Next-Generation Sequencing Results
on the AutoGen FlexSTAR+

White Paper

Executive Summary

Next-Generation Sequencing results from genomic DNA extracted from whole blood samples using the Flexigene precipitation chemistry on the AutoGen FlexSTAR+

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Introduction

Claritas Genomics is a commercial genetic testing laboratory specializing in diagnosis of rare pediatric genetic disease. Claritas is accredited by CLIA, ISO 15189, New York, California, and many other states to perform clinical whole exome sequencing (WES), the Claritas Clinical Exome (CCE), and phenotype defined region of interest (ROI) panels derived from the CCE. The Claritas approach performs WES on two independent Next-Generation Sequencing platforms, NextSeq by Illumina and Ion Torrent Proton by Thermo Fisher Scientific. Results are combined to increase assay sensitivity and provide rapid orthogonal confirmation of variants thereby decreasing the Sanger sequencing confirmation burden by nearly 95%.

The majority of CCE tests run by Claritas are derived from genomic DNA (gDNA) extracted from 2ml whole blood samples received in EDTA blood collection tubes. Claritas WES tests are validated with gDNA extracted by AutoGen instruments for large volume blood samples. Recently, Claritas upgraded from an older AutoGen model (AGFSTAR) to AutoGen's newest model, the FlexSTAR+ for whole blood extractions. The new AutoGen FlexSTAR+ has increased throughput capacity and requires fewer manual technician interventions thereby reducing hands-on-time and decreasing potential sample handling errors.

The new instrument was validated and put into service in the clinical laboratory. Claritas analyzed NGS results from libraries constructed from 54 blood samples extracted in November 2016 on the new AutoGen FlexSTAR+ and results were within expected quality metrics. The new AutoGen FlexSTAR+ has replaced the AGFSTAR as their primary blood extraction instrument.

Materials and Methods

A total of 54 samples of 2ml fresh whole blood preserved in EDTA were extracted using the newest automated model, the FlexSTAR+ ("FlexStar Plus"). The FlexSTAR+ offers the same reliability and chemistry but now has the ability to automate the DNA extraction process from primary tube sampling to final DNA elution into storage tubes. The method used is a precipitation based chemistry called FlexiGene and is made by QIAGEN (FlexiGene DNA Handbook, 2010).

Blood samples were loaded into the on-board sample carousel, the 1-2 ml extraction protocol was selected from the menu list and 2mls of donor blood were automatically transferred into AutoGen's 5-hole tube units. During the first step of the automated extraction process (using the FlexiGene chemistry), lysis buffer was added to the sample. Cell nuclei and mitochondria were pelleted by centrifugation. Many contaminants including RNA were eliminated at this step. The pellet was resuspended and incubated in denaturing buffer which contains a chaotropic salt and protease. This step efficiently removes contaminants such as proteins. Following protein digestion, DNA was precipitated by the addition of isopropanol, recovered by centrifugation, washed in 70% ethanol, and dried. DNA was resuspended in the elution buffer, and then automatically transferred into commercially available DNA storage racks. All donor IDs, locations of samples and DNA are recorded in the sample tracking software throughout the entire process.

Extracted gDNA was used to make whole exome libraries for sequencing on the Illumina NextSeq and Thermo Fisher Ion Torrent Proton instruments (Chennagiri, et al., 2016). Claritas currently uses the Agilent Clinical Research Exome hybridization-capture baits (50 ng gDNA input) for sequencing on the Illumina NextSeq. The NextSeq has two flow cell sizes and barcoded libraries are multiplexed to achieve ~100x coverage with 2 x 150 bp paired end sequencing. Ion Torrent Ampliseq Exome libraries are constructed from 100 ng gDNA, barcoded and multiplexed at 2 libraries per chip to achieve coverage in excess of 100x on a Proton PI semiconductor sequencing chip.

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Results

The size and integrity of gDNA extracted by the AutoGen FlexSTAR+ was examined by analyzing 15 samples with Genomic ScreenTape on an Agilent TapeStation (Figure 1). DNA size and integrity were very good as evidenced by a single high molecular weight band of >48.5kb for all samples. The 100bp fragment in each sample serves as the lower molecular weight marker added to each sample for calibration purposes. Extracted gDNA from the 2ml whole blood samples was resuspended in ~600 L Tris-EDTA (TE) and quantitated with Qubit BR reagents (Thermo Fisher). Yield and concentration of gDNA from the AutoGen FlexSTAR+ was 117±55 ng/µL, n=54. The average recovery of ~70 µg gDNA per 2ml whole blood from the AutoGen FlexSTAR+ is far more than enough to perform any genetic testing performed at Claritas.

NextSeq sequencing results were examined from 54 libraries constructed from the AutoGen FlexSTAR+ gDNA extracted in November 2016. As expected, Illumina NextSeq sequencing results were of high quality (Figure 2). Mean target coverage was 127x and over 72 million unique reads per library. In addition to coverage, library uniformity is an important consideration and describes evenness of sequencing coverage. 95% of target bases were covered at 20x or greater and 92% of target bases were covered at 30x or greater. These results suggest there was no significant bias in extracted gDNA composition.

Next we examined Proton sequencing results from 24 AmpliSeq Exome libraries (Figure 3). Mean target coverage was 122x. Ampliseq libraries are composed of PCR amplified amplicons and uniformity is measured as percent of amplicons sequenced at ≥20% of the mean coverage and was measured at 93% with target base coverage at 20x of 93% and 30x at 90%. Mean read length of 182 bp and 44 million reads per sample were as expected. These results also suggest there are no significant gDNA extraction biases.

DNA prepared from whole blood extracted by the FlexSTAR+ also contains mitochondrial DNA. Many rare mitochondrial variants are causal for pediatric rare disease and of interest to clinical diagnostics. We have used the Takara LA PCR™ kit to perform long range PCR from 1-10 ng extracted DNA to amplify two pairs of fragments that redundantly cover the entire 16569 bases of the human mitochondria (Figure 4). The fragments are sized 8.9, 8.0, 9.1 and 9.2kb and all migrated in the expected size ranges. Long range PCR products are used as templates for Sanger sequencing and next-generation sequencing assays.

Conclusions

Genomic DNA extracted using FlexiGene precipitation chemistry on the AutoGen FlexSTAR+ produced high quality gDNA of high molecular weight and free of contaminants and inhibitors. It was used by Claritas Genomics for clinical whole exome sequencing from two completely different and complementary NGS platforms. High sequence quality was generated independent of DNA selection method or NGS technology. The DNA was also successfully used for Sanger sequencing, long range PCR, and other genomic assays. The instrument's increased extraction capacity, improved sample handling capabilities, and robust chemistry, makes the AutoGen FlexSTAR+ a valuable tool in our laboratory.

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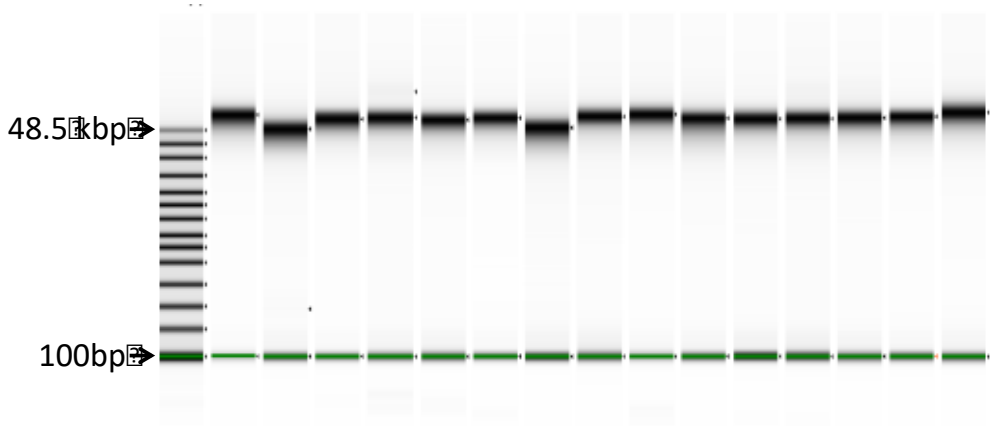

Figure 1. Fifteen gDNA samples extracted on an AutoGen FlexSTAR+ instrument were analyzed with Genomic DNA Screen Tape on an Agilent TapeStation. 100bp calibration fragments were added to all samples.

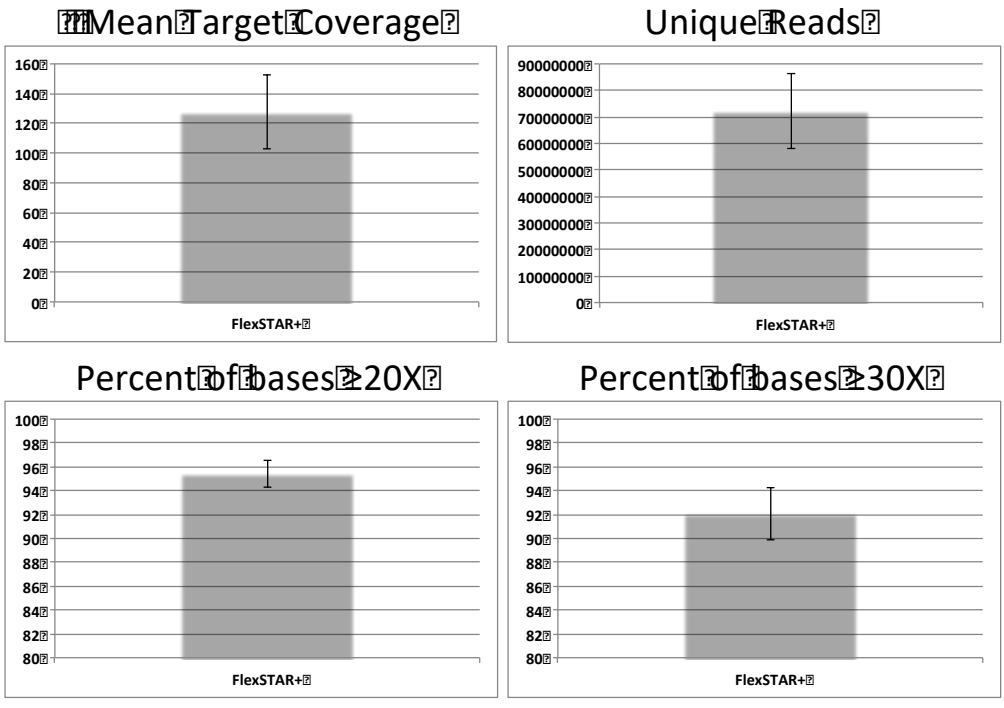

Figure 2. Illumina NextSeq sequencing metrics from Agilent Clinical Research Exome libraries constructed from AutoGen FlexSTAR+ extracted gDNA.

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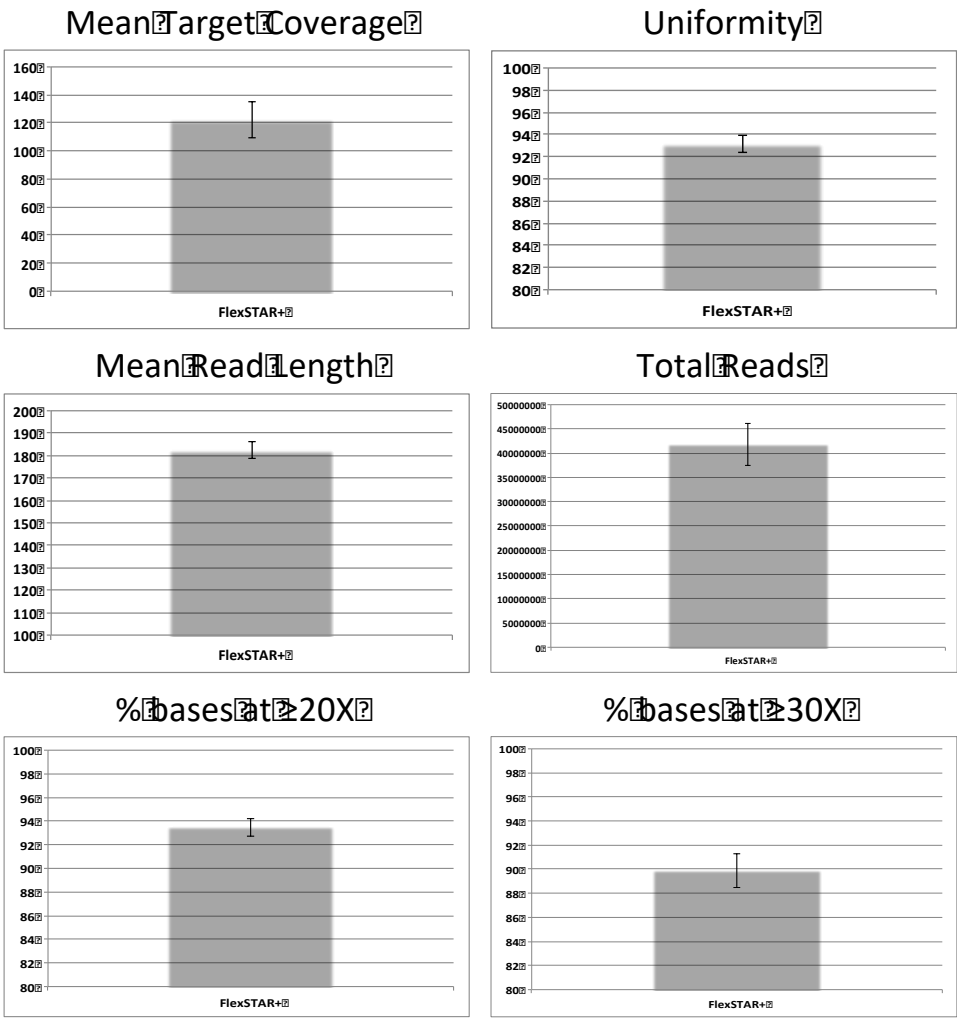

Figure 3. Ion Torrent Proton sequencing metrics from AmpliSeq Exome libraries constructed from AutoGen FlexSTAR+ extracted gDNA.

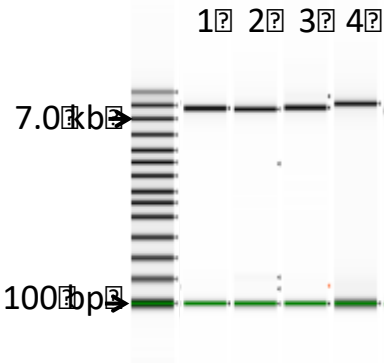

Figure 4. Agilent Genomic TapeStation of long range PCR products. Lane 1-4: 8.9, 8.0, 9.0 and 9.1kb amplified fragments.

Sources:
Chennagiri, N., White, E. J., Frieden, A., Lopez, E., Lieber, D. S., Nikiforov, A., et al. (2016). Orthogonal NGS for High Throughput Clinical Diagnostics. Scientific Reports , 6 (24650), 1-7.
FlexiGene DNA Handbook. (2010, May). QIAGEN.

About AutoGen

AutoGen is a leading provider of automated nucleic acid extraction workflows that allows lab professionals to produce premier quality and value-added extraction results. Our workflows provide solutions that are the best fit for our customers' laboratory needs and budget, and our customers include biorepositories, contract research organizations, academic research laboratories, pharmaceutical companies, clinical diagnostic laboratories, and government institutions all over the world. We strive to provide quality instrumentation and chemistries, as well as dedicated technical support – all with a level of post-sale service that is truly unmatched. Visit www.AutoGen.com to learn more.

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