GenapSys™ Sequencing Platform: Small RNA Sequencing

- **Interrogate thousands of small RNAs in parallel with greater sensitivity and dynamic range vs orthogonal methods**
- **Sample batching to enable cost-effective small RNA sequencing**
- **Utilize an affordable, personal NGS platform that offers a low price per run and low price per sample**
- **Leverage a range of library prep kits as part of a simple, flexible workflow solution**

Introduction

Small RNAs are important non-coding regulators in diverse biological settings. MicroRNAs (miRNAs) that are the most widely studied small RNAs regulate protein-coding expression post-transcriptionally. miRNA expression profiles in tissues or cell populations are highly informative to reveal cellular states, such as in human cancers, and to identify cellular mechanisms. Small RNAs, including miRNAs, are single-stranded non-coding RNAs between 17-34 nucleotides in length and are involved in the regulation of numerous cellular processes. The GenapSys™ Sequencing Platform offers the most affordable platform for small RNA sequencing.

Next-Generation Sequencing (NGS) offers a digital readout as compared to alternative analog techniques. Historically, high-throughput RT-PCR or microarray technology were the typical methods to study these species. Both RT-PCR and microarray gene expression measurements are compromised by limited dynamic range and the need for *a priori* knowledge of the various species resulting in missing both low and high expressors as well as novel species altogether. By comparison, NGS offers an ability to count individual transcripts enabling a large dynamic range. Researchers can choose the level of sensitivity that is needed for their experiment allowing for optimization for sensitivity or the number of samples that can be processed per run. The GenapSys Sequencing Platform enables the ability to generate over 10 million sequence reads per run allowing the interrogation of millions of unique small RNA and microRNA sequences. Additionally, investigation of samples without a reference sequence or other baseline information is possible with NGS, enabling the discovery of novel species.

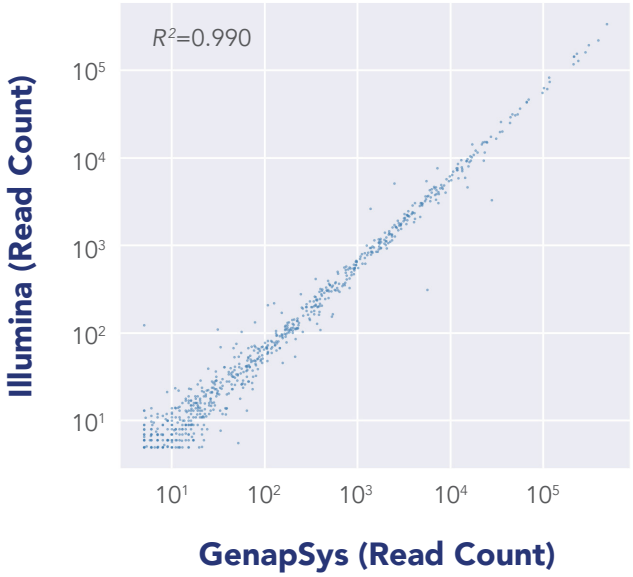

Fig. 1 Strong correlation between GenapSys and Illumina sequencing of a small RNA library. A library was generated with the Universal Human miRNA Reference RNA (Agilent) using the QiaSeq miRNA library prep kit. The final PCR step was performed with a GenapSys-customized PCR primer instead of the library PCR primer from the kit.

Streamlined small RNA sequencing workflow on the GenapSys Sequencing Platform

Library prep
Generate sequencing library with a GenapSys-customized PCR primer.

Clonal Amplification
Clonally amplify libraries on the GenapSys Sequencing Prep System.

Sequencing
Load amplified beads into the chip and sequence using the GenapSys Sequencer.

Data Analysis
Generate FASTQ with GenapSys analysis software.

Technology

The GenapSys Sequencer employs a novel electrical detection method that is capable of generating highly accurate DNA sequence information. With a CMOS based detector, simple fluidics, and low computational requirements, the GenapSys instrument is small, affordable, and accessible even to novice genomic scientists. Inside the sequencing chip are millions of individual sensors, each loaded with a single bead coated in thousands of clonal copies of a particular DNA sequence. Individual nucleotides are flowed across the chip in succession and successful incorporation is detected by changes in impedance as the complementary DNA strand grows.

Experimental Methods

The small RNA library preparation workflow begins with total RNA extracted from the sample of interest. The GenapSys workflow is compatible with a range of different library prep kits, such as the QIAseq® miRNA Library Kit (Qiagen 331502), the NEBNext® Small RNA Library Prep Set (NEB E7330), and the Lexogen Small RNA-seq Library Prep Kit. The final PCR step is performed with a GenapSys customized PCR primer, which enables the library to be compatible with the platform.

For this study, the miRNA-seq library was generated using 100 ng of the Universal Human miRNA Reference RNA from Agilent (PN 750700), which is a standard composed of total RNA from nine human tissues or cell lines specifically selected and formulated for optimal miRNA representation. The QIASeq miRNA Library Prep Kit (PN 331502) was used according to manufacturer's typical recommendations, with the exception of the final PCR step. The final PCR was performed with a set of GenapSys customized PCR primers (Table 1) instead of the library PCR primer set provided as part of the miRNA kit. All library prep kits with the sRNA specific adapter region (shown in red in Table 1) are compatible with these primers. This is needed to ensure that the final libraries will be compatible with the GenapSys Sequencer. PCR amplification was performed with the NEBNext Ultra™ II Q5® Master Mix (M0544). The library was clonally amplified and sequenced on the GenapSys Sequencing Platform, and FASTQ data was accessible with the GenHub cloud interface. The same library was also run on an Illumina® NextSeq™ platform for performance comparison.

Bioinformatics analysis of the GenapSys and Illumina data was done using identical pipelines, as follows. The human transcriptome FASTA file (hg38, release 33) was downloaded from GenCode. The index files were generated using Salmon (v1.1.0) based on the miRNA sequences in the human transcriptome FASTA file. Adapter sequences in the fastq files were removed using cutadapt. Quantification of RNA was performed using Salmon (v1.1.0) based on the adapter-removed fastq files. The correlation between samples was calculated using Pearson's coefficient method for miRNAs with a minimum of five read counts.

Table 1: PCR primers for final index PCR step of sRNA-seq library prep. The GenapSys-customized PCR primer set is compatible with sRNA-library prep kits that have the sRNA-specific adapter, shown in red. We recommend 8 bp long unique dual indexes for the sRNA-seq libraries, as against single index 6 bp barcode libraries.

Index PCR Primers	Sequence
sRNA_Index1_PCR-pri1	5'-CAAGCAGAAGACGGCATACGAGAT[Index1]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T -3'
sRNA_Index2_PCR-pri2	5'- AATGATACGGCGACCACCGAGATCTACAC[Index 2]ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTCAGAGTTCTACAGTCCG*A -3'

Results

The abundance of the different sRNA species in the sample were plotted for the GenapSys data and Illumina data. The data shows high correlation between the two abundances with a R^2 value > 0.99 (Fig. 1). Additionally, strong concordance was observed between the top 25 most expressed miRNA species between the two sequencing platforms with some minor variations in the respective abundance order (Table 2). These results indicate that the GenapSys sequencing technology enables accurate sequencing of small RNA species.

Conclusion

The GenapSys Sequencing Platform enables elucidation of complex small RNA and microRNA species on an affordable, personal sequencer. This combination puts the researcher in charge of their small RNA sequencing workflow and obtain results like never before. Sample batching enables a cost-effective price per sample while benefiting from the power of NGS in labs of all sizes.

To learn more about the GenapSys Sequencing Platform, visit GenapSys.com

Table 2: Top 25 microRNAs in the Universal Human Reference RNA and abundance ranking by sequencing platform.

Ensembl ID	MicroRNA ID	Rank in GenapSys	Rank in Illumina
ENSG00000283990	MIRLET7A3	1	1
ENSG00000207971	MIR125B1	2	2
ENSG00000198987	MIR16-2	3	3
ENSG00000284329	MIR9-3	4	4
ENSG00000199161	MIR126	5	8
ENSG00000284182	MIR143	6	5
ENSG00000284520	MIRLET7B	7	7
ENSG00000199072	MIRLET7F1	8	6
ENSG00000208008	MIR125A	9	9
ENSG00000199121	MIR26B	10	11
ENSG00000199017	MIR1-1	11	10
ENSG00000207654	MIR128-1	12	13
ENSG00000199075	MIR26A1	13	12
ENSG00000207752	MIR199A1	14	14
ENSG00000199179	MIRLET7I	15	15
ENSG00000199030	MIRLET7C	16	16
ENSG00000284440	MIR122	17	17
ENSG00000199153	MIR30D	18	18
ENSG00000207864	MIR27B	19	19
ENSG00000284190	MIR21	20	21
ENSG00000198972	MIRLET7E	21	20
ENSG00000284459	MIR24-1	22	22
ENSG00000283926	MIR192	23	24
ENSG00000199085	MIR148A	24	25
ENSG00000199150	MIRLET7G	25	23

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