Determining the Molecular Weight Cut-Off of Plasma Circulating DNA Using Two Commercially Available Kits

M. Abdalla[1], M. Simkin[1], Y. Haj-Ahmad[1,2].
[1]Norgen Biotek Corporation, Thorold, Ontario, Canada
[2] Centre for Biotechnology. Brock University, St. Catharines, Ontario, Canada

INTRODUCTION

Circulating plasma DNA has been extensively studied for its diagnostic potential (1), however, the abundance of many DNA fragments is often not properly reflected in downstream analyses, due to the molecular weight cut off of the plasma DNA isolation method used. Molecular weight cutoffs of certain isolation methods have been found to be a source of bias in determining the origin of circulating DNA (2). For example, apoptosis has been found to produce fragments of ~180 bps, whereas necrosis results in higher molecular weight fragments. When only high molecular weight fragments are isolated by a given system, valuable low molecular weight biomarkers go undetected (3). Therefore, a robust circulating plasma DNA isolation system is required to identify low molecular weight biomarkers in the plasma of many patients. The objective of this study is to compare two commercially available plasma DNA isolation kits to determine their respective molecular weight cutoffs for circulating plasma DNA recovery.

MATERIALS AND METHODS

Blood Collection and Plasma Preparation

Human blood was drawn directly into Heparin tubes in one single seating from the same individual. Plasma was prepared according to standard procedure. Norgen's FastRunner DNA Ladder (Cat# 12800), and UltraRanger 1kB DNA Ladder (Cat# 12100) were then pooled, and 10 µl/isolation of this sample was spiked into the plasma prior to aliquoting.

Plasma DNA Purification

DNA was isolated from 200 µL of spiked plasma using two kits: Norgen's Plasma/Serum Circulating DNA Isolation Mini Kit (Slurry Format; Cat# 50600) and Competitor's DNA Blood Mini Kit according to manufacturer's protocols.

Determination of Percent Recovery

Fifteen percent of each purified plasma DNA sample was loaded on a 1X TAE 1.8% agarose gel. Percent recovery was determined based on gel densitometry, using AlphaEaseFC™ (AlphaImager 2200— AlphaInnotech). The Area Under the Curve (AUC) was determined for input and test bands using the pixel density of each band.

RESULTS AND DISCUSSION

Two commercially available kits were compared in this study for their ability to recover all sizes of DNA fragments from plasma, using a plasma sample spiked with two DNA ladders. DNA was purified from the plasma using both kits, and the elutions were run on a 1.8% 1X TAE agarose gel (Figure 1).

Norgen's kit was found to recover all sizes of DNA (even as low as 50 bps), while Competitor's kit only successfully recovered high molecular weight bands (4000 bps and above), as well as contaminating genomic DNA.

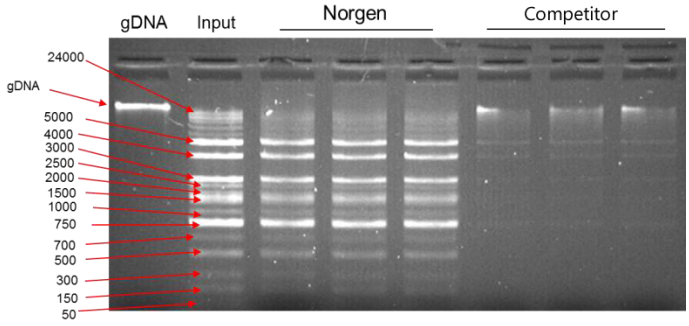

Figure 1. Plasma DNA Recovery on a 1.8% 1X TAE agarose gel. Human plasma was spiked with DNA ladders with bands ranging from 24000-50bps.

Using gel densitometry, based on the AUC generated by the input pooled ladders, relative percent recovery values were determined, and graphed in Figure 2. Norgen's kit was found to have a high percent recovery of all bands, with the highest percent recovery in the low MW bands (300 and 500 bps).

3430 Schmon Parkway
Thorold, ON Canada L2V 4Y6
Phone: 905-227-8848 • Fax: 905-227-1061
Toll-Free (North America): 1-866-667-4362
www.norgenbiotek.com

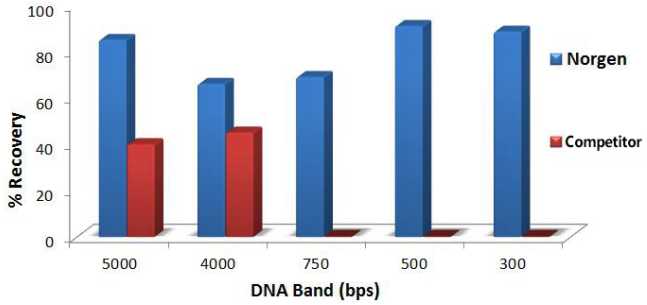

Figure 2. Plasma DNA Recovery from Human Plasma Spiked with DNA Ladders. Percent recovery was determined based on gel densitometry (from Figure 1).

CONCLUSIONS

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

1. Norgen's proprietary resin has a higher affinity for all sizes of DNA, including very low MW fragments. This affinity was found to be evident in the percent recovery of the smaller bands of the DNA ladders.

2. Norgen's kit recovered less contaminating genomic DNA from plasma compared to Competitor's kit.

3. Norgen's Plasma/Serum Circulating DNA Isolation Mini Kit (Slurry Format) is the most ideal kit for circulating plasma DNA isolation, as it reduces DNA fragment size bias in downstream analyses.

REFERENCES

1. Sozzi G *et al.* 2003. *J Clin Oncol.* 21: 21.
2. Jahr S *et al.* 2001.. *Cancer Res.* 61: 1659-1665.
3. Chan KCA *et al.* 2003. *Cancer Res.* 63: 2028-2032.

3430 Schmon Parkway
Thorold, ON Canada L2V 4Y6
Phone: 905-227-8848 • Fax: 905-227-1061
Toll-Free (North America): 1-866-667-4362
www.norgenbiotek.com