С



Supplementary figure 1: Structural conservation across ParB/Srx superfamily and multiple sequence alignment of Osa-like members of the superfamily. (a) Electron density map (2Fo-Fc) for a selected region of Osa contoured at 1.0 o. (b) Structural superposition of ParB/Srx core region of Osa, hSrX and PfuParB. (c) Multiple sequence alignment of Osa-like fertility inhibition factors within the ParB/Srx superfamily showing conserved and predicted active site residues marked with red stars. Protein sequences are labeled by their gene names (where available), species abbreviations and Genbank index (GI) numbers. The alignment is colored at 95% consensus using the following scheme: : h, hydrophobic (ACFGHILMTVWY), I, aliphatic (ILAV) and a, aromatic (FYH) residues shaded yellow; p, polar (CDEHKNQRST) residues colored blue; s, small (ACDGNPSTV) and u, tiny (GAS) residues colored green, big residues (QRKEILMWYF) shaded grey; o, alcohol group (ST) containing residues shaded orange and absolutely conserved residues are shaded red. Species abbreviations are as follows: Bcen : *Burkholderia sence*; Blat : *Burkholderia lata*; Burk : *Burkholderia vietnamiensis*; Hinf : *Haemophilus influenzae*; Sent : *Salmonella enterica*; Sfle : *Shigella flexneri*; Tden : *Treponema denticola*; Vsp. : *Vibrio sp.*



hSrx

а

hSrx + ATP + Mg²⁺





Supplementary figure 2: Comparison of active site of hSrx with the predicted functional site of Osa, and associated biochemical activities.(a) ATP binding pocket of hSrx without ATP, with bound ATP in the active site of hSrx (PDB:3cyi) is shown along with the predicted ATP binding pocket of Osa. (b) DNase activity of P1ParB using 90-mer ssDNA substrate. (c) ATPase and (d) DNase activity of Osa in the presence and absence of EDTA. (e) Conserved phosphate binding site in the crystal structures of ParB/Srx superfamily proteins are shown. (f) ATPase activity of Osa in absence or presence of 1 µg of plasmid pCAMBIA1301 in the reaction mix.



Supplementary figure 3: Multimerization is conserved across the ParB/Srx superfamily. (a) Gel filtration chromatogram showing multiple peaks containing pure Osa, which was purified to homogeneity even before loading onto Superdex-S200 column. Corresponding eluted fractions were run on SDS-PAGE (inlet). (b) Blue-Native PAGE of purified Osa shows multimerization in the absence of substrate DNA. (c) Gel-shift assay with Osa and Ice1056Fin showing supershifting of DNA seen with uncut plasmid (pRSF-Duet1) as non-specific DNA, linearized dsDNA (pCAMBIA1301) with T-DNA borders, and 90mer ssDNA with left and right borders substrates. The reaction carried out for 15 minutes on ice and ran on 1% and 2.5% Agarose gels for dsDNA and ssDNA, respectively. Samples loaded in lanes 1-4 had 0.5, 1, 2.5, 10 µg, respectively, of corresponding FIN proteins. The concentrations used were 50 and 500 ng for dsDNA and ssDNA, respectively.



Supplementary figure 4: ATPase activity of purified VirB/D4 T4SS components; VirD4, VirB4, VirB11 and the relaxosome ATPase VirC1. [γ -P32]ATP was used as substrate in the assay and the reaction products were run on TLC.

a OSA-associated

KikATrbM Histone_HNS GDResolvase+HTP RepA_C KfrA-HTH SNase KfrB* ParB-OSA ArdC_DUF1738 2 DUF3560 SSB KorAHTH nuc_Plasmid IncW pIE321_189009816
SSB DUF3560 2 2 Ardc_DUF1738 2 ParB-OSA KfrB* SNase KfrA-HTH RepA_C COResolvase+HTH
SSB DUF3560 2 2 ArdC_DUF1738 2 ParB-OSA KfrB* SNase KfrA-HTH RepA_C CDResolvase+HTH 2 Phage_Integrase
ParB-OSA KfrB* SNase KfrA-HTH 2 2 2 C CResolvase+HTH _Oligella ureolytica_517401865
KorAHTH SSB DUF3560 ? KfrB* SNase KfrA-HTH ? ? ? Transposase nuc1 Plasmid pHH2-227 373158784 ? KfrB* SNase KfrA-HTH ? ? ? Transposase
T4SS and Relaxase (RCRE) system associated
KfrA-HTH MRR ? Kees_DUF2688 ? KorAHTH VbhA DOC+KfrB* ? <th?< th=""> ? <th?< th=""> ? <th< td=""></th<></th?<></th?<>
PhdreFM_antitox PIN ? VirB6_TrbL ? VirB8_Tral KfrB* VirB9_Tral VirB11 ? TrfA ? ParA_TraL_MobD _Bordetella sp. FB-8_518782582
VirD4TraG VirD2TraIRCRE TraJ-RHH TraK_HTH ParA_traL_MobD TraM X? KfrB* KorB ParA ? KleE_2xTM RepA_C XfasM23_2255_Xylella fastidiosa Plasmid pXFAS01_182682763
KleA KleE_2xTM KorAHTH Para_Trat_MobD KorB KfrA+HTH KfrB* Tratk_PRtase Tradk_MobD Tradk_HTH Tradk_PRtase Tradk_MobD Tradk_HTH Tradk_PRtase Tradk_MobD Tradk_HTH Tradk_PRtase Tradk_MobD Tradk_HTH Tradk_PRtase Tradk_MobD Tradk_HTH Tradk_HTH Tradk_PRtase Tradk_PRtase </td
RepC Kies_DUF2688 KleE_2XTM KorAHTH ParA_TraL_MobD KfrA-HTH KfrB* ? TraM ParA_TraL_MobD VirD2TraIRCRE VirD4TraG Vanthomonas avononodis 515736195 Station
VIRD4TraG VIRD2TraIRCRE TraJ-RHH TraK_HTH ParA_traL_MobD TraM TraN_PRTase KfrB* KfrA-HTH ? KOrB RarA_traL_MobD KorAHTH KleE_2xTM KleA
CYCME_3042_Cycloclasticus zancles 7-ME plasmid p7ME01_529066522
GDResolvase+HTP HTH KraC_DNA_primase (?) raE_TopolsomerasineF_SignalPeptidas (?) VirD4TraG VirD2TraIRCRE
traB_Plasmid QKH54_108524641 VirD4TraG riaF_SignalPeptida? 2 DiraE_Topolsomeras 2 DUF1738+Toprim 2 KfrB*
_Klebsiella pneumoniae_490300773 DUF2748 GDResolvase 7 KfrB* TrbL VirB10 TrbH VirB9_TraO VirB8 7 VirB6_TrbL VirB5
L665_2587_Ralstonia solanacearum SD54_525464989 Phage_Integrase 2 DDE_Tnp_IS240 DDE_Tnp_IS240 Transposase KfrB* 2 Troi_VirB10 DDE_Tnp_IS240 ? DDE_Tnp_IS240
SeSA_B0074_Salmonella enterica subsp. enterica serovar Schwarzengrund Plasmid pCVM19633_110_194733875
Kirb Plasmid pMBUI1_406693608
VirD4TraG /raF_SignalPeptids [®] // 2/TaF_Topoisomeras [®] // 2/UF1738+Toprim / KfrB* // 2/ 2/ 2/ VirB10 _Enterobacteriaceae_489109916
PTox3, T4SS and Relaxase (RCRE) associated
KirALTraL_Mobb TraKHTH RHH TruBVirB4 TruBVirB4 TruBVirB4 TruBVirB3 VirB1 VirB10Trb1 VirB9_Tra0 VirB8 pDTG1p74_Pseudomonas putida plasmid pDTG1_32469950 2 PTox3+Kfr8* KfrB PLD KikATrbM VirB11 VirB10Trb1 VirB9_Tra0 VirB8
VirB9_Tra0 VirB10Trb1 VirB11 VirB1SLT KikATrbM PLD PTox3+KfrB* _Pseudomonas monteilii_515086186
PTox2 and T4SS associated
PTox2+Krf8* C2 VirD4TraG VirB1 VirB0 VirB9_TraO VirB8_TraJ VirB6_TrbL Neut_2626_Nitrosomonas eutropha C91 Plasmid2_114326648
PTox1-associated or fused and T4SS and Mob associated
VIEB_IFD_ VIEB_IFD_ VIEB_IFD_ VIEB_IFD_ VIEB_IFD_ VIEB VIEBU VIEB VIEBU VIEB VIEBU V
KorB ? MobClear Relin VirD2TraiRCRE ? KikATrbM OmpA KfrB* PTox1 KfrB VirD4TraG ? VirB11 Trb1_virb10 VirB9_Tra0 VirB8_Tra1 traP_Plasmid pSB102_15919974
ParA_Tral_MobD KorB WirD2TralRCRE KikATrbM PTox1 KfrB* VirD4TraG VirB11 VirB9_TraO VirB8_TraJ ? XAC29_22584_Xanthomonas axonopodis Xac29-1 Plasmid pXAC47_470464946 VirD4TraG VirB11 Trb1_VirB10 VirB8_TraJ ? VirB6_TrbL
Fused to PTox1
KfrB fused to Zeta toxin and in the neighborhood of T4SS
DAM X 2 2 Epsilon2AT Zeta_toxin+KftB epsilon_1_AT Zeta_toxin+KftB 2 CDResolvase+HTF CRISPR_Cas2 VirB1SLT KikATrbM VirB6_TrbL pEP5289_p12_Neisseria gonorrhoeae Plasmid pEP5289_295788821
VIrD4TraG VIrD2TraIRCRE TraJ-RHH TraK_HTH ParA_TraL_MobD TraM TraN KfrB* (? KorB ParA_TraL_MobD KorAHTH Zeta_toxin (? KleE_2xTM Zeta toxin_Escherichia coli_518680379
Epsilon2AT PIN ?? ?? Epsilon2AT Zetatoxin+KfrB* ?? Coze-sranktaweturoc TrwBVirB4

Zeta toxin_Xanthomonas axonopodis_492671913

Relaxase (RCRE) associated

VIRD2TraIRCRE TraJ-RHH TraK_HTH ParA_TraL_MobD TraM TraN KfrB* KorB ParA_TraL_MobD KorAHTH Zeta_toxin (? KleE_2xTM G717_05107_Escherichia coli HVH 42 (4-2100061)_534699869
VIND2TRAIRCRE 2 TraJ_RHH TraK_HTH ParA_TraL_MobD TraM 2 KfrB* KfrA-HTH KorB ParA ParA_TraL_MobD KorAHTH KieFDUF2761 KleE_2XTM upf54.8_Plasmid pKJK5_111038104
TraN KfrB* KfrA-HTH KorB ParA_TraL_MobD ParA KorAHTH KIEE_2xTM KIEA
VIRDZTRAIRCRE ? TraJ-RHH TraK_HTH PARA_TRAL_MODD TraM TRAN PRTase KfrB* KorB ParA KleE_2xTM ? ? Antirestrict
virD2TraIRCRE TraH TraF.HH TraK_HTH ParA_TraL_MobD TraM 2 KfrB*
Fused to Zeta toxin
Zeta_toxin+KfrB*
Zeta toxin_Scytonema hofmanni_516351700
Zetatoxin+KfrB* 2 2 2 2 GDResolvase+HTH Enbinemolium venenhauum E1E7E0206
ParA_TraL_MobD 2 2 2 GDResolvase 2 2 Zetatoxin+KfrB* Epsilon2AT TraJ-RHH VirC1ParA 2 AvrX VirC1ParA
- F313 Venthermore site: Discover a Ven

orf213_Xanthomonas citri Plasmid pXcB_38639493

TraH ? ? ? ? Peptidase_M48 Epsilon2AT Zeta_toxin+KfrB* ? ? ? PIN

RBRH_00639_Burkholderia rhizoxinica HKI 454 Plasmid pBRH02_330399541

ZetaToxin and Relaxase (RCRE) associated

integrase 2X2 2 2eta_toxin+KfrB* 2 VirO2TraIRCRE ViraJ-RHH+TraJ-RH MPTase ParA_TraL_MobD KorB _Gilvimarinus chinensis_519055168
PLDC_2 2 Cas_ Cas4 Ajs_4239_Acidovorax sp. JS42 Plasmid pAOV001_121582542 2 DOC+KfrB VbhA 2 2 Cas_ Cas4 Ajs_extractiona_496535437 2 Cas_ Cas4 2 2 Cas_ Cas4 Puptidase_M48 2 2 Cas_ Cas4 2 2 Cas_ Cas4 Alide2_4789_Alicycliphillus denitrificans K601 Plasmid pALIDE201_330827365 VbhA DOC+KfrB Zeta_toxin+KfrB 2 Bac_DNA_binding 2 Asp_ protease_2
DOC associated in Toxin-Antitoxin systems with VbhA as antitoxin, also with Relaxase (RCRE)s virb2tralRCRE 2 Tra3-RHH Tra4_HTH ParA KorAHTH 2 KleE_2xTM kfrB-Plasmid pAKD4_290791091 TelA 7 KorAHTH ParA KorAHTH 2 KleE_2xTM Fic_Enterobacteriaceae_489109871 TelA 12 KorAHTH ParA Tra4_HTH Tra4_MobD Tra4_KrB_ TraN KfrA-HTH 2 Tra4_HTH Vrb2TraIRCRE Fic_enterobacteriaceae_489109871 TelA 12 KorAHTH ParA_Tra4_MobD Tra4_KrB_ TraN KfrA-HTH 2 Tra4_MobD Tra4_HTH Tra4_KrB_HTH Tra4_
DOC associated, most in a TA system with VbhA as antitoxin <u>UMWPC</u> VX contresters 2 D DOC+KfrB* AWRS Toxin 2 V VirB5 RPSIO7_mp1020_Ralstonia solanacearum PSIO7 Plasmid mpPSIO7_300694007 SN22+Resedur39 D DOC+KfrB* DOC+KfrB* D D DOC+KfrB* D DOC+KfrB* D D D DOC+KfrB* D D D D D D D D D D D D D D D D D D D
abhydrolase associated, most in a TA system with VbhA VirB5 RepC 2 2 2 bhydrolase+Kff9 _Acinetobacter baumannii_446686322 Toprim 2 GDResolvase KfrB 2 2 bhydrolase+KfrB MPTase _Acinetobacter lwoffii_490408714
TA-system with HEPN HEPN+KfrB* NT ? ? ? Radical_SAM SY1_16470_Fretibacterium fastidiosum_479198637
Fused to DCM and DAM and helicase. Note the several tandem KfrB domain containing genes
Fused to Toprim and in the neighborhood of a Relaxase (RCRE)

CDResolvase+HTH ? RelE_ParE ? F1738+Toprim+Kfr ? ? VirD2TraIRCRE ? Replicase+PriCT ? VirC1ParA

_Limnobacter sp. MED105_495528408

Fused to DUF4314, which is also associated with DNA modification systems

 ParA_Tral_MobD
 ?
 TraJ-RHH
 DUF4314+KfrB*
 VirD2TraJRCRE+KfrP
 ?
 KfrB
 ?
 SRAP

Secondary_structure		_ 		
nuc_Sent(PpSa)_976156	70	ΤΡΟΑΤΚSΤ	NANRLE	R D V E - R A I I E A Q Q V R E A V - G R E K <mark>A</mark> R A D E F H R H A E P G E T <mark>Y</mark> R <mark>G</mark> R V I G R T N S
kfrB_(BInc)_157277541	2	KHRLLVMN	GQRIVQ	T E N Q - G A W T N Q K V D K A G - A L K P G I Y N I Y M A Q K A D K S Q R H D G S I V H A D S G
SY1_00310_Ffas_479197652	156	ARQANLRA	GLEHFQ	<mark>E</mark> L Q R - E A L F P E A V K L E K E - I Q E L <mark>N</mark> L I E A P K I F V E P R E D G - Q T <mark>Y</mark> K <mark>G</mark> E <mark>I I</mark> H V <mark>N</mark> E <u>E</u> Q G -
SY1_10/90_Ffas_4/9198297	158		RERQRE	A L M P - E A V S S E L A G M I T E D - T G V Q E P T I N L H P Y T K G - Q W F T G K I I H T D A A H G R
Trac_CG10_493733315	419	REGLPVVR	RQISLA	LLME-LSAPSTMRTPTDIAREVIGETTVVKYAKENGQYVGPIIGQAAS
RPS107_mp1020_RS01(mpPS107)_300694007	397	GPGGPGTE	AGPVVD	E A R E T K Q S E L N V H A - A L D A G G Y T V S E T S G K Q D - R Q Y S G P V V A A S T L
MI du2051_0557_MI du(UMRADU2)_170745044	249	HQLLVILN	ESKLVD	TRIG-GEWVSRHVGQRG-GEPRGIYDLIGAARPALNAIS-QAYAGNVLHVSPS
WD 010030871 Bcn 518782582	12	LNAKAQLS	EQLHKG	Q V P K - G N V A L D E S K K V I D M A - A G Q K G L I V K D A G Q I K - Q D F K G Q V V A T S N H
Alide2 4702 Aden(nALTE201) 330827368	130			N TO CEVEN THE CENTRY OF CONTRACT OF CONTR
Msin34 2889 Malu(pMsin01) 254003174	241	F N A K A E L S	GULHKG	X Y E = G S V I V = DE S K K V I D M A - A S I K G L V V = K D A Q E L V Q D V K G E V V A I S S Π K D A Q E L V Q D V K G E V V A I S S Π
WP 017182896 Sxen 515750296	338		GSRIHE	
Mrad2831 6335 Mrad(pMRAD02) 170745042	12	OSKAEDRT	KPTDTP	
Neut 2626 Neut(Plasmid2) 114326648	193	TMEKEAOD	ΤΤΚΥΤΟ	F R L P - K P D W F T A R N L V S S - A D R K G F V Y A K P N - A O Y A G K V L M M S D T
XALg_3207_Xalb(plasmII)_386081571	175	SIEAYH-S	TPDRLA	
upf54.8_(рКЈК5)_111038104	2	KORLLVMN	GORIVO	TEOG-GAWTNEKVDKAG-ALKPGTYNLYMAKEADKG-OKHDGMTVHSDSB
kfrB_Bper(pBP136)_116006738	2	KERLLVMN	GQRIVQ	A E K D - G A W T N Q K V D K A G - A L K P G I Y N L Y T A Q A A D K K - Q T Y A G V I V H A D A T
kfrB_Ubac(pAKD4)_290791091	2	KQRLLVMN	GQRLVQ	S E Q G - G Q W A T D K V E K A G - T I K P G I Y N I H L S T K A D K S - Q S H D G V I V H A D K D
G717_05107_Eco1_534699869	3	KQRVLVMN	GQRVLQ	S E E A - G K W K N D N V D R A G - S I K P G I Y P L Y L S K G P D K T - S S Y N G P I L H A D K Q
XfasM23_2255_Xfas(pXFAS01)_182682763	2	KHRVLVMN	GQKIVQ	N E Q T P G K W H T E H V D K A G - L L K P G I Y N I Y A A T V A D K A - K E H E G T I V H V D K Q
Ms1p34_2857_Mg1u(pMs1p01)_254003142	237	GKSIRLGA	SGGGVV	Q V N R - D P F T P T G D R V P N V T I D - A I E A <mark>S</mark> I M A A A N I H F A K P D R T <mark>Y</mark> V <mark>G</mark> T <mark>L I</mark> H Q A D K S
TraB_Kpne_490300773	30	SMKICVLN	GSRQVD	K V V G – G E – – – – – – W Q T Q K V L P E A – G L P K <mark>G</mark> I Y Q L S E A Q – – Q A S K N V Y P – H S H V <mark>G</mark> Q V L H V <mark>D</mark> D R – – –
Tras_pokins4_108524641	22	SVKVVVLN	GSRQID	Q V V P - G A G Q N G E A G W V T M K V L P E N - G L P K G I H Q L H D A T N P A K N V H P - Q S F G G Q V L H V D K K
pP9014_p17_Pdam(pP9014)_253723656	×0	KQRLLVMN	GQRIIQ	TDNS-GAWTNQKVEKAG-ALKPGIYNLYMAKEADKS-KSHDGMIVHADTN
pDTGIp74_Pput(pDTGI)_32469950	80	DFKASFKG	ALELSK	K E H A - G A - A A G K P A E P E L A N - S K P K D M P A Y E R A V A G - K T Y E G K V V G F K E N Q
Trao Bamy 492083554	15	EFKASFKS	ALELSK	KEHA - GA - AA GK PAEMELAK - SK PMDM P AYEKAVAG - KIYEGKVVG - KENQ
Zeta toxin shof 516351700	167	SLKICHMN	COTTVO	
orf213 xcit(nxcB) 38639493	263	Q Q A V V I E N	BVBDVA	
MAK3 34c Sent(pMAK3) 160431694	70	TPDATKST		
SeSA B0074 Sent(pCVM19633 110) 194733875	5	SRKVCVIN	GSROLD	
L665 2587 Rsol 525464989	22	SRKTCVIN	GSROLD	
trap_pMOL98_254966497	20	HELLLIAN	ESRLLD	K V V D - G A W O S N Y T G S R G - G L P K G L Y D L T G A E R P G K T G A T - K S E E G N V L H V D K K
pEP5289_p14_Ngon(pEP5289)_295788823	284	OORVVTNS	KGVTLE	TKSA-NELWSKVEOIPVT-GMKAGIYLLGOAKKAETG-OTYSGEIIYKDAA
consensus/85%				pa.G.lls.p
Secondary_structure				
Secondary_structure nuc_Sent(Ppsa)_976156	YVI	QADDSRPG	T <mark>I I</mark> L	L HERAAVSGAEKVKMNDHAEISYPHGRAGIVRNPQAAQHQR 185
Secondary_structure nuc_sent(PpSa)_976156 kfrB_(BInC)_157277541	Y <mark>VI</mark> SIY	Q A D D S R P G Q Q V G K	T <mark>I I</mark> L N <mark>F V</mark> M	L H E R A A Y S G A E K V K M N D H A E I S Y P H G R A G I V R N P Q A A Q H Q R 185 M A A R S D F D
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 \$71_00310_Ffas_479197652	Y <mark>VI</mark> SIY YCL	Q A D D S R P G Q Q V G K Q R I G S	T I I L N F V M G L Y V	L H E R A A V S G A E K V K M N D H A E I S Y P H G R A G I V R N P Q A A Q H Q R 185 M H A R S D F D
Secondary_structure nuc_sent(PpSa)_976156 kfrB_(BInc)_157277541 syl_00310_ffas_479197652 syl_10790_ffas_479198297	YVI SIY YCL CIQ	Q A D D S R P G Q Q V G K Q R I G S Q T A S R	T <mark>I I L</mark> N F V M G L Y V L F T V	L H E R A A V S G A E K V K M N D H A E I S V P H G R A G I V R N P Q A A Q H Q R 185 M H A R S D F D K V P E I G S A K S I T V D A S G K A Q V S A E S V K L S R G R 113 V H R L D R L D
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 SY1_00310_Ffas_479197652 SY1_10790_Ffas_479198297 Trac_CG10_493733315 Drc20_mc300_pca1(erpcr07)_200604007	Y V I S I Y Y C L C I Q Q I V	Q A D D S R P G Q Q V G K Q R I G S Q T A S R Q N V G R Q	T <mark>I I L</mark> N F V M G L Y V L F T V T A I I	L H E R A A Y S G A E K V K M N D H A E I S Y P H G R A G I V R N P Q A A Q H Q R 185 M H A R S D F D K V P E I G S A K S I T Y D A S G K A Q Y S A E S V K L S R G R 113 V H R L D R L D V S P E
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 sY1_00310_Ffas_479197652 sY1_10790_Ffas_479198297 Trac_CG10_49373315 RPSI07_mp1020_Rso1(mpPS107)_300694007 Nrs42927 6232_Nrs4(cmpap02)_170745044	Y V I S I Y Y C L C I Q Q I V H V A	Q A D D S R P G Q Q V G S Q Q I S Q Q T A S Q Q N V G R Q Q N V G R Q	T <mark>I I L</mark> N F V M G L Y V L F T V T A I I W Q V V I	L H E R A A V S G A E K V K M N D H A E I S Y P H G R A G I V R N P Q A A Q H Q R 185 M H A R S D F D K V P E
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 syl_00310_Ffas_479197652 syl_10790_Ffas_479198297 TraC_CG10_493733315 RPS107_mp1020_Rso1(mpPS107)_300694007 Mrad2831_6337_Mrad(pMRAD02)_170745044 PBPU_00639_PcFbi(PBPUDQ)_33030541	Y V I S I Y Y C L C I Q Q I V H V A H V A	Q Q D D S R P G K Q Q V G K Q Q V S R Q R I S R Q N V G G R Q H I G R Q H I T A D - Q R	T I I L N F V N G L Y V L F T V T A I I W Q V V I G K A T V V K	L H E R A A V S G A E K V K M N D H A E I S V P H G R A G I V R N P Q A A Q H Q R 185 M H A R S D F D
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 svl_00310_Ffas_479197652 svl_10190_Ffas_479198297 trac_CG10_49373315 RPSI07_mp1020_Rso1(mpFs107)_300694007 Mrad2831_6337_Mrad(pMRA022)_170745044 RBRH_00639_Brhi(pBRH02)_330399541 wp_010930871 psn_518787582	Y V I S I Y Y C L C I Q Q I V H V A H V F A L V	Q Q Q V G K Q Q Q V G K Q Q R I A S R Q T A Q R Q Q H I T A D - Q R Q L T A D - Q M Q Q L S G M Q C L S	T I I I N F V L F T V T A I I W Q V V G K A T V V V A V F V A V F	L H E R A A V S G A E K V K M N D H A E I S V P H G R A G I V R N P Q A A Q H Q R 185 M H A R S D F D
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 syl_00310_Ffas_479197652 syl_10310_Ffas_479198297 Trac_cGlo_493733315 RPS107_mp1020_Rso1(mpPS107)_300694007 Mrad2831_6337_Mrad(pMRAD02)_170745044 RBRH_00639_Bcrhi(pBR402)_33039541 wP_019939871_Bsp_518782582 alide2 4292_aden(nat_IDF201)_330827368	Y V I S I Y Y C L C I Q Q I V H V A H V F A L V C V V	Q Q D D S R P G K Q Q V G K R Q Q V S R Q Q T A S R Q Q T A D - Q R V S D M Q V S D M V S D M V S D M V S D M V S D M	T I I L N F V G F V T F T W Q V V Q V V V A V V A V V F I V	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 sv1_00310_Ffas_479197652 sv1_10790_Ffas_479198297 traC_CG10_49373315 RPSIO7_mp1020_Rso1(mpFsD07_300694007 mrad2831_6337_mrad(pMRAD02)_170745044 RBRH_00639_Brhi(pBRH02)_330399541 wp_01993987_LBsp518782582 Al7de2_4792_Aden(pALIDE201)_330827368 Msin34_2888_M01u(msin01)_74003174	Y V I S I Y C I Q Q I V H V A H V F A L V C V V V V V V	Q Q Q Q Q Q Q Q Q Q Q Q Q Q	T I I I G L Y V L F T Y W Q V V I G K A T V V K V A V F V A V F V A V F	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 SY1_00310_Ffas_479197652 SY1_10790_Ffas_479198297 TraC_C610_49373315 RPSI07_mp1020_Rso1(mpPSI07)_300694007 Mrad2831_6337_Mrad(pMRAD02)_170745044 RBRH_00639_BFhi(pBR402)_330399541 WP_019939871_Bsp_518782582 Alide2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mg1u(pMsip01)_254003174 Wp_017182866_sxen_515570296	Y S I C L S Y C L Q Q I V H V F A L V C A L V C A L V C A L V C A L V	G A D U	T F V V G L Y V L A I I W Q A V V K V A V F V F K I	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 SYL_00310_Ffas_479197652 SYL_101790_Ffas_479198297 TraC_CG10_49373315 RPSI07_mp1020_Rso1(mpS107)_300694007 Mrad2831_6337_Mrad(pMRAD02)_170745044 RBRH_00639_BF1(pBRH02)_330399541 wp_01993987_LBsp518782582 A17de2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mg1u(pMsip01)_254003174 wp_ad2831_6335_mrad(pMRAD02)_170745042	Y S Y C L Q Y S Y C L Q V A V F H V V V A L V V G V V G T V	9 9 9 9 9 9 9 9 9 9 9 9 9 9	T F V N G F V N G F V N V F T V W Q V V K V A V F V A V F V F I V V F K V N - E - F K G V A F	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 svl_00310_Ffas_479197652 svl_10190_Ffas_479198297 Trac_CG10_49373315 RPSI07_mp1020_Rso1(mpPS107)_300694007 mrad2831_6337_mrad(pMRAD02)_170745044 RBRH_00639_Brhi(pBRH02)_330399541 wP_019939871_BSp_518782582 A1ide2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mglu(pMsip01)_254003174 wP_01718286_sxen_15750296 Mrad2831_6335_mrad(pMRAD02)_170745042 Mrad2831_6335_mrad(pMRAD02)_170745042	Y V I Y S Y C L Q V C Q V A F L V V V V V V V V V V V V V V V V V V V	G K K G K K G K K G K K K G K K K G K K K G K K K G K K K G K	T I I L G F V T A I I W Q A I I W Q A I I W Q A V V A V V A V V A V N A V S A V S A V S A V	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInC)_157277541 SV1_00310_Ffas_479197652 SV1_10700_Ffas_479198297 TraC_CG10_439733315 RPS107_mp1020_Rso1(mpS107)_300694007 Mrad2831_6337_Mrad(pMRAD02)_170745044 RBRH_00639_BFni(pBRH02)_330399541 wp_019393871_Bsp518782582 Alīde2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mg1u(pMs1p01)_254003174 wp_0127182896_Sxen_515750296 Mrad2831_6335_Mrad(pMRAD02)_170745042 Neut_2626_Neut(Plasmit)_380801571	Y V I S I Y L V C I V V V F V V V V V V V V V V V V V V V V	G G G S R Q R R R G G S R Q R R M G N Q R M G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N Q N G N G	T F V V L F V V L A I I Y V I G K A T V V I G K A T V V I G K A T V V I V A V F V	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 svl_00310_Ffas_479197652 svl_10790_Ffas_479197652 svl_00310_Ffas_479197652 rrac_CG10_49373315 RPSI07_mp1020_Rso1(mpFs107)_300694007 mrad2831_6337_mrad(pMRA002)_170745044 RBRH_00639_Brhi(pBRH02)_330399541 wp_0193987Lssp181782582 Al7ide2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mg1u(pMsip01)_254003174 wp_017182896_sxe_515750296 Mrad2831_6335_Mrad(pMRA002)_170745042 Mrad2831_6335_Mrad(pMRA002)_170745042 Neut_2626_Neut(Plasmid2)_114326648 XALq_3207_xalb(plasmi12)_386081571 upf54.8_(pKixS)_111038104	Y V I S I Y C I V	GKSRQRRMGMQZRGKGK GGSRQRRMGMQZRGKGK D	T	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BIRC)_157277541 SY1_00310_Ffas_479197652 SY1_10790_Ffas_479197652 SY1_10790_Ffas_479198297 Trad_CG10_49373315 RPSI07_mp1020_Rso1(mpRAD02)_170745044 RBRH_00639_Brhi(pBRAD02)_170745044 MP_019939871_Bsp_518782582 Alide2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mg1u(pMsip01)_254003174 WP_01718286_Sxen_515750296 Mrad2831_6335_Mrad(pMRAD02)_170745042 Neut_2626_Neut(Plasmid2)_114326648 XALq_3207_Xalb(plasmi1)_386081571 upf54.8_(pK)s5)_111038104 kfrB_Bper(pBP136)_116006738	Y S Y C Q H H A C A Y G F H Y S V V I V L Q V V V V V V V V V V V V V V V V V	GKSRQRRMGN GKSRQRRMGN SRGSRQRRMGN SRGGSRQRRMGN ADV SRGGSRQRRMGN QRTAT SRGGSRQRRMGN SRGGSRQRRMGN SRGGSRQRRMGN SRGGSRQRRMGN SRGGSRQR SRGQSQ <td>T F V N F V V F V V F V V F V V F V V V V F V V F V S V F V V F V V F V V F V V F V V F V V</td> <td>L H E R A A V S G A E K V K</td>	T F V N F V V F V V F V V F V V F V V V V F V V F V S V F V V F V V F V V F V V F V V F V V	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 sv1_00310_Ffas_479197652 sv1_10790_Ffas_479197652 sv1_00310_Ffas_479197652 rrac_CG10_49373315 RPSIO7_mp1020_Rso1(mpFsIO7)_300694007 mrad2831_6337_mrad(pMRA02)_170745044 RBRH_00639_Br1(pBRH02)_330399541 wp_01993987_lsp518782582 Al7de2_4792_Aden(pALIDE201)_330827368 Mrip34_2889_Mg1u(pMRA02)_170745042 Mrip34_2889_Mg1u(pMRA02)_170745042 Mrad2831_6335_mrad(pMRA02)_170745042 Neut_2662_Meut(Plasmid2)_114326648 XALq_3207_Xhl(plasmid2)_114326648 XALq_3207_Xhl(plasmid2)_1386081571 upf54.8_(pKr)51_11038104 kfrB_ubac(pAr64)_230791091	Y S Y C Q H H A C A Y G F H Y S V C Q H H A C A Y G F H Y S V Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y	GKSRQRRMGMQ7RKGKK GKSRQRRMGMQ7RKGKK GL	T - - F I I T - - - F Y Y N - - - F Y Y V - - - Y Y X V - - - Y Y X V - - - Y X X V - - - Y X X V - - - Y X X V - - - - X X V - - - - X X V - - - - X X V - - - - X X V - - - - X X V - - - - X X V - - - - - <t< td=""><td>L H E R A A V S G A E K V K</td></t<>	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 sv1_00310_Ffas_479197652 sv1_10790_Ffas_479198297 Trac_CG10_49373315 RPSIO7_mp1020_Rso1(mpPSIO7)_300694007 Mrad2831_6337_Mrad(pMRAD02)_170745044 RBRH_00639_Brhi(pBRH02)_330399541 wP_0193987L_B8p_518782582 A1ide2_4792_Aden(pARLD02)_1303827368 Msip34_2889_Mg1u(pMsip01)_254003174 wP_01718286_sxen_515750296 Mrad2831_6335_Mrad(pMRAD02)_170745042 Mrad2831_635_Mrad(pMRAD02)_170745042 Mrad2831_635_Mrad(pMRAD02)_170745042 Mrad2831_635_Mrad(pMRAD02)_170745042 Mrad2831_635_Mrad(pMRAD02)_170745042 Mrad2831_635_Mrad(pMRAD02)_170745042 Mrad2831_635_Mrad(pMRAD02)_170745042 Mrad2831_635_Mrad020_170745042 Mrad2831_635_Mrad020_170745042 Mrad2831_635_Mrad020_170745042 Mrad2831_635_Mrad020_170745042 Mrad2831_635_Mrad020_170745042 Mrad2831_635_Mrad020_170745042 Mrad2831_635_Mrad020_170745042 Mrad2831_635_Mrad020_170745042 Mrad2831_635_Mrad020_17074504	YSYCQHHACAYGFHYSNHS YSYCQHHACAYGFHYSNHS YSYCQHHACAYGFHYSNHS	GKKORCQRRMGNQQCQKKGKKKK PGGSNCQRTMGNQQCKGQCQQQQQ SR	T N G F Y Y T N G F Y Y V Y I V G V F X Y R V C X F Y R V C X	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInC)_157277541 SY1_00310_Ffas_479197652 SY1_10790_Ffas_479198297 TraC_CG10_49373315 RPS107_mp1020_Rso1(mpFs107)_300694007 Mrad2831_6337_Mrad(pMRA002)_170745044 RBRH_00639_Brhi(pBRH02)_330399541 wp_01993871_Bsp518782582 A17de2_4792_Aden(pALIDE201)_330827368 Mrsj934_2889_Mg1u(pMs102)_154003174 wp_01932889_Mg1u(pMs102)_154003174 wp_01932889_Mg1u(pMs102)_170745042 Neut_2626_Neut(Plasmid2)_114326648 XALq_3207_Xalb(plasmi1)_380605171 upf54_8_(pK3K5)_111038104 kfrB_UBac(pAKD4)_290791091 G717_05107_EC0_534(pps469) XfasW2_2255_Xfas(pXFAS01)_182682763	I Y L Q V A F V V L Y V V Y Y Y Y Y Y Y Y Y Y Y Y Y Y	GKOSRQRRRMQZRKGKKKK R - GSSRQRRRMQZRKGKKKKK D	I I	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB.(BInc)_157277541 sv1_00310_Ffas_479197652 sv1_10790_Ffas_479197652 sv1_10790_Ffas_479198297 trac_CG10_49373315 RPSI07_mp1020_Rso1(mpFs107)_300694007 mrad281_6337_mrad(pMRA002)_170745044 RBRH.00639_Brhi(pBRH02)_330399541 wp_0198987_Bsp518782582 A1ide2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mg1u(pMsip01)_254003174 wp_01718286_Sxen_515750296 Mrad2831_6335_Mrad(pMRA002)_170745042 Mrad2831_6335_Mrad(pMRA002)_170745042 Mrad2831_6335_Mrad(pMRA002)_170745042 Mrad2831_6335_Mrad(pMRA002)_170745042 Mrad2831_6335_Mrad(pMRA002)_170745042 Katg_3207_xa1b(p1asmi12)_14326648 XALq_3207_xa1b(p1asmi12)_1386081571 upf54.8_(pAts04)_20791091 g717_05107_Eco1_53469869 xfasW3.2255_xfas(pXFAS01)_182682763 Msip34_2857_Mg1u(pMsip01)_254003142	Y S Y C Q H H A C A Y G F H Y S N H S A F V V L Y V V Y Y Y Y Y Y Y Y Y Y Y Y Y Y	GKGSRQRRMGMQQRKGKKKKK PGGSRQDTDGQQQQGGGQGQGGGGGG DG	I I	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInC)_157277541 SY1_00310_Ffas_479197652 SY1_10790_Ffas_479197652 SY1_10790_Ffas_479198297 TraC_CG10_49373315 RPSI07_mp1020_Rso1(mpRsD02)_170745044 RRRH_00639_BFni(pBRH02)_330399541 wp_019939871_Bsp518782582 A17de2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mg1u(pMsip01)_254003174 wp_017182896_Sxen_515750296 Mrad2831_6335_mrad(pMRAD02)_170745042 Neut_2626_Neut(Plasmit)_380681571 upf54.8_(pK3K5)_111038104 kfrB_Bper(pBP136)_116006738 kfrB_ubac(pAK04)_290791091 G717_05107_Ecol_534699869 XfasM33_2255_Xfas(pXFAS01)_182682763 Msip34_2857_MG1u(pMsip01)_254003142 TraB_KDPUE_40300773 TraB_KDPUE_40300773	Y S Y C Q H H A C A Y G F H Y S N H S A I H H	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	I I	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 sv1_00310_Ffas_479197652 sv1_10790_Ffas_479197652 sv1_00310_Ffas_479197652 rrac_CG10_49373315 RPSI07_mp1020_Rso1(mpFs107)_300694007 mrad2831_6337_mrad(pMRA002)_170745044 RBRH_00639_Brni(pBRH02)_330399541 wp_01993987_Bsp18782582 Al7de2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mglu(pMsip01)_254003174 wp_017182896_sxen_515750296 Mrad2831_6335_Mrad(pMRA002)_170745042 Neut_2626_Neut(Plasmid2)_114326648 XALq_3207_Xalb(plasmid2)_114326648 XALq_3207_Xalb(plasmid2)_114326648 KrB_Ubac(pArbA)_290791091 G717_05107_Ecol_53469869 XfasW3_2255_Xfas(DxFAS01)_182682763 Msip34_2857_Mglu(pMsip01)_254003142 traB_KDne_490300773 traB_KDne_490310773	Y S Y C Q H H A C A Y G F H Y S N H S A I H H Y Y S Y C Q H H A C A Y G F H Y S N H S A I H H Y Y S Y C Q H H A C A Y G F H Y S N H S A I H H Y	GKGRQRRMGMG7RKGKKKKKKK PGGSRQRTGQDFGQGQGQGQGQGGGGGGGGGGGGG DG	T I I T N - - F Y I - - - F Y Y I - - - Y Y Y I - - - Y Y X I - - - Y Y X I - - - - - Y X I Y X Y X Y X Y X I Y X Y X Y X Y X I Y X Y Y X Y X Y X I Y Y Y Y Y X Y X Y Y Y X Y	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInC)_157277541 SV1_00310_Ffas_479197652 SV1_10790_Ffas_479197652 SV1_10790_Ffas_479198297 Trad_CG10_49373315 RPS107_mp1020_Rso1(mpRa002)_170745044 RRRH_00639_BFni(pBRH02)_330399541 wp_019393871_Bsp518782582 A17de2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mg1u(pMs1p01)_254003174 wp_017182886_Sxen_515750296 Mrad2831_6335_Mrad(pMRA002)_170745042 Neut_2626_Neut(Plasmit)_318081571 up754.8_(pKJK5)_111038104 kfrB_Bper(pBP136)_116006738 kfrB_0ber(pBP136)_116006738 kfrB_0ber(pBP136)_116006738 kfrB_0ber(pBP136)_16006738 kfrB_0ber(pBP136)_16006738 kfrB_0ber(pBP136)_16006738 kfrB_0ber(pBP136)_16006738 kfrB_0ber(pBP136)_16006738 kfrB_0ber(pBP136)_16006738 kfrB_0ber(pBP136)_16006738 kfrB_0ber(pBP136)_1254003142 TraB_NcPR64_00300773 traB_0C454_108524641 pP9014_p17_Pdam(pP9014)_253723656 pDTG174_Pout(pD161)_32469950	<pre>Y > Y > Q Q H H A O A Y G F H Y S N H S A I H H K Y Y > Y > Q Q H H A O A Y G F H Y S N H S A I H H K Y Y = Y = Y = Y = Y = Y = Y = Y = Y = Y</pre>	GKKORCQRRMGMQQCKKGKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK	T I I T - - F Y - - F Y T - - - F Y T - - - - - - V - - - - - - - V - - - - - - - - - V -	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(BInc)_157277541 sv1_00310_Ffas_479197652 sv1_10790_Ffas_479197652 sv1_10790_Ffas_479197652 rrac_CG10_49373315 RPSIO7_mp1020_Rso1(mpFsIO7)_300694007 mrad2831_6337_mrad(pMRA002)_170745044 RBRH_00639_BFni(pBRH02)_330399541 wp_0199398_Tlsp518782582 Alīde2_4792_Aden(pALIDE201)_330827368 Mrad2831_6355_mrad(pMRA002)_170745042 Neut_2626_Neut(Plasmid2)_114326648 XALq_3207_XA1b(plasmid2)_114326648 XALq_3207_XA1b(plasmid2)_114326648 XALq_3207_XA1b(plasmid2)_114326648 XALq_3207_XA1b(plasmid2)_114326648 XALq_3207_XA1b(plasmid2)_114326648 XALq_3207_XA1b(plasmid2)_116006738 kfrB_Ubac(pAR04)_200791091 G717_05107_Ecol_534698669 XfasW3_2255_Xfas(0xFAS01)_182682763 Msip34_2857_Mg1u(pMsip01)_254003142 traB_Kpne_490300773 traB_kpne_490310773 traB_kpne_490310773 traB_kpne_1074_17_Pdam(pP014)_253723656 pDTG1p74_Pput(nWAF7)_0527612	Y S Y C Q H H A C A Y G F H Y S N H S A I H H K Y Y Y S Y C Q H H A C A Y G F H Y S N H S A I H H K Y Y	GKGRQRRMGMQ7RKGKKKKKKKKK PGGSRQQDTDGQQPGQGQGGGGGGGGGGG DIG	T - - F I I T - - - F Y I - - - - F Y I - - - - - Y I I - - - - - - I I I V V I I - - - I <td< td=""><td>L H E R A A V S G A E K V K</td></td<>	L H E R A A V S G A E K V K
Secondary_structure nuc_Sent(PpSa)_976156 kfrB_(EBnc)_157277541 SV1_00310_Ffas_479197652 SV1_10300_Ffas_479197652 SV1_10790_Ffas_479198297 Trad_CG10_439733315 RPS107_mp1020_Rsol(mpS107)_300694007 Rrad2831_6337_Mrad(pMRA002)_170745044 RBRH_00539_BFni(pBRH02)_330399541 WP_019393871_Bsp518782582 Alīde2_4792_Aden(pALIDE201)_330827368 Msip34_2889_Mg1u(pMs1p01)_254003174 WP_017182886_Sxen_515750296 Mrad2831_6335_Mrad(pMRA002)_170745042 Neut_2626_Neut(Plasmit)_380681571 upf54.8_(pKJN5)_111038104 kfrB_Bper(pBP136)_116006738 kfrB_ubac(pAK04)_290791091 G71_05107_Ecol_534699869 XfasM32_2255_Xfas(pXFAS01)_182682763 Msip34_2857_Mg1u(pWsip01)_253723656 pTG1p74_put(pNG1)_32469950 orf31_Pput(pNA47)_9057612	Y S Y C Q H H A C A Y G F H Y S N H S A I H H K V Y H Y V Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y	GKGNRQRRMGNQRKKKKKKKKKKKKK GGSNQDT DGQGQGGGGGGGGGGGGGGGG D	I I	L H E R A A V S G A E K V K
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Supplementary figure 5: Operon associations of KfrB and multiple sequence alignment. In the gene neighborhood illustrations (**a**), genes are shown as box-arrows with the arrow head pointing to the 3' gene in the gene neighborhood. KfrB containing genes are colored red. Operons are labeled with gene names, species abbreviations and/or plasmid name and Genbank index numbers (GI) of the KfrB containing genes (marked as asterisk in the gene neighborhood). Gene neighborhoods are grouped based on similarity of associations. In the alignment (**b**), proteins are denoted by their gene names, species abbreviations and Genbank index numbers (GI). Additionally those derived from plasmids are denoted with the plasmid name in brackets. The alignment was colored at 85% consensus. Secondary structure elements are shown above the alignment. The consensus abbreviations and colouring scheme are as in Supplementary figure 1c. Species abbreviations are as follows: Aden : *Alicycliphilus denitrificans*; Blnc : *Birmingham IncP-alpha*; Bper : *Bordetella pertussis*; Brhi : *Burkholderia rhizoxinica*; Bsp. : *Bordetella* sp.; CGlo : *Candidatus Glomeribacter*, E : Enterobacteriaceae ; Ecol : *Escherichia coli*; Ffas : *Fretibacterium fastidiosum*; Mglu : *Methylovorus glucosetrophus*; Mrad : *Methylobacterium radiotolerans*; Neut : *Nitrosomonas eutropha*; Ngon : *Neisseria gonorrhoeae*; Pamy : *Pseudomonas amygdali*; Pdam : *Photobacterium damselae*; PpSa : Plasmid pSa; Pput : *Pseudomonas putida*; Rsol : *Ralstonia solanacearum*; Sent : *Salmonella enterica*; Shof : *Scytonema hofmanni*; Spla : Synthetic plasmid; Sxen : *Sphingobium xenophagum*; Ubac : uncultured bacterium; Xalb : *Xanthomonas albilineans*; Xcit : *Xanthomonas citri*; Xfas : *Xylella fastidiosa*.

а



Supplementary Figure 6a: Uncropped blots of Fig. 4a showing that Osa interacts with VirB11 independent of T4S machine and VirD4. After blocking, membranes i) and ii) were cut into two portions. The top and bottom portions of both i) and ii) were detected for VirB4 and FLAG-Osa respectively.



Supplementary Figure 6b: Uncropped blots of Fig. 4b showing that Osa interacts with VirB11 independent of T4S machine and VirD4. After blocking, membranes were either retained as a single membrane (ii) or cut into two (i) or three (iii) portions. i) The top and bottom membrane portions were detected for VirB4 and VirB11 respectively. ii) The membrane was detected for FLAG-Osa. Since the IgG heavy chain (used in the experiment) got detected at higher intensity even before FLAG-Osa could be detected, the blot was cut into two halves and the top portion was washed. The bottom portion was over exposed to detect for FLAG-Osa. iii) The top portion was used for detection of VirB4, the middle for detection of VirB11 and the bottom piece for detection of FLAG-Osa.





Supplementary Figure 6c : Uncropped blots of Fig. 4c showing Osa interacts with VirB4 and VirB11 in vitro.

No	Dlagmid	Strain	Description
110.	riasiiiu	Strain	Description
1	pKA165 (pvirB::osa)	<i>E. coli</i> DH5α	<i>osa</i> cloned under <i>virB</i> promoter, using <i>NdeI/Xho</i> I
2	pKA197	<i>E. coli</i> DH5α	FLAG-osa cloned under virB
	1		promoter, using <i>NdeI/Xho</i> I
3	pET28b::osa	<i>E. coli</i> DH106	osa cloned into $pET28b(+)$ under
-	I	and BL21(DE3)	<i>NdeI/XhoI</i> with N-terminal His ₆
		nlvsS	
4	nSounosa	A tumefaciens	osa cloned under virB promoter using
-	poorposu	I RA4404	HindIII/ KnnI
5	nSounosa-K100A	<i>"</i>	osa-K100A cloned under virB
5			promoter using <i>Hind</i> III/KnnI
6	\mathbf{pSoup} \mathbf{pSoup} $\mathbf{P1}$	"	$a_{Sa} R 1404$ cloped under vir B promoter
U	p50up0sa-K1+0A		using HindIII/KpmI
7	pSoup::osa-KR (100A)	"	$asa_{R}/1004_{R}/1004$ cloped under virB
/	P140A		promoter using HindIII/KnnI
8	nSoun: osa-nenta (D98A-	"	promoter using minum Kpm promoter using minum Kpm
0	$K100\Delta_D136\Delta_T139\Delta_$		<i>R140A</i> cloned under virB promoter
	R100A-D150A-1155A-		using HindIII/KnnI
0	$nSoup: fiw\Delta$	"	fiwA cloned under virB promoter using
,	psoupnwA		HindIII/KppI
10	nSoun…ICE1056Fin	"	<i>ica</i> 1056 <i>fin</i> cloned under vir B promoter
10	psoupeE10501		using HindIII/KpnI
11	nSoup: fin A	"	find cloned under virB promoter using
11	psoupipA		HindIII/KnnI sites
12	nSounnifC	"	nifC cloped under virB promoter using
14	psouppnc		HindIII/Knnl sites
13	pET28aice1056fin	E coli	Ice1056Fin in pET28a under
15	pE128aee1050111	E. CON BI 21 Codon Dlus	Neol/Yhol sites with C His, tog
14	pFTM/1osa-	<i>E coli</i> Rosetta	MBP tagged O_{s2} in pETM/1 under
14	pL1114103a-	(DF3) pL vsS	Ncol/BamHI
15	pFTM/1osa-K100A	<i>E coli</i> Rosetta	MBP tagged O_{82} -K100A in pETM/1
15	pL1114103a-1(100/1	(DF3) pL vsS	under NcoI/KnnI
16	pETM/1osa P1/0A	(DL3) pLyss "	MBD tagged Osa $P140A$ in $pETM41$
10	pE1104105a-K140A		under Ncol/KnnI
17	pETM/1osa-KP	"	MBP tagged O_{S2} -K100A-R140A in
17	(K100A - R140A)		nFTM/1 under NcoI/KnnI
18	$(\mathbf{K}_{100} \mathbf{A} - \mathbf{K}_{140} \mathbf{A})$	"	MBP tagged $O_{S2} K 100A P 140A$
10	$(D08 \land K100 \land D136 \land$		D126A T120A D140A in $pETM41$
	(D / 0A - R 1 / 0A - D 1 / 0A -		under NcoI/KnnI
10	$r T M / 1 \cdots v r C 1$	E coli T7	MBP tagged VirC1 in pETM41 under
17	pE110141VIIC1	E. $Ourress I^q$	Ncol/Knyl
20	pFTM/1virC2	"	MBP tagged VirC2 in pETM41 under
40	PE114171411C2		NcoI/KnnI

Supplementary table 1. Plasmids and strains used in this study.

21	InfR-Co::virD2	E. coli Rosetta	VirD2 in InfR-Co in the second
		(DE3) pLysS	cassette with C-His ₆ tag
22	pETM41::Δ1-86virD4	"	MBP tagged VirD4 (87-543) in
			pETM41under <i>NcoI/Kpn</i> I
23	pETM41::Δ1-424virB4	E. coli T7	MBP tagged VirB4 (425-743) in
		Express I ^q	pETM41under <i>NcoI/Kpn</i> I
24	pETM41::virB11	E. coli Rosetta	MBP tagged VirB11in pETM41 under
		(DE3) pLysS	NcoI/KpnI
25	InfR-Co::P1 parB	E. coli T7	His ₆ -tagged P1 ParB in InfR-Co first
		Express I^q	cassette
26	InfR-Co::RK2 korB	E. coli Rosetta	His ₆ -tagged RK2 KorB in InfR-Co
		(DE3) pLysS	first cassette

pETM41 was obtained from EMBL protein expression and purification core facility. InfR-Nhis, InfR-Chis and InfR-Co are in-house modified vectors from pRSF-Duet1 (Novagen) cotaining In-Fusion (Clonetech) cloning cassettes. InfR-Nhis and InfR-Chis both have an In-Fusion cloning cassette with TEV protease cleavable His₆-tag at the N and C-terminus, respectively. InfR-Co has two In-Fusion cassettes with both TEV protease cleavable N-terminal His₆-tag in the first cassette and C-terminal His₆-tag in the second cassette.

Primer	Seequence	Purpose
osa-R140A-FP	GGCATGACCGCTTCAATGTGG	To generate <i>osa-R140A</i>
osa-R140A-RP	ATCAGTGAAACACACCGCGAAA	"
	TG	
osa-K100A-FP	AAAGACCGGGCATATCCGTCG	To generate osa-K100A mutant and
		osa-K100A-R140A mutant
osa-K100A-RP	AGGCATGTCCTGCTTCAACGG	"
osa-D98A-	ATGCCTAAAGCCCGGGCATAT	To generate osa-D98A-K100A-
InK100A-FP		<i>R140A</i> from <i>Osa-K100A-R140A</i>
osa-D98A-	GTCCTGCTTCAACGGGCAGGC	"
InK100A-RP		
osa-D136A-	TTCACTGCTGGCATGGCCGCTTC	To generate osa-D98A-K100A-
T139A-InR140A-	Α	D136A-T139A-R140A from osa-
FP		D98A-K100A-R140A
osa-D136A-	ACACACCGCGAAATGGCCCAGA	"
T139A-InR140A-	AT	
RP		
fiwA-FP	AGACCATGGCTATGCTGACACG	PCR amplification of <i>fiwA</i> from
	GTTGAAG	pRP4* and cloning into pAK165
fiwA-RP	AGACTCGAGTTATCAGAGTCCG	"
	CGGCCTTT	
fipA-FP	AGACCATGGCTATGATGGAGCA	PCR amplification of <i>fipA</i> from
C A DD	GATCGAC	pKM101+ and cloning into pAK165
fipA-RP	AGACICGAGITATCACAGIGIA	
	AGCAGGCG	
ice1050fin-FP	AGACCATGGCTATGATAAAACT	PCR amplification of <i>ice1030fin</i> from $ICE H$: $105 C^{\#}$ and alaring inter
	GALLELI	nom ICEHIN1056 and cloning into
ico1056fin DD		PAR103
ICC1050IIII-IXI	TATTTTTCTCC	
nifC_FP		PCR amplification of <i>nifC</i> of
pite-m	CLACCTT	plasmid E' from <i>E</i> coli Rosetta-
	CERCETT	gami (DF3) plys
		(Novagen) and cloning into nAK165
nifC-RP	AGAGTCGACTTATTACAGATCT	"
phe fu	CCGTACAGGC	
ice1056fin -	AGACCATGGCTATGATAAAACT	PCR amplification of <i>ice1056fin</i>
pET28a-FP	GACCCCT	from ICE <i>Hin</i> 1056 and cloning into
I ····		pET28a
ice1056fin -	AGACTCGAGCAAGGGGTATTTT	1 ''
pET28a-RP	TCTCC	
VirD4 (87-543)	TACTTCCAGTCCATGGGACTGC	virD4 cloning into InfR-Co
NhisFP	GCAATCAGAAG	for overexpression and purification
VirD4 (87-543)	AGAGTTATCAACATATGTTATTA	

Supplementary table 2. List of oligos used in this study.

NhisRP	CTGGCGTTCAAAAGC	
VirBA (A25-7A3)	TACTTCCAGTCCATGGATGTTGG	virB4 cloning into InfR-Co for
NhisFP	CATGACGG	for overexpression and purification
VirBA (A25-7A3)		"
$\mathbf{Nh}_{0}\mathbf{DD}$		
NIIISNE Vir P 11NhisNeoIE		wir B11 cloning into InfP Co for
VIIDIIINIIISINCUIF D	ATCCCCAACTAC	for eveneumossion and purification
r V:-D11Nb: MdaID		"
VIEBTINNISNUEIK	AGAGITATCAACATATGTTATIG	
VircINhisNcoIFP		virC1 cloning into InfR-Nhis for
		for overexpression and purification
VircINhisNdeIRP	AGAGITATCAACATATGITATTA	
	AGCCTCCAAGATTT	
Virc2 NhisNco1FP	TACTTCCAGTCCATGGCAATTCG	virC2 cloning into InfR-Nhis for
	CAAGCCCGCGTT	for overexpression and purification
Virc2 NhisNde1RP	AGAGTTATCAACATATGTTATTA	17
	CCAATTCCTCGATG	
VirD2	TACTTCCAGTCCATGGCTCCCGA	<i>virD2</i> cloning into InfR-Co for
NhisNco1FP	TCGCGCTCAAGTA	for overexpression and purification
VirD2	AGAGTTATCAACATATGTTATTA	11
NhisNde1RP	GGTCCCCCGCGCC	
Osa NcoI FP	TTTCAGGGCGCCATGGCTTTGCT	Osa, osa-K100A, osa-R140A, osa-
	ACGGCGGCGGTGTCG	<i>RK</i> and <i>osa-penta</i> cloning into
		pETM41 for overexpression and
		purification
Osa BamHI RP	GCTCGAATTCGGATCCCTAGAT	"
	CTTCCTGCATTGCTCA	
P1ParB	TACTTCCAGTCCATGGCTTCAAA	<i>plparB</i> cloning into InfR-Co for
NcoInhisFP	GAAAAAC	for overexpression and purification
P1ParB	GTTAATTAACTCGAGGGTACCT	"
KpnINhisRP	CAAGGCTTCGGCT	
KorB NcoINhisFP	TACTTCCAGTCCATGGCTACTGC	<i>rk2korB</i> cloning into InfR-Co for
	GGCTCAAG	for overexpression and purification
KorB KpnINhisRP	GTTAATTAACTCGAGGGTACCT	"
	CAGCCCTCGATGAGC	
FLAG-Osa-FP	GCGCTAACGATGCATATGGATT	For in vivo protein-protein
	ACAAGGATGACGATGACAAGTT	interaction studies
	GCTACGGCGG	
FLAG-Osa-RP	AAAGCGCAGGGACTCGAGTCAC	"
	TATTCTAGAATCTTCCTGCATTG	
90-mer oligo with	TTGGCAGGATATATTGTGGTGT	For ssDNA nuclease assays, and for
RB and LB	AAACATTGTCGTTTCCCGCCTTC	T-DNA-VirD2 complex preparation
	AGTTTAAACTATCAGTGTTTGAC	
	AGGATATATTGGCGGGTAAACC	
	TAAGAGAAAAGAGCGTTTA	

^{*}DSM collection No. 3876, ⁺DSM collection No. 9496, [#]Integrative Conjugative Element ICE*Hin*1056 isolated from *Haemophilus influenzae*. DSM - Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (<u>www.dsmz.de</u>).

Name of the	Buffer composition	pН	NaCl	Imidazole	βME	DTT	Glycerol	PMSF
butter			(mM)	(mM)	(mM)	(mM)	(%)	(mM)
Lysis buffer	50 mM Tris-HCl	8.0	500		3			1
1^*								
Lysis buffer	25 mM Tris-HCl	"	150		2		10	1
$2^{\#}$								
А	"	"	500	10	2			
В	"	"	"	500	2			
С	"	"	200		5			
D	"	"	750			2		
Е	"	"	500		5			
$F^{@}$	"	"	1000		5			
G	"	"	500			2		
$\mathrm{H}^{\$}$	20 mM Tris-HCl	7.4	100					
Ι	25 mM Tris-HCl	8.0	10			2		
J	"	"	1000			2		
Κ	50 mM Tris-HCl	"	300	10			10	1
L	50 mM Tris-HCl	"	"	1000			10	1
Μ	50 mM Na-	"	50				10	
Ν	phosphate 50 mM Na- phosphate	"	1000				10	

Supplementary table 3. Composition of buffers used for protein purification.

*-contains 1 mM Benzamidine-HCl, [#]-contains 1 mM Benzamidine-HCl, 10 μg/ml DNase I and 100 μg/ml Hen Egg White Lysozyme, [@]-contains 20 mM D-maltose, ^{\$}-contains 1 mM EDTA.