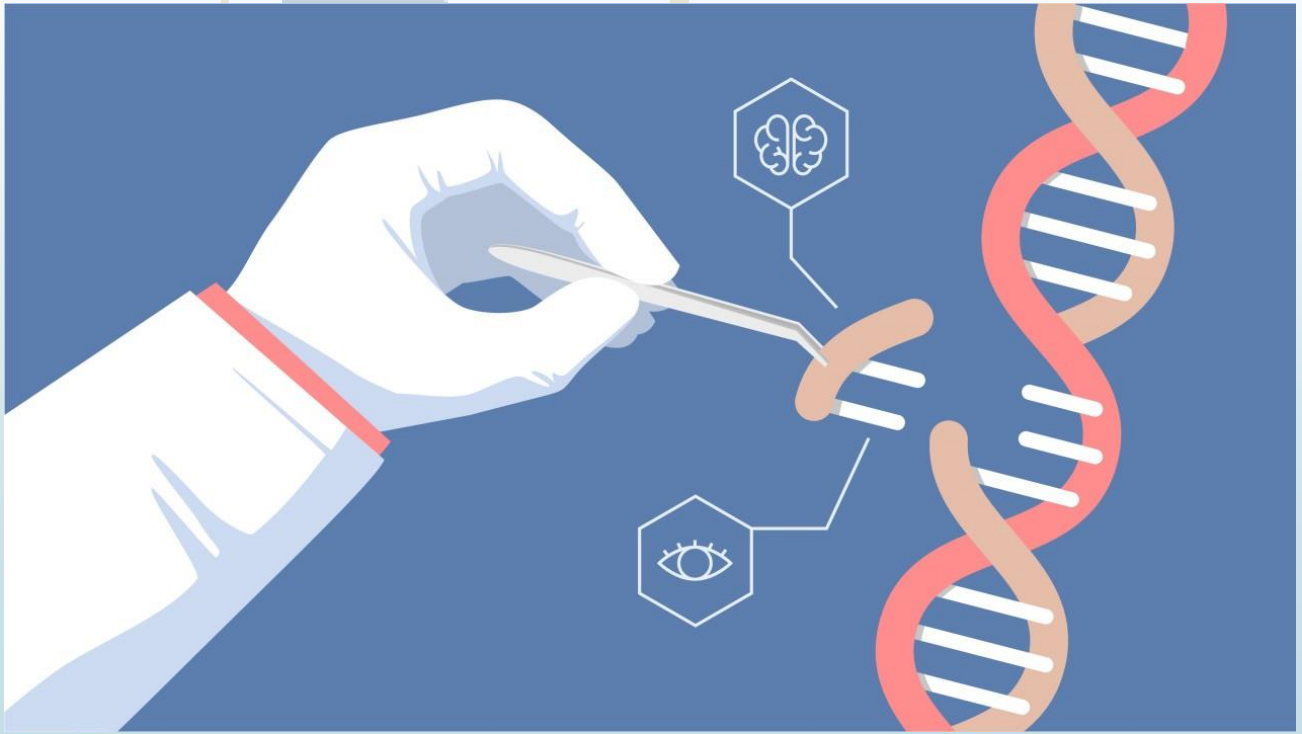
Chapter 3: Genetic Variation and Polymorphism

- Document 1: Mutations and the Environment
- Document 2: Mutation and Multiple Alleles
- Document 3: Polymorphic Genes in a Population
- Document 4: Detection of Genetic Polymorphism
- Document 5: Genetic Identity of Individuals

Document 5

Genetic Identity of Individuals



- Because of the large number of polymorphisms observed in humans, each of us is genetically unique (with the exception of identical twins).
- Genetic variation can be used to identify individuals.
- **How can the genetic identity of an individual be determined?**

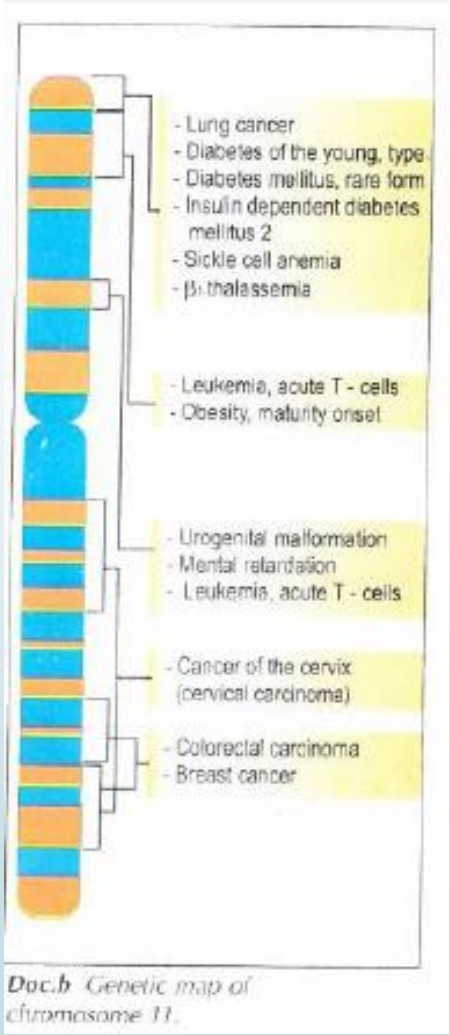
I. Localization of a Gene on a Chromosome

- A specific gene could be located on a chromosome by using a specific method called :**FISH technique.**

➢ **FISH:** Fluorescence In Situ Hybridization, is a method used to locate a specific gene (having a known sequence of nucleotides) on a chromosome, by using a probe that binds to a specific gene.

→ Scientists can determine the locus (position) of a gene on a chromosome.

→FISH allows to construct a human genetic map. Doc.b, p.67 (The indicated genes are involved in normal cell function, and only in case of mutation the disease would appear).



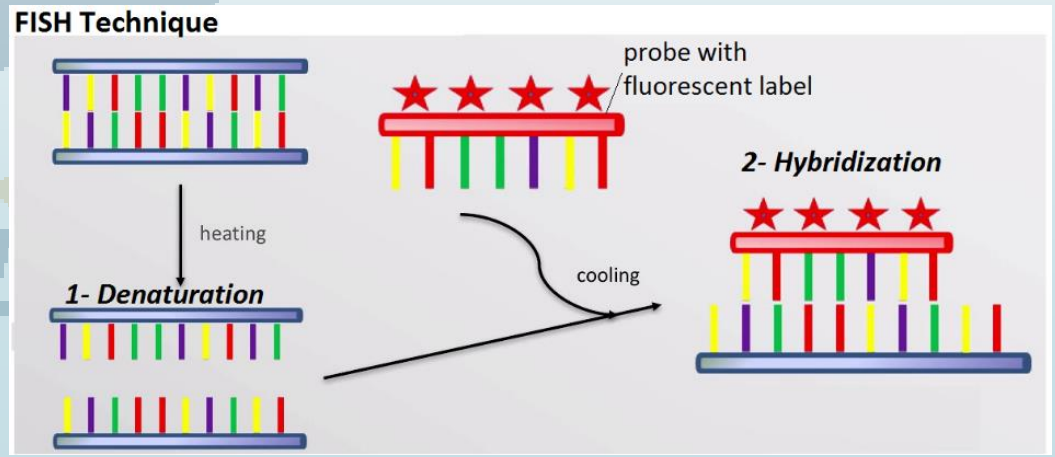
Doc.b Genetic map of chromosome 11.

➢ FISH technique involves 2 main steps:

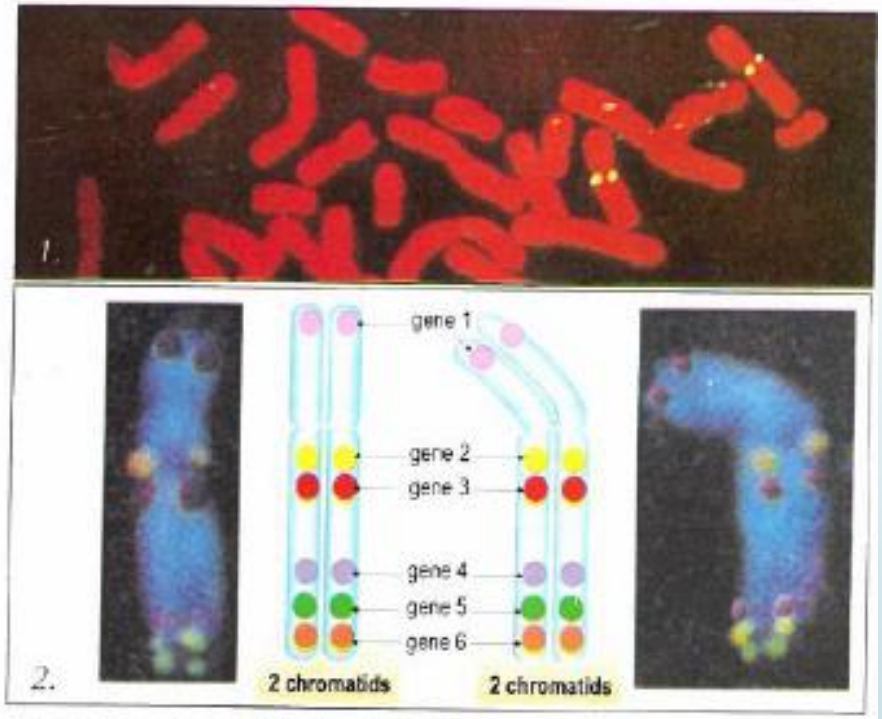
1- Denaturation: Is the separation of the 2 DNA strands by breaking the hydrogen bonds between nitrogenous bases by heating.

***Probe:** Is a synthesized fluorescent short single stranded DNA, that is complementary (binds) to one of the strands of a known specific gene.

2- Hybridization: Is the binding of the radioactive probe to its complementary sequence of DNA.



➢ Application of FISH technique



Doc.a *Genes located by the FISH technique.*
1. A mono-locus probe specific for a given gene.
2. Two human metaphase chromosomes labeled by six different "mono-locus" probes.

II. DNA Fingerprint

- Each diploid human cell has ≈ 30,000 to 40,000 genes distributed on 46 chromosomes. Each gene has at least 2 alleles that may be different or identical.

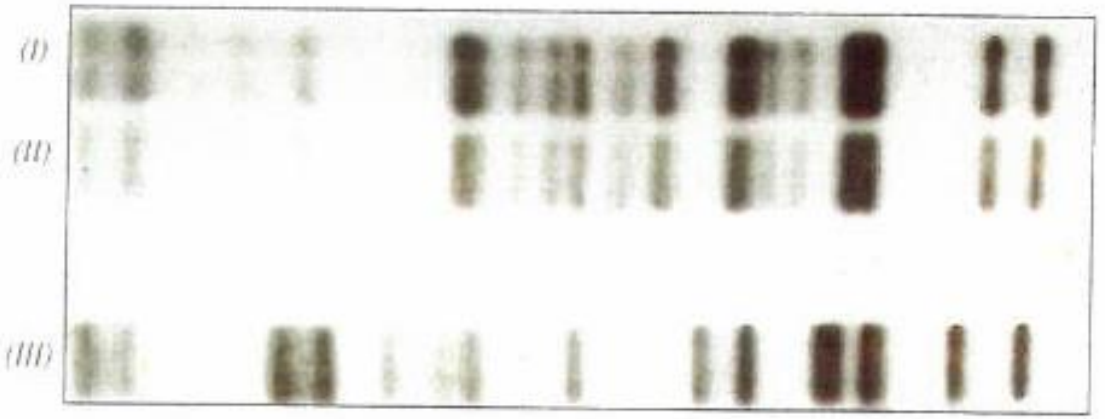
➢ **DNA Fingerprint:** is the pattern of bands obtained after applying Jeffrey's technique, used to study specific gene in DNA (not all sequences as RFLP).

- Each individual has a specific DNA finger print, and is uniquely characteristic for that individual. Doc.d, p.69 (next slide)

- Only identical twins have identical DNA fingerprints.

II. DNA Fingerprint

Doc.d *DNA fingerprints of identical twins (brothers I and II) and that of their sister (III).*



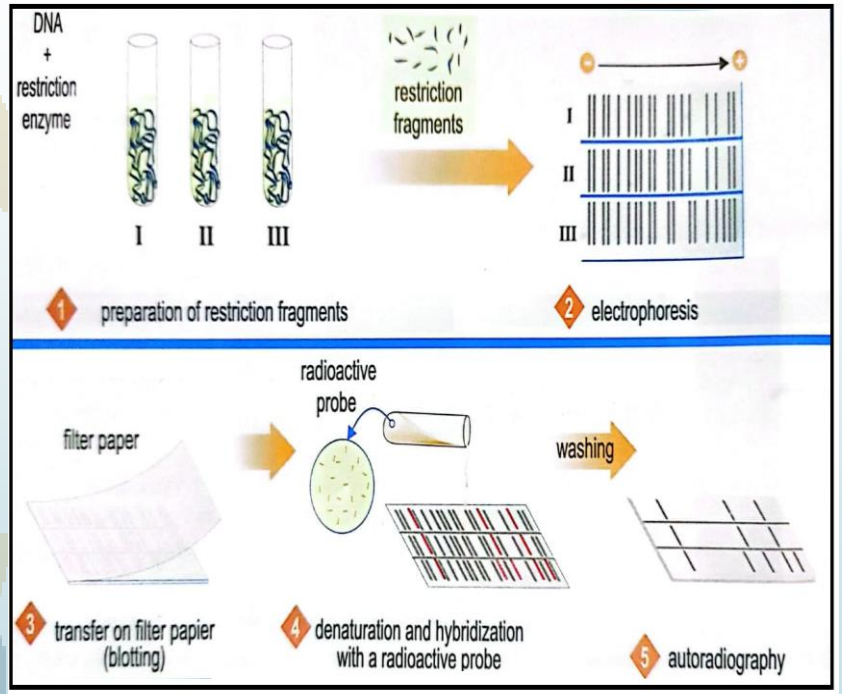
- Referring to Doc.d. draw out a conclusion.

Identical twins have the same DNA fingerprints and related individuals have common bands in their DNA fingerprints.

➢ Steps of Jeffrey's method (technique) Doc.c ,p.68

- DNA is first cut with a RE.
- DNA fragments are separated by electrophoresis.
- Fragments are then transferred (blotted) and fixed onto a solid membrane (filter paper). This technique is called "Southern blotting".
- On the membrane, the denatured fragments are hybridized to a radioactive labeled DNA probe. This is followed by washing to remove unbound probes.
- Finally, autoradiography is done. The hybridization of the DNA sequence with the probe is visualized by a technique known as autoradiography.

  → The resulting pattern of bands is the **" DNA finger print".**



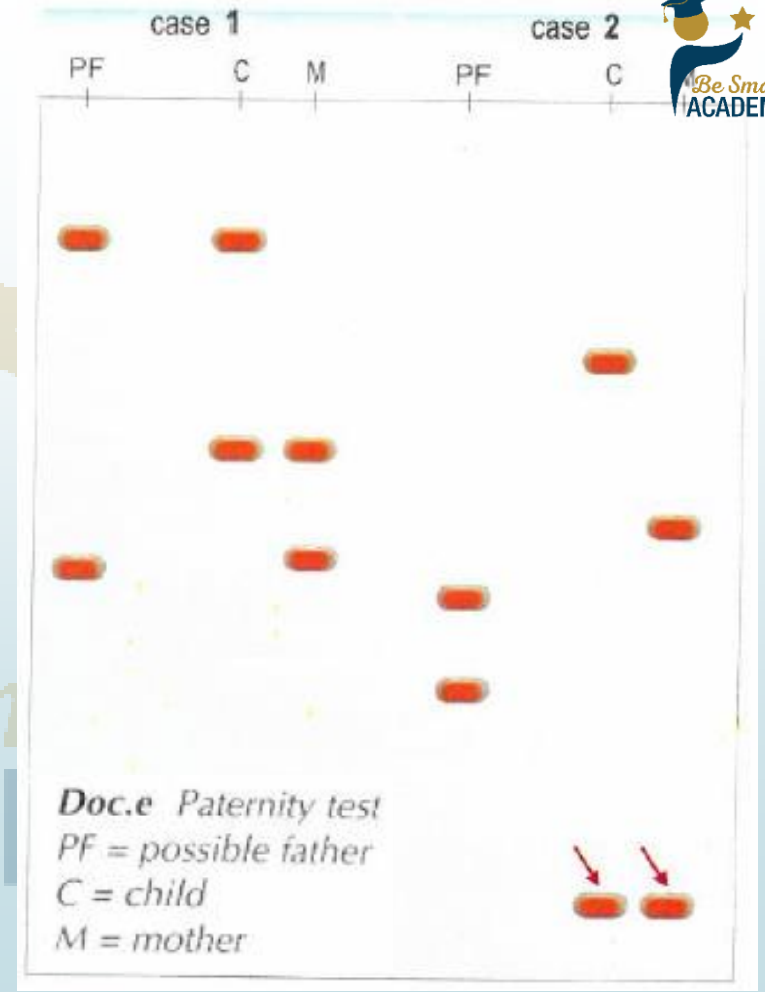
Doc.c

- **Application of DNA finger print**

- Paternity test
- Study genetic abnormalities
- forensic science

III. DNA finger print in Paternity Test

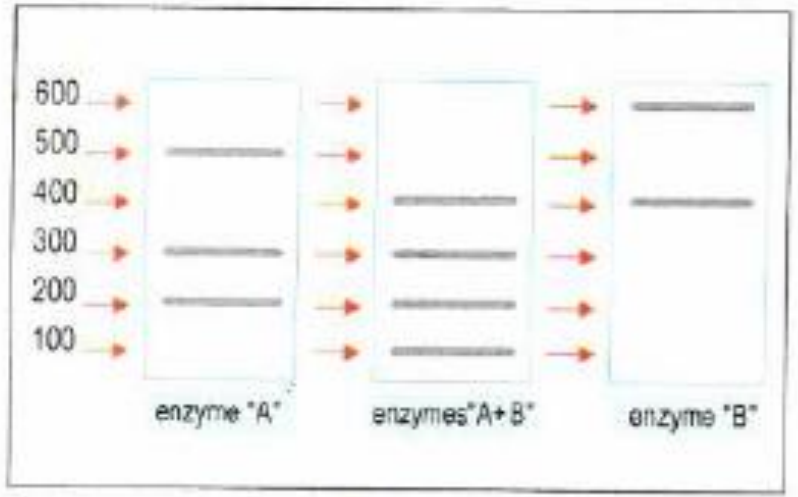
- The uniqueness of our DNA finger print can be used to determine parenthood, where each band of the child' DNA finger print should be shared with either the father's or the mother's DNA finger print as in case 1 Doc.e, p.69.



➢ Exercises:

☐ *Exercise III*

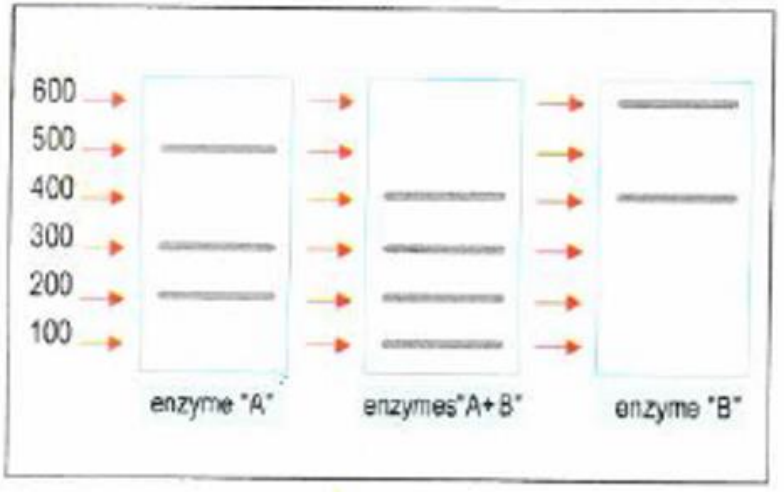
Two restriction enzymes (A and B) were used to cut a piece of DNA. The results are represented in the document below.



Determine the size of the DNA piece in base pairs (bp) and locate the respective restriction sites of enzymes A and B.

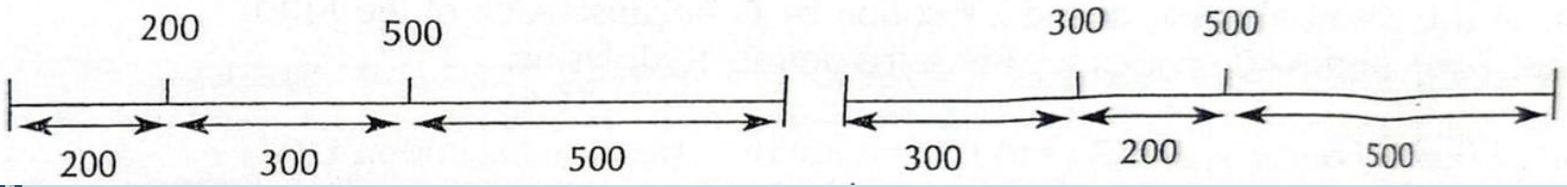
***Solution:**

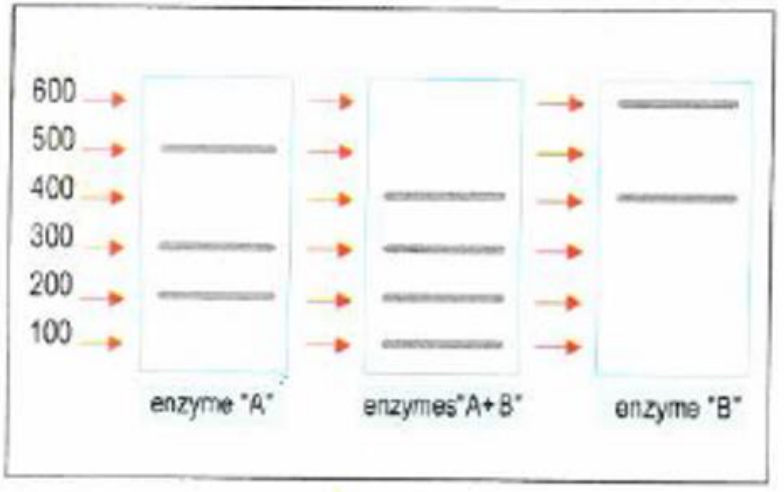
Since 3 bands were obtained when the DNA is cut with RE A, then the size of the DNA segment is:
200+300+500=1000 bp (when cut with enzyme A).
Or 400 +600= 1000 bp (when cut with enzyme B).



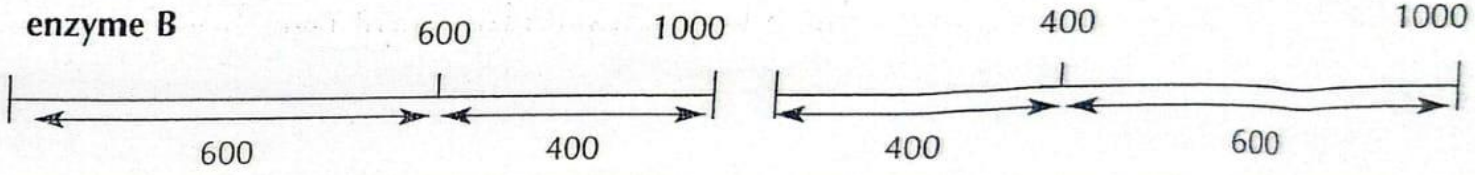
Enzyme A cuts the DNA on 2 sites:200 bp and 500 bp
or 300 bp and 500 bp

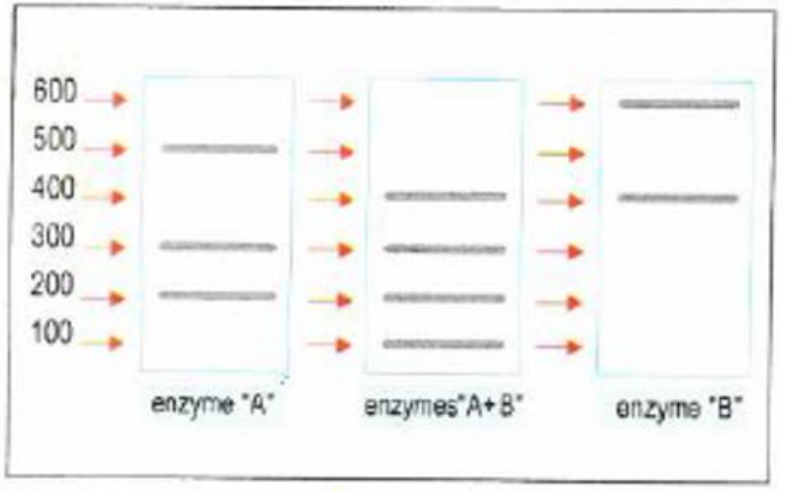
enzyme A



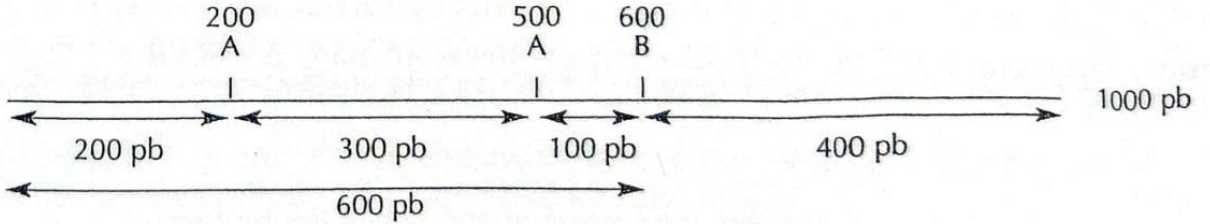


Enzyme B cuts the DNA on one site only:400 bp or 600 bp.

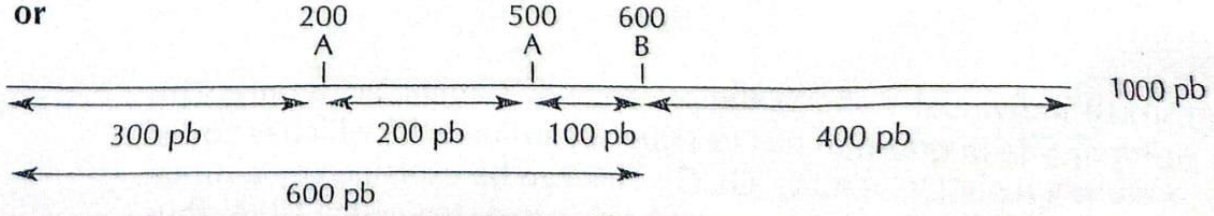




Segments obtained when using enzymes A and B are:

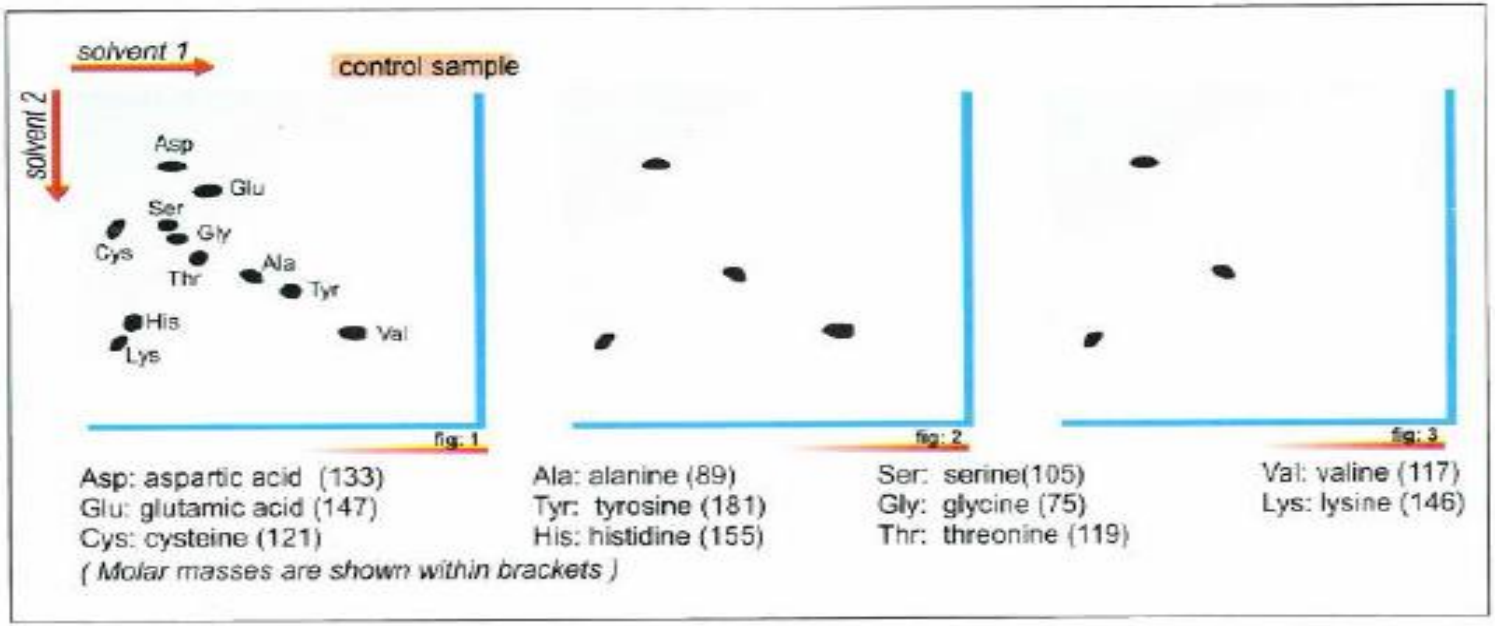


or



Exercise VII

A bidimensional chromatography is performed using a mixture of different human amino acids; the chromatogram of figure 1 is thus obtained. The figure 2 chromatogram shows the result of a tetrapeptide hydrolysis of an individual X, whereas figure 3 chromatogram shows the result of the hydrolysis of the same tetrapeptide of another individual Y.



a- What is the molar mass of this tetrapeptide (figure 2)?

b- Can one determine the exact sequence of this tetrapeptide? Justify the answer.

c- How can you explain the chromatogram of individual Y?

Exercise VII solution:

a- When comparing the position of the 4 amino acids (figure 2) with the reference chromatogram, we can find that the tetrapeptide contains: Asp + Ala + Lys + Val.
The molar mass of the tetrapeptide = 133 + 89 + 146 + 117 = 485.

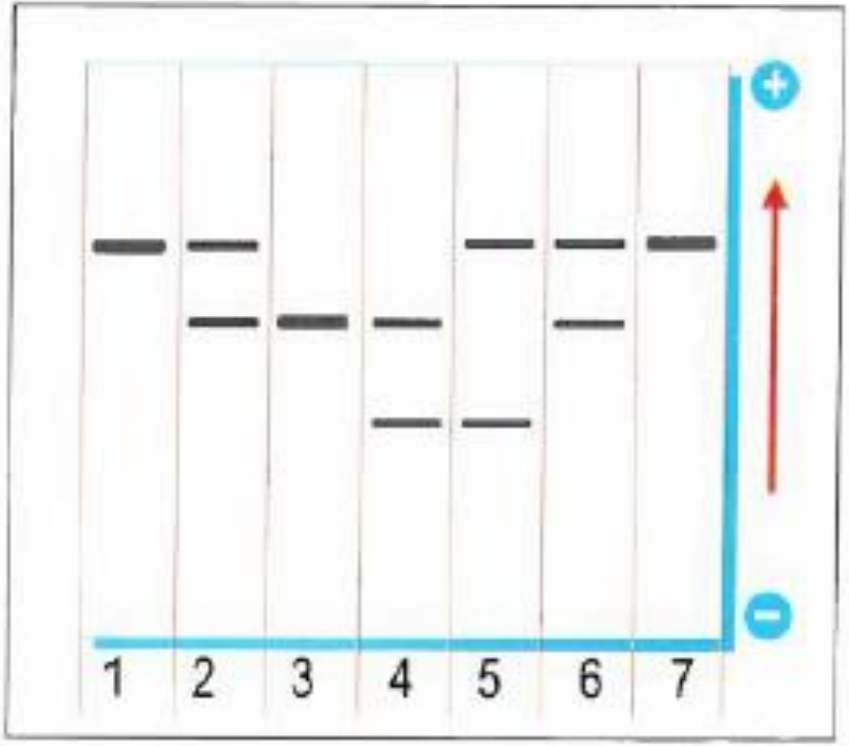
b-No, using the chromatography techniques we can only determine the different kinds of a.a found in a protein and not their sequence.

c- It could be due to a mutation in the gene coding for this tetrapeptide resulting in a stop codon and incomplete protein which explain the presence of 3 a.a instead of 4, or may be one of the a.a exists in 2 copies.

☐ *Exercise X*

The next document shows a series of electrophoreses of the hemoglobin of seven individuals. The differences in migration are due to the differences in the amino acid sequences of the two polypeptide chains of β-globin, which form the hemoglobin molecule along with the two α-globin chains.

a. How many possible alleles of the β-globin gene are shown by these electrophoreses?
b. How many alleles of the β-globin gene are present in an individual?
c. How can you explain the results obtained with the hemoglobin of subjects 1, 3 and 7?



Exercise X solution:

a- Determine ……

Since the β globin protein appears in 3 different positions on the gel electrophoresis, then it is coded by 3 different alleles.

b- Indicate …….

Each individual has 2 alleles coding for β-globin gene.

c- - Individuals 1,3 and 7 show 1 thick band of β-globin protein, which means that they have 2 identical alleles coding for this protein, Thus 1 type of β-globin chain is produced.

*These individual are pure or homozygous since they have 2 same alleles.

- Individuals 2,4,5 and 6 show 2 bands at 2 different positions, then they have 2 different alleles, so they are heterozygous or hybrid.

Selected Exercises of Official Exams

Official Exam2011 (2)

Exercise 2 (5 points)

The Xeroderma pigmentosum is a disease that results in skin lesions which can develop into cancerous tumors and eye lesions. We are interested in the causes of this disease and the relative influence of genes and environment on its appearance.
The body cells have, in their nucleus, enzymes that can repair DNA whenever this latter shows alterations. One of these enzymes is the ERCC3 which is coded by the gene G-ERCC3.
We present in document 2 the nucleotides sequence of a fragment of the non-transcribed strand of the gene G-ERCC3 of a healthy individual(allele G1) and the sequence of the equivalent fragment of an individual affected by xeroderma pigmentosum (allele G2).

DNA alteration

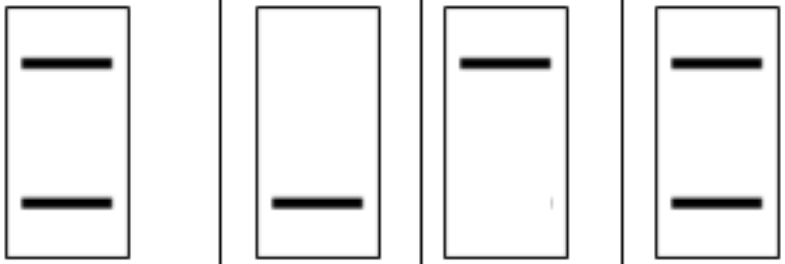
NUCLEOTIDE POSITION 1	NUCLEOTIDE POSITION 2: U	NUCLEOTIDE POSITION 2: C	NUCLEOTIDE POSITION 2: A	NUCLEOTIDE POSITION 2: G	NUCLEOTIDE POSITION 3
U	UUU, UUC: phenylalanine; UUA, UUG: leucine	UCU, UCC, UCA, UCG: serine	UAU, UAC: tyrosine; UAA, UAG: stop	UGU, UGC: cysteine; UGA: stop; UGG: tryptophane	U C A G
C	CUU, CUC, CUA, CUG: leucine	CCU, CCC, CCA, CCG: proline	CAU, CAC: histidine; CAA, CAG: glutamine	CGU, CGC, CGA, CGG: arginine	U C A G
A	AUU, AUC, AUA: isoleucine; AUG: methionine	ACU, ACC, ACA, ACG: threonine	AAU, AAC: asparagine; AAA, AAG: lysine	AGU, AGC: serine; AGA, AGG: arginine	U C A G
G	GUU, GUC, GUA, GUG: valine	GCU, GCC, GCA, GCG: alanine	GAU, GAC: asparatic acid; GAA, GAG: glutamic acid	GGU, GGC, GGA, GGG: glycine	U C A G

A: Adenine U: Uracile G: Guanine C: Cytosine

Document 1

Allele	nucleotides sequence of the fragment
G1	1 12 …AAG AAG AGC AAC…
G2	1 12 …AAG AAG AGA AAC…

Document 2

	Reference electrophoresis	Individual A	Individual B	Individual C
ERCC3 (coded by allele G1) ERCC3 (coded by allele G2)				

Document 3

1- Determine using the genetic code table (doc.1) the amino acid sequence of the portion of each of the enzymes ERCC3 coded by the allele G1 and by the allele G2.

We can separate, by electrophoresis, the enzyme ERCC3 coded by the allele G1 and enzyme ERCC3 coded by allele G2. Electrophoresis is performed for three different individuals: A, B and C. Individual A is affected with Xeroderma pigmentosum, and individuals B and C are not. The results are presented in document 3.

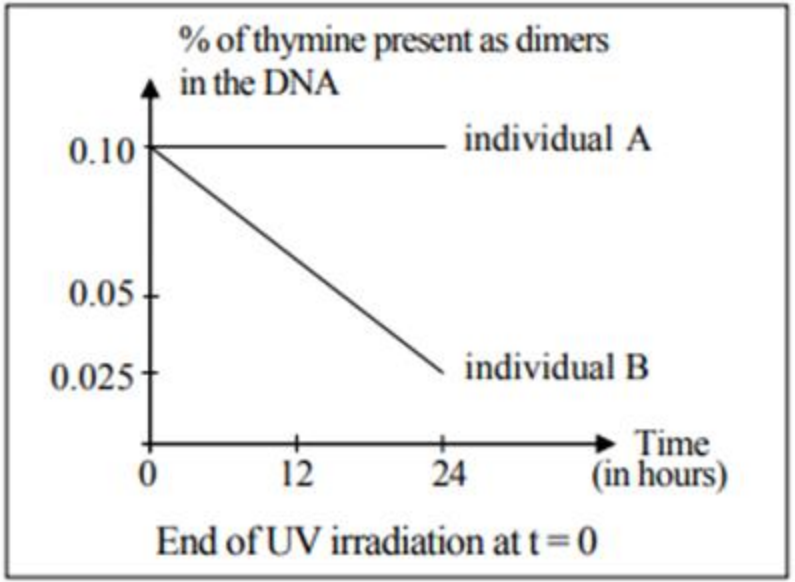
2- Write the genotypes of individuals A, B and C. Justify the answer.

3- Specify the dominant allele and the recessive one. Justify the answer.

Part of the EX	Answer key
	Exercise 2 (5 points)
1	mRNA resulting from the transcription of the allele G1: AAG AAG AGC AAC Amino acid sequence of the polypeptide coded by the allele G1: Lysine – Lysine – Serine – Asparagine mRNA resulting from the transcription of the allele G2: AAG AAG AGA AAC Amino acid sequence of the polypeptide coded by the allele G2: Lysine – Lysine – Arginine – Asparagine Or We can obtain it directly from the non transcribed strand of DNA by replacing T by U. thus, we obtain the same sequence for both the mRNA and the DNA non-transcribed strand. Amino acid sequence of the polypeptide coded by the allele G1: Lysine – Lysine – Serine – Asparagine Amino acid sequence of the polypeptide coded by the allele G2: Lysine – Lysine – Arginine – Asparagine

2	The genotype of individual A G2//G2 **(0.25 pt)** because the result of his electrophoresis shows one type of enzyme ERCC3 that is coded by allele G2. **(0.25 pt)** The genotype of individual B is G1//G1 **(0.25 pt)** because the result of his electrophoresis shows one type of enzyme ERCC3 that is coded by allele G1. **(0.25 pt)** The genotype of individual C is G1//G2 **(0.25 pt)** because the result of his electrophoresis shows the two types of enzymes. **(0.25 pt)**
3	The allele G1 is dominant **(0.25 pt)** and the allele G2 is recessive **(0.25 pt)** because individual C who is heterozygous of genotype G1//G2 is not affected by Xeroderma Pigmentosum, Allele G2 is masked and not expressed phenotypically in the presence of allele G1 which dominates allele G2**(0.25 pt)**.

Upon exposure to ultra violet sunlight rays, the DNA of skin cells undergo alterations, particularly the formation of dimers between two successive thymines T-T. We measure the evolution of the percentage of dimers in the two individuals A and B after being subjected to irradiation with ultraviolet rays. The measured results are presented in document 4.



Document 4

4- Analyze the obtained results in document 4.

5- Based only on the previous given:

5-1- Explain the results of document 4.

5-2- Specify the factors that determine the development of the studied disease. Justify the answer.

4	The percentage of thymine dimers in the DNA remains constant (0.10%) through the 24 hours in individual A affected by xeroderma, while it decreases from 0.10% to 0.025% through the 24 hours in the healthy individual B after their exposition to ultraviolet irradiation.
5-1	Individual A (doc. 3) affected with xeroderma has no functional enzyme ERCC3 which is responsible of repairing the DNA alterations . The thymine dimers formed due to the exposition to ultra violet radiation cannot be repaired in this individual and thus the percentage of dimers T-T remains stable **(0.25 pt).** In the healthy individual B, which possesses functional ERCC3 enzyme, the altered DNA formed by ultraviolet irradiation is gradually repaired by this enzyme thus the percentage of thymine dimers decreases **(0.25pt)**
5-2	Two factors determine the development of Xeroderma pigmentosum: - The genetic factor**(0.25pt)**: the disease develops only in homozygous individuals with two mutant alleles of a gene coding for the enzyme ERCC3 involved in the repair of DNA damage**(0.25pt);** - The environmental factor**(0.25 pt)**: exposure to sun ultraviolet rays provokes the alteration of DNA**(0.25 pt)**.

Selected Exercises of Official Exams (2013-2-)

Exercise 2 (5 points)

Origins of Phenylketonuria

In hepatic cells, the enzyme phenylalanine hydroxylase, PAH, is responsible for the transformation of phenylalanine into tyrosine. Its absence or its inactivity results in the accumulation (increase in the amount) of phenylalanine in the blood which becomes toxic at a dose exceeding 20mg/dL which leads to the destruction of the nerve cells in individuals affected with phenylketonuria. This disease has different origins and is manifested by irreversible mental retardation.

1- Pick out the consequence of the high amount of phenylalanine in the blood.

Nucleotides position 1	Nucleotides position 2 U	Nucleotides position 2 C	Nucleotides position 2 A	Nucleotides position 2 G	Nucleotides position 3
U	UUU, UUC: phenylalanine; UUA, UUG: leucine	UCU, UCC, UCA, UCG: serine	UAU, UAC: tyrosine; UAA, UAG: non-sens	UGU, UGC: cysteine; UGA: non-sens; UGG: tryptophane	U C A G
C	CUU, CUC, CUA, CUG: leucine	CCU, CCC, CCA, CCG: proline	CAU, CAC: histidine; CAA, CAG: glutamine	CGU, CGC, CGA, CGG: arginine	U C A G
A	AUU, AUC, AUA: isoleucine; AUG: methionine	ACU, ACC, ACA, ACG: threonine	AAU, AAC: asparagine; AAA, AAG: lysine	AGU, AGC: serine; AGA, AGG: arginine	U C A G
G	GUU, GUC, GUA, GUG: valine	GCU, GCC, GCA, GCG: alanine	GAU, GAC: aspartic acid; GAA, GAG: glutamic acid	GGU, GGC, GGA, GGG: glycine	U C A G

A : Adenine U : Uracil G : Guanine C : Cytosine.

Document 1

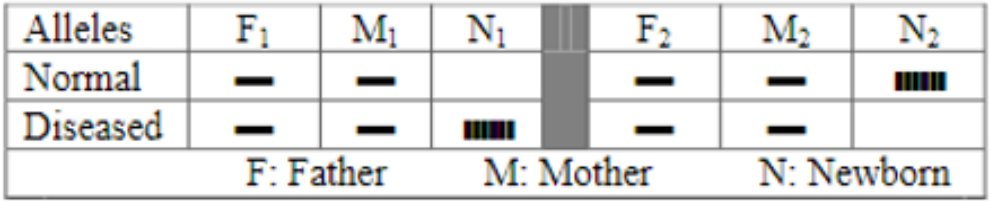
Document 2 represents a part of the gene coding for the enzyme PAH of a healthy individual and that of the equivalent fragment of an individual suffering from phenylketonuria.

2- Determine, using the genetic code table (document 1), the sequence of amino acids of the part of the enzyme PAH coded by each of these two alleles.

3- Explain how the modification in the nucleotide sequence of the allele leads to the appearance of phenylketonuria.

Alleles	Nucleotide sequence of the non-transcribed strand of DNA from codon 277 to codon 283
Normal	TAT ACC CCC GAA CCT GAC ATC
Diseased	TAT ACC CCC AAA CCT GAC ATC

Document 2



Document 3

Two normal couples had two newborns with high plasma concentration of phenylalanine that exceeds 20mg/dL.

4- Indicate if the allele of the disease is dominant or recessive. Justify the answer.

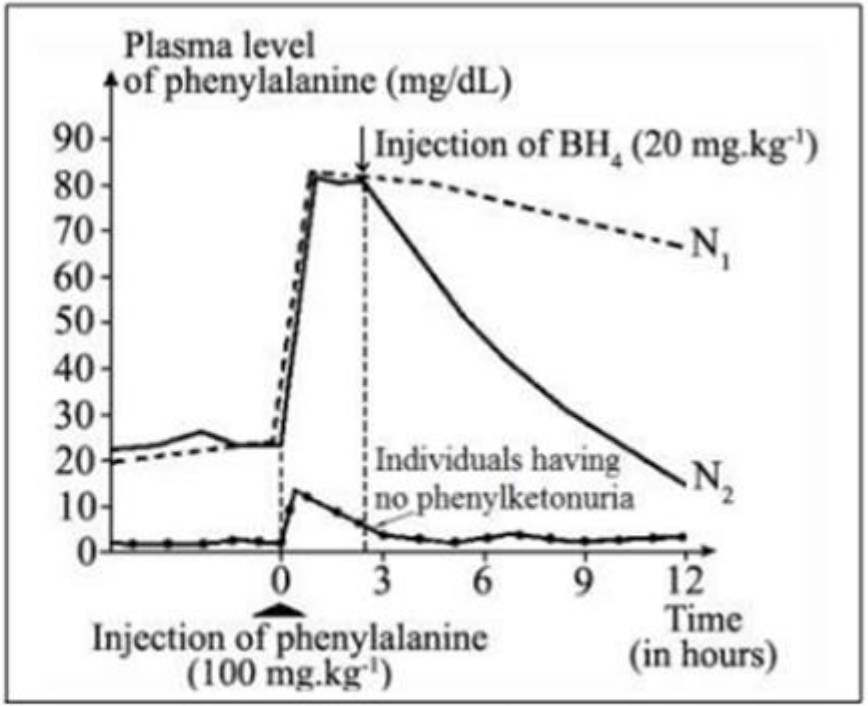
1	It is toxic, leads to the destruction of the nerve cells and is manifested by irreversible mental retardation.
2	Portion of the amino acids sequence of the enzyme: We establish the mRNA sequence by replacing T by U Normal mRNA: UAU ACC CCC GAA CCU GAC AUC Amino acids sequence : Tyr-Thr-Pro-Glu-Pro-Asp-Ile Diseased m RNA: UAU ACC CCC AAA CCU GAC AUC Amino acids sequence : Tyr-Thr-Pro-Lys-Pro-Asp-Ile
3	The mutation by substitution at the level of the first nucleotide of the 280th codon of the DNA where G is replaced by A is transcribed at the level of mRNA by a new codon which is translated into a new amino acid, lysine instead of the glutamic acid. This new amino acid sequence affects the tridimensional structure of the enzyme PAH which becomes inactive (nonfunctional). Since this enzyme is responsible for the transformation of phenylalanine into tyrosine. This transformation doesn't occur any more leading thus to the accumulation of phenylalanine which in high amount becomes toxic and causes phenylketonuria.
4	The allele of the disease is recessive with respect to the normal allele. Since normal parents gave birth to an affected child, thus they carry the allele of the disease that is masked in the parents. Let N be the symbol of the normal allele. Let m be the symbol of the allele coding for the disease.

Alleles	F_1	M_1	N_1	F_2	M_2	N_2
Normal	—	—		—	—	▮▮▮▮
Diseased	—	—	▮▮▮▮	—	—	
	F: Father		M: Mother		N: Newborn	

Document 3

In order to determine the origin of the disease in these two newborns, N_1 and N_2, these couples consulted a doctor who recommended DNA analysis for all the family members. The obtained results are presented in document 3.

Moreover, the doctor proposed another test, where he injected the newborns with phenylalanine followed by injection of BH_4, an organic substance normally present in the organism and that is indispensable for the normal activity of PAH. The obtained results are presented in document 4.



Document 4

5- Indicate the possible origin of the disease in the case of the newborn (N1). Justify the answer by referring to documents 3 and 4.

6- Determine, by referring to documents 3 and 4, the possible origin of the disease in the case of the newborn (N2).

5	The origin of the disease in the case of N1 is a mutation that leads to the synthesis of an inactive PAH (non-functional). Document 3 shows that affected N1 is homozygous of genotype m//m. And document 4 shows that a slight decrease in the plasma level of phenylalanine in N1 from 80 to 70 mg/dL after the injection of 20 mg/Kg of BH4. This implies that even in the presence of functional BH4, the PAH remains nonfunctional.
6	Document 3 shows that the affected newborn N2 is homozygous of genotype N//N. His allele codes for a normal PAH. Document 4 shows that in N2, the constant plasma level of phenylalanine of 80 mg/dL decreases after the injection of 20 mg/Kg of BH4to 15 mg/dL value that is inferior to the reference level of 20 mg/dL. Thus BH4 acts in N2 by decreasing the plasma level of phenylalanine toward its normal value. The PAH in the newborn N2 is functional but needs the presence of BH4 to be activated. Hence, his disease in N2 can be due to the absence of BH4 or to the presence of non-functional BH4.