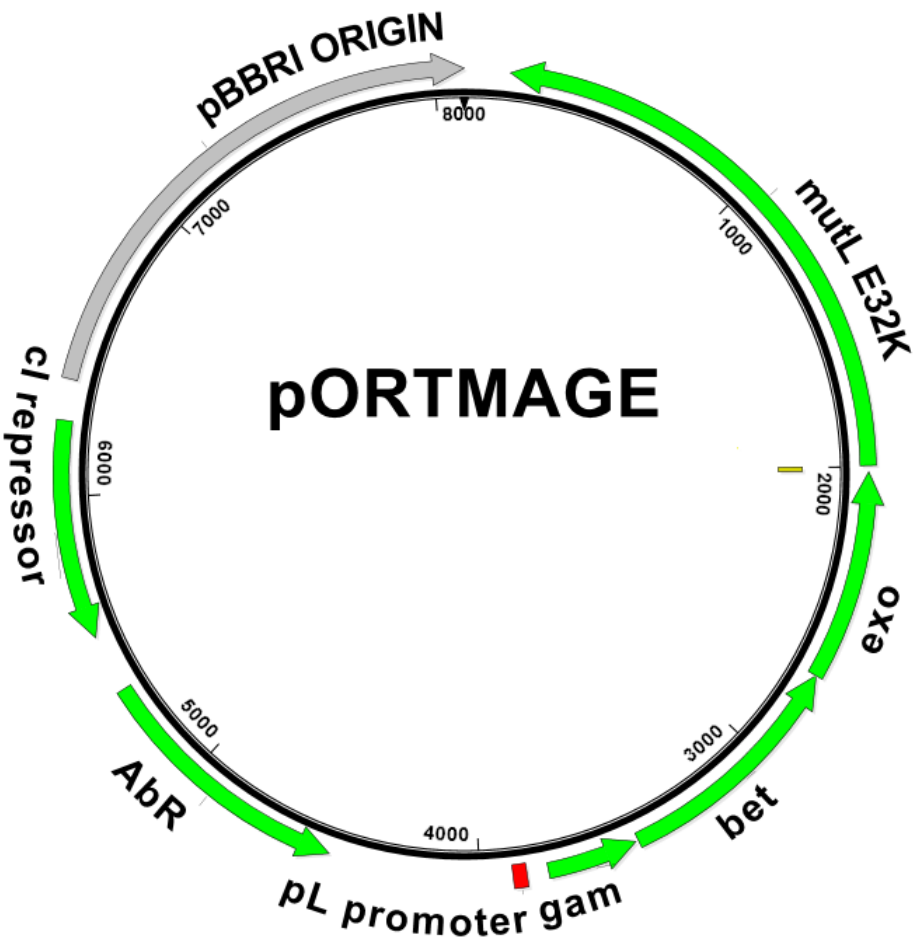
pORTMAGE & pSEVAMAGE Protocol

This page aims to help genome-engineering endeavors using the pORTMAGE or pSEVAMAGE plasmid sets, with a detailed description of our tried-and-tested experimental procedure.

Building on the prior development of Multiplex Automated Genome Engineering (MAGE) in *Escherichia coli* by Harris H. Wang and Farren J. Isaacs (Programming Cells by Multiplex Genome Engineering and Accelerated Evolution Nature 460 (7257): 894–98.) we present plasmid-based, portable Multiplex Automated Genome Engineering (pORTMAGE and pSEVAMAGE), a system that allows highly efficient modification of a wide variety of bacteria without producing any undesired, off-target effects.

Reference: Nyerges, Ákos, Bálint Csörgő, István Nagy, Balázs Bálint, Péter Bihari, Viktória Lázár, Gábor Apjok, et al. 2016. "**A Highly Precise and Portable Genome Engineering Method Allows Comparison of Mutational Effects across Bacterial Species**." Proceedings of the National Academy of Sciences, February, 201520040. doi: 10.1073/pnas.1520040113. www.pnas.org/cgi/doi/10.1073/pnas.1520040113

Using a dominant negative mutant protein of the methyl-directed mismatch repair (MMR) system, the plasmids allow transient suppression of DNA repair in enterobacterial species, which is necessary for efficient oligonucleotide integration. By integrating all necessary components into the Standard European Vector Architecture format, the pSEVAMAGE vector set facilitates rapid MAGE applications in a diverse set of enterobacterial species.


pBBRI ORIGIN
mutL E32K
exo
bet
pL promoter gam
AbR
cI repressor
pORTMAGE
8000
1000
2000
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4000
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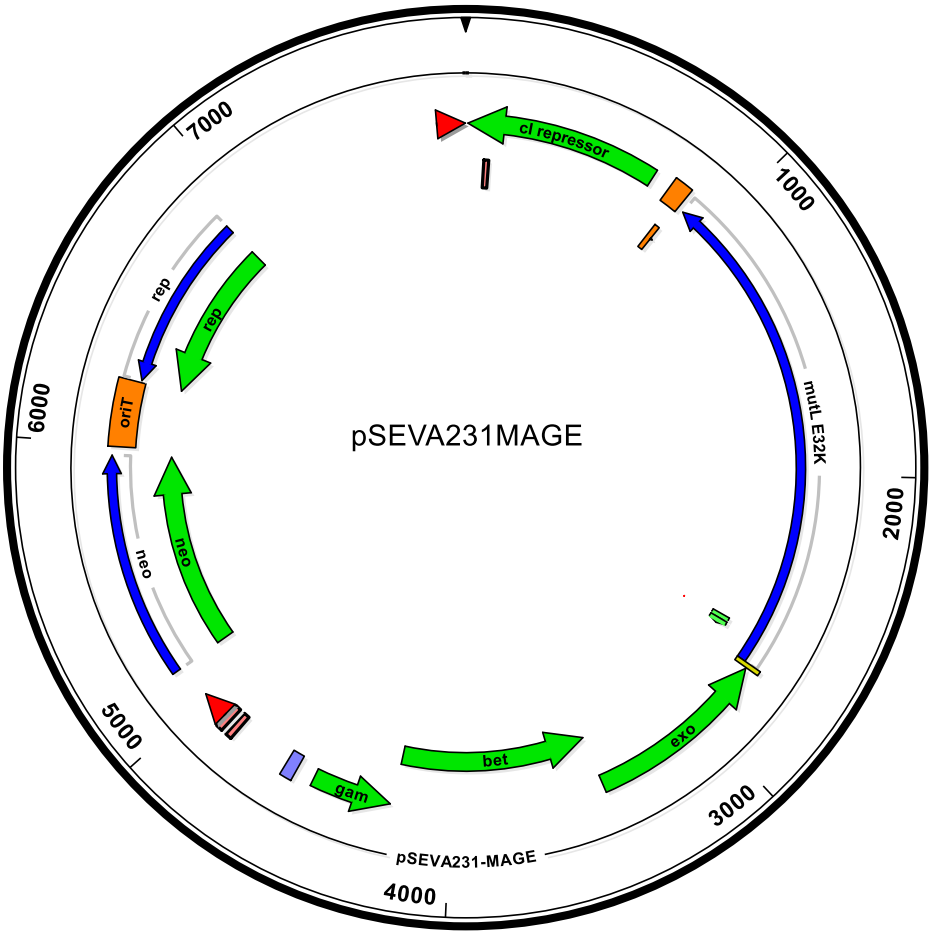
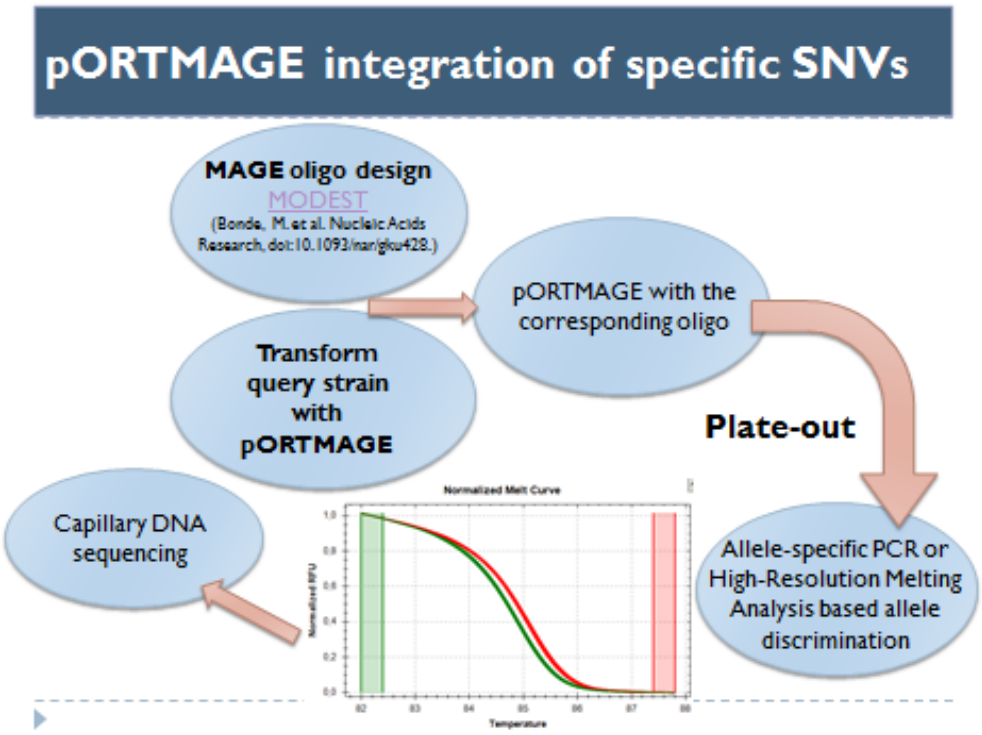


pORTMAGE and pSEVAMAGE allows efficient modification of multiple loci, without prior modification of the host genome. Due to the conserved nature of the bacterial MMR system, they simultaneously allow genome editing and mutant library generation in a wide range of biotechnologically and clinically relevant enterobacterial species.

The pORTMAGE and pSEVAMAGE plasmids easily incorporated into the standard workflow of MAGE (for background information and detailed design of MAGE experiments, see **References 1-6**) and both plasmids are available via the Standard European Vector Architecture (SEVA) (**http://wwwuser.cnb.csic.es/**) ; Addgene (ID# 72677; 72680; 72679; 72678) and directly from our laboratory (For direct strain request and further questions, please contact: nyerges.akos{at}brc.mta.hu).

Experimental workflow for pORTMAGE and pSEVAMAGE



Cells and reagents:

- Target species, transformed with the corresponding pORTMAGE, pSEVA221MAGE or pSEVA231MAGE plasmid:

Plasmids		
Plasmid	**Marker gene**	**Note**
pORTMAGE2	AmpR	derived from pSIM8 (from S. Datta et al. / Gene 379 (2006) 109–115)
pORTMAGE3	KanR	KanR derivative of pORTMAGE2
pORTMAGE4	CmR	CmR derivative of pORTMAGE3
pSEVA221MAGE	KanR	pSEVA221 based derivative of pORTMAGE3
pSEVA231MAGE	KanR	pSEVA231 based derivative of pORTMAGE3

- Oligonucleotides for MAGE (for design: see **Reference 1, 2, 4** and **6**):

Ordered with standard desalting from IDT and designed according to the general guideline (**Reference 2**): 90 nt length, 2 phosphorotioate bonds at each termini, minimized secondary structure (dG >-12 kcal/mol) and should be screened for mistargets on the corresponding genome to avoid false hybridization.

- Chilled water (+4°C) for electrocompetent cell preparation
- Luria-Bertani-Lennox (LB^L) broth: 10 g of tryptone, 5 g of yeast extract, 5 g of sodium chloride per 1L of water
- Antibiotic-supplemented LB^L broth and agar plates with the corresponding antibiotic
- Terrific-broth (TB): yeast extract 24 g, tryptone 12 g, K_2HPO_4 9.4 g, KH_2PO_4 2 g per 1L of water

Labware:

- Shaking water bath (42°C)
- Shaking incubator for flasks (30°C)
- BioRad MicroPulser or BTX (Harvard Apparatus) CM-630 Exponential Decay Wave Electroporation System
- Refrigerated centrifuge with swing-out rotor
- Electroporation cuvettes with 1 mm (VWR Signature Electroporation Cuvette) gap
- 150 ml glass flask

MAGE cycling protocol:

- Start with a freshly streaked, individual colony from an LB^L agar + antibiotic plate (grown at 30°C, overnight)
- Inoculate into a glass flask, containing 2 ml LB^L aliquot, supplemented with the corresponding antibiotic
- Incubate in a shaking incubator at 250 rpm at 30 °C overnight
- From the stationary phase culture, dilute cells 100-fold in LB^L, supplemented with the corresponding antibiotic in a 150 ml glass flask
- Incubate in a shaking incubator at 250 rpm at 30 °C
- Upon reaching OD_{550} 0.55-0.65, transfer cells to the 42°C shaking water bath to induce λ Red protein expression for 15 min at 250 rpm
- Place cells immediately on ice and chill for at least 5 min
- Prepare electrocompetent cells by washing and pelleting them twice in 10 ml ice-cold dH_2O at 3800 rpm for 7 min in a refrigerated centrifuge with swing-out rotor at 4 °C
- Suspend cells in 160 μl dH_2O and keep on ice until electroporation
- For electroporation, mix 40 μl of competent cells with 1 μl 100 μM MAGE oligo (resuspended in TE buffer)
- Electroporate cells in a pre-chilled 1 mm gap cuvette (e.g. VWR Signature Electroporation Cuvette) using a BioRad MicroPulser or BTX (Harvard Apparatus) CM-630 Exponential Decay Wave Electroporation System (parameters: 1800 V, 25 μF, 200 Ω)
- Immediately after electroporation, add 1 ml room temperature TB medium
- Transfer cell suspension immediately to 4 ml prewarmed (30 °C) TB medium, in a 150 ml glass flask
- Allow to recover for 60 min at 30°C at 250rpm
- Add 5 ml room-temperature LB^L medium supplemented with the corresponding antibiotic
- Grow cells at 30 °C until mid-logarithmic state (OD_{550} 0.55-0.65) at 250 rpm
- At this point, cells could be either subjected to additional MAGE cycles or assayed for phenotype and genotype analysis

References and further information:

1. Wang, Harris H., Farren J. Isaacs, Peter A. Carr, Zachary Z. Sun, George Xu, Craig R. Forest, and George M. Church. 2009. "Programming Cells by Multiplex Genome Engineering and Accelerated Evolution." Nature 460 (7257): 894–98. doi:10.1038/nature08187.
2. Gallagher, Ryan R., Zhe Li, Aaron O. Lewis, and Farren J. Isaacs. 2014. "Rapid Editing and Evolution of Bacterial Genomes Using Libraries of Synthetic DNA." Nature Protocols 9 (10): 2301–16. doi:10.1038/nprot.2014.082. **Detailed resource for MAGE experiment design**
3. Carr, Peter A., Harris H. Wang, Bram Sterling, Farren J. Isaacs, Marc J. Lajoie, George Xu, George M. Church, and Joseph M. Jacobson. 2012. "Enhanced Multiplex Genome Engineering through Co-Operative Oligonucleotide Co-Selection." Nucleic Acids Research 40 (17): e132–e132. doi:10.1093/nar/gks455.
4. Bonde, Mads T., Mads V. Anderson, Annika I. N. Wallin, Harris H. Wang, and Morten O. A. Sommer. 2014. "MODEST: A Web-Based Design Tool for Oligonucleotide-Mediated Genome Engineering and Recombineering." Nucleic Acids Research, May, gku428. doi:10.1093/nar/gku428. **Online tool for MAGE oligo design** http://modest.biosustain.dtu.dk/doc/modest_online.html
5. Nyerges, Ákos*, Bálint Csörgő*, István Nagy, Dóra Latinovics, Béla Szamecz, György Pósfai, and Csaba Pál. 2014. "Conditional DNA Repair Mutants Enable Highly Precise Genome Engineering." Nucleic Acids Research 42 (8): e62–e62. doi:10.1093/nar/gku105.
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7. Silva-Rocha, Rafael, Esteban Martínez-García, Belén Calles, Max Chavarría, Alejandro Arce-Rodríguez, Aitor de las Heras, A. David Páez-Espino, et al. 2013. "The Standard European Vector Architecture (SEVA): A Coherent Platform for the Analysis and Deployment of Complex Prokaryotic Phenotypes." Nucleic Acids Research 41 (D1): D666–75. doi:10.1093/nar/gks1119. http://www.cnb.csic.es/~seva/?page_id=25