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# DNA Profiling from Dentine and It's Role in Signifying Individuality

Bansal. S. K.
*Department of Forensic Science, Chandigarh University, Mohali, Punjab, 140413, India*

***Abstract:*** ***Forensic dentistry is the field which is a combination of forensic and dentistry. In this we go for principles used in dentistry or dentistry for the use in judiciary. It is one field in which we collectthe evidence related to dentistry and analyze them for the purpose of investigation. In a crime scene or a disaster situation a lot of destruction occurs. It becomes important to determine the identity of an individual and we can say it is the identity of the deceased. This study is being conducted based on previous research and literature presented by various research scholars. When it comes to a crime scene where we do not get the victim or culprit but we find the dentalevidence and also in cases of mass disasters, catastrophic events, industrial disasters where identification is necessary if we found the dental evidence, they can help a lot in determining age, gender partial identity can be known through this evidence. Many methods are there to determine identity through dental evidence and also apart from dental evidence we have orthometric methods but dental evidence is found to be more cheap, easy, fast methods. In this we have discussed how DNA is extracted from the teeth and it's further processing so that it will help in establishing someone's identity.***
***Keywords: Dentistry, Forensic Dentistry, Dental Evidence, Extraction, DNA Profiling etc.***

## I. INTRODUCTION

Teeth are used as a way of personal identification from a very long time. dentistry is one suchfield that helps us in determining a lot from just teeth. dentistry when combined with forensic becomes a very important field of forensic science that is forensic dentistry. forensic dentistrynowadays serving a vital purpose in our legal and judicial matters.

Not every time it is possible that one will get the direct evidence from the crime scene i.e a complete dead body or a person leaving his major identity, at that time even if we get a small tooth it can act as a pin pointing proof in those cases. With the enhancement of technologies like PCR we nowadays are able to amplify the small amount of DNA and find out a lot about the person. This study is a review of how the DNA is extracted from the teeth and how a profileis generated and in return how it helps in individualization.

Dentition is the most essential part of the body that is used for cutting and chewing the food. The teeth in humans are generally 32 in number and these are present in both the mandibular and maxillary arches. The gums are structured in such a way that the teeth seem to be embeddedin a socket so due to this reason they are called thecodont.

It is diphyodont which means that it is of 2 types- milk dentition that are also called as temporary / primary / deciduous - dentition and for second one is permanent / secondary dentition. Milk dentition has only 20 numbers of teeth and it as a dental formula - 2102/2102- ICPM. It does not have premolars and the third molar. Milk teeth when visually seen are smallin size as compared to permanent teeth. They help in chewing of food and also help for maintaining space for the upcoming permanentones. When we see permanent dentition in permanent dentition we have 32 number of teeth and they have dental formula of 2123/2123- ICPM. They start to occur with the shedding of last primary teeth. They are large in size as compared to deciduous dentition. Their role is to help in chewing of the food, aesthetics, and speech.

A tooth has 2 parts- crown and root. Crown is the visible portion that seems to be bulging abovethe gingiva and the root is the one that is inserted in the socket like structure. Apart from these2 parts our teeth also contain three hard tissues called enamel, dentine, and cementum. Enamelis the covering of the teeth that covers it from outside and is the toughest and rigid structure ofthe body.

Tissue named dentine present below enamel ; it constitutes of bulk of the different parts of dentine i.e. crowns and roots . In the middle portion of the teeth pulp is present it is the softy tissue that contains blood vessels and nerves. The place where roots are present a bony thin layer is present that is termed as cementum.

Human dentition is also called as heterodont because it contains teeth which can be differentlycategorized as they have different morphology that is outer look and the functions to be performed. The four kinds of teeth we have in our mouth are-

1) INCISORS
2) CANINES
3) MOLARS
4) PREMOLARS

 

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## II. MASS DISASTER CONDITION

Mass disaster is a condition in which the number of people dying or the person injured is above the set criteria in which medical aid and forensic investigation is required. There aretwo types of mass disasters, natural and man-made. Natural includes earthquakes, tsunamisetc. Man-made disasters include industrial accidents etc. Forensic experts in this kind of disasters have a major role – firstly they have to identify the cause of disaster and second are victim identification. In victim identification different fields of forensics are applied that is forensic anthropology, dentistry, medicine etc. In mass disasters victim identification is one of the crucial tasks.

Nowadays forensic dentistry is considered more because of its advantages like-

*1)* It provides accurate results.
*2)* Rapid and cheap method of identification.
*3)* Easy to perform.
*4)* Cost effective.

The main objective of our research is to study about how DNA profile is extracted from adental source that can be used as a source of individualisation in many cases.

## III. DNA ISOLATION

*A. Day 1*

*1)* Take the teeth and remove contaminants by either cleaning it or by sterilising it withthe help of Sodium Hypo-chloride solution (NaOCl)- 0.5% w/v, which means 0.5 gmin 100 ml of distilled water.
*2)* Leave the teeth sample for 30 min with mixing. Then discard the NaOCl solution andrinse it with distilled water.
*3)* Repeat the same step for 2-3 times until the teeth sample is decontaminated.
*4)* Rinse the tooth sample with 70% ethanol. discard ethanol and air-dry tooth sample atroom temperature all the (ethanol will evaporate)
*5)* Break the tooth sample using sterile plyer (autoclave it)
*6)* Take a large centrifuge tube and break the tooth sample so that everything would beinside the centrifuge tube.
*7)* Add sufficient amount of lysis buffer- I (completely dipped). mix properly.
*8)* Incubate for 2 hrs at 4 degree Celsius, then transfer to 65-degree Celsius hot oven for15 min. mix completely and carefully and made it to come on room temperature.
*9)* Centrifuge at 6000 rpm at 4 degree Celsius for 15 min.
*10)* If there is no clear pellet or supernatant is there. so, remove water and transfer thetooth part to another test tube.
*11)* Add lysis buffer -II and then add SDS (1ml) and proteinase K(5ml)
*12)* INCUBATION (37 degree Celsius) (water bath) (leave it overnight)

*B. Day 2*

*1)* Bring the sample from water bath
*2)* To the content add saturated phenol
*3)* Mix properly
*4)* Centrifugation (6000rpm) (15mins.) (4 degree)
*5)* Transfer aqueous layer to another tube and discard organic layer
*6)* Transfer aqueous layer + phenol: chloroform (1:1)
*7)* Mix properly
*8)* Centrifugation (6000rpm) (15mins.) (4 degree)
*9)* Transfer aqueous layer to another tube and discard organic layer
*10)* Transfer aqueous layer + chloroform + Isoamyl alcohol (24:1)
*11)* Mix properly
*12)* Centrifugation (6000rpm) (15mins.) (4 degree)
*13)* Transfer aqueous layer to another tube and discard organic layer
*14)* Transfer aqueous layer + chilled propanol + sodium acetate solution
*15)* Mix properly. DNA will be precipitated.

 

*16)* Centrifugation (6000rpm) (1min) (4degree)
*17)* Discard supernatant, we have DNA as pellet
*18)* To DNA pellet + 70% ethanol (1ml)
*19)* Mix gently by tapping
*20)* Centrifugation (6000 rpm) (1min) (4 degree)
*21)* Discard supernatant and air dry the DNA sample at room temperature
*22)* Add distilled water or TE buffer
*23)* Dissolve the DNA sample and store at 4 degrees.

| LYSIS BUFFER I | | LYSIS BUFFER II | |
|---|---|---|---|
| Tris Cl | 100mM | Tris Cl | 50mM |
| EDTA | 50mM | EDTA | 50Mm |
| NaCl | 30mM | $MgCl_2$ | 30Mm |
| pH (buffer)= 7.9 – 8.2 (should be maintained) | | | |
| PHENOL AND PROTEIN SOLVENT= specific gravity more than water | | | |
| SDS (SODIUM DOCILE SULPHATE) AND SLS (SODIUM LAURYL SULPHATE)- USED | | | |
| CHLOROFORM (protein solvent) (less corrosive) | | | |
| ISOAMYL ALCOHOL= solvent for carbohydrates and lipids | | | |
| CHILLED PROPANOL AND SODIUM ACETATE SOLUTION= (make ppt of DNA) | | | |
| ETHYL ALCOHOL= purification and dehydration | | | |

Table 1 – Preparation of lysis buffer I and II

## IV. QUALITY AND QUANTITY ANALYSIS

### A. *Quality Analysis*

$$\frac{optical\ density\ at\ 260\ nm}{optical\ density\ at\ 280\ nm}$$

*1)* It has no unit, because it is in ratio
*2)* Protein contaminated < 1.6- 1.8 < Phenol and Organic compound contaminated

### B. *Quantity Analysis*

OD at 260 nm * 50(constant) * dilution factor
Dilution Factor

$$\frac{Initial\ volume}{final\ volume}$$

## V. RESTRICTION ENZYME

*1)* To cut the DNA from the particular place or from thesite of interest.
*2)* It is done by restriction enzymes, that is EXONUCLEASE ANDENDONUCLEASE.

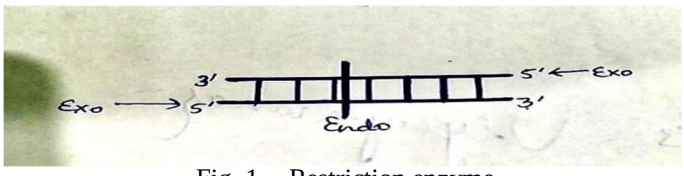


Fig. 1 - Restriction enzyme

RESTRICTION ENZYMES(CLASS 2) EXAMPLES

*a)* Eco RI
*b)* Hind III
*c)* Hin *f*

Restriction Endonuclease

| CLASS | SPECIFICITY | CLEAVE UPTO BASE PAIR |
|---|---|---|
| Class I | Not specific | ± 50 base pairs |
| Class II | Specific | Cleave DNA from locus |
| Class III | Not specific | ± 10 base pairs |

Table 2 – Different classes of endonuclease

## VI. ELECTROPHORESIS

It is defined as the separation or migration of charges in the influence of electricfields.

*A. Casting of Agarose Gel*

*1)* Agarose gel is almost transparent in nature and it is carried out at room temperature.
*2)* We have, CASTING PLATE AND COMB (used to prepare wells in gel)
*3)* Close the 2 open ends with cello tapes
*4)* Place the comb on the casting plate
*5)* There should be a gap between base and comb, otherwise it touches the base to makeholes.
*6)* Agarose (powder) + buffer + heat (50-60 degree)
*7)* Agarose (derived from red algae) and 0.8% gel which means, 0.8gm agarose in 100mlof buffer. (for genomic DNA)
*8)* pour it in casting plate
*9)* Keep at room temp to slightly cool down and solidify. (takes 45mins)
*10)* Gel is being casted and wells created.
*11)* Gel gets solidify, open the tapered ends
*12)* Casting plate with gel, put in electrophoresis tank
*13)* Make sure gel completely dipped in buffer
*14)* Take DNA sample by micro pipettes
*15)* As, DNA has no colour we need to add dye (loading dye) in it. TE and other buffershave no colour
*16)* Add the DNA sample in wells.
*17)* Low voltage power supply (100-120 volts) (30- 40 mins.)
*18)* Loaded DNA sample will move

| TAE BUFFER | | TBE BUFFER | |
|---|---|---|---|
| Tris chloride | 40mM | Tris chloride | 10mM |
| Acetic acid | 40mM | Borate acid(powder) | 10mM |
| EDTA | 1mM | EDTA | 20mM |
| LOADING DYE<br>Bromophenol blue (1 mg powder) + Glycerine (10ml) | | | |

Table 3 - Preparation of buffer solution .

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## VII. STEPS OF PCR

There are 3 steps of PCR method

### *A. Denaturation*

Temperature - 94°C for 1 minute

At this temperature in denaturation, the bonds of hydrogen breaks and the double strandedDNA will become single stranded.

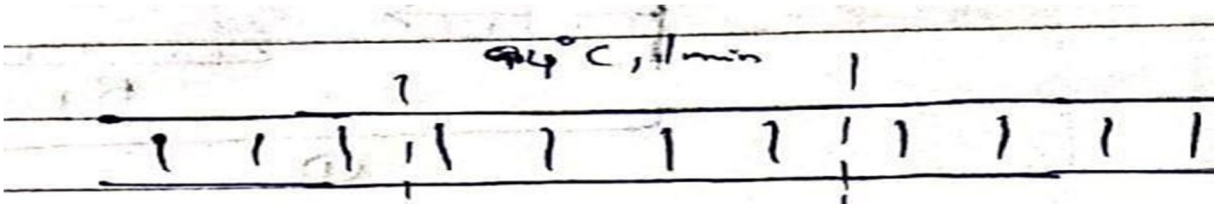


Fig.2- Denaturation

### *B. Annealing*

Temperature - 65°C for 45 seconds.

The single stranded DNA is then binded with the primers with their complementary sequences. Here, the primers are always used in the pair forms that are termed as forward andreverse.

The size of prime may vary (from up to 85 base pairs) these primers are also defined as the known sequences of the single stranded DNA and thus they have the ability to bind with thecomplementary strand of the DNA.

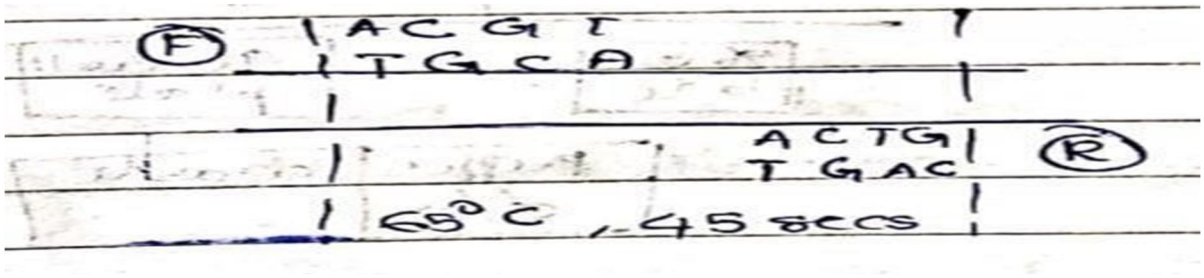


Fig.3 - Annealing

### *C. Polymerization Or Extension*

Temperature - 72°C for 45 seconds

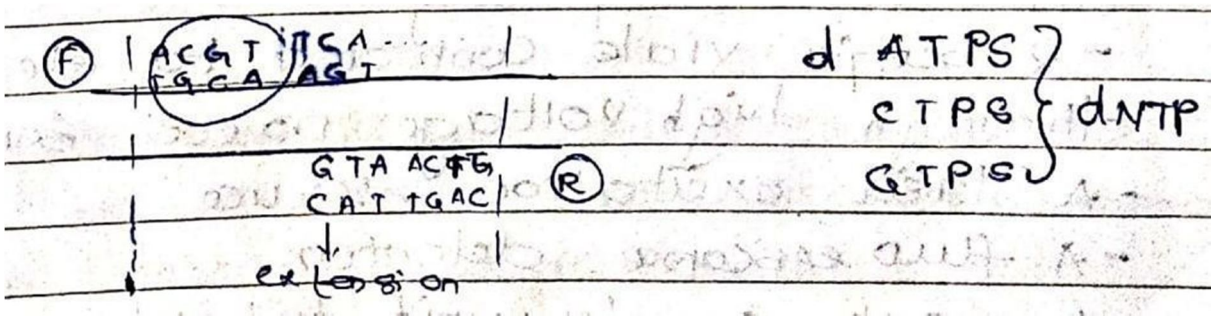


Fig.4 -Polymerisation

*1)* At 4°C for 30 seconds, the same steps are repeated.

*2)* Formula – $2^n$

$2^1 = 1$

$2^2 = 4$

$2^3 = 8$ and so on ….

 

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## VIII. MULTIPLEX PCR

Here we can use more than one DNA fragment at the same time.

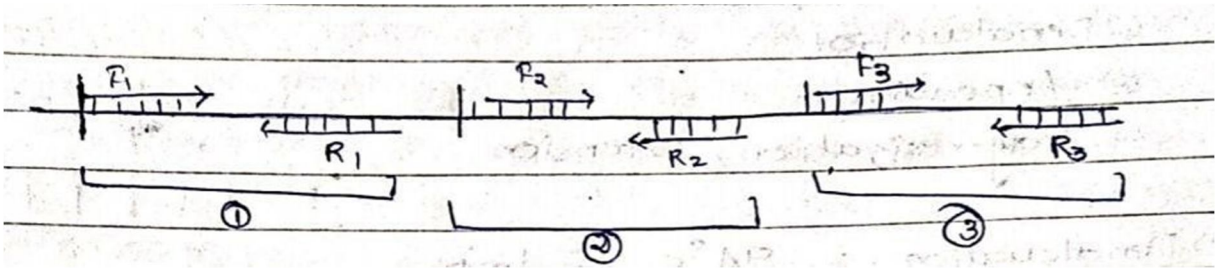


Fig.5 - Multiplex PCR

## IX. ANALYSIS OF DNA FRAGMENTS BY CAPILLARY ELECTROPHORESIS

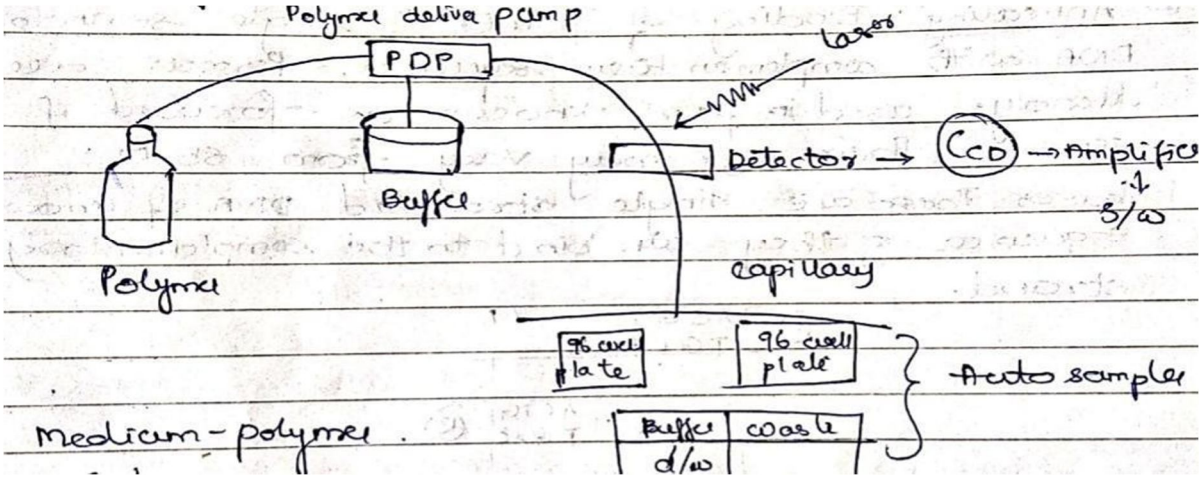


Fig.6 -Capillary Electrophoresis

THE 1° COMPONENTS OF ELECTROPHORESIS BY CAPILLARY ACTION INCLUDES –

*1)* Capillary of narrow size .
*2)* 2 buffer glass tubes connected to electrodes through power unit source of high energy .
*3)* Laser is used as source of excitation .
*4)* Detector – fluorescence is used .
*5)* Autosampler – sample is hold by this unit .
*6)* A PC system with related accessories to control sample injection and deletion.
*7)* Capillaries are typically made up of glass with 50 internal diameters.
*8)* Prior to electrophoresis the capillary is loaded with polymer with the help of apolymer delivery pump (PDP) followed by DNA sample injection.
*9)* The capillaries can be of various sizes depending on the size of amplicon to beanalyzed .
    36 cm = 700 base pairs
    50 cm = 1200 base pairs
    80 cm = 2000 base pairs

*10)* Detection of samples performed automatically, by the instrument through measuringthe time span between the sample injection and deletion.
*11)* At the end of capillary , detector is kept, which contains a laser excitationsource.
*12)* DNA fragments along with fluorescent dye move through the detector and they getilluminated.

 

13) Smaller DNA fragments will reach the detector first followed by larger fragments.
14) The data from capillary electrophoresis definition are plotted against fluorescenceintensity for detection purpose.
15) Fluorescent emission signals from the dye attached to DNA fragments can be used for quantification and detection of DNA fragments.
16) Once the DNA sample is injected into the capillary, the higher potential difference iscreated (up to 50 kb ) that the DNA sample starts moving from cathode to anode .
17) Once the DNA sample reaches the detector, it is then detected by fluorescentdetectors

## X. FLUORESCENT DETECTORS

1) Fluorescence based detection assessments are commonly used in DNA typingmethods.
2) In this process STR markers are used.
3) Fluorescent dyes attached to primers get incorporated into the amplicons throughmultiplex PCR.
4) Different types of fluorescent dyes can be simultaneously used for the detection ofseveral DNA fragments.
5) Amplified DNA products are visualized as characteristic electropherograms afterdetection.
6) Fluorescence measurement involves excitation of a dye molecule followed by itsdetection from the emitted light.
7) A dye molecule which is capable of fluorescence is called as FLUOROPHORES.
8) Fluorophores can be different in size and shape and also in chemical activity.
9) Only those fluorophores are utilized in DNA fingerprinting ,Range for fluorescence = 400 nm – 600 nm .
10) The base source provides excitation energy through or to the fluorophores and the electrons move from ground state to excitation transition state.
11) While coming back to the ground state, these electrons releases energy in the form ofemittance.
12) Optical filters can be used to detect characteristic emission spectra.
13) Fluorescent detector is a photosensitive device which can measure the intensity ofemittance through a PMT OR CCD which amplifies these electrical signals.
14) The light intensity is typically recorded.
15) The data is processed to specific software to provide tangible data.

## XI. CODIS DATABASE

EXAMPLE –
(D) (3) (S) (1358) – D = DNA, 3 = CHROMOSME NUMBER, S = SINGLE COPY1358 = ACCESSING NUMBER.
(2) (p) (25). (3) – 2= NUMBER OF CHROMOSOME, p = LOCATION, 25 = MAJORBAND, 3= INTER BAND

| LOCUS | CHROOMOSOMAL LOCATION | REPEAT MOTIF | ALLELICRANGE |
|---|---|---|---|
| TPOX | 2p 25.3 | AATG | 4-16 |
| D3S1358 | 3p 21.31 | TCTA | 6-26 |
| FGA | 4q 31.3 | CTTC | 12-51 |
| D5S818 | 5q 23.2 | AGAT | 4-29 |
| CSF1P0 | 5q 23.1 | AGAT | 5-17 |
| D7S820 | 7q 21.11 | GATA | 5-16 |
| D8S1179 | 8q 24.13 | TCTA | 6-20 |
| TH01 | 11p 15.5 | TCAT | 3-14 |
| VWA | 12p 13.31 | TCTA | 8-225 |
| D13S317 | 13q 31.1 | TATC | 5-17 |
| D16S539 | 16q 24.1 | GATA | 4-17 |
| D18S51 | 18q 21.33 | AGAA | 5-40 |
| D21S11 | 21q 21.1 | TCTA | 12-43 |

Table 4 - chromosomal position

1) Combined DNA index system
2) Largest DNA database
3) Officially, launched by FBI on 13th October 1998
4) 3 sub classes – LDIS (LOCAL) SDIS (STATE) NDIS (NATIONAL)
5) TPOX – THYROID PEROXIDASE $10^{TH}$ INTRON (gene in $2^{nd}$ chromosome produce thyroid peroxidase enzyme) (the $10^{th}$ intron of thyroid peroxidase enzymes producing the gene)
6) TH01 – TYROSIN HYDROXYLASE $1^{ST}$ INTRON
7) FGA – ALPHA FIRBRINOGEN $3^{RD}$ INTRON
8) VWA – VON WILLEBRAND FACTOR $40^{TH}$ INTRON (named after syndrome)
9) CSF1P0 – C- fms PROTO- ONCOGENES $6^{TH}$ INTRON .

| DYE | NOMENCLATURE | EXCITATION nm ( λ) | EMISSION nm ( λ) |
|---|---|---|---|
| 5- FAM | 5—CARBOXY FLUOROSCENE | 493 nm | 522nm |
| JOE | 6-CARBOXY-2,7DIMEOXY4,5-DICHLOROFLUORESCENE | 528nm | 544nm |
| HEX | 4,7,2',4',5',7'-HEXACHLORO-6-CARBOXYFLUORESCENE | 535nm | 553nm |
| RHODAMINERED | RHODAMINE RED | 580nm | 590nm |
| FL | FLUORESCENE | 490nm | 520nm |

Table 5 - Different fluorescent dyes

There are 'n'number of techniques, for this purpose some of them which aremainly used in forensic dentistry are-

*A. Polymerase Chain Reaction Technique (PCR Technique)*

Kary Mullis had developed a technique which is termed as PCR which got the establishment in the year 1983.this technique came out to be the revolutionary one in science as this techniquehas the ability to amplify very little amount of DNA and make multiple copies of it. These shorttarget sequence DNA are processed using oligonucleotide primer that are specific for a particular sequence along with thermostable taq DNA polymerase is used (5). This is the mostpowerful tool used in molecular biology. As we have studied that our teeth can bear extremelyhigh temperature, in many cases where all other body parts get destroyed and no such remain is found we can look after for the teeth as it contains the pulp from which DNA can be extractedand further can be processed using PCR and identification can be determined from this. Thus, in some cases , this can help in the identification or the individualization , like where the bodyis completely, or in cases of some disasters etc. we can also go for dentine and cementum but according to a study conducted in the year 2003 it was found that this dentine a cementum in PCR amplification show like amplification whereas pulp of the teeth shows the most strongestkind of amplification (6).

*B. RFLP Process*

Restriction endonuclease is that enzyme which cuts the DNA into pieces or fragments and thusthe possession of digestion also takes place by the process of quantification and isolating thesefragments of DNA.

The function of restriction endonuclease

1) It is a biological scissor, cuts the DNA from particular location and also helps in finding the sequence of DNA
2) These biological scissors, usually chops the DNA particularly from the region which are not going to find in the sequence of tandem repeat.
3) Thus, after cutting down of DNA sequences, they are having different lengths and also develops DNA of different sizes and is been referred as VNTR
4) In VNTR, it contains 15-65 base pairs of different sizes with sequence of short repeat
5) With this method, poor quality of DNA is developed abs thus it would not be used for the forensic analysis.

*C. Mt DNA analysis (Mt. =Mitochondrial and Nclr. = nuclear )*

Mitochondrial DNA Is off 16569 base pairs whereas nuclear DNA 3.2 billion base pairs there is a great difference between Mt. DNA and Nclr. DNA. Basically mother inherits the Mt . DNAto the child. We can say that the analysis of Mt. DNA can be used for those analyses where therestriction fragment length polymorphism STR analysis does not give appropriate results. biological evidence in which absence of nucleated cells at that point Mt. DNA turns out to be a boon to us. choosing the Mt. DNA is beneficial as it has many copies in the body when compared to nuclear DNA. This Mt. DNA technology is very helpful in case of cement time and end time dentine as it contains DNA to such an extent that it can be further amplified so that it can be useful for human identification. a study was published in 2007 which says that inthe samples of the past it is hard to find nuclear DNA in that instances Mt.DNA is used.

*D. Amp FLP (amp = amplified , FLP = fragment length polymorphism )*

This technique discovered and introduced in the beginning of 1990' s. In this polymerase chainreaction was used for the duplication of DNA samples. It is very useful in creating phylogenetictrees on the basis of comparison made by DNA of an individual. It is much faster than RFLP and is counted in a low-cost technology, so used by countries with low income.

## XII. DISCUSSION

DNA fingerprinting plays a vital role in cases where it is difficult to find out the complete body,in those instances forensic dentistry plays a major role. In situations like mass disasters, decomposed bodies, and structural failure we do not find other parts of the body where the dental evidence plays a major role. Some cases are mentioned below. In the year 1995 sweet and sweet presented a case in which the scenario was that the person who was the victim had been incinerated and when the body was found it showed that her bodywas carbonated or carbonized completely in such situations DNA extraction from the body is not recommended the body was in the end identified by DNA extraction from the third molars. In the year 2004 a disastrous Tsunami came in Indian ocean on 26th December at that time individualization & identification of the Victim has become a hectic task other dental method fail but DNA profiling from dentine has played a major role and help to establish the identity of the deceased. In Egypt when the Queen's mummy was in questionable state for many years, in that case the molar tooth which was said to be belonging to the queen was sent for DNA profiling & it helpedin establishing the Identity of the Queen's mummy. (7)

## XIII. CONCLUSION

For human identification there are several sources like human skull, long bones, DNA etc. but when it comes to situations like mass disasters, industrial accident where there is possibility thatthat the other factors are slightly destroyed due to these catastrophic events a method of identification through dentition is considered, as the enamel is the hardest tissue of our body thatis the outer covering of our teeth that don't get destroyed even in the worse condition. It is alsoconsidered nowadays because it is the fastest and cheapest method of identification than the other techniques. And today this field of forensic dentistry which serves the most important purposes in case of identification in several cases is now also being started to be used in severalcountries of the world including India. We can say that this technique not only gives us the appropriate results but can also be considered as the most sophisticated technique. Forensic caseswhere the identity of the dead individual is important to locate them a forensic odontologist is the one who deals with such types of identification procedures. Hence dentition becomes the most appropriate technique for the identification of individuals in case of mass disasters.

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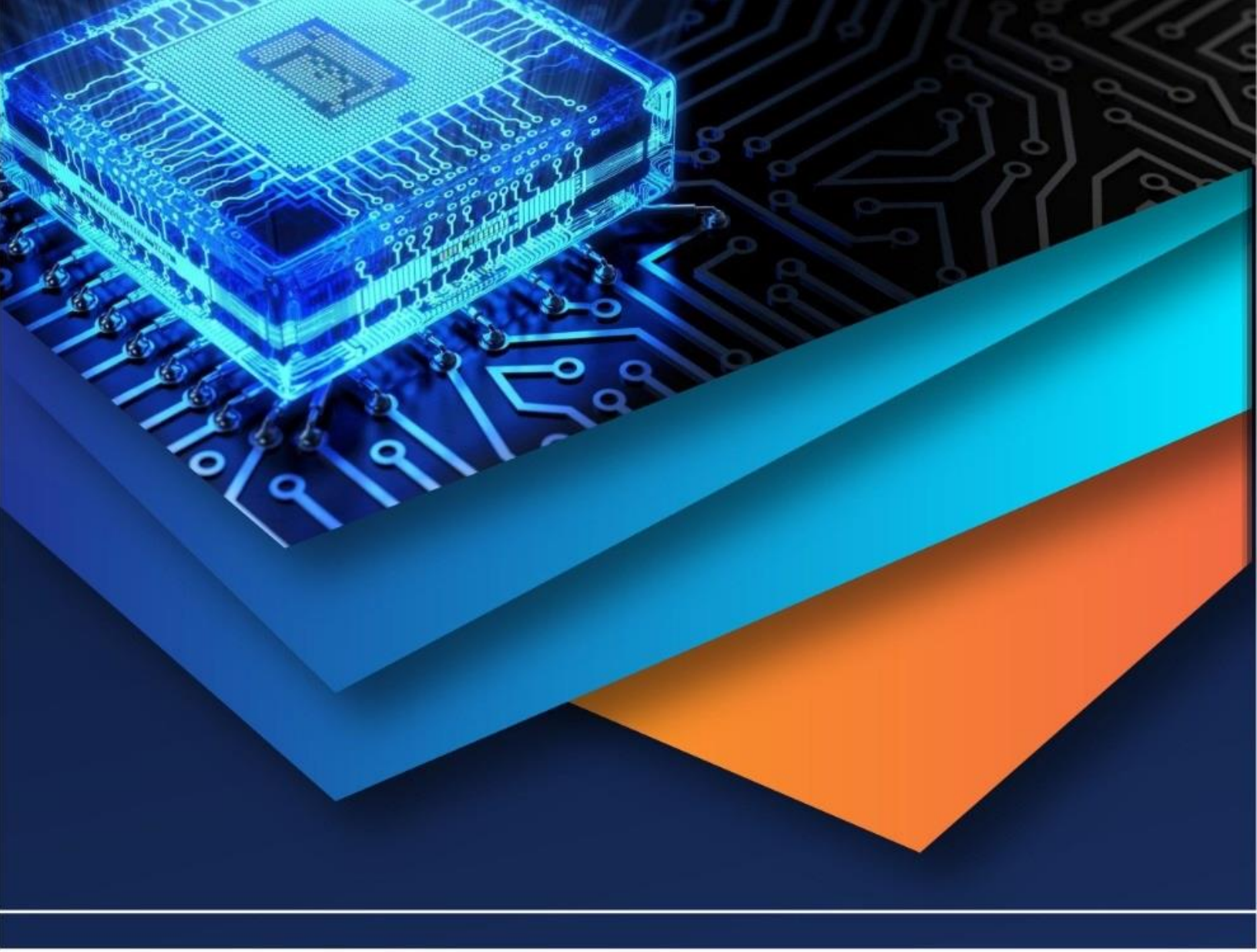

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