

Analýza DNA v kriminalistice

Odbor kriminalistické techniky a expertíz
Krajské ředitelství PČR Jihomoravského kraje

I.Kraus

Zdroje biologického materiálu k profilování DNA v kriminalistice

- krev
- sperma
- kost
- zub
- vlas s kořínkem
- vlas bez kořínku (stvol)
- sliny s obsahem jaderných buněk
- moč
- výkaly
- nečistoty zpod nehtů
- svalovina
- nedopalek cigarety
- poštovní známka
- chlopeň poštovní obálky
- lupy
- otisky prstů
- osobní věci: žiletka, žvýkačka, náramkové hodinky, ušní maz, zubní kartáček, spodní prádlo, hygienická vložka, hřeben

Type of Sample**Amount of DNA**

Liquid blood

20 000–40 000 ng/mL

Blood stain

250–500 ng/cm²

Liquid semen

150 000–300 000 ng/mL

Post-coital vaginal swab

10–3000 ng/swab

Plucked hair (with root)

1–750 ng/root

Shed hair (with root)

1–10 ng/root

Liquid saliva

1000–10 000 ng/mL

Oral swab

100–1500 ng/swab

Urine

1–20 ng/mL

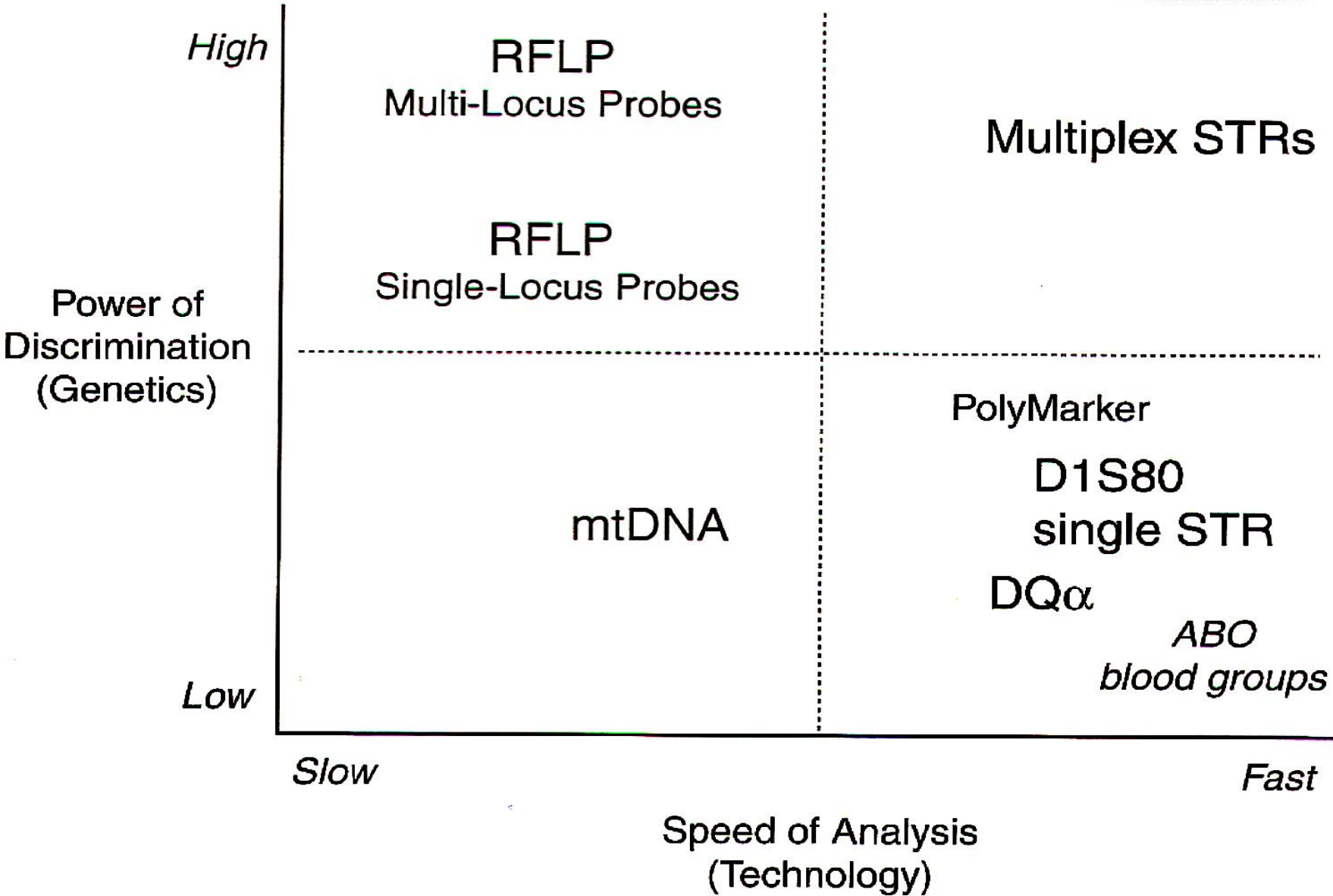
Bone

3–10 ng/mg

Tissue

50–500 ng/mg

Markers Used
(Biology)



Characteristic	RFLP Methods	PCR Methods
Time required to obtain results	6–8 weeks with radioactive probes; ~1 week with chemiluminescent probes	1–2 days
Amount of DNA needed	50–500 ng	0.1–1 ng
Condition of DNA needed	High molecular weight, intact DNA	May be highly degraded
Capable of handling sample mixtures	Yes (single-locus probes)	Yes
Allele identification	Binning required since a distribution of sizes are observed	Discrete alleles obtained
Form used in analysis	DNA must be double-stranded for restriction enzymes to work	DNA can be either single-stranded or double-stranded
Power of discrimination	~1 in 1 billion with 6 loci	~1 in 1 billion with 8–13 loci (requires more loci)
Automatable and capable of high-volume sample processing	No	Yes
Commonly used DNA markers	D1S7, D2S44, D4S139, D5S110, D7S467, D10S28, D17S79	DQA1, D1S80, STR loci: TH01, VWA, FGA, TPOX, CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11

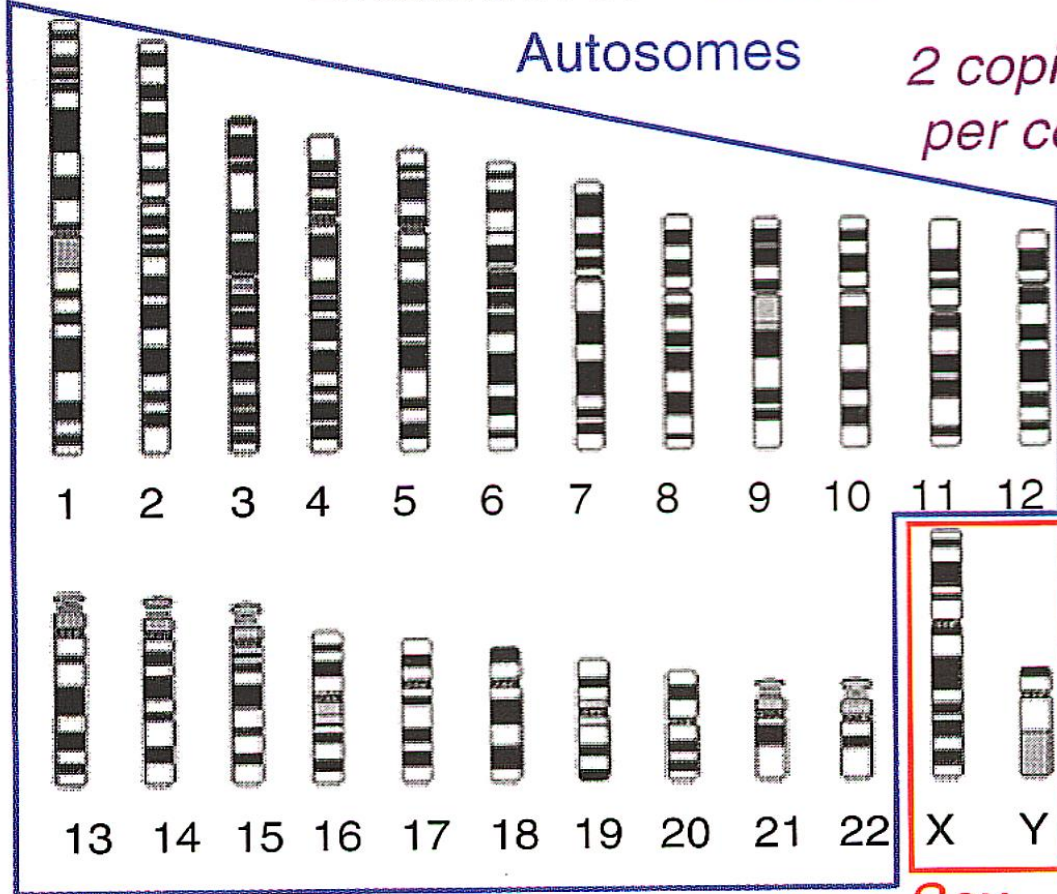
Human Genome

23 Pairs of Chromosomes + mtDNA

Located in cell nucleus

Autosomes

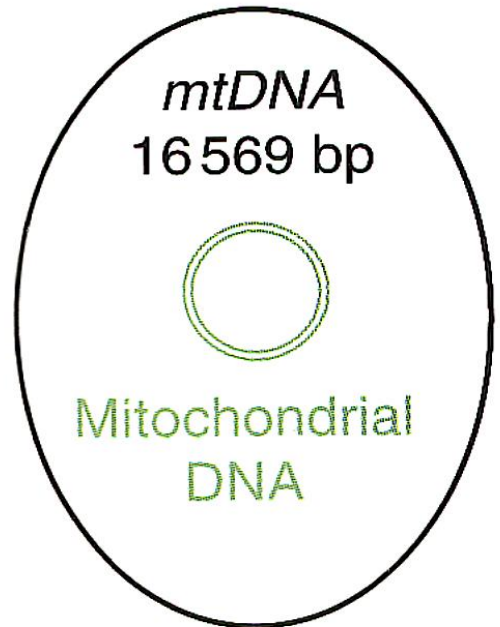
2 copies per cell



Nuclear DNA
3.2 billion bp

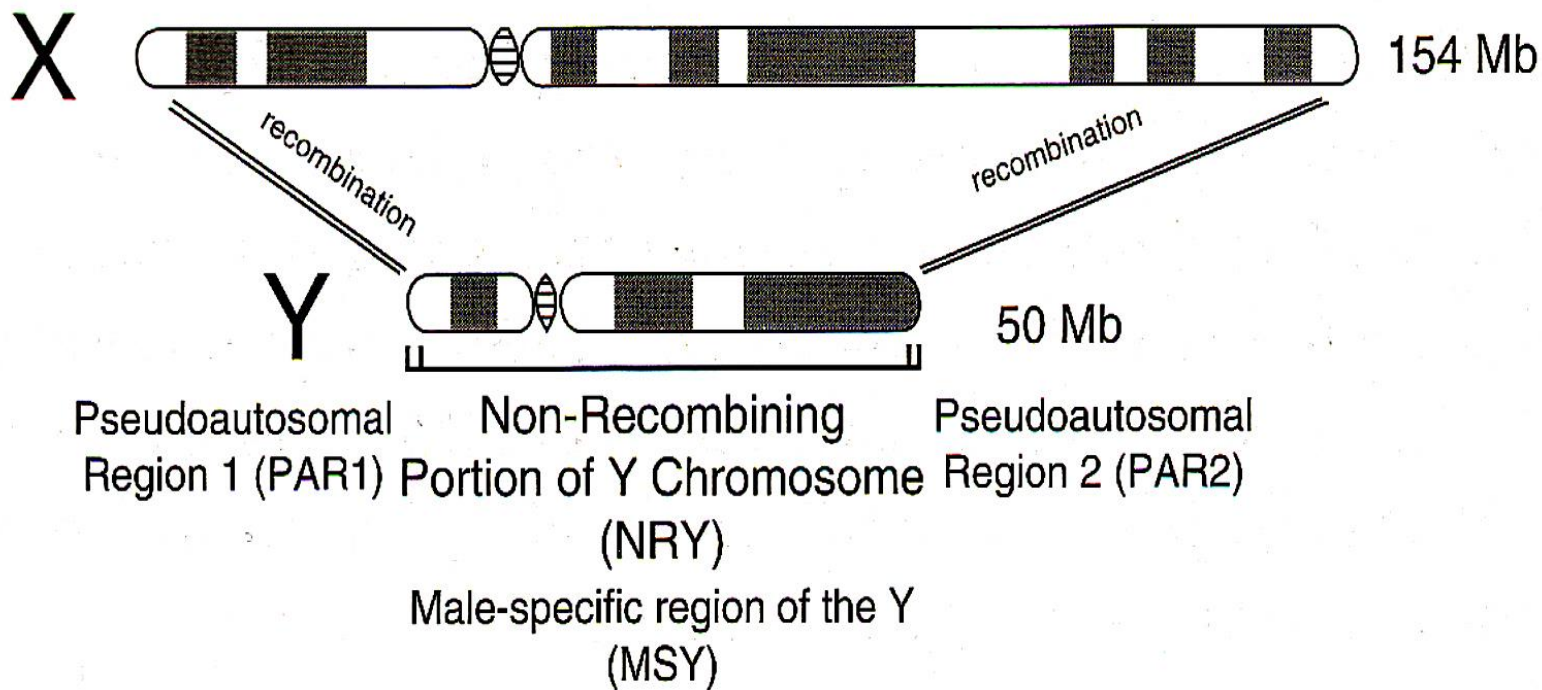
Sex-chromosomes

*Located in mitochondria
(multiple copies in cell cytoplasm)*

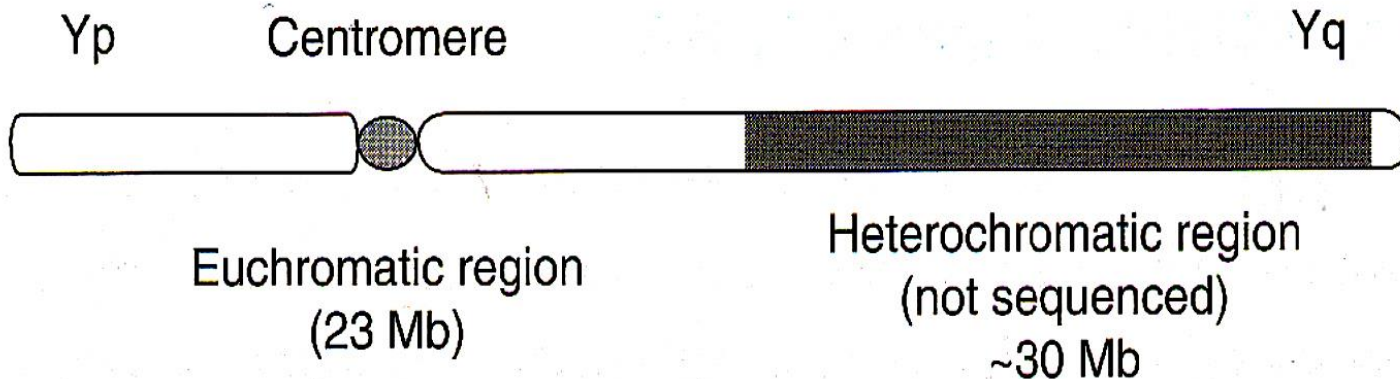


100s of copies per cell

(a)



(b)



Lidská mt DNA

← kontrolní oblast →

342 b

~ 1 100 bp

268 b

HV 1

HV 2

1

1 6024

1 6365

73

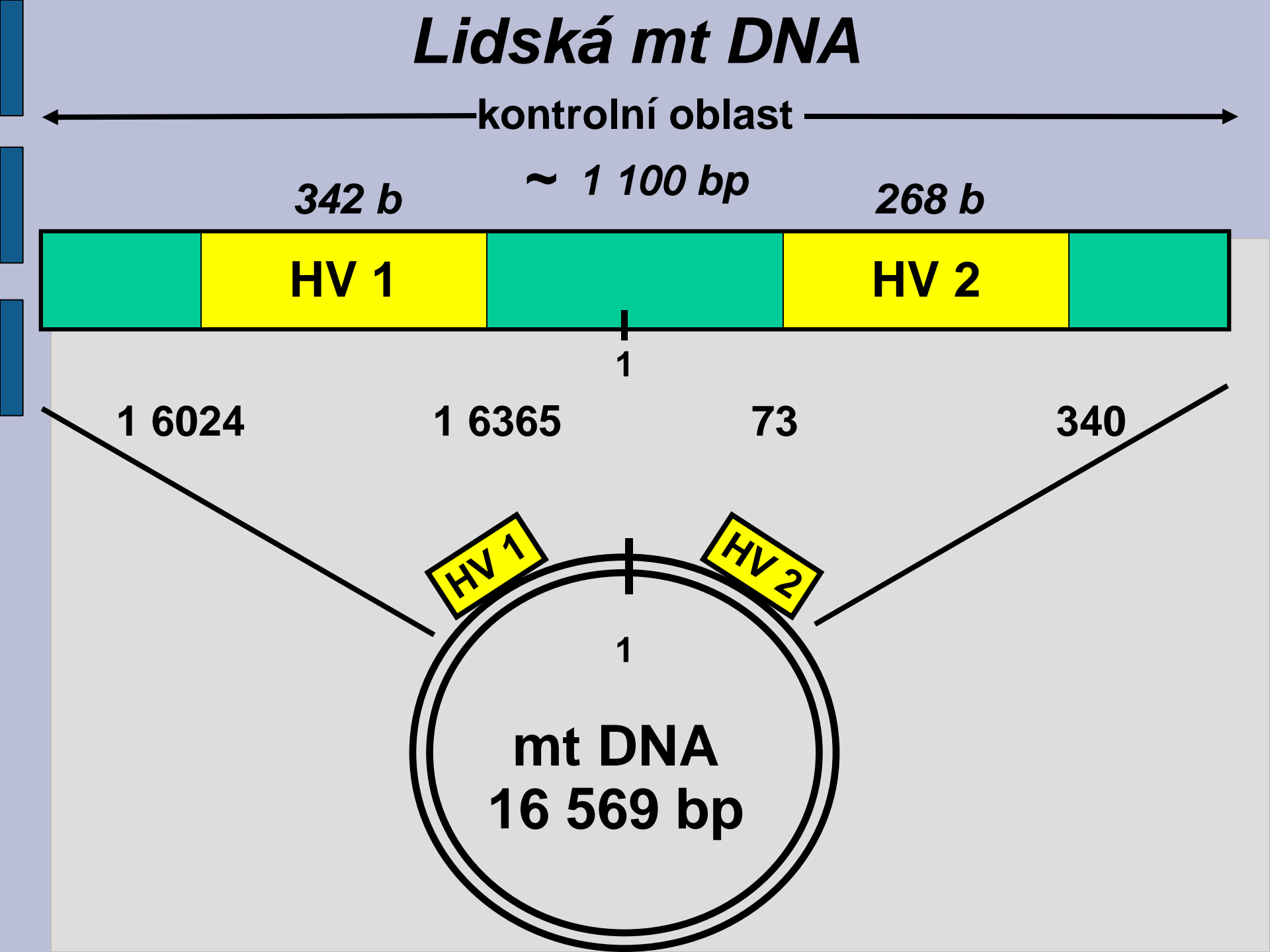
340

HV 1

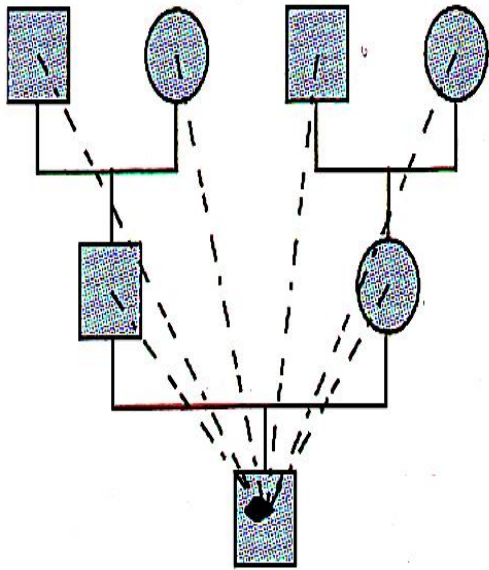
HV 2

1

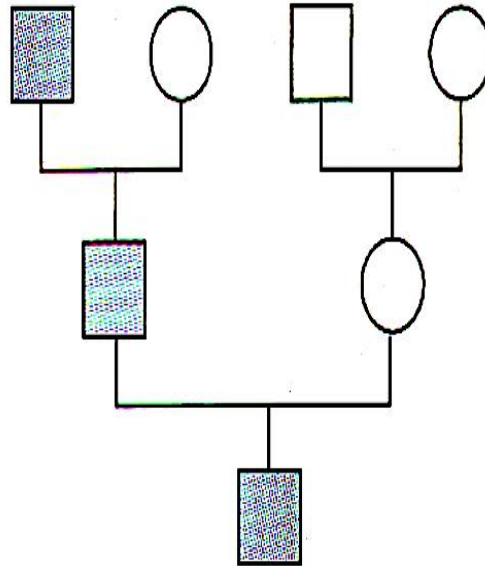
mt DNA
16 569 bp



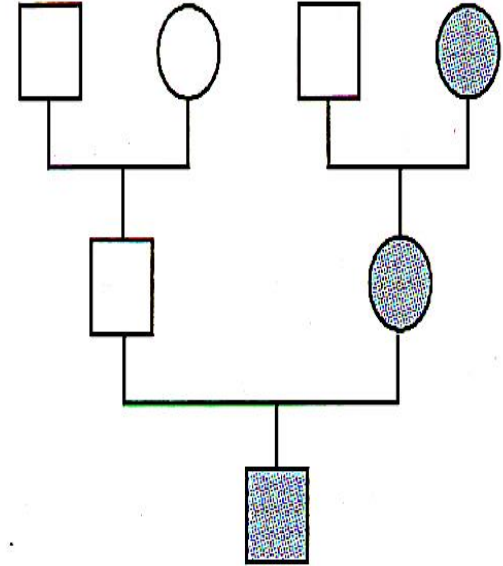
Lineage Markers



Autosomal
(passed on in part,
from all ancestors)



Y Chromosome
(passed on complete,
but only by sons)



Mitochondrial
(passed on complete,
but only by daughters)

Sample Obtained from
Crime Scene or Paternity
Investigation

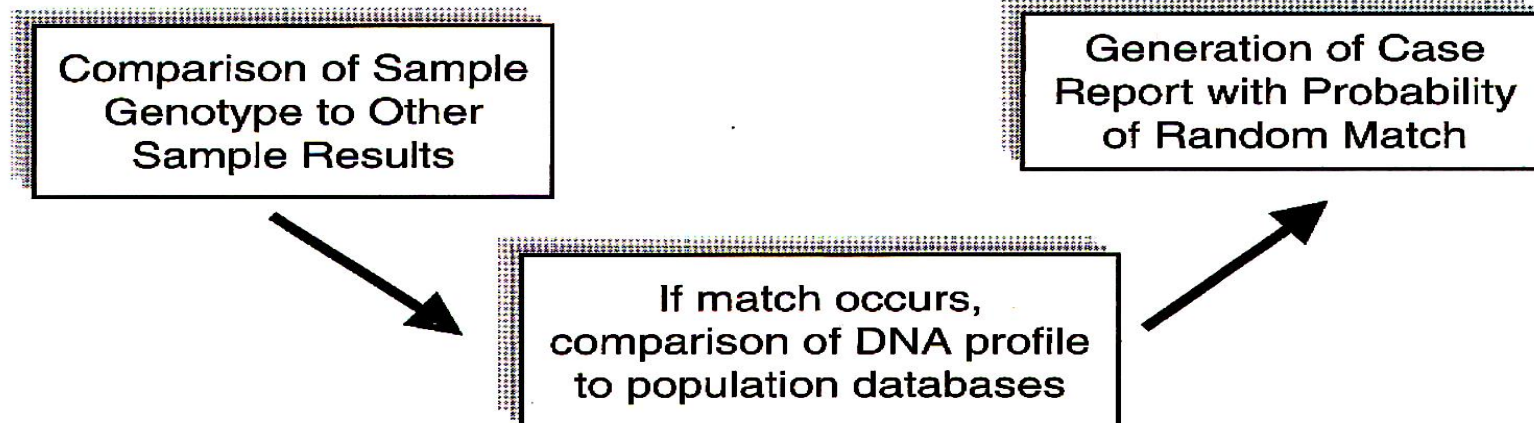
BIOLOGY

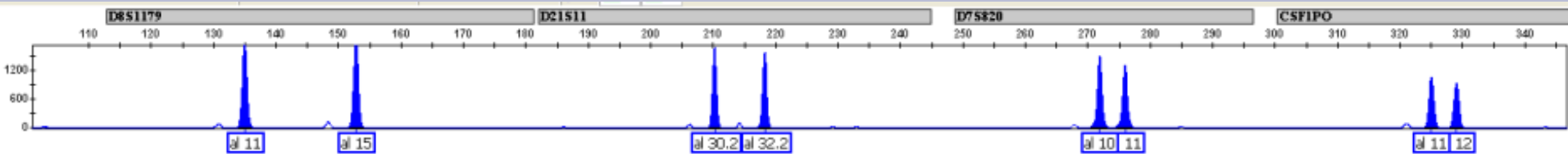


TECHNOLOGY



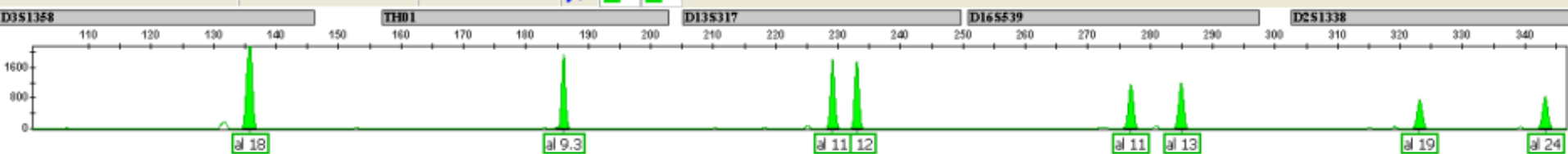
GENETICS





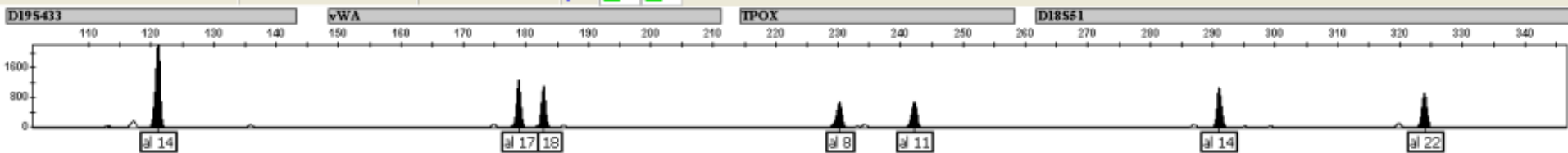
_2010-03-30_15-06_3080_J110-011180-VK03.f; J110-011180-VK03

Identifiler_v1



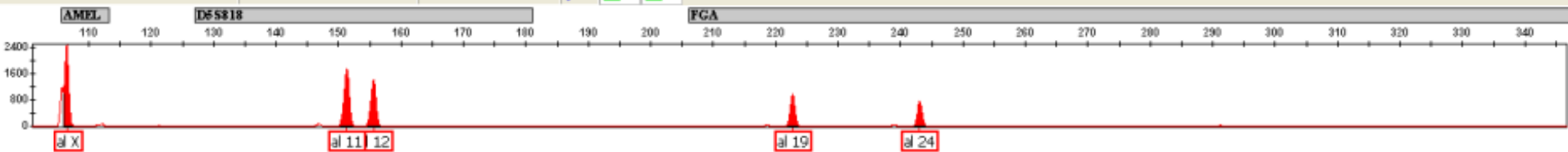
_2010-03-30_15-06_3080_J110-011180-VK03.f; J110-011180-VK03

Identifiler_v1

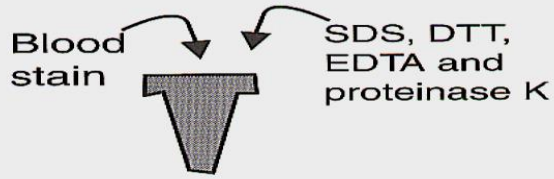


_2010-03-30_15-06_3080_J110-011180-VK03.f; J110-011180-VK03

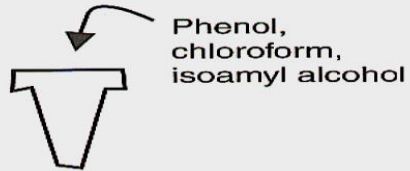
Identifiler_v1



ORGANIC



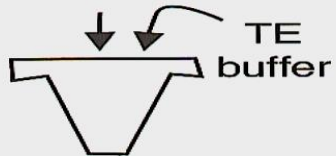
INCUBATE (56 °C)



VORTEX



TRANSFER aqueous (upper) phase to new tube



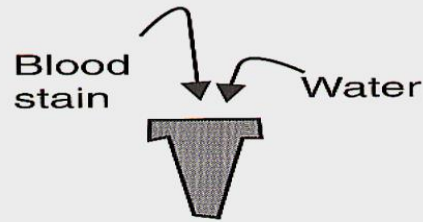
CONCENTRATE sample (Centricon/Microcon-100 or ethanol precipitation)



QUANTITATE DNA

PERFORM PCR

CHELEX



INCUBATE (ambient)



REMOVE supernatant



INCUBATE (56 °C)

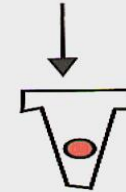
INCUBATE (100 °C)



QUANTITATE DNA

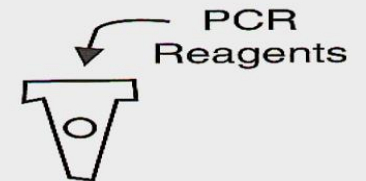
PERFORM PCR

FTA Paper



WASH Multiple Times with extraction buffer

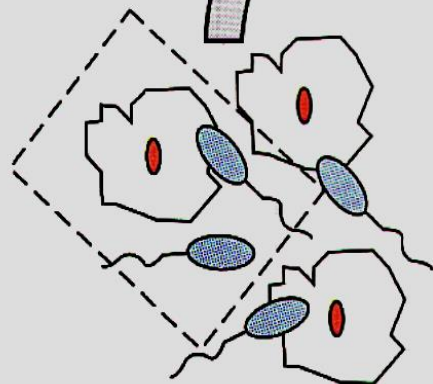
REMOVE supernatant



(NO DNA QUANTITATION REQUIRED)

PERFORM PCR

Remove a portion of the mixed stain



Perpetrator's sperm mixed with victim's epithelial cells

SDS, EDTA and proteinase K
(cell lysis buffer)

Incubate at 37 °C

Centrifuge

sperm pellet

REMOVE supernatant

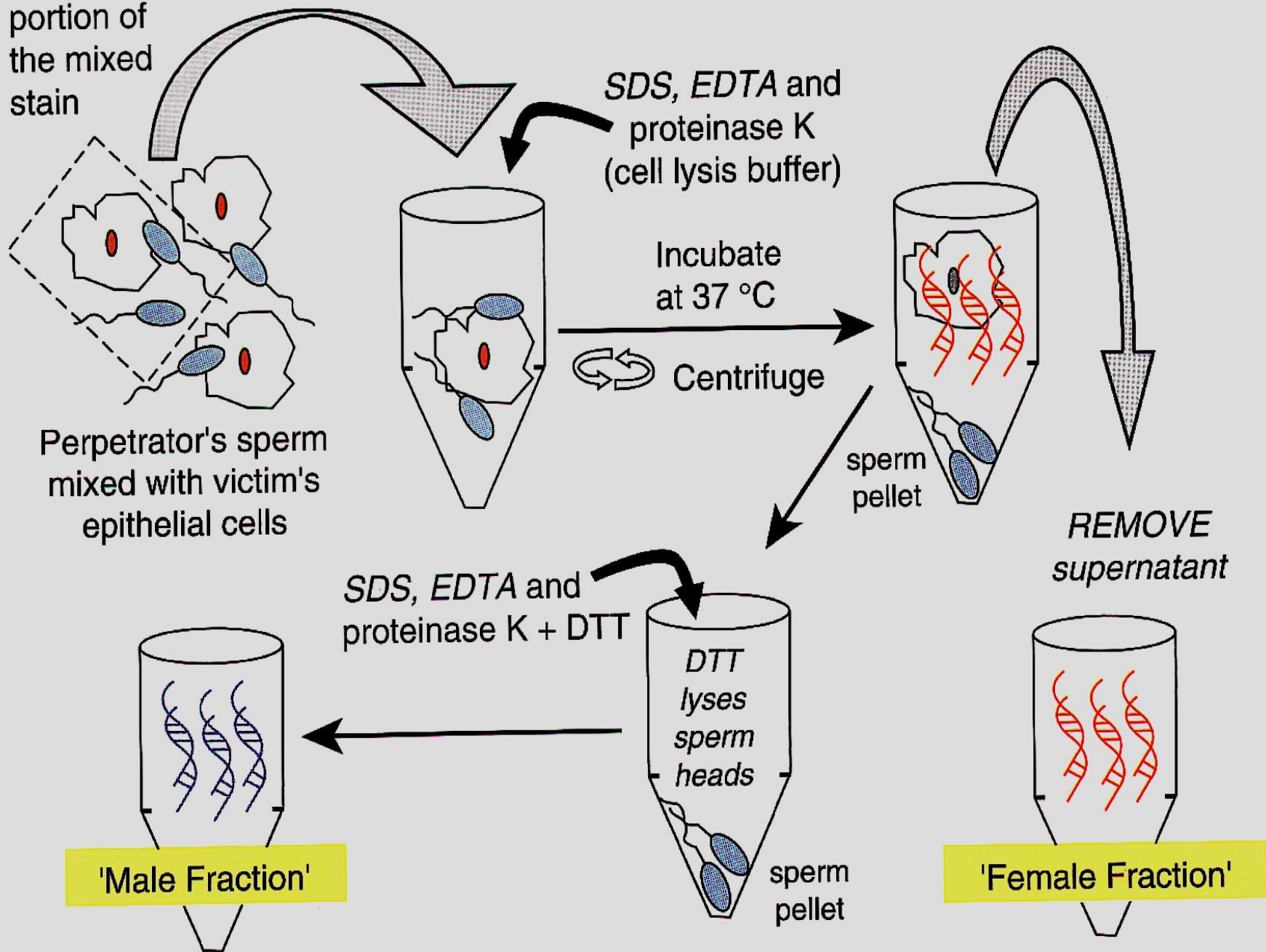
SDS, EDTA and proteinase K + DTT

DTT lyses sperm heads

sperm pellet

'Male Fraction'

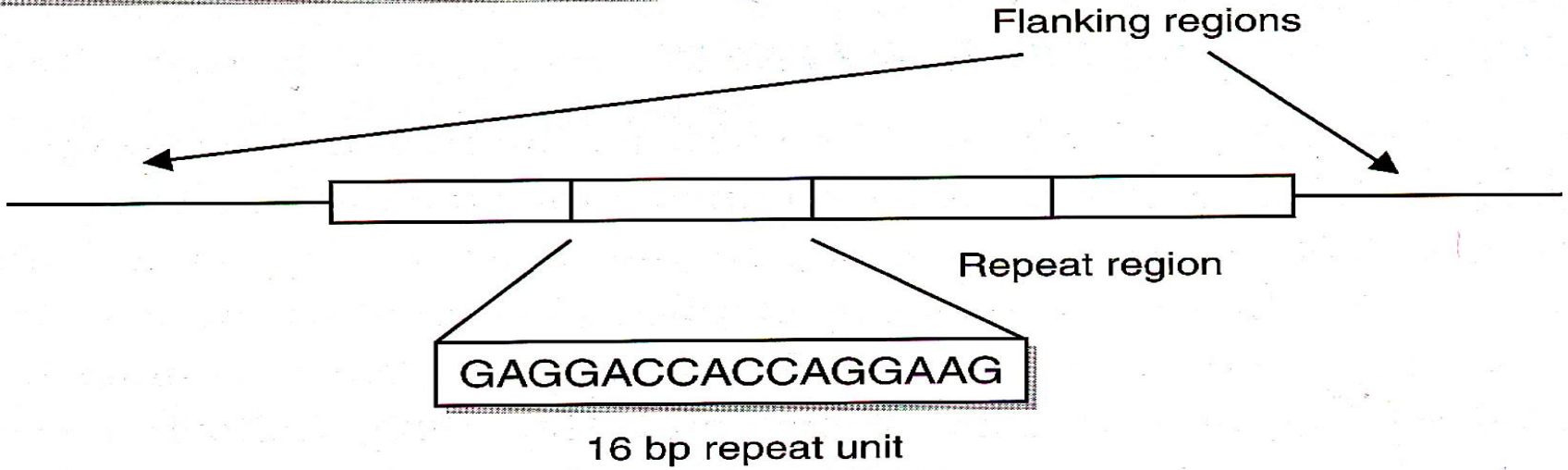
'Female Fraction'



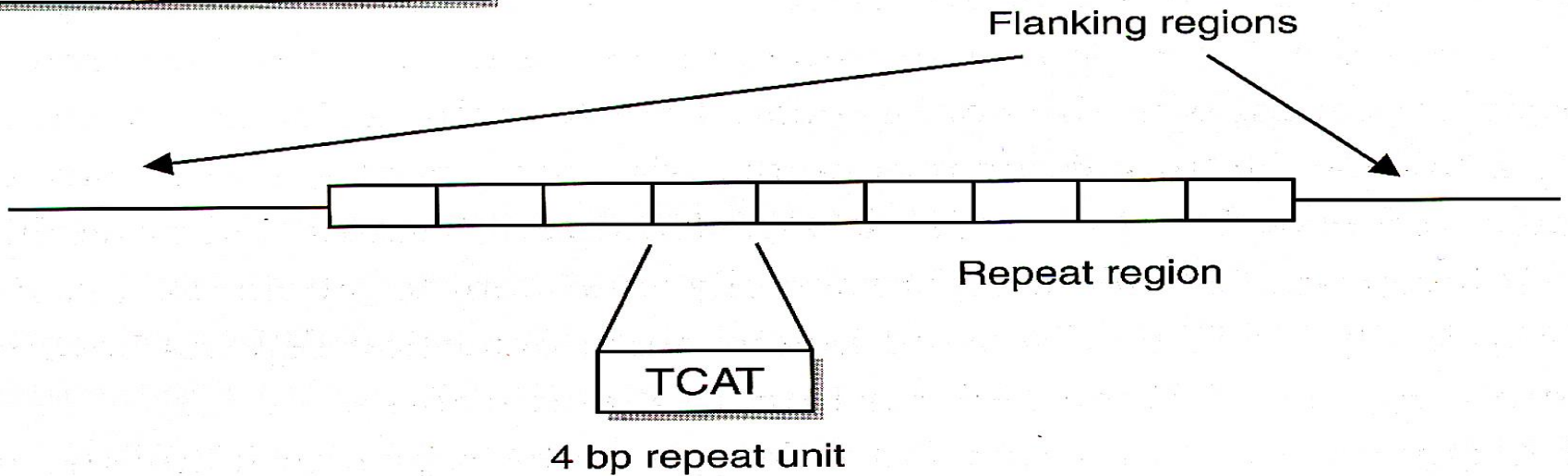
Step in Protocol	AmpF/STR® kits (Applied Biosystems)	GenePrint® STR kits (Promega Corporation)
Initial Incubation	95°C for 11 minutes	95°C for 11 minutes
Thermal Cycling	28 cycles	30 cycles ^a
Denature	94°C for 1 minute	94°C for 30 seconds (cycle 1–10) 90°C for 30 seconds (cycle 11–30)
Anneal	59°C for 1 minute	60°C for 30 seconds
Extend	72°C for 1 minute	70°C for 45 seconds
Final Extension	60°C for 45 minutes	60°C for 30 minutes
Final Soak	25°C (until samples removed)	4°C (until samples removed)

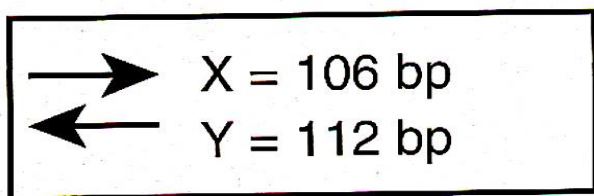
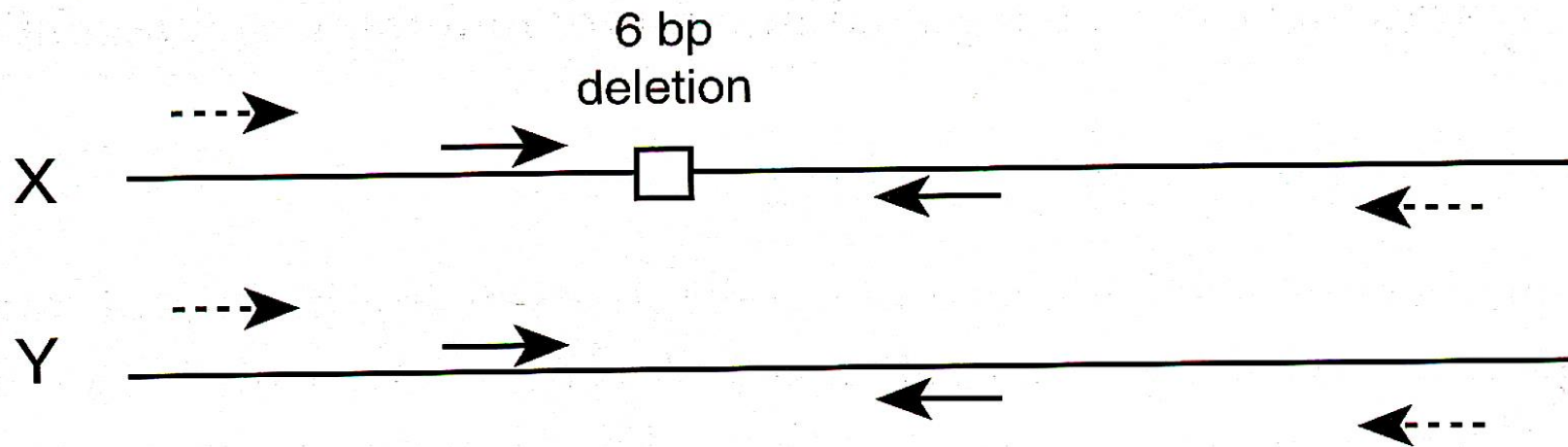
^aThe first 10 cycles are run with a denaturation temperature of 94°C and the last 20 cycles are run at 90°C instead. The Promega PowerPlex 1.1, 2.1, and 16 kits also use specific ramp times between the different temperature steps that differ from the conventional 1°C/second.

Minisatellite Marker (D1S80)

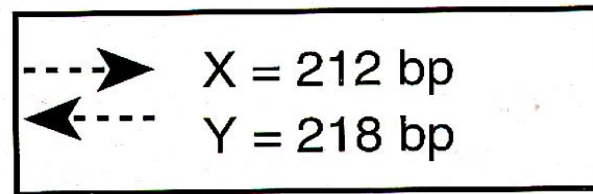


STR Marker (TH01)



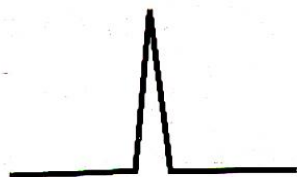


*AmpFISTR kits
and PowerPlex 16*



PowerPlex 1.1

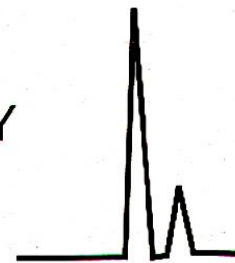
Female: X, X



Male: X, Y

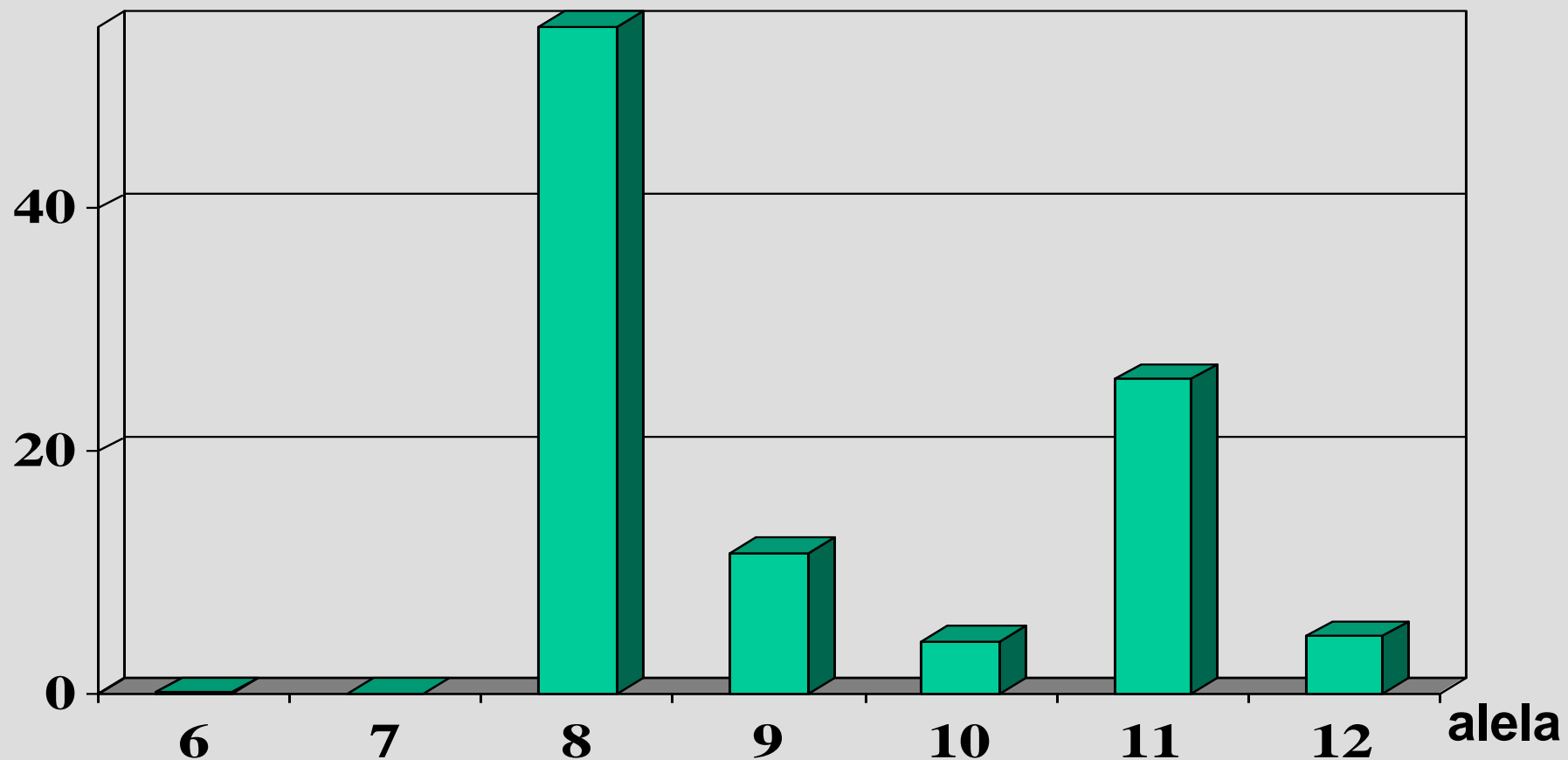


1:1 Mixture: 3X + 1Y



Rozložení alel lokusu *TPOX* (thyroid peroxidáza, 10.intron, 2p25.3, GAAT) v bílé americké populaci

%



Rozložení alel lokusu D2SD1338 v bílé americké populaci

%

20

10

0

16

17

18

19

20

21

22

23

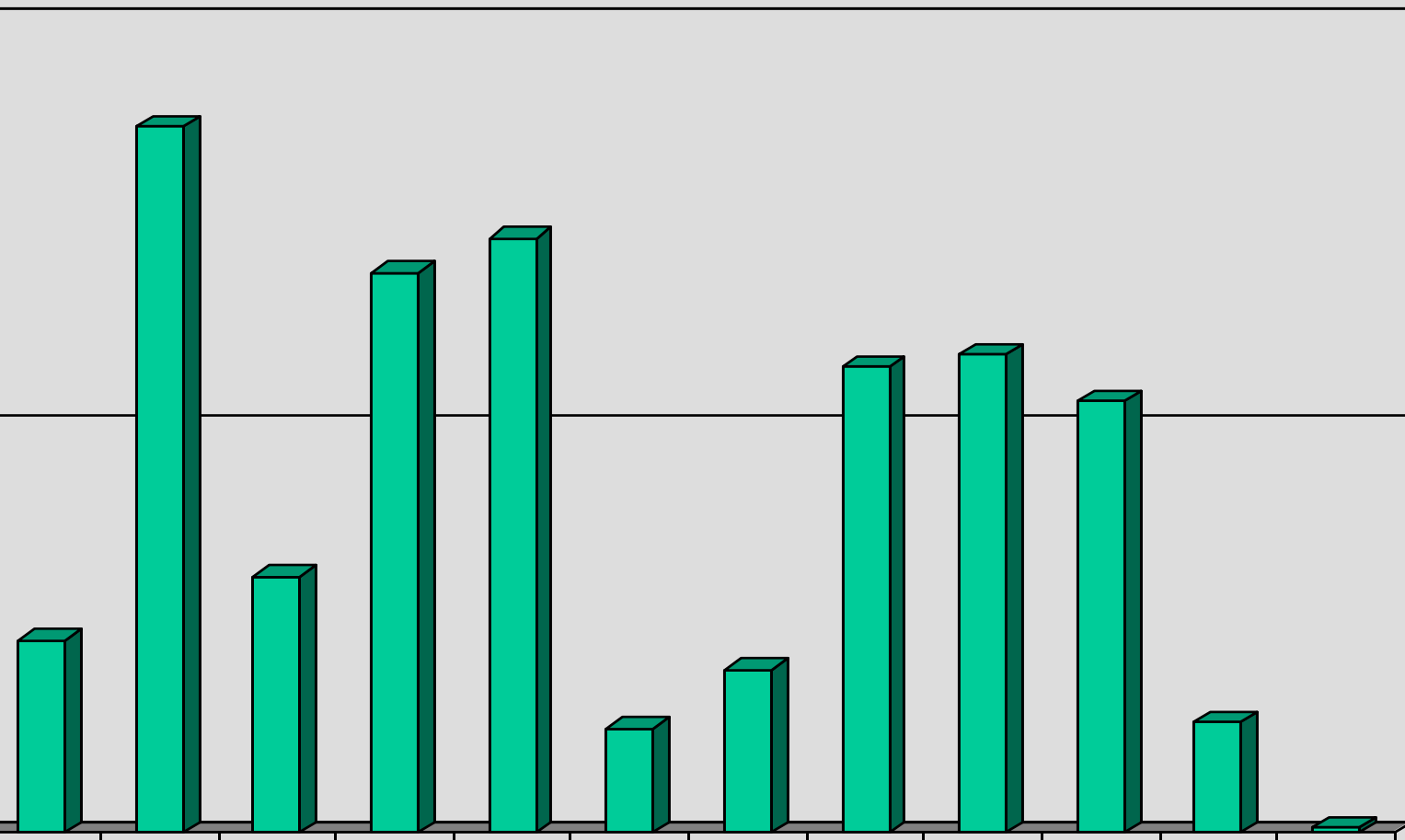
24

25

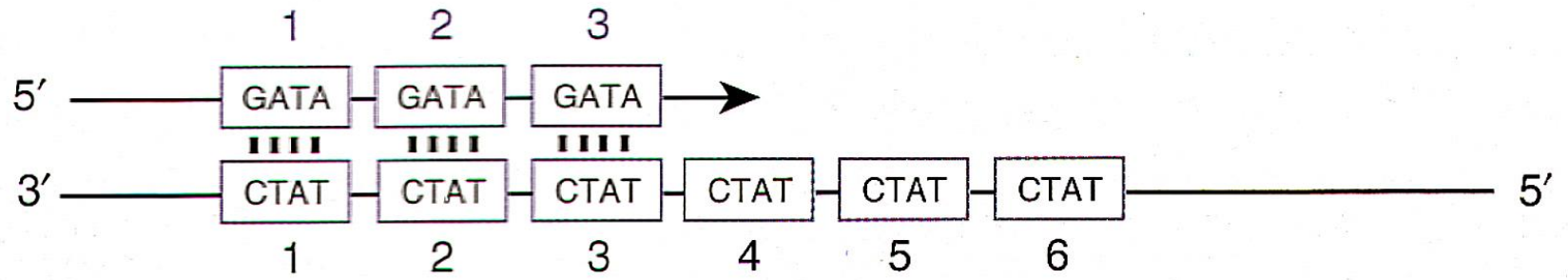
26

27

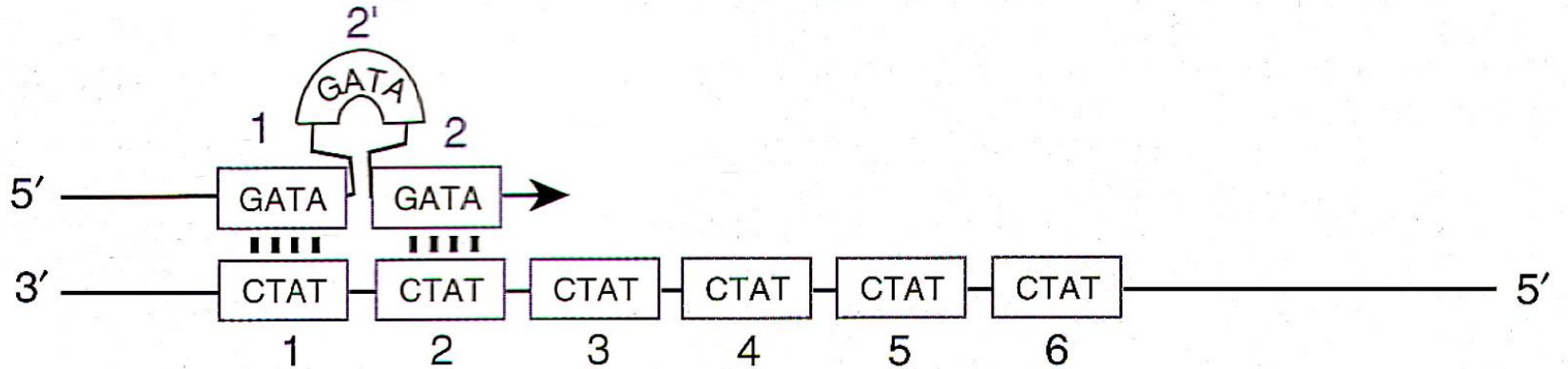
alela



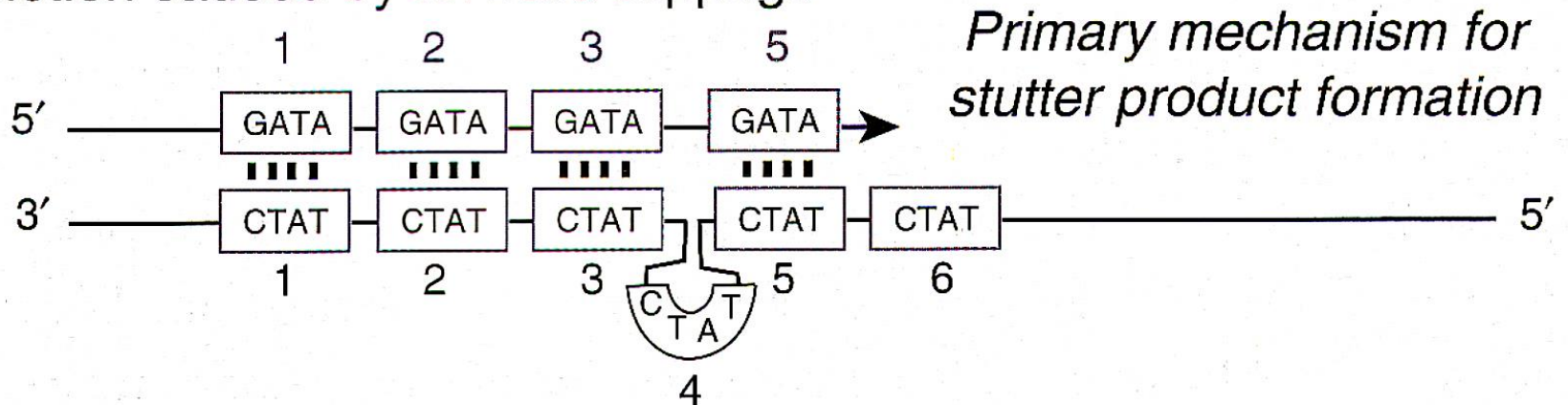
(a) Normal replication



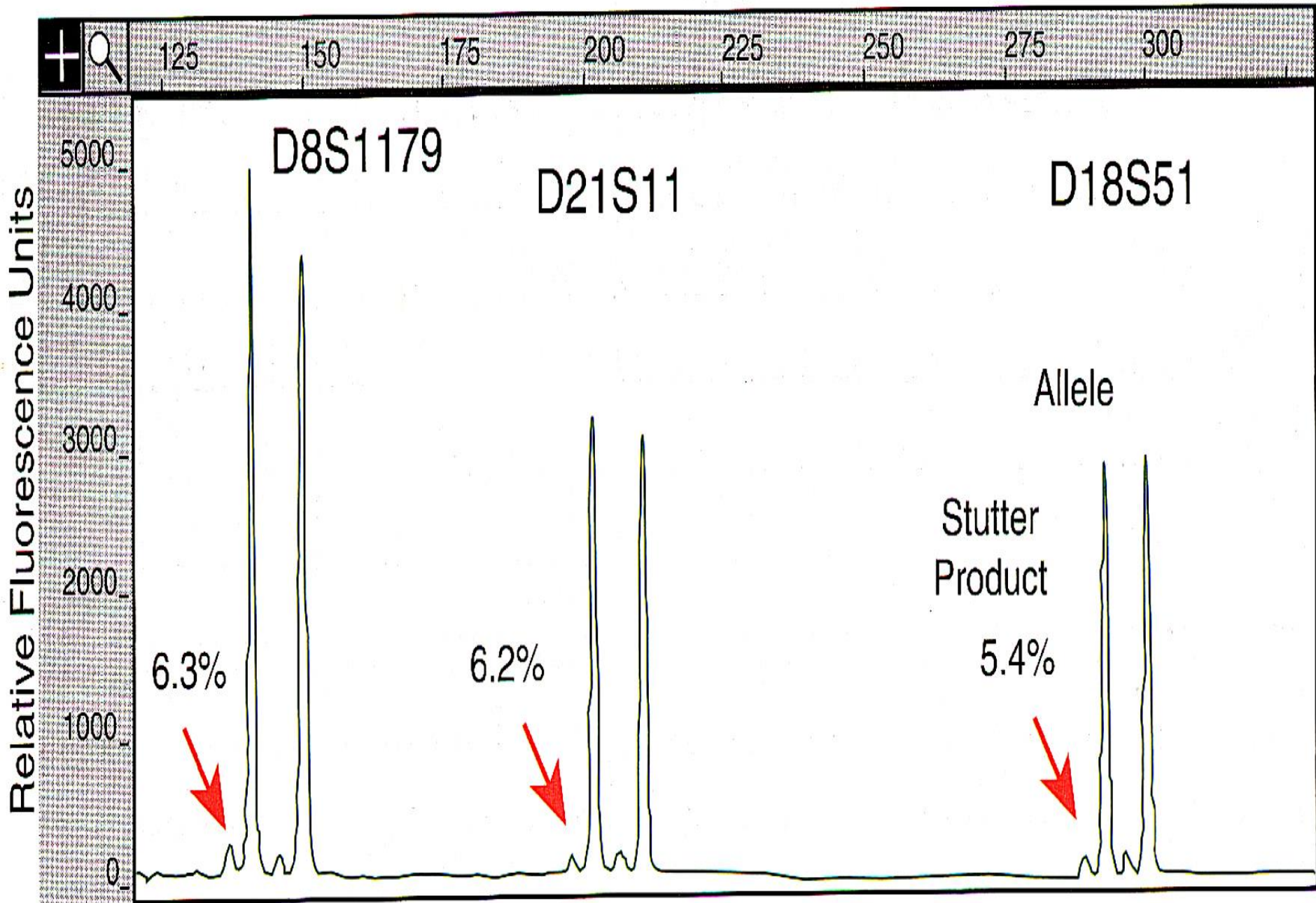
(b) Insertion caused by backward slippage



(c) Deletion caused by forward slippage

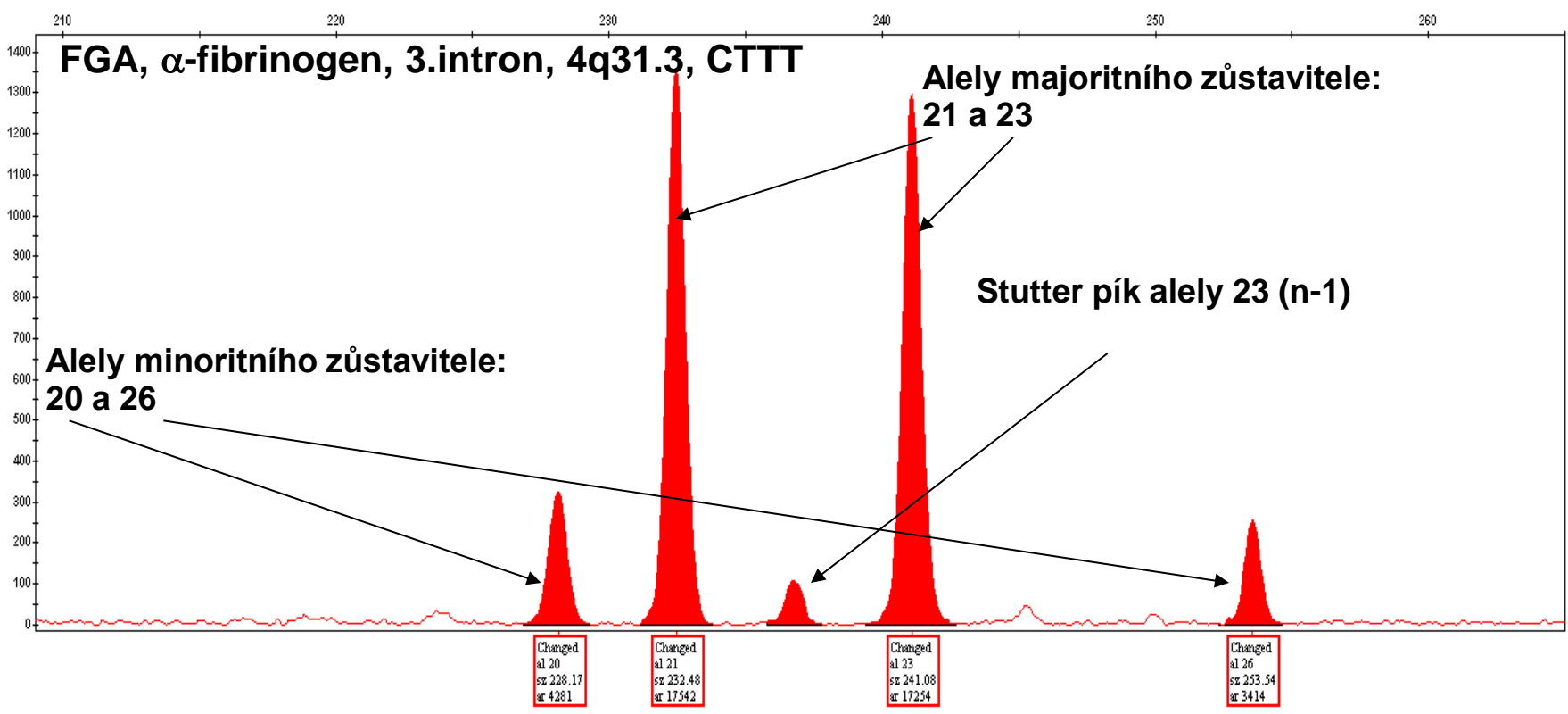


DNA Size (bp)



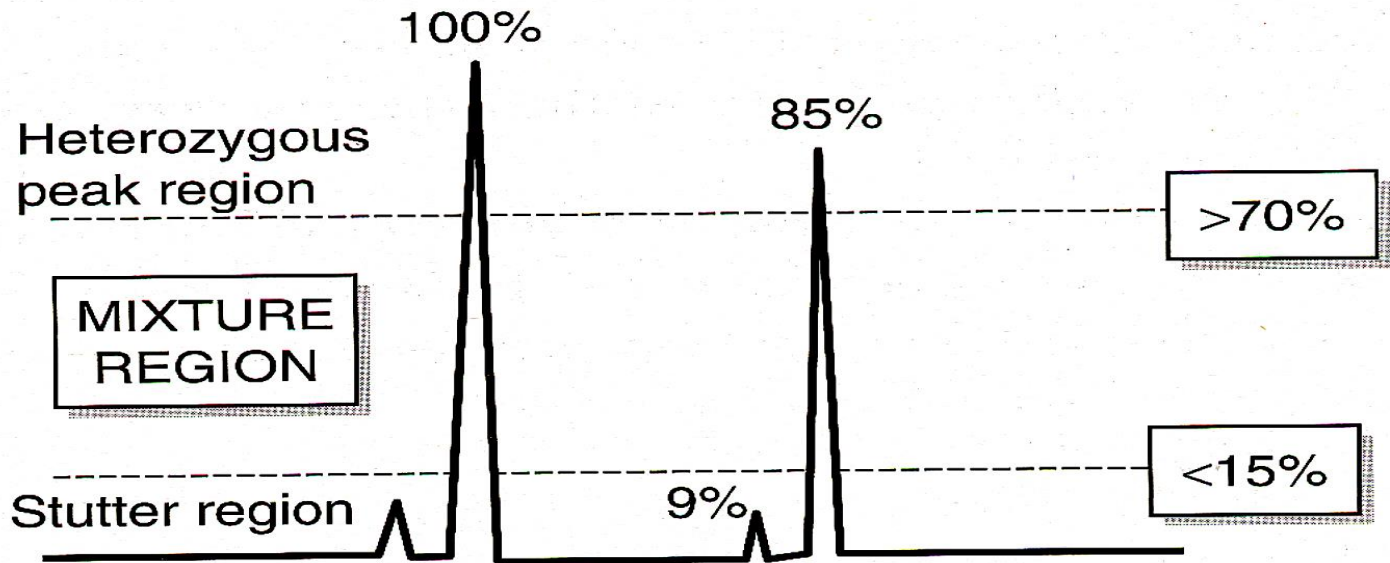
Sample File	Sample Name	Panel	SQ0	OS	SQ
1_JI08-01133-FU06.fsa	JI08-01133-FU06	Identifler_v1			

FGA

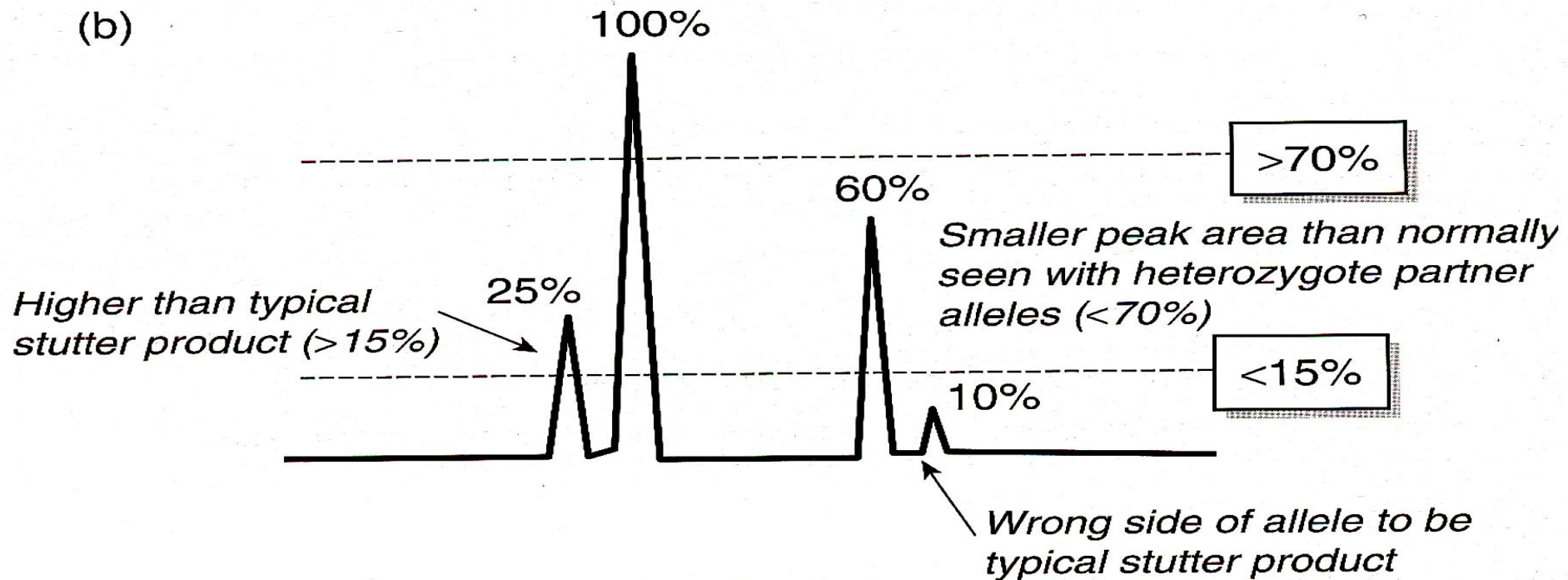


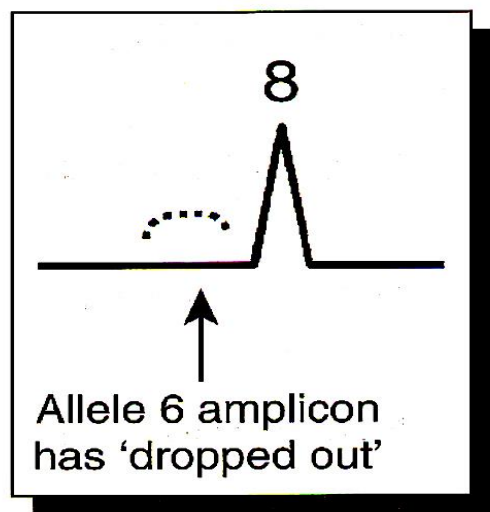
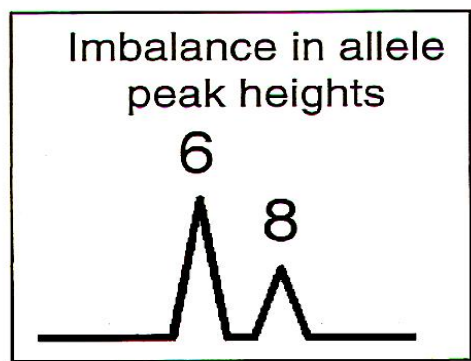
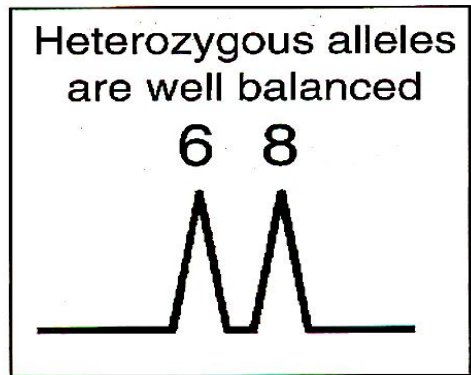
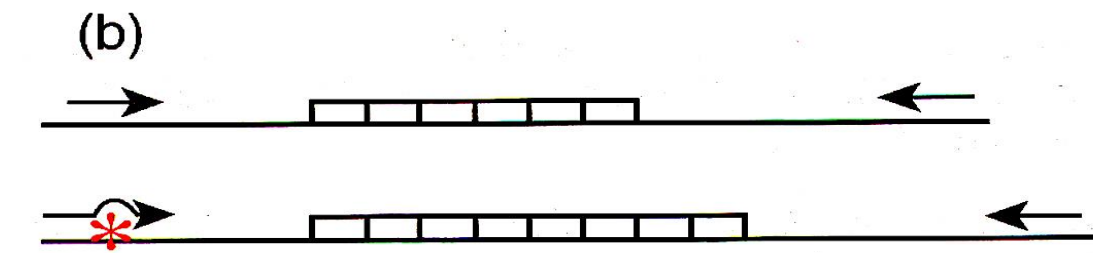
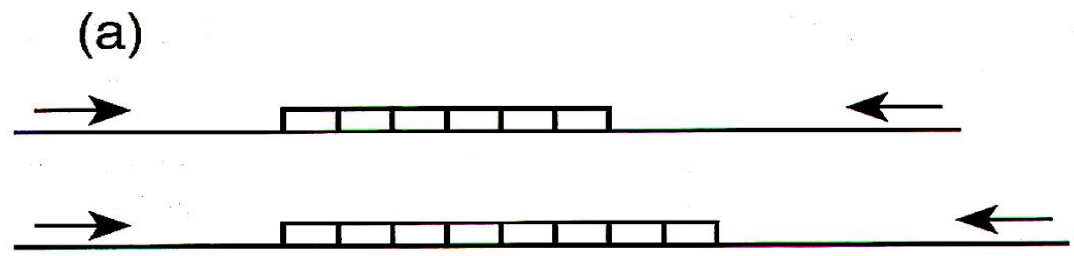
Dye/Sample Peak	Sample File Name	Size	Height	Area	Data Point
R,47	1_JI08-01133-FU06.fsa	228.17	326	4281	6340
R,48	1_JI08-01133-FU06.fsa	232.48	1358	17542	6409
R,49	1_JI08-01133-FU06.fsa	236.72	107	1407	6477
R,50	1_JI08-01133-FU06.fsa	241.08	1297	17254	6547
R,51	1_JI08-01133-FU06.fsa	253.54	257	3414	6754

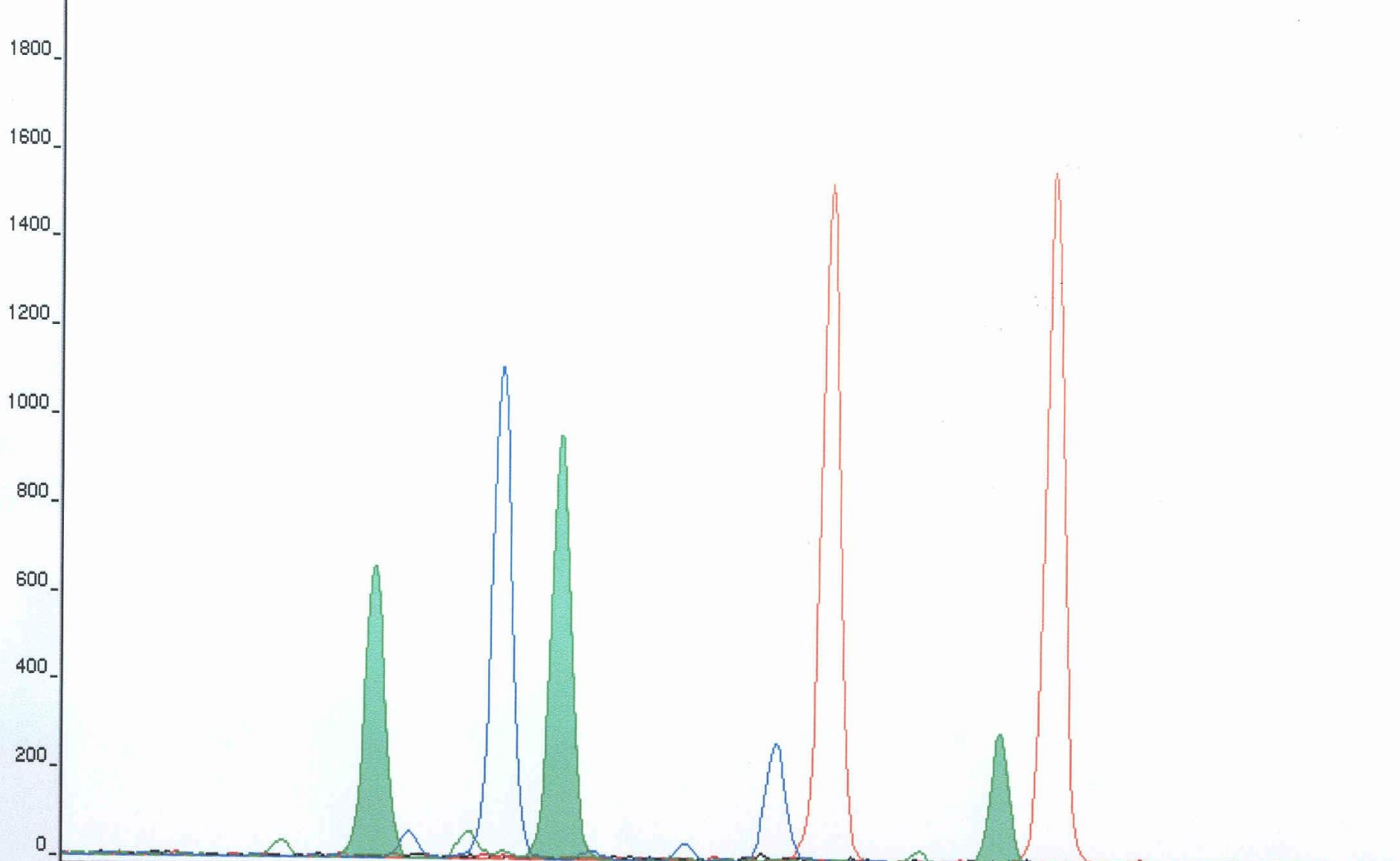
(a)



(b)







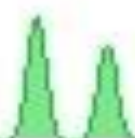
BGYRO X: 312 Y: 1701

Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
G, 187	20.12	319.81	663	5283	5486
G, 189	20.35	328.10	963	7855	5549
G, 190	20.92	347.54	297	2454	5705

18/19



23/24

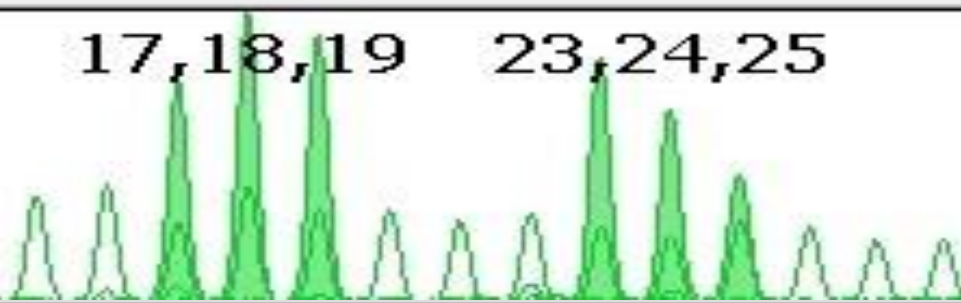


17/25



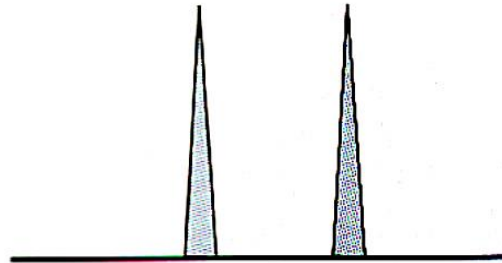
17,18,19 23,24,25

51% !



Female-Male Mixture Performance with Autosomal vs. Y Chromosome DNA Markers

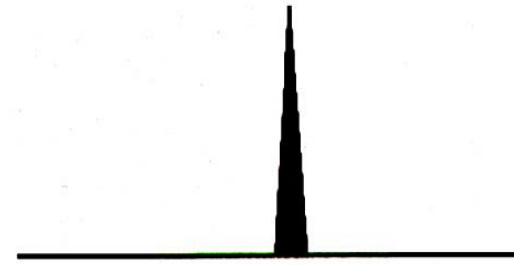
Female Victim
DNA Profile



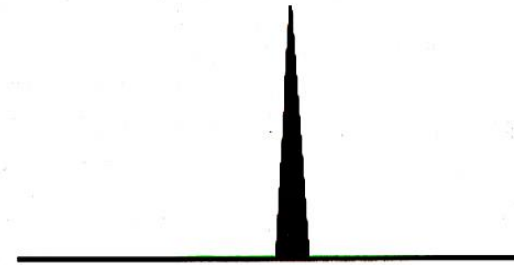
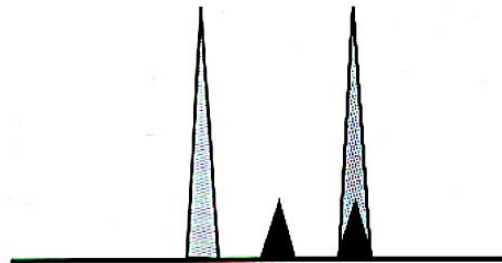
No signal observed



Male Perpetrator
DNA Profile



DNA Profile from
Crime Scene



Autosomal STR
Profile

Y Chromosome STR
Profile

Possible Forensic Source	PCR Inhibitor	Reference
Blood	Heme (hematin)	Akane <i>et al.</i> (1994)
Tissue and Hair	Melanin	Eckhart <i>et al.</i> (2000)
Feces	Polysaccharides	Monteiro <i>et al.</i> (1997)
Feces	Bile salts	Lantz <i>et al.</i> (1997)
Soil	Humic compounds	Tsai and Olson (1992)
Urine	Urea	Mahony <i>et al.</i> (1998)
Blue jeans	Textile dyes (denim)	Shutler <i>et al.</i> (1999)

Stochastický efekt

