DNA genotek

Comparison of DNA yield from saliva and blood

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Saliva collected in Oragene®/saliva samples† yields large quantities of high quality genomic DNA that is equivalent to DNA extracted from blood.

Introduction

Blood collection for genomic DNA presents a number of disadvantages: it is invasive and inconvenient for the donor, requires a trained medical professional, must be refrigerated for transportation and storage and it is difficult to transport across borders. All of these factors can add significant cost to any genetic study and impact compliance rates.

Saliva collection provides a non-invasive alternative source of genomic DNA for use in genetic analysis. The Oragene self-collection kit is specifically designed for collecting and preserving DNA in saliva for long periods of time at room temperature. The collection can be performed unsupervised, and the ability to store and transport the collected samples at ambient temperatures makes it an attractive choice for wide-ranging genetic analysis projects.

It is therefore important to assess the yield and quality of genomic DNA extracted from saliva samples collected with the Oragene self-collection kit in comparison to DNA from blood. This technical bulletin provides quantitative information about the amount and quality of human genomic DNA in Oragene/saliva samples, as compared to DNA extracted from blood.

Materials and methods

Sample collection

Paired blood and saliva samples were collected from more than 100 donors. After providing consent, each donor was first asked to deliver 2 mL saliva into an Oragene collection device according to the standard instructions included with the kit. The saliva collection was unsupervised. Next, a phlebotomist drew approximately 8 mL of blood from each donor using a BD Vacutainer EDTA tube (BD Cat. No. 36643).

DNA purification

Saliva

After collection, the Oragene/saliva samples were mixed by manual shaking for 15 seconds and the samples were stored at room temperature. Several days later, 500 µL aliquots of each sample were purified according to the prepIT®•L2P 0.5 mL manual purification protocol[1].

Blood

Immediately after collection, blood samples were placed on a rocker and allowed to mix for 30 minutes to avoid the formation of microclots. Buffy coat was prepared by spinning the collected sample at 2500 × *g* for 10 minutes, then the top plasma layer was discarded and the buffy coat was transferred to a microcentrifuge tube. The buffy coat was purified using the Qiagen® QIAamp® blood mini kit (Cat. No. 51106).

The purified DNA, from both blood and saliva, was stored at -20°C. RNAse digestion and DNA analysis was done on a small subset of paired samples to highlight differences and similarities of DNA purified from blood and saliva.

Double-RNase digestion

RNase digestion was performed as previously described[2]. Briefly, paired blood and saliva samples from 5 donors were thawed and 2 × 25 µL aliquots of DNA were taken from each. Aliquots were diluted to 250 µL with TE buffer. To one aliquot of both blood

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER

and saliva, RNase A and RNase T1 were added to a final concentration of 10 µg/mL and 25 units/mL, respectively. All samples were incubated at 37°C for 30 minutes. Next, NaCl was added to a final concentration of 0.1 M and the DNA was precipitated with 500 µL (2 volumes) of 95% ethanol. The DNA was collected by centrifugation and resuspended in 25 µL of TE buffer. The samples were allowed to sit at room temperature overnight to ensure that the DNA was fully dissolved.

DNA analysis

The absorbance spectrum from 220 to 320 nm was measured using a Varian Cary 1E spectrophotometer. The A_{260}/A_{280} ratio was corrected by subtracting the A_{320} value from the A_{260} and A_{280} values. The nucleic acid concentrations were determined using the corrected A_{260} value, where 1 ODA_{260} unit is equal to 50 µg/mL. The DNA concentrations were determined by fluorescence using SYBR® Green I (Invitrogen, Cat. No. S-7563) and a Rotor-Gene® 6000 real-time thermal cycler (Corbett Research) according to the DNA Genotek relative fluorescence (RFL) assay[3]. The Molecular weight of the DNA and the presence of RNA were determined by gel electrophoresis. A total of 50, 100, 200, and 400 ng of DNA, as determined by RFL, were run on a 0.8% agarose gel and the nucleic acid was visualized by ethidium bromide staining followed by UV trans-illumination. PCR using primers specific for the Amelogenin gene was performed as previously described using 50 ng of DNA isolated from blood or saliva as a template. The molecular weight of the PCR product was determined by gel electrophoresis on a 1% agarose gel.

Results

The corrected A_{260} reading is a measure of the total nucleic acid in a sample. While the corrected A_{260}/A_{280} ratio is a measure of sample purity. For both saliva and blood, the A_{260}/A_{280} ratio of the isolated DNA was between 1.8 and 2.0 (Table 1), indicating a solution that is mainly composed of nucleic acids.

For some donors, the isolated saliva DNA may also contain some degraded RNA. Like DNA, the concentrations of RNA in saliva can vary from donor to donor. This is evident when the DNA concentrations, as determined by absorbance and fluorescence, are directly compared (Table 2).

For DNA purified from blood, there is little difference between concentrations calculated from absorbance and from fluorescence; however, for untreated saliva DNA, these differences are significant. When the saliva DNA is treated with RNase A/T1, the DNA concentrations as measured by absorbance are reduced significantly and are comparable to the concentrations determined by fluorescence for both treated and untreated saliva samples.

	A_{260}/A_{280}		A_{260}/A_{280}		A_{260}/A_{280}
B53–	1.9	S53–	2.0	S53+	1.8
B54–	1.9	S54–	2.0	S54+	1.8
B59–	1.9	S59–	2.0	S59+	1.8
B65–	1.8	S65–	1.8	S65+	1.8
B73–	1.9	S73–	1.9	S73+	1.8

***Table 1**: Corrected A_{260}/A_{280} ratios of 5 paired blood (B)/saliva (S) samples, both untreated (–) and treated (+) with RNase A/T1.*

	Blood, –RNase ng/ µL		Saliva, –RNase ng/µL		Saliva, +RNase ng/µL	
Donor	Abs	Fluor	Abs	Fluor	Abs	Fluor
53	73.50	73.24	636.11	170.20	201.82	194.29
54	28.60	30.21	389.99	121.31	183.48	171.85
59	29.19	30.31	391.83	87.17	89.99	85.51
65	35.81	36.76	56.71	40.62	43.79	44.41
73	16.66	22.00	369.71	288.84	301.19	277.64

***Table 2**: Raw DNA concentrations (ng/µL) of untreated (–) blood and untreated (–)/RNase treated (+) saliva DNA as determined by absorbance (Abs) and fluorescence (Fluor). Note these are from equal sample volumes of blood and saliva.*

The differences in the amount of RNA present in DNA isolated from blood and saliva can also be seen visually on an agarose gel. When large amounts of RNA are present in a DNA sample, it can usually be seen as a "cloud" below ~560 bp. For DNA isolated from blood only a faint band can be seen when more than 400 ng of Total DNA is run (Figure 1A). However, from the same donor, a large bright band is seen for the same amount of DNA isolated from saliva (Figure 1C). This band disappears when the DNA is treated with RNase A/T1 confirming that the band is partially degraded RNA (Figure 1D).

The presence of large amounts of degraded RNA in some DNA samples does not affect the quality of DNA but does impact quantification methodologies. The DNA from both the blood and saliva samples is of high molecular weight (>23 kb).

Smearing is seen in lanes where >200 ng of DNA has been loaded. This smearing effect is seen for both blood and saliva (Figure 1) suggesting that the smear is an indication of overloading of the agarose gel and is not an indication of poor quality DNA.

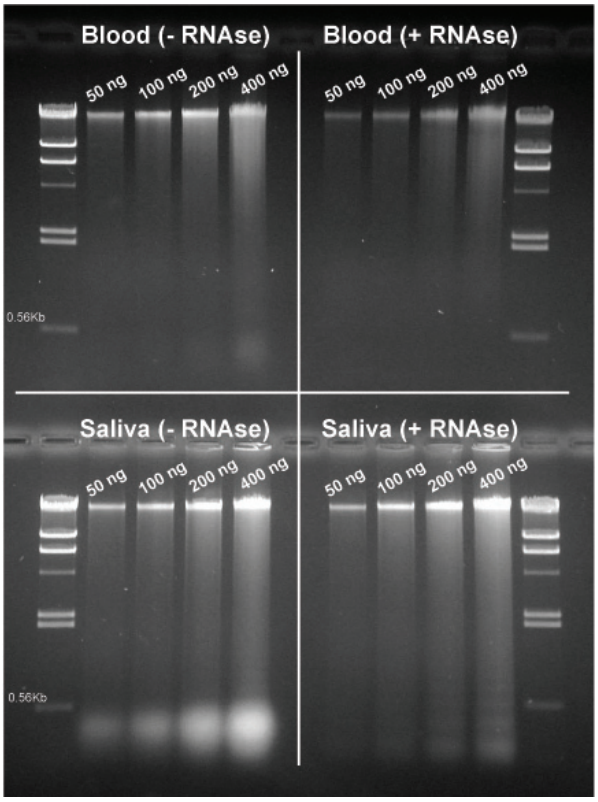

***Figure 1**: Agarose gel electrophoresis of increasing amounts of human genomic DNA extracted from blood and saliva. Samples were untreated or treated with RNAse A/T1.*

DNA purified from blood and DNA purified from saliva collected with the Oragene kit is of equal quality, and the presence of RNA in saliva does not affect downstream applications (e.g., PCR). Using primers specific for Amelogenin, a sex determining gene, both blood and saliva DNA generate identical PCR products (Figure 2). A single band is seen for females at 977 bp and 2 bands are seen for males at 977 and 790 bp.

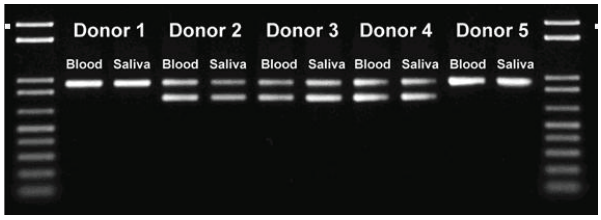

***Figure 2**: Agarose gel electrophoresis of Amelogenin PCR products.*

Conclusions

Saliva collected in the Oragene self-collection kit is a non-invasive source of high quality genomic DNA. In comparison to blood, saliva samples can yield more DNA (Table 2), with no compromise in quality (Table 1). It is important to note that fluorescence is a more accurate method for determining DNA concentration in saliva samples as it is specific for double-stranded DNA.

The DNA recovered from saliva is high molecular weight and performs identically to DNA isolated from blood in downstream applications (Figure 2).

References

1 Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.
2 RNA removal by double-RNase digestion. DNA Genotek. PD-PR-040.
3 DNA quantification of Oragene/saliva samples using SYBR Green I Dye and a micro-plate reader. DNA Genotek. PD-PR-075.