ANALYSIS OF MUTATION RATE OF 17 Y-CHROMOSOME SHORT TANDEM REPEATS LOCI USING TANZANIAN FATHER-SON PAIRED SAMPLES

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH MOLECULAR BIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

In the present study, 100 unrelated father-son buccal swab sample pairs from consented Tanzanian population were examined to establish mutation rates using 17 Y-STRs loci DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a, DYS385b, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, and Y-GATA-H4 of the AmpFlSTRYfiler kit used in forensics and paternity testing. Prior to 17 Y-STRs analysis, father-son pair biological relationships were confirmed using 15 autosomal STRs markers and found to be paternally related. A total of four single repeat mutational events were observed between father and sons. Two mutations resulted in the gain of a repeat and the other two resulted in a loss of a repeat in the son. All observed mutations occurred at tetranucleotide loci DYS389II, DYS385a and DYS385b. The locus specific mutation rate varied between 0 and 1.176 $x10^{-3}$ and the average mutation rate of 17 Y-STRs loci in the present study was $2.353x10^{-3}$ ($6.41x10^{-4}$ - $6.013x10^{-3}$) at 95% CI. Furthermore the mean fathers' age with at least one mutation at son's birth was 32 years with standard error 2.387 while the average age of all fathers without mutation in a sampled population at son's birth was 26.781 years with standard error 0.609. The results shows that fathers' age at son's birth may have confounding effect on Y-STRs mutation rate analysis though this age difference is statistically not significant using un paired samples t-test ($p = 0.05$). As a consequence of observed mutation rates in this study, the precise and reliable understanding of mutation rate at Y chromosome short tandem repeats loci is necessary for a correct evaluation and interpretation of DNA typing results in forensics and paternity testing involving males. The criterion for exclusion in paternity testing should be defined, so that an exclusion from paternity has to be based on exclusion constellations at the minimum of two 17 Y-STRs loci.

DECLARATION

I, Fidelis Charles, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Fidelis Charles Date

(MSc. Candidate)

The above declaration is confirmed;

_____ _____

Prof. Gerald Misinzo Date

(Supervisor)

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Above all, I thank The Almighty God for bestowing upon me good health, patience and will for completion of this work.

DEDICATION

This work is dedicated to my lovely wife Husna Charles Bugoye and my lovely son Derick Bugoye for their support, patience and for hard times they endured during my absence. To my late parents, Mr. and Mrs. Charles Bugoye, for bringing me up to who I am today, may God rest you in eternal peace.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

bp	base pairs
CA	Califonia
CI	confidence interval
DNA	deoxyribonucleic acid
EDTA	ethylenediamine tetra acetic acid
Fig.	figure
GCLA	Government Chemist Laboratory Agency
GLP	good laboratory practice
km	kilometers
Mbp	mega base pairs
Min.	minute
MLP	multi locus probe
MRCC	Medical Research Coordinating Committee
MSY	male specific Y-chromosome
NIMR	National Institute for Medical Research
NRY	non-recombining Y-chromosome
PAR	pseudo-autosomal region
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
SLP	single locus probe
SNP	single nucleotide polymorphism
SRY gene	sex determining gene on the Y chromosome
STRs	short tandem repeats
SWGDAM	Scientific Working Group on DNA Analysis Methods

UK	United Kingdom
VNTR	variable number of tandem repeat
Y-STRs	Y- chromosome short tandem repeats

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Research on application of Y-chromosome short tandem repeats (Y-STRs) have proven beneficial in a number of fields including paternity, anthropology and genealogical studies (Kayser *et al.,* 1997). The very useful application of Y-STR systems is due to their potential in detecting and discriminating male DNA. Human Y-chromosomal short tandem repeats polymorphisms or microsatellites are useful in resolving and relating male lineages in forensics especially in sexual assault cases where there is a large proportion of mixed male/female stains (Redd *et al*., 2002), genealogical (Kayser *et al*., 2007), evolutionary studies (Jobling and Tyler-Smith, 2003) and anthropological applications (Knijiff *et al*., 2000).

The interpretation of DNA evidence in forensic analysis and paternity testing is based on the similarities or differences at genetic loci used. In parenthood testing, the difference at inheritable genetic marker loci between the putative father and the offspring are attributed to non-biological paternity and therefore leads to exclusion of biological paternity. On the other hand, the spontaneous mutations in the germline of the putative father at any genetic marker locus used in the analysis can lead to an erroneous exclusion because such mutation results in differences between the parent and offspring. Since new alleles occur due to the mutation events, there is natural correlation between the degree of polymorphism and the underlined mutations rate of any given locus i.e. the higher the mutation rate, the more variable the locus is (Kayser *et al*., 2001).

In forensic DNA typing applications, the highly polymorphic loci are usually preferred due to their high power of discrimination. Therefore, short tandem repeat (STR) loci or

microsatellites are evolved to be the marker of choice in forensics because of their high power of discriminating and easy of analysis. For criminal and paternity testing investigation, which involve males with deceased alleged father, Y-STRs are used as the marker of choice (Roewer, 2000). Y-STRs are preferred because they are transmitted without recombination from fathers to sons and therefore are able to characterise paternal pedigree. In additional, Y-STRs are suitable for sexual assault investigations as they provide male specific DNA profiles which avoid problems of mixed stain interpretation (Kayser *et al.,* 1997). However, since highly polymorphic Y-STR loci applied to forensic investigation are constantly evolved through mutation process, the evaluation and interpretation of the genetic profiles requires precise knowledge on mutation rate at each loci used. Reliable estimations of mutation rates for these loci are a valuable in assisting the interpretation of Y-STRs test results. While there are a big number of articles reporting mutation rates for the minimal haplotype loci in different populations, only a few articles have reported results with the 17 Y-STRs loci mutation rates using African populations.

Since the release of commercially available kits, an increasing number of forensic DNA testing laboratories in the world are adopting Y-STRs analysis into their routine casework. However, there is no available data for estimating the average Y-STRs mutation rate of father-son pairs for sub-Saharan African populations including Tanzanians.

1.2 Problem statement and justification

Y-Chromosome short tandem repeats currently are used as markers of choice in criminal investigations of crime against human males, sexual assaults cases, disaster victim identification and paternity testing of male offspring with a deceased alleged father. In Tanzania, the numbers of cases which require the use of Y-STRs for paternity and criminal investigation are increasing. According to Tanzania police report between 2008

and 2010, the reported crimes against albino were 30, rape cases were 13 728, baby dumping were 358, baby thefting were 219 and reported murder cases were 8004 (Tanzania Police Report, 2010).

Since polymorphic Y-STRs loci used in forensic DNA investigations constantly evolves through mutational process, precise and reliable knowledge of mutation rate at each Y-STRs locus is essential for a correct evaluation and interpretation of typing results in forensic case work and specially those involving kinship genetic studies. There are different Yfiller Amplification kits used in forensic laboratories, but in Tanzania, the forensic human DNA analysis for criminal investigation and paternity testing involving males is only done at Government Chemist Laboratory Agency (GCLA) using 17 Y-STRs loci Amp FlSTRYfiler kit (Applied Biosystems, Foster City, CA). The DNA evidence report using 17 Y-STRs loci that lack statistical value for average mutation rate cannot be placed to strengthen the DNA evidence against the defendant. Since there has been no data for average Y-STRs mutation rates across father -son pairs for Tanzanian population, the purpose of this research was to determine the average estimate of 17 Y-STRs loci mutation rate in father-son paired samples and compute the summary statistics which can be used for correct evaluation and interpretation of results in criminal investigations, disaster victims' identification and paternity testing involving males.

1.4 Study objective

1.4.1 Overall objective

The goal of this research study was to determine the average estimate of 17 Y-STRs loci mutation rate using Tanzanian father-son paired samples.

1.4.2 Specific objectives

i. To determine the characteristics of locus specific mutation of 17 Y-STRs loci using Tanzanian father-son paired samples collected in Dar es Salaam, and

ii. To determine the average estimates of 17 Y-STRs locus specific mutation rate using Tanzania father-son paired samples collected in Dar es Salaam.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Forensic DNA typing

Forensic DNA typing technique was earliest used in the United Kingdom (UK) in the year 1985 for human identification (Jeffrey *et al.,* 1985). The first technique to be used was Multi Locus Probe (MLP), a variety of restriction fragment length polymorphism (RFLP). Multi Locus Probe method produces results which can be visualized as a set of parallel bands on a photographic plate (Jeffrey *et al.,* 1985). In cases where a suspect is identified, a adequate number of bands of that person's DNA can be compared to evidence sample from the scene of crime. The results of this comparison may help to establish whether the defendant committed the crime or not. On the other hand, the MLP method required large biological sample to give reliable results (Jeffrey *et al.,* 1985). Another variant of MLP, the Single Locus Probes (SLP) technique replaced the MLP. In 1988, SLP technique was introduced to forensic casework in the UK. Single Locus Probes was identified as the restriction fragment length polymorphism (RFLP) technique (Gill *et al.,* 1990). Specific loci on DNA were target by probes and the results of probing produced one or two bands depending upon whether that person is homozygous or heterozygous (Kloosterman, 2002). Single Locus Probes enabled scientist to give an estimate of the frequency of the precise DNA profile in the population. This method is identical to MLP, however the probe used is specific for only one point in the genome. Single Locus Probes became the method of choice in forensic science society until 1993 when the first fluorescent STR marker kit became available. Regardless of the potential usefulness of SLP in discriminating individuals in the population, forensic scientists my encounter difficulties when trying to

recover DNA from the crime scene with low quality of DNA or damaged DNA due to degradation (Kloosterman, 2002).

2.2 Short tandem repeats (STRs)

Microsatellites or short tandem repeats (STRs) are the most frequently used genetic markers in forensic DNA fingerprinting (Butler, 2005). The number of repeats units within each particular STRs locus varies between individuals leading to large degree of polymorphism throughout the population. Short Tandem Repeats loci consist of simple tandemly repeated sequences of 2-6 base pairs in length, which are extensively distributed in the human genome (Kimpton *et al*., 1993). Short Tandem Repeats loci are considered excellent genetic markers for human identification as compared to variable number of tandem repeat (VNTR) method. Variable Number Tandem Repeats method analyses samples with large amount of DNA. Samples from the crime scene contain low quality and small amount of DNA or the DNA can be in a degraded state. Recovering intact loci using VNTR is not possible. Short Tandem Repeats alleles are about 100 – 400 base pairs as compared to VNTR which are about 400 – 1000 base pairs. The small size of STR markers makes them more appropriate for degraded DNA which is common in forensic case work (Tully *et al*., 1993).

The di-nucleotide repeats are the most common among the STRs loci; however tetra nucleotide repeats have become more popular in forensic science community (Bacher *et al.*, 1999). Tetranucleotide STRs loci have insignificant slippage, reduced stutter and the alleles are more easily resolved. Stutter products are DNA amplification which arises during PCR process due to strand slippage (Butler, 2005; Walsh *et al*., 1996). Stutter products depending on the STR locus and it can be larger as much as 15% of the allele product quantity with tetranucleotide repeats. The stutter percentage for di- and tri-

nucleotide repeats can be more than 30% of the true allele's peak height, therefore it turn out to be more difficult to interpret DNA typing results from mixed samples (Butler, 2005).

2.3 Y-Chromosome

Structurally, Y-chromosome is the smallest in human genome, about 60 Mb in length representing around 2% – 3% of a haploid genome, and is composed of two pseudo autosomal regions (PAR 1 and 2) which are situated in the short (Yp) and long(Yq) arms (Quintana-Murci and Fellous, 2001). In these regions, the Y-chromosome pairs and exchanges genetic material with the X chromosome during male meiosis in the same manner as autosomal genes. PAR1 and PAR2 represent the 5% of the whole chromosome (Fig. 1). In each male there is only one Y-chromosome. The male determining gene (SRY gene) resides in the male specific (MSY) or non-recombining Y region (NRY) near the distal end of the short arm. There is growing indication that the MSY region of chromosomes may have some capability to recombine within itself (Buckleton *et al.,* 2005).

Approximately 95% of the length of the Y-chromosome is made by the non-recombining Y region (NRY), in this region there is no X-Y crossing during meiotic stage of cell division. The non-recombining region of the Y-chromosome includes the euchromatic and heterochromatic portion (Fig. 1). The heterochromatic portion is considered genetically inert while the euchromatic portion has several highly repeated sequences but also contains some genes responsible for essential biological functions (Quintana-Murci and Fellous, 2001).

The sequences in the NRY region of the Y-chromosome is inherited in a patrilineal mode and do not recombine during meiosis they are passed down from one generation to another without any changing, with the exception of when there is occasional mutation (de Knijff, 2000). This non-independent segregation of genetic markers on the Y-chromosome, which is in sharp contradistinction to the independently segregating behaviour of commonly, used autosomal STR markers, results in reduced genetic inconsistency (Hall and Ballantyne, 2003). In Y-chromosome markers the genetic information is referred to as a haplotype rather than genotype because there is only a single allele per individual. Y-chromosome contains the large number of polymorphisms including variable number and short tandem repeats (Jobling *et al.,* 1998). It is responsible for vital biological roles such as sex determination and male fertility. It has also become a powerful tool in studying human populations and evolutionary application pathways. Since the non-recombining part of the Y chromosome retains a record of the mutational events that have occurred along male lineages during the course of evolution, therefore the study of the different mutations this molecule has accumulated along its evolution may be highly informative in deducing the histories of human populations (Quintana-Murci and Fellous, 2001).

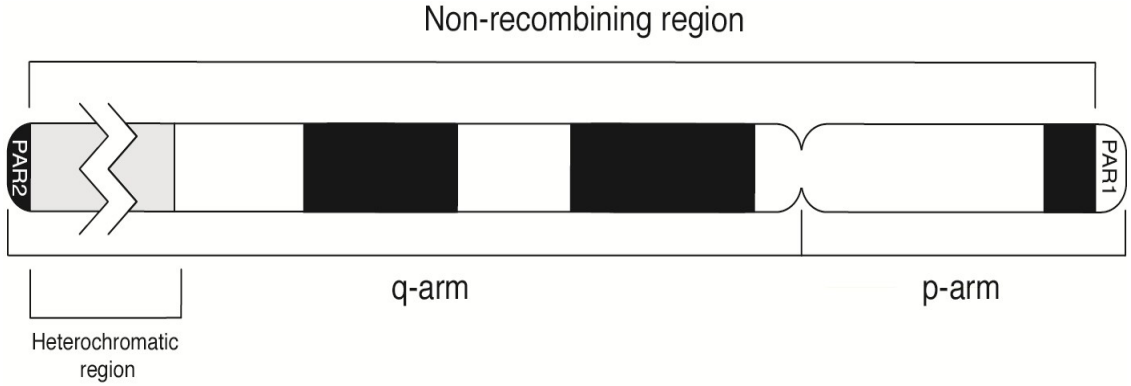

Figure 1: Simplified Y-chromosome structure. The Y chromosome has three regions; pseudo- autosomal region 1(PAR1) and PAR2 in the short (p) and long (q) arms, heterochromatic and the non-recombining (Jobling and Tyler-Smith, 2003).

2.4 Y-Chromosome short tandem repeats (Y-STRs)

The number of Y-chromosome short tandem repeats (Y-STRs) loci available for use in human identification testing has increased noticeably since the turn of the century. In the 1990s only few Y-STR markers were characterized and available for use. The first STR locus to be recognized on the Y-chromosome was DYS19 (Roewer *et al.,* 1992). A series of highly polymorphic Y-specific microsatellites have been identified and tested on different population samples. These markers show high levels of heterogeneity within and between populations and thus very useful for population genetic, evolutionary and forensic applications (de Knijff *et al.,* 1997). Y-STRs have various applications in forensic DNA analysis. The advantages of Y-STR analysis over autosomal STRs includes: a) male profile can be obtained in the presence of large amounts of female DNA; b) differential extraction of sperm and non-sperm fraction is not necessary; c) analysis of azoospermic semen samples from vasectomised males is feasible; d) the number of male contributors often can be determined in multiple rape cases because of the haploid nature of the Y-STRs; 5) rapid exclusion of suspects can occur; e) interpretation is simplified due to single allele per locus profile; f) in deficient paternities and g) multi generation male lineage studies can be performed (Shewale *et al*., 2004).

A core set of Y-STR loci was selected for human identity testing in 1997 that continue to serve as 'minimal haplotype' loci (Kayser *et al*., 1997; Pascali *et al*., 1998). The minimal haplotype is defined by the single copy Y-STR loci DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and highly polymorphic multi copy loci DYS385 a/b (Schneider *et al*., 1999). In 2004 the Scientific Working Group on DNA Analysis Methods (SWGDAM) agreed to adopt the 11Y-STR loci for forensic casework analysis. The decision was based on accessibility to the scientific community and the large amount

of published performance and database information for most to these loci. The committee encourages further study of additional loci as to their suitability for forensic applications. The first nine loci comprise the minimal haplotype complement of markers, plus two other additional markers DYS 438 and DYS 439 (Ayub *et al*., 2000).

The limitation of Y-STRs compared with autosomal STRs is a reduced power of discrimination due to a lack of recombination throughout most of the Y-chromosome (Mulero *et al*., 2006). But this has an advantage because male relatives share for several generations an identical Y-STR profile, the Y-chromosome analysis has a potential to make inferences on the population of origin of a given DNA profile (Roewer, 2009). Due to the duplicated, palindromic regions of the Y-chromosome, some Y-STR loci occur more than once and when amplified with a locus specific set of primers produce more than one PCR product. This fact can lead to confusion in terms of counting the number of loci present in a haplotype. A single set of primers can produce two amplicons, which may be thought of as 'two loci' for a Y-chromosome haplotype. For example the Y-STR locus DYS385 is present in two regions along the long arm of the Y-chromosome. These duplicated regions are situated about 40 000 bp apart and can generate two different alleles when amplified with a single set of primers. The two alleles are characteristically labeled 'a' and 'b' with 'a' designation going to the smaller sized allele. It also possible to have both 'a' and 'b' alleles be the same size in which case only a single peak would appear in an electropherogram. Due to the presence of two alleles, this duplicated locus is usually referred to as DYS 385a/b. Two PCR products can also be generated at the locus DYS389I using a single set of primers resulting in DYS389II which is a subset of DYS389I (Butler, 2005). In some cases duplications or even triplications of Y-STR locus have been reported, particularly for DYS 19. It is significant to keep this fact in mind so that two peaks at the DYS 19 locus are not automatically interpreted as coming from a mixture of

two males. Both of these issues, mutations and duplications of loci impact analysis and therefore confusing mixture interpretation and analysis of additional Y-STR loci can be helpful in these situations (Butler, 2005).

2.5 Mutation rate at Y-Chromosomal short tandem repeats (Y-STRs)

DNA evidence in forensic and paternity testing are carried out by analysis of short tandem repeat DNA loci and the interpretation of results depends on similarities or differences at genetic marker loci between the father and offspring and the observed differences are attributed to non-biological paternity (Heyer *et al*., 1997). The Y-chromosome in human is predominantly exposed to high mutation rates due to the environment in which it is contained. The Y-chromosome is solely passed through sperm which experience numerous cell divisions throughout gametogenesis and each cell division offers additional opportunity to gather base pair mutations. Moreover, sperm are reserved in the extremely oxidative environment of the testis, which boosts further mutation (Graves, 2006). This spontaneous mutation in the germ line of father at any genetic maker locus used in the DNA analysis can lead to incorrect exclusion because such mutation results in differences between parent and offspring. Since polymorphic STRs which are normally used for forensics are constantly evolved through the mutational process, therefore the accurate and reliable interpretation of DNA profiles require the knowledge of mutation rate at each locus used (Heyer *et al*., 1997).There are different methods to find out the mutation rate at Y-STRs loci (Heyer *et al*., 1997), but the analysis of father-son pairs of confirmed biological paternity can only reveal reliable results (Kayser and Sijantila, 2001). Different Y-STR loci have different mutation rate, this is for the reason that the mutation rate normally depends on the molecular structure of the particular genetic marker (Kayser *et al.,* 2000).

Similar rate of mutation and features have been observed, for both autosomal and Y-chromosomal STRs, the exclusion criteria for the paternity testing should be the same.

Also, in any given number of loci and meiosis, the mutations at two loci within a single germline transmission are likely statistically (Kayser *et al*., 2000).Normally, the general practice for the paternity testing is that, a difference at one or two out of 6 – 15 STR loci commonly analyzed is attributed to mutation rather than non-paternity where's different more than two loci are interpreted as non-biological paternity (Kayser and Sijantila, 2001). There has been no enough data comparing mutations rates across populations using 17 Y-STRs markers used in routine forensic case-work. However the average mutation rate of $3.5x10^{-3}$ ($2–5.8\ x10^{-3}$) at 95% CI were reported for Moroccan (Laouina *et al*., 2011).

These average mutation rate may be determined by dividing the number of mutated samples by the total number of meiosis (Lee *et al*., 2005), and that mutation rates are positively correlated with population diversity and not with sample size or number of Y-STRs loci used (Laouina *et al.,* 2011).

2.6 Determination of Y-STRs Mutation Rate

The knowledge of Y-chromosomal microsatellites mutation and estimates of the mutation rates is requisite for the accurate interpretation of Y-chromosomal microsatellite data in parenthood testing and forensic casework (Jobling *et al.,* 1997; Kayser *et al.,* 1997). Also these reliable estimates of the mutation rates of Y-chromosomal microsatellites are the requirement for dating the origin of Y-chromosomal lineages defined by single-nucleotide polymorphisms (SNPs), as attempted in molecular and anthropology application studies (Zerjal *et al.,* 1997; Bianchi *et al.,* 1998; Lahermo *et al.,* 1999). For autosomal STRs loci, relative mutation rates have been approximate by the use of statistical models to

population genetic data (Di Rienzo *et al.,* 1994; Chakraborty *et al.,* 1997). But, those mutation rates estimates depend on a number of suppositions, rather than on true data such as effective population size or population separation time. There are various methods to estimate the mutation rate at Y-STRs loci (Heyer *et al*., 1997), but the analysis of father-son pairs of previously confirmed biological relationship can only reveal reliable results (Kayser and Sijantila, 2001). And these average mutation rates was determined by dividing the number of mutated samples by the total number of meiosis (Lee *et al.,* 2005).

2.7 17 Y-STRs loci specific mutation Characteristics

Y-chromosomal microsatellites are principally useful in paternity testing of deficiency cases, which involve a deceased alleged father and a male child, because characterization of the genotype of the deceased alleged father can be replaced by the analysis of any of his living paternal relatives (Kayser *et al.,* 1997). Also the locus specific mutation rate estimates are necessary in such paternity testing in order to assess the likelihood of a potential false exclusion.

According to previous research study findings, multiple Y-STR locus specific mutations on single germline transmission are statistically expected, therefore these multiple mutation on a particular germline transmission can be misinterpreted as false exclusion of paternity. Apart from multiple mutation on a single germline transmission, another mutational feature of Y-chromosomal microsatellites that has consequences for data assessment and interpretation in forensic case analysis is the duplication or triplication of a larger Y-chromosomal region that includes the microsatellite locus, followed by a change in the number of repeats within the microsatellite. These duplication or triplication mutational features have been found in various research conducted using different Y-STRs loci in different populations. In previous research findings it observed that one father-son

pair with three instead of two male-specific alleles at DYS385, and additional cases of multiple alleles, at DYS19, DYS390 and DYS391, have been testified (Santos *et al.,* 1996a, b*;* Kayser *et al.,* 1997; Redd *et al.,* 1997). In forensic-case analysis, these additional alleles are normally interpreted as mixed profiles and, thus, will lead to erroneous conclusions (Kayser *et al.,* 2000).

2.8 Effects of fathers' Age on Y-STRs Mutation Rate Analysis

According to research findings, father's age appears to influence the mutation rate; this difference in mutation rate between older men and younger men is associated with different numbers and types of cell divisions in germ cell genesis. Research finding revealed that sperm cells of older men have undergone more divisions than the cells of younger men (Brinkman *et al.,* 1998).

The effect of the father's age on the mutation rate was modelled using a Poisson distribution, where the mutation rate was estimated as an exponential function of the age of the father. The previous research findings showed that the mutation rate increased with increasing age of the father , these research findings are signifying that age is a factor that should be taken into account not only when determining Y-STR mutation rates but also when comparing estimated mutation rates from different studies (Goedbloed *et al*., 2009). Numerous earlier studies investigated the age effect on the mutation rate for all or some of the Y-STRs, Some of these studies found the average age of the fathers with mutations being older than that of fathers without at least one mutation (De Souza *et al*., 2005) others observed the reverse effect, i.e. fathers without any mutations being older than that of fathers with at least one mutation (Kayser *et al*., 2000; Turina *et al*., 2006) and some found no age difference between mutated and no mutated fathers (Dupuy *et al*., 2004; Lee *et al*., 2007; Sanchez-Diz *et al*., 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was carried out in Dar es Salaam and laboratory analytical procedures were performed at the Government Chemist Laboratory Agency (GCLA), Dar es Salaam ,Tanzania where all forensic DNA and paternity testing analysis from all 30 Tanzania administrative regions is normally done. Dar es Salaam is found in the eastern part of the Tanzanian mainland at $6^{o}51'$S latitude and $39^{o}18'$E longitude with an area of 1350 square kilometers (km^2), stretching about 100 km between the Mpiji river to the north and beyond the Mzinga river in the south and the Indian Ocean borders it to the east (Fig. 2). Samples were collected from participants residing in 10 wards (Fig. 2).

3.2 Study Design

The present research was a cross-sectional study that was conducted from September 2014 to June 2015.

3.3 Sample Size

The sample size was estimated by using the formula $n = Z^2SD^2/e^2$ for the unknown population (Kothari, 2004), where n = size of sample, z = standard variate at 95% confidence level (1.96), SD = the standard deviation of mutation (15%) (Kyosov, 2009) and e = acceptable error (0.025). Using the above formula, the required sample size was found to be 138 father-son pairs.

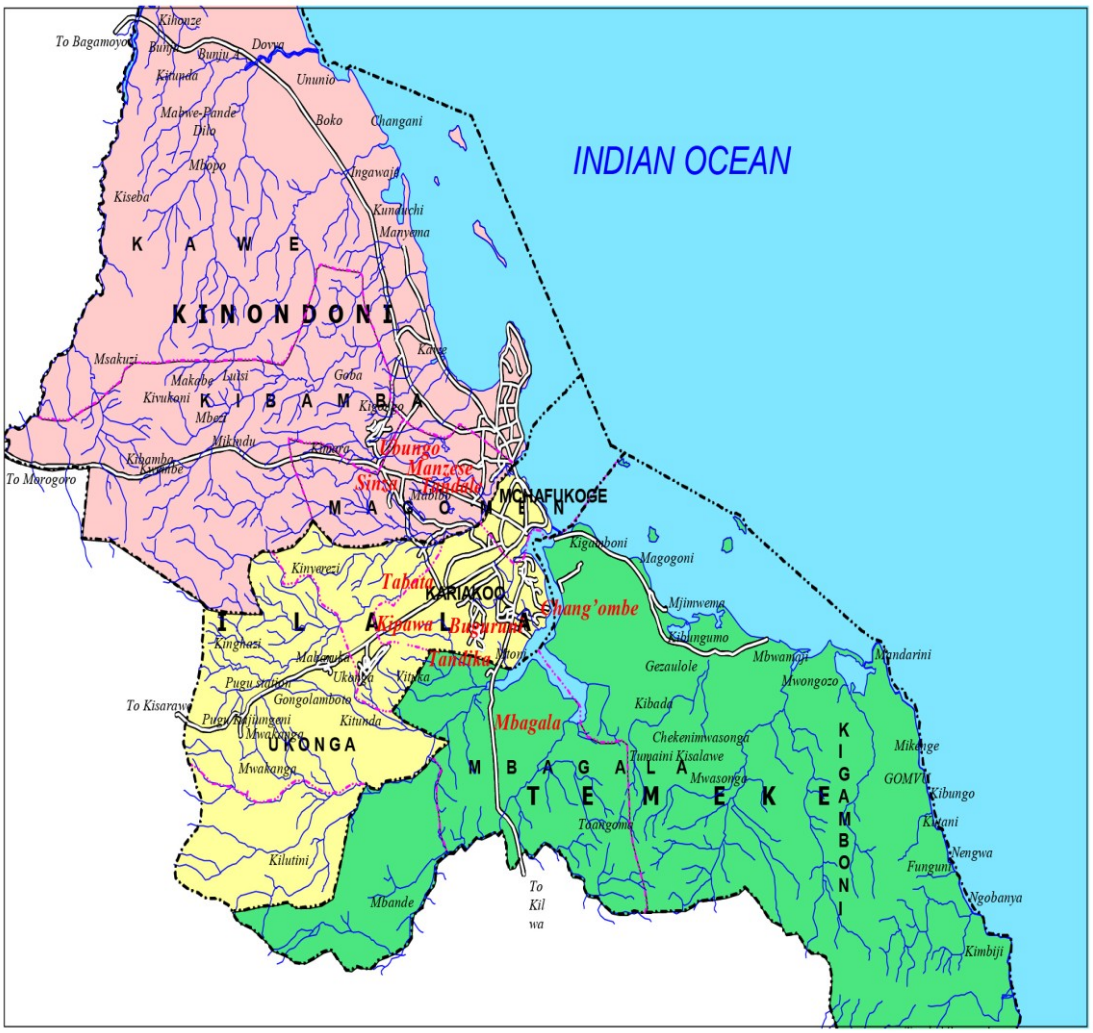

Figure 2: Map of Dar Es Salaam city showing sampling locations where samples were obtained. Samples were collected from study participants in 10 different wards indicated in bold red colour . The wards are located in all the three districts of Dar es Salaam (Adapted from; Dar es Salaam City Profile, 2004).

3.4 Sampling and Sample Handling

Buccal swab samples were randomly collected from participants in 10 wards, including Sinza, Tandale, Ubungo, Manzese, Mbagala, Changómbe, Tandika, Tabata, Buguruni and Kipawa (Fig. 2). These 10 wards were randomly selected out of 84 wards found in Dar es Salaam. Mother and father-son pairs of unrelated individuals were consented and sampled through random home visits. Mother samples were used purposely to confirm the biological relationship of the father-son paired samples. The collected samples were air

dried, labelled, packaged and sent to the Human DNA laboratory at GCLA for analysis. The collected buccal swabs were coded, hereafter no information existed that can link a sample to a specific individual. Each sample collected was given specific sample ID, the place where sample have been collected and the date were recorded in sample register book. This was done to ensure the objectivity, integrity and confidentiality of the obtained results. All the samples collected were processed and analysed at GCLA according to standard operating procedures and confirmed father-son biological relationship with autosomal STRs was one of the inclusion criteria. All samples from individuals were handled by qualified personnel with adequate knowledge on good laboratory practices (GLP), quality control and safety knowledge. All items used to collect individual sample, transport and for laboratory analysis associated with this study were clean and sterile.

3.4.1 Inclusion criteria

The samples meeting the following inclusion criteria were included in present study; (i) apparently health individual, (ii) consent to participate and (iii) willingness to be sampled.

3.4.2 Exclusion criteria

Samples meeting the exclusion criteria below were excluded from the study; (i) a refusal to participate or unwillingness to be sampled (ii) participants under the age of 1 year, or (iii) related individuals (father-son pairs from two brother families).

3.5 Ethical Considerations

Before conducting this research study, ethical clearance permit with reference number NIMR/HQ/R.8a/VOL.IX/1826 was obtained from Medical Research Coordinating Committee (MRCC) of National Institute for Medical Research (NIMR) (Appendix 1). Permission to conduct the research on human DNA and use of the forensic Human DNA

laboratory at GCLA was granted by the Chief Government Chemist as indicated in the Human DNA regulation Act, 2009. The DNA sample contain the genetic privacy of an individual, therefore before collecting buccal swabs from an individual, he/she were required voluntarily to participate in the study by filling a consent form (Appendix 2) after all the benefits, risks and alternative of the study have been clearly explained and understood.

Research was conducted according to the approved protocol as stipulated in Human DNA regulation Act, 2009. After the DNA analysis and results from each sample were obtained, samples were destroyed according to the Human DNA regulation Act, 2009.

3.6 DNA Extraction

DNA extraction was done using chelex method in which single stranded DNA were produced. Chelex is composed of styrene divinyl benzene copolymers containing paired iminodiacetate ions that act as chelating groups in binding polyvalent metal ions. Each buccal swab was transferred to an appropriately labelled 1.5 ml eppendorf tube. To the buccal swabs in each of the Eppendorf, 1,000 µl deionized water was added and incubated at room temperature for 40 minutes. Each sample was then vortexed for 15 seconds and centrifuged for 3 minutes at 1,400 rpm. Then 980 µl of supernatant were removed and 50 µl of chelex were added, followed by the addition of 130 µl of deionized water to make the final chelex of 5%. Each sample was briefly mixed and incubated at 56 ^{o}C for 1 hour. After incubation, the samples were vortexed and centrifuged to remove drops from the inside of the lid. Then, the samples were boiled for 8 minutes to ensure complete lysate. After boiling, each sample was vortexed and centrifuged at 14 000 rpm for 3 minutes. Recovered DNA was then temporarily stored at 2 – 8 ^{o}C before DNA amplification.

3.7 Short Tandem Repeats Amplification

3.7.1 15-Autosomal STRs loci DNA amplification

Polymerase chain reaction of autosomal STRs from all collected samples was carried out using GeneAmp PCR System 9700(Applied Biosystems, Foster City, CA). A total of 15 autosomal STRs loci including CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX and vWA and the gender amelogenin were typed using AmpF/STR Identifiler PCR amplification kit (Applied Biosystems, Foster City ,CA). In each 0.2 ml PCR tube, 3 µl of DNA extract were mixed with 10 µl AmpF/STR PCR reaction mix, 5 µl AmpF/STR Identifiler primer set, 0.5 µl AmpliTaq Gold and 9 µl of deionized water. The amplification condition include: denaturation and enzyme activation at 95 ^{o}C for 11 minutes, 28 cycles of 94 ^{o}C for 1 minute, 59 ^{o}C for 1 minute and 72 ^{o}C for 1 minute followed by 60 minutes at 60 ^{o}C (Table 1).

Table 1: Amplification condition for 15 autosomal STRs loci

Initial incubation step	Denature	Anneal	Extend	Final extension	Final hold
1 CYCLE	28 CYCLES			1 CYCLE	HOLD
95°C 11 min.	95°C 1 min.	95°C 1 min.	95°C 1 min.	95°C 60 min.	4 °C $^{\infty}$

Note: The 15 autosomal STRs loci was amplified using AmpFlSTR® Identifiler ®PCR Amplification kit using the conditions shown above.

3.7.2 17 Y-STRs loci DNA amplification

Polymerase chain reactions for 17 Y-STRs loci of father-son paired samples were carried out using GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). A total of 17 Y-STRs loci including DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a, DYS385b, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, and Y-GATA-H4 were typed using the AmpFlSTRYfiler™ kit PCR Amplification Kit (Applied Biosystems, Foster City, CA). In each of the 0.2 ml PCR tube, 10 µl of DNA extract were mixed with 9.2 µl AmpFlSTRYfiler PCR reaction mix, 5 µl AmpFlSTRYfiler primer set, 0.8 µl AmpliTaq Gold® DNA polymerase. The amplification condition used included the following: denaturation and enzyme activation at 95 ^{o}C for 11 minutes, 30 cycles of 94 ^{o}C for 1 minute, 61 ^{o}C for 1 minute and 72 ^{o}C for 1 minute followed by 80 minutes at 60 oC.

Table 2: Amplification condition for Y-STRs loci

Initial incubation step	Cycle (30 cycles)			Final Extension	Final hold
	Denature	Anneal	Extend		
95 ^{o}C 11 min.	94 ^{o}C 1 min.	61 ^{o}C 1 min.	72 ^{o}C 1 min.	60 ^{o}C 80 min.	4 ^{o}C

Note: The 17 YSTRs loci was amplified using AmpFlSTR Yfiler PCR Amplification kit using condition shown above.

3.8 Capillary Electrophoresis

3.8.1 Capillary electrophoresis for autosomal 15 STRs loci

Capillary electrophoresis of amplified PCR products for autosomal 15 STRs loci were performed using the 3130 xl Genetic analyzer (Applied Biosystems, Foster City, CA). During the process, a reaction mix were prepared containing 9.25 µl of Hi-Di formamide

(Applied Biosystems, Foster City, CA), and 0.5 µl Gene Scan 500HD Liz size standard. Afterwards 8.5 µl of the reaction mix were loaded into MicroAmp optical 96-well plate (Applied Biosystems Foster City, CA). Two µl of PCR products were then added into each well of interest followed by addition of 2 µl of allelic ladder into each run. The plates containing the prepared samples were heated at 95 ^{o}C for 5minutes and snap-cooled on ice for 5 minutes prior to loading to the instrument. Electrophoresis was carried out using 36 cm capillary array containing POP-4 polymer and 1x Genetic analyzer buffer with EDTA. Samples were injected using module G5, i.e. 5 second injection, 15 kv and 60 ^{o}C oven temperature. Fluorescently labelled products were separated and detected using a 3130 xl Genetic analyzer (Applied Biosystems, Foster City, CA) data collection software v 3.0 and data were analysed using Gene Mapper IDX analysis software v 1.0 (Applied Biosystems, Foster City, CA). The 15 STRs DNA profiles from the Gene Mapper IDX analysis software v 1.0 were recorded in a table of locus-specific alleles using Microsoft Excel spreadsheet and the biological relation of father-son pair of the collected sample were confirmed by direct observation. Those father-son paired sample with correct match for all 15 STRs loci were confirmed for biological relationship and further analysed for 17 YSTRs loci.

3.8.2 Capillary electrophoresis for 17 Y- STRs loci

After having the 15 autosomal STRs DNA paternity testing results that confirm father-son paired biological relationship, the capillary electrophoresis for 17 Y-STRs loci were performed. Before sample loading, a reaction mix which contains 8.7 µl of Hi-Di formamide (Applied Biosystems, Foster City, CA) and 0.3 µl Gene Scan 500HD Liz size standard were prepared. Then 9 µl of the reaction mix was loaded into MicroAmp optical 96-well plate (Applied Biosystems, Foster City, CA), 1µl of PCR products were added into each well of interest and 1 µl of allelic ladder was added into each run. The prepared

plate containing samples were heated at 95 ^{o}C for 3 minutes and snap-cooled on ice for 3 minutes prior loading to the instrument. Electrophoresis was carried out using 36 cm capillary array containing POP-4 polymer and 1x Genetic analyzer buffer with EDTA. Samples were injected using module G5, i.e. 5 second injection, 15 kv and 60 ^{o}C oven temperature. Fluorescently labelled products were separated and detected using 3130 xl Genetic analyzer (Applied Biosystems, Foster City, CA) using data collection software v 3.0 and data were analysed using Gene Mapper IDX analysis software v 3.2 (Applied Biosystems, Foster City, CA).

CHAPTER FOUR

4.0 RESULTS

4.1 Confirmation of Father-son Biological Relationship

The autosomal 15-STRs DNA analysis of the buccal swab samples collected from the previously consented father, mother and sons were analysed using the Gene Mapper IDX analysis software v 3.2. The DNA paternity typing results which confirm father-son pairs biological relationship were obtained and recorded in a table of locus-specific alleles using Microsoft Excel spread sheet (Appendix 3).

4.2 17 Y-STRs locus specific Mutation characteristics

In present study 100 father-son paired samples with confirmed biological relationship was used, this is due to time and budget limitation, however mutation rate analysis of father-son pair using Y-STRs does not depend on sample size and number loci used (Laouina *et al.,* 2011), also sample size of 100 -150 individuals is found to be sufficient to study the genetic loci (Chakraborty, 1992).

The 17 Y-STRs DNA analysis of DNA sample from previously confirmed father-son pairs using 15-Autosomal STRs analysis using the buccal swab samples collected from the previously consented father and sons were analysed using the Gene Mapper IDX analysis software v 3.2. The 17 Y-STRs DNA typing results obtained were recorded in a table of locus-specific alleles using Microsoft Excel spread (appendix 4). These 17 Y-STRs DNA typing results reveal four father-son pair's mutation characteristics events which were observed on father-son paired sample numbers: F0074/C074, F082/C082, F003/C003 and F012/C012 (Table 3). These mutation events were indicated by the observed allele's difference between father and son based on Y-STRs loci compared, the observed

mismatch between father-son at any of 17 Y-STRs loci used are identified as the mutational events because the biological relationships of father-son were previously confirmed using 15-STRs autosomal DNA analysis (appendix 3).

Table 3: **Mutation count and Y-STRs loci mutation characteristics events as revealed by direct observation on father-son paired samples of previously confirmed biological relationship.**

Sample ID's	Loci	Repeat sequence[a]	Father's profile	Son's profile	Mutation characteristics	Mutation count
F/C003	DYS385b	GAAA	16	17	Gain	1
F/C012	DYS385a	AAGG	15	16	Gain	1
F/C074	DYS385a	AAGG	16	15	Loss	1
F/C082	DYS389II	CTGT/CTAT	31	30	Loss	1

[a]Repetitive sequence structure previously reported by Gusmao and Carracedo (2003).

The results in table 3 above, shows highly polymorphic Y-STR locus DYS385a/b, if considered as a single locus (Kayser *et al*., 2000) was observed to contains three mutation characteristics events in which single repeat mutation events were observed at loci DYS385a and DYS385b while single repeat mutation event was observed at the other DYS385a locus, therefore the more mutation was observed to occur at locus DYS385a/b among all 17 Y-STRs loci analysed followed by locus DY389II. In addition, all observed locus specific mutations events in this study were characterised by tetranucleotide repetitive sequence motif in which the locus DY389II, a compound microsatellite consists of more than one tetranucleotide repetitive sequence motif CTGT/CTAT while the loci DYS385a and DYS385b each consists of different tetranucleotide repetitive sequence motif AAGG and GAAA respectively. Also, Kayser *et al*., 2000 refers locus DYS385 as single locus DYS385a/b, therefore all mutation events observed in this study may also

characterised as the compound microsatellites with more than one tetranucleotide repeat motif.

In addition, the mutation analysis on the 17 Y-STRs loci (Table 3) shows two mutation characteristics events on single repeat loss and two mutation characteristics events on single repeat gain. The single mutation gains were observed at loci DYS385a and DYS385b from father-son pairs for sample number F012/C012 and F003/C003 respectively as well as single mutation loss were observed at loci DYS385a and DYS389II from father-son pairs of sample number F074/C074 and F082/C082 respectively. These single mutation characteristics events observed during data analysis using Gene Mapper IDX analysis software v 1.0 are illustrated using electropherogram results (Fig. 3, Fig. 4, Fig. 5 and Fig. 6).

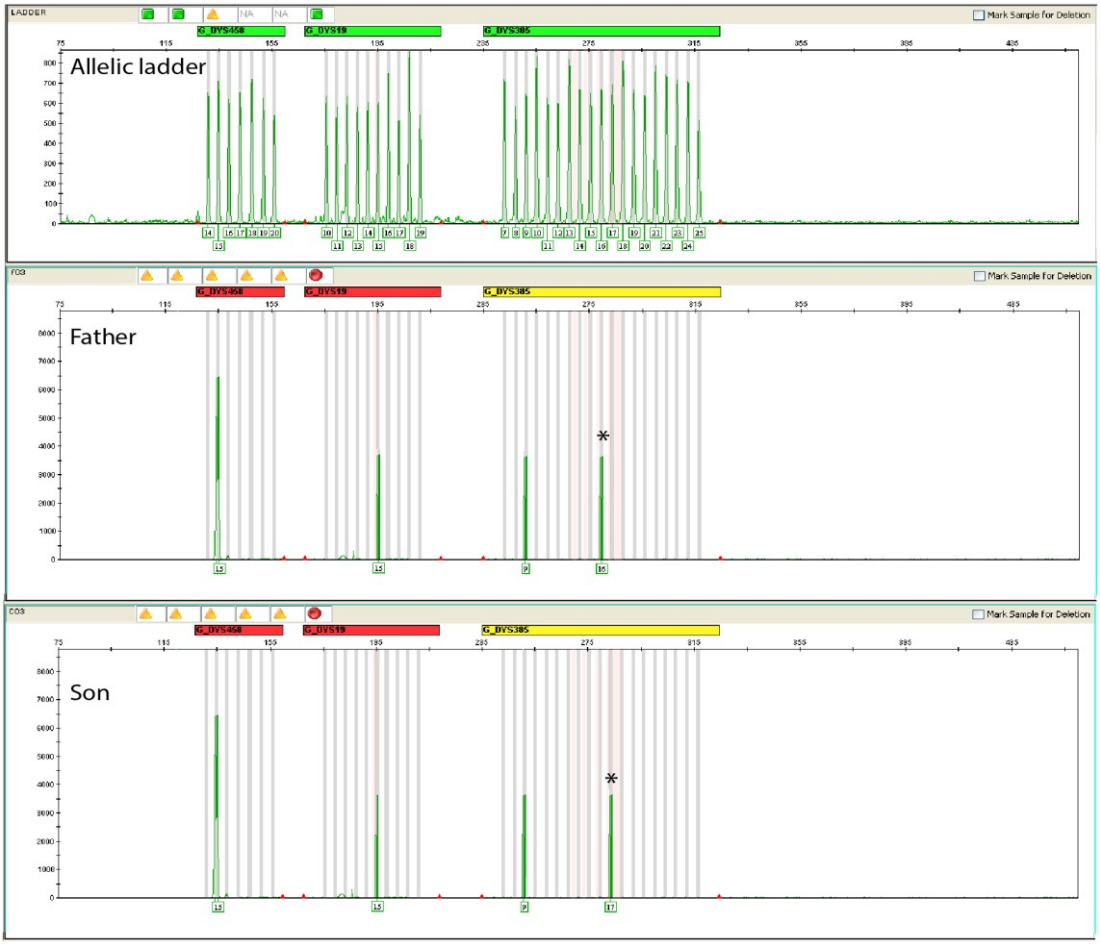

Figure 3: An electropherogram indicating a single repeat gain mutation from father to son at tetranucleotide locus DYS385b. The ladder (top panel) shows all possible alleles at loci DYS468 (alleles 14 to 20), DYS19 (alleles 10 to 19) and DYS385a/b (alleles 7 to 25). The middle panel shows fathers' alleles including allele 15 at locus DYS468, allele 15 at locus DYS19 and alleles 9 and 16 at locus DYS385a/b, respectively. The bottom panel shows son's alleles that are similar to those of fathers with exception of a gain mutation for allele 16 to 17 at DYS385b locus.

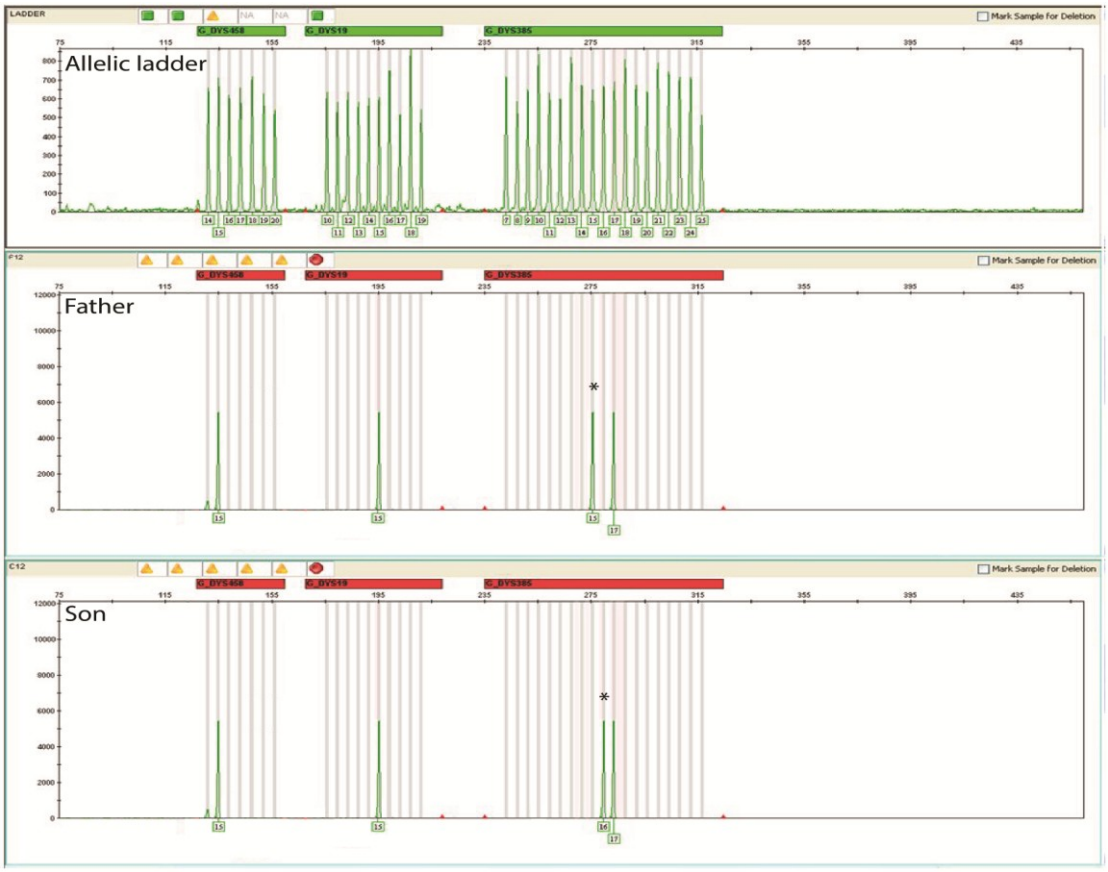

Figure 4: An electropherogram indicating a single repeat gain mutation from father to son at tetranucleotide locus DYS385a. The allelic ladder (Top panel) shows all possible alleles at loci DYS468 (alleles 14 to 20), DYS19 (alleles 10 to 19) and DYS385a/b (alleles 7 to 25). The middle panel shows fathers' alleles including allele 15 at locus DYS468, allele 15 at locus DYS19 and alleles 15 and 17 at locus DYS385a/b respectively. The bottom panel shows son's alleles that are similar to those of fathers with exception of a gain mutation for allele 15 to 16 at DYS385a locus.

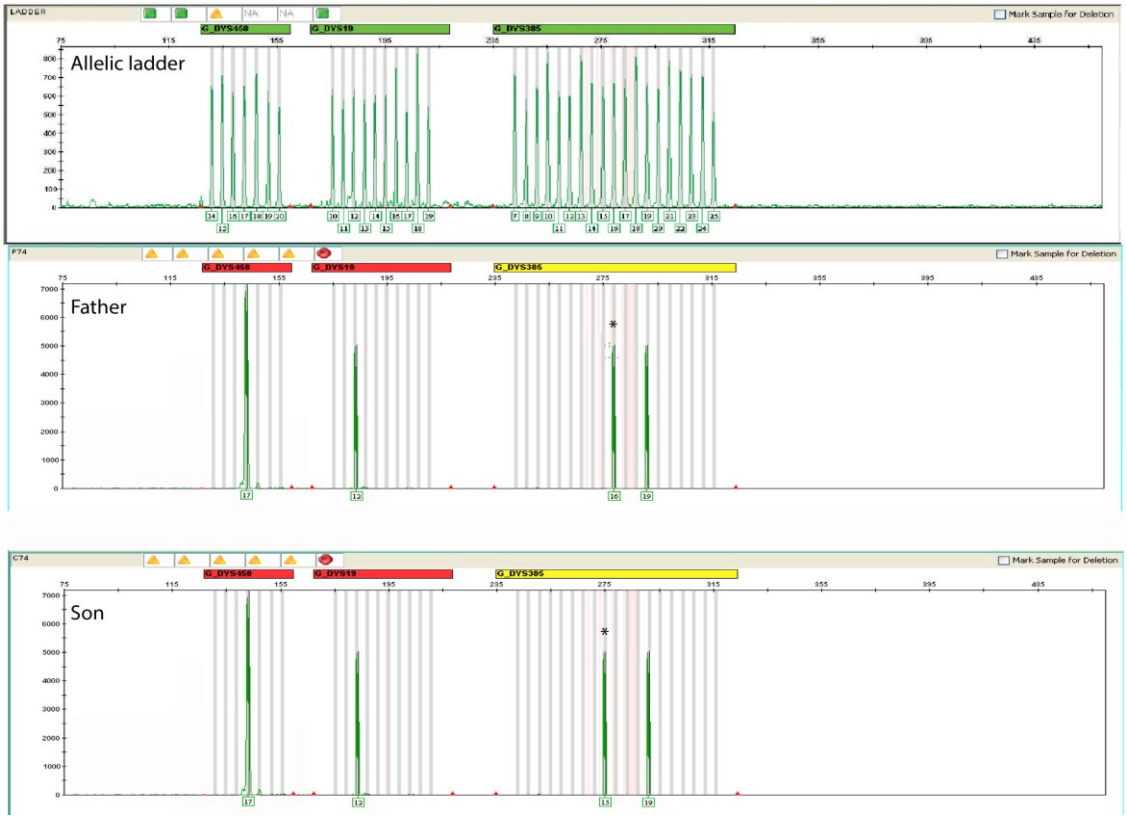

Figure 5: An electropherogram indicating a single repeat loss mutation from father to son at tetranucleotide locus DYS385a. The ladder (Top panel) shows all possible alleles at loci DYS468 (alleles 14 to 20), DYS19 (alleles 10 to 19) and DYS385a/b (alleles 7 to 25). The middle panel shows fathers' alleles including allele 17 at locus DYS468, allele 12 at locus DYS19 and alleles 16 and 19 at locus DYS385a/b respectively. The bottom panel shows son's alleles that are similar to those of fathers with exception of a loss mutation for allele 16 to 15 at DYS385a locus.

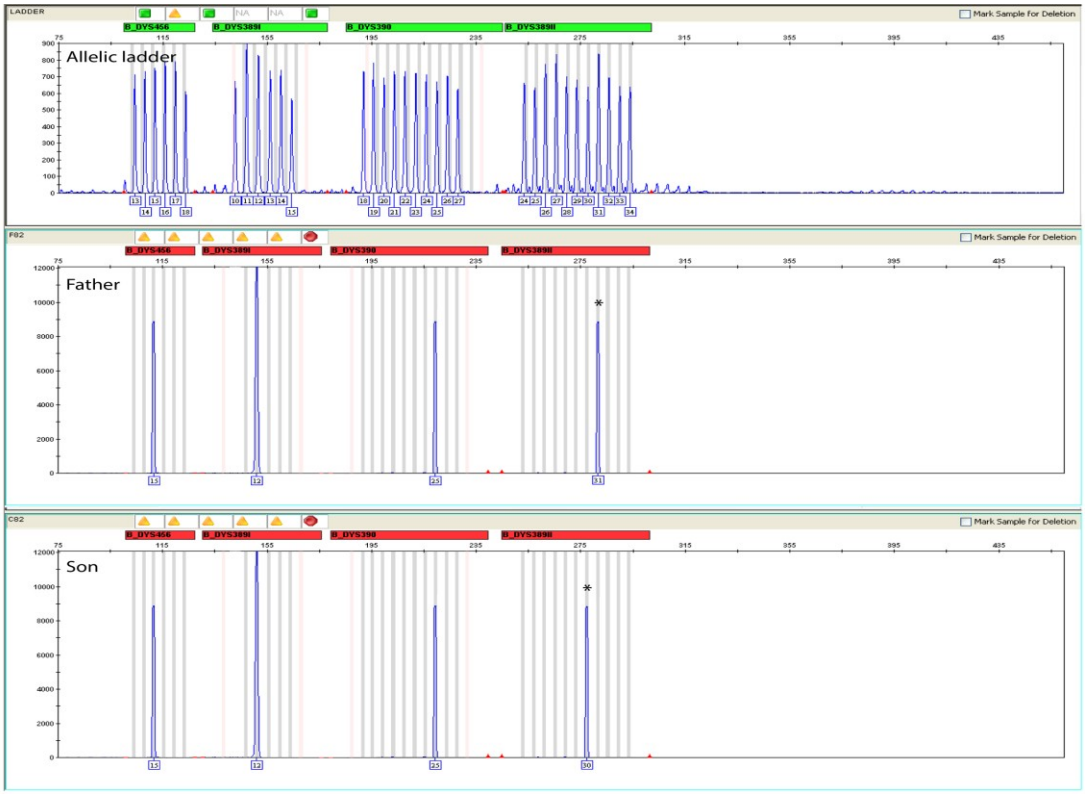

Figure 6: An electropherogram indicating a single repeat loss mutation from father to son at tetranucleotide locus DYS389II. The ladder (Top panel) shows all possible alleles at loci DYS456 (alleles 13 to 18), DYS389I (alleles 10 to 25) DYS390 (alleles 18 to 27), and DYS389II (alleles 24 to 34). The middle panel shows fathers' alleles including allele 15 at locus DYS456, allele 12 at locusDYS389I, alleles 25 at locus DYS390 and allele 31 at locus DYS389II. The bottom panel shows son's alleles that are similar to those of fathers with exception of a loss mutation for allele 31 to 30 at DYS389II.

4.3 17 Y-STRs locus specific mutation rate analysis

There are different methodologies to estimate the mutation rate at Y-STRs loci, but the analysis of father-son pairs of confirmed biological paternity can only reveal reliable results. In this research study, the average mutation rate was determined by dividing the number of mutated samples by the total number of meiosis (Lee et *al.,* 2005) and the confidence interval was calculated using binomial distribution. In present study, the locus

specific mutation rate and its confidence interval were calculated using Excel spread sheet statistical software (statpages.org/confint.html). The results show the locus specific mutation rate estimate were between 0 and 1.176 $x10^{-3}$ and average mutation rate estimate was 2.353 x 10^{-3} (Table 4). This indicates, depending on the Y-STR locus analysed, approximately to one of every thousand father-son pairs show a mutation and on average the Y-STR mutation occurs in about two of every thousand father-son pairs analysed.

4.4 The effect of Fathers' age on 17 Y-STRs Mutation rate analyses

In present study, the confounding effect of the father's age on the mutation rate was modeled using t-test analysis, where the average of fathers' age with at least one mutation were compared with average fathers' age without mutation (Appendix 5). The research findings of this study shows the average fathers' age with at least one mutation at son's birth were 32 years with standard error of 2.387 while the average age of all fathers without mutation in a sampled population at son's birth were 26.781 years with standard error of 0.609. The results clearly shows that fathers' age at son's birth may have confounding effect on Y-STRs mutation rate analysis though this age difference is statistically not significant using unpaired samples t-test ($p = 0.05$).

Table 4: **Mutation count, mutation rate and 95% confidence interval (CI) for the 17 Y-STRs loci studied using Tanzanian father-son paired samples.**

Loci	Repetitive DNA sequence[a]	Mutation count	Allele transmission	Mutation rate	95% CI
DYS19	CTAT/CTAC	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS389I	AAGG/GAAA	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS389II	CTGT/CTAT	1	1700	$5.88x10^{-4}$	$1.5x10^{-5}$ – $3.273x10^{-3}$
DYS390	CTGT/CTAT	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS391	CTGT/CTAT	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS392	ATT	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS393	GATA	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS385a	AAGG	2	1700	$1.176x10^{-3}$	$1.43x10^{-4}$ – $4.243x10^{-3}$
DYS385b	GAAA	1	1700	$5.88x10^{-4}$	$1.5x10^{-5}$ – $3.273x10^{-3}$
DYS438	TTTTC/TTTTA	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS439	GATA	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS437	TCTA/TCTG	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS448	AGAGAT	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS458	GAAA	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS456	AGAT	0	1700	0.000	0.000 – $2.168x10^{-3}$
DYS635	TCTA/TGTA	0	1700	0.000	0.000 – $2.168x10^{-3}$
Y GATA- H4	TAGA	0	1700	0.000	0.000 – $2.168x10^{-3}$
Average		**4**	**1 700**	**$2.353x10^{-3}$**	**$6.41x10^{-4}$ - $6.013x10^{-3}$**

[a]Repetitive sequence structure previously reported by Gusmao and Carracedo (2003).

CHAPTER FIVE

5.0 DISCUSSION

5.1 The 17 Y-STRs Locus Specific Mutation Characteristics

Analysis of locus specific mutation characteristics using 17 Y-STRs loci in Tanzanian father-son pairs of DNA confirmed biological paternity revealed four mutations events which were identified on DYS385a, DYS385b and DYS389II among 17Y-STRs loci analysed. However, no mutation event were observed for DYS19, DYS389I, DYS390, DYS391, DYS392, DYS393, DYS437, DYS348, DYS439, DYS448, DYS456, DYS458, DYS635, and Y-GATA-H4 loci analysed. The observed locus specific mutation rate ranged between 0 for DYS19, DYS389I, DYS390, DYS391, DYS392, DYS393, DYS437, DYS348, DYS439, DYS448, DYS456, DYS458, DYS635, Y-GATA-H4 loci and 1.765 x 10^{-3} ($1.43x10^{-4}$– $4.243x10^{-3}$) for DYS385a locus at 95% CI. Among 100 father-son pairs analysed at the same 17 Y-STRs loci, there were no observation of multiple Y chromosome microsatellite mutation within the same germline transmission or non-uniform alleles such as microvariants, duplication and triplication that have been previously reported by Laouina *et al.,* 2011 using Moroccan population.

The highly polymorphic Y-STR locus DYS385 was observed to have a higher mutation rate compared to all other Y-STRs loci analysed. In this study, the observed higher specific locus mutation rate for Y-STR locus DYS385a/b (if treated as single locus) was 1.765 x 10^{-3} followed by mutation rate of $5.88x10^{-4}$ for locus DY389II.

All observed mutation events were characterised by single step mutations, these results are in accordance with the generally accepted mutation model for microsatellites, in which the

alleles are known to mutate primarily through the gain and loss of single repeat units (Weber and Wong, 1993; Di Rienzo *et al*., 1994; Zhivotovsky and Feldman, 1995).

In addition, two tetranucleotide microsatellites loci DYS385 and DY389II appeared to consist of higher average mutation rates among all 17 Y-STRs analysed compared to all other trinucleotide and dinucleotide microsatellite loci. Similar locus specific mutation characteristics was found in Moroccan's population in a sample of 252 father-son pairs using 17 Y-STRs loci by Laouina *et al.,* 2011 in which average locus specific mutation rate was higher at tetranucleotide microsatellites loci. This higher mutation rate on tetranucleotide microsatellites were also observed by Kayser *et al.,* 2000 using 15 Y-STRs loci for a total of 4999 male germline transmission from father-son pairs of previously confirmed paternity. Furthermore, the single loss mutation characteristics event observed in this study was in agreement with research results found by Farfan *et al.*, 2009 where three single-step loss mutations were observed at DYS389II loci, during mutations analysis at 17 Y-STR loci in father–son pairs from Southern Spain.

5.2 Average Estimates of 17Y-STRs Locus Specific Mutation Rate

In this research study, Tanzanian father-son paired samples collected in Dar es salaam covering 1700 meioses obtained at 17 Y-STRs loci were used to estimate 17Y-STRs locus specific mutation rate. The observed average estimates of 17 Y-STRs locus specific mutation rate ranged from 0 to 1.765×10^{-3} (1.43×10^{-4} - 4.243×10^{-3}) at 95% CI. The higher average locus specific mutation rate was found at DYS385a locus while mutation rates of 5.88×10^{-4} (1.5×10^{-5} – 3.273×10^{-3}) at 95% CI were observed for both DYS385b and DYS389II loci. Furthermore, no mutation observed for DYS19, DYS389I, DYS390, DYS391, DYS392, DYS393, DYS437, DYS348, DYS439, DYS448, DYS456, DYS458, DYS635, and Y-GATA-H4 loci analysed.

However, the average mutation rate across all markers in this study was $2.353x10^{-3}$(6.41×10^{-4} - $6.013x10^{-3}$) at 95% CI. This overall average mutation rate is nearly similar to those reported by Sanchez-Diz *et al.,* 2008 in which he found mutation rate of 2.2×10^{-3} in 701 father-son pairs in Iberian and Latin America groups. Dupuy *et al.,* 2004 used 1766 father-son pairs to analyse 9Y-STRs alone in Norway's population and found the average mutation rate of $2.3x10^{-3}$. A similar mutation rate of $2.3x10^{-3}$ was found by Lee *et al.,* 2007 in collection of Y-STRs mutation events for Korean population using a high number of loci, 22 Y-STRs in a sample size equal to 369 father-son pairs. Furthermore, the average mutation rate estimated in this study is not significantly different from the average mutation rate of autosomal STR loci commonly used in forensics as previously reported from family analysis. In their findings, the autosomal STRs mutation rate of 2.1×10^{-3} was reported by Brinkman *et al.,* 1998, mutation rate of 2.7×10^{-3} was reported by Henke and Henke*,* 1999 whereas mutation rate of 0.6×10^{-3} was reported by Sijantila *et al.,* 1999.

The average mutation rate for 17Y-STRs loci found in this research study is greater than those calculated by Vieira- *et al.,* 2009 in a sample of 95 father-son pairs from Portugal (1.85×10^{-3}).Similarly, Farfan *et al*., 2009 using 17 Y-STRs loci from southern Spain population found the mutation rate to be 1.563×10^{-3} (0.322×10^{-3} - 4.559×10^{-3}) at 95% CI. In contrast, the average mutation rate found in this study is less than those calculated by Decker *et al.,* 2007 for Caucasian and Asians populations cited up, in U.S. admixed sample of 399 father-son pairs using 17 Y-STRs, the mutation rate found was $3.13x10^{-3}$.

The results of present study are in general agreement with the fore mentioned research findings in which all the same 17 Y-STRs set or other number Y-STRs loci used the average mutation rate observed were in the order of 10^{-3} though number of father-son pairs varied between the mentioned studies above. Since there was no significant

differences in mutation rate observed, therefore the mutation rates analysis does not depend on the sample size or number of Y-STRs loci used but population diversity.

5.3 Father's age and 17 Y-STR mutation rates

The research findings of the present study shows the average fathers' age with at least one mutation at son's birth were 32 years with standard error of 2.387 while the average age of all fathers without mutation in a sampled population at son's birth were 26.781 years with standard error of 0.609. The results clearly shows that fathers' age at son's birth may have confounding effect on Y-STRs mutation rate analysis though this age difference is statistically not significant using un paired samples t-test ($p = 0.05$).

A number of previous studies investigated the age effect on the mutation rate for all or some of the Y-STRs studied shows contradictory results. Some studies found the average age of the fathers with mutations being older than that of fathers without, others observed the reverse effect and some found no age difference between mutated and non-mutated fathers. The results of present study is in agreement with previous three research findings reported the age difference between fathers' age with at least one mutation and fathers age without mutation statistically not significant (Sanchez- Diz *et al.,* 2008; Lee *et al.,* 2007). Also, the findings of this study are different from those obtained by De Souza *et al.,* 2005; Gusmao *et al*., 2005 who found fathers age with at least one mutation were older than fathers age without mutation. Similarly, the results of present study in not in agreement with reported results by Kayser *et al.* (2000) and Turina *et al*., (2006) in which the fathers without mutation were older than fathers with mutation.

However, the present study results shows undoubtedly the age of the mutated father from our study is marginally older than that without mutation, this finding is in agreement with

Goedbloed *et al*., 2009 who also found relatively older average age of mutated fathers in her study while other studies did not observe mutated fathers being older than non-mutated ones (Dupuy *et al*., 2004; Sanchez –Diz *et al*., 2008; Lee *et al.,* 2007). Therefore results of this study shows fathers' age at son's birth may have confounding effect on Y-STRs mutation rate analysis though this age difference is statistically not significant using un paired samples t-test at ($p = 0.05$).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The present study shows the locus specific mutation rate estimate of 17 Y-STRs loci were between 0 and 1.176 $x10^{-3}$ and average mutation rate estimate was 2.353 x 10^{-3}. Considering the results obtained in the present study, it can be concluded that, the reliable knowledge about mutation rates of 17 Y-STRs loci used in forensics and paternity testing involving males is very important for a correct interpretation of results. Additionally, locus specific mutations across the different 17 Y-STR loci appear to be independent of each other but depend on repetitive nucleotide sequence structure of the Y-STR locus analysed. Furthermore there is undoubted evidence that single-repeat mutation is strongly favoured over multiple-repeat mutation in the same germline transmission which was not observed in this study. Finally, the mutation rate increased with increasing age of the father at son's birth (though statistically found no significant), suggesting that age is a factor that must be taken into consideration not only when approximating Y-STRs loci mutation rates but also when comparing evaluated mutation rates from different studies.

6.2 Recommendations

As a consequence of observed mutation rates in this study, the criterion for exclusion in paternity testing should be defined, so that an exclusion from paternity has to be based on exclusion constellations at the minimum of two 17 Y-STRs loci. This will assist the experts in forensic DNA testing laboratories to decide the general practice of distinguishing between exclusion and mutation in Y-STRs based analysis. Moreover, similar recommendation pertaining to mutation rates should be implied for both Y-STRs and autosomal STRs loci given that the mutation rate findings of this study was nearly

similar to the reported autosomal STRs mutation rate by Brinkman *et al.,* (1998), as well as reported mutation rate by Henke and Henke (1999).

In order to circumvent practical problem of Y-STRs mutations particularly in paternity testing, the Y-STRs loci can be combined with or substituted with single nucleotide polymorphism (SNPs) which has considerably lower mutation rate. In addition, the GCLA should establish the Human DNA data base; the data base will also include the Y-STRs mutation data that will enrich our knowledge about Y-STRs mutation rates and enhance correct evaluation and interpretation of DNA typing results. Finally, it would be significant to characterize more consented Tanzanian father-son pairs with confirmed biological relationship using larger sample size in order to estimate more reliable locus specific mutation rates and characteristics for most widely used 17 Y-STRs markers in both population and forensic genetics.

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APPENDICES

Appendix 1: Ethical clearances certificate with reference number NIMR/HQ/R.8a/VOL. IX/1826 obtained from Medical Research Coordinating Committee (MRCC) of NIMR.

THE UNITED REPUBLIC OF TANZANIA

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Ministry of Health and Social Welfare
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Fax: 255 22 2110986

25th September 2014

Mr Fidelis Charles Bugoye
Government Chemist Laboratory Agency
P O Box 164
DAR ES SALAAM, Tanzania

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Analysis of Mutation Rate of 17-Y Chromosome Short Tandem Repeats Loci Using Tanzanian father/son Paired Samples Collected in Dar es Salaam, (Bugoye F C *et al*), has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Dar es Salaam Region.

Approval is for one year: 25th September 2014 to 24th September 2015.

Name: Dr Mwelecele N Malecela

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Dr Donan Mmbando

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL WELFARE

CC: **RMO**
DED
DMO

Appendix 2: Study consent form (English and Swahili versions)

SOKOINE UNIVERSITY OF AGRICULTURE
FACULTY OF VETERINARY MEDICINE
Department of Veterinary Microbiology and Parasitology
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INFORMED CONSENT AGREEMENT

TITLE: Analysis of mutation of 17 Y-Chromosome short tandem repeat loci using Tanzanian father-son paired samples.

RESEACHERS: Fidelis Charles and Prof. Gerald Misinzo

PURPOSE: The goal of this research study is to give the average estimate of 17 Y-STRs mutation rate of Tanzanian father-son paired samples.

DESCRIPTION OF THE RESEARCH:

DNA is the deoxyribonucleic acid which is found in every cell of an individual's body and it is inherited from one generation to the next. DNA is unique to each individual. Therefore is useful tool in forensic investigations, paternity testing and disaster victim's identification. DNA testing is a valuable powerful tool for criminal investigations: many people have been exonerated from the crimes they did not commit and criminals have been convicted for the crimes.

At each stage of development, all the cells forming the body contain the same DNA, half from the father and half from the mother. This fact allows the relationship testing to use all types of all samples including loose cells from the cheeks collected using buccal swabs, blood or other types of samples.

IMPORTANCE OF Y-CHROMOSOME MUTATION RATE DATA

Y-Chromosome short tandem repeats (Y-STRs) currently are used as markers of choice in criminal investigations of crime against human male, sexual assaults cases, disaster victim identification and paternity testing of male offspring with a deceased alleged father. DNA evidence in forensics and paternity testing are carried out by analysis of short tandem repeat DNA loci and the interpretation of results depends on similarities or differences at genetic marker loci between the father and offspring and the observed differences are attributed to non-biological paternity.

The spontaneous mutation in the germ line of father at any genetic marker locus used in the DNA analysis can lead to erroneous exclusion because such mutation results in differences between parent and offspring. Since polymorphic short tandem repeats (STRs) which are normally used for forensics are constantly evolved through the mutational process, the interpretation of DNA profiles require the knowledge of mutation rate at each locus used.

The DNA evidence using YSTRs loci that lack statistical value for average mutation rate cannot be placed to strengthen the DNA evidence against the defendant.

PROCEDURE

To involve yourself in this study you will be required to contribute a small sample of saliva from the mouth for DNA extraction and analysis. DNA sample will be collected using sterile buccal swabs. Your sample will then be organized in a safe and sterile environment and be transported at the Government chemist laboratory agency for DNA analysis.

RISKS

This procedure is completely safe and is not associated with any known health risks, the sterile tools will be used to avoid contamination

BENEFITS

This study may eventually lead to more effective law enforcement in the country; the results from this study will be used to solve more truthful criminal cases including; murder, rape, burglary and thefts. Also the study will be used in the identification of victims of mass disasters and civil cases such as paternity cases involving males.

CONFIDENTIALITY

Your samples will be collected and labeled using code in such a way that they cannot be traced back to you by the sampling officer handling your sample. Your participation in this study will also be kept confidential and will not be used in a manner that ever discloses your identity. However, the overall results of this study may be shared with members of the scientific community at large and will become a matter of public record, but the names will not be disclosed.

ALTERNATIVES/RIGHT TO REFUSE OR WITHDRAW

You can make a decision today not to involve yourself in this study. You may also withdraw your sample from the study at any time. Your DNA sample will only be used for the purposes stated herein and will be destroyed once it has been tested.

FINANCIAL COSTS TO THE SUBJECT

There is no financial cost to you for participating in the study.

PAYMENT FOR PARTICIPATION

You will receive no payment for participating in the study.

INDIVIDUAL(S) TO CONTACT

If you have any questions about the conduct or results of this study, you may contact: Dr. Gerald Misinzo 0767058805, Fidelis Charles Bugoye 0713 889262 all from Sokoine University of Agriculture.

If you have any questions about your rights as a research subject, you can contact:
Prof. Gerald Misinzo (PhD), senior lecture, department of veterinary microbiology and parasitology- Sokoine University of Agriculture , P.O.box 3019 Morogoro.
The Chairman of the National Health Research Ethics Committee can also be contacted at +255222121400 concerning your rights as a research volunteer.

You will receive a copy of this consent form if you agree to participate in this research study.

_____ Participant initials.

CONSENT

I have read and understand this consent form and I have had a chance to ask questions about this research study. I also understand that when I sign my name below, I am agreeing to participate in this research study.

…….

Signature of Participant/or Thumb print Date

…….. …….

Participant Name (please print) and Age Date

…… …….

Signature of parent (if participant is under the age of 18) Date

……... …….

Signature of Primary Investigator Date

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FOMU YA KUKUBALI KUSHIRIKI KWENYE UTAFITI.

JINA LA UTAFITI: Kuchunguza kasi ya mabadiliko ya vinasaba kutoka kwa baba kwenda kwa mtoto wa kiume kwa kutumia vitambuzi vya vinasaba aina 17 kwa sampuli za baba na mtoto wa Tanzania.

WATAFITI: Watafiti ni Fidelis Charles na Prof. Gerald Misinzo .

LENGO LA UTAFITI:

Dhumuni la utafiti huu ni kupata takwimu za kasi ya mabadiliko ya vinasaba kutoka kwa baba kwenda kwa mtoto wa kiume wa Tanzania ili ziweze kutumika kwa kutambua waharifu, watu waliokufa kwenye majanga na uhalali wa baba kwa mtoto wa kiume.

MAELEZO KUHUSU UTAFITI

DNA (vinasaba) ni kifupi cha Deoxyribonucleic acid ambayo ni jina la molekuli ndani ya seli za viumbehai pamoja na wanyama, mimea, bakteria na virusi. Inabeba ndani yake taarifa zote za urithi wa kiumbe hai kutoka kwa wazazi.

Vinasaba (DNA) vya binadamu zinatofautiana, kama alama za vidole (Fingerprint) isipokuwa mapacha. Kutokana na utofauti huo kipimo cha DNA kinaweza kutumika kutambua waharifu kama vile majambazi, wabakaji na wezi. Vinasaba (DNA) vya binadamu imeundwa na muungano wa vinasaba kutoka kwa wazazi, nusu inatoka baba na nusu inatoka kwa mama. Vilevile kipimo cha DNA kinaweza kutumika kumtambua baba halisi wa mtoto.

Kipimo cha vinasaba kinapotumika kwenye uchunguzi wa makosa ya jinai kinaweza kumtambua mharifu aliyehusika kwenye tukio la wizi, ubakaji, ujambazi au uporaji. Vilevile kipimo hiki kinaweza kumsaidia mtuhumiwa kuachiwa huru kutokana na kuhusishwa na kosa ambalo hakutenda. Kipimo cha vinasaba (DNA) kinaweza kutumika kutambua wahanga wa majanga kama vile moto, ajari za majini na mafuriko.

UMUHIMU WA TAKWIMU ZA KASI YA MABADILIKO YA VINASABA KUTOKA KWA BABA KWENDA KWA MTOTO WA KIUME

Mpangilio wa vinasaba wa seli za mwili huwa unatofautiana kati ya mtu mmoja na mwingine na kutofautiana huko kwa vinasaba ni sifa mojawapo muhimu inayotumiwa katika kutambua wahalifu na kujua uhalali wa baba kwa mtoto. Vilevile kuna ya mpangilio wa vinasaba vinavyopatikana kwa mwanaume na vyeenye jinsia ya kiume ambazo urithiwa kwa wanaume tu, yaani kutoka kwa baba kwenda kwa mtoto wa kiume toka kizazi kimoja hadi kingine ambazo kwa kawaida huwa zinafanana kutoka kwa baba hadi kwa mtoto wakiume tu. Mabadiliko ya vinasaba vya jinsia ya kiume huweza kutokea wakati wa uundwaji wake na hali hii huweza kusababisha maeneo machache ya mpangilio wa vinasaba kutofautiana kati ya baba na mtoto wa kiume.

Hivyo ukusanyaji wa sampuli za vinasaba kutoka kwa baba na moto wake wa kiume kwa jamii ya watanzania na kuzifanyia uchunguzi wa kimaabara utasaidia kupata takwimu za kasi ya mabadiliko ya mpagilio wa vinasaba vya jinsia ya kiume na zitakuwa chombo muhimu kwenye uchunguzi wa makosa ya jinai na utambuzi wa wahanga katika majanga mbalimbali.

TARATIBU ZA KUSHIRIKI

Kushiriki kwenye utafiti huu utatakiwa kutoa mate au mpanguso wa mate ndani ya shavu la kinywa. Sampuli ya mate itachukuliwa kwa kutumia pamba iliyoko kwenye kijiti.

Sampuli itahifadhiwa kwenye mazingira salama na kusafirihwa kwenye maabara ya Mkemia Mkuu wa Serikali kwa uchunguzi.

THADHARI

Utaratibu wa kuchukua sampuli ni salama na hauna madhara yoyote.

FAIDA

Matokeo ya utafiti huu yatasaidia vyombo vya dola kuweza kukamilisha uchunguzi wa makosa ya jinai kwa kutambua wahusika halisi na kutoa hukumu iliyo sahihi.

USIRI

Sampuli yako itapewa alama ambayo mtu yeyote hawezi kuifuatilia. Baada ya uchunguzi wa kimaabara na majibu kupatikana sampuli yako itaharibiwa.

MALIPO YA USHIRIKI

Hakutakuwa na malipo ya aina yoyote ili kushiriki katika utafiti huu.

MAWASILIANO BINAFSI

Kama una maswali kuhusu utafiti huu au matokeo ya utafiti huu, unaweza kuwasiliana na: Prof. Gerald Misinzo 0767058805: Fidelis Charles 0713 889262 wote kutoka Chuo Kikuu cha Sokoine-Morogoro.

Kama una maswali kuhusu haki yako kama mshiriki , unaweza kuwasiliana na:

Prof. Gerald Misinzo (PhD), Mhadhili mwandamizi katika idara ya mirobiolojia na parasaitolojia ya mifugo na, Chuo kikuu cha kilimo Sokoine, S.L.P 3019 Morogoro .

Mwenyekiti wa Kamati ya Kuzingatia Maadili kutoka Taasisi ya Utafiti wa Magonjwa ya Binadamu ya Tanzania (NIMR) anaweza pia kutaarifiwa kuhusu haki zako (au mwanao) kama mshiriki katika utafiti kwa namba ya simu +255-22-2121400.

Utapata nakala ya fomu hii yakibali endapo unakubali kushiriki katika utafiti huu.

…….Herufi za mshiriki.

Nimesoma na kuelewa yote yaliyoandikwa kwenye fomu hii na nimepata muda wa kuuliza maswali. Natambua kuwa kwa kuweka saini yangu au alama ya dole gumba kwenye fomu hii nimekubali kushiriki kwenye utafiti huu.

…… …….

Saini ya mshiriki/au dole gumba Tarehe

…… …….

Jina la Mshiriki na umri Tarehe

…… …….

Saini ya mzazi ikiwa mshiriki ana umri chini ya miaka 18 Tarehe

……... … …….

Saini ya Mtafiti.

Appendix 3: Autosomal 15 STRs loci DNA typing results. The results confirmed father-son pairs biological relationship.

Sample ID	D8	D2	D7	CSF	D3	THO1	D13	D16	D2	D1	VWA	TPOX	D18	D5	FGA
FOO1	13,13	28,30	9,12	11,11	15,16	7,9	11,12	9,10	17,21	12,13	14,15	10,11	18,19	12,12	22,27
MOO1	15,16	30,31	8,10	8,10	15,16	6,8	11,13	12,12	17,19	12,13	15,16	8,8	18,19	11,12	23,24
C001	13,15	28,30	8,12	10,11	15,16	7,8	12,13	10,12	17,21	12,12	15,16	8,10	18,18	12,12	22,24
FOO2	11,14	29,31	10,10	11,11	16,16	7,9	12,13	9,11	19,20	13,13.2	15,17	9,11	16,17	11,13	23,28
MOO2	13,15	31,30	10,12	11,11	16,16	7,8	12,13	9,12	19,21	13,13	17,15	9,8	16,19	8,12	24,24
C002	13,14	28,31	10,10	10,11	16,16	7,7	12,13	9,11	19,26	13,13.2	17,17	9,11	16,17	8,11	24,28
FOO3	15, 15	28,30.2	8, 10	8, 12	15, 16	7, 9	11, 12	9, 11	19, 23	13, 16.2	11, 17	9, 11	12, 16	12, 13	21, 23
MOO3	11, 16	28, 31	8, 11	10, 12	15, 17	6, 6	11, 12	9, 12	16, 17	14, 15	15, 18	8, 9	16, 16	11, 13	22, 23
C003	11, 15	28, 31	8, 8	12, 12	15, 17	6, 9	11, 12	9, 12	16, 19	13, 15	11, 15	9, 9	16, 16	11,12	22, 23
FOO4	12, 14	29, 32	8, 9	7, 10	15, 16	7, 9	12, 13	11, 14	17, 17	11, 13	15, 15	8, 8	17, 19	9, 13	21, 23
MOO4	12, 12	28, 29	8, 8	11,11	15, 17	6, 8	10, 11	11, 13	18, 19	11, 12	15, 19	6, 8	18, 19	12, 12	22, 25
C004	12, 14	29, 32	8, 9	10, 11	15, 15	6, 7	11, 12	11, 11	17, 19	12, 13	15, 19	8, 8	17, 18	12, 13	21, 25
FOO5	14,16	28,30	10,11	11,12	16,17	6,8	11,11	9,11	21,22	12.2,14.2	14,16	8,11	14,17	12,13	23,24
MOO5	15,16	28,28	11,12	10,10	15,16	6,8	11,13	9,10	17,21	10,14	15,16	12,13	15,16	8,10	23,26
C005	15,16	28,28	10,11	10,11	15,17	8,8	11,13	9,9	21,22	10,14.2	15,16	8,12	15,17	8,12	24,26
FOO6	15,16	27,31.2	11,11	10,11	16,16	7,7	8,11	9,12	22,23	12,13	18,18	8,8	16,18	12,13	21,28
MOO6	14,14	28,32.2	8,10	10,11	15,18	7,9.3	12,13	11,12	17,19	13,13.2	16,17	8,9	16,18	8,12	22,23
C006	14,15	31.2,32.2	10,11	11,11	16,18	7,7	8,12	9,11	17,23	13,13	16,18	8,8	16,18	8,12	21,23
FOO7	11,12	30,34	8,11	10,12	16,18	7,9	12,13	9,13	22,23	12,13.2	16,17	6,8	13,16	12,12	22,27
MOO7	12,15	28,30	10,11	9,13	14,17	8,9	11,14	11,11	19,27	14.2,15	15,20	6,9	15,16	11,11	20,26
C007	11,15	28,30	8,11	9,10	14,16	9,9	12,14	11,13	22,27	12,15	16,20	8,9	13,16	11,12	22,26
FOO8	12, 13	28, 33.2	8, 10	10, 10	14, 17	6, 9	8, 12	8, 12	19, 22	12, 14	15, 19	6, 11	12, 16	10, 11	23, 24
MOO8	11,12	28,29	10,9	11,10	16,17	8,9	13,14	11,13	19,23	11,12	15,17	8,8	16,18	11,12	21,25
C008	11, 12	28, 29	9, 10	9, 10	16, 17	8, 9	12, 13	12,13	19, 19	12, 14.2	15, 16	8, 11	15, 16	11, 12	24, 25
FOO9	11, 13	28, 30	11,12	11, 12	17, 18	6, 6	11, 12	11, 11	24, 27	15, 16.2	14, 15	9, 10	14, 19	13, 13	24, 31.2
MOO9	11,13	28, 35	8, 10	11, 11	16, 17	6, 7	11, 14	10, 13	17, 23	14, 14.2	16, 16	8, 11	19, 20	11, 11	23, 29
C009	11, 13	28, 28	8, 11	11, 12	16, 17	6, 6	11, 11	10, 11	17, 24	14, 15	15, 16	11, 10	14, 20	11, 13	24,23
FO10	14,15	29,30	10,11	10,11	15,16	7,8	11,12	10,13	19,22	12,13	15,19	6,8	13,17	9,13	24,26
MO10	11,14	30,31	8,9	10,12	16,18	6,8	12,13	11,12	19,20	11,12	16,18	8,8	15,16	12,12	21,26
C010	11,14	30,30	8,10	10,12	16,18	8,8	11,12	10,12	20,22	11,13	15,16	6,8	15,17	9,12	26,26
FO11	14, 14	28, 31.2	11, 11	10, 11	15, 16	7, 9	11, 11	11, 12	18, 19	14.2, 15	16, 19	8, 11	15, 20	12, 13	19, 26
MO11	11, 16	28, 32.2	10, 11	11, 12	15, 15	7, 9	12, 14	10, 12	22, 22	12, 14	11, 16	9, 11	12, 13	11, 12	20, 20
C011	11, 14	28, 28	10, 11	10, 12	15, 16	9, 9	11, 14	10, 12	18, 22	12, 15	11, 16	9, 11	12, 15	11, 13	19,20
FO12	13,14	29,29	10,10	12,14	16,17	7,9	12,12	12,13	22,26	13,14	11,17	8,11	17,21	12,13	22,23
MO12	14,14	28,28	10,12	10,12	17,18	6,9	12,12	9,10	19,22	12.2,13	15,17	10,11	16,19	11,12	20,22
C012	14,14	28,29	10,12	10,14	17,17	7,9	12,12	9,13	22,22	12.2,14	11,17	11,11	17,19	12,12	22,22

FO13	15, 15	29,29	7, 11	9, 11	15, 17	6, 8	12, 12	9, 12	16, 18	14, 15.2	17, 18	8, 12	14, 18	12, 13	20, 24
MO13	14, 17	30, 31	10, 10	10, 10	14, 17	8, 9	9, 14	9, 10	23, 23	13, 15.2	16, 18	6, 9	16, 18	13, 13	21, 24
C013	14, 15	29, 31	10, 11	9, 10	15, 17	8, 8	9, 12	9, 9	16, 23	14, 15.2	16, 17	6, 12	14, 18	12, 13	21, 24
FO14	13,15	27,28	8,10	10,10	14,15	7,8	12,12	9,13	22,22	13,14.2	15,17	9,11	14,20	12,12	22,23
MO14	13,14	28,33	8,10	10,11	16,17	7,8	12,12	11,12	19,19	11,13	15,19	8,11	17,18	11,13	22,25
C014	13,14	27,28	8,10	10,10	15,17	7,8	12,12	12,13	19,22	11,14.2	15,19	11,11	14,18	11,12	22,25
FO15	10, 16	27, 30	9, 11	11, 11	15, 17	6, 7	11, 13	9, 9	19, 21	14, 14	15, 15	7, 8	13, 17	8, 13	21, 21
MO15	15, 16	28, 31.2	9, 10	12, 12	15, 18	7, 8	11, 11	8, 12	18, 21	14, 14.2	15, 15	8, 8	17, 19	12, 13	17, 21
C015	15, 16	27, 28	9, 11	11, 12	17, 18	7, 8	11, 13	9, 12	19, 21	14, 14.2	15, 15	7, 8	13, 19	13, 13	17, 21
FO16	13,15	30,31	10,11	10,12	15,16	7,8	11,12	11,11	17,23	11,13	15,17	11,11	16,20	12,13	22,23
MO16	11,16	27,32.2	11,12	10,12	14,15	6,8	11,12	10,12	19,22	12,14	14,15	11,11	15,22	13,13	22,24
C016	13,16	31,32.2	11,12	10,10	15,16	8,8	12,12	10,11	17,22	11,12	14,17	11,11	15,16	12,13	22,22
FO17	15, 16	30, 31	8, 11	10, 12	14, 16	6, 7	10, 13	11, 12	18, 19	11, 15.2	15, 18	10, 11	17, 20	12, 13	20, 22
MO17	11,15	30,31	8,11	11,12	14,16	6,8	11,13	10,12	19,22	11,14	15,16	8,6	17,19	12,13	20,24
C017	11, 16	30, 31	8, 11	12, 12	14, 16	6, 7	11, 13	10, 11	19, 22	11, 12.2	16, 18	6, 10	17, 19	12, 12	20, 23
FO18	11,16	30,31	11,12	11,12	15,17	7,8	12,13	9,12	19,21	14,16	15,15	10,12	16,16	11,12	20,24
MO18	13,14	28,28	7,10	11,12	14,16	6,7	8,12	9,10	17,27	11,14	17,17	9,9	13,14	8,11	22,28
C018	11,14	28,31	10,12	11,12	14,15	6,8	12,13	9,9	17,19	11,14	15,17	9,12	14,16	8,12	24,28
FO19	12,14	31,32.2	8,12	11,12	15,18	7,7	12,12	12,12	21,22	12.2,14.2	17,17	8,11	12,19	11,13	22,23
MO19	12,14	28,28	10,11	7,12	16,17	8,8	10,13	11,12	22,23	13,16.2	13,20	9,11	12,19	12,13	21,21
C019	12,14	28,31	8, 10	11,12	16,18	7,8	10,12	12,12	21,23	12.2,13	17,20	11,11	12,12	11,13	21,22
FO20	12,15	29,32.2	13,13	9,12	15,17	5,8	9,12	9,12	19,19	12,13	16,18	8,9	16,17	12,13	23,28
MO20	14,15	31,31.2	9,10	8,12	16,18	7,8	12,13	10,12	17,23	14,16	17,19	8,9	15,16	10,12	23,25
C020	15,15	29,31.2	10,13	8,9	16,17	7,8	12,13	9,10	17,19	12,16	16,19	9,9	15,16	10,12	25,28
FO21	13,14	32,32.2	11,12	9,11	15,16	6,8	8,12	11,12	21,24	13,15.2	16,16	8,11	17,18	12,13	22,25
MO21	10,13	29,30	10,12	11,12	14,14	6,8	11,12	10,12	19,21	12,17.2	16,19	9,11	14,17	12,13	19,23
C021	13,14	32,30	10,12	11,12	14,16	6,6	12,12	12,12	21,19	13,17.2	16,19	9,11	14,17	13,12	23,25
FO22	12,12	28,30.2	10,12	10,13	15,17	8,9.3	12,13	12,13	21,22	11,13	15,17	8,9	13,19	8,11	20,24
MO22	10,13	29,30.2	11,12	9,10	14,15	7,8	11,12	10,11	21,24	12,13	14,15	8,8	13,17	8,12	22,24
C022	12,13	30,30.2	10,12	10,10	15,15	8,8	12,13	11,13	21,24	13,13	15,19	8,9	17,19	8,13	24,30
FO23	13,14	27,28	9,11	12,13	14,16	8,9	11,14	9,11	22,24	13,13	17,20	11,11	16,21	12,13	23,24
MO23	13,14	27,28	10,12	9,12	16,16	6,8	11,12	9,9	22,22	13,14.2	14,16	8,9	15,16	11,13	21,23
C023	13,14	27,27	9,12	12,13	14,16	8,9	11,12	9,9	22,22	13,14.2	16,17	9,11	16,21	11,13	21,23
FO24	14,16	28,29	10,14	7,11	15,16	7,7	12,12	9,10	17,25	12,13	15,16	8,11	15,17	8,12	20,23
M024	13,13	28,31	10,11	11,12	15,16	6,8	11,11	11,13	22,25	13,13	17,18	8,11	14,15	11,11	24,24
C024	13,14	28,29	10,11	11,12	15,15	7,8	12,11	11,10	17,22	13,13	17,16	8,11	14,15	8,11	24,23
FO25	15,12	28,35	9,10	9,12	14,16	7,7	11,12	11,12	21,21	13,15	16,19	6,9	15,20	12,12	23,26
MO25	12,14	31,31	10,10	10,10	14,16	8,9	11,13	9,11	19,23	11,14	16,17	11,11	17,18	12,13	22,24
C025	14,15	31,35	10,10	10,12	14,14	7,8	11,12	9,12	21,23	13,14	16,19	9,11	15,18	12,13	22,26
FO26	13,13	31.2,34	9,10	7,12	14,15	6,7	11,12	9,11	20,21	13,13.2	14,15	8,10	16,18	12,13	21,22
MO26	14,14	27,32.2	8,10	11,12	14,16	7,7	11,13	11,11	22,22	11,12	17,18	11,12	15,19	12,13	29,29
C026	13,14	31.2,32.2	8,9	7,12	14,16	6,7	12,13	11,11	21,22	11,13.2	15,18	8,11	15,18	12,13	21,29
FO27	15,15	28,28	10,10	9,10	15,17	7,8	12,12	11,11	19,27	13,13	13,16	11,12	17,23	12,13	25,28

MO27	12,12	31,31.2	10,10	12,12	14,16	6,9,3	11,12	11,12	17,23	13,14	15,20	6,9	16,20	8,11	22,24
C027	12,15	28,31	10,10	9,12	14,17	6,8	11,12	11,12	17,27	13,14	13,15	6,12	16,17	8,12	24,28
FO28	13,15	28,30	10,10	11,12	14,15	6,8	12,12	10,10	19,19	13,16	16,18	9,9	17,22	8,11	21,22
MO28	12,14	30,31	11,13	9,12	15,15	6,10	11,14	9,10	22,23	11,12	15,16	10,11	12,17	12,13	22,22
C028	12,15	28,30	10,13	9,11	15,15	10,8	12,14	10,10	19,22	11,13	16,18	9,11	17,22	11,12	22,21
FO29	14,15	28,28	8,12	10,12	14,16	7,7	12,12	11,13	16,17	12,14	16,18	8,8	16,19	10,12	22,31.2
MO29	12,14	28,35	8,11	11,11	15,16	7,7	11,13	11,13	17,21	15,16	14,18	8,11	12,18	12,13	21,30
C029	14,15	28,35	8,11	10,11	15,16	7,7	12,13	11,11	16,17	14,15	14,18	8,11	16,18	12,13	21,31.2
FO30	11,15	27,28	9,12	10,12	15,18	6,8	11,12	11,12	18,22	13,13.2	15,16	9,9	14,15	6,12	22,24
MO30	11,12	29,34.2	12,12	10,12	15,15	6,8	12,14	9,9	16,16	13,13	15,17	8,8	16,17	12,13	21,23
C030	11,11	27,34.2	12,12	10,12	15,18	6,8	12,14	9,12	16,22	13,13	16,17	8,9	14,16	12,12	21,24
FO31	14,15	28,28	8,12	10,12	14,16	7,7	12,12	11,13	16,17	12,14	16,18	8,8	16,19	10,12	22,31.2
MO31	12,14	28,35	8,11	11,11	15,16	7,7	11,13	11,13	17,21	15,16	14,18	8,11	12,18	12,13	21,30
C031	14,15	28,35	8,11	10,11	15,16	7,7	12,13	11,11	16,17	14,15	14,18	8,11	16,18	12,13	21,31.2
FO32	10,14	29,35	10,13	10,12	16,18	7,8	12,14	10,12	21,23	13,15.2	16,16	9,11	12,18	11,12	22,23
MO32	14,16	31,35	8,10	11,12	16,16	6,7	12,14	9,12	19,20	13,14	15,16	9,9	13,16	12,13	24,26
C032	10,16	31,35	8,10	10,11	16,16	6,7	14,14	12,12	20,23	13,14	16,16	9,9	16,18	12,13	22,24
FO33	12,13	31,36	10,11	10,12	14,15	6,7	12,14	10,11	18,21	15,15.2	15,16	8,11	17,17	13,13	19,13
MO33	13,15	28,30	10,12	10,10	15,16	7,8	11,12	11,13	16,19	13.2,14	16,18	6,8	16,21	8,13	22,24
C033	12,13	28,36	10,11	10,10	15,16	6,8	11,14	11,13	16,21	14,15	16,18	8,8	17,21	8,13	19,22
FO34	14, 15	27, 34	8, 10	10, 12	15, 16	8, 9	11, 12	11, 13	19, 20	12.2, 12.2	18, 19	9, 11	14, 15	9, 12	22, 22
MO34	12,15	27,30	10,12	7,12	14,16	8,9	11,14	12,13	16,20	11.2,13	15,16	8,9	14,15	9,13	19,22
C034	12, 14	27, 28	10, 12	7, 10	14, 15	8, 9	11, 12	12, 13	16, 19	11.2, 12.2	15, 19	9, 9	15, 16	9, 10	22, 24
FO35	14, 14	29,35	8,8	11,12	14,16	9,9	10,11	10,11	17,22	13.2,14	16, 17	10,11	18,19	12, 13	22, 25
M035	12,14	27,28	8,13	10,11	14,15	7,8	10,11	10,13	18,22	14,16.2	15,16	8,11	17,18	11,12	19,22
C035	14,14	28, 35	8,10	10, 11	14, 17	8, 9	11, 11	10,11	22,24	14,16.2	16, 18	8, 11	18,20	12,13	19,22
FO36	15, 15	27, 31.2	8, 11	10, 12	15, 17	6, 7	11, 13	9, 11	19, 22	14, 15	15, 16	8, 11	15, 18	10, 13	20, 22
MO36	14, 16	27, 35	10, 10	10, 12	14, 17	9, 9	12, 13	10, 13	17, 19	13, 14.2	13, 17	10, 11	16, 17	8, 13	23, 24
C036	15, 16	27, 31.2	10, 11	10, 12	15, 17	7, 9	12, 13	9, 10	19, 22	13, 15	16, 17	10, 11	16, 18	8, 13	20, 24
FO37	13,13	30,30	10,11	11,12	18,18	8,8	10,12	9,12	19,20	13,13	14,17	8,9	15,20	12,13	20,23
MO37	13,14	29,32.2	10,11	8,11	15,15	7,7	11,12	12,13	17,22	11,12	16,17	8,10	17,20	12,12	20,26
C037	13, 13	29,30	10,11	8,11	15, 18	7,8	11,12	12,13	17,19	12,13	16,17	8,10	20,20	12,13	20,26
FO38	14,16	29,29	8,10	11,12	16,17	7,8	11,12	9,9	17,23	13,14	16,18	11,11	15,17	10,12	20,22
MO38	13,15	28,29	10,11	10,11	15,17	6,8	12,13	9,12	19,22	11,13	15,14	8,11	15,18	11,12	20,22
C038	13,16	29,30	10,12	11,11	17,17	6,7	12,12	9,11	22,23	13,14	14,18	6,11	15,15	12,13	21,22
FO39	14,14	29,31.2	10,10	12,13	16,17	7,7	13,13	11,13	21,23	13,13	16,19	11,11	13,15	11,12	21,25
MO39	14,15	29,31	8,10	10,11	14,16	7,7	12,12	11,12	19,19	13,14	15,18	9,11	16,22	8,13	21,22
C039	14,15	29,31.2	10,10	11,12	14,17	7,7	12,13	11,13	19,21	13,13	18,19	11,11	15,22	8,12	21,21
FO40	14,15	30,34	9,12	11,12	16,17	6, 7	13, 13	10,11	17,22	15,16.2	18,18	11,11	16,17	11,12	22,24
MO40	12,14	29,33.2	10,11	9,10	14,15	6,6	11,12	9,11	16,19	12, 13	17,18	8,11	16,19	10,13	21,24
C040	15, 14	30,33.2	10,12	9,11	15, 16	6, 7	11,13	9,11	17,16	12,15	17,18	8,11	17,16	10,12	21,22
FO41	13,16	28,30	9,11	8,10	16,17	6,9	10,11	9,11	21,22	13,14	15,15	8,9	16,17	13,14	21,21

MO41	14,14	28,31.2	8,10	11,12	15,15	8,8	11,11	9,9	19,24	13,13.2	18,19	6,9	15,18	10,12	19,22
C041	13,14	28,28	10,11	8,12	15,17	8,9	11,11	9,9	19,21	13,13.2	15,18	6,9	17,18	10,13	19,21
FO42	13,15	28,33.2	9,10	10,12	16,17	7,9	12,12	12,13	19,25	13,14	11,16	6,11	10.2,17	11,12	20,24
MO42	11,15	28,28	10,12	11,12	16,17	7,8	10,13	11,11	20,23	14,17.2	14,17	6,8	16,18	9,12	24,25
C042	13,15	28,33.2	10,10	10,11	16,17	7,9	12,13	11,12	23,25	14,14	11,17	6,6	10.2,18	9,11	24,25
FO43	14,15	29,31	10,11	8,12	14,16	7,9.3	11,12	11,11	19,23	13,15	15,18	8,9	12,16	12,12	21,22
MO43	16,17	31,32.2	8,10	10,12	15,15	7,9.3	12,13	9,9	17,24	13.2,14.2	18,19	10,11	12,18	12,13	21,23
C043	14,16	31,32.2	10,11	8,10	14,15	7,9.3	11,12	9,11	17,23	13.2,15	18,18	8,10	16,18	12,12	21,22
FO44	12,12	27,32	8,12	11,12	15,15	7,7	11,12	11,14	19,23	14.2,15.2	15,15	8,10	15,16	10,13	22,25
MO44	11,14	30,30	11,12	9,11	14,16	7,9.3	11,12,	10,12	19,24	13.2,14	15,16	8,8	15,17	9,13	25,25
C044	12,14	30,32	11,12	9,12	14,15	7,7	12,12	11,12	19,19	14,14.2	15,15	8,10	15,17	13,13	22,25
FO45	13,15	30,32	11,12	12,13	14,15	6,7	12,13	9,12	22,24	11,13	17,17	11,12	14,18	10,12	24,27
MO45	13,14	27,28	10,11	11,11	14,14	7,7	11,12	10,11	17,22	10,13	15,16	11,11	14.2,15	12,13	22,13
C045	13,15	28,30	10,11	11,12	14,14	7,7	11,13	11,12	22,22	11,13	16,17	11,12	15,18	10,12	22,27
FO46	13,15	27,32.2	9,11	10,12	16,17	6,7	11,13	11,11	19,26	11,14	13,15	9,10	16,19	11,12	22,28
MO46	12,15	29,31	8,8	11,11	16,16	6,7	11,11	9,10	17,19	10,13	14,16	8,10	10.2,13	11,12	23,26
C046	12,15	31.32.2	8,11	10,11	16,16	7,7	11,11	10,11	17,26	13,14	15,16	8,10	10.2,16	12,12	23,28
FO47	12,14	29,30	8,10	8,11	15,17	7,9	11,11	11,11	22,24	13.2,14	17,18	9,11	13,17	12,13	22,22
MO47	12,13	28,31	8,11	10,12	16,17	1,8	11,13	12,12	21,23	14,15	17,19	9,11	15,18	12,12	20,26
C047	12,15	28,29	8,10	10,11	17,17	7,9	11,12	11,12	21,24	10,14	17,19	6,9	15,17	12,13	20,22
FO48	11, 17	28, 29	10, 11	11, 11	15, 15	6, 8	12, 12	9, 12	16, 20	14, 14.2	15, 19	6, 11	15, 17	11, 13	22, 25
MO48	13, 16	28, 29	10, 12	10.211	16, 17	7, 8	12, 12	11, 13	19, 19	13.2, 14	16, 16	9, 9	15, 17	11, 13	22, 24
C048	13, 17	28, 29	10, 11	11, 11	15, 17	6, 8	12, 12	12, 13	19, 20	13.2, 14.2	16, 19	6, 9	15, 17	13, 13	22, 24
FO49	13, 15	32.2,32.2	8, 8	10, 12	16, 17	7, 9	11, 12	11, 12	18, 22	10, 13.2	14, 16	9, 11	18,2O	11, 13	22, 26
MO49	13,14	30,31	8,10	10,11	16,17	8,9	12,13	11,12	18,21	13,14	16,18	9,11	18,19	10,11	24,25
C049	13, 15	30, 32.2	8, 11	10, 12	16, 17	8, 9	11, 12	9, 11	18, 21	13, 13.2	16, 17	9, 11	18, 18	11, 13	24, 26
FO50	14,15	30,30	10,11	11,12	14,15	7,9	11,13	11,11	17,27	10,11.2	14,20	6,11	16,17	12,13	23,26
MO50	13,14	28,28	8,10	9,11	15,16	7,8	13,13	9,12	21,22	10,12.2	15,17	9,10	12,19	8,10	21,25
C050	13,14	28,30	10,11	11,12	14,15	7,7	11,13	9,11	21,27	10,11.2	14,15	10,11	12,16	10,12	23,25
FO51	12, 13	27, 32.2	9.11	10, 11	16, 17	7, 8	11, 12	9,12	16, 22	10, 15	17, 18	9, 11	15, 15	11, 12	21,44.2
MO51	12, 15	28, 31	8, 10	12, 12	15, 16	7, 9	12, 13	11, 11	19, 20	10, 14	15, 18	8, 11	10.2,15	11, 12	22, 25
C051	12, 12	27, 28	10, 11	11,12	16, 16	7, 9	11, 13	9, 11	16, 20	14, 15	1818	8, 9	15, 15	12, 12	25,44.2
FO52	14,15	27,29	11,12	12,12	14,16	8,8	12,12	12,12	16,18	12,13	18,20	8,11	18,20	12,12	22,25
MO52	13,14	30,36	9,10	10,11	16,17	9,9	13,14	10,12	18,24	13,14	15,16	8,10	18,20	8,12	26,28
C052	13,14	27,36	10,12	10,12	16,16	8,9	12,13	10,12	16,18	13,14	16,20	10,11	20,20	8,12	22,26
FO53	11,12	25,30	9,11	10,11	17,17	7,8	13,14	9,13	19,23	13.2,14	16,16	8,9	15,17	12,12	17,25
MO53	11,13	29,31	10,12	11,13	15,16	8,9	14,14	9,11	23,24	14,15	16,17	9,10	15,18	12,13	17,26
C053	11,12	29,30	10,11	11,12	15,17	7,8	14,14	11,13	19,23	13,2,14	16,16	8,9	15,19	12,12	17,23
FO54	12, 13	29, 31	9, 10	11,12	14, 16	8, 9	11, 13	12, 13	16, 27	12.2, 14.2	16, 17	10, 11	13, 18	12, 12	25, 25
MO54	13,14	28,29	10,11	11,13	14,16	8,9	11,12	13,13	16,20	14,15	16,18	9,11	16,19	10,12	23,26
C054	13, 13	28, 31	9, 10	11,12	14, 15	8, 8	11, 11	13,13	16, 20	12.2, 14	16, 17	9, 10	16, 18	10, 12	23, 25

FO55	14,16	28,32.2	10,12	9,12	15,15	6,7	12,13	12,13	21,22	13,14	11,19	10,11	15,17	11,13	25,26
MO55	12,15	28,29	10,11	10,11	14,18	8,8	12,13	9,11	17,20	15,15.2	14,19	8,11	16,16	8,13	19,20
C055	12,14	28,32.2	10,11	10,12	14,15	7,8	12,13	11,12	20,22	13,15.2	11,19	10,11	16,17	8,11	20,26
FO56	11,14	29,29	8,8	9,12	15,16	8,8	11,14	11,12	19,22	14,15.2	16,18	9,11	18,18	8,13	23,24
MO56	11,13	27,28	8,9	12,13	15,17	7,8	11,15	10,13	19,21	15.2,16	15,16	9,10	18,20	8,11	22,23
C056	11,13	27,29	8,11	12,13	15,15	7,8	11,13	10,12	19,19	14,15.2	16,18	9,9	18,21	8,13	22,24
FO57	13,15	28,30	10,10	9,12	16,17	7,8	12,12	11,12	22,26	12,13	15,19	8,11	15,16	8,11	24,24
MO57	14,15	31,31	9,9	10,12	16,16	8,8	12,12	8,9	21,22	11,14	14,15	6,9	10.2,15	11,12	24,24
C057	15,15	30,31	9,10	12,12	16,17	8,8	12,12	8,11	21,26	13,14	14,15	6,8	15,16	8,12	24,24
FO58	12, 14	28, 30	8, 10	10, 12	16, 16	6, 7	11, 11	11, 12	24, 24	13.2,14	15, 18	8, 9	13, 14	8, 10	24, 27
MO58	14, 15	30, 31	8, 10	12, 12	15, 17	7, 9.3	12, 13	11, 11	17, 23	13, 14.2	16, 17	8, 9	16, 20	11, 12	27, 27
C058	12, 15	30, 31	10, 10	10, 12	16, 17	7, 9.3	11, 13	11, 11	23, 24	13.2,14.2	15, 17	8, 9	13, 20	8, 12	27, 27
FO59	14, 15	28, 29	8, 8	9, 10	15, 17	7, 8	12, 13	9, 11	19, 21	12, 16	16, 20	8, 9	15, 15	8, 12	19, 23
MO59	13,15	28,30	9,10	9,11	16,17	7,8	10,12	11,13	18,21	12,14.2	16,15	8,9	12,15	9,12	20,23
C059	15, 15	28, 29	8, 10	9, 10	17, 18	7, 8	12, 13	9, 13	21, 22	14.2, 16	15, 20	9, 10	12, 15	12, 13	23, 23
FO60	12,13	29,35	9,12	9,12	15,17	6,7	12,14	9,11	16,21	14,14.2	15,19	6,11	18,19	12,13	19,22
MO60	12,13	28,30	8,10	12,13	17,18	6,8	11,13	9,12	16,20	14,16	15,18	9,10	18,20	10,11	19,22
C060	12,13	28,35	8,9	12,12	17,17	6,9.3	11,12	9,11	16,22	13.2,14	15,15	9,11	18,19	10,13	19,24
FO61	13,15	28,28	8,8	11,12	15,16	8,9	12,13	11,11	17,20	13.2,14	16,16	9,9	13,16	12,12	19,20
MO61	11,15	30,35	8,11	10,12	16,17	9,9	11,13	9,10	17,17	13,14	16,17	11,11	17,18	12,12	20,23
C061	15,15	28,35	8,8	10,12	16,17	8,9	13,13	10,11	17,20	14,14	16,17	9,11	13,18	12,12	20,20
FO62	12,13	28,32.2	8,13	10,10	14,17	8,9	12,13	10,10	19,24	13,13.2	16,17	10,11	10,18	8,13	21,23
MO62	14,16	30,30	9,10	10,11	13,15	7,8	12,14	11,13	16,20	14.2,16.2	18,19	8,10	14.2,17	8,12	24,28
C062	13,16	30,32.2	8,10	10,10	14,15	8,8	12,12	10,13	16,24	13,14.2	17,19	8,11	14.2,18	12,13	21,24
FO63	12,12	29,32	10,10	11,12	15,15	7,8	11,12	9,11	19,19	11,13	16,18	9,10	15,17	11,12	21,22
MO63	12,14	30,32	10,12	10,11	15,16	8,9	11,13	9,11	17,20	13,14	16,17	8,11	9,18	12,12	20,21
C063	12,15	32,34.2	10,11	10,12	15,17	8,8	11,12	9,9	17,19	13,13	16,17	8,10	9,17	12,13	19,21
FO64	12,15	30,31	8,10	12,13	16,16	7,9	12,14	11,12	16,24	12.2,15	15,19	9,11	17,17	11,13	24,25
MO64	14,14	31.2,31.2	8,10	10,11	16,18	6,7	11,12	11,12	22,24	12.2,15.2	18,18	8,11	17,18	12,12	21,29
C064	14,15	30,31.2	8,8	11,13	16,18	6,7	12,12	11,12	22,24	15,15.2	18,19	8,11	17,17	12,13	21,24
FO65	12,14	29,32.2	9,10	8,12	16,17	7,8	11,13	11,11	19,23	11,13	16,18	8,10	11,16	12,15	22,25
MO65	13,15	28,35	8,11	10,11	17,18	7,9	12,13	12,13	21,26	12.2,14	16,17	9,9	15,19	10,12	19,23
C065	13,14	29,35	10,11	8,10	16,17	7,7	11,13	11,12	23,26	12.2,13	16,17	8,9	11,19	12,12	19,22
FO66	12,15	28,29	8,12	10,12	15,15	8,8	11,12	9,13	21,22	12,14	15,15	8,10	19,19	11,13	22,24
MO66	13,15	27,28	8,10	12,12	14,17	6,9	9,11	10,13	19,21	12.2,15.2	15,17	6,11	17,17	8,11	24,24
C066	13,15	28,29	8,12	10,12	15,17	6,8	11,12	13,13	19,21	12,12.2	15,15	8,11	17,19	8,13	22,24
FO67	15,12	28,35	9,10	9,12	14,16	7,7	11,12	11,12	21,21	13,15	16,19	6,9	15,20	12,12	23,26
MO67	12,14	31,31	10,10	10,10	14,16	8,9	11,13	9,11	19,23	11,14	16,17	11,11	17,18	12,13	22,24
C067	14,15	31,35	10,10	10,12	14,14	7,8	11,12	9,12	21,23	13,14	16,19	9,11	15,18	12,13	22,26
FO68	14,15	28,28	10,11	10,12	14,15	6,7	11,13	13,13	17,21	13,15.2	15,17	8,11	13,13	11,12	22,23
MO68	13,14	27,28	10,11	11,12	15,16	7,8	11,12	12,13	17,19	13,15	15,16	11,12	13,17	11,12	22,24
C068	14,15	28,28	9,11	10,11	15,17	6,7	12,13	13,14	17,22	13,13	15,15	8,11	13,16	12,13	21,22
FO69	14,14	30.2,31	8,10	10,12	16,17	7,9	12,12	10,10	15,18	11,13	13,17	6,11	16,19	11,13	28,30.

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MO69	13,15	31,32	10,11	11,12	16,17	7,8	11,12	10,11	16,18	13,14	12,13	9,10	13,18	11,12	24,26
C069	14,15	30.2,31	8,10	10,12	16,17	8,9	12,13	10,11	18,19	13,13.2	13,17	10,11	13,19	8,11	25,28
FO70	14,15	35,36	8,9	8,10	15,16	7,7	12,12	9,12	17,27	12,15.2	14,18	8,10	10.2,18	8,12	22,24
MO70	12,14	28,28	10,11	10,11	17,17	7,9	11,13	10,12	17,18	13.2,13.2	15,16	6,10	16.2,19	12,13	18,22
C070	12,14	28,35	8,10	8,11	15,17	7,7	11,12	9,12	18,27	12,13.2	15,18	8,10	10.2,16.2	12,13	18,22
FO71	14, 15	28, 29	7, 10	10, 12	16, 17	6, 8	10, 11	10, 14	18, 23	12, 13	15, 18	8, 11	16, 19	11, 12	22, 24
MO71	13,15	27,29	8,10	11,12	13,17	7,8	10,12	11,13	17,19	12,13	16,18	9,11	16,19	10,12	21,22
C071	14, 15	28, 29	9, 10	10, 12	16, 17	6, 8	11, 12	10, 11	18, 19	11.2, 13	15, 16	11, 11	12, 19	11, 12	22, 22
FO72	13, 14	32.2, 35	11, 12	11, 12	16, 16	6, 9	12, 13	13, 13	24, 25	12, 15	14, 17	8, 10	15, 17	9, 13	21, 27
MO72	12, 13	29, 30	9, 10	12, 12	14, 18	6, 9	12, 13	9, 12	16, 20	11, 13.2	14, 14	10, 10	15, 17	9, 12	17, 21
C072	13, 13	29, 35	10, 11	11, 12	14, 16	6, 9	12, 13	9, 13	16, 25	11, 15	14, 17	8, 10	15, 17	9, 13	21, 27
FO73	14,15	28,29	8,10	10,11	16,17	6,7	11,13	11,13	16,21	12,13	16,17	8,11	16,18	12,12	22,23
MO73	12,16	28,28	8,8	12,12	16,16	6,8	11,12	10,13	20,22	10,14	17,17	8,11	16,17	12,12	22,23
C073	14,16	28,29	8,8	10,12	16,17	6,8	11,11	10,13	16,20	13,14	16,17	8,11	17,18	12,12	23,23
FO74	13,14	30,31.2	10,10	7,10	13,16	7,9	11,13	9,11	19,21	13,15.2	15,17	6,8	15,17	11,12	23,25
MO74	14,15	28,35	8,11	8,12	16,18	7,8	10,13	11,13	17,18	13,14	16,17	6,8	16,18	8,12	24,24
C074	14,15	28,30	8,10	10,12	13,16	7,7	13,13	11,11	17,19	14,15.2	17,17	6,8	16,17	11,12	24,25
FO75	11,15	31,35	9,12	12,12	16,18	7,8	11,11	9,10	16,21	14,15	15,16	8,11	15,15	12,13	21,23
MO75	13,14	28,30	8,10	10,10	15,16	7,8	11,11	9,10	19,21	10,13	14,16	8,9	13,15	10,11	20,23
C075	14,15	28,35	10,12	10,12	16,16	8,8	11,12	9,9	21,22	13,15	16,18	8,11	15,15	11,12	23,24
FO76	13,14	28,29	8,10	9,12	14,16	6,6	12,13	9,11	19,20	11,12	15,19	8,11	16,17	12,12	23,24
MO76	14,15	28,30	10,11	9,10	15,16	7,8	13,14	10,11	19,20	11,12	16,18	8,9	15,16	10,11	21,24
C076	14,14	28,30.2	8,10	10,12	16,17	6,8	13,13	11,12	19,21	11,13	16,19	6,8	15,17	11,12	22,24
FO77	13,15	28,31.2	8,10	10,12	16,17	8,9	11,14	11,11	17,25	13.2,15	16,18	9,11	14,25	11,13	19,27
MO77	11,13	27,28	8,9	11,11	15,16	8,10	11,12	10,11	17,20	13.2,14	16,18	8,11	14,19	11,12	22,26
C077	11,15	28,28	8,8	11,12	15,17	8,9	12,14	11,11	17,22	13.2,13.2	13,18	9,11	14,19	11,11	26,27
FO78	14, 15	28, 29	7, 10	10, 12	16, 17	6, 8	10, 11	10, 14	18, 23	12, 13	15, 18	8, 11	16, 19	11, 12	22, 24
MO78	14,16	28,30	10,11	10,11	15,16	7,8	10,12	11,12	17,18	13,14	14,15	10,11	13,17	10,11	21,22
C078	14, 15	28, 29	9, 10	10, 12	16, 17	6, 8	11, 12	10, 11	18, 19	11.2, 13	15, 16	11, 11	12, 19	11, 12	22, 22
FO79	11,14	30,32.2	9,12	8,10	15,18	8,9	11,11	12,12	21,23	13,13	13,17	8,8	12,16	8,13	23,24
MO79	11,12	27,29	8,9	8,10	15,17	7,8	11,12	11,12	16,20	13,14	13,15	8,9	10,12	8,10	23,26
C079	12,14	29,30	9,10	8,10	15,15	7,8	11,12	12,12	16,23	13,14	13,16	8,8	12,12	8,12	23,30
FO80	14,14	28,30	8,9	9,11	15,17	6,9	12,12	9,11	20,26	13,14	15,17	9,9	14,17	10,11	22,22
MO80	11,13	31.2,32.2	10,10	9,12	14,16	7,7	12,14	8,11	19,20	14,14.2	14,15	9,9	13.2,19	11,12	22,26
C080	13,14	30,32.2	8,10	9,11	16,17	7,9	12,12	8,9	19,20	13,14	14,17	9,9	14,19	11,12	22,22
FO81	14, 14	28, 29	10, 10	9, 12	15, 16	6, 8	12, 12	10, 13	19, 20	13.2, 15	14, 15	9, 9	16, 16	8, 12	22, 22
MO81	12, 16	29, 29	7, 11	10, 12	15, 15	8, 8	11, 13	11, 13	16, 22	11, 13	15, 18	6, 8	15, 17	12, 13	21, 24
C081	12, 14	28, 29	7, 10	12, 12	15, 15	8, 8	11, 12	10, 13	16, 20	13, 13.2	14, 15	8, 9	15,16	12, 13	21, 22
FO82	13,16	28,31	10,12	8,11	16,17	6,7	10,13	11,12	19,21	12,13	16,19	9,10	18,19	12,12	22,24
MO82	12,14	30,32.2	8,10	7,9	14,17	8,8	11,14	11,11	16,20	12,14	14,15	8,11	13,19	10,11	22,23
C082	14,16	28,30	10,10	8,9	14,16	7,8	11,13	11,12	19,20	12,13	15,16	10,11	13,18	10,12	22,24

FO83	10,13	28,28	10,11	10,11	16,17	7,7	11,13	12,12	19,23	12,14	16,19	8,9	12,15	10,12	20,21
MO83	11,14	27,29	8,13	11,12	15,15	6,6	12,12	11,13	17,22	14,14	20,21	8,10	16,17	11,11	24,25
C083	10,14	28,29	8,10	11,12	15,17	6,7	11,12	12,13	17,19	12,14	19,21	9,10	12,17	11,12	21,24
FO84	13,16	30,30	10,12	10,12	16,17	8,9	12,13	9,9	16,17	14,15	15,18	11,11	16,21	13,13	22,24
MO84	14,14	30,32.2	8,8	9,11	15,15	9,9.3	12,13	11,12	17,23	11,13.2	15,17	6,11	18,19	11,13	24,26
C084	14,16	30,30	8,12	9,10	15,17	8,9.3	12,13	9,11	17,17	13.2,14	17,18	11,11	16,19	11,13	22,24
FO85	12,15	26,30	8,10	11,12	15,16	7,9	8,12	10,12	21,24	12,14.2	18,18	9,11	18,20	11,12	23,26
MO85	14,15	28,28	8,10	8,12	15,16	7,8	12,12	11,12	23,23	13,13	16,18	8,11	17,19	12,12	21,23
C085	14,15	28,30	8,10	12,12	15,15	8,9	8,12	10,12	23,24	12,13	16,18	11,11	19,20	12,12	23,26
FO86	12,13	28,29	8,10	10,12	15,16	6,8	10,12	11,11	16,22	13,13.2	16,18	6,11	16,17	10,12	22,24
MO86	13,14	27,28	9,10	10,12	14,17	7,9	11,13	11,12	22,25	14,14	17,19	8,11	16,17	11,12	23,25
C086	13,13	27,28	8,9	10,10	15,17	6,7	12,13	11,11	16,25	13,2,14	18,19	6,8	17,17	10,11	22,23
FO87	15,16	30,33.2	8,10	10,10	16,16	7,8	12,12	11,12	22,27	13.2,14.2	18,19	8,10	15,17	13,13	19,21
MO87	11,15	29,30	9,11	10,12	11,17	7,9	8,11	9,11	18,22	14,15	18,18	11,11	14,19	9,12	21,22
C087	15,16	30,33.2	8,11	10,12	16,17	8,7	8,12	9,12	18,27	13.2,14	18,19	8,11	15,19	9,13	19,22
FO88	12, 15	29, 32.2	8, 10	7, 12	17, 17	7, 7	11, 12	9, 12	19, 23	13, 15.2	17, 18	11, 13	15.2, 18	10, 12	23, 23
MO88	14,17	28,29	9,11	11,12	14,15	8,8	12,14	9,11	20,21	12.2,14	15,16	10,11	16,18	10,12	21,23
C088	14, 15	29, 31	9, 10	7, 11	14, 17	7, 8	12, 13	9, 14	21, 23	12.2, 15.2	16, 17	11, 11	15.2, 18	10, 11	23, 24
FO89	14,15	28,28	8,11	11,13	15,16	7,8	12,12	11,11	21,24	12,13	13,16	10,11	15,17	11,13	23,25
MO89	15,16	28,29	9,11	10,12	15,16	7,8	10,12	9,12	19,21	11,12	13,15	10,11	12,16	11,13	21,25
C089	15,15	28,30	10,11	10,13	16,16	8,8	12,12	10,11	20,21	12,12	13,16	9,11	12,17	12,13	23,25
FO90	15,15	30,30	8,10	10,11	15,16	8,9	12,13	10,11	22,23	13,14	15,16	8,11	16,16	8,12	21,27
MO90	14,15	30,33.2	9,10	11,12	14,15	6,6	10,12	11,12	21,22	11,14	16,19	6,9	17,21	8,12	22,22
C090	15,15	30,33.2	8,9	10,11	15,16	6,8	10,13	11,11	21,22	11,14	15,16	9,11	16,17	8,12	21,22
FO91	16, 17	28, 30	10, 10	10, 12	16, 17	7, 8	11, 13	11, 12	16, 20	13, 14	16, 17	8, 9	16, 19	12, 12	24, 25
MO91	14,15	29,30	9,10	8,10	14,16	7,7	11,12	9,11	15,16	13,13	15,16	8,11	15,16	11,12	20,24
C091	14, 16	30, 31.2	10, 10	10, 12	16, 17	7, 7	11, 12	9, 11	16, 19	13, 14	15, 17	8, 11	16, 19	11, 12	24, 25
FO92	11, 12	28, 30	10, 11	8, 10	15, 17	7, 8	11, 14	8, 11	22, 24	11, 15.2	16, 18	8, 11	13, 16	11, 13	19, 24
MO92	12, 14	30, 31.2	10, 10	11, 11	14, 15	7, 9	11, 14	9, 10	19, 22	13,13	15, 18	10, 11	16, 18	12, 12	24, 24
C092	11, 14	28, 30	10, 11	8, 11	14, 15	7, 8	11, 14	8, 10	22, 24	11, 13	18, 18	8, 11	16, 18	12, 13	19, 24
FO93	14,16	27,30	8,8	10,12	15,16	7,8	11,11	9,11	17,23	14,16.2	14,15	11,11	15,17	8,12	20,26
MO93	11,16	27,31.2	9,10	9,11	16,16	7,8	11,12	9,12	16,23	13,14	16,17	11,11	13,16	8,10	22,26
C093	11,14	27,30	8,10	10,11	15,16	7,7	11,11	9,12	23,23	13,14	15,16	11,11	15,16	8,10	20,22
FO94	14, 14	29,35	8,8	11,12	14,16	9,9	10,11	10,11	17,22	13.2,14	16, 17	10,11	18,19	12, 13	22, 25
MO94	12,14	28,35	8,10	10,11	13,14	8,9	10,11	10,11	19,22	13,14	16,17	8,11	15,18	10,12	19,24
C094	14,14	28, 35	8,10	10, 11	14, 17	8, 9	11, 11	10,11	22,24	14,16.2	16, 18	8, 11	18,20	12,13	19,22
FO95	14, 16	28, 31	9, 11	10, 10	15, 16	7, 8	11, 14	10, 12	18, 20	9, 14	13, 15	8, 8	17, 18	11, 13	21, 24
MO95	14, 14	31, 35	10, 11	12, 12	15, 15	6, 7	11, 14	11, 12	23, 24	13.2, 14	17, 18	8, 8	11, 20	13, 13	21, 26
C095	14, 14	28, 31	10, 11	10, 12	15, 15	7, 8	11, 14	10, 12	18, 23	14, 14	15, 18	8, 8	18, 20	11, 13	21, 21
FO96	13,14	27,28	8,11	11,12	15,17	7,8	11,13	9,10	18,20	14,15	15,17	7,8	18,19	11,12	21,22

MO96	10, 15	28, 29	8, 10	12, 13	15, 18	7, 7	11, 12	9, 13	19, 23	13, 14	15, 18	8, 8	13, 14	10, 10	24, 24
C096	15, 15	28, 29	8, 10	11, 13	15, 18	7, 9	12, 14	13, 13	19, 22	13, 14	15, 17	8, 11	14, 16	10, 10	24, 30.2
FO97	11, 17	28, 29	10, 11	11, 11	15, 15	6, 8	12, 12	9, 12	16, 20	14, 14.2	15, 19	6, 11	15, 17	11, 13	22, 25
MO97	13, 16	28, 29	10, 12	10.2 11	16, 17	7, 8	12, 12	11, 13	19, 19	13.2, 14	16, 16	9, 9	15, 17	11, 13	22, 24
C097	13, 17	28, 29	10, 11	11, 11	15, 17	6, 8	12, 12	12, 13	19, 20	13.2, 14.2	16, 19	6, 9	15, 17	13, 13	22, 24
FO98	14,15	29,29	8,8	11,12	14,14	9,9	12,13	10,11	17,20	12,14	18,20	9,12	18,19	12,13	24,24
MO98	13,14	28,31	10,12	11,12	14,15	7,7	12,13	9,11	18,23	14,14	15,16	8,9	15,16	8,12	22,25
C098	14,14	28,29	8,12	12,12	14,15	7,9	12,13	9,11	18,20	12,14	15,18	9,12	16,19	8,12	22,24
FO99	12,14	31.2,32.2	8,9	10,12	14,15	8,9	11,12	10,11	17,17	12,13	16,18	7,11	16,17	11,13	19,26
MO99	14,14	28,28	9,11	10,11	14,17	8,9.3	10,12	11,11	18,25	13,14	16,18	8,11	13,16	13,14	24,26
C099	12,14	28,32.2	9,11	11,12	14,15	9,9.3	11,12	11,11	17,18	13,14	16,18	8,11	16,17	11,14	24,26
F100	13,15	29,33.2	10,11	12,12	16,18	7,8	12,14	10,13	19,21	11,16.2	15,19	9,12	15,16	10,15	20,22
M100	15,16	28,29	8,8	12,12	17,18	7,7	11,12	12,12	17,17	13,13	15,16	8,11	17,18	8,11	21,29
C100	15,15	29,33.2	8,11	12,12	17,18	7,7	12,14	10,12	17,21	11,13	15,16	9,11	15,17	10,11	20,21

Appendix 4: The 17 Y-STRs loci typing results. The results show four father-son pair's mutation events on father-son paired sample numbers: F003/C003, F012/C012, F074/C074 and F082/C082.

Sample ID	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385a/b	DYS393	DYS391	DYS439	DYS635	DYS392	Y_GATA H4	DYS437	DYS438	DYS448
F001	16	13	21	30	16	13	17,18	15	10	12	21	11	11	14	11	21
C001	16	13	21	30	16	13	17,18	15	10	12	21	11	11	14	11	21
F002	18	13	21	31	16	13	17,21	13	11	10	22	14	12	14	11	21
C002	18	13	21	31	16	13	17,21	13	11	10	22	14	12	14	11	21
F003	16	13	21	30	15	13	9,**16**	13	10	9	21	12	11	14	11	21
C003	16	15	21	31	15	15	9,**17**	13	10	10	21	11	11	14	11	21
F004	16	13	24	31	16	13	15,16	13	11	10	21	11	11	16	10	20
C004	16	13	24	31	16	13	15,16	13	11	10	21	11	11	16	10	20
F005	15	14	25	31	17	13	16,17	14	11	9	21	15	10	14	12	20
C005	15	14	25	31	17	13	16,17	14	11	9	21	15	10	14	12	20
F006	16	11	23	31	17	15	18,19	12	11	13	24	12	11	17	11	22
C006	16	11	23	31	17	15	18,19	12	11	13	24	12	11	17	11	22
F007	16	12	24	30	18	14	16,17	12	10	12	23	13	11	17	11	20
C007	16	12	24	30	18	14	16,17	12	10	12	23	13	11	17	11	20
F008	15	14	21	30	17	13	17,18	15	10	12	21	11	11	14	11	21
C008	15	14	21	30	17	13	17,18	15	10	12	21	11	11	14	11	21
F009	16	13	24	31	16	13	15,16	13	11	9	21	14	12	16	10	20
C009	16	13	24	31	16	13	15,16	13	11	9	21	14	12	16	10	20
F010	15	13	24	31	15	13	15,16	14	11	11	21	15	11	14	10	19
C010	15	13	24	31	15	13	15,16	14	11	11	21	15	11	14	10	19
F011	15	13	21	29	16	13	9,16	12	10	9	21	11	12	14	8	18
C011	15	13	21	29	16	13	9,16	12	10	9	21	11	12	14	8	18
F012	15	12	21	30	15	15	**15,**17	14	10	12	22	11	11	14	11	21
C012	15	13	21	30	19	13	**16,**17	14	10	10	22	14	11	14	11	21
F013	13	14	24	31	17	13	11	13	10	12	22	11	13	14	10	22
C013	13	14	24	31	17	13	11	13	10	12	22	11	13	14	10	22
F014	15	14	21	30	17	16	16,17	15	10	10	22	11	11	14	11	21
C014	15	14	21	30	17	16	16,17	15	10	10	22	11	11	14	11	21
F015	16	13	21	30	16	13	17,21	13	11	10	22	15	12	14	11	21
C015	16	13	21	30	16	13	17,21	13	11	10	22	15	12	14	11	21
F016	16	13	21	31	15	13	9,17	13	10	12	21	17	11	14	11	21
C016	16	13	21	31	15	13	9,17	13	10	12	21	17	11	14	11	21
F017	15	13	21	31	17	13	16,17	14	10	13	21	15	11	14	12	21
C017	15	13	21	31	17	13	16,17	14	10	13	21	15	11	14	12	21
F018	15	13	24	32	16	14	16,18	14	10	11	21	15	10	15	9	19

C018	15	13	24	32	16	14	16,18	14	10	11	21	15	10	15	9	19
F019	15	14	21	30	17	13	17,18	15	10	12	21	11	11	14	11	21
C019	15	14	21	30	17	13	17,18	15	10	12	21	11	11	14	11	21
F020	15	13	21	32	18	13	17,18	13	10	9	21	15	12	14	12	21
C020	15	13	21	32	18	13	17,18	13	10	9	21	15	12	14	12	21
F021	15	13	21	27	16	13	14,17	15	10	9	23	10	12	14	11	20
C021	15	13	21	27	16	13	14,17	15	10	9	23	10	12	14	11	20
F022	16	13	24	30	16	13	18,21	13	11	12	24	15	11	14	11	20
C022	16	13	24	30	16	13	18,21	13	11	12	24	15	11	14	11	20
F023	13	14	24	31	17	13	16,17	13	10	12	20	10	11	14	10	22
C023	13	14	24	31	17	13	16,17	13	10	12	20	10	11	14	10	22
F024	16	13	24	30	16	13	18,21	13	11	12	24	15	11	14	11	20
C024	16	13	24	30	16	13	18,21	13	11	12	24	15	11	14	11	20
F025	15	13	21	31	16	13	16,17	13	10	12	21	15	12	14	12	20
C025	15	13	21	31	16	13	16,17	13	10	12	21	15	12	14	12	20
F026	14	11	25	33	15	14	8,21	13	10	11	24	10	11	15	11	20
C026	14	11	25	33	15	14	8,21	13	10	11	24	10	11	15	11	20
F027	14	13	21	31	16	14	17,19	15	13	12	21	11	12	14	11	19
C027	14	13	21	31	16	14	17,19	15	13	12	21	11	12	14	11	19
F028	13	13	21	31	16	16	15,16	14	13	12	23	11	12	14	11	20
C028	13	13	21	31	16	16	15,16	14	13	12	23	11	12	14	11	20
F029	15	13	21	28	18	15	11,13	14	12	12	21	12	11	15	10	21
C029	15	13	21	28	18	15	11,13	14	12	12	21	12	11	15	10	21
F030	15	13	21	31	19	14	17,19	13	10	11	21	15	12	14	13	22
C030	15	13	21	31	19	14	17,19	13	10	11	21	15	12	14	13	22
F031	17	14	23	27	17	13	15,16	14	11	11	21	10	11	14	10	20
C031	17	14	23	27	17	13	15,16	14	11	11	21	10	11	14	10	20
F032	15	14	21	31	16	13	15,17	13	11	12	21	16	12	14	11	21
C032	15	14	21	31	16	13	15,17	13	11	12	21	16	12	14	11	21
F033	15	14	26	30	15	15	14,20	13	11	10	21	12	11	14	10	21
C033	15	14	26	30	15	15	14,20	13	11	10	21	12	11	14	10	21
F034	16	12	20	30	16	17	18	13	11	11	22	10	11	14	11	21
C034	16	12	20	30	16	17	18	13	11	11	22	10	11	14	11	21
F035	17	14	21	31	17	12	17	14	11	11	21	14	12	14	12	21
C035	17	14	21	31	17	12	17	14	11	11	21	14	12	14	12	21
F036	17	12	20	30	15	13	13,17	12	10	11	21	11	12	14	11	21
C036	17	12	20	30	15	13	13,17	12	10	11	21	11	12	14	11	21
F037	15	14	20	32	15	15	17,19	11	11	10	21	10	11	14	11	21
C037	15	14	20	32	15	15	17,19	11	11	10	21	10	11	14	11	21
F038	15	14	20	31	16	12	15,16	11	10	11	21	11	11	14	9	21
C038	15	14	20	31	16	12	15,16	11	10	11	21	11	11	14	9	21
F039	15	12	20	31	17	12	15	11	10	11	21	11	12	14	12	20
C039	15	12	20	31	17	12	15	11	10	11	21	11	12	14	12	20
F040	16	12	25	28	19	13	14,21	13	11	10	24	14	11	14	11	19

C040	16	12	25	28	19	13	14,21	13	11	10	24	14	11	14	11	19
F041	15	14	25	30	15	15	15,18	13	13	10	22	12	11	14	10	21
C041	15	14	25	30	15	15	15,18	13	13	10	22	12	11	14	10	21
F042	17	13	21	31	16	13	15,16	14	13	12	20	11	12	14	11	19
C042	17	13	21	31	16	13	15,16	14	13	12	20	11	12	14	11	19
F043	13	9	21	28	15	15	11,13	14	13	11	22	11	10	14	10	20
C043	13	9	21	28	15	15	11,13	14	13	11	22	11	10	14	10	20
F044	14	14	24	29	17	13	17,21	13	10	11	22	12	9	14	11	21
C044	14	14	24	29	17	13	17,21	13	10	11	22	12	9	14	11	21
F045	15	12	23	31	16	13	9,19	12	10	10	21	11	11	14	10	19
C045	15	12	23	31	16	13	9,19	12	10	10	21	11	11	14	10	19
F046	16	12	24	29	17	15	16,17	13	14	13	21	11	11	16	10	21
C046	16	12	24	29	17	15	16,17	13	14	13	21	11	11	16	10	21
F047	16	12	24	28	19	15	16,17	13	11	11	21	11	11	15	10	21
C047	16	12	24	28	19	15	16,17	13	11	11	21	11	11	15	10	21
F048	17	13	21	30	16	15	15,17	14	13	12	22	11	10	14	11	20
C048	17	13	21	30	16	15	15,17	14	13	12	22	11	10	14	11	20
F049	15	12	21	31	17	13	16,17	15	10	9	22	14	12	14	11	21
C049	15	12	21	31	17	13	16,17	15	10	9	22	14	12	14	11	21
F050	15	13	21	31	17	15	16,17	13	10	11	20	12	12	14	11	21
C050	15	13	21	31	17	15	16,17	13	10	11	20	12	12	14	11	21
F051	15	12	25	28	18	14	14,22	13	10	11	22	11	11	14	11	19
C051	15	12	25	28	18	14	14,22	13	10	11	22	11	11	14	11	19
F052	15	12	22	30	19	16	19,21	11	11	9	20	10	10	16	10	21
C052	15	12	22	30	19	16	19,21	11	11	9	20	10	10	16	10	21
F053	17	11	24	31	16	15	14,16	13	10	9	19	15	10	16	12	21
C053	17	11	24	31	16	15	14,16	13	10	9	19	15	10	16	12	21
F054	15	13	22	31	16	12	18,21	13	10	9	19	14	12	14	12	17
C054	15	13	22	31	16	12	18,21	13	10	9	19	14	12	14	12	17
F055	15	13	22	31	16	13	19,20	13	10	10	19	10	12	14	12	18
C055	15	13	22	31	16	13	19,20	13	10	10	19	10	12	14	12	18
F056	15	14	21	30	16	15	19,21	14	11	12	21	10	11	14	10	21
C056	15	14	21	30	16	15	19,21	14	11	12	21	10	11	14	10	21
F057	15	13	21	30	17	15	19	14	10	12	22	11	11	14	11	21
C057	15	13	21	30	17	15	19	14	10	12	22	11	11	14	11	21
F058	15	13	21	31	16	13	17	15	10	12	20	11	11	14	11	21
C058	15	13	21	31	16	13	17	15	10	12	20	11	11	14	11	21
F059	18	15	21	31	17	13	19,20	13	10	10	21	10	13	14	11	18
C059	18	15	21	31	17	13	19,20	13	10	10	21	10	13	14	11	18
F060	15	13	21	30	17	15	10,19	14	10	12	22	11	11	14	11	21
C060	15	13	21	30	17	15	10,19	14	10	12	22	11	11	14	11	21
F061	15	13	21	31	17	13	16,19	13	11	12	21	11	12	14	12	21
C061	15	13	21	31	17	13	16,19	13	11	12	21	11	12	14	12	21
F062	15	14	21	32	17	13	17	13	10	12	21	11	12	14	12	21

C062	15	14	21	32	17	13	17	13	10	12	21	11	12	14	12	21
F063	16	12	25	31	19	13	14,19	13	11	11	24	14	11	14	11	19
C063	16	12	25	31	19	13	14,19	13	11	11	24	14	11	14	11	19
F064	14	14	24	30	17	13	14,15	13	10	10	22	15	9	14	11	20
C064	14	14	24	30	17	13	14,15	13	10	10	22	15	9	14	11	20
F065	15	13	22	31	17	15	16,17	13	10	11	22	11	12	14	12	21
C065	15	13	22	31	17	15	16,17	13	10	11	22	11	12	14	12	21
F066	14	13	21	30	16	16	15,17	14	13	12	24	11	12	14	11	20
C066	14	13	21	30	16	16	15,17	14	13	12	24	11	12	14	11	20
F067	16	13	21	31	15	16	17,19	15	12	12	21	11	11	14	12	21
C067	16	13	21	31	15	16	17,19	15	12	12	21	11	11	14	12	21
F068	16	13	22	31	16	13	13	13	10	12	19	12	12	14	10	21
C068	16	13	22	31	16	13	13	13	10	12	19	12	12	14	10	21
F069	15	13	21	32	17	13	15,16	13	10	12	21	11	12	14	11	21
C069	15	13	21	32	17	13	15,16	13	10	12	21	11	12	14	11	21
F070	15	13	24	32	16	18	18,22	13	10	12	21	14	10	14	10	20
C070	15	13	24	32	16	18	18,22	13	10	12	21	14	10	14	10	20
F071	16	13	21	31	17	17	17,19	15	12	12	21	11	11	17	12	21
C071	16	13	21	31	17	17	17,19	15	12	12	21	11	11	17	12	21
F072	14	13	20	28	16	12	16,18	14	10	9	21	14	12	14	11	19
C072	14	13	20	28	16	12	16,18	14	10	9	21	14	12	14	11	19
F073	17	13	20	30	16	13	16,18	14	10	11	21	10	11	14	11	21
C073	17	13	20	30	16	13	16,18	14	10	11	21	10	11	14	11	21
F074	15	13	21	31	17	12	**16**,19	14	10	12	21	11	11	14	11	21
C074	15	13	21	30	17	12	**15**,19	14	10	12	21	11	11	14	11	21
F075	15	15	21	31	17	13	13,17	13	10	9	21	10	12	14	11	20
C075	15	15	21	31	17	13	13,17	13	10	9	21	10	12	14	11	20
F076	17	15	20	32	16	17	17	14	10	11	22	9	11	14	11	21
C076	17	15	20	32	16	17	17	14	10	11	22	9	11	14	11	21
F077	16	13	21	29	16	12	9,16	14	10	11	21	11	11	14	11	21
C077	16	13	21	29	16	12	9,16	14	10	11	21	11	11	14	11	21
F078	17	14	24	31	16	12	15,16	14	11	11	22	11	11	14	11	21
C078	17	14	24	31	16	12	15,16	14	11	11	22	11	11	14	11	21
F079	14	13	23	30	19	14	14,18	12	10	11	22	11	11	14	10	20
C079	14	13	23	30	19	14	14,18	12	10	11	22	11	11	14	10	20
F080	14	13	22	30	17	16	16,17	14	10	12	23	11	11	14	11	21
C080	14	13	22	30	17	16	16,17	14	10	12	23	11	11	14	11	21
F081	16	13	21	30	16	15	15,18	15	9	12	21	11	12	14	13	21
C081	16	13	21	30	16	15	15,18	15	9	12	21	11	12	14	13	21
F082	15	12	25	**31**	17	15	14,18	13	11	13	23	11	11	14	11	19
C082	15	12	25	**30**	17	12	14,18	13	11	13	23	11	11	14	11	19
F083	17	14	24	31	19	12	14,17	13	10	11	20	11	11	15	9	19
C083	17	14	24	31	19	12	14,17	13	10	11	20	11	11	15	9	19
F084	16	12	24	29	17	15	15,17	13	12	13	21	11	11	16	10	21

C084	16	12	24	29	17	15	15,17	13	12	13	21	11	11	16	10	21
F085	13	14	24	32	18	12	11,12	13	10	12	23	11	12	16	10	22
C085	13	14	24	32	18	12	11,12	13	10	12	23	11	12	16	10	22
F086	15	13	22	31	16	13	19,21	13	10	10	19	10	12	14	10	18
C086	15	13	22	31	16	13	19,21	13	10	10	19	10	12	14	10	18
F087	15	12	20	31	15	15	16,17	11	10	10	22	10	12	14	11	21
C087	15	12	20	31	15	15	16,17	11	10	10	22	10	12	14	11	21
F088	16	12	20	30	15	12	13,20	11	10	9	21	15	12	14	11	21
C088	16	12	20	30	15	12	13,20	11	10	9	21	15	12	14	11	21
F089	16	13	22	28	16	13	13	13	10	12	19	12	12	14	10	21
C089	16	13	22	28	16	13	13	13	10	12	19	12	12	14	10	21
F090	15	11	20	30	15	15	16,18	11	10	10	21	11	12	14	11	21
C090	15	11	20	30	15	15	16,18	11	10	10	21	11	12	14	11	21
F091	16	13	21	30	16	13	17	14	10	12	21	11	10	14	11	21
C091	16	13	21	30	16	13	17	14	10	12	21	11	10	14	11	21
F092	15	8	21	28	15	15	16,17	13	14	11	21	11	11	14	10	21
C092	15	8	21	28	15	15	16,17	13	14	11	21	11	11	14	10	21
F093	16	12	20	31	15	15	16	11	11	11	21	10	12	14	11	21
C093	16	12	20	31	15	15	16	11	11	11	21	10	12	14	11	21
F094	15	14	24	30	15	15	17,19	13	13	13	22	11	11	14	10	21
C094	15	14	24	30	15	15	17,19	13	13	13	22	11	11	14	10	21
F095	15	11	20	31	16	12	16,17	11	10	11	21	11	11	14	11	21
C095	15	11	20	31	16	12	16,17	11	10	11	21	11	11	14	11	21
F096	15	14	24	32	16	13	8,14	14	11	10	20	10	12	14	9	22
C096	15	14	24	32	16	13	8,14	14	11	10	20	10	12	14	9	22
F097	16	13	21	31	16	13	17,18	15	10	12	21	11	11	14	11	21
C097	16	13	21	31	16	13	17,18	15	10	12	21	11	11	14	11	21
F098	15	15	21	28	17	13	14,21	13	10	10	21	10	12	14	11	20
C098	15	15	21	28	17	13	14,21	13	10	10	21	10	12	14	11	20
F099	15	12	21	32	17	13	17,18	15	10	13	22	11	12	14	11	21
C099	15	12	21	32	17	13	17,18	15	10	13	22	11	12	14	11	21
F100	16	13	21	30	17	13	17	15	10	9	22	14	10	14	11	18
C100	16	13	21	30	17	13	17	15	10	9	22	14	10	14	11	18

Appendix 5: Age of participants with and without mutation on17 Y-STRs loci

Fathers' age (Years) with at least one mutation	Fathers' age (Years) without mutation
25,40,32,31	19,20,26,25,30,21,25,26,24,30,32,30,21,28,30,30,24,25,34,20 35,18,19,18,19,20,32,36,32,33,34,32,21,20,25,34,29,28,35,25 24,26,25,24,26,25,34,23,25,24,28,29,25,26,23,24,27,28,36,26 37,25,34,20,34,18,19,18,19,39,32,33,32,33,36,32,42,34,20,18 18,19,26,19,20,32,33,32,33,36,25,20,24,18,29,19
Average = 32.000	**Average = 26.781**
Standard error = 2.387	**Standard error = 0.609**
P Value = 0.060	