

Paternity Test Through Kinship Analysis and Cell Free Fetal DNA (Cff-DNA) as a Forensic Identification Technique

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Abstract

The application of kinship analysis and cell-free fetal DNA (cff-DNA) analysis can be found in forensic identification processes, mainly on (civil or criminal) parentage testing, disaster victim identification, missing person identification, and familial searching (Butler, 2006; 2015). So far, paternity tests using kinship analysis and cell-free fetal DNA (cff-DNA) as a forensic identification examination are still not widely known.

This study aims to analyze the application of kinship analysis and cff-DNA analysis in paternity examination tests. This study is observational laboratory research with a one-shot research design. The extracted DNA sample was measured for its DNA contents and purity. The average DNA contents were 575 ± 4.33 ng/ μ l with the purity range of 1.035-1.82 while the average DNA contents for maternal plasma DNA (cff-DNA) were 75 ± 2.31 ng/ μ l with the purity range of 1.12-1.56. The highest allele frequency was on allele 31 of the D21S11 locus (64.375%). All the examined STR CODIS loci showed that allele sharing was dominated by two allele sharing with a percentage higher than 50%.

The 13 STR CODIS loci has the highest percentage of two allele sharing. Based on this finding, it is recommended that paternity tests can be performed through kinship line by using siblings' DNA in case the DNA from the parents are unavailable, and the use of cff-DNA as a non-invasive method in paternity test examination.

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Introduction

A paternity test is a valid legal procedure in determining parentage. Up until today, the solution for paternity problems is often determined by assessing similar aspects or the dissimilar aspects of a child and his/her alleged father. The similar aspects here refer to the characteristics of iris color, hair, unique gesture and way of speaking, and body stature.¹

The unavailability of information originating from both parents during the identification process is one of the problems in forensic DNA

analysis, especially in a paternity test.^{2,3} Therefore, a comparison originating from close family or relatives such as biological siblings is required as one of the solutions in the forensic DNA analysis process.

Paternity tests can be conducted before an individual is born. DNA test can be conducted by using chorionic villi sampling (CVS) at the 10th-13th week of pregnancy or by using amniocentesis at the 14th-24th week of pregnancy. Inside a mother's body, molecules are transferred from the mother to the fetus and vice versa. Studies on this two-way molecular transfer have been developed and one of the topics with the most rapid development is cell-free fetal DNA (cff-DNA).⁴

The principles of forensic DNA examination are based on the comparison of alleles originating from the victims or perpetrators with the comparing sample from their family or relatives (kinship analysis). It is common in cases

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such as unborn child disputes, paternity disputes, or in forensic DNA analysis on mass disaster victims or war victims. In these conditions, a comparison originating from a close family line is required as a means in the forensic DNA analysis process, for example, the siblings of the victim or perpetrator in case the information from parents or descendants is unavailable.

Objectives to explain the application of kinship analysis and cell-free fetal DNA (cff-DNA) analysis can be found in forensic identification processes, particularly on parentage testing (civil or criminal), disaster victim identification, missing person identification, and familial searching.^{5,6} So far, paternity tests using kinship analysis and cell-free fetal DNA (cff-DNA) as a forensic identification examination are still not widely known.

Materials and methods

Research Samples

This research was observational laboratory research with a one-shot study design. The respondents of this study consisted of 20 women in the second and third trimesters of pregnancy and their two biological children. This study obtained ethical clearance from the Faculty of Dentistry Universitas Airlangga Number 275/HRECC.FODM/VI/2020. This study was conducted at the Human Genetics Study Group, the Institute of Tropical Diseases, Universitas Airlangga.

Sample Preparation

Twenty samples of respondents' peripheral blood were stored in tubes and labeled A (mother), B (Child 1), and C (Child 2) to indicate the blood samples from mothers and biological children.

DNA Extraction

For pregnant women's blood samples: the blood samples were centrifuged at 1600 rpm for 10 minutes. The supernatant was moved into Eppendorf tubes and was centrifuged at 1600 rpm for 10 minutes. The supernatant was removed and the DNA was extracted while for children 1 and 2's blood sample, the DNA was extracted. The DNA extraction process of the 20 peripheral blood samples was carried out using the DNAzol method.⁷ 50 µl distilled water was added to the isolated DNA pellets.

DNA Amplification

The DNA amplification was conducted through the Polymerase Chain Reaction (PCR) process (PowerPlex® 21Systems, Promega,

USA) targeting specific DNA sequences to produce a number of replications of the isolated DNA. The amplification of the 80 samples used 13 primers of Short Tandem Repeats [STR]-Combined DNA Index System [CODIS] (TPOX, D3S1358, FGA, D5S818, CSFIPO, D7S820, D8S1179, THOI, vWA, D13S317, D16S539, D18S51, D21S11) and Amelogenin (Amel) x:106bp,y:112bp. The amplification adjustment for D3S1358, FGA, D8S1179, D18S51, and D21S11 was: 96 °C- 2 minutes, followed by [94 °C-1 minute, 60 °C-1 minute, 70 °C-1.5 minutes for 10 cycles], [90 °C-1 minute, 64 °C- 1 minute, 70 °C-1.5 minutes for 25 cycles]. The adjustment for CSF1PO was 96 °C-2 minutes, followed by [94 °C-1 minute, 64 °C-1 minute, 70 °C-1.5 minutes for 10 cycles], [90 °C-1 minute, 64 °C- 1 minute, 70 °C-1.5 minutes for 30 cycles]. The amplification adjustment for D5S818, D7S820, and D13S317 was 96 °C- 1 minute, followed by [94 °C-30 seconds, 60 °C-30 seconds, 70 °C-45 seconds for 10 cycles], [90 °C-30 seconds, 64 °C- 30 seconds, 70 °C-45 seconds for 30 cycles]. The amplification adjustment for D16S539 was 96 °C-1 minute, followed by [94 °C-1 minute, 59 °C-1 minutes, 72 °C-1.5 minutes for 25 cycles] and 72 °C-1 minutes. All DNA templates were stored at 4 °C temperature.⁸

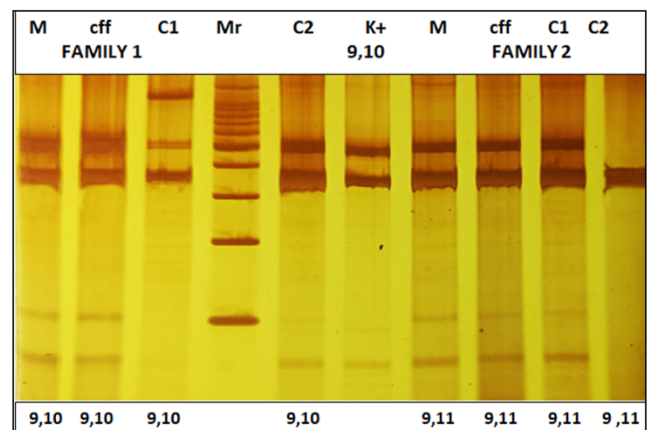


Figure 1. The PCR visualization of CSF1PO locus (321 bp-357 bp) Mr (marker 100 bp), M (mother), cff (cell-free fetal), C1 (Child 1), C2 (Child 2).

Electrophoresis Gel

The visualization of PCR results was conducted through vertical electrophoresis using 6% polyacrylamide agarose gel [PAGE] [Bio-Rad Mini-PROTEAN®] with Silver nitrate staining (Figure 1).

Allele Sample

The reading of PCR visualization on the electrophoresis gel resulted in alleles at each locus with K562 as the control (Table 1).

The alleles were matched with family members (the father, the mother, and the children) and the value of allele frequency (Table 2).

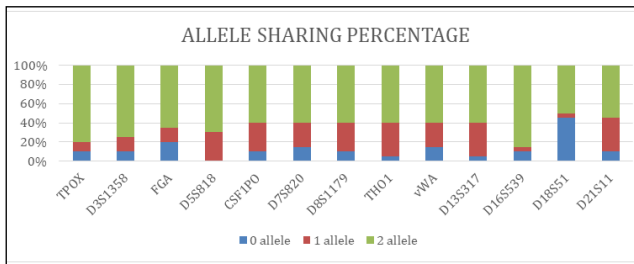


Figure 2. Percentage of allele sharing on biological children.

The analysis was based on the frequency of allele sharing based on kinship analysis between the biological siblings at every STR CODIS locus by assessing the use of allele sharing (Figure 2).

Results

The extracted DNA samples were measured for their DNA contents and purity range. The average value of biological children's DNA contents was 675 ± 5.35 with a purity range of 1.05-1.86. The average value of pregnant women's DNA contents was 4.75 ± 3.31 ng/ μ l with the purity range of 1.02-1.78 while the average value of maternal plasma DNA (cff-DNA) was 75 ± 2.31 ng/ μ l with the purity range of 1.12-1.56.

The PCR amplification through the primers of 13 STR-CODIS loci and the visualization of PCR results through Polyacrylamide Agarose Gel (PAGE) with silver nitrate staining are presented in the figure 1.

Discussion

The genetic materials of an individual are inherited from his/her parents with each of his/her parents contributing 50% of genetic materials (as presented in Table 1). Since the nuclear DNA is inherited from the father and the mother, it can be said that nuclear

DNA is inherited according to the Mendelian way. The first Mendel's law (the segregation of allelic genes) discusses the principles of allelic segregation during gamete formation. Gamete formation occurs through meiosis where the homologous pairs are decoupling and segregated. The alleles of a gene freely segregate from diploid to haploid.^{9,10,11} Table 2 presents the frequency of the allele in this study, namely on D21S11 allele 31 (64.375%).

In genetics, alleles are the alternative forms of genes in certain loci that are related to the expression of certain traits (phenotype). Alleles are formed as the result of variations in nitrogen base sequences caused by mutation. In an individual, allele pairs determine the genotype of the individual. The term 'allele' exists as the result of the use of the term *allelomorph* in Mendel's *Principles of Heredity*.^{5,9,12}

Individuals with the same allele at a locus are said to have a homozygote genotype while individuals with different alleles are heterozygote genotypes. Since genotypes are expressed into certain phenotypes, alleles may cause different appearances among individuals within a population.^{5,6}

A paternity test is a DNA test aimed to determine whether a man is the biological father of a child. Family dispute cases, in the form of doubting parents, are increasingly popular in Indonesian society.²

Therefore, a paternity test uses parents as the comparison where the results are statistically close to 100% or about 99.99%.¹³ The unavailability of information originating from a father, a mother, or a child that can be used as a comparison in forensic DNA examination becomes a problem in forensic DNA examination.¹⁴ Unlike DNA testing with parental DNA as the comparison, the accuracy rate of personal identifications using siblings' information is not close to 100%.¹⁵

The finding of this study indicates that the alleles of STR CODIS loci with the

highest percentage are TPOX allele 9 [34.375%], D3S1358 allele 17[35%], FGA allele 21[35.625%], D5S818 allele 11[32.5%], CSF1PO allele 9 [35.75%], DS820 allele 9[38.12%], D8S1179 allele 12[50%], TH01 allele 9[27%], vWA allele 18[22.25%], D13S317 allele 8[43.75%], D16S539 allele 10[29.375%], D18S51 allele 16[34.375%], and D21S11 allele 31[64,375%] (Table 2).

The principle of identification examination through DNA is based on the process of allelic comparison between the victim or the perpetrator and alleles from the family line, especially parents, following the Mendelian laws.^{16,17,18} In case the parental or descendant line is unavailable, a comparison with close family line is needed as one of the methods taken in an identification examination through DNA, namely biological siblings (i.e. full brother or full sister). The use of siblings as the comparison is one of the identification methods known as kinship analysis. Similar to a paternity test, the kinship analysis of an identification process using siblings as the comparison also has a possibility of mismatched profile in the examined DNA loci.^{10,15,19,20,21}

Allele sharing plays a significant role in kinship analysis. Wenk, Traver, and Chiafari (1996) stated that in determining full siblings, allele sharing was very useful in defining kinship relationships when the two alleles were interrelated.²² Statistically, full siblings had the probability of two exact alleles as much as 0.25 (25%). This value was as high as not having a shared allele (zero allele

sharing) while the probability of one shared allele was 50%.¹⁵ The findings of this study support the theory proposed by O'Connor (2011) above. All examined STR CODIS loci show that 2-allele sharing is dominant with an average value higher than 50% (Figure 2).

Allele sharing is a genetic variation an individual inherits from his/her parents. All individuals are part of a population as the result of mating between individuals and have the same gene pool. A gene pool is a collection of genes/alleles in a population.¹⁰

Hardy-Weinberg equilibrium principle states that the frequency of genes and genotypes will remain constant through generations in an equilibrium population. This can be found in a large population with randomized marriage and there is no attempt to regulate certain traits.^{11,23,24,25}

Conclusions

All examined STR-CODIS loci have the highest percentage of two allele sharing (percentage above 50%. Based on this finding, it is recommended that paternity tests can be conducted using sibling line or kinship line in case the parental line is unavailable. This study also recommends the use of cell-free fetal DNA as a non-invasive method in paternity examination.

Declaration of Interest

The authors declare that there are no conflicts of interest.

| Family | Ko de | STR CODIS | | | | | | | | | | | | | |
|--------|-------|-----------|----------|--------|---------|---------|---------|----------|-------|-------|----------|----------|---------|---------|----------|
| | | TPO X | D3S13 58 | FGA | D5S81 8 | CSF1P O | D7S82 0 | D8S1 179 | THO 1 | vWA | D13S 317 | D16S 539 | D18S 51 | D21 S11 | Ame 1 |
| 1 | A1 | 8,9 | 16,17 | 21,2 4 | 11,12 | 9,10 | 9,11 | 12,13 | 8,9 | 15,18 | 8,8 | 11,11 | 15,16 | 29,3 1 | 106, 112 |
| | B1 | 8,10 | 16,16 | 21,2 4 | 11,12 | 8,10 | 9,11 | 12,13 | 9,10 | 16,17 | 8,8 | 11,11 | 15,16 | 30,3 1 | 106 |
| | C1 a | 8,8 | 16,16 | 21,2 1 | 12,12 | 8,10 | 9,11 | 12,12 | 9,10 | 15,17 | 8,8 | 11,11 | 15,16 | 30,3 1 | 106, 112 |
| 2 | C1 b | 8,9 | 16,16 | 21,2 4 | 11,12 | 9,10 | 9,11 | 12,13 | 9,10 | 15,17 | 8,8 | 11,11 | 15,15 | 31,3 1 | 106, 112 |
| | A2 | 9,10 | 15,17 | 21,2 2 | 10,12 | 9,10 | 8,8 | 11,12 | 11,13 | 19,20 | 8,9 | 10,11 | 14,15 | 29,3 1 | 106, 112 |
| | B2 | 8,9 | 16,17 | 21,2 4 | 9,10 | 8,9 | 9,10 | 10,11 | 9,10 | 18,19 | 8,10 | 11,13 | 15,15 | 30,3 1 | 106 |

| | | | | | | | | | | | | | | | |
|----|----------|-------|-------|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----------|-------------|
| | C2 a | 8,9 | 17,17 | 21,2 1 | 10,10 | 9,9 | 8,9 | 11,11 | 9,11 | 19,19 | 8,8 | 11,11 | 15,15 | 31,3 1 | 106 |
| | C2 b | 8,9 | 15,17 | 21,2 2 | 10,12 | 8,9 | 8,9 | 11,11 | 9,11 | 19,19 | 8,9 | 11,11 | 15,15 | 31,3 1 | 106 |
| 3 | A3 | 8,9 | 15,17 | 20,2 4 | 9,11 | 8,8 | 8,9 | 9,10 | 8,9 | 15,17 | 8,11 | 9,11 | 14,16 | 29,3 2 | 106, 112 |
| | B3 | 9,10 | 16,17 | 22,2 4 | 11,11 | 9,10 | 9,10 | 11,12 | 8,10 | 18,19 | 9,10 | 9,10 | 16,16 | 30,3 2 | 106 |
| | C3 a | 9,10 | 16,17 | 24,2 4 | 11,11 | 8,9 | 9,9 | 10,11 | 8,8 | 17,18 | 9,11 | 9,9 | 16,16 | 32,3 2 | 106, 112 |
| | C3 b | 9,10 | 16,17 | 24,2 4 | 11,11 | 8,9 | 8,9 | 10,12 | 8,9 | 17,18 | 8,9 | 9,9 | 16,16 | 29,3 0 | 106, 112 |
| 4 | A4 | 9,9 | 16,18 | 22,2 4 | 8,9 | 9,10 | 8,11 | 9,12 | 11,13 | 20,21 | 9,11 | 10,11 | 15,16 | 31,3 2 | 106, 112 |
| | B4 | 9,10 | 17,18 | 21,2 2 | 10,12 | 9,11 | 9,10 | 12,13 | 11,12 | 16,18 | 9,11 | 10,10 | 16,17 | 29,3 1 | 106 |
| | C4 a | 9,9 | 18,18 | 22,2 2 | 9,10 | 9,9 | 9,11 | 12,12 | 11,11 | 16,20 | 9,9 | 10,10 | 16,16 | 31,3 1 | 106, 112 |
| | C4 b | 9,9 | 18,18 | 21,2 4 | 9,10 | 9,11 | 8,9 | 12,12 | 11,11 | 16,20 | 9,9 | 10,10 | 16,16 | 31,3 1 | 106 |
| 5 | A5 | 8,10 | 16,17 | 20,2 1 | 11,13 | 8,12 | 9,11 | 10,12 | 8,12 | 16,17 | 9,11 | 9,11 | 14,17 | 29,3 1 | 106, 112 |
| | B5 | 8,9 | 17,17 | 22,2 4 | 9,11 | 9,10 | 9,11 | 9,12 | 9,11 | 18,20 | 11,11 | 8,10 | 16,17 | 30,3 1 | 106 |
| | C5 a | 8,8 | 17,17 | 21,2 2 | 11,11 | 9,12 | 9,9 | 12,12 | 8,9 | 16,18 | 11,11 | 8,9 | 14,16 | 31,3 1 | 106, 112 |
| | C5 b | 8,8 | 17,17 | 21,2 2 | 11,11 | 9,12 | 9,9 | 12,12 | 8,9 | 16,18 | 11,11 | 8,9 | 17,17 | 31,3 1 | 106 |
| 6 | A6 | 7,10 | 15,18 | 21,2 1 | 9,11 | 8,9 | 8,10 | 11,12 | 8,9 | 19,20 | 8,9 | 11,13 | 14,15 | 29,3 1 | 106, 112 |
| | B6 | 10,11 | 16,17 | 22,2 3 | 11,11 | 10,11 | 9,11 | 12,13 | 10,13 | 18,19 | 10,12 | 10,12 | 15,15 | 30,3 1 | 106 |
| | C6 a | 7,11 | 16,18 | 21,2 2 | 11,11 | 8,11 | 8,9 | 12,12 | 8,10 | 19,19 | 9,10 | 11,12 | 15,15 | 30,3 1 | 106, 112 |
| | C6 b | 10,11 | 16,18 | 21,2 3 | 11,11 | 8,11 | 9,10 | 12,12 | 8,10 | 19,19 | 9,10 | 11,12 | 15,15 | 31,3 1 | 106, 112 |
| 7 | A7 | 9,10 | 16,17 | 20,2 1 | 10,12 | 10,11 | 8,11 | 11,13 | 11,12 | 17,19 | 8,8 | 11,11 | 14,16 | 29,3 1 | 106, 112 |
| | B7 | 8,9 | 15,17 | 22,2 4 | 11,13 | 9,10 | 9,10 | 10,12 | 9,10 | 17,20 | 8,9 | 10,10 | 16,16 | 30,3 1 | 106 |
| | C7 a | 8,9 | 17,17 | 21,2 2 | 11,12 | 10,10 | 9,11 | 11,12 | 9,11 | 17,17 | 8,8 | 10,11 | 16,16 | 31,3 1 | 106 |
| | C7 b | 8,9 | 15,17 | 21,2 2 | 11,12 | 9,11 | 8,9 | 11,12 | 9,11 | 17,19 | 8,8 | 10,11 | 16,16 | 31,3 1 | 106, 112 |
| 8 | A8 | 9,10 | 17,19 | 22,2 4 | 10,12 | 9,11 | 9,11 | 12,13 | 11,12 | 15,17 | 8,9 | 9,11 | 14,17 | 31,3 2 | 106, 112 |
| | B8 | 8,9 | 16,18 | 21,2 3 | 9,11 | 10,11 | 9,11 | 12,13 | 10,11 | 20,21 | 8,10 | 8,10 | 16,17 | 29,3 1 | 106 |
| | C8 a | 8,9 | 17,18 | 21,2 4 | 9,10 | 9,11 | 9,9 | 12,12 | 11,11 | 15,21 | 8,8 | 8,9 | 14,16 | 31,3 1 | 106, 112 |
| | C8 b | 8,9 | 17,18 | 21,2 4 | 9,10 | 9,11 | 9,9 | 12,13 | 11,11 | 15,21 | 8,9 | 8,9 | 17,17 | 31,3 1 | 106, 112 |
| 9 | A9 | 11,13 | 16,18 | 22,2 3 | 11,12 | 8,10 | 9,11 | 11,12 | 8,13 | 16,17 | 9,11 | 11,13 | 14,15 | 29,3 1 | 106, 112 |
| | B9 | 11,12 | 17,17 | 21,2 4 | 10,11 | 9,10 | 9,11 | 12,13 | 9,10 | 18,20 | 9,11 | 10,12 | 15,15 | 30,3 1 | 106 |
| | C9 a | 11,11 | 16,17 | 21,2 4 | 11,11 | 8,9 | 9,9 | 12,12 | 8,9 | 16,18 | 9,9 | 11,12 | 15,15 | 31,3 1 | 106 |
| | C9 b | 11,11 | 16,17 | 21,2 4 | 11,11 | 8,9 | 9,9 | 12,12 | 8,9 | 16,18 | 9,9 | 11,12 | 15,15 | 31,3 1 | 106, 112 |
| 10 | A1 0 | 10,11 | 15,17 | 20,2 4 | 9,11 | 8,12 | 9,11 | 11,13 | 8,11 | 15,18 | 9,11 | 10,11 | 15,16 | 31,3 2 | 106, 112 |
| | B1 0 | 9,10 | 16,18 | 19,2 2 | 11,13 | 8,10 | 11,11 | 10,12 | 8,9 | 16,17 | 11,11 | 10,10 | 16,17 | 29,3 1 | 106 |
| | C1 0a | 10,10 | 15,18 | 20,2 2 | 11,11 | 8,8 | 11,11 | 11,12 | 8,8 | 15,17 | 11,11 | 10,10 | 16,16 | 31,3 1 | 106, 112 |
| | C1 0b | 9,11 | 16,17 | 20,2 2 | 9,13 | 8,8 | 11,11 | 11,12 | 8,8 | 15,17 | 11,11 | 10,10 | 16,16 | 31,3 1 | 106, 112 |
| 11 | A1 1 | 11,13 | 15,17 | 19,2 1 | 10,12 | 9,10 | 8,8 | 12,13 | 8,9 | 15,17 | 9,11 | 9,11 | 14,17 | 29,3 1 | 106, 112 |

| | | | | | | | | | | | | | | | |
|----|----------|-------|-------|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----------|-------------|
| | B1 1 | 11,12 | 16,18 | 21,2 3 | 9,11 | 9,11 | 8,8 | 12,13 | 10,13 | 18,19 | 11,11 | 8,10 | 16,17 | 30,3 1 | 106 |
| | C1 1a | 11,11 | 15,18 | 21,2 1 | 9,10 | 9,9 | 8,8 | 12,12 | 8,10 | 17,18 | 11,11 | 8,9 | 14,16 | 31,3 1 | 106, 112 |
| | C1 1b | 11,11 | 16,17 | 21,2 1 | 9,10 | 9,11 | 8,8 | 12,13 | 8,10 | 17,18 | 11,11 | 8,9 | 17,17 | 31,3 1 | 106, 112 |
| 12 | A1 2 | 11,13 | 16,17 | 20,2 4 | 10,12 | 9,11 | 8,9 | 12,13 | 11,12 | 20,21 | 8,8 | 11,13 | 14,15 | 29,3 1 | 106, 112 |
| | B1 2 | 11,12 | 16,16 | 19,2 2 | 9,11 | 10,11 | 8,10 | 12,13 | 9,10 | 16,18 | 8,8 | 10,12 | 15,15 | 30,3 1 | 106 |
| | C1 2a | 11,11 | 16,16 | 20,2 2 | 9,10 | 9,11 | 8,8 | 12,12 | 9,11 | 16,20 | 8,8 | 11,12 | 15,15 | 30,3 1 | 106 |
| | C1 2b | 11,11 | 16,16 | 20,2 2 | 9,10 | 9,11 | 8,9 | 12,13 | 9,11 | 16,20 | 8,8 | 11,12 | 15,15 | 31,3 1 | 106 |
| 13 | A1 3 | 9,10 | 16,17 | 22,2 4 | 10,12 | 8,10 | 8,11 | 11,12 | 8,13 | 15,18 | 8,9 | 10,11 | 15,16 | 29,3 1 | 106, 112 |
| | B1 3 | 8,9 | 17,17 | 21,2 3 | 9,10 | 9,10 | 9,10 | 12,13 | 9,10 | 16,17 | 8,10 | 10,10 | 16,17 | 30,3 1 | 106 |
| | C1 3a | 8,9 | 17,17 | 21,2 4 | 10,10 | 8,9 | 9,11 | 12,12 | 8,9 | 15,17 | 8,8 | 10,10 | 16,16 | 31,3 1 | 106, 112 |
| | C1 3b | 8,9 | 17,17 | 21,2 4 | 10,12 | 8,9 | 8,9 | 12,12 | 8,9 | 15,17 | 8,9 | 10,10 | 16,16 | 31,3 1 | 106, 112 |
| 14 | A1 4 | 8,9 | 15,18 | 22,2 3 | 9,11 | 8,12 | 9,11 | 11,13 | 8,11 | 16,17 | 8,8 | 9,11 | 14,17 | 29,3 1 | 106, 112 |
| | B1 4 | 9,10 | 16,17 | 21,2 4 | 11,11 | 8,10 | 9,11 | 10,12 | 8,9 | 18,20 | 8,8 | 8,10 | 16,17 | 30,3 1 | 106 |
| | C1 4a | 9,10 | 16,18 | 21,2 4 | 11,11 | 8,8 | 9,9 | 11,12 | 8,8 | 16,18 | 8,8 | 8,9 | 14,16 | 30,3 1 | 106, 112 |
| | C1 4b | 9,10 | 16,18 | 21,2 4 | 11,11 | 8,8 | 9,9 | 11,12 | 8,8 | 16,18 | 8,8 | 8,9 | 17,17 | 31,3 1 | 106 |
| 15 | A1 5 | 9,9 | 17,19 | 21,2 4 | 8,9 | 9,10 | 9,11 | 12,13 | 11,13 | 15,18 | 9,11 | 9,11 | 14,17 | 28,3 0 | 106, 112 |
| | B1 5 | 9,10 | 16,18 | 21,2 4 | 10,12 | 9,11 | 11,11 | 12,13 | 11,12 | 16,17 | 9,11 | 8,10 | 16,17 | 30,3 1 | 106 |
| | C1 5a | 9,9 | 17,18 | 21,2 1 | 9,10 | 9,9 | 11,11 | 12,12 | 11,11 | 15,17 | 9,9 | 8,9 | 14,16 | 30,3 0 | 106, 112 |
| | C1 5b | 9,9 | 17,18 | 21,2 4 | 9,10 | 9,11 | 11,11 | 12,13 | 11,11 | 15,17 | 9,9 | 8,9 | 17,17 | 30,3 1 | 106 |
| 16 | A1 6 | 9,10 | 16,18 | 21,2 2 | 8,9 | 8,12 | 8,9 | 11,12 | 8,12 | 19,20 | 9,11 | 10,11 | 15,16 | 31,3 2 | 106, 112 |
| | B1 6 | 8,9 | 17,17 | 21,2 4 | 10,12 | 8,10 | 8,10 | 10,11 | 9,11 | 18,19 | 11,11 | 10,10 | 16,17 | 29,3 1 | 106 |
| | C1 6a | 8,9 | 16,17 | 21,2 1 | 9,10 | 8,8 | 8,8 | 11,11 | 8,9 | 19,19 | 11,11 | 10,10 | 16,16 | 31,3 1 | 106, 112 |
| | C1 6b | 8,9 | 16,17 | 21,2 2 | 9,10 | 8,8 | 8,9 | 11,11 | 8,9 | 19,19 | 11,11 | 10,10 | 16,16 | 31,3 1 | 106, 112 |
| 17 | A1 7 | 11,13 | 15,17 | 20,2 4 | 10,12 | 9,10 | 9,11 | 9,10 | 11,13 | 17,19 | 8,8 | 9,11 | 14,17 | 29,3 1 | 106, 112 |
| | B1 7 | 11,12 | 16,18 | 19,2 2 | 9,10 | 9,11 | 9,11 | 11,12 | 9,10 | 17,20 | 8,8 | 8,10 | 16,17 | 30,3 1 | 106 |
| | C1 7a | 11,11 | 15,18 | 20,2 2 | 10,10 | 9,9 | 9,9 | 10,11 | 9,11 | 17,17 | 8,8 | 8,9 | 14,16 | 31,3 1 | 106 |
| | C1 7b | 11,11 | 16,17 | 20,2 2 | 10,12 | 9,11 | 9,9 | 10,12 | 9,11 | 17,19 | 8,8 | 8,9 | 17,17 | 31,3 1 | 106, 112 |
| 18 | A1 8 | 10,11 | 16,17 | 22,2 4 | 8,9 | 8,12 | 9,11 | 11,13 | 8,9 | 16,17 | 8,9 | 11,13 | 14,15 | 29,3 1 | 106, 112 |
| | B1 8 | 9,10 | 16,16 | 21,2 3 | 10,12 | 9,10 | 11,11 | 10,12 | 9,10 | 18,20 | 8,10 | 10,12 | 15,15 | 30,3 1 | 106 |
| | C1 8a | 10,10 | 16,16 | 21,2 4 | 9,10 | 9,12 | 11,11 | 11,12 | 9,10 | 16,18 | 8,8 | 11,12 | 15,15 | 30,3 1 | 106, 112 |
| | C1 8b | 9,11 | 16,16 | 21,2 4 | 9,10 | 9,12 | 11,11 | 11,12 | 9,10 | 16,18 | 8,9 | 11,12 | 15,15 | 31,3 1 | 106, 112 |
| 19 | A1 9 | 11,13 | 15,18 | 21,2 4 | 9,11 | 8,12 | 9,11 | 12,13 | 11,13 | 19,20 | 8,11 | 9,11 | 14,17 | 31,3 2 | 106, 112 |
| | B1 9 | 11,12 | 16,17 | 21,2 4 | 11,13 | 8,10 | 11,11 | 12,13 | 9,10 | 18,19 | 9,10 | 8,10 | 16,17 | 29,3 1 | 106 |
| | C1 9a | 11,11 | 16,18 | 21,2 1 | 11,11 | 8,8 | 11,11 | 12,12 | 9,11 | 19,19 | 9,11 | 8,9 | 14,16 | 31,3 1 | 106 |
| | C1 9b | 11,11 | 16,18 | 21,2 4 | 9,13 | 8,8 | 11,11 | 12,13 | 9,11 | 19,19 | 8,9 | 8,9 | 17,17 | 31,3 1 | 106, 112 |

| | | | | | | | | | | | | | | | |
|----|------|------|-------|-------|-------|------|-----|-------|------|-------|------|-------|-------|-------|---------|
| 20 | A20 | 9,9 | 17,19 | 21,22 | 10,12 | 9,10 | 8,8 | 11,12 | 8,13 | 17,19 | 8,9 | 11,13 | 14,15 | 29,31 | 106,112 |
| | B20 | 9,10 | 16,18 | 21,24 | 9,11 | 9,11 | 8,8 | 10,11 | 9,10 | 17,20 | 8,10 | 10,12 | 15,15 | 30,31 | 106 |
| | C20a | 9,9 | 17,18 | 21,21 | 9,10 | 9,9 | 8,8 | 11,11 | 8,9 | 17,17 | 8,8 | 11,12 | 15,15 | 31,31 | 106,112 |
| | C20b | 9,9 | 17,18 | 21,22 | 9,10 | 9,11 | 8,8 | 11,11 | 8,9 | 17,19 | 8,8 | 11,12 | 15,15 | 31,31 | 106,112 |

Table 1. STR allele profile of 20 families.

| Allele | Frequency | Allele | Frequency |
|----------------|-----------|----------------|-----------|
| TPOX | | THOI | |
| 7 | 0,01250 | 8 | 0,25250 |
| 8 | 0,17500 | 9 | 0,27000 |
| 9 | 0,34375 | 10 | 0,10750 |
| 10 | 0,18125 | 11 | 0,27000 |
| 11 | 0,22500 | 12 | 0,05000 |
| 12 | 0,03125 | 13 | 0,05000 |
| 13 | 0,03125 | | |
| D3S1358 | | vWA | |
| 15 | 0,09375 | 15 | 0,12750 |
| 16 | 0,33125 | 16 | 0,10750 |
| 17 | 0,35000 | 17 | 0,19500 |
| 18 | 0,20625 | 18 | 0,22250 |
| 19 | 0,01875 | 19 | 0,19500 |
| | | 20 | 0,10250 |
| | | 21 | 0,05000 |
| FGA | | D13S317 | |
| 19 | 0,03750 | 8 | 0,43750 |
| 20 | 0,08750 | 9 | 0,25625 |
| 21 | 0,35625 | 10 | 0,06250 |
| 22 | 0,22500 | 11 | 0,23750 |
| 23 | 0,05000 | 12 | 0,00625 |
| 24 | 0,24375 | | |
| D5S818 | | D16S539 | |
| 8 | 0,02500 | 8 | 0,13125 |
| 9 | 0,21250 | 9 | 0,16875 |
| 10 | 0,25625 | 10 | 0,29375 |
| 11 | 0,32500 | 11 | 0,28125 |
| 12 | 0,14375 | 12 | 0,08750 |
| 13 | 0,03750 | 13 | 0,03750 |
| CSFIPO | | D18S51 | |
| 8 | 0,23250 | 14 | 0,13750 |
| 9 | 0,35750 | 15 | 0,31875 |
| 10 | 0,20250 | 16 | 0,34375 |
| 11 | 0,15750 | 17 | 0,20000 |
| 12 | 0,05000 | | |
| D8S1179 | | D21S11 | |
| 9 | 0,02500 | 28 | 0,00625 |
| 10 | 0,08750 | 29 | 0,12500 |
| 11 | 0,23125 | 30 | 0,16875 |
| 12 | 0,50000 | 31 | 0,64375 |
| 13 | 0,15625 | 32 | 0,05625 |
| D7S820 | | | |
| 8 | 0,25000 | | |
| 9 | 0,38125 | | |
| 10 | 0,05625 | | |
| 11 | 0,3125 | | |

Table 2. STR Allele Frequencies of the Sample (N=2080).

References

- Yudianto A. Pemeriksaan Identifikasi Forensik Molekuler. Surabaya: Global Persada Press; 2015:1-45.
- Syukriani YF. DNA Forensik. Jakarta: Sagung Seto; 2012:45-60.
- Lu D, Liu Q, Wu W, Zhao H. Mutation analysis of 24 short tandem repeats in Chinese Han population. Int J Legal Med. 2012;126(2):331-5.
- Prastowo W, Lyrawati D, Andarini S, Mintaroem K. Allele frequencies of STR CODIS 13 of Madura Ethnic from Bangkalan and Probolinggo. Res J Life Sci. 2018; 5(2):116-20. <https://doi.org/10.21776/ub.rjls.2018.005.02.5>
- Butler JM. Genetics and genomics of core STR loci used in

- human identity testing. *J Forensic Sci.* 2006;1–48
6. Butler JM. The future of forensic DNA analysis. *Philos Trans R Soc Lond B Biol Sci.* 2015;370(1674): 20140252. <https://doi.org/10.1098/rstb.2014.0252>
 7. Venkanna N, Ravi NV, Naveen, Kiran KD, Purna CGK, Lakshmi NM. Single and double incompatibility at vWA and D8S1179/D21S11 loci between mother and child: Implications in kinship analysis. *Clinica Chimica Acta.* 2008; 395: 162–5
 8. Omran GA, Ruddy GN, Jobling MA. Genetic variation of 15 autosomal STR loci in upper (Southern) Egyptians. *Forensic Science International: Genetics.* 2009; 3(2): e39-44. <https://doi.org/10.1016/j.fsigen.2008.05.007>
 9. Elvita A, Feldi W, Husni W, Maimanah, Mitha P, Rani PS, Sofi S, Ucky S, Wella Y, Yaoli S, *Genetika dasar.* FK UNRI; 2008:45-60. <http://yayanakhyar.wordpress.com>
 10. Mangoendidjojo W. *Genetika Populasi.* Gadjah Mada University Press; 2014:45-60.
 11. Artadana IBM, Wina DS. Dasar-dasar Genetika Mendel dan Pengembangannya. *Graha Ilmu;* 2018:45-60
 12. Butler JM, Hill CR. Biology and genetics of new autosomal STR loci useful for forensic DNA Analysis. *Forensic Sci Rev.* 2012;24(1):15-26
 13. Untoro E, Atmadja DS, Pu CE, Wu FC. Allele Frequency of CODIS 13 in Indonesian Population. *Legal Medicine.* 2009;11(1):S203–S205. <https://doi.org/10.1016/j.legalmed.2009.01.007>
 14. Kido AR, Susukida MO, Fujitani N, Kimura H, Hara M. Allele frequency distributions of four STR loci VWA, TH01, TPOX and F13A01 in three Asian populations (Japanese, Bangladeshis and Indonesians). 2003;1239: 105–8.
 15. Connor O. Interpretation of DNA Typing Results for Kinship Analysis. National Institute of Standards and Technology USCIS Working Group on DNA Policy Washington, DC; 2011:1-28.
 16. Leclair B, Fregeau CJ, Bowen KL, Fournay RM. Enhanced Kinship Analysis and STR-based DNA Typing for Human Identification in Mass Fatality Incidents: The Swissair Flight 111 Disaster. *J Forensic Sci.* 2004; 49(5):939-53. www.astm.org
 17. Reid TM, Baird ML, Reid JP, Lee SC, Lee RF. Use of sibling pairs to determine the familial searching efficiency of forensic databases. *Forensic Science International: Genetics.* 2008;2(4):340–2. <https://doi.org/10.1016/j.fsigen.2008.04.008>
 18. Hares DR. Selection and implementation of expanded CODIS core loci in the United States. *Forensic Science International: Genetics.* 2015;17:33–4. <https://doi.org/10.1016/j.fsigen.2015.03.006>
 19. Maeda K, Murakami C, Irie W, et al. The case of 2 siblings that identified not only by DNA profiling. *Forensic Science International: Genetics Supplement Series.* 2015;5:e555–e556. <https://doi.org/10.1016/j.fsigs.2015.09.219>
 20. Marano LA, Fridman C. DNA phenotyping: current application in forensic sciences. *Research and Reports in Forensic Medical Science.* 2019;9:1-8; <https://doi.org/10.2147/RRFMS.S164090>.
 21. Sosiawan A, Yudianto A, Furqoni AH, Nzilibili SM Indah N. Full-Sibling Allelic frequency and Sharing among Madurese: STR Technique by 12 locus and the sex-typing amelogenin gene. *Egyptian Journal of Forensic Sciences.* 2019; 9:38. <https://doi.org/10.1186/s41935-019-0143-5>
 22. Wenk RE, Traver M, Chiafari FA. Determination of sibship in any two persons. *Transfusion.* 1996;36(3):259–62. <https://doi.org/10.1046/j.1537-2995.1996.36396182146.x>
 23. Chomczynski P, Mackey K, Drews R, Wilfinger W. DNAzol: a reagent for the rapid isolation of genomic DNA. *BioTechniques.* 1997;22(3):550–3
 24. Fung WK, Yang CT, Guo W. EasyDNA: user-friendly paternity and kinship testing program. *International Congress Series.* 2004; 1261:628-630. doi: 10.1016/S0531-5131(03)01486-9.
 25. Krenke BE, Viculis L, Richard ML, et al. Validation of a male-specific, 12-locus fluorescent short tandem repeat (STR) multiplex. *Forensic Sci Int.* 2005;148(1):1–14.