TrimGen GENETIC DIAGNOSTICS

DNA extraction with the BuccalQuick™ kit

D Roy[1], E Vaillancourt[1], H Doong[2], CJ Harder[1]
[1]Spartan Bioscience Inc.
[2]TrimGen Corporation

BuccalQuick™ is designed to efficiently extract genomic DNA from a buccal swab or brush. The extraction is performed in a single tube and the whole process is completed in under 10 minutes. No centrifugation or further purification steps are required. DNA from a single swab is sufficient for 30-50 PCR reactions.

Introduction

Whole blood is the most common source of human genomic DNA for genetic testing. The disadvantages of using blood include invasive collection, need for a trained phlebotomist, special storage, and time-consuming DNA extraction (Ref 1). In contrast, DNA from saliva or buccal cells gives the same results as DNA from blood and may be collected non-invasively from the inside of the cheek by non-technical personnel (Ref 2, 3).

The BuccalQuick DNA Extraction Kit (TrimGen, Cat. No. BQ-50) includes, sterile Easy-Swab™ sample collection swabs (Cat. No. SW-100). Swab-Pack™ (Cat. No. SP-50) swab holders with foam slits are available for secure storage and transport from the site of collection to the site of analysis. Dry swabs are stable at room temperature for up to 3 months (Ref 4).

The BuccalQuick Kit comes with BQ-Solution and BQ-Mix that enable PCR-ready DNA to be extracted from the swabs in 5 min without heat treatment.

The purpose of this study was to determine the performance of DNA from the BuccalQuick kit with the Spartan DX desktop DNA analyzer.

Materials and Methods

DNA collection and extraction

Buccal cells were collected from the cheeks of 10 individuals using Easy-Swab sample collection swabs. Prior to sample collection, subjects were asked to rinse their mouths with water. After collection, the swabs were immersed in BQ-Solution and DNA was extracted as per the manufacturer's instructions. In brief, the buccal samples were incubated at room temperature for 5 min in BQ-Solution, and then 50 µl of BQ-Mix were added. The final volume was 350 ml. Samples were stored at -20°C.

Real-time PCR

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

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 (Table 2). 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, and real-time PCR was performed using the Spartan DX instrument.

DNA analysis

Fluorescence data from the Spartan DX were downloaded to a computer and graphed using Spartan Analyzer software.

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	2 µl
Sterile water	
Total reaction volume	**20 µl**

Table 1. Components of PCR amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	150 s	1
Denaturation	95°C	30 s	60
Annealing/extension	58°C	55 s	60

Table 2. Cycling parameters.

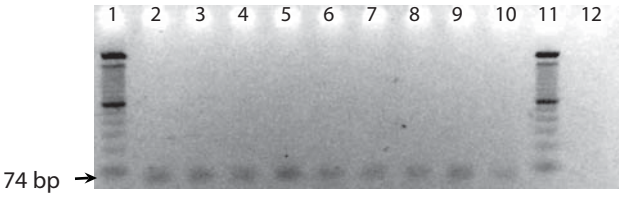

Figure 2. Gel electrophoresis results. Shown is 100 bp ladder (Lane 1 & 11) and PCR amplification results (Lanes 2-10).

In addition, real-time PCR results were confirmed by agarose gel electrophoresis using 10 µl of the amplification products (Figure 2).

Results

Real-time results for the *CHRM3* gene were positive for all 10 samples. The cycle threshold values ranged from 21-27 cycles. Results are shown in Figure 1. Confirmatory gel electrophoresis showed amplification of the expected 74-bp fragment for all samples. Total time to collect and purify one buccal swab was 5 min. Total run time on the Spartan DX was 88 min.

Discussion and Conclusions

The results show that DNA collected and extracted with the BuccalQuick kit is suitable for real-time PCR with the Spartan DX. DNA collection and extraction took 5 min, and real-time PCR took 88 min. Therefore, it is possible to go from sample-to-result, in less than 2 hours, using a patient-friendly, non-invasive method of DNA collection.

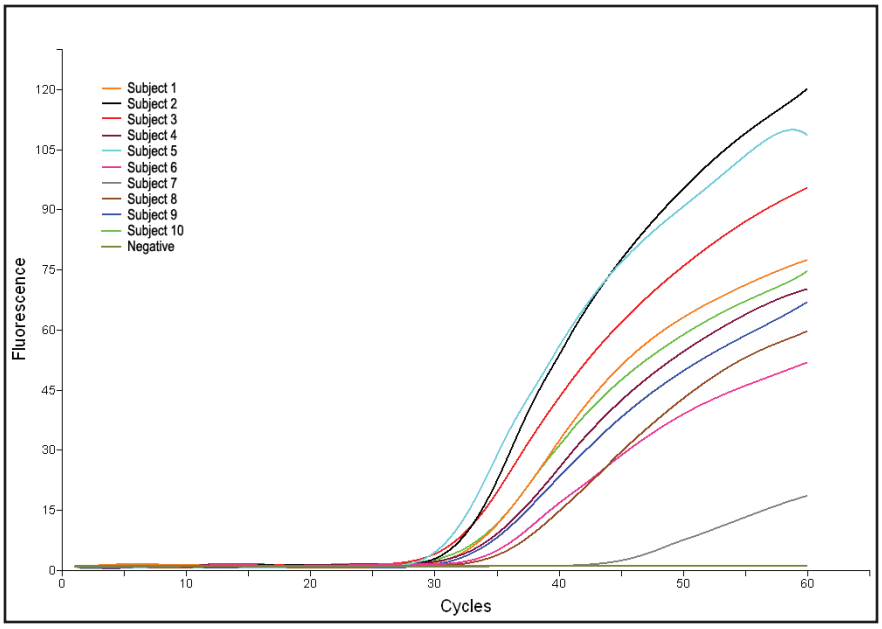

Figure 1. Real-time PCR results for 10 individuals.

References

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