

## CARTOGRAPHY OF METHICILLIN-RESISTANT *S. AUREUS* TRANSCRIPTS: DETECTION, ORIENTATION AND TEMPORAL EXPRESSION DURING GROWTH-PHASE AND IN STRESS CONDITIONS

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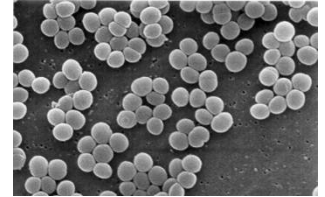
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## Methicillin-resistant *Staphylococcus aureus* (MRSA) :

- Gram-positive bacterium
- Responsible for a wide spectrum of infections (localized to life-threatening diseases)

—> **Prototype of a nosocomial pathogen**

- Approximately 30% of healthy carriers (*S. aureus*) : Archer *et al.*, 1998
- Spreading of methicillin-resistant *S. aureus* (MRSA) : Boyce *et al.*, 1992
- Outbreaks in the community : Jones *et al.*, 2002



—> **Particular capacity to survive and adapt to drastic environmental changes**



—> **Important plasticity**

—> **Precise regulation of gene expression**

RNAs are recognized as major regulators of gene expression (Toledo-Arana *et al.*, 2005)

➔ **Key role of small RNAs (sRNAs)**

Small RNAs = heterogeneous group of molecules that act by various mechanisms to modulate a wide range of physiological responses

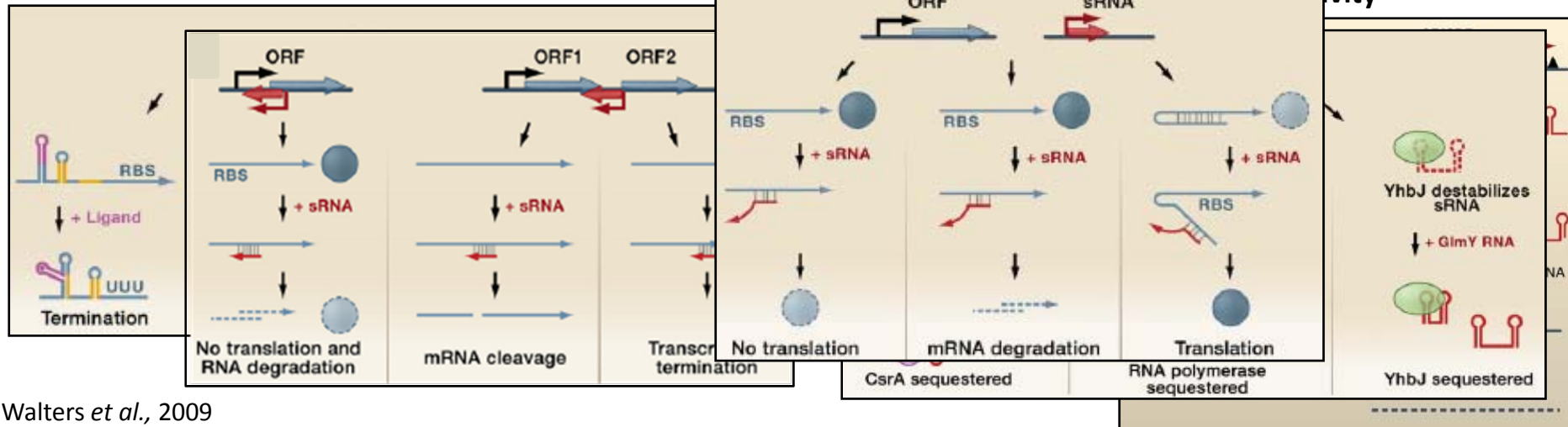
Riboswitches  
Cis-acting regulator

Trans-encoded base pairing

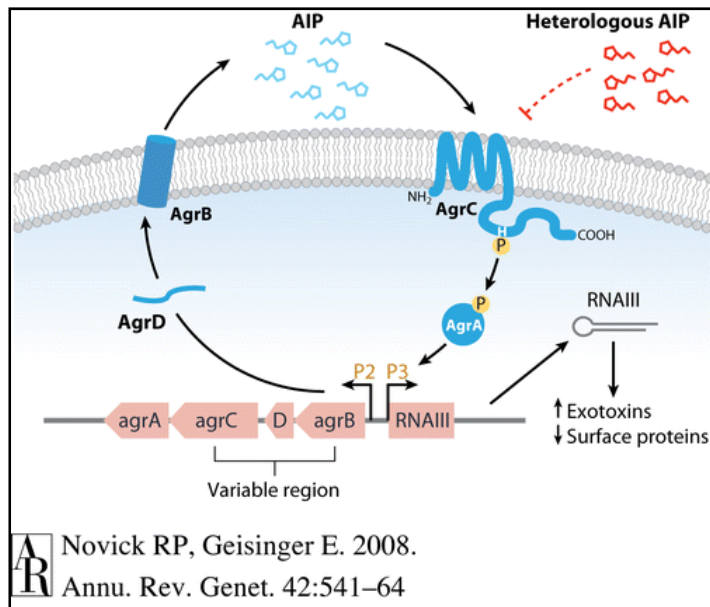
CRISPR and STAR

*Staphylococcus aureus* repeats

Cis-encoded base pairing

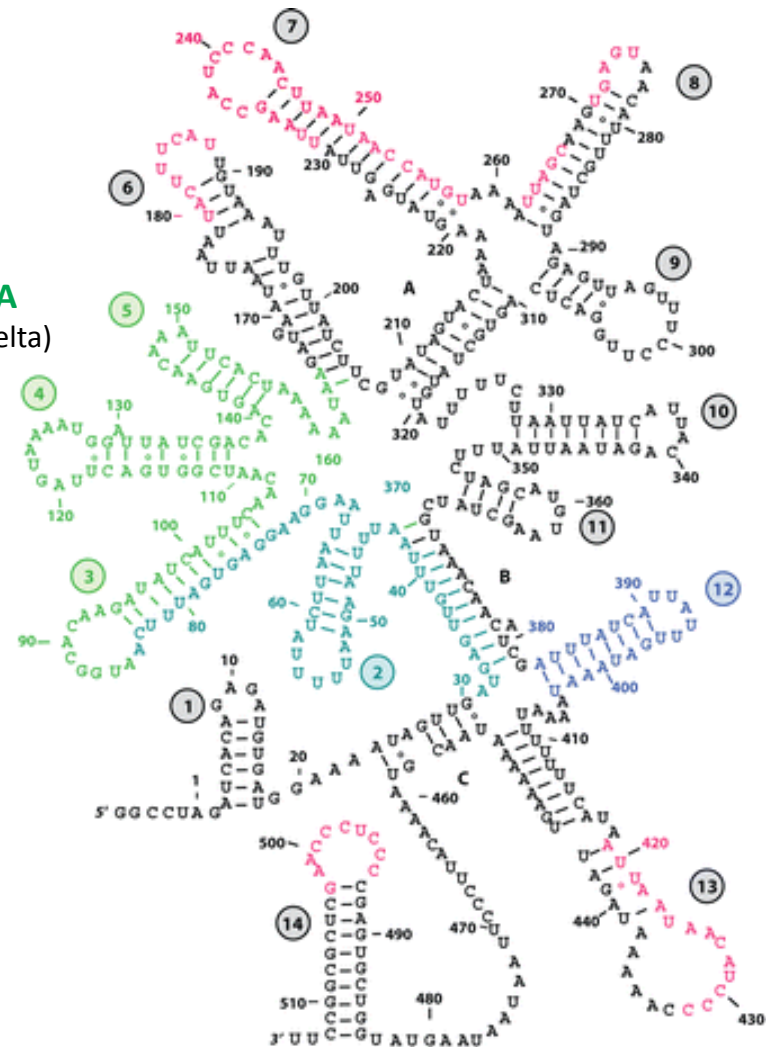


**RNAIII example** : A link between quorum-sensing and virulence



Interaction with SD sequence (*spa*, *rot*, SA1000)

**hld mRNA**  
(hemolysin delta)



Antisense activator *hla* mRNA translation

## sRNAs and *Staphylococcus aureus* : State of the art

➤ RNAIII and virulence – Novick *et al.* (1993)

Experimental discovery

➤ 6 non coding RNAs from pathogenicity islands – Pichon & Felden (2005)

Bioinformatic predictions and experimental confirmation

➤ Approximately 126 stable RNAs respond to SOS, heat and cold shocks, and stringent conditions - Roberts *et al.* & Anderson *et al.* (2006)

Affymetrix array

➤ 7 novels small RNAs and riboswitches - Marchais *et al.* (2009)

Phylogenetic analysis and experimental confirmation

➤ 11 small non coding RNAs - Geissmann *et al.* (2009)

Bioinformatic predictions and experimental confirmation

➤ 142 small non-protein-coding RNAs – Abu-Qatouseh *et al.* (2010)

Sequencing

**Identification of differentially expressed small non-protein-coding RNAs in *Staphylococcus aureus* displaying both the normal and the small-colony variant phenotype**

Luay F. Abu-Qatouseh • Suresh V. Chinni • Jochen Seggewiß • Richard A. Proctor • Jürgen Brosius • Timofey S. Rozhdestvensky • Georg Peters • Christof von Eiff • Karsten Becker

## sRNAs and *Staphylococcus aureus* : Aims of the study

**Link between genomic content & epidemicity / virulence / adaptability**

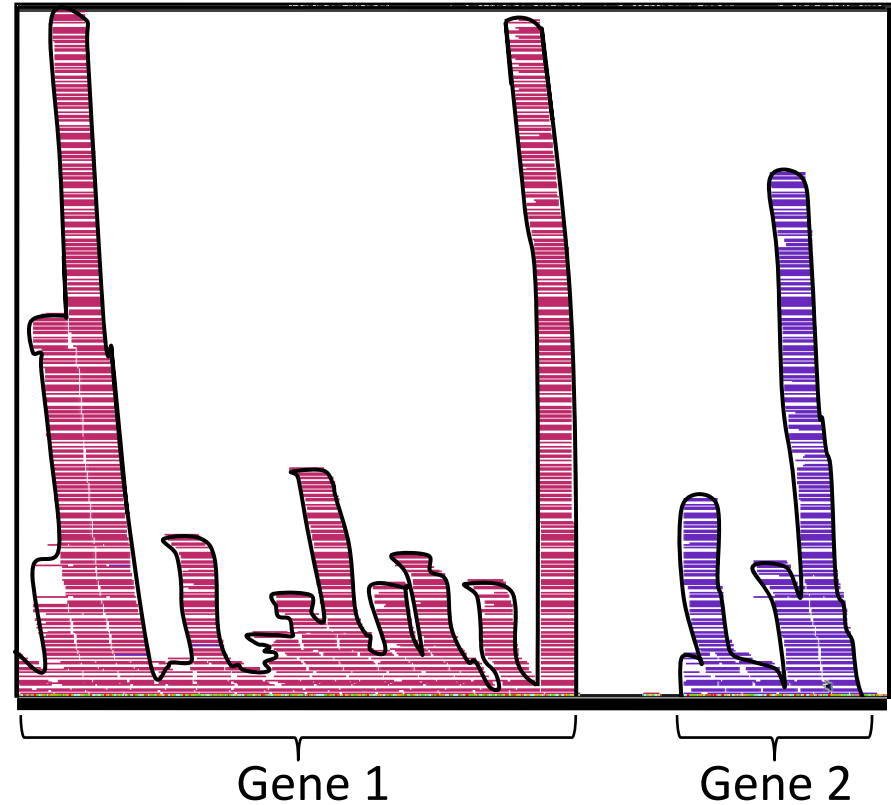
**Identify transcriptomic features responsible for the success of *S. aureus* in epidemiological and clinical settings**

↳ **Investigation by transcriptomic analysis at the genome scale**



## Procedure:

1. Culture of *S. aureus* N315
2. RNA extraction and purification
3. High-throughput sequencing (Illumina)
4. Sequenced read mapping to annotated genome



For 1 gene :



Numerous reads overlapped

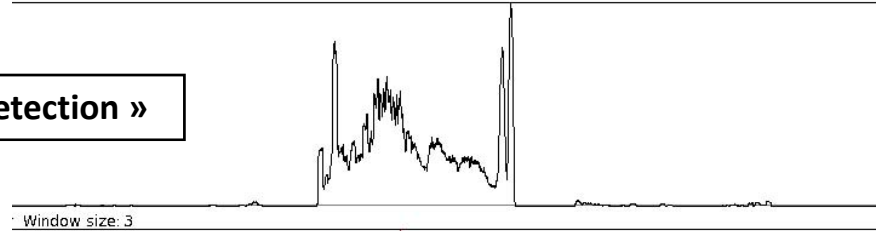


1 signal corresponds to sequencing coverage

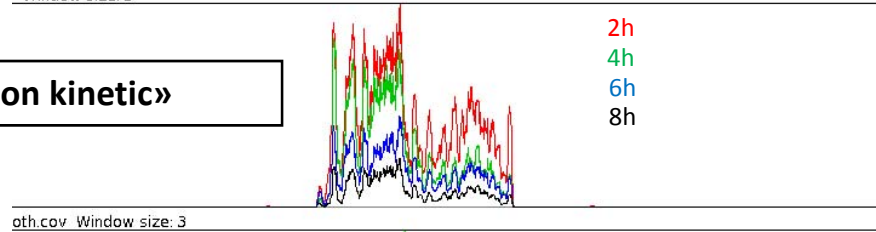
# Transcriptomic by high-throughput sequencing (HTS)

Artemis Entry Edit: NC\_002745\_marie.gbk  
Create Run Graph Display  
771..2699854 = complement (114963..115046)

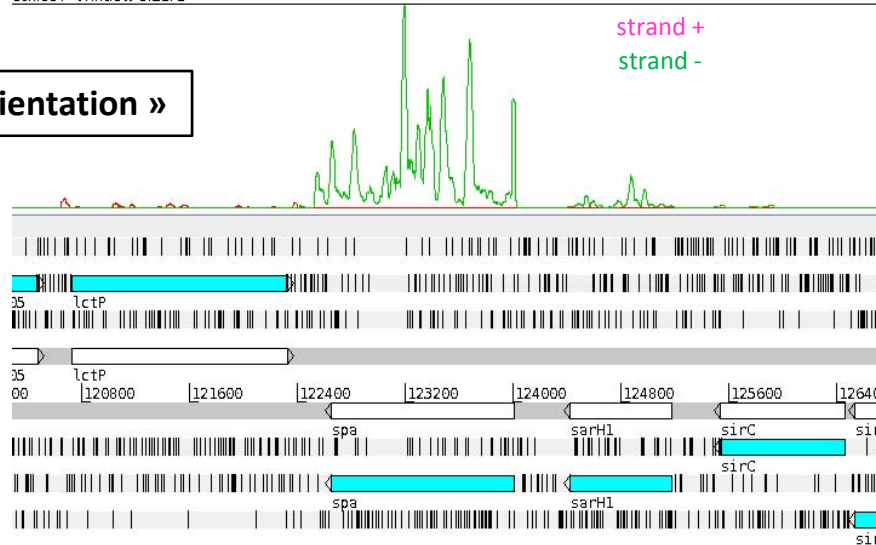
Run 1 : « detection »



Run 2 : « expression kinetic »



Run 3 : « orientation »



Culture conditions :

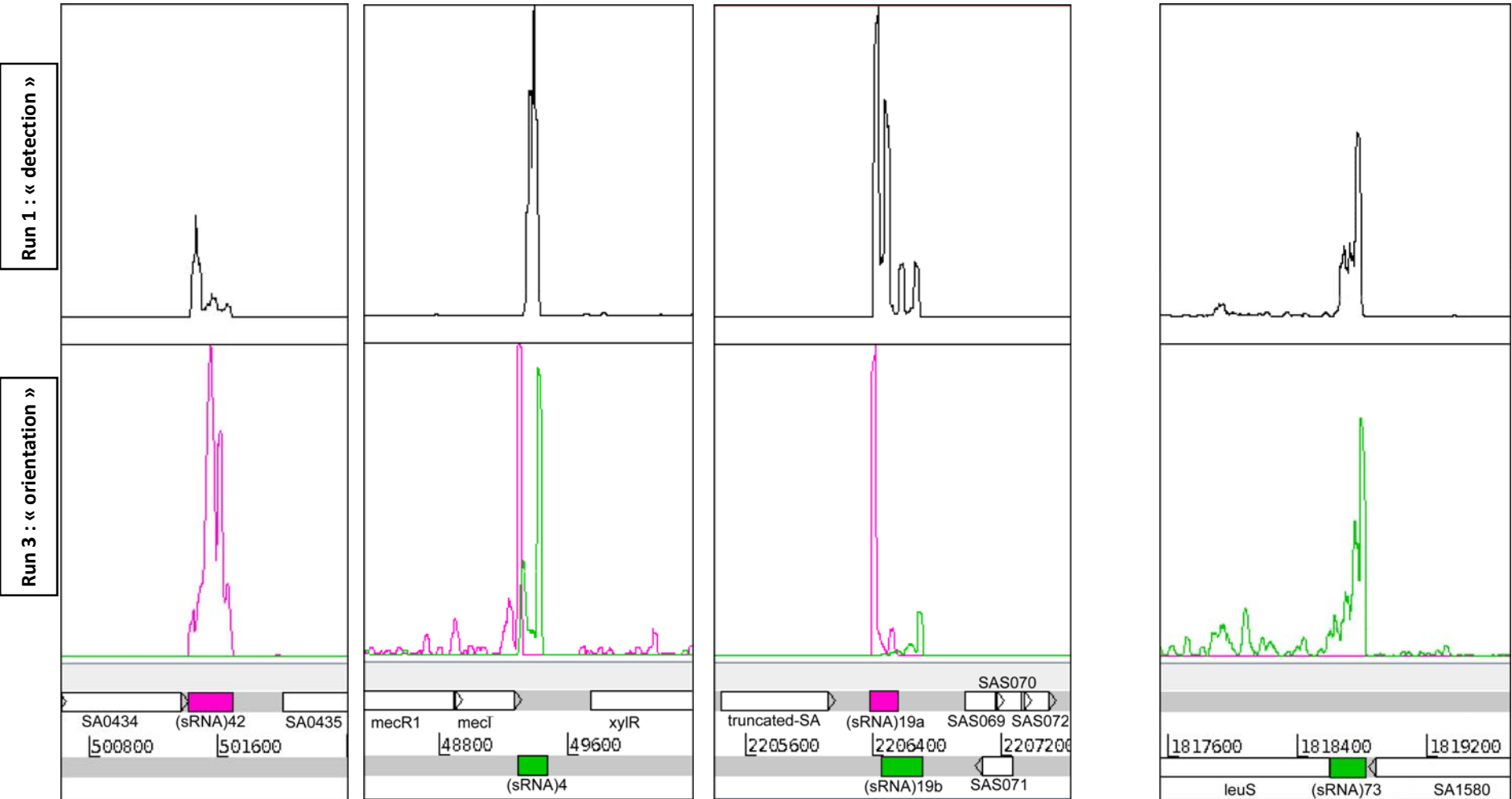
4h of growth  
Rich medium

Growth kinetic  
Rich medium

4h of growth  
Rich medium

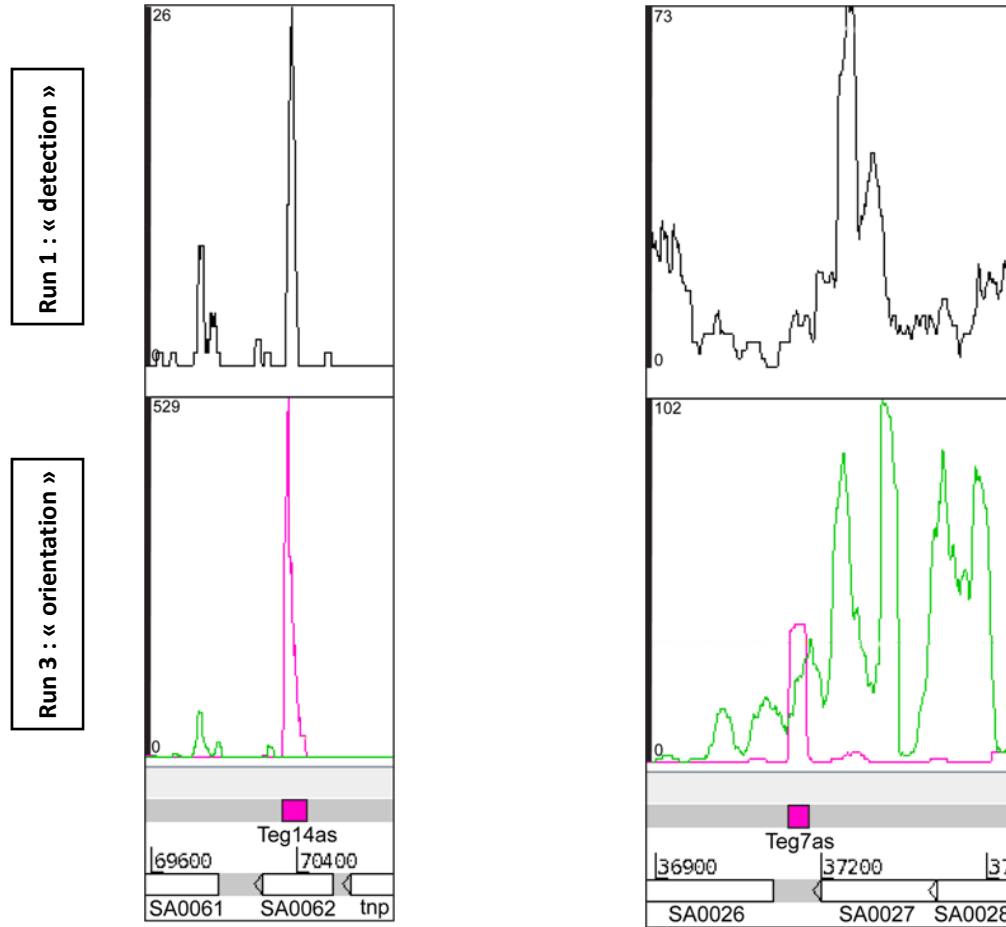


## • Intergenic signals: Potential small RNAs



strand +  
strand -

- Antisense RNA molecules :



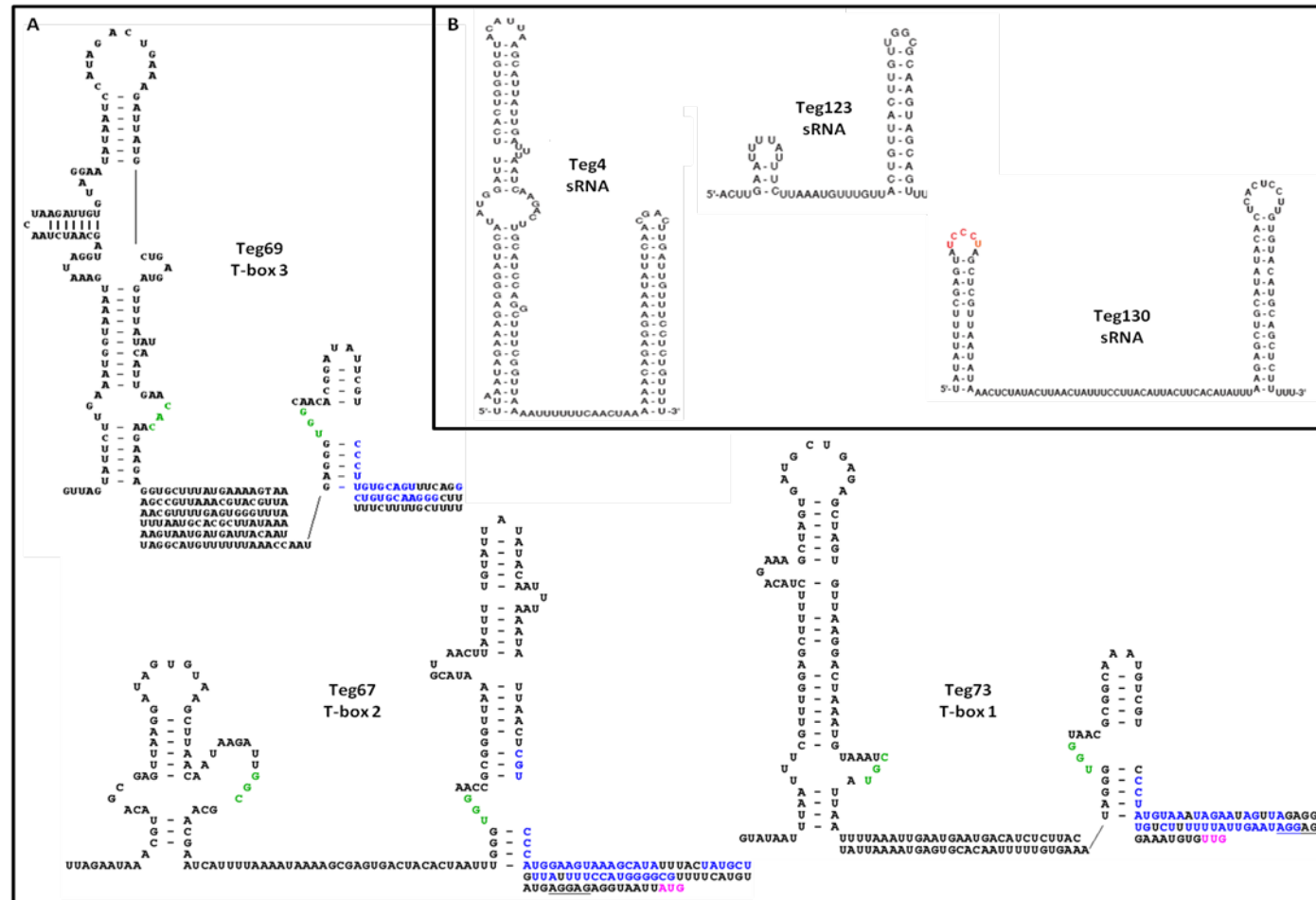
Antisense to annotated gene

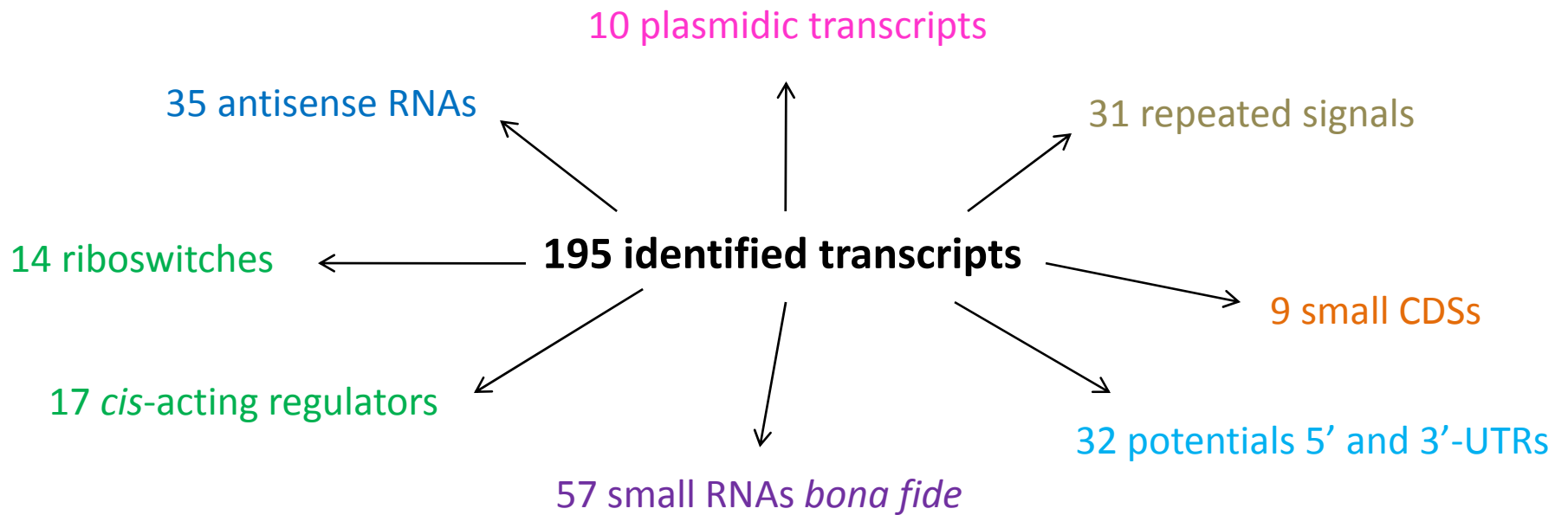
Antisense to potential operon

# Bioinformatic annotations of intergenic transcripts

Performed by Christine Gaspin and Pascale Romby

- Genomic location
- Previous identification in literature
- Putative Shine-Dalgarno sequence, promoter, rho-independant terminator, ...
- Comparative analysis and conservation study
- Presence of UCCC motif
- RFAM annotation
- GC %
- QRNA
- Structure predictions





Named « Teg » (for Transcripts from Experimental method from Geneva)



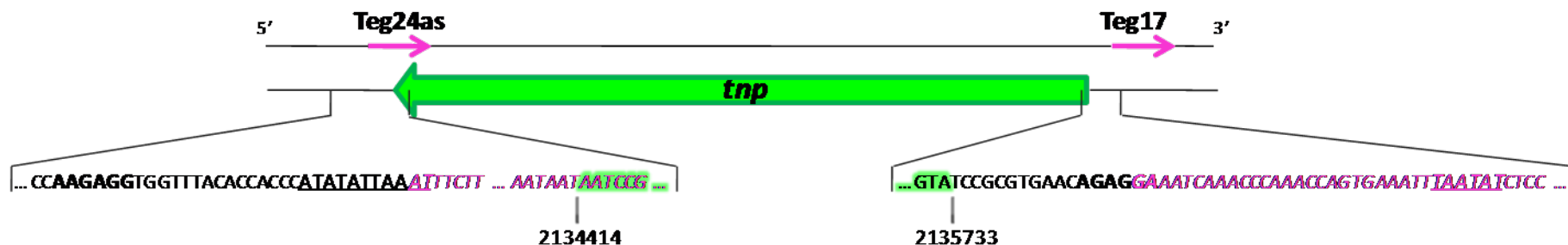
Represent almost 10% of the intergenic regions

# sRNAs and transposition

Specific organization of these transcripts : linked by pairs (7 duplication in the genome)

1 RNA antisense to **3'-end and 3'-UTR of *tnp***

1 RNA antisense to **5'-UTR of *tnp***



Hypothesis :

Insertion sequence may activate downstream gene

→ antisense transcripts could regulate this mechanism

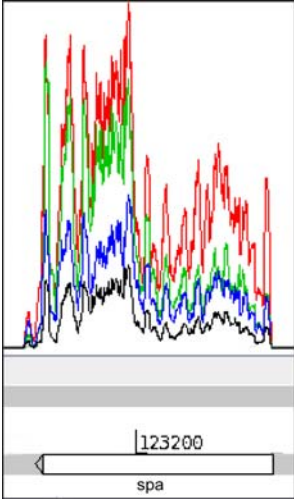
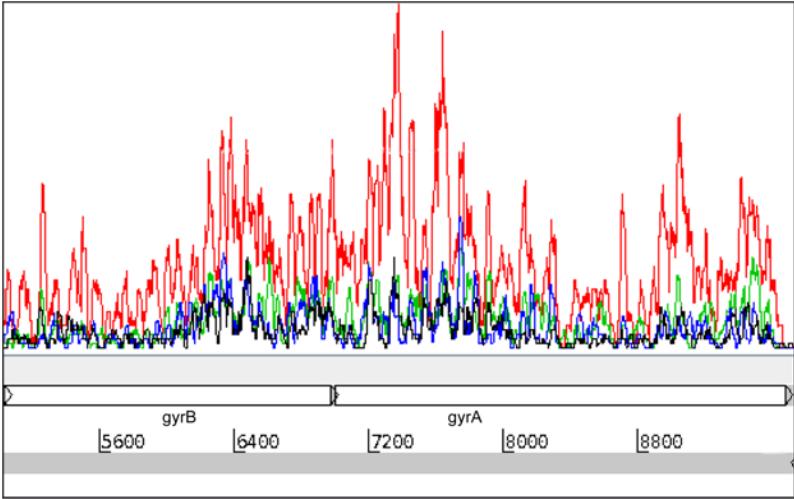
Sequestration of transcription and translation initiation domains

Small RNAs → transposon mechanism and mobility

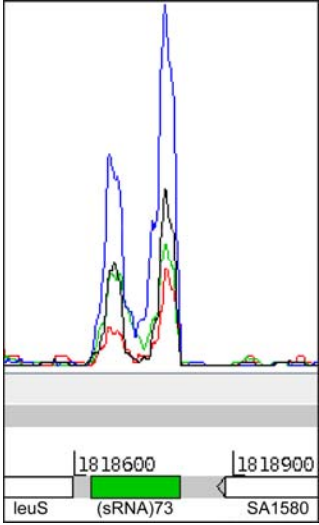
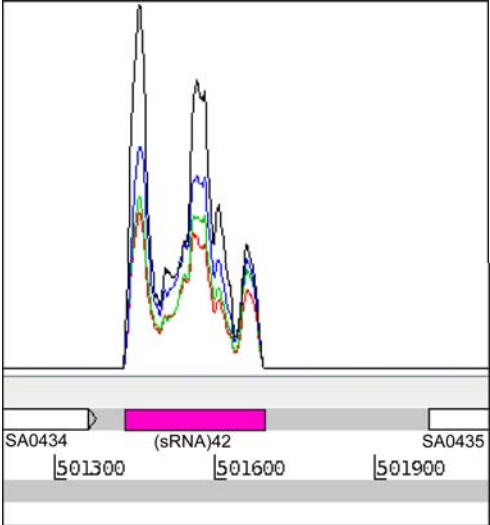
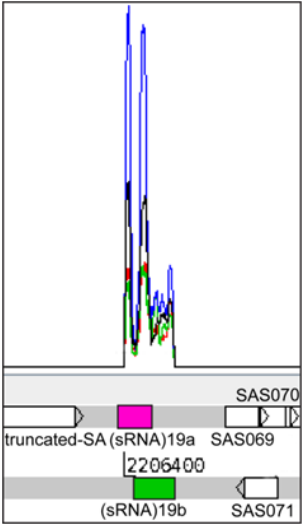
gene activation, gene inactivation and/or the genetic rearrangement

Regulation cascades

## Kinetic expression



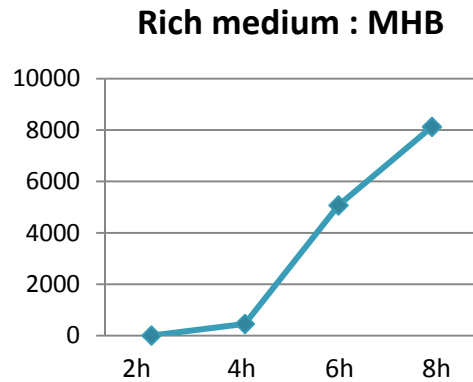
2h  
4h  
6h  
8h



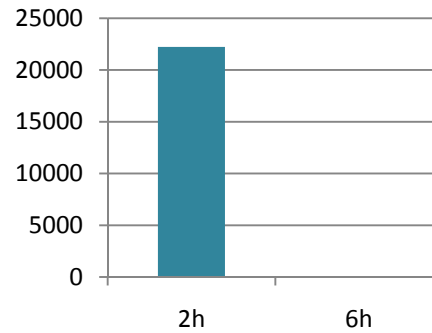
# sRNA expression by qRT-PCR

 Kinetic expression in rich medium

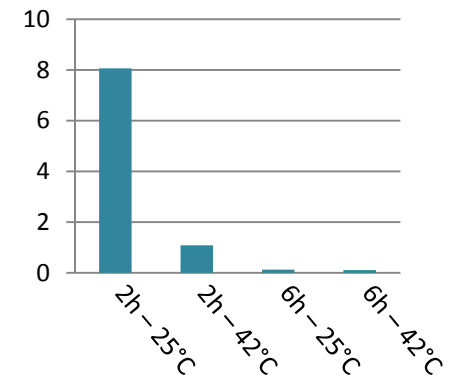
 Expression in stress condition



### Oxydative stress : pestanal

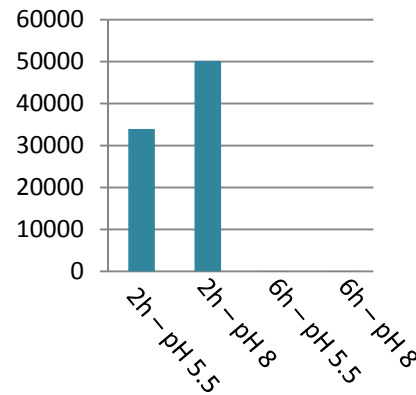


### Thermic stress

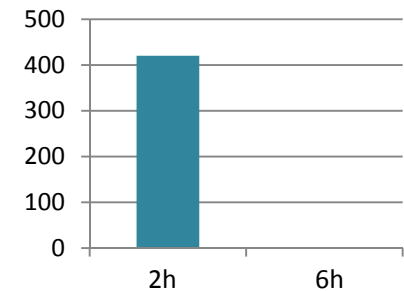


 Expression in stationary phase (most often)

### pH stress



### Stringent medium : NZM



 sRNAs induction in stress condition

# sRNA expression by qRT-PCR

Transcripts tested	MHB	Oxidative stress		pH stress				Thermal stress				Stringent medium	
		2h	6h	2h		6h		2h		6h		2h	6h
				pH 5.5	pH 8.0	pH 5.5	pH 8.0	25°C	42°C	25°C	42°C		
<b>Riboswitches</b>													
Teg1	m-e ; s	++	+	++	++	+	+	+	+	0	0	++	-
Teg76	s	ND	ND	++	++	+	+	ND	ND	ND	+	ND	ND
<b>cis-acting regulators</b>													
Teg35	m-e ; s	+	ND	++	++	++	++	ND	ND	0	+	+	0
Teg38	m-e ; s	ND	0	++	++	+	+	+	+	--	-	+	-
Teg45	m-e ; s	ND	+	++	++	+	+	++	++	--	0	+	-
Teg56	m-e ; s	ND	0	++	++	+	+	ND	ND	--	+	-	-
Teg69	s	++	+	+	+	0	0	-	--	-	-	-	-
Teg70	m-e ; s	--	+	++	++	+	+	++	++	--	+	ND	--
Teg73	m-e ; s	ND	+	++	++	+	+	ND	ND	--	--	ND	--
<b>Small RNAs bona fide</b>													
Teg4	m-e ; s	++	+	+	+	-	-	ND	ND	ND	--	-	-
Teg19b	s	--	++	++	++	++	++	ND	ND	+	++	ND	+
Teg24	m-e ; s	++	+	++	++	+	0	-	-	+	0	-	0
Teg42	m-e ; s	++	+	++	++	+	+	+	0	-	-	++	0
Teg47	s	+	+	+	+	--	++	++	++	+	+	ND	-
Teg60	s	+	+	-	-	+	+	--	-	-	-	not tested	
Teg91	m-e ; s	++	+	+	0	+	+	ND	-	-	-	not tested	
<b>5'UTRs and 3'UTRs</b>													
Teg21	m-e ; s	++	+	++	++	--	+	++	++	+	+	+	-
Teg26	m-e ; s	++	+	++	++	--	+	++	++	+	+	-	-
Teg28	m-e ; s	ND	+	++	++	ND	+	++	++	+	+	ND	-
Teg55	m-e ; s	ND	+	-	-	+	+	--	-	-	-	-	0
Teg57	m-e ; s	ND	-	++	++	+	+	+	+	--	+	0	-
Teg61	m-e ; s	ND	0	+	+	+	+	+	+	-	-	not tested	
Teg72	s	ND	+	-	-	+	+	-	-	-	-	not tested	
<b>Plasmidic transcript</b>													
Teg2pl	m-e ; s	+	0	+	+	-	+	-	-	-	-	-	-
<b>Repeated regions</b>													
Teg17	m-e ; s	++	+	+	-	-	++	++	++	++	+	ND	-
Teg18	s	ND	+	+	+	--	+	+	+	+	+	+	+



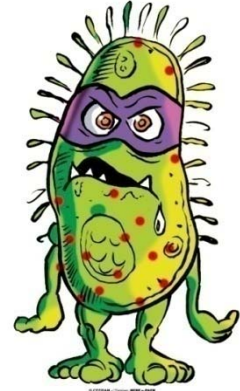
## Discovered transcripts :

- Homogeneous repartition along the chromosome and the plasmid of the bacterium
- Highly conserved across sequenced *S. aureus* strains
- Some are conserved in other bacteria
  
- Localized in biologically /clinically relevant regions  
( >50 transcripts localized near virulence genes or in pathogenicity islands)
  
- Expression :
  - temporally regulated during growth phase
  - altered by various stress conditions

Thanks for your attention



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Dr. Alex Scherl

Dr. Laurent Farinelli

