Extraction of the DNA and profiling from Chewed Gutkha

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Abstract— Extraction of *DNA* and its profiling is used in forensic laboratories for establishing origin of biological fluids found at the crime scene. A forensic analyst routinely encounters a variety of challenging biological and non-biological samples, many of which contain DNA which has been exposed to environmental insults.

In one murder case we received chewed *GUTKHA* sample. Hence isolation of DNA from chewed *GUTKHA* was carried out by using different extraction methods, with varying time of exposure of the samples. After the standardization of extraction method from *GUTKHA*, we applied the method for the case in which we had received Gutkha as a vital piece of evidence found at the crime scene by the team of forensic experts. DNA was quantified by Real Time PCR (RT-PCR) using Quantifiler ™ Kit. Amplifiable DNA was used for further processing i.e. PCR amplification and genotyping. DNA from eight accused samples was extracted using Himedia Blood Genomic DNA Extraction Kit. Pattern generated by the *GUTKHA* sample was then compared with the reference samples.

In the present study it was observed that as compared to DNA from saliva samples, the DNA from *GUTKHA* is highly stable, if preserved under cool, dry conditions. Saliva degrades at the lapse of time and thus the quality and quantity of DNA obtained gets affected.

Collection and preservation of various biological materials from crime scene thus plays an important role in the isolation of DNA from forensic samples for establishing the link between crime and the offender.

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Index Terms— **GUTKHA, forensic identification, DNA quantitation, Polymerase chain reaction and DNA genotyping.**

I. Introduction

The discovery of variable number of tandem repeats (VNTRs) in 1985's permitted investigators to perform almost unbelievable feats of identification. It is now possible to extract DNA from virtually all biological specimens which have greatly expanded the potential for individual identification.

Various types of crime cases are referred to forensic laboratory. In some of these cases, there could be a lapse of time between the crime committed and the detection of crime and collection of evidence and thus delay in submission of the samples for DNA analysis. During this period the important biological evidence gets exposed to adverse physical and environmental conditions. In one such case we received chewed *GUTKHA* samples as an important evidential sample. Therefore, the present work was taken up to study the effect of storage time on the quality and quantity of DNA from *GUTKHA.* After standardization, it was applied to the case work.

II. Procedure for Paper Submission

A. Review Stage

Subm **Materials and Methods**

Twenty GUTKHA samples were collected from the volunteers by taking their consent to participate in the study. All samples used in these studies were prepared in the laboratory. Organic extraction method, Inorganic extraction method, Hi-media kit and Magnetic bead extraction methods were used for extraction of DNA from chewed GUTKHA.

Extracted DNA was quantified on Agarose gel as well as on real time PCR.

The samples were exposed to different time and temperatures. Table 1 shows exposure chart to which each sample collected for studies was exposed.

Table 1: Time and temperatures exposure chart for samples in the study

SAMPLE	EXPOSURE	STORAGE
GUTKHA sample	Room temperature	⇒ Fresh ⇒ 3 days ⇒ 8 days
GUTKHA mixed ➢ with earth ➢ on painted wall	Room temperature Dried over night	8 days
Cheek cells	Room temperature	Fresh

Chewed Gutkha which the offender could have spitted on the sun-mica top of the table where the deceased was murdered was referred to our laboratory for analysis along with other exhibits. DNA was extracted from the blood samples of seven suspected offender's by using Himedia Blood Genomic DNA Extraction Kit. DNA from Gutkha was extracted using magnetic bead extraction method. DNA was quantified using Quantifiler™ Human Identification Kit. 1ng DNA was used for PCR processing. The AmpFlSTR® Identifiler and Yfiler Kit were used.

Results and Discussions

Assessment of the quality and quantity of DNA extracted is a crucial step in implementing molecular techniques for identification of an individual.

DNA from K562 of different concentration, cheek cells (fresh) DNA by inorganic extraction method and fresh Gutkha DNA by organic extraction method on 1% agarose gel. A high molecular weight DNA migrates very slowly and appears as a single band near the origin of the gel. The approximate concentration of DNA in the sample was calculated by visually comparing the intensity of the band obtained by the sample with that of standard DNA. The efficient recovery of high molecular weight DNA is essential for a successful DNA typing in forensic science specimens.

There was no in change in the concentration of DNA for gutkha samples after 3-days of storage period when compared with that of fresh cheek cells and gutkha. These results indicated that DNA is stable in saliva (gutkha) after three days of storage at room temperature.

It was also observed that there was decrease in the DNA concentration with the increase in storage time. At higher temperatures DNA is unstable. Therefore both time and temperatures have adverse effects on the yield and quality of DNA.

The DNA extracted from blood samples of 7 Accused and from Reddish mass (GUTKHA) found on the crime scene were typed at 15 STR loci, gender specific Amelogenin locus and male specific 16 Y-STR loci using PCR amplification technique. The results of DNA typing for 15 STR loci and male specific Y-STR loci for each of the above exhibits are summarized in Table 2 and 3 respectively.

Table: 2 Results of DNA typing for each of above individuals are summarized below

Locus Name	Accused 1	Accused 2	Accused 3	Accused 4	Accused 5	Accused 6	Accused 7	GUTKHA	Deceased's banian
D8S1179	14,15	10,13	13,14	14,16	12,15	11,13	10,11	11,13	12,17
D21S11	27,32.2	29,30	30,33.2	31.2,33.2	30.2,32.2	29,30	28,29	29,30	28,28
D7S820	8,11	11,12	11,12	10,11	10,10	8,8	8,12	8,8	10,10
CSF1PO	12,12	8,11	10,12	10,12	11,12	10,11	12,12	10,11	11,12
D3S1358	17,18	14,18	18,18	15,16	16,18	16,17	15,18	16,17	14,17
TH01	7,9	7,9	6,9	6,9	6,7	8,8	6,6	8,8	5.3,6
D13S317	12,12	10,11	11,12	11,12	8,12	8,12	7,8	8,12	12,12
D16S539	10,11	11,13	11,12	10,12	12,13	11,13	9,11	11,13	11,11
D2S1338	21,23	18,24	17,20	20,23	22,23	20,20	18,23	20,20	21,23
D19S433	10.2,15.2	15.2,16.2	14,14	13,15	13,15	13,14	14,14	13,14	13,13
vWA	16,16	17,17	16,18	16,18	18,19	17,17	17,17	17,17	16,17
TPOX	8,11	8,9	8,11	8,8	8,9	6,9	8,10	6,9	8,8
D18S51	15,18	12,13	13,14	17,20	12,14	16,16	14,16	16,16	-
Amelogenin	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	11,12	10,12	11,12	12,12	11,12	11,12	12,12	11,12	13,13
FGA	23,25	21,23	20,24	22,23	19,23	20,25	22,23	20,25	23,24

For all the 15 different STR loci analyzed with PCR, accused 1, 2, 3, 4, 5 and 7 failed to match with all the obligate alleles present in the crime scene exhibit GUTKHA. For all the 15

different STR loci analyzed with PCR, the DNA profile obtained from Deceased's banian failed to match with all the obligate alleles present in the crime scene exhibit GUTKHA.

For all the 15 different STR loci analyzed with PCR, accused 6 matched all the obligate alleles present in the crime scene exhibit GUTKHA.

Table 3: Results of DNA Y-STR typing are summarized below:

Locus name	Accused 1	Accused 2	Accused 3	Accused 4	Accused 5	Accused 6	Accused 7	GUTKHA	Deceased's banian
DYS456	15	15	16	14	17	17	15	17	15
DYS3891	13	14	14	13	14	15	13	15	13
DYS390	22	23	22	24	22	24	24	24	25
DYS3891l1	30	29	28	31	31	32	30	-	31
DYS458	17	15	16	15	17	15	16	15	16
DYS19	15	14	17	17	15	16	15	16	16
DYS385	15,16	18,19	15,16	11,13	14,14	11,14	15,15	11,14	11,13
DYS393	12	14	12	13	12	14	13	14	13
DYS391	10	10	10	10	11	10	11	10	11
DYS439	11	12	11	10	12	10	13	10	10
DYS635	20	25	20	23	21	23	23	23	23
DYS392	11	10	11	9	11	11	11	11	11
GATA_H4	12	13	11	12	12	12	11	12	13
DYS437	14	14	14	14	14	15	15	15	14
DYS438	9	11	9	11	10	11	10	11	11
DYS448	18	19	19	20	19	20	20	20	20

Male haplotypes obtained from Deceased's banian, accused 1, 2, 3, 4, 5 and 7 failed to match with the male haplotypes present in the crime scene exhibit GUTKHA. Male haplotypes of accused 6 matched with the male haplotypes present in the crime scene exhibit GUTKHA.

Conclusions

This study demonstrated that

1. A high molecular weight DNA can be obtained from Gutkha by any extraction method. Hence all the methods of extracting DNA are of great use to forensic laboratories.

2. Under dry and cool conditions, DNA is a relatively stable molecule; therefore storage of samples is a crucial step in preservation of forensic samples.

3. Collection of specimen from the crime scene is an integral part of DNA profiling. A good understanding about the analysis principles and techniques of DNA profiling is essential to avoid DNA degradation and poor handling of crucial and important criminal evidence.

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REFERENCES

[1] Bhasin MK and Chahal SMS, A Laboratory manual for human blood analysis. 1st edition. Kamla-Raj Enterprises, Delhi (1996).
[2] Butler JM, Forensic DNA Typing, 2nd edition, Elsevier, Academic Press (2002).
[3] Culliford BJ, The examination and typing of blood stains in the crime laboratory. Published by "U.S. Department of Justice Law Enforcement Assistance Administration". Washington D.C (1971).
[4] Gill P, Jeffrey AJ, et al., Forensic application of DNA fingerprints. "Nature". 318. 577-579 (1985).
[5] Jobling MA and GilL P, Encoded Evidence: DNA in forensic Analysis. "Nature reviews/Genetics". 5. 739-751 (2004).
[6] Maniatis T, et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. (1982)
[7] Nederhand RJ, Logistics and quality control for DNA sampling in large multicenter studies. "Journal of Thrombosis and Homeostasis". 1. 987-991 (2003).
[8] Stoney DA , Reporting of Highly Genetic Typing Results: A practical Approach. "Journal of Forensic Sciences".37(2). 373-386 (1992).

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