A Simplified Approach to High-throughput miRNA Expression Analysis

MicroRNAs (miRNAs) are a class of small, non-coding RNAs that regulate gene expression of target mRNAs and have been implicated in different disease states offering the potential for discovery of novel biomarkers and new therapeutic insights (Bartel, 2004). Despite the widespread interests in the potential clinical application of miRNAs, currently available detection platforms are often limited in the range of targets and can exhibit variation in the results, leaving researchers few options for a comprehensive, reproducible, and reliable method for miRNA quantification.

The HTG EdgeSeq™ miRNA Whole Transcriptome Assay (WTA) offers a fast, easy to use solution that allows researchers to interrogate the human miRNA transcriptome in a single reaction from a variety of solid and liquid sample types.

Panel Overview

The HTG EdgeSeq miRNA WTA was developed to assist researchers looking at differential expression of miRNAs *(Table 1)*. The panel can simultaneously interrogate 2,083 targets, using common sample input types, in less than three days. miRNAs continue to play an important role in numerous disease and the panel allows researchers get a more comprehensive information about miRNA-disease associations *(Figure 1)*.

Table 1: *HTG EdgeSeq miRNA WTA Overview*

Number of Targets	2,083*
Sample Types and Recommended Input Amount	FFPE: 8-9 mm^2 Plasma: 15 µL Serum: 15 µL PAXgene: 500 µL Extracted RNA: 35 ng
Time to Results for 96 Samples	36 hours
Data Analysis	HTG EdgeSeq Reveal Software

** The current probe list is based on miRbase version 20.*

Figure 1: *The HTG EdgeSeq miRNA WTA includes targets miRNAs associated with numerous diseases listed, making it applicable for biomarker discovery and signature development.*

HTG EdgeSeq
miRNA Whole Transcriptome Assay

Small Sample Input

The HTG EdgeSeq technology enables miRNA transcriptome analysis using low sample amounts, as low as 15 µL of biofluids or 1 slide of formalin-fixed, paraffin embedded (FFPE) tissue with a minimum tissue area of 8-9 mm^2. Peer reviewed publications, including Yeri *et al.*, 2018, have shown that the HTG EdgeSeq miRNA Whole Transcriptome Assay was able to generate expression profiles using RNA extracted from FFPE tissue. They compared 10 ng and 20 ng of RNA per sample well and found no statistically detectable differences in the number of miRNAs detected, with greater than 95% concordance to RNA-Seq.

Compatible with biofluids and FFPE

HTG EdgeSeq extraction-free chemistry can detect miRNA targets without the requirement for RNA extraction, adaptor ligation, cDNA synthesis or pre-amplification of the sample by PCR.

The HTG EdgeSeq chemistry enables precise measurements of miRNA transcriptome with high reproducibility using biologically relevant sample types including fixed tissue samples, biofluids such as plasma, serum and PAXgene, cell lines and extracted RNA. Godoy *et al.*, 2019 have reported generating reproducible miRNA profiles from crude plasma which have lower concentration of miRNA compared to extracted samples. The authors conclude that the HTG EdgeSeq miRNA WTA offers the ability to detect a large number of miRNAs in either isolated RNA samples or crude plasma with relatively low detection bias *(Figure 2)*.

Panel Performance

Sensitivity and Specificity

HTG EdgeSeq offers excellent sensitivity due to its ability to detect miRNAs with low detection bias *(Figure 2)* when compared with other platforms including small RNA-Seq (Godoy *et al.*, 2019). These biases, which can affect reproducibility and sensitivity, can be introduced by RNA extraction, PCR amplification of targets and ligation, none of which are part of the HTG EdgeSeq workflow (Brown *et al.*, 2018). In addition, the extraction-free sample prep workflow retains small and fragmented miRNAs, further increasing sensitivity with low sample input and reducing bias associated with low expressors (Wong *et al.*, 2019). The HTG EdgeSeq miRNA Whole Transcriptome Assay has comparable sensitivity and specificity to small RNA-Seq, as measured by AUC—Area Under the Curve *(Table 2)*. The AUC was calculated by comparing the separation between present and absent miRNAs in a pool of defined targets.

Table 2. *Comparison of performance characteristics*

	Small RNA-Seq	HTG EdgeSeq	FirePlex	nCounter
Bias (% RNAs within 2-fold of the median)	31	**76**	57	47
Specificity and Sensitivity (AUC)	**0.99**	0.97	0.94	0.81
Reproducibility of synthetic equimolar samples (% CV)	8.2	**6.9**	22.4	NA
Reproducibility of RNA isolated from plasma (% CV)	33.4	**14.4**	NA	NA
Reproducibility of crude plasma (% CV)	NA	**17.8**	43.2	NA

Note: *Bold indicates best performer for each performance characteristic. Replicate data was not available for nCounter platform. NA, not applicable. Godoy et al., 2019*

Reproducibility

In addition to being sensitive, an assay must generate the same answer repeatedly and ensure the accuracy of that data. As mentioned above, the introduction of bias can greatly affect reproducibility from platform to platform. A well described source of bias for miRNA expression profiling is variability in the different methods of miRNA isolation. Our extraction-free chemistry enables direct assessment of miRNAs from crude samples without isolation or amplification, reducing bias and increasing reproducibility. Reproducibility of the HTG EdgeSeq miRNA Whole Transcriptome Assay compared to small RNA-Seq (Illumina), nCounter (nanoString) and FirePlex (Abcam) miRNA assay as shown (*Figure 2* and *Table 2*). Data generated by Godoy *et al.*, 2019 show that the HTG EdgeSeq platform has lower

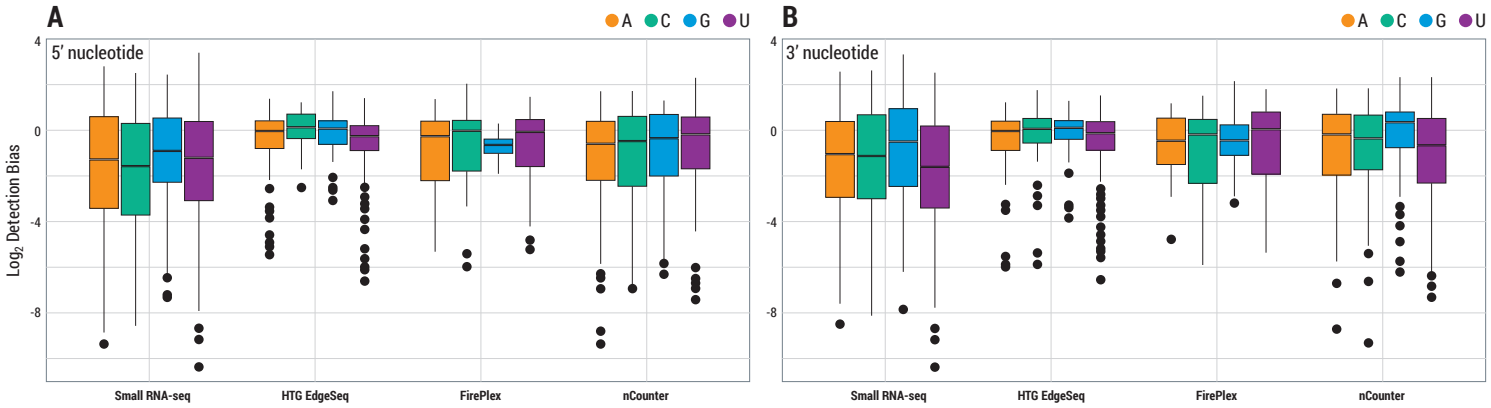

Figure 2: *Detection bias (ratio of observed to expected signal) is plotted grouped by either the 5′ (A) or 3′ (B) nucleotide. Boxes represent median and interquartile ranges. Godoy et al., 2019*

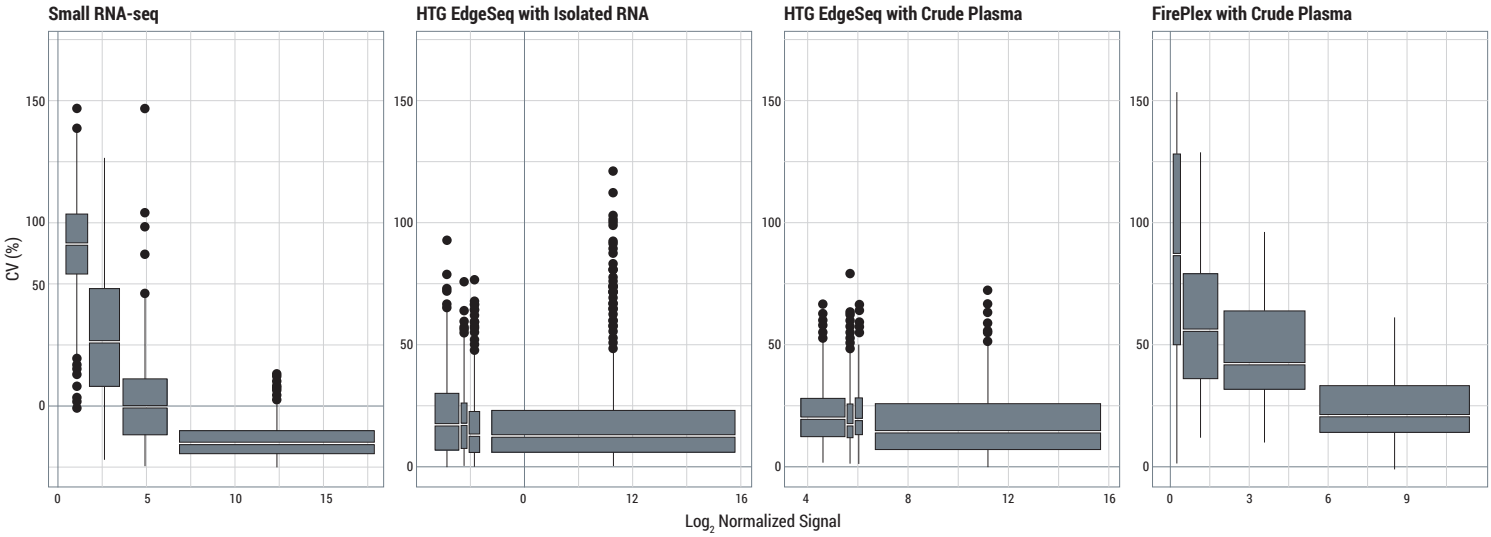

Figure 3: *Reproducibility of HTG EdgeSeq using human plasma. Coefficient of variation for technical replicates for FirePlex (22.4%), small RNA-Seq (8.2%), and HTG EdgeSeq (6.9%). Godoy et al., 2019*

% CV for all evaluation methods, including synthetic miRNA pools, miRNA extracted from plasma and miRNA from crude plasma (no extraction). Together, these data demonstrate the reproducibility of the HTG EdgeSeq platform, regardless of sample type.

Accuracy

Accuracy of the HTG EdgeSeq miRNA WTA (HTG EdgeSeq) was evaluated by comparison to quantitative PCR (qPCR), digital PCR and Microarray (*Figure 4)*. These data, published by Songia *et al.*, 2018, used human plasma samples to generate and compare miRNA expression profiles. Pearson's coefficient calculations between the four platforms tested here showed high correlation to the HTG EdgeSeq miRNA Whole Transcriptome Assay.

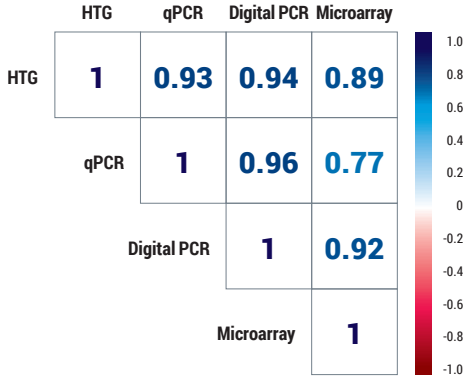

Figure 4: *Correlation matrix of the Pearson's coefficients among the different technologies. Songia et al., 2018*

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Panel Products

Kit Configurations and Catalogue Numbers

Kit Name	Configuration	Illumina Sequencers	Thermo Fisher Scientific Ion Torrent Sequencers
HTG EdgeSeq miRNA WTA	2 x 8	916-001-208	916-001-308
HTG EdgeSeq miRNA WTA	4 x 8	916-001-008	916-001-108
HTG EdgeSeq miRNA WTA	1 x 24	916-001-224	916-001-324
HTG EdgeSeq miRNA WTA	4 x 24	916-001-024	916-001-124
HTG EdgeSeq miRNA WTA	1 x 96	916-001-096	916-001-196

About HTG

Our mission is to illuminate the transcriptome with targeted gene expression profiling and empower actionable insights for clinical research. HTG's comprehensive gene expression solutions have enabled generation of biomarker signatures, link expression profiling to map novel pathways and decode complexities of diseases.

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