# DNA TYPING IN COLOMBIA - PREVIOUS EXPERIENCES AND FUTURE OUTLOOK

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## Introduction:

DNA typing methods have made a strong impact in Medical and Forensic Genetics. Different genetic markers and various technologies are available today that allow us to identify a suspect based on the evidence left at the crime scene or to confirm or exclude a paternity. However, different issues have to be addressed to apply this powerful technology to aid justice achieve its goals in different countries. Although the technology is at hand in several developing countries, rules and regulations need to be implemented to use DNA typing in properly in the legal setting. Of particular importance are accreditation issues, quality control programs, and the need for qualified personnel performing the tests. In addition regulations regarding human rights and mandatory testing for suspects need to be addressed. In the following sections, we will try to provide an overview of the DNA typing status in Colombia.

Colombia is a very rich country. Fourteen percent (14%) of the world's bio-diversity is found in Colombia. Its population of 41 million people is composed of a great majority of Caucasian-Mestizo, African descent individuals (1.5%) and the Amerindian population composed of 83 ethnic groups (1.3%) (1). Colombia possesses in its territory the largest number of bird and amphibian species in the world, the second place in plants and flowers species, the third place for reptiles, and the fourth place for mammalian species in the world. On the other hand, Colombia is one of the most violent countries in the World. The following sections, briefly describe some of the crime statistics in Colombia.

## Homicide

Based on the PHO/UN (Pan American health organization/United Nations) world health statistics, Colombia has one of the highest homicide rates in the world. For 1992, this rate was estimated in 76/100.000 individuals. This number is highly contrasting with the homicide rates for other Latin American countries like Brazil (24.6/100.000) or Mexico (20.6/100.000). In 1998 a total of 21.160 homicides were registered in Colombia. Just between January and April of that year, 8.123 homicides were committed and only 25% of the suspects were apprehended. Impunity is a common factor.

## Kidnapping

An average of 2000 kidnapping cases/year with an average of 6 new cases/day occurs in Colombia. Extortion and political motives are the main causes for kidnapping. 60% of the cases are attributed to the guerrilla and the remaining to paramilitary forces and non-organized crime (Fundación País Libre, Colombia). Some cases involve small children where extortion is the main motive. In the remaining cases, children are sold for adoption or used for pornographic and prostitution practices.

## Massacres

Massacres are common crime events in Colombia (violent death of more than 3 persons under the same circumstances of time, place and cause of death); between 1996 and the beginning of 1999 a total of 708 massacres with 3.356 victims were registered, with an average of 1 massacre every 1.5 days (Defensoría del Pueblo, República de Colombia). The massacres are carried out mainly by paramilitary forces, and in a lesser extent by the guerrilla, urban militias and other groups.

**Rapes and Sexual assault**

In 1998 a total of 11.791 sexual assault cases were registered with a rate of 27/100.000 people. Of those, 25.17% took place in Bogotá, the capital city. (Instituto Nacional de Medicina Legal y Ciencias Forenses, INMLCF, Bogotá, Colombia).

**Missing People**

There are 2.858 registered missing people in the Colombian Government database as of April 1999 (Fiscalía General de la Nación, República de Colombia). The great majority of cases are related to political reasons carried out by the military and paramilitary forces.

**Paternity Testing**

There are in average 6.000 new cases of parentage disputes registered officially every year. In 1997 a total of 5.662 cases were registered, 6.527 in 1998 and 3.425 cases as of June 1999 (Instituto Colombiano de Bienestar Familiar, República de Colombia). However, these numbers could underestimate the magnitude of demand since private cases are not included in these statistics.

**DNA typing methods in Colombia**

Different DNA typing methods have been implemented by our laboratory to complement serological markers such as blood groups and HLA serological typing used in paternity testing cases. In addition, these DNA typing methods have also been used in forensic casework.

We first introduced HLA class II (DRB, DQA1, and DQB1) PCR based DNA typing methods and VNTR-RFLP methods in 1991. In 1994, due to the need of large scale testing for paternity cases, HLA class II (DRB, DQA1, and DQB1) typing based on PCR methods was applied. At the beginning of 1996, near 7000 samples had been analyzed with these methods along with other serological and DNA markers. Large population databases were established for these genetic markers. VNTR-RFLP methods were reserved only for selected cases due in part to the elevated cost of reagents in Colombia.

In 1994, we also introduced PCR-based typing for dimorphic human specific Alu insertions for both anthropological studies as well as in paternity testing. Their use was replaced in 1995 when Short Tandem Repeats (STR) typing was introduced by our laboratory.

As commercial STR kits became available, they were introduced to increase the number of loci analyzed. Today, STR typing is widely used in both paternity and forensic settings. A total of 17 STR loci (CSF1PO, TPOX, TH01, F13AO1, FES/FPS, VWA, D16S539, D7S820, D13S317, F13B, LPL, D12S1090, D3S1744, D18S849, FGA, D9S304 and D1S533) and D1S80 are routinely used in our laboratory in forensic and Paternity testing studies.

Population databases have been developed in our laboratory for 17 STR loci and D1S80 in the Caucasian-mestizo populations, 9 STR loci for Black population and 6 STR loci for Amerindian population (2, 3). Additional loci are currently analyzed to increase the number in both, Black and Amerindian populations for our anthropological and forensics programs.

Since most of the forensic cases analyzed by our laboratory involved skeletal human remains, usually only STR and/or mtDNA analysis can be performed in these samples. mtDNA analysis was introduced in 1997 for forensic cases and anthropological studies and have been increasingly used in our forensic testing program. Currently, population databases for mtDNA polymorphism are under development for all the Colombian ethnic groups.

We have selected two cases to illustrate the application of DNA typing in our laboratory. The first case occurred in 1996 and the second one at the end of 1998.

**Case 0141. Human remains DGE**

A male Caucasian individual was kidnapped in a northeast town of Colombia In December 1996. One year later, based on an anonymous tip, the authorities recovered the human remains of a male Caucasian between 26-30 years of age found in a riverbank that could correspond to the kidnapped individual. The body had been cut into pieces with a machete. No skull or jawbone was found to be used for dental identification.

**Materials and methods**

Blood samples were obtained from the alleged father and mother. In addition, two dental pieces (reference samples) that have been removed by a dentist and were stored by his parents before the kidnapping took place were also sent for analysis, as well as a sample of compact bone from the recovered remains.

DNA from the compact bone was obtained by cleaning the outer surface and inner bone canal with a sanding drill. 3 gr of bone were pulverized in a Micro-Mill blender. The sample was decalcified with EDTA pH 8.0 for 48 hours, washed and lysed in a Buffer containing SDS and Proteinase K. The aqueous sample was extracted twice with phenol-chlorophorm, once with cholorphorm/Isoamilalcohol and once with 1-Butanol and washed and concentrated in 1X TE pH 7.5 using centricon 100 (Amicon).

DNA from tooth was obtained by cleaning the outer tooth surface with a sanding drill and exposing the root canal. The pulp was lysed in a Buffer containing SDS and Proteinase K. The aqueous sample was extracted twice with phenol-chlorophorm, once with cholophorm/Isoamilalcohol and once with 1-Butanol and washed and concentrated in 1X TE pH 7.5 using centricon 100 (Amicon).

Genomic DNA was obtained from the blood samples of the alleged parents with the Wizard DNA isolation kit (Promega Corporation, Madison, WI).

All samples were analyzed with a battery of 9 STR (CSF1PO, TPOX, TH01, F13AO1, FES/FPS, vWA, D12S1090, D3S1744 and D18S849). The results obtained are summarized in Table 1.

A complete match was obtained between the genetic profile from the skeletal remains and that obtained from the tooth material. The paternity and maternity were compatible with all markers analyzed, with a Combined Paternity Index of 99.95% (Combined Paternity Index 2288), based on Colombian allele frequencies. The identity index for the genetic profile obtained was $1:2.7x\ 10^{11}$.

**Case J.D.S. Kidnapping**

In 1996 a newborn baby was kidnapped from a maternity clinic in Santa Fe de Bogotá. This case became the most publicized case in the country. During the next two years, eleven additional children were recovered by the authorities, however, the missing child was not among them. In December 1998 the authorities recovered a 2 years old child that could correspond to the kidnapped one. A woman was apprehended as the alleged kidnapper.

**Materials and Methods**

Blood samples were obtained from the alleged kidnapper, the child and the alleged mother. A total of 17 STR and D1S80 loci (STR-F13AO1, FES/FPS, VWA, CSF1PO, TPOX, TH01, D16S539, D7S820, D13S317, F13B, LPL, *GenePrint*™ STR systems, Promega Corporation, Madison, WI) (D12S1090, D3S1744, D18S849, FGA, D7S820, D9S304, D1S533 and D1S80, Lifecodes Corporation, Stamford, CT) were typed. In addition, molecular HLA I (HLA-A, HLA-B) and class II (HLA-DRB, DQB1) (Lifecodes Corporation, Stamford, CT) and mtDNA analysis of the HV1 and HV2 regions were also analyzed.

The FFv, CTT, SilverSTR™ , Multiplex I and Multiplex II were amplified using triplex and quadriplex systems, while LPL, F13B and D1S80 were individually amplified. The amplification conditions were

identical to those proposed by the manufacturers of the PE480 thermocycler. All amplifications were carried out in a MJ Research PTC100VG thermal cycler. The amplification products were resolved in a 4% Acrylamide-Bis-Acrylamide denaturing gel and silver nitrate stained. Allelic designations were based on the recommendations by the DNA commission of the International Society for Forensic Haemogenetics (ISFH) with the aid of allelic ladders provided in each kit.

HLA class I and class II typing was carried out following the manufacturer's recommendations (Lifecodes Corporation, Stamford, CT), followed by SSOP hybridization with alkaline-phosphatase labeled probes and chemiluminescent detection.

The mtDNA HV1 and HV2 regions were amplified with the F15996-H16401 and L29-H408 primer pairs (4). The amplified products were purified with Wizard® PCR preps (Promega Corporation, Madison WI). The sequencing reaction was carried out with CY5.5 labeled primers and the 7-Deaza-dGTP-thermosequenase cycle sequencing kit (Visible Genetics, Toronto, Canada) in both directions and analyzed in a MicroGene Blaster Automated sequencer (Visible Genetics, Toronto, Canada).

The results obtained from the analysis of the 17 STR, D1S80, and HLA typing analysis demonstrated exclusion (5 STR and HLA typing) between the kidnapped child with the alleged kidnapper (Tables 2, 3 and 4).

The alleged mother was compatible for all STR's, D1S80, HLA and mtDNA analysis with the child. The combined Maternity Index was 240.791 (mtDNA not included) and the Probability of Maternity of 99.9996%. The kidnapper later confessed the crime and motives.

**Regulations**

A law created in 1968 determined that all evidence, including biological evidence will be admissible in parentage testing disputes in Colombia. For many years, paternity testing was conducted only by a government agency, the Instituto Colombiano de Bienestar Familiar (ICBF). This agency was responsible for all official parentage disputes in Colombia. In 1994, a contract between the ICBF and the Universidad Nacional de Colombia, introduced large scale DNA typing for parentage testing disputes and for forensic cases under our direction. Later on, another official laboratory started DNA analysis in some forensic cases and lately few other laboratories from the official sector have started to use DNA typing in paternity testing analysis.

Currently there are no regulations in Colombia for DNA typing in forensic and paternity testing. There is an increasing need for both external quality control and quality assurance programs for laboratories performing DNA testing in Colombia. Very few laboratories performing DNA typing participate in external quality control programs at least once a year. These programs in part provide assurance about the efficiency of the participating laboratories, but due to the lack of a regulating agency, no restrictions are imposed to a deficient performance. Thus, the need for an accreditation program should be a priority and some efforts are under way to establish such a program.

Despite of the lack of a government regulating agency, the judicial system with recent decisions from both the Supreme Court of Justice and the Constitutional Court of Colombia have accepted the technology and its applications. In that regard, our laboratory has been involved in a continuous teaching and training program for legal and non-legal personnel. We have aimed some of our efforts to teach and illustrate Judges, officials from the Attorney General's Office and lawyers about the uses and limitations of DNA technology, as well as interpretations guidelines. However, there is still a lot of work to be done nationwide. In addition, for the last 2 years we have served as advisors and trainers in the establishment of a state of the art DNA typing Laboratory for the Fiscalia General de la Nación (equivalent to the Attorney General Office). For them, a ten months training program was conducted on DNA isolation methods from different sources, STR and mtDNA typing, as well as in interpretation guidelines. The laboratory is expected to start operations in January 2000.

Recently, discussions about the development of DNA Databases for forensic purposes have taken place. Among them, a database for missing individuals is a priority due to the magnitude of the problem. However, relatives of missing individuals and non-government organizations have serious concerns about the establishment of a database controlled by a government institution. On the other hand and due to the high criminality rate, the development of databases from convicted felons could have a strong impact on crime prevention programs. However, these kind of programs are not a priority and there is not legislation to regulate which crimes should be included in the database efforts, if the testing should be mandatory or not, as well as regulation issues related to privacy and human rights.

Table 1. Analysis of STR's in the identification of human remains. (0141)

| | F13AO1 | FESFPS | VWA | CSF1PO | TPOX | TH01 |
|---|---|---|---|---|---|---|
| A. Father | 6 / 3.2 | 13 / 11 | 16 / 14 | 12 / 11 | 8 / 8 | 7 / 6 |
| A. Mother | 5 / 4 | 11 / 10 | 17 / 16 | 13 / 11 | 10 / 8 | 9.3 / 6 |
| Tooth | 4 / 3.2 | 11 / 11 | 17 / 14 | 13 / 11 | 10 / 8 | 9.3 / 6 |
| Bone remains | 4 / 3.2 | 11 / 11 | 17 / 14 | 13 / 11 | 10 / 8 | 9.3 / 6 |

| | D12S1090 | D3S1744 | D18S849 |
|---|---|---|---|
| A. Father | 19 / 12 | 18 / 18 | 17 / 15 |
| A. Mother | 21 / 12 | 20 / 18 | 17 / 15 |
| Tooth | 12 / 12 | 20 / 18 | 17 / 15 |
| Bone remains | 12 / 12 | 20 / 18 | 17 / 15 |

Table 2. Analysis of STR's and D1S80 in a kidnapping case. (JDS)

| | F13AO1 | FESFPS | VWA | CSF1PO | TPOX | TH01 |
|---|---|---|---|---|---|---|
| A. Mother | 6 , 5 | 12 , 11 | 19 , 19 | 13 , 10 | 8 , 8 | 9.3 , 6 |
| Child | 5 , 3.2 | 12 , 11 | 19 , 14 | 13 , 11 | 8 , 8 | 9.3 , 6 |
| Kidnapper | 7 , 6 | 13 , 12 | 17 , 16 | 12 , 10 | 12 , 8 | 9 , 8 |

| | D12S1090 | D3S1744 | D18S849 | LPL | F13B | D1S80 |
|---|---|---|---|---|---|---|
| A. Mother | 22 , 20 | 18 , 17 | 16 , 16 | 12 , 10 | 10 , 10 | 18 , 18 |
| Child | 20 , 20 | 19 , 18 | 16 , 14 | 12 , 10 | 10 , 7 | 24 , 18 |
| Kidnapper | 21 , 20 | 19 , 16 | 16 , 16 | 12 , 12 | 10 , 9 | 30 , 24 |

| | D16S539 | D7S820 | D13S317 | FGA | D1S533 | D9S304 |
|---|---|---|---|---|---|---|
| A. Mother | 13 , 13 | 10 , 10 | 12 , 9 | 23 , 20 | 14 , 8 | 8 , 8 |
| Child | 13 , 12 | 10 , 8 | 12 , 11 | 28 , 20 | 12 , 8 | 8 , 8 |
| Kidnapper | 13 , 12 | 13 , 9 | 13 , 12 | ND | ND | ND |

ND: Not done

STR loci showing exclusions between the kidnapped child and the kidnapper are highlighted. Table 3. HLA Class I and Class II molecular typing. Case JDS.

Alleged Mother

| HLA-A | HLA-B | DRB1 | DRB3 | DRB4 | DQB1 |
|---|---|---|---|---|---|
| 02 | 4402 | 0701 | | 01 | 0201 |
| 02 | 3511/3514 | 1305 | 02 | | 0301 |

Child

| HLA-A | HLA-B | DRB1 | DRB4 | DQB1 |
|---|---|---|---|---|
| 02 | 4402 | 0701 | 01 | 0201 |
| 3001 | 5801/5803 | 0102 | | 0501 |

Alleged kidnapper

| HLA-A | HLA-B | DRB1 | DRB4 | DQB1 |
|---|---|---|---|---|
| 02 | ND | 0802/0804 | | 0402 |
| 68012 | ND | 04 | 01 | 0302 |

ND: Not done

HLA loci showing exclusions between the kidnapped child and the kidnapper are highlighted.

Table 4. HV1 and HV2 mtDNA analysis. Case JDS.

| | 16047 | 16111 | 16145 | 16155 | 16232 | 16249 | 16290 | 16299 | 16319 | 16146 | 16153 | 16235 | 16263 | 309 | 315i |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Anderson | G | C | G | A | C | T | C | A | G | T | A | A | A | - | - |
| A Mother | - | T | A | G | T | C | T | G | A | C | G | G | G | C | C |
| Child | - | T | A | G | T | C | T | G | A | C | G | G | G | C | C |

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