May 2020

Edition no. 67
(replaces Pathchat 25 of March 2015)

AMPATHCHAT

Dr Irma Ferreira (PhD in Biochemistry)

Paternity testing

INTRODUCTION

The term paternity testing, parental testing and human identity testing are interchangeable. Paternity testing refers to the use of genetic fingerprinting to determine a biological parent-child relationship. A paternity test establishes proof as to whether a man is the biological father of an individual, and a maternity test establishes whether a woman is the biological mother of an individual.

While older methods include ABO blood group typing, the analysis of various proteins and enzymes, or using human leukocyte antigen antigens, DNA testing is currently the most accurate and widely used technology to determine parentage. In a DNA paternity test, the probability of paternity is 0% when the alleged father is biologically unrelated to the child, and greater than 99.8% when the alleged father is biologically related to the child. The current techniques for paternity testing are polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Indications for testing include the following:

- Men denying paternity when faced with maintenance claims
- Women with multiple sex partners who are uncertain about the paternity of a child
- Investigating the likelihood that babies were accidentally switched at birth
- Cases of legacies
- Cases of fraud, e.g. Road Accident Fund and immigration
- The identification of human remains after disasters, e.g. Twin Towers, earthquakes, etc.
- Criminal investigations

The process of DNA paternity testing in a nutshell

The first step in a DNA paternity test is the collection of the biological source material (buccal swab, saliva, blood) followed by DNA extraction and analysis using the PCR technique for the presence of a set of specific DNA regions. The resulting PCR products are separated with capillary electrophoresis and detected. The resulting DNA profile of the child is then compared to the DNA profile of the mother and the alleged father.

Failure to match the DNA profiles of a child and alleged father is known as exclusion. If a match results, a comparison of the DNA profile of the alleged father is made to a population database, which is a collection of DNA profiles obtained from unrelated individuals of a particular ethnic or population group.

HOW DOES THIS REALLY WORK?

Basic DNA principles

Chromosomes in the nucleus consist of DNA, which are found in all cells of the body. Paternity testing can therefore use a variety of specimen types for collection, including cells from the cheeks using buccal swabs, blood or any other types of specimens.

Humans have 22 matched pairs of autosomal chromosomes and two sex-determining chromosomes. Paternity testing is performed with markers on the autosomal chromosomes and gender is determined with markers on the sex chromosomes. One chromosome in each chromosomal pair is derived from each parent at the time of conception. Chromosomal DNA has coding and non-coding regions. The coding regions are genes, which have protein-coding regions and intervening regions. These intervening regions contain repeated DNA sequences. The number of repeats varies among individuals. Variability in these regions can be used to distinguish one DNA profile from another. The markers used in paternity testing utilise these repeated sequences, also known as short tandem repeats (STRs).

DNA markers

The areas of repeated DNA sequences are scattered over the chromosomes. A marker by itself is not unique to an individual and therefore the more markers used in DNA analysis, the greater the odds for a unique pattern. The chance that two people will have the same number of repeats at all areas is exceedingly small. A number of commercial kits containing these DNA markers are available. Ampath utilises the kit supplied by The Promega Corporation. This kit contains 27 markers.

Markers are named according to their location. If a marker is part of a gene, the gene name is used in the designation. Markers outside gene regions are designated by their chromosomal position, e.g. in D5S818 – D is DNA, 5 is the chromosome 5, S is the single copy sequence and the number indicates the order in which it was discovered.

Detection

Once the DNA in question has been amplified using the PCR technique, STR analysis examines how many DNA repeats there are in specific loci, or locations, on a DNA strand. The markers for each person (STR alleles) are visualised as peaks on an electropherogram generated by the ABI Prism 3500 Genetic Analyser (Figure 1). These alleles are numerically labelled.

Each person's DNA contains two copies of these alleles (markers), one copy inherited from the father and one from the mother. Depending on which alleles were inherited from the parents, the alleles at each person's DNA location could differ in length. The combination of allele sizes found in each person makes up their unique genetic profile.

In paternity testing, the alleles from the child are compared to those of the alleged parents to determine if either or both parents have contributed the alleles present in the child. Assume that a child has a 10 and 11 allele for a particular locus (marker) and the mother has a 10 and a 12 allele for this system, the mother must have contributed the 10 allele and the child must have inherited the 11 allele from the father. Any man who does not possess an 11 allele could thus not be the child's father.

The two exclusion rule is commonly accepted in paternity testing laboratories. Thus if two markers (loci) do not match between an alleged father and a child, the alleged father is excluded as being the biological father.

Paternity Index

Each locus (marker) is assigned with a Paternity Index, which is a statistical measure of how powerful a match at that particular marker is, which indicates paternity (Table 1). The Combined Paternity Index is generated by multiplying the Paternity Index of each marker. This indicates the overall probability of an alleged father being the biological father of the child, compared to any random unrelated man from the population of the same race. The Combined Paternity Index is utilised to calculate the probability of paternity. This determines the degree of relatedness between the alleged father and the child.

The South African National Accreditation System (SANAS) considers a probability greater than 99.8% as proof of paternity. A significant index is usually not given, as the percentage depends on the arithmetic. The probability percentage is calculated by dividing the index by the (index + 1).

SPECIMEN REQUIREMENT AND COLLECTION

At least 1 ml whole blood (EDTA), a blood spot on a filter card or a buccal swab can be used. There is no special requirement for blood samples. In the case of a buccal swab, the patient should not eat 30 minutes prior to collection. Specimens are transported at ambient temperature.

CHAIN-OF-CUSTODY

Chain-of-custody refers to chronological documentation or a paper trail. In the case of paternity testing, this refers to sample collection, consent, transport, analysis and reporting of the results. To satisfy the chain-of-custody legal requirements, all tested parties have to be properly identified, and their samples collected by a third-party professional who is not related to any of the tested parties and has no interest in the outcome of the test. The DNA paternity tests that follow a strict chain-of-custody can generate legally admissible results.

QUALITY CONTROL

Ampath is SANAS-accredited to perform paternity testing.

CONTACT DETAILS

Irma Ferreira: 012 678 1355.

REFERENCES AND RECOMMENDED READING

Butler J.M. 2005 Forensic DNA typing (2nd ed.). Elsevier Academic Press.
en.wikipedia.org/wiki/DNA_profiling
www.dnaforensic.org/dna_typing/dnatyping_3.html
www.scientific.org/tutorials/articles/riley/riley.html
www.cstl.nist.gov/strbase/pub_pres/NEAFS_STRs.pdf

Figure 1: DNA profile of a male individual

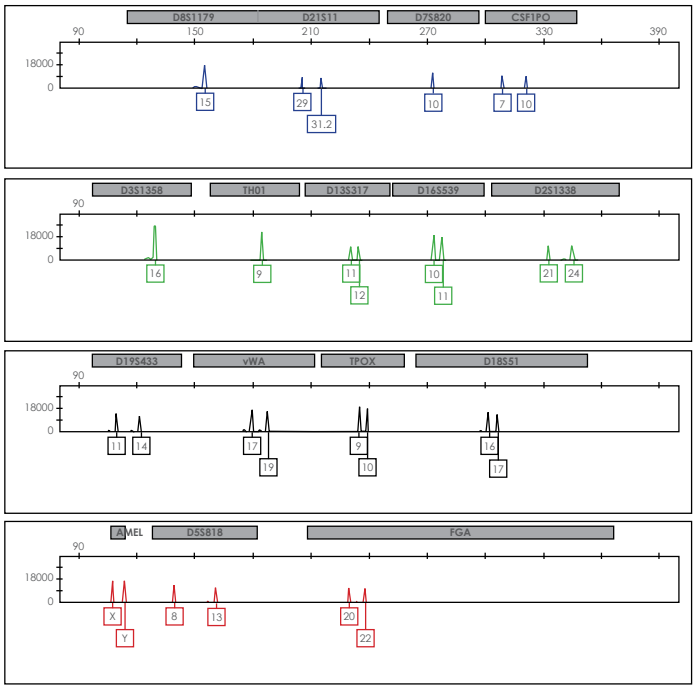

Table 1: DNA paternity report – inclusion

STR locus	Mother	Child	Alleged father	Paternity Index
D8S1179	14 12	12 13	13 14	2.092
D21S11	34 34	34 28	28 34	1.567
D7S820	10 11	11 11	11 10	2.212
CSF1PO	12 10	10 11	11 11	5.208
D3S1358	15 18	18 17	17 17	5.319
TH01	8 6	6 8	8 7	1.166
D13S317	14 11	11 12	12 12	2.646
D16S539	11 10	10 11	11 14	0.971
D2S1338	22 20	20 18	18 21	12.500
D19S433	13.2 13.2	13.2 12	12 13	4.762
vWA	20 15	15 18	18 16	3.333
TPOX	11 12	12 11	11 11	3.390
D18S51	15 20	20 14	14 17	7.353
D5S818	12 13	13 11	11 13	2.304
FGA	21 19	19 22	22 24	2.924
Amelogenin	X X	X X	X Y	
Combined Paternity Index: 20 046 118.95				
Probability of paternity: 99.999995				