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# Tissue Microarrays Reach New Markets

## Emphasis on Potential Clinical Applications and Enhancing RNA Sample Quality

Nina Flanagan

Researchers are beginning to look at tissue microarrays (TMA) from different perspectives, including one with a strong emphasis on potential clinical applications. There is also a great deal of interest in enhancing RNA sample quality and developing high-throughput analysis of protein expression in various tissues.

Cytomyx ([www.cytomyx.com](http://www.cytomyx.com)) is expanding applications of its biorepository through a recent collaboration with **Origene Technologies** ([www.origene.com](http://www.origene.com)). The biorepository holds more than 150,000 well-characterized clinical specimens. Clients search the database and may request either frozen or paraffin-embedded tissue samples or microarrays, in addition to RNA, DNA, and/or protein extracts. These solutions provide early safety and efficacy profiles of potential drugs in development.

The collaboration involves the co-development of new tools to profile gene expression and biomarker validation in various cancers. Cytomyx will provide highly characterized RNA samples from its biorepository, and Origene will use these to develop a new line of its Rapid-Scan Gene Expression Panels.



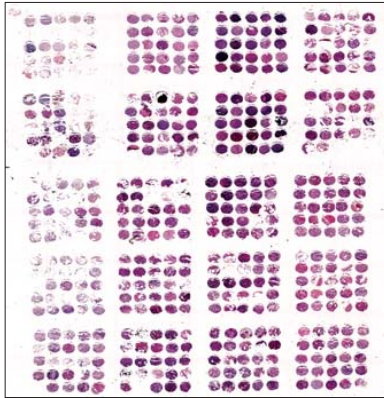
“We wanted their expertise in manufacturing types of cDNAs used in quantitative PCR and target validation,” states Mike Kerins, CEO of Cytomyx. “These panels are designed to obtain information on genes that have been differentially regulated in order to look into different stages of disease progression and understand whether there is a stage-specific aspect to the gene-regulation pattern.” Initial panels include colon and lung cancer.

“Our TMA exactly matches the layout of Origene’s panel, so if the customer wants to look for phenotypic expression

of a certain gene, a specific biomarker, we have not only the disease-matched tissue, but also individual donor-matched material,” says Glenn Gershon, vp biological operations at Cytomyx. “It eliminates one more potential variable in the process of going from gene expression to phenotypic expression.”

### Measuring RNA Quality

Frozen tissue repositories provide samples for proteomics and genomics research, so it’s important to assess sample quality. This is especially true with RNA since it is sensitive to handling and



**An H&E of the TARP2 tissue microarray from the National Cancer Institute. It contains 50 cores of normal tissue, 25 cores each of brain tumors and melanoma, 50 cores each of lymphoma and ovarian cancer, and 75 cores each of breast cancer, colon cancer, lung cancer, and prostate cancer.**

freezing. Asterand ([www.asterand.com](http://www.asterand.com)) has its own biorepository with a global network of about 80 sites. “One way we can determine how well our sites are following our SOPs is to check the quality of RNA of the frozen tissue,” states James Eliason, Ph.D., CSO, R&D at Asterand. “That’s the molecule that tends to go the fastest if not handled properly.”

The RNA is extracted using the standard TRIzol® reagent (Invitrogen; [www.invitrogen.com](http://www.invitrogen.com)) and then analyzed using the Agilent ([www.agilent.com](http://www.agilent.com)) Bioanalyzer 2100, which provides a score for RNA integrity. “Up until then we had developed our own system. In some ways it was quite similar, but ours was graded into six steps, based on the same features as the Agilent algorithm. We’ve been trying to understand how our grading correlates with Agilent’s algorithm and convert all our data in our database so we can also supply the RIN.” Dr. Eliason says there is fairly good correlation between their grading and the RNA Integrity Number (RIN) proposed by Agilent. He adds that there is a difference in quality based on the type of tissue recovery.

“We find we get high-quality RNA from over 60% of our surgical samples,



**The AmpliChip CYP450 Test analyzes variations in two genes that play a major role in the metabolism of many widely prescribed drugs. Roche Diagnostis is working on development of AmpliChip microarrays and other gene-based assays for earlier and more specific detection of disease.**

but only about 35% from the post-mortem samples.” This is because surgical samples are obtained and frozen quickly, whereas the post-mortem tissues may be affected by long-ailing periods where the DNA is degrading for an extended time.

Some of the critical issues in preserving RNA quality include the time between obtaining samples and freezing them, as well as variation in different tissue types. Tumors often yield more RNA than normal tissues; for example breast tumors yield 10 times more RNA than normal breast tissue. In addition, Dr. Eliason says, customer requests are becoming more complex, with more demand overall for tissue samples. “If you want to have specific diagnostics and medications, you have to go to the human disease.”

#### **A Role in Translational Medicine**

The shifting focus from genomics to proteomics has brought the challenge of new methods to study protein expression within tissues. “Right now proteomics still does not have robust clinical applications,” explains Stephen Hewitt, Ph.D., chief, tissue array research program, NCI. “Protein arrays

are great technology, but there really is not yet a good way to introduce these into routine clinical practice in the U.S. However, the discoveries that we make through these technologies can still be applied to clinical care by just changing assays slightly.”

Dr. Hewitt’s research group is developing a high-throughput technology for construction of tissue and protein arrays. They have a method of transferring proteins from a formalin-fixed, paraffin-embedded (FFPE) tissue section to a stack of membranes that is probed with antibodies to detect individual epitopes. This converts a traditional tissue section into a multiplex platform for expression profiling. A single tissue can be transferred to up to ten membranes, each of which is probed with different antibodies, detected with fluorescent secondary antibodies, and quantified by a microarray scanner. Total protein can be determined on each membrane.

The researchers are also developing protocols to optimize RNA recovery from formalin-fixed paraffin embedded tissues primarily for expression array analysis. “We find that most of the protocols are not completely optimized, and we were startled to see some of the early data about the quantity versus the quality of RNA that one recovered from FFPE tissue. We’ve been doing a number of experiments looking at where different aspects are damaging the RNA, for example, fixation damages RNA and paraffin embedding impedes recovery,” notes Dr. Hewitt.

“Agilent’s RIN is the current gold standard,” according to Dr. Hewitt, but most labs do not interpret the information the same. “We anticipate isolating RNA from FFPE tissues and applying different quality measurements to judge the performance of the assays, either chip-based or RT-based assays,” Dr. Hewitt explains.

“We have a great deal of data that we’re preparing that shows you really

can get high-quality microarrays from FFPE tissue. We're also able to use a lot less tissue to ask a lot more questions with the system we're developing."

### TMA's Key to Human Proteome Project

Researchers at Uppsala University in Sweden are involved in a project screening all human proteins. Caroline Kampf, Ph.D., senior scientist, department of genetics and pathology at Rudbeck Laboratory, is part of a group developing a high-throughput antibody-based tissue profiling strategy. The goal is to create an atlas of protein-expression patterns in normal human tissues and cancer tissues representing the 20 most common cancer types ([www.proteinatlas.org](http://www.proteinatlas.org)).

The atlas will serve as a general knowledge base with regard to structural and temporal expression of human proteins in various cells and tissues.

A set of standardized tissue microarrays was produced to allow for rapid screening of different cells and tissues using immunohistochemistry. The group then developed a high-throughput strategy for the systemic generation of their mono-specific antibodies. These were used to create this atlas containing protein-expression patterns from 48 normal human tissues and 20 different types of cancer.

"We have produced eight tissue microarrays, two normal and six cancer tissues. These contain 48 different normal human tissues and cancer tissues from 216 different tumors. We are screening our antibodies against all eight TMAs, and then analyzing them by pathology," explains Dr. Kampf. "We have 576 images per antibody per protein, so it's a lot of images to look through."

The combination of its TMAs with image analysis is providing information on the fraction of positive cells, staining intensity, and subcellular localization. It also provides a strategy for HT analysis of protein expression in various tissues.



### Clinical Applications of Microarrays

Researchers at Roche Molecular Systems ([www.roche-diagnostics.com](http://www.roche-diagnostics.com)) are developing several microarray technologies for clinical applications, including genotyping, re-sequencing, and gene-expression studies of disease tissue.

The AmpliChip CYP450 is a genotyping test for drug metabolism variations that impact individuals' responses to approximately 20% of drugs on the market. "The AmpliChip CYP450 test has applications in coronary arterial disease, psychiatry, and potentially in cancer," asserts Walter Koch, Ph.D, vp, head of research.

"One of the genes we tested, *CYP2D6*, has its enzyme activity lacking in about 7% of all Caucasians because they've inherited two nonfunctional alleles from their parents." Dr. Koch explains that if a breast cancer patient lacks this enzyme, she won't benefit from Tamoxifen because the *CYP2D6* gene catalyzes its activation. In addition, many antidepressants and anti-psychotics are substrates for this enzyme.

However, Dr. Koch says in this case there are more apt to be adverse reactions to the medications. This gene is also important for other agents, such as beta-blockers, anti-arrhythmics, and common pain medications like codeine.

Another application being developed determines the sequence of the most frequently mutated gene in cancer, the *P53* tumor suppressor gene. "This is a

### Tissue Microarray Suppliers

Company & Website	
Ardais	<a href="http://www.ardais.com">www.ardais.com</a>
Asterand	<a href="http://www.asterand.com">www.asterand.com</a>
Beecher Instr.	<a href="http://www.beecherinstruments.com">www.beecherinstruments.com</a>
Biogenex	<a href="http://www.biogenex.com">www.biogenex.com</a>
Chemicon	<a href="http://www.chemicon.com">www.chemicon.com</a>
Cybrdi™	<a href="http://www.cybrdi.com">www.cybrdi.com</a>
Cytomyx	<a href="http://www.cytomyx.com">www.cytomyx.com</a>
Folio Biosciences	<a href="http://www.foliobio.com">www.foliobio.com</a>
HistoBest	<a href="http://www.histobest.com">www.histobest.com</a>
Imgenex	<a href="http://www.imgenex.com">www.imgenex.com</a>
Invitrogen	<a href="http://www.invitrogen.com">www.invitrogen.com</a>
ISU Abxis	<a href="http://www.abxis.com">www.abxis.com</a>
Lifespan Biosciences	<a href="http://www.lsbio.com">www.lsbio.com</a>
Oligene	<a href="http://www.oligene.com">www.oligene.com</a>
Pathology Devices	<a href="http://www.pathologydevices.com">www.pathologydevices.com</a>
Roche Molecular Systems	<a href="http://www.roche-diagnostics.com">www.roche-diagnostics.com</a>
SuperBioChips Labs	<a href="http://www.tissue-array.com">www.tissue-array.com</a>
Tissue Array Networks	<a href="http://www.tissue-array.net">www.tissue-array.net</a>
Tristar Technology Group	<a href="http://www.tristargroup.us">www.tristargroup.us</a>
US Biological	<a href="http://www.usbio.net">www.usbio.net</a>
US Biomax	<a href="http://www.biomax.us">www.biomax.us</a>

slightly different use, instead of looking for variation distributed across a couple of genes, now we're determining the exact sequence of the entire gene," Dr. Koch notes.

*P53* is a master regulator of cell biology. "It turns out that when the gene is mutated the protein isn't functioning normally, and these mutations are

associated with poor prognosis as the cells are often resistant to chemotherapy. This can be important in making decisions about what therapy to choose in a particular cancer or in some cases, even driving therapy to an earlier stage.”

A third application is using gene-expression profiling to categorize or sub-divide leukemias into molecular subtypes, most of which have different

associated prognostic outcomes. “We see a host of other cancer diagnostics that will employ this approach,” states Dr. Koch. “We know that the specific program of genes that are up- and down-regulated within a tumor can be informative about the molecular etiology of that cancer and that it is associated with both prognosis and treatment decisions.”

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