Utilization of conjugative CRISPR/Cas9 system to eliminate antibiotic resistance plasmids in *Pseudomonas aeruginosa*

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INTRODUCTION

Pseudomonas aeruginosa

- One of the most common bacteria related to **nosocomial infections**
- Worldwide public health threat due to the appearance of **antibiotic resistances**
- Intrinsic, adaptive and acquired mechanisms that confer resistances
- Acquisition of antibiotic resistance genes via horizontal gene transfer (**HGT**), being conjugation the most predominant mechanism

CRISPR/Cas9 system

- **Adaptive immune system** of prokaryotes
- 2 main components:
  - **Cas9** → Endonuclease
  - **Single guide RNA (sgRNA)** → Fusion of trans-activating crRNA and CRISPR RNA
- Potential tool to **cure antibiotic resistance plasmids**

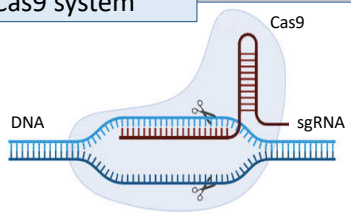


Fig. 1. Schematic representation of CRISPR/Cas9 technology.

HYPOTHESIS AND OBJECTIVES

A single conjugative plasmid with CRISPR/Cas9 system could be used as a strategy to cure antibiotic resistance plasmids and prevent their acquisition in *P. aeruginosa.*

1. To construct a conjugative plasmid with CRISPR/Cas9 technology and another one used as negative control
2. To validate the constructed plasmid's ability to conjugate and re-sensitize
3. To validate the constructed system to limit HGT
4. To assess *in vivo* the conjugative CRISPR/Cas9 system activity

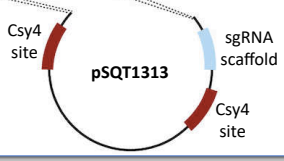
MATERIAL AND METHODS

1. Plasmids constructions

1.A. **Identification** of the target sequences with PAM located downstream
5' GACACTTCAACGATCCCCACCGG 3'
PAM

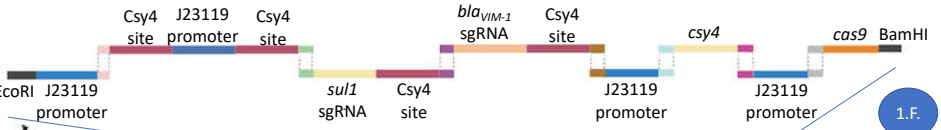
1.B. **Synthesis** of target sequences with complementary overhangs to sticky ends of pSQT1313

1.C. **Ligation** of the targets sequences into different digested pSQT1313 plasmids

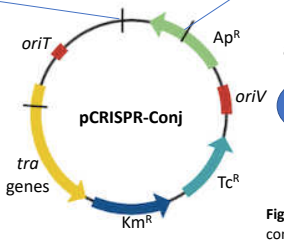


1.D. Individual amplifications of J23119 promoters, sgRNAs with Csy4 sites, *csy4* and *cas9* by **PCR** to add overlapping sequences

1.E. **Gibson Assembly** of the overlapping fragments previously obtained



1.F. **Digestion** of the assembled product and RK2 plasmid, which acts as backbone, and ligation



1.G. **Generation** of **pCas9**, which will be used as negative control

Fig. 2. Scheme of the procedure to construct pCRISPR-Conj and pCas9.

2. Curing assay

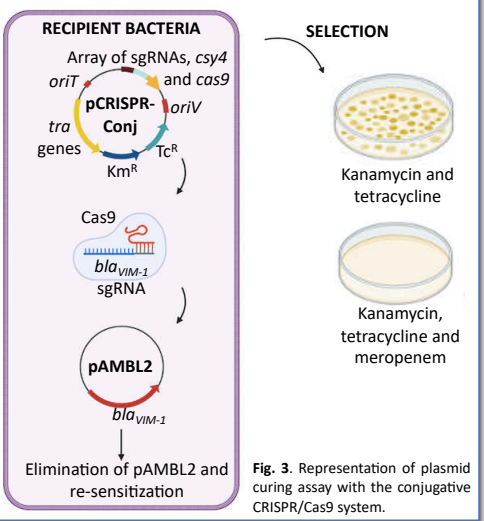


Fig. 3. Representation of plasmid curing assay with the conjugative CRISPR/Cas9 system.

3. HGT limitation assay

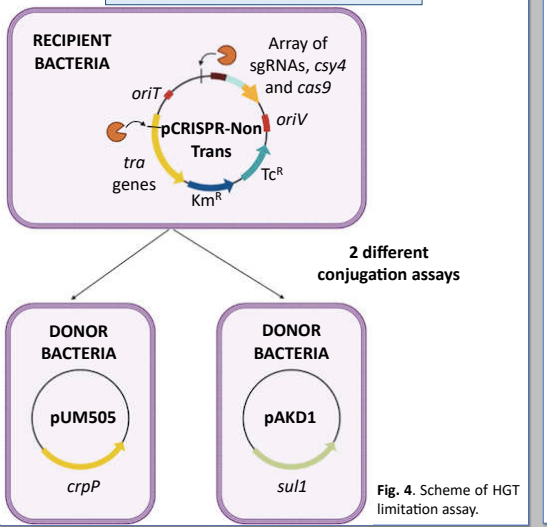


Fig. 4. Scheme of HGT limitation assay.

4. *In vivo* assessment

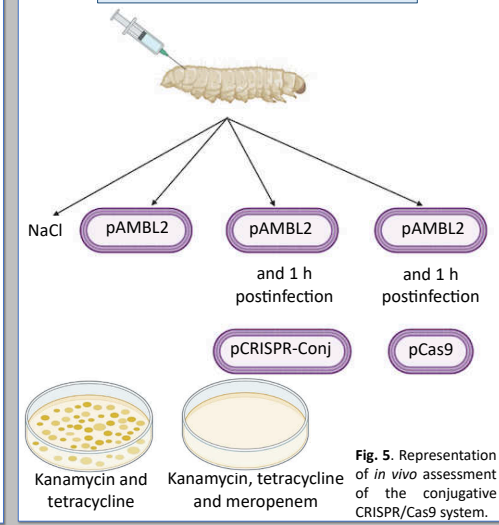


Fig. 5. Representation of *in vivo* assessment of the conjugative CRISPR/Cas9 system.

EXPECTED RESULTS

- It will be demonstrated if **pCRISPR-Conj is able to conjugate and cure antibiotic resistance plasmids *in vitro* and *in vivo*→ ~90%** of curing efficiency
- It will be shown whether **pCRISPR-Conj limits HGT→ 100-500-fold reduction** in **conjugation frequency**
- **Future applications** of the constructed system:
  - Reduce therapy's limitation and prolong the effective lifespan of conventional antibiotics
  - Cut down the exposure of patients to antibiotics
  - It would not be necessary to look into new antibiotics
  - Reduce antibiotic resistant *P. aeruginosa* in the human skin microbiota and in water-reservoirs
  - Decrease the amount of antibiotic resistant *P. aeruginosa* from water-reservoirs

DISSEMINATION PLAN

- **Publication of the methodology and results** in renowned scientific journals related to genetic engineering, medical research and antimicrobial resistances
- **Presentation via poster** at relevant national and international conferences

REFERENCES

1. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnol Adv. 2019;37(1):177–92. doi:10.1016/j.biotechadv.2018.11.013 **2.** Gholizadeh P, Köse Ş, Dao S, Ganbarov K, Tanomand A, Dal T, et al. How CRISPR-Cas system could be used to combat antimicrobial resistance. Infect Drug Resist. 2020;13:1111–21. doi:10.2147/idr.s247271 **3.** Kim JS, Cho DH, Park M, Chung WJ, Shin D, Ko KS, et al. Crispr/cas9-mediated re-sensitization of antibiotic-resistant escherichia coli harboring extended- spectrum β-lactamases. J Microbiol Biotechnol. 2015;26(2):394–401. doi:10.4014/jmb.1508.08080. **Images created with** BioRender.com.