DNA extraction from Whatman FTA® cards

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FTA cards enable human genomic DNA to be collected from blood or saliva and stored at room temperature. DNA extracted from FTA cards is compatible with the Spartan DX™.

Introduction

FTA® is an acronym for Fast Technology for Analysis of nucleic acids. FTA cards enable non-invasive collection of human genomic DNA from blood or saliva samples. The cards are impregnated with a solution that lyses cell membranes and denatures proteins upon contact. This protects the nucleic acids from UV damage, and microbial and fungal degradation. Infectious pathogens are also rendered inactive. Once dried, samples are stable on FTA cards up to 5 years for buccal cells, and up to 14 years for blood (Ref 1). The purpose of this study was to determine the performance of human genomic DNA extracted from FTA cards on the Spartan DX desktop DNA analyzer.

Materials and Methods

DNA collection and extraction

Blood was collected from 8 individuals by pricking the skin and applying drops of blood to FTA paper from an FTA Starter Pack (Whatman, Cat. No. WB120061). After drying the blood spots, DNA was extracted as per the manufacturer's instructions. In brief, a Harris Uni-Core punch supplied with the Starter Pack was used to punch 1.2-mm discs of dried blood from the FTA cards. The discs were incubated for 5 min at room temperature with 200 µl of FTA Purification Reagent. This incubation step was repeated three times with fresh Purification Reagent each time. Next, the discs were washed for 5 min with 200 µl of TE buffer. This wash step was repeated once. After washing, the discs were placed directly in the PCR tubes.

Real-time PCR

Oligonucleotide primers and probe were designed against a conserved region of the human muscarinic acetylcholine receptor subtype M3 gene (*CHRM3*) (Ref 2). The forward primer sequence was 5'-ttg ggt cat ctc ctt tg-3', the reverse primer was 5'-gca cag ttc tct ttc ca-3', and the probe sequence was 5'-tcc ttt ggg ctc ctg cca tct-3'. The TaqMan® probe consisted of a FAM fluorophore at the 5' end and a Black Hole Quencher® (BHQ-1) at the 3' end. The expected amplicon size was 74 base pairs.

Components of the PCR amplification mixtures are listed in Table 1, and cycling parameters are listed in Table 2. Note that a two-temperature cycling program was performed by combining the annealing and extension steps. Reactions were performed in 0.2 ml thin-wall, flat-cap PCR tubes (VWR, Cat. No. 53550-106). Reactions were topped with 15 µl of mineral oil (Biotools, Cat. No. 20.032) to prevent evaporation. Real-time PCR was performed using the Spartan DX instrument.

DNA analysis

Fluorescence results from the Spartan DX were graphed using Spartan Analyzer software supplied with the instrument. In addition, real-time PCR results were confirmed by agarose gel electrophoresis using 5 µl of the amplification products (data not shown).

Results

Real-time PCR results for the *CHRM3* gene were positive for all 8 samples. Cycle threshold (Ct) values ranged from 30-36 cycles. A representative result is shown in Figure 1. Confirmatory gel electrophoresis showed amplification of the expected 74-bp fragment for all samples. The presence of the FTA card punch in the PCR tube made it impractical to determine PCR efficiency. DNA extraction time was 1h 35 min, and real-time PCR run time was 63 min for 45 cycles.

Component	Final amount
10X PCR Reaction Buffer (no $MgCl_2$) (Invitrogen)	1 X
$MgCl_2$ (Invitrogen)	5.75 mM
dNTP mix (Invitrogen)	0.2 mM
Platinum TAQ Polymerase (Invitrogen)	1 U
TaqMan probe (Biosearch)	0.1 µM
PCR primers (Biosearch)	0.5 µM each
Template DNA	1 sample disc*
Sterile water	
Total reaction volume	**25 µl**

* 1.2 mm sample disc removed from FTA card using a Harris Uni-Core Punch.

Table 1. Components of PCR amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	150 s	1
Denaturation	95°C	30 s	50
Annealing/extension	58°C	55 s	50

Table 2. Cycling parameters.

Discussion and Conclusions

The results show that DNA extracted from whole blood using Whatman FTA cards is suitable for real-time PCR with the Spartan DX. FTA cards are a simple and non-invasive method for non-technical personnel to collect DNA samples. The Spartan DX is designed for on-demand, non-batched DNA testing. By combining FTA cards with the Spartan DX, DNA testing is faster and more convenient than with batched instruments and conventional blood sample tubes.

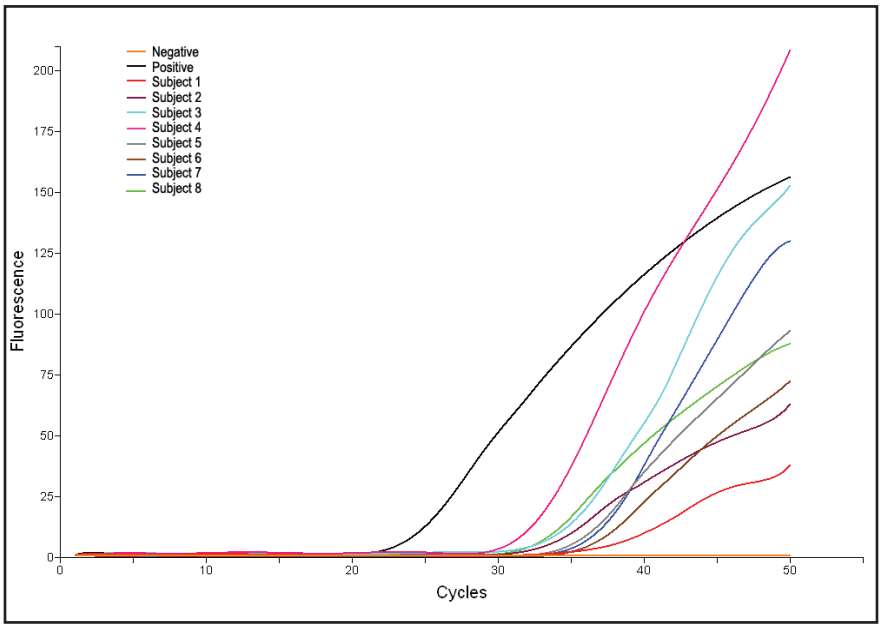

Figure 1. Real-time PCR results for 8 individuals.

References

1. Whatman. *FTA® Nucleic Acid Collection, Storage and Purification.* Online FTA for DNA Archiving - Blood Buccal Longevity Study. Lit. #51653.

2. Prevost SL, Arbour NA, Sowers B, Harder CJ. (2007). Fluorophores compatible with the Spartan DX™. *Spartan Bioscience.* AN 021 Ver 1.1 September 07.