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WHITE PAPER

Genomic DNA Extracted from Heparinized Whole Blood Using AutoGen FlexSTAR

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Executive Summary

The American Type Culture Collection, ATCC®, routinely extracts genomic DNA from heparinized whole blood using an automated extraction instrument. However, that instrument was prone to operation failures and high repair turnaround times. To solve this problem, ATCC® turned to AutoGen's FlexSTAR instrument. This change allowed them to develop a better extraction protocol, leading to higher-quality genomic DNA extractions with less hands-on effort and instrument downtime.

Introduction

The American Type Culture Collection, ATCC®, a nonprofit organization founded in 1925, is the world's most diverse biological resource center. In addition to its core business, ATCC® has established and managed bioprocessing and biosample repositories to support public health initiatives for the U.S. government, academia, pharmaceutical industries, and research foundations. As a part of their bioprocessing effort, ATCC® routinely extracts genomic DNA from whole blood received in heparinized blood collection tubes. An automated DNA extraction robot is used for whole-blood genomic DNA extraction due to the large volume of starting material. However, the organization had major concerns with the original automated instrument because of its frequent operation failure and high turnaround time for repair. To continue their service for heparinized whole-blood DNA extraction, ATCC® decided to invest in AutoGen's FlexSTAR. This instrument offers reliable whole-blood genomic DNA extraction with less hands-on time. However, a process modification was required to standardize the method for whole-blood genomic DNA extraction using heparin tubes. FlexSTAR was proven effective at extracting high-quality genomic DNA with minimal hands-on effort and instrument downtime.

FlexSTAR+

Materials and Methods

A total of fourteen FlexSTAR runs were executed to standardize whole-blood genomic DNA extraction using heparin tubes. The DNA extraction process was tested based on different starting volumes (1-4 mL of blood) and different starting cell numbers (5 – 15 x 106 cells). The FlexSTAR frozen blood DNA extraction kit was used as the reagent source. The mechanism of DNA extraction is based on a precipitation-based DNA extraction chemistry called "FlexiGene" (FlexiGene DNA handbook, 2010).

Before loading the blood samples to FlexSTAR, frozen samples were thawed in a 37°C water bath and diluted with 1X D-PBS. For the experiments where different volumes of blood were chosen as the starting material, samples were diluted, as described in **Table 1**. For the experiments where the starting material was based on the different cell numbers (5, 10, 12, or 15 x 10^6 cells), thawed blood was aliquoted according to the cell number and diluted with 1X D-PBS until the total volume of the sample reached 5 mL in each processing tube. Blood samples were then loaded into the on-board sample carousel, and the frozen blood protocol was selected from the menu list.

Table 1. Whole blood preparation for FlexSTAR run

Whole Blood (mL)	1X D-PBS (mL)	Total volume (mL)
1	4	5
2	3	5
3	2	5
4	1	5

DNA was extracted and purified through multiple steps during FlexSTAR operation. Lysis buffer was added to the sample, and cell nuclei and mitochondria were pelleted by centrifugation. The pellet was then resuspended and treated with protease to remove protein contaminants. Genomic DNA was precipitated by the addition of isopropanol and recovered by centrifugation. The recovered DNA was washed with 70% ethanol to remove adherent impurities. Finally, the DNA was resuspended in an elution buffer. The quality of the extracted DNA was checked by measuring the A260/280 ratio using the NanoDrop® ND-1000 UV-Vis Spectrophotometer.

Results

As heparin was used as a blood additive, several modifications were introduced throughout the extraction protocol to achieve the industrial standard of DNA purity (A260/280 = 1.7-1.9). The major process improvements were to increase protease incubation time from 20 minutes to 60 minutes, decrease elution buffer volume from 1.10 mL to 0.40 mL, and exclude RNase from the original protocol provided by AutoGen.

Our data indicates that the yield of DNA (µg) extracted was proportionally increased with the volume of starting material **(Figure 1, left panel)**, which is evident from the R2 value being very close to 1 **(Figure 1, right panel)**. Gel electrophoresis analysis also indicates a gradual increase in DNA yield with increased starting material (data not shown). The purity of the extracted DNA, as measured by the A260/280 ratio, was also within the acceptable range (1.7-1.8).

Initial Blood Volume (mL)	DNA Yield (µg)	Purity (A260:A280)
1	31.37 ± 1.94	1.83 ± .012
2	64.95 ± 2.33	1.82 ± .010
3	94.41 ± 6.31	1.81 ± .010
4	118.40 ± 12.38	1.78 ± .013

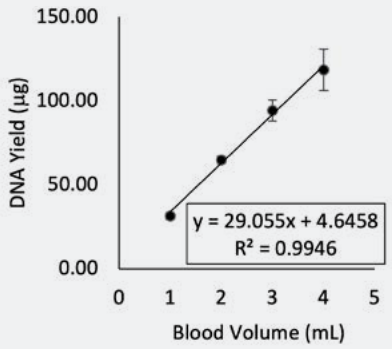

Figure 1. Heparinized whole blood volume vs. DNA yield

The genomic DNA was also extracted from heparinized whole blood based on the starting cell count. DNA yield (µg) increased as the cell count increased while maintaining an R2 value close to one. Initially, the heparinized whole blood sample did not have an R2 value close to one (R2 = 0.8705, data not shown). Hence, the protocol for genomic DNA extraction for the heparinized whole blood had to be optimized to obtain a high quality and quantity of DNA **(Figure 2).**

Starting Whole Blood Cell Count (x 10^6)	DNA Yield (µg)	Purity (A260:A280)
5	28.96 ± 12.0	1.84 ± .008
10	64.89 ± 3.29	1.82 ± .006
12.5	89.23 ± 1.85	1.82 ± .007
15	106.2 ± 4.33	1.81 ±.025

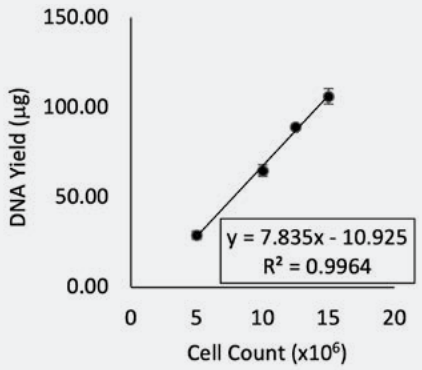

Figure 2. Heparinized whole blood cell count vs. DNA Yield.

Conclusions

Genomic DNA from human whole blood preserved in heparin was extracted using FlexiGene precipitation chemistry on the AutoGen FlexSTAR instrument. The DNA produced was of high quality, having an A260/280 ratio of around 1.80, where the DNA was free of contaminants and inhibitors. The FlexiGene protocol has been optimized by modifying incubation time for the protease, decreasing elution volume, excluding RNase during extraction, and utilizing the maximum amount of blood and cells that each FlexSTAR tube can process while extracting quality DNA. Overall, the optimized protocol can be used for samples containing heparin blood additives to ensure high quality of DNA.

About autogen

AutoGen is a leading provider of automated nucleic acid extraction workflows that allow 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 budgets, 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.

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