

NUKLEINSKE KISLINE

zaporedja, analize

- iskanje zaporedij v GenBank, razlike med zaporedji cDNA in genomskega zaporedjem, EST kloni;
- nukleotidno zaporedje in komplementarno zaporedje; format zaporedij (FASTA); vzorci v nukleotidnih zaporedjih; restrikcijske endonukleaze- prepoznavna mesta; restrikcijske karte;
- genski kod, okviri prevajanja (+1, +2, +3, -1, -2, -3) in identifikacija odprtih bralnih okvirjev; uporaba genskega koda; razlike med organizmi; optimizacija zaporedij za izražanje v različnih gostiteljih;
- načrtovanje začetnih oligonukleotidov za verižno reakcijo s polimerazo; izračun Tm;
- napoved struktur RNA;
- plazmidne mape.

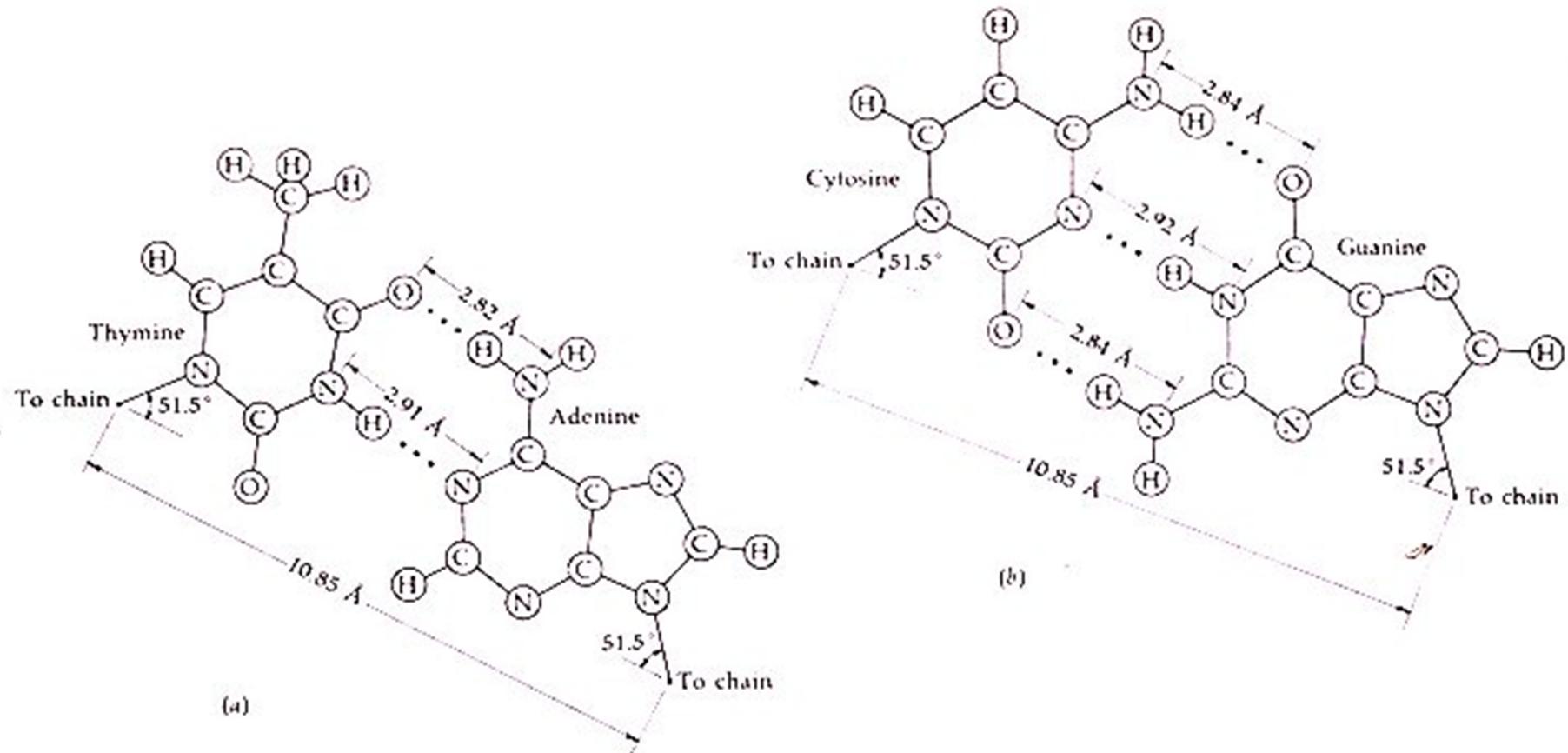
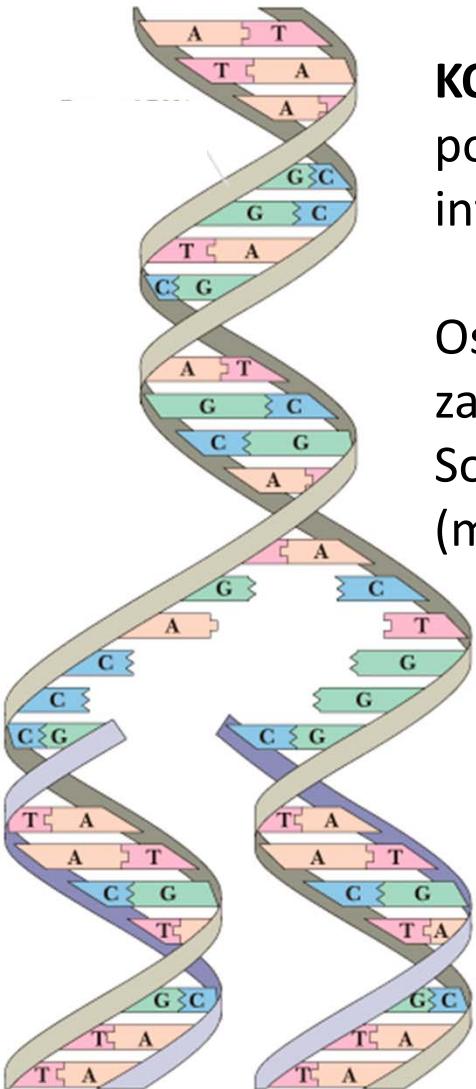


FIGURE 9.4 Dimensions and hydrogen bonding of (a) thymine to adenine and (b) cytosine to guanine in double helix of DNA. (From M. H. F. Wilkins and S. Arnott, *J. Molecular Biol.*: 11: 391, 1965.)

LASTNOSTI DVOJNEGA HELIKSA



KOMPLEMENTARNOST BAZ

pomembna pri prenosu genetske informacije

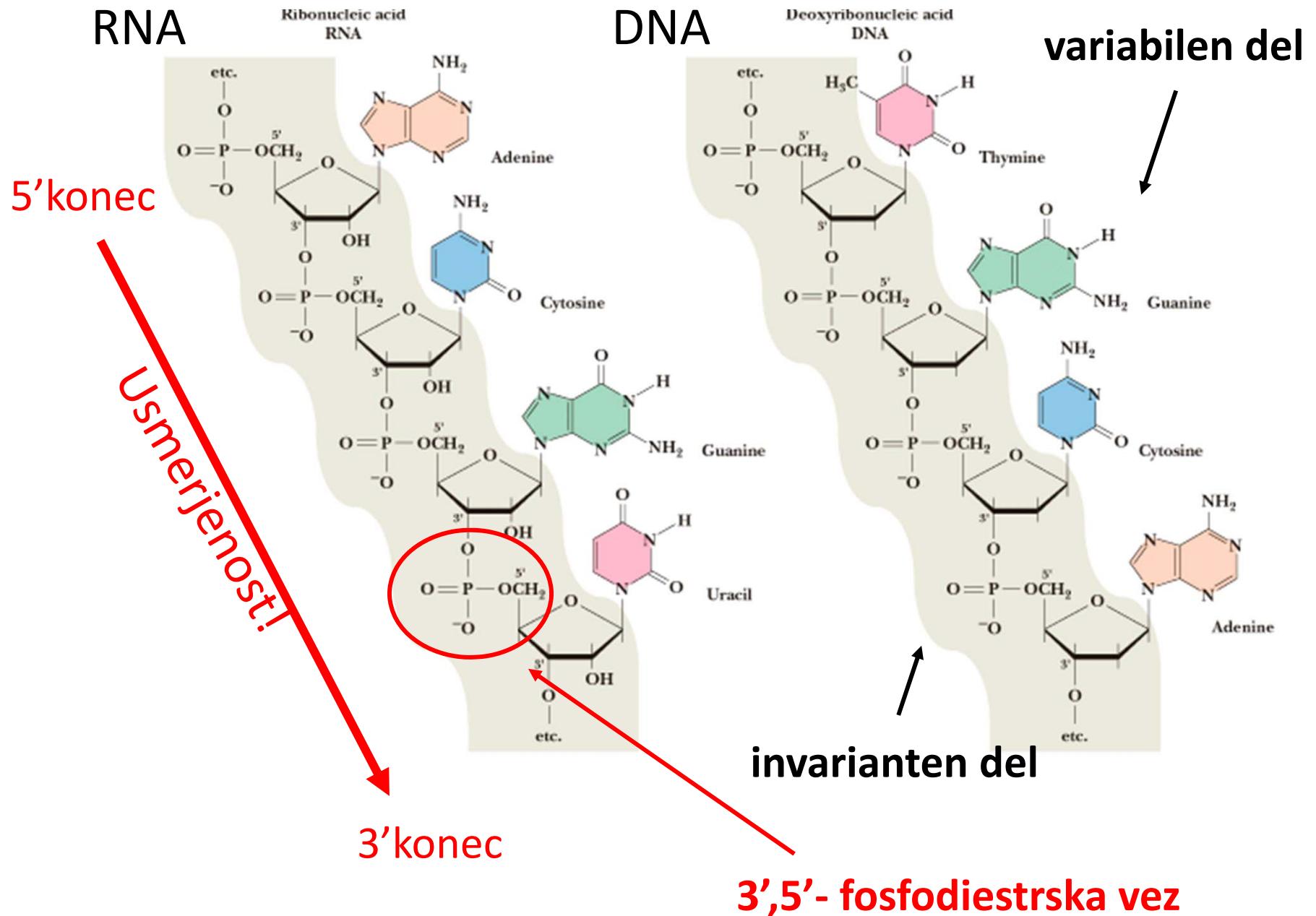
Osnova **HIBRIDIZACIJE**- pomembna metoda za detekcijo specifičnih koščkov DNA:
Southern, Northern blotanje; Genski čipi (mikromreže); PCR

devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

~~Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.~~

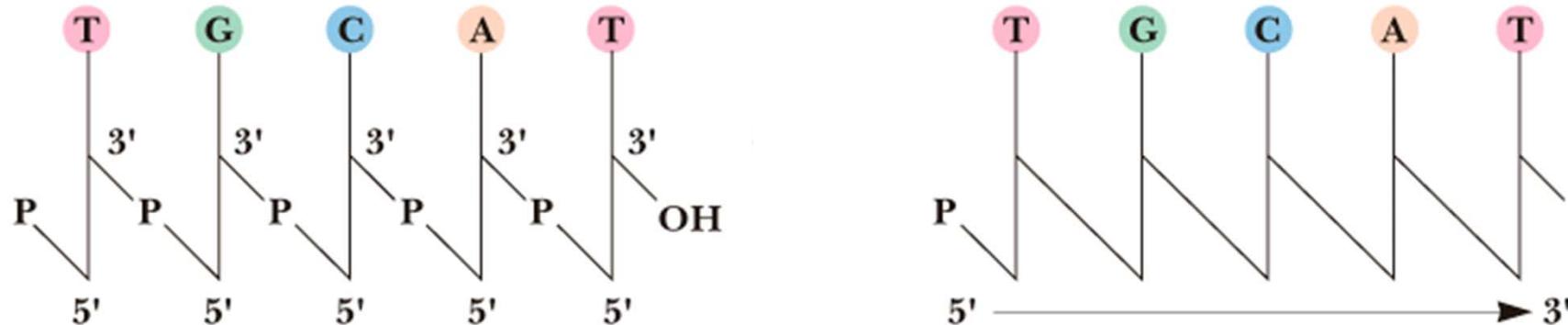
NUKLEINSKE KISLINE



Predstavitev nukleinskih kislin

- **Enočrkovni simboli:** A, C, G, T
- **Predstavitev negotovosti na posameznem mestu:** zaradi napake ali dejansko obstaja več možnosti (različne variante zaporedij, prepoznavna mesta za transkripcijske faktorje, restrikcijske endonukleaze, itn.)

SHEMATSKA PREDSTAVITEV



Oligonukleotid: TGCAT

Zaporedje nukleinskih kislin zapisujemo od 5' proti 3' koncu!

>gi|14270147|emb|AL365230.8 | Human DNA sequence from clone RP11-384G23
on chromosome 13, complete sequence

CTTCCTTAATTAGAGCTTCAGTCCTGAGTAAATCTAGGCAGTAAATAGGCCTGGGCTTAAAATAATT
TTTCCTATTGTGTAACAATAAGAGAATTGATAGATTGATATTGTGGGTTGTATGCACTCTGCCTTGG
GTGAAAGTCAGGACAACCCTAGCTATTGCCATGATCCAGGGCCTAGGTGATGGCTCTTCTTAGGGG
CAGTATTGAGGTCAGAATATAAAGAAAGGGCAAAAGGAATAAAATAAAATTAGTCCAAACAATTGTAC
AAATAACCCCAAATATGGTGGTGGTCTCTTCAAAACTGAGTATAAGTTAACACACCTCACTGTGGTA
GAACGGAAACTTCTGAGCTCAGATCAAAGTTCCAAGGGAGTGTCAAAATGCAATATAACTTATTGGGA
TTTCTAAAATTAGCTTCAGTTGATAGTTGTTAAAATAAGACCCCTAGTTCTGGTCCAACTAGTGGGTT
ATCTCTTACATTGAAGATCCTATTTTATTCTACCTCAGCCTTCATAATTAAAATTCTTCAACAGATT
TGCAAAGTATACCCATAAAATATAAAATAATGTGATGGAATAATTAAGTCATACTTGGAAAAACAGTATT
ATGAATCCAATGTATAGACAGTAAATAAGCCAATAACTTAAACTCAACTAGAACATTAATTAGAATATAA
ACCTTGCTAAAACAATAGATAACATTATTGGGCACTACTTGCATCTAACACTATGTTAGATGTCTT
ACATCTTAATGTGTGTTACTACCACACATTAATCCTGTGATAGTAACCTTTATCCCCATTACATAG
ATAAATAAACGGAGACTTATAGAATGTCTTAGTCCATTCTGGCTATAACAAAGTACCCCTAGACTGGG
TAATTATAATCAACAAAAATCTATTGCTCACAGTTCTGGAGGCTGGGAAGTCCAAGATCAAGGCACCAC

IUPAC nomenklatura

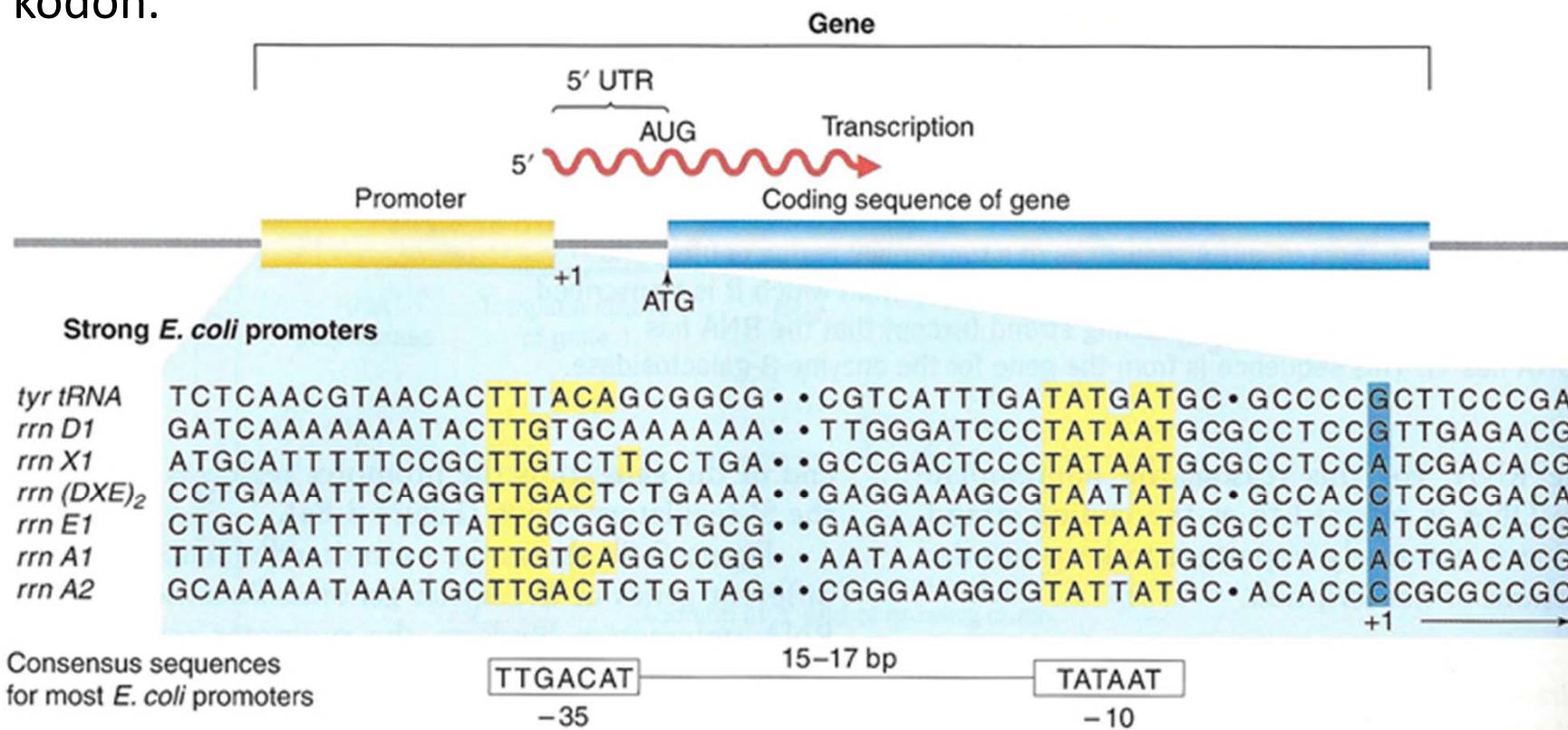
Symbol ^[2]	Description	Bases represented				
A	Adenine	A				1
C	Cytosine		C			
G	Guanine			G		
T	Thymine				T	
U	Uracil				U	
W	Weak	A			T	2
S	Strong		C	G		
M	aMino	A	C			
K	Keto			G	T	
R	puRine	A		G		3
Y	pYrimidine		C		T	
B	not A (B comes after A)		C	G	T	
D	not C (D comes after C)	A		G	T	4
H	not G (H comes after G)	A	C		T	
V	not T (V comes after T and U)	A	C	G		4
N or -	aNy base (not a gap)	A	C	G	T	

Vir: Wikipedia

Signali za transkripcijo in translacijo (prokarionti)

Signali za transkripcijo: promotorsko področje, začetek transkripcije, konec transkripcije (terminator), drugi regulacijski elementi (represorji, enhancerji).

Signali za translacijo: mesto vezave na ribosom, startni kodon, genski kod, stop kodon.



Operon	-35 region	-10 region	Initiation (Pribnow box)	Initiation site (+1)
<i>lac</i>	ACCCCAAGGCTTTACACTTATGCTTCCGGCTCGTATGTTGTGTGGATTGTGAGCGG			
<i>lacI</i>	CCATCGAATGGCGCAAAACCTTCGCCTGATGGCATGATAGCGCCCGGAAGAGAGTC			
<i>galP2</i>	ATTTATTCCATGTACACTTTCGCATCTTGTTATGCTATGGTTATTTCATACCAT			
<i>araBAD</i>	GGATCCCTACCTGACGCTTTTATCGCAACTCTACTGTTCTCCATAACCGTTTT			
<i>araC</i>	GCCGTGATTATAGACACTTTGTTACGCCTTTGTCATGGCTTGTCGGCTTGGTCCCCTTTG			
<i>trp</i>	AAATGAGCTGTTGACAATTAAATCATCGAACTAGTTAACTAGTACGCAAGTTCACGTA			
<i>bioA</i>	TTCCAAAAACGTGTTTTTGTGTTAAATTCGGTGTAGACTTGTAAACCTAAATCTTTT			
<i>bioB</i>	CATAATCGACTTGAAACCAAATTGAAAAGATTAGGTTACAAGTCTACACCGAAT			
tRNA ^{Tyr}	CAACGTAACACACTTACAGCGGCCGTCATTGATATGATGCGCCCCGCTTCCCATA			
<i>rrnD1</i>	CAAAAAAAATACTTGTGCAAAAAATTGGGATCCCTATAATGCGCCTCCGTTGAGACGA			
<i>rrnE1</i>	CAATTTCCTATTGCGGCCCTGCGGAGAACCTCCCTATAATGCGCCTCCATCGACACGG			
<i>rrnA1</i>	AAAAATAATGCTTAGCTGTAGCGGGAAAGGCGTATTATGCACACCCCCGGCGCCGCTG			

Consensus sequence:	-35 region						-10 region						Initiation site				
	T	T	G	A	C	A	...	16-19 bp...	T	A	T	A	A	T	...	5-8 bp...	A
	69	79	61	56	54	54			77	76	60	61	56	82			C 55 G 42

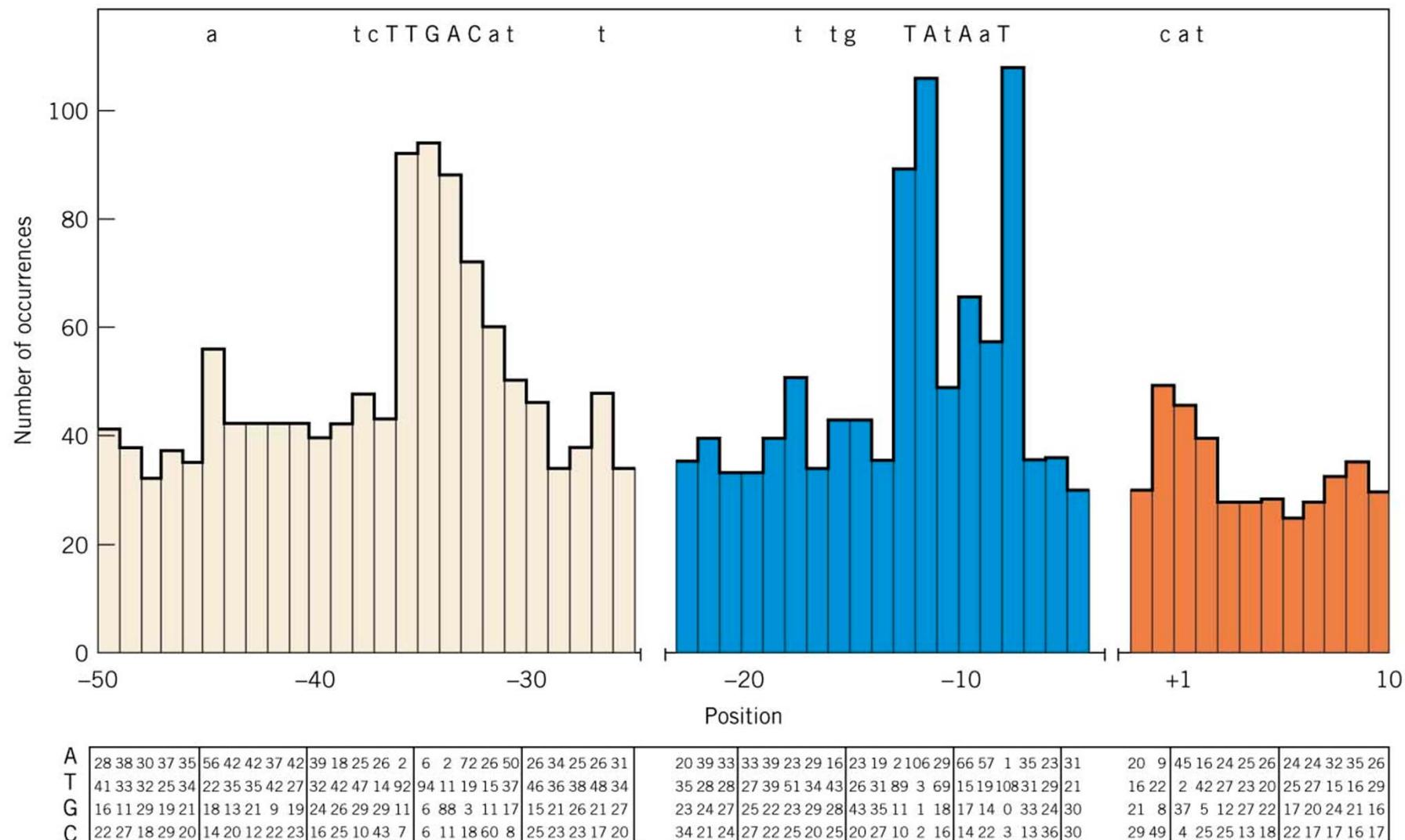
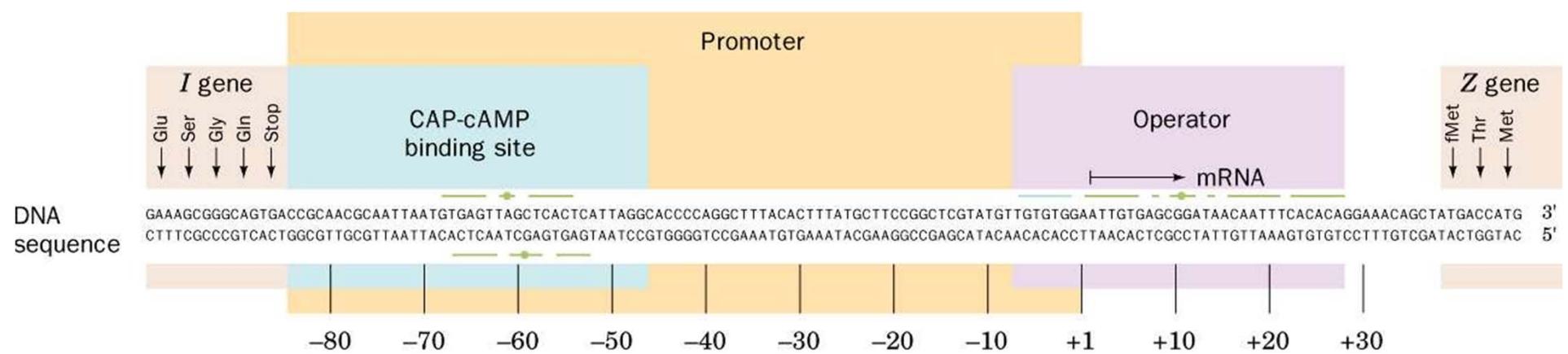


Figure 5.1. A consensus sequence for prokaryotic promoters. Redrawn from Hawley, D. K. and McClure, W. R. *Nucleic Acids Res.* 11:2237, 1983.

Textbook of Biochemistry With Clinical Correlations, Sixth Edition, Edited by Thomas M. Devlin. Copyright © 2006 John Wiley & Sons, Inc.



SHINE-DALGARNOVO ZAPOREDJE

5' -mRNA-3'

araB

gale

lacI

lacZ

Q β fagna replikaza

φX174 fagni A protein

R17 fagni plaščni protein

ribosomalni protein S12

ribosomalni protein L10

trpE

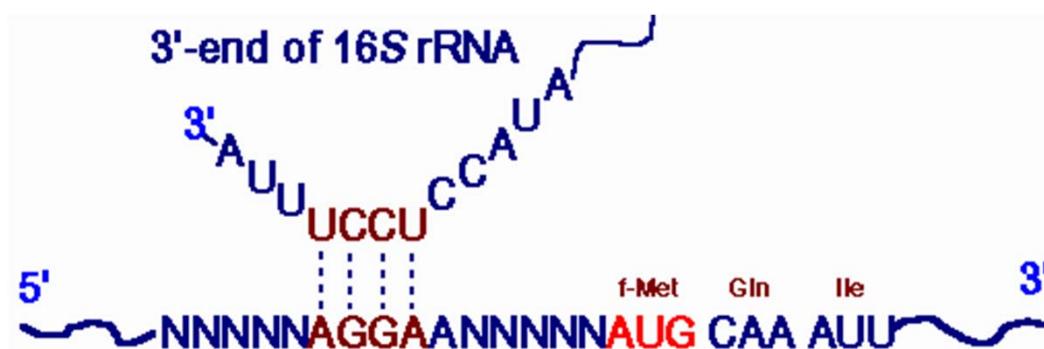
trpL

STARTNI KODON

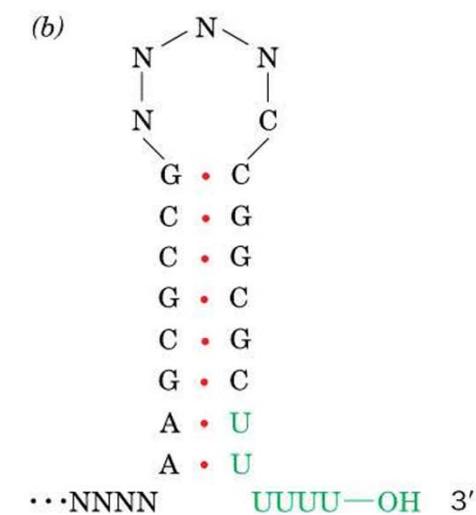
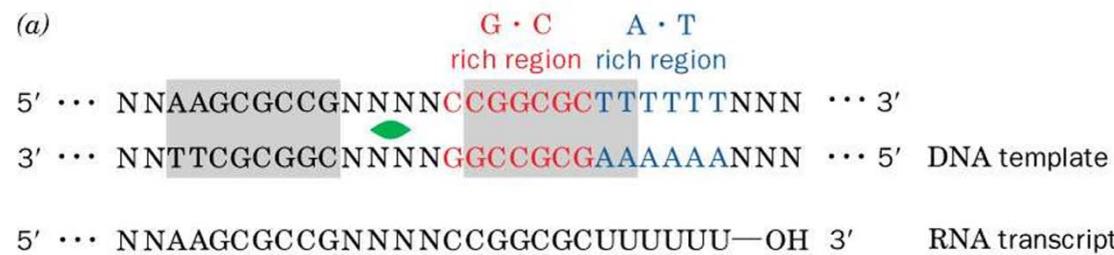
UUUGGAUGGAGUGAAACGAUGGCGAUU
 AGCCUAAUGGAGCGAAUUAUGAGAGUU
 CAAUUCAGGGGUGGUGAUUGUGAAACCA
 UUCACACAGGGAAACAGCUAUGACCAU
 UAACUAAGGAUGAAAUGCAUGUCUAAG
 AAUCUUGGAGGCUUUUUAUGGUUCGU
 UCAACCGGGUUUGAAGCAUGGCUUCU
 AAAACCAGGAGCUUUUAAUGGCAACA
 CUACCAGGAGCAAAGCUAAUGGCUUUA
 CAAAAUUAGAGAAUAAACAAUGCAAACA
 GUAAAAAAGGUAUCGACAAUGAAGCA

3' -konec 16S rRNA

3' -AUUCCUCCACUAG- 5'



E. coli terminator



CEPITEV DNA IN RNA Z NUKLEAZAMI

NUKLEAZE

- katalizirajo hidrolizo fosfodiestske vezi
- razgradnja nukleinskih kislin kot del normalnega celičnega delovanja

Deoksiribonukleaze DNaze

Ribonukleaze RNaze

Eksonukleaze cepijo s koncem

5'-eksonukleaze, 3'-eksonukleaze

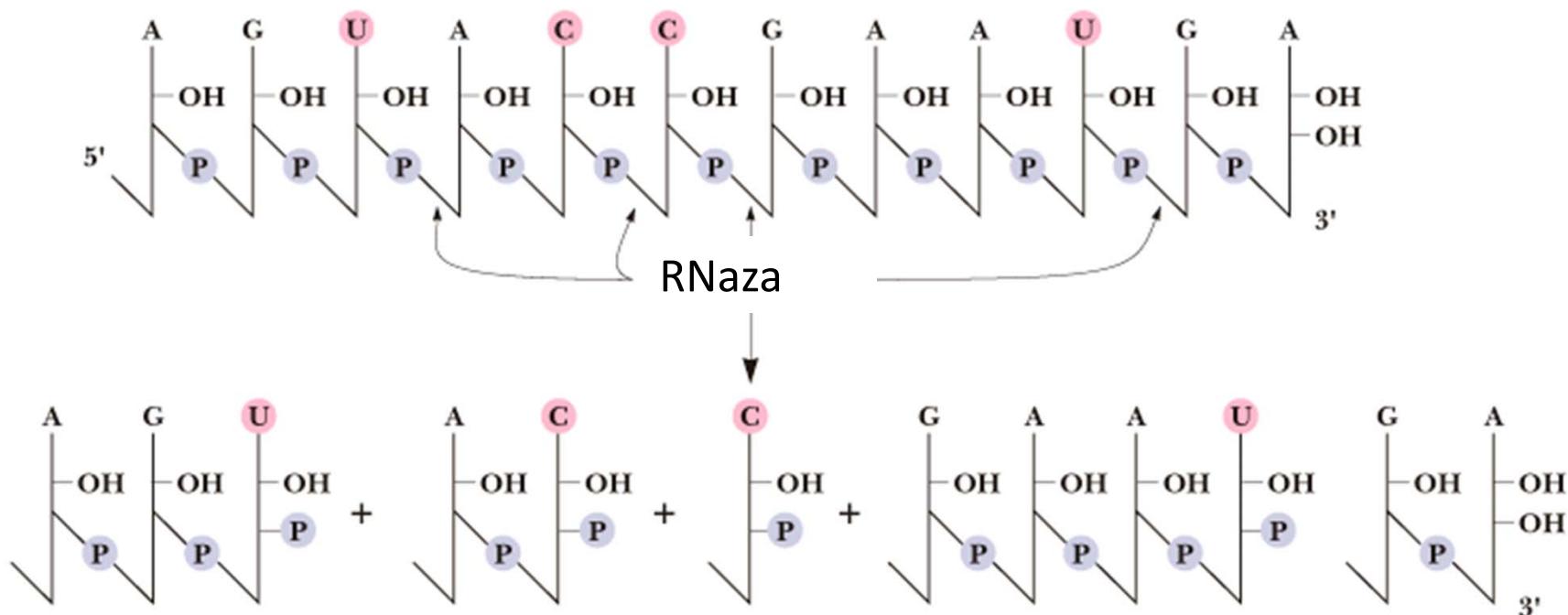
Endonukleaze cepijo znotraj verige

Tip a, tip b glede na to, kje cepijo fosfodiestsko vez

Restriktivne endonukleaze koristno orodje v molekularni biologiji; specifično prepozna 4-6 nukleotidov v zaporedju

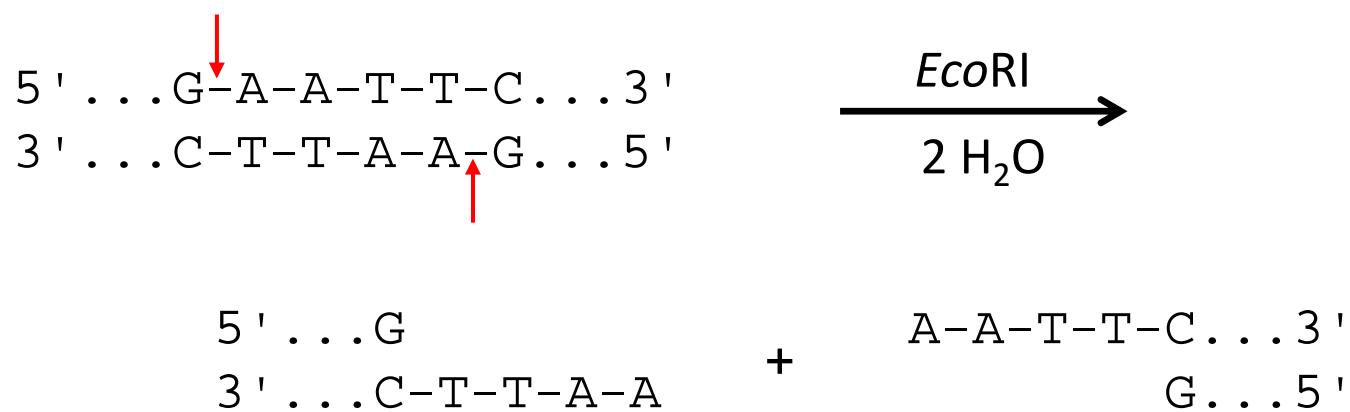
Encim	Substrat	Tip	Specifičnost
<i>Standardne nukleaze</i>			
Fosfodiesteraza iz klopotače	DNA, RNA	ekso (a)	s 3' konca, ni
Fosfodiesteraza iz vranice	DNA, RNA	ekso (b)	s 5' konca, ni
Ribonukleaza A iz treb. slinavke	RNA	endo (b)	pirimidin na 3' strani
Vranična DNaza	DNA	endo (b)	ni
<i>Restriktijske endonukleaze</i>			
<i>EcoRI</i>	DNA	endo (a)	GAATTC
<i>BalI</i>	DNA	endo (a)	TGGCCA
<i>TaqI</i>	DNA	endo (a)	TCGA
<i>HinfI</i>	DNA	endo (a)	GANTC s krepkim označeno mesto cepitve

Pankreasna ribonukleaza (RNaza): tip b. Endonukleaza, cepi za pirimidinskimi bazami. V endoplazemskem retikulumu, transportiran tudi v črevesje, kjer sodeluje pri prebavi hrane.



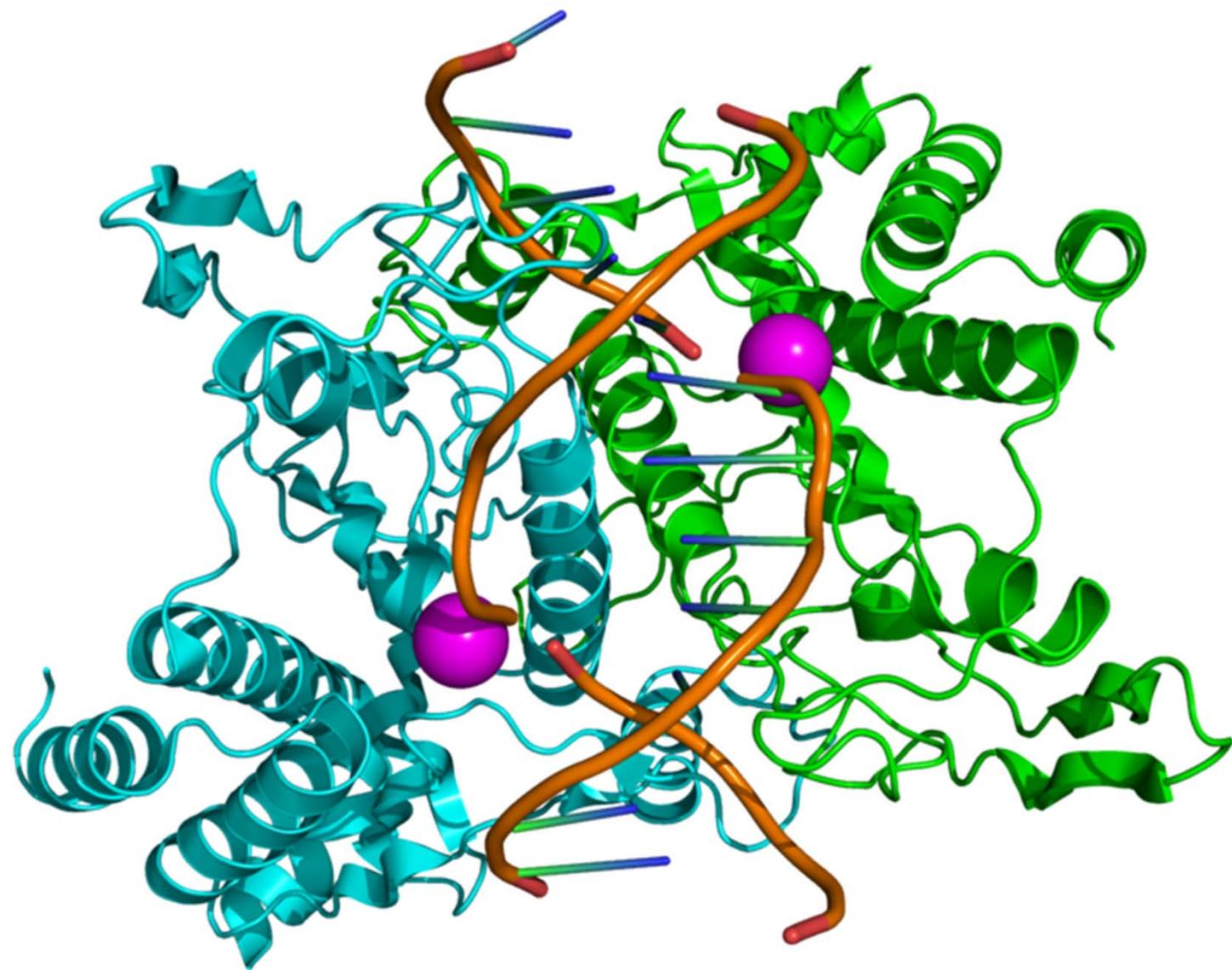
RESTRIKCIJSKE ENDONUKLEAZE

- izjemno uporabni encimi
- endonukleaze
- pri bakterijah obramba pred vdorom tuje DNA
- prepoznajo 4-6 nukleotidov dolgo zaporedje in cepijo to ali v bližini tega mesta
- prepoznavno mesto ima 2-kratno os simetrije
- po cepitvi nastanejo “topi” ali “lepljivi” konci
- *EcoRI* je prvi znani restrikcijski encim iz *E. coli* (sev RI)



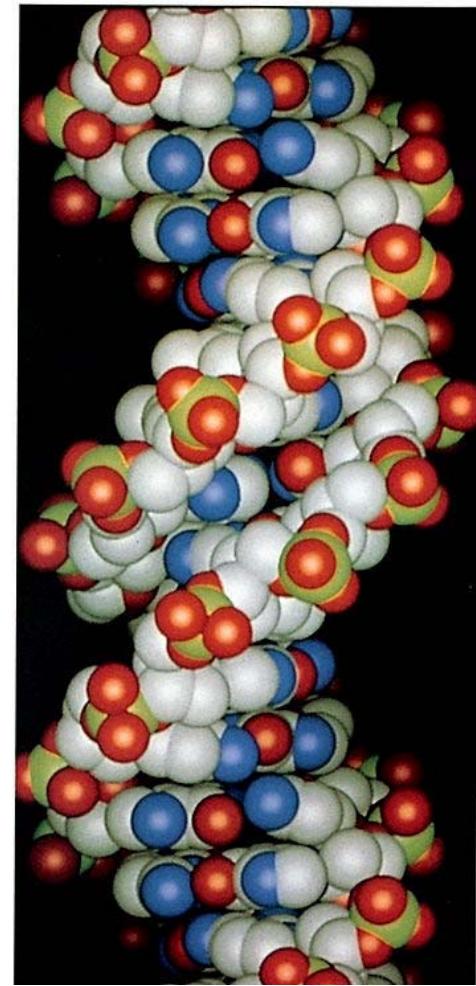
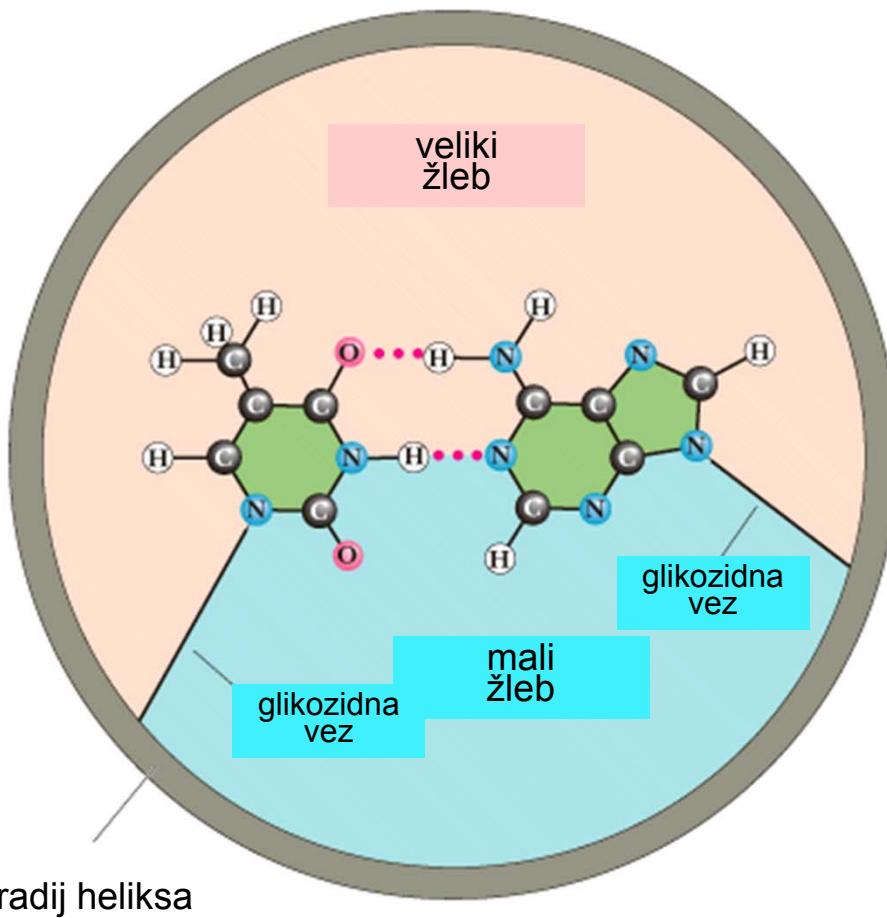
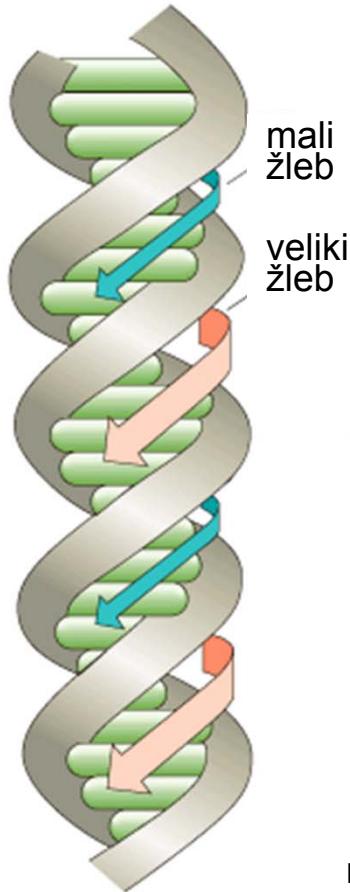
- prepoznavna mesta so palindromi
Npr. PERICA REŽE RACI REP

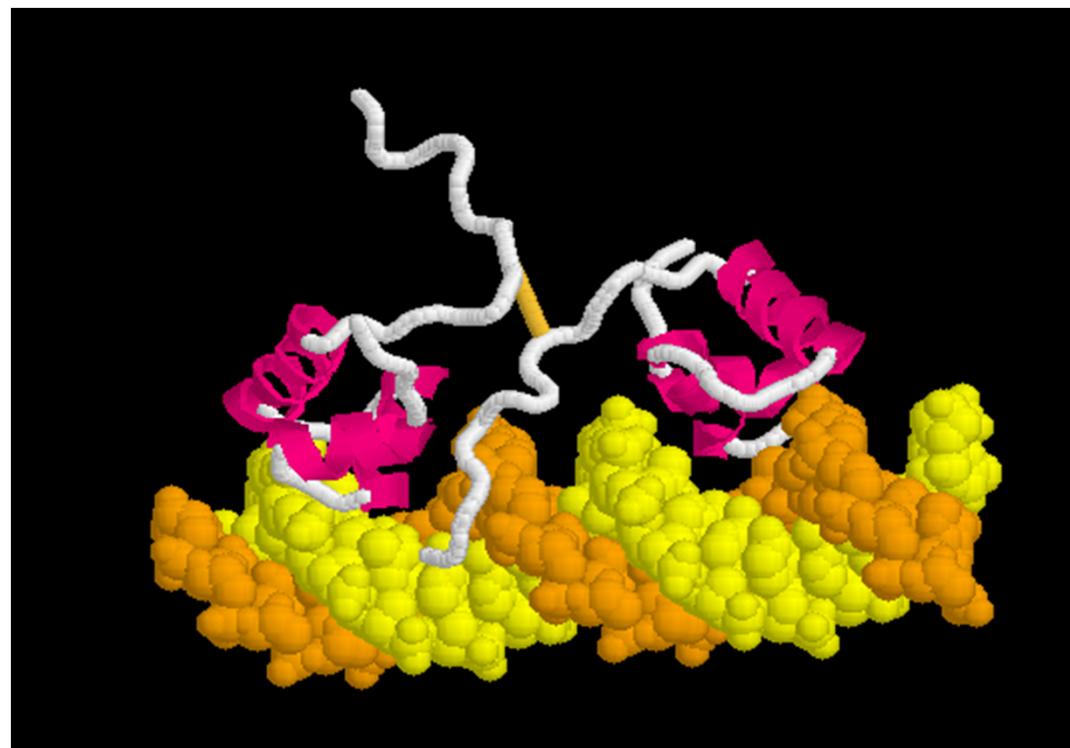
<i>Microorganisms</i>	<i>Restriction enzymes</i>	<i>Cleavage sites</i>	<i>Cleavage products</i>	
<i>Bacillus amyloquefaciens H</i>	<i>Bam</i> HI	5-GGATCC-3 3-CCTAGG-5	5-G 3-CCTAG	GATCC-3 G-5
<i>B. globigii</i>	<i>Bgl</i> II	5-AGATCT-3 3-TCTAGA-5	5-A 3-TCTAG	GATCT-3 A-5
<i>Escherichia coli RY13</i>	<i>Eco</i> RI	5-GAATTC-3 3-CTTAAG-5	5-G 3-CTTAA	AATTC-3 G-5
<i>Haemophilus influenzae Rd</i>	<i>Hin</i> dIII	5-AAGCTT-3 3-TTCGAA-5	5-A 3-TTCGA	AGCTT-3 A-5
<i>H. parainfluenzae</i>	<i>Hpa</i> I	5-GTTAAC-3 3-CAATTG-5	5-GTT 3-CAA	AAC-3 TTG-5
<i>Klebsiella pneumoniae</i> OK 8	<i>Kpn</i> I	5-GGTACC-5 3-CCATGG-3	5-GGTAC 3-C	C-3 CATGG-5
<i>Streptomyces albus G</i>	<i>Sal</i> I	5-GTCGAC-3 3-CAGCTG-5	5-G 3-CAGCT	TCGAC-3 G-5
<i>Staphylococcus aureus</i> 3AI	<i>Sau</i> 3AI	5-GATC-3 3-CTAG-5	5- 3-CTAG	GATC-3 5



EcoRI

Vir: Wikipedia







The Nobel Prize in Physiology or Medicine 1978

Werner Arber

Daniel Nathans

Hamilton O. Smith

"for the discovery of restriction enzymes and their application to problems of molecular genetics"



"Their research opens up the possibility to copy human beings in the laboratory, to construct geniuses, to massproduce workers, or to create criminals." This is a quotation from the presentation on Swedish television of this year's laureates in medicine.



Dr. Arber, Dr. Nathans, Dr. Smith: **The discovery of restriction enzymes started off an avalanche in molecular genetics.** Their application made possible the detailed chemical analysis of the organisation of the genetic material, and this has in particular in higher organisms given unexpected but far reaching results. At long last we are in a position to tackle successfully the basic problem of cell differentiation. Your work has pioneered this development.

Recognition Sequence	Enzymes
AA/CGTT	Ad I
A/AGCTT	Hind III
AAT/ATT	Ssp I
/AATT	Tsp509 I
A/CATGT	Pci I
A/CCGGT	Age I
ACCTGC(4/8)	BfuA I
ACCTGC(4/8)	BspM I
A/CCWGGT	SexA I
A/CGCGT	Mlu I
ACGGC(12/14)	BceA I
A/CGT	HpyCH4 IV
ACN/GT	HpyCH4 III
(10/15)ACNNNNGTAYC(12/7)	Bae I
(9/12)ACNNNNNCTCC(10/7)	BsaX I
A/CRYGT	Afl III
A/CTAGT	Spe I
ACTGG(1/-1)	Bsr I
ACTGGG(5/4)	Bmr I
A/GATCT	Bgl II
AGC/GCT	Afe I
AG/CT	Alu I

RESTRIKCIJSKE ENDONUKLEAZE izredno uporabne za klonirnje DNA v molekularni biologiji

(0/2)CATCC	BstF5 I
(13/9)CATCC	Fok I
(14/10)CATCGC	BtgZ I
C/ATG	CviA II
/CATG	Fat I
CATG/	Nla III
(0/2)CATTGC	BsrD I
CAYNN/NNRTG	Msl I
(10/12)CCACNNNNNTTG	CspC I
(-1/1)CCAGT	Bsr I
CCANNNNN/NNNNNTGG	Xcm I
CCANNNNN/NTGG	BstX I
CCANNNNN/NTGG	PflM I
CCATC(4/5)	Bcc I
C/CATGG	Nco I
CCCAGC(-5/-1)	BseY I
(4/5)CCCAGT	Bmr I
CCCAGC(4/6)	Fau I
CCC/GGG	Sma I
C/CCGGG	Xma I
CCGC(-3/-1)	Aci I
CCGC/GG	Sac II
-----	---

Recognition Sequence	Enzymes
AGC/GCT	Afe I
AG/CT	Alu I
AGG/CCT	Stu I
AGT/ACT	Sca I
AT/CGAT	BspD I
AT/CGAT	Cla I
ATG/CAT	BfrB I
ATGCA/T	Nsi I
AT/TAAT	Ase I
ATTT/AAAT	Swa I
(11/13)CAANNNNNGTGG(12/10)	CspC I
C/AATTG	Mfe I
CACGAG(-5/-1)	BssS I
CACGTC(-3/-3)	BmgB I
CAC/GTG	Pml I
CACNNN/GTG	Dra III
CACNN/NNGTG	Ale I
(0/2)CACTGC	Bts I
CAGCAG(25/27)	EcoP15 I
CAG/CTG	Pvu II
CAGNNN/CTG	AlwN I
CASTGNM	TspR I

NEBcutter V2.0 - Windows Internet Explorer
http://tools.neb.com/NEBcutter2/ NEBcutter V2.0

File Edit View Favorites Tools Help
Google web2 cutter Išči Skupna raba Dodatno » Prijava Convert Select

NEBcutter V2.0

NEW ENGLAND BioLabs[®] inc.
[\[NEB homepage\]](#)

Program Guide Help Comments

This tool will take a DNA sequence and find the large, non-overlapping open reading frames using the E.coli genetic code and the sites for all Type II and commercially available Type III restriction enzymes that cut the sequence just once. By default, only enzymes available from NEB are used, but other sets may be chosen. Just enter your sequence and "submit". Further options will appear with the output. **The maximum size of the input file is 1 MByte, and the maximum sequence length is 300 Kbases.**

[What's new in V2.0](#) [Citing NEBcutter](#)

Local sequence file: Browse... Standard sequences:
GenBank number: [\[Browse GenBank\]](#) # Plasmid vectors
or paste in your DNA sequence: (plain or FASTA format) # Viral + phage

TTACTTACCAAGAAAACCTTACCAAGCTTGTGAGGGTCCAAATTGGAACGATAAACATCATTAGAA
AGGGAAAAAAAGATAATTCATGCAACTGTTGTTTAGAGCTCTTGGCTGCTGTTGGGCTGAGA
AGTGTCCAGGGTTGTGATTAATTATGCAACAATTATGTCAGCTTTCAGGAAGATAGGGGAAG
GCAGAGAACTTGTCTAGGATCTGTTTTCAGCTCAAATAATCTTATGCCAAAGGGGAAGATTTGGA
GTGGCATATACTGTATTCCTCTGTAGGTTTACTATACAGTTCTGCTTACATGACGCTTAGTT
TAACAGGAAGCGAAGACTTAATGTAATAATCACATGTCATCAAAGATGGGAGCTCATTTCAGTTGAGTAA
AATGTCATTCAGGTTTTTTCTTAAACTTGCTCAACTTCATAAAAATAGGAGGAAGTTCTCT
TCGGGCAATAGGTCTAACCTGAAATCTACCTGAGCAATGCTTATGCAATGCAITCAITATTTCTG

Submit More options Set colors

The sequence is: Linear Circular Enzymes to use: NEB enzymes
 All commercially available specificities
 All specificities
 All + defined oligonucleotide sequences
 Only defined oligonucleotide sequences
[\[define oligos\]](#)

Minimum ORF length to display: 100 a.a.

Name of sequence: (optional)

Earlier projects:

Note: Your earlier projects will be deleted 2 days after they were last accessed.

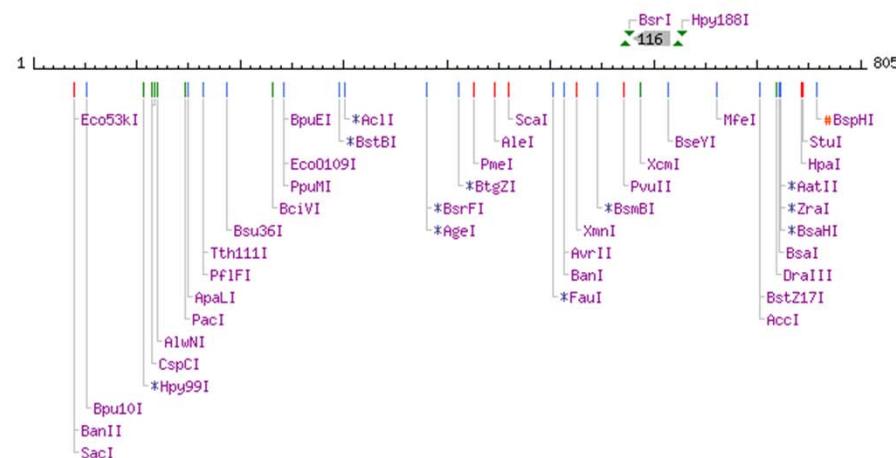
Linear Sequence: unnamed sequence
[Help](#) [Comments](#)

Display:

- NEB single cutter restriction enzymes
- Main non-overlapping, min. 100 aa ORFs

GC=36%, AT=64%

Cleavage code	Enzyme name code
✗ blunt end cut	Available From NEB
▼ 5' extension	Has other supplier
▲ 3' extension	Not commercially available
■ cuts 1 strand	*: cleavage affected by CpG meth. #: cleavage affected by other meth. (enz.name): ambiguous site



Main options

[New DNA](#)
[Custom digest](#)
[View sequence](#)
[ORF summary](#)
[Save project](#)
[Print](#)

Availability
[All commercial](#)
[All](#)

Display
[2 cutters](#)
[3 cutters](#)

Zoom
[Zoom in](#)
[More...](#)

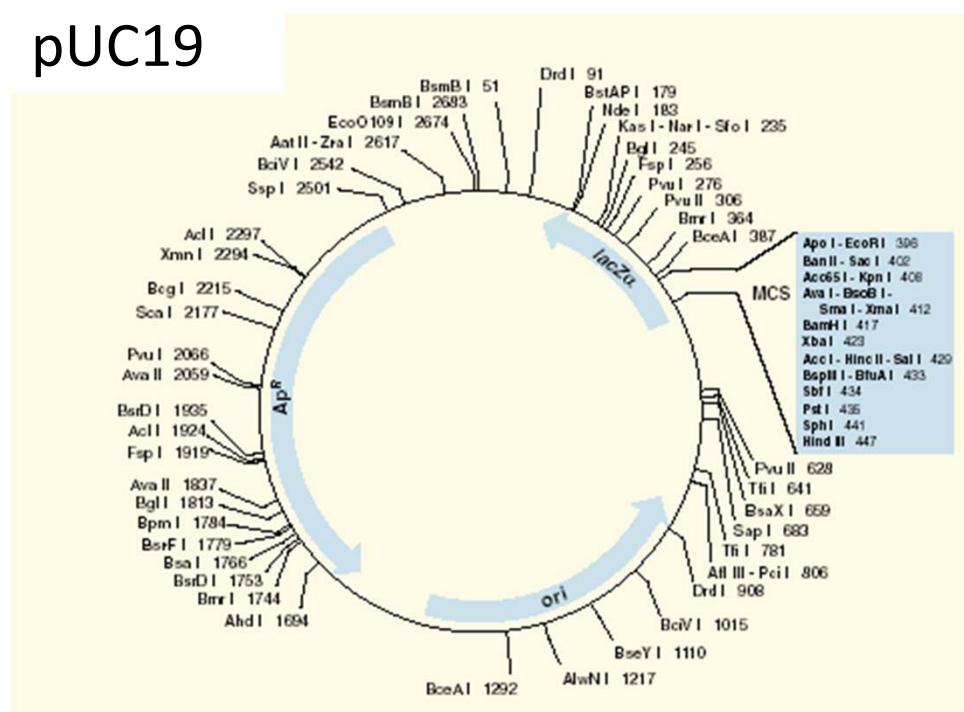
List
[0 cutters](#)
[1 cutters](#)
[All sites](#)
[Save all sites](#)
[Flanking enzymes](#)

RESTRIKCIJSKA ANALIZA

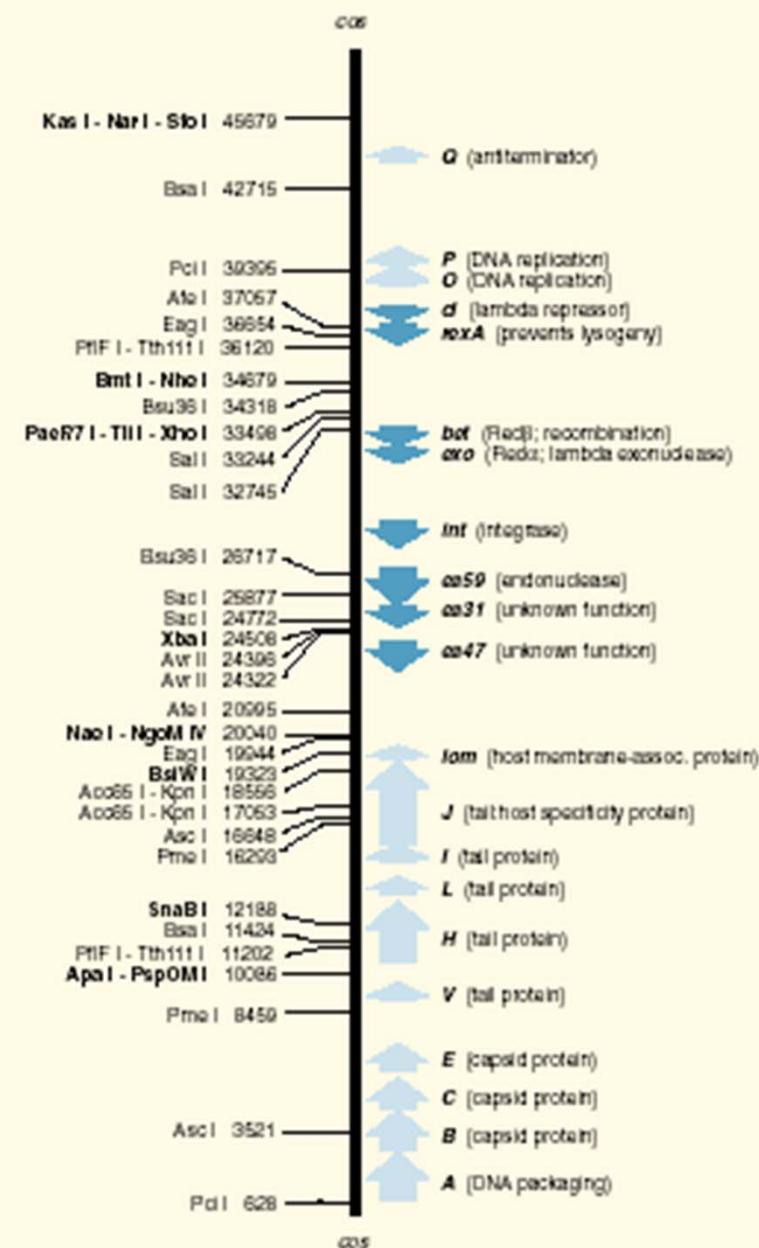
mapiranje DNA

Katera prepoznavna mesta prisotna
Katera nastopajo le enkrat ali nekajkrat

pUC19



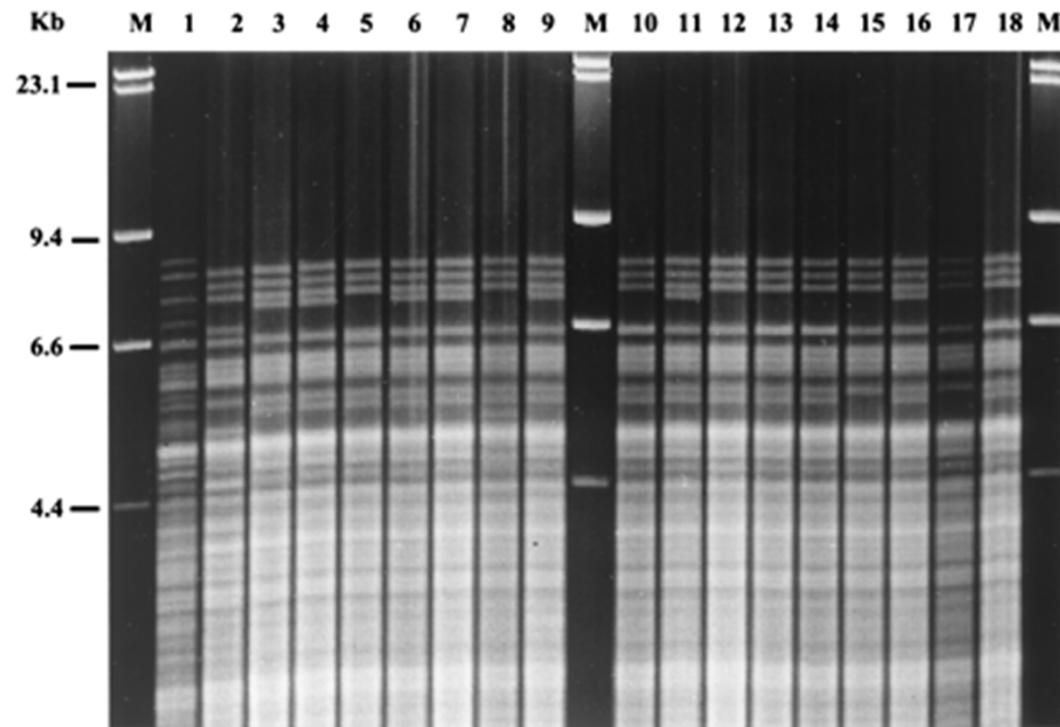
bakteriofag λ



RESTRIKCIJSKA ANALIZA

iskanje prstnega odtisa “fingerprinting”

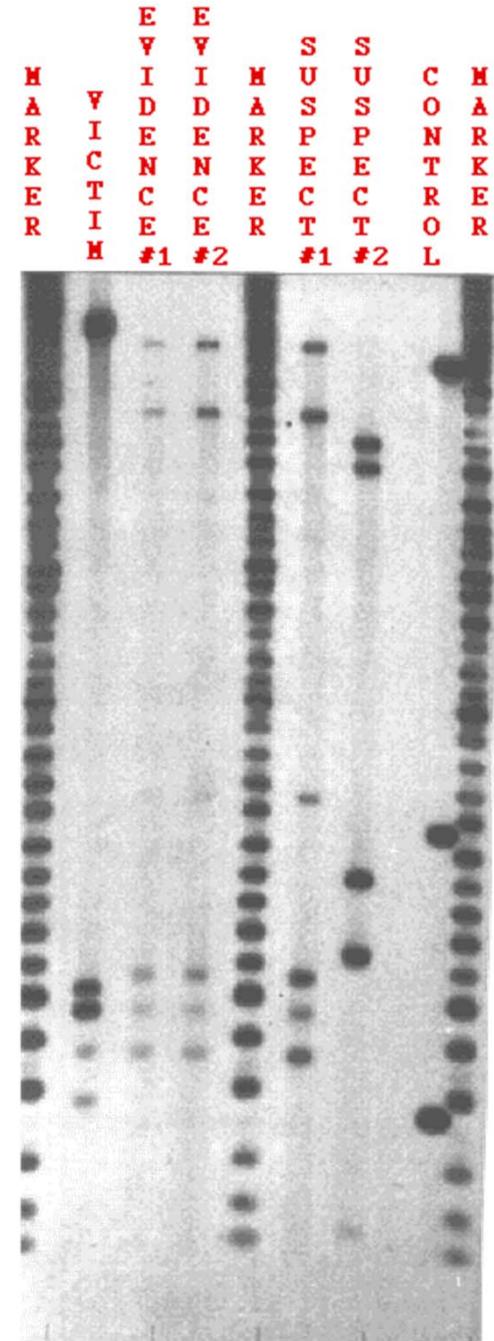
Restriction Fragment Length Polymorphism (RFLP)

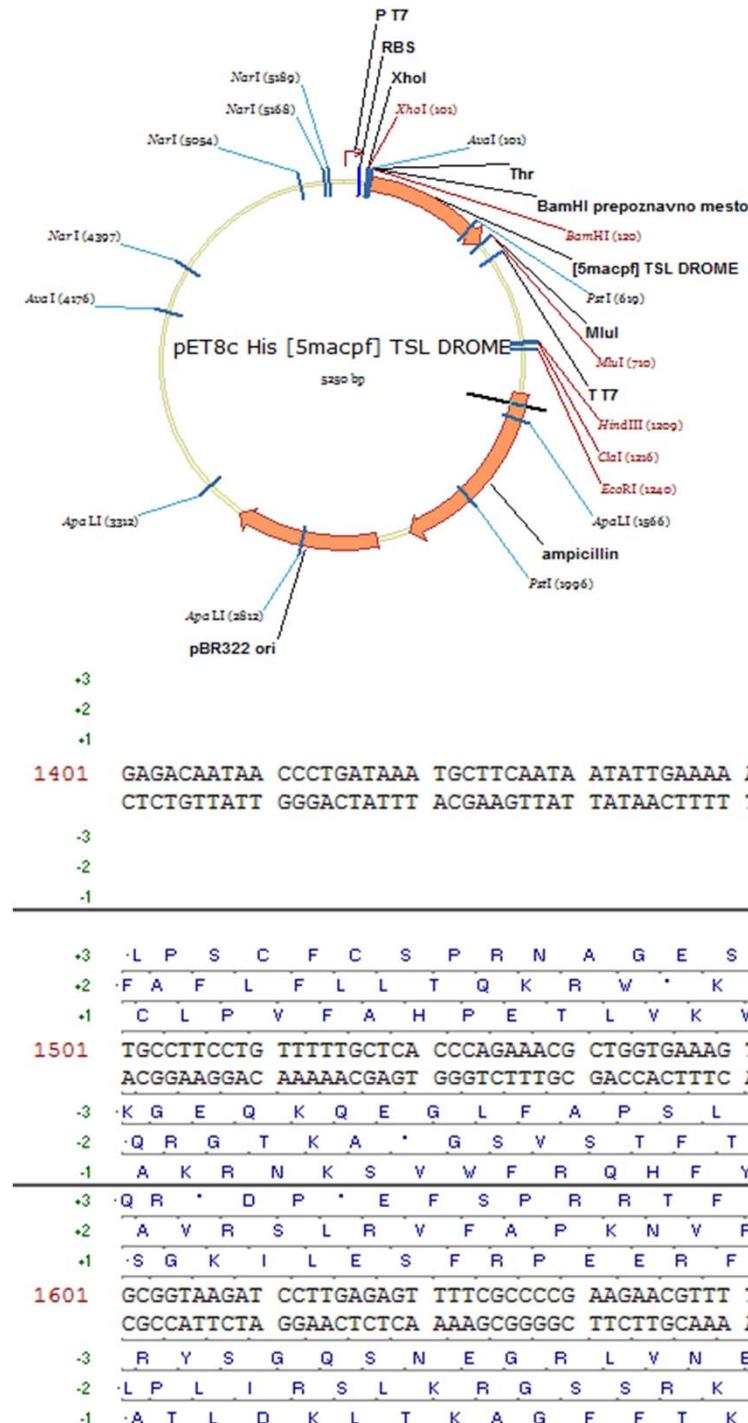


cepitev kromosomske DNA

B. bronchiseptica izolatov s *HinfI*

Sacco RE et al. (2000) J Clin Microbiol 38, 4387-4394

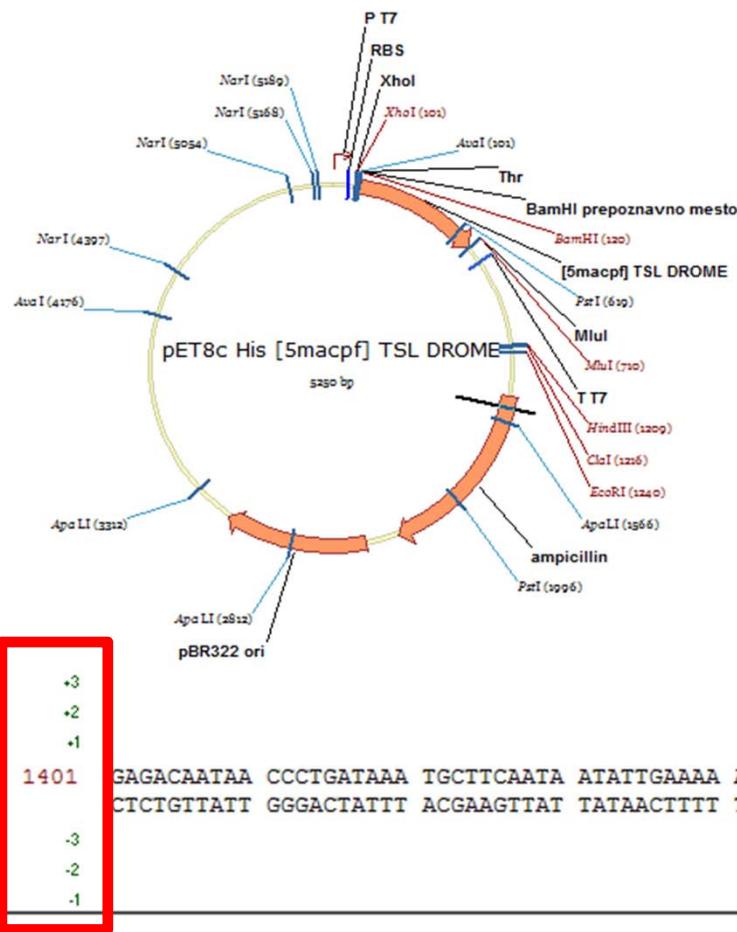




ODPRT BRALNI OKVIR

(ang. open reading frame, ORF)

Del nukleotidnega zaporedja, ki kodira protein in ne vsebuje stop kododnov (nukleotidno zaporedje med začetnim in stop kodonom).



+3 E Y S T F P C R P Y S L F C G I L
 +2 * V F N I S V S P L F P F L R H F
 +1 M S I Q H F R V A L I P F F A A F

 1401 SAGACAATAA CCTGATAAA TGCTTCAATA ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTGCCCTTA TTCCCTTTTGCGGCATT
 CTCTGTTATT GGGACTATTTACCGAAGTTAT TATAACTTTTCCTTCTCAT ACTCATAAGT TGTAAAGGCA CAGCGGAAT AAGGGAAAAACCGCGTAAA

 -3 S Y E V N G H R G * E R K Q P M K
 -2 L I * C K R T A R I G K K A A N Q
 -1 H T N L M E T D G K N G K K R C K

 ApaLI
 +3 L P S C F C S P R N A G E S K R C * R S V G C T S G L H R T G S Q Q
 +2 F A F L F L L T Q K R W * K * K M L K I S W V H E W V T S N W I S T
 +1 C L P V F A H P E T L V K V K D A E D Q L G A R V G Y I E L D L N S

 1501 TGCCCTCCGT TTTTGCTCA CCCAGAAACG CTGGTGAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAAACA
 ACGGAAGGAC AAAAACGAGT GGGTCTTGC GACCACTTTC ATTCTCTACG ACTTCTAGTC AACCCACGTG CTCACCCAAT GTAGCTTGAC CTAGAGTTGT

 -3 K G E Q K Q E G L F A P S L L L H Q L D T P H V L P N C R V P D * C
 -2 Q R G T K A * G S V S T F T F S A S S * N P A R T P * M S S S R L L
 -1 A K R N K S V W F R Q H F Y F I S F I L Q T C S H T V D F Q I E V A
 -3 Q R * D P * E F S P R R T F S N D E H F * S S A M W R G I I P C * R
 -2 A V R S L R V F A P K N V F Q * * A L L K F C Y V A R Y Y P V L T P
 -1 S G K I L E S F R P E E R F P M M S T F K V L L C G A V L S R V D A

 1601 CGGGTAAAGAT CCTTGAGAGT TTTCGCCCG AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTTGGCGCG GTATTATCCC GTGTTGACGC
 CGCCATTCTA GGAACCTCTCA AAAGCGGGGC TTCTTGCAAA AGGTTACTAC TCGTGAAAT TTCAAGACGA TACACCGCGC CATAATAGGG CACAACTGCG

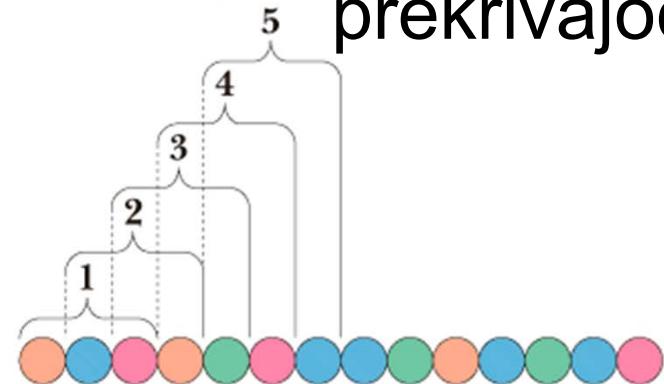
 -3 R Y S G Q S N E G R L V N E L S S C K * L E A I H R P I I G H Q R R
 -2 L P L I R S L K R G S S R K G I I L V K L T R S H P A T N D R T S A
 -1 A T L D K L T K A G F F T K W H H A S K F N D * T A B R Y * G T N V G

GENETSKI KOD

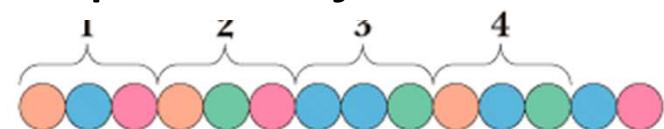
LASTNOSTI

1. Triplet: tri baze na mRNA določajo 1 aminokislino
2. Neprekričajoč: zaporedje treh baz kot celota (KODON). En kodon se uporabi za en korak pri translaciji
3. Ni ločil: med kodoni ni baz, ki bi ločevale kodo (pik, vejic)
4. Degeneriran: ena aminokislina ima lahko več kodonov
5. Univerzalni: isti kod se uporablja pri vseh organizmih. Izjeme so mitohondriji in nekatere alge

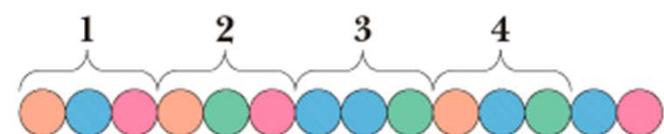
prekrivajoč



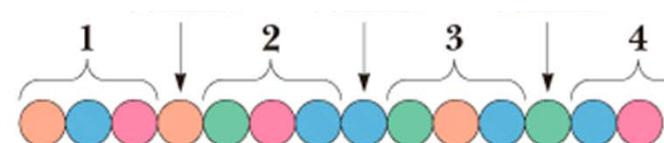
neprekričajoč



neprekinjen



prekinjen





The Nobel Prize in Physiology or Medicine 1968

Robert W. Holley

Har Gobind Khorana

Marshall W. Nirenberg

"for their interpretation of the genetic code and its function in protein synthesis"

Dr. Holley, Dr. Khorana, Dr. Nirenberg. At the end of his Nobel lecture, Edward Tatum in 1958 looked into his crystal ball and tried to predict some of the future developments in molecular biology. He suggested among other things that the solution of the genetic code might come during the lifetime of at least some of the members of his audience. **This appeared to be a bold prophecy at that time. In reality it took less than three years before the first letters of the code were deciphered and, because of the ingenuity of you three, the nature of the code and much of its function in protein synthesis were known within less than eight years.** Together you have written the most exciting chapter in modern biology.

	U	C	A	G	
	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
	AUU Ile AUC Ile AUA Ile AUG Met*	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

LASTNOSTI GENETSKEGA KODA

- vse aminokisline več kodonov, razen Trp in Met po enega
- trije STOP kodoni: UGA, UAG, UAA
- kodoni, ki določajo isto aminokislino podobni v zaporedju
- 2. baza pirimidin- običajno nepolarna aminokislina
- 2. baza purin- običajno polarna aminokislina
- pri degeneriranosti običajno prvi dve bazi enaki, tretja drugačna

Vendar... nekatere izjeme

Mitochondrion	UGA	AUA	CUN ^a	AG _G ^A	CGG
Mammalian	Trp	Met ^b		Stop	
Baker's yeast	Trp	Met ^b	Thr		
<i>Neurospora crassa</i>	Trp				
<i>Drosophila</i>	Trp	Met ^b		Ser ^c	
Protozoan	Trp				
Plant					Trp
"Standard" code	Stop	Ile	Leu	Arg	Arg

^aN represents any of the four nucleotides.

^bAlso acts as part of an initiation signal.

^cAGA only; no AGG codons occur in *Drosophila* mitochondrial DNA.

Source: Mainly Breitenberger, C.A. and RajBhandary, U.L., *Trends Biochem. Sci.* **10**, 481 (1985).

Codon Usage Database

Data source

[NCBI-GenBank](#) Flat File Release 160.0 [June 15 2007].

Data amount

35,799 organisms

3,027,973 complete protein coding genes (CDS's)

[Announcement](#)

QUERY Box for search with Latin name of organism

Case: sensitive insensitive

Input a scientific name (or its [regular expression](#)) for an organism and press "Submit" or return key. Use **Latin name** such as "Marchantia polymorpha", "Saccharomyces cerevisiae" etc., not "liverwort", "yeast" etc.

Alphabetical lists of all organisms

[A](#) [B](#) [C](#) [D](#) [E](#) [F](#) [G](#) [H](#) [I](#) [J](#) [K](#) [L](#) [M](#)

[N](#) [O](#) [P](#) [Q](#) [R](#) [S](#) [T](#) [U](#) [V](#) [W](#) [X](#) [Y](#) [Z](#)

[Chloroplast](#) [Mitochondrion](#)

[Others \(intials are not capital\)](#)

E

- Epizootic haematopoietic necrosis virus* [gbvrl]: 6
Equine rotavirus strain H-1 [gbvrl]: 3
Entosiphon sulcatum [gbpln]: 3
Enterobacteria phage ES18 [gbphg]: 82
Eupatorium cannabinum [gbpln]: 2
Enterobacteria phage WPhi [gbphg]: 52
Equid herpesvirus 1 [gbvrl]: 280
Equid herpesvirus 4 [gbvrl]: 106
Equid herpesvirus 5 [gbvrl]: 1
Enterobacteria phage phiP27 [gbphg]: 68
Echovirus 1 [gbvrl]: 1
Echovirus 9 [gbvrl]: 2
Erythrobacter longus [gbbct]: 8
Echinometra lucunter [gbinv]: 1
Ectothiorhodospira shaposhnikovii [gbbct]: 12
European elk papillomavirus [gbvrl]: 13
Epicoccum nigrum [gbpln]: 1
Euptelea pleiosperma [gbpln]: 2
Enterobacteria phage PRD1 [gbphg]: 42
Ensifer adhaerens [gbbct]: 5
Enterobacteria phage T2 [gbphg]: 36
Enterobacteria phage T4 [gbphg]: 579
Enterobacteria phage T6 [gbphg]: 30
Enterobacteria phage D108 [gbphg]: 5
Enterobacteria phage Mu [gbphg]: 115
Enterobacteria phage P1 [gbphg]: 282
Enterobacteria phage P2 [gbphg]: 46
Enterobacteria phage P4 [gbphg]: 26
Enterobacteria phage P7 [gbphg]: 125
Enterobacteria phage Tula [gbphg]: 8
Enterobacteria phage Ox2 [gbphg]: 12

Escherichia coli APEC O1 [gbct]: 4890 CDS's (1598551 codons)

fields: [triplet] [frequency: per thousand] ([number])

UUU 22.6(36127)	UCU 8.9(14277)	UAU 16.5(26306)	UGU 5.4(8659)	UUU 17.6(714298)	UCU 15.2(618711)	UAU 12.2(495699)	UGU 10.6(430311)
UUC 16.4(26141)	UCC 8.9(14300)	UAC 12.1(19400)	UGC 6.5(10463)	UUC 20.3(824692)	UCC 17.7(718892)	UAC 15.3(622407)	UGC 12.6(513028)
UUA 13.6(21712)	UCA 8.3(13256)	UAA 1.9(2979)	UGA 1.0(1536)	UUA 7.7(311881)	UCA 12.2(496448)	UAA 1.0(40285)	UGA 1.6(63237)
UUG 13.2(21043)	UCG 8.9(14247)	UAG 0.2(375)	UGG 15.2(24367)	UUG 12.9(525688)	UCG 4.4(179419)	UAG 0.8(32109)	UGG 13.2(535595)
CUU 12.1(19336)	CCU 7.6(12136)	CAU 13.0(20809)	CGU 20.3(32489)	CUU 13.2(536515)	CCU 17.5(713233)	CAU 10.9(441711)	CGU 4.5(184609)
CUC 11.0(17619)	CCC 5.8(9208)	CAC 9.4(15104)	CGC 20.8(33305)	CUC 19.6(796638)	CCC 19.8(804620)	CAC 15.1(613713)	CGC 10.4(423516)
CUA 4.0(6381)	CCA 8.8(14004)	CAA 14.8(23716)	CGA 4.0(6336)	CUA 7.2(290751)	CCA 16.9(688038)	CAA 12.3(501911)	CGA 6.2(250760)
CUG 50.2(80301)	CCG 21.9(35017)	CAG 29.3(46806)	CGG 6.2(9895)	CUG 39.6(1611801)	CCG 6.9(281570)	CAG 34.2(1391973)	CGG 11.4(464485)
AUU 29.9(47799)	ACU 9.3(14904)	AAU 18.6(29672)	AGU 9.3(14939)	AUU 16.0(650473)	ACU 13.1(533609)	AAU 17.0(689701)	AGU 12.1(493429)
AUC 24.2(38750)	ACC 22.4(35864)	AAC 21.4(34283)	AGC 15.7(25115)	AUC 20.8(846466)	ACC 18.9(768147)	AAC 19.1(776603)	AGC 19.5(791383)
AUA 5.3(8501)	ACA 8.2(13139)	AAA 33.5(53487)	AGA 3.0(4804)	AUA 7.5(304565)	ACA 15.1(614523)	AAA 24.4(993621)	AGA 12.2(494682)
AUG 27.5(43892)	ACG 14.6(23347)	AAG 11.2(17837)	AGG 2.0(3180)	AUG 22.0(896005)	ACG 6.1(246105)	AAG 31.9(1295568)	AGG 12.0(486463)
GUU 18.7(29962)	GCU 16.0(25516)	GAU 32.4(51782)	GGU 24.3(38834)	GUU 11.0(448607)	GCU 18.4(750096)	GAU 21.8(885429)	GGU 10.8(437126)
GUC 15.0(23912)	GCC 25.3(40403)	GAC 19.0(30356)	GGC 27.9(44562)	GUC 14.5(588138)	GCC 27.7(1127679)	GAC 25.1(1020595)	GGC 22.2(903565)
GUA 11.2(17875)	GCA 20.8(33292)	GAA 38.7(61906)	GGA 9.1(14474)	GUA 7.1(287712)	GCA 15.8(643471)	GAA 29.0(1177632)	GGA 16.5(669873)
GUG 25.4(40582)	GCG 31.6(50463)	GAG 18.3(29279)	GGG 11.4(18190)	GUG 28.1(1143534)	GCG 7.4(299495)	GAG 39.6(1609975)	GGG 16.5(669768)

Coding GC 51.42% 1st letter GC 58.42% 2nd letter GC 40.94% 3rd letter GC 54.90%

Homo sapiens [gbpri]: 93487 CDS's (40662582 codons)

fields: [triplet] [frequency: per thousand] ([number])

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Optimalni kodoni v organizmih, ki hitro rastejo (npr. mikroorganizmi *E. coli* ali *S. cerevisiae*) odražajo tRNA sestavo. Optimalni kodoni omogočajo hitrejšo translacijo in večjo točnost. Pri genih, ki se izražajo več, je zvišana selekcija na nivoju translacije.

Pri organizmih, ki počasi rastejo (npr. človek) takšne optimizacije na nivoju kodonov ni.

RAZVITJE DVOJNEGA HELIKSA OMOGOČAJO

proteini

toplota, kisline, baze, denaturanti

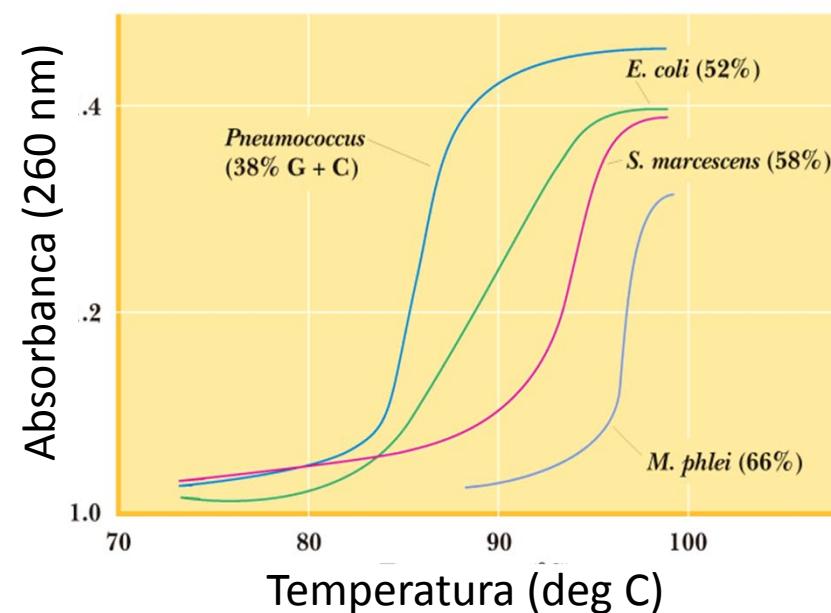
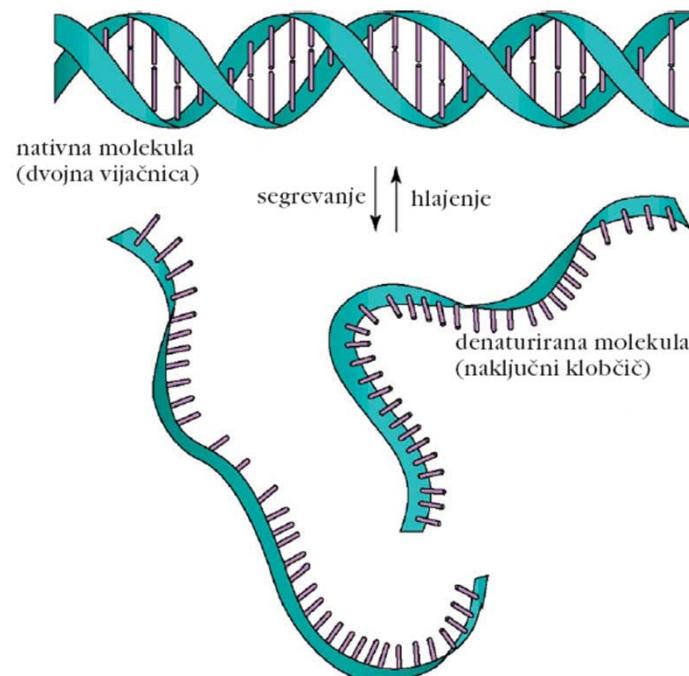
Segrevanje raztopine DNA do 80°C ("taljenje") - UV absorbacija naraste za 30-40%

Hiperkromni efekt odraža razvitje dvojnega heliksa DNA

Vzporedno pakirani bazni pari v nativni DNA absorbirajo manj svetlobe

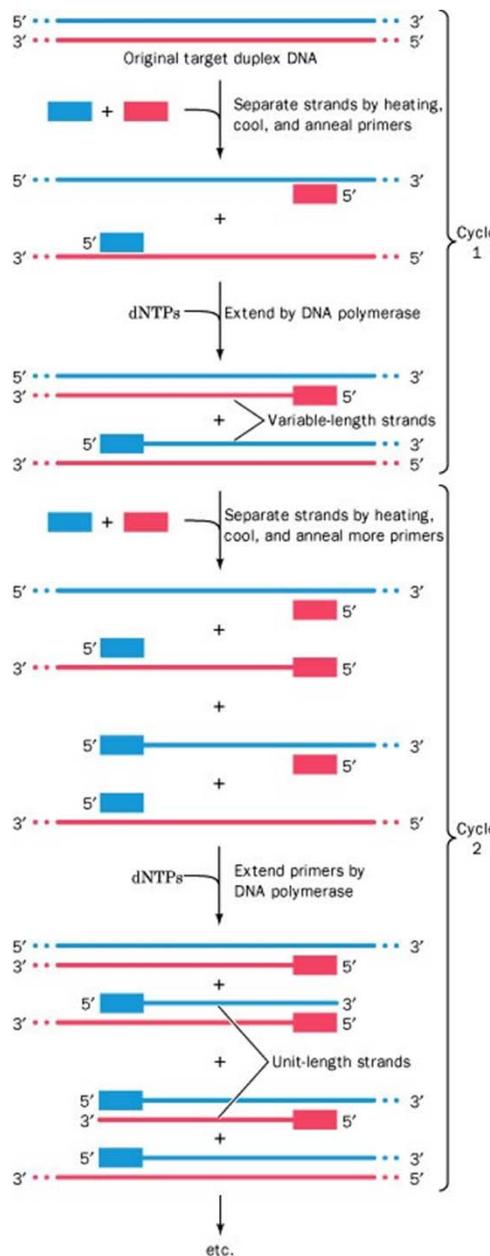
Če ponovno znižamo temperaturo, se tudi absorbanca zmanjša- ponovno pakiranje baznih parov ("renaturacija, prileganje").

Osnova **HIBRIDIZACIJE**- pomembna metoda za detekcijo specifičnih koščkov DNA: Southern, Northern blotanje; Genski čipi (mikromreže); PCR



VERIŽNA REAKCIJA S POLIMERAZO

ang. polymerase chain reaction, PCR



Uporaba:

- Priprava DNA fragmentov za kloniranje
- Detekcija DNA v vzorcih
- Določanje zaporedij
- Uvedba točkovnih mutacij

Poteka v ciklih:

Denaturacija: 94 °C

Hibridizacija: prileganje oligonukleotidov na matrično DNA (tipično 45-60 °C)

Polimerizacija: podaljševanje začetnega oligonukleotida (72 °C)

NAČRTOVANJE ZAČETNIH OLIGONUKLEOTIDOV

1. Iskanje ustreznih mest na DNA (dolžina fragmenta, kaj želiš pomnožiti)
2. Poravnavanje zaporedij in priprava optimalne poravnave (ang. **alignment**)
3. Iskanje ohranjenih delov

<i>lac</i>	ACCCCAGGCTTTACACTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGG
<i>lacI</i>	CCATCGAACGGCGAAACCTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTC
<i>galP2</i>	ATTTATTCCATGTCAACACTTTCGCATCTTGTTATGCTATGGTTATTTCATACCAT
<i>araBAD</i>	GGATCCTACCTGACGCTTTTATCGCAAACCTCTACTGTTCTCCATACCGTTTT
<i>araC</i>	GCCGTGATTATAGACACTTTGTTACCGCGTTTGTCAATGGCTTGGTCCCCGCTTTG
<i>trp</i>	AAATGAGCTGTTGACAATTAAATCATCGAACTAGTTAACTAGTACGCAAGTTCACGTA
<i>bioA</i>	TTCCA AAAACGTGTTTTGTTGTTAAATT CGGTGTAGACTTGAAACCTAAATCTTT
<i>bioB</i>	CATAATCGACTTGTAAACCAAAATTGAAAAGATTAGGTTACAAGTCTACACCGAAT
<i>tRNA^{Tyr}</i>	CAACGTAACACCTTACAGCGGCCGTCAATTGATATGATGCGCCCGCTTCCGATA
<i>rrnD1</i>	CAAAAAAAATACTTGTGCAAAAAAATTGGGATCCCTATAATGCGCCCTCCGTTGAGACGA
<i>rrnE1</i>	CAATTTCCTATTGCGGCCGTGGAGAACTCCCTATAATGCGCCCTCCATCGACACGG
<i>rrnA1</i>	AAAAATAAAATGCTTGACTCTGTAGCGGGAAAGGCGTATTATGCACACCCCCGCGCCGCTG

Ključno: izbira optimalnih začetnih **oligonukleotidov** (ang. primer):

- Dolžina okoli 20 nukleotidov
- T_m okoli 55-75 °C
- Brez neželenih sekundarnih struktur
- Specifičnost samo za izbrano DNA
- Zadnji nukleotid G ali C
- Nekomplementarnost samemu sebi ali drugemu oligonukleotidu

4 bp, delta G = -6.6 kc/m (**bad!**) (worst= -36.6)
5' GGGAAAATTCCAGGATCTAT 3'
|||||

3' TATCTAGGA~~CCTT~~AAAAGGG 5'

4 bp, delta G = -5.4 kc/m (**bad!**) (worst= -36.6)
5' GGGAA~~A~~TTCCAGGATCTAT 3'
|||||

3' TATCTAGGAC~~C~~TAAAAGGG 5'

https://bioweb.uwlax.edu/GenWeb/Molecular/seq_anal/primer_design/primer_design.htm

Izračun T_m

Za kratke oligonukleotide, < 14 nukleotidov, v prisotnosti 50 mM soli

$$T_m = 4 \text{ } ^\circ\text{C} \times (\text{število G in C v oligonukleotidu}) + 2 \text{ } ^\circ\text{C} \times (\text{število A in T v oligonukleotidu})$$

Za daljše oligonukleotide (N je dolžina oligonukleotida)

$$T_m = 64.9 \text{ } ^\circ\text{C} + 41 \text{ } ^\circ\text{C} \times (\text{število G in C v oligonukleotidu} - 16.4)/N$$

Za daljše oligonukleotide ob upoštevanju koncentracije soli

$$T_m = 81.5 \text{ } ^\circ\text{C} + 16.6 \text{ } ^\circ\text{C} \times (\log_{10}[\text{Na}^+] + [\text{K}^+]) + 0.41 \text{ } ^\circ\text{C} \times (\%GC) - 675/N$$

3D STRUKTURA RNA

Razlike med DNA in RNA

- riboza namesto deoksiriboze: zato je RNA manj stabilna in se hitreje razgradi. Ker je DNA bolj stabilna je nosilec genetske informacije.
- uracil namesti timina

DNA je zvita v dvojni heliks (sekundarna struktura)

RNA je zvita v nekaj različnih strukturnih elementov, ki so razmeščeni preko celotnega zaporedja:

Lasnice

Povežejo komplementarne regije “stem-loop” strukture

Desno sučno dvojni heliksi

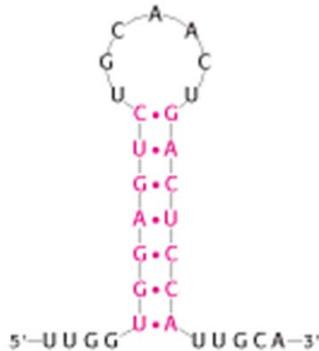
Posledica interakcij med deli zaporedja. Analogno kot pri DNA.

Antiparalelen heliks. Interakcije iste kot pri DNA. Parjenje baz A::U, C::G.

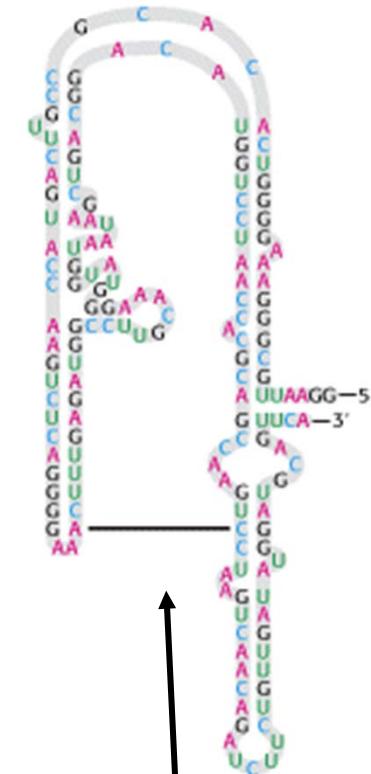
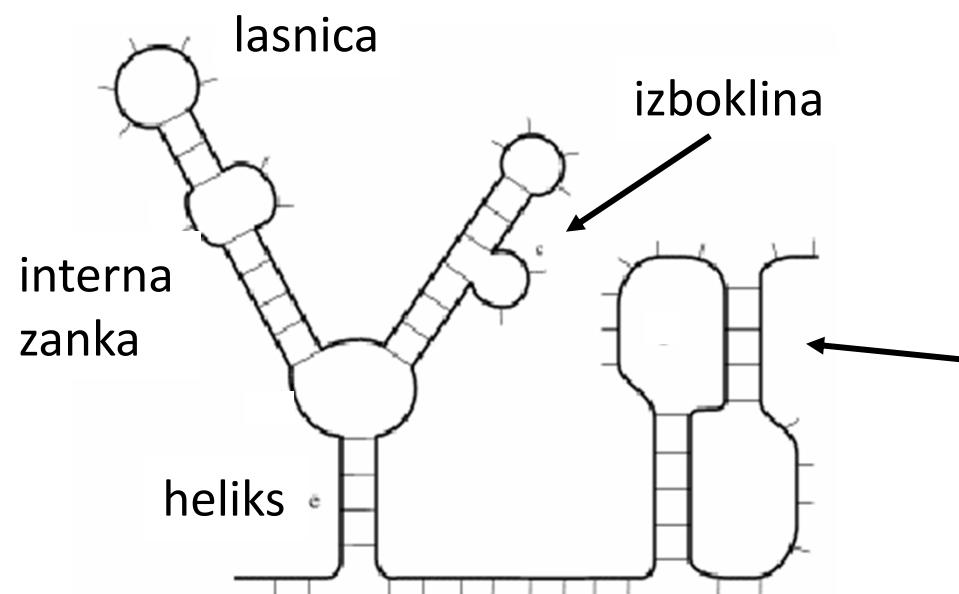
Interne zanke in izbokline

strukturni elementi, ki prekinejo dele z dvojnim heliksom

5'-U UGG UGGAGUCUGCAACU GACUCCA UUGCA-3'

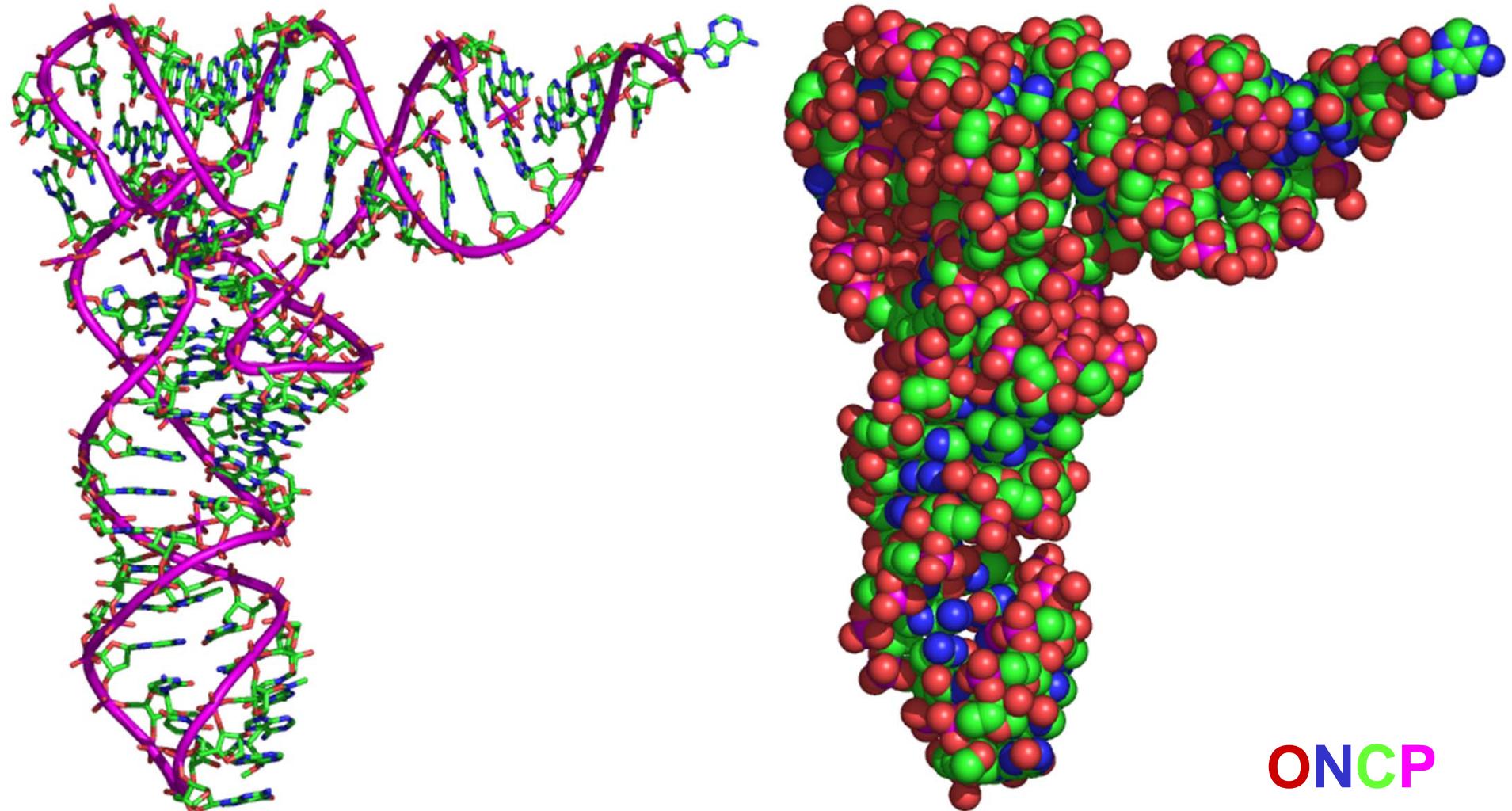


lasnica



kompleksne interakcije med
nukleotidi, ki so oddaljeni v
zaporedu
(stabilizirane z vodikovimi vezmi
in kovinskimi ioni, npr. Mg²⁺)

kvasna fenilalanin tRNA

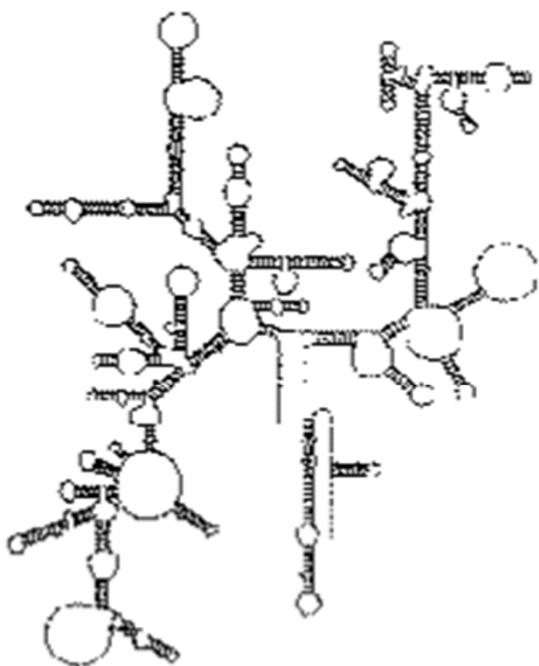


H. Shi and P.B.Moore, *RNA* 6: pp. 1091, 2000

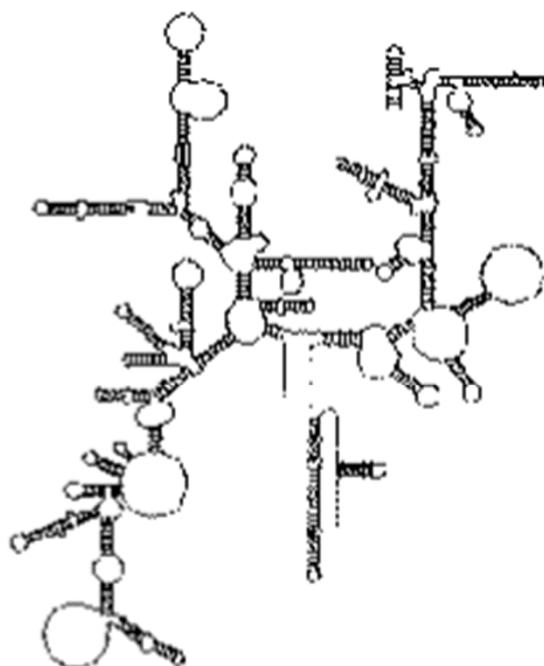
Ribosomalna RNA- rRNA

- Ribosomi: velika in mala podenota
- rRNA molekule: do 2/3 ribosoma, proteini 1/3
- Komplementarnost znotraj verige omogoča obsežno parjenje baz
- Ohranjena sekundarna zgradba, ne pa zaporedje
- Pri vseh organizmih podobna prostorska zgradba in funkcija rRNA - se morata ohranjati zaradi funkcionalnosti
- rRNA dober marker za ugotavljanje filogenetskih odnosov

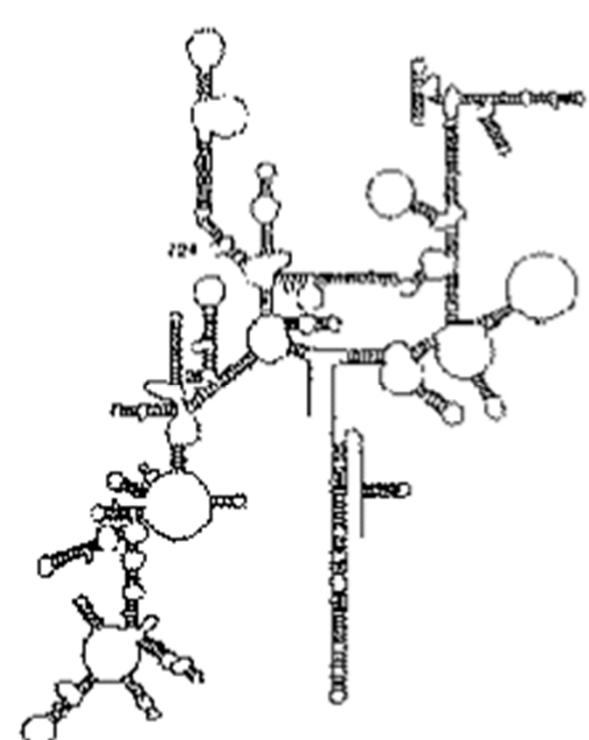
E.coli

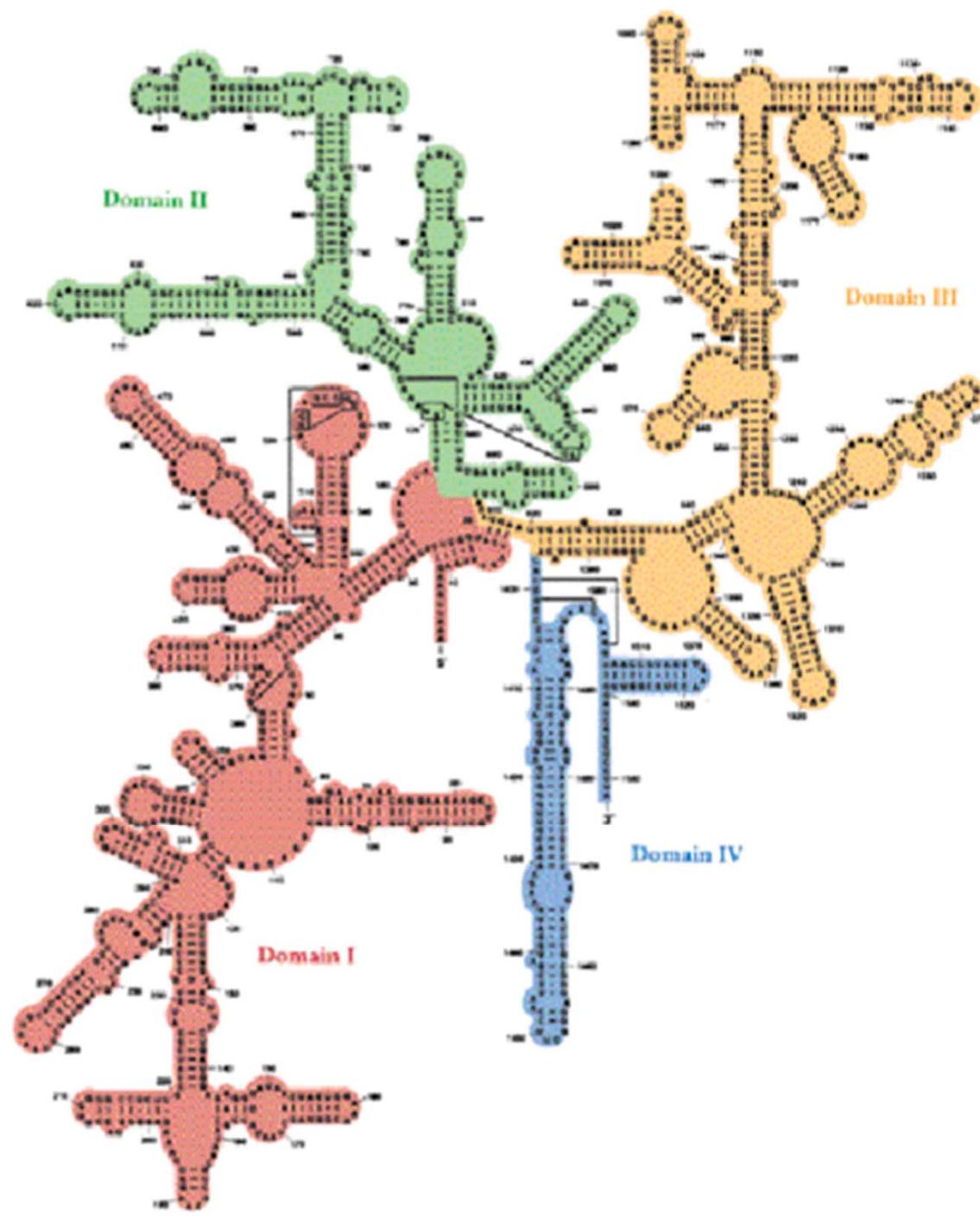


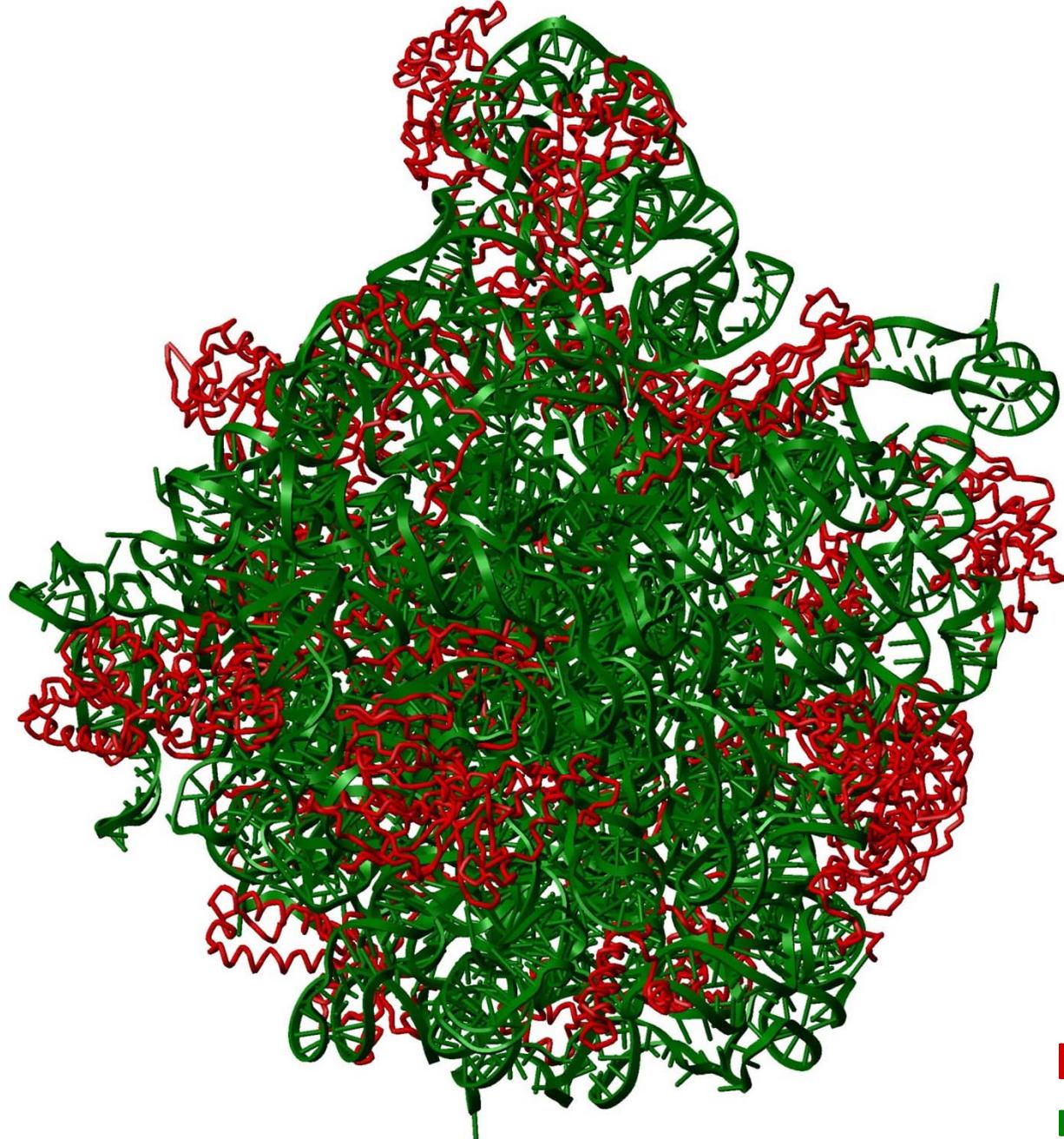
arheja



kvasovka







protein
rRNA