Supplementary material

Protein interactions within and between two F-type type IV secretion systems

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Content

Table S1. Strains and plasmids used in this study

	Relevant description	Reference or source
Neisseria gonorrheae		
MS11	WT *N. gonorrhoeae*	(Swanson, 1972)
AY529	MS11 transformed with pAY28	This study
AY534	MS11 transformed with pAY27	This study
PK127	MS11Δ*atlA*	(Kohler et al. 2007)
MR535	MS11Δ*traK*	(Ramsey et al. 2014)
PK186	MS11Δ*traG*	(Kohler et al. 2013)
AY534Δ*atlA*	PK127 transformed with pAY27	This study
AY534Δ*traK*	MR535 transformed with pAY27	This study
AY534Δ*traG*	PK186 transformed with pAY27	This study
Escherichia coli		
JM101	F´ *traD36 proA*$^{+}$*B*$^{+}$ *lacI*^{q} Δ*(lacZ)M15/* Δ*(lac-proAB) glnV thi*	New England Biolabs
BTH101	(F-, *cya-99, araD139, galE15, galK16, rpsL1 (Str r), hsdR2, mcrA1, mcrB1*)	Euromedex
10-beta	Δ*(ara-leu) 7697 araD139 fhuA* Δ*lacX74 galK16 galE15 e14-* ϕ*80*d*lacZ*Δ*M15 recA1 relA1 endA1 nupG rpsL* (Str^{R}) *rph spoT1* Δ*(mrr-hsdRMS-mcrBC)*	New England Biolabs
BL21(DE3)	F-, *lon-11, Δ(ompT-nfrA)885, Δ(galM-ybhJ)884, λDE3 [lacI, lacUV5-T7 gene 1, ind1, sam7, nin5], Δ46, [mal*$^{+}$*]$_{K-12}$*(λ^{S}), *hsdS10*	Novagen
Origami2(DE3)	*Δ(ara-leu)7697 ΔlacX74 ΔphoA Pvu*II *phoR araD139 ahpC galE galK rpsL* F′[*lac*$^{+}$ *lacI*^{q} *pro*] (DE3) *gor522*::Tn*10 trxB* (Str^{R}, Tet^{R})	Novagen
Plasmids		
pMR100	plasmid for constructing C-terminal linker-FLAG3 fusions	(Ramsey et al. 2014)
pAY25	pMR100 with TraH-FLAG3	This study
pMR68	*iga-trpB* complementation construct contains the tetracyline-inducible promoter	(Ramsey et al. 2012)
pAY27	pMR68 with TraH-FLAG3 under control of the tetracycline inducible promoter	This study
pAY28	pAY25 with sequence downstream of *traH* added after FLAG3 tag, used to place the FLAG3 tag at the *traH* native site	This study
pUT18C	Bacterial two-hybrid vector designed to express a given polypeptide fused in frame at its N-terminal end with T18 fragment; ColE1 ori, Amp^{R}	(Karimova et al., 2001)
pUT18	Bacterial two-hybrid vector designed to express a given polypeptide fused in frame at its C-terminal end with T18 fragment; ColE1 ori, Amp^{R}	(Karimova et al., 2001)
pUTM18C	As pUT18C but designed to insert the TM domain 1 of *E. coli* oppB between the cloned polypeptide and the T18 fragment; ColE1 ori, Amp^{R}	(Ouellette et al., 2014)
pKT25	Bacterial two-hybrid vector designed to express a given polypeptide fused in frame at its N-terminal end with T25 fragment; p15 ori, Km^{R}	
p25N	Bacterial two-hybrid vector designed to express a given polypeptide fused in frame at its C-terminal end with T25 fragment; p15 ori, Km^{R}	(Claessen et al., 2008)
pSTM25	Bacterial two-hybrid vector designed to express a given polypeptide fused in frame at its N-terminal end with TM domain 1 of *E. coli* oppB and T25 fragment, p15 ori, Sm^{R}	(Ouellette et al., 2014)
pKTM25	Bacterial two-hybrid vector designed to express a given polypeptide fused in frame at its N-terminal end with TM domain 1 of *E. coli* oppB and T25 fragment, p15 ori, Km^{R} The TM region was PCR amplified from pSTM25	This study

	with primer pair 1/2 cut with SalI/EcoRI and cloned into SalI/EcoRI digested pKT25	
pUTM18CltgX$_N$	LtgX$_N$ was PCR amplified using primer pair 3/4 and cloned into pUTM18C	This study
pKTM25ltgX$_N$	LtgX$_N$ was PCR amplified using primer pair 3/4 and cloned into pKTM25	This study
pUTM18CYag$_N$	Yag$_N$ was PCR amplified using primer pair 5/7 and cloned into pUTM18C	This study
pKTM25Yag$_N$	Yag$_N$ was PCR amplified using primer pair 5/7 and cloned into pKTM25	This study
pUT18TraL$_N$	TraL$_N$ was PCR amplified using primer pair 7/8 and cloned into pUT18	This study
p25NTraL$_N$	TraL$_N$ was PCR amplified using primer pair 7/8 and cloned into p25N	This study
pUT18CTraE$_N$	TraE$_N$ was PCR amplified using primer pair 9/10 and cloned in pUT18C	This study
pKT25TraE$_N$	TraE$_N$ was PCR amplified using primer pair 9/10 and cloned in pKT25	This study
pUT18CTraK$_N$	TraK$_N$ was PCR amplified using primer pair 11/12 and cloned in pUT18C	This study
pUTM18CTraK$_N$	TraK$_N$ was PCR amplified using primer pair 11/12 and cloned in pUTM18C	This study
pKT25TraK$_N$	TraK$_N$ was PCR amplified using primer pair 11/12 and cloned in pKT25	This study
pKTM25TraK$_N$	TraK$_N$ was PCR amplified using primer pair 11/12 and cloned in pKTM25	This study
pUT18CTraB$_N$	TraB$_N$ was PCR amplified using primer pair 13/14 and cloned into pUT18C	This study
pKT25TraB$_N$	TraB$_N$ was PCR amplified using primer pair 13/14 and cloned into pKT25	This study
pUTM18CDsbC$_N$	DsbC$_N$ was PCR amplified using primer pair 15/16 and cloned into pUTM18C	This study
pKTM25DsbC$_N$	DsbC$_N$ was PCR amplified using primer pair 15/16 and cloned into pKTM25	This study
pUT18CTraV$_N$	TraV$_N$ was PCR amplified using primer pair 17/18 and cloned into pUT18C	This study
pUTM18CTraV$_N$	TraV$_N$ was PCR amplified using primer pair 17/18 and cloned into pUTM18C	This study
pKT25TraV$_N$	TraV$_N$ was PCR amplified using primer pair 17/18 and cloned into pKT25	This study
pKTM25TraV$_N$	TraV$_N$ was PCR amplified using primer pair 17/18 and cloned into pKTM25	This study
pUT18CTraC$_N$	TraC$_N$ was PCR amplified using primer pair 19/20 and cloned into pUT18C	This study
pUT18TraC$_N$	TraC$_N$ was PCR amplified using primer pair 19/21 and cloned into pUT18C	This study
pKT25TraC$_N$	TraC$_N$ was PCR amplified using primer pair 19/20 and cloned into pKT25	This study
p25NTraC$_N$	TraC$_N$ was PCR amplified using primer pair 19/21 and cloned into pUT18C	This study
pUTM18CTraW$_N$	TraW$_N$ was PCR amplified using primer pair 22/23 and cloned into pUTM18C	This study
pKTM25TraW$_N$	TraW$_N$ was PCR amplified using primer pair 22/23 and cloned into pKTM25	This study
pUT18CTraU$_N$	TraU$_N$ was PCR amplified using primer pair 24/26 and cloned into pUT18C	This study
pUTM18CTraU$_N$	TraU$_N$ was PCR amplified using primer pair 24/26 and cloned into pUTM18C	This study
pKT25TraU$_N$	TraU$_N$ was PCR amplified using primer pair 24/26 and cloned into pKT25	This study

pKTM25TraU$_N$	TraU$_N$ was PCR amplified using primer pair 24/26 and cloned into pKTM25	This study
pUTM18CTrbC$_N$	TrbC$_N$ was PCR amplified using primer pair 26/28 and cloned into pUTM18C	This study
pKTM25TrbC$_N$	TrbC$_N$ was PCR amplified using primer pair 26/28 and cloned into pKTM125	This study
pUT18CTraN$_N$	TraN$_N$ was PCR amplified using primer pair 28/30 and cloned into pUT18C	This study
pUTM18CTraN$_N$	TraN$_N$ was cut out from pKT25TraN$_N$ with XbaI/BamHI and cloned into XbaI/BamHI digested pUTM18C	This study
pKT25TraN$_N$	TraN$_N$ was PCR amplified using primer pair 29/30 and cloned into pKT25	This study
pKTM25TraN$_N$	TraN$_N$ was cut out from pUTM18CTraN$_N$ with XbaI/BamHI and cloned into XbaI/BamHI digested pKTM25	This study
pUTM18CTraF$_N$	TraF$_N$ was PCR amplified using primer pair 32/34 and cloned into pUTM18C	This study
pKTM25TraF$_N$	TraF$_N$ was PCR amplified using primer pair 32/34 and cloned into pKTM25	This study
pUT18CTraH$_N$	TraH$_N$ was PCR amplified using primer pair 34/35 and cloned into pUT18C	This study
pUTM18CTraH$_N$	TraH$_N$ was PCR amplified using primer pair 34/35 and cloned into pUTM18C	This study
pKT25TraH$_N$	TraH$_N$ was PCR amplified using primer pair 34/35 and cloned into pKT25	This study
pKTM25TraH$_N$	TraH$_N$ was PCR amplified using primer pair 34/35 and cloned into pKTM25	This study
pUT18TraG$_N$	TraG$_N$ was PCR amplified using primer pair 36/37 and cloned into pUT18	This study
p25NTraG$_N$	TraG$_N$ was PCR amplified using primer pair 36/37 and cloned into p25N	This study
pUT18CAtlA$_N$	AtlA$_N$ was PCR amplified using primer pair 38/39 and cloned into pUT18C	This study
pUT18AtlA$_N$	AtlA$_N$ was PCR amplified using primer pair 40/41 and cloned into pUT18	This study
pUTM18CAtlA$_N$	AtlA$_N$ was PCR amplified using primer pair 40/42 and cloned into pUTM18C	This study
pKT25AtlA$_N$	AtlA$_N$ was PCR amplified using primer pair 40/42 and cloned into pKT25	This study
p25NAtlA$_N$	AtlA$_N$ was PCR amplified using primer pair 38/43 and cloned into pKT25	This study
pKTM25AtlA$_N$	AtlA$_N$ was PCR amplified using primer pair 40/42 and cloned into pKTM25	This study
pUT18CTraB$_F$	TraB$_F$ was PCR amplified using primer pair 44/45 and cloned into pUT18C	This study
pKT25TraB$_F$	TraB$_F$ was PCR amplified using primer pair 44/45 and cloned into pKT25	This study
pUT18CTraC$_F$	TraC$_F$ was PCR amplified using primer pair 46/48 and cloned into pUT18C	This study
pUT18TraC$_F$	TraC$_F$ was PCR amplified using primer pair 46/48 and cloned into pUT18	This study
pKT25TraC$_F$	TraC$_F$ was PCR amplified using primer pair 46/48 and cloned into pKT25	This study
p25NTraC$_F$	TraC$_F$ was PCR amplified using primer pair 46/47 and cloned into p25N	This study
pUT18CTraE$_F$	TraE$_F$ was PCR amplified using primer pair 49/50 and cloned into pUT18C	This study
pKT25TraE$_F$	TraE$_F$ was PCR amplified using primer pair 49/50 and cloned into pKT25	This study

pUTM18CTraF$_F$	TraF$_F$ was PCR amplified using primer pair 51/52 and cloned into pUTM18C	This study
pKTM25TraF$_F$	TraF$_F$ was PCR amplified using primer pair 51/52 and cloned into pKTM25	This study
pUTM18CTraH$_F$	TraH$_F$ was PCR amplified using primer pair 53/54and cloned into pUTM18C	This study
pKTM25TraH$_F$	TraH$_F$ was PCR amplified using primer pair 53/54 and cloned into pKTM25	This study
pUT18CTraK$_F$	TraK$_F$ was PCR amplified using primer pair 55/56 and cloned into pUT18C	This study
pUTM18CTraK$_F$	TraK$_F$ was PCR amplified using primer pair 55/56 and cloned into pUTM18C	This study
pKT25TraK$_F$	TraK$_F$ was PCR amplified using primer pair 55/56 and cloned into pKT25	This study
pKTM25TraK$_F$	TraK$_F$ was PCR amplified using primer pair 55/56 and cloned into pKTM25	This study
pUTM18CTraU$_F$	TraU$_F$ was PCR amplified using primer pair 57/58 and cloned into pUTM18C	This study
pKTM25TraU$_F$	TraU$_F$ was PCR amplified using primer pair 57/58 and cloned into pKTM25	This study
pUT18CTraV$_F$	TraV$_F$ was PCR amplified using primer pair 59/60 and cloned into pUT18C	This study
pUTM18CTraV$_F$	TraV$_F$ was PCR amplified using primer pair 59/60 and cloned into pUTM18C	This study
pKT25TraV$_F$	TraV$_F$ was PCR amplified using primer pair 59/60 and cloned into pKT25	This study
pKTM25TraV$_F$	TraV$_F$ was PCR amplified using primer pair 59/60 and cloned into pKTM25	This study
pUTM18CTraW$_F$	TraW$_F$ was PCR amplified using primer pair 61/62 and cloned into pUTM18C	This study
pKTM25TraW$_F$	TraW$_F$ was PCR amplified using primer pair 61/62 and cloned into pKTM25	This study
pUTM18CTrbC$_F$	TrbC$_F$ was PCR amplified using primer pair 63/64 and cloned into pUTM18C	This study
pKTM25TrbC$_F$	TrbC$_F$ was PCR amplified using primer pair 63/64 and cloned into pKTM25	This study
pCOLADuet-1	Protein expression vector. Kan^R	Novagen
pET22b	Protein expression vector. Amp^R	Novagen
pET22bTraV$_N$	TraV$_N$ and pET22b were PCR amplified using primer pair 67/68 and primer pair 65/66 respectively. The PCR products were combined using the NEBuilder HiFi DNA Assembly Cloning Kit (New England Biolabs.).	This study
pET22bTraV$_F$	TraV$_F$ and pET22b were PCR amplified using primer pair 75/76 and primer pair 73/74 respectively. The PCR products were combined using the NEBuilder HiFi DNA Assembly Cloning Kit (New England Biolabs.).	This study
pCOLATraW$_N$	TraW$_N$ and pCOLADuet were PCR amplified using primer pair 71/72 and primer pair 69/70 respectively. The PCR products were combined using the NEBuilder HiFi DNA Assembly Cloning Kit (New England Biolabs.).	This study
pCOLATrbC$_F$-SP	TrbC$_F$-SP and pCOLADuet were PCR amplified using primer pair 77/78 and primer pair 69/70 respectively. The PCR products were combined using the NEBuilder HiFi DNA Assembly Cloning Kit (New England Biolabs.).	This study

Version Controlled Strains:

Version-controlled cell repositories (Tellechea-Luzardo et al., 2020) to facilitate reproduction and derivative work from this paper can be found here:

$TraV_N$: https://cellrepo.herokuapp.com/repositories/53?branch_id=68&locale=en

$TraV_F$: https://cellrepo.herokuapp.com/repositories/56?branch_id=75&locale=en

$TraV_N$

$TraV_F$

This repository focuses on TraV proteins and describes the plasmids containing them.

Table S2. Primers used in this study. Restriction sites used for cloning are underlined

1. CCGACAACGACGTCAACAG
2. CCAGGGTTTTCCCAGTCA
3. GGAAGGATCCCTGTTATAAGGAAGCCGGCAGCAA
4. GCTACGAATTCCTACGAAGAGCGTGTTTTTAATCGTT
5. GGAAGGATCCCATGATTCTGACAGCCTGTGCC
6. GCTACGAATTCTTACCAAGTGATTACAACGGTTCTC
7. CAAGATCTAGAGATGAGAGATTATCATGTGCC
8. GCTACGAATTCGAATTTAGAAACTCCCGTTTAT
9. GGAAGGATCCCATGTTGAGTGAATTGGCGATA A
10. GCTAC GAATTCTCAATTACCTCGATTACCGCC
11. GGAAGGATCCCGCGCAACGAGTGCCGGCAACA
12. GCTACGAATTCTCAACCTCCCGGACGGCTGAT
13. GGAAGGATCCCATGAGGGTGAAAGTAAACAAATT
14. GCTACGAATTCCTATTTGGTTTCGCCGGTAT
15. GGAAGGATCCCGATGTCAAAACTGTTGGAAGCACT
16. GCTACGAATTCTCATTCTGCTCCCGCTCCATTTAA
17. CAA GATCTAGAGTCAACCTTAACCATGTCCGGT
18. AAGGGGATCCCTATCGAACGGTACCGGGAAT
19. GCTACTCTAGAGATGGGCGTCCTGTCAAAATTT
20. AAGGGGATCCTTAGCGGGTTGCGGCACGGTCCCTT
21. AATTCGGATCCTCGCGGGTTGCGGCACGGTCCCTT
22. GGAAGGATCCCTCGACACCCCCTGTCGAGA
23. GCTACGAATTCTCACGGTTTCATGACCTCTACAC
24. GCTACTCTAGAGGCTGAAGCTGTTCCTACT
25. AAGGGGATCCTCAATTAGTCTTAAATTTATATGCACCA
26. GGAAGGATCCCGCGGATGTAGAGGCGGC
27. GCTACGAATTCTCATTTTCTGCCTTCCAGAACGG
28. GCTACGGATCCCGCCGCACTCAGGGAA
29. AAGGCCCGGGGTTATTGATATTCATAATAGTTCTTCACTTCCAA
30. GCTACTCTAGAGGCCGCACTCAGGGAAA
31. AAGGGGATCCTTATTGATATTCATAATAGTTCTTCACTTCCAAATTTTTCC
32. GGAAGGATCCCGCGGATGATGGCATGGGAT
33. GCTACGAATTCTTAATACAATGTCCCGACAGGGCG
34. GGAAGGATCCCGGCATAGAGAAGAACATGGCT
35. GCTACGAATTCTTAGAAACGGTTCATCGAATCAAA
36. GCTACTCTAGAGATGGCTGTCGAATACTTTACTTTC
37. CTACGGTACCCGTTCCTTTGGGCGGAATACAGC
38. CAAGATCTAGAGATGTGGCGTGGAATATCAAGTGGA
39. TTCGCGGTACCTTAAAATCCTCTCTGCCTAAAGAA

40. GGAAGGATCCCATGTGGCGTGGAATATCAAGT
41. GCTACGAATTCAGAAATCCTCTCTGCCTAAAGAAATT
42. GCTACGAATTCTTAAAATCCTCTCTGCCTAAAGAAATT
43. CTACGGTACCCGAAATCCTCTCTGCCTAAAGAAATT
44. GGAAGGATCCCATGGCCAGTATCAATACCATTGTG
45. GCTACGAATTCTTATTTGCCATCGTTGCCCC
46. GGAAGGATCCCGTGAATAACCCACTTGAGGCC
47. GCTACGAATTCGATGCCACACTCCTGTATTTCT
48. GCTACGAATTCTCATGCCACACTCCTGTATTTCT
49. GGAAGGATCCCATGGAACACGGTGCCCG
50. GCTACGAATTCTTATTTTTTCTCATCGTCTG
51. GGAAGGATCCCAAAGATGCAGGCTGGCAGT
52. GCTACGAATTCTTAAAAATTGGGTTTAAAATCTTCAGAAACGTTCAG
53. GGAAGGATCCCGATGTGAACAGCGACATGAATCAG
54. GCTACGAATTCTCACAGCGTGCTCCCTC
55. GGAAGGATCCCGCAAACGGTACGCTGGC
56. GCTACGAATTCTCAGTTGCCCTCCCCG
57. GGAAGGATCCCGATTCTGCCTGTGAGGGGC
58. GCTACGAATTCTCACAGGAAGACACAGTTACGTTTACG
59. GGAAGGATCCCAGTACGGAATTTGAGTGTAACGC
60. GCTACGAATTCTTAATTAATACGTGGTTTTCCCCACG
61. GGAAGGATCCCGCCGATCTTGGTACCTGGG
62. GCTACGAATTCTCATTTTCTGCCCTCCTCTG
63. GGAAGGATCCCTCAGAAAACGTGAACACTCCTG
64. GCTACGAATTCTCATTTCCCGGAATCTCCTTTC
65. TACCGTTCGACTCGAGCACCACCACCAC
66. TACCGGACATATCCATGGCCATCGCCGG
67. GGCCATGGATATGTCCGGTATCGGCGGTAG
68. GGTGCTCGAGTCGAACGGTACCGGGAATAC
69. CTCGAGTCTGGTAAAGAAAC
70. TGTATATCTCCTTCTTATACTTAAC
71. GTATAAGAAGGAGATATACAATGTCCCACAAGAAAAAAATAG
72. GTTTCTTTACCAGACTCGAGCGGTTTCATGACCTCTAC
73. ACGTATTAATCTCGAGCACCACCACCAC
74. ATTCCGTACTATCCATGGCCATCGCCGG
75. GGCCATGGATAGTACGGAATTTGAGTGTAAC
76. GGTGCTCGAGATTAATACGTGGTTTTCCC
77. GTATAAGAAGGAGATATACAATGTCAGAAAACGTGAAC
78. GTTTCTTTACCAGACTCGAGTTTCCCGGAATCTCCTTTC

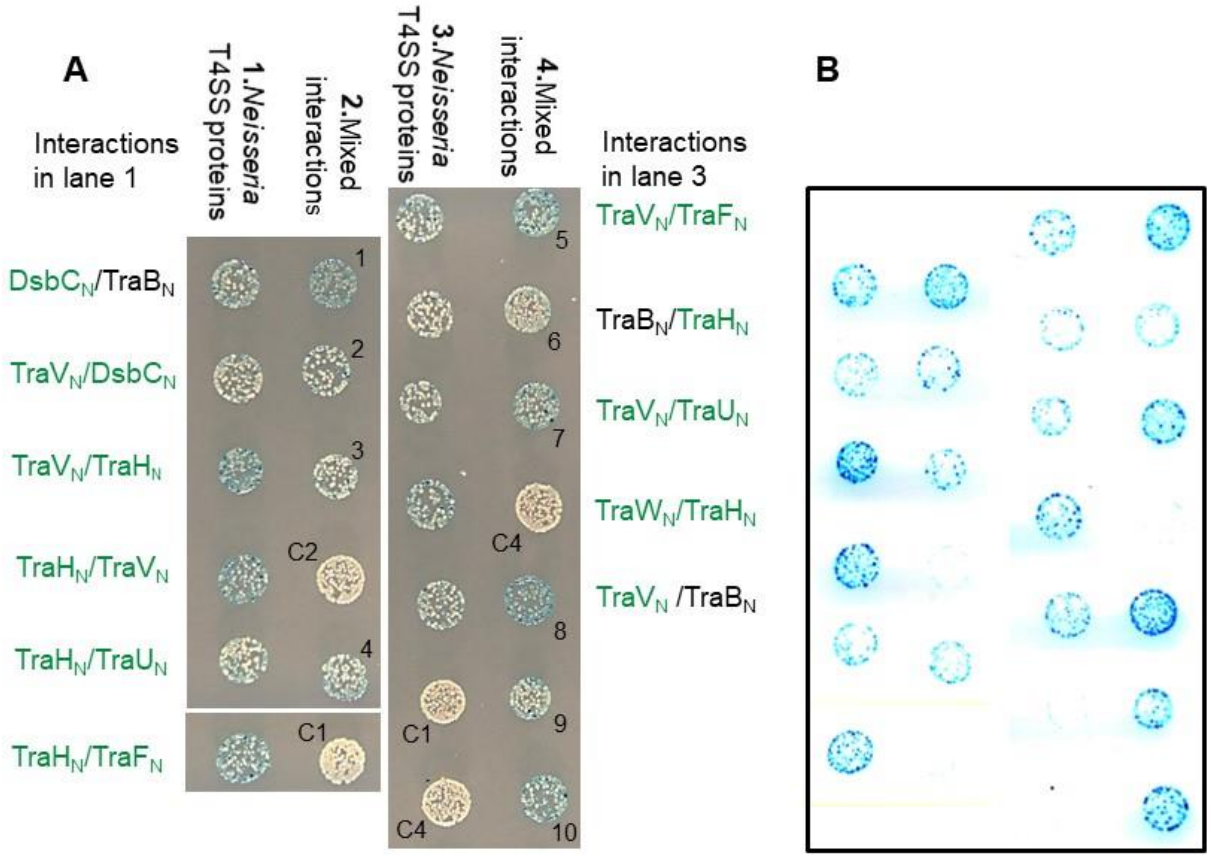


Fig.S1. Bacterial two-hybrid interactions between *Neisseria* proteins (lane 1 and 3) and corresponding mixed interactions defined as an interaction between a protein from the *N. gonorrhoeae* T4SS and a protein from the T4SS encoded by the F-plasmid. The figure shows colonies of *E. coli* BTH101 transformants carrying plasmids encoding the proteins indicated in the order T18/T25. Protein names in green indicate that the gene encoding the protein was cloned in a BACTH-TM system vector, black names indicate that the gene was cloned in the BACTH system vector. C1, C2, C3, C4 and C5 are vector controls; respectively pUT18C/pKT25, pUTM18C/pKTM25, pUT18/pKT25, pUT18C/pKTM25 and pUTM18C/pKT25. The mixed interactions are: 1. $DsbC_N/TraB_F$, 2. TM-$TraV_F/DsbC_N$, 3 TM-$TraV_F/TraH_N$, 4. $TraU_F/TraH_N$, 5. TM-$TraV_N/TraF_F$, 6. $TraB_F/TraH_N$, 7. $TraH_N/TraB_F$. 8. TM-$TraV_N/TraB_F$, 9. $TraB_F$/TM-$TraV_N$, 10. TM-$TraV_F/TraB_N$. B. Image obtained by scanning the agar plate.

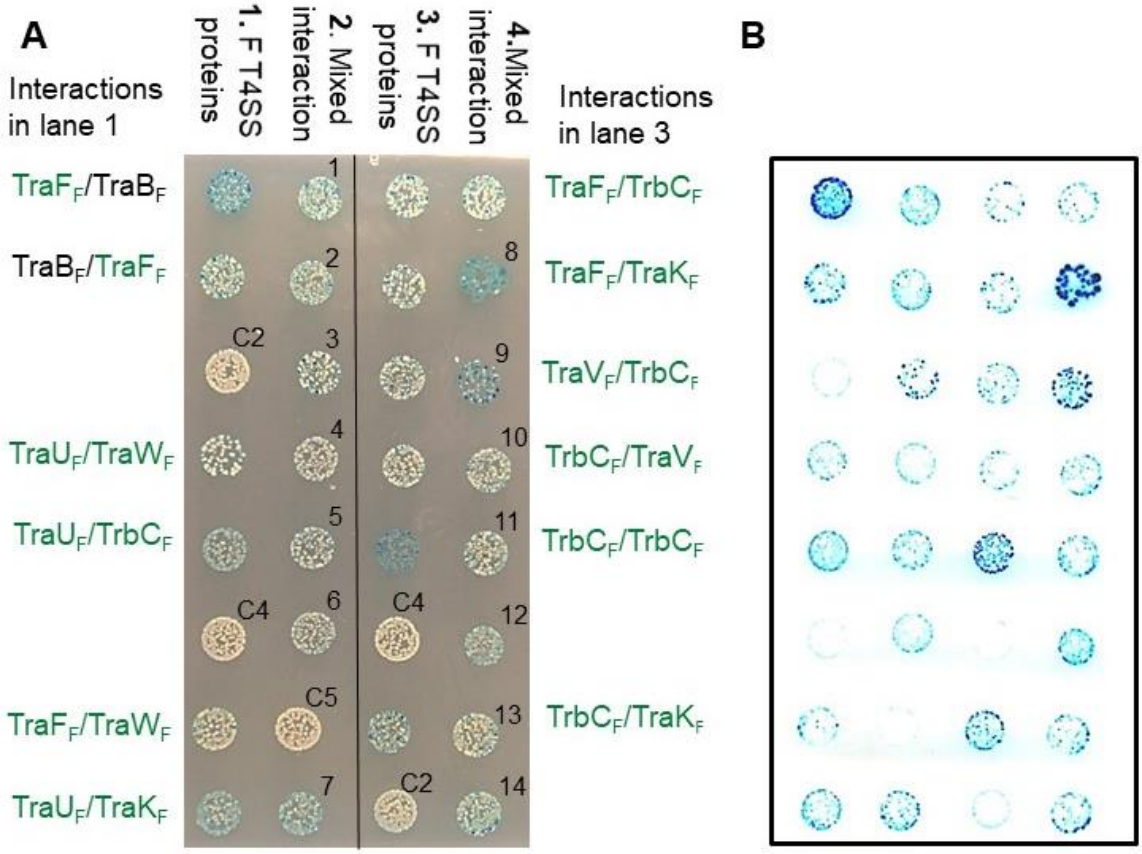


Fig.S2. Bacterial two-hybrid interactions between F-plasmid proteins (lane 1 and 3) and corresponding mixed interactions defined as an interaction between a protein from the *N. gonorrhoeae* T4SS and a protein from the T4SS encoded by the F-plasmid. The figure shows colonies of *E. coli* BTH101 transformants carrying plasmids encoding the proteins indicated in the order T18/T25. Protein names in green indicate that the gene encoding the protein was cloned in a BACTH-TM system vector, black names indicate that the gene was cloned in the BACTH system vector.C2, C3, C4 and C5 are vector controls; respectively pUT18C/pKT25, pUTM18C/pKTM25, pUT18/pKT25, pUT18C/pKTM25 and pUTM18C/pKT25. The mixed interactions are: 1 TraF$_F$/TraB$_N$, 2. TraF$_N$/TraB$_F$, 3. TraB$_F$/TraF$_N$, 4. TraU$_F$/TraW$_N$ 5. TrbC$_F$/TraU$_N$, 6. TaU$_N$/TrbC$_{F,,}$, 7. TraU$_N$/TM-TraK$_{F,}$ 8. TraF$_N$/TM-TraK$_F$ 9. TM-TraV$_N$/TrbC$_F$ 10. TrbC$_F$/TM-TraV$_N$ 11. TrbC$_F$/TrbC$_N$, 12. TrbC$_N$/TrbC$_F$, 13. TrbC$_N$/TM-TraK$_F$, 14.TM-TraK$_N$/TrbC$_F$. B. Image obtained by scanning the agar plate.

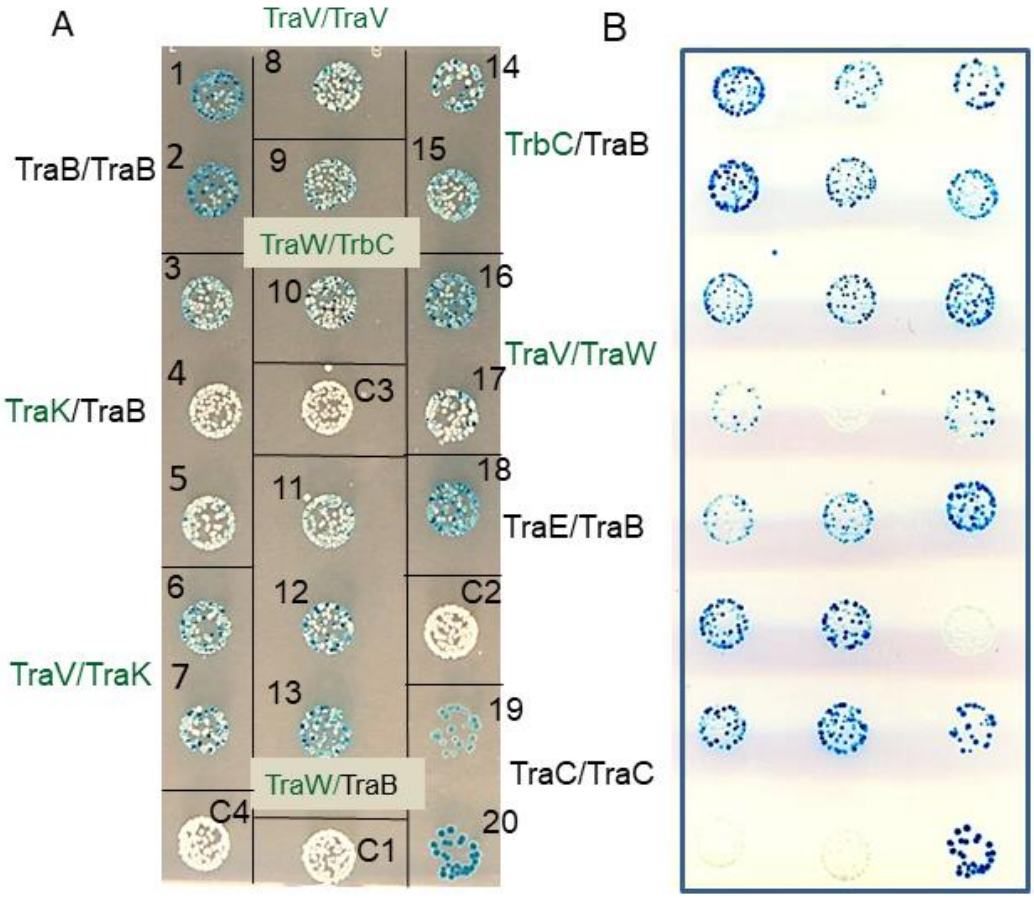
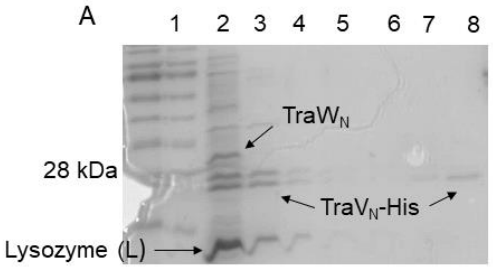


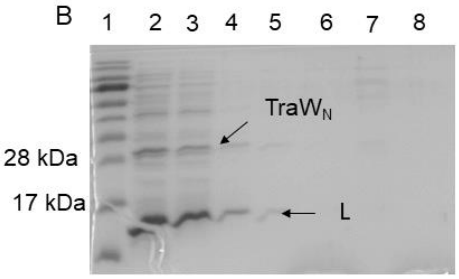
Fig. S3. Mixed interactions (defined as interactions between a protein from the *N. gonorrhoeae* T4SS and a protein from the F-plasmid T4SS) corresponding to interactions observed with both *N. gonorrhoeae* and F-plasmid proteins. **A**. Photograph of colonies of *E. coli* BTH101 transformants carrying plasmids encoding the proteins indicated in the order T18/T25. Protein names in green indicate that the gene encoding the protein was cloned in a BACTH-TM system vector, black names indicate that the gene was cloned into the BACTH system vector. C1, C2 and C4 are vector controls; respectively pUT18C/pKT25, pUTM18C/pKTM25 and pUT18C/pKTM25. The following interactions are shown: 1. $TraB_N/TraB_F$. 2. $TraB_F/TraB_N$, 3. $TraB_N$/TM-$TraK_F$, 4. TM-$TraK_F/TraB_N$, 5.TM-$TraK_N/TraB_F$, 6. TM-$TraV_F$/TM-$TraK_N$, 7. TM-$TraV_N$/TM-$TraK_F$, 8. TM-$TraV_F$/TM-$TraV_N$, 9. TM-$TrbC_F$/TM-$TraW_N$, 10. TM-$TraW_N$/TM-$TrbC_F$, 11. TM-$TraW_F/TraB_N$, 12. $TraB_F$/TM-$TraW_N$, 13. TM-$TraW_N/TraB_F$, 14. TM-$TrbC_F/TraB_N$, 15. TM-$TrbC_N/TraB_F$, 16. TM-$TraV_N$/TM-$TraW_F$, 17. TM-$TraV_F$/TM-$TraW_N$, 18. $TraE_N$,/$TraB_F$ 19. pUT18$TraC_N$/pKT25$TraC_F$, 20. pUT18C$TraC_N$/pKT25$TraC_F$. B. Image obtained by scanning the agar plate

A



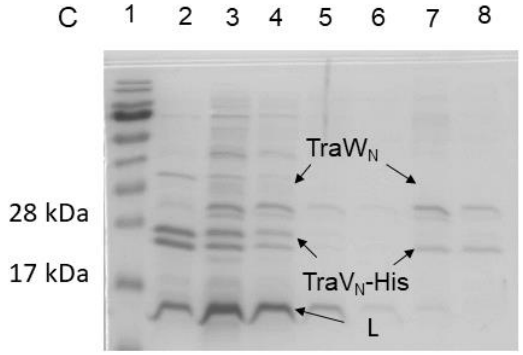
1. Standard
2. TraV$_N$/TraW$_N$ loading †
3. TraV$_N$ run through
4. TraV$_N$ wash 1
5. TraV$_N$ wash 2
6. TraV$_N$ wash 3
7. TraV$_N$ elution 1 125 mM imidazole
8. TraV$_N$ elution 2 250 mM imidazole

B



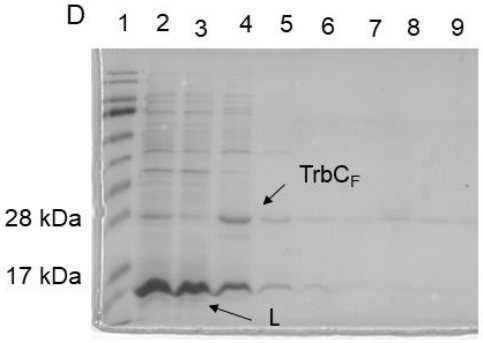
1. Standard
2. TraW$_N$ loading
3. TraW$_N$ run through
4. TraW$_N$ wash 1
5. TraW$_N$ wash 2
6. TraW$_N$ wash 3
7. TraW$_N$ elution 1 125 mM imidazole
8. TraW$_N$ elution 2 250 mM imidazole

C



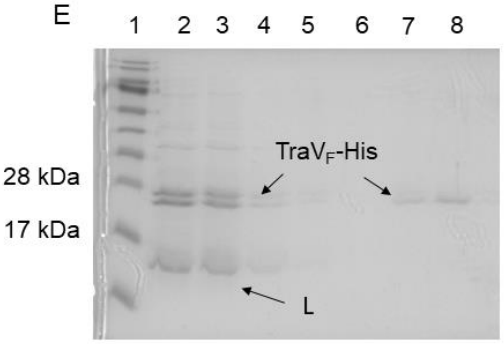
1 Standard
2. TraV$_N$ loading †
3. TraV$_N$ /TraW$_N$ run through
4. TraV$_N$ /TraW$_N$ wash 1
5. TraV$_N$ /TraW$_N$ wash 2
6. TraV$_N$/TraW$_N$ wash3
7. TraV$_N$ / TraW$_N$, elution 125 mM imidazole
8. TraV$_n$/TraW$_N$ elution 250 mM imidazole

D

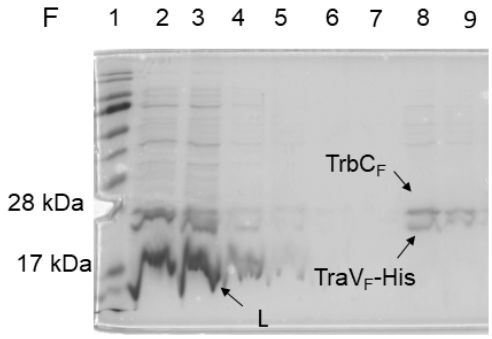


1. Standard
2. TrbC$_F$ loading
3. TrbC$_F$ run through
4. TrbC$_F$ wash 1
5. TrbC$_F$ wash 2
6. TrbC$_F$ wash 3
7. TrbC$_F$ wash 4
8. TrbC$_F$ elution 1 125 mM imidazole
9. TrbC$_F$ elution 2 250 mM imidazole

E



1. Standard
2. TraV$_F$ loading
3. TraV$_F$ run through
4. TraV$_F$ wash 1
5. TraV$_F$ wash 2
6. TraV$_F$ wash 3
7. TraV$_F$ elution 1 125 mM imidazole
8. TraV$_F$ elution 2 250 mM imidazole



1 .Standard
2. TraV$_F$/TrbC$_F$ loading
3. TraV$_F$ /TrbC$_F$ run through
4. TraV$_F$ /TrbC$_F$ wash 1
5. TraV$_F$ /TrbC$_F$ wash 2
6. TraV$_F$/TrbC$_F$ wash 3
7. TraV$_F$/TrbC$_F$ wash 4
8. TraV$_F$ /TrbC$_F$, elution 1 125 mM imidazole
9. TraV$_F$/TrbC$_F$ elution 2 250 mM imidazole

Fig. S4. Pull-down experiments. A. Pull-down results for lysate from *E. coli* BL21(DE3)(pET22bTraV$_N$) mixed with lysate from *E. coli* BL21(DE3)(pCOLADuet). † By a mistake TraV$_N$/TraW$_N$ loading is shown on Gel A while the TraV$_N$ loading is shown on Gel C. **B**. Pull-down results for lysate from *E. coli* BL21(DE3)(pCOLADuetTraW$_N$) mixed with lysate from *E. coli* BL21(DE3)(pET22b). **C**. Pull-down results for lysate from *E. coli* BL21(pET22bTraV$_N$) mixed with lysate from *E. coli* BL21(DE3)(pCOLADuetTraW$_N$). **D**. Pull-down results for lysate from *E. coli* Origami-2(DE3)(pCOLADuetTrbC$_F$) mixed with lysate from *E. coli* BL21(DE3)(pET22b). **E.** Pull-down results for lysate from *E. coli* BL21(pET22bTraV$_F$) mixed with lysate from *E. coli* Origami-2(DE3)(pCOLADuet). **F.** Pull-down results for lysate from *E. coli* BL21(pET22bTraV$_F$) mixed with lysate from *E. coli* Origami-2(DE3)(pCOLADuetTrbC$_F$). Only a partial resin binding of His-TraV$_N$ and His-TraV$_F$ was observed probably due to some His-TraV$_N$ and His-TraV$_F$ being trapped in inclusion bodies (**A**, **C** and **E**).

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