

# Methods of dna identification



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To isolate DNA from blood, saliva, buccal swab and betel quid by phenol-chloroform method and chelex method and compare the efficacy of both the methods. To carry out restriction digestion of the DNA samples isolated from above mentioned sources using the restriction enzyme EcoRI (G| AATTC) and identify individuals based on the pattern of restriction banding and to ascertain the applicability of the restriction digestion in forensics

**MATERIALS AND METHODS:** Blood, saliva, betel quid and buccal swab were collected from 15 patients and DNA isolation was done by phenol-chloroform method and chelexmethod. DNA fingerprinting was carried out using EcoRI restriction enzyme.

**RESULTS:** DNA could be extracted from residues of saliva, DNA fingerprinting done with the isolated DNA was able to match with those of individuals. Chelex method was found to be more efficient than the Phenol-chloroform method

**KEY WORDS:** Betel Quid, Chelex method, DNA, DNA fingerprinting, Phenol chloroform method

## Introduction

DNA fingerprinting has ascertained an increasingly imperative role towards decision making in judiciary. DNA tests have helped convict suspects, to exonerate suspects or overturned previous convictions. Scientific evidences such as fingerprints, blood, semen, shreds of clothing, hair, weapons, tire tracks, and other physical evidence at the crime scene can be a more riveting to a tribunal than the testimony of an eyewitness. DNA is more

suitable because DNA remains stable in challenging environments where such evidence is found. The DNA molecule holds an impressive dependability to withstand time. <sup>1</sup>

DNA profiling compares the DNA fragment lengths and patterns. The isolated DNA from the samples is fragmented using a restriction enzyme. Then the length of the resulting fragments is determined by electrophoresis and compared by a visual interpretation of the pattern of DNA bands. <sup>2</sup>

DNA can be sourced from fresh blood, fresh or dried human buccal swabs, soft tissue, saliva and salivary stains. Optimizing the methodology in DNA extraction from various sources have been tried by many studies. Minute quantities of saliva allows establishing a DNA profile. <sup>3</sup> DNA has been proven to be isolated from cell samples from objects that was in contact with the body and from sources like chewing gums, cigarettes, bite marks in foods, among others.

Restriction fragment length polymorphism (RFLP) analysis provides details of the DNA which is referred as a DNA fingerprint. As DNA is unique to every individual, analyzing the sequence helps in identification of specific patterns of each individual. DNA profile is considered as valid evidence in the court of law for paternity disputes and human identification. Standardization of DNA extraction technique will improve the reliability and speed up sample processing time. <sup>4-6</sup>

Limited availability of biological samples in a crime scene challenges the procedure of extraction, characterization and analysis of DNA. Furthermore,

difficulty arises in retrieving DNA from stains and degraded samples which provide contaminated or poor quality DNA. Hence, purification of DNA from samples is still a significant step in obtaining useful genotypes.

Notwithstanding, tremendous advances have been made in the recent times in DNA testing. <sup>7</sup>

Chewed betel quid (BQ) stains are encountered frequently on crime scenes in Southeast Asian countries. Though the quid presents as an important biological evidence, the forensic analysis using betel quid as an evidence has been impeded due to difficulty in extraction of human DNA . Hence, constituting a definite method for extracting DNA from chewed Betel quid residues is of paramount importance. <sup>8</sup>

Saliva found on victims of several violent crimes is a potential source of DNA. They can be recovered from bite marks, cigarette butts, betel quid, postage stamps, envelopes and other objects. However , salivary stains usually dry up easily becoming invisible, making recognition and collection difficult.

Among the various biological sources available, salivary analysis have great discriminatory power and can be incorporated into a criminal investigation . Improvisation of DNA extraction procedures will improve its reliability and also help to expedite the process. The present study aims to isolate DNA from blood, saliva (under different conditions) by phenol chloroform method and chelex method and to find the efficacy of these methods in extraction of DNA from traces of saliva. <sup>9, 10</sup>

## ISOLATION OF DNA FROM BLOOD AND SALIVA BY PHENOL CHLOROFORM

## METHOD :

The DNA was extracted with an equal volume of phenol: chloroform: isoamyl alcohol. This mixture was centrifuged at 10000rpm for 5 minutes. The aqueous phase was collected and extracted with chloroform: isoamyl alcohol mixture and centrifuged at 10000rpm for 5 minutes. The supernatant was transferred to a new microfuge tube and 0.6 volume of isopropanol was added. The spongy white precipitate was transferred to a microfuge tube and added equal volume of ethanol was added. Then it was centrifuged at 10000rpm at room temperature for 10 minutes. The supernatant was drained and to the pellet 100 $\mu$ L of TE buffer was added stored at 4°C.

## ISOLATION OF DNA FROM BLOOD AND SALIVA BY CHELEX METHOD:

0.5 ml of whole blood was collected in 2 ml tube and the cells are harvested by centrifugation at 3000 rpm for 3 min. at 4°C. The supernatant was discarded. 0.8 ml TBP buffer was added to the collection tube, vortexed gently, then centrifuged at 3000 rpm for 3 minutes, supernatant was discarded. The next step was continued if the blood pellet looks mauve 0.5 ml of TBM buffer was added to the tube, and vortexed followed by addition of 3  $\mu$ L of proteinase K and incubated at 55°C for 30 minutes. The sample was centrifuged for 2 minutes at 5000 rpm and the supernatant saved to 2 ml tube and then added 260  $\mu$ L of absolute ethanol. The mixture was applied to EZ-10 column, centrifuged at 8000 rpm for 1 minute; discarded the flow in the collection tube. 500  $\mu$ L of wash solution was added and centrifuged at 8000 rpm for 1 minute. This step was repeated spin at 8000 rpm for an

additional minute to remove residual amount of wash solution. The column was placed into a clean 1.5 ml microfuge tube and 30  $\mu$ L of elution buffer was added into the center part of membrane. The tube was incubated at 50°C for 2 minutes centrifuged at 10,000 rpm for 1 minute to elute the DNA from the column

1. The standards and samples were removed from the freezer and thawed. In a separate sterile 1.5 ml microfuge tube for each standard/sample, 10  $\mu$ L of DNA was mixed with 990  $\mu$ L of D. I. water and vortexed. The solution was allowed to stand for 10 minutes to ensure the complete diffusion of DNA throughout the solution. This represents a 1:100 dilution of the standards and the DNA samples.

#### B. DNA quantification

1. The DNA sample was briefly vortexed and the solution was transferred to the cuvette of the spectrophotometer with care not to create bubbles. The cuvette is inserted into the spec ensuring the correct face of the cuvette is in line the light beam. An absorbance reading appears on the screen. Reading is continued until all standards and samples have been quantified. The concentration of DNA in the sample is determined according to the conversion factor ( $A_{260}$  of 1.0 = 50  $\mu$ g ml<sup>-1</sup> DNA). The concentration of DNA in the sample can be read as  $\mu$ g/mL using the conversion factor and dilution factor.

#### 2. RESTRICTION DIGESTION:

Restriction enzyme buffer was vortexed before pipetting to ensure that it was well-mixed and was added to the tube. Appropriate amount of DNA to

be cut was vortexed before pipetting to ensure that it was well-mixed and was added to the tube. After vortexing the enzyme to ensure that it was well-mixed 1  $\mu$ L of enzyme EcoRI was added. The mixture is placed in thermal cycler (Eppendorf) for 2-3 hour incubation at 37°C. To heat inactivate the enzyme the mixture is maintained at 80°C for 20 min. The mixture is kept at 4°C until the reaction mixture is out of the thermal cycler.

## Agarose Gel Electrophoresis Protocol

### Preparation of the agarose gel

1. 25 g Agarose powder was taken in 500 ml flask and 125 ml of TAE Buffer was added to it. The mixture is melted in hot water bath till a clear solution forms. The solution is allowed to cool to a temperature of 50-55°C by periodic swirling to achieve even cooling. To it ethidium bromide solution was added. The ends of the casting tray are sealed with two layers of tape. The combs are placed in the gel casting tray. The melted agarose solution was poured into the casting tray and allowed to cool until it is solid. The comb and the tape are removed carefully. The gel is placed in the electrophoresis chamber. 2-3 mm of TAE Buffer is added over the gel.

### Loading the gel

6  $\mu$ l of 6X Sample Loading Buffer is added to each DNA sample containing tubes. 20  $\mu$ l of each sample is pipetted into separate wells in the gel. 10  $\mu$ l of the DNA ladder standard is pipetted into one well of each row on the gel.

### Running the gel

The lid is placed on the gel box, the electrode wires are connected to the power supply. The power supply is turned on to about 100 volts. To ensure the correct direction of the current, the movement of the blue loading dye is checked. The power supply is continued till the blue dye approaches the end of the gel. The wires are disconnected from the power supply. The lid is removed from the electrophoresis chamber. Using gloves, gel is carefully removed and observed in a transilluminator for the DNA bands.

## RESULTS:

Isolation of DNA was done from blood, fresh saliva, saliva stored at -20°C, saliva stored at 37°C for 24hrs, buccal swab and betel quid by both the phenol-chloroform method and the chelex method. Gel electrophoresis of the isolated genomic DNA was carried out on 0.8% agarose gel. (figure 1)

After restriction digestion electrophoresis gel is prepared to run and to identify the number of bands. DNA samples obtained from blood were labelled as A<sub>b</sub> and subsequently as B<sub>b</sub>, C<sub>b</sub>, D<sub>b</sub>, E<sub>b</sub> as shown in table 2. DNA obtained from fresh saliva were labelled as A<sub>s</sub>, B<sub>s</sub>, C<sub>s</sub>, D<sub>s</sub>, E<sub>s</sub>. DNA obtained from saliva stored at -20 degree were labeled as A<sub>fs</sub>, B<sub>fs</sub>, C<sub>fs</sub>, D<sub>fs</sub>, E<sub>fs</sub>. DNA obtained from saliva stored at room temperature were labelled as A<sub>ds</sub>, B<sub>ds</sub>, C<sub>ds</sub>, D<sub>ds</sub>, E<sub>ds</sub>.

DNA obtained from blood of 5 individuals was made to run in the well marked 1 to 5 in a uniform manner i.e. DNA obtained from the first individual named as A<sub>b</sub>, was made to run in well No. 1. DNA obtained from second individual named as B<sub>b</sub> was made to run in well No. 2. DNA obtained from third individual named as C<sub>b</sub> was made to run in well No. 3. DNA obtained from

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fourth individual named as D<sub>b</sub> was made to run in as well No- 4. DNA obtained from Fifth individual named E<sub>b</sub> was made to run in well No. 5. (table 2)

But while running DNA obtained from saliva of different sources the order was changed randomly. For example DNA isolated from fresh saliva for the first individual (A<sub>s</sub>) instead of being run in the first well ie well No -6 was made to run in the third well( well no 8) and DNA isolated from saliva stored at -20 degree for the first individual(A<sub>fs</sub>) instead of being run in the first well ie well No-11was made to run in the third well (well No. 13)and DNA isolated from saliva stored at room temperature for the first individual(A<sub>ds</sub>) instead of being run in the first well ie well No-16 was made to run in the fifth well (well No. 20). Likewise DNA isolated from different sources of saliva of different individuals made to run in different wells and the number of bands produced is identified .

From the figure 1 it could be identified that the well number 1, 8 , 13, 20 corresponding to DNA isolated from the first individual from various sources named A<sub>b</sub> A<sub>s</sub> A<sub>fs</sub> A<sub>ds</sub> identified by the yellow arrow has uniformly three bands.

For the well number 2, 7, 14, 19 corresponding to DNA isolated from the second individual from various sources named B<sub>b</sub> B<sub>s</sub> B<sub>fs</sub> B<sub>ds</sub> identified by the blue arrow has uniformly 6 bands .

various

DNA isolated from the fifth individual from various sources named  $E_b$ ,  $E_s$ ,  $E_{fs}$ ,  $E_{ds}$  identified by the green arrow has uniformly 4 bands. From the above figure it could be identified that the well number 1, 10 corresponding to DNA isolated from different source for the first individual named  $A_b$ ,  $A_{bs}$ , identified by the yellow arrow has uniformly four bands.

For the well number 2 and 6 corresponding to DNA isolated from second individual from blood and buccal swab named  $B_b$ ,  $B_{bs}$  identified by the blue arrow has uniformly 6 bands. For the well number 3 and 7 corresponding to DNA isolated from third individual from blood and buccal swab named  $C_b$ ,  $C_{bs}$  identified by the red arrow has uniformly 5 bands. For the well number 4 and 8 corresponding to DNA isolated from fourth individual from blood and buccal swab named  $D_b$ ,  $D_{bs}$ , identified by the aqua arrow has uniformly 7 bands.

For the well number 5 and 9 corresponding to DNA isolated from fifth individual from blood and buccal swab named  $E_b$ ,  $E_{bs}$ ,  $E$  identified by the green arrow has uniformly 8 bands.

This shows that DNA obtained from an individual from blood and buccal swab produce uniform banding pattern. This shows that DNA obtained from an individual from various source produce uniform banding pattern.

Identification of individual from traces of saliva which could be used for forensic application - Extraction of DNA from Buccal swab. Restriction digestion with Ecor-1 from extracted DNA obtained from above mentioned source has been done for identifying individuals. Blood was used as a control and compared with DNA bands from buccal swab. A total of 10 wells were

created. DNA obtained from blood were labeled as A<sub>b</sub>, B<sub>b</sub>, C<sub>b</sub>, D<sub>b</sub>, E<sub>b</sub> as shown in tab 3. DNA obtained from Buccal swab were labeled as A<sub>bs</sub>, B<sub>bs</sub>, C<sub>bs</sub>, D<sub>bs</sub>, E<sub>bs</sub>. DNA obtained from blood from 5 individuals was made to run in the well marked 1 to 5 in a uniform manner.

But while running DNA obtained from buccal swab the order was changed randomly. For example DNA isolated from buccal swab for the first individual (A<sub>bs</sub>) instead of being run in the first well ie well No -6 was made to run in the fifth well( well no 10). Likewise DNA isolated from buccal swab of different individuals was made to run in different wells and the number of bands produced is identified

Different methods of DNA extraction is been followed in that, most widely used is phenol chloroform method . Many new methods of DNA extraction have been tried. The chelex method is one among then . To know the efficacy of the chelex method it was compared with that of phenol chloroform method. Of the two methods studied the chelex method proved to be more easy to handle and less time consuming in addition to yields higher amount of DNA and is proved by quantification with U. V spectrometer as shown in fig. 2.

#### DISCUSSION:

Forensic odontology is a branch of forensics which analyses stains and organic liquids from the oral cavity or its contents, bite mark comparison, investigation of trauma and oral injuries such as personal injury cases, and dental malpractice. The fundamental requirement of a criminal investigation is that the victim and aggressor should be positively identified. Forensic

dentistry aids in the forensic process by comparing the deceased's dentition with that of previous dental records or by facilitating to shape the profile of an individual in terms of age at the time of death, sex and phylogeny to aid in identification. <sup>11, 12</sup>

Saliva has been a potential source of identification and is usually found in bite marks, cigarette butts, betel quid, postage stamps, envelopes and other objects. The first phase of the study intended to isolate DNA from saliva (under different conditions), by phenol-chloroform method and chelex method and compare the yield with that of blood . The second objective was to find out efficacy of these methods in extraction of DNA from traces of saliva ie from Buccal swab, and from Betel quid and which could be used for forensic application. <sup>8</sup>

The presence of residues are considerably important as biological evidences, but forensic analysis of such evidences has been hindered by failures in extraction of human DNA. Consequently, it is indispensable in forensic science to establish a reliable method for extracting DNA from samples collected at the crime site. The most important objective was whether individuals can be identified from samples of different source and to ascertain the applicability of the restriction digestion in forensics. <sup>13, 14</sup>

Blood was taken as control, saliva was divided into 3 parameters ie from fresh saliva, from saliva stored at -20 degree for 24 hr from saliva stored in room temperature for 24 hr's were obtained . Identification of individual has been done with restriction enzyme EcoRI. . The isolated DNA was digested using the restriction enzyme EcoRI(G| AATTC)The digested DNA was run on <https://assignbuster.com/methods-of-dna-identification/>

1% agarose gel electrophoresis and the bands produced in each individuals DNA were scored and is proved that identification of individual can also be done by DNA fingerprinting or profiling.

Agarose gel electrophoresis separates DNA fragments according to their size. The most important objective was whether individuals can be identified from samples of different source and to ascertain the applicability of the restriction digestion in forensics. <sup>16</sup>

DNA fingerprinting is a technique that is used to represent like and unlike DNA that is present in different individuals. Nucleotide sequences which show significant variation from one individual to another are taken into consideration. <sup>17</sup> The most important objective of the study was to ascertain whether individuals can be identified from samples of different source and to ascertain the applicability of the restriction digestion in forensics and the last objective was to Compare the DNA yield from manual and kit method.

To prove that DNA could be extracted from traces of saliva , Buccal swab and Beetal quid was used . DNA could be extracted from buccal swab, beetal quid and quantification was done with U. V spectrometer. Comparison of DNA isolated from all the samples collected from all the individual using two different procedures has been done and comparison of yield of different sources showed the kit method to be more effective .

Use of biological evidences like saliva, buccal swab and betel quid are compromised due to the quandary in extraction of human DNA. The present study had proved to establish a reliable method for extracting DNA from

samples collected from different sources of saliva and from traces of salivary stains which was comparable to blood in proving identification. Samples collected from different sources of saliva and from traces of salivary stains can also be assessed by DNA fingerprinting or profiling which is based on the fact that DNA is unique to every individual .