Investigating the Simultaneous Extraction of miRNA and DNA from Forensically Relevant Body Fluids

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Abstract
It was recently recognized that microRNAs (miRNA) may serve as potential biomarkers for body fluid identification, thereby providing tissue source information in addition to DNA profiling. This study investigated simultaneous extraction of both DNA and RNA from forensically relevant body fluids.
Following ethical approval from the Institutional Review Board and informed volunteer consent, venous blood, semen, saliva and urine were collected from 5 volunteers (n=20). Two commercially available kits were investigated namely; ZR-Duet™ DNA/RNA MiniPrep kit (ZYMO) and AllPrep™ DNA/RNA Mini Kit (Qiagen). The manufacturers guidelines were followed using 200µl of sample. A series of dilutions of each body fluid were co-extracted for their RNA/DNA content. The quality/quantity of each extract was analyzed using a Biotek EON Spectrophotometer. RQ-PCR was performed on a selection of the RNA samples targeting miR-16 to determine if a miRNA signal was present. In parallel, STR analysis was performed on a selection of the DNA samples.
The results showed that quantifiable amounts of both DNA and RNA were obtained from all body fluids using both kits. The results were variable depending on the body fluid and the kit used. The diluted samples produced varying concentrations at much lower levels, as expected. Overall, the Zymo Kit provided higher concentrations of both DNA and RNA when compared to the Qiagen Kit. Finally, miRNA signals and full DNA profiles were obtained from all samples profiled. This research highlights the potential of miRNAs in forensic investigations with the ability to extract both miRNA and DNA from a single sample.

Introduction

In the field of Forensic Science, it is essential that from biological stains, both the type of tissue and the individual who deposited the stain be identified. There are many different methodologies available for the preliminary identification of biological stains in criminal investigations, both in the laboratory and at the crime scene. However, despite the advantages of many of these methods, many infer disadvantages, which are difficult to overcome.
Currently, microRNAs (miRNA) have been suggested as potential biomarkers for body fluid identification (Zubakov, 2010). However, depending on the amount of biological evidence left behind, if there are only trace amounts, miRNA analysis will consume the sample and as a result eliminate the possibility of obtaining a DNA profile (Hanson,2012). Therefore, there is a great need for a method, which performs both RNA and DNA extraction simultaneously from one sample. There have been limited advancements in this area with only a few methods currently offered for this purpose. As a result, there is still much research and technology to be developed and it is crucial that these methods are tested extensively and compared to one another.

MessengerRNA (mRNA) can be used as a valuable tool to identify specific body fluids (Zubakov, 2010). However, mRNA would only be useful if the sample was not degraded and was abundant in quantity (Zubakov, 2010). This is not advantageous to the forensic science field because most samples are degraded and are minute in quantity (Haas, 2013). Recent research however, has shown that microRNA (miRNA) shows great potential for the identification of body fluids in forensically relevant samples, due to their small size, stability and robustness (Zubakov, 2010). MiRNA does not degrade easily and can be analyzed months, and in some cases, years later (Haas, 2013). However, it is crucial that we can extract both RNA and DNA from the one sample simultaneously, thereby allowing us to identify both the 'who' and 'what' of the sample.

Due to the trace amounts of samples available in forensic investigations, it is often difficult if not impossible to extract both the RNA and DNA portions (Haas, 2015). Scientists faced with this challenge have therefore developed commercial kits that analyze both RNA and DNA simultaneously within one sample. In addition, it is a necessity that the sensitivity and accuracy be analyzed using different kits. Commercial kits such as ZR-Duet™ DNA/RNA MiniPrep kit and the Qiagen AllPrepTM DNA/RNA Mini Kit have been created which have the ability to co-extract RNA and DNA. However, these kits have not been compared in regards to their sensitivities. Upon comparison of these kits, the accuracy, and the amount of both RNA and DNA each kit yields will be analyzed.

Materials and Methods

Sample Selection:

All urine, semen, saliva and blood samples were obtained from participants with written, informed consent following the approval from the University of New Haven (UNH) Institutional Review Board (IRB). Each body fluid consisted of five different participants, thus there were five samples of each body fluid and four body fluids all together. Participation was on a volunteer basis with no preliminary testing. The samples were collected in a collection tube and

stored at 4°C until DNA/RNA was extracted from each sample.

Procedure for ZR Duet DNA/RNA MiniPrep Kit:

Each different body fluid, semen, saliva, urine and blood was processed separately at different times to ensure minimal contamination. Before the procedure, all equipment and the fume hood was cleaned with industrial grade ethanol. The fume hood was also cleaned via UV light for 20 minutes. The appropriate amounts of 96-100% ethanol were added to the concentrates supplied by the kit (see ZR Duet DNA/RNA MiniPrep Kit Protocol). Next, using a collection tube, 200µl of sample was added to a labeled collection tube with the number of both samples matching up. 600µl of DNA/RNA Lysis Buffer was added to the sample and mixed using a pipette. This was then transferred this into Zymo-Spin IIIC Column in the collection tube and centrifuged at ≥12,000xg for 1 minute. The maximum capacity of the column was 800µl and the column was reloaded as necessary. The flow-through was saved for RNA extraction. RNA extraction was the first extraction and then DNA extraction was performed. 2 volumes of ethanol (95-100%) were added to the flow-through in the collection tube (capacity 2ml) from the prior step. It was mixed thoroughly via pipetting. This was then transferred into column in a collection tube. It was then centrifuged at ≥12,000xg for 1 min. The flow-through was discarded. Then, 400µl RNA Prep Buffer was added to the column and centrifuged at ≥12,000xg for 1 minute. The flow-through was discarded. 700µl RNA Wash Buffer was added to column and centrifuged at ≥12,000xg for 30 seconds. The flow-through was discarded and this step was repeated with 400µl RNA Wash Buffer. This was then centrifuged the column in an emptied collection tube at ≥12,000xg for 2 minutes. The column was removed carefully from collection tube and transferred it into an RNase-free tube. 40µl of DNase/RNase-Free Water was directly added to the column matrix and it was left for 1 minute at room temperature. Lastly, it was centrifuged at 10,000xg for 30 seconds. The eluded RNA was stored at -20°C until it was later quantified.

For the DNA Extraction, first the column was placed into a new collection tube and 400µl DNA Prep Buffer was added to the column and centrifuged at ≥12,000xg for 30 seconds. Then, 700µl DNA Wash Buffer was added to the column and the centrifuged at ≥12,000xg for 30 seconds. The flow-through was discarded and the step was repeated with 400µl DNA Wash Buffer. Next, this was centrifuged in an emptied collection tube at ≥12,000xg for 2 minutes. The column was removed carefully from collection tube and transferred into a clean micro-centrifuge tube. 100µl DNase/RNase-Free Water was added directly into the column matrix and it was left for 5 minutes at room temperature. Then, it was centrifuged at top speed for 30 seconds. The eluded DNA was stored at ≤-20°C until it was later quantified.

Procedure for the Qiagen AllPrepTM DNA/RNA Mini Kit:

Each different body fluid, semen, saliva, urine and blood was processed separately at different times to ensure minimal contamination. Before the procedure, all equipment and the fume hood was cleaned with industrial grade ethanol. The fume hood was also cleaned via UV light for 20 minutes. The appropriate amounts of 96-100% ethanol were added to the concentrates supplied by the kit (see Qiagen AllPrepTM DNA/RNA Mini Kit Protocol). Next, using a collection tube, 200µl of sample was added to a labeled DNA column with the number of both samples matching up. 350µl of Lysate was added. The mixture was mixed via pipetting and then the mixture was centrifuged for 30s at ≥10,000. AllPrep DNA spin column was placed in a new 2ml collection tube and store at room temp for later DNA purification. The flow-through was saved for RNA Purification.

For RNA Purification, 550µl of 70% ethanol was added to the flow-through and mixed well by pipetting. 700µl of the sample was transferred into an RNeasy spin column placed in a 2ml collection tube. It was then centrifuged for 15s at ≥10,000 RPM. The flow-through was discarded. This step was repeated until the entire sample had been transferred and pipetted. 700µl of Buffer RW1 was added to the RNeasy spin column and centrifuged for 15 seconds for ≥10,000 RPM. The flow-through was then discarded. 500µl Buffer RPE was added to the RNeasy spin column. This was then centrifuged for 15 seconds for ≥10,000 RPM. The flow-through was discarded. Then, 500µl Buffer RPE was added to the RNeasy spin column and centrifuged for 2minutes at ≥10,000 RPM. RNeasy spin column was placed into a new 2ml collection tube and centrifuged for 1minute. The RNeasy spin column was placed into a new 1.5ml collection tube and 40µl RNase Free water was added into the spin column and centrifuge 1min at ≥10,000 RPM. The eluted RNA was stored at -20°C until it was later quantified.

For DNA Purification, first, 500µl Buffer AW1 was added to the AllPrep DNA spin column and centrifuged for 15 seconds at ≥10,000 RPM. The flow-through was discarded. Then, 500µl Buffer AW2 was added to the AllPrep DNA spin column. Centrifuge for 2min at full speed. Lastly, the AllPrep DNA spin column was placed in a new 1.5ml collection tube and 100µl of Buffer EB was directly added to the spin column membrane and the lid was closed. This was left for 1 minute and then centrifuged for 1 min at ≥10,000. The eluded DNA was stored at ≤-20°C until it was later quantified.

DNA and RNA Quantification:

All eluded DNA and RNA samples including the diluted samples, were quantified using the BioTek™ Eon™ Microplate Spectrophotometer, which was connected to the Generation 5, Version 2.01 computer program. First, a completely blank module was put through the spectrophotometer to calibrate the plate and the reading system. After that, three full plates were read followed by another blank to recalibrate the machine. Lastly, the next two full plates were read. The data was saved onto a flash drive where the NG/UL and the 260/280 values were

averaged and recorded onto a data table. The data was then put into a graph to show the quantity that was extracted from the original samples.

DNA Amplification:

The 9700 microRNA cDNA Archive Plate was used for DNA amplification. A master mix was made and added to each well and then the 10x RT Buffer, Nuclease Free Water, RNase inhibitor, Multiscribe, and Stem Loop Primer was added. Then the mixture was put into the machine and went through several cycles. It went through a 30 minute period at 16°C, a 30 minute period at 42°C, and a 5 minute period at 85°C and then stayed at 4°C.

RNA Amplification:

The 7900HT 96 Well microRNA TaqMan Plate was used for the miRNA amplification. First, a master mix was made and put into 8 wells. Then a proportional amount of Nuclease Free Water was added to each well. Also, miRNA PDAR was added to each well. The mixture then ran several cycles, which included an Rxn Volume, a target cycle, and a control cycle.

Results

Quantifiable amounts of miRNA and DNA were found in both the Zymo and the Qiagen kits. The concentrations obtained however, were highly variable depending on the particular body fluid and the particular kit used. The results obtained from the diluted samples produced varying concentrations at much lower levels, as expected. Overall, the Zymo Kit proved to obtain higher concentrations of both DNA and RNA when compared to the Qiagen Kit. Finally, miRNA signals and full DNA profiles were obtained from all samples selected for miRNA/DNA profiling.

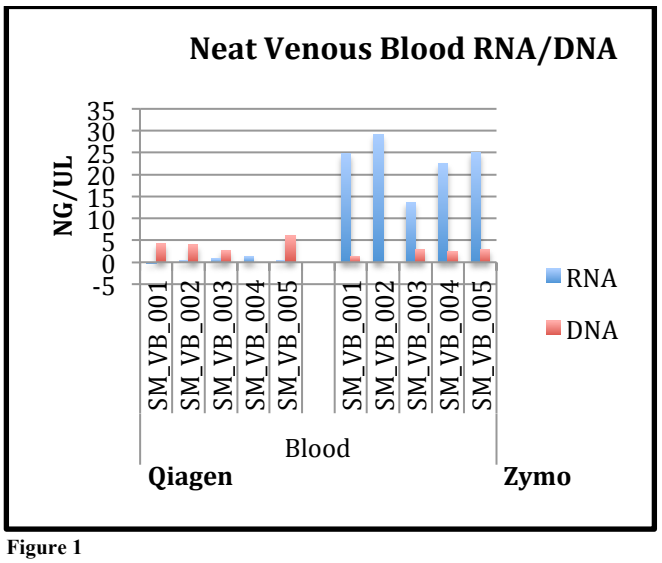

Figure 1

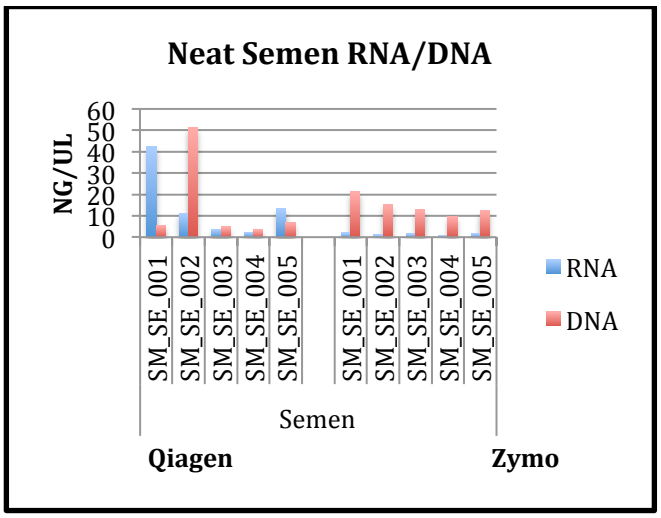

Figure 2

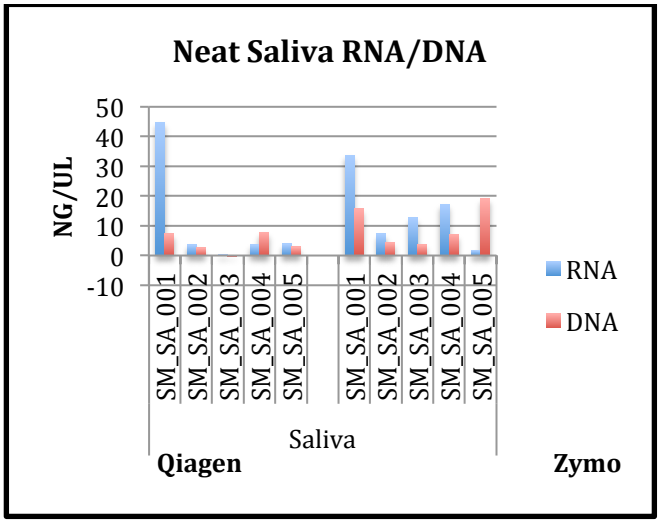

Figure 3

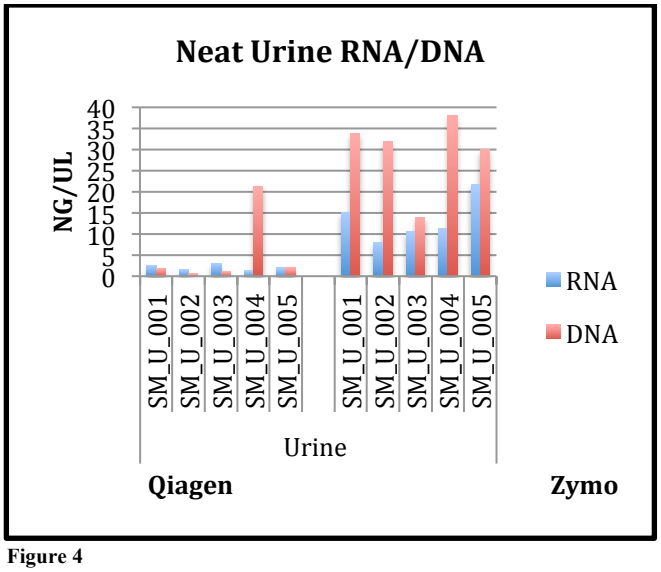

Figure 4

As for the dilution series, it is also clear that the Zymo kit performed better than the Qiagen kit. Quantifiable amounts of all fluids were available from each dilution. However, as expected, as the fluid was progressively diluted, the quantifiable amount decreased dramatically. The concentrations obtained from each kit were highly variable, depending on the kit and the dilution factor that was used.

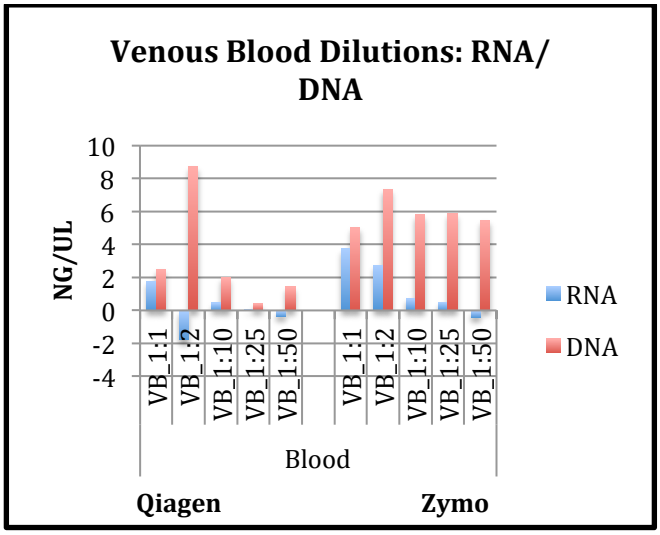

Figure 5

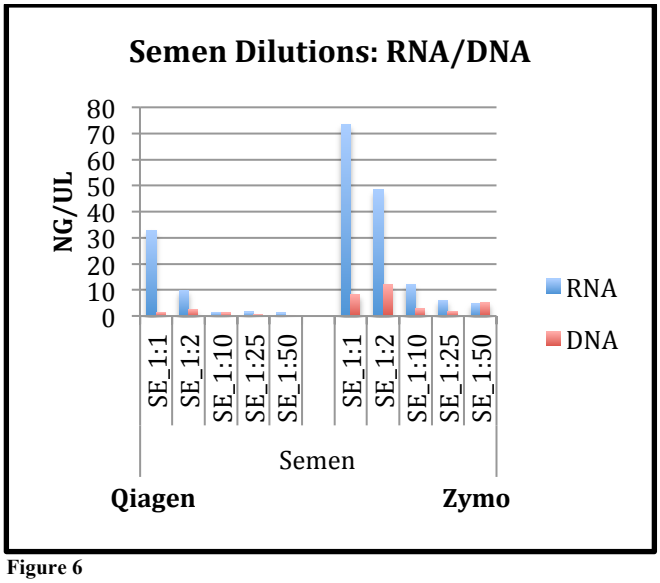

Figure 6

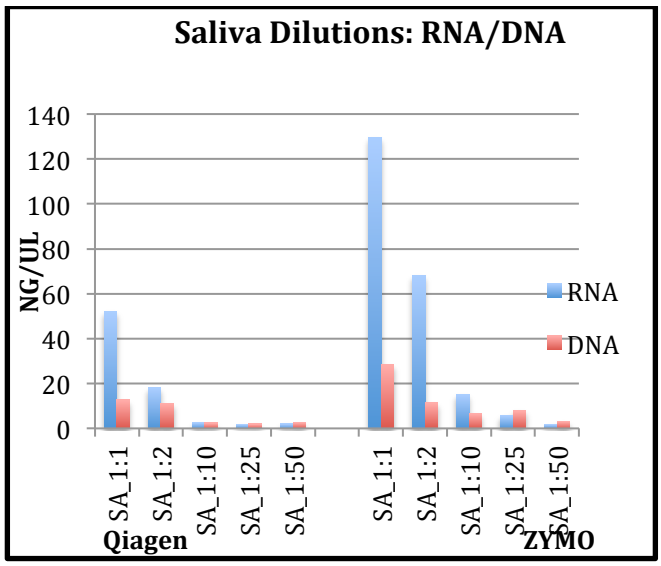

Figure 7

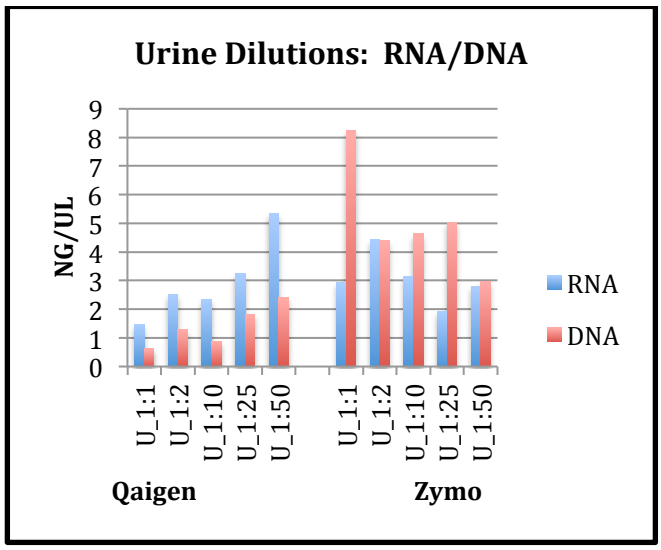

Figure 8

Discussion

In this study quantifiable amounts of miRNA and DNA were found in both the Zymo and the Qiagen kits. The concentrations obtained however, were highly variable depending on the particular body fluid and the particular kit used. The results obtained from the diluted samples produced varying concentrations at much lower levels, as expected. Overall, the Zymo Kit proved to obtain higher concentrations of both DNA and RNA when compared to the Qiagen Kit. Finally, miRNA signals and full DNA profiles were obtained from all samples selected for miRNA/DNA profiling. Due to the trace amounts of samples available in forensic investigations, it is often difficult if not impossible to extract both the RNA and DNA portions (Haas, 2015). Scientists faced with this challenge have therefore developed commercial kits that analyze both RNA and DNA simultaneously within one sample. It was necessary that the sensitivity and accuracy be analyzed using different kits.

Conclusion

This study reveals the ability to successfully co-extract both RNA and DNA from forensically relevant body fluids, suggesting the Zymo kit as a superior method for this purpose. This research highlights the potential of miRNAs for the identification of forensically relevant body fluids as it has shown possible the ability to extract both miRNA profiles and DNA profiles from a single sample, which could prove crucial to a forensic investigation.

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Biography:

My name is Sarah Markland and I am from Long Island, New York. I am a second year junior studying Forensic Science and Biology-Premedical with a minor in Chemistry here at the University of New Haven. I am currently involved in many Campus Organizations such as the Forensic Science and Chemistry Club, Regenerative Medicine Club, Model United Nations, HOSA, Biology Club, and the Political Science Organization. I am also a Lab Assistant in Microbiology. Additionally, I am volunteering for Yale-New Haven Hospital and will soon be working to get my EMT Certification. My dream in l life is to attend medical school and become a Medial Examiner.

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