Plasmids used in this work

| Plasmid name | Relevant characte-ristic [a] | Drug resistan-ce | Reference, source or short description/construction |
|---|---|---|---|
| pUC19 | - | Ap | (1) |
| pACYC184 | - | Cm, Tc | (2) |
| pBR322 | - | Ap, Tc | (3) |
| pZS*24-MCS-1 | - | Km | (4) |
| pNM481, pNM482 | *'lacZ* | Ap | (5) |
| pEsp1396 | *R M C* | - | Plasmid which occurs naturally in *Enterobacter* sp. RFL1396 and codes for *Esp*1396I RM system |
| pEsp1396IRM5.6 | *R M C* | Ap | *Bgl*II-linearized pEsp1396 was ligated with the *Bam*HI-cleaved pUC19 |
| pACNM481, pACNM482 | *'lacZ* | Tc | The DNA fragment harboring '*lacZ* gene was subcloned from high copy number plasmids pNM481 or pNM482 to low copy number vector pACYC184 to yield plasmids pACNM481 and pACNM482, respectively (*Bsp*119I-*Eco*RI DNA fragment of 3.55-kb either from pNM481 or from pNM482 replaced the *Bst*1107I-*Eco*RI DNA fragment of pACYC184; *Bsp*119I-generated cohesive DNA ends were blunted) |
| pBR-C | *C* | Ap | *Mun*I-*Sca*I DNA fragment of 0.54-kb from pEsp1396IRM5.6 was ligated with the *Eco*RI-*Eco*32I DNA fragment of 4.17-kb from pBR322 |
| pEspM::Lac [b] | *M'::'lacZ* | Tc | *Psu*I DNA fragment of 0.21-kb from pEsp1396IRM5.6 was ligated into *Bam*HI-cleaved pACNM481 |
| pEspCR::Lac | *C R'::'lacZ* | Tc | *Mun*I-*Xho*I DNA fragment of 0.96-kb from pEsp1396IRM5.6 was ligated into *Eco*RI-*Sal*I cleaved pACNM482 |
| pEspC::Lac | *C'::'lacZ* | Tc | *Mun*I-*Xba*I DNA fragment of 0.33-kb from pEsp1396IRM5.6 was ligated into *Eco*RI-*Sma*I cleaved pACNM481 (sticky DNA ends produced by *Xba*I were blunted) |
| pEsp$^{+4}$CR::Lac | *R'::'lacZ* | Tc | Obtained after the digestion of pEspCR::Lac with *Xba*I (which possesses a unique target within the *esp1396IC*) followed by filling-in and re-circularization. The *esp1396IC* gene in pEsp$^{+4}$CR::Lac contains four extra nucleotides resulting in +1 frameshift mutation |
| pEsp$^{+12}$CR::Lac | *R'::'lacZ* | Tc | Obtained after the insertion of octanucleotide GAGATCTC into *Xba*I-digested and blunted pEspCR::Lac. The *esp1396IC* gene in pEsp$^{+12}$CR::Lac contains 12 extra nucleotides resulting in four additional amino acid residues within the regulatory protein |
| pZS-C [c] | *C* | Km | The gene for C.*Esp*1396I was amplified using primers 5'-CAG GTA CCG AAT AGA AAA TAT TAG TTA TGG AA-3' and 5'-GAC GCG TTT AGT CAT GCT TTA AAA TCT CC-3' (*Kpn*I and *Mlu*I targets introduced into primer sequences are underlined), *Taq* DNA polymerase, plasmid pEsp1396IRM5.6 as template and following reaction conditions: initial denaturation, 95$^{o}$C, 2 min; denaturation, 95$^{o}$C, 1 min; annealing, 58$^{o}$C, 1 min; extension, 72$^{o}$C, 30 seconds; number of cycles, 30. PCR fragment of 0.2-kb was cleaved with *Kpn*I and *Mlu*I and inserted into pZS*24-MCS-1 pre-cleaved with *Kpn*I-*Mlu*I. The inserted sequence was verified by dideoxy sequencing (6). |

[a] *R, M, C* - genes for *Esp*1396I Enase, Mtase and regulatory protein, respectively

[b] Introduction of pEspM::Lac into *E. coli* was carried out using the host cells which expressed the *Esp*1396I repressor gene located within the plasmid pBR-C

[c] Gene for regulatory protein is under the control of promoter $P_{lac/ara-1}$

References


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