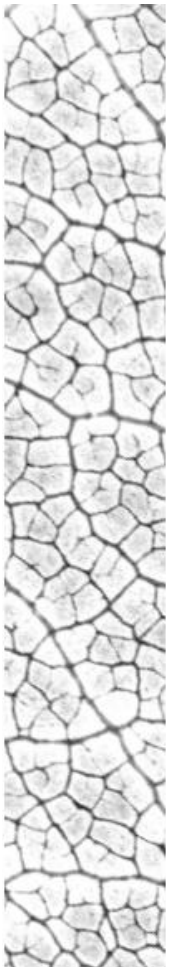

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Use the Variable Number of Tandem Repeats (VNTR) in DNA fingerprinting and its application biological sciences

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Abstract
DNA fingerprinting is an important tool in criminal investigations. Typing of Variable Number Tandem Repeat (VNTR) is a genotyping tool that provides a numeric and simple format of data based on the repetitive sequences number. The present study uses DNA fingerprinting by amplify certain specific VNTR regions present at a particular locus of different chromosomes. The biological samples include available materials such as hair and blood present on a scene of crime, blood, buccal and along with fingernail. By amplifying he specific VNTR fragments of different DNA samples & comparing them on agarose gel electrophoresis, we obtained DNA profile of individuals, which in turn helps in criminal identification. It can also indicate whether the individuals are homozygous or heterozygous.

Keywords: DNA fingerprinting, VNTR, Molecular biology, RFLP

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INTRODUCTION

Chemical and biological analysis of the general composition of nucleic acids (RNA and DNA): they are nucleotides composed (Blackburn *et al*., 2006). A nucleotide consists of a group of phosphate, nitrogenous base together and a five- carbon sugar (ribose in RNA and deoxyriboses in DNA), in DNA molecule has one of four (adenine, guanine, thymine, and cytosine) nitrogenous bases for each nucleotide; the two rings of atoms structure (Purina bases) are the first and the single ring of atoms (Pyrimidines bases) are the latter (Pray, 2008). Dr. Jeffrey was first described the DNA fingerprinting in 1985, who found that the DNA in a certain regions has sequences that were repeated over many times to each other, in a sample, the present number of repeated sections could differ from individuals (Parker et al., 1998;Cunningham and Meghen, 2001). These repeat regions of DNA became known as variable number of tandem repeats (VNTRs) (Vergnaud and Pourcel, 2006). The technique used to analysis the VNTRs was called restriction fragment length polymorphism (RFLP) due to restriction enzyme used to cut the DNA regions surrounding the VNTRs (Sreenan *et al*., 1997).

Number of laboratories across the world widely applied the DNA test using different techniques in manin Europe, Asia and United States; the DNA typing methods that used for testing the human identity and forensic DNA programs that in investigated the crime scene and testing of paternity as well (Butler, 2007;Shewale and Liu, 2013). Furthermore, numerous features of the variability mechanisms, its processes of evolution involvement and its biological roles yet remain obscure (Supply *et al*., 2006). VNTR is a genotyping tool that provides data in a simple, obscure format based on the number of repeated sequences (Ramazanzadeh and McNerney, 2007). Invented of numerous molecular technique by genome using including the gold standard (RFLP typing), (spoligotyping), (MIRU typing) and also used of different techniques in laboratories (Varma-Basil *et al*., 2011).

MATERIALS AND METHODS

Samples were collected from the crime scene then analyzed, store them within secure. The biological samples include available materials such as hair and blood present on a scene of crime, blood, buccal and along with fingernail.

Amplification of specific VNTR region by PCR

Aim: to amplify specific VNTR (variable number tandom repeat) of the isolated DNA fragments by polymerase chain reaction.

Polymerase chain reaction is a process by which minute quantities of DNA can be amplified into large

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number of copies for detection of many diseases and other purposes (Hamzah and Hasso, 2019; Musimba, et al, 2015).

In DNA fingerprinting it is used to amplify certain specific VNTR regions present at a particular locus of different chromosomes. By amplifying he specific VNTR fragments of different DNA samples & comparing them on agarose gel electrophoresis, we can make DNA profile of individuals, which in turn helps in criminal identification. It can also indicate whether the individuals are heterozygous or homozygous.

The chemical reactions involved following steps:

Denaturation:

The double strand during the denaturation dissolved open to single DNA stranded, stop all the enzymatic reactions.

Annealing:

Constantly formed of ionic bonds and broken between the primer and the template single stranded at 54°C. The primers that fit exactly (more stable bonds) last and on that DNA double stranded in little piece (primers and template), attach the polymerase and starts the template copying. When there are built in a few bases, the strong ionic bond between the primer and template, that would not break anymore.

Extension:

The ideal working temperature at 72°C for the polymerase. Where the primers are a built in few bases, a stronger ionic attraction have already to the template than the these attractions forces breaking. Primers get loose again if are with no exact match position (due to the temperature is higher) and the fragment gives no extension.

Coupled the bases to the primer on the 3' to 5' side; bases are added complementary to the template.

Holding temp.at 4°C

Principle:

Used of Polymerase chain reaction to nucleotide sequence amplification from chromosome 1 to insertion of a sequence of short DNA called variable number tandem repeats. Although different DNA of individuals is more similar, there are many human chromosome regions that show more of diversity. Such variable sequence is called a polymorphic and support the basis for diagnosis of genetic disease, paternity testing and forensic identification.

Requirements:

• Chemicals:

Temples –G-DNA, Primer mix (forward & backward primers): (D1S80 locus), Master Mix (dNTP, metal ions, taq polymerase), DNA samples from cheek & hair cells, nuclease free water.

Thermal cycler.

Procedure:

- 2.5 µl of different DNA samples extracted from cheek & hair blood, cells were taken in PCR tubes.
- 2.5 µl of primer mix was added to the above PCR tubes containing the samples.
- 12.5 µl of master mix was added to the above tubes containing the mixture of DNA & primer.
- 7.5 µl of nuclease free water was added to each of the tubes to make up the volume to 25µl.
    - The tubes were then placed in thermal cycler where the necessary inputs were given.

LabnetMultigen II Thermal cycler programmed as follows:

- Pre-PCR cycle: Initial denaturation of template DNA at 94°C/ 3 minutes and press ENTER.
- PCR cycle: Denaturation for 45 sec at 94°C.

Primer annealing for 45 sec at 68°C.

Extension at 72°C. For 1 minute.

Restriction Fragment Length Polymorphism (RFLP) analysis of DNA

Restriction fragment length polymorphism (RFLP) was homologous DNA sequences differences that might examine by different lengths presence after DNA samples digestion with specific restriction end nucleases. RFLP, is specific to a restriction enzyme combination / single clone as a molecular marker.

The common markers of RFLP are highly co-dominant and locus-specific (heterozygous sample for detected both alleles.

In RFLP test, cut the organism DNA is into fragments using restriction enzymes. Produced a large number of DNA short fragments.

Requirements:

- Samples of DNA
- Gene (Hind 111, Eco R1, Bam1)
- Assay buffer
- Dist. H2O
- Incubator
- 7ul of DNA sample was taken
- 2ul of Hind111 gene was added
- 2.5ul of 10X assay buffer was added and made the volume 25ul by adding 13.5ul dist. water
- The RFLP sample then incubated at 37 C for 1hr

After incubation Agarose gel electrophoresis was performed

Note:

The amplified VNTR regions were run on agarose gel electrophoresis. The bands were then visualized under U.V transilluminator.

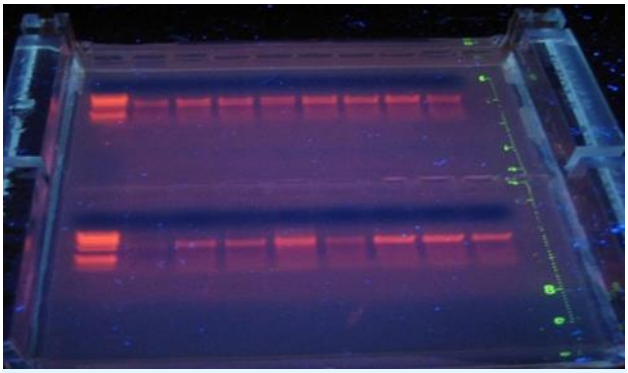

Fig. 1. DNA samples Wells 1, 9: Standard DNA, Wells: 2, 3, 4, -Hair DNA

Table 1. Standard DNA concentration 1mg/ml.

S. No.	Conc. Of Standard DNA (ug/ml)	O. D. at 595nm
1.	200	0.10
2.	400	0.22
3.	600	0.38
4.	800	0.46
5.	1000	0.58
6.	1200	0.71
7.	1400	0.83
8.	1600	0.97
9.	1800	1.11
10.	2000	1.18

RESULTS and DISCUSSION

Variable number tandem repeat (VNTR)

Wells 6, 7, 8 Blood DNA, Wells 12, 13, 14: Buccal DNA samples, Wells 15, 16: Other samples DNA was isolated from different hair samples and four different blood samples, buccal, samples . They were then run on agarose gel electrophoresis and the above bands were observed indicating the presence of DNA in the samples. Our results were agreed with Butler (2009) who discussed the individuals identify, scan 13 loci of DNA, or regions, that personally varied and the data use to produce profile of DNA for individual (DNA fingerprint), extremely, there is a few chance that the same DNA to another person for a 13 regions set particularly. DNA was extracted from different part of the body to personal identity using VNTR method, this results agreed with Hammond *et al.* (1995) ; Sumi *et al.* (2005) who find the DNA extracting and testing it for the presence of a specific DNA regions set (markers) for the identify of individuals.

There are variable regions to individual DNA generation profile, by using samples from bone, blood, hair, buccal and other tissue of body (Lee *et al.*, 2006 ; Silva *et al.*, 2007).

DNA samples	7ul
Hind 111	2ul
Assay buffer	2.5ul
Nucleus. H2O	13.5ul
Total volume	25ul

Table 2. The following values were obtained for the samples

S.No.	Sample	OD at 595nm	Con.
1.	STND	0.10	1000
2.	Hair	0.51	880
3.	Hair	0.63	180
4.	Hair	0.82	1390
5.	Other sample	0.59	1020
6.	Blood	0.82	1390
7.	Blood	0.54	930
8.	Blood	0.48	940
9.	STND	00.10	1000
10.	OTHER SAMPLE	-	-
11.	OTHER SAMPLES		
12.	Buccal	0.72	1840
13.	Buccal	0.74	2011
14.	Buccal	0.64	1658

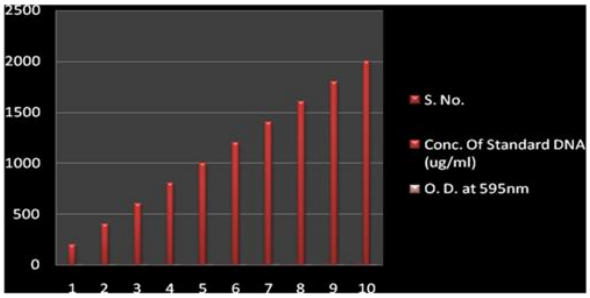

Fig. 2. Agarose gel electrophoresis for PCR products

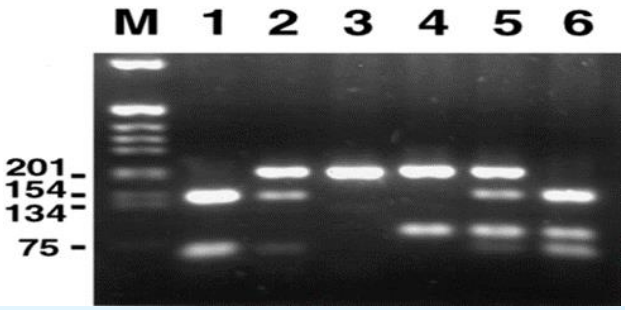

Fig. 3. VNTR fingerprints for D1S80 locus among different samples

Quantitative analysis: Reliable DNA purity and concentration measurement is important for numerous applications is in molecular biology.

The result of samples gene amplification and subsequent agarose gel electrophoresis showed the bands.

The result of samples was: 1,2 – Blood samples 3,4-hair sample 5,6-Buccal sample

The above bands were observed in UV transilluminator after amplification of the VNTR regions by PCR and running them on agarose gel electrophoresis.

From the above band pattern it can be conclude that all of the hair ,blood , and buccal cell samples were from different individuals as the fingerprints were not matching with each other. It can also be said that all of the individuals analyzed were heterozygous. 16–64 base pairs sequence for each repeating unit. specific restriction enzymes used for the VNTR sites flanking, in

different individuals, fragments of variable lengths can be obtained due to each subject has a different number of repeats. Also, can be obtained the fragments and multiplied by PCR using complementary primers to the VNTR flanking sequences. DNA with electrophoretic patterns differing in 6 or more bands from other bands are considered as unrelated. This results agreed with (Nübel *et al.*, 1996). Kim *et al.* (2019) developed a new kit for test human genomic DNA for forensic studies that get results agreed with the results of the present study. The current results agreed with other results obtained by Anchordoquy *et al.* (2003);Rogers *et al.* (2007) that noted the profile of DNA for individual, DNA is mostly isolated from the cheek / buccal cells since it contains the maximum amount of DNA.

CONCLUSION

Typing of Variable Number Tandem Repeat (VNTR) is a genotyping process that provides a numeric and simple format of data based on the repetitive sequences number, this process can helps in criminal identification. It can also indicate whether the individuals are homozygous or heterozygous.

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