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Der Pharmacia Lettre, 2016, 8 (14):73-79
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Mutations in mtDNA D-loop region of mtDNA in various tissues of Papuan individuals

Johnson Siallagan,[1] Agnes Maryuni,[1] Jukwati,[2] Rosye H. R. Tanjung[3] and Yohanis Ngili[1]

[1]Department of Chemistry, Faculty of Mathematics and Natural Science, University of Cenderawasih, Jayapura, Indonesia
[2]Study Program of Chemistry, Faculty of Teacher Training and Education, University of Cenderawasih, Jayapura, Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Cenderawasih, Jayapura, Indonesia

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ABSTRACT

High mutation rate of mtDNA causes the difference in the nucleotide sequence of mtDNA between individual (high degree of polymorphism). At the mtDNA there are areas that do not encode controller (noncoding region), which is known by the local displacement loop (D-loop), which has two areas with high variations which hypervariable region I (HVR1) and hypervariable region II (HVR2). But there is no information on whether the nucleotide sequence of mtDNA D-loop is the same for the different cells in certain individuals. The purpose of this study to obtain nucleotide sequence information area mtDNA D-loop different cells on each individual to five individuals with different ages. Stages of research performed includes preparation of template mtDNA by way of cell lysis. Amplification fragments of mtDNA D-loop with the method of Polymerase Chain Reaction (PCR) using the primers M1 and HV2R. Analysis of the results of PCR with the aid of agarose gel electrophoresis using standard pUC19 which is cut by the restriction enzyme HinfI (pUC19/HinfI). The results of the analysis of nucleotide sequences using DNASTAR seqman program with rCRS as references show that for three different cells, ie blood cells, epithelial cells, and hair cells, in individuals in individuals Papua, position and type of mutation of each individual are the same or homology. Meanwhile, nucleotide sequence analysis of blood cells and hair cells of individuals with different ages also shows the position and type of the same mutation. Thus, the nucleotide sequence of mtDNA D-loop in different cells are blood cells, epithelial, and each individual hair to show the same mutation. This is because the cells are derived from a single egg that has one type of mtDNA then differentiated in line with the development of the embryo. In the next phase of development into an adult human, this differentiation does not lead to any changes in the nucleotide sequence of mtDNA in blood cells, epithelial, and hair in a single individual. Thus, all three cell types can be said to represent the whole cell types that exist in the human body. Hopefully, the results of this study can be useful to facilitate the identification process in the field of forensics.

Keywords: mtDNA mutations, D-Loop, mtDNA, various tissues, the population of Papua

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INTRODUCTION

One of the unique properties of mitochondrial DNA (mtDNA) is relatively higher mutation rate than nuclear DNA. The high mutation rate in mtDNA caused by mtDNA does not have a repair system for the replication process. DNA polymerase γ has no proofreading activity that does not have the ability to correct errors.

Mitochondrial DNA is unique, unlike nuclear DNA because mitochondrial DNA is inherited through the maternal lineage. The egg cell has a high number of copies of mtDNA ($\geq 10^5$) while sperm cells have low mtDNA copy number (50-75) and is present in the tail. At the time of fertilization of the egg, the sperm tail section separated so that no mtDNA into the egg. Because recombination does not occur, then the mtDNA is haploid, handed down from mother to all his descendants. Mitochondrial DNA also is unique and distinct from nuclear DNA because it has a

high mutation rate, which is about 5-10 times the nuclear DNA because the mitochondrial DNA do not have a repair mechanism, does not have a histone protein as a protector, and has a high content of free radicals [1- 3].

DNA replication is not always accurate so there will be mutations that will be passed down from one generation to the next, so far away kinship between two individuals, the greater the number of differences in mutation. Variations base or polymorphism can occur in coding regions and noncoding regions in the displacement loop (D-loop) can be used to distinguish one individual to another [4-5]. However, polymorphisms in the D-loop region is higher than the coding region polymorphisms caused by the mutation rate is higher. D-loop regions have two areas of high variation which hypervariable region I (HVR1) and hypervariable region II (HVRII). High mutation rate of mtDNA causes the many differences in the nucleotide sequences of mtDNA between individual (high degree of polymorphism). However, no information on whether the nucleotide sequence of mtDNA D-loop is the same for the different cells in certain individuals.

MATERIALS AND METHODS

Characteristics of five individuals sampled were healthy and did not have a kinship between one individual to another individual. The sample consisted of three different cells (blood, epithelium and hair) from five individuals of different ages. Sampling and epithelial hair done by researchers. While blood sampling on five individuals to use health care services.

Template of mtDNA and lysis of hair root cells, epithelial and blood

Template mtDNA prepared using cell lysis. Cell lysis is performed in a lysis buffer consisting of 50 mM Tris-HCl pH 8.5; 1 mM EDTA pH 8.0; and 0.5% Tween-20 [6-7]. Hair root cell lysis begins with a small cut 5-7 strands of hair at the roots (whitish) using a knife that has been sterilized with 70% alcohol. Pieces of hair root is then inserted into a micro tube of 1.5 mL lysis buffer containing 30 mL and 10 mL proteinase K 20 mg/mL 260 mL ddH_2O added. Then incubated for 1 h at 50 °C and continued for 10 min in boiling water bath. The mixture was centrifuged using mikrosentrifuga cell extracts with a speed of 12000 rpm for 3 min. Supernatants are a source of mtDNA template for PCR reaction [8-10].

Epithelial cell lysis begins by cutting small pieces of filter paper containing epithelial cells in a 1.5 mL micro tube. The next process is done the same as in the hair root cell lysis. Blood cell lysis begins by taking 100 mL of blood with a micro pipette and then inserted into the tube and then added 1.5 mL 500 mL TE buffer, homogenized with vortex 30 seconds and then centrifuged at 8000 rpm 1 min, the supernatant was discarded, the process is done to obtain a clean white pellets, prepared in lysis. Lysis do the same as the roots of the hair follicle cell lysis, it's just a mixture of cell extracts using mikrosentrifuga centrifuged at a speed of 8000 rpm for 3 min [11].

1 kb fragment amplified mtDNA D-loop PCR

1 kb fragment amplified region mtDNA D-loop for all the samples was done by using Polymerase Chain Reaction (PCR) using the primers M1 and HV2R developed by [12]. PCR reactions were performed in 0.5 mL micro-tubes containing 50 uL reaction mixture consisting of 1.25 units of Taq DNA polymerase enzyme, 5 mL lysis results snippets, 20 pmol of each primer M1 and HV2R, 5 uL 10X PCR buffer (Tris -HCl 100 mM pH 9; 500 mM KCl; 15 mM MgCl2), 0.2 mmol of dNTPs consisting of dATP, dTTP, dGTP, dCTP, and sterile ddH2O. The PCR process is carried out in a Thermal Cycler Automatic machine as many as 30 cycles, each cycle consisting of template denaturation step at 94 °C for 1 min, stage annealing at 50 °C for 1 min, and a primer elongation stage at 72 °C for 5 min. Stages PCR performed consisted of denaturation at 94 °C for 1 min, annealing at a temperature of 50 °C for 1 min, and primer extension by DNA polymerase at 72 °C for 1 min. PCR reactions were performed by 30 cycles, and to enhance the reaction, at the end of the cycle added one stage polymerization at 72 °C for 5 min.

Determination of nucleotide sequence

Determination of nucleotide sequence begins with the preparation of sequencing reaction. PCR Results of that have been extended to 700 ng included in a 1.5 mL micro tube. Primer M1 prepared with a concentration of 10 pmol/mL to 1.5 mL micro tube. For one sequencing reaction primer required 3 mL with a concentration of 10 pmol/mL. Nucleotide sequence fragments of 1 kb region mtDNA D-loop of about 1021 bp PCR product was determined by dideoxy method [13-14].

RESULTS AND DISCUSSION

In this study analyzed 12 samples of cells derived from individuals with different ages. Five individuals do not have a kinship between one individual to another individual. MtDNA template is prepared using cell lysis. Cell lysis is performed in a lysis buffer containing Tween-20 (Merck). Tween-20 is a non-ionic detergent in the solution to form micelles. The molecular structure of Tween-20 has a hydrophilic portion composed of ester or alcohol and hydrophobic parts which are hydrocarbons. Hydrophobic interaction of micelles of Tween-20 with compounds of

cell membrane phospholipids make membrane phospholipids soluble compounds forming micelles mix with Tween-20.

Sequencing 1 kb fragment of mtDNA D-loop

The sequencing results obtained 1 kb fragment mtDNA D-loop around 200-600 bp on 12 samples of cells. Given that the amount of variation HV1 region mutation (*polymorphic*) is higher than the regions HVR2, an observation which focuses on the overall HVR1 and most HVR2 been quite representative in determining the nucleotide sequence of the D-loop region of mtDNA. Results of sequencing to mtDNA of 12 samples of cells from five different individuals whose age is obtained in the form electropherogram. In individuals aged 30 years showed peaks same electropherogram blood cells, epithelial cells, and hair cells.

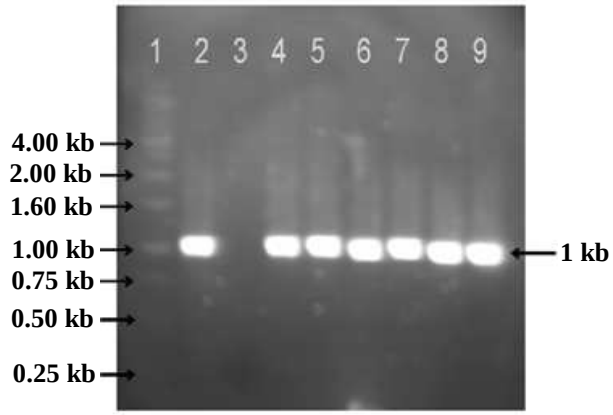

Fig 1. Results PCR fragment D-loop. Fragment amplified using primers M1 and M2. Lane 1: Marker 1 kb ladder, lane 2 is the control (+), lane 3 is the control (-), and lanes 4-9 are samples of human tissues Individual of Papua, Indonesia (0.982 kb)

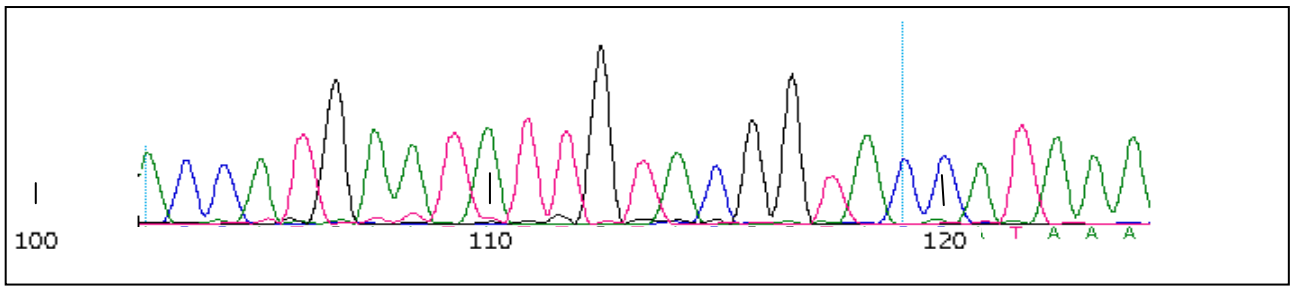

Fig 2. Results of such sequencing electropherogram D-loop region of mtDNA in blood cells of individuals aged 30 years (Papua 1)

MtDNA D-loop mutations of different cell samples for one individual

Results of the analysis of mutations in blood cells, epithelial cells, and hair cells showed the same mutation in individuals aged 30 years. Electropherogram region showed mtDNA D-loop mutations T16140C transition and transition C16174T same mutation in blood cells, epithelial, and individual hairs. Similarly, electropherogram region D-loop mtDNA that showed mutations transversion A16182C and A16183C, and mutation transition T16189C same blood cells, epithelial cells, and hair cells in individuals aged 30 years compared to a primary standard (rCRS) and a secondary standard that is derived from a sample on another individual. Additional samples are used as a secondary standard has the same nucleotide positions with the analyzed samples mutations. The secondary standard is used to compare the mutations that occur in the analyzed samples with mutations that occur in other samples to rCRS at the same nucleotide position.

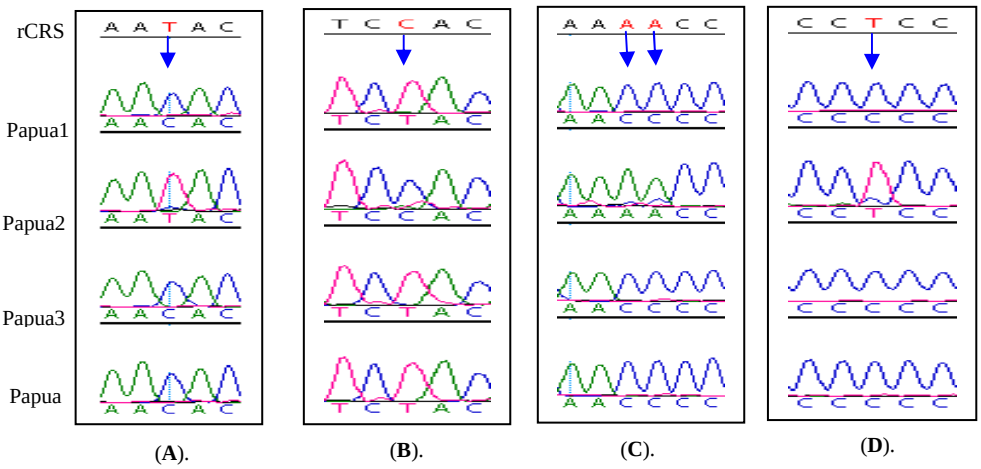

Fig 3. Electropherogram D-loop region that indicates a) .mutasi T16140C transition, b). C16174T transition mutations, c). transversion mutation A16182C and A16183C, and d). T16189C same transition mutation in blood cells, epithelial cells, and hair cells in individuals Papua than primary standard (rCRS) and secondary standards

Thus, individuals aged 30 years have five same mutation in blood cells, epithelial cells, and hair cells. One of them C16189T transition mutation causing poly C were also similar in blood cells, epithelial cells, and hair cells. Poly C nucleotide sequences showed that the nucleotide sequence further can not be read again with the same kind of sequencing primer is primer M1. Therefore, do sequencing using primer M2 generate the same six mutations in blood samples and hair Papua human individual.

Meanwhile, eight other individuals have mutations in the D-loop region the same blood cells, hair cells, and epithelial cells. Electropherogram D-loop region showed the same T16086C transition mutation in blood cells, epithelial cells, and hair cells in individuals older than 40 years compared to a standard primary and secondary standards are derived from the sample on another individual.

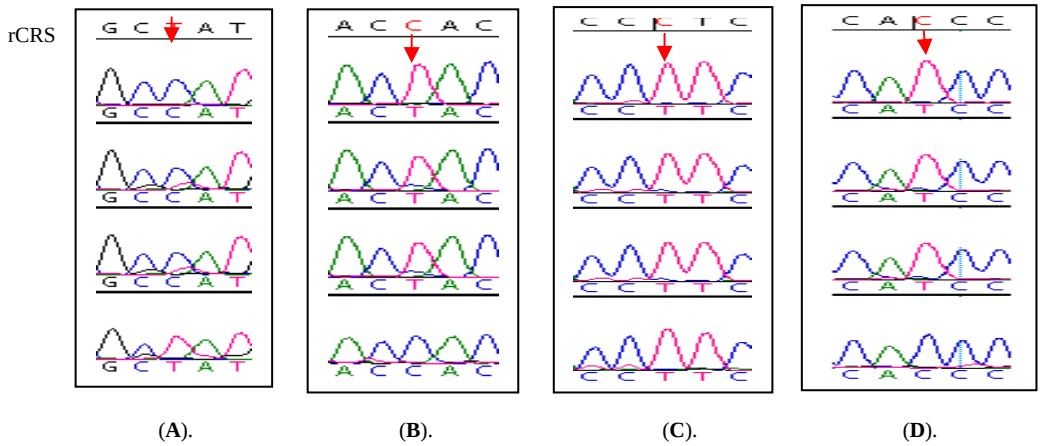

Fig 4. Electropherogram results of sequencing the human Papua in D-loop region that indicates a). T16086C transition mutation, and b). C16148T transition mutations, c). C16223T transition mutation and d). C16259T same transition mutation in blood cells, epithelial cells, and hair cells in the human individual Papua than rCRS as a primary standard and the other sample is used as a secondary standard

Similarly, the D-loop region electropherogram showing the transition mutation C16259T and C16278T same on blood cells, epithelial cells, and hair cells in individuals 40 years of age compared with CRS as a primary standard and the other sample is used as a secondary standard. Electropherogram D-loop region that shows another mutation on a sample of individuals aged 40 years. Thus, individuals older than 40 years had eight mutations in the D-loop region the same blood cells, epithelial cells, and hair cells.

D-loop mutations of different cell samples for some individuals

Electropherogram D-loop region of mtDNA in different cells for individuals aged 30 years and 40 years, shows that each individual has the same mutation in blood cells, epithelial cells, and hair cells. Individuals 30 years of age have the same five mutations in blood cells, hair cells and epithelial cells, one of which T16189C transition mutation causing poly C were also similar in blood cells, epithelial cells, and hair cells. Poly C nucleotide sequences showed that the nucleotide sequence further can not be read again with the same kind of sequencing primer is primer M1.

Therefore, do sequencing using primer M2 produced six same mutation in blood cells and hair cells of individuals aged 30 years.

Based on the analysis of mutations in blood cells and hair cells of individuals aged 30 years were sequenced with primer M2, found a mutation that is complementary to A16189G transition from T16189C transition mutation. The type of mutation that is dominant in both samples are transition mutations, the insertion mutation 16301C same blood cells and hair. Thus, the D-loop region of mtDNA has been successfully determined 11 mutation variants that showed the same mutation on a sample of blood cells and hair cells of individuals aged 30 years. Similarly, individuals older than 40 years had eight mutations in the D-loop region the same blood cells, epithelial cells, and hair cells. Electropherogram D-loop region showed the same T16086C transition mutation in blood cells, epithelial cells, and hair cells in individuals older than 40 years compared to a primary standard (rCRS) and secondary standards are derived from the sample on another individual.

Meanwhile, the D-loop region electropherogram showing the same mutation in blood cells and hair cells are found in individuals ages 10, 20 and 80 years. Individuals 10 years of age have the same three mutations in blood cells and hair cells. Electropherogram region D-loop that shows a mutation transition C16147T, an insertional mutations at position 16183C, and one mutation transition T16189C each showed the same mutation in blood cells and hair cells in individuals aged 10 years compared to a primary standard (rCRS) and sample another function as a secondary standard.

One 16183C insertional mutations cause readings nucleotide sequence at CRS sequence one base shifted to the left, so the mutation indicated as a deletion mutation T16190 shifted its readings into T16189C transition mutation that causes the nucleotide sequence of poly C. However, the three mutations in samples showed the same in blood cells and hair cells of individuals aged 10 years.

One 16136G insertional mutations cause subsequent reading of the nucleotide sequence in the CRS sequence one base shifted to the left, so the mutation shown as a transition mutation T16190C shifted its readings into T16189C transition mutation that causes the nucleotide sequence of poly C. However, five mutations in samples showed mutations the same blood cells and hair cells of individuals aged 20 years. Meanwhile, seven people of Papua 3 shows the same mutation in blood cells and hair cells. Electropherogram region D-loop that showed mutations transition C16067T, mutations transition G16129A mutation transition T16136C, mutations transition T16140C, and mutations transition C16223T same blood cells and hair cells in individuals aged 80 years compared to a primary standard (CRS) and standard secondary derived from another individual samples were used as a secondary standard.

Table 1. Total mtDNA D-loop mutations in the samples analyzed on Papuan Populations. Mutations with CRS as standard on twelve samples comprising cells from blood cells, epithelial cells and hair cells from five individuals

Nucleotide positions	16067	16086	16129	16136	16136	16140	16147	16148	16169	16174	16182	16183	16183	16189	16223	16259	16271	16278	16319	16399	16519	16526
rCRS	***C***	***T***	***G***	-	***T***	***T***	***C***	***C***	***C***	***C***	***A***	-	***A***	***T***	***C***	***C***	***T***	***C***	***G***	***A***	***T***	***G***
Blood	.	.	.	.	.	.	t	.	.	.	.	.	c	c	.	.	.	.	.	.	.	.
Hair	.	.	.	.	.	.	t	.	.	.	.	.	c	c	.	.	.	.	.	.	.	.
Blood	.	.	.	g	.	c	.	.	a	.	.	.	-	c	.	.	.	.	.	.	.	.
Hair	.	.	.	g	.	c	.	.	a	.	.	.	-	c	.	.	.	.	.	.	.	.
Blood	.	.	.	.	.	c	.	.	.	t	c	c	.	c	.	.	.	.	.	.	.	.
Epithelium	.	.	.	.	.	c	.	.	.	t	c	c	.	c	.	.	.	.	.	.	.	.
Hair	.	.	.	.	.	c	.	.	.	t	c	c	.	c	.	.	.	.	.	.	.	.
Blood	.	c	.	.	.	.	.	t	.	.	.	.	.	.	t	t	.	t	a	g	.	a
Epithelium	.	c	.	.	.	.	.	t	.	.	.	.	.	.	t	t	.	t	a	g	.	a
Hair	.	c	.	.	.	.	.	t	.	.	.	.	.	.	t	t	.	t	a	g	.	a
Blood	t	.	a	.	c	c	.	.	.	.	.	.	.	.	t	.	c	.	.	.	c	.
Hair	t	.	a	.	c	c	.	.	.	.	.	.	.	.	t	.	c	.	.	.	c	.

Showed the same mutation G16129A transition in blood cells and hair cells of individuals aged 80 years, this type of mutation is also found in patients with osteosarcoma (Guang Guo, 2006). Mutation G16129A transition mentioned above are only found in individuals aged 80 years showed the same mutation in blood cells and hair cells. Similarly, electropherogram showing the transition mutation T16271C and T16519C showed the same mutation in blood cells and hair cells of individuals aged 80 years. Thus, in individuals aged 80 years was found seven mutations in the same blood cells and hair cells.

The position and type of mutations in blood cells and hair cells of individuals Papua 4 above is the same. Thus, the nucleotide sequence of the D-loop region of mtDNA in 12 cells were analyzed, with CRS as a standard showed the same mutation in different individual cells (Table 1).

In mtDNA mutations are nucleotide differences found in the samples compared to standard rCRS because rCRS not necessarily wild type. The type of mutation that is dominant on twelve samples from five individuals is a mutation of transition that the mutation caused by the change of purine bases into purine bases others are adenine into guanine, and or changes of pyrimidine bases into pyrimidine else is thymine be cytosine, or vice versa. Transversion mutation is always less than the transition mutation. This is because the reaction phase transversion mutation is longer than the transition mutation.

The results of the analysis determining the nucleotide sequence was determined by Sanger dideoxy method for all sample of approximately 5,500 bp, has been successfully obtained. The results of the analysis of nucleotide sequences using the program seqman DNASTAR with rCRS as references show that for three different cells, ie blood cells, epithelial cells, and hair cells, in individuals aged 30 years and 40 years, the position and type of mutation of each individual is same. Meanwhile, nucleotide sequence analysis of blood cells and hair cells of individuals aged 10, 20 and 80 years also shows the position and type of mutation that sama.Urutan nucleotide same mtDNA D-loop in different cells of each individual. This is caused because the cells are derived from a single egg that has one type of mtDNA then differentiated in line with the development of the embryo. In the next development phase into adult human, this differentiation does not lead to any changes in the nucleotide sequence of mtDNA in hair cells, epithelial, and blood in a single individual. Thus, all three cell types can be said to represent the whole cell types that exist in the human body. Based on the foregoing, it can be proposed to use one of the blood cells or epithelial cells or hair cells in the forensic identification purposes. It is based on the findings of this study that the nucleotide sequence of mtDNA D-loop the same on all three of these cells to a variety of individuals with different ages [15-19].

CONCLUSION

PCR amplification of the D-loop region of mtDNA of 12 samples of five different individual, was observed on an agarose gel as a single band of DNA which is estimated 1 kb. Nucleotide sequence was determined by Sanger dideoxy method for all sample of approximately 5,500 bp, has been successfully obtained. The results of the analysis of nucleotide sequences using the program seqman DNASTAR with rCRS as references show that for three different cells, ie blood cells, epithelial cells, and hair cells, in individuals aged 30 years and 40 years, the position and type of mutation of each individual is same or homology. Meanwhile, nucleotide sequence analysis of blood cells and hair cells of individuals aged 10, 20 and 80 years also shows the position and type of the same mutation. On the basis of the above, it can be proposed to use one of the blood cells or epithelial cells or hair cells in forensic purposes because the nucleotide sequence of mtDNA D-loop the same on all three of these cells to a variety of individuals with different ages. Hopefully, the results of this study can be useful to facilitate the identification process in the field of forensics.

Acknowledgements
The authors gratefully acknowledgement the support of this work by Competitive Research Grant from the Directorate of Research and Community Services, the Ministry of Research, Technology and Higher Education in 2016 to JS. Thanks for Biochemistry laboratory facilities at the Bandung Institute of Technology (ITB) that has helped in the process of PCR amplification and sequencing analysis.

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