



ADNAN MENDERES ÜNİVERSİTESİ
ZİRAAT FAKÜLTESİ
TARIMSAL BİYOTEKNOLOJİ BÖLÜMÜ



**GOSSYPIUM HIRSUTUM (PAMUK)'DA
LİF KALİTESİNİ KONTROL EDEN
GHMYB-R2R3 TRANSKRİPSİYON FAKTÖRLERİNİN
DOĞRUDAN BAĞLANDIĞI GENLERİN
İN SILICO YÖNTEMLERLE
TAHMİN EDİLMESİ VE BELİRLENMESİ**

Dr. Murat Kemal AVCI



**Türk Biyokimya Derneği / Biyokimya Günleri Kongresi
02-05 KASIM 2016**

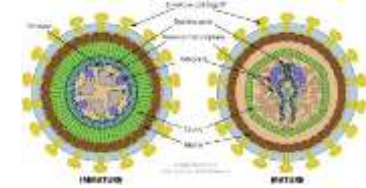
Sivas-2016

İÇERİK

- MYB protein ailesi...
 - MYB-R2R3 transkripsiyon Faktörleri...
- *Gossypium hirsutum*
- Lif ve lif kalitesi
- DNA-Protein etkileşimleri
- Materyal-Metod
- Sonuçlar ve Tartışma
- Sonuç

1. MYB PROTEİN AİLESİ

- Transkripsiyon faktörleri...
 - Pleotropik regülatörler....
- Bütün Ökaryotlarda görölmektedir..
 - Hayvanlar,
 - Bitkiler, → Pamuk (*Gossypium hirsutum*)
 - Diğer ökaryotlar



○ MYB ismi:

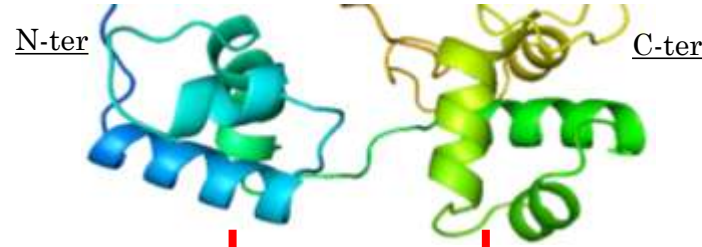
- Tanımlanan ilk MYB geni → *v-MYB* oncogeni

[Avian Myeloblastosis Virus (AMV)]
(MYB-3 ASIL / Klempnauer et al., 1982).

- COLORED1 (C1) geni → *Zea mays*, keşfedilen ilk bitki MYB geni...
(MYB-Review-1 / Paz-Ares et al. 1987)

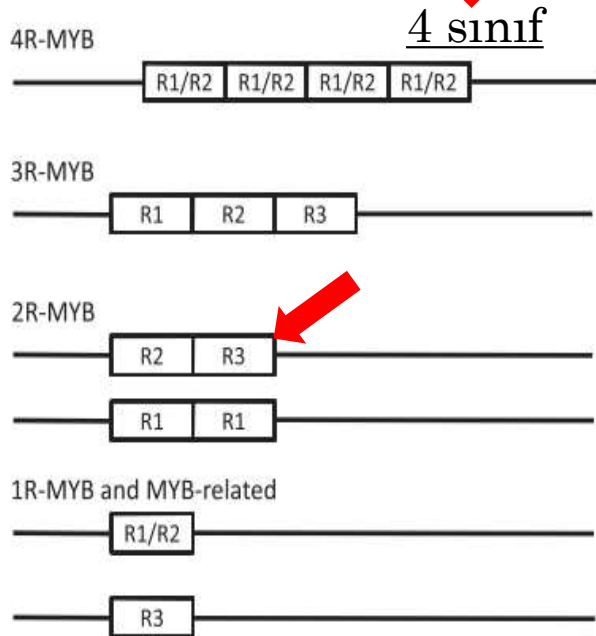
MYB PROTEİNLERİ

(İki farklı kısımdan oluşmaktadır)



N-terminal
Korunmuş MYB DNA-bağlanma
domaini

C-terminal
Düzenleyici domain
(Regulatör bölge)

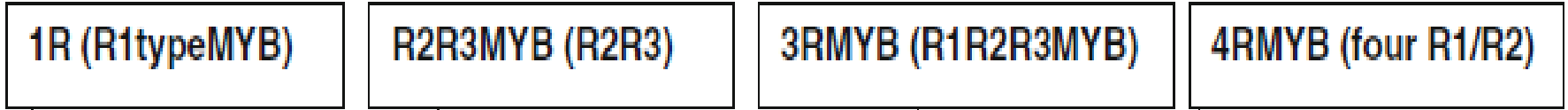
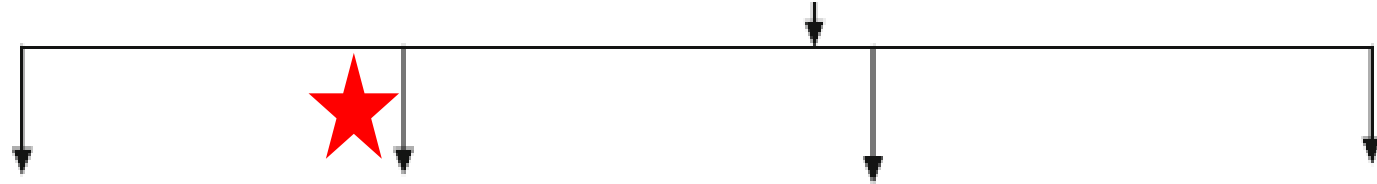


BİTKİLERDE MYB PROTEİNLERİ

- Kuraklık stresi
- Tuz stresi
- Sıcaklık stresi
- Pi eksikliği
- Metal Stresi
- Şeker stresi
- Işık stresi
- Yaranlama
- Diğer stresler
 - Anoksia (oksijen eksikliği)
 - Nitrojen eksikliği
 - Bor stresi vs.. vs..

MYB-R2R3 PROTEİNLERİ

MYB Protein Alt Aileleri



- Cellular morphogenesis
- Secondary metabolism
- Organ morphogenesis
- Phosphate starvation
- Chloroplast development
- Circadian regulation

- Primary metabolism
- Cell fate and identity
- Secondary metabolism
- Developmental processes
- Responses to biotic and abiotic stresses

Cell cycle control

Unknown

Domainlerin yapısı → 22 alt grup

Arabidopsis.....120 üye

Rice-----90 üye [Katiyar et. al., 2012]

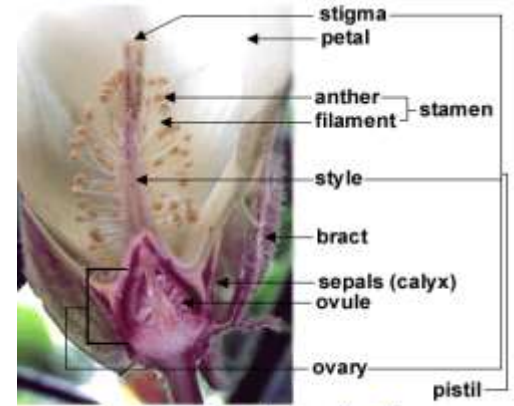
2R-MYB → Bitkilere özgü metabolizmalarda temel görevler üstlenirler.

PAMUK ta → LİF KALİTESİ

PAMUK (GOSSYPIMUM HIRSUTUM)

Lif ve Lif Kalitesi

- 1) Lif uzunluđu
- 2) Lif inceliđi
- 3) Lif mukavemeti
- 4) Lif olgunluđu
- 5) Yabancı madde miktarı
- 6) Lifin kıvrımlıđı
- 7) Lifin rengi
- 8) Lifin yumuşaklık ve sertlik derecesi
- 9) Liflerde rutubet
- 10) Liflerde yapışkanlık
- 11) Lifin nepsleşme durumu



Anatomy of a mature cotton flower

- Bu etmenlerden **ilk beş tanesi** iplik üretiminde hayati önem taşımakla birlikte diđer etmenler de üretilen ipliđin kalitesini etkilemektedir [4] –
- MYB-R2R3 proteinleri
 - Pleiotropik regülator

DNA-PROTEİN ETKİLEŞİMLERİ

- EMSA _ Electrophoretic Mobility Shift Assay
- ChIP_ Chromatin Immunoprecipitation Assay
- RT-qPCR
- Vs....

4. AMAÇ

- Pamukta
- «Lif kalitesi» ni etkilemekle birlikte bir çok genin de kontrolünden sorumu
- MYB-R2R3 transkripsiyon faktörleri..
- Doğrudan bağlandığı genlerin,
- Biyoinformatik veriler ve in silico analizler yardımıyla
- Tahmin edilmesi ve
- Belirlenmesi.

4. MATERYAL-METOD

○ Biyoinformatik araçlar

- NCBI,
- UNIPROT,
- Protein Data Bank (PDB),
- ClustalW,
- ExPasy-ProtParam,
- MODBASE →, Modeller 9.12 version
- StructuralBiology Knowledgebase

- MEGA.6,
- PyMOL

4. MATERYAL-METOD

03.11.2016

○ Organizma/Gen/Protein

- *Gossypium hirsutum* (Upland cotton)
- *Ghmyb109* gene (NCBI ID: CAD71140.1)
- GhMYB109 Transcription factor (UNIPROT ID: [Q70SX1](#))

Referans Proteinler:

- P27900 (UniProt ID) → [Arabidopsis thaliana](#) [AtMYBGL1]
- Q9SEI0 (UniProt ID) → [Arabidopsis thaliana](#) [AtWER=AtMYB66]
- 1H88 (PDB ID) → [Mus musculus](#) [c-Myb, protooncogene product]
- 2K9N (PDB ID) → [Trichomonas vaginalis](#) [tvMyb1]





1H88

Mus musculus

[c-Myb, protooncogene product]



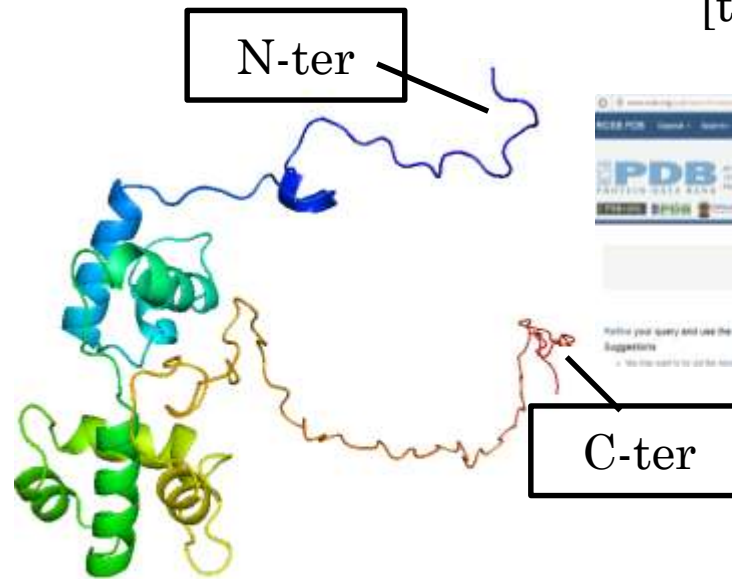
2K9N

Trichomonas vaginalis

[tvMyb1]

GhMYB109

?



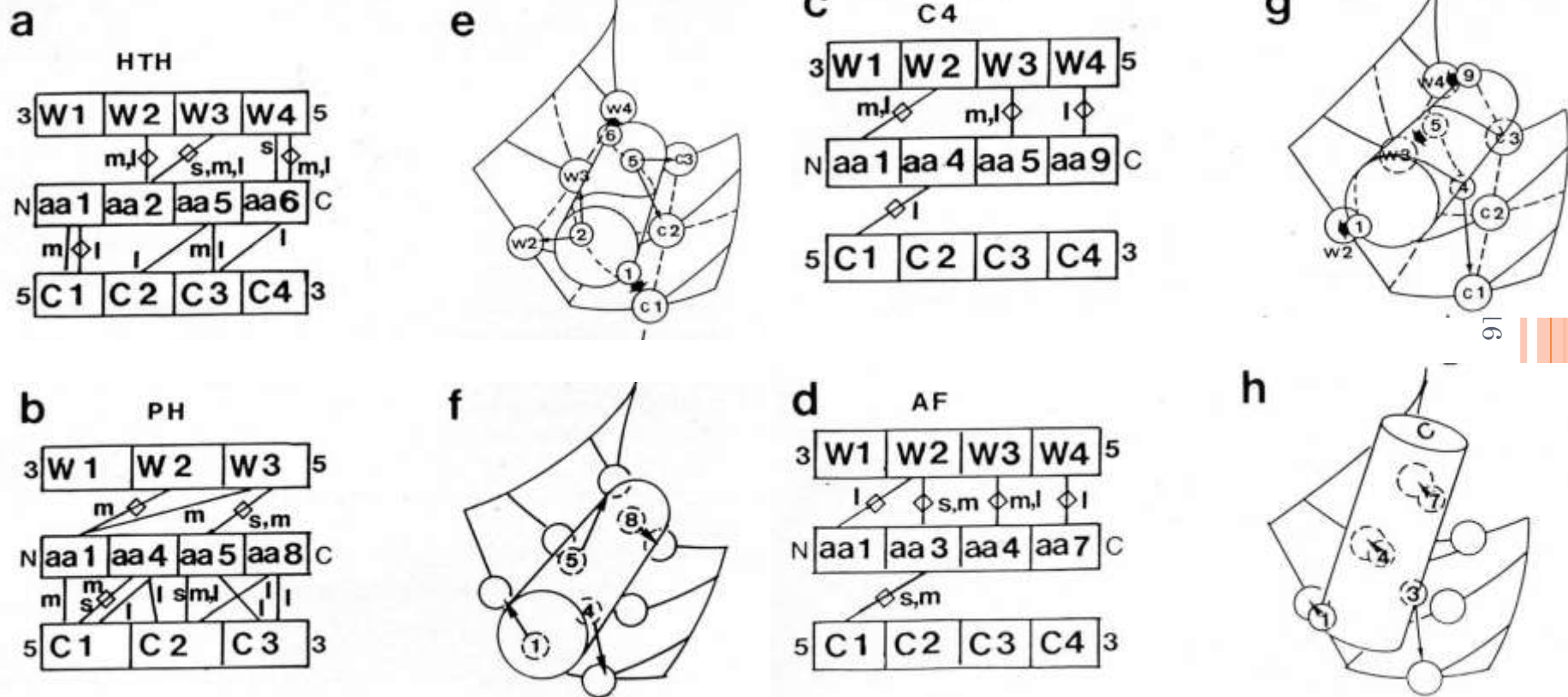
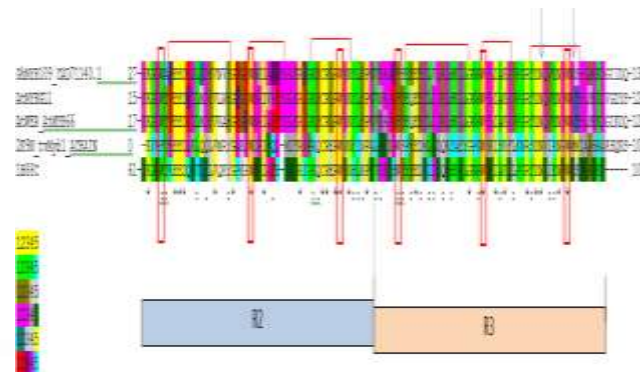
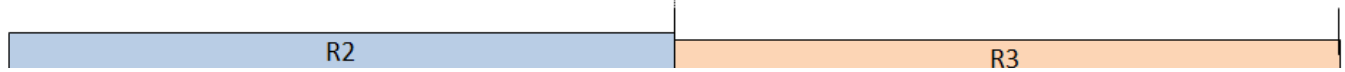


Fig. 2. Stereochemical charts (a–d) and base contacts (e–h) of HTH (a and e), PH (b and f), C4 (c and g) and AF (d and h) families, as deduced from molecular structures determined by NMR and crystallography. (a–d) Sketches of the DNA major groove with the bases W1–W4 (top) and C1–C4 (bottom), to which a recognition helix (in the central line) binds. The sizes of residues (small, s; medium, m; large, l) used for the contacts are also shown. In many cases more than one contact is possible. The optimal contacts are noted by a diamond; other potential contacts are indicated by a line. For quantitating the quality of an interaction (see text), 10 stereochemical merit points are given to the contacts marked with diamonds, while five are given to the other contacts. No stereochemical points are allotted otherwise. (e–h) The helix–groove geometry that generates the stereochemical charts depends upon patterns of interaction between residues and bases.



GhMYB109_CAD71140.1 27-KKGIWAMEEDKLLIDYVNVHGGKQWNKIANRTGLKRSKGKSCRLFWMNYLSPNVKKGFSEEEEDLVIRLHKLLGNRWBLIAKRVPGRTDNQVKNYWNSHLRKKLGIIDQ-135
 AtMYBGL1 15-KKGIWTVVEEDNILMDYVLNHTGGQWNRIVRKTGLKRCGKSCRLFWMNYLSPNVKNGFTEQEEDLIIRLHKLLGNRWBLIAKRVPGRTDNQVKNYWNTLSKKLVDYS-123
 AtWER_AtMYB66 17-KKGIWTVVEEDKILMDYVKAHGKGFWRNRIAKKTGLKRCGKSCRLFWMNYLSPNVKNRNFTEQEEDLIIRLHKLLGNRWBLIAKRVPGRTDNQVKNYWNTLSKKLGIKDQ-125
 2K9N_tvMyb1_ACHAIN 0 -KVFTEEEEDLKLQQLVMRYGAKIWIIRISQL-MITRNRQCREFRWNNYINPALRTDFWSPPEEDMLLDQKYAEYGPKNKISKFLKNRSDNNIRNFWMMIARHRAKHQKS-107
 1H88C 61-KGWIKEEDQVILVQKYGPKRWSYIAKH-LKGRIGKQCREFRWNHNLNPEVKKSWTEEDSIIIQAHKLLGNRWBLIAKRLPGRTDNQIKNWSLDRKK----- 103

12345
 12345
 12345
 1234



27-KKGIWAMEEDKLLIDYVNVHGGKQWNKIANRTGLKRSKGKSCRLFWMNYLSPNVKK
 15-KKGIWTVVEEDNILMDYVLNHTGGQWNRIVRKTGLKRCGKSCRLFWMNYLSPNVKN
 17-KKGIWTVVEEDKILMDYVKAHGKGFWRNRIAKKTGLKRCGKSCRLFWMNYLSPNVKN
 0 -KVFTEEEEDLKLQQLVMRYGAKIWIIRISQL-MITRNRQCREFRWNNYINPALRT
 61-KGWIKEEDQVILVQKYGPKRWSYIAKH-LKGRIGKQCREFRWNHNLNPEVKK

.2016

PNVKKGFSEEEEDLVIRLHKLLGNRWBLIAKRVPGRTDNQVKNYWNSHLRKKLGIIDQ-135
 PNVNKGNFTEQEEDLIIRLHKLLGNRWBLIAKRVPGRTDNQVKNYWNTLSKKLVDYS-123
 PNVKRNFTTEQEEDLIIRLHKLLGNRWBLIAKRVPGRTDNQVKNYWNTLSKKLGIKDQ-125
 PALRTDFWSPPEEDMLLDQKYAEYGPKNKISKFLKNRSDNNIRNFWMMIARHRAKHQKS-107
 PEVKKSWTEEDSIIIQAHKLLGNRWBLIAKRLPGRTDNQIKNWSLDRKK----- 103

Tablo: 5 MYB Proteinin benzerlik oranları (Clustal W).

	1.	2.	3.	4.	5.
1: GhMYB109_CAD71140.1	100.00	47.93	54.17	28.97	42.40
2: AtMYBGL1	47.93	100.00	59.50	28.97	45.13
3: AtWER_AtMYB66	54.17	59.50	100.00	28.04	45.22
4: 2K9N_tvMyb1_ACHAIN	28.97	28.97	28.04	100.00	38.24
5: 1H88C	42.40	45.13	45.22	38.24	100.00

Percent Identity Matrix - created by Clustal2.1

Hek-likler arası açılar (derece)						
	R2			R3		
	H1-H2	H1-H3	H2-H3	H1-H2	H1-H3	H2-H3
1 GhMYB109	130,55	91,6	104,9	140,11	83,56	96,46
2						
3						
4 2K9N	139,78	87,57	114,43	130,2	94,08	116,12
5 1H88	134,69	82,67	101	142,57	82,05	109

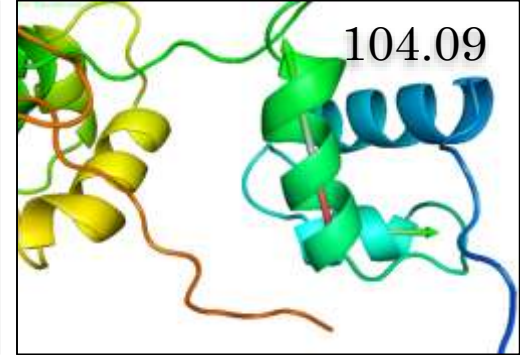
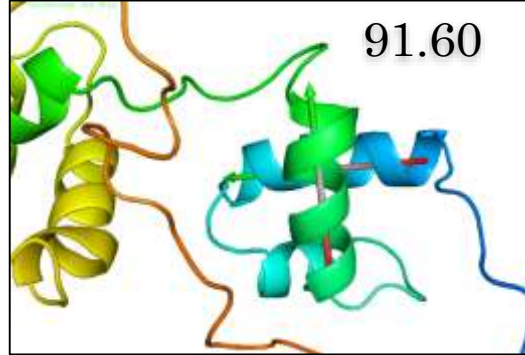
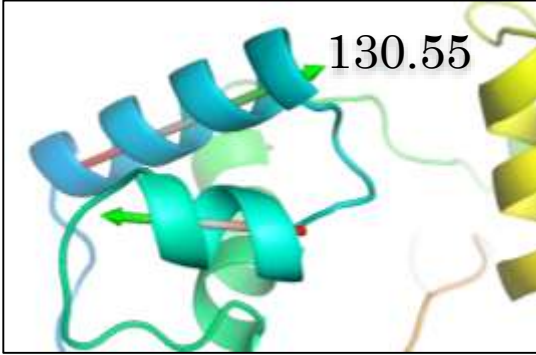
R2

Heliks1- Heliks2

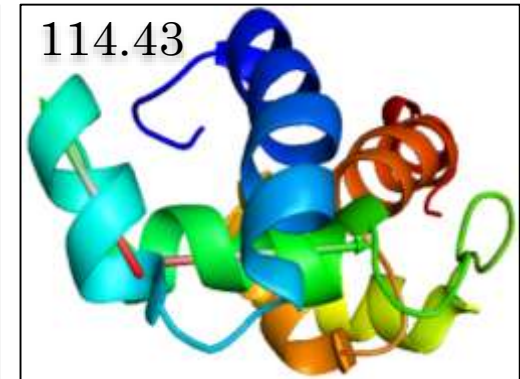
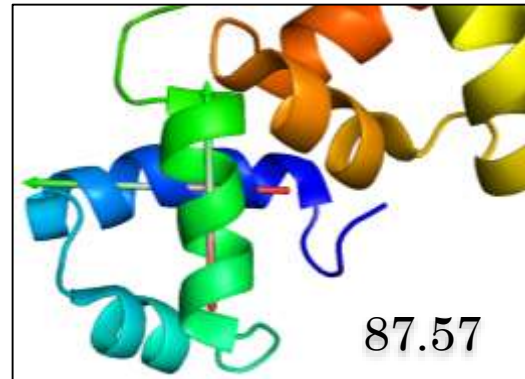
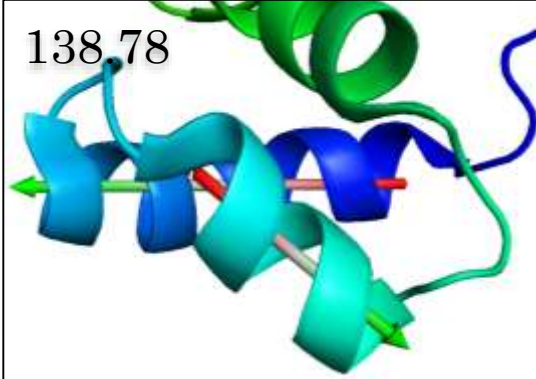
Heliks1- Heliks3

Heliks2- Heliks3

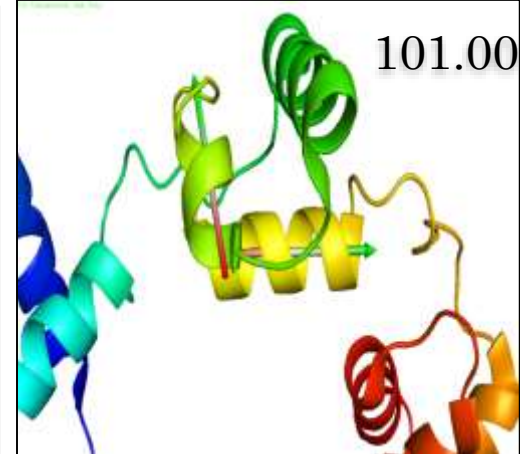
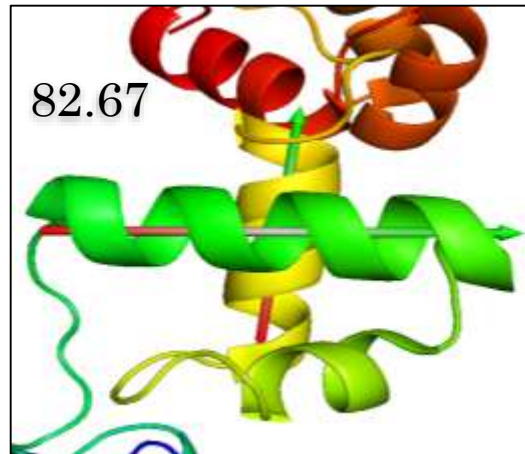
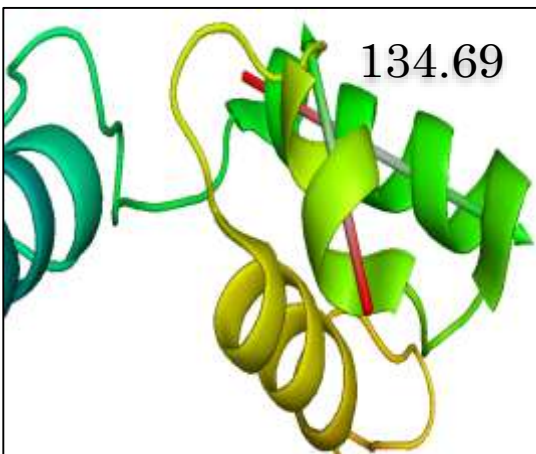
GhMYB109



2K9N



1H88



03.11.2016

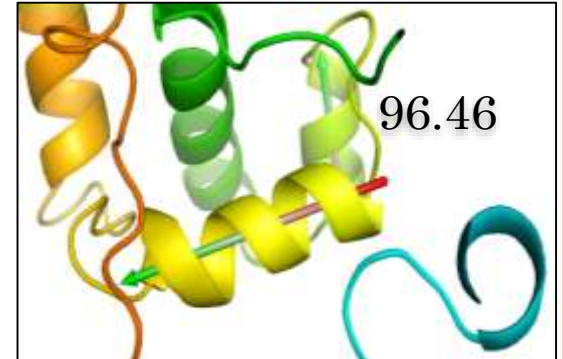
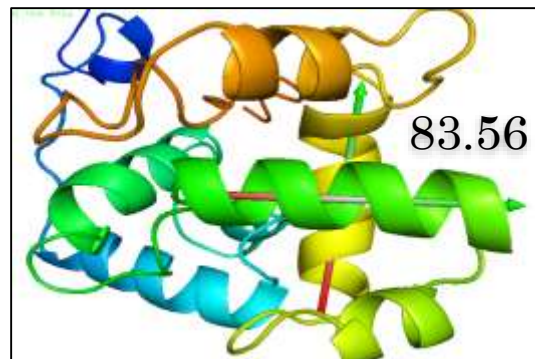
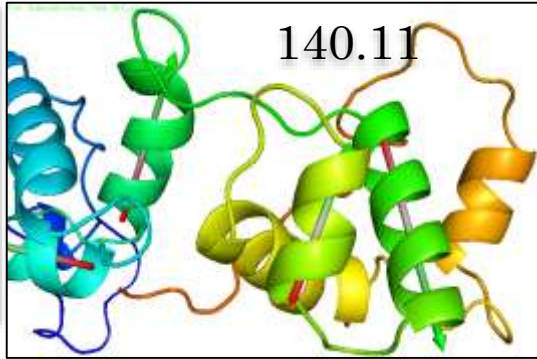
R3

Heliks1- Heliks2

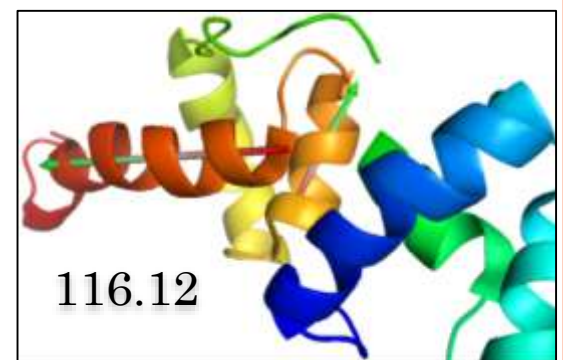
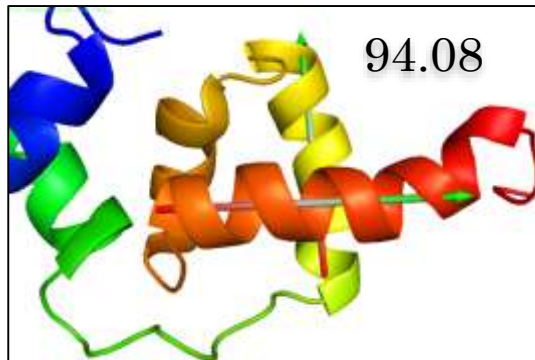
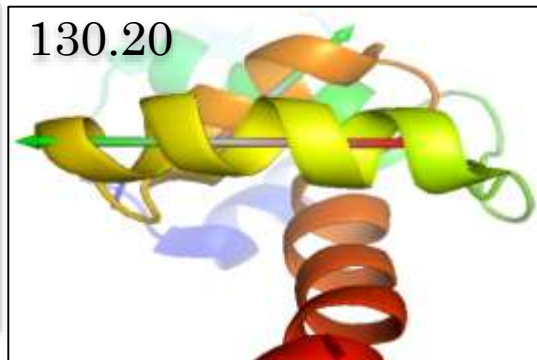
Heliks1- Heliks3

Heliks2- Heliks3

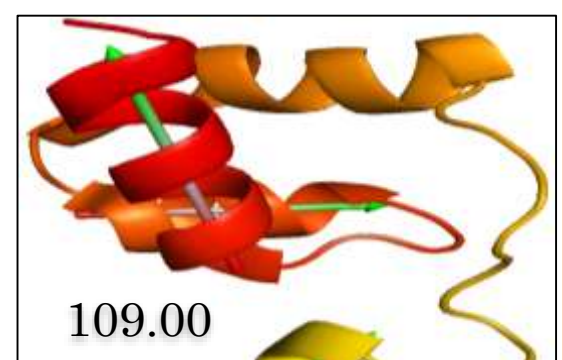
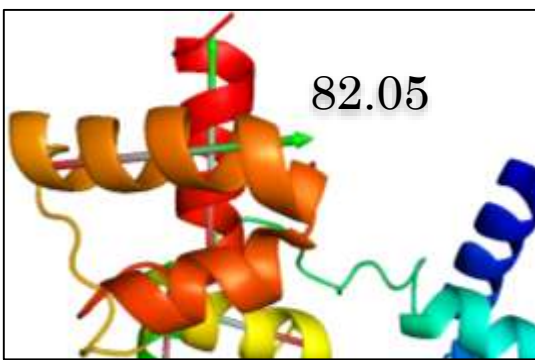
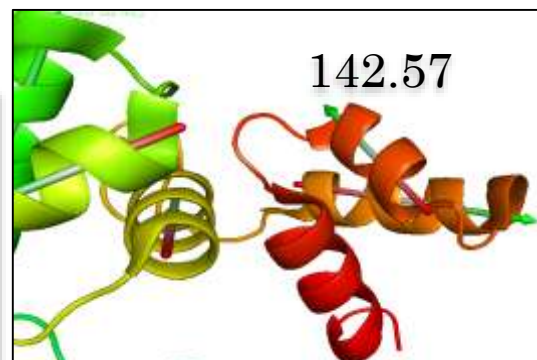
GhMYB109



2K9N

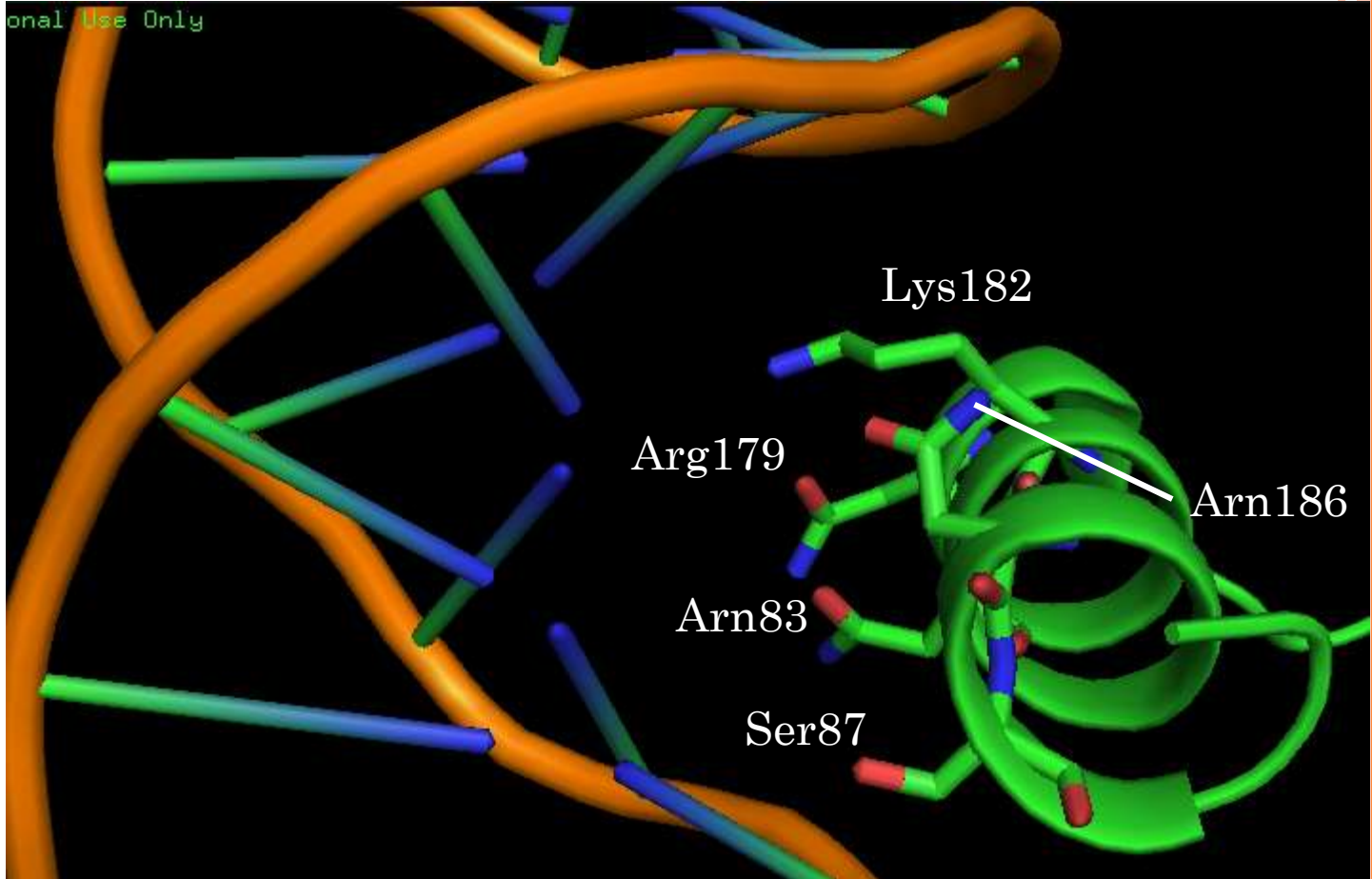


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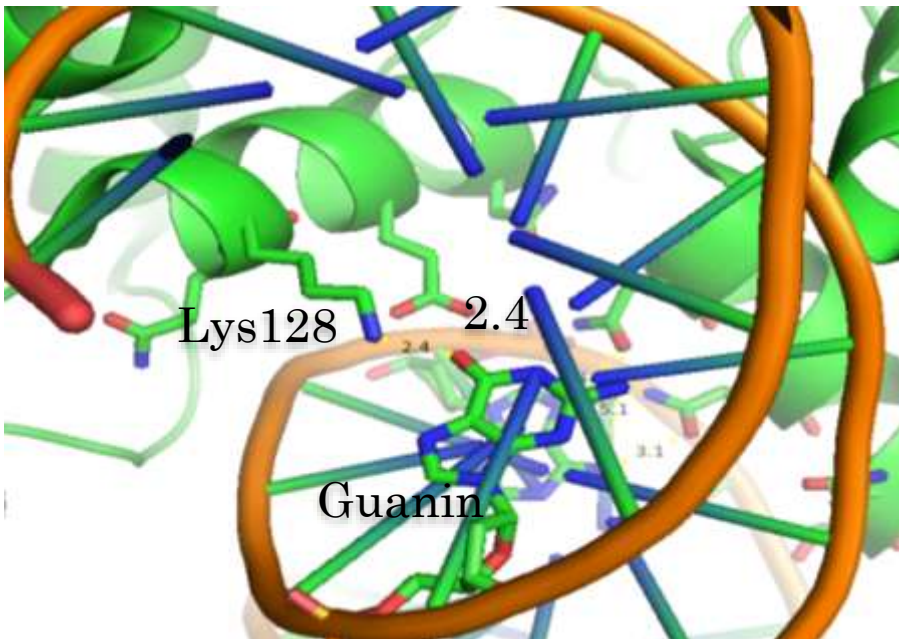
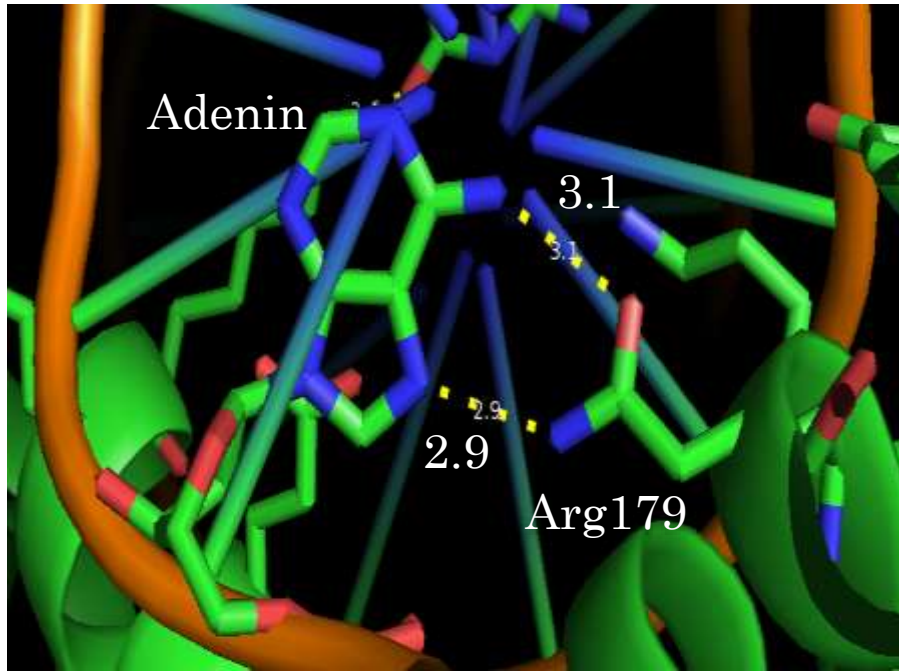


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MOL Viewer
131 136 174 181 186 1191
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- Asn-179
- Lys-182
- Asn-183
- Asn-186
- Ser-187

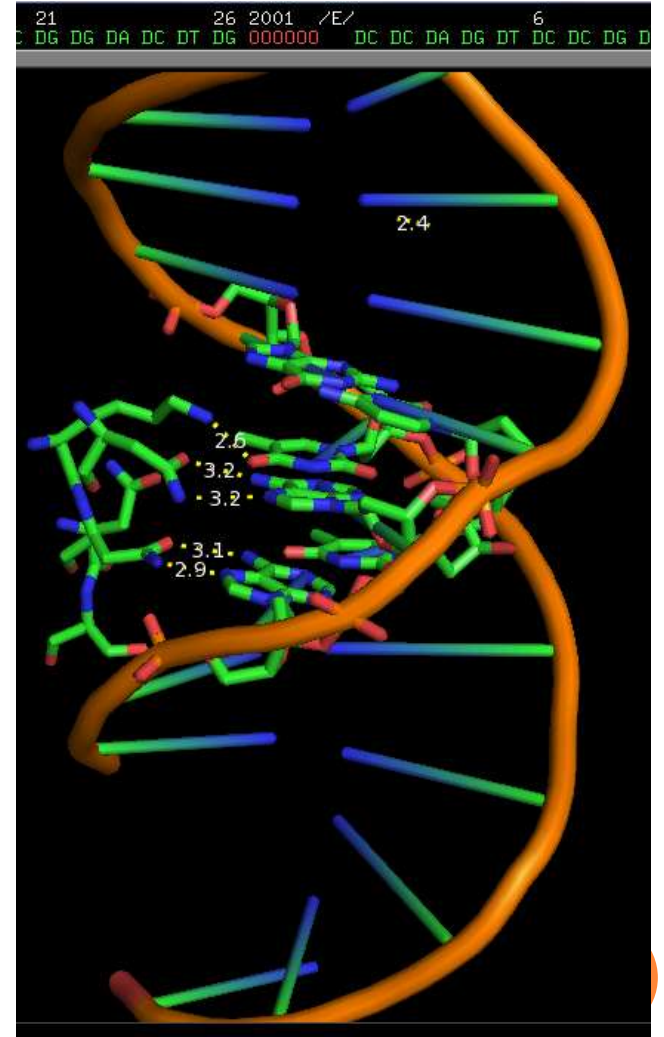
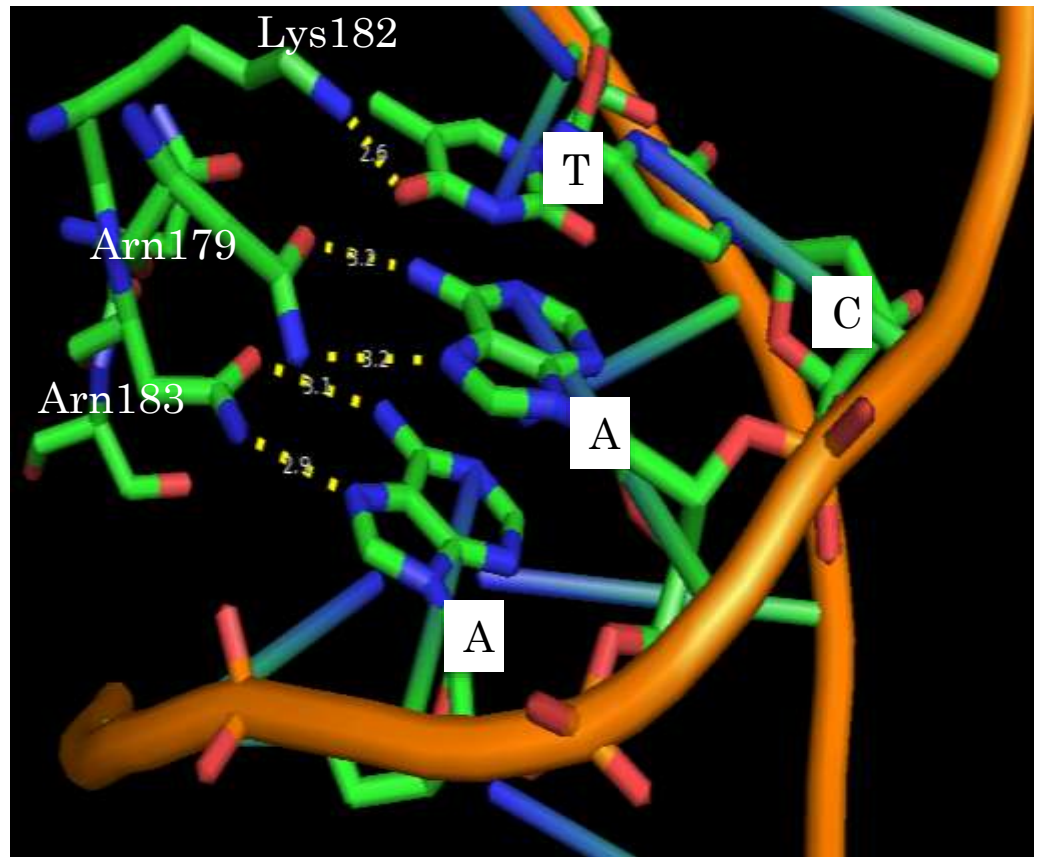


○ Asparajinler Adenin N6 ve N7 ile çift bağ...



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DT DT DA DA DC DG DG DA DC DT DG 000000 DC DC DA DG DT DC DC DC DT DT DA DA
  
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a

	small	medium	large	aromatic
A	Cys,Ser, Thr 10	Asn 15 Asp 9 His 8	Gln 15 Glu 9 Arg,Lys 3 Met 5	Tyr 5 Trp 5
T	Ala 10 Cys,Ser, Thr	Val,Ile 12 Asn 10 His 8	Leu,Met 12 Gln 10 Arg,Lys 5	Tyr,Phe 12 Trp 12
G	Cys,Ser, Thr 10	His 12 Asn 10	Arg,Lys 15 Gln 10	Tyr 5
C	Val 8 Cys,Ser, Thr 10	Asp 12 Asn 10 His 8 Ile 8	Glu 12 Gln 10 Leu,Met 8	Tyr,Phe 8 Trp 8

Arg, Lys

5'-AAC-3'

3'-TTG-5

BLAST ANALİZLERİ
Aday gen listeleri....

Deney tasarımları...

b

aa	C(i)—C(i+1)	C(i)—W(i+1)	W(i+1)-W(i)	W(i+1)-C(i)
Asp, Glu	A C C	A A C	A A C	C A C
Asn, Gln Ser, Cys Thr, Tyr	A A A T T G G C C C	A C G A C A C T G	A A A T G	A T G C C
Lys, Arg	G T A G G	G G G T A	G T A	G G G
Leu, Val Ile	T T C	T C C		

TEŞEKKÜRLER...