Applied Biosystems

From genes to diagnostics: integrating technology makes it possible

Molecular profiling and classification of human diseases hold great promises in revolutionizing the future of clinical medicine. Applied Biosystems' integrated gene expression technologies allow acceleration of the path from discovery to clinical practice in a time- and cost-effective manner.

The assessment of disease risk and the means of selecting appropriate medical treatments are continually evolving and becoming increasingly based on molecular classification. Disease risk assessment is moving from population-based models to predictive, individual models while treatment selection is becoming more targeted and less empirical. As a result, the need for new biomarkers that can distinguish distinct forms of disease continues to grow. One of the principal challenges researchers face in the discovery and development of genomic biomarkers is the ability to replicate study results. For example, it is not uncommon for different researchers studying the same disease to use different microarray platforms during the discovery phase of their research. A byproduct of this practice is the generation of multiple nonoverlapping candidate gene sets that negatively impact downstream development because there is little consensus on which final set of candidate genes should be moved forward in development. Implementation of a completely integrated platform for both discovery and development would not only streamline research, but would also allow accurate, direct comparisons of data from different studies.

To make such an integrated workflow possible, Applied Biosystems offers powerful platform technologies that address all stages of genomic discovery—from sample preparation, to whole genome analysis, to downstream validation studies of promising biomarkers with clinical potential. These platforms include the ABI PRISM™ 6100 PrepStation and Tempus™ Blood RNA Tubes for clinical research sample preparation, the Applied Biosystems Expression Array System for gene expression analysis of whole genomes, TaqMan® Gene Expression Assays for validation of candidate genes using quantitative real-time PCR, and the TaqMan® Low Density Arrays for screening on the Applied Biosystems 7900HT Real-Time PCR System.

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Preparing samples with confidence

Optimal clinical research sample preparation is essential to achieving unsurpassed experimental results when evaluating biomarkers of disease. The company's ABI PRISM 6100 Prep Station, a semiautomated platform, can produce high-quality total RNA from whole blood or isolated blood cells from various animal species and can purify up to 96 samples of total RNA or genomic DNA in less than one hour. The automated purification procedure generates RNA that is free of PCR inhibitors, RNases, and contaminating genomic DNA and is completely compatible with commonly used anticoagulants, such as citrate, heparin and EDTA.

For researchers working with whole blood, Applied Biosystems Tempus RNA evacuated blood collection tubes contain a reagent that stabilizes large quantities of high-quality RNA for up to five days at room temperature, allowing for both the isolation and transportation of the collected blood.

From tens of thousands to hundreds

The Applied Biosystems Expression Array System, comprised of the Applied Biosystems Genome Survey Microarray and 1700 Chemiluminescent Microarray Analyzer, allows gene expression patterns to be surveyed and measured across the whole genome to select which sets of genes are suitable for downstream studies. These smaller subsets, comprised of tens to a few hundred genes, typically contain the clinically relevant candidate biomarkers whose expression profiles can be used to classify cancers.

Each Genome Survey Microarray uses 60-mer, quality-assured, DNA probes for optimal specificity, which can detect a fully curated and annotated set of approximately 29,098 human genes derived from data from both public sources and Celera Genomics. The 60-mer oligonucleotides (oligos) and chemiluminescent technology provide a tenfold increase in sensitivity compared to other commercially available microarray products that use shorter oligos or fluorescence detection approaches. Long oligos allow tighter binding to the target, which leads to the detection of more genes with greater selectivity and specificity. This is imperative for researchers working

APPLICATION NOTES

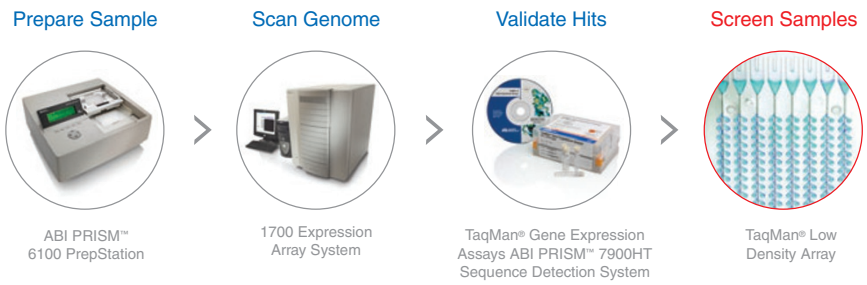

Figure 1 | The Applied Biosystems gene expression solution allows researchers to identify, validate and screen clinically relevant candidate biomarkers.

with small, difficult-to-obtain samples, such as those required for cancer research.

The Applied Biosystems 1700 Chemiluminescent Microarray Analyzer uses a state-of-the-art charge-coupled device (CCD) camera that precisely images the chemiluminescent signal that results when labeled transcripts are hybridized to a microarray. It is powered by an Oracle® database that contains the latest gene annotation data for comprehensive gene expression studies.

The power of quantitative real-time PCR

The performance capabilities and ease-of-use of real-time PCR chemistries and instrumentation has led to the widespread use of real-time reverse transcription PCR (RT-PCR) as a preferred method for quantifying gene expression[1]. In particular, following whole genome scans using the Expression Array System, Applied Biosystems has dramatically increased the ability of researchers to identify candidate genes with real-time PCR using its TaqMan Gene Expression Assays products.The assays are comprised of more than 500,000 gene-specific TaqMan probe and primer sets that allow researchers to quickly and easily perform quantitative gene expression studies on human, mouse and rat genes. They cover all known human genes, focusing on genes in the public domain with associated RefSeq transcripts from the National Center for Biotechnology Information (NCBI), and aim to offer a minimum of one assay per RefSeq transcript as an off-the-shelf product. The TaqMan Gene Expression Assays are available for nearly all exon-exon junctions of human, rat and mouse gene transcripts, giving researchers flexibility to select an assay designed within a specific location of a gene transcript. The state-of the-art genome sequence–aided design also includes a comprehensive, automated bioinformatics design pipeline that masks single nucleotide polymorphisms (SNPs), repeats and sequence discrepancies, and selects against regions of high homology to design a specific, high-performing gene expression assay for a gene of interest.

Built on the Applied Biosystems 5′ nuclease assay chemistry, the TaqMan Gene Expression Assays take advantage of the 5′ nuclease activity of AmpliTaq® Gold DNA Polymerase to cleave a TaqMan probe during PCR. A TaqMan probe is an oligo that hybridizes to an internal region of the PCR product and contains a fluorescent reporter dye attached to the 5′ end and a nonfluorescent quencher (NFQ) moiety coupled to the 3′ end. Each assay consists of two unlabeled PCR primers and a FAM™ dye–labeled TaqMan probe with a minor groove binder coupled to the NFQ. The minor groove binder enhances melting temperature (T_m) by binding in the minor groove of a DNA duplex, allowing the use of probes as short as 13 nucleotides. Shorter probes such as these can be advantageous as they offer superior primer/probe design control for improved specificity and greater flexibility when designing assays for closely related sequences[2].

The most informative subset: how low can you go?

Once the candidate markers for a disease are validated with TaqMan Gene Expression Assays and quantitative real-time PCR, a smaller subset of significant marker genes that are identified can be routinely screened in downstream studies using Applied Biosystems TaqMan Low Density Arrays. These arrays provide a more efficient and standardized way for researchers to generate data and interpret their clinical research results versus hybridization microarrays, and eliminate the need for liquid handling robots, thereby reducing reagent consumption and minimizing laboratory costs.

From 10,000 genes to a six-gene model: a case study

Last year a team of scientists from Applied Biosystems and Stanford University published a study in the *New England Journal of Medicine* demonstrating how quantitative real-time PCR and Applied Biosystems TaqMan Gene Expression Assays can be used to develop practical gene expression tests for use in clinical research and biomarker development[3]. Using quantitative real-time PCR, a set of six genes that predict survival in diffuse large B-cell lymphoma (DLBCL) patients was identified.

Using the Applied Biosystems TaqMan Gene Expression Assays products on an Applied Biosystems 7900HT Real-Time PCR System, quantitative real-time PCR was used to measure the expression profiles of mRNA from 36 genes (selected from more than 10,000 candidate genes) in DLBCL tumor samples from 66 patients. The researchers performed a univariate analysis of genes that were correlated with overall survival, either positively or negatively. The six genes that were demonstrated to be the strongest predictors were *LMO2*, *BCL6*, *FN1*, *CCND2*, *CCL3* (also known as *SCYA3*) and *BCL2*. The team built a multivariate model based on expression levels of the six genes and validated the model by applying it to previously published data from two independent sources. The researchers determined that the six-gene model's predictive value was powerfully independent of the International Prognostic Index (IPI), a well-established predictor of patient outcome because, using the model, they were able to further refine the survival prediction of patients

within each IPI risk category (low, medium and high). In addition, identifying patients with medium- and high-risk IPI scores coupled with a high-risk expression profile gave the researchers the ability to identify a group of patients whose survival was especially short (approximately 30% of all patients). Independently, the genes could not predict how long a patient survived after treatment; however, the six genes taken together were able to predict how long each of the 66 patients survived. Based on this study, the authors concluded that quantitative real-time PCR using Applied Biosystems TaqMan Gene Expression Assays proved to be an easier and more suitable approach for these types of studies than hybridization arrays.

Although the traditional uses for biomarkers include screening and monitoring disease status, future clinical applications are emerging, such as those demonstrated by the collaborative work and scientific findings described here.

Making the impossible possible

Using the Applied Biosystems suite of enabling gene expression technology platforms, researchers can perform efficient genomic discovery and biomarker development studies that provide insight to pathways involved in disease and therapeutic response. This results in the rapid identification of potential diagnostic tools. Applied Biosystems' platform technologies, with their seamless integrated workflow, accelerate the path to discovery by allowing scientists to accomplish their research objectives in a time- and cost-effective manner. Gene expression studies that used to take months to complete can now be accomplished in a fraction of that time using these platforms. Researchers can now relate the results they generate with the Applied Biosystems technology platforms back to the biology of the genes and the transcripts that originate from the marker genes. This means that researchers can interpret their results in an integrated, coordinated manner through the different steps of the process. From sample preparation to whole-genome analysis to validation studies of promising genes and potential diagnostic tools, Applied Biosystems makes it all possible.

The Applied Biosystems TaqMan Expression Assays can be ordered through an enhanced e-commerce site (http://store.appliedbiosystems.com/). The web interface offers robust search options that allow researchers to identify the assays relevant to their particular research. Customers are provided with RefSeq, Celera and reagent identification numbers as well as other important biological information and may browse an online catalog free of charge.

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