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► **To cite this version:**

Nicolas Charbonnel, Damien Balestrino, Geneviève Bricheux, Richard Bonnet, Ousmane Traoré, et al.. A conjugation-based delivery vehicle with CRIPSR-Cas system targeting carbapenemase encoding genes. 15ème Congrès National de la SFM, Sep 2019, Paris, France. hal-04939950

HAL Id: hal-04939950

<https://hal.science/hal-04939950v1>

Submitted on 11 Feb 2025

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A conjugation-based delivery vehicle with CRISPR-Cas system targeting carbapenemase encoding genes

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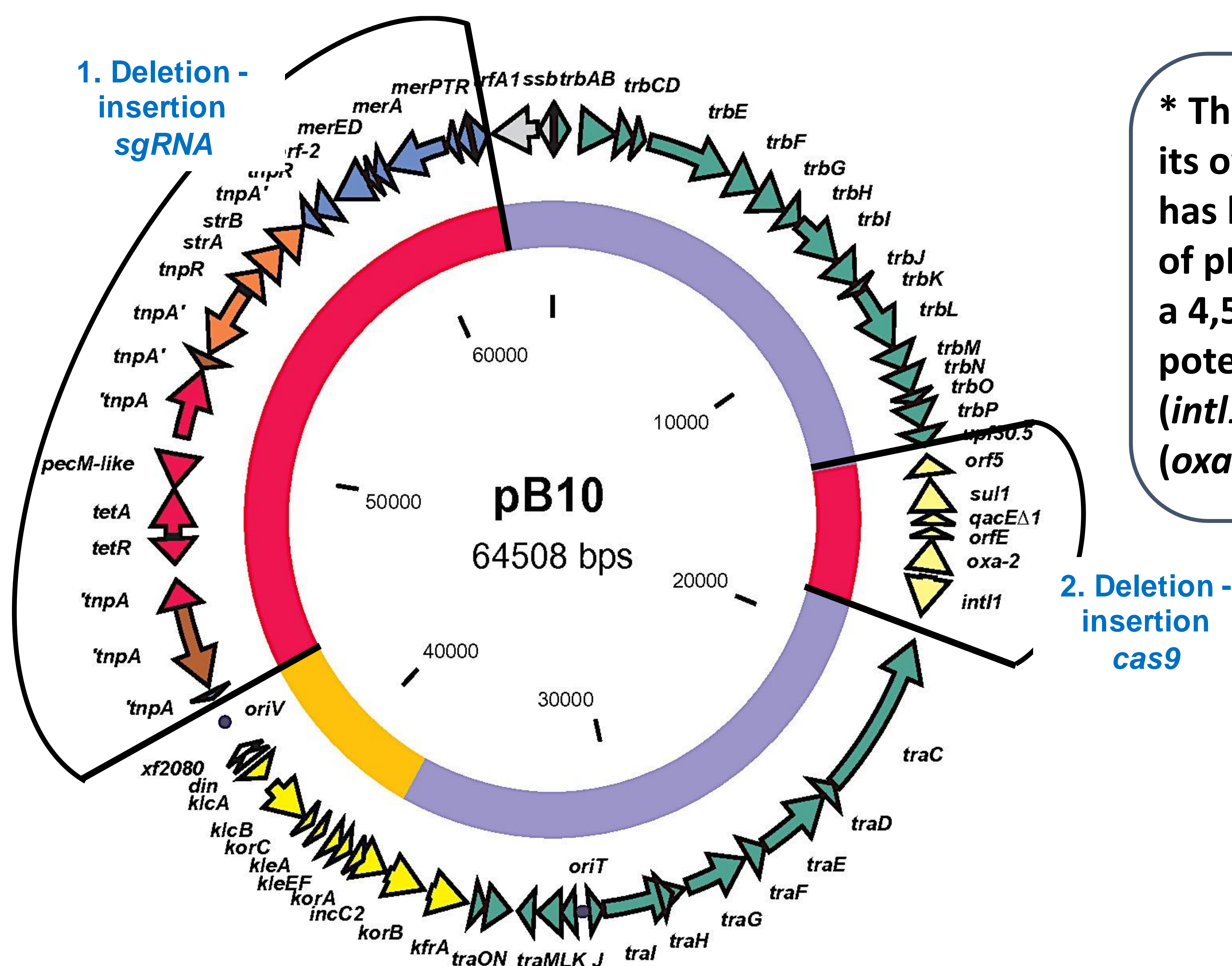
Background

The emergence of bacterial strains resistant to antibiotics is a major health threat. In view of that situation, and face to the lack of development of new antibiotics, various approaches have been proposed to reduce the emergence and spreading of antibiotic resistances. CRISPR-Cas system can be programmed to specifically target any DNA based on the information provided in the CRISPR array and can be exploited to target bacterial populations that carry antibiotic resistance genes.

The aim of this project is to develop genome-editing approach that imposes pressure on carbapenemase-encoding genes to reduce their prevalence in complex bacterial communities by using the CRISPR-Cas system.

The broad host range conjugative plasmid pB10 plasmid (IncP-1 group) isolated from a wastewater treatment plant is being used to carry the Cas9 encoding gene and the sgRNA targeting the carbapenemase encoding genes.

* pB10 plasmid contains numerous antibiotic resistance genes (pink segments) have been deleted independently of the mating and replication encoding regions. Allelic exchange enabled us to delete an 18,844 bp region containing genes coding for tetracycline, ampicillin and mercury resistances (region 1).



* The Cas9 encoding gene with its own promoter (5,673 bp) has been inserted in region 2 of pB10 (see Fig) by replacing a 4,537 bp-region harboring a potential integrase element (*int1*) and resistance cassettes (*oxa-2*, *qacE*, *sul*).

* Mating assays performed after each step indicated that the replacements had occurred on all copies of the plasmid (5-7 copies / cell) and that this slight version of the plasmid (48 kb) was still able to conjugate at the same rate

Region 1 will be replaced by the sgRNA encoding gene created by successive PCRs together with a fluorescent protein (GFP) encoding gene. The sgRNA have been designed by comparing the available carbapenemase-encoding genes to design representative sequences of the different groups of carbapenemases, OXA-48, NDM and KPC-type carbapenemases, which represent the most frequently isolated ones in *Enterobacteriaceae*.

Conclusion

The construction of this pB10 derivative constitutes the important first step of a strategy aiming to reduce the level of carbapenem resistance. The efficiency of this construct to diffuse and to target the genome of carbapenemase-encoding bacteria will be assessed in complex bacterial communities with high level of mating such as biofilms formed in hospital effluents.