TECHNICAL REPORT

Combining NuGEN's Whole Transcriptome Amplification with the Fluidigm BioMark™ Real-Time PCR System for Ultimate Flexibility in Transcriptome Analysis

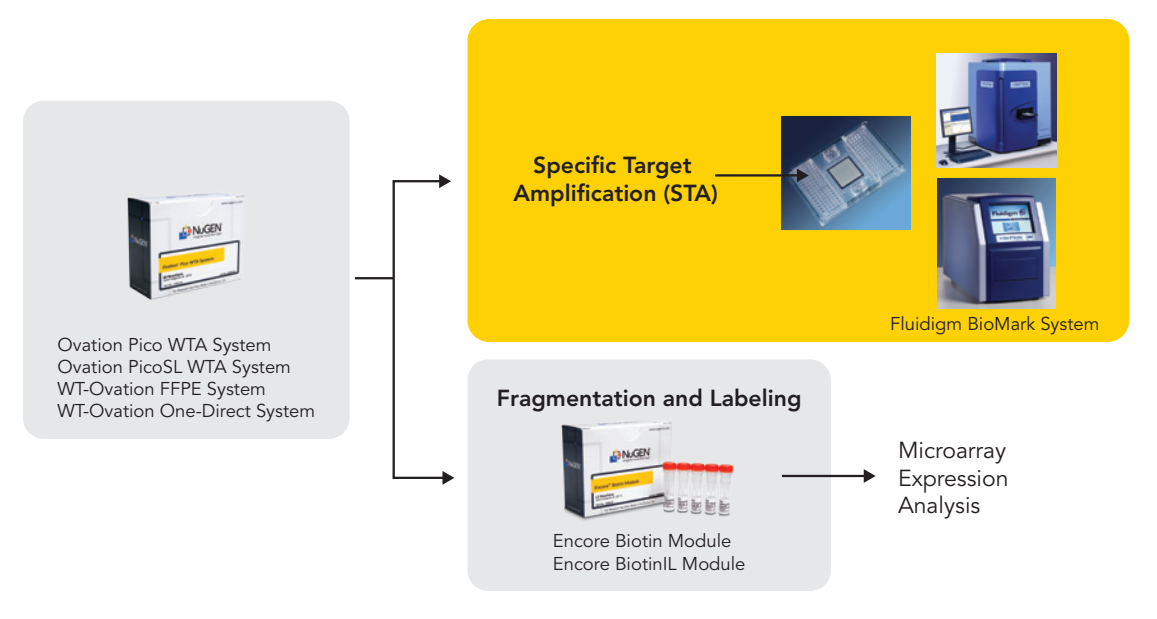
Introduction

Gene expression analysis is currently used routinely for basic research and translational applications. As researchers continue to adopt this type of analysis for broader use, their requirements for sample preparation and analysis solutions have evolved to include a desire for increased flexibility in conducting analysis on multiple platforms, and higher sample throughput, reduced starting materials to preserve precious clinical samples, as well as reduced cost/data point.

To better support the evolving needs of researchers, NuGEN and Fluidigm collaborated with mutual customers to demonstrate a workflow that combines NuGEN's innovative sample preparation reagents with the Fluidigm BioMark System for high-throughput, nanoliter volume qPCR. This workflow utilizes the Ribo-SPIA® Whole Transcriptome Amplification (WTA) technology from NuGEN, and the Dynamic Array™ IFCs from Fluidigm, providing versatility for greatly reduced sample input, while maintaining high data quality, increased versatility of the number of genes analyzed downstream, and flexibility in the type of analysis conducted.

This workflow employs one of NuGEN's Ovation® RNA Amplification Systems for small and degraded samples. The resulting amplified antisense single-stranded cDNA representing the entire transcriptome can then be subject to Specific Target Amplification (STA) System for a selected subset of genes of interest for expression quantitation on the Fluidigm BioMark System. Alternatively or in parallel, the amplified cDNA from the NuGEN system can be fragmented and labeled by one of the Encore Modules for whole transcriptome analysis on microarrays.

Figure 1. Integrated NuGEN and Fluidigm Workflow for Ultimate Flexibility in Input Sample Choice and Downstream Analysis



NuGEN® imagine more from less™

By using NuGEN's WTA system in tandem with Fluidigm's STA, two major experimental concerns are addressed:

1. **Variability in the amount and type of starting material.** This workflow enables the analysis of extremely small or degraded samples.
2. **Depleting precious clinical samples.** The amplified cDNA from WTA may be preserved for future analysis on additional panels of genes or on other expression profiling platforms such as microarrays, providing more molecular insight into the same samples.

Detailed methods and results from this proof-of-principal study are described in this Application Note as an illustration of the integrated workflow from sample to analytical results.

Results

Using WTA Followed by STA Generates Highly Reproducible TaqMan® Results

Many clinical studies rely on biopsies that are limited in quantity and quality. NuGEN's Ovation Systems utilize an isothermal linear amplification Ribo-SPIA technology that enables analysis of samples down to a few cells in quantity, or highly degraded samples such as those obtained from FFPE blocks.

To examine the suitability of conducting whole transcriptome amplification prior to TaqMan analysis on the Fluidigm BioMark system, a mouse model system was used and samples were obtained from three distinct groups of animals:

Group A: Naïve animals as control

Group B: Sick animals

Group C: Sick animals treated with the compound

This animal model has been characterized extensively by our customer with great knowledge of the gene expression profile changes related to the treatment. A panel of 48 genes was selected based on prior analysis with microarrays and analyzed against triplicate samples from each biological group.

Four workflows were compared in this study on three 48.48 Dynamic Array chips:

cDNA: The isolated total RNA was reverse transcribed to cDNA and directly analyzed at either 250 ng/µL or 500 ng/µL concentration without any pre-amplification.

WTA: Ovation Pico WTA System (input of 500 pg – 50 ng total RNA) was used initially to amplify the total RNA. The amplified cDNA (output of 6–10 µg cDNA) was diluted 1:20 and analyzed on the Dynamic Array chips.

WTA+STA: The total RNA was amplified first with the Ovation Pico WTA System followed by STA with gene-specific pre-amp primers recommended by Life Technologies. The resulting cDNA was then analyzed on the BioMark System.

STA: The total RNA was used for gene-specific pre-amp directly without WTA.

As shown in the Heat Map (**Figure 2**), consistent detection of the 48 genes (in 48 columns) in biological triplicates (various rows) is apparent for the WTA+STA and STA alone workflow. Both the cDNA alone and direct WTA samples failed to generate consistent results. It is possible the concentration of individual genes was below the detection limit with the cDNA and WTA methods (see **Figure 2**).

The reproducibility was further quantified by looking at specific example genes. Three genes were selected that represent down-regulation in Group B samples

FIGURE 2. **Heat Map of 48 Genes TaqMan Assays Across Biological Triplicates from A, B, and C Groups**

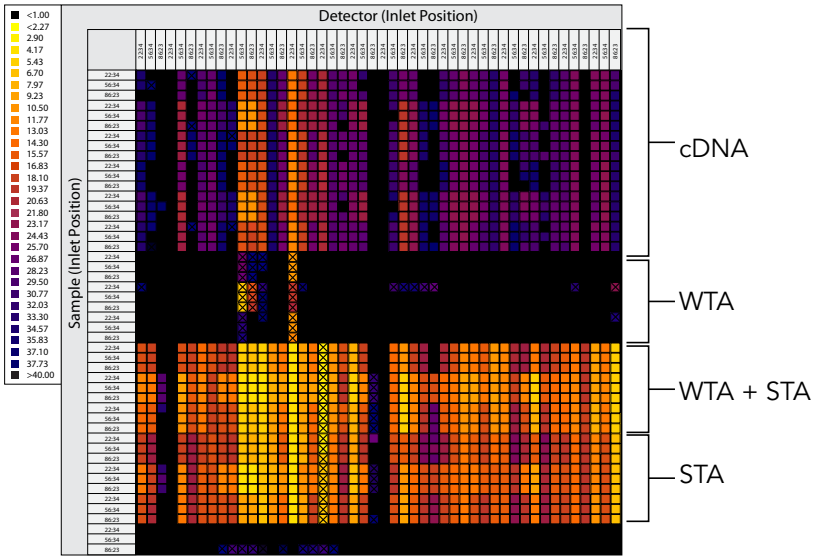
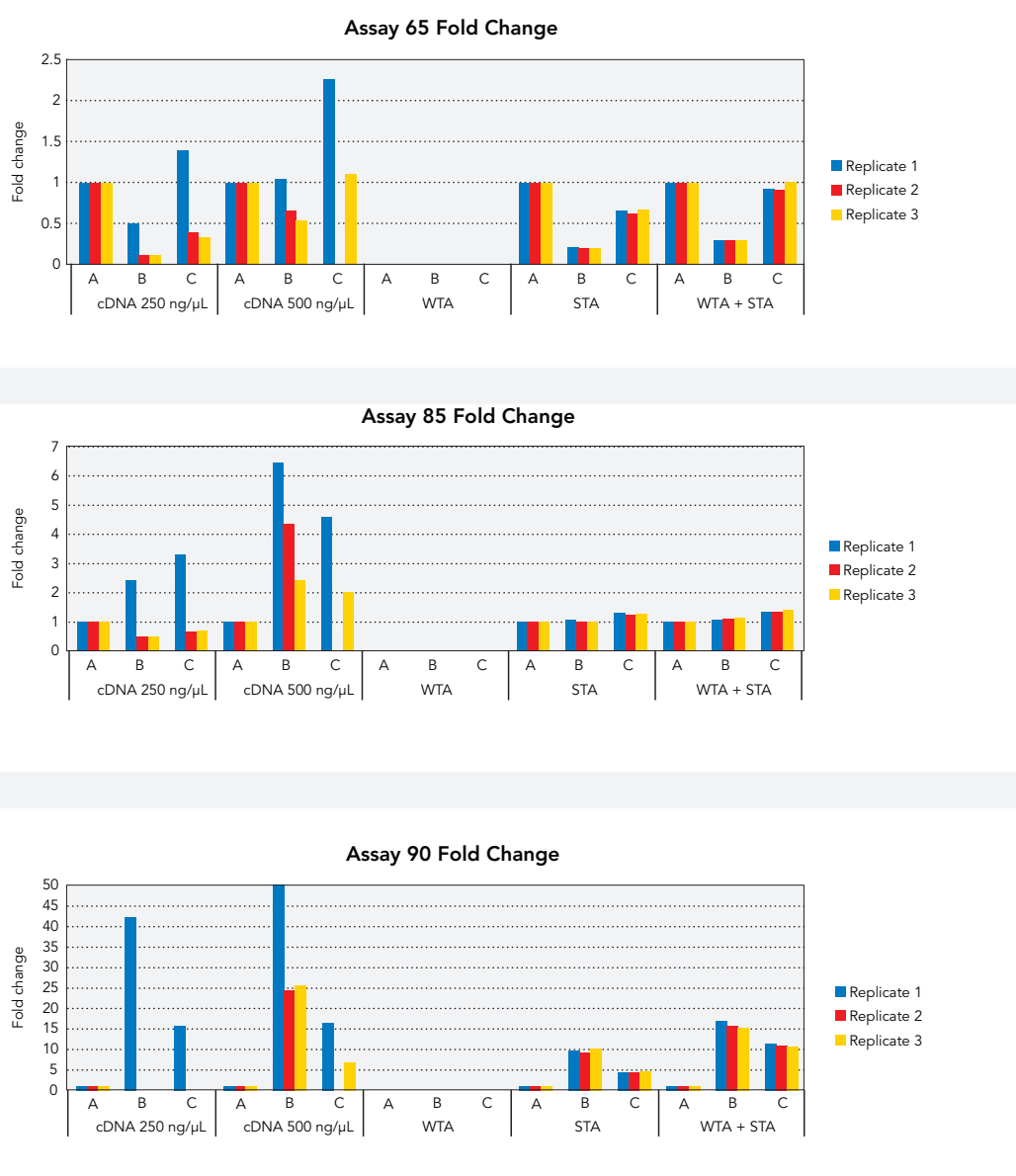


FIGURE 3. **TaqMan Fold Change Across Example Assays and Biological Replicates**



(Assay 65 in **Figure 3**), up-regulation (Assay 90) and no change (Assay 85). As shown in **Figure 3** and consistent with the heat map, both WTA+STA and STA alone samples demonstrated tight reproducibility across biological replicates whereas the other workflows did not generate the same consistent results.

To validate the accuracy of the results, selected assays (Assay 49 and Assay 57 normalized to control Assay 96) from Group A samples were also run on the 7900 System (from Life Technologies). As shown in **Figure 4**, the replicate normalized C_t values are consistent across the groups and compared to the 7900 System results for both the WTA+STA and STA alone workflows illustrating the accuracy of the results.

In conclusion, the use of WTA with NuGEN's Ovation Pico WTA Systems produces accurate and reproducible results compared to performing gene-specific STA alone and comparable to the data obtained on the 7900 System. This WTA+STA workflow enables flexible and large-scale

expression analysis from limited clinical samples on multiple analysis platforms or addition of new panels of genes from extremely small amounts of starting materials.

Using WTA Followed by STA Generates Highly Reproducible SYBR® Green Results

As an alternative to TaqMan, SYBR Green assays can also be run on the BioMark System. A similar panel of SYBR Green assays was analyzed by first filtering by those assays that produced a single melting curve to eliminate those assays that produced multiple PCR products in the reaction. As a result, a significantly lower number of genes were included in the final analysis.

Similar results were obtained as the TaqMan assay described above. More consistent detection of gene expression levels across replicates was evident for both the WTA+STA and STA alone workflows as shown in **Figure 5**.

Conclusions

Data obtained using the mouse model system demonstrated that a workflow employing NuGEN's Ribo-SPIA isothermal linear whole transcriptome amplification prior to gene-specific amplification and detection on the BioMark system generated highly reproducible and accurate TaqMan and SYBR Green results. The results were comparable to those obtained with STA alone on the BioMark system and to reference TaqMan assays on the 7900 Systems.

Related NuGEN Products

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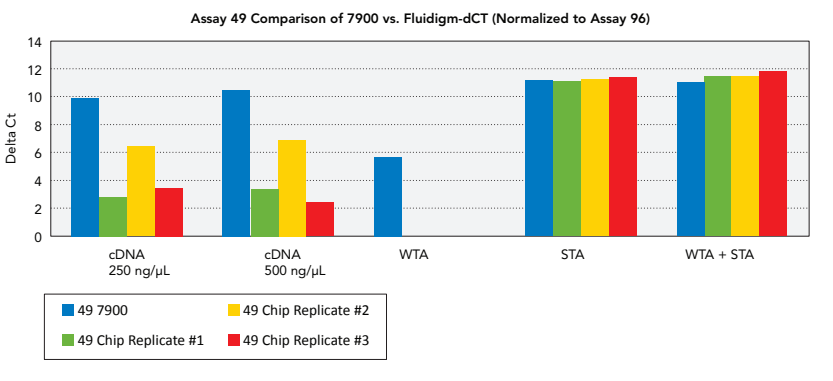
FIGURE 4. **Example TaqMan Assays Comparing BioMark Results with the 7900 System Data**



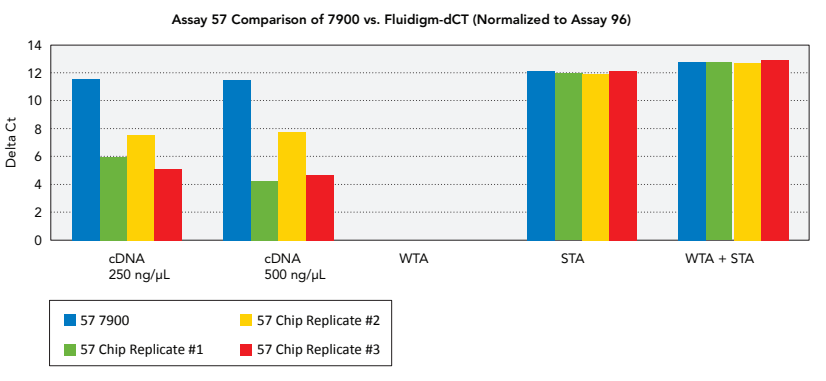
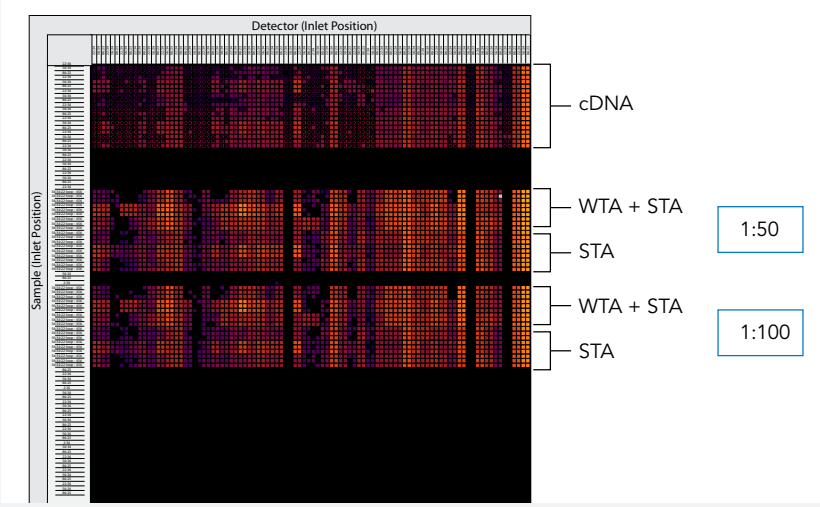


FIGURE 5. **Heat Map of SYBR Green Assays Across Biological Triplicates from A, B, and C Groups**



Product Name	Starting Material	Part Number	Number of Reactions
Ovation Pico WTA	500 pg – 50 ng purified total RNA Direct lysate from 50–20,000 cells	3300	12, 60, or 96
Ovation PicoSL WTA	500 pg – 50 ng purified total RNA Direct lysate from 50–20,000 cells	3310	48
WT-Ovation FFPE V2	50 ng of total RNA from FFPE samples	3400	12, 60, or 96
WT-Ovation One-Direct	10–500 pg purified total RNA Direct lysate from a few cells	3500	12
Encore Biotin Module	Fragment and biotinylate 5 µg of cDNA targets for analysis on Affymetrix GeneChip Expression Arrays	4200	12, 60, or 96
Encore BiotinIL Module	Fragment and biotinylate 2–4 µg of cDNA targets for analysis on Illumina Expression BeadChips	4210	48

Related Fluidigm Products

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Product Name	Description	Part Number
BioMark Real-Time PCR Reader	Real-time cycler and reader for high-throughput qPCR for gene expression, genotyping and digital PCR	BMK-BMK
96.96 Dynamic Array Chip for Gene Expression or 48.48 Dynamic Array Chip for Gene Expression	Integrated Fluidic Circuits (IFCs) for 96 or 48 samples and assays	BMK-M-96.96 BMK-M-48.48

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