ÇUKUROVA'DA HEMOGLOBİNOPATİLERİN MOLEKÜLER TANISI

Ahmet GENÇ

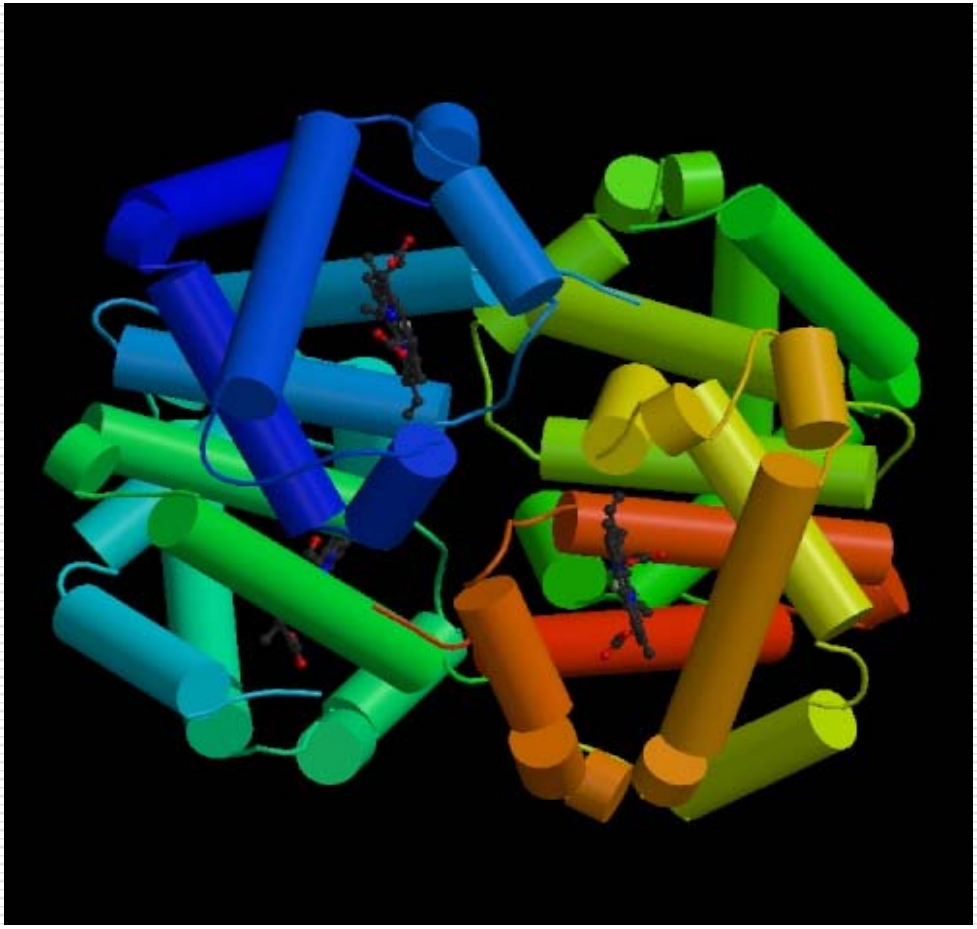
Çukurova Üniversitesi Tıp Fakültesi
Biyokimya Anabilim Dalı
Doktora Öğrencisi
ahmetgenc_@hotmail.com

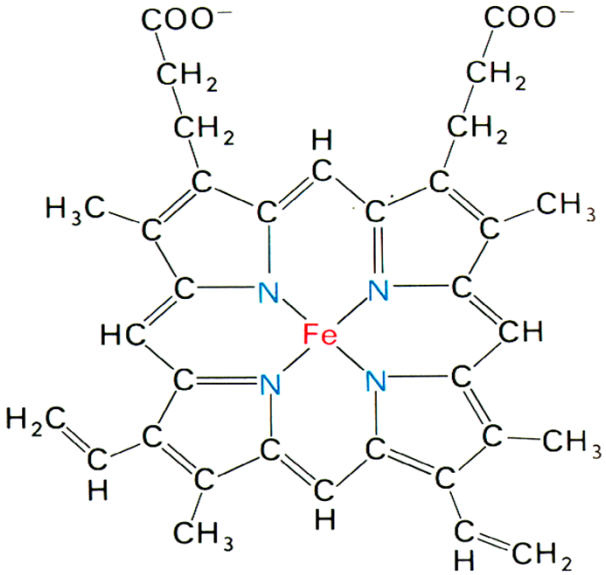
Bu tez çalışması, Çukurova Üniversitesi Bilimsel Araştırma Projeleri Birimi tarafından TF2005YL2 nolu proje ile desteklenmiştir.

SUNU AKIŞI

- Hemoglobinin yapısı
- Hemoglobinopatiler
- Anormal Hemoglobinler
- Talasemi
- Tarama ve Tanı Yöntemleri
- Moleküler Yöntemler (ARMS, RFLP, DNA Dizi Analizi)

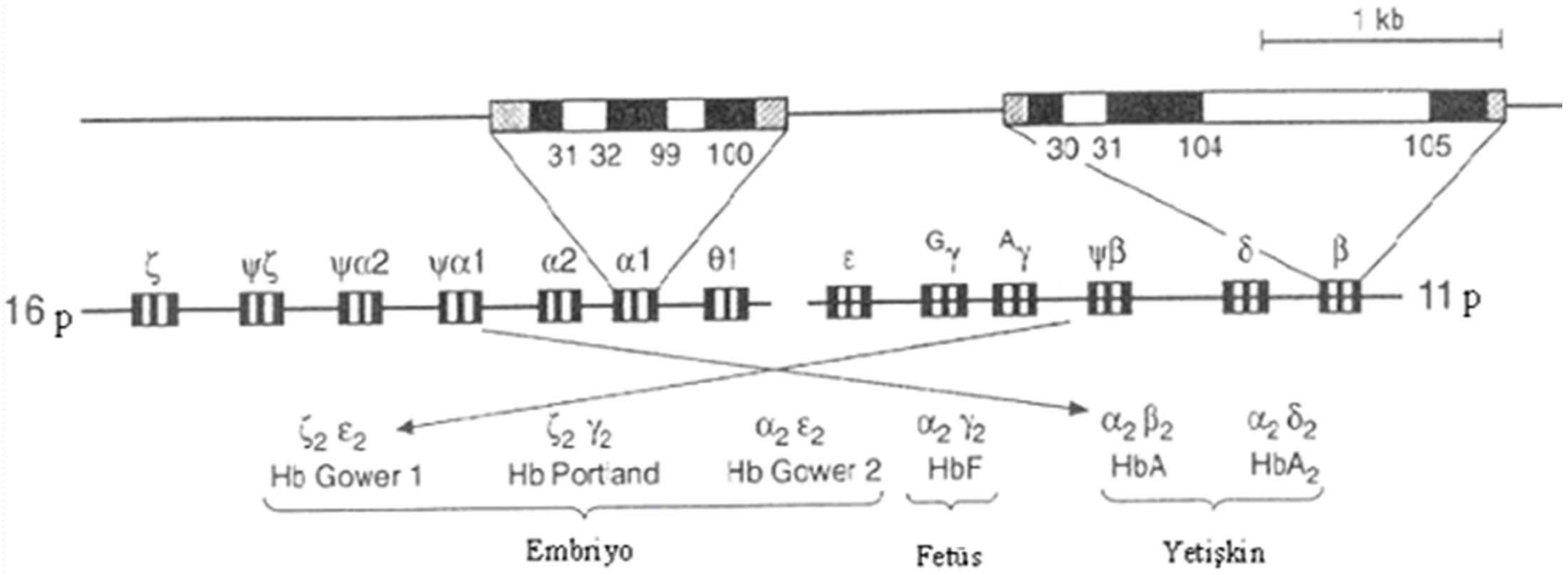
Hemoglobin molekülünün yapısı



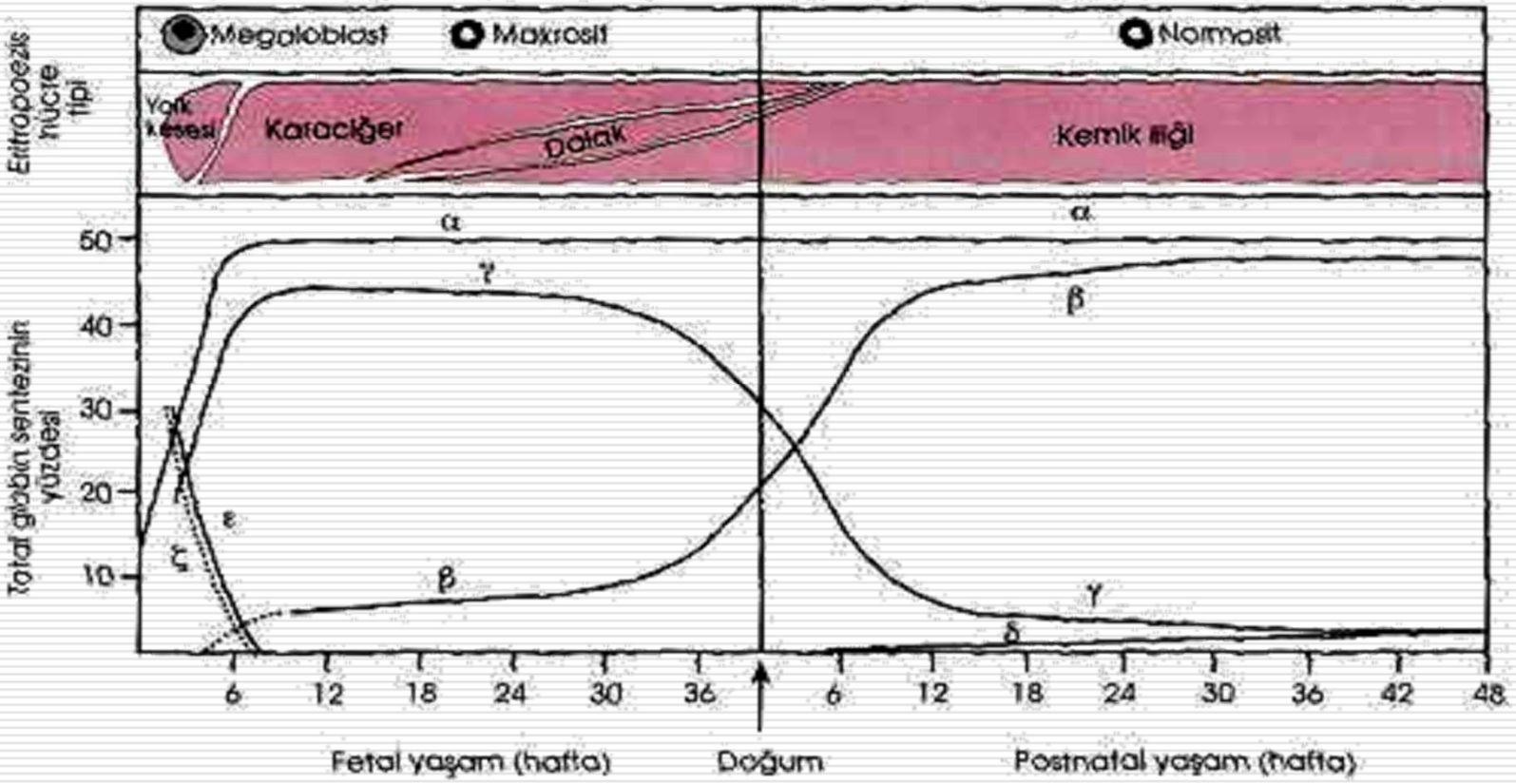


Heme
(Fe–protoporphyrin IX)

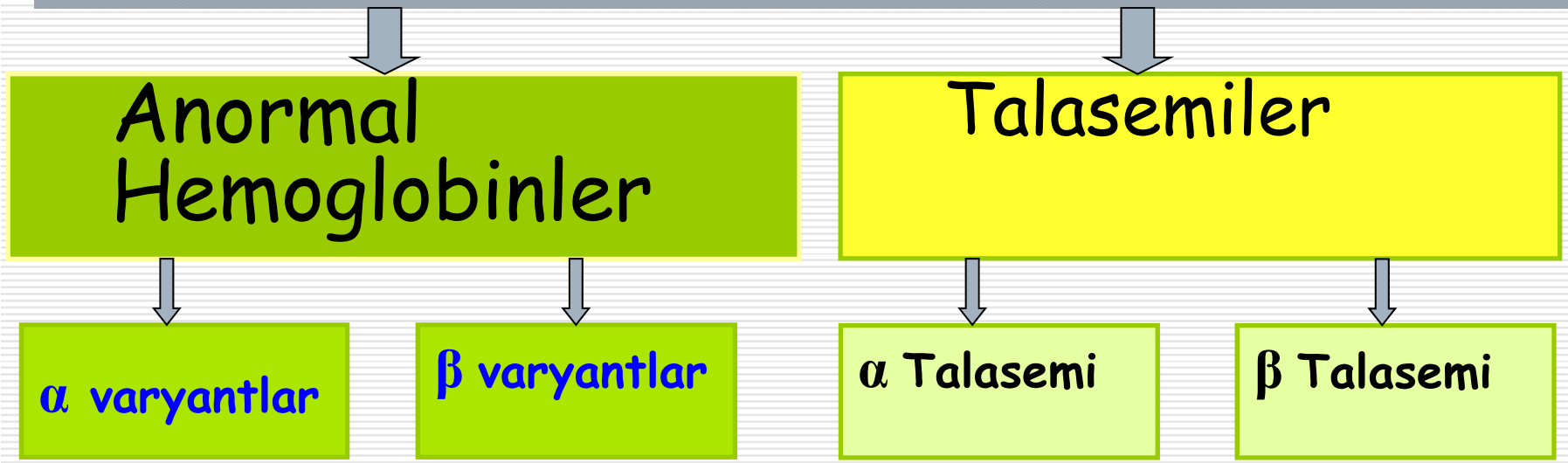
Alfa ve beta benzer globin genleri ve sentezinden sorumlu olduğu hemoglobin türleri



Embriyonik ve fetal gelişimin farklı evrelerinde globin sentezi



HEMOGLOBİNOPATİLER



ANORMAL HEMOGLOBİNLER (978*)

- HbS: *β6 (GAG→GTG); Glu→Val*
  - Eritrositler oksijen azlığında sonucunda polimerleşerek eritrositlerin orak şeklini almasına (sickling) neden olmaktadır

- HbE: Hb E *β26(GAG→AAG); Glu→Lys*
  - β^E-globin mRNA'nın normal splicinginde azalma görülmektedir
  - Mikrositoz ve hipokromi ile hafif derecede anemi meydana gelmektedir
  - HbE homozigotları, asemptomatiktirler ve sadece hafif anemik olmalarına rağmen, HbE mutasyonu farklı β-talasemi alleleriyle birlikte anormal fenotip oluşturmaktadır.

* http://globin.cse.psu.edu/

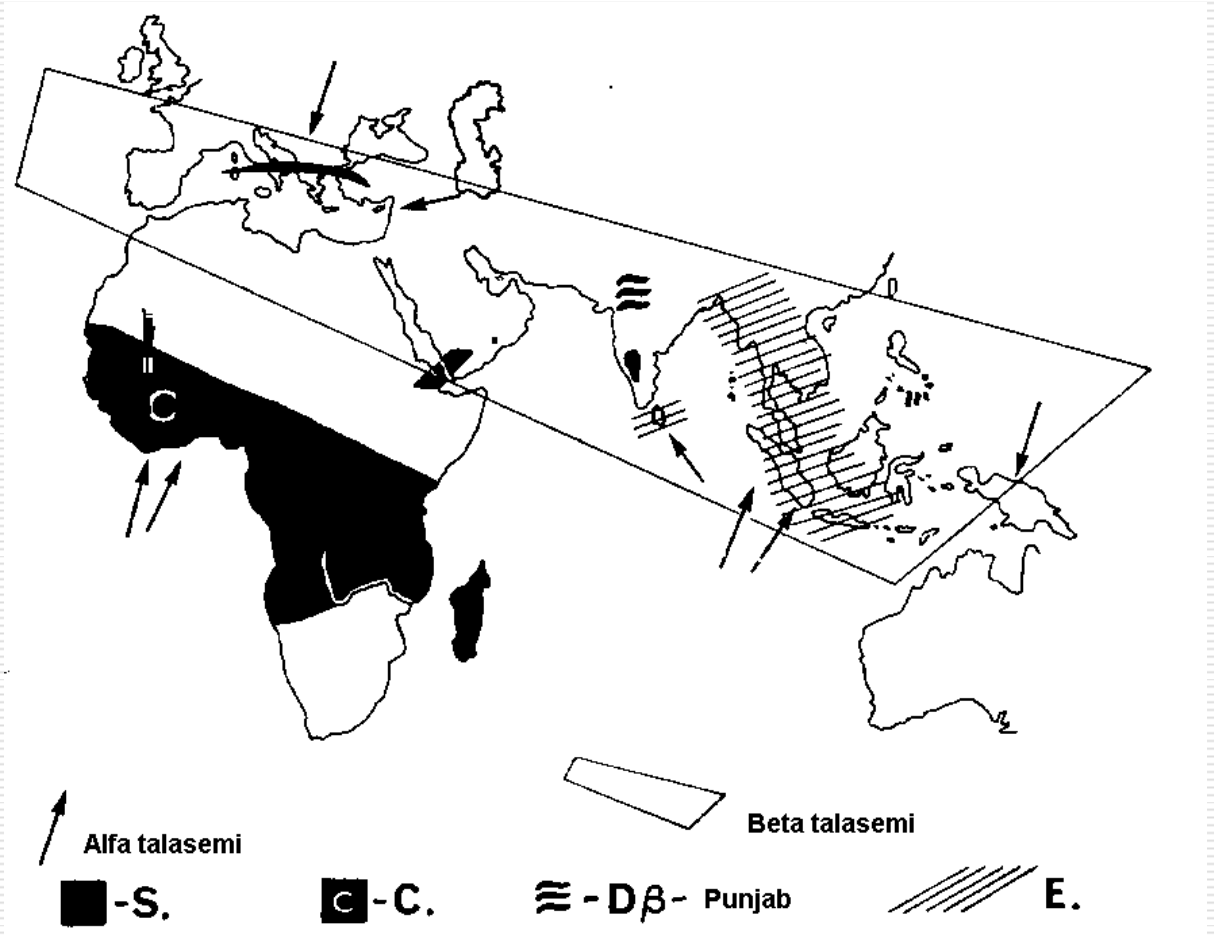
ANORMAL HEMOGLOBİNLER

- HbC: *β6(GAG→AAG) ;Glu→Lys*
  - HbA'dan daha az çözünür; kılcal damarlarda deformasyonu azaltıp hafif hemolitik anemiye yol açarak eritrositlerde kristalleşme eğilimi gösterir
  - Homozigot C ise, splenomegali ile hafif hemolitik anemi ile karakterizedir

- HbD: *β121 (GAG→AAG);Glu→Gln*
  - Taşıyıcılarında klinik, hematolojik yada fizyolojik olarak bir anormalik gözlenmez
  - Homozigot olgularda orta derecede hemolitik anemi tespit edilmektedir

Hb Adı	Mutasyon	Bulunduğu yöre	Yazarlar
Hb Adana*	Alfa 2, 59Gly→Asp	Adana	Çürük ve ark.
Hb Ankara*	Beta 10 Ala→Asp	Kastamonu	Arcasoy ve ark.
Hb J Antakya*	Beta 65 Lys→Met	Antakya	Huisman ve ark.
Hb Beograd	Beta 121 Glu→Val	Göçmen	Arcasoy ve ark.
Hb Brockton	Beta 138 Ala→Pro	İstanbul	Ulukutlu ve ark.
Hb C	Beta 6 Glu→Lys	İzmir, Adana	Göksel/Kılınç/Özsoylu
Hb D-Los Angeles	Beta 121 Glu→Gln	Birden fazla bölgede	Dinçer/Çavdar/Özsoylu
Hb E	Beta 26 Glu→Lys	Birden fazla bölgede	Aksoy/Arcasoy/Altay
Hb E Saskaton	Beta 22 Glu→Lys	Kayseri	Prozorova/Gürgey
Hb F-Başkent*	Gama 128 Ala→Thr	Ankara	Altay ve ark.
Hb G-Coushatta	Beta 22 Glu→Ala	Kastamonu, Denizli	Dinçol/Sözmen
Hb Hakkari*	Beta 31 Leu→Arg	Hakkari	Gürgey ve ark.
Hb Hamadan	Beta 56 Gly→Arg	Batı Trakya	Dinçol ve ark
Hb City of Hope	Beta 69 Gly→Ser		Kutlar ve ark.
Hb İstanbul*	Beta 92 His→Gln	Göçmen	Aksoy ve ark.
Hb J-İran	Beta 77 His→Asp	Ankara	Arcasoy ve ark.
Hb Knossos	Beta 27 Ala→Ser		Kutlar ve ark.
Hb Köln	Beta 95 Val→Met	Malaya	Gürgey ve ark.
Hb Lepore-Boston	Fusion Hb	Diyarbakır, K. Kıbrıs	Çavdar ve ark.
Hb M-İwate	Alfa 87 His→Try	Bursa	Özsoylu ve ark.
Hb Moabit	Alfa 86 Leu→Arg	Denizli	Knuth ve ark.
Hb N-Baltimore	Beta 95 Lys→Glu	Antalya	Bircan ve ark.
Hb O-Arab	Beta 121 Glu→Lys	Kütahya,Kıbrıs,Trakya	Altay/Cin/Aksoy
Hb O-Padova	Alfa 30 Glu→Lys	Adana	Kılınç ve ark.
Hb Q-İran	Alfa 75 Asp→His	Adana, Batı Trakya	Aksoy ve ark
Hb P-Nilotic	Alfa 2 (Beta-Delta)2	Orta Anadolu	Altay ve ark.
Hb Ube-2	Alfa 68 Asn→Asp	Kayseri	Bilgin ve ark.
Hb Summer Hill	Beta 52 Asp→His	Kuzey Kıbrıs	Cin ve ark.
Hb J-Anatolia	Alfa 61 Lys→Thr		Giardano ve ark.
Hb Strumia	Alfa 112 His→Arg	Bursa	Akar ve ark.
Hb Çapa*	Alfa 94 Asp→Gly	Kars	Dinçol ve ark.
Hb J-Meerut	Alfa 120 Ala→Glu		Yalçın ve ark.
Hb J-Paris I	Alfa 12 Ala→Asp	Çukurova	Dikmen ve ark.

DÜNYADA YAYGIN OLARAK GÖRÜLEN ANORMAL HEMOGLOBİNLER ve TALASEMİLER.



Talasemiler

Alfa talasemiler

- Delesyonel α talasemi mutasyonları >40
- Nondelesyonel α talasemi mutasyonları >25

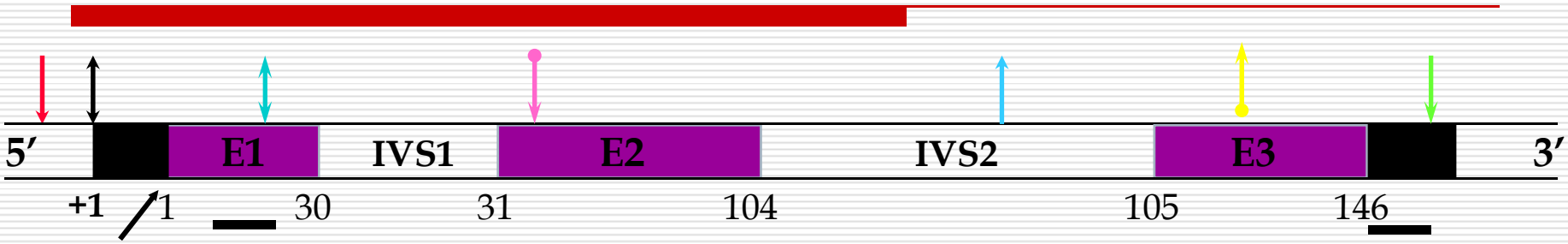
Beta talasemiler

- Nokta mutasyonları >200
- Küçük delesyonlar ~13

β-Talasemiler

- **İki fenotip:**
  - **β^0-talasemi: β-globin zincir sentezi olmamakta**
    - Ekzon/intron birleşme yerindeki mutasyonlar & büyük delesyonlar
  - **β^+-talasemi: Az β-globin zincir sentezi**
    - İntron ve regülatör bölgelerindeki mutasyonlar

β-Globin Gen Mutasyonları: >200



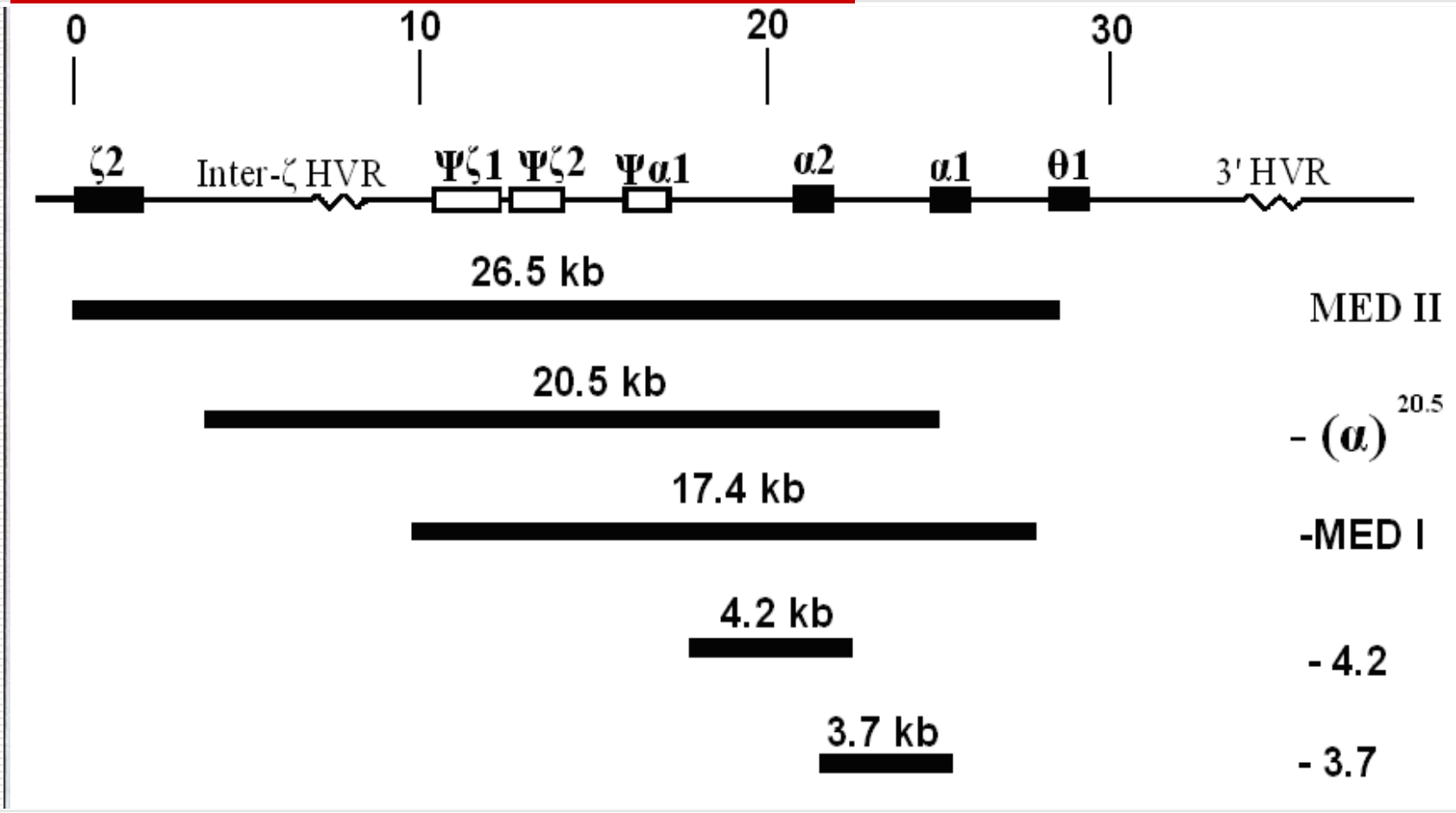
- Transkripsiyon
- RNA Processing
- Cap Site +1 (A→C)
- RNA Cleavage
- Başlangıç kodonu ATG→ AGG, ACG, GTG
- Frameshift
- Nonsense Kodon
- Unstable Globin
- Küçük Delesyon

Ülkemizde β-Talasemi Mutasyonları ve Görülme Sıklığı

Mutasyon	Sıklığı	Tipi	Mutasyon	Sıklığı	Tipi
IVSI-110 (G→A)	39.4	β^+	IVSII-848 (C→A)	0.4	β^+
IVSI-6 (T→C)	10.1	β^+	IVSI-116 (T→G)	0.2	β^o
FSC8 (-AA)	5.5	β^o	-101 (C→T)	0.1	β^+
IVSI-1 (G→A)	5.0	β^o	CD27 (G→T)	0.1	β^+
IVSII-745	5.0	β^+	-28 (A→C)	0.1	β^+
IVSII-1 (G→A)	4.7	β^o	IVSI-130 (G→A)	0.1	β^o
CD39 (C→T)	3.8	β^o	FSC36/37 (-T)	0.1	β^o
-30 (T→A)	3.1	β^+	290 bp deletion	0.1	β^o
FSC5 (-CT)	2.1	β^o	IVSII-654 (C→T)	0.1	β^+
FSC8/9 (+G)	1.3	β^o	Cd15(TGG→TAG)	0.1	β^o
FSC44 (-C)	1.3	β^o	Cd15(TGG→TGA)	0.1	β^o
IVSI-5 (G→C)	1.1	β^+	FSC74/75 (-C)	0.1	β^o
-87 (C-G)	0.8	β^+	FSC22-24 (-7 bp)	0.1	β^o
Poly A (TAA→TGA)	0.5	β^+	3′-UTR (-13 bp)	0.1	β^+
FSC6 (-A)	0.4	β^o	Unknown	9.0	

En yaygın 6 mutasyon %69.7 sıklıkta, 12 mutasyonun görülme sıklığı ise %82.4

Delesyonel Alfa Talasemiler



TARAMA VE TANI YÖTEMLERİ NELER OLMALI ?

Kan sayımı

- RBC
- Hb
- Hct
- MCV
- MCH
- MCHC

Hematolojik Değerler	Normal Sınırlar
RBC 10^{12}/L	4,10-5,10
Hb g/dL	12,3-15,3
Hct %	36,0-45,0
MCV fL	80,0-96,1
MCH pg	27,5-33,2
MCHC g/dL	33,4-35,2
Hb Elektroforezi	HbAA
Hb A_2 %	2,5-3,5
Hb F %	<1,0

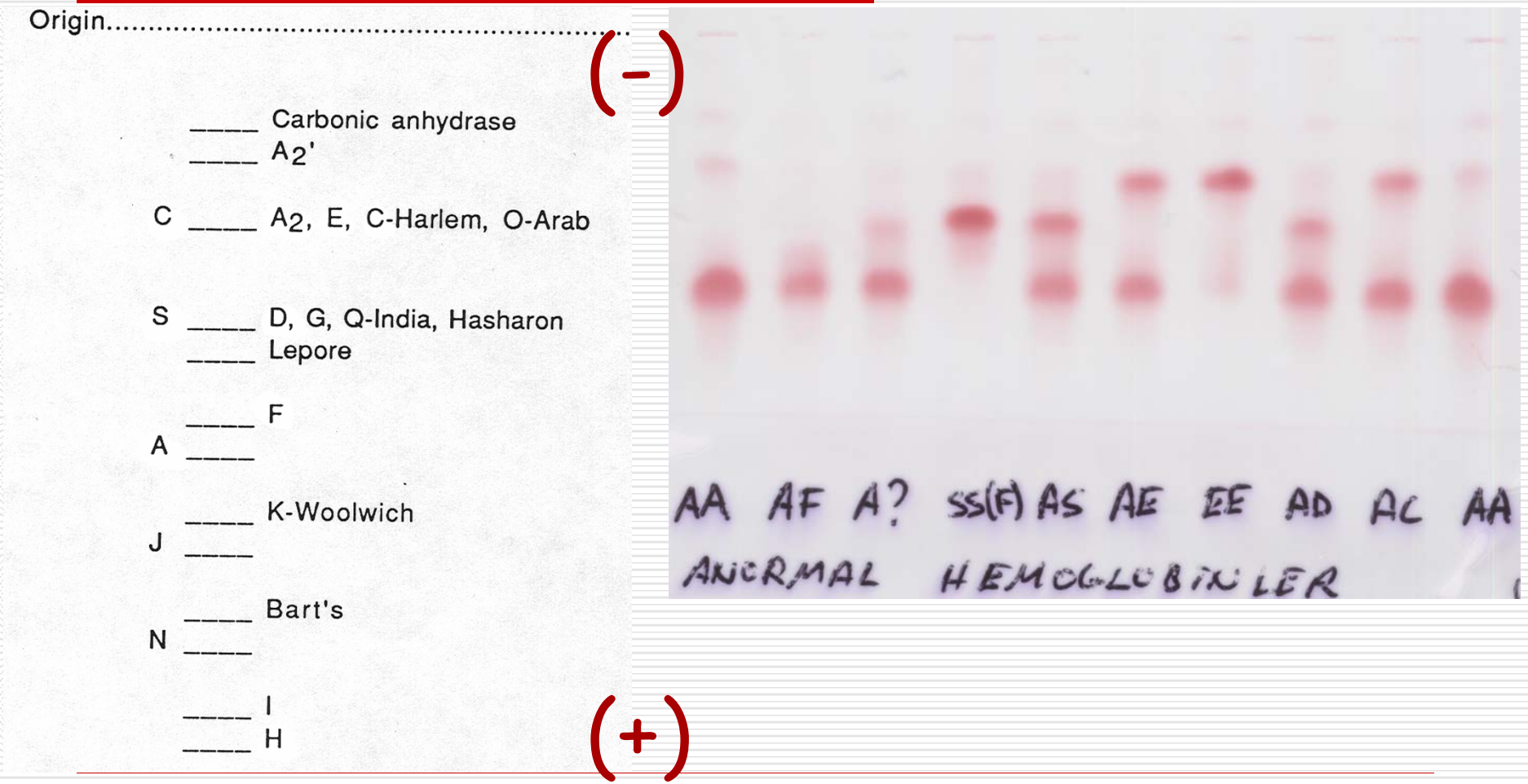
Hb tiplendirmesi

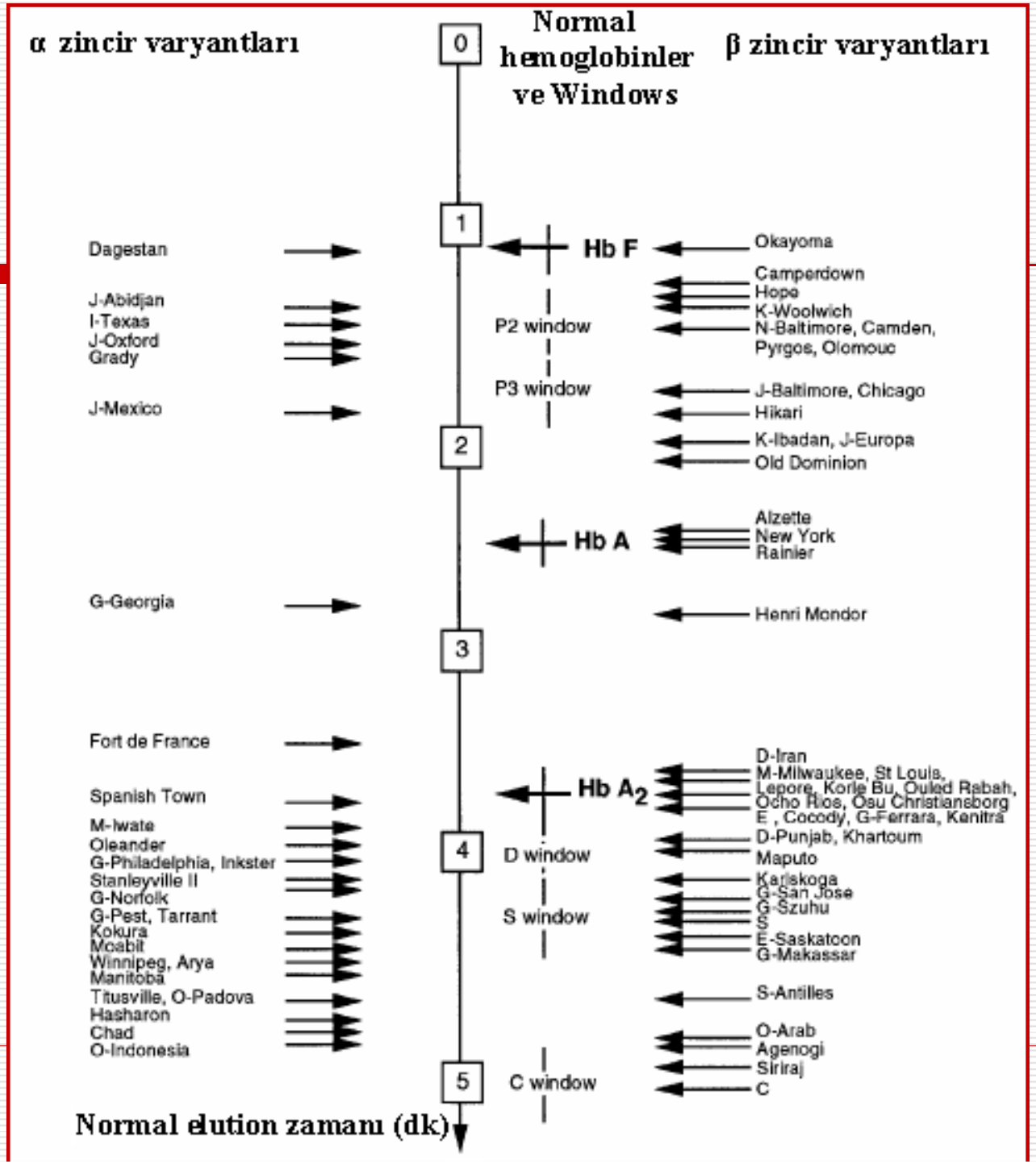
- Elektroforez
- HPLC
- IEF
- Oraklaşma testi
- HbA2 (%2,5-3,7)
  - > %3,7 β talasemi
  - > %2,5 α talasemi
- HbF

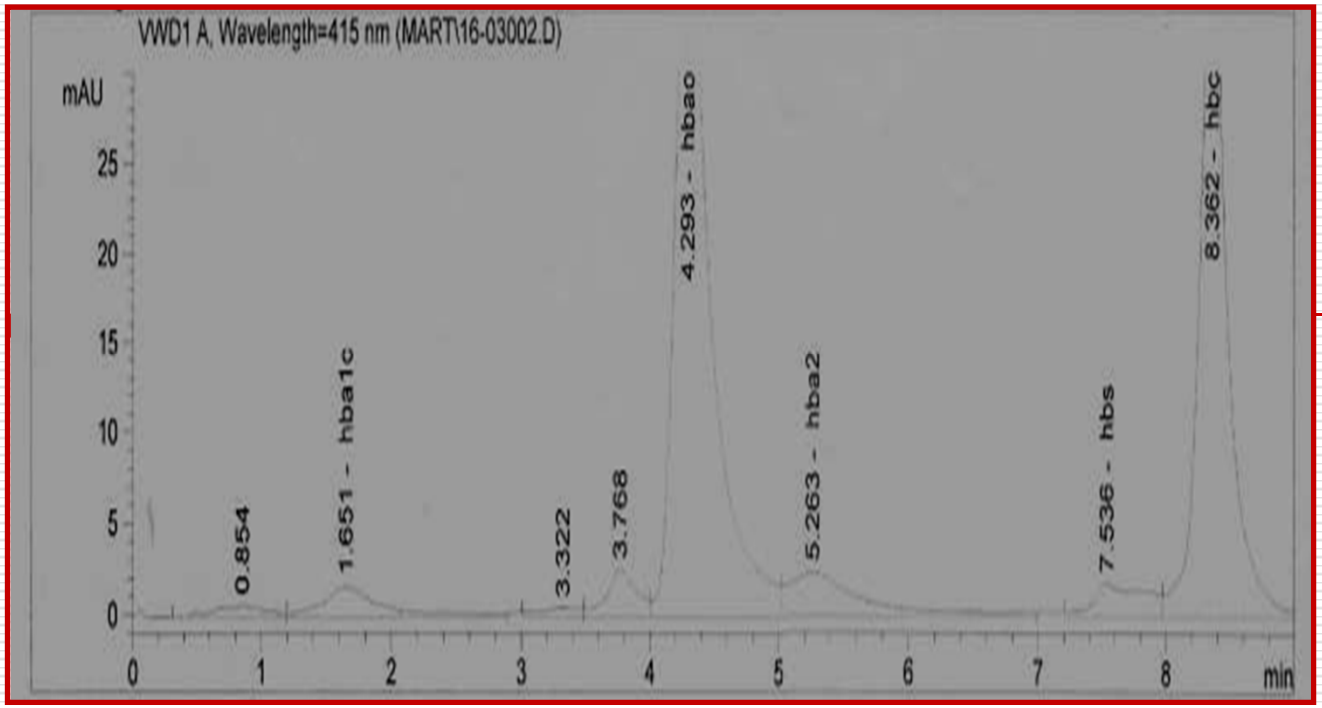
Hemoglobin F (Hb F)

- Bazı β-talasemi taşıyıcılarında miktarı artar > % 1
- Bazı orak hücre anemililerde miktarı artar > % 1
- HPFH'li vakalarda seviyesi artar
  (Herediter Persistance Fetal Hemoglobin)

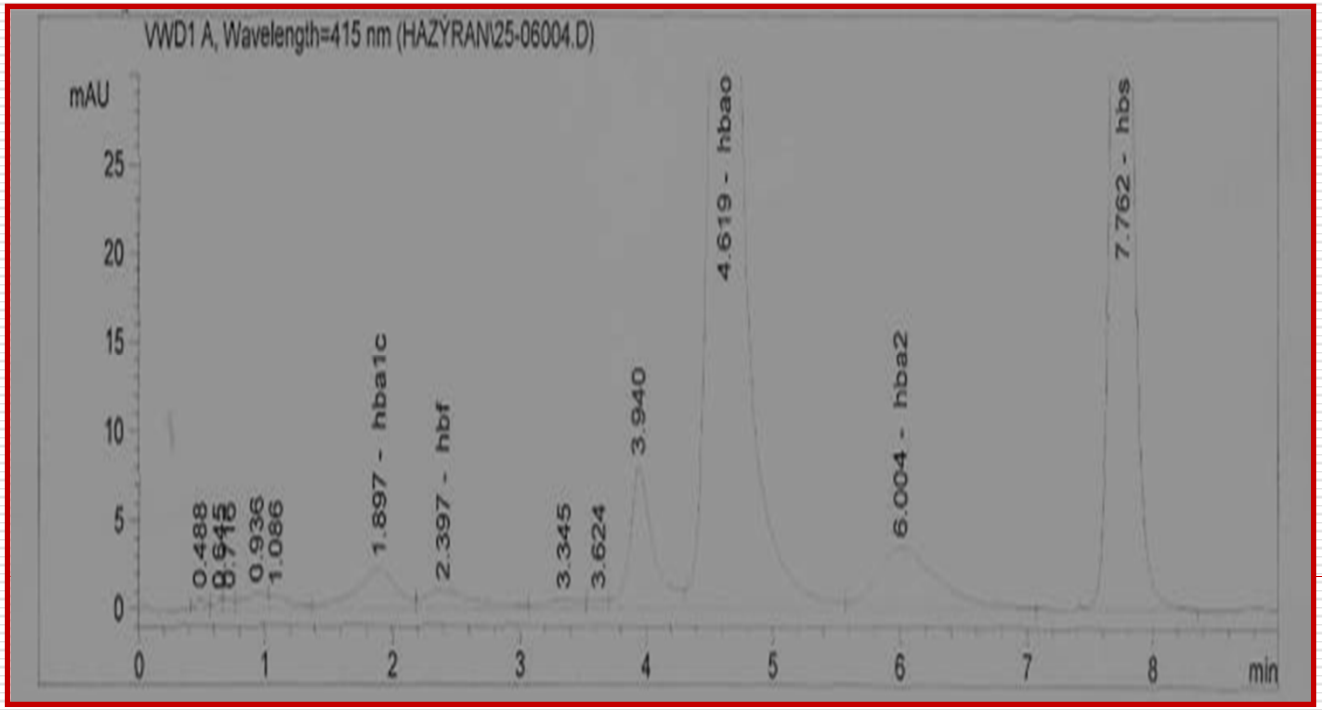
Hb Elektroforezi



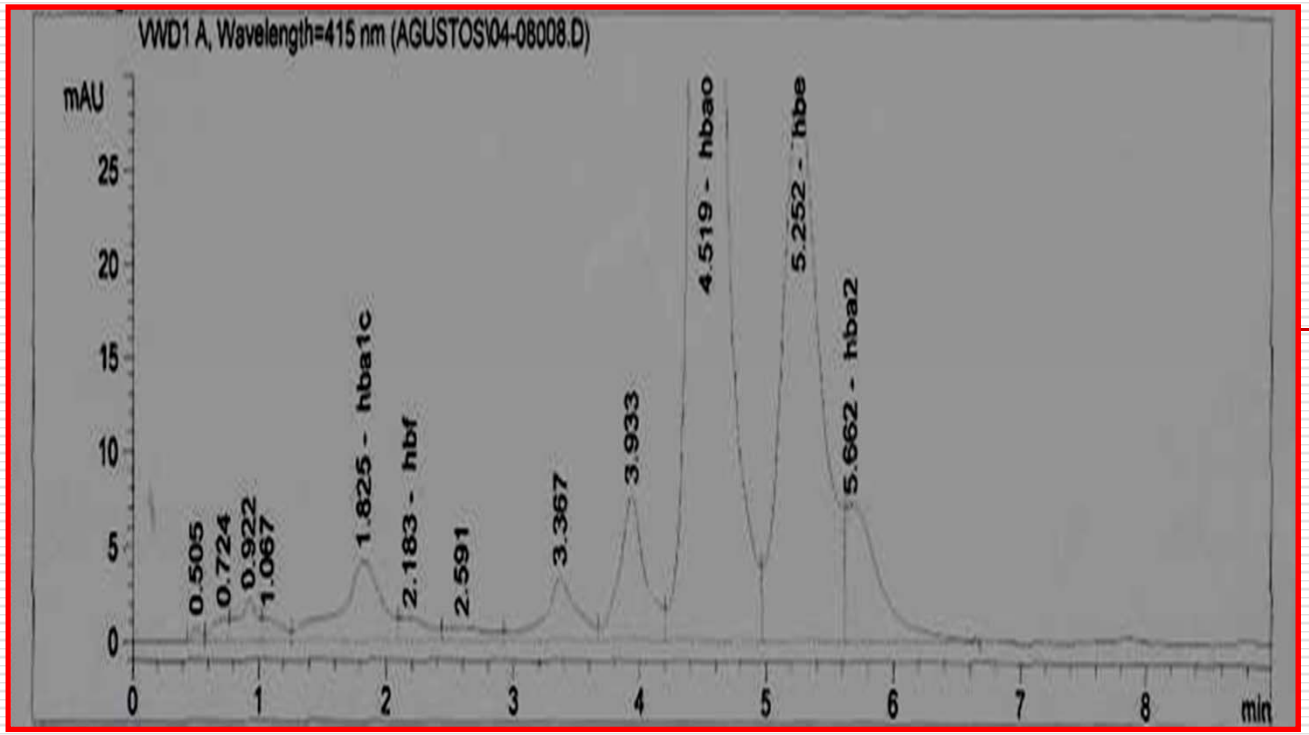

α zincir varyantları
Normal hemoglobinler ve Windows
β zincir varyantları
0
1
2
3
4
5
Hb F
P2 window
P3 window
Hb A
D window
S window
Hb A2
C window
Dagestan
J-Abidjan
I-Texas
J-Oxford
Grady
J-Mexico
G-Georgia
Fort de France
Spanish Town
M-Iwate
Oleander
G-Philadelphia, Inkster
Stanleyville II
G-Norfolk
G-Pest, Tarrant
Kokura
Moabit
Winnipeg, Arya
Manitoba
Titusville, O-Padova
Hasharon
Chad
O-Indonesia
Okayama
Camperdown
Hope
K-Woolwich
N-Baltimore, Camden, Pyrgos, Olomouc
J-Baltimore, Chicago
Hikari
K-Ibadan, J-Europa
Old Dominion
Alzette
New York
Rainier
Henri Mondor
D-Iran
M-Milwaukee, St Louis, Lepore, Korle Bu, Ouled Rabah, Ocho Rios, Osu Christiansborg
E , Cocody, G-Ferrara, Kenitra
D-Punjab, Khartoum
Maputo
Kariskoga
G-San Jose
G-Szuhu
S
E-Saskatoon
G-Makassar
S-Antilles
O-Arab
Agenogi
Siriraj
C
Normal elution zamanı (dk)


VWD1 A, Wavelength=415 nm (MART\16-03002.D)
mAU
25
20
15
10
5
0
0.854
1.651 - hba1c
3.322
3.768
4.293 - hbao
5.263 - hba2
7.536 - hbs
8.362 - hbc
0
1
2
3
4
5
6
7
8
min

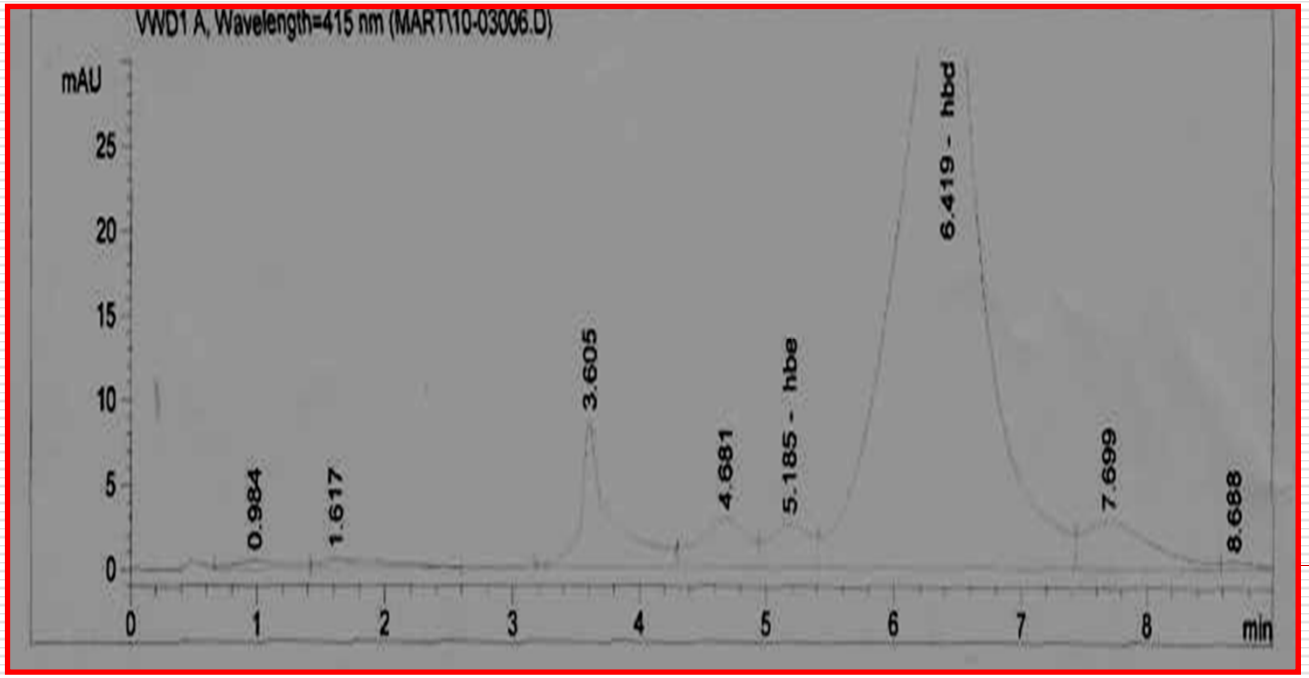
HbAC


VWD1 A, Wavelength=415 nm (HAZYRAN\25-06004.D)
mAU
25
20
15
10
5
0
0.488
0.645
0.936
1.086
1.897 - hba1c
2.397 - hbf
3.345
3.624
3.940
4.619 - hbao
6.004 - hba2
7.762 - hbs
0
1
2
3
4
5
6
7
8
min

HbAS



HbAE



HbEE-Saskaton

TARAMA VE TANI YÖTEMLERİ NELER OLMALI ?

1. aşama

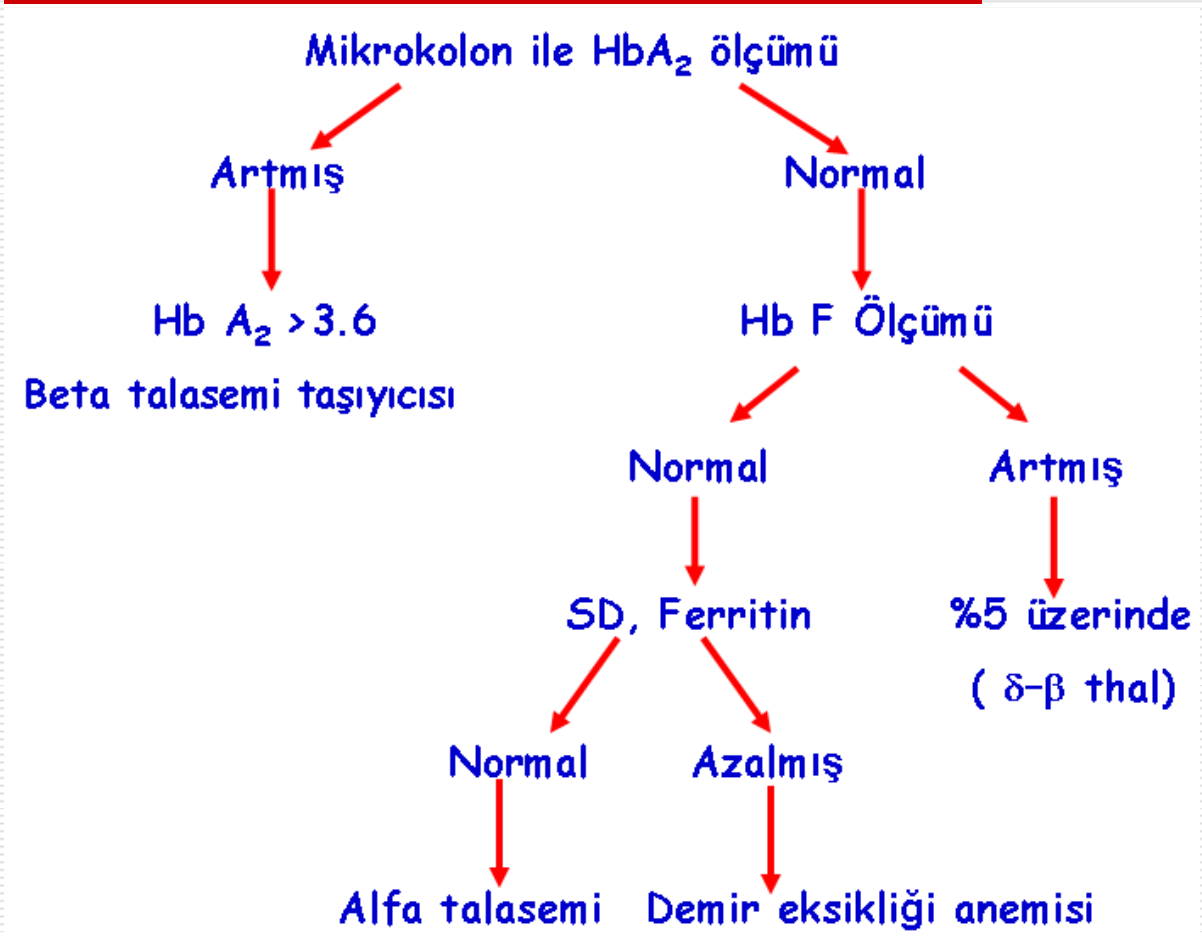
MCV → Azalmış → <78fL

MCH → Azalmış → <27 pg

Hb Elektroforezi → Hb A_2↑, HbF↑ → 2. Aşama

Hb Elektroforezi → Anormal Hb → Rapor edilir

2. aşama

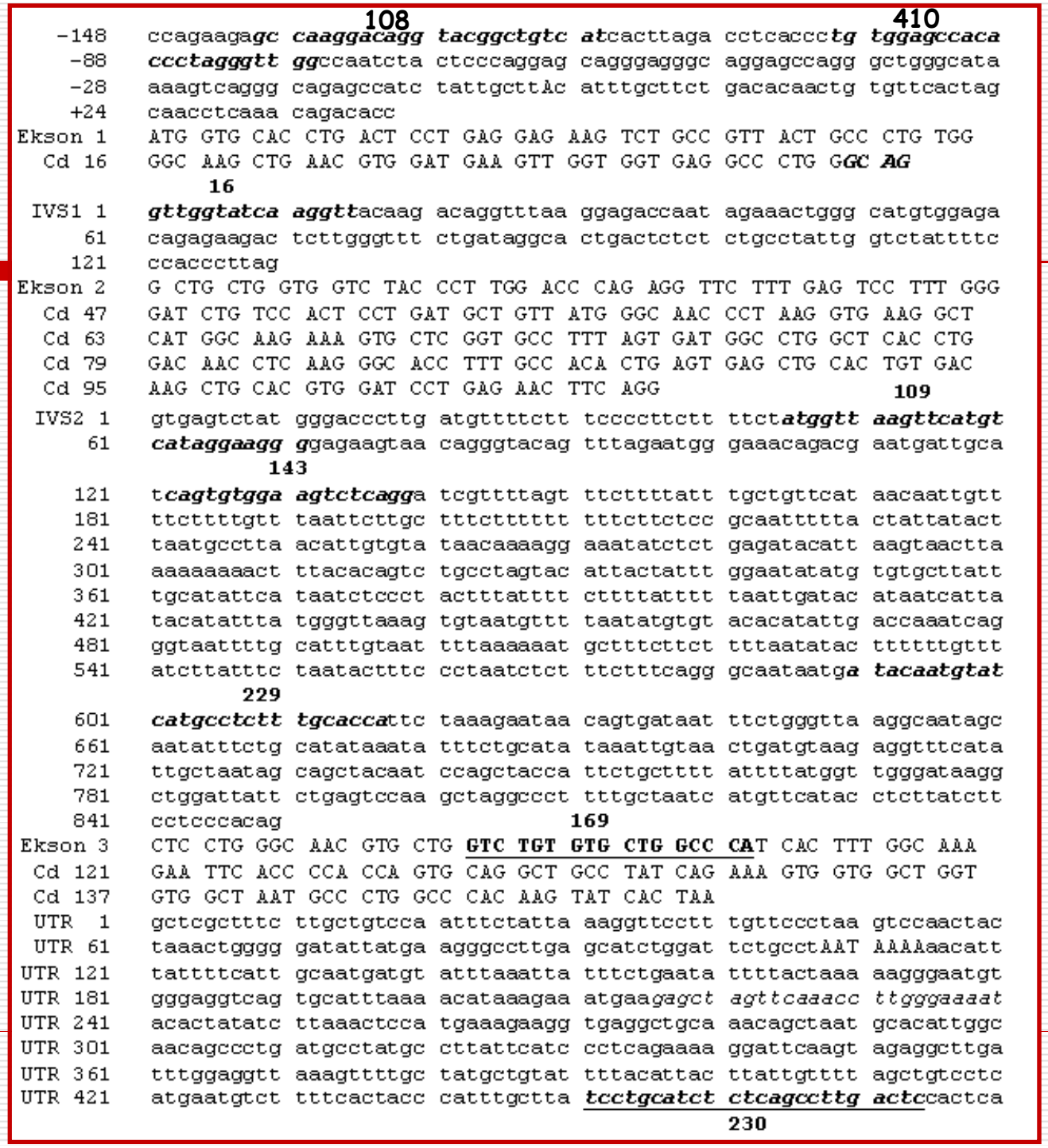

Mikrokolon ile HbA2 ölçümü
Artmış
Normal
Hb A2 >3.6
Beta talasemi taşıyıcısı
Hb F Ölçümü
Normal
Artmış
SD, Ferritin
%5 üzerinde
(δ-β thal)
Normal
Azalmış
Alfa talasemi
Demir eksikliği anemisi

Adı Soyadı	RBC 10^{12}/L	Hb g/dL	Hct %	MCV fL	MCH Pg	MCHC g/dL	Hb Elektr.	Mutasyon
O. B.	5.10	**15.9**	46.1	**90.3**	31.2	34.6	**AS**	**AS**
K. B.	4.87	**14.1**	42.0	**86.2**	29.0	33.7	**AS**	**AS**
Ü. Ö.	5.57	**15.6**	45.7	**81.9**	28.0	34.2	**AS**	**AS**
R. K.	5.73	**16.4**	48.0	**83.7**	28.6	34.2	**AS**	**AS**
E. A.	5.13	**14.4**	42.8	**83.5**	28.0	33.6	**AS**	**AS**
M. K.	5.69	**14.9**	46.0	**80.8**	26.2	32.4	**AD**	**AD**
H. K.	4.43	**12.5**	38.2	**86.2**	28.1	32.7	**AD**	**AD**
M. G.	5.63	**16.1**	47.8	**84.8**	28.5	33.6	**AD**	**AD**
E. H.	3.89	**11.4**	35.6	**91.5**	29.3	32.0	**AD**	**AD**
T. H.	5.11	**16.6**	50.2	**98.3**	32.5	33.1	**AD**	**AD**
B. B.	4.36	**11.0**	34.0	**77.8**	25.1	31.8	**AE**	**AE**
E. O.	4.47	**10.7**	34.4	**77.0**	24.0	31.1	**AE**	**AE**
Ö. Ö.	3.85	**10.0**	30.5	**79.1**	25.9	32.8	**AE**	**AE**
M. E.	6.09	**15.7**	44.7	**73.4**	25.8	35.2	**AE**	**AE**
G. E.	4.66	**11.4**	34.9	**75.0**	24.4	32.6	**AE**	**AE**
N. S.	6.06	**16.5**	46.7	**77.0**	27.2	35.3	**AC**	**AC**
C. D.	8.7	**16.1**	45.2	**77.4**	27.6	35.6	**AC**	**AC**
D. K.	5.0	**12.9**	38.2	**76.3**	25.9	33.9	**AC**	**AC**
H. K.	5.84	**16.6**	45.9	**78.6**	28.4	26.2	**AC**	**AC**
A. K.	5.75	**16.3**	45.5	**79.1**	28.3	35.7	**AC**	**AC**

Adı Soyadı	RBC 10^{12}/L	Hb g/dL	Hct %	MCV fL	MCH Pg	MCHC g/dL	Hb Elektr	HbF %	Mutasyon
S. S.	3.07	**7.6**	24.9	**81.1**	24.8	30.5	**SS(F)**	6.5	**SS**
C. K.	3.58	**9.9**	30.5	**85.4**	27.8	32.6	**SS(F)**	9.0	**SS**
Z. K.	2.83	**8.9**	26.0	**91.8**	29.2	33.0	**SS(F)**	7.9	**SS**
G. A.	2.70	**5.8**	21.0	**77.7**	21.5	27.6	**SS(F)**	5.1	**SS**
O. Z.	3.20	**9.9**	28.1	**87.7**	30.8	35.1	**SS**	1.9	**SS**
G. G.	4.16	**8.5**	29.5	**70.9**	20.6	30.7	**SS(F)**	3.4	**S/IVSI-1**
G. A.	5.03	**10.5**	33.0	**65.6**	20.9	31.8	**SF**	15.8	**S/IVSI-5**
İ. T.	6.10	**13.1**	39.5	**67.6**	22.5	37.5	**SS**	1.7	**S/IVSI-6**
H. Ö.	4.55	**8.8**	29.4	**64.5**	19.4	30.1	**SS**	2.0	**S/IVSI-110**
A. Ç.	3.71	**9.1**	28.6	**77.1**	24.4	31.6	**SS(F)**	7.7	**S/IVSI-110**
S. K.	3.26	**7.3**	22.8	**70.0**	22.3	31.8	**SF**	17.0	**S/Cd 39**
A. K.	2.75	**6.0**	19.4	**70.5**	21.6	30.7	**SF**	10.0	**S/Cd 39**
S. B.	2.99	**9.5**	28.9	**96.4**	31.7	32.9	**SS**	0.9	**S/Fsc 5**
N. H.	4.24	**11.4**	32.8	**77.5**	26.8	34.6	**DD**	0.4	**DD**
S. D.	4.67	**11.7**	39.2	**73.3**	25.1	34.2	**DD**	1.7	**DD**
C. G.	6.06	**12.3**	39.8	**65.6**	20.3	30.9	**DD**	1.4	**D/IVSI-1**
S. A.	5.34	**11.1**	33.9	**63.4**	20.8	32.9	**DD**	1.1	**D/IVSI-110**
M. B.	5.98	**12.1**	38.6	**64.6**	20.3	31.3	**DD**	1.7	**D/IVSI-110**
B. G.	4.67	**8.5**	28.1	**60.1**	18.2	30.4	**EE**	1.2	**E/IVSI-6**
B. D.	4.26	**10.4**	35.4	**83.2**	24.5	29.4	**EF**	10.0	**E/IVSI-110**

ANORMAL HEMOGLOBİNLER

Adı Soyadı	RBC 10^{12}/L	Hb g/dL	Hct %	MCV fL	MCH Pg	MCHC g/dL	Hb Elektr.	Mutasyon
A. S.	5.99	**15.1**	44.6	**74.5**	25.3	33.9	**AE**	**AE-Saskatoon**
S.A.	4.28	**12.4**	36.3	**84.9**	29.0	34.1	**EE**	**EE-Saskatoon**
T. B.	4.46	**11.0**	34.1	**76.5**	24.7	32.3	**AS**	**AG-Coushatta**
Z. B.	4.64	**12.5**	38.2	**82.4**	26.9	32.7	**AS**	**AG-Coushatta**


108
410
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-88 ccctagggtt ggccaatcta ctcccaggag cagggagggc aggagccagg gctgggcata
-28 aaagtcaggg cagagccatc tattgcttAc atttgcttct gacacaactg tgttcactag
+24 caacctcaaa cagacacc
Ekson 1 ATG GTG CAC CTG ACT CCT GAG GAG AAG TCT GCC GTT ACT GCC CTG TGG
Cd 16 GGC AAG CTG AAC GTG GAT GAA GTT GGT GGT GAG GCC CTG GGC AG
16
IVS1 1 gttggtatca aggttacaag acaggtttaa ggagaccaat agaaactggg catgtggaga
61 cagagaagac tcttgggttt ctgataggca ctgactctct ctgcctattg gtctattttc
121 ccacccttag
Ekson 2 G CTG CTG GTG GTC TAC CCT TGG ACC CAG AGG TTC TTT GAG TCC TTT GGG
Cd 47 GAT CTG TCC ACT CCT GAT GCT GTT ATG GGC AAC CCT AAG GTG AAG GCT
Cd 63 CAT GGC AAG AAA GTG CTC GGT GCC TTT AGT GAT GGC CTG GCT CAC CTG
Cd 79 GAC AAC CTC AAG GGC ACC TTT GCC ACA CTG AGT GAG CTG CAC TGT GAC
Cd 95 AAG CTG CAC GTG GAT CCT GAG AAC TTC AGG
109
IVS2 1 gtgagtctat gggacccttg atgttttctt tccccttctt ttctatggtt aagttcatgt
61 cataggaagg ggagaagtaa cagggtacag tttagaatgg gaaacagacg aatgattgca
143
121 tcagtgtgga agtctcagga tcgttttagt ttcttttatt tgctgttcat aacaattgtt
181 ttcttttgtt taattcttgc tttcttttct tttcttctcc gcaattttta ctattatact
241 taatgcctta acattgtgta taacaaaagg aaatatctct gagatacatt aagtaactta
301 aaaaaaaact ttacacagtc tgcctagtac attactattt ggaatatatg tgtgcttatt
361 tgcatattca taatctccct actttatttt cttttatttt taattgatac ataatcatta
421 tacatattta tgggttaaag tgtaatgttt taatatgtgt acacatattg accaaatcag
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541 atcttatttc taatactttc cctaatctct ttctttcagg gcaataatga tacaatgtat
229
601 catgcctctt tgcaccattc taaagaataa cagtgataat ttctgggtta aggcaatagc
661 aatatttctg catataaata tttctgcata taaattgtaa ctgatgtaag aggtttcata
721 ttgctaatag cagctacaat ccagctacca ttctgctttt attttatggt tgggataagg
781 ctggattatt ctgagtccaa gctaggccct tttgctaatc atgttcatac ctcttatctt
841 cctcccacag
169
Ekson 3 CTC CTG GGC AAC GTG CTG GTC TGT GTG CTG GCC CAT CAC TTT GGC AAA
Cd 121 GAA TTC ACC CCA CCA GTG CAG GCT GCC TAT CAG AAA GTG GTG GCT GGT
Cd 137 GTG GCT AAT GCC CTG GCC CAC AAG TAT CAC TAA
UTR 1 gctcgctttc ttgctgtcca atttctatta aaggttcctt tgttccctaa gtccaactac
UTR 61 taaactgggg gatattatga agggccttga gcatctggat tctgcctAAT AAAAaacatt
UTR 121 tattttcatt gcaatgatgt atttaaatta tttctgaata ttttactaaa aagggaatgt
UTR 181 gggaggtcag tgcatttaaa acataaagaa atgaagagct agttcaaacc ttgggaaaat
UTR 241 acactatatc ttaaactcca tgaaagaagg tgaggctgca aacagctaat gcacattggc
UTR 301 aacagccctg atgcctatgc cttattcatc cctcagaaaa ggattcaagt agaggcttga
UTR 361 tttggaggtt aaagttttgc tatgctgtat tttacattac ttattgtttt agctgtcctc
UTR 421 atgaatgtct tttcactacc catttgctta tcctgcatct ctcagccttg actccactca
230

ARMS

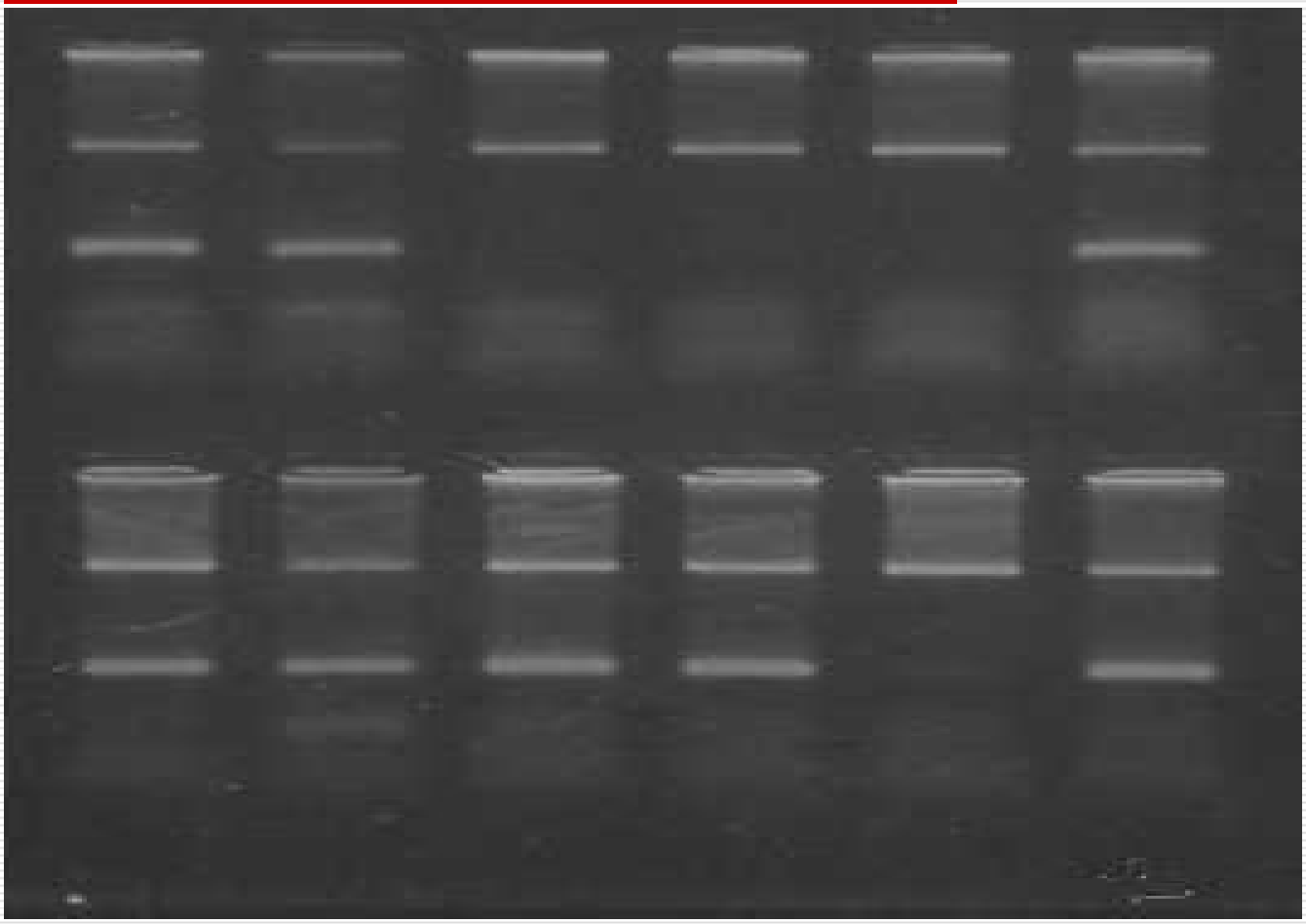
- Nokta mutasyonlarının saptanması için kullanılan yaygın bir tekniktir
- ARMS yöntemi iki tanımlayıcı reaksiyondan oluşur
  - 1. reaksiyon: Mutasyonu taşımayan normal primerler kullanılarak amplifikasyon yapılır
  - Bu reaksiyonda, normal DNA dizisine özgül ARMS primerleri içerir ve belirli bir yerdeki mutant DNA'yı amplifiye edemez, sadece normal (mutasyon içermeyen) DNA dizisini amplifiye edebilir
  - 2. reaksiyon: Araştırılacak mutasyona özgü primerler (mutant primer)
  - İkinci reaksiyonda mutanta özgül primerleri içerir ve normal DNA dizisini amplifiye edemez, sadece DNA'daki belirli bir mutasyonu amplifiye edebilmektedir
- Sonuç olarak normal bir birey yalnızca normal reaksiyonda PCR ürünü oluştururken, heterozigot bir birey her iki reaksiyonda PCR ürünü oluşturur. Mutant birey ise yalnızca mutant reaksiyonda PCR ürünü oluşturur.

HbS (A→T)	(M*)	5'-CCC ACA GGG CAG T AA CGG CAG ACT TCT GCA-3'
HbS (A→T)	(N*)	5'-CCC ACA GGG CAG TAA CGG CAG ACT TCT GCT-3'
HbD (G→C)	(M*)	5'-ATA GGC AGC CTG CAC TGG TGG GGT GAG TTG -3'
HbD (G→C)	(N*)	5'-ATA GGC AGC CTG CAC TGG TGG GGT GAG TTC-3'
HbE (G→A)	(M*)	5'-TAA CCT TGA TAC CAA CCT GCC CAG GGC GTT-3'
HbE (G→A)	(N*)	5'-TAA CCT TGA TAC CAA CCT GCC CAG GGC GTC-3'
IVSI-110 (G→A)	(M*)	5'-ACC AGC AGC CTA AGG GTG GGA AAA TAC ACT-3'
IVSI-110 (G→A)	(N*)	5'-ACC AGC AGC CTA AGG GTG GGA AAA TAC ACC-3'
IVSI-1 (G→A)	(M*)	5'-TTA AAC CTG TCT TGT AAC CTT GAT ACG AAT-3'
IVSI-1 (G→A)	(N*)	5'-TTA AAC CTG TCT TGT AAC CTT GAT ACG AAC-3'
IVS1-5 (G→C)	(M*)	5'-CTC CTT AAA CCT GTG TTG TAA CCT TGT TAG-3'
IVS1-5 (G→C)	(N*)	5'-CTC CTT AAA CCT GTC TTG TAA CCT TGT TAC-3'
Fsc 5 (-CT)	(M*)	5'-ACA GGG CAG TAA CGG CAG ACT TCT CCG CGA-3'
Fsc 5 (-CT)	(N*)	5'-ACA GGG CAG TAA CGG CAG ACT TCT CCG CTC-3'

*M: Mutant primer *N: Normal primer

HbS mutasyonun ARMS ile belirlenmesi. 1 anne, 2 baba, 3 ve 4 CVS, 5 negatif kontrol ve 6 pozitif kontrol

1M 2M 3M 4M 5M 6M

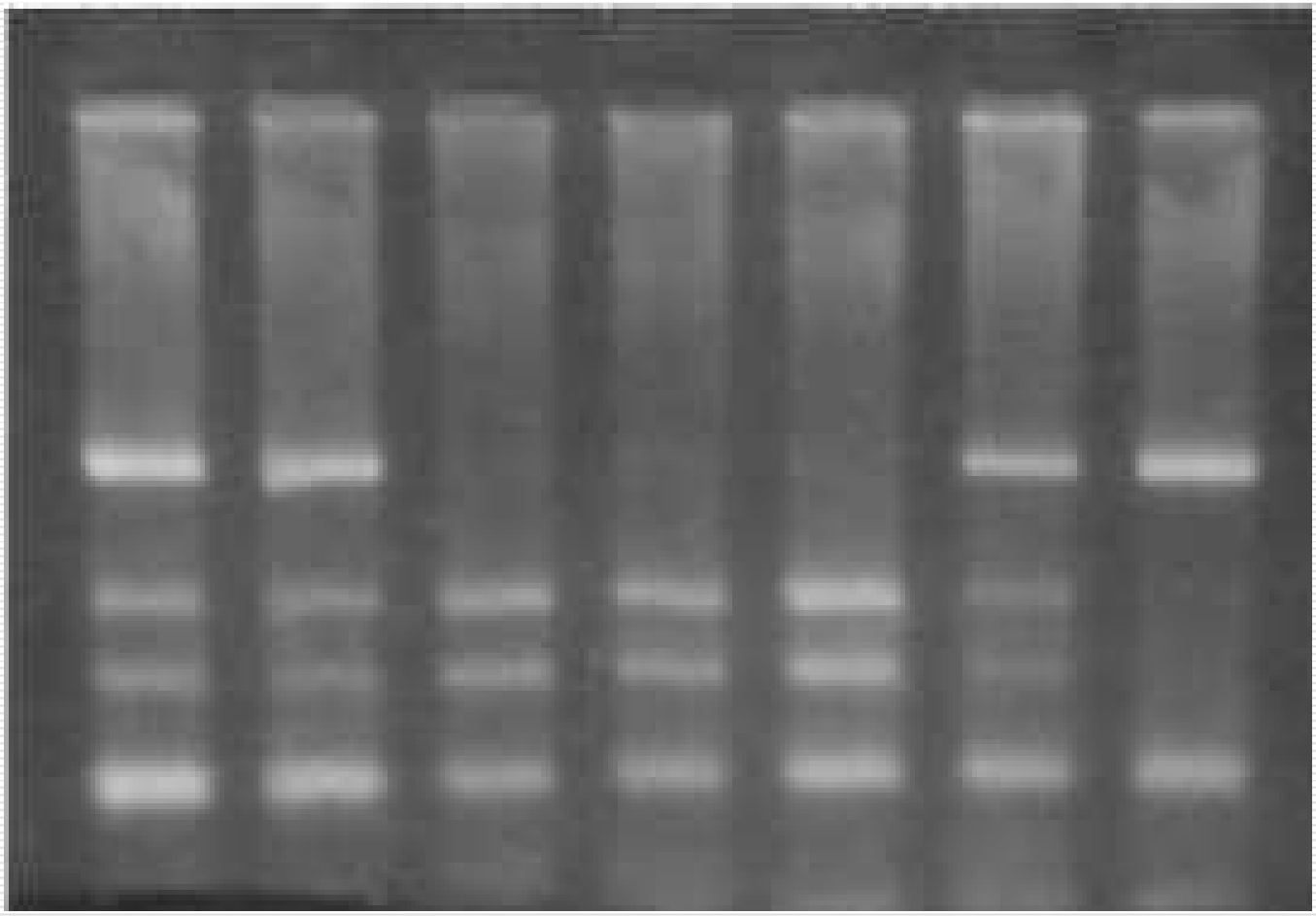


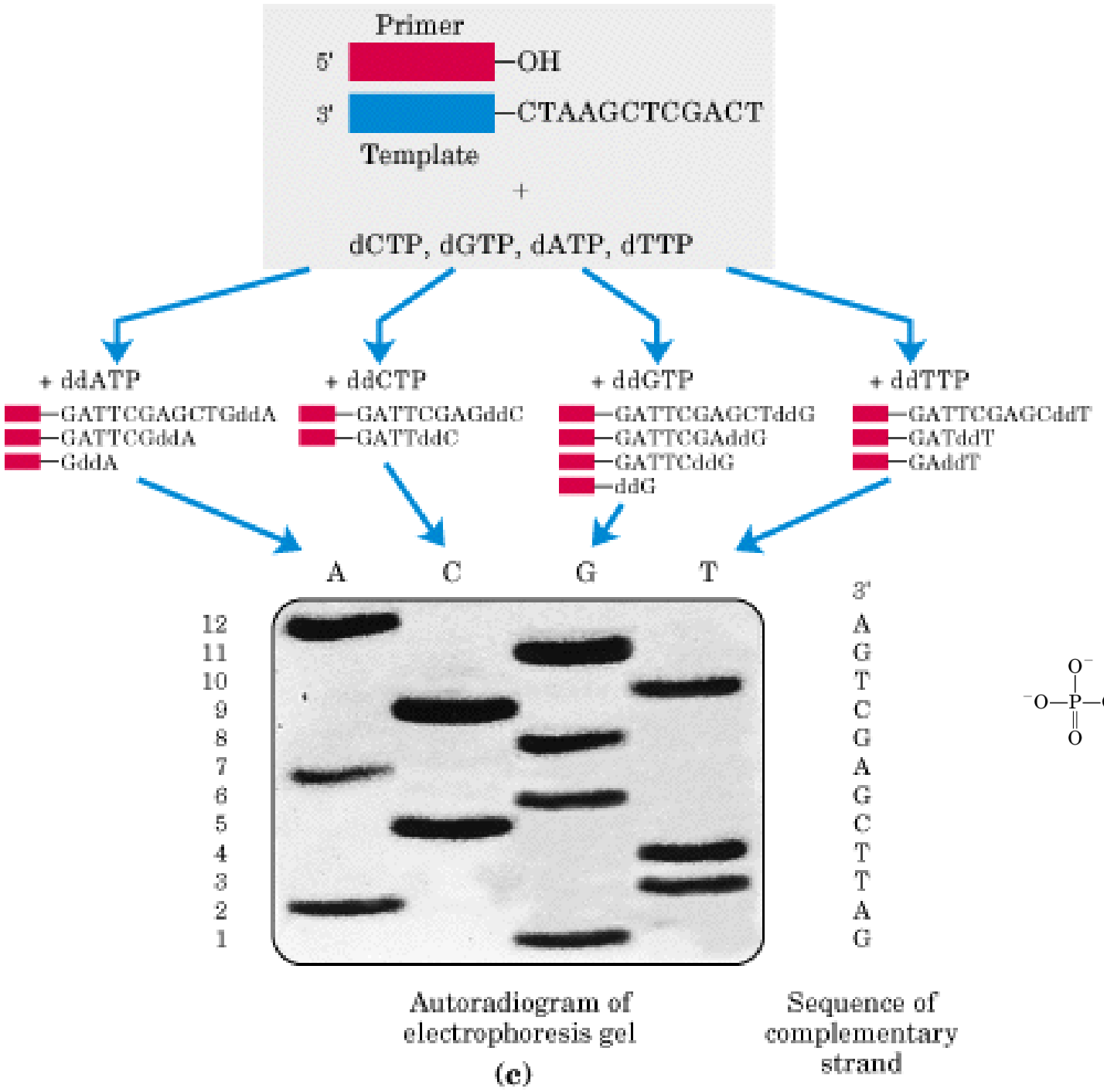
1N 2N 3N 4N 5N 6N

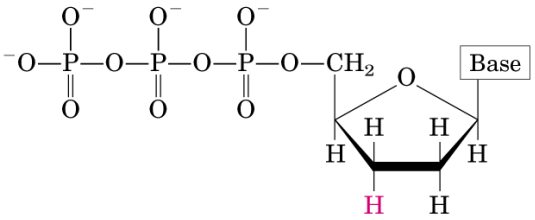
RFLP

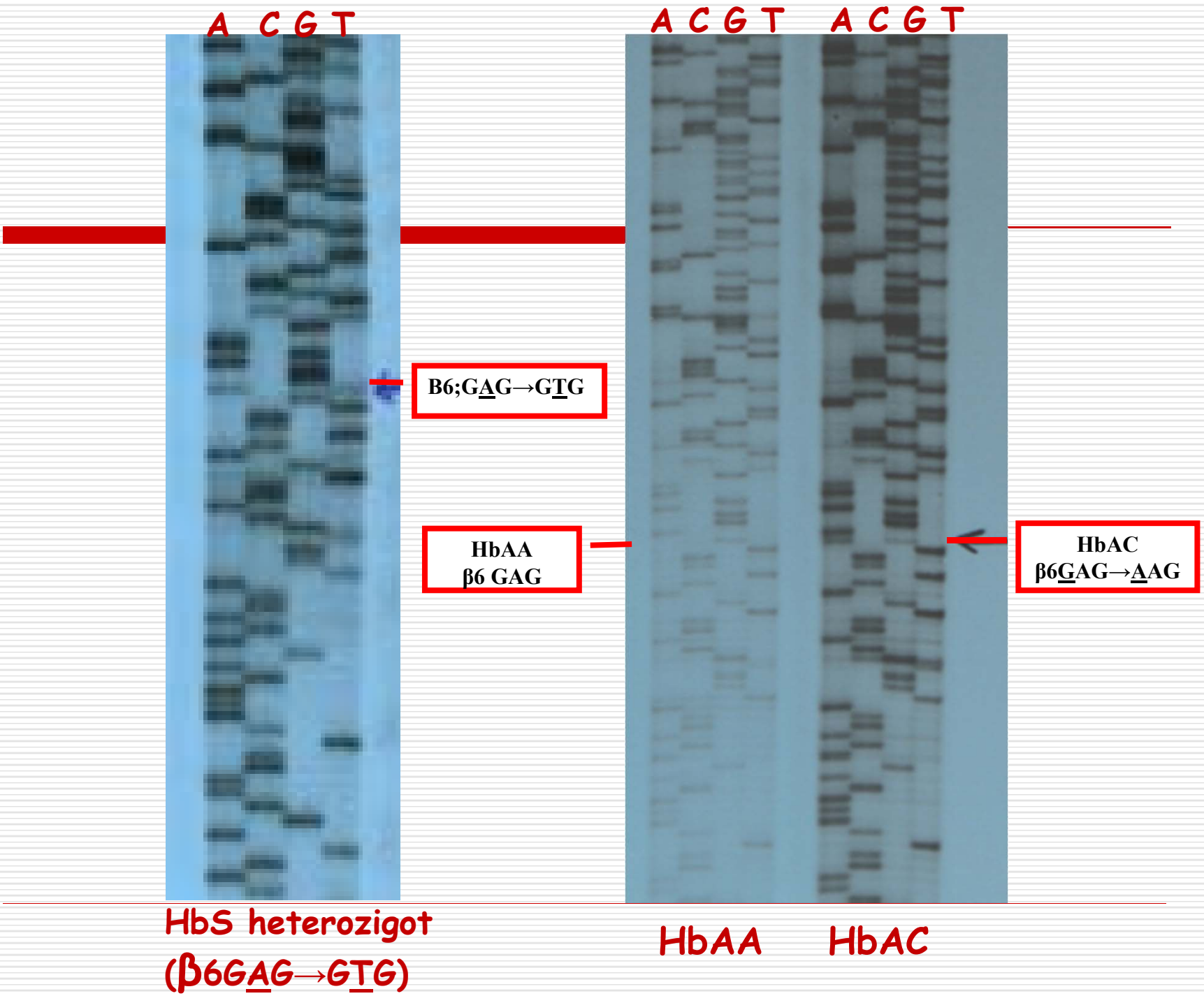
Anne Baba CVS KONTROL

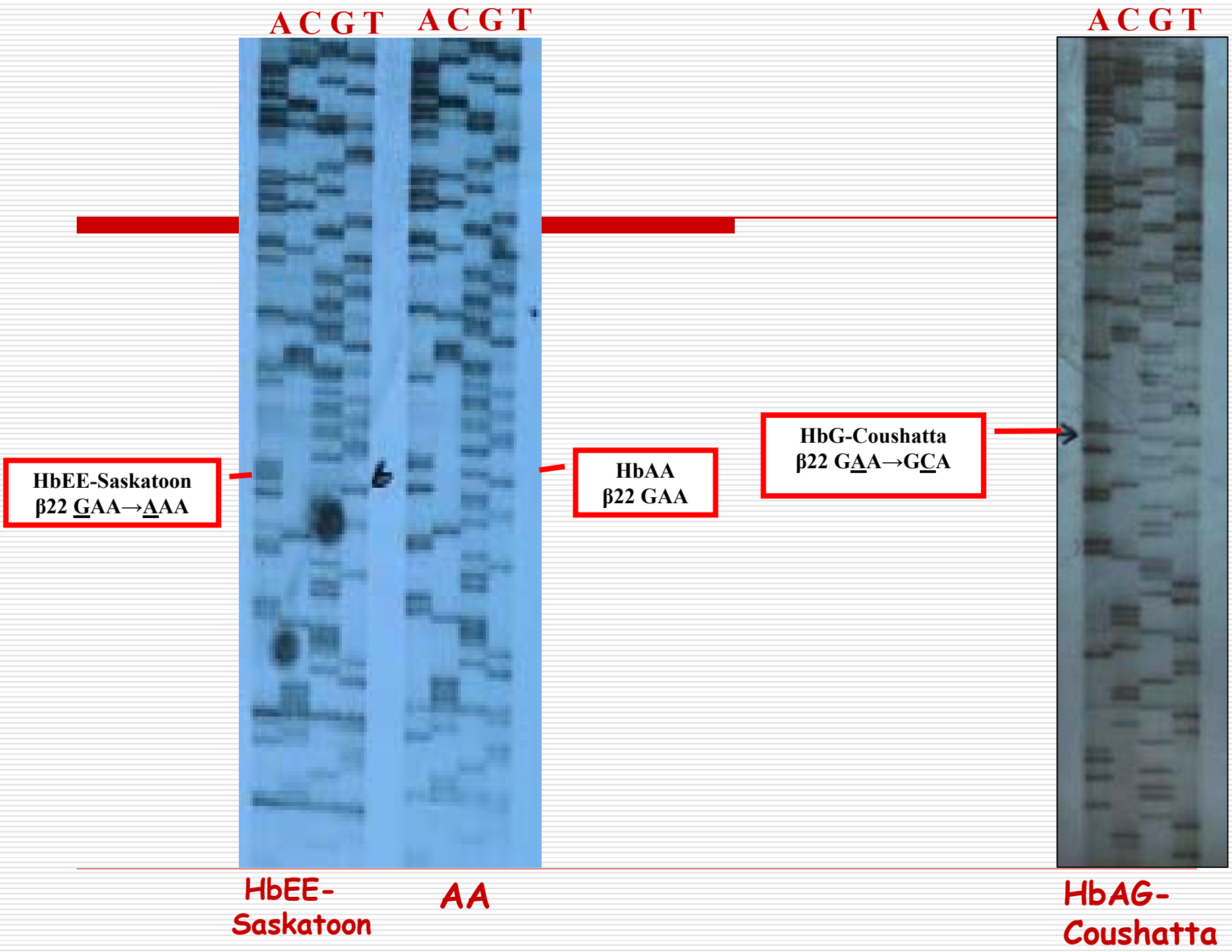
AS AS AA AA AA AS SS

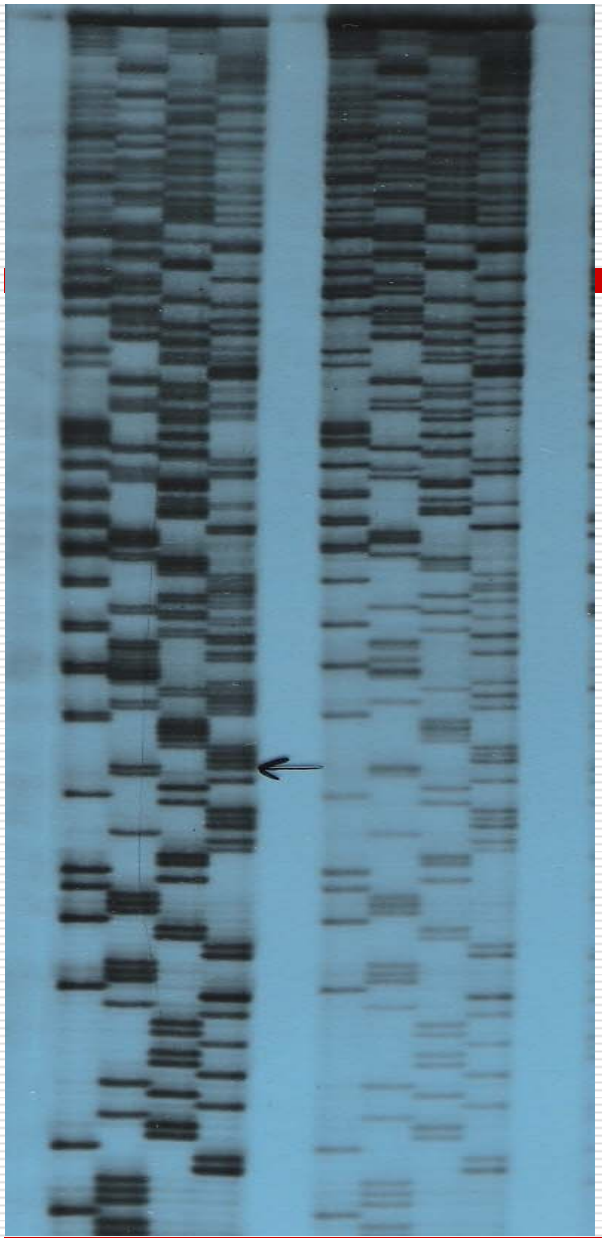



Primer
5'
OH
3'
CTAAGCTCGACT
Template
+
dCTP, dGTP, dATP, dTTP
+ ddATP
GATTCGAGCTGddA
GATTCGddA
GddA
+ ddCTP
GATTCGAGddC
GATTddC
+ ddGTP
GATTCGAGCTddG
GATTCGAddG
GATTCddG
ddG
+ ddTTP
GATTCGAGCddT
GATddT
GAddT
A
C
G
T
12
11
10
9
8
7
6
5
4
3
2
1
3'
A
G
T
C
G
A
G
C
T
T
A
G
Autoradiogram of electrophoresis gel
(c)
Sequence of complementary strand


Base
ddNTP analog
(b)


A C G T
A C G T A C G T
B6;GAG→GTG
HbAA
β6 GAG
HbAC
β6GAG→AAG
HbS heterozigot
(β6GAG→GTG)
HbAA HbAC


A C G T A C G T
A C G T
HbEE-Saskatoon
β22 GAA→AAA
HbAA
β22 GAA
HbG-Coushatta
β22 GAA→GCA
HbEE-Saskatoon
AA
HbAG-Coushatta



A/FSC 44(-C) HbAA