Verification of miRNA Expression using Nuclease Protection and Targeted Next-Generation Sequencing (NGS)

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HTG Molecular Diagnostics, Inc. | Tucson, AZ | Presented at ESHG 2016

Abstract

Introduction: miRNAs are short, ~22nt RNA sequences that mediate gene transcription and downstream cell behavior. The HTG EdgeSeq miRNA Whole Transcriptome Assay (WTA) enables users to measure the expression of 2,083 human miRNA transcripts using next generation sequencing. The extraction-free, sample preparation HTG EdgeSeq chemistry is compatible with formalin-fixed, paraffin embedded tissue (FFPE), plasma, serum, whole blood in PAXgene, cell lines, and purified RNA.

Materials and Methods: Two studies were conducted to characterize the performance of the HTG EdgeSeq WTA assay in FFPE and plasma sample types. The sample input studies evaluated a range of sample input amounts for both plasma and FFPE sample types. Optimal input range was established based on sample quality (using process controls) and performance (technical correlation and detection of low expression). Reproducibility studies across technical replicates were performed for both sample types.

Results: Read depth for FFPE (defined as the total aligned counts at the sample level) ranged from 2,048 to 5 million at the highest sample inputs. The percentage of probes expressing moderate (≥100 counts) and high (≥1,000 counts) expression was highly reproducible across sample plates. The sensitivity of probes expressing moderate (c. 100) and high (c. 1,000) counts was similar. Lower expression was more variable between both sample types. The correlation between replicate samples was summarized by the percentage of pairs with Pearson correlation > 0.90 for FFPE and plasma sample types.

Conclusions

The HTG EdgeSeq miRNA Whole Transcriptome Assay allows for highly reproducible expression with low sample input volumes for both plasma and FFPE samples. The assay detects expression of 2000 miRNAs in different sample types tested, is linear over a wide range of sample inputs, and has average Pearson correlation between replicate pairs of ≥0.93. It is amenable to small clinical specimens—requires very little sample input (~1-2 μl FFPE tissue, 12.5 μl blood).

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Table 1. Sensitivity

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<th>Sample Type</th>
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<td>250</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>Plasma</td>
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<td>2000</td>
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Sample Input Dynamic Range

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Detection of miRNA Probes Across Processors and Days

Figure 1. Detection of miRNA Probes Across Processors and Days

Reproducibility Study/Samples

Table 2. Reproducibility Study

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Figure 2. Reproducibility Study

Reproducibility

Figure 3. Reproducibility

Sensitivity

Figure 4. Sensitivity

Supplementary Figure 1. Supplementary Figure 1