
Supplementary information

Mating pair stabilization mediates bacterial conjugation species specificity

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Supplementary Information

Mating pair stabilisation mediates bacterial conjugation species specificity

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Supplementary Table 1. Bacterial strains used in this study.

Strain	Description	Source
Cloning strains		
CC118 λ pir	Expresses the Pi protein for the replication of plasmids with the R6K origin.	Lab collection
<i>E. coli</i> 1047 pRK2013	Triparental conjugation helper strain. Kanamycin resistant	Lab collection
<i>Klebsiella pneumoniae</i> strains		
ICC8001	Parental wild type (WT) strain of <i>K. pneumoniae</i> ATCC43816 serially passaged <i>in vitro</i> on Rifampicin (100 μ g/ml) followed by two passages in BALB/c mice.	Lab collection
Donor strains		
ICC8005	Donor strain for conjugation assays. Tagged with constitutive Biofab promoter-driven <i>lacI</i> construct at 3' end of <i>glmS</i> gene	This study
GFP-D	ICC8005 carrying pKpGFP	This study
GFP-DD	ICC8005 carrying pKpGFP-D	This study
GFP-D Δ <i>traN</i>	ICC8005 carrying pKpGFP Δ <i>traN</i>	This study
GFP-DD Δ <i>traN</i>	ICC8005 carrying pKpGFP-D Δ <i>traN</i>	This study
GFP-D <i>traN</i> _{R100-1}	ICC8005 carrying pKpGFP <i>traN</i> _{R100-1}	This study
GFP-DD <i>traN</i> _{R100-1}	ICC8005 carrying pKpGFP-D <i>traN</i> _{R100-1}	This study
GFP-DD <i>traN</i> _{Ch1}	ICC8005 carrying pKpGFP <i>traN</i> _{Ch1} . Tip region of TraN corresponds to TraN _{R100-1} .	This study
GFP-D <i>traN</i> _F	ICC8005 carrying pKpGFP <i>traN</i> _F	This study
GFP-DD <i>traN</i> _F	ICC8005 carrying pKpGFP-D <i>traN</i> _F	This study
GFP-DD <i>traN</i> _{Ch2}	ICC8005 carrying pKpGFP <i>traN</i> _{Ch2} . Tip region of TraN corresponds to TraN _{pSLT} .	This study
GFP-DD <i>traN</i> _{Ch3}	ICC8005 carrying pKpGFP <i>traN</i> _{Ch3} . Tip region of TraN corresponds to TraN _{MV1} .	This study
Recipient strains		
Δ 35	ICC8001 Δ <i>ompK35</i>	Lab collection
Δ 35/36 _{ST258}	ICC8001 Δ <i>ompK35</i> expressing ST258 variant of <i>ompK36</i>	Lab collection
Δ 35/ Δ 36	ICC8001 Δ <i>ompK35</i> Δ <i>ompK36</i>	Lab collection
Δ 35/36 _{ST258} Δ GD	ICC8001 Δ <i>ompK35</i> , <i>ompK36</i> _{ST258} with L3 GD deletion	Lab collection
Δ 35/36 _{WT} +GD	ICC8001 Δ <i>ompK35</i> , <i>ompK36</i> _{WT} with L3 GD insertion	Lab collection
ICC8006	Recipient strain used for live microscopy experiment. Tagged with Biofab-promoter driven <i>dTomato</i> construct at 3' end of <i>glmS</i> site	This study
ICC8007	ICC8001 Δ <i>ompA</i>	This study
ICC8008	ICC8001 Δ <i>ompW</i>	This study
ICC8009	ICC8001 Δ <i>phoE</i>	This study
<i>Escherichia coli</i> strains		
DH5 α R100-1	<i>E. coli</i> donor for R100-1	This study
MG1655	<i>E. coli</i> K-12 strain	Lab collection
ICC8010	MG1655 Δ <i>ompA</i>	This study
ICC8011	MG1655 Δ <i>ompF</i>	This study
ICC8012	MG1655 Δ <i>ompC</i>	This study
Others		
SV3081	<i>Salmonella enterica</i> serovar Typhimurium LT2 recipient strain. Cured of pSLT	Gift from Josep Casadesus
ATCC 13047	<i>Enterobacter cloacae</i> recipient strain.	Gift from Avinash Shenoy

Supplementary Table 2.xlsx. List of IncF conjugative plasmids.

Plasmids were grouped and colored according to *traN* variant. The percentage identity of each *traN* to the reference *traN* as determined by tBLASTn is listed.

Supplementary Table 3. Conjugative IncF plasmids used and generated in this study.

Plasmid	Description		Source
pKpQIL	IncFII _K <i>bla</i> _{KPC-2} -encoding plasmid. Shares sequence with pKpQIL-UK (Genbank accession no. KY798507)		Lab collection
R100-1	Derepressed variant of R100 (IncFII)		Provided by Fernando de la Cruz
pOX38	F plasmid derivative		Lab collection
pSLT	IncFII _S <i>S. Typhimurium</i> virulence plasmid		Lab collection
Synthetic reporter plasmids			
ID	Plasmid	Description	Source
pICC4000	pKpGFP	pKpQIL with <i>P</i> _{lac-sfGFP} construct inserted at disrupted <i>aadA</i> gene. Parental reporter plasmid.	This study
pICC4003	pKpGFP-D	pICC4000Δ <i>finO</i> . Derepressed reporter plasmid	This study
pICC4005	pKpGFP <i>traN</i> _{R100-1}	pICC4000Δ <i>traN</i> :: <i>traN</i> _{R100-1}	This study
pICC4006	pKpGFP-D <i>traN</i> _{R100-1}	pICC4000Δ <i>finO</i> Δ <i>traN</i> :: <i>traN</i> _{R100-1}	This study
pICC4007	pKpGFP-D <i>traN</i> _{Ch1}	pICC4000Δ <i>finO</i> Δ <i>traN</i> :: <i>traN</i> _{Ch1}	This study
pICC4008	pKpGFP <i>traN</i> _F	pICC4000Δ <i>traN</i> :: <i>traN</i> _F	This study
pICC4009	pKpGFP-D <i>traN</i> _F	pICC4000Δ <i>finO</i> Δ <i>traN</i> :: <i>traN</i> _F	This study
pICC4010	pKpGFP-D <i>traN</i> _{Ch2}	pICC4000Δ <i>finO</i> Δ <i>traN</i> :: <i>traN</i> _{Ch2}	This study
pICC4011	pKpGFP-D <i>traN</i> _{Ch3}	pICC4000Δ <i>finO</i> Δ <i>traN</i> :: <i>traN</i> _{Ch3}	This study
pICC4002	pKpQILΔ <i>finO</i>	Derepressed pKpQIL. No <i>P</i> _{lac-sfGFP} construct.	This study

Supplementary Table 4. Plasmids and vectors used for mutagenesis.

Vector	Description	Source
pACBSR	SmR; expresses I-SceI and lambda-red induced by L-Ara.	Lab collection
pSEVA612S	GmR; integrative plasmid (ori R6K) that harbours the oriT for conjugation.	Lab collection
pSEVA471	SmR; for selecting recipient cells in conjugation assays	Lab collection
pSEVA612S-Kp-N2-sfGFP	pSEVA612S derivative; inserts Biofab promoter driven <i>sfGFP</i> construct at 3' end of <i>glmS</i>	Lab collection
pET-28a(+)	KanR; used as template to amplify <i>lacI</i> gene	Lab collection
pCSCMV-dTomato	Used as template to amplify <i>dTomato</i>	Lab collection
pSEVA612SbiofablacI	pSEVA612S derivative; inserts Biofab promoter driven <i>lacI</i> construct at 3' end of <i>glmS</i>	This study
pSEVA612SPlacsfGFP	pSEVA612S derivative; inserts <i>lac</i> promoter driven <i>sfGFP</i> at disrupted <i>aadA</i> gene on pKpQIL	This study
pSEVA612SΔfinO	pSEVA612S derivative, deletes <i>finO</i> from pKpQIL	This study
pSEVA612SΔtraN	pSEVA612S derivative, deletes <i>traN</i> from pKpQIL	This study
pSEVA612Sdtomato	pSEVA612S derivative; inserts Biofab promoter driven <i>dTomato</i> construct at 3' end of <i>glmS</i>	This study
pSEVA612STraNR100	pSEVA612S derivative; substitutes the ORF of <i>traN</i> in pKpQIL with the ORF for <i>traN</i> from R100-1	This study
pSEVA612STraNCh1	pSEVA612S derivative; substitutes the ORF of <i>traN</i> in pKpQIL with a chimeric <i>traN</i> expressing tip of TraN _{R100-1}	This study
pSEVA612SΔompAKP	pSEVA612S derivative; deletes <i>ompA</i> from <i>K. pneumoniae</i>	This study
pSEVA612SΔompW	pSEVA612S derivative; deletes <i>ompW</i>	This study
pSEVA612SΔphoE	pSEVA612S derivative; deletes <i>phoE</i>	This study
pSEVA612STraNF	pSEVA612S derivative; substitutes the ORF of <i>traN</i> in pKpQIL with the ORF for <i>traN</i> from F	This study
pSEVA612SΔompAEC	pSEVA612S derivative; deletes <i>ompA</i> from <i>E. coli</i>	This study
pSEVA612STraNCh2	pSEVA612S derivative; substitutes the ORF of <i>traN</i> in pKpQIL with a chimeric <i>traN</i> expressing tip of TraN _{pSLT}	This study
pSEVA612STraNCh3	pSEVA612S derivative; substitutes the ORF of <i>traN</i> in pKpQIL with a chimeric <i>traN</i> expressing tip of TraN _{MV1}	This study
pSEVA612SΔompF	pSEVA612S derivative; deletes <i>ompF</i> from <i>E. coli</i>	This study
pSEVA612SΔompC	pSEVA612S derivative; deletes <i>ompC</i> from <i>E. coli</i>	This study

Supplementary Table 5. Primers used in this work.

Primer	Sequence (5' - 3')	Description
pSEVA612S_F	ATTACCCTGTTATCCCTATACTG	Amplifies linear pSEVA612S
pSEVA612S_R	TAGGGATAACAGGGTAATCCG	
lacIvector_F	TAAGGATCCAACAGGGTTC	Amplifies linear pSEVA612S with homology regions flanking the 3' end of <i>glmS</i> and Biofab promoter from pSEVA612S-Kp-N2-sfGFP
lacIvector_R	TTTTTTTTTACCTCCTTAAACTCC	
lacI_F	TTTAAGGAGGTAAAAAAAAGTGGTGA ATGTGAAACCAGTAAC	Amplifies <i>lacI</i>
lacI_R	AGAACCCTGTTGGATCCTTATCACTGC CCGCTTTCCAG	
glmS_F	GGTCAGGATGCGTCTATCG	Checks for insertion at 5' end of <i>glmS</i>
glmS_R	CCTGAGTCAGTTTGTGTCATC	
aadAUPHR_F	GGATTACCCTGTTATCCCTACAAACGC GAAGGCCGGTG	Amplifies upstream homology region flanking disrupted <i>aadA</i> on pKpQIL
aadAUPHR_R	TTTTCTCGACGCGCGAGGCCAAGCGA TC	
aadADNHR_F	GTACAAATAAGCAGATCAGTTGGAAGA ATTTG	Amplifies downstream homology region flanking disrupted <i>aadA</i> on pKpQIL
aadADNHR_R	TATAGGGATAACAGGGTAATGCAAGAT TCCACTATCAAAC	
plac_F	GGCCTCGCGCGTCGAGAAAATTTATC AAAAAGAGTG	Amplifies lac promoter
plac_R	CTTTACGCATACGTATCCTCCAAGCCT G	
sfGFP_F	GAGGATACGTATGCGTAAAGGCGAAG AG	Amplifies <i>sfGFP</i>
sfGFP_R	ACTGATCTGCTTATTTGTACAGTTCAT CCATACC	
finOUPHR_F	GGATTACCCTGTTATCCCTACCCGTGG TATCCGGAATATTC	Amplifies upstream homology region of <i>finO</i>
finOUPHR_R	GTAAATATAAAACAATTGCCTATCGTT CAGTTAATAAG	
finODNHR_F	GGCAATTGTTTTATATTTACCCATTCT GATAATTATACCTGGG	Amplifies downstream homology region of <i>finO</i>
finODNHR_R	TATAGGGATAACAGGGTAATCGGCAA CATCGTCTCCCC	
finOext_F	GTTCTATGCTGTGCACCTGG	Checks for deletion of <i>finO</i>
finOext_R	GTTATGATGCCGCAGCCTG	
traNUPHR_F	GGATTACCCTGTTATCCCTACCGCCAG TTTATCGATAATCTG	Amplifies upstream homology region of <i>traN</i> from pKpQIL
traNUPHR_R	GCAGCATGGTTTCTGCCCTCCCTCATC C	
traNDNHR_F	GAGGGCAGAAACCATGCTGCCTAATA AAGAG	Amplifies downstream homology region of <i>traN</i> from pKpQIL
traNDNHR_R	TATAGGGATAACAGGGTAATGGAATAG CGGCATGCTCAG	
traNext_F	GGAGAAAGTGGCACAAACCG	Checks for deletion or substitution of <i>traN</i> on pKpQIL
traNext_R	CTTCCCGACGTCCCTTTGAC	
dTomato_F	TTTAAGGAGGTAAAAAAAATGGTGA GCAAGGGCGAG	Amplifies <i>dTomato</i>
dTomato_R	AGAACCCTGTTGGATCCTTATTACTTG TACAGCTCGTCCATG	

traNvector_F	AGGACAGTAAACCATGCTGCCTAATAA AGAG	Amplifies linear pSEVA612S with homology regions flanking <i>traN</i> from pKpQIL
traNvector_R	TACGTTTCATTTCTGCCCTCCCTCATC C	
traNR100_F	GAGGGCAGAAATGAAACGATTTTACC TCTG	Amplifies <i>traN</i> from R100-1
traNR100_R	GCAGCATGGTTTACTGTCCTGCCTGTT TC	
KPCtraN_F	AGGGATGAGGGAGGGCAGAAATGAAG ACGGTTATTTCCG	Amplifies <i>traN</i> from pKpQIL
KPCtraN_R	TCTTTATTAGGCAGCATGGTTTATTGC GCGGATTGCTG	
KPC169vector_F	ACCCTGGTGATGGAAGAAAC	Amplifies linear pSEVA612S with portion of <i>traN</i> from pKpQIL flanking the variable region
KPC169vector_R	CCGTGTGCAAAAATTTTCC	
traNR100-160_F	TGGAAAATTTTTGCACACGGACTGCCA GTATCACCGGG	Amplifies variable region from <i>traN</i> of R100-1
traNR100-160_R	GTTTCTTCCATCACCGGGTCAGCGTA AAGGTGGAAGC	
ompAUPHR_F	GGATTACCCTGTTATCCCTAGGAGTTA ACCGCTGACGAAC	Amplifies upstream homology region flanking <i>ompA</i> from <i>K. pneumoniae</i>
ompAUPHR_R	CGGTTATAACTTTTTGCGCCTCATTAT CATCC	
ompADNHR_F	GGCGCAAAAAGTTATAACCGATAAAAA AACCCGCTTC	Amplifies downstream homology region flanking <i>ompA</i> from <i>K. pneumoniae</i>
ompADNHR_R	TATAGGGATAACAGGGTAATCCCGCTA CATTGAGGCCAG	
ompAext_F	CTTACGCTGCATGTATCAG	Checks for deletion of <i>ompA</i> in <i>K. pneumoniae</i>
ompAext_R	CAGGTAGGATCGTCGAC	
ompWUPHR_F	GGATTACCCTGTTATCCCTACCGGTTT TCATAAATAGTGC	Amplifies upstream homology region flanking <i>ompW</i>
ompWUPHR_R	ACAGAAGAATATCCACTTCCTCATTAT GG	
ompWDNHR_F	GGAAGTGGATATTCTTCTGTAACTGG CCAACG	Amplifies downstream homology region flanking <i>ompW</i>
ompWDNHR_R	TATAGGGATAACAGGGTAATGGCCAG GGGAGACCTATG	
ompWext_F	CTGAGGACTTAGTGTGATC	Checks for deletion of <i>ompW</i>
ompWext_R	CCCTGCTCAACATGTATCAC	
phoEUPHR_F	GGATTACCCTGTTATCCCTAAGGCGAT GGTGGCGGGCA	Amplifies upstream homology region flanking <i>phoE</i>
phoEUPHR_R	CTGCGGTTAATATTCAGTCCTGGTGAT TTATTTATACGCG CTATTCAATTGCG	
phoEDNHR_F	GGAAGTGGATATTCTTCTGTAACTGG CCAACG	Amplifies downstream homology region flanking <i>phoE</i>
phoEDNHR_R	TATAGGGATAACAGGGTAATATTGATA GCGGATCGGAC	
phoEext_F	CGGCGTTAAAAAACCTCC	Checks for deletion of <i>phoE</i>
phoEext_R	CTGCCGAAGGAGTATAAC	
traNF_F	GAGGGCAGAAATGAAACGATTTTACC TCTG	Amplifies <i>traN</i> from F
traNF_R	GCAGCATGGTTTACTGTCCTGCCTGTT TC	
ompAECUPHR_F	GGATTACCCTGTTATCCCTAGACTGAA GAAGAGCATGC	

ompAECUPHR_R	AGACGAGAACTTTTTGCGCCTCGTTATC	Amplifies upstream homology region flanking <i>ompA</i> from <i>E. coli</i> MG1655
ompAECDNHR_F	GGCGCAAAAAGTTCTCGTCTGGTAGA AAAAC	Amplifies downstream homology region flanking <i>ompA</i> from <i>E. coli</i> MG1655
ompAECDNHR_R	TATAGGGATAACAGGGTAATGAAAGC GGTTGGAAATGG	
traNpSLT_F	TGGAAAATTTTTGCACACGGACGGCC ACCATCACCGGC	Amplifies variable region from <i>traN</i> of pSLT
traNpSLT_R	GTTTCTTCCATCACCAGGGTCAGCGT GAACGTCGTTCTCCC	
traNMV1_F	TGGAAAATTTTTGCACACGGACGGCC AGCATTACCGGC	Amplifies variable region from <i>traN_{MV1}</i>
traNMV1_R	GTTTCTTCCATCACCAGGGTAACGGTG AAGCTGTAGCGC	
ompFUPHR_F	GGATTACCCTGTTATCCCTACGATCAT CCTGTTACGGAATATTAC	Amplifies upstream homology region flanking <i>ompF</i> from <i>E. coli</i> MG1655
ompFUPHR_R	AGGTGTGCTATATTTATTACCCTCATG GTTTTTTTTATG	
ompFDNHR_F	GTAATAAATATAGCACACCTCTTTGTTA AATGCCGAAAAAACAGGACTTTG	Amplifies downstream homology region flanking <i>ompF</i> from <i>E. coli</i> MG1655
ompFDNHR_R	TATAGGGATAACAGGGTAATCGCCAG TGCCCCCGGAG	
ompFext_F	GCAGACACATAAAGACAC	Checks for deletion of <i>ompF</i>
ompFext_R	GAGATTGCTCTGGAAG	
ompCUPHR_F	GGATTACCCTGTTATCCCTAGTGAAAT AGTTAACAAGCG	Amplifies upstream homology region flanking <i>ompC</i> from <i>E. coli</i> MG1655
ompCUPHR_R	TCAATCGAGAGTTATTAACCCTCTGTT ATATGC	
ompCDNHR_F	GGTTAATAACTCTCGATTGATATCGAA CAAAGGGC	Amplifies downstream homology region flanking <i>ompC</i> from <i>E. coli</i> MG1655
ompCDNHR_R	TATAGGGATAACAGGGTAATGATTCAC CAGCGGCCCGA	
ompCext_F	GTATCATATTCGTGTTGG	Checks for deletion of <i>ompC</i>
ompCext_R	GTACGCTGAAAACAATG	

Supplementary Table 6. Cryo-EM and Refinement Statistics of OmpK36-TraN complex.

Parameter	OmpK36-TraN
Data collection and processing	
Voltage (kV)	300
Electron exposure (e ⁻ Å ⁻²)	50
Pixel size (Å)	1.08
Particle images (n)	359,314
Point group	C1
Map resolution (Å)	
Software (final reconstruction)	cryoSPARC
Model:map FSC (0.5)	2.7
Map:map FSC (0.143)	2.6
Refinement and Model validation	
Bond lengths rmsd (Å)	0.002
Bond angles rmsd (°)	0.441
Clashscore	3.61
Ramachandran Favored (%)	96.39
Ramachandran Outlier (%)	0.1
MolProbity score	1.39
Deposition ID	
PDB (model)	7SZI
EMDB (map)	EMD-25567

Supplementary Table 7. Cysteine residues in predicted disulphide bonds in TraN.

TraN_pKpQIL		TraN_R100-1		TraN_F	
CYS #1	CYS #2	CYS #1	CYS #2	CYS #1	CYS #2
114	417	114	392	114	378
129	372	129	347	129	333
139	319	139	294	139	280
203 ^a	213 ^a	244 ^a	250 ^a	294	316
242 ^a	250 ^a	308	330	342	367
333	355	356	381	349	358
381	406	363	372	384	389
388	397	398	403	435	446
423	428	449	460	445	460
474	485	459	474	483	491
484	499	497	505	499	530
522	530	513	544		
538	569				

^aCysteine residues in the tip domain of TraN

Supplementary Table 8.xlsx. Cysteine residues in plasmid ORFs.

The table lists all annotated open reading frames in plasmids containing the reference *traN* genes and the corresponding number of cysteine residues encoded.

Supplementary Video. Live microscopy of pKpGFP-D conjugation.

Donor cells carrying pKpGFP-D, (GFP-DD) were mixed at a 1:1 ratio with WT recipients expressing dTomato (red) and observed by live microscopy. Transconjugant cells which have acquired the plasmid appear green. Each frame was captured 10 min apart. The video plays at 2400X actual speed.