Genomic DNA Isolation from 1 µL – 100 µL of Whole Blood using Norgen's Blood Genomic DNA Isolation Micro Kit

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INTRODUCTION

Blood is an excellent sample for diagnostic purposes. It is rich in protein and nucleic acid-based biomarkers that can be used to detect a panel of diseases long before any physical symptoms become apparent. Studies have shown that cancer biomarkers, such as differentially methylated target genes, are detectable in blood samples and have proven to be extremely sensitive and specific for given cancer types1. These biomarkers can be cell associated, such as those found in leukocytes[2], or cell-free, such as those found in plasma or serum samples. For this reason, blood is often the sample of choice for biomarker or diagnostic research.

Investigators utilizing blood in their research have unique needs, based on their downstream applications and sample volume. For some studies, less blood may be processed in order to preserve precious samples. On the other hand, some studies require a high yield of DNA from their samples, and thus require higher volumes of sample to be processed. The method used for blood DNA isolation can heavily influence the results of research-based or diagnostic tests associated with blood. Investigators must ensure that their blood DNA isolation method is flexible, i.e. a linear increase in blood volume being processed leads to a linear increase in DNA concentration and yield. A robust blood DNA isolation kit eliminates sample processing biases, and increases data reproducibility.

The purpose of this study is to test Norgen's Blood Genomic DNA Isolation Micro Kit (Cat# 52100) over a range of different blood input volumes (1 µL - 100 µL) for its ability to isolate high quality and high quantities of genomic DNA.

METHODS AND MATERIALS

Sample collection

Blood was collected in Ethylenediaminetetraacetic acid (EDTA) tubes, from one healthy individual, by a trained professional. The sample was frozen at -70°C until processed.

Blood DNA extraction

DNA was extracted from the thawed blood samples using Norgen's Blood Genomic DNA Isolation Micro Kit (Cat# 52100) as per the manufacturer's instruction. Briefly, Proteinase K was added to a microcentrifuge tube, followed by 1, 10, 25, 50 or 100 µL of blood. The volume of each was raised to 100 µL using nuclease-free water.

Lysis Solution was then added, and samples were vortexed and incubated at 55°C for 10 minutes. Next, ethanol was added to each sample, and samples were bound, washed and eluted as per the manufacturer's protocol.

Spectrophotometry

Blood DNA quantity was measured using the UltraSpec 2100 Pro (Fisher Scientific). Fifty microliters of each DNA elution was diluted with 450 µL of nuclease-free water, and OD measurements were taken using the cuvette-based spectrophotometry method.

Real-Time PCR

The purified DNA was then used as the template in a realtime TaqMan® PCR reaction. Briefly, 5 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007), 0.4 µL of 25 µM GAPDH primer pair mix, 0.2 µL of 25 µM TaqMan® probe, and nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 40 cycles of 95°C for 15 seconds for denaturation, 60°C for annealing and extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

RESULTS AND DISCUSSION

lood is an excellent resource for research and diagnostic purposes. Systemic diseases can be detected through a simple blood test, and changes in DNA (both cellular and cell-free) can be detected from blood, giving rise to its high potential for screening for a panel of diseases.

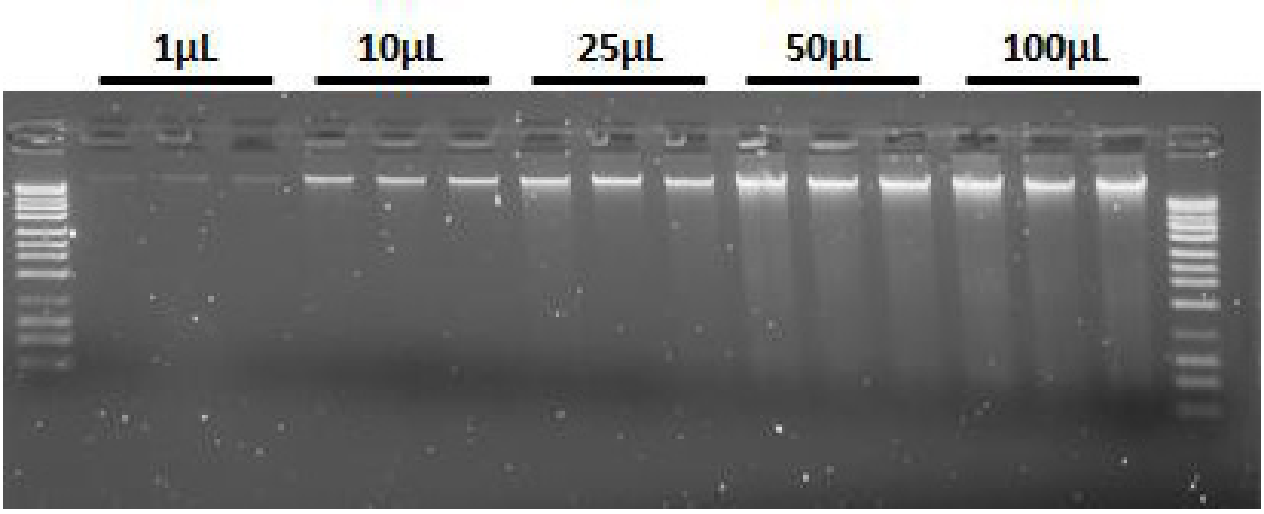

Figure 1. Resolution of DNA isolated from buffy coats using Norgen's Blood Genomic DNA Isolation Mini Kit. Fifteen microliters of 200 µL elutions were run on 1X TAE 1.0% agarose gel. Marker= Norgen's UltraRanger DNA Ladder.

he key to the success of any study utilizing blood is a reliable blood DNA isolation method that performs optimally from a range of sample input volumes. One can determine the flexibility of a kit by increasing the sample input volume in order to see the linearity of the increase in DNA yield. In this study, DNA was isolated from 1, 10, 25, 50 and 100 µL of blood using Norgen's kit. Fifteen microliters of each 100 µL elution was then run on a 1X TAE 1.0% agarose gel to visually inspect the isolated genomic DNA **(Figure 1)**. It was found that the yield was proportional to the blood input volume.

In order to determine the yield and purity of DNA isolated from each volume of blood using Norgen's kit, samples were measured using a cuvette-based spectrophotometry method **(Figure 2)**. The quantification did not agree with the gel, particularly at low input volumes, since the yield was lower than the detection limit of the device, however DNA was still visible on the gel. On the other hand, OD260/280 ratio was found to be > 1.7 from all samples **(Figure 3)**.

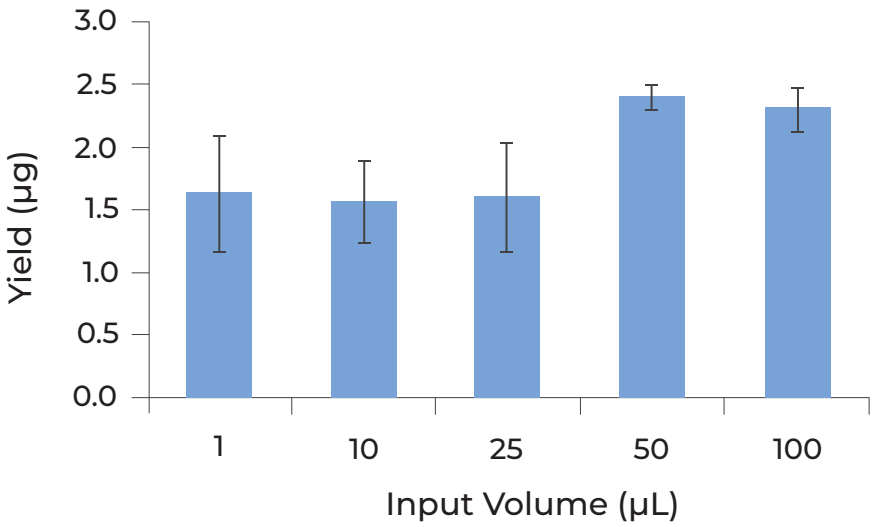

Figure 2. The difference in DNA yield from buffy coats processed using Norgen's Blood Genomic DNA Isolation Mini Kit. Twenty-five microliters of each sample was diluted in 475 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific).

DNA quality was determined through the use of a TaqMan® Real-Time PCR method. Five microliters of each sample was used in the reaction, and the Ct values were then graphed **(Figure 4)**. Successful PCR amplification was observed for all samples with an almost linear decrease in Ct with increasing blood input volume, which indicates that the kit has a linear dynamic range for blood input ranges of 1 – 100 µL.

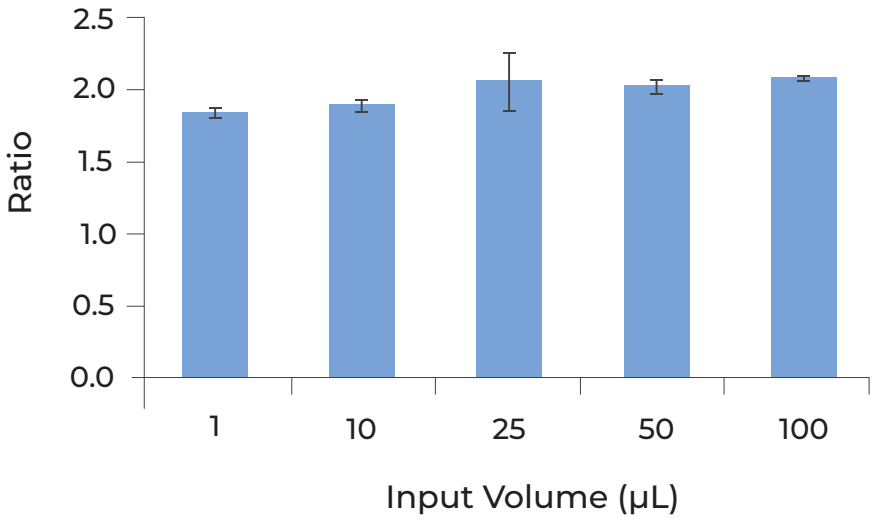

Figure 3. OD260/280 ratios of DNA isolated from buffy coats processed using Norgen's Blood Genomic DNA Isolation Mini Kit. Twenty-five microliters of each sample was diluted in 475 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific). One elution was performed.

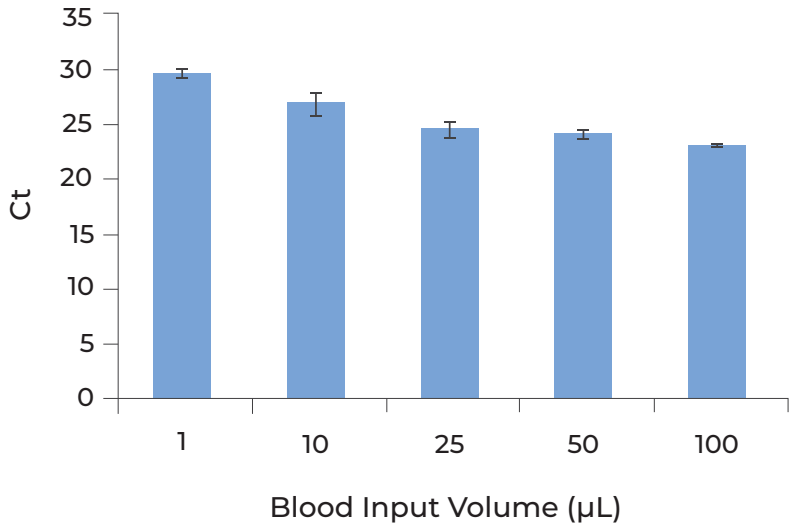

Figure 4. The difference in Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated from buffy coats. One, three, six and nine microliters of each elution were used in a 20 µL qPCR reaction involving GAPDH primers.

CONCLUSION

From the data presented in this report, the following can be concluded:

1. Isolated genomic DNA has good purity and integrity with positive PCR amplification from all samples over the blood input range tested (1 µL -100 µL).

2. Norgen's Blood Genomic DNA Isolation Micro Kit displayed a good performance with a linear dynamic blood input range of 1 – 100 µL.

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