

Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Miroslav Plohl

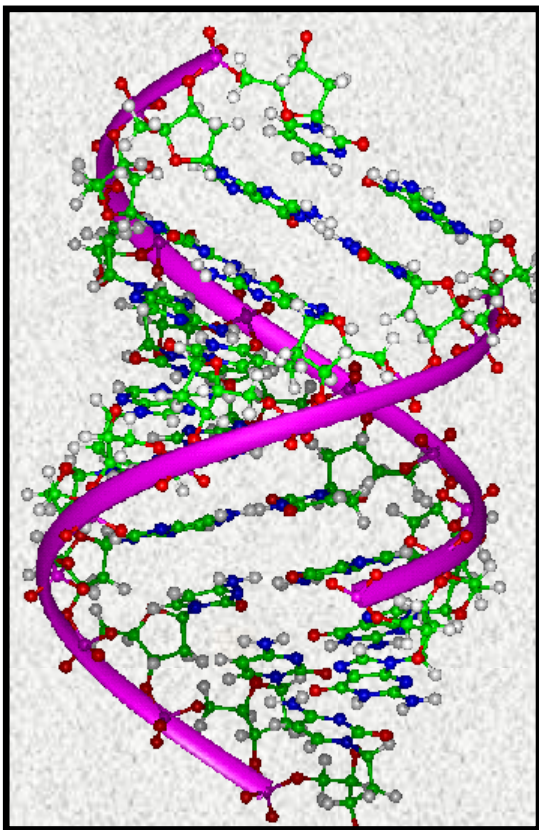
Emeritus Instituta Ruđer Bošković
Miroslav.Plohl@irb.hr



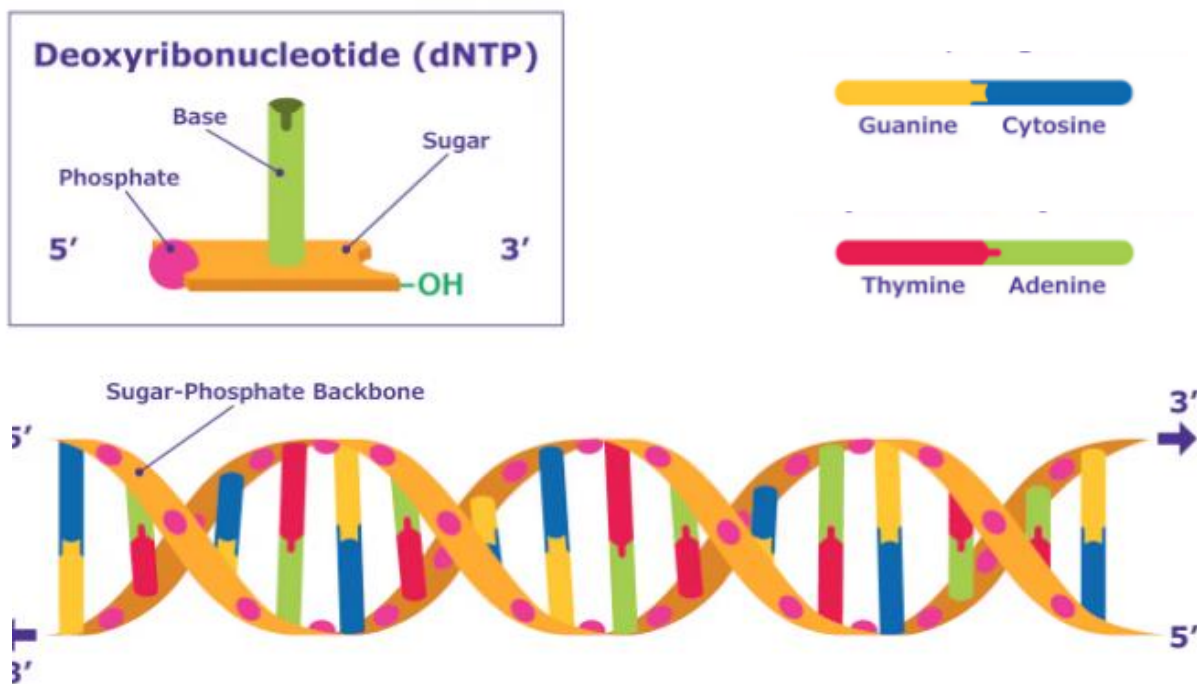
Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Osnove nasljeđivanja biološke informacije

Molekula DNK sadrži informaciju i predstavlja „nacrt” koji opisuje neki organizam.



4 baze (A, G, T, C) → komplementarno se sparuju u dvostrukoj uzvojnici:
Uvijek A = T; G ≡ C



Strukturu molekule DNK su na osnovu podataka Rosalind Franklin i Maurice Wilkins prvi deducirali James Watson i Francis Crick, **1953. godine.**

Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

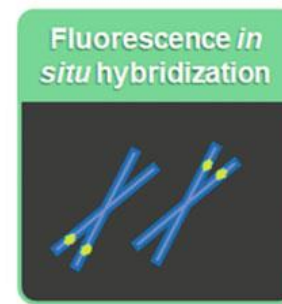
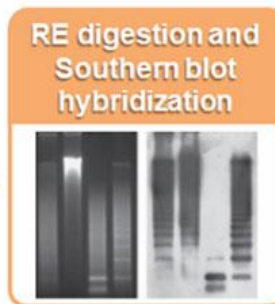
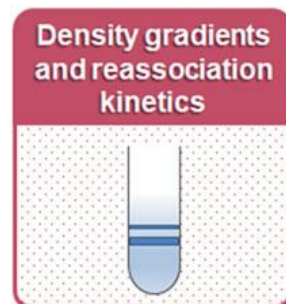
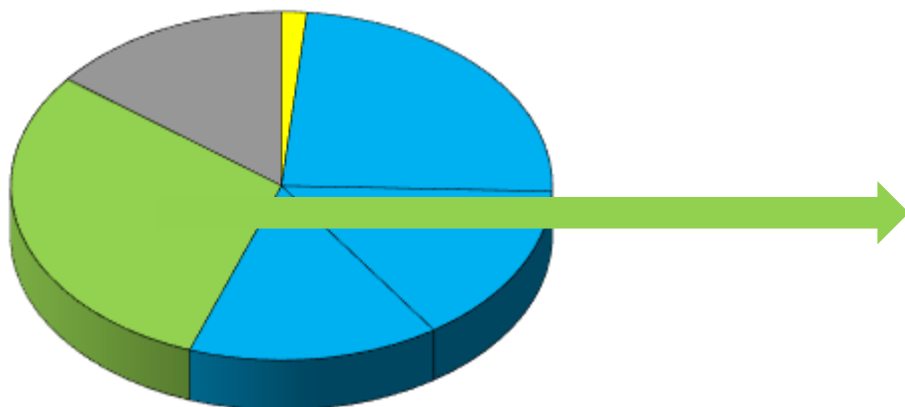
Što su satelitne DNK? (nas su zainteresirale oko 1985. ...)

Molekula DNK sadrži informaciju i predstavlja „nacrt” koji opisuje neki organizam.

Gen je segment molekule DNK koji određuje neko svojstvo ili funkciju.

Genom: sveukupna DNK u stanici

Geni: < 2%



Otkrivene oko 1960. – dodatna (satelitna) frakcija pri izolaciji genomske DNK



Jedinice ponavljanja (monomeri) obično se razlikuju <5% u nukleotidnoj sekvenci

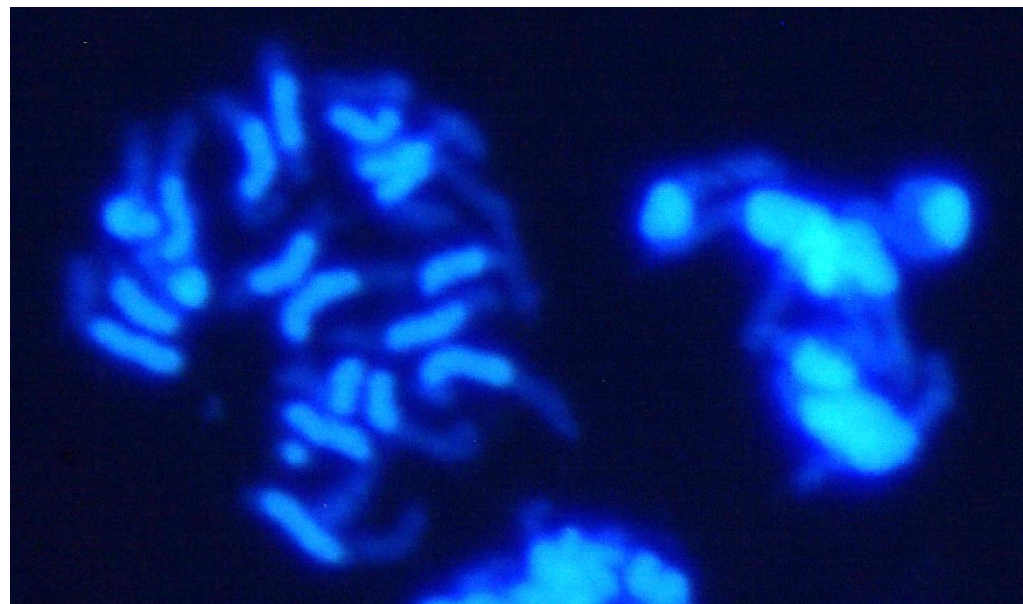
Ostatak: ‘dark matter’, nekodirajuća DNK, najčešće segmenti ponovljeni u velikom broju kopija; **uzastopno** i **raspršeno** ponovljene sekvence

Satelitna DNK brašnara

Prva sekvenciranja DNK u Hrvatskoj: 1986/87 na Institutu Ruđer Bošković

Organizam: žuti brašnar *Tenebrio molitor*, sakvencirali satelitnu DNK

Vera Gamulin
Đurđica Ugarković
Miroslav Plohl

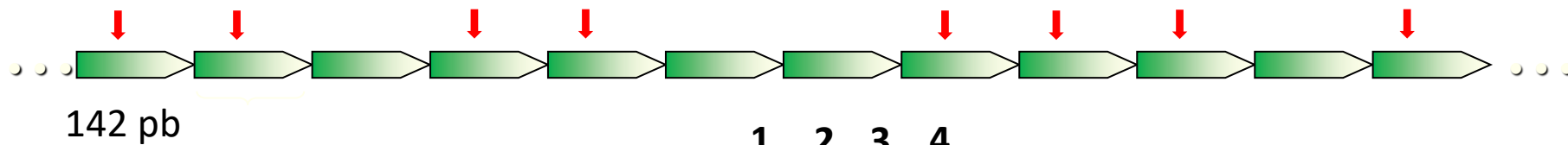


Prva procjena: oko 50% genoma izgrađuje samo jedna satelitna DNK!

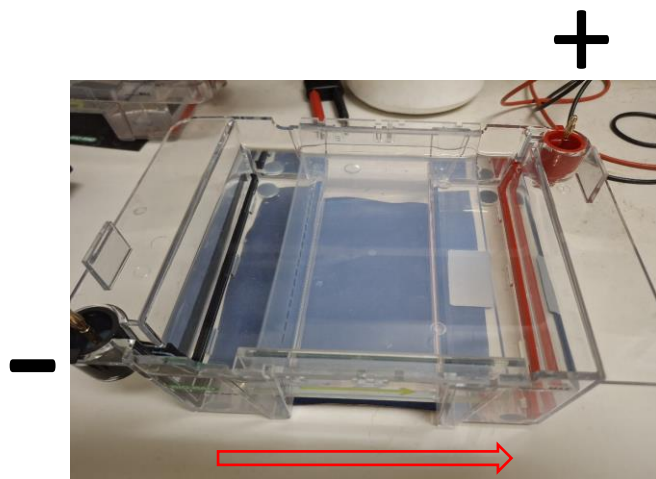
Petitpierre E, Gatewood JM, Schmid CW (1988) Satellite DNA from the beetle *Tenebrio molitor*. Experientia 44:498-499

Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

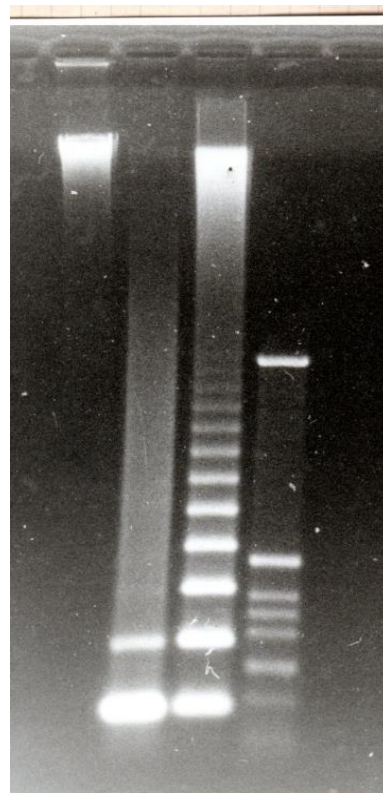
Satelitna DNK brašnara



Satelitna DNK brašnara *Tenebrio molitor*



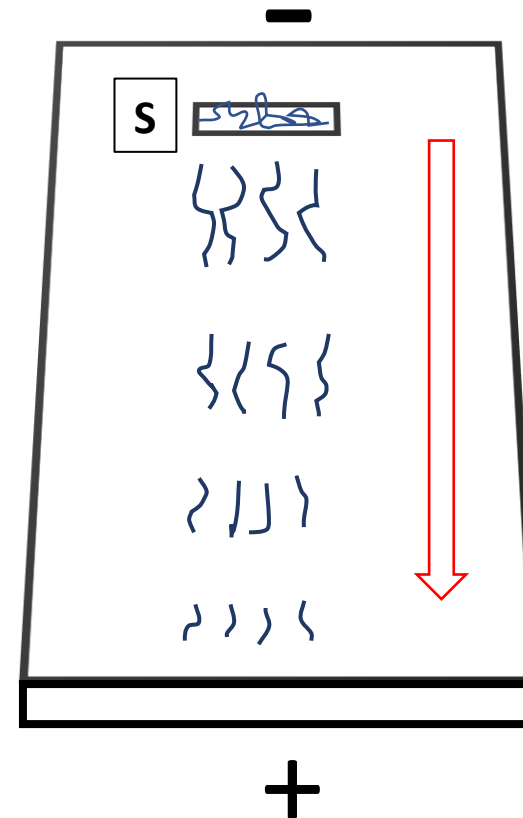
1 2 3 4



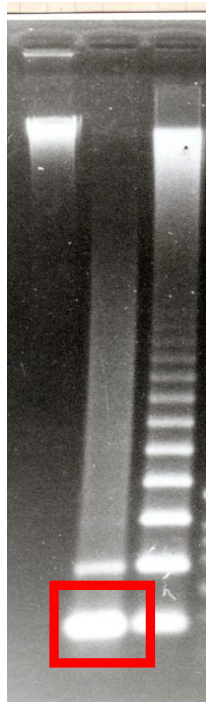
S



+



Satelitna DNK brašnara



sekvenciranje

kloniranje

142 pb

Sekvenciranje prema Sangeru
(dideoksi sekvenciranje, 1977.)

Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Sekvenciranje prema Sangeru (dideoksi sekvenciranje, 1977.)

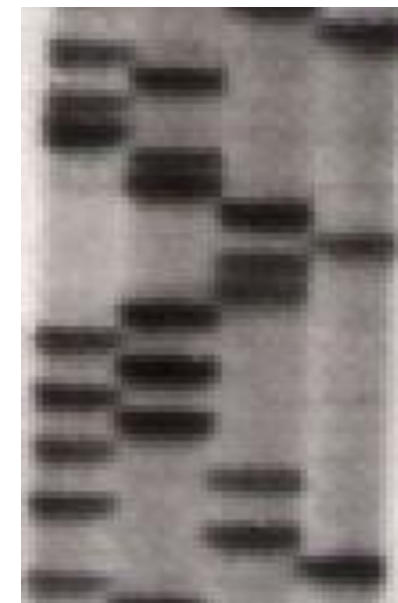
...T A C T A G T **A T G C T C T A T A T A**...
A T G A T C A →

DNA sequence

na sekvencu se vezuje klica, a
DNA polimeraza sintetizira
novi lanac

... <u>A T G A T C A</u>	T
... <u>A T G A T C A</u>	A A
... <u>A T G A T C A</u>	A A C
... <u>A T G A T C A</u>	A A C G
... <u>A T G A T C A</u>	A A C T A
... <u>A T G A T C A</u>	A A C T T G
... <u>A T G A T C A</u>	A A C T T C A
... <u>A T G A T C A</u>	A A C T T C C T
... <u>A T G A T C A</u>	A A C T T C C A A
... <u>A T G A T C A</u>	A A C T T C C A A T
... <u>A T G A T C A</u>	A A C T T C C A A A A
... <u>A T G A T C A</u>	A A C T T C C A A A G T

A T G C



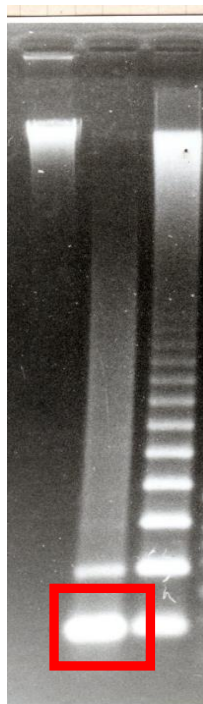
100-250 REG. VOLTA 250V A DISEG 2LEV 22μ/1000V
 TRAF 500VA 220/2x750
 220V

0.1μ/1000V
 MAX 3000V
 A
 V
 9V MOTOR 9V
 220/2x15V
 +12V
 0
 -12V
 COMPARATOR
 220V/AUTO
 U_{ref}
 I_{ref}
 potenziometro struje/napona

NAPAJANJE	220 V/50 Hz
POTROSNJA	500 VA max
KORIŠNA IZLAZNA SNAGA	450 W max
REGULACIJA:	
- RUCNA	0 - 150 mA 0 - 3000 V
AUTOMATSKA	0 - 100 mA 0 - 3000 V
MAXIMALNA DOZVOLJENA STRUJA	160 mA
ODSTUPANJE OD ZADANE VRIJEDNOSTI	± 4,25 mA
VRIJEME REGULACIJE	do 5 sekundi
MAX. KONTINUIRANO VRIJEME RADA	5000 sati
ZASTITA:	
- ZASTITA MREZNOC DIJELA	rastelni osigurac 2,5 A brzi
- ZASTITA IZLAZNOC DIJELA	elektronska na 20 o/o vise od max. vrijednosti rastelni osigurac 200 mA brzi
TEMPERATURA OKOLINE	u radu 15 do 40 st C skladištenja -20 do 45 st C
VLAZNOST ZRAKA	u radu do 80 o/o rel. skladištenja do 95 o/o rel. (bez kondenziranja)

Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

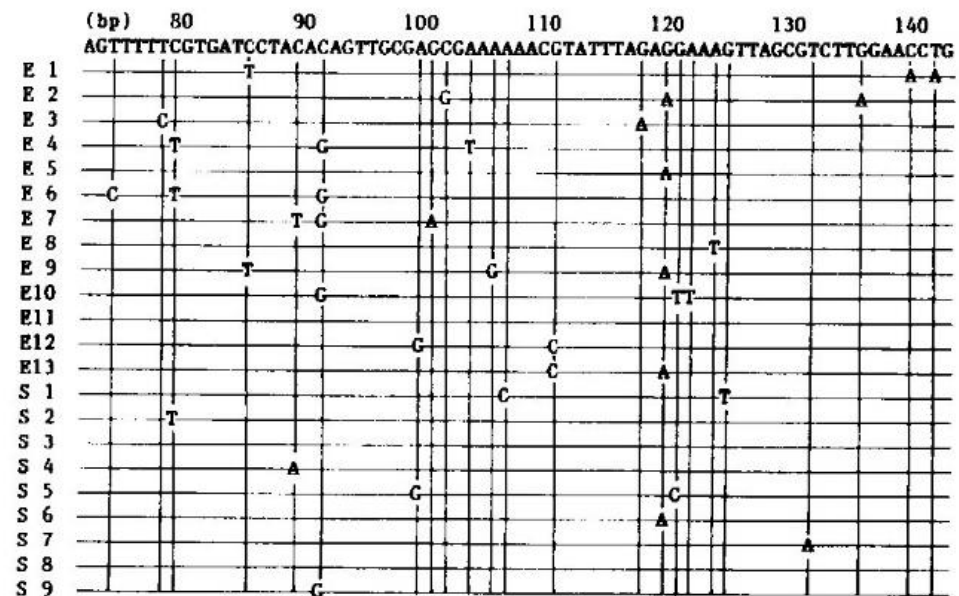
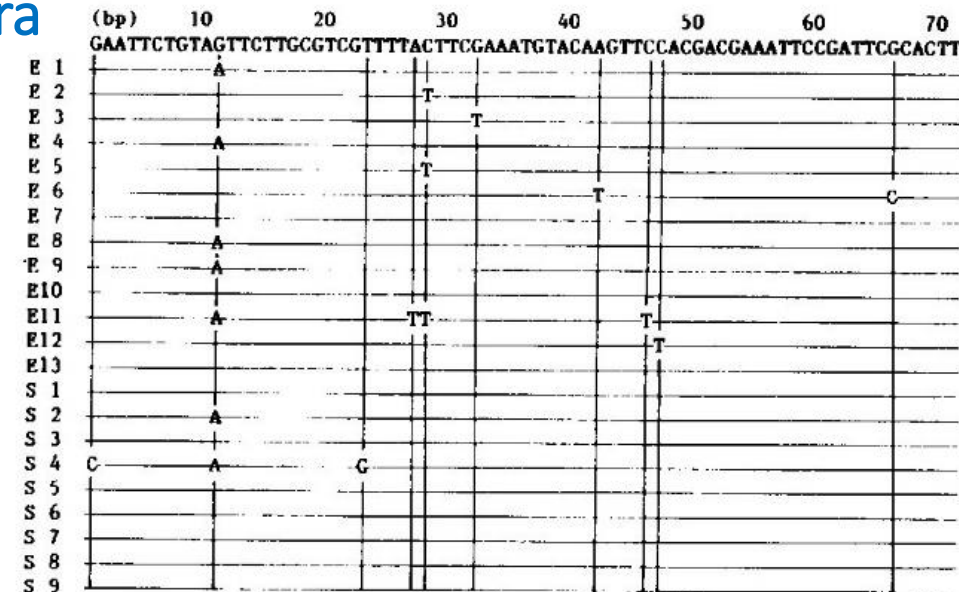
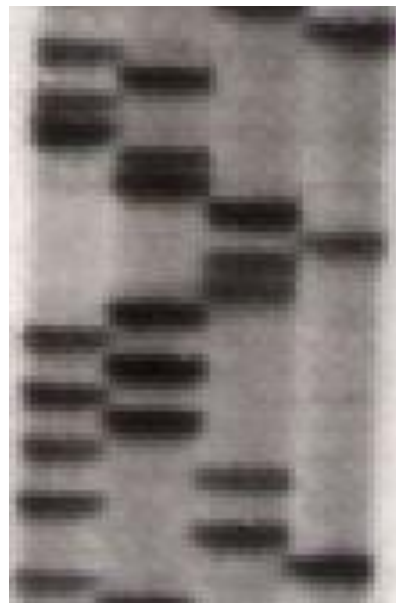
Satelitna DNK brašnara



sekvenciranje

kloniranje

A T G C

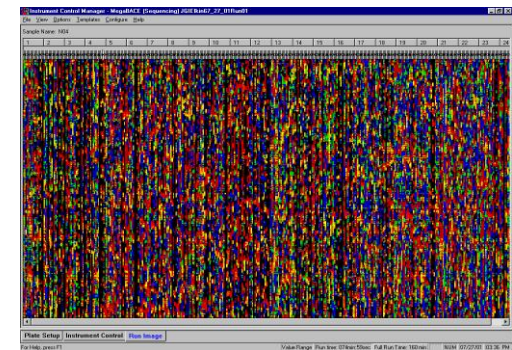
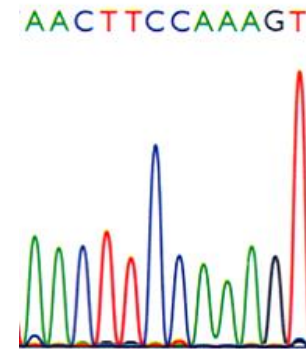
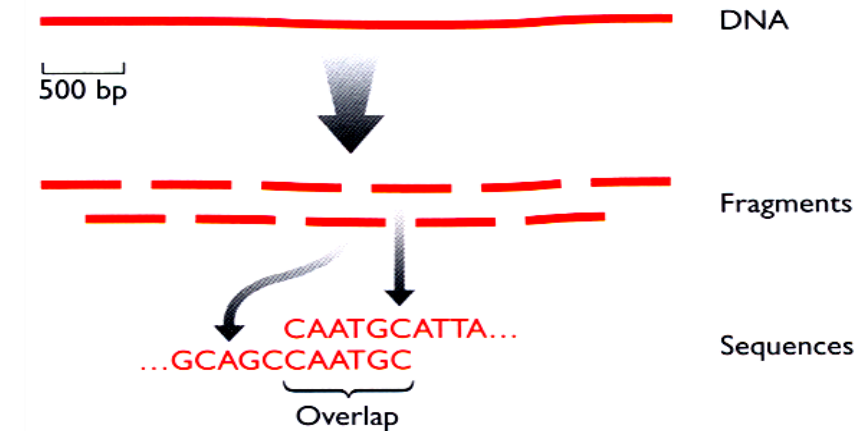


Đ. Ugarković, M. Plohl, V. Gamulin: Sequence variability of satellite DNA from the mealworm *Tenebrio molitor*, *Gene*, 83, travanj 1989, 181-183.

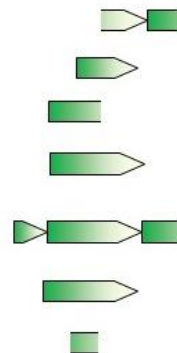
Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Satelitne DNK i razvoj tehnika sekvenciranja

Automatizacija, kapilarni sekvenatori, baze podataka, informatička analiza itd...



B





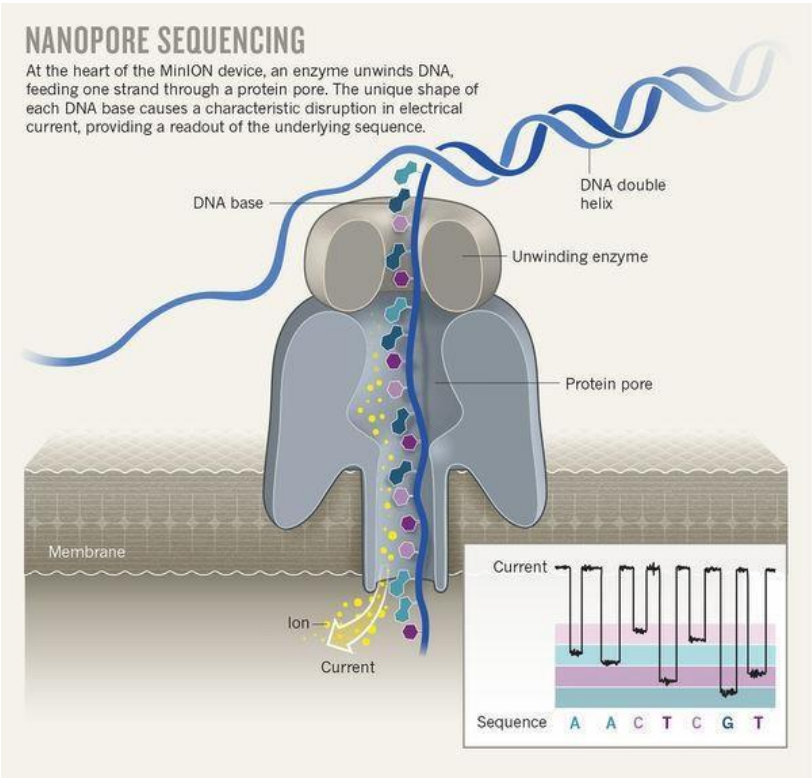
Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Satelitne DNK i razvoj tehnika sekvenciranja

Sekvenciranje nove generacije (NGS) i sekvenciranje dugih segmenata DNK (treća generacija)

Specialized bioinformatics tools: **Assembly-free strategies** for repetitive DNA analyses

Cluster		Genome Proportion [%]	Genome Proportion Adjusted [%]	Size real	Satellite probability	Consensus length	Consensus	Graph layout
1	CL1	9.300	32.000	13887	0.0416	166	AGCCGTAAGTGGAGAAATTAATTAATCAAAATTTATAAAAGTAAAAATAGTTCTAATTCAAAAACCTATTAGGAA TACTGCGATTTTTTGTACTTCAATGATTCCTGGATCATTTTGCATCGGCAAGGTCCAATAGTTCCACTGTTTCTGAA TAGTAT	
2	CL2	4.100	4.100	6177	0.0331	225	TATTATAATTCGAAGAAAAACGGCTTATTTCGAACGATTCCTAGCTTCGAACGACCAAGCTTTACAAAACATAGTAA AACGCTTTATTCTGGCTCAAAAGAACGATTGATGCAATATTTGGAAAGCAAGGCGCAATTTTCTGAAGATTTTTTGTCTTA GAATCCAGTAAACCTTATATAATCAGACGAAAAAGCTCTAGTTGCTGCTCAAAAAGCGATATA	
4	CL4	1.400	1.400	2147	0.2700	1110	CTTAAATCGAACCTAGTGCATCTTTTCTACTTTTAAACACTCTAGAGGCTGTATAGCATAGAAATGCTCATAAAGC TTTGATAGTGGAGTAAAMAAAAATTTATTTTTCTATTTTGTGAGGTAAAGTTGCTGACCATTAATTTTATGCA AAAAATTTCAAAAATTTAAATCTCATGAAGAGTTAATAATAGTACGATGAGTGGAGGCGAGATTCCTAGGCAAAACCGGT TTTGTCTACGACTTTTGTAGTTTAAAGTTATGAATTTTAAAGTTTAACTTTAAATGCTCTGTACCCGGAACCGGT CCCGAGCGGCTCCGGGTTTGAACGAGAAATAGATTAATTTAAAGGCTATCCGATGCTTTATTGAATTTGCTCTAAGT CTATAGTTTCCGAGAAAAAGCAAAAAAGGTTGCTATTTTCTACTTTTCTAATTTTAAACGCGGATTACGGCGAA CTATAAGAGTTAGAGAAAAAGCGTAAAAAGGTTAGAGGCGGTATAAAAGCCCTACAACTGGCATAGTTTCAAGGCG ATATCTCGCAAAAAATTTTCTAATTTTCAACGCTGATTACGGCCAACTAAAGAGCTAGAGGAAACGGTTTACTCCA TTGTAAGAGCACATCAAAATCTATAAGATAGGATGGGTTGAATTTGATTTTGAACACTTTAAAAATGGCCATTTT AGGGGGGGGTGTAAACAAATTTTCTATTTTCTATTTTCTCTATTACGCGTAAACTTTCGAAAAATGGGCACTATT AATCTTCTACTATGCAAAATGATCCGGGAATCGATTGGGCTCGAGAAATCGCAAAATCTTAATAGTTTTTATGATTT GAATTTTTTAAATTTTGAACCTACTTTTGTGATTTTCTAAAAATTTTCTGAAAACTAAGTGGAGAGCAATAATCTTTT TTGAAACGATAGACAAATTAAGAGCTTTCCACAGATTAATTAACCTTATGGGTTTATCTCTAATAGTTTCCGGAGTATT GCTCGATAAATGTTCCGGGATCCGGAATCGCAAAATCGGACCAAAATTTGAAAAATCAAAATTT	
33	CL33	0.081	0.081	122	0.0884	112	GAGTTTATTTTGAGCAATCTTTGCTTCGAAATGAACAACTCTCATAAACTCACTGAAAAAGCTCTTAATCTCTCTCA AAGAAAGATTTTTGTACAGTTTGGATGAAA	



Nanopore technology:
Telomere to telomere assembly

Satelitom – ukupan broj satelidnih DNK u nekoj vrsti

Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Satelitne DNK i razvoj tehnika sekvenciranja

Vrsta	Broj satelitnih DNK	% genoma	Najzastupljenija satelitna DNK (%)
<i>Crassostrea gigas</i>	52	6.33	1.29
<i>Cydalima perspectalis</i>	1	0.14	0.14
<i>Diatraea postlineella</i>	2	0.06	0.04
<i>Eneoptera surinamensis</i>	45	14	1.41
<i>Gryllus assimilis</i>	13	4	1.35
<i>Hippodamia variegata</i>	30	14.93	9.37
<i>Locusta migratoria</i>	60	2.14	0.97
<i>Locusta migratoria</i>	62	2.39	0.98
<i>Megaleporinus macrocephalus</i>	164	13.47	2.78
<i>Meloidogyne arenaria</i>	81	5.07	0.31
<i>Meloidogyne floridensis</i>	38	1.55	0.14
<i>Meloidogyne incognita</i>	56	3.52	0.24
<i>Meloidogyne javanica</i>	73	4.27	0.21
<i>Oedaleus decorus</i>	58	2.53	0.62
<i>Ostrinia nubilalis</i>	4	0.27	0.15
<i>Pontastacus leptodactylus</i>	258	27.57	10.91



Tenebrio molitor, satelitom: 11 satDNK, 28% genoma
Glavna, 142 pb monomer, tek 26,5%.

Sekvenciranje 3. generacije PacBio nije uspjelo posložiti oko 50% genoma – problem su satelitne DNK

B. Oppert, ... E. Šatović-Vukšić, M. Plohl, (11 autora) The genome of the yellow mealworm, *Tenebrio molitor*: it's bigger than you think. *Genes (Basel)*, 14(12), prosinac 2023, 2209,

Satelitom – ukupan broj satelidnih DNK u nekoj vrsti

E. Šatović-Vukšić, M. Plohl, Satellite DNAs-From Localized to Highly Dispersed Genome Components, *Genes (Basel)*, 14(3), ožujak 2023, 742.



Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Filozofska promišljanja o ciljnosti (svrhovitosti)

Opća teorija sustava (1968),

Ludwig von Bertalanffy:

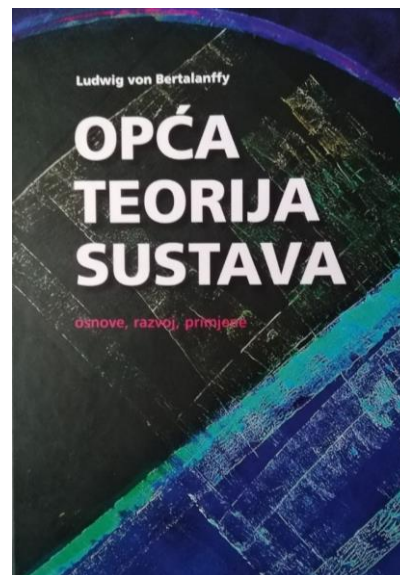


Doktorat iz biologije i iz filozofije znanosti,
ali i dobro poznavanje matematike, biokemije,
biofizike, termodinamike, teorije informacije,
medicine, psihologije i psihijatrije, lingvistike...

Ludwig von Bertalanffy
Beč 1901. – Buffalo (SAD) 1972.

'...genom kao cjelina proizvodi organizam kao cjelinu.
Određeni geni, međutim, u prvom redu određuju smjer
razvoja određenih značajki – tj. djeluju kao glavni dijelovi'

'Mendelovi zakoni bili su korijeni genetike, ali –
nasljednost, križna razmjena, učinak položaja koda i
još mnogo toga – bio je to samo mali dio genetičkog iskustva...'



Uloge satelitnih DNK:

Organizacija i evolucija genoma
Struktura interfazne jezgre
Izgrađuju centromere (dioba kromosoma)
Telomere (kromosomski krajevi)
Specijacija – nove vrste
Regulacija aktivnosti gena
Reakcije na promjene u okolišu
Formiranje i održavanje heterokromatina
Epigenetska regulacija genomske funkcije
Stabilnost genoma
Nastanak tumora
Sprječavanje nastanka tumora
Biljezi nekih bolesti
U forenzici, istraživanje demografije
.....

Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Miroslav Plohl

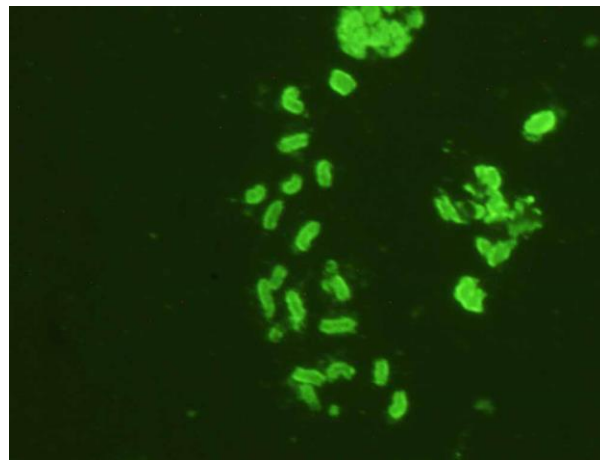
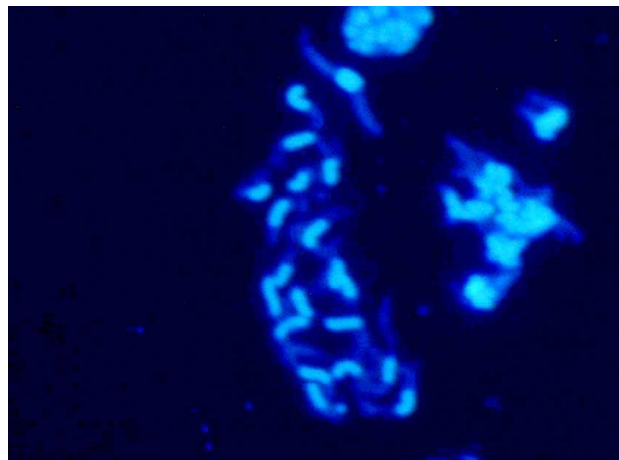
Emeritus Instituta Ruđer Bošković
Miroslav.Plohl@irb.hr





Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Satelitna DNK brašnara



Tenebrio molitor satelitna DNK (zeleno)

boja: FITC (fluorescein isothiocyanate)

Kromosomi su obojeni fluorescencijskom bojom DAPI (4',6-diamidino-2-phenylindole)

S vremenom analizirali cijeli niz satelitnih DNK različitih vrsta:
kukaca kornjaša, nematoda, školjkaša

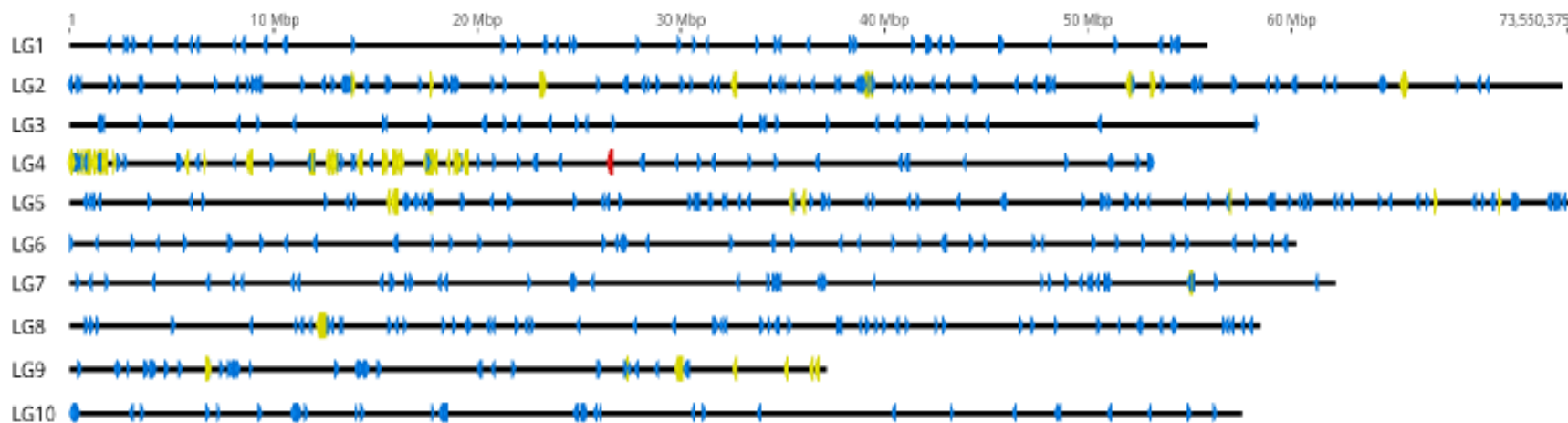
Đ. Ugarković, M. Plohl, V. Gamulin: Sequence variability
of satellite DNA from the mealworm *Tenebrio molitor*,
Gene, 83, travanj **1989**, **181-183**.

Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Nova paradigma: satelitne DNK raspršene po cijelom genomu

Pacific oyster *Crassostrea gigas* – dispersed, relatively short arrays in the euchromatin of all chromosomes; not in heterochromatin

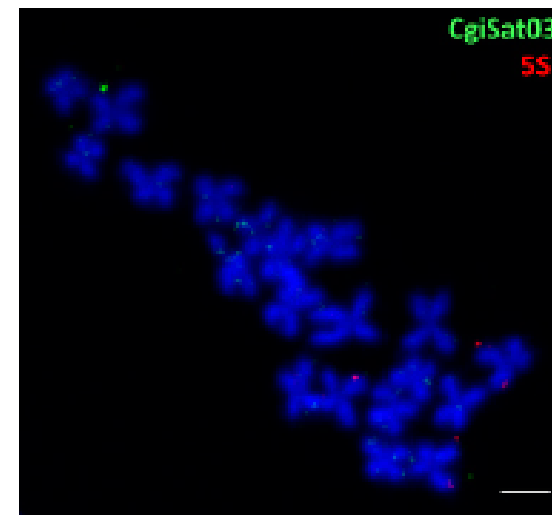
Satellitome: 52 satDNAs



3 satellite DNAs annotated on the chromosomes of the last genome assembly (Peñaloza et al. GigaScience 2021)

CgiSat03 (blue), CgiSat27 (yellow) and CgiSat22 (red)

- only two pairs of heterochromatin blocks



M. Tunjić-Cvitanić, J.J. Pasantes, D. García-Souto, T. Cvitanić, M. Plohl, E. Šatović-Vukšić: Satellitome analysis of the Pacific Oyster *Crassostrea gigas* reveals new pattern of satellite DNA organization, highly scattered across the genome, *Int. J. Mol. Sci.*, 22 (13), lipanj 2021, 6798

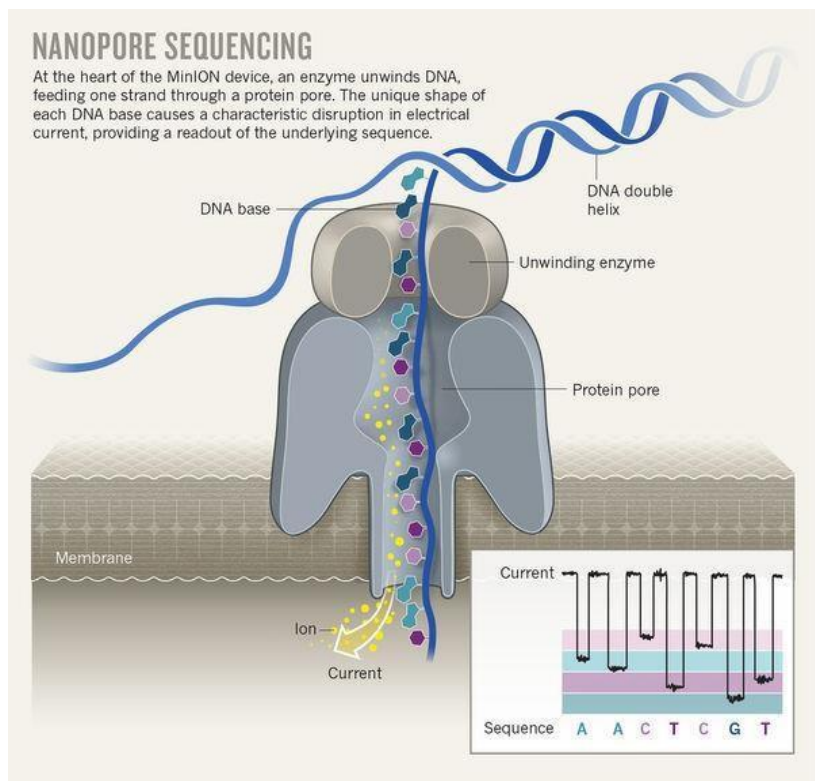


Razvoj tehnika sekvenciranja i istraživanje satelitnih DNK

Satelitne DNK i razvoj tehnika sekvenciranja

Third Generation (long-Read) Sequencing (PacBio and Oxford Nanopore): reads $> 100\,000$ bp; read and assembly of large repetitive regions, e.g. Centromeres ($> \text{Mb}$) (extensive use 2015 – on)

Possible nearly gap-free end-to-end chromosome reconstruction



The complete sequence of a human genome. Science 2022

Nanopore technology

Telomere to telomere assembly - T2T consortium

21 years after the first presentation of „completed” human genome!

T2T sequences of other genomes are in progress.