DNAgard® Saliva *HT:* DNA purification from saliva samples and PCR amplification

INTRODUCTION

Salivary DNA is becoming increasingly popular for genetic analysis because saliva collection is painless and non-invasive. DNAgard® Saliva *HT* is designed for safe collection and automated sample processing of salivary DNA. This technical bulletin provides evidence that the stabilizing chemistry in DNAgard® Saliva *HT* preserves salivary DNA integrity for at least 44 days under high temperature conditions, equivalent to 10 months at 22°C. Further, DNA isolated from saliva samples stored in DNAgard® Saliva *HT* is shown to be compatible with long range PCR and qPCR.

MATERIALS AND METHODS

Saliva Collection and Storage

Saliva samples from five (5) donors were collected into conical tubes. Samples from each individual donors were mixed before allocating into DNAgard® Saliva *HT* solution (DGS-*HT*) or competitor O's solution (CO) at a 1:1 (vol:vol) ratio in duplicates. In addition, equal volume of saliva were stored alone as the non–protected control (NP). All samples were stored at 45°C for five (5) weeks and moved to 60°C for an additional nine (9) days.

DNA Purification

500 µL aliquots of each sample were removed and processed on a MagNA Pure Compact Instrument using MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume (Roche, Cat. No. 03730972001) according to the manufacturer's instructions.

DNA Yield and Purity

The purified DNA was quantified using a Quant-iT™ PicoGreen® dsDNA Assay kit *(*Thermo, Cat. No. P7589*)*, and the purity evaluated by measuring the A_{260}/A_{280} ratio using a Take3 microplate (Biotek). Approximately 5% of purified DNA from each sample was analyzed by agarose gel electrophoresis and stained with ethidium bromide.

Long-range PCR

A 3.9 kb fragment of the human GAPDH gene was amplified using KOD Xtreme™ Hot Start PCR kit (Novagen) on the iCycler Thermal Cycler (Bio-Rad). Primers specific to the human GAPDH gene were synthesized by Integrated DNA Technologies Inc. (Table 1). Long-range PCR of 50µL containing reagents at the final concentrations outlined in Table 2 were performed using the program recommended by the manufacturer (Table 3). Approximately 5% of the purified DNA from each sample was used in each reaction. The final amounts of DNA template from each donor are listed in Table 4. 40% of the PCR products from each reaction were visualized on a 1% agarose gel stained with ethidium bromide

Primer	Sequence
GADPH Forward	5'- CGG GTC TTT GCA GTC GTA TG -3'
GADPH Reverse	5'- CCA GCA AGA ATG TCT CAC CT -3'

Table 1: *Primer sequences for long-range PCR.*

KOD Xtreme™ Hot Start PCR kit	Final Concentration
2X Xtreme Buffer	1x
dNTPs	0.4 mM
Primers	0.3 µM
KOD Xtreme™ Hot Start DNA Polymerase	1 U
Template DNA	varies

Table 2: *Final composition of long-range PCR assays.*

Cycle	Temperature	Time
1x	94°C	2 minutes
40x	98°C	10 seconds
40x	54.8°C	30 seconds
40x	68°C	3:54minutes

Table 3: *Thermal cycling parameters for long-range PCR.*

	DNA template (ng)	
	Donor 2	Donor 5
CO-1	28.3	23.1
CO-2	58.3	30.7
NP-1	11.5	7.2
NP-2	13.5	4.4
DGS-*HT* 1	44.0	18.5
DGS-*HT* 2	45.9	17.1

Table 4: *Amount of DNA template used in long-range PCR.*

qPCR

Approximately 1% of the purified DNA from each sample was amplified in duplicate using iQ™ SYBR® Green Supermix (Bio-Rad, Cat. No. 170-8880) on a CFX96 Real-Time PCR Instrument (Bio-Rad) with thermal cycling conditions stated in Table 5. Primers specific to the human RNase P gene were synthesized by Integrated DNA Technologies Inc. (Table 6). qPCR compositions are listed in Table 7.

Cycle	Temperature	Time
1x	95°C	3 minutes
40x	95°C	15 seconds
40x	60°C	1 minute

Table 5: *Thermal cycling parameters for qPCR.*

Primer	Sequence
RNase P Forward	5'- AGA TTT GGA CCT GCG AGC G -3'
RNase P Reverse	5'- GAG CGG CTG TCT CCA CAA GT -3'

Table 6: *Primer sequences for qPCR.*

iQ™ SYBR® Green Supermix	Final Concentration
2x qPCR mix	1x
Primers	0.3 µM
Template DNA	varies

Table 7: *Final composition of qPCR assays.*

RESULTS

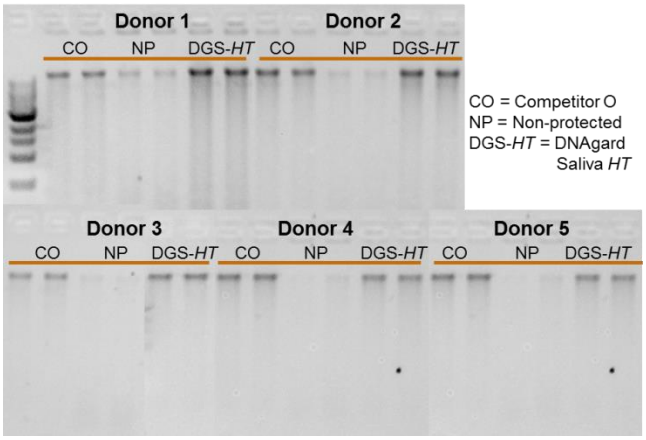

Figure 1: *Agarose gel of DNA purified from saliva stored using Competitor O, DNAgard® Saliva HT, or non-protected saliva samples. DNA was visualized on a 1% agarose gel stained with ethidium bromide.*

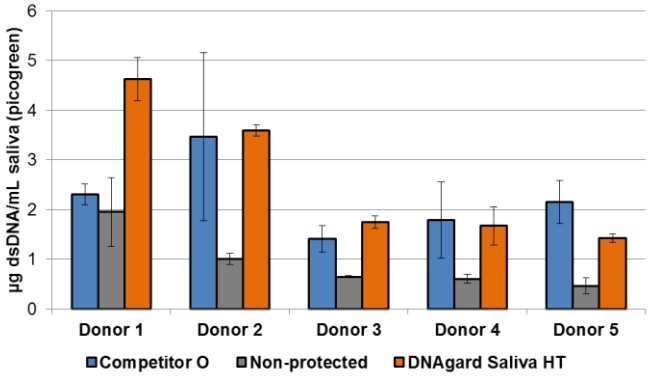

Figure 2: *dsDNA yield of DNA isolated from saliva stored using Competitor O, DNAgard® Saliva HT, or non-protected saliva samples. DNA concentration was measured using Quant-iT™ PicoGreen® dsDNA Assay.*

		DNA Yield (µg/mL saliva)		
	A_{260}/A_{280} ratio	Picogreen	Spectrophotometry	qPCR
Competitor O	1.73 ± 0.71	2.18 ± 0.96	5.07 ± 3.34	4.62 ± 1.09
Non-protected	2.20 ± 0.44	0.91 ± 0.60	4.93 ± 2.34	0.01
DNAgard® Saliva *HT*	2.16 ± 0.88	2.56 ± 1.33	11.54 ± 9.47	5.43 ± 1.05

Table 8: *Summary of results.*

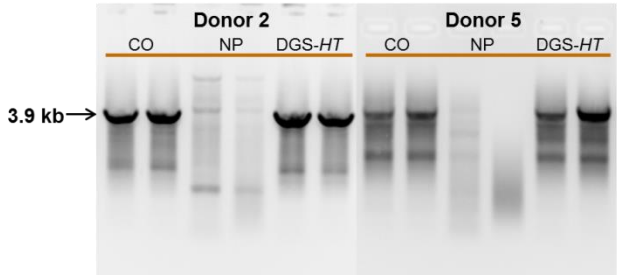

Figure 3: *Examples of long-range PCR products from two donors.*

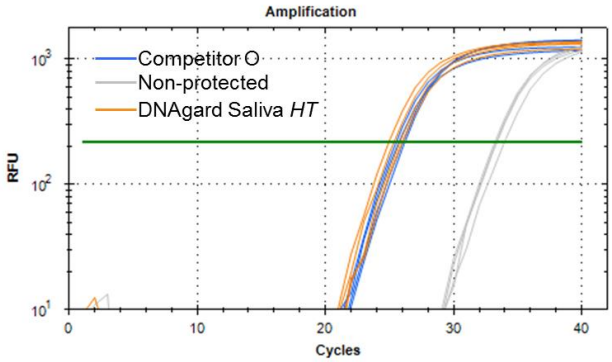

Figure 4: *Example of amplification curve from donor 3.*

SUMMARY

Accelerated-aging studies of saliva samples stored in DNAgard® Saliva *HT* indicate that the DNA remained stable for at least 10 months at room temperature (Figure 1) calculated using the Arrhenius equation. The Arrhenius equation assumes that the rate of a chemical reaction reduces by half when the temperature is reduced by 10°C. Samples purified after storage in DNAgard® Saliva *HT* exhibit equal or better high molecular weight human genomic DNA (Figure 1), dsDNA yield and quality (Figure 2 and Table 8) when compared to samples stored in Competitor O's solution. Table 8 summarizes these results. DNAgard® Saliva *HT* stabilized DNA gives equal performance to Competitor O-stabilized DNA in long-range PCR (Figure 3), and qPCR (Figure 4). Therefore, DNAgard® Saliva *HT* stabilizer preserves high quality DNA that is suitable for long-range PCR and qPCR applications.

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