Mobilization of the ColE1 plasmid with synthetic constructs

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Abstract

ColE1 is a plasmid widely used in cloning and expression vector technology. Its mobilization region consists of five genes, four of them involved in the mobilization process. A combination of these genes, either synthetically constructed or wild-type, were assembled in different vectors by isothermal assembly.

Their mobilization frequencies were tested using different conjugative plasmids in conjugation assays. Results were compared with the wild-type ColE1 mobilization frequencies. The degree of complementation between the synthetic plasmids and the wild-type ColE1 depends on the conjugative plasmid used.

Introduction

ColE1 is the prototype of a super-family of mobilizable plasmids found in gram-positive and gram-negative bacteria (Francia et al, 2004). It is mobilized by a wide array of conjugal plasmids, which attracted considerable scientific attention. The return of persistent bacterial infections, due to the development of multi-drug resistance, re-ignited the interest in the study of conjugation, and therefore, this plasmid.

Its mobilization region (Figure 1) consists of a cluster of five genes (*mbe*A, *mbe*B, *mbe*C, *mbe*D and *mbe*E) with two of them (*mbe*B and *mbe*D) completely overlapping mbeA (Boyd et al, 1989). Due to that complex structure, genetic manipulation of *mbe*B or *mbe*D gene without affecting *mbe*A is complicated.

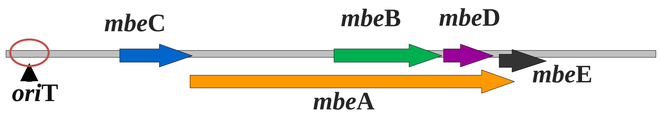

Figure 1. *Genetic structure of the ColE1 mobilization region.*

Figure 2. *Synthetic construct of the ColE1 mobilization region. Blue bars indicate restriction sites.*

The construct comprised four synthetic genes encoding proteins identical to *mbe*A, *mbe*B, *mbe*C and *mbe*D. Contrary to the wild type organization, *mbe*B and *mbe*D were not overlapping *mbe*A, but were located downstream, as shown in Figure 2. Unique restriction sites, upstream and downstream each gene, allow easy manipulation.

Surprisingly, it proved impossible to clone the construct shown in Figure 2 (ColE1-MOB^S) using pSU18 as cloning vector (Figure 3). Nevertheless, ColE1-MOB^S was successfully cloned as functional independent fragments, resulting in plasmids pAV18 (oriT-*mbe*C^S-*mbe*A^S), pAV19 (*mbe*B^S) and pAV23 (*mbe*D^S) (Figures 4, 5 and 6, respectively)(Varsaki A., unpublished data). The fragment (*mbe*B^S + *mbe*D^S) proved impossible to clone, for reasons beyond our understanding.

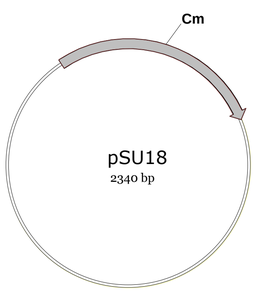

Figure 3. *Map of pSU18 plasmid.*

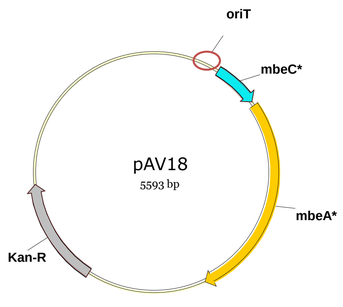

Figure 4. *Map of pAV18 plasmid.*

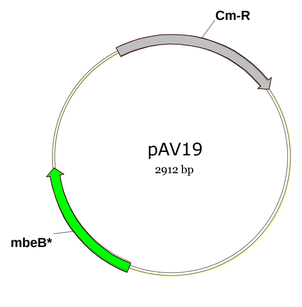

Figure 5. *Map of pAV19 plasmid.*

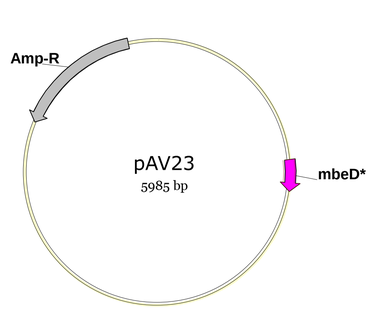

Figure 6. *Map of pAV23 plasmid.*

Objectives

- **Built** a plasmid containing the wild-type (mbeB+mbeD), compatible with pAV18.
- **Test** if it complements pAV18.
- **Compare** the conjugation frequencies with wild-type strains and strains lacking those genes.

Materials & Methods

- **Construction of pMCR1 plasmid (Figure 7).**
  Plasmid pSU18 was selected as vector and the wild-type genes *mbe*B and *mbe*D as insert. Cloning was performed by Gibson Isothermal Assembly and following standard cloning procedures (Sambrook et al. 1989)

- **Conjugation assays.**
  Were performed in order to test the degree of complementation between pAV18 and pMRC1. Conjugative plasmids used were R388, pRL443, R6K-drd1, R64-drd11 and R751. Donors were strain DH5α carrying the appropriate plasmids and strain HMS174 was used as receptor. For the assays, standard procedures were followed (Varsaki et al. 2003).

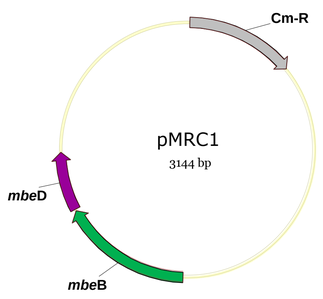

Figure 7. *Map of pMRC1 plasmid.*

Results

The mobilization frequencies of the synthetic constructs and the ColE1 plasmid were calculated by conjugation assays, using various conjugative plasmids. Values are shown in Figures 8-12. Results are the average of at least 6 repetitions.

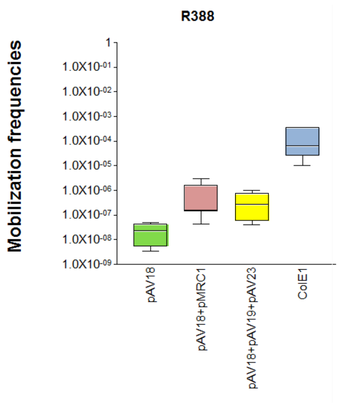

Figure 8. *Mobilization frequencies using the R388 conjugative plasmid.*

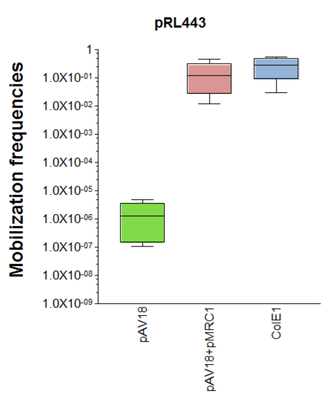

Figure 9. *Mobilization frequencies using the pRL443 conjugative plasmid.*

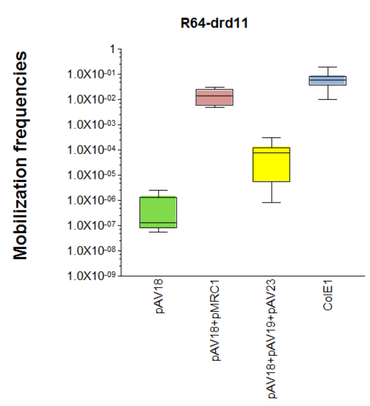

Figure 10. *Mobilization frequencies using the R64-drd11 conjugative plasmid.*

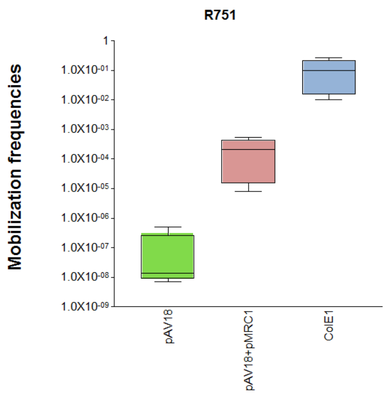

Figure 12. *Mobilization frequencies using the R751 conjugative plasmid.*

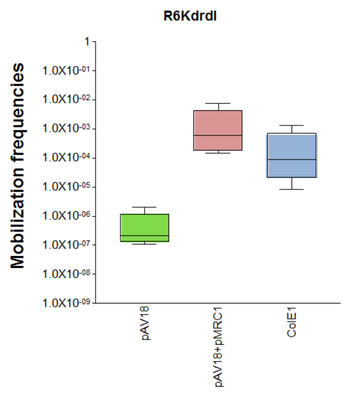

Figure 11. *Mobilization frequencies using the R64drdI conjugative plasmid.*

Conclusions

- The degree of complementation of pMRC1 to mobilize pAV18 depends on the conjugative plasmid used. Best functionalities were exhibited with plasmids pRL443, R64-drd11 and R6Kdrd1.

- Wild-type genes *mbe*B and *mbe*D (pMRC1) were more efficient at mobilizing the synthetic construction pAV18 than their synthetic version (pAV19+pAV23).

Acknowledgements

- I would like to thank the Intergenomics group because they are awesome.
- Fernando de la Cruz for the opportunity.
- Athanasia Varsaki for being my tutor.
- And specially Carolina for her patience and teachings.

References

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