High Efficiency Quantitative PCR from Urine RNA

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INTRODUCTION
In recent years attention has been turning to the use of non-invasive samples for genetic and diagnostic analysis, including the use of urine. Norgen Biotek Corp. has developed a simple method for the concentration, preservation, storage and isolation of RNA from urine using Individual Urine RNA Concentration and Preservation Devices. Donors simply collect their urine and transfer it directly into the Urine Concentration Tube, and then mix the urine with Norgen’s resin that is inside the tube. All the macromolecules present within the urine will bind to the resin in the presence of the buffer located within the Urine Concentration Tube. The resin is then allowed to precipitate by gravity for 10 minutes, then the urine supernatant is discarded and Norgen’s Urine Preservative is added. The preservative is an aqueous storage buffer designed for rapid preservation of urine RNA from fresh, concentrated urine specimens.

This buffer stabilizes the RNA for long-term storage at ambient temperature. Since the buffer prevents the growth of microorganisms and inactivates viruses it also allows the samples to be handled and shipped safely. The RNA subsequently isolated from the preserved samples is of a high quality and can be used directly in sensitive downstream diagnostic assays such as real-time PCR. Real-time PCR is one major application that is used in quantification of gene copy numbers, viral quantitation, pathogen detection as well as array verification and genotyping. In this study RNA was collected and isolated from 20 urine samples using Norgen’s Urine RNA Concentration, Preservation and Isolation kit, and was subsequently used as the template in Real-Time PCR reactions to detect the 5S gene. Figure 1 shows both the successful and consistent amplification of all 20 RNA samples (Panel A), and the melt curve indicates real amplification from all templates (Panel B). Figure 2 shows a graph of the Ct values from all the RNA samples, and the consistent values indicate that the RNA samples were all of a similar quality. Therefore, all 20 RNA samples were amplifiable on real-time PCR and the average Ct value was 18±1.2.

RESULTS AND DISCUSSION
Urine RNA must be pure and free from inhibitors in order for the RNA to be used in sensitive downstream applications. Here, RNA was collected and isolated from 20 urine samples using Norgen’s Urine RNA Concentration, Preservation and Isolation kit, and was subsequently used as the template in Real-Time PCR reactions to detect the 5S gene. Figure 1 shows both the successful and consistent amplification of all 20 RNA samples (Panel A), and the melt curve indicates real amplification from all templates (Panel B). Figure 2 shows a graph of the Ct values from all the RNA samples, and the consistent values indicate that the RNA samples were all of a similar quality. Therefore, all 20 RNA samples were amplifiable on real-time PCR and the average Ct value was 18±1.2.

MATERIALS AND METHODS
RNA Isolation
Twenty different urine samples were collected using Norgen’s Urine RNA Concentration, Preservation and Isolation Kit. RNA was purified from 30 mL of the concentrated urine/preservative mix according to the supplied protocol and was eluted into 150 µL elution volumes.

Quantitative PCR Amplification
Two microliter of each RNA urine sample was then used as the template in a reverse transcription PCR using a reverse specific primer for 5S gene. Five microliters from each reverse transcription PCR were used as a template in a real-time PCR reaction using 2X SYBR Green/Fluorescein qPCR Master Mix (Norgen Biotek, Cat# 28109). 5S specific primers were used in the reaction amplified on the iCycler iQ real-time system (Bio-Rad).

Figure 1. Amplification of the 5S Gene from 20 Urine RNA samples. (A) Real-time PCR amplification and (B) melt-curve analysis. RNA samples are in Red color and the no template control is colored in Blue. All the RNA templates were successfully amplified with high efficiency.
**Figure 2**: Ct values of the real-time amplification of 5S gene from the 20 Urine RNA samples. The average Ct value is 18±1.2.

**CONCLUSION**

RNA isolated using Norgen’s Urine RNA Concentration, Preservation and Isolation Kit is of a very high quality, and is compatible with PCR applications, including real-time PCR. All 20 saliva RNA samples were amplified with similar Ct values, indicating the consistency of the RNA isolation.