Introduction
RNA quality plays a major role in the generation of accurate quantitative results from gene expression analysis experiments. cDNA made from RNA that has been degraded will not become amplified or labeled to the same degree as cDNA derived from intact, undegraded RNA. The Experion™ automated electrophoresis system provides an effective method for determining both the quality and quantity of RNA to be used in gene expression analysis experiments using as little as 20 ng total RNA. Here we purposely degrade RNA to varying extents and demonstrate the effects of RNA degradation on quantitative results from gene expression analysis experiments using quantitative PCR (qPCR) and microarray analysis.

Methods
RNA samples (1 mg/ml) prepared from control human liver tissue and from human liver carcinoma cell line HEPG2 were obtained from Ambion, Inc. (now Applied Biosystems, Inc.). RNA was analyzed using the Experion system and the RNA StdSens analysis kit.

Microarray Analysis
Microarrays consisting of long oligonucleotides (MWG-Biotech Inc., Illinica, Ill., and Qiagen Inc.) to detect ~2,000 human genes (1). Five different control liver RNA samples (1 mg/ml) prepared from control human liver tissue and from human liver carcinoma cell line HEPG2 were obtained from Ambion, Inc. (now Applied Biosystems, Inc.). Fluorescently labeled cDNA (5 µg each of Cy3 and Cy5 label) was hybridized to the microarrays as described in Gingrich et al. (2006).

Results
Analysis of RNA With the Experion Automated Electrophoresis System
RNA is susceptible to degradation by endogenous cellular RNases as well as by chemical or heat treatment. To mimic and accelerate these natural processes, we degraded intact commercial RNA preparations over time by incubating them at 90°C in TE buffer. The degree of degradation was monitored using the Experion system (Figure 1). As shown, the 28S/18S rRNA ratio and the size distribution of the smear indicate that RNA was degraded both as RNA became amplified and as RNA remained unamplified (Figure 2). The degradation rate of RNA was calculated for each RNA sample at each time point using the Experion system (Figure 3).

Effects of RNA Degradation on qPCR
To examine the effects of RNA degradation on quantitation of specific gene transcripts, qPCR was performed on aliquots of RNA that had been degraded to various extents. As seen in Figure 2, for each qPCR experiment, the detection of amplified product was seen at successively later PCR cycles, as the RNA is degraded. In qPCR experiments, the Ctt (threshold cycle) number is used to compare the difference in quantity of the amplifying transcript, with a difference of 1 cycle reflecting a 2-fold difference in starting transcript level (assuming 100% amplification efficiency). The Ctt values of the qPCR reactions from the five gene transcripts degraded at different points in time are shown in Table 1. In order to graphically present these data, the proportion of amplifiable RNA remaining was plotted as a function of degradation time (Figure 3). It is clear from the results that comparing qPCR results derived from RNA samples in different states of degradation will result in very different quantitative conclusions. These differences can be as great as 1,000-fold, as seen in Figure 3 with samples subjected to 7 hr of heat degradation.

Conclusions
The Experion automated electrophoresis system provides a quick and effective way to characterize RNA samples prior to gene expression analysis. As RNA becomes degraded, quantitative expression levels determined by qPCR decrease. This can lead to erroneous conclusions regarding levels of gene expression when comparing samples that are degraded to different extents. When degraded RNA is used in gene expression experiments employing microarrays, genes with high levels of expression can still be characterized with respect to relative expression levels. However, degradation compromises the ability to detect differences in expression of genes expressed at low levels. Effects of RNA Degradation on the Ability to Detect Changes in Gene Expression Using Microarrays

For a downloadable version of this poster, visit us on the Web at 6000 James Watson Drive Bio-Rad Laboratories, Inc. Life Science Group

References

Table 1: Percent of RNA remaining in qPCR. cDNA was prepared from five RNA samples that were degraded at varying times. RNA samples were analyzed in duplicate using the Experion system with the RNA StdSens analysis. Data shown are representative of three independent times at each condition. Conditions: 0 hr = intact; 1,000 µg = degraded over 5 hr.

<table>
<thead>
<tr>
<th>Condition</th>
<th>1 hr degradation</th>
<th>5 hr degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>5 hr</td>
<td>100%</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 3. Time courses of degradation of specific liver carcinoma transcript sequences (pairs of spots on the far left and far right of the microarray). The gene expression microarray results obtained from these subarrays (top) show that, excluding the control spots on the bottom two rows of each subarray, the signal in the microarray hybridized with probe derived from intact RNA was uniformly higher than that of the microarray hybridized with probe derived from the degraded RNA. This difference in hybridization signal is reflected in the scatter plots for the entire microarray (bottom) and correlates with the decrease in amplifiable transcripts expected from the degraded RNA sample as evaluated on the Experion system (see Figure 3).

A number of specific gene array elements are highlighted in the scatter plots to demonstrate the utility of data derived from degraded RNA. For example, there are a number of array elements that contain the actin transcript (shown in pink, Figure 5, bottom). The data indicate that for a highly expressed gene such as actin, the relative expression levels between two samples can still be determined with degraded RNA, but with significantly higher variability. Also indicated in Figure 5(bottom) are signal levels from other transcripts present in only a single array element. The signal levels and data ratio for these genes are shown in Table 2. For actin and other genes that are highly expressed, the array data still show a comparable level of differential expression. In contrast, for genes expressed at lower levels, it is no longer possible to detect the transcript when the RNA is degraded, as the signals generated by these spots are too close to the background noise level of the microarray. For genes with low expression levels, the relative level of expression for intact and degraded RNA is not as detailed, indicating a poor signal from degraded RNA hydrolyzed RNA samples hybridized with probes derived from degraded RNA.

Table 2: Signal levels and ratios of genes expressed to different levels in microarrays. I, intact; D, degraded.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Signal Level</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC</td>
<td>10,000</td>
<td>500</td>
</tr>
<tr>
<td>ATP</td>
<td>5,000</td>
<td>10</td>
</tr>
<tr>
<td>HPRT</td>
<td>100</td>
<td>1</td>
</tr>
</tbody>
</table>

Conclusions
Effects of RNA Degradation on the Ability to Detect Changes in Gene Expression Using Microarrays

We further examined the effect of RNA degradation on differential gene expression data derived from spotted oligonucleotide microarrays. In these experiments, differential gene expression was demonstrated from a pair of microarrays in which gene expression levels were compared from control human liver tissue to those from a human liver carcinoma cell line. In one experiment, the RNA from both sources was intact; in the other microarray, both RNA samples were degraded for 3 hr by heating to 90°C in TE. The RNA degradation level was first evaluated using the Experion system (Figure 4).

The gene expression microarray results obtained from these RNA samples are shown in Figure 5. Equivalent microarray subarrays (top) show that, excluding the control spots on the bottom two rows of each subarray, the signal in the microarray hybridized with probe derived from intact RNA was uniformly higher than that of the microarray hybridized with probes derived from the degraded RNA. This difference in hybridization signal is reflected in the scatter plots for the entire microarray (bottom) and correlates with the decrease in amplifiable transcripts expected from the degraded RNA sample as evaluated on the Experion system (see Figure 3).

A number of specific gene array elements are highlighted in the scatter plots to demonstrate the utility of data derived from degraded RNA. For example, there are a number of array elements that contain the actin transcript (shown in pink, Figure 5, bottom). The data indicate that for a highly expressed gene such as actin, the relative expression levels between two samples can still be determined with degraded RNA, but with significantly higher variability. Also indicated in Figure 5(bottom) are signal levels from other transcripts present in only a single array element. The signal levels and data ratio for these genes are shown in Table 2. For actin and other genes that are highly expressed, the array data still show a comparable level of differential expression. In contrast, for genes expressed at lower levels, it is no longer possible to detect the transcript when the RNA is degraded, as the signals generated by these spots are too close to the background noise level of the microarray. For genes with low expression levels, the relative level of expression for intact and degraded RNA is not as detailed, indicating a poor signal from degraded RNA hydrolyzed RNA samples hybridized with probes derived from degraded RNA.