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Advances in Microgenomics: Less is More

Precise Analysis of Small Cell Samples

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Once considered a limitation, small cell samples have now led to new technology advances that are providing researchers with faster and more accurate molecular information. These advances include separation methods to obtain pure cell populations, novel gene expression techniques, and accurate quantitation of material during probe development.

Using these technologies together, researchers are now able to obtain important information even from a single cell. Overall, this is reducing costs, improving data quality, and making R&D labs more quantitative.

Microgenomics is the new catch phrase for the precise molecular analysis of very small populations of cells that have been excised, or microdissected from biopsies.

Realizing that there was no technology to allow such analysis of solid tumor biopsies, Thomas M. Baer, Ph.D., founder and chairman of **Arcturus Biosciences** (www.arctur.com), developed the first Laser Capture Microdissection (LCM) platform with several colleagues at the National Institutes of Health (NIH).

LCM uses a nondamaging infrared laser to adhere specific cells to a thermoplastic film. This ensures integrity of biological molecules like RNA, DNA, and proteins, which are extracted from the targeted cells.

The PixCell® Ile is a manually controlled system mounted on an inverted microscope platform, and is geared toward labs with low- to medium-throughput needs. It microdissects single cells efficiently and uses a direct point-and-shoot process.

AutoPix® automatically microdissects cells from up to three slides per session and can pool microdissected cells from several samples, isolate up to eight unique cell types in one session, and capture rare cells dispersed across several



NanoDrop's retention system in action.

slides, according to the company. It can dissect from frozen, formalin-fixed paraffin-embedded tissue, or cytology smears, and can identify and capture fluorescently labeled cells automatically.

Veritas™ Microdissection, the company's latest platform, is a high throughput, fully automated system that performs

both laser capture and laser cutting microdissection using two lasers. Using the IR laser and UV laser technologies, coupled with image-recognition software, Veritas Microdissection can harvest single cells and large cell populations quickly.

“Our customers wanted something useful for proteomics applications. When you’re dealing with mass spectrometry or Western blot, you need to microdissect tens of thousands of cells,” explains Dr. Baer.

Veritas Microdissection is routinely employed in the preclinical research phase of the drug discovery and development process. It goes hand-in-hand with routine preclinical testing of lead compounds for efficacy, toxicity, and adverse effects.

Scientists can perform a risk assessment of dosage, for example, in a mouse or rat targeted with a lead compound, by looking at the histopathology of a variety of tissues affected by this compound.

Single stained tissue sections from various organs help identify changes in individual cell types that have known drug effects, rather than “grinding up” whole tissues. Ultimately, this process facilitates biomarker identification and confirmation, helping to narrow the search for which genes are changed or affected and which biomarkers may be tested in the clinic.

Studies have reportedly shown that LCM increases the sensitivity of point mutation analysis of specific genes and identifies differentially expressed genes not visible when standard whole tissue is used. LCM can also enhance the user’s ability to locate up- and down-regulated proteins in studies using 2-D gel electrophoresis, mass spectroscopy, and SELDI.

In addition, it has proven useful in providing rapid cell-based analysis of disease

progression, which may ultimately lead to personalized medicine and diagnostics.

Making Every Drop Count

Samples derived from LCM demand proper preparation for expression arrays. Accurate quantitation from limited volumes is needed for robust quality control assessment during several stages of probe development.

The NanoDrop® ND-1000 Spectrophotometer, developed by **NanoDrop Technologies** (www.nanodrop.com), fills this need by requiring only 1 µL of sample to generate a full wavelength spectrum without the use of cuvettes or capillaries.

The sample is pipetted directly onto the measurement surface where it is retained between two optical fibers by surface tension. This patented retention system utilizes two different path lengths (1 mm and 0.2 mm) during each measurement cycle to achieve an extensive dynamic range that eliminates the need to perform dilutions for RNA/DNA or proteins, the firm says.

This technology allows researchers to measure samples up to 50-fold higher in concentration than can be measured using classical 1-cm cuvette-based systems. Sound process control, critical to the success and reliability of these experiments, can now be implemented and monitored using this micro-spectrophotometry technology in concert with other microgenomic technologies.

Concentrations of nucleic acid intermediates are measured at several stages during microarray probe preparation to determine how to proceed with a specific sample. Investigators often had to forgo such quality control steps due to severe limitation of microgenomic sample volume. Quality and quantity of amplified RNA intermediates are assessed using the

system’s nucleic acids module.

The condition of the final product is determined using the microarray module which displays the full UV/Vis spectrum of the sample as well as the calculated concentrations of both the nucleic acid and dye components of a labeled probe.

The ND-1000 allows researchers to check the efficiency of the labeling process and to monitor the quality of hybridization probes or labeled proteins while saving sample volume. This increases confidence that a particular array will be successful and helps reduce time and costs associated with failed arrays.

“We are a genomics core facility that receives RNA samples from a multitude of sources and in varying quality and quantities,” explains J. Russ Carmical, Ph.D., quality assurance manager at **SeqWright** (www.seqwright.com).

“It’s imperative that we can accurately measure nanogram quantities of RNA. In addition, accurate quantity assessment allows us to normalize the amount of input RNA for both microarray and real-time PCR experiments. The ND-1000 has proven indispensable in our sample quality assessment.”

David Ginzinger, Ph.D., director of scientific operations, **Applied Biosystems** (www.appliedbiosystems.com), says this spectrophotometer has become a tool to assess the appropriate control gene to use in RT-PCR. He says he uses it to obtain precise measurements of RNA. “Prior to having the ND-1000, we didn’t have any ability to measure how much was there,” he states.

Most importantly, Dr. Ginzinger says, the system’s main value is that “it gives you the ability to know whether a data point is real. This is more important than any money or amount of time saved. It’s not trivial because any data points that can be

relied upon, the more of those you have, the better your study is going to be.”

Thomas Schmittgen, Ph.D., an associate professor at Ohio State University, also uses the ND-1000 for quantifying RNA in samples prior to doing RT-PCR. He says he has completed a study comparing it to a standard cuvette-based spectrophotometer and a fluorescent 96-well plate method.

“We found the ND-1000 performs well with low RNA levels, and performed the best out of the three methods in terms of reproducibility of PCR data and RNA measurements.”

Drs. Ginzinger and Carmical say that because they have a lot of high throughput projects, a NanoDrop model with multiple channels would be ideal. Although the company won’t say whether this is being developed, Lynne Kielhorn, business development director, says they will be launching a fluorometer this year for fluorescent assays using the same sample handling (1 μ L) technology as the ND-1000.

Assessing RNA Quality

The importance of accurately measuring the concentration of RNA is key to whether a sample can be further used for microarray analysis or RT-PCR. Assessing RNA integrity is critical in obtaining

meaningful gene expression data.

In response to customer demands for a tool to help characterize aspects of RNA analysis that reflect sample quality, **Agilent Technologies** (www.agilent.com) has developed a new algorithm for its 2100 bioanalyzer called RIN (RNA Integrity Number).

Currently, RNA samples are gauged according to ribosomal peak heights on an electropherogram, specifically the ratio between 18S and 28S ribosomal peaks. This is visually interpreted, but samples can often have a wide range of ribosomal ratios. “We know this can be very misleading,” states Tony Owen, Agilent’s manager of the liquid-phase analysis business.

“There was an obvious need to develop software that would take into account multiple parameters in the evaluation of RNA QC and would give consistent results between researchers.”

So, instead of “eyeballing” data, the software automatically assigns a quality number to the electropheric trace. Developed with neural networks and adaptive learning trained on 1,200 eukaryote total RNA samples, the algorithm analyzes the entire electropherogram and offers a measure of RNA quality on a scale of 1 (most degraded) to 10 (most intact).

“This makes it simple to use and a universal tool for RNA quality control,” adds Owen. The training set of the RIN neural network can be extended in the future, allowing inclusion of more tissue types and organisms. This enables comparison of samples and repeatability of experiments. It also saves costs in expensive downstream experiments (microarrays quantitative real-time PCR, etc.) by ensuring only good quality RNA is used.

Potential customers include research biochemists and molecular biologists isolating RNA for further study (microarray, realtime-PCR, RT-PCR), and also for companies producing RNA since the software provides a quality control standard. “Several production companies are already quoting RIN numbers,” Owen says.

The software will only be available with the 2100 bioanalyzer, but the company says it expects to make it available for the new 5100 Automated Lab-on-a-Chip system when an RNA assay is developed for it. RIN is currently available for free as a test version.

“We are evaluating the inputs from over 750 users and are finalizing the software for full release in spring, 2005,” explains Owen. **GEN**