Comparative Study of DNA Isolated from Whole Blood Using Norgen's Blood Genomic DNA Isolation Mini Kit and Competitors' Kits

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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 and sample volume. 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 efficient in isolating high amounts of DNA with high purity and compatibility with sensitive downstream applications. A robust blood DNA isolation kit eliminates sample processing biases, and increases data reproducibility.

The purpose of this study is to compare four commercially available blood DNA isolation methods: Norgen's Blood Genomic DNA Isolation Mini Kit (Cat# 46300), Competitor A's DNA Blood Mini Kit), Competitor B's Blood kit and Competitor B's Dx Blood, for their ability to isolate high quality and high quantities of genomic DNA from increasing volumes of blood.

MATERIALS AND METHODS

Sample collection

Ten milliliters of blood was collected in Ethylenediaminetetraacetic acid (EDTA) tubes, from one healthy individual, by a trained professional. The sample was frozen at -70ºC until processed.

Blood DNA extraction

DNA was extracted from 200 µL of the thawed blood sample using Norgen's Blood Genomic DNA Isolation Mini Kit (Cat# 46300), Competitor A's DNA Blood Mini Kit, Competitor B's Blood kit and Competitor B's Dx Blood, as per the manufacturers' instructions.

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 real-time TaqMan® PCR reaction. Briefly, 1, 3, 6 & 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 25mM GAPDH primer pair mix, 0.2 µL from a 25mM 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

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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.

The key to the success of any study utilizing blood is a reliable blood DNA isolation method that provides high quality DNA. In this study, DNA was isolated from 200 µL of blood using Norgen's kit, Competitor A and two Competitor B kits. 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)**. All kits were able to isolate intact genomic DNA of > 24kbp in size, with higher yields from Norgen's Blood Genomic DNA Isolation Mini kit and Competitor B's Dx Blood kit.

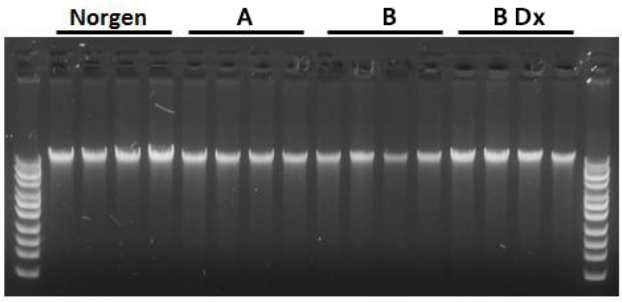

Figure 1. Resolution of DNA isolated using Norgen's Blood Genomic DNA Isolation Kit (Norgen) and Competitor A's DNA Blood Mini Kit (A), Competitor B's Blood kit (B) and Competitor B's Dx Blood (B Dx). Fifteen microliters of 200 µL elutions were run on 1X TAE 1.0% agarose gel. Marker= Norgen's UltraRanger DNA Ladder.

In order to determine the yield and purity of the isolated DNA, samples were measured using a cuvette-based spectrophotometry method (UltraSpec 2100 Pro; Fisher Scientific) **(Figure 2 and Figure 3)**. Norgen samples were found to have higher DNA yields and OD260/280 ratios than the other competitors with an average recovery of 4 µg of DNA and OD260/280 ratio of 2.

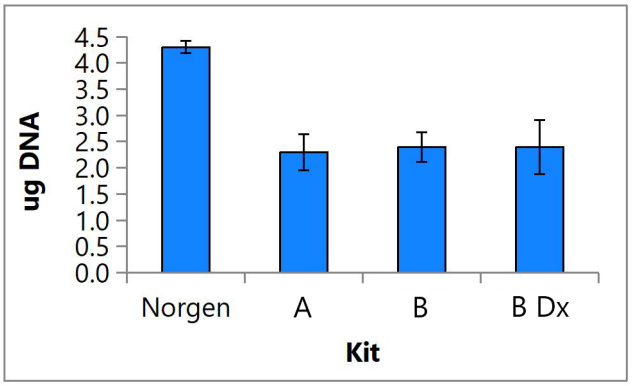

Figure 2. The difference in yield of blood DNA isolated using Norgen's Blood Genomic DNA Isolation Mini Kit (Norgen) and Competitor A's DNA Blood Mini Kit (A), Competitor B's Blood kit (B) and Competitor B's Dx Blood (B Dx). Fifty microliters of each sample was diluted in 450 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific).

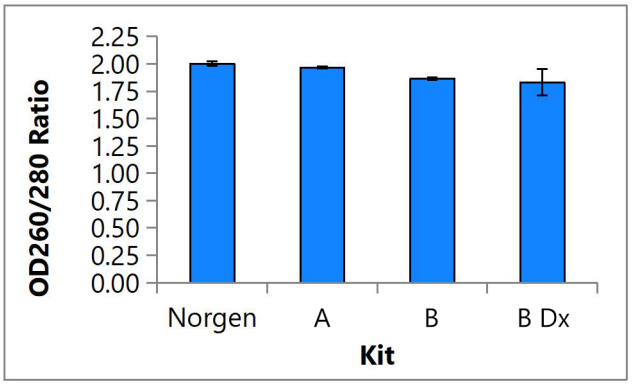

Figure 3. The difference in OD260/280 of blood DNA isolated using Norgen's Blood Genomic DNA Isolation Mini Kit (Norgen) and Competitor A's DNA Blood Mini Kit (A), Macherey-Nagel's Competitor B's Blood kit (B) and Competitor B's Dx Blood (B Dx). Fifty microliters of each sample was diluted in 450 µL 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, 3, 6 & 9 µL of each sample was used in the reaction. The Ct values were then graphed **(Figure 4)**.

Successful amplification was obtained from all samples for all the input volumes tested. Norgen's kit showed the lowest average Ct value at 9 µL template input compared to the other kits, indicating the higher purity of the isolated DNA and absence of PCR inhibitors.

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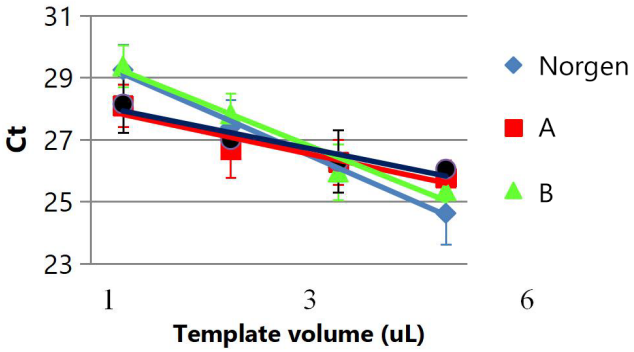

Figure 4. The difference in Ct values between DNA isolated using Norgen's Blood Genomic DNA Isolation Mini Kit (Norgen), Competitor A's DNA Blood Mini Kit (A), Competitor B's Blood kit (B) and Competitor B's Dx Blood (B Dx), in a Taqman® qPCR reaction. Different template volumes (1, 3, 6 & 9 µL) microliters of each elution were used in a 20µl 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 isolates the highest yields of DNA, when compared to the leading competitors' kits.
2. Norgen's Blood Genomic DNA Isolation Mini Kit outperforms competitors' kits in sensitive downstream applications, such as qPCR.
3. Norgen samples are free from inhibitors, as 9 µL of elution was used in each PCR reaction, with no delay in Ct values when compared to competitors' kits.

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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