Automated DNA Extraction

APPLICATION NOTE **DANASALIVA Sample Collection Kit/ZiXpress32**

Robot ZiXpres32 protocol for DNA Extraction from preserved Saliva Samples in DANASALIVA Sample Collection MICROBIOME Kit

Introduction

Extraction of nucleic acids is a vital part of life science and clinical research. In molecular biology, nucleic acids must be isolated as a starting point for hundreds of downstream applications, such as diagnosis, experimental research, and product development.

Automated extraction systems tend to be specifically designed instruments to simplify and increase the output of nucleic acid extraction. Not only automated machines do decrease required working time and labor costs, but also increase safety, quality, and reliably high yield.

We tested the efficiency of ZiXpress32 Robot (ZINEXTS,Taiwan) (Fig.1)

Fig1. ZiXpress 32 Robot

This reliable platform offers users the freedom to operate with optimized reagent kits or non-prefilled consumables and is an open magnetic pillar aNAP platform. Compact and sleek instrumentation used to purify 1 to 32 samples per run.

In this application note we describe an efficient protocol for DNA extraction of preserved saliva samples using the ZiXPresss 32 Robot and evaluate the extracted DNA efficacy for a broad range of common downstream applications, such as end-point PCR, qPCR, and Microbiome analysis.

Materials and Methods

The automated DNA extraction workflow was performed using the ZiXpress32 Robot. The system was configured for DNA extraction workflows using a modification of ZiXpress Whole Blood Genomic DNA Extraction Kit (ZINEXTS,Taiwan).

Saliva sample collection

A saliva sample of 2 ml was collected from 3 healthy donors using our DANASALIVA Sample Collection MICROBIOME Kit (Fig.2) and stored at room temperature.

Fig.2 DANASALIVA Sample Collection MICROBIOME Kit

Automated Human Genomic DNA extraction for PCR assays

Human genomic DNA was isolated from 200 µl of preserved saliva sample using the ZiXpress Whole Blood Genomic DNA Extraction Kit 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 20 minutes) from the existing default blood protocol on the robot.

We process 3 samples from donors 1 and 2, and 2 samples for donor 3 according to the plate configuration.

Automated Microbial DNA Extraction for microbiome analysis

Sample Preparation:
Briefly, 800 µl of preserved saliva sample + 25 µl of proteinase K were added to a bead microtube containing glass 0.1 mm particles. The mixture was incubated at 70ºC for 10 minutes. Following the incubation, the microtubes were homogenized by bead beating for 10 minutes at maximum speed on the Vortex Genie2 using a horizontal adapter. Next, the microtubes were centrifuged for 5 minutes and we used 200 µl of the supernatant for the Microbial DNA extraction.

Reagents Plate Preparation:
We used the ZiXpress Whole Blood Genomic DNA Extraction Kit changing the Lysis Buffer A for the Viral Lysis Buffer (DANAGEN-BIOTED,Spain) and we used the new SALIVA protocol on the robot.

We process 3 samples from donors 1 and 2, and 2 samples for donor 3 according to the plate configuration.

Manual Microbial DNA Extraction for microbiome analysis

Manual Microbial DNA was isolated from 800 µl of saliva sample following the recommended protocol of our DANAGENE MICROBIOME SALIVA DNA Kit.
All samples were done in duplicate.

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.

Gel Electrophoresis

For visual analysis of Human Genomic DNA and PCR, size was assessed using the Agilent 4150 TapeStation.

End-point PCR analysis

The purified DNA was used as a template in an End-point PCR reaction for the Rh factor determination following the protocol of the Determination of the Rh factor 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 SolutionsTM, Spain).

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

16S rRNA Gene Sequencing

Genomic DNA amplification was conducted using the 16S Barcoding Kit (SQK-RAB204; Oxford Nanopore Technologies, Oxford, UK) with the following PCR conditions:

Initial denaturation at 95°C for 1 minute, 25 cycles of 95°C for 20s, 55°C for 30s, and 65°C for 2 minutes, followed by a final extension at 65°C for 5 minutes.

Amplifies were purified using Agencourt AMPure XP beads (Beckman Coulter) and quantified by fluorometric quantification with Qubit (Thermo Fisher Scientific).

A total of 100 ng of DNA was used for library preparation and sequenced in MiniON flow cells (FLO-FLG001; Oxford Nanopore Technologies) according to the manufacturer's protocol.

Results and Discussion

Detection of Total DNA

DNA quantity was assessed using both capillary electrophoresis (Fig.3) and spectrophotometry (Qubit) (Fig.4).

We can observe the presence of Genomic DNA without degradation and a similar yield in each replica form the same donor (D1, D2 and D3)

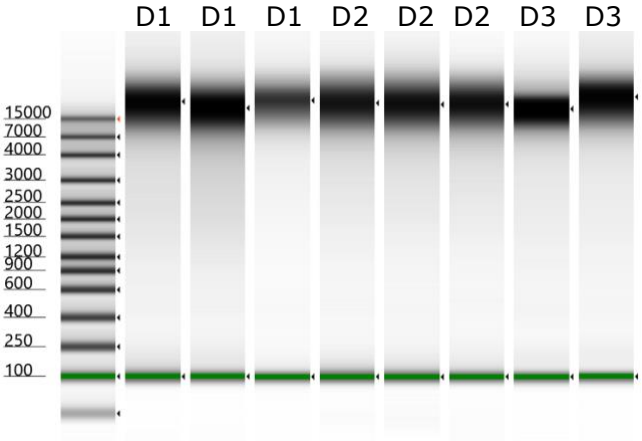

Figure 3. Capillary electrophoresis of Total DNA purified from preserved saliva samples

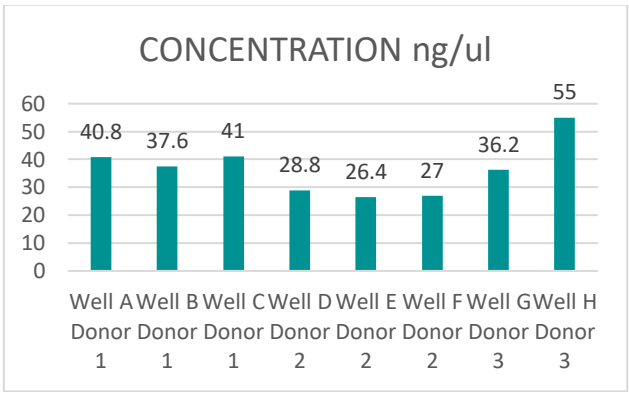

Figure 4. DNA concentrations of Total DNA obtained by spectrophotometric analysis (Qubit)

Determination of Rh factor by End-point PCR

Rh+ individuals will produce 2 fragments of different sizes (1200 and 600 bp) and Rh- individuals a single band corresponding to the CcEe gene fragment (1200 bp).

We can observe that the obtained DNA has a good quality for PCR assays.
Donor 1 and 2 are Rh+ and Donor 3 is Rh- (Fig5).

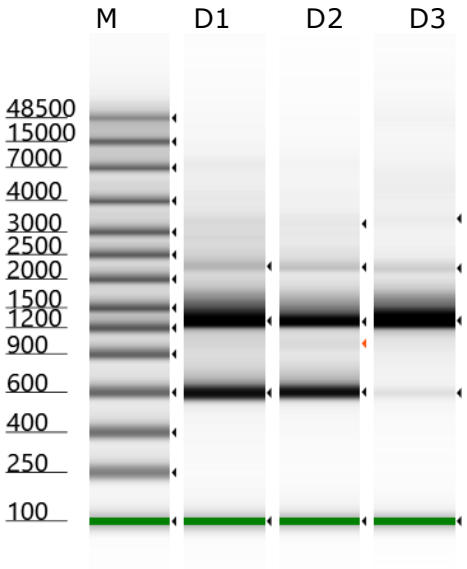

Figure 5. Capillary electrophoresis of End-point PCR

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.

We observed a little variation in Ct (Fig.6).

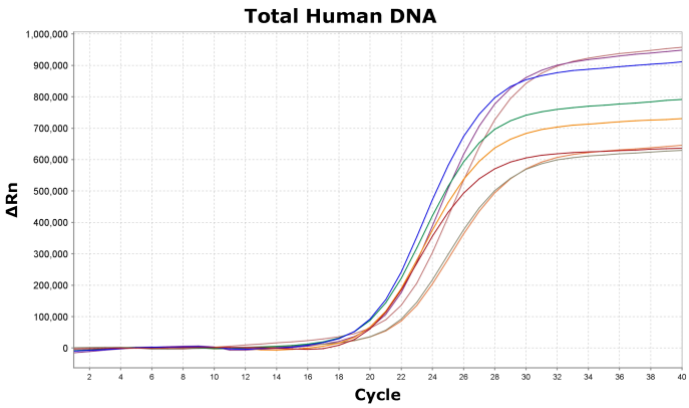

Figure 6. Amplification of Total Human DNA by qPCR analysis

Microbiome Analysis

To verify that the automated extracted DNA using the robot has the same quality as manual extracted DNA, we conducted a comparative microbiome analysis from preserved saliva samples.

We can observe very good and similar results manual versus automated DNA isolation (Fig.7).

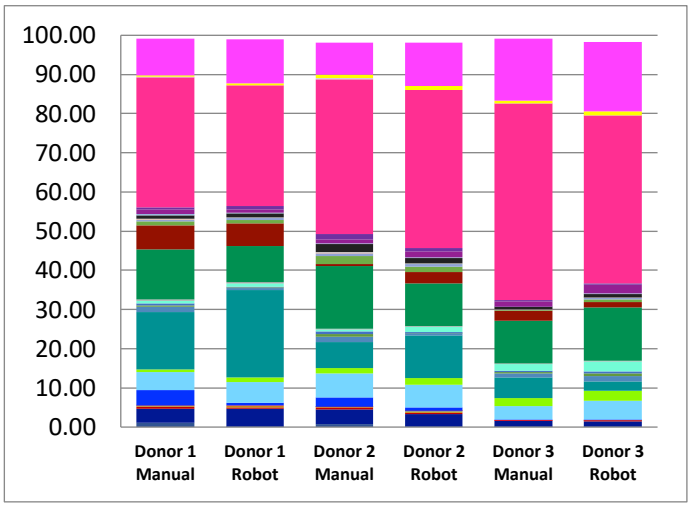

Figure 7. Microbial composition (Genus) of preserved saliva samples at room temperature

Conclusion

This application note demonstrates a fully automated Total DNA purification workflow on the ZiXpress32 Robot from preserved saliva samples in our DANASALIVA Sample Collection MICROBIOME kit.

The total extracted DNA obtained was of high purity and suitable for a broad range of common downstream applications, such as end-point PCR, qPCR, and library preparation for NGS.

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