

## REPRODUCIBILITY OF MICROARRAY AND QUANTITATIVE PCR RESULTS FROM NANOGRAM SAMPLES OF RNA AMPLIFIED USING THE OVATION™ AMINOALLYL SYSTEM

### BACKGROUND

Messenger RNA from nanogram amounts of total cellular RNA must be amplified before differential gene expression analysis can be performed on microarrays. The amplification process must be exceptionally reproducible; otherwise signal intensity differences observed after microarray hybridization may be due to amplification-to-amplification variation, rather than from real differences in gene expression.

The Ovation™ Aminoallyl System uses the Ribo-SPIA™ process, an extraordinarily fast and simple linear amplification method for generating micrograms of cDNA from a few nanograms of total RNA. (For more information, visit the NuGEN website at [www.nugeninc.com](http://www.nugeninc.com)). This study examines the reproducibility of the Ribo-SPIA™ process by measuring the outcome of parallel amplification of independent biological replicates followed by microarray hybridization.

### MATERIALS AND METHODS

Eight separate 20 ng total RNA samples were amplified with the Ribo-SPIA™ process using the reagents and protocols provided in the Ovation™ Aminoallyl System kit (NuGEN cat. #2101-12). Four samples were Universal Human Reference RNA (UHR, Stratagene cat. #74000), a mixture of total RNA from ten cancer cell lines, and four samples were a mixture of colon, heart, liver, and skeletal muscle (CHLS) RNA, obtained as individual RNAs from BD Biosciences-Clontech.

To assess amplification-to-amplification consistency, the yield of amplified cDNA product from each of the

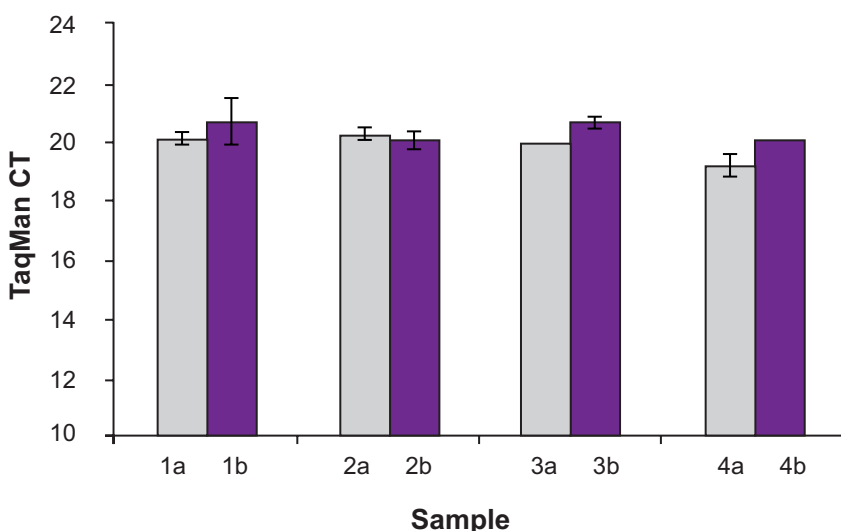
eight samples was determined spectrophotometrically. Then a portion of each amplified cDNA product was diluted 1:10 and duplicate TaqMan assays were performed for the GAPDH gene according to the Applied Biosystems User Guide [Forward Primer: GAGTCAACGGATTTGGTCGTATT; Probe: CCTGGTCACCAGGGCTGCTT; Reverse Primer: GAATTTGCCATGGTGAAT; amplicon size 143 bp located 1,040 bp from the poly (A) tail at the 3' end].

Two micrograms of each of the four UHR amplified cDNA samples were coupled to Cy5 and the four amplified CHLS cDNA samples to Cy3 (Cy3 Mono-Reactive Dye Pack, Amersham, cat. #PA23001; Cy5 Mono-Reactive Dye Pack, Amersham, cat. #PA25001) following the recommendations in the Ovation™ User Guide. The UHR and CHLS cDNA samples were then paired

and mixed together to create a total of four UHR-CHLS samples. Labeled UHR-CHLS samples were co-purified over QIAquick columns (Qiagen, cat. #28104) and concentrated using Microcon YM-30 columns (Millipore, cat. #4241).

To evaluate microarray result reproducibility between samples, an amount equivalent to 2 µg of each of the four cye dye-labeled UHR-CHLS cDNA samples was hybridized to a 22,000-feature, 60-mer oligonucleotide microarray (Agilent cat. #G4110A) at 60°C for 16 to 18 hours and washed using protocols provided by the array manufacturer.

Hybridized arrays were scanned on a GenePix 4000B Scanner (Axon Instruments) and fluorescent intensities determined using GenePix 4.1 software. Correlation of the average log intensities



**Figure 1. Sample-to-Sample Amplification Reproducibility Measured by TaqMan Analysis.** The amount of an amplified sequence of GAPDH gene was measured in each of eight amplified cDNA samples generated from 20 ng total RNA [four UHR (a) and four CHLS (b)]. The average cycle threshold (CT) of two replicates per sample is shown. Bars indicate one standard deviation. The average cDNA yield from all samples was 5.3 µg ± 0.5.

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