SPIA® cDNA Targets Provide Higher Sensitivity and Specificity in Microarray Analysis Compared to Targets Derived from senseRNA

Leah R. Turner1, Wieland Keilholz1, David D’Ambrosio2, Qi Wang3, Michael Moreau3 and Andrew Brooks2

1NuGEN Technologies, San Carlos, CA 94070; 2Bionomics Research and Technology Center, Rutgers University, New Brunswick, NJ 08901

ABSTRACT

Gene expression analysis of clinical samples in the study and diagnosis of disease continues to be a significant challenge to researchers and clinicians. Limitations in the amount and integrity of genomic material obtained from samples are most significant for the formalin-fixed, paraffin-embedded (FFPE) samples widely used in cancer research. In the current study, two sample prep systems, the Ovation® FFPE WTA System (NuGEN Technologies) and the Sensation™ RNA Amplification Kit (Genisphere®) were evaluated for their suitability for generating reliable biological information from compromised FFPE RNA samples. The NuGEN Ovation FFPE WTA System provides many advantages including existing automation protocols, significantly shorter and simpler workflow (from RNA to arrays in one day), compatibility with multiple array platforms (including both sense and antisense probe based arrays) and, as demonstrated in this study, higher specificity and sensitivity which leads to the acquisition of richer biological information. The combined advances in processivity, specificity and sensitivity for compromised samples make diagnostic applications an achievement that can be attained in the near future.

RESULTS

All samples yielded sufficient material for fragmentation, labeling and hybridization (Table 1). Principal Component Analysis revealed that the NuGEN cDNA targets allowed clearer discrimination between tissue types (Figure 1). Expression profiles from the NuGEN samples exhibit greater separation in the second and third components of the PCA, while the Genisphere profiles are clustered quite tightly together, making sample discrimination more difficult. Following 1-way ANOVA of individual FFPE tissues versus the pool of the other three tissues, the significantly changed gene sets from each comparison were used to assess the relative sensitivity of cDNA vs senseRNA-derived targets. While there was significant overlap in genes identified by both methods (Figure 2A), the NuGEN targets identified more significantly changed genes with greater dynamic range and improved tissue-specificity compared to the senseRNA-derived targets (Figures 2B and 2C). Annotations for the significantly changed genes are shown in the tables below the graphs in Figure 2.

Table 1: Average yield for 3 replicates of each input.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>RIN Score</th>
<th>NuGEN Avg yield (µg)</th>
<th>Genisphere Avg yield (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>2.6</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Liver</td>
<td>2.5</td>
<td>2.8 ± 0.0</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>Lymph</td>
<td>2.6</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Lung</td>
<td>1.4</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.2</td>
</tr>
</tbody>
</table>

Table 1: Average yield for 3 replicates of each input.

CONCLUSIONS

cDNA targets prepared with the NuGEN Ovation FFPE WTA System exhibit higher sensitivity and specificity than targets derived from the senseRNA produced with the Genisphere Sensation RNA Amplification kit, allowing for the acquisition of richer biological information by the Ovation-produced targets. A significant overlap of changed genes between assay types was observed, however:

• Ovation assays exhibit a greater dynamic range, therefore greater sensitivity
• Genes found only by Ovation are compressed in the Sensation assay
• Genes found only by Ovation contain additional informative biological information
• Genes found only by Sensation are largely uninformative

Figure 1: PCA plot of 4 FFPE tissues amplified with NuGEN or Genisphere kits. Samples are color coded by tissue type: Liver (red), Lung (blue), Lymph (green) and Spleen (Purple). 50 ng and 100 ng input samples cluster together (3 replicates of each). Genisphere amplified samples are circled on the plot on the right.

Figure 2: Data shown here is for liver vs the pool of the other three tissues and is representative of all comparisons. Significantly changed genes identified by each method show significant overlap (A). The changed genes were also plotted by fold change in each assay (x axis = Genisphere; y axis = NuGEN). Genes identified in the NuGEN samples only (B) show greater dynamic range and more specific tissue and disease association than changed genes identified only in the Genisphere samples (C). Please note the different axes.

MATERIALS AND METHODS

Total FFPE RNA with RIN scores of <3 were amplified at two input levels (50 and 100 ng) in triplicate using either the NuGEN kit or the Genisphere kit. Products from the NuGEN amplification were directly fragmented and labeled using the Encore® Biotin Module (NuGEN Technologies). Genisphere kit. Amplification products from these samples through a simple, robust and easily automated process using as little as 50 ng of total FFPE RNA, for analysis on at least one GeneChip array.

INTRODUCTION

Formalin-fixed paraffin-embedded (FFPE) tissue fixation techniques have been the clinical sample archival storage method of choice for many years. The majority of clinical samples are fixed in this way and while this method renders the samples amenable to traditional approaches of characterization, they become difficult to access for gene expression analysis, due to quite tightly together, making sample discrimination more difficult.

The combined advances in processivity, specificity and sensitivity for compromised samples make diagnostic applications an achievement that can be attained in the near future.