

JC1	MCSLLKKIVFSALLAAGPATSF AA Q R V P A T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	61
SK-93-1035	MCSLLKKIVFSALLAAGPATSF AA Q R V P A T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	61
DGI2	MCSLLKKIVFSALLAAGPATSF AA Q R V P T T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	61
PID18	MCSLLKKIVFSALLAAGPATSF AA Q R V P T T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	61
PID2059	MCSLLKKIVFSALLAAGPATSF AA Q R V P A T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	61
MS11	MCSLLKKIVFSALLAAGPATSF AA Q R V P A T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	61
PID1	MCSLLKKIVFSALLAAGPATSF AA Q R V P A T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	61
PID332	MCSLLKKIVFSALLAAGPATSF AA Q R V P A T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	61
NCCP11945	MCSLLKKIVFSALLAAGPATSF AA Q R V P A T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	61
FA19	MCSLLKKIVFSALLAAGPATSF AA Q R V P A T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	61
MC	...LLKKIVFSALLVAGP T T T S F A A Q R V P A T E N T P H V V S I S K R N L S R I A I E G G R I S S W K F M E	58
JC1	GDLELQKDTT T TGQLFVRS L T S N P T N L F V I S E E G K T Y L L V L K P T S K Q G D N I V D V A G A N R R E	122
SK-93-1035	GDLELQKDTT T TGQLFVRS L T S N P T N L F V I S E E G K T Y L L V L K P T S K Q G D N I V D V A G A N R R E	122
DGI2	GDLELQKDTT T TGQLFVRS L T S N P T N L F V I S E E G K T Y L L V L K P T S K Q G N N I V D V A G A N R R E	122
PID18	GDLELQKDTT T TGQLFVRS L T S N P T N L F V I S E E G K T Y L L V L K P T S K Q G N N I V D V A G A N R R E	122
PID2059	GDLELQKDTT T TGQLFVRS L T S N P T N L F V I S E E G K T Y L L V L K P T S K Q G D N I V D V A G A N R R E	122
MS11	GDLELQKDTT T TGQLFVRS L T S N P T N L F V I S E E G K T Y L L V L K P T S K Q G D N I V D V A G A N R R E	122
PID1	GDLELQKDTT T TGQLFVRS L T S N P T N L F V I S E E G K T Y L L V L K P T S K Q G D N I V D V A G A N R R E	122
PID332	GDLELQKDTT T TGQLFVRS L T S N P T N L F V I S E E G K T Y L L V L K P T S K Q G D N I V D V A G A N R R E	122
NCCP11945	GDLELQKDTT T TGQLFVRS L T S N P T N L F V I S E E G K T Y L L V L K P T S K Q G D N I V D V A G A N R R E	122
FA19	GDLELQKDTT T TGQLFVRS L T S N P T N L F V I S E E G K T Y L L V L K P T S K Q G D N I V D V A G A N R R E	122
MC	GDLELQKDTT T TGQLFVRS L T S S P T N L F V V S E E G K T Y L L V L K P T S K Q G D N I V D V A G A N R H E	119
JC1	AAVLASQTGMPV T MN S T E Y V R A I K K M M T S M M R G T T G D M G I N H S H E Y Q T V P L W K D T L F I Q N	183
SK-93-1035	AAVLASQTGMPV T MN S T E Y V R A I K K M M T S M M R G T T G D M G I N H S H E Y Q T V P L W K D T L F I Q N	183
DGI2	AAVLASQTGMPV T MN S T E Y V R A I K K M M A S M M R G T T G D M G I N H S H E Y Q T V P L W K D T L F I Q N	183
PID18	AAVLASQTGMPV T MN S T E Y V R A I K K M M A S M M R G T T G D M G I N H S H E Y Q T V P L W K D T L F I Q N	183
PID2059	AAVLASQTGMPV T MN S T E Y V R A I K K M M A S M M R G T T G D M G I N H S H E Y Q T V P L W K D T L F I Q N	183
MS11	AAVLASQTGMPV T MN S T E Y V R A I K K M M A S M M R G T T G D M G I N H S H E Y Q T V P L W K D T L F I Q N	183
PID1	AAVLASQTGMPV T MN S T E Y V R A I K K M M A S M M R G T T G D M G I N H S H E Y Q T V P L W K N T L F I Q N	183
PID332	AAVLASQTGMPV T MN S T E Y V R A I K K M M A S M M R G T T G D M G I N H S H E Y Q T V P L W K N T L F I Q N	183
NCCP11945	AAVLASQTGMPV T MN S T E Y V R A I K K M M A S M M R G T T G D M G I N H S H E Y Q T V P L W K N T L F I Q N	183
FA19	AAVLASQTGMPV T MN S T E Y V R A I K K M M A S M M R G T T G D M G I N H S H E Y Q T V P L W K N T L F I Q N	183
MC	AAVLAS R TGMPV T MN S T E Y V R T I K K M M T S M M R G T T G D M G I N H S H E Y Q T I P L W K N T L F I Q N	180
JC1	GSYTAADM Q GLS F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R P G G	244
SK-93-1035	GSYTAADM Q GLS F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R P G G	244
DGI2	GSYTAADM Q GLS F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R P G G	244
PID18	GSYTAADM Q GLS F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R P G G	244
PID2059	GSYTAADM Q GLS F S F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R P G G	244
MS11	GSYTAADM Q GLS F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R P G G	244
PID1	GSYTAADM Q GLS F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R P G G	244
PID332	GSYTAADM Q GLS F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R P G G	244
NCCP11945	GSYTAADM Q GLS F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R P G G	244
FA19	GSYTAADM Q GLS F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R P G G	244
MC	GSYTAADM Q GLS F TL T NL G T K Q L E I R E Q E F Y R Q G V L A V A V R K Q I L Q P G E I T D V F I I S R L G G	241

Figure S1. Alignment of the amino acid sequence of TraK from eight sequenced *Neisseria gonorrhoeae* strains carrying the GGI and two *N. gonorrhoeae* strains (JC1 and PID2059) for which we sequenced the *traK* locus. A representative *N. meningitidis* strain (MC: α 275) is included for reference. In some strains of *N. meningitidis*, including α 275, an insertion sequence is present at the start of the *traK* gene (1). Non-conserved amino acids are highlighted in gray.

DGI2	MMRLKALSITAAAILILSGCSTLTMSGIGGSEKFRCPNARNSDDPYCESISSNYKASVAGV	60
FA19	MMRLKALSITAAAILILSGCSTLTMSGIGGSEKFRCPNARNSDDPYCESISSNYKASVAGV	60
MS11	MMRLKALSITAAAILILSGCSTLTMSGIGGSEKFRCPNARNSDDPYCESISSNYKASVAGV	60
NCCP11945	MMRLKALSITAAAILILSGCSTLTMSGIGGSEKFRCPNARNSDDPYCESISSNYKASVAGV	60
PID1	MMRLKALSITAAAILILSGCSTLTMSGIGGSEKFRCPNARNSDDPYCESISSNYKASVAGV	60
PID18	MMRLKALSITAAAILILSGCSTLTMSGIGGSEKFRCPNARNSDDPYCESISSNYKASVAGV	60
PID332	MMRLKALSITAAAILILSGCSTLTMSGIGGSEKFRCPNARNSDDPYCESISSNYKASVAGV	60
SK-93-1035	MMRLKALSITAAAILILSGCSTLTMSGIGGSEKFRCPNARNSDDPYCESISSNYKASVAGV	60
MC	MMRLKALSITAAAILILSGCSTLTMSGIGGSEKFRCS NAW NSDDPYCESISSNYKASVAGV	60
DGI2	LKDGMRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
FA19	LKDGMRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
MS11	LKDGMRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
NCCP11945	LKDGMRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
PID1	LKDGMRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
PID18	LKDGMRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
PID332	LKDGMRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
SK-93-1035	LKDGMRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
MC	LKDGMRQVGQPYTDQSVRTLMLTQAYSSGTPVRSQSEIARIWVAPYLDTDGDLNDQSFT	120
DGI2	YVVLNQGDLIAHNGQOIINEYRPIRLLGDGAKTSGSSVETSPTNSNNTNDRIPDVGVNL	180
FA19	YVVLNQGDLIAHNGQOIINEYRPIRLLGDGAKTSGSSVETSPTNSNNTNDRIPDVGVNL	180
MS11	YVVLNQGDLIAHNGQOIINEYRPIRLLGDGAKTSGSSVETSPTNSNNTNDRIPDVGVNL	180
NCCP11945	YVVLNQGDLIAHNGQOIINEYRPIRLLGDGAKTSGSSVETSPTNSNNTNDRIPDVGVNL	180
PID1	YVVLNQGDLIAHNGQOIINEYRPIRLLGDGAKTSGSSVETSPTNSNNTNDRIPDVGVNL	180
PID18	YVVLNQGDLIAHNGQOIINEYRPIRLLGDGAKTSGSSVETSPTNSNNTNDRIPDVGVNL	180
PID332	YVVLNQGDLIAHNGQOIINEYRPIRLLGDGAKTSGSSVETSPTNSNNTNDRIPDVGVNL	180
SK-93-1035	YVVLNQGDLIAHNGQOIINEYRPIRLLGDGAKTSGSSVETSPTNSNNTNDRIPDVGVNL	180
MC	YVVLNQGDLIAH NQ OI I DEYRPIRLL GN GAKT SDSTVDPS STNSNNTNDRIPDVGVNL	180
DGI2	HLQETSGIPGTVR	193
FA19	HLQETSGIPGTVR	193
MS11	HLQETSGIPGTVR	193
NCCP11945	HLQETSGIPGTVR	193
PID1	HLQETSGIPGTVR	193
PID18	HLQETSGIPGTVR	193
PID332	HLQETSGIPGTVR	193
SK-93-1035	HLQETSGIPGTVR	193
MC	N LQETSGIPG T T R	193

Figure S2. Alignment of the amino acid sequence of TraV from eight sequenced *Neisseria gonorrhoeae* strains carrying the GGI. A representative *N. meningitidis* strain (MC: α 275) is included for reference. Non-conserved amino acids are highlighted in gray.

DGI2	MRVKVNKFKLSAARKRQLA IAGMVAGSMAA I VGS AIYVIEGNKKKSAEVTQVQNK RQILRTDFTSPQAGI	70
NCCP11945	MRVKVNKFKLSAARKRQLA IAGMVAGSMAA I VGS AIYVIEGNKKKSAEVTQVQNK RQILRTDFTSPQAGI	70
PID18	MRVKVNKFKLSAARKRQLA IAGMVAGSMAA I VGS AIYVIEGNKKKSAEVTQVQNK RQILRTDFTSPQAGI	70
SK-93-1035	MRVKVNKFKLSAARKRQLA IAGMVAGSMAA I VGS AIYVIEGNKKKSAEVTQVQNK RQILRTDFTSPQAGI	70
PID1	MRVKVNKFKLSAARKRQLA IAGMVAGSMAA I VGS AIYVIEGNKKKSAEVTQVQNK RQILRTDFTSPQAGI	70
PID332	MRVKVNKFKLSAARKRQLA IAGMVAGSMAA I VGS AIYVIEGNKKKSAEVTQVQNK RQILRTDFTSPQAGI	70
MS11	MRVKVNKFKLSAARKRQLA IAGMVAGSMAA I VGS AIYVIEGNKKKSAEVTQVQNK RQILRTDFTSPQAGI	70
FA19	MRVKVNKFKLSAARKRQLA IAGMVAGSMAA I VGS AIYVIEGNKKKSAEVTQVQNK RQILRTDFTSPQAGI	70
MC	MRVKVNKFKLSAARKRQLA IAGMVAGSMAA I VGS AIYVIEGNKKKSAEVTQVQNK RQILRTDFTSPQAGI	70
DGI2	TDNSLWMNTESSKIEYANRK I SELETMVQELKEKENS NPDT SKGLGPDGLGKPPA ISGIGDNGR LPPAP	140
NCCP11945	TDNSLWMNTESSKIEYANRK I SELETMVQELKEKENS NPDT SKGLGPDGLGKPPA ISGIGDNGR LPPAP	140
PID18	TDNSLWMNTESSKIEYANRK I SELETMVQELKEKENS NPDT SKGLGPDGLGKPPA ISGIGDNGR LPPAP	140
SK-93-1035	TDNSLWMNTESSKIEYANRK I SELETMVQELKEKENS NPDT SKGLGPDGLGKPPA ISGIGDNGR LPPAP	140
PID1	TDNSLWMNTESSKIEYANRK I SELETMVQELKEKENS NPDT SKGLGPDGLGKPPA ISGIGDNGR LPPAP	140
PID332	TDNSLWMNTESSKIEYANRK I SELETMVQELKEKENS NPDT SKGLGPDGLGKPPA ISGIGDNGR LPPAP	140
MS11	TDNSLWMNTESSKIEYANRK I SELETMVQELKEKENS NPDT SKGLGPDGLGKPPA ISGIGDNGR LPPAP	140
FA19	TDNSLWMNTESSKIEYANRK I SELETMVQELKEKENS NPDT SKGLGPDGLGKPPA ISGIGDNGR LPPAP	140
MC	TDNSLWMNTESSKIEYANRK I SELETMVQELKEKENS NPDT SKGLGPDGLGKPPA ISGIGDNGR LPPAP	140
DGI2	PAGTLPNGAPPADRPIERKI VSGSMSEAE LQP GTTGSVNTGNPNENVRVSPQLKEAEWVKSTPRMNIEV	210
NCCP11945	PAGTLPNGAPPADRPIERKI VSGSMSEAE LQP GTTGSVNTGNPNENVRVSPQLKEAEWVKSTPRMNIEV	210
PID18	PAGTLPNGAPPADRPIERKI VSGSMSEAE LQP GTTGSVNTGNPNENVRVSPQLKEAEWVKSTPRMNIEV	210
SK-93-1035	PAGALPNGAPPADRPIERKI VSGSMSEAE LQP GTTGSVNTGNPNENVRVSPQLKEAEWVKSTPRMNIEV	210
PID1	PAGTLPNGAPPADRPIERKI VSGSMSEAE LQP GTTGSVNTGNPNENVRVSPQLKEAEWVKSTPRMNIEV	210
PID332	PAGTLPNGAPPADRPIERKI VSGSMSEAE LQP GTTGSVNTGNPNENVRVSPQLKEAEWVKSTPRMNIEV	210
MS11	PAGTLPNGAPPADRPIERKI VSGSMSEAE LQP GTTGSVNTGNPNENVRVSPQLKEAEWVKSTPRMNIEV	210
FA19	PAGTLPNGAPPADRPIERKI VSGSMSEAE LQP GTTGSVNTGNPNENVRVSPQLKEAEWVKSTPRMNIEV	210
MC	PAGTLPNGAPPADRPIERKI VSGSMSEAE LQP GTTGSVNTGNPNENVRVSPQLKEAEWVKSTPRMNIEV	210
DGI2	VEDGGKVVVSQGKFRARDSY IPSGTFFRS VLLGGVDAPTGGEAQNASPHVLMRVTDFAQLPNRFKYNFR	280
NCCP11945	VEDGGKVVVSQGKFRARDSY IPSGTFFRS VLLGGVDAPTGGEAQNASPHVLMRVTDFAQLPNRFKYNFR	280
PID18	VEDGGKVVVSQGKFRARDSY IPSGTFFRS VLLGGVDAPTGGEAQNASPHVLMRVTDFAQLPNRFKYNFR	280
SK-93-1035	VEDGGKVVVSQGKFRARDSY IPSGTFFRS VLLGGVDAPTGGEAQNASPHVLMRVTDFAQLPNRFKYNFR	280
PID1	VEDGGKVVVSQGKFRARDSY IPSGTFFRS VLLGGVDAPTGGEAQNASPHVLMRVTDFAQLPNRFKYNFR	280
PID332	VEDGGKVVVSQGKFRARDSY IPSGTFFRS VLLGGVDAPTGGEAQNASPHVLMRVTDFAQLPNRFKYNFR	280
MS11	VEDGGKVVVSQGKFRARDSY IPSGTFFRS VLLGGVDAPTGGEAQNASPHVLMRVTDFAQLPNRFKYNFR	280
FA19	VEDGGKVVVSQGKFRARDSY IPSGTFFRS VLLGGVDAPTGGEAQNASPHVLMRVTDFAQLPNRFKYNFR	280
MC	VEDGGKVVVSQGKFRARDSY IPSGTFFRS VLLGGVDAPTGGEAQNASPHVLMRVTDFAQLPNRFKYNFR	280
DGI2	ECFVTGQAYGDISSERAY IRLQNLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTKQGQLLANALMSG	350
NCCP11945	ECFVTGQAYGDISSERAY IRLQNLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTKQGQLLANALMSG	350
PID18	ECFVTGQAYGDISSERAY IRLQNLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTKQGQLLANALMSG	350
SK-93-1035	ECFVTGQAYGDISSERAY IRLQNLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTKQGQLLANALMSG	350
PID1	ECFVTGQAYGDISSERAY IRLQNLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTKQGQLLANALMSG	350
PID332	ECFVTGQAYGDISSERAY IRLQNLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTKQGQLLANALMSG	350
MS11	ECFVTGQAYGDISSERAY IRLQNLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTKQGQLLANALMSG	350
FA19	ECFVTGQAYGDISSERAY IRLQNLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTKQGQLLANALMSG	350
MC	ECFVTGQAYGDISSERAY IRLQNLSCVGTDGRAIDMPVKGYVAGEDGKTGVRGNLVTKQGQLLANALMSG	350
DGI2	VISGMGKGVSEAFKVTNNTAFGSTTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVPVVEVNAGRQ	420
NCCP11945	VISGMGKGVSEAFKVTNNTAFGSTTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVPVVEVNAGRQ	420
PID18	VISGMGKGVSEAFKVTNNTAFGSTTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVPVVEVNAGRQ	420
SK-93-1035	VISGMGKGVSEAFKVTNNTAFGSTTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVPVVEVNAGRQ	420
PID1	VISGMGKGVSEAFKVTNNTAFGSTTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVPVVEVNAGRQ	420
PID332	VISGMGKGVSEAFKVTNNTAFGSTTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVPVVEVNAGRQ	420
MS11	VISGMGKGVSEAFKVTNNTAFGSTTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVPVVEVNAGRQ	420
FA19	VISGMGKGVSEAFKVTNNTAFGSTTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVPVVEVNAGRQ	420
MC	VISGMGKGVSEAFKVTNNTAFGSTTTSIRGSDQYRAGIASGIGGAADRLAEYYIKLADKVPVVEVNAGRQ	420
DGI2	VDVVL TQGI EIDTGETK 437	
NCCP11945	VDVVL TQGI EIDTGETK 437	
PID18	VDVVL TQGI EIDTGETK 437	
SK-93-1035	VDVVL TQGI EIDTGETK 437	
PID1	VDVVL TQGI EIDTGETK 437	
PID332	VDVVL TQGI EIDTGETK 437	
MS11	VDVVL TQGI EIDTGETK 437	
FA19	VDVVL TQGI EIDTGETK 437	
MC	VDVVL TQGI EIDTGETK 437	

Figure S3. Alignment of the amino acid sequence of TraB from eight sequenced *Neisseria gonorrhoeae* strains carrying the GGI. A representative *N. meningitidis* strain (MC: α 275) is included for reference. Non-conserved amino acids are highlighted in gray.

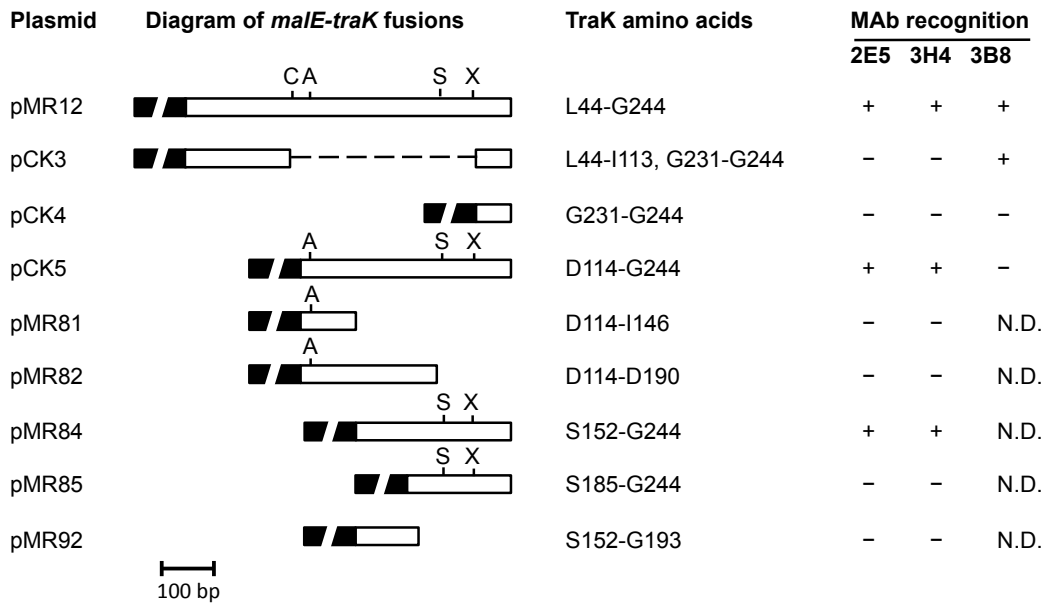


Figure S4. Characterization of monoclonal antibodies for TraK. Epitopes for three monoclonal antibodies (2E5, 3H4, and 3B8) were determined using MBP-TraK fusions. A series of plasmids was constructed in which different lengths of *traK* were fused to *malE*. The MBP-TraK fusion proteins were expressed in *E. coli*, and Western blots were performed on *E. coli* lysates with each monoclonal antibody. In the plasmid diagrams, the broken black rectangle represents *malE*, while the white rectangle indicates the portion of the *traK* sequence expressed in each fusion. Several restriction sites in TraK are indicated for reference: ClaI (C), AgeI (A), StuI (S), XmaI (X). The TraK amino acids expressed from each fusion are also indicated. The reactivity of the monoclonal antibodies to each fusion protein was determined by immunoblot. +, detection in immunoblot; -, no detection in immunoblot; N.D., reactivity not determined.

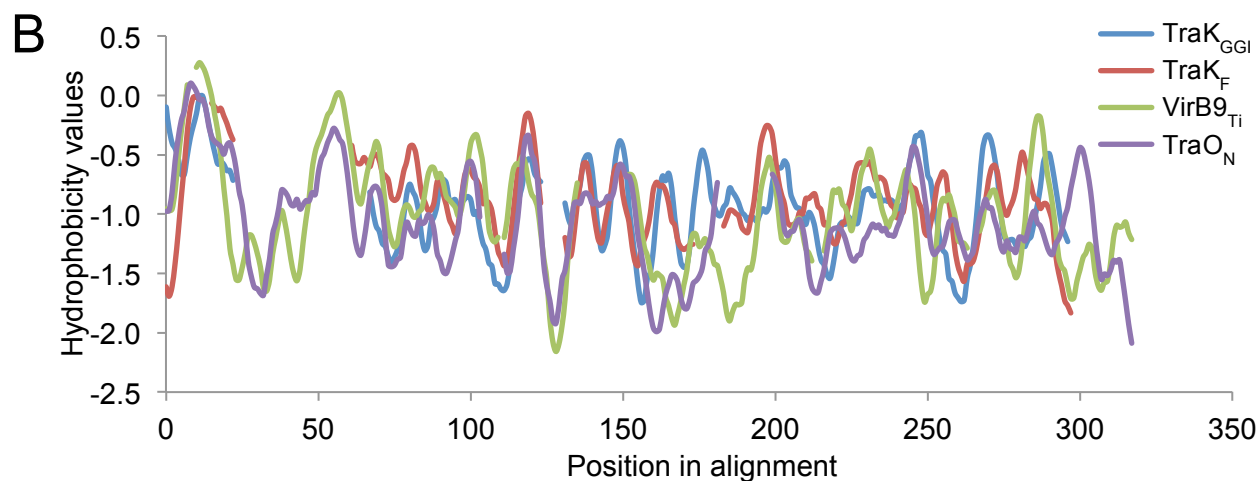
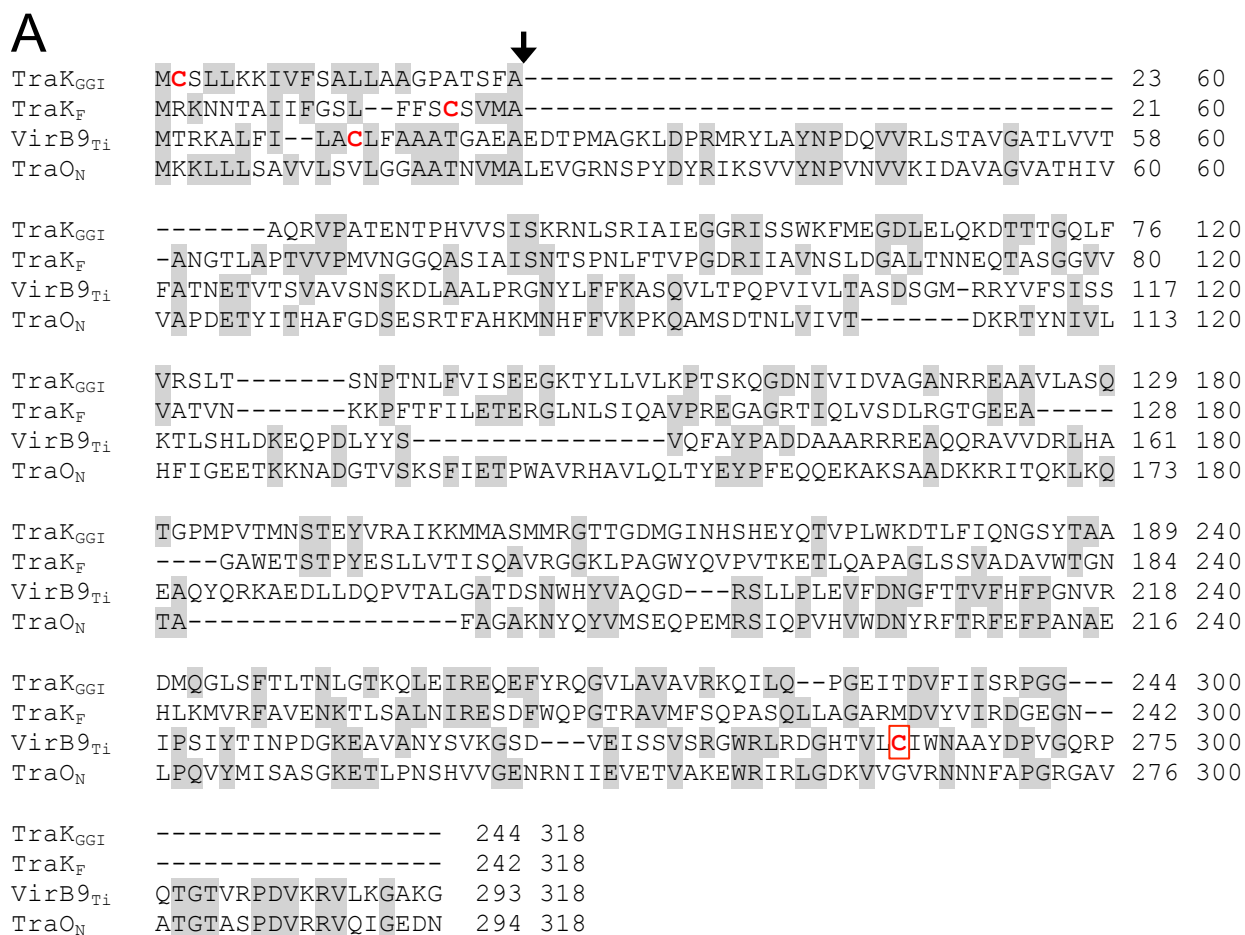


Figure S5. Alignment of TraK_{GGI} homologs. **(A)** Alignment of gonococcal TraK (TraK_{GGI}, NCBI Accession No. AAW83065) with homologs from F plasmid (TraK_F, NCBI Accession No. BAA97947), pKM101 (TraO_N, NCBI Accession No. AAA86458), and *A. tumefaciens* (VirB9 encoded by pTi15955, NCBI Accession No. CAA29979) using AlignMe (Alignment of Membrane Proteins) (2, 3). Identical residues are highlighted in gray. The arrow indicates the predicted Sec-dependent signal peptide cleavage site (SignalP) (4). Cysteines are in red, with the VirB9_{Ti} cysteine that contributes to the disulphide bridge with VirB7_{Ti} also boxed. The first column of numbers on the right is the amino acid number in each protein. The second column of numbers is the position in the overall alignment. **(B)** Hydrophobicity plot of the alignment (2, 3).

A

	*			
TraV _{GGI}	MMRLKALSITAAIILILSGCSTL-----TMSGIGGSEKFRCPNARNSDDPYCESIS	51	60	
TraV _F	MKQTSFFIPLLGLTLLLYGCAGTSTEFECNATTSDTCTMTMEQANEKAKKLLERSSEAKPVAA	60	60	
VirB7 _{Ti}	----MKYCLLCLVVALSGCQTNDTIASCKGPIFPLNVGRWQPTPSDLQLRNSGGRYDGA-	55	60	
TraN _N	----MRSLLLMGVLLISACSSGHKPPPEPDWSNTVPVNKTIPV-----DTQGGRNES-	48	60	
TraV _{GGI}	NY-----KASVAGVLKDGMRQVGQPYTDQSVRTLMLTQAYSSGT-----	91	120	
TraV _F	SLPRLAEGNFRTMPVQTVTATTPSGSRPAVTAHPEQKLLAPRPLFTAAREVKTIVVPVSSV	120	120	
VirB7 _{Ti}	-----	55	120	
TraN _N	-----	48	120	
TraV _{GGI}	-----PVRSQSEIARIWVAPYLDTDGDLNDQSFYVVLNQGDWLI AHNGQOIINEYRP	144	180	
TraV _F	TPVTPPRPLRTGEQTAALWIAPIYIDNQDVYHQSSVFFVIKPSAWGKPRIN-----	171	180	
VirB7 _{Ti}	-----	55	180	
TraN _N	-----	48	180	
TraV _{GGI}	IRLLGDGAKTSGSSVETSPTNSNNTNDRI PDVGVNLHLQETSGIPGTVR	193	229	
TraV _F	-----	171	229	
VirB7 _{Ti}	-----	55	229	
TraN _N	-----	48	229	

B

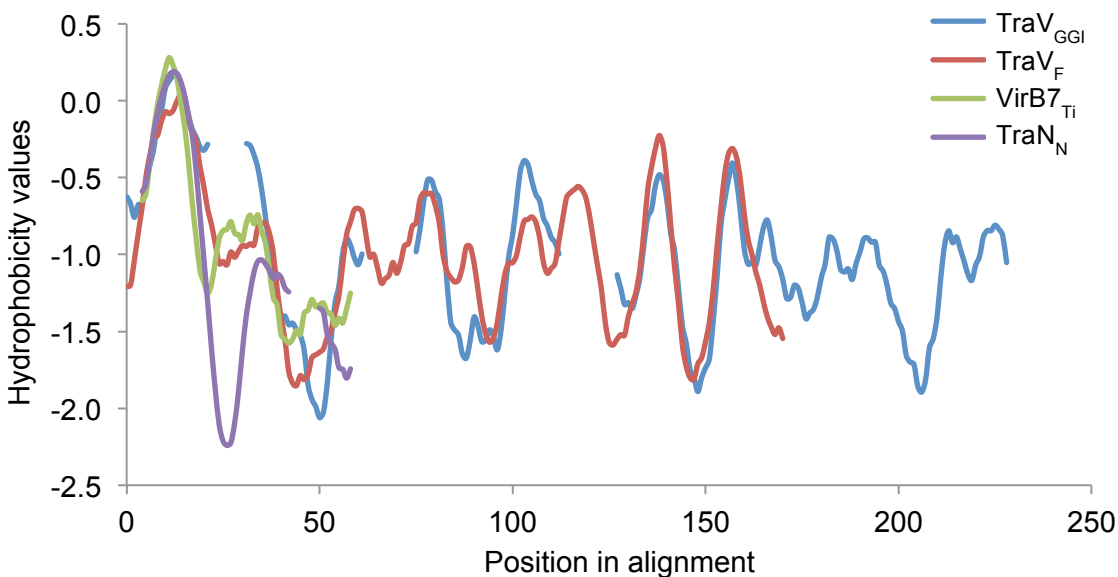


Figure S6. Alignment of TraV_{GGI} homologs. **(A)** Alignment of gonococcal TraV (TraV_{GGI}, NCBI Accession No. AAW83068) with homologs from F plasmid (TraV_F, NCBI Accession No. BAA97952), pKM101 (TraN_N, NCBI Accession No. AAA86456), and *A. tumefaciens* (VirB7 encoded by pTi15955, NCBI Accession No. CAC15164) using AlignMe (Alignment of Membrane Proteins) (2, 3). Identical residues are highlighted in gray. Cysteines are shown in red, with the N-terminal lipidated cysteines indicated by an asterisk. Cys24 in VirB7_{Ti}, which contributes to the disulphide bridge with VirB9, is boxed (5). The first set of numbers on the right is the amino acid number in each protein. The second set of numbers of the position in the overall alignment. **(B)** Hydrophobicity plot of the alignment generated using AlignMe (2, 3).

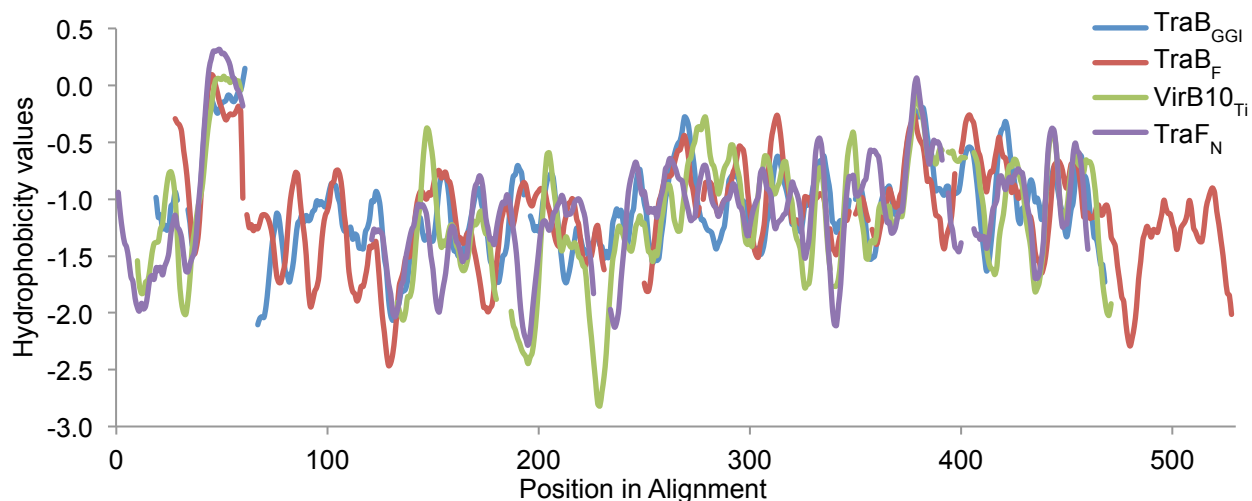
B

Figure S7. (A) Alignment of gonococcal TraB (TraB_{GGI}, NCBI Accession No. AAW83066) with homologs from F plasmid (TraB_F, NCBI Accession No. BAA97948), pKM101 (TraF_N, NCBI Accession No. AAA86459), and *A. tumefaciens* (VirB10 encoded by pTi15955, NCBI Accession No. CAA29981) using AlignMe (Alignment of Membrane Proteins) (2, 3). Identical residues are highlighted in gray. The alpha helix predicted to span the inner membrane (α 1) is labeled and underlined (predicted using the TMHMM Server v. 2.0) (6). The highly conserved GxxGxxG motif involved in channel gating in *A. tumefaciens* is indicated with asterisks (7). The alpha helices that form the outer membrane pore (α 2 and α 3) in the TraF_N crystal structure are indicated by boxes (8). The predicted alpha helical regions in VirB10_{Ti}, TraB_{GGI}, and TraB_F are underlined (Phyre2) (9). The alignment was generated by manually dividing the amino acid sequences into two regions (after the α 1 helix) and aligning the two regions separately. **(B)** Hydrophobicity plot of the alignment generated using AlignMe (2, 3).

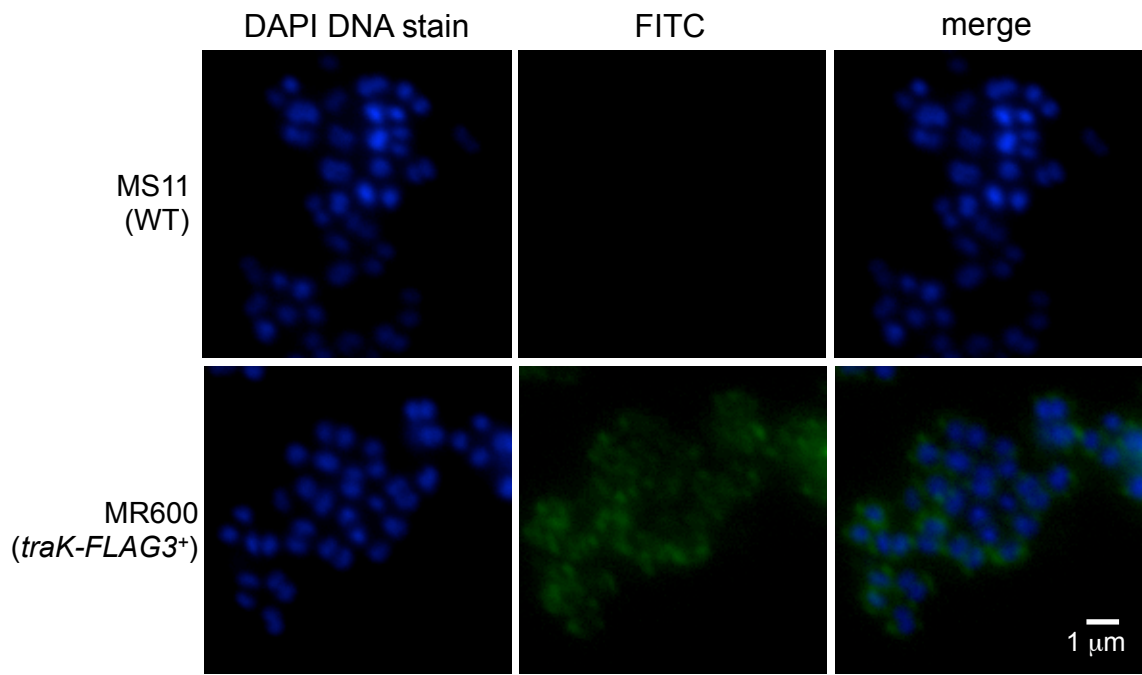


Figure S8. Immunofluorescence microscopy for TraK-FLAG3 in MS11 (WT) and MR600 (*traK-FLAG3⁺*). The DAPI DNA stain was used to counterstain the cells. A FITC-conjugated secondary antibody was used to detect TraK-FLAG3.

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