

# DNA Database of the Nicaraguan Population: Allele Frequencies of Importance in Forensic Genetics

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SCIENTIFIC-TECHNICAL DEVELOPMENT IN THE FIELD OF NATURAL SCIENCE, specifically the discovery of human DNA polymorphism, has allowed us to identify people by their genetic fingerprint, i.e. their DNA, unique to every individual on earth. Its use in criminal investigations and forensic medicine has brought about the creation of DNA databases for discrete groups, populations and entire nations. In Nicaragua, the Molecular Biology Center of the Universidad Centroamericana has been a pioneer in this area of research, providing support for criminal investigations and resolving innumerable cases of paternity disputes. In this report we present the achievements of ten years of research, highlighting the technical aspects and, in particular, the application of the *AmpFISTR Identifiler* system, as well as future prospects for scientific investigation in this area.

**Keywords:** AmpFISTR Identifiler / DNA databases / genetic diversity / population genetics / Nicaragua, STRs

## 1. Introduction

Scientific-technical advances in forensic biology, made possible by the discovery of human DNA polymorphism and the implementation of the polymerase chain reaction technique (PCR, polymerase chain reaction; Jeffreys, Wilson, Neumann & Keyte, 1988; Monckton & Jeffreys, 1993), brought about the ability to identify people by their genetic fingerprint, i.e. their DNA, unique to every person on earth. This was quickly followed by the initiative to organize DNA databases for the purpose of supporting criminal investigations which, under other circumstances, might go unsolved.

In the year 2000, the Center for Molecular Biology of the Universidad Centroamericana in Nicaragua, (CBM-UCA in Spanish), established the use of molecular markers for identifying individuals, which placed the Center at the vanguard of this area of knowledge, countrywide. These scientific processes made it possible to provide support for criminal investigations and resolve numerous cases of paternity disputes.

Given the publicity inherent in certain criminal cases of great interest to the public, the Molecular Biology Center has received attention from the media, primarily to inform and educate the public on the techniques utilized and their scientific value. One example of such a case was that in which support was given to the National Police during their investigation of the highly publicized assassination of four police officers on Nicaragua's Caribbean coast. The Center has also solved cases of rape, more often than not involving adolescent victims as well as girls and boys. With time, the identification of biological samples by comparing DNA has gradually become more associated with police investigations than with academia; today, it has become an important tool in the field of forensics nationally.

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Although it is possible to determine the individual source of biological samples such as saliva, semen and blood, it is impossible to identify the individual in question with near absolute certainty without having previously determined the genetic profile (a collection of data consisting of molecular markers) of the individual. Victims of rape who survive often do not recognize or remember their assailant, except in cases of incest in which the assailant is present in the family environment. In either case, positively identifying the perpetrators of crimes such as these must be done through DNA testing. And in turn, the arduous labor of identifying the perpetrators through DNA testing must include a DNA database.

In this report we present the achievements of ten years of research carried out by the Molecular Biology Center, focusing on two important events: the creation of the 2004 database using the *GenePrint*® *STR Systems (Silver Stain Detection)* of the *Promega Corporation* and the amplified and improved database using automatic sequencing and the *AmpFLSTR*® *Identifiler*™ PCR Kit of *Applied Biosystems*.

## **2. DNA database for population studies**

Different types of genetic databases exist which are of great interest and importance, including DNA databases to identify persons who are reported missing, databases for criminal investigations and population databases (see Box 1). The population databases of human DNA markers used in genetic forensics (autosomal STR's, Y chromosome STR's and hypervariable regions of mitochondrial DNA) are also essential for conducting adequate biostatistical evaluation of the validity of the test.

**Box 1. Nuclear DNA vs. Mitochondrial DNA**

Deoxyribonucleic acid, more frequently abbreviated as DNA, is the biochemical component charged with transmitting and regulating the life of all species of plants and animals on earth. It contains the genetic information used in the development and function of living organisms and some viruses, and is responsible for their hereditary transmission. DNA is composed of one sugar (deoxyribose), a nitrogenous base (which can be Adenine [A], Thymine [T], Cytosine [C], Guanine [G]), and one phosphate group. In living organisms, DNA presents as a double chain of nucleotides, in which both strands are united by connections called hydrogen bridges. Within the cells, DNA is organized in structures called chromosomes. Each person has 23 pairs of chromosomes, of which 22 are autosomal, leaving one pair of sex chromosomes.

Two types of DNA exist, depending on location and structure: nuclear DNA and mitochondrial DNA.

- **Nuclear DNA**, to which reference is usually made when speaking of DNA, without further specification, located inside the cell nucleus.
- **Mitochondrial DNA**, located inside the mitochondria, a cell organelle.

Nuclear DNA is divided into coding DNA, equivalent to 3% of all DNA, and non-coding DNA (97%). Non-coding means that it is not transcribed into RNA and therefore does not code for any particular protein. Nevertheless, this DNA is essential for forensic investigation as an instrument for individualization. Non-coding DNA sub-divides into repetitive and non-repetitive DNA. In forensic and population genetics, the more important of the two is repetitive DNA. Tandem-repetitive DNA: These are repetitions organized in a repetitive manner in such a way that identical or near identical sequences follow one after the other. There are three types of tandem-repetitive sequences (STRs or short tandem repeats): satellites, minisatellites and microsatellites. Of these three, it is the microsatellites that are used to identify genes.

All chromosomes, with the exceptions of chromosomes X and Y, are known as *autosomal*. Given their high variability, the study of autosomal markers is primarily employed in the field of forensic genetics and biological studies of paternity. The Y chromosome represents only 2% of the human genome. It is one of the smallest chromosomes. Ninety-five percent of the Y chromosome does not recombine (does not exchange genetic material), and is therefore transmitted intact from father to son.

The STR markers of the Y chromosome allow us to study the strictly paternal line of genetic transmission (see Box 2). They are also used in genetic investigations of semen residue in some cases of sexual assault. In cases of sexual and physical assault, it is often difficult to distinguish the female victim's DNA profile from that of the (male) aggressor. This obstacle may be overcome using the Y-chromosome markers to generate male specific profiles.

**Box 2. Use of the Y chromosome in solving paternity cases**

A woman became pregnant and gave birth after having been raped. In the laboratory, blood samples from the mother, her child and two men suspected of the rape were analyzed in order to determine which of the two men might be the biological father of the child. Markers of the *AmpFlSTR® Identifiler™* system for all four individuals were analyzed. Y chromosome analyses of the two men and the child were also done. Results obtained are presented in the tables below.

**Analysis 1. *AmpFlSTR® Identifiler™* system**

Sample	D8S1179	D21S11	D7S820	CSF1P0	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	D5S818	FGA
S1*	12-12	29-31	7-11	10-10	15-16	6-7	9-9	10-12	17-22	13-15.2	16-18	8-12	14-21	9-11	21-25
S2*	10-15	30-33.2	9-12	10-13	15-17	6-7	9-12	8-11	22-23	15-16	16-17	11-12	16-18	11-12	20-22
M*	12-13	29-31	10-11	10-12	15-16	6-9	9-10	10-13	17-20	14-15.2	16-16	11-12	14-21	9-11	20-21
C*	12-13	31-31	7-10	10-10	16-16	7-9	9-9	10-12	17-22	15.2-15.2	16-16	8-11	14-14	9-11	20-21

\* S1=suspect 1; S2=suspect 2; M=mother; C=child.

Through the autosomal markers, it is revealed that there is a probability of 99.999998% that suspect 1 is the biological father of the child, a virtually proven paternity.

**Analysis 2. Y Chromosome. *AmpFlSTR® Yfiler™* system**

Sample	DYS 456	DYS 389I	DYS 390	DYS 389II	DYS 19	DYS 385 a/b	DYS 393	DYS 391	DYS 439	DYS 635	DYS 392	GATA H4	DYS 437	DYS 438	DYS 448
S 1	16	14	23	32	16	13-18	12	10	11	20	11	10	14	10	20
S 2	14	12	25	28	17	13-17	12	10	11	21	11	11	16	9	19
child	16	14	23	32	16	13-18	12	10	11	20	11	10	14	10	20

Observe the total similarities or “coincidences” between suspect 1 and the child. This indicates that subject 1 and the child come from the same Y lineage, which in this case of rape points to suspect 1.

Mitochondrial DNA (mtDNA) is comprised of the genetic material of the mitochondrial cells. It is a bicatenary molecule, circular, closed and without extremities. In human beings it has 16,569 base pairs, with a coding region that contains 37 genes (Jarovsky, Shiozawa, Rosalino & Barros, 2006). There are two principal regions of mtDNA: a larger coding region and a smaller region known as the control region, associated with the regulation of replication and genetic transcription. This control region measures 1.2 Kb and is highly polymorphic. Internally there are two hypervariable sequences: hypervariable region 1 and hypervariable region 2 (HV1 and HV2) which serve in human identification.

The mtDNA presents certain characteristics that make it particularly useful for identification and molecular evolution analysis, including: the high number of copies possessed by each individual, transmission only through the mother, the fact that the genome has been

completely characterized, the presence of numerous polymorphisms and their rapid rate of evolution.

### 3. DNA database of the Nicaraguan population, results obtained

All laboratories involved in forensics and in human identification must have a database with the allele frequencies of the population in which DNA analyses are being done. The database of the Nicaraguan population is an indispensable instrument for the statistical validation of forensic and paternity findings. The Molecular Biology Center began its data collection for the Nicaraguan population database in 2000.

#### 3.1. 2004 Database and 2010 Update

##### *Methodology (2004 Database)*

*Samples:* 111 samples were collected from the Nicaraguan Red Cross from blood donors.

*DNA Extraction:* Obtained through Chelex® 100 method following protocol described by Walsh, Metzger and Higuchi (1991).

*DNA Amplification:* Amplification and allele determination for CTT, FFv and DDD sets of markers, were carried out using the GenePrint System protocol from Promega.

*Polyacrylamide Gel Electrophoresis:* For allele assignment, a denaturing polyacrylamide gel was used with Gibco-BRL® Model SA32 sequencing equipment. Following the electrophoresis process, the gels were stained with a tincture of silver nitrate and DNA bands were developed with sodium carbonate. Allele assignment was done visually using an ultraviolet light transilluminator.

##### *Methodology for 2010 Update*

*Samples:* 400 blood samples were collected on FTA® paper (Whatman Inc., Clifton, NJ) from unrelated individuals.

*DNA Extraction:* Blood samples were allowed to dry on FTA® paper for 24 hours. DNA extraction was done according to manufacturer's instructions (Whatman Inc., Clifton, NJ). FTA® Cards are impregnated with a chemical formula that breaks down cell membranes. Nucleic acids are immobilized and protected from UV light damage, microbes and fungal attack. The sample is easy to apply and dries at room temperature. A small disk is cut from the paper (1.2 mm or 2.0 mm) and run through a wash solution, and is then ready to be analyzed.

*DNA Amplification:* Amplification was carried out using the AmpFLSTR® Identifiler™ PCR Kit from Applied Biosystems (Life Technologies, USA.) This system has 15 autosomal markers with fluorochromes (fluorescent dyes) (D8S1179, D21S11, D7S820, CSF1P0, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA).

*Capillary Electrophoresis:* The amplified samples were processed in the *Applied Biosystems 3130 Genetic Analyzer* which detects the alleles using *Applied Biosystems GeneMapper™ ID v3.1* software. This software carries out automated genotyping for forensic, paternity and database analysis.

*Population Analysis:* Allele frequencies, power of discrimination (PD) and power of exclusion (PE) were calculated using the *PowerStats* program (*Promega, 1999*). Hardy-Weinberg equilibrium, observed heterozygosity and expected heterozygosity were calculated using *Genepop v4.0* software (*Rousset, 2008*).

#### 4. Results

The results of the two allele databases are as follows.

##### 4.1. Database using GenePrint® STR Systems (Silver Stain Detection)

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The allele frequencies for all multiplex markers were obtained in the group of 111 DNA samples (2004): CCT (CSF1PO, TPOX, THO1), FFT (F13A01, FESFPS, vWA) and DDT (D16S539, D7S820, D13S317). The most frequent alleles are shown in Box 3 below.

**Box 3.** Most frequent alleles for markers CSF1PO, TPOX, THO1, F13A01, FESFPS, vWA, D16S539, D7S820 and D13S317

Marker	CSF1PO	TPOX	THO1	F13A01	FESFPS	vWA	D16S539	D7S820	D13S317
MFA*	10,11,12	8,9,11	6,7,9,3	3,2,5,6,7	10,11,12	15,16,17,18	9,10,11,12	10,11,12	9,10,11,12

\*MFA, most frequent alleles

The percentages of heterozygotes and homozygotes were determined for each one of the markers (Box 4).

**Box 4.** Percentages of heterozygotes and homozygotes

Marker	CSF1PO	TPOX	THO1	F13A01	FESFPS	vWA	D16S539	D7S820	D13S317
Heterozygotes	72.1	70.3	79.3	68.4	68.5	77.5	74.8	73.0	81.1
Homozygotes	27.9	29.7	20.7	31.6	31.5	22.5	25.2	27.0	18.9

The power of exclusion (PE) for the markers under consideration are shown in Box 5 below. The combined PE for all seven markers was 0.996.

**Box 5.** Power of exclusion (PE) using markers CSF1PO, TPOX, THO1, F13A01, FESFPS, vWA, D16S539, D7S820 and D13S317

Marker	CSF1PO	TPOX	THO1	F13A01	FESFPS	vWA	D16S539	D7S820	D13S317
PE	0.398	0.460	0.599	0.428	0.553	0.695	0.506	0.476	0.619

The power of discrimination (PD) for the markers under consideration are shown in Box 6 below. The combined PD for all seven markers was 0.999.

**Box 6.** Power of discrimination (PD) using markers CSF1PO, TPOX, THO1, F13A01, FESFPS, vWA, D16S539, D7S820 and D13S317

Marker	CSF1PO	TPOX	THO1	F13A01	FESFPS	vWA	D16S539	D7S820	D13S317
PD	0.858	0.759	0.846	0.918	0.834	0.834	0.927	0.919	0.947

The analysis of the Hardy-Weinberg equilibrium or principal was done using Fisher's Exact Probability Test in which a P value > than 0.05 indicates that there is no deviation from the Hardy-Weinberg equilibrium for the markers in question. For the first study done in 2004, the markers show no overall deviation from the Hardy-Weinberg equilibrium in our population. The only marker that did show deviation was F13A01, which gave a P value of 0.002. This could be due to a non random pairing in the population, or immigrations. The P value of Fisher's Exact Probability Test for each marker is shown in Box 7 below.

**Box 7.** P value for each marker

Marker	CSF1PO	TPOX	THO1	F13A01	FESFPS	vWA	D16S539	D7S820	D13S317
P value	0.119	0.051	0.146	0.002	0.120	0.325	0.071	0.361	0.704

**4.2. Database using the AmpFLSTR® Identifiler™ system**

Four hundred samples were analyzed for which allele frequencies were obtained. The most frequent alleles for each marker are shown in Box 8 below.

**Box 8.** Most frequent alleles using AmpFLSTR® Identifiler™ system

Marker	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	D5S818	FGA
MFA*	13,14	29,30	10,11,12	10,11,12	15,16	6,7,9,3	11,12	10,11,12	19,20,23	13,14	16,17	8,11	14,15,17	11,12	24,25

\*MFA, most frequent alleles

The percentages of heterozygotes and homozygotes were determined for each marker. See Box 9 below.

**Box 9.** Percentages of heterozygotes and homozygotes using the AmpFLSTR® Identifiler™ system

Marker	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	D5S818	FGA
Hetero	77.95	85.09	76.71	72.36	69.88	71.12	81.37	78.57	85.40	81.99	73.60	68.94	89.41	74.53	86.08
Homo	22.05	14.91	23.29	27.64	30.12	28.88	18.63	21.43	14.60	18.01	26.40	31.06	10.59	25.47	13.92

The combined power of discrimination (PD) for the 15 markers using the AmpFLSTR® Identifiler™ system was 99.9999999999%. Box 10 below shows the PD for each marker.

**Box 10.** Power of discrimination (PD) for each marker using the AmpFLSTR® Identifiler™ system

Marker	D8S1179	D21S11	D7S820	CSF1P0	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	D5S818	FGA
PD	0.9268	0.9122	0.9248	0.8650	0.8803	0.9031	0.8438	0.9273	0.9683	0.9459	0.8983	0.8543	0.9696	0.8810	0.9715

The combined power of exclusion (PE) was 0.999998. The PE of each marker is shown in Box 11 below.

**Box 11.** Power of exclusion (PE) for each marker using the AmpFLSTR® Identifiler™ system

Marker	D8S1179	D21S11	D7S820	CSF1P0	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	D5S818	FGA
PE	0.5616	0.5617	0.5394	0.4657	0.4263	0.4458	0.6246	0.5728	0.7028	0.6364	0.4862	0.4121	0.7833	0.5018	0.7162

104 According to results of the study done in 2010, the markers do not show overall deviation from the Hardy-Weinberg equilibrium in our population. The only marker that showed deviation was D19S433, with a P value of 0.0307. The P values of Fisher’s Exact Probability Test for each marker are shown in Box 12 below.

**Box 12.** P values of Fisher’s Exact Probability Test for each marker using the AmpFLSTR® Identifiler™ system

Marker	D8S1179	D21S11	D7S820	CSF1P0	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	D5S818	FGA
P value	0.3484	0.7361	0.0884	0.7679	0.2407	0.0901	0.1712	0.4871	0.1474	0.0307	0.1887	0.1712	0.7717	0.8936	0.216

These results, obtained using the *AmpFLSTR® Identifiler™* system, show that these genetic markers are efficient tools for showing probability for use in administering justice. Blood or other human body fluids serve as “clues” in paternity investigations and the identification of individuals.

## 5. Discussion

The first country to create a DNA database was the United Kingdom. The government ordered the creation of the UK’s first National DNA Database in 1999 (Wallace, 2006) and today it is the most organized and the largest database in existence. With the technical support of this database, more than 40,000 cases were solved in 2008 and 2009. As a general rule of police investigations in that country, samples of DNA are taken from everyone accused of a crime. In the United States, a database was created called CODIS (Combined DNA Index System), initially sponsored and funded by the FBI. The original DNA data collection was intended for convicted sex offenders, but with time CODIS became a database of 5.6 million DNA profiles, primarily of convicted felons. In Spain, the DNA database of the police was created by law in 2007, uniting the files of both the police and the Civil Guard. According to its founders, the law creating the database would facilitate the solving of nearly 5,000 criminal

cases annually, including homicides, acts of terrorism, armed assault and sex crimes. Many countries, including New Zealand, France and India, have their own DNA databases on a smaller scale of magnitude.

In the case of Nicaragua, the database organized by the Molecular Biology Center (CBM-UCA), was not created for the purpose of criminal investigation but rather for population characterization. Nevertheless, the same database is useful as a reference for biological data and principally as a statistical tool in criminal cases. At the request of the police and the Office of Legal Medicine, the CBM-UCA has resolved cases of rape and murder, as well as paternity disputes. The CBM-UCA has recommended to the investigative offices that a biological (samples and tissues) and DNA database should be created. The technical capacities, experience and infrastructure of the CBM-UCA are of much usefulness for these government institutions.

The results of the experiments presented in this paper represent enormous advancement in genetic research in Nicaragua. The first report on DNA data specific to the Nicaraguan population was published in 2004 by the Molecular Biology Center of the Universidad Centroamericana, in which the results of an analysis of blood samples supplied by the Nicaraguan Red Cross, using the *GenePrint® Silver STR Systems*, were presented (Flores-Obando, Budowle & Huete-Pérez, 2004).

Later, the Center purchased a state of the art genetic analyzer, the *ABI 3130* made by *Applied Biosystems*. By 2010, seven new markers were incorporated into the analysis process, for a total of fifteen markers, in addition to the sex marker. This not only reduced the time it took to produce results, it also provided greater resolution and precision. This system allows the Center to routinely solve up to 200 samples per week, which represents an unparalleled tool for solving the paternity disputes brought to the Center by the Ministry of the Family, as well as for criminal investigations.

Thus far we have pointed out the advantages of creating and utilizing genetic databases for solving crimes, as well as for proving the innocence of those who have been unjustly accused. In terms of the general public, the most frequent use of these databases is almost exclusively identified with crime investigation. Nevertheless, other uses of critical importance exist, such as identifying victims and the “disappeared” during or following political, civil and environmental crises around the world, (war, dictatorships, earthquakes, tsunamis, etc.). At the same time, one must not fail to mention some of the difficulties which might arise from the creation and use of these databases, such as issues related to ethics, human rights and possible abuse that could occur at the hands of some individuals or powerful State institutions. One particular worry involves the use of the genetic information contained in DNA for purposes beyond that of forensics such as for the benefit of the interests of insurance companies (health and otherwise), to mention just one instance. One can also imagine difficult situations in countries in which ethnic differences could lead to social conflict.

Another application for the use of DNA databases is aimed at studying the origins of population groups. The CBM-UCA is developing a project for studying the diverse indigenous

groups in Nicaragua for the purpose of investigating the origins of the first populations to have inhabited the country. Using mitochondrial and Y chromosome DNA, it is possible to trace the ethnic origins of populations (Armour et al., 1996). It is with great interest that the CMB-UCA is investigating the genetic structure of the principal ethnic groups that make up the Nicaraguan nation, and in particular, their identity from the anthropological point of view and their genetic relationship to other populations in the Americas, through molecular markers used in the population databases. This objective is of profound importance given the difficulties that face these human groups and the marked tendency for these groups to disappear.

106 Nicaragua is geographically located in a position that connects both North and South America. Many aboriginal groups migrated through our territory and some of them settled in the region. With the aid of the genetic databases, it is possible to determine the genetic structure of the populations, genes susceptible to disease and individual genetic profiles of the Nicaraguan population. For this purpose, we must study groups whose genetic ethnicity has been conserved, and who still inhabit certain regions of the country, such as the *Matagalpa* in the central region, the *Sutiava* in the west and the *Mayagna* in the Nicaragua's Caribbean region. With the database expanded to include the study of genetic diversity, the relationship between the indigenous study group and other related ethnic groups in the country and in Mesoamerica can be determined through the use of molecular phylogenetic methods.

Population DNA databases have aided criminal investigations enormously in the countries where they have been created. While much debate exists regarding their usefulness and applicability, as well as the legal fundamentals and the management of this powerful instrument, there is no doubt that the implementation of these databases has revolutionized the field of legal medicine. In Nicaragua, it falls on the National Police to further explore the need to organize a database of criminals and of samples obtained in police investigations. At the same time, in order to be of the highest caliber, the forensic work must be carried out according to the highest possible standards. In particular, one must consider the validity of maintaining biological samples for long periods of time given that these samples can degrade with time, invalidating the analytical process (Alaeddini, Walsh & Abbas, 2009). In particular, the techniques based on the amplification of DNA fragments and the determination of the sizes and repetitions can be negatively affected by decomposition.

The existing databases containing information regarding the genetic structure of the Nicaraguan population are currently useful as a statistical support for forensic investigation, but they do not contain information on criminals, nor suspects, *per se*. However, this statistical support has resulted enormously advantageous for solving all the paternity cases and criminal investigations carried out at the CBM-UCA.

As the scientific-technical revolution advances, more and more dilemmas appear in terms of social, economic and ethical concerns. The vast majority of scientists adheres to the position of the World Bioethics Congress (2004), which firmly states that the human genome—every single human genetic information—belongs to all of humanity, and as such, cannot be patented. Nicaragua could not only benefit from incorporating these technologies and

applications, but could also contribute to expanding this world heritage. Since the creation of the Molecular Biology Center of the Universidad Centroamericana in 1999, the possibility of conducting molecular research in the country in different fields of knowledge, was born. During the last 10-15 years, molecular research, which constitutes the foundation for the study of genomics, has been consolidated. The experience of other countries, such as Mexico, which established the *Mexican Genome Program*, gives rise to the hope that the incorporation of Nicaragua and the rest of the Central American region into the society of knowledge will not remain simply a dream (Huete-Pérez, 2009).

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