

Isolation of High Quality Urinary DNA using Norgen's Urine DNA Isolation Kit

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INTRODUCTION

There has been a great expectation to exploit cancer signatures in different bodily fluids as non-invasive or minimally invasive cancer molecular markers (1). The advantage for using urine as a source for cancer biomarkers is that it can be obtained in large quantities without using invasive procedures. In addition, repeated sampling from the same individual is simple, which facilitates longitudinal studies. Furthermore, urine is considered a specific filtrate of blood; therefore protein components of urine are qualitatively similar to those of blood (2).

There are many advantages favoring the use of urinary DNA for cancer biomarker discovery over blood and tissue samples, including: (1) urine-based tests are noninvasive; (2) urine is noninfectious for HIV and less infectious for many other pathogens; (3) the concentrations of urinary DNA is similar to that in plasma; (4) additional urine can be easily collected for analysis and; (5) DNA isolation from urine is technically much easier because of its low protein concentration (1000-fold lower than blood) (3).

Norgen's Urine DNA Isolation Kit provides an innovative and rapid method for the isolation and purification of total DNA from urine, including both the low molecular weight apoptotic nucleic acid and the high molecular weight genomic DNA. The procedure is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The isolated DNA can be used in a number of downstream applications including real time PCR, methylation-sensitive restriction enzyme PCR, methylation-specific and expression array analysis.

Despite the current battery of advanced and powerful molecular tools, the isolation of genetic materials from bodily fluids, especially urine, remains difficult. This is due to the presence of different inhibitors found naturally in urine (i.e. urea and salts). Norgen's Urine DNA Isolation kit has been developed to specifically target the isolation of high quality urinary DNA free from such inhibitors.

In this application note, Norgen's Urine DNA Isolation Kit is used to isolate total urinary DNA from different urine samples. The quality of the isolated urinary DNA is demonstrated through different downstream applications including PCR amplification of the circulating *K-ras* gene

and real-time PCR of the *5S rRNA* gene. In addition, the urinary DNA isolated using Norgen's Urine DNA Isolation kit was used to amplify the 13 STRs (Short-Tandem Repeats) usually used in DNA fingerprinting and forensics.

MATERIALS AND METHODS

Urine DNA Isolation

Total DNA was isolated from 1.75 mL urine samples using Norgen's Urine DNA Isolation kit as per the provided protocol (**Figure 1**). Briefly, 1.75 mL of urine was transferred into a 2 mL Eppendorf tube. Next, 250 μ L of Binding Solution I was added to the urine sample, and 650 μ L of the urine was then loaded onto an assembled column and centrifuged for 1 minute at 6,700 x g (~10,000 rpm). The flow-through was discarded and the column reassembled. The remaining urine was then loaded onto the column through 2 additional spins for 1 minute at 6,700 x g. Next, 35 μ L of both Proteinase K and Pronase were added onto an assembled column and centrifuged for 1 minute at 5,000 x g. The column with the collection tube was then incubated for 20 minutes at 60°C. After incubation, 450 μ L of Binding Solution II was mixed with the lysate in the collection tube. The lysate/Binding Solution II mix were then transferred back onto the re-assembled column and centrifuged for 1 minute at 6,700 x g (~10,000 rpm). The column was then washed by applying 450 μ L of Wash Solution I to the column, centrifuging for 1 minute and then discarding the flow-through. The column was then washed a second time by applying 450 μ L of Wash Solution II to the column, centrifuging for 1 minute and then discarding the flow-through. The column was then washed a third time by applying 450 μ L of 95% ethanol to the column and centrifuging for 1 minute, and then discarding the flow-through.

The column was then centrifuged for 2 minutes to dry the resin, followed by incubating the column for an additional 3 minutes at 60°C to thoroughly dry the resin. For total urinary DNA elution the column was placed into a fresh 1.5 mL elution tube and 75 μ L of the elution buffer was applied to the column. The column was then centrifuged for 2 minutes at 200 x g (~2000 rpm), followed by a 1 minute spin at 14,000 x g. The column was then transferred into a new 1.5 mL elution tube and 75 μ L of the elution buffer was applied to the column. The column was then centrifuged for 1 minute at 14,000 x g. Purified DNA was then stored at -20°C for several days or at -70°C for long term storage.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to visualize the purified urine DNA. Generally, one tenth of the eluted DNA was run on a 1.2% agarose gel in Tris-Acetate-EDTA buffer at 100V. Pictures of the gel were taken after 15 minutes, in order to visualize the different species of DNA that had been isolated.

Real Time and End-Point PCR Assay

DNA isolated from urine was used as template for Real Time PCR with primers specific to the *S15* housekeeping gene (5S-F 5'-GCCATACCACCTGAACG-3' and 5S-R 5'-AGCCTACAGCACCCGGTATT-3'). Furthermore, the isolated urinary DNA was used as a template for end-point PCR with primers specific to the circulating *k-ras* oncogene (K-ras-F 5'-ACTGAATATAAAGTGTGGTCCAT GGAGCT-3' and K-ras-R 5'-TTATCTGTATCAAAGAATG GTTCTGCACCA-3').

Short Tandem Repeats Amplification Assay

Purified DNA was also used as a template to amplify the 13 STR loci commonly used in DNA fingerprinting and DNA forensics. The 13 STR loci were amplified using Promega's PowerPlex® 1.1 and 2.1 Systems.

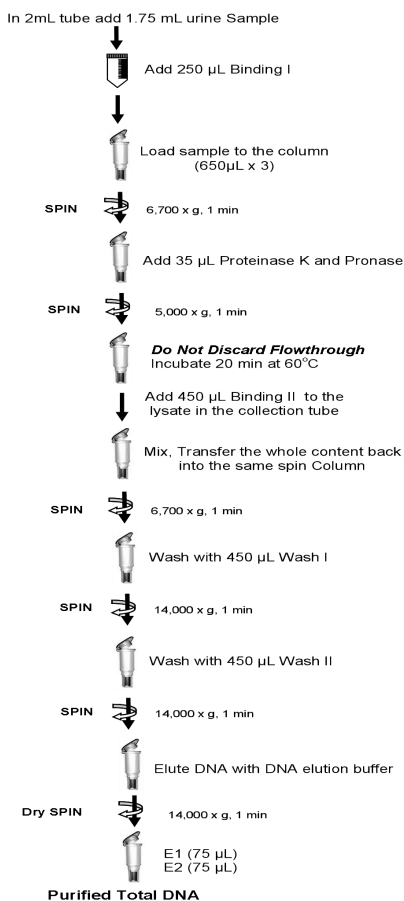


Figure 1. Procedure flowchart for the purification of DNA using Norgen's Urine DNA Isolation kit.

RESULTS AND DISCUSSION

Despite the well-known problems that accompany the isolation of different macromolecules from urine, Norgen's Urine DNA Isolation Kit has been optimized to specifically isolate high quality DNA from urine. Total urinary DNA was isolated from two urine samples collected from two volunteers. The time of sample collection was optimized to be the first midstream void. By observing the DNA isolated from the two urine samples, it can be observed that the total DNA content in urine varies from one sample to another (**Figure 2**). This is mainly due to the age, sex, time of sample collection, diet, exercise and various other factors that affect the urinary content of nucleic acids.

Downstream applications of the purified DNA demand that the DNA be of the highest quality. Quality of purified nucleic acids can be determined through the use the isolated urinary DNA in many downstream applications that regularly use urinary DNA.

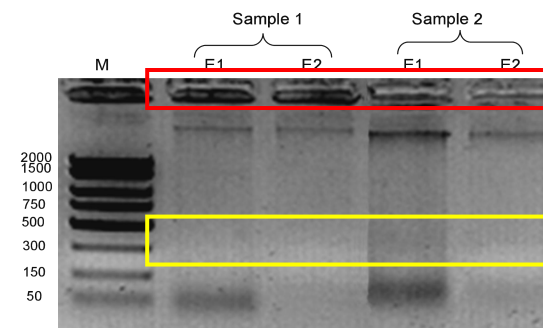


Figure 2. Isolation of High Quality Urinary DNA from Two Different Urine Samples. DNA was isolated from 1.75 mL urine samples collected from two volunteers using Norgen's Urine DNA Isolation Kit. Norgen's kit allowed for the isolation of high quality DNA from two urine samples. The red rectangle indicates the high molecular weight gDNA, whereas the yellow rectangle indicates the low molecular weight apoptotic nucleic acid. M: 10 µL Norgen's FastRunner DNA Ladder.

The quality of DNA isolated by Norgen's kit was further demonstrated by real time PCR amplification of the *5S* gene using the DNA isolated from urine as a template (**Figure 3**). The *5S* gene was successfully amplified from both elutions, indicating the high quality of the isolated urinary DNA.

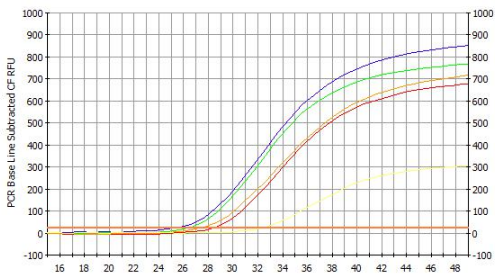


Figure 3. Isolation and Detection of DNA from 1.75 mL Urine Samples. Total genomic DNA was isolated from two different 1.75 mL urine samples using Norgen's Urine DNA Isolation Kit. The isolated DNA was then subjected to quantitative PCR using human 5S rRNA gene primers to detect the genomic DNA. The red line in the PCR baseline graph above corresponds to the first elution from the DNA isolated from the first urine sample, the green line corresponds to the second elution from the DNA isolated from first urine sample, the blue line corresponds to the first elution from the DNA isolated from the second urine sample, whereas the orange line corresponds to the second elution from the DNA isolated from the second urine sample. The yellow line corresponds to the No Template Control (NTC). The isolated DNA was eluted into two separate elutions of 75 μ L each.

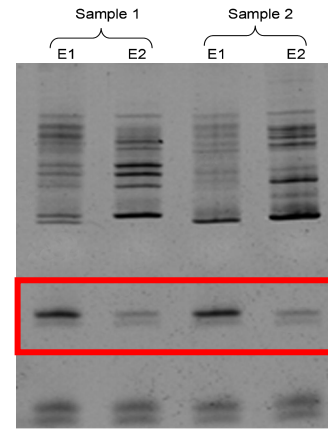


Figure 5. Detection of STRs Differences Between Two Different Populations Based on Urine DNA

Total urinary DNA was isolated from two urine samples collected from a Serbian volunteer (sample 1) and an Egyptian volunteer (sample 2). Urinary DNA isolated from the two samples was used as a template to amplify and analyze the difference in the STRs between these two different populations. The red rectangle shows the PCR product of the Amelogenin gene which is used as a sex determinant.

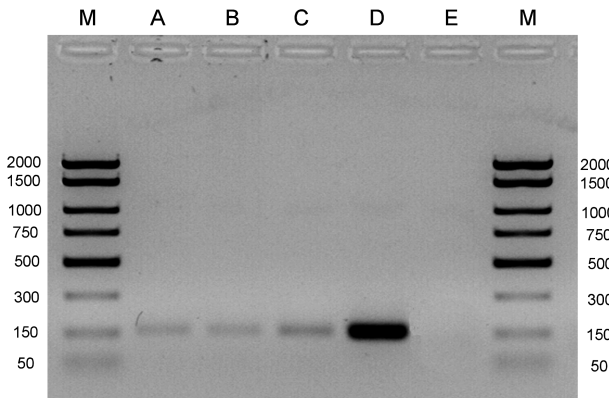


Figure 4. Circulating DNA isolated from urine can be used in PCR Reactions. DNA isolated from three different urine samples was used as the template in a PCR reaction to amplify the *K-ras* gene. Lanes A - C contain the expected 157 bp product, and correspond to the first elution from each sample. Lane D is the positive control of 293 HEK DNA, while Lane E is the negative control. Lane M is Norgen's FastRunner DNA Ladder.

It has previously reported that some circulating DNA crosses the kidney barrier and appears in urine (transrenal DNA) (3). In these studies, *K-ras* mutations were present in the urine of patients with pancreatic or colorectal carcinomas; Y chromosome-specific sequences were found in urine of women pregnant with a male fetus or in urine of women who have received blood from male donors. Therefore, to validate the presence of the circulating *K-ras* in urine, *K-ras* was amplified from DNA isolated from three different urines samples. **Figure 4** shows the amplification of the *K-ras* oncogene from total urinary DNA isolated using Norgen's Urine DNA Isolation Kit from 3 different urine samples. The PCR product was detected in all 3 DNA samples, thereby confirming that the circulating DNA isolated from urine was of a high purity.

The most sensitive downstream application for any DNA isolated from urine is DNA fingerprinting. Also, urinary DNA is commonly used in forensics such as criminal identification and paternity testing. These two applications require a very efficient methodology to isolate DNA from the smallest urine volume. Also, these downstream applications require the isolation of high quality DNA. We have previously shown that DNA can be isolated from as little as 25 μ L of urine (Norgen Application Note 10). Furthermore, the DNA isolated from 25 μ L of urine was used as a template to amplify the y-chromosome from

male volunteers (4). In this experiment, we used the DNA isolated from a Serbian and an Egyptian volunteer as a template for the detection of differences in the STRs between the Serbian and the Egyptian populations. **Figure 5** shows the successful amplification and the differences in the STRs between the two populations. Also, **Figure 5** shows the PCR product of the Amelogenin gene located on the X and the Y chromosome. Amelogenin is usually used for gender identification in the field of forensic DNA testing as it provides primary information regarding samples found at the scene of the crime during a criminal investigation. Through the test, it is possible to confirm whether biological remains found at the crime scene belong to males or females. Since this gene is located on both the X and the y chromosome, it appears as a single band when the sample is from a female suspect or as two bands if the sample is from a male suspect. In **Figure 5**, the PCR product of the Amelogenin gene appears as two bands in both cases, therefore confirming that the samples were collected from two males. Furthermore, this also demonstrates that the quality of the DNA isolated from urine using Norgen's Urine DNA Isolation kit is compatible with the identification of the different STRs commonly used in DNA fingerprinting as well as in the field of forensics.

CONCLUSIONS

Through analysis of the performance of Norgen's Urine DNA Isolation Kit in the isolation of total DNA from different urine samples, a number of conclusions regarding Norgen's kit can be made:

1. Norgen's kit allows for the isolation of high quality total circulating urinary DNA within 30 minutes from small urine volumes. Norgen's kit allows for rapid and convenient DNA isolation.

2. Norgen's kit allows for the isolation of high yields of DNA. High yields of DNA could be isolated from the urine samples using Norgen's kit, and therefore could be visualized on agarose gels. These gels demonstrated the variations in DNA content between different samples, which is due to factors such as age, gender, diet, exercise and time of sample collection.

3. Norgen's kit allows for the isolation of high quality urine DNA. Norgen's Urine DNA Isolation Kit was able to isolate high quality urinary DNA that is free from the contaminants usually found in urine, as demonstrated by the successful amplification of the 5S *rRNA* gene and the *K-ras* oncogene without any observed PCR inhibition.

4. Urine DNA isolated using Norgen's kit can be used for highly sensitive downstream applications. It was demonstrated that the urinary DNA isolated from urine samples can be used for the purpose of DNA fingerprinting, paternity testing and forensics for the identification of criminal suspects.

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4. Norgen Application Note 10: Isolation of DNA from as Little as 25 μ L of Urine Using Norgen's Urine DNA Isolation Kit



