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RESEARCH ARTICLE

DNA EXTRACTION AND GENE AMPLIFICATION FROM SALIVA DEPOSITED ON A PLASTIC CUP USING DOUBLE SWAB TECHNIQUE

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ABSTRACT

Background and Objectives: The positive identification of living or deceased persons using the unique traits and characteristics of the teeth and jaws is a cornerstone of forensics. Establishment of offender's identity is essential in criminal investigations. Our study aims, to extract DNA from saliva deposited on a solid object (plastic cup), simulating saliva deposited on objects in real situations and to amplify STRs from salivary DNA for purpose of forensic individualization.

Methods: Direct buccal swab samples were collected from five unrelated, healthy volunteers with no genetic and blood disorders. The volunteers were asked to drink fruit juice from a plastic cup and it was allowed to dry for sixty minutes. With a swab immersed in sterile distilled water the surface of the plastic cup was wiped for 15 seconds using moderately strong pressure and circular motions followed by a dry swab. The DNA is extracted using PCR technique and the Quality and quantity analysis of the DNA thus obtained will be performed by gel electrophoresis and spectrophotometry respectively.

Results: Concentration of DNA isolated from direct buccal swab samples ranged from 8.5 mg/ml to 12.5 mg/ml with mean of 10.8 mg/ml, while those from five double swab samples ranged from 5.5 mg/ml to 10.5 mg/ml, with mean of 8.5 mg/ml. AMG and F13 could be amplified in all five (100%) of saliva samples deposited on the cup. All the amplified samples of double swab matched positively with corresponding direct buccal swab samples, giving 100% matching and overall success of 100%.

Conclusion: Saliva is a very useful DNA source due to the fact that it contains leukocytes and exfoliated epithelial cells from the host. PCR allows replication of thousands of copies of a specific DNA sequence in vitro, enabling the study of small amounts of DNA. Our results show high reliability of PCR technique in amplifying gene loci from salivary samples. Based on our findings we concluded that even though amplification of DNA from salivary samples is a technique sensitive procedure, requiring strict control of various factors, it has a potential to be utilized in forensics.

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INTRODUCTION

The positive identification of living or deceased persons using the unique traits and characteristics of the teeth and jaws is a cornerstone of forensics. Establishment of offender's identity is essential in criminal investigations. Offender's saliva can also be recovered and typed victims body and objects of crimes like bite marks, cigarette butts, postage stamps, envelopes and other objects. The Double swab technique is effective for recovering salivary DNA from skin. In this study, this technique is used to recover salivary DNA from a solid object (plastic cup). Forensic DNA profiling methods using the polymerase chain reaction (PCR) technique to amplify small amounts of recovered DNA at specific genetic loci are sensitive techniques. Studies have shown that it is possible to discriminate one individual from all others with a high level of confidence by starting with only 1ng (1billionth of a gram) or less of target DNA. In addition to identifying the perpetrators of crimes, these same methods have been used to identify deceased victims when virtually no physical evidence remains (Sweet, 2001; Anzai- Kanto *et al.,* 2005). Our study aims, to extract DNA from saliva deposited on a solid object (plastic cup), simulating saliva deposited on objects in real situations and to amplify STRs from salivary DNA for purpose of forensic individualization.

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MATERIALS AND METHODS

Direct buccal swab samples were collected from five unrelated, healthy volunteers with no genetic and blood

disorders. The volunteers were asked to drink fruit juice from a plastic cup and it was allowed to dry for sixty minutes. With a swab immersed in sterile distilled water the surface of the plastic cup was wiped for 15 seconds using moderately strong pressure and circular motions. The swabs were rotated along its long axis allowing each side of the swab to come into contact with the target surface. This was followed by a second, dry sterile cotton swab to absorb the water left behind on the surface by the initial swab and to collect additional salivary cells. The swabs were completely air dried and transferred to micro centrifuge tubes. 160 µL of lysis buffer was added followed by 30 µL proteinase K and it was incubated at 56°C for 2 hrs. It was then washed repeatedly with sterile water for recovering the DNA and the cotton swabs were removed from the wash solution. To this wash solution, 400 µL of phenol-chloroform was added and it was centrifuged at 12000 rpm for 10 minutes. The supernatant was transferred to a fresh micro centrifuge tube to which 1 ml of ethanol was added and centrifuge again 12000 rpm for 10 minutes. Supernatant solution was again decanted and the pellet was rinsed in 70% alcohol followed by drying the pellet at 56°C for 4 hours. Quality and quantity analysis of the DNA thus obtained will be performed by gel electrophoresis and spectrophotometry respectively. This resultant DNA solution was stored at -20°C until PCR analysis. The integrity of high molecular weight DNA is an important factor during extraction steps. Integrity was checked by electrophoresis on 0.8% Agarose for STRs.

Selection criteria of the STR markers

Selection of the STR markers was based on the global surveys carried out by Lezaun et al. 1997 and Destro- Bisol 2000 Which suggested that these STRs are one of the most polymorphic markers. Studies by Agarwal et al. on nine endogamous groups also suggested that these markers can be employed for individualization purposes based on their high heterozygosity estimates (Silva *et al.,* 2006; Sweet *et al.,* 1996; Coomey *et al.,* 1991).

Table 1. Primer sequences for STRS LOCI

S.No.	Locus	Primer Sequences
1	AMG	Amel A-5'CCCTGGGCTCTGTAAAGAATAGTG3' Amel B- 5' ATCAGAGCTTAAACTGGGAAGCTG3'
2	F13	5' GAGGTTGCACTCCAGCCTTT 3' 5' ATGCCATGCAGATTAGAAA 3'
3	D4S	5' TCAGTCTCTCTCTTTCTCCTTGCA 3' 5' TAGGAGCCTGTGGTCCTGTT 3'

PCR amplification
STR was genotyped by using single locus PCR

Amelogenin gene, is located on the X chromosome (Xp22.1 - p22.3) and Y chromosome (Yp11.2). It is employed for human identity testing, criminal and parentage testing cases. F13 is present on chromosome 6p24.2-p23 (F13A1) and has application in forensic analysis and paternity determination. D4S represents more than 6 percent of the total DNA in cells. Highly accurate comprehensive sequence for chromosomes 4 represents its efficiency for the Human Genome Project, in that it makes possible more detailed and conclusive analyses (Sweet *et al.,* 1997; Pang and Cheung, 2007; Perez-lezaun *et al.,* 1997).

RESULTS AND DISCUSSION

Concentration of DNA isolated from direct buccal swab samples ranged from 8.5 mg/ml to 12.5 mg/ml with mean of 10.8 mg/ml, while those from five double swab samples ranged from 5.5 mg/ml to 10.5 mg/ml, with mean of 8.5 mg/ml. Quality of DNA obtained was assessed by calculating the ratio of optical density readings at 260nm and 280nm. OD260/OD280 ratio of DNA samples from direct buccal swab ranged from 1.3 to 1.6 with a mean of 1.46. In double swab OD260/OD280 ratio ranged from 1.2 to 1.5 with mean of 1.36. Quality of DNA extracted through double swab in all 5 samples was below optimal range.

Table 2. OD260/OD280 ratio of the DNA isolates from the Double swab on the cup and Direct Buccal swab

S.no	Sample no.	Double swab nucliec acid protein ratio	Buccal swab nucliec acid protien ratio
1	1	1.4	1.6
2	2	1.5	1.5
3	3	1.3	1.4
4	4	1.2	1.3
5	5	1.4	1.5

Results of the DNA typing of the three STRs (AMG, F13, and DS4) that were used for typing the DNA amplified from the salivary samples deposited on the cup using PCR.

AMG and F13 could be amplified in all five (100%) of saliva samples deposited on the cup. All the amplified samples of double swab matched positively with corresponding direct buccal swab samples, giving 100% matching and overall success of 100%. D4S could be amplified in three out of five (60%) of saliva samples deposited on the cup. In two samples where D4S could not be amplified the corresponding direct buccal swab saliva sample was also not able to amplify the gene. All the amplified samples of double swab matched positively with corresponding direct buccal swab saliva samples, giving 100% matching and overall success of 60%.

Table 3 PCR amplification results of the various STRs and the VNTR for salivary samples deposited on Plastic cup and collected by double swab method

S.No		AMG		F13		D4S	
		A1	A2	A1	A2	A1	A2
1	BS	785	953	204	204	181	181
	DS	785	953	204	204	181	181
2	BS	785	953	204	204	NA	NA
	DS	785	953	204	204	NA	NA
3	BS	785	953	204	204	181	181
	DS	785	953	204	204	181	181
4	BS	785	953	204	204	181	181
	DS	785	953	204	204	181	181
5	BS	785	953	204	204	NA	NA
	DS	785	953	204	204	NA	NA

A1 – Allele 1; A2 – Allele 2; BS – Buccal swab; DS – Double swab; NA – Not Amplified

Conclusion

Saliva is a very useful DNA source due to the fact that it contains leukocytes and exfoliated epithelial cells from the host. PCR allows replication of thousands of copies of a specific DNA sequence in vitro, enabling the study of small amounts of DNA. The double swab technique of collection of

deposited salivary samples may afford an advantage over the traditional techniques in this aspect as using a wet swab in the first step loosens the salivary epithelial cells which are then collected by gentle swabbing in the second step. This ensures collection of maximum cells with minimum physical pressure (Destro-Bisol *et al.,* 2000a; Agrawal and Khan, 2005). Though the amplification of STRs from salivary samples was inconsistent, the amplified DNA samples, both from directly collected as well as deposited saliva showed excellent DNA matching. Our results show high reliability of PCR technique in amplifying gene loci from salivary samples. Even though our samples showed high positive matching, the reliability of salivary DNA is not absolute. Based on our findings we concluded that even though amplification of DNA from salivary samples is a technique sensitive procedure, requiring strict control of various factors, it has a potential to be utilized in forensics. However, the technique has to standardize by further studies on a larger and more varied sample size so as to produce predictable results.

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