Direct testing of human buccal cells

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The EPICENTRE BuccalAmp™ DNA Extraction Kit enables human genomic DNA to be collected and extracted in 5 min. The Spartan DX™ personal DNA analyzer provides real-time PCR results with the human DNA in 49 min. These two technologies combined enable human genetic testing in less than 1 hour.

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 give 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 BuccalAmp DNA Extraction Kit (EPICENTRE, Cat. # MB71030) includes individual, sterile Catch-All™ Sample Collection Swabs (Cat. # MB030BR). The swabs are provided in positive-seal, hard plastic tubes for secure storage and transport from the site of collection to the site of analysis. Dry brush samples are stable for up to 1 week at temperatures up to 37°C (Ref 4).

The BuccalAmp Kit comes with QuickExtract™ DNA Extraction Solution (Cat. # MB7901S) that enables PCR-ready DNA to be extracted from the swabs with only 3 min of heat treatment. Yields range from 0.5-7 µg of DNA from each buccal sample.

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

Materials and Methods

DNA collection and extraction

Buccal cells were collected from the cheeks of 10 individuals using Catch-All swabs. Prior to sample collection, the subjects were asked to rinse their mouths with water. After collection, the swabs were immersed in QuickExtract DNA Extraction Solution and DNA was extracted as per the manufacturer's instructions with a final volume of 500 µl. In brief, the buccal samples were heated at 65°C for 1 min and then 98°C for 2 min. Samples were stored at -20°C.

Real-time PCR

Oligonucleotide primers were designed against a conserved region of the human *dopamine-D2 receptor* (*hDRD2*) gene (Ref 5). The forward primer sequence was 5'-tga act tgg cca cgt tac atg-3', and the reverse primer was 5'-cta ctg tgg gca ttg cac ttt at-3'. The expected amplicon size was 124 bp.

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 (Fisher Scientific, Cat. No. 08-408-214). 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 Microsoft Excel®. In addition, real-time PCR results were confirmed by agarose gel electrophoresis using 10 µl of the amplification products (Figure 2).

Component	Final amount
2X PCR TAQurate™ GREEN Real-Time PCR Master Mix (EPICENTRE)	1 X
PCR primers (IDT)	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	94°C	135 s	1
Denaturation	94°C	45 s	35
Annealing/extension	53°C	45 s	35

Table 2. Cycling parameters.

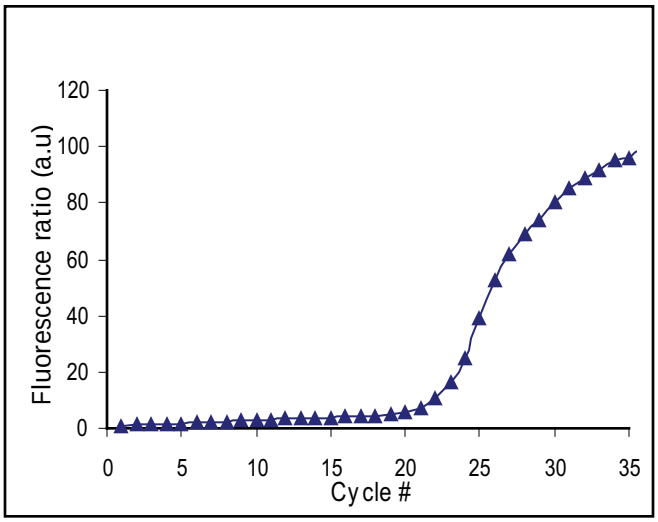

Figure 1. Real-time PCR result.

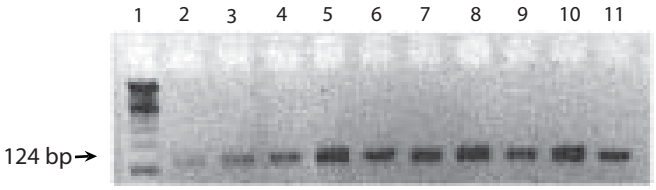

Figure 2. Gel electrophoresis results. Shown is 100 bp ladder (Lane 1) , PCR amplification results (Lanes 2-11).

Results

Real-time results for the *hDRD2* gene were positive for all ten samples. The cycle threshold values ranged from 21-27 cycles. A representative result is shown in Figure 1. Confirmatory gel electrophoresis showed amplification of the expected 124 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 49 min.

Discussion and Conclusions

The results show that DNA collected and extracted with the BuccalAmp kit is suitable for real-time PCR with the Spartan DX. The time for DNA collection and extraction was 5 min and the time for real-time PCR was 49 min. Therefore, it is possible to go from sample-to-result using a patient-friendly, non-invasive method of DNA collection, in less than an hour.

References

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