Total RNA/DNA Purification and Detection from Blood Preserved on a Neoteryx™ Mitra Microsampling Device

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INTRODUCTION

Blood is a very common sample used in clinical analysis. In particular, the DNA and RNA within can be used in the genotyping of an individual to detect various polymorphisms, and also be used to identify biomarkers in individuals to indicate clinical problems such as cancer and heart disease. Blood is usually drawn from a patient into a specialized blood collection tube, based on the nature of analysis (such as metabolite analysis or cell count). Alternative blood collection devices could also be used to overcome some of the inconvenience of blood tube collection, including the need of venepuncture, the potential of bodily fluid leakage as well as storage/shipping space requirement. A commonly used method is dried blood spotting (DBS) on special absorbent paper, drawn by lancet from the finger, heel or toe. Most current DNA or RNA clinical analysis utilizes different forms of blood tubes for input (including specialized tubes that are designed to preserve DNA or RNA such as PAXgene and Tempus). However, increasing numbers of DNA/RNA tests have been developed to utilize alternative collection device such as DBS. Here, we demonstrated that high quality DNA and RNA can be isolated from a Neoteryx Mitra™ Microsampling device. The isolated DNA and RNA can be used in different downstream molecular tests including Single Nucleotide Polymorphism (SNP) analysis as well as RNA expression analysis by Next Generation Sequencing (NGS).

MATERIALS AND METHODS

DNA Purification

Human blood was collected onto Neoteryx Mitra™ Microsampling Devices (Part# 10005). The capacity of each device is about 20 µL of blood. The blood DNA was isolated using Norgen's Blood DNA Isolation Micro Kit (Cat# 52100) with some modifications. One or two tips of the Neoteryx Mitra™ Microsampling device were dispensed into a 1.5 mL microfuge tube. Next, 200 µL of PBS was applied to the tip(s) and vortexed for 10 seconds. This was followed by adding 20 µL of Proteinase K along with 300 µL of Lysis Buffer B, then vortexed for 10 seconds. The solution was then incubated at 56°C for 20 minutes. The lysate was then transferred to a clean microfuge tube, where 250 µL of 96 – 100% ethanol was added and vortexed to mix. The Lysate-Ethanol Mix was then applied to a DNA Micro Spin Column and centrifuged at 8,000 RPM (~ 6,000 x g) for 1 minute. The column was then washed with 500 µL Wash Buffer WN and centrifuged at 8,000 RPM (~ 6,000 x g) for 1 minute. The column was further washed twice with 500 µL of Wash Solution A and centrifugation at 14,000 RPM (~ 14,000 x g) for 1 minute. The column was then dry spun at 14,000 RPM (14,000 x g) for 2 minutes. DNA was then eluted with 30 µL of Elution Buffer B and incubated for 1 minute at room temperature, then centrifuged for 1 minute at 8,000 rpm (~ 6,000 x g). Twelve isolations were performed using one or two Mitra tips (total of 24 isolations).

RNA Purification

Human blood was collected onto Neoteryx Mitra™ Microsampling Devices (Part# 10005). The capacity of each device is about 20 µL of blood. The blood RNA was isolated using Norgen's Total RNA Purification Plus Micro Kit (Cat# 48500) with some modifications. One or two tips of the Neoteryx Mitra™ Microsampling device were dispensed into a 1.5 mL microfuge tube. Next, 300 µL of Buffer RL was added and mixed by vortexing, then incubated at 42°C for 30 minutes. Vortexing was done every 10 minutes in between. The tube was then centrifuged at 14,000 RPM (14,000 x g) for 1 minute and the supernatant collected into a fresh microfuge tube. The lysate was passed through a gDNA Removal Column and centrifuged at 14,000 RPM (14,000 x g) for 1 minute and the flowthrough was retained. Next, 200 µL of 96-100% ethanol was added to the flowthrough and then mixed by vortexing. The Lysate-Ethanol Mix was then added to an RNA Micro Spin Column and centrifuged at 6,000 RPM (~3,500 x g) for 1 minute. The column was then washed three times with 400 µL of Wash Solution A at 14,000 RPM (14,000 x g) for 1 minute. A dry spin was then performed at 14,000 RPM (14,000 x g) for 2 minutes. The RNA was then eluted with 20 µL of Elution Solution A and centrifuged at 2000 RPM (~200 x g) for 1 minute, followed by 14,000 RPM (14,000 x g) for 1 minute.

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Twelve isolations were performed using one or two Mitra tips (total of 24 isolations).

qPCR and RT-qPCR Analysis

DNA (2 µL) was used in a 15 µL qPCR reaction using Norgen's 2x PCR Master Mix (Cat# 28007) with primers specific to the gene encoding human 5S rRNA. cDNA was synthesized using 3 µL of the isolated RNA using Norgen's TruScript Reverse Transcriptase system (Cat# 54440) and subsequently probed in a qPCR reaction with primers specific to human S15 (mRNA) and miR-21 (microRNA).

Sanger Chain Terminator Sequencing of the Human VKORC1 polymorphism

Two µL of DNA was used in Norgen's workflow for traditional Sanger Chain Terminator Sequencing using the ABI Big Dye Terminator chemistry on an ABI Genetic Analyzer 3130XL. The human VKORC1 polymorphism was studied. A "G" to "A" mutation within the VKORC1 gene is shown to be involved in Warfarin sensitivity

Small RNA-Sequencing

The Small RNA Libraries were prepared using 3 µL of isolated RNA and sequenced using Illumina MiSeq Reagent Kit 150 cycles with v3 chemistry. The sequencing was performed at 1 x 70 cycle as per standard operating procedure. The output was analyzed using Norgen's standard Small RNA Seq-Workflow (https://norgenbiotek.com/services/small-rna-and-microrna-next-gen-sequencing)

RESULTS AND DISCUSSION

Section 1: DNA Purification and Detection

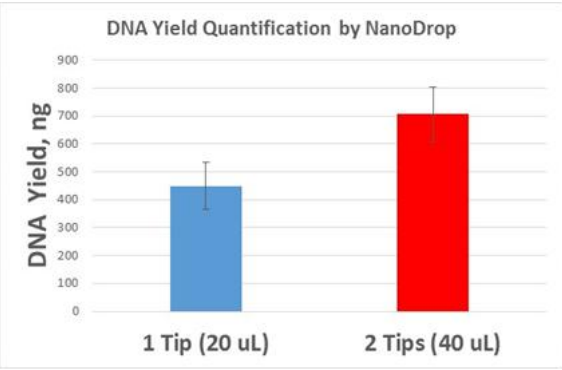

Figure 1. DNA Quantification by NanoDrop. A graph detailing the spectrophotometric results of 2 µL of DNA isolated from 1 or 2 tips of the Neoteryx Mitra™ Microsampling device (12 replicates each). Each sample was measured for DNA concentration using NanoDrop. The yield of DNA using 2 tips is seen to be around double of that using 1 tip, with over 700 ng of DNA recovered.

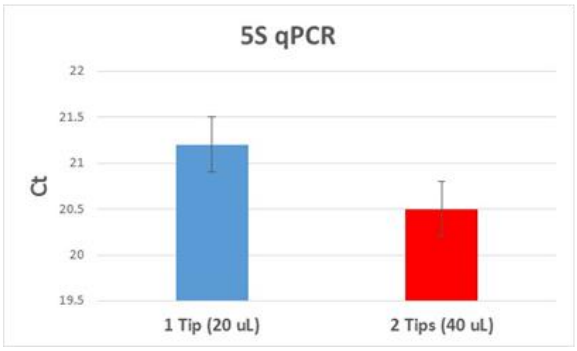

Figure 2. DNA Detection by qPCR. The above graph depicts the results from qPCR reactions using 2 µL of purified DNA with primers specific to the gene encoding the human 5S rRNA. The genomic copy of the human 5S rRNA was successfully amplified from all samples (both 1-tip and 2-tip isolations). 5S rRNA qPCR showed a good concentration effect as 2-tip isolation had in general 1 Ct lower than that of 1-tip isolation (1 Ct unit = 1 doubling event).

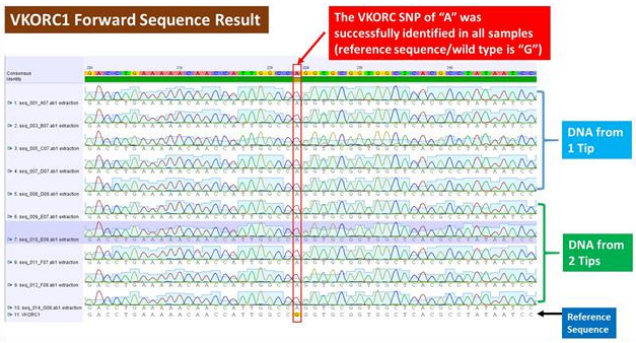

Figure 3. DNA Detection by Sanger Chain Terminator Sequencing of the Human VKORC1 Polymorphism. The diagram showed the result from using 2 µL of DNA purified in Norgen's workflow for traditional Sanger Chain Terminator Sequencing using the ABI Big Dye Terminator chemistry on an ABI Genetic Analyzer 3130XL. The human VKORC1 polymorphism was studied. A "G" to "A" mutation within the VKORC1 gene is shown to be involved in Warfarin sensitivity. The donor of the blood sample has a confirmed homozygous mutant genotype ("AA") for the VKORC1. Ten samples of DNA (5 each isolated from 1 or 2 Mitra™ tips, respectively) were used for the profiling. In VKORC1 Forward Sequencing, all 10 samples showed the genotype of "A" at the SNP site as expected, showing the high quality of the DNA purified.

Section 2: RNA Purification and Detection

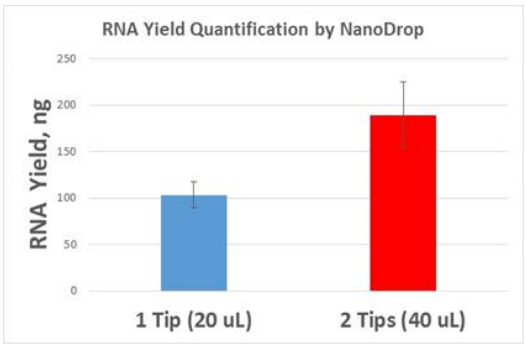

Figure 4. RNA Quantification by NanoDrop. A graph detailing the spectrophotometric results of 2 µL of RNA isolated from 1 or 2 tips of

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the Neoteryx Mitra™ Microsampling device (12 replicates each). Each sample was measured for RNA concentration using NanoDrop. The yield of RNA using 2 tips is seen to be around double of that using 1 tip, with close to 200 ng of RNA recovered.

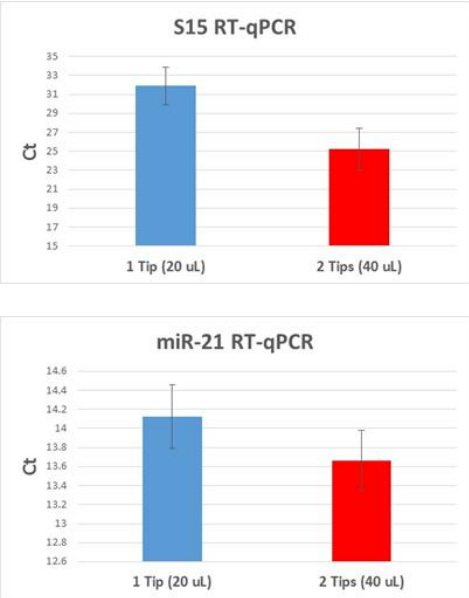

Figure 5. RNA Detection by RT-qPCR. The figures above depict the results of using 3 µL of purified RNA in a 10 µL reverse transcription reaction using Norgen's TruScript Reverse Transcriptase system and reverse primer specific to mRNA (oligo dT) or human miR-21 microRNA. The cDNA synthesized was used directly in a 20 µL qPCR reaction using primers specific to human S15 transcript (mRNA) or human miR-21, respectively. Both S15 and miR-21 were successfully amplified from all samples (both 1-tip and 2-tip isolations). Both S15 and miR-21 RT-qPCR showed a good concentration effect as 2-tip isolation had in general lower Ct value than that of 1-tip isolation, suggesting consistent isolation of high quality Total RNA (both large mRNA and microRNA) from the Neoteryx Mitra™ Microsampling devices.

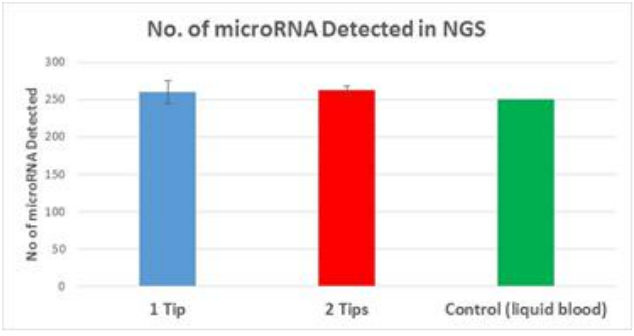

Figure 6. RNA Detection by Illumina Next Generation Sequencing (Small RNA-Seq). RNA isolated using the Neoteryx Mitra™ Microsampling device was used in Norgen's Small RNA-Sequencing Workflow for profiling of small RNA (including microRNA) abundance (https://norgenbiotek.com/services/small-rna-and-microrna-next-gen-sequencing). The Small RNA Libraries were sequenced using Illumina MiSeq Reagent Kit 150 cycles with v3 chemistry. The sequencing was performed at 1 x 70 cycle as per standard operating procedure on a MiSeq system. Each library attained an average minimum of 2 million raw reads with over 75% of processed reads (≥ 1.5M) mapped to known microRNAs. All samples were then normalized to RPM (Reads per Million). Any microRNA that had a read count of > 5.0 was considered detected. The total number of microRNA detected was almost identical among all three inputs (1-Tip, 2-Tips or 40 µL Liquid Blood control). This suggests that RNA diversity could be well-preserved and recovered from the Neoteryx Mitra™ Microsampling device.

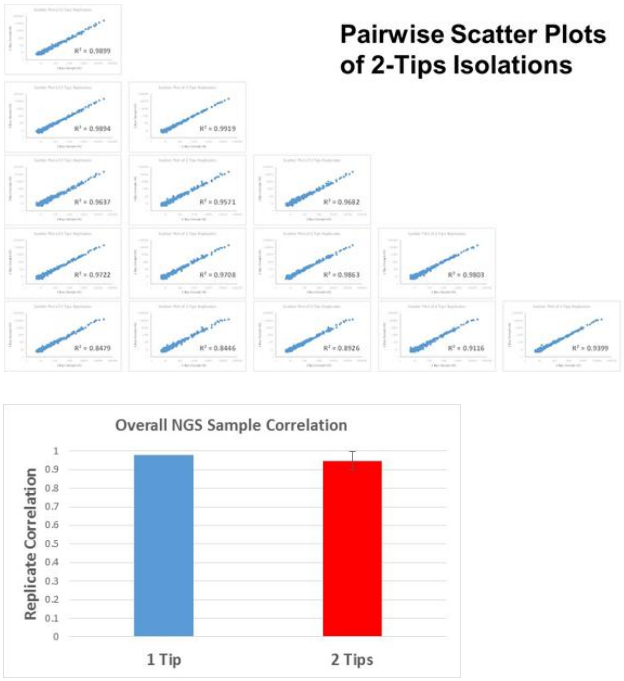

Figure 7. Pairwise Scatter Plots of 2-Tips Isolation. The expression level (read count) of each microRNA detected among each sample in the Small RNA-Seq run was compared using Scatter Plot analysis. The Scatter Plot analysis on the top panel showed that the expression level of the microRNAs recovered from 2 tips of the Neoteryx Mitra™ Microsampling device had high correlation between each of the 6 replicates. In summary in the lower panel microRNA level correlated highly among replicates of 1-Tip or 2-Tips isolation (>97% and >94%, respectively).

CONCLUSIONS

1. Both total RNA (large mRNA and microRNA) as well as total DNA were shown to be preserved in the Neoteryx Mitra Microsampling Device.
2. Specially optimized protocols using Norgen's patented silicon carbide nucleic acid technology were developed to isolate and purify RNA or DNA from the Neoteryx Mitra Microsampling Device
3. Proportional amounts of RNA or DNA were successfully purified from 1 or 2 Neoteryx Mitra Microsampling Devices.
4. The isolated RNA or DNA was of the highest quality. All nucleic acids purified were used successfully in various

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downstream applications including qPCR and Sanger sequencing for DNA, as well as RT-qPCR and Next Gen Sequencing (Small RNA-Seq) for RNA

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