# Forensic Genetics and Legal Medicine 2019-2020

## 15° April 2020

## DNA polymorphisms in forensics

## Forensic genetics

- ✓ identification of human (and non human) biological stains in criminal investigations
- ✓ “criminal DNA databases”
- ✓ paternity and kinship testing
- ✓ positive identification of: putrified, dismembered, skeletonized, carbonized, etc. bodies (missing person and disaster victim identification)
- ✓ investigative leads: determining tissue origin of a stain, ancestry inference, “genetic phenotyping”;
- ✓ contribution to the determination of the cause of death (“molecular autopsy”)


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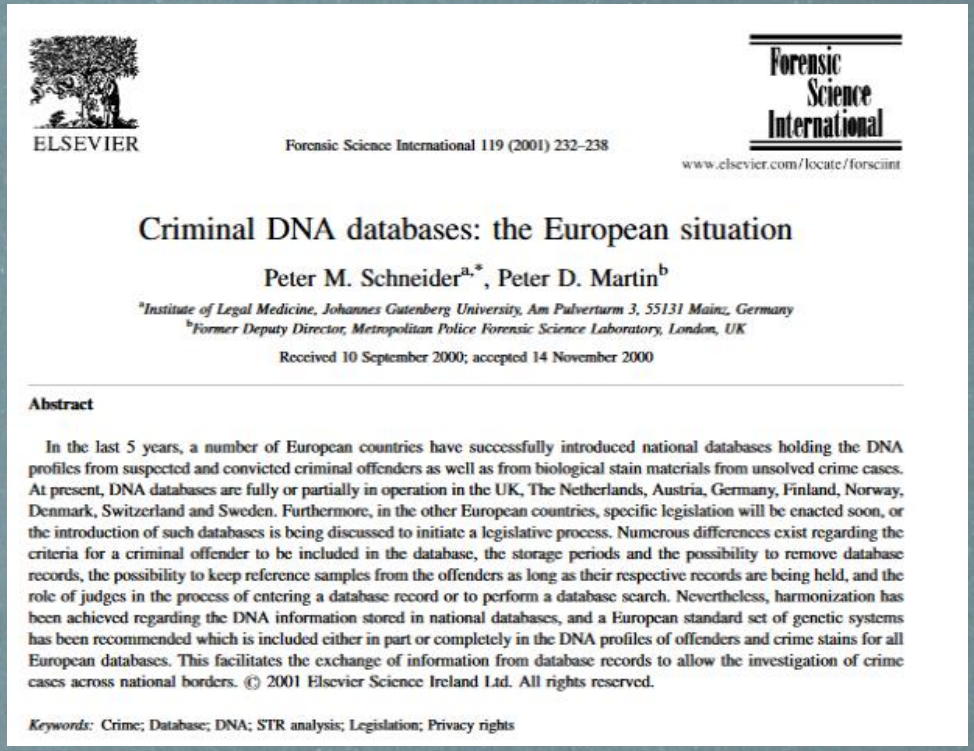

ELSEVIER

Forensic Science International 119 (2001) 232–238

Forensic Science International

www.elsevier.com/locate/forsciint

# Criminal DNA databases: the European situation

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**Abstract**

In the last 5 years, a number of European countries have successfully introduced national databases holding the DNA profiles from suspected and convicted criminal offenders as well as from biological stain materials from unsolved crime cases. At present, DNA databases are fully or partially in operation in the UK, The Netherlands, Austria, Germany, Finland, Norway, Denmark, Switzerland and Sweden. Furthermore, in the other European countries, specific legislation will be enacted soon, or the introduction of such databases is being discussed to initiate a legislative process. Numerous differences exist regarding the criteria for a criminal offender to be included in the database, the storage periods and the possibility to remove database records, the possibility to keep reference samples from the offenders as long as their respective records are being held, and the role of judges in the process of entering a database record or to perform a database search. Nevertheless, harmonization has been achieved regarding the DNA information stored in national databases, and a European standard set of genetic systems has been recommended which is included either in part or completely in the DNA profiles of offenders and crime stains for all European databases. This facilitates the exchange of information from database records to allow the investigation of crime cases across national borders. 

*Keywords:* Crime; Database; DNA; STR analysis; Legislation; Privacy rights

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Forensic Science International: Genetics 18 (2015) 21–32

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## Molecular approaches for forensic cell type identification: On mRNA, miRNA, DNA methylation and microbial markers

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ARTICLE INFO

*Keywords:*
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Body fluid identification
mRNA
miRNA
DNA methylation
Microbial species

ABSTRACT

Human biological traces have the potential to present strong evidence for placing a suspect at a crime scene. In cases, the activity that led to deposition of an individual's cellular material is increasingly disputed, for which the identification of cell types could be crucial. This review aims to give an overview of the possibilities of the employment of mRNA, miRNA, DNA methylation and microbial markers for tissue identification in a forensic context. The biological background that renders these markers tissue-specificity is considered, as this can affect data interpretation. Furthermore, the forensic relevance of inferring certain cell types is discussed, as are the various methodologies that can be applied. Forensic stains can carry minute amounts of cell material that may be degraded or polluted and most likely cell material of multiple sources will be present. The interpretational challenges that are imposed by this compromised state will be discussed as well.



Forensic Science International: Genetics 18 (2015) 33–48

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## Forensic DNA Phenotyping: Predicting human appearance from crime scene material for investigative purposes☆

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*Keywords:*
Appearance
Externally visible traits
Physical traits
Forensic DNA Phenotyping
FDP
DNA intelligence

ABSTRACT

Forensic DNA Phenotyping refers to the prediction of appearance traits of unknown sample donors, or unknown deceased (missing) persons, directly from biological materials found at the scene. "Biological witness" outcomes of Forensic DNA Phenotyping can provide investigative leads to trace unknown persons, who are unidentifiable with current comparative DNA profiling. This intelligence application of DNA marks a substantially different forensic use of genetic material rather than that of current DNA profiling presented in the courtroom. Currently, group-specific pigmentation traits are already predictable from DNA with reasonably high accuracies, while several other externally visible characteristics are under genetic investigation. Until individual-specific appearance becomes accurately predictable from DNA, conventional DNA profiling needs to be performed subsequent to appearance DNA prediction. Notably, and where Forensic DNA Phenotyping shows great promise, this is on a (much) smaller group of potential suspects, who match the appearance characteristics DNA-predicted from the crime scene stain or from the deceased person's remains. Provided sufficient funding being made available, future research to better understand the genetic basis of human appearance will expectedly lead to a substantially more detailed description of an unknown person's appearance from DNA, delivering increased value for police investigations in criminal and missing person cases involving unknowns.

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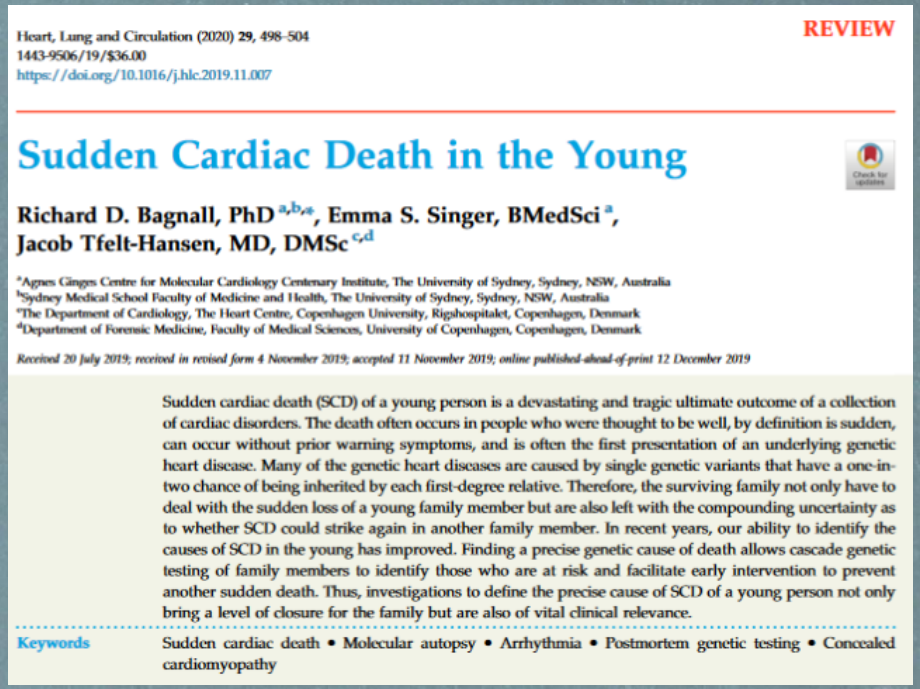

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1443-9506/19/$36.00
https://doi.org/10.1016/j.hlc.2019.11.007

REVIEW

# Sudden Cardiac Death in the Young

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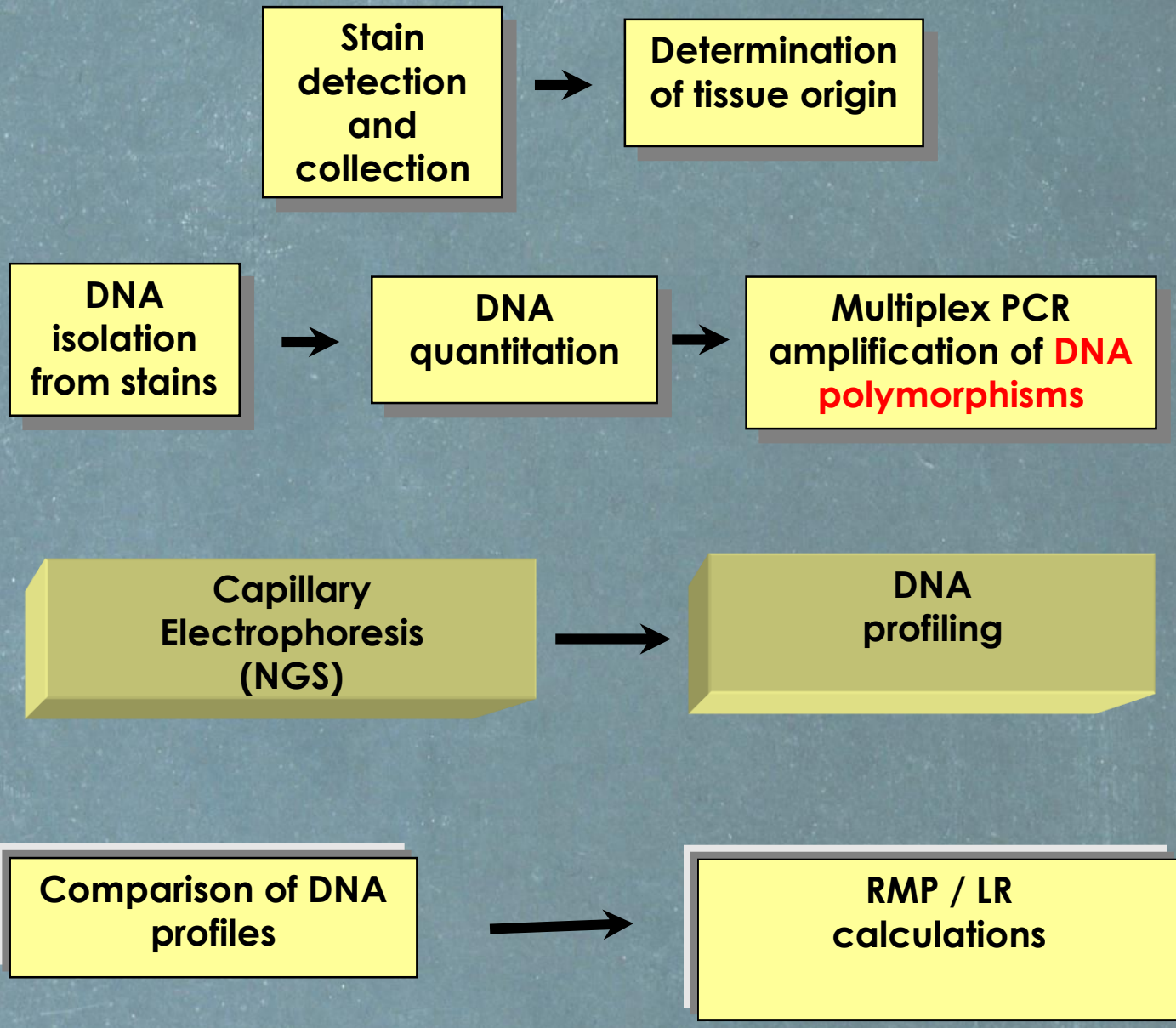
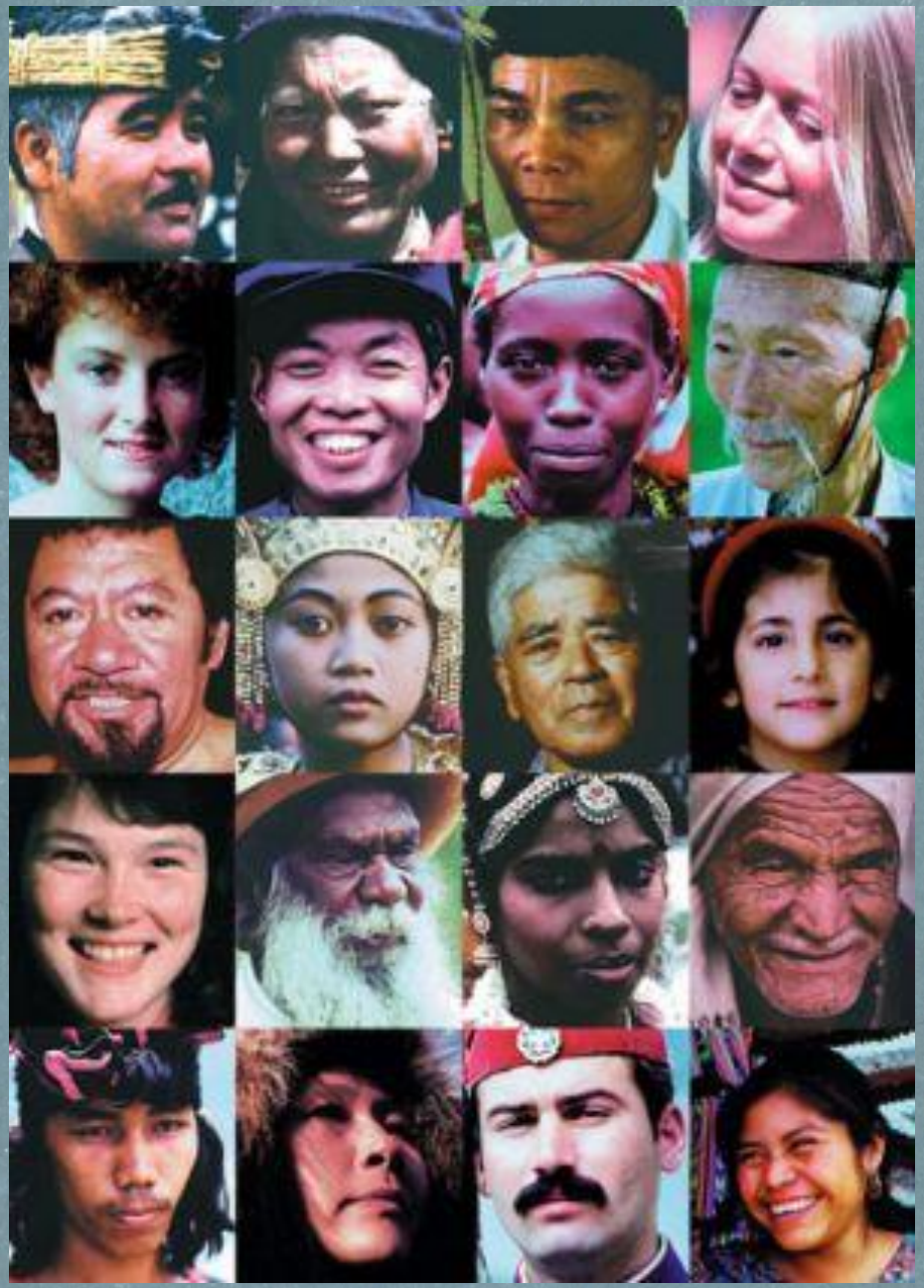
Sudden cardiac death (SCD) of a young person is a devastating and tragic ultimate outcome of a collection of cardiac disorders. The death often occurs in people who were thought to be well, by definition is sudden, can occur without prior warning symptoms, and is often the first presentation of an underlying genetic heart disease. Many of the genetic heart diseases are caused by single genetic variants that have a one-in-two chance of being inherited by each first-degree relative. Therefore, the surviving family not only have to deal with the sudden loss of a young family member but are also left with the compounding uncertainty as to whether SCD could strike again in another family member. In recent years, our ability to identify the causes of SCD in the young has improved. Finding a precise genetic cause of death allows cascade genetic testing of family members to identify those who are at risk and facilitate early intervention to prevent another sudden death. Thus, investigations to define the precise cause of SCD of a young person not only bring a level of closure for the family but are also of vital clinical relevance.

**Keywords** Sudden cardiac death • Molecular autopsy • Arrhythmia • Postmortem genetic testing • Concealed cardiomyopathy


Stain detection and collection
Determination of tissue origin
DNA isolation from stains
DNA quantitation
Multiplex PCR amplification of DNA polymorphisms
Capillary Electrophoresis (NGS)
DNA profiling
Comparison of DNA profiles
RMP / LR calculations



# Polymorphism

many shapes

- ✓ **The occurrence together in a population of two or more alternative genotypes**
- Genotype: combination of alleles present at a locus
- Allele: one of the alternative versions of a DNA sequence that may occupy a given locus
- Locus: the position of a DNA sequence on a chromosome

Genotypes and the environment in which they are espressed deterimine the observable biochemical, physiological, and morphological characteristics of individuals (**phenotypes**)

# Types of DNA Polymorphisms

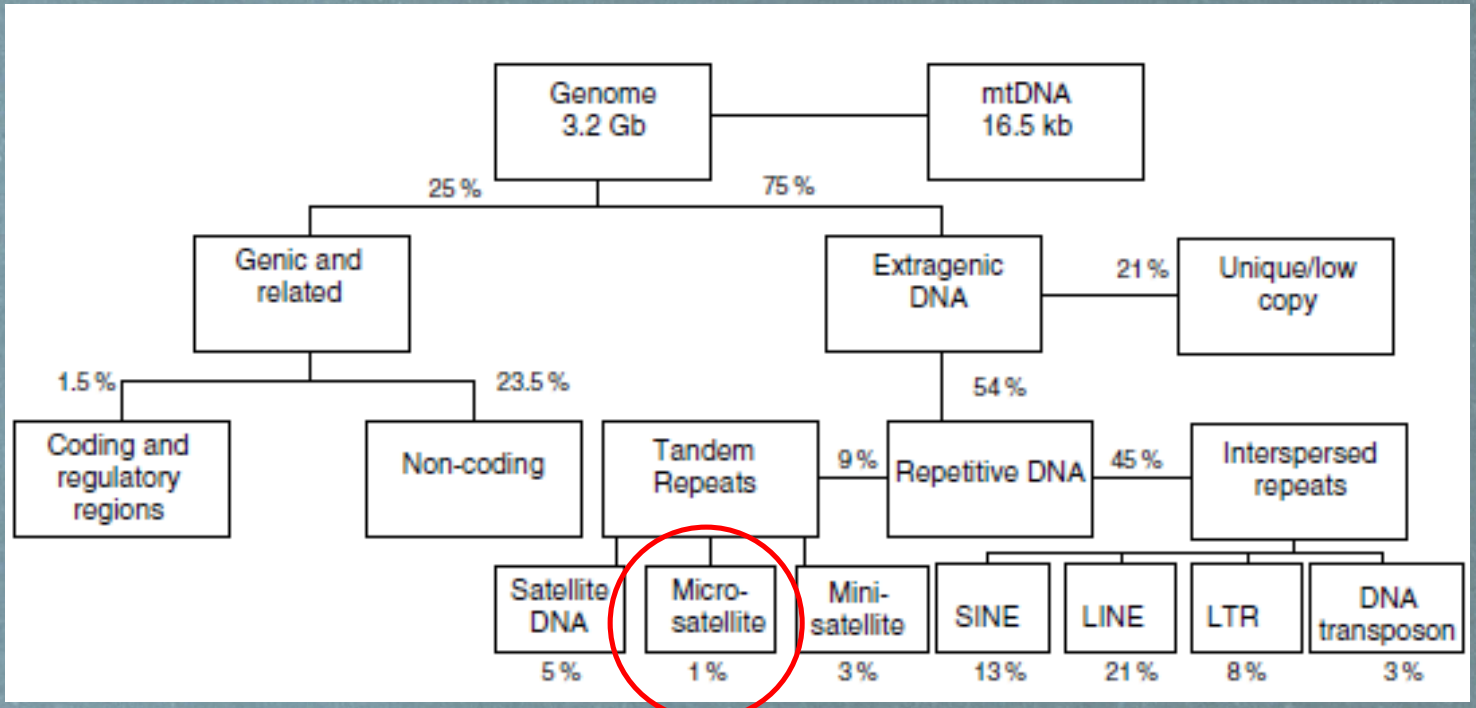
✓ human genomes are 99.9% identical

✓ 0.1% differences (4-5 x $10^6$ bases):

• single nucleotide polymorphisms, > 1.000.000, 1 every ~ 2 kb (Sachidanandam et al, Nature 2001)

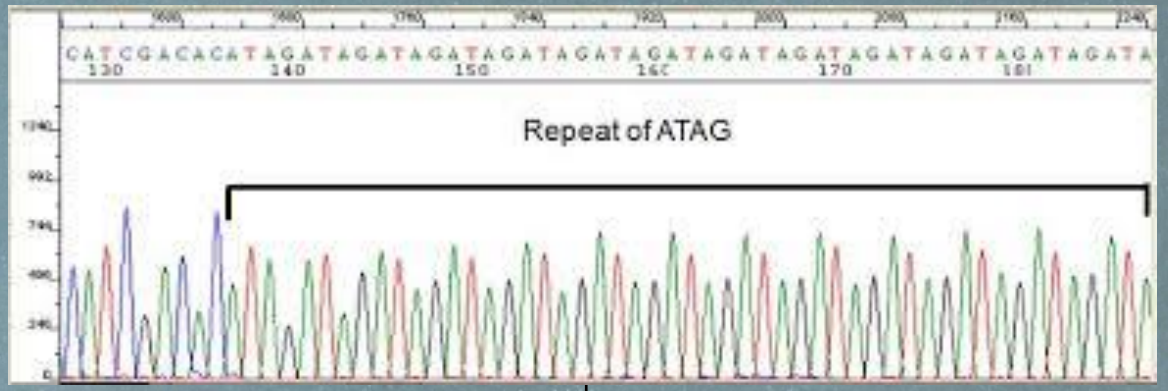
• insertion/deletion polymorphisms, > 400.000, 1 every ~ 7,2 kb (Mills et al, Genome Res 2006)

• repetitive DNA



✓ the most common polymorphisms in forensic genetics are a class of repetitive DNA called **microsatellites** or **short tandem repeat (STR)** loci

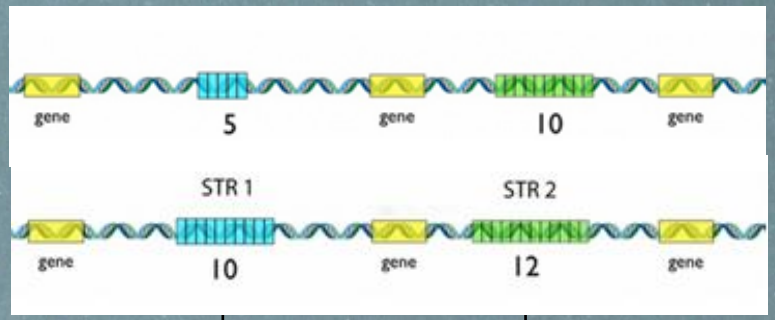
- about 7 x 10^5 STRs are dispersed in the human genome (Willems et al., Genome Research 2014)
- tandemly repeated repetitive units ("motif") of STRs range between 2 and 7 bases (bp); most of the STRs used in forensic have 4 bp motifs
- lenght of a repetitive block can vary between ~ 50 to 300 bp
- the number of repetition of the motif for a specific STR varies in the population
- STR alleles are defined by the number of repetitions of the motif



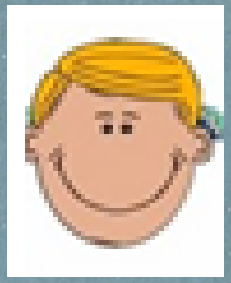

Need for a standard nomenclature!!!

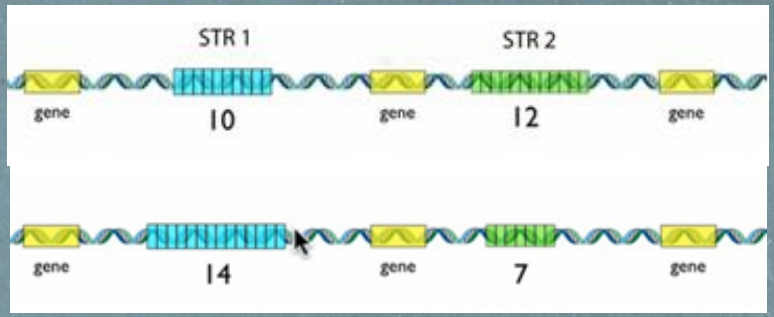
- STR alleles are inherited as mendelian traits

**Mother**




**Child**





# ISFG nomenclature guidelines for STRs (Bär et al, Forensic Sci Int 1997)

https://www.isfg.org/Publication;Bär1997

✓ DNA sequences are read in the 5′ to 3′ direction. The choice of the strand also influences the sequence designation. To avoid confusion, the following guidelines should be followed:

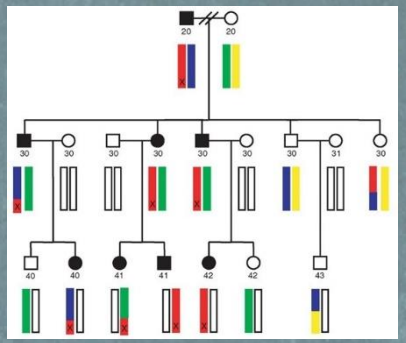
• for STRs within protein coding genes as well as in the intron of the genes e.g. vWA* locus), the coding strand should be used. The same applies to pseudogenes (e.g. SE33 locus), where the strand with the sequence similar to the coding strand of the “original” gene should be used

• for repetitive sequences without any connection to protein coding genes (D#S## loci**), the sequence originally described in the literature or the first public database entry shall become the standard reference (and strand) for nomenclature

• if a nomenclature is already established in the forensic field but not in accordance with the aforementioned guideline, the nomenclature shall be maintained to avoid unnecessary confusion

• for those situations where two or more nomenclatures already exist, priority should be given to the nomenclature that more closely adheres to the guidelines described here.

* many STRs were identified in linkage mapping studies for the identification of disease genes and carry the name of the specific gene (e.g. vWA: von Willebrand factor gene)
** # indicates chromosome number, ## is a progressive number



It is sometimes possible to define different repetitive motifs, even though the choice of the strand is clear. In the two following examples, the initial point for reading the repeat motif is different for the same sequence:

1. 5′–GGTCATCATCATGG–3′3×TCA

2. 5′–GGTCATCATCATGG–3′3×CAT

The recommendation is that the repeat sequence motif must be defined so that the first 5′-nucleotides that can define a repeat motif are used. Thus only the first edition is correct.

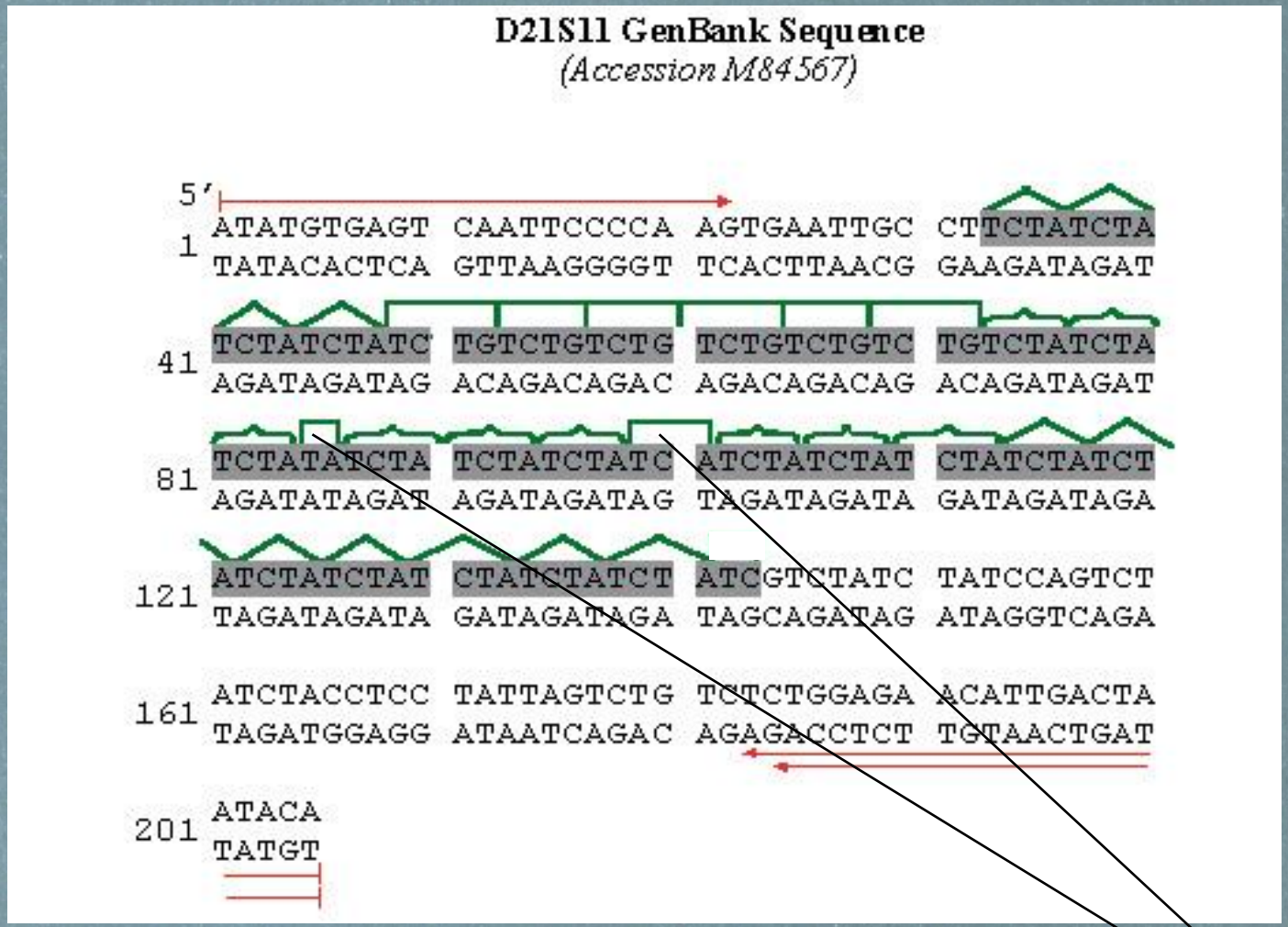
✓ Since the polymorphisms concerned are defined by variations in the number of repeats, allele designation basically should observe this structural principle.

• For simple systems, this is straightforward.

TPOX

| Allele Name | Repeat Sequence |
|---|---|
| 6 | AATGAATGAATGAATGAATGAATG |
| 7 | AATGAATGAATGAATGAATGAATGAATG |
| 8 | AATGAATGAATGAATGAATGAATGAATGAATG |
| 9 | AATGAATGAATGAATGAATGAATGAATGAATGAATG |
| 10 | AATGAATGAATGAATGAATGAATGAATGAATGAATGAATG |
| 11 | AATGAATGAATGAATGAATGAATGAATGAATGAATGAATGAATG |
| 12 | AATGAATGAATGAATGAATGAATGAATGAATGAATGAATGAATGAATG |
| 13 | AATGAATGAATGAATGAATGAATGAATGAATGAATGAATGAATGAATGAATG |

• For systems composed of repeat regions where the sequence may vary, designation of alleles should refer to the total number of full repeats, although the sequence can be different.




$[TCTA]_n + [TCTG]_n$

non-repertitive intervening sequence

• The designation of incomplete repeat motifs should include the number of complete repeats and, separated by a decimal point, the number of basepairs in the incomplete repeat (e.g. .1, .2, .3).

**TH01**

allele

aatg aatg aatg aatg aatg aatg aatg aatg aatg aatg - 10

aatg aatg aatg aatg aatg aatg atg aatg aatg aatg - 9.3

1998 Italian serial killer case in which investigators were initially unable to link two crimes because the two different labs who performed the analysis used unconsistent nomenclature

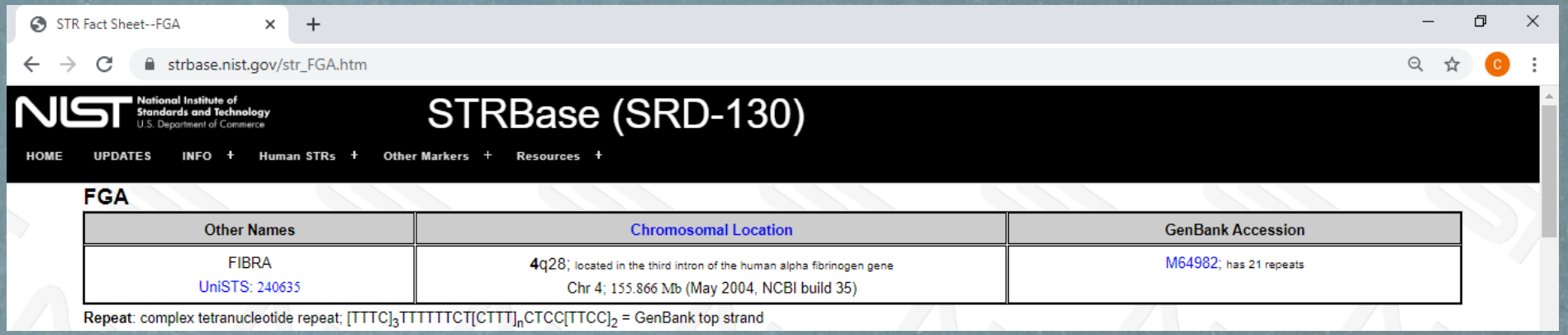
| | H'Didou | | Gallarini |
|---|---|---|---|
| Amelogenina | X;Y | Amelogenina | non eseguito |
| CSF1PO | 10;10 | CSF1PO | 10;10 |
| D13S317 | 11;12 | D13S317 | 11;12 |
| D16S539 | 11;12 | D16S539 | non eseguito |
| D18S51 | 14;20 | D18S51 | 14;20 |
| D21S11 | 59;61 | D21S11 | 27;28 |
| D3S1358 | 15;15 | D3S1358 | 15;15 |
| D5S818 | 11;12 | D5S818 | 11;12 |
| D7S820 | 11;11 | D7S820 | negativo |
| D8S1179 | 12;15 | D8S1179 | 12;15 |
| FGA | non eseguito | FGA | 20;24 |
| FIBRA | 20;24 | FIBRA | non eseguito |
| Penta D | non eseguito | Penta D | non eseguito |
| Penta E | non eseguito | Penta E | non eseguito |
| TH01 | 6;9 | TH01 | 6;9 |
| TPOX | 8;9 | TPOX | 8;9 |
| vWA | 17;20 | vWA | 17;20 |

Two different names for the same STR

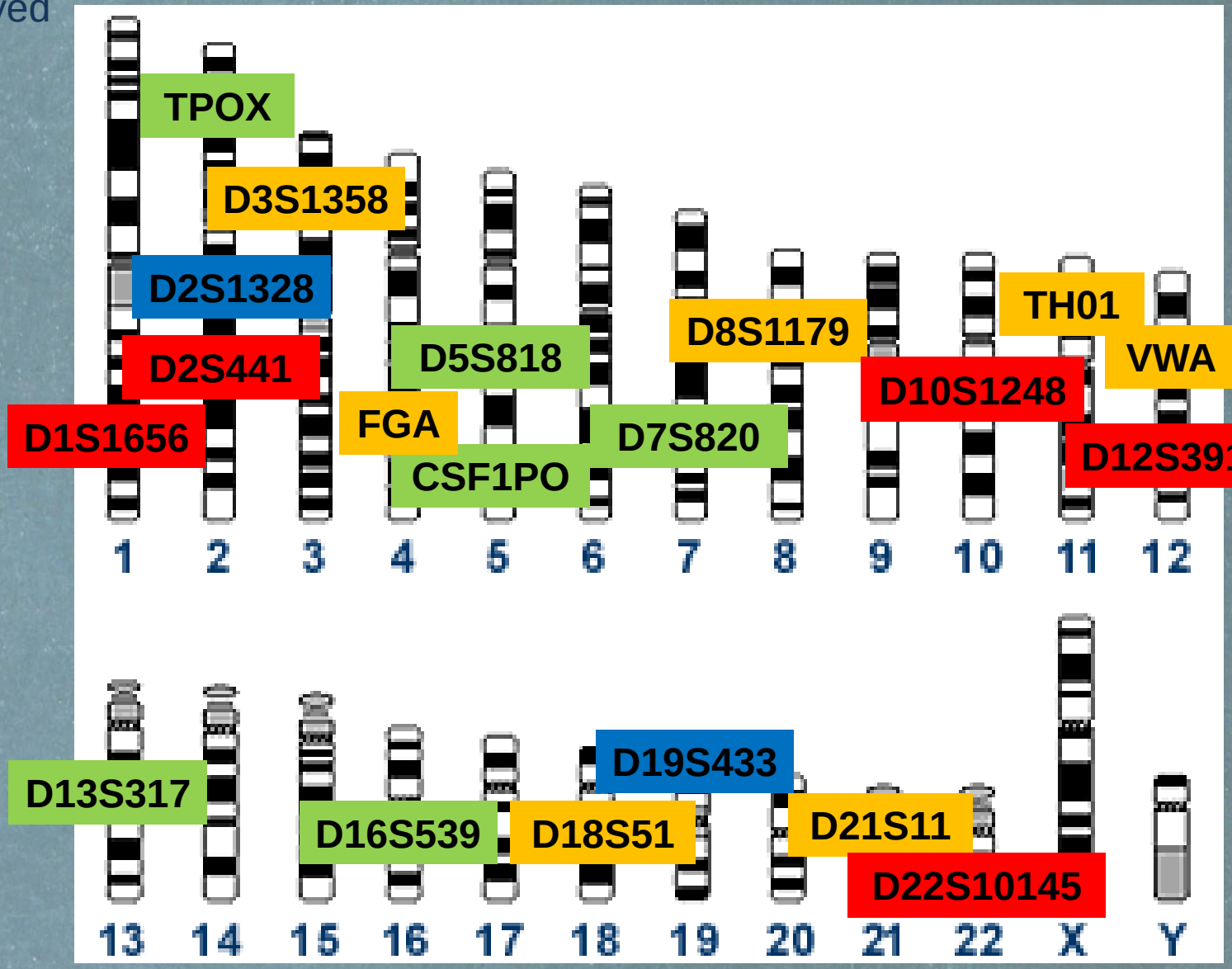
Two different nomenclatures

Nomenclature according to Urquhart et al Int J Legal Med 1994

Nomenclature according to Moller et al Int J Legal Med 1994



Need for data exchange between forensic laboratories requires that not only the same STR nomenclature is used all over the world, but also that largerly overlapping sets of STRs are employed




+ CODIS (USA) + ESS (Europe) + + + Expanded CODIS

- ✓ The Combined DNA Index System (CODIS) loci are the 13 core STR loci included in the U.S. National Criminal DNA database introduceded in the late ‘90s

- ✓ In 2001 the EU Council established an European Standard Set (ESS) of loci, mostly based on loci previously included in the UK National DNA database (active since 1995) to enable the comparison of DNA profiles from different countries

- ✓ In 2009 the ESS was expanded to include 5 additional STRs

- ✓ In 2017 the CODIS loci were expanded to include the 13 original core loci, all ESS loci, plus 2 additional loci

- ✓ Some countries, such as Gernany, use in their criminal DNA national databases and, consequently, in routine forensic investigations, STR markers that are not widely applied elsewhere (e.g. SE33)

- ✓ For kinship/paternity testing it is generally recommended to employ widely used forensic STR markers (CODIS/ESS loci) for which relevant information (population diversity, mutation rate) is easily derived from the literature

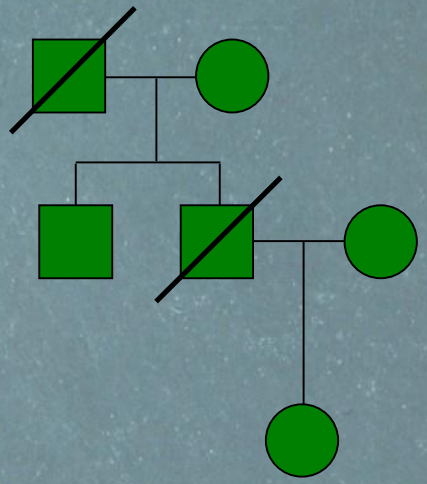
# Y-chromosomal STRs (Y-STRs)

- ✓ Y chromosome is specific for male sex, therefore analysis of Y-STRs allows investigators to identify a DNA profile of the male contributor in male/female mixed stains when female contribution is overwhelming

- ✓ Due to their peculiar transmission pattern (father transmits his Y chromosome to all sons without recombination) Y-STRs are also useful in kinship testing of alleged paternal relatives

- ✓ Several Y-STRs were validated for forensic purposes, first in in-house PCR multiplexes, then throuogh commercially available kits

# X chromosomal STRs (X-STRs)



- ✓ Due to their peculiar transmission pattern (males are hemizygous and transmit their X chromosome to all daughters without recombination) X-STRs are particularly useful in selected kinship cases (e.g. alleged maternal grandmother and niece, when sample from putative father is missing)
- ✓ Several X-STRs were validated for forensic purposes, first in in-house PCR multiplexes,

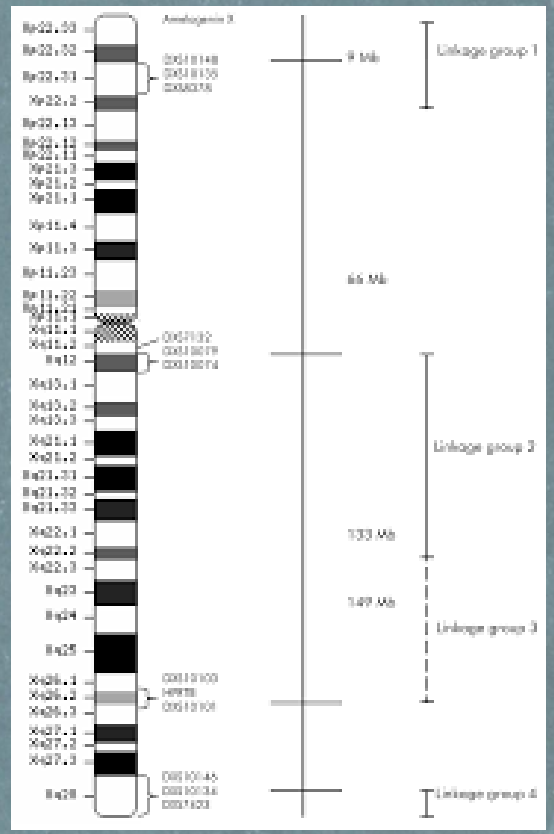
Int J Legal Med (5006) 120:315–318
DOI 10.1007/s00414-006-0115-9

SHORT COMMUNICATION

**Development of two multiplex PCR systems for the analysis of 12 X-chromosomal STR loci in a northwestern Italian population sample**

C. Robino · A. Giolitti · S. Gino · C. Torre

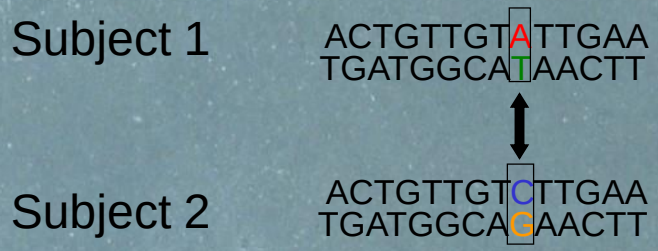
- ✓ A set of 12 X-STRs arranged in four clusters of linked markers is commercially available



# Non-STR forensic DNA polymorphisms

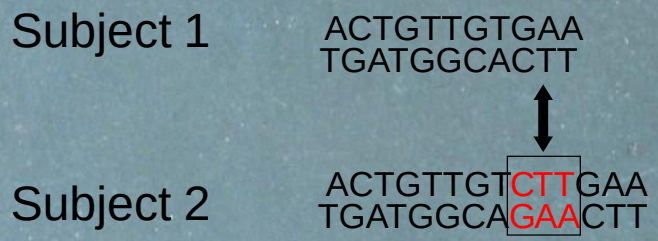
✓ **SNPs**

- interindividual difference does not consist in variable number of repertitions, but in the substitution of single bases in the DNA sequence




✓ **Indels**

- interindividual difference consist in presence/absence of sequence stretch of variable lenght



# STRs vs Non-STR forensic DNA polymorphisms

✓ STRs remain, at present, the forensic markers of choice for identification, kinship, and criminal DNA databasing purposes

- STRs are multiallelic (heterozygosity >70%) and therefore highly informative even in limited numbers
- SNPs and Indels are normally biallelic (in thebest case scenario, with two alleles each with 50% frequency in the population, heterozygosity will be 50%)
- ~ 50 SNPs/Indels are necessary to achieve the same informativity of ~15 STRs in identification cases (>>50 in paternity and kinship cases)
- It is easier to arrange 15 markers in a multiplex PCR reaction rather than 50

Probability of identity CODIS loci

| Locus | African-American | U.S. Caucasian | U.S. Hispanic | Native American |
|---|---|---|---|---|
| CSF1PO | 0.079 | 0.132 | 0.141 | 0.123 |
| D2S1338 | 0.023 | 0.027 | 0.038 | 0.043 |
| D3S1358 | 0.097 | 0.076 | 0.112 | 0.158 |
| D5S818 | 0.104 | 0.147 | 0.115 | 0.110 |
| D7S820 | 0.085 | 0.063 | 0.083 | 0.081 |
| D8S1179 | 0.074 | 0.064 | 0.089 | 0.104 |
| D13S317 | 0.132 | 0.079 | 0.056 | 0.056 |
| D16S539 | 0.077 | 0.097 | 0.090 | 0.082 |
| D18S51 | 0.033 | 0.031 | 0.031 | 0.046 |
| D19S433 | 0.042 | 0.087 | 0.049 | 0.044 |
| D21S11 | 0.037 | 0.044 | 0.047 | 0.074 |
| FGA | 0.034 | 0.035 | 0.032 | 0.031 |
| TH01 | 0.109 | 0.079 | 0.097 | 0.134 |
| TPOX | 0.089 | 0.188 | 0.168 | 0.159 |
| vWA | 0.066 | 0.066 | 0.080 | 0.103 |

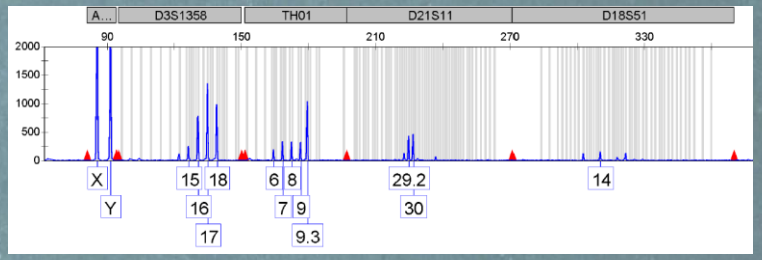
Best biallelic 0.375

Probability of paternity exclusion CODIS loci

| Locus | African-American | U.S. Caucasian | U.S. Hispanic | Native American |
|---|---|---|---|---|
| CSF1PO | 0.545 | 0.496 | 0.450 | 0.409 |
| D2S1338 | 0.748 | 0.725 | 0.671 | 0.399 |
| D3S1358 | 0.591 | 0.630 | 0.495 | 0.510 |
| D5S818 | 0.506 | 0.440 | 0.525 | 0.601 |
| D7S820 | 0.591 | 0.582 | 0.574 | 0.492 |
| D8S1179 | 0.580 | 0.680 | 0.599 | 0.601 |
| D13S317 | 0.383 | 0.487 | 0.638 | 0.370 |
| D16S539 | 0.649 | 0.566 | 0.567 | 0.428 |
| D18S51 | 0.760 | 0.731 | 0.767 | 0.329 |
| D19S433 | 0.601 | 0.531 | 0.678 | 0.360 |
| D21S11 | 0.737 | 0.708 | 0.586 | 0.399 |
| FGA | 0.760 | 0.766 | 0.739 | 0.309 |
| TH01 | 0.492 | 0.566 | 0.618 | 0.646 |
| TPOX | 0.521 | 0.329 | 0.392 | 0.687 |
| vWA | 0.709 | 0.625 | 0.555 | 0.528 |

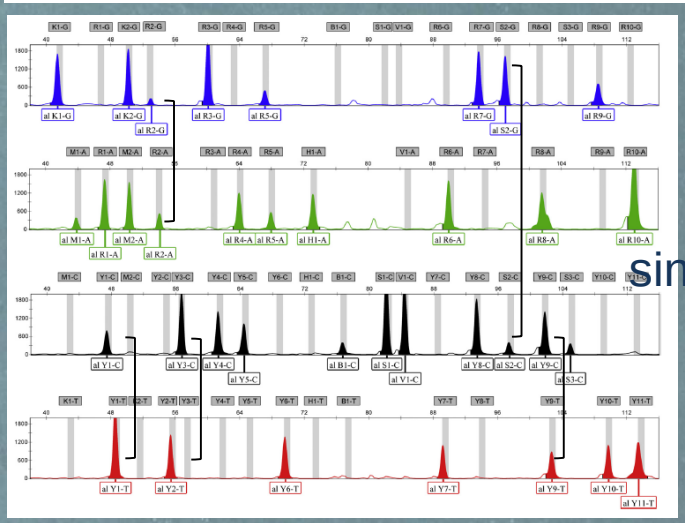
Best biallelic 0.250

- Being multiallelic STRs easily detected mixed stains (> alleles at multiple loci)




mixed

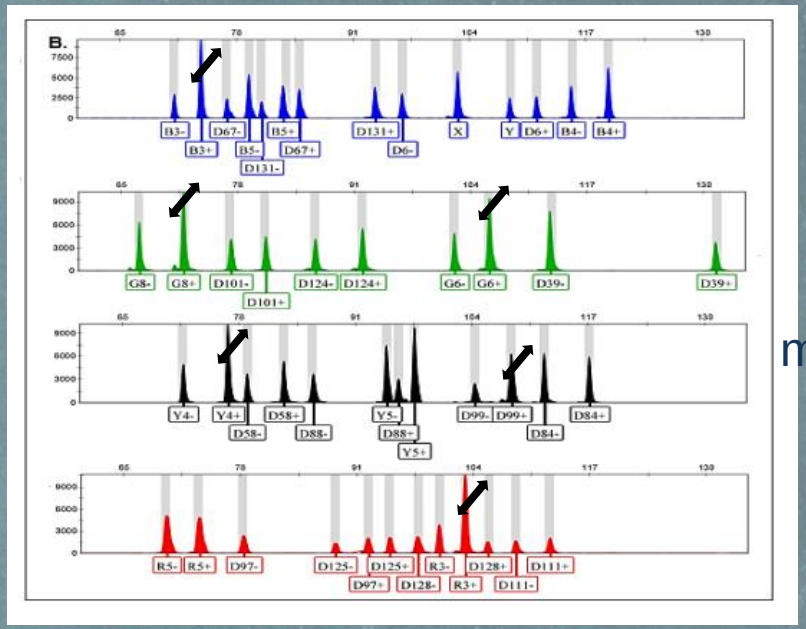
- Base incorporation in SBE* is not sensitive to original DNA quantity, so SNPs cannot easily identify mixed stains (unless triallelic SNPs are included in the assay)



single source

* the standard technique used to type SNPs by capillary electrophoresis platforms

- Although Indels are biallelic, typing is fully based on PCR and therefore quantitative within single loci (as in the case of STRs): imbalance in peak height ratio at multiple Indel loci detects mixed samples




mixed

## STRs vs Non-STR forensic DNA polymorphisms

- ✓ SNPs and Indels have properties that can make them particularly useful in specific situations

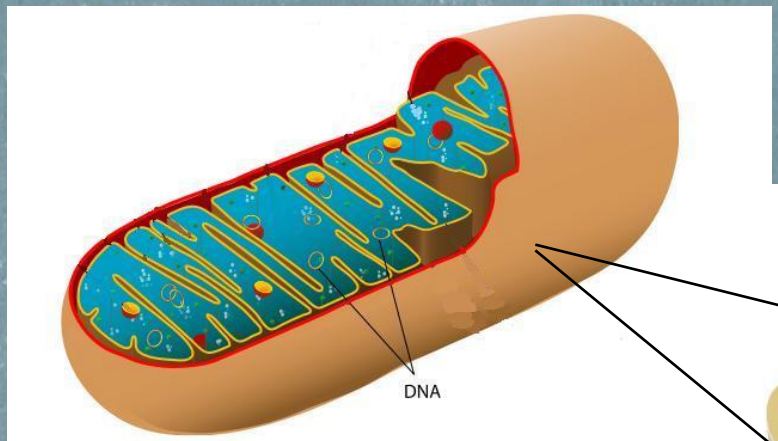
- Amplicon size for SNP typing is << then that required for many STRs; also for Indels, the shorter lenght of the polymorphic region enebles the cre.ation of PCR assays with amplicon size that, on average is shorter compared to STR kits → Analysis of degraded DNA

- SNPs/Indels have decidedly lower mutation rates (~1 x $10^7$ – $10^8$) compared to STRs (~1 x $10^3$) → Paternity and Kinship testing

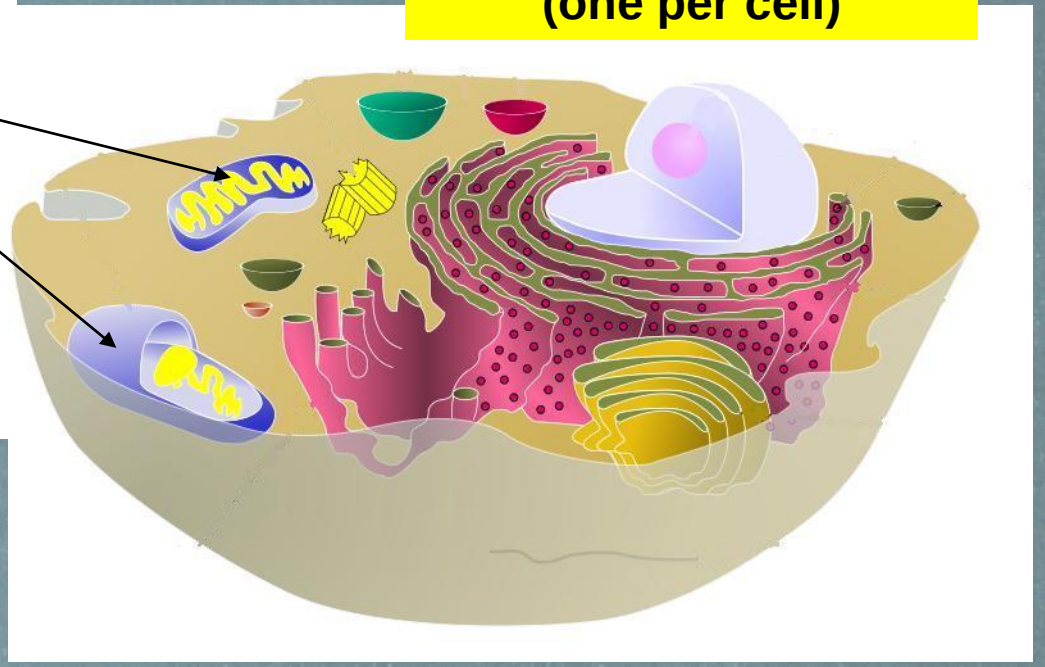
↓

In a paternity test with 20 STRs investigating 40 meiotic transmissions (20 paternal + 20 maternal) there is a ~40 x $10^3$ risk (1 every 25 tests) to observe a mutation that can complicate data interpretation!

# Mitochondrial DNA (mtDNA)




**The Nucleus = control center for the cell (one per cell)**

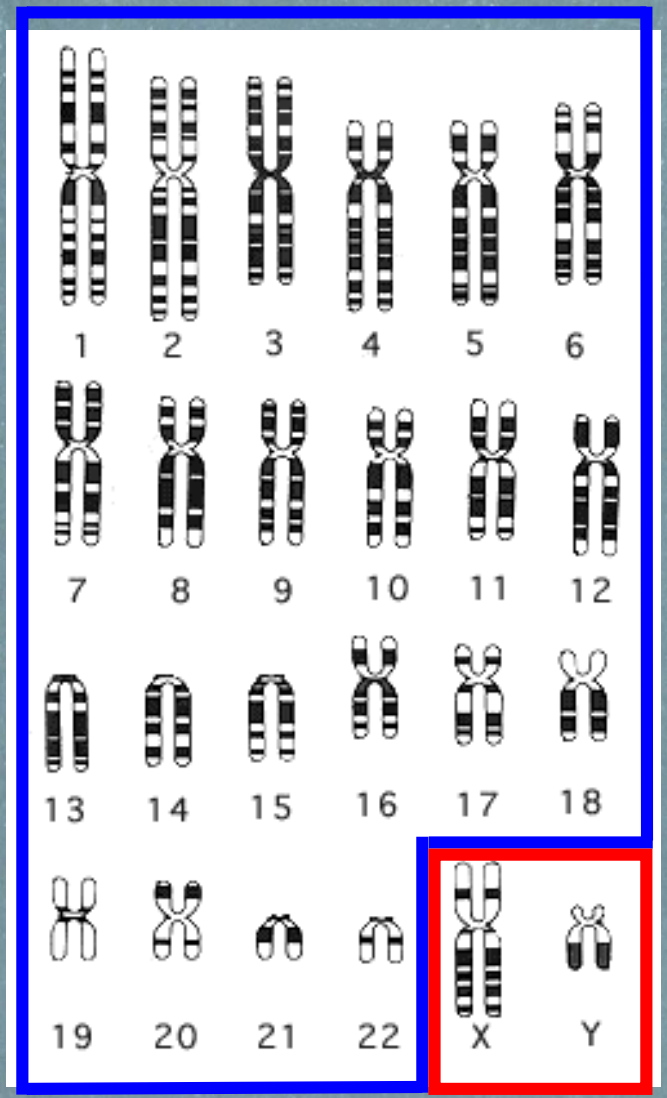


**Mitochondria = the power houses for the cell (hundreds per cell)**

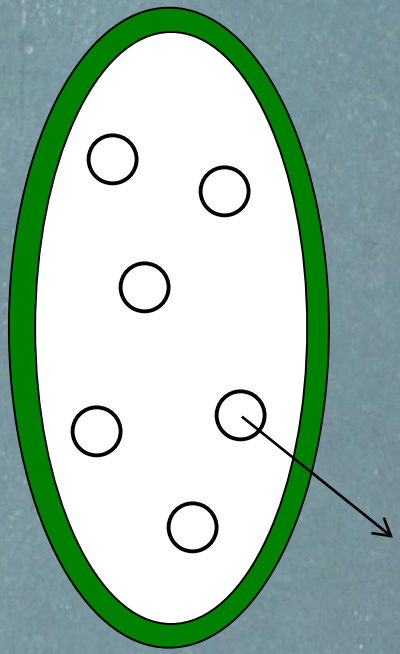
✓ each mitochondrion contains several copies of the same round mitochondrial DNA (**mtDNA**), which is only 16,569 bp long, compared to 3 x $10^9$ bp of nuclear DNA)

✓ mtDNA is “haploid” (one copy only of each information) and not diploid like nuclear DNA

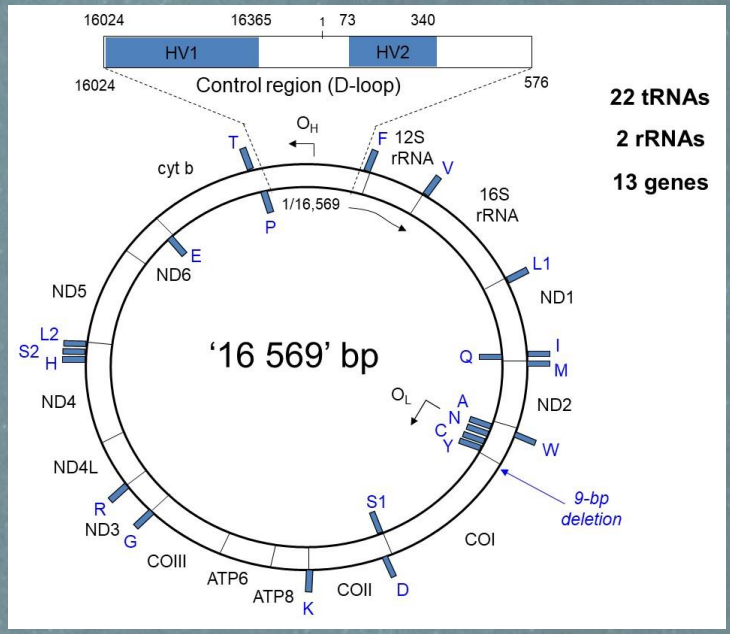
**Autosomes** – 22 pairs –
2 copies per cell



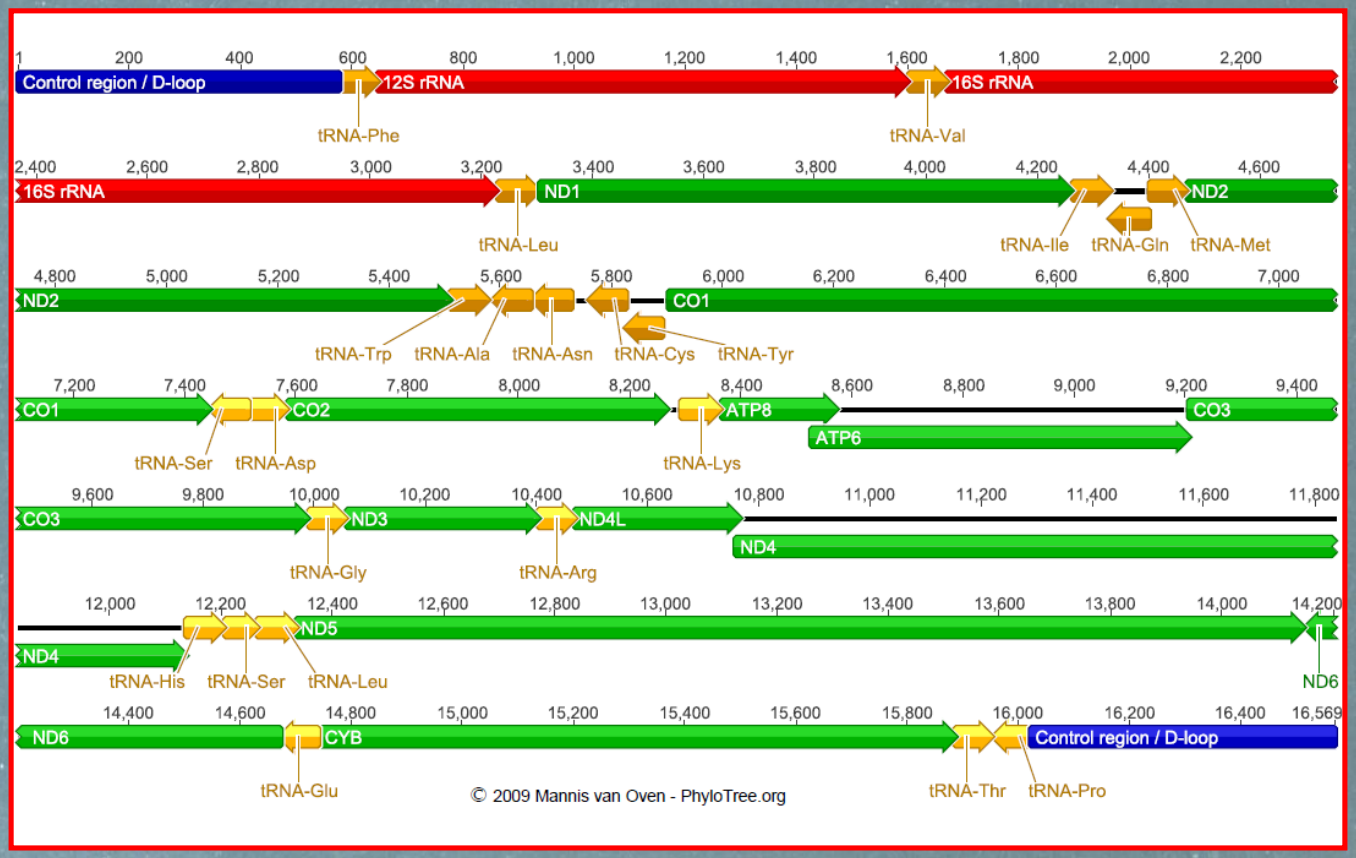

**Sex Chromosomes (XX or XY)**



**mitochondria** – several mitochondria in cell cytoplasm
- 100s of mtDNA copies per cell




mtDNA encodes for 22 transfer RNAs and 2 ribosomal RNAs plus 13 proteins, but most of human mitochondrial variation is concentrated in the non-coding region called d-loop



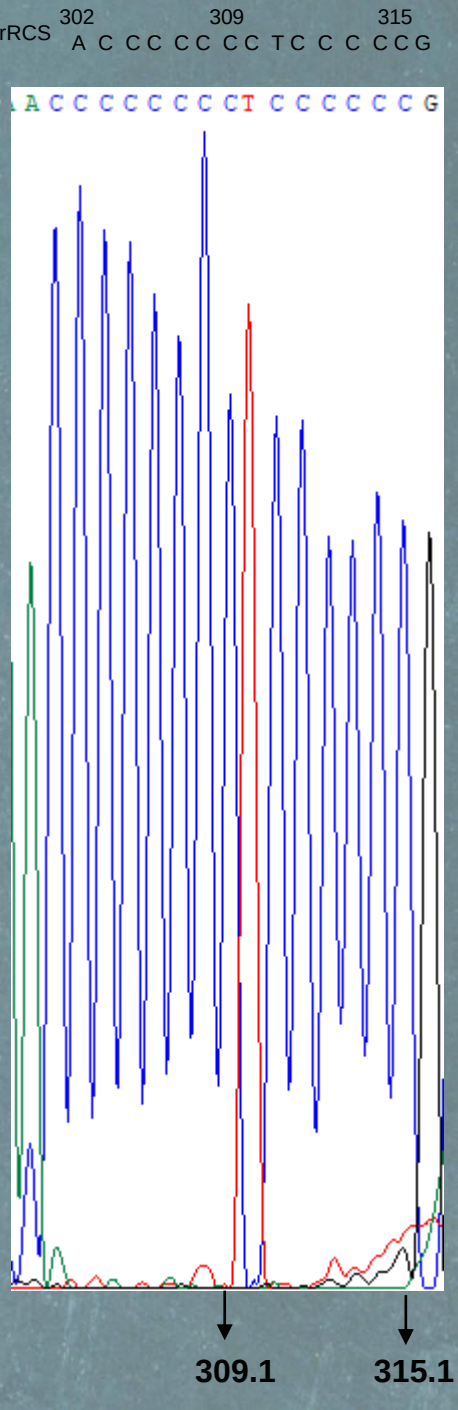

✓ mtDNA base positions are identified with standard numbers from 1 to 16569 (with position 1 being arbitrarily located in correspondance of a MboI restriction enzyme site on the L-strand of the d-loop)

✓ the array of base substitutions typical of a single individual (mtDNA haplotype) is identified by comparing all individuals with a reference mtDNA sequence called the revised Cambridge reference sequence (rCRS, NC001807), corresponding to the first human mtDNA completely sequenced in 1981 and then revised in 1999.

Standardized reporting of mtDNA variation (ISFG nomenclature) is fundamental in forensic science as it enables the comparison of mtDNA data from different studies or forensic cases.

ISFG recommendations https://www.isfg.org/Publication;Parson2014

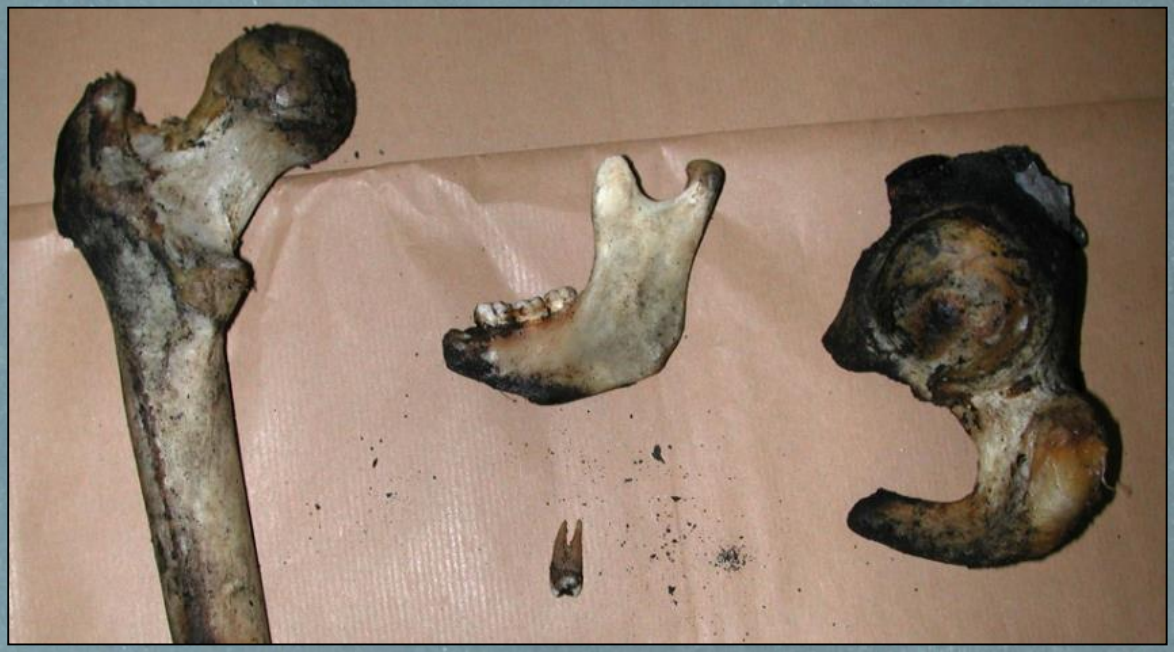
- ✓ When a difference between an individual's sequence and that of the rCRS is observed, only the site position number and the nucleotide differing from the reference standard are recorded. For example, at site 73 the rCRS has an A; however, a large portion of the population carries a G at site 73. Such an individual's mtDNA sequence is described as 73G.
- ✓ Insertions are described by first noting the site immediately 5' to the insertion, followed by a decimal point and a '1' (for the first insertion), a '2' (if there is a second insertion), and so on, and then by the nucleotide that is inserted. In the case of homopolymeric tracts, where the exact position at which the insertion has occurred is unknown, the assumption is always made that the insertion has occurred at the highest numbered end of the homopolymeric region.
- ✓ Deletions are recorded by listing the missing site followed by "DEL", "del", or "−" (Parson et al., Forensic Sci Int Genet 2014)



## Forensic applications of mtDNA typing

- ✓ Challanging samples with very little and/or highly degraded DNA

- Within a human cell, there are thousand of copies of mtDNA per copy of nuclear DNA, therefore it is much more likely to find non-fragmented copies of mtDNA rather than nuclear DNA



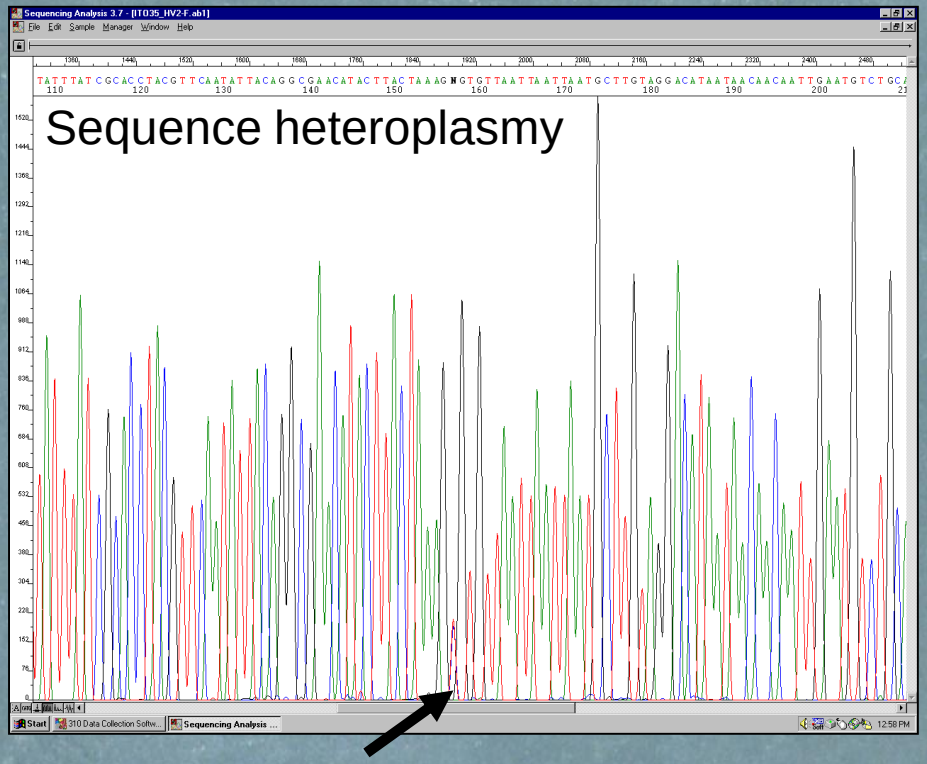
- ✓ Analysis of anucleated cells
- mtDNA is cytoplasmatic

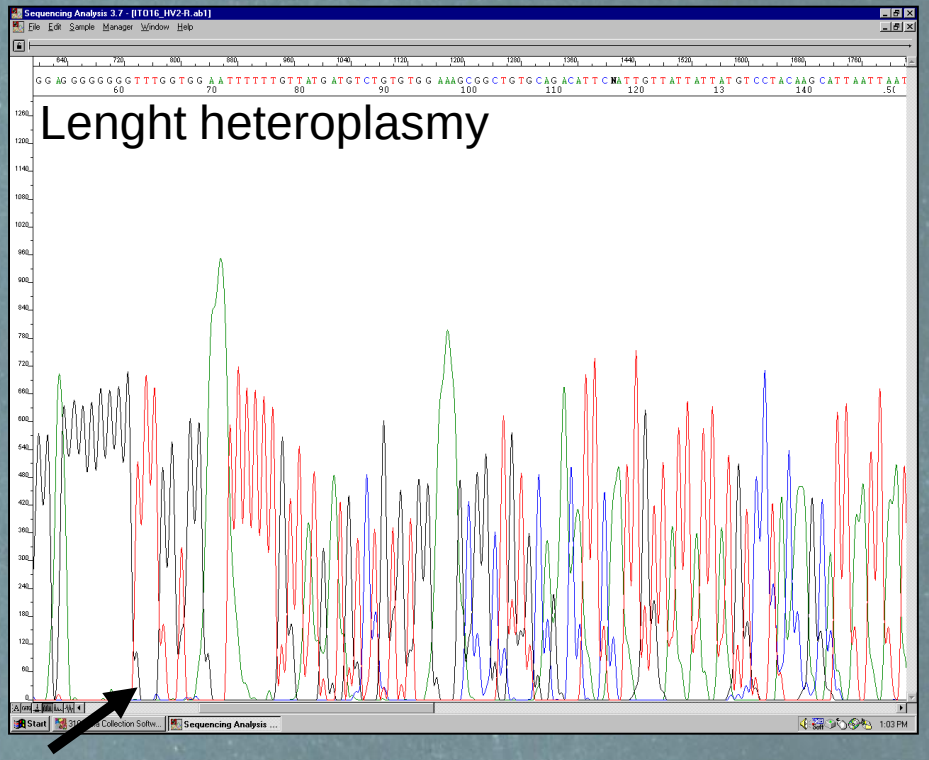

nucleated cells in hair are exclusively present in the root

Most of the hair found at the crime scene are shed hair, spontaneously falling at the end of their life cycle (telogen phase)

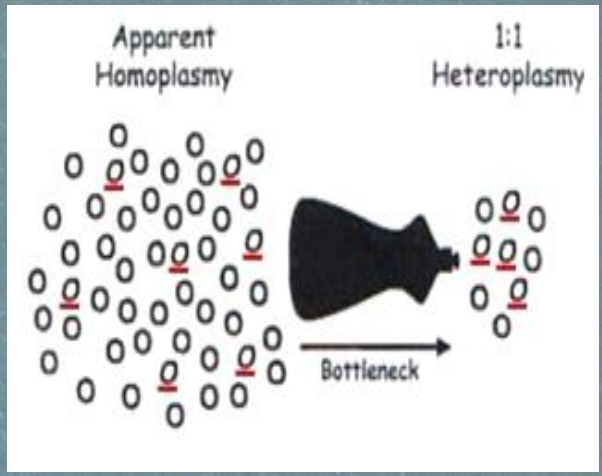
In telogen phase even root cells are completely keratinized and devoided of nuclei

✓ mtDNA **heteroplasmy** (presence of different mtDNA sequences within an individual, in all or some specific tissue)

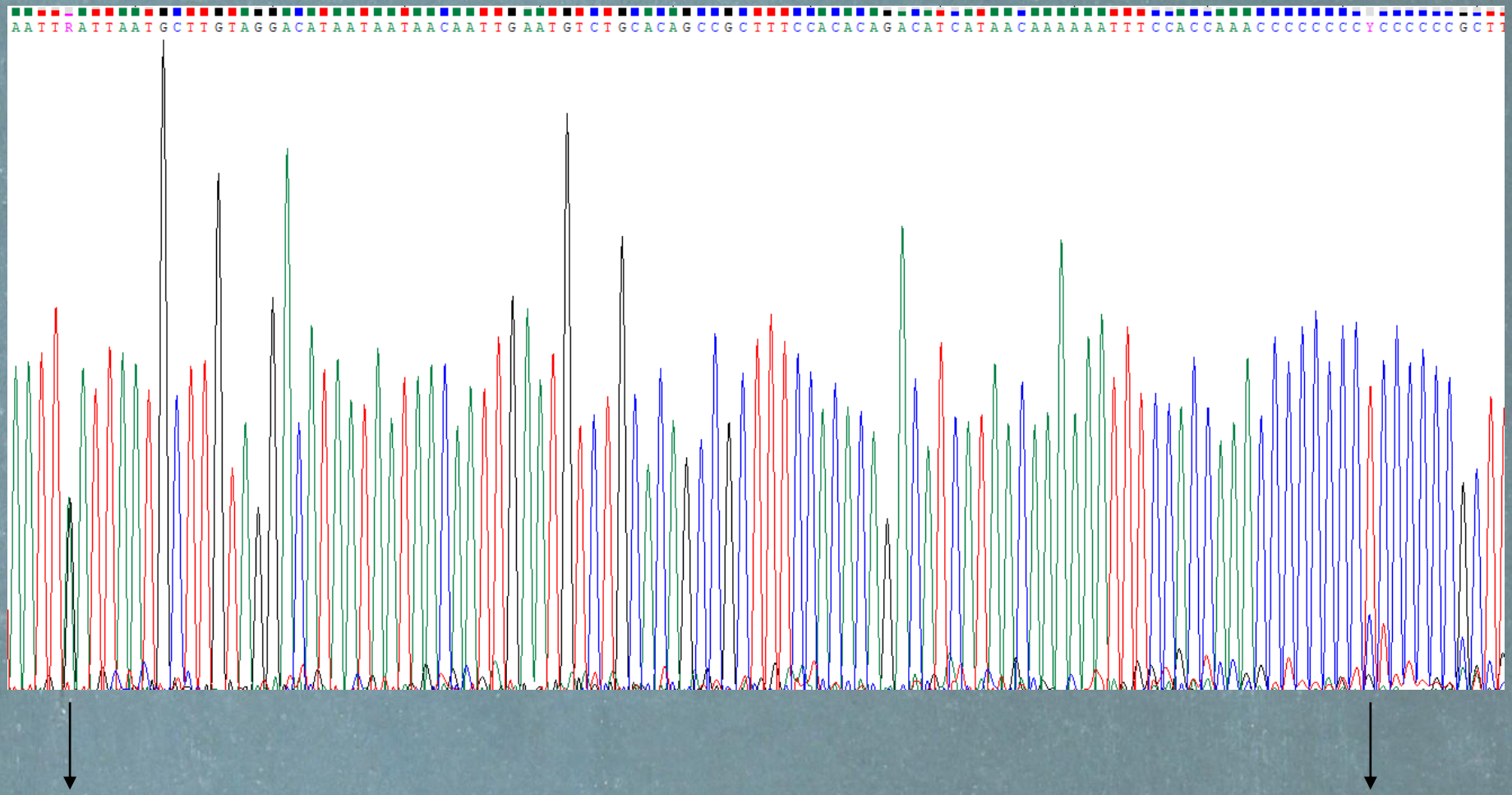



Observation of heteroplasmy is particularly common in hair sample, since hair mtDNA derive from a very small number of parent cells (those present in each root).
A mutation occurring in a parent cell is not masked by overwhelming «wild-type» sequences as (often) in other tissue types.



# Designation of heteroplasmic positions according to ISFG nomenclature

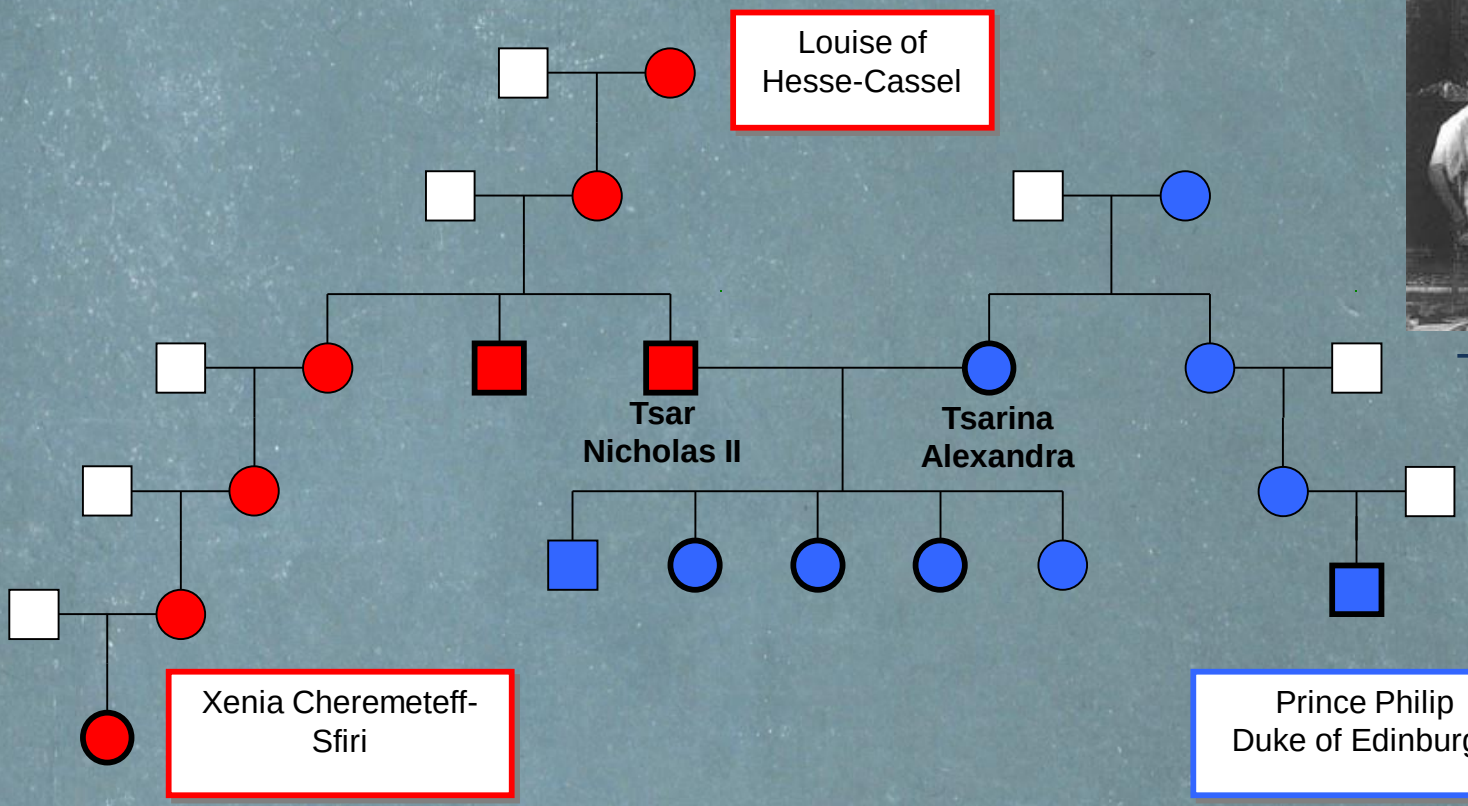



At position 214, subject carries a combination of A (as rCRS) and G, according to IUPAC code, the substitution is scored as 214R (capital letter)

A combination of 309.1C and 309.2C is observed, and scored as 309.2c (small letter). Small letters are also used for heteroplasmic mixtures of deleted/undeleted bases

✓ matrilinear transmission of mtDNA can be used in complex kinship cases and historical studies

- The zygote's cytoplasm is completely derived from the mother's egg, as a consequence all subjects having a common maternal ancestor) share the same mtDNA haplotype




The Romanoff