

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

Salim Aissaoui and Bonnie LaFleur

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

Introduction

MicroRNAs (miRNA) are short, ~22nt RNA sequences that modulate gene transcription and downstream cell behavior. There are several platforms used for quantitation 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 microRNA quality control (miRQC) study (Mastdagh, 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

Sample Type	Input Amount
Plasma / Serum	≥ 15 µL
PAXgene	500 µL
FFPE Tissue	5 µm section
Cells	≥ 3,000 cells
Extracted RNA From Frozen	10 - 25 ng

- 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 Mastdagh, et. al, manuscript and shows which of the common reference samples were used for each performance metric.

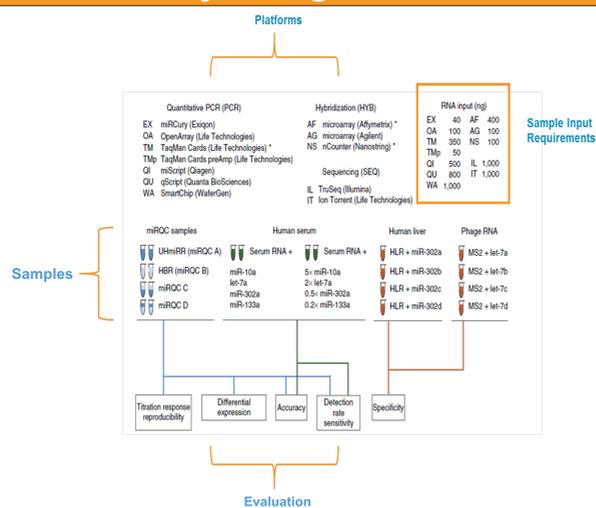


Figure 1. Schematic from Nature manuscript.

Titration 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 20 probes with a difference in expression (\log_2) 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 $A_i > B_i > C_i > D_i$ for probe i . Mastdagh, et. al., pointed out that the percent of titrating probes is expected to decrease as the expression difference decreases.

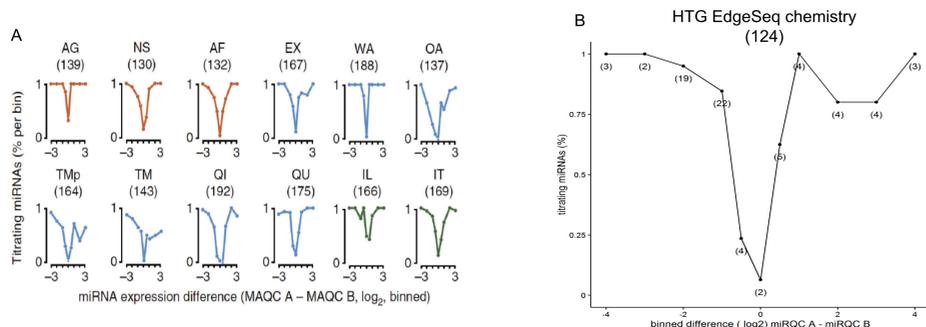


Figure 2. Percent titrating probes and difference between miRQC A and B. The left figure (A) is from the published manuscript and the right figure (B) is the resulting figure from the HTG EdgeSeq miRNA Whole Transcriptome Assay

Observations:

- Sixty-four percent (124/192) of the HTG EdgeSeq miRNA WTA miRNA \log_2 expression differences (miRQC A – miRQC B) are between -3 and 3.
- This is similar to other HYB platforms and directly comparable to NS, who showed 66% (130/196) of titrating miRNA differences between -3 and 3.
- The “shape” of the HTG curve is very similar to the other HYB techniques (AG, NS and AF).

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 $4 \times 196 = 784$. For HTG, the number of possible double positives was $4 \times 192 = 768$.

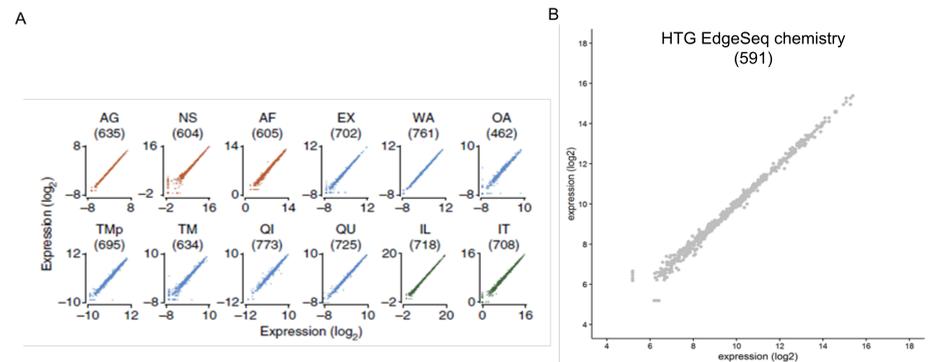


Figure 3. Correlation between replicates over all four miRQC titrations for original platforms (A) and the HTG EdgeSeq miRNA Whole Transcriptome Assay (B).

Observations: HTG correlation between replicates is quite comparable to both HYB and SEQ platforms visually. The number of probes included in the assessment is proportionally identical to the NanoString (NS) and Affymetrix (AF) platforms, 77% (591/768) for HTG EdgeSeq miRNA WTA and 77% for NS and AF (604/784 and 605/784, respectively).

Accuracy and Precision

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

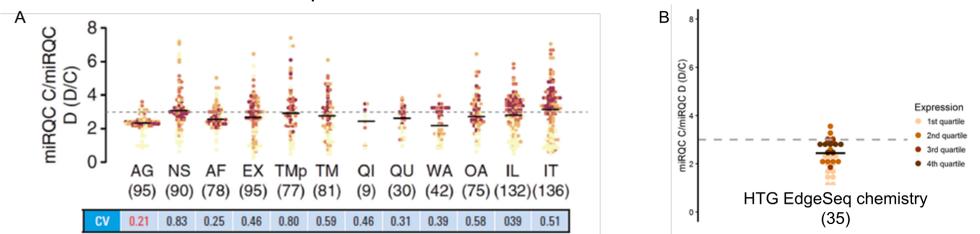


Figure 4. Fold change for miRQC B-specific miRNAs; HTG EdgeSeq miRNA Whole Transcriptome Assay has a median fold change of 2.44 and CV of 0.26. These are shown for titrations for original platforms (A) and HTG EdgeSeq miRNA Whole Transcriptome Assay (B).

Observations: The median fold change difference for HTG EdgeSeq miRNA Whole Transcriptome Assay was 2.44, very close to the expected threefold change. The CV of 0.26 was very similar to the AF and Agilent (AG) HYB platforms.

Accuracy and Precision

Figure 5 shows the volcano plot showing mean expression difference between miRQC A and miRQC C (x-axis) and percentage of false positives on the \log_{10} scale (y-axis).

Observations: HTG EdgeSeq miRNA Whole Transcriptome Assay demonstrated a 14% false positive rate, similarly as good as SEQ platforms, and nearly the same as NS. The HTG data are presented with no filtering (several platforms applied a data filter).

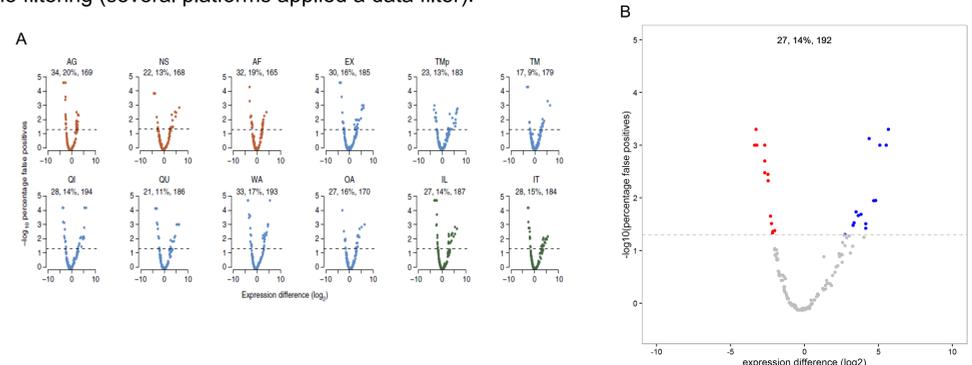


Figure 5. Mean expression differences and % false positives for titrations for original platforms (A) and HTG EdgeSeq Whole Transcriptome Assay (B).

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-reactivity) 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.

Reference: Mastdagh, P., Hartmann, N., Baeriswyl, L., Andreasen, D., Bernard, N., Chen, C., Cheo, D., D'Andrade, P., DeMayo, M., Dennis, L. et al. (2014) Evaluation of quantitative miRNA expression platforms in the microRNA quality control (miRQC) study. *Nat. Methods*, 11, 809–815.

For Research Use Only. Not for use in diagnostic procedures.

In the U.S. and other applicable jurisdictions, HTG EdgeSeq is a trademark of HTG Molecular Diagnostics, Inc. Any other trademarks or trade names used herein are the intellectual property of their respective owners.