

Research into Personalized Treatment for Brain and Pancreatic Cancer Using the TaqMan® Low Density Arrays and the Applied Biosystems 7900HT Fast Real-Time PCR System

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APPLICATIONS

- Correlating gene expression patterns with patient responses
- Gene expression analysis in needle biopsies, blood, and paraffin-embedded archival tissue samples
- Screening a large number of patient samples against a fixed set of targets

TECHNOLOGIES

- TaqMan® Low Density Array
- TaqMan® Gene Expression Assays
- Applied Biosystems 7900HT Fast Real-Time PCR System

Dr. Martin Johnson at the University of Alabama is a passionate researcher striving to improve treatment for patients with pancreatic and brain tumors, two of the most commonly known incurable cancers. Dr. Johnson explains, "Right now, the treatments we have were developed empirically, and for these two types of cancer, the approach has been disappointing. Median survival is one year or less, and there has been very little improvement in overall median survival." Dr. Johnson hopes that combining information from the human genome project with microfluidic gene-expression analysis cards such as TaqMan® Low Density Arrays will finally improve outcomes for these cancer patients.

He and his colleagues are currently conducting research studies of the molecular profiles of tumor samples from the few patients who respond well to certain types of treatment. Once genes are identified that correlate with a favorable treatment response, Dr. Johnson believes it will be possible to improve treatment at the individual level.

Molecular Profiling May Indicate New Uses for Existing Treatments

Molecular profiling with the TaqMan® Array led Dr. Johnson to hypothesize that the chemotherapy drug Capecitabine (Xeloda®), currently approved only for treatment of breast and colorectal cancer, would also be effective against brain tumors. Subsequent clinical trials confirmed that hypothesis. One patient had a complete remission, and Dr. Johnson is now applying custom TaqMan® Gene Expression Assays to further investigate the specific expression patterns in the regressed tumor from that patient.

Paraffin-Embedded Tissue Samples Unlocked as a Source of Gene Expression Data

In the past, researchers have been frustrated by attempts to profile gene expression in archived cancer tissue. “Almost all archived tissue samples are embedded in paraffin. The RNA is degraded and crosslinked, and this significantly limits our ability to examine changes in gene expression,” Dr. Johnson explains. Yet these archived cancer tissues represent a vast and valuable resource due to accompanying data about how the patient responded to treatment.

“We needed a technology that was PCR-based, sensitive enough, and reproducible enough—and this is where the fluorescent chips fell short,”

says Dr. Johnson. “[With TaqMan® Arrays,] the amplicon is so small that the results we obtain from RNA isolated from paraffin-embedded tissue correlates well with that obtained from fresh tissue,” he adds.

Needle Biopsies Sufficient for Tumor-Cell Expression Profiling

Many of the research samples profiled in Dr. Johnson’s lab come from needle biopsies taken for diagnosis, which provide minuscule amounts of sample material “You don’t get gram-sized pieces of tumor from a needle biopsy,” says Dr. Johnson. Furthermore, once the samples are depleted, it’s impossible to obtain more. He emphasizes that, “these samples are absolutely irreplaceable.”

Therefore, Dr. Johnson is especially enthusiastic about the fact that he can run a TaqMan® Array with significantly less RNA. “A needle biopsy doesn’t



The Applied Biosystems 7900HT combined with TaqMan® Gene Expression Assays and the TaqMan® Low Density Array enables high-throughput and flexibility while streamlining reaction setup time.

provide enough sample to run a DNA chip, but there is plenty to run a microfluidic card," he says.

Blood Samples Reveal Leukemia-Cell Expression Profiles

Dr. Johnson also uses TaqMan® Arrays to run assays on research blood samples. For example, as part of his research, he has developed an array to look at cytokine levels in samples from children receiving whole-body irradiation to treat acute lymphocytic leukemia. By drawing blood samples before, during, and after treatment, Dr. Johnson's

Using the TaqMan® Array, Dr. Johnson's research has shown for the first time that valuable paraffin-embedded samples can be mined for gene expression data.

research looks at how the expression of 94 cytokines changes over the course of treatment in an individual patient.

"This was never possible before that I know of," he says. "Using this new technology, only 1-2 nanograms of total RNA are required to quantify each gene, so for hundreds of genes I can easily get what I need from 1 mL of blood."

Microarray Expression Analysis Can Easily be Validated by TaqMan® Arrays

TaqMan® Arrays may also be used instead of individual TaqMan® Gene Expression Assays to validate the research results derived from DNA microchips. "Now you are confirming DNA microchip results using real-time quantitative PCR, where you might identify 20-30 genes and check 5 to see if they have differential expression. Now you could run an array and build a complete microfluidic card to look at the expression of every gene."

Key Results From Individuals Can Easily be Tested in Large Populations

Dr. Johnson also likes the ability to easily follow up on results with essentially the same technology if the amount of sample becomes so limited that there

isn't even enough to run a TaqMan® Array." You can take that one gene that is overexpressed or underexpressed, and use real-time PCR to follow up on results in larger populations," he says.

TaqMan® Arrays Increase Precision of Expression Profiles

Dr. Johnson says one difficulty with microarray studies is running statistical analysis on the tremendous amount of data that is generated, especially because there are so many fluctuating variables in the studies. "There is such variation in the subject population," he says, pointing out that there is no standard cancer patient. "They almost all receive slightly different treatment regimens and have tumors at different stages, sizes, and locations."

"With commercially available fluorescent chips, the standard deviation can be as high as 50%," he says. Adding this variability to the heterogeneous patient study population increases the difficulty of statistical analysis. "[But with the TaqMan® Array,] the error between replicates is so low. We can run the same sample, and every time we get the same answer," says Dr. Johnson. With a 5-7% error rate, Dr. Johnson only needs to run three replicates per TaqMan® Array.

Dr. Johnson believes it will be possible to improve personalized treatment of brain and pancreatic cancer patients.

“One of the smartest things ABI did was to look at all the expressed human proteins and design primers and probes.”

Dr. Martin Johnson

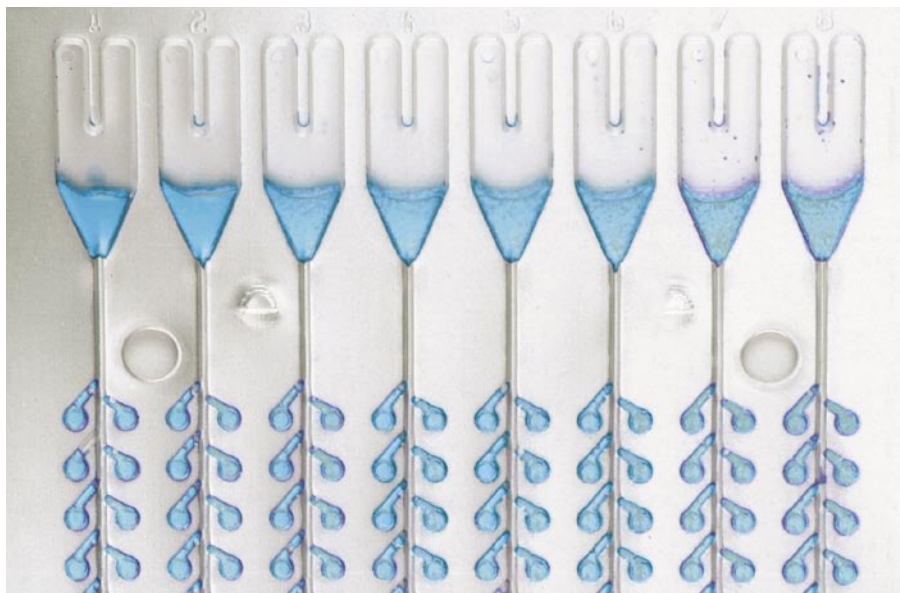
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Primer and Probe Design Nearly Eliminated from Experimental Process

Dr. Johnson's lab has been able to increase its data yield using predesigned primers and probes. “One of the smartest things ABI did was to look at all the expressed human proteins and design primers and probes. Of the 600 or so genes that I've put on cards, only six or seven weren't available,” he says. TaqMan® Gene Expression Assays were used to design an array including all the genes in the hedgehog molecular pathway.

TaqMan® Arrays increase sensitivity and precision over DNA microarrays, and design time is minimal due to the vast pool of available Inventoried TaqMan® Gene Expression Assays. Dr. Johnson sees this pool as an essential system component of his research.

TaqMan® Arrays can be used to validate gene expression results obtained by DNA microarrays. Furthermore, key TaqMan® Array results obtained from individual test cases can easily be tested in the larger population by real-time PCR.



TaqMan® Low Density Array: Streamlines reaction setup time, eliminates the need for liquid handling robotics, and provides standardization when screening selected panels of genes.

TaqMan® Arrays are an excellent research tool and an improvement over DNA microchip analysis for the modern gene expression analysis laboratory.

TaqMan® Low Density Arrays can be used in research studies on previously untapped sample sources

to reveal more refined expression patterns that determine pancreatic and brain cancer patients' responses to various treatments. Such new information will ultimately lead to better application of current treatments and to prolonged survival.

Get more information at www.7900HT.com

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Printed in the USA. 06/2006 Publication 117M114-01



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