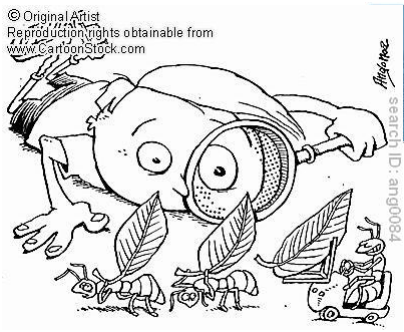
FORENSICS



Forensics

Forensic science is the application of a broad spectrum of sciences to answer questions of interest to the legal system.

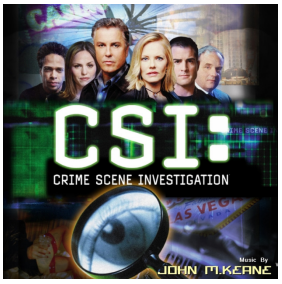
This may be in relation to a crime or to a civil action

Forensic comes from the Latin word "forensis" meaning forum (place of assembly; public)

Nowadays it is a synonym for "legal" or "related to courts"

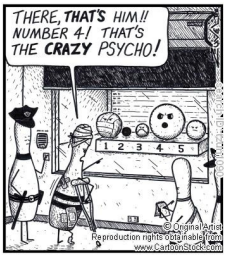
Forensics

Biology/genetics



- Chemistry
- Pathology
- Toxicology
- Dentistry
- Anthropology
- Engineering

DNA & crime scene



*Identify potential suspects

*Exonerate persons wrongly accused of crimes

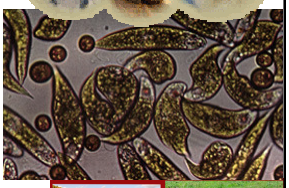
*Identify crime and catastrophe victims

*Establish paternity and other family relationships

DNA & crime scene

*Identify endangered and protected species as an aid to wildlife officials (could be used for prosecuting poachers)

*Detect bacteria and other organisms that may pollute air, water, soil, and food



*Match organ donors with recipients in transplant programs

*Determine pedigree for seed or livestock breeds

*Authenticate consumables such as caviar and wine

Advantages of DNA testing

1.DNA typing can routinely provide exclusion probabilities on the order of one in billions.
(HLA -1 in several millions, Blood group testing 1 in several thousand)

2. Exquisite sensitivity, small quantity, possibility of amplification – other labs- error exclusion

3. applicable to such samples as hairs, semen, urine and saliva – any tissue, any organ – nucleus containing cells

4. Resistance to degradation – acid, base, salt, bacterial contamination

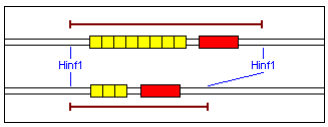
Forensics

Biology/genetics

Techniques: ~~RFLP~~, PCR, STR analysis, mtDNA & chromosome X/Y analysis

DNA polymorphism Analysis
STR

- 2-5 bp repeated numerous times – head to tail
- Different number of copies of the repeat element can occur in the population



"gatagatagatagata"

4 head-tail copies of the tetramer "gata".

Extend possible reference samples beyond a single generation (benefits missing persons cases and genetic genealogy)

STR Analysis in forensics

tetra- or penta-nucleotide repeats (4 or 5 repeat units),

give a high degree of error-free sequence read-outs

survive degradation in non-ideal conditions

Shorter repeat sequences tend to suffer from artifacts such as stutter and preferential amplification

longer repeat sequences will suffer more highly from environmental degradation and do not amplify by PCR as well as shorter sequences.

A piece of History -DNA Fingerprinting

1. Amplification of locus (loci) of interest
2. Restriction of the locus (loci)
3. Electrophoresis
4. Southern blot hybridization
   (probe specific to the polymorphism of interest)
5. Read-out

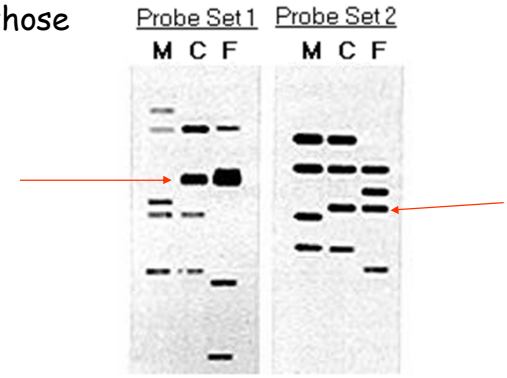
Single locus analysis

Multilocus analysis
application of a mixture of single locus probes or application of a single probe that identifies multiple similar sequence polymorphisms

DNA Fingerprinting

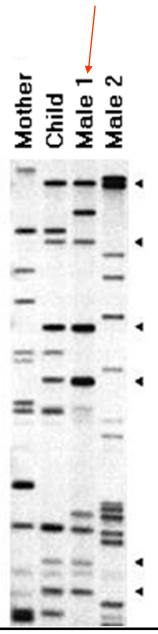
Minisatellite fingerprinting to demonstrate kinship using mixtures of two or three single locus probes (probe sets 1 and 2).

The loci detected in the child (C) are clearly a composite of those present in the mother (M) and father (F).

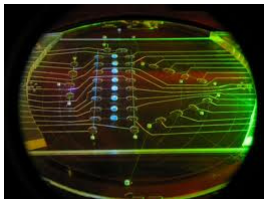


DNA Fingerprinting

Microsatellite fingerprinting to establish parentage.
The probe, (CAG)5, recognizes a large number of loci

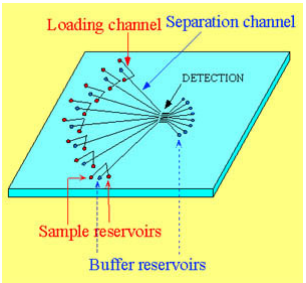


STR analysis



1.gel electrophoresis,
2.capillary electrophoresis ,
3.microchip capillary electrophoresis
4.capillary array electrophoresis
5. electronic stringency hybridization microarrays
(Radtkey et al; NAR 2000)
6. mass spectrometry

Microchip capillary electrophoresis

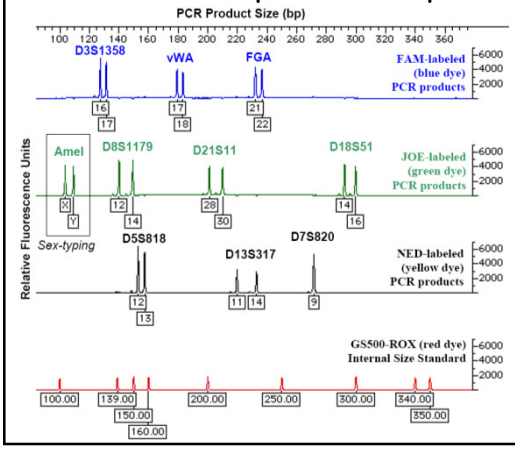


Low fabrication costs
High-throughput
Ultra-fast separation
Short separation distances
High lane densities
Almost – no sample handling –
reduction of costs and human errors

STR typing - gel or capillary electrophoresis with laser-induced fluorescence detection

distinguishable fluorescent tags and/or non-overlapping PCR product sizes.

Fluorescently labeled primers are incorporated into amplified PCR products



the different STR alleles can be characterized by size and color

internal standard- bottom panel is used to size the PCR products

DNA & crime scene

*Identify potential suspects

*Exonerate persons wrongly accused of crimes

*Identify crime and catastrophe victims

*Establish paternity and other family relationships

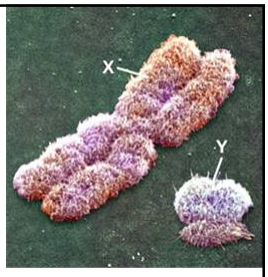
↓

gDNA

X/Y chromosome testing

MtDNA

Why Y chromosome?



* 98% of violent crime is done by men

- Easy isolation of male component
– no additional extraction

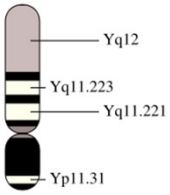
* applicable in material with male/male mixtures

Disadvantage: combination of Y- STRs are the same in the family : father, son, uncle

Male children can be tied to fathers in motherless paternity cases

Lack of recombination enables comparison of male individuals separated by large periods of time

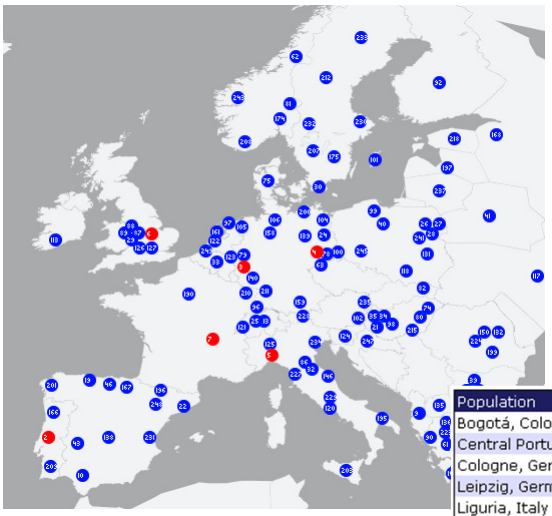
Y chromosome



*AMELY (amelogenin,Y-chromosomal)
*ANT3Y (adenine nucleotide translocator-3 on the Y)
ASMTY (which stands for acetylserotonin methyltransferase)
*AZF1 (azoospermia factor 1)
*AZF2 (azoospermia factor 2)
*BPY2 (basic protein on the Y chromosome)
*CSF2RY (granulocyte-macrophage colony-stimulating factor receptor, alpha subunit on the Y chromosome)
*DAZ (deleted in azoospermia)
*IL3RAY (interleukin-3 receptor)
*PRKY (protein kinase, Y-linked)
*RBM1 (RNA binding motif protein, Y chromosome, family 1, member A1)
*RBM2 (RNA binding motif protein 2)
*SRY (sex-determining region)
*TDF (testis determining factor)
*TSPY (testis-specific protein)
*UTY (ubiquitously transcribed *TPR gene on Y chromosome)
*ZFY (zinc finger protein)

Y-Chromosome Haplotype Reference Database

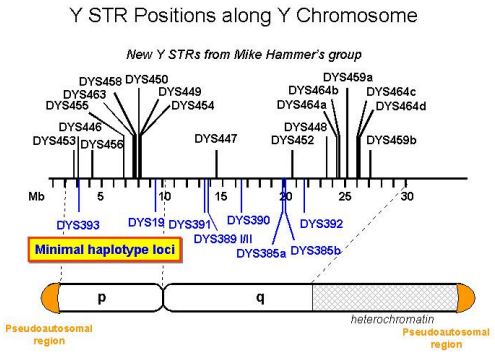
www.YHRD.org



haplotype?

Population	#	Metapopulation
Bogotá, Colombia [European]	1 / 147	Eurasian MP / European MP
Central Portugal	1 / 230	Eurasian MP / European MP
Cologne, Germany	1 / 135	Eurasian MP / European MP
Leipzig, Germany	1 / 661	Eurasian MP / European MP
Liguria, Italy	1 / 81	Eurasian MP / European MP
London, UK	1 / 285	Eurasian MP / European MP
Lyon, France	1 / 125	Eurasian MP / European MP

Y chromosome in forensics



DYS19-DYS389I-DYS389II-DYS390-DYS391-DYS392-DYS393 -DYS385a,b

The Meaning of a Y-Chromosome Match

The Y-STR profile of the crime sample matches the Y-STR profile of the suspect (at *xxx* number of loci examined).

Therefore, *we cannot exclude the suspect* as being the donor of the crime sample.

In addition, we cannot exclude all patrilineal related male relatives and an unknown number of unrelated males as being the donor of the crime sample.

Males can share Y-haplotypes without being related

- an unknown number of males from the same region cannot be excluded. A more accurate answer can only be obtained if

(1) we have detailed knowledge of the population structure of the region of interest,

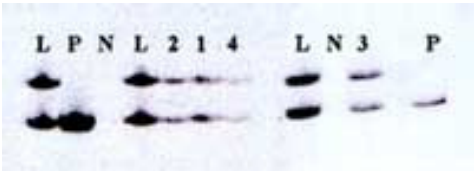
(2) the Y-STR frequencies therein are known

(3) we have knowledge about the family structure of the suspect.

Gender discrimination

Use of genes that differ on X or Y chromosome

AMGXY – amelogenin, shorter sequence on X chromosome (6nt deletion, intron 1)



L=allelic ladder; P=positive control; N=negative control;

* tooth enamel protein

Sex-typing Markers

Locus	Primers	Product Size	Comments
Amelogenin	5'-CCCTGGGCTCTGTAAAGAATAGTG-3' 5'-ATCAGAGCTTAAACTGGGAAGCTG-3'	X = 106 bp Y = 112 bp	may be multiplexed with STRs
Amelogenin	5'-ACCTCATCCTGGGCACCCTGG-3' 5'-AGGCTTGAGGCCAACCATCAG-3'	X = 212 bp Y = 218 bp	may be multiplexed with STRs or D1S80
Amelogenin	5'-CTGATGGTTGGCCTCAAGCCTGTG-3' 5'-TAAAGAGATTCATTAACTTGACTG-3'	X = 977 bp Y = 788 bp	may be analyzed with agarose gels
Centromeric alphoid repeat	5'-TATTTGGACTCTCTCTGAGGA-3' (X3) 5'-TTCTACTACAAGGGTGTTGCA-3' (X4) 5'-GTGTATTCACCTCCGGGAG-3' (Y3) 5'-ACAAAAGGTTCAATTCTGTGAG-3' (Y4)	X = 157 bp Y = 200 bp	both X- and Y-sequences were coamplified
Centromeric alphoid repeat	5'-AATCATCAAATGGAGATTTG-3' (X1) 5'-GTTCAGCTCTGTGAGTGAAA-3' (X2) 5'-ATGATAGAAACGGAAATATG-3' (Y11) 5'-AGTAGAATGCAAAGGGCTC-3' (Y22)	X = 170 bp Y = 130 bp	separate PCR reactions were performed
ZFX/ZFY zinc finger gene	5'-CTGGAGAGCCACAAGCTGAC-3' 5'-TTGCTGTGGACTGCCAAGAG-3'	X/Y = 209bp after *Hae*III cuts X = 172 +37 Y = 88 +84 +37	reverse dot blot typing assay; may be used with AmpliType PM and HLA-DQA1
SRY 93	5'-ATAAGTATCGACCTCGTCGGAAG-3' 5'-GCACTTCGCTGCAGAGTACCGAAG-3'	Y = 93 bp	can be multiplexed with amelogenin

DNA & crime scene

*Identify potential suspects

*Exonerate persons wrongly accused of crimes

*Identify crime and catastrophe victims

*Establish paternity and other family relationships

gDNA

X/Y chromosome testing

mtDNA

Location and Copy Number of mtDNA

• Found within the mitochondria in the cellular cytoplasm.

• On average 4-5 copies of mtDNA molecules per mitochondria (range of 1-15 mtDNA copies).

• Number of mitochondria vary by cell type (e.g., muscles have more…).
• Generally, hundreds of mitochondria per cell.

Comparison of Human nucDNA and mtDNA

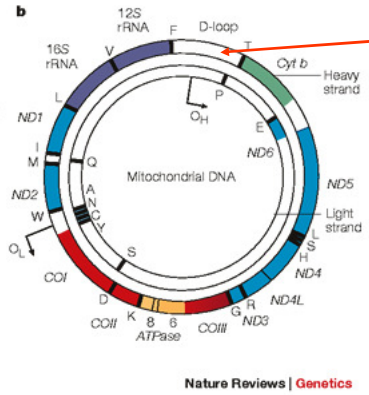
Characteristics	Nuclear DNA (nucDNA)	Mitochondrial DNA (mtDNA)
Size of genome	~3.2 billion bp	~16 569 bp
Copies per cell	2 (1 allele from each parent)	Can be > 1000
Percent of total DNA content per cell	99.75%	0.25%
Structure	Linear; packaged in chromosomes	Circular
Inherited from	Father and Mother	Mother
Chromosomal pairing	Diploid	Haploid
Generational recombination	Yes	No
Replication repair	Yes	No
Unique	Unique to individual (except identical twins)	Not unique to individual (same as maternal relatives)
Mutation rate	Low	At least 5–10 times nucDNA
Reference sequence	Described in 2001 by the Human Genome Project	Described in 1981 by Anderson and co-workers

Mitochondrial DNA

-No spaces between genes

-The only gene-free region (D-loop, control region) is required for the initiation of transcription

VERY VARIABLE

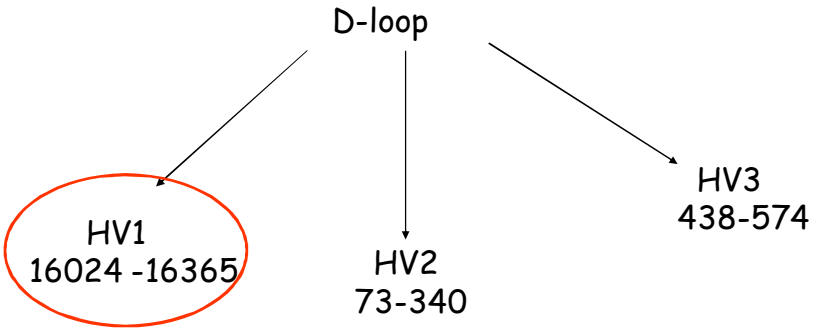


Initiation of mtDNA replication& transcription

Taylor et al; 2005

Identification of the suspect mtDNA

Typically only 610 bases examined



- Substitution : transition 75%, transversion 15%
- insertion 6%
- Deletion 4%

Family relations, identification
Difficulty: heteroplasmic character

mtDNA Is Not Always 16,569 bp …

- Dinucleotide repeat at positions 514-524 (near end of control region)

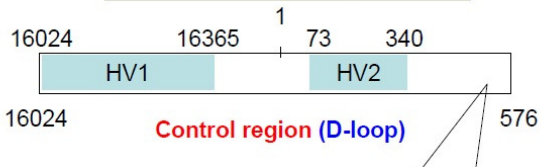
Usually ACACACACAC or (AC)5 in most individuals
Can vary from (AC)3 to (AC)7

- Other insertions and deletions may occur
  - 9 bp deletion (positions 8277 to 8285) in some individuals from Asia and Pacific Islands (haplogroup B) and Africans (haplogroup L).

mtDNA Is Not Always 16,569 bp …

Control Region (16024-576)

1,122 nucleotide positions



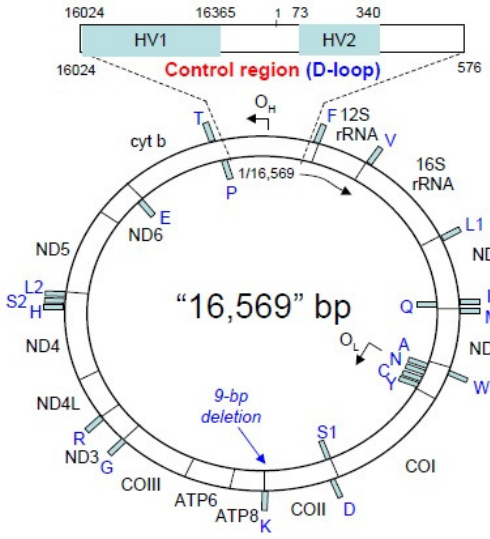
Forensic Focus

Typically only **610 bases examined**

– (HVI: 16024-16365; HVII: 73-340)

$(AC)_3$
$(AC)_4$
$(AC)_5$
$(AC)_6$
$(AC)_7$

mtDNA Is Not Always 16,569 bp …



22 tRNAs
2 rRNAs
13 genes

Figure 10.1, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Process for Evaluation of mtDNA Samples

Shaft (Telogen)

DNA Extraction

PCR Amplification

Usually performed with 34-38 cycles
Some protocols may go to 42 cycles
for highly degraded specimens

PCR Clean-up

{EXO/SAP}

DNA Sequencing

Centricon

Process for Evaluation of mtDNA Samples

Question Sample

Extract mtDNA from evidence (Q) sample

PCR Amplify HV1 and HV2 Regions

Sequence HV1 and HV2 Amplicons (both strands)

Confirm sequence with forward and reverse strands

Note differences from Anderson (reference) sequence

Extract mtDNA from reference (K) sample

PCR Amplify HV1 and HV2 Regions

Sequence HV1 and HV2 Amplicons (both strands)

Confirm sequence with forward and reverse strands

Note differences from Anderson (reference) sequence

Compare Q and K sequences

Performed separately and preferably after evidence is completed

Compare with database

The reference sequence

Original Reference Sequence

• Human mtDNA was first sequenced in 1981 in Frederick Sanger's lab located in Cambridge, England.

• Authors for this paper (Nature 1981, 290:457-465) were listed in alphabetical order so Stan Anderson was the first author.

• This sequence has come to be referred to as the "Anderson" sequence (GenBank accession: M63933). • This first sequence is sometimes called the Cambridge Reference Sequence (CRS).

The reference sequence

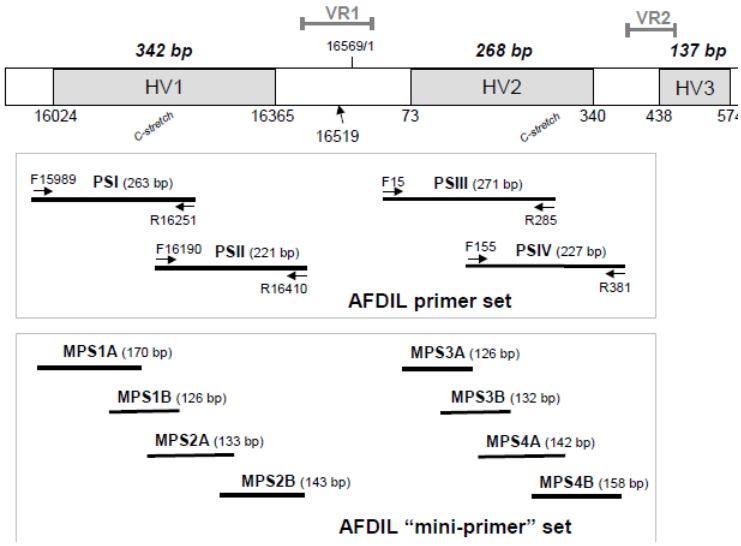
Re-Sequencing of CRS

• The 1981 sequence was derived primarily from a placenta of an individual with European ancestry; however, some HeLa and bovine sequence was used to fill in gaps due to early sequencing procedures performed.

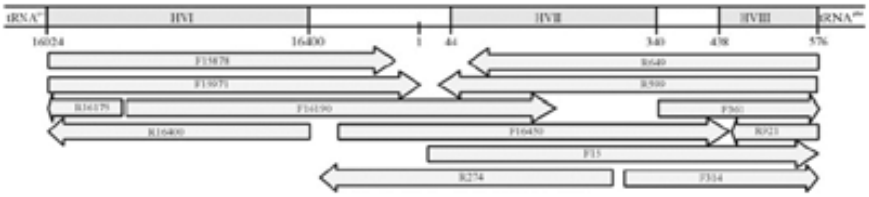
• Re-analysis of original placental material by Andrews et al. (1999) found 11 nucleotides that differed from Anderson et al. (1981) sequence.

• This revised Cambridge Reference Sequence (rCRS) is now the accepted standard for comparison

Common mtDNA primer sets



mtDNA sequencing

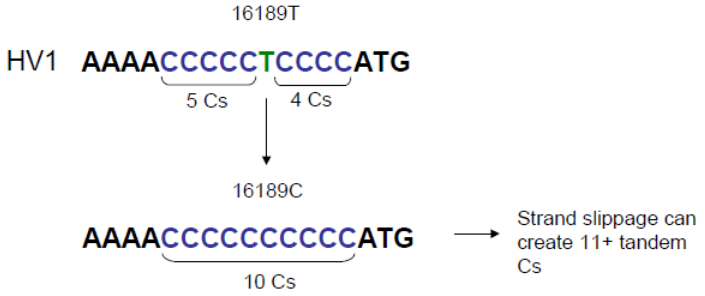


13 primers – 11 for routine sequencing

Why the Redundancy?

mtDNA sequencing

- Homopolymeric stretches of Cytosines (C-stretches).



mtDNA sequencing

Primer strategies typically used with C-stretch containing samples

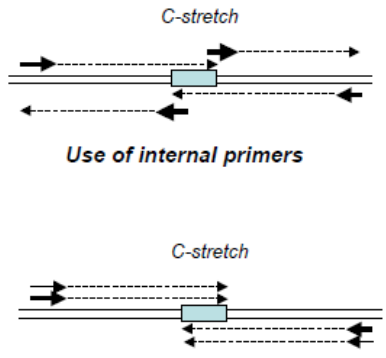
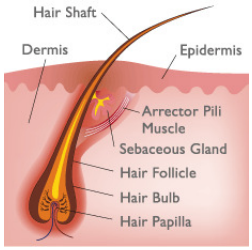


Figure 10.7, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

mtDNA and forensics



Commonly used :

-In hair shaft analysis

-Low amounts of gDNA/Higher copy number per cell

Mostly : highly degraded samples

mtDNA and forensics

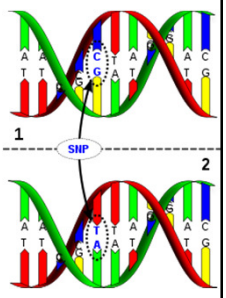
Labor intensive
Expensive

- the low power of discrimination obtained when common HVI/HVII types are encountered in casework

- a lack of high quality population reference databases from many regions of the world

-technical reason: melanin is a PCR inhibitor

SNPs in forensics



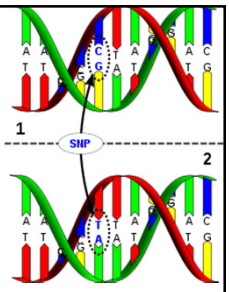
1. Zero mutation rate (as compared to STRs)

2. Possibility of accurate automated typing
   * qualitative analysis (A or G allele)
   in contrast to quantitive STR testing (copy number)

3. Suitable for testing of degraded material :
   *45-55bp (60-130 bp average)
   *100-450 bp in STR analysis

4. Cheap, automated, accurate, fast genotyping

SNPs in forensics



Disadvantages:

-Many more SNPs required (~60) for identification as compared to STRs (~15)

-Population specific data required (good databases)

-Costs issue – standardized STR methodology is very cheap

Forensic databases

The Combined DNA Index System, CODIS

DNA profiles of convicted individuals

DNA profiles from crime scene evidence

Computerized automatic searches

Forensic databases

CODIS – initiated 1994

By 2003 :

more than a million DNA profiles in its Convicted Offender Index

48,000 DNA profiles collected from crime scenes but which have not been connected to a particular offender.

The National DNA Index (NDIS) contains over 10,718,700 offender profiles and 427,500 forensic profiles as of April 2012.

Forensic databases

The ENFSI DNA Working Group (1994) - members from State, Government, Police or University laboratories whose primary function is the analysis of DNA for law enforcement.

The objectives:

-exchanging and disseminating information on forensic applications

-to discuss, share and compare forensic DNA analytical methods, protocols and research

-to establish forensic DNA analysis quality assurance guidelines and quality controls for Europe

-to cooperate with other national and international organizations in developing European standards for forensic DNA analyses

Forensic databases

Poland

2004 – final decision on initiation/shape of polish forensic database

-Suspects and convicts
-Unidentified individuals and those who are trying to hide it
-Evidence of unsolved cases
-Unidentified victims

Instytut Ekspertyz Sadowych
Wydzial Biologii Centralnego Laboratorium Policji

Work within a frame of ENFSI

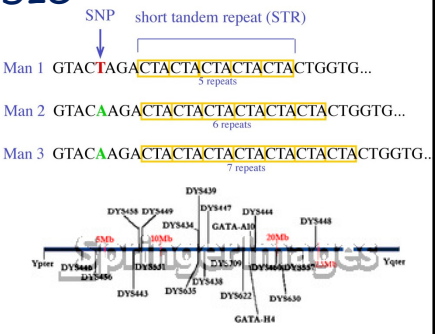
CODIS

over 170 public law enforcement laboratories participate in NDIS across the United States.

Internationally, more than 40 law enforcement laboratories in over 25 countries use the CODIS software for their own database initiatives

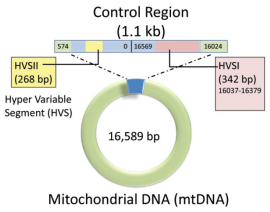
Data submitted to CODIS

DNA data generated through PCR Short Tandem Repeat (STR) technology,



Y chromosome STR (Y STR) technology

Mitochondrial DNA (mtDNA) technology are accepted at NDIS



!!!!!!!!!!!!!!!!!

The National DNA Index no longer searches DNA data developed using RFLP technology

Data requirements for the DNA records

DNA data submitted to NDIS must be:

1. generated in accordance with the FBI Director's Quality Assurance Standards;

2. generated by a laboratory that is accredited by an approved accrediting agency;

3. generated by a laboratory that undergoes an external audit every two years to demonstrate compliance with the FBI Director's Quality Assurance Standards;

Data requirements for the DNA records

The DNA data must be:

1. one of the categories of data acceptable at NDIS, such as convicted offender, arrestee, detainee, legal, forensic (casework), unidentified human remains, missing person or a relative of missing person;

2. must meet minimum loci requirements for the specimen category;

3. The DNA PCR data must be generated using PCR accepted kits;

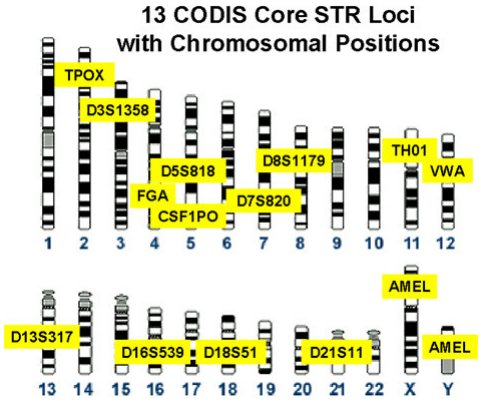
4. Participating laboratories must have and follow expungement procedures in accordance with federal law.

Matching in CODIS

Locus	Forensic Unknown	Candidate Offender	Match Stringency
D8S1179	13	13, 14	Moderate
D21S11	28, 31.2	28, 31.2	High
D7S820	12	10, 12	Moderate
CSF1PO	10, 12	10	Moderate
D3S1358	15, 17	15, 17	High
TH01	8	7, 8	Moderate
D13S317	9, 12	9	Moderate
D16S539	11, 12	12	Moderate
VWA	17	15, 17	Moderate
TPOX	8, 11	8	Moderate
D18S51	24	16, 24	Moderate
D5S818	9, 12	12	Moderate
FGA	24, 25	24, 25	High

The 13 core CODIS loci

CSF1PO
FGA
THO1
TPOX
VWA
D3S1358
D5S818
D7S820
D8S1179
D13S317
D16S539
D18S51
D21S11

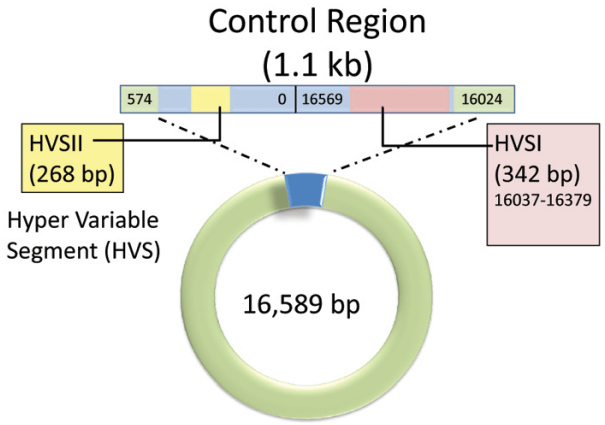


the 13 core CODIS loci are required for submission of convicted offender, arrestee, detainee, and legal profiles.

The 13 core CODIS loci and Amelogenin are required for relatives of missing person profiles

mtDNA data submission

Hypervariable region I ("HV1"; positions 16024-16365) and hypervariable region II ("HV2"; positions 73-340) are required for the submission of mtDNA data to NDIS



Mitochondrial DNA (mtDNA)

Polish reality

Polska baza DNA mizerna. Nie pomaga w łapaniu przestępców. "Kpina"

Lubię to! Wyślij +1 0

psm, PAP | 30.11.2011 , aktualizacja: 30.11.2011 06:25 A A A

Sprzęt do identyfikowania kodu DNA Fot. Jakub Ociepa / Agencja Gazeta

PRZEGLĄD PRASY. Przestępcy zwykle zostawiają na miejscu zbrodni ślady DNA. Dzięki nim można powiązać sprawę z konkretną osobą, o ile jej dane są w bazie. Ale w Polsce dzieje się tak wyjątkowo rzadko - ujawnia "Rzeczpospolita".

W Wielkiej Brytanii w latach 2001 - 2011 dane z bazy pozwoliły wykryć prawie 400 tys. sprawców przestępstw.

Dzięki polskiej, która działa od czterech lat, zaledwie 56. Takie liczby przytacza prof. Józef Wójcikiewicz z Katedry Kryminalistyki i Bezpieczeństwa Publicznego UJ. - Polska baza danych DNA to kpina. Na koniec ubiegłego roku było w niej tylko 0,07 proc. populacji - ocenia profesor.

http://wiadomosci.gazeta.pl/wiadomosci/1,114883,10731464,Polska_baza_DNA_mizerna__Nie_pomaga_w_lapaniu_przestepcow_.html

Polish reality

Policja 997

wersja tekstowa

Strona główna / Inne / Tylko służba /

Tylko służba

Baza DNA – sukces czy niewypał? (Nr 84 / 03.2012)

Na początku roku w mediach rozgorzała dyskusja na temat funkcjonowania polskiej bazy DNA. Pojawiły się liczne zastrzeżenia związane z jej efektywnością, liczbą przechowywanych profili oraz z działaniem organów ścigania.

Rzeczywiście, dane statystyczne nie prezentują się dobrze. Zgodnie ze stanem na czerwiec 2011 roku w polskiej bazie było zarejestrowanych ponad 29 tys. profili, w tym 2203 profile nieznanych sprawców przestępstw. Jest to wielkość porównywalna do tego, z czym możemy się spotkać na Słowacji czy w Norwegii, z tym że populacja tych krajów stanowi niewiele ponad 10 proc. populacji Polski. Takie dane z całą pewnością nie mogą więc być satysfakcjonujące, ale oprócz liczby mieszkańców danego kraju należy wziąć pod uwagę populację przestępców.

– Średnio jeden zarejestrowany profil przypada na 255 Europejczyków, w Wielkiej Brytanii jeden na 15 Brytyjczyków. U nas natomiast tylko jeden na 1600 osób – opowiada podinsp. Maria Walczuk, ekspert Zakładu Biologii Centralnego

http://gazeta.policja.pl/wai/997/986/76082/Baza_DNA__sukces_czy_niewypal_Nr_84__032012.html

Romanov Family

July 16-17, 1918

1991

1. (STR) analysis and confirm that a family group was present in the grave.

1. Analysis of mtDNA reveals an exact sequence match between the putative Tsarina and the three children

2. Amplified mtDNA extracted from the remains of the Tsar has been cloned to demonstrate heteroplasmy at a single base within the mtDNA control region. One of these sequences matches two living maternal relatives of the Tsar.

Marie Antoinette & Louis XVII

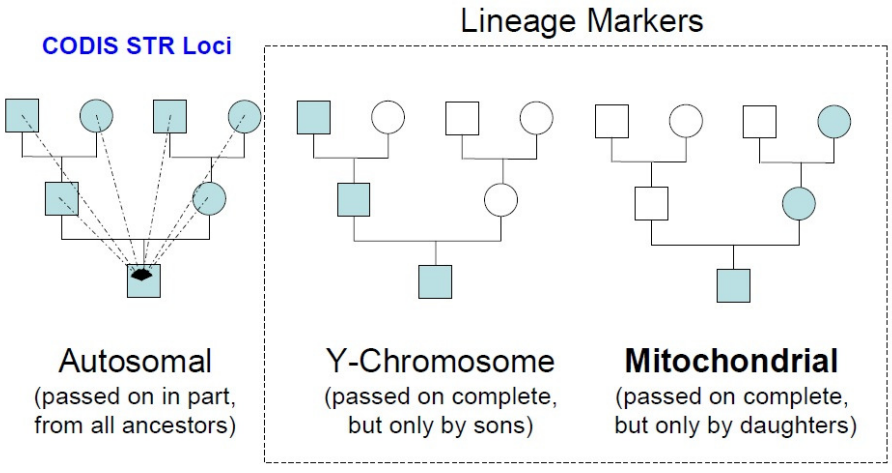
In 2000, comparison with DNA reclaimed from the hair of Marie Antoinette confirmed the heart as royal

the DNA test - mtDNA

the comparison only proved that the two samples shared the same maternal ancestry

may have been that of another young royal, for instance that of Louis XVI's first son

DNA in forensics- the summary



SNPs? NGS?