New technologies in nucleic acid detection and screening

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The second annual IBC *'Nucleic Acid Detection and Screening Technologies'* conference (Tucson, Arizona; 23–25 April 2001) featured 16 scheduled presentations in the categories of detection enhancement, applications, and DNA and protein arrays. In addition, several posters were selected for oral presentation. Highlights of ten of the excellent presentations will be covered here, with an emphasis on emerging new technologies; a common theme at the meeting being miniaturization, speed and parallelism.

Technological advances

The plenary address, entitled 'New technologies for deciphering the genome', was delivered by Lloyd M. Smith (University of Wisconsin, Madison, WI, USA). Smith described his laboratory's interest in MS and DNA arrays, and also his involvement with Third Wave Technologies (Madison, WI, USA) and the company's Cleavase® technology. The difficulty in using single nucleotide polymorphisms (SNPs) as genetic markers for widespread screening were highlighted, because of the cost and complications involving amplification (i.e. PCR) and multiplexing. Because of the limited visible spectrum, in which only 5–6 distinct fluorophores can be resolved, MS presents an appealing alternative with potentially much greater multiplexing capacity because of the wide mass-range. SNP-typing with a Surface Invader™ assay (Third Wave Technologies) can potentially accommodate up to 10^6 SNPs in a single step and opens up the possibility of large-scale population SNP typing without PCR. Smith also described limitations with matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS), whereby the signal intensity decreases as a function of the nucleic-acid length. A new technique of charge-reduction electrospray MS (CREMS), however, allows significant improvement in signal uniformity across a broad mass-range. In particular, a new instrument design implements a piezo drop-on-demand sample delivery, whereby spectra are collected from single 100 pl drops and provide a 50–100-fold increase in signal intensity.

Probes and genotyping

Tim Tieman (Clinical Microsensors, Pasadena, CA, USA) contrasted DNA 'discovery' activities where thousands of assays are necessary, cost per datapoint is a key factor and comprehensive coverage is vital, with DNA 'diagnostic' activities where only tens of assays are run, panel cost is more important than datapoint cost and the market is heavily regulated, to illustrate the distinctions between these two businesses. The company's eSensor™ platform is a homogeneous electrochemical detection scheme applicable to any amplified nucleic-acid target, and gives a digital readout without interference from contaminants (e.g. buffers, proteins and reaction components). The current integrated system accommodates 48 chips, each with 36 distinct sensors. Two DNA probes are used for each target: an immobilized capture probe and a ferrocene-labeled signaling probe. The immobilized probe is connected to an electrode through phenyl acetylene 'wires' embedded in a self-assembled monolayer. An insulating monolayer protects the electrode from interferents. A genotyping example was shown that used two types of ferrocene labels, each with a different redox potential. Other examples included SNP detection, gene detection and quantitative genotyping of HIV, where <10% wildtype sequence was readily measured (conventional gel techniques only detect 30% differences in sequence).

The Multiplexed Molecular Profiling assay (MMP) was described by Matthew Rounseville (High-Throughput Genomics, Tucson, AZ, USA); this assay handles 16 RNA targets from 96 samples with material from as few as 1000 cells (no target amplification is needed). Each microtiter well contains a 4×4 array of immobilized capture probes. A key component of the nuclease protection assay is the combined lysis and hybridization buffer. The process involves lysis and solution-hybridization with DNA protection probes, S1 nuclease digestion, base hydrolysis and transfer to a functionalized MMP 96-well plate, followed by horseradish peroxidase chemiluminescence detection. Four weeks are needed to completely set up the assay. The conversion of RNA to DNA is stoichiometric and signal attenuation can be achieved to widen the dynamic range. Examples of MMP in drug development include monitoring drug effects, dose responses and compound screening. An ELISA example was shown to demonstrate possible proteomic applications of the MMP assay.

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Bead technologies and DNA detection

James Jacobson (Luminex, Austin, TX, USA) described 'analyte profiling' with the LabMap suspension array system, which uses derivatizable color-coded microspheres, a flow-analyzer and a high-speed digital signal processor, as well as the necessary hardware, software and reagents. Currently, 100 uniquely colored beads are available, and 1,000 are expected to be available soon. Each new color allows introduction of yet another level of multiplexing. Hybridization kinetics for the 5.6 μm diameter beads are similar to solution hybridization and, therefore, assay times are significantly reduced compared with conventional microarrays. Several examples were shown for SNP detection [e.g. Factor V and cystic fibrosis (CF)] and expression profiling. In the case of CF, only a small volume (a few μl) of patient sample yielded results, in <15 min, using a 5-plex assay that covers the majority of mutations that cause CF in North America. The suspension arrays allow simple assay reconfiguration and elaboration, in contrast to a conventional two-dimensional (2D) microarray.

Details of a universal electrocatalytic DNA detector based on ruthenium-mediated guanine oxidation were given (Natasha Popovich, Xanthon, Research Triangle Park, NC, UCA). This approach does not require labels or target amplification, and sample preparation is minimal. In fact, assays can be performed directly from cell lysates. The electrodes have been miniaturized to 200 μm, which still produces nA currents and allows conventional potentiostat designs to be used, thus simplifying the instrumentation. In a microplate format, each well contains up to seven electrodes bearing immobilized DNA capture probes (or analogue DNA capture probes with enhanced biophysical properties) on an engineered surface. The prototype instrument can read a complete 96-well plate (672 electrodes) in five minutes, and its associated software produces easy-to-understand results.

The randomly self-assembled BeadArray technology was explained by Kevin Gunderson (Illumina, San Diego, CA, USA) in which 5 μm diameter beads fit into the etched wells of a drawn optical-fiber bundle. The individual optical fibers become light pipes with a density of up to four million individual capillaries per cm^2, which far exceeds the density of conventional DNA microarrays. Total internal reflection within each light pipe minimizes crosstalk and ensures efficient signal transfer. A key challenge is decoding the random array after assembly with encoded beads. Beads can be encoded with dyes or with DNA (i.e. optically- or sequence-encoded). In the case of DNA encoded beads, sequential hybridization allows binary sorting. A prototype assay model consists of 2,000 bead types with a 50,000-core fiber bundle (1 mm in diameter), which allows for 20-fold redundancy. Examples of application of this technology included SNP genotyping with ZipCode arrays and Oligonucleotide Ligation Assays (OLA), splice variant profiling and expression profiling.

Applications

Paul Rochelle (Metropolitan Water District of Southern California, La Verne, CA, USA) discussed the water industry's requirement for rapid methods of pathogen and polymorphism detection and determination of viability, infectivity and virulence; these rapid methods do not yet exist. In Southern California, 0.5 billion gallons of water are treated within 2 h and transported to the furthest customer in <24 h. However, microbial analysis requires 28 h for bacteria, 3–5 days for protozoa and 14–48 days for viruses. Further challenges include extremely dilute samples confounded by numerous inhibitors to PCR. In the USA alone, 80 million cases of illness are attributed to contaminated food and/or water each year. Worldwide, a child dies every eight seconds because of microbial contaminants in water, and half of the world's diseases are transmitted by water. Clearly, the challenge exists to create rapid tools for pathogen detection in water supplies. Ideally, the industry needs a 'Star-Trek Tricorder', which gives an instantaneous and comprehensive analysis. Until these devices are available, microarray technologies offer the most promise.

Spyro Mousses (National Institutes of Health, Cancer Genetics Branch, Bethesda, MD, USA) presented work on tissue microarrays where up to 1,000 tissue samples are placed on a microscope slide for the *in situ* analysis of morphology, nucleic acid [by *in situ* hybridization (ISH)] and protein [by immunohistochemistry (IHC)] at the cellular level. Tissue microarrays enable the determination of the cellular localization of targets and also integrate DNA, RNA and protein information for a comprehensive molecular profile. Applications were shown for heregulin (HER-2) and S6 kinase frequency and their clinicopathological associations in breast cancer.

Protein arrays were introduced as tools to explore the human proteome by Ian Humphery-Smith (Glaucus Proteomics, Utrecht, The Netherlands). Key challenges for proteomics include small or hydrophobic proteins, or those present in low abundance. 2D electrophoresis appears to be inadequate to cope with many of these challenges, in addition to the technique's inability to display the entire proteome. Antibody arrays present a viable alternative approach but require the parallel generation and screening of large numbers of new antibodies in an extremely high-throughput mode. In particular, the new antibodies must be screened for cross-reactivity by emulating the antigenic diversity encoded by a genome. Recombinant proteins must be carefully purified and properly immobilized to a suitable surface. The absence of a PCR equivalent for proteins is a major complication of this technique, which also needs to incorporate

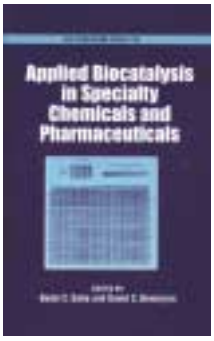
reproducibility. Of significant importance is the ease of replication, quality assurance and scalability.

PROfusion™ technology

Robert Kuimelis (Phylos, Lexington, MA, USA) described PROfusion™ technology, which covalently joins proteins to their encoding mRNA during *in vitro* translation, thus effectively linking phenotype and genotype. Massive protein libraries (10^{13} members) based on an 8.5 kDa single-domain antibody-mimic scaffold were subjected to directed protein evolution using PROfusion™ technology coupled with the PCR amplification of enriched binding sequences. After 8–10 rounds of selection and enrichment, a functional screen identified numerous high-affinity and specific binders against numerous protein targets. These binders were immobilized to a solid-phase in an oriented manner to generate affinity protein microarrays. These protein microarrays were stable for at least eight weeks, functioned in human serum, and detected subnanomolar levels of protein target. An example was shown using an immobilized binder in a microarray format to selectively capture a cytokine from serum followed by label-free detection using matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOF MS). The entire process of mRNA–protein fusion formation, *in vitro* selection, screening and protein production is currently being automated and 'industrialized' so that antibody-mimics can be prepared against a vast number of protein targets both quickly and cost-effectively.

Conclusion

Nucleic acid amplification, detection and screening is central to all molecular biological research. Emerging technologies are driven by the field's need for faster, easier, cheaper and more robust approaches. The Tucson meeting showcased the most recent advances in the field, primarily from the corporate sector, and emphasized real-world applications of these new technologies. Other topics presented at the meeting included sample preparation and nucleic acid purification, DNA probe design and bioinformatics.



Applied Biocatalysis in Specialty Chemicals and Pharmaceuticals

by Saha, B.C. and Demirjian, D.C., eds, ACS/Oxford University Press, 2000, 292 pages, hardback, £79.50, ISBN 0-8412-3679-8

The past several years have seen the continuing expansion of applied biocatalysis. As a result, more than two dozen commercial processes for the synthesis of fine chemicals and pharmaceuticals now use biological catalysts. The expanding role of biocatalysis has been clearly reflected by the growing number of publications on this subject – it has been estimated that 15% of papers on asymmetric synthesis use biological catalysts. This wealth of information was aptly summarized in two recent books: *Biocatalysis for Fine Chemical Synthesis* (published by J. Wiley & Sons in 1999) and *Industrial Biotransformations – A Comprehensive Handbook* (published by Weinheim: Wiley-VCH in 2000).

The book being reviewed here is based on a recent symposium on *Advances in Applied Biocatalysis* held at the *217th National Meeting of the American Chemical Society* in Anaheim in March 1999. The book represents a compilation of 16 manuscripts organized into three sections: biocatalyst discovery, characterization and engineering; applications: specialty chemicals; and applications: pharmaceuticals.

Content

It has been widely recognized that the current expansion of industrial biocatalysis is attributed to recent advancements in molecular biology, HTS and bioengineering. This notion is well reflected in the first section of the book. The screening of large collections of isolated enzymes or biocatalytic libraries requires the development of new and efficient HTS methods. In Chapter 3, Moris-Varas and co-authors present an effective tool for the rapid evaluation of enantioselectivity of hydrolytic enzymes. The method, developed for screening of a library of lipases against numerous substrates in a microplate format, is a general one and is applicable to screening of other hydrolases.

Directed evolution is undoubtedly the most powerful technique for improving the functional characteristics of an enzyme. The advantage of directed evolution over the more traditional protein engineering techniques is that it allows many characteristics of a protein to be improved without any knowledge of the protein's structure, function or mechanism of action. Chapter 6 of the book describes the application of this technique to β-glucosidase, lysozyme, xylanase and aminopeptidase resulting in the generation of mutants with improved thermostability.

Metabolic engineering has been successfully applied to the production

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