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MICROSCOPES REDEFINED

Microdissection to Microarrays: A Complete Solution for Gene Expression Profiling of Precious Tissue Samples

APPLICATION NOTE #12

INTRODUCTION

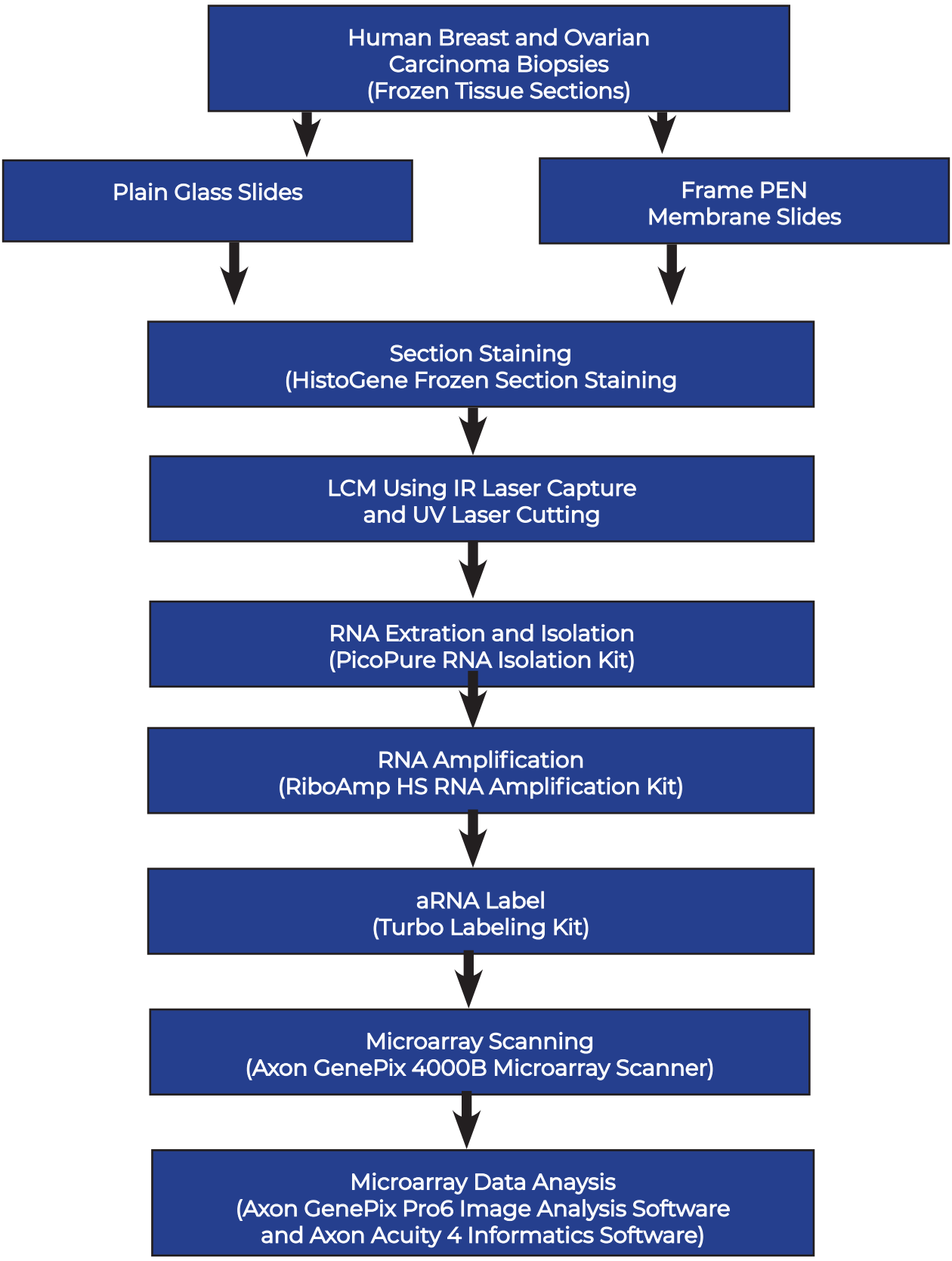
Transcriptional profiling of tissue biopsies using microarrays has become a powerful tool for studying biological mechanisms and their alterations leading to diseases. Laser capture microdissection (LCM) has proven its value as a technique for the isolation of desired cell populations from tissue biopsies, thereby improving the sensitivity of microarray analysis.[1,2] Historically, 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 system for Microgenomics® provides researchers with a complete and robust solution for microarray analysis from small samples, including systems for (a) microdissecting pure cell populations from tissue biopsies, (b) isolating high quality RNA from microscopic samples, (c) amplifying picogram amounts of total RNA isolated from frozen tissue, (d) non-enzymatic labeling of amplified antisense RNA (aRNA) for microarray hybridizations, and (e) scanning arrays and analyzing microarray data. This integrated platform includes LCM instrumentation to capture specific cells of interest using a nondamaging near-infrared (IR) laser and incorporates protocols and procedures to analyze the quality of samples at various stages during the process.

The microgenomics platform ensures 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 picograms (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 %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 laser-cutting (LC) on the LCM microdissection instrument. Subsequent to LCM, RNA was extracted, linearly amplified and labeled for microarray hybridization and analysis. (See Figure 1.)

Microgenomics Workflow (Figure 1)

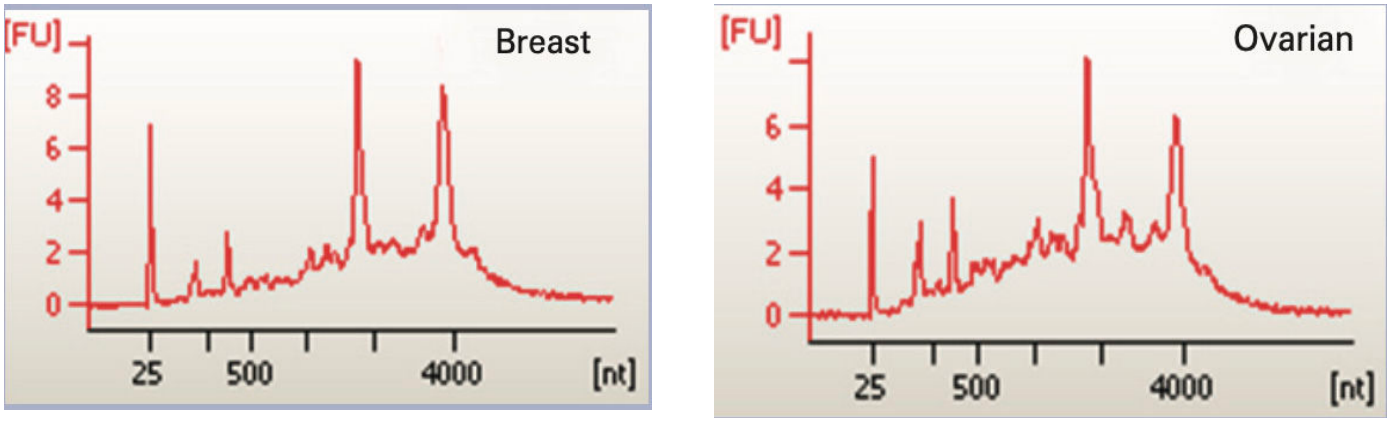


Schematic of the Microgenomics Workflow

MATERIALS AND METHODS

Eight-micron-thick sections from frozen human breast and ovarian carcinoma biopsies were mounted on either plain glass or frame PEN membrane slides. Following staining with the HistoGene® LCM Frozen Section staining kit, the slides were loaded onto the LCM microdissection instrument. Areas of carcinoma cells were microdissected from the sections and collected onto 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 PicoPure® RNA Isolation Kit. The total RNA recovered was quantified using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA) and quality was assessed using an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Isolated RNA was amplified using the RiboAmp® RNA amplification kit, labeled using Turbo LabelingTM Cy3 and Cy5 kits, and hybridized in duplicate onto Agilent 4 x 44K human gene expression microarrays. Universal Human RNA (Stratagene, La Jolla, CA, USA) was used as control for the 2-color hybridizations. The arrays were processed following 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.

RNA Quality Assessment of Microdissected Material (Figure 2)



Bioanalyzer profiles of total cellular RNA isolated from human 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 LCM instrument.

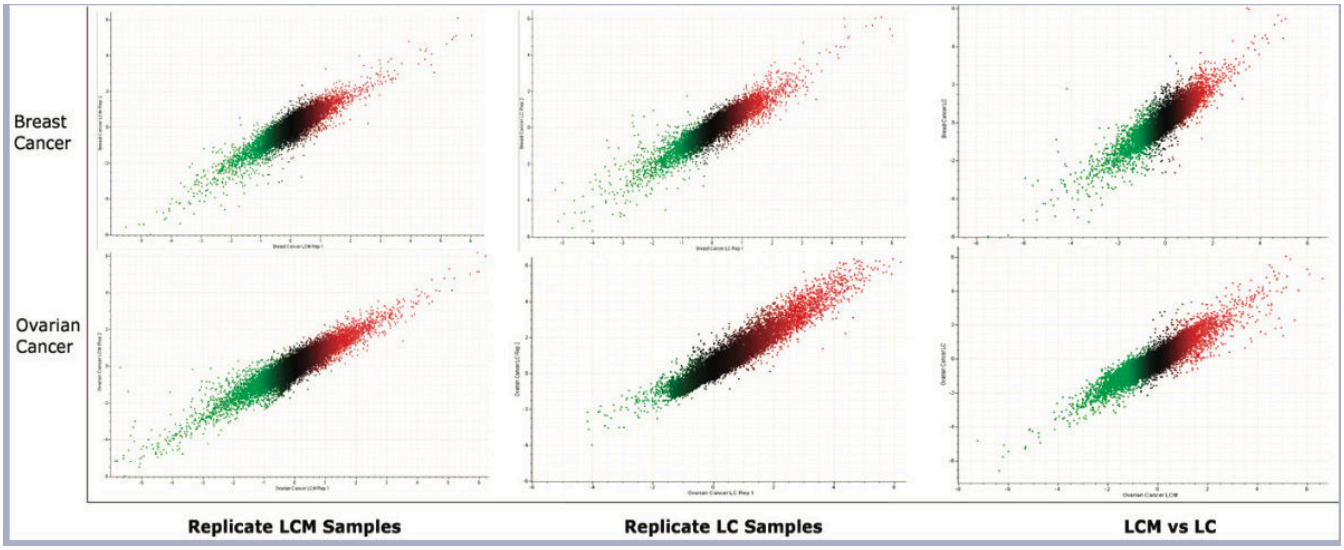
RESULTS

Linear amplification of total cellular RNA from laser capture microdissected or laser cut carcinoma samples (Figure 2) yielded 30–50 micrograms of amplified RNA (aRNA). aRNA labeling showed high labeling efficiency for Cy3 and Cy5 dyes. (See Table 1.) Analysis of gene expression ratios showed a high level of reproducibility within and among collection types for both tissues. (See Figure 3.) Unsupervised hierarchical clustering of 1730 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.

Amplified RNA (aRNA) Yield and Labeling Efficiency (Table 1)

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	24.19
		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	46.70	34.31	21.07
Human Universal RNA	Frozen	2 ng	66.89	33.93	26.45
* Frequency of incorporation of dye per 1000 nucleotides					

table showing RNA input used for amplification, rnA yield after two rounds of amplification using the riboAmp Kit protocol, and frequencies of incorporation (FoI) of cy3/cy5 dyes after using the turbo labeling kit on RNA from LCM and LC samples from human breast and ovarian carcinoma.



Scatter plots of normalized gene expression intensity ratios comparing within and between sample collection methods for human breast cancer and ovarian cancer biopsies. normalized log2 (cy5/cy3) ratio of median intensities show a high correlation among replicate LCM and LC samples (r=0.945–0.961, n=41092).

CONCLUSION

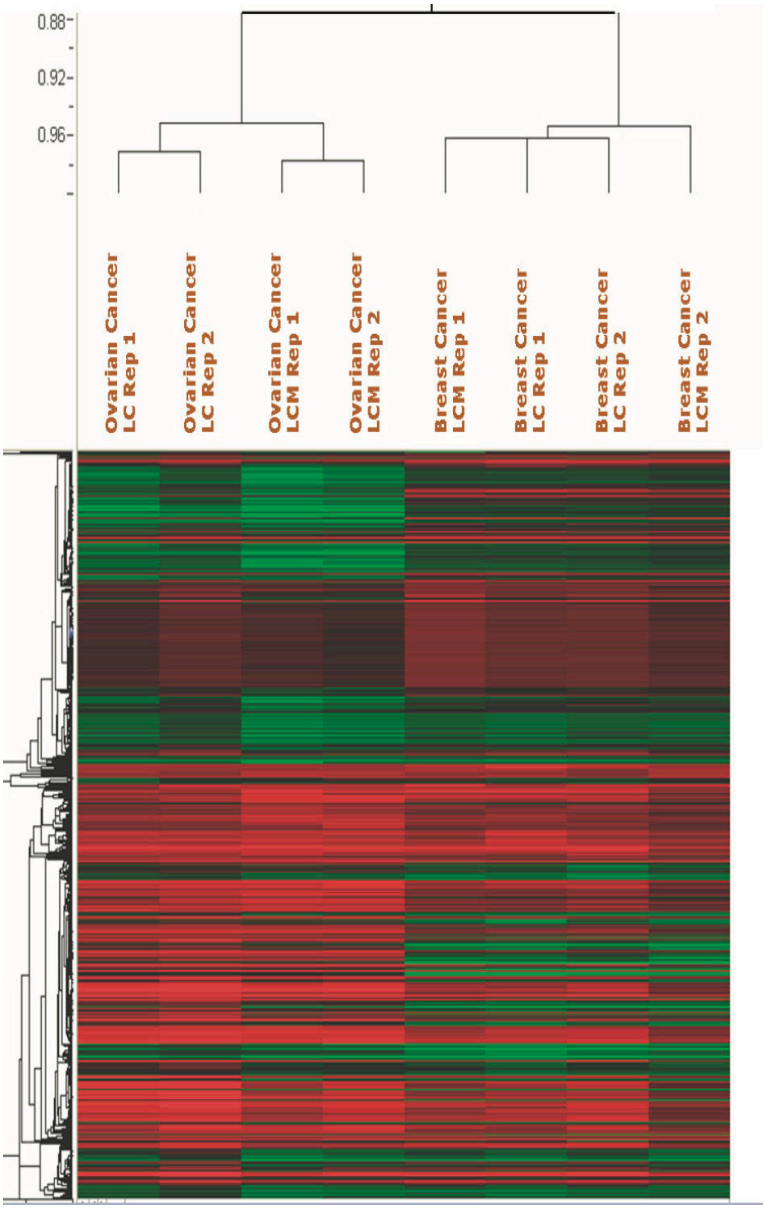
This study has proven the ability to generate high fidelity gene expression data from small quantities of RNA isolated from pure cell populations obtained using IR-enabled laser capture microdissection and UV laser cutting. The data obtained have been used to identify differentially expressed genes in two cancer types, demonstrating biological relevance. We believe that this method, provided through the complete System for Microgenomics, will prove to be a valuable tool in enabling drug discovery and clinical diagnostics.

REFERENCES

1. MichaelR.Emmert-Buck,RobertF. Bonner, Paul D. Smith, Rodrigo F. Chuaqui, Zhengping Zhuang, Seth R. Goldstein, Rhonda A. Weiss, Lance A. Liotta, Laser Capture Microdissection, Science, Vol. 274, 8 November, 1996.

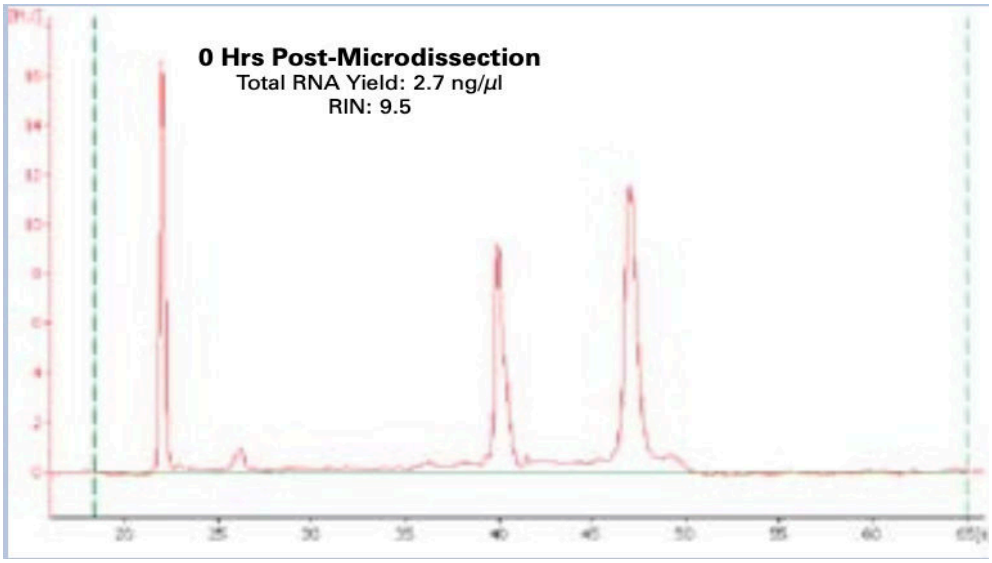
2. XJMa,RSlaunga,JTTuggle,JGaudet, E Enright, P McQuary, T Payette, M Pistone, K Stecker, BM Zhang, YX Zhou, H Varnholt, B Smith, Gadd M, Chatfield E, Kessler J, TM Bare, MG Erlander and DC Sgrio. Gene Expression Profiles of Human Breast Cancer Progression. Proceedings of the National Academy of Science 100:5974-5979, 2003.

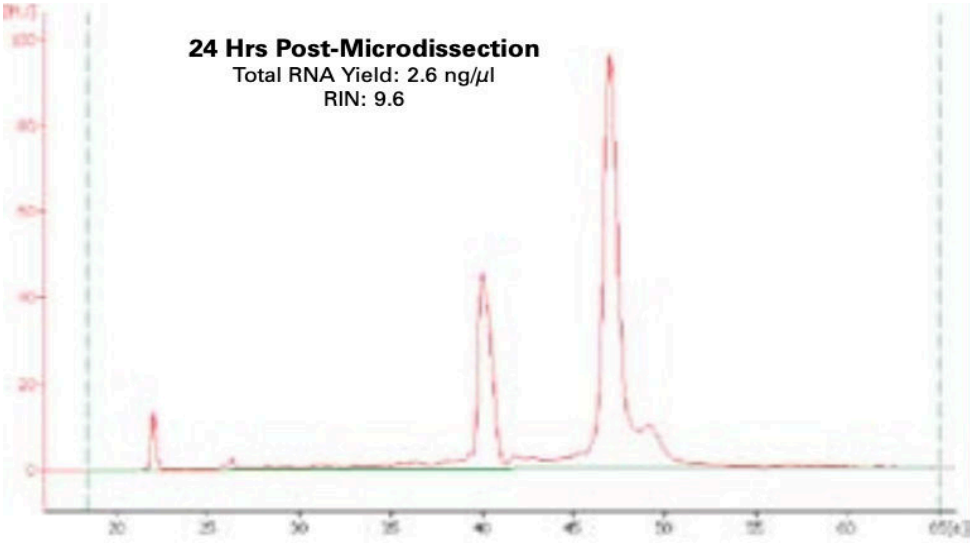
Hierarchical Cluster of Microarray Data (Figure 4)

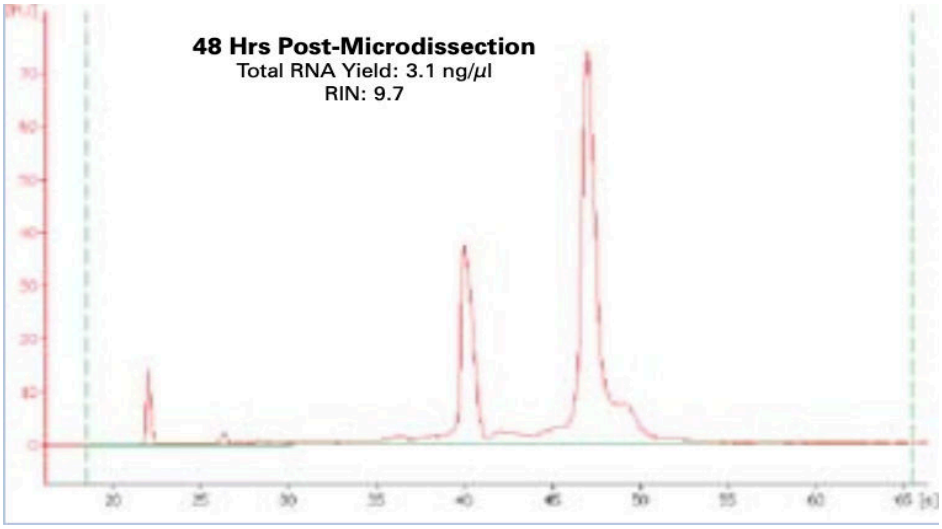


Unsupervised hierarchical clustering of 1730 genes with ≥ 4-fold differential expression from ovarian and breast carcinoma cells dissected using lc as well as lcM. clustering shows high concordance of differentially expressed genes within each tumor type.

Total RNA Assessment, Post-Microdissection (Figure 7)







Live cells were microdissected using the LCM instrument, and total RNA was isolated at 0 hrs, 24 hrs and 48 hrs post-microdissection. Total RNA quality (Agilent Bioanalyzer) and total RNA yield (NanoDrop ND1000) were measured, both demonstrating that the microdissection process does not damage the RNA.

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LCM-MKT-DOC-1700117

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