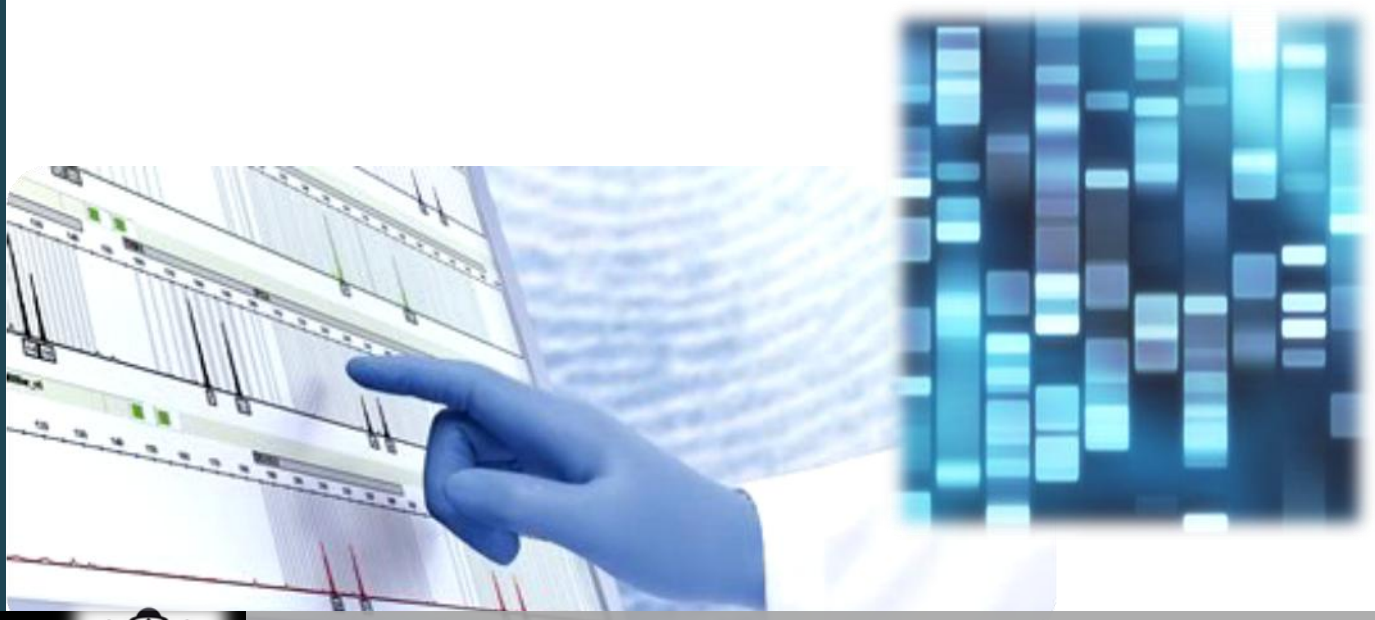
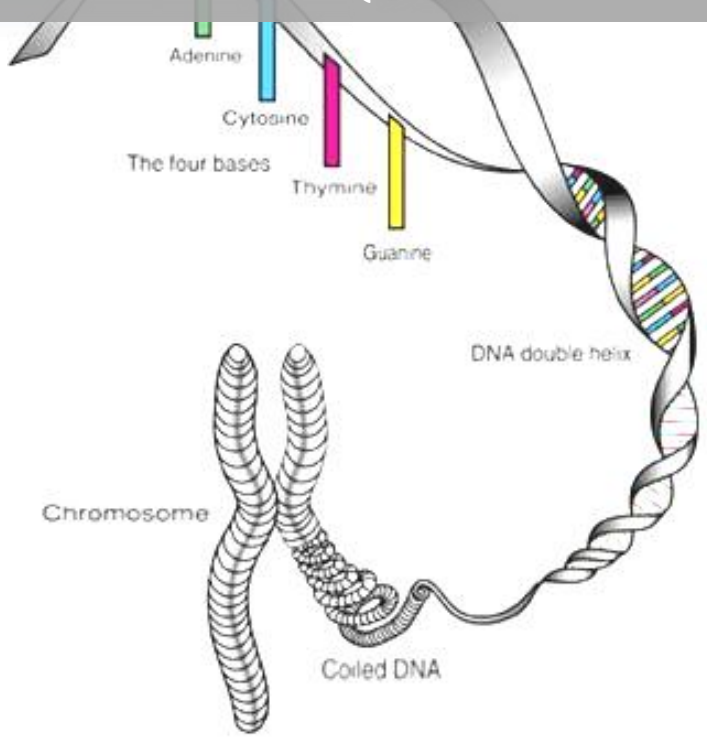
Ernst-Moritz-Arndt- Universität Greifswald 17/7/2013

Institut für Rechtsmedizin

Forensische Molekulargenetik



CHARACTERIZATION OF HUMAN POPULATIONS USING MOLECULAR GENETICS TECHNIQUES



MASTER EN BIOTECNOLOGÍA DEL MEDIO AMBIENTE Y LA SALUD

Master of Biotechnology of Environment and Health

CENTRO INTERNACIONAL DE POSTGRADO
CAMPUS DE EXCELENCIA INTERNACIONAL

Master Thesis | Cristina Cano García

An expert is one who knows more and more about less and less until they know absolutely everything about nothing…

(First part by Nicholas Butler, Bartlett's 585:10)

ACKNOWLEDGEMENTS

Nunca dije que el camino hasta aquí fuera sencillo, de hecho me imaginé muchas veces el final de este ciclo, porque acostumbro demasiado a vivir como 2 meses por delante de la situación real, pero mis expectativas se quedaron algo cortas… Pudieron ser cosas relacionadas con estar lejos de casa, los idiomas (en el lab con mi limitado inglés y en la calle con mi inexistente alemán), la vida independiente… o quizás una mezcla de todo junto. El trabajo durante estos 4 meses (y se dice pronto) ha sido enriquecedor en muchos aspectos, dónde por un lado he tenido la suerte de conocer a mucha gente interesante y sobre todo buenos compañer@s y amig@s que espero tengamos la oportunidad de volver a reencontrarnos en algún momento, pero sobre todo, aprender nuevos conocimientos y una nueva forma de trabajar.

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ABSTRACT

Studies and new technologies applied to Y chromosome analysis have allowed a great development for haplotype discrimination and identification of individuals at forensic, anthropological and evolutionary studies. Such analyses are more commonly used in paternity tests and forensic casework such as sexual abuse. The implementation of new *Y-STR* markers is a necessary objective to improve the use of the techniques and their applications. In this recent study, the commercial kit *PowerPlex*® *Y23* was used for typing 23 *Y-STR* (*DYS19, DYS385a / b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS481, DYS533, DYS549, DYS570, DYS576, DYS635, DYS643 and Y-GATAH4*) simultaneously for a total of 175 samples from a German population in *Mecklenburg-Vorpormmern* and 178 samples for the province of *Asturias*.

To complement these analyses some statistics have been calculated such as the locus allele frequencies in the two populations and the power of discrimination (*PD*). Similarities and differences connected with the analyzed locus have been found. For both populations, but especially the German population, the locus *DYS481* has the highest discriminatory power, whereas in the Asturian is DYS570. On the other hand, others loci are less informative and with lower allelic diversity as in the case of *Y-GATAH4* or *DYS437*. There are also cases of bimodal behavior where *DYS392* allele frequencies are different depending on the population. The high discrimination power of the *PowerPlex*® Y23 kit is notable, but at the same time, it becomes necessary to implement databases and the incorporation of higher markers studied to characterize the male populations and for future researchers.

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Los estudios y nuevas tecnologías aplicadas en el análisis del cromosoma Y han permitido un gran desarrollo para la discriminación de haplotipos y la identificación de individuos a nivel forense, antropológico y en estudios evolutivos. Dichos análisis son más comúnmente usados en tests de paternidad y en trabajo social forense como puede ser en abusos sexuales. La implementación de nuevos marcadores *Y-STR* es un objetivo necesario para poder mejorar el uso de las técnicas y sus aplicaciones. En el reciente estudio, se ha utilizado el Kit comercial *PowerPlex*® Y23 para tipar 23 *Y-STR* (*DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS481, DYS533, DYS549, DYS570, DYS576, DYS635, DYS643 y Y-GATAH4*) de forma simultánea para un total de 175 muestras de una población alemana en *Mecklenburg-Vorpormmern* y 178 muestras para la provincia de Asturias.

Para complementar esos análisis se han hallado una serie de estadísticos como son las frecuencias alélicas por locus en las dos poblaciones y el poder de discriminación. Se han encontrado similitudes y diferencias atentiendo al locus analizado. Para ambas poblaciones, pero especialmente la población alemana, el locus *DYS481* presenta el mayor poder de discriminación, mientras que en la población asturiana es *DYS570*. Por el contrario otros loci son menos informativos y con una diversidad alélica mucho menor como es el caso de *Y-GATAH4* o *DYS437*. Existen también casos de comportamiento bi-modal muy destacados para *DYS392* dónde las frecuencias alélicas se encuentran bien diferenciadas dependiendo de la población. Queda vigente el alto poder de discriminación del kit *PowerPlex*® Y23 pero al mismo tiempo se hace necesaria la implementación de bases de datos y la incorporación de mayores marcadores en estudio para poder caracterizar a las poblaciones de varones y para futuras investigaciones.

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ABBREVIATION

CE	**C**apillary electrophoresis
Ct	Cycle threshold
DNA	Deoxiribonucleic acid
INE	Instituto Nacional de estadística
IPC	Internal positive control
MV	Mecklenburg-Vorpommer
PCR	Polymerase Chain Reaction
PD	Power of Discrimination
qRT-PCR	quantitative Real Time PCR
Rn	Flourescent signal
RNA	Ribonucleic acid
SE	Standard error
SNP	Single Nucleotide Polymorphism
STR	Short Tandem Repeats

INTRODUCTION

The beginning of genetics studies

Genetics, as was classically defined by biologist William Bateson, is a science that studies the processes and mechanisms of inheritance from parents to offspring (*Bateson 2002*). Nowadays, this includes the molecular structure and function of individual genes, and their distribution and effects on organisms and populations. Summaryzing, every topic connected to living systems and their variation and heredity of information is a matter dealt by Genetics.

A landmark for the progress of Genetics was the discovery of the molecule which stores inheritable information, the *DNA* or Deoxiribonucleic acid. This molecule is responsible for encoding the genetic instructions used by the living organism for its function, which will be transcribed into an *RNA* molecule (Ribonucleic acid) and finally synthesised into proteins. The actual discovery predates the first notions of its importance, as it was the Swiss scientist Friedrich Miescher who isolated *DNA* in 1869, calling this white and acidic chemical "nuclein" *(Dahm 2008*). In 1911, Thomas Hunt Morgan suggested chromosomes as the carriers of genetic information, and Oswald Avery, Colin McLeod and Maclyn McArthy assigned this function uniquely to DNA (*Sturtevant 1965*). Finally, it was not until 1953 when James Watson and Francis Crick suggested the accepted molecular structure for DNA (*Watson and Crick 1953*), which reported them the 1962 Nobel Prize for Physiology or Medicine together with Maurice Wilkins.

The biochemical foundations of DNA

The *DNA* structure is formed by a double-helix which consists of two long chains of biopolymers constituted by nucleotides. Each nucleotide is composed by a nucleotidic base that can be adenine, cytosine, guanine or thymine (*A, C, G* and *T* respectively) attached to a phosphate group and a sugar (deoxyribose). Bases of both chains pair according to their molecular affinities, which makes the *DNA* resistant to natural degradation or cleavage, and ensures the existence of a duplicate of all the information.

This encoded information is passed on from generation to generation and, in the case of organisms with sexual reproduction, combined so each biological parent contributes in the same extent. However, more than 90% of this information is represented by non-coding regions and these parts of the genome are not mainly controlled by the forces of natural selection (*McPherson et al. 2001*). Mutation in the remaining 10% becomes the main source of variability for natural populations, with the known exceptions of base changes that affect non-coding but regulatory regions (*Lee and Young 2013*).

Person individualization and forensic analysis

All the aforementioned create some particularities which make *DNA* an important tool to work for assessing natural population variation and, at the same time, for characterizing individuals. In fact, *DNA* analysis has become the main standard procedure used in laboratories for criminal forensic casework, such as paternity tests and sample individualization. In this regard, the classical serological markers were used for these purposes decades ago have been substituted, as in some cases, the analysis of these markers was difficult due to the limited and degraded state of the samples involved in real cases (*Carracedo and Sanchez-Diz 2005*). Other advantages that *DNA* markers have over classical markers are related to their population variance. This can be very high in some cases, which eases the identification of a person, even when he or she might be a member of a big admixed population. Moreover, the *DNA* information is the same in all cells, and this makes the extraction and sampling more successful and easy to do. Finally, DNA can be analyzed in degraded material because of its natural resistance for degrading conditions *(Carracedo and Sanchez-Diz 2005*).

Technology for performing *DNA* typing has had a rapid development since the 1990s to the point where nowadays it is possible to obtain results in a few hours or to get samples with a small amount of biological material (*Butler 2005c*). With the new sequencing machinery and typing methodologies, different populations have been studied and standard protocols in worldwide laboratories have been validated, that define the current *state-of-the-art* techniques. In fact, the variety of genetics techniques is really broad and constantly evolving. For example, multi-locus and single-locus *DNA* probes analysed by Southern blotting were the

reference technique for paternity testing and identification until 15 years ago (*Southern 1975*), when they were replaced by short-tandem-repeat (*STR*) analysis due to their simplicity and lack of problems for replication of results (*Carracedo and Sanchez-Diz 2005*). Currently, all of the *DNA* analyses routinely carried out in a forensic laboratory involve a polymerase chain reaction (*PCR*) step, which amplifies the *DNA* quantities allowing working with small source samples (*Alvarez-García et al. 1996)*, and the subsequent analysis of *STR* of Single Nucleotide Polymorphism (*SNP*) markers.

Mini and micro Satellites

The complete first *STRs* analyses were made by gel electrophoresis systems until the introduction of the fluorescent technology combined with the capillary electrophoresis, which was the main revolution in this field. This allowed to type large quantity of *STRs* automatically and to interpret those using bioinformatics programs. In fact, there are several commercially available kits for analying *STRs* with the use of reference allelic ladders (*Welch et al. 2012*). These allelic ladders are essential for the analysis, and provide a consistent currency that helps to interpret the results and make them compatible with the *DNA* databases *(Butler 2005f*). In this report the *PowerPlex® Y23 System* (*Promega Co.*) was used for typing 23 different loci from Y chromosome.

The aforementioned non-coding regions are very important for forensic and genetic research. Approximately a 30% of the non-coding *DNA* consists of repetitive sequences that can be divided in tandem repetitive sequences and interspersed elements (*Xing et al. 2007*). The latter are the most useful and common for forensic analysis (*Livne et al. 2010*). They can divided into mini-satellites (J*effreys et al. 1985*) and Microsatellites, which are also called *STRs* (*Tautz 1989*).

One of the differences between them is length (*Fig. 1*); *mini-satellites* consist of motifs of 15-50bp and the total range can be of nearly 500-20Kb, meanwhile the *STRs* are characterized by small motifs (2-6bp) with a total ranging of 50-500bp. Other very important difference is the distribution in the genome because mini-satellites appear commonly in sub-telomeric regions and *STRs* are widely

distributed in the entire human genome (*Beckman and Weber 1992*). These repeat regions vary among individuals making them useful markers for human identification and anthropological or forensic studies. However, until the introduction of *STRs* analysis by *PCR*, single-locus mini-satellite analysis was very popular and widely used in the main forensic laboratories (*Thompson et al. 2012*).

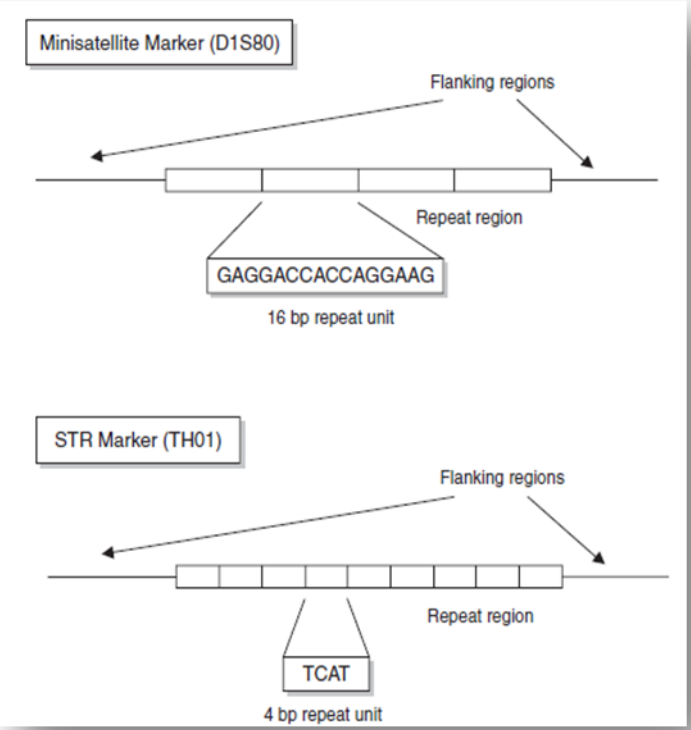


Figure 1. *Scheme which represents an example of a Minisatellite and Microsatellite (STR). The number of tandem repeat units and their length can be used to differentiate both. (Butler 2005a)*

The Y chromosome and its characteristics

Focus on the Y-chromosome (*Fig. 2*), there are many interesting loci information available for the anthropological and forensic fields. The basic use of Y chromosome is for paternity testing, and for some criminal casework (such as sexual assault) where the determination of male presence is necessary when the typing of *STR*-loci fail due to high quantities of female *DNA* and minor amounts of DNA male (*Figure 3*). In this case, the testing of specific *Y-STRs* permits to

determinate the male without having to perform a differential lysis or other methods.

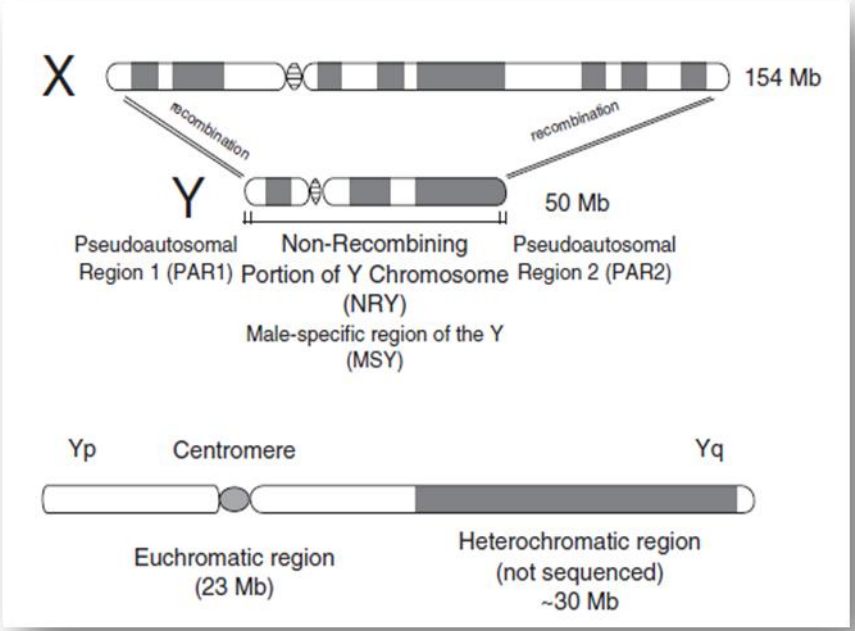


Figure 2. *General structure of X and Y chromosome (part at the top). The PAR1 and PAR2 of the Y chromosomet can be able to recombine with the X chromosome. NRY corresponds with the non-recombining part and MSY with male-specific region.* *(Butler 2005f)*

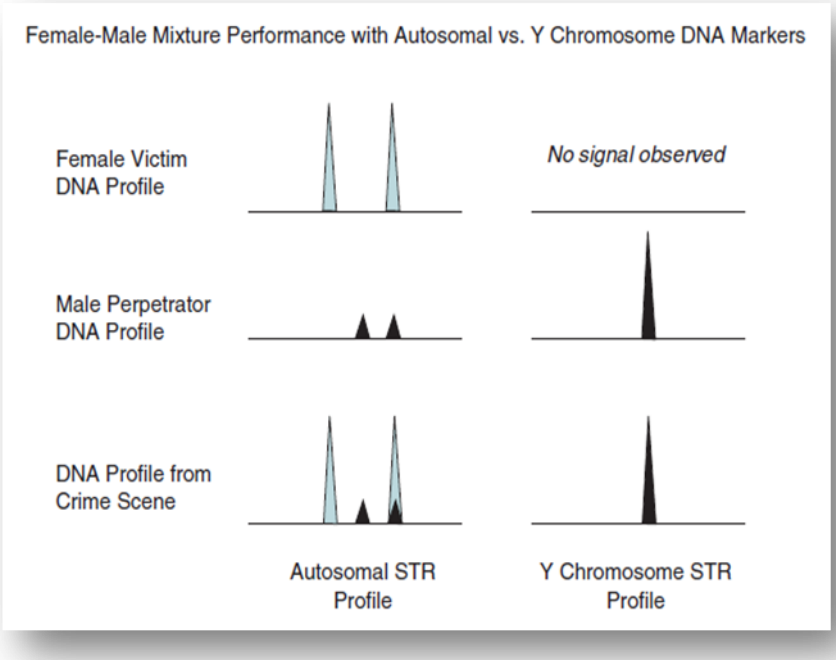


Figure 3. *Scheme which illustrates the types of autosomal or Y-STR profiles that might be observed in one criminal or sexual assault evidence where mixtures of high amounts of female DNA may mask the STR profile of the perpetrator. (Butler 2005f)*

The Y chromosome, together with the mitochondrial *DNA* are called "lineage-markers" because the polymorphism information, such as (Single Nucleotide Polymorphism) *SNP* and *STR,* is inherited from generation to generation without changing (except for mutational events). Paternal lineages can be studied with the Y chromosome as well as maternal lineages can be followed with the *DNA* of the mitochondria. (*Butler 2005f*). In Y chromosome studies there are two categories of *DNA* markers to analyze its diversity; bi-allelic and multi-allelic loci, with usually the first being SNPs and Alu insertions (*Y-SNPs*) and the second being STRs (*Y-STRs)*. To check the diffences between *SNPs* and *STRs* in detail, the **Table 1** can be consulted.

Characteristics	***STRs***	***SNPs***
Occurrence in human genome	1 in every 15 kb	~1 in every 1 kb
General informativeness	High	Low: Only 20-30% as informative as *STRs*
Marker type	Di-, tri-, tetra-, pentanucleotide repeat markers with **many** alleles	Mostly bi-allelic markers with **six** possibilities: A/G, C/T, A/T, C/G, T/G, A/C
Currently detection methods	Gel or Capillary electrophoresis	Sequence analysis: microchip hybridation
Major advantage to forensic application	Many alleles enabling higher success rates for detecting and deciphering mixtures	*PCR* products can be made small potentially enabling higher success rates with degraded DNA samples

Table 1. *Comparison of STRs and SNPs markers which shows some characteristics (Butler 2005e).*

Mutation rate is usually different between *SNPs* and *STRs*, which affect their practical usefulness. Bi-allelic markers are characterized on low mutation rates (~10^{8} per generation), that enable their use for anthropological studies dealing with long-range historical or evolutionary timeframes (*Butler 2005e*). Multi-allelic loci have a much higher mutation rate of approximately 10^{-3} per generation per mini-satellite loci (*Jobling and Tyler-Smith 1997*) and ~3.78 x 10^{-4} Y-*STRs* (*Ballantyne et al. 2010*) and this enables them to be useful for paternity testing and

other studies focused in generational or short-time historical timeframes. This differentiation enables for the distinction between haplogroups, which are evolutionary constructs defined by the combination of *Y-SNPs*, widely used in anthropological genetics; and haplotypes, which are the combinations of *Y-STRs*. (*Knijff 2000*). .

On the other hand, there are also some *loci-STRs* more interesting for forensic purposes. The size, structure or length might influence the mutation rates, and also, the mutations are more frequent in the male germ line *(Brinkmann et al. 1998; Carracedo and Sanchez-Diz 2005*). In context of forensic analysis, the short sizes are more desirable because small fragments could be amplified easier than larger fragments if the samples are degraded *(Alvarez-García et al. 1996*) as well as *SNPs*.

Y-Chromosome and Forensic Casework

The most used *Y-STRs* for forensic analysis are the trinucleotide repeats such as the system *DYS392* and the tetranucleotide repeats such as *DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391* and *DYS393*. These *STRs* are commonly called minimum *Y-STR* haplotype (*Kayser et al. 1997*) but, recently there have been many other *STRs* described (*Ballantyne et al. 2010*) and applied in new available commercial kits. In fact, only about 30 *Y-STRS* were available at the beginning of 2002 (*Butler 2005f*) and since that moment more than 400 have been introduced (*http://www.jogg.info*). The number of *Y-STR* studied is still growing and there are some references databases available online (More information, *Table Ap. 1 and Tab Ap.2* can be consulted in *Appendix of Introduction*).

It seems clear that is really necessary to promote analyses related with the Y chromosome in order to characterize male populations. New technologies and new kits are being developed for making easier the obtention of results in the forensic field. For this purpose, the *Y-STRs* have an important role due to their higher discrimination power and their possibilities for quick and reliable analysis.

MATERIALS AND METHODS

Extraction of human DNA samples

DNA samples were taken from the buccal cells of unrelated male volunteers from the North-Eastern German population of Mecklenburg-Vorpommern (N=175) and the Northern Spanish population of Asturias (N=178) (Global Tables can be consulted in *Appendix*), who allowed to use their samples for scientific purposes according to the German *Sendiagnostikgesetz* and the Spanish *Biomedical Spanish Research Law of July 14/2007* . For the sample collection, Dacron Swabs (*Deltalab*) were used for the Asturian population, while *Fab-Swabs (Colorprint)* were used for collection in Germany.

C) *Maiximiliam Dörrbecker cc* (3.06.2013) *http://goo.gl/Xm6GG*

B) *©Netmaps, 2013* (3.06.2013) *www.mapasmurales.es*

A) *(3.06.2013) http://goo.gl/Lzei8i*

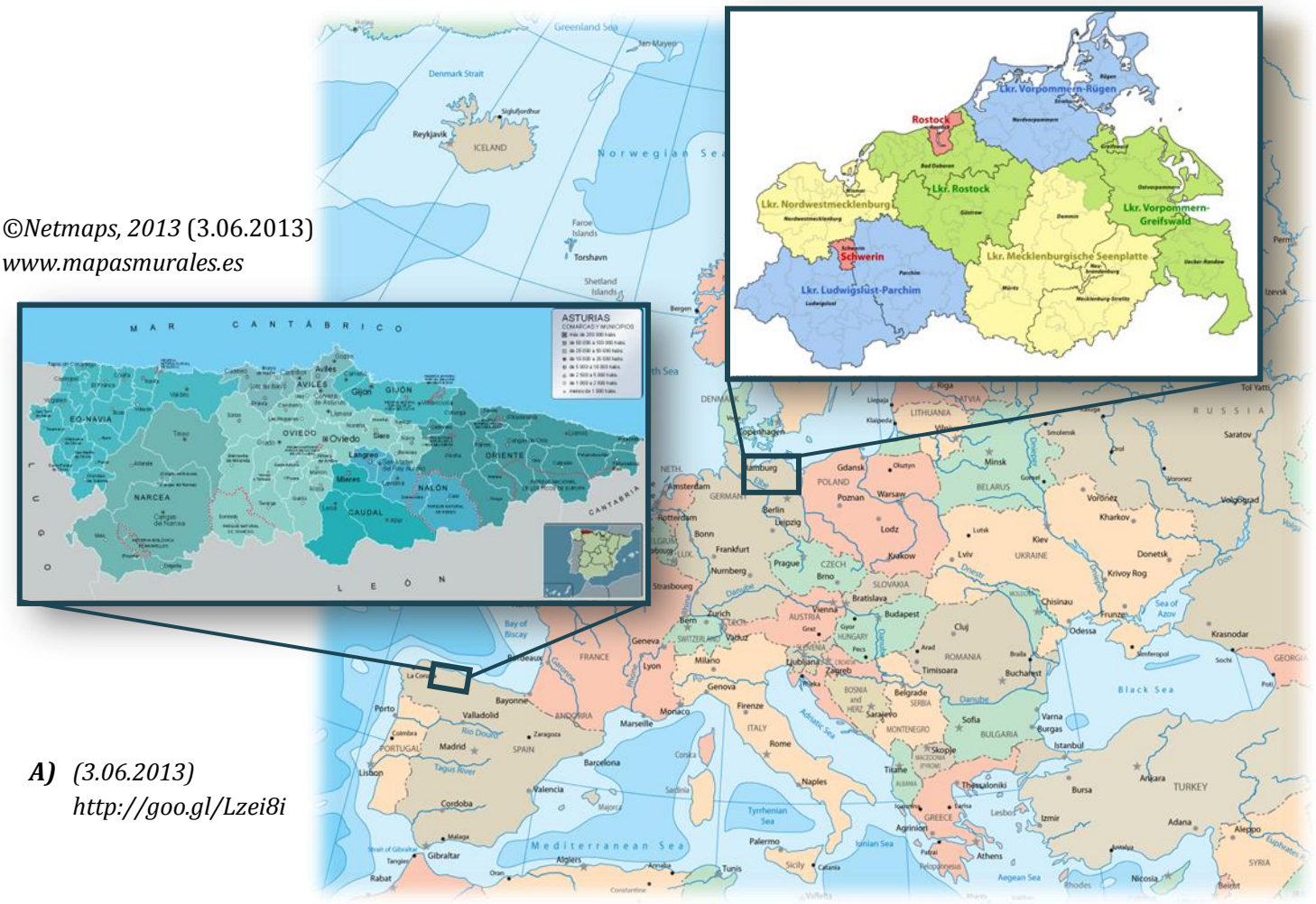


Figure 4. *Maps of the sampling populations.* ***A)*** *General map of European.* ***B)*** *Detailed map of Asturias province in Spain.* ***C)*** *Detailed map of Mecklenburgische-Vorpommern in Germany.*

The two studied populations belong to Western Europe (*Fig. 4A*). The German population belongs to Mecklenburg-Vorpommer (*MV*) (*Fig. 4C*), which is one of the 16 federal states in Germany. *MV* is the sixth largest German area, and the least densely populated with approximately 72,7 residents per km^2. The total surface is approximately 23.189 km^2 with 1,60 million inhabitants (*Bundesrat 2013*). *MV* is limited by the Baltic Sea in the north, and shares borders with the states of Schleswig-Holstein and Lower Saxony in the West, the state of Brandeburg in the South and the Republic of Poland in the East. The capital city is Schwerin and the major cities are Rostock, Neubrandenburg, Stralsund, Greifswald and Wismar.

Asturias (formally The Principality of Asturias) is one of the provinces of the North-west of Spain (*Fig. 4B*) which has an area of 10.603,57 km^2 with 1.081.487 inhabitants, and a population density of 102 residents per km^2 (*INE, 2011*). 50 % of its population lives in the three most populated cities, which are Gijon, Oviedo (the capital) and Aviles. It is the most mountainous region in Spain and one of the most mountainous of Atlantic Europe, and internally divided by deep river valleys (*Delgado-Viñas 2007*). Geographically, Asturias shares borders with the province of Lugo in the West, the autonomous community of Cantabria in the East, the province of León in the South and is limited by the Cantabrian Sea in the north.

It is known that the population of Asturias experienced a major boost after the modern implementation of coal mining facilities, especially after the 1950s (*Arbaiza-Vilallonga 1994*). To ensure a proper representation of the autochtonous Asturian population, sampling included an interview in which volunteers had to declare at least two generations of Asturian ancestry to be included in the study (paternal grandparents born in Asturias).

DNA of *MV* samples were extracted using the *DNA IQ™ Reference Samples Kit for Maxwell® 16* according to the manufacturer's protocol (*Promega Co),* The *DNA* of Asturias samples were extracted using *Chelex-100®* following the protocol of *Walsh et al* (1991).

Quantification of the DNA in the samples

In some cases, it was also necessary to quantify the concentration of human *DNA* in the sample. The methodology employed was based on a *TaqMan*® assay probe (The details and Figures of the *TaqMan*® assay antd their evaluation can be consulted in *Appendix of Material and Methods - Quantification*).

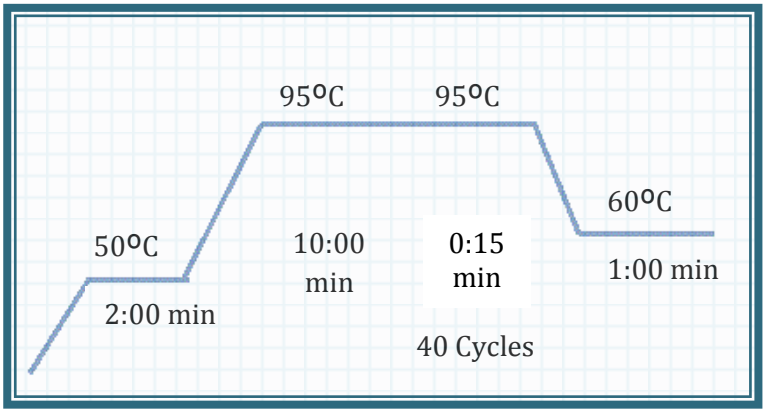


Figure 5. *Diagram representing the used program for the qRT-PCR.. Information extracted from Manufacture's User Manual Protocol. (Applied Biosystems 2006)*

The information connected with the quantitative Real Time PCR (*qRT-PCR)* cycle program can be consulted in the **Figure 5** and the chemical compounds concentrations used are in the **Table 2.**

Volume	Reagents
12,5 µl	*Quantifiler® PCR Reaction-Mix* (*Applied Biosystem*)
10,5 µl	*Quantifiler® Human Primer-mix* (*Applied Biosystem*)
2 µl	DNA (or *HPLC* in the negative control)

Table 2. *Summary PCR compounds used for the Quantifiler Human DNA Quantification Kit®*

The *qRT-PCR* machine used was a 7500 Real Time *PCR* System® (*Applied Biosystem*). For determining the results of the quantification the 7500 System *SDS* software (*Applied Biosystems 2006*) was used. This software assesses the relative quantity of a target nucleic acid sequence in a sample. Changes in fluorescent signal (*Rn*) are analyzed cycle-per-cycle, which are a measurement of the amount of *PCR* product amplified.

To evaluate the performance of the *qRT-PCR* assay and to include an objective measure of potential *PCR* inhibition, we introduced an *IPC* that is amplified simultaneously in the same assay with the samples. *IPC* brings an amplification result in another dye layer and can be evaluated independently from the signal of the unknown sample (*Köchl et al. 2005*).

Amplification and determination of Y-STR

The polymerase chain reaction or *PCR* is a biochemical technology to amplify a single locus or several loci in order to obtain high copy number of a sequence. This method consists of cycles where the *DNA* strands are separated for the activity of the Polymerase. *PCR* can be modified to perform a wide array of genetics studies.

For *STR* characterization of *VM* and Asturian populations of the Y-Chromosome *PowerPlex® Y23 System* (*Promega Co.*) was used. It is a polymerase chain reaction-based amplification kit which can target 23 *Y-STR* loci: *DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS481, DYS533, DYS549, DYS570, DYS576, DYS365, DYS643* and *Y-GATA-H4*. (See more details in Table 3). This large battery of loci could be determinate because of the primer sequences and configuration which are be able to type simultaneously and compatible with the same common technologies employed in forensic laboratories (*Davis et al. 2013*). First of all, *DNA-Exitus Plus® AppliChem* is used on every working-surface to maintain a clean area and to avoid contamination. To sterilize the working-equipment, first of all, *UV DNA/RNA* Cleaner Hood (*Kisker-Biotech*) was used for 20 minutes. Then, the *DNA* samples were amplified using reagents contained in the *PowerPlex® Y23 System Kit* (*Promega Co.*).

STR Locus	Label	Repeat Sequence 5'→3'
DYS576	Fluorescein	AAAG
DYS389I/II	Fluorescein	(TCTG)(TCTA)
DYS448	Fluorescein	AGAGAT
DYS19	Fluorescein	TAGA
DYS391	JOE	TCTA
DYS481	JOE	CTT

DYS549	JOE	GATA
DYS533	JOE	ATCT
DYS438	JOE	TTTTC
DYD437	JOE	TCTA
DYS570	TMR-ET	TTTC
DYS635	TMR-ET	TSTA compound
DYS390	TMR-ET	(TCTA)(TCTG)
DYS439	TMR-ET	AGAT
DYS392	TMR-ET	TAT
DYS643	TMR-ET	CTTTT
DYS393	CXR-ET	AGAT
DYS458	CXR-ET	GAAA

Table 3. *Locus of the different system placed in the Y-Chromosome and their specific tandem sequence of each other. The labeled color column is representing with their particular fluorescence in the results. (Promega Corporation 2012)*

Primer Pair Mix, included as reagents of the kit, were used. The *2800M Positive Control DNA* tube was standardized to a final 1:20 dilution. The manufacturer's protocol was modified with a final volume in every tube of 12,5µl instead of 25µl. The resume of the *PCR* chemical compounds details are in the **Table 4**.

Volume	**Controls**
4 µl	*PowerPlex® 2800M -Positive Control-* (*Applied Biosystem*)
4,75 µl	*HPLC* Water for Positive Control
8,75 µl	*HPLC* Water -Negative Control-
Volume	**Samples**
2,5 µl	*PowerPlex® Y23 Master Mix* (*Applied Biosystem*)
1,25 µl	*PowerPlex® Primer Pair Mix* (*Applied Biosystem*)
6,75 µl	*HPLC* Water
2 µl	DNA

Table 4. *Final concentrations used for every sample in the PowerPlex® Y23 PCR method. Master-Mix and Primer-Mix were added into the samples including control with a final concentration of 3,75 µl.*

Furthermore, during *PCR* a positive and negative control was included for every reaction. The samples were run in a thermal cycler *Analytikcjena FlexCycler v. 2007* using a *PCR* Protocol represented in the **Figure 6**. The complete *PCR* program lasted for approximately 1 hour.

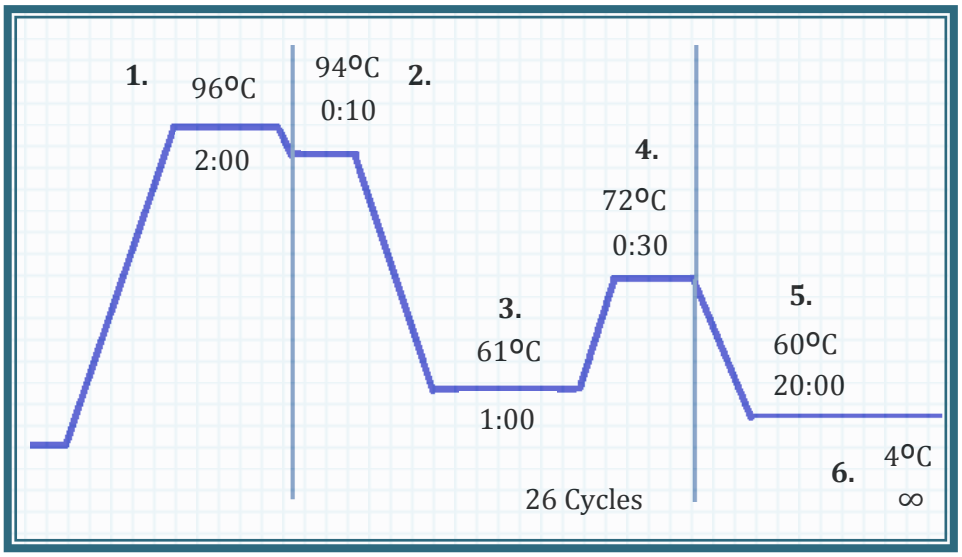


Figure 6. *Complete Thermocycler program of PCR for PowerPlex® Y23 . The time in minutes are represented under the temperature. The numbers represent the different stages:* ***1.*** *Initial denaturation.* ***2.*** *Denaturation.* ***3.*** *Annealing.* ***4.*** *Elongation.* ***5.*** *Final Elongation and* ***6.*** *Soak. Numbers* ***2. 3.*** *and* ***4.*** *correspond with the 26 cycles of the PCR. Information extracted from the Manufacture's User Manual Protocol (Promega Corporation 2012)*

Analysis of the amplification product with Capillary Electrophoresis

Capillary electrophoresis (*CE*) supposes a modern development in the classic field of electrophoresis. One of the main characteristics of this method is the possibility to automate the injection, separation and detection steps. Moreover, only very low volumes are required for each of the samples, which allow overcoming of the typical limitations of forensic specimens. In most cases, the only drawback concerns the difficulty to process a high number of samples when a single capillary is used (*Butler 2005b*). (More details of Capillar electrophoresis can be consulted in Appendix of Material and Methods-Capillar electrophoresis)

Prior to injecting of each sample, the capillary is filled with a new polymer solution into the capillary. The detection of the samples is automated and is measured the time between the injection and the detection with the laser that is situated next to the end of the capillary. In fact, the laser is shined over the window detection of the capillary where *DNA* fragments passed on it. The fluorescent emission signals from the dyes attached to the *DNA* molecules are detected and quantified because the more fluorescent is emitted; the more concentration of DNA there is in the sample.

The analysis of the amplified *PCR* products was performed on an *ABI PRISM® 310* GeneticAnalyzer (*Applied Biosystem*). A Master Mix was made with formamide and CC5 internal Lane Standard 500. The final concentration for each sample is represented in the **Table 5**.

Volume	Master-Mix Tube
12 µl	Formamide *(Applied Biosistem)*
1 µl	CC5 Internal Lane Standard 500 (*Promega Co.*)
Volume	**Probe Tube**
12 µl	Master-Mix
0,5 µl	Allelic ladder (*Promega Corporation 2012*) or amplified sample

Table 5. *Volume for capillary electrophoresis. Final concentration of the chemical compounds that were added into the samples. Information extracted from the Technical Manual Protocol (Promega Corporation 2012)*

Afterwards, data and sample's names are introduced into the sample sheet on the computer. When the Sample Sheet is completed, an injection list is created and all the information is imported into it. There are several data that are introduced into the injection list; the most important of them are summarized in the **Table 6**.

In addition, for introducing the data in the sample sheet the *Data Collection 3.1.0.* (*Promega Corporation 2012*) software was used and afterwards the *Gene Mapper® ID 3.2 (Promega Corporation 2012*) software for analysing the results of the capillary electrophoresis.

Parameter	Electrophoresis Conditions
Injection Time	5 seconds
Injection Voltage	15 kV
Module	GS STR POP4 G5 (ABI PRISM) (*Applied Biosystem*)
Run temperature	60ºC
Run time	27 minutes

Table 6. *Different parameters for the injection list.. Information extracted from the Technical Manual Protocol (Promega Corporation 2012) (Butler 2005d; Promega Corporation 2012)*

The software for statistics methods

In order to analyse the data for each population, several specific software were needed. For general STR analyses, the suite *MicroSatellite Toolkit*® (*Park 2001*) was used. The statistics computed with this software were the Allelic Frequency and its associated standard error, and the Power of Discrimination statistic. Bar-charts were constructed for result representation.

The *allelic frequency* statistic is a measurement of the occurrence of one allele of a given locus in a given population used for comparing populations in genetics and forensic studies *(Hidding and Schmitt 2000; Rodig et al. 2007; Zarrabeitia et al. 2003)*. It can be calculated with the formula:

$$f_{ij} = \frac{N_{ij}}{n}$$

f_{ij}= Allelic frequency

n= Total sampling

N_{ji}= Frequency of the allele

i=Allele

j= System-STR

Its *standard error* is related to the population size, and its calculated with the formula:

$$SE=\sqrt{\frac{f_{ij}\left(1-f_{ij}\right)}{N}}$$

SE= Standard error

f_{ij}= Allelic Frecueny

N= Total sampling

Finally, the *Power of Discrimination* (*PD*) is the microsatellite version of the classic "Unbiased Gene Diversity" statistic (*Nei 1973*). It is defined as the probability that, at a single locus, any two alleles, chosen at random from the population, are different to each other. It is usually computed at a per-locus basis, but it can also be averaged over an entire population, giving an estimate of its genetic variability. This measure is considered unbiased only if no relatives or inbred individuals are included in the population, as these individuals would share alleles by common ancestry, and thus the number of independent observations used to estimate allelic frequencies would be less than the total sample size. In this case, the formula is:

$$PD=1-\sum_{i=1}^{n_j} f_{ij}^2$$

PD= Power of Discrimination

f_{ij}= Allelic Frecueny

n= Total sampling

nj= number of the j-allelic system

RESULTS

Bar charts were used for representing the complete 23 *Y-STR* set of *MV*, Asturias and a combined dataset for *allelic frequency*. In all graphics the *standard error* of the frequency estimate was also represented. Every locus was analysed independently to resolve its particular characteristics. On the other hand, the *PD* was computed for each locus and each population.

The sample *Abst181-10* of the *MV* population was excluded, and so were the samples *ID356*, *ID505*, *ID590* and *ID633* of the Asturias population, due to the presence of double peaks typed in some loci. Double peaks can be caused by cross-contamination. Additionally, every allele with a non-standard size value (i.e. decimals) was also excluded from the analysis.

- ***Locus DYS19***

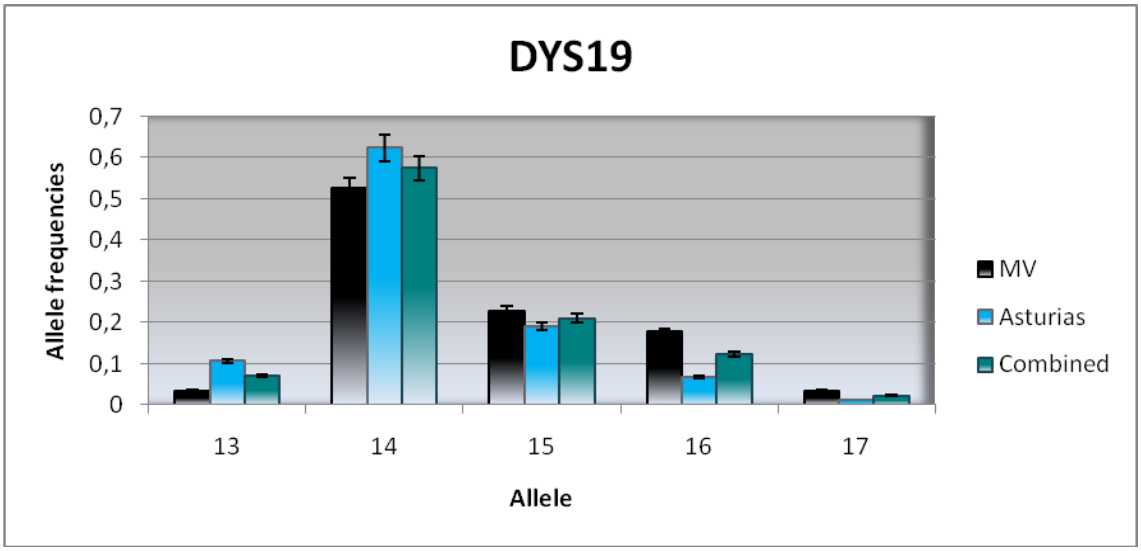


Figure 7. *Bar chart from the locus-system DYS19 representing the number of the alleles and their frequencies in the two populations and both combined.*

The locus *DYS19* has a diversity of alleles that ranges from 13 to 17 (*Fig. 7*). The allele 17 and 13 are not so common in the populations although the allele 13 is more frequent in the Asturian population (0.1067 ±0.023 and 0.0343±0.014). For this locus, the highest frequency is in the allele 14 in both populations although is

quite higher in Asturias (0.6236 ±0.036) (*Table 7.*) The alleles 15 and 16 have low frequencies but the *MV* population is higher in both.

DYS19	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ji}	***SE***
13	6	0.0343	±0.014	19	0.1067	±0.023	25	0.0705	±0.014
14	92	0.5257	±0.038	111	0.6236	±0.036	203	0.5747	±0.026
15	40	0.2286	±0.032	34	0.191	±0.029	74	0.2098	±0.022
16	31	0.1771	±0.029	12	0.0674	±0.019	43	0.1223	±0.017

Table 7. *Summary of the main parameters calculated for the MV and Asturias population and Combined in DYS19.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus DYS385a***

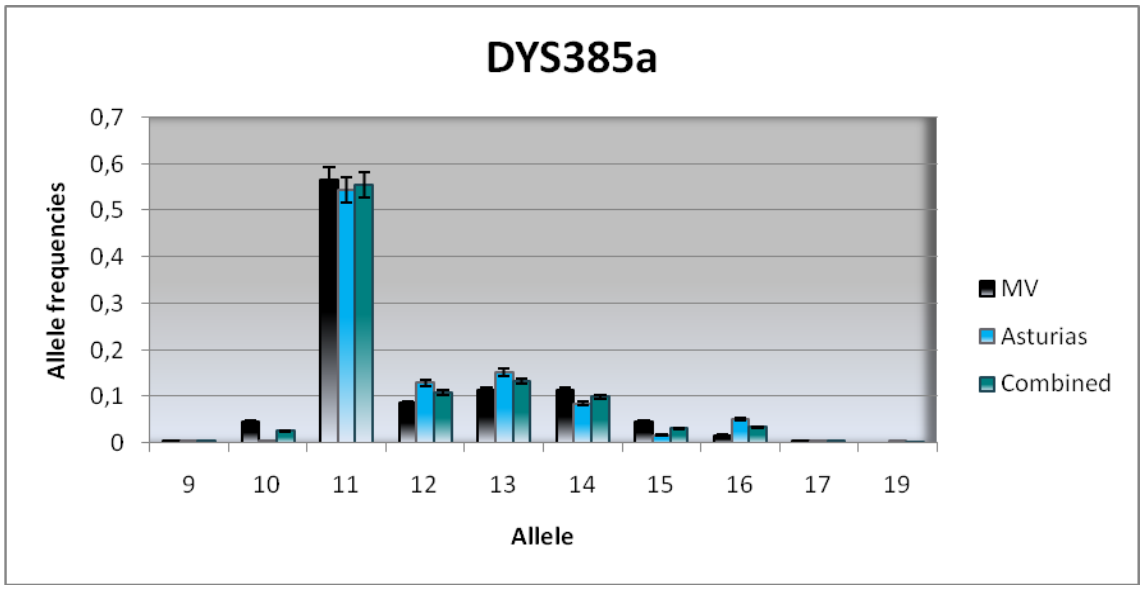


Figure 8. *Bar chart from the locus-system DYS389a representing the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS385a* has a diversity of alleles that ranges from 9 to 19 (*Fig. 8*). The alleles 9, 17 and 19 have a very low frequency in the population with one individual in some of them or each other. The most common allele corresponds with the allele 11 for both populations (with a frequency of 0.5657±0,016 for MV

and 0.5449±0,037 for Asturias) (*Table 8*). Generally the rest of alleles have low frequencies for both.

DYS385a	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***
9	1	0,0057	±0,006	1	0,0056	±0,006	2	0,0057	±0,004
10	8	0,0457	±0,016	1	0,0056	±0,006	9	0,0257	±0,008
11	99	0,5657	±0,037	97	0,5449	±0,037	196	0,5553	±0,026
12	15	0,0857	±0,021	23	0,1292	±0,025	38	0,1075	±0,016
13	20	0,1143	±0,024	27	0,1517	±0,027	47	0,133	±0,018
14	20	0,1143	±0,024	15	0,0843	±0,021	35	0,0993	±0,016
15	8	0,0457	±0,016	3	0,0169	±0,010	11	0,0313	±0,009
16	3	0,0171	±0,010	9	0,0506	±0,016	12	0,0339	±0,010
17	1	0,0057	±0,006	1	0,0056	±0,006	2	0,0057	±0,004
19	0	0	±0	1	0,0056	±0,006	1	0,0028	±0,003

Table 8. *Summary of the main parameters calculated for the MV and Asturias population and combined in DYS385a.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus DYS385b***

The locus *DYS385b* has a diversity of alleles that ranges from 11 to 21 (*Fig. 9*). The allele 14 occurs with the higher frequency in both populations (*Table 9*). *MV* has 99 individuals with 0.5657±0,037 and Asturias 98 and a frequency of 0.5632±0,037.

The alleles 11, 19, 20 and 21 have in general very low frequencies. For the *MV* population, the second highest allele is 15 with 0.2±0,030 and 35 individuals where the distance from the Asturian population with only 19 individuals and 0.1092±0,023 is significant.

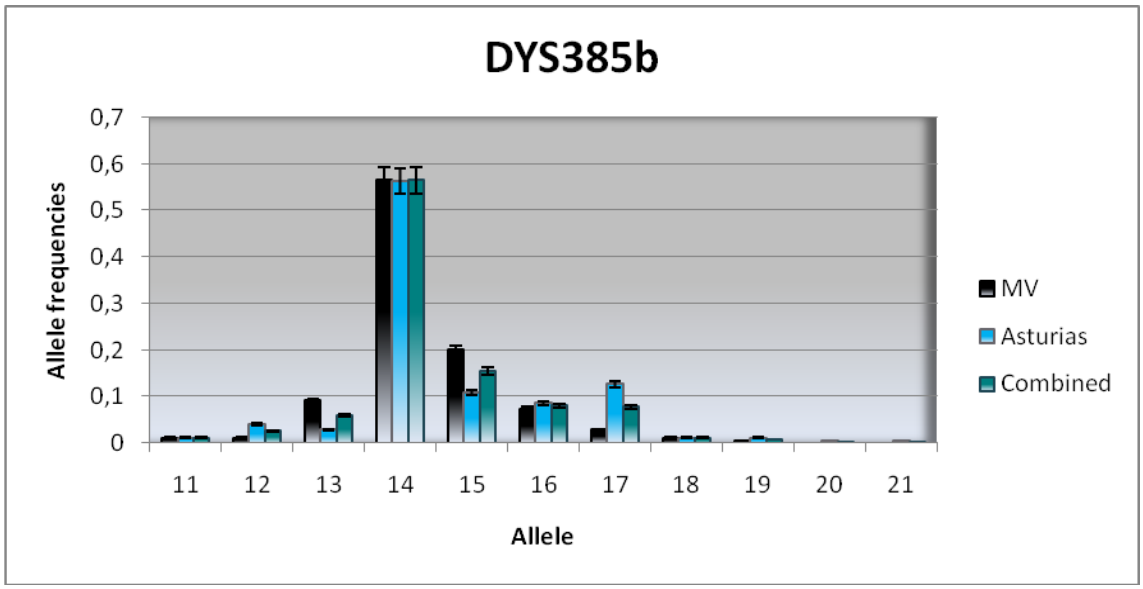


Figure 9. *Bar chart from the locus-system DYS389b representing the number of the alleles and their frequencies in the two populations combined.*

DYS385b	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***
11	2	0,0114	±0,008	2	0,0115	±0,008	4	0,0115	±0,006
12	2	0,0114	±0,008	7	0,0402	±0,015	9	0,0258	±0,008
13	16	0,0914	±0,022	5	0,0287	±0,013	21	0,0601	±0,013
14	99	0,5657	±0,037	98	0,5632	±0,037	197	0,5645	±0,026
15	35	0,2	±0,030	19	0,1092	±0,023	54	0,1546	±0,019
16	13	0,0743	±0,020	15	0,0862	±0,021	28	0,0802	±0,014
17	5	0,0286	±0,013	22	0,1264	±0,025	27	0,0775	±0,014
18	2	0,0114	±0,008	2	0,0115	±0,008	4	0,0115	±0,006
19	1	0,0057	±0,006	2	0,0115	±0,008	3	0,0086	±0,005
20	0	0	±0,000	1	0,0057	±0,006	1	0,0029	±0,003
21	0	0	±0,000	1	0,0057	±0,006	1	0,0029	±0,003

Table 9. *Summary of the main parameters calculated for the MV and Asturias population and combined in DYS385b.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

❖ ***Locus DYS389I***

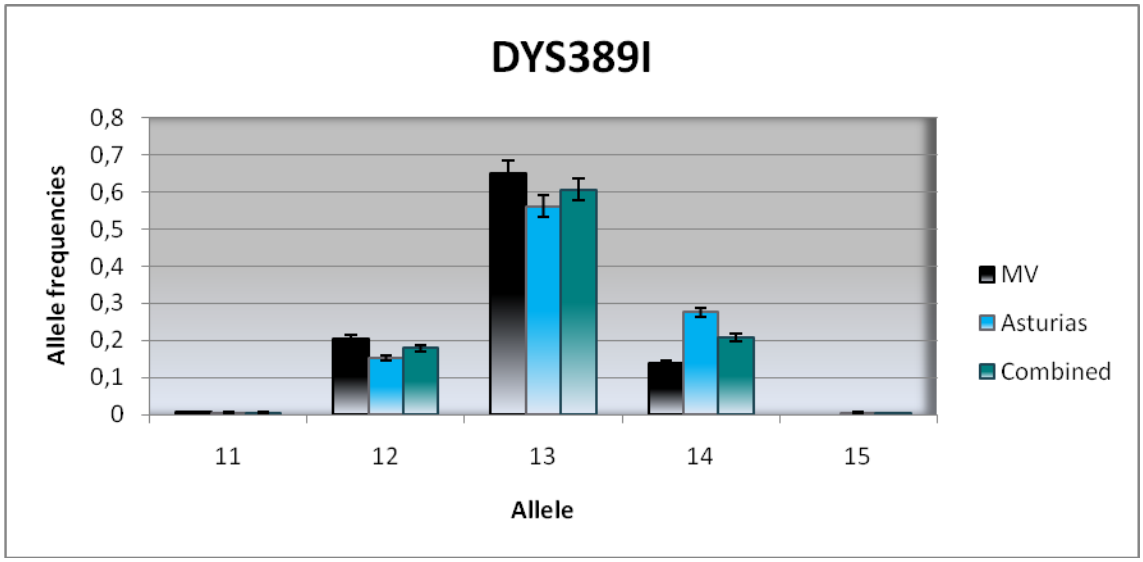


Figure 10. *Bar chart from the locus-system DYS389I representing the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS389I* has a diversity of alleles that ranges from 11 to 15 (*Fig. 10*) although the allele 15 is not presented in the *MV* population and there is only one individual of Asturias. The highest frequency corresponds with the allele 13 for the two populations (114 and 100 individuals present this allele in both populations, *MV* and Asturias, respectively) (*Table 10.*). The other alleles (11, 12 and 14) the populations have similar allelic frequencies.

DYS389I	***MV***			***Asturias***			***Comb.***		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	F_{ij}	***SE***
11	1	0.0057	±0.006	1	0.0056	±0.006	2	0.0057	±0.004
12	36	0.2057	±0.031	27	0.1517	±0.027	63	0.1787	±0.020
13	114	0.6514	±0.036	100	0.5618	±0.037	214	0.6066	±0.026
14	24	0.1371	±0.026	49	0.2753	±0.033	73	0.2062	±0.022
15	0	0	±0.000	1	0.0056	±0.006	1	0.0028	±0.003

Table 10. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS389I.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

❖ ***Locus DYS389II***

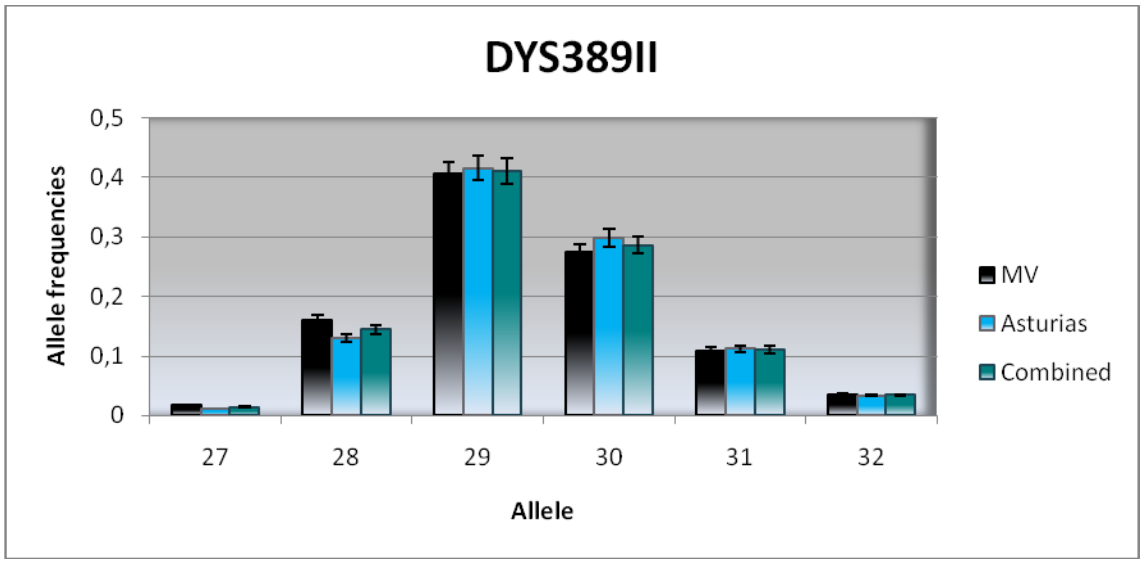


Figure 11. *Bar chart from the locus-system DYS389II representing the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS389II* has a diversity of alleles from 27 to 32 (*Fig. 14*). The allele 27 has a very low frequency. The allelic frequency of the allele 28 and especially 31 and 32 alleles are very similar between the populations (*Table 13*). The highest frequency is in the allele 29 (with 0.4057±0.037 and 0.4157±0.037 for *MV* and Asturias respectively). The allele 30 is also common in the Asturias population.

DYS389II	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ji}	***SE***
27	3	0.0171	±0.010	2	0.0112	±0.008	5	0.0142	±0.006
28	28	0.16	±0.028	23	0.1292	±0.025	51	0.1446	±0.019
29	71	0.4057	±0.037	74	0.4157	±0.037	145	0.4107	±0.026
30	48	0.2743	±0.034	53	0.2978	±0.034	101	0.286	±0.024
31	19	0.1086	±0.024	20	0.1124	±0.024	39	0.1105	±0.017
32	6	0.0343	±0.014	6	0.0337	±0.014	12	0.034	±0.010

Table 11. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS389II.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

❖ ***Locus DYS390***

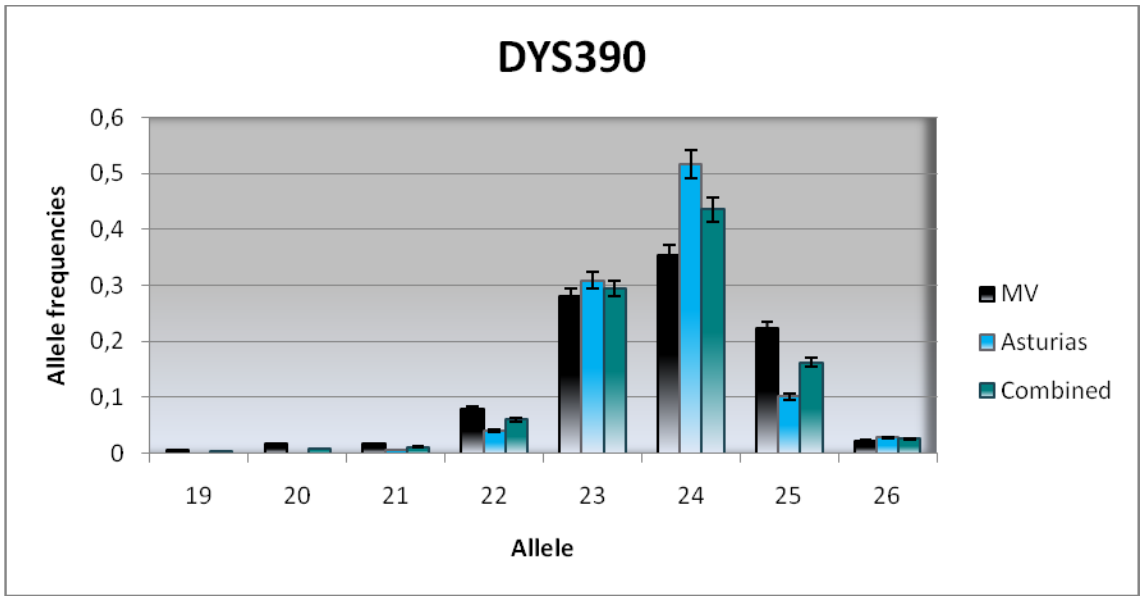


Figure 12. *Bar chart from the locus-system DYS390 representing the number of the alleles and their frequencies in the two population combined.*

The locus *DYS390* has a high diversity in the number of alleles (from 19 to 26) (*Fig. 12*). The allele 19 is not present in the Asturian population and has a low frequency in *MV* (0.0057±0.006) (*Table 12*). The alleles 20, 21 and 22 are also very low but slightly more common in comparison to the Asturian population.

DYS390	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ji}	***SE***
19	1	0.0057	±0.006	0	0	±0.000	1	0.0029	±0.003
20	3	0.0171	±0.010	0	0	±0.000	3	0.0086	±0.005
21	3	0.0171	±0.010	1	0.0056	±0.006	4	0.0114	±0.006
22	14	0.08	±0.021	7	0.0393	±0.015	21	0.0597	±0.013
23	49	0.28	±0.034	55	0.309	±0.035	104	0.2945	±0.024
24	62	0.3543	±0.036	92	0.5169	±0.037	154	0.4356	±0.026
25	39	0.2229	±0.031	18	0.1011	±0.023	57	0.162	±0.020
26	4	0.0229	±0.011	5	0.0281	±0.012	9	0.0255	±0.008

Table 12. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS390.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

The allele 26 has also a low frequency but quite more common for the Asturian population in this case. The highest frequency corresponds with 0.5169±0.037 for Asturias and 0.3543±0.036 for *MV* in the allele 24. The frequency of the allele 23 is higher in Asturias too, but the 25 is more common for *MV* population (with 0.229±0.031 against 0.1011±0.023).

- ***Locus DYS391***

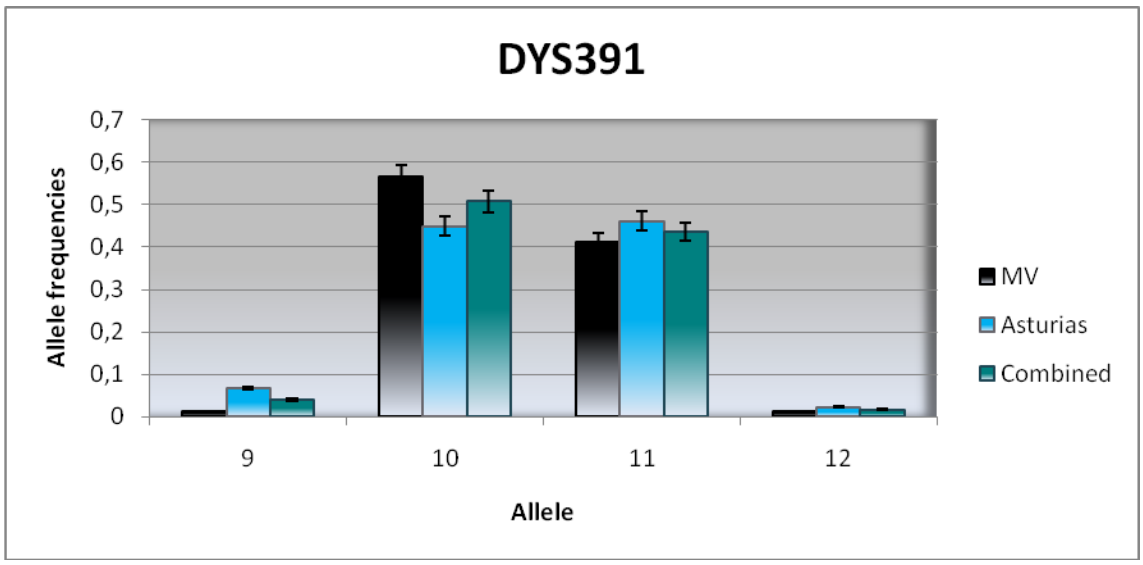


Figure 13. *Bar chart from the locus-system DYS391 reprsenting the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS391* has a diversity of alleles that ranges from 9 to 12 (*Fig. 13*). This locus is one of the least diversity systems. In fact, the alleles 9 and 12 have also a low frequency in the populations (0.0114±0.008 in *MV* for both alleles and 0.0674±0.019 and 0.0225±0.011 for Asturias). The allele 10 and 11 are the highest in frequency, for *MV* the allele 10 is more common and for Asturias is the allele 11 without a significant difference (*Table 13*).

DYS391	*MV*			*Asturias*			*Comb.*		
Allele	*Indiv*	f_{ij}	*SE*	*Indiv*	f_{ij}	*SE*	*Indiv*	f_{ji}	*SE*
9	2	0.0114	±0.008	12	0.0674	±0.019	14	0.0394	±0.010
10	99	0.5657	±0.037	80	0.4494	±0.037	179	0.5076	±0.027
11	72	0.4114	±0.037	82	0.4607	±0.037	154	0.4361	±0.026
12	2	0.0114	±0.008	4	0.0225	±0.011	6	0.017	±0.007

Table15. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS391.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus DYS392***

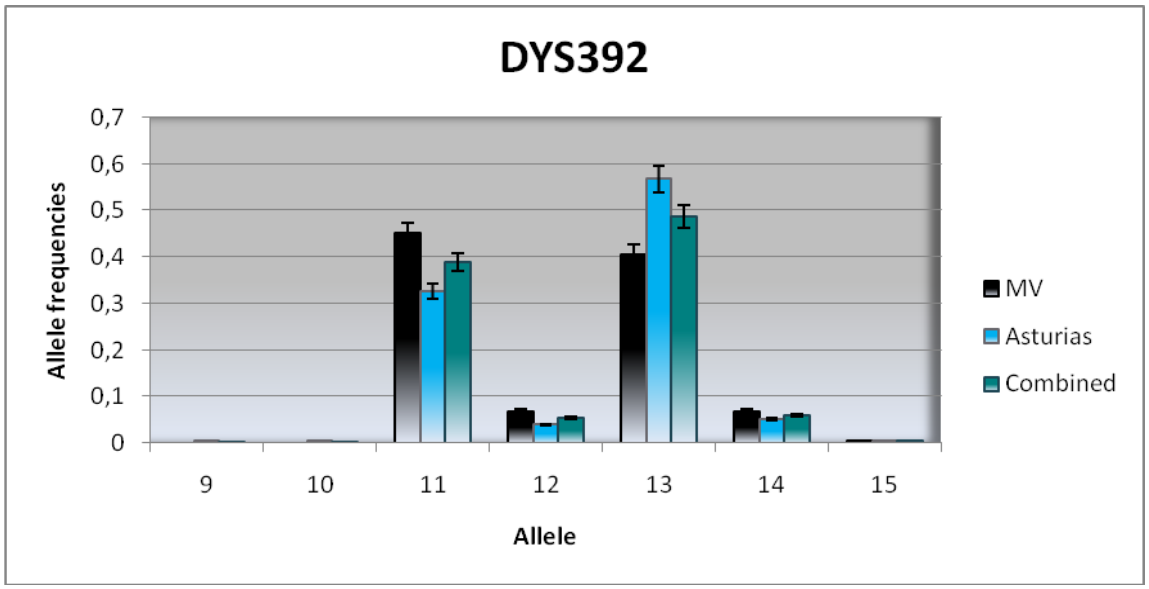


Figure 14. *Bar chart from the locus-system DYS392 representing the number of the alleles and their frequencies in the two populations combined.*

DYS392	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***
9	0	0	±0,000	1	0.0056	±0,006	1	0,0028	±0,003
10	0	0	±0,000	1	0.0056	±0,018	1	0,0028	±0,003
11	79	0,4514	±0,038	58	0.3258	±0,035	97	0,3886	±0,026
12	12	0,0686	±0,019	7	0.0393	±0,037	19	0,0539	±0,012
13	71	0,4057	±0,037	101	0.5674	±0,024	172	0,4866	±0,027
14	12	0,0686	±0,019	9	0.0506	±0,006	21	0,0596	±0,013
15	1	0,0057	±0,006	1	0.0056	±0,000	2	0,0057	±0,004

Table 14. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS392.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

The locus *DYS392* has a diversity of alleles that ranges from 9 to 15 (*Fig14*). For Asturias the highest frequency (0.5674±0,024) that corresponds with the allele 13 as most common (*Table 14*) while for *MV* population the most common is 11 (0.4514±0,038). The alleles 9, 10 and 15 are not common for both populations. On the other hand, the alleles 12 and 14 have also low frequency but higher in *MV* than Asturian population (for example 0.0686±0,019 in *MV* than 0.016±0,006 of Asturias).

- ***Locus DYS393***

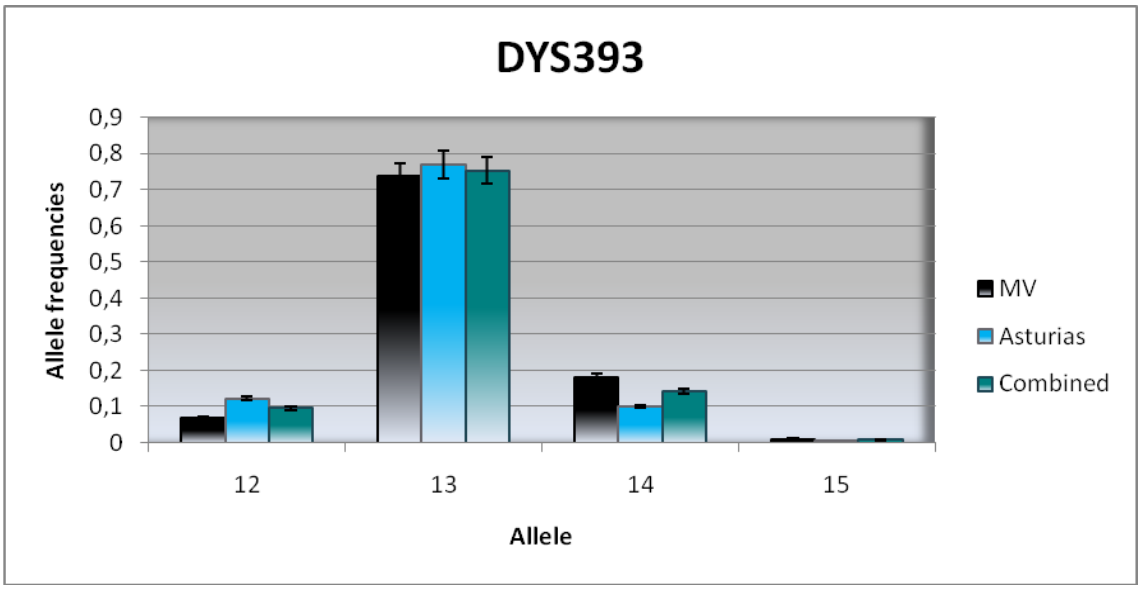


Figure 15. *Bar chart from the locus-system DYS393 representing the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS393* has a diversity of alleles that ranges from 12 to 15 (*Fig. 15*). This locus has a low diversity of alleles. The highest frequency in both populations corresponds with the allele 13 (higher frequency in Asturias with 0.7697±0,032) (*Table 15*). The allele 15 is not common in the two populations and the allele 12 has a higher frequency in Asturias (0.1236±0,025) and in the allele 14 the highest frequency is in *MV* (0.1829±0,029)

DYS393	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***
12	12	0,0686	±0,019	22	0,1236	±0,025	34	0,0961	±0,016
13	129	0,7371	±0,033	137	0,7697	±0,032	266	0,7534	±0,023
14	32	0,1829	±0,029	18	0,1011	±0,023	50	0,142	±0,019
15	2	0,0114	±0,008	1	0,0056	±0,006	3	0,0085	±0,005

Table 15. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS393.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus DYS437***

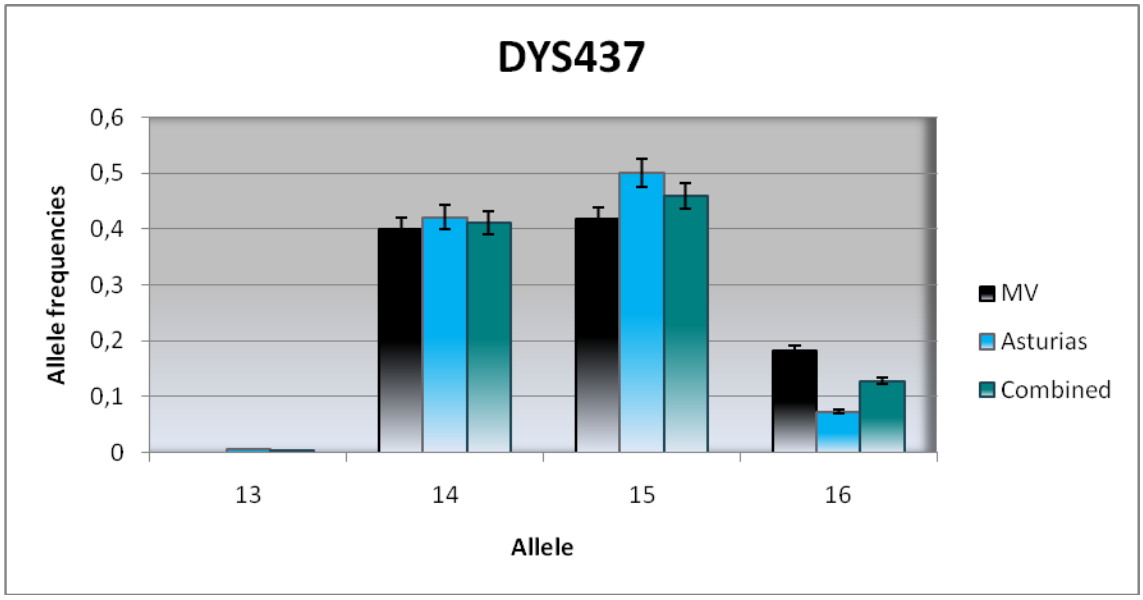


Figure 16. *Bar chart from the locus-system DYS437 representing the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS437* has alleles from 13 to 16 (*Fig. 16*). The allele 13 is not present in the *MV* population and with only one individual in Asturias (*Table 16*). The most common allele is the 15 and more Asturian than *MV* population. The allele 14 is also very frequent in *MV* (with 0.4±0.037 and 0.4171±0.037 correspond with the alleles 14 and 15 respectively). The allele 14 is also quite frequent in Asturias although with lower frequency (0.037±0.020). The allele 16 has the lowest frequency for both populations.

DYS437	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ji}	***SE***
13	0	0	±0.000	1	0.0056	±0.006	1	0.0028	±0.003
14	70	0.4	±0.037	75	0.4213	±0.037	145	0.4107	±0.026
15	73	0.4171	±0.037	89	0.5	±0.037	162	0.4586	±0.027
16	32	0.1829	±0.029	13	0.073	±0.020	45	0.1279	±0.018

Table 16. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS437.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus DYS438***

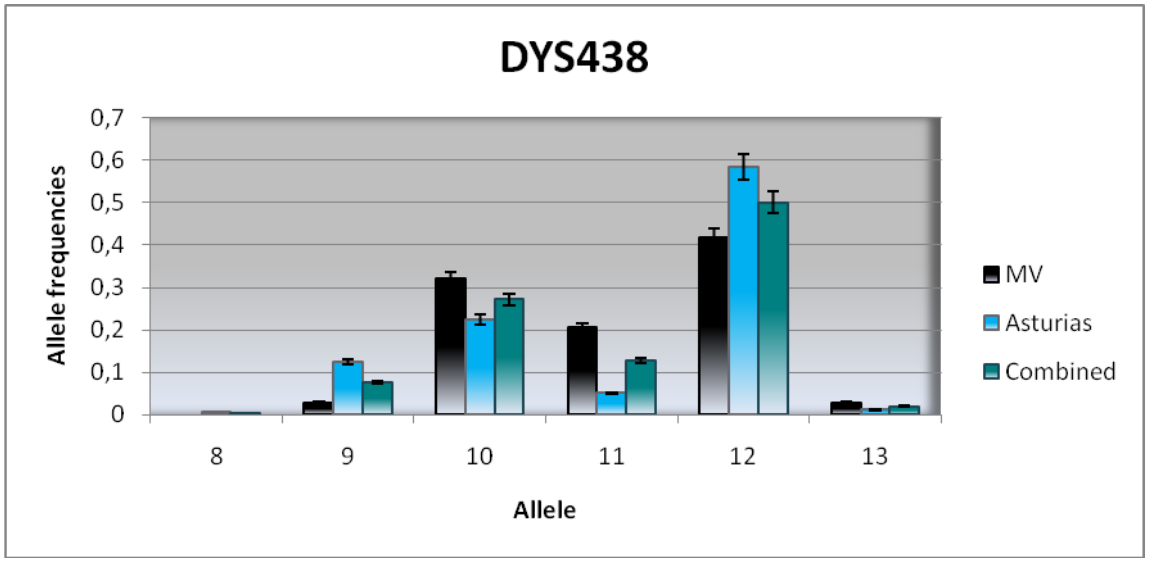


Figure 17. *Bar chart from the locus-system DYS438 representing the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS438* presents the number of alleles from 8 to 13 (*Fig. 17*). Only one individual presents the allele 8 in the Asturian population (*Table 17*) and the allele number 13 has a low frequency in both populations. The most common is the allele number 12. Although for *MV,* the allele 10 has also quite similar frequency (the highest is 12 with 0.4171±0.037 of frequency and the second one is 0.32±0.035 with 73 and 56 individuals respectively for *MV*).

DYS438	MV			Asturias			Comb.		
Allele	Indiv	f_{ij}	SE	Indiv	f_{ij}	SE	Indiv	f_{ji}	SE
8	0	0	±0.000	1	0.0056	±0.006	1	0.0028	±0.003
9	5	0.0286	±0.013	22	0.1236	±0.025	27	0.0761	±0.014
10	56	0.32	±0.035	40	0.2247	±0.031	96	0.2724	±0.024
11	36	0.2057	±0.031	9	0.0506	±0.016	45	0.1281	±0.018
12	73	0.4171	±0.037	104	0.5843	±0.037	177	0.5007	±0.027
13	5	0.0286	±0.013	2	0.0112	±0.008	7	0.0199	±0.007

Table 17. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS438.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus DYS439***

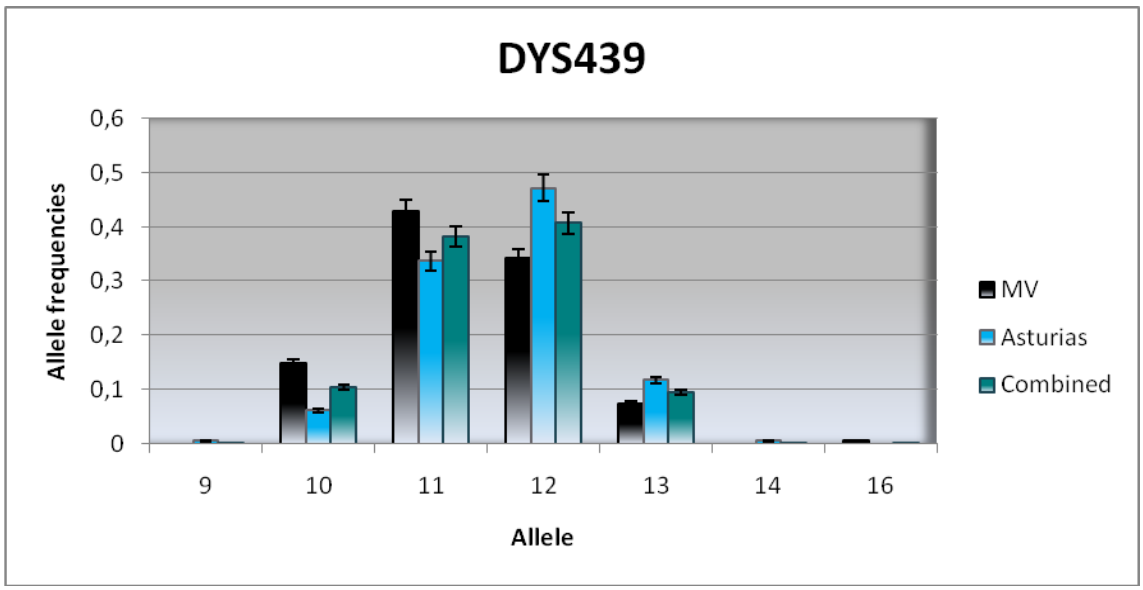


Figure 18. *Bar chart from the locus-system DYS439 representing the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS438* presents the number of alleles from 9 to 16 (*Fig. 18*). In this locus there is not frequency in the population for the allele number 15, and the frequencies of the alleles 14, and 16 are very low for both populations (*Table 18*). The highest frequency corresponds with the allele 12 for the Asturian population

(0.4719±0,037) and for MV the highest frequency is 0.4286±0,027 with the number of allele 11.

DYS439	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	***f_{ij}***	***SE***	***Indiv***	***f_{ij}***	***SE***	***Indiv***	***f_{ij}***	***SE***
9	0	0	±0,000	1	0,0056	±0,006	1	0,0028	±0,003
10	26	0,1486	±0,027	11	0,0618	±0,018	37	0,1052	±0,016
11	75	0,4286	±0,037	60	0,3371	±0,035	141	0,3828	±0,026
12	60	0,3429	±0,036	84	0,4719	±0,037	144	0,4074	±0,026
13	13	0,0743	±0,020	21	0,118	±0,024	34	0,0961	±0,016
14	0	0	±0,000	1	0,0056	±0,006	1	0,0028	±0,003
16	1	0,0057	±0,006	0	0	±0,000	1	0,0029	±0,003

Table 18. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS439.* ***Indiv*** *is the number of individuals per allele in the population.* ***f_{ij}*** *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus DYS448***

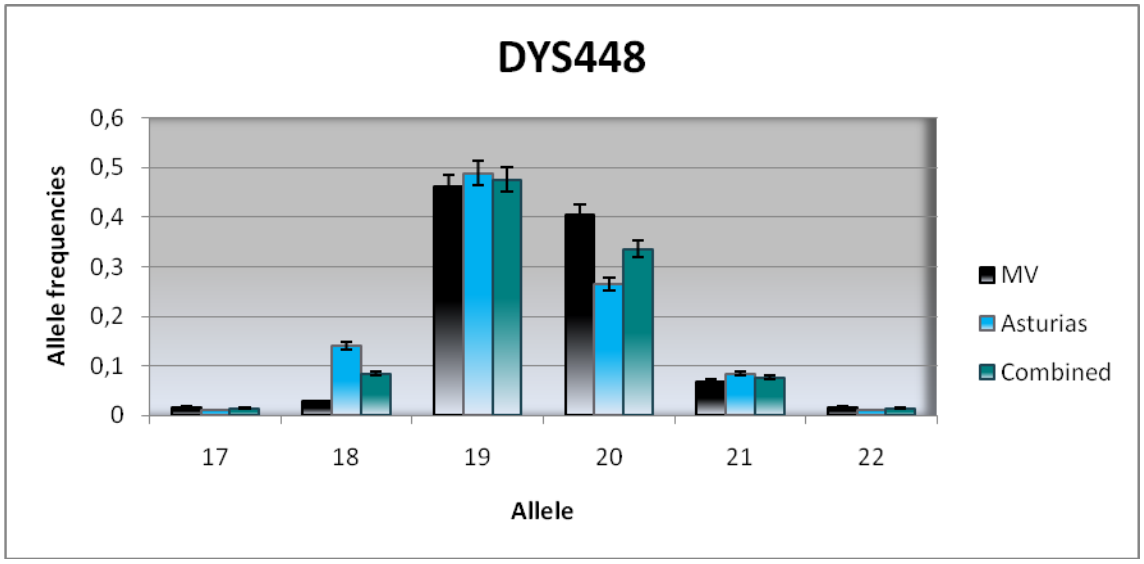


Figure 19. *Bar chart from the locus-system DYS448 where is represented the number of the alleles and its frequency in the two populations combined.*

The locus *DYS448* has a diversity of alleles from 17 to 22 (*Fig. 19*). The alleles 17 and 22 have the lower frequency in comparison to the allele 19. In *MV* population

the allele 20 is also common (0.4057±0.037 if it is compared with the 0.0264±0.033 against the Asturias population) (*Table 19*.).

DYS448	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ji}	***SE***
17	3	0.0171	±0.010	2	0.0112	±0.008	5	0.0142	±0.006
18	5	0.0286	±0.013	25	0.1404	±0.026	30	0.0845	±0.015
19	81	0.4629	±0.038	87	0.4888	±0.037	168	0.4758	±0.027
20	71	0.4057	±0.037	47	0.264	±0.033	118	0.3349	±0.025
21	12	0.0686	±0.019	15	0.0843	±0.021	27	0.0764	±0.014
22	3	0.0171	±0.010	2	0.0112	±0.008	5	0.0142	±0.006

Table 19. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS448.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus DYS456***

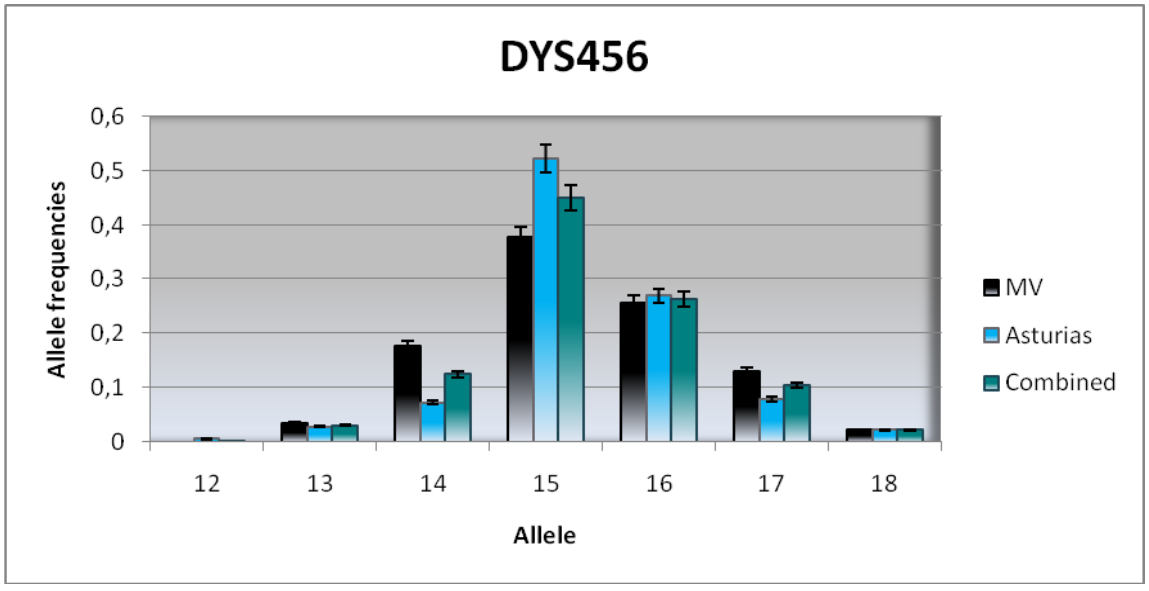


Figure 20. *Bar chart from the locus-system DYS456 representing the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS456* has a diversity of alleles that ranges from 12 to 18 (*Fig. 20*). The allele number 12 is not common for both populations and the allele 18 has a very low frequency with few individuals (4 individuals for *MV* and Asturias) (*Table 20*). The allele 15 is the most common allele in both populations but with higher

frequency for Asturias (0.5225±0,037). The allele 16 has a very similar frequency between populations (0.2571±0,033 for *MV* and 0.2697±0,033 for Asturias) and the allele 14 and 17 are more common in *MV* than Asturias.

DYS456	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	***f_{ij}***	***SE***	***Indiv***	***f_{ij}***	***SE***	***Indiv***	***f_{ij}***	***SE***
12	0	0	±0,000	1	0,0056	±0,006	1	0,0028	±0,003
13	6	0,0343	±0,014	5	0,0281	±0,012	11	0,0312	±0,009
14	31	0,1771	±0,029	13	0,073	±0,020	44	0,1251	±0,018
15	66	0,3771	±0,037	93	0,5225	±0,037	159	0,4498	±0,026
16	45	0,2571	±0,033	48	0,2697	±0,033	93	0,2634	±0,023
17	23	0,1314	±0,026	14	0,0787	±0,020	37	0,105	±0,016
18	4	0,0229	±0,011	4	0,0225	±0,011	8	0,0227	±0,008

Table 20. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS456.* ***Indiv*** *is the number of individuals per allele in the population.* ***f_{ij}*** *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus DYS458***

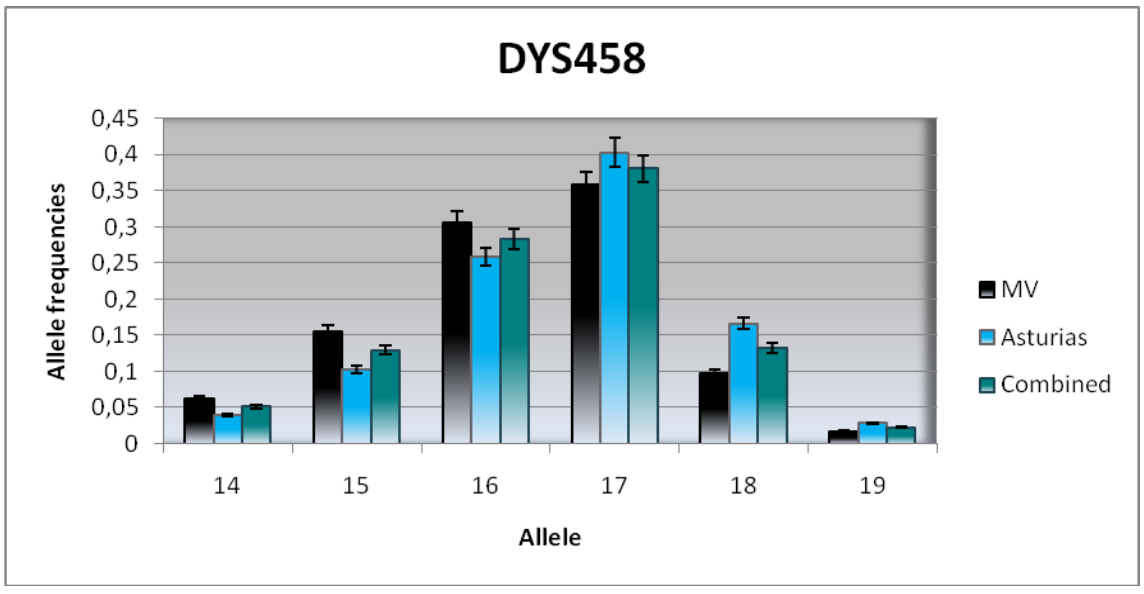


Figure 21. *Bar chart from the locus-system DYS458 representing the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS458* has a diversity of alleles that ranges from 14 to 19 (*Fig. 21*). Allele 17 occurs with the highest frequency for both populations (0.3584±0,036 in *MV* population and 0.4023±0,037 in Asturias) (*Table 21*), although in *MV* the alleles occur with higher frequencies than Asturias, with the exception of the allele 18 where in Asturian population is higher (0.1667±0,028 against 0.0983±0,023).

DYS458	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	***f_{ij}***	***SE***	***Indiv***	***f_{ij}***	***SE***	***Indiv***	***f_{ij}***	***SE***
14	11	0,0636	±0,018	7	0,0402	±0,015	18	0,0519	±0,012
15	27	0,1561	±0,027	18	0,1034	±0,023	45	0,1298	±0,018
16	53	0,3064	±0,035	45	0,2586	±0,033	98	0,2825	±0,024
17	62	0,3584	±0,036	70	0,4023	±0,037	132	0,3803	±0,026
18	17	0,0983	±0,023	29	0,1667	±0,028	46	0,1325	±0,018
19	3	0,0173	±0,010	5	0,0287	±0,013	8	0,023	±0,008

Table 21. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS458.* ***Indiv*** *is the number of individuals per allele in the population.* ***f_{ij}*** *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus DYS481***

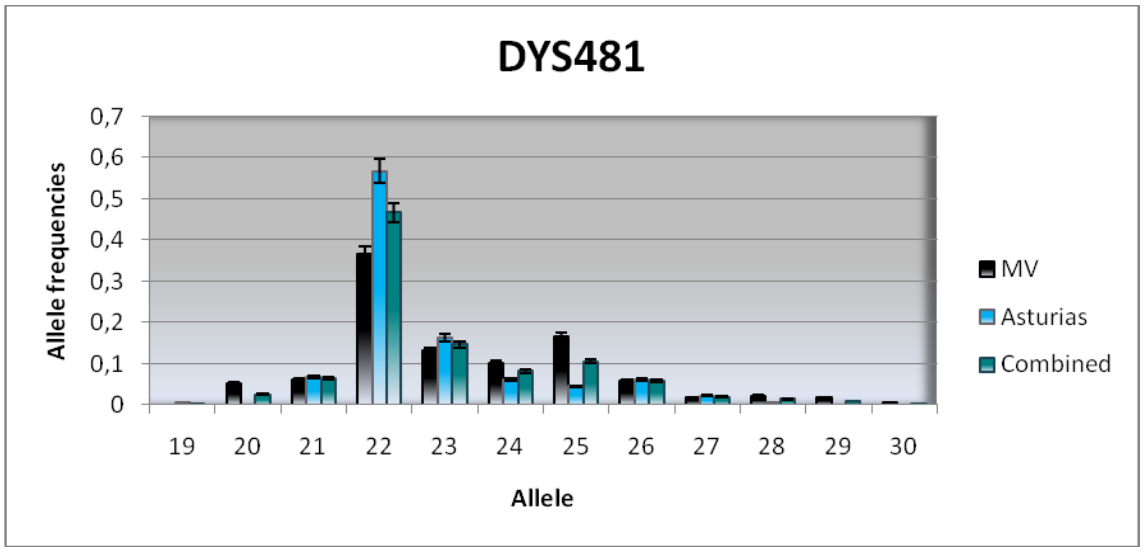


Figure 22. *Bar chart from the locus-system DYS481 representing the number of the alleles and their frequencies in the two populations combined.*

The locus *DYS481* has one of the biggest diversity in alleles. It has alleles from 19 to 30 although not all of them are presented completely in the population (*Fig. 22*). In fact, the Asturian population does not have any allele for 20, 29 and 30, and *MV* population has some individuals for all of them (*Table 22*). However, both populations have a very high proportion of the population with the allele 22 and obviously the frequency is higher for the Asturian population because the frequencies of the alleles in *MV* are more spread.

DYS481	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ji}	***SE***
19	0	0	±0.000	1	0.0056	±0.006	1	0.0028	±0.003
20	9	0.0514	±0.017	0	0	±0.000	9	0.0257	±0.008
21	11	0.0629	±0.018	12	0.0674	±0.019	23	0.0651	±0.013
22	64	0.3657	±0.036	101	0.5674	±0.037	165	0.4666	±0.027
23	23	0.1314	±0.026	29	0.1629	±0.028	52	0.1472	±0.019
24	18	0.1029	±0.023	11	0.0618	±0.018	29	0.0823	±0.015
25	29	0.1657	±0.028	8	0.0449	±0.016	37	0.1053	±0.016
26	10	0.0571	±0.018	11	0.0618	±0.018	21	0.0595	±0.013
27	3	0.0171	±0.010	4	0.0225	±0.011	7	0.0198	±0.007
28	4	0.0229	±0.011	1	0.0056	±0.006	5	0.0142	±0.006
29	3	0.0171	±0.010	0	0	±0.000	3	0.0086	±0.005
30	1	0.0057	±0.006	0	0	±0.000	1	0.0029	±0.003

Table 22. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS481.* ***Indiv*** *is the number of individuals per allele in the population.* $\boldsymbol{f_{ij}}$ *is the allelic frequency.* ***SE*** *is the standard error.*

❖ *Locus DYS533*

The locus *DYS533* has a number of alleles from 9 to 14 (*Fig. 23*). Most common allele in both populations is 12. The allele 11 and 13 the *MV* and Asturian populations have very similar frequencies (*Table 23*). For example the allele 13 has a frequency of 0.1143±0.024 in *MV* and 0.1067±0.023 for Asturias (with 19 and 20 individuals respectively). Asturias does not present the allele 14 in the population and the *MV* is not high (0.0171±0.010 and 3 individuals).

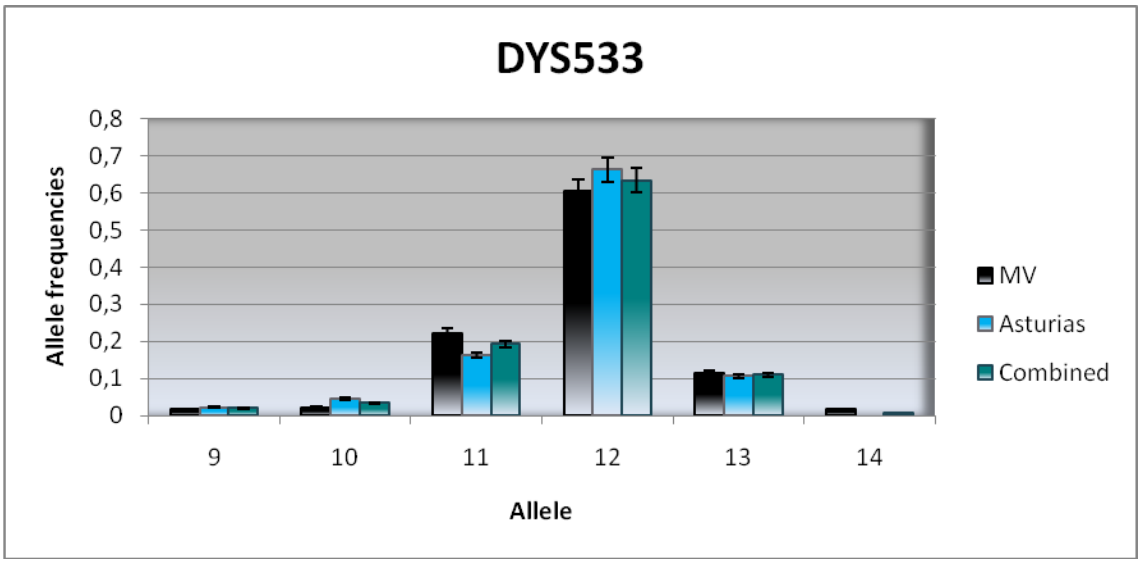


Figure 23. *Bar chart from the locus-system DYS533 representing the number of the alleles and their frequencies in the two populations combined.*

DYS533	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	***f_{ij}***	***SE***	***Indiv***	***f_{ij}***	***SE***	***Indiv***	***f_{ji}***	***SE***
9	3	0.0171	±0.010	4	0.0225	±0.011	7	0.0198	±0.007
10	4	0.0229	±0.011	8	0.0449	±0.016	12	0.0339	±0.010
11	39	0.2229	±0.031	29	0.1629	±0.028	68	0.1929	±0.021
12	106	0.6057	±0.037	118	0.6629	±0.035	224	0.6343	±0.026
13	20	0.1143	±0.024	19	0.1067	±0.023	39	0.1105	±0.017
14	3	0.0171	±0.010	0	0	±0.000	3	0.0086	±0.005

Table 23. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS533.* ***Indiv*** *is the number of individuals per allele in the population.* ***f_{ij}*** *is the allelic frequency.* ***SE*** *is the standard error.*

❖ *Locus DYS549*

The locus *DYS549* presents a diversity that ranges from the allele from 9 to 15. There are two higher allelic frequencies in *MV* and Asturias (*Fig. 24*). *MV* has the highest frequency for the allele 12 and Asturias for the allele 13. Although in both population the allele 12 is the most frequent one (with 147 individuals and a frequency of 0.4171±0.026, the second one is 0.3678±0.026 with 130 individuals

in total) (*Table 24*). The allele 9 does not occur in the sample of individuals of *MV* population and the frequency of the allele 15 is very low for both populations.

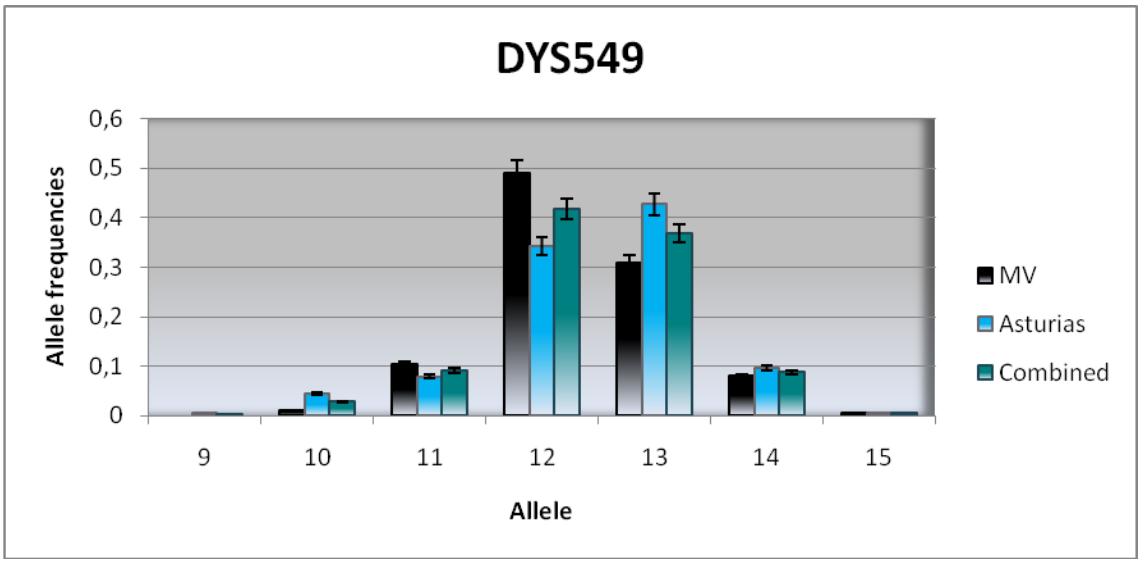


Figure 24. *Bar chart from the locus-system DYS549 representing the number of the alleles and their frequencies in the two populations combined.*

DYS549	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ji}	***SE***
9	0	0	±0.000	1	0.0056	±0.006	1	0.0028	±0.003
10	2	0.0114	±0.008	8	0.0449	±0.016	10	0.0282	±0.009
11	18	0.1029	±0.023	14	0.0787	±0.020	32	0.0908	±0.015
12	86	0.4914	±0.038	61	0.3427	±0.036	147	0.4171	±0.026
13	54	0.3086	±0.035	76	0.427	±0.037	130	0.3678	±0.026
14	14	0.08	±0.021	17	0.0955	±0.022	31	0.0878	±0.015
15	1	0.0057	±0.006	1	0.0056	±0.006	2	0.0057	±0.004

Table 24. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS549.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

❖ *Locus DYS570*

The locus *DYS570* has a high diversity in the number of alleles (14 to 24) (*Fig. 25*). The frequencies of the alleles 14, 15, 23 and 24 are low in both populations.

The alleles 21 and 22 are presenting individuals (8 and 1 in *MV* and 1 and 5 in Asturias, respectively) (*Table 25*) and are higher in *MV.*

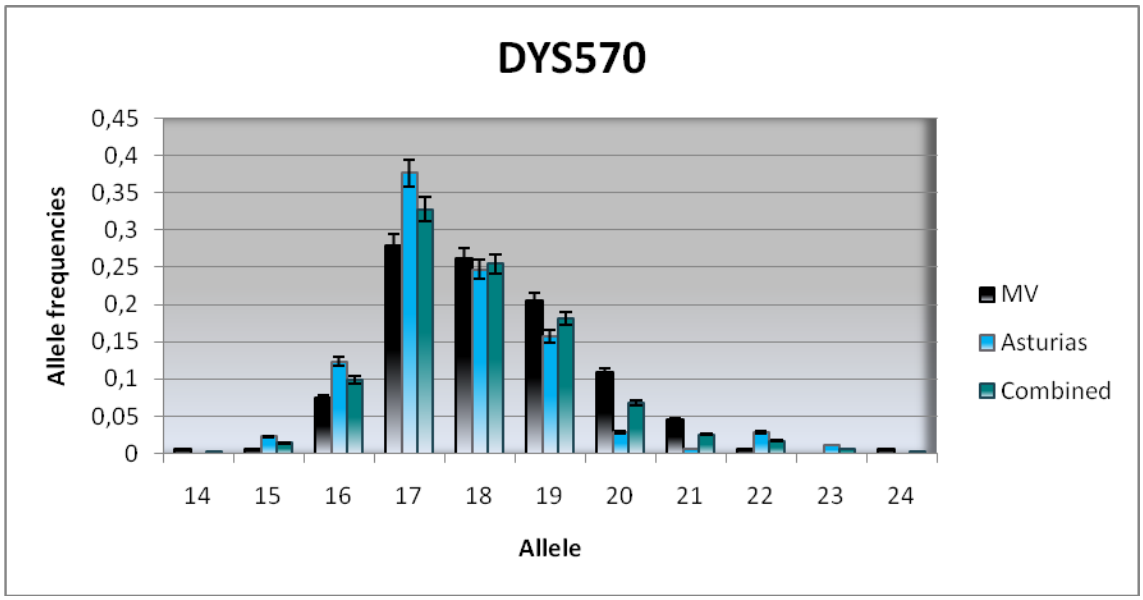


Figure 25. *Bar chart from the locus-system DYS570 representing the number of the alleles and their frequencies in the two populations combined.*

DYS570	*MV*			*Asturias*			*Comb.*		
Allele	*Indiv*	f_{ij}	*SE*	*Indiv*	f_{ij}	*SE*	*Indiv*	f_{ji}	*SE*
14	1	0.0057	±0.006	0	0	±0.000	1	0.0029	±0.003
15	1	0.0057	±0.006	4	0.0225	±0.011	5	0.0141	±0.006
16	13	0.0743	±0.020	22	0.1236	±0.025	35	0.0989	±0.016
17	49	0.28	±0.034	67	0.3764	±0.036	116	0.3282	±0.025
18	46	0.2629	±0.033	44	0.2472	±0.032	90	0.255	±0.023
19	36	0.2057	±0.031	28	0.1573	±0.027	64	0.1815	±0.021
20	19	0.1086	±0.024	5	0.0281	±0.012	24	0.0683	±0.013
21	8	0.0457	±0.016	1	0.0056	±0.006	9	0.0257	±0.008
22	1	0.0057	±0.006	5	0.0281	±0.012	6	0.0169	±0.007
23	1	0	±0.000	2	0.0112	±0.008	3	0.0056	±0.004
24	1	0.0057	±0.006	0	0	±0.000	1	0.0029	±0.003

Table 25. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS570.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

The allele 22 is also presenting in Asturias but not with high differences. The highest frequency is in the allele 17 (more for the Asturian population with 116 individuals and 0.3764±0.036 of allelic frequency). This allele is also the highest frequency for *MV* but the allele 18 is very similar too.

- ***Locus DYS576***

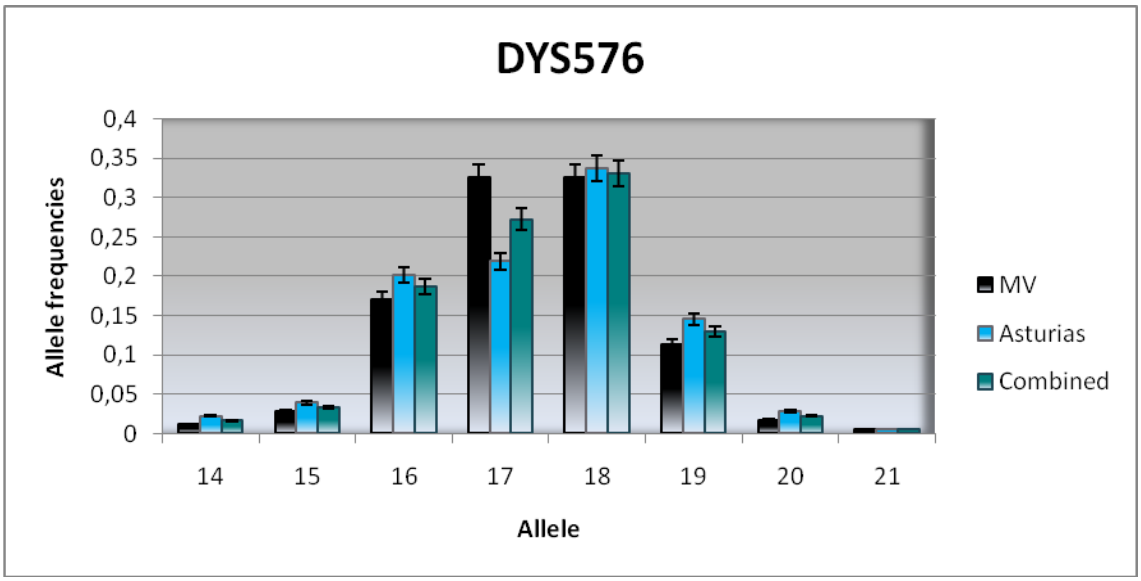


Figure 26. *Bar chart from the locus-system DYS576 representing the number of the alleles and theirs frequencies in the two populations combined.*

DYS576	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ji}	***SE***
14	2	0.0114	±0.008	4	0.0225	±0.011	6	0.017	±0.007
15	5	0.0286	±0.013	7	0.0393	±0.015	12	0.0339	±0.010
16	30	0.1714	±0.028	36	0.2022	±0.030	66	0.1868	±0.021
17	57	0.3257	±0.035	39	0.2191	±0.031	96	0.2724	±0.024
18	57	0.3257	±0.035	60	0.3371	±0.035	117	0.3314	±0.025
19	20	0.1143	±0.024	26	0.1461	±0.026	46	0.1302	±0.018
20	3	0.0171	±0.010	5	0.0281	±0.012	8	0.0226	±0.008
21	1	0.0057	±0.006	1	0.0056	±0.006	2	0.0057	±0.004

Table 26. *Summary of the main parameters calculated for the MV and Asturias population and the combinaded in DYS576.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

The Locus*DYS576* has a diversity of alleles that ranges from 14 to 21 (*Fig. 26*). The most common allele is 18 in both populations combined (117 individuals and a frequency of 0.3314 ± 0.025), although the allele 17 occurs as often as allele 18 in *MV* (0.3257±0.035) for the allele 17 (*Table 26.*). The frequencies of the alleles 14, 15, 20 and 21 do not occur in high frequencies in both populations.

- ***Locus DYS635***

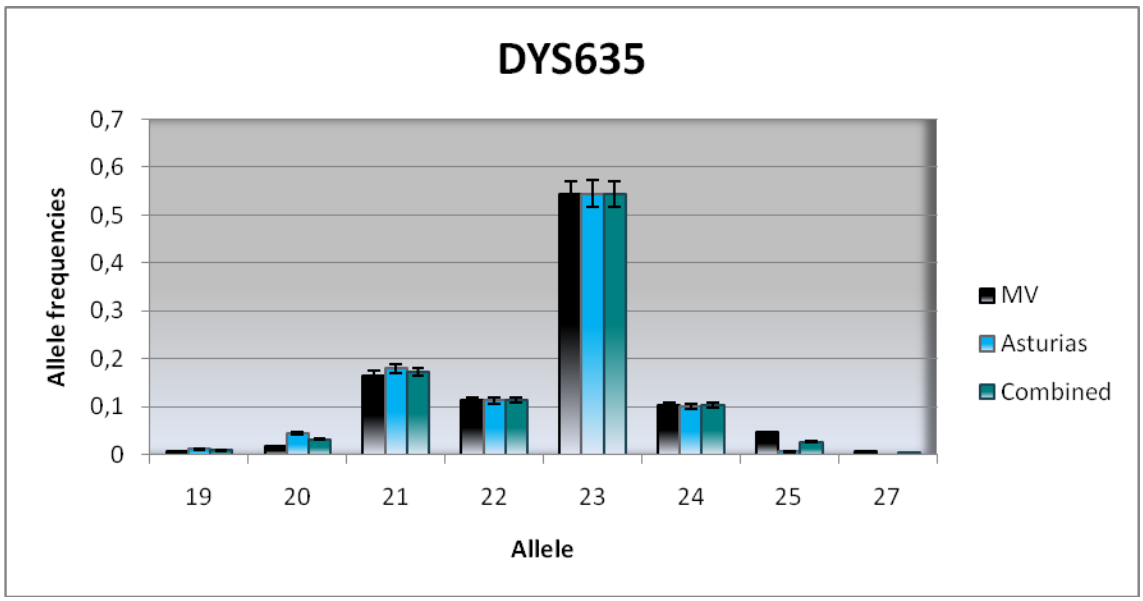


Figure 27. *Bar chart from the locus-system DYS635 where is represented the number of the allele and its frequency in the two populations combined.*

DYS635	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ji}	***SE***
19	1	0.0057	±0.006	2	0.0112	±0.008	3	0.0085	±0.005
20	3	0.0171	±0.010	8	0.0449	±0.016	11	0.031	±0.009
21	29	0.1657	±0.028	32	0.1798	±0.029	61	0.1727	±0.020
22	20	0.1143	±0.024	20	0.1124	±0.024	40	0.1133	±0.017
23	95	0.5429	±0.038	97	0.5449	±0.037	192	0.5439	±0.027
24	18	0.1029	±0.023	18	0.1011	±0.023	36	0.102	±0.016
25	8	0.0457	±0.016	1	0.0056	±0.006	9	0.0257	±0.008
27	1	0.0057	±0.006	0	0	±0.000	1	0.0029	±0.003

Table 27. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS635.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

The locus *DYS635* presents number of alleles from 19 to 27, but the allele 26 is not present in any of the populations (*Fig. 27*). The allele 27 is only in one individual of *MV* (*Table 27*). The allele 23 is the most common in both populations.. The main difference is in the alleles 20 and 25 where the 20 occurs more frequent in Asturian population than in *MV* (with the frequency of 0.0449±0.016 against 0.0171±0.010) and the frequency of the allele 25 is higher in *MV* (0.0457±0.016 against 0.0056±0.006).

- ***Locus DYS643***

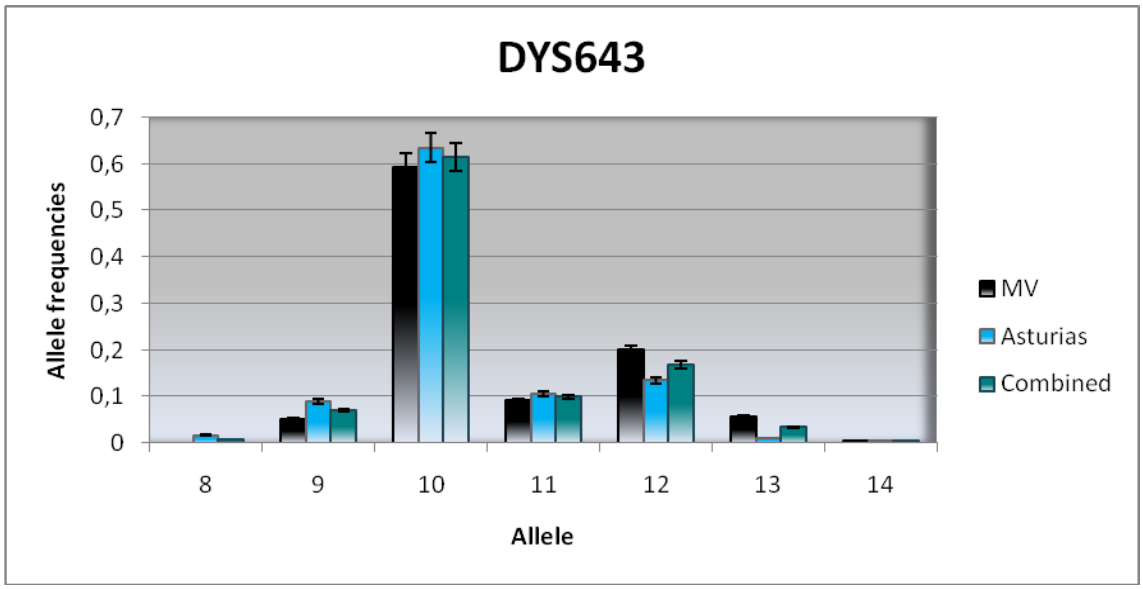


Figure 28. *Bar chart from the locus-system DYS643 representing the number of the alleles and their frequencies in the two populations combined.*

The Locus *DYS643* has a diversity of alleles that ranges from 8 to 14 (*Fig. 28*). The highest frequency is 0,5943±0,037 for the allele 10 in *MV* and 0,6348±0,036 for the same allele in Asturias. The differences in comparison to other alleles are remarkable. The alleles 8 and 14 are not frequent in both populations. The allele 13 and 12 has a low frequency in *MV* but is lower for the Asturian population (such as, in the allele 13 in *MV* is 0,0571±0,018 and in Asturias is 0,0112±0,008)(*Table 28*).

DYS643	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	***f_{ij}***	***SE***	***Indiv***	***f_{ij}***	***SE***	***Indiv***	***f_{ij}***	***SE***
8	0	0	±0,000	3	0,0169	±0,010	3	0,0084	±0,005
9	9	0,0514	±0,017	16	0,0899	±0,021	25	0,0707	±0,014
10	104	0,5943	±0,037	113	0,6348	±0,036	217	0,6146	±0,026
11	16	0,0914	±0,022	19	0,1067	±0,023	35	0,0991	±0,016
12	35	0,2	±0,030	24	0,1348	±0,026	59	0,1674	±0,020
13	10	0,0571	±0,018	2	0,0112	±0,008	12	0,0342	±0,010
14	1	0,0057	±0,006	1	0,0056	±0,006	2	0,0057	±0,004

Table 28. *Summary of the main parameters calculated for the MV and Asturias population and the combined in DYS643.* ***Indiv*** *is the number of individuals per allele in the population.* ***f_{ij}*** *is the allelic frequency.* ***SE*** *is the standard error.*

- ***Locus YGATAH4***

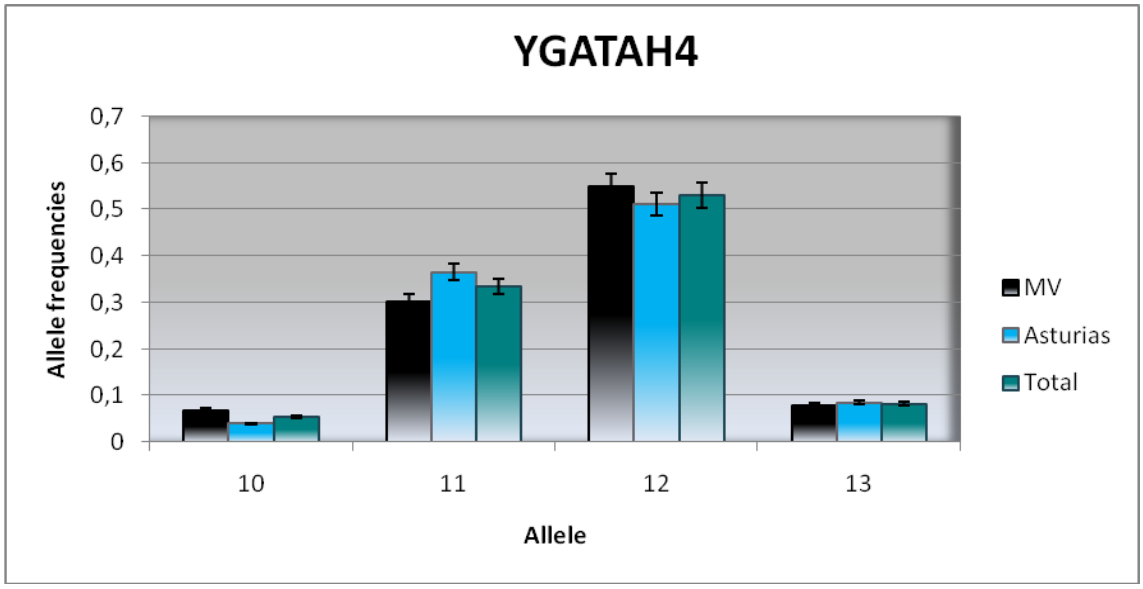


Figure 29. *Bar chart from the locus-system YGATAH4 representing the number of the alleles and their frequencies in the two populations combined.*

The Locus *YGATAH4* has a diversity of alleles that ranges from 10 to 13 (*Fig. 29*). The highest frequency occurs in the allele 12 for both populations. The other alleles have similar frequencies, for example in the allele 13 the frequency in *MV* is 0.08±0,021 and in Asturias is 0.0843±0,021 (*Table 29*).

YGATAH4	*MV*			*Asturias*			*Comb.*		
Allele	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***	***Indiv***	f_{ij}	***SE***
10	12	0,0686	±0,019	7	0,0393	±0,015	19	0,0539	±0,012
11	53	0,3029	±0,035	65	0,3652	±0,036	118	0,334	±0,025
12	96	0,5486	±0,038	91	0,5112	±0,037	187	0,5299	±0,027
13	14	0,08	±0,021	15	0,0843	±0,021	29	0,0821	±0,015

Table 29. *Summary of the main parameters calculated for the MV and Asturias population and the combined in YGATAH4.* ***Indiv*** *is the number of individuals per allele in the population.* f_{ij} *is the allelic frequency.* ***SE*** *is the standard error.*

The *power of discrimination* is summarized in the **Table 30**. The highest *PD* occurs for the locus *DYS481* (0.804) in *MV* and *DYS576* (0.778) in the Asturian population. On the other hand, the lowest *PD* occurs in the locus *DYS393* in both populations (with 0.420 in *MV* and 0.384 in Asturias)

Locus	***MV***	***Asturias***	***Locus***	***MV***	***Asturias***
DYS19	0.641	0.562	**DYS448**	0.619	0.668
DYS385a	0.646	0.657	**DYS456**	0.746	0.645
DYS385b	0.628	0.648	**DYS458**	0.744	0.735
DYS389 I	0.517	0.589	**DYS481**	0.805	0.640
DYS389 II	0.725	0.712	**DYS533**	0.573	0.523
DYS390	0.743	0.628	**DYS549**	0.650	0.687
DYS391	0.513	0.584	**DYS570**	0.795	0.759
DYS392	0.626	0.571	**DYS576**	0.748	0.778
DYS393	0.421	0.384	**DYS635**	0.656	0.649
DYS437	0.636	0.570	**DYS643**	0.596	0.562
DYS438	0.684	0.593	**YGATAH4**	0.600	0.600
DYS439	0.675	0.650			

Table 30. *Summary representing the* ***PD*** *of MV and Asturian populations*

DISCUSSION

The multiplex analysis of Y-chromosome short tandem repeats (*Y-STRs*) using the *PowePlex® Y23* kit was carried out using samples from two populations; Mecklenburg-Vorpommern (*MV*) and Asturias, from Germany and Spain respectively. This kit can work with 23 *Y-STRs* automatically and in one *PCR* program. Hence, it is a useful tool for quickly obtaining *STR* information of male individuals.

The allelic frequencies show that there are some differences between the Asturian and *MV* populations, although, these differences are more present in particular loci.

The locus *DYS481* is the most polymorphic one in both populations. The allele 22 is the most common (although it is more frequent in Asturias). In fact, *MV* has the maximum power of discrimination (*PD*) for this locus (0.805). These data are similar to other German populations, such as West Saxony *(Geppert et al. 2009*). This locus could be useful for distinguishing individuals in Germanic populations in routine forensic casework and paternity testing thanks to the high *PD*. In the Asturian population, locus *DYS576* has the highest *PD* (0.778). This value is also very high in populations such as Lithuania, Estonia and Latvia, although the highest power of discrimination corresponds with the locus *DYS481* (Lessig et al. 2009). In addition, *DYS570* presents also higher values of *PD* that are common again in these mentioned populations (Lessig et al. 2009) and the discrimination is remarkable in both studied populations.

On the other hand, the lowest polymorphic loci are *DYS437, YGATAH4* and *DYS393*. Locus *DYS437* presents only frequencies for alleles 13, 14, 15 and 16 in both populations. Comparing with other researchers, the highest frequency occurs in allele 15 as with the North Portugal population. Allele 13 does not occur in *MV* or in Portugal either *(Gusmão et al. 2002*), but the Asturian population presents one individual with this allele.

In *DYS393* the most common allele is 13 and the remaining alleles have a similar frequency in both populations. In other countries, such as Asian populations like

China, the number of alleles is low for this locus, something shared with loci such as *DYS389II.* This result is completely different in our populations, where the most common allele for the locus *DYS389II* is 29 in both and the rest of alleles present a similar distribution. These differences could be based in the existence of geographic borders because they are completely different regions; the shorter alleles in those loci are more frequent in Asia and the longer in European populations *(Gusmão et al. 2002; Hidding and Schmitt 2000*) On the other hand, Vietnamese populations present a number of alleles akin to European populations in some loci, in this case, the most common allele for this population is also 29 (*Dewa et al. 2003*). Related with *DYS389II*, there is *DYS389I*. This locus possesses two primary repeats regions that are flanked on one side by a similar sequence. *DYS389II* is included in *DYS389I* as a repeat region (*Butler 2005f*).

Another particularity exists with the locus *DYS385a* and *DYS385b, which* is a multi-copy marker. These two different regions can generate two different alleles when they are amplified with a single set of primers. These two loci are called "a" and "b" depending on their size, "a" is the smaller and "b" is bigger. If regions *a* and *b* have the same sizes only a single peak would appear *(Butler 2005f*). As a consequence, these loci can be used as a single system or not, depending on the references (*Hidding and Schmitt 2000; Rodig et al. 2007)*. If it is compared to each other, it is remarkable that *DYS385b* is more polymorphic because the highest frequency is for the allele 14, but also the allele 15 is remarkable in *MV* population while *DYS385a* has as the most common allele 11 but, the rest of frequencies are very low in comparison.

For the locus *DYS392* and other loci, the two populations show more differences. *DYS392* usually shows a bimodal allele frequency. This means that two different alleles occur with high frequency in populations (*Knijff et al. 1997*). The allele 13 is the most common for the Asturian population, but in this case, *MV* presents the allele 11 as more common. In fact, the allele 13 is the most common allele in this locus for the Spanish population where have shown a longitudinal decrease of frequencies from the west to the east of Europe. Other populations like Italy have the same results *(Rodríguez et al. 2009*) and in this case more similar to the *MV* population shown in this analysis. The Neolithic diffusion could explain this

distribution of the allele into the European populations. Then, the allele 13 of this locus could be present in the proto-European gene pool *(Quintana-Murci et al. 1999; Quintana-Murci et al. 2003*). The clinal frequency has a similar effect for other loci, such as *DYS438*, where the most common allele for the Spanish population is the allele 12 and it could be due to this Neolithic effect again (*Rodríguez et al. 2009*). According to the results, both populations have the 12 allele as the most common, sharing these results with Portuguese populations (Gusmão et al. 2002), although *MV* has also higher frequencies for the alleles 10 and 11.

More differences between populations can be found in the loci *DYS549* and *DYS439* with this bimodal effect in the frequencies. In *DYS549* the highest frequency is presented in the allele 12 for *MV* and the allele 13 for Asturian population. Similar range of common alleles occurred also in other populations independent of the distance, such as the Japanese population (*Asamura et al. 2008*). On the other hand, locus *DYS439* presents high polymorphism in its number of alleles; there are some alleles where their frequencies occur in few individuals such as 9, 14 and 16. It is remarkable that in this locus the allele 15 does not occur in any of the populations and only one individual shows the allele 16 (*MV*). This could be due to its mutation rate of 3.84×10^{-3} per allele per generation (Ballantyne et al. 2010). It seems that in both populations the alleles 15 or 16 do not occur frequently.

Continuing with this bimodal effect, the locus *DYS391* has two clear differences in frequencies with allele 10 as most common in *VM* population, and allele 11 in Asturias. One characteristic of this locus is its high mutation rate (3.23×10^{-3} per allele per generation), which is even higher for locus *DYS19* ($4{,}37 \times 10^{-3}$ per allele per generation) according to Ballantyne et al. (2010). The allele distribution is not the same in the two populations as *DYS391*. In fact, there is one allele as the highest in some populations (the allele 14) (Gusmão et al. 2002) while the rest of frequencies are very similar and lower. This allele also corresponds with the normal allele frequency for the European population (*Knijff et al. 1997*). But other worldwide comparative studies have found out that a great heterogeneity exists for this locus *(Santos et al. 1996*).

The loci *DYS393 DYS533*, *DYS635* and *DYS643* are the markers that provide less information about intrapopulation differences for these two populations. *DYS393* has the lowest *PD* for both. In Asturias is even lower than in *MV* (0.384 for Asturias and 0.421 for *MV*). The most common allele is the 13. This locus has also a longitudinal decrease frequency depending on the country. There is also a tendency to have shorter alleles for Asiatic populations but, inside Europe, populations are more similar to each other (*Hidding and Schmitt 2000*).

The loci *DYS635* and *DYS643* have very similar results. In *DYS635* the highest frequency is represented by the allele 25 in both populations although a wide diversity exists in the allele numbers. This allele range is common and found out in other German populations, Hispanic and US Caucasian (*Geppert et al. 2009*). *DYS643* presents a more reduced diversity in allele number but most of the individuals are concentrated in allele 10, and for the rest, both populations have a similar distribution. In the case of *DYS533* the diversity of alleles is lower with the same most common allele for both populations (12) and a similar *PD* (around ~0.55) forming one of the less informative locus. This allele is also common for Caucasian populations (*Butler et al. 2006*).

Otherwise, the loci, *DYS456*, *DYS576*, *DYS448*, *DYS390* and *DYS458* present a similar allelic diversity in the allele's number, but in these cases, the *MV* population has more differences in frequencies in comparison with the Asturian population. *DYS576* is one marker that presents a high polymorphism together with other loci *(Geppert et al. 2009*) such as *DYS481* discussed above.

DYS456 presents a similar result in *MV* but not with two clear peaks of frequency. But, in the allelic frequency of this locus, *MV* has the population more distributed through the different alleles. However, both populations share the same common allele (15). *DYS448* presents also two high frequencies for *MV* and one clear high frequency for Asturias. This locus needs to be used in combination with other loci, such as *DYS446*, *DYS447* or *DYS449* for increasing the discrimination and haplotype diversities in German populations *(Rodig et al. 2007*).

For *DYS390* the range of alleles is higher and the behavior is different depends on the population. Although for both populations the allele 24 is more common,

there is a big difference in frequencies, because *MV* is more homogeneously distributed for this locus and presents alleles in the complete set of allele diversity. The alleles 19, 20, 21 and 26 have very low frequencies where *MV* is always quite higher than the Asturian population. At least *DYS458* presents a high *PD* in both populations because the frequencies are distributed homogeneously with a great diversity in the allele's number. Again in this case, the Asturian population presents the highest frequency for the most common allele (17) while *MV* has also two similar frequencies in two different alleles (16 and 17).

These results between populations show that the analyses of Y-STR loci are able to enhance the allelic diversity and haplotype resolution. DNA Typing can reveal haplotypes for complete individualization of male lineages. It is important to take in consideration that mutations can be responsible for modifications in the normal allelic frequency of populations. Similar Y-chromosome haplotypes can occur in widely separated populations without any direct ancestral connection, due to mutation events, but the simultaneous typing of many markers reduces this possibility *(Knijff et al. 1997*). The sample sizes of the analyzed populations can helpful to be more discriminative and ease intra- or inter-population comparisons. For this reason, it should be considered that complete haplotype discrimination may be more difficult within one selected local population by increasing the number of tested males due to unknown deeper relationships among them. Another important aspect to considerer is the genetic background of the samples analysed (*Rodig et al. 2008*). In fact, it is expected that different populations especially from different geographic regions of the world might require a different set of minimal Y-STR loci for obtaining the maximal haplotype resolution.

The kit *PowerPlex® Y23* helps to increase the haplotype discrimination capacity of Y-STRs working with 23 Y-STRs that can be analysed at the same time. To continue developing these results and for future uses in population studies, the design of new markers could be undertaken, which highlight different specificities depending on the population in order to increase the individual discrimination power.

REFERENCES

Afonina I, Zivarts M, Kutyavin IV, Lukhtanov EA, Gamper HB, and Meyer RB. 1997. Efficient of PCR with short oligonucleotides conjugated to a minor groove binder. Nucleic Acids Res 25(13):2657-2660.

Alvarez-García A, Muñoz I, Pestoni C, Lareu MV, Rodríguez-Calvo MS, and Carracedo A. 1996. Effect of environmental factors on PCR-DNA analysis from dental pulp. International Journal Legal Medicine 109:125-129.

Applied Biosystems. 2006. Quantifiler Kits User's Manual. USA.

Arbaiza-Vilallonga M. 1994. Movimientos migratorios y economías familiares en el norte de España (1877–1910). . Revista de Demografía Histórica. p 93-124.

Asamura H, Fujimori S, Ota M, Oki T, and Fukushima H. 2008. Evaluation of miniY-STR multiplex PCR systems for extended 16 Y-STR loci. International Journal Legal Medicine 122:43–49.

Ballantyne KN, Goedbloed M, Fang R, Schapp O, Lao O, Wollstein A, Choi Y, van Duijn K, Vermeulen M, Brauer S et al. . 2010. Mutability of Y-Chromosomal Microsatellites: Rates, Characteristics, Molecular Bases, and Forensic Implications. Amer J Hum Genet 87:341-353.

Bateson P. 2002. William Bateson: a biologist ahead of his time. Journal of Genetics 81(2):49-58.

Beckman JS, and Weber JL. 1992. Survey of human and rat microsatellites. Genomics 12:1616-1622.

Brinkmann B, Klintschar M, Neuhuber F, Hühne J, and Rolf B. 1998. Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. Amer J Hum Genet 62:1408–1415.

Butler JM. 2005a. Commonly used STR markers and Commercial Kits. In: Elsevier, editor. Forensic DNA Typing Biology, Technology and Genetics of STR Markers.

Butler JM. 2005b. DNA Separation methods: Slab Gel and Capillary Electrophoresis. In: Elsevier, editor. Forensic DNA Typing Biology, Technology and Genetics of STR Markers.

Butler JM. 2005c. Introduction. In: Elsevier, editor. Forensic DNA Typing Biology, Technology and Genetics of STR Markers.

Butler JM. 2005d. Intrumentation for STR Typing. In: Elsevier, editor. Forensic DNA Typing Biology, Technology and Genetics of STR Markers. Second ed.

Butler JM. 2005e. Single Nucleotide Polymorphisms and other Bi-allelic markers. In: Elsevier, editor. Forensic DNA Typing Biology, Technology and Genetics of STR Markers.

Butler JM. 2005f. Y Chromosome DNA Testing. In: Elsevier, editor. Forensic DNA Typing Biology, Technology and Genetics of STR Markers.

Butler JM, Decker AE, Vallone PM, and Kline MC. 2006. Allele frequencies for 27 Y-STR loci with US Caucasian, African American and Hispanic samples. Forensic Sci Int 156:250-260.

Carracedo A, and Sanchez-Diz P. 2005. Forensic DNA-Typing Technologies: A review. In: Press H, editor. Forensic DNA Typing Protocols. Totowa, New Jersey.

Dahm R. 2008. Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. Hum Genet 122(6):565-581.

Davis C, Ge J, Sprecher C, Chidambaram A, Thompson J, Ewing M, Fulmer P, Rabbach D, Storts D, and Budowle B. 2013. Prototype PowerPlex Y23 System: A concordance study. Forensic Science International: Genetics 7:204-208.

Delgado-Viñas C. 2007. Breve historia crítica y análisis comparativo de las políticas aplicadas en Europa a las áreas de montaña.

Dewa K, Tuyen NQ, Rand S, Hohoff C, and Brinkmann B. 2003. 13 Y-chromosomal STRs in a Vietnamese population. International Congress series 1239:315-317.

Förster T. 1948. Zwischenmolekulare Energiewanderung und Fluoreszenz. Annals of Physics 2:55-75.

Geppert M, Edelmann J, and Lessig R. 2009. The Y-chromosomal STRs DYS481, DYS570, DYS576 and DYS643. Legal Med 11:S109–S110.

Gusmão L, Alves C, Beleza S, and Amorim A. 2002. Forensic evaluation and population data on the new Y-STRs DYS434, DYS437, DYS438, DYS439 and GATA A10. International Journal Legal Medicine 116(1):139–147.

Hidding M, and Schmitt C. 2000. Haplotype frecuencies and populations data of nine Y-chromosomal STR polymorphism in a German and Chinese population. Forensic Sci Int 113:47-53.

Jeffreys AJ, Wilson V, and Thein SL. 1985. Hypervariable minisatellite regions in human DNA. Nature 314:67-73.

Jobling MA, and Tyler-Smith C. 1997. The Y-chromosome in forensic analysis and paternity testing. International Journal Legal Medicine 110:118-124.

Kayser M, Cagliá A, Corach D, Fretwell N, Gehrig C, and Graziosi G. 1997. Evaluation of Y-chromosomal STRs: a multicenter study. International Journal Legal Medicine 110:125-133.

Knijff P. 2000. Message through bottlenecks: on the combined, use of slow and fast evolving polymorphic markers in the human Y chromosome. Amer J Hum Genet 67:1055-1061.

Knijff P, Kayser M, Caglia A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herrmann S, Herzog B et al. . 1997. Chromosome Y microsatellites: Population genetic and evolutionary aspects International Journal Legal Medicine 110:134-140.

Köchl S, Niederstätter H, and Parson W. 2005. DNA Extraction and Quantitation of Forensic Samples Using the Phenol–Chloroform Method and Real-Time PCR. In: Press H, editor. Forensic DNA Typing Protocols.

Kutyavin IV, Lukhtanov EA, Gamper HB, and Meyer RB. 1997. Oligonucleotides with conjugated dihydropyrroloindole tripeptides: base composition and backbone effects on hybridization. Nucleic Acids Res 25(18):3718-3723.

Lakowicz JR. 1983. Energy Transfer. Principlles of Fluorescent Spectroscopy Plenum Press(303-339).

Lee TI, and Young RA. 2013. Transcriptional Regulation and Its Misregulation in Disease Cell 152(6):1237-1251.

Lessig R, Edelmanna J, Dresslera J, and Krawczakb M. 2009. Haplotyping of Y-chromosomal short tandem repeats DYS481, DYS570, DYS576 and DYS643 in three Baltic populations. Forensic science International: Genetics Supplement series 2(1):429-430.

Livne AM, Edlund H, and Allen M. 2010. Forensic analysis of autosomal STR markers using Pyrosequencing. Forensic Science International: Genetics 4(2):122-129.

Martens H, and Naes T. 1989. Multivariate Calibration. London: Wiley.

McPherson JD, Marra M, Hillier L, Waterston RH, Chinwalla A, Wallis J, Sekhon M, Wylie K, Mardis ER, Wilson RK et al. . 2001. The International Human Genome Mapping Consortium. Nature 409(934).

Nei M. 1973. Analysis of gen diversity in subdivided populations. Proceeding of the National Academics of Science 70:3321-3323.

Park SDE. 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection: University of Dublin.

Promega Corporation. 2012. Power Plex Y23 System. Technical Manual. USA.

Quintana-Murci L, Semino O, Minch E, Passarino G, Brega A, and Santachiara-Benerecetti AS. 1999. Further characteristics of proto-European Y chromosomes Europ J Hum Genet 7:603-608.

Quintana-Murci L, Veitia R, Fellous M, Semino O, and Poloni ES. 2003. Genetic structure of Mediterranean populations revealed by Y-chromosome Europ J Hum Genet 7:603-608.

Rodig H, Grum M, and Grimmecke H-D. 2007. Population study and evaluation of 20 Y-chromosome STR loci in Germans. International Journal Legal Medicine 121:24-27.

Rodig H, Roewer L, Gross A, Richter T, Knijff P, Kayser M, and Brabetz W. 2008. Evaluation of Haplotype discrimination capacity of 35 Y-chromosomal short tandem repeat loci. Forensic Sci Int 174(3):182-188.

Rodríguez V, Tomàs C, Sánchez JJ, Castro JA, Ramon M, Barbaro A, Morling N, and Picornell A. 2009. Genetic sub-structure in western Mediterranean populations revealed by 12 Y-chromosome STR loci. International Journal Legal Medicine 123:137-141.

Roewer L, Krawczak M, Willuweit Mk, Nagy M, Alves C, Amorim A, Anslinger K, Augustin C, Betz A, Bosch E et al. . 2001. Online reference database of European Y-chromosomal short tandem repeat (STR) haplotypes. Forensic Sci Int 118:106-113.

Santos RF, Gerelsaikhan T, Munkhtuja B, Oyunsuren T, Epplen JT, and Pena SDJ. 1996. Geographic differences in the allele frequencies of the human Y-linked tetranucleotide polymorphism DYS19. Hum Genet 97 309–313.

Southern EM. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503-517.

Sturtevant A. 1965. A history of Genetics. New York, USA.

Tautz D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 17:6463-6471.

Thompson R, Zoppis S, and McCord B. 2012. An overview of DNA typing methods for human identification: past, present, and future. London, UK: Humana Press.

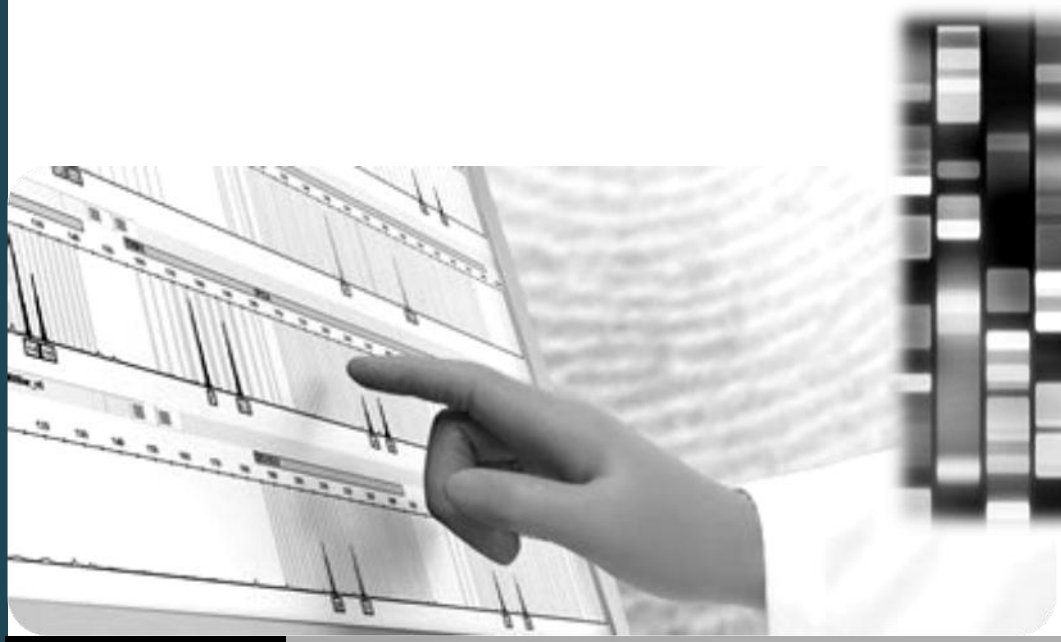
Walsh PS, Metzger DA, and Higuchi R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10(4):506-513.

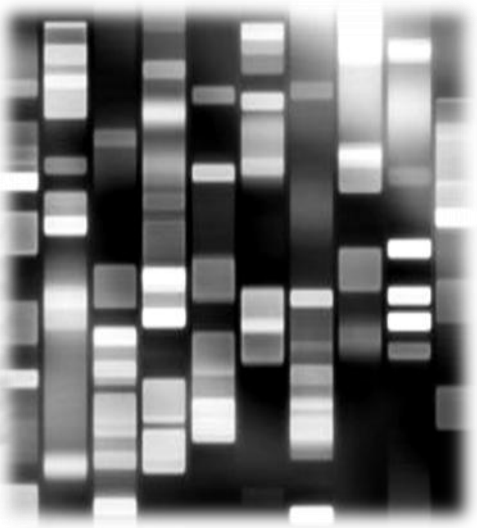
Watson JD, and Crick FHC. 1953. A Structure for Deoxyribose Nucleic Acid. Nature 171(4356):737-738.

Welch LA, Gill P, Phillips C, Ansell R, Morling N, Parson W, and Bastisch I. 2012. European Network of Forensic Science Institutes (ENFSI): evaluation of new commercial STR multiplexes that include the European Standard Set (ESS) of markers. Forensic Sci Int 6(6):819-826.

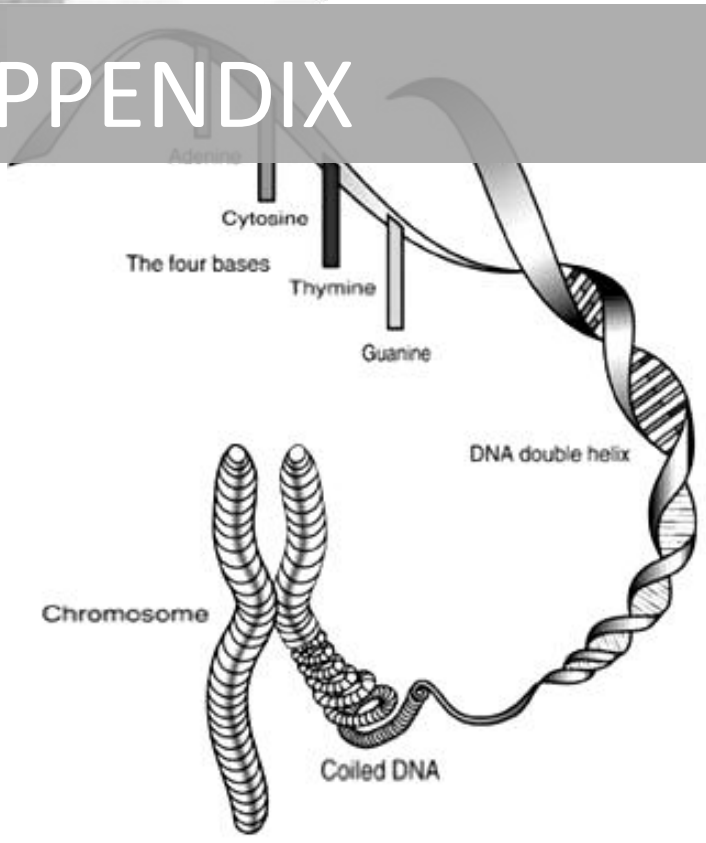
Xing J, Witherspoon DJ, Ray DA, Batzer MA, and Jorde LB. 2007. Mobile DNA elements in primate and human evolution. Amer J Phys Anthrop 135(S45):2-19.

Zarrabeitia MT, Riancho JA, Gusmão L, Lareu MV, Sañudo C, Amorim A, and Carracedo A. 2003. Spanish population data and forensic usefulness of a novel Y-STR set (DYS437, DYS438, DYS439, DYS460, DYS461, GATAA10, GATAC4, GATAH4). International Journal Legal Medicine 117(5):306-311.





APPENDIX


Adenine
Cytosine
The four bases
Thymine
Guanine
DNA double helix
Chromosome
Coiled DNA

Appendix of introduction

One example is the *http://ystr.charite.de*. This database represents the biggest collection of male-specific genetic profiles for worldwide populations. The importance of the public databases in routine forensic casework and the awareness of genetic researchers is promoting a rapid growth of them (Roewer et al. 2001). Additionally, there are many possible uses for the anthropological and forensic communities embedded in these databases, from estimation of rare haplotypes to the worldwide Y-STR profile search, which can establish connections between different geographic regions or give information about the mutability of a given locus (*Roewer et al. 2001*).

Population Group	No. of samples (as of June 2004)	Markers Tested	References
224 worldwide populations	24474	Minimal haplotype loci	*www.yhrd.org*
94 European populations	13892	Minimal haplotype loci	*(Roewer et al. 2001)* *www.ystr.org*
30 regional U.S. populations	1075 total 599 African-Americans 628 European-Americans 478 Hispanic-Americans	Minal haplotype loci	*www.ystr.org/usa*
22 Asian populations	2576	Minimal haplotype loci	*www.ystr.org/asia*
U. S. groups	4623 total 2239 African-Americans 1826 Caucasians 454 Hispanics 104 Native Americans	Y-PLEX 6 loci	*www.reliagene.com*
U. S. groups	3406 total 1605 African-Americans 1243 Caucasians 454 Hispanics 104 Native Americans	SWGDAM-recommended loci (with Y-PLEX 6 and Y-PLEX 5 kits)	*www.reliagene.com*
U. S. groups	2443 total 577 African-Americans 595 Caucasians 630 Hispanics 357 Native Americans	SWGDAM-recommended loci + DYS437 (with PowerPlex Y kit)	*www.promega.com*

	284 Asian-Americans		
Genealogists from around the world	2300	Uo to 49 Y-STRs (run by genetic genealogy companies)	*www.ybase.org*
Genealogists from around the world	5300 records 4169 unique haplotypes	Up t0 37 Y-STRs (run by FamilyTree DNA)	*www.ysearch.org*
Genealogists from around the world	8735 haplotyes associated with 296424 inidivual antecessor	Allele frequencies for 37 Y-STRs (run by Sorenson Genomics/Relative Genetics)	*www.smgf.org*

Table Ap. 1. *Summary of different references online about studies with Y-Chromosome populations. It is shown the number of individuals, countries, markers used and the main websites. (Butler 2005f)*

Different uses	Advantage
Historical and genealogical researches	In many countries males inherit the Surname of the family and it could be easier to build genealogy and establish the origins.
Human migrations and evolutionary studies	Lack of recombination allows the comparison between males separated by large periods of time.
Paternity testing	When the father is not present could use the samples of a relative and even in cases of motherless.
Missing persons in investigation	Patrilineal samples can be useful for making the references with the missing person.
Casework on sexual assault evidence	Male-specific amplifaction

Table App2. *Summary of some ares of uses in Y chromosome. (Butler 2005f)*

Appendix of Material and Methods

Quantification:

For this assay, probes were covalently attached to a fluorophore (***R***), which was 6-*FAM™* dye (6-Carboxyfluorescein) or *VIC®* dye, in the 5'-end, and attached to a Minor Groove Binder (***MGB***) and Non-fluroescent quencher (***NFQ***) in the 3'-end.

Probes were employed in a Quantitative Real Time Polymerase Chain Reaction (*qRT-PCR*) framework, using *AmpliTaq Gold®* Polymerase (***P***). During the process, the *TaqMan® MGB* probe anneals to a complementary sequence between the forward and reverse primers like a normal *PCR* cycle. All these components were included in the *Quantifiler Human DNA Quantification Kit®* (*Applied Biosystem*), and their working characteristics can be found in **Figure 1**.

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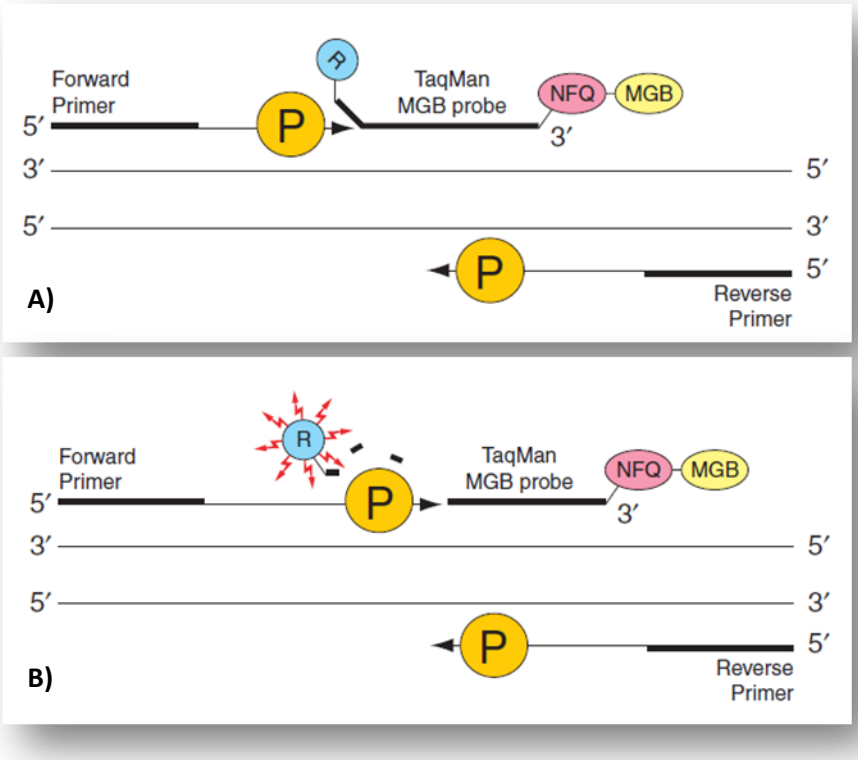

Figure 1. A) *Step of the qRT-PCR where the AmpliTaq® Polymerase is going next to the reporter dye or R.* ***B)*** *The reporter dye is leaving from the TaMan® MGB probe and this distance belong to emit fluorescence in the reaction. Manufacture's User Manual Protocol (Applied Biosystems 2006)*

During one of the PCR cycles, the *TaqMan® MGB* probe anneals specifically between the forward and reverse primers sequence sites. While the probe is intact (*Fig. 1A*) the reporter dye and the

quencher dye produce a supression in the reporter fluorescence (*Förster 1948; Lakowicz 1983)*. Additionally, the ***MGB*** quencher increases the melting temperature (T_m) without increasing probe length (Afonina et al. 1997; Kutyavin et al. 1997) which allows the design of shorter probes.

The *AmpliTaq Gold*® polymerase cleaves only probes that are hybridized to the target sequence (*Fig. 1B*). The 5' to 3' exonuclease activity of the polymerase degrades the probe that is annealed to the sequence. This degradation of the probe releases the fluorophore and a bigger distance to the quencher allows the fluorescence by the reporter (*Fig.1C*).

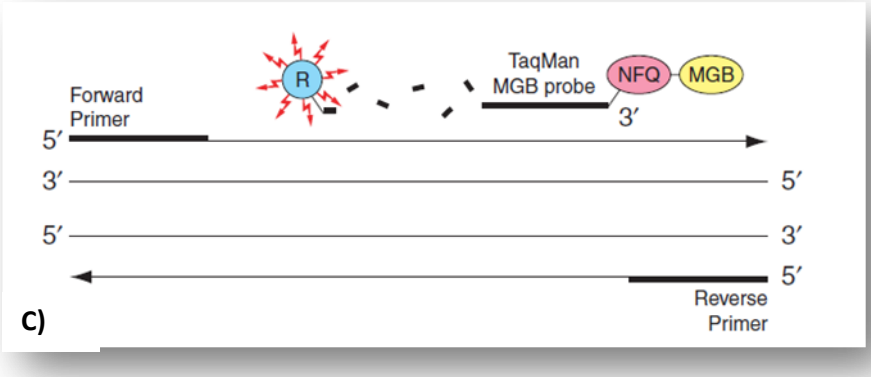

Figure 1. C) *Final end of Polymeration. The fluorescence detected in the qRT-PCR is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Manufacture's User Manual Protocol (Applied Biosystems 2006)*

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To evaluate the performance of the *qRT-PCR* assay and to include an objective measure of potential *PCR* inhibition, we introduced an *IPC* that is amplified simultaneously in the same assay. Initially, *Rn* appears as a flat line because the fluorescent signal is below the detection limit of the sequence detector. The line can be used to identify the dynamic range of the assay and can be used to quantify the amount of initial target *DNA* from an unknown sasmple. (*Köchl et al. 2005*). In these initial cycles of PCR, in which only little change in fluorescence occurs, are used to define the baseline for the amplification plot. The threshold fluorescence signal is the point at which a reaction reaches a fluorescent intentisty above background. The cycle at which the sample reaches this level is called the cycle threshold (*Ct*) (*Köchl et al. 2005*).

The first phase is the Exponential stage where the signal is detected and increases in direct proportion of the *PCR* product. But, as soon as PCR product is increasing, the ratio of AmpliTaq® Gold is decreasing. When the template concentration reaches 10^{-8}M, the *PCR* product gives up accumulating exponentially (*Fig. 2*). The second phase is the Linear where the rise of the amplification graph decreases steadily. In this phase the amplification approximates an arithmetic progression, rather

than an exponential increase (*Applied Biosystems 2006*). The last phase is the plateau when the PCR stops. The *Rn* signal remains more or less constant and the concentration of the template reaches a steady phase at about 10^{-7}M (*Martens and Naes 1989*).

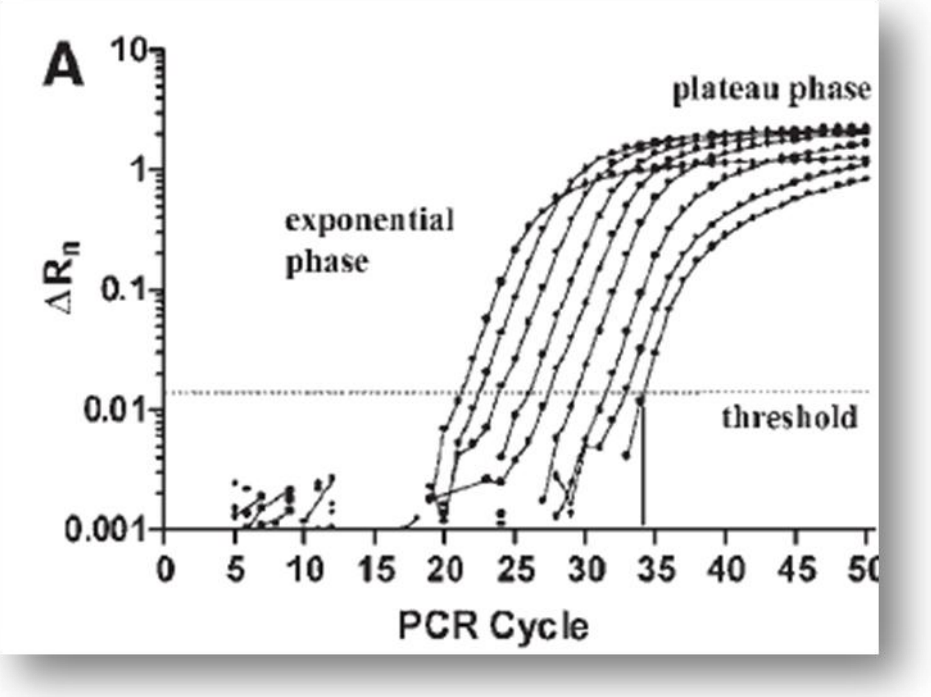

Figure 2. *Amplification plots of a dilution series of the DNA standard (genomic DNA) showing the changes in fluorescence vs PCR cycle number (logarithmic view). Linear phase is not shown (Köchl et al. 2005).*

Capillary electrophoresis:

The basic elements of the Capillary Electrophoresis process include a narrow capillary, two electrodes which are connected to a high-voltage supply, two buffer vials, a laser excitation source, a fluorescence detector and auto-sampler to put the samples in it (*Figure 3*). Normally, capillaries are made of fused silica with an inner diameter of 50, 75 or 100 μm and a length of 25-75 cm which is filled with a viscous polymer solution that acts like other electrophoresis gels creating a sieving environment for the *DNA* samples. In addition, long *DNA* molecules are slower than smaller *DNA* fragments. For this reason, this methodology leads to a separation between size-bands of *DNA (Butler 2005b)* .

It is important to take in consideration two factors that are correlated with capillary length: the peak resolution and the separation time, because the longer the capillary is the better is the resolution

and the greater is the separation time too. The length of the capillary will depend on the necessities for the *DNA* identification *(Butler 2005b).*

Additionally, polymer choice has to be considered. Polymer POP-4 was used in this study, as it is the most commonly used for Y-*STR*. Polymer POP-6 is more viscous and able to improve the resolution with longer run times. It was not used, and it is more suitable for DNA sequencing applications (*Butler 2005d*).

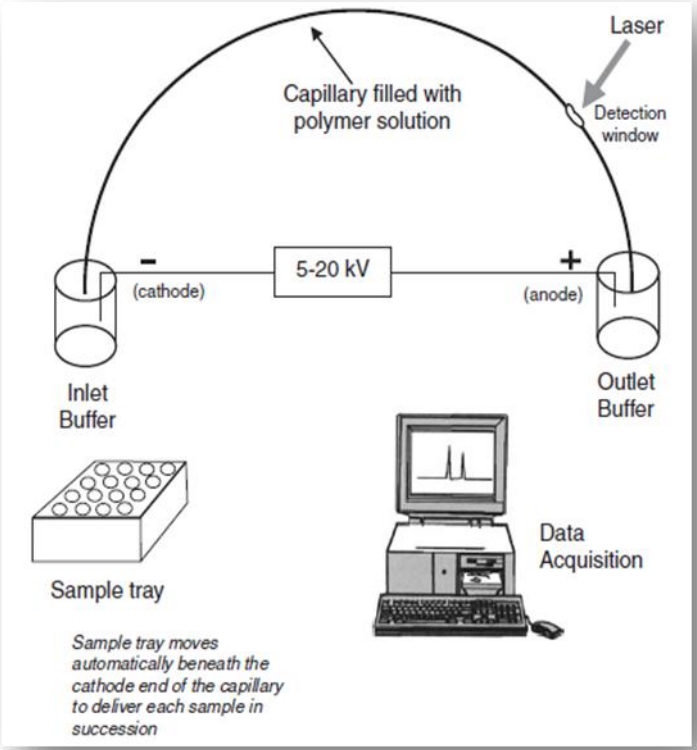

Figure 3. *Scheme of the capillary electrophoresis used for DNA analysis. Samples are placed into the sample-tray and injected into the capillary by high voltage. The DNA samples move through the capillary and the fragments are separated by size. DNA fragments are dye-labeled and analyzed as the pass by the detection window and they are excited by the laser. The monitoring of this process is measured with specific software on a computer (Butler 2005b).*

Summary table of the German population (Mecklenburgische-Vorpommern)

General data		STR loci																						
Abst.	**Region**	**DYS 576**	**DYS 389 I**	**DYS 448**	**DYS 389 II**	**DYS 19**	**DYS 391**	**DYS 481**	**DYS 549**	**DYS 533**	**DYS 438**	**DYS 437**	**DYS 570**	**DYS 635**	**DYS 390**	**DYS 439**	**DYS 392**	**DYS 643**	**DYS 393**	**DYS 458**	**DYS 385a/b**		**DYS 456**	**YGATA H4**
033-10	Bergen	17	13	20	29	16	10	20	12	12	11	14	19	23	24	11	11	10	13	17	11	15	16	13
034-10	Rostock	18	13	19	29	15	11	20	11	11	10	14	19	22	23	10	14	11	14	17	11	14	14	12
040-10	Neubrandenburg	17	14	20	31	15	10	26	12	11	11	14	20	22	23	11	12	12	14	17	14	16	14	10
041-10	Greifswald	16	12	22	29	16	11	23	12	12	11	14	19	23	25	11	11	10	13	16	11	14	17	13
044-10	Pasewalk	18	13	19	29	14	11	21	13	12	12	15	17	24	24	12	13	10	13	17	11	14	15	12
045-10	Wolgast	17	13	19	29	14	11	23	12	12	12	15	18	23	24	12	13	10	13	16	11	15	17	12
046-10	-	18	13	20	29	14	10	22	14	11	12	15	17	23	24	12	13	11	13	17	11	13	15	12
047-10	Neubrandenburg	16	13	19	30	14	11	22	14	12	12	15	17	23	24	13	13	10	13	18	12	14	15	12
048-10	Greifswald	19	14	19	30	14	11	22	13	12	12	15	17	24	24	12	13	12	13	16	11	15	15	11
049-10	-	18	13	19	29	14	11	22	13	12	12	15	16	23	24	12	13	10	13	17	12	14	16	12
051-10	Greifswald	18	14	19	30	15	10	21	11	13	10	14	18	22	23	10	14	10	14	17	11	13	13	11
053-10	Krakow am See	18	13	19	29	15	10	22	12	12	12	15	18	23	23	12	13	10	13	16	11	14	14	12
058-10	Friedland	17	13	19	30	14	11	22	13	12	12	15	16	23	24	16	13	10	13	16	11	14	15	12
060-10	Neubrandenburg	17	12	20	30	15	10	28	12	11	10	14	20	21	20	11	12	13	14	15	16	16	14	10
062-10	-	16	12	21	28	15	10	21	12	9	10	16	17	21	22	11	11	11	14	16	14	14	15	12
065-10	-	17	13	20	30	17	10	25	13	14	10	15	19	21	23	11	12	13	14	15	15	16	17	11
066-10	-	17	13	20	30	14	10	24	12	11	10	16	19	21	22	11	11	12	13	15	13	15	15	11
071-10	Greifswald	17	13	19	29	14	11	22	13	12	12	15	19	23	23	12	13	10	13	18	10	14	17	11
073-10	Wismar	17	14	19	30	14	10	20	11	11	10	14	17	22	23	10	14	11	14	17	11	13	13	12
077-10	Pasewalk	18	13	20	30	16	10	23	13	12	11	14	17	23	24	10	11	10	13	15	12	14	15	13
080-10	Stralsund	16	12	20	28	15	10	25	13	11	10	16	20	22	23	11	11	13	13	15	14	15	14	11
082-10	Greifswald	16	13	21	30	14	10	22	12	14	9	15	14	24	23	12	11	10	12	14	13	17	15	11
083-10	Neubrandenburg	18	13	19	28	13	10	22	12	12	12	15	16	23	24	12	13	10	13	19	11	14	16	11
086-10	Wolgast	19	13	19	29	14	10	22	13	11	12	15	18	23	24	12	13	10	13	18	11	12	15	11
089-10	Wolgast	18	13	20	30	15	11	24	12	13	12	14	19	24	25	11	11	10	13	17	12	14	15	12
093-10	Neubrandenburg	17	13	19	28	14	10	22	13	12	12	15	19	23	22	11	13	10	14	17	11	14	16	11

094-10	-	18	12	20	28	14	10	24	12	11	10	16	19	21	22	12	11	12	12	15	13	15	15	12
095-10	Bergen	17	13	19	29	14	11	22	12	12	13	14	17	23	25	11	13	10	13	16	11	14	15	11
100-10	Bergen	17	13	20	30	16	10	25	12	12	11	14	18	23	25	12	11	10	13	16	11	14	17	12
102-10	Anklam	19	13	19	32	15	10	23	12	12	10	14	20	24	24	10	11	10	13	15	12	14	14	12
108-10	-	18	13	19	29	14	11	22	12	12	12	15	18	24	23	12	13	11	13	16	11	15	16	11
109-10	-	17	13	20	30	17	11	22	12	12	11	14	18	23	25	10	11	10	13	14	12	13	16	13
112-10	Greifswald	18	14	18	30	14	11	22	13	12	12	15	17	25	24	12	13	9	13	17	11	14	16	12
113-10	Pasewalk	15	12	19	28	15	10	23	13	12	9	16	16	22	24	12	11	9	12	16	10	19	13	12
114-10	-	16	13	19	29	15	11	25	13	12	12	14	17	23	25	11	13	10	13	17	11	14	16	12
125-10	-	16	13	19	29	15	11	22	14	12	12	14	21	23	25	11	14	10	13	18	13	15	15	12
129-10	Stralsund	20	14	19	31	14	11	22	12	13	12	15	18	23	24	12	13	10	12	16	11	14	16	12
130-10	Neubrandenburg	17	13	20	29	15	11	24	12	13	12	14	18	24	25	10	11	10	13	17	13	14	16	10
132-10	Pasewalk	20	13	19	29	14	11	22	13	12	12	14	19	23	25	12	13	11	13	18	11	15	18	12
134-10	Demmin	18	13	20	30	16	10	26	12	12	11	14	17	24	25	12	11	10	13	16	11	14	17	12
136-10	-	17	12	19	27	15	10	23	14	12	12	15	16	23	23	11	13	10	13	15	12	13	16	11
142-10	Greifswald	14	12	22	28	16	10	21	13	10	10	16	18	20	21	12	11	13	14	15	13	14	15	11
143-10	Anklam	18	14	21	31	15	10	24	13	12	11	14	19	23	25	11	11	10	13	17	11	14	15	12
144-10	Greifswald	17	12	19	27	15	11	22	14	12	9	16	18	22	24	12	11	9	12	16	15	17	13	11
146-10	Greifswald	19	13	19	29	14	11	22	12	12	12	15	17	23	23	12	13	10	13	17	10	15	16	12
147-10	-	18	13	17	30	15	11	23	13	12	12	15	18	23	24	11	13	10	13	15	11	14	17	11
149-10	Greifswald	19	13	20	29	16	10	25	12	12	11	14	17	24	25	11	11	10	13	16	11	14	17	12
151-10	Bergen	19	13	19	29	14	11	22	14	12	12	15	17	23	24	13	13	10	14	17	11	15	15	12
153-10	Neubrandenburg	18	13	20	31	17	10	23	13	12	11	14	19	23	25	10	11	10	13	14	11	14	16	13
154-10	-	18	13	19	30	15	11	21	14	12	12	15	16	23	23	12	13	10	13	17	12	14	15	12
156-10	Stralsund	18	13	19	29	14	10	22	14	12	12	15	17	23	24	11	13	10	13	17	11	16	16	12
158-10	-	18	12	21	31	14	10	25	12	11	10	16	20	21	22	11	11	12	13	15	14	14	14	11
159-10	Stralsund	16	13	20	29	15	10	25	11	12	10	16	19	21	22	11	11	12	13	16	13	14	14	10
164-10	Röbel	18	13	20	31	15	11	23	13	12	11	14	18	23	25	10	11	10	13	16	11	13	15	13
165-10	Demmin	18	14	19	30	16	10	24	13	12	11	14	20	23	25	11	11	10	14	16	11	14	17	12

171-10	Güstrow	18	13	21	30	15	10	24	12	13	11	14	18	23	25	10	11	10	13	16	11	14	15	13
175-10	Wolgast	17	14	20	32	16	11	29	10	13	10	15	18	23	24	12	11	10	13	19	14	15	15	11
178-10	-	17	12	20	29	14	11	25	12	12	10	16	18	22	23	11	11	12	13	17	13	13	14	11
179-10	Wolgast	16	13	19	30	14	11	22	14	12	12	15	18	23	24	12	13	9	13	17	11	14	15	12
180-10	-	15	14	19	30	15	11	20	11	11	10	14	19	21	23	10	15	11	14	16	11	13	13	12
181-10	Altentreptow	17	12	21	29		10	21	13	11	10	16	17	21	23	11	11	11	14	19	16	16	14	12
188-10	Neubrandenburg	16	12	20	28	14	10	25	12	11	10	16	19	20	23	11	11	12	13	14	14	14	14	11
189-10	Greifswald	16	13	20	29	14	10	22	12	12	12	15	17	23	26	12	14	12	13	17	11	14	16	12
192-10	Greifswald	19	13	20	31	16	11	29	11	13	10	15	18	23	24	13	11	10	13	16	15	15	15	11
194-10	Greifswald	18	13	19	29	14	11	22	11	13	12	15	17	23	24	13	13	10	13	18	11	15	16	12
196-10	Bergen	17	13	20	30	16	10	24	12	12	12	14	18	23	25	10	11	11	12	15	11	14	17	12
201-10	Bergen	17	13	20	29	16	10	25	12	12	11	14	20	23	25	11	11	10	13	16	11	14	16	12
204-10	-	16	13	19	29	14	11	22	13	12	12	15	18	23	24	12	13	10	13	17	11	15	16	12
001-11	Rostock	18	13	20	30	16	10	25	12	12	11	14	20	24	25	10	11	10	13	16	10	14	16	12
005-11	Waren	18	13	19	30	14	11	22	12	13	12	15	17	23	24	12	13	10	13	17	11	14	15	11
008-11	Greifswald	17	13	20	31	16	9	23	12	12	11	14	19	23	25	10	11	10	13	14	11	14	16	13
012-11	-	17	13	19	29	14	11	22	12	12	12	14	19	23	23	12	13	10	13	16	11	15	15	12
015-11	Greifswald	18	13	20	29	16	10	24	13	12	11	14	18	23	24	11	11	10	13	16	11	14	17	12
020-11	-	17	12	20	28	14	10	27	13	11	10	16	21	23	23	11	11	12	13	15	14	14	15	11
029-11	Wolgast	19	13	20	30	15	10	22	11	12	11	14	17	23	24	10	11	10	13	15	12	14	17	12
035-11	Anklam	17	13	20	30	15	11	24	12	13	11	14	18	25	25	10	11	10	13	17	11	14	15	12
035-11	Ueckermünde	17	12	20	30	16	10	26	12	11	10	14	20	21	19	11	12	13	14	15	15	15	14	10
039-11	Malchin	17	13	20	32	16	11	30	11	13	10	15	18	23	24	13	11	10	13	16	14	15	15	11
040-11	-	16	12	20	28	14	10	27	12	11	10	16	20	24	22	11	11	12	12	16	14	14	14	11
042-11	Pasewalk	17	12	20	29	14	10	25	12	11	10	16	19	21	23	11	11	12	13	15	13	14	14	11
047-11	-	16	12	20	28	14	10	25	13	11	10	16	19	21	22	12	11	12	14	16	12	14	14	11
050-11	Greifswald	18	14	17	29	15	11	23	13	12	12	15	16	23	24	11	13	10	13	17	11	14	15	11
054-11	Calw	17	13	19	30	14	10	22	13	12	11	15	17	23	25	11	13	10	13	18	11	14	17	12
055-11	Neustrelitz	19	13	19	29	14	11	22	13	12	12	15	16	23	23	12	13	10	13	17	11	14	16	11

067-11	Anklam	17	13	19	30	14	11	24	13	12	12	15	16	25	24	12	14	10	13	17	11	15	15	12
071-11	Anklam	19	13	20	29	16	10	25	12	12	11	14	17	23	25	11	11	10	13	16	11	14	17	12
072-11	Waren	14	11	21	27	15	10	21	12	9	10	16	17	20	22	11	11	11	14	16	14	14	15	12
073-11	Wismar	18	14	20	30	14	10	20	12	11	9	14	18	23	23	11	14	12	13	18	11	15	14	10
076-11	-	17	13	20	31	13	10	22	12	11	10	14	19	21	24	12	11	12	13	15	17	17	15	12
079-11	Stralsund	21	13	21	29	14	10	24	13	11	10	14	18	21	23	12	11	9	12	17.2	13	15	15	11
081-11	Greifswald	18	13	20	30	16	10	24	12	12	11	14	17	23	25	12	11	10	13	16	10	14	16	12
085-11	-	19	13	19	30	14	12	23	12	11	12	15	17	25	24	11	13	10	14	18	11	14	14	11
094-11	Greifswald	19	13	19	30	14	11	22	12	12	12	16	17	23	24	11	13	10	13	18	11	13	16	12
096-11	Greifswald	17	13	19	29	14	11	22	13	12	11	15	17	25	23	12	13	10	13	17	11	16	15	12
104-11	-	18	14	18	30	15	11	22	12	10	10	14	16	22	23	12	13	12	14	18	13	17	15	11
105-11	Demmin	16	12	20	29	14	10	25	12	11	10	16	22	22	21	11	11	12	13	17	14	14	15	10
108-11	-	18	14	19	30	14	11	22	14	12	12	15	21	23	24	11	13	10	13	17	11	14	15	12
109-11	-	16	12	19	28	17	11	23	12	10	10	15	19	21	26	11	11	14	13	16	13	16	14	10
112-11	Demmin	17	13	18	29	14	11	22	15	12	13	15	17	24	23	12	13	10	13	17	12	14	17	12
113-11	-	17	12	20	29	14	11	25	12	12	10	16	18	22	23	11	11	12	13	17	13	13	14	11
114-11	-	17	12	20	28	14	11	25	12	11	10	16	21	21	22	11	11	12	13	17	14	15	14	11
117-11	Greifswald	16	13	19	29	16	10	25	12	12	11	14	20	23	26	11	11	10	13	16	11	14	17	12
118-11	Greifswald	16	14	19	30	14	11	20	10	12	10	14	20	23	23	10	14	11	14	18	12	13	14	12
120-11	Stralsund	20	13	19	29	14	11	22	11	12	12	14	17	24	24	10	13	9	14	17	12	14	15	12
124-11	Stralsund	16	13	19	29	15	11	20	11	11	10	14	18	21	24	10	14	11	14	16	11	13	14	12
131-11	Greifswald	18	13	19	29	14	11	23	13	14	12	15	17	23	23	12	13	10	13	17	11	14	16	12
132-11	-	18	13	19	29	14	11	23	12	12	12	15	15	23	25	12	13	9	13	17	11	16	15	12
139-11	-	18	14	20	32	17	10	26	13	12	10	14	19	21	23	12	12	12	15	15	13	15	15	11
140-11	Rostock	18	12	19	29	14	11	22	13	12	13	15	19	25	24	11	13	10	13	16	11	14	15	12
141-11	Ribnitz-Damgarten	17	13	20	29	16	10	25	12	12	12	14	18	23	25	11	11	10	13	17	10	12	15	11
143-11	-	16	12	20	28	14	10	26	12	12	10	15	20	21	22	11	11	12	13	16	12	15	15	12
145-11	Ueckermünde	17	13	19	29	14	11	24	13	12	12	15	18	25	24	12	14	10	13	17	11	15	15	12
146-11	Greifswald	17	12	19	29	16	10	23	12	11	10	15	19	19	23	11	12	12	14	16	14	16	14	12

163-11	Pasewalk	17	13	18	29	14	11	21	12	12	13	16	18	23	24	13	13	10	12	16	10	13	16	13
171-11	Neubrandenburg	18	13	19	29	14	11	22	13	12	12	15	17	23	23	11	13	11	13	17	11	14	16	13
172-11	Stralsund	18	13	20	29	16	10	25	12	12	11	14	19	23	25	11	11	10	13	16	11	14	16	12
176-11	Pasewalk	16	13	19	30	14	10	22	13	12	12	15	17	23	24	12	13	10	13	18	11	14	15	13
177-11	Pasewalk	18	13	19	29	14	10	21	11	12	12	15	18	23	24	12	13	11	13	17	11	15	16	11
178-11	Koserow	18	13	19	29	14	11	22	12	12	13	15	17	23	24	11	14	10	13	18	11	14	16	12
183-11	Reichenbach	16	12	22	29	15	10	21	12	11	10	16	18	21	21	12	11	12	15	15	13	16	15	11
186-11	Greifswald	16	13	19	29	14	11	22	12	12	12	15	19	23	23	12	13	10	13	19	11	13	15	12
187-11	Demmin	17	12	21	30	13	10	26	12	10	10	14	20	21	24	12	11	12	13	17	16	17	15	12
191-11	-	19	13	19	31	16	11	25	13	11	11	14	18	23	25	10	11	10	13	15	11	14	15	13
193-11	Neubrandenburg	18	13	19	30	14	10	21	13	13	12	16	17	23	24	12	13	10	13	17	11	14	16	11
001-12	Gützkow	17	13	20	29	16	10	25	13	12	11	14	19	23	25	11	11	10	13	16	11	14	18	12
002-12	Anklam	17	13	20	30	15	10	26	14	11	10	14	19	22	23	11	12	12	14	14	13	16	13	11
003-12	Malchin	18	13	19	31	13	10	22	13	13	10	14	17	22	23	12	11	13	14	16	15	16	17	11
007-12	Neubrandenburg	18	12	17	28	14	10	22	13	13	12	15	18	23	24	11	13	9	13	17	11	13	15	12
008-12	-	18	12	20	28	14	10	26	11	11	10	16	20	22	22	12	11	12	14	16	13	14	14	11
011-12	Greifswald	19	13	19	29	14	11	22	13	12	12	15	18	23	24	11	13	10	13	17	11	14	17	11
013-12	-	17	13	20	32	17	10	25	12	12	11	14	19	23	25	10	11	10	13	17	9	14	15	12
015-12	Rostock	19	12	21	28	16	10	23	12	11	10	15	17	22	25	12	11	13	13	17	13	16	15	10
021-12	-	18	12	20	28	14	10	26	12	12	10	16	19	22	23	11	11	12	13	15	14	15	14	11
026-12	Anklam	18	13	19	29	14	11	22	13	12	12	15	17	23	25	13	13	10	13	17	11	14	16	12
031-12	-	18	13	20	29	15	12	22	13	12	12	15	17	23	24	13	13	9	13	17	11	11	14	12
033-12	Pasewalk	18	13	19	29	14	10	22	13	12	12	15	17	24	24	11	13	10	13	16	11	14	15	12
038-12	-	15	13	19	28	14	11	20	13	12	12	15	17	24	24	11	13	10	13	16	11	14	15	12
041-12	-	18	13	19	28	14	10	22	12	12	12	15	17	23	24	13	13	10	14	16	11	14	15	12
042-12	-	17	14	21	31	15	10	23	11	13	11	14	18	23	23	11	11	10	13	15	11	14	16	11
043-12	Levin	18	13	20	31	14	11	24	12	13	11	14	19	27	25	10	11	10	13	17	11	14	16	12
044-12	Greifswald	17	13	20	31	15	10	28	12	11	10	14	20	21	20	11	12	13	14	15	15	15	14	10
048-12	Ueckermünde	19	14	20	30	13	9	29	11	11	10	14	24	21	24	10	11	12	13	17	13	14	17	12

050-12	Stralsund	17	13	19	29	15	11	24	13	13	12	15	16	23	24	13	13	10	13	17	11	14	17	11
053-12	-	16	13	19	29	15	10	20	11	11	10	14	19	21	23	10	14	11	14	17	11	14	14	12
055-12	-	17	12	20	28	14	10	25	14	11	10	16	21	22	23	11	11	12	13	15	14	15	15	10
058-12	Wolgast	17	14	20	31	16	10	28	12	12	10	14	18	21	23	11	12	12	14	14	14	15	15	11
061-12	-	16	14	19	30	14	11	22	14	12	12	15	17	23	23	11	13	10	13	17	11	14	17	12
062-12	Greifswald	17	13	18	29	14	11	23	12	12	12	14	17	24	25	11	13	10	12	16	11	14	16	12
070-12	Neustrelitz	19	14	19	31	14	10	22	12	13	12	14	18	23	24	12	13	10	12	16	11	13	16	12
071-12	-	17	13	19	29	14	10	22	12	12	12	16	17	23	24	13	13	10	13	17	11	14	15	13
075-12	Greifswald	17	12	20	28	14	10	25	12	11	10	16	21	21	23	11	11	12	13	16	14	14	14	11
076-12	Neustrelitz	19	13	20	31	15	10	25	12	12	11	14	19	23	25	11	11	10	14	16	11	14	17	12
077-12	Greifswald	19	13	19	29	16	10	23	12	12	11	14	17	24	24	11	11	10	13	14	11	14	16	12
080-12	-	18	13	19	29	14	11	23	12	12	12	15	17	23	25	11	13	10	13	17	11	14	15	12
081-12	Pasewalk	17	13	20	28	16	10	25	12	12	11	14	21	23	24	11	11	10	13	16	11	14	15	12
085-12	Pasewalk	17	13	19	29	14	10	22	13	12	12	15	20	25	24	12	13	12	13	17	11	14	16	12
087-12	-	18	14	19	30	14	11	22	13	12	12	15	17	22	24	12	13	10	13	17	11	14	18	12
089-12	Güstrow	17	14	21	30	14	10	23	11	11	9	15	18	21	23	13	11	10	12	14	14	18	17	11
090-12	Greifswald	16	13	19	30	14	10	22	13	12	12	15	17	23	24	12	13	10	13	18	11	14	15	13
091-12	-	18	13	20	29	16	11	23	12	12	11	14	20	23	25	10	11	10	13	16	11	15	16	12
096-12	Wolgast	18	14	20	32	15	10	27	12	12	10	14	18	21	23	11	12	12	14	14	15	16	15	11
099-12	-	16	13	19	29	14	11	24	12	12	12	15	18	23	23	12	13	10	13	17	11	14	15	12
101-12	Barth	15	12	20	28	14	10	24	12	11	10	16	19	21	22	11	11	12	13	14	13	14	14	11
102-12	Greifswald	15	12	21	28	15	10	22	13	9	10	16	21	22	22	12	11	11	14	16	14	15	16	12
103-12	Rostock	18	13	20	31	16	10	25	12	12	11	14	18	23	25	10	11	10	13	17	10	14	15	12
105-12	Teterow	17	13	19	29	14	11	22	13	13	12	16	18	23	24	11	13	10	13	16	11	15	16	12
107-12	-	16	13	19	30	14	10	22	13	12	12	15	17	23	26	12	13	10	13	17.2	11	11	16	12
108-12	Pasewalk	18	13	19	29	14	10	21	11	12	12	15	18	23	24	12	13	11	13	17	11	15	16	11
110-12	Greifswald	19	13	20	29	14	10	22	13	12	12	15	17	23	23	11	13	10	13	17	12	14	16	12
118-12	-	18	13	19	28	14	11	22	12	13	12	15	16	23	23	12	12	10	13	18	11	14	18	12
125-12	Stralsund	16	12	20	28	14	10	26	12	11	10	16	18	24	23	12	11	12	13	15	14	14	14	11

130-12	Greifswald	17	13	20	31	15	10	28	12	11	10	14	20	21	20	11	12	13	14	15	15	15	14	10
137-12	Rostock	17	13	20	30	14	10	22	14	12	12	15	18	23	24	11	13	10	13	18	11	14	15	12
142-12	Greifswald	18	13	20	29	15	10	25	12	12	11	14	19	23	25	11	11	10	13	16	11	14	16	12
143-12	Stralsund	17	13	20	30	13	10	22	12	12	10	14	19	22	23	13	11	13	13	15	16	18	15	12
155-12	Greifswald	16	12	19	28	14	10	22	12	12	12	15	16	23	23	11	13	10	13	17	11	14	16	12

Summary table of the Asturias population (North of Spain)

General data	STR loci																						
ID.	DYS 576	DYS 389 I	DYS 448	DYS 389 II	DYS 19	DYS 391	DYS 481	DYS 549	DYS 533	DYS 438	DYS 437	DYS 570	DYS 635	DYS 390	DYS 439	DYS 392	DYS 643	DYS 393	DYS 458	DYS 385a/b		DYS 456	YGATA H4
ID 000	17	13	20	31	13	10	26	14	11	10	14	18	20	24	11	11	11	13	19	16	19	15	11
ID 022	17	13	20	31	13	10	26	13	10	10	14	18	21	24	11	11	11	13	17	16	17	15	11
ID 026	16	14	18	30	14	11	22	14	12	12	14	17	23	24	12	13	9	13	16	12	14	15	12
ID 040	19	13	19	29	14	11	23	13	11	12	15	17	23	25	11	13	10	13	17	11	14	16	12
ID 049	18	13	19	29	14	10	23	13	12	12	15	18	23	23	11	13	10	13	16	11	14	15	11
ID 051	18	13	19	28	14	11	23	12	12	12	15	17	23	24	12	13	10	13	16	11	14	15	12
ID 058	17	14	20	30	13	9	28	11	11	10	14	23	21	23	11	11	12	13	17	14	16	15	12
ID 069	17	13	19	29	14	11	22	13	12	12	15	17	23	24	11	13	10	12	17	11	14	17	12
ID 072	16	13	21	29	15	9	23	12	13	9	14	17	22	23	11	11	11	12	15	12	17	16	12
ID 107	17	14	21	30	17	10	22	9	12	10	15	20	21	23	12	11	12	13	18	11	12	15	13
ID 110	18	13	19	29	14	10	22	13	12	12	15	17	23	24	12	13	12	13	17	11	14	16	13
ID 111	19	14	19	30	14	11	21	11	13	12	14	18	23	24	12	13	10	13	17	11	14	16	12
ID 112	18	13	19	30	14	11	22	13	12	12	15	17	23	24	12	13	11	13	17	11	15	15	12
ID 113	16	14	18	30	14	11	22	14	12	12	14	17	23	24	12	13	9	13	16	12	14	15	12
ID 115	14	12	21	28	16	11	21	12	9	10	16	18	22	22	11	11	11	14	17	14	14	15	11
ID 114	16	13	19	29	14	10	21	15	13	12	15	17	24	24	11	12	11	13	16	11	14	15	12
ID 118	19	13	19	29	14	11	22	14	12	12	15	17	24	23	12	13	10	13	16	11	14	15	12
ID 119	16	13	19	30	15	10	22	12	12	9	14	19	21	23	11	13	10	13	19	14	16	15	11
ID 120	18	13	19	30	14	11	23	13	13	12	15	18	23	24	11	13	10	13	16	11	14	15	12
ID 122	18	14	19	30	13	11	23	12	11	12	15	17	23	24	13	13	9	13	18	11	14	17	12
ID 124	19	12	20	29	14	10	22	12	12	10	14	18	19	24	12	11	11	13	15	17	20	16	13
ID 127	16	13	19	29	14	11	22	13	12	12	15	17	23	24	12	13	10	13	17	11	14	18	12
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