HTG EdgeSeq miRNA Whole Transcriptome Assay Platform Performance Metrics Utilizing the MicroRNA (miRQC) Study Specimens

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HTG Molecular Diagnostics, Inc. | Tucson, AZ | Presented at ESHG 2017

Introduction

MicroRNAs (miRNAs) are short, ~22nt RNA sequences that modulate gene transcription and downstream cell behavior. There are several platforms used for quantification of low abundance miRNA targets found in circulating human bodily fluids, the miRQC manuscript included 12 such platforms from quantitative PCR (PCR), hybridization (HYB), and sequencing (SEQ) based technologies. The HTG EdgeSeq miRNA Whole Transcriptome Assay (WTA) is a new technology that enables users to measure the expression of human miRNA transcripts using NGS.

We obtained control samples used in the miRNA quality control (miRQC) study (Mastadgh, et. al., Nature, 2014) to measure performance of the miRNA WTA using the methods described in the Nature publication. Sixteen mandatory and four optional human serum RNA control samples included in the miRQC study. These were prepared using HTG protocols; including RNA at 25 ng. These control samples were constructed to evaluate a series of metrics that measured platform performance: reproducibility, detection rate sensitivity, accuracy, specificity, and differential expression.

HTG EdgeSeq System Workflow

**HTG EdgeSeq Whole Transcriptome Assay**

Measure 2,083 human miRNA transcripts in a NGS-based assay using the HTG EdgeSeq technology

- Extraction-free
- Workflow in as little as 36 hours
- Small sample pre-preparation
- Automated using the HTG EdgeSeq system

Overview and Study Design

Figure 1 describes the 12 platforms by general technology type, as well as the recommended RNA input (ng) recommended for each of these 12 platforms. This figure is from the Mastadgh, et. al, manuscript and shows which of the common reference samples were used for each performance metric.

The data evaluated by the HTG EdgeSeq miRNA WTA are based on a common set of 192 probes. The assay included both 5’ and 3’ variants for 19 probes, and these were the higher prevalent variant per miRBase was selected.

Titrination Response

Figure 2 describes the percent of titrating probes (y-axis) that show a difference (x-axis) between the miRQC A samples (100% Universal Human miRNA Reference RNA) and miRQC B samples (100% human brain RNA). As an example from HTG data, there were 26 probes with a difference in expression (log2) of miRQC A minus miRQC B of ≥2; 19 of those probes titrated. A probe is deemed to be titrating if the expression levels of miRQC samples A, B, C, and D plot in the expected order, such as A>B>C>D for probe i. Mastadgh, et. al., pointed out that the percent of probes that titrate expected to decrease as the expression difference decreases.

**Reproducibility**

Figure 3 depicts the correlation between all 4 miRQC samples and their replicates; the number shown in parentheses above each plot is the number of probes with double positives. A double positive is a probe that was detected in both technical replicates of each of the four samples (miRQC A, B, C, and D). For all platforms other than HTG, the number of possible double positives was 4 X 196 = 784. For HTG, the number of possible double positives was 4 X 192 = 768.

**Accuracy and Precision**

Figure 4 shows fold change expression differences between miRQC C and D. The dotted line is the threshold change expression. Figure 5 shows the median deviation from the expected ratio between miRQC C and miRQC D across platforms.

**Conclusions**

Additional data analyses from these samples showed that the HTG EdgeSeq miRNA Whole Transcriptome Assay demonstrated excellent reproducibility with an area to the left of the cumulative distribution curve (ALC) of 0.108, ranking 2nd across all platforms. Specificity (cross-readability) was moderate at 29.2%. The 14% false positive rate was equivalent to that seen in two other platforms using PCR- and SEQ-based technologies, with only 4 platforms performing better.

With the lowest RNA input of all 12 comparator platforms, the HTG EdgeSeq miRNA Whole Transcriptome Assay produced reproducible, accurate, and sensitive results using the miRQC study samples.