Genomic DNA Isolation from Buffy Coat using Norgen's Blood Genomic DNA Isolation Mini 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.

Whole blood can be fractionated by centrifugation into 3 main components: the upper plasma, the middle buffy coat containing leukocytes, and the bottom erythrocytes. The buffy coat is about 1% of the total volume of the blood sample. It is a very thin, delicate portion and is used in diagnostic labs and procedures. The amount of white blood cells/leukocytes in blood is highly variable between samples. There is great inter-individual variation as well as intra-individual variation (physiological variability)[3]. Physiological variability, or variability within the same individual at different periods, can be stimulated by factors such as diet, medication, infection or activity[3]. This can cause either an increase or decrease in white blood cell concentration within the bloodstream, causing differences in DNA concentration or yield[3].

Investigators have unique needs based on which portion of the blood sample is being utilized in their study. The portion of blood used can affect the results from blood DNA isolation kits, ultimately influencing the results of research-based or diagnostic tests associated with blood. Investigators must ensure that their blood DNA isolation method is flexible, i.e. it can work efficiently on isolating blood from both whole blood and buffy coats. A robust blood DNA isolation kit eliminates sample processing biases, and increases data reproducibility while using different portions of blood.

The purpose of this study is to isolate blood genomic DNA from the buffy coat portion of separated blood using Norgen's Blood Genomic DNA Isolation Mini Kit (Cat# 46300).

MATERIALS AND METHODS

Sample collection

Fresh blood was collected in EDTA tubes, from two healthy individuals, by a trained professional. The blood was then centrifuged for 10 minutes and separated into three components: plasma, buffy coat, and erythrocytes. The upper plasma portion was then removed and the buffy coat beneath was carefully withdrawn using a pipette and placed in a separate tube.

Blood DNA extraction

DNA was extracted from the buffy coat sample using Norgen's Blood Genomic DNA Isolation Mini Kit (Cat# 46300), as per the manufacturer's instruction. Briefly, Proteinase K was added to a microcentrifuge tube, followed by 200 µL of buffy coat. 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, 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). 25 µL of each DNA elution was diluted with 475 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, 1 µL, 3 µL, 6 µL and 9 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 from a 25µM 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;

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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 beneficial for studies utilizing blood to use a blood DNA isolation method that performs optimally when any portion of blood is used, as the samples they have available may be of different types. In this study, DNA was isolated from the buffy coats from two different donors using Norgen's Blood Genomic DNA Isolation Mini Kit. Fifteen microliters of each 200 µL elution was then run on a 1X TAE 1.0% agarose gel to visually inspect the isolated gDNA **(Figure 1)**. Isolated DNA shows high integrity of > 24kbp.

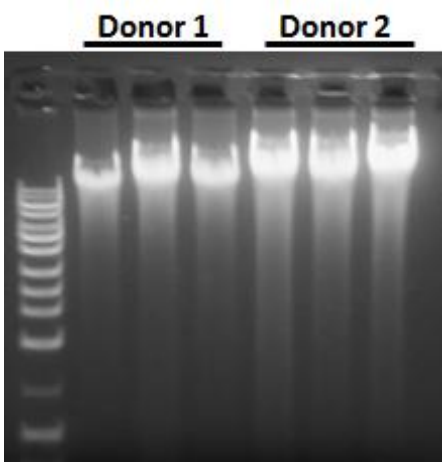

Figure 1. Resolution of DNA isolated from buffy coats using Norgen's Blood Genomic DNA Isolation Mini Kit. Fifteen microliters of 200 µL elutions were run on 1X TAE 1.0% agarose gel. Marker= Norgen's UltraRanger DNA Ladder.

In order to determine the yield and purity of the isolated DNA from both donors, samples were measured using a cuvette-based spectrophotometry method (UltraSpec 2100 Pro; Fisher Scientific). DNA yield was determined **(Figure 2),** as well as OD260/280 ratio **(Figure 3).** DNA yield was between 17.1 ug and 48.5 ug from the two donors, with OD260/280 ratio of > 1.8 from all samples.

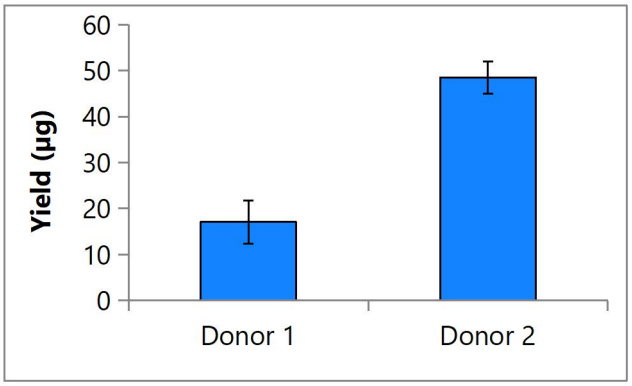

Figure 2. The difference in DNA yield from buffy coats processed using Norgen's Blood Genomic DNA Isolation Mini Kit. Twenty-five microliters of each sample was diluted in 475 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific).

DNA quality was determined through the use of a TaqMan® Real-Time PCR method. In order to assess sample inhibition, 1 µL, 3 µL, 6 µL and 9 µL of each sample was used in the reaction. The Ct values were then graphed **(Figure 4)**. The positive amplification from all samples at all the input volumes (including the 9 µL input), as well as the proportional decrease in Ct value with increasing the template input volume indicates the high quality of the isolated DNA and absence of PCR inhibitors.

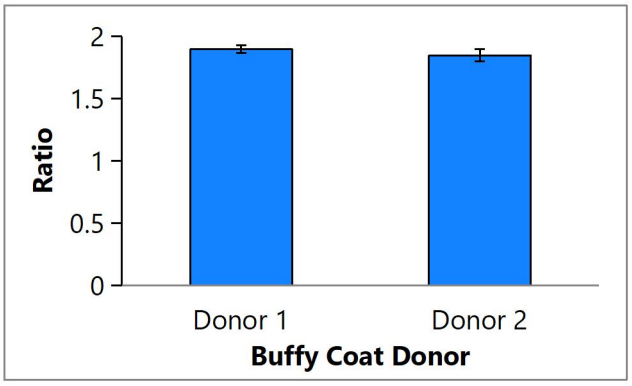

Figure 3. OD260/280 ratios of DNA isolated from buffy coats processed using Norgen's Blood Genomic DNA Isolation Mini Kit. Twenty-five microliters of each sample was diluted in 475 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific). One elution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

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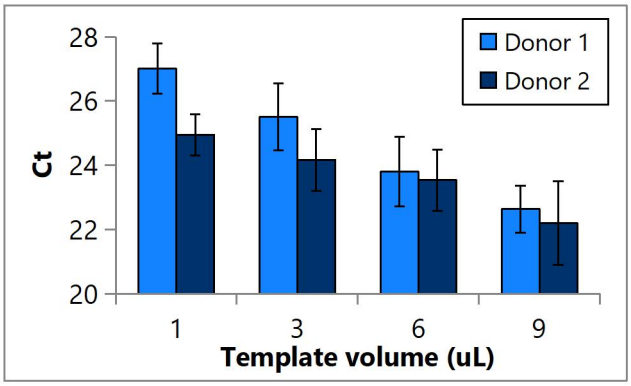

Figure 4. The difference in Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated from buffy coats. One, three, six and nine microliters of each elution were used in a 20 µL qPCR reaction involving GAPDH primers.

CONCLUSIONS

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

1. Norgen's Blood Genomic DNA Isolation Mini Kit can isolate DNA from buffy coat with high yield of up to 50 µg.
2. DNA isolated from buffy coat using Norgen's Blood Genomic DNA Isolation Mini Kit is of a high purity and quality, and is free of PCR inhibitors.

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