Single Elution vs. Re-Elution: Implications on Concentration and Purity using the 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 investigate the effect of re-elution using different elution volumes on concentration, yield and purity of blood genomic DNA when isolated using Norgen's Blood Genomic DNA Isolation Micro Kit (Cat# 52100). Re-elution involves reapplying the initial elution back onto the column to elute a second time.

MATERIALS AND METHODS

Sample collection

Blood was collected from a single individual into a citrate tube by a trained professional. The sample was frozen at -70°C 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 55°C for 10 minutes. Next, 250 µL ethanol was added to each sample, which was followed by the pooling of all lysates into a 50CC tube. Samples were then bound, washed and eluted as per the manufacturer's protocol. Five different elution volumes were used in two sets of samples: 15 µL, 20 µL, 30 µL, 50 µL, and 100 µL. For one set of samples, a single elution was performed using the volumes indicated above. For the second set of samples, the initial elutions were performed using the volumes indicated above, and then the elutions were re-loaded onto the column to perform a second re-elution.

Spectrophotometry

Blood DNA quantity was measured using the UltraSpec 2100 Pro (Fisher Scientific). Five µL 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

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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 elutions 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 using elution volumes of 15 µL, 20 µL, 30 µL, 50 µL, and 100 µL. For one set of samples a single elution was performed, while for a second set of samples a re-elution was performed by loading the first elution back onto the column. 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)**. DNA integrity was consistent in all samples with no significant effect of re-elution on concentration as visualized on the gels.

In order to determine the concentration and yield of isolated DNA, samples were measured using a cuvette-based spectrophotometry method **(Figure 2 & 3).** In general, re-elution slightly improved the DNA concentration as well as yield. The OD260/280 ratio was found consistently to be greater than 1.7 from all the single elution and re-elution samples with equal or higher ratios from the re-elution compared to the single elution **(Figure 4).**

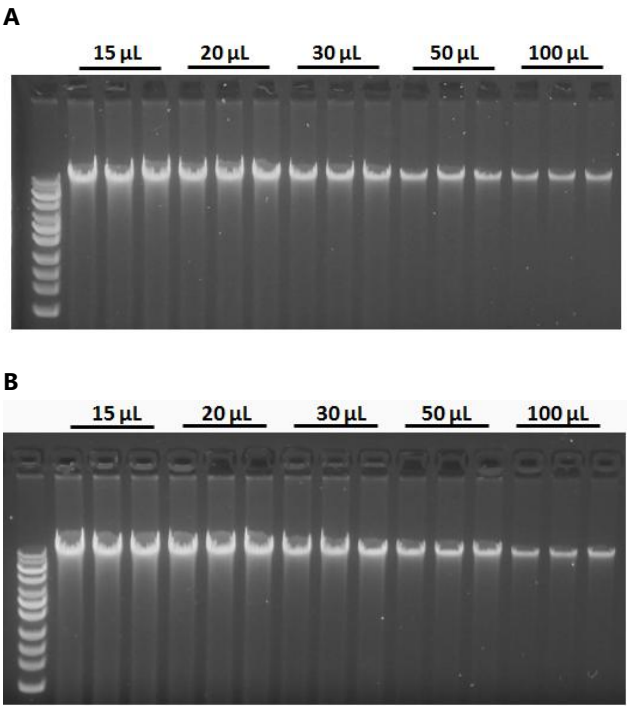

Figure 1. Resolution of DNA isolated from blood with a single elution (A) and re-elution (B) using Norgen's Blood Genomic DNA Isolation Micro Kit. Five microliters of the various elutions were run on 1X TAE 1.0% agarose gel. The ladder is Norgen's UltraRanger DNA Ladder.

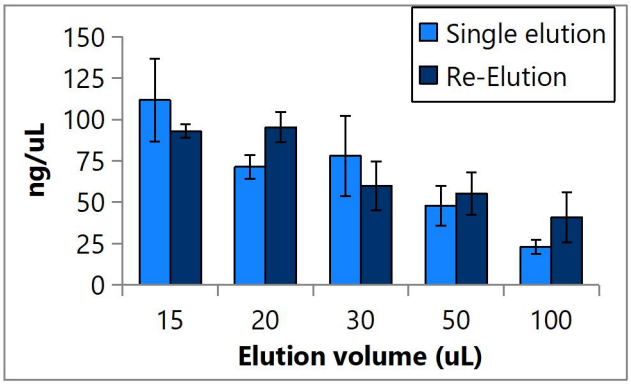

Figure 2. A comparison of DNA concentration between a single elution and re-elution using Norgen's Blood Genomic DNA Isolation Micro Kit. Five microliters of the 15 uL and 20 uL samples were diluted in 495 µL of nuclease-free water, while 10 µL of the 30 µL, 50 µL and 100 µL samples were diluted in 490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

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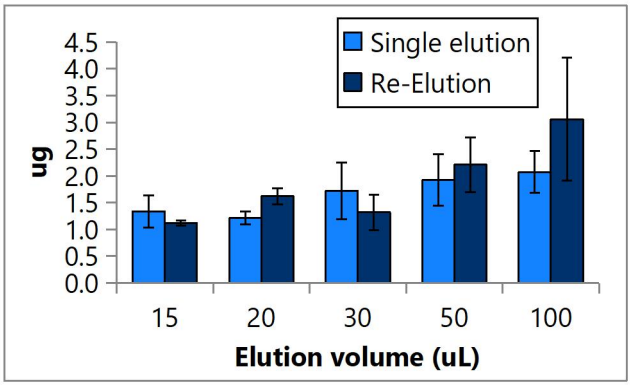

Figure 3. A comparison of DNA yield between a single elution and re-elution using Norgen's Blood Genomic DNA Isolation Micro Kit. Five microliters of the 15 uL and 20 uL samples were diluted in 495 µL of nuclease-free water, while 10 µL of the 30 µL, 50 µL and 100 µL samples were diluted in 490 µL of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

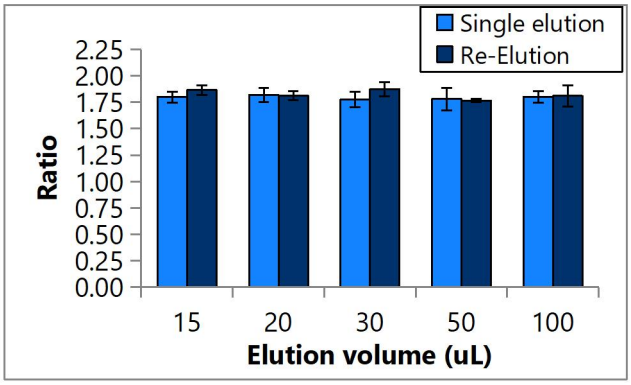

Figure 4. A comparison of OD260/280 ratios between a single elution and re-elution using Norgen's Blood Genomic DNA Isolation Micro Kit. Five microliters of the 15 uL and 20 uL samples were diluted in 495 µL of nuclease-free water, while 10 µL of the 30 µL, 50 µL and 100 µL samples were diluted in 490 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 resulting Ct values were graphed **(Figure 5)**. Successful qPCR amplification was observed from all elution volumes of the single elution and re-elution with no significant difference between the Ct values of the corresponding elution volumes.

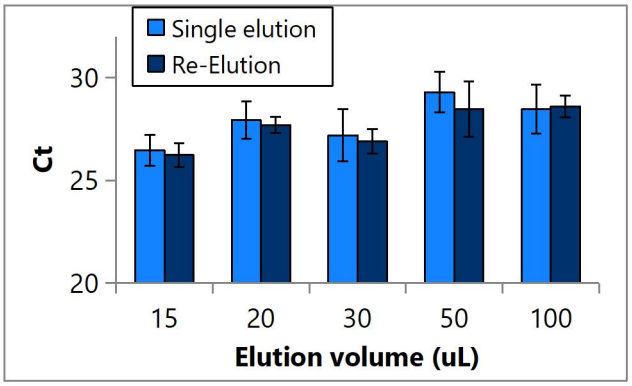

Figure 5. The difference in Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated from blood with a single elution and re-elution. Two microliters of each elution were used in a 20 µl qPCR reaction involving GAPDH primers.

CONCLUSIONS

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

1. Re-elution can slightly enhance DNA concentration and yield without affecting purity or PCR efficiency.
2. Eluted DNA from all elution volumes tested, whether from the single elution or the re-elution conditions, has good integrity, high purity and good PCR performance.

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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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