Isolation of DNA from as Little as 25 µL of Urine Using Norgen's Urine DNA Isolation Kit

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INTRODUCTION

The use of DNA for diagnostics has seen a steady increase in recent years. The means of obtaining, isolating and analyzing the DNA have been extensively studied. A number of methods for the isolation of the DNA are extremely intrusive, such as the use of amniocenteses for the isolation of fetal blood, or biopsies to isolate genetic material associated with tumors for cancer diagnosis and monitoring. These methods are often painful and can lead to possible complications. Thus there exists a need to develop a less intrusive and safer way to isolate DNA for diagnostic uses.

Circulating cell-free DNA has been detected in the serum and plasma of both healthy and diseased individuals (1), and its use in diagnostics has been investigated. This circulating DNA has been found to contain disease markers including sequence alterations associated with various forms of cancer including lung cancer (2), head and neck cancer (3), renal cancer (4), pancreatic cancer (5) and breast cancer (6). Many of these mutations were found to be identical to those present in the primary tumour, verifying that some of the circulating DNA is indeed of tumour origin and not of blood cell origins (7).

The source of the circulating cell-free DNA in blood has been extensively studied, and appears to be the result of cell death. Upon cell death, cellular components are dismantled and usually phagocytosed by macrophages or neighboring cells. The nuclear DNA is often degraded by a range of enzymes, producing nucleosomes and their oligomers. However, it has been found that some of this DNA from dying cells escapes intracellular degradation and phagocytosis, and is able to circulate in the bloodstream (8). The cell death may result from apoptosis, or be associated with disease, such as the increased proliferation and cell death associated with malignant, benign or pre-neoplastic cells (7). Indeed, the DNA content of plasma appears to reflect the amount of cell death occurring in the whole body, and is increased in individuals with cancer (8).

Recently, it has been demonstrated that small amounts of this cell-free circulating DNA are able to pass the kidney barrier and end up in urine (i.e. trans-renal DNA) (7,8,9). Urine DNA has been found to be comprised of two main types of DNA from two different sources. The larger species is generally greater than 1 kb in size, and appears to derive mainly from cells shed into the urine from the urinary tract (7). The second species is smaller, generally between 150 and 250 bp, and is mostly recovered from the supernatant. Researchers have found that this smaller species of urine DNA derives from the circulation (7), and thus contains DNA that has passed the kidney barrier.

The circulating DNA from the bloodstream that passes into the urine can be isolated and used in many different applications in diagnostics. The DNA can be used for molecular diagnostics and prognosis, including cancer testing (7,8), prenatal diagnosis, transplantation monitoring, (8) and doping control in sports (10). Furthermore, the larger DNA obtained from cells shed into the urine can be used to diagnose cancer by detecting tumours arising at or near the urinary tract (7).

There are a number of advantages to using the DNA found in urine for diagnostics, including the fact that sample collection of urine is absolutely non-invasive, and the isolation of DNA from urine is technically easier than from blood due to the low protein content of urine. Furthermore, urine is non-infectious for HIV and less infectious for many other pathogens (8). However, the current methods for isolating DNA from urine have a number of drawbacks associated with them. First of all, the methods are generally long and tedious, and only result in trace amounts of DNA being isolated (7, 11). Furthermore, large amounts of urine are required for the procedure, however only very small amounts of DNA are isolated. Also, the procedures often involve the use of expensive or harmful chemicals (12).

In this study, the Urine DNA Isolation Kit from Norgen Biotek was evaluated for its ability to isolate DNA from small amounts of urine. The kit provides a fast and simple

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procedure for isolating both species of DNA from 2 mL of urine. The kit is based on spin column chromatography, using Norgen's proprietary resin as the separation matrix. Preparation time for a single sample is stated as less than 90 minutes, and purified samples can be used in various downstream applications including PCR. The kit was evaluated based on these claims, in order to see if it provides a good alternative to the traditional methods of DNA isolation from urine.

MATERIALS AND METHODS

DNA Isolation Using Norgen's Columns

DNA was isolated from two different samples of human male urine using Norgen's Urine DNA Isolation Kit as per the provided protocol. Briefly, 2 mL of urine was collected into a sterile tube, and Stabilizer was added to the sample. The pH of the sample was then adjusted to 3.5 using Binding Solution I, and Binding Solution II was also added to the sample. The column was activated using the Activation Buffer, and the urine sample was subsequently bound to the column. Incubation Solution was then spun into the column, and the column incubated at 60°C for 1 hour. The Incubation Solution was then spun from the column, and the column washed twice with Wash Solution. Lastly, the purified DNA was eluted using the provided Elution Buffer.

The procedure was also performed in order to isolate DNA from smaller volumes of urine. Using a human male urine sample, 25 µL, 50 µL, 100 µL, 250 µL, 500 µL, 750 µL and 1 mL of urine were used for the input. The samples were prepared in the same manner as above, and the urine DNA was isolated using the same protocol.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to visualize the purified urine DNA. Generally, forty percent of the eluted DNA was run on a 2% agarose gel in Tris-Acetate-EDTA buffer at 100V. Pictures of the gel were taken after 5, 10 and 15 minutes, in order to visualize the different species of DNA that had been isolated.

Polymerase Chain Reaction

Y-chromosome-specific sequences of the urine DNA were targeted by PCR as described by Botezatu *et al* (8). In order to perform nested PCR, the primers (Y1) 5′ – TCCACTTTATTCCA GGCCTGTCC - 3′ and (Y2) 5′ – TTGAATGGAATGGG AACGAATGG - 3′ were initially used to amplify a 154 bp sequence, followed by the use of the internal primers (nY1) 5′ – GTCCATTACACTACA TTCCC -3' and (nY2) 5′ – AATGCAAGCGAAAGG AAAGG - 3′ to amplify a nested 77 bp sequence.

Target sequences were amplified in a 100 µL reaction, using 10 µL of purified urine DNA as the template, and 5 µ mol/L of each relevant primer. For the nested PCR reaction, 10 µ L of the first reaction was used as the template in the next 100 µ L reaction, using the nested primers. PCR conditions were used as outlined by Botezatu *et al* (8). For analysis of the PCR products, aliquots of both PCRs were loaded onto a 2% agarose gel in TAE buffer and electrophoresed for about 1 hour to allow complete separation of the marker.

RESULTS AND DISCUSSION

In order for urine DNA to be used for diagnostics, it is important that both species of DNA are recovered during the isolation procedure. Norgen's Urine DNA Isolation Kit was tested to determine which types of DNA were being isolated using the standard provided protocol. DNA was isolated from 2 mL samples of human urine obtained from 2 different males, using the kit and the provided protocol. Forty % of each elution was then run on a 2% agarose gel in TAE buffer. Three different pictures of the gel were taken, corresponding to a running time of 5 minutes, 10 minutes and 15 minutes (Figure 1). From the various gel pictures, it can be seen that the kit is indeed isolating both the higher MW DNA (greater than 1 kb in size) and the lower MW DNA (150 – 250 bp).

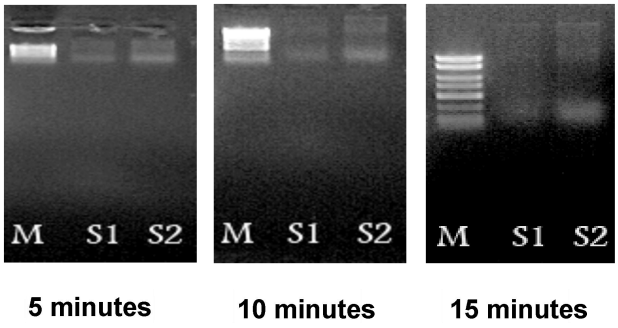

Figure 1. Agarose gel electrophoresis of urine DNA isolated using Norgen's Urine DNA Isolation Kit. Lane M corresponds to the marker, while Lanes S1 and S2 represent the two different urine samples.

When running the agarose gels for both samples 1 and 2, it was found that the amount of DNA isolated from the urine samples differed (data not shown). This is to be expected, as the amount of DNA that is extractable from urine varies greatly from individual to individual. It will depend on a number of factors, including gender, health, storage

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conditions of the urine, bacterial contamination and content, and the release of nucleases from hydrolyzed cells (13). In some cases, the amount of DNA isolated will not be enough to detect on a gel. However, the amount should be sufficient for downstream applications including PCR.

In order to demonstrate that the DNA isolated using the kit is of a high quality, the DNA was used as a template for PCR reactions. Y-chromosome-specific sequences were targeted using a nested-PCR procedure. The results of

the nested PCR reaction can be seen in Figure 2, which depicts the results when the urine DNA isolated from Sample 2 was used as the PCR template. Lane 1 in Figure 2 represents the 154 bp PCR product, while in Lane 2 the 154 bp product can be seen along with the nested 77 bp product. These results indicate that the isolated DNA is of a high quality, and can be used in downstream applications involved in diagnostics, including PCR. Furthermore, these results indicate that sufficient amounts of DNA are isolated from 2 mL of urine for downstream applications.

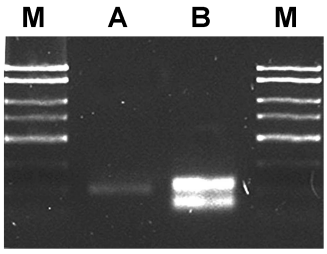

Figure 2. PCR amplification of urine DNA isolated using Norgen's Urine DNA Isolation Kit. M corresponds to the marker, A is the initial PCR product, and B is the nested PCR product.

Next, DNA was isolated from various amounts of urine, to determine the smallest amount of urine that could be used for the procedure. As stated in the Materials and Methods section, DNA was isolated from 25 µL, 50 µL, 100 µL, 250 µL, 500 µL, 750 µL, and 1 mL of urine. The purified DNA was then used as the template for the nested PCR reaction for the detection of Y-chromosome-specific sequences. Figure 3 shows the results when DNA was isolated from varying amounts of Sample 2. For all the input amounts of urine, the initial PCR of the 154 bp product is very faint. However, the nested PCR reaction results in a much brighter and more concentrated 154 bp product in all cases, as well as the 77 bp product. It can be seen that the nested PCR works very well when DNA was isolated from as little as 25 µL (Figure 3; Lanes 1 and 2). Thus, the Norgen Urine DNA Isolation Kit can be used to isolate DNA from as little as 25 µL of urine, and the DNA can be used in PCR reactions, indicating its high quality and biological activity.

CONCLUSIONS

Norgen's Urine DNA Isolation Kit provides a very attractive alternative to current methods for isolating urine DNA. The procedure is simple and rapid, relying on the use of spin-columns. The DNA is of a high quality, and can be used directly in PCR, which is a common downstream application for diagnostics. Furthermore, very small amounts of urine can be used for the isolation of DNA. The provided protocol suggests the use of 2 mL of urine, however we have shown that as little as 25 µL of urine can provide enough DNA for PCR detection. Lastly, it was shown that the kit allows for the isolation of both the large and small species of DNA present in the urine, which again is important for downstream diagnostic applications of the urine DNA.

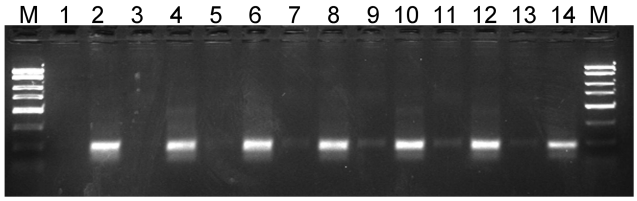

Figure 3. Isolation and detection of DNA from as little as 25 µL of urine. Lanes 1 and 2 correspond to DNA isolated from 25 µL of urine, Lanes 3 and 4 from 50 µL, Lanes 5 and 6 from 100 µL, Lanes 7 and 8 from 250 µL, Lanes 9 and 10 from 500 µL, Lanes 11 and 12 from 750 µL, Lanes 13 and 14 from 1 mL. Lane M is a marker. Lanes 1,3,5,7,9,11 and 13 are the results of the first PCR, and show the 154 bp PCR product. Lanes 2,4,6,8,10,12 and 14 are the results of the nested PCR reaction, and contain the 154 bp product along with the nested 77 bp product.

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