ECCO-2017

Brno, Czech Rep.

Promoting the phylogenetic analysis for the identification of isolates and genetic detection methods in laboratories of routine control

Antonio Martínez-Murcia[1,2]
[1]Genetic PCR Solutions™
[2]University Miguel Hernández,
Orihuela, Alicante, Spain

Molecular clocks

Conserved molecules
Ancestral functions
Constant mutation rates?

Protein-coding genes

High mutation rates

Degenerative code

Fast clocks

Short-term phylogenies

rRNA-coding genes

Low mutation rates

Mosaic of signatures

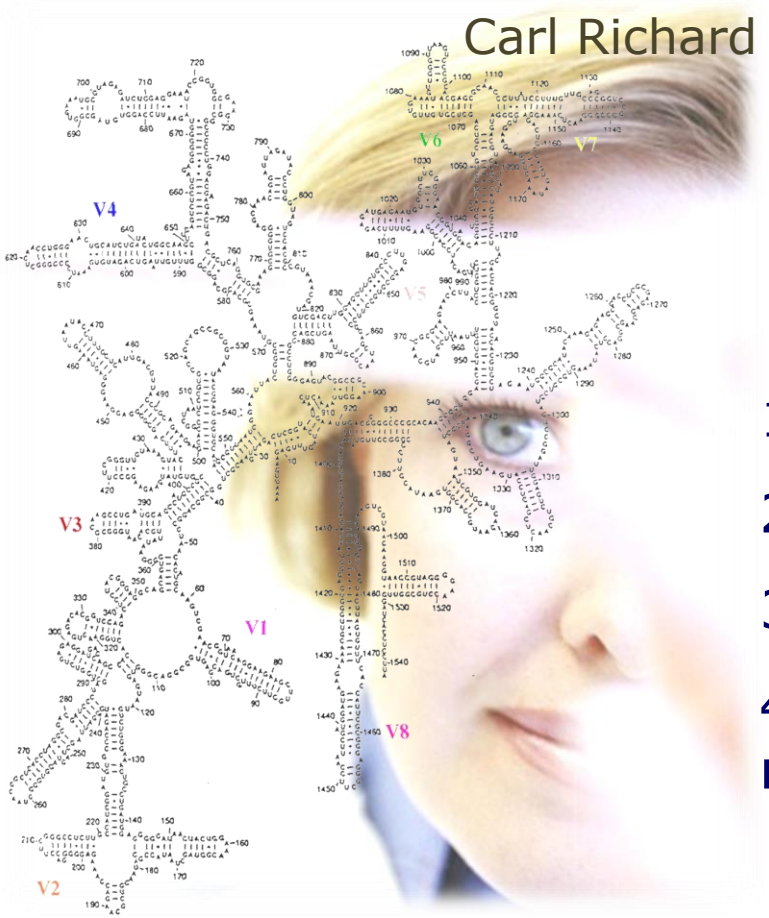
Slow clocks

Long-term phylogenies

Molecular Chronometers

±Constant Mutation Rates

Ribosomal RNA
Carl Richard Woese 1928-2012



1) Universaly distributed

2) Ancestral function/structure

3) Highly conserved

4) Mosaic of different variation rates= signature regions

Woese, Carl R. (1987). «Bacterial evolution» *Microbiological Reviews* **51** (2): pp. 221–271.

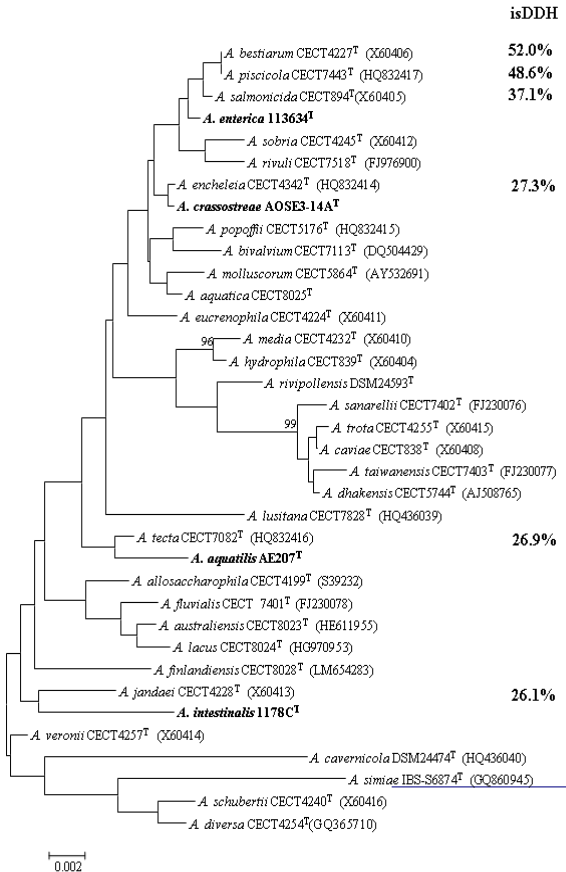
INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, July 1992, p. 412–421
0020-7713/92/030412-10$02.00/0

Vol. 42, No. 3

Phylogenetic Interrelationships of Members of the Genera *Aeromonas* and *Plesiomonas* as Determined by 16S Ribosomal DNA Sequencing: Lack of Congruence with Results of DNA-DNA Hybridizations

A. J. MARTINEZ-MURCIA, S. BENLLOCH, AND M. D. COLLINS*

Department of Microbiology, AFRC Institute of Food Research, Reading Laboratory, Earley Gate, Whiteknights Road, Reading, RG6 2EF, United Kingdom



Curr Microbiol
DOI 10.1007/s00284-012-0253-x

Aeromonas cavernicola sp. nov., isolated from fresh water of a brook in a cavern

Antonio Martínez-Murcia · Roxana Beaz-Hidalgo · Pavel Svec · Ma José Saavedra · Ma José Figueras · Ivo Sedlacek

Received: 27 Aug[illegible]ber 2012

16S rDNA RESOLUTION TO SPLIT *Aeromonas* spp. IS LIMITED

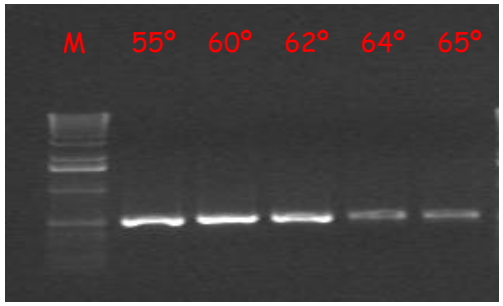
- Extremely conserved : but good marker above the genus level

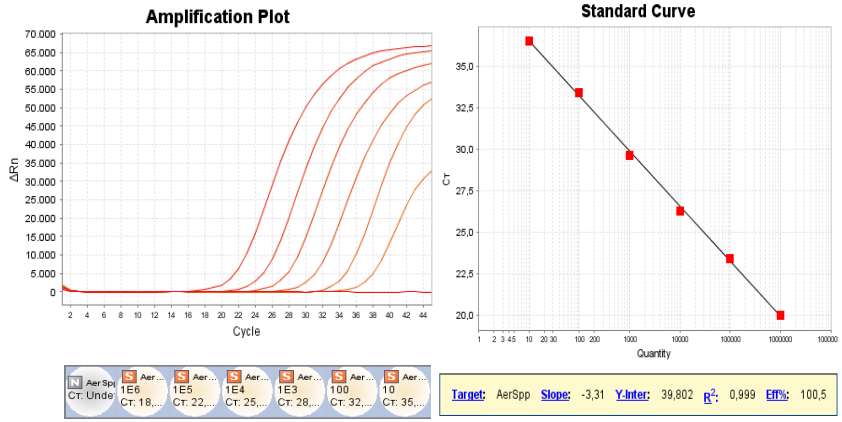
- Some variable regions (viz. V3 stem-loop) are identical between different species = convergences (chronometric distortions)

- Microheterogeneities: differences between operons

- Intra-species diversity > inter-species

16S rRNA. Signature Regions
Aeromonas specific





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INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, July 1993, p. 626–629
0020-7713/93/030626-04$02.00/0

Vol. 43, No. 3

Letter to the Editor

Evidence from *Aeromonas* for Genetic Crossing-Over in Ribosomal Sequences

In a recent issue of the *International Journal of Systematic Bacteriology*, Martinez-Murcia and colleagues (8) report complete 16S rRNA sequences from *Aeromonas*, each consisting of 1,502 bp which offer no problems of homology

ric. Departures from this relationship are caused by factors such as inconstant evolution rates and parallel or convergent evolutionary changes. Their figure is poorly ultrametric and seemed to me to be more discrepant than chance effects

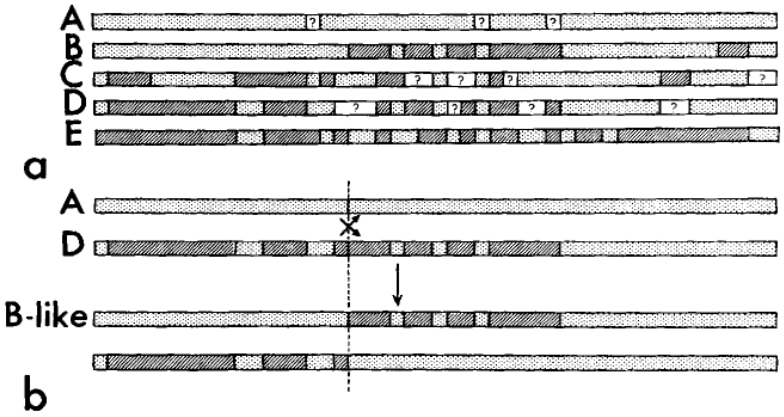
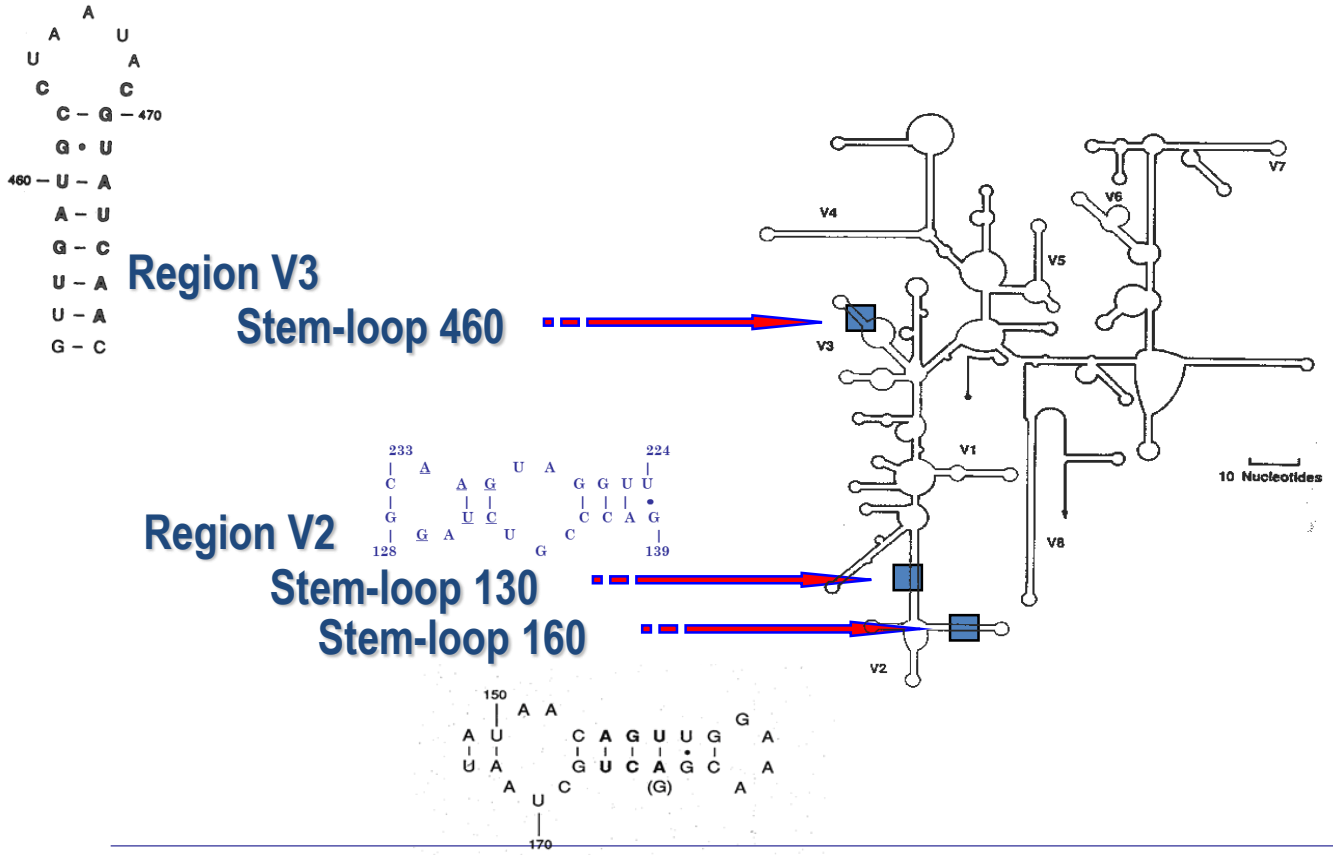


FIG. 2. (a) The major patterns of sequences (see text). The sets of sequences are as follows: A, sequences 1 and 6; B, sequences 4, 13, and 5; C, sequences 2, 3, 7, 8, and 11; D, sequences 9 and 10; E, sequence 12. Shading corresponds to that in Fig. 1. Sites where sequences vary considerably are indicated with a question mark. (b) Illustration of how a crossover at the dashed line (as shown by arrows) between a sequence of pattern A and one of pattern D could yield a B-like sequence.

P. H. A. Sneath
Microbiology Department
Leicester University
Leicester LE1 9HN
United Kingdom

16S rRNA Variable Regions
Aeromonas

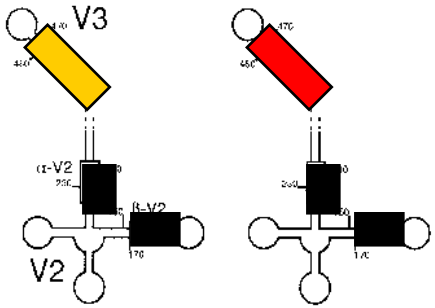


A.hydrophila

A.media

A.caviae

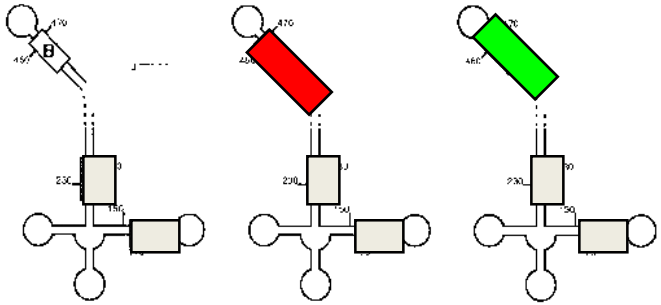
A.trota



A.schubertii

A.jandaei

A.veronii



A.sobria

A.salmonicida

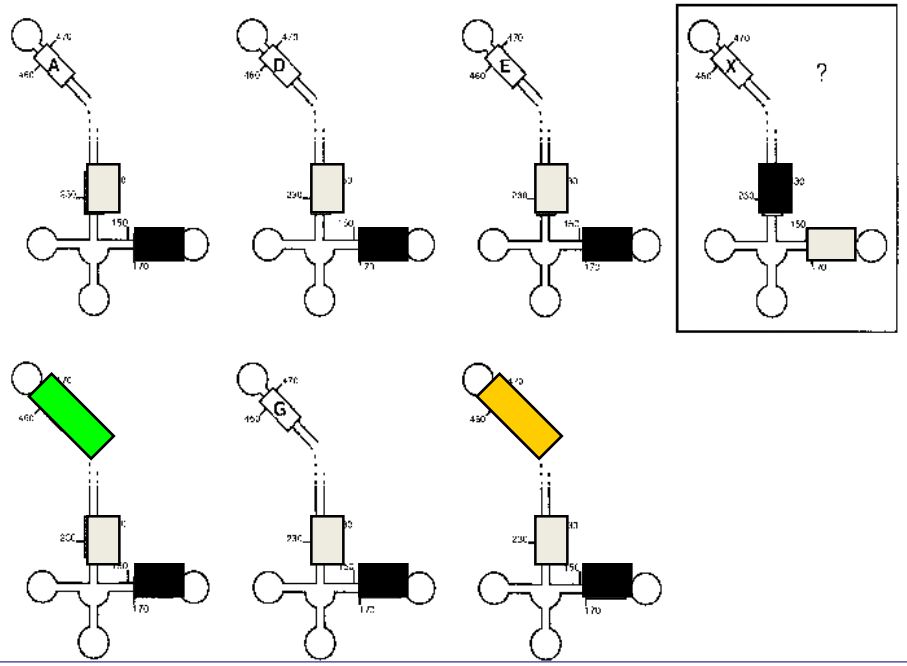
A.encheleia

A.popoffii

A.allosaccharophila

A.bestiarum

A.eucrenophila



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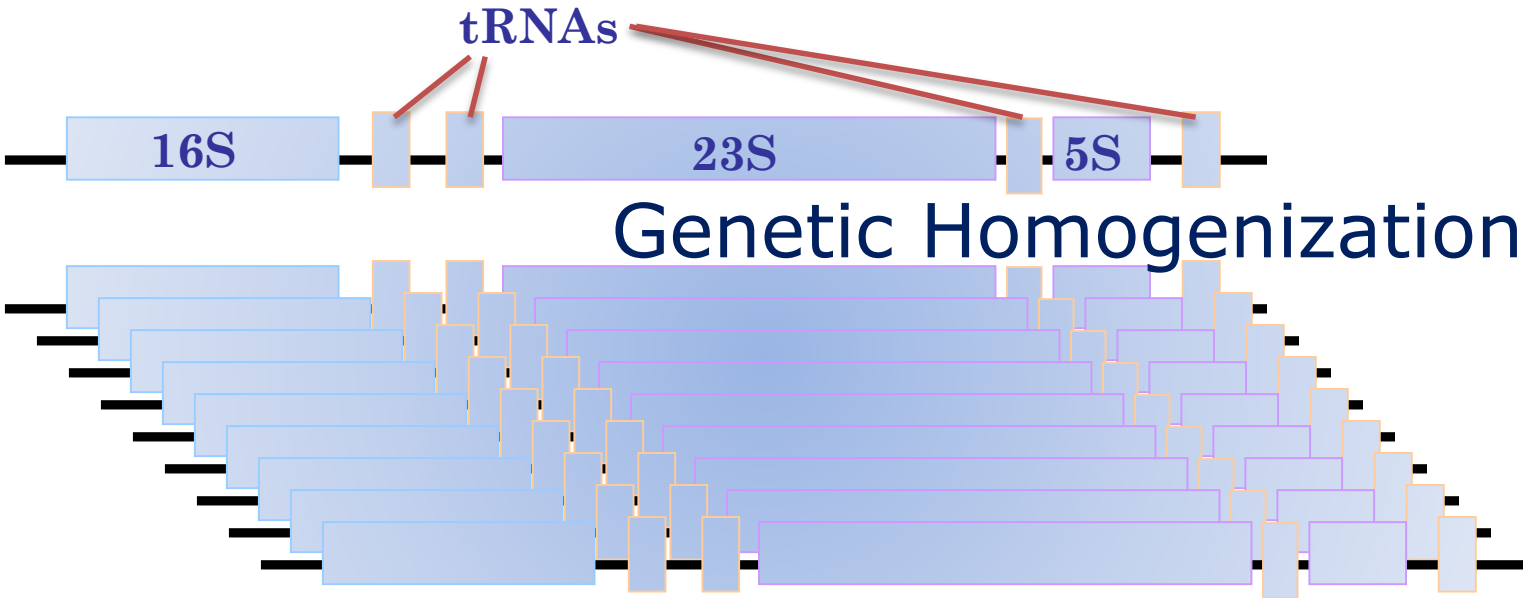
rrn multigene family



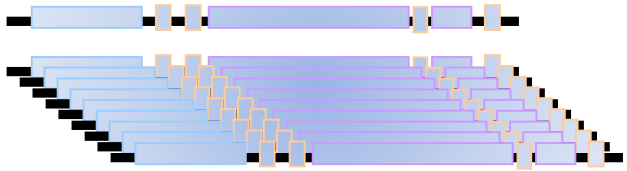
Genetic Homogenization

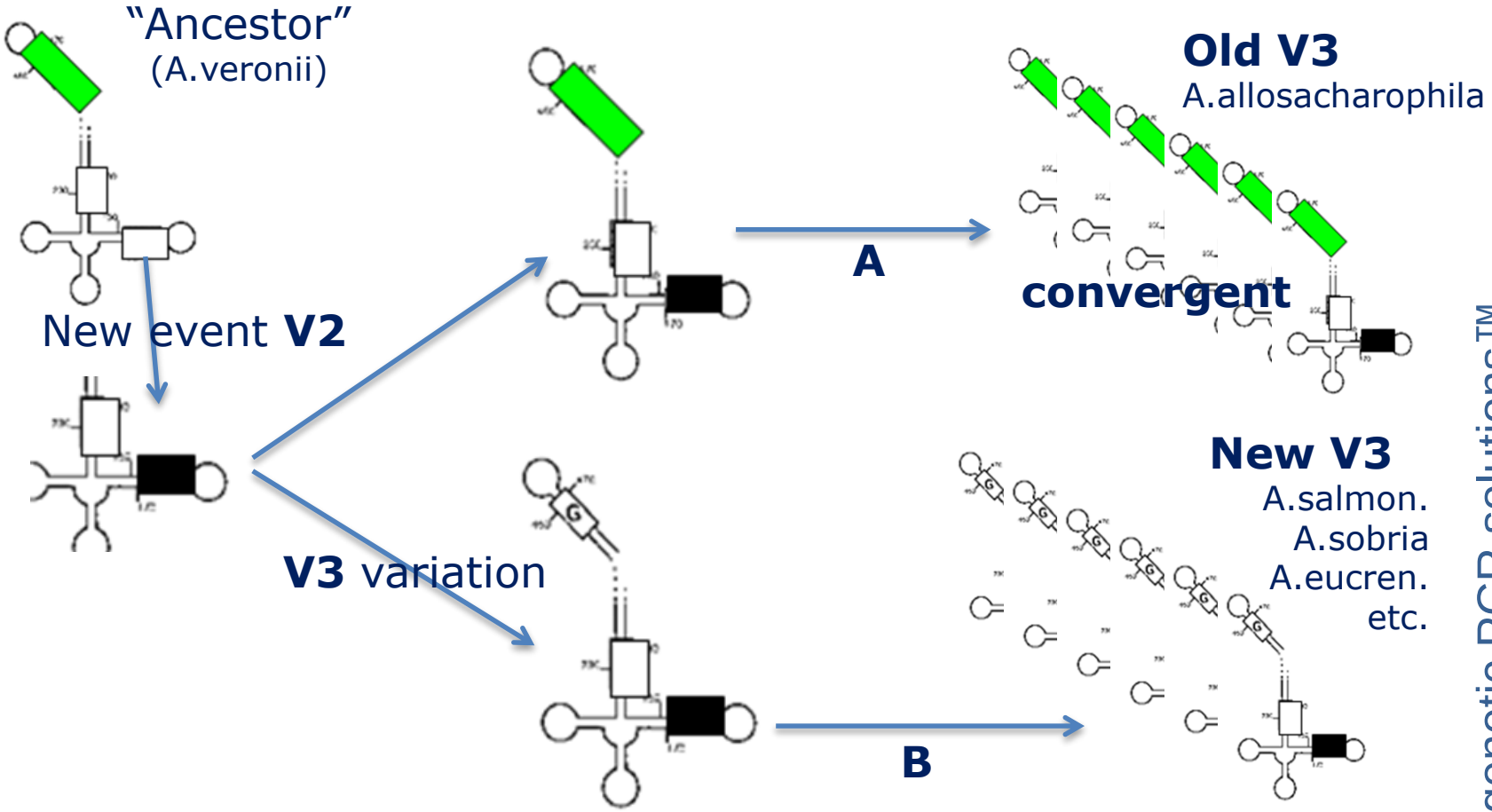
Aeromonas – 9 rRNA copies?

Concerted Evolution

How is this drived?

rrn multigene family





Template leader (A or B) for Genetic Homogenization?

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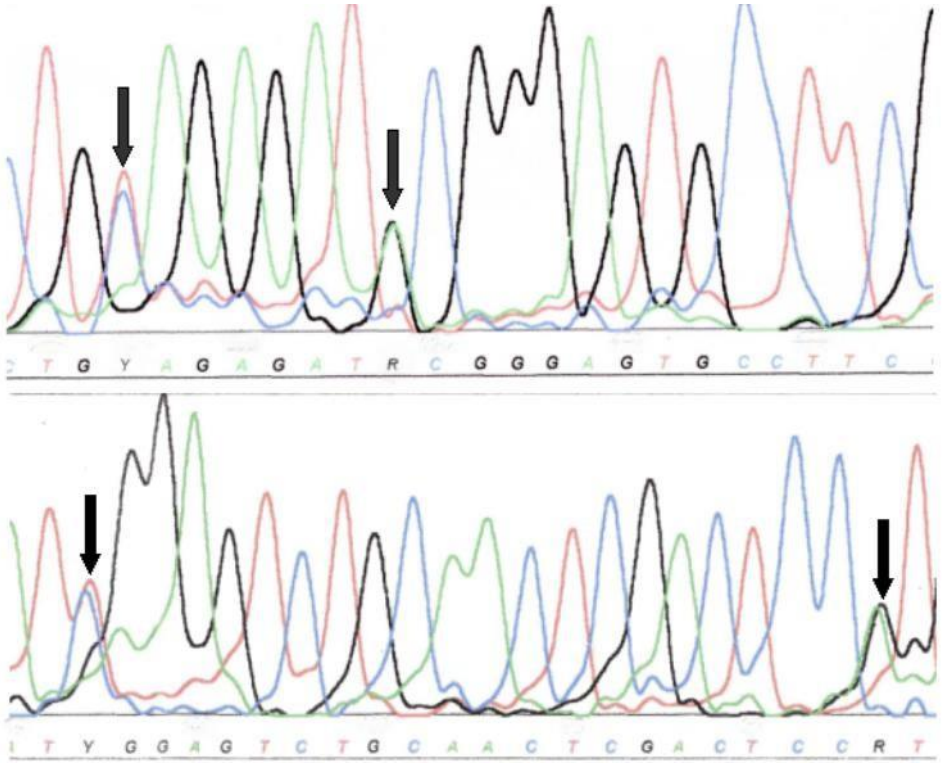
- Intra-species diversity > inter-species

Inter-cistron diversity: microheterogeneities



Variable positions

Species	No of Strains	1011	1018	1308	1329
A. caviae	12	T/C	A/G	G/A	C/T
A. veronii	6	T/C	A/G	C/T	G/A
A. sal./ A. best.	11	T/C	A/G		

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Conserved molecules
Ancestral functions
Constant mutation rates?

Protein-coding genes

High mutation rates

Degenerative code

Fast clocks

Short-term phylogenies

rRNA-coding genes

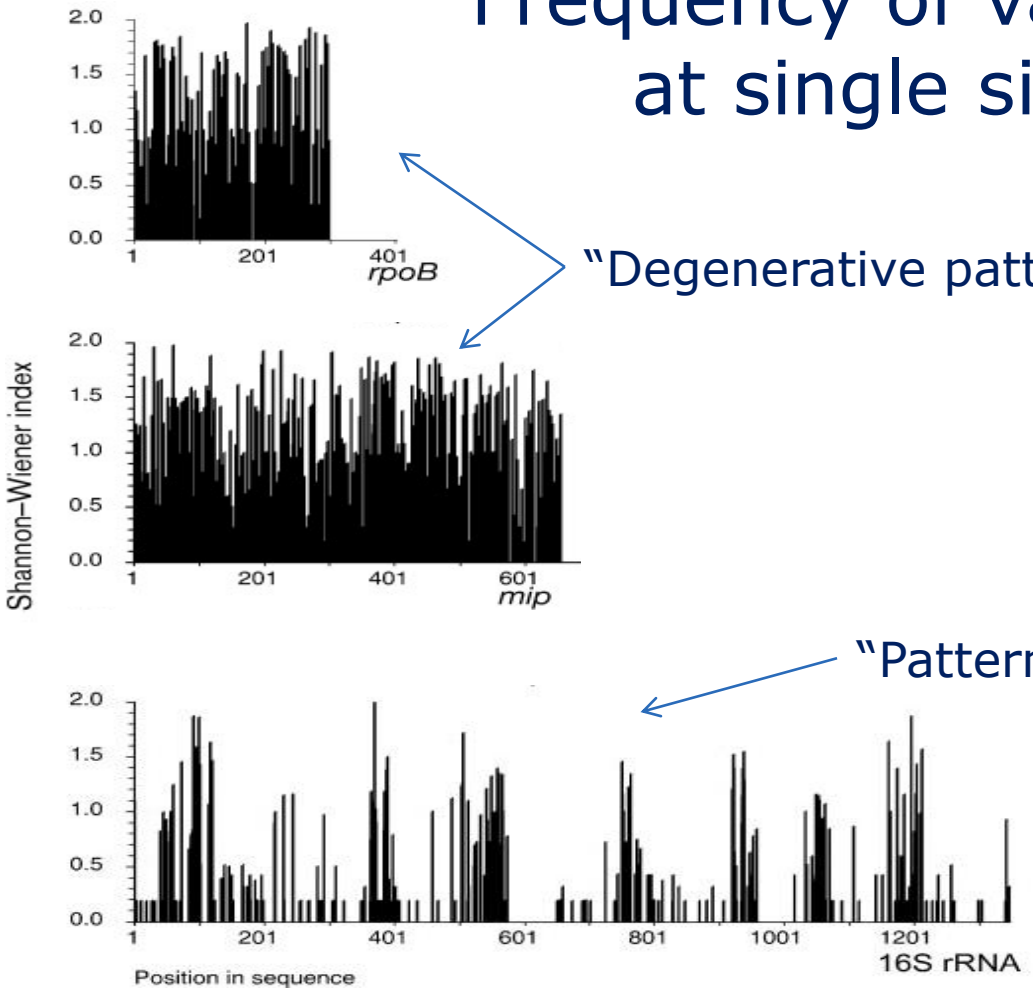
Low mutation rates

Mosaic of signatures

Slow clocks

Long-term phylogenies

Frequency of variation at single sites



Carl-Johan Rubin, et al., 2005. IJSEM.

Protein-coding genes

Accessory
Non-fundamental
Virulence
Resistance, etc..

Highly variable
Transference
"Plasmids"

House-Keeping
Replication
Transcription
Translation, etc.

Relatively variable
Transference is rare
"Chromosome"

International Journal of Systematic and Evolutionary Microbiology (2003), 53, 875–883 DOI 10.1099/ijs.0.02443-0

Phylogenetic analysis of members of the genus *Aeromonas* based on *gyrB* gene sequences

M. A. Yáñez,[1,2] V. Catalán,[2] D. Apráiz,[2] M. J. Figueras[3] and A. J. Martínez-Murcia[1]

[1]Unidad de Diagnóstico Molecular, EPSO, Universidad Miguel Hernández, Ctra Beniel Km 3, E-03312 Orihuela (Alicante), Spain

[2]Labaqua SA, Alona 33, E-03007 Alicante, Spain

[3]Unidad de Microbiología, Departamento de Ciencias Médicas Básicas, Facultad de Medicina y Ciencias de la Salud, Universidad Rovira y Virgili, Reus, E-43201 Tarragona, Spain

Correspondence
A. J. Martínez-Murcia
ammurcia@umh.es

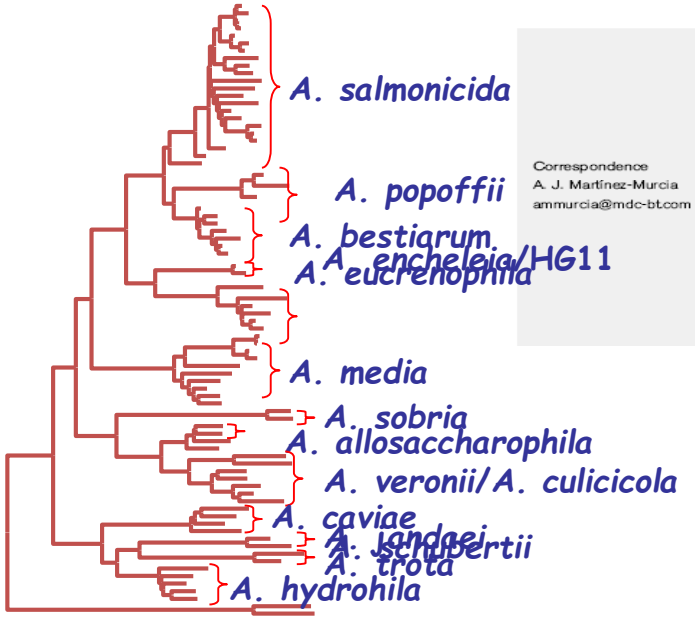
- What really matters is **clustering of strains** (species level)
- The balance with bona-fide strains is relevant

- Different similarity ranges Δ ≠ genes : housekeeping selection (gradient of chronometricity)

- Housekeeping genes (DNA/RNA processing) evolve in concert: same species-clustering

- Different similarity ranges Δ ≠ taxa : "A global MLPA species definition cannot be based on absolute values"

International Journal of Systematic and Evolutionary Microbiology (2004), 54, 1511–1519 DOI 10.1099/ijs.0.03048-0



Correspondence
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ammurcia@mdc-btcom

Phylogenetic analysis of the genus *Aeromonas* based on two housekeeping genes

L. Soler,[3†] M. A. Yáñez,[2,4†] M. F. Chacon,[3] M. G. Aguilera-Arreola,[3,5] V. Catalán,[4] M. J. Figueras[3] and A. J. Martínez-Murcia[1]

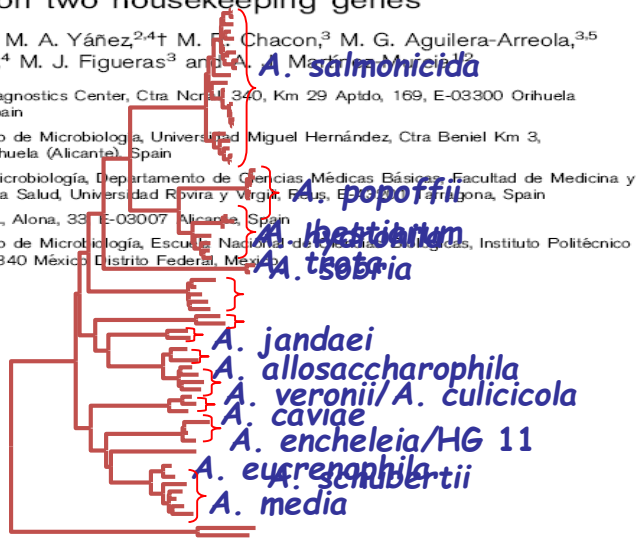
[1]Molecular Diagnostics Center, Ctra. Nacional 340, Km 29 Aptdo, 169, E-03300 Orihuela (Alicante), Spain

[2]Departamento de Microbiología, Universidad Miguel Hernández, Ctra Beniel Km 3, E-03312 Orihuela (Alicante), Spain

[3]Unidad de Microbiología, Departamento de Ciencias Médicas Básicas, Facultad de Medicina y Ciencias de la Salud, Universidad Rovira y Virgili, Reus, Spain

[4]Labaqua, S.A., Alona, 33, E-03007 Alicante, Spain

[5]Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, 11340 México D.F. Distrito Federal, México



gyrB

Sequence similarity: 86,3 -100%

4 pairs with identical sequence

Intra-species 0 - 2,3 % (1,6%)

Inter-species >3%

rpoD

Sequence similarity: 81,7 -100%

4 pairs with identical sequence

Intra-species 0 - 2,6 (1,6%)

Inter-species > 3%

Especies	rDNA 16S	*gyrB*	*rpoD*
A. cavie/A. trota	1	70	79
A. hydrophila/A. media	3	61	68
A. jandaei/A. culicicola	1	60	58

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- Different similarity ranges Δ ≠ genes : housekeeping selection (gradient of chronometricity)
- Housekeeping genes (DNA/RNA processing) evolve in concert: same species-clustering
- Different similarity ranges Δ ≠ taxa : "A global MLPA species definition cannot be based on absolute values"

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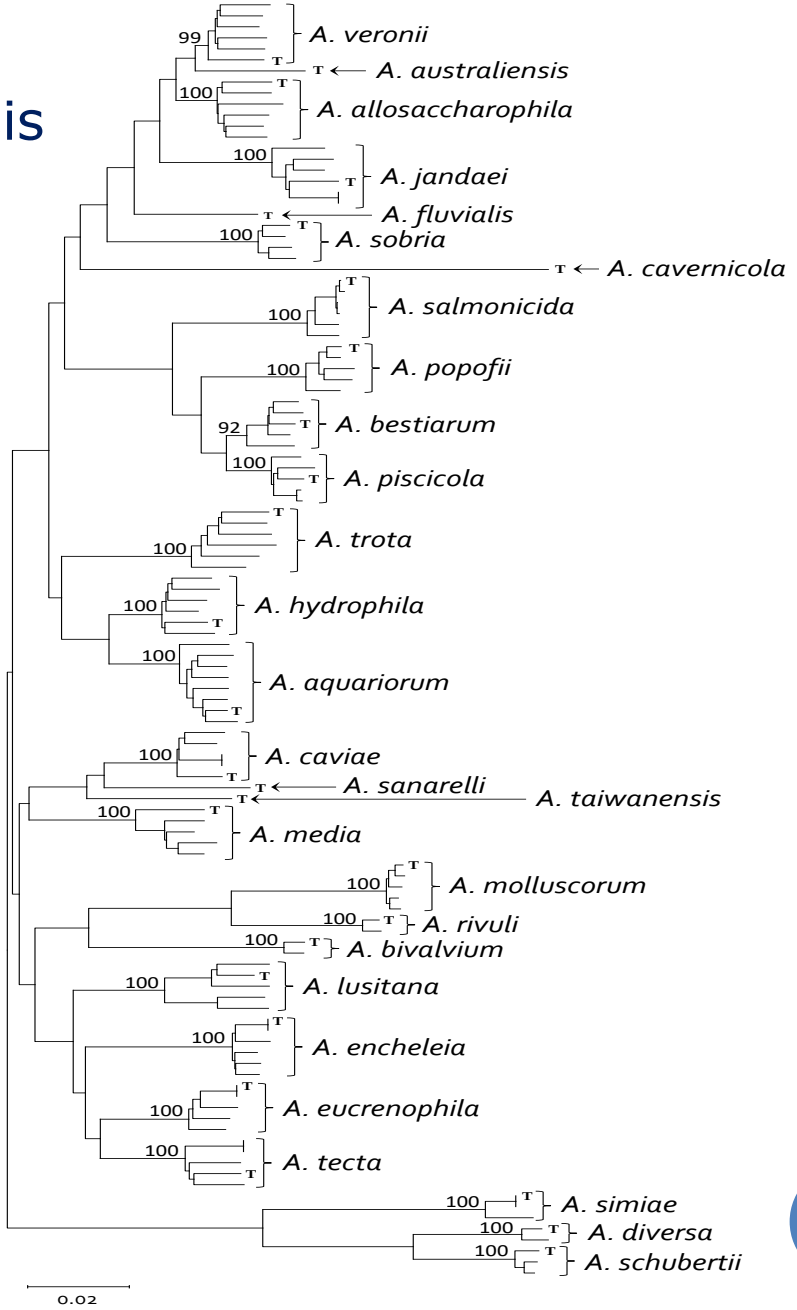
MultiLocus **P**hylogenetic **A**nalysis
MLPA

gyrB, rpoD, recA, dnaJ, gyrA, dnaX
4204 bp.

Systematic and Applied Microbiology 34 (2011) 189–199



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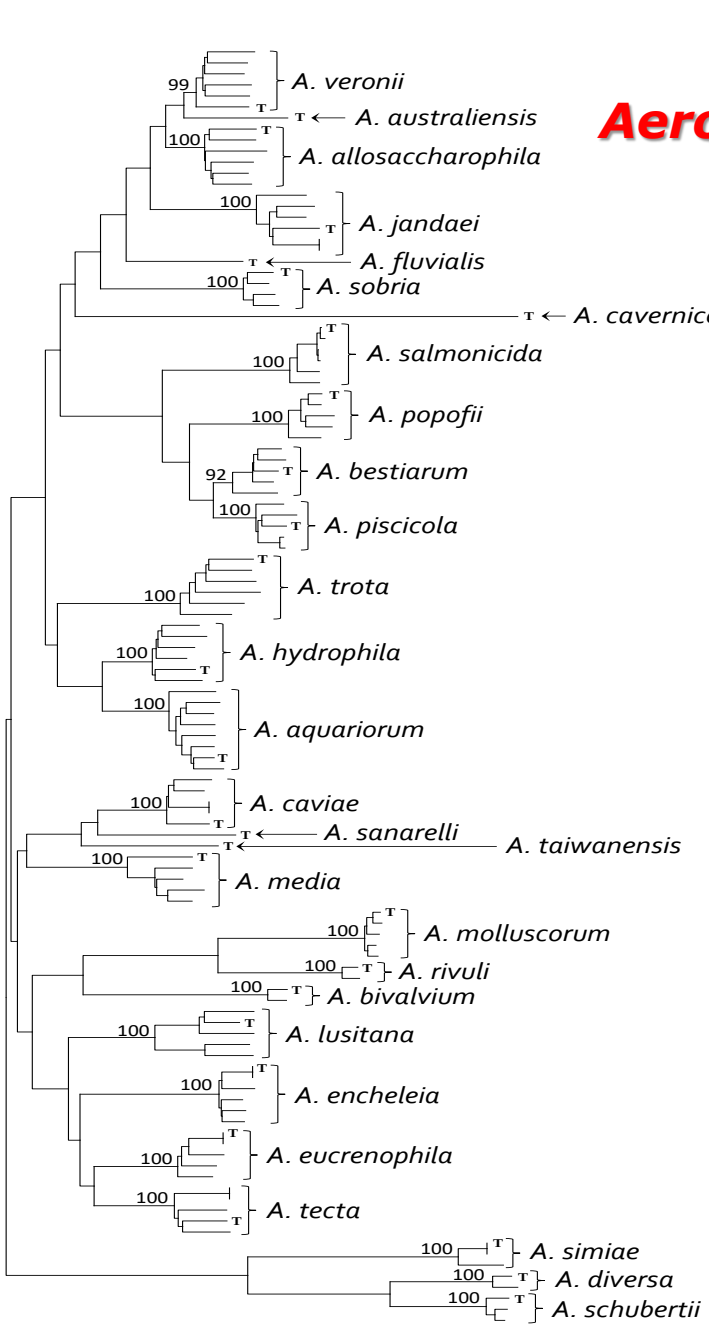
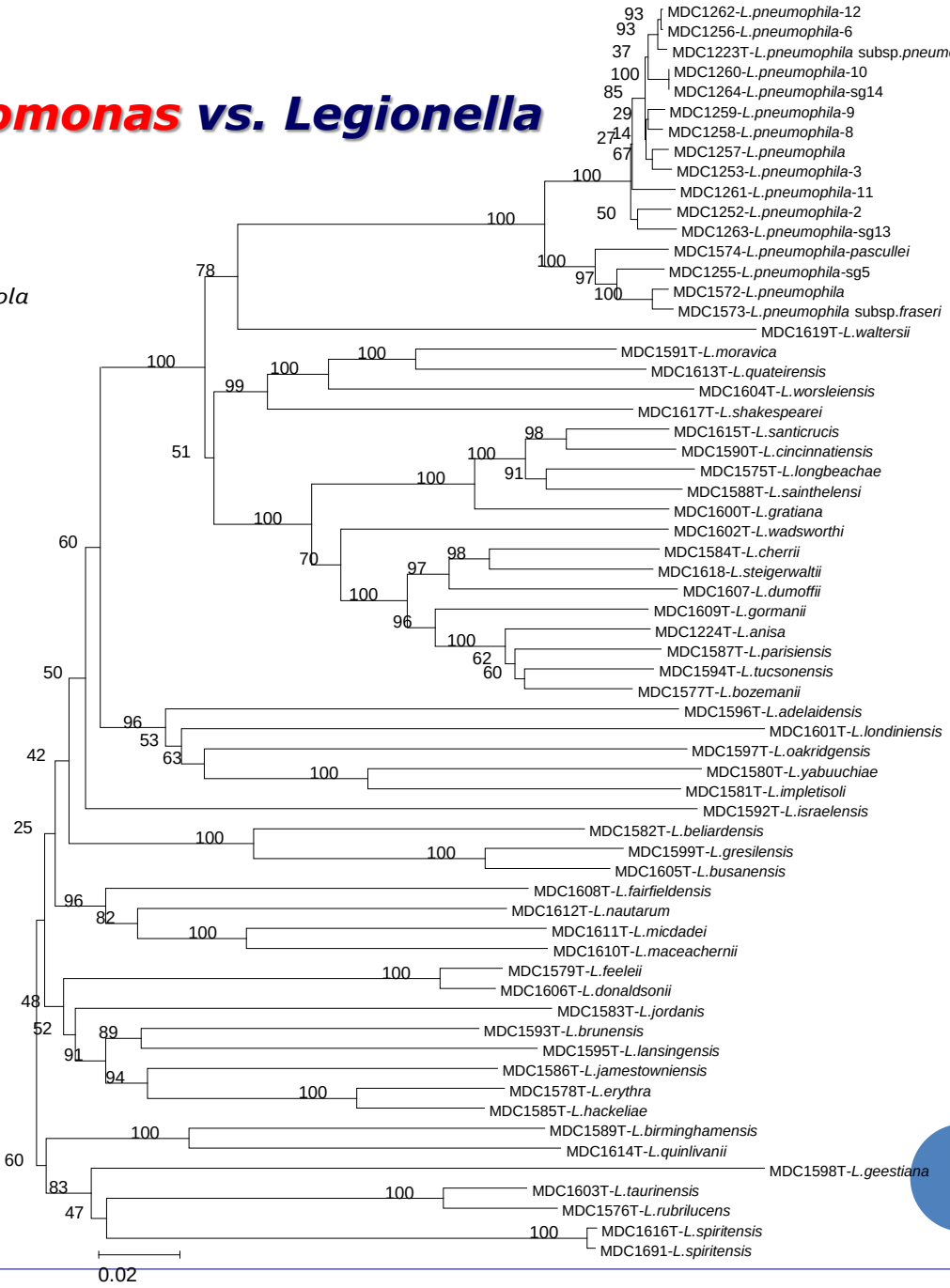
- Housekeeping genes (DNA/RNA processing) evolve in concert: same species-clustering

- Different similarity ranges Δ ≠ taxa : "A global MLPA species definition cannot be based on absolute values"

Aeromonas vs. Legionella





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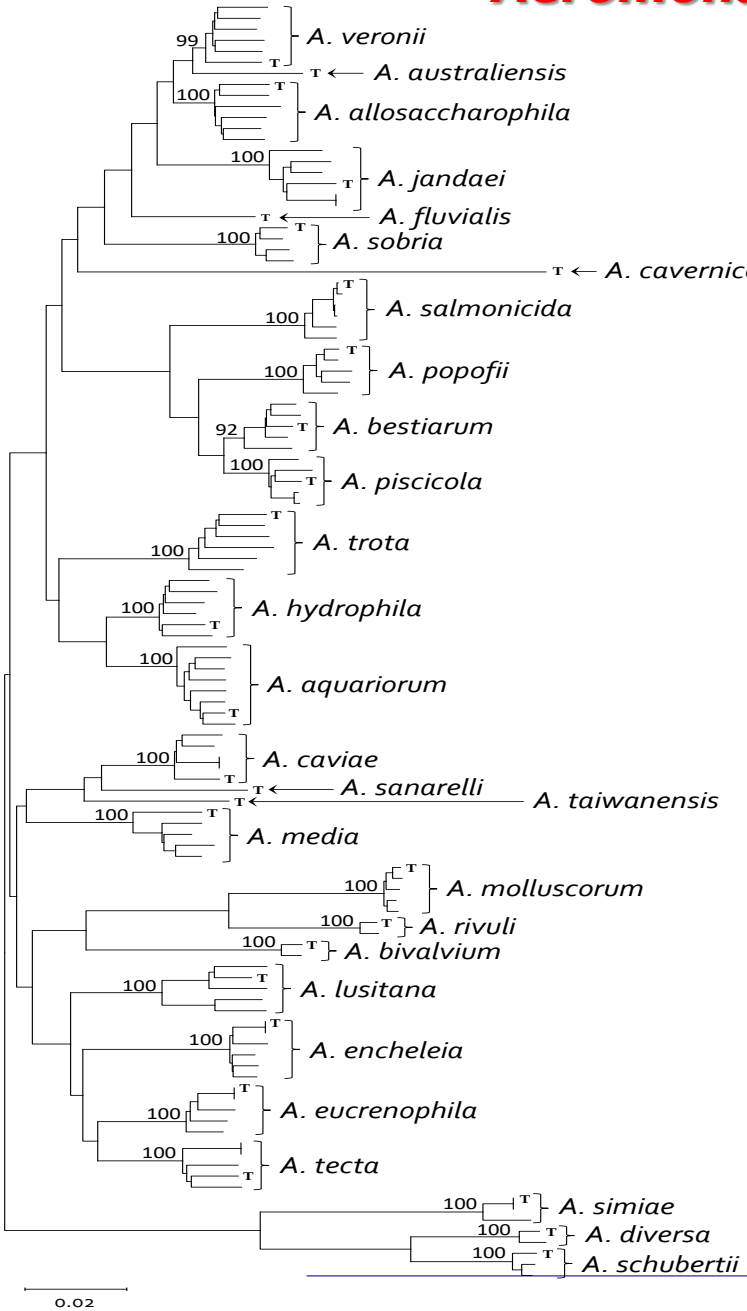
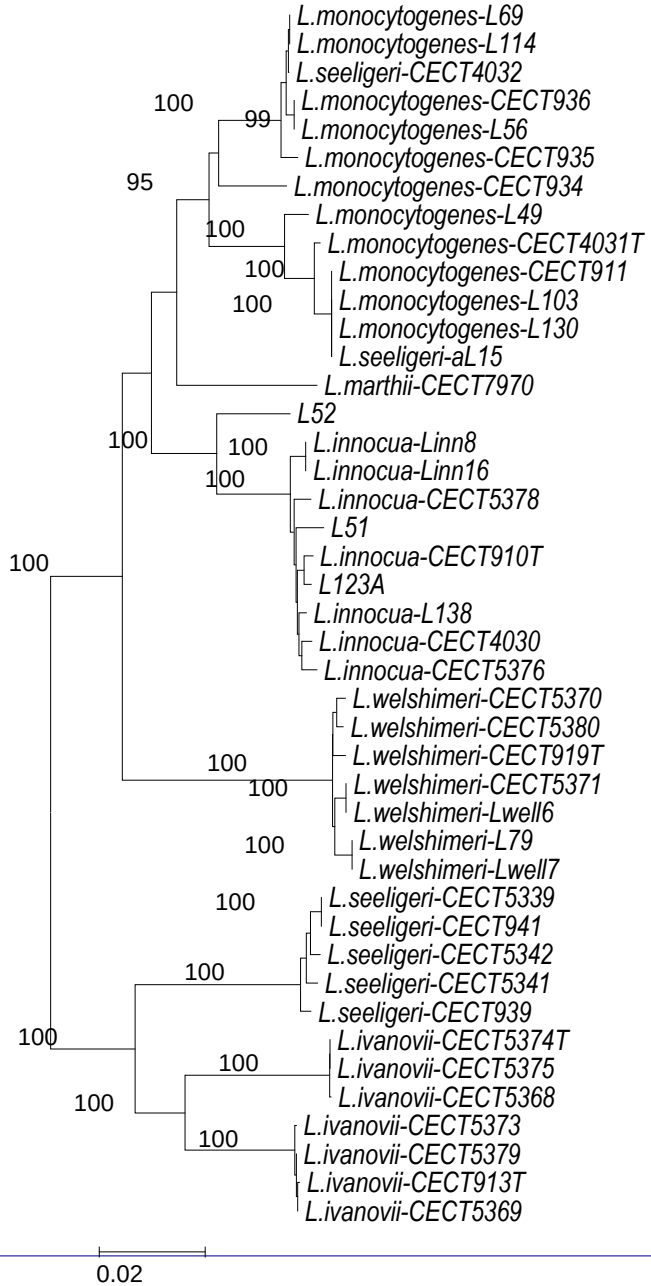
Aeromonas vs. Listeria





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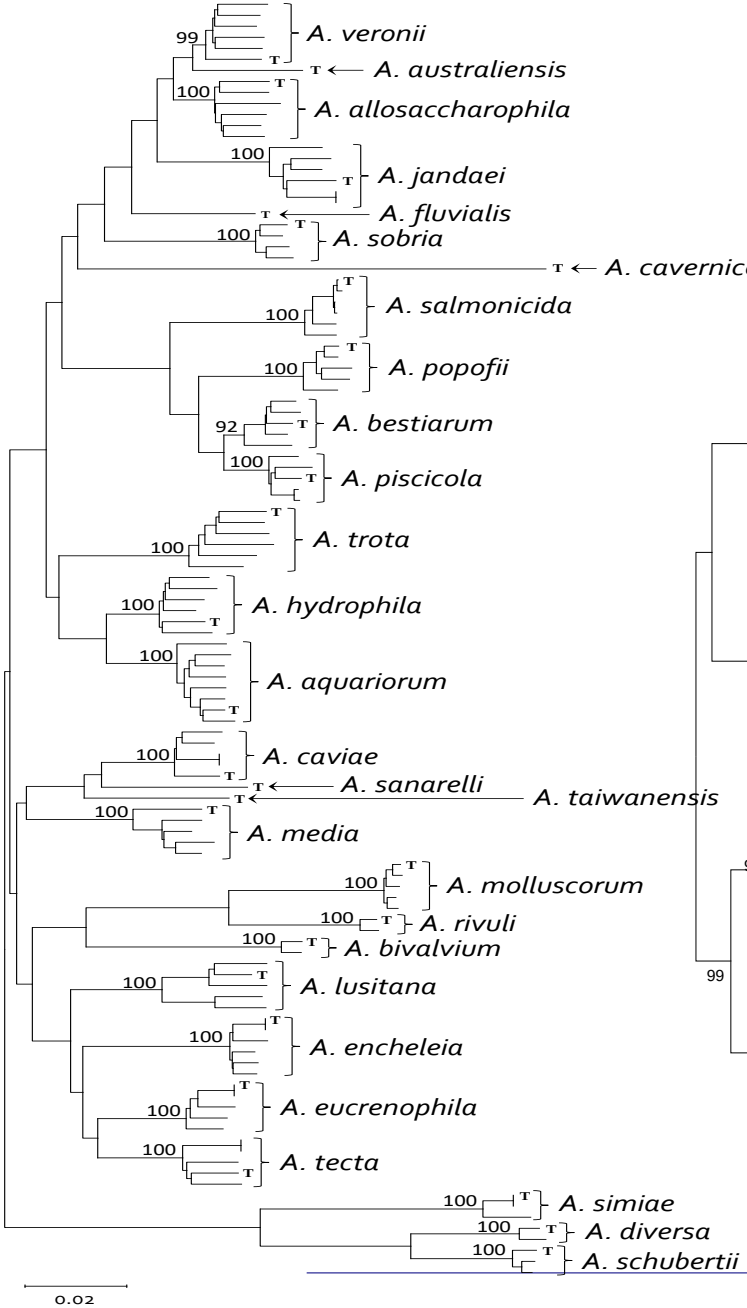
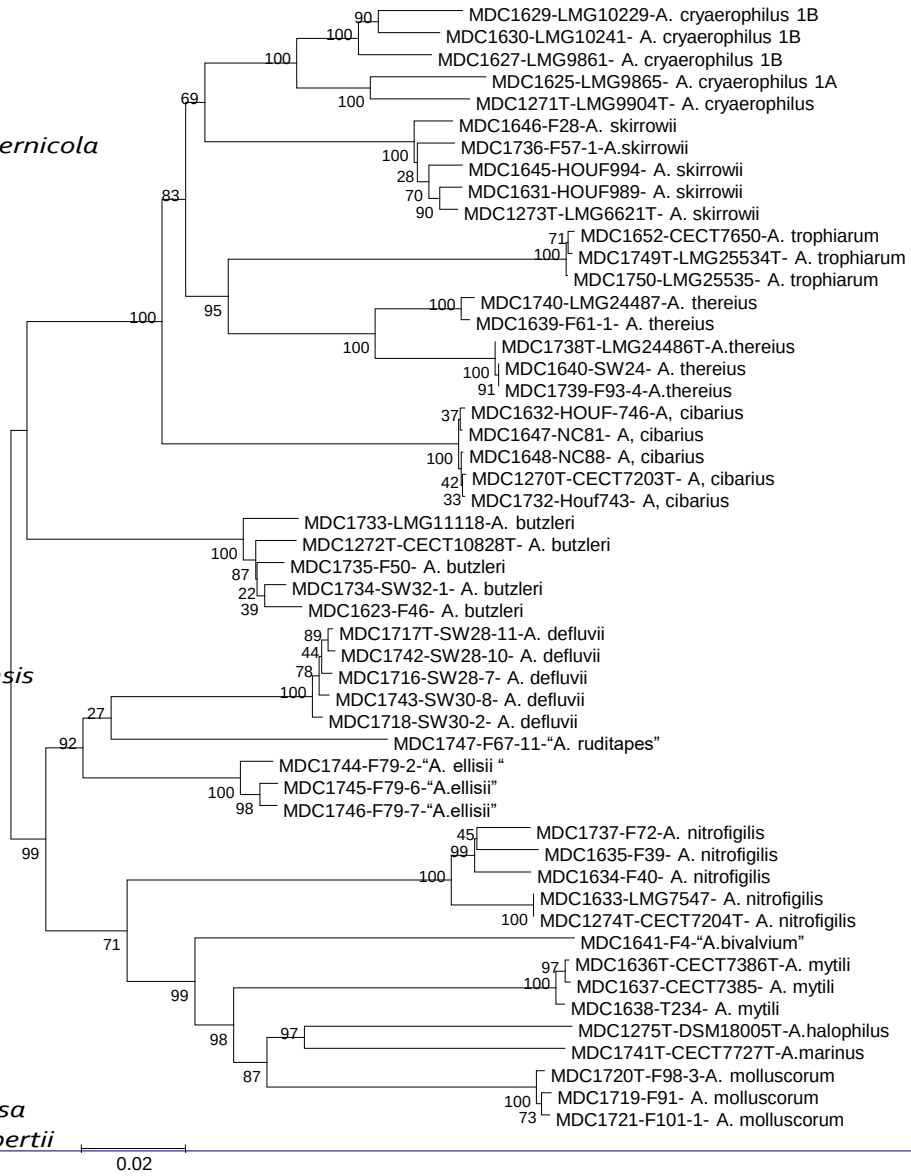
MLPA - Arcobacter

gyrB-gyrA-rpoB-atpA-groEL

3142 bp





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Next Generation Sequencing (NGS) = full genomes

- the average nucleotide identity (ANI) among shared genes
- the genome-to-genome distance, referred as *in silico* DDH (*is*-DDH)
- the **"multi-locus phylogenies"** from "determined" genes (HK, RG, EC)

RESEARCH ARTICLE

CrossMark click for updates

Bioinformatic Genome Comparisons for Taxonomic and Phylogenetic Assignments Using *Aeromonas* as a Test Case

Sophie M. Colston,[a] Matthew S. Fullmer,[a] Lidia Beka,[a] Brigitte Lamy,[b,c] J. Peter Gogarten,[a] Joerg Graf[a]

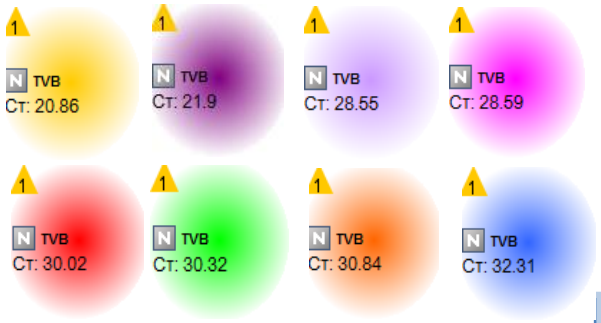
Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut, USA[a]; Laboratoire de Bactériologie-Virologie, UMR 5119, Equipe Pathogènes et Environnements, Université Montpellier, Montpellier, France[b]; Laboratoire de Bactériologie, Centre Hospitalier Universitaire de Montpellier, Montpellier, France[c]

Most of the general relationships observed in our study were consistent with those reported in the published literature. The

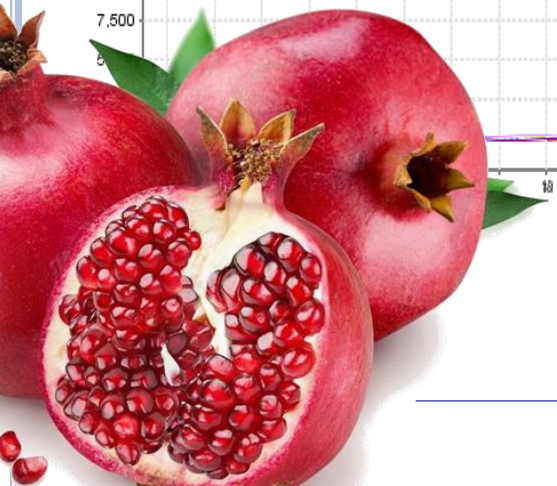
supported the MLPA are in concert to the entire genomes,
the MLPA-tree is like "the mirror" of the full genomic relationships

Total Bacteria (rRNA) dtec-qPCR

Negative controls



Co. A
Co. B
Co. B-DNA_Free
Co. C
Co. D
Co. E
Co. F
MixStable (GPS)



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Accummulated number of *Aeromonas* sequence NCBI entries.

Drawbacks:
-wrong species
-wrong names
-low quality

(≥ 200 bp)

Gene	<2005	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	08/2016*
atpD	0	0	0	0	0	0	0	115	115	115	115	126	435 (10157)
cpn60	0	3	3	4	4	74	84	177	316	317	404	405	405 (8802)
dnaJ	0	0	0	27	27	62	83	221	378	378	403	416	724 (2398)
dnaK	0	0	0	0	0	0	0	0	194	194	194	200	242 (8908)
dnaX	0	0	0	0	0	0	0	115	116	116	141	154	462 (873)
gltA	0	0	0	0	0	0	0	83	321	386	451	460	515 (7119)
gyrA	13	13	13	13	13	18	19	132	136	136	161	174	482 (6765)
gyrB	159	174	288	306	383	599	663	1299	2095	2210	2539	2579	2915 (34494
mdh	0	0	0	0	0	0	36	36	152	152	153	224	224 (8777)
metG	0	0	0	0	0	0	0	82	132	152	220	229	316 (685)
ppsA	0	0	0	0	0	0	0	87	87	107	178	187	274 (720)
radA	0	0	0	0	0	0	0	0	192	192	192	192	230 (262)
recA	1	1	1	60	60	90	118	333	516	536	634	761	1084 (30628
rpoB	0	53	67	73	78	80	87	109	338	344	346	353	396 (31989)
rpoD	77	77	160	171	177	263	484	934	1192	1372	1498	1537	2028 (10034
tsf	0	0	0	0	0	0	0	0	195	195	195	195	231 (342)
zipA	0	0	0	0	0	0	0	0	195	195	195	195	231 (236)
rrs >200 bp	510	719	942	1125	1464	2389	2647	3339	4225	4938	5522	6245	6776 (5.8x10^6
rrs >500 bp	410	595	800	964	1175	1816	2043	2690	3456	4111	4645	5295	5800
rrs >1000 bp	212	264	397	489	605	1023	1146	1699	1984	2400	2761	3164	3506
rrs >1450 bp	114	128	225	252	316	485	545	642	731	856	922	1012	1090

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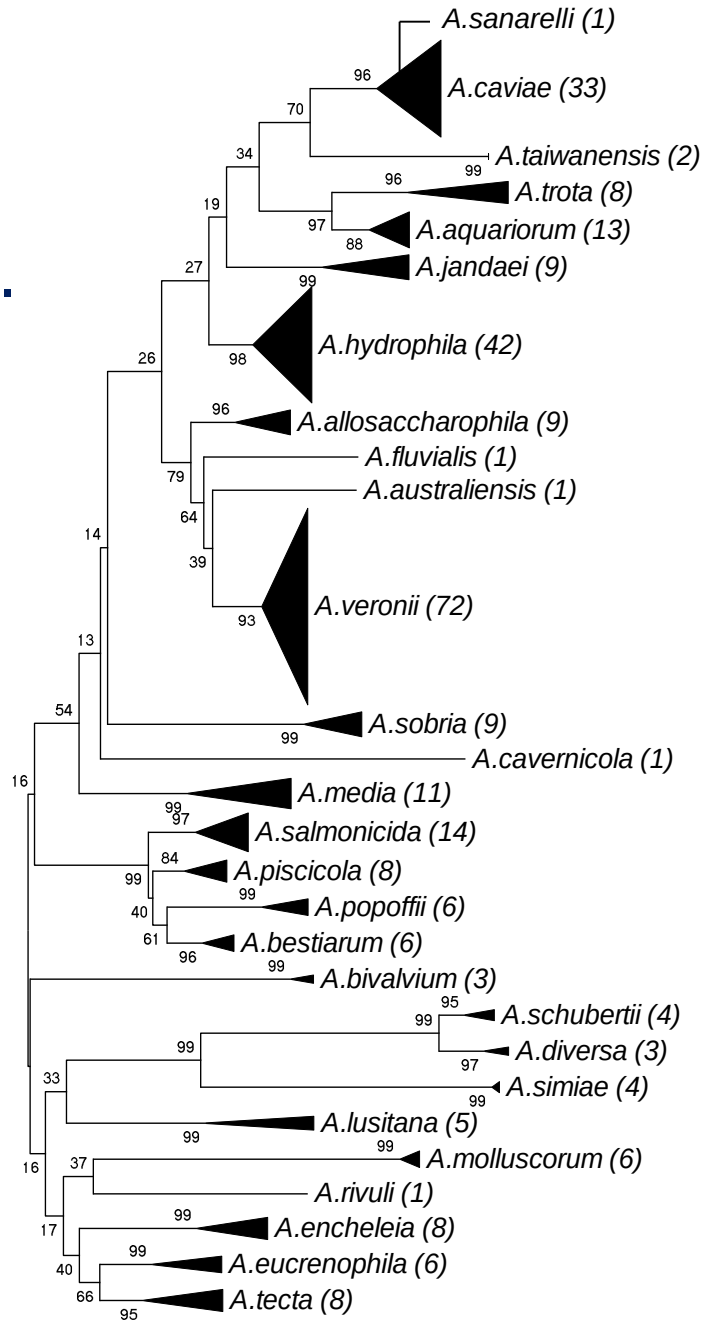
Phylogenetic NJ-tree
759 bp - *gyrB* sequences
294 "bona-fide" Aeromonas spp.
selected from EMBL
(Martinez-Murcia and co-workers)



<**pragmaticu**> (pragmatic)

From Greek *pragma* ("deed")
philosophers/politicians concerned more with
real-world application of ideas
versus theory or dogma

Laboratories in Spain

Clinical	Food	Microbiological	Analytical
693	458	537	2.296

Universities and R&D in Spain

Universities	Universities + R&D
82	58

"WEBOMETRICS 2016, SCIMAGO INSTITUTION RANKINGS 2015"
"Universidad Española en Cifras". Año 2014. Curso 2014-2015. CRUE 2016.

Systematics – diversity → new taxa

- Taxonomy – classification frame (robust)
- Nomenclature – scientific names
- Identification/Detection – taxa location (pragmatic)

Pragmatic Identification

Universal target
Axenic sample- pure culture
Result: taxon

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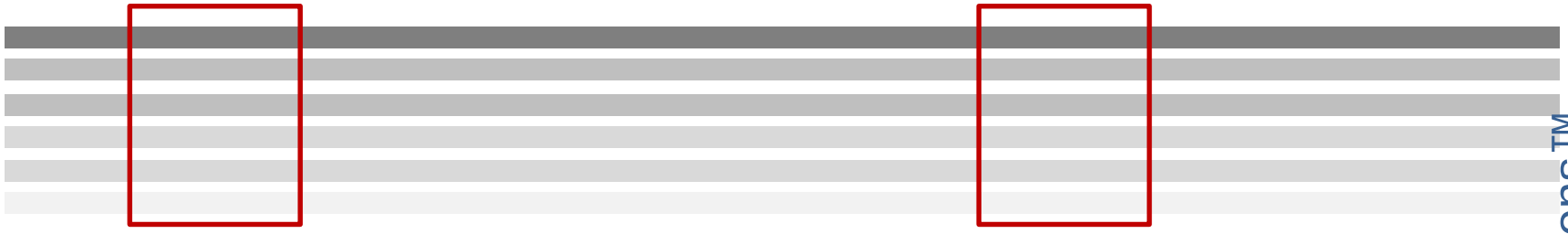
Yamamoto and Harayama, 1995
gyrB "universal primers"

5' - GAA GTT ATT ATG ATC GTT TTA **CAY GCN GGN GGN AAR TTY GA** - 3'



5' - AGC AGG GTA CGG ATG TGC GAG **CCR TCN ACR TCN GCR TCN GTC AT** - 3'

Permutations with repetition: n^r

n; number of nucleotides (G, A, T, C = 4)

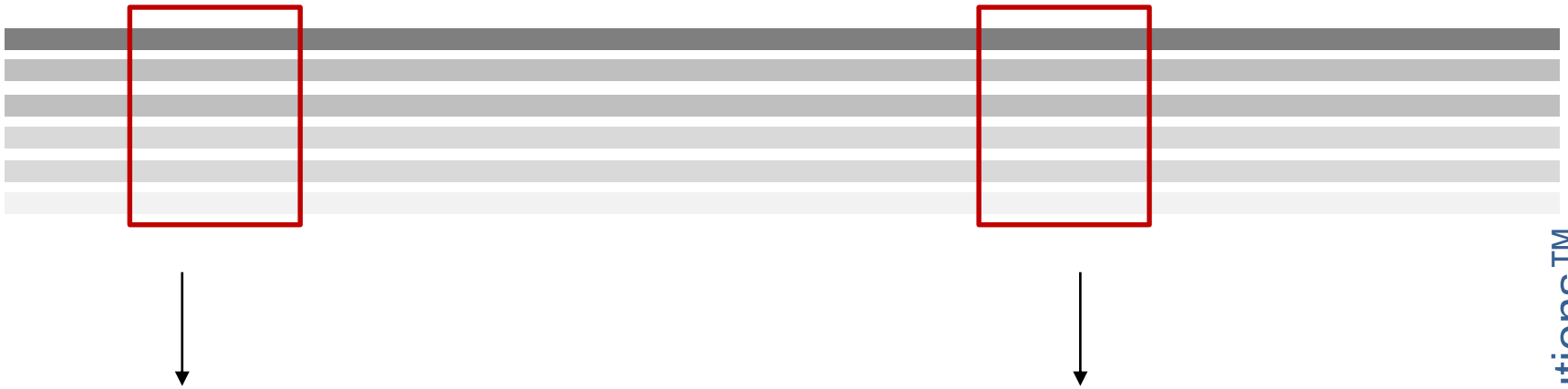
r; number of degenerations

Comb. = **262.144**

genetic PCR solutions™

www.geneticpcr.com

GPS™
gyrB group-specific primers



5′ XXXXNXXXXXNXXXXXXXXXX 3′	5′ XXNXXXXXXXXXXXXNXXXX 3′	Bacterial Group A
5′ XXXXXXXNXXXXXXXXNXXXX 3′	5′ XXXXXXXXXXNNXXXXXXXXX 3′	Bacterial Group B
5′ XNXXXXXXXXXXXNXXXXXX 3′	5′ XXXNXXXXXXXXXXXXXNXX 3′	Bacterial Group C
5′ NXXXXXXXXXNXXXXXXXXX 3′	5′ XXNXNXXXXXXXXXXXNXX 3′	Bacterial Group D

Comb. = **256**

2 N/primer

Comb. = **4.096**

3 N/primer

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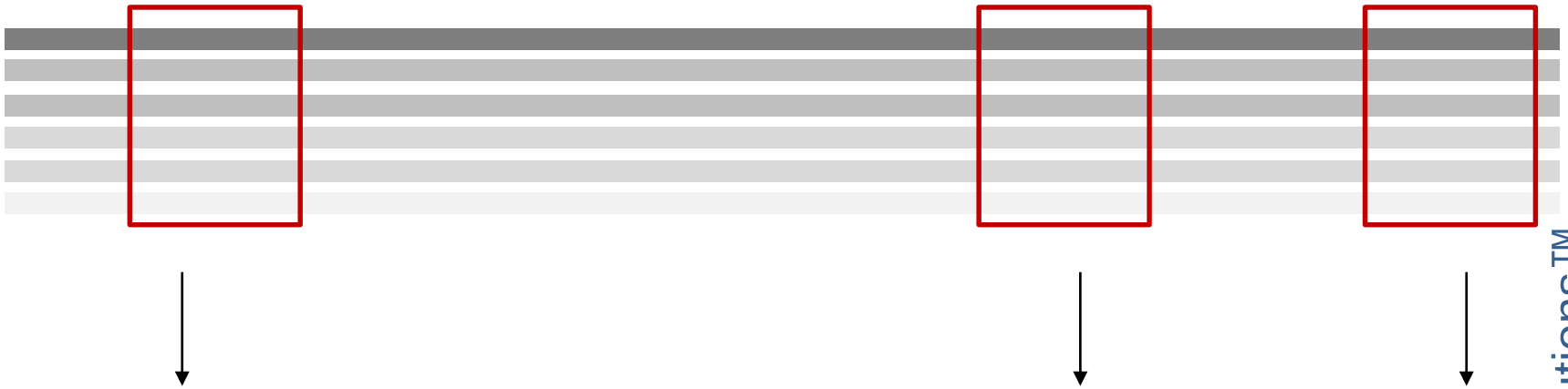
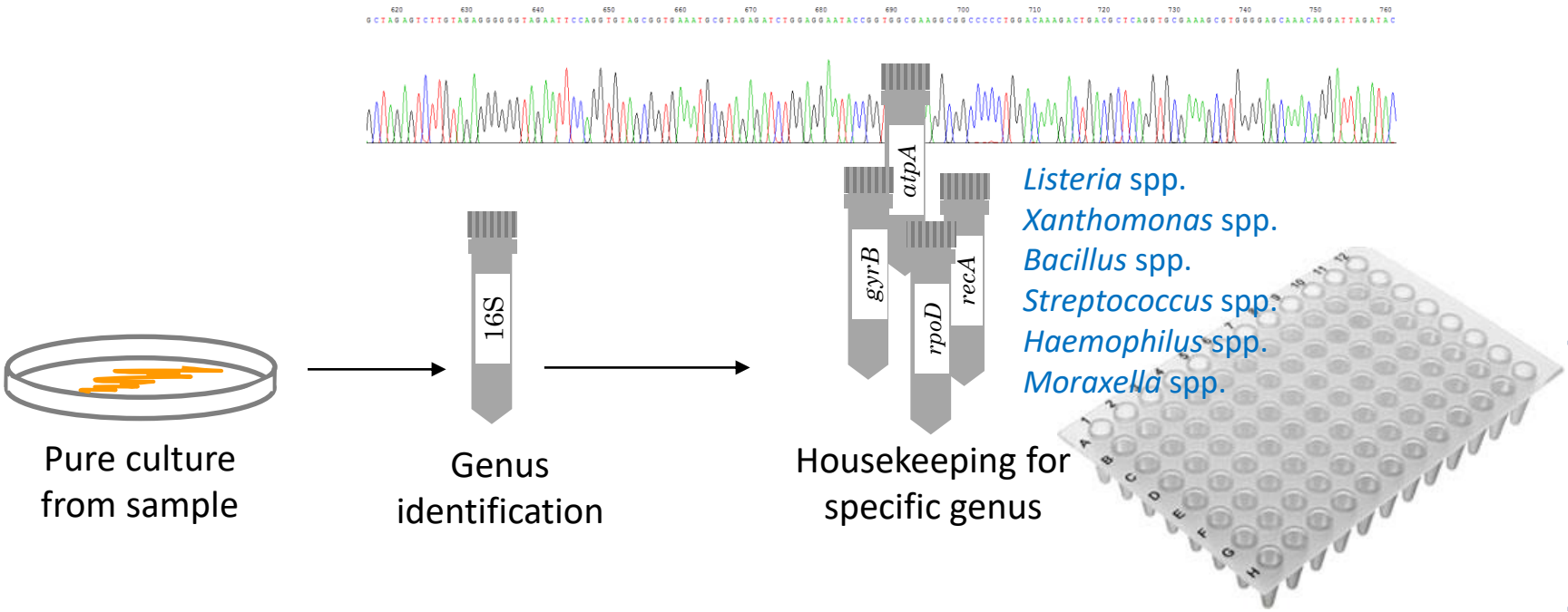
GPS™
gyrB enterobacteriaceae-specific primers



Diseño 1	Code	Sequence (5'->3')	Strand	Length	Tm °C	GC%
Forward	Fam.Ent-gyrB-0F		Plus	20	57.3	50
Reverse	Fam.Ent-gyrB-9R		Minus	20	53.2-59.3	40-55

Diseño 2	Code	Sequence (5'->3')	Strand	Length	Tm °C	GC%
Forward	Fam.Ent-gyrB-0F		Plus	20	57.3	50
Reverse	Fam.Ent-gyrB-16R		Minus	19	56.7-61	52.6-63.2

The MLPA kit from GPS™



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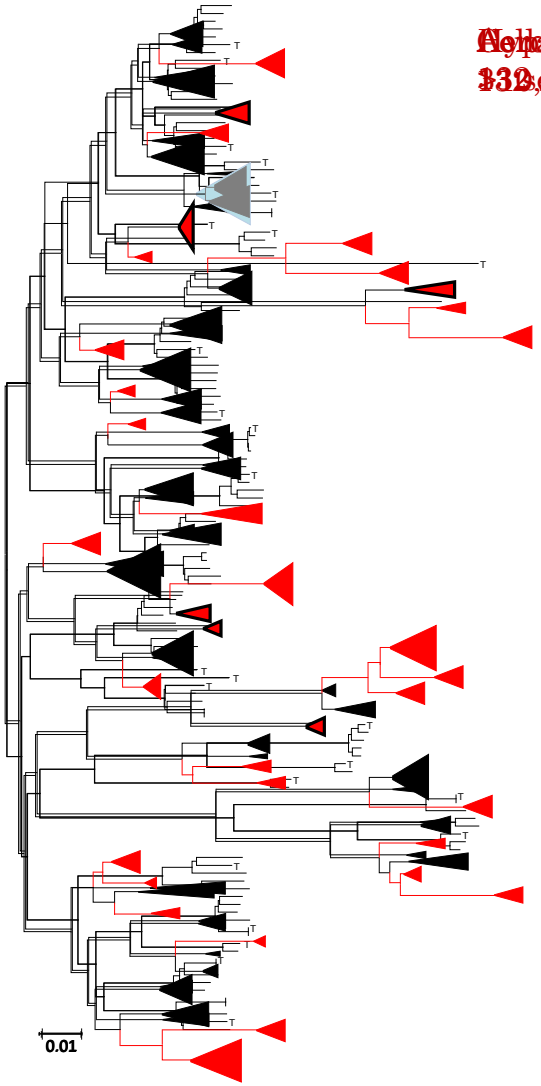
Possible evolution of phylogenetic tree with addition of sequence diversity



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Systematics – diversity → new taxa

- Taxonomy – classification frame (robust)
- Nomenclature – scientific names
- Identification/Detection – taxa location (pragmatic)

Reliable Detection

Specific taxon or strain
Non axenic –heterogeneous
Result: positive/negative

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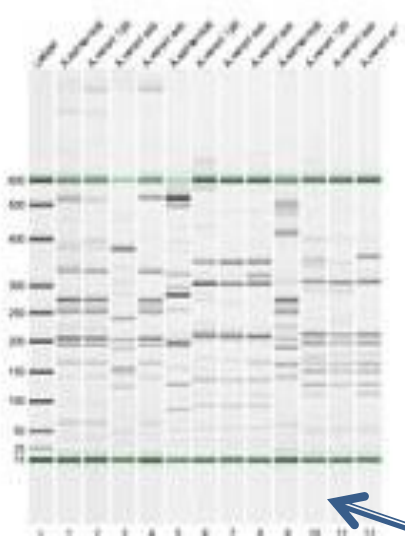
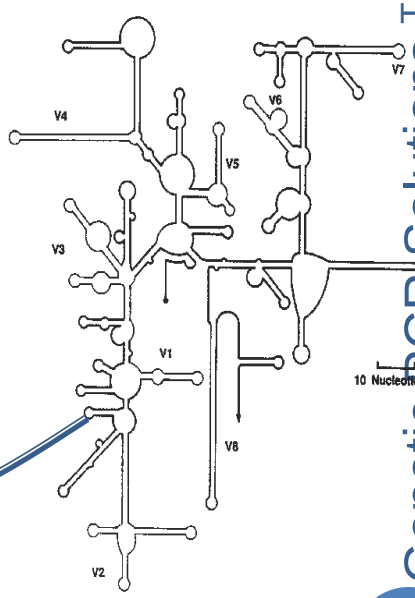
A simple message

Genetic patterns: a “bar-codes” like



- **Accuracy**, genetics is unequivocal
- **Sensibility**, single cell detection
- **Fast**, results in a few hours



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Major hopes of the qPCR

- Validated platforms, should be affordable:
  - **easy** to perform (automated)
  - **not expensive**

- But must be **innovative** (?)
  - Theoretical --- make it possible!
  - Complex --- make it simple!

Major drawbacks of the qPCR

Concentration of sample

- Increase sensibility (the true LOD)
- Increase qPCR inhibitors

DNA/RNA purification

- Decrease qPCR inhibitors
- Low yields, decrease sensibility

Non-Viable cells and free-DNA

- False positives
- False quantification

Multiplexing

- Unreliable & unpredictable

PRODUCTS AND SERVICES
(CORE BUSINESS)

- qPCR **GPS™** kits, or newly designed “a la carta”, for your specific pathogen
- Phylogenetic identification services
- Customer tech-training and protocols by using **GPS™** kits
- Validation to standards of UNE-EN ISO/IEC 17025

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qPCR **GPS™** kits

> 200 pathogen targets

Dried-ice transport is **not** needed

Format F-100 **CONTENT**
(100 reactions of 20 µl)

- TargetSpecies **dtec-qPCR-mix** 100 rxn. (AMBER TUBE)
- **MixStable** qPCR.5X (BLUE CAP)
- qPCR-mix resuspension **buffer** (WHITE CAP)
- **Standard template** (+) control (RED CAP) 10^9 target copies
- DNase/RNase free **water** (GREEN CAP)

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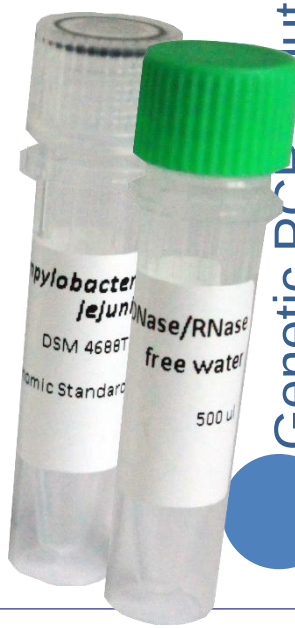
GPS™ designs*

Phylogenetic considerations for
full "in silico" specificity

- Inclusivity
- Exclusivity
- Genetic maker selection
- Updated diversity



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* For microbial control

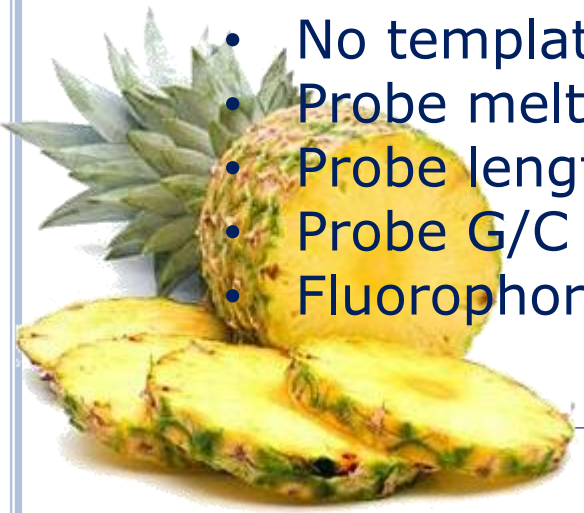
GPS™ designs

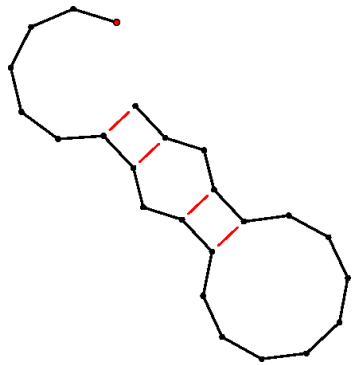
Structural considerations for a "healthy" PCR thermodynamic

```
5'                TCTGATTATTGAAAGTACCCG
                  :|::||
3' CCATAGAAGAGAGGGGTTA
```

```
5' TCTGATTATTGAAAGTACCCG
     |||:   | |||:
3'   CCATAGAAGAGAGGGGTTA
```

- Minimise primer dimers
- Minimise primer hairpins
- Optimal Tm
- Optimal G+C content
- 3'-end GC clamp Short amplicons
- No template secondary structure
- Probe melting point
- Probe length
- Probe G/C content
- Fluorophore/Quencher (background)





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qPCR kits Quality Control

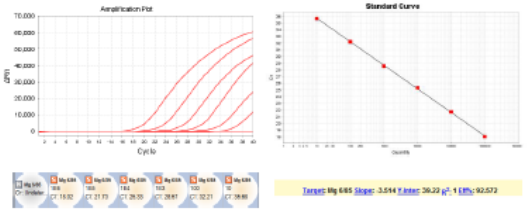
Calibration curve: 10^6-10 copies
Each batch

GPS™ certifies reagents to meet the quality standards and the specifications stated on the handbook.

QUALITY CERTIFICATION

Mg 6/85 dtec-qPCR Test

Genetic PCR Solutions™ certifies that, reagents of the Mg 6/85 dtec-qPCR Test (RUO*), to detect ***Mycoplasma gallisepticum*** **vacunal 6/85** by qPCR, have been manufactured and tested using internal analysis systems and is hereby certified to meet the quality standards and the specifications stated on the product handbook.



Mg 6/85 GPS™ *Quality Control*. Left) Graph with a decimal dilution standard template amplification (10-10^6 copies); Right) and the corresponding calibration curve with stats.

*RUO: Research Use Only

Code: 0415104
Batch: 0815104
Validation date: 25/08/2015
G. Bru, GPS™ Technician

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Genetic PCR Solutions ™

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SISTEMA DE GESTIÓN DE LA CALIDAD oca UNE-EN ISO 9001 CERT
CERTIFICADORA ACREDITADA POR ENAC

ISO 9001 BUREAU VERITAS Certification

ISO 13485 BUREAU VERITAS Certification

GPS™ R&D PROJECTS

AQUAVALENS

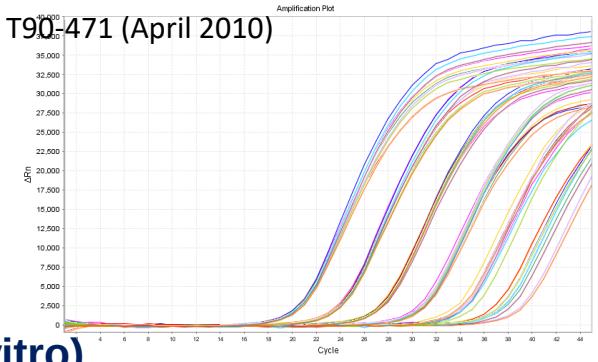
Project acronym: **Aquavalens**

"Protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation".

KBBE.2012.2.5-01. 7th Framework Program, European Union.

Consortium: 39 members, 13 countries, 2013-2018. Budget: ca. 9 M €

Internal **GPS™** qPCR kits Validation to standards of UNE-EN ISO/IEC 17025 and French Standard NF T90-471 (April 2010)



Terms of validation

Specificity of the qPCR:

- **Inclusiveness / Exclusiveness (in silico & in vitro)**

Quantitative PCR phase (calibration and statistical analysis n≥10):

- **Standard curve validation principle** (10^6 to 10 copies).
- **Standard curve evaluation protocol.**
- ***Estimating the regression line (R^2).***
- ***Validation of the linear model.***
- ***Validation of efficiency (yield of pCR).***

Reliability of the analysis:

- **Repeatability.**
- **Reproducibility.**

Sensibility of the method:

- **Limit of detection (LOD)**
- **Limit of quantification (LOQ)**

Parameter	Acceptance criteria	
Specificity	Inclusiveness: positive amplification in all strains within the taxon level.	
	Exclusiveness: Negative amplification in all species upper the taxon level.	
Standard Curve*	$-3{,}587 < a < -3{,}103$	
	$F_{assay} < F_{fisher}$	
	$90\ \% < e < 110\ \%$	
Reliability*	Repeatability	
	CV < 10 %	
	Reproducibility	
	CV < 10 %	
LD* (10 copies)	10 copies	Posit ≥ 90 %
LQ* (10 copies)	10 copies	$t_{value} < t_{Student}$

(*n=10 experiments)

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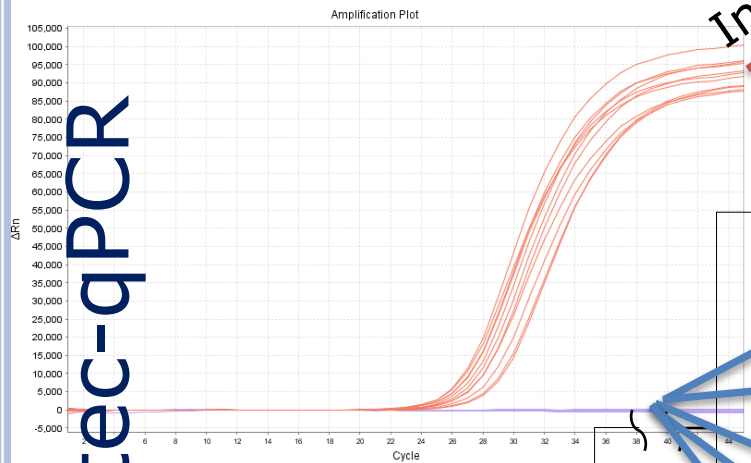
MLPA-Listeria

recA-gyrB-rpoB-atpA-cpn60
3659 bp
unpublished

LisMon dtec-qPCR



Inclusivity

Exclusivity

L.monocytogenes-L130
L.seeligeri-aL15
L.monocytogenes-L103
L.monocytogenes-CECT911
L.monocytogenes-CECT4031T
L.monocytogenes-L49
L.monocytogenes-CECT934
L.monocytogenes-CECT935
L.monocytogenes-CECT936
L.monocytogenes-L56
L.monocytogenes-L69
L.monocytogenes-L114
L.marthii-CECT7970
L52
L.innocua-Linn8
L.innocua-Linn16
L.innocua-CECT5378
L.innocua-CECT910T
L123A
L.innocua-L138
L.innocua-CECT4030
L.innocua-CECT5376
L.welshimeri-CECT5370
L.welshimeri-CECT5380
L.welshimeri-CECT919T
L.welshimeri-CECT5371
L.welshimeri-Lwell6
L.welshimeri-L79
L.welshimeri-Lwell7
L.seeligeri-CECT5339
L.seeligeri-CECT941
L.seeligeri-CECT5342
L.seeligeri-CECT5341
L.seeligeri-CECT939
L.ivanovii-CECT5374T
L.ivanovii-CECT5375
L.ivanovii-CECT5368
L.ivanovii-CECT5373
L.ivanovii-CECT5379
L.ivanovii-CECT913T
L.ivanovii-CECT5369
L.roucourtidae-CECT7972
L.grayi-CECT942
L.grayi-CECT931T
L.grayi-CECT4181

95
100
100
100
100
100
100

0.01
0.02

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Validation results summary

Parameter	*Salmonella enterica*	*Vibrio cholerae*	*Pseudomonas aeruginosa*	*Arcobacter* spp.	Campylobacter coli
Specificity	Strain of *Salmonella enterica* subsp. *enterica* sv. Typhi	4 strains of *Vibrio cholerae*	2 strains of *Pseudomonas aeruginosa*	20 strains from all species of *Arcobacter* spp.	5 strains of *Campylobacter coli.*
	Tested with 7 related genera	5 strains of *Vibrio* spp. and 7 related genera	12 strains of *Pseudomonas* spp., and 7 related genera	8 related genera	13 strains of Campylobacter *jejuni* and 7 related genera.
Standard Curve*	Y = -3.309*X + 38.162 a = -3.309 R^2 = 0.992	Y = -3.306*X + 37.468 a = -3.306 R^2 = 0.997	Y = -3.335*X + 36.584 a = -3.335 R^2 = 0.997	Y = -3.353*X + 38.314 a = -3.353 R^2 = 0.995	Y = -3.508*X + 40.092 a = -3.508 R^2 = 0.995
	F_{assay} = 0.54868 F_{fisher} = 5.31766	F_{assay} = 1.10849 F_{fisher} = 5.31766	F_{assay} = 2.16309 F_{fisher} = 5.31766	F_{assay} = 0.33538 F_{fisher} = 5.31766	F_{assay} = 0.06562 F_{fisher} = 5.31766
	Efficiency (*e*) = 100.56 %	Efficiency (*e*) = 100.65 %	Efficiency (*e*) = 99.45 %	Efficiency (*e*) = 98.74 %	Efficiency (*e*) = 92.76 %
Reliability*	**Repeatability**	**Repeatability**	**Repeatability**	**Repeatability**	**Repeatability**
	Conc. / CV (%)	Conc. / CV (%)	Conc. / CV (%)	Conc. / CV (%)	Conc. / CV (%)
	10^6 copies / 0.40	10^6 copies / 1.52	10^6 copies / 0.88	10^6 copies / 0.44	10^6 copies / 0.30
	10^5 copies / 0.22	10^5 copies / 0.12	10^5 copies / 0.64	10^5 copies / 0.13	10^5 copies / 0.42
	10^4 copies / 0.10	10^4 copies / 1.04	10^4 copies / 0.60	10^4 copies / 0.31	10^4 copies / 0.07
	10^3 copies / 0.44	10^3 copies / 1.15	10^3 copies / 0.38	10^3 copies / 0.54	10^3 copies / 0.24
	10^2 copies / 0.76	10^2 copies / 0.47	10^2 copies / 0.11	10^2 copies / 0.61	10^2 copies / 1.75
	10^1 copies / 3.76	10^1 copies / 1.67	10^1 copies / 2.53	10^1 copies / 2.07	10^1 copies / 1.65
	5 copies / 3.63	5 copies / 3.22	5 copies / 2.63	5 copies / 3.24	5 copies / 1.39
	Reproducibility	**Reproducibility**	**Reproducibility**	**Reproducibility**	**Reproducibility**
	Conc. / CV (%)	Conc. / CV (%)	Conc. / CV (%)	Conc. / CV (%)	Conc. / CV (%)
	10^6 copies / 1.92	10^6 copies / 2.85	10^6 copies / 0.90	10^6 copies / 0.92	10^6 copies / 2.85
	10^5 copies / 1.69	10^5 copies / 1.67	10^5 copies / 0.89	10^5 copies / 1.31	10^5 copies / 2.19
	10^4 copies / 1.83	10^4 copies / 2.18	10^4 copies / 1.17	10^4 copies / 1.39	10^4 copies / 2.21
	10^3 copies / 2.35	10^3 copies / 2.70	10^3 copies / 0.49	10^3 copies / 1.16	10^3 copies / 2.41
	10^2 copies / 1.99	10^2 copies / 2.39	10^2 copies / 0.45	10^2 copies / 1.05	10^2 copies / 3.58
	10^1 copies / 0.54	10^1 copies / 3.66	10^1 copies / 0.56	10^1 copies / 2.38	10^1 copies / 3.86
	5 copies / 1.04	5 copies / 2.98	5 copies / 0.77	5 copies / 0.52	5 copies / 5.22
LD* (10 copies)	Posit. = 10 (100 %)	Posit. = 10 (100 %)	Posit. = 10 (100 %)	Posit. = 10 (100 %)	Posit. = 10 (100 %)
LQ* (10 copies)	*t* value = 0.050 $t_{student}$ = 2.262	*t* value = 0.790 $t_{student}$ = 2.262	*t* value = 0.074 $t_{student}$ = 2.262	*t* value = 0.072 $t_{student}$ = 2.262	*t* value = 0.13753 $t_{student}$ = 2.26216

Overview about validation of real-time PCR kits for bacterial pathogens. *: (n=10), number of assays to determine each parameter

Innovations by **GPS™**

MONODOSE GPS™ dtec-qPCR

single qPCR Tests for **specific pathogen detection**.
Ready-to-use and dehydrated

Just add your sample

NO ice on transport
NO Rupture of the enzyme by freezing/thawing
NO Risks of cross-contamination
NO Fluorophore deterioration by UV light
NO Time consuming

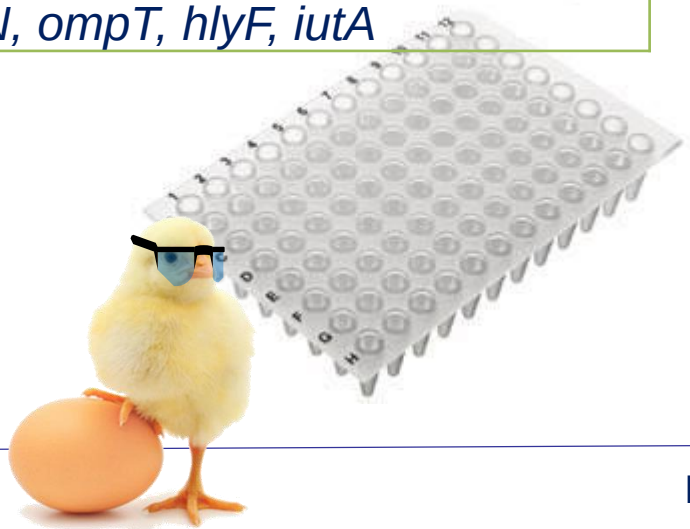
Innovations by **GPS™**

qPCR **GPS™** kits: *Same qPCR protocol*

"qPCR panels"
(avoids multiplexing)

Escherichia coli Virulence Genes
dtec-qPCR Panel-11

iucC, tsh, cvi, iss, fimC, fyuA, iroN, ompT, hlyF, iutA



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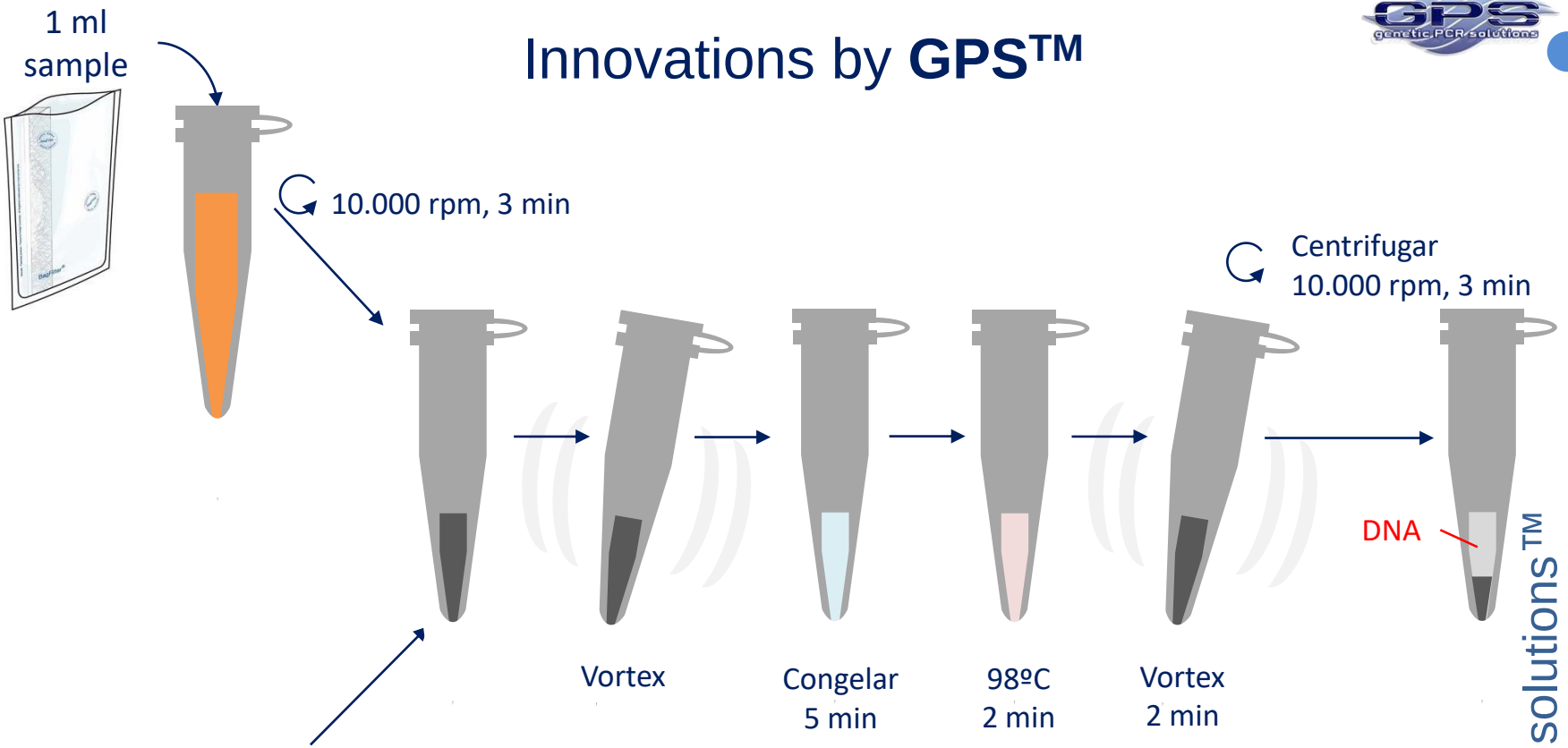
Innovations by **GPS™**



Protocol completed < 15 minutes

FastExt®

GPS™ Fast DNA extraction buffer

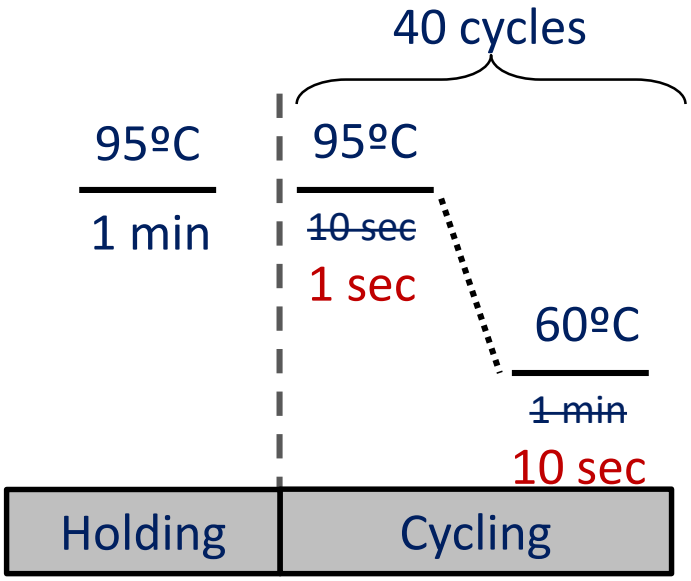
genetic PCR solutions™

www.geneticpcr.com

FastCycling protocol

Validated on **MONODOSE format**
Foodborne poisoning Panel-4
(SalSpp, LisMon, Stx1, Stx2)
and ECO157

Protocol	Reaction time	Xxpress time	StepOne time
GPS™ standard	53 m	62 m	1 h 28 m
GPS™ **Fast-Cycling**	**8 m**	**17 m**	**45 m**



Validación **GPS™** interna de kits para qPCR según la norma internacional

UNE-EN ISO/IEC 17025

Términos de validación

Las validaciones se han realizado siguiendo las directrices de la norma internacional UNE-EN ISO 17025. Diversos aspectos de los ensayos se basaron en las normativas UNE-EN ISO 6579:2002 (*Salmonella*), UNE-EN ISO 11290-1:2004 (*Listeria monocytogenes*) y UNE-EN ISO 16654 (*E.coli* O157). Para cuestiones relacionadas con la PCR se consultó la UNE-EN ISO 22118:2011.

Especificidad de la qPCR:

-Inclusividad / Exclusividad (in silico & in vitro).

Fase cuantitativa de la PCR (calibración y análisis estadístico n≥10):

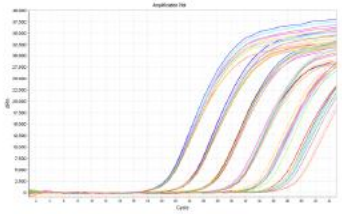
-Principio de validación de la curva estándar (10^6 a 10 copias).
-Protocolo de evaluación de la curva estándar.
-Estimación de la regresión lineal (R^2).
-Validación del modelo lineal.
-Validación de la eficiencia (rendimiento de la PCR).

Confiabilidad del análisis:

-Repetibilidad.
-Reproducibilidad.

Sensibilidad del método:

-Límite de detección (LOD) .
-Límite de cuantificación (LOQ).



genetic PCR solutions™

by GENETIC ANALYSIS STRATEGIES S.L.
CEEI Elche, Pol. Ind. Carrús Ronda Vall d'Uxó, 125 03206-Elche (Alicante)
☎ *Phone: +34-965429901* *Fax: +34-966661040*
Web: www.geneticpcr.com ✉ *e-mail: info@geneticpcr.com*

genetic PCR solutions™

One-day Food testing

Detección de patógenos de alimentos en un día

Genetic PCR Solutions ™

www.geneticpcr.com

Validation of qPCR detection

225 ml Peptone (*Salmonella enterica, E.coli*)
225 ml Fraser (*Listeria monocytogenes*)

		Chicken				Spinach			
	Target	8 h	12 h	16 h	18 h	8h	12 h	16 h	18 h
1-10 UFC	SalSpp	27	18	17	17	29	22	22	21
	LisMon	Nd	32	27	23	Nd	36	32	30
	Stx1	25	14	13	15	27	14	14	13
	Stx2	23	14	13	14	24	14	14	13
	eae	22	13	12	14	23	13	13	13

Average Ct values for n experiments.
Nd, non detected

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Volume reduction of Fraser medium

Listeria monocytogenes qPCR

spinach samples

< 10 cels

LisMon dtec-qPCR	12 h	16 h
225 ml Fraser	29.5	24.8
180 ml Fraser	28.8	25.3
135 ml Fraser	27.3	22.0

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