Fully Automated DNA isolation and NGS Library Preparation

H. Zhu[1], B.J. Kim[1], R. Yasmin[1], S. Valiyaparambil[2], B. Marzullo[2], M.J. Buck[2] and R. Montagna[1]
[1]Rheonix, Inc., Ithaca, NY, [2]State University of NY at Buffalo, Buffalo, NY

Introduction:

The total cost of next generation sequencing (NGS) continues to decline but still considerable time and cost is required to isolate the DNA and prepare sequence-ready libraries from a variety of human-source samples. The goal of the present study was to simplify and fully automate these crucial steps in order to reduce the time and cost as well as the level of training required to complete these critically important tasks.

Methodology:

A microfluidic cartridge (Fig. 1) and workstation (Fig. 2) were designed to fully automate DNA isolation and sequence-ready NGS library preparation from a variety of different human samples, including whole blood, buccal swabs, saliva, and formalin fixed paraffin embedded (FFPE) tissue blocks. The workstation's software was programmed to isolate the DNA using Rheonix nucleic acid purification reagents and then prepare sequence-ready libraries using either the Nextera Flex Kit (Illumina, San Diego, CA) or the ThruPLEX Plasma-seq kit (Takara Bio, Ann Arbor, MI). Finally, the resulting NGS libraries were sequenced on Illumina sequencers and the quality metrics compared to libraries generated with standard approaches.

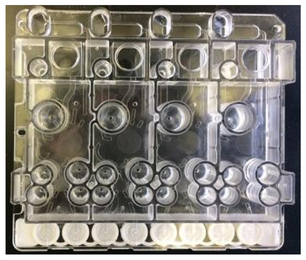


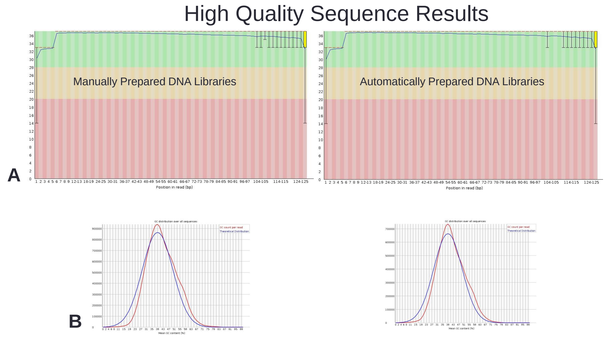
Figure 1: The Rheonix CARD® Cartridge is an injection molded plastic microfluidic device that can process four individual samples. All pumps, valves, microchannels, reagent and reaction reservoirs are contained within the cartridge.

Figure 2: The Rheonix Encompass Optimum™ workstation's software can automatically control one to six CARD cartridges (i.e., 1-24 individual samples) without user intervention. Pneumatic signals are used to actuate pumps and valves and move paramagnetic magnets in and out of position. The workstation's robotic liquid handler delivers samples and reagents as necessary.

Results:

Nextera Flex preparation of DNA libraries from Whole Blood

Human samples/Process	% GC	Insert. size	Median Coverage	% Aligned
On bench Whole Blood	48%	323	14.0X	99.80%
ON CARD Whole Blood	47%	305	10.0X	99.80%



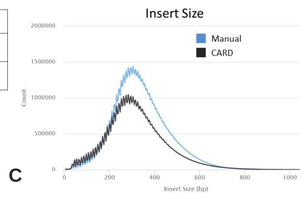


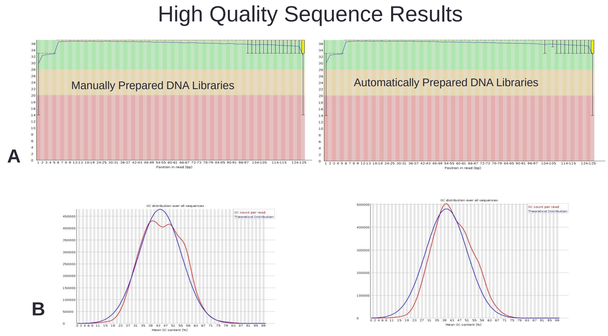
Figure 3: Two libraries were generated from human whole blood (commercially purchased), one manually on the bench top following the manufacturer's instructions and another on the fully automated *Encompass Optimum*™. The libraries were QC'd to verify concentration (Qubit) and fragment size (Bioanalyzer). Post QC libraries were pooled and sequenced on an Illumina HiSeq.

A: Quality Scores
B: Percent GC content
C: Insert size distribution

The results demonstrate that libraries automatically prepared using the Rheonix *Encompass Optimum*™ are comparable to manually prepared libraries with similar quality scores, size distribution, GC content and percent alignment to the reference genome

Nextera Flex preparation of DNA libraries from Buccal Swabs

Human samples/Process	% GC	Insert. size	Median Coverage	% Aligned
On bench Buccal Swab	52%	309	7.0X	99.40%
ON CARD Buccal Swab	49%	305	7.0X	98.40%



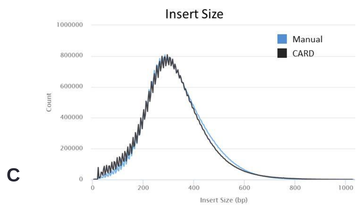


Figure 4: Two libraries were generated from human buccal swabs samples (collected locally), one manually on the bench top following the manufacturer's instructions and other on the fully automated *Encompass Optimum*™. The libraries were QC'd to verify concentration (Qubit) and fragment size (Bioanalyzer). Post QC libraries were pooled and sequenced on HiSeq

.

A: Quality Scores
B: Percent GC content
C: Insert size distribution

The results demonstrate that libraries automatically prepared using the Rheonix *Encompass Optimum*™ are comparable to manually prepared libraries with similar quality scores, size distribution, GC content and percent alignment to the reference genome

Nextera Flex preparation of DNA libraries from Saliva

Human samples/Process	% GC	Insert. size	Median Coverage	% Aligned
On bench Saliva	49%	323	7.0X	94.90%
ON CARD Saliva	48%	299	6.0X	83.10%

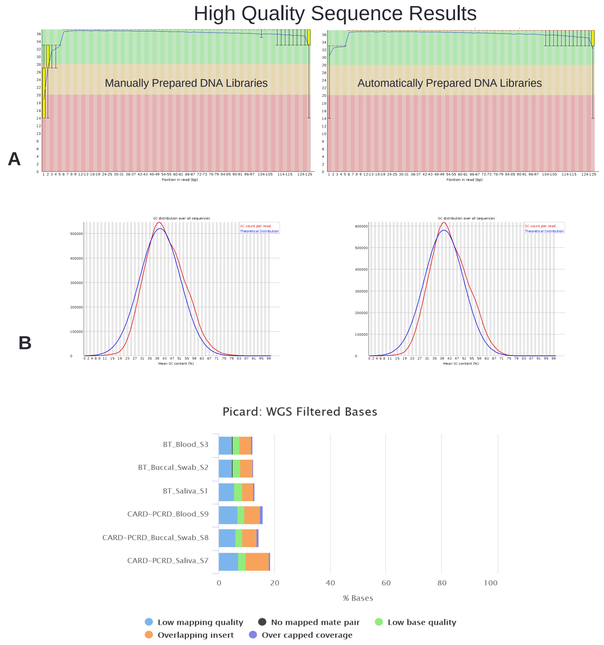


Figure-6: The histogram show Whole Genome Sequence indicating % base mapping.

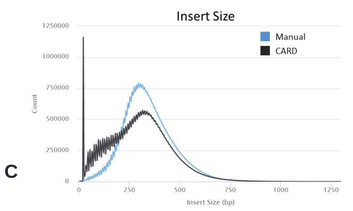


Figure 5: Two libraries were generated from human saliva samples (collected locally), one manually on the bench top following the manufacturer's instructions and other on the fully automated *Encompass Optimum*™. The libraries were QC'd to verify concentration (Qubit) and fragment size (Bioanalyzer). Post QC libraries were pooled and sequenced on a HiSeq sequencer

A: Quality Scores
B: Percent GC content
C: Insert size distribution

The results demonstrate that libraries automatically prepared using the Rheonix *Encompass Optimum*™ are comparable to manually prepared libraries with similar quality scores, size distribution, GC content.

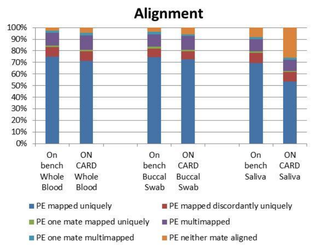
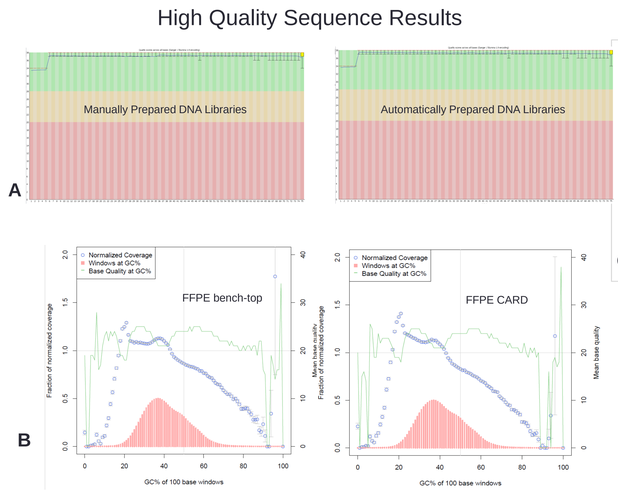


Figure 7: Shows the alignment of Whole Genome Sequence from whole blood, buccal swab and saliva processed on bench top and *Encompass Optimum*™. The results are comparable among samples processed manually and on CARD with the deviation of ON CARD Saliva sample most likely due to microbial content of the saliva samples.

ThruPlex Plasma-Seq preparation of DNA libraries from FFPE



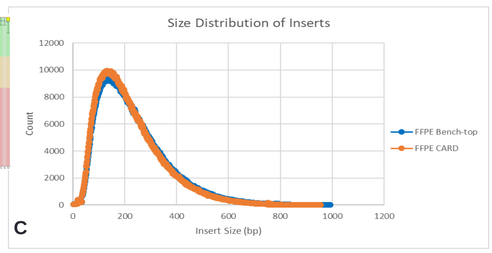


Figure 8: Four FFPE ThruPLEX Plasma-seq libraries were generated, two manually on the bench top following the manufacturer's (Takara Bio, Ann Arbor, MI) instructions and two on fully automated *Encompass Optimum*™. The libraries were QC'd to verify concentration (Qubit) and fragment size (Bioanalyzer). Post QC libraries were pooled and sequenced on a MiSeq, 2x75. Output performance metrics were analyzed following established ThruPLEX Plasma-seq metrics.

A: Quality Scores
B: Percent GC content
C: Insert size distribution

The results demonstrate that the automatically-prepared libraries were comparable with the manually-prepared libraries similar quality scores, size distribution and GC content.

Conclusion:

The data presented here indicate that the sequence-ready DNA libraries automatically prepared using the Rheonix *Encompass Optimum™* workstation are comparable to libraries manually prepared on the bench top. The ability to automatically isolate nucleic acid and prepare sequence ready libraries on a single instrument that requires very little "hands on" effort reduces the total time from approximately 1.5 day to about 4.5 - 6 hours, depending upon the starting sample type. In addition, the automatic preparation of DNA libraries will also reduce the overall cost of next generation sequencing. Finally the ability of *Encompass Optimum™* workstation to automatically process a variety of sample types will also allow a broad application of NGS including detection of genetic variants in germ line and somatic cells. Therefore, the integrated automated nucleic acid isolation and NGS library preparation will not only reduce the total time and cost of these prerequisite steps, but will also appeal to third party payers as the clinical utility of NGS data justifies expanded diagnostic applications.

Acknowledgments:

We thank Takara Bio (Ann Arbor, MI) for providing the ThruPlex kits and Takara Bio scientists Emmanuel Kamberov, Kyle Thurston, Marta Gonzalez, Julie LaLiberte, Datta Mellacheruvu and Andrew Farmer for performing the DNA sequencing and analysis of the DNA libraries prepared from the FFPE samples.