Fully Automated DNA Isolation & NGS Library Preparation

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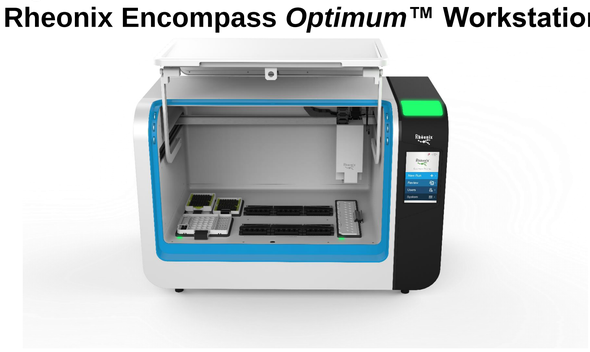
Background

Over the past 15 years, the massively parallel next generation sequencing (NGS) technology has revolutionized the field of genomics by its capability to provide large amounts of sequence data. In order to decrease the time and cost of NGS library preparation we have developed the microfluidic Rheonix CARD® cartridge (Figure 1) that, under the software control of the Rheonix Encompass *Optimum*™ workstation (Figure 2), can automatically isolate DNA and prepare sequence-ready NGS libraries for analysis on Illumina instruments.

Figure 1
Rheonix CARD® Cartridge

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

Figure 2
Rheonix Encompass *Optimum*™ Workstation



Under the control of its software, the Rheonix Encompass Optimum™ workstation 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 place. The workstation's robotic liquid handler delivers samples and reagents where necessary.

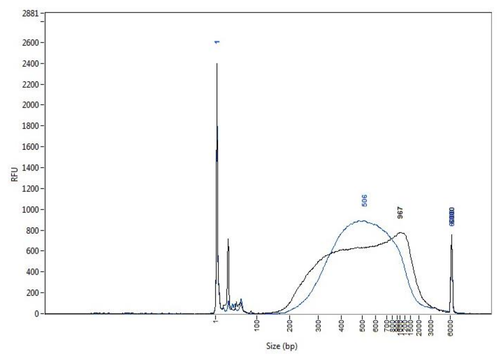
Methods

Buccal swabs were obtained from volunteers and used in studies to compare the recovery and purity of isolated DNA, as well as usefulness of NGS libraries prepared manually and automatically. Both approaches performed the steps of the Invitrogen Dynabeads SILANE genomic DNA Kit for DNA isolation and Illumina Nextera DNA Preparation Kit for NGS library preparation prior to sequencing on Illumina HiSeq2500 instruments. The automated DNA isolation process included sample lysis, bead binding, bead washing, bead drying and DNA elution; while the NGS library preparation process included tagmentation, tagmented DNA clean up, low cycle PCR and PCR product clean up.

Results

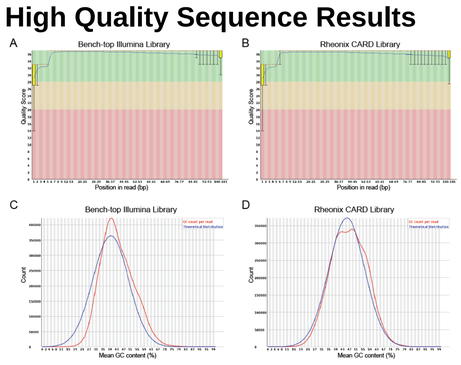
In the DNA isolation step, the automated process yielded **25 µl** of DNA at **24.8±0.04 ng/µl** and the manual method yielded **25 µl** of DNA at **40.5±0.99 ng/µl**. Both types of preparations had similar purity based on A260/A280 values. Once libraries were prepared, both the automatically and manually prepared libraries had similar concentrations of approximately **3.75 ng/µl**. The size distribution of automatically prepared NGS libraries had fragment sizes ranging from **880-1163 bp** with an average of **1086 for the 8 NGS libraries** (Figure 3). Comparison of the sequence data resulting from both the manually and automatically prepared NGS libraries gave indistinguishable sequence metrics; similar sequence quality scores and GC content (Figure 4), as well as insert sizes (Figure 5). Significantly, the NGS libraries prepared with the Rheonix CARD outperformed the manually prepared libraries for coverage uniformity (Figure 6).

Figure 3
Size Distribution of NGS Library



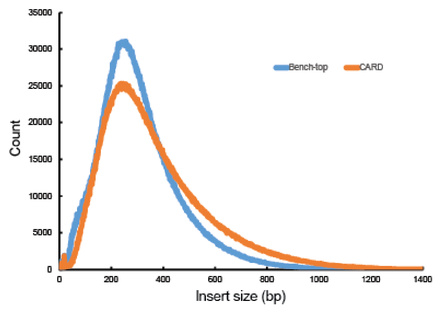
Library size distribution with Fragment Analyzer (Advanced Analytical). Relative Fluorescent Units (RFU) are plotted versus base pair size of NGS library. Fragment size is determined by a ladder and the lower marker (LM) and upper marker (UM). The peak of library fragment size is shown. Black line is 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



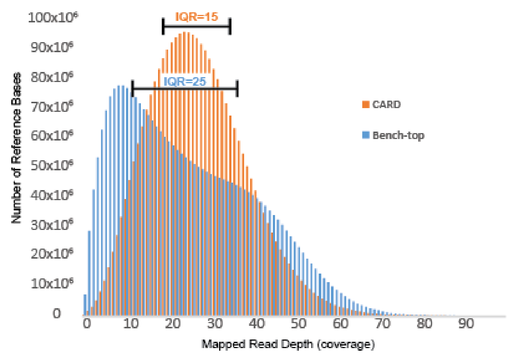
(A, B) Average sequence quality for each base pair for the first read in a 100 bp paired end sequencing reaction for the standard bench-top protocol (A) or with the Rheonix CARD cartridge (B). Quality scores are on the y-axis for bp position in the read, x-axis. The red line is median value, yellow box 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.

Conclusions

The ability of Encompass *Optimum*™ to automatically isolate DNA and prepare sequence-ready DNA libraries will help to reduce the overall cost of NGS for both research and clinical applications. By integrating DNA isolation and library preparation processes on a single instrument, we were able to reduce the time to result from 1.5 - 2.5 days to less than five hours. Therefore, multiple pieces of equipment can be eliminated and individuals with limited technical training can successfully isolate DNA and prepare sequence-ready DNA libraries. Finally, the Rheonix CARD cartridge system can isolate and purify DNA from a broad spectrum of clinical and nonclinical samples, thus providing a versatile system for multiple applications.

Acknowledgements

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