Supplementary materials for

A novel plasmid entry exclusion system in pKPC_UVA01, a promiscuous conjugative plasmid carrying the \textit{bla}\textsubscript{KPC} carbapenemase gene

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Table S1. Characteristics of plasmids having the novel \( \text{trbK} \) entry exclusion gene

| Plasmid | Accession number | Plasmid Inc type | Size (kb) | Major resistance genes |
|---------|------------------|------------------|-----------|------------------------|
| K. pneumoniae plasmid pKPC_UVA01 | CP017937.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| C. freundii plasmid pKPC_CAV1857-43 | CP037739.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| C. freundii complex sp. plasmid pKPC-9d88 | CP026239.1 | pKPC_UVA01 type | 53 | blatem, blakPC-2 |
| K. pneumoniae strain AR_0126 plasmid tig00000003 | CP021743.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| K. pneumoniae plasmid pKPC_CAV1193 | CP013325.1 | pKPC_UVA01 type | 49 | blatem, blakPC-2 |
| C. freundii plasmid pKPC_CAV1193 | CP011656.1 | IncM1/ pKPC_UVA01 type | 129 | blatem, blakPC-2, blashv-30, aac(6')-Ib-cr |
| K. pneumoniae plasmid pCAV1344-40 | CP011620.1 | pKPC_UVA01 type | 39 | blatem |
| C. freundii plasmid pKPC_CAV1321-45 | CP011608.1 | pKPC_UVA01 type | 45 | blatem, blakPC-2 |
| E. hormaechei plasmid pKPC_CAV1668 | CP011582.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| K. pneumoniae plasmid pKPC_CAV1392 | CP011575.1 | pKPC_UVA01 type | 43 | blatem, blakPC-3 |
| E. hormaechei plasmid pCAV1311-34 | CP011570.1 | pKPC_UVA01 type | 33 | blatem |
| E. hormaechei plasmid p34983-43.621kb | CP010379.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| E. hormaechei plasmid p34977-43.621kb | CP010374.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| C. freundii plasmid p1C44 | CP054297.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| K. pneumoniae plasmid p2K44 | CP054293.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| K. pneumoniae plasmid p3K44 | CP054288.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| E. coli plasmid p5E44 | CP054283.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| C. koseri genome assembly PRJEB6512_assembly_1 | LK931338.1 | pKPC_UVA01 type | 33 | None |
| L. adercarboxylata plasmid p16005813B | MK036884.1 | pKPC_UVA01 type | 45 | blaimp-8, aac(6')-Ib-cr |
| K. pneumoniae strain AR_0129 plasmid tig00000003 | CP021716.1 | pKPC_UVA01 type | 43 | blatem, blakPC-3 |
| E. coli strain plasmid pECAZ161_KPC | CP019010.1 | CoIRNAI/ pKPC_UVA01 type | 55 | blatem, blakPC-3 |
| K. pneumoniae plasmid pKPC_Kp46 | KX348146.1 | pKPC_UVA01 type | 49 | blatem, blakPC-3, blaoxa-9, aac(6')-Ib-cr |
| S. marcescens plasmid pSUNAM836 | CP012686.1 | pKPC_UVA01 type | 26 | blatem |
| E. cloacae plasmid p35734-109.753kb | CP012163.1 | pKPC_UVA01 type | 109 | blatem, blakPC-3, blaoxa-9, aac(6')-Ib-cr |
| E. hormaechei plasmid p34399-43.500kb | CP010387.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| K. pneumoniae plasmid pCRE-195-5 | CP061394.1 | pKPC_UVA01 type | 43 | blatem, blakPC-2 |
| Organism                     | Plasmid                                  | Accession Number | Inc/CP Type | Type | β-Lactamase(s)                           |
|-----------------------------|------------------------------------------|------------------|-------------|------|-----------------------------------------|
| *K. quasipneumoniae*        | plasmid pKp4101                          | CP047282.1       | IncC/pKPC_UVA01 | 187  | *bla*TEM, *bla*CMY-4, *bla*OXA-204, *aac*(6')-Ib-cr |
| *E. hormaechei*             | plasmid pKPC_49790_VIM_1                 | CP059426.1       | pKPC_UVA01   | 55   | *bla*TEM, *bla*VIM-1, *qnr*S1, *aac*(6')-Ib-cr |
| *E. hormaechei*             | plasmid pKPC_48212_VIM                  | CP059417.1       | pKPC_UVA01   | 55   | *bla*TEM, *bla*VIM-1, *qnr*S1, *aac*(6')-Ib-cr |
| *K. pneumoniae* ABFQB       | plasmid pKPC-e4b7                        | CP036440.1       | pKPC_UVA01   | 51   | *bla*KPC-3, *qnr*S1                      |
| *K. pneumoniae*             | KPNIH45 plasmid pKPC-e4b7                | CP036448.1       | pKPC_UVA01   | 51   | *bla*KPC-3, *qnr*S1                      |
| *C. freundii*               | plasmid pKPC-e4b7                        | CP036437.1       | pKPC_UVA01   | 51   | *bla*KPC-3, *qnr*S1                      |
| *E. hormaechei*             | plasmid unnamed3                        | CP027143.1       | pKPC_UVA01   | 52   | *bla*KPC-2                              |
| *K. pneumoniae*             | plasmid pMNCRE53_3                       | CP018436.1       | pKPC_UVA01   | 52   | *bla*KPC-2, *qnr*S1                      |
| *K. pneumoniae*             | plasmid pMNCRE78_3                       | CP018432.1       | pKPC_UVA01   | 52   | *bla*KPC-2, *qnr*S1                      |
| *K. pneumoniae*             | plasmid pMNCRE69_3                       | CP018426.1       | pKPC_UVA01   | 52   | *bla*KPC-2, *qnr*S1                      |
| *K. pneumoniae*             | plasmid pKPC_CAV1042-44                  | CP018668.1       | pKPC_UVA01   | 43   | *bla*TEM, *bla*KPC-2                     |
Table S2. Primers used in this study

| Primer | Sequence(5’-3’)* | Reference/Accession number |
|--------|-------------------|----------------------------|
| RepA1_F | ATGGACTGATCAAGCTGGCG | This study; CP009465 |
| RepA1_R | TTTGACGCGGCCATATACGG | This study; CP009465 |
| TrbK_F_HindIII | CGAAGCTTCAGCAAGCAGACTAATACGG | This study; CP009465 |
| TrbK_R_XbaI | CGTCTAGAGCAAAAACATGCAAAACAGAA | This study; CP009465 |
| TrbK_116CT_R_XbaI | CGTCTAGAGCAGGGTCCAGATTAGG | This study; CP009465 |
| TrbK_232CT_R_XbaI | CGTCTAGAGCAGGCTTACCAGATTAGG | This study; CP009465 |
| TrbJ_F_HindIII | CGAAGCTTCAGTTGCAATACGG | This study; CP009465 |
| TrbJ_R_XbaI | CGTCTAGAGTAATTTATCCCCCTATATGAAATACGC | This study; CP009465 |
| ATPase_F_HindIII | CGAAGCTTCCGCTTGAGCAATCTTT | This study; CP009465 |
| TrbK_FRT_P1 | CGTTGACTAAAAATATTGTCTCTTTTAAGTGCTAATGCAGGC | This study; CP009465 |
| TrbK_FRT_P2 | CAAGATGCTAAAAATCAACATTGTCTGTATTTTCCCATATGAAATACGC | This study; CP009465 |
| TrbJ_FRT_P1 | TGGCTGGTATCTTTCTCTCTCTGTATTTATGCAGGCTGCC | This study; CP009465 |
| TrbJ_FRT_P2 | CTGACGGCAATTAATTTGAGTTGCAAGCAGACTATTG | This study; CP009465 |
| TrbL_FRT_P1 | GCCGTGCTTTCAAGTTCGGGTAGTGACATTTATGTGTCG | This study; CP009465 |
| TrbL_FRT_P2 | CCCGCAGCATTGATTGTGCTTTTACACCAACATGGT | This study; CP009465 |
| TrbL_F | GCTGATGCGGTGCCAGCATT | This study; CP009465 |
| TrbL_R | TCACCCCGCAGCATTGATGT | This study; CP009465 |
| TrbL_HindIII_F | CGAAGCTTCAGGCCGAAAAATCAACACA | This study; CP009465 |
| TrbL_XbaI_R | CGTCTAGAGCAAAAACATGCAAAACAGCAAT | This study; CP009465 |
| AbR_nest_F1 | GCCAATATCACTACGTTAAGCCACAGTCAGAAAACTGTCGGCAGATAGACGCGGAGGATTGAGTGG | This study; CP009465 |
| AbR_nest_R1 | GCCAATATCACTACGTTAAGCCACAGTCAGAAAACTGTCGGCAGATAGACGCGGAGGATTGAGTGG | This study; CP009465 |
| AbR_nest_F2 | AAGATCTTGAAGTCTGTTATGTGCAGCAACGAGGCAGGCGAGATAGACGCGGAGGATTGAGTGG | This study; CP009465 |
| AbR_nest_R2 | CACCCCGCATTCAACAACAACACTTGTGTTATTTTCTCTCACCAGACGTGGCATCGTCAGTTAAGAAA | This study; CP009465 |
| TrbK_F_EcoRI | GCAGAATGCGAGCAAGAGCGGCTTAAAAGAA | This study; CP009465 |
| trbK_F_RT | TTGATGCAGCATAAGCCACTG | This study; CP009465 |
| trbK_R_RT | CGAGGGGTCCACGATTAGG | This study; CP009465 |
* Restriction enzyme cutting sites are underlined, **bold** nucleotide are for gene-specific sequences and plain nucleotides with bold characters are homologous arm from the deletion target region.
Figure S1. Amino acid sequence alignment of putative entry exclusion protein of pKPC_UVA01 with that of IncP plasmid RP4 (A), IncP plasmid R751 (B) and IncN plasmid R46 (C). Identical amino acids are shaded in black.
**Fig. S2.** Nucleotide sequence synthesized with an early termination codon at the C-terminal region of *trbK*. HindIII and XbaI restriction sites are underlined. The early termination codon is shown in red-bold characters (GG>AA changes). *trbK* coding region is shaded grey.
**Fig. S3.** Relative *trbK* expression from different *trbK* constructs in *E.coli*. The RT-qPCR primers position were indicated by two forward and reverse facing arrows. Relative expression values are the mean of 3 biological replicates with standard errors. The red asterics in pJIMK130 indicates the position of the early termination codon.
Fig. S4. Relative *trbK* expression from BW25113Rf(pJIMK131) after induction with different dosages of arabinose (A). Conjugation transfer frequency of pKPC_UVA01 plasmid from J53 to recipient BW25113Rf(pJIMK131) after induction with different dosages of arabinose (B). The results are the mean of 3 independent experiments with standard errors.