Supporting Information for the manuscript entitled:

Campylobacter jejuni PglH is a Single Active Site Processive Polymerase that Utilizes Product Inhibition to Limit Sequential Glycosyl Transfer Reactions

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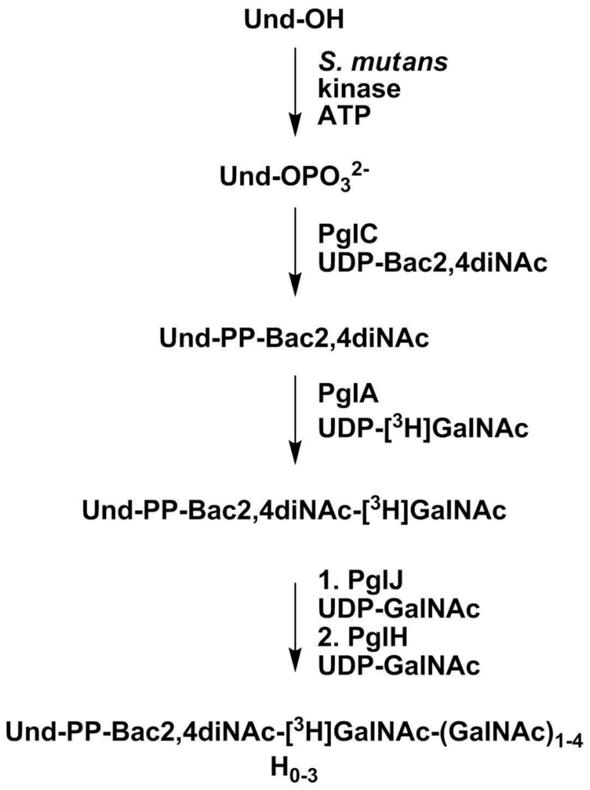
Supporting Scheme 1. Biosynthesis of the Pgl enzyme substrates and products



Supporting Scheme 2. Design of the dual label interference assay.

Und-PP-Bac2,4diNAc-([^{3}H]GalNAc)$_2$ **[^{3}H]H$_0$**

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Und-PP-Bac2,4diNAc-(***[^{14}C]GalNAc***)$_3$ **[^{14}C]H$_1$**

$$\Big\downarrow\begin{array}{l}\text{PglH}\\ \text{UDP-GalNAc}\end{array}$$

Und-PP-Bac2,4diNAc-([^{3}H]GalNAc)$_2$-(GalNAc)$_3$ **H$_3$ from [^{3}H]H$_0$**

OR

Und-PP-Bac2,4diNAc-(***[^{14}C]GalNAc***)$_3$-(GalNAc)$_2$ **H$_3$ from [^{14}C]H$_1$**

Supporting Figure 1

PglH mutagenesis forward primers in bold capital is the mutation

E41A: 5’-gtattattaaatttcatgcaggag**C**atctttttataagcttgaaaatg-3’
E49A: 5’-ctttttataagcttg**C**aaatgaagttaaagttacaagtttgg-3’

E171A: 5’-ccttgtcattttagcgatg**C**aatttcttttgattc-3’
E179A: 5’-gattctagttttg**C**aaaggaaaatttggttctttttatagg-3’

E265A: 5’-gcctttgttcttttgtag**C**gggtttgccaacggttttaattg-3’
E273A: 5’- gccaacggttttaattg**C**aagtttgtattttgagg-3’

E308A: 5’-gatgggcttttggtaggttgtgatgatgcaatag**C**acttgctaaaaaacttgaac-3’
E316A: 5’- ggcttttggtaggttgtgatgatgaaatagcacttgctaaaaaacttg**C**acttgttttaaatgatg-3’

E346A: 5’- ctcatatcaaag**C**agaatggcttaagc-3’
E354A: 5’- ggcttaagcttatagccg**C**ggttaaaaatgccc-3’

R191A: 5’-ggaaaatttggttctttttataggg**GC**tttagatcacaacaaaaaccctgtaatgtttttaaaagc-3’

Supporting Figure 2 EX_7E motifs in the PglC, A, J and H enzymes

PglC

1 MYEKVFKRIF DFILALVLLV LFSPVILITA LLLKITQGSV IFTQNRPGLD EKIFKIYKFK
61 TMSDERD**EKG ELLSDE**LRLK AFGKIVRSLS LDELLQLFNV LKGDMSFVGP RPLLV**EYLSL**
121 **YNEE**QKLRHK VRPGITGWAQ VNGRNAISWQ KKFELDVYYV KNISFLLDLK IMFLTALKVL
181 KRSGVSKEGH VTTEKFNGKN

PglA

1 MRIGFLSHAG ASIYHFRMPI IKALKDRKDE VFVIVPQDEY TQKLRDLGLK VIVYELSRAS
61 LNPFVVLKNF FYLAKVLKNL NLDLIQSAAH KSNTFGILAA KWAKIPYRFA LVEGLGSFYI
121 DQGFKANLVR FVINNLYKLG FKFAHQFIFV NESNAEFMRN LGFKESKICV IKSVGINLKK
181 FFPIYVESEK KELFWKKLNI DKKPIVLMIA RALWHKGVKE FYESATMLKD KANFVLVGGR
241 D**ENPSCASLE** FLNSGVVHYL GARSDIVELL QNCDIFVLPS YK**EGFPVSVL E**AKACGKAIV
301 VSDCEGCVEA ISNAYDGLWA KTKNAKDLSE KISLLLEDEK LRLNLAKNAA QDALQYDENN
361 IAQRYLKLYD RVIKNV

Pgl J

1 MQKLGIFIYS LGSGGAERVV ATLLPILSLK FEVHLILMND KISYEIPECQ IHFLECSKPS
61 ENPILKFLKL PFLALKYKKL CRNLGIDTEF VFLNRPNYIA LMARMFGNKT RLVINECTTP
121 SVMYMKNNFN SLVNKFLISL LYPKADLILP NSKGNLEDLV QNFSISPKKC EILYNAIDL**E**
181 **NIGQKALE**DI ALKDKFILSV GRLDKGKNHA LLIRAYARLK TDLKLVILGE GVLKDELLAL
241 IKELNLEEKV LLLGFDNNPY KYMAKCEFFA FASVF**EGFSN VLIE**SLACSC AVVCTDHKSG
301 ARELFGDDEF GLLVEVDNEN SMFQGLKTML EDDKLRKAYK NKAKTRAKAF DKVKIARDAL
361 KYLLG

Pgl H

1 MKISFIIATL NSGGAERVLV TLVNALCKEH EVSIIKFHAG **ESFYKLENE**V KVTSLEQFRF
61 DTLYHKIASR FKKFFALRKA LKESKADVFI SFLDTTNIAC IAAKIGFKTP LIISEHSNEA
121 YLKPKIWRFL RRVSYPFCDA LSVLGSSDKV YYERFVKRVR LLLNPCHFSD **EIPFDSSFE**K
181 ENLVLFIGRL DHNKNPAMFL KAIAHLDKNL QENYKFVIAG DGELRQELEY KVKSLGIKVD
241 FLGRVENVKT LYEKAKVLCL CSFV**EGLPTV LIE**SLYFEVC RISSSYYNGA KDLIKDNHDG
301 LLIGCDD**EIA LAKKLE**LVLN NENFRKELVN NAKQRCKDFE ISHIK**EEWLK LIAE**VKNA

Supporting Figure 3

Threaded model in which the amino acid sequence of PglH has been threaded into the structure of PimA. E265 (red spheres), E273 (red spheres) and R191 (orange spheres) were all inactive and appear at the hypothesized active site, while all other alanine mutants E41, E49, D170, E171, E179, D306, D307, E308, E316, E346, and E354 (yellow ball and stick) had no major effect on activity.