

Fig.S1

N400	(1)	MKIVFITTTVA	SSIYGFRAV	IKKLIGKNHQ	VYAFVSEFSD	NELDIIREMG	VTPVTYRSNR	SGVNPFSDIK	STFLIFKALKK	ISPDLVFPY	FAKPVIFGTF
053442	(1)	M-----	-----I	-----	-----	-----T	-----	-----L	-----E	-----	-----
8013	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
FAM18	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
alpha14	(1)	M-----	-----I	-----	-----	-----T	-----	-----L	-----E	-----	-----
Z2491	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
961-5945	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
M6190	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
MC58	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
ATCC13091	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
FA19	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
SK-93-1035	(122)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----V	-----
ST-640	(1)	M-----	-----I	-----	-----	-----T	-----	-----L	-----E	-----	-----
ST-3787	(1)	M-----	-----I	-----	-----	-----	-----	-----L	-----E	-----	-----
FA1090	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
SK-92-679	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----V	-----
35/02	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
NCCP11945	(34)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
F62	(34)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
1291	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----V	-----
PID1	(1)	M-----	-----	-----	-----	-----	-----	-----L	-----E	-----	-----
PID332	(1)	M-----	-----	-----	-----	-----	-----	-----EL	-----E	-----	-----

N400	(101)	AAKLAGVPRI	VGMLEGLGFA	FTPQPEGIPL	KTKIIKGILI	ALYRITLPLM	ESLIVLNPDD	KDELLHQYGI	KIKNIHILGG	IGLDLRQYPY	SEADIPDEKE
053442	(101)	-----	-----	-----	-----	-----A	-----	-----TDK	-----	-----S	-----
8013	(101)	-----	-----	-----	-----	-----H-A	-----	-----TDK	-----	-----	-----
FAM18	(101)	-----	-----	-----	-----	-----A	-----	-----TDK	-----	-----	-----
alpha14	(101)	-----	-----	-----	-----	-----H-A	-----	-----TDK	-----	-----	-----
Z2491	(101)	-----	-----	-----	-----	-----T-A	-----	-----TDK	-----	-----	-----
961-5945	(101)	-----	-----	-----	-----	-----A	-----	-----TDK	-----	-----S	-----
M6190	(101)	-----	-----	-----	-----	-----A	-----	-----TDK	-----	-----S	-----
MC58	(101)	-----	-----	-----	-----	-----A	-----	-----TDK	-----	-----	-----
ATCC13091	(101)	-----	-----R	-----	-----	-----A	-----	-----TDK	-----	-----	-----
FA19	(101)	-----	-----	-----	-----	-----A	-----	-----TDK	-----	-----	-----
SK-93-1035	(222)	-----	-----	-----	-----	-----A	-----	-----TDK	-----	-----S	-----
ST-640	(101)	-----	-----	-----	-----	-----A	-----	-----D	-----	-----	-----
ST-3787	(101)	-----	-----	-----	-----	-----A	-----	-----	-----	-----	-----
FA1090	(101)	-----	-----	-----	-----	-----A	-----	-----	-----	-----	-----
SK-92-679	(101)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
35/02	(101)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
NCCP11945	(134)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
F62	(134)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
1291	(101)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
PID1	(101)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
PID332	(101)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

N400	(201)	PVKFLFIGRF	LKEKGIDDFI	RAAEQVKGKY	PDTVFTALGA	IDKPRGSG..	.DLEQPAARD	IIRFPGFVNN	VSEVIKEHHI	FVLPSYYREG	VPRSTQEAMA
053442	(201)	-----	-----	-----D	-----	-----S-AG-GG	-----RL	-----	-----	-----	-----
8013	(201)	-----	-----	-----D	-----	-----S-AG-..	AIWNALPPAI	LSVSPVL	-----	-----	-----
8013	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----	M-
FAM18	(201)	-----	-----	-----D	-----	-----S-AG-G.	AIWNALPPAI	LSVSPVL	-----	-----	M-
FAM18	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----	M-
alpha14	(201)	-----	-----	-----D	-----	-----S-AG-G.	-----RL	-----	-----	-----	-----
Z2491	(201)	-----	-----	-----D	-----	-----S-G-GG	-----RL	-----	-----	-----	-----
961-5945	(201)	-----	-----	-----D	-----	-----S-G-GG	G-----RL	-----	-----	-----	-----
M6190	(201)	-----	-----	-----D	-----	-----S-G-GG	-----RL	-----	-----	-----	-----
MC58	(201)	-----	-----	-----D	-----	-----S-G-G.	-----RL	-----	-----	-----	-----
ATCC13091	(201)	-----	-----	-----D	-----	-----S-.GG	AIWNGLPPAI	LSVS-VL	-----	-----	-----
ATCC13091	(1)	-----	-----	-----	-----	-----	-----	-----	M-----A	-----	-----
FA19	(201)	-----	-----	-----	-----	-----S-RG-GG	GGFRTLYRPR	YYP--R-CEQ	CFRSD-GASY	IRIAVL	-----
SK-93-1035	(322)	-----	-----	-----W	-----E-I-V	-----S-AG-GG	GGFRTLYR-R	YYP--R-CKQ	CFRSNQGAPY	IRIAVLL	-----
SK-93-1035	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----	M-
ST-640	(201)	-----	-----	-----D	-----	-----S-..GG	AIWNALPPAI	LSVS-VLWTM	FPK	-----	-----
ST-640	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----	M-
ST-3787	(201)	-----L	-----	-----D	-----	-----S-..GG	AIWNALPPAI	LSVS-VLWTM	FPK	-----	-----
ST-3787	(1)	-----	-----	-----	-----	-----	-----	-----	M-----A	-----	-----
FA1090	(201)	-----	-----	-----	-----	-----S-G-G.	-----RFI	-----	-----A	-----	-----
SK-92-679	(201)	-----	-----	-----	-----	-----S-G-G.	-----RFI	-----	-----A	-----	-----
35/02	(201)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
NCCP11945	(234)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
F62	(234)	-----	-----	-----	-----	-----	-----	-----	-----M	-----	-----
1291	(201)	-----	-----	-----E	-----	-----	-----	-----	-----M	-----	-----
PID1	(201)	-----	-----	-----E	-----	-----	-----	-----	-----M	-----	-----
PID332	(201)	-----	-----	-----E	-----	-----	-----	-----	-----M	-----	-----

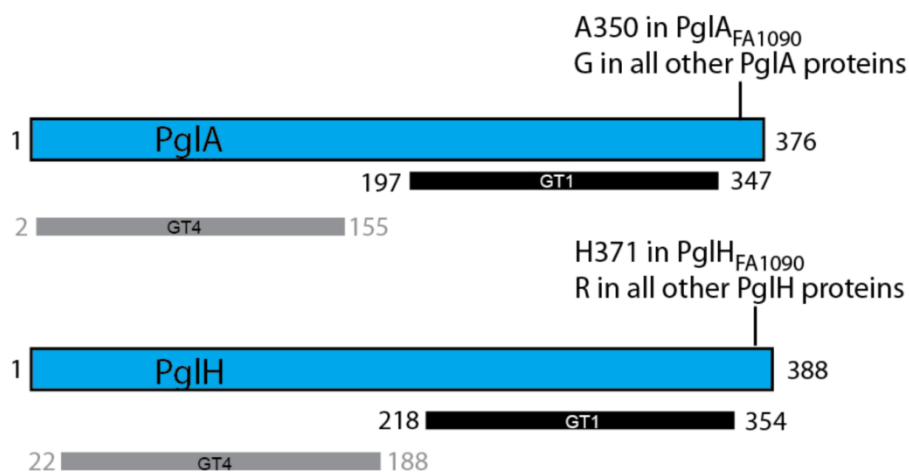


N400	(298)	VGRAVITTDV	PGCRETVA DK	VNGFLIEPWN	PRILAEKMIY	FIENRAAVRL	MGNASYAIAK	DKFDAEKVDL	KFLDILKA	-----	-----
053442	(300)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
8013	(299)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
FAM18	(3)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
alpha14	(298)	-----	-----	-----	-----	-----E-I	-----	-----	-----L	-----	-----
Z2491	(300)	-----	-----	-----	-----	-----E-I	-----	-----	-----L	-----	-----
961-5945	(301)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
M6190	(300)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
MC58	(299)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
ATCC13091	(33)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
SK-93-1035	(3)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
ST-640	(3)	-----	-----	-----	-----	-----E-I	-----	-----	-----L	-----	-----
ST-3787	(28)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
FA1090	(299)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
SK-92-679	(298)	-----	-----	-----	-----	-----E	-----	-----	-----L	-----	-----
35/02	(298)	-----	-----	-----	-----	-----	-----	-----	-----L	-----	-----
NCCP11945	(332)	-----	-----	-----	-----	-----	-----	-----	-----L	-----	-----
F62	(332)	-----	-----	-----	-----	-----	-----	-----	-----L	-----	-----
1291	(298)	-----	-----	-----	-----	-----	-----	-----	-----L	-----	-----
PID1	(298)	-----	-----	-----	-----	-----	-----	-----	-----L	-----	-----
PID332	(298)	-----	-----	-----	-----	-----	-----	-----	-----L	-----	-----

### **Fig. S1. Amino acid sequence alignment of PglA proteins**

PglA from N400 was used as consensus sequence. Amino acids differences are shown in red and identical amino acids are represented by minus signs. The critical amino acid, alanine 350, in FA1090 is boxed. Alignment was made with AlignX in Vector NTI Advance 11 (Invitrogen) that uses a modified ClustalW algorithm (24). *N. gonorrhoeae* strains are shown in black (N400, FA19, SK-93-1035, FA1090, SK-92-679, 35/02, NCCP11946, F62, 1291, PID1, PID332). *N. meningitidis* strains are shown in green (05332, 8013, FAM18, alpha14, Z2491, 961-5945, M6140, MC58, ATCC13031). *N. lactamica* strains are shown in blue (ST-640, ST-3787). The strains 8013, FAM18, ST-640 and ST3787 were in a phase off configuration but nevertheless show some PglA activity (Fig. 6).

Fig.S2



**Fig. S2. The PglA and PglH glycosyltransferase family domains**

Cartoon of the PglA (376 aa) and PglH (388 aa) glycosyltransferases in blue. The CAZy glycosyltransferase family 1 (GT1) domains are shown below in black and were identified by searching the Pfam database using PglA<sub>FA1090</sub> and PglH<sub>FA1090</sub> sequences (22). The CAZy glycosyltransferase family 4 (GT4) domains were also identified by searching Pfam and are shown below in grey, although it was insignificant for PglH<sub>FA1090</sub>. The position of alanine 350 in PglA<sub>FA1090</sub> and histidine 371 in PglH<sub>FA1090</sub> are shown.

Table S1. *Neisseria* strains and *pgl* genotypes

	Accession nr	<i>pglG</i>	<i>pglH</i>	<i>pglA</i>	polyG	<i>pglE</i>	<i>pglB</i>	Origin
<b><i>Neisseria gonorrhoeae</i></b>								
FA1090	<a href="#">NC_002946.2</a>	ON	ON	ON	11G	ON (-PV)	<i>pglB</i>	DGI
F62	<a href="#">ADAA01000005.1</a>	ON	ON	ON (-PV)	4G	ON (-PV)	<i>pglB</i>	UI
PID1	<a href="#">ABZM01000034.1</a>	OFF	ON (-PV)	ON (-PV)	4G	OFF	<i>pglB</i>	PID
FA6140	<a href="#">ABZI01000024.1</a>	OFF	ON (-PV)	OFF	16G	ON	<i>pglB</i>	ES
PID24-1	<a href="#">ABZN01000022.1</a>	OFF	ON (-PV)	OFF	11G	OFF	<i>pglB</i>	PID
DGI18	<a href="#">ABZH01000024.1</a>	OFF	ON (-PV)	OFF	13G	OFF	<i>pglB</i>	DGI
PID332	<a href="#">ABZO01000041.1</a>	OFF	ON	ON (-PV)	4G	OFF	<i>pglB</i>	PID
35/02	<a href="#">ABZG01000029.1</a>	OFF	ON (-PV)	ON (-PV)	4G	OFF	<i>pglB</i>	
SK-93-1035	<a href="#">ABZQ01000041.1</a>	ON	ON (-PV)	OFF	15G	OFF	<i>pglB</i>	DGI
SK-92-679	<a href="#">ABZP01000038.1</a>	OFF	ON (-PV)	ON	11G	OFF	<i>pglB</i>	DGI
FA19	<a href="#">ABZI01000029.1</a>	OFF	ON (-PV)	OFF	16G	OFF	<i>pglB</i>	UI
PID18	<a href="#">ABZL01000024.1</a>	OFF	ON	ON (-PV)	4G	OFF	<i>pglB</i>	PID
DGI2	<a href="#">ACIG01000103.1</a>	OFF	ON (-PV)	ON (-PV)	4G	OFF	<i>pglB</i>	DGI
MS11	<a href="#">ABZK01000025.1</a>	-	-	ON (-PV)	4G	OFF	<i>pglB</i>	UI
NCCP11945	<a href="#">NC_011035.1</a>	-	-	ON (-PV)	4G	OFF	<i>pglB</i>	
1291	<a href="#">ABZF01000025.1</a>	-	-	ON (-PV)	4G	OFF	<i>pglB</i>	GU
<b><i>Neisseria lactamica</i></b>								
ST-3787/ATCC 23970	<a href="#">ACEQ02000033.1</a>	OFF	OFF	ON	8G	ON	<i>pglB</i>	C
ST-640	<a href="#">NC_014752.1</a>	OFF	ON	OFF	10G	ON	<i>pglB</i>	C
Y92-1009	<a href="#">CACLO1000022.1</a>	OFF	ON	ON	11G	OFF	<i>pglB</i>	C
NS19	<a href="#">AEP101000013.1</a>	OFF	OFF	OFF	11G	OFF	<i>pglB</i>	C
<b><i>Neisseria meningitidis</i></b>								
H44/76	<a href="#">AEQZ01000037.1</a>	-	-	ON	11G	OFF	<i>pglB</i>	IMD
MC58	<a href="#">NC_003112.2</a>	-	-	ON	11G	OFF	<i>pglB</i>	IMD
ATCC 13091	<a href="#">AEEF01000085.1</a>	ON	OFF	OFF	13G	OFF	<i>pglB</i>	
K1207	<a href="#">ADWM01000120.1</a>	OFF	ON (-PV)	ON	11G	OFF	<i>pglB2</i>	IMD
S0108	<a href="#">ADWN01000126.1</a>	ON	ON (-PV)	OFF	13G	OFF	<i>pglB2</i>	IMD
Z2491	<a href="#">NC_003116.1</a>	OFF	ON	ON	14G	ON	<i>pglB</i>	IMD
053442	<a href="#">NC_010120.1</a>	ON	OFF	ON	12G	OFF	<i>pglB2</i>	IMD
NS44	<a href="#">AEPJ01000151.1</a>	ON	OFF	ON	8G	OFF	<i>pglB2</i>	
FAM18	<a href="#">NC_008767.1</a>	OFF	ON (-PV)	OFF	11G	OFF	<i>pglB2</i>	IMD
ALPHA14	<a href="#">NC_013016.1</a>	OFF	ON (-PV)	ON	9G	OFF	<i>pglB</i>	HC
8013	FM999788	-	-	OFF	8G	OFF	<i>pglB2</i>	IMD
M6190	<a href="#">AEQF01000040.1</a>	OFF	ON (-PV)	ON	14G	OFF	<i>pglB2</i>	IMD
ES14902	<a href="#">AEQI01000038.1</a>	OFF	ON (-PV)	ON	14G	OFF	<i>pglB2</i>	IMD
M0579	<a href="#">AEQH01000026.1</a>	OFF	OFF	OFF	9G	OFF	<i>pglB2</i>	IMD
OX99.30304	<a href="#">AEQE01000110.1</a>	OFF	OFF	OFF	10G	OFF	<i>pglB2</i>	HC
961-5945	<a href="#">AEQK01000132.1</a>	ON	ON	ON	17G	ON	<i>pglB2</i>	IMD
M13399	<a href="#">AEQG01000039.1</a>	-	-	ON	11G	OFF	<i>pglB</i>	IMD
N1568	<a href="#">AEQD01000080.1</a>	-	-	OFF	11G	OFF	<i>pglB2</i>	IMD
CU385	<a href="#">AEQJ01000044.1</a>	-	-	ON	11G	OFF	<i>pglB</i>	IMD
M01-240013	<a href="#">AEQL01000047.1</a>	-	-	OFF	10G	ON	<i>pglB</i>	IMD
<b><i>Neisseria flavescens</i> NRL30031/H210</b>								
<i>Neisseria flavescens</i> SK114	<a href="#">ACQV01000018.1</a>	ON	ON (-PV)	-	-	-	<i>pglB2</i>	C
<i>Neisseria subflava</i> NJ9703	<a href="#">ACEO02000013.1</a>	ON	ON (-PV)	-	-	-	<i>pglB2</i>	C
<i>Neisseria mucosa</i> C102	<a href="#">ACRG01000017.1</a>	ON	ON (-PV)	-	-	-	<i>pglB2</i>	C
<i>Neisseria mucosa</i> ATCC 25996	<a href="#">ACDX02000011.1</a>	ON	ON (-PV)	-	-	-	<i>pglB2</i>	C
<i>Neisseria sicca</i> DS1	<a href="#">AEPG01000397.1</a>	ON	ON (-PV)	-	-	-	<i>pglB2</i>	C
<i>Neisseria sicca</i> 4320	<a href="#">AEPF01000056.1</a>	ON	ON (-PV)	-	-	-	<i>pglB2</i>	C
<i>Neisseria sicca</i> ATCC 29256	<a href="#">ACKO02000014.1</a>	ON	ON (-PV)	-	-	-	<i>pglB2</i>	C
<i>Neisseria polysaccharea</i> NS342	<a href="#">AEPH01000240.1</a>	ON	ON (-PV)	-	-	OFF	<i>pglB</i>	C
<i>Neisseria polysaccharea</i> ATCC 43768	<a href="#">ADBE01000085.1</a>	-	-	OFF	10G	ON	<i>pglB</i>	C
<i>Neisseria cinerea</i> ATCC 14685	<a href="#">ACDY02000005.1</a>	ON	ON (-PV)	-	-	-	<i>pglB</i>	C
<i>Neisseria</i> sp. oral taxon 014 str. F0314	<a href="#">ADEA01000027.1</a>	ON	ON (-PV)	-	-	-	<i>pglB2</i>	C
<i>Neisseria elongata</i> subsp. <i>glycolytica</i> ATCC 29315	<a href="#">ADBF01000031.1</a>	ON	OFF	-	-	-	<i>pglB</i>	C
<i>Neisseria bacilliformis</i> ATCC BAA-1200	AFAY01000015.1	ON	ON (-PV)	-	-	-	<i>pglB</i>	C

(-PV), not phase variable; DGI, Disseminated Gonococcal Infection; UI, Uncomplicated Infection; PID, Pelvic Inflammatory Disease; ES, Epidemic strain; GU, Gonococcal Urethritis; IMD, Invasive Meningococcal Disease; HC, healthy carrier; C, Commensal strain.

Table S2. QuikChange primers

AA mutations	Primers	Sequence 5'-3'
<b>FA1090</b>		
SRGGGG → PRGSG	PRGS-f	GGGCGCAATCGACAAACCACGCGGGAGCGGGGATTTAGAACGCTTTATCG
	PRGS-r	CGATAAAGCGTTCTAAATCCCCGCTCCCGCGTGGTTTGTTCGATTGCGCCC
RFI → QPA	QPA-f	GGGGGGGGGAGATTTAGAACAGCCTGCCGCCCGCGATATTATCCG
	QPA-r	CGGATAATATCGCGGGCGGCAGGCTGTTCTAAATCTCCCCCCCC
GGGG → GSG	GSG-f	CGACAAATCACGCGGGAGCGGAGATTTAGAACGC
	GSG-r	GCGTTCTAAATCTCCGCTCCCGCGTGATTGTTCG
SRGGGGDLERFI → PRGSGDLEQPA	PRGSGDLEQPA-f	CGCGGGAGCGGGGATTTAGAACAGCCTGCCGCCCGCGATATTATCCG
	PRGSGDLEQPA-r	CGGATAATATCGCGGGCGGCAGGCTGTTCTAAATCCCCGCTCCCGCG
A → E	A275E-f	CAATGTTTCCGAAGTGATAAAGGAACATCATATATTCGTATTGCCGTC
	A275E-r	GACGGCAATACGAATATATGATGTTCTTTATCACTTCGGAAACATTG
A → T	A146T-f	GCCCTGTACCGCATTACCCTGCCGATG
	A146T-r	CATCGGCAGGGTAATGCGGTACAGGGC
A → G	A350G-f	CCGTCCGCTGATGGGGAATGCAAGTTATGC
	A350G-t	GCATAACTTGCATTCCCATCAGGCGGACG
<b>N400</b>		
G → A	G350A-f	CCGTCCGCTGATGGCGAATGCAAGTTATGC
	G350A-r	GCATAACTTGCATTCCCATCAGGCGGACGG
GSG → GGGG	GGGG-f	CGCAATCGACAAACCACGCGGGGGGGGGGAGATTTGGAACAGCCTGCC
	GGGG-r	GGCAGGCTGTTCCAAATCTCCCCCCCCCGCGTGGTTTGTTCGATGCG
E → A	E275A-f	CAATGTTTCCGAAGTGATAAAGCGCATCATATATTCGTATTGCCGTC
	E275A-r	GACGGCAATACGAATATATGATGCGCTTTTATCACTTCGGAAACATTG