

Precise and portable method for broad-host bacterial genome engineering

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ASK ME FOR a
FREE ALIQUOT
of pPORTMAGE!

Please write me if you have any
questions!
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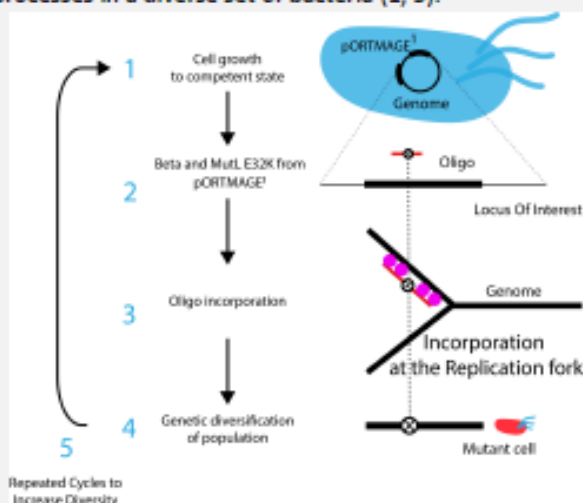
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Introduction

Recently developed tools for bacterial genome engineering (pPORTMAGE and DiVERGE) allow the rapid analysis of mutational effects and the reconstruction of evolutionary processes in a diverse set of bacteria (1, 3).



pPORTMAGE

High-Throughput *in vivo* mutation incorporation in a diverse set of Gram-negative bacteria

pPORTMAGE can rapidly insert mutations into the genome of a diverse set of Enterobacterial species (1).

As a practically useful application, pPORTMAGE allows the analysis of antibiotic resistance-conferring mutations in pathogens that are immediate global health threats.

pPORTMAGE-based mutagenesis is applicable to numerous species, including ESCAPE pathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella*, *Shigella* species, and their Multidrug-Resistant (MDR) variants.



pPORTMAGE is freely available:
www.addgene.org/Csaba_Pal

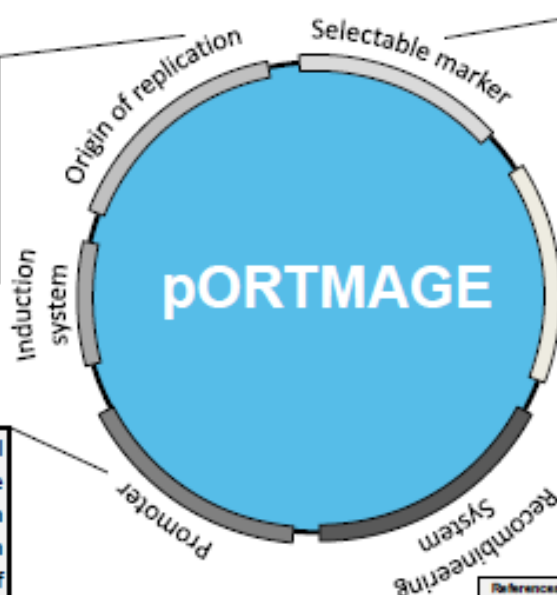


My goals

- 1, improve recombineering by using novel recombinases
- 2, generalized expression system that functions within a diverse set of species
- 3, apply novel selectable markers

The establishment of pPORTMAGE-variants with a diverse set of broad-host-range replication origins will ensure seamless plasmid maintenance in Gram-negative species that are immediate health threats.

In order to adapt pPORTMAGE to novel hosts, new expression platforms are needed. One solution to this problem can be to apply broad-host expression platforms to drive the production of recombinases and MutL (4).



Novel selectable markers to extend the host-range of pPORTMAGE to Multi Drug Resistant strains:

- Gentamycin^R
- Hygromycin^R
- Blasticidin^R
- Tellurite^R

Mutant homologues of EcMutL to extend host-specificity of Mismatch Repair Control.

Application of recently described, novel recombinases (2).

References

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3. Nyerges, Á. et al.: Directed evolution of multiple genomic loci allows the prediction of antibiotic resistance, *Proc Natl Acad Sci USA*, 2016 Jun 5, pii: 201601648.
4. Kuwahara, M. & Saito, H. M. A portable expression resource for engineering cross-species genetic circuits and pathways. *Nat Commun* 6, (2015)