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Deutscher Akademischer Austausch Dienst
German Academic Exchange Service

6S RNA IN LACTIC ACID BACTERIA

Philipps



Universität
Marburg

Pablo Cataldo

Bled, February 2019



**PhD Internal Scholarship 2016- 2021
CONICET- ARGENTINA**

**Supervisors: Dr. Elvira Hébert
Dr. Lucila Saavedra
(CERELA-CONICET)**



**Short- term Grant (09/2018- 02/2019)
DAAD - GERMANY**

**Supervisor: Dr. Marcus Lechner
Department of Pharmaceutical Chemistry
Philipps-Universität Marburg- Germany**

LACTIC ACID BACTERIA - RELEVANT FEATURES

LACTIC ACID BACTERIA

GRAS

Coccus and rod-shaped

High nutritional demands

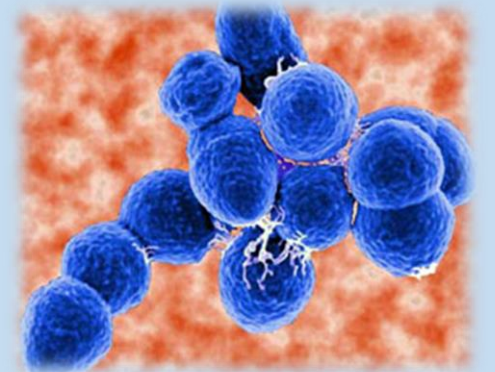
Gram (+)

Strictly fermentative metabolism

Catalase (-)

Non sporulating

Aerotolerant



LACTIC ACID BACTERIA - RELEVANT FEATURES

Properties and technological applications

Fermented food and beverages

- *Lactococcus lactis*
- *Lactobacillus delbrueckii*
- *Streptococcus thermophilus*
- *Lactobacillus paracasei*
- *Lactobacillus rhamnosus*



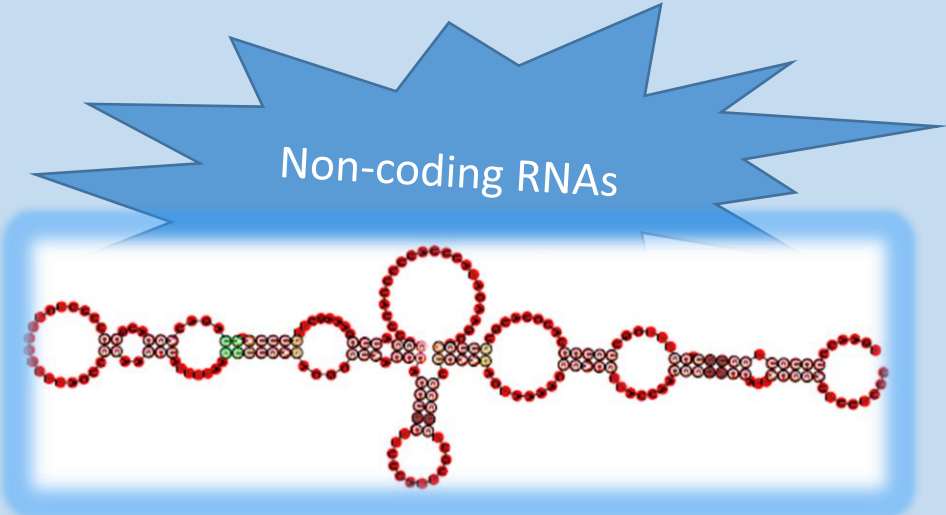
Opportunistic pathogens

- *Abiotrophia defectiva*
- *Aerococcus sanguinicola*
- *Streptococcus pneumoniae*
- *Enterococcus gallinarum*





Stress resistance



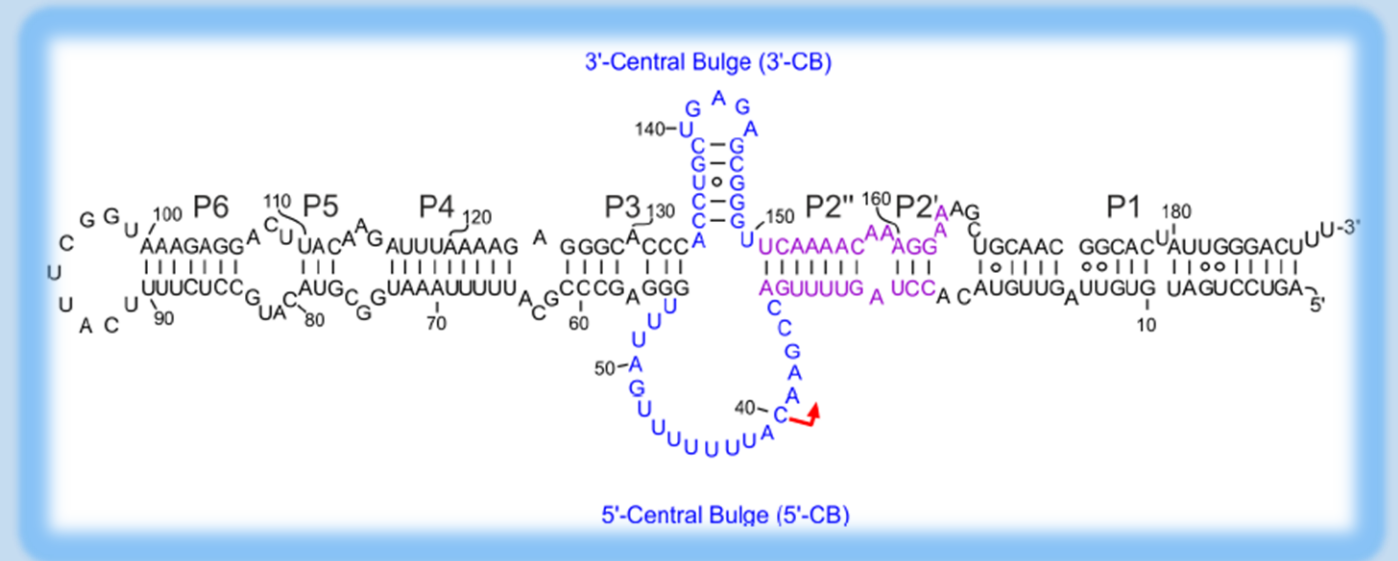
Main objective

Identification and characterization of 6S RNAs and pRNAs in LAB species

6S RNA

Main characteristics and mechanism

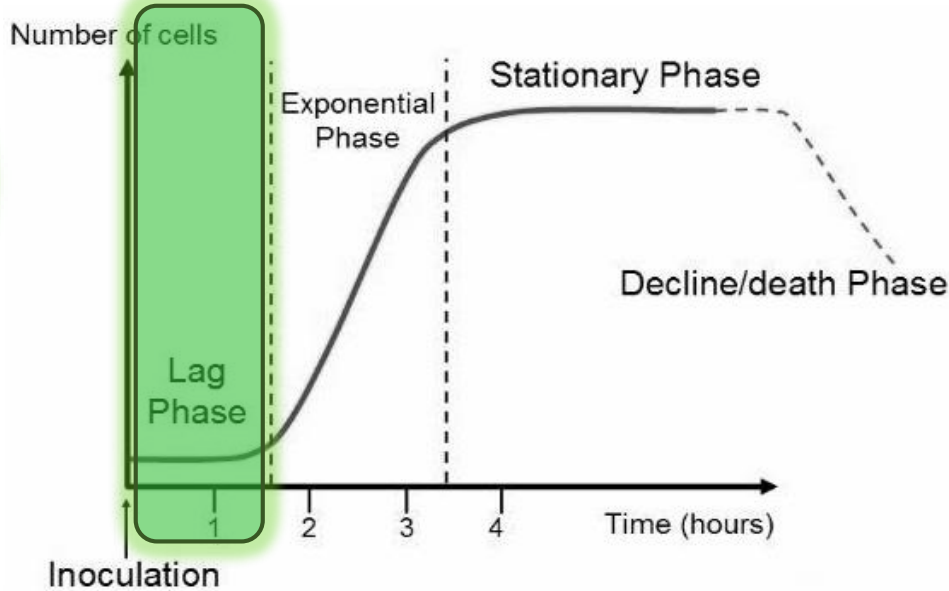
- Small regulatory non-coding RNA
- 160-200 nt in length
- Adopts a rod-shaped structure with a large internal loop flanked by largely helical arms on both sides
- Model organisms: *Escherichia coli* – *Bacillus subtilis*



(Beckmann et al., 2012).

6S RNA

Main characteristics and mechanism



RNAP



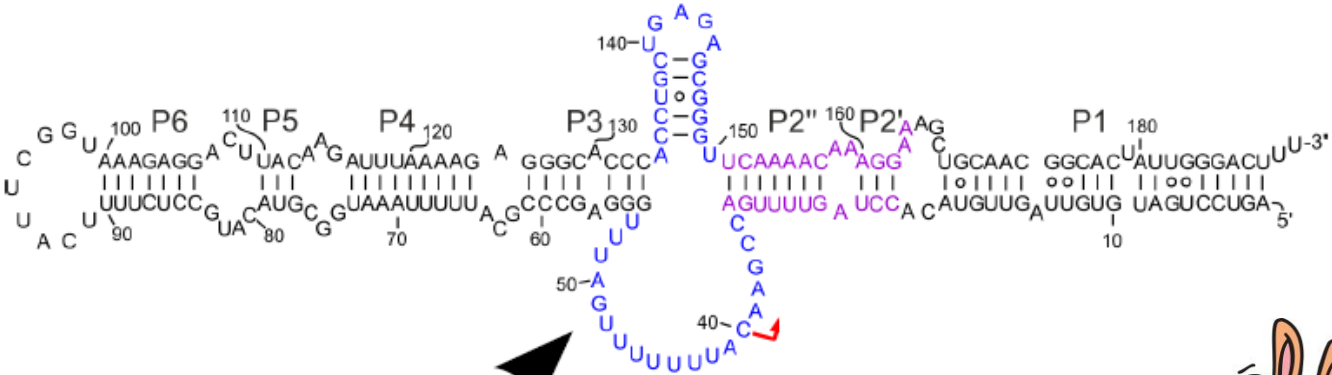
promoter

(non-)coding region

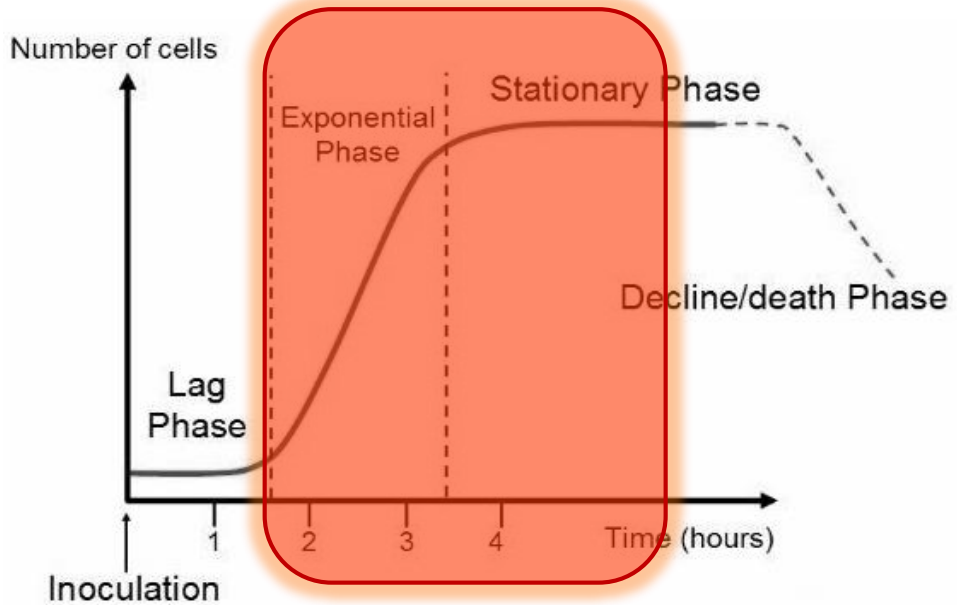
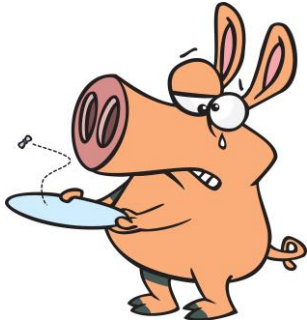
Main catabolic pathways

6S RNA

Main characteristics and mechanism



RNAP



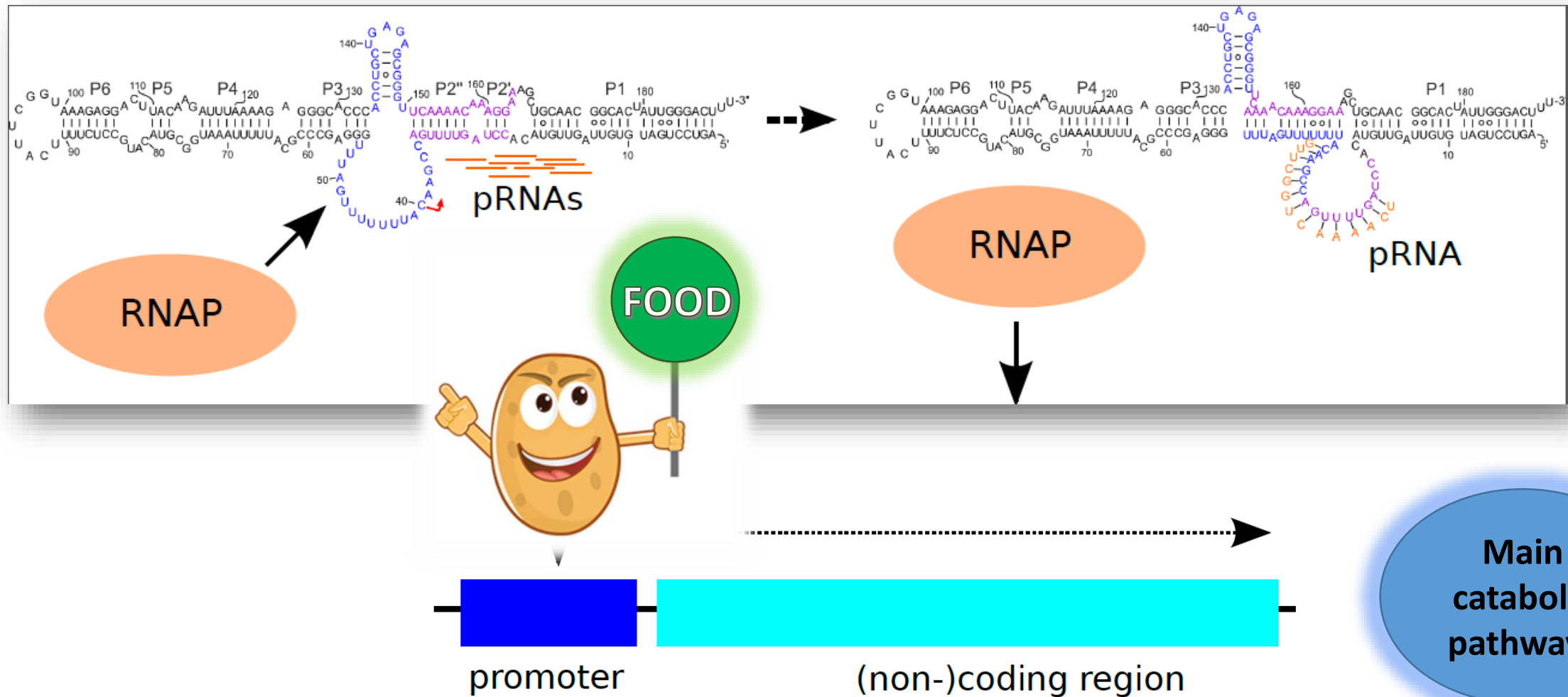
promoter

(non-)coding region

Altered housekeeping transcription


6S RNA

Main characteristics and mechanism: OUTGROWTH




LACTIC ACID BACTERIA AND 6S RNA


What is known so far?

  **JOURNALS**
investing in science

FEMS Microbiology Reviews, fux028, 41, 2017, S220-S243
doi: 10.1093/femsre/fux028
Review Article

REVIEW ARTICLE
The Evolution of gene regulation research in *Lactococcus lactis*
Jan Kok*, Lieke A. van Gijtenbeek, Anne de Jong, Sjoerd B. van der Meulen, Ana Solopova and Oscar P. Kuipers

 ORIGINAL RESEARCH
published: 14 September 2017
doi: 10.3389/fmicb.2017.01704



Early Transcriptome Response of *Lactococcus lactis* to Environmental Stresses Reveals Differentially Expressed Small Regulatory RNAs and tRNAs

Sjoerd B. van der Meulen^{1,2}, Anne de Jong^{1,2} and Jan Kok^{1,2*}

¹Department of Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, Netherlands; ²Top Institute Food and Nutrition, Wageningen, Netherlands

Published online 25 January 2011

Nucleic Acids Research, 2011, Vol. 39, No. 7 e46
doi:10.1093/nar/gkr012

A simple and efficient method to search for selected primary transcripts: non-coding and antisense RNAs in the human pathogen *Enterococcus faecalis*

Aymeric Fouquier d'Héroue^{1,2}, Françoise Wessner¹, David Halpern¹, Joseph Ly-Vu¹, Sean P. Kennedy¹, Pascale Serror¹, Erik Aurell² and Francis Repoila^{1,*}

¹INRA, UMR1319 Micalis, Domaine de Vilvert, F-78352 Jouy-en-Josas, France and ²Department of Computational Biology, Royal Institute of Technology, AlbaNova University Center, SE-10691 Stockholm, Sweden

Received October 4, 2010; Revised December 30, 2010; Accepted January 4, 2011

RNA BIOLOGY
2016, VOL. 13, NO. 3, 353-366
<http://dx.doi.org/10.1080/15476286.2016.1146855>

 Taylor & Francis
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RESEARCH PAPER

 OPEN ACCESS

Transcriptome landscape of *Lactococcus lactis* reveals many novel RNAs including a small regulatory RNA involved in carbon uptake and metabolism

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^aDepartment of Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands; ^bTop Institute Food and Nutrition (TIFN), Wageningen, The Netherlands

GENOMES SELECTION

Lactobacillales order

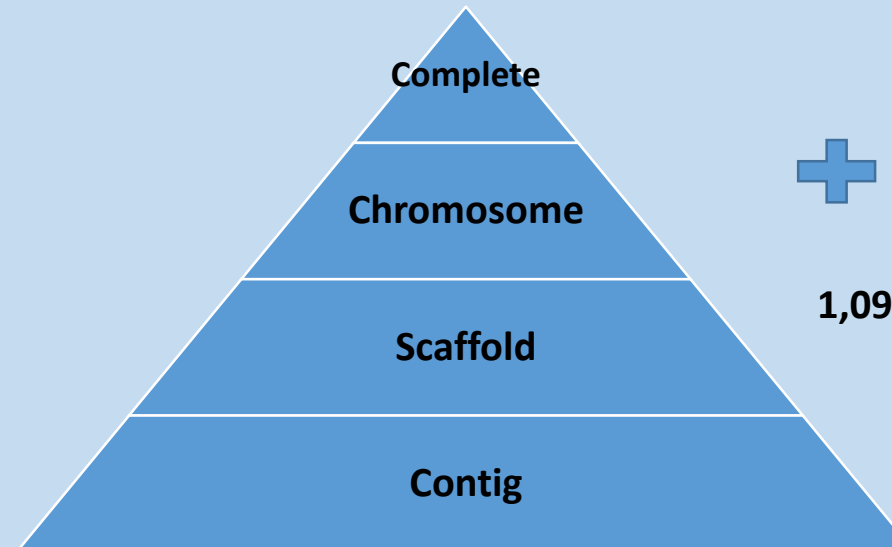
6 Families

Family	Genus	stains / available
Aerococcaceae	<i>Abiotrophia</i>	1 / 2
	<i>Aerococcus</i>	8 / 61
	<i>Dolosicoccus</i>	2 / 3
	<i>Eremococcus</i>	1 / 2
	<i>Facklamia</i>	3 / 9
	<i>Globicatella</i>	1 / 4
Carnobacteriaceae	<i>Agitococcus</i>	1 / 1
	<i>Alkalibacterium</i>	1 / 8
	<i>Allofustis</i>	1 / 1
	<i>Atopobacter</i>	1 / 1
	<i>Atopococcus</i>	1 / 1
	<i>Carnobacterium</i>	9 / 41
	<i>Dolosigranulum</i>	10 / 12
	<i>Granulicatella</i>	1 / 7
	<i>Jeotgalibaca</i>	1 / 4
	<i>Lacticigenium</i>	1 / 1
	<i>Marinilactibacillus</i>	1 / 5
<i>Trichococcus</i>	7 / 15	
Enterococcaceae	<i>Bavariicoccus</i>	1 / 1
	<i>Enterococcus</i>	114 / 2105
	<i>Melissococcus</i>	2 / 14
	<i>Tetragenococcus</i>	5 / 19
Lactobacillaceae	<i>Lactobacillus</i>	460 / 1680
	<i>Pediococcus</i>	25 / 61
	<i>Sharpea</i>	1 / 4
Leuconostocaceae	<i>Convivina</i>	1 / 1
	<i>Fructobacillus</i>	5 / 9
	<i>Leuconostoc</i>	23 / 118
	<i>Oenococcus</i>	3 / 208
	<i>Vagococcus</i>	4 / 6
Streptococcaceae	<i>Weissella</i>	23 / 43
	<i>Floricoccus</i>	2 / 2
	<i>Lactococcus</i>	44 / 168
	<i>Streptococcus</i>	328 / 12076

Genera with potential pathogenic species

Genera with technological and health positive features

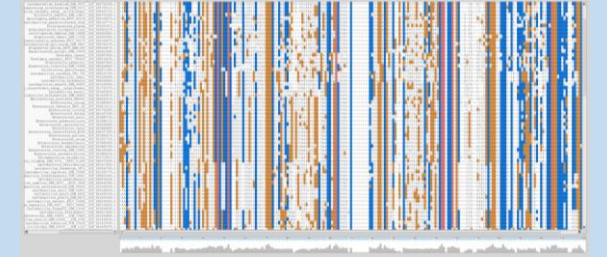
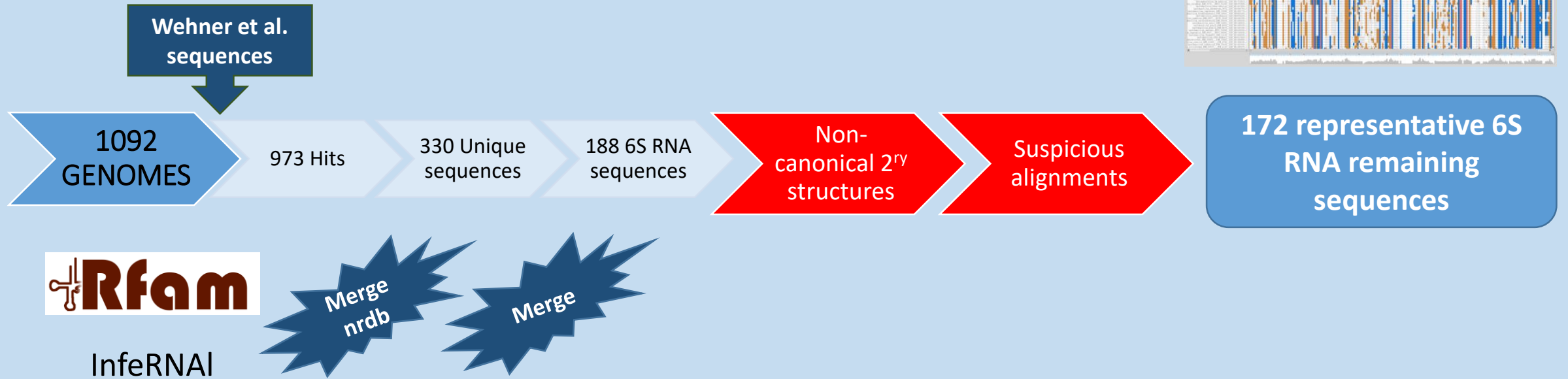
“The most complete genome” criteria



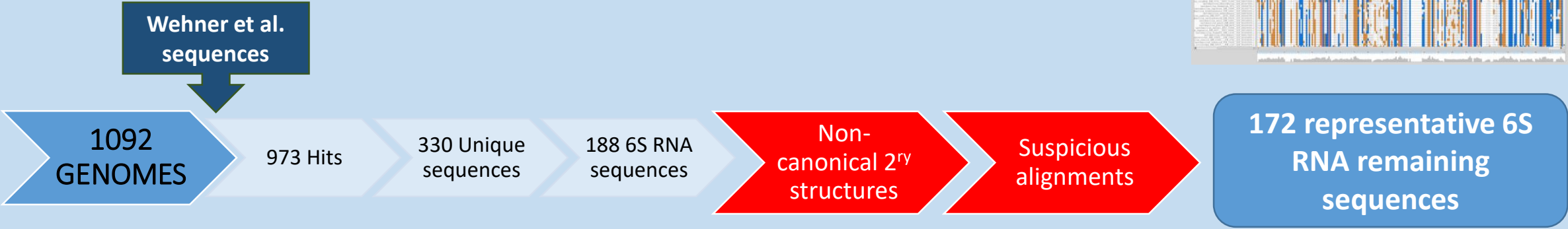
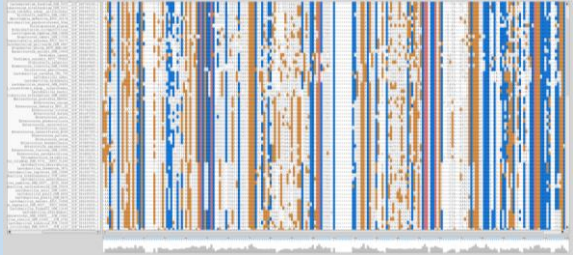
+ 13 strains (CERELA-CONICET)

1,092 genomes were considered in this study

6S RNA alignment + Clustering



6S RNA alignment + Clustering



InfeRNAI

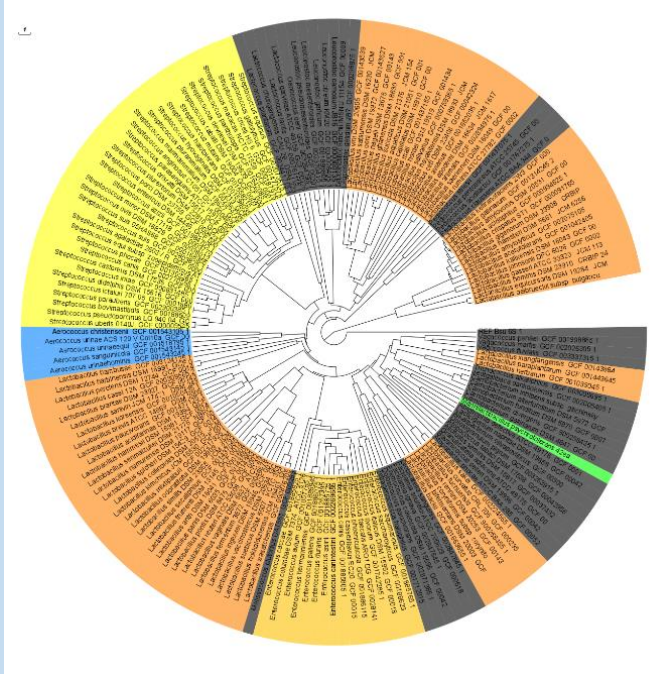


RNAclust

Representative 6S RNA sequences

Outgroup:
- 6S-1 from *Bacillus subtilis* 168

LocARNA
Simultaneously folded and aligned (or match) RNAs based on their sequence and structure features.



Wehner, S., Damm, K., Hartmann, R. K., & Marz, M. (2014). Dissemination of 6S RNA among bacteria. *RNA biology*, 11(11), 1467-78.

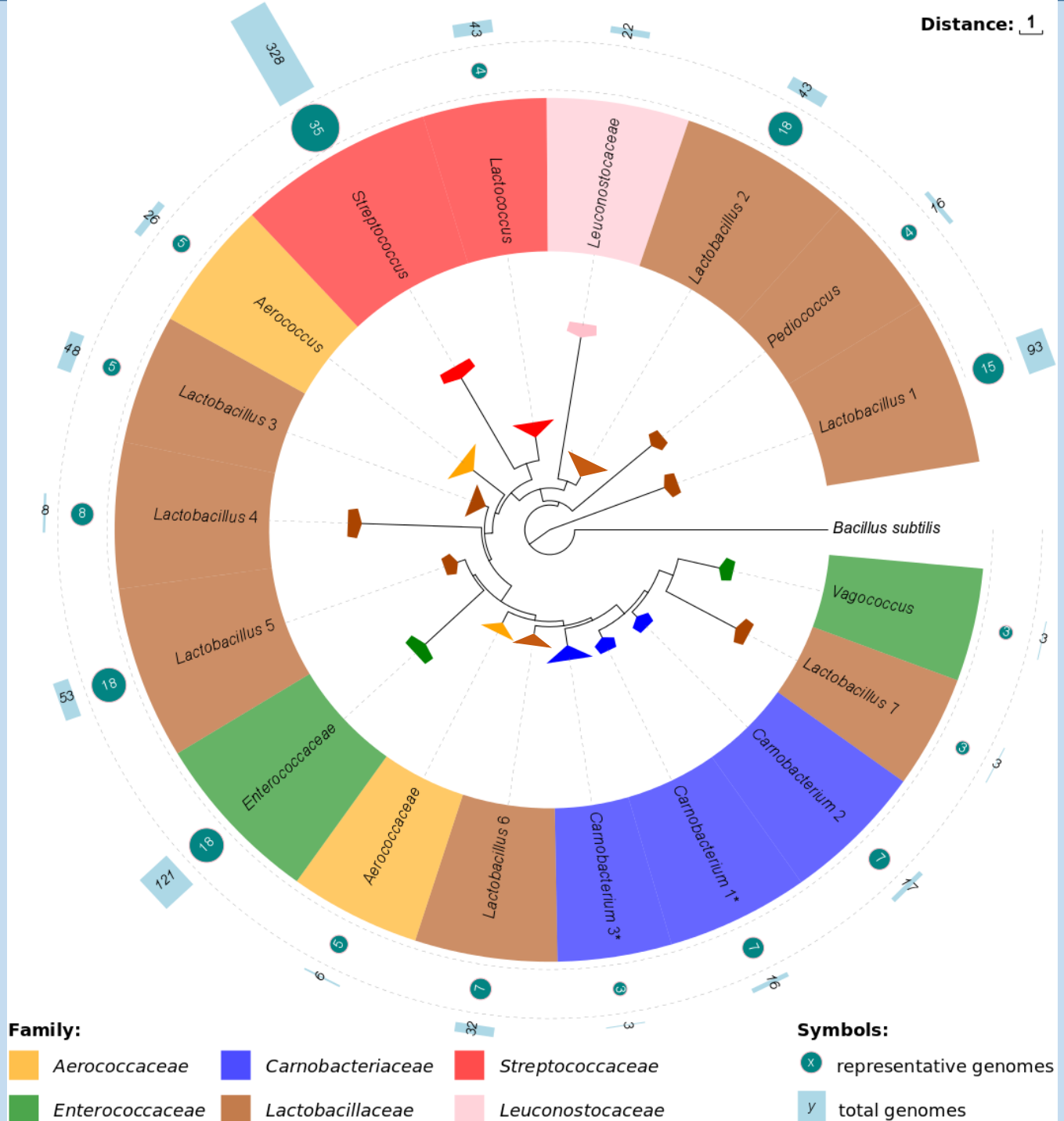
Distance: 1

6S RNA -

Weisella

Fructobacillus

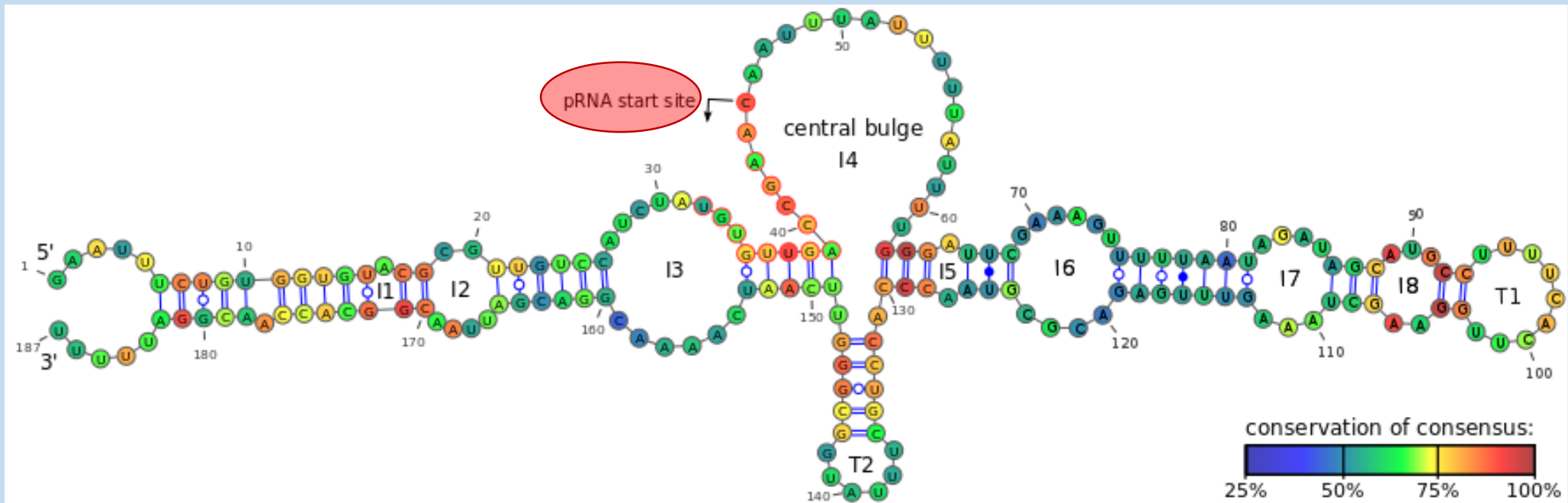
Lactobacillus



Genetic heterogeneity within the *Lactobacillaceae* family

6S RNA secondary structure analysis

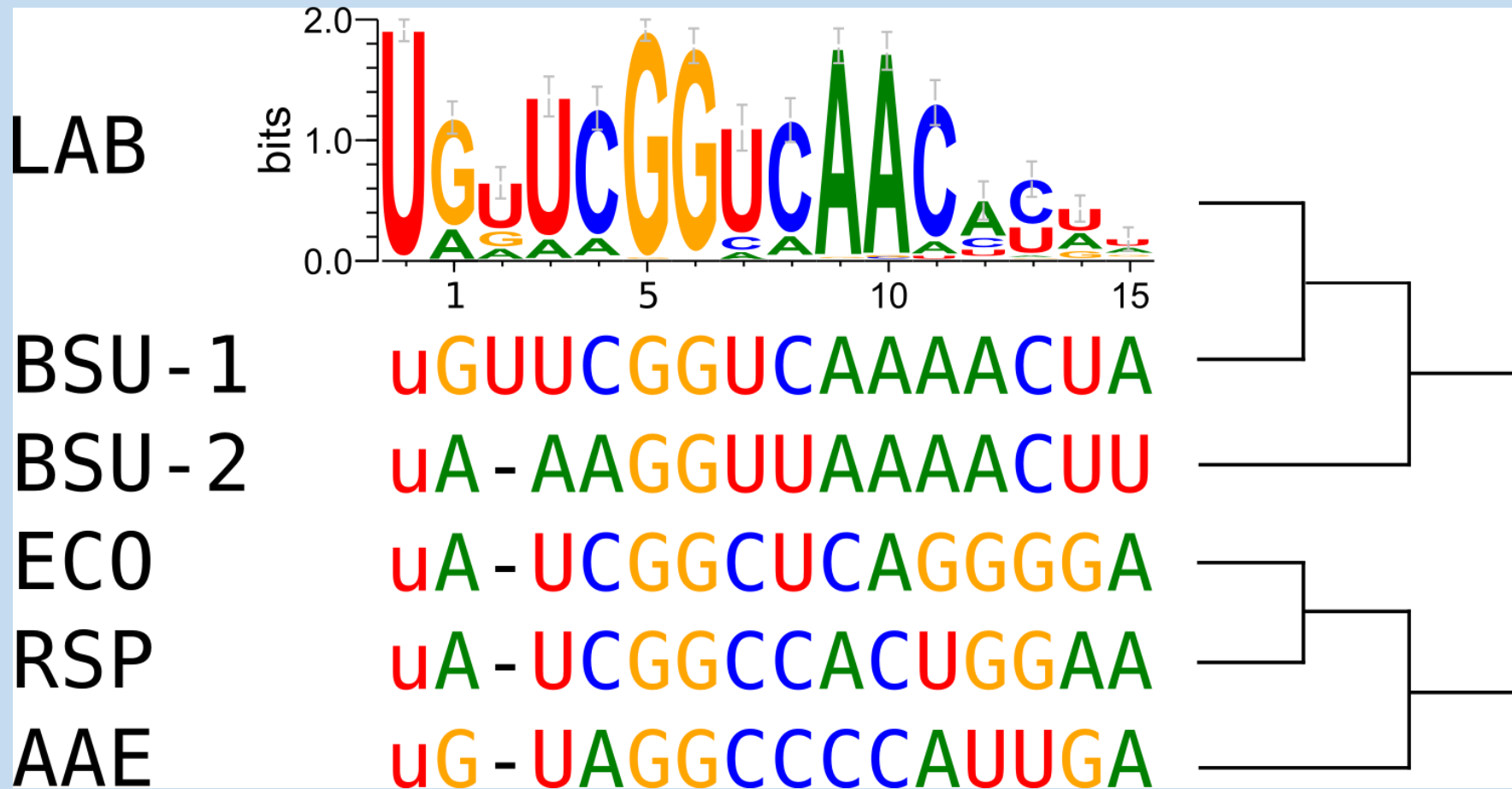
6S RNA Consensus secondary structure for LAB (VARNA)



Canonical secondary structure

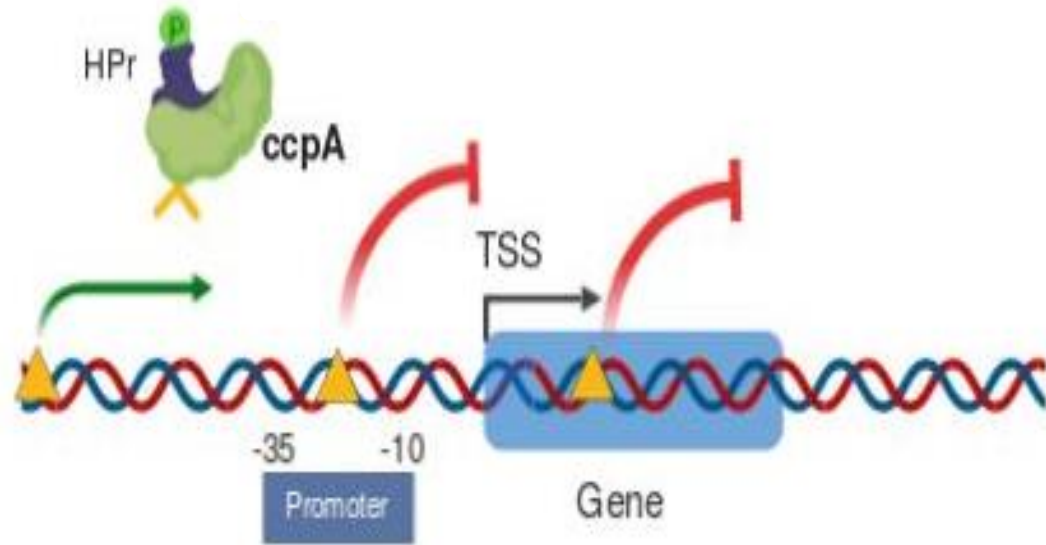
Well conserved central region

pRNAs prediction and analysis



pRNA Motif = New criteria for 6S RNA detection and function?

CRE Sites



- Degenerate pseudo-palindromes
- Conserved in low GC % Gram (+)
- Are recognized by ccpA

Key role in C catabolite repression

RNA BIOLOGY
2016, VOL. 13, NO. 3, 353–366
<http://dx.doi.org/10.1080/15476286.2016.1146855>



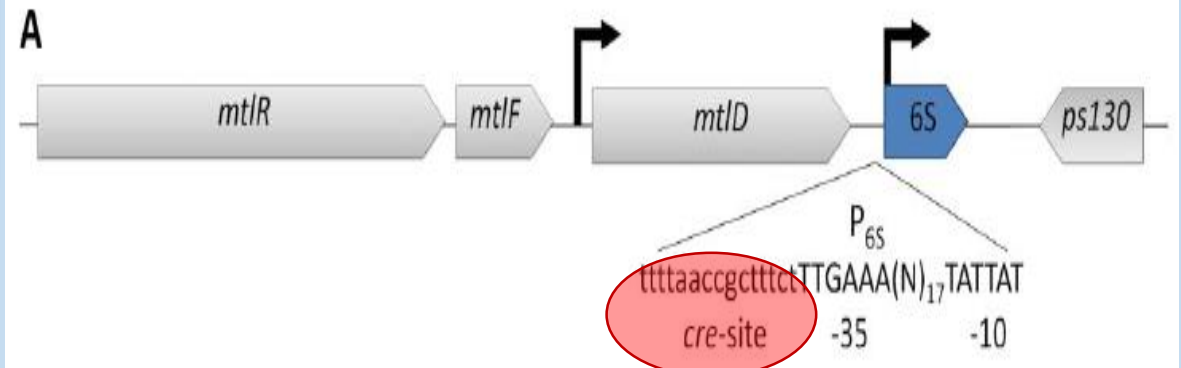
RESEARCH PAPER

OPEN ACCESS

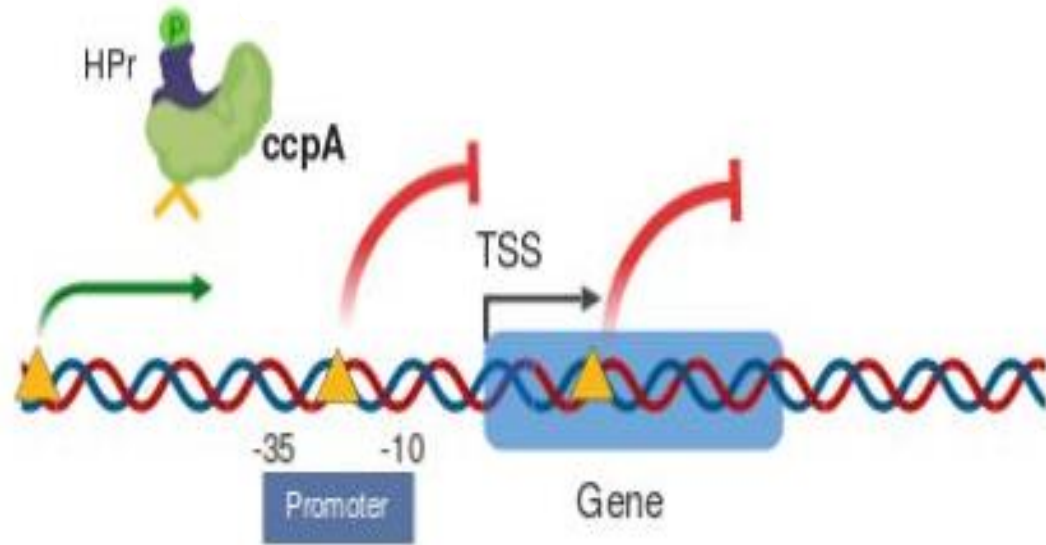
Transcriptome landscape of *Lactococcus lactis* reveals many novel RNAs including a small regulatory RNA involved in carbon uptake and metabolism

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^aDepartment of Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands; ^bTop Institute Food and Nutrition (TIFN), Wageningen, The Netherlands



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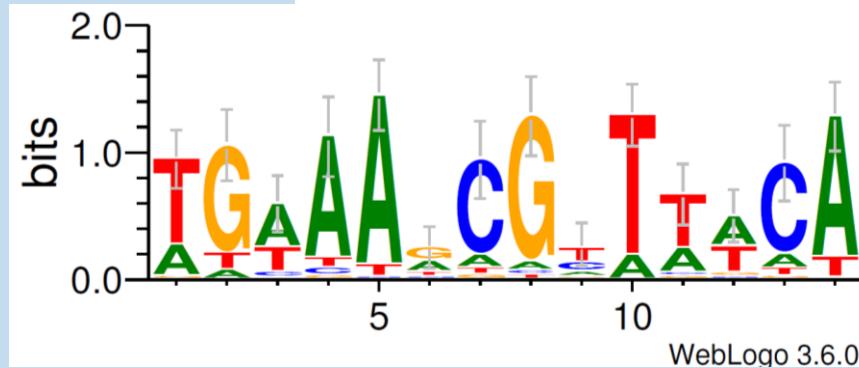
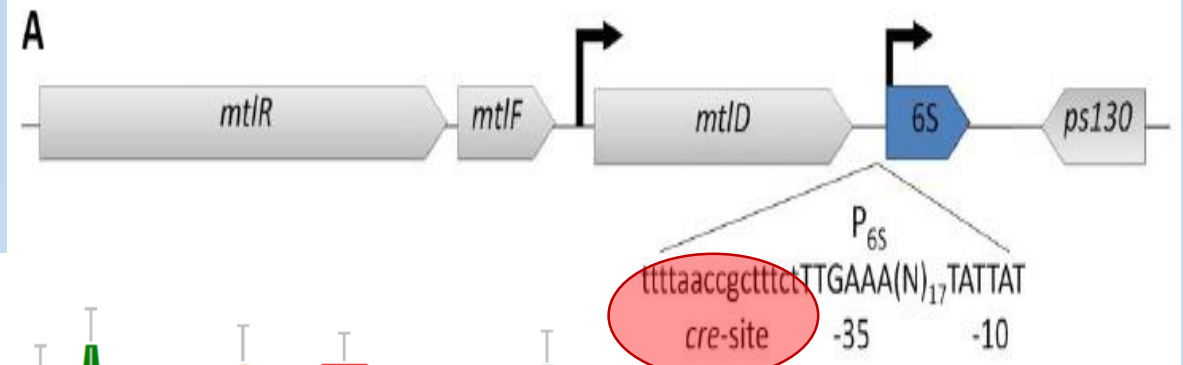
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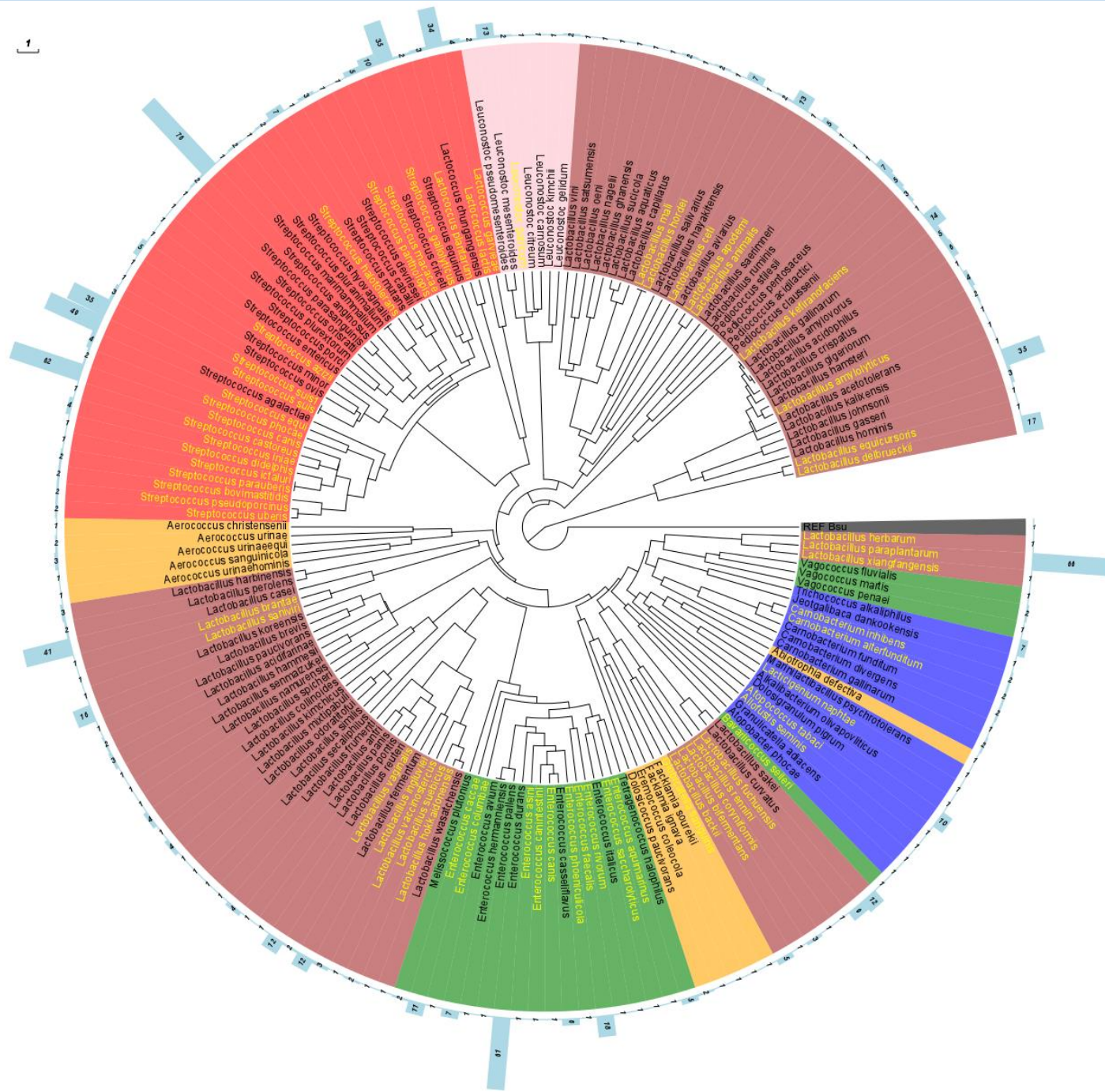
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CRE Sites analysis

200 nt upstream
6S RNA + 6S RNA

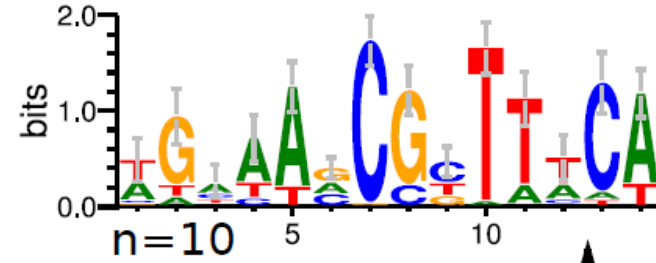
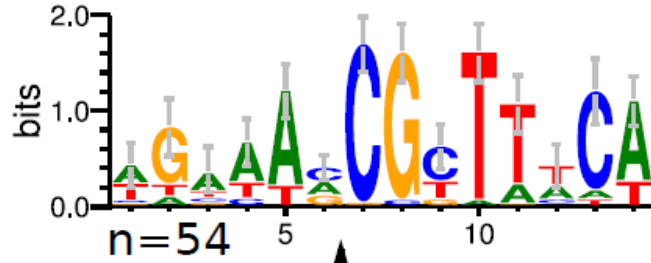


Present in all 6
LAB families

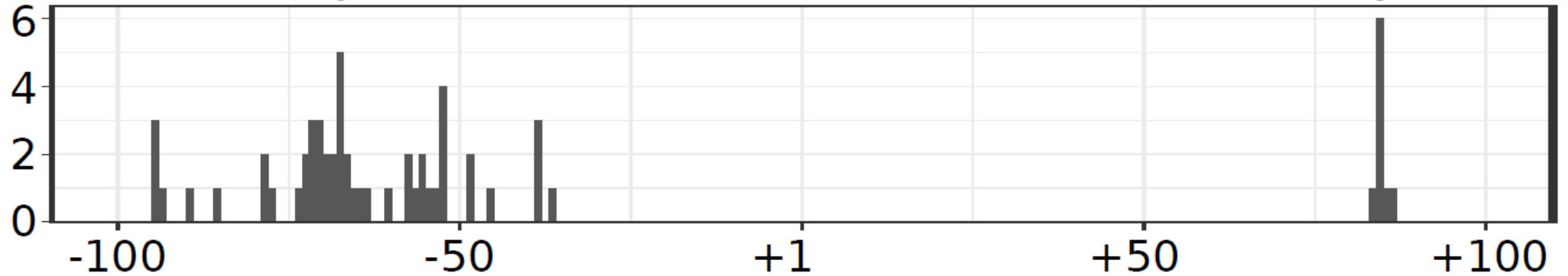
CRE Sites analysis

Putative
activator role

Putative
repressor role?



genomes

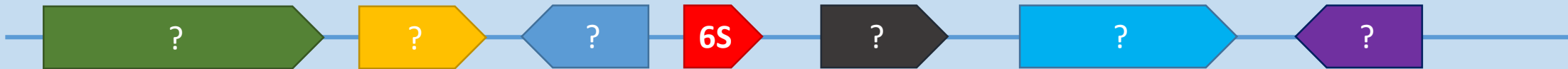


usp

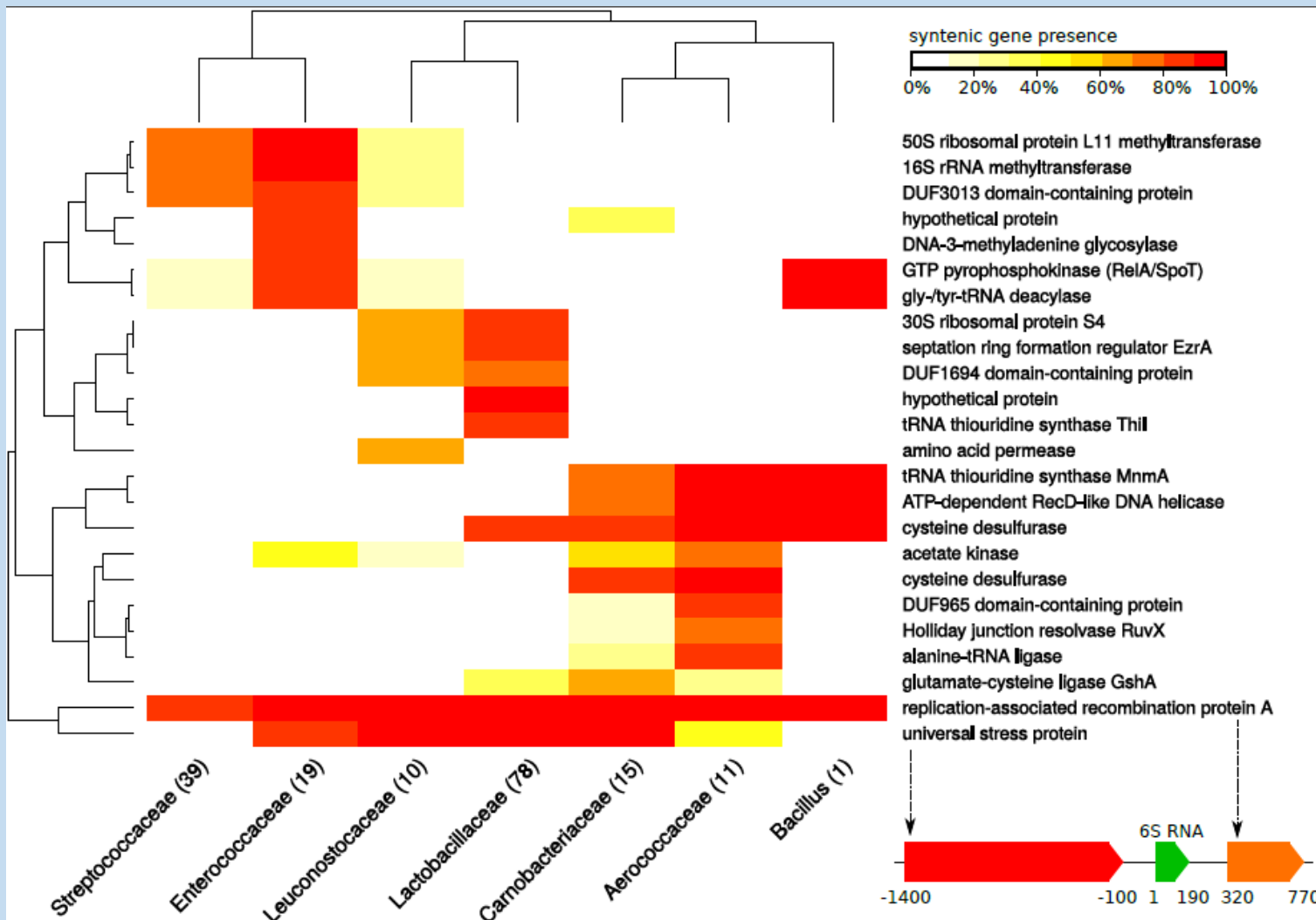
6S RNA

position of the strongest cre-site

Synteny Analysis



Proteinortho

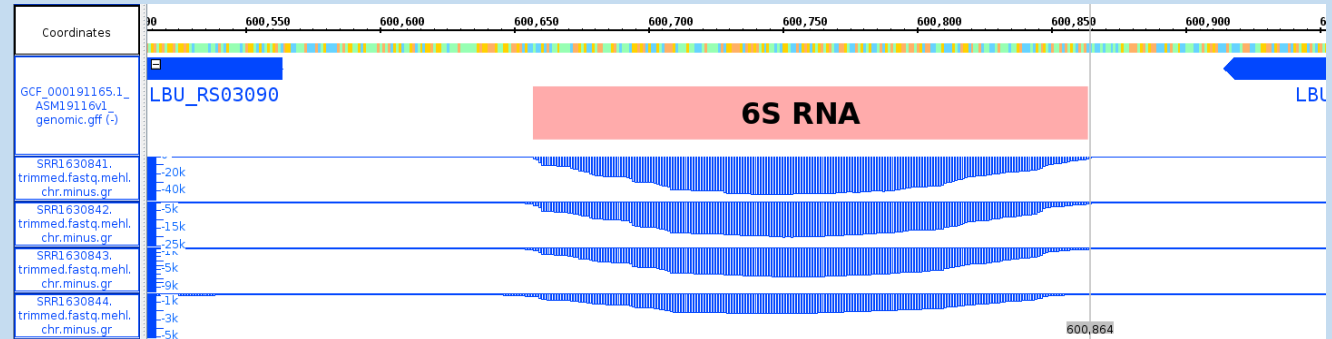


- Universal stress protein
- Replication-associated recombination protein A

EXPERIMENTAL EVIDENCE

RNA-Seq data NCBI

Microorganism	Annotated	Overlapping CDS	Genomic coordinates	Expression
Lactobacillus delbrueckii subsp. bulgaricus ATCC 11842	NO	NO	608737-608909	3K
Lactobacillus delbrueckii subsp. bulgaricus 2038	NO	NO	600657- 600860	45-50 K
Lactobacillus delbrueckii subsp. bulgaricus STCC BAA-365	NO	NO	602886- 603062	15-20 K
Lactobacillus paracasei L9	NO	YES	1303993- 1304183	5-20 K
Lactobacillus salivarius UCC118	NO	NO	1104567- 1104719	200- 300 k
Lactobacillus rossiae DSM 15814	NO	NO	-	-
Oenococcus oeni AWRI551/2/419	NO	NO	1227746-1227906	20-40 k
Lactobacillus plantarum LY- 78	YES	NO	3020579-3020748	1-3 k
Lactobacillus salivarius Ren	YES	NO	1062337-1062523	1-1,5 k
Lactobacillus salivarius UCC118	NO	NO	1104567-1104719	200- 300 k
Lactobacillus gasserii ATCC 33323 = JCM 1131	NO	NO	1232586-1232770	9 k
Lactobacillus gasserii JV-V03	NO	NO	1232583-1232738	9-10 k
Lactobacillus reuteri SD2112	NO	NO	1454619-1454801	9-35 k
Lactobacillus rhamnosus JCM1553	NO	NO	1138692-1138877	10-35 k
Lactobacillus helveticus CNRZ32	NO	NO	898359-898553	35-40 k
Lactobacillus crispatus ST1	NO	NO	778792-778973	110-120 k
Lactobacillus amylovorus GRL118	NO	NO	764895-764077	80-90 k
Lactobacillus acidophilus NCFM	NO	NO	767003-767196	50-60 k
Lactobacillus sakei 23K	NO	NO	824318-824492	38-55 k
Lactococcus lactis subsp. lactis G423	YES	NO	34144-34331	4-5 k
Lactococcus lactis subsp. cremoris NCDO712	NO	YES	33764-33918	15-75 k
Lactococcus lactis subsp. cremoris NZ9000	NO	YES	32196-32395	5-300 k
Enterococcus faecium	YES	NO	2.363.352-2.363.525	50-9 k
Enterococcus faecalis ATCC29212	YES	NO	956568-956747	800-1200 k
Streptococcus mutans UA159	NO	NO	1929963-1930135	1-7 k
Streptococcus anginosus J4211	NO	NO	282932-283097	2-4,5 k
Oenococcus oeni AWRI551/2/419	NO	NO	1227746-1227906	20-40 k
Pediococcus pentosaceus SD-2015	NO	NO	1292009-1292180	2K k

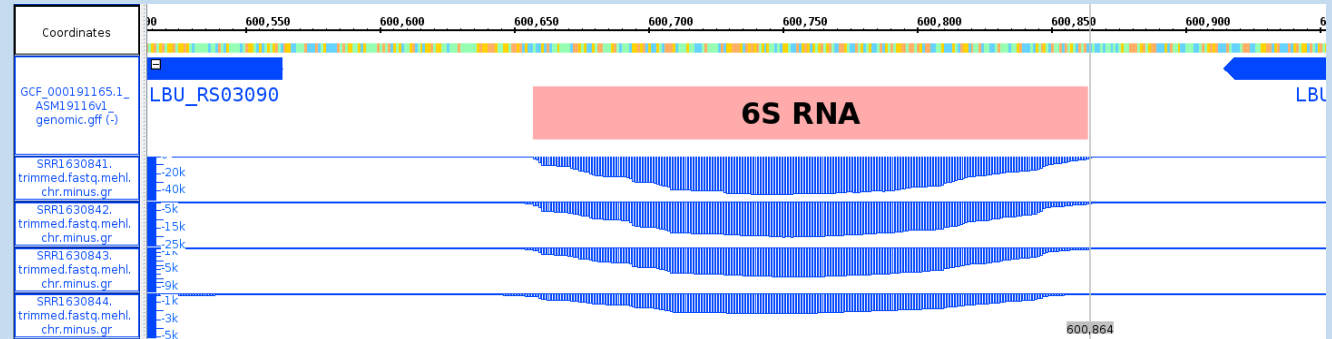


28 Data sets
22 LAB species

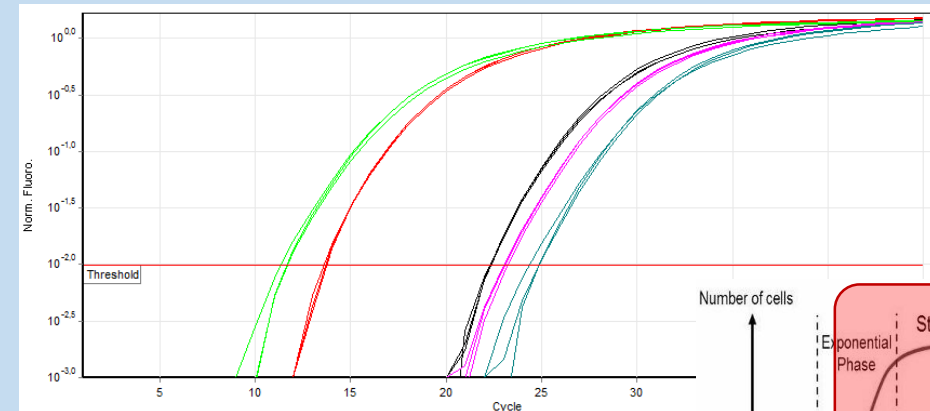
EXPERIMENTAL EVIDENCE

RNA-Seq data NCBI

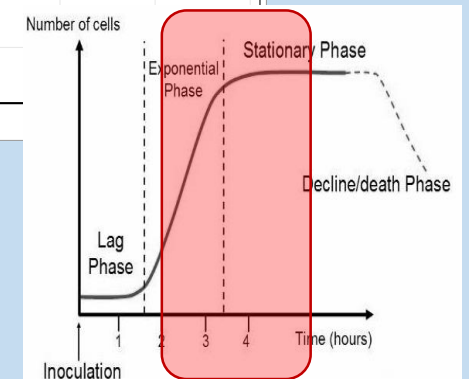
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Lactobacillus reuteri SD2112	NO	NO	1454619-1454801	9-35 k
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Real-time qPCR

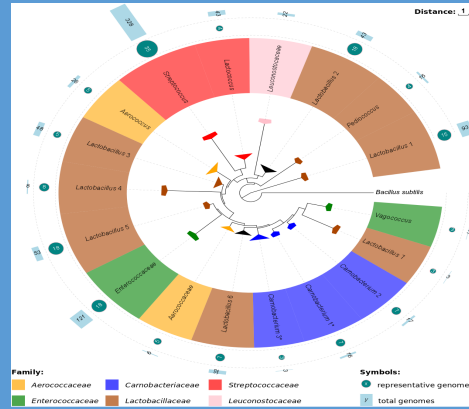


Lactobacillus brevis CRL 2013

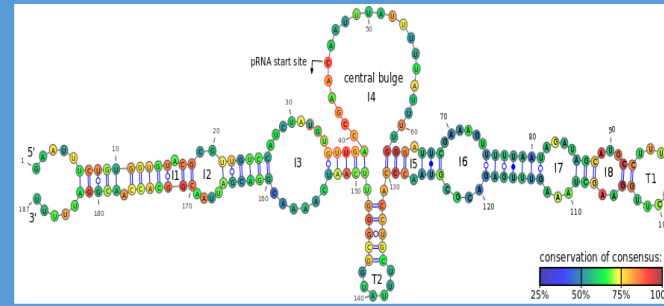


CONCLUSIONS

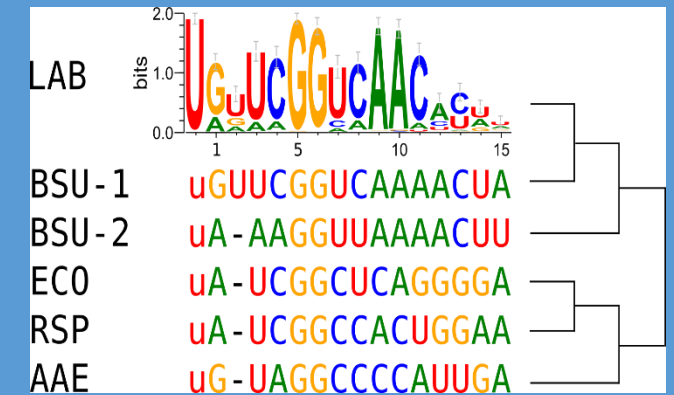
Distribution



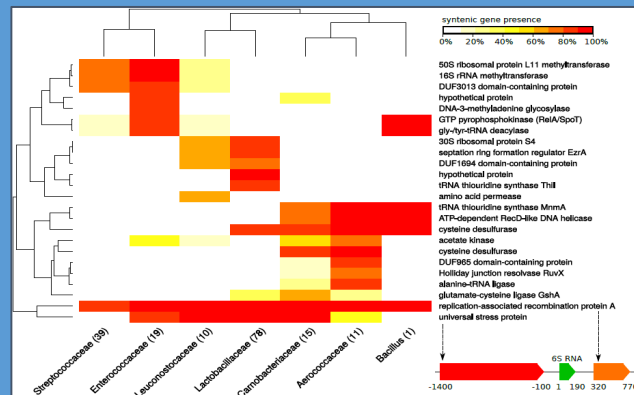
Canonical structure



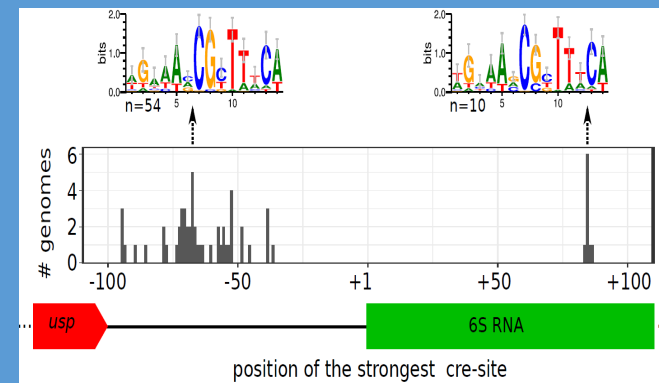
Conserved pRNA



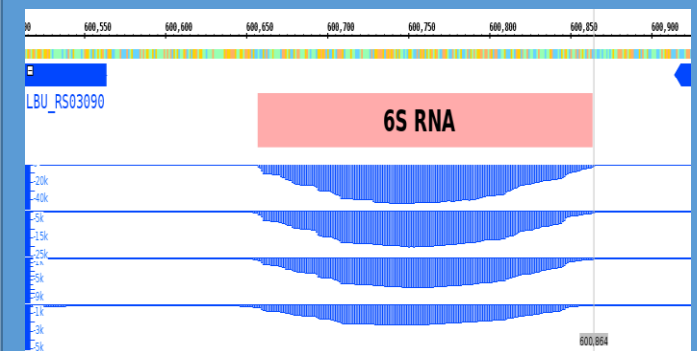
Synteny



cre sites



Experimental



Acknowledgements



- Dr. Marcus Lechner
- Marietta Thüring
- Paul Klemm

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Deutscher Akademischer Austausch Dienst
German Academic Exchange Service

C E R E L A



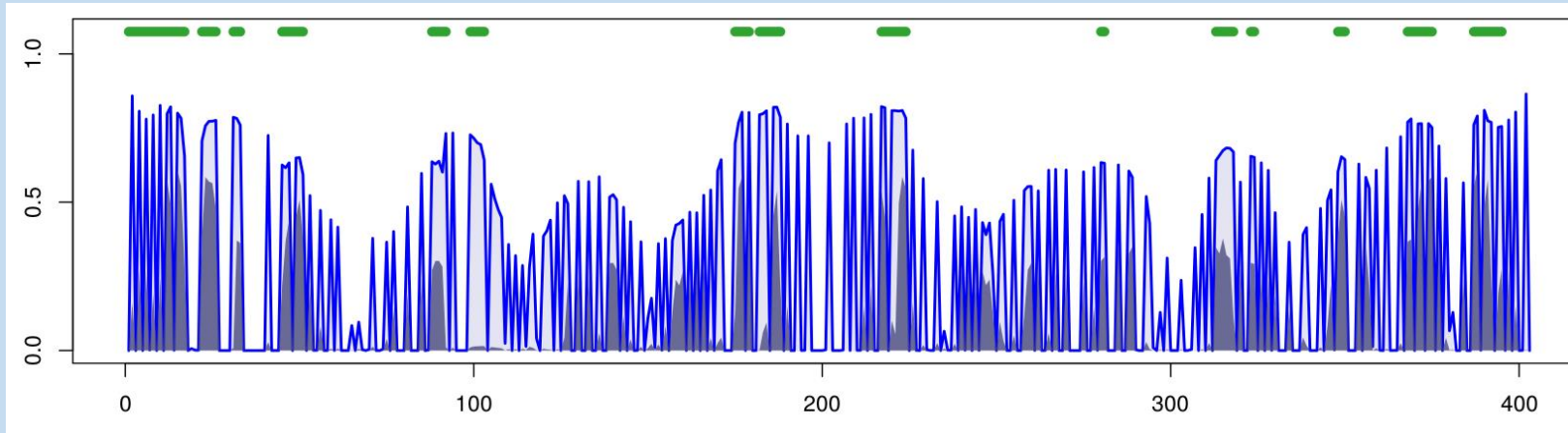
C O N I C E T

Thank you for your attention

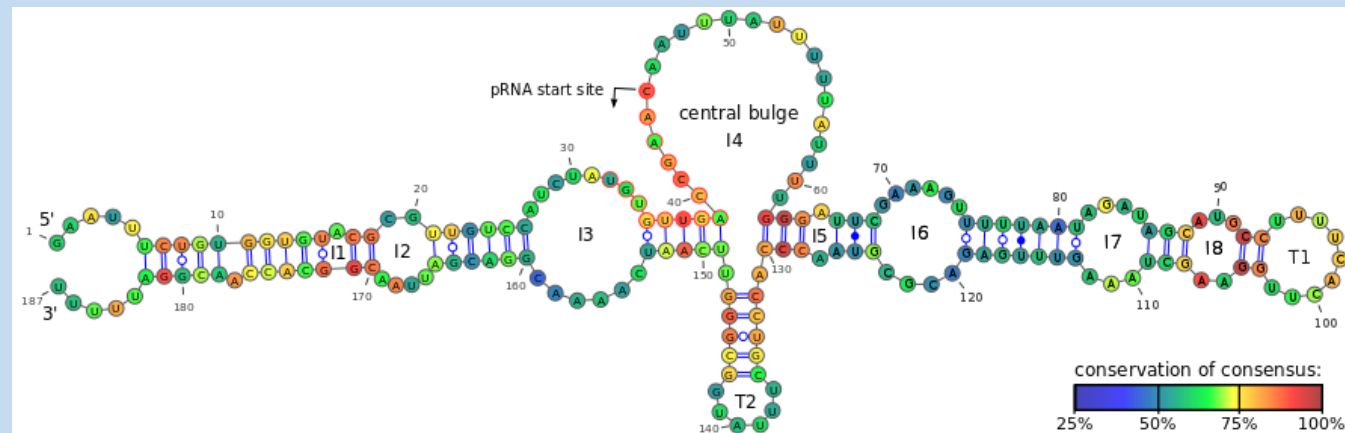
6S RNA in lactic acid bacteria

6S RNA secondary structure analysis

Reliability plot



6S RNA Consensus secondary structure for LAB (VARNA)



6S RNA in lactic acid bacteria

pRNAs prediction

