Mitochondrial DNA in Human Identification

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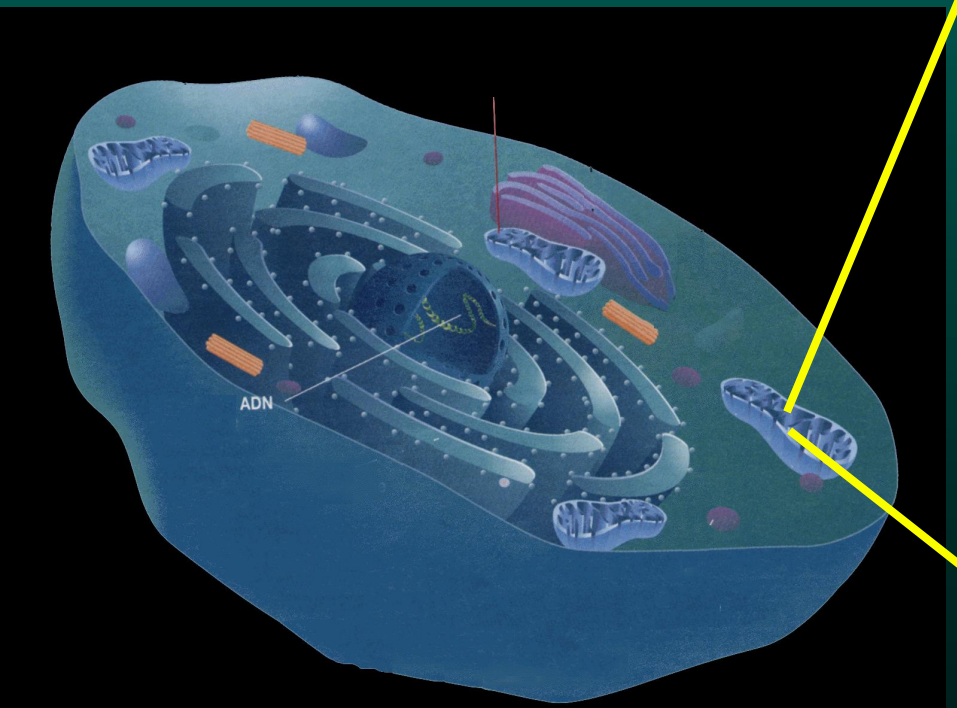
Agenda

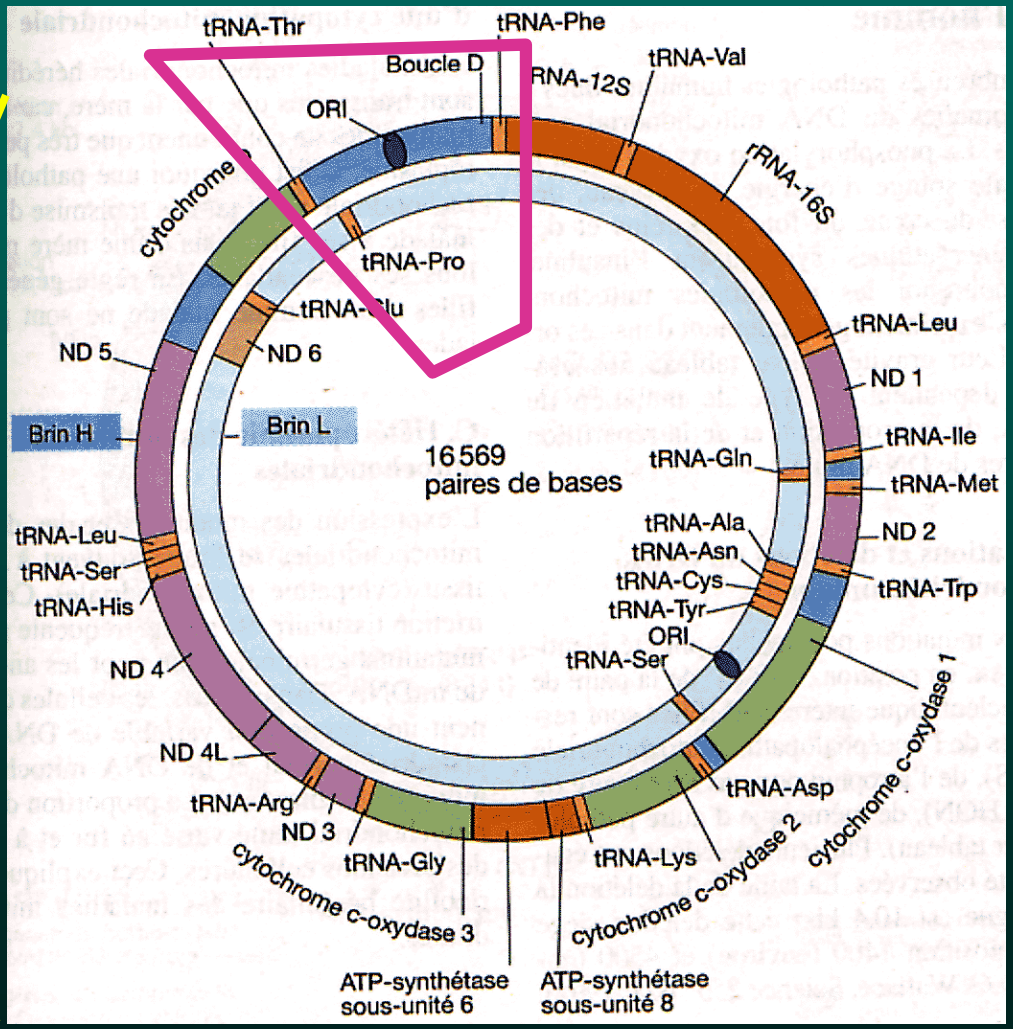
- PCR
- Electrophoresis and Purification of PCR product
- Sequence Reaction
- Purification of the sequence reaction
- Electrophoresis on ABI Genetic Analyzer
- Analysis of the results

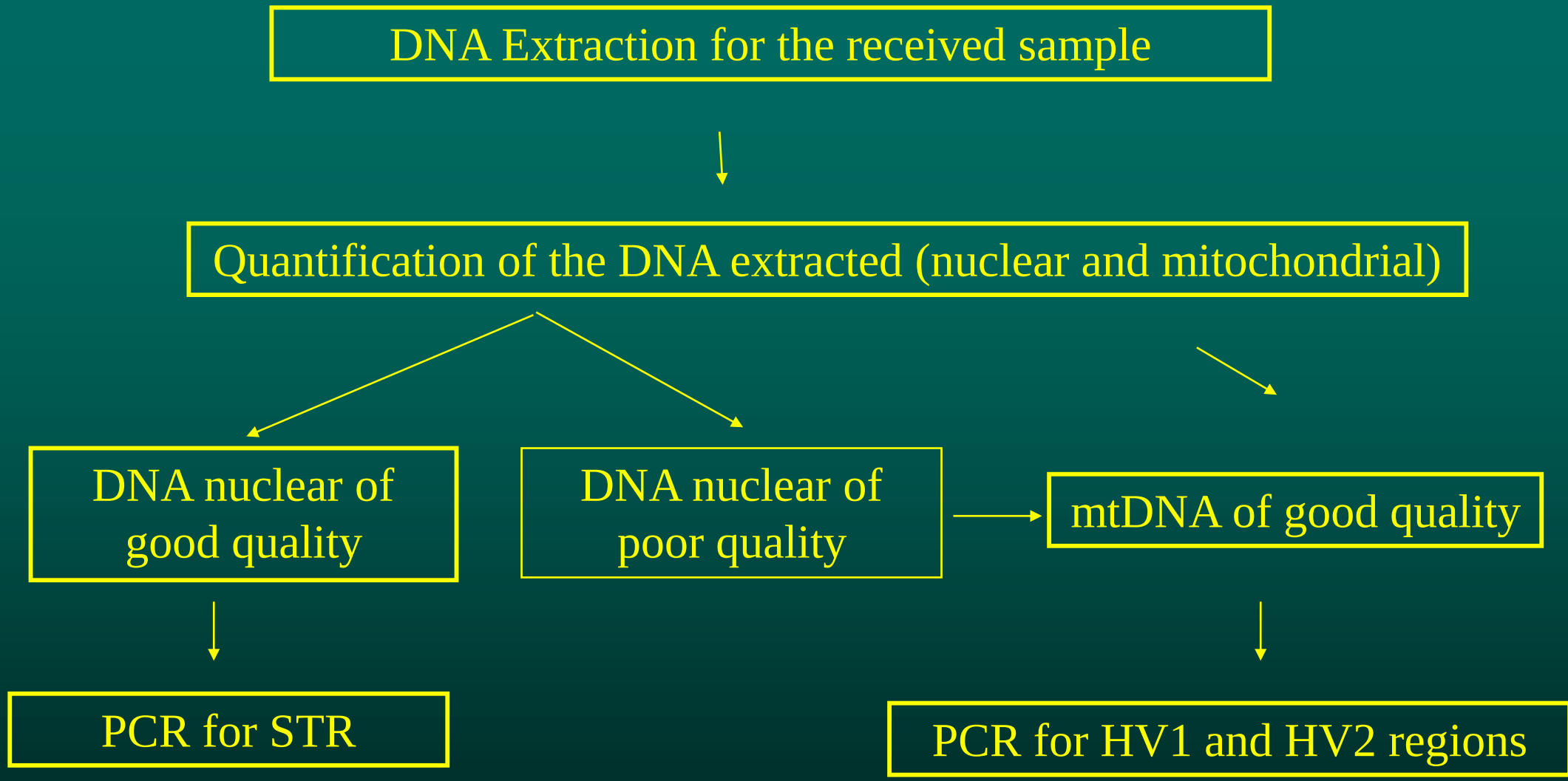
Mitochondrial DNA

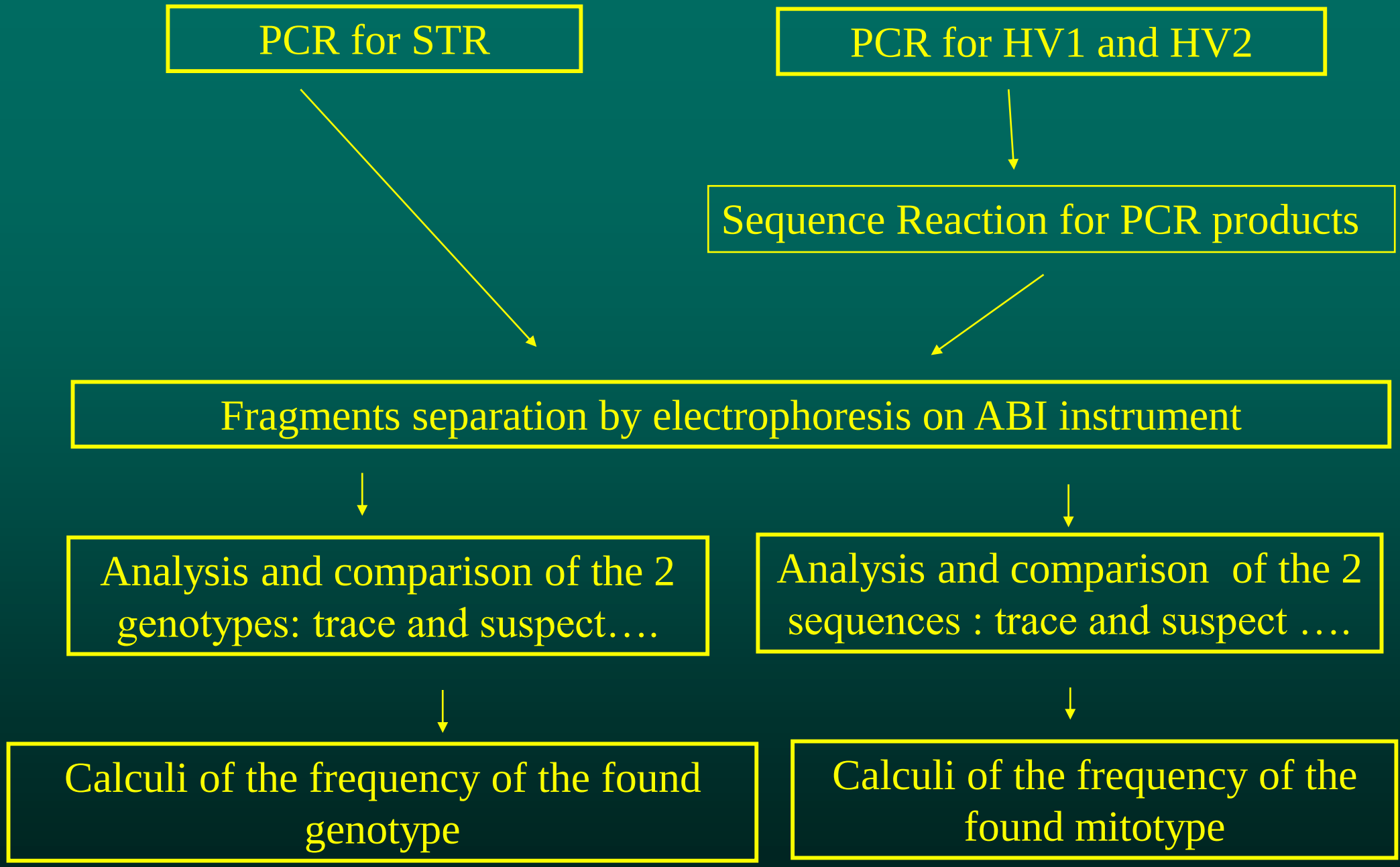
Variable from an individual to another

- 16569 bp/mitochondria






DNA Extraction for the received sample
Quantification of the DNA extracted (nuclear and mitochondrial)
DNA nuclear of good quality
DNA nuclear of poor quality
mtDNA of good quality
PCR for STR
PCR for HV1 and HV2 regions


PCR for STR
PCR for HV1 and HV2
Sequence Reaction for PCR products
Fragments separation by electrophoresis on ABI instrument
Analysis and comparison of the 2 genotypes: trace and suspect….
Analysis and comparison of the 2 sequences : trace and suspect ….
Calculi of the frequency of the found genotype
Calculi of the frequency of the found mitotype

2 different PCR

- PCR n° 1 helps to amplify HV2 domain, 360 pb using these primers:

L048: 5'- CTCACGGGAGCTCTCCATGC-3'

H408: 5'-CTGTTAAAAGTGCATACCGCCA -3'

- PCR n° 2 helps to amplify HV1 domain, 468 pb using these primers:

L15933: 5'- CAGTCTTGTAAACCGGAGATG-3'

H16401: 5'-TGATTTCACGGAGGATGGTG -3'

PCR Mix

		ini. conc.	**PCR I** DNA extracted rom rich biological material	**PCR II** DNA extracted from poor biological material
DNA			1 µl of DNA at 100 ng/µl	2 to 5 µl of initial DNA
Buffer 10X	Gold	10X	5 µl	2.5 µl
dNTP		2.5mM	5 µl	2.5 µl
BSA		20 mg/ml	---	1.25 µl
primer F		100 ng/µl	1 µl	1 µl
primer R		100 ng/µl	1 µl	1 µl
MgCl2		25mM	3 µl	2 µl
Taq polymease	Gold	5 U/µl	0.2 µl	0.5 µl
H2O			33.8 µl	12.25 or 9.25 µl

PCR conditions

	Time	θ
	12 min	95°C
	1 min	95°C
35 to 40 cycles	1 min	56°C
	1 min	72°C
Hold	10 min	72°C
Hold	forever	4°C

Agarose gel preparation

Weigh & make agarose solution

1g of agarose + 100 ml of TBE (1%)

Microwave, cool & add stain

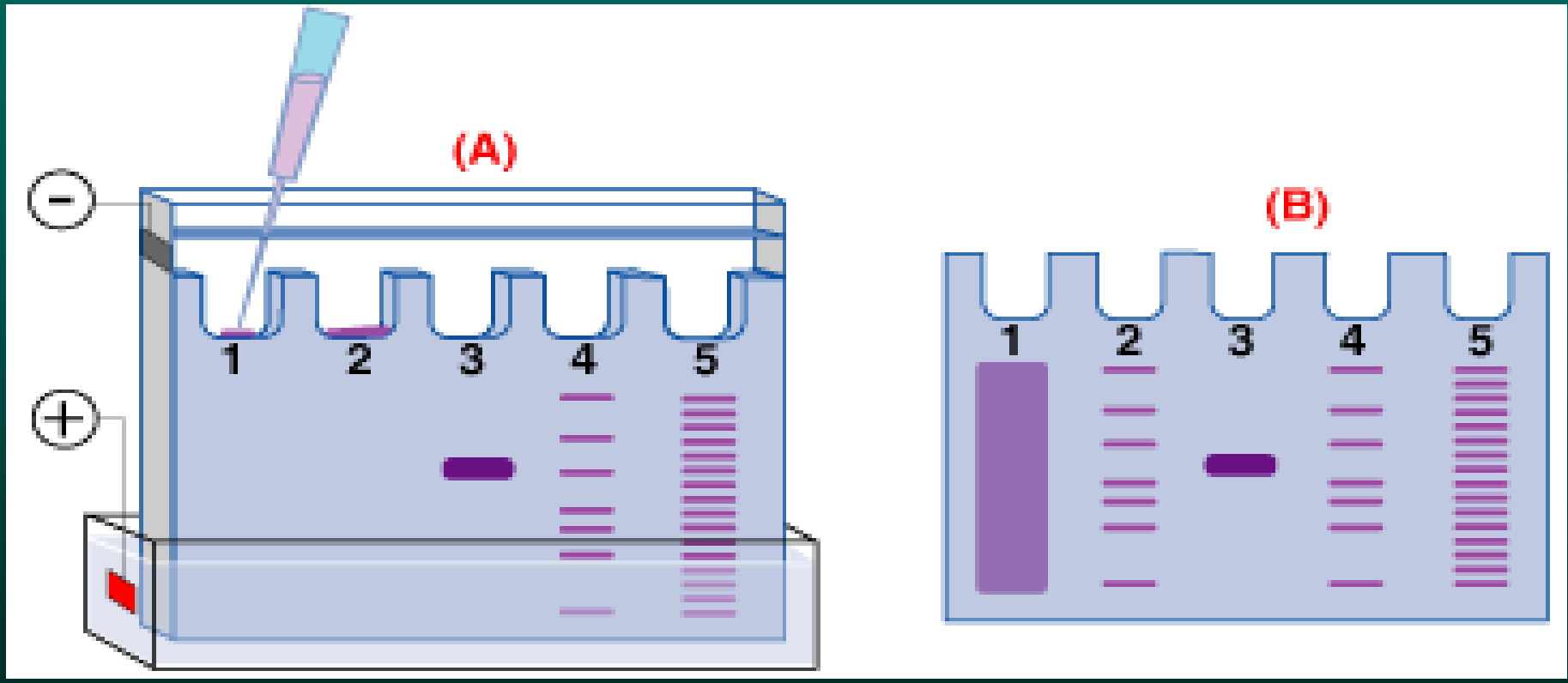
(5% = 5 µl of Ethidium Bromide or Syber Safe for 100 ml solution)

Pour gel & wait to set

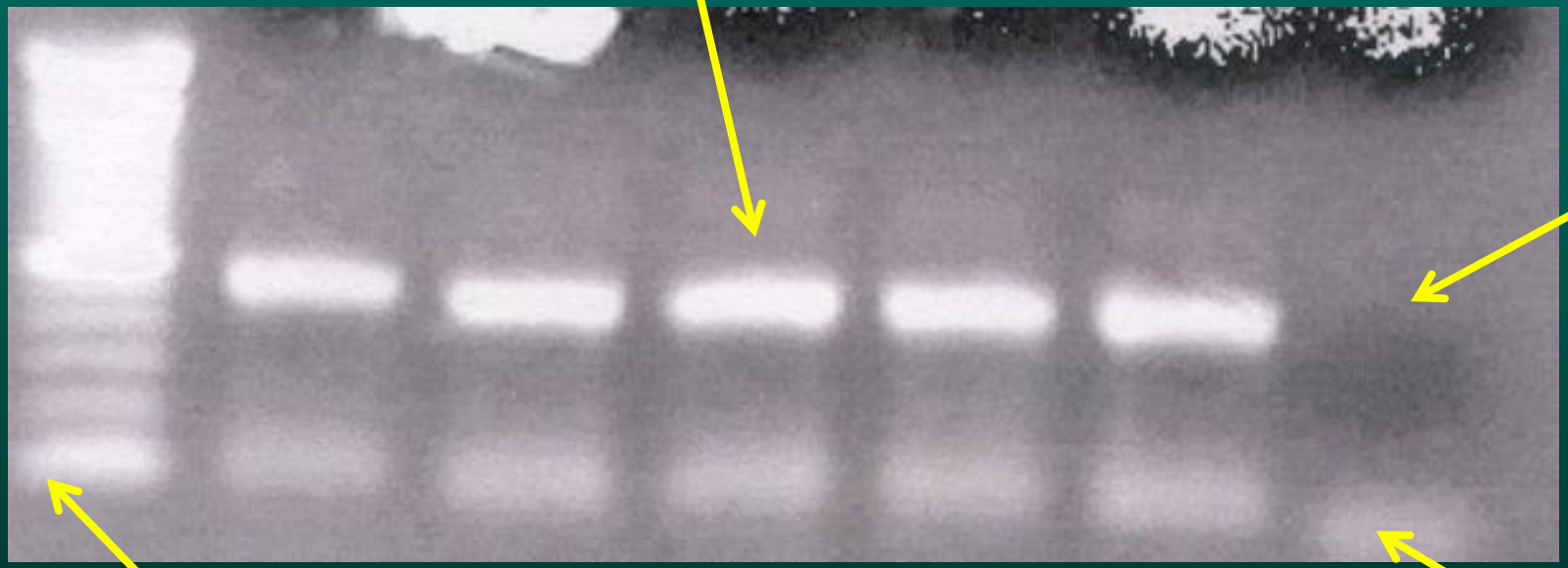
Load samples + Size Marker

Run the gel

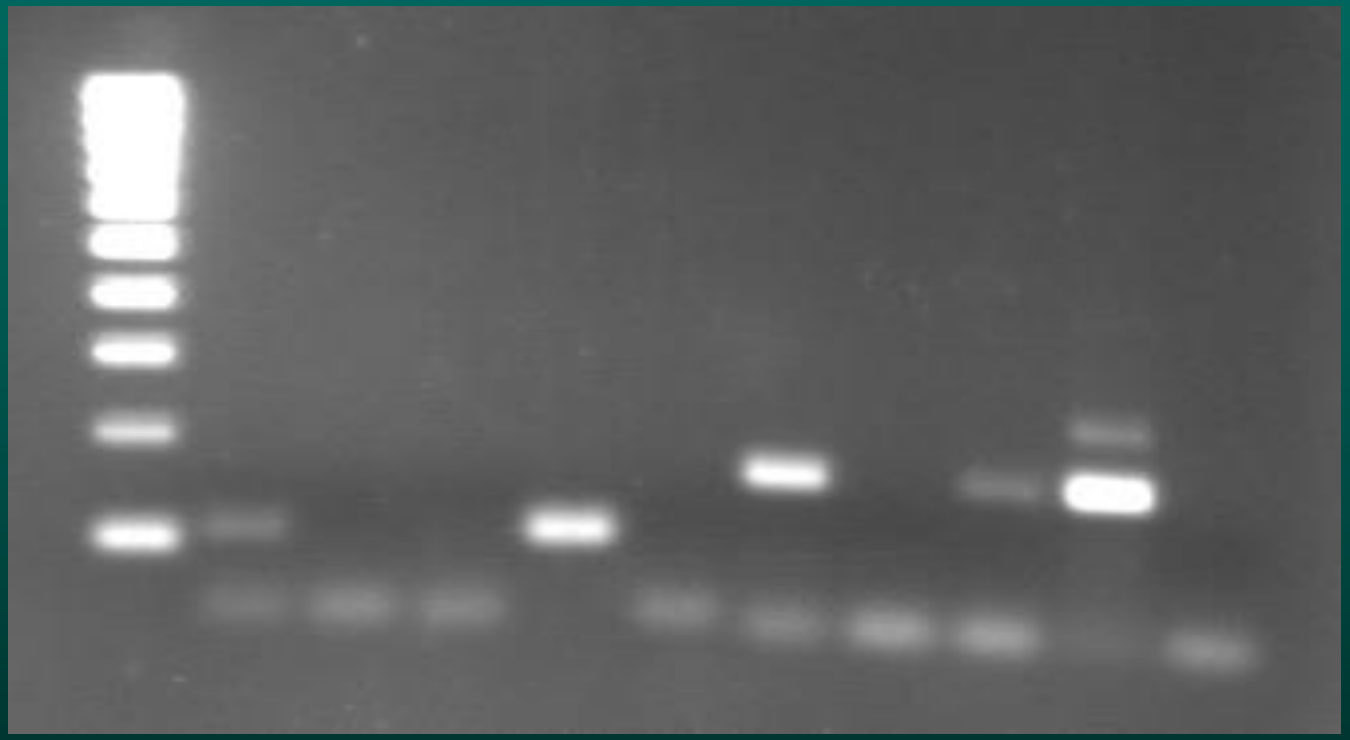
Analyze


(A)
1
2
3
4
5
(B)
1
2
3
4
5

Electrophoresis



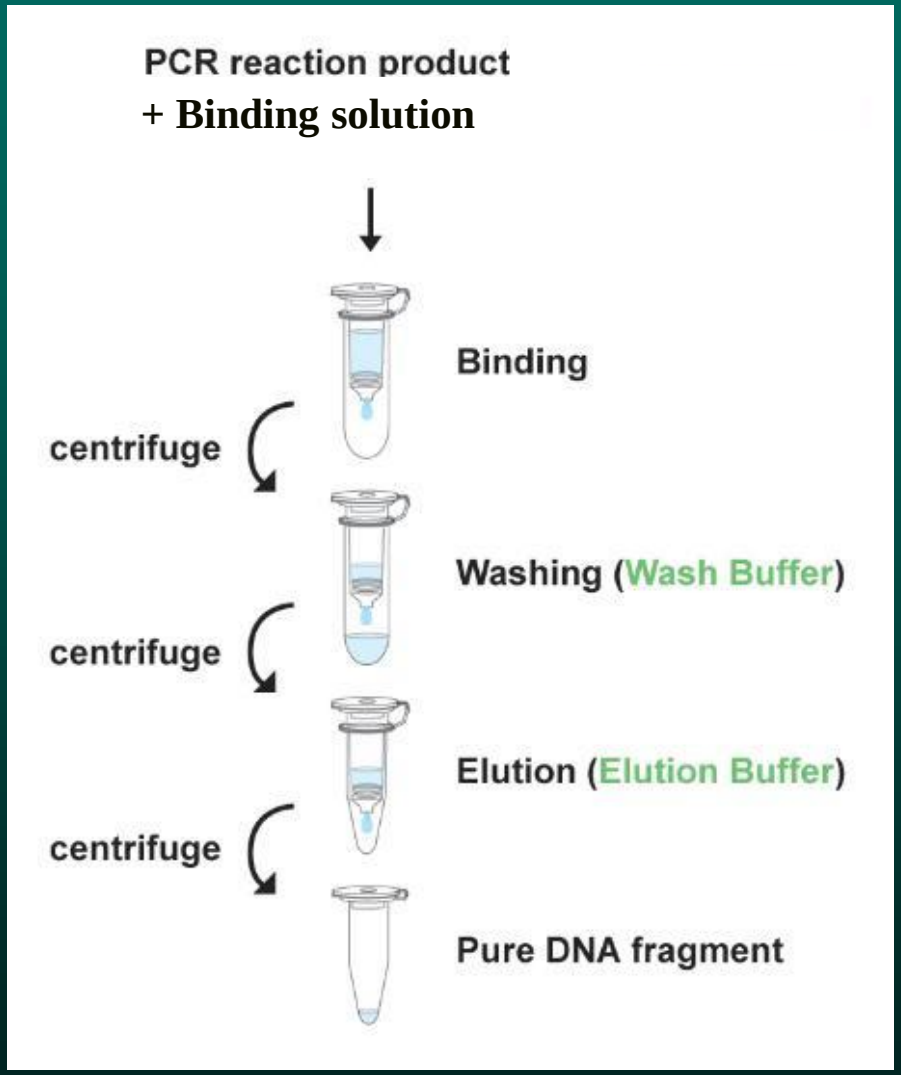
Unspecific bands



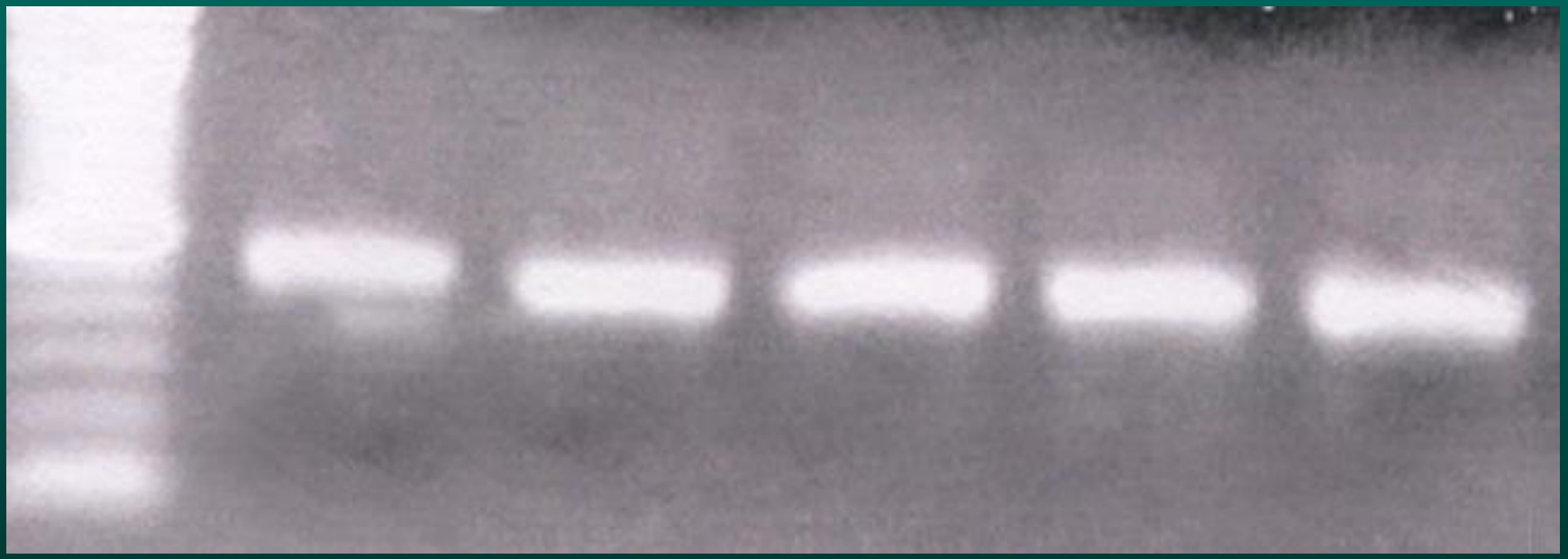
1- Increase the annealing temperature

2- Decrease MgCl2 final concentration

PCR Purification



Electrophoresis



Sequence Reaction Mix

Product	**Quantity(µl)**
Big Dye Terminator v1.1	2
primer (1.6 picomole/µl) (forward **or** reverse)	1
H_2O qsp 7.5µl	Depending on the vol X of the PCR
PCR purified	X µl
final Vol	7.5 µl

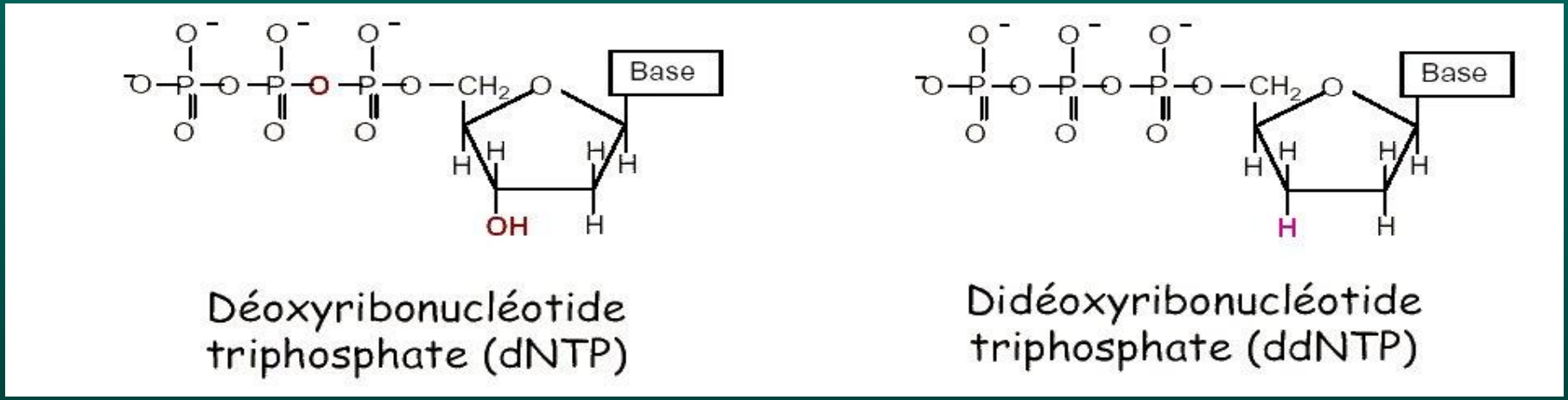
Sequence Reaction conditions

25 cycles :

- 96°C -10’’ (denaturation)
- 50°C - 5’’ (hybridisation or annealing)
- 60°C - 4’ (elongation or polymerisation)

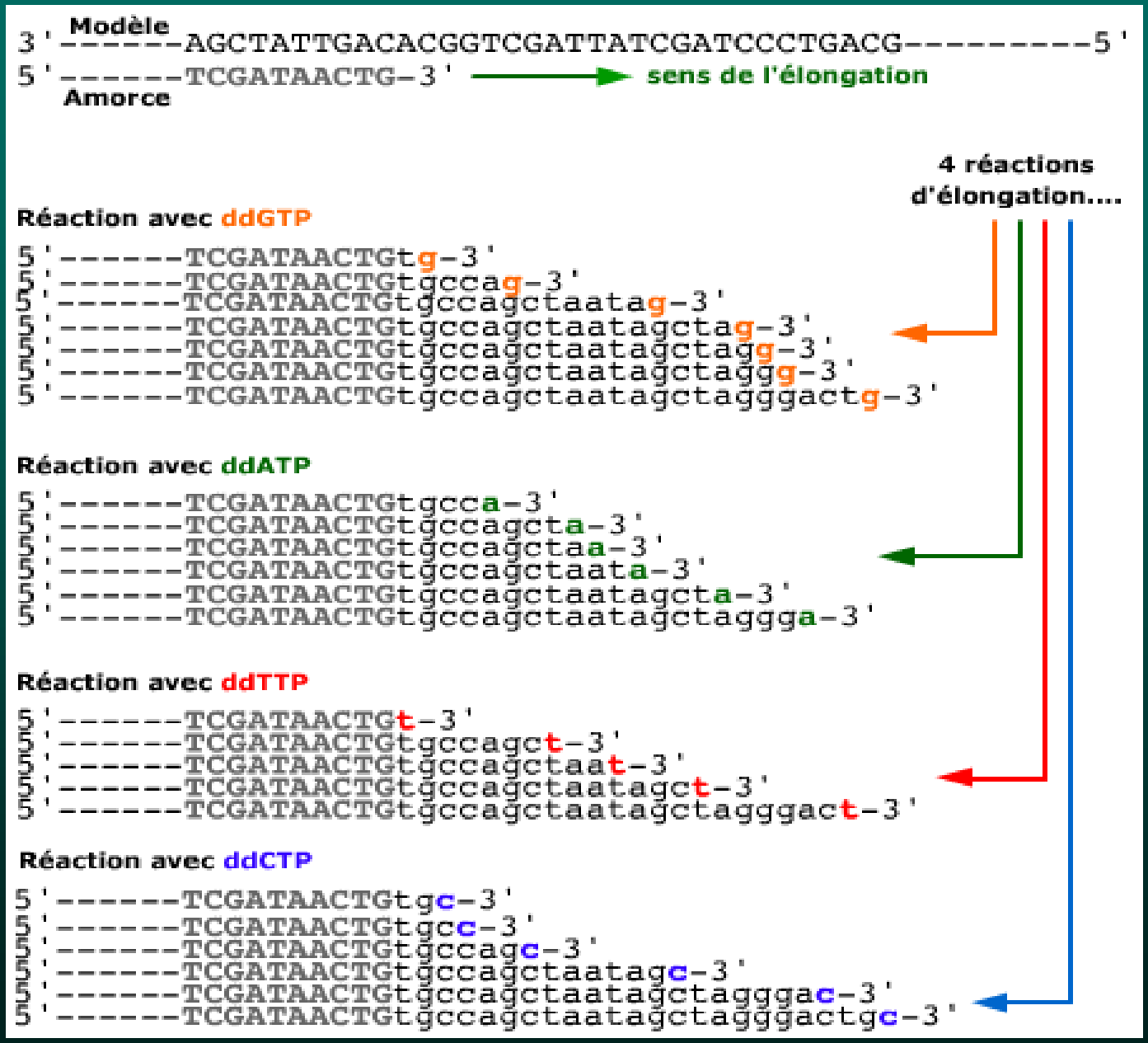
Sequencing

Sanger method



- ddNTP : can not realize phosphodiester linkage.
- ddNTP* : labeled with 4 different fluorescence dyes.
- DNA polymerase incorporate dNTP or ddNTP*.

Sequencing


Modèle
3'------AGCTATTGACACGGTCGATTATCGATCCCTGACG---------5'
5'------TCGATAACTG-3'
Amorce
sens de l'élongation
4 réactions d'élongation....
Réaction avec ddGTP
5'------TCGATAACTGtg-3'
5'------TCGATAACTGtgccag-3'
5'------TCGATAACTGtgccagctaatag-3'
5'------TCGATAACTGtgccagctaatagctag-3'
5'------TCGATAACTGtgccagctaatagctagg-3'
5'------TCGATAACTGtgccagctaatagctaggg-3'
5'------TCGATAACTGtgccagctaatagctagggactg-3'
Réaction avec ddATP
5'------TCGATAACTGtgcca-3'
5'------TCGATAACTGtgccagcta-3'
5'------TCGATAACTGtgccagctaa-3'
5'------TCGATAACTGtgccagctaata-3'
5'------TCGATAACTGtgccagctaatagcta-3'
5'------TCGATAACTGtgccagctaatagctaggga-3'
Réaction avec ddTTP
5'------TCGATAACTGt-3'
5'------TCGATAACTGtgccagct-3'
5'------TCGATAACTGtgccagctaat-3'
5'------TCGATAACTGtgccagctaatagct-3'
5'------TCGATAACTGtgccagctaatagctagggact-3'
Réaction avec ddCTP
5'------TCGATAACTGtgc-3'
5'------TCGATAACTGtgcc-3'
5'------TCGATAACTGtgccagc-3'
5'------TCGATAACTGtgccagctaatagc-3'
5'------TCGATAACTGtgccagctaatagctagggac-3'
5'------TCGATAACTGtgccagctaatagctagggactgc-3'

Sequence Reaction Purification

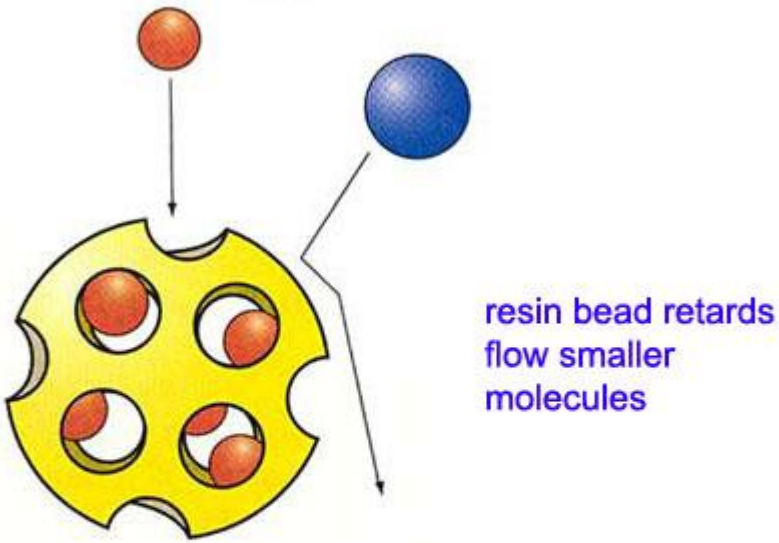
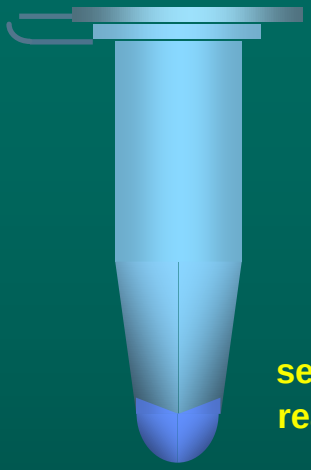


Figure **Gel filtration chromatography. (a)** Principle of the method. A resin bead is schematically represented as a "whiffle ball" (yellow). Large molecules (blue) cannot fit into the beads, so they are confined to the relatively small buffer volume outside the beads. Thus, they emerge quickly from the column. Small molecules (red), by contrast, can fit into the beads and so have a large buffer volume



primer → A G C T A T ...

Taq

DNA T C G A T A

sequence reaction

A G C T A T

A G C T A

A G C T

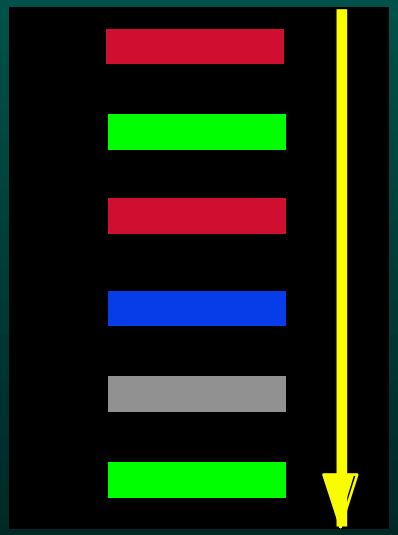
A G C

A G

A

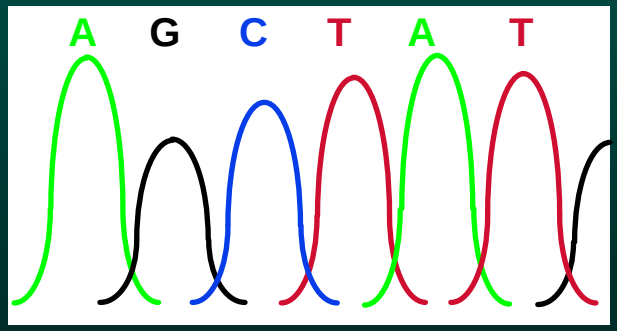
Loading

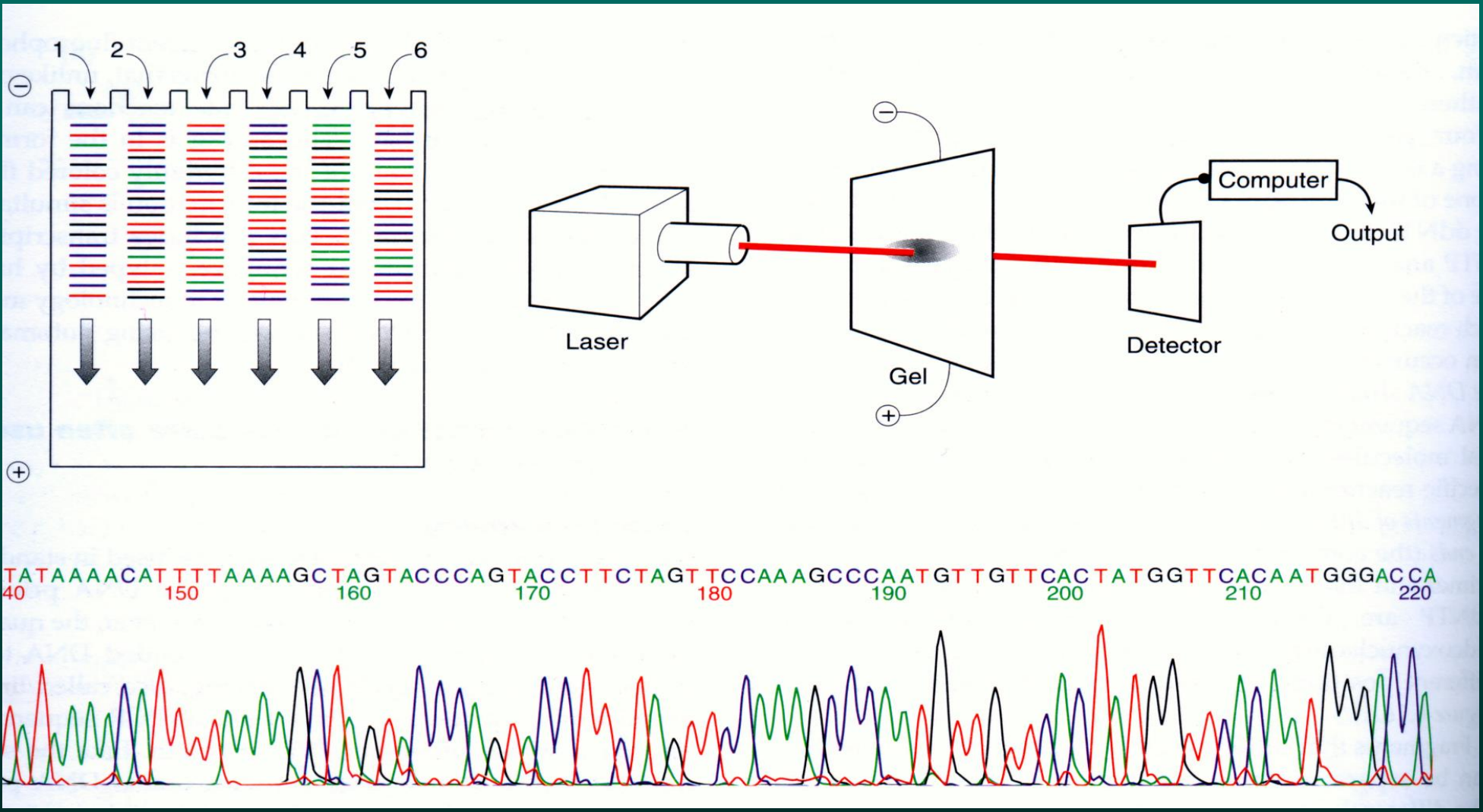
Electrophoresis



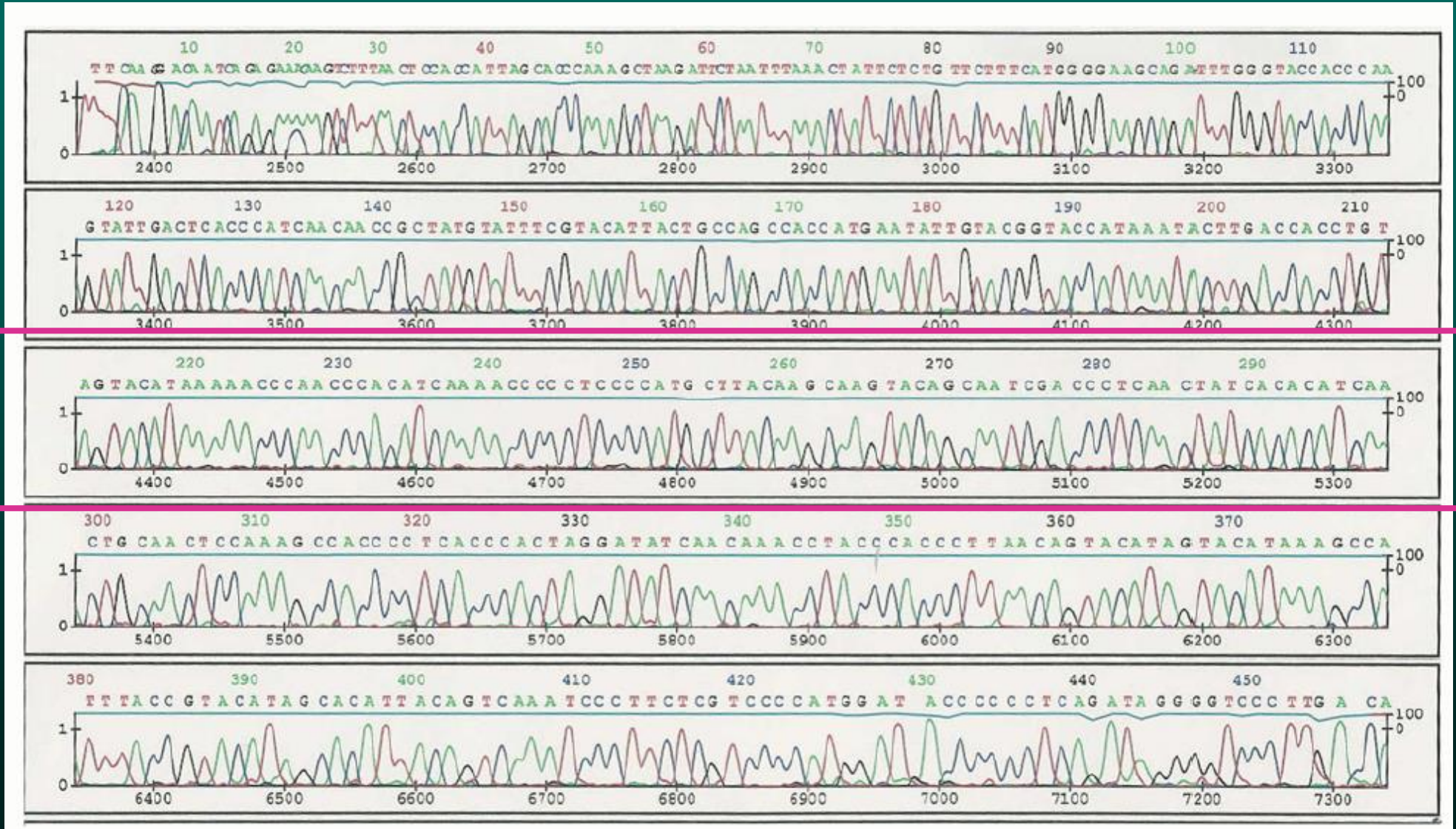
detection

Automatic analysis

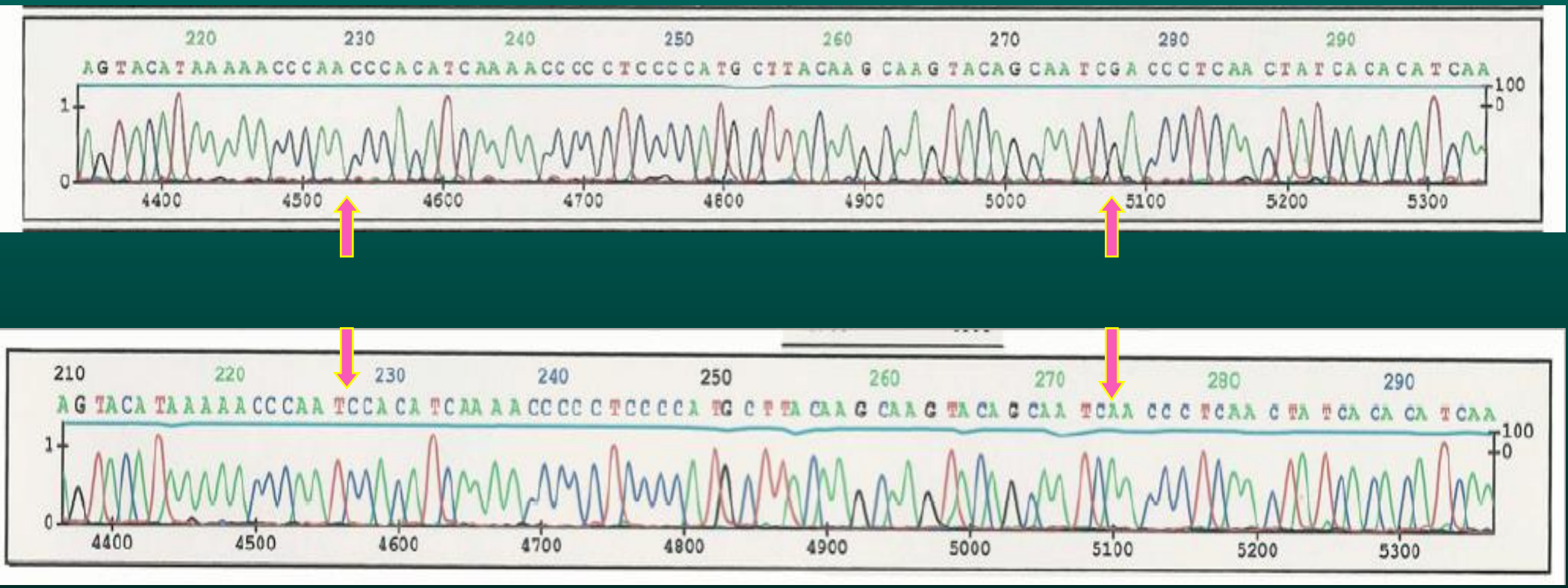



1
2
3
4
5
6
Laser
Gel
Detector
Computer
Output
TATAAAACATTTTAAAAGCTAGTACCCAGTACCTTCTAGTTCCAAAGCCCAATGTTGTTCACTATGGTTCACAATGGGACCA
40
150
160
170
180
190
200
210
220

mtDNA sequence



Comparison of two mtADN sequences



Results

Trace 1	Trace 2	suspect
HV1 (De 15970 à 16413)	HV1 (De 15970 à 16413)	HV1 (De 15970 à 16413)
-	-	**C16069T**
T16093C	-	-
-	-	**T16126C**
T16224C	-	-
A16293G	-	**A16293C**
T16311C	-	-
	-	**G16319A**
HV2 De 59 à 416	HV2 De 59 à 416	HV2 De 59 à 416
A73G	**A73G**	-
-	-	**G185A**
A197G	-	-
A263G	**A263G**	**A263G**
310+1C	-	-
315+1C	-	**315+1C**