USE OF BUCCAL EPITHELIAL CELLS FOR PCR AMPLIFICATION OF LARGE DNA FRAGMENTS

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ABSTRACT

The analysis of human DNA is widely employed in the genetic studies of families and populations, and in most cases is performed with samples obtained from peripheral blood. The use of buccal epithelial cells as a source of DNA for PCR amplifications has several advantages over blood sampling but has only been used to amplify small fragments of DNA. Its use in forensic analysis has been limited to cases where the sampling of peripheral blood is not feasible. In the present study we show that buccal epithelial cells are a reliable source of DNA for the PCR amplification of high molecular mass fragments, which could be used in large-scale population sampling. Since most PCR gender-typing systems rely on the amplification and electrophoretic separation of the amelogenin gene, our results show that buccal epithelial cells may be the preferred source of DNA for gender -typing analysis. **(J Forensic Odontostomatol 2000; 18:6-9)**

Keywords: PCR, buccal epithelium, amelogenin.

INTRODUCTION

The analysis of human DNA is widely employed in genetic studies of families and populations and in most cases is performed with samples obtained from peripheral blood. Blood sampling is however an invasive procedure and sample collection may involve ethical problems in cases such as extreme illness, elderly persons and babies. Additionally, it requires medical supervision and specific equipment, which contribute to the increased overall costs of the procedure.

The polymerase chain reaction (PCR) provides a rapid and sensitive approach for the analysis of polymorphisms and mutations of the human genome. The use of buccal epithelial cells as a source of DNA for PCR reactions has been limited to studies of infectious agents present in oral mucosa;[1,2] and in forensic analysis, where the sampling of peripheral blood is not feasible.[3,4] Invariably, these studies report the amplification of small fragments of DNA (< 300 base pairs), which are suitable for the identification of viral and bacterial sequences, as well as the analysis of highly polymorphic loci in the human genome. However, amplification of larger DNA fragments may be desirable in some instances such as PCR-RLFP,[5] mismatch cleavage mutation analysis,[6,7] cloning, and sequencing of amplified sequences.[8] In the present study, we show that buccal epithelial cells are a reliable source of DNA for the PCR amplification of high molecular mass fragments, which could be used in large-scale population sampling and epidemiological studies, as well as in forensic analysis. As will be seen our results show that buccal epithelial cells may be the preferred source of DNA for gender-typing analysis.

MATERIALS AND METHODS

Sampling

A group of 83 consenting female subjects undertook a mouthwash containing 5 mL of 3% sucrose rather than water in order to prevent osmotic imbalance that would cause rupture of cells and loss of genomic DNA, for 2 min. A sterile wood spatula was then

used to scrape the buccal oral mucosa and the tip of the spatula shaken into the retained mouthwash solution. Buccal epithelial cells were pelleted by centrifugation at 2000 rpm for 10 min, the supernatant was discarded and the cell pellet resuspended in 500 uL of extraction buffer [10 mM Tris-HCl (pH 7.8), 5 mM EDTA, 0.5 % SDS] with antibiotics. The samples were then frozen at –20°C until used for DNA extraction.

DNA extraction

After defrosting, the samples were incubated at 37°C with 100 ng/mL proteinase K*, and agitated overnight. DNA was then purified by sequential phenol/chloroform extraction and salt/ethanol precipitation and dissolved in 70 µL TE buffer [10 mM Tris (pH 7.8), 1 mM EDTA]. The concentration was estimated by measurements of OD 260.

Polymerase chain reaction

Amplification reactions were performed with 300 to 700 ng DNA in a volume of 50 µL in reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM $MgCl_2$, deoxyribonucleotides (200 µM each), 1 µM primers, 2U *Taq* DNA polymerase** and five pairs of primers were used. The primer sequences are as follows:

AMX1- 5' GGATTGGTTGTTACAGATGCC 3'
AMX2- 5' TTACTCACAGGCATGGCAAAAGCTGC 3'
AMX3- 5' CATTTCAGAACCATCAAGAAATGGG 3'
AMX4- 5' CTTTACAGAGCCCAGGGCATTG 3'
AMX5- 5' CCTCCCTGTAAAAGCTACCACC 3'
AMX6- 5' AATGTCTACATACCGGTGGCC 3'
AMX7- 5' GTAGAACTCACATTCTCAGGC 3'
AMX8- 5' GGCTTCAAAATATACTCACCACTTCC 3'
AMX9- 5' CCAGCCCCAGCCTGTTCAGCCAC 3'
AMX10- 5'TGTCTGCTAATGGTACTTTTTTAG 3'

Samples were heated initially to 95°C for 5 min, each cycle comprising denaturation at 95°C for 50 sec, primer annealing at 67°C for 1 min and polymerization at 72°C for 2 min. Samples were subjected to 35 cycles of amplification followed by a final extension of 72°C for 7 min. Amplification was carried out in a *Perkin-Elmer GeneAmp 2400 thermal cycler*#. Amplification products were visualized by electrophoresis on vertical 5% polyacrylamide gels in 1 X TBE (89 mM Tris-Borate, 89 mM boric acid, 2 mM EDTA), followed by silver staining¶.

RESULTS AND DISCUSSION

The use of buccal epithelial cells as a source of DNA for PCR amplifications has several advantages over blood sampling. The collection of material is fast and inexpensive, buccal samples can easily be obtained from people who are reluctant to donate blood, consent becomes simplified, there is no need of medical supervision during sampling, and the risk of contamination is reduced.[9,10]

The amplifications produced specific reaction products of 1690 base pairs [bp] (AMX1-AMX2), 1986 bp (AMX3-AMX4), 1550 bp (AMX5-AMX6), 879 bp (AMX7-AMX8), and 2039 bp (AMX9-AMX10), spanning the whole amelogenin X gene.

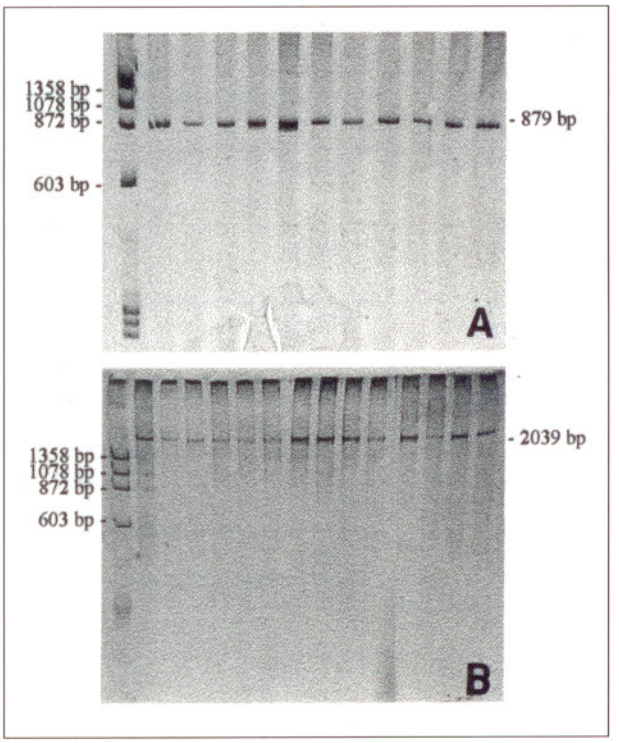

Figure 1. *Polyacrylamide gel electrophoresis showing PCR products from total genomic DNA derived from buccal epithelium. A: 879 bp product of amplification with primers AMX7 and AMX8. B: 2039 bp product of amplification with primers AMX9 and AMX10. Lane 1- ϕX-174-RF DNA Hae III digest molecular weight marker (Pharmacia).*

* Sigma Chemical Co., St. Louis, MO, USA
** Amersham Pharmacia Biotech, Uppsala, Sweden
# Perkin-Elmer, Perkin-Elmer Co., Norwalk CT 06859, USA
¶ Bio-Rad Silver Stain Kit, Bio-Rad Laboratories, 200 Alfred Nobel Dr, Hercules CA 94547, USA

Fig. 1 illustrates typical PCR products from total genomic DNA derived from buccal epithelial cells showing that buccal epithelial cells can be used for the PCR amplification of large DNA fragments. The amount of DNA extracted ranged from 2.1 µg to 360 µg, which is sufficient to enable PCR amplification, with a success rate of identification of the gender gene, in this case the female, of around 90% of large fragments ranging from 879 to 2039 base pairs (Table 1). Failure to amplify DNA may result from degradation or from the presence of inhibitors which interfere with PCR reaction and can in most cases be overcome by adding bovine serum albumin to the PCR reaction,[11-13] or by repurification of DNA with Chelex extraction.[14] The use of small volumes of DNA extract is also recommended in order to reduce the amount of inhibitors.[3,14]

Primers	Fragment size (bp)	% positive
AMX1-AMX2	1690	91.5
AMX3-AMX4	1986	92.5
AMX5-AMX6	1550	88
AMX7-AMX8	879	88
AMX9-AMX10	2039	88

Table 1: *Positive amplification percentages of amelogenin X gene of 83 individuals.*

Gender determination can be a valuable piece of information in forensic investigations. Most PCR gender-typing systems rely on the amplification and electrophoretic separation of the amelogenin gene which produce small PCR products with 106-112 bp[15,16] or 330-218 bp[14] from the X and Y chromosomes respectively. However, the main difference between X and Y loci is a 177 bp insertion in the X gene[17] and the X and Y sequences can be amplified using a single set of primers which produce a 977 bp and 780 bp fragments,[18] which can be clearly distinguished in agarose or polyacrylamide gels. The results presented here show that buccal epithelial cells may be the preferred source of DNA for gender-typing analysis. Finally, it is worth mentioning that DNA extracted from this source has been routinely used in our laboratory for RFLP and direct sequencing of PCR products.

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