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Feature Article

Advances in Protein Arrays Overcome Obstacles

New Improvements Spur Growth Despite Early Skepticism

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Protein microarrays are emerging as one of the most active areas in biotechnology today. Steady advances are overcoming initial skepticism as to their feasibility and utility. Industry sources project dramatic growth spurred on by new improvements in the field such as parallel multiplex screening of thousands of interactions and multianalyte diagnostic assays.

There are two basic types of protein arrays, says John L. Tonkinson, Ph.D., director of business development, Epitome Biosystems (www.epitomebiosystems.com).

Microscale immunoassays use a capture antibody that is attached to a surface such as a chip or bead. The goal is to provide a quantitative measurement from a sample containing the antigen of interest. A second type of protein microarray uses the antigen as a coating to look for protein-protein interactions or for the presence of antibodies in the sample.

Significant hurdles have been encountered in the field specifically related to microscale immunoassays. For example, where does one get good antibodies to use as content on arrays?

Also, parameters have been difficult to establish relative to specificity and sensitivity. Cross-reactivity and matrix effects can severely limit the utility of such assays. Finally, standardization of assay conditions so that all antibody pairs work together in a multiplex assay has been extremely difficult.

Systematic and Scalable Approaches

Epitome is tackling these problems using its EpiTag technology that measures proteins by zeroing in on selected peptides within the protein. Dr. Tonkinson reports, Unlike traditional assays, we do not need to clone or purify proteins to generate antibodies or to use as antigen standards.

Rather, we develop antibodies against unique linear peptide sequences found within every protein. Starting with the genomic database, in silico techniques are used to identify these tags. Antibodies are then raised against synthetic peptides that comprise these unique sequences and antigen affinity purified to yield mono-specific type reagents.

Basically, we transform the generation of antibodies from an iterative, low efficiency process to a predictable approach requiring only the protein sequence. We use these antibodies as binders on arrays. Before assaying relevant biological samples (e.g., tissue and serum) we enzymatically digest the sample in order to liberate the EpiTag from the protein allowing for a good antibody/antigen interaction.

We can also do the reverse and attach the peptide to a chip in order to perform a competitive assay, eliminating the need for a detection antibody or sample labeling.

This approach provides a systematic and scalable means for generating highly quantitative and specific information

from protein arrays. An immunoassay involves trying to find the proverbial needle in the haystack. We make the haystack more consistent and orderly so the needle is much easier to find.

Another advantage is that this technology allows us to identify multiple tags in proteins to build in redundant measurements to a single protein. This is very significant because it can ensure that changes in signal on an array actually correlate to a change in protein concentration and not measurement artifact.

The company expects its first commercial products to be available in the second quarter of 2006. Its technology will be applied to multiple areas including assays for biomarkers useful in clinical trials and basic research and development.

Arraying Autoimmune Diseases

Although cancer and heart disease rank as the top two most common diseases in the U.S., autoimmune diseases come in a surprising third. There are more than 80 clinically distinct autoimmune disorders affecting at least 5% of the U.S. population. Almost 75% of the afflicted are young women.

Jens Beator, Ph.D., project manager, Whatman Schleicher & Schuell (www.schleicher-schuell. com) notes, Most laboratory analyses start essentially with a yes/no assay. If its a yes, then further tests try to pinpoint which disease it is. Although, multiple analytes need to be tested, the current diagnostic paradigm is one analyte per tube. This is a slow and expensive process. However, protein arrays are becoming an increasingly useful tool.

Dr. Beator continues, Our company is developing a multiple analyte test for autoimmune diseases. Using the microarray principle we spot 1015 analytes in one assay, and we are able to screen 16 different patients on a single slide.

This slide can diagnose collagenosis and vasculitis-related illnesses such as systemic lupus erythematosus (SLE) and Sjgrens Syndrome. This represents a technology leap enabling affordable diagnostic profiling.

The company uses clinically validated antigens that are already established for disease diagnostics. They bind analytes to glass slides coated with proprietary nitrocellulose polymers. Unlike other surfaces, this nitrocellulose retains arrayed proteins in a quantitative and reproducible fashion, explains Dr. Beator.

Our company has a long and productive history of providing top-quality nitrocellulose surfaces and other membranes to the scientific and diagnostic communities. We are pursuing the application opportunities of this new technology with our own R&D projects in addition to being a provider.

Dr. Beator reports that the long-term scope of the companys new venture is to reach reference laboratories, medical schools, and the pharmaceutical industry with protein microarray-based diagnostic technologies. They currently are working to clinically validate their assay.

Functional Approaches

Detecting the quantity of a protein is one thing, knowing if its intact and functional is another. Proteins are much more difficult to work with than DNA, says Jeremy Gillespie, Ph.D., business segment manager, Invitrogen (www.invitrogen.com).

This has slowed the development of protein microarrays. But, we have begun to develop microarrays in which we can look for measures of protein function such as interactions with other proteins, DNA, signaling pathways, and with small molecules. Such microarrays may dramatically accelerate drug target identification, as well as selection and validation.

A functional microarray requires that the coated proteins maintain stability. Dr. Gillespie explains, Arrays can contain more than 3,000 different human proteins. So, the first thing we do is to express and then purify each protein. We use the baculovirus system to generate proteins.

Next we coat these onto glass slides. Weve tracked stability over time and found most proteins are stable for at least one year. Part of our processing practices involves purifying and arraying under cold temperatures followed by immediate storage in a freezer.

Dr. Gillespie believes that as functional arrays become more widely used, they will help to more fully realize the true potential of protein microarrays. There used to be a lot of pessimism about the feasibility of using protein arrays. But with new advances in protein expression, purification, and coating techniques things are looking up.

Invitrogen recently introduced its high density ProtoArray Human Protein Microarray. The functional array includes pharmaceutically relevant protein classes such as kinases, membrane-associated, cell-signaling, and metabolic proteins. The assay can be completed in less than four hours as contrasted to weeks required for similar experiments, according to the company. They also plan to launch a human proteome-wide array in 2006.

Microfluidic Microarrays

Akonni Biosystems (www. akonni.com) is coupling microfluidic technologies with protein microarrays. The company has created a novel diagnostic approach that uses microarrays. They use precision robotic methods to coat arrays with micro-gel-drops within a microfluidic Lab-on-a-card.

These TruArrays rapidly screen samples for hundreds of disease markers using hundreds of molecular biosensors in an array the size of a fingernail.

The system consists of a portable reader device and credit-card sized disposable test array. Samples such as blood, saliva, or urine are directly applied. The card is then placed into the reader, which performs a complex battery of tests and provides results within only a few minutes.

According to the company, applications include screenings for multiple infectious diseases, diagnosing a cancer type (such as breast or prostate), and identifying individuals predisposed to Alzheimers or susceptible to adverse drug reactions.

In contrast to traditional microarray glass slides, Akonnis card automates all of the processes because each gel-drop serves as a micro test tube that can be built to perform any diagnostic biochemical test. The company suggests that its integrated microfluidic card approach is easier, less expensive, faster, and more accurate than currently available technologies.

Human Protein Atlas

The recently launched web-based database called the Human Proteome Atlas (www.proteinatlas.org) is stirring some excitement in the field, according to Mathias Uhlen, Ph.D., professor department Biotechnology of Albanova Center, Royal Institute Technology (Stockholm, Sweden).

Our database provides >300,000 high-resolution images that detail protein expression patterns in a variety of human cells and tissues. These images are obtained using immunohistochemically stained tissue sections and tissue microarrays.

A pathologist annotates each image. You can click on different proteins and see how they are expressed (i.e., by name or id number) or by clicking on individual chromosomes. The Atlas can be searched in several ways. You can see how a protein is expressed in the body or if you are interested in, for example, colon cancer, you can determine what proteins are expressed specifically in this disease.

Dr. Uhlen says that quality-assured antibodies were used to generate the data. Antibodies are important tools to understand our biology. Proteins are the essential building blocks of life. My vision is that in the next 10 years we will have antibodies to all human proteins.

Our database images were produced from an initiative by the nonprofit Swedish Human Proteome Resource (www.hpr.se) program that was established to systematically explore the human proteome using antibody probes .

The effort combines high throughput generation of affinity-purified (mono-specific) antibodies with protein profiling obtained in tissue arrays.

It is the hope of Dr. Uhlen that companies will eventually join the initiative to provide their antibodies for further tissue

probing. This is a win-win situation because arrays need antibodies and companies can validate the quality of their antibodies.
