

ORIGINAL ARTICLE

Kinship Analysis on Paternity Test Through Str Codis Locus [CSF1PO, TH01, TPOX & vWA] From Maduranese Siblings in Surabaya

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ABSTRACT

Introduction: Paternity test is a DNA examination to determine whether a man is a biological father of a child through DNA pattern comparison between the child and the suspected father to verify DNA inheritance proof. **Methods:** This study was conducted through analysis of kinship analysis application on paternity test through STR CODIS examination in siblings using peripheral bloods samples of forty samples Madurese 3 generations. The examined locuses were CSF1PO, TH01, TPOX, vWA. **Results:** DNA samples isolates found that the mean level of DNA was $595.35 \pm 4.15 \text{ ng}/\mu\text{l}$, whereas the range of purity was 1.17 – 1.29. From this study, alleles from maduranese family population in Surabaya was variable, the highest percentage was CSF1PO locus allele 9 [33.75%], TH01 allele 8 [26.25%], TPOX allele 9 [32.5%] and vWA allele 17 [21.25%]. This study also revealed shared alleles on one sibling allele, the highest was 2 shared alleles on CSF1PO [70%], TH01 [100%], TPOX [70%] and vWA locus [90%]. **Conclusion:** TH01 locus had 100% 2 shared alleles so that it was recommend using STR locus to identify kinship on Madurese in Surabaya.

Keywords: Kinship analysis, Paternity test, Madurese, STR CODIS, Siblings

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INTRODUCTION

A paternity test is a DNA examination to determine whether a man is the biological father of a child. Every child inherits their parents' DNA (genetic material) from their biological parents, therefore some parts or all parts of the parents' DNA are inherited to their children's DNA. A paternity test is also used for various cases that are related to forensic identification: natural disaster, bomb explosion, murder, severe fire cases, finding a missing person, or incest (1,2). Currently, the most common DNA paternity test is through Short Tandem Repeats because of its high discrimination value, mainly when it is combined with STR loci number 6, 9, 13, or 15 and recommended by the Federal Bureau Investigation (FBI) through the use of 13 types of Short Tandem Repeats (STRs) loci that are known as Combined DNA Index System (CODIS) (2).

The accuracy level of identification using 13 types of loci is 100%, therefore it can be used in the paternity test to confirmed the suspected child or father. STR is a nuclei DNA that is passed from the father or the mother in accordance with Mendelian law (3,4). In this study, the population was Madurese who lived in Surabaya. The northern part of Surabaya is directly bordered to Madura Island where most Madurese are adventurous and have a good work ethic, and those traits encourage them to migrate from where they come from to make a living, especially since Suramadu Bridge was established, giving faster access to Surabaya (5). The purpose of this study is to prove the use of paternity test using sibling kinship on Forensic identification.

MATERIALS AND METHODS

Population and Sample

The population in this study was Madurese who lived in Surabaya that was involved in the same-ethnicity marriage from 3 previous generations. DNA samples were taken from families that consisted of parents and children. This study was an observational

research so that it did not have any inclusion and exclusion criteria (s) because it used the sample criteria (s). This study was ethically approved by Dentistry Faculty Universitas Airlangga Reference No.: 275/HRECC.FODM/VI/2020.

Sample preparation

Forty peripheral blood samples were taken from volunteers and were stored on blood collection tubes that were labeled as F [father], M [mother], and S [child].

DNA extraction and amplification through PCR

DNA extraction process from all 40 samples (10 pairs) was carried under the DNAzol protocol (McClintock 2014). Isolated DNA pellets were added with 50 µl of aquadest (6). DNA amplification utilized the PCR-STR process (PowerPlex® 21Systems, Promega, USA) with certain targeted DNA sequences to produce a number of copies from the isolated DNA. Amplification of all 40 samples was done using 4 autosomal STR loci (CSF1PO, TH01, TPOX, vWA) and amelogenin gene loci for sex (6-8).

Electrophoreses

The amplification result of PCR was then vertically electrophoresed with polyacrylamide agarose gel [PAGE] 6% [Bio-Rad Mini-PROTEAN®] using Silver Nitrate staining [Fig. 1].

RESULTS

Figure 1 shows DNA band contrast visualization of using locus CSF1PO establishment has responded within locus CSFIPO band limits formation (321bp-357bp) with Ma [100bp marker]. The sample of locus CSF1PO comes from a DNA kinship paternity test from volunteers that consisted of father, mother, and children. Visualization of PCR amplification results through Polyacrylamide Agorose Gel Electrophorese [PAGE].

Table I shows the spectrophotometer of measured DNA concentration and purity of sample both Psoas and Masseter Muscle that come from STR CODIS loci: CSF1PO, TPOX, TH01, vWA, and amelogenin locus from volunteer samples from 10 families that consisted of father, mother, and children.

Table II shows the STR allele frequency from STR CODIS loci: CSF1PO, TPOX, TH01, and vWA from 10 family samples that consisted of father, mother, and two children that are presented in percentage.

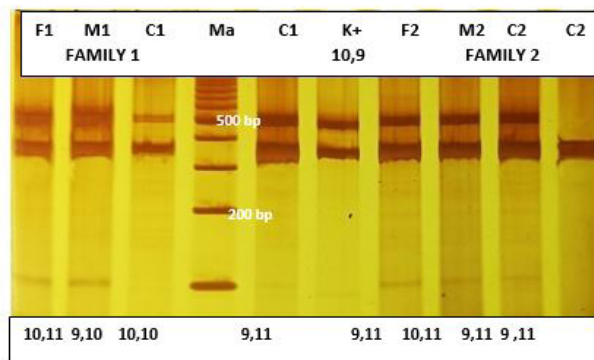


Figure 1 : Visualization of PCR result of STR CODIS CSF1PO locus [321 bp – 357 bp], Ma [marker 100 bp], F [Father], M [Mother], and C [Child]

DNA band contrast visualization of using locus CSF1PO establishment has responded within locus CSFIPO band limits formation (321bp-357bp) with Ma [100bp marker]. The sample of locus CSF1PO comes from DNA kinship paternity test from volunteers that consisted of father, mother, and children. Visualization of PCR amplification result through Polyacrylamide Agorose Gel Electrophorese [PAGE].

DISCUSSION

This study was started from DNA isolation from the peripheral blood of the participants using DNAzol method. DNA sample isolates level and purity were then measured using Spectrophotometer. The mean level of DNA samples was 595.35 ± 4.15ng/µl and its purity range was 1.17 – 1.29. Afterward, PCR amplification was conducted using primer on STR CODIS loci [CSF1PO, TH01,TPOX, vWA, and Amelogenin], and its visualization of the PCR amplification results used Polyacrylamide Agorose Gel Electrophorese [PAGE] with silver nitrate staining, as shown in Figure 2. In genetic, an allele is an alternative shape of a gene on a locus (6,7-9). Because genotype is then expressed as a phenotype, allele might cause different appearances in different individuals on a population. Forensic DNA examination that uses parents as a comparison will give an almost 100% result or approximately 99.99%. In this study, alleles that came from the Madurese family in Surabaya were highly variable, with the highest percentage was from CSF1PO locus allele 9 [33.75%], TH01 allele 8 [26.25%], TPOX allele 9 [32.5%], and vWA allele 17 [21.25%] (10). In previous conditions, a comparison through kinship might be considered for forensic DNA analysis, for example through the victim’s or the suspect’s siblings if parental nor child comparison was not available. The identification process using kinship comparison will face a mismatch probability in the DNA locus profile. It has been known that kinship analysis (siblings DNA) in

Tabel 1 : STR allele genotypes in 10 families

Volunteer	Code	STR Locus				
		CSF1PO	THOI	TPOX	vWA	Amelogenin
Family 1	F [Father]	9.10	8.9	8.9	15.18	106.112
	M [Mother]	8.10	9.10	8.10	16.17	106
	C [Child]	8.10	9.10	8.8	15.17	106.112
	C [Child]	9.10	9.10	8.9	15.17	106.112
Family 2	F [Father]	9.10	11.13	9.10	19.20	106.112
	M [Mother]	8.9	9.10	8.9	18.19	106
	C [Child]	9.9	9.11	8.9	19.19	106
	C [Child]	8.9	9.11	8.9	19.19	106
Family 3	F [Father]	8.8	8.9	8.9	15.17	106.112
	M [Mother]	9.10	8.10	9.10	18.19	106
	C [Child]	8.9	8.8	9.10	17.18	106.112
	C [Child]	8.9	8.9	9.10	17.18	106.112
Family 4	F [Father]	9.10	11.13	9.9	20.21	106.112
	M [Mother]	9.11	11.12	9.10	16.18	106
	C [Child]	9.9	11.11	9.9	16.20	106.112
	C [Child]	9.11	11.11	9.9	16.20	106
Family 5	F [Father]	8.12	8.12	8.10	16.17	106.112
	M [Mother]	9.10	9.11	8.9	18.20	106
	C [Child]	9.12	8.9	8.8	16.18	106.112
	C [Child]	9.12	8.9	8.8	16.18	106
Family 6	F [Father]	8.9	8.9	7.10	19.20	106.112
	M [Mother]	10.11	10.13	10.11	18.19	106
	C [Child]	8.11	8.10	7.11	19.19	106.112
	C [Child]	8.11	8.10	10.11	19.19	106.112
Family 7	F [Father]	10.11	11.12	9.10	17.19	106.112
	M [Mother]	9.10	9.10	8.9	17.20	106
	C [Child]	10.10	9.11	8.9	17.17	106
	C [Child]	9.11	9.11	8.9	17.19	106.112
Family 8	F [Father]	9.11	11.12	9.10	15.17	106.112
	M [Mother]	10.11	10.11	8.9	20.21	106
	C [Child]	9.11	11.11	8.9	15.21	106.112
	C [Child]	9.11	11.11	8.9	15.21	106.112
Family 9	F [Father]	8.10	8.13	11.13	16.17	106.112
	M [Mother]	9.10	9.10	11.12	18.20	106
	C [Child]	8.9	8.9	11.11	16.18	106
	C [Child]	8.9	8.9	11.11	16.18	106.112
Family 10	F [Father]	8.12	8.11	10.11	15.18	106.112
	M [Mother]	8.10	8.9	9.10	16.17	106
	C [Child]	8.8	8.8	10.10	15.17	106.112
	C [Child]	8.8	8.8	9.11	15.17	106.112

Table II : STR allele frequency in 10 families

Locus	Allele	n	Percentage [%]
CSF1PO	8	21	26,25
	9	27	33,75
	10	17	21,25
	11	11	13,75
	12	4	5
THO1	8	21	26,25
	9	20	25
	10	11	13,75
	11	20	25
	12	4	5
	13	4	5
	7	2	2,5
TPOX	8	21	26,25
	9	26	32,5
	10	18	22,5
	11	11	13,75
	12	1	1,25
	13	1	1,25
	15	10	12,5
	16	11	13,75
vWA	17	17	21,25
	18	14	17,5
	19	15	18,75
	20	9	11,25
	21	4	5

forensic DNA identification is used for various cases in the forensic field, such as parentage tests (civil or criminal), disaster victim identification, missing person identification, or familial searching (1-3). In kinship analysis, the “shared allele” plays a major role. Shared alleles in a sibling’s verification are important when two alleles are involved. According to this result, we recommended THO1 locus as a specific marker for research on Madurese DNA in Surabaya siblings’ shared allele on Madurese in 12 STR loci. STR CODIS loci allele frequency on Madurese in Bangkalan and Probolinggo revealed that THO1 had the highest frequency [76%] of all 13 STR CODIS loci that were examined (9,10).

CONCLUSION

Forensic DNA examination on paternity test is based on allele comparison between parents and their child. Hence, kinship analysis might be an alternative for the research, mainly from the parental side which is in accordance with Mendelian law. The result of this study revealed that THO1 locus had 100% 2 shared alleles, and we recommend it as an STR locus to identify kinship on Madurese in Surabaya.

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