

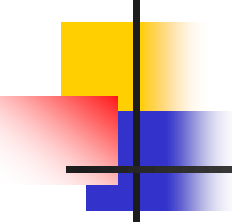


# **Aspecte de filogenie moleculară la microorganisme**

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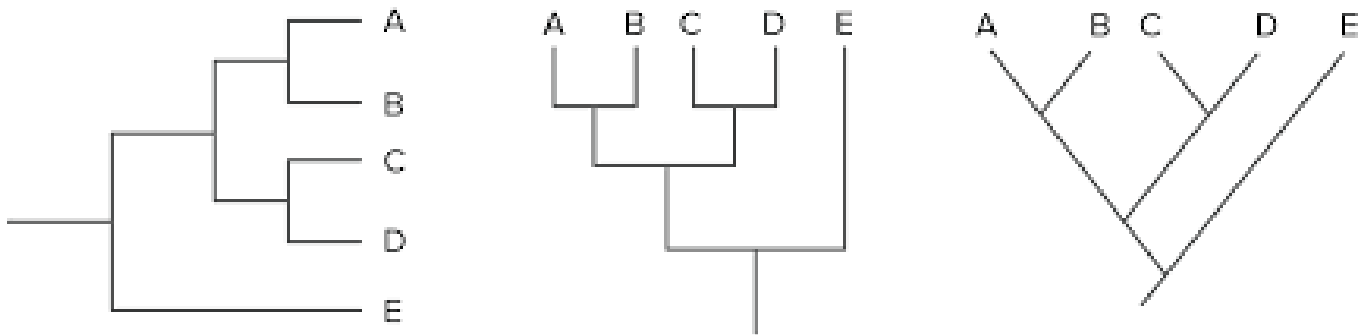
**concepte si tehnici utilizate**

**Lector dr. Ana-Maria Tănase**



**Filogenia** este ramura biologiei care prezinta relatii evolutive intre diferitele specii pe baza similaritatilor si diferentelor privind caracteristicile fenotipice si genetice.

**Arbori filoogenetici-** reprezentari grafice ale acestor relatii evolutive





# **marker filogenetic - conceptul de cronometru molecular**

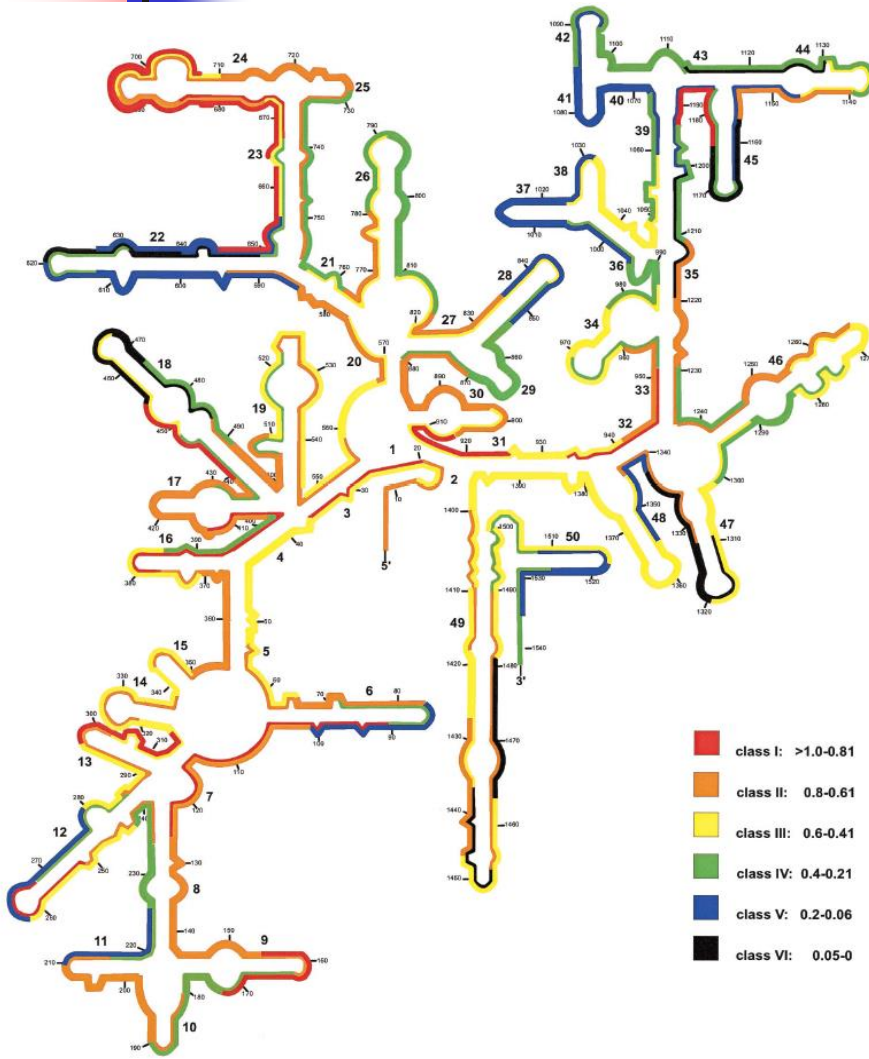
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## **Caracteristici**

- **constanță funcțională**
- **distribuție ubicvitară**
- **dimensiune relativ mare (conținutul informațional)**
- **regiuni structurale conservate dar și foarte variabile**
- **lipsa transferului lateral de gene**

Gene candidat : 16S; 23S; rpoB, gyrB, ITS,

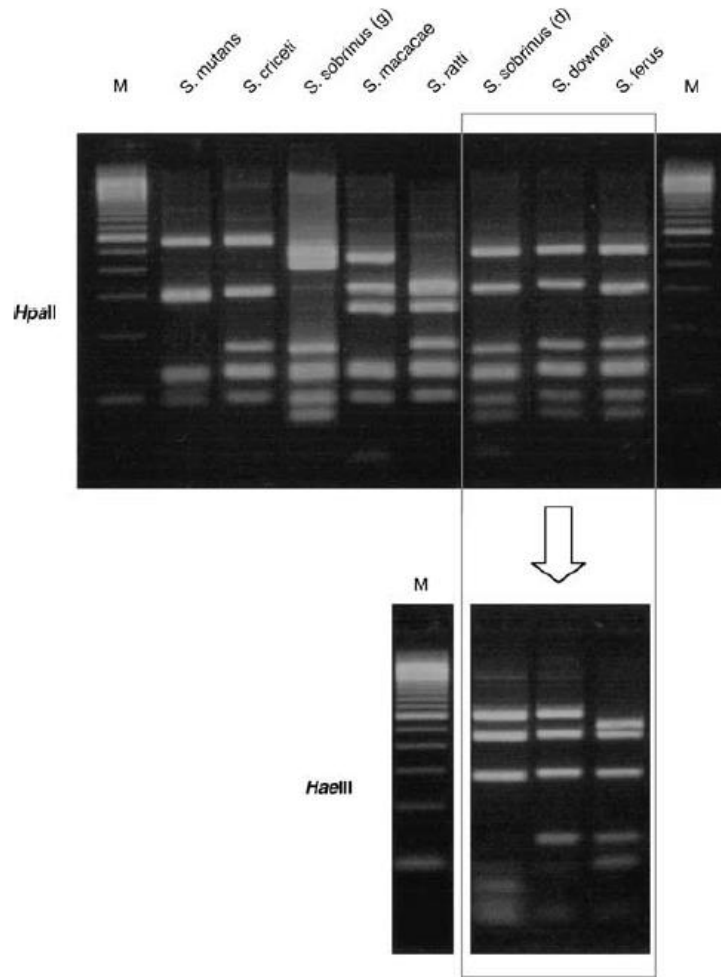
# De ce 16S ARN ribozomal?



## Comportament de "ceas molecular"

1. Prezența universală și evoluția relativ lentă a acestor molecule, dar supuse presiunii selective
2. Toate tipurile de ARN ribozomal sunt implicate în sinteza proteică și reprezintă până la 60% din ribozom
3. Transferul lateral de gene este foarte puțin probabil datorită funcției înalt conservate

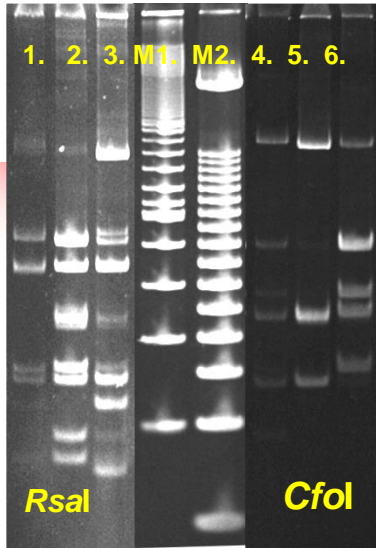
# ARDRA (Amplified Ribosomal DNA Restriction Analysis) - RFLP



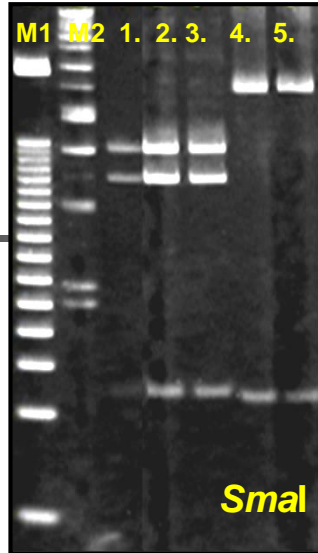
Metoda comparativă care necesită utilizarea unor tulpini de referință din Colectii de Microorganisme care să fie înrudite din punct de vedere filogenetic cu tulpinile analizate

Schema de identificare a 8 specii de *Streptococci* prin tehnica ARDRA: restricție cu *Hpa* II care nu a putut diferenția între cele 3 specii care la restricție cu *Hae*III au dat profile diferite. M-100bp DNA Ladder, Invitrogen Corp.(Sato, 2003)

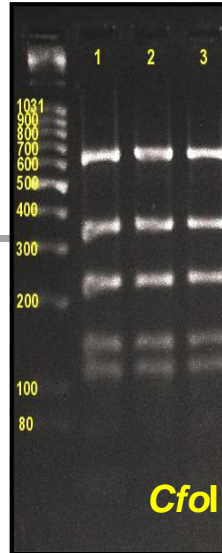
# Profile ARDRA



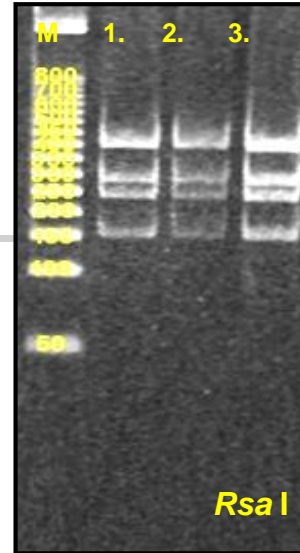
1- *Ch.sp.* ATCC 51823,  
2-S2, 3- S5B, M1-1kb  
DNA Step Ladder  
(PROMEGA), M2-  
100bp DNA Step  
Ladder (PROMEGA),  
4- *Ch.sp.* ATCC 51823 ,  
5-S2, 6- S5B



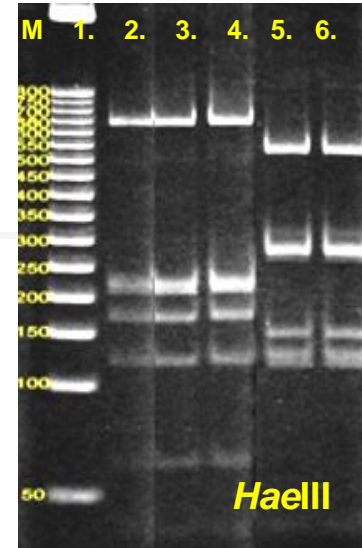
M1-50bp DNASTep  
Ladder (PROMEGA),  
M2- 1kb DNA ladder  
(PROMEGA),  
1-*P.aeruginosa* ATCC  
27853, 2-K1A, 3-K2,  
4-S2, 5-S5B



M-100bp  
Ladder  
(FERMENTAS),  
1-  
*P.aeruginosa*  
ATCC 27853,  
2-K1A, 3-K2



M-50bp DNA Step  
Ladder  
(PROMEGA),  
1-*P.aeruginosa*  
ATCC 27853, 2-  
K1A, 3-K2



M-50bp DNA Step  
Ladder (Promega),  
1-*Ps. aeruginosa* ATCC  
27853, 2-K1A,  
3-K2, 4-S2, 5-S5B

Sau3AI (17)		Sau3AI (277)		CfoI (574)		Sau3AI (833)		DdeI (1,147)		Sau3AI (1,290)	
DdeI (26)		HaeIII (210)		MspI (610)		BamHI (833)		CfoI (1,105)		HaeIII (1,214)	
HaeIII (49)		AluI (244)		SmaI (611)		Sau3AI (842)		MspI (1,159)		AluI (1,274)	
DdeI (8)		CfoI (217)		AluI (647)		AluI (858)		AluI (1,065)		MspI (1,300)	
AluI (82)		DdeI (292)		DdeI (753)		DdeI (846)		HaeIII (1,170)		HaeIII (1,385)	
Sau3AI (384)		MspI (500)		CfoI (576)		HaeIII (846)		HaeIII (926)		MspI (1,381)	
CfoI (574)		AluI (647)		HaeIII (892)		CfoI (855)		ApaI (928)		SmaI (1,382)	
DdeI (753)		HaeIII (985)		DdeI (1,117)		HaeIII (892)		HaeIII (985)		AluI (1,436)	
Sau3AI (384)		MspI (500)		CfoI (576)		DdeI (846)		HaeIII (926)			
AluI (82)		DdeI (292)		AluI (647)		CfoI (855)		ApaI (928)			
CfoI (217)		MspI (500)		HaeIII (892)		HaeIII (985)		DdeI (1,117)			
DdeI (292)		Sau3AI (384)		DdeI (753)		Sau3AI (842)		HaeIII (1,205)			
Sau3AI (384)		MspI (500)		CfoI (576)		AluI (858)		MspI (1,170)			
DdeI (292)		AluI (244)		AluI (647)		DdeI (846)		HaeIII (1,214)			
HaeIII (210)		HaeIII (210)		HaeIII (210)		HaeIII (210)		HaeIII (210)			
Sau3AI (301)		Sau3AI (301)		Sau3AI (301)		Sau3AI (301)		Sau3AI (301)			
DdeI (319)		DdeI (319)		DdeI (319)		DdeI (319)		DdeI (319)			
Sau3AI (384)		Sau3AI (384)		Sau3AI (384)		Sau3AI (384)		Sau3AI (384)			
MspI (500)		MspI (500)		MspI (500)		MspI (500)		MspI (500)			
CfoI (576)		CfoI (576)		CfoI (576)		CfoI (576)		CfoI (576)			
AluI (647)		AluI (647)		AluI (647)		AluI (647)		AluI (647)			
DdeI (753)		DdeI (753)		DdeI (753)		DdeI (753)		DdeI (753)			
HaeIII (892)		HaeIII (892)		HaeIII (892)		HaeIII (892)		HaeIII (892)			
HaeIII (985)		HaeIII (985)		HaeIII (985)		HaeIII (985)		HaeIII (985)			
DdeI (1,117)		DdeI (1,117)		DdeI (1,117)		DdeI (1,117)		DdeI (1,117)			
HaeIII (1,205)		HaeIII (1,205)		HaeIII (1,205)		HaeIII (1,205)		HaeIII (1,205)			
Sau3AI (1,302)		Sau3AI (1,302)		Sau3AI (1,302)		Sau3AI (1,302)		Sau3AI (1,302)			
AluI (1,436)		AluI (1,436)		AluI (1,436)		AluI (1,436)		AluI (1,436)			

Mapping all cutsites.

Cutters : AluI, ApaI, BamHI, CfoI, DdeI, HaeIII, MspI, Sau3AI & SmaI

Non-Cutters : EcoRV & NotI

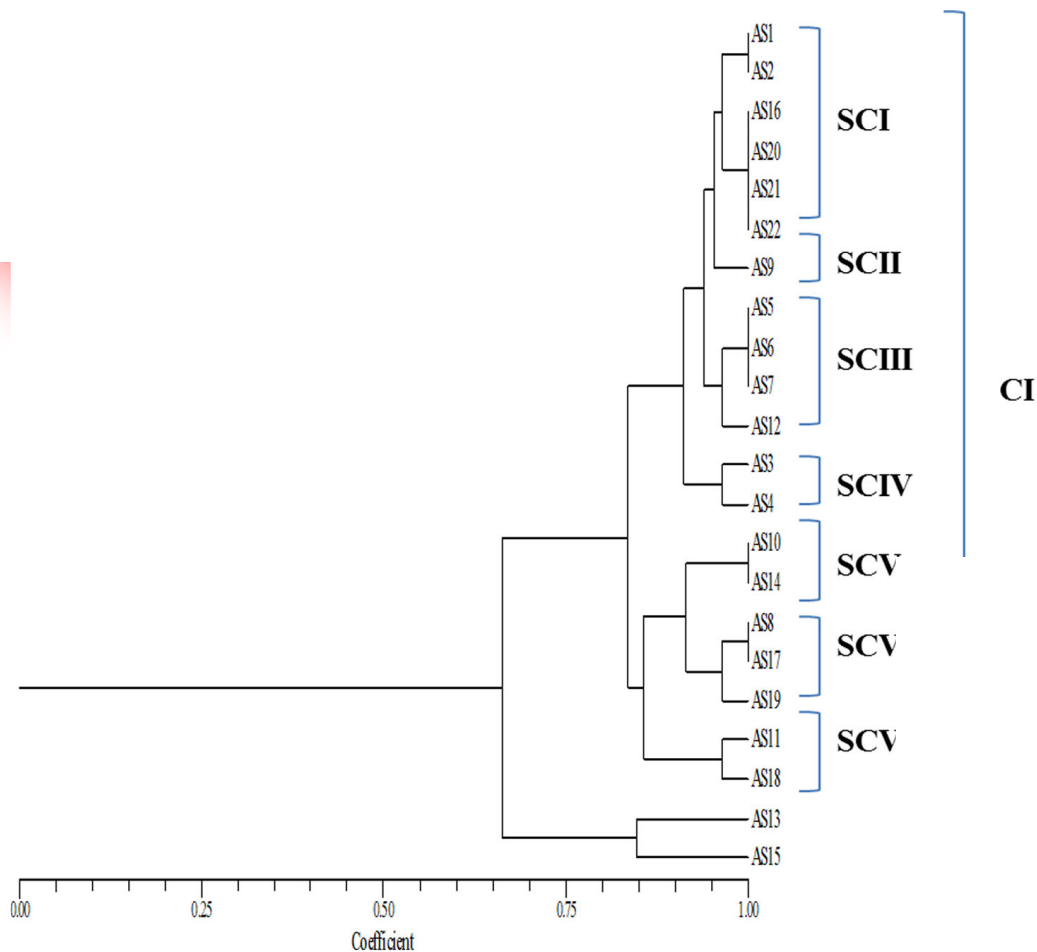
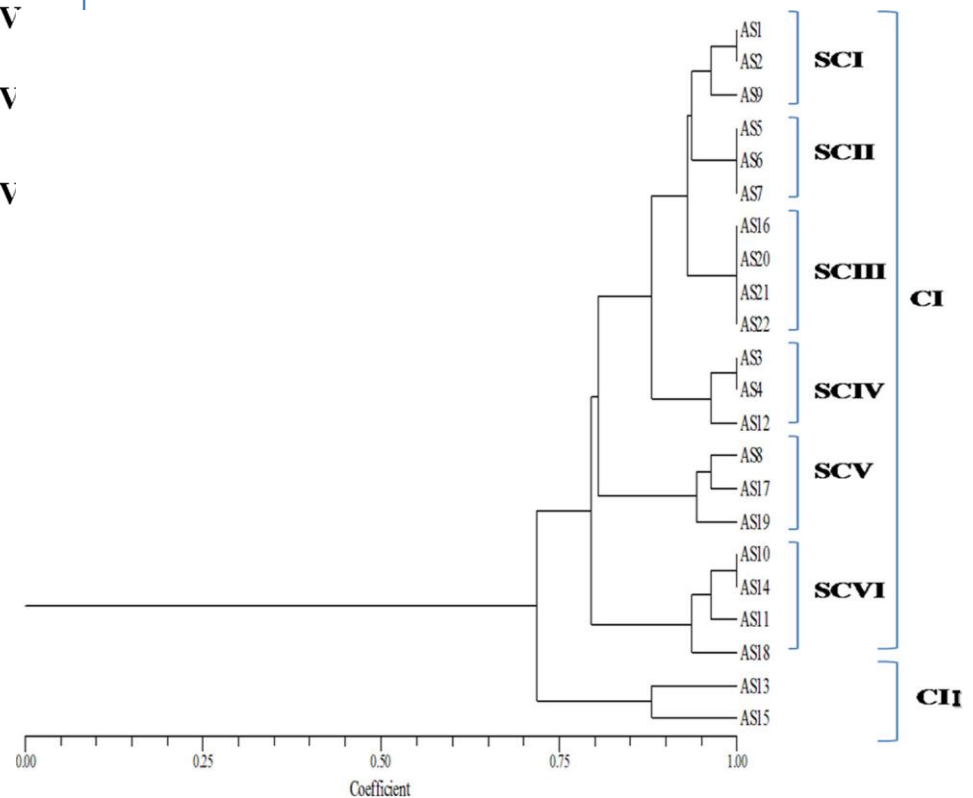


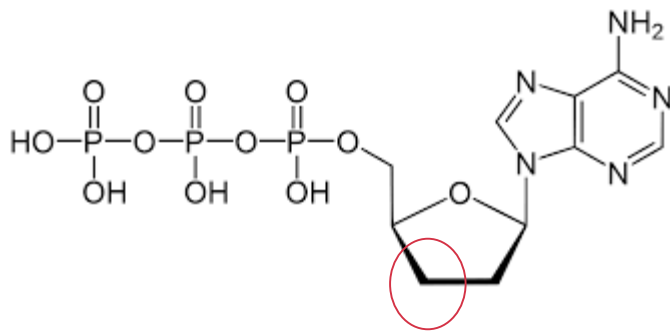
Fig. 2 Combined UPGMA dendrogram of *Pseudomonas* specific gene of ARDRA with *AluI*, *RsaI* and *BamHI* (Rajwar, 2016)

Fig. 1 Combined UPGMA dendrogram of 16S rDNA on the basis of ARDRA with *AluI*, *RsaI* and *BamHI*



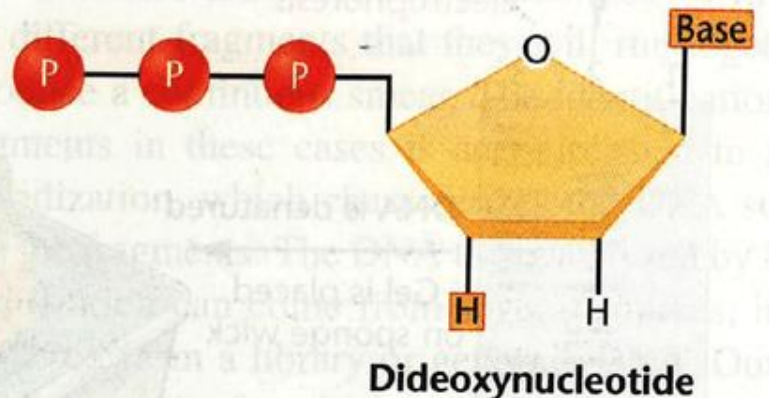
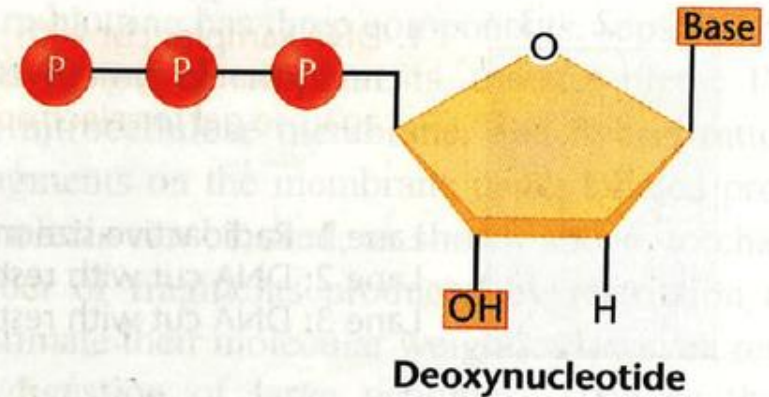
<https://www.youtube.com/watch?v=ONGdehkB8jU>

## Secvențiere Sanger – metoda dideoxy



### Componente:

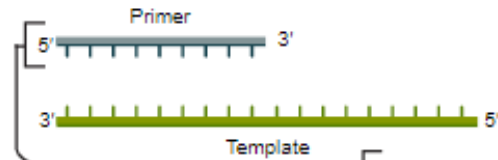
- ADN de secvențiat (amplicon)
- primer doar unul singur
- ADN polimerază
- amestec de dNTPs
- ddNTPs





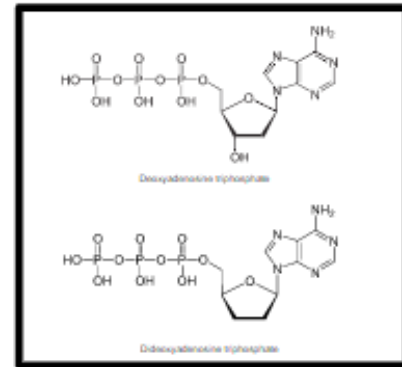
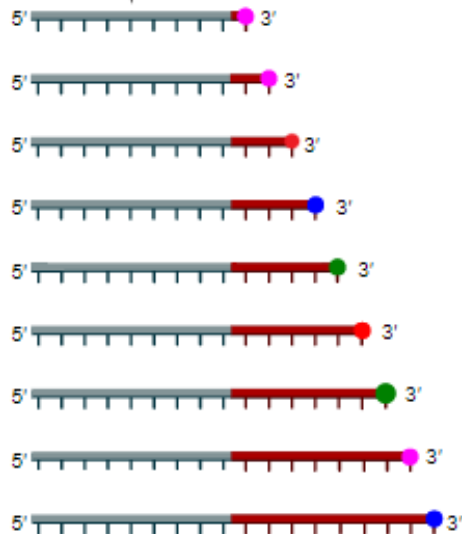
### ① Reaction mixture

- Primer and DNA template
- DNA polymerase
- ddNTPs with flourochromes
- dNTPs (dATP, dCTP, dGTP, and dTTP)

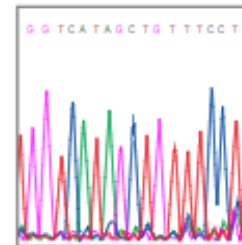
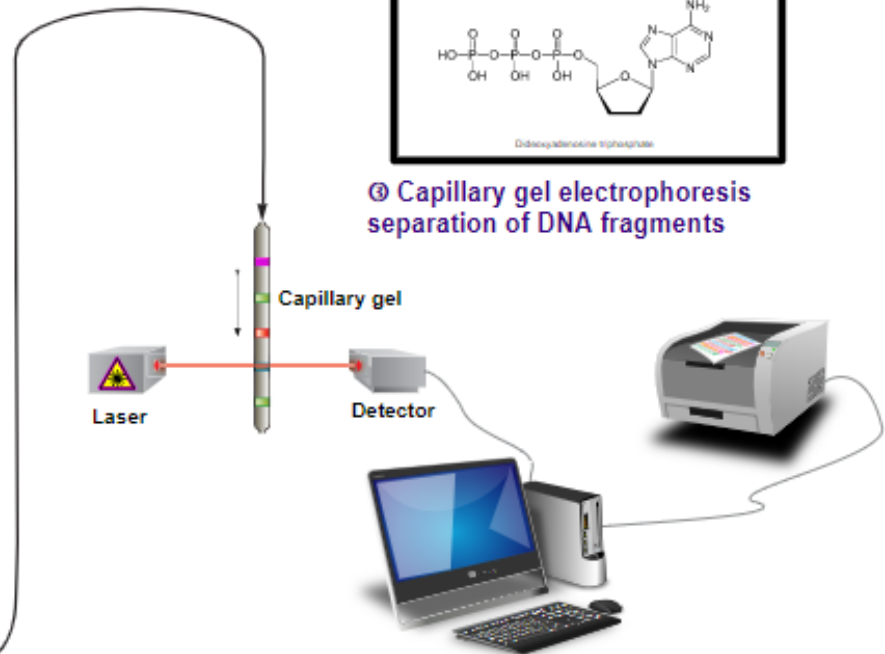


- ddNTPs
- ddTTP
- ddCTP
- ddATP
- ddGTP

### ② Primer elongation and chain termination



### ③ Capillary gel electrophoresis separation of DNA fragments

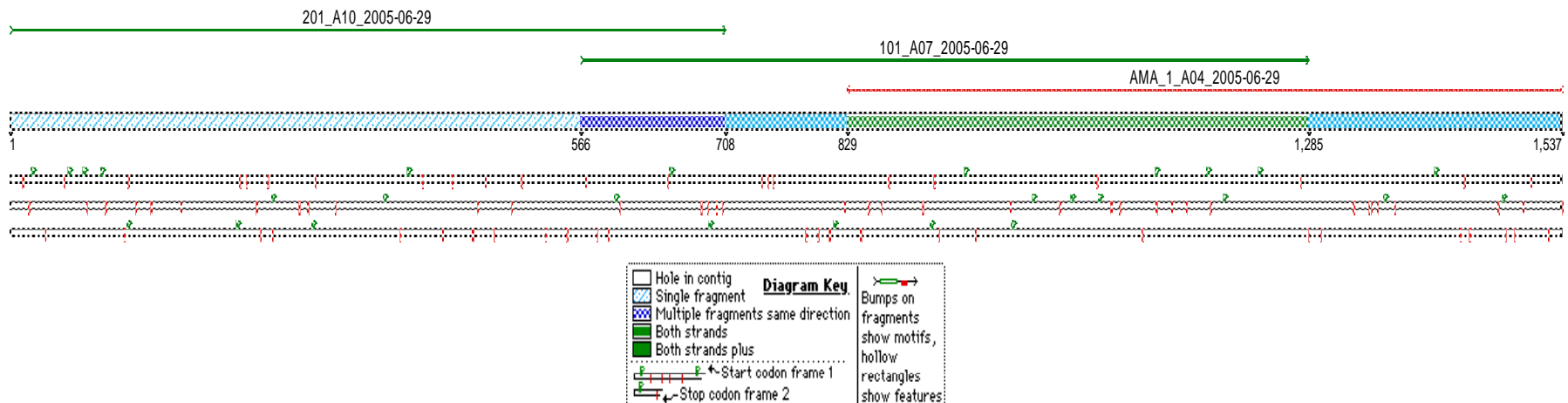
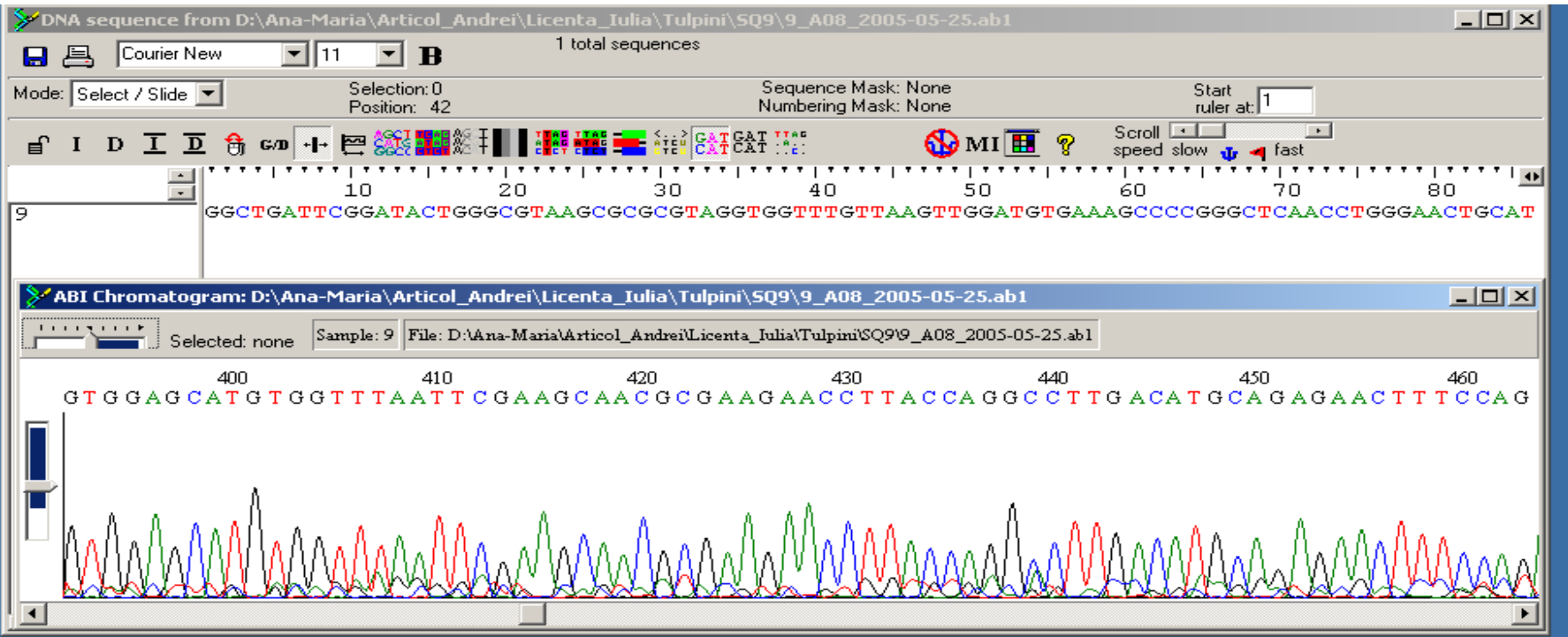


Chromatograph

### ④ Laser detection of flourochromes and computational sequence analysis

# Secventierea genei pentru ARNr 16S

BioEdit Sequence  
Alignment Editor



# National Center for Biotechnology Information NCBI - BLAST

<http://www.ncbi.nlm.nih.gov/>

ncbi.nlm.nih.gov/guide/sequence-analysis/#howtos

Apps Dezvolta-ti vointa p... Abonare la Newslet... Cum să ne eliberăm... Whole-Genome Se... Library Genesis: Sci... Editorial Manager® EU-US Training 201... Paired t-test - Han... Other bookmarks

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NCBI National Center for Biotechnology Information

All Databases Search

## Sequence Analysis

All Databases Downloads Tools How To

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- [Submit sequence data to NCBI](#)
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- [Submit multiple query sequences in a single BLAST search](#)
- [Design PCR primers and check them for specificity](#)
- [Run BLAST software on a local computer](#)
- [Automate BLAST searches performed on NCBI servers](#)
- [Compare your sequence to the RefSeqGene/LRG standard](#)

### Quick Links

- [BLAST \(Stand-alone\)](#)
- [Basic Local Alignment Search Tool \(BLAST\)](#)
- [Conserved Domain Search Service \(CD Search\)](#)
- [Genome Workbench](#)
- [Primer-BLAST](#)
- [ProSplign](#)
- [Splign](#)

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- Domains & Structures
- Genes & Expression
- Genetics & Medicine
- Genomes & Maps
- Homology
- Literature
- Proteins
- Sequence Analysis

## Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) ?

[Clear](#)

Query subrange ?

From

To

Or, upload file

Alegeți fișierul Nu s-au ales fișiere ?

Job Title

Enter a descriptive title for your BLAST search ?

## Choose Search Set

Database

☒ Representative genomes only ☐ All genomes ?

Organism

Optional

Enter organism name or id--completions will be suggested

☐ Exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown. ?

Entrez Query

Optional

Enter an Entrez query to limit search ?

## Program Selection

Optimize for

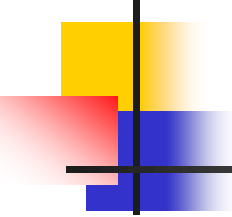
- ☒ Highly similar sequences (megablast)  
☐ More dissimilar sequences (discontiguous megablast)  
☐ Somewhat similar sequences (blastn)

Choose a BLAST algorithm ?

**BLAST**

Search **database Representative genomes** using **Megablast (Optimize for highly similar sequences)**

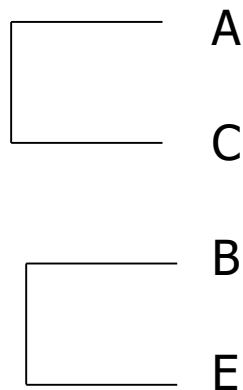
☐ Show results in a new window



## Distance matrix UPGMA (unweighted pair group method with arithmetic mean- matrix de similaritate)

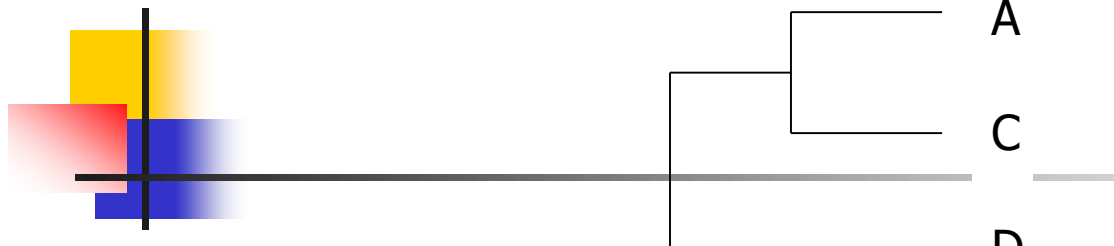
```

A   A T C G T G G T A C T G
B   C C G G A G A A C T A G
C   A A C G T G C T A C T G
D   A T G G T G A A A G T G
E   C C G G A A A A C T T G
F   T G G C C C T G T A T C
  
```



Differences between sequences

	A	B	C	D	E	F
A		9	2	4	9	10
B			9	6	2	10
C				5	9	10
D					6	10
E						10
F						



	A/C	B	D	E	F
A/C		9	4.5	9	10
B			6	2	10
D				6	10
E					10
F					



	A/C	B/E	D	F
A/C		9	4.5	10
B/E			6	10
D				
F				

	A/C/D	B/E	F
A/C/D		7.5	10
B/E			10
F			

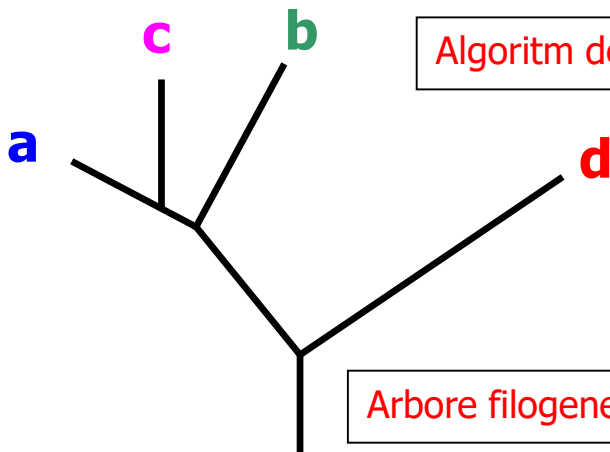
# Principalele etape ale reconstrucției de arbori filogenetici

1. Determinarea secvenței
2. Alinierea secvenței
3. Analiza datelor prin încadrarea la nivel de grup
4. Reconstrucția filogenetică a arborilor

specia a	ACGUGCUCUUCAAGCUACGUUGGAACACACUGGCGCAAUUU
specia b	.....CC.....
specia c	.....C.....
specia d	.....AAA.....

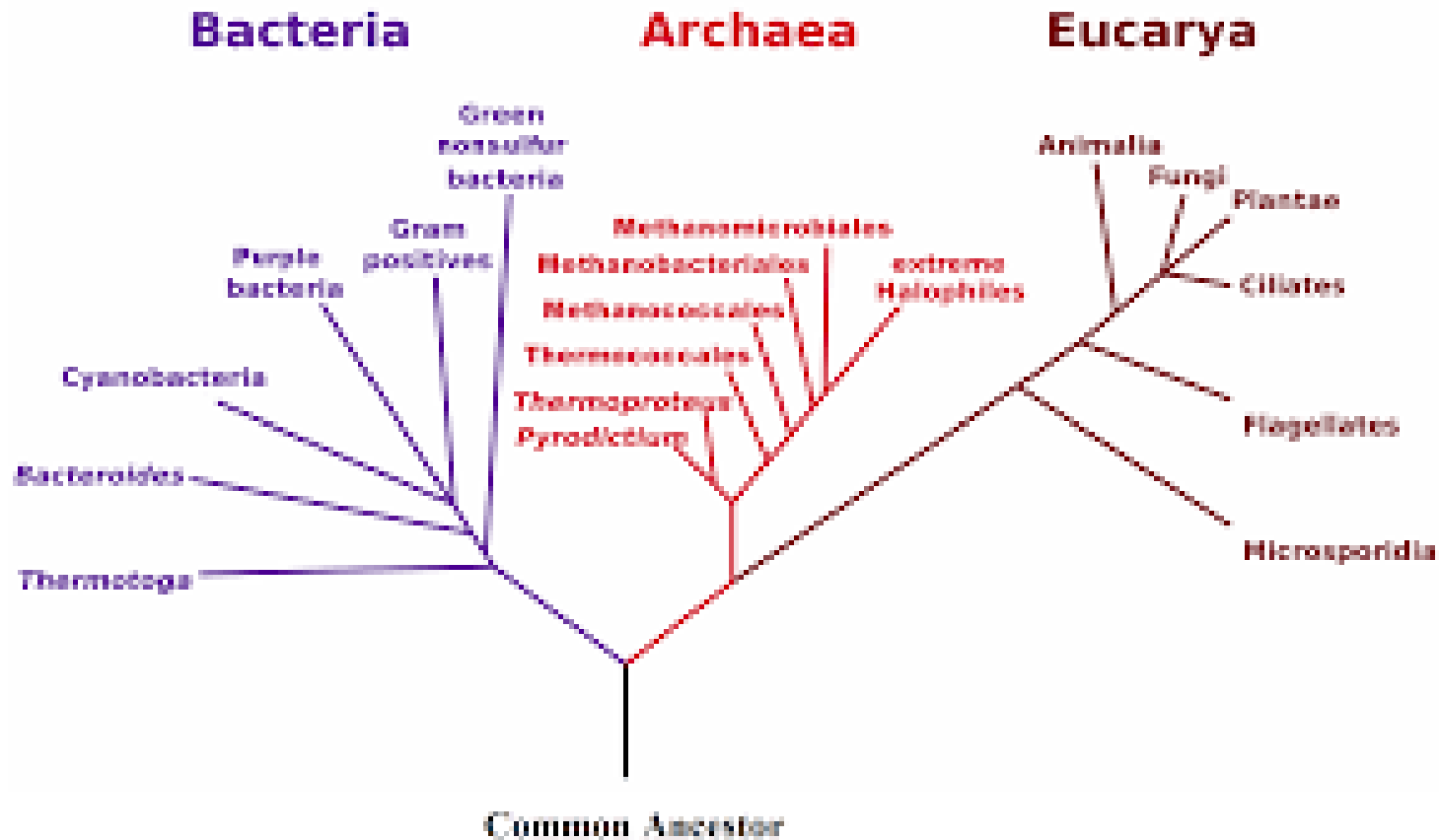
Analiza comparativă a secvențelor

Algoritm de calcul



Arbore filogenetic

# Tree of life – arborele vieții





# Alinierea secventelor

GKS2-189	Odata	AGAGUUUGAUCAUUGGCUCAAGGACGAACCGUUGCGACGUGCUUAAACACAUGCCAGUCGAGCGAUUGAAACACCUUCCGGUGUGAA
GKS2-103	Odata	AGAGUUUGAUCAUUGGCUCAAGGACGAACCGUUGCGCGCGUGCUUAAACACAUGCCAGUCGAGCGAUUGAAACACCUUCCGGUGUGAA
FukuN30	Odata	AGAGUUUGAUUUAUUGGCUCAAGGACGAACCGUUGCGCGCGUGCUUAAACACAUGCCAGUCGAGCGAUUGAAACACCUUCCGGUGUGAA
ACK-M1	Odata	AUCCUGGCGUCAGACGAACCGUUGCGCGUGCUUAAACACAUGCCAGUCGAGCGAUUGAAACACCUUCCGGUGUGAAUUAAGCGGUCCAU
UniAct63	Odata	AUCCUGGCGUCAGACGAACCGUUGCGCGUGCUUAAACACAUGCCAGUCGAGCGAUUGAAACACCUUCCGGUGUGAAUUAAGCGGUCCAU
FukuN15	Odata	AGAGUUUGAUCCUUGGCUCAAGGACGAACCGUUGCGCGCGUGCUUAAACACAUGCCAGUCGAGCGAUUGAAACACCUUCCGGUGUGAA
FukuN44	Odata	AGAGUUUGAUCAUUGGCUCAAGGACGAACCGUUGCGCGCGUGCUUAAACACAUGCCAGUCGAGCGAUUGAAACACCUUCCGGUGUGAA
FukuN8	Odata	AGAGUUUGAUCAUUGGCUCAAGGACGAACCGUUGCGCGCGUGCUUAAACACAUGCCAGUCGAGCGAUUGAAACACCUUCCGGUGUGAA
AF141592	Odata	GACGAACCGUUGCGCGCGUGCUUAAACACAUGCCAGUCGAGCGAUUGAAACACCUUCCGGUGUGAAUUAAGCGGCGAACCGGUGAGCG
AF141426	Odata	ACGAACCGUUGCGCGCGUGCUUAAACACAUGCCAGUCGAGCGGUGAAACACCUUCCGGUGUGAAUUAAGCGGCGAACCGGUGAGGA
GKS2-189	Odata	---A-CGGC-G-CG-U-G-A-G-G-CA-U-G-A-A-G-G-CC-----UU-CG-----G-G-U-U-G-U-A-AA-C-CUC
GKS2-103	Odata	---A-CGGC-G-CG-U-G-A-G-G-CA-U-G-A-A-G-G-CC-----UU-CG-----G-G-U-U-G-U-A-AA-C-CUC
FukuN30	Odata	---A-CGGC-G-CG-U-G-A-G-G-CA-U-G-A-A-G-G-CC-----UU-CG-----G-G-U-U-G-U-A-AA-C-CUC
ACK-M1	Odata	---A-CGGC-G-CG-U-G-A-G-G-CA-U-G-A-A-G-G-CC-----UU-CG-----G-G-U-U-G-U-A-AA-C-CUC
UniAct63	Odata	---A-CGGC-G-CG-U-G-A-G-G-CA-U-G-A-A-G-G-CC-----UU-CG-----G-G-U-U-G-U-A-AA-C-CUC
FukuN15	Odata	---A-CGGC-G-CG-U-G-A-G-G-CA-U-G-A-A-G-G-CC-----UU-CG-----G-G-U-U-G-U-A-AA-C-CUC
FukuN44	Odata	---A-CGGC-G-CG-U-G-A-G-G-CA-U-G-A-A-G-G-CC-----UU-CG-----G-G-U-U-G-U-A-AA-C-CUC
FukuN8	Odata	---A-CGGC-G-CG-U-G-A-G-G-CA-U-G-A-A-G-G-CC-----UU-CG-----G-G-U-U-G-U-A-AA-C-CUC
AF141592	Odata	---A-CGGC-G-CG-U-G-A-G-G-CA-U-G-A-A-G-G-CC-----UU-CG-----G-G-U-U-G-U-A-AA-C-CUC
AF141426	Odata	---A-CGGC-G-CG-U-G-A-G-G-CA-U-G-A-A-G-G-CC-----UU-CG-----G-G-U-U-G-U-A-AA-C-CUC



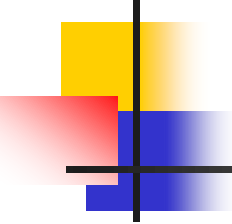
## **ARBORI FILOGENETICI**

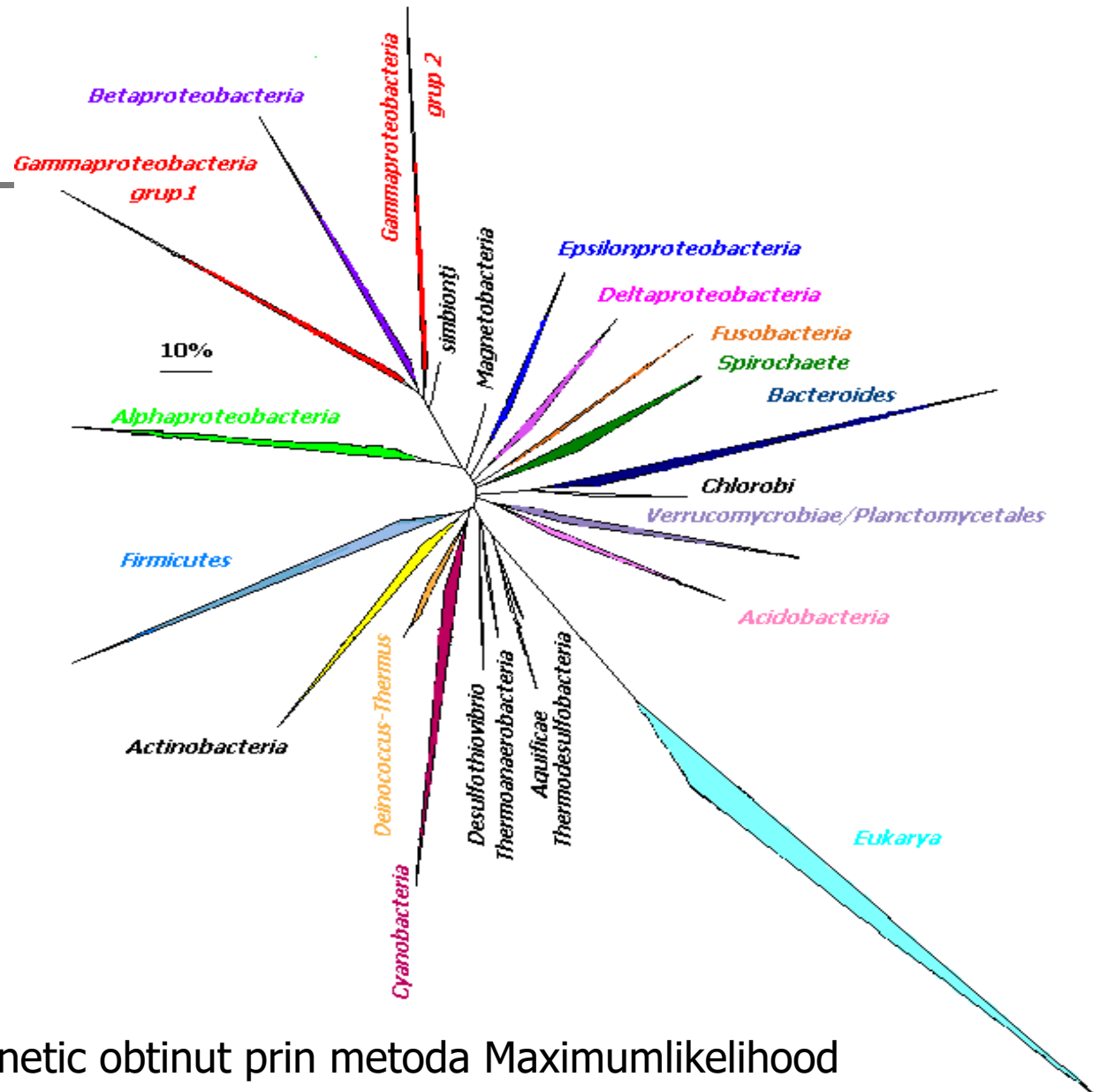
reprezentări grafice a relațiilor filogenetice dintre grupuri taxonomice

---

- **Alinirea secvențelor este absolut necesară deoarece numai modificările apărute față de secveța unui ancestor pot fi utilizate pentru a trage concluzii asupra filogeniei secvențelor.**
- **Numărul și poziția diferențelor apărute la nivelul regiunilor variabile ale secvențele aliniate, reprezintă baza deducție relațiilor dintre ele.**
- **Algoritmi matematici au fost elaborați pe baza unor mode matematice statistice de evoluție**
- **Alura ramurilor arborelui reprezintă modalitatea de evoluție, iar lungimea ramurilor indică distanța filogenetică între organismul repectiv și ancestorul său.**
- **Există două categorii de prezentare : arbori radiali și dendrogramă**
- **Avantajul arborilor radiali: organismele înrudite filogenetic pot fi mult mai bine evidențiate.**

# Cele mai uzuale metode de reconstructie de arbori filogenetici

- 
1. Distance Matrix
  2. Maximum Parsimony
  3. Maximum Likelihood



Arbore filogenetic obtinut prin metoda Maximumlikelihood