Fully Automated Molecular Diagnostics*

Rheonix, Inc.

***NOT YET CLEARED BY FDA FOR DIAGNOSTIC PURPOSES**

Capabilities

The Rheonix Encompass Optimum™ Workstation automatically processes 3 or 6 Rheonix CARD® cartridges during each run. Each CARD cartridge processes four individual samples, therefore, a total of up to 24 individual specimens can be automatically processed at one time.

Under the control of software the system automatically performs cell lysis, DNA (or RNA) extraction, multiplex PCR amplification and detection of the amplicons on an integrated DNA array.

A variety of raw specimens can be placed into the system which will then automatically introduce them to the CARD cartridges. The types of specimens that can be processed include:

- Cell culture fluids
- Saliva
- Serum
- Plasma
- Urine
- Vaginal swabs
- Endocervical swabs
- Buccal swabs
- Tissue – fresh and FFPE
- Food and Beverages

A broad array[1] of assays have been reduced to practice, including:

- Detection of DNA or RNA Targets
- SNP Detection
- Infectious Disease (ID) targets
- User-defined assays
- "Dual assays" that simultaneously detect ID nucleic acids and host antibodies.
- Foodborne pathogens
- Beer spoilage organisms

[1]Additional products in the pipeline:

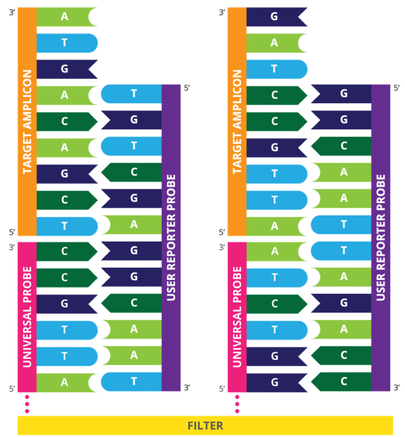
- STI TriPlex Assay undergoing clinical studies for 510(k) clearance
- Listeria Pattern Recognition Assay

User-defined Assays

The Rheonix Universal CARD® cartridge allows end users to automatically functionalize the integrated DNA array to permit multiplex PCR assays to be easily designed. Moreover, under the control of the Encompass Optimum's software, the functionalization of the DNA arrays occurs automatically and simultaneously while the samples are prepared and subjected to PCR. Therefore, when the amplicons have been generated, the integrated DNA array is ready and available for hybridization and detection.

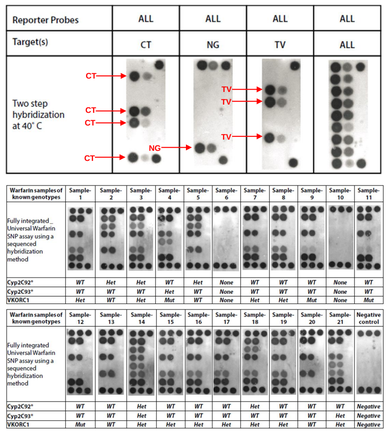
The Universal CARD array has a lawn of 20 unique oligonucleotides (16 mers) that are spotted at defined locations. The user designs "Reporter" probes that are complementary at their 3' termini to the membrane bound "Universal" probes and complementary at their 5' termini to the amplicons of interest (Figure 1). A number of assays, including detection of up to 20 targets, as well as SNPs and sexually transmitted infections have been reported[1] (Figure 2).

Figure 1:
Configuration of Universal CARD array



Schematic representation of Universal CARD that includes twenty (20) universal probes (only two shown) consisting of 20 nucleotides (only 6 shown). After user designs reporter probes that are complementary at their 3' termini to the universal probes and complementary at their 5' termini to the target amplicons, the system automatically delivers the reporter probes to the array which is now ready to hybridize to the target amplicons generated during PCR.

Figure 2
Use of Universal CARD

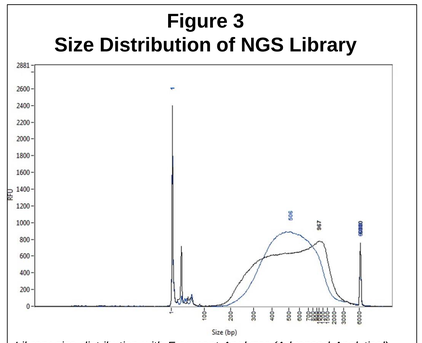


TOP: DNA from Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG) and Trichomonas vaginalis (TV) was automatically isolated and PCR amplified on the Universal CARD and amplicons detected on the array automatically functionalized as depicted in Figure 1.Location of specific probes is noted in red.
*BOTTOM: DNA obtained from four samples of known genotype at the CYP2C9*2, CYP2C9*3 and VKORC1 loci (as determined by Sanger sequencing) were automatically analyzed on the Universal CARD.*

Automated NGS Library Prep

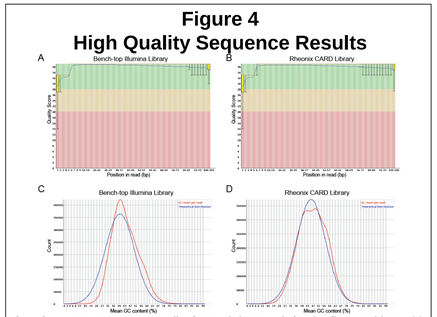
In order to simplify and reduce the cost and labor of NGS library preparation, the Rheonix Encompass Optimum was programmed to perform the Nextera DNA Preparation NGS library preparation. Starting with buccal swabs obtained from volunteers (IRB approved), DNA was isolated and NGS libraries prepared. As compared to benchtop prepared libraries, the automatically prepared libraries gave indistinguishable quality metrics when analyzed on Illumina HiSeq instruments. The size distribution (Figure 3), Quality scores (Figure 4) as well as Size distribution of inserts (Figure 5) and coverage (Figure 6) were excellent. Other library prep kits are currently being implemented.

Figure 3
Size Distribution of NGS Library



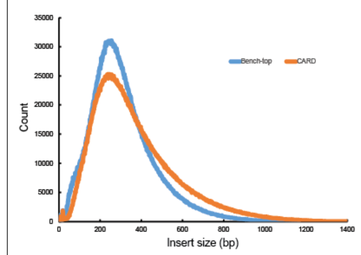
Library size distribution with Fragment Analyzer (Advanced Analytical). Relative Fluorescent Units (RFU) are plotted versus base pair size of NGS library. Fragment size is determined by a ladder and the lower marker (LM) and upper marker (UM). The peak of library fragment size is shown. Black line is a representative of a CARD cartridge generated NGS library and blue line is a representative of bench-top generated NGS library. Size distributions appear optimal for Illumina sequencing.

Figure 4
High Quality Sequence Results



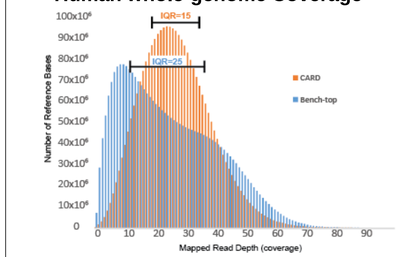
***(A, B)** Average sequence quality for each base pair for the first read in a 100 bp paired end sequencing reaction for the standard bench-top protocol (A) or with the Rheonix CARD cartridge (B). Quality scores are on the y-axis for bp position in the read, x-axis. The red line is median value, yellow box represents inter-quartile range (25-75%), upper and lower whiskers represent 10% and 90% points, and the blue line represents the mean value. **(C, D)** GC content across the whole length of each sequence read compared to a modelled normal distribution of GC content. Red line GC count per read from the sequencer compared to simulated normal distribution (blue line).*

Figure 5
Size Distribution of Inserts



Sequence insert sizes were determined from paired end reads by aligning to the human genome.

Figure 6
Human whole-genome Coverage



Histogram of coverage per base was determined from aligned sequencing results. IQR: Inter-Quartile Range, is the difference in sequencing coverage between the 75th and 25th percentiles of the histogram and is used as a measure of statistical variability. The ideal coverage histogram will take the form of Poisson-like distribution with a small standard deviation.

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