**SUPPLEMENTARY MATERIAL**

# Periplasmic expression of a restriction endonuclease in *Escherichia coli* and its effect on the antiviral activity of the host

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## SUPPLEMENTARY METHODS

### Quantification of Gfp fluorescence

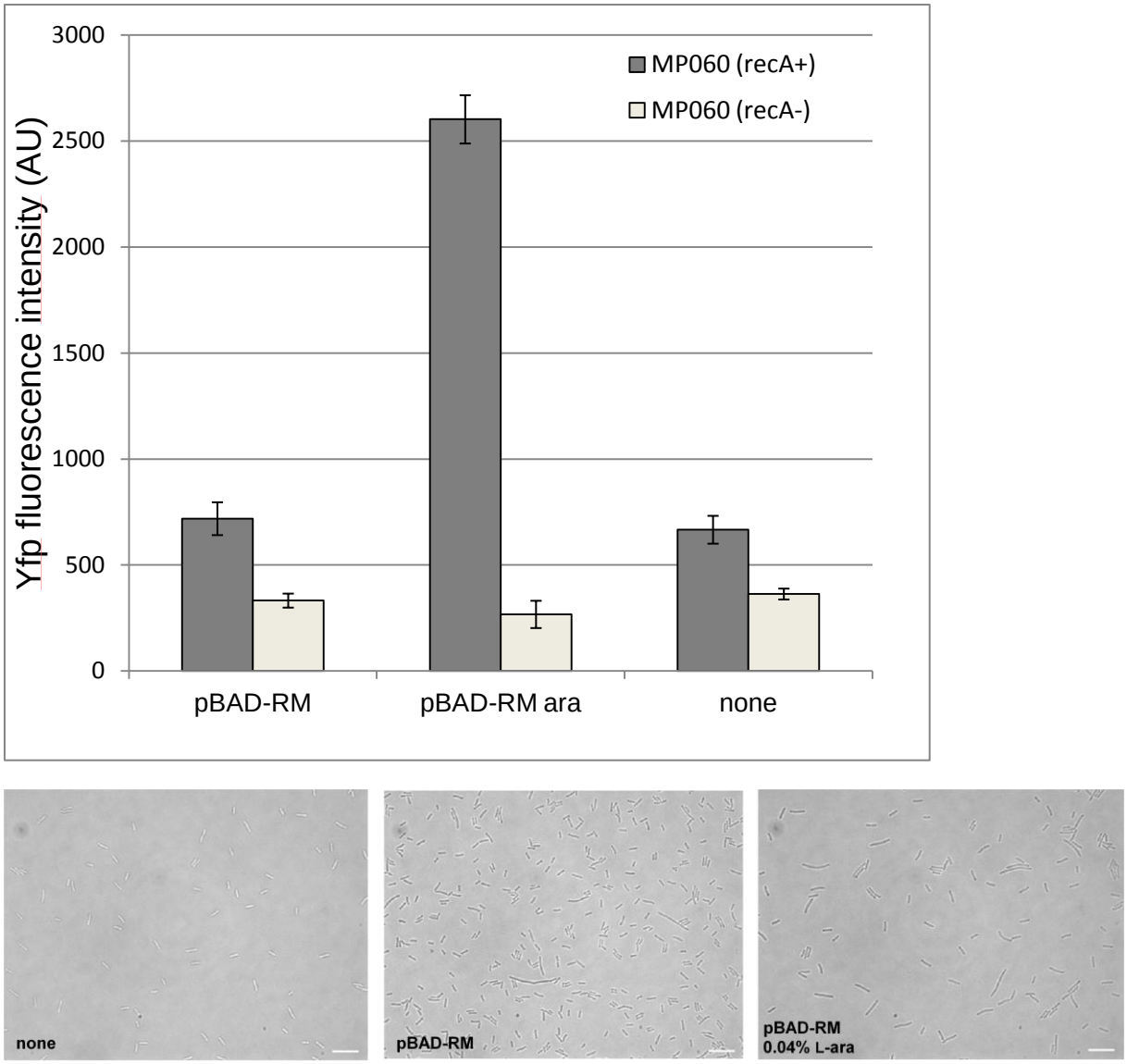
Relative fluorescence intensity of the *E. coli* MP060 and MP064 cells (Pleska et al., 2016) were quantified using a Varioskan® Flash Spectral Scanning Multimode Reader spectrophotometer (Thermo Scientific) at excitation and emission wavelengths of 485 and 510 nm, respectively, using 96-well black plates (200 μl sample). Samples were assayed in two duplicate repetitions.

### Preparation of bacteria for microscopy

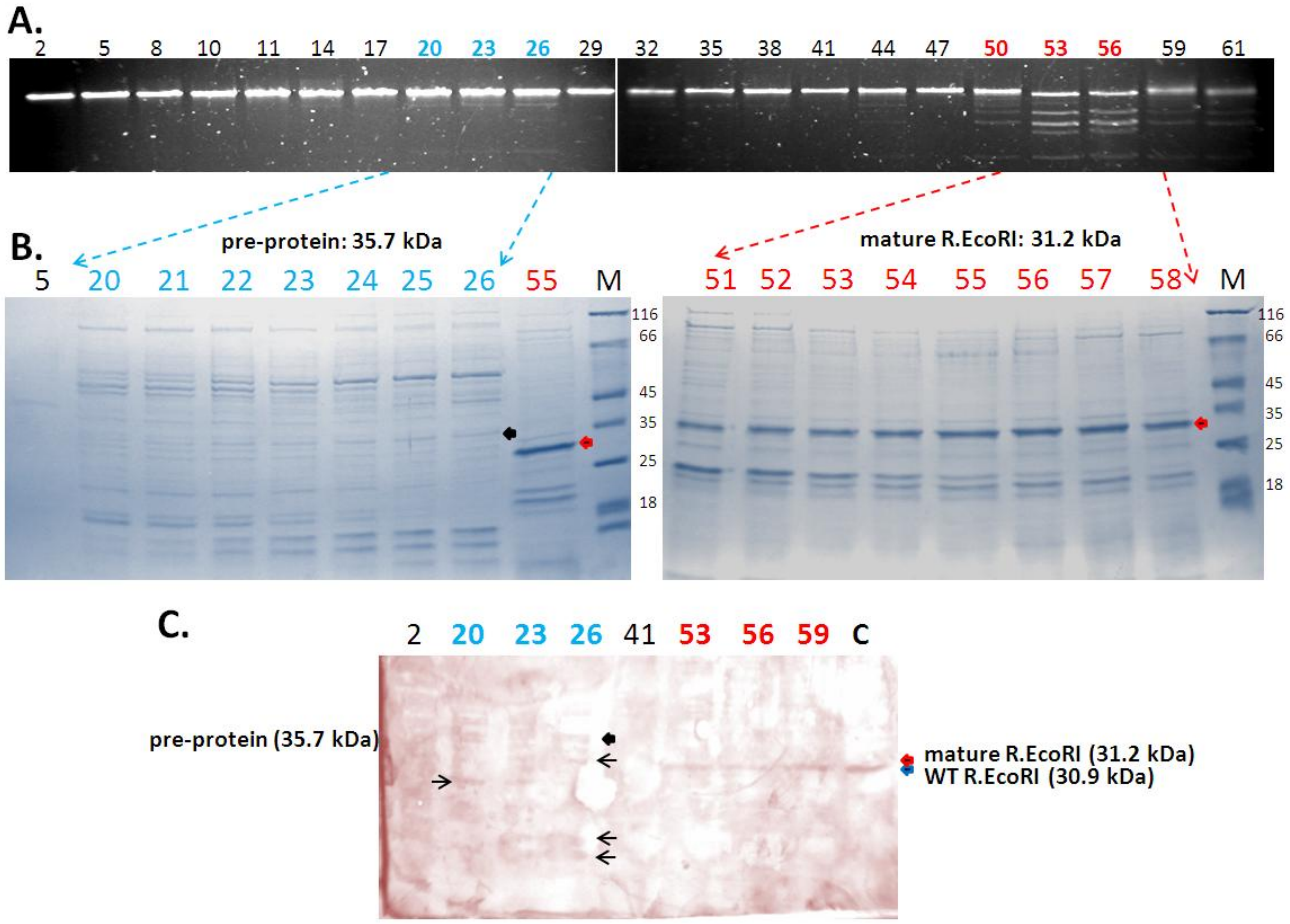
Samples of *E. coli* MP060 cell cultures (0.1 ml) bearing a *gfp*-tagged SOS reporter were studied with fluorescence microscopy. Samples were immobilized on 1-mm 1.5% agarose pads dissolved in LB medium and visualized using a Leica DMI4000B microscope fitted with a DFC365FX camera (Leica). Leica filter for green fluorescent protein (GFP) was used. The cells were observed under 1000 × magnification. Images were collected and processed using LAS AF 3.1software (Leica).

### EcoRI restriction endonuclease activity determination

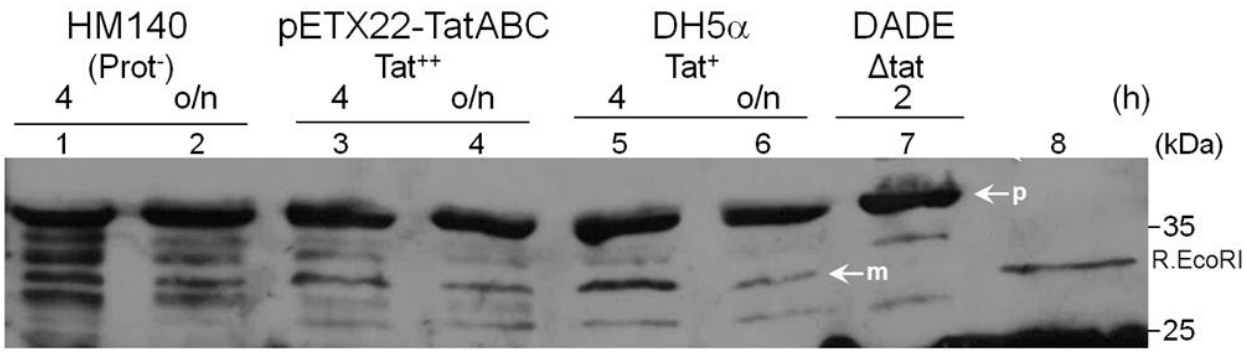
The occurrence of EcoRI restriction endonuclease activity in *E. coli* strains was tested by the modified lysozyme and Triton X-100 method (Belavin et al., 1988). Bacterial cells were collected from Petri dishes and transferred into 20 μl of incubation mixture A containing 20 mM Tris–HCl, pH 8.0, 1000 mM NaCl, 12.5 mM EDTA, 10 mM 2-mercaptoethanol (ME), and lysozyme at a concentration of 10 g/L. The sample was incubated for 30 min at room temperature and then 20 μl of incubation mixture B containing 20 mM Tris–HCl, pH 8.0, 2% Triton X-100 and 10 mM β-mercaptoethanol was added for 60 min at 4 C. The restriction endonuclease activity was assayed in 20 μl of reaction mixture containing 0.4 μg λ DNA, 2 μl restriction buffer Orange (Fermentas) and 2 μl of bacterial lysate cleared by centrifugation, for 1.5 hour at 37 C.



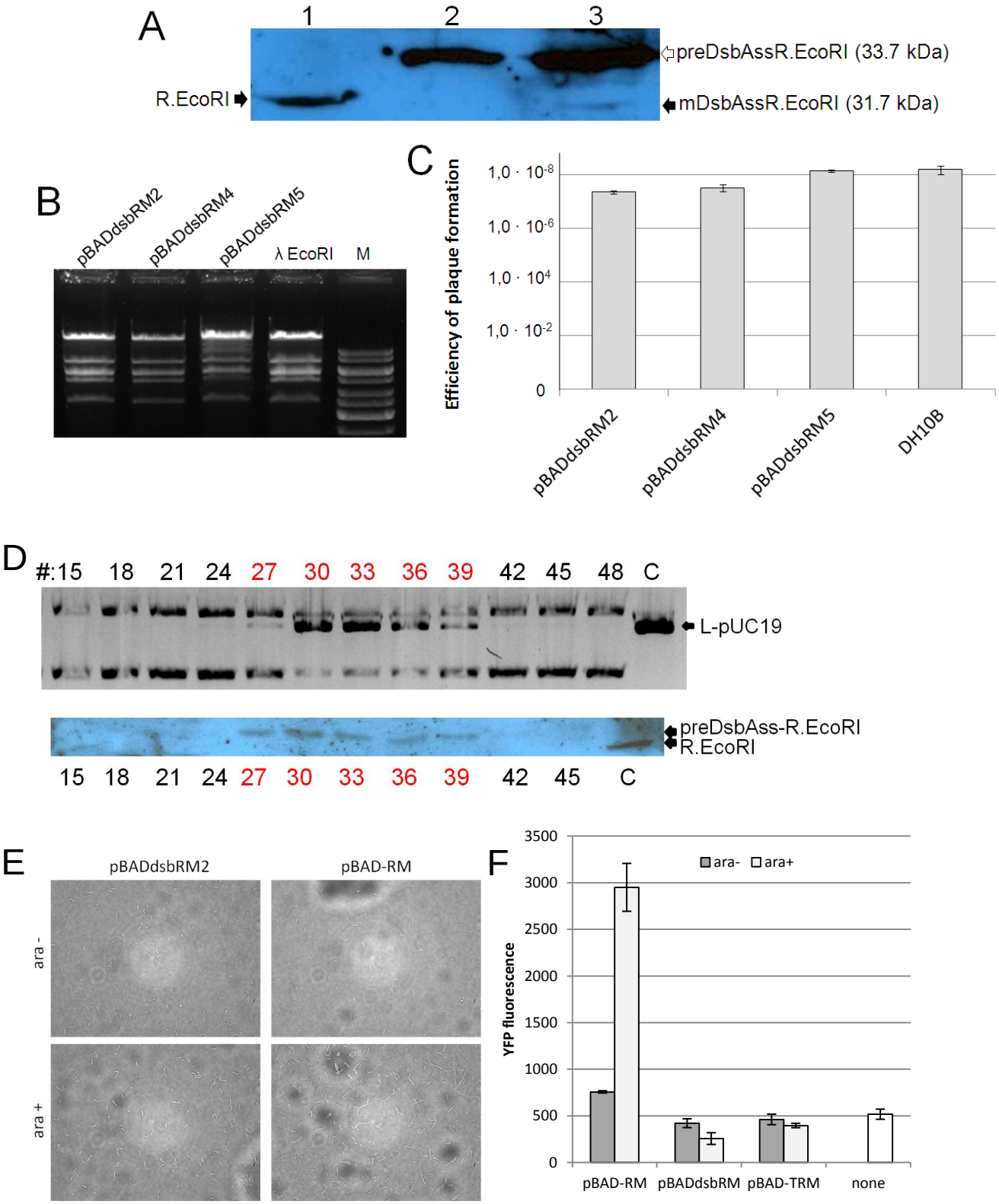

**Supplementary Fig. 1. The quantification of the self-restriction caused by EcoRI RM system overexpression in *Escherichia coli* cells.** (A) SOS-response level measured by *yfp*-reporter fluorescence. Plasmid pBAD-RM was transformed to SOS-proficient and deficient reporter *E. coli* strains, MP060 and MP064, respectively, in which gene coding YFG is under control of *PsulA* promoter, up-regulated by SOS–response (Pleska et al., 2016). Promoter *P*araBAD in pBAD-RM was induced with 0.04% L-arabinose through 2 hours. Results shown are the mean values from twice repeated measurements triplicated. (B) Monitoring of SOS-expressed cells by microscopy. Image of the MP060 cells alone and pBAD-RM bearing plasmid cells, without or with induction (under the same conditions as above). Filamentous cells are seen mainly in case of the induced culture. The scale bar indicates 10 μm.




**Supplementary Fig. 2**. **Biochemical and immunological identification of mature R.EcoRI in a lysate of DH5α expressing the *torA::ecoRIR* gene. (A)** Endonucleolytic assay of successive protein fractions collected after elution from the P11 column. (B) SDS-10% PAGE electrophoresis of selected fractions eluted from P11 column coomassie brillant blue stained. **(C)** Western blotting of the selected endonucleolytically active fractions with anti-R.EcoRI (alkaline phosphatese/NBT/BCIP developed). Lane C –WT R.EcoRI preparation. Red arrows – indicate mature R.EcoRI position; black arrows – indicate ssTorA-R.EcoRI pre-protein position; blue arrow - indicates WT R.EcoRI position. Thin black arrows – products of R.EcoRI degradation.




**Supplementary Fig. 3.** Export of ssTorA-R.EcoRI after expression in HM140 strain deficient in known cell envelope proteases and co-expression of the *tatABC* genes. HM140 cells carrying pBad-TRM were grown in LB and induced with 0.04% arabinose for 4 h and overnight at 30$^{o}$C (lanes 1 and 2, respectively). Cells were pelleted, lysed in Laemmli buffer and analyzed by SDS-10% PAGE. The gel was immunoblotted with antibodies against R.EcoRI and the positions of ssTorA-R.EcoRI pre-protein (p) and mature R.EcoRI (m) are indicated by arrows. DH5α cells carrying pBad-TRM and pEXT-TatABCs were grown in LB and induced with 0.04% arabinose for 2 h at 37$^{o}$C. Cells were pelleted, washed and resuspended in fresh LB containing 1mM IPTG for *tatABC* induction of expression. Samples of cells were removed for further analysis after 4 h and overnight incubation with IPTG (lanes 3 and 4, respectively). Lanes 5 and 6, samples of cells obtained after 4 h and overnight induction of expression of genes carried by pBad-TRM in wt Tat *E. coli* cells (DH5α), respectively. Lane 7, sample obtained after 4 h arabinose induction of expression in Δ*tat E. coli* cells (DADE) carrying pBad-TRM. Lane 8, sample of purified R.EcoRI protein.




**Supplementary Fig. 4. Restriction effect of DsbAss-R.EcoRI overproduction**. **A**. Detection of DsbAssR.EcoRI by western blotting. *E. coli* DH10B bearing pBADdsbRM plasmid was induced with 0.1% L-arabinose through 2 hours (lane 3). Lane 1. purified R.EcoRI protein, lane 2, non-induced cells. **B**. R. EcoRI specific activity in above mentioned

strains. **C.** Efficiency of plaque formation of lambda phage after plating on three randomly selected positive recombinant with pBADdsbRM plasmid. **D**. Immunological confirmation of the preDsbAss-R.EcoRI hybrid activity. Top**:** Endonucleolytic assay (λ DNA) of successive protein fractions collected after elution from the P11 column. C – control, pUC18 DNA digested by R.EcoRI. Bottom: Western blotting of the selected endonucleolytically active fractions with anti-R.EcoRI. **E**. Monitoring of SOS-expressed cells by light microscopy. Image of the MP060 cells pBADdsbRM and pBAD-RM bearing plasmids, without or with induction by 0.1% L-arabinose through 2 h. The scale bar indicates 10 μm. **F**. SOS-response level measured by *yfp*-reporter fluorescence. Plasmid pBAD-RM was transformed to SOS-proficient reporter *E. coli* strains, MP060 (Pleska et al., 2016). Promoter *P*araBAD in pBAD-RM was induced with 0.1% L-arabinose through 2 hours. Results shown are the mean values from twice repeated experiments.

**Supplementary Table S1**
Plasmids and oligonucleotides used in this study.

| Name | Relevant characteristic(s) | Source or gene specificity |
|---|---|---|
| Plasmids | | |
| pACYC184 | P15A ori replicon, $Cm^R$ $Tc^R$ | Chang and Cohen, 1978 |
| pBAD24 | Arabinose inducible expression vector, pBR322, $Ap^R$ | Guzman et al. 1995 |
| pBADdsbA | pBAD24 derivative with cloned a 66 bp *dsbA* gene leader sequence (NC_000913)with unique BglII site at its distal part, inserted between NdeI–SalI sites | This study |
| pBADdsbEcoRI' | pBADdsbA derivative with translational gene fusion *dsbA'-ecoRIR'* (R.EcoRIΔ70-277aa), constructed by an insertion of 205 bp PCR fragment carrying deletion mutant of *ecoRIR* between BglII-HindIII sites | This study |
| pBADdsbRM | pBADdsbEcoRI' derivative carrying the *dsbA'ecoRIR-ecoRIM* R-M system created by insertion of a 1690 bp fragment coding for rest part of *ecoRIR* and *ecoRIM* | This study |
| pBADecoM | pBAD24 derivative with *ecoRIM* gene cloned between NcoI-HindIII | This study |
| pBAD-RM | pBAD24 derivative with EcoRI M-R genes cloned between NcoI-HindIII L-arabinose iducible | This study |
| pBadT | pBAD24 derivative with cloned a 145 bp PCR fragment of the *torA* signal sequence with unique BglII site at its distal end, inserted between NcoI-SalI sites | This study |
| pBadTR | pBadTR' derivative (5470 bp) carrying gene fusion *torA'-ecoRIR* ($R.EcoRI^+$), constructed by insertion into HindIII site of 635 bp PCR fragment comprised of a distal part of the *ecoRIR* gene | This study |
| pBadTR' | pBadT derivative with translational gene fusion *torA'-ecoRIR'* (R.EcoRIΔ70-277aa), constructed by an insertion of 205 bp PCR fragment carrying deletion mutant of *ecoRIR* between BglII-HindIII sites | This study |
| pBadTR'G | pBadTR' derivative (5595 bp) with translational gene fusion *torA'-ecoRIR' -gfp* created by insertion into the HindIII site of a of 770 bp PCR fragment coding for *gfp* | This study |
| pBadTRM | pBadTR derivative carrying the *torA'ecoRIR-ecoRIM* R-M system created by insertion of a 1690 bp PCR fragment coding for rest part of *ecoRI* and | |

| | | |
|---|---|---|
| | *ecoRIM* | This study |
| pEXT22-TatABCs | IPTG inducible vector for expressing *E.coli* TatABC with a Streptag, $Kan^R$ | Barrett et al. 2003 |
| pGreenTIR | Source of the *gfp* reporter gene with a double mutation (F64L/S65T) | Miller and Lindow, 1997 |
| pIM27 | Restriction defective pIM-RM derivative, deletion of HindIII-BglII fragment of *ecoRIR*, $Cm^R$ | Mruk et al. 2011 |
| pIM27Tc | pIM27 derivative with the tetracycline resistance gene *tetA* inserted into ScaI site of the *cat* gene, $Tet^R$, $Cm^S$ | This study |
| pKRP12 | Source of the tetracycline resistance gene *tetA* | Reece and Philips, 1995 |
| pSalectDmdNK+1 | pBR322 derivative vector for β-lactamase fusion reporter of the periplasmic transport via the Tat secretion system export, $Cm^R$ | Lutz et al. 2002 |
| pSalectEcoRI | pSALect derivative with NdeI/SpeI *ecoRIR* in-frame fusion insertion between the N-terminal *torA* leader and C-terminal *bla* gene | This study |
| pUC18 | $P_{lac}$ promoter expression vector, $Ap^R$ | Yanisch-Perron et al. 1985 |

| Oligonucleotides | (5'→3') | Relevant characteristics |
|---|---|---|
| Gfpdown | CAGTGCCAAGCTTGCATGCTT | Distal end of *gfp* with HindIII site |
| Gfpup | CCCAAGCT**TCA**AAAGGAGAAGAACTTTTCACT | Proximal end of *gfp* with HindIII site (2nd codon is TCA for serine in bold) |
| MecoBAD | CACC**ATG**GCTAGAAATGCAACA | Proximal end of *ecoRIM* gene with NcoI site ATG start codon is in bold) |
| MecoEnd | GACGAAGCTTATGATCTCAAGAAA | Distal end of *ecoRIM* gene with HindIII site |
| P1467 | TTAAATCTTGATCTC | Centrally located, reversed to *ecoRIR* start |
| REcoBgl2 | GGAAGA**TCT**AATAAAAAACAGTCAAATAGG | Proximal end of *ecoRIR* gene for the translational fusion with *torAss* flanked by BglII site (2nd codon is TCT for serine in bold) |
| REcoHind | CCCAAGCTTATATCACTTAGATGTAAGCTGTTC | Distal part of of *ecoRIR* gene with HindIII site |
| torA.for | CATGCC**ATG**GCGAACAATAACGATCTCTTCAG | Proximal end of the *torA* signal sequence with NcoI site (ATG start codon is in bold) |
| torA.rev | ACGCGTCGACAGATCTCGCCGCTTGCGCCGCAGTCGCA | Distal end of signal sequence of *torA* with SalI and BglII sites |
| ecoNde | CATGGACAT**ATG**TCTAATAAAAAACAGTC | Proximal end of *ecoRIR* with NdeI site and ATG codon (underlined and in bold, respectively) |

| | | |
|---|---|---|
| ecoSpe | AGGAACTAGTCTTAGATGTAAGCTGTTC | Distal end of *ecoRIR* with SpeI site to be fused with *bla* |
| dsbAf | GGGAATTCCAT**ATG**AAAAAGATTTGGCTGGCGCTGGC TGGTTTAGTTTTAG | Proximal part of *dsbA* leader sequence with EcoRI site and initiation codon ATG (in bold) |
| dsbAr | CGCGTCGAC**AGATCT**ATACTGCGCCGCCGATGCGCTA AACGCTAAAACTAA | Distal part of *dsbA* leader sequence with SalI site (underlined) and BglII (in bold) |

**Supplementary Table S2.** Restriction activity of the TorAss-R.EcoRI hybrid producing strains express as $\lambda b2_{vir}$ titer.

| **Bacterial strain** | **$\lambda b2_{vir}$ titer** |
|---|---|
| DH5α pIM-27 (EcoRIR$^-$) | $3.6 \times 10^8$ |
| DH5α pBAD-RM (EcoRIR-M)$^+$ after 3h of L-ara induction (0.03%) | $2.4 \times 10^7$ |
| DH5α pBad-TRM after 3h of L-ara induction (0.03%) | $1.2 \times 10^8$ |
| DH5α pACYCΔeco; pBadTR non-induced | $2.3 \times 10^8$ |
| DH5α pACYCΔeco; pBadTR after 1h of L-ara induction (0.03%) | $5.4 \times 10^7$ |
| DH5α pACYCΔeco; pBadTR after 2h of induction | $3.1 \times 10^7$ |
| DH5α pACYCΔeco; pBadTR after 3h of induction | $2.3 \times 10^7$ |

**Supplementary References**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