Supplementary Information

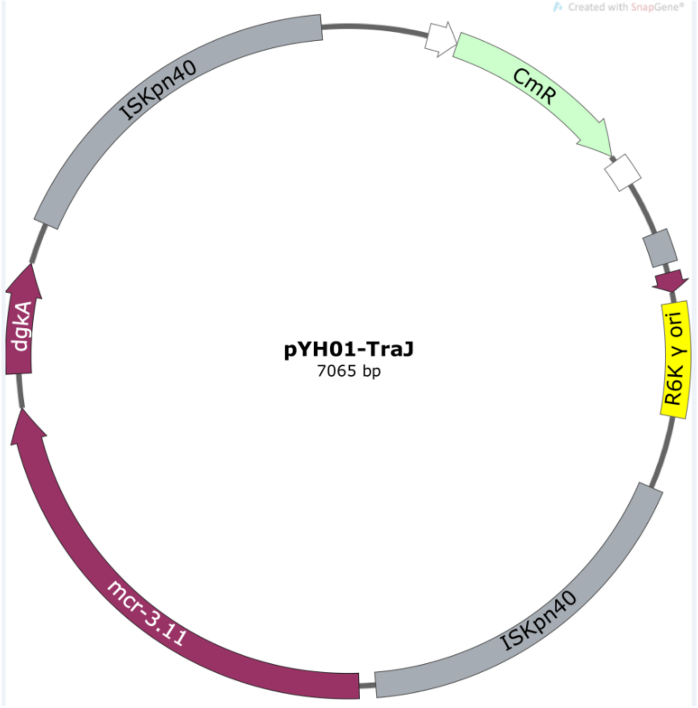

Figure S1: The plasmid profile of pYH01-TraJ

Table S1. Target site test primers used in this study.

Target sites	primers	Sequence (5'→3')
NO1	TF1	CAGTGTCGAGGGCTTCATCA
NO2	TF2	CTCACGTACCTGAGAGTAGTC
NO3	TF3	CAAAGCGATGGGAGCAATGA
NO4	TF4	TGCTGAATGCGATGAAGCAC
NO5	TF5	CGTTGGGTGTAGGAATGACA
NO6	TF6	ACGGTTTCCTTGATGAAATCCTC
NO7	TF7	CGGTTTTGCTGGATTCGGTT
NO8	TF8	GGATCACCACAAAGAACACC
NO9	TF9	GACAAAAGGTGCACTCATCC
NO10	TF10	TGCGGCTACACTATTTCCCTG
NO11	TF11	TATAGCCGCTCACCTGCACA
NO12	TF12	CGGATAACGGTATTTCCATGCT
NO13	TF13	ACACGGCAACACATCCCATC
NO14	TF14	GTCCTGACTGACAGCTCGAA
NO15	TF15	GAAGCAGTGGGGAAGAAGTG
NO16	TF16	CGTCACCAATCACGACACTG
NO17	TF17	GGCAGAAGAGCTTAAGGATGT
NO18	TF18	GCGAGTGGTGAATGTGTCGA
NO19	TF19	GTCAGGTAATCGACTATTCCGT
NO20	TF20	CGTATGGCGCTGATCGTTGA
NO21	TF21	TTGGTAATTGCCGGGATCATCG
NO22	TF22	ATGACGATCAGGGTCGTCTG
NO23	TF23	CAGCTTGGCGTTGTTATCTACC

Table S2. Plasmid construction and AP-PCR primers used in this study

Primers	Sequence (5'→ 3')	Reference
pYH-AflII	TACGC**CTTAAG**CGGTAAGCTAGACAAAACGGA	This sdudy
pYH-BglII	TACGC**AGATCT**TGGTCGGAGATATGGGTGTAG	This sdudy
pJS05-AflII	TACGC**CTTAAG**TCTCCGTCTCGAGGTTGATC	This sdudy
pJS05-BglII	CTCCGACCA**AGATCT**CCATGTCAG	This sdudy
traJ-1	TACGC**ACTAGTT**CGTCTGGAAGGCAGTACACCTT	This sdudy
traJ-2	TACGC**GTCGAC**CCAGTCGGTAGATATTCCAC	This sdudy
IS*Kpn40*-F1	AGCCTTAATTGCTCGCTTCG	This sdudy
IS*Kpn40*-F2	ATCAGAACGCTCAAGGAGCA	This sdudy
CTI-3U	AGTGTGGGGTGCATTGACAG	This sdudy
MCR3-F	TGGTCGGAGATATGGGTGTAG	This sdudy
MCR3-R	CGGTAAGCTAGACAAAACGGA	This sdudy
AB1	GGCCACGCGTCGACTAGTACNNNNNNNNNNACGCC	AB1
AB2	GGCCACGCGTCGACTAGTACNNNNNNNNNNCCTGG	AB2
AB3	GGCCACGCGTCGACTAGTACNNNNNNNNNNCCTCG	AB3
ABS	GGCCACGCGTCGACTAGTAC	ABS

Restriction sites are underlined.

Table S3. The whole genetic structure IS*Kpn40-mcr-3.11-dgkA*-IS*Kpn40* in the genome verified primers used in this study

Target sites	Upstream primers	Sequence (5'→3')	Downstream primers	Sequence (5'→3')
NO1	trg-F	CAGTGTCGAGGGCTTCATCA	trg-R	GTTCAGCCCGGATCAATATG
NO2	ybcY-F	CTCACGTACCTGAGAGTAGTC	ybcY-R	CATTCTCGAAATGCTCTGAG
NO 3	yjfk-F	CAAAGCGATGGGAGCAATGA	yjfk-R	ATATGAAGTGATGTCAGCGG
NO 4	narQ-F	TGCTGAATGCGATGAAGCAC	acrd-R	AAATGGGGCGATCAATAAAG
NO 5	ydgJ-F	CGTTGGGTGTAGGAATGACA	blr-R	GACAAGAACGATCCAACCTG
NO 6	fliZ-F	ACGGTTTCCTTGATGAAATCCTC	fily-R	TTAAGCAGACCTTCATCTGC
NO 7	pagA-F	CGGTTTTGCTGGATTCGGTT	pgaa-R	ATCAGTGAAAGCCCAGGAGA
NO 8	acrF-F	GGATCACCACAAAGAACACC	acrF-R	GTAGTGCCGTTAGGGATTG
NO 9	slp-F	GACAAAAGGTGCACTCATCC	slp-R	ATCAAGGAAGCCGCTTTGTC
NO 10	ygeQ-F	TGCGGCTACACTATTTCCCTG	glyu-R	CCGCTCCAATTTATCTTCGC
NO 11	aer-F	TATAGCCGCTCACCTGCACA	pat-A	GTTCAAAATATGCTCCGGAG
NO 12	ydiO-F	CGGATAACGGTATTTCCATGCT	ydio-R	ACGCGTGTAAGTGGTAGTGG
NO 13	maa-F	ACACGGCAACACATCCCATC	ylac-R	TAGCAAACGCGGAAATCCAG
NO 14	yclT-F	GTCCTGACTGACAGCTCGAA	osmb-R	GTCCCGTTTAGACCAGTTAG
NO 15	glf-F	GAAGCAGTGGGGAAGAAGTG	glf-R	GCATTCCCTTGGAAGTTTGG
NO 16	yrdA-F	CGTCACCAATCACGACACTG	rrsd-R	GAGCCATGATCAAACTCTTC
NO 17	ugd-F	GGCAGAAGAGCTTAAGGATGT	wzzb-R	GTTATAGCCAGCAATTTGCC
NO 18	yjbI-F	ATCAAAGCGAGTGGTGAATGT	yjbi-R	CATAACAGCTGTCAAATTCG
NO 19	hha-F	GTCAGGTAATCGACTATTCCGT	maa-R	GAGCGATACAACTCACCAGC
NO 20	upp-F	CGTATGGCGCTGATCGTTGA	uraa-R	GGCGCGTCATAGTATTATCC
NO 21	yihO-F	TTGGTAATTGCCGGGATCATCG	yiho-R	CGGGAAAAAATCGCCTATGG
NO 22	yhdV-F	ATGACGATCAGGGTCGTCTG	yhdw-R	ATAATGCTTCCTGTGAGGCG
NO 23	xylF-F	CAGCTTGGCGTTGTTATCTACC	xylf-R	TGAAGAAACACAAATGTCGC
MCR-3	MCR-3D	GGCTATGAGAAAGACACCAATCC	MCR-3U	ATTGCGAGCGCGATTATCAT

Table S4. Plasmids and genome sequences bearing *mcr-3 and its variants containing* one or more copies of IS*Kpn40*, searched in Genbank database.

Plasmids/ Strains	Size (bp)	Location	Specials	Source	Region	IS*Kpn40* (up)	*dgkA*	IS*Kpn40* (down)	DR	*mcr-*type	Acc. No.
pECSC102	60744	IncP	*Salmonella enterica*	Poultry -	China	1	1	1	ATTT/GAAT	*mcr-3.11*	MG552133
pMYKLB95-1	99493		*Klebsiella pneumoniae*		China	1	1	1	AAAT/ATTC	*mcr-3.11*	MH341574
Strain KLB38			*Klebsiella pneumoniae*		China	1	1	1	ATTC.AAAT	*mcr-3.11*	MK112271
Strain KLB49			*Klebsiella pneumoniae*		China	1	1	1	ATTT/GAAT	*mcr-3.11*	MK112272
pVE769	88108		*Escherichia coli*	Food	Vietnam	0	1	1	/CACC	*mcr-3.2*	AP018353
pVE155	87280		*Escherichia coli*	Food	Vietnam	0	1	1	/CACC	*mcr-3.1*	AP018354
pJSWP006	123579	IncF	*Escherichia coli*		Japan	1	1	1	/CACC	*mcr-3.1*	AP018939
GSH8M-1	4611279		*Aeromonas caviae*	Plant	Japan		1			*mcr-3*	AP019195
ASNIH7	4817060		*Aeromonas sp*		USA		1			*mcr-3.18*	CP026226
pMCR3_025943	50520		*Escherichia coli*		China					*mcr-3.5*	CP027203
17ISAe	4652208		*Aeromonas veronii*	Discus	Korea		1			*mcr-3*	CP028133
FC951	4666657		*Aeromonas veronii*		India		1			*mcr-3.19*	CP032839
pCHL5009T	101860		*Escherichia coli*		New Zealand	1	1		/GTTT	*mcr-3.1*	CP032937
pCP53-mcr	231859		*Escherichia coli*		China		1	1	/AAAT	*mcr-3.19*	CP033094
Aeromonas veronii	36959		*Aeromonas veronii*		China		1			*mcr-3.6*	MH481281
pHS5257MCR3.1	7698		*Salmonella*		Brasil		1	1	/CATT	*mcr-3.1*	MK652761
plasmid pT3	79293		*Escherichia coli*		Switzerland		1			*mcr-3*	MK656937
p08-5333.1	178506		*Salmonella enterica*		Canada		1	1	/GTTT	*MCR-3.1*	CP039562
pWJ1	261119		*Escherichia coli*		China		1	1	/GAAT	*mcr-3*	KY924928
pMCR3_WCHEC-LL123	52208		*Escherichia coli*		China		1			*MCR-3.5*	MF489760

strain 172	268746	*Aeromonas veronii*		China		1			*mcr-3*	MF495680
Aeromonas caviae	26269	*Aeromonas caviae*		China					*mcr-3.10*	MG214531
Escherichia coli	20376	*Escherichia coli*		Brazil		1			mcr-3	MG491669
pHN8	53148	*Escherichia coli*		China	1	1		ATTT/	mcr-3	MG780294
p17S-208	260339	*Escherichia coli*	Swine	South Korea	1	1	1	TTTA/CACC	mcr-3	MH077952
p131681	201750	*Salmonella enterica*		Canada		1	1	/CACC	mcr-3.2	MH114596

Table S5. Insertion sites of IS*Kpn40-mcr-3.11-dgkA*-IS*Kpn40* in the genome of *E.coli* MG1655 (*recA*∷Km).

Transposition Events	Insertion sites	Function
NO1	trg	methyl-accepting chemotaxis protein Ⅲ, ribose and galactose sensor receptor
NO2	ybcy	pseudogene, DLP12 prophage; methyltransferase homology; Phage or Prophage Related
NO3	*yjfK*	conserved protein, DUF2491 family
NO4	*narQ/ acrD*	sensory histidine kinase in two component regulatory system with Nar (NarL)/aminoglycoside；multidrug efflux system
NO5	*ydgJ/ blr*	Putative oxidoreductase/betalactam resistance membrane protein; divisome-associated protein
NO6	*fliZ/ fliY*	RpoS antagonist; putative regulator of FliA activity/cystine transporter subunit
NO7	*pgaA*	biofilm adhesin polysaccharide PGA secretin; OM porin; polybeta-1,6-N-acetyl-D
NO8	*acrF*	glucosamine export protein
NO9	*slp*	outer membrane lipoprotein
NO10	*ygeQ*	pseudogene
NO11	*patA/aer*	putrescine:2-oxoglutaric acid aminotransferase, PLPdependent/ fused signal transducer for aerotaxis sensorycomponent ；methyl accepting chemotaxis component
NO12	*ydiO*	putative acyl-CoA dehydrogenase
NO13	*ylaC*	inner membrane protein, DUF1449 family
NO14	*osmB*	lipoprotein
NO15	*glf*	UDP-galactopyranose mutase, FAD/NAD(P)-binding
NO16	*yrdA/rrsD*	hypothetical protein/16S ribosomal RNA of rrnD operon

NO17	*wzzB*	regulator of length of O-antigen component of lipopolysaccharide chains
NO18	*yjbI*	pseudogene, pentapeptide repeat-related
NO19	*hha*	modulator of gene expression, with H-NS
NO20	*upp*	uracil phosphoribosyltransferase
NO21	*yihO*	putative transporter
NO22	*yhdV*	putative outer membrane protein
NO23	*xylF*	D-xylose transporter subunit