Beta Thalasemmia Diagnostics

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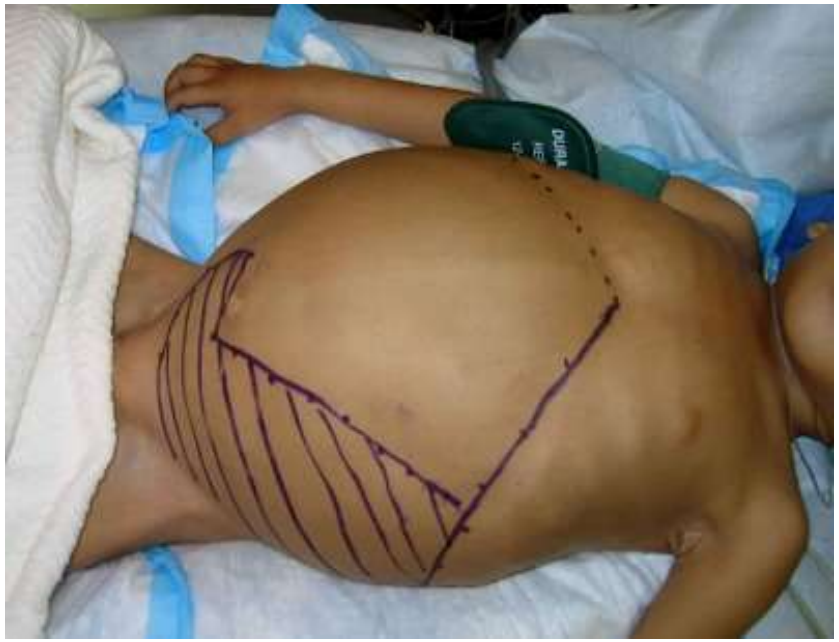
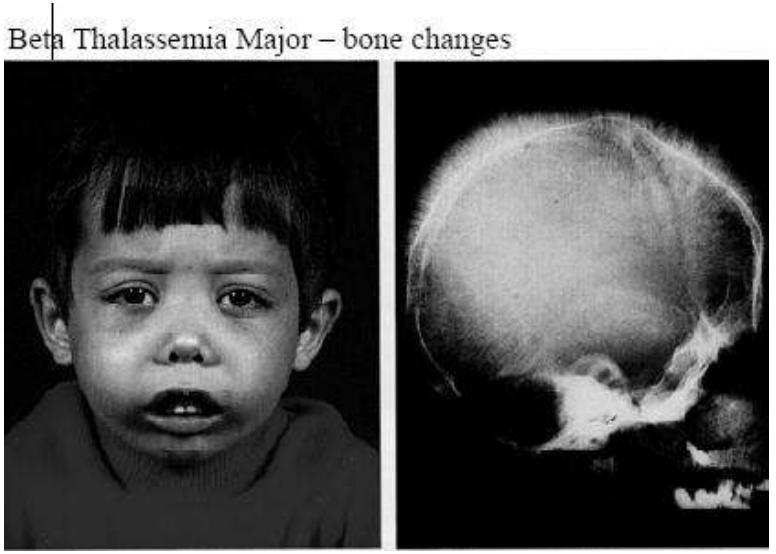
Thalassemia Disorder

Thalassemia is inherited disorders characterized reduced or absent amounts of hemoglobin, the oxygen-carrying protein inside the red blood cells.



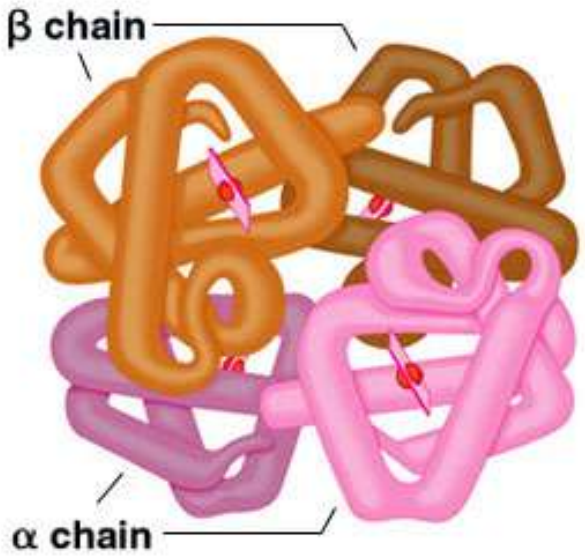


Symptoms of Beta Thalassemia

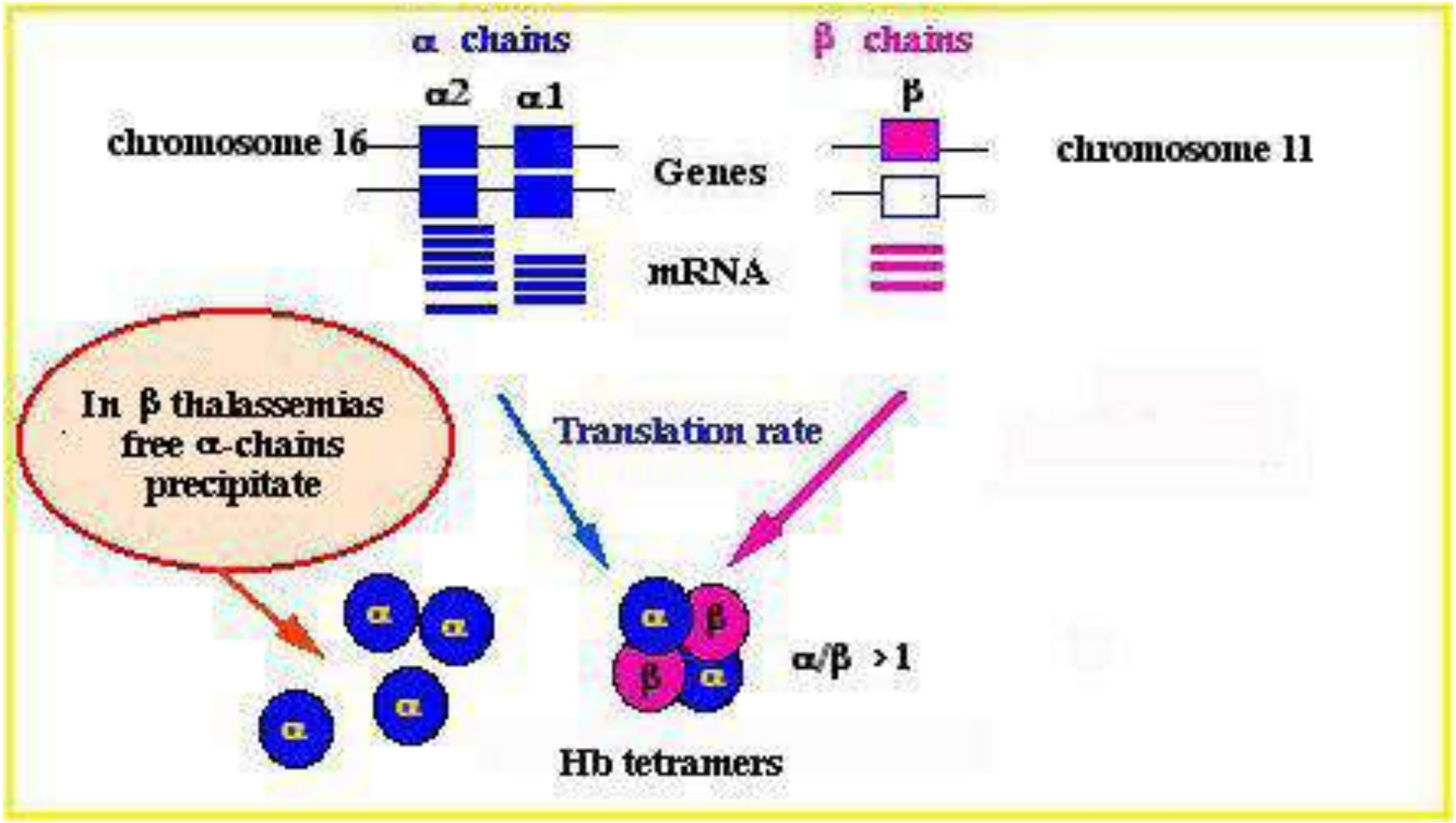
- ***It is characterize by severe anemia that can begin months after birth***
- ***Paleness***
- ***Delays in growth and development***
- ***Bone marrow expansion.***
- ***Untreated Beta Thalassemia major can lead to child death due to heart failure.***

Two Basic Groups of Thalassemia Disorder

- Adult hemoglobin composed two alpha and two beta chains.
- Alpha thalassemia usually caused by gene deletion; Beta thalassemia usually caused by mutation.
- Results in microcytic, hypochromic anemias of varying severity.



Genetics of Thalassemia



Genetics of Thalassemia

Normal
(4 alpha genes)
M: 14.5-16.5; F: 13-15

'Silent' Carrier
(3 alpha genes)
M: 13-15.5; F: 11.5-13.5

Heterozygous alpha 0 Trait
(2 alpha genes)
M: 12-14; F: 10.5-12.5

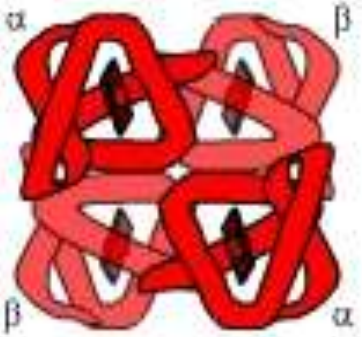
Homozygous alpha plus trait
(3 alpha genes)
M: 12-14; F: 10.5-12.5

Hemoglobin H Disease
(1 alpha gene)
M: 10-12; F: 8.5-10.5

Alpha Thalassemia Major
(0 alpha genes)
Severe Anemia in Fetus

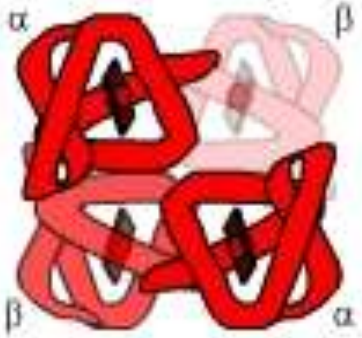
Genetics of Thalassemia

Without a mutation enough Hemoglobin



No thalassemia carrier

With one mutation less Hemoglobin



β-thalassemia carrier without illness, but less hemoglobin (slight aneamia)

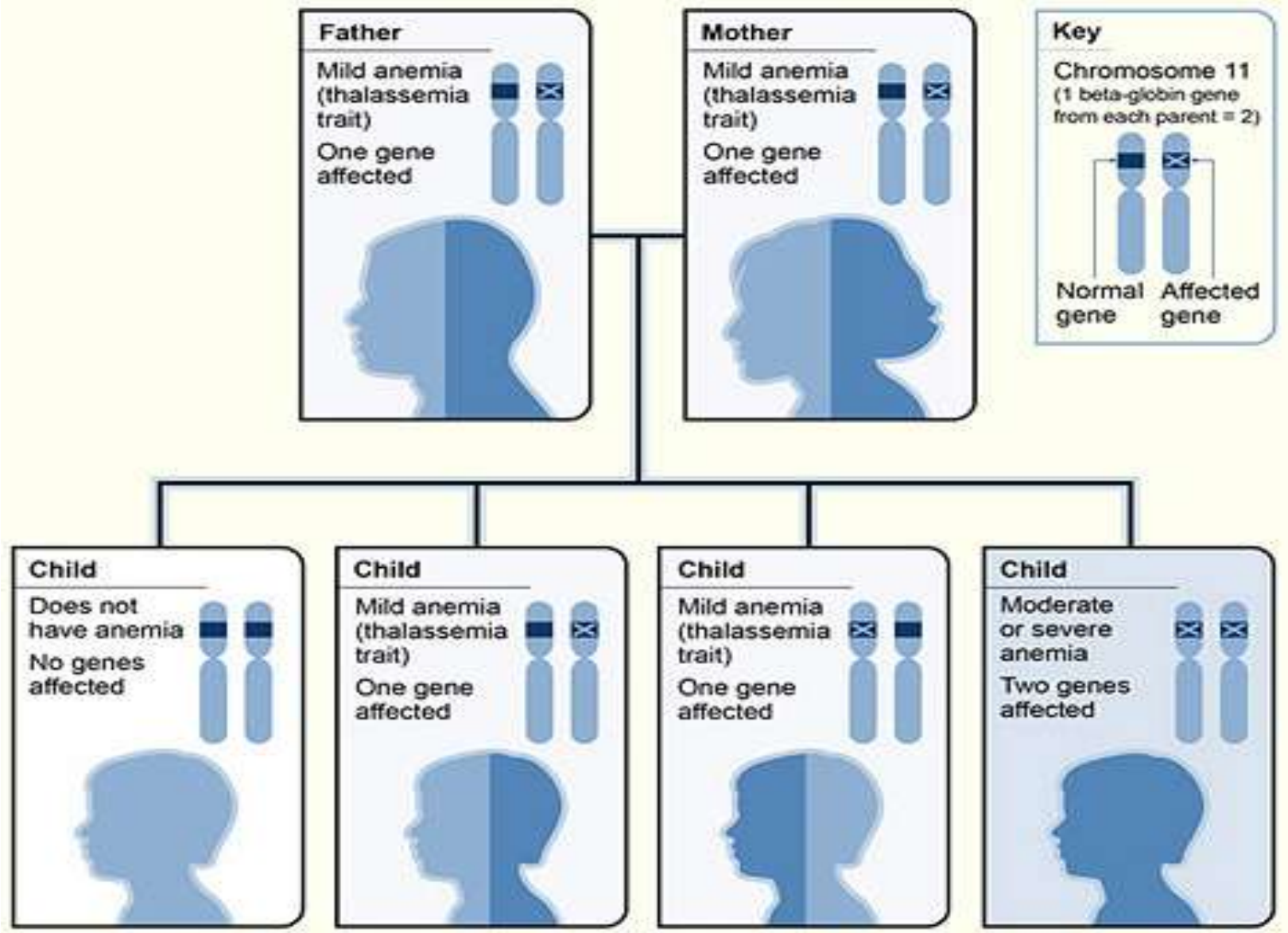
With two mutations no β-globin

β-thalassemia major patient with severe aneamia

Genetics of Thalassemia

Common genotypes	Name	Phenotype
β/β	Normal	None
β/β^0 β/β^+	Beta thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic hypochromic anemia
β^+/β^+ β^+/β^0 β^E/β^+ β^E/β^0	Beta thalassemia intermedia	Variable severity Mild to moderate anemia Possible extramedullary hematopoiesis Iron overload
β^0/β^0	Beta thalassemia major (Cooley's Anemia)	Severe anemia Transfusion dependence Extramedullary hematopoiesis Iron overload

Inheritance Pattern for Thalassemia

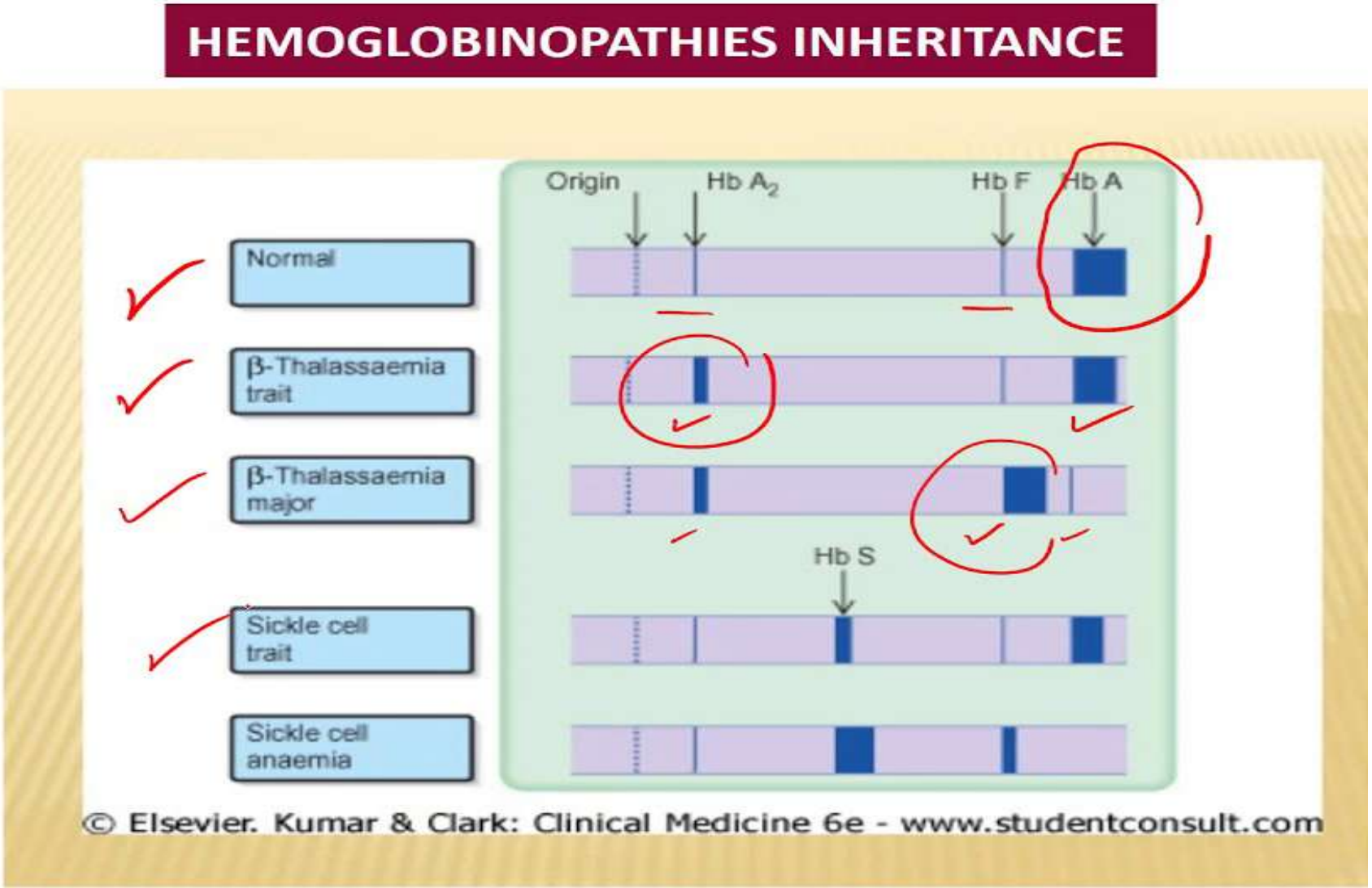


Diagnostics: Thalassemia

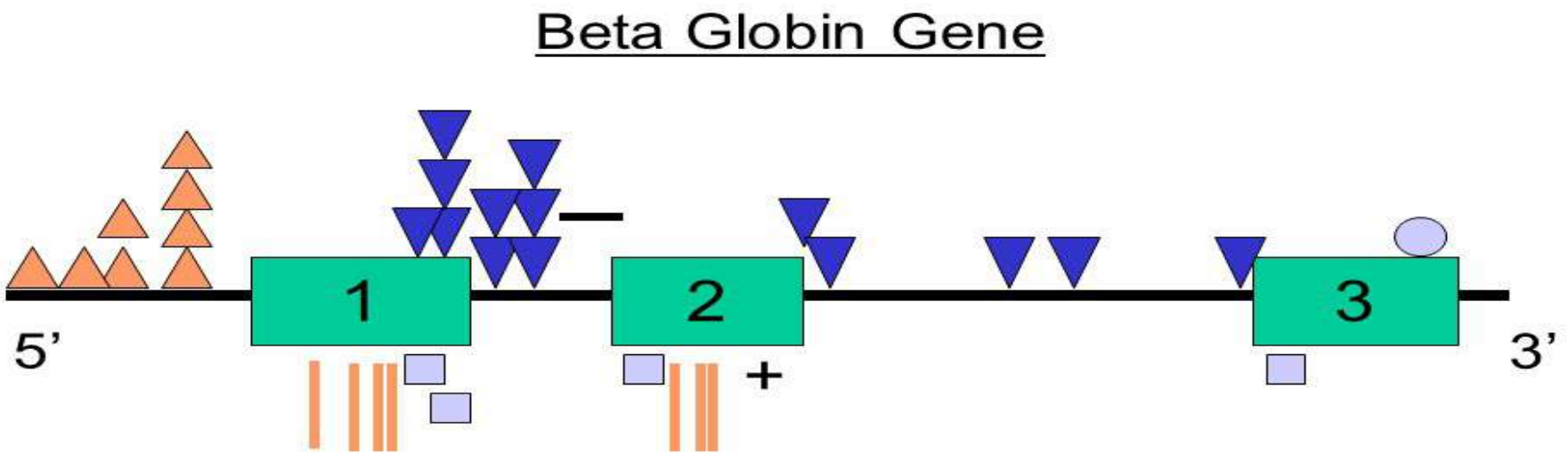
- Diagnostic assessment
  - Hgb electrophoresis
  - Prenatal diagnosis

RBCs	Normal /Low
Hgb	Low
MCV	Low
MCH	Low
MCHC	Normal /Low
Serum iron	High

Diagnostics: Thalassemia



HBB gene - Mutations



Transcription

Nonsense

Splicing

\+ Insertion

Frameshift deletion

Deletion

Poly A site

Beta Thalassemia Mutations

Table 1. Position and SNP ID of the mutations observed at the nucleotide level, the respective consequences at the amino acid level, the types of mutations, and the number of individuals.

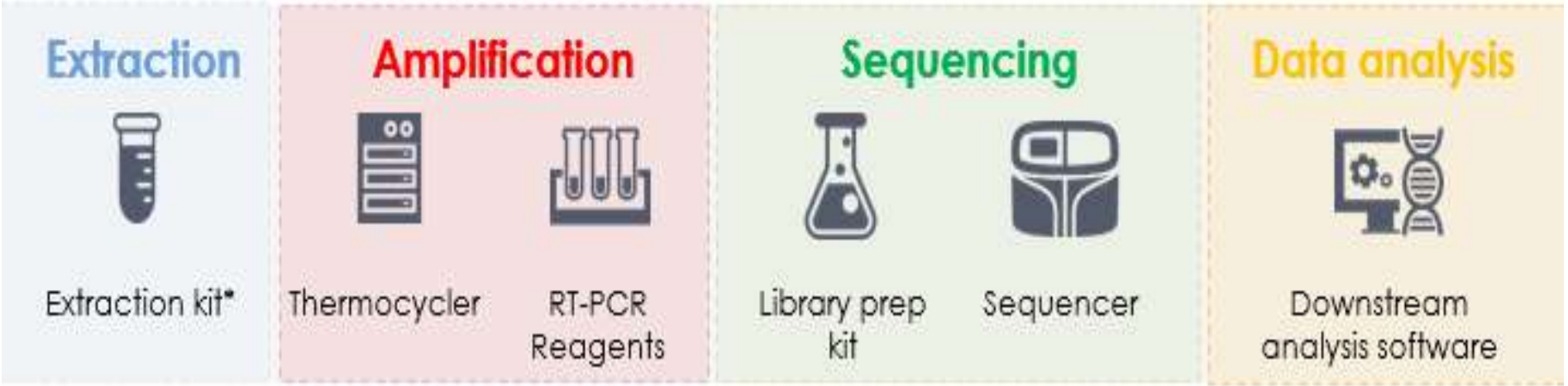
Position	SNP ID	Nucleotide change	AA alteration	Type of mutation	N° Individuals	Ref.
5246840	rs36020563	G/A	His144His	Synonymous	1	[21]
5246870	rs113082294	C/G	Val134Val	Synonymous	9	[22]
5246883	rs111645889	G/A	Ala130Val	Missense	1	[23]
5246890	rs33971634	G/A	Gln128	Stop gained	1	[24]
5246908	rs33946267	C/G	Glu122Gln	Missense	3	[25]
5246947	rs33958637	T/G	Asn109His	Missense	1	[26]
5246948	rs193922562	G/A	Gly108Gly	Synonymous	1	[27]
5247876	rs145669504	G/T	Leu82Leu	Synonymous	5	[28]
5247992–5247996	rs281864900	CAAAG/C	Phe42fs	Frameshift	5	[29]
5248004	rs11549407	G/A	Gln40	Stop gained	1	[30]
5248029	rs1135071	C/A	Arg31Ser	Splice region and missense	1	[31]
5248030	rs33943001	C/G	#	Splice acceptor and intron variant	1	[32]
5248159	rs33971440	C/T	#	Splice donor and intron variant	1	[33]
5248162	rs35578002	G/T	Glu30Gly	Splice region and synonymous variant	1	[34]
5248173	rs33950507	C/T	Glu27Lys	Missense	14	[35]
5248200	rs33986703	T/A	Lys18	Stop gained	6	[36]
5248205	rs63750783	C/T	Trp16	Stop gained	2	[37]
5248232	rs334	T/A	Glu7Val	Missense	137	[38]
5248233	rs33930165	C/T	Glu7Lys	Missense	17	[39]
5248236	rs33912272	G/A	Pro6Ser	Missense	1	[40]

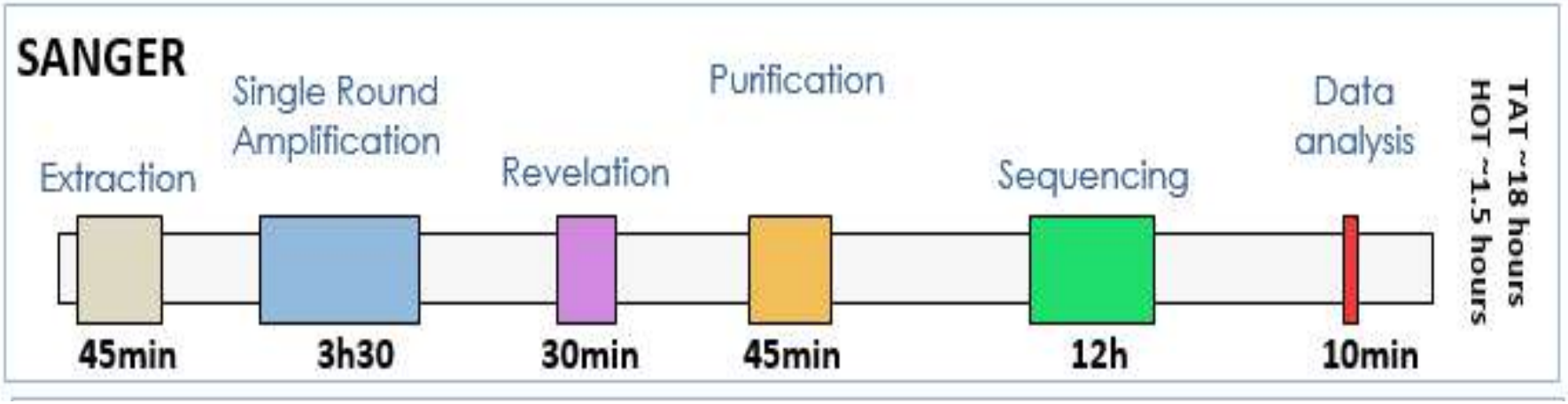
#—Intronic variant mutations

https://doi.org/10.1371/journal.pone.0174637.t001

Carlice-dos-Reis T, Viana J, Moreira FC, Cardoso GdL, Guerreiro J, et al. (2017) Investigation of mutations in the HBB gene using the 1,000 genomes database. PLOS ONE 12(4): e0174637. https://doi.org/10.1371/journal.pone.0174637
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0174637

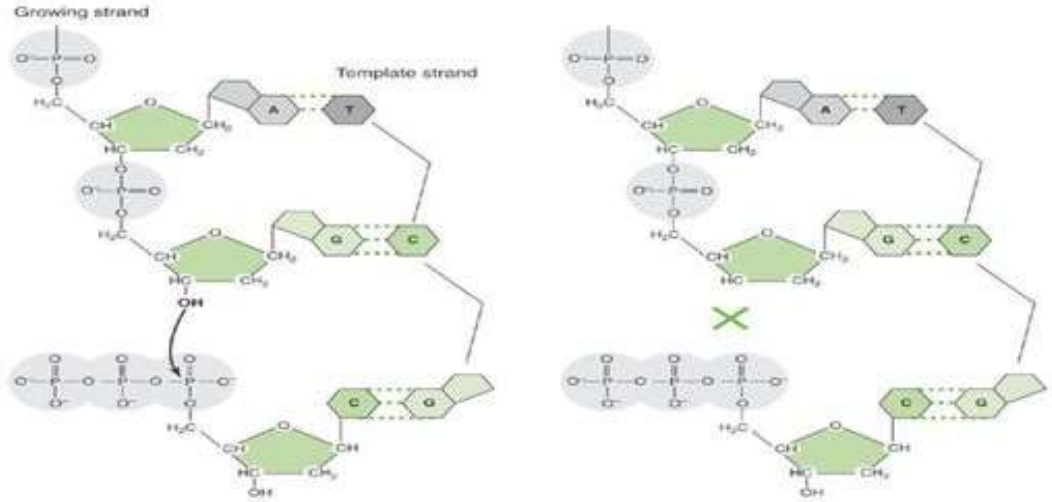
HBB gene - Sequencing





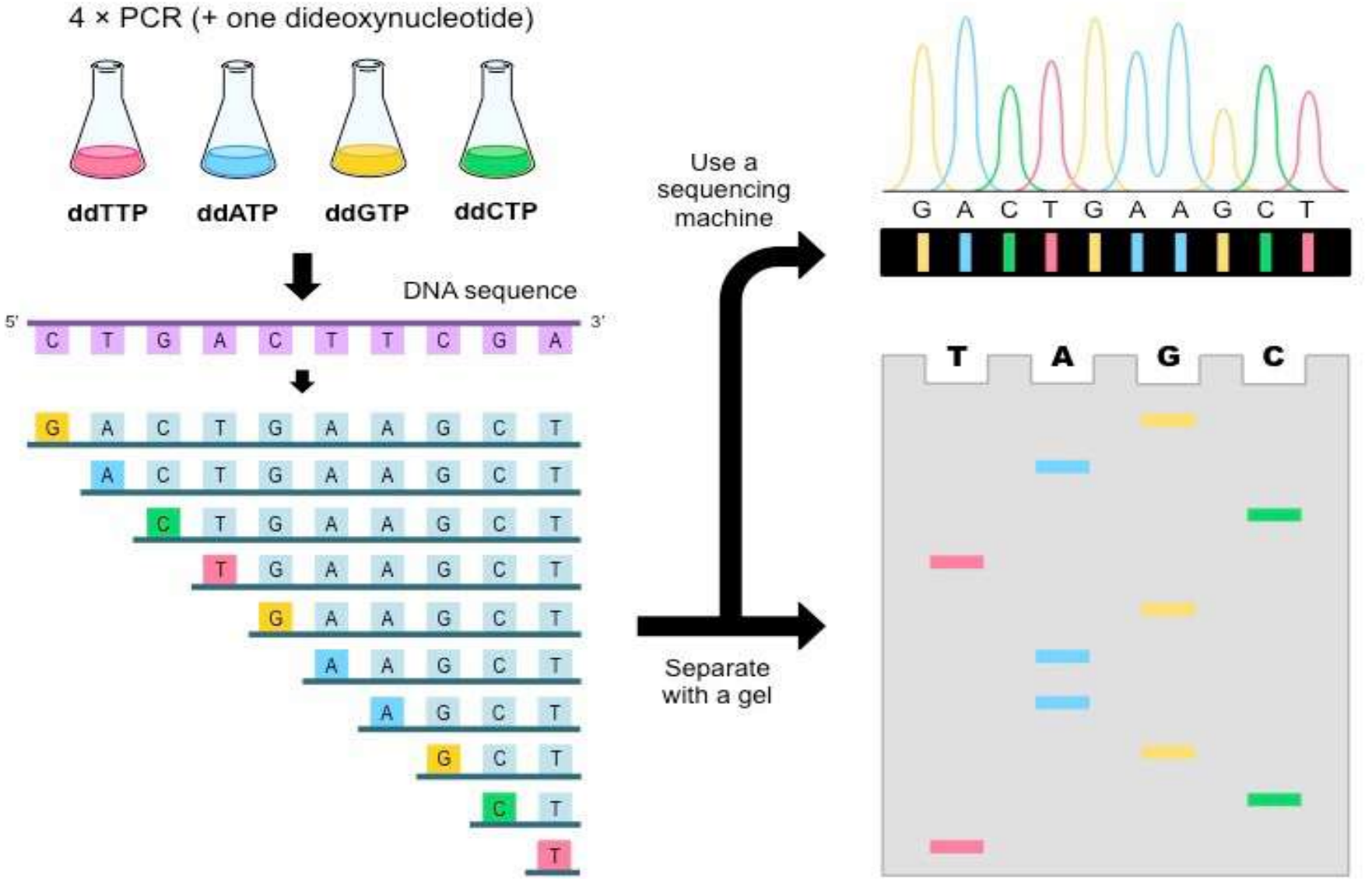
HBB gene - Sequencing

The 3′-OH group necessary for formation of the phosphodiester bond is missing in ddNTPs.

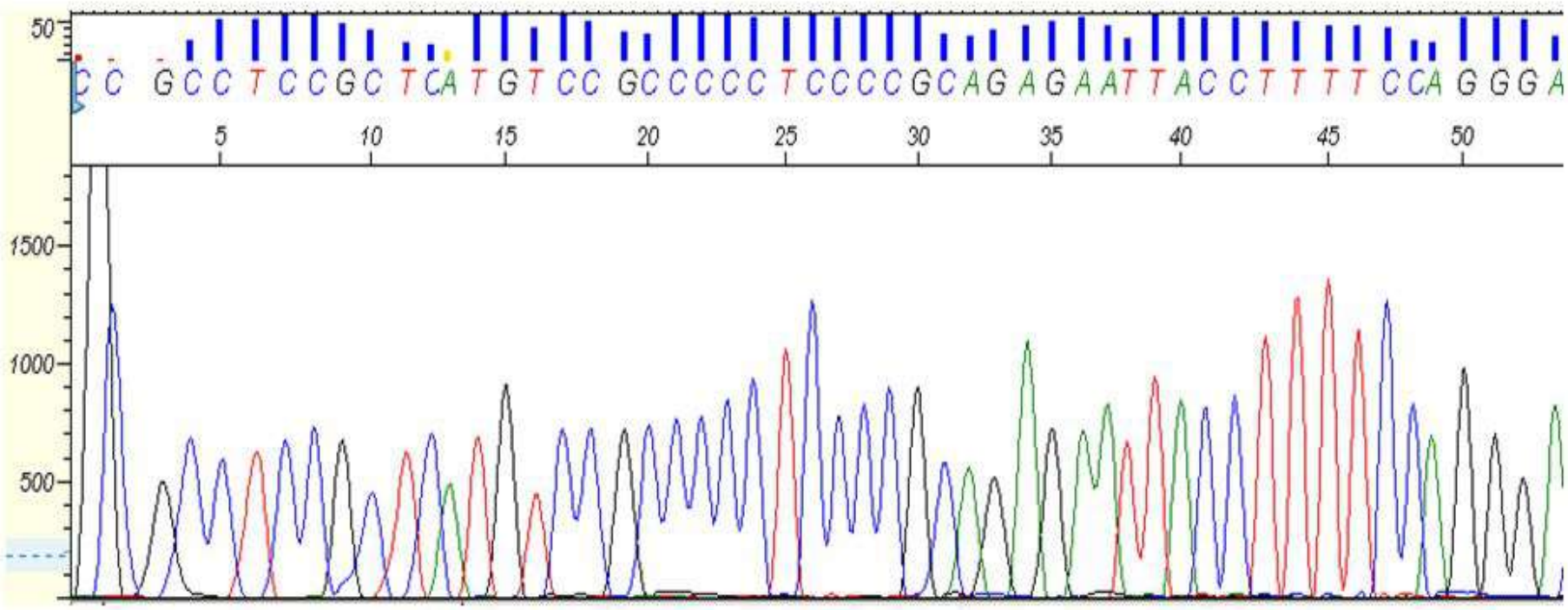


Chain terminates at ddG

HBB gene - Sequencing

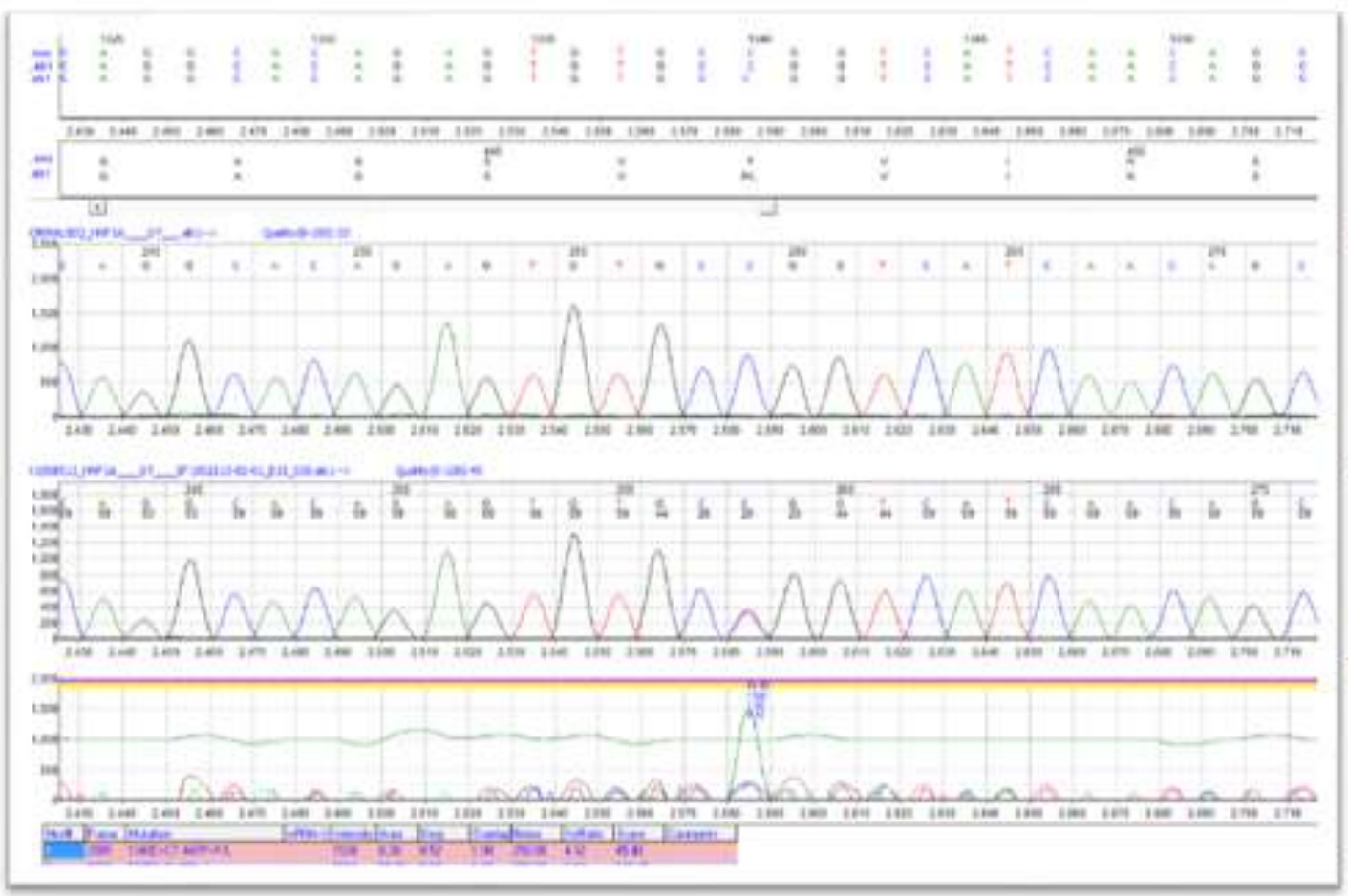


HBB gene - Sequencing



BigDye Direct sequencing and POP-7 polymer

HBB gene - Sequencing



Nucleotide and protein sequence information

'Normal' reference sequence

Patient's sequence

Difference between normal and patient sequences

HBB gene - Sequencing

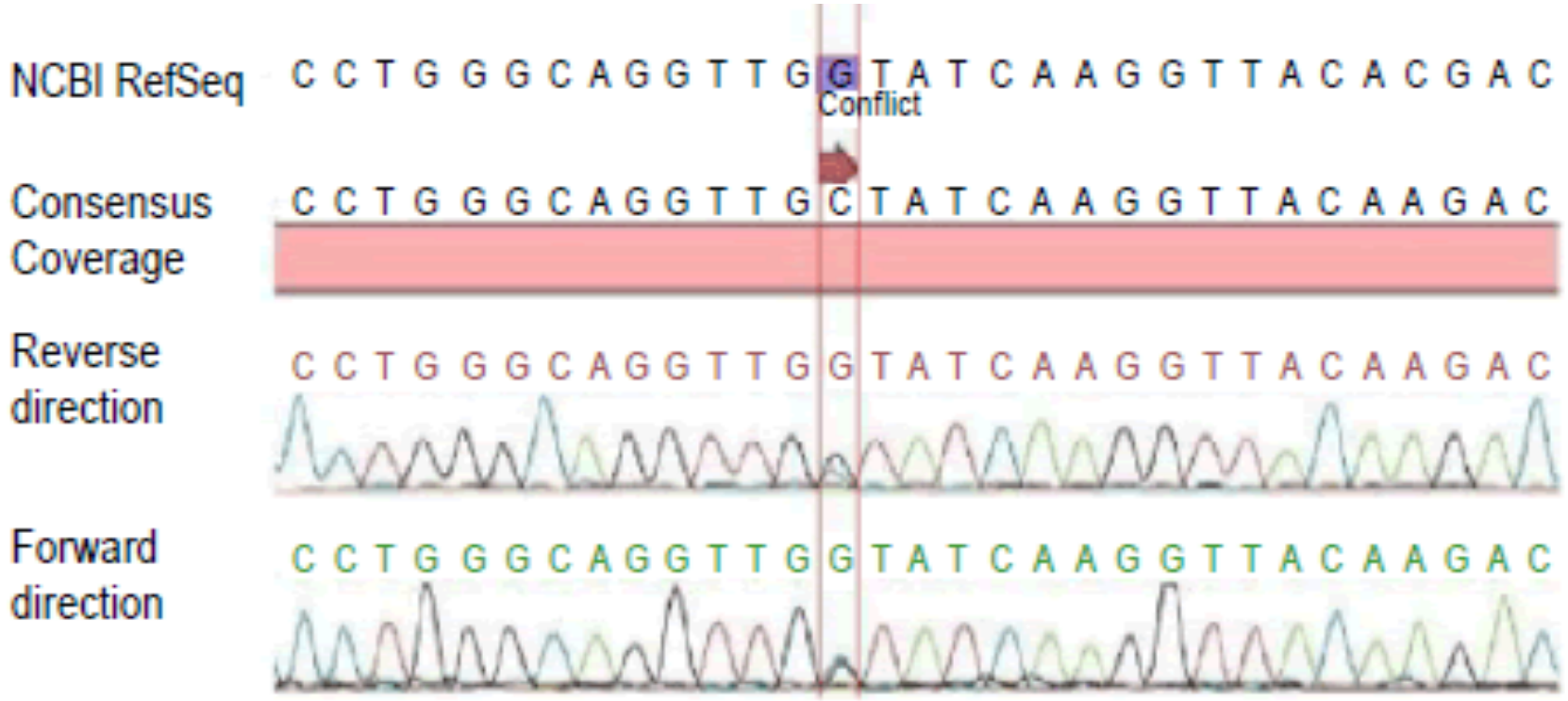


Figure 3: Electropherogram of bidirectional sequencing analysis demonstrated heterozygous mutation [c.92+5G>C (IVS I-5 G>C)] for β-thalassemia in Fetal DNA (amniotic fluid) sample.

A DISEASE MAY BE RARE
BUT HOPE SHOULD NOT BE

Thank You