

Supplemental table 1. Features of pKPI-6 ORFs (GenBank accession no. AB616660)

ORF	Position (bp)		Strand	Gene	Length		Description	Identity (%)	Overlap <sup>b</sup>		Function/product
	start	stop			(aa) <sup>a</sup>	Source			(aa)	Accession no.	
1	1	720	+	<i>repA</i>	239	<i>K. pneumoniae</i> (plasmid 12)	Initiator Replication protein	100	239/239	YP_002286934	Initiation of plasmid replication
2	2417	2986	-	<i>resP</i>	189	<i>K. pneumoniae</i> subsp. <i>ozaenae</i> (pKO6)	resolvase, Site-specific recombinases	100	189/189	AGW99991	Resolvase
3	3379	4392	-	<i>intI1</i>	337	<i>Shigella flexneri</i> 2b (plasmid R100)	IntI (E2) integrases, site-specific tyrosine recombinases	100	337/337	NP_052698	Class 1 integrase
4	4494	5114	+	<i>aacA4'</i>	210	uncultured bacterium (pKSP212)	aminoglycoside (6')-N-acetyltransferase	97	204/210	ADC80806	Aminoglycoside acetyltransferase
5	5191	5931	+	<i>bla<sub>IMP-6</sub></i>	246	<i>Serratia marcescens</i>	metallo-β-lactamase	100	246/246	BAB15941	Metallo-β-lactamase
6	6076	6855	+	<i>aadA2</i>	267	Gamma proteobacteria	Streptomycin 3'-adenyltransferase	100	267/267	WP_000729308	Aminoglycoside adenylyltransferase
7	6996	8111	+	<i>tnpA</i> of IS91 family	371	<i>Psychromonas ossibalaenae</i>	<i>tnpA</i> of IS91 family	55	198/359	WP_019617277	Transposase of IS91 family
8	8481	8828	+	<i>qacEA1</i>	115	<i>Citrobacter freundii</i> (pT-OXA-181)	Quaternary ammonium compound resistance	100	115/115	AGE11240	Quaternary ammonium compound resistance
9	8822	9661	+	<i>sulI</i>	279	<i>Shigella flexneri</i> 2b (plasmid R100)	dihydropteroate synthase	100	279/279	NP_052895	Dihydropteroate synthase
10	9789	10289	+	<i>orf5</i>	166	<i>Desulfurispirillum indicum</i> S5	GCN5-like N-acetyltransferase	100	166/166	YP_004111325	Unknown
11	10766	11560	+	<i>tnpA</i> of IS6100	264	<i>E. coli</i> (pEK499)	transposase for insertion sequence IS6100	100	264/264	YP_003108355	Transposase of IS6100
12	11821	13035	+	<i>EcoRII</i>	404	<i>K. pneumoniae</i> NK245 (pK245)	N-terminal domain of type IIE restriction endonuclease <i>EcoRII</i>	100	404/404	YP_001966230	Restriction endonuclease type II
13	13069	14502	-	<i>EcoRIImet</i>	477	<i>E. coli</i> (pMUR050)	DNA cytosine methylase	100	477/477	YP_724464	DNA methylase
14	14884	15090	-	hypothetical protein	68	<i>K. pneumoniae</i> (plasmid 12)	hypothetical protein	100	68/68	YP_002287015	Unknown
15	15095	15604	-	<i>mrr</i>	169	<i>E. coli</i> (pNDM-BTR)	Restriction endonuclease	100	169/169	YP_008574960	Restriction endonuclease
16	15792	16103	-	hypothetical protein	103	<i>Salmonella typhimurium</i> (plasmid R46)	unknown	100	103/103	NP_511182	Unknown
17	16139	16453	-	<i>kikA</i>	104	<i>K. pneumoniae</i> (plasmid 12)	conjugal transfer protein TrbM	100	104/104	YP_002287012	Killing of <i>Klebsiella</i> during conjugation
18	16450	16794	-	hypothetical protein	114	<i>K. pneumoniae</i> (plasmid 12)	unknown	100	114/114	YP_002287011	Unknown
19	16810	17115	-	<i>korB</i>	101	<i>E. coli</i> 517-2H1 (pLEW517)	histone-like proteins of HNS family	100	101/101	YP_001096375	Initiation of kik mediated killing
20	17224	17958	+	<i>traL</i>	244	<i>K. pneumoniae</i> (plasmid 12)	Lytic Transglycosylase	100	244/244	YP_002287009	Conjugal transfer
21	17955	18248	+	<i>korA</i>	97	<i>K. oxytoca</i> KOX105 (pKOX105)	Initiation of kik mediated killing	100	97/97	YP_003675738	Initiation of kik mediated killing
22	18258	18551	+	<i>traM</i>	97	<i>K. pneumoniae</i> (plasmid 9)	Conjugal transfer	100	97/97	YP_002286912	Conjugal transfer
23	18601	18918	+	<i>traA</i>	78	<i>E. coli</i> L46 (pEC_L46)	Type IV secretory pathway	100	78/78	YP_003829295	Conjugal transfer
24	18918	21518	+	<i>traB</i>	866	<i>K. pneumoniae</i> KP96 (pKP96)	conjugal transfer protein	100	866/866	YP_002332880	Conjugal transfer
25	21536	22249	+	<i>traC</i>	237	<i>K. pneumoniae</i> (plasmid 9)	Type IV secretion system proteins	100	237/237	YP_002286909	Conjugal transfer
26	22257	22484	+	<i>eex</i>	75	<i>E. coli</i> L46 (pEC_L46)	entry exclusion protein	100	75/75	YP_003829298	Entry exclusion
27	22500	23540	+	<i>traD</i>	346	<i>K. pneumoniae</i> (plasmid 12)	TrbL/VirB6 plasmid conjugal transfer protein	100	346/346	YP_002286993	Conjugal transfer
28	23611	23769	+	<i>traN</i>	52	<i>K. pneumoniae</i> (plasmid 12)	metallophosphatase superfamily	100	52/52	YP_002286992	Conjugal transfer
29	23759	24457	+	<i>traE</i>	232	<i>K. pneumoniae</i> (plasmid 9)	VirB8 protein	100	232/232	YP_002286905	Conjugal transfer
30	24468	25352	+	<i>traO</i>	294	<i>E. coli</i> L46 (pEC_L46)	type IV conjugative transfer system protein	100	294/294	YP_003829302	Conjugal transfer
31	25352	26512	+	<i>traF</i>	386	<i>Photobacterium damsela</i> subsp. <i>piscicida</i> PT99-018 (pP99-018)	Bacterial conjugation TrbI-like protein	100	386/386	YP_908595	Conjugal transfer
32	26554	27549	+	<i>traG</i>	331	<i>E. coli</i> (pMUR050)	conjugation	100	331/331	YP_724501	Conjugal transfer
33	27549	28082	+	<i>nuc</i>	177	<i>K. oxytoca</i> E718 (pKOX_R1)	endonuclease	100	177/177	YP_006501590	Periplasmic endonuclease
34	28256	28750	-	<i>ΔfipA</i> (1)		<i>E. coli</i> 345-2RfC (plasmid N3)	fertility inhibition of IncP plasmids protein			YP_004558188	Fertility inhibition of IncP plasmids (truncated)
35	29019	29261	+	<i>relaxase</i>	80	<i>E. coli</i> A2363 (pAPEC-O2-R)	relaxase	100	80/80	YP_190207	Unknown
36	29293	29919	+	<i>tetR</i>	208	<i>Providencia stuartii</i> MRSN 2154 (pMR0211)	tetracycline repressor protein	100	208/208	YP_005351739	tetracycline resistant regulatory protein
37	30049	31248	+	<i>tetA</i>	399	<i>K. pneumoniae</i> KP96 (pKP96)	tetracycline resistance protein	100	399/399	YP_002332890	tetracycline resistant protein A
38	31280	31978	-	<i>pecM</i>	232	<i>E. coli</i> (pHKU1)	EamA-like transporter family	100	232/232	YP_008574853	Unknown
39	32165	32301	-	<i>ΔfipA</i> (2)		<i>E. coli</i> 345-2RfC (plasmid N3)	fertility inhibition of IncP plasmids protein			YP_004558188	Fertility inhibition of IncP plasmids (truncated)
40	32301	35543	-	<i>traI</i>	1080	<i>K. pneumoniae</i> KP96 (pKP96)	conjugal transfer protein	100	1080/1080	YP_002332893	Conjugal transfer
41	35543	37072	-	<i>traJ</i>	509	<i>K. pneumoniae</i> KP96 (pKP96)	conjugal transfer protein	100	509/509	YP_002332894	Conjugal transfer
42	37074	37490	-	<i>traK</i>	138	<i>K. pneumoniae</i> (plasmid 12)	conjugal transfer protein	100	138/138	YP_002286951	Conjugal transfer
43	38020	38400	+	<i>stbA</i>	126	<i>K. pneumoniae</i> (plasmid 12)	prevention of the induction of SOS-responses	99	125/126	YP_002286950	Prevention of SOS response
44	38409	39125	+	<i>stbB</i>	238	<i>K. pneumoniae</i> (plasmid 12)	plasmid stability protein	100	238/238	YP_002286949	Stable plasmid inheritance
45	39127	39495	+	<i>stbC</i>	122	<i>Salmonella typhimurium</i> (plasmid R46)	stable plasmid inheritance	100	122/122	NP_511206	Stable plasmid inheritance
46	39677	40021	+	<i>orfD</i>	114	<i>Salmonella typhimurium</i> (plasmid R46)	hypothetical protein	100	114/114	NP_511207	Unknown
47	40132	40452	-	<i>cagAII</i>	106	<i>K. pneumoniae</i> (plasmid 12)	member of CUP-controlled regulon; similar to RecX	100	106/106	YP_002286945	Prevention of RecA overproduction
48	40530	40769	-	<i>cagEIII</i>	79	<i>Salmonella typhimurium</i> (plasmid R46)	member of CUP-controlled regulon	100	79/79	NP_511213	Unknown
49	40779	41183	-	<i>ardR</i>	134	<i>E. coli</i> L46 (pEC_L46)	antirestriction protein	100	134/134	YP_003829321	Regulation of <i>cag</i> genes
50	41241	41666	-	<i>ardB</i>	141	<i>E. coli</i> L46 (pEC_L46)	Antirestriction protein	100	141/141	YP_003829320	Inhibition of host type I restriction enzymes
51	42348	43223	-	<i>bla<sub>CTX-M-2</sub></i>	291	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium	Beta-lactamase	100	291/291	P74841	Extended-spectrum-β-lactamase
52	43480	44742	-	<i>tnpA</i>	420	<i>E. coli</i> L46 (pEC_L46)	IS <i>Ecp1</i> transposase	100	420/420	YP_003829282	Transposase of IS <i>Ecp1</i>
53	45005	45379	+	<i>ΔmucB</i>	124	<i>K. pneumoniae</i>	DNA polymerase V subunit	99	123/124	WP_020320119	UV protection (truncated)
54	45494	46321	+	<i>mpr</i>	275	<i>K. oxytoca</i> KOX105 (pKOX105)	zinc metalloproteinase	100	275/275	YP_003675760	Unknown
55	46336	46677	-	<i>ardK</i>	113	<i>Salmonella typhimurium</i> (plasmid R46)	control of expression of genes fused to CUP sequences	100	113/113	NP_511219	Regulation of <i>cag</i> genes
56	46761	46952	-	hypothetical protein	63	Enterobacteriaceae	unknown	100	63/63	WP_016750023	Unknown

<sup>a</sup> aa, amino acids.<sup>b</sup> Overlap indicates the number of overlapping amino acids/total number of amino acids.

Supplemental table 2. Primers used in this study.

Primer set No.	Primer	Sequence	Amplicon size (bp)	References
For PCR scanning of In722				
A	intl1-1014R intl1-F	CTACCTCTCACTAGTGAGGG GCATCCTCGGTTTTCTGG	644	Kouda <i>et al.</i> Kouda <i>et al.</i>
B	intl1-501R aac6'lb-R	CACGATGATCGTGCCGTGAT CCCAAGCCTTTGCCAGTTG	1009	Kouda <i>et al.</i> Kouda <i>et al.</i>
C	aac6'lb-F imp-6-500R	GAGTGGTGGGGCGGAGAAGA GGCAACCAAACCACTACGTT	1038	Kouda <i>et al.</i> this study
D	imp-1-381F aadA2-447R	CGGTAAGGTTCAAGCCACAA AGCTTCAAGGTTTCCCTC	1009	Kouda <i>et al.</i> this study
E	aadA2-212F transposase-315R	AATTTGGAACCAACTATCAGAG AAAGACTCCGCAGAGATG	1083	this study this study
F	transposase-81F tamspase-1241R	TTCACCATCAAGTGTAGCTG GCCGCTATTCGTTCTGTG	1153	this study this study
G	transposase-569F qacEdelta1-R	GCGTCGCTAAATATTTAGCTC AGCAATTATGAGCCCATAC	1043	this study Kouda <i>et al.</i>
H	qacEdelta1-269F sul1-527R	GTATGGGGCTCATAATTGCT ATCCCCGGATCGAGGATGAG	600	Kouda <i>et al.</i> Kouda <i>et al.</i>
I	sul1-435F orf5-107R	GCTCGACGAGATTGTGCGGT GTTCCCTTGGCGGACATCCA	640	Kouda <i>et al.</i> Kouda <i>et al.</i>
J	sul1-815F orf5-R	GAGACCGAGGGTTAGATCAT ATTTGAGTTCCTAGGCGTTC	650	Kouda <i>et al.</i> Kouda <i>et al.</i>
For PCR scanning of pKPI-6				
1	repA-40F resP-51F	GTCTAACGAGCTTACCGAAG CCAGGTTGACGAACTAACA	2898	Aurora <i>et al.</i> this study
2	resP-237R imp-1-500R	TAATGACTTGACCCAATGAAC GGCAGCCAAACCACTACGTT	2942	this study Kouda <i>et al.</i>
3	imp-1-381F qacEdelta1-R	CGGTAAGGTTCAAGCCACAA AGCAATTATGAGCCCATAC	3259	Kouda <i>et al.</i> Kouda <i>et al.</i>
4	qacEdelta1-269F IS6100-631R	GTATGGGGCTCATAATTGCT ATCCCTTGATCGTGGCATAG	2639	Kouda <i>et al.</i> this study
5	Sul815F EcoRII-1325F	GAGACCGAGGGTTAGATCAT AGTTCGGTAACTCCGTAGTGG	3703	Kouda <i>et al.</i> this study
6	EcoRII-686F kikA-248F	TTCTATTGGTTGAGGAAGTGC GCGAAGGTGGAAGTAACCAG	3701	this study this study
7	mrr-kikA-F traL-593R	CCATAGAATAAGGTTTTCTGTAGTG CCCTTCGCTGTTTCTGTCT	2254	this study this study
8	traL-173F traB-2574R	ATAATTTAGTGGGCCTGGGTC GAGCCATACTTCAGGGTC	3961	this study this study
9	traB-2360F traO-305R	TGTCGATTACCGAACACTC ATCACCAGGTTGGTATCACTC	3476	this study this study
10	traE516F nuc526R	AGCAACGATACGCTTTACTAC TGGAACGGTAGTCTCTCC	3806	this study this study
11	nuc-321F tetA-888R	TACTGACAGTGATTTCCCTATCC GAAGGCAAGCAGGATGTAGC	3068	this study this study
12	tetR-179R tral-1110F	ACCGAATGCGTATGATTCTC ACCTGAAGGCGGTACAGAAG	4258	this study this study
13	tral-1773R traK-286F	GCGAGCGTTATGCTTCTC TGCATGAATCACTCGCTATTG	3434	this study this study
14	traJ-135R cagAII-195F	TAACCAGATACGGAATCAGG ATTTGACTATCTGACCCAAGAC	3322	this study this study
15	cagAII-282R ISEcpI-470F	ATTACGCTCAATACCGTGG CCTAAGAACTGGGAAACCG	4122	this study this study
16	ISEcpI-1117R repA-553R	AGTCCAAGGAATCAAACCTTG ACGGTCATTTAACCAAGCATG	4163	this study Aurora <i>et al.</i>

Supplemental table 3. Result of susceptibility testing

Strain	Name	Place	Year	ABPC	PIPC	CEZ	CAZ	CPR	CMZ	CPDX	AZT	IPM	MEPM	A/C	C/S	P/T	AMK	GM	MINO	CPFX	IPM <sup>#</sup>	MEPM <sup>#</sup>	PAPM <sup>#</sup>	BIPM <sup>#</sup>	DRPM <sup>#</sup>
<i>K. pneumoniae</i>	a26	Hiroshima	2009	>16	>64	>16	>16	>16	>32	>4	>16	1	>8	16	>32	≤16	≤4	4	>8	>2	1	32	1	1	32
<i>K. pneumoniae</i>	MS5263	Amagasaki	2011	>16	>64	>16	>16	>16	>32	>4	>16	2	>8	>16	>32	>64	≤4	>8	>8	≤0.25	2	256	8	2	256
<i>K. pneumoniae</i>	MS5265	Amagasaki	2011	>16	>64	>16	16	>16	>32	>4	16	≤0.5	8	16	>32	≤16	≤4	2	8	>2	0.25	32	0.5	≤0.125	64
<i>K. pneumoniae</i>	MS5266	Amagasaki	2011	>16	>64	>16	16	>16	>32	>4	8	2	>8	16	>32	≤16	8	2	8	≤0.25	1	32	2	1	64
<i>K. pneumoniae</i>	MS5283	Kinki area	2006	>16	>64	>16	>16	>16	>32	>4	>16	2	>8	>16	>32	>64	≤4	>8	>8	≤0.25	2	128	8	0.5	128
<i>K. pneumoniae</i>	MS5287	Kinki area	2008	>16	>64	>16	16	>16	>32	>4	4	≤0.5	8	16	>32	≤16	≤4	4	8	>2	0.5	32	1	1	32
<i>K. pneumoniae</i>	MS5288	Kinki area	2009	>16	>64	>16	8	>16	32	>4	8	≤0.5	>8	8	32	≤16	≤4	4	4	≤0.25	0.5	16	0.5	0.5	8
<i>K. pneumoniae</i>	MS5289	Kinki area	2010	>16	>64	>16	>16	>16	>32	>4	>16	1	>8	16	>32	≤16	≤4	4	8	>2	1	32	1	0.5	32
<i>K. pneumoniae</i>	MS5290	Kinki area	2010	>16	>64	>16	>16	>16	>32	>4	16	1	>8	16	>32	≤16	≤4	8	4	>2	1	64	4	2	32
<i>K. pneumoniae</i>	MS5291	Kinki area	2010	>16	16	>16	>16	≤8	>32	>4	≤1	2	>8	16	>32	≤16	≤4	4	8	>2	1	128	8	4	64
<i>K. pneumoniae</i>	MS5292	Kinki area	2010	>16	>64	>16	4	>16	32	>4	4	1	>8	8	>32	≤16	≤4	4	4	≤0.25	0.5	16	0.5	0.5	8
<i>K. pneumoniae</i>	MS5293	Kinki area	2009	>16	>64	>16	>16	>16	>32	>4	>16	1	>8	>16	>32	≤16	≤4	2	>8	>2	0.5	32	1	1	16
<i>K. pneumoniae</i>	MS5294	Kinki area	2009	>16	>64	>16	>16	>16	>32	>4	>16	≤0.5	>8	16	>32	≤16	≤4	4	4	>2	2	32	4	0.25	32
<i>K. pneumoniae</i>	MS5295	Kinki area	2010	>16	>64	>16	>16	>16	>32	>4	16	≤0.5	>8	16	>32	≤16	≤4	4	4	>2	1	32	2	0.5	32
<i>K. oxytoca</i>	MS5282	Kinki area	2009	>16	>64	>16	>16	>16	>32	>4	>16	1	>8	16	>32	≤16	8	8	>8	>2	0.5	32	1	0.25	16
<i>E. coli</i>	MS5264	Amagasaki	2011	>16	>64	>16	>16	>16	>32	>4	>16	1	>8	>16	>32	>64	≤4	>8	2	>2	0.5	32	1	0.25	64
<i>E. coli</i>	MS5267	Amagasaki	2011	>16	>64	>16	16	>16	>32	>4	>16	1	8	16	>32	≤16	8	8	8	>2	0.25	8	0.25	0.25	4
<i>E. coli</i>	MS5275	Kinki area	2006	>16	>64	>16	>16	>16	>32	>4	>16	1	>8	16	>32	≤16	≤4	≤1	4	>2	0.25	16	0.5	≤0.125	8
<i>E. coli</i>	MS5276	Kinki area	2006	>16	>64	>16	>16	>16	>32	>4	>16	1	>8	>16	>32	≤16	≤4	≤1	>8	>2	0.5	16	0.5	≤0.125	8
<i>E. coli</i>	MS5277	Kinki area	2009	>16	>64	>16	16	>16	>32	>4	>16	1	>8	16	>32	≤16	≤4	4	4	0.5	0.5	16	1	≤0.125	8
<i>E. coli</i>	MS5278	Kinki area	2010	>16	>64	>16	8	>16	>32	>4	>16	1	8	16	>32	≤16	≤4	8	2	≤0.25	0.25	2	≤0.125	≤0.125	1

Susceptibility tests were performed by MicroScan WalkAway (Siemens Healthcare Diagnostics, Tokyo, Japan), NMIC6.31J.

# Susceptibility tests were performed by the microdilution method (7).

ABPC; Ampicillin, PIPC; Piperacillin, CEZ; Cefazolin, CAZ; Ceftazidime, CPR; Cefpirome, CMZ; Cefmetazole, CPDX; Cefpodoxime, AZT; Aztreonam, IPM; Imipenem, MEPM; Meropenem, A/C; Amoxicillin/Clavulanate, C/S; Cefoperazone/Sulbactam, P/T; Piperacillin/Tazobactam, AMK; Amikacin, GM; Gentamicin, MINO; Minocycline, CPFX; Ciprofloxacin, PAPM; Panipenem, BIPM; Biapenem, DRPM; Doripenem