A breakthrough in genomics/proteomics array technology

A new high-throughput array technology uses nuclease protection to provide DNA, RNA and protein multiplexed profiling with the sensitivity and reproducibility of conventional biochemical assays.

Dr Bruce Seligmann, High Throughput Genomics

The sequencing of the human genome and related efforts have unleashed the potential for discovering thousands of new drug discovery targets and - from these efforts - new therapeutic treatments and drugs. However, the work has only just begun. High sample throughput and gene throughput assays are necessary to identify potential gene and protein targets, validate these targets with numerous human tissues and cells treated in specific ways, and then exploit the validated targets by way of high throughput screening to discover drug leads.

But no matter how many targets are discovered, the bottlenecks created by drug metabolism, safety and clinical trials still limit the number of successful drugs reaching the marketplace. Genomics and proteomics offer the potential to address these later but highly problematic phases of drug development. What is required are methods to measure gene expression, protein translation and protein function, with high throughput, sensitivity and reproducibility, to provide highly quantitative and reliable results.

High Throughput Genomics (HTG) was founded to develop a universal tool-set to improve current methods and additionally satisfy unmet needs encountered at all levels of drug discovery, development and clinical practice. While other methods are available and in common use for the discovery and validation of gene targets, a lack of sensitivity and reproducibility has meant that low abundance targets which change by only a small but significant amount in disease cannot be identified or distinguished from assay noise. The same limitations prevent certain types of samples - such as laser biopsies - from being tested across a large number of genes. Low sample-throughput also limits the number of samples that can be tested during target validation to understand how gene expression profiles change during the course of disease and therapy, and to characterise potentially useful cell lines, treatment protocols and animal models.

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Lack of sample throughput, as well as sensitivity and reproducibility, all prevent current methods from being used to provide high content High Throughput Screening (HTS) of expression profiles to discover potential drugs which mechanistically regulate gene expression, and subsequent thorough characterisation of specificity and potential safety issues across a wide spectrum (ideally the whole genome) of expressed genes. The capability to pursue these approaches will lead to the discovery of entirely new classes of drugs, potentially with the effectiveness of steroids (which act through such a mechanism) but without their side effects.

A quantitatively reproducible, sensitive assay of gene and protein profiles would also provide a valuable tool for the optimisation of safety and metabolism properties of potential drugs before testing in animals or man, addressing the first two major bottlenecks of successful drug discovery. Likewise, the ability to measure profiles of expressed genes will afford the possibility to develop a new generation of quantitative diagnostic tests for detecting the propensity towards disease, the onset and stage of a disease, and providing the physician with molecular data about how the body is responding to drug therapy in a simple, quantitative read-out of normal and abnormal range values, akin to blood chemistry tests.

HTG is focusing its efforts on developing hardware that will provide “push-button” Microfluidic Array Diagnostic Systems (MADS) to be operated by healthcare professionals in the emergency room, urgent care centres, critical care units and medical/surgical floors, in the doctor’s office, and by patients at home or by the general public for immediate diagnosis. As drugs are developed for illnesses where early use is much more effective - such the common cold - early diagnosis becomes even more beneficial and cost-effective both to the individual and society.

MMP arrays

The first of HTG’s Multiplexed Molecular Profiling (MMP) products - the ArrayPlate™ (Figure 1) - was released recently. MMP multiplexes assays by permitting the testing of many targets simultaneously using a spatial array; the number of targets can be further increased through multiplexing the assay of more than one target within each element of the array. Finally, the ArrayPlate™ adds a further layer of sample multiplexing by providing clients with the MMP arrays in 96- or 384-well microplates.

This is not the first time that arrays have been printed in microplates, but there is little practical value in an array microplate format if the sample size required for analysis is so large that the samples cannot also be processed in microplates. Lack of sample throughput, as well as sensitivity and reproducibility, all prevent current methods from being used to provide high content High Throughput Screening (HTS) of expression profiles to discover potential drugs which mechanistically regulate gene expression, and subsequent thorough characterisation of specificity and potential safety issues across a wide spectrum (ideally the whole genome) of expressed genes. The capability to pursue these approaches will lead to the discovery of entirely new classes of drugs, potentially with the effectiveness of steroids (which act through such a mechanism) but without their side effects.

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This is not the first time that arrays have been printed in microplates, but there is little practical value in an array microplate format if the sample size required for analysis is so large that the samples cannot also be processed in microplates. To date, array methods have been inherently low in sensitivity and reproducibility, making them a low sample throughput platform for providing “Yes/No” rather than highly quantitative answers. Consequently, real-time PCR, one gene per well, has been the most sensitive and most quantitative method for measuring RNA expression.

This has all changed with the introduction of the ArrayPlate™. HTG has developed a single-addition-only (no separations required) protocol and reagents for processing samples for simultaneous measurement of DNA, RNA and protein, and a universal assay platform for making these determinations as well as measuring protein function (for example, enzyme activity).
The sensitivity of DNA, RNA and protein measurement enables samples of as few as 1,000 cells to be used without the need for amplification. Measurement of results is made by imaging the entire plate to provide a simultaneous read-out of all the data-points; for example, 1,536 data points from a 96-well plate with 16 gene targets per well, or 11,904 data points from a 96-well plate, 124 genes per well. Commercial automation and imagers can be used to handle and read the ArrayPlate™, although HTG also markets its own Omix Imager™.

The sensitivity of DNA, RNA and protein measurement enables samples of as few as 1,000 cells to be used without the need for amplification (Figure 2). The use of any number of amplification steps enables even smaller samples to be analysed. This represents a dramatic breakthrough in the simultaneous measurement of DNA genotype and RNA expression across a profile of gene targets. The key was the discovery that one of the three dominant methods of molecular biology - the gold standard gel-based nuclease protection assay - could be applied to array measurement. HTG owns two issued patents covering the use of nuclease protection with arrays.

Nuclease protection

Nuclease protection is based on the incorporation of short synthetic sequences of DNA complementary to RNA targets of interest at the time cells or tissues are lysed and processed. The appropriate synthetic DNA nuclease protection probe binds to its target RNA, and protects that portion of the RNA from being destroyed by nucleases. In fact, nucleases are added to completely destroy all the non-target single-stranded RNA and DNA (including all excess nuclease protection probes). In the ArrayPlate™ assay, the “protected” nuclease protection probes - which are a stoichiometric representation of the original amount and profile of RNA expression - are measured quantitatively.

A further advantage of this assay - besides its simple protocol, high sensitivity and high quantitative reproducibility - is that each well can contain control genes against which changes in target genes can be compared, and variations between samples (cell number or amount of tissue) can be normalised. In fact, the ArrayPlate™ assay can measure DNA simultaneously in the same well as RNA, and therefore DNA can be used for normalisation and determination of the absolute number of RNA molecules per cell of each gene measured.

Finally, custom ArrayPlate™ assays for a few to hundreds of genes can be developed and validated using actual samples in a matter of just two weeks from selecting the gene set, and can then be modified “on-the-fly” by insertion or replacement of genes requiring only validation of the new reagent set for the new gene(s). All reagents are handled batch-wise, so the work required to assay or validate an assay for four genes is the same as that required for 100 genes. The speed with which assays can be established and changed represents a breakthrough that will enable drug discovery and clinical diagnostic applications to keep pace with rapid advances in elucidating the human genome.

Expression profiling ArrayPlate™ technology permits the expression profile of a focused set of four to 124 genes to be measured economically and quantitatively for a large number of samples. Sample sizes of 30,000 down to 1,000 cells or tissue equivalents (0.01 - 0.03 µg total RNA) permit the preparation and treatment of samples in microplates, so that the overall assay is high throughput - not just the ArrayPlate™ measurement. Quantitative reproducibility of 3% to 13% CV at 30,000 cells per sample for a set of genes is typical of the data obtained; this permits use of the assay not only for target validation, but also for the first time for high throughput screening of a complete profile of genes, and quantitative follow-up dose-response curves of activity and specificity.

As shown in Figure 3 (gene expression dose response curves, in this case for steroids), medicinal chemists can derive the quantitative structure activity relationship (QSAR) data from the ArrayPlate™. By using a set of drug metabolism or safety genes, QSAR data can be obtained for drug safety and metabolism - not only in humans but also in animals - and these properties of a compound series can then be optimised before testing in animals or man. This is a breakthrough in drug development that will lead to higher-quality clinical candidates and greater success in overcoming the bottlenecks of animal/human metabolism and safety.
A diagnostic platform

Selection of diagnostic gene sets converts the ArrayPlate™ assay into a diagnostic platform, capable of measuring single nucleotide polymorphisms (SNPs) with high accuracy, sensitivity and sample throughput. Such diagnostic assays have the potential to revolutionise clinical development and health care through selection and stratification of patients for preferred treatments, and through profiling of determination of individual drug metabolisation characteristics before selecting dosing levels. HTG is adapting the technology to flow-through microchips to provide an even more versatile and convenient diagnostic platform - the push-button MADS for hands-on use by health professionals and patients at home.

The three markers of cell function - DNA genotype, RNA expression and protein translation - can be measured from the same treated sample using ArrayPlate™ assays. Figure 4 shows a time-course of gene expression and protein synthesis or secretion from inflammatory cells after stimulation with bacterial lipopolysaccharide to simulate a bacterial challenge. Protein measurements are made using a multiplexed ELISA ArrayPlate™. Assays can also be used to measure enzyme activity, and protocols are being developed for measurement of receptor binding and cell function, making the ArrayPlate™ a truly universal genomics and proteomics platform.

Future developments

The technology is being adapted to a high sample throughput platform (ArrayPlate 1000™) for measuring 1,000 genes at a time in target discovery applications; this platform is in the final stages of validation. A follow-up product - the ArraySlide 10,000™ - is also in development. These products exploit the breakthrough in sensitivity and reproducibility to provide investigators with an assay which can use very small samples and yet identify slight (10-20%) changes in low abundance genes or cells which fall within the "noise" of current array methods used to identify disease-causing gene targets.

To date, validated uses of ArrayPlate™ assays include: target validation and treatment protocol optimisation, cell culture monitoring, high throughput screening, metabolism and safety assessment, SNP analysis, and the diagnostic detection and identification of diseased cells. Many of these results can be reviewed at the HTG website (www.htgenomics.com).

Note: High Throughput Genomics (HTG) can develop and validate a custom ArrayPlate™ assay using client samples within four weeks of gene selection for sets of up to 100 or more genes. Samples can be lysed, frozen and shipped by the client to HTG for assay - assuring client control. Custom and standard kits (ArrayPlates™, reagents and protocols) are available for sale: HTG will also test samples sent by clients and enter into drug discovery and technology, and diagnostic development collaborations. For more information about HTG’s products and services, please contact Michael Cusack (e-mail: mcusack@htgenomics.com).

Bruce Seligmann, President and CEO at High Throughput Genomics Inc, has been responsible for the discovery and development of the MMP technology and ArrayPlate™ products. Previously, Dr Seligmann was a Senior Staff Fellow at the NIH, NIAID, Team Leader and Senior Research Fellow with Ciba-Geigy, VP of Research and Development at the combinatorial chemistry company, Selectide, and subsequently Center Director (after the acquisition of Selectide by Marion Merrill Dow, now Aventis). He was also founder and CEO/Chairman of SIDDGO and HTG, and negotiated the strategic sale of SIDDGO and divestiture of HTG to shareholders in order to separate the low-value combinatorial chemistry business of SIDDGO from the high-value genomics/proteomics business of HTG. Dr Seligmann has been responsible for several potential drug products that are in clinical development, and is an internationally recognised scientist and manager of successful technology and corporate development. He is on the Board of the Arizona Cancer Center.