



**Diversity and mobility of conjugative elements in a population of
Streptomyces isolated from a rhizospheric micro-habitat**

Caroline CHOUFA

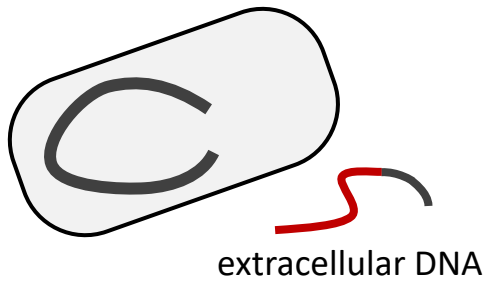
UMR UL-INRAE 1128

Dynamique des Génomes et Adaptation Microbienne

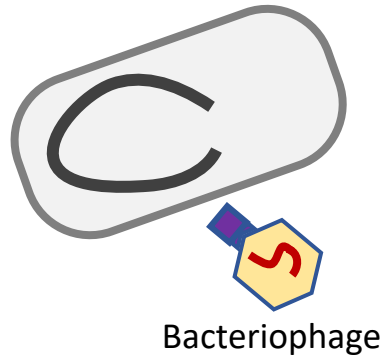
Université de Lorraine

Mechanisms of horizontal gene transfer

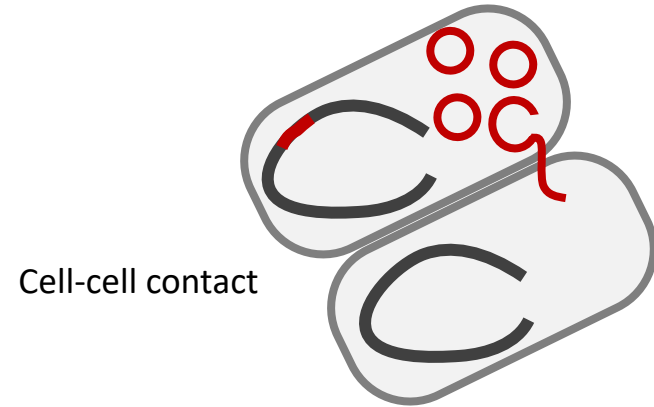
Natural Transformation



Transduction



Conjugation



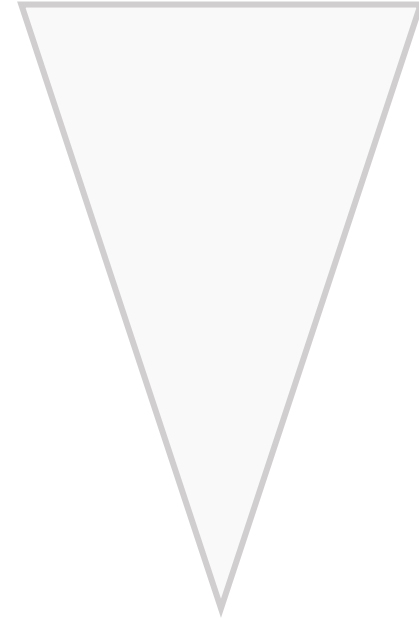
Mechanisms that promote bacterial diversity

Genetic diversity and evolution

of natural populations of bacterial at micro-scale

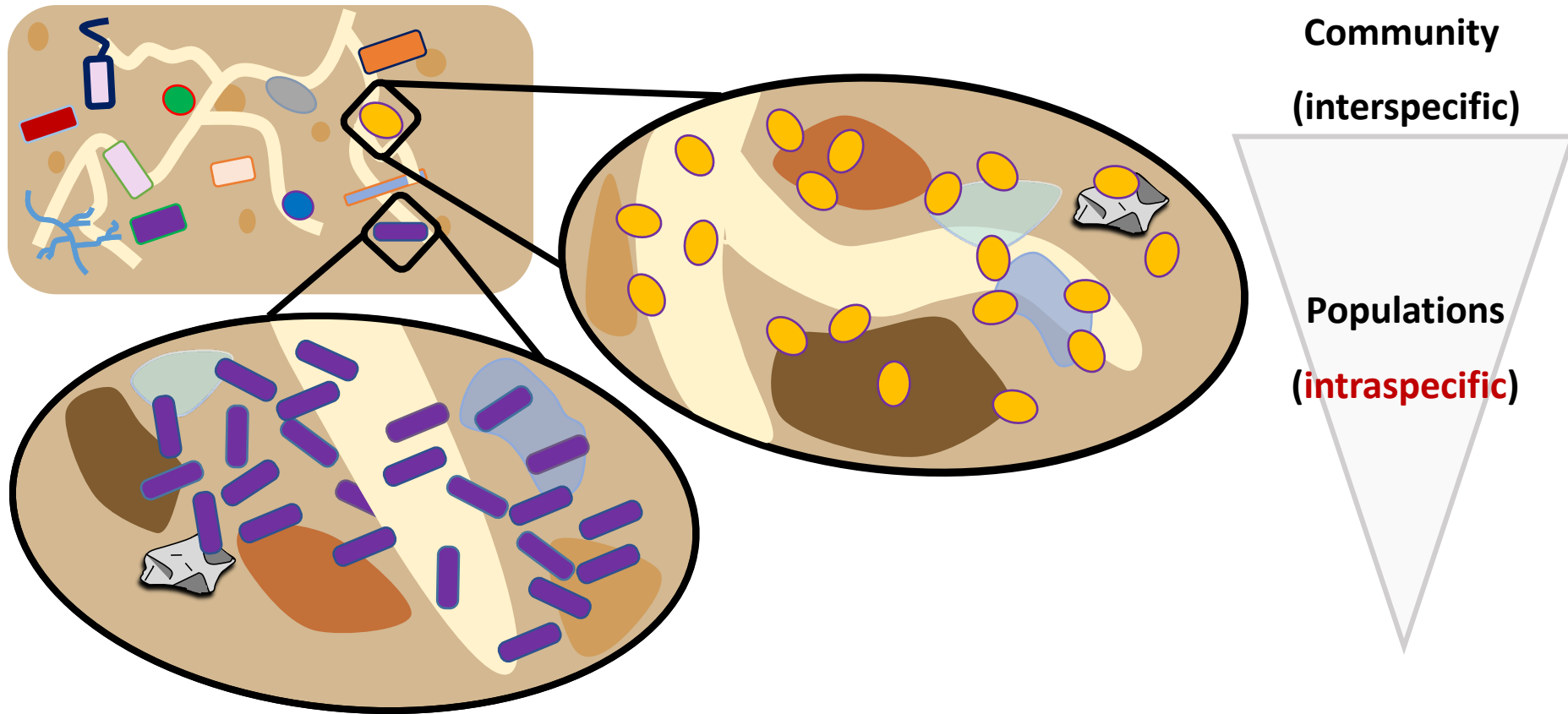


Community
(interspecific)



Genetic diversity and evolution

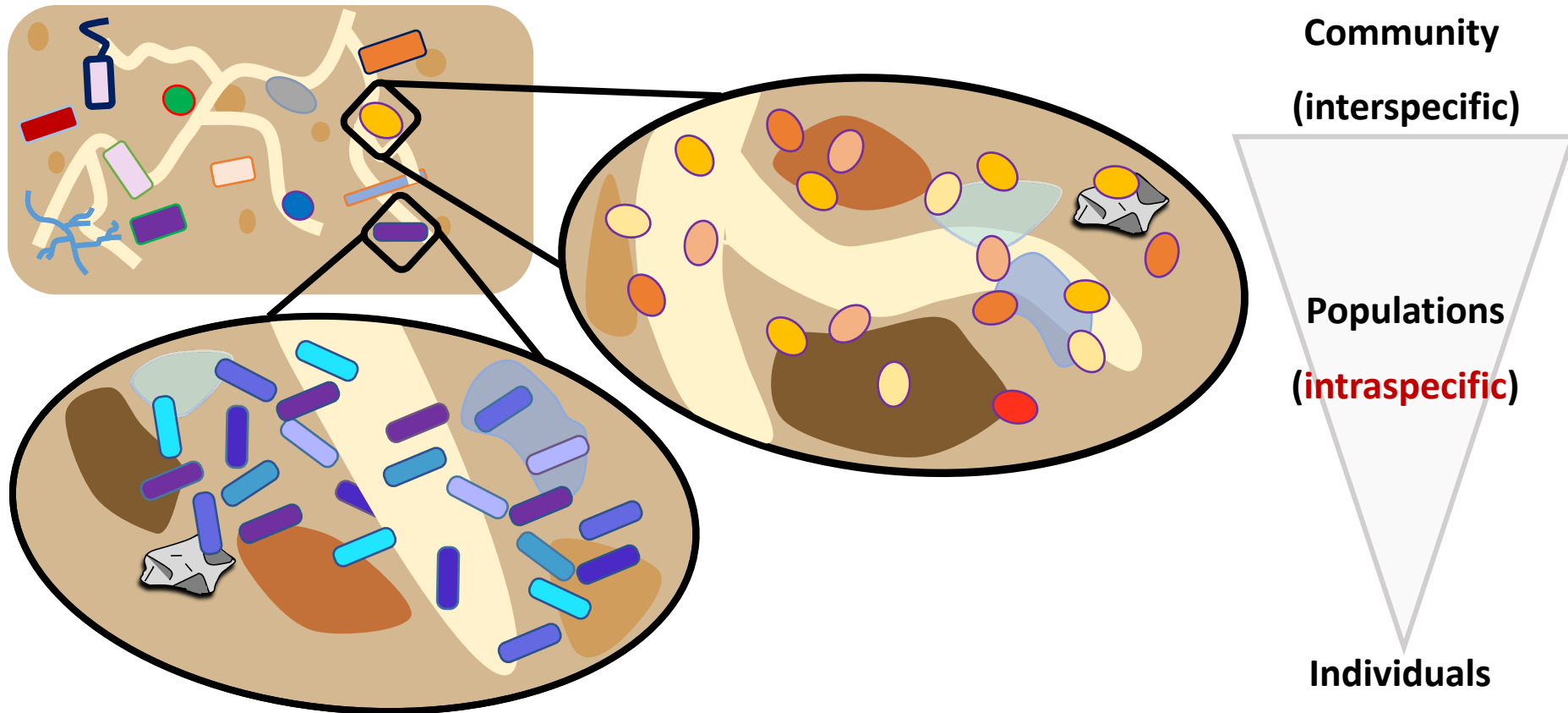
of natural populations of bacterial at micro-scale



Bacterial population

Sympatric individuals of the same species

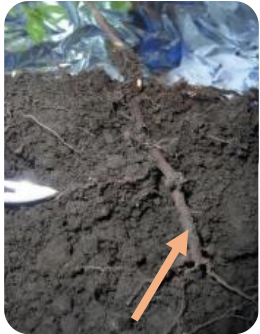
Genetic diversity and evolution of natural populations of bacterial at micro-scale



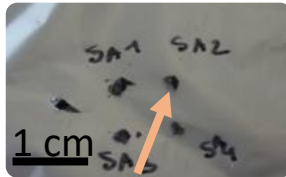
What is the extent of horizontal transfer within a natural population?

Collection of a natural rhizospheric population of *Streptomyces*

Isolation from a grain of soil



Rhizosphere



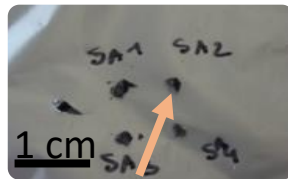
Grain of soil

Collection of a natural rhizospheric population of *Streptomyces*

Isolation from a grain of soil



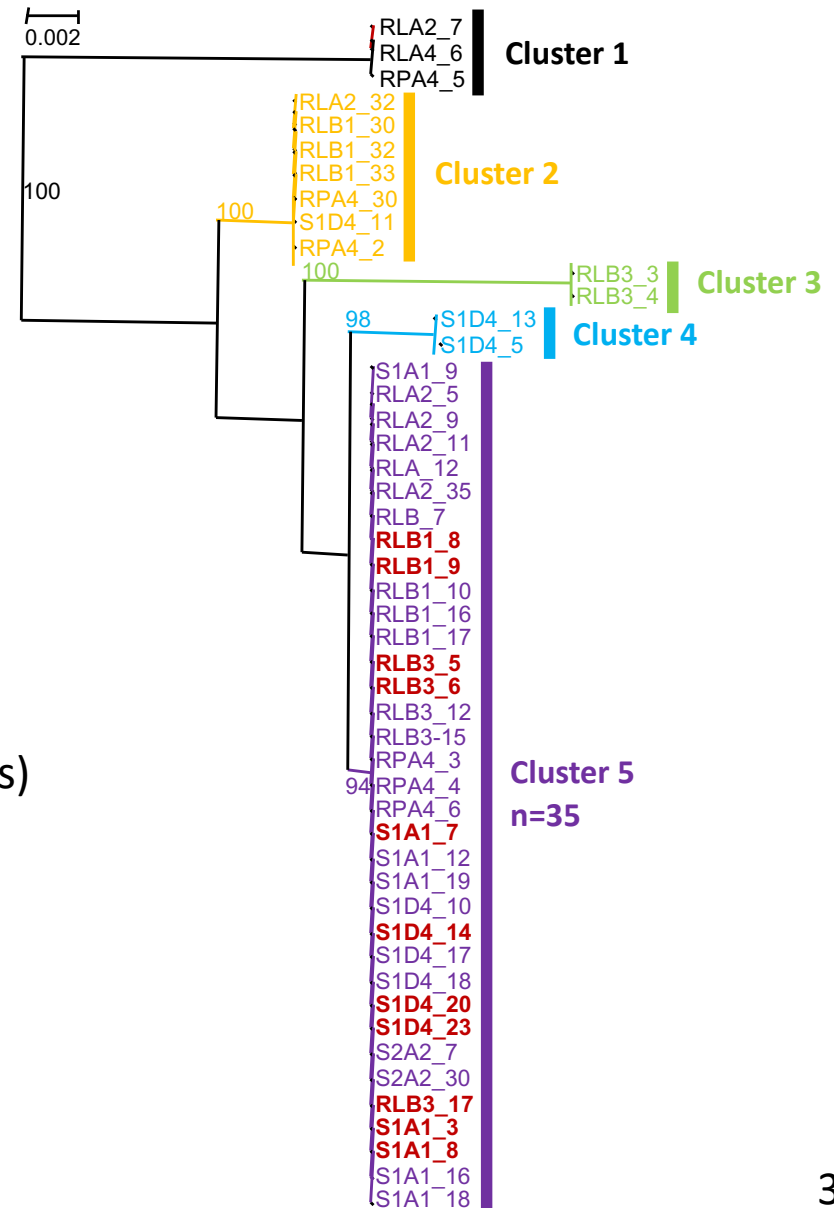
Rhizosphere



Grain of soil

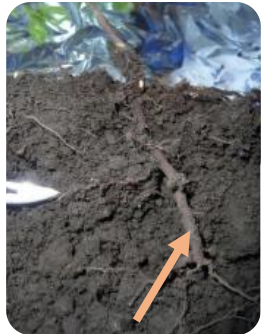
Selection of taxonomically closely related strains

- Identical 16S ribosomal DNAs
- Validated by MLSA (MultiLocus Sequence Analysis)

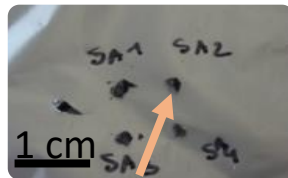


Collection of a natural rhizospheric population of *Streptomyces*

Isolation from a grain of soil



Rhizosphere



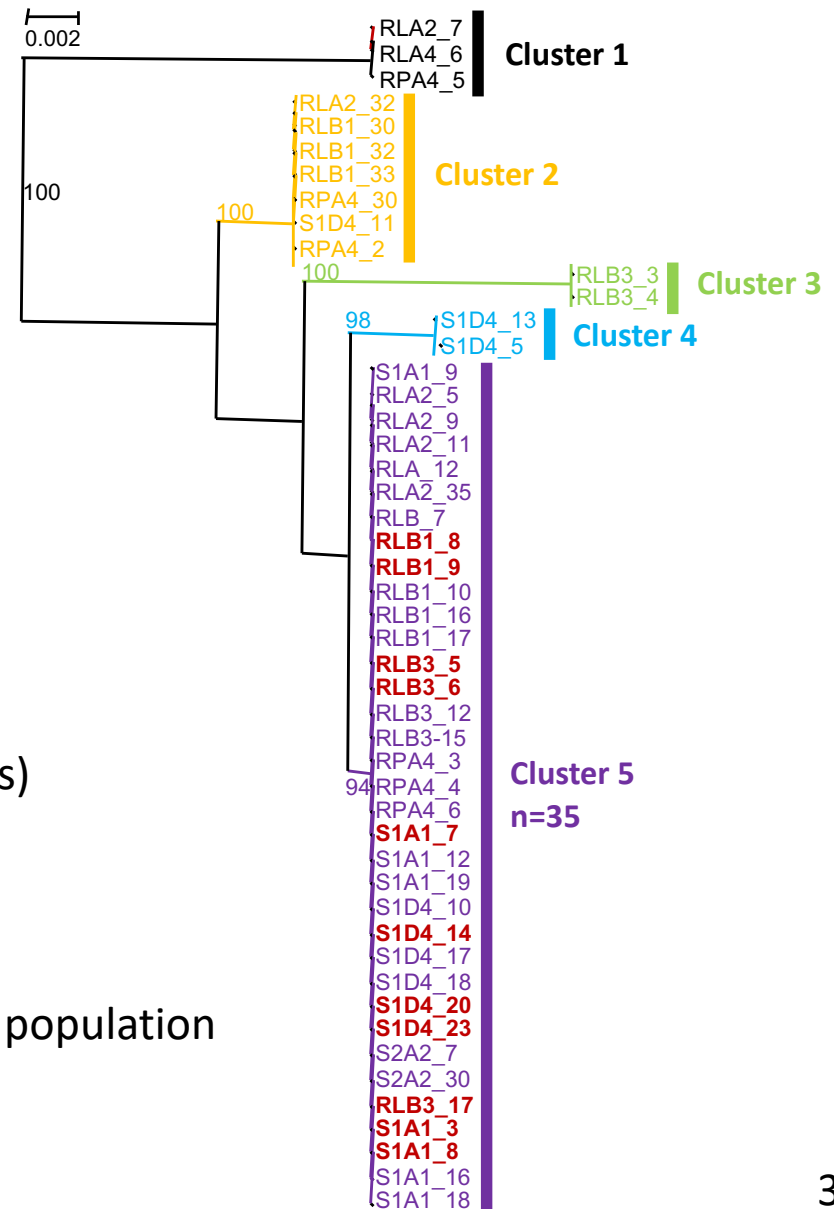
Grain of soil

Selection of taxonomically closely related strains

- Identical 16S ribosomal DNAs
- Validated by MLSA (MultiLocus Sequence Analysis)

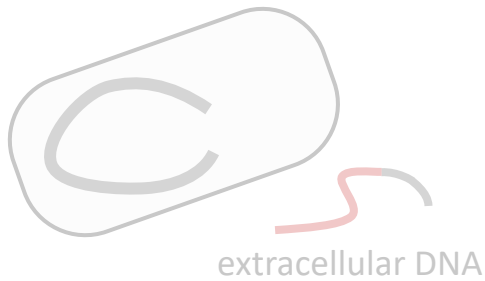
Analysis of the population diversity (11 isolates)

- Sequencing of **11 genomes** representative of the population
- Linear chromosomal of *ca.* 12 Mb



Mechanisms of horizontal gene transfer in *Streptomyces*

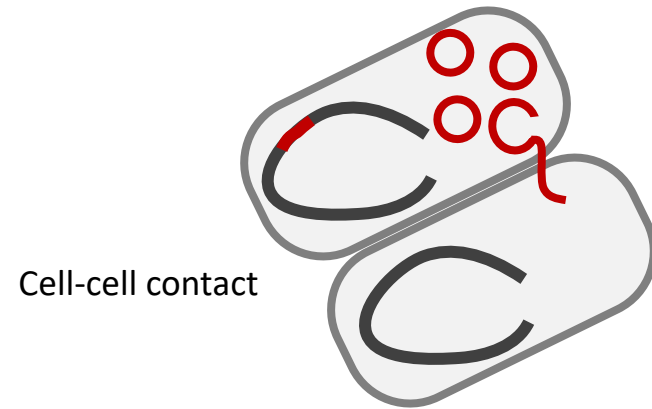
Natural Transformation



Transduction



Conjugation

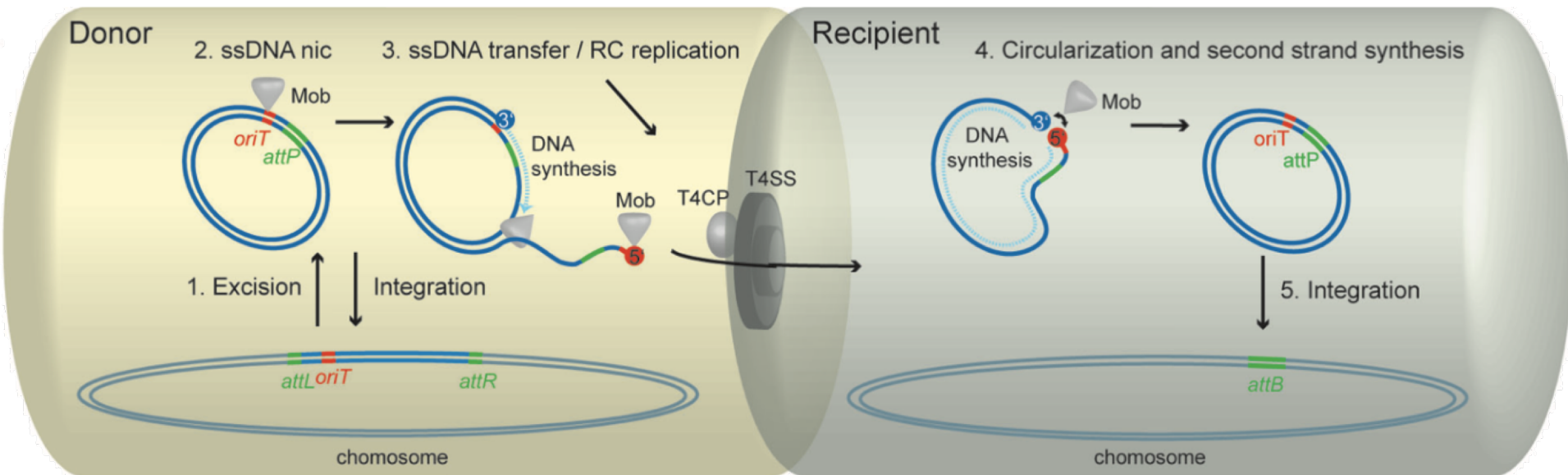


Conjugation

The main mechanism that would promote gene flows

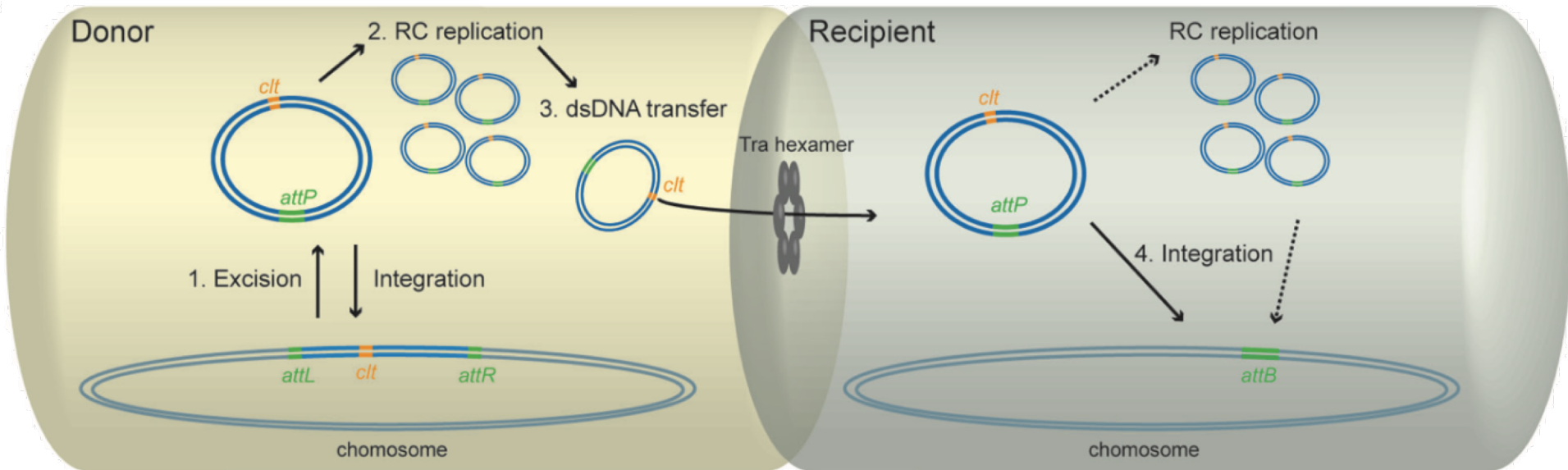
Two mechanisms of conjugation in *Streptomyces*

- **Type IV secretion system dependent (T4SS)**
=> ICE (Integrative and Conjugative Elements)

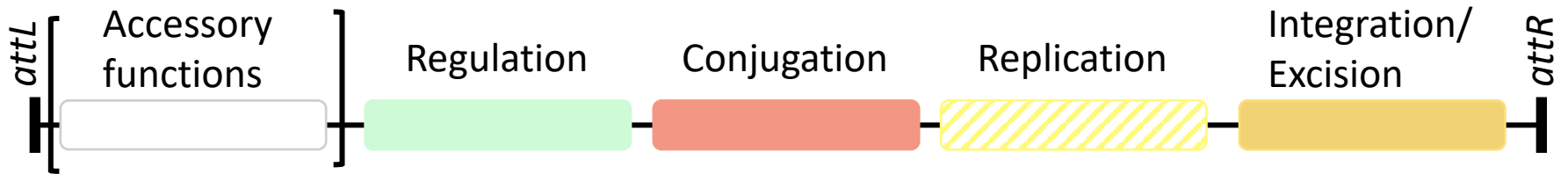


Two mechanisms of conjugation in *Streptomyces*

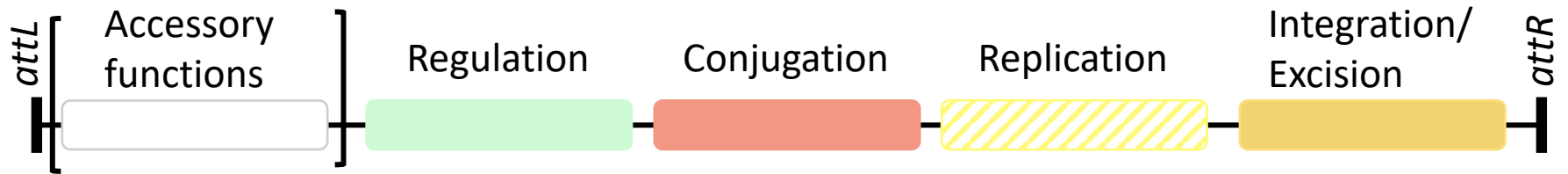
- **TraB protein dependent** (cell division and sporulation FtsK/SpoIIIE-homolog protein)
=> AICE (**A**ctinomycete **I**ntegrative and **C**onjugative **E**lements)



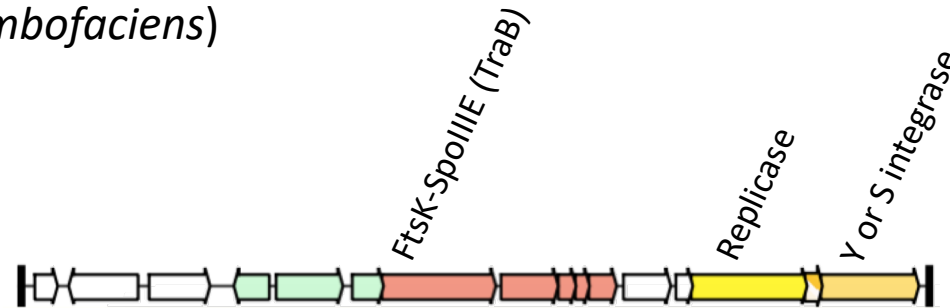
Search for conjugative elements in *Streptomyces* genome



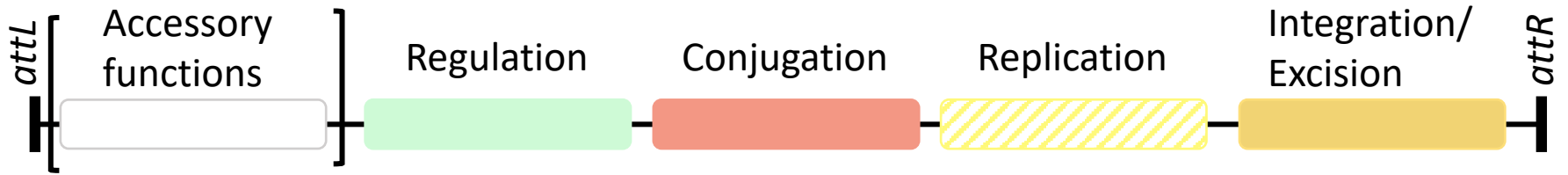
Search for conjugative elements in *Streptomyces* genome



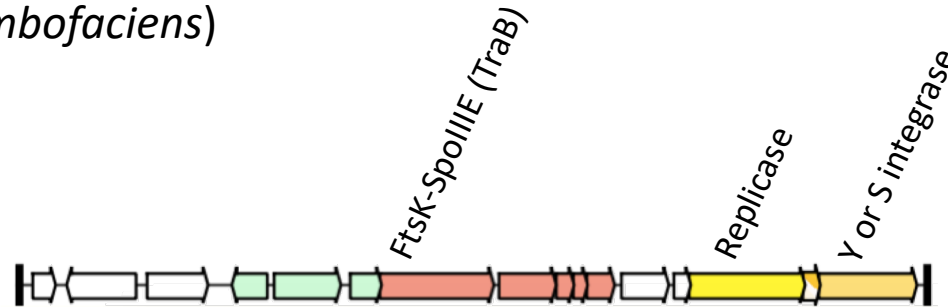
- AICE (e.g. pSAM2 *S. ambofaciens*)



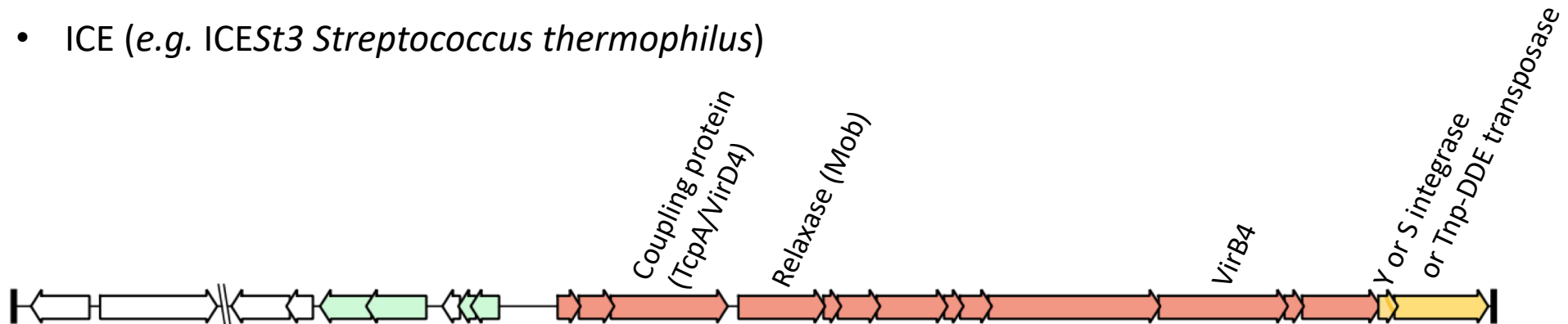
Search for conjugative elements in *Streptomyces* genome



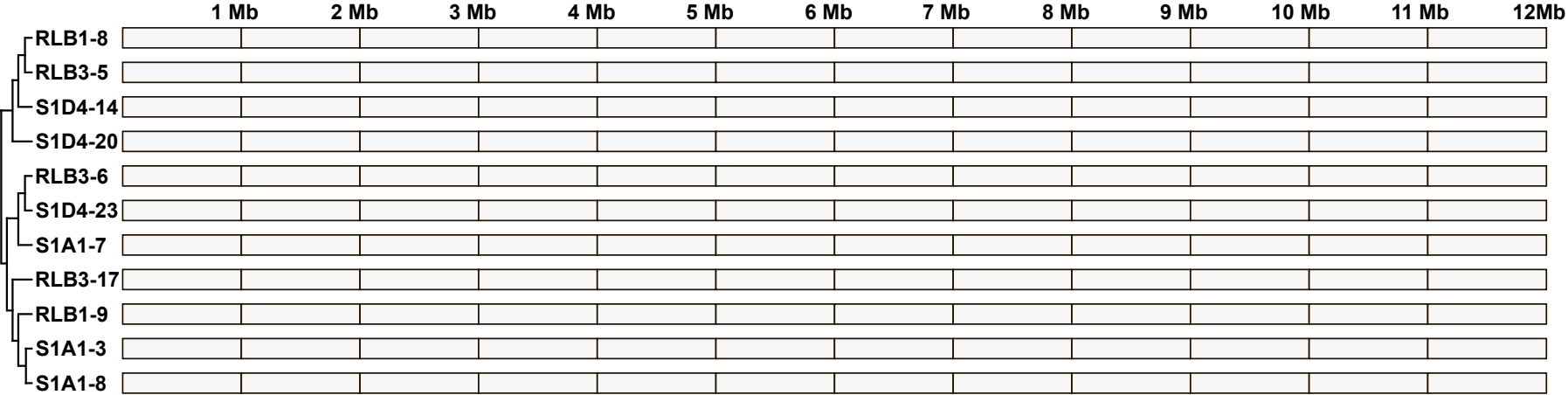
- AICE (e.g. pSAM2 *S. ambofaciens*)



- ICE (e.g. ICESt3 *Streptococcus thermophilus*)

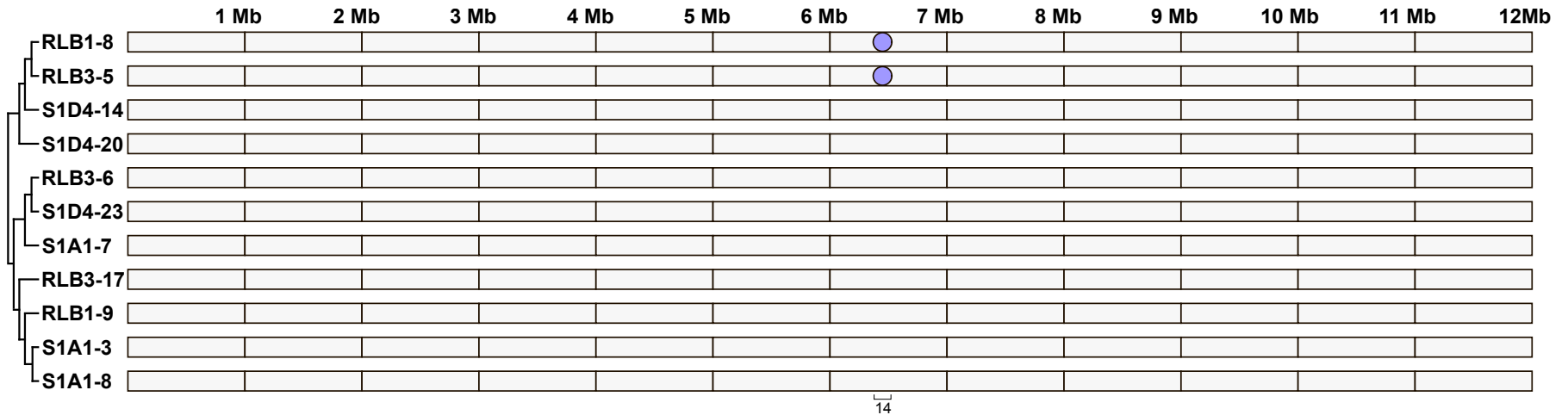


Prevalence of integrative and conjugative elements in the population



Prevalence of integrative and conjugative elements in the population

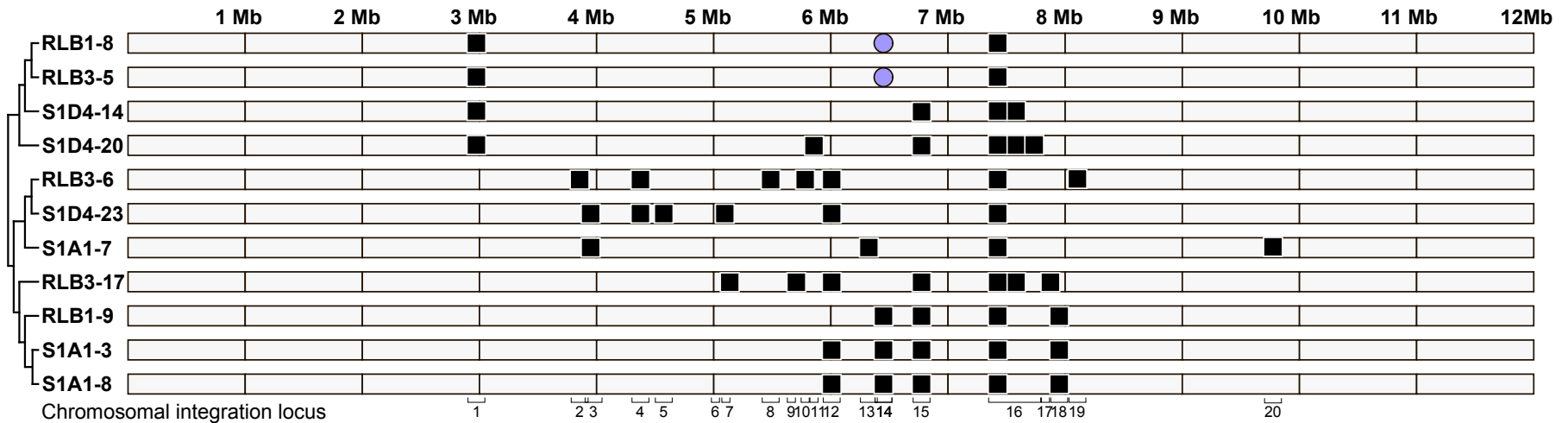
- 2 ICEs 100% identical in sequence



14

Prevalence of integrative and conjugative elements in the population

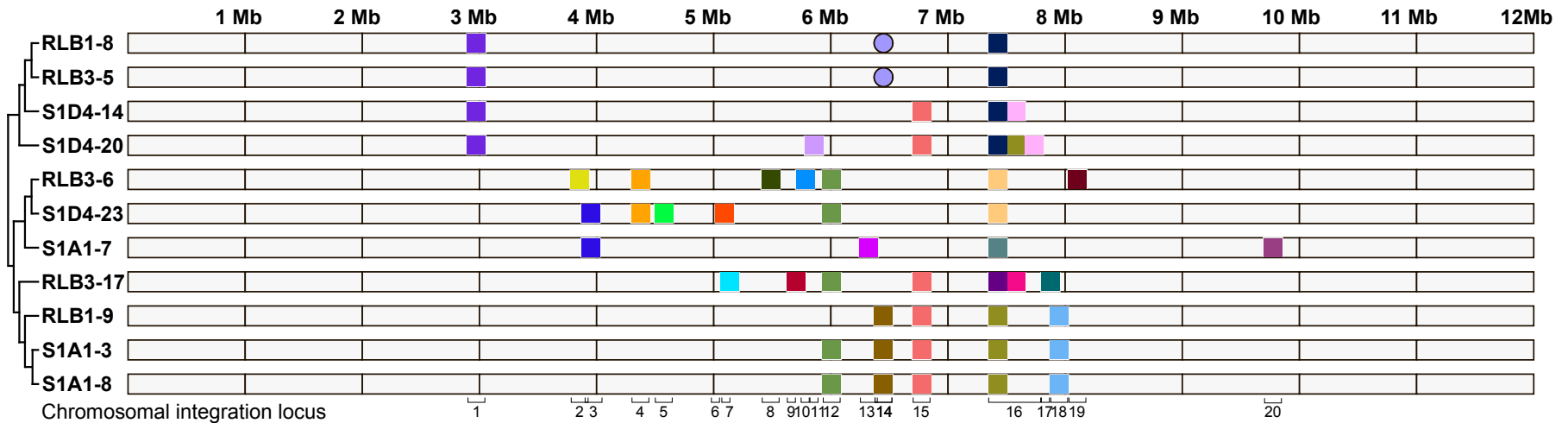
- 2 ICEs 100% identical in sequence
- 52 AICEs



Prevalence of integrative and conjugative elements in the population

- 2 ICEs 100% identical in sequence
- 52 AICEs divided into 26 families

AICEs diverge in size (11.9 to 44.1 kb) and in gene content (15 to 41 CDSs)



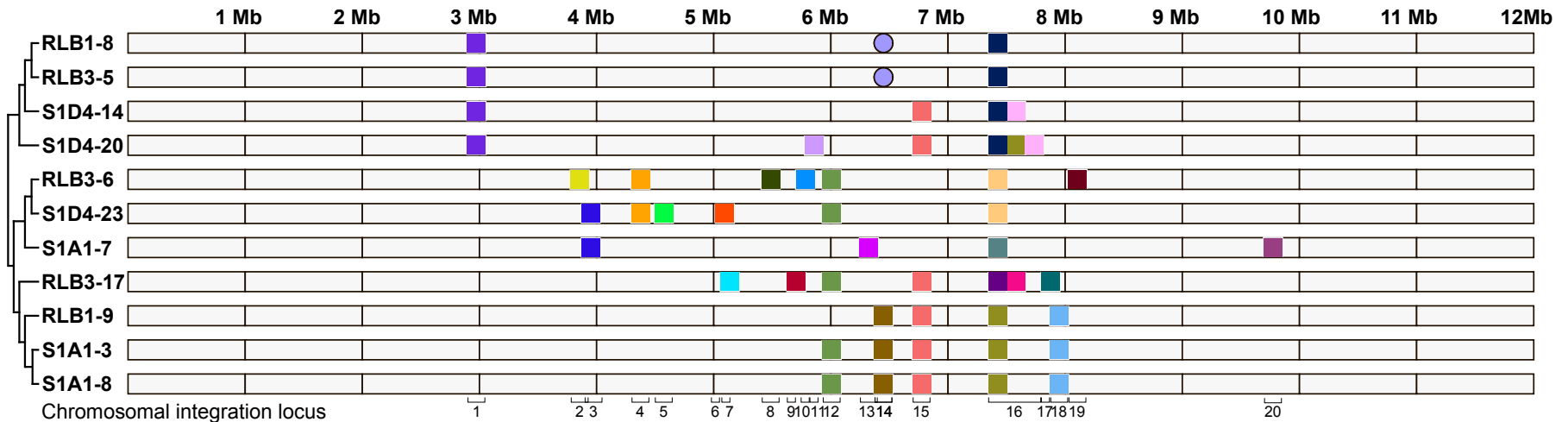
Prevalence of integrative and conjugative elements in the population

- 2 ICEs 100% identical in sequence

- 52 AICEs divided into 26 families

AICEs diverge in size (11.9 to 44.1 kb) and in gene content (15 to 41 CDSs)

- 54 conjugative elements contain the signature genes

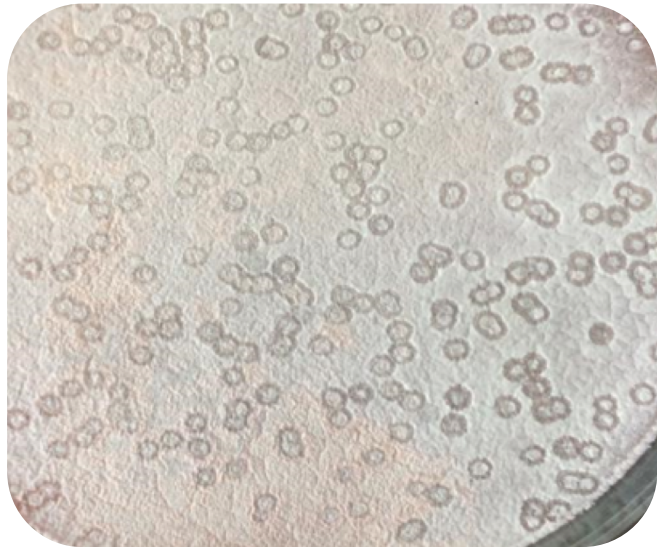
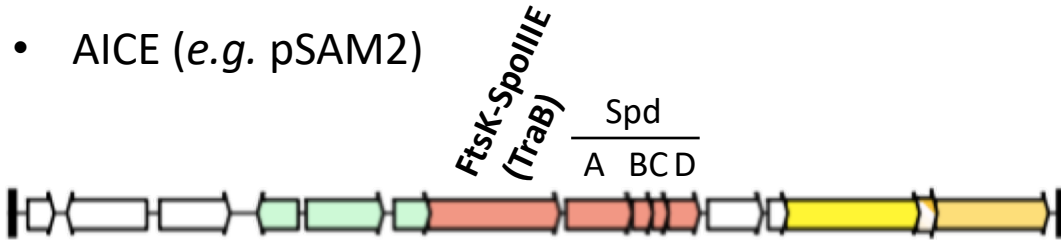


There is considerable diversity in AICEs.

Their distribution suggests an effective dynamic within the population.

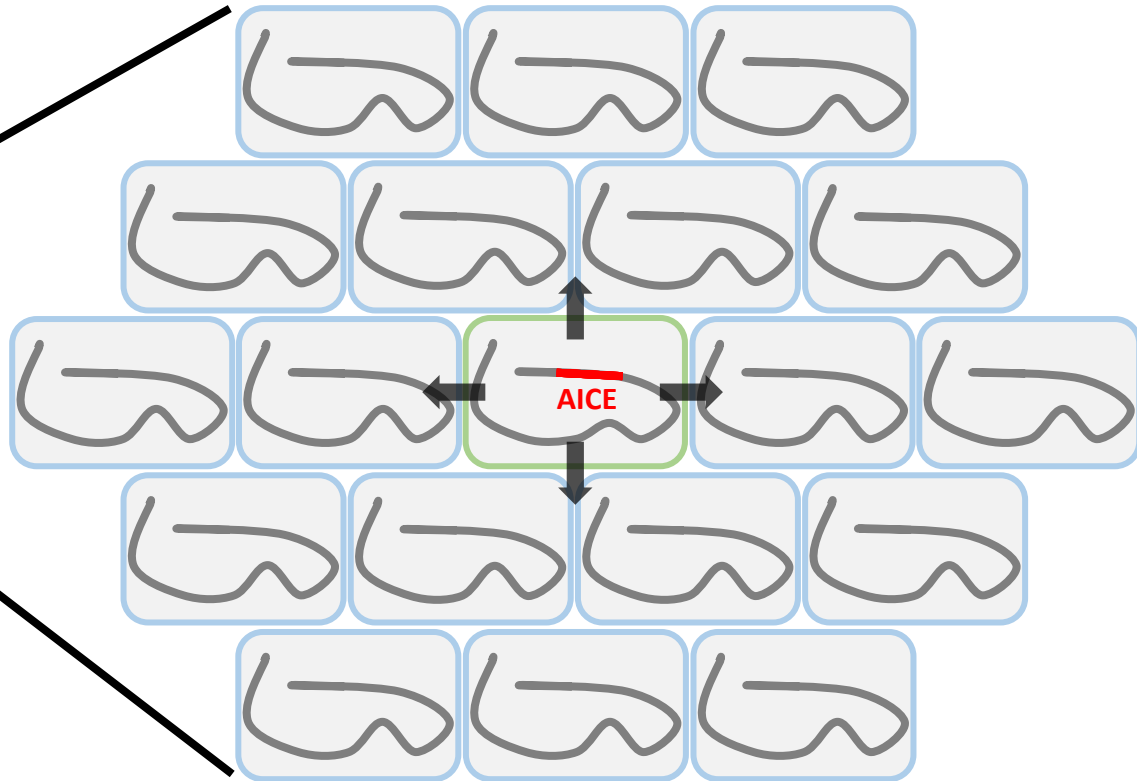
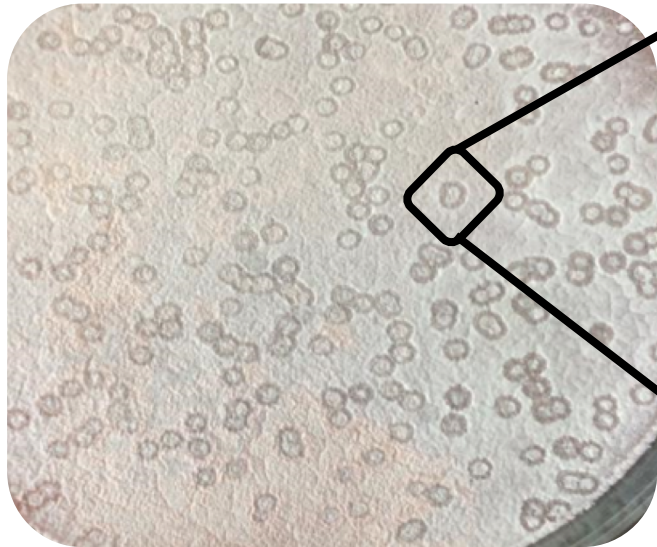
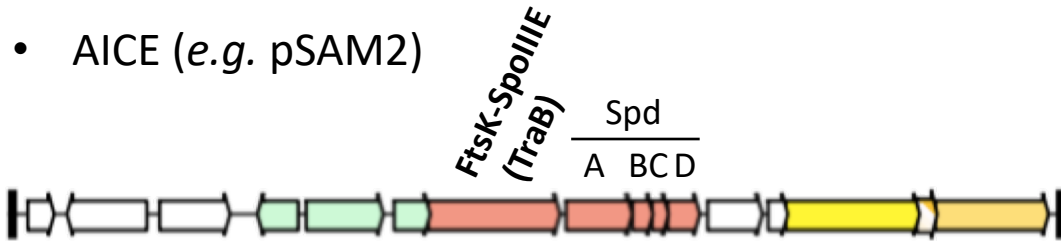
AICE transfers are visible to the naked eye: pock phenomenon

- AICE (e.g. pSAM2)



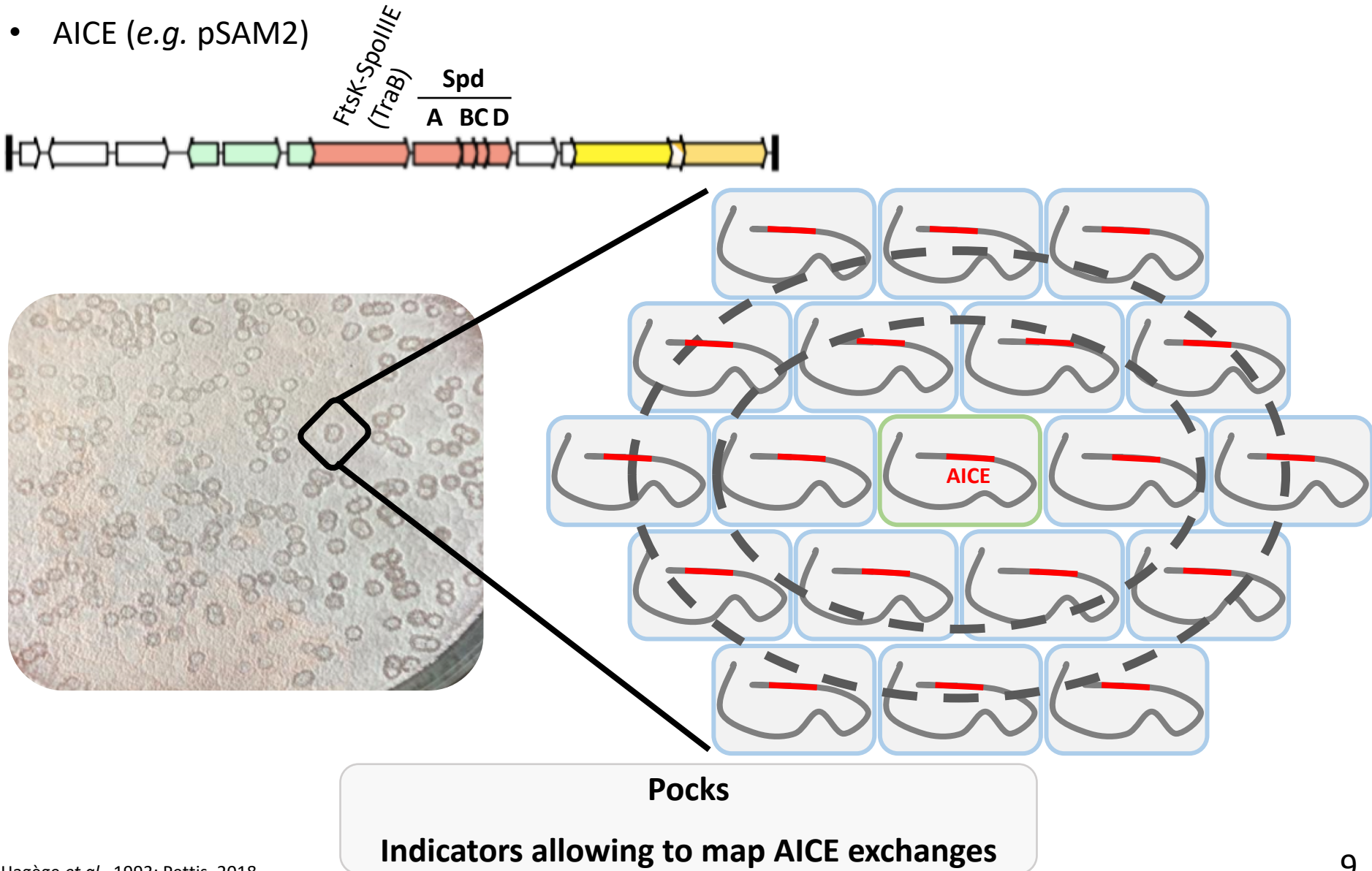
AICE transfers are visible to the naked eye: pock phenomenon

- AICE (e.g. pSAM2)



AICE transfers are visible to the naked eye: pock phenomenon

- AICE (e.g. pSAM2)



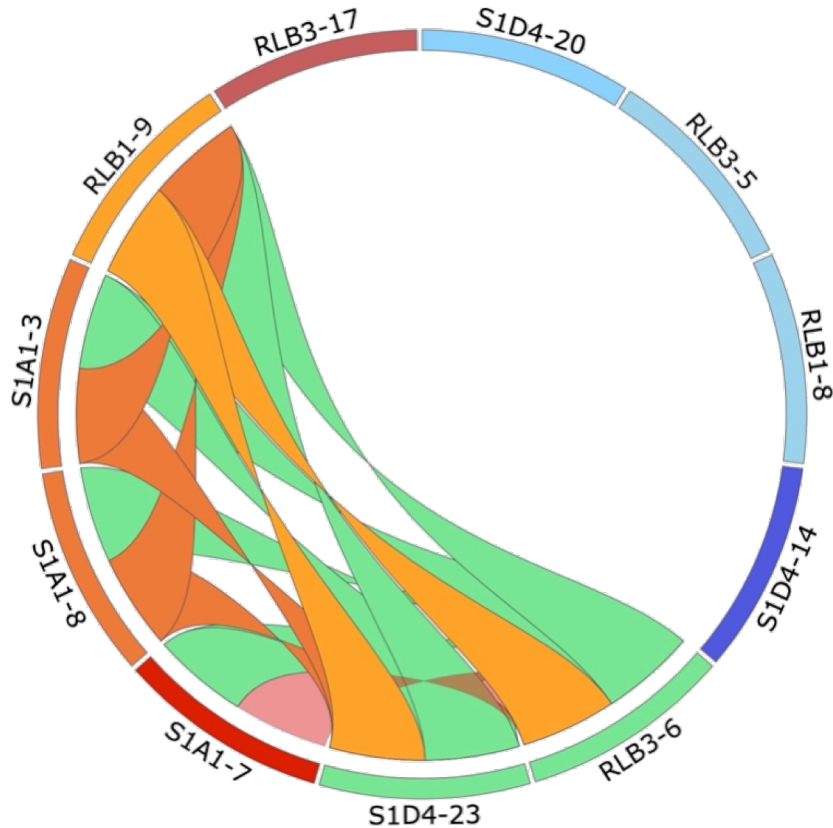
Pocks

Indicators allowing to map AICE exchanges

Mapping the flow of AICEs in the population

- **13/110 mating pairs form pocks**

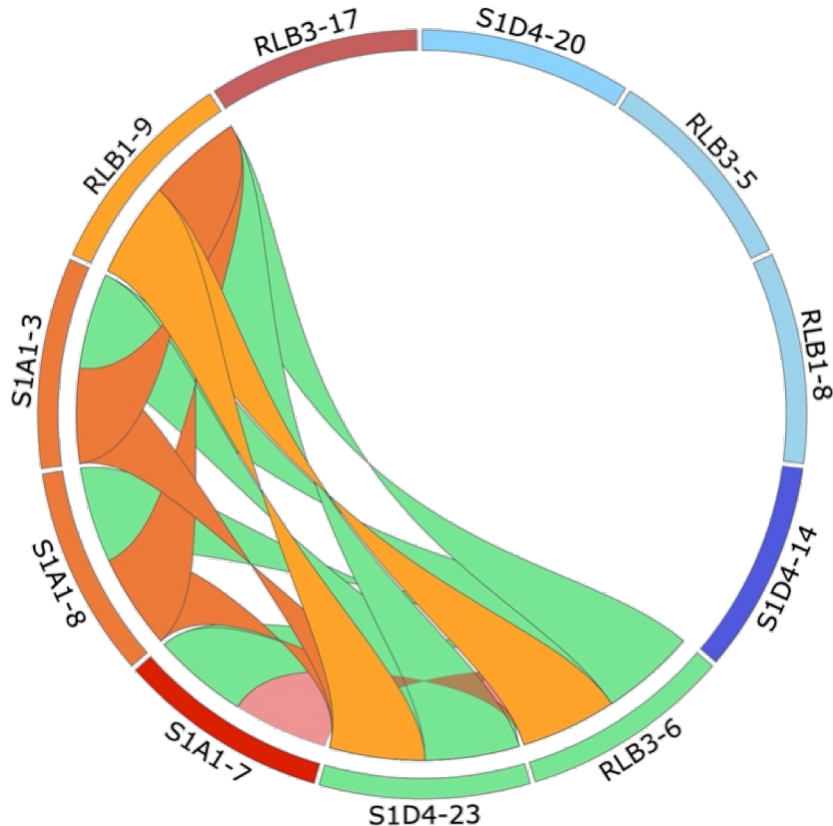
AICEs can transfer between individuals of the population



Mapping the flow of AICEs in the population

- 13/110 mating pairs form pocks

AICEs can transfer between individuals of the population

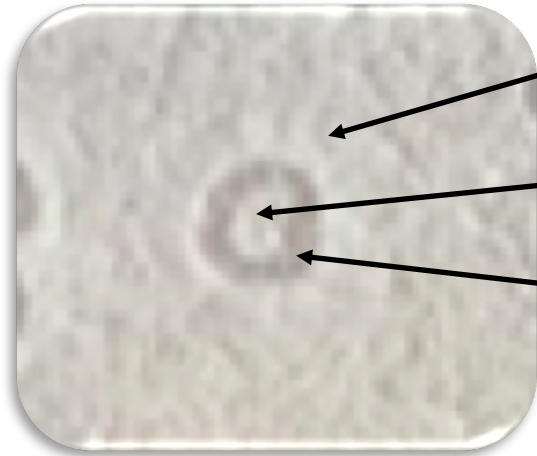


- **Transfers are not panmictic**
 - Spd proteins can be absent
 - Experimental conditions are not optimal

- The absence of pocks does not exclude the conjugation

Identify AICEs responsible for the formation of pocks

Validation of AICE transfers

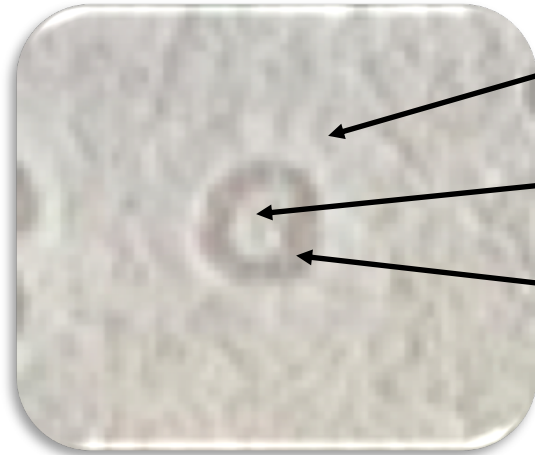


(R) Recipient lawn (Kan^R or Apra^R)

(D) Donor

(T) Transconjugants

Validation of AICE transfers

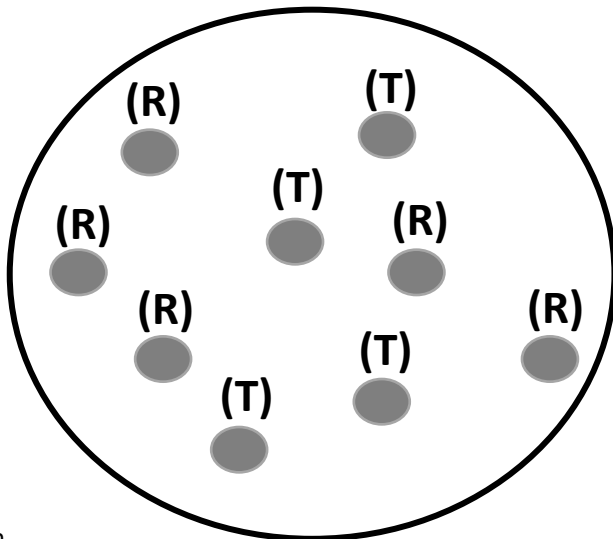


(R) Recipient lawn (Kan^R or Apra^R)

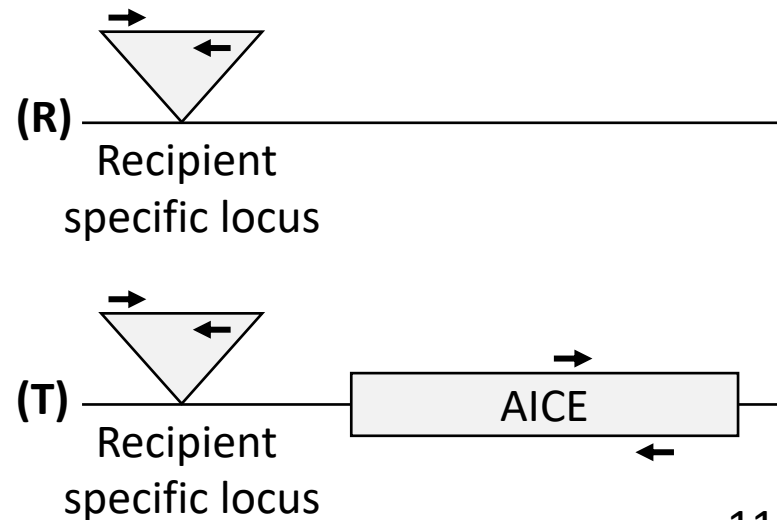
(D) Donor

(T) Transconjugants

- Isolation and selection of transconjugants from pocks

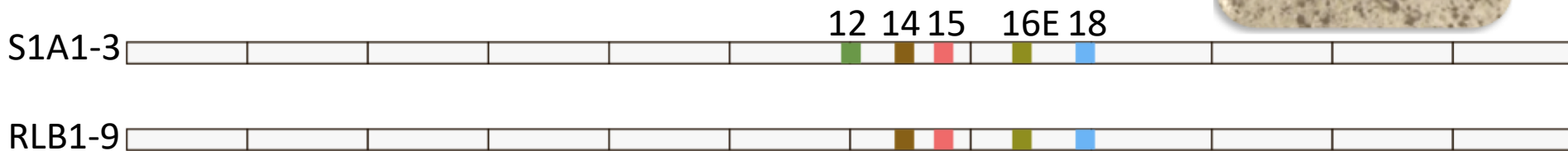
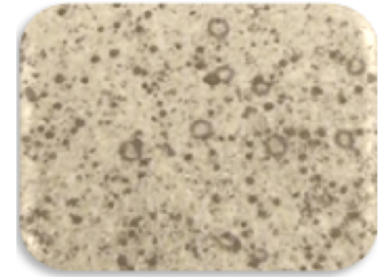


- PCR screening of transconjugants



Confirmation of AICE transfer

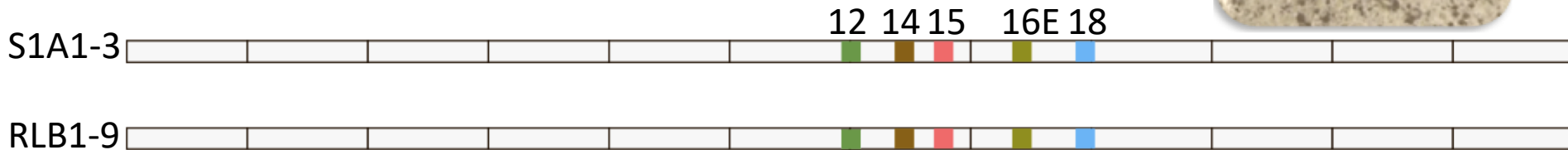
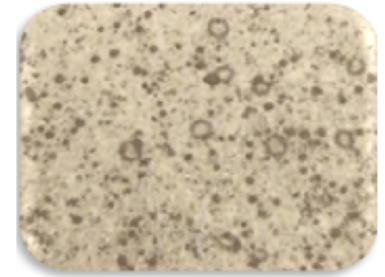
- Identification of two strains that differ by one AICE



=> Transfer of **AICE12** from S1A1-3 to RLB1-9

Confirmation of AICE transfer

- Identification of two strains that differ by one AICE

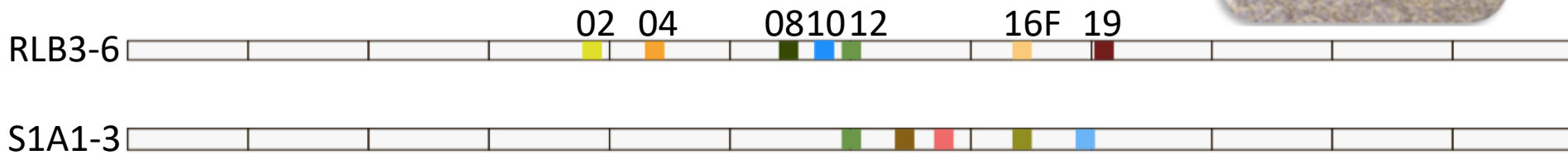
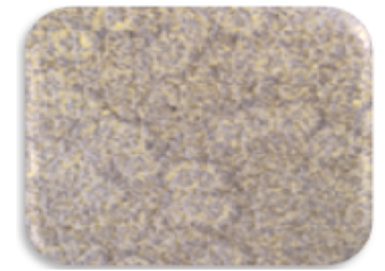


=> Transfer of **AICE12** from S1A1-3 to RLB1-9

- Identification of two strains that differ by several AICEs

Which AICE can transfer?

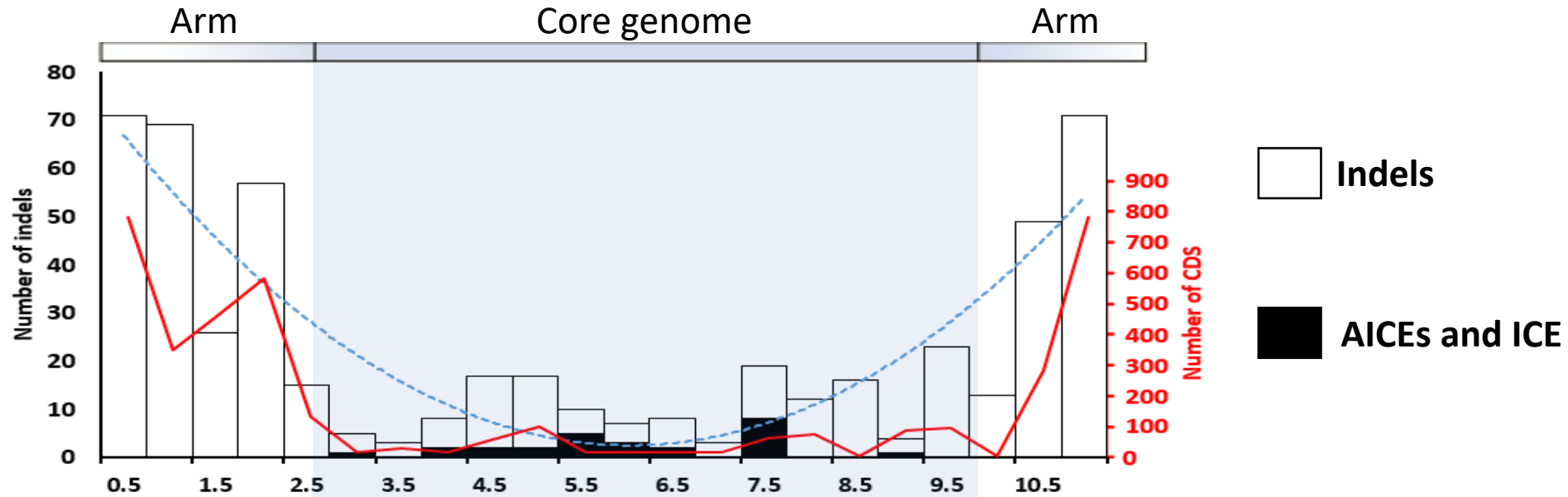
Do co-transfers of several AICEs occur?



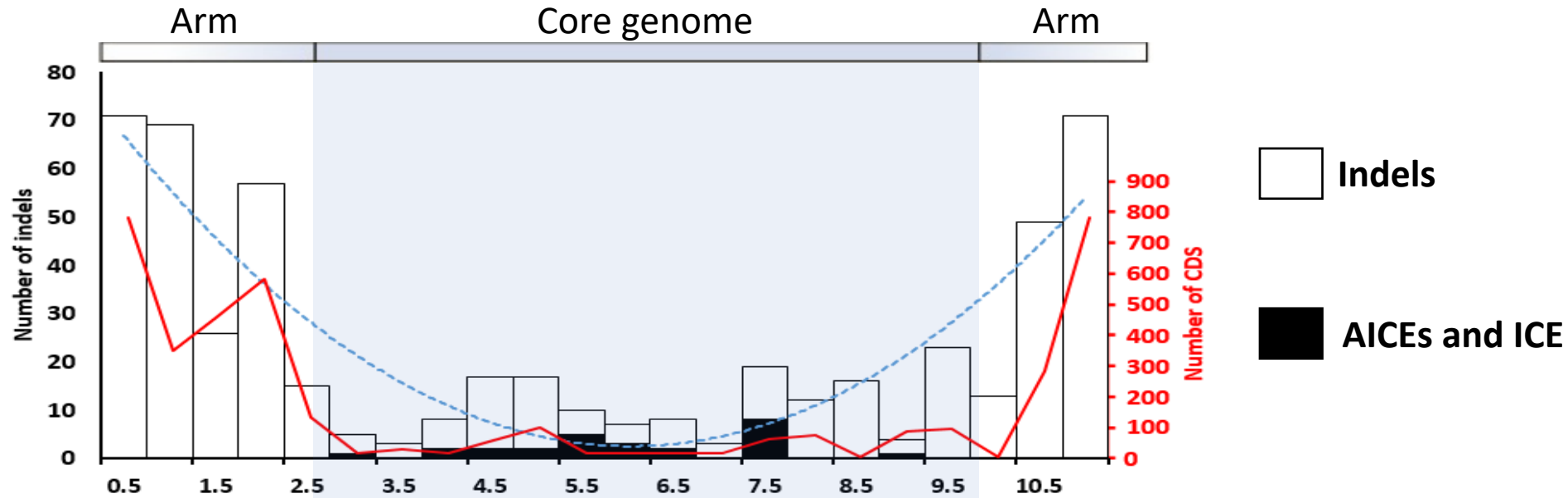
=> Transfer of **AICE10** from RLB3-6 to S1A1-3

**What is the impact of AICE transfers
in the population diversification ?**

Distribution of Indels along a consensus genome



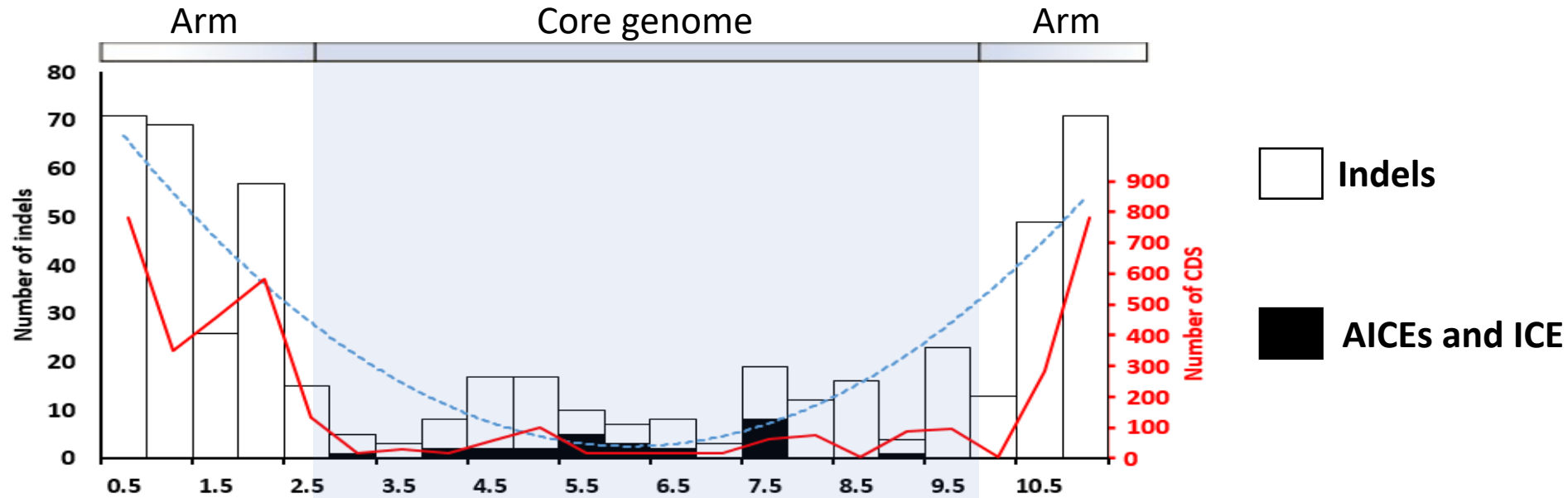
Distribution of Indels along a consensus genome



Indels are more located in chromosomal extremities

AICEs are more located in the chromosomal middle

Distribution of Indels along a consensus genome



Indels are more located in chromosomal extremities

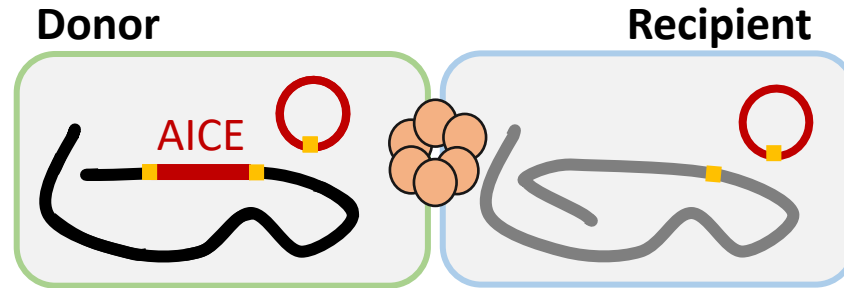
AICEs are more located in the chromosomal middle

Do AICEs promote genetic diversification of the population?

Are they responsible for the gradient of gene flux towards the terminal ends ?

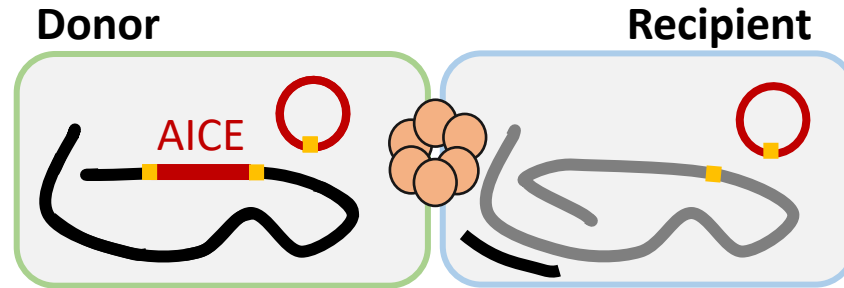
Mobilization of chromosomal DNA in *trans* by AICEs

The DNA translocase TraB is capable of mobilizing chromosomal DNA in *trans*,
i.e., not physically linked to the element.



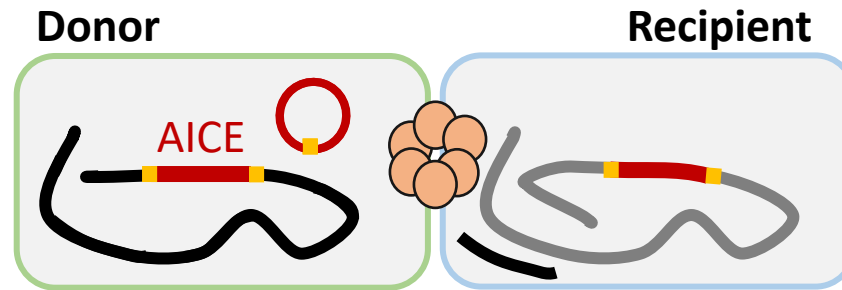
Mobilization of chromosomal DNA in *trans* by AICEs

The DNA translocase TraB is capable of mobilizing chromosomal DNA in *trans*,
i.e., not physically linked to the element.



Mobilization of chromosomal DNA in *trans* by AICEs

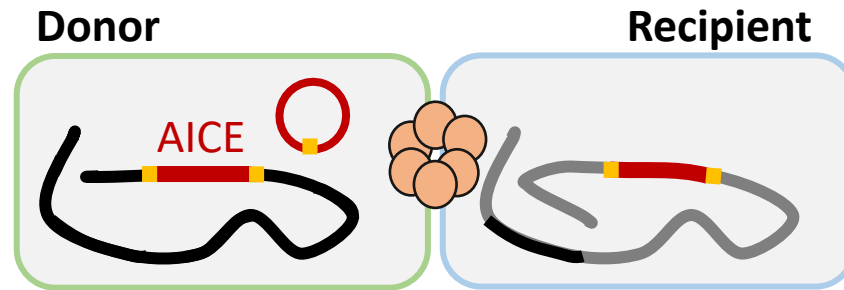
The DNA translocase TraB is capable of mobilizing chromosomal DNA in *trans*,
i.e., not physically linked to the element.



Perspectives

Extent of the mobilization of chromosomal DNA in *trans* by AICEs

The **trans mobilization** of DNA fragments during **mating** could contribute to the **genetic diversification** of a **population**



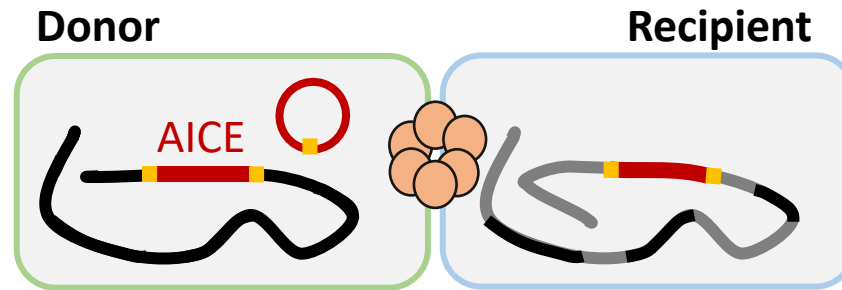
Trans-mobilisation

- **What is its extent?**

Perspectives

Extent of the mobilization of chromosomal DNA in *trans* by AICEs

The **trans mobilization** of DNA fragments during **mating** could contribute to the **genetic diversification** of a **population**

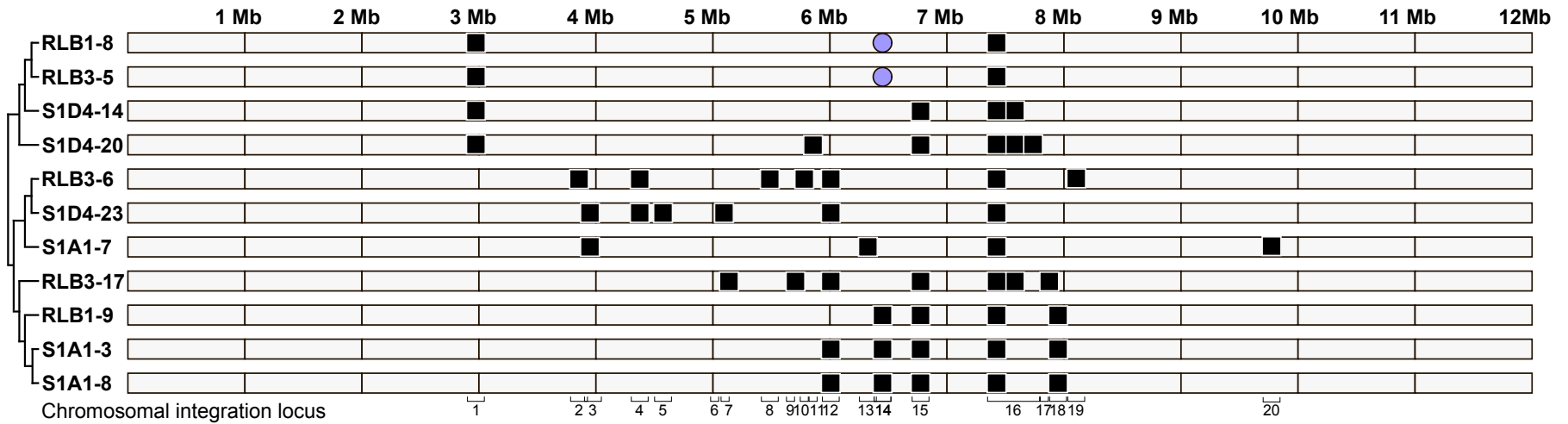


Trans-mobilisation

- **What is its extent?**
- **Does it contribute to the acquisition of new functions in the recipient?**

Perspectives

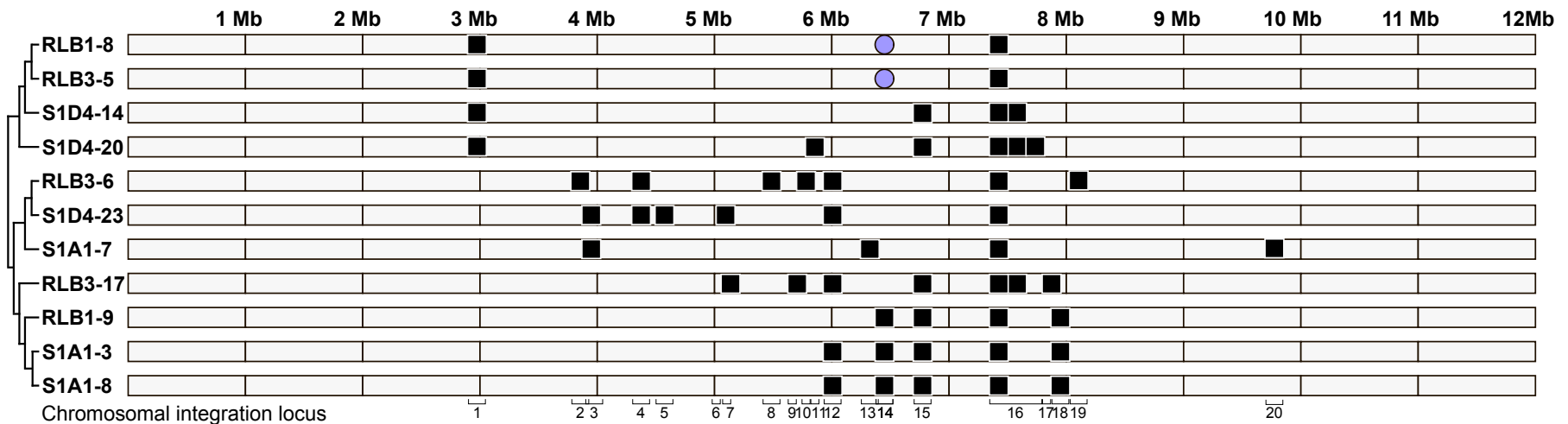
Is the T4SS-conjugation dependent effective in *Streptomyces*



Perspectives

Is the T4SS-conjugation dependent effective in *Streptomyces*

- Some scarce T4SS-ICEs have been predicted in silico in actinobacteria.
- The excision of two ICEs from *Frankia sp. Cci3* was confirmed by PCR
- The T4SS tranfer has never been experimentally demonstrated



**Is the transfer of the ICE14 of *Streptomyces sp. RLB1-8*
or *Streptomyces sp. RLB3-5* effective?**

UMR UL-INRA 1128, Nancy, France

Dynamique des Génomes et Adaptation Microbienne

- Abdoul-Razak Tidjani, PhD
- Anthony Gauthier, Technicien
- Harb Manar, ATER
- Cyril Bontemps, MCF
- Pierre Leblond, Pr

Université d'Exeter, Royaume-Uni

European Centre for Environment and Human Health

- Michiel Vos , *Senior Lecturer*

INRA IAM, Champenoux, France

- Claire Veneault-Fourrey, DR INRA

Société PAT (*Plant Advanced Technologies*)

- Frédéric Bourgaud, Vice-Président de Recherche



Merci pour votre attention
