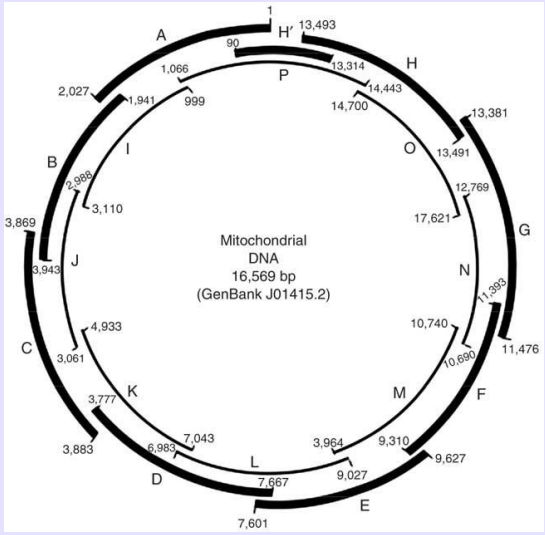
DNA mitocondriale: analisi di misture

Monica Omedei, Shady Giulia Amayeh, Marta Bernocco, Sarah Gino

Polimorfismi del DNA mitocondriale

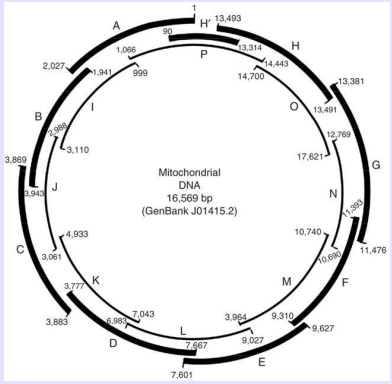
- **Ogni cellula contiene nel citoplasma circa mille mitocondri**
- **Ogni mitocondrio contiene 5-10 molecole di DNA circolare (16569 bp)**
- **I polimorfismi del mtDNA possono essere studiati a scopo identificativo in presenza di cellule prive di nucleo (formazioni pilifere senza bulbo o bulbo in fase telogen) oppure in presenza DNA nucleare altamente degradato o in piccole quantità**
- **I polimorfismi analizzati risiedono nella regione di controllo (tratto non codificante) del mtDNA**
- **Le sequenze caratterizzate vengono comparate con una sequenza umana di riferimento (revised Cambridge ReferenceSequence, rCRS)**



Polimorfismi del DNA mitocondriale

MA …

- **Il DNA mitocondriale è ereditato non per via mendeliana, ma citoplasmatica: poiché il citoplasma dello zigote deriva quasi totalmente dalla cellula uovo, ciascun individuo riceve il solo mtDNA materno, quindi tutti gli individui imparentati per linea materna condividono il medesimo corredo di polimorfismi (aplotipo). Risulta dunque meno informativo rispetto agli STR, ma utile per indagini di consanguineità ove si ipotizza una stessa progenitrice**
- **Nello stesso individuo e spesso anche nello stesso tessuto, soprattutto se composto da poche cellule con un'origine pressoché clonale, possono essere presenti sequenze diverse (eteroplasmia)**



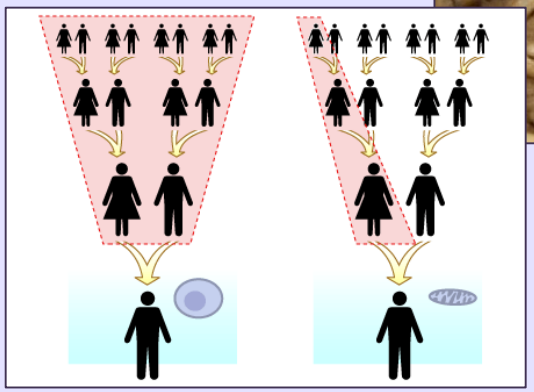
APPLICAZIONI DEL mtDNA:

- **DNA antico e/o degradato**

APPLICAZIONI DEL mtDNA:

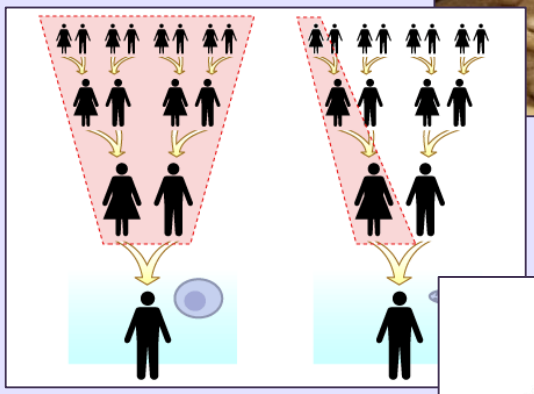
- **DNA antico e/o degradato**

- **Parentele deficitarie**



APPLICAZIONI DEL mtDNA:

- **DNA antico e/o degradato**



- **Parentele deficitarie**

- **Ricostruzioni filogenetiche**

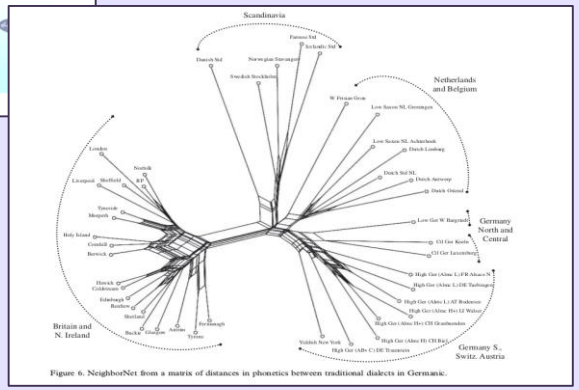
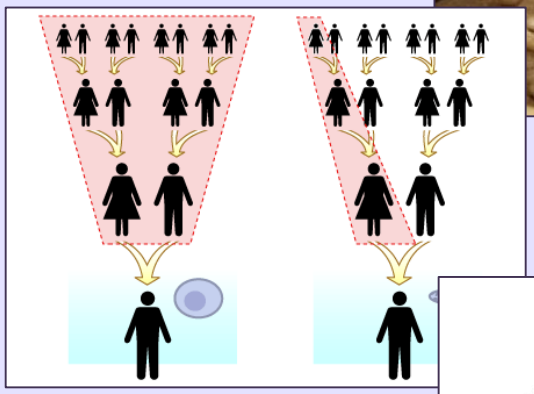


Figure 6. NeighborNet from a matrix of distances in phonetics between traditional dialects in Germanic.

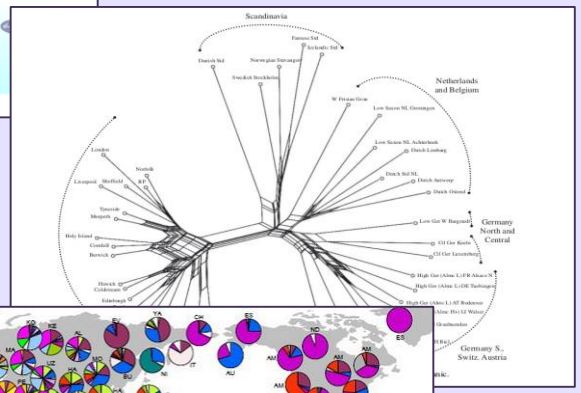
APPLICAZIONI DEL mtDNA:

- **DNA antico e/o degradato**

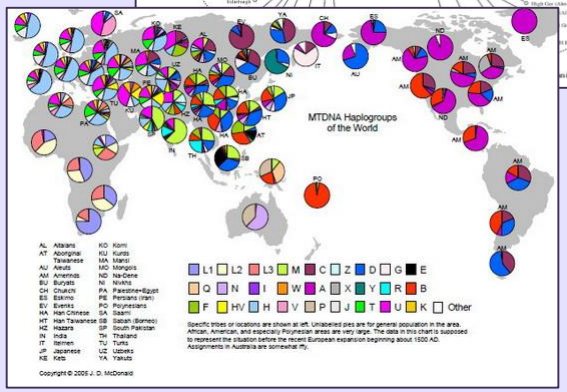
- **Parentele deficitarie**



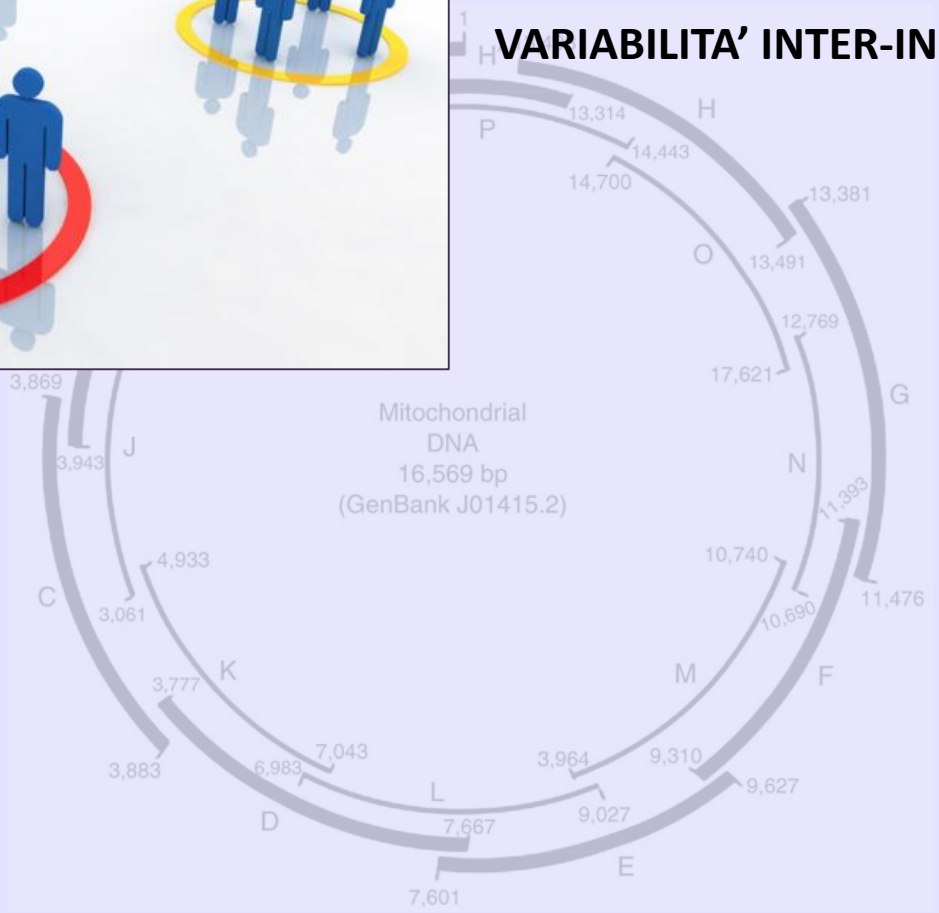
- **Ricostruzioni filogenetiche**



- **Stima della provenienza geografica**



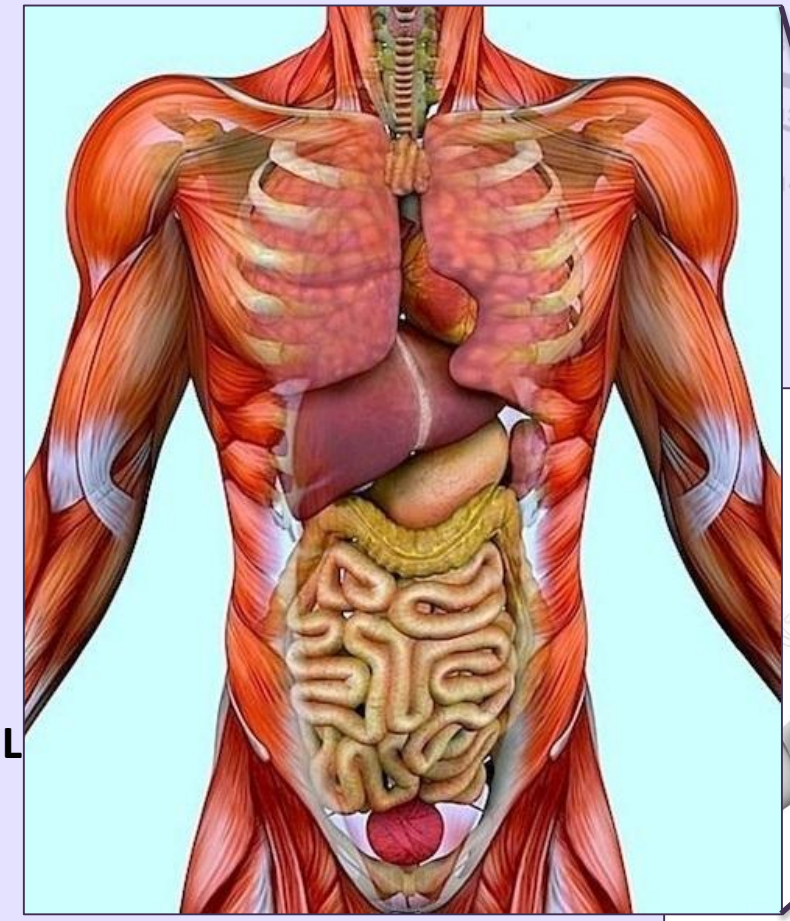
VARIABILITA’ INTER-INDIVIDUALE

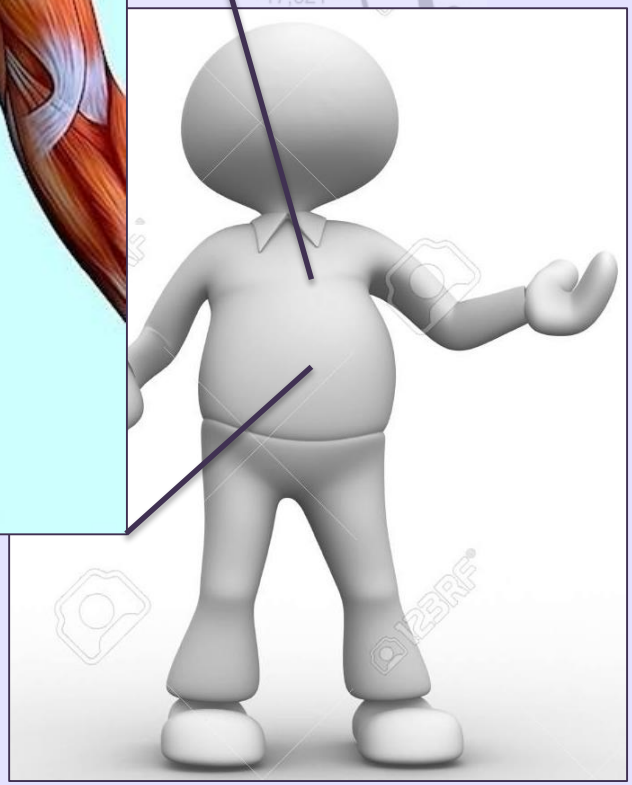


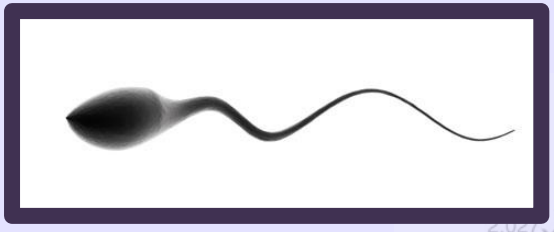
VARIABILITA’ INTER-INDIVIDUALE

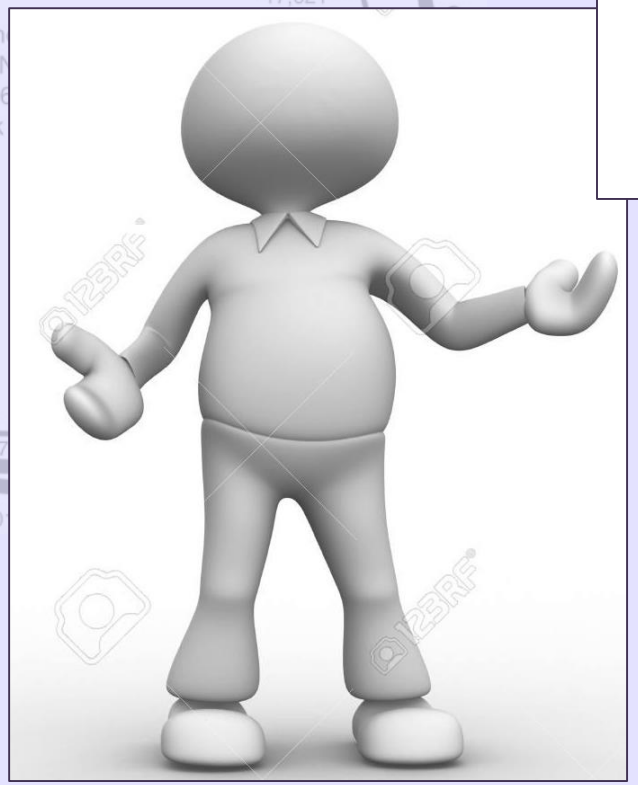
VARIABILITA’ INTRA-INDIVIDUALE

VARIABIL

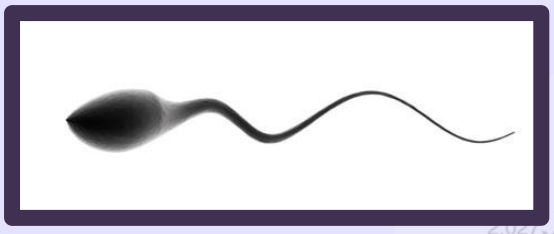


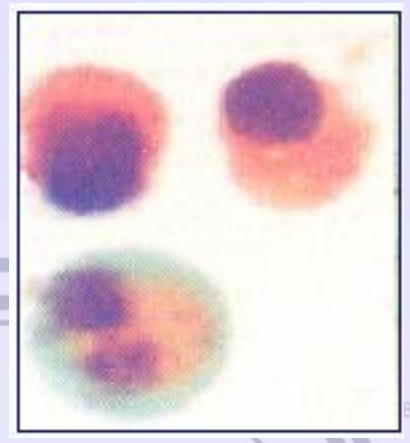




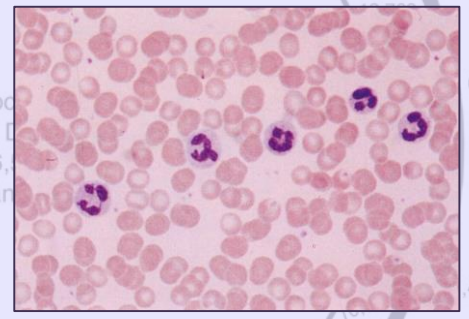


VARIABILITA’ INTRA-INDIVIDUALE

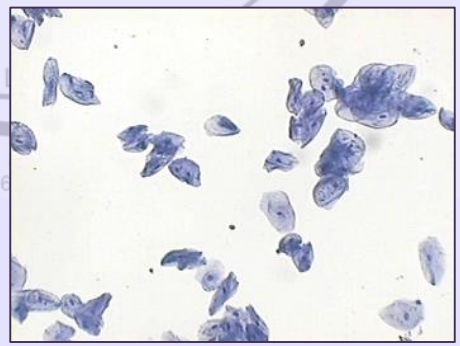




ELEMENTI DELLA LINEA GERMINATIVA



LEUCOCITI



CELLULE DI SFALDAMENTO

ghep-isfg

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Forensic Science International 160 (2006) 157–167
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Results of the 2003–2004 GEP-ISFG collaborative study on mitochondrial DNA: Focus on the mtDNA profile of a mixed semen-saliva stain

7 campioni costituiti da:

- **5 SANGUE**
- **1 SALIVA + SEMEN (1:3 ratio)**
- **1 CAPELLI**

Sequenziamento HV1 e HV2

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70% osserva solo mtDNA SALIVA

Sequenziamento HV1 e HV2

ghep-isfg

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Finally and concerning to mixture interpretation, it seems evident that the inferior participation in the analysis of the M6 sample shows the majority idea that mtDNA in not the marker of choice in the resolution of profiles in mixed samples (saliva:semen). STRs are most clearly the method of choice. Nevertheless, in a number of cases, it could be interesting to know the mitochondrial DNA (mtDNA) haplotypes that contributed to the mixture (e.g. degraded reference samples, exclusion of a maternal relationship between the victim and suspect in rape cases...) and in this type of cases we consider that the interpretation should be making with extreme care.

7 c

- **5**
- **1**
- **1**

DNA

Sequenziamento HV1 e HV2

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Forensic Science International 168 (2007) 42–56
Forensic Science International
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Analysis of body fluid mixtures by mtDNA sequencing: An inter-laboratory study of the GEP-ISFG working group

M. Montesino [a], A. Salas [b], M. Crespillo [c], C. Albarrán [d], A. Alonso [d], V. Álvarez-Iglesias [b], J.A. Cano [e], M. Carvalho [f], D. Corach [g], C. Cruz [h], A. Di Lonardo [i], R. Espinheira [h], M.J. Farfán [j], S. Filippini [i], J. García-Hirschfeld [d], A. Hernández [k], G. Lima [l], C.M. López-Cubría [e], M. López-Soto [j], S. Pagano [m], M. Paredes [c], M.F. Pinheiro [l], A.M. Rodríguez-Monge [a], A. Sala [g], S. Sóñora [m], D.R. Sumita [n], M.C. Vide [f], M.R. Whittle [n], A. Zurita [k], L. Prieto [a,*]

3 campioni costituiti da:

- **MISTURE SANGUE + SEMEN (1/1, 1/10, 1/20)**
- **MISTURE SALIVA + SEMEN (1/1, 1/10, 1/20)**

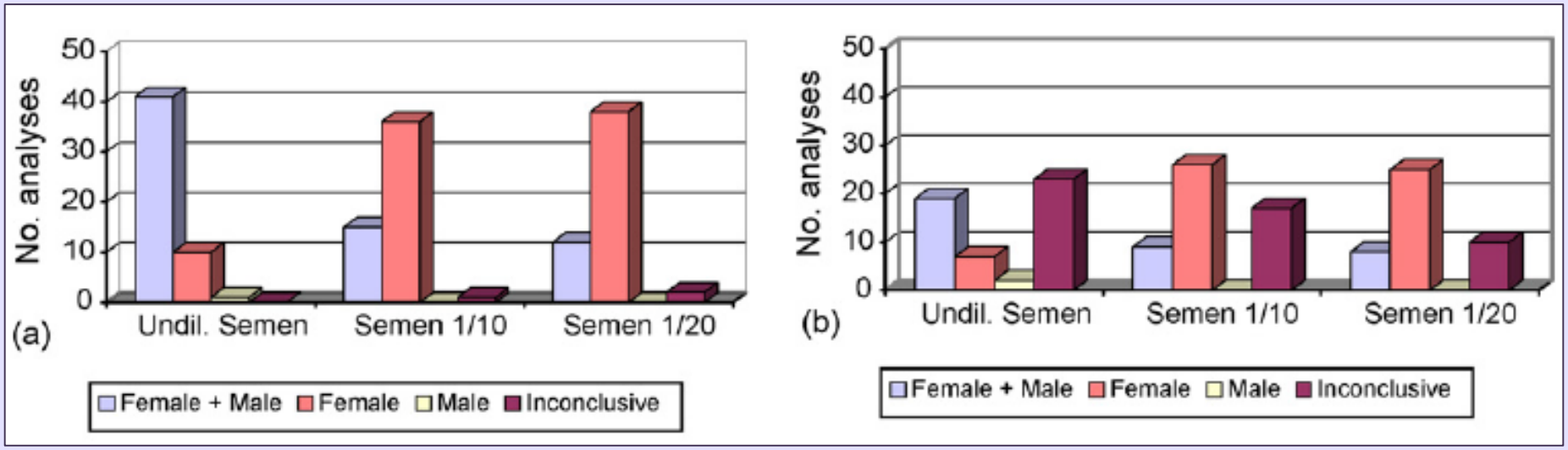
Minisequenziamento per stimare i rapporti

Provenienti da 3 diverse coppie con 1-3 varianti puntiformi differenti.

MA ATTENZIONE I RAPPORTI CALCOLATI IN BASE AL VOLUME E NON SU QUANTIFICAZIONE

Lisi differenziale:

FRAZIONE EPITELIALE **FRAZIONE SPERMATICA**



RISULTATI:

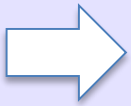
- **L'estrazione differenziale non è un metodo di elezione in caso di analisi di misture su mtDNA**
- **Visibile il LIQUIDO SEMINALE solo quando il rapporto è 1/1, sia nei misti con SALIVA che con SANGUE**
- **mtDNA BLOOD > SALIVA**

ESPERIMENTO CONDOTTO

- **2 DONATORI SELEZIONATI**

REGION	POSITION	DONOR A	DONOR B	PRIMER LENGHT
HV1	16127	C	T	23 bp
	16312	C	T	43 bp
HV2	72	G	A	19 bp
	93	A	G	28 bp

- **FLUIDI / TESSUTI RACCOLTI**



SANGUE

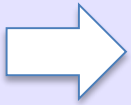
SALIVA

LIQUIDO SEMINALE

CUTE

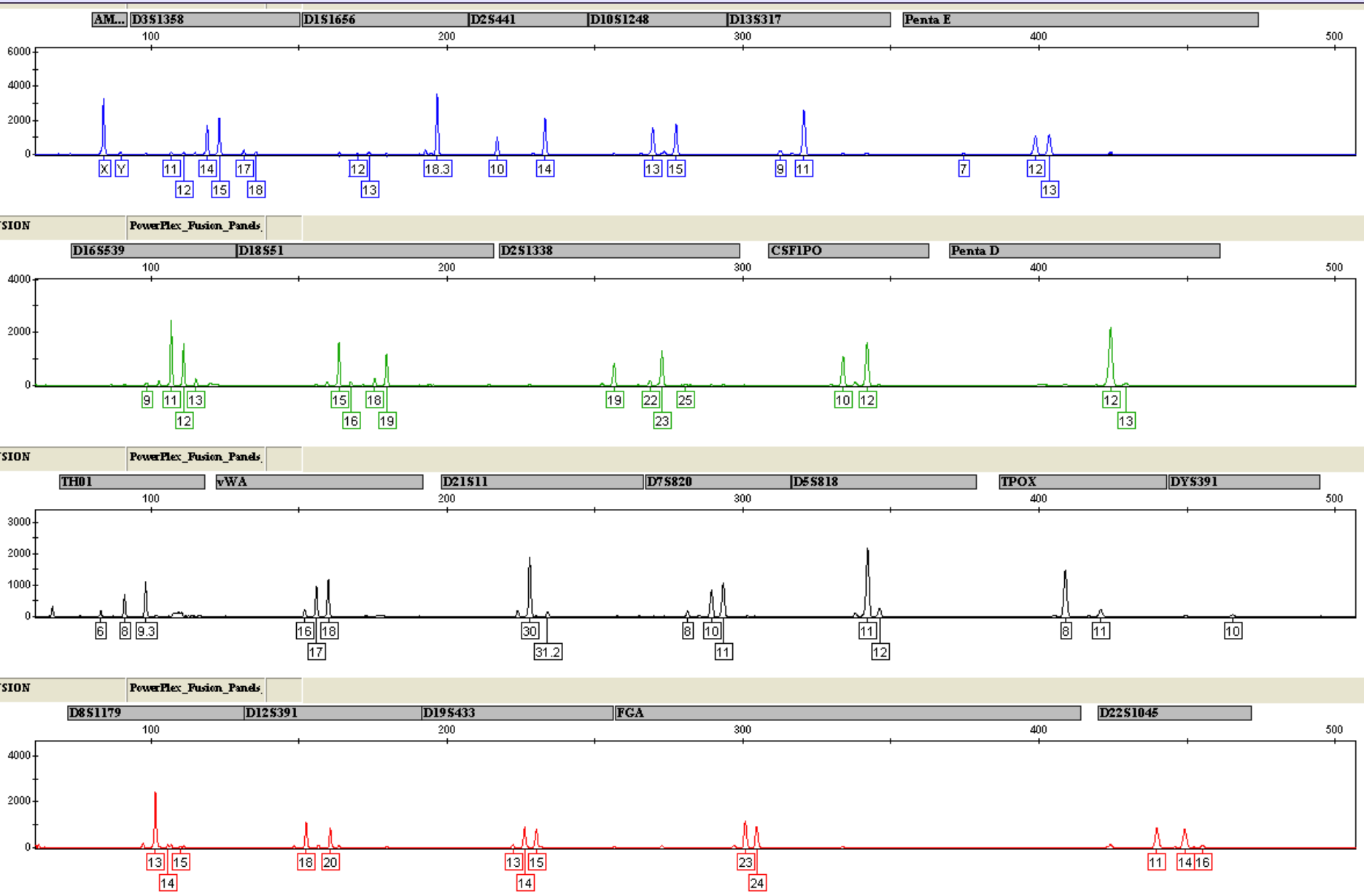
- **QUANTIFICAZIONE IN REAL-TIME**

- **RAPPORTI**



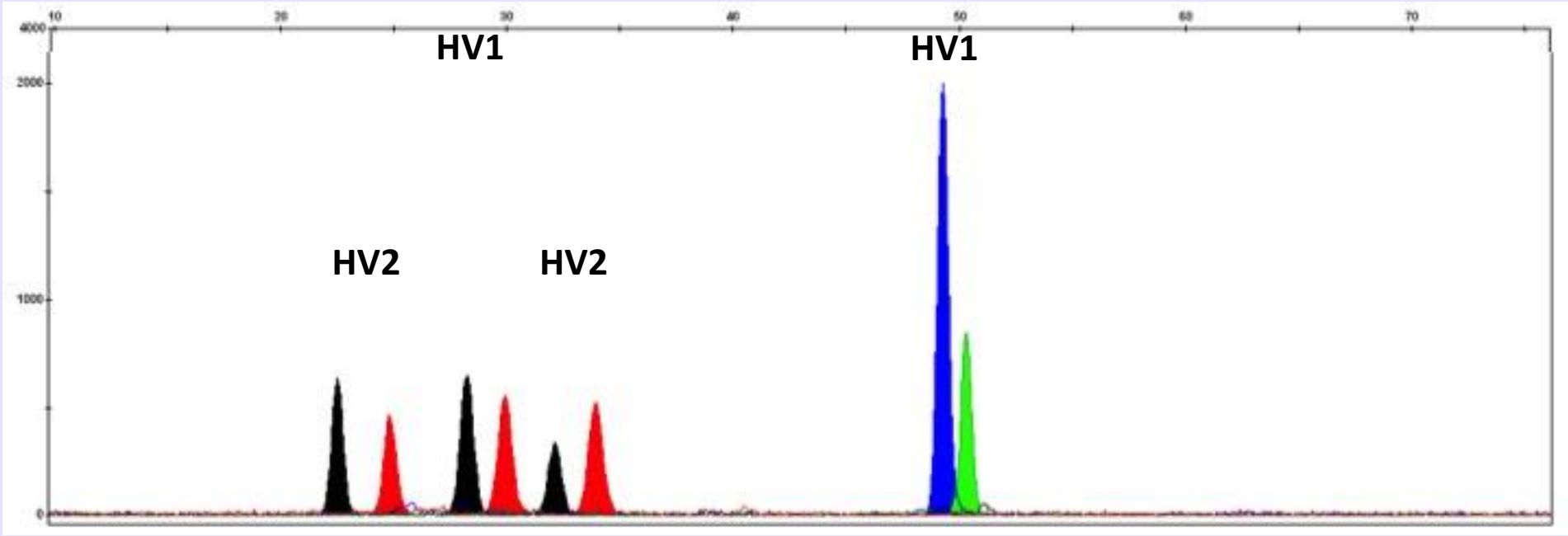
1 : 1
1 : 10
1 : 20
10 : 1
20 : 1

- **AMPLIFICAZIONE STR**
- **MINISEQUENZIAMENTO CON LE MEDESIME PROPORZIONI IMPIEGATE IN PCR - STR**

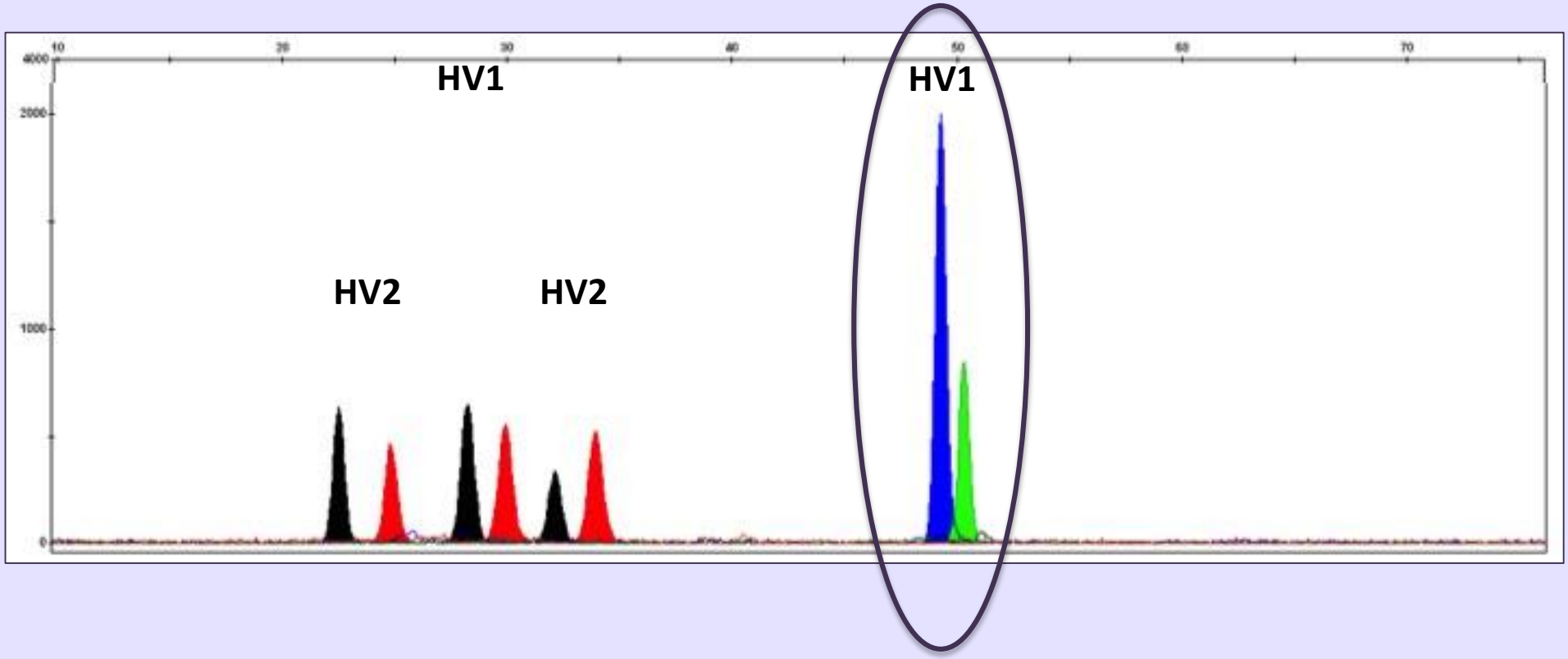

AM...
D3S1358
D1S1656
D2S441
D10S1248
D13S317
Penta E
100
200
300
400
500
6000
4000
2000
0
X
Y
11
14
17
12
15
18
12
13
18.3
10
14
13
15
9
11
7
12
13
SION
PowerPlex_Fusion_Panels
D16S539
D18S51
D2S1338
CSF1PO
Penta D
4000
2000
0
9
11
13
12
15
16
18
19
19
22
25
23
10
12
12
13
SION
PowerPlex_Fusion_Panels
TH01
vWA
D21S11
D7S820
D5S818
TPOX
DYS391
3000
2000
1000
0
6
8
9.3
16
18
17
30
31.2
8
10
11
11
12
8
11
10
SION
PowerPlex_Fusion_Panels
D8S1179
D12S391
D19S433
FGA
D22S1045
4000
2000
0
13
15
14
18
20
13
15
14
23
24
11
14
16


LE PROPORZIONI CHE VEDIAMO NELL'EPG MISTO
COME SI RIVELANO NEL mtDNA

RISULTATI

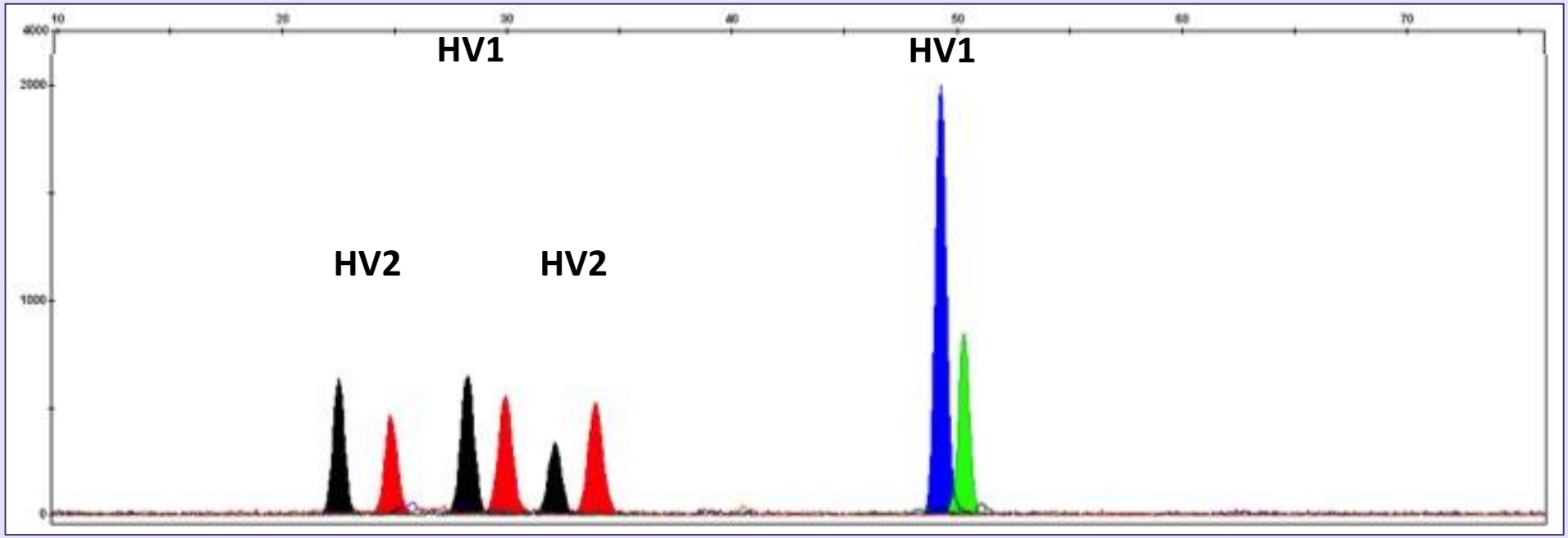


RISULTATI



Test di Normalità Shapiro-Wilk

RISULTATI



DONOR A T T C A

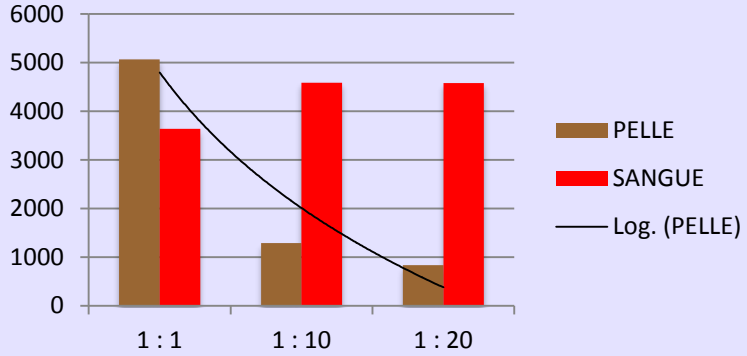
DONOR B C C T G

MEDIA DELLE ALTEZZE

T - TEST

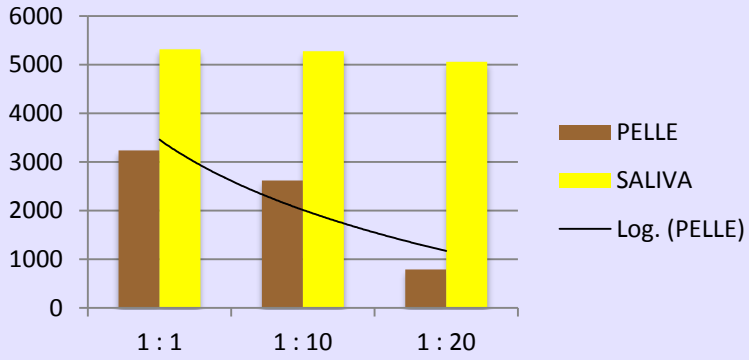
All'aumento del fluido "sangue" o "saliva" non corrisponde una proporzionato aumento dei picchi corrispondenti

cute-sangue crescente

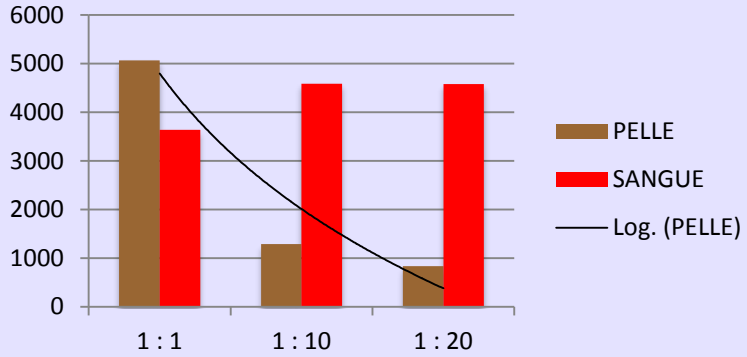


mtDNA cute > mtDNA SANGUE/SALIVA

cute-saliva crescente



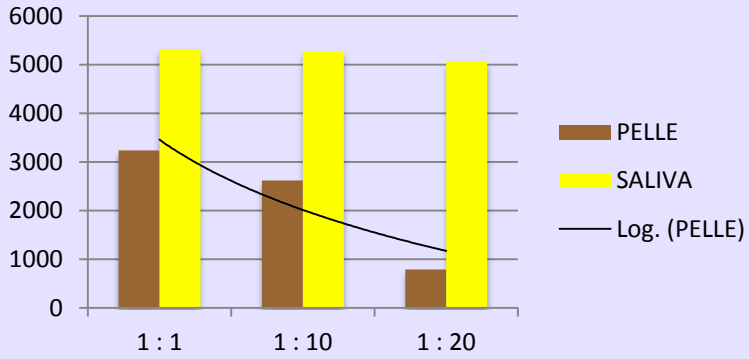
cute-sangue crescente



All'aumento del fluido "sangue" o "saliva" non corrisponde una proporzionato aumento dei picchi corrispondenti

mtDNA cute > mtDNA SANGUE/SALIVA

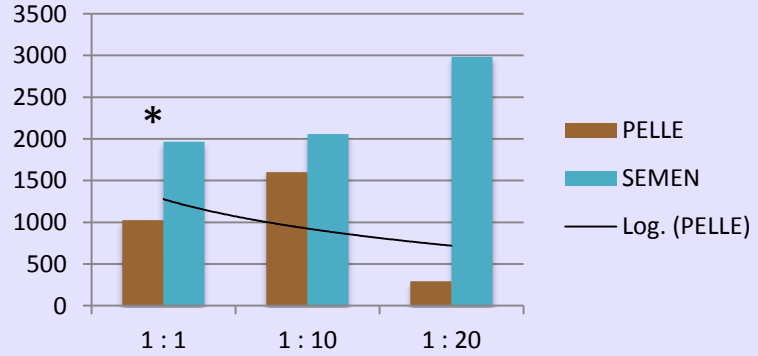
cute-saliva crescente



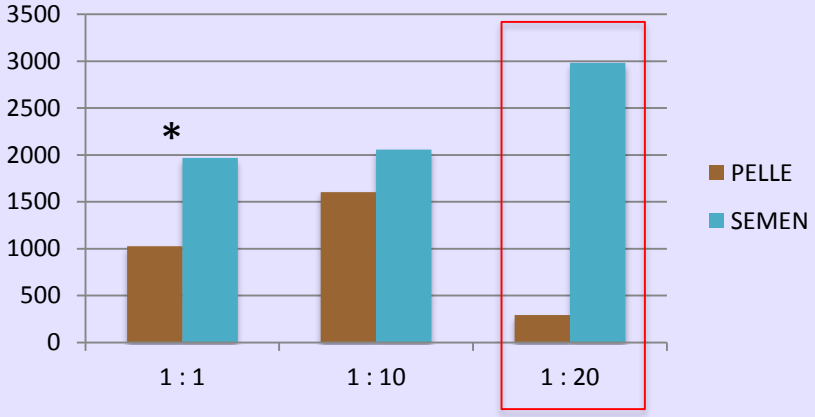
All'aumento del fluido "semen" corrisponde un proporzionato aumento dei picchi corrispondenti, ad eccezione del rapporto 1:1

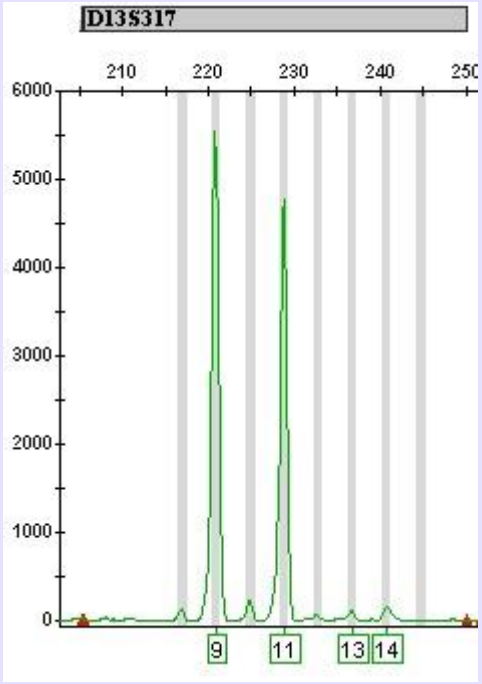
mtDNA cute ≅ mtDNA SEMEN

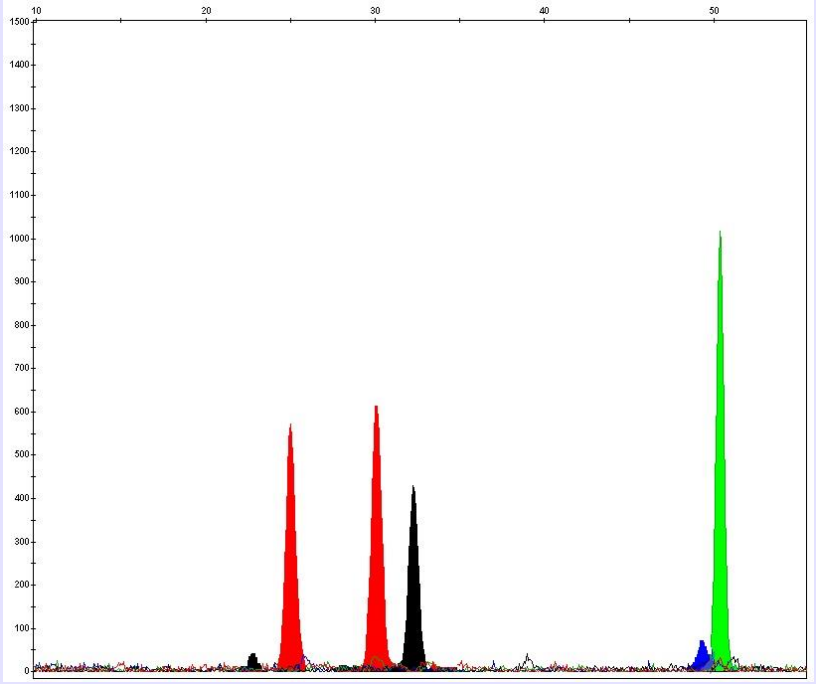
cute-semen crescente

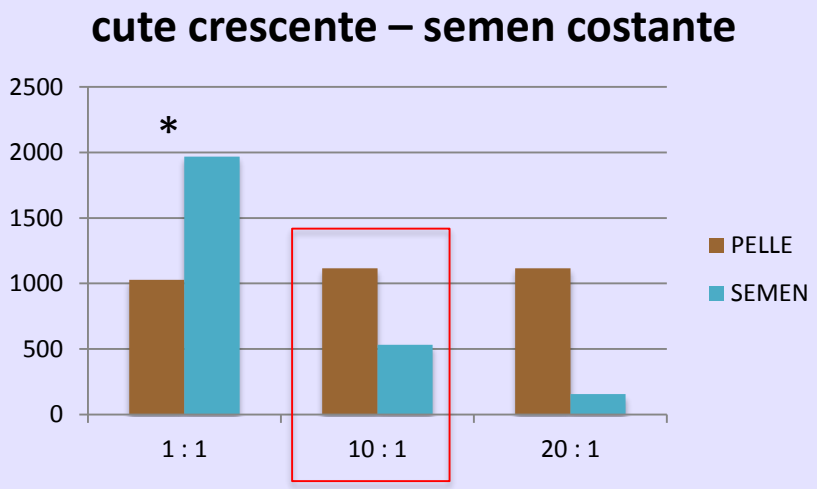


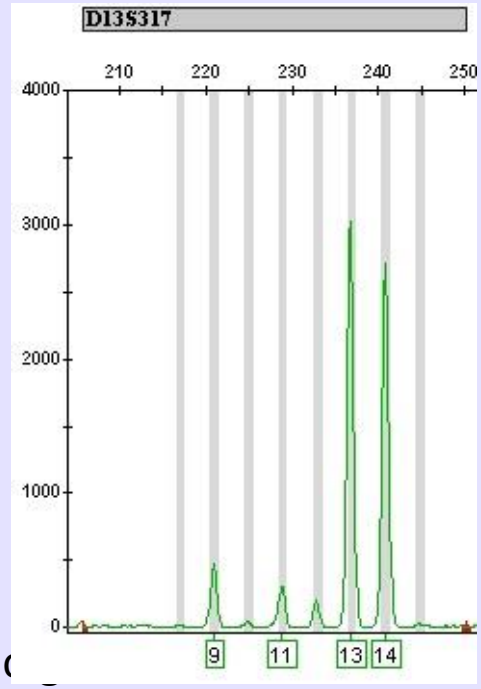
cute costante - semen crescente

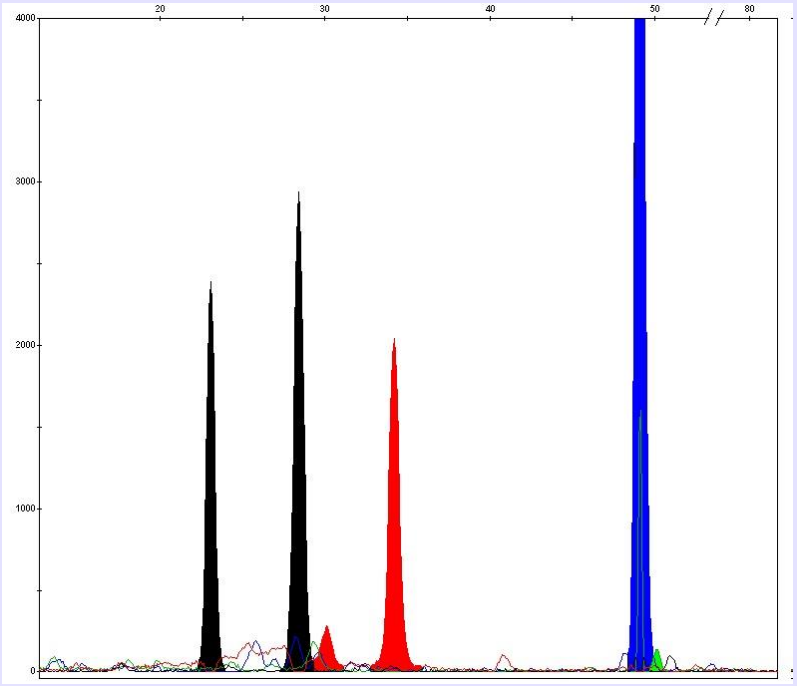







cute crescente – semen costante
2500
2000
1500
1000
500
0
*
1 : 1
10 : 1
20 : 1
PELLE
SEMEN


D13S317
210
220
230
240
250
4000
3000
2000
1000
0
9
11
13
14


20
30
40
50
80
4000
3000
2000
1000
0

Le mettiamo

Conclusioni

- **Necessità di conoscere la natura dei fluidi/tessuti al fine di poter interpretare al meglio i risultati ottenuti sia con lo studio di marcatori STR autosomici sia con lo studio dei polimorfismi del mtDNA**

- **Necessità di approfondire lo studio di misture con mtDNA**