface-enhanced laser time-of-flight (SELDI) spectrometry.

Microarrays for genomics and diagnostics

Several speakers described the applications of microarrays for genomics and diagnostics. Elizabeth Winzeler (The Scripps Research Institute, San Diego, USA) used a system based on screening of individual yeast cells as a tool for drug discovery. Using this approach, she has identified inhibitors of specific cellular processes and targets and found that these are active not only in yeast but also on a broad range of pathogens. The biggest advantage of the cell-based screening method is the ability to screen thousands of targets simultaneously; the assays may not be very sensitive, however. Using Affymetrix yeast gene chips® and yeast grown in rich media, she has identified new, not-yet-annotated open reading frames and further validated their expression at the mRNA level by northern-blot analysis. Using different growth conditions and nocodazole treatment (which destabilizes microtubules) she has monitored phenotypes and systematically identified potential new open reading frames. In addition, she presented results on identification of DNA-damage response genes by treating yeast cells with ultra-violet light.

Currently, many researchers use low-throughput analyses such as restriction-fragment length polymorphism and

single strand conformational polymorphism for genotyping, but to analyze large numbers of SNPs from many samples, array-based and/or mass spectrometry techniques are needed. Charles Cantor (Sequenom, San Diego, USA) presented an approach to gene discovery that involves fully automated genotyping by mass spectrometry. Using this method he has identified apolipoprotein E as a marker for Alzheimer's disease, confirming previous work. He noted that using this method, 190,000 validated assays can be performed and 1.3 million single nucleotide polymorphisms (SNPs) can be tested per year. His group are also looking at various disease markers and biochemical risk factors in the general population. He detailed a strategy for detecting genes involved in cardiovascular disease by measuring adenine:guanine ratios in high-density lipoprotein and low-density lipoprotein genes in DNA pools from twins. In order to screen for genetic markers for use in the clinic and for population studies, they have collected and deposited DNA samples from about 10,000 healthy patients in their proprietary DNA bank.

Towia Libermann (Harvard Medical School, Boston, USA) demonstrated the power of the 'multiplexed molecular profiling technology' platform in the identification of targets for epithelial-specific transcription factors involved in epithelial cell differentiation and proliferation, using prostate cancer as a model. His group has discovered a new family of human Ets-related transcription factors that include ESE-1, ESE-2, ESE-3, and prostate down-regulated expressed factor (PDEF) and have characterized them and determined their mRNA localization using *in situ* hybridization. In an attempt to identify target genes for the Ets transcription factors, LNCaP prostrate cancer cell lines were stably transfected with PDEF. The overexpression of PDEF was confirmed by GeneChip® analysis and validated by real-time PCR. They analyzed the regulatory pathway after treating the cell line with interleukin-6, leading to the isolation of nuclear factor κB as a potential therapeutic target for prostate cancer. Libermann also presented data on genotyping of type 1 diabetes patients using the Nanogen® microelectronic chip technology platform; his group has found SNPs that were associated with Ets genes.

Emerging technologies

There were several presentations on cutting-edge technologies. M. Richard Shen (Illumina, San Diego, USA) presented data on high-throughput genotyping using arrays of oligonucleotides on microspheres (beads) attached to high-density optical fibers. Illumina™ manufactures these oligonucleotide-coated bead arrays by a self-assembly process that takes place in a random fashion on the end of a fiber-optic bundle. Approximately 2,000 different oligonucleotides can be attached to each bead, meaning that 2,000 assays can be performed per array. The bead-bound oligonucleotides hybridize to complementary sequences that are generated in genotyping assays, in which the ligation of two labeled oligonucleotides is determined by the allele present. This technology is cost-effective, robust, accurate, and flexible, but whether it will change the field of microarrays in the near future remains to be seen.

David Ward (Yale University Medical School, New Haven, USA) discussed the rolling-circle DNA amplification method and 'lollypop probe' applications for the detection of point mutations *in situ*. In this method, a circle of DNA, a short DNA primer (complimentary to a portion of the circle) plus a polymerase (together, a 'lollypop') generate a single-stranded concatameric DNA molecule that is composed of thousands of tandemly repeated copies of the circle. Unlike other amplification techniques, this method produces a single amplified product that remains linked to the DNA primer, and it is thus well suited to solid-phase formats, for example for generating localized signals at specific microarray locations. Interestingly, this method has been adopted for real-time quantitation purposes and could detect fewer than ten molecules. Using *in situ* hybridization and cytometry, together with rolling-circle amplification, Ward has determined the localization of antigens in the spirochete that causes Lyme disease (*Borrelia burgdorferi*).

In conclusion, the meeting provided an overview of the current status of gene and protein chips and their applications in diagnostics and genomics. Identification and validation of marker genes for particular diseases and determination of their clinical implications continue to be major bottlenecks in drug discovery.

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