Isolation of DNA from Hair Follicles and Hair Shafts using Norgen's Dried Blood Spot Genomic DNA Isolation Kit

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INTRODUCTION

Hair can be used for a variety of procedures—from DNA isolation and identification to genotyping and forensics. There are two parts to hair from which DNA can be extracted and isolated: the hair follicle and the hair shaft. Isolation can be carried out from the hair follicle to isolate both cellular and mitochondrial DNA (mtDNA)[1]. The hair shaft, however, usually contains mtDNA and may contain very little nuclear material[1]. Shed hairs which are commonly found at crime scenes are of the latter type[1,2]. The hair shaft of shed hair undergoes keratinization, hardening and causing degradation of the nucleus[1]. On the other hand, mtDNA remains sufficiently intact to undergo isolation and downstream application[1]. Higher DNA yields can be obtained from unwashed hair due to the presence of surrounding cells attached to the hair shafts and can be a source of genomic DNA.

The purpose of this study is to isolate genomic DNA from hair follicles and hair shafts using Norgen's Dried Blood Spot Genomic DNA Isolation Kit (Cat# 36000).

MATERIALS AND METHODS

Sample collection

Hair samples were collected from 3 different donors, ensuring that each hair that was extracted contained a follicle and sufficient length for the shaft samples.

DNA extraction

DNA was extracted from the hair follicle and hair shaft samples using Norgen's Dried Blood Spot Genomic DNA Isolation Kit as per the manufacturer's instruction, with slight modifications. Briefly, a hair follicle or 3 pieces of hair shaft approximately 1 cm in length were added to a 3x3mm diameter tube. Next, 100 µL of Digestion Buffer was added to the tube and vortexed for 10 seconds. The tubes were then incubated at 85ºC for 10 minutes and given a quick spin in a centrifuge. Proteinase K was then added to the microcentrifuge tube. Next, 300 µL of Lysis Solution was added and the samples were vortexed and incubated at 55ºC for 10 minutes. Next, 250 µL ethanol was added to each sample, and samples were bound, washed and eluted as per the manufacturer's protocol.

Real-Time PCR

The purified DNA was then used as the template in a real-time TaqMan® PCR reaction. Briefly, 5 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007), 0.4 µL of a 25µM GAPDH primer pair mix and 0.2 µL of the TaqMan® probe. The volume was brought up to 20 µL using nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 40 cycles of 95°C for 15 seconds for denaturation, 60°C for annealing and extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

RESULTS AND DISCUSSION

Hair follicles and hair shafts are important and common sources of biological evidence in forensic applications and identification purposes; however their use in these applications depends on the successful isolation of DNA from these sources. Since the amount of DNA present in hair follicles and hair shafts is very limited, PCR is the only viable detection method for such isolations. Here, DNA was isolated from both hair follicles and hair shafts from three different donors using Norgen's Dried Blood Spot Genomic DNA Isolation Kit.

DNA quality was determined through the use of a TaqMan® Real-Time PCR method. Five µL of each DNA isolation was used in the reaction. The Ct values were then graphed **(Figure 1)**. The average Ct values for DNA isolated from both the hair follicle and the hair shaft are illustrated in Figure 2. Successful amplification was obtained from all

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samples, with lower Ct values obtained from the hair follicle when compared to the hair shaft. Since hair follicle contains more cells than the hair shaft, this explains the higher recovery and therefore lower Ct from DNA isolated from the follicle.

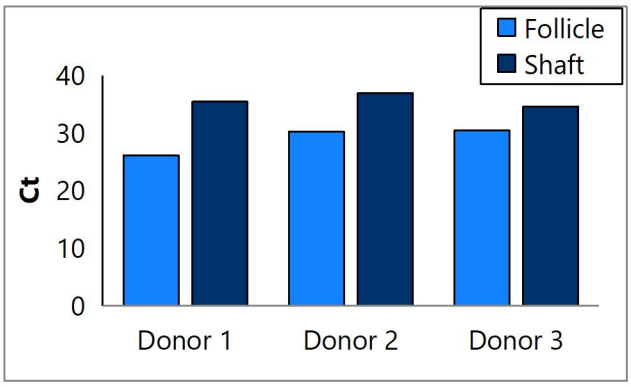

Figure 1. The difference in Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated using Norgen's Dried Blood Spot Genomic DNA Isolation Kit from hair follicles and hair shafts collected from 3 donors. Five microliters of each elution were used in a 20 µL qPCR reaction involving GAPDH primers.

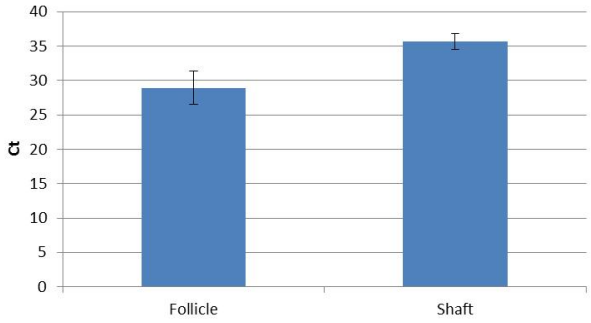

Figure 2. The average Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated using Norgen's Dried Blood Spot Genomic DNA Isolation Kit from hair follicles and hair shafts collected from 3 donors. Five microliters of each elution was used in a 20 µL qPCR reaction involving GAPDH primers.

CONCLUSIONS

From the data presented in this report, the following can be concluded:

- Norgen's Dried Blood Spot Genomic DNA Isolation Kit was able to isolate DNA from both the hair follicle and hair shaft, with a higher DNA yield obtained from the hair follicle.

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