Supplementary data

Low level expression of the Type II restriction-modification system confers potent bacteriophage resistance in *Escherichia coli*

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Supplementary Figure S1. Genetic map of pACYCeco and pIM-RM plasmids carrying EcoRI R-M system.
**Supplementary Figure S2.** Potential for EcoRI endonuclease production and *in vitro* restriction activity detected at cell lysates carrying pBAD-RM plasmid.

(A) Production level of M.EcoRI and R.EcoRI proteins after 1, 2, 3 and 4 hours of 0.04% arabinose induction of *ecoRIRM* operon expression.

(B) Digestion of lambda phage DNA with serial dilutions of bacterial lysate after 2 hours of induction. Bacterial lysate was prepared by Belavin method (Belavin et al., 1988). The reaction mixture contained 0.4 μg λ DNA and the appropriately diluted lysate. Digestion was carried out in optimal buffer for R.EcoRI at 37°C for 30 min. K - λ DNA digested with 10 u of R.EcoRI (Fermentas).
Supplementary Figure S3. Relative restriction of above constructs expressed in Xer/cer mutant strains as value of efficiency of plaque forming units.
**Supplementary Table S1.** Plasmids used in this study.

| Name            | Relevant characteristic(s) or sequence                                                                 | Source                        |
|-----------------|--------------------------------------------------------------------------------------------------------|-------------------------------|
| pACYC184        | P15A derivative, Tc<sup>R</sup>, Cm<sup>R</sup>                                                                 | Chang and Cohen, 1978         |
| pACYC-35T       | pACYCp derivative with single deletion mutation in -35 sequence (TTAAGG→aTAAGG)                          | this work                     |
| pACYC-35T2      | pACYCp derivative with double deletion mutation in -35 sequence (TTAAGG→aaAAGG)                          | this work                     |
| pACYCara        | pACYCcer derivative with *araC*-P<sub>araBAD</sub> promoter cloned into Smal site                         | this work                     |
| pACYCblaRBS     | pACYCpR derivative with partial *bla* RBS region of pBR322                                               | this work                     |
| pACYCcer        | pACYCeco derivative with broad deletion (1476-3909 bp[SmaI]) encompassing *tetA* gene and P<sub>L1-nutL</sub> region, while retaining all Xer/cer recombination region, Cm<sup>R</sup> | this work                     |
| pACYCΔnutL      | pACYCeco derivative with deletion including *nutL* region                                                | this work                     |
| pACYCΔnutLacZ   | pACYCΔnut derivative with insertion of a promoterless *lacZ* gene (SmaI-DraI of pRS415) between MluI-BglII (deletion of P<sub>R</sub> promoter and a proximal part of *ecoRIR* gene) | this work                     |
| pACYCeco        | Wild type EcoRI R-M system with additional upstream *P<sub>L1-nutL</sub>* region of λ phage in pACYC184, Cm<sup>R</sup>Tc<sup>R</sup> | Katna et al., 2010            |
| pACYCecoARAlacZM| pACYCara derivative with *ecoRIRK113A* gene and *lacZ* gene inserted in SpeI site of *ecoRIM* as translational fusion, subcloned frompACYClacZM  | this work                     |
| pACYCecoARAlacZR| pACYCara derivative with PCR created *lacZ* gene inserted in BglIII site of *ecoRIR* gene as translational fusion | this work                     |
pACYClacZ  pACYCeco derivative with promoterless *lacZ* gene (SmaI-DraI of pRS415) inserted between MluI-BglII (deletion of the *Pr* promoter and a proximal part of *ecoRIR* gene)  this work

pACYClacZM  pACYCeco derivative with *ecoRIRK113A* gene and *lacZ* gene inserted in SpeI site of *ecoRIM* as translational fusion, subcloned from pIM-RMI lacM  this work

pACYClacZR  pACYCeco derivative with PCR created *lacZ* gene inserted in BglIII site of *ecoRIR* gene as translational fusion  this work

pACYCxerCD  pACYCcer derivative with deletion of *Pcer* promoter and binding sites for ArgR and PepA (box1) retaining PepA box2, *xerC* and *xerD* binding sites  this work

pACYCpR  pACYCeco derivative with minimal *Pr* promoter (deletion downstream of XbaI site:1429-4042 bp)  this work

pACYCpR*  pACYCp with triple mutation of -35 sequence of *Pr* (TTAAGG→TTGACA)  this work

pACYCpR*RBS  pACYCpR* derivative with partial *bla* RBS region of pBR322  this work

pACYCtet  pACYCpR derivative with *PtetA* promoter instead of *Pr*  this work

pBAD24  pBR322 ori replicon with arabinose *ParaBAD* inducible promoter, *Ap*<sup>R</sup>  Guzman et al., 1995

pBAD24N  pBAD24 derivative with *E. coli* λ phage N antitermintor protein gene cloned in XbaI site, under *ParaBAD* control  this work

pBADecoM  pBAD24 derivative with *ecoRIM* gene cloned between NcoI-HindIII sites  this study

pBAD-RM  *ecoRIRM* genes cloned under *araC*-*ParaBAD* control unit(insertion of NcoI site overlapping translation initiation site of *ecoRIR* gene (CCATGG) caused substitution of the second codon Ser to Ala (TCT→GCT) in R. EcoRI protein  this work

pBR322  pMB1 replicon Tet<sup>R</sup>, *Ap*<sup>R</sup>  Bolivar et al., 1977

pIM-RM  Wild type EcoRI R-M system in pACYC184 backbone; *Cm*<sup>R</sup>  Mruk et al., 2011
| Vector | Description | Source |
|--------|-------------|--------|
| pIM27  | Restriction defective pIM-RM derivative with deleted HindIII-BglII fragment of *ecoRIR* | Mruk et al. 2011 |
| pIM-RM113A | pIM-RM derivative with mutagenized *ecoRIR* gene (K113A) causing production of inactive EcoRI ERase | this work |
| pIM-RMkan | pIM-RM derivative with HincII fragment with *kan* kanamycine resistance gene inserted in PvuII-PvuII promoter-proximal part of the *cat* gene, Km<sup>R</sup> Cm<sup>S</sup> | this work |
| pIM-RMlacZM | pIM-RMspeIM derivative with PCR created *lacZ* gene inserted in SpeI site of *ecoRIM* gene as translational fusion | this work |
| pIM-RMlacZR | pIM-RM derivative with PCR created *lacZ* gene inserted in BglII site of *ecoRIR* gene as translational fusion | this work |
| pIM-RMspeIM | pIM-RM derivative with HincII fragment with *tetA* tetracycline resistance gene inserted in PvuII-PvuII promoter-proximal part of the *cat* gene, Tet<sup>R</sup> Cm<sup>S</sup> | this work |
| pINTtsCm | λ <sup>−</sup> Int-delivery pSC101Ts replicon containing thermoinducible expression cassette cI857-P<sub>K::-int</sub>, Cm<sup>R</sup> | Hasan et al., 1994 |
| pKRP11 | The source of *kan* kanamycine resistance gene | Reece and Phillips, 1995 |
| pRS415 | The source of *lacZ* gene for transcriptional/translational fusion | Simons et al., 1987 |
| pUC18 | *P<sub>lac</sub>* expression vector, Ap<sup>R</sup> | Yanisch-Perron et al., 1985 |
**Supplementary Table S2.** List of oligonucleotides used in this study.

| No | Name     | Sequence (5’→3’) | Comment                                                                 |
|----|----------|------------------|------------------------------------------------------------------------|
| 1  | 35dT (f) | TAAGGGATTATGGTAAATCAAACG | Primers 1 and 34 were used to PCR mutagenization by deletion of the -35 sequence of P<sub>R</sub> promoter (ΔT)TAAGG, (pACYC-35T) |
| 2  | 35dT2 (f) | AAGGGATTATGGTAAATCAAACG | Primers 2 and 34 were used to PCR mutagenization of the -35 sequence of P<sub>R</sub> promoter by deletion (ΔTT)AAGG, thus only -10 extended promoter TGTATAATA remained (pACYC-35T2) |
| 3  | blaRBS (f) | ATAAATGCTTCAATAATATTGGAACATGGATT CATGTCTAAT | Primers 3 and 29 used to exchange downstream part of P<sub>R</sub> promoter (between NdeI site and GAA - SD sequence) by part of corresponding fragment from bla gene (underlined); ATG initial codon bold (pACYCblaRBS) |
| 4  | crRNAf   | TCGGAATTACTGGGCGTAAAG | Primers 4 and 5 specific to 16S rrn genes, were used for RT-qPCR |
| 5  | crRNAr   | CCTCCAGATCTCTACGCATTTC | |
|   | Primer Code | Sequences | Description |
|---|-------------|-----------|-------------|
| 6 | ecoMfor     | GCATTTGCTATGTAGAGAATAAAGAA | Primers 6 and 7 specific to *ecoRIM* gene were used for RT-qPCR |
| 7 | ecoMrev     | AGATCAATGCTCTCCGAACTG |             |
| 8 | ecoRfor     | GGAGATCAAGATTTAATGGCTGC | Primers 8 and 9 specific to *ecoRIR* gene were used for RT-qPCR |
| 9 | ecoRrev     | CAACCCTCCATCTGGTCTT |             |
| 10| Enullf      | TGCTGAAGCCGACACCAAGG | Primers 10 and 11 used to alter 113 lysine (AAA) to alanine residue (GCA) of *ecoRIR* gene |
| 11| Enullr      | TG**T**Cggcttcagcaaca |             |
| 12| lacBf       | GGAATCTATTTACGATCTCACTG GCC | Primers 12 and 13 used for PCR production of *lacZ* gene to translational fusion with *ecoRIR* gene from BglII site (underlined). Note that LacZ is lacked by first 3 natural amino acids, thus *lacZ* gene starting from ATT (in bold) |
| 13| lacBr       | GCAGATCTGGCCTGCCCCGGTTAT |             |
| 14| lacMf       | GGAC**T**GTATTTACGATCTCACTG GCC | Primers 14 and 15 used for creation of *lacZ* gene to translational fusion with *ecoRIM* gene from SpeI site (underlined). Note that LacZ is lacked by first 3 natural amino acids, thus *lacZ* gene starting from ATT (in bold), |
| 15| lacMr       | GGAC**T**GTGGCCTGCCCCGGTTAT |             |
| 16| MecoEnd (r) | GACGAAGCTTTATGATCTCAAGAAA | Primers 16 and 27 used for PCR production of the complete |
|   | Primer | Sequence |
|---|--------|----------|
| 17 | Nend (r) | TCTAAGCTTCTAGATAAGAGGAATC |
| 18 | Nfor (f) | AAGTCTAGAAAGCTAACTGACAGGAGA |
| 19 | nutL (r) | GGGCAAATCCCTGTGTTGGTTGGGG |
| 20 | nutR (f) | GGGTGTCAGTGCCTGCTGCTG |
| 21 | PcerL (r) | GGGCTGACTTCAGGTGCTACATTGG |
| 22 | PcerR (f) | GGGTCTGACCATCGTGGTGCTAGGG |
| 23 | Peco35 (f) | CTagatacaattgacagattatggtaaatcacaagctatgtaaatcatactatcgaca |
| 24 | Peco35 (r) | CTagatacaattgacagattatggtaaatcacaagctatgtaaatcatactatcgaca |

Promoterless *ecoRIRM* operon to clone in pBAD24 vector under arabinose $P_{araBAD}$ inducible promoter, between NcoI-HindIII sites of (pBAD-RM). Primers 17 and 18 were used to PCR creation of *E. coli* $\lambda$ phage N antiterminator protein gene to clone in XbaI site (underlined) of pBAD24 vector, under arabinose $P_{araBAD}$ inducible promoter (pBAD24N). Primers 19 and 20 used to deletion of *nutL* region of pACYCeco by PCR. SmaI site (underlined) in junction was created (pACYCΔnut). Primers 21 and 22 used to PCR deletion of 2433 bp fragment of pACYCeco carrying *tetA* gene and $P_{L1-nutL}$ region. SmaI site (underlined) was created in junction (pACYCcer). Primers 23 and 24 were used to anneal and insert between XbaI and NdeI sites (underlined) of pACYCeco, to change -35 sequence (in bold) of the minimal $P_{R}$ promoter (TTAAGG→TGACA), (pACYCp*).
|    | Primer | Sequence                                                                 | Description                                                                                      |
|----|--------|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| 25 | PecoF (f) | CTAGATACAATTTAAGGATTATGTAATCAAANCGTATGTTAAATCTATCGACAGA                  | Primers 25 and 26 were used to anneal and insert between XbaI and NdeI sites (underlined) of pACYCeco, to create the minimal P<sub>R</sub> promoter region, -35 and -10 sequences in bold, (pACYCp) |
| 26 | PecoR (r) | TATGTCGATGATTAACATACGTTTGATTTACCATAAATCCCTATTATGATAT                    |                                                                                                 |
| 27 | Pmlu (f) | GGGATCGCGCTGAACGCGTTTTAGC                                                | 27 and 21 used to delete P<sub>cer</sub> promoter and binding sites for ArgR and PepA (box1), while retaining PepA box2, xerC and xerD binding sites, and also P<sub>R</sub> promoter (pACYCxerCD) |
| 28 | PEcoNco (f) | CATGCATGGCTAATAAAAAACAGTCA                                               | Primer specific to a proximal part of ecoRI gene, NcoI sites underlined, ATG bold, substitution Ser2Ala (TCT→GCT) |
| 29 | PRNde (r) | CATATGTCGAGATTAACATACGT                                                  | Primer 29 was used in pair with primer 3                                                        |
| 30 | SpeMf   | GGTNTTACTAGTACGGCGTGCGTTTTAGC                                            | Primers 30 and 31 used to create SpeI site (underlined) in ecoRIM gene. Position of A nucleotide (in bold) is 253 nt of the gene |
| 31 | SpeMr   | CGCCTACTAGTATACCCCCAGTGTTATTATTC                                         |                                                                                                 |
| 32 | TETp1 (f) | CTAGATTATGTGGCAGCCTGTATCATGATAAGCGTTAATGCGGTAGTTATTAC                   | Primers 32 and 33 were used to anneal and insert between XbaI and NdeI sites (underlined) of pACYCeco, to create the construct pACYCtet, when ecoRIM genes are under control of constitutive P<sub>tetA</sub> promoter |
| 33 | TETp2 (r) | TATGATAAAACTACCCGATTAAAGCTTTATGGATGATAAAGCTGAACATATAAT                    |                                                                                                 |
|   | Xbaleft (r) | TTGTATCTAGAAATTTTTATCTGATTAATAAG | Primer 34 is a pair for 1 and 2, XbaI sequence is underlined |
|---|-------------|----------------------------------|-------------------------------------------------------------|
| 34| XecoMBad    | AGCCATGGCTAGAAATGCAACAAACAAAG   | Primers 34 and 17 used to PCR creation of *ecoRM* gene clone into NcoI-HindIII sites of pBAD24 |
**Supplementary Table S3.** Comparison of efficiency of plaque forming units of $\lambda_{vir}$ phage after infection of *E. coli* ER1992 bacteria with constructs derived from pACYCeco plasmid

| Plasmid         | PFU               | EOP      | Restriction relative to pIM-RM |
|-----------------|-------------------|----------|---------------------------------|
| pACYCeco        | $(5.2 \pm 0.9) \times 10^3$ | $1.6 \times 10^{-5}$ | 1250                            |
| pACYCcer        | $(5.7 \pm 1.9) \times 10^3$ | $1.8 \times 10^{-5}$ | 1111                            |
| pACYCpR*        | $(6.7 \pm 4.0) \times 10^3$ | $2.1 \times 10^{-5}$ | 952                             |
| pACYCpR         | $(8.3 \pm 3.3) \times 10^3$ | $2.6 \times 10^{-5}$ | 769                             |
| pACYCxerCD      | $(1.6 \pm 0.1) \times 10^4$ | $5.0 \times 10^{-5}$ | 400                             |
| pIM-RMkan       | $(5.3 \pm 0.9) \times 10^4$ | $1.6 \times 10^{-4}$ | 125                             |
| **pIM-RM**      | $(7.0 \pm 0.9) \times 10^6$ | 0.02     | 1                               |
| pACYCblaRBS     | $(1.2 \pm 0.7) \times 10^7$ | 0.04     | 0.5                             |
| pBAD-RM         | $(4.2 \pm 0.4) \times 10^7$ | 0.13     | 0.15                            |
| pIM27           | $(3.2 \pm 1.5) \times 10^8$ | 1        | 0.02                            |
SUPPLEMENTARY REFERENCES

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