Il genetista forense: dalla scena del crimine alle indagini di laboratorio

Mimmo Turano
- PLS -
Napoli 28 aprile 2021

IL PROFILO GENETICO INDIVIDUALE O GENOTIPO

Indagini di filiazione

Profilo genetico del figlio

Profilo genetico del padre

Profilo genetico della madre

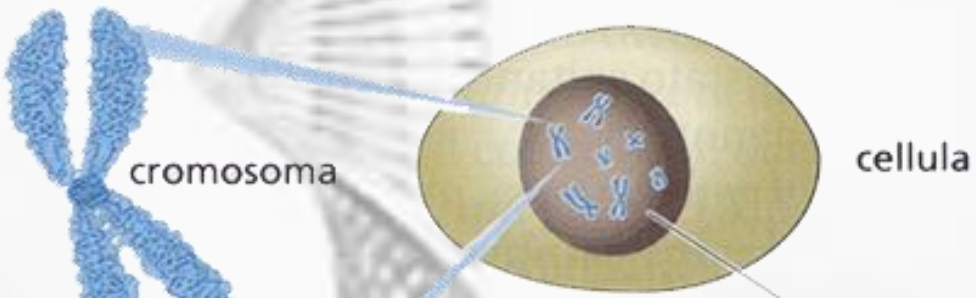
Investigazioni criminali

Profilo genetico da Tracce biologiche

Profilo genetico di banche dati

Profilo genetico di indagati

IL DNA

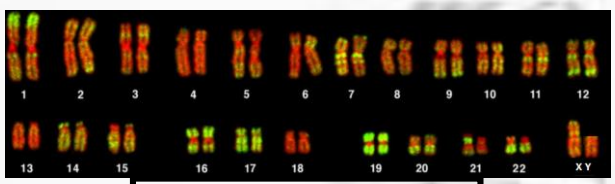


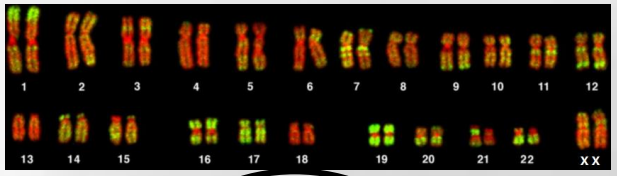
AACAGAGATGGCATTGTACTTGGGAA

GGTTTAAGCCTACAGACAGAAGAAGCTTACAGAGATGGTGAAACAGAGATGGTGACCCTATCA

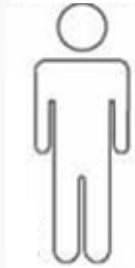
CATACGGTTAAAGCCAATACAGACAGAAGAAGCTTACAGAGATGGTGAAACAGAGATGCGTAAAA…

I CROMOSOMI





46



46

23 **23**

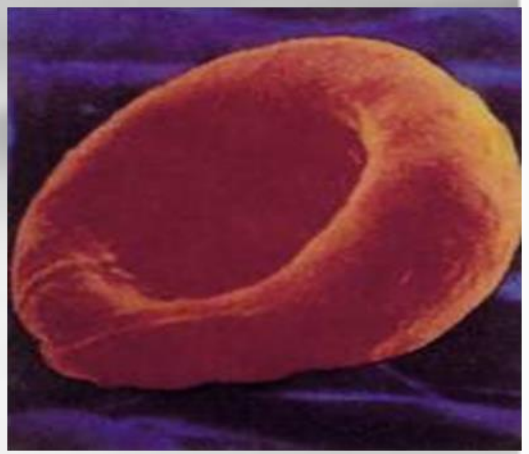
23 **23**

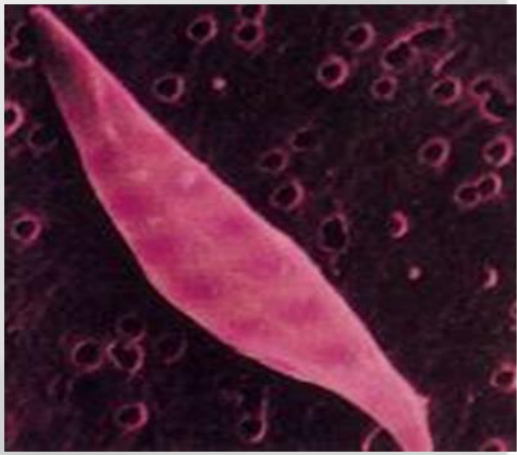
LA VARIABILITA' GENETICA INDIVIDUALE

MUTAZIONE

…CGAATGAGCGACG…

…CGAATGGGCGACG…





MUTAZIONI E POLIMORFISMI

POLIMORFISMI

POLIMORFISMI DI SEQUENZA

..AACCGCATAAGG..

..AACCGTATAAGG..

POLIMORFISMI DI LUNGHEZZA

ACAGAAG AATG AATG AACAGAG

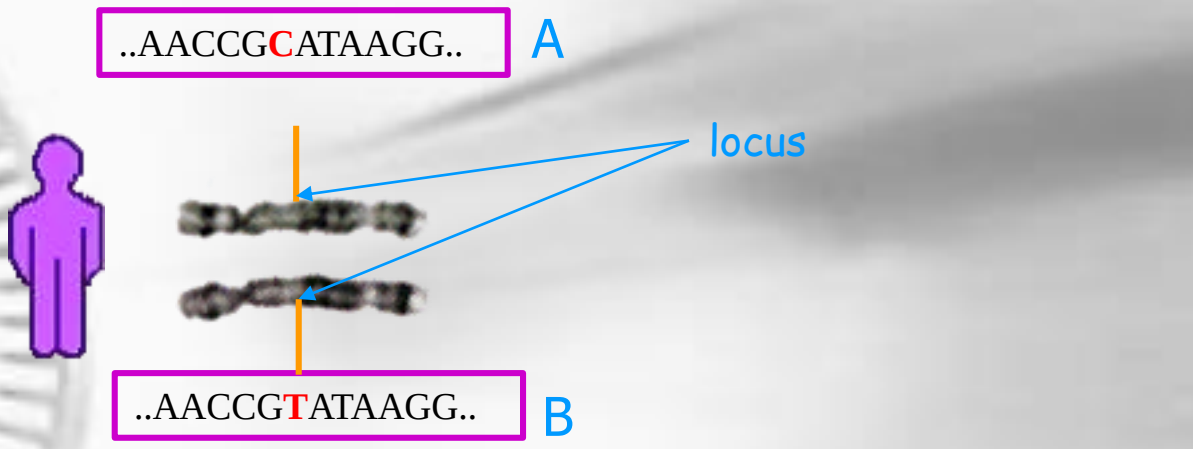
ACAGAAG AATG AATG AATG AATG AATG AACAGAG

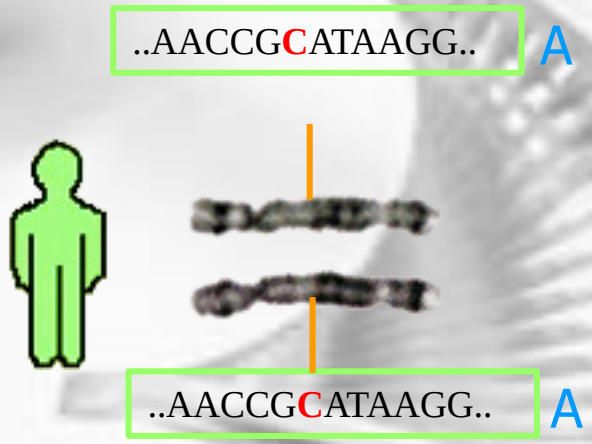
POLIMORFISMI DI SEQUENZA

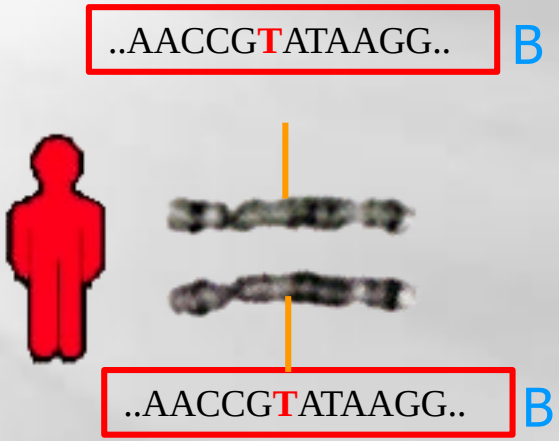
SINGLE NUCLEOTIDE POLYMORPHISMS (SNP)

Polimorfismi biallelici

GENOTIPI:
(AA; BB; AB)







POLIMORFISMI DI SEQUENZA

SINGLE NUCLEOTIDE POLYMORPHISMS (SNP)

GENOTIPO:
AB

ATCGAAGTTC**C**CGAAATTAGC CGAATGAGCGACG TTGGCCAATTTGGATCAAAACGGC

ATCGAAGTTC**T**CGAAATTAGC CGAATGAGCGACG TTGGCCAATTTGGATCAAAACGGC

GENOTIPO:
AA

ATCGAAGTTC**C**CGAAATTAGC CGAATGAGCGACG TTGGCCAATTTGGATCAAAACGGC

ATCGAAGTTC**C**CGAAATTAGC CGAATGAGCGACG TTGGCCAATTTGGATCAAAACGGC

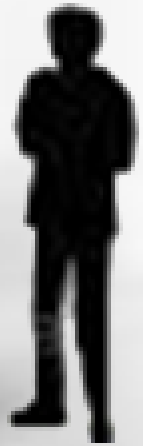
GENOTIPO:
BB

ATCGAAGTTC**T**CGAAATTAGC CGAATGAGCGACG TTGGCCAATTTGGATCAAAACGGC

ATCGAAGTTC**T**CGAAATTAGC CGAATGAGCGACG TTGGCCAATTTGGATCAAAACGGC

POLIMORFISMI DI LUNGHEZZA

I MICROSATELLITI O SHORT TANDEM REPEAT (STR)

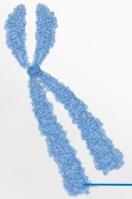


Cromosoma paterno

ATCGAAGTTCCCGAAATTAGC**GATGATGATGAT**CGAATGAGCGACGTTGGC…

ALLELE 4

Cromosoma materno



ATCGAAGTTCCCGAAATTAGC**GATGATGATGATGATGAT** CGAATGAGCGA…

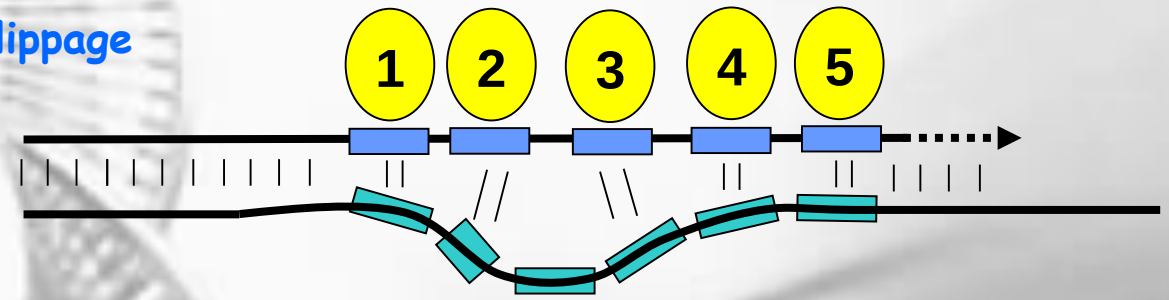
ALLELE 6

locus

GENOTIPO 4,6

STR

Slippage



Allele 3

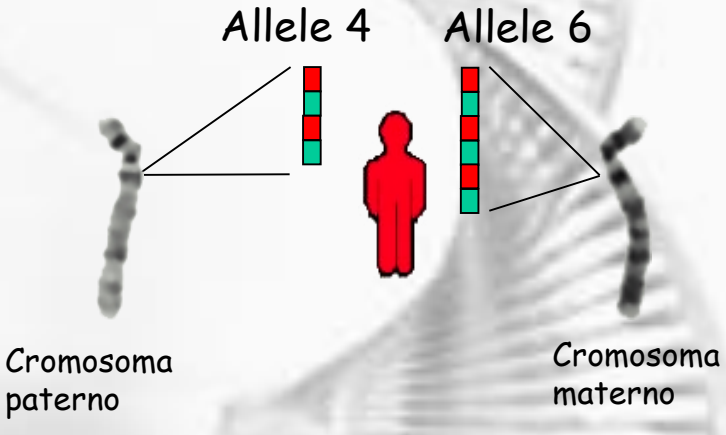
Allele 4

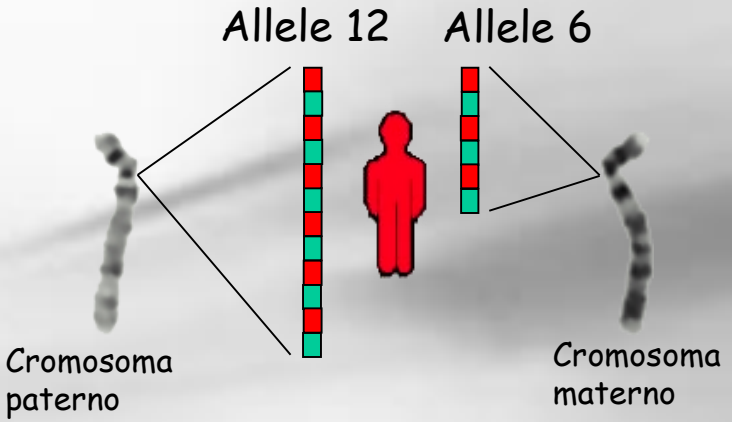
Allele 80

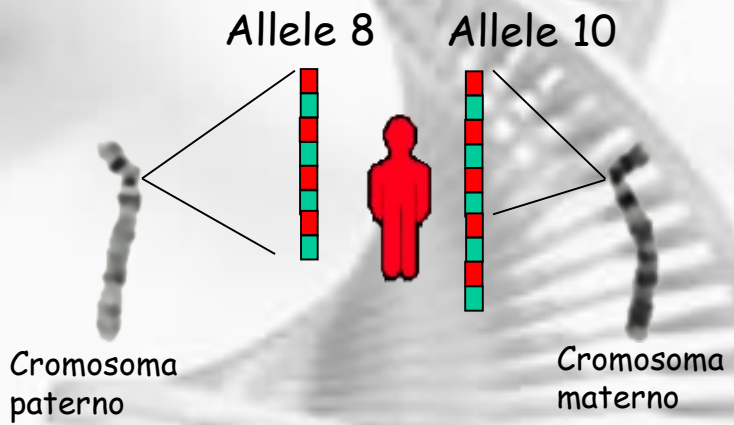
Genotipi del locus FGA:
80 alleli, 3240 genotipi

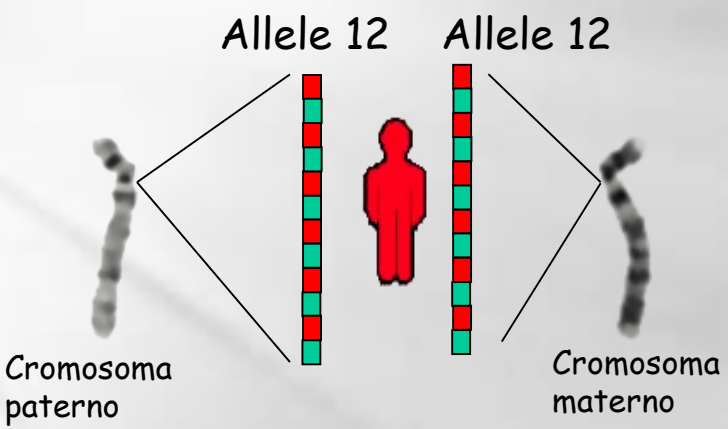
GENOTIPI:
n (n+1)/2

3240 GENOTIPI



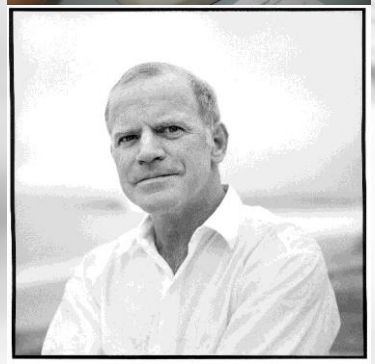




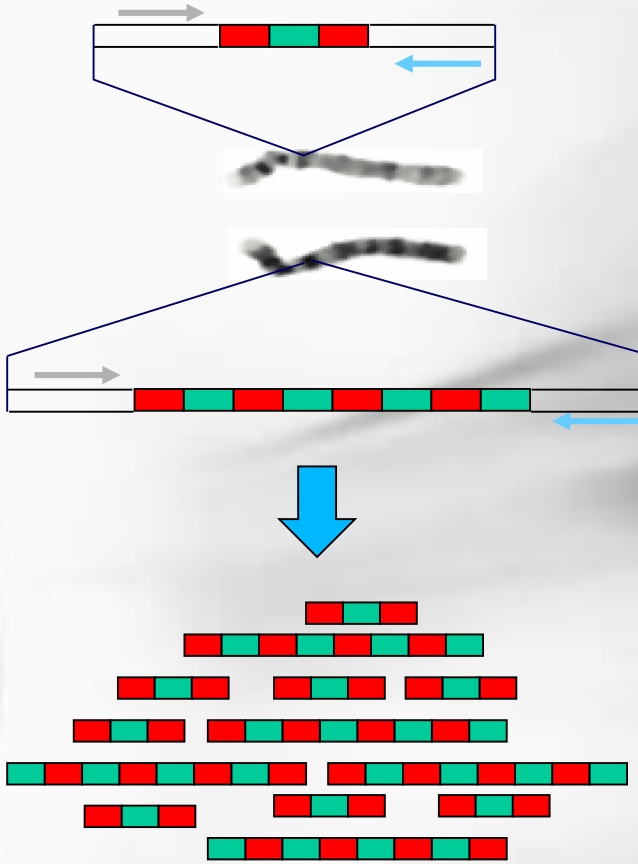


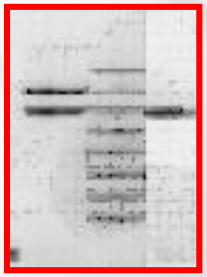
E COSI' VIA…….

PCR DI UN SINGOLO LOCUS STR



Kary Mullis

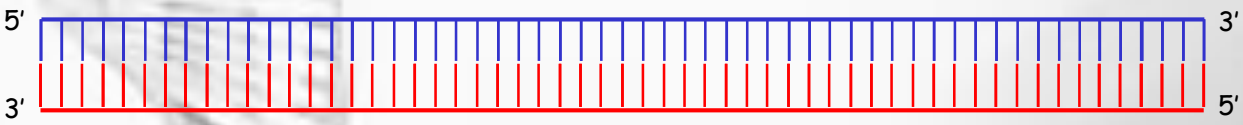


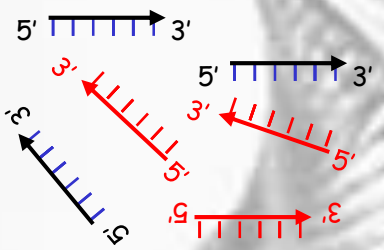


Eterozigoti :
I due alleli hanno lunghezza diversa

Omozigoti:
entrambi gli alleli hanno la stessa lunghezza

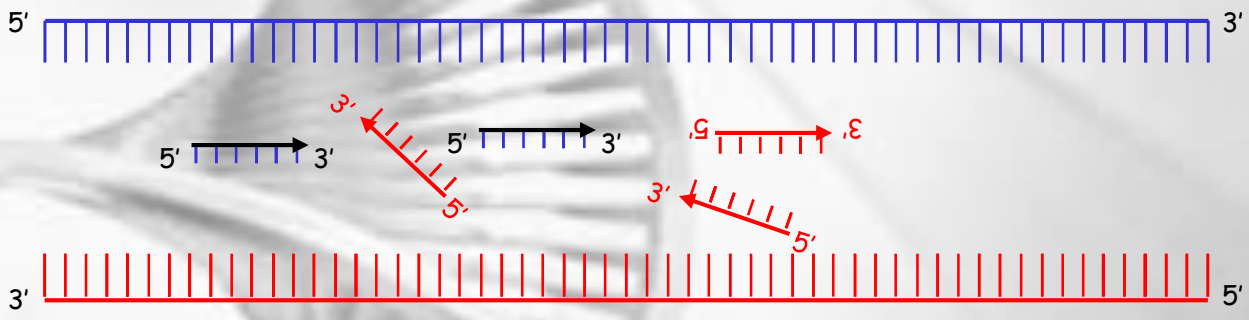
POLYMERASE CHAIN REACTION (PCR)





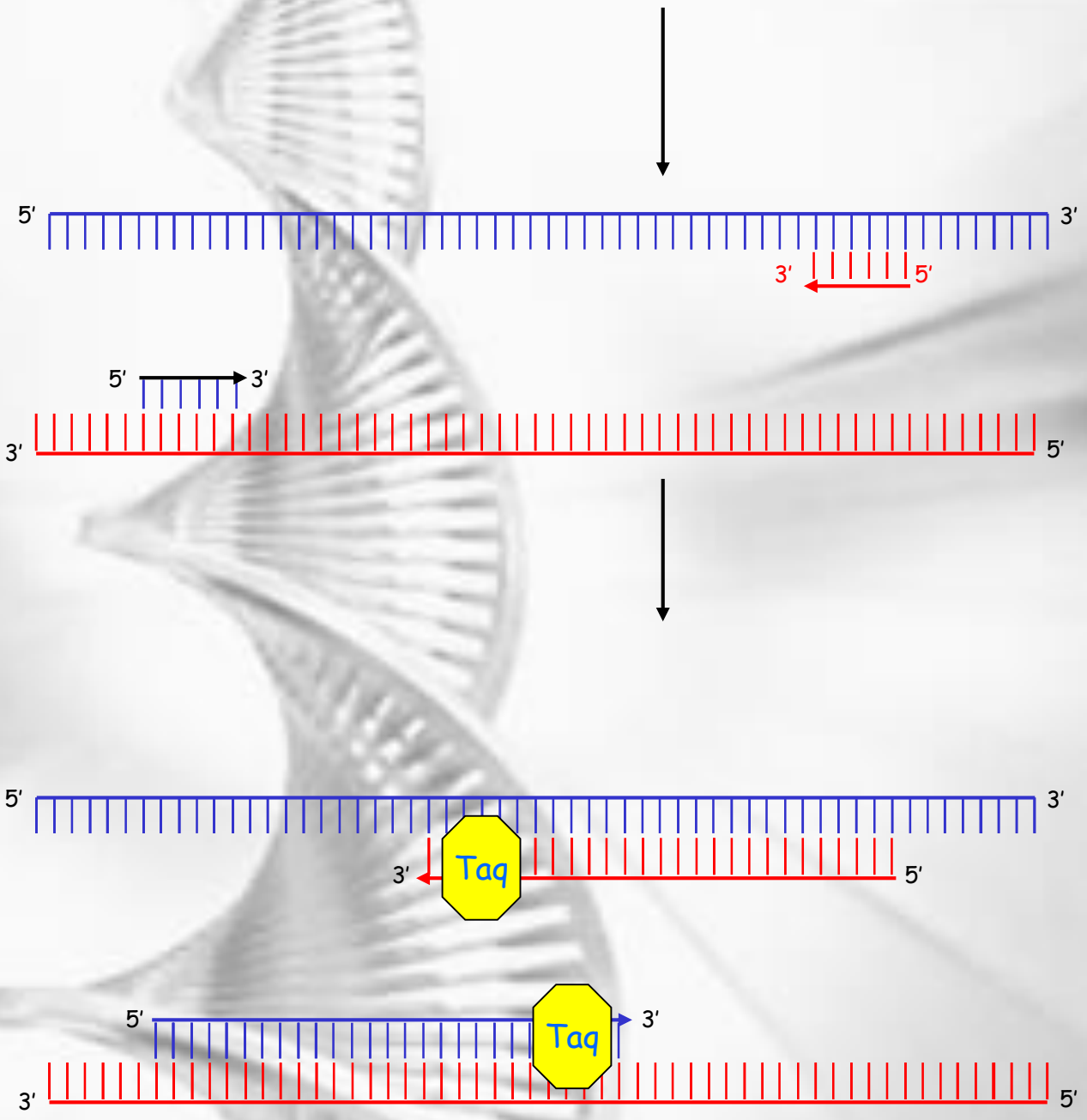
- Primers
- dNTPs (dATP, dGTP, dTTP, dCTP)
- Template (DNA o cDNA)
- Buffer
- Thermal Stable DNA Polymerase (Taq)

UNIRE I COMPONENTI NEL TUBINO DA PCR



DENATURATION

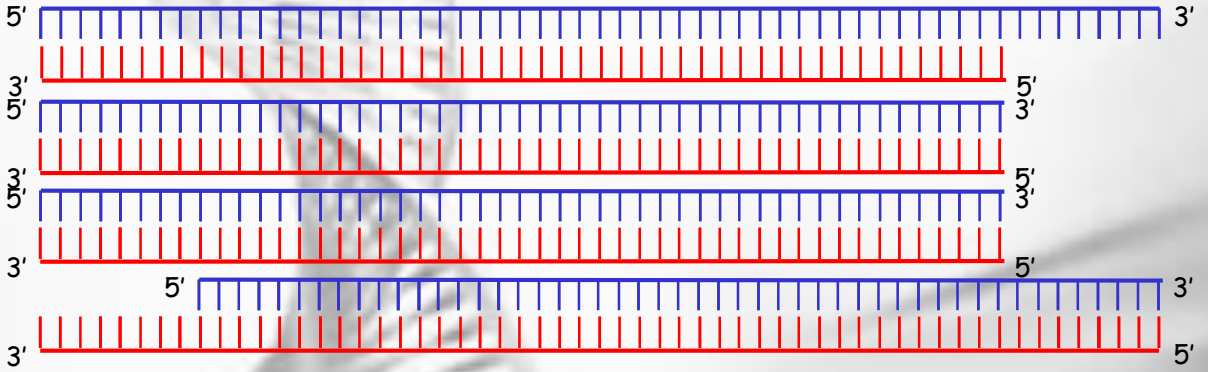
95 °C



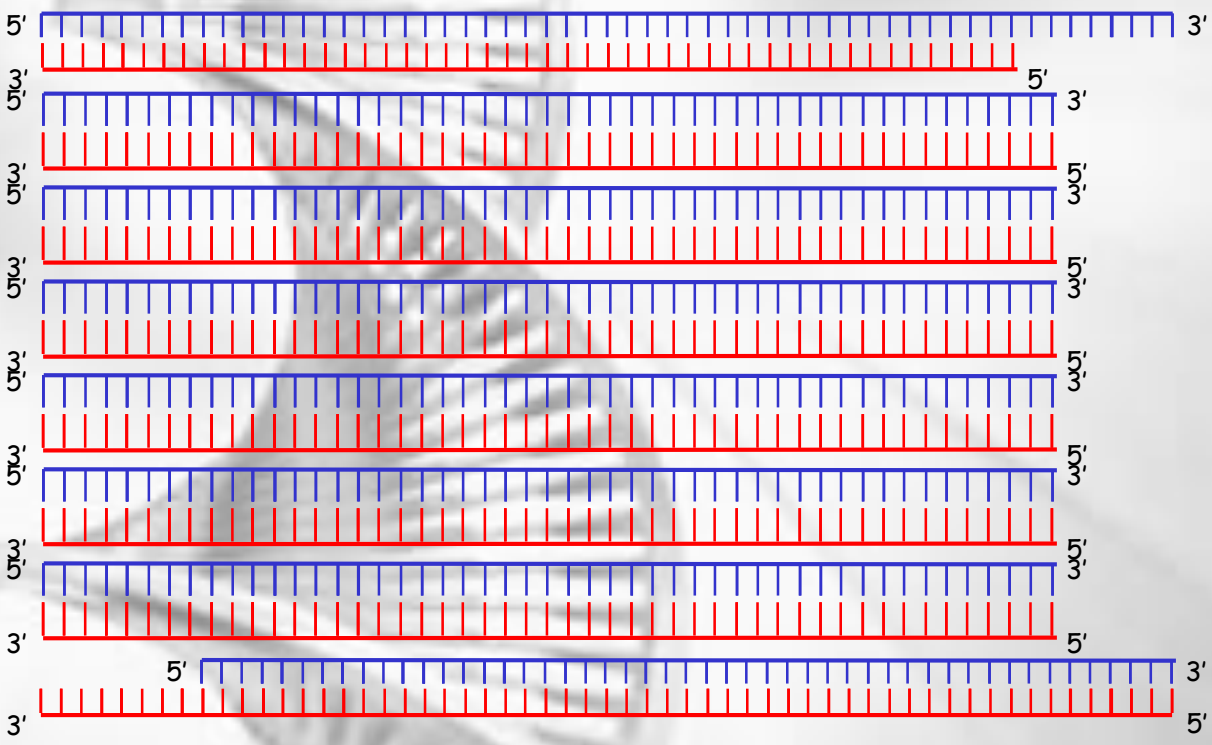
ANNEALING
VARIABLE TEMP.

EXTENSION
72 °C

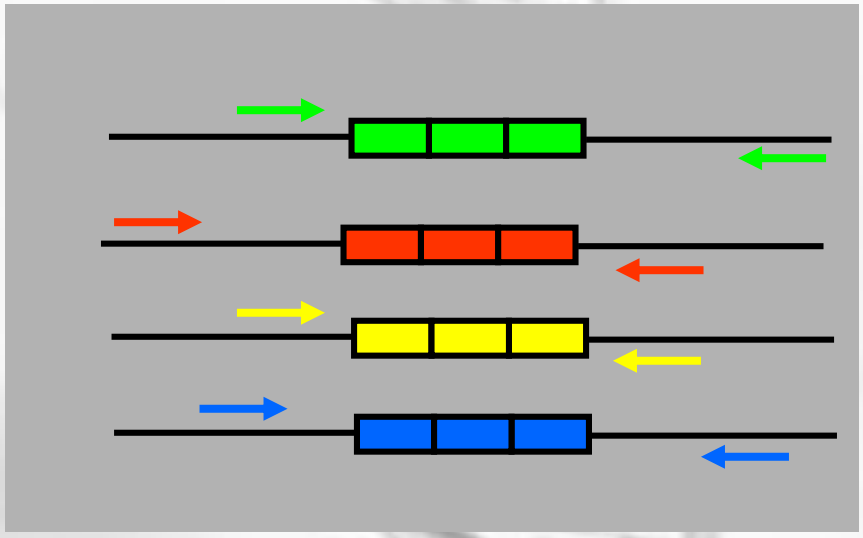
Ciclo 2 → 4 molecole



Ciclo 3 → 8 molecole



MULTIPLEX PCR



Coppie di primer compatibili

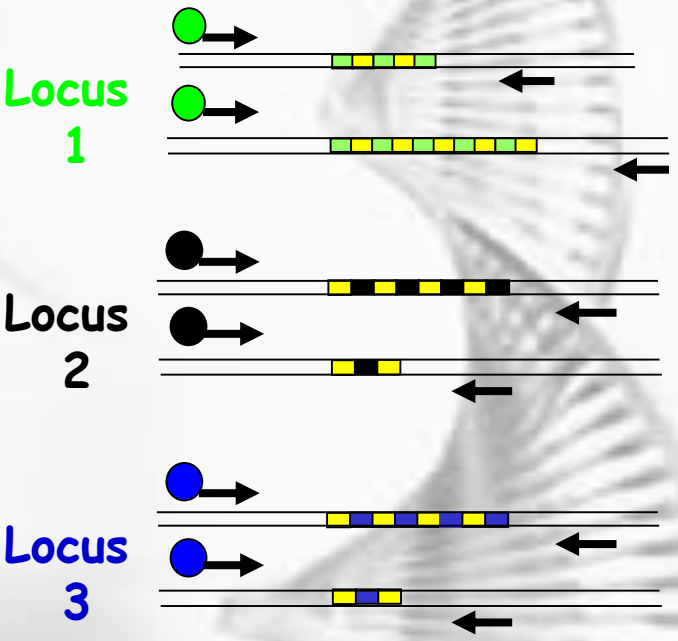
Metodo adeguato per la separazione degli ampliconi

IL SISTEMA CODIS (1996-1997)
COmbined DNA Index System

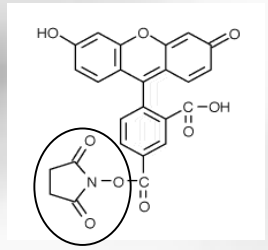
LOCUS	Sequence	chromosome position	GenBank accession	Alleles
TPOX	gaat	2p25.3	M68651	15
D3S1358	[tctg][tcta]	3p21.3	NT_00597	24
FGA	cttt	4q31.3	M64982	80
D5S818	agat	5q23.2	G08446	15
CSF1PO	taga	5q33.1	X14720	20
D7S820	gata	7q21.11	G08616	30
D8S1179	[tcta][tctg]	8q24.13	G08710	17
TH01	tcat	11p15.5	D00269	20
vWA	[tctg][tcta]	12.p13.31	M258S8	28
D13S317	tatc	13q31.1	G09017	17
D16S539	gata	16q24.1	G07925	19
D18S51	agaa	18q21.33	L18333	51
D21S11	[tcta][tctg]	21q21.1	AP000433	82

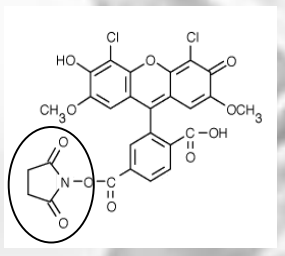
Random match probability = $1/10^{12}$

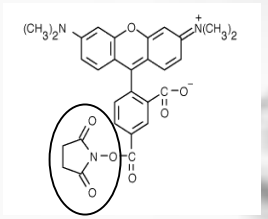
ELETTROFORESI CAPILLARE

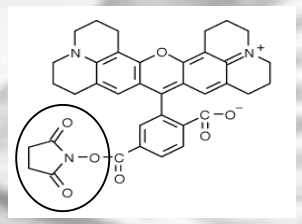


FAM (Blue) JOE (Green)

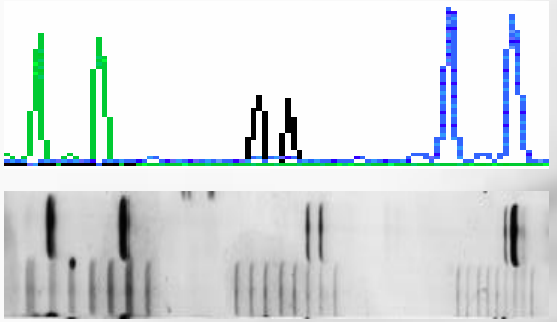


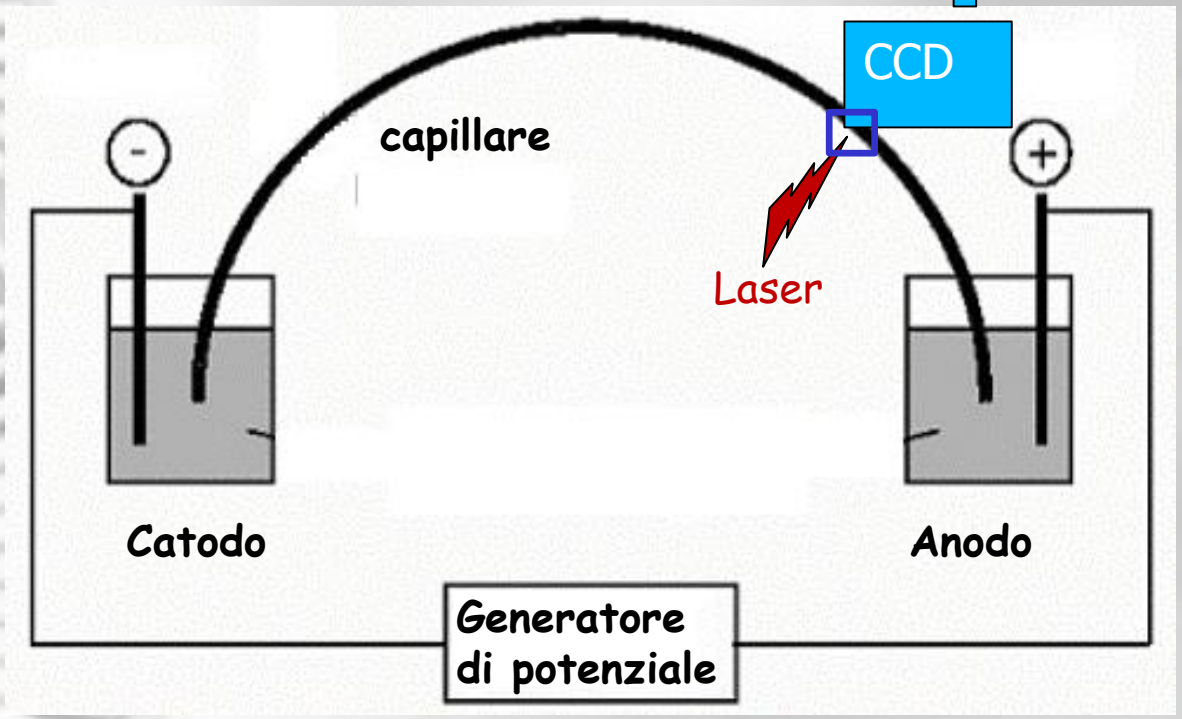




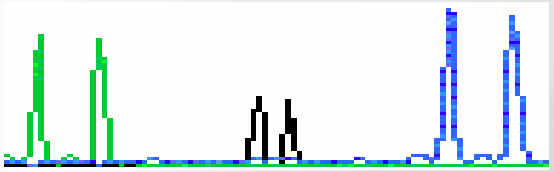


TAMRA (Yellow) ROX (Red)





Frammenti amplificato marcati



Capillare

Rilevamento del segnale

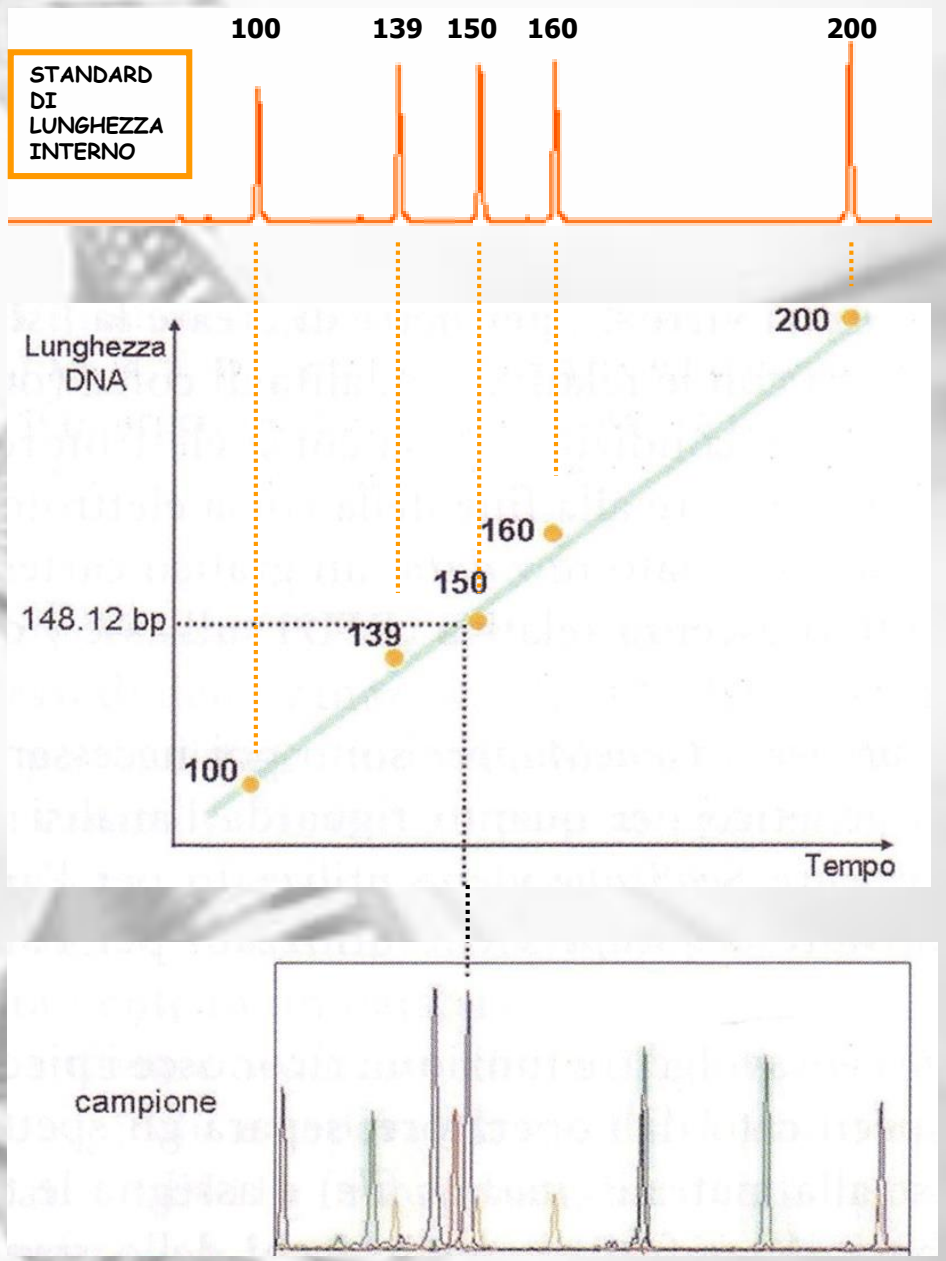
LASER

CCD

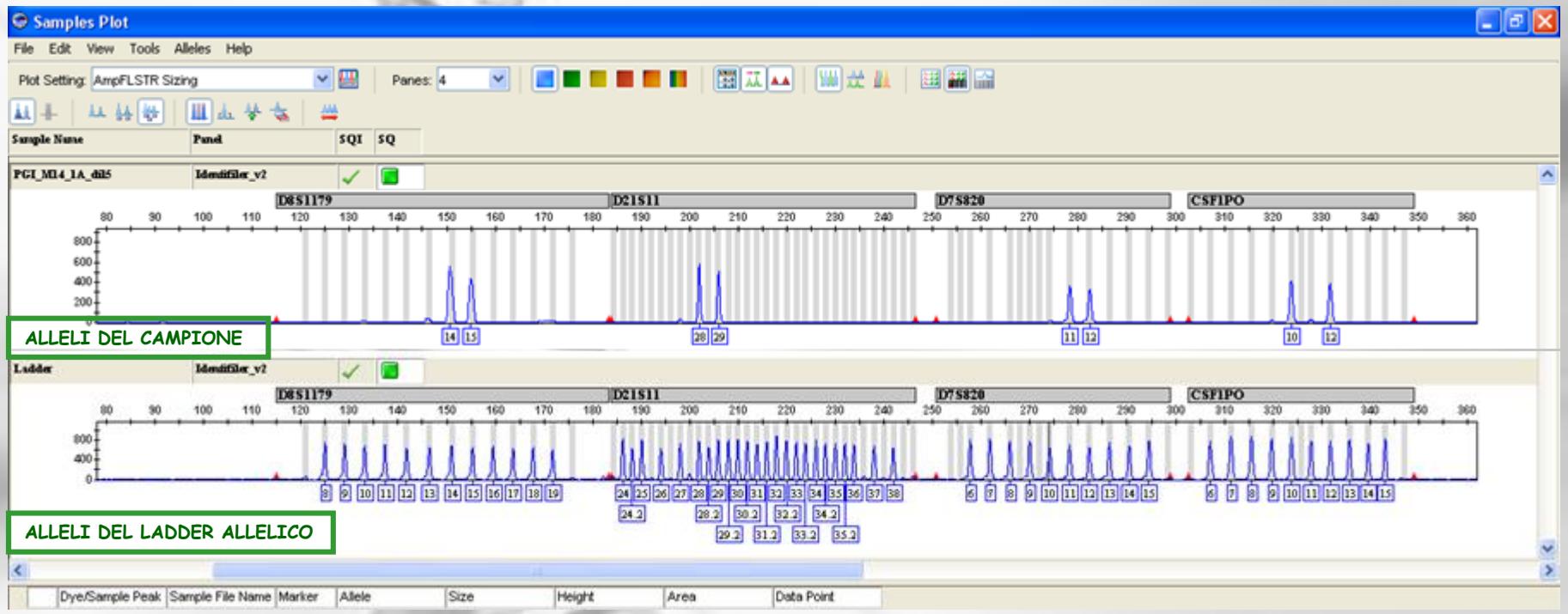
Eccitazione

Emissione di segnali luminosi

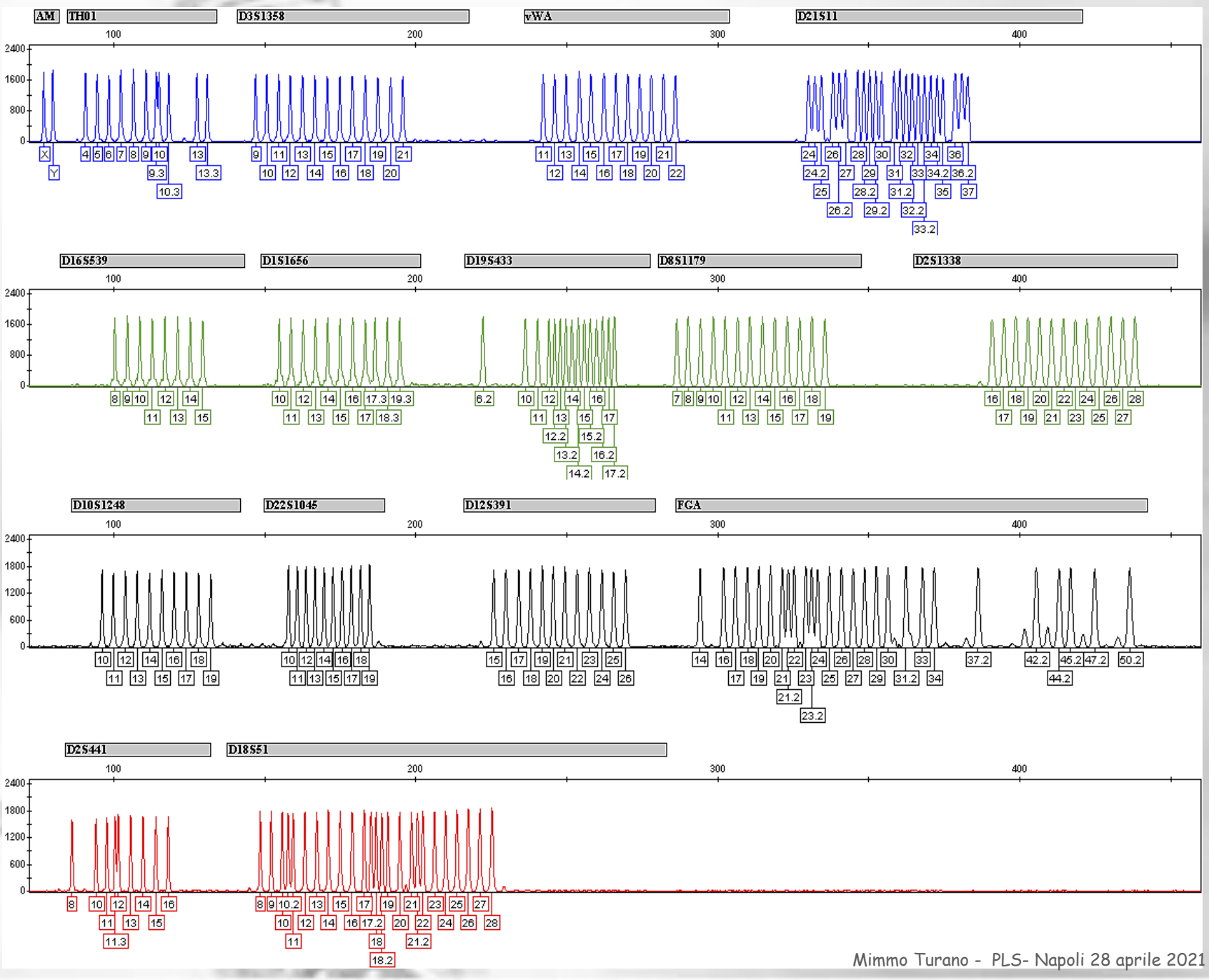
ASSEGNAZIONE DEL PESO MOLECOLARE DEI PICCHI DEL CAMPIONE



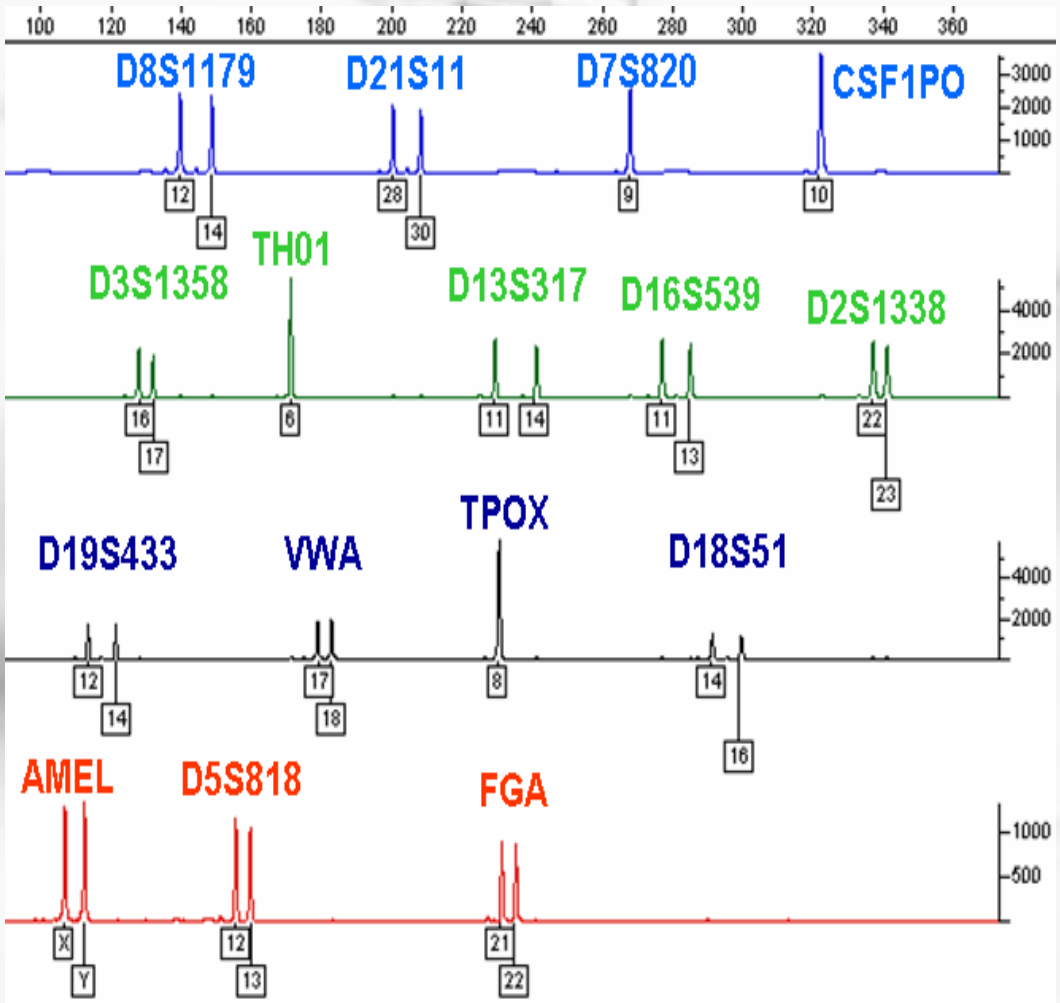
ASSEGNAZIONE ALLELICA



FRAMMENTI DI UN LADDER ALLELICO COMMERCIALE



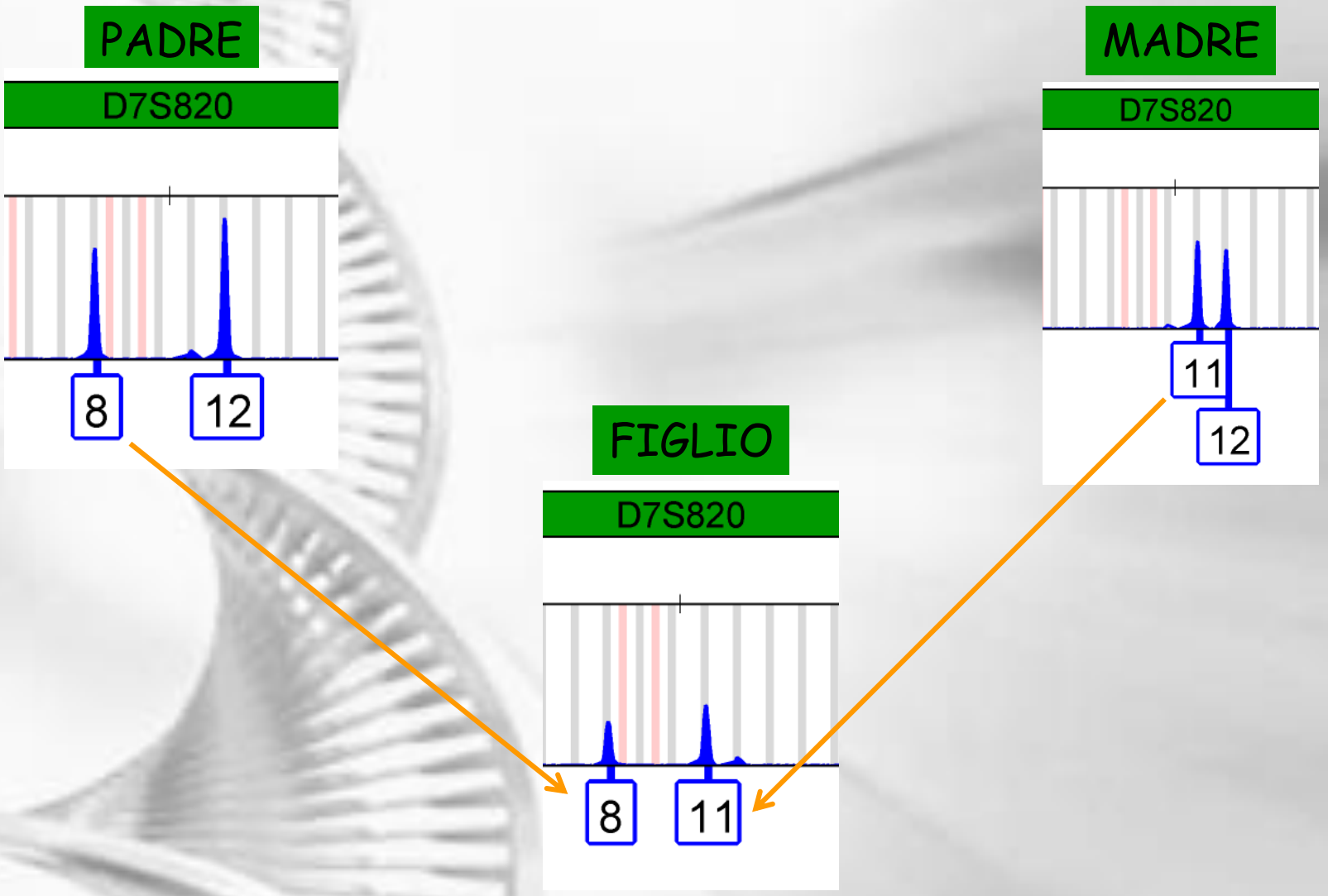
RISULTATO



AmpFlSTR®Identifiler™

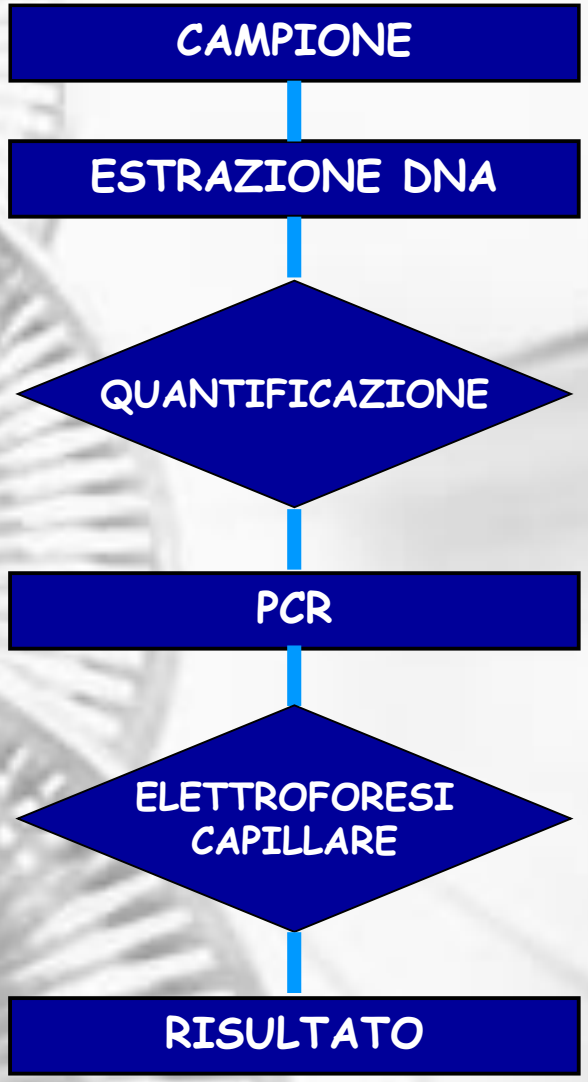
LOCUS	ALLELI	
D8S1179	12	14
D21S11	28	30
D7S820	9	9
CSF1PO	10	10
D3S1358	16	17
TH01	6	6
D13S317	11	14
D16S539	11	13
D2S1338	22	23
D19S433	12	14
vWA	17	18
TPOX	8	8
D18S51	14	16
AMEL	X	Y
D5S818	12	13
FGA	21	22

ATTRIBUZIONE DI PATERNITA'



RISULTATO: PROFILO GENETICO

LOCUS STR	PADRE	FIGLIA	MADRE
D8S1179	13 / 14	13 / 13	13 / 13
D21S11	30 / 32	30 / 34	29 / 34
D7S820	10 / 12	10 / 12	10 / 10
CSF1PO	10 / 12	10 / 10	10 / 11
D3S1358	15 / 18	15 / 18	15 / 15
TH01	8 / 9	8 / 11	11 / 11
D13S317	9 / 11	11 / 11	8 / 11
D16S539	9 / 9	9 / 11	11 / 11
D2S1338	19 / 20	20 / 20	17 / 20
D19S433	15 / 15	13 / 15	13 / 13
vWA	14 / 16	16 / 16	14 / 16
TPOX	8 / 11	8 / 11	8 / 11
D18S51	12 / 13	12 / 12	12 / 18
D5S818	11 / 12	11 / 12	12 / 12
FGA	22 / 25	22 / 24	21 / 24
AMELOGENIN	X / Y	X / X	X / X


CAMPIONE
ESTRAZIONE DNA
QUANTIFICAZIONE
PCR
ELETTROFORESI CAPILLARE
RISULTATO

ALCUNE METODICHE DI ESTRAZIONE DEL DNA DA CAMPIONI BIOLOGICI

ESTRAZIONE ORGANICA

SDS e Proteinase K

↓

Fenolo-cloroformio

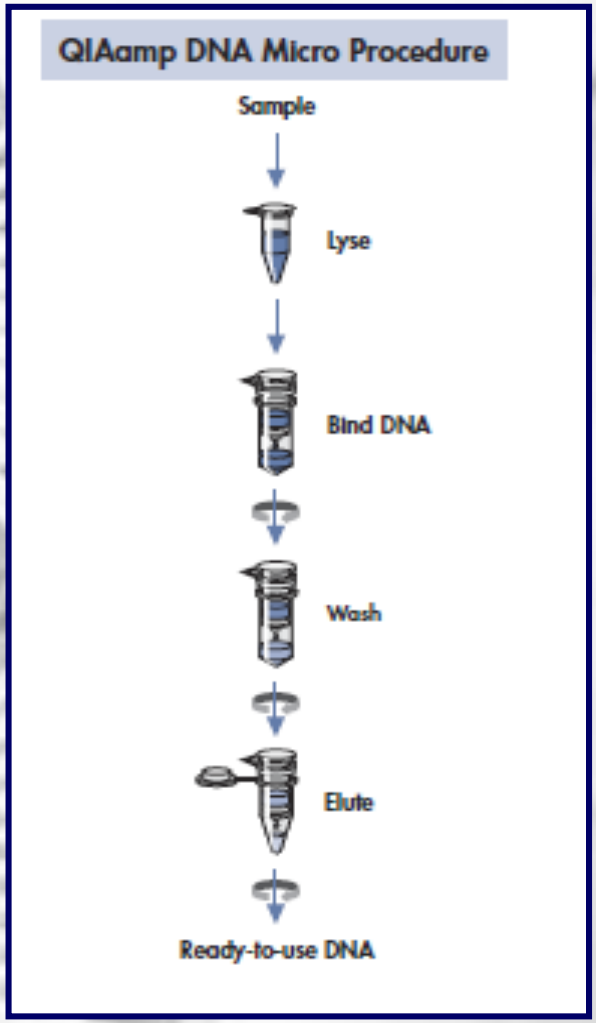
↓

Precipitazione in alcool

↓

Lavaggi e risospensione

SPIN COLUMNS



ESTRAZIONE CON RESINE CHELANTI

+5-10% Chelex
+ Proteinase K

↓

56 °C 30' (lisi)

↓

90 °C 10' (inatt. P. K)

↓

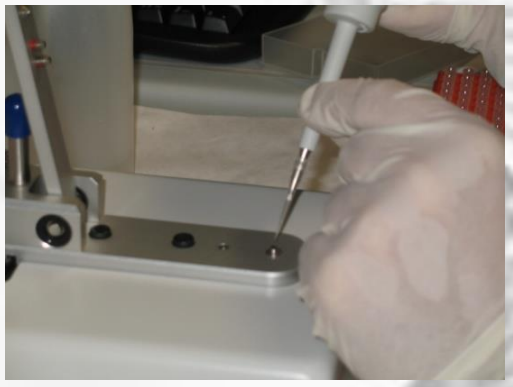
14000rpm 5'

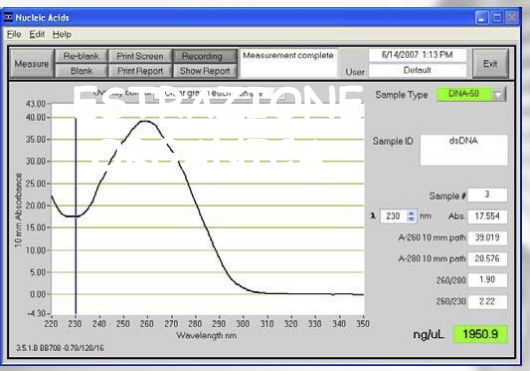
↓

DNA nel surnatante'

QUANTIFICAZIONE DNA

SPETTROFOTOMETRICA



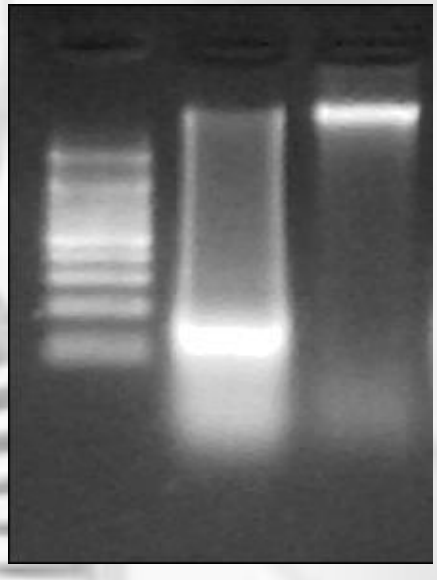


260/280 = 1.8

RNA

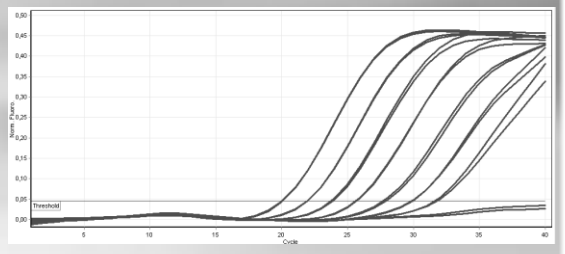
Proteine
Fenolo

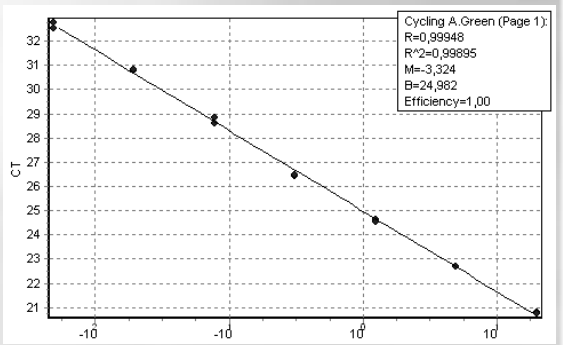
ELETTROFORETICA



MEDIANTE REAL-TIME PCR

20 ng	5 ng	1,25 ng	312,5 pg
78,1 pg	19,5 pg	4,8pg	

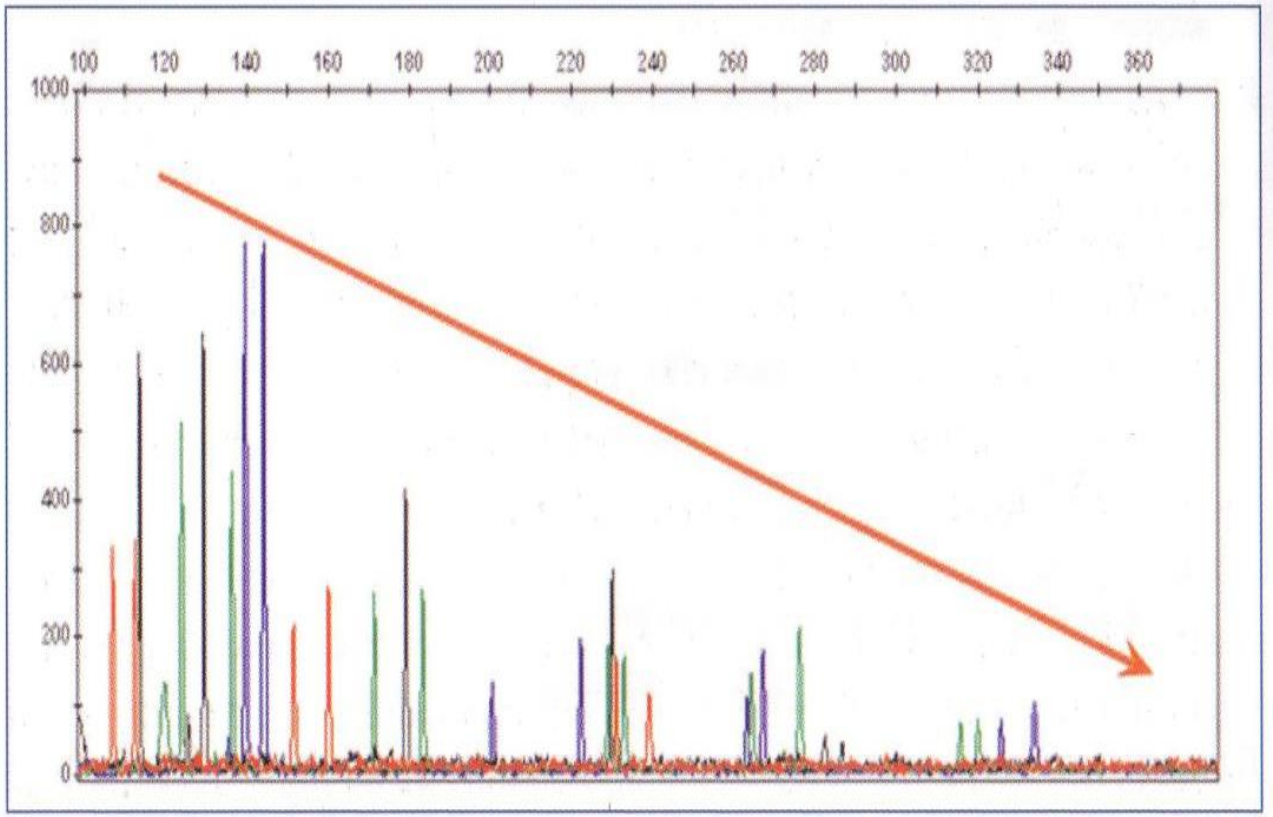




FATTORI CHE INFLUENZANO NEGATIVAMENTE LA PCR

Degradazione

Presenza di inibitori

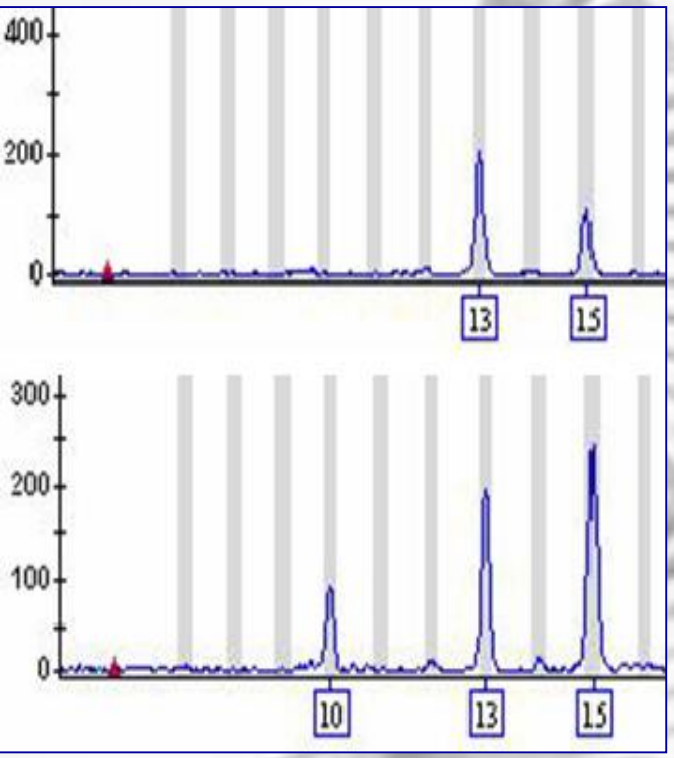


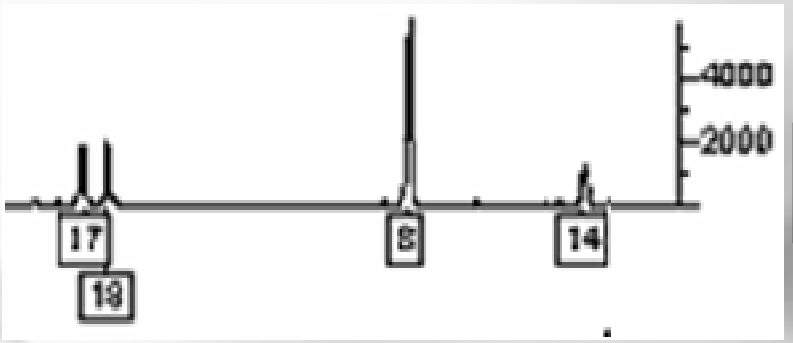
Impatto della degradazione del DNA sulla reazione di amplificazione degli STRs

FATTORI CHE INFLUENZANO NEGATIVAMENTE LA PCR

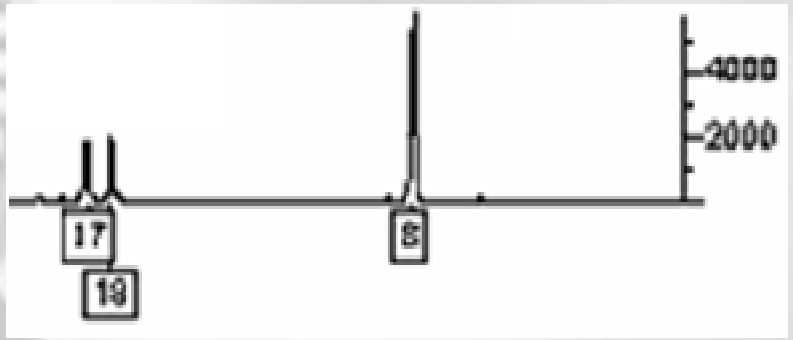
Scarsa quantità di DNA

DROP-IN ALLELICO





SBILANCIAMENTO ALLELICO



DROP-OUT ALLELICO

DROP-OUT DEL LOCUS

RANDOM MATCH PROBABILITY

Locus **D17S79**

Alleli	Frequenze
1	0,01
2	0,003
3	0,007
4	0,004
5	0,015
6	0,223
7	0,199
8	0,263
9	0,2
10	0,029
11	0,032
12	0,01
13	0,006

FREQUENZE GENOTIPICHE = $(p+q)^2 = p^2 + q^2 + 2pq$

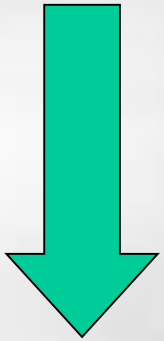
Genotipo
omozigote 6/6 : 0,223 X 0,223 = 0,0497

Genotipo
eterozigote 8/9 : 0,263 X 0,2 X 2 = 0,1052

RANDOM MATCH PROBABILITY

Alleli	D1S7	D2S44	D17S79	D4S139
1	0,004	0,005	0,01	0,004
2	0,006	0,003	0,003	0,01
3	0,009	0,016	0,007	0,006
4	0,012	0,024	0,004	0,014
5	0,011	0,046	0,015	0,033
6	0,014	0,034	0,223	0,024
7	0,01	0,123	0,199	0,04
8	0,029	0,106	0,263	0,047
9	0,021	0,084	0,2	0,054
10	0,014	0,049	0,029	0,071
11	0,028	0,083	0,032	0,108
12	0,031	0,039	0,01	0,19
13	0,046	0,041	0,006	0,129
14	0,067	0,039		0,095
15	0,057	0,087		0,036
16	0,061	0,089		0,036
17	0,069	0,075		0,103
18	0,055	0,022		
19	0,06	0,018		
20	0,063	0,008		
21	0,079	0,008		
22	0,077			
23	0,077			
24	0,032			
25	0,019			
26	0,05			

5/22 D1S7 : 0.011 X 0.077 X 2 = 0.001694
6/8 D2S44 : 0.034 X 0.106 X 2 = 0.007208
5/9 D17S69 : 0.015 X 0.200 X 2 = 0.006000
12/12 D4S139 : 0.190 X 0.190 = 0.036100



0.001694 X 0.007208 X 0.006000 X 0.036100 = 2 X 10-9
(= 1/5 mld)

E se consideriamo 16 o addirittura 24 loci ?

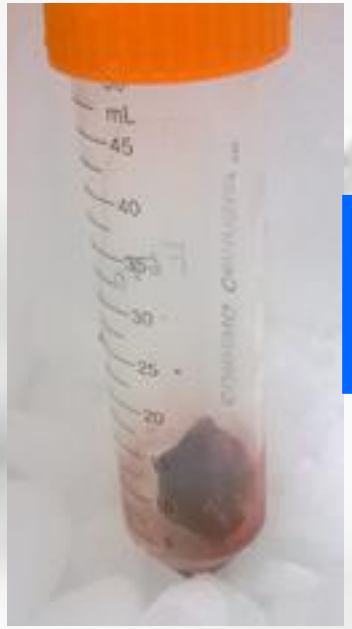
UNA PERIZIA PENALE

CONFERIMENTO INCARICO DI PERIZIA

Proceda il consulente, esaminati gli atti, effettuati i necessari prelievi biologici sul padre, sulla figlia e sul prodotto abortivo in sequestro ed effettuate le necessarie indagini di laboratorio:

- all'estrazione del profilo genetico (DNA) rilevabile dai predetti campioni;

- alla comparazione dei profili genetici estratti, evidenziando se essi siano compatibili e in che grado e se la paternità del prodotto del concepimento sia da attribuire al padre e con quale grado di certezza e/o compatibilità.



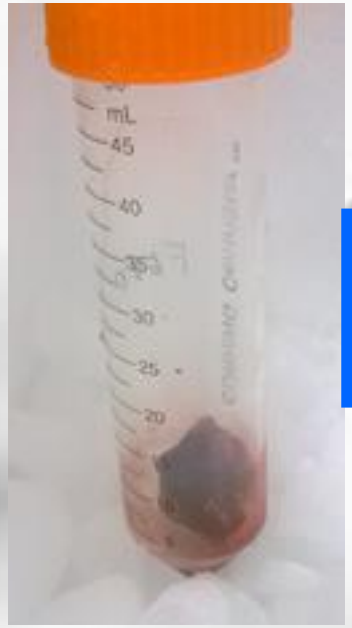
Estrazione DNA
da 1 pezzetto di tessuto
Amplificazione ed
Elettroforesi capillare

LOCI STR	FETO
D8S1179	14/15
D21S11	28/29
D7S820	11/12
CSF1PO	10/12
D3S1358	15/18
TH01	6/9
D13S317	11/11
D16S539	10/11
D2S1338	18/24
D19S433	13/13.2
vWA	14/19
TPOX	8/9
D18S51	14/17
D5S818	11/13
FGA	19/22
AMELOGENIN	X/X

Prelievo salivare sulla madre

Estrazione DNA
Amplificazione
Elettroforesi capillare
e
Confronto dei profili

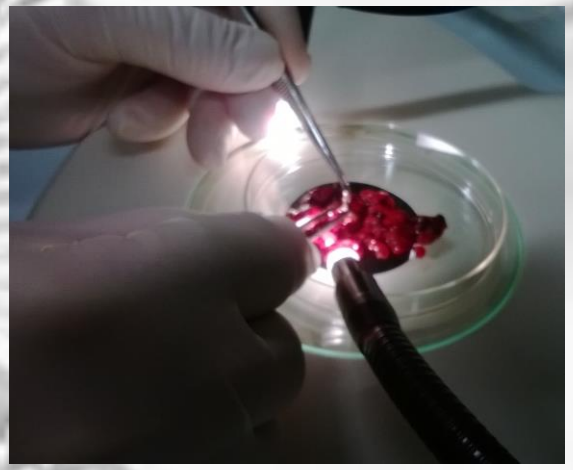
LOCI STR	FETO	SALIVA
D8S1179	14/15	14/15
D21S11	28/29	28/29
D7S820	11/12	11/12
CSF1PO	10/12	10/12
D3S1358	15/18	15/18
TH01	6/9	6/9
D13S317	11/11	11/11
D16S539	10/11	10/11
D2S1338	18/24	18/24
D19S433	13/13.2	13/13.2
vWA	14/19	14/19
TPOX	8/9	8/9
D18S51	14/17	14/17
D5S818	11/13	11/13
FGA	19/22	19/22
AMELOGENIN	X/X	X/X

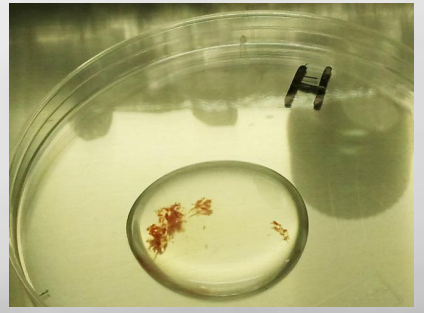


Estrazione DNA
da altri 5 pezzetti tissutali
Amplificazione ed
Elettroforesi capillare

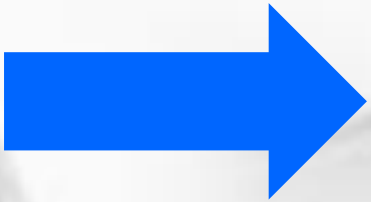
LOCI STR	FETO	SALIVA
D8S1179	14/15	14/15
D21S11	28/29	28/29
D7S820	11/12	11/12
CSF1PO	10/12	10/12
D3S1358	15/18	15/18
TH01	6/9	6/9
D13S317	11/11	11/11
D16S539	10/11	10/11
D2S1338	18/24	18/24
D19S433	13/13.2	13/13.2
vWA	14/19	14/19
TPOX	8/9	8/9
D18S51	14/17	14/17
D5S818	11/13	11/13
FGA	19/22	19/22
AMELOGENIN	X/X	X/X

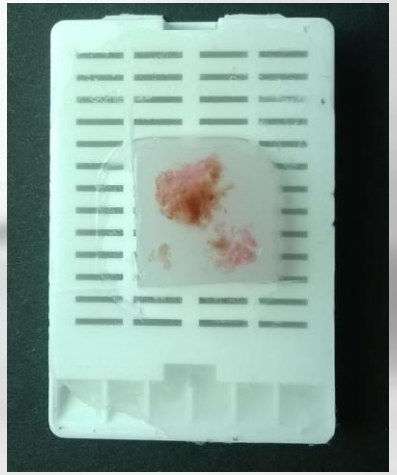
RICERCA DI VILLI CORIALI

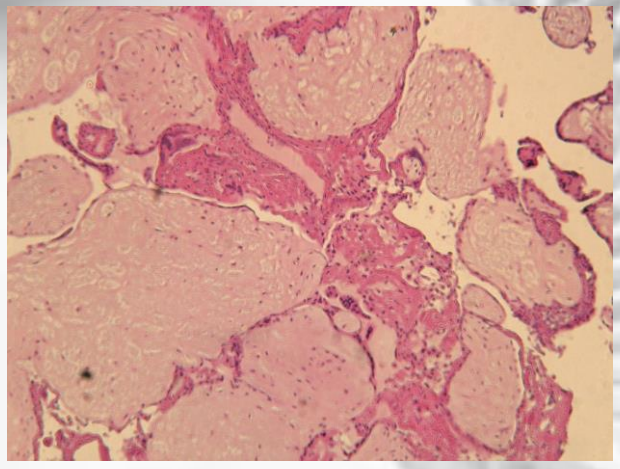


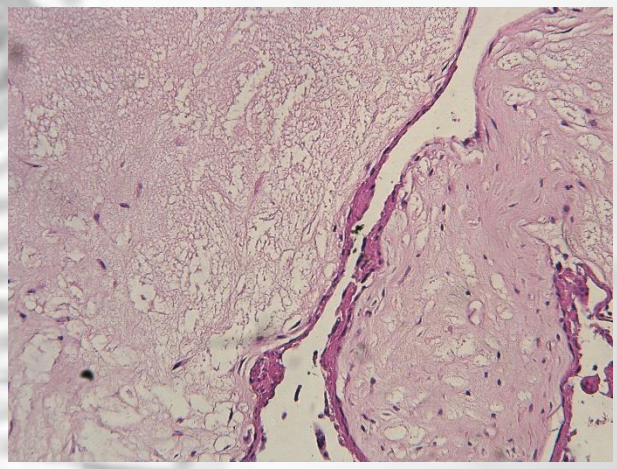


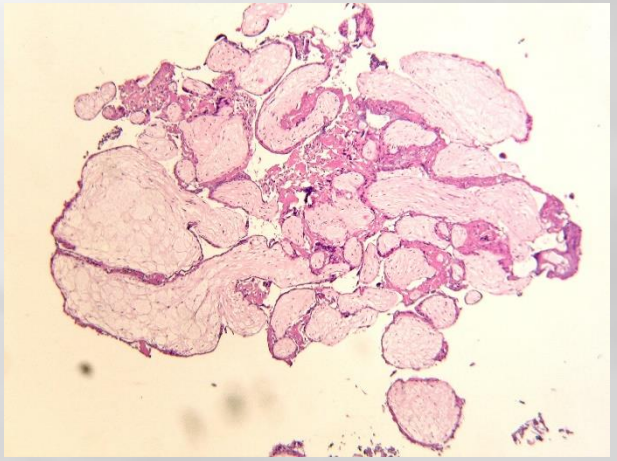
IMMUNOISTOCHIMICA

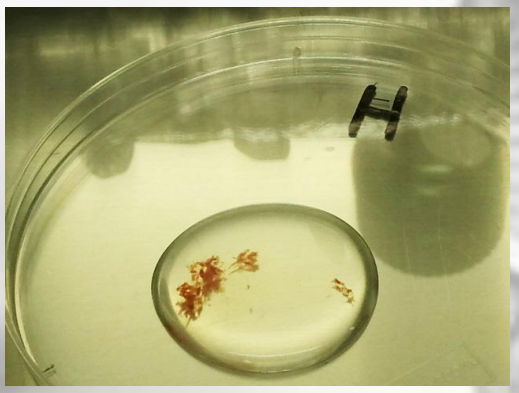












Estrazione DNA
Amplificazione ed
Elettroforesi capillare

LOCI STR	VILLO	SALIVA
D8S1179	15/15	14/15
D21S11	28/29	28/29
D7S820	8/11	11/12
CSF1PO	10/10	10/12
D3S1358	15/18	15/18
TH01	6/6	6/9
D13S317	11/11	11/11
D16S539	11/11	10/11
D2S1338	18/24	18/24
D19S433	13.2/13.2	13/13.2
vWA	14/19	14/19
TPOX	8/9	8/9
D18S51	15/17	14/17
D5S818	13/13	11/13
FGA	22/22	19/22
AMELOGENIN	X/X	X/X

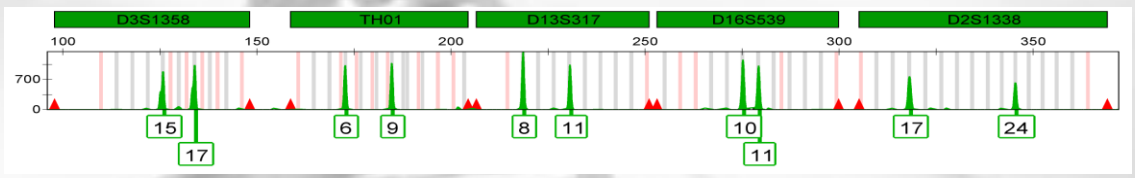
Prelievo salivare sull'indagato

Estrazione DNA
Amplificazione ed
Elettroforesi capillare

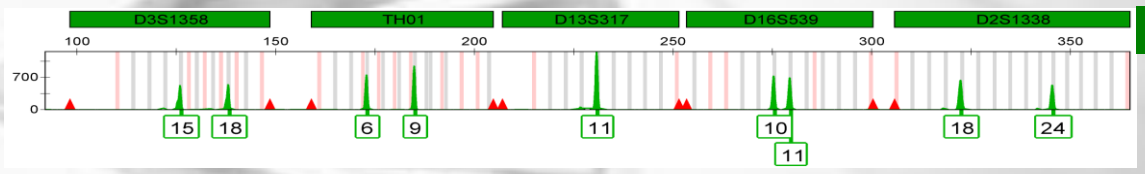
LOCI STR	INDAGATO
D8S1179	13/15
D21S11	28/29
D7S820	8/12
CSF1PO	10/11
D3S1358	15/17
TH01	6/9
D13S317	8/11
D16S539	10/11
D2S1338	17/24
D19S433	13/13.2
vWA	18/19
TPOX	8/11
D18S51	15/17
D5S818	10/13
FGA	22/24
AMELOGENIN	X/Y

LOCI STR	FETO	PADRE	FIGLIA
D8S1179	15/15	13/15	14/15
D21S11	28/29	28/29	28/29
D7S820	8/11	8/12	11/12
CSF1PO	10/10	10/11	10/12
D3S1358	15/18	15/17	15/18
TH01	6/6	6/9	6/9
D13S317	11/11	8/11	11/11
D16S539	11/11	10/11	10/11
D2S1338	18/24	17/24	18/24
D19S433	13.2/13.2	13/13.2	13/13.2
vWA	14/19	18/19	14/19
TPOX	8/9	8/11	8/9
D18S51	15/17	15/17	14/17
D5S818	13/13	10/13	11/13
FGA	22/22	22/24	19/22
AMELOGENIN	X/X	X/Y	X/X

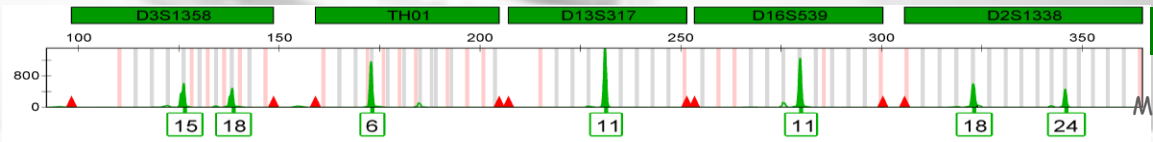
L'INDAGATO E' IL PADRE DEL FETO!!!



PADRE



FIGLIA



FETO

Laboratorio di identificazione e tracciabilità molecolare

UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II
DIPARTIMENTO DI BIOLOGIA

CORSO DI PERFEZIONAMENTO DIAGNOSTICA E GENETICA FORENSE A.A. 2020/2021

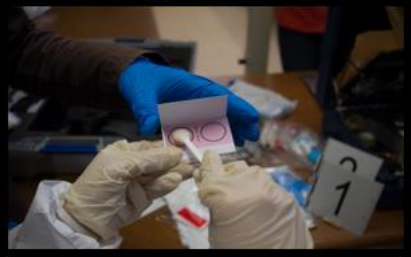
Scadenza del bando 04/12/2020

Il corso si propone l'obiettivo di fornire le competenze teoriche, pratiche e strumentali per l'esecuzione delle principali analisi forensi in ambito biologico

Il corso, articolato in moduli mensili di 4-5 incontri per un totale di 14 CFU, è riservato a laureati triennali e magistrali e prevede 56 ore di didattica frontale e 56 ore di attività pratiche in laboratorio, svolte da docenti universitari ed esperti del settore.

Verranno trattate le analisi del DNA e di molecole utili nella diagnostica forense per:

- **identificazione di autori di reati**
- **ricostruzione di rapporti parentali**
- **tipizzazione di specie animali e vegetali**



L'importo del contributo di iscrizione al Corso è di € 850 versabile anche in due rate di € 425 ciascuna.
Il bando e la domanda possono essere scaricati sul sito WEB del corso

(http://genetica-forense.it/)

sul sito WEB dell'Ateneo (https://www.unina.it/didattica/post-laurea/perfezionamento) e sul sito WEB del Dipartimento di Biologia (http://www.dipartimentodibiologia.unina.it/corsi-di-perfezionamento/).
Per ulteriori informazioni rivolgersi alla segreteria scientifica ed organizzativa:

mimmo.turano@unina.it ; Tel. 081 679076 **aldo.donizetti@unina.it ; Tel. 081 679082**

GRAZIE PER L'ATTENZIONE