A COMPARISON OF DIFFERENTIAL GENE EXPRESSION LEVELS IN NON-AMPLIFIED AND AMPLIFIED RNA USING THE OVATION™ AMINOALLYL SYSTEM

BACKGROUND

Global gene expression analysis using limited amounts of RNA prepared from biopsies, stem cells and other types of small samples requires high fidelity amplification of mRNA prior to microarray or other forms of analysis. Maintenance of the relative expression levels of mRNA found in vivo within the amplified products is essential to achieving reliable gene expression measurements.

This is the third report on results from a series of studies designed to examine the precision, accuracy and reproducibility of the RNA amplification process used in the Ovation™ Aminoallyl System. Using real-time PCR, we examine the accuracy of the Ovation™ Aminoallyl System by measuring relative expression levels of a panel of genes in both amplified and non-amplified RNA.

MATERIALS AND METHODS

Independent 20 ng total RNA samples were amplified with the Ribo- SPIA™ process using the reagents and protocols provided in the Ovation™ Aminoallyl System (NuGEN, cat. #2101-12). The samples were Universal Human Reference RNA (UHR, Stratagene cat. # 74000), a mixture of total RNA from ten cancer cell lines, and skeletal muscle total RNA, obtained from BD Biosciences-Clontech. Duplicate reactions were used for each sample type.

An aliquot of each amplified cDNA was diluted 1:100 with 1x TE for realtime PCR analysis (SYBR Green). In parallel, total RNA was reverse transcribed and used as non-amplified material for real-time PCR analysis.

Real-time PCR was performed first on a panel of 34 genes. This panel was subsequently expanded to include a total of 68 genes with different abundance levels. Primers were designed using PrimerExpress, Version 2.0.0 (Applied Biosystems) and synthesized by Integrated DNA Technologies, Inc. (IDT).

Contact NuGEN Technical Services **(techserv@nugeninc.com)** for details on primer sequences. Real-time QPCR analysis was performed according to QIAGEN's QuantiTect SYBR Green PCR handbook.

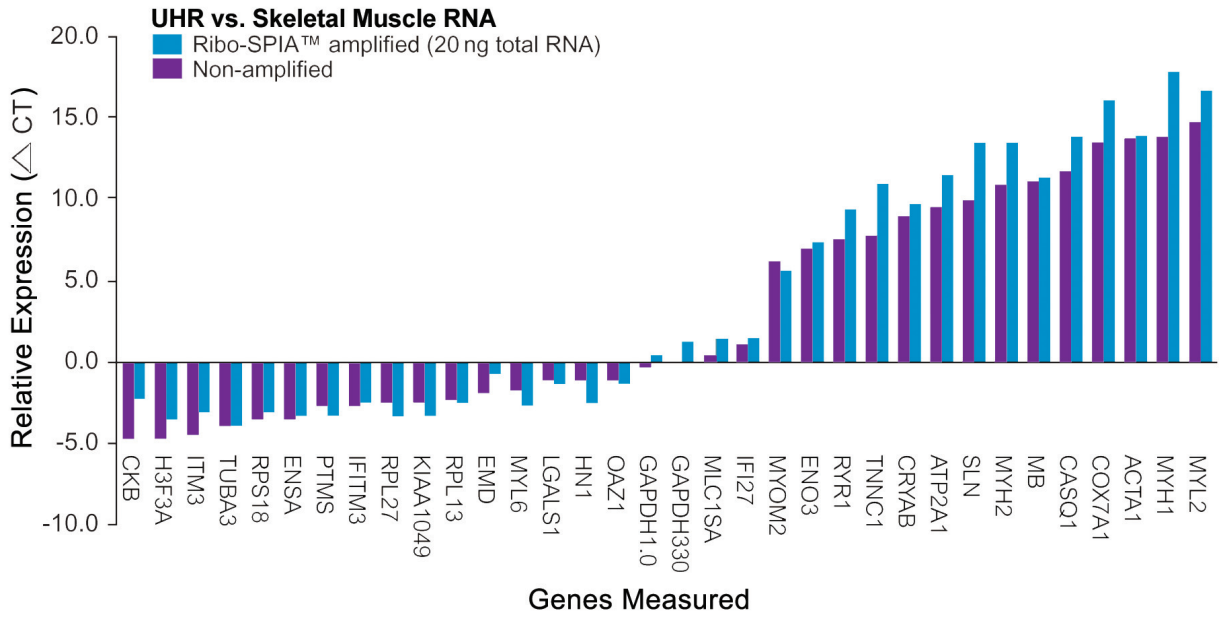

Figure 1. **Ribo-SPIA™ Amplification Maintains Relative Expression Levels**
The graph shows the changes in expression of selected genes both before (non-amplified) and after Ribo-SPIA™ amplification as measured by real-time PCR. The high level of agreement in changes in expression between the two samples demonstrate that Ribo-SPIA™ amplification accurately maintains the relative expression levels of different transcript species.

RESULTS AND CONCLUSIONS

The Ovation™ Aminoallyl System provided a high degree of fidelity of representation of relative gene abundance levels following amplification that was independent of sample type and gene abundance levels (Figure 1).

The panels of genes selected for measurement by real-time PCR in this study spanned low to high abundance levels of differentially expressed genes in both UHR and skeletal muscle RNA.

Correlation in differential gene expression between the two tissues in non-amplified and amplified RNA is presented in Figure 2.

Relative expression using the expanded panel of 68 genes is presented as the difference in cycle thresholds (ΔCT) in real-time PCR.

Linear regression analysis revealed a high level of correlation (R^2 = 0.95) indicating that relative gene expression levels are maintained with amplification using the Ovation™ Aminoallyl System.

Maintenance of relative gene abundance levels across a broad spectrum of genes is essential in any gene expression methodology requiring amplification of the transcript RNA.

The Ovation™ System's novel amplification process maintains the relative gene expression level, providing assurance that expression data following amplification is a true picture of the state of gene expression in the starting material.

System Specifications

Cat No.: 2101-12, 12 reactions

Input: 5-100 ng total RNA

Yield: 5-10 µg single stranded cDNA

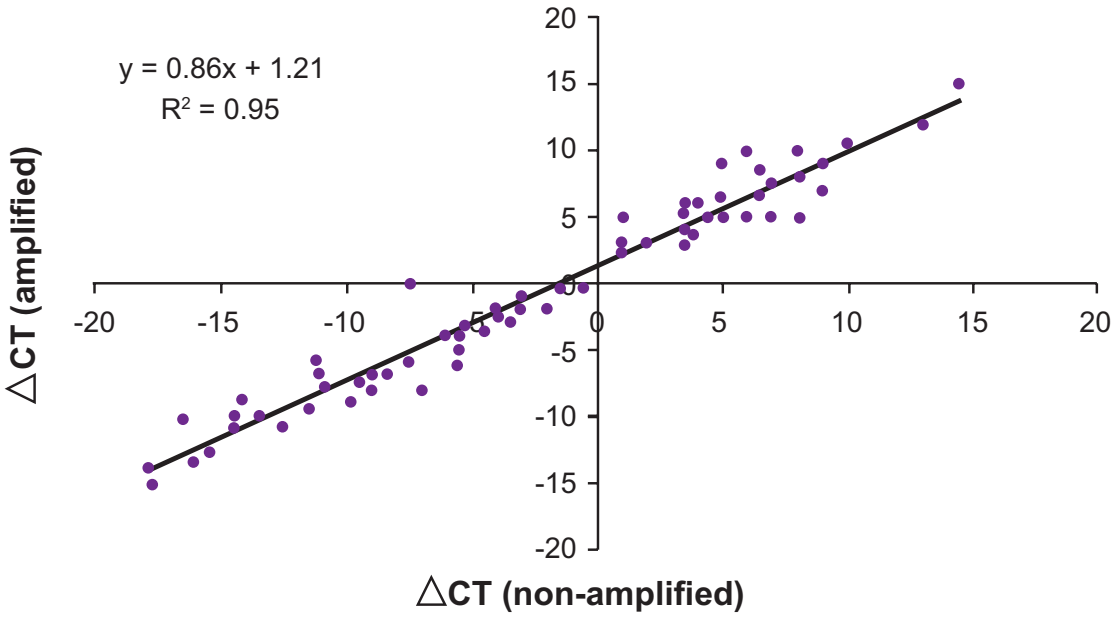

Figure 2. **Correlation of Gene Expression Levels in Non-Amplified and Ovation™ Aminoallyl System-amplified RNA.** Differences in relative gene expression between UHR and skeletal muscle RNA are presented as delta (Δ) CT. Log2 values of ΔCT for non-amplified RNA and amplified RNA are plotted on the x and y axis, respectively. A high level of correlation (R^2=0.95) was obtained.

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