# Microdissection to microarrays: a complete solution for gene expression profiling of precious tissue samples

## Introduction

Transcriptional profiling of tissue biopsies using microarrays has become a powerful tool for studying biological mechanisms and their alterations leading to diseases. Laser capture microdissection (LCM) has proven its value as a technique for the isolation of desired cell populations from tissue biopsies, thereby improving the sensitivity of microarray analysis [1,2]. Until recently, the ability to utilize pure cell populations obtained through LCM for microarray analysis has been severely limited by the need for several micrograms of total RNA to perform hybridizations.

The Applied Biosystems™ Complete Systems for microgenomics platform provides researchers with a complete and robust solution for microarray analysis from small samples, including systems for microdissecting pure cell populations from tissue biopsies, isolating high-quality RNA from microscopic samples, amplifying picogram amounts of total RNA isolated from frozen tissue, and nonenzymatic labeling of amplified antisense RNA (aRNA) for microarray hybridizations. This integrated platform includes LCM instrumentation to capture specific cells of interest using a non-damaging near-infrared (IR) laser and incorporates protocols and procedures to analyze the quality of samples at various stages during the process.

The microgenomics platform permits high-efficiency recovery of quality total RNA from as little as a single cell obtained through LCM. High-sensitivity linear amplification of mRNA is possible from as little as 100 pg (10 LCM cells) of total RNA, generating enough aRNA for replicate microarray hybridizations. Linear amplification is followed by non-enzymatic labeling, which allows the use of unlabeled nucleotides during amplification. This significantly reduces the required amount of starting material and results in higher aRNA yields and higher percent present (%P) calls during microarray analysis. The complete microgenomics platform has been validated for use with all common microarray platforms.

Here we report gene expression profiling of human ovarian cancer and human breast cancer cells captured using LCM and ultraviolet (UV) laser cutting (LC) on the Applied Biosystems™ ArcturusXT™ microdissection instrument. Subsequent to LCM, RNA was extracted, linearly amplified, and labeled for microarray hybridization and analysis (Figure 1).

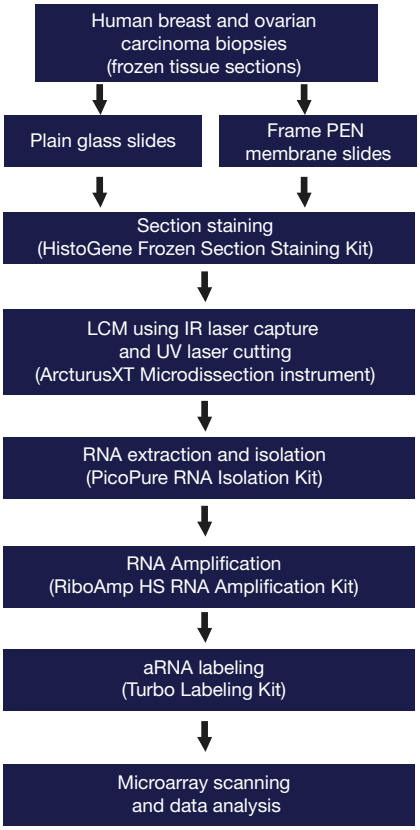


**Figure 1. The microgenomics workflow.**

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## Materials and methods

T sections (8 µm thick) from frozen human breast and ovarian carcinoma biopsies were mounted on either plain glass or frame polyethylene naphthalate (PEN) membrane slides. Following staining with the Applied Biosystems™ HistoGene™ LCM Frozen Section Staining Kit, the slides were loaded onto the ArcturusXT microdissection instrument. Areas of carcinoma cells were microdissected from the sections and collected onto Applied Biosystems™ CapSure™ LCM Caps using LCM only or a combination of LCM and LC. Extraction and isolation of total RNA from each microdissected sample was completed using the Applied Biosystems™ PicoPure™ RNA Isolation Kit. The total RNA recovered was quantified using a Thermo Scientific™ NanoDrop™ ND-1000 Spectrophotometer and quality was assessed using an Agilent 2100 Bioanalyzer™ system (Figure 2). Isolated RNA was amplified using the Applied Biosystems™ RiboAmp™ RNA Amplification Kit, labeled using Applied Biosystems™ Turbo Labeling™ Cy®3 and Cy®5 Kits, and hybridized in duplicate onto Agilent 4 x 44K Human Gene Expression Microarrays. Stratagene Universal Human Reference RNA was used as a control for the two-color hybridizations. The arrays were processed following the manufacturer's recommendations and scanned using an Axon GenePix™ 4000B scanner. Microarray data acquisition and normalization, as well as correlation and cluster analyses, were performed using Axon GenePix™ Pro 6 and Axon Acuity™ 4 software.

## Results

Linear amplification of total cellular RNA from laser capture microdissected

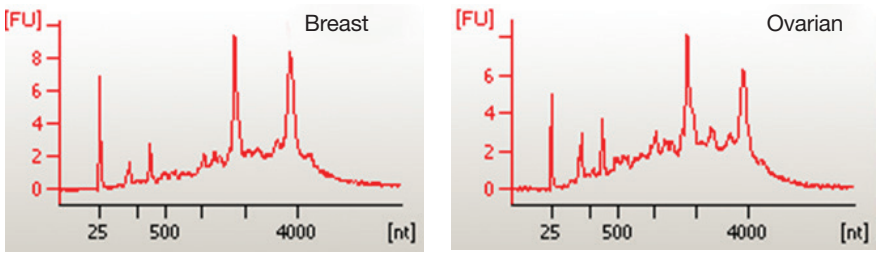


**Figure 2. RNA quality assessment of microdissected material.** Profiles on the Agilent 2100 Bioanalyzer system of total cellular RNA isolated from breast (left) and ovarian (right) cancer samples. The distinct 18S and 28S peaks demonstrate the high-quality total RNA obtained from material microdissected using the ArcturusXT instrument.

**Table 1. aRNA yield and labeling efficiency.** Table shows RNA input amounts used for amplification, aRNA yields after two rounds of amplification using the RiboAmp Kit protocol, and frequency of incorporation (FOI) of Cy®3 and Cy®5 dyes after using the Turbo Labeling Kit on RNA from LCM and LC samples from breast and ovarian carcinomas.

| Tissue type | Sample collection | RNA input for amplification | aRNA yield after 2 rounds of amplification (µg) | Cy3 FOI* | Cy5 FOI |
|---|---|---|---|---|---|
| Breast cancer | LCM | 1 ng | 52.14 | 33.78 | 24.65 |
| | | 1 ng | 32.48 | 32.24 | 24.92 |
| | LC | 1 ng | 31.13 | 33.98 | 24.13 |
| | | 1 ng | 34.94 | 32.72 | 23.50 |
| Ovarian cancer | LCM | 1 ng | 43.04 | 31.91 | 24.85 |
| | | 1 ng | 34.95 | 34.44 | 25.15 |
| | LC | 1 ng | 51.46 | 31.46 | 24.33 |
| | | 1 ng | 45.70 | 34.31 | 21.07 |
| Universal Human Reference RNA | Frozen | 2 ng | 65.89 | 33.93 | 26.45 |

* Frequency of incorporation of dye per 1,000 nucleotides.

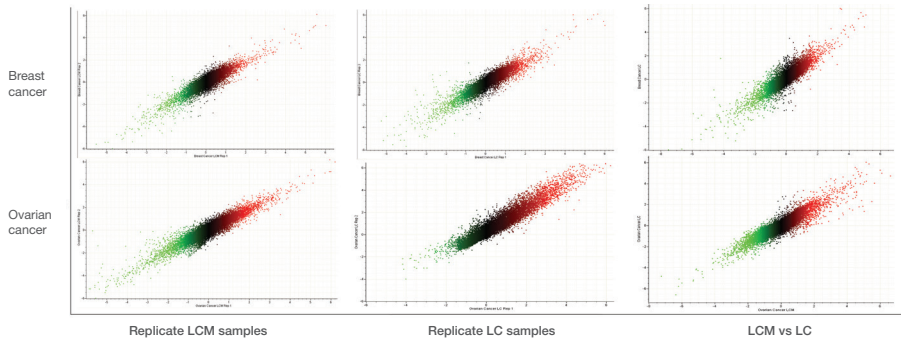


**Figure 3. Microarray scatter plots.** Comparison of normalized gene expression intensity ratios within and between sample collection methods for breast and ovarian cancer biopsies. Normalized $\log_2$ (Cy5/Cy3) ratios of median intensities show a high correlation among replicate LCM and LC samples (R = 0.945–0.961, N = 41,092).

or laser cut carcinoma samples (Table 1) yielded 30–50 µg of aRNA. The aRNA labeling showed high labeling efficiency for Cy3 and Cy5 dyes (Table 1). Analysis of gene expression ratios showed a high level of reproducibility within and among collection types for both tissues (Figure 3). Unsupervised hierarchical clustering of 1,730 genes showing ≥4-fold differential expression showed high concordance within each tissue type, irrespective of collection method used (R ≥0.95, Figure 4). Clustering analysis classified the cancer types into two distinct clusters, highlighting the differences in gene expression patterns between the two cancer types.

## Conclusion

This study shows that LCM using both IR and UV lasers enables the isolation of small quantities of RNA from pure cell populations to provide high-fidelity gene expression data. The data obtained have been used to identify differentially expressed genes in two cancer types, demonstrating biological relevance. We believe that this method, provided using the complete system for microgenomics, will prove to be a valuable tool in enabling drug discovery and clinical research.

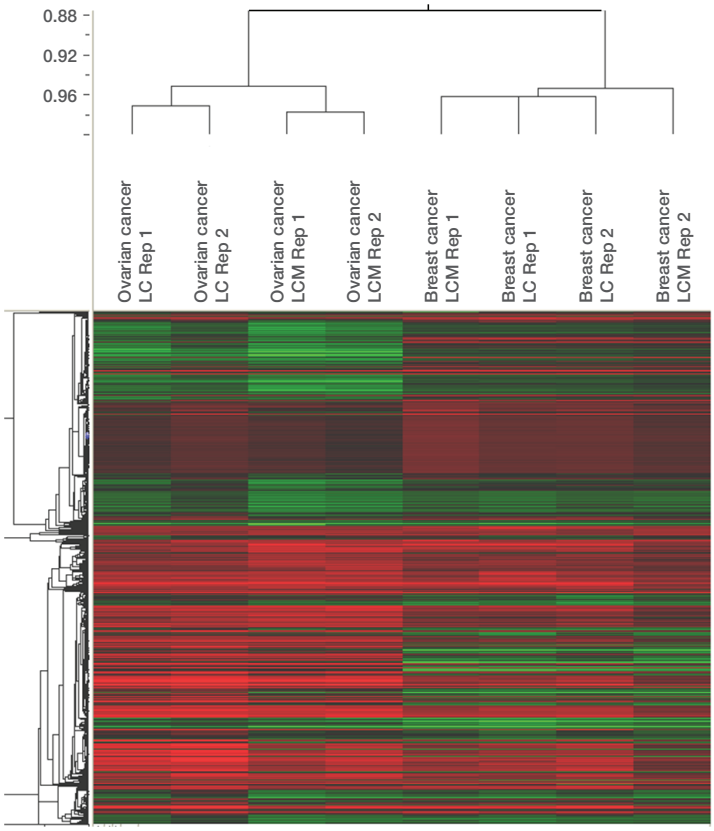


**Figure 4. Hierarchical cluster of microarray data.** Unsupervised hierarchical clustering of 1,730 genes with ≥4-fold differential expression from ovarian and breast carcinoma cells dissected using LC or LCM. Clustering shows high concordance of differentially expressed genes within each tumor type.

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## References

1. Emmert-Buck MR, Bonner RF, Smith PD et al. (1996) Laser capture microdissection. *Science* 274(5289):998–1001.
2. Ma XJ, Salunga R, Tuggle JT et al. (2003) Gene expression profiles of human breast cancer progression. *Proc Natl Acad Sci USA* 100(10):5974–5979.


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