Effect of Elution Volume on DNA Recovery and Quality 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 types[1]. 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. Some blood samples are very small in size, and therefore require careful handling especially for precious samples. There are numerous sensitive downstream applications that require high concentrations of DNA in order to be completed successfully such as microarrays, southern blotting, and genotyping. Investigators must ensure that their blood DNA isolation method can provide DNA at the desired concentration and purity to suit their downstream application. Therefore, an isolation method with a flexible elution volume can be used to obtain the desired concentration of DNA particularly from blood samples that contain low DNA content.

The purpose of this study is to determine the relationship between concentration, yield and purity of blood DNA isolated by Norgen's Blood Genomic DNA Isolation Micro Kit (Cat# 52100) using a range of elution volume.

MATERIALS AND METHODS

Sample collection

Blood was collected from a single healthy individual in citrate tubes by a trained professional. The sample was frozen at -70ºC until processed.

Blood DNA extraction

DNA was extracted from the thawed blood sample 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 100 µL of blood. Next, 300 µL of Lysis Solution was added, and samples were vortexed and incubated at 55ºC for 10 minutes. Next, 250 µL ethanol was added to each sample, which was followed by the pooling of all lysates into a 50 CC tube. Samples were then bound, washed and eluted as per the manufacturer's protocol. Five different elution volumes were used: 15 µL, 20 µL, 30 µL, 50 µL, and 100 µL.

Spectrophotometry

Blood DNA quantity was measured using the UltraSpec 2100 Pro (Fisher Scientific). Five microliters of the 15 µL and 20 µL elution samples was diluted with 495 µL of nuclease-free water, while 10 µL of the 30 µL, 50 µL and 100 µL DNA elution samples was diluted with 490 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 real-time TaqMan® PCR reaction. Briefly, 2 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 a 25 µM GAPDH primer pair mix and 0.2 µL of the TaqMan® probe. The volume was brought up to 20 µL using 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

Blood 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.

It is advantageous to use an isolation method that can provide high concentrations of DNA without compromising purity or downstream applications. A kit that can perform elution at a wide range of volumes, while maintaining robustness and consistency, can be efficient in providing higher DNA concentrations when required. In this study, DNA was isolated from blood collected from the same donor on citrate, using Norgen's Blood Genomic DNA Isolation Micro kit. The samples were then eluted in volumes of 15 µL, 20 µL, 30 µL, 50 µL, and 100 µL. Following the isolation, five microliters of each elution volume was run on a 1X TAE 1.0% agarose gel to visually inspect the isolated genomic DNA (**Figure 1**). It was found that the concentration was inversely proportional to the elution volume, and that the DNA integrity was consistent from all samples.

In order to determine the concentration and yield of DNA isolated by the kit at the different elution volumes, samples were measured using a cuvette-based spectrophotometry method (**Figures 2 & 3).** The data demonstrated that the elution volume was inversely proportional to concentration, while directly proportional to the yield. The OD260/280 ratio was found to be consistent with a high purity of DNA of greater than 1.7 at all the elution volumes **(Figure 4)**.

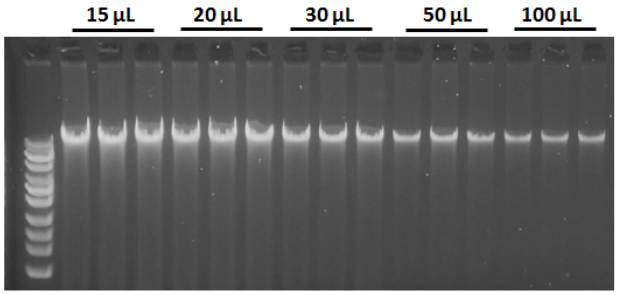

Figure 1. DNA isolated from blood and eluted with volumes of 15 µL, 20 µL, 30 µL, 50 µL, and 100 µL using Norgen's Blood Genomic DNA Isolation Micro Kit. Five microliters of the various elutions were run on 1X TAE 1.0% agarose gel. The used ladder is Norgen's UltraRanger DNA Ladder.

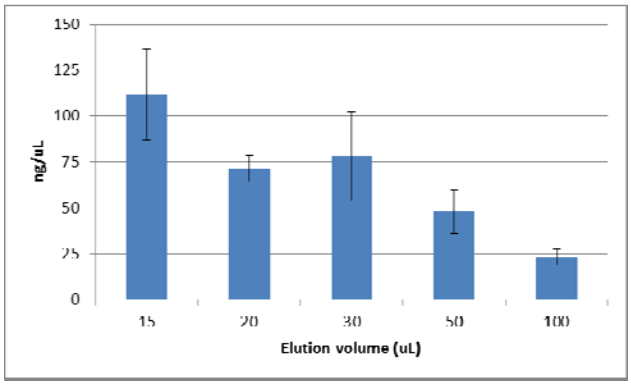

Figure 2. A comparison of DNA concentration from blood processed with elution volumes of 15 µL, 20 µL, 30 µL, 50 µL, and 100 µL using Norgen's Blood Genomic DNA Isolation Micro Kit. Five microliters of the 15 µL and 20 µL samples were diluted in 495 µL of nuclease-free water, while 10 µL of the 30 µL, 50 µL and 100 µL samples were diluted in 490 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific).

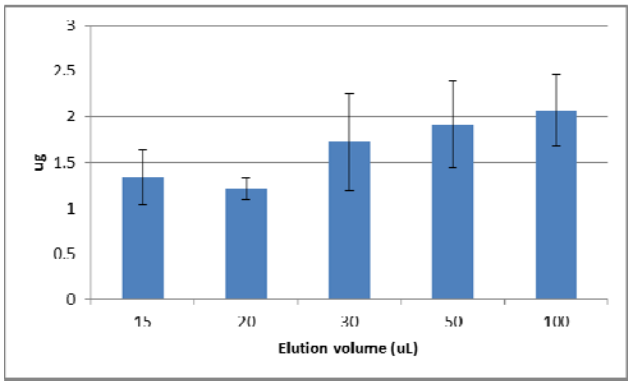

Figure 3. A comparison of DNA yield from blood processed with elution volumes of 15 µL, 20 µL, 30 µL, 50 µL, and 100 µL using Norgen's Blood Genomic DNA Isolation Micro Kit. Five microliters of the 15 uL and 20 uL samples were diluted in 495 µL of nuclease-free water, while 10 µL of the 30 µL, 50 µL and 100 µL samples were diluted in 490 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific).

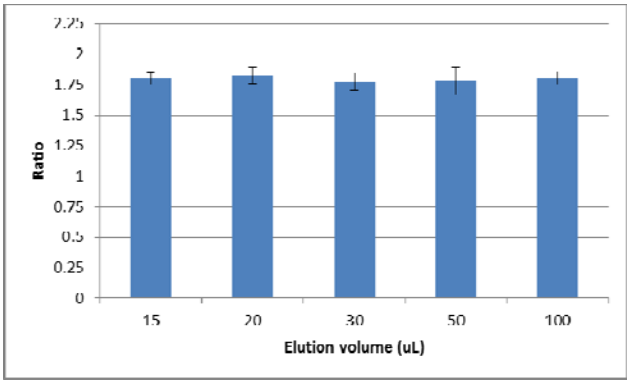

Figure 4. OD260/280 ratios of DNA isolated from blood eluted with volumes of 15 µL, 20 µL, 30 µL, 50 µL and 100 µL and processed using Norgen's Blood Genomic DNA Isolation Micro Kit. . Five microliters of the 15 uL and 20 uL samples were diluted in 495 µL of nuclease-free water, while 10 µL of the 30 µL, 50 µL and 100 µL samples were diluted in 490 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. The Ct values were then graphed (**Figure 5)**. Successful qPCR amplification was observed from all elution volumes. The Ct value increased with increasing elution volume, as expected, due to the reduction in concentration.

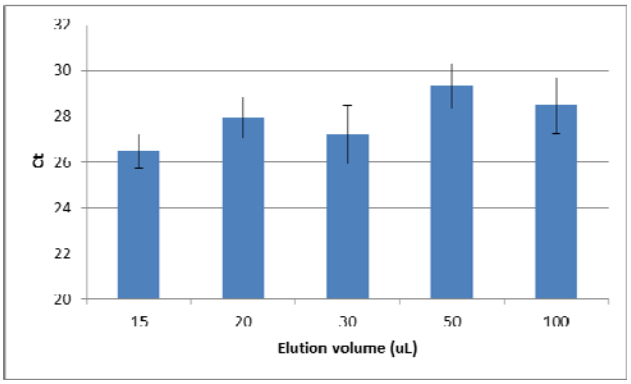

Figure 5. The difference in Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated from blood eluted using 15 µL, 20 µL, 30 µL, 50 µL and 100 µL elution volumes. Two microliters of each elution were used in a 20 µl qPCR reaction involving GAPDH primers.

CONCLUSIONS

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

1- Norgen's Blood Genomic DNA Isolation Micro Kit has a dynamic elution range over 15-100 µL with a higher concentration and lower yield observed with lower elution volumes.

2- DNA from all the elution volumes tested has good integrity, high purity and can be used successfully in downstream PCR amplifications.

REFERENCES

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2. Koestler DC, Marsit CJ, Christensen BC, Accomando W, Langevin SM, Houseman EA, et al. 2012. Peripheral blood immune cell methylation profiles are associated with nonhematopoietic cancers. Cancer Epidemiol Biomarkers Prev; 21(8):1293-302.

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