Sample Collection & Preservation kits

APPLICATION NOTE

DANASALIVA Sample Collection Kit
DANAGENE SALIVA DNA Kit
DANAGENE SPIN SALIVA DNA Kit

Saliva as a sample type for genomic applications

Introduction

Saliva collection provides a non-invasive alternative source of genomic DNA for use in genetic analysis.

Saliva offers several appealing features for both researchers and clinicians. It is a non-invasive and needle-free method, that still enables collection of a high quantity of white blood cells, source of high-quality DNA. The collection can be performed unsupervised, and the ability to store and transport the collected samples at room temperature makes it an attractive choice for wide range of genetic analysis projects.

A very similar workflow in the lab can be used to extract DNA from saliva as is used for blood, producing the same resulting genomic DNA, with similar 260/280 ratios and similar molecular weight.

In this application note we evaluate the different DANAGEN solutions for working with saliva samples:

- The **DANASALIVA Sample Collection Kit** is specifically designed for collecting and preserving saliva samples for long periods of time at room temperature.
- The **DANAGENE Saliva DNA kit** is designed to isolate genomic DNA from fresh or preserved saliva samples using a "salting-out" method.
- The **DANAGENE SPIN Saliva DNA kit** is designed to isolate genomic DNA from preserved saliva samples using a MicroSpin column method.

Fig.1 DANASALIVA Sample Collection Kit/DANAGENE Spin Saliva DNA Kit

Material and Methods

Saliva sample collection

A saliva sample of 2 ml was collected from 2 healthy donors using our DANASALIVA Sample Collection Kit (Fig.1) and stored at room temperature for several days.

Manual Human Genomic DNA extraction for PCR assays

Method 1: Human genomic DNA was isolated from 400 µl of preserved saliva sample following the specific protocol of **DANAGENE Saliva DNA kit**.
Method 2: Human genomic DNA was isolated from 400 µl of preserved saliva sample following the specific protocol of **DANAGENE SPIN Saliva DNA kit** (Fig.1).
For each donor, three samples were processed.

Automated Human Genomic DNA extraction for PCR assays

Method 3: Human genomic DNA was isolated from 200 µl of preserved saliva sample using the ZiXpress Whole Blood Genomic DNA Extraction Kit using the **ZiXpress32 Robot** (ZINEXTS,Taiwan) (Fig.2) with the following modifications:

a) For the reagent plate preparation, we used the Viral Lysis Buffer (DANAGEN-BIOTED,Spain) instead of the Lysis buffer A.

b) For the robot protocol extraction setting, we edited a new protocol called SALIVA. We only changed the lysis buffer incubation time (10 minutes instead of 20 minutes) from the existing default blood protocol on the robot.

For each donor, four samples were processed.

Fig2. ZiXpress 32 Robot

Quality and Quantification of extracted DNA

For DNA quantification, DNA concentration was determined fluorometrically on the Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) using the QUBIT dsDNA BR Assay Kit.

For DNA quality, DNA purity was determined via 260/280 and 260/230 ratios measured on the NanoDrop (Thermo Fisher Scientific, USA).

Gel Electrophoresis

For visual analysis of Human DNA and PCR size and integrity, 20 µl of DNA from the final elution were loaded onto a 1% agarose TAE gel and run for 30 minutes at 125 V.

End-point PCR analysis

The purified DNA was used as a template in an End-point PCR reaction for the Alu human polymorphism determination following the protocol of the Determination of the Alu polymorphism by PCR (DANAGEN-BIOTED, Spain).

Real-Time PCR analysis

The purified DNA was used as a template in a Real-Time PCR reaction for the detection of Human Genomic DNA using the cfhDNA MONODOSE dtec-qPCR Kit (Genetic PCR Solutions™, Spain).

The target is a multiple-copy gene, 200 copies per genome, with a slow evolutionary rate.

Results and Discussion

Detection of Human Genomic DNA

DNA quantity was assessed using both fluorescence (Qubit. Figure 3) and gel electrophoresis (Figure 4). The shown results are the average of three samples and all samples were eluted/hydrated in 50 µl Tris HCl 5 mM pH 8.5.

We can observe the DNA concentration of method 1 (salting-out) is double that method 2 (MicroSpin column) and method 3 (Robot).

The amount of DNA that can be obtained from saliva is variable between individuals, as seen with donor 1, that obtained a higher quantity of DNA.

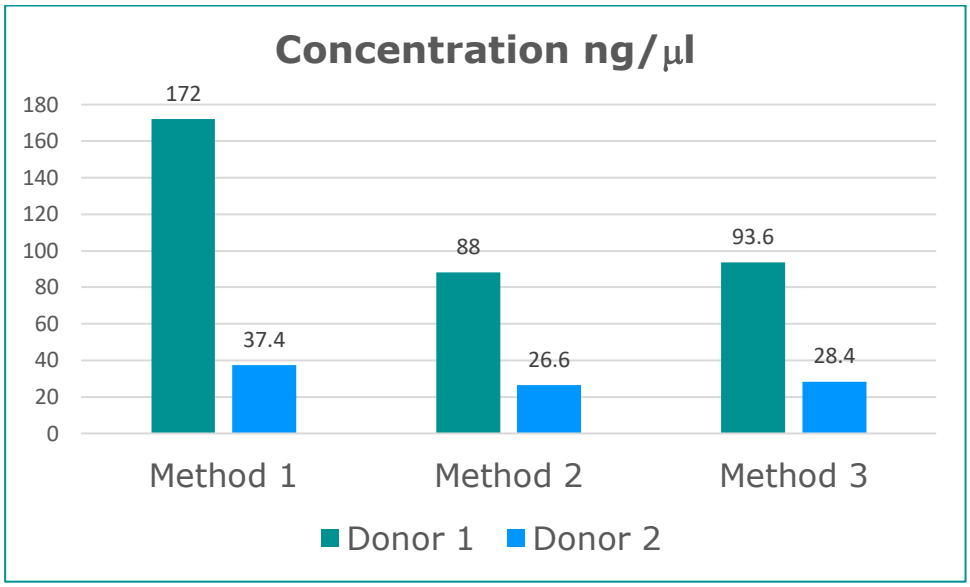

Figure 3. DNA concentrations of total DNA obtained by spectrophotometric analysis (Qubit)

The DNA obtained is high molecular weight (>23kb). The presence of large amounts of degraded RNA in donor 1 does not affect the quality of DNA but it may impact quantification methodologies (Nanodrop measures not shown). Smearing is seen in lanes (donor 1) where > 200 ng of DNA has been loaded, suggesting an overloading of the agarose gel rather than poor-quality DNA.

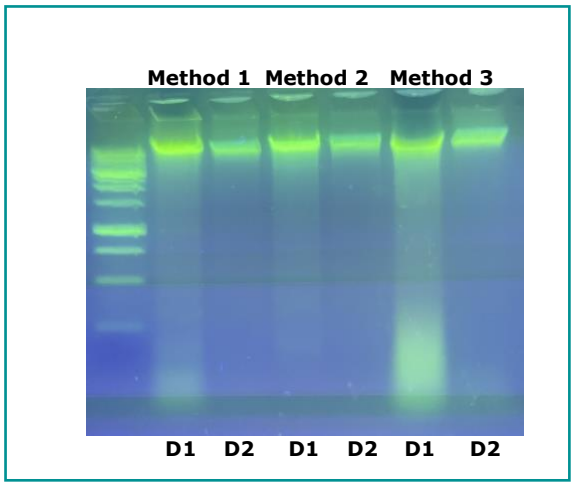

Figure 4. Gel electrophoresis of genomic DNA purified form preserved saliva samples

DNA quality was assessed by spectrophotometry (Nanodrop. Figure 5). All total nucleic acid isolated from all samples displayed A260/280 ratios > 1.75, demonstrating the high purity of the preparations. The A260/230 values are also good for method 2 (MicroSpin column) and lower than methods 1 and 3. The results are the average of three samples.

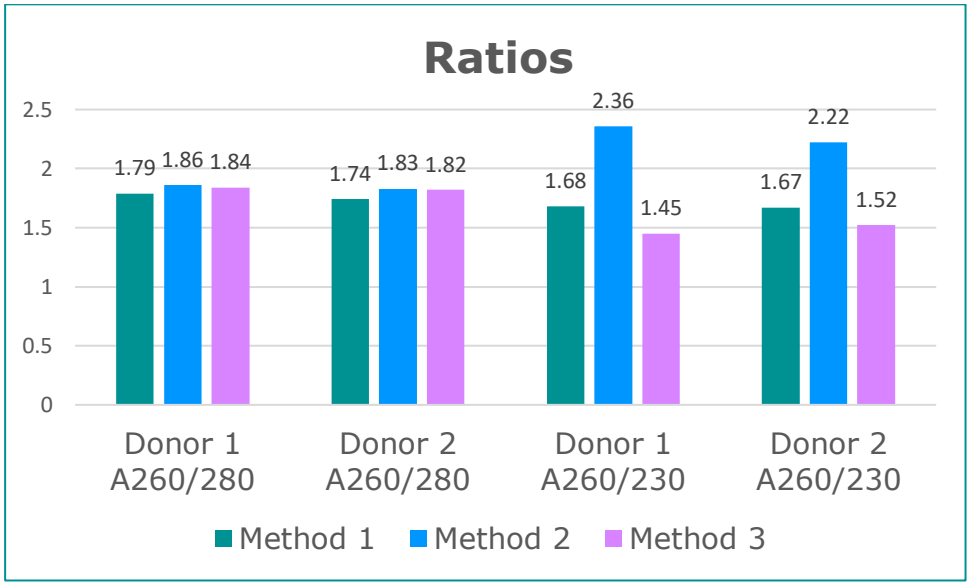

Figure 5. 260/280 and 260/230 ratios by spectrophotometric analysis (Nanodrop)

Determination of Alu polymorphism by PCR

The Alu polymorphism studied here is an insertion found in intron 8 of the tissue plasminogen activator gene (TPA). This region is about 260-270 nucleotides long and the insertion is approximately 300 base pairs long, so the insertion will result in an increase of 570 base pairs.

It can be tested whether a person has an Alu insertion at the TPA locus by PCR amplification. If a person is homozygous for the insertion, agarose gel electrophoresis of the PCR product will produce a single band of 570 base pairs. If a person is heterozygous, having the insertion in one of the homologous chromosomes but not in the other, 2 bands will appear on the gel, one of 570 base pairs and another of 260 base pairs. If a person does not present this particular insertion on either of the homologous chromosomes, the PCR will result in a single 260 base pair band.

In Figure 6, the results of PCR amplification of the TPA locus obtained following the three different extraction methods are shown. All three methods produced good PCR results. We observed that donor 1 is heterozygous for the Alu insertion, presenting 2 bands (260 and 570 base pairs), while donor 2 is homozygous for the Alu insertion.

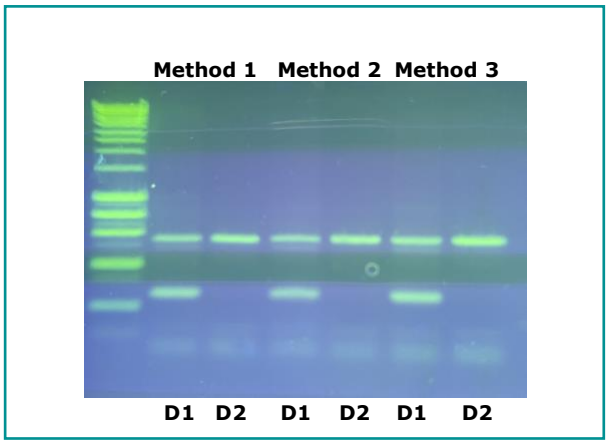

Fig.6 PCR analysis for the ALU insertion in intron 8 of the tissue plasminogen activator gene

Real Time PCR assay for Total Human DNA

To verify that the extracted DNA was of high purity, we also performed a Real Time PCR assay. To do so, we used the cfhDNA MONODOSE dtec-qPCR Kit (Genetic PCR Solutions TM, Spain). The target is a multiple-copy gene, 200 copies per genome, with a slow evolutionary rate.
Although we observed some variability in Cts, the amplification was successful with DNA from all three different methods (Fig.7).

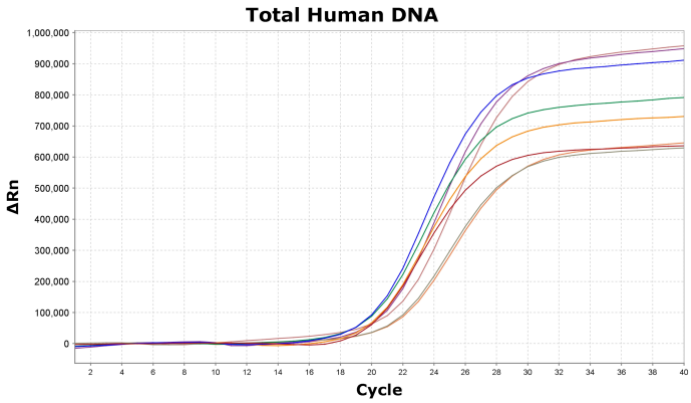

Figure 7. Amplification of Total Human DNA by qPCR analysis

Conclusion

Saliva collected in the DANASALIVA Sample Collection Kit is a non-invasive source of high quality of genomic DNA allowing a flexible extraction pipeline, regardless of the extraction method, manual or automatic, good results are obtained.

The DNA recovered from saliva is high molecular weight and performs identically to DNA isolated from blood for a broad range of common downstream applications, such as end-point PCR, qPCR, and further molecular detection methods.