TAXONOMI

Fra fænotypi til molekylær biologi

Mogens Kilian

Institut for Medicinsk Mikrobiologi og Immunologi

Aarhus Universitet

AARHUS UNIVERSITET

Carl von Linné

1707-78

Ordo I.

PRIMATES.

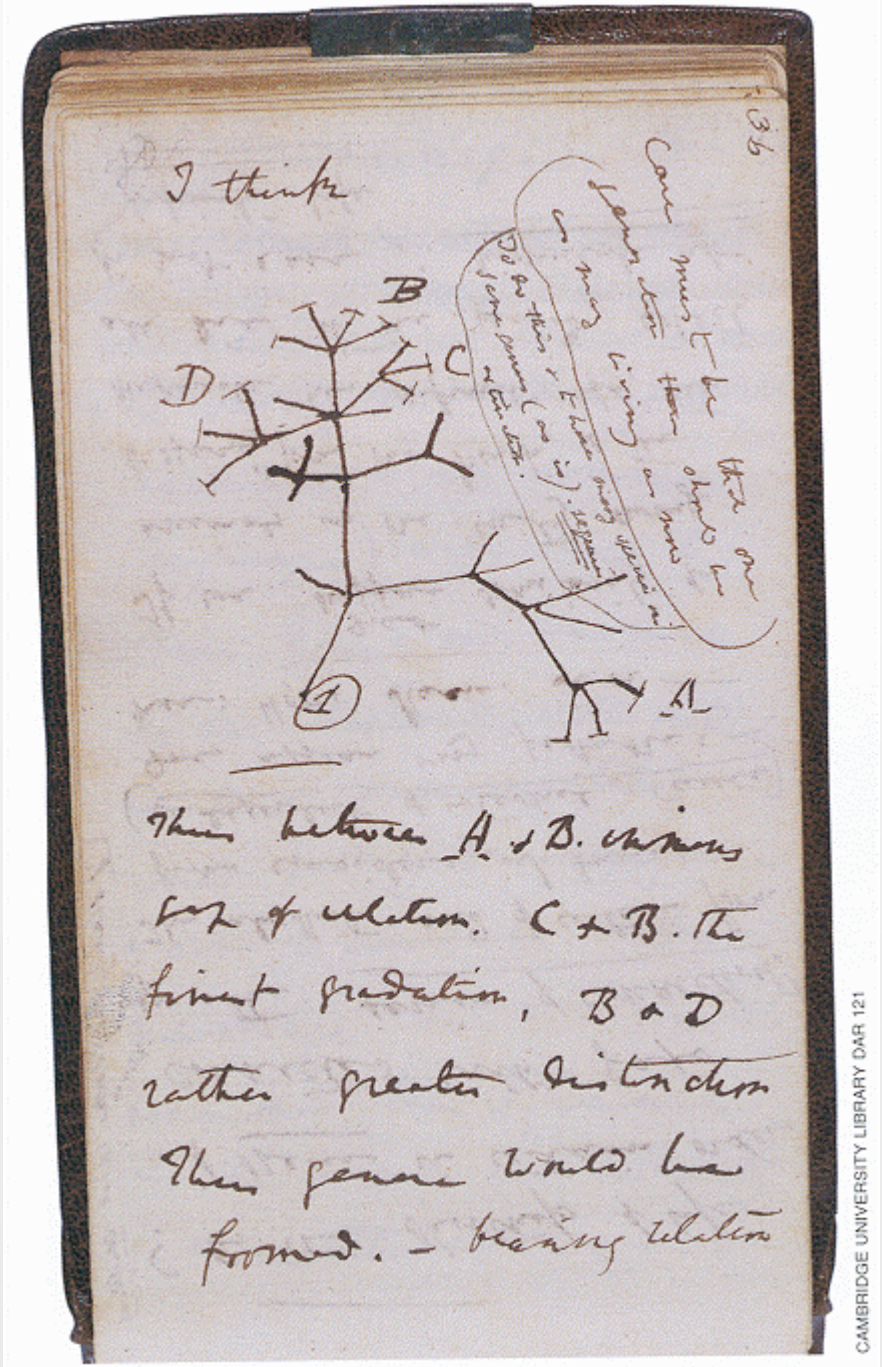
Dentes primores superiores IV paralleli.
Mammæ pectorales, binæ.

1. HOMO nosce Te ipsum.

Taxonomi

- Klassifikation
- Navngivning
- Identifikation

- International Code of Nomenclature of Bacteria (1930 -)



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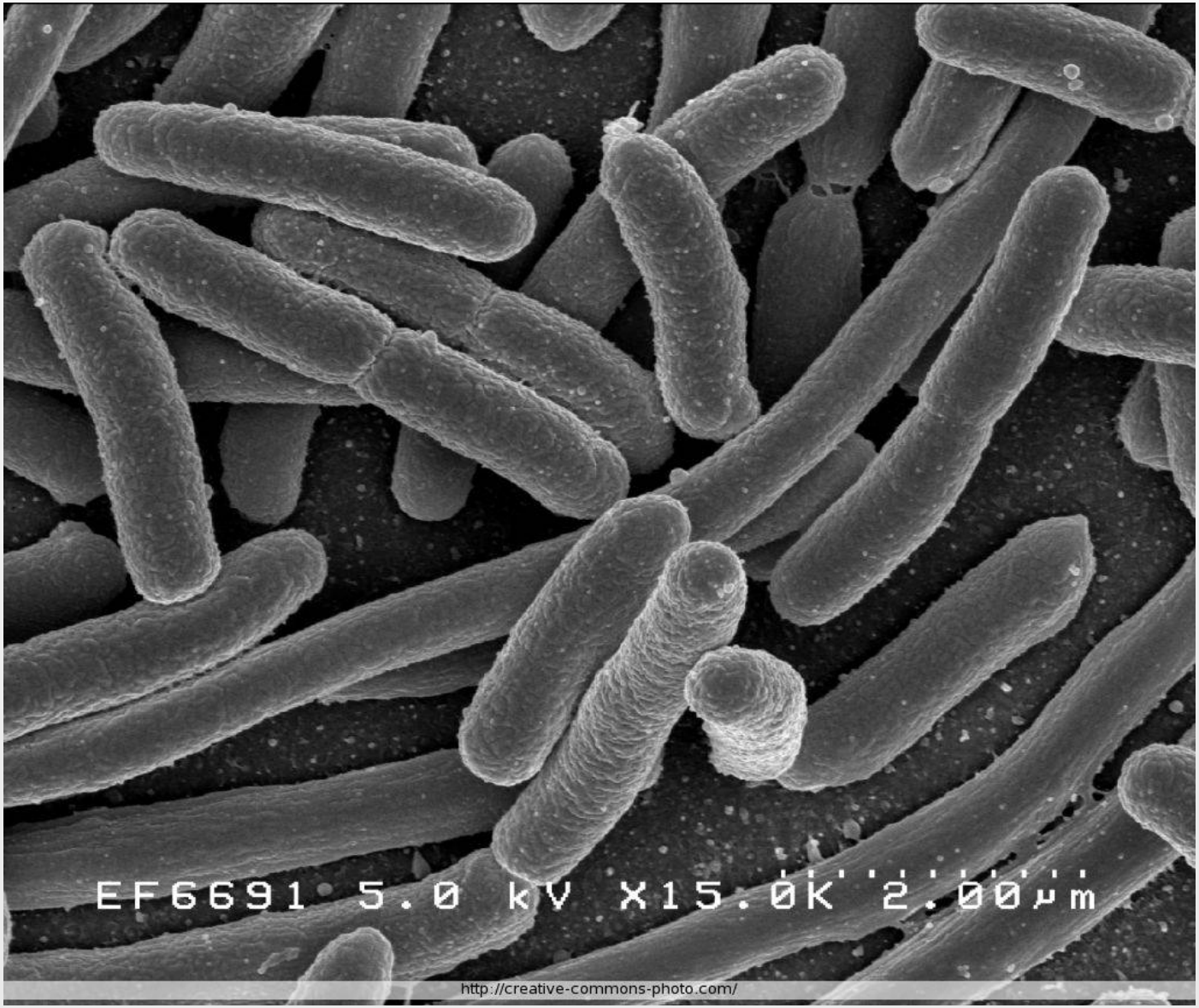
Evolution over 3,5 milliarder år


EF6691 5.0 kV X15.0K 2.00µm
http://creative-commons-photo.com/

Genetisk diversificering af bakterier

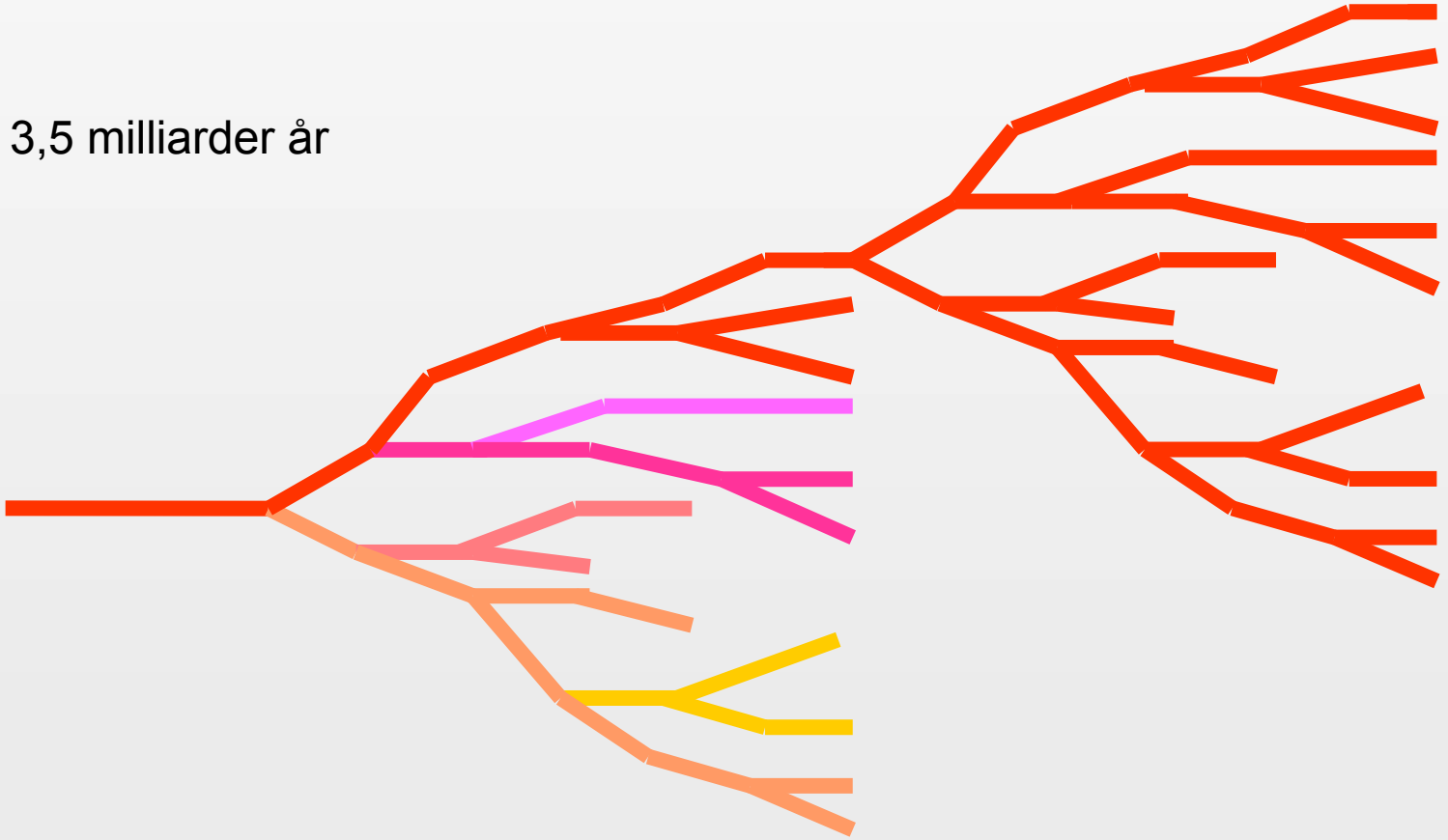
3 milliarder år

Genetisk diversificering af bakterier

Udvikling af arter

Diversificeringen fortsætter

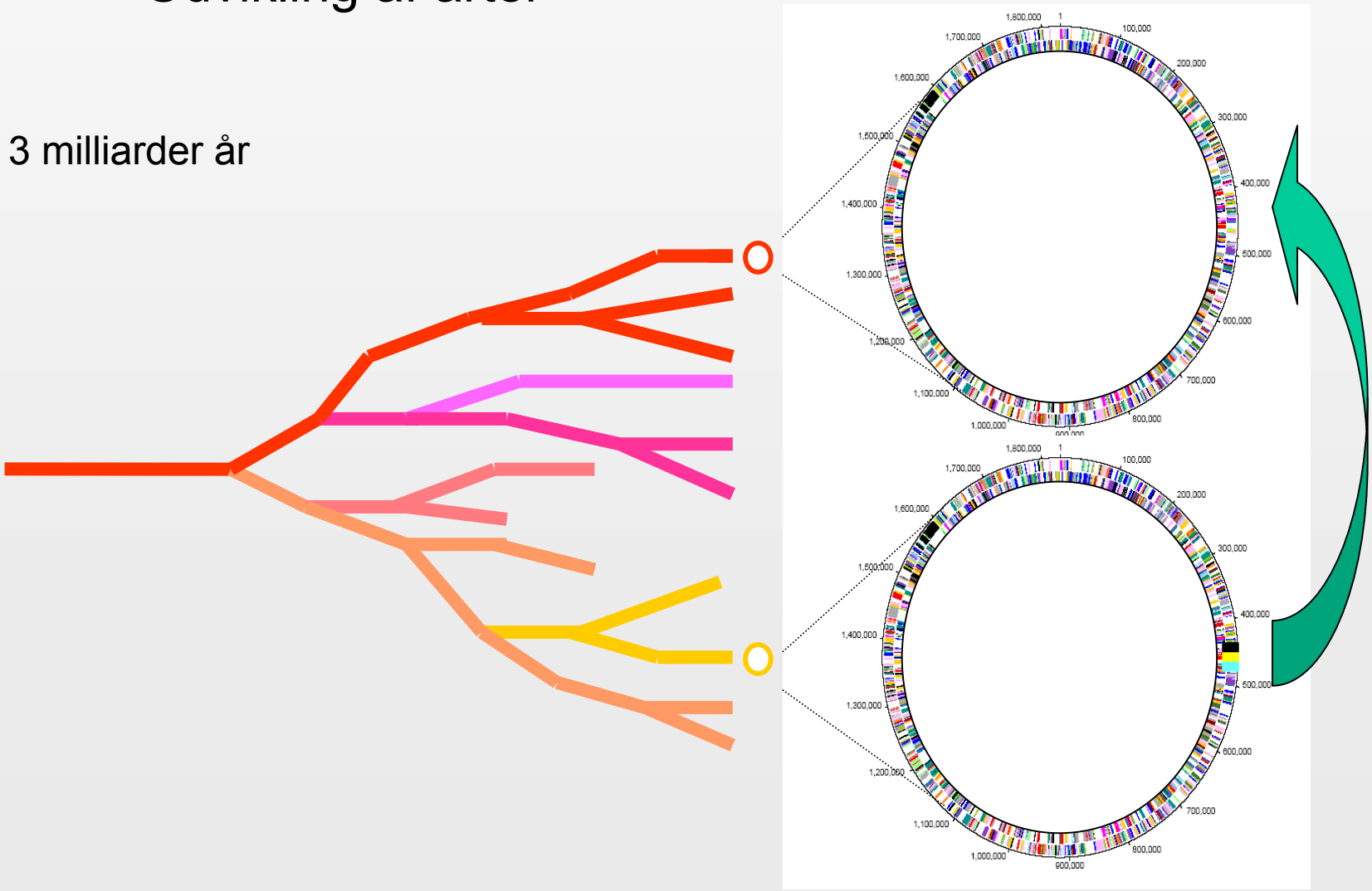
3,5 milliarder år



Genetisk diversificering af bakterier

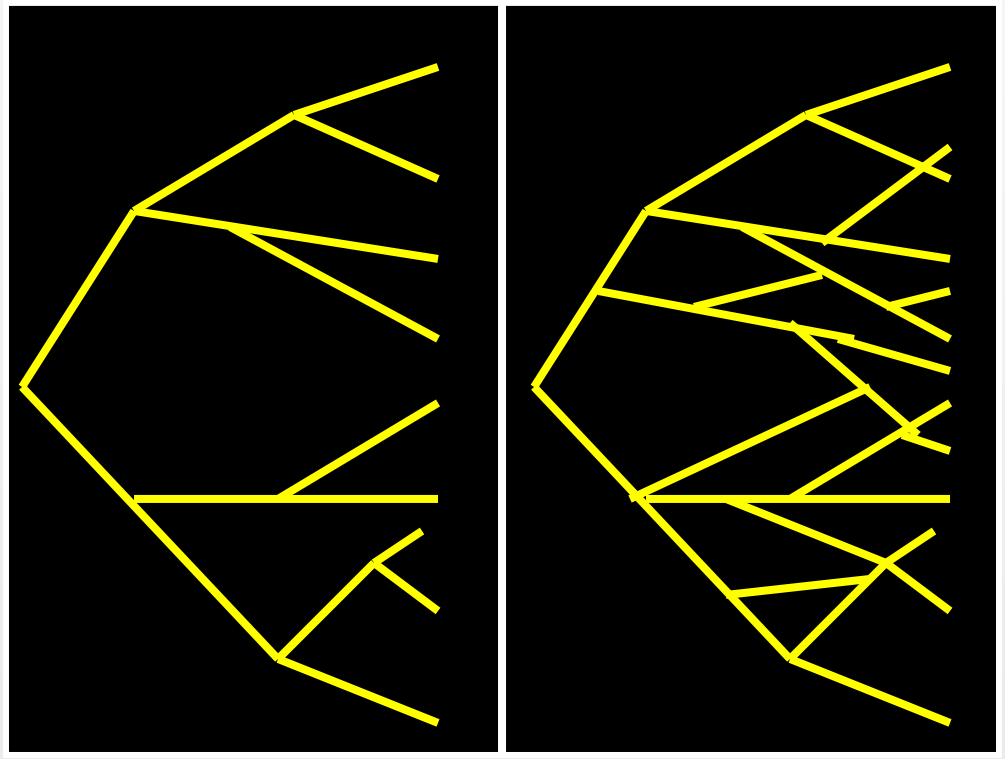
Udvikling af arter

3 milliarder år



GENETISKE POPULATIONS-STRUKTURER

CLONAL **PANMIX**

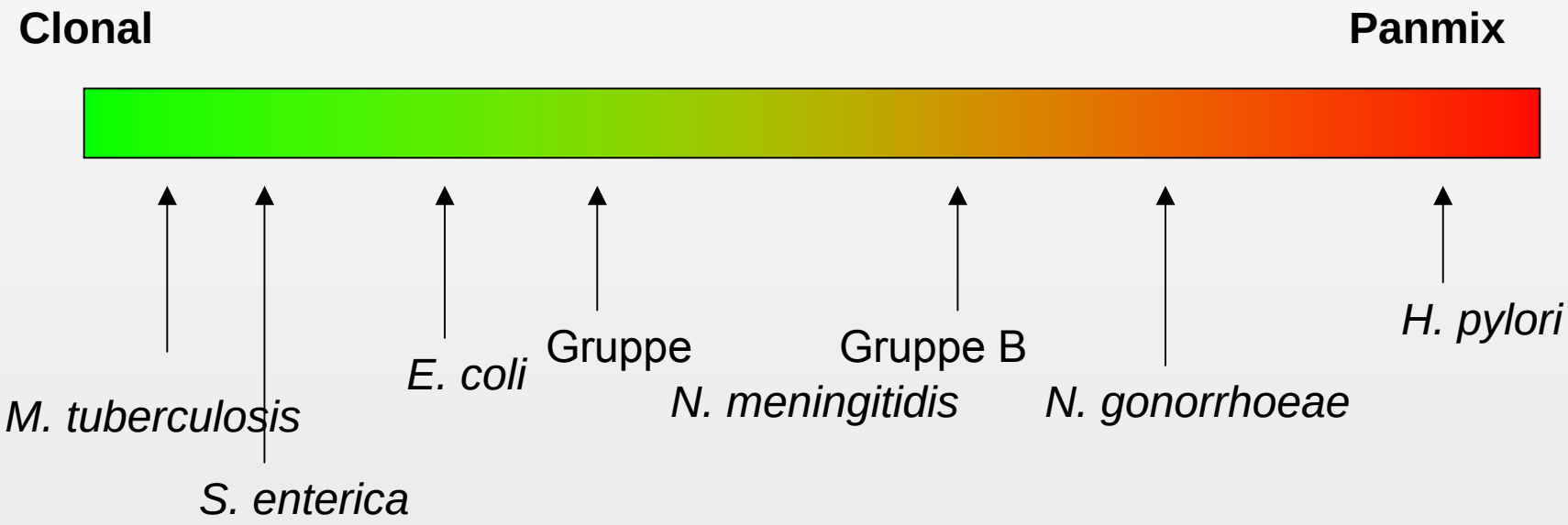


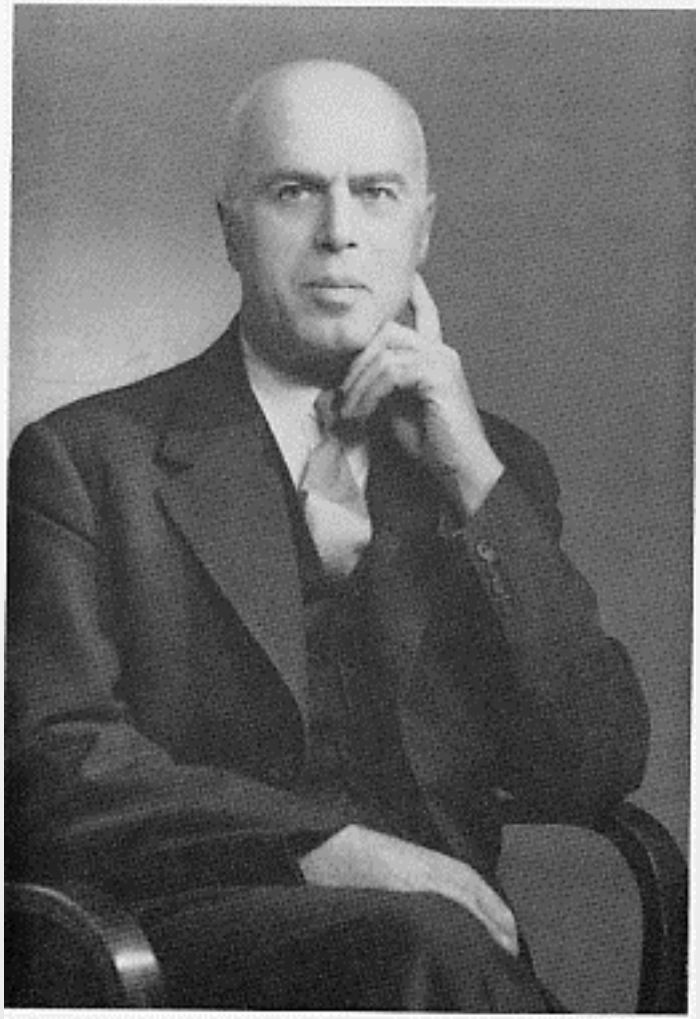
Koblings-

uligevægt

Tilfældig eller nær tilfældig kombination af egenskaber (alleller)

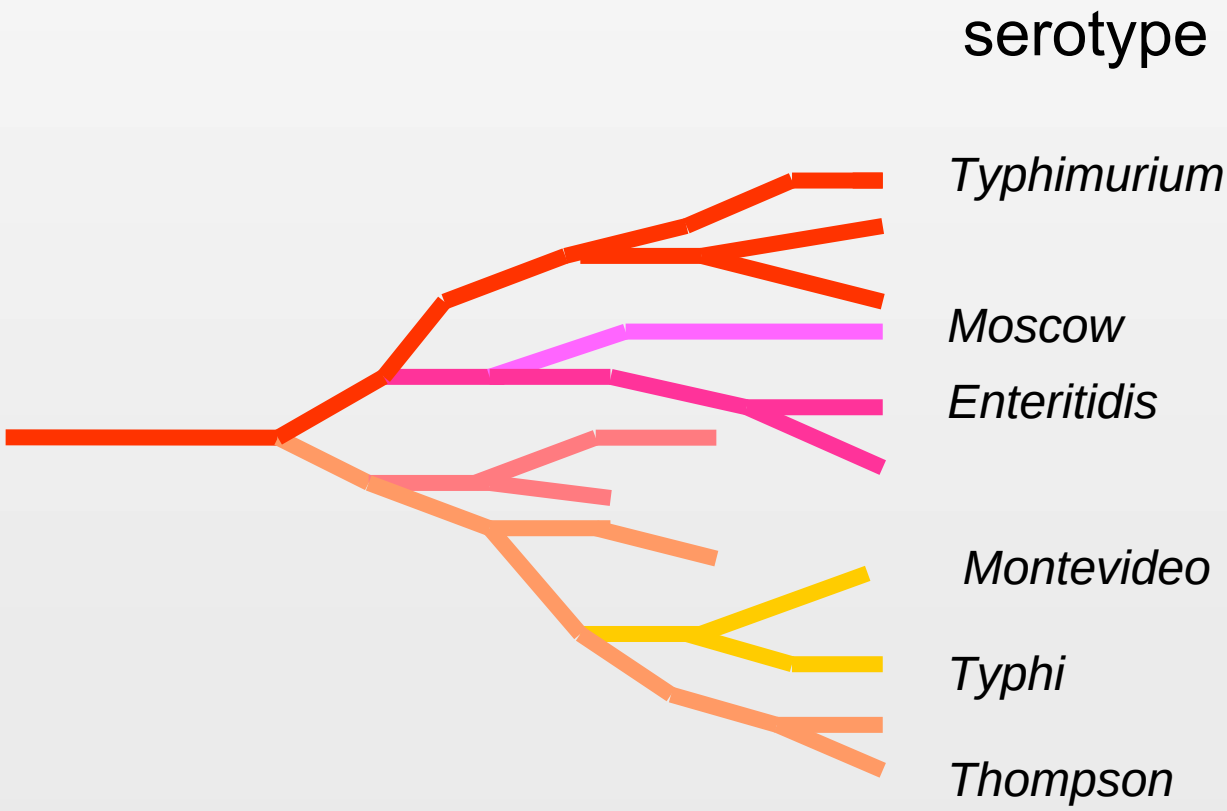
Genetisk Populations-strukturer Blandt Bakteriearter



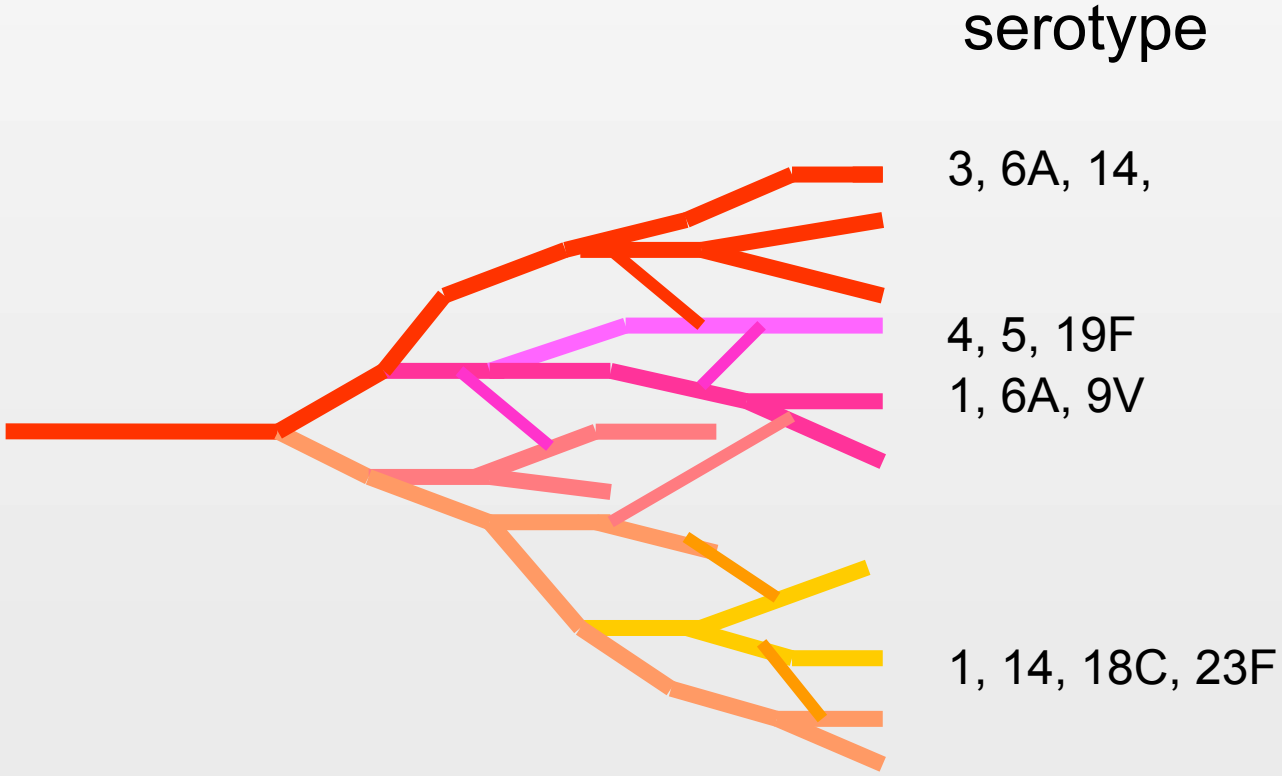


F. Kauffmann:
Classification of bacteria.
A realistic scheme with special reference to the classification of *Salmonella*- and *Escherichia*-species.
Munksgaard, 1975.

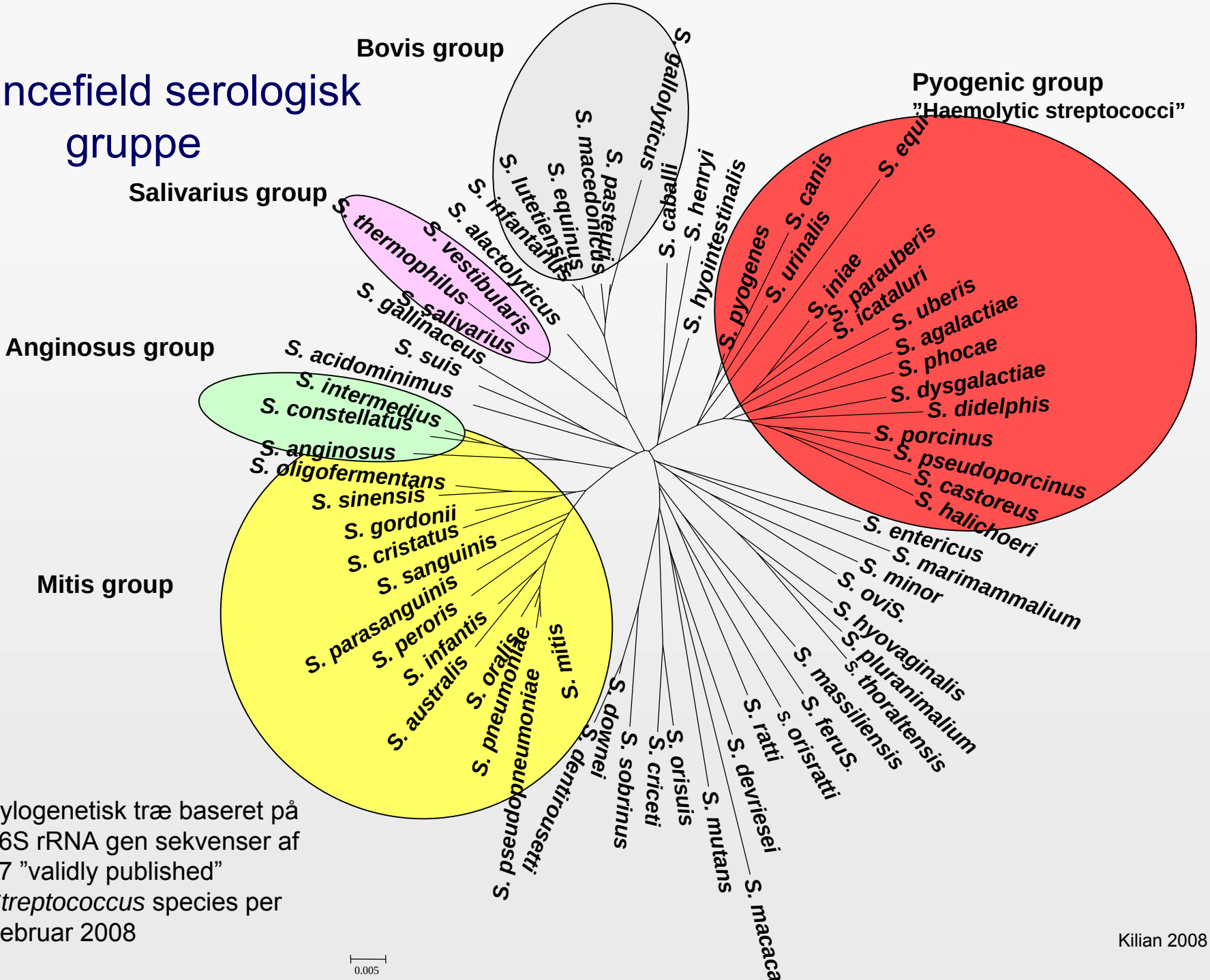
Udviklingslinier indenfor *Salmonella*



Udviklingslinier indenfor *S. pneumoniae*



Lancefield serologisk gruppe



Fylogenetisk træ baseret på 16S rRNA gen sekvenser af 67 "validly published" *Streptococcus* species per Februar 2008

Kilian 2008

Taxonomi baseret på enkelt egenskab: antigen

Gruppe A streptokokker:

S. pyogenes

S. castoreus

S. dysgalactiae subsp. equisimilis

S. orisratti

S. anginosus

S. constellatus subsp. constellatus

Gruppe B streptokokker:

S. agalactiae

S. halichoeri

Taxonomi baseret på enkelt egenskab: antigen

Gruppe C streptokokker:

S. equi subsp*. equi*

S. equi subsp*. zooepidemicus*

S. equi subsp. *ruminatorum*

S. dysgalactiae subsp*. dysgalactiae*

S. dysgalactiae subsp. *equisimilis*

S. phocae

S. marimammalium

S. parasanguinis

S. anginosus

S. constellatus subsp. *constellatus*

S. constellatus subsp*. pharyngis*

Taxonomi baseret på enkelt egenskab: morfologi

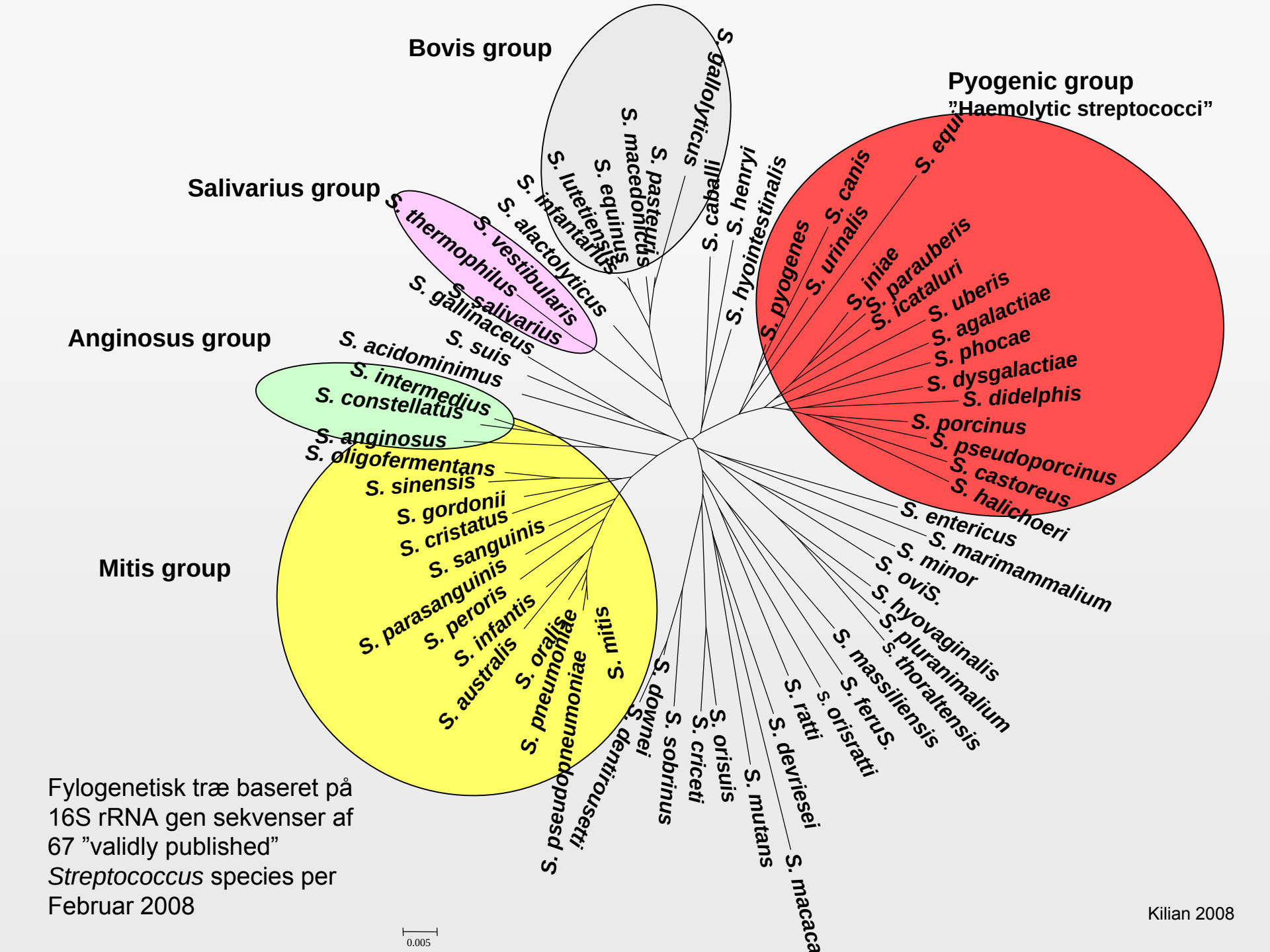
Streptococcus brevis

Streptococcus longus

Streptococcus longissimus

Streptococcus conglomeratus

Diplococcus pneumoniae



Fylogenetisk træ baseret på 16S rRNA gen sekvenser af 67 "validly published" *Streptococcus* species per Februar 2008

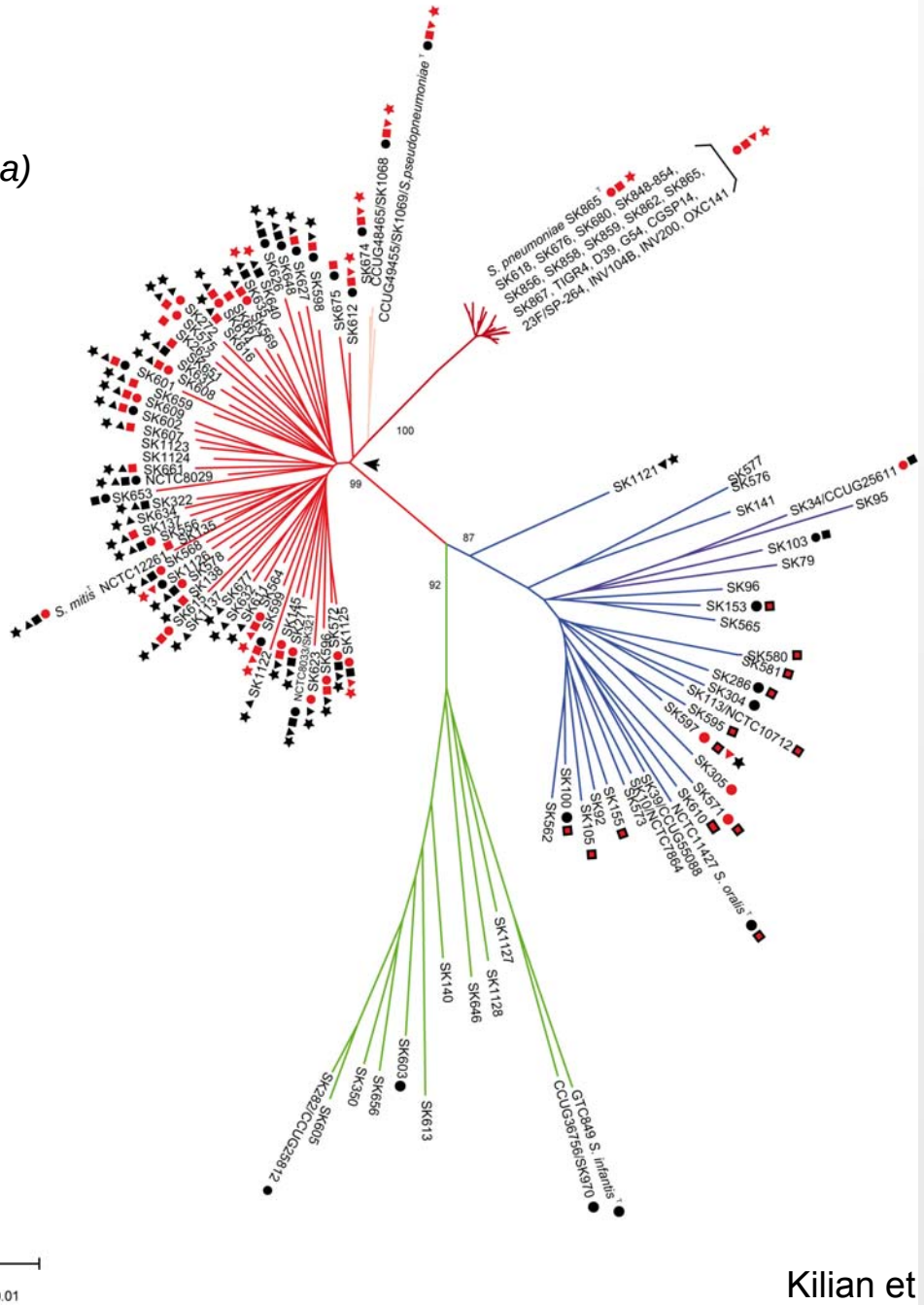
Kilian 2008

cap locus

IgA1 protease gene *(iga)*

Autolysine *(lytA)*

Pneumolysine *(ply)*



Red signatures: gene present

Black signatures: gene absent

Kilian et al. PLoS ONE 2008

Selected phenotypic properties among *S. pneumoniae, S. pseudopneumoniae*, and *S. mitis* strains illustrating genome reduction in *S. mitis*

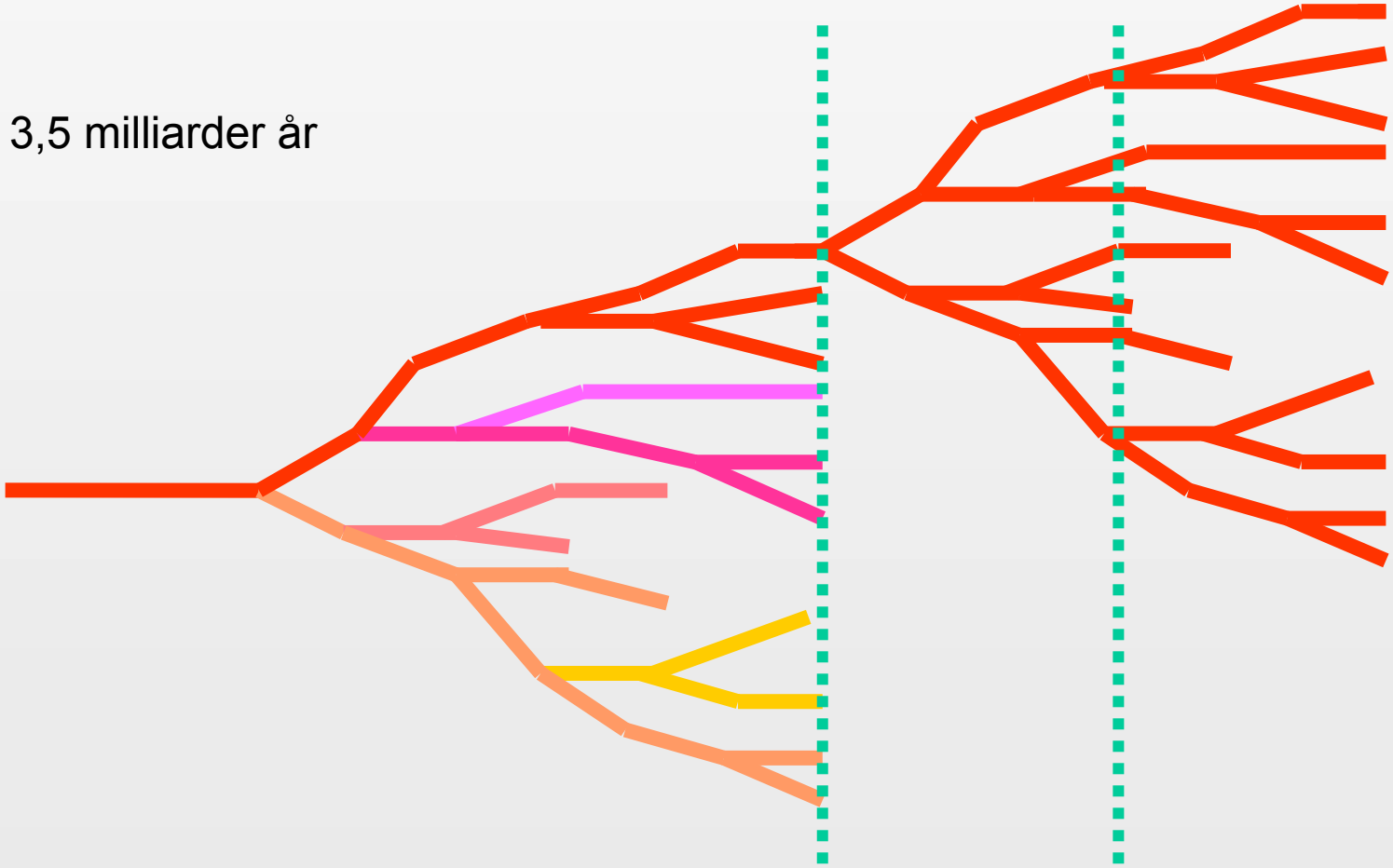
	S. pneumoniae N = 17	*S. pseudo-pneumoniae* N = 3	*S. mitis* N = 54
Aesculine hydrolysis	47 %	0 %	7 %
IgA1 protease	100 %	100 %	55 %
Optochin susceptibility	100 %	67 %	4 %
Bile solubility	94 %	33 %	7 %
Neuraminidase	100 %	100 %	69 %
α-galactosidase	88 %	33 %	27 %
Inuline	71 %	33 %	4 %

Genetisk diversificering af bakterier

Udvikling af arter

Diversificeringen fortsætter

3,5 milliarder år



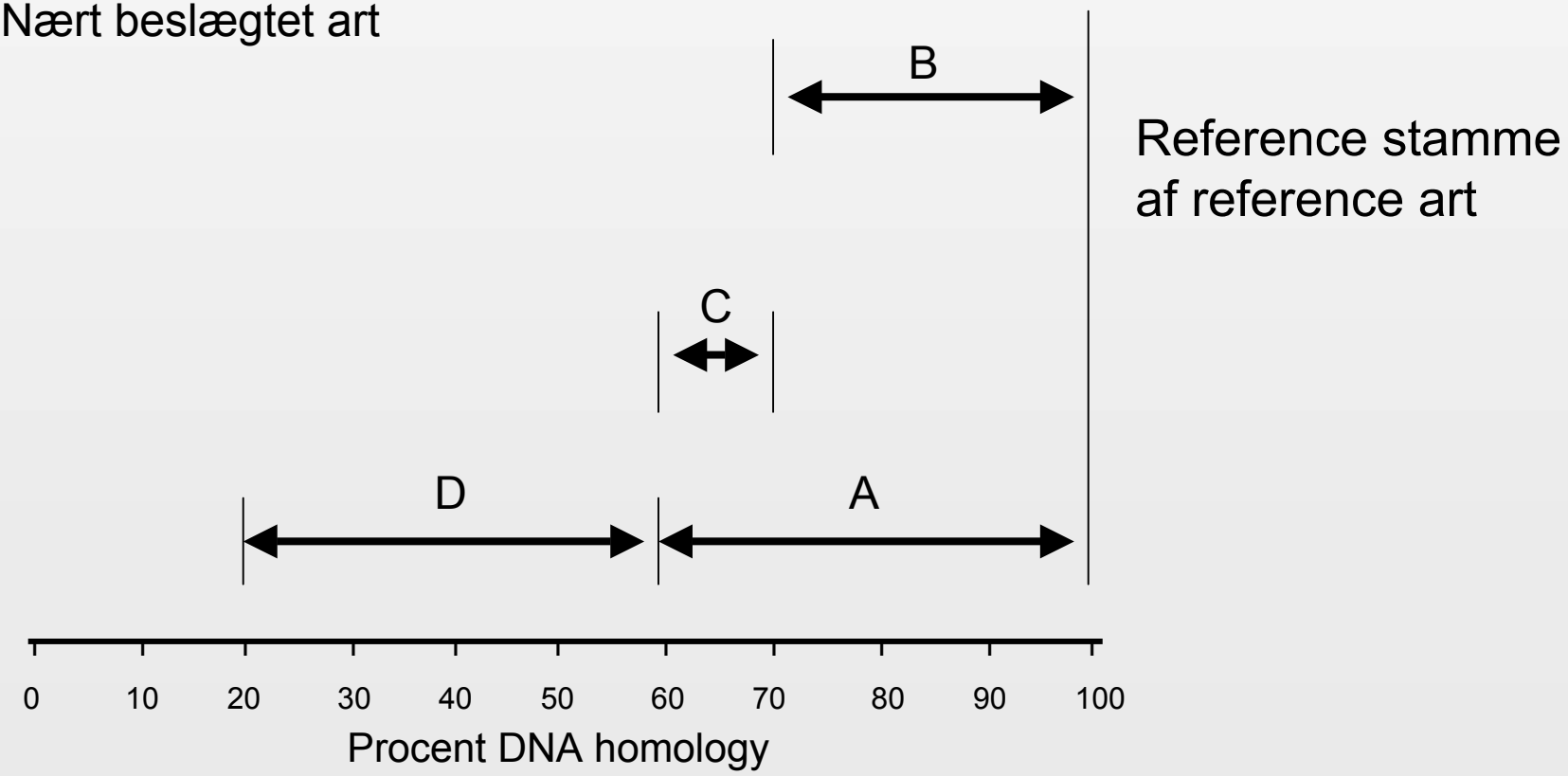
Taxonomisk niveau baseret på DNA homologi data

A: Samme art

B: Samme subspecies

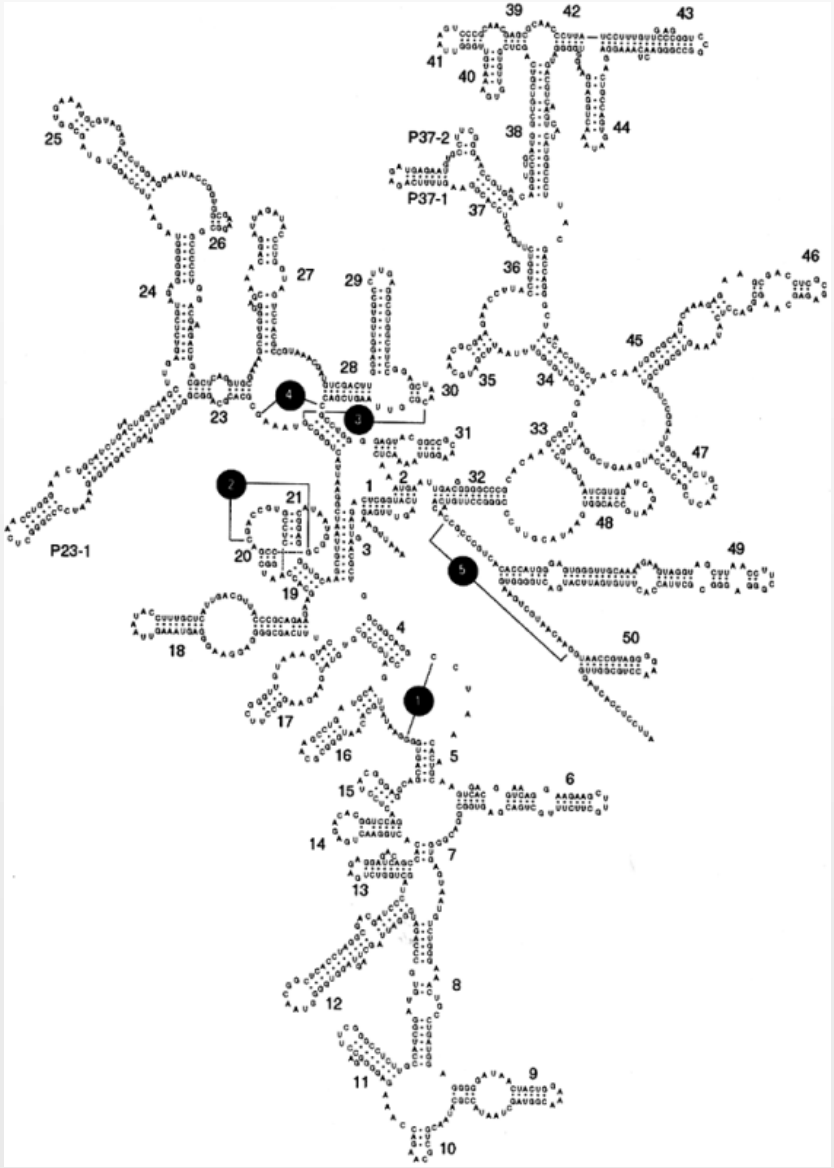
C: Anden subspecies indenfor samme art

D: Nært beslægtet art



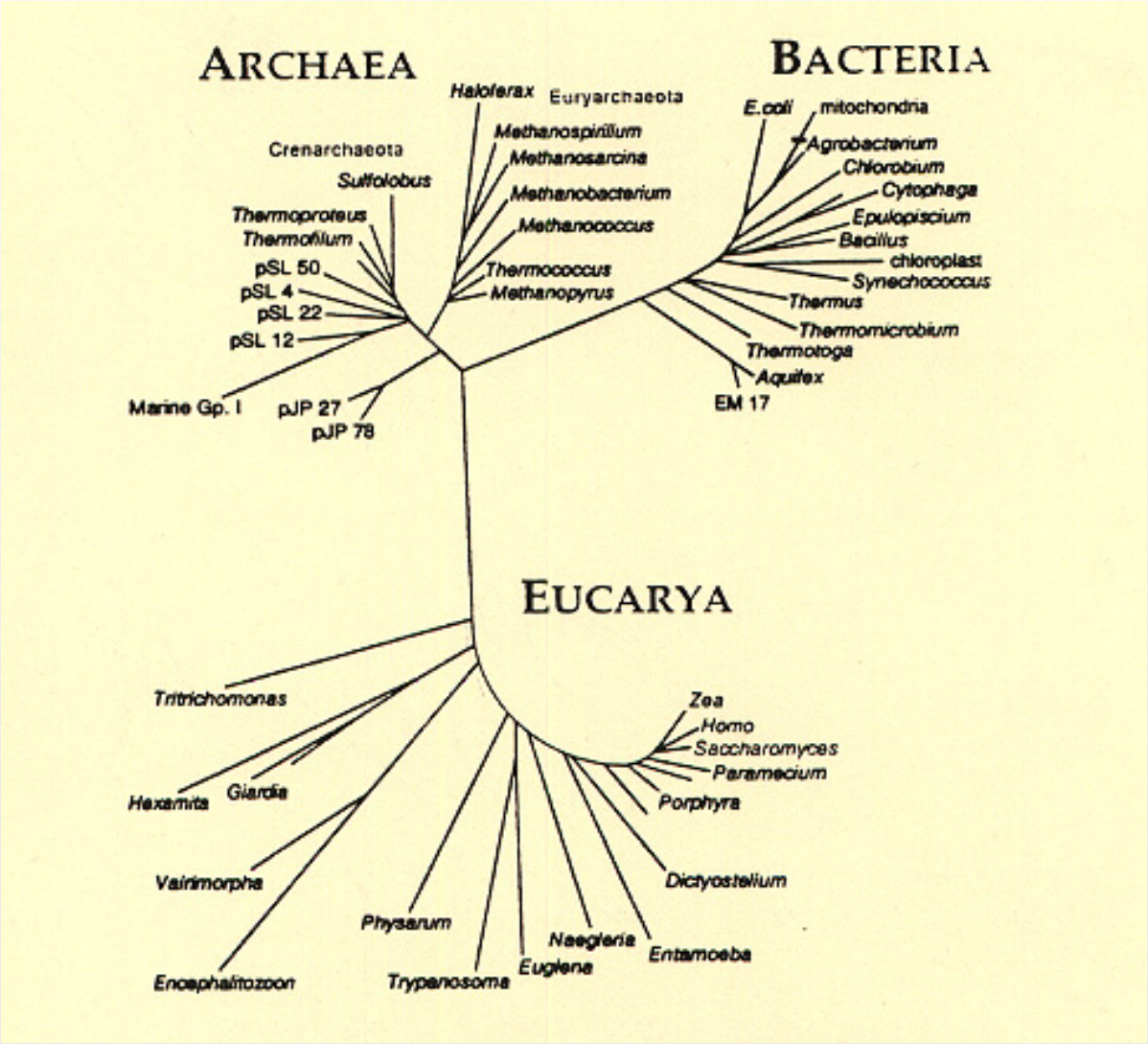
”It is important to remember that few bacteria have read Fig. III.1; therefore, the exact limits chosen for a given group of organisms will have to remain at the discretion of the individual investigators”

John L. Johnson , Bergey’s manual of Systematic Bacteriology 1984



16S rRNA

Universal tree of life



Taxonomiske niveauer defineret ud fra phylogenetiske træer baseret på 16S rRNA gen sekvenser

- Phyla – Klasse – Subklasse – Orden – Suborden - Familie – Subfamilie - Tribus – Subtribus - Genus – Species- Subspecies

Udvalgte Phyla

Actinobacteria

Bacterioidetes

Chlamydiae

Deferribacteres

Firmicutes

Fusobacteria

Proteobacteria (alpha

Spirochaetes

TM7

Aerococcus

Bacillus

Clostridium

Enterococcus

Lactobacillus

Streptococcus

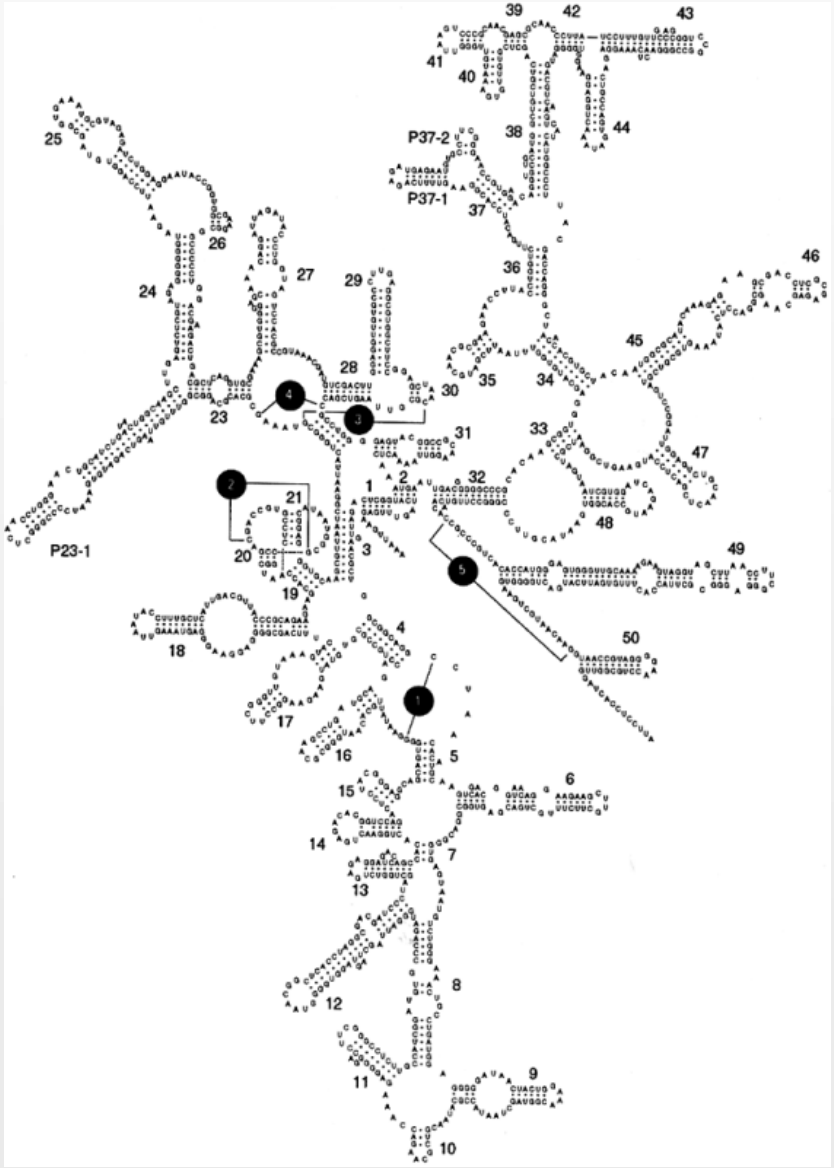
Staphylococcus

Veillonella

Pasteurellaceae

1. *Pasteurella*
2. *Haemophilus*
3. *Actinobacillus*

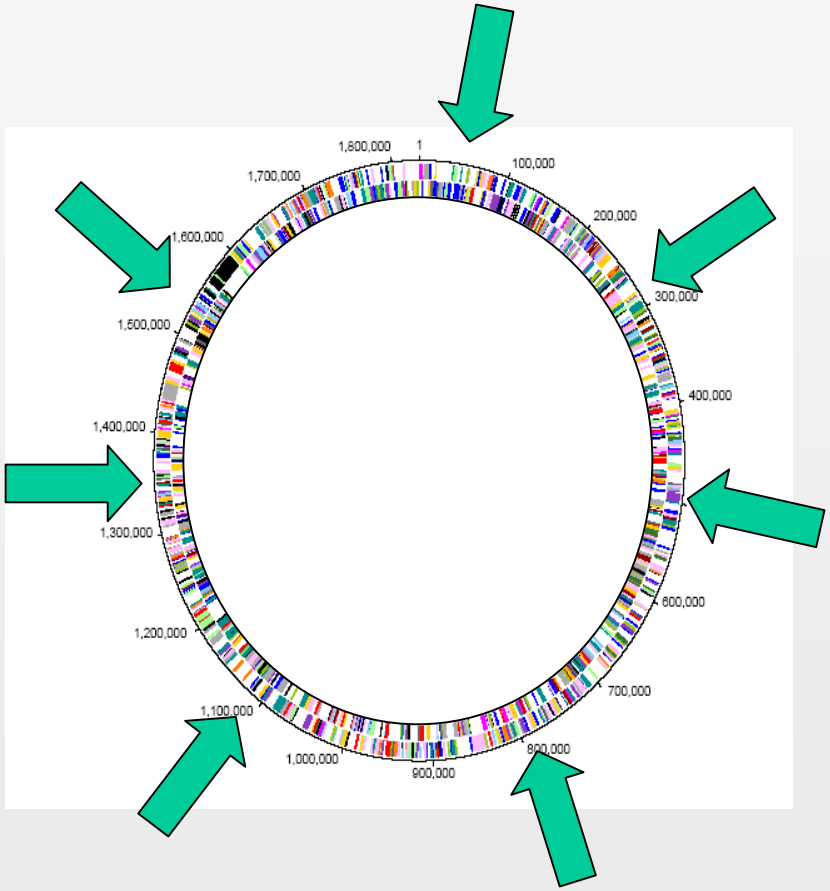
1. *Pasteurella*
2. *Actinobacillus*
3. *Haemophilus*
4. *Lonepinella*
5. *Mannheimia*
6. *Phocoenobacter*
7. *Gallibacterium*
8. *Histophilus*
9. *Volucribacter*
10. *Avibacterium*
11. *Nicoletella*
12. *Aggregatibacter*
13. *Bibersteinia*
14. *Chelonobacter*
15. *”Basfia”*

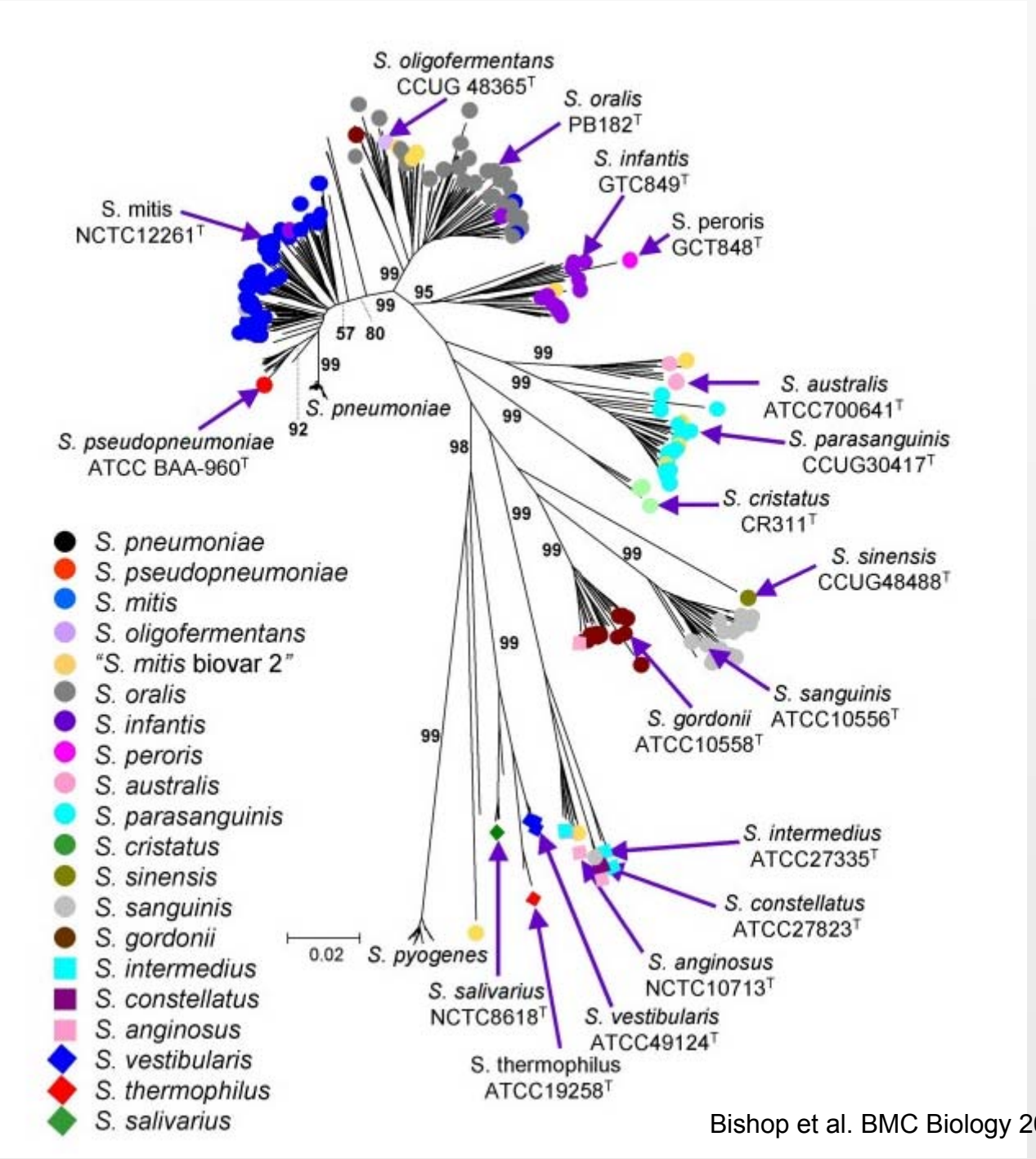


Potentielle problemer ved anvendelse i taxonomi på detailniveau:

- Rekombination
- Forskelle i operons indenfor samme genom
- Stor grad af sekvens-konservering indenfor nogle bakteriegrupper
- Sekventeringsfejl!

Fylogeni ved hjælp af MultiLocus Sekvens Analyse (MLSA)





Bishop et al. BMC Biology 2009;Jan 26; 7:3

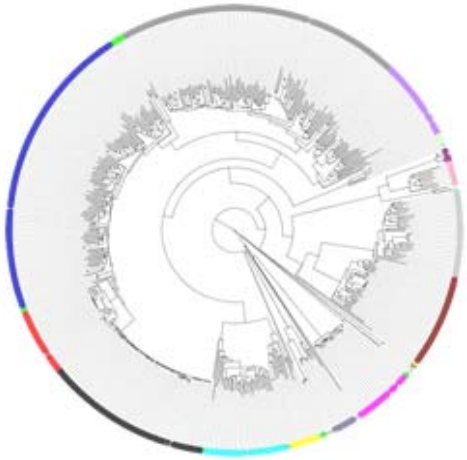
eMLSA.net

Databases

Viridans group streptococci

Burkholderia spp.

Assigning bacterial strains to species via the Internet – Electronic taxonomy



www.eMLSA.net provides a portal for the electronic taxonomy of bacteria, providing a common format and software for assigning strains to species via the Internet.

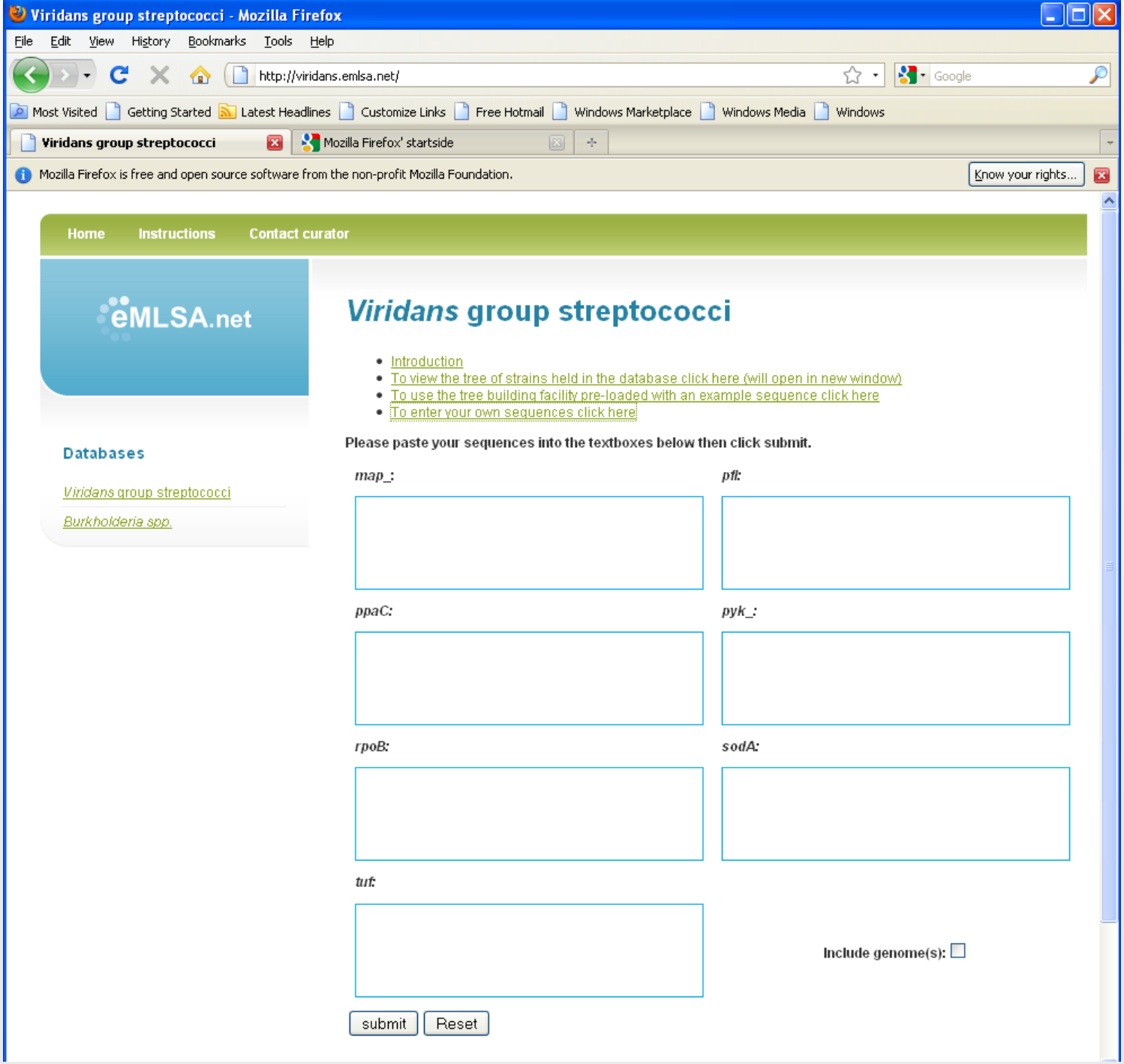
Electronic taxonomy contrasts with the current approach for distinguishing species within a genus, and for defining new species, which is based on polyphasic taxonomy, an approach that incorporates all available phenotypic and genotypic data into a consensus classification (Vandamme et al., 1996).

For further information and instructions please see the Instruction pages.

Software Requirements

Browser:	JAVA:
You are currently using Firefox 3.5 on Windows This is fine.	You are currently using version: 1.6.0_13 This is fine.

If you experience problems using the site please see the Troubleshooting guide here.


Viridans group streptococci - Mozilla Firefox
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Instructions
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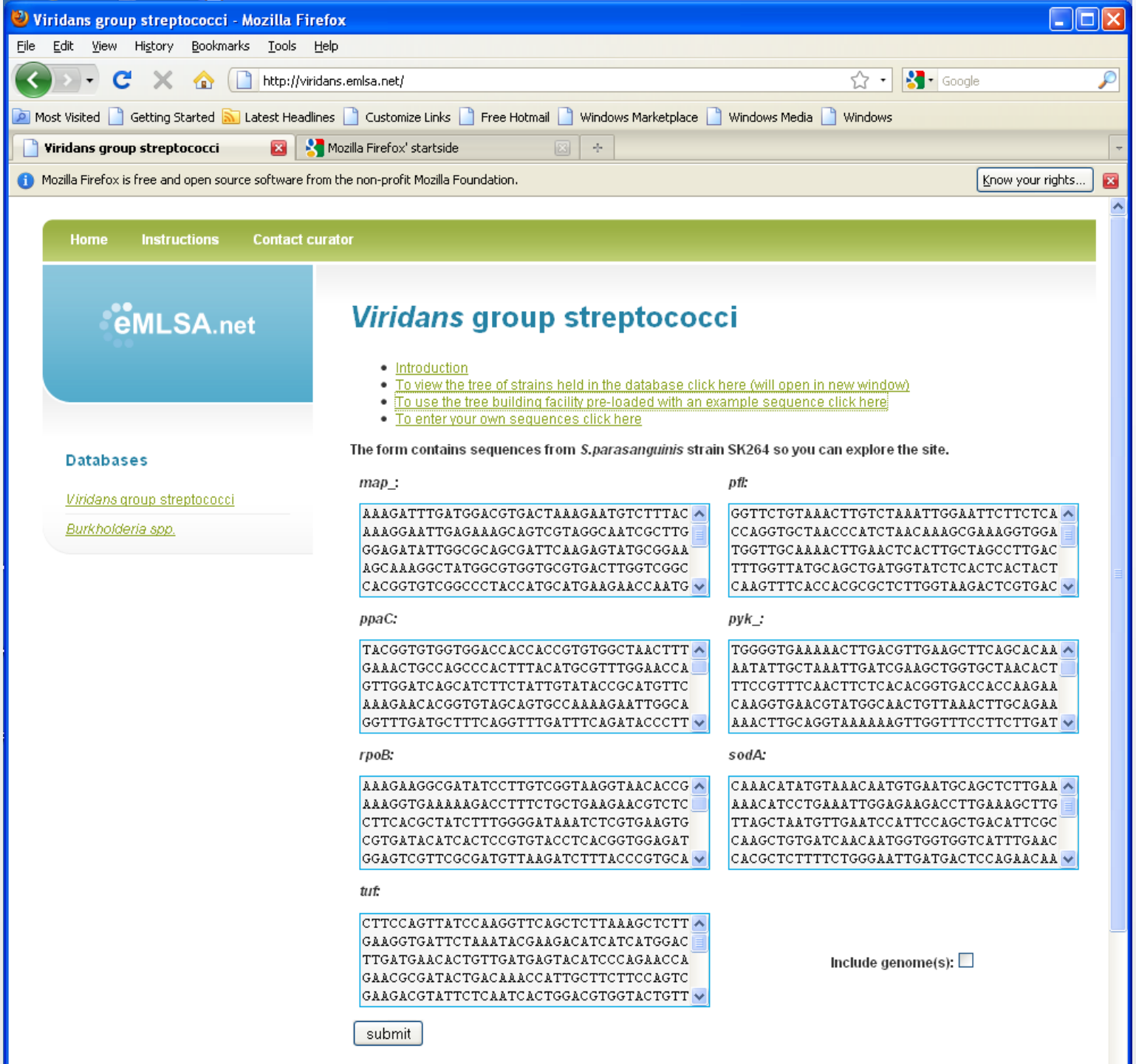
Databases
Viridans group streptococci
Burkholderia spp.
Viridans group streptococci
Introduction
To view the tree of strains held in the database click here (will open in new window)
To use the tree building facility pre-loaded with an example sequence click here
To enter your own sequences click here
Please paste your sequences into the textboxes below then click submit.
map_:
pfl:
ppaC:
pyk_:
rpoB:
sodA:
tuf:
Include genome(s):
submit
Reset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Databases
Viridans group streptococci
Burkholderia spp.
Viridans group streptococci
Introduction
To view the tree of strains held in the database click here (will open in new window)
To use the tree building facility pre-loaded with an example sequence click here
To enter your own sequences click here
The form contains sequences from S.parasanguinis strain SK264 so you can explore the site.
map_:
AAAGATTTGATGGACGTGACTAAAGAATGTCTTTAC
AAAGGAATTGAGAAAGCAGTCGTAGGCAATCGCTTG
GGAGATATTGGCGCAGCGATTCAAGAGTATGCGGAA
AGCAAAGGCTATGGCGTGGTGCGTGACTTGGTCGGC
CACGGTGTCGGCCCTACCATGCATGAAGAACCAATG
pfl:
GGTTCTGTAAACTTGTCTAAATTGGAATTCTTCTCA
CCAGGTGCTAACCCATCTAACAAAGCGAAAGGTGGA
TGGTTGCAAAACTTGAACTCACTTGCTAGCCTTGAC
TTTGGTTATGCAGCTGATGGTATCTCACTCACTACT
CAAGTTTCACCACGCGCTCTTGGTAAGACTCGTGAC
ppaC:
TACGGTGTGGTGGACCACCACCGTGTGGCTAACTTT
GAAACTGCCAGCCCACTTTACATGCGTTTGGAACCA
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pyk_:
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GGAGTCGTTCGCGATGTTAAGATCTTTACCCGTGCA
sodA:
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CAAGCTGTGATCAACAATGGTGGTGGTCATTTGAAC
CACGCTCTTTTCTGGGAATTGATGACTCCAGAACAA
tuf:
CTTCCAGTTATCCAAGGTTCAGCTCTTAAAGCTCTT
GAAGGTGATTCTAAATACGAAGACATCATCATGGAC
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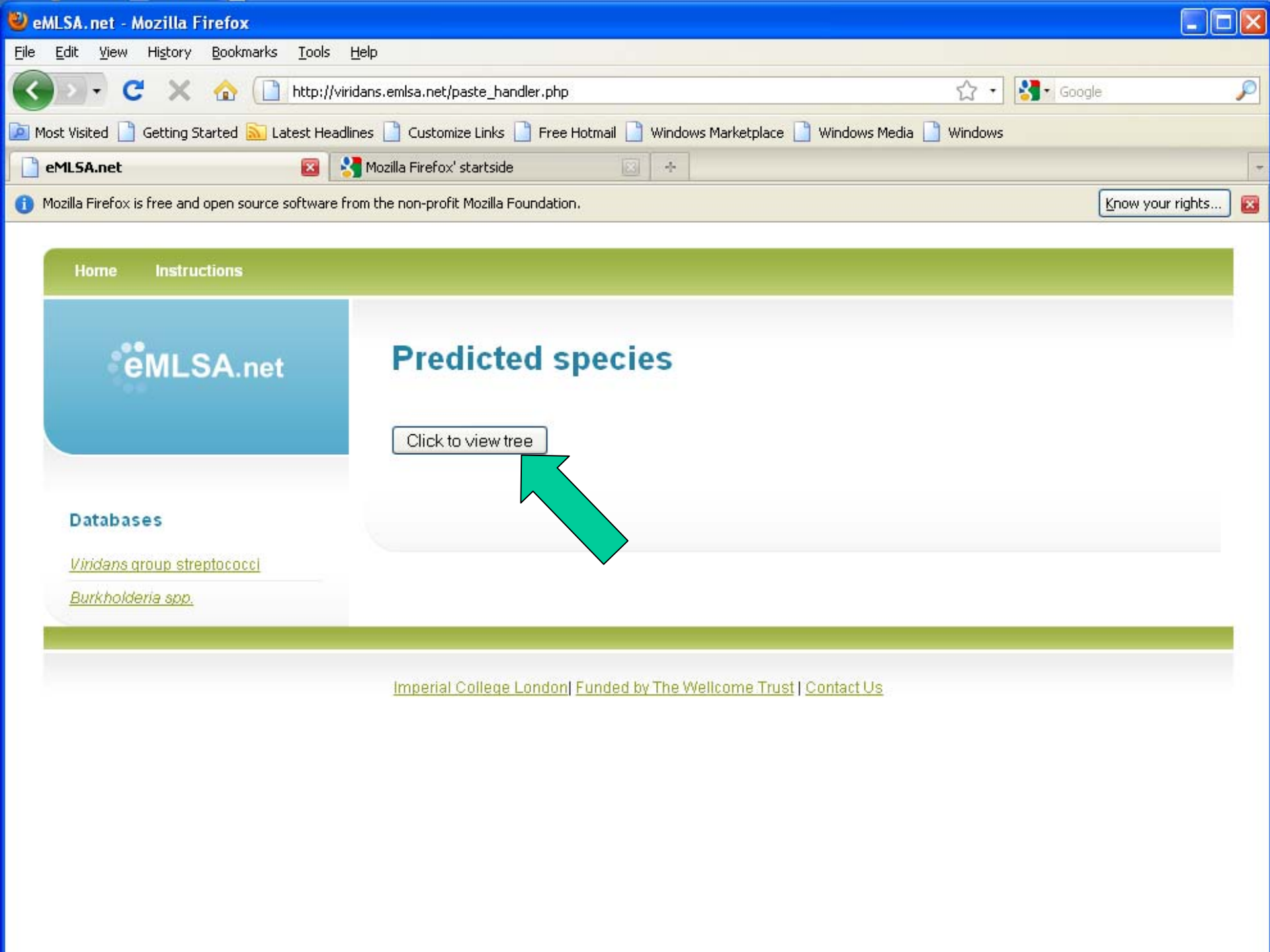
Include genome(s):
submit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Predicted species
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Burkholderia spp.
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Home Instructions

eMLSA.net

Databases

Viridans group streptococci

Burkholderia spp.

Navigation

Reference Tree View

Locus view

Database Query

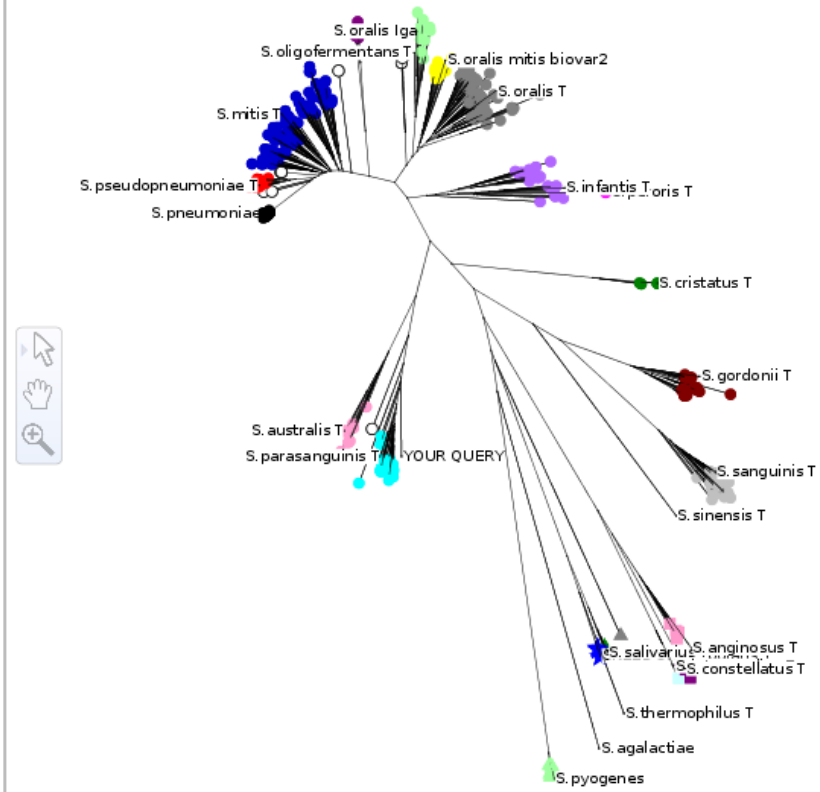
Download Data

Species assigned to the five database sequences that are most similar to the concatenated query sequence.

S.parasanguinis (SK971) 96.28%
S.parasanguinis (SK148) 96.25%
S.parasanguinis (SK438) 96.25%
S.parasanguinis (SK21) 95.92%
S.parasanguinis (SK236) 95.66%

Refresh the tree

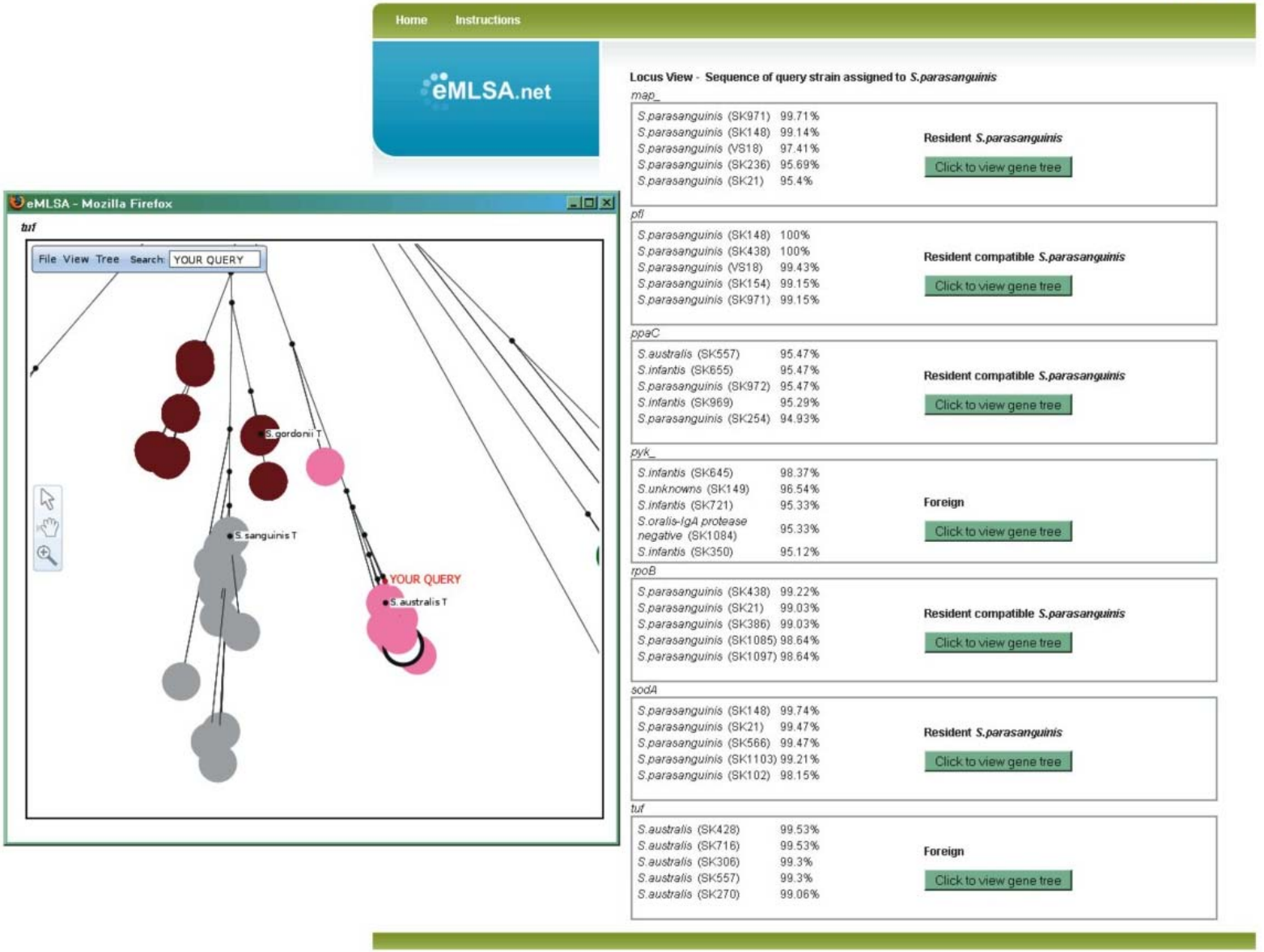
File View Tree Search:

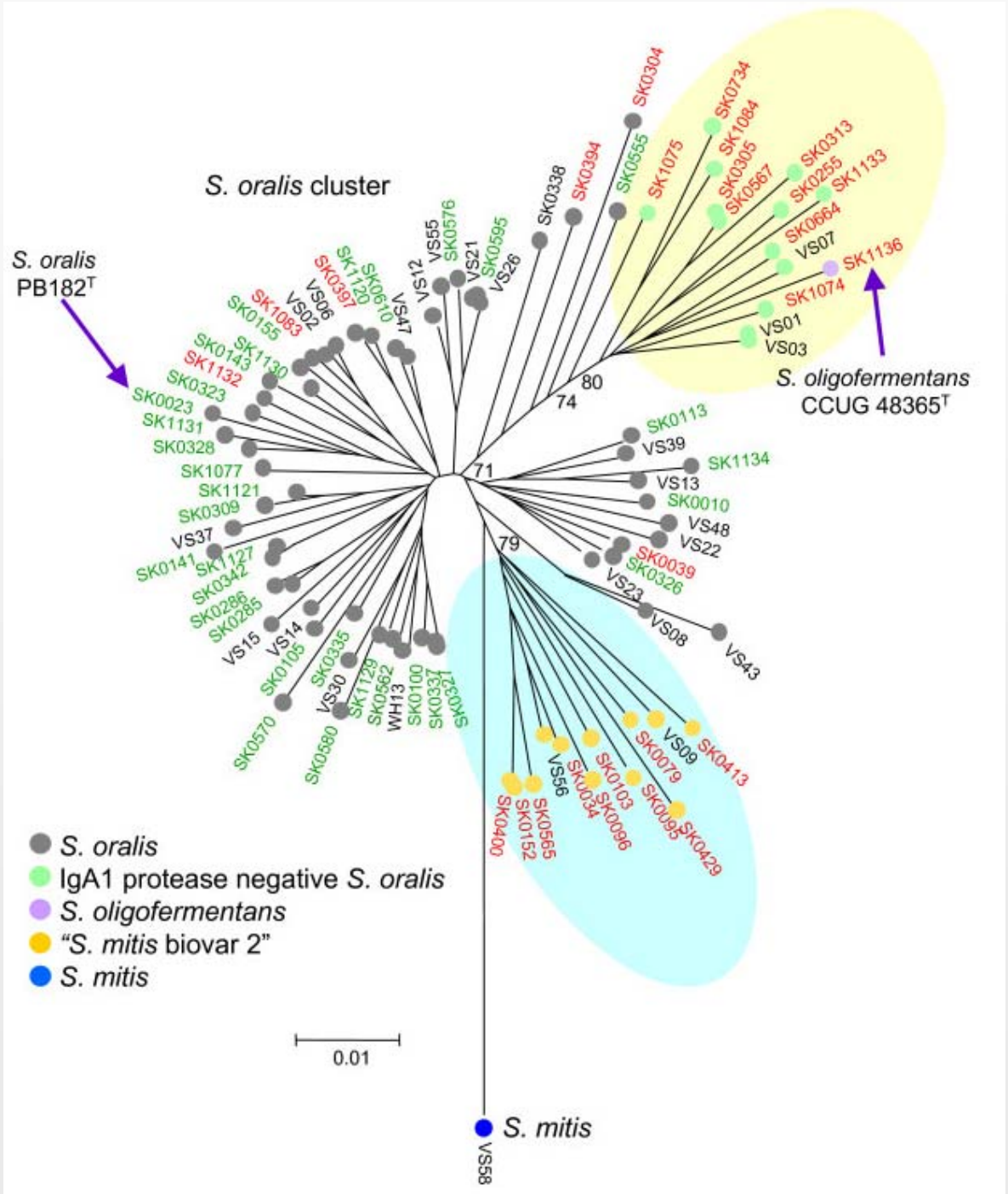


http://www.biomedcentral.com/?highres=y - Figure 8 - Windows Internet Explorer

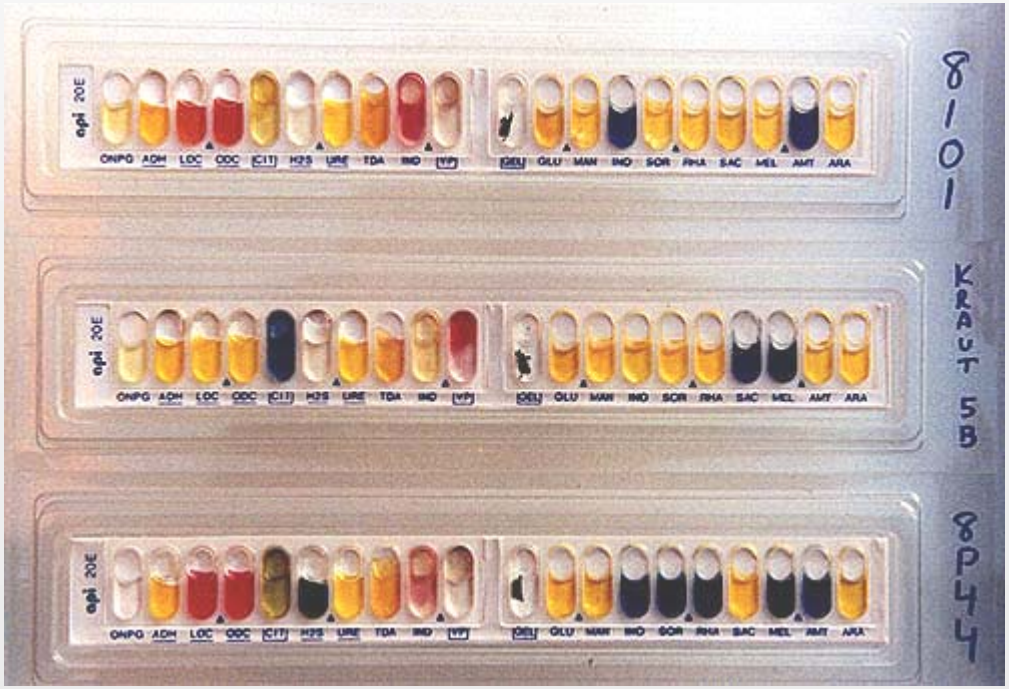
Figure 8.

Resolution: standard / **high**




S. oralis cluster
S. oralis
PB182T
S. oligofermentans
CCUG 48365T
S. mitis
80
74
71
79
0.01
S. oralis
IgA1 protease negative S. oralis
S. oligofermentans
"S. mitis biovar 2"
S. mitis

Identifikation



Academics and Research
Art Conservation Research
Feed & Forage
Food & Beverage
Milk&Dairy Analysis
Solid Fat Content Analysis (official methods)
Iodine Value Analysis
Trans Fat Content
Droplet Sizes in Emulsions
Bacteria Identification
Oil and Moisture in Seeds (official methods)
Forensics Science
Fuel Ethanol Applications
Paper & Pulp Analysis
Pharmaceutical Applications
Polymers & Plastics
Process Control
Semiconductors
Textile Industry

Bacteria Identification

The FT-IR spectroscopic analysis of microorganisms allows a fast and reliable identification of microorganisms at low running expenses.

Due to the large variety of analytical questions and the high number of different microorganism species many analytical methods are applied in microbiology. The differentiation of microorganisms by morphological characteristics and their identification using biochemical staining tests are typically applied techniques. In recent years more and more molecular biological methods have been used. Apart from the identification of the microorganism medical applications require the determination of its pathogenity and resistance against antibiotics. In biotechnology monitoring of the production of certain natural materials or recombinant proteins is of interest.

FT-IR spectroscopy is a method that combines high sensitivity with a broad field of applications. Using FT-IR spectroscopy biomolecules like proteins, lipids, carbohydrates and DNA/RNA can be identified and quantified very sensitively. When cultivating microorganisms under standardized conditions a characteristic pattern of these biomolecules is obtained, allowing a reliable identification of the strain.

Sample preparation is quick and easy: The microorganisms are harvested from the cultivation medium, suspended in water and then transferred on a special IR-transparent, reusable sample plate. The-measurement is performed after drying of the samples. The IR-method allows in-depth differentiation, often down to strain level and furthermore it can be used for additional examinations, e.g. to monitor the production of certain metabolites.

Language
English

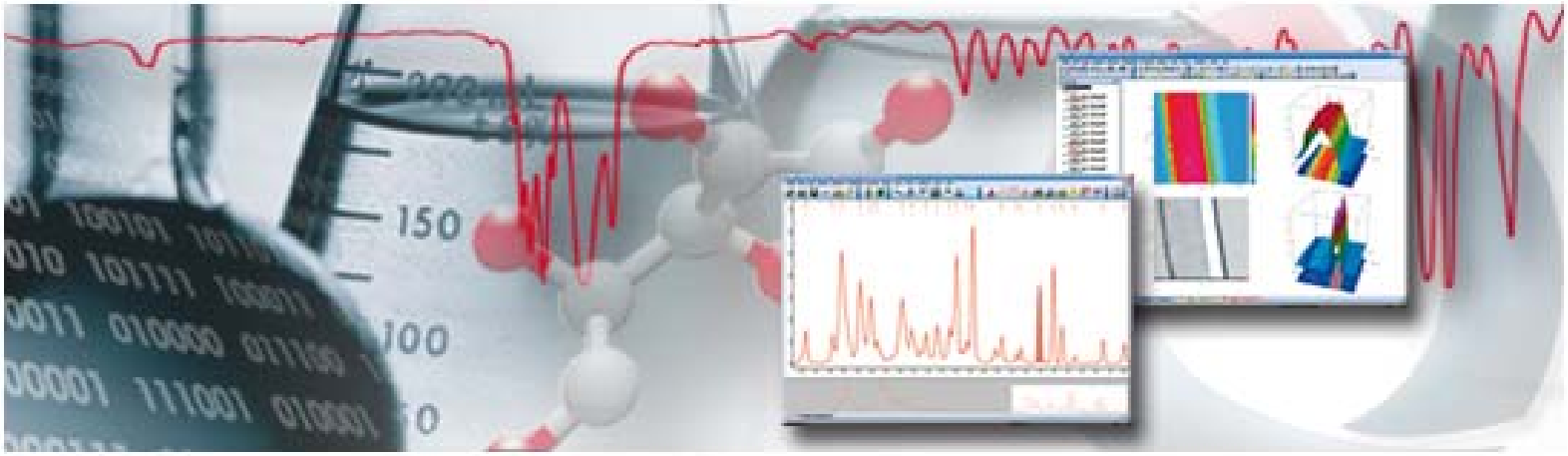
Search

News

- Japan Patent Office Revokes FT-IR-ATR Imaging Patent
- TANDEM II Fully Integrated On-line Pharmaceutical Tablet Characterization PAT Tool
- LANCIR II Dedicated NIR Analyzer for Real-time Pharmaceutical Blending Measurements

Upcoming Events

- NIR Training
- WIRMS
- AOAC

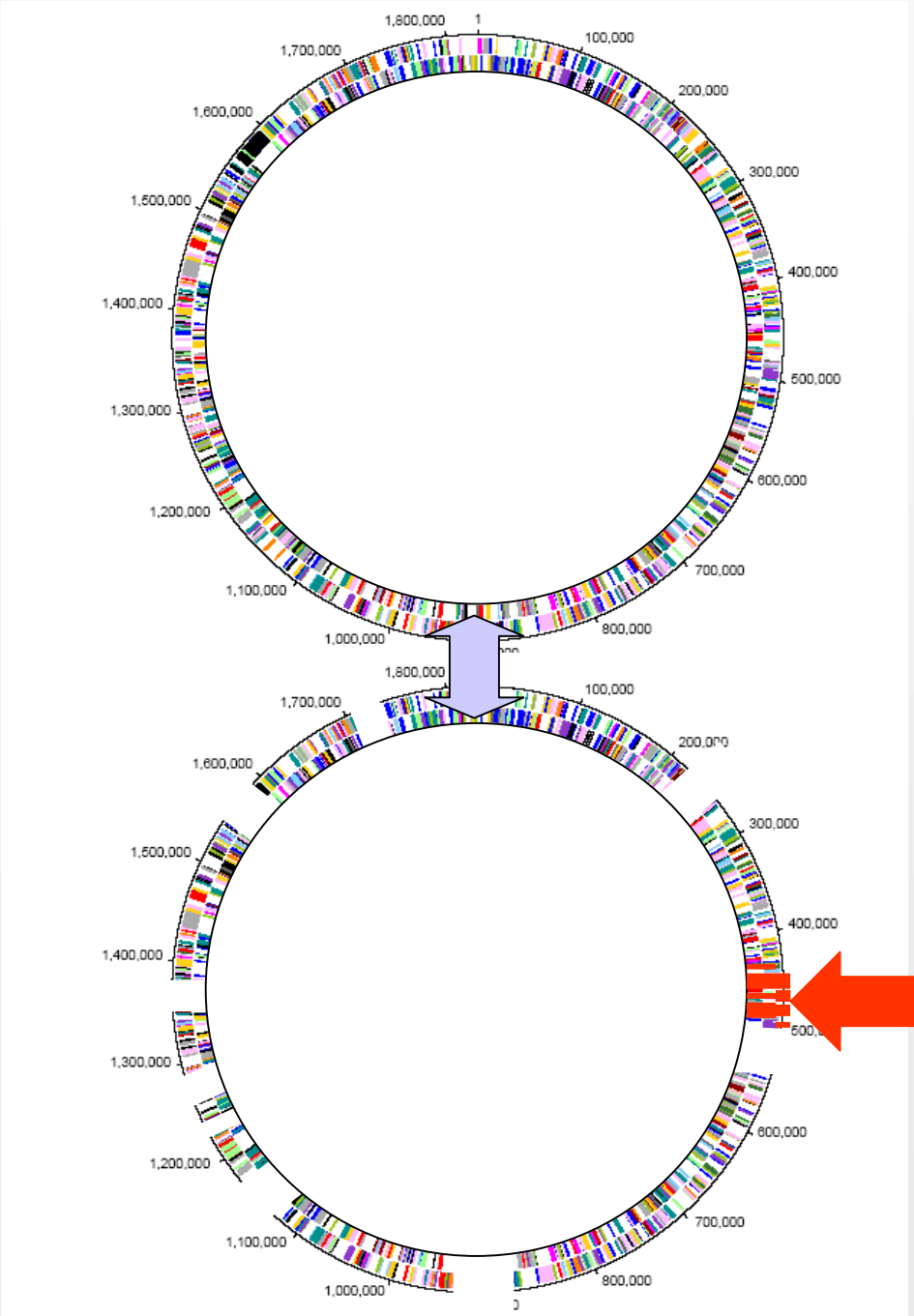


Bruker Optics releases the new 6.5 version of OPUS software

OPUS, the "all-in-one" IR and Raman spectroscopy software consists of a suite of software packages that cover both standard and specialized applications.

Easy-to-use

OPUS offers an extensive set of spectral processing routines such as the spectrum calculator, absorbance-to-transmission conversion, automatic baseline correction, peak picking and many more. All the functions are set-up such that multiple spectra can be manipulated at the same time. Drag & drop handling of spectral data and a variety of interactive functions make OPUS extremely easy-to-use. Interactive spectra manipulation and evaluation functions, such as peak pick, baseline correction, integration and spectral subtraction for semi-quantitative work are also part of the standard package. OPUS provides spectral processing routines that meet the demands of analytical and research laboratories as well as process control. The new version of OPUS also includes a Comprehensive FT-IR tutorial and improved help files.



1. Core genom

   Husholdningsgener

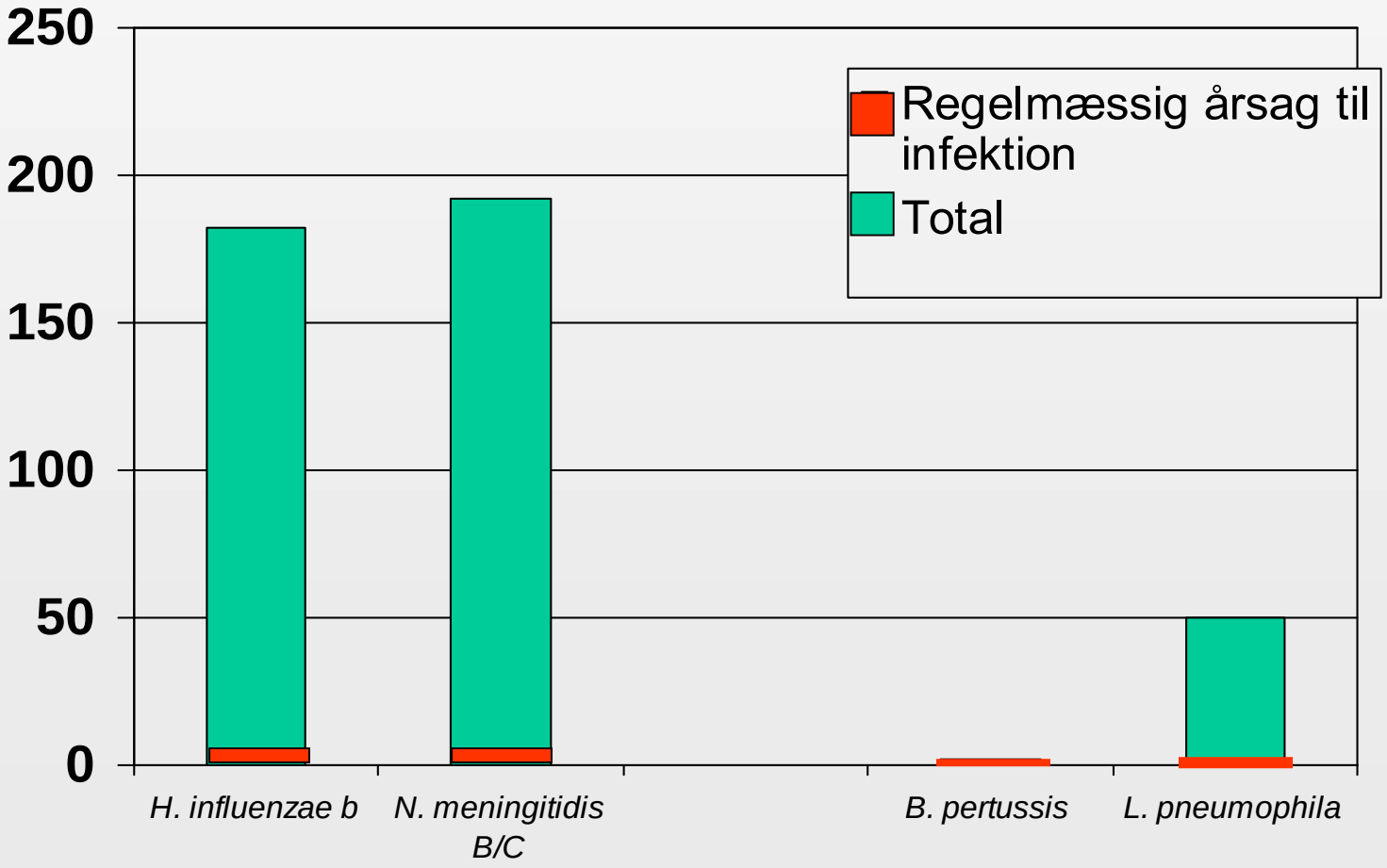
2. Variabelt forekommende gener

   30% af genomet

Patogenitets-øer. Kan bevæge sig på tværs af udviklingslinier

Trandposomer eller plasmider med virulensgener

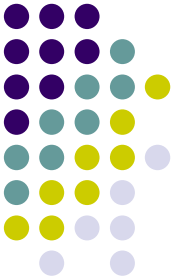
Totale antal kloner af udvalgte patogene bakterier og antallet af kloner, som regelmæssigt forårsager infektion



J. Craig Venter INSTITUTE

PFGRC

National Institute of Allergy and Infectious Diseases
National Institutes of Health



Diagnostic microarray for Bacterial pathogens

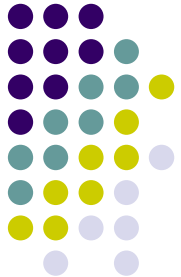
- Marker Design & Validation Efforts

Leka Papazisi

Scott Peterson

April 2007

PFGRC's strategy in diagnostics



Questions to be addressed:

1. What is the genomic background of the putative infectious agent[s] in the sample?
2. What is the virulence potential of the putative infectious agent[s] in the sample?
3. What is the antimicrobial resistance profile of the putative infectious agent[s] in the sample?
4. Does the samples contain engineered/weaponized infectious agent[s]?

- <u>Bottom line</u> – How dangerous and how can the infection be treated ?

Design of Gene specific 70mers based on the Unique Genetic Regions [UGRs, Strain Specific]

BLAST the DNA sequence BD agent genomes

1. Other related species [if there are additional]
2. NR database

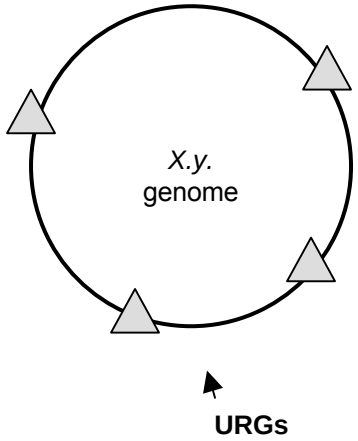
Filter against Human, Swine, Cow etc genomes

Identify the unique regions/genes [**UGRs**]

Create the UGRs data set[s]

From each bacterial genome

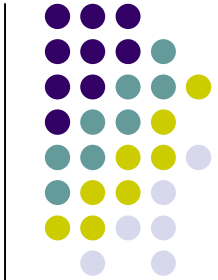
1. **Select a set of UGRs which are [somehow] uniformly distributed across the species genome**
2. **Create a list of potential 70mer** oligos from uniformly distributed regions
3. Design/chose at **least two specific 70mers/ VAG**



Select at least two specific70mers/ UGR to be included in the **Diagnostic Microarrays tests**

Recognizing the Genomic Background Profile

Design of Gene specific 70mers based on the specific virulence factors - Virulence Associated Genes [VAGs]



Identify **VAGs from the literature**

e.g. Cholera Toxin [CT]; anthrax protective antigen [PAG] or Shiga toxins [STX]

Design scripts to pull the VAG DNA sequences from the NCBI database

Create the data set for all of the bacterial VAGs from the NIAID/BD list A,B and C

- **Create a list of potential 70mer** oligos from each VAG
- Design/chose at **least two specific 70mers/ VAG**

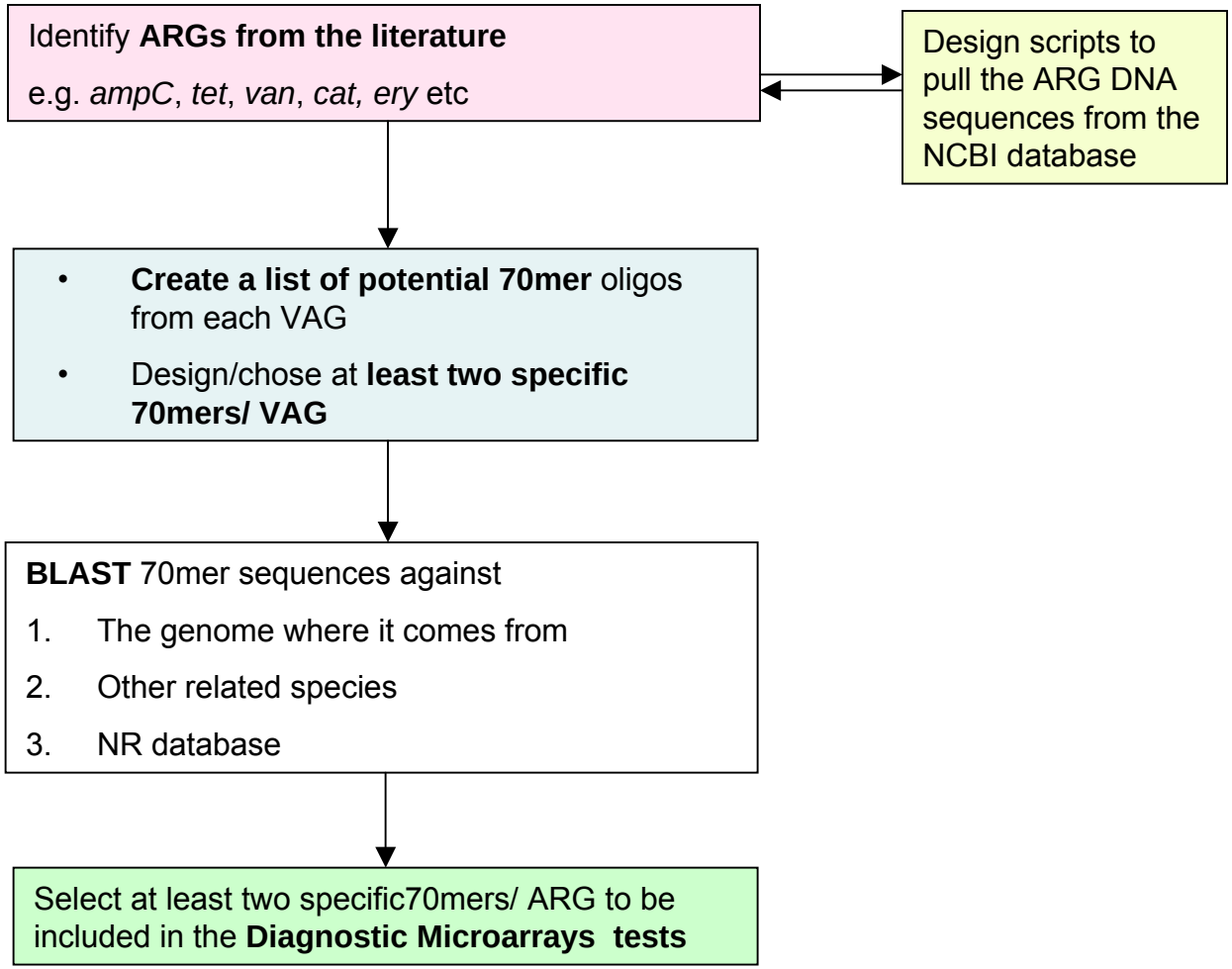
BLAST 70mer sequences against

1. The genome where it comes from
2. Other related species
3. NR database

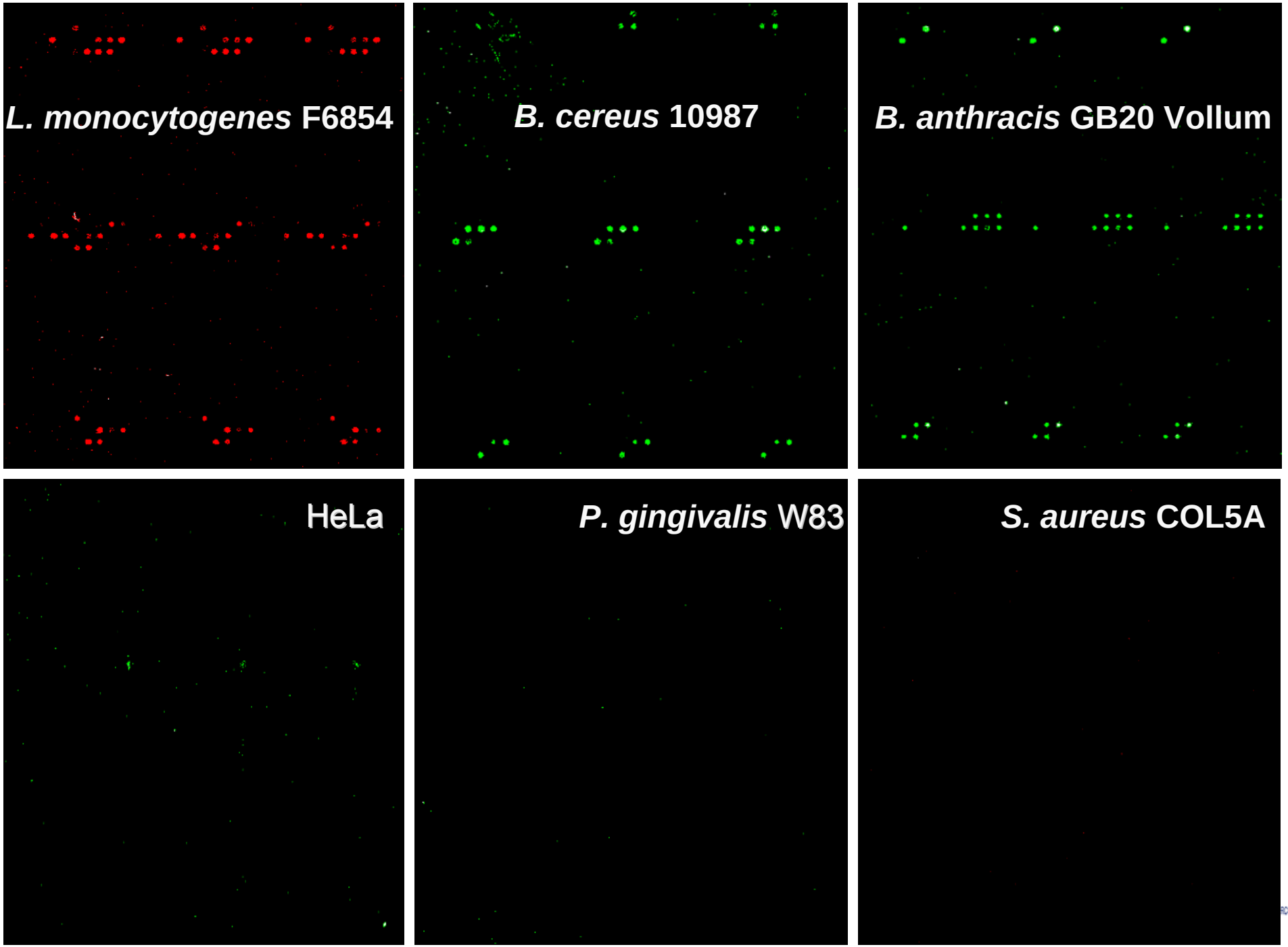
Select at least two specific70mers/ VAG to be included in the **Diagnostic Microarrays tests**

Recognizing the Virulence Potential

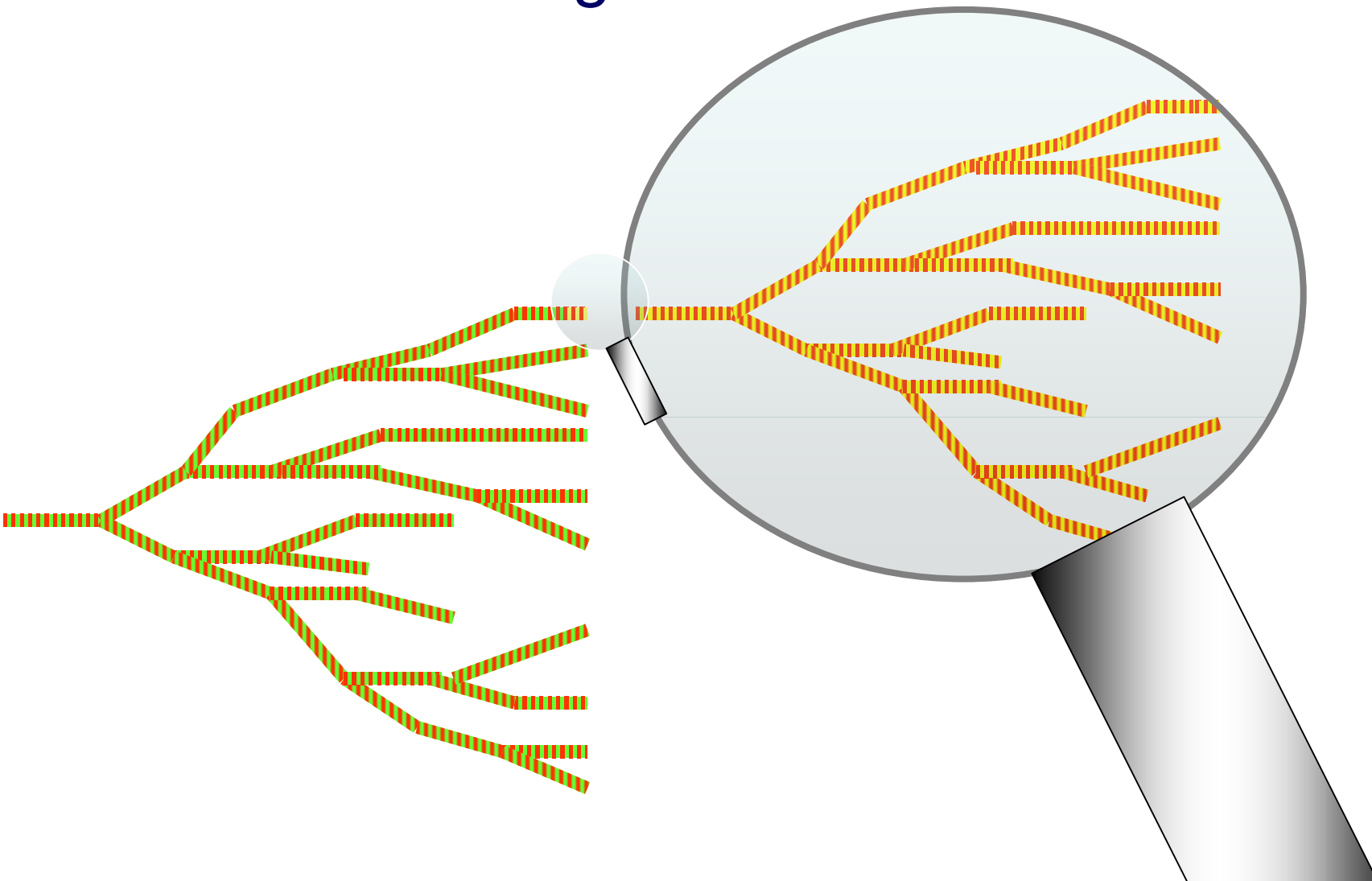
Design of Genome/Gene specific 70mers based on Antibiotic Resistance Genes [ARGs]



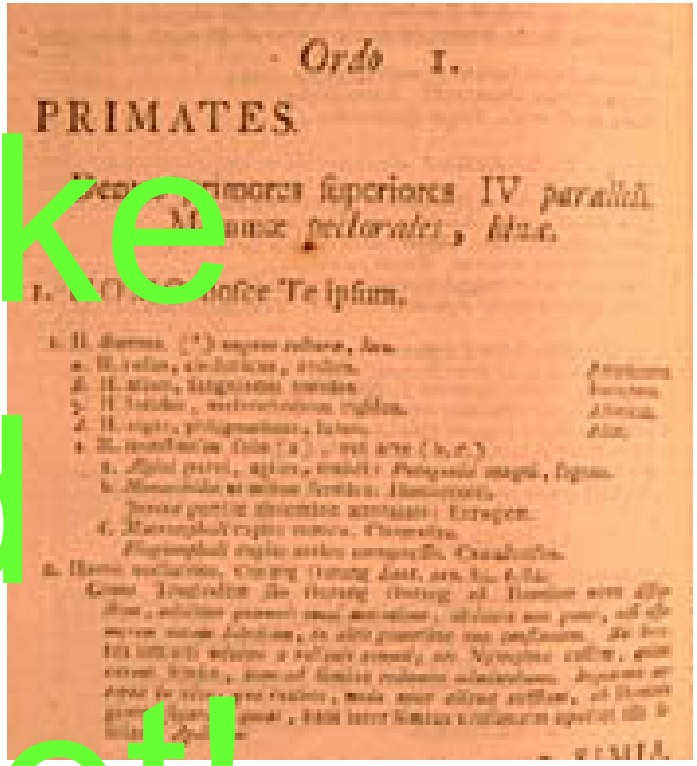
Recognizing the Antimicrobial Resistance Profile


L. monocytogenes F6854
B. cereus 10987
B. anthracis GB20 Vollum
HeLa
P. gingivalis W83
S. aureus COL5A

Coevolution og Genetisk Diversifisering af den Humane Population og de Associerede Mikroorganismer



Til lykke med jubilæet!


Ordo I.

PRIMATES.

Carl von Linné

1707-78