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A Method for the Ultra Rapid Isolation of PCR-Ready DNA from Urine and Buccal Swabs

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Abstract

Isolation of DNA from buccal swabs for the analysis of genomic DNA is becoming common for research and forensic purposes. Here, we demonstrate that urine provides a good alternative for obtaining small amounts of PCR-ready DNA.

We developed a rapid and safe method for extracting DNA from both sources using components of Qiagen's PCR cleanup kit™. PCR amplification on the isolated DNA gave consistent and good results. In addition, conditions for the storage of unprocessed urine and buccal swabs are given. One isolation takes less than 10 minutes and is enough for at least ten PCR reactions.

Introduction

PCR amplification of genomic DNA is a widely used method for research and forensic purposes. Blood has been the traditional source for obtaining genomic DNA. However, the method for extracting DNA from blood has several drawbacks, including the inconvenience of drawing blood and the risk of exposure to blood-borne pathogens. In addition, DNA may be extracted from hair follicles, semen and buccal swaps. However, the methods for extracting genomic DNA from these sources usually takes more than 2 hours (Grimberg *et al.*, 1989; Kendall *et al.*, 1991; Parzer and Mannhalter, 1991; Richards *et al.*, 1993).

In the present study we aimed at obtaining a reliable and fast method for obtaining PCR-ready genomic DNA from human buccal swabs and urine. In addition, we evaluated the stability of genomic DNA in unprocessed urine and buccal swabs at different storage conditions.

Results and Discussion

In the present study we aimed at obtaining a reliable and fast method for obtaining PCR-ready genomic DNA from human buccal swabs and urine. Using components of Qiagen's PCR cleanup kit™ we developed a reliable protocol that can be performed in less than 10 minutes per reaction.

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Yield

The yield of both methods for extracting DNA was evaluated using gel electrophoresis. Small scale DNA extraction from urine and buccal swabs resulted in 1-50 ng of genomic DNA per isolation. Medium scale DNA extraction from urine resulted in 10-500 ng of genomic DNA per isolation. The size of the isolated DNA ranged from 100bp to about 10kbp (Figure 1A) indicating considerable fragmentation of the isolated genomic DNA.

Performance of the Isolated DNA in a PCR Assay

To check the quality of the DNA isolated from the small scale DNA isolation from urine and buccal swaps, we amplified the region of the ACE gene containing the ACE I/D polymorphism (Figure 1B) in a PCR assay. Both methods resulted in strong amplification of the ACE gene and gave identical results. Therefore, urine is a good alternative for obtaining PCR-ready genomic DNA. Although the yield varied considerably between individual DNA isolations, all DNA isolations performed similarly well in the PCR assay. As only 5 μl of the isolated DNA was used, at least 10 PCR reactions can be performed on one small scale DNA isolation.

The independence of the PCR reaction on the amount of starting DNA possibly reflects saturation of the employed PCR reaction. Although both methods of isolation performed well in the ACE genotyping PCR reaction, the variations in DNA yield may prove problematic for more critical PCR reactions.

Stability of the DNA

Storage for 1 month at -20°C did not change the PCR performance of DNA obtained from urine and buccal swabs (Figure 1C). Therefore, the isolated DNA can be stored at -20°C for later use.

Buccal swabs can be stored in buffer PB for up to 48 hours at 4°C without changing its performance in the PCR assay. The performance of PCR assay decreased when buccal swabs were stored in buffer PB for 24 and 48 hours at 20°C (Figure 3A). Therefore, buccal swabs are best stored in buffer PB at 4°C.

In addition, we investigated whether DNA can be extracted from urine samples stored in the freezer. DNA extracted from urine stored for 1 month at -20°C performed equally well as fresh urine (Figure 2A). However, DNA extracted from urine stored for 18 months at -20°C performed less well in the PCR assay. No DNA could be extracted from urine stored for 3 years at -20°C. Although the DNA in urine seems to degrade over time, urine stored up to 18 months at -20°C may be used for PCR amplification.

As it may be more convenient to store urine in a liquid form, we investigated whether DNA can be extracted from urine samples stored in a refrigerator and at room temperature. Storage of urine for 24 and 48 hours at 4°C

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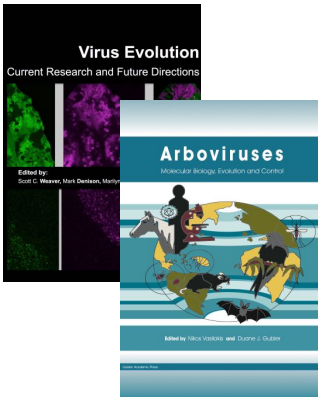

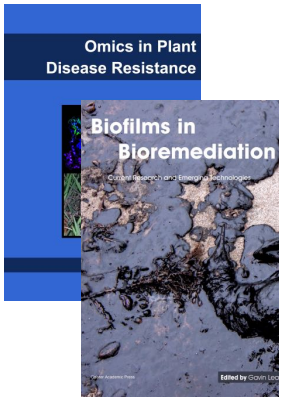

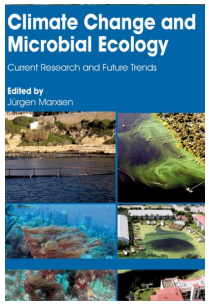

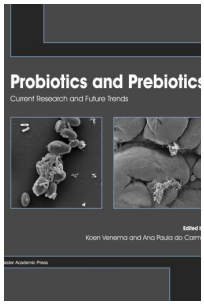

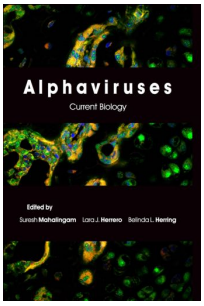

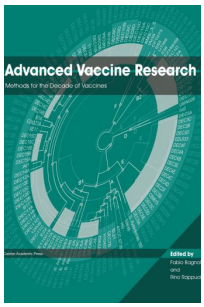

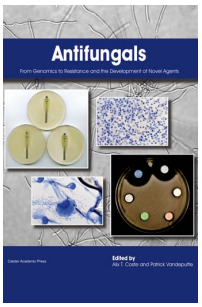

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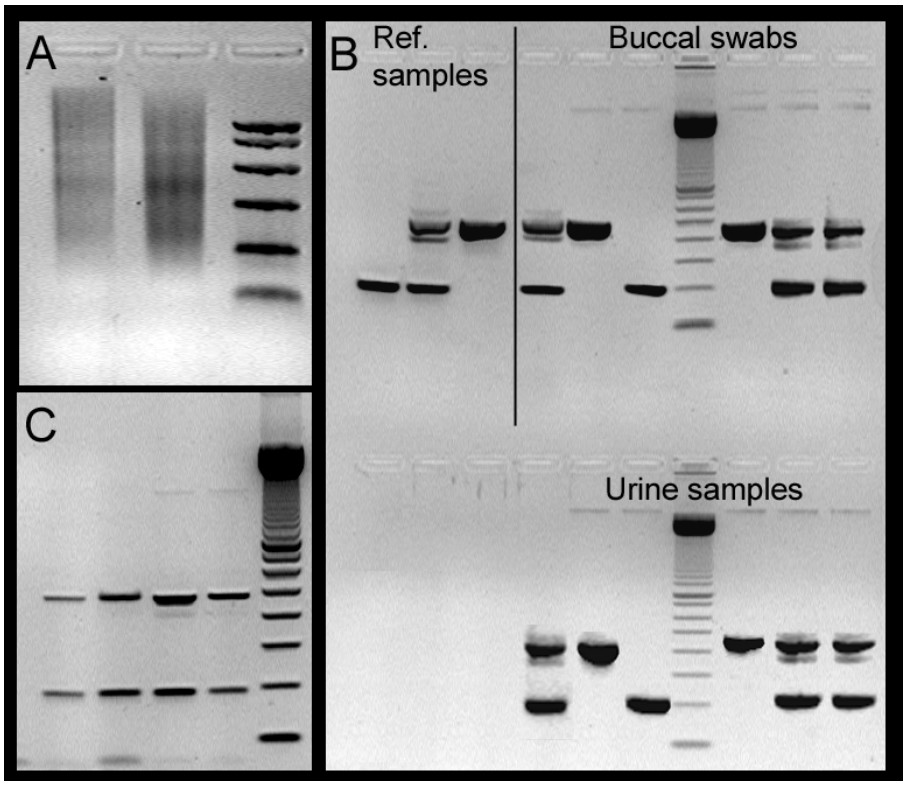

Figure 1. Quality and performance of the isolated DNA in a PCR assay. A) lanes 1-2; Medium scale DNA isolation from urine resulted in DNA fragments ranging from 100bp to 10kbp. lane 3; size marker (50, 150, 300, 500,750 and 1000bp). B) Lanes 1-3; Amplification of the reference samples confirmed the DD (lane 1), ID (lane 2) and II genotype (lane 3). (lane 4,5,6,8,9,10 upper and lower panel; pat 1-6) Analysis of six patients using either buccal swabs or urine as a DNA source. Lane 7; 100 bp marker. C) Amplification products of freshly isolated DNA (lane 1 and 3, buccal swab and urine respectively) and of DNA stored at -20°C for 1 month (lane 2 and 4, buccal swab and urine respectively). Lane 5; 100 bp marker.

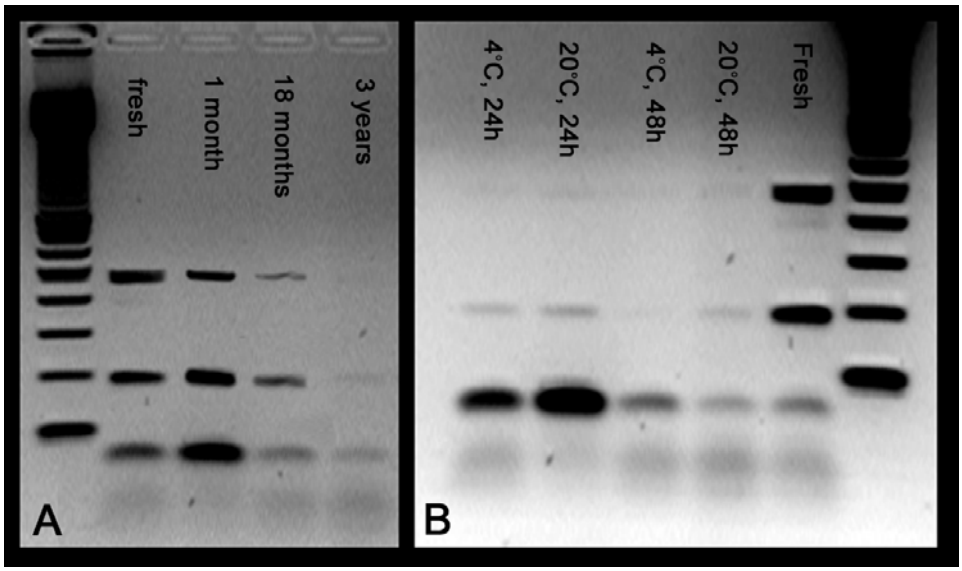

Figure 2. Stability of DNA in urine. A) Stability of DNA in frozen urine samples. Fresh urine and urine stored for 1 month at -20°C performed equally well in the PCR assay. Storage for 18 months at -20°C decreased the yield of the PCR assay considerably. Storage for 3 years at -20°C, did not result in successful amplification of the ACE gene. B) Storage of urine for 24 and 48 hours at 4°C and 20°C did not result in successful amplification of the ACE gene.

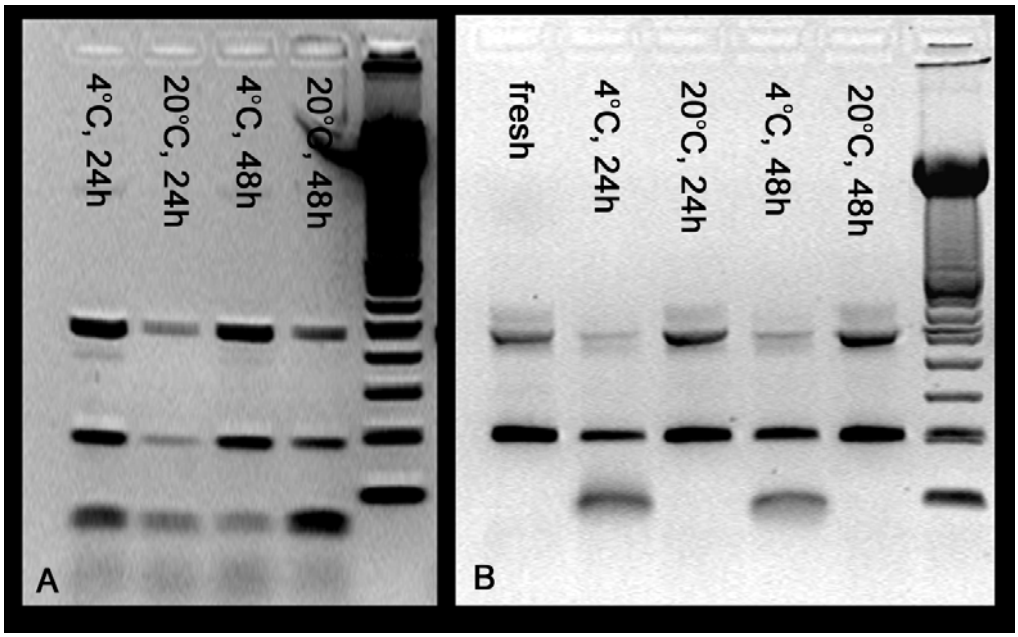

Figure 3. Stability of Buccal swabs and urine in buffer PB. A) Buccal swabs stored at 4°C for 24 and 48 hours (lane 1 and 3) performed equally well in the PCR assay. Storage at 20°C for 24 and 48 hours decreased the performance in the PCR assay (lane 2 and 4). B) Storage of urine diluted in buffer PB at 20°C for 24 and 48 hours performed equally well as fresh urine (lane 3 and 5). Storage at 4°C decreased the performance in the PCR assay (lane 2 and 4).

and 20°C did not result in successful amplification of the ACE gene (Figure 2B), indicating degradation of genomic DNA.

As DNA is apparently degraded in urine at temperatures at 4°C or higher, we investigated whether the addition of buffer PB stabilizes the DNA. Storage of urine diluted in buffer PB at 20°C for 24 and 48 hours performed equally well as fresh urine (Figure 3B). Surprisingly, storage of the urine mixture at 4°C decreased its performance in the PCR assay. Possibly, the extensive precipitation of urine components at 4°C interferes with the extraction procedure of genomic DNA.

In conclusion, we developed a reliable and very fast method for obtaining PCR-ready genomic DNA from both human buccal swabs and urine. Unprocessed urine may be stored for up to 18 months at -20°C and up to 48 hours at room temperature when stabilized by buffer PB. Buccal swabs may be stored in buffer PB for up to 48 hours at 4°C. One small scale isolation takes less than 10 minutes and is enough for at least ten PCR reactions.

Experimental Procedures

Small Scale DNA Isolation from Buccal Swabs Lysates
Human buccal swabs were obtained using plastic shafts and cotton tips. The swabs were immediately placed in a 12 ml centrifuge tube containing 500 µl buffer PB (Qiagen, Hilden, Germany). Tubes were thoroughly vortexed for 1 minute and the swabs were subsequently removed. Swab lysates were either used immediately or stored (see Stability of the DNA). The swab lysate was passed through a Qiagen spin column (Qiagen PCR cleanup kit). The column was washed with 750 µl buffer PE (Qiagen) and genomic DNA was eluted in 50 µl EB elution buffer (Qiagen). DNA solutions were stored at –20°C.

Small Scale DNA Isolation from Urine
Human urine was collected. 100 µl urine was transferred into an eppendorf vial and 500 µl of buffer PB (Qiagen) was added. Tubes were thoroughly vortexed for 1 minute. Urine solutions were either used immediately or stored (see Stability of the DNA). The urine solution was passed through a Qiagen spin column (Qiagen PCR cleanup kit) by centrifugation (10.000g, 1min.). The column was washed with 750 µl buffer PE (Qiagen) and genomic DNA was eluted in 50 µl EB elution buffer (Qiagen). DNA solutions were stored at –20°C.

Medium Scale DNA Isolation from Urine
One ml urine was transferred into an 12 ml centrifuge tube and 5 ml buffer PB (Qiagen) was added. Tubes were thoroughly vortexed for 1 minute. Cellular debris was pelleted by centrifugation at 10.000g for 5 minutes. All of the supernatant was passed through a Qiagen spin column (Qiagen PCR cleanup kit) using a vacuum manifold (QIAvac 24, Qiagen, Germany). Columns were subsequently centrifuged at 10.000g for 1 minute. The column was washed with 750 µl buffer PE (Qiagen) and genomic DNA was eluted in 50 µl EB elution buffer (Qiagen). DNA solutions were stored at –20°C.

Amplification of the Polymorphism of the Human Angiotensin Converting Enzyme (ACE) Gene

The method used for genotyping of the ACE I/D polymorphism was described by Rigat and colleagues (Rigat *et al*., 1990). In brief, 5μl of the DNA solutions obtained from either buccal swabs or urine were used for each polymerase chain reaction (PCR). In addition 3 reference samples containing 50 ng of purified human DNA of known genotype were analysed. PCR cycling was performed in a Perkin Elmer thermocycler 9600 using a protocol of 5 sec. denaturation, 5 sec. annealing and 30 sec. extension for 35 cycles. 10μl of PCR amplification product was analysed on a ethidium bromide stained agarose gel.

Stability of the DNA

Buccal swabs and urine were stored at different conditions to investigate the stability of the DNA. Buccal swabs were stored in buffer PB for 24 and 48 hours at both 4°C and 20°C. Urine was stored for 24 and 48 hours at both 4°C and 20°C. Frozen urine was stored for two weeks, 18 months and 3 years at -20°C. DNA solutions were stored for 1 month at -20°C.

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