

Microgenomics: Into the Mainstream

by Philippe Desjardins and Steven Blakely

Laser capture microdissection (LCM) technology permits the acquisition of desired cells under direct microscopic visualization, while preserving biological molecules of interest, including RNA, DNA and proteins

The desire to perform precise molecular analysis on progressively smaller, pure cell populations has developed into the burgeoning field of microgenomics. Investigators have tended to forgo the use of microgenomic technologies due to a lack of process control needed to ensure reliability of such studies. Key advances in microgenomic technologies were needed to overcome the limitations posed by researching minute samples, including: precise cell separation methods, refined high quality RNA isolation and amplification protocols, expression array analysis, and novel quality control systems. For the first time, concerted use of the latest microgenomic technologies provides unprecedented morphologic information critical to development, pathology, oncogenesis, and other processes of a desired target cell mass. These advances have expanded the researcher's ability to reliably

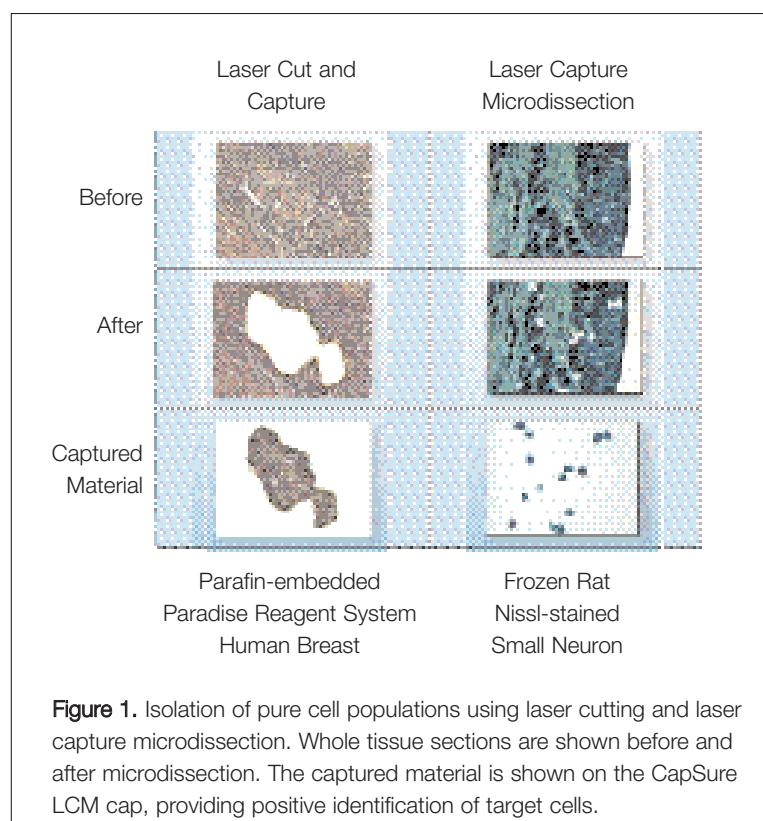


Figure 2. RiboAmp HS RNA Requirements for Two Rounds of Amplification

	Minimum	Recommended
Total Cellular RNA	100-500 pg	500-1ng
LCM Cells	10-50 cells	50-250 cells

Figure 2. Minimum and Recommended Total RNA Inputs for RiboAmp HS Amplification Kit. Minimum RNA input is the smallest amount tested in which useful levels of amplification can be achieved. Different sources of total RNA contain varying amounts of mRNA; consequently, the total RNA input needed to obtain microgram quantities of aRNA depends on the source of total RNA.

access critical expression information from an extraordinarily small number of cells, even from a single cell.

Laser capture microdissection (LCM) technology permits the acquisition of desired cells under direct microscopic visualization, while preserving biological molecules of interest, including RNA, DNA and proteins. LCM, developed in a collaborative effort between the National Institutes of Health and Arcturus Bioscience (Mountain View, CA), uses an infrared laser to adhere specific cells to a thermoplastic film without damaging the nucleic acids and proteins within the targeted tissue. The company recently expanded their LCM offering with the introduction of the Veritas platform, incorporating a UV laser that accelerates the speed at which pure cell populations can be

determine the quality and quantity of material at critical steps during probe development for expression array analysis. The quality assessment of RNA is regularly performed using lab-on-a-chip technologies developed by Agilent Technologies (Palo Alto, CA).

However, quantitation of minute samples during probe development has proven either difficult or impossible with conventional technologies. A recent development in spectrophotometry allows for simple, accurate generation of a full wavelength absorbance analysis using only 1 μ l of sample. A patented retention system, developed by NanoDrop Technologies (Wilmington, DE), uses the natural surface tension properties of the sample to hold it in place during the measurement cycle, eliminating the

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collected. Advances in RNA isolation and amplification methods have also greatly improved the ability to generate adequate amounts of material for high quality expression analysis.

While these advances have shown great promise, the success and dependability of these technologies depend greatly on the ability to

need for cuvettes or capillaries. This method has streamlined process control for microgenomic studies.

Methods

The PixCell Iie and the Veritas laser microdissection platforms from Arcturus Bioscience are routinely used to acquire between 50–4,000

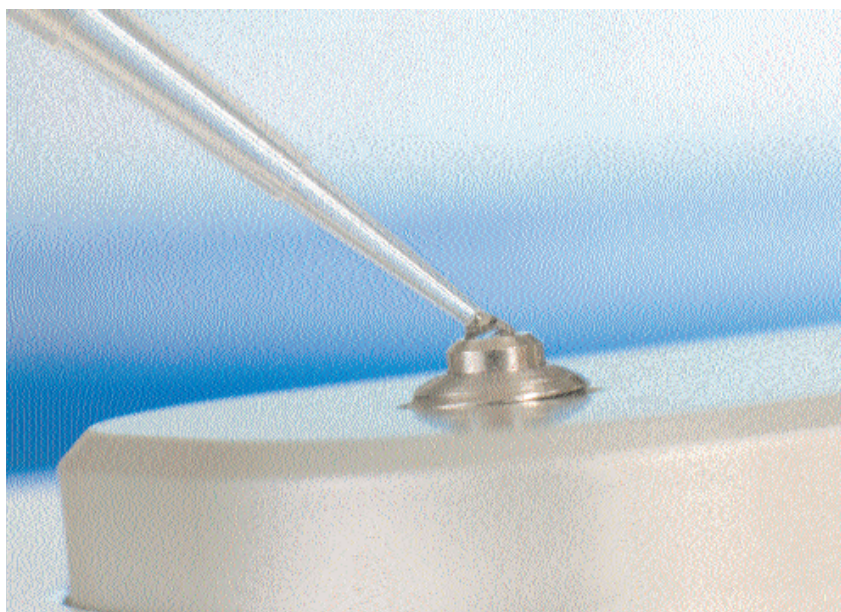


Figure 3a. Loading 1 μ l of sample.



Figure 3b. 1 μ l sample 'bridging' the optical fibers.

cells. LCM allows for the microdissection of a single cell from a heterogeneous cell population, while the UV laser cutting feature of the Veritas provides speed and precision when capturing larger groups of desired cells (Figure 1).

Proper preparation of high quality RNA is critical for adequate production of probe for expression arrays. Isolation and amplification systems, such as the PicoPure RNA isolation kit and the RiboAmp HS RNA

Amplification kit (Arcturus Bioscience), are specifically designed for maximum extraction, isolation and amplification efficiency from limited starting material (Figure 2).

Quantity and purity of the resulting total RNA, as well as amplified RNA product, may be determined using the ND-1000 spectrophotometer (NanoDrop Technologies). In order to take a measurement, 1 μ l of sample is pipetted directly onto the

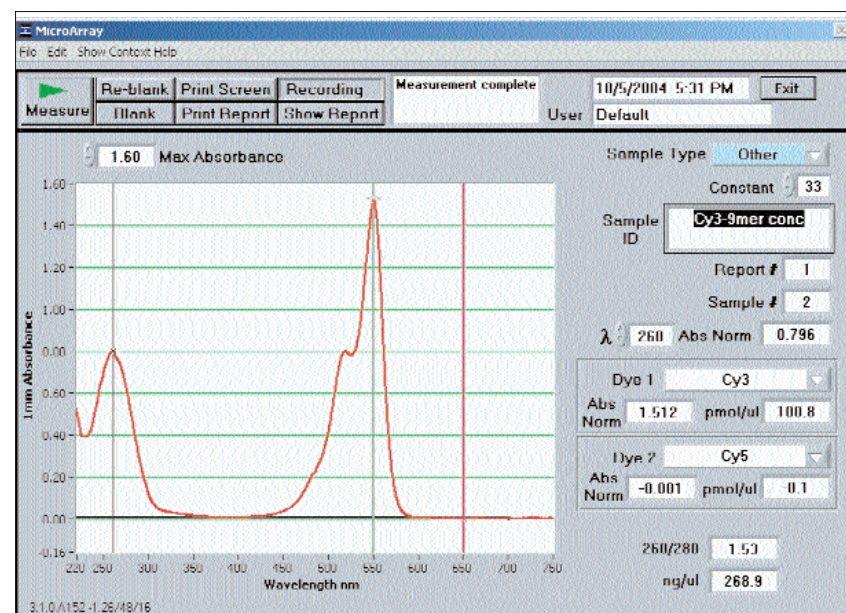


Figure 4. The NanoDrop microarray module displays the full-spectrum of a labeled nucleic acid probe with associated absorbances and calculated concentrations.

lower optical (measurement) surface (Figure 3a). An upper optical fiber automatically engages the sample, forming a liquid column of mechanically-controlled path length (1 mm) and is held in place by the sample's surface tension (Figure 3b).

Preparing the instrument for another measurement simply involves cleaning the sample off with a lab wipe before loading the next sample. Following dye incorporation, the condition of the final product is assessed using the microarray module of the NanoDrop spectrophotometer. This module 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 (Figure 4).

The RNA 6000 PicoLabChip system on the Agilent 2100 bioanalyzer is commonly used to determine RNA probe quality. Using micro-spectrophotometer technology to assess probe quantity and purity, together with lab-on-a-chip technology to assess probe quality, provides unprecedented process control with minimal consumption of limited product.

Conclusion

The development of several micro-genomic technologies may now be used in concert to overcome the inherent limitations posed by extremely minute samples. Although

advanced cell separation methods, such as LCM and expression analysis using microarray technology, laid the foundations of the microgenomics field, adequate quality control techniques were needed to ensure reliable results. Combined use of novel micro-spectrophotometry technology and pre-existing lab-on-a-chip technology enables unprecedented quality control, while taking advantage of breakthroughs in the processing of small, pure cell populations. These quality control assurances provide the confidence needed for scientists to explore the use of microgenomic technologies as a viable means of acquiring expression data from extremely limited cell material.

About the authors

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