Application of Y –STR typing in human identification: A case study

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Y-STRs are specialized class of short tandem repeats (STRs) located on human Y (male) chromosomes and are passed unchanged (barring a mutation) from one generation to the next. They are widely used in population genetic studies, forensics, Paternity and genealogical DNA testing and unknown male identification. A common Problem with this kind of analysis is the decomposition of DNA by bacteria and other microorganisms, the simultaneous exposure to environmental agents results in DNA degradation in postmortem tissues.

Materials and Methods:

The DNA extracted from the blood samples of the members of two families by(usingQIAamp® DNA Mini Kit (QIAGEN, USA), as well as from bone sample of the corpse by decalcifying steps , then followed by organic method for DNA extraction.STR amplification and typing:using an AmpFlSTR®Identifiler® plus PCRAmplification Kit (AppliedBiosystems).andAmpFlSTR®Yfiler® PCR Amplification Kit.We separate amplicons using a geneticanalyzer (ABI 3130; AppliedBiosystems).Then analyzed the amplicons with the suitable software (GeneMapper*ID*, version 3.2; Applied Biosystems) using standard procedures.

Results:

The corpse was the son of the second family not the first one**.**

Conclusion:

In forensic genetics, applications Y-STRs are useful for discrimination of paternal linkage rather than for individual identification.The male specific part of the human Y chromosome is widely used in forensic DNA analysis, particularly in cases where standard autosomal DNA profiling is not informative.

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Introduction

DNA fingerprinting is proving to be of great importance in the establishmentof the paternity of an individual. The forensic DNA analysisis commonly used to detect the criminal activities such as homicide, rape, it is also used in cases to establish the paternity of disputed offspring or, to know the identity of dead person and in cases of babyswapping[1,2]. The composition of the DNA molecule essentially does not vary from cell to cell.Therefore, the DNA in blood is identical to that in other biological materials such as hair, semen, skin, and bonemarrow[3]. The studies of blood grouping, which commonly employ the ABO system, cannot establish paternity but can conclusively exclude an alleged father from being a candidate. In any case ,the child must inherit his or her blood type from the mother and/or father.Thus, if the child's blood type differs from both the mother's and the alleged father's types, the man could not possibly be a parent of child. A typical population frequency for conventional blood typing might be 1 in 200, and for DNA 1 in 5,000,000. Meaning,that only 1 in 5,000,000 people would have the same DNA profile. Adequate samples for DNA typing can be collected from blood, blood stain and oral swab easily. DNA typing compares strands of genetic material between the child and alleged father from various locations of the genetic material allows accuracy ratings of 99.9 percent[4]. DNA typing allows an alleged father to be excluded with 100 percent certainty[5].

The male-specific part of the human Y chromosome is widely used in forensic DNA typing, particularly in cases where standard autosomal DNA profiling is not informative. Haplotypes composed of Y-chromosomal short tandem repeat polymorphisms (Y-STRs) are used to characterize paternal linkages of unknown male trace donors, especially suitable when males and females have contributed to the same trace, such as in sexual assault cases. Y-STR typing applied in crime scene investigation can exclude male suspects from involvement in crime, identify the paternal lineage of male perpetrators, highlight multiple male contributors to a trace, and provide investigative leads for finding unknown male perpetrators(6). Y-STR

analysis is applied in paternity disputes of male offspring and other types of paternal kinship testingincluding historical cases, as well as in special casesof missing person and disaster victim identification involving men. Y-chromosome polymorphisms are applied for inferring the paternal bio-geographic ancestry of unknown trace donors or missing persons, in cases where autosomal DNA profiling is uninformative. Because the fact of "Y-STR haplotypes are shared between paternally related men belonging to the same paternal lineage", Y-STR haplotyping is suitable for solving paternity disputes of male offspring, other types of paternal kinship questions, and for familial searching. It is also suitable to male identification cases involving human remains such as in disaster victim and missing person identification where only distant relatives are available[7].

Aim of the work:

In the present study we highlighted the accuracy of Y –STR typing as a tool in human identification because of its lower mutability rate than the mt DNA which is always transferred to all the children from the mother, thus making it more stable generation after generation.

The story of the case study:

The story begins with a report to the prosecution of finding a body of a young man in the second decade of the age , sinking on a place on the banks of the riverin a small village located in upper Egypt overlooking in the Nile River,in one day of the winter season of 2017. The body was transferred to the morgue with an order to know the cause of death if it is done before drowning and the tool used to create it.The body was photographed and the pictures were shown to the two families who both are reporting that they having a lost son month ago in the second decade of his life. Since the condition of the corpse was severely deformed as the fisheat most of the corpse body, especially the head and legs.Blood samples were collected by venipuncture in EDTA tubes from all the members of the two families for the forensic labs necessary tests,especially DNA as well as a bone sample of the dead body to make comparison with the family members after doing X-ray to the body . All

samples have been send to the central medical labs in the Egyptian Forensic MedicoLegalAuthority- Cairo Governorate..

Materials and Methods:

Bone preparation

The bone fragments(part of a rib) from the corpse were cleaned with sterilized metal brush, washed with tap water and then with distilled water and let it to dry in air. The washed bone fragments exposed to NaOCl 10% for a few minutes, washed twice with distilled water, let it todry in air, and milled (6×90s cycles) in liquid nitrogen using cryogenic mill (6850 Freezer Mill SpexCertiPrep, USA).

DNA extraction:

Bone sample: 500 mg of bone powder was incubated with 2.5% NaOCl for 4 h, then centrifuged. The pellet was washed with water and 95% ethanol, suspended in absolute ethanol, centrifuged and dried overnight. The dried material was sonicated in 95% ethanol, vortexed and allowed to settle. The precipitant was washed with 2.5% NaOCl and water and then exposed to decalcification steps by adding 0.5 M EDTA with pH 8.0, followed by centrifugation, repeat the step of decalcification with the same manner for 5 days. Then, the decalcifiedmaterial was incubated overnight at 56°C in the extraction buffer (0.5 M EDTA, 1% lauryl sarcosyl) and 20 µl of 20 mg/ml proteinase K, then concentrated using Centricon Plus-20 and purified with Centricon 30 centrifugal filter unit (Millipore). Add appropriate volume (20–50 ul) of sterile water, resuspend the sample and store it at −20°C until use.

Blood samples: DNA was extracted from the blood samples by usingQIAamp® DNA Mini Kit (QIAGEN, USA), following the manufacturer protocol**.** During all steps of extraction, appropriate controls and decontamination processes were followed.

DNA quality:

Recovery of DNA and degree of extract contamination by polymerase chain reaction (PCR) inhibitors were evaluated using Real-Time PCR with Quantifiler Human kit in accordance with the manufacturer's protocol[8]. All samples give agood amount of DNA.

STR amplification and typing:

An AmpFlSTR® Yfiler® PCR Amplification Kit (Applied Biosystems ®) as per the manufacturer's instructions .AmpFlSTR® Identifiler® plus PCR Amplification Kit (Applied Biosystems®)by using GeneAmp-9700 thermal cycler (Applied Biosystems) .Amplification conditions: an initial denaturation at 95 °C for 11 min; 28 cycles of 94 °C for 1 min,59°C for 1 min and 72°C for 1 min, and a final extension of 60 °C for 60 min to enable full PCR products .Amplicons were separated using a genetic analyzer (ABI 3130; Applied Biosystems), with the following conditions1.5ul of each PCR reaction

product was added to 24.5ul of deionized formamide (Sigma, USA) and 0.5 ul of GeneScan 500 LIZ size standard (Applied Biosystems). PCR products were denatured at 95 °C for 3 min and chilled for 3 min. Electrophoresis was performed using the Performance Optimized Polymer 4 (Applied Biosystems) and analyzed with the appropriate software (GeneMapper *ID*, version 3.2; Applied Biosystems) using standard procedures[9].

Statistical analysis

In 1980,population allele frequency for the STR loci was calculated according to Botstein et al[10]. Paternity index which measures the weight of the scientific evidence obtained from the paternity test was calculated using the method described by Brenner andMorris . Paternity index (PI) was calculated for each STR locus, then the combined paternity index (CPI) was estimated by multiplying the individual paternity index with the others. For simply, the paternity index was used to give the meaning ofcombined paternity index. Probability of paternity (POP), a conditional probability of whether an alleged father is the biological father of a child, was calculated using the followingequation: CPI · 100/CPI + (1 _ 0.5), and 0.5 is the prior probability[11].

Results:

After an X-ray,the body isfor a young man in the second decade of life, and no gunshot wounds or stabs to his body, the cause of death was drowning, excluding other causes of death before drowning.

Analysisof the DNA profiles (Autosomal and Y STR profiles)of the blood samples of all members of the two families and the bone of the corpse shown in Tables 1and2 .The analysis revealed that the first family showed no resemblance in their alleles with the alleles of the corpse.On the other hand, the analysis of the second family revealed that the corpsewas the son of themby finding a full match between his half of all alleles in the15 loci in the STR profile and the half of all alleles of the father and the rest of his alleles were fully matchedwiththe mother in the second family. As well as the Y filer profile of the corpse is similar to that of the fatherof the second family not of thefirst one.

Discussion:

DNA was extracted using organic extraction method (bone of the corpse) and DNA was extracted from the all blood samples of both families (usingQIAamp® DNA Mini Kit (QIAGEN, USA).The amplified products were separated and detected using

Genetic analyzer .On the basis of above observation it can be concluded that(corpse) is the son of the second family. Because Y-STR haplotypes are shared between paternally related men belonging to the same paternal lineage, Y-STR

haplotyping is suitable for solving paternity disputes of male offspring, other types of paternal kinship questions, and for familial searching. It is also suitable to male identification cases involving human remains such as in disaster victim and missing person identification where only distant relatives are available. The present study has a similar results with some researchers as Burgarella C. &Navascues M.(2010), as well as Vanek D., et al, (2009) where they revealed that, the Y-STR test can be used for searching and identification of legitimate male family members (familial searching) among the doubtful pedigree lineages which is failed to be identified by autologous STR markers just after first generation[12,13].

There are also many studies agreed with the present results ,in year 2012, Ge J .,et al. ,and in year 2014, Ge J. ,Sun H. and their colleagues revealed that, Y-STR analysis can be used to separate male DNA from other male or female DNA whose autologous STR markers coincide or match at certain loci. And they mentioned that, the Y-STR data has been

approved by the FBI, USA, for use by forensic laboratories generating DNA profiles for inclusion to the databases such as the US National DNA Index System (NDIS) and the Combined DNA Index System (CODIS)[14,15].But in another case at a year of 2015 , an unusual case was encountered where six brothers were accused of gagging and raping a woman. The Y-STR profiles of the accused brothers did not match with the male profile generated from the victim's garment and vaginal swab, but an unusual mutation was observed among the brother's Y-STR profile which was also analyzed further with the father's Y-STR profile. The Y-STR haplotype of all six brothers was found to be the same as that of their father except at locus DYS458 where unusual results were observed.This allelic variation at single locus could have arisen due to mutation during spermatogenesis. The mutation is very interesting in the perspective that there is gain as well as loss of one repeat at that particular locus in one generation(16).

Conclusion:

For human identification purposes, it is important to have DNA markers that exhibit the highest possible variation in order to discriminate between samples. It is often challenging to obtain PCR amplification products from forensic samples because either the DNA in those samples is degraded, or mixed, such as in a sexual assault case.The smaller size of STR alleles make STR markers better candidates for use in forensic applications, in which degraded DNA is common. PCR amplification of degraded DNA samples can be better accomplished with smaller target product sizes.Because of their smaller size, STR alleles can also be separated from other chromosomal locations more easilyto ensure closely linked loci are not chosen. Closely linked loci do not follow the predictable pattern of random distribution in the population, making statistical analysis difficult.STR alleles also have lower mutation rates, which makes the data more stable and predictable.Because of these characteristics, STRs with higher power of discrimination are chosen for human identification in forensic cases on a regular basis. It is used to identify victim, perpetrator, missing persons, and others.

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Table 1: DNA profiles (STR Identifier) of both families and the corpse.

Loci	corpse	Family1 Father1	Family1 Mother1	Family2 Father2	Family2 Mother2
D8S1179	13,14	10,12	12,15	14,15	13,16
D21S11	28,32.2	30,34.2	28.30	30.32.2	28,31
D7S820	9,11	8,11	10,12	9,12	11,12
CSF1Po	10,13	11,11	10,11	10,12	12,13
D3S1358	16,17	15,16	17,18	15,16	14,17
THO1	9,9	9,9.3	8,9	9,9	7,9
D13S317	12,12	12,13	11,12	10,12	12,12
D16S539	11,12	9,11	9,11	9,11	11,12
D2S1338	16,20	18,25	23,25	16,14	20,23
D19S433	12,13	13,13	14,15	12,13	13,14
VWA	16,18	17,17	16,16	16,18	16,16
TPOX	8,8	9,9	8,11	8,8	8,9
D18S51	12,17	14,17	13,21	11,12	15,17
AMEL.	X,Y	X,Y	X,X	X,Y	X,X
D5S818	12,12	9,12	11,12	9,12	12,12
FGA	19,24	21,22	21,23	20,24	19,22

Table 2:Y Filer of both fathers from two families and the corpse.

Loci of Y filer	Corpse filer	Father1 filer	Father2 filer
DYS456	15	14	15
DYS3891	14	13	14
DYS390	24	23	24
DYS38911	31	30	31
DYS458	17	20	17
DYS19	14	14	14
DYS385a\b	15,17	13,18	15,17
DYS393	13	12	13
DYS391	10	9	10
DYS439	12	11	12
DYS635	21	23	21
DYS392	13	11	13
YGATA H4	11	11	11
DYS437	14	14	14
DYS438	10	10	10
DYS448	18	20	18

تطبيق تقنية التكرار المترادف القصير للكروموسوم الذكورى Y
فى الاستعراف: دراسة حالة

أمانى موسى

تعتبر تقنية التكرار المترادف القصير للكروموسوم الذكورى y فئة متخصصة من تقنية التكرار المترادف القصير، فهى تعتمد على الكروموسوم y؛ حيث ينتقل هذا الكروموسوم عبر الأجيال بدون طفرات (لا يتغير).

وتستخدم هذه التقنية بتوسع فى الدراسات الجينية للسكان والتحاليل الجنائية، واختبارات إثبات الأبوة، وفى الاستعراف على الذكور مجهولى الهوية، إضافة إلى علم الأنساب الوراثى. وتعد المشكلة المشتركة فى هذه التقنية، كيفية المحافظة على الحمض الوراثى DNA من الفساد؛ حيث يتعرض للتعفن بسبب البكتريا والكائنات الدقيقة. كما ينتج عن التعرض المستمر للعوامل البيئية تكسير الحمض النووى الوراثى DNA فى أنسجة الجثث.