Table 1. Oligonucleotides used for sequencing and cloning. [1]

Primer name	Oligonucleotide sequence from 5' to 3'	Target cloning vector	Description
*ape2*Up	tacgcggctttacatatggccaatttttaaggg	pET30a(+) pET28a(+)	Up-stream primer for *ape2* from *N. gonorrhoeae; Nde* I
*ape2*Dwn	tgatgtgtttgAAGCTTtggctgtgtacttga	pET30a(+) pET28a(+)	Down-stream primer for *ape2* from *N. gonorrhoeae; Hin*d III
*ape2*Up(g)	tacgcggctttacatatgGgccaatttttaaggg	pET30a(+)	Up-stream primer for *ape2* from *N. gonorrhoeae;* frame-shift from G insertion at 4th bp*; Nde* I
ape2-g-removeFwd	gaaggagatatacatatgccaatttttaagggtttggacatg	pACPM17g	Up-stream primer for site-directed mutagenesis of pACPM17g
ape2-g-removeRev	catgtccaaacccttaaaaattggcatagtgtatatctccttc	pACPM17g	Down-stream primer for site-directed mutagenesis of pACPM17g
*ape2*UppET32	**gacgacgacaagat**ggctttatttatgccaattttta	pET32 Ek/LIC	Up-stream primer for ape2 from *N. gonorrhoeae*; **Ek/LIC overhang**
*ape2*DwnpET32	**gaggagaagcccggt**gtttggAAGCTTtggctgtgt	pET32 Ek/LIC	Down-stream primer for ape2 from *N. gonorrhoeae*; **Ek/LIC over-hang**; *Hin*d III
*ape2*UppBADHisA	tacccggctttactcgagccaatttttaagg	pBADHis-A	Up-stream primer for *ape2* from *N. gonorrhoeae; Xho* I
*ape2*DwnpBADHisA	tgatgtgtttgAAGCTTtggctgtgtacttga	pBADHis-A	Down-stream primer for *ape2* from *N. gonorrhoeae; Hin*d III
*ape2*UppBAD18Cm	actggctagc**aggagg**aattcaccatgccaatttttaagggtttggacatgaaaaac	pBAD18-Cm	Up-stream primer for *ape2* from *N. gonorrhoeae*; **RBS**; *Nhe* I
*ape2*DwnpBAD18Cm	atcgaagcttca**ATGATGATGATGATGATG**tggctgtgtacttgatggttgcgtactcg	pBAD18-Cm	Down-stream primer for *ape2* from *N. gonorrhoeae*; **His6-Tag/Stop codon**; *Hin*d III
*ape2*UppACPM19	actgaagctt**aggagg**aattcaccatgccaatttttaagggtttggacatgaaaaac	pACPM19	Up-stream primer for *ape2* from *N. gonorrhoeae*; *Hin*d III; **RBS**

*ape2*UppACPM20	actg<u>gctagc</u>**aggagg**aattcaccat ggtgtggttcagccaaaaccccatca acgcc	pACPM20	Up-stream primer for *ape2* from *N. gonorrhoeae* encoding an N-terminal 26 amino acid truncation; *Nhe*I; **RBS**
*ape2*DwnpACPM19	cgat<u>aagctt</u>*tca***atgatgatgatga tgatg**tggctgtgtacttgatggttg cgtac	pACPM19 pACPM20	Common down-stream primer for pBAD18-Cm based constructs; *Hin*d III; **Stop, His_6-tag**
*ape2*UppACPM22	actg<u>ctcgag</u>tactggcagcagacc	pACPM22	Up-stream primer for *ape2* from *N. gonorrhoeae* encoding an N-terminal 36 amino acid truncation*; Xho*I
*ape2*DwnpACPM21	cagt<u>aagctt</u>tcatggctgtgtactt gatggttg	pACPM21 pACPM22	Common down-stream primer for pBADHis-A based constructs; *Hin*d III

[1]Incorporated restriction sites are indicated by underlined typeface and new features introduced by the primers are indicated in bold typeface and explained in the description. Mismatches with the template sequence are indicated by capital letters.

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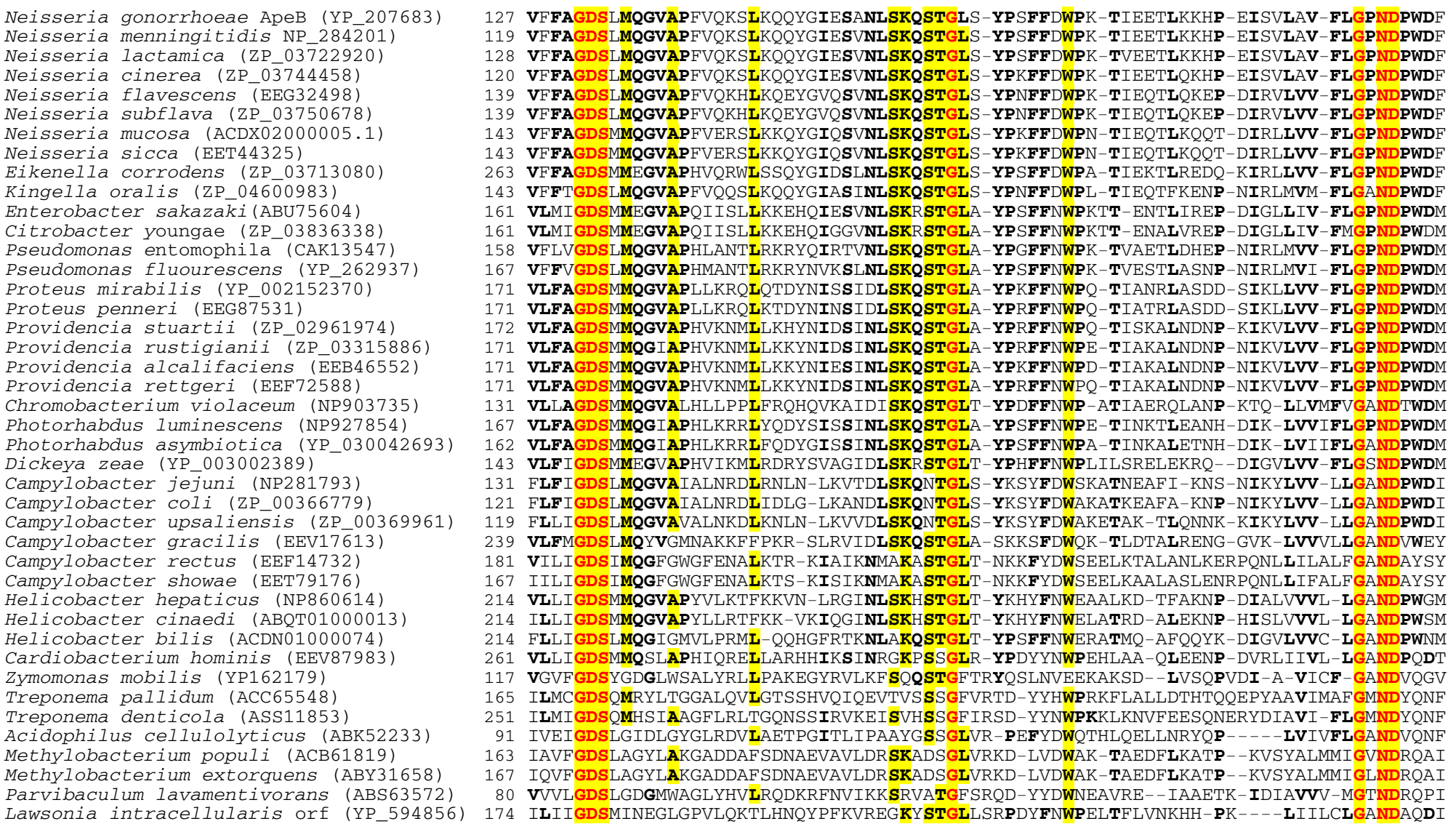
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Neisseria menningitidis NP_284201) 119 VFFAGDSLMQGVAPFVQKSLKQQYGIESVNLSKQSTGLS-YPSFFDWPK-TIEETLKKHP-EISVLAV-FLGPNDPWDF
Neisseria lactamica (ZP_03722920) 128 VFFAGDSLMQGVAPFVQKSLKQQYGIESVNLSKQSTGLS-YPSFFDWPK-TVEETLKKHP-EISVLAV-FLGPNDPWDF
Neisseria cinerea (ZP_03744458) 120 VFFAGDSLMQGVAPFVQKSLKQQYGIESVNLSKQSTGLS-YPKFFDWPK-TIEETLQKHP-EISVLAV-FLGPNDPWDF
Neisseria flavescens (EEG32498) 139 VFFAGDSLMQGVAPFVQKHLKQEYGVQSVNLSKQSTGLS-YPNFFDWPK-TIEQTLQKEP-DIRVLVV-FLGPNDPWDF
Neisseria subflava (ZP_03750678) 139 VFFAGDSLMQGVAPFVQKHLKQEYGVQSVNLSKQSTGLS-YPNFFDWPK-TIEQTLQKEP-DIRVLVV-FLGPNDPWDF
Neisseria mucosa (ACDX02000005.1) 143 VFFAGDSMMQGVAPFVERSLKKQYGIQSVNLSKQSTGLS-YPKFFDWPN-TIEQTLKQQT-DIRLLVV-FLGPNDPWDF
Neisseria sicca (EET44325) 143 VFFAGDSMMQGVAPFVERSLKKQYGIQSVNLSKQSTGLS-YPKFFDWPN-TIEQTLKQQT-DIRLLVV-FLGPNDPWDF
Eikenella corrodens (ZP_03713080) 263 VFFAGDSMMEGVAPHVQRWLSSQYGIDSLNLSKQSTGLS-YPSFFDWPA-TIEKTLREDQ-KIRLLVV-FLGPNDPWDF
Kingella oralis (ZP_04600983) 143 VFFTGDSLMQGVAPFVQQSLKQQYGIASINLSKQSTGLS-YPNFFDWPL-TIEQTFKENP-NIRLMVM-FLGANDPWDF
Enterobacter sakazaki(ABU75604) 161 VLMIGDSMMEGVAPQIISLLKKEHQIESVNLSKRSTGLA-YPSFFNWPKTT-ENTLIREP-DIGLLIV-FLGPNDPWDM
Citrobacter youngae (ZP_03836338) 161 VLMIGDSMMEGVAPQIISLLKKEHQIGGVNLSKRSTGLA-YPSFFNWPKTT-ENALVREP-DIGLLIV-FMGPNDPWDM
Pseudomonas entomophila (CAK13547) 158 VFLVGDSLMQGVAPHLANTLRKRYQIRTVNLSKQSTGLA-YPGFFNWPK-TVAETLDHEP-NIRLMVV-FLGPNDPWDM
Pseudomonas fluourescens (YP_262937) 167 VFFVGDSLMQGVAPHMANTLRKRYNVKSLNLSKQSTGLA-YPSFFNWPK-TVESTLASNP-NIRLMVI-FLGPNDPWDM
Proteus mirabilis (YP_002152370) 171 VLFAGDSMMQGVAPLLKRQLQTDYNISSIDLSKQSTGLA-YPKFFNWPQ-TIANRLASDD-SIKLLVV-FLGPNDPWDM
Proteus penneri (EEG87531) 171 VLFAGDSMMQGVAPLLKRQLKTDYNINSIDLSKQSTGLA-YPRFFNWPQ-TIATRLASDD-SIKLLVV-FLGPNDPWDM
Providencia stuartii (ZP_02961974) 172 VLFAGDSMMQGVAPHVKNMLLKHYNIDSINLSKQSTGLA-YPRFFNWPQ-TISKALNDNP-KIKVLVV-FLGPNDPWDM
Providencia rustigianii (ZP_03315886) 171 VLFAGDSMMQGIAPHVKNMLLKKYNIDSINLSKQSTGLA-YPRFFNWPE-TIAKALNDNP-NIKVLVV-FLGPNDPWDM
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Providencia rettgeri (EEF72588) 171 VLFAGDSMMQGVAPHVKNMLLKKYNIDSINLSKQSTGLA-YPRFFNWPQ-TIAKALNDNP-NIKVLVV-FLGPNDPWDM
Chromobacterium violaceum (NP903735) 131 VLLAGDSMMQGVALHLLPPLFRQHQVKAIDISKQSTGLT-YPDFFNWP-ATIAERQLANP-KTQ-LLVMFVGANDTWDM
Photorhabdus luminescens (NP927854) 167 VLFAGDSMMQGIAPHLKRRLYQDYSISSINLSKQSTGLA-YPSFFNWPE-TINKTLEANH-DIK-LVVIFLGPNDPWDM
Photorhabdus asymbiotica (YP_030042693) 162 VLFAGDSMMQGIAPHLKRRLFQDYGISSINLSKQSTGLA-YPSFFNWPA-TINKALETNH-DIK-LVIIFLGANDPWDM
Dickeya zeae (YP_003002389) 143 VLFIGDSMMEGVAPHVIKMLRDRYSVAGIDLSKRSTGLT-YPHFFNWPLILSRELEKRQ--DIGVLVV-FLGSNDPWDM
Campylobacter jejuni (NP281793) 131 FLFIGDSLMQGVAIALNRDLRNLN-LKVTDLSKQNTGLS-YKSYFDWSKATNEAFI-KNS-NIKYLVV-LLGANDPWDI
Campylobacter coli (ZP_00366779) 121 FLFIGDSLMQGVAIALNRDLIDLG-LKANDLSKQNTGLS-YKSYFDWAKATKEAFA-KNP-NIKYLVV-LLGANDPWDI
Campylobacter upsaliensis (ZP_00369961) 119 FLLIGDSLMQGVAVALNKDLKNLN-LKVVDLSKQNTGLS-YKSYFDWAKETAK-TLQNNK-KIKYLVV-LLGANDPWDI
Campylobacter gracilis (EEV17613) 239 VLFMGDSLMQYVGMNAKKFFPKR-SLRVIDLSKQSTGLA-SKKSFDWQK-TLDTALRENG-GVK-LVVVLLGANDVWEY
Campylobacter rectus (EEF14732) 181 VILIGDSIMQGFGWGFENALKTR-KIAIKNMAKASTGLT-NKKFYDWSEELKTALANLKERPQNLLILALFGANDAYSY
Campylobacter showae (EET79176) 167 IILIGDSIMQGFGWGFENALKTS-KISIKNMAKASTGLT-NKKFYDWSEELKAALASLENRPQNLLIFALFGANDAYSY
Helicobacter hepaticus (NP860614) 214 VLLIGDSMMQGVAPYVLKTFKKVN-LRGINLSKHSTGLT-YKHYFNWEAALKD-TFAKNP-DIALVVVL-LGANDPWGM
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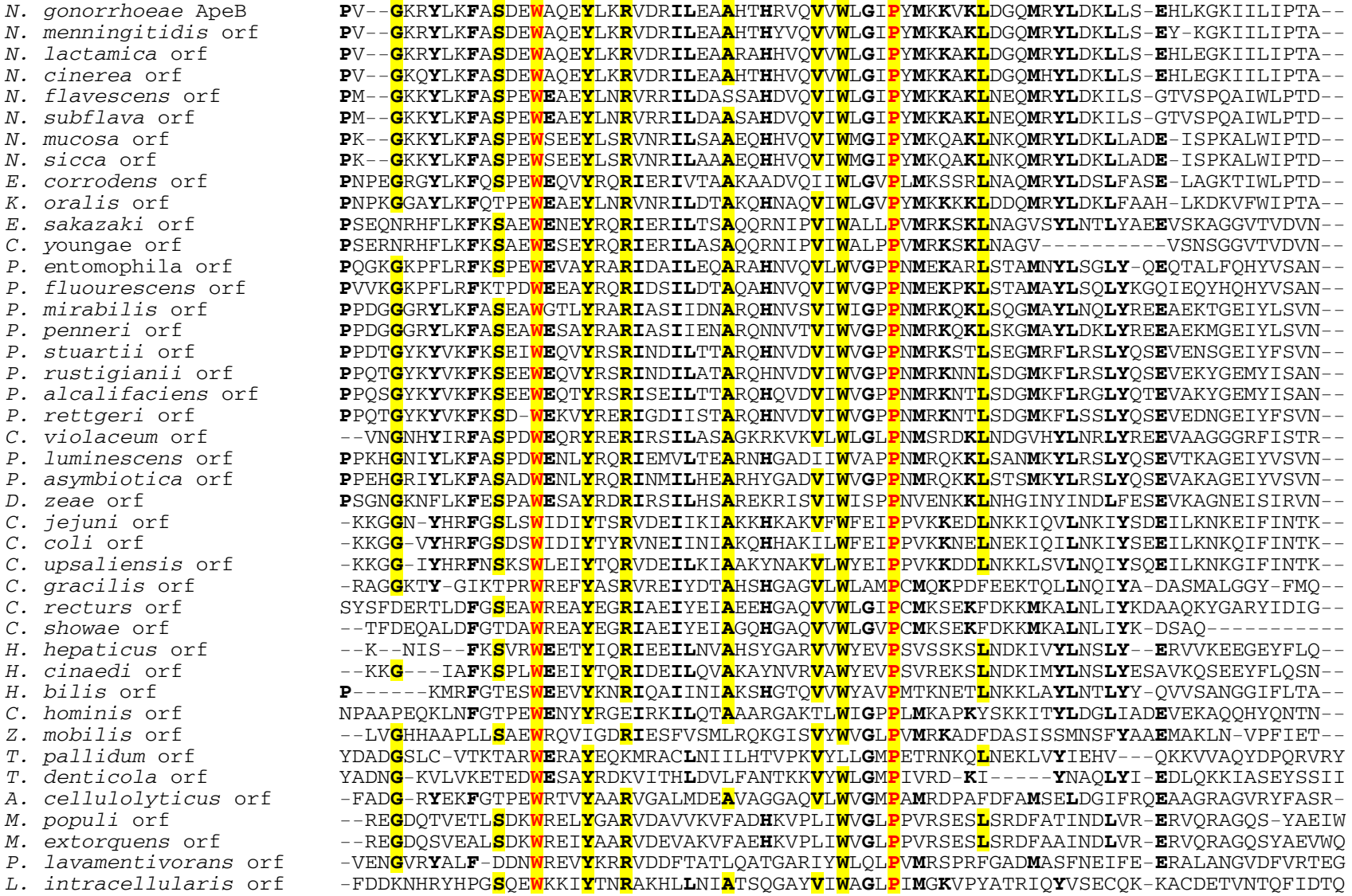
Helicobacter bilis (ACDN01000074) 214 FLLIGDSLMQGIGMVLPRML-QQHGFRTKNLAKQSTGLT-YPSFFNWERATMQ-AFQQYK-DIGVLVVC-LGANDPWNM
Cardiobacterium hominis (EEV87983) 261 VLLIGDSMMQSLAPHIQRELLARHHIKSINRGKPSSGLR-YPDYYNWPEHLAA-QLEENP-DVRLIIVL-LGANDPQDT
Zymomonas mobilis (YP162179) 117 VGVFGDSYGDGLWSALYRLLPAKEGYRVLKFSQQSTGFTRYQSLNVEEKAKSD--LVSQPVDI-A-VICF-GANDVQGV
Treponema pallidum (ACC65548) 165 ILMCGDSQMRYLTGGALQVLGTSSHVQIQEVTVSSSGFVRTD-YYHWPRKFLALLDTHTQQEPYAAVIMAFGMNDYQNF
Treponema denticola (ASS11853) 251 ILMIGDSQMHSIAAGFLRLTGQNSSIRVKEISVHSSGFIRSD-YYNWPKKLKNVFEESQNERYDIAVI-FLGMNDYQNF
Acidophilus cellulolyticus (ABK52233) 91 IVEIGDSLGIDLGYGLRDVLAETPGITLIPAAYGSSGLVR-PEFYDWQTHLQELLNRYQP-----LVIVFLGANDVQNF
Methylobacterium populi (ACB61819) 163 IAVFGDSLAGYLAKGADDAFSDNAEVAVLDRSKADSGLVRKD-LVDWAK-TAEDFLKATP--KVSYALMMIGVNDRQAI
Methylobacterium extorquens (ABY31658) 167 IQVFGDSLAGYLAKGADDAFSDNAEVAVLDRSKADSGLVRKD-LVDWAK-TAEDFLKATP--KVSYALMMIGLNDRQAI
Parvibaculum lavamentivorans (ABS63572) 80 VVVLGDSLGDGMWAGLYHVLRQDKRFNVIKKSRVATGFSRQD-YYDWNEAVRE--IAAETK-IDIAVVV-MGTNDRQPI
Lawsonia intracellularis orf (YP_594856) 174 ILIIGDSMINEGLGPVLQKTLHNQYPFKVREGKYSTGLLSRPDYFNWPELTFLVNKHH-PK----LIILCLGANDAQDI


N. gonorrhoeae ApeB PV--GKRYLKFASDEWAQEYLKRVDRILEAAHTHRVQVVWLGIPYMKKVKLDGQMRYLDKLLS-EHLKGKIILIPTA--
N. menningitidis orf PV--GKRYLKFASDEWAQEYLKRVDRILEAAHTHYVQVVWLGIPYMKKAKLDGQMRYLDKLLS-EY-KGKIILIPTA--
N. lactamica orf PV--GKRYLKFASDEWAQEYLKRVDRILEAARAHHVQVVWLGIPYMKKAKLDGQMRYLDKLLS-EHLEGKIILIPTA--
N. cinerea orf PV--GKQYLKFASDEWAQEYLKRVDRILEAAHTHHVQVVWLGIPYMKKAKLDGQMHYLDKLLS-EHLEGKIILIPTA--
N. flavescens orf PM--GKKYLKFASPEWEAEYLNRVRRILDASSAHDVQVIWLGIPYMKKAKLNEQMRYLDKILS-GTVSPQAIWLPTD--
N. subflava orf PM--GKKYLKFASPEWEAEYLNRVRRILDAASAHDVQVIWLGIPYMKKAKLNEQMRYLDKILS-GTVSPQAIWLPTD--
N. mucosa orf PK--GKKYLKFASPEWSEEYLSRVNRILSAAEQHHVQVIWMGIPYMKQAKLNKQMRYLDKLLADE-ISPKALWIPTD--
N. sicca orf PK--GKKYLKFASPEWSEEYLSRVNRILAAAEQHHVQVIWMGIPYMKQAKLNKQMRYLDKLLADE-ISPKALWIPTD--
E. corrodens orf PNPEGRGYLKFQSPEWEQVYRQRIERIVTAAKAADVQIIWLGVPLMKSSRLNAQMRYLDSLFASE-LAGKTIWLPTD--
K. oralis orf PNPKGGAYLKFQTPEWEAEYLNRVNRILDTAKQHNAQVIWLGVPYMKKKKLDDQMRYLDKLFAAH-LKDKVFWIPTA--
E. sakazaki orf PSEQNRHFLKFKSAEWENEYRQRIERILTSAQQRNIPVIWALLPVMRKSKLNAGVSYLNTLYAEEVSKAGGVTVDVN--
C. youngae orf PSERNRHFLKFKSAEWESEYRQRIERILASAQQRNIPVIWALPPVMRKSKLNAGV----------VSNSGGVTVDVN--
P. entomophila orf PQGKGKPFLRFKSPEWEVAYRARIDAILEQARAHNVQVLWVGPPNMEKARLSTAMNYLSGLY-QEQTALFQHYVSAN--
P. fluourescens orf PVVKGKPFLRFKTPDWEEAYRQRIDSILDTAQAHNVQVIWVGPPNMEKPKLSTAMAYLSQLYKGQIEQYHQHYVSAN--
P. mirabilis orf PPDGGGRYLKFASEAWGTLYRARIASIIDNARQHNVSVIWIGPPNMRKQKLSQGMAYLNQLYREEAEKTGEIYLSVN--
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P. stuartii orf PPDTGYKYVKFKSEIWEQVYRSRINDILTTARQHNVDVIWVGPPNMRKSTLSEGMRFLRSLYQSEVENSGEIYFSVN--
P. rustigianii orf PPQTGYKYVKFKSEEWEQVYRSRINDILATARQHNVDVIWVGPPNMRKNNLSDGMKFLRSLYQSEVEKYGEMYISAN--
P. alcalifaciens orf PPQSGYKYVKFKSEEWEQTYRSRISEILTTARQHQVDVIWVGPPNMRKNTLSDGMKFLRGLYQTEVAKYGEMYISAN--
P. rettgeri orf PPQTGYKYVKFKSD-WEKVYRERIGDIISTARQHNVDVIWVGPPNMRKNTLSDGMKFLSSLYQSEVEDNGEIYFSVN--
C. violaceum orf --VNGNHYIRFASPDWEQRYRERIRSILASAGKRKVKVLWLGLPNMSRDKLNDGVHYLNRLYREEVAAGGGRFISTR--
P. luminescens orf PPKHGNIYLKFASPDWENLYRQRIEMVLTEARNHGADIIWVAPPNMRQKKLSANMKYLRSLYQSEVTKAGEIYVSVN--
P. asymbiotica orf PPEHGRIYLKFASADWENLYRQRINMILHEARHYGADVIWVGPPNMRQKKLSTSMKYLRSLYQSEVAKAGEIYVSVN--
D. zeae orf PSGNGKNFLKFESPAWESAYRDRIRSILHSAREKRISVIWISPPNVENKKLNHGINYINDLFESEVKAGNEISIRVN--
C. jejuni orf -KKGGN-YHRFGSLSWIDIYTSRVDEIIKIAKKHKAKVFWFEIPPVKKEDLNKKIQVLNKIYSDEILKNKEIFINTK--
C. coli orf -KKGG-VYHRFGSDSWIDIYTYRVNEIINIAKQHHAKILWFEIPPVKKNELNEKIQILNKIYSEEILKNKQIFINTK--
C. upsaliensis orf -KKGG-IYHRFNSKSWLEIYTQRVDEILKIAAKYNAKVLWYEIPPVKKDDLNKKLSVLNQIYSQEILKNKGIFINTK--
C. gracilis orf -RAGGKTY-GIKTPRWREFYASRVREIYDTAHSHGAGVLWLAMPCMQKPDFEEKTQLLNQIYA-DASMALGGY-FMQ--
C. recturs orf SYSFDERTLDFGSEAWREAYEGRIAEIYEIAEEHGAQVVWLGIPCMKSEKFDKKMKALNLIYKDAAQKYGARYIDIG--
C. showae orf --TFDEQALDFGTDAWREAYEGRIAEIYEIAGQHGAQVVWLGVPCMKSEKFDKKMKALNLIYK-DSAQ----------
H. hepaticus orf --K--NIS--FKSVRWEETYIQRIEEILNVAHSYGARVVWYEVPSVSSKSLNDKIVYLNSLY--ERVVKEEGEYFLQ--
H. cinaedi orf --KKG---IAFKSPLWEEIYTQRIDEILQVAKAYNVRVAWYEVPSVREKSLNDKIMYLNSLYESAVKQSEEYFLQSN--
H. bilis orf P------KMRFGTESWEEVYKNRIQAIINIAKSHGTQVVWYAVPMTKNETLNKKLAYLNTLYY-QVVSANGGIFLTA--
C. hominis orf NPAAPEQKLNFGTPEWENYYRGEIRKILQTAAARGAKTLWIGPPLMKAPKYSKKITYLDGLIADEVEKAQQHYQNTN--
Z. mobilis orf --LVGHHAAPLLSAEWRQVIGDRIESFVSMLRQKGISVYWVGLPVMRKADFDASISSMNSFYAAEMAKLN-VPFIET--
T. pallidum orf YDADGSLC-VTKTARWERAYEQKMRACLNIILHTVPKVYLLGMPETRNKQLNEKLVYIEHV---QKKVVAQYDPQRVRY
T. denticola orf YADNG-KVLVKETEDWESAYRDKVITHLDVLFANTKKVYWLGMPIVRD-KI-----YNAQLYI-EDLQKKIASEYSSII
A. cellulolyticus orf -FADG-RYEKFGTPEWRTVYAARVGALMDEAVAGGAQVLWVGMPAMRDPAFDFAMSELDGIFRQEAAGRAGVRYFASR-
M. populi orf --REGDQTVETLSDKWRELYGARVDAVVKVFADHKVPLIWVGLPPVRSESLSRDFATINDLVR-ERVQRAGQS-YAEIW
M. extorquens orf --REGDQSVEALSDKWREIYAARVDEVAKVFAEHKVPLIWVGLPPVRSESLSRDFAAINDLVR-ERVQRAGQSYAEVWQ
P. lavamentivorans orf -VENGVRYALF-DDNWREVYKRRVDDFTATLQATGARIYWLQLPVMRSPRFGADMASFNEIFE-ERALANGVDFVRTEG
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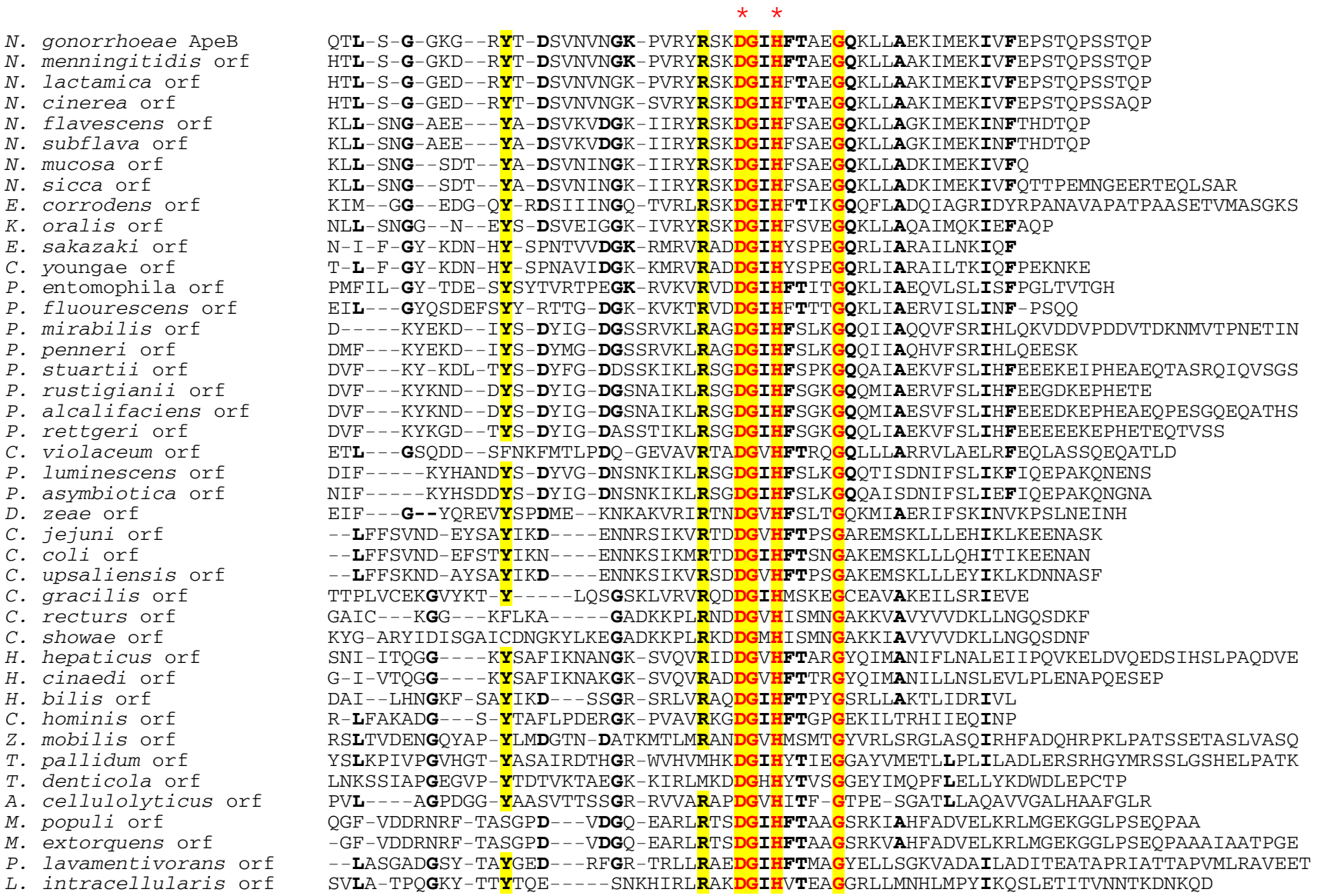
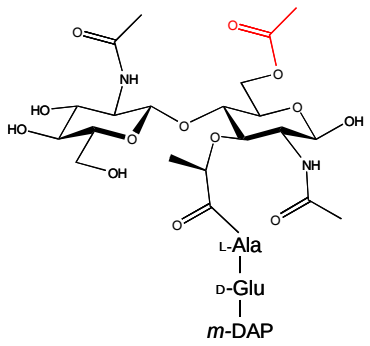
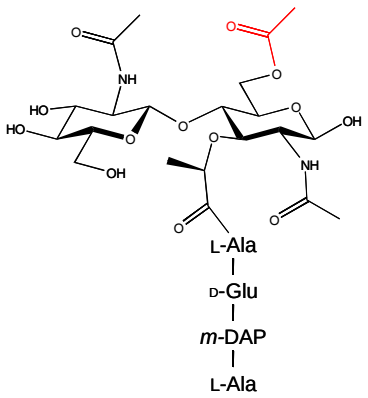


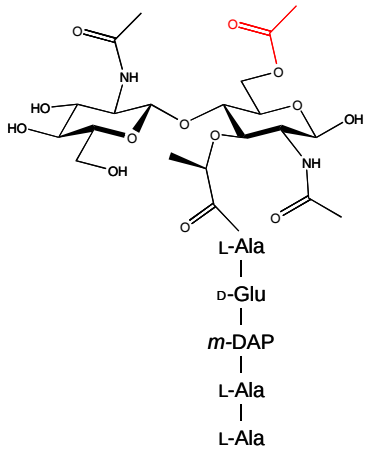
Figure 1. Sequence alignment of *N. gonorrhoeae* Ape2/PatB with hypothetical proteins identified in the nucleotide sequence database. The residues in bold face and yellow highlight denote greater than 50% and 80% identity, respectively, while those in red are invariant amongst all sequences. The potential catalytic residues are denoted by the red asterisks. The accession numbers of the hypothetical proteins are provided in parenthesis.



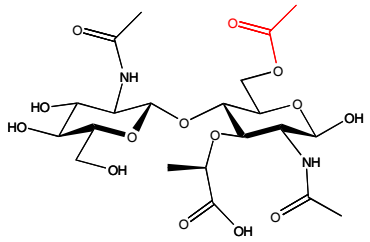
G-*O*AcM(Tri)



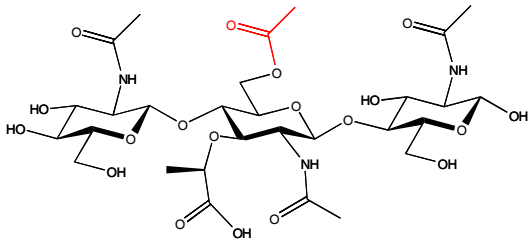
G-*O*AcM(Tetra)



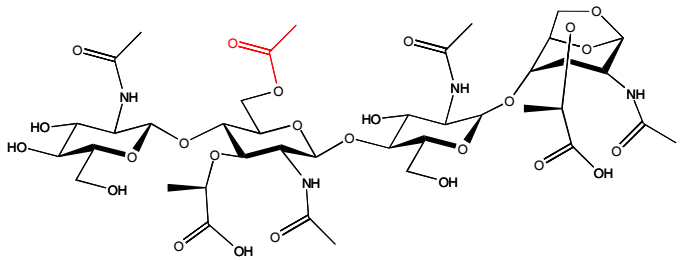
G-*O*AcM(Penta)



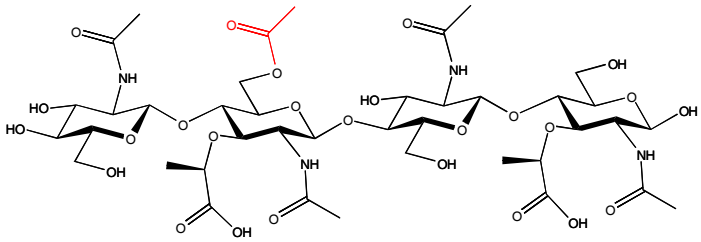
G-*O*AcM



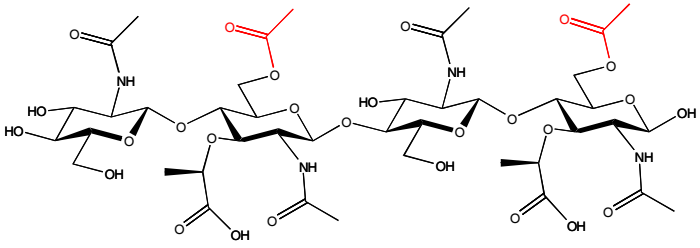
G-*O*AcM-G



G-*O*AcM-G-anhM



G-*O*AcM*-G-M*



G-*O*AcM-G-*O*AcM

Fig. 2. Structures of unique muropetides and muroglycans identified in PG digests from PatB overproduced in permeabilized cells of *E. coli* Top10[pACPM19]. O-Linked acetate is in red and the asterisks denote that the O-acetate groups could be on either of the MurNAc residues. G, GlcNAc; M, MurNAc; anhM, 1,6-anhydroMurNAc; Tri, tripeptide; Tetra, tetrapeptide; Penta, pentapeptide.