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 present 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) prepared from control human liver tissue and from human liver carcinoma cell line HEPG2 were obtained from Ambion, Inc. (Invi. Applied Biosystems, Inc.). RNA was analyzed using the Experion System and the RNA StdSens kit.

**Microarray Analysis**

Microarrays consisting of long oligonucleotides (MWG-Biotech Inc., Illinoinc., Il., and IgaGen Inc.) to detect ~2,000 human genes and a series of control spots were fabricated as described in Katak et al. (2004). Fluorescence labeled cDNA (5 µg each of Cy3 and Cy5 label) was hybridized to the microarray 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 decreases over time as the smear of RNA decreases as degradation progressed.

Effects of RNA Degradation on qPCR

To examine the effects of RNA degradation on quantitative results of specific gene transcripts, qPCR was performed on equivalent amounts of RNA that had been degraded to varying extents. As seen in Figure 2, for each qPCR experiment, the detection of amplified product is seen at successively later cycles as the RNA is degraded over time. In qPCR experiments, the C_{T} (threshold cycle) number is used to compare the efficiency of RNA hybridization and the difference in the number of starting transcript with a difference of 1 cycle reflecting a 2-fold difference in starting transcript level (assuming 100% amplification efficiency). The C_{T} 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 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.

**Table 1.** Table of signal levels and ratios of genes expressed to different levels in microarrays.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Control Liver</th>
<th>Tumor</th>
<th>Control Liver</th>
<th>Tumor</th>
<th>Control Liver</th>
<th>Tumor</th>
<th>Control Liver</th>
<th>Tumor</th>
<th>Control Liver</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>hspc085</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Cyt c</td>
<td>8,167</td>
<td>1,339</td>
<td>6.10</td>
<td>6.10</td>
<td>6.10</td>
<td>6.10</td>
<td>6.10</td>
<td>6.10</td>
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<td>6.10</td>
</tr>
<tr>
<td>D14683</td>
<td>40,772</td>
<td>16,253</td>
<td>2.51</td>
<td>2.51</td>
<td>2.51</td>
<td>2.51</td>
<td>2.51</td>
<td>2.51</td>
<td>2.51</td>
<td>2.51</td>
</tr>
<tr>
<td>Actin</td>
<td>6,892 (avg)</td>
<td>7,665 (avg)</td>
<td>0.94 (avg)</td>
<td>0.94 (avg)</td>
<td>0.94 (avg)</td>
<td>0.94 (avg)</td>
<td>0.94 (avg)</td>
<td>0.94 (avg)</td>
<td>0.94 (avg)</td>
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</table>

**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.

**References**


